APPROACHES TO DESIGN AND SYNTHESIS OF ANTIPARASITIC DRUGS

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PREFACE

Since parasitism provides a survival advantage to organisms in nature, the prevalence of parasitic organisms in the world is widespread. Humans alone, for example, host around one hundred kinds of eukaryotic parasites. Many of these parasites cause disease in the host, some in normal course while others only when the 'normal' host-defence system is disturbed, for instance cryptosporidium is a major cause of wasting syndrome, and *Pneumocystis carinii* that of pneumonia in AIDS patients. The diseases caused by parasites in humans are many and varied. By taking appropriate public health measures parasitic diseases have been almost eliminated from developed countries, but continue to be a major health problem in developing tropical countries. Six major tropical parasitic diseases, viz. malaria, filariasis, schistosomiasis, African trypanosomiasis, Chagas' disease and leishmaniasis, together account for more than one million deaths annually and are the cause of morbidity in hundreds of millions more.

Antiprotozoal agents were the earliest chemotherapeutic agents to be discovered by design around the beginning of the 20th century - methylene blue as an antimalarial in 1891, iodinated 8-hydroxyquinolines for amoebiasis in 1904, trypan red for trypanosomiasis in 1907 and trypan blue for babesia in 1909. These discoveries provided useful chemical leads which in turn led to the development of important antiprotozoal drugs, particularly antimalarials, in the 1920s and 30s, and some of these drugs are in use even today. However, by the time the modern era of chemotherapeutic research got into its full stride in the wake of the discovery of sulfonamides and penicillin antibiotics in 1930s and 1940s respectively, parasitic diseases had been more or less controlled through public health measures in the developed countries where most of the research on new drugs was being carried out. Not much attention was, therefore, paid to the discovery of antiparasitic drugs. Of the new drugs discovered and introduced between 1988 and 1992 the Annual Reports on Medicinal Chemistry list 48 cardiovasculars and 44 antibacterials, but only 4 antiparasitic drugs, though parasitic diseases affect a much larger proportion of the human population than cardiovascular and bacterial diseases.

Another cause of concern is the increasing incidence of development of resistance by parasites to existing drugs, particularly antiprotozoals, which has resulted in the depletion of the already limited armamentarium of antiparasitic drugs. The major gaps in the chemotherapy of parasitic diseases are: non-availability of an

orally active, safe antileishmanial drug; low safety margin of primaquine, the only antirelapse antimalarial available; rising incidence of chloroquine/multidrug-resistant *P. falciparum* and emergence of pockets of chloroquine-resistant *P. vivax*; inadequacy of existing antitrypanosomal, antiamoebic/antigiardia drugs; non-availability of a really effective and safe macrofilaricide; and limited number of antihelmintic drugs. In view of the logistic difficulties faced by most developing countries in substantially improving public health measures in the foreseeable future, prophylactic and curative measures will have to be their mainstay for the prevention and control of parasitic diseases for many years to come. There is, therefore, urgent need to fill these therapeutic gaps. This will require much greater investment in research and development of antiparasitic drugs. It is worth mentioning here that as a result of the dramatic developments in molecular biology in recent years our understanding of the biology of parasites, of the pathophysiology of parasitic diseases, of host-parasite interactions and of the mechanisms evolved by parasites to evade attacks by the host defence system in order to survive and proliferate, has grown enormously. These advances have opened up a range of new possibilities for treatment of parasitic diseases. The big challenge is to convert this new knowledge to new chemotherapeutic agents.

In this book Dr. Satyavan Sharma has presented a comprehensive and up to date account of the chemotherapy of parasitic diseases, both human and veterinary. The book starts with an Overview of parasitic diseases in Chapter 1. The body of the book is divided into two parts, anthelmintic drugs (Chapter 2-12) and antiprotozoal drugs (Chapter 13-21). Both parts start with chapters highlighting the 'Biochemical Targets' available for chemotherapeutic interference. Individual chapters deal with one chemical class of compounds and describe their origin, structure-activity relationship, mode of action, and methods of synthesis and their status both in clinical and veterinary practice. The book will be useful to a wide spectrum of readers viz., students embarking on a research career in parasitic chemotherapy, clinicians (and veterinarians) and clinical pharmacologists desiring detailed information about the drugs currently in use, and pharmaceutical technologists wanting to update their knowledge of the methods of manufacture.

Sharma's involvement with parasitic chemotherapy started in 1969 when he joined me at the Central Drug Research Institute as a research fellow to work on the *"Chemotherapy of Filariasis"* for his Ph.D. degree. His special contribution in this field was the optimization of the activity of benzimidazoles, and one of the compounds synthesized by him, 82-437, has shown very significant oral macrofilari-

cidal activity in experimental animals. After preclinical toxicity studies this candidate drug was under Phase 1 clinical studies at the time of his untimely death in a road accident on 19th March, 1993. His research career was wholly devoted to parasitic chemotherapy; indeed, he was one of the few fighters against this sadly neglected group of infections. This single-author book, which is becoming a rarity these days, reflects the dedication to and deep involvement of Satyavan Sharma in the subject. Satyavan had often discussed the scope and progress of the book with me, and had requested me to write the Foreword. Little did I realise at that time that he would be snatched away so soon. Editing the book has been very satisfying. I have thereby honoured the memory of a very valued student and colleague, whose promising career has been cut short.

I would like to record my special thanks to Mr. Ramesh Sharma, Satyavan's younger brother, who undertook the onerous task of getting the manuscript ready for the press; he was wholly responsible for the coordination between editor, artist and typist and also did much of the checking. Drs. Ram Pratap and Amita Dave did most of the proof reading and reference checking, and their help has been most valuable. The typing on the computer was done by Mr. V.K. Kanal and the computer-graphics was provided by *Multi Media Computer Point*, Lucknow. My most grateful thanks to them.

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CHAPTER 1

PARASITIC DISEASES : AN OVERVIEW

1. INTRODUCTION

Parasitic diseases continue to be the major public health problems in tropical developing countries. These are responsible for a high degree of morbidity, mortality and socio-economic under-development in these regions. According to WHO estimates the annual death toll due to parasitic diseases is nearly 2.5 million throughout the world [1,2]. The biggest killers of these are malaria, amoebiasis and hookworm infections responsible for killing 1,000,000-2,000,000; 40,000-110,000 and 50,000-60,000 patients, respectively, every year [2]. In view of the detrimental impact of parasitoses on human health and resulting economic losses, WHO has included five parasitic diseases, viz. filariasis, schistosomiasis, malaria, trypanosomiasis and leishmaniasis and one mycobacterial disease, viz. leprosy, amongst its priority research areas.

Parasitic infections are distributed world-wide but they are particularly endemic in the tropical zones of the globe. The main cause of the widespread prevalence of parasitic infections in the tropics is the climate; high temperature and humidity are ideal for parasite growth. This when combined with low standards of living, poor sanitation, lack of personal hygiene, inadequate prophylactic measures and abundance of disease carriers provide ideal situation for survival, dissemination and propagation of the parasites. Although most parasitic infections can be effectively prevented by proper prophylaxis, strict sanitary regulations and vector control, the implementation of these measures, pose practical problems in developing countries due to climatic factors and economic constraints. Immunotherapy and vaccination may emerge as useful tools to control and eradicate parasitic infections, but these are still in their early stages of development. Therefore, chemotherapy is the main tool available to combat parasitic diseases in humans and domestic animals.

Parasitic diseases are caused by the invasion of humans and animals by several species of protozoans and helminths. The pathogenic protozoans may invade the blood circulation, liver, spleen, or external organs such as mouth, gastrointestinal tract and vagina. A major population of the helminths,on the other hand, parasitize the gastrointestinal tract while some live in the blood circulation, lymphatics, connective and subcutaneous tissues, eyes, lungs, and liver. Most of the extra-intesti-

nal protozoal infections are acquired by the bites of an insect vector which injects infective larvae of the parasite while feeding on the blood of the victim. The intestinal protozoal diseases are contracted by ingesting protozoal cysts through food, drink and faecal contact. The helminth diseases in man may be acquired either by ingestion of helminth eggs through food, drinks or soiled hands or penetration of infective larvae through skin. The latter mode of infection may occur either by the bites of an arthropod vector or exposure of legs and arms to soil and water contaminated with infective larvae [3-6].

2. THE HELMINTH INFECTIONS

A variety of helminths belonging to the class nematoda (roundworms), trematoda (flatworms or flukes) and cestoda (tapeworms) are known to infect humans and domestic animals. The diseases caused by these worms are not only responsible for occasional deaths and wide range of health problems in man, but also exert detrimental effect on the nutritional and immune status of the host resulting in low resistance against other infections. The presence of helminth infections in livestock leads to decrease in output of animal products (milk, fat, butter, meat, eggs, wool and leather etc.) and has, therefore, strong socio-economic impact in countries with agro- and dairy-based industries [7].

The economic losses incurred by helminth infections have been assessed in several ways. In ascariasis the loss is due to the carbohydrate depletion by *Ascaris* worms in the patients. It has been estimated that a patient with 20 adult worms of *Ascaris lumbricoides* may lose 2.8 g of carbohydrate daily [8] which amounts to 2800 kg of carbohydrate per 1 million cases per day. Thus the world-wide loss of carbohydrate for 1100 million patients carrying ascariasis would be nearly 3080 tonnes per day. Stephenson and coworkers [9] have shown that ascariasis is not only associated with poor growth and protein-caloric malnutrition in pre-school children, but also reduces absorption of macronutrients and vitamin A. The authors also showed that economic loss due to ascariasis in Kenya in 1976 was about US$ 5 million which could have been saved by the use of an anthelmintic costing about US$ 1 million only.

The hookworms are other important human parasites which cause high degree of economic losses among the victims. An early estimate [10] showed that Japan suffered an economic loss of US$ 60 million per year due to hookworm infections that could have been prevented by treating the patients with anthelmintics costing

only US$ 7 million. Since hookworms survive on the direct blood feed from the hosts, their presence in human leads to heavy blood loss resulting in hypochromic anaemia. It has been estimated [11,12] that one *Ancylostoma duodenale* sucks 0.15-0.23 ml of blood per day. Thus, for 1 million patients carrying an average of 100 hookworms, the total blood loss would be 15,000-23,000 liter per day. In case of *Necator americanus* which consumes about 0.03 ml of blood daily from its host, the total blood loss for 1 million cases with an average of 400 hookworms would be nearly 12,000 liter daily.

The high degree of physical deformity (hydrocoele and elephantiasis of legs and arms) caused as a result of lymphatic filariasis and blindness due to onchocerciasis still pose major medical challenge in different countries of Asia, Africa and Latin America. Similarly, the grave clinical manifestations produced by tapeworm infections, schistosomiasis, trichinosis and hydatidosis continue to be major health problem for millions of people living both in the developing and developed nations of the world. Recent studies carried out by Stephenson and coworkers [13,14] in Kenyan subjects show that by deworming the infected population, it is possible to improve the growth and physical fitness in children and productivity in adults.

Worm infections are also known to exert detrimental effect on the health and productivity of cattle, equines, sheep, goats, pigs and fowls, thereby considerably hampering the yield of various animal products like milk, eggs and wool etc. Urquhart [15] has earlier estimated that the potential loss due to uncontrolled nematode parasites in ruminants was nearly US$ 160 million. By treating the infected ruminants with anthelmintics costing US$ 20,000, this loss was reduced to around US$ 30 million. In Great Britain, about US$ 100 million is lost annually due to liverfluke infections in sheep and cattle [16]. Similarly, in Florida, USA, the economic loss from liver condemnation was more than US$ 500,000 per year which was due to liver fluke infections in cattle [17]. Stephenson *et al.* [18] have shown that pigs infected with *Ascaris* spp. and on low protein diet consumed 6.8 kg of food to gain 1 kg as compared to control pigs which needed only 3.3 kg of food to gain 1 kg of weight. It has been estimated that the total loss due to parasitic infections in livestock in USA is more than US $ 3 billion per year [19]. Thus eradication of parasitoses from livestock is economical as it increases the productivity of the animals [20].

These are only a few examples which clearly demonstrate that treatment of helminth diseases in man and domestic animals is not only essential to have a healthier society but also to raise the living standards by boosting socio-economic

status of the people living in the tropics. A brief outline of the helminth diseases occurring in man and animals is given below.

2.1 Helminth diseases of human

Worm infections are amongst the earliest diseases known to mankind. Their impact on human health and its serious dimension was highlighted in the classical paper of Stoll "This Wormy World" published in 1947 [21]. Estimating the world population at that time as 2.1 billion, Stoll reported that nearly 650 million people were infected with the intestinal roundworm, *Ascaris lumbricoides*. The incidence (in million) of other intestinal nematode infections was as follows: hookworm disease (450), trichuriasis (350) and strongyloidiasis (35). With the increase in world population the prevalence of these worm infestations has proportionally increased. According to recent estimates [22,23] in a global population of 4.3 billion there are 1100-1300 million cases of ascariasis while the incidence of hookworm infection may touch the 1000 million mark. Similarly the number of cases suffering from trichuriasis, enterobiasis and strongyloidiasis may range from 500-1000, 300-500 and 50-100 million, respectively. In addition, nearly 400 million people around the world are known to suffer from the debilitating effects of filariasis, while there are 130 million cases of tapeworm infections and nearly 200 million people are infected with shistosomiasis in different parts of the world [24-26]. These figures indicate that helminthiasis is undoubtedly a wide-spread parasitic disease of the tropics. The following are the important helminths which are pathogenic to human.

2.1.1 Nematode (roundworm) infections

2.1.1.1 Ascariasis

It is caused by *Ascaris lumbricoides*, the adult worms of which live in the lumen of small intestine of man. The infection is acquired by consuming fruits, salad, vegetables and drinks contaminated with *Ascaris* eggs. Poor sanitation and lack of personal hygiene are the main reasons for the wide-spread prevalence of the disease. That is why ascariasis is primarily seen in people living in slums and rural areas where human excreta are disposed in the vicinity of residential localities. Children are the major victims of the disease.

Ascariasis has a worldwide distribution affecting nearly 1000-1300 million people with nearly 20,000 patients dying every year [1,2,26]. In addition to the protein-energy malnutrition caused in children, ascariasis is also associated with a series of pathogenic effects. The main clinical manifestations of the disease during migra-

tion of larvae from the gut to lungs are atypical pneumonia with inflammation of lung and liver cells, fever and eosinophilia. The adult worms may occasionally wander into liver, appendix and oesophagus or may obstruct the intestinal tract causing grave colic pains.

2.1.1.2 Hookworm infections

The hookworm disease is caused by the blood sucking nematodes, *Ancylostoma duodenale, A. ceylanicum* and *Necator americanus*, commonly known as hookworms, in the intestine of human. The infection normally takes place when farmers working in coffee, banana, sugarcane, sweet potato, rice and maize fields expose their bare feet to the soil fertilized with human excreta where the infective larvae penetrate the skin and enter the blood circulation. These larvae eventually grow into adult hookworms and live in the intestine where they engulf the intestinal villi into their buccal cavities and survive on direct blood feed from the host.

Hookworm infections are usually found amongst the field workers and poor masses of the tropics. The most common clinical symptom of the disease is hypochromic anaemia resulting from heavy blood loss. This leads to general weakness, fatigue, anorexia and poor health. Hookworm infection is also known to cause various gastrointestinal disturbances and epigastric pain. Children, with heavy worm burden, show poor mental and physical growth.

2.1.1.3 Trichuriasis

The disease is caused by *Trichuris (Trichocephalus) trichiura,* commonly known as whipworms, which live embedded in the intestine especially in the large bowel and caecum of man. The infection is cosmopolitan and is found more in children than adults. The usual mode of infection is the consumption of water and vegetables contaminated with the ova of *T. trichiura.* The disease is usually asymptomatic in the case of light infection; however, heavy infections may lead to anaemia, eosinophilia, abdominal pain, diarrhea, mucoid stool and occassional prolapse of the rectum.

2.1.1.4 Strongyloidiasis

Like hookworms, strongyloidiasis is also caused by penetration of the human skin by filariform larvae of *Strongyloides stercoralis.* The parasites possess slender and thread-like structures, hence called threadworms. They live burried in the intestinal mucosa of human.

The movement of larval and adult parasites produces several pathological changes like inflammation of the cells, allergic reactions and eosinophilia. The clinical manifestations of the disease include attacks of diarrhea, diffused abdominal pain, epigastric discomfort and hunger pains, which may lead to false diagnosis of peptic ulcer. Heavy infections may cause malabsorption, flatulence and abdominal distension.

2.1.1.5 Enterobiasis

It is a common helminth infection of man found mostly in children and is caused by *Enterobius (Oxyuris) vermicularis* called pinworms. The adult worms live attached to the mucosa of the lower ileum, caecum and terminal parts of the colon. Man acquires the infection by ingestion of eggs of *E. vermicularis*, which reach the mouth through soiled hands or while handling contaminated clothings and bathroom fixtures. Since the eggs are resistant to desiccation, the infection also occurs by consuming raw vegetables, food and drinks contaminated with pinworm eggs.

E. vermicularis does not produce significant pathological changes in the host except intense pruritis caused by the migrating gravid females and eggs laid by them on the perianal region. Scratching of the perianal skin may lead to dermatitis, eczema and secondary bacterial infections. The patient may also suffer from anorexia, restlessness, insomnia and mild to acute abdominal pain. Occasionally, vulvovaginitis may also occur in young girls.

2.1.1.6 Trichostrongyloidiasis

A number of species of *Trichostrongylus* (pseudohookworms) are known to parasitize the small intestine of sheep, goats, camels and occasionally humans in the tropics. *Trichostrongylus orientalis* is the main etiological agent in humans. The adult worms live embedded through their heads in the mucosa of the duodenum and jejunum. According to an early estimate [21] more than 5 million people suffered from trichostrongyloidiasis in Asia, Russia and CIS (former USSR) alone.

Human acquires the infection when the semi-filariform larvae of *Trichostrongylus* species enter the body through skin or mouth (while consuming contaminated drinks). The use of night-soil as fertilizer in some countries and resistant nature of the eggs provide a strong basis for propagation of this infection in farming communities.

Trichostrongyloidiasis is generally symptomless and little is known about its

pathology. However, severe infections may give rise to mild anaemia as the worms may suck blood with their capillary heads embedded in the mucosa.

2.1.1.7 Capillariasis (wasting disease)

This is a relatively new enteric helminth disease of human caused by a minute whipworm, *Capillaria philippinensis*, which was responsible for severe enteritis with high mortality in the Province of Ilocos Norte on the north west coast of Luzon of Philippines in 1967. Later the infection was reported from other adjacent provinces and from southern Thailand [27].

The adult worms of C. *philippinensis* are found embedded in the mucosa of the small intestine. Some worms may also be seen moving free in other parts of the alimentary canal such as larynx, oesophagus, stomach and colon. The infection occurs when infected fresh water fish and crustaceans are eaten raw by man. The infection causes a syndrome which resembles with that of autoinfected and disseminated strongyloidiasis giving rise to abdominal pain, vomiting, malaise, nausea and anorexia. In chronic cases, there is cachexia with muscle weakness, muscle wasting and depletion of minerals. In addition, there is protein losing enteropathy with extreme malabsorption of sugars and fat. There is continuous weight loss and often leads to death of the patient within 2-4 months [28].

2.1.1.8 Intestinal angiostrongyliasis

It is a newly discovered intestinal helminth infection of man found in Latin America. The causative agents of the disease, *Angiostrongylus costaricensis* and A. *cantonensis*, produce tumor-like lesions in the colon. Chemotherapy is usually not very satisfactory. Sometimes surgical intervention may be required.

2.1.1.9 Trichinosis

This is a disease caused by *Trichinella spiralis* which is essentially a nematode parasite of rats. The adult worms live in the small intestine of man. The infection also occurs in cats, dogs, pigs, polar bears, seals and whales. It is a widespread infection occuring throughout the temperate regions of the world and is found especially in people eating undercooked pork and pork products. The disease has been reported from several parts of United States, Hawai, Alaska, South America, Africa, Europe, Russia, CIS (formerly known as USSR) and Asia.

Trichinosis is transmitted to humans, when they eat infected pork. The hogs and rats serve as the reservoirs of *T. spiralis*. However two hosts are required to

complete the life cycle of the worm. This may be a hog-to-man, rat-to-hog or hog-to-hog cycle for the *T. spiralis* development. Humans, hogs and rats are infected by eating infected pork containing the cysts of *T. spiralis*. On reaching the digestive tract, the larvae emerge from the cysts and mature into adult males and females within a few days. The fertilized females liberate 1000-1500 larvae in the intestinal mucosa. The larvae reach the different parts of the body through blood circulation and finally encyst in the muscles of diaphragm, chest wall, neck, limbs, larynx, tongue and eyes. These cysts survive for several years and develop into adults on getting favourable conditions.

The hogs become infected when they feed on the garbage containing infected pork scraps (hog-to-hog cycle). The hogs may also acquire the infection by eating rats infected with *T. spiralis* while rats get the disease by eating the infected pork (hog-to-rat cycle). The hog-to-man cycle arises when man eats the infected pork and pork products.

The presence of larvae and cysts of *T. spiralis* may cause localised inflammation, necrosis, damage of muscle fibres and increased eosinophil counts. Sometimes death may occur due to toxemia, trichinous encephalitis or myocardial damage caused by invasion of musculature by the larvae.

The clinical symptoms of the disease are variable and depend upon the intensity of the infection. The presence of adult and larval parasites may give rise to abdominal pain, nausea, vomiting, diarrhea and blood in stool. The migration of larvae leads to high fever, edema of face, eyelids, muscular pain in chest wall, cough, dyspnoea and stiffness of limbs. In severe cases, neurotoxic symptoms, myocardiasis, meningitis and encephalitis may also be observed in some patients.

2.1.1.10 Creeping eruption

Creeping eruption or larva migrans in man is caused by the presence of the larvae of dog and cat hookworms, *Ancylostoma caninum* and *A. braziliense* in the skin. Some other nematodes like *Uncinaria stenocephala* (European dog hookworm) and *Gnathostoma spinigerum* also produce somewhat similar cutaneous lesions. The creeping eruption is prevalent in different regions of the warm climates, especially in the Americas, Africa and Asia. It is estimated that nearly 10 million people around the world suffer from this disease [24].

Both wild and domestic cats and dogs are the natural reservoir hosts of *A. braziliense*. The female worms produce eggs which pass out with the stool and de-

velop into filariform infective larvae in the soil. Man gets infected when these larvae penetrate the skin. The larvae produce serpiginous tunnels under the skin and do not undergo further development.

The epidemiology and life cycle of *G. spinigerum* is different. The adult worms live in the alimentary canal of cats, dogs and tigers. The eggs produced by the adult females come out with the faeces and become embryonated in the soil. They hatch upon coming in contact with water and give rise to cylindrical, ensheathed and rhabditiform larvae. These larvae are ingested by copepods where further development of the parasite takes place. When fish, frogs, birds and snakes eat these infected copepods, they become infected and third stage larvae are generated. Human acquires the infection by eating the above infected intermediate hosts.

After infecting the humans, the larvae move under the skin at the rate of 2-3 cm per day producing linear, erythematous and serpiginous tunnels. This causes inflammation and pruritus and may also damage lungs, kidneys, eyes and other parts of the body. In the case of gnathostomiasis, the patient may show edema, eosinophilia and leukocytosis. Some fatal cases of eosinophilic myeloencephalitis caused by the migration of immature worms of *G. spinigerum* in brain [29] and urinary gnathostomiasos [30] have also been reported. The larvae of *G. spinigerum* can also cause abdominopleuropulmonary syndrome which may resemble like acute appendicitis or pleurisy.

2.1.1.11 Visceral larva migrans

This form of tissue helminthiasis is caused by the migration of the larvae of dog and cat ascarids, *Toxocara canis* and *Toxocara cati* in the visceral tissues of humans. The disease is more common in children than the adults who often come in contact with the eggs of *T. canis* or *T.catti* while playing on the ground polluted by cat and dog faeces. The infection by *Toxocara* in dogs is cosmopolitan; however, surveys indicate that it is prevalent in the U.S.A., Britain, Africa and some parts of Asia [31,32]. In humans the rate or incidence of visceral larva migran is low; nevertheless nearly 10,000 people are estimated to carry this disease around the world [24].

After reaching the intestine of human, the eggs liberate larvae which penetrate the bowel wall and enter the portal blood system from where they are carried to the liver, lungs and different parts of the body. The larva seldom develops into the adult in the human intestine as man is not its natural host. In cats and dogs, the larvae develop into male and female adults.

The early phase of toxocariasis in man shows low to high eosinophilia which disappears as the infection grows chronic. Attacks of fever, malaise, nausea, vomiting, cough, abdominal pain, anorexia, weight loss and muscle and joint pain may be observed occasionally. In chronic cases, the patient may report of some eye problem like weak sight and impared vision resulting due to migration of larvae in the eyes. Liver complications like hepatitis and lung problems like cough and asthmatic attacks may occur due to migration of larvae to liver and lungs, respectively. Similarly, involvement of brain in toxocariasis can give rise to an epilepsy like syndrome.

2.1.1.12 Filariasis

Filariasis is one of the most widespread parasitic diseases of the tropics affecting nearly 300-400 million people around the world [24,33]. According to recent estimates by the WHO, the world-wide prevalence of filariasis is about 280-290 million [1,2]. The main disease causing worms in humans are *Wuchereria bancrofti, Brugia malayi, Onchocerca volvulus, Loa loa, Dipetalonema perstans, Dipetalonema streptocerca* and *Mansonella ozzardi*. The haematophagous arthopods, mosquitoes and flies, serve as the intermediate hosts in the life cycle of the parasite. The transmission of the infection to humans occurs when the mosquitoes feed on the blood of man. After reaching the blood circulation of man, the infective larvae undergo several moultings and develop into adult male and female worms living in lymph nodes, lymphatic vessels, connective tissues and other organs of the body. The female worms produce microfilariae which migrate to blood stream and are sucked by mosquitoes and flies where the life cycle of the parasite gets completed.

The early phase of the infestation by *W. bancrofti* and *B. malayi* is characterised by high fever, chills, enlargement of lymph nodes, pain and swelling in testes and thickening of the spermatic cord. Later the blockade of the lymphatic circulation occurs which leads to hydrocele and chyluria in the patients. In chronic cases, the obstruction of the lymphatic system may take place causing massive enlargement of legs (elephantiasis), arms, scrotum and breasts. The clinical characteristics of *L. loa* infection include appearance of painful Calabar swellings on face, limbs, head, wrist and forearms. Sometimes the adult worm may migrate in the eye ball giving rise to blindness and nervous disorders.

The *O. volvulus* infection, also called river blindness, is the most serious form of human filariasis responsible for blindness in a large population of the African con-

tinent. The early stage of ocular onchocerciasis, caused by the presence of micro-filariae in the eyes, is marked by pain in the eyes, photophobia and lacrimation which gradually leads to conjunctivitis with eventual loss of vision. The infection also give rise to dermal problems and genital elephantiasis.

The filariases caused by *M. ozzardi* and *Dipetalonema* spp. are usually non-pathogenic. However, *M. ozzardi* may occasionally cause hydrocele and enlargement of lymph nodes while *D. perstans* may be associated with fever, abdominal pain, itching and edema of scrotum in the patients.

2.1.1.13 Guinea worm infections

The guinea worm infection is a very old parasitic disease caused by *Dracunculus medinensis* which are enlongated thread-like filarial worms living in the deep connective and subcutaneous tissues of man. The infection has been reported from different parts of Africa, South America and Asia; however, it is endemic in Cameroon, Lake Chad, Sudan, Uganda and India.

Humans become infected with guinea worms by drinking water contaminated by infected *Cyclops*. On reaching the intestine, the *Cyclops* get digested by gastric juice liberating free larvae which pierce the intestinal mucosa and reach the connective tissues where they live and attain sexual maturity in about a year.

After fertilization the female worms migrate under the skin and produce a dermal blister which causes irritation. When the patient scratches the skin or the blisters come in contact with water, they burst liberating numerous motile rhabditiform larvae which are soon engulfed by the *Cyclops*; thus the life cycle of the parasite is completed.

The early stage of the infection produces no pathological sign. The worms require 8-12 months of incubation period before they are sexually mature. Symptoms appear only after the female is fertilized and is ready to discharge larvae. This stage is marked by appearance of reddish papular lesions on legs and arms which cause itching, fever, giddiness, utricaria and allergic reactions. Repeated scratching of skin or contact with water ruptures the blisters which release milky fluid with larvae which may lead to secondary infections. The migration of adult guinea worms to other parts of the body may result in neurological damage, joint swelling and arthritis.

2.1.1.14 Tropical pulmonary eosinophilia (TPE)

This is an allergic manifestation produced by the presence of various helminth

parasites such as *Ascaris lumbricoides, Trichinella spiralis, Strongyloides stercoralis, Toxocara* spp., *Brugia malayi* or *Dirofilaria* spp. in humans. The disease has been reported from different parts of Asia and Africa.

Since the disease is associated with the respiratory system, its histopathology is confusing. However, various lesions in the lungs may be seen with chest X-ray. The clinical symptoms of the disease may range from mild to severe attacks of cough, asthma and bronchitis. The eosinophil counts in blood may rise upto 20-90% and the leukocytes may increase upto 60,000/cu mm. The infection is usually taken to be confirmatory if the eosinophil counts exceeds 3000/cu mm and the total leukocyte count is more than 10,000 cells/cu mm in the blood. X-ray picture of lungs may show chronic bronchitis and other signs which are sometimes mistaken for Loeffler's syndrome or tuberculosis. In such cases, the antigen of *D. immitis* may be used to diagnose tropical eosinophilia [34,35].

2.1.2 Trematode (flatworm, fluke) infections

2.1.2.1 Schistosomiasis

Schistosomiasis is a major helminth disease of man caused by the invasion of the blood circulatory system by four species of blood flukes, viz. *Schistosoma haematobium, S. mansoni, S. japonicum* and *S. intercalatum*. The adult worms of human schistosomes have separate sexes but they are dioecious (existing together as males and females). The male worm has a groove (gynecophoral canal) along its ventral side in which it carries the female worm during most of its life span.

The adult worms of *S. haematobium* cause urinary schistosomiasis (bilharziasis) and live in the portal system, pelvic veins, particularly in the vesical and pelvic plexus and occasionally in the veins of the colon and rectum. The worms excrete their eggs in the urine of man but rarely in the faeces. The other three schistosomes (*S. mansoni, S. japonicum, S. intercalatum*) are responsible for intestinal bilharziasis and live in the portal blood system, mesentric veins and haemorrhoidal plexus. Unlike the *S. haematobium* worms, these three blood flukes pass their eggs in the faeces and rarely in urine.

Schistosomiasis has a wide geographical distribution. It has been reported from various parts of Africa, Asia and South America affecting nearly 200 million people around the world [2] of which 20 million people are estimated to suffer from schistosomiasis in Egypt alone [36]. It has also been reported that *S. mansoni* infects nearly 70 million people throughout the world [37].

Humans acquire schistosomiasis while working in rice fields, lakes, ponds, canals and water streams where the cercariae present in the water penetrate the skin and enter the blood stream. Drinking of water containing cercariae of schistosomes may also infect humans. After reaching the blood circulation, the cercariae undergo some changes and eventually mature into male and female adults feeding on portal blood. When the female worms get fertilized they deposit their eggs on the walls of the urinary bladder (*S. haematobium*) or intestine (*S. mansoni, S. japonicum, S. intercalatum*) which are later expelled with urine and faeces respectively.

When the eggs come in contact with water, they liberate free swimming embryos called miracidium which seeks its intermediate host, usually a fresh water or amphibious snail, and gets itself attached to the host's body. If the miracidium does not find a snail it dies soon. After attaching to the body of a snail, the miracidium penetrates the musculature and reaches different parts of the body and produces a mother sporocyst. The mother sporocyst then produces several daughter sporocysts which later produce a large number of fork-tailed infective larvae called cercariae. These cercariae then leave the snail and swim freely in the water searching for their definitive host, the humans, to complete the life cycle.

The pathological changes and clinical characteristics of schistosomiasis depends upon the nature and site of infestation in humans. The early phase of the disease is marked by schistosoma dermatitis and katayama fever (fever, chills, malaise). The main clinical manifestations of the disease are:

(a) Urinogenital schistosomiasis: It is the most common form of schistosomiasis along the Nile Valley in Egypt which involves different parts of the male and female urinogenital system. The main clinical symptoms may include formation of kidney stones, renal failure, haematuria, renal and ureteric colic, fistula of bladder, penis and vagina, generation of ulcer leading to the formation of pus tracks in scrotum and penis, swelling of testes and cancer of the bladder [38].

(b) Hepatosplenic schistosomiasis: This is marked by inflammation and fibrosis of the spleen. The liver also shows periportal cellular infiltration and fibrosis followed by wasting and swelling of the cells. The main diagnostic clinical symptoms of hepatosplenic schistosomiasis are weakness, weight loss, epigastric discomfort, diarrhea, enlargement of liver and spleen, swelling of limbs and clubbing of fingers.

(c) Alimentary schistosomiasis: This condition of the disease is characterised by lesions in different parts of the alimentary tract such as stomach, colon, intestine, ap-

pendix or rectum. The main clinical symptoms produced are peptic like ulceration, fistula of rectum and colon, rectal prolapse, rectal and anal polyposis leading to intestinal obstruction due to large group of polyps, bouts of diarrhea and other gastrointestinal disturbances.

(d) **Cardiopulmonary schistosomiasis:** The ova and worms of schistosomes in the lungs cause cardiopulmonary schistosomiasis. The penetration of worms into lungs causes fibrosis and obstruction of pulmonary blood circulation. The main clinical manifestations of this form of schistosomiasis are fatigue, palpitation, cardiac pain, giddiness and pulmonary hypertension.

2.1.2.2 Fasciolopsiasis (intestinal fluke infections)

A number of trematode parasites are known to infest the gastrointestinal tract of humans and animals, hence commonly known as the intestinal flukes. Although several intestinal flukes such as *Fasciolopsis buski, Heterophyes heterophyes, Metagonimus yokogawi, Echinostoma ilocanum* and *Gastrodiscoides hominis* are parasitic to man, the present section is intended to deal only with the disease caused by the giant intestinal fluke, *F. buski.*

Fasciolopsiasis in man is acquired by eating raw stems, bulbs or fruits of some water plants (water chestnut or water caltrop) infected heavily with the matacercariae of *F. buski.* These plants grow in ponds and other water reservoirs polluted with human excreta.

The adult worms which are hermaphroditic, lay large number of eggs in the intestine of humans that are passed out with the faeces. These eggs hatch in fresh water and develop into miracidium and later enter the snail's body forming mother sporocysts, rediae, daughter rediae and finally cercariae. The cercariae encyst on different parts of the edible aquatic plants from where they reach the human body and the life cycle of the intestinal fluke is completed.

The infection is prevalent in Central and South China, Vietnam, Taiwan, Burma, Thailand, Bangla Desh and some parts of India and eastern Asia. The main clinical symptoms of the disease are abdominal pain and diarrhea with nausea, vomiting and anorexia. Children show edema of the face; sometimes death may also occur following cachexia.

2.1.2.3 Liver fluke infections

Several species of trematodes infect the bile duct and liver of man and ani-

mals. The main liver flukes infecting man are *Fasciola hepatica, F. gigantica, Clonorchis sinensis, Opisthorchis felineus* and *Dicrocoelium dendriticum*.

The liver rot disease, caused by *F. hepatica*, is normally a disease of sheep, goats and cattle who acquire the infection by grazing on aquatic plants containing encysted metacercariae. Humans are infected by ingesting these encysted metacercariae through drinking water. The life cycle of *F. hepatica* is similar to that of *F. buski*. The eggs excreted with the faeces of cattle, goats, sheep and humans hatch in water giving rise to miracidia which infect a fresh water snail. After undergoing some development in the snail, the parasite emerges out as cercariae which encyst on aquatic plants as metacercariae. After reaching the intestine of the definite host, the metacercariae come out of the cyst and migrate through the intestinal wall and reach the liver parenchyma and bile ducts.

The Chinese liver fluke disease is caused by *C. sinensis*. Man is usually infected by eating raw, inadequately cooked or salted fish containing the metacercariae of *C. sinensis*. The adult worms live in the bile duct and liver of cats, dogs, pigs and humans. They produce operculated eggs contanining fully developed miracidia. The eggs are carried down through the bile to the duodenum from where they come out with the faeces. These eggs when ingested by the snail, develop into mother sporocysts, rediae and finally cercariae. After leaving the snail, the cercariae swim in the water where they are swallowed by fishes. The cercariae may also penetrate the skin of the fish below the scales and reach the musculature. Here the cercariae get encysted and wait for the definite host to complete their life cycle.

The lancet fluke infection is caused by *D. dendriticum* in sheep, goats, deer and humans. The adult worms live in the bile duct of the host giving rise to various liver troubles. The disease is acquired by ingestion of metacercariae while snails and ants serve as the intermediate hosts in the worm's life cycle.

Since the seat of predilection of the liver flukes is liver or biliary passage, the clinical manifestations produced by them chiefly relate to liver and gastric problems. The early stage of the infection is marked by epigastric pain, fever and eosinophilia. Later the patient experiences diarrhea, anorexia, prolonged fever and abdominal pain. In chronic cases, the disease may lead to jaundice, cirrhosis of the liver and biliary duct, ascites and cachexia. Sometimes the patient may die of serious liver complications.

2.1.2.4 Lung fluke infections

Several species of *Paragonimus*, the lung flukes, infect the lungs, pleura, bronchi and sometimes liver and spleen of humans, dogs, cats, pigs, and some wild animals. The most common lung fluke infecting man is *Paragonimus westermani*. The infection is more prevalent in countries of the Far East, North and South America and Africa.

Human acquires lung fluke infection by eating raw crabs and cray fishes infected with metacercariae. The adult worms live in the lungs of humans and produce eggs which are excreted with sputum or swallowed to be passed out with faeces. The eggs hatch in water and liberate miracidia which later infect the snails and undergo futher development to mother sporocysts, rediae and cercariae. The cercariae then attack their second intermediate hosts, the fresh water crabs and cray fishes where they encyst in the musculature and eventually develop into metacercariae. When humans eat these crabs and fish raw, the cysts dissolve and liberate the metacercariae in the duodenum. The liberated metacercariae penetrate the intestinal wall and migrate through different parts of the body to lungs where they develop into adults.

Migration of the young flukes through different parts of the body produces tunnels and cystic cavities giving rise to inflammatory reactions. The main clinical manifestations of the disease are cough with gelatinous blood, stained sputum and discomfort in the chest. Mild anaemia, fever, body pain, adbominal pain and diarrhea with occasional bloody mucus may also be observed. The parasite may migrate to the brain resulting in frequent Jacksonian type epilepsy.

2.1.3 Cestode (tapeworm) infections

2.1.3.1 Intestinal cestode infections

These are common parasitic infections of the tropics resulting from the invasion of the gastrointestinal tract of humans by *Taenia saginata* (beef tapeworm), *Taenia solium* (pork tapeworm), *Diphyllobothrium latum* (fish tapeworm), *Hymenolepis nana* (dwarf tapeworm), *Echinococcus granulosus* and *E. multilocularis*. The intestinal cestode infections have a world-wide distribution, though they are more prevalent in the tropical and sub-tropical regions. The incidence of tapeworm infections has always been high [21,39]; it has been estimated that nearly 80 million people carry *T. saginata* and there are 2 million cases of *D. latum* infestation around the world [24].

The tapeworms are hermaphroditic parasites which live in the alimentary canal of humans by attaching themselves to the mucosa of the intestinal wall. Humans acquire the infection by eating raw or poorly cooked beef, pork or fish infected with the larval cysts of tapeworms (measly meat). The infection may also be acquired by ingesting tapeworm eggs through drinking water, eating raw fruits and poorly cooked vegetables.

The cestodes have a simple life cycle consisting of one or two hosts. The adult tapeworms living in the intestine of the definite host (generally man) produce mature eggs which are shed out from gravid proglottids and pass out with the stool. These eggs are then taken up by the intermediate hosts (cattle, pigs and fishes). On reaching the alimentary canal of the intermediate host, the eggs liberate onchospheres which bore through the intestinal wall and enter the blood circulation from where they are carried to the different parts of the body. On reaching the musculature, the worms develop into their second larval stage called *Cysticercus* or bladder worms. These bladder worms get encysted and live in the muscles. When humans eat poorly cooked meat of cattle, pigs or fishes,the scolices emerge out of the bladder worm and attach to the intestinal wall and slowly develop into adult tapeworms. This completes the life cycle of the parasite.

The tapeworm infections generally do not produce significant pathological changes; however, some of them may cause occasional appendicitis or increased eosinophil counts. The clinical symptoms of the disease are nausea, vomiting, general weakness, weight loss, abdominal pain and diarrhea. In the case of *D. latum* infection, the patient exhibits 'bothriocephalus anaemia', a condition which resembles clinically with vitamin B_{12} deficiency (pernicious anaemia). This is due to the strong affinity of *D. latum* worms for vitamin B_{12}, an important component for blood synthesis in the host. The bladder worms may migrate in different parts of the body and may cause serious clinical complications (cysticercosis) characterised by nervous system disorders and different problems of eyes, liver and brain.

2.1.3.2 Hydatid disease

This is one of the most serious tapeworm infections caused by the hydatid cysts, formed by the larval stage of *Echinococcus granulosus* and *E. multilocularis* in man and domestic animals. The disease is prevalent wherever man is closely associated with cats, dogs and sheep. The adult worms live in the alimentary canal of the definite host (cats, dogs, wolves, foxes and jackals) and shed eggs from gravid pro-

glottids which come out with the faeces. Sheep and cattle get infected by grazing on grass and vegetation contaminated with *Echinococcus* eggs. Dogs and cats get infected when they feed on the discarded viscera of slaughtered sheep and cattle already infected with *Echinococcus*. Humans acquire the infection by ingestion of *Echinococcus* eggs through fruits, vegtables, salad or water.

On reaching the duodenum of the intermediate host (man, sheep, cattle and camel), the shell of the egg is digested liberating onchospheres which penetrate the intestinal wall and reach the blood circulation. The onchospheres are then carried to different parts of the body and get encysted. The liver is the most favoured site where the majority of the cysts settle down. However, other parts of the body such as lungs, spleen, brain, heart, kidneys, bones, abdominal cavity and musculature may also contain the hydatid cysts.

The main pathological changes during hydatid disease is inflammation and necrosis of tissues around the cysts. Usually the hydatid disease is symptomless in its early stage; however, various clinical symptoms may appear later which depend upon the number, size and site of the cysts. The presence of hydatid cysts in liver causes nausea, vomiting, vague abdominal pain and bulging of right hypochondrium or epigastrium due to hepatic enlargement. If the cysts are located in lungs, they give rise to recurrent pyrexia and coughing paroxysms. The patient may also exhibit biliary colic and jaundice. Sometimes the cysts rupture into the peritoneal cavity, lungs, kidneys and other organs of the body releasing hydatid fluid which may give rise to anaphylactic shock with vasomotor collaps, edema, utricaria and respiratory discomfort which may be fatal in few cases.

2.2 Helminth diseases of animals

Like humans, the domestic animals are also infected by a large variety of intestinal and tissue-dwelling helminths giving rise to considerable morbidity and mortality resulting in serious economic losses.

2.2.1 Intestinal nematodes (roundworms)

The gastrointestinal tract is the most common seat for a variety of roundworms in domestic and wild animals. The high prevalence of gastrointestinal nematodiasis is due to the simpler life cycle of the parasite with no intermediates host and easy access of eggs and larvae to the grazing animals.

2.2.1.1 Ruminants

The important nematodes which invade the gastrointestinal tract of cows, bullocks, oxes and calves are *Haemonchus contortus, H. Placei, Trichostrongylus axei, T. colubriformis, Ostertagia ostertagi, Cooperia punctata, C. pectinata, Nematodirus battus, Bunostomum plebotomum, Strongyloides papillosus, Trichuris ovis, Chabertia ovina* and *Oesophagostomum radiatum.*

The main nematodes which inhabit the digestive tract of sheep, goats and deer are *Gongylonema pulchrum, Gaigeria pachyscelis, Haemonchus contortus, H. placei, H. similis, Trichostrongylus axei, T. colubriformis, Ostertagia ostertagi, Cooperia punctata, C. onchophora, Nematodirus battus, Oesophagostomum columbrianum, Bunostomum trigonocephalum, Chabertia ovina, Strongyloides papillosus, Trichuris ovis* and *Capillaria longipes.*

The adult worms and developing forms of the nematodes may inhabit the abomasum (eg. *Haemonchus, Trichostrongylus* etc.) or intestine (eg. *Cooperia, Bunostomum* etc.) of cattle, sheep and goats. The presence of nematodes in the intestine may cause different types of gastrointestinal disturbances such as diarrhea, depletion of intestinal minerals and depressed enzymatic activities. The infestations may also be associated with anaemia and mucoid hyperplasia. The economic loss during intestinal nematode infections in ruminants may be due to decreased levels of mineral causing reduced skeletal growth, weight loss, poor quality of meat and wool and less production of milk in dairy cattle.

2.2.1.2 Horses

The digestive tract of horses, mules and donkeys are parasitized by a number of nematodes, the most notable being *Parascaris equorum, Strongyloides westeri, S. edentatus, S. equinus, S. vulgaris, Oxyuris equi, Oesophagodontus robustus, Trichostrongylus axei, Habronema microstoma* and *Gongylonema pulchrum.* These nematodes are responsible for much morbidity in equines. For example, *S. Westeri* may cause acute diarrhea in foals and donkeys. The migratory larvae of *P. equorum* are responsible for coughing and circulating eosinophilia in the animals. The adult worms of *P. equoroum* may cause diarrhea, malaise, debility and catarrhal enteritis.

2.2.1.3 Pigs

Infections by intestinal roundworms is prevalent in pigs throughout the world. The presence of nematodes in the digestive tract of pigs is associated with

much morbidity and occasional mortality leading to heavy economic losses.

The common intestinal nematodes infecting pigs are *Ascaris suum, Strongyloides westeri, S. ransomi, Trichinella spiralis, T. suis, Oesophagostomum dentatum, Necator suillus, Ancylostoma duodenale, Trichostrongylus colubriformis, T. axei* and *Hyostrongylus rubidus*.

The pathology and clinical manifestations may depend on the type of the parasite infecting the animal. For example, migration of larave of *A. suum* through gut wall, peritoneal cavity, liver and lungs may cause tissue damage and haemorrhage. Animals with heavy worm burden may die due to severe lung damage. There is also poor weight gain in pigs infected with *Ascaris*. Similarly, *Oesophagostomum* spp. (nodular worms) live in the large intestine of pigs; their larval forms induce nodule formation which may cause anorexia, blood mixed stool and enteritis leading to occasional death. The cysts of *T. spiralis* present in the muscles of different parts of the body not only decrease the quality of pork but also serve as a potential source of infection for man and domestic animals.

2.2.1.4 Cats and dogs

The common roundworms invading the digestive tract of cats and dogs are the ascarids, *Toxascaris leonina, Toxocara canis* and *Toxocara cati*. In addition some hookworms, *Ancylostoma caninum, A. tubaeforme, A. braziliense, A. ceylanicum* and *Uncinaria stenocephala* and whipworms, *Trichuris vulpis, T. serrata* and *T. campanula* may also infect cats, dogs and other carnivors. Other roundworms found in the gastrointestinal tract of cats and dogs are *Strongyloides stercoralis, S. cati, T. spiralis, N. americanus* and *Gnathostoma spinigerum*.

The intestinal nematode infections in cats and dogs occur throughout the world. The presence of ascarids may be responsible for gastrointestinal disturbances and poor growth in the animals. The hookworms and some whipworms (such as *T. vulpis*) are blood feeders and, therefore, may cause anaemia and haemorrhage. The adult worms of *Trichuris* spp. may be responsible for mucosal damage resulting due to tunneling into mucosa of large intestine.

2.2.1.5 Poultry

The infestations by intestinal roundworms in fowls is responsible for a number of gastrointestinal problems. The common nematodes of the digestic tract of poultry are *Heterakis gallinarum, H. indica, Ascaridia galli, Capillaria* spp., *Strongyloides avium* and *Trichostrongylus tenuis*.

2.2.2 Extra-intestinal nematodes

2.2.2.1 Filariasis and heartworm infections

As in humans filariasis has high prevalence in domestic animals. The micro-filariae live in the peritoneal blood and skin while the adult filariids reside in the blood vessels, lymphatic system, subcutaneous tissues and body cavities. In addition, the heartworm of the dog, *Dirofilaria immitis*, is found in the right ventricle and adjacent pulmonary arteries of carnivorous animals.

The important filariids infecting animals are *Setaria cervi, Parafilaria bovicola, Stephanofilaria* spp. *Onchocerca armillata* and *Elaeophora poeli* (cattle); *Setaria equina* (horses), *Setaria digitata* (sheep, goats), *Setaria congolensis* (pigs) and *Dipetalonema dracunculoides* (cats, dogs).

2.2.2.2 Lungworm infections

Some strongyloides belonging to the genus *Dictyocaulus* are known to inhabit the lungs of cattle, sheep, horses and other animals and produce pulmonary complications. Some important lung worms of domestic animals are: *Dictyocaulus viviparus* (cattle), *D. filaria* (sheep, goats), *D. arnfieldi* (horses), *Metastrongylus* spp. (pigs), *Filaroides osleri, F. hirthi* (cats, dogs) and *Syngamus* spp. (poultry). Of these, *D. viviparus* and *D. filaria* cause parasitic bronchitis (husk disease) especially in young calves and spring-born lambs. Occasionally, the worms may block lungs of animals which may be fatal.

2.2.2.3 Other extra-intestinal nematode infections

In addition to the filariids and lung worms, a few nematodes also invade extra-intestinal organs of domestic animals. The most notable parasites of this class are *Stephanurus dentatus* (kidney worm) of pigs, *Thelazia capillaria, T. lacrymalis* and *T. californiensis* which parasitize the conjunctival sac and lachrymal ducts of dogs, sheep, deer and cattle, and *Capillaria plica* living in the urinary tract of cats and dogs.

2.2.3 Trematode infections

Several species of trematodes, commonly known as flat worms or flukes, infest the digestive tract, liver, lungs and blood circulation of sheep, goats, cattle and other animals producing a broad spectrum of morbidity and occasional mortality.

2.2.3.1 Intestinal flukes

The important flukes which invade the gastrointestinal tract of ruminants are *Fasciolopsis buski, Gastrodiscoides hominis, Paramphistomum cervi, Metagonimus yokogawai, Heterophyes heterophyes* and *Echinostoma* spp.

2.2.3.2 Liver flukes

These are particularly common in cattle sheep and goats producing significant economic losses. The major liver flukes which infect ruminants are *Fasciola hepatica, F. gigantica, Fascioloides magna, Dicrocoelium dendriticum, Clonorchis sinensis* and *Opisthorchis* spp. These parasites usually cause hepatic problems in the animals.

2.2.3.2 Lung flukes

Several species of *Paragonimus*, commonly known as lung flukes, infect lungs, pleura and bronchi of cats, dogs and pigs. The most common lung flukes are *Paragonimus westermani* and *P. kellicoti.*

2.2.3.3 Blood flukes

Blood fluke infections in cattle, sheep and goat are common in Africa, China and India. Some important flukes invading the blood circulatory system of cattle, sheep and goats are *Schistosoma bovis, S. mattheei, S. nasalis, S. indicum, S. japonicum* and *S. spindale.*

2.2.4 Cestode infections

The tapeworm infections are very common in grazing animals. This is because of the fact that tapeworm eggs are easily taken up by animals while feeding on the vegetation contaminated with human excreta. For some cestodes like *Taenia* spp., *Diphyllobothrium latum* and *Hymenolepis nana*, cattle, pigs and fishes serve as the intermediate hosts, while for others cats, dogs, foxes and jackals serve as the definite host. The presence of cystic forms of the cestodes in the muscles of cattle and pigs give rise to 'measly beef' and 'measly pork', respectively, which are unsuitable for human consumption. The adult tapeworms live in the gastrointestinal tract and are responsible for various digestive system problems.

The important tapeworms inhabiting the digestive tract of animals are *Moniezia expansa, M. benedeni* and *Thysaniezia giardi* (ruminants), *Anoplocephala perfoliata, A. magna,* and *Paranoplocephala mamillana* (horses), *Dipylidium caninum,*

Echinococcus spp., *Diphyllobothrium latum* and *Spirometra erinacei* (cats, dogs); and *Raillietina* spp., *Hymenolepis* spp., *Dicranotaenia* spp. and *Choanotaenia infundibulum* (birds). A few tapeworms such as *Stilesia hepatica* and *Thysanosoma actinioides* are found in the bile duct of cattle.

The larval forms of various cestodes are found in the musculature of some animals. Thus, the bladder worms of *T. saginata (Cysticercus bovis, C. inermis)* and *T. solium (C. cellulosae)* are seen in the muscles of different organs of cattle and pigs, respectively. Similarly larval stages of *Echinococcus granulosus* and *E. multilocularis* infect the muscles of sheep and goats, while larvae of *T. ovis, T. hydatigena, E. granulosus* and *S. erinacei* may occur in the muscles of cats, dogs and other carnivors.

3.   THE PROTOZOAL INFECTIONS

A large number of protozoans are known to invade the gastrointestinal tract, liver, spleen, brain, blood circulation and other organs of humans and domestic animals producing a wide-spectrum of morbidity and considerable mortality. In humans, the gastrointestinal tract is the seat of predilection of several amoebae, flagellates, ciliates and sporozoates causing from mild gastrointestinal disturbances to fatal clinical conditions like liver abscess and amoebic meningoencephalitis. The important intestinal protozoal diseases of man are amoebiasis, giardiasis, trichomoniasis, naegleriasis and toxoplasmosis. Of these, amoebiasis is estimated to affect nearly 480 million people around the world of which nearly 40,000-110,000 patients die every year [1,2]. Giardiasis is another widespread disease infecting nearly 200 million people around the globe [1].

The extraintestinal organs like liver, spleen, CNS and blood circulatory system are also invaded by a number of parasitic protozoans through the bites of a variety of mosquitoes, flies and bugs. The major extraintestinal protozoal infections of man are malaria, trypanosomiasis (Chaga's Disease and African sleeping sickness) and leishmaniasis, all of which are notorious for their detrimental effects on human health. The medical problems associated with these vector-borne diseases are many and varied [40]. The clinical manifestations may range from fever and dermatological problems to grave clinical complications involving CNS, liver and tubular organs, many of which are often difficult to treat resulting in high rate of mortality, especially in patients suffering from malnutrition. The cost of treatment may also sometime become a limiting factor in curing the advanced cases of protozoan diseases [41]). It has been estimated

that nearly 800 million people suffer from malaria with an annual death toll touching the 1,500,000 mark. Similarly the number of persons infected with trypanosomiasis and leishmamiasis are 25 and 1.2 million, of which nearly 65,000 and 1,000, respectively, die every year [1].

The presence of pathogenic protozoans in domestic animals also produces debilitating effects and, therefore, eradication of these parasites is essential for better health of livestock and improved socio-economic status of the farmers. The economic importance of protozoal infections may be judged by the fact that more than US$ 280 million are spent worldwide in poultry industry alone for prophylactic treatment of chicken against coccidiosis [42]. Another fatal protozoal infection of animals is *Theileria parva parva* responsible for East Coast fever in cattle. The pathogenicity of theileriasis is so pronounced that the mortality among the infected dairy herds may reach upto 100% [43]

The economic importance of other protozoal diseases in domestic animals has received much attention as well. It has been estimated that nearly 1.2 billion cattle in the world are potentially exposed to the risk of babesiasis caused by different species of *Babesia*. The highest economic loss due to *Babesia* spp. in cattle occurs in the U.S.A., Australia, South Africa and South America [44,45]. Trypanosomiasis is another important disease of cattle. The impact of this infection is spread over 10 million square kilometers area of Africa extending from the Sahara to Limpopo and threatens the health of over 25 million cattle and 35 million people. This may be responsible for severe economic losses, which is evident from the fact that livestock in Africa are treated with more than 25 million doses of various trypanosomicidal drugs every year [46]. Trypanosomiasis in cattle may, therefore, be one of the reasons why Africa produces about 70 times less animal protein per unit area than Europe [47].

3.1 Human protozoal diseases

The gastrointestinal tract of humans is the natural seat of prediliction for a number of parasitic amoebae (*Entamoeba histolytica, E. hartmanni, E. coli, E. polecki, Iodamoeba buetschlii, Endolimax nana* and *Dientamoeba fragilis*), flagellates (*Giardia lamblia, Trichomonas vaginalis, T. hominis, Chilomastix mesnili, Retortamonas intestinalis* and *Enteromonas hominis*), ciliates (*Balantidium coli*) and sporozoates (*Isospora hominis, I. belli*). Some of these are nonpathogenic while others may produce a variety of clinical manifestations. The protozoans which belong to the class *Plasmodium, Trypanosoma*

and *Leishmania* invade the extraintestinal organs such as liver, spleen, CNS and blood circulatory system, are usually pathogenic and produce serious medical problems, which may be life threatening.

The major protozoa parasitic to humans are briefly described below.

3.1.1 Intestinal protozoans

3.1.1.1 Entamoeba histolytica

This is the most common intestinal parasite responsible for causing amoebiasis in humans. The disease has a cosmopolitan distribution, and is endemic in some parts of the world. *E. histolytica* lives in the intestine (colon in particular) in all the five developmental stages of its life cycle, namely the trophozoite, precyst, cyst, metacyst and metacyst trophozoite forms. The parasite enters a new host by the oral route as a cyst. The amoebic cysts, discharged in the patient's stool are spread by pests, water streams and man himself through nightsoil fertilization of agricultural lands. Transmission of the disease occurs by consuming water, food and vegetables contaminated by cysts. Direct faecal contact, person-to-person contact, flies and cockroaches may also help in transmitting the amoebic cysts.

Amoebiasis is marked by two phases of the infection: (a) intestinal amoebiasis characterised by dysentery and diarrhea, nondysenteric colitis, amoeboma (amoebic granuloma) and amoebic appendicitis; and (b) extraintestinal amoebiasis (hepatic amoebiasis) marked by liver abscess [48].

3.1.1.2 Entamoeba coli

It is a common but nonpathogenic amoeba present in humans which is often confused with *E. histolytica*.

3.1.1.3 Iodamoeba buetschlii

These are also nonpathogenic amoeba that inhabits the intestine of man. Both trophozoite and encysted stages are seen in their life cycle.

3.1.1.4 Dientamoeba fragilis

This intestinal protozoa is probably responsible for causing mild form of diarrhea in man. The infection is associated with recurring episodes of abdominal discomfort which usually cease after treating the patient with an amoebicide.

3.1.1.5 Trichomonas vaginalis

It is a pathogenic flagellate responsible for causing vaginitis or urethritis in women and men, respectively, and has world wide distribution. Although *T. vaginalis* is a parasite of the intestinal tract, vaginal trichomoniasis is not acquired by faecal contamination. Coitus is probably the main source of transmission of the infection. That is why it is usually advisable to treat both sexual partners. The infection is also transmitted to humans by douche equipments, clothings and towels.

The infection is established when the pH of vagina goes below 3.8 as *T. vaginalis* is unable to survive at the normal acidity of vagina which ranges from pH 3.8 to 4.4. Vaginal trichomoniasis is characterised by vulval pruritus with profuse and irritating vaginal discharge leading to excoriation of the vulva and dermatitis on thigh skin. In men, chronic urethritis may be observed.

3.1.1.6 Trichomonas hominis

It is a nonpathogenic protozoa of the large intestine of man also distributed throughout the world. The flagellate is present only in the trophozoite form. The parasite may be seen in very large numbers in stool during diarrhea of nonparasitic etiology.

3.1.1.7 Giardia lamblia

This is pathogenic flagellate which usually inhabits the duodenum and upper jejunum but may also be found in the gall bladder. The parasite occurs both in the trophozoite and encysted forms. The infection is acquired by consuming food or drink contaminated with cysts and, therefore, it is more prevalent in children than the adults. The clinical manifestations of the disease may include epigastric pain, nausea, flatulence and diarrhea. Acute giardiasis may be associated with steatorrhea and weight loss [49].

3.1.1.8 Balantidium coli

This is a pathogenic ciliate which parasitizes the colon of humans. The infection is reported to occur throughout the world producing diarrhea and dysentery. Since *B. coli* penetrates mucosa producing necrosis and ulcerations, it is usually associated with secondary infections. Pigs are the main reservoirs of *B. coli*, which also serve as the source of infection to man.

3.1.1.9 Isospora spp.

The pathogenic protozoans of this class are *Isospora belli* and *I. hominis* which inhabit the small intestine of man. The infection may give rise to abdominal pain, diarrhea, nausea, anorexia and headache.

3.1.2 Extra-intestinal protozoans
3.1.2.1 Naegleria and acanthamoeba spp.

These are free living soil amoebae which occasionally infect the respiratory and nervous systems of man. *Naegleria* spp. may cause primary amoebic meningoencephalitis which is invariably fatal. The parasite enters the nasal cavity and invades the brain via the olfactory nerve. *Acanthamoeba* (*Hartmannella*) spp., on the other hand, can cause granulomatous amoebic encephalitis and keraritis. It is interesting to note that *Naegleria* spp. affects robust young persons, while *Acanthamoeba* (*Hartmannella*) spp. usually infect immune depressed persons [50,51]. The infection may be acquired while swimming in lakes and pools.

3.1.2.2 Toxoplasma gondii

It is a widely distributed pathogenic parasite of man and animals. Cats serve as the definite host. The sexual part of the life cycle of the parasite takes place in the intestinal cells of the felines. These animals pass out immature oocysts in their faeces which are taken up by sheep, pigs and cattle. Man becomes infected by eating poorly cooked or raw meat of sheep, pigs and cows containing toxoplasma cysts. Sometimes oocyts from cat's faeces may also be ingested directly. The infection may also be acquired through the transplacental route. The intermediate hosts (man, pigs, ruminants) harbour extraintestinal sexual forms, namely tachyzoites (proliferative forms) and bradyzoites (encysted forms).

Toxoplasmosis produces a wide-spectrum of pathological and clinical manifestations. The disease appears in two forms: congenital and acquired. Congenital toxoplasmosis is usually fatal. The infection in newborns is marked by the presence of hydorcephalus, encephalitis, bilateral retinochoroiditis, hepatosplenomegaly and jaundice. In some cases the disease may be inactive at birth, but may develop as the child grows older. Such children may show esotropia, strabismus, microphthalmia, and cataract.

Acquired toxoplasmosis is characterised by retinochoroiditis, lymphadenopathy and fever. In adult patients, retinochoroiditis, mononucleosis like syndrome, fever and malaise may be frequently observed. The disease is also known to recrudesce in immunosuppressed patients.

3.1.2.3 Pneumocystis carinii

This is an opportunistic parasite causing diffuse interstitial pneumonia in infants and children. However, clinically significant parasitic pneumonia is mainly seen

in patients with lowered immunity, especially those suffering from AIDS, leukemia and lymphoma. The initial stage of the disease is characterised by anorexia, weight loss and dyspnea with bluish colouration around mouth and nostrils. Later respiratory problems become pronounced with marked tachypnea, cyanosis and spells of nonproductive (with pleurisy) cough. This leads to diffused interstitial and alveolar infiltrates resembling pulmonary edema. The patient usually dies if not treated promptly.

3.1.2.4 Cryptosporidium spp.

This is a mild type of pathogen. Although the infections due to *Cryptosporidium* usually subside without therapy, it may produce chronic debilitating diarrhea, especially in patients with AIDS and low immunological profile.

3.1.2.5 Plasmodium spp.

A number of species of *Plasmodium* invade the blood and liver causing malaria in man and animals. The important pathogens of malaria in man are *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*. The disease is transmitted to man by female mosquitoes belonging to the genus *Anopheles* and is endemic in several parts of Asia, Africa and South America.

The life cycle of *Plasmodia* involve the liver and erythrocytes of humans and the stomach wall of mosquitoes. The sporozoites released into the blood by the bite of a mosquito are taken up by the hepatocytes and develop into schizonts. In *P. vivax* there is also a dormant tissue stage, termed hypnozoite, which mature to schizonts at different intervals, and are responsible for relapsing fever episodes. The tissue schizonts produce merozoites which are released into the circulation and infect erythrocytes and generate more merozoites as a result of an asexual cycle. Some of the merozoites, thus formed, differentiate into male and female gametocytes which are taken up by the mosquitoes while sucking human blood. In the mosquito, these gametocytes fuse to form a zygote, thereby completing the sexual cycle of the parasite. The zygote give rise to sporozoites, which are stored in the salivary gland and are infective. These are released into blood through a mosquito bite.

The main clinical manifestations of malaria are periodic fever with chills, splenomegaly, anaemia and leukopenia. The liver is occasionally enlarged. *P. vivax, P. ovale* and *P. malariae* are usually associated with chronic illness, while falciparum malaria may be fatal if not treated promptly. *P. falciparum* gives rise to cerebral malaria due to agglutination and adherence of parasitized red blood cells to the capil-

laries in the brain. This leads to blockade of the blood circulation causing progressive increase in headache with little or no fever. The final stage of the disease is characterised by coma and death of the patient.

3.1.2.6 Trypanosoma spp.

Several haemoflagellates of the genus *Trypanosoma (Trypanozoon)* are known to invade the central nervous system, blood and tissues causing acute and chronic protozoal diseases, collectively termed as 'trypanosomiasis'.

Gambian sleeping sickness is caused by *Trypanosoma brucei gambiense*. The disease is transmitted to man by the tsetse flies, *Glossina palpalis*. The Rhodesian sleeping sickness is caused by *T. brucei rhodesiense* which are transmitted to man by the bites of tsetse fly *Glossina morsitans*. Both these forms of African trypanosomiasis are endemic in different parts of Africa.

The parasites of *T. b. gambiense, T. b. rhodesiense* invade the CNS of man leading to 100% mortality. The early phase of the infection is marked by fever, headache, malaise and anaemia. The later stage of the disease is related to involvement of CNS and is characterised by meningoencephalitis, meningomyelitis and cachexia. The patient becomes emaciated, falls in coma and finally dies.

Chaga's disease or American trypanosomiasis is caused by the invasion of the blood and tissues by the trypanosome *T. cruzi*. The trypanosomes inhabit the blood circulation, while their leishmanial forms reside in the tissues. The main victims of this disease are children and young adults. Transmission of Chaga's disease takes place through the kissing bugs, *Triatoma* spp.

Chaga's disease is widespread in several parts of South America; it is endemic in Brazil, Argentina, Chile and Venezuela. The acute phase of the disease is marked by high fever, anorexia, vomiting, diarrhea and enlargement of the liver and spleen. The patients also suffer from various eye problems. There is a continous damage of tissues in various organs like heart, liver and spleen. If the heart is affected by *T. cruzi*, the patient may die of heart failure. Another serious clinical feature of Chaga's disease is dilation of tubular organs such as oesophagus (mega-oesophagus) and colon (megacolon) in some patients.

3.1.2.7 Leishmania spp.

A number of intracellular protozoan parasites belonging to the genus *Leishmania* infect humans through female sand flies of the genus *Phlebotomus, Sergentomyia*

and *Lutzomyia*. Depending upon the nature of infection, human leishmaniasis can be divided into three forms: (a) Visceral leishmaniasis (Kala-azar) and post kala-azar dermal leishamaniasis (PKDL) caused by *Leishmania donovani* complex; (b) cutaneous leishmaniasis caused by *L. tropica* complex and *L. mexicana* complex and, (c) mucocutaneous leishmaniasis due to *L. brasiliensis*.

Kala-azar (visceral leishmaniasis) gives rise to several clinical manifestations, the most notable being diarrhea, vomiting, cough, dizziness, weight loss, anaemia, malaise, bleeding gums, skin darkening, wasting and enlargement of spleen, liver and lymph nodes. The clinical features of PKDL include the cutaneous type of leishmaniasis with hypopigmentation and erythematous patches on face, limbs and body trunk.

The main clinical symptoms of cutaneous leishmaniasis are related to dermal problems like skin lesions and ulcerations. The mucocutaneous leishmaniasis is the severe and destructive form of cutaneous leishmaniasis that may occasionally cause destruction or disfiguration of tissues of nasal septum, lips and larynx.

3.2 The protozoal diseases of animals

A large number of parasitic protozoans infect cattle, horses, pigs, sheep, goats and birds causing a serious threat to the livestock health. The important protozoal diseases of domestic animals are described below.

3.2.1 Coccidiosis

This is a serious parasitic disease of birds, especially poultry, though coccidiosis in cattle, sheep, goats, horses, swine and rarely in humans has also been reported. [52,53]. Coccidiosis in domestic fowl is responsible for considerable economic loss [53]. The coccidia are microscopic sporozoa belonging to the genera *Eimeria* and *Isospora*.

Birds acquire coccidiosis when they ingest sporulated oocysts along with feed and water. The disease is exacerbated due to poor nutritional conditions of chicken. In addition, environmental and climatic factors also add to the spread of the infection. Faecal discharge of birds help oocysts to sporulate and become infective under warm and humid conditions. Crowding of birds due to intensive rearing creates favourable conditions for sudden outbreaks of severe forms of coccidiosis.

Coccidiosis is usually marked by haemorrhagic enteritis resulting in devastating bloody caecal coccidiosis (*E. tenella*) and intestinal coccidiosis (*E. necatrix*) in

chicken. The disease is further complicated by secondary bacterial infections. Other clinical manifestations of the disease include poor weight gain (weight loss), weakness and emaciation. In severe infection the animals die even before distinct clinical symptoms appear.

3.2.2 Cryptosporidiosis

The disease is caused by a coccidian like protozoa, *Cryptosporidium* spp. and is widespread in animals, though only in immune-compromised humans. *Cryptosporidium* has been shown to be the main cause of respiratory infections in turkeys and chicken [54,55].

3.2.3 Sarcocystosis

Infections due to *Sarcocystis* is quite common in domestic animals [52]. The infection in pregnant animals such as cattle, sheep and pigs may often cause abortion.

3.2.4 Histomoniasis (blackhead disease)

This disease is caused by the presence of *Histomonas meleagridis* in the cecum and liver of turkeys, chicken, peafowls, guineafowls, pheasants and partirides. The amoeboid stage of the parasite causes enterohepatitis in turkeys and chicken and, therefore, is responsible for high economic losses in the turkey industry. The birds acquire the infection by ingesting either the trophozoites or the embryonated eggs of caecal worms, *Heterakis gallinarium* containing *H. meleagridis*.

3.2.5 Leucocytozoonosis

This is also an important protozoal disease of turkeys, ducks, geese and chicken caused by invasion of blood cells and other organs of these birds by *Leucocytozoon* spp.

The vectors of the disease are *Simulium* spp. and other arthropods. The important parasites are *L. smithi* (turkeys), *L. caulleryi* (chicken) and *L. simondi* (geese and ducks) which cause high rates of mortality in infected birds. This results in significant economic losses in poultry industry in Japan and other parts of Southeast Asia [6].

3.2.6 Theileriasis

Several pathogenic protozoans belonging to the family *Theileriidae* invade the blood cells (lymphocytes and erythrocytes) of cattle, sheep ad goats throughout the

world. The disease is transmitted by ticks and is of significant economic importance due to high mortality rates (upto 100%) in the untreated animals. The important parasites infecting cattle are *Theileria parva parve* (East coast fever), *T.p. lawrenci* (corridor disease), *T. annulata* (tropical theileriasis or Mediterranean Coast fever) and *T. mutans* (tzaneen disease). Of these East Coast fever affects the cattle in South, East and Central Africa, while corrider disease is endemic in East and Central Africa. Tropical theileriasis may be lethal to animals in Mediteranean regions extending from Morocco to the Middle East and also from Russia and CIS (formerly USSR) to the Indian subcontinent. *T. hirci*, which infects sheep and goats, occurs in Africa, Asia and Southern Europe [56,57].

The clinical symptoms of acute and subacute forms of theileriasis in cattle are fever at irregular intervals, enlarged lymph nodes, diarrhea, anaemia, dyspnea, leukopenia and weakness.

3.2.7 Babesiasis

Like theileriasis, babesiasis is also a tick-borne disease caused by invasion of blood cells of domestic animals and humans by protozoans of the family *Babesiidae* [58,59]. The important *Babesia* spp. which infect animals are: *Babesia ovis, B. divergens, B. bigemina* (cattle), *B. ovis, B. motasi* (sheep, goats), *B. equi, B. caballi* (horses), *B. perroncioti, B. trautmani* (pigs), *B. gibsoni, B. canis* (dogs) and *B. felis* (cats). Babesiasis is a highly pathogenic disease characterised by haemolytic anaemia, jaundice and haemoglobinuria, causing occasional death of the animals. The erythrocytes parasitized with *B. bovis* may adhere to the capillary endothelium. This can block blood vessels in brain, liver, kidneys and lungs producing necrosis of adjacent tissues [60].

3.2.8 Trypanosomiasis

This is another major protozoal disease of domestic animals prevalent primarily in tropical Africa. The infection is mainly transmitted by tsetse flies, though salivarian or stercoranian methods of infection are also known. Some blood sucking insects such as *Tabanus* and *Stomoxys* flies, and also vampire bats may transmit trypanosomiasis in animals. Infections with *Trypanosoma equiperdum* in horses and donkeys may also be transmitted during coitus.

The major trypanosomes infecting domestic animals are: *Trypanosoma vivax* and *T. congolense* (cattle and horses), *T. brucei* and *T. evansi* (horses), and *T. suis* (pigs). All these parasites invade the blood cells in the early stage. Later the trypano-

somes enter the CNS causing high mortality. Typical clinical symptoms of trypanosomiasis are characterised by wasting with progressive deterioration of general health leading to extreme emaciation, collaps and death of the animals.

In addition to the pathogenic trypanosomes, some nonpathogenic species such as *T. lewisi* and *T. rangeli* also infect rats, cats, dogs, monkeys and humans.

3.2.9 Anaplasmosis

This is a disease of sheep and cattle prevalent in many parts of Asia, Africa and Southern U.S.A. The disease is caused by *Anaplasma ovis* (sheep) and *A. marginale* (cattle) and is responsible for heavy mortality in ruminants. Since anaplasmosis is a tick-borne disease, it is usually grouped with babesiasis and theileriasis. However, some workers consider *Anaplasma* to belong to order *Rickettsiales* and, therefore, should not be treated as protozoa at all [61].

4.   DRUGS FOR PARASITIC DISEASES

The modern era of chemotherapy started with the discovery of antiparasitic agents around the beginning of the 20th century. However, during the period 1930-1965, often referred to as the golden-age of medicinal science [16], parasitic chemotherapy did not receive the attention it deserved considering the extensive morbidity and mortality (and the associated socio-economic loss) caused by them (vide infra). Thus, while the progress during this period in antibacterial chemotherapy was spectacular, rather few new classes of antiparasitic drugs were added [62]. The drugs used at present to treat the major parasitic infections in humans and domestic animals are listed in Table 1 and 2, respectively [63-70]. A perusal of the table would show that parasitic chemotherapy at present is rather inadequate. There are big gaps in the therapeutic armementarium for these infections. There are very few drugs available for systematic protozoal and helminth infections; no suitable antileishmanicidal or macrofilarial drug is available. Primaquine, the only drug available for relapse of *vivox* malaria, is not very safe to use. There are very few drugs available against multidrug resistant *P. falciparum*. These gaps needed to be filled urgently.

Recent advances in molecular and cellular biology and in instrumentation techniques have provided new insights into the etiology, physiology and pathology of these diseases and elucidated complex molecular structures and their function, uncovering new targets for drug-interference and design. Developments in new instrumentation techniques have also provided new non-invasive approaches to

Table 1: Drugs for parasitic diseases of human

Disease	Causative Agent	Drugs Available
Helminth diseases		
Ascariasis	*Ascaris lumbricoides*	Santonin, piperazine, pyrantel, levamisole, thiabendazole, mebendazole, albendazole
Hookworm infection	*Ancylostoma duodenale, A. ceylanicum, Necator americanus*	Tetrachloroethylene, bephenium, thiabendazole, pyrantel, albendazole, mebendazole
Whipworm infection	*Trichuris trichiura*	Dichlorovos, thiabendazole mebendazole, oxantel
Threadworm infection	*Strongyloides stercoralis*	Pyrviniumpamoate, thiabendazole, mebendazole, levamisole
Pinworm infection	*Enterobius vermicularis*	Piperazine, pyrvinium pamoate pyrantel, thiabendazole, albendazole, mebendazole
Pseudohook worm infection	*Trichostrongylus orientalis*	Pyrantel, levamisole, mebendazole, thiabendazole
Wasting disease	*Capillaria philippinensis*	Thiabendazole, mebendazole
Angiostrongyliasis	*A. cantonensis*	Thiabendazole, mebendazole
Trichinosis	*Trichinella spiralis*	Thiabendazole, corticosteroids
Creeping eruption	*Ancylostoma caninum, A. braziliensis, Gnathostoma spinigerum*	Thiabendazole, mebendazole
Visceral larva migrans	*Toxocara canis, T. cati*	Diethylcarbamazine, thiabendazole, mebendazole

Disease	Causative Agent	Drugs Available
Filariasis	*Wuchereria bancrofti, Brugia malayi, Loa loa, Onchocerca volvulus, Mansonella ozzardi, Dipetalonema perstans*	Suramin, diethylcarbamazine mebendazole, levamisole, ivermectin
Guinea worm infection	*Dracunculus medinensis*	Thiabendazole, niridazole, metronidazole
Tropical eosinophilia	*Intestinal roundworms, filarial worms*	Levamisole, diethylcarbamazine
Schistosomiasis	*S. mansoni, S. japonicum, S. haematobium, S. intercalatum*	Hycanthone, niridazole, oxamniquine, emetine, dehydroemetine, praziquantel
Intestinal fluke infection	*Fasciolopsis buski*	Hexylresorcinol, tetrachloroethylene, niridazole, praziquantel, niclosamide
Liver fluke infection	*Fasciola hepatica, F. gigantica, Clonorchis sinensis, Opisthorchis felineus, Dicrocoelium dendriticum*	Emetine, dehydroemetine, bithionol, praziquantel, hexachlorophene, dithiazanine iodide
Lung fluke infection	*Paragonimus westermani*	Bithionol, hetol, praziquantel
Tapeworm infection	*Taenia solium, T.saginata, Diphyllobothrium latum, Hymenolepis nana*	Niclosamide, praziquantel, dichlorophene, mebendazole, paromomycin sulphate
Hydatid disease	*Echinococcus granulosus, E. multilocularis*	Praziquantel

Disease	Causative Agent	Drugs Available
Protozoal diseases		
Amoebiasis	*E. histolytica*	Iodoquinol, diloxanide furoate, chloroquine, emetine, dehydroemetine, metronidazole, tinidazole, tetracycline, paromomycin sulphate
Dientamoeba diarrhea	*D. fragilis*	Iodoquinol, tetracycline, paromomycin sulphate
Vaginal trichomoniasis	*T. vaginalis*	Metronidazole, tinidazole
Giardiasis	*G. lamblia*	Quinacrine hydrochloride, metronidazole, tinidazole, furazolidone, paromomycin
Balantidiasis	*B. coli*	Iodoquinol, tetracycline, metronidazole, nitrimidazine
Isosporiasis	*I. belli, I. hominis*	Cotrimoxazole, fansidar, amprolium
Primary amoebic meningoencephalitis	*Naegleria spp., Acanthamoeba spp.*	Amphotericin B
Toxoplasmosis	*T. gondii*	Pyrimethamine with sulfadiazine, cotrimoxazole, clindamycin, spiramycin
Pneumocytis pneumonia	*P. carinii*	Pentamidine, pyrimethamine plus sulfadiazine, cotrimoxazole
Cryptosporidium infection	*Cryptosporidium* spp.	Spiramycin, amprolium
Malaria	*Plasmodium vivax, P. falciparum, P. ovale, P. malariae*	Quinine, chloroquine, amodiaquine, amopyroquine, hydroxychloroquine, primaquine, mefloquine, proguanil, chlorproguanil, cycloguanil, sulfadiazine, sulfalene, sulfadoxine, dap

Disease	Causative Agent	Drugs Available
		sone, acedapsone, pyrimethamine, trimethoprime, tetracycline, doxycycline, minocycline, clindamycine and qinghaosu.
African sleeping sickness	*Trypanosoma brucei-gambiense, T.b. rhodesiense*	Suramine, pentamidine, DFMO (for early stage) and tryparsamide, Mel B, Mel W, melarsen sodium, nitrofurazone (for late stage of the disease with CNS involvement).
American trypanosomiasis (Chaga's disease)	*Trypanosoma cruzi*	Nifurtimox, benznidazole
Leishmaniasis	*Leishmania donovani, L. tropica, L. mexicana, L. brasiliensis*	Pentostam, glucantime, urea stibamine, pentamidine, WR-6026, allopurinol, allopurinol riboside, amphotericin B

Table 2: Drugs for parasitic diseases of domestic animals

Disease	Causative Agent	Drugs Available
Helminth diseases		
Intestinal roundworm infections	Nematodes	Thiabendazole, cambendazole, parbendazole, mebendazole, flubendazole, flubendazole, fenbendazole, oxfendazole, albendazole, febantel, thiophanate, tetramisole, levamisole, piperazine, pyrantel, morantel, dichlorovos, metrifonate, haloxon, coumaphos, crufomate, closantel, disophenol, ivermectin
Filariasis	Nematodes	Diethylcarbamazine, suramin, levamisole, fenbendazole, ivermectin, CGP6140.
Heartworm infections	*Dirofilaria immitis*	Diethylcarbamazine, iacetarsamide, melarsoprol, dithiazanine, levamisole, ivermectin.
Lungworm infections	*Dictyocaulus, Filaroides* and *Metastrongylus* spp. etc.	Mebendazole, thiabendazole, fenbendazole, albendazole, febantel, levamisole, ivermectin.
Kidney worm infections	*Stephanurus dentatus*	Fenbendazole, flubendazole, levamisole, ivermectin
Nematode infection of urinary tract	*Capillaria plica*	Fenbendazole, albendazole
Nematode infection of conjunctival sac and lachrymal ducts	*Thelazia spp.*	Levamisole, febantel, ivermectin

Disease	Causative Agent	Drugs Available
Intestinal fluke infections	*Fasciolopsis buski, Metagonimus, Hetero-phyes, Paramphis-tomum* spp. etc.	Hexachlorophene, bithionol, bithionol sulphoxide, niclosamide, oxyclozanide, rafoxanide, praziquantel.
Liver fluke infections	*Fasciola, Dicrocoelium, Clonorchis, Opisthor-chis* spp.	Carbon tetrachloride, hexachloroethene, oxyclozanide, niclofolan, nitroxynil, brotianide, rafoxanide, closantel, clorsulon, diamphenetide, albendazole, triclabendazole, thiabendazole, cambendazole, mebendazole, fenbendazole, febantel, praziquantel.
Lung fluke infections	*Paragonimus* spp.	Hetol, bithionol
Blood fluke infections	*Schistosoma* spp.	Tartar emetic, stibophene, sodium antimony dimercaptosuccinate, lucanthone, hycanthone, niridazole, haloxon, trichlorphon, amoscanate, praziquantel.
Tapeworm infections	Cestodes	Arecoline hydrobromide di-n-butyltin, dichlorophene, bunamidine, niclosamide, terenol, nitroscanate, praziquantel, fenbendazole, albendazole, oxfendazole, luxabendazole, paromomycine sulphate.

Protozoal diseases

Coccidiosis	*Eimeria, Isospora* spp.	Sulpha drugs (sulphaquinoxaline, sulphadimethoxine, sulphaguanidine, sulphamethazine etc.), pyrimethamine, ormetoprim, ethopabate, amoprolium, arprinocid, clopidol, decoquinate,

Disease	Causative Agent	Drugs Available
		dinitrolmide, halofuginone, lasalocid, monensin, narasin, nicarbazin, prinicin, robenidine, roxarsone, nitromide, furazolidone, salinomycin, clazuril, toltrazuril, diclazuril.
Cryptosporidosis	*Cryptosporidium* spp.	Probably none
Sarcocystosis	*Sarcocystis* spp.	Amoprolium, Sulfadiazine plus pyrimethamine.
Blackhead disease	*Histomonas meleagridis*	Dimetridazole, ipronidazole, ronidazole, carnidazole, furazolidone.
Leucocyto-zoonosis	*Leucocytozoon* spp.	Combination of pyrimethamine with sulfa drugs.
Theileriasis	*Theileria* spp.	Tetracyclines (oxytetracycline, chlortetracycline, rolitetracycline), menoctone, BW-993C (clexon), BW-720C (buparvaquone), halofuginone lactate.
Babesiasis	*Babesia* spp.	Trypan blue, acriflavine hydrochloride, quinuronium sulphate, diminazene, pentamidine, phenamidine, imicarbalide, imidocarb, primaquine, oxytetracycline, chlortetracycline, clindamycin.
Trypanosomiasis	*Trypanosoma* spp.	Tartar emetic, suramin, quinapyramine, homidium chloride, diminazene, isometamidium chloride.
Anaplasmosis	*Anaplasma* spp.	Tetracycline, oxytetracycline, chlortetracycline, imidocarb dipropionate, gloxazone.

studying the effects of drugs *in vivo* and thus aid in drug-screening and development. These developments provide great opportunities for new drug development. Similarly, the lead-optimization approach in drug research has further been made more scientific and less time consuming by using well defined methods of QSAR proposed by Hansch and Fujita [71], Free and Wilson [72] and Bocek and co-workers [73].

For example a number of biochemical targets such as glucose metabolism, neuromuscular transmission, tubulin polymerisation and interference with ion-flux in helminths appear suitable for rationale design of new anthelmintics [74]. Similarly, inhibition of DNA functions, antagonism of folate, coenzyme A and Q, hypoxanthine-guanine phosphoribosyl transferase (HGPRT), catalase and glutathione peroxidase in protozoans have been used to develop better agents. In addition, design of suicide enzyme inhibitors, prodrugs and agents that would interfere with lipid and polyamine metabolism has helped to throw light on newer template structures for generating potent antiparasitic agents. More recently computer assisted molecular modelling and drug design is being used to solve intricate problems of conformational analysis and other structural requirements for a fruitful prediction and selection of an active molecule of desired biological profile. Nevertheless, intuition and experience of a medicinal chemist would continue to guide to arrive at new molecules that may turn into potential drugs if subjected to reliable *in vitro* and *in vivo* screening systems. It is hoped that with the help of the knowledge regarding disease process(es) of various protozoal and helminth infections, the biochemistry of parasites and newer strategies of drug design would lead us to develop ideal drugs for filariasis, trichinosis, guinea worm infections, hydatidosis, malaria, leishmaniasis, trypanosomiasis and a few protozoal diseases of the gastrointestinal tract [40,75-77] which still pose a challenge to scientists engaged in drug development for parasitic diseases.

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CHAPTER 2

BIOCHEMICAL TARGETS FOR ANTHELMINTIC ACTIVITY

1. INTRODUCTION

A number of biochemical pathways of the helminths concerned with their growth, multiplication and survival in the host offer useful targets for chemotherapeutic intervention and drug-design. The main physiological needs of the adult helminths are related either to maintaining adequate energy levels by glucose metabolism or to maintain their position in the host's body via neuromuscular coordination. Some other biochemical pathways important to the worms are mediated through polyamines and tubulines or are related to evasion of host's defence mechanisms by antioxidant enzymes. Interference with any of the above biological processes of the worm may lead to its paralysis and elimination or death.

The helminths draw energy predominantly from catabolism of carbohydrates using a sequence of enzymatic reactions in their cytoplasm and mitochondria. While doing so, the worms maintain their position against peristalsis and flow of intestinal fluids in the alimentary canal by neuromuscular coordination. For intestinal worms, the expulsion of the parasite may result from muscular paralysis making the worms unfit to hold *in situ* against peristalsis. On the other hand, for killing the extra-intestinal helminths a drug must inhibit their life supporting processes, the function of which are mainly to maintain an advantageous feeding site and utilization of the food for generation of energy. Since most of the helminths operate an anaerobic life and require little or no oxygen for their survival, their carbohydrate metabolism differs considerably from that of the host [1-3].

Some of the above biochemical pathways are found exclusively in the helminths and offer unique targets for chemotherapeutic attack. This subject has been reviewed by several workers [4-12]. The present chapter will highlight only the vulnerable biochemical sites of helminths for chemotherapeutic attack and effective drug design.

2. NEUROMUSCULAR ACTIVITY

The study of neuromuscular coordination in helminths would require under-

standing of the structure and function of their neuromuscular apparatus [13] which is given below.

2.1   Neuromuscular junction

At the neuromuscular junction in vertebrates, the end plate of the nerve fibre invaginates into the muscle fibre. The invagination caused by the nerve ending on the membrane layer is called *synaptic gutter* or *synaptic trough* and the space between the terminal axon and muscle fibre membrane is known as *synaptic cleft*. There are several folds of muscle membrane around the synaptic gutter which are known as *subneural clefts*. These provide a large surface area for the action of neurotransmitters. Acetylcholine (1) is synthesized in the cytoplasm of the nerve axon and stored in the *synaptic vesicles* (Chart 1).

$$Me_3N^+\!\!-\!\!(CH_2)_2OAc \underset{\text{Ach Synthetase}}{\overset{\text{AchE}}{\rightleftharpoons}} Me_3N^+\!\!-\!\!(CH_2)_2OH + Ac\text{-}CoA$$
$$X^- \qquad\qquad\qquad\qquad\qquad X^-$$
$$\mathbf{1} \qquad\qquad\qquad\qquad\qquad\qquad \mathbf{2}$$
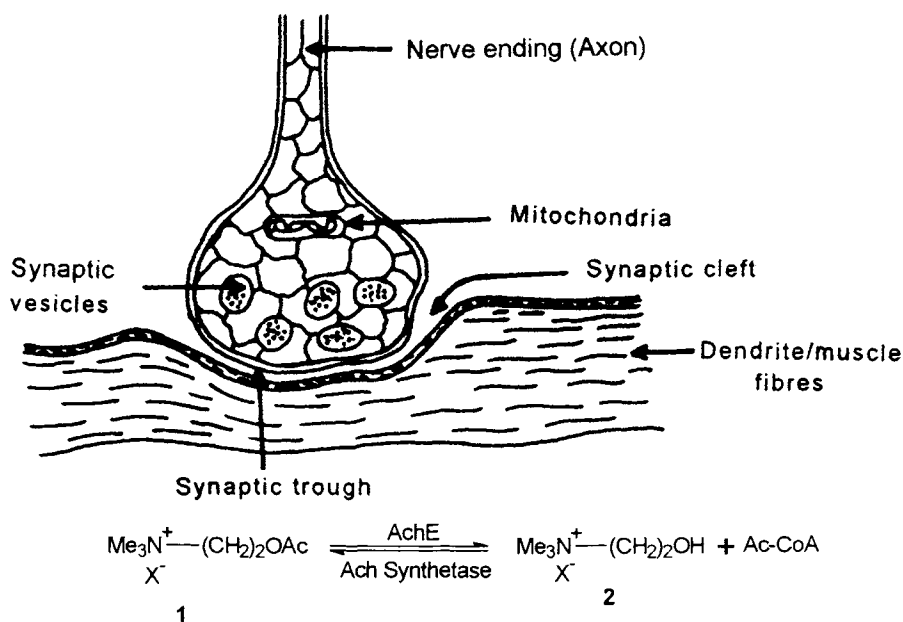
Chart 1:   Schematic presentation of structure and function of neuromuscular junction

2.2   Membrane potential

The concentrations of Na^+ and K^+ ions considerably differ across the nerve fibre membrane. This generates a net difference in the charge concentration on the two sides and produces a potential difference across the membrane, a situation usually termed as *membrane potential* or *resting potential*. The membrane potential

of the somatic muscle of *Ascaris* is around -30 mV. In case of a mammalian nerve fibre, this value has been found to be -90 mV [14,15].

2.3 Transmission of the nerve impulse

When a nerve impulse reaches the neuromuscular junction, it rapidly changes the permeability of the fibre membrane and allows influx of Na^+ or Ca^{2+} ions from the extracellular fluids. This increases the net positive charge inside the cell membrane and causes depolarisation of the membrane. The Ca^{2+} ions cause the synaptic vesicles to rupture and to release acetylcholine (1) into the synaptic cleft.

The released acetylcholine (ACh) then reacts with its receptor protein on the muscle fibre membrane and makes it permeable. This allows the influx of Na^+ as well as other ions into the muscle fibre resulting in increase in ion concentrations and subsequent lowering of the resting potential of the membrane. The change in membrane potential to a threshold voltage is responsible for eliciting action and tone in the muscle fibre. Meanwhile acetylcholine present in the subneural junction is converted into choline (2) and acetic acid by an enzyme, acetylcholine esterase (AChE). The conversion of acetylcholine (ACh) into choline reestablishes the membrane potential and allows it to recover from the first impulse. The generated choline reacts with acetyl CoA in the presence of acetylcholine synthetase and gives back acetylcholine which induces a fresh impulse in the muscle layers.

Thus, the motility of helminths is controlled by their neuromuscular system. Accordingly, the tone and activity of the longitudinal muscle fibres are controlled by neurotransmitters. There are two types of neurotransmitters, namely acetylcholine which works as an *excitatory* transmitter, and 4 (or γ)-aminobutyric acid (GABA) which acts as an *inhibitory* transmitter.

The presence of the inhibitory neurotransmitter GABA and the structure and function of GABA receptors in helminths have not been fully worked out. However, studies on *Ascaris* muscles have shown that GABA depolarises muscle cells [16], causes relaxation of muscle strips [17] and increases chloride conductance in isolated muscle cells [18]. This indicates that some helminths might operate a GABA-mediated control mechanism of their muscle, which is evident from the fact that some drugs like ivermectin act as GABA agonist. Ivermectin increases the activity of the inhibitory neurotransmitter GABA causing paralysis of the worms.

2.4 Possible targets for anthelmintic action

Although little is known about the neuromuscular system in helminth parasites, on the basis of the mammalian system the following classes of compounds may be envisaged to interfere with different states of the neuromuscular coordination.

2.4.1 Cholinergic agonists

Compounds having structural similarity with acetylcholine (1) such as bephenium (3) or thenium (4) are reported to exert their action on helminths by competing for the acetylcholine receptor site. The difference between these compounds and acetylcholine is that the former are not biodegraded by AChE and, therefore, the depolarisation effect persists very long. The net results is the contraction of the muscle causing spastic paralysis of the worm.
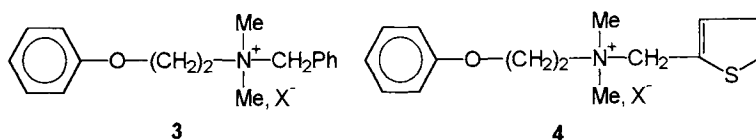
2.4.2 Cholinergic blocking agents

The compounds of this class may not bear any obvious similarity with the acetylcholine molecule but would exert the anthelmintic action by blocking the acetylcholine receptor site. Consequently, acetylcholine is unable to increase the permeability of the membrane to initiate a depolarisation effect.

2.4.3 AChE inhibitors

The neuromuscular transmission in helminths can also be effectively interrupted by inhibiting AChE, the enzyme responsible for converting acetylcholine into choline. Inhibition of AChE activity increases the concentration of acetylcholine with successive impulses. The accumulated acetylcholine stimulates the muscle fibres which do not come to the resting stage. Organophosphates are known to be potent inhibitors of AChE activity.

2.4.4 Muscle hyperpolarisers (Inhibitory neurotransmitter mimetics)

Compounds of this class mimic the action of inhibitory neurotransmitters like GABA. These compounds increase the membrane potential causing hyperpolarisation of the muscle fibre membrane. This leads to flaccid paralysis of the worm. Piperazine is a potent muscle hyperpolariser of nematode muscles.

3. GLUCOSE METABOLISM

Glucose is the main energy generating substrate in most of the helminths and, therefore, carbohydrate metabolism in worms has attracted much attention.

3.1 Glucose metabolism in the cytosol (cytoplasm)

Detailed studies carried out on glucose catabolism in adult *Ascaris suum* have shown that glucose is metabolised using the classical Embden-Meyerhof-Parnas pathway to form phosphoenol pyruvate (PEP) (Chart 2). Further metabolism of PEP is mediated by phosphoenol pyruvate carboxykinase (PEP-CK) and pyruvate kinase (PK). The ultimate fate of PEP is decided by the relative activities of these kinases as well as of lactate and malate dehydrogenases (LDH and MDH) [19]. Thus, *Schistosoma mansoni* [20], *Chandlerella hawkingi* [21] and *Brugia pahangi* [22] exclusively produce lactate as the end product and, therefore, are called homolactate fermenters. The above parasites possess very active PK and LDH [22-24]. Consequently, PEP is converted into lactate via pyruvate in these parasites.

Contrary to the above situation, helminths like *Ascaris lumbricoides*, *Hymenolepis diminuta* [23] and *Fasciola hepatica* [25] possess PEP-CK and MDH in high concentrations. Thus the metabolism of PEP is shifted to give malate in these worms.

Recently it has been shown that strain differences in helminths may lead to differences in end products. Kolhagen and coworkers [26] found a variation from 30-60% in the production of lactate in different strains of *H. diminuta*. It has been reported by McManus [27] that carbohydrate metabolism in adult schistosomes may exclusively lead to lactate formation and that homolactate fermentation is not *per se* essential for generating ATP in these worms.

3.2 Glucose metabolism in mitochondria

Malate, produced in the cytostol, enters the mitochondria where it undergoes dismutation [28]. One half of the malate is oxidized and decarboxylated to pyruvate by malic enzyme (ME) [29,30], while the other half undergoes dehydration to form fumarate which is reduced to succinate through an electron-transport-associated fumarate-reductase (FR) [31-33]. Metabolism of both the halves of malate produces one mole of NADH each. From pyruvate and succinate, the metabolism proceeds through the sequence of reactions as described by Komuniecki *et al.* [34]. During the entire metabolism in mitochondria, ATP is produced at various steps either by substrate level phosphorylation or by anaerobic phosphorylation at site I [28,30,35,36]

CH2OH

ATP   ADP

D-Glucose

Glucose--6 phosphate (G-6-P)

OPO3H2

H2C   O   H

H HO

CH2OH

OH H

Fructose-6-phosphate (F-6-P)

ATP

ADP

OHC—CH—CH2—OPO3H2
          OH

Glyceraldehyde-3-phosphate

Pi   NAD

NADH

CH2OPO3H2
CHOH
O=C—OPO3H2

1,3-Diphosphoglycerate (1,3-DPG)

ADP

ATP

HOOC—CH—CH2—OPO3H2
          OH

3-Phosphoglycerate (3-PG)

O
HOOC—C—CH2—COOH

Oxaloacetate (OAA)

NAD

NADH

HOOC—CH—CH2—COOH
          OH

Malate (MAL)

Fructose-1,6-diphosphate (FDP)

HO—CH2—CO—CH2—OPO3H2

Dihydroxyacetone phosphate (DHAP)

HOOC—CH—CH2—OH
          OPO3H2

2-Phosphoglycerate (2-PG)

HOOC—C=CH2
          OPO3H2

Phosphoenol pyruvate (PEP)

CH3—CO—COOH

Pyruvate (PYR)

NAD

NADH

CH3—CH—COOH
          OH

Lactate (LAC)

Chart 2:   Glucose metabolism in cytosol of helminths.

52

(Chart 3). The end products of the glucose metabolism in ascaris mitochondria are acetate, propionate and the higher volatile fatty acids such as 2-methylbutyrate and 2-methylvalerate.
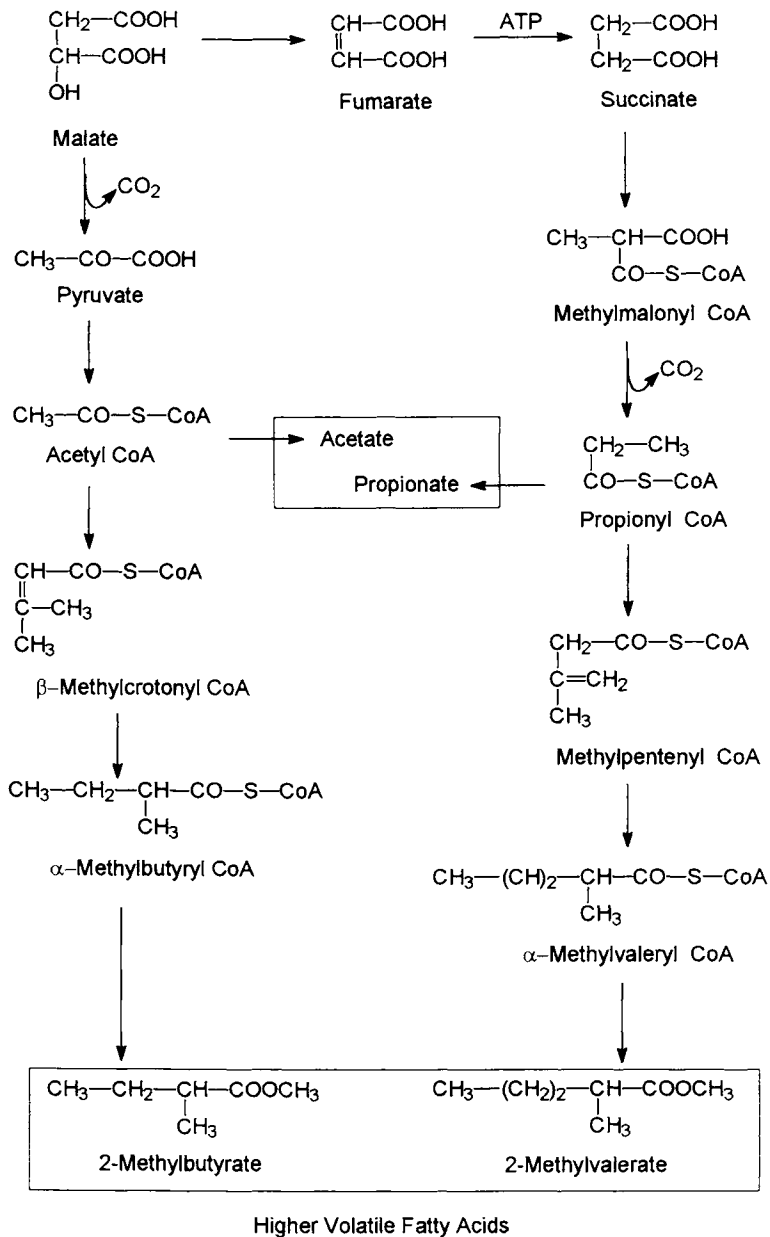
Chart 3: Glucose metabolism in Ascaris mitochondria.

3.3   Possible targets of anthelmintic action

Since a variety of enzymatic reactions control the energy metabolism in para-sitic helminths, the following biochemical targets may be utilized for effective design of new anthelmintics.

3.3.1 Inhibition of glucose uptake

Blocking the uptake of glucose, the primary source of worm's energy, would lead to starvation and ultimate death of the parasite. This may be achieved by modulating the cytosolic or mitochondrial enzymes. Praziquantel (5) and mebenda-zole (6) have been found to inhibit glucose uptake in various helminths [9]. The inhi-bition of glucose uptake by praziquantel in *H. diminuta* may be mediated through modulation of mitochondrial enzymes [37]. Amoscanate (7) has also been found to block uptake of glucose by *B. pahangi* and *L. carinii* [38].
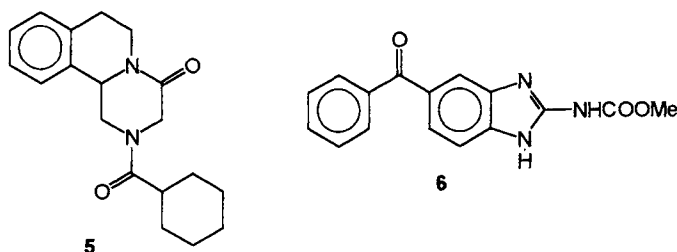
3.3.2 Inhibition of glycolytic enzymes

The effective inhibition of glycolysis may be achieved by blocking rate limit-ing steps involving phosphorylation of various intermediate metabolites. Such reac-tions are catalysed by hexokinase, phosphofructokinase (PFK), pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK). Inhibition of hexokinase stops the entry of glucose into the Embden-Meyerhof-Parnas pathway in the cytosol, thereby making the glucose catabolism nonfunctional. Other kinases also play im-portant roles in the glycolysis and, therefore, may serve as useful targets for drug design. In addition, malate and lactate dehydrogenases, which reoxidise NADH in the cytosol leading to proper functioning of the glycolysis, have also been reported as possible biochemical targets for anthelmintic action [39]. However, these enzymes are common to host and parasite, and it would be difficult to design molecules which have selective action on parasitic enzymes.

3.3.3 Inhibition of glycogen metabolism

Glycogen serves as the reserve carbohydrate which is utilized by the parasite for energy production during starvation. However, even under normal conditions when there is excess of sugar, a constant turn-over of glycogen continues. The presence of surplus glucose or ample supply of other fuels is marked by high concentration of glucose 6-phosphate and consequent high energy charge. These factors turn on glycogen synthase resulting in conversion of glucose into glycogen, which is stored in liver and muscles. When fuel is required by the body as a result of low energy charge or less supply of glucose in the blood, glycogen phosphorylase is activated and glycogen synthase is inhibited causing break down of reserve glycogen to yield glucose in liver and glucose 6-phospate in muscles for glycolysis and subsequent energy production.

The two enzymes involved in the synthesis and break down of glycogen exist in active and inactive forms. Thus, glycogen synthase is present as glycogen synthase-I (independent form or active) and glycogen synthase-D (dependent form or inactive) of which the former can be inactivated by protein kinase which, in turn, is activated by cyclic AMP. Similarly, glycogen phosphorylase exists as phosphorylase-a (highly active) and phosphorylase-b (poorly active) forms. The formation of active phosphorylase-a occurs in the presence of phosphorylase-b kinase which *per se* is activated by a cAMP dependent protein kinase (Chart-4).

Glycogen Phosphorylase-b

Phosphorylase-a kinase

Protein kinase

Phosphorylase-b kinase

cAMP

Glycogen Phosphorylase-a

Glycogen ⟺ Glucose

Glycogen Synthase - I

Protein kinase ◄------- cAMP

Glycogen Synthase - D
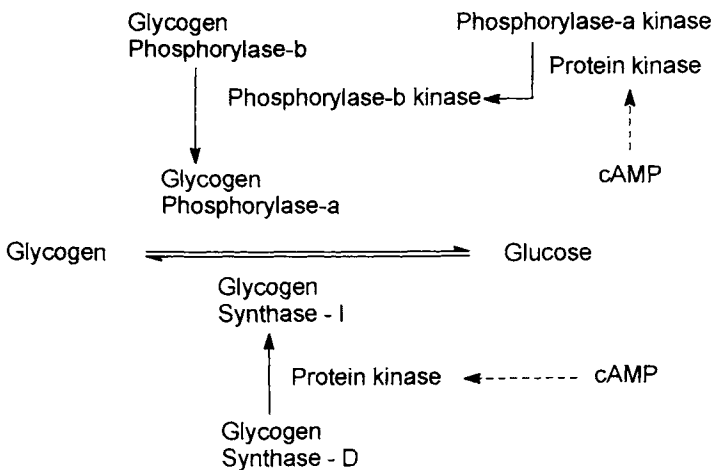
Chart 4: Regulation of glycogen metabolism in mammalian liver.

Inhibition of either glycogen synthase or glycogen phosphorylase is expected to disturb the glycogen equilibrium in the biophase. This can be effectively achieved by changing either the level of cAMP or inactivation of protein kinase. Amoscanate (7) and levamisole (8) have been shown to interfere with the metabolism of glycogen [38,40-42].

7   8
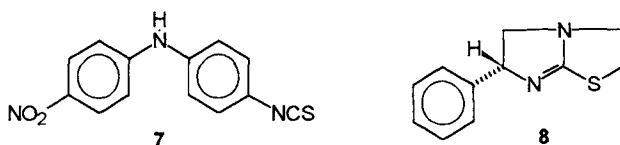
3.3.4 Inhibition of mitochondrial reactions

The formation of succinate from fumarate by fumarate reductase (FR) and anaerobic phosphorylation of ADP to ATP are amongst the important reactions taking place in the mitochondria to provide energy to the helminths. The FR system of helminths differs from succinate dehydrogenase (SDH) of mammalian tissues in several ways. For example: (a) FR requires NAD while SDH utilizes flavin nucleotide (FAD) as the coenzyme; (b) FR acts only in one direction but SDH is a reversible enzyme; (c) FR acts as the terminal electron acceptor under anaerobic conditions while SDH has no such property. Levamisole was shown to inhibit the FR in *Ascaris* [43].

The anaerobic phosphorylation of ADP to ATP in mitochondria offers an excellent site for chemotherapeutic attack. The uncoupling of oxidative phosphorylation would lead to inhibition of ATP synthesis and subsequent starvation of the worms. A number of uncouplers of oxidative phosphorylation are now known, the first being 2,4-dinitrophenol (9) described by Loomis and Lipmann in 1948 [44]. Niclosamide (10) and other salicylanilide anthelmintics have been found to inhibit anaerobic incorporation of ^{32}P into ATP and affect net mitochondrial production of ATP [45].

9   10
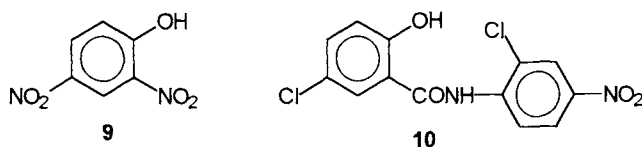
4. EVASION OF HOST'S DEFENCE MECHANISMS

Since the parasites live inside the host's body at the expense of the latter, they have developed effective mechanisms to overcome the host's defence system for their survival, growth and reproduction which are broadly described under the following two heads.

4.1 Protection from immune attack

Various helminths such as the filariids and schistosomes, which live in the lymph nodes, musculature and blood circulatory system, are known to have evolved various mechanisms to prevent themselves from the host's immune attack. Most of the tissue-dwelling helminths studied have been found to carry host originated proteins on their surface and thereby disguise the host's immune surveillance [46]. In addition, the parasites may also secrete immune modulatory molecules, or may be involved in the induction of suppressor cells and proteinase inhibitor-mediated retardation of humoral and cellular immune effector arms, which are used by the host to eliminate foreign bodies [47]. Consequently, interference with the parasite's evading mechanisms provides a promising target for anthelmintics design. Compounds which facilitate the phagocytosis of the parasite by the immune system, and prevent the parasite evasion process, are termed opsonisers. Diethylcarbamazine (11) is the best example of this class. The drug is known to alter the surface of the microfilariae in such a way that they are recognised as foreign bodies by the host and are phagocytised by the reticuloendothelial system (RES) of the liver [48]. Later it was shown that the drug enhances the antibody mediated cell adhesion to the microfilariae [49]. This calls for design of compounds that may initiate cell mediated destruction of the parasites.

$$CH_3-N\underset{}{\overset{}{\bigcirc}}N-\overset{\overset{\displaystyle O}{\parallel}}{C}-N\overset{\displaystyle Et}{\underset{\displaystyle Et}{}}$$
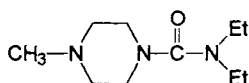
11

4.2 Protection from host oxidants

Another effective method employed by the host for destroying foreign bodies involves generation of reactive oxygen species (ROS) such as O_2^-, H_2O_2 and $\cdot OH$. This represents a powerful effector mechanism against parasites by virtue of their cytotoxicity and ability to damage membranes, nucleic acids and proteins resulting in death of the cell or the entire organism. These species are produced during normal cellular mechanisms, especially by macrophages; however, their production is

greatly increased in the presence of xenobiotics or parasitic infections. The mechanism of increased formation of ROS, usually termed as 'respiratory burst', is the defence mechanism of the host wherein the phagocytes kill a variety of infectious agents.

In order to survive in the host, the parasites have armed themselves with antioxidant enzymes that neutralize the toxic effects of the ROS generated by the host's phagocytes. All the protozoan and helminth parasites studied so far have been found to possess one or more of the three major antioxidant enzymes, superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-px) [50].

The mechanism of scavenging or quenching the ROS by antioxidant enzymes is given in chart 5. Under a parasitic burden, the phagocytes undergo respiratory burst releasing hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-)', which further interact and give rise to singlet oxygen ($^1O_2$) and hydroxyl radicals ($^{\cdot}OH$) by the Haber-Weiss reaction. The superoxide anions undergo dismutation in the presence of superoxide dismutase to form hydrogen peroxide and oxygen. The parasites may quench the cytotoxicity of H_2O_2 in several ways. These include converting H_2O_2 into water either by the enzymes catalase or glutathione peroxidase. The enzymes, myeloperoxidase (MPO) and eosinophil peroxidase (EPO), secreted by neutrophils and eosinophils, respectively, catalyse the conversion of H_2O_2 and halides (Cl^-, Br^-, I^-) into water and hypohalous acids that may further react with amines to yield more stable and toxic products [50].
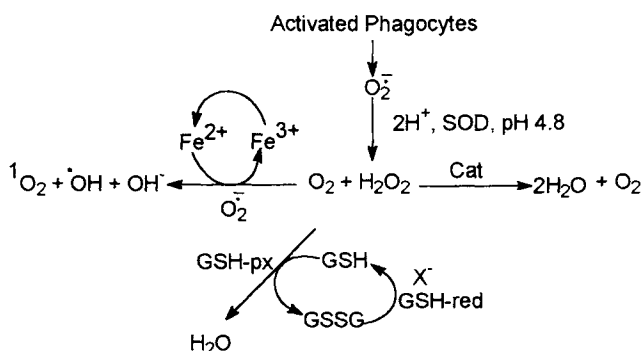
Chart 5 : Scavenging of ROS by antioxidant enzymes in helminths.

Thus, inhibition of the activity of catalase and glutathione peroxidase of the parasites, responsible for scavenging the H_2O_2 generated by host phagocytes, offers excellent targets in helminth chemotherapy. Inhibition of the above enzymes would

cause accumulation of toxic H_2O_2 leading to progressive damage and eventual death of the pathogen. Recently the benzimidazole anthelmintics, CDRI 82-437 (**12**) and 81-470 (**13**), which exhibit potent macrofilaricidal and enteric nematodicidal activities, respectively [51,52], have been found to inhibit the antioxidant enzymes of the helminths. Compound **12** inhibits catalase and glutathione peroxidase [53a-c], while **13** interferes with the activity of superoxide dismutase [54].

12

13
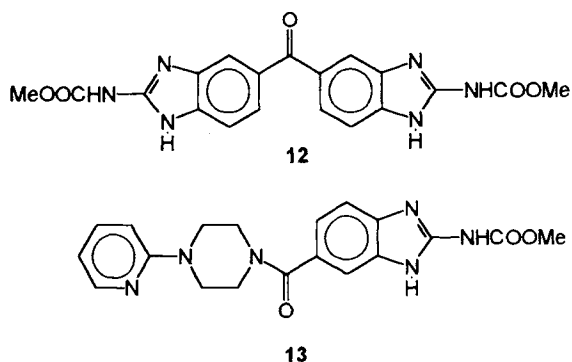
5.   OTHER MECHANISMS

In addition to the various biochemical reactions operating during neuromuscular coordination, glucose metabolism and evasion of host's defences there are several other biochemical processes in the parasites which provide excellent targets for the design of effective anthelmintics.

5.1   Transport processes

Tapeworms (cestodes) and flatworms (trematodes) absorb nutrients they need for their survival through their tegument. Consequently, these helminths possess a variety of transport systems. They may absorb nutrients and other vital ingredients by simple and facilitated diffusion as well as by active and co-transport mechanisms. Even in enteric roundworms (nematodes) which have a well defined digestive tract and take nutrients through the gut, the transcuticular absorption of different molecules can not be ruled out [55]. Thus, transport of molecules across the helminth tegument appears to play an important role in the worm's physiology and, therefore, provides an useful target for interference. Obviously, compounds that cause a change in the topography of the helminth's cuticle may be expected to disturb the transcuticular transport of various molecules and ions. The antischistosomal activity

of benzylic diamines (R_1R_2N-CH_2-C_6H_4-O-$(CH_2)_n$-O-C_6H_4-CH_2-NR_1R_2; where n=2-10) has been shown to be due to their ability to inhibit the mediated transport of glucose [56]. Similarly, triphenyltin chloride (Ph_3SnCl) and mercuric *p*-hydroxybenzoate make the filarial worms, *Setaria cervi*, immotile by inhibiting transcuticular absorption of methylglucose [57].

5.2 Tubulin polymerisation

Tubulin, a globular polypeptide of 50-55 K daltons, is an important protein of the cytoskeleton and mitotic spindle of all living cells. The most abundant source of tubulin is the brain of vertebrates. The tubulins isolated from brain and other parts of the body have been found to be a dimer of α- and β-tubulins with a closely related amino acid sequence and molecular weight of 100-110 K daltons. The α- and β-tubulins may generally assemble in αα, αβ, or ββ ways to form the dimeric tubulins. Polymerisation of the dimer protein gives microtubules. This is achieved by constituting protofilaments of tubulin polypeptides aligned in rows where α-tubulin of one dimer is joined with the β-tubulin of the next. Usually 13 such protofilaments are arranged side by side around a central core giving rise to a hollow cylindrical structure called microtubule [58] (Chart 6).
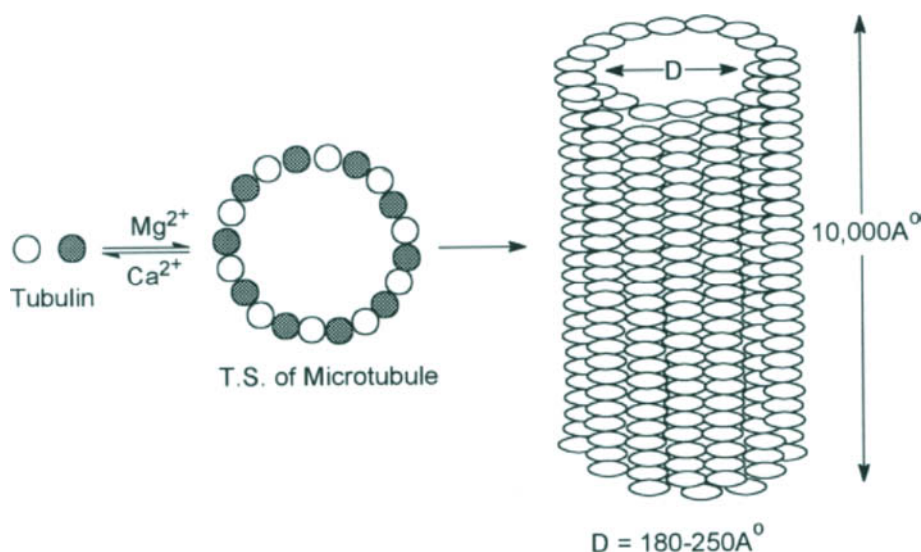
Chart 6: Tubulins and microtubules.

The length of the microtubule cylinder may measure upto 10,000 Å, but the diameter is usually 180-250 Å. The polymerisation of tubulins to microtubules takes place at $37^{o}C$ in presence of Mg^{2+}ions, endogenous cofactors such as GTP and microtubule-associated proteins (MAPs). The depolymerisation of microtubules occurs at temperatures lower than $37^{o}C$ and in presence of Ca^{2+} ions. The assembly and disassembly of microtubules proceeds in a nucleated fashion and is associated with a number of cellular functions. The formation of microtubules are required to control various cell activities such as cytoplasmic movement, cell division, cell shape and substrate and vesicle transport etc. Thus, interruption of the microtubulin assembly by a chemotherapeutic agent would result in several cellular dysfunctions leading to death of the parasites. Several drugs are known to bind with tubulin and block its polymerisation into microtubules. This results in gradual disappearance of microtubules from the cells. Consequently cytoplasmic movement and transport of nutrients are disturbed. These abnormal conditions cause death of the cell [59,60].

Benzimidazoles were the first anthelmintics which were shown to bind with tubulin and inhibit formation of microtubules in various helminths [61]. Later Borgers and De Nollin [62] showed disintegration of microtubules in the intestinal cells of *Ascaris* after mebendazole (6) treatment. Further, it was also demonstrated that the benzimidazole anthelmintics possess high selectivity to bind 250-400 times faster to parasitic tubulin than the mammalian tubulin [63,64].

5.3 Polyamine metabolism

Polyamine biosynthesis is associated with regulation of a number of metabolic functions including growth of cells in most of the living organisms. In mammals, ornithine is the precursor of aliphatic polyamines. Putrescine, formed by decarboxylation of the former by ornithine decarboxylase, is the first amine formed in polyamine biosynthesis. Putrescine gives rise to the other two polyamines, spermine and spermidine by successive addition of 3-aminopropyl residues derived from S-adenosyl-L-methionine (SAM) in the presence of different enzymes [44] (Chart 7).

Polyamines regulate a number of biochemical functions in mammals as well in the parasites. The presence of these amines has been demonstrated in some helminths. Others, such as the buffalo filarial worm, *Setaria cervi*, which lack the enzymes essential for the biosynthesis of polyamines, depend on their host to meet the requirement of these amines [65a]. Consequently, the inhibition of the uptake of polyamines, their biosynthesis or metabolic functions also provide useful targets for design of potential anthelmintics [65a,b].

HN_2

H_2N—$(CH_2)_3$—CH—$COOH$
       |
       NH_2
Ornithine

CH_2—$\overset{+}{\underset{|}{S}}$—$CH_2$—$CH_2$, X^-
      Me
      $HOOC$—CH
              |
              NH_2

$-CO_2$ | Ornithine
        | decarboxylase

H_2N—$(CH_2)_4$—NH_2

Putrescine

S-Adenosyl-L-methionine
(SAM)

SAM decarboxylase

HN_2

Spermine | Aminopropyl
synthase |
         | Transferase

CH_2—$\overset{+}{\underset{|}{S}}$—$(CH_2)_3$—$NH_2$, X^-
      Me

H_2N—$(CH_2)_4$—NH—$(CH_2)_3$—NH_2

Spermine

S-Adenosyl-3-methylmercapto-
propylamine

H_2N—$(CH_2)_3$—NH—$(CH_2)_4$—NH—$(CH_2)_3$—NH_2

Spermidine
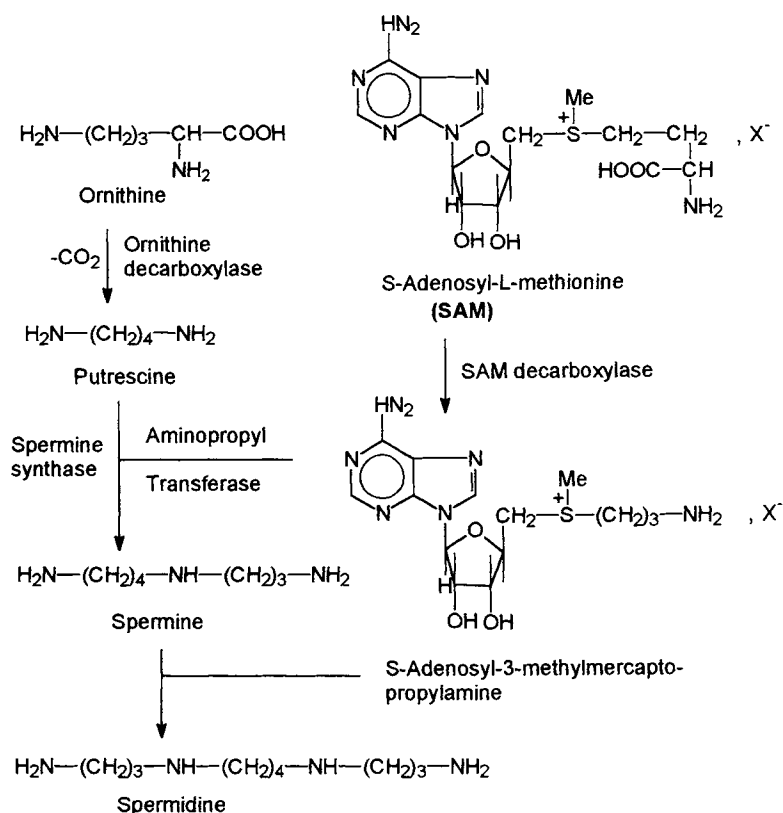
Chart 7: Polyamine biosynthesis.

5.4 Reproductive system

The chemotherapy of filariasis, a major helminth infection of the tropics, is unsatisfactory because none of the drugs available in clinical practice can provide radical cure of the disease. Radical cure of filariasis involves killing of both the microfilariae and adult worms with least toxicity to the host. However, sudden death of micro- and macrofilariae may lead to anaphylactic reactions due to the high titre of proteins liberated from decaying worms. This situation can become grave especially in patients with a heavy worm burden. In order to circumvent this problem, interference with the reproductive system of the adult female worms has been considered as an useful target for chemotherapy. The sterilization of the adult female filariids will not only bring down the blood microfilariaemia and interrupt the transmission cycle, but will also help the host to assimilate the degenerating worms dying at different intervals and thus prevent the anaphylactic reaction [66].

Knowledge of helminth endocrinology is rudimentary at present, though presence of a hormonal system similar to that of insects has been suggested [67]. It has been further pointed out that ecdysteroids and juvenile hormones, which control metamorphosis in insects, may also play a similar role in the growth of nematodes. This is based on the fact that ecdysteroids have been detected in nematodes, cestodes and trematodes [68-71].

Several authors have reported a quantitative estimation of ecdysteroids in various helminths. In the female dog heartworm, *Dirofilaria immitis*, the ecdysteroids were present in a concentration of 1.9 ng/g [72]. Of this the ratio of ecdysone (14) and 20-hydroxyecdysone (15) was 60 and 40%, respectively. In the male worm, the content of free ecdysteroid was found to be 3.0 ng/g. These ecdysteroids are found both in free and conjugated forms [73]. It has been assumed that free ecdysteroids are generated from the polar conjugated forms by enzymatic hydrolysis during embryogenesis in the eggs of female insects [67]. In nematodes such as *D. immitis* and *A. suum*, the free and conjugated ecdysteroids are usually concentrated in the reproductive organs; however, these may also be found in other parts of the body like gut and carcass [73].

14   15
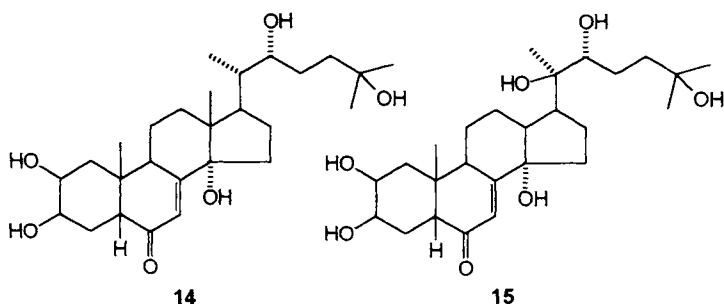
The concentration of ecdysteroids in the adult sheep tapeworm, *Moniezia expansa* has been found to be 0.3-0.7 ng/g. Free and conjugated ecdysteroids have also been reported from *Hymenolepis diminuta* [74]. Such steroids may be found in schistosomes too [69].

The biosynthesis of ecdysteroids in filarial worms has been studied [75]. It has been shown that *D. immitis* and *Brugia pahangi* can not synthesize ecdysteroids from cholesterol. Further, no evidence was available to support the conversion of ecdysone (14) to 20-hydroxyecdysone (15). Not withstanding the fact that the basic studies regarding endocrinology of female worms are still not elaborate, interference of ecdysteroid biosynthesis and function in female helminths may provide

novel and useful targets for chemotherapeutic attack.

It has been shown that 5-fluorodeoxyuridine acts synergistically with low doses of methotrexate and induces immediate, long lasting sterilization of adult female worms of *B. pahangi* in jirds. Possibly thymidylate synthase is involved in chemosterilization of the filariids [76]. Recently a number of benzimidazoles, quinolines, pyrroles, nitriles and tetrahydropyrimidines have been found to sterilize the female adult worms of *Litomosoides carinii* in cotton rats. Many of the compounds studied also killed the microfilariae in blood and adult worms in the musculature at a dose of 30-100 mg/kg given parenterally or orally for 5 days [66]. Some brassino steroids (16) and aminoalkyl steroids (17) have been found to exhibit macrofilaricidal action and also inhibit production of microfilariae by adult female worms of *B. pahangi in vitro* [77].

16 R = OH or H
R$_1$ = OH or H
R$_2$ = CH$_3$ or H
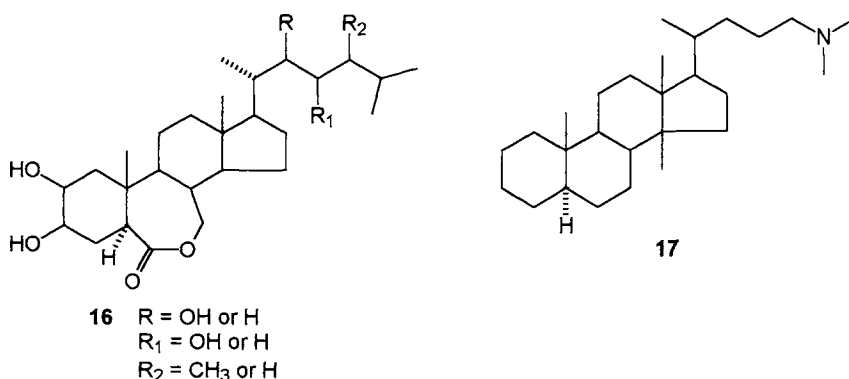
5.5   Less explored biochemical targets

The nutritional effects of vitamin A (retinol) and its role in controlling various physiological functions in mammalian cells is well-known. However, the role of retinol and retinoic acid in helminths is not fully understood. Comley and Jaffe [78] have shown the uptake of retinol and its formation from β-carotene by *Brugia malayi*. It has also been found that retinol contents in *Onchocerca volvulus* is 8 times higher than that of surrounding host tissues [79]. More recently, Sani and Vaid [80] have demonstrated a competition between ivermectin and retinol for retinol binding proteins in some filarial worms but not in the host. Thus interaction between chemical agents and retinol/retinoic acid binding proteins may offer an unexplored target for design of anthelmintics.

Chitin biosynthesis provides yet another target for drug design. The presence of chitin in membranes of young embryos of *Onchocerca gibsoni* [81] and the absence

of chitin biosynthesis in mammals would suggest that interference with chitin synthesis in helminths is a useful target for chemotherapeutic attack.

Destruction of the apical tegumental layer of helminths by inducing vacuolization may also serve as a selective method of killing the parasites. Praziquantel is known to vacuolize the tegument of *S. mansoni, Dicrocoelium dendriticum* and *H. nana* which causes disruption of the outer layer [82].

A few other less studied biochemical approaches such as purine and pyrimidine metabolism, protein biosynthesis and lipid metabolism in helminths also provide targets for antiparasitic drug design [83]. Like protozoal parasites, some helminths such as *S. mansoni* (adult and larval forms) lack *de novo* purine biosynthesis and, therefore, depend entirely on the salvage mechanism for their purine requirements. Similarly amino acid metabolism and biosynthesis of proteins has also been not worked out in many parasites [83a]. Although the helminths meet their requirements of amino acids by absorbing freely from the host, they may also synthesize some amino acids. For example, *Fasciola hepatica*, schistosomes and other trematodes produce proline by a reaction sequence given in Chart 8. Similarly *H. diminuta* can
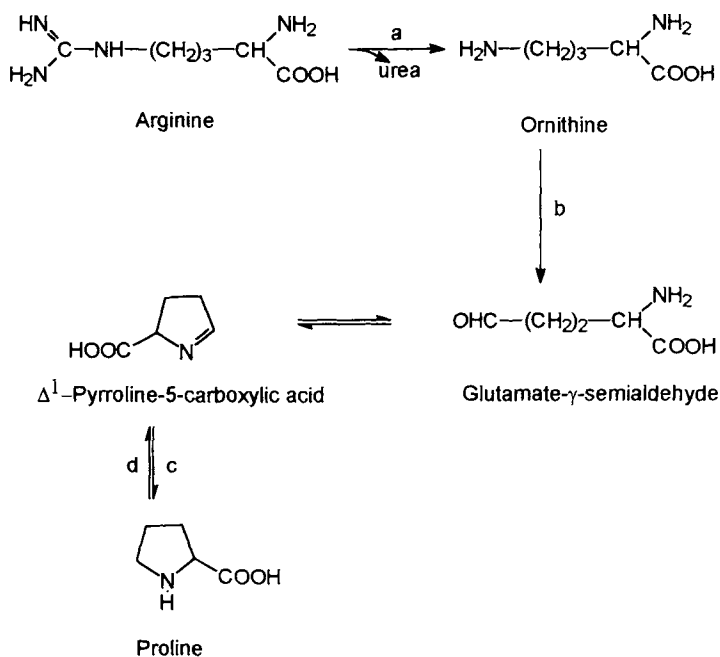
Chart 8: Proline biosynthesis in trematodes (abbreviations: a, arginase; b, ornithine-α-ketoacid transferase; c, pyrroline-5- carboxylate reductase; d, proline oxidase).

synthesize small amounts of serotonin from tryptophan, while *F. hepatica* can produce this neuromuscular stimulator from 5-hydroxytryptophan [83]. Some nematodes like *Ascaris* and *Dirofilaria immitis* are capable of synthesizing long chain fatty acids from simple precursors. Though these parasites may convert acetyl CoA into various intermediates for sterol biosynthesis such as mevalonate and farnesol, the formation of cholesterol is rarely achieved [83]. These aspects, therefore, need detailed exploration before it may be concluded that lipid, purine or amino acid metabolism can be used as definite leads for the design of anthelmintics.

Recently Liu and Weller [84] have reviewed the arachidonic acid metabolism in filarial parasites and other helminths. Arachidonic acid (AA) is a 20 carbon polyunsaturated fatty acid derived from dietary fatty acids. In human tissues, AA is usually present in the esterified form such as glycerolipids, phospholipids and neutral lipids. The free AA, released by phospholipases, undergoes various enzymatic oxygenations to form local mediators such as prostaglandins and leukotrienes, which are collectively known as eicosanoids (Chart 9). These eicosanoids are associated with platelet aggregation, vasodilation, leukocyte inflammatory and immune functions and cellular adhesion [85].
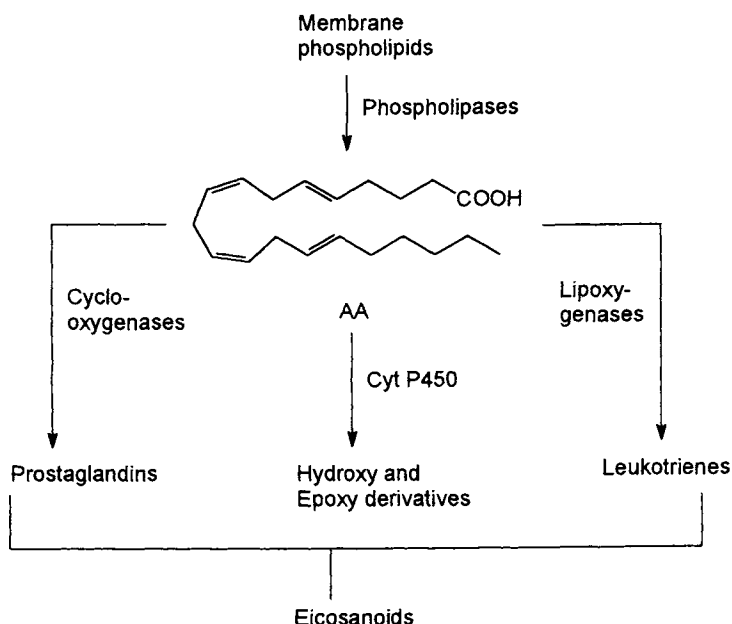
Chart 9: Arachidonic acid (AA) metabolism.

In general, helminth parasites including filariids, can not synthesize AA *de novo* and, therefore, utilize polyunsaturated fatty acids of the host. In fact, it has been shown that *Brugia malayi* preferentially incorporates exogenous AA and metabolises it into eicosanoids such as prostacycline and PGE_2. The filarial worms can also utilize the endogenous reserve of AA to metabolise it into prostaglandins [84,86,87]. Consequently, interference with the helminthic eicosanoid biosynthesis provides a new area for the design of anthelmintic agents. According to recent information the microfilarial action of diethylcarbamazine (DEC) has been related to interference with the AA metabolism to prostaglandins both in microfilariae and host [88]. This warrants a detailed study of the eicosanoid biosynthesis in different helminth parasites with special reference to enzymes involved in such biological reactions. Some recent reviews on this aspect are available [89,90].

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CHAPTER 3

NATURAL PRODUCTS

1.   INTRODUCTION

The use of "herbal preparations" in the treatment of some human parasitic infections has been described in traditional systems of medicine like *Ayurveda*, Traditional Chinese Medicine and *Unani*, practiced since immemorial times. The drugs used in traditional systems thus provide useful leads for development of modern drugs. Consequently a variety of medicinal plants have been subjected to detailed chemical and biological investigations culminating in the discovery of novel anthelmintic natural products of medicinal and veterinary importance [1].

2.   DRUGS FOR ROUNDWORM INFECTIONS

2.1   Santonin (1)

Santonin (**1a,b**), an effective drug for treating intestinal roundworms, was first isolated from unexpanded flower heads of *Artemesia* plants (Levant Wormseed) grown in Russian and Chinese Turkestan and the Southern Ural region. The structure of this compound was established by Clemo *et al.* [2,3] and Ruzicka and Steiner [4]. Santonin exists in two forms, α-santonin and β-santonin, the stereochemistry of which has been worked out by several workers [5-7].

2.2   Kainic acid (2) and ascaridol (3)

Kainic acid (**2**) is another ascaricide showing nearly ten times higher activity than santonin [8]. It was first isolated from the red algae, *Digenea simplex* by Murakami *et al.* [8,9] and the structure was established by Watase and coworkers [10-12]. Another effective human ascaricide is ascaridol (**3**), which is a terpene peroxide constituting 60-80% of the oil of chenopodium isolated from the Jerusalem Oak (*Chenopodium ambrosioides*), a plant also known as American wormseed. The leaves, seeds, flowers and sometimes roots of this plants have been used in Latin America and China during the eighteenth and nineteenth centuries as intestinal anthelmintics [1]. Ascaridol has been characterised chemically as 1-methyl-4-(1-methylethyl)-2,3-dioxabicyclo[2.2.2]oct-5-ene (**3**).
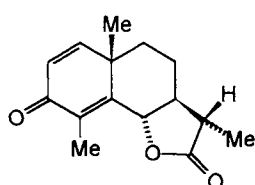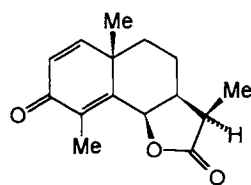
1a (α-Santonin)   **1b** (β-Santonin)
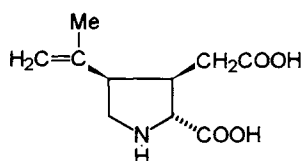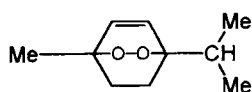
2 (Kainic acid)   **3** (Ascaridol)

2.3   Diospyrol (5)

Diospyrol (5), a polyhydroxybinaphthyl compound, occurs in fresh fruits of *Diospyros mollis*, a tall shrub which grows widely in South-East Asia. The extract of fresh fruits of the plant has been used since long in Thailand both as an anthelmintic and as a black dye. Loder *et al.* [13] isolated diospyrol from dried ripe fruits of *D. mollis* and assigned its structure as **4**. Later diospyrol was isolated from other *Diospyros* species and its chemical constitution was studied in detail. The correct structure of diospyrol was found to be **5**. A few structural analogues of diospyrol have also been synthesized [14,15]. Diospyrol is highly sensitive to air oxidation and, therefore, turns black when exposed to air. However, it can be preserved for a long period when kept in an evacuated vial.
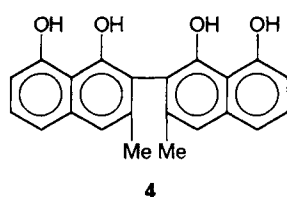
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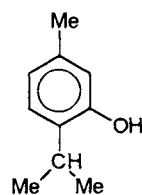
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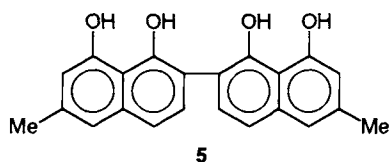
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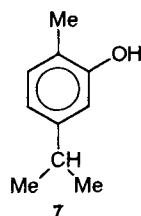
7

2.4 Thymol (6) and carvacrol (7)

Thymol (6) is another naturally occuring phenol, which has been widely used to treat several helminth infections in man during early periods of this century. Thymol was first isolated in 1719 by Neumann from the plants of *Thymus vulgaris* and its antihookworm activity was discovered by some Italian physicians while combating the hookworm epidemic, which struck the workers building the St. Gotthard Tunnel in the Alps during 1879-1880 [16]. The drug has also been found to occur in varying amounts in the essential oil of several plants belonging to the genera *Thymus* (Thyme), *Origanum* (Origanum) and *Carum* (Ajowan). A number of the above plants constitute a rich source of thymol; this product is often associated with its isomer, carvacrol (7).

2.5 Avermectins

During early 1980s, scientists at Merck Laboratories introduced avermectins in the chemotherapy of roundworm infections of humans and animals. The avermectins are structurally interrelated macrolide lactone antibiotics produced by the actinomycete *Streptomyces avermitilis* [17]. Eight such avermectins have been isolated and characterised. Ivermectin (8), a mixture of two avermectins containing at least 80% of 22,23-dihydroavermectin B_{1a} and less then 20% of 22,23-dihydroavermectin B_{1b}, was found to be the most active and, therefore, was chosen for detailed biological evaluations in humans and domestic animals [18]. The other avermectin of interest is abamectin (9) which contains at least 80% of avermectin B_{1a} and not more than 20% of avermectin B_{1b} [18,19]. Of these two, ivermectin has emerged as the most powerful veterinary antiparasitic drug and agricultural pesticide. It has been found to have high activity against lymphatic filariasis and river blindness in humans. Abamectin also possesses marked anthelmintic activity in domestic animals [18-20].

Dutton and coworkers [21] have isolated about 36 avermectins (10) which are produced through mutational biosynthesis by *Streptomyces avermitilis*, a mutant strain ATCC-53568. These antibiotics exhibit broad-spectrum of antiparasitic activity. Several avermectin homologues (11) were produced by *S. avermitilis* in presence of externally supplied sodium 2-methylpentanoate and sodium 2-methylhexanoate. The homologues, thus produced, carry 2-pentyl and 2-hexyl groups, respectively, at the C-25 position of the aglycone moiety. These antibiotics are designated as avermectin "c" and "d", respectively, which possess high anthelmintic and insecticidal activities [22].

74
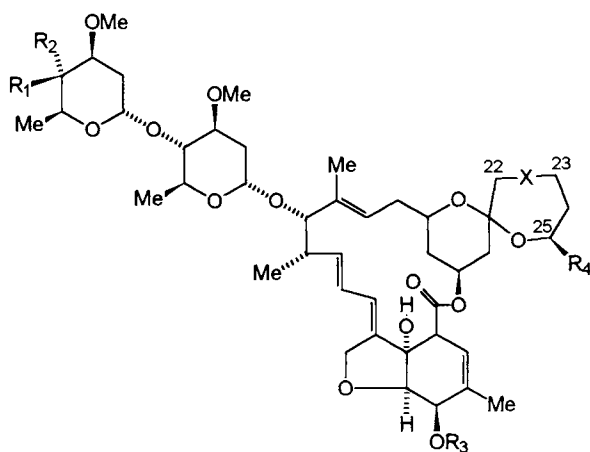
Avermectin	R_1	R_2	R_3	R_4	C22-X-C23
A_{1a}	H	OH	CH_3	$CH(CH_3)C_2H_5$	-CH=CH-
A_{1b}	H	OH	CH_3	$CH(CH_3)_2$	-CH=CH-
B_{1a}	H	OH	H	$CH(CH_3)C_2H_5$	-CH=CH-
B_{1b}	H	OH	H	$CH(CH_3)_2$	-CH=CH-
A_{2a}	H	OH	CH_3	$CH(CH_3)C_2H_5$	$-CH_2-CHOH-$
A_{2b}	H	OH	CH_3	$CH(CH_3)_2$	$-CH_2-CHOH-$
B_{2a}	H	OH	H	$CH(CH_3)C_2H_5$	$-CH_2-CHOH-$
B_{2b}	H	OH	H	$CH(CH_3)_2$	$-CH_2-CHOH-$
Ivermectin 8	H	OH	H	$CH(CH_3)C_2H_5$ 80% $CH(CH_3)_2$ 20%	-CH=CH-
Abamectin 9	H	OH	H	-do-	$-CH_2-CH_2-$
10	H	OH	H, alkyls	alkyls, cycloalkyls heteroaryls	-CH=CH- $-CH_2-CHOH-$
11	H	OH	H, Me	C_3H_7, C_4H_9	-CH=CH- $-CH_2-CHOH-$
12	NH_2	H	H	$CH(CH_3)_2$ $CH(CH_3)C_2H_5$	-CH=CH- -CH=CH-
13	$NHCH_3$	H	H	-do-	-CH=CH-

Mrozik *et al.* [23] have identified a series of novel 4"-amino-4"-deoxyavermectins with excellent insecticidal activity. The most effective members of this class are 12 and 13 which show 1,500 fold higher activity than avermectin B_1 (abamectin) against beet armyworm, *Spodoptera exigua* and other lepidopteran larvae.

2.6 Milbemycins

These are a family of novel macrolide antibiotics with high order of insecticidal and ascaricidal properties. The milbemycins are structurally related to the avermectins except for the fact that they have no substitution at position 13 and, therefore, may be called 13-deoxyavermectin aglycones. Originally nine milbemycins were isolated from the broth of *Streptomyces hygroscopicus*, subsp. *aureolacrimosus* [24,25]. Various other milbemycin derivatives were produced by fermentation of different strains of *Streptomyces* such as strain MA-5920 (prepared by fusion of *S. avermitilis* protoplast with *S. hygroscopicus* protoplast) [26], E-225 [27] and *S. eurythermus* [28]. The structure of the milbemycins is represented by the general formula **14**; the important members of this class are milbemycin-D (**15**) and nemadectin (**16**) [29,30].

14

15 R = CHMe$_2$, R$_1$ = H
16 R = C(Me)=CHCHMe$_2$,
R$_1$ = OH

17a R = OH, R$_1$ =Me
b R = R$_1$ = H
c $\begin{array}{c}R\\R_1\end{array}$ = CH$_2$
d R = H, R$_1$ = Me

18a R = H
b R = OH
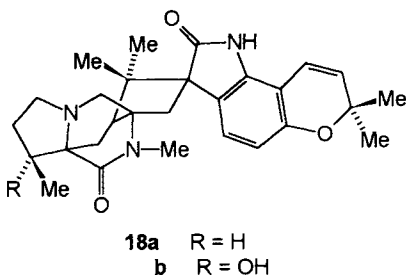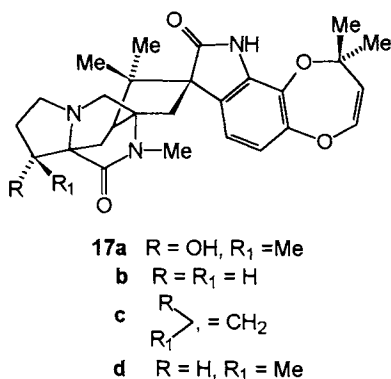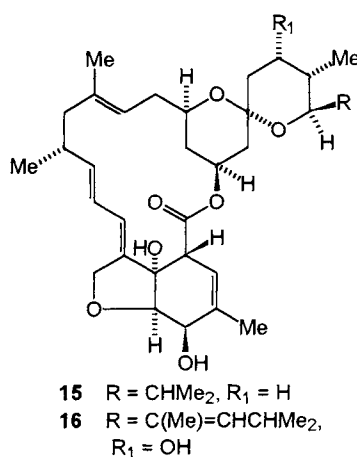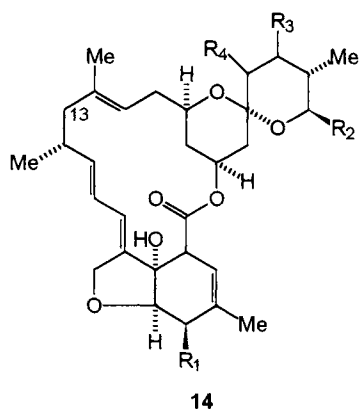
2.7 Paraherquamide (17a)

This is an indole alkaloid isolated from *Penicillium paraherquei* [31]. A number of naturally occuring and semi-synthetic analogues (17b-d and 18a, b) of paraherquamide possess marked anthelminitic activity [32,33].

3. DRUGS FOR TAPEWORM INFECTIONS

3.1 Aspidium oleoresin

The tapeworm infection is a widespread helminth disease which has attracted the attention of native practitioners and physicians. The first so called "effective" herbal preparation for eradicating tapeworms from humans became available in 1775 known as "Madame Nauffer's Tapeworm Cure" [1]. The active ingredient of this remedy was male fern. Since then the extract of the rhizome of male fern (*Dryopteris filix mas*), called aspidium oleoresin, has been used as a folk remedy and also as a drug in clinical medicine to treat tapeworm infections in humans.

Aspidium oleoresin contains a number of phloroglucinol derivatives, which were first isolated by Boehm [34]. The main constituents of the extract are filixic acid (19), aspidine (20a), flavaspidic acid (20b) and filicinic acid (21) with interrelated chemical structures [35-40]. A number of synthetic derivatives of these phlorogluci-nols have also been prepared but none was found suitable for clinical management of cestode infections [41]. Another phloroglucinol derivative, desaspidine (22), has been isolated from the rhizomes of *Dryopteris dilatata, D. austriaca* and *D. caucasia* [42-44]. This product has been found to be a better taenicide than aspidium oleoresin [45].

19 (Flixic acid)

20a R = CH₃ (Aspidine)
b   R = H (Flavaspidic acid)

21 (Filicinic acid)

22 (Desaspidine)
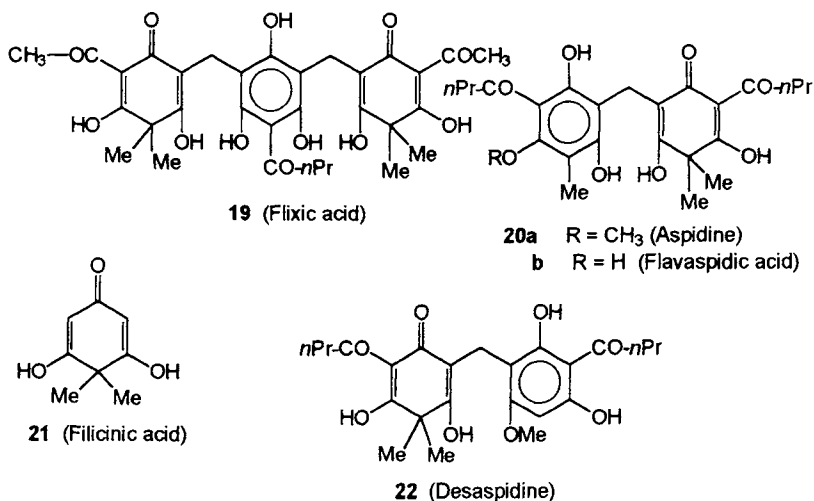
3.2 Kamala and kousso

Kamala obtained from *Mallotus philippinensis,* and Kousso obtained from *Hagenia abyssinica* (= *Brayera anthelmintica*) also show activity against tapeworms infecting man and animals. The extracts of these plants contain phloroglucinols very similar to those present in male fern. The active principle of kamala is rottlerine 23 [46-48], while kousso contains a mixture of α- and β-kosins (**24a,b**) [49,50].

23 (Rottlerine)

24a   R = Me, R_1 = H (α-Kosin)
  b   R = H, R_1 = Me (β-Kosin)
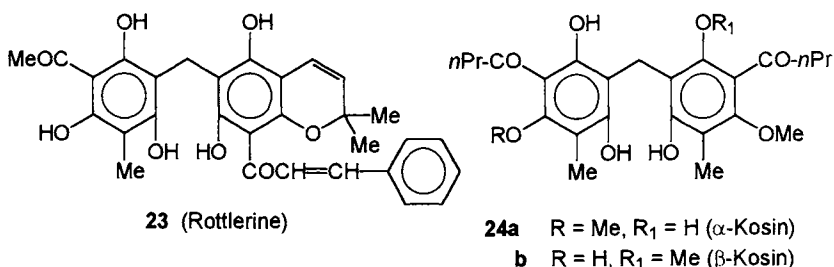
3.3 Arecoline and related anthelmintics

Arecoline (**25a**) is an old anthelmintic that has been used as a taenicide for cats, dogs and poulty since 1921. It is isolated from the seeds of the betel nut palm, *Areca catechu* [36]. Attempts to carry out SAR in arecoline analogues showed that N-alkyl homologues are inactive except for the propyl derivative which exhibits weak anthelmintic activity [51a]. The root bark of the pomegranate tree, *Punica granatum,* yields pelletierine (**25b**) whose structure is related to arecoline. The antitapeworm activity of this plant was known since the Middle Empire of ancient Egypt. Pelletierine tannate has been used to treat tapeworm infections in man with varying degree of success [52]. The pumpkin seeds are also known to possess activity against *T. sagniata* in man since long. The active principle of the seed is cucurbitin (**26**).

25a   25b   26
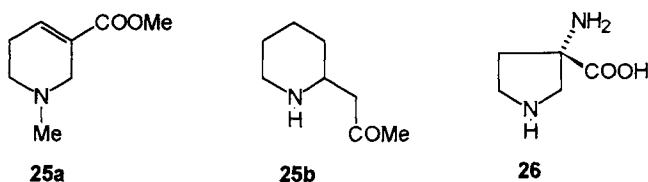
In addition to the above natural products, the ipecac alkaloid, emetine (**6,** chapter 15) and the antibiotic paromomycin (**18,** chapter 15), which are primarily used as antiprotozoal drugs, have also been found to be effective in the chemotherapy of trematode infections in humans.

3.4 Streblus asper

The crude extract of the stem bark of *Strebulus asper*, a traditionally used medicinal plant of India, has been found to possess potent macrofilaricidal activity against *Litomosoides carinii* and *Brugia malayi* in experimental animals. The active principles of the extract are two cardiac glycosides KO29 (asperoside, **27a**) and KO30 (strebloside, **27b**). Of these KO29 is a better macrofilaricide than KO30. At an oral dose of 50 mg/kg given for 5 days, KO29 killed adult worms of *L. carinii* (90%), *A viteae* (70%) and *B. malayi* (70%) in rodents. Futher work on the segregation of cardiotonic and macrofilaricidal activities by molecular modification in KO29 is in progress [51b].
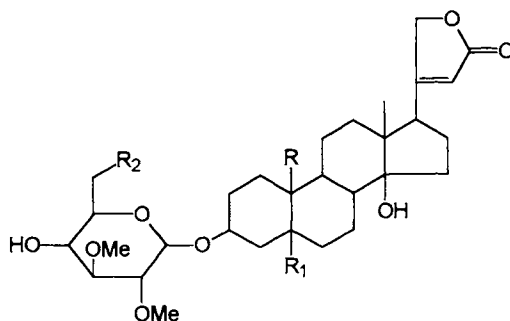
27a   R = Me, R_1 = H, R_2 = OH (Asperoside)
  b   R = CHO, R_1 = OH, R_2 = H (Strebloside)

4.   SAR IN NATURAL PRODUCTS

Although a number of natural products have enjoyed wide usage in the treatment of various helminth diseases of man and domestic animals prior to 1960, with the advent of more effective and safer synthetic anthelmintics most of them were eventually abandoned and are now of historical value only [52]. For example, santonin was included in 25th edition of the United States Dispensary (1955), but was removed from the U.S. Medical Compendium of National Formulary (1960). Similarly, aspidium oleoresin was included in the U.S. Pharmacopeia of 1960 and the full clinical usage of this drug was available in the U.S. Dispensary only until 1973 [1]. This makes the description of SAR and synthesis of most of the anthelmintic natural products less meaningful and is, therefore, not discussed in the present text. However, the SAR profile of avermectins and milbemycins is discussed, of which the former have in recent years been used extensively in the treatment of onchocerciasis and lymphatic filariasis in humans [20].

4.1   Avermectins

The avermectin molecule presents a complex chemical framework in which a large variety of structural modifications are possible [53-66]. However, the available data on SAR in avermectins indicate that molecular variations at positions 5,13,22,23 and 26 appear to be detrimental to antiparasitic response. Accordingly, a variety of structural analogues of avermectins have been synthesized and evaluated for their biological activity. The SAR which has emerged out of this study, may be summarised as follows:

(i) Of the 4''-O-acylated and 5-O-substituted derivatives of avermectins, the 4''-O-substitued derivatives retained biological activity while 5-O-substituted analogues showed weak or no activity. This would indicate that the presence of a hydroxy or methoxy group at 5-position is essential for antiparasitic activity of avermectins [53]. It also became evident that the sugar moiety may be modified with retention of activity [54-56].

(ii) The presence of two sugar moieties at position 13 is essential for biological activity. This was established by the fact that removal of one sugar from avermectins gave the corresponding monosaccharides (28) showing 1/2 to 1/4 activity of the parent drug [53]. Further removal of the sugar residue from monosaccharides give the aglycone (29) with still lower activity. The aglycone of dihydroavermectin B$_1$ has been found to be 30 folds less active than the parent drug [53]. However, it is interesting to note that removal of the hydroxy group from the 13-position of the aglycone yields 13-deoxyavermectin aglycones (30) (milbemycins), some of which show potent anthelmintic activity [30,53,57,64].

(iii) The avermectins of the A series with a methoxy at 5-position show less activity than those belonging to the B series having a 5-hydroxy function [53].

(iv) Reduction of the double bond at 22,23-position of avermectins leading to the formation of corresponding 22,23-dihydroavermectins do not significantly change the biological profile except for the fact that reduced products show a better safety index.

(v) The aliphatic nature of the ring having 5-OH/OMe is also essential for antiparasitic activity as conversion of this ring into an aromatic system exhibited no activity [53,57].

28 (Monosaccharide)

1% H$_2$SO$_4$

29 R = OH (Aglycone)
30 R = H (Milbemycins)
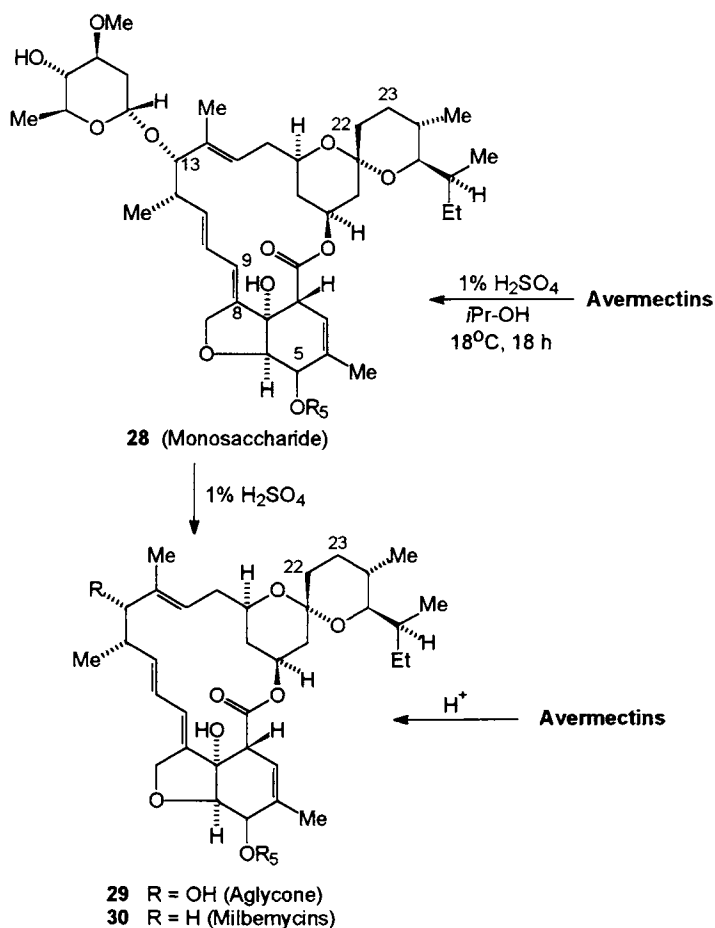
4.2 Milbemycins

Like avermectins, the milbemycins have also undergone extensive molecular modifications. A number of synthetic milbemycins have been found to possess a wide-spectrum of parasiticidal and insecticidal activities [67-83]. The structure-activity analysis would indicate that the milbemycins having different functional groups at positions 13,14 and 5 generally retain biological activity. The important milbemycin derivatives thus prepared include 13-substituted milbemycins of the type **31** [64,73-75] and **32** [76-78], 14-substituted milbemycins (**33**) [79], 5-keto/oximinomilbemycins (**34**) [81,82], **35** [80] and **36** [83].

A few semi-synthetic analogues of nemadectin (**16**) have also been prepared [84]. In general, most of the structural modifications yielded milbemycin derivatives
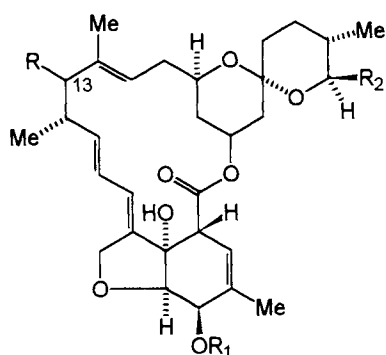
31

R = halogen, methoxy, H
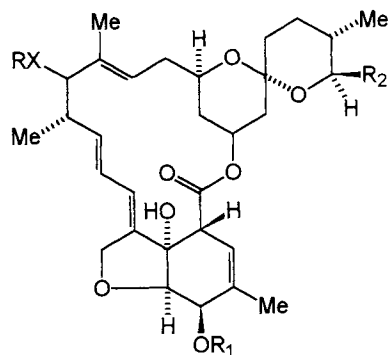
32

X = O, S
R = alkyl, aralkyl, acyl

33

$X = \begin{smallmatrix} ^{15}CH \\ \| \\ _{14}CH-CH_2COOR \end{smallmatrix}$
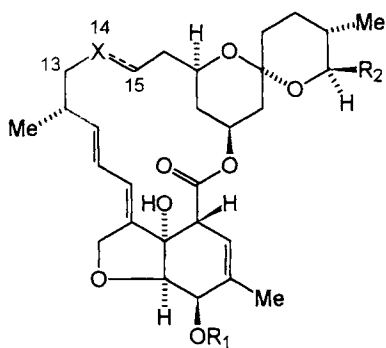
$X = {_{14}}C{=}CH_2$
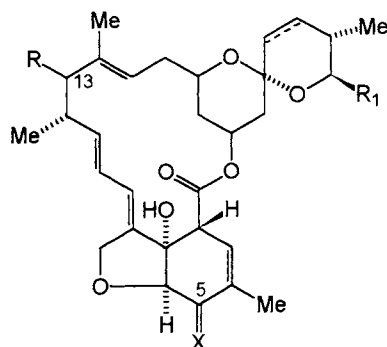
34

R = H, halogen, OH
X = O, NOH, NNR$_2$R$_3$
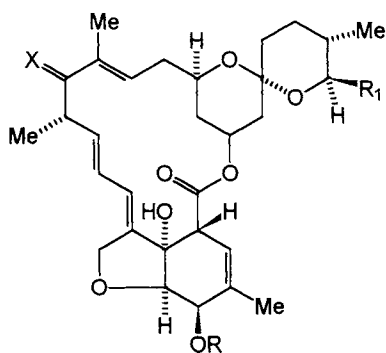
35

X = CH$_2$, O
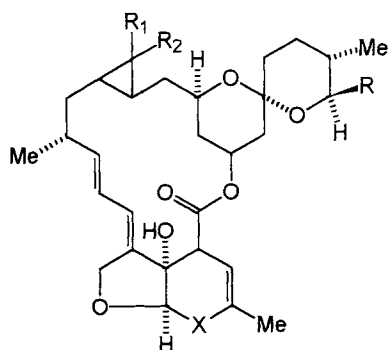
36

R$_1$ = H, alkyl, R$_2$ = H, halogen, alkyl
X = CO, C = NOR$_1$
= CHOR$_1$

with promising anthelmintic activity. For example, **31** (R=H, R$_1$=H, R$_2$=i-Pr) eradicates all the major nematodes from naturally infected sheep at a dose of 0.4 mg/kg [85].

Similarly, a 80:20 mixture of the milbemycin oximes **34** (R=H, R$_1$=Et, X=NOH) and **34** (R=H, R$_1$=Me, X=NOH) were found to be highly effective against precardiac and microfilarial stages of *D.immitis* and *T. canis* in dogs at a dose of 500 µg/kg. This combination was equally effective against adult hookworms, *A. caninum* in dogs [86,87]. Milbemycin oxime, called "Interceptor" has been found to be 97.8% active against *A. caninum* in dogs at a dose of 0.5 mg/kg [88].

5.   SYNTHESIS OF ANTHELMINTICS

5.1   α- and β-Santonins

The synthesis of α- and β-santonins (**1a,b**) has been described by Abe and coworkers [89]. The key intermediate for preparing santonins utilizes eusantonin (**38**) which is obtained by condensing 3-keto-4,9-dimethyl-1,2,3,7,8,9-hexahydronaphthalene (**37**) with diethyl methylmalonate. The two different routes to arrive at α- and β-santonins are given in scheme 1.

A stereocontrolled total synthesis of α-santonin (**1a**) and the less stable β-santonin (**1b**) has been developed by Marshall and Wuts [90] which is outlined in scheme 2.

5.2   Kainic acid (2)

In the first method for preparing kainic acid the key intermediate is 4,5-disubstituted piperidin-2-one (**60**) which was obtained by two different methods (Scheme 3) [91]. The piperidin-2-one (**60**) is brominated to form the monobromo derivative (**65**) which undergoes ring transformation to yield 2,3,4-trisubstituted pyrrolidine (**66**). Further reactions of **66** resulting in the formation of L-α-kainic acid are described in scheme 4.

Oppolzer and Andres [92] have synthesized α-kainic acid (**2**) by intramolecular Ene reaction. The starting material of this method is the amido ester (**70**) which is converted into **2** by the reaction sequence shown in scheme 5.
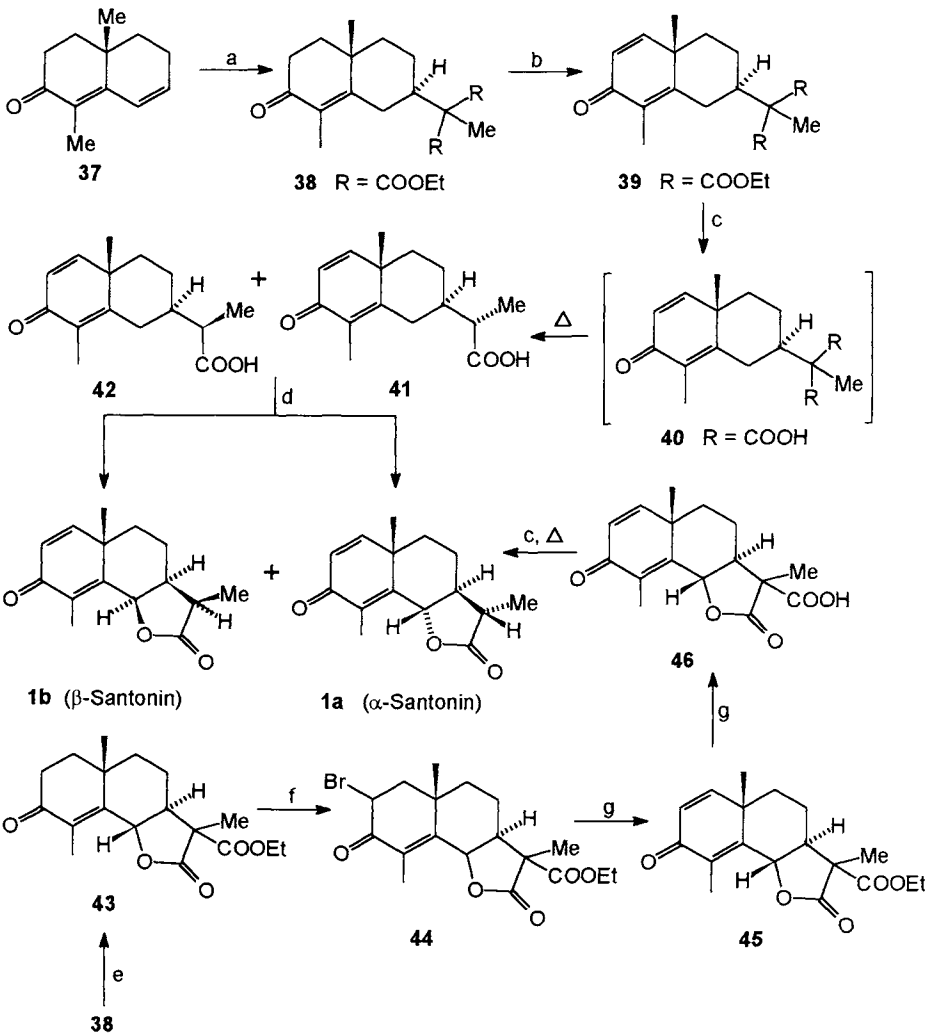
Scheme 1

Reagents: (a) MeCH(COOEt)$_2$, (b) SeO$_2$, (c) Collidine, (d) SeO$_2$-AcOH, (e) (i) Ac$_2$O-H$_2$SO$_4$; (ii) performic acid, (f) Br$_2$, (g) KOH.

84
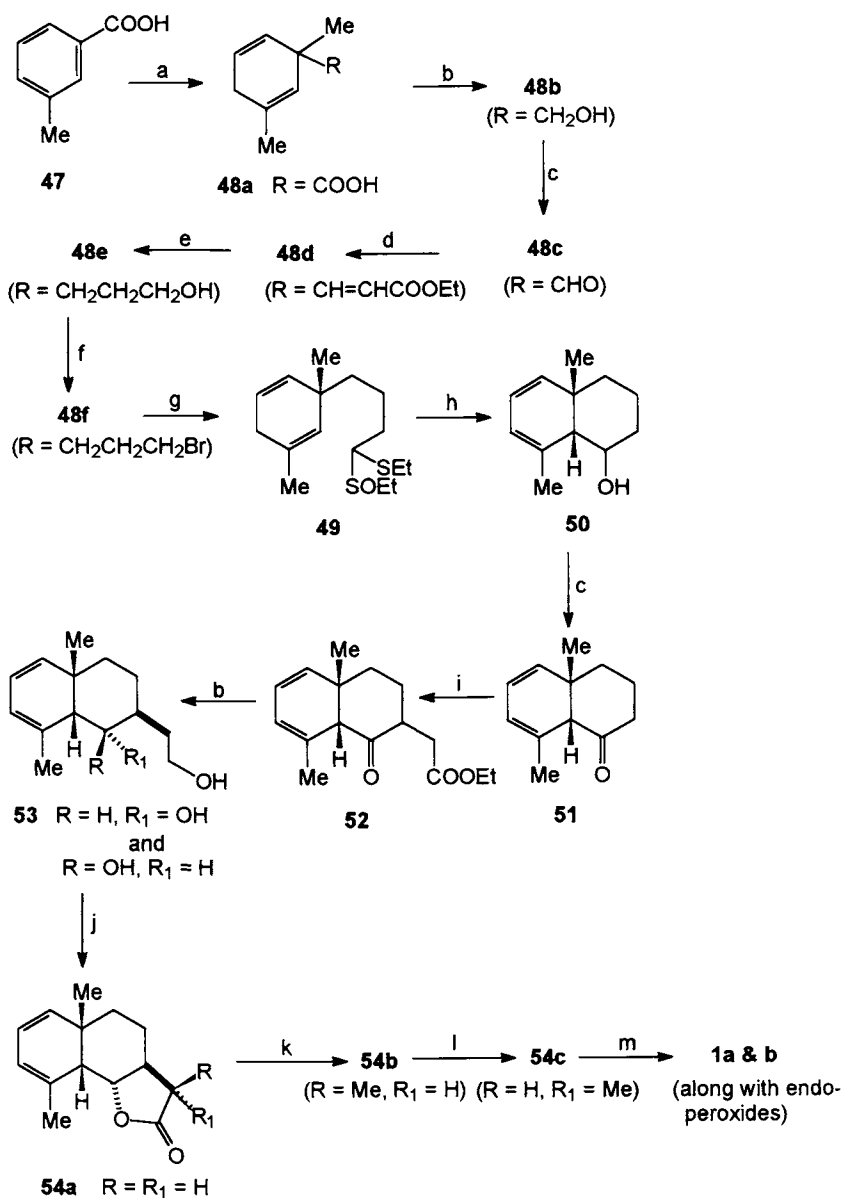
Scheme 2

Reagents: (a) Li, NH$_3$, MeI, (b) LAH, (c) Me$_2$S, NCS, (d) NaH, (EtO)$_2$POCH$_2$COOEt, (e) Li, NH$_3$, (f) Ph$_3$P, NBS, (g) EtSCH$_2$SOEt, BuLi, (h) HClO$_4$, (i) LiNPr$_2$, ICH$_2$COOEt, (j) Ag$_2$CO$_3$, Celite, (k) LiNPr$_2$, MeI, (l) LiNPr$_2$, (m) O$_2$.
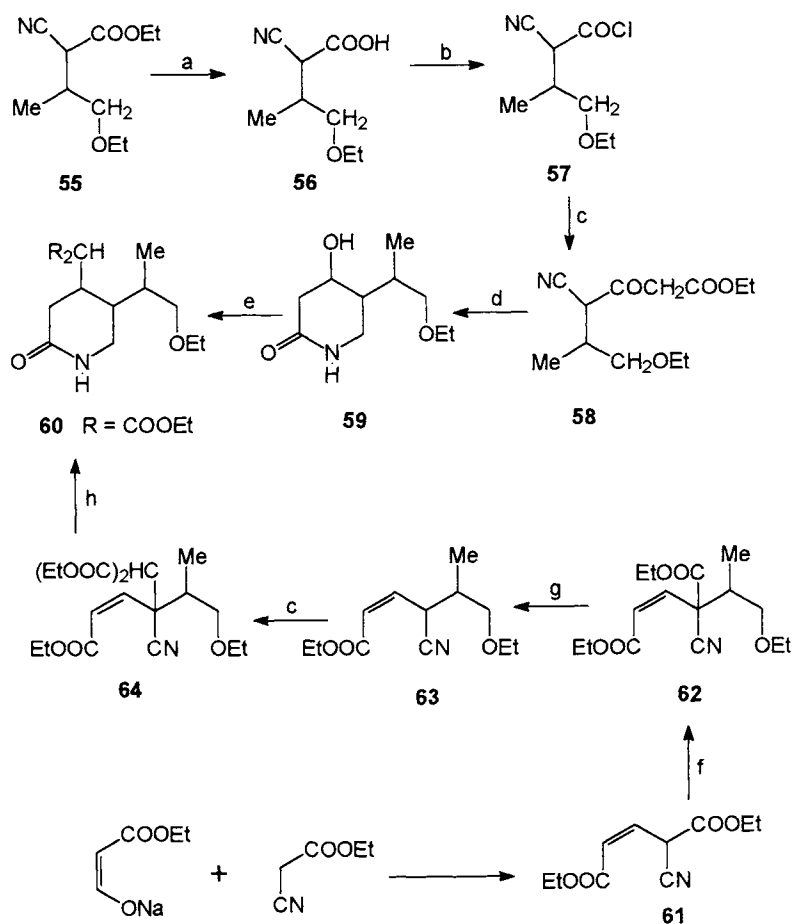
Scheme 3

Reagents: (a) H$^+$, (b) SOCl$_2$, (c) CH$_2$(COOEt)$_2$, heat, (d) Ra-Ni, H$_2$, (e) (i) Ac$_2$O, (ii) CH$_2$(COOEt)$_2$, (f) I-CH(Me)CH$_2$OEt, (g) (i) H$^+$, (ii) heat, (h) Ra-Ni, H$_2$.
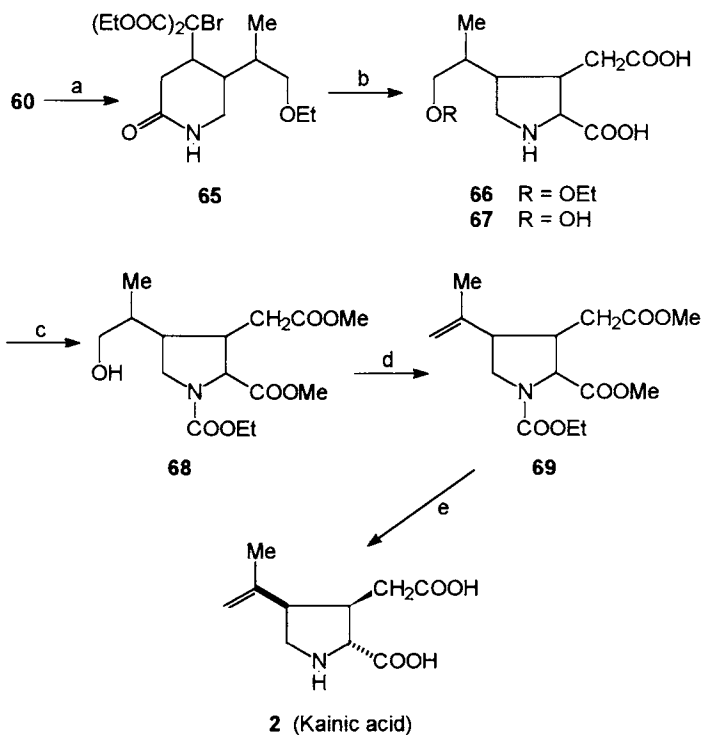
Scheme 4

Reagents: (a) Br_2, (b) HBr, (c) (i) MeOH-HCl, (ii) ClCOOEt, (d) (i) PBr_3, (ii) Pyridine, heat, (e) KOH.
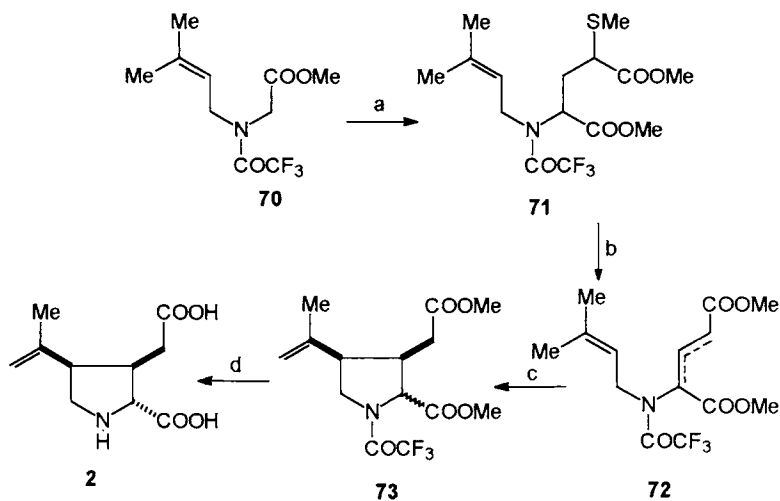
Scheme 5

Reagents: (a) Lithium N-isopropylcyclohexylamide, MeS-C(=CH$_2$)COOMe, (b) *m*-chloro-perbenzoic acid, Δ 130°C, (c) heat, 180°C,36 h, (d) (i) OH⁻, (ii) H⁺.

5.3 Ascaridol (3)

Ascaridol is obtained by treating α-terpinene (74) with oxygen in presence of light and chlorophyll [93] (**Caution!** this product explodes on heating or on treatment with acids) [94].
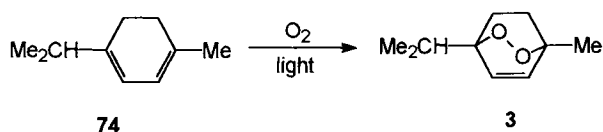
5.4 Thymol (6)

Several methods are available for the synthesis of thymol.

(a) **From m-cresol:** Treatment of m-cresol (75) with isopropanol at high temperatures in presence of H_2SO_4 or H_3PO_4 gives thymol in good yields [95-97]. Reaction of 75 with isopropanol in presence of aluminium at 170°C followed by hydrolysis of the resulting product also affords 6 in 33-39% yield [98]. Thymol may be obtained by heating m-cresol with prop-1-ene in presence of $FeSO_4$ [99] or acetone in presence of Raney nickel and aluminum silicates [100,101] or frankonite [102] (Scheme 6).
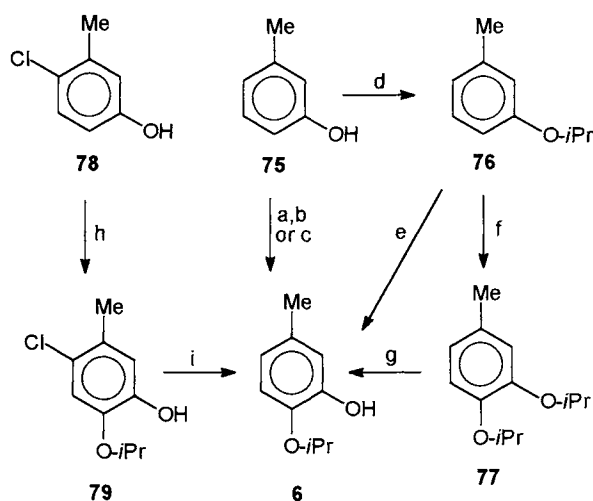
Scheme 6

Reagents: (a) Me_2CHOH in presence of H_2SO_4, H_3PO_4 or Al; (b) Me-CH= CH_2 and $FeSO_4$; (c) Me_2CO, Ra-Ni and aluminum silicates; (d) Me-CH=CH_2; (e) $BF_3.Et_2O$;(f) Me_2CHOH, H_3PO_4; (g) $ZnCl_2$; (h) Me_2CHOH, H_3PO_4 or $ZnCl_2$; (i) Fe, NaOH.

In a slight variation, *m*-cresol is treated with prop-1-ene to get *m*-cresyliso-propyl ether (**76**) in 80% yield which is isomerised in the presence of BF_3. Et_2O to form thymol in 90-91% yields [103]. Further reaction of **76** with isopropanol gives the ether **77** which can be converted into thymol [104] (Scheme 6).

Alkylation of 4-chloro-5-methylphenol (**78**) with isopropanol in the presence of H_3PO_4 or $ZnCl_2$ gives chlorothymol (**79**) which can be converted into thymol [105] (Scheme 6).

(b) From *p*-cymene: *p*-Cymene (**80**) occurs in oil of thyme and eucalyptus. This has been widely used to synthesize thymol as shown in scheme 7 [106-109].
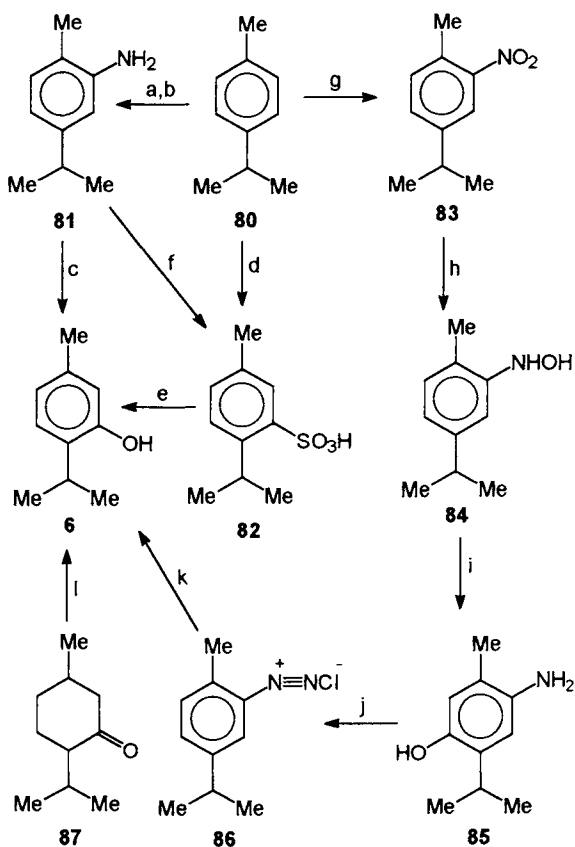
Scheme 7

Reagents: (a) HNO_3; (b) Red., (c) 5 steps, (d) 4 steps, (e) NaOH, (f) Cu, H_2SO_4, (g) HNO_3, (h) Al-Hg, (i) dil. H_2SO_4, (j) $NaNO_2$, HCl, (k) Zn dust, EtOH, (l) $FeCl_3$.

(c) **From piperitone (87):** This is isolated from the essential oils of *Cymbopogon sennaarensis, Andropogon iwarancusa, Mentha, Eucalyptus dives,* and Pippermint. Oxidation of piperitone (87) with $FeCl_3$ gives thymol in 48-90% yields [110,111] (Scheme 7).

(d) **From l-menthone (88):** Bromination of l-menthone (88) gives dibromomenthone (89), which is dehydrohalogenated by heating with quinoline to get thymol (6) [112].
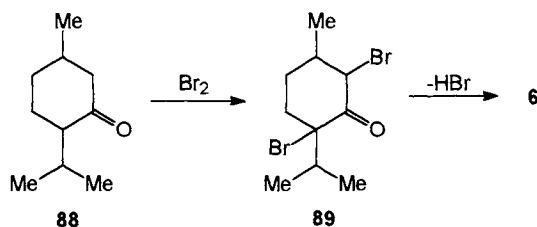
5.5 Avermectins

The preparation of avermectins has been carried out by fermentation, microsomal oxidation of early fermentation compounds and by semi-synthetic methods [57,113]. Approaches to synthesize some avermectins such as abamectin [114] and ivermectin [115-117] have also been published. Recently the total synthesis of avermectin B_{1a} [118], avermectin A_{1a} [119] and the aglycones of avermectin A_{1a} and B_{1a} [120,121] have been reported.

5.5.1 Total synthesis of avermectin B_{1a}

The synthetic strategy for obtaining avermectin B_{1a} involves preparation of the northern segment (C_{11}-C_{28} unit) and the southern segment (C_1-C_{10} unit), followed by coupling of the above two segments, macrolactonisation, stereocontrolled glycosylation and adjustment of the functionality [118].

(a) Synthesis of the C_{11}-C_{28} unit (northern segment)

This is obtained in three phases: first the C_{11}-C_{14} unit is generated from (S)-malic acid (90) (Scheme 8); the C_{15}-C_{21} unit is obtained from D-glucose (Scheme 9) and finally the C_{11}-C_{28} unit is build up starting from L-isoleucine (96) (Scheme 10).

C11-C14 unit (A)
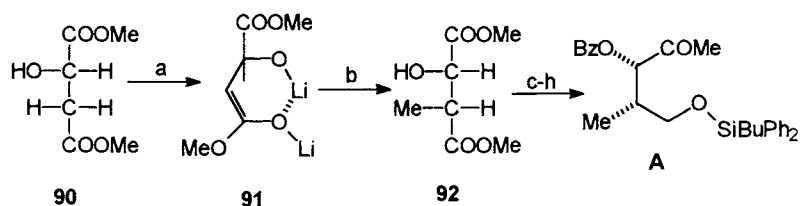
Scheme 8

Reagents: (a) 2LDA, THF,-78°C, (b) MeI, (c) KOH, (d) cyclohexanone, BF3.Et2O, (e) BH3.Me2S and BF3.Et2O, (f) Ag2O, BzBr (g) MeLi, THF,-78°C, (h) t-BuPh2SiCl.

C15-C21 unit B
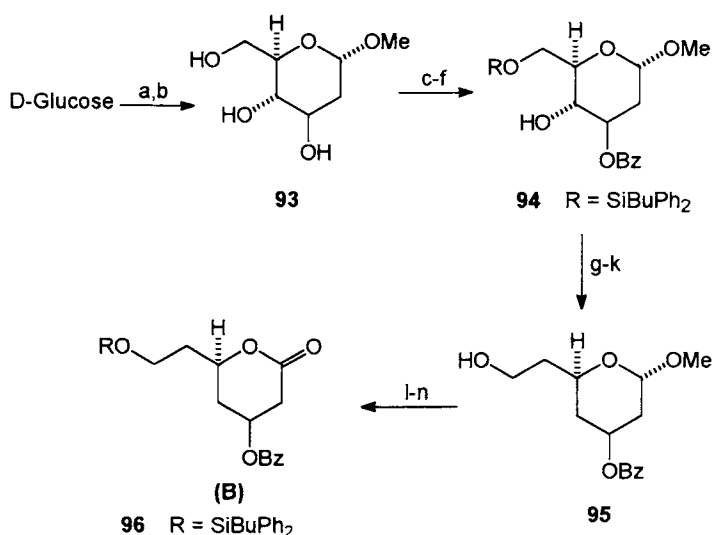
Scheme 9

Reagents: (a) Hg(OAc)2, (b) NaBH4, (c) PhCHO, H+, (d) KH, BzBr, (e) TsOH, H2O, MeOH, (f) t-BuPh2SiCl, Py (g) NaH, CS2, MeI,(h) Bu3SnH, Py, (i) Bu4NF, (j) PCC, Ph3P=CH2, (k) 9-BBN, NaOH, (l) AcOH, (m) t-BuPh2SiCl, Py, (n) PCC.

C22-C28 unit (C)
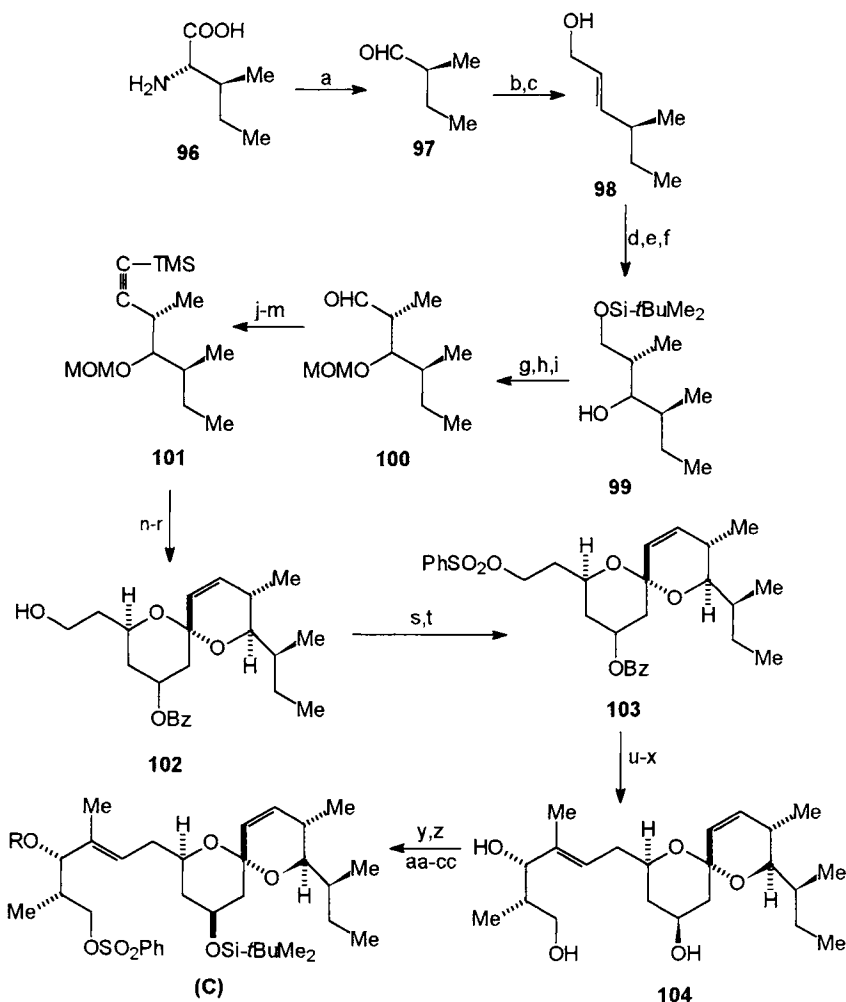
Scheme 10

Reagents: (a) ninhydrin, heat, (b) Ph₃P=CHCO₂Me, (c) DIBAL (d) Ti(O*i*-Pr)₄, D-diethyl tartrate, (e) Me₂Cu(CN)Li₂, Et₂O, (f) *t*-BuPh₂SiCl, (g) MOMCl, DMAP, DIPEA, (h) Bu₄NF, (i) PCC, (j) Ph₃P, CBr₄, (k) BuLi, THF, (l) Me₃SiBr, (m) Me₃SiBr, Et₃N, DMAP, (n) BuLi, Et₂O-78° and add (B),(o) PPTS, (p) Pd-BaSO₄, H₂ (q) BF₃.Et₂O, (r) Bu₄NF, THF, (s) PSSPh, Ph₃P, (t) MCPBA, (u) BuLi, THF, -78°, add (A), (v) Na-Hg, MeOH, KH₂PO₄, (w) Bu₄NF, (x) Li-NH₃, (y) *t*-BuCOCl, Et₃N, (z) *t*-BuMe₂SiCl, DMAP, DMF, (aa) NaOMe, (bb) PhSSPh, Bu₃P, THF, (cc) MCPBA.

92

(b)   Synthesis of C₁-C₁₀ unit (southern segment)

This segment (D) has been prepared starting from the benzyl ether of the allylic alcohol **105** which, in turn, was synthesized from diacetone glucose by Prashad and Fraser-Reid [122] and Hanessian *et al.* [118] as described in scheme 11.
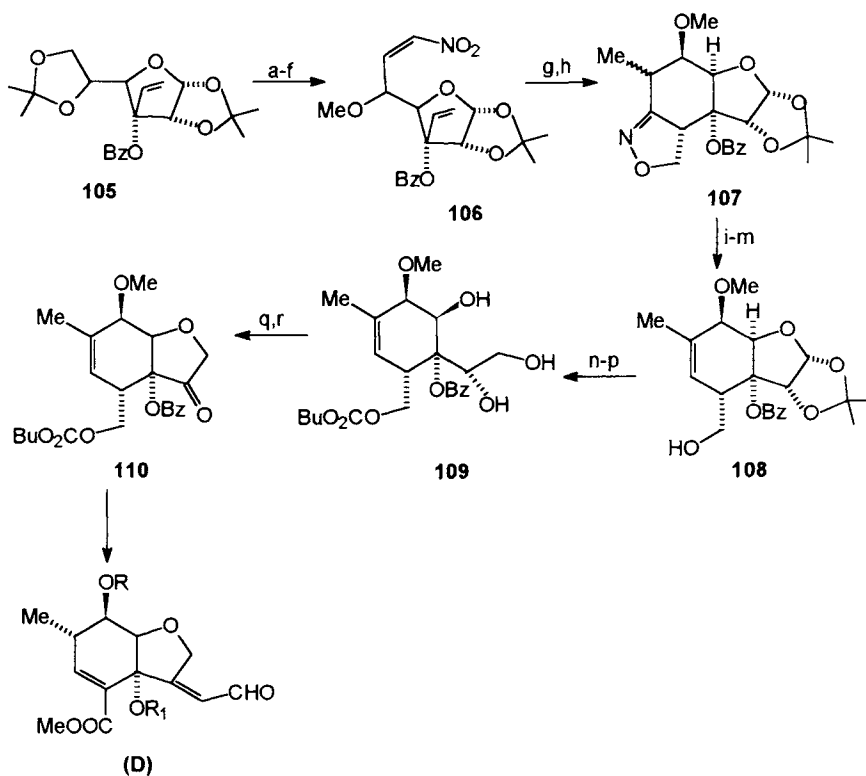
Scheme 11

Reagents: (a) H⁺, (b) *t*-BuMe₂SiCl, Et₃N, (c) NaH,MeI, (d) Bu₄NF, (e) (COCl)₂, Me₂SO,Et₃N, (f) MeNO₂, (g) MeLi, (h) PhNCO,Et₃N, (i) Ra-Ni, H₂, (j) LAH, (k) Ph₃CCl,MsCl, (l) NaOAc, HMPA, (m) Camphor-10-sulphonic acid, (n) *t*-BuOCOCl, (o) 0.5% H₂SO₄, (p) NaBH₄, (q) TsCl, (r) (COCl)₂,Me₂SO, Et₃N.

(c) Coupling of the northern and sourthern segments (C+D) (Scheme 12)

111

112

113 (Avermectin B₁a)
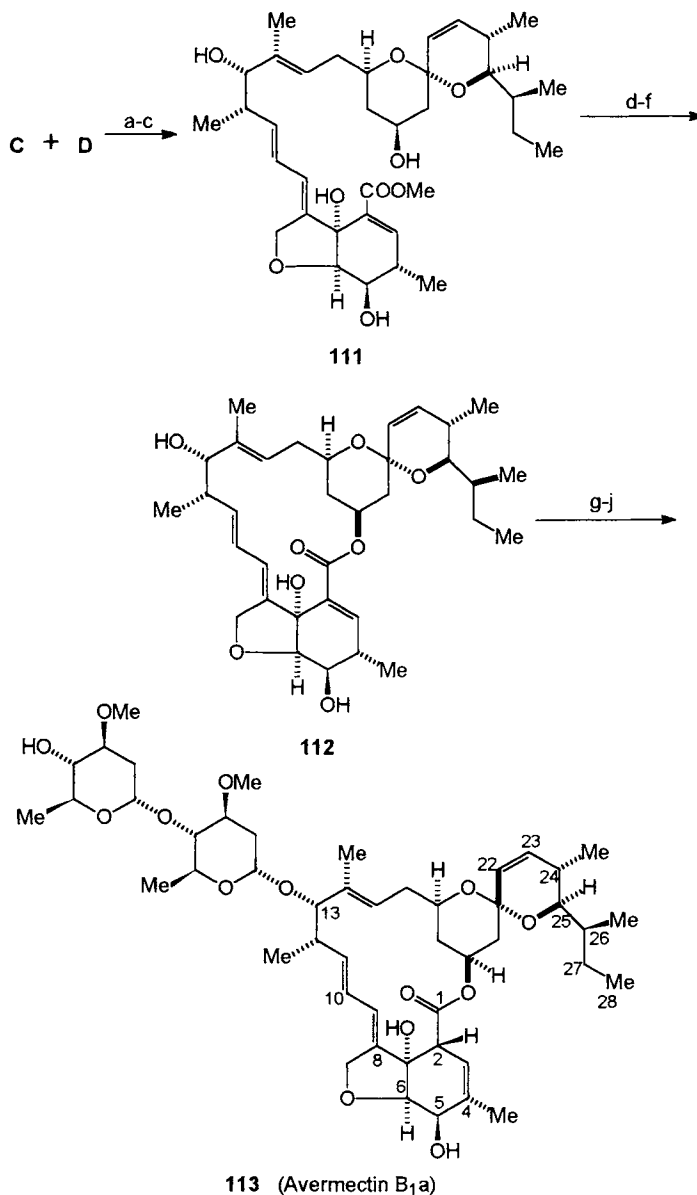
Scheme 12

Reagents: (a) BuLi, THF, -78°, (b) SOCl₂, Py, Na-Hg,MeOH, (c) Bu₄NF, THF, (d) KOH, THF, (e) DCC, DMAP, (f) t-BuMe₂SiCl, (g) 2-pyridylthioglycoside of disaccharide, CF₃SO₃Ag, (h) Me₃SiCl, Et₃N, DMAP, (i) LDA, Me₃SiCl, THF, -78°C, (j) Bu₄NF, THF.

5.5.2 Total synthesis of avermectin A$_{1a}$

The synthetic plan to achieve the total synthesis of avermectin A$_{1a}$ involves the preparation of the aldehyde fragment (A) from D-glucal tripivalate (**114**), and the ketone fragment (B) from D-ribose aldehyde(**125**). Coupling of (A) and (B) gives the macrolactone (C). The resulting lactone is reacted with disaccharide (D) to form avermectin A$_{1a}$ [119].

Step 1: Synthesis of fragment (A) (Scheme 13).

114

115a X = CH$_2$
b X = Me, H

116a R = Pv

116b R = H
c R = OTN

117b
R = CHO

117a R=CN

118a X / Y = O

118b
(X = OH, Y = H)

118c
(X = OTBS, Y = H)

119

120a R = TBS, R$_1$ = H
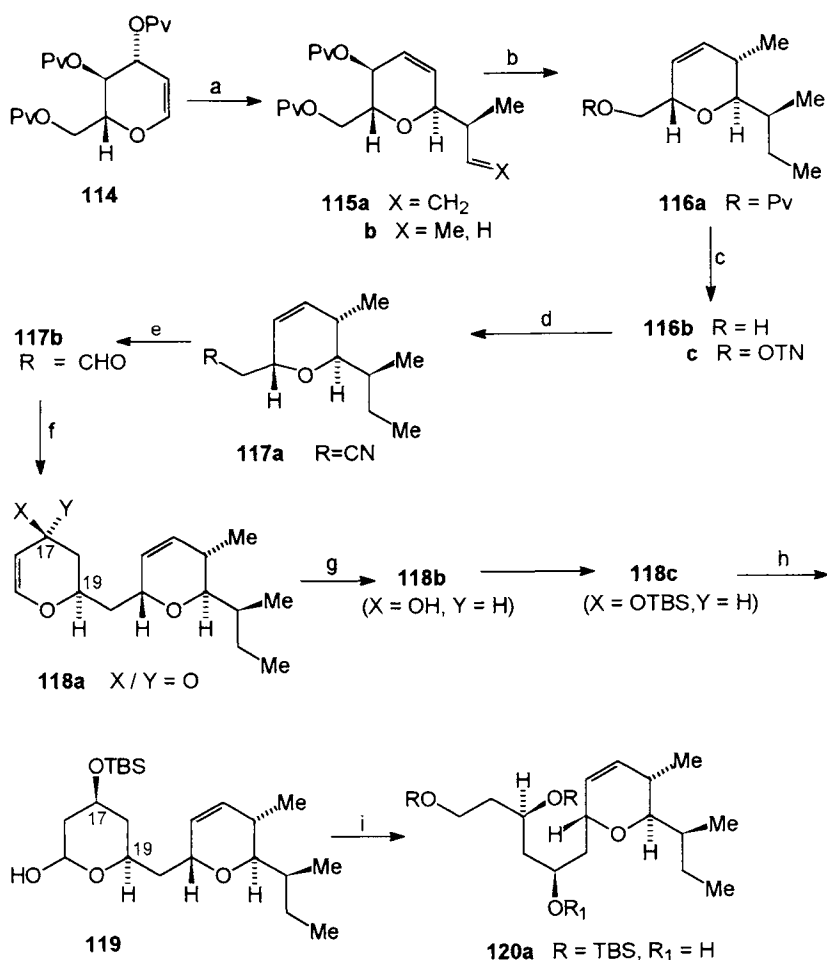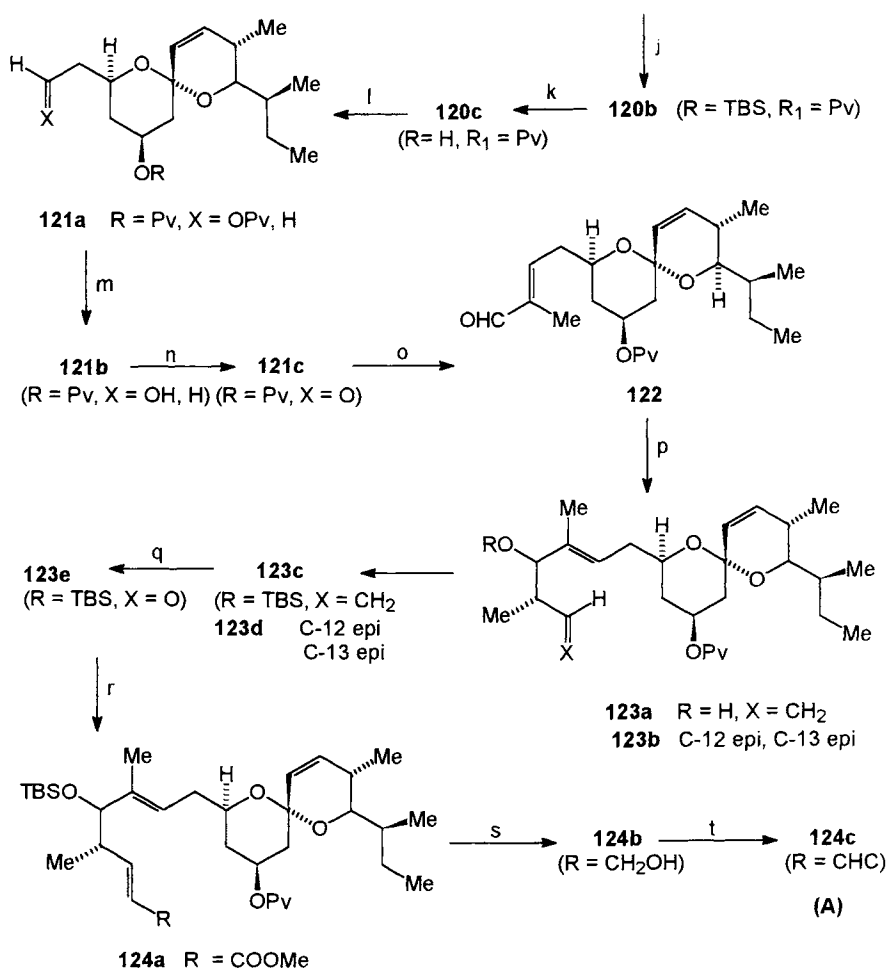
Scheme 13

Reagents: (a) Ph₃SiCH₂CH=CHMe (*cis*), (b) Li dimethylcuprate, (c) (i) LiOH (ii) Tf₂O, (d) NaCN, (e) DIBAH, (f) Me₃SiO-C (=CH₂)-CH=CHOMe, (g) NaBH₄, Cerium (III), (h) (i) NBS (ii) Ph₃SnH, (i) LiBH₄, (j) *t*-BuCOCl, DMAP, (k) HF, (l) HgO-I₂, CCl₄ (m) KEt₃BH, (n) Swern oxidation, (o) Ph₃P=C(Me)CHO, (p) Crotyl borane, (q) (i) OsO₄(ii) LTA, (r) MeOCOP(Me)Ph₃, (s) DIBAH, (t) oxidation.

Step 2: Synthesis of ketone fragment (B) (Scheme 14)
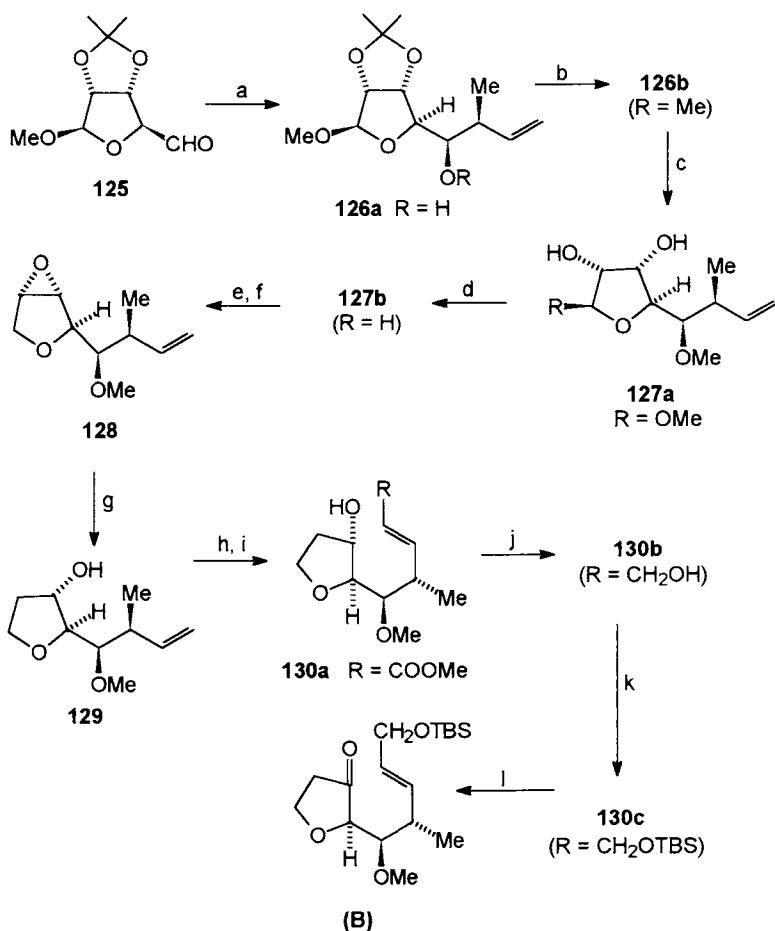
Scheme 14

Reagents: (a) Trimethyl crotylsilane, BF₃.Et₂O, (b) NaH, MeI, (c) HCl, MeOH, (d) Gray's method, (e) α-acetoxypivolyl chloride, (f) amberlite, (g) LiEt₃BH, (h) (i) O₃ (ii) Zn-AcOH, (i) MeOCOP(Me)Ph₃, (j) DIBAH (k) t-BuMe₂SiCl, imidazole, (l) oxidation.

Step 3: Coupling of fragments A & B (Scheme 15)

A + B ⟶   a ⟶ **131b** (R = H)

b

131c (R = CHO) ⟶ c

132b (R = COOH, R₁ = Pv) ⟵ d

e

132c (R = COOMe, R₁ = Pv) ⟶ f ⟶ **132d** (R = COOH, R₁ = H) ⟶ g

133a R = TBS

Scheme contd....

(C)
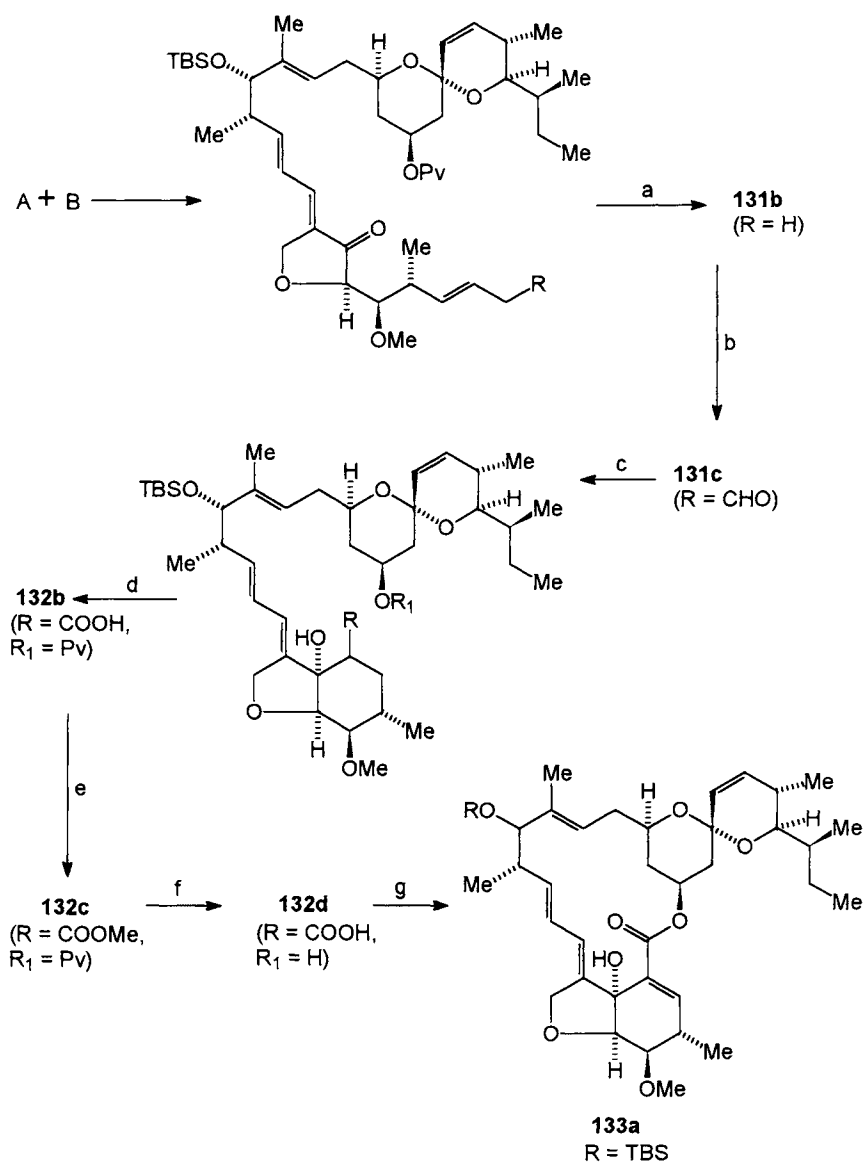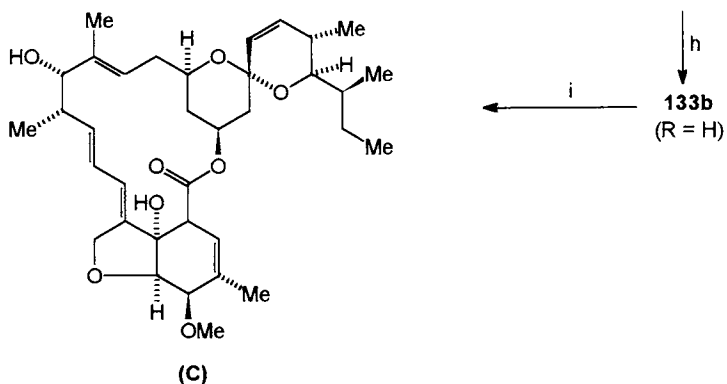
Scheme 15

Reagents: (a) HF, (b) PCC, (c) (i) Me$_3$Al-LiSPh (ii) MCPBA, (d) Sodium chlorite, (e) CH$_2$N$_2$, (f) LiOH, (g) N-Methylpyridinium chloride, Et$_3$N, (h) Bu$_4$NF, (i) lithium diisopropylamide, -78°.

Step 4: Synthesis of disaccharide (D) from dihydropyrone (134) (Scheme 16)

138a
X = I
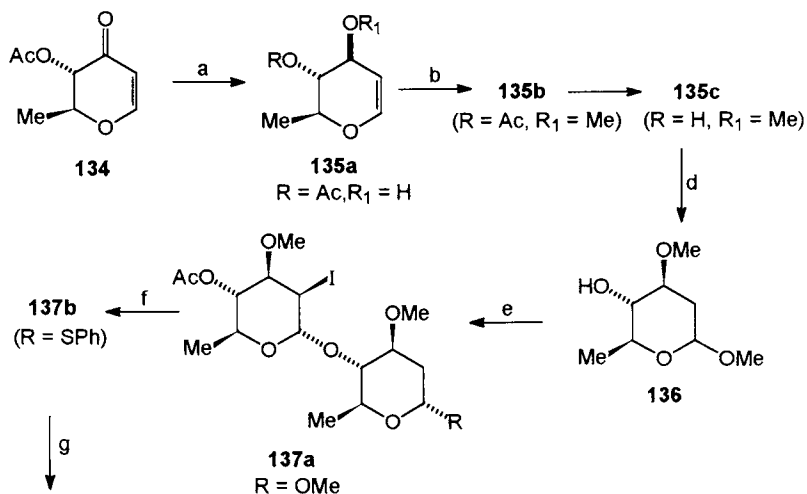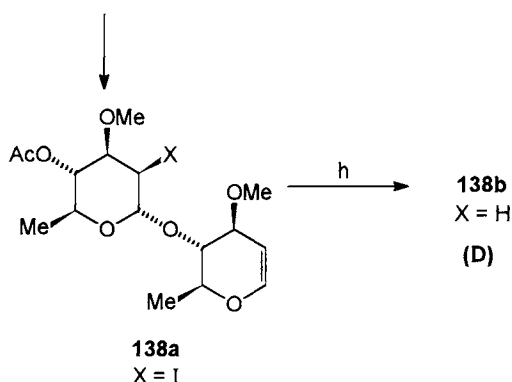
Scheme 16

Reagents: (a) NaBH$_4$, CeCl$_3$, CH$_2$Cl$_2$-EtOH (2:1), (b) Ag$_2$O, MeI (c) MeOH, K$_2$CO$_3$, NaHCO$_3$, CH$_2$Cl$_2$ (d) (i) anhy. MeOH, NBS, CH$_2$Cl$_2$, (ii) n-Bu$_3$SnH, AIBN, CH$_3$CN, (e) CH$_3$CN, **135b**, N-iodosuccinimide, (f) dichloroethane, (trimethylsilyl)- thiophenol, ZnI$_2$, TBAI, (g) (i) CH$_2$Cl$_2$, MCPBA, (ii) C$_6$H$_6$, heat, (h) C$_6$H$_6$, n-Bu$_3$SnH, AIBN.

Step 5: Coupling of lactone (C) with disaccharide (D)

139b (X = H)
(C$_4$"-acetate of
Avermectin A$_1$a)
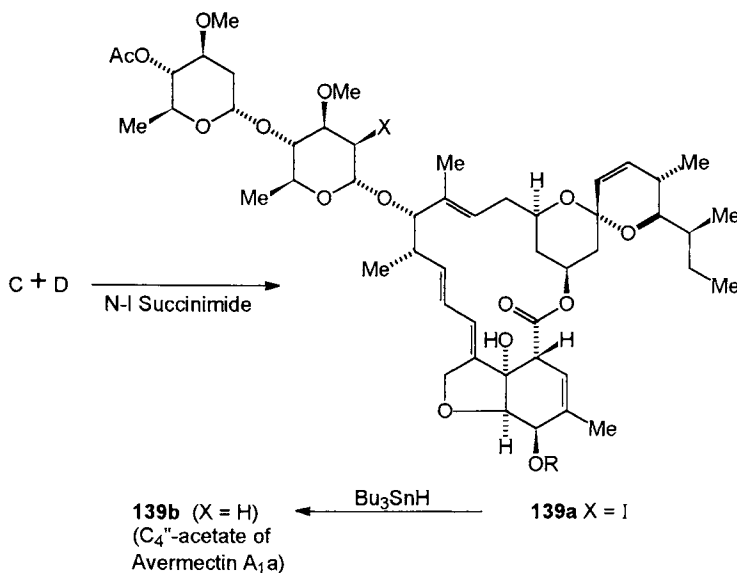
5.6 Milbemycins

Mrozik *et al.* [67] have converted the 22,23-dihydroavermectins B_{1a} and B_{1b} (**140b**, R=OH) into 13-deoxy-22,23-dihydroavermectin B_{1a} and B_{1b} aglycones (**140a**, R=H) corresponding to 26-ethylmilbemycin-α_3 and milbemycin-B41D, respectively. Later the total synthesis of milbemycin-β_3 (**141**) was developed [123,124].

5.6.1 Total synthesis of milbemycin-β3 (141)

140a R = H; R$_{13}$ = H
  b R = H; R$_{13}$ = disaccharide

141 R = H
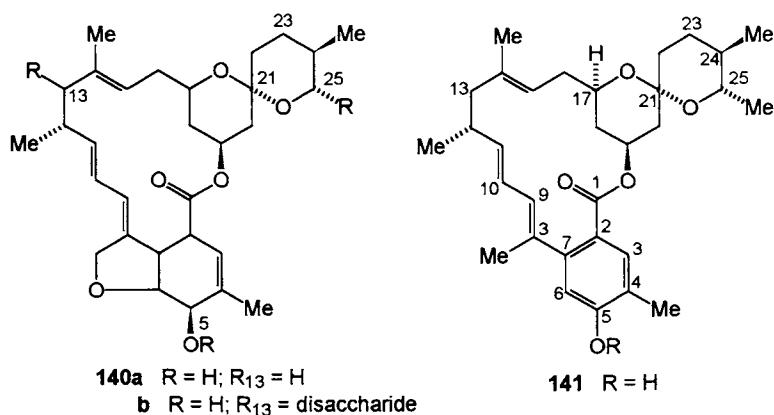
Smith III and coworkers [123] have achieved the total synthesis of **141** through union of northern and southern hemispheres (A and B) *via* Horner-Wittig coupling followed by macrocyclic lactonisation to form the desired product.

(a) Synthesis of northern hemisphere (A) (Scheme 17)

142a R = H

143

144

145

146a R = α-COOEt
b R = β-COOEt

147a R = α-Me
b R = β-Me

A
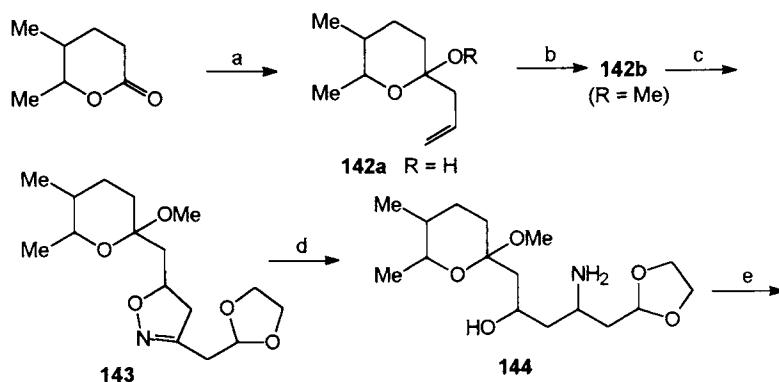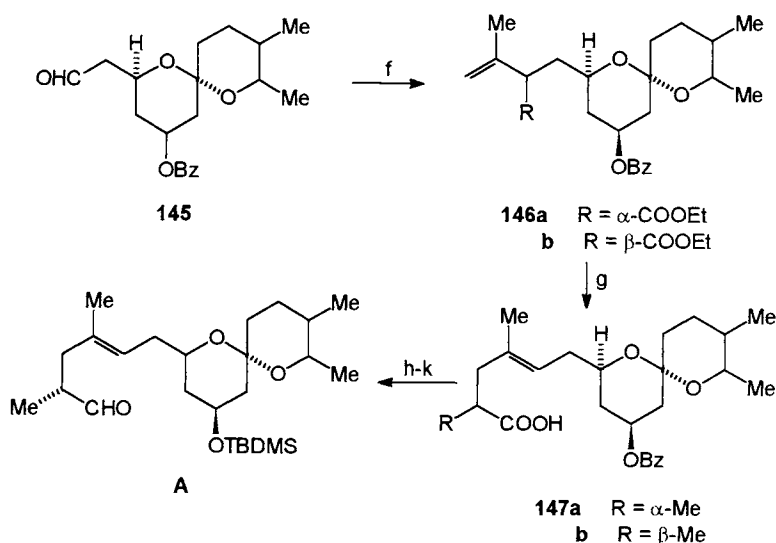
Scheme 17

Reagents: (a) allyl Grignard, -78°C, THF, (b) CH(OMe)₃, CeCl₃.7H₂O, (c) nitrile oxide derived from ethylene ketal of 3-nitropropanal, (d) LAH, (e) KH, Bzl, MeI, TsOH, (f) isopropenyl Grignard,-78°C, (g) Ireland-Claisen rearrangement, (h) Li,NH₃, (i) excess TBDMSCl, DMAP, (j) LAH, (k) Collin's oxidation.

(b) Synthesis of southern hemisphere (B) (Scheme 18)

148

149a (R = H)

149b (R = COMe)

150

151 (E + Z)

B (E + Z)
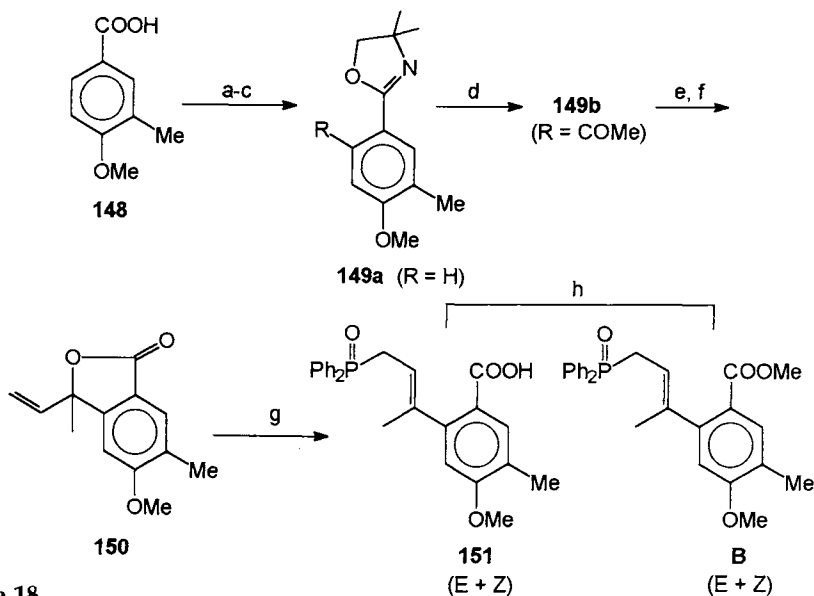
Scheme 18

Reagents: (a) SOCl₂, (b) 2-amino-2-methylpropanol, (c) SOCl₂, (d) s-BuLi, Ac₂O (e) Vinyl-Grignard, (f) H₂SO₄ (3M), (g) LiPPh₂, (h) CH₂N₂.

(c) Coupling of (A) and (B) (Scheme 19)
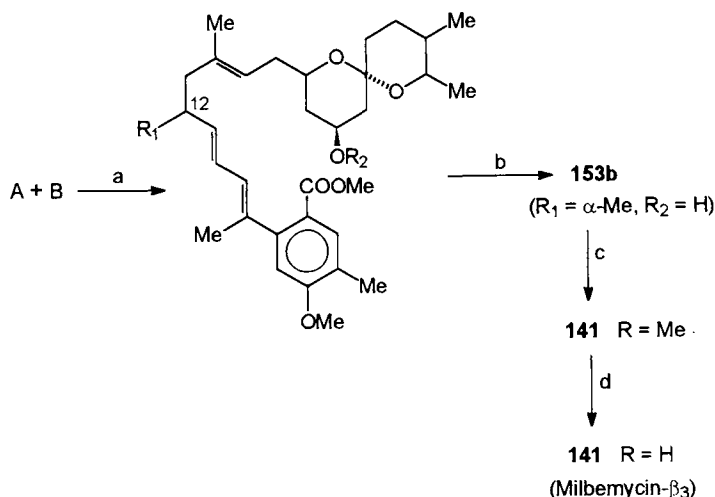
Scheme 19
Reagents: (a) hexamethylenedisilamide, -78°C, (b) n-Bu$_4$NF, THF, (c) KH, (d) NaSEt, DMF.

The total synthesis of milbemycin-β₃ developed by Williams *et al.* [124] involves construction of three units; the spiroketal moiety (A), carbon chain with a remote chiral centre at C-12 (B) and the substituted benzoic acid (C). Unit (A) is prepared starting from citronellol (**154**), while unit (B) was prepared starting from (-)-(3S)-citronellal (**162**) (Scheme 20). A and B were joined after transmetalation of the tetrahydropyranyl ether **166** to give **167** (Scheme 21), which is allowed to react with the aldehyde A to give **168**. Further steps are shown in scheme 22.

(a) Synthesis of unit (A) (Scheme 20)
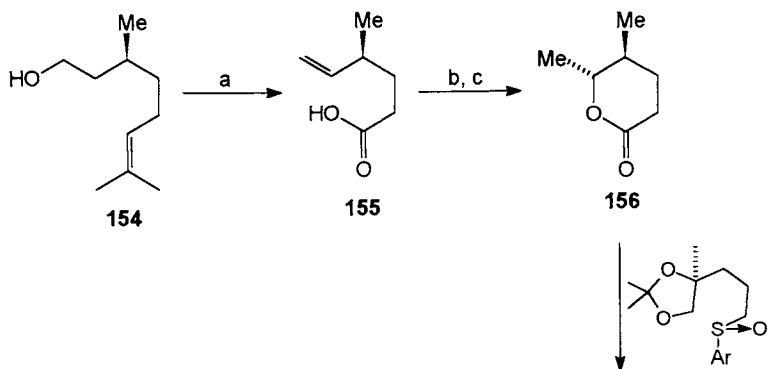
Scheme contd....
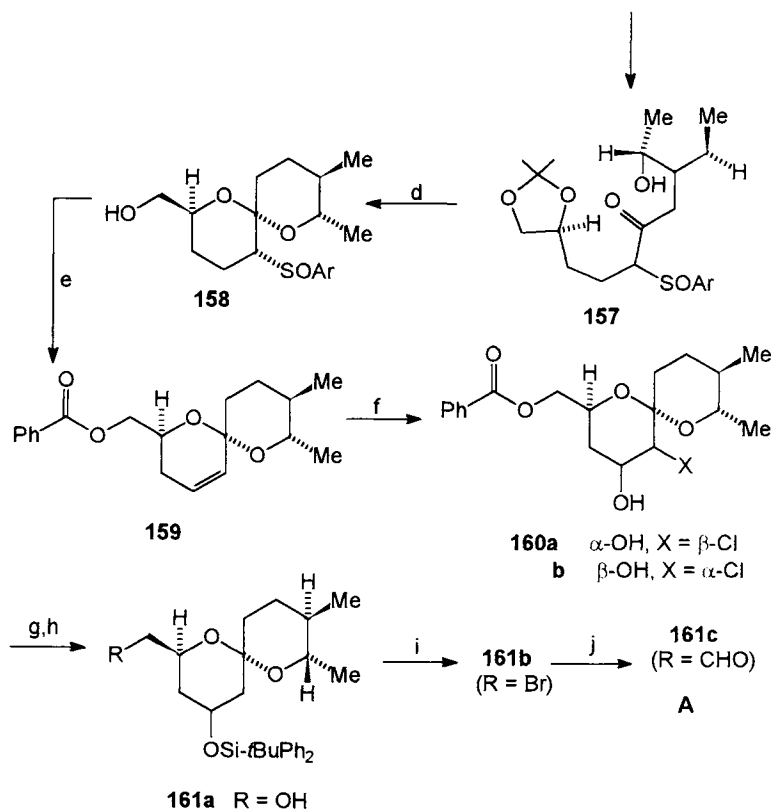
Scheme 20

Reagents: (a) dehydration, oxidation of trisubstituted olefin, Zone's oxidation, (b) I$_2$,MeCN, (c) n-Bu$_3$SnH, (d) cat.MsOH, (e) PhCOCl, toluene, (f) t-BuOCl (g) n-BuSnH, (h) Ph$_2$-t-BuSiCl, DMAP, (i) LiOH, THF, (j) Swern oxidation (Me$_2$SO, oxalyl chloride).

(b) Synthesis of Unit B (Scheme 21)

Scheme contd....

167 ← **166** ← **165a** X = H
b X = Br ⎤ B
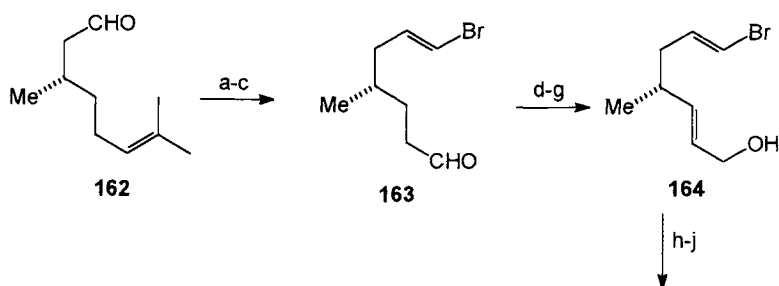
Scheme 21

Reagents: (a) LiCHBr₂,-78°C, (b) O₃, (c) Zn-AcOH,(d) piperidine, (e) PhSeCl, -110°C, (f) LiAl (Ot-Bu)₃H, (g) MCPBA, (h) MeLi, 0°C, (i) AlMe₃, Cp₂ZrCl₂, (j) I₂,THF,-30°C, (k) transmetalation of THP ether, (l) BuLi, THF, -100°C.

(C) Coupling of A with ether of B (Scheme 22)

A + 167 ⟶

168

169 →c, d→ **170** →e→ **171** →f→ **172**

↓ g,h

Scheme contd....

Milbemycin-β_3
(140, R = H)

173

Reagent I
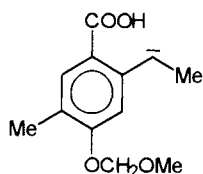
Reagent II

Scheme 22

Reagents: (a) CS_2,THF (b) NaH,MeI (c) n-Bu$_3$SnH, 80°C, (d) PPTS,MeOH (e) Swern oxidation (f) reagent I, (g) reagent II, (h) NaI, Cat.HCl.

5.7 Arecoline (25) and cucurbitin (26)

Arecoline is prepared from bis(2-cyanoethyl)amine (174) which is a by-product of the alanine synthesis [125] (Scheme 23).
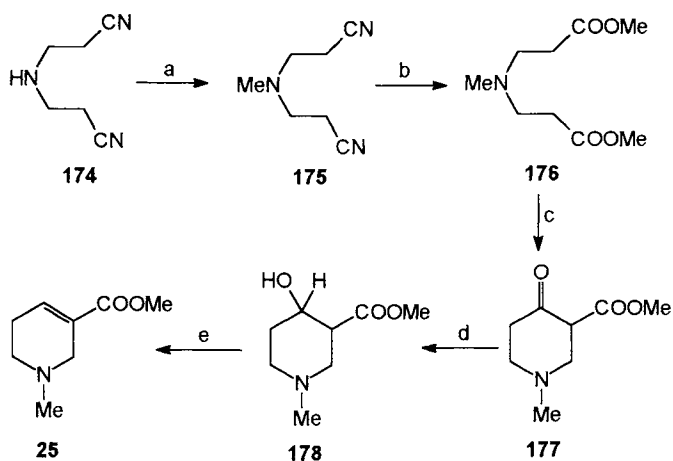
Scheme 23

Reagents: (a) Methylation, (b) MeOH, H^+, (c) Dieckmann condensation, (d) PtO$_2$, H$_2$, (e) SOCl$_2$ or POCl$_3$.

An improved method to synthesize **25** involves the reduction of the methylated nicotinic acid ester **179** with sodium borohydride [126a].
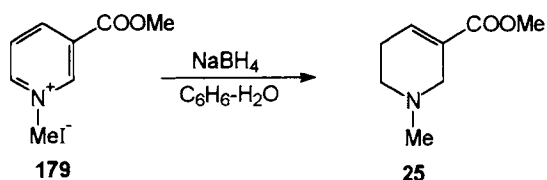
The synthesis of (±)-cucurbitin (**26**) may be achieved starting from 1-carbethoxy-3-pyrrolidone (**180**). Reaction of **180** with KCN and ammonium chloride in aqueous methanol gives an adduct which is refluxed with 48% HBr to afford cucurbitin hydrobromide. Resolution of (±)-cucurbitin with (+)-camphoric acid yields (-)-**26**-(+)-camphorate (m.p. 195-96°C) from which free base is liberated to give (-)-cucurbitin (**26**) [126b].
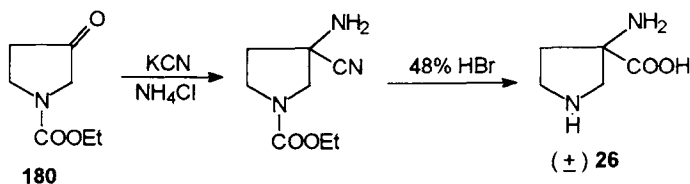
6.   BIOLOGICAL ACTIVITY

The natural products have shown better activity against nematodes as compared to trematodes and cestodes and some of them have been used both in veterinary and clinical medicine. Modern therapy, however, does not include many natural products because of their low therapeutic index and the availability of more effective and safer synthetic drugs.

6.1   Natural Products in veterinary medicine

6.1.1   Avermectins

This is one of the most effective natural products developed so far. Although a number of compounds of the avermectin family exhibit a high order of activity against various nematodes of domestic animals, ivermectin has been picked-up for marketing as a veterinary anthelmintic. Ivermectin has been found to be highly effective against the adult worms and larvae of various roundworms parasitizing the gastrointestinal tract of cattle, sheep, goats, horses, pigs, cats and dogs. The drug

also shows high activity both against microfilariae and adult filariids of which the latter reside in the body cavities, lymphatic and subcutaneous tissues, while the former are found in the peripheral blood circulation and skin of the domestic animals. The activity of ivermectin against lungworms in different economically important animals has been reviewed [18, 19, 127]. The drug is either administered subcutaneously (as injection) or orally (as paste or drench).

(a) Cattle : The recommended dose of ivermectin in cattle is 0.2 mg/kg, s.c. which kills 90-100% of the gastrointestinal nematodes (adults and larval forms) of *Haemonchus placei, Ostertagia ostertagi, O. lyrata, Trichostrongylus axei, T. colubriformis, Cooperia oncophora, C. punctata, C. peptinata, Nematodirus helvetionis, Oesophagostomum radiatum* and *Bunostomum phlebotomum*. Many of the above nematodes are equally susceptible to oral administration of ivermectin [18,19]. Usually the drug is well tolerated by cattle and can be safely used in pregnant animals too. However, some side effects may be seen while treating cattle having a large number of larvae of *Hypoderma* spp. present in the oesophageal wall or spinal canal [128].

Recently a slow release bolus of ivermectin has been found to be more than 99% effective against trichostrongyles and other gastrointestinal nematodes in grazing animals [129,130]. Consequently it has been suggested that treatment of cattle with ivermectin is economical as it keeps them free of helminths leading to better output of animal products [131].

Ivermectin has been found to be highly effective in eliminating the tissue-dwelling adult filariids of *Parafilaria bovicola* with a single injection of 0.2 mg/kg [18,132]. The larval and adult forms of the lungworms, *Dictyocaulus viviparus* are highly susceptible to ivermectin as 0.2 mg/kg, s.c. of the drug kills 90-100% of these worms in cattle [18,133].

(b) Sheep : A single oral dose of 0.2 mg/kg of ivermectin has been found to be almost 90-100% effective against the mature and immature forms of the gastrointestinal nematodes, *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus axei, T. colubriformis, Cooperia curticei, Gaigeria pachyscelis, Oesophagostomum columbianum, Nematodirus battus, N. spathiger, Strongyloides papillosus, Chabertia ovina* and the lungworms, *Dictyocaulus filaria, D. arnfieldi*, and *Mullerius* spp. The above dose level of ivermectin is also very effective against the adult worms of *H. placei, C. oncophora* and *T. ovis* in sheep [18,133].

(c) Pigs : The standard dose of ivermectin is 0.3 mg/kg given as a solution through subcutaneous injection. The drug exhibits high activity against the adult and immature *Ascaris suum, Hyostrongylus rubidus, Oesophagostomum* spp., and the adult forms of *Strongyloides ransomi* and *Metastrongylus* spp. [18, 134]. Ivermectin has also been found to be active against *Trichuris suis* and *T. spiralis* [128].

(d) Equines : An oral dose of 0.2 mg/kg of ivermectin has been found to be highly active against a variety of small and large strongyles found in the intestine of the equines. The drug kills the developing and adult forms of *Strongylus vulgaris, S. edentatus, Cyathostomum* spp., *Cylicocyclus* spp., *Cylicostephanus* spp., *Cylicodontophorus* spp., *Gyalocephalus* spp. and *Oxyuris equi.* However, the drug is only effective on the adult stages of *S. equina, Parascaris, equorum, Trichostrongylus axei* and *Strongyloides westeri* [18]. In donkeys, a s.c. dose of 200 μg/kg was found to be 100% effective against naturally occurring strongyles [135].

Ivermectin shows high activity against the microfilariae of *Onchocerca* spp. in horses and adult worms of *Setaria equina* in ponies. The adult and immature forms of *D. arnfieldi* may also be eradicated from equines at an oral dose of 0.2 mg/kg [18, 133].

(e) Dogs : At a subcutaneous dose of 0.2 mg/kg, ivermectin exhibits a high order of activity against the larval and adult forms of *Toxocara canis, Ancylostoma caninum, A. braziliense, Uncinaria stenocephala* and *Strongyloides stercoralis,* and against the immature stages of *Toxascaris leonina* and *Trichuris vulpis* [18].

For the treatment of extra-intestinal nematode infections in dogs, oral administrations of the drug have been recommended. Thus, a single oral dose of 0.05 mg/kg of ivermectin is active against the third and fourth stage larvae and microfilariae of *Dirofilaria immitis.* A higher dose of 0.25 mg/kg, p.o. is required to kill the microfilariae of *Dipetalonema reconditum* in dogs [18].

6.1.2 Milbemycins

A few members of the milbemycin family, namely milbemycin-D (15), nemadectin (16) and milbemycin oxime, called "interceptor" have shown great promise in the treatment of various intestinal and tissue-dwelling nematodes parasitizing dogs and sheep [85-88].

6.1.3 Arecoline

This alkaloid is an old veterinary anthelmintic which has been used to treat tapeworm infections in cats and dogs [136,137]. Arecoline is given orally as its hydrobromide salt at a dose of 1-1.5 mg/kg. Since it is a bitter tasting salt, it is given as a 1.5% solution in 15% sucrose. At this dose level, about 80% of the dogs will purge out the paralysed worms of *Taenia* spp. and *Echinococcus granulosus* within 2 hours. If no purgation occurs within 2 hours, a half dose of the drug may be given. Arecoline hydrobromide is not recommended to cats, pregnant bitches and pups below 6 months of age [137,138].

6.2   Natural products in clinical medicine

Although a large number of natural products have been known to be effective against different gastrointestinal helminths of man [52,139], their use in clinical medicine could not be extended beyond early sixties because of the advent of more effective and safer drugs of synthetic origin. With the exception of some antibiotics like paromomycin and avermectins,most of the natural products discussed in this chapter are only of historical value. A brief description of the clinical profile of a few such anthelmintics is given in table 1 [52,140,141].

6.2.1 Ivermectin

Extensive clinical trials carried out in different parts of the world have proved this drug to be one of the most effective anthelmintics for treating different forms of filariasis in humans and animals [19,20]. The antifilarial profile of ivermectin is as follows:

(a) Onchocerciasis : The drug has been evaluated against *O. volvulus* in man in different parts of Africa and other regions of the tropics with very promising results [19,20]. Ivermectin has been found to cause high reduction in skin microfilarial counts at a single oral dose of 100-200 µg/kg [142-146]. The effective dose for adults and children is 200 and 150 µg/kg, respectively [20]. The drug is primarily microfilaricidal with no apparent effect on adult worms of *O. volvulus*. However, it interferes with the production and release of microfilariae from the uterus of female adult worms [147-150]. A partial action on adult worms of *O. volvulus* was observed at 12 monthly doses of 150 µg/kg in man [10]. At therapeutic dose ivermectin is well tolerated; however, about 33-60% of the treated patients may develop fever, headache, pruritus, edema, myalgias and arthralgias, which are of mild to moderate nature and subside within a few days. These side effects were more pronounced at

200 µg/kg as compared to 100 or 150 µg/kg dose levels [20, 145,151].

Ivermectin possesses high activity against ocular onchocerciasis. At a dose of 150-200 µg/kg the drug caused gradual clearance of microfilariae from the eyes with marked improvement in the ocular status. The side effects of the drug were minor causing transient inflammation in the anterior eyes [152-154].

Ivermectin has been evaluated in 50,000 patients in Central America and Africa to ensure its value in mass treatment of onchocerciasis [155,156]. In all the trials about 9% of the treated cases developed adverse reactions of which only 0.24% were severe and 2.4% were of moderate nature. The frequency of the side effects was proportional to the microfilarial load under the skin. Further trials of ivermectin in 66,894 subjects have established that ivermectin is a safe drug for the mass treatment of onchocerciasis and is also well accepted by patients due to less side effects and mild Mazzotti reaction occurring only in a few individuals [20,157]. Chijioke and Okonkwo [158] have treated 7556 Nigerian patients with *O. volvulus* using ivermectin. Of these 992 patients (13.1%) complained of Mazzotti type side effects (pruritus, edema, headache and worsening of rash) which were more pronounced in patients with higher worm load. Use of ivermectin has also been shown to cause mild prolongation of prothrombin time in 6.7% of patients as compared to 1.4% with placebo [159]. Although ivermectin is an useful drug for large scale treatment of onchocerciasis in man due to its ability to produce less intense Mazzotti reaction than DEC, the former causes migration of microfilarial of *O. volvulus* (dose : 122-200 µg/kg) from subepidermal layers to deeper layers of the dermis, subcutaneous fat, connective tissues and lymph nodes. No microfilaria migrated upwards the dermis [160].

Since ivermectin primarily kills the microfilariae of *O. volvulus* in man, it has been compared with DEC in patients with onchocerciasis [147,161-165]. The drug (single dose of 200 µg/ml) was compared with DEC (a total of 1.3 g as one week's course) in all the trials; it was found that ivermectin and DEC were almost equipotent in clearing microfilariae of *O. volvulus* from the skin. However, the former produced milder and transient Mazzotti reaction than DEC. Thus ivermectin may be regarded as a superior drug to DEC in terms of activity, tolerance and safety for the chemotherapy of human onchocerciasis [20].

(b) Lymphatic filariasis : Ivermectin has been found to clear blood microfilariaemia when given at single oral doses ranging from 25-200 µg/kg to patients infected with *W. bancrofti* [163,166]. In another clinical trial carried out in French Polynesia, it was shown that oral doses of 50, 100, 150 or 200 µg/kg of ivermectin caused very high

reduction in microfilarial counts in patients of lymphatic filariasis caused by *W. bancrofti* var. *pacifica*. Side effects were similar to DEC but were severe in subjects with a heavy infection [167]. It has also been suggested that ivermectin, given at a single oral dose of 100 µg/kg body weight once every year, may be the best candidate available for mass therapy [161]. A single dose of 100 or 200 µg/kg of ivermectin has also been found to be highly active against microfilariae of *Brugia malayi* in patients. Mild side effects like fever, headache and myalgia were seen [168].

Ottesen and coworkers [169] have compared ivermectin (two dose levels of 20 and 125 µg/kg) with DEC (6 mg/kg for 13 days) in 40 patients with bancroftian filariasis and found that both the low and high doses of ivermectin cleared microfilariaemia from blood from all the patients while DEC cleared 11 out of 14 patients. After 6 months microfilarial counts rose to 19% in ivermectin treated cases and 6% in patients treated with DEC. In another comparative trial against *W. bancrofti*, DEC (6 mg/kg) and ivermectin (100 µg/kg) were administered orally to 58 patients. It was observed that ivermectin is superior to DEC in single doses in terms of immediate microfilarial clearance but not in terms of sustained decrease of microfilariae for 6 months [170]. It has, therefore, been concluded that a single dose (100 µg/kg) of ivermectin is an effective alternative to the standard course (total dose 75 mg/kg over a period of 6-9 months) of DEC for the control of recurrent microfilariaemia due to *W. bancrofti* in man [171a]. However, Richards, Jr. and his colleagues [171b] are of the view that it is premature to conclude that ivermectin is superior to DEC for the treatment of *W. bancrofti* in man. They found that DEC causes more damage to adult worms of *W. bancrofti* and also caused greater reduction in microfilarial counts than ivermectin. Thus it is suggested that the effect of ivermectin on adult worms of *W. bancrofti* should be studied. Data regarding cost, ease of drug delivery, availability, safety and efficacy of DEC and ivermectin should also be generated. These informations would help to judge the value of these two drugs in individual and mass therapy of lymphatic filariasis.

(c) **Other filarial infections** : Ivermectin has been evaluated against *L. loa* and concomitant *O. volvulus* and *Mansonella perstans* infections in man [172,173]. At a single oral dose of 200 µg/kg, ivermectin caused 80% reduction in blood microfilarial counts in patients with *L. loa*. The same dose exhibited poor activity against *M. perstans*. However, Nutman *et al.* [174] have reported successful treatment of a patient with *M. ozzardi* infection by ivermectin. More recently a combination of ivermectin (200 µg/kg for 1 or 2 days) with DEC (6 mg/kg for 12 days) has been found to suppress the microfilarial levels in blood for 2 years. Thus this combination may be use-

ful in reducing the transmission of lymphatic filariasis [175]. A dose of 0.2 mg/kg of ivermectin given every 3 months can be used to prevent the transmission of *L. loa* in endemic areas [176].

6.2.2 Paromomycin sulphate

This antibiotic, produced by *Streptomyces rimosus* var. *paromomycinus*, has been used earlier to treat tapeworm infections in man. A dose of 30-50 mg/kg of the drug given daily for 1-5 days to adults (in capsules) and children (in flavoured syrup) has been found to produce 89-100% cures against *Taenia solium*, *T. saginata* and *Hymenolepis nana* [141]. Further work carried out on the efficacy of paromomycin sulphate has established this drug to provide above 90% cures against *T. solium*, *T. saginata* and *H. nana* infections at a dose of 40 mg/kg given daily for 5-7 days or a single dose of 75 mg/kg (max. 4 g) [177,178]. The drug has also been found to cure patients infected with *Diphyllobothrium latum* when given as 250 mg capsule every 15 minutes until 4 g/patient had been consumed [141].

Paromomycin sulphate is poorly absorbed through the gastrointestinal tract. The common side effects of the drug are abdominal pain and diarrhea. Nausea, vomiting and dizziness may also be seen occasionally [179].

7.   MODE OF ACTION

7.1   Avermectins

The mode of action of avermectin B_{1a} and ivermectin has been reviewed in [18,134,180-183]. Avermectin B_{1a} and ivermectin are potent inhibitors of the neuromuscular transmission causing paralysis of the susceptible organisms [184-188]. These drugs appear to stimulate the release of GABA from nerve endings (GABA agonist) and enhance the binding of GABA to its post synaptic receptor. In other words, ivermectin mimics the action of the inhibitory neurotransmitter, GABA; this effect can be blocked by the GABA antagonist picrotoxin [184].

The paralysing action of ivermectin on the nematodes is due to its ability to activate a membrane chloride conductance in neurons of the nerve cord either directly or by enhancing the presynaptic release of GABA [180, 189] which eventually results in opening of chloride channels.

It has now been well established that avermectins open a chloride ion channel in invertebrates which is distinct from GABA-gated chloride channels [190,191]. Turner and Schaeffer [192] have shown that ivermectin specifically increases mem-

brane chloride ion permeability mediated through GABA-independent chloride channels at low concentrations $(10^{-10}M)$ and GABA-dependent channels at higher concentrations $(10^{-7}M)$.

Recently it has been shown that ivermectin prevents moulting of L_3 larvae of *W. bancrofti* to L_4 *in vitro* at the concentrations ranging from 0.1-1000 mg/ml. The inhibition of moulting of L_3 to L_4 of *W. bancrofti* is 20 times higher for ivermectin than DEC [193]. Ivermectin also appears to inhibit the intrinsic exsheathing process of the microfilariae of *B. malayi* in mosquito hosts thereby blocking the transmission of filariasis [194].

7.2   Paromomycin

The mode of action of this antibiotic is not clear. However, Garin *et al.* [195] have suggested that the drug may exert its anthelmintic action by affecting the ultrastructure of the tegumental membrane of *T. saginata*. The change in surface topography may make the tapeworm susceptible to the host's digestive action [196].

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CHAPTER 4

ORGANOMETALLICS

1.   INTRODUCTION

Organometallic compounds of group V elements (arsenic, antimony, bismuth and phosphorus) were amongst the earliest agents used in the chemotherapy of parasitic infections. The Greek physician Hippocrates (460- 377 B.C.) recommended the use of an ointment containing arsenic trisulphide for the treatment of ulcerative abscesses. Galenus (138-201 A.D.) prescribed the use of similar arsenic preparations for skin and respiratory infections. Avicenna (980-1037 A.D.) recommended the use of arsenic compounds for the treatment of infections. Fowler's solution (potassium arsenate, K_3AsO_4) and orpiment (auripigmentum, As_2S_3), used since long as pesticides, may be regarded as the earliest arsenicals given in combination with some dyestuffs to treat trypanosomiasis in animals [1]. Following these early developments a number of organo-arsenicals, -antimonials and -phosphates were found to possess a wide-spectrum of antiparasitic activities. Despite the high toxicity, some of these organo-metallics find use to the present day for treatment of some protozoal and helminth diseases of humans and domestic animals [2,3].

2.   ORGANOARSENICALS

Taking the lead that some biologically effective dyes such as Afridol violet and Chlorazol Fast Pink BK contain arylsulphonate (Ar-SO$_3$H) groups and that the activity of such compounds might be increased by replacing sulphur by arsenic, Thomas introduced the first arylarsonate, atoxyl, in the treatment of human trypanosomiasis in 1905 [4]. The structure of atoxyl proposed by Thomas was C_6H_5-NHAsO(OH)$_2$, which was later corrected to 4-aminobenzenearsonate (1a) by Ehrlich and Bertheim [5]. Although atoxyl was soon abandoned due to its high toxicity, it provided a useful 'lead' to prepare more effective arylarsonates. The important arylarsonates that emerged are tryparsamide (1b), carbarsone (1c), glycobiarsol (1d), orsanine (2), nitarsone (3) and roxarsone (4) [6-8]. Of these arsenicals, atoxyl (1a) was used in the treatment of *Eimeria* infection in poultry, while tryparsamide (1b) was used to treat trypanosomiasis in humans. Carbarsone (1c) possesses activity against *Histomonas, Balantidia, Eimeria* and *Entamoeba.* Similarly, the bismuth ester of

benzenearsonic acid, glycobiarsol (1d) is effective against *Entamoeba* and *Trichomonas*. Orsanine (2) and acetarsone (4b) are no longer used in clinical practice, but nitrasone (3) and roxarsone (4a) may be used to treat *Histomonas* and *Eimeria* infections in animals [8a]. The other noteworthy organoarsenicals which exhibited marked antiprotozoal activities, are butarsen (4c) and balarsen (4d). Of these, butarsen was effective only against early stages of sleeping sickness [8b], while balarsen exhibited potent amoebicidal activity in humans [8c].

1 a R = H; R₁ = H
  b R = CH₂CONH₂; R₁ = H
  c R = CONH₂; R₁ = H
  d R = COCH₂OH; R₁, Bi = O

3

4a R = NO₂
b R = NHAc

4c

4d

2
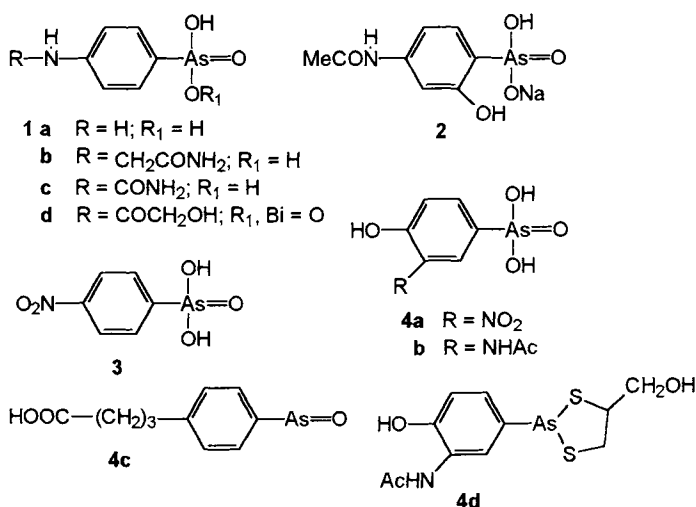
Dimers of 4-aminophenylarsonate (1a), such as difetarsone (5) were also active, but are now obsolete due to high toxicity [9]. However, substitution of one of the hydrogens of the amino function in 1a by a triazine ring was found to reduce toxicity of the resulting triazinoarsonate called melarsen. Consequently a series of melarsen derivatives were synthesized by Friedheim [10-12] of which melarsen sodium (6), Mel-B (7a), Mel-W (7b) and F-151 (8) have been used to treat African trypanosomiasis and filariasis in humans [3]. The fact that pentavalent arsenicals are believed to exert their antiparasitic response after getting changed to the trivalent form, has led to the synthesis of some trivalent arsenic compounds. The most noteworthy compound of this class is arsenamide (thiacetarsamide, 9) which shows high activity against *Wuchereria bancrofti* in humans and *Dirofilaria immitis* in dogs [3,13].

5

6

7a R = H, R$_1$ = CH$_2$OH
b R = R$_1$ = COOK

8 (F-151)

9
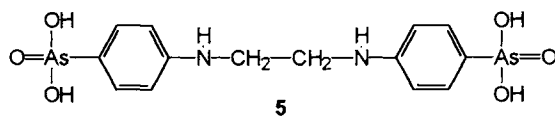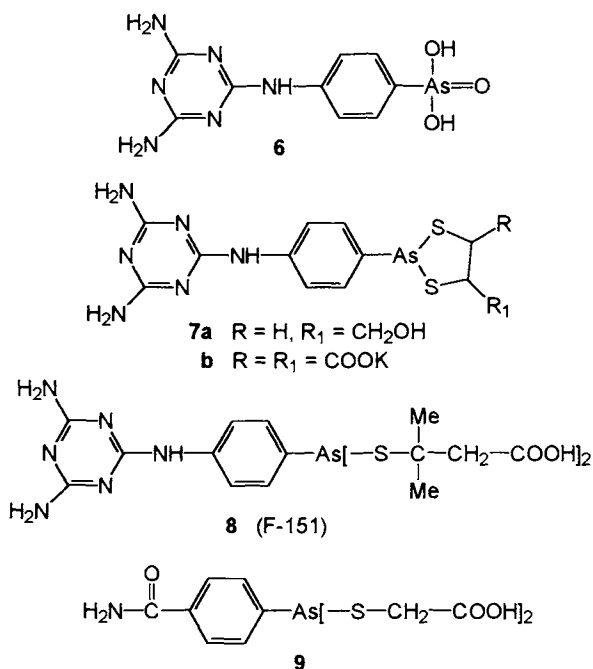
3. ORGANOANTIMONIALS

Like arsenic salts, the history of antimony compounds as therapeutic agents dates back to medieval age when Paracelus (1493-1541) recommended metallic antimony and its salts as a cure for many diseases. Although the above panacea made Paracelsus the father of iatrochemistry, the therapy was later rejected by patients due to toxic effects [1,9]. The interest in antimony compounds revived during 1918-1920 when the medicinal value of tartar emetic (**10**), a mordant prepared in 1847 by boiling antimony trioxide and cream of tartar in water [14], was established by Christopherson [15] and Rogers [16].

Following the discovery of antileishmanial and antifilarial activities of tartar emetic [3], a number of organoantimonials were prepared as possible antiparasitic agents.

10

11a R = H
b R = COMe
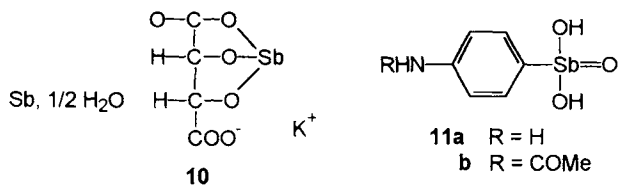
The modern use of antimonials in chemotherapy is now largely restricted to leishmaniasis, although earlier they were used in some helmintic infections also like schistosomiasis and filariasis. These antimony compounds may be broadly divided in two classes: (a) those containing a carbon-antimony bond such as 4-aminoben-zenestibonic acid (Stibamin, **11a**), and 4-acetylaminobenzenestibonic acid (Stibacetin, **11b**); (b) compounds containing antimony-oxygen or antimony-sulphur bonds thereby forming an antimoniate ester. The therapeutic agents of the latter class included both trivalent and pentavalent antimonials such as triostam (**12a**), pentostam (**12b**), meglumine antimonate (glucantime, **13**), urea stibamine (**14**), stibocaptate (**15**), sodium antimony dimethylcystein (**15b**), stibophen (**16**) and MSb B (**17**). Of these, compounds **10-14** find use in the treatment of protozoal infections while **15-17** along-with **10** have been found effective against filariasis and schistosomiasis [3].

12a   12b

13   14

15

15a   16

17 (M-SbB)
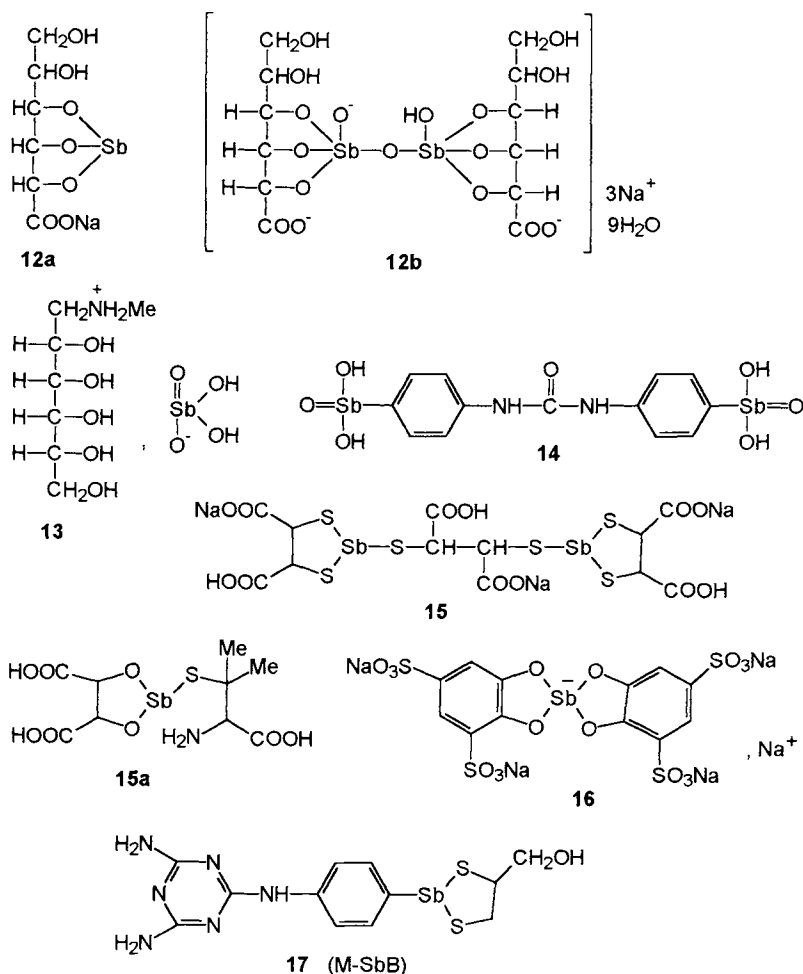
4.   ORGANOPHOSPHATES

The organophosphorus compounds are primarily used as agricultural pesti-
cides. Compounds of this class are potent inhibitors of acetylcholinesterase (AChE),
and were originally synthesized during World War II for chemical warfare as nerve
poisons. However, after the 1950s the organic phosphates emerged as an important
class of pest control agents. Today about three dozen safe and effective pesticides are
available which were selected after screening more than 50,000 compounds having
the general formula $RR_1P(=O)X$; where R and R_1 may be alkoxy, aryloxy, amino,
mercapto etc. and X is halo, cyano, carboxyl, oxy, phosphono, phenoxy, thiophe-
noxy, and thiocyanato [9,17,18]. Extension of the biological screening of these or-
ganophosphates to other parasites revealed some of them to possess useful anti-
filarial activity. These include metrifonate (18), fenthion (19), haloxon (20a),
coumafos. (20b), crufomate (21), naphthalofos (22), dichlorovos (23), fomophos (24)
and chlorpyrifos (25), which exhibit marked microfilaricidal activity with little or no
action on adult worms [19-21]. Despite the fact that the therapeutic indices of these
compounds were reasonably high, only metrifonate (18) finds some use in the treat-
ment of human filariasis.

18

19

20a   R = Cl, X = O
  b   R = H, X = S

21

22

23

24

25
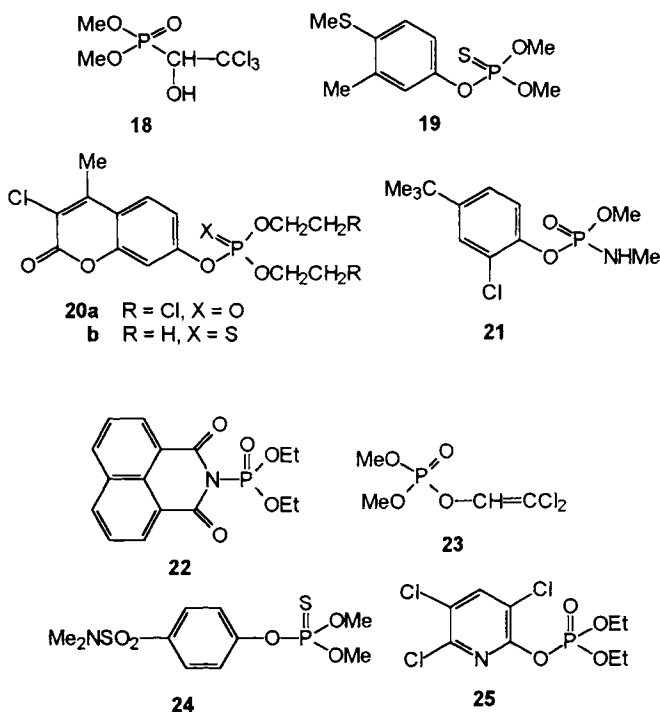
Some organophosphates have also been found effective against various tapeworms and flukes in animals; chlorpyrifos (25) was used to eliminate *Echinococcus granulosus* and *Taenia hydatigena* from dogs, while cyclophosphamide (26) and a few other organophosphorus compounds (27-29a,b) exhibit marked activity against different tapeworms in experimental animals [22]. Bromphenphos (30) kills the liver flukes, *Fasciola hepatica* in sheep and cattle at a dose of 12-16 mg/kg [23].
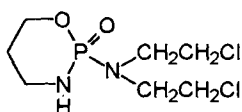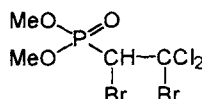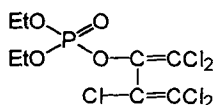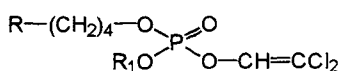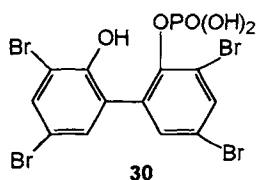
5.   COMPOUNDS FROM OTHER METALS

A few salts of bismuth such as bismuth subcarbonate, subgallate, subnitrate and subsalicylate or milk of bismuth, find use in the treatment of nonspecific diarrhea and for skin protectants [9]. However, their use in the chemotherapy of parasitic diseases is limited. The only noteworthy example is glycobiarsol (1d), an anhydride of a bismuth oxide and phenylarsonic acid, which has been found to be active against *Entamoeba* and *Trichomonas* spp. [8].

The arsenates of lead, zinc, copper and calcium have been used to eradicate tapeworm infections from cattle, sheep and poultry. Similarly copper sulphate, copper carbonate and potassium permanganate have been used to treat *Moniezia expansa* infection in sheep [22].

Some tin salts and organotin compounds have been evaluated against different parasitic infections in humans and animals. Stannoxyl, a combination of tin and

tin oxide, has been used to treat *Taenia solium, T. saginata* and *Diphyllobothrium latum* infections in adults and children [24]. Another mixture prepared from pure tin, tin oxide and tin chloride (cestodin) can be used to remove *Hymenolepis nana* from children. Cestodin was found to be well tolerated producing more than 72% cure rates [25].

A number of organotin compounds of the general formula R_2SnX_2 have been evaluated against animal cestodiasis with encouraging results. The most effective compounds of this class are di-*n*-butyltin maleate (**31**), di-*n*-butyltin dilaurate (**32**), diphenyltin oxide (**33a**), di-*n*-butyltin oxide (**33b**), diphenyltin dichloride (**34a**) and di-*n*-butyltin dichloride (**34b**), which show varying degree of activities against mature and immature forms of different tapeworms parasitizing laboratory and domestic animals [22]. Of these, di-*n*-butyltin dilaurate (butynorate, **32**) is currently used to treat *Eimeria* infection in poultry [8].
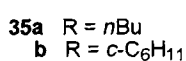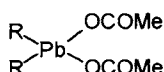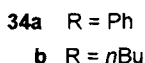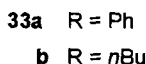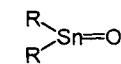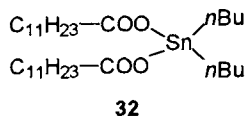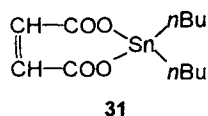
Some organolead compounds are known to possess better antitapeworm activity than organotin compounds. These are di-*n*-butyllead diacetate (**35a**) and dicyclohexyllead diacetate (**35b**) showing high activity against *Choanotaenia, Raillietina, Hymenolepis, Moniezia* and *Avitellina* spp. in chicken and sheep and low toxicity [22].

6. SYNTHESIS OF ORGANOMETALLICS

6.1 Atoxyl (Arsanilic acid, 1a)

Arsanilic acid, a drug used to treat *Eimeria* infection, also serves as a versatile starting material for preparing various organoarsenical drugs. It has been prepared by heating aniline with arsenic acid [26].

$$C_6H_5\text{---}NH_2 \ + \ AsO_4H_3 \longrightarrow 4\text{-}H_2N\text{---}C_6H_4\text{---}AsO_3H_2 \quad (\textbf{1a})$$
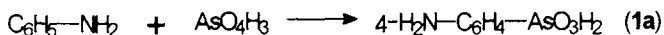
6.2 Tryparsamide (1b)

This is prepared by treating the sodium salt of arsanilic acid (1a) with chloroacetamide in presence of sodium hydroxide [27,28].

$$4\text{-}H_2N\text{—}C_6H_4\text{—}AsO_3HNa \ + \ ClCH_2CONH_2 \longrightarrow$$

$$4\text{-}H_2NCOCH_2NH\text{—}C_6H_4\text{—}AsO_3H_2 \xrightarrow{\ NaOH\ } 1b$$
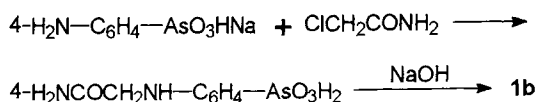
6.3 Carbarsone (1c)

Carbarsone has been prepared by reacting the sodium salt of arsanilic acid (1a) with potassium cyanate or cyanogen bromide followed by hydrolysis [29].

$$4\text{-}H_2N\text{—}C_6H_4\text{—}AsO_3HNa \ + \ KCNO \ (or \ BrCN) \longrightarrow 4\text{-}H_2NCONH\text{—}C_6H_4\text{—}AsO_3H_2$$
$$1c$$
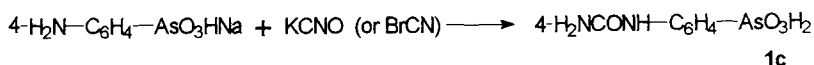
6.4 Nitrasone (3)

The preparation of this compound has been reported by Jacobs *et al.* [30] and by Ruddy and Starkey [31]. An aqueous solution of 4-nitrobenzenediazonium borotetrafluoride (36) is treated with sodium arsenite in the presence of sodium hydroxide and cuprous chloride to give disodium 4-nitrobenzenearsonic acid, which is acidified with hydrochloric acid to afford nitrasone in 71-79% yields [31].

$$4\text{-}O_2N\text{—}C_6H_4\text{—}\overset{+}{N_2}B\overset{-}{F_4} + NaAsO_2 + Cu_2Cl_2 \xrightarrow{\ NaOH\ } 4\text{-}O_2N\text{—}C_6H_4\text{—}AsO_3Na_2$$

$$\downarrow HCl$$

$$4\text{-}O_2N\text{—}C_6H_4\text{—}AsO_3H_2$$
$$(3)$$
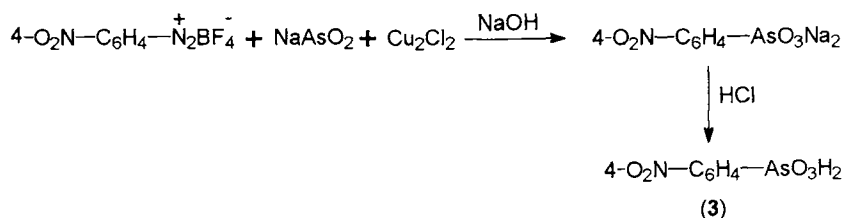
6.5 Roxarsone (4a)

It is prepared by treating sodium *p*-hydroxyphenylarsonate (37) with a mixture of nitric acid and sulphuric acid at $0^{\circ}C$ [32].

$$HO\text{—}C_6H_4\text{—}AsO_3H_2 + HNO_3 + H_2SO_4 \longrightarrow HO\text{—}C_6H_3(O_2N)\text{—}AsO_3H_2$$
$$37 \qquad\qquad\qquad\qquad\qquad\qquad\qquad 4a$$
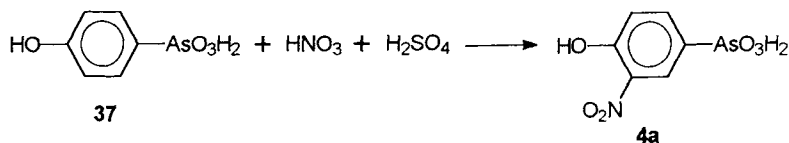
6.7 Melarsen compounds

The starting material for preparing various melarsen derivatives is cyanuryl chloride (38), which is treated with the sodium salt of arsanilic acid (1a) to form 2-(4-substituted phenyl)cyanuryl chloride (39a). Reaction of the latter with aqueous ammonia and sodium hydroxide affords 4-melaminylphenylarsonic acid disodium salt (melarsen sodium, 6) [33,34]. Further reaction of 6 with ammonium thioglycolate gives the thioglycolate complex, which is acidified with acetic acid to pH 6.5-7.0 and then treated with 3-hydroxypropane-1,2-dithiol in ethanol to yield Mel-B (7a) [35,36] (Scheme 1).
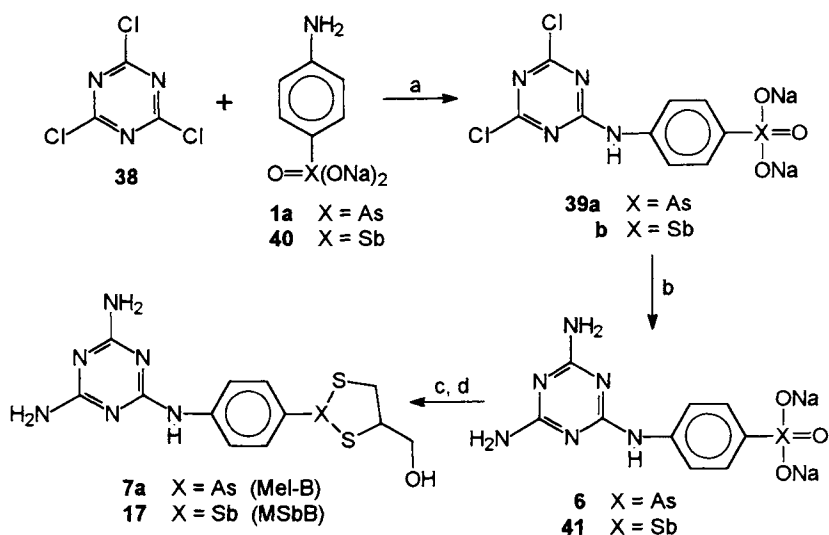
Scheme 1
Reagents: (a) NaOH, (b) aq. NH₃, NaOH, (c) HS-CH₂COONH₄, (d) HS-CH₂CH(SH)-CH₂OH.

The above scheme is used to prepare MSbB (17) also. Reaction of 38 with sodium stibanilate (40) gives 39b, which is allowed to react with ammonia to form 41. Compound 41 is converted to 17 as described for Mel-B [37,38].

6.8 Arsenamide (9)

Arsenamide has been prepared starting from 4-dichloroarsenobenzoyl chloride (42). Reaction of 42 with aqueous ammonia gives 4-dichloroarsenobenzamide (43) which is heated with thioglycolic acid to yield arsenamide in 98% yield [39,40].

$$\underset{\textbf{42}}{\underset{\text{AsCl}_2}{\overset{\text{COCl}}{\bigcirc}}} + \text{NH}_3 \longrightarrow \underset{\textbf{43}}{\underset{\text{AsCl}_2}{\overset{\text{CONH}_2}{\bigcirc}}} \xrightarrow{\text{HSCH}_2\text{COOH}} \underset{\textbf{9}}{\underset{\text{As(SCH}_2\text{COOH})_2}{\overset{\text{CONH}_2}{\bigcirc}}}$$
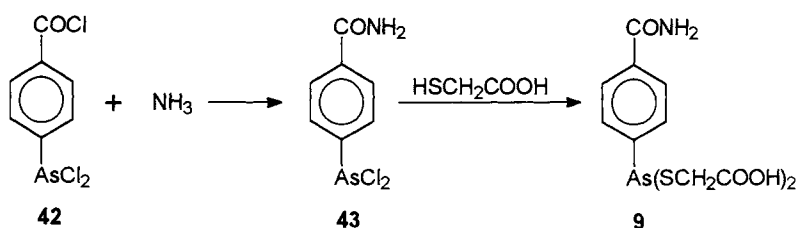
6.9 Tartar emetic (10)

Tartar emetic is manufactured by heating an aqueous solution of potassium bitartrate (44) with metallic antimony in the presence of nitric acid to oxidise the metal [41]. Alternatively, an aqueous solution of 44 is heated with antimony oxide in the presence of excess oxalic acid maintaining the pH of the solution around 1-3 [42].

$$\text{KOOC}-(\text{CHOH})_2-\text{COOH} + \text{Sb or Sb}_2\text{O}_3 \longrightarrow \textbf{10}$$

6.10 Sodium stibogluconate (trivalent and pentavalent forms) (12)

Trivalent stibogluconate (12a) may be prepared by treating an aqueous solution of calcium gluconate (45) with sodium carbonate. The resulting sodium gluconate (46) is reacted with antimony trichloride and sodium hydroxide to yield sodium stibogluconate (12a) [43]. In an alternate approach, aqueous calcium gluconate (45) is treated with oxalic acid to get free gluconic acid (47) to which antimony trichloride and sodium hydroxide are added to get the desired product 12a [44].

$$[\text{HO}-\text{CH}_2-(\text{CHOH})_4-\text{COO}]_2\text{Ca} \xrightarrow{\ a\ } \text{HO}-\text{CH}_2-(\text{CHOH})_4-\text{COONa}$$

45	46
$\downarrow$ d	$\downarrow$ b, c

$$\underset{\textbf{47}}{\text{HO}-\text{CH}_2-(\text{CHOH})_4-\text{COOH}} \xrightarrow{\ b,\ c\ } \begin{array}{l}\text{Sodium antimony (III)}\\ \text{gluconate (12a)}\end{array}$$
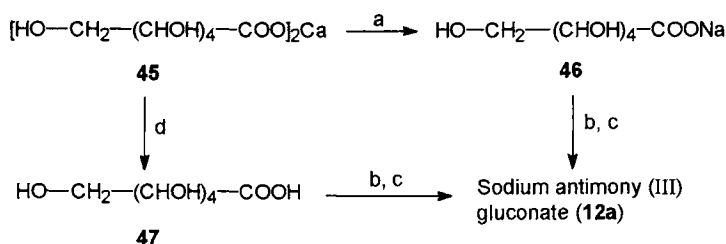
Reagents: (a) Na$_2$CO$_3$; (b) SbCl$_3$; (c) NaOH; (d) Oxalic acid.

The pentavalent sodium stibogluconate (12b) is obtained by treating an aqueous solution of gluconic acid (47) with moist Sb$_2$O$_5$; the resulting mixture is neutralised with sodium hydroxide to get sodium antimony (V) gluconate (12b) with antimony content ranging form 28-29.5% [45].

6.11 Glucantime (13)

This antimonial is prepared by hydrolysing antimony pentachloride to antimonic acid followed by reaction with N-methylglucamine (48) [46].

$$\text{HOCH}_2\text{—(CHOH)}_4\text{—CH}_2\text{NHMe} \xrightarrow{\text{Sb(OH)}_3} \textbf{13}$$
$$\textbf{48}$$
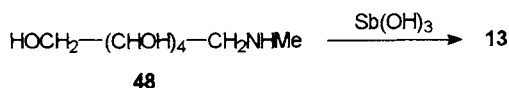
6.12 Urea stibamine (14)

Dutta *et al.* [47] synthesized urea stibamine by condensation of 4-aminophenylstibonic acid (49a) with urea at 80°C in the presence of small amounts of 4-acetylaminophenylstibonic acid (49b).

$$\text{RHN—C}_6\text{H}_4\text{—SbO}_3\text{H}_2 + \text{OC(NH}_2)_2 \longrightarrow \textbf{14}$$
$$\textbf{49a} \quad R = H$$
$$\textbf{b} \quad R = Ac$$
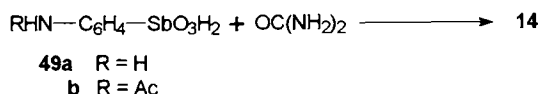
6.13 Stibocaptate (15)

It is synthesized by stirring a mixture of one mole of $Sb(OH)_3$ and 1.5 mole of dimercaptosuccinic acid (50) in the presence of 3 moles of sodium bicarbonate [48,49]. In another method, disodium dimercaptosuccinate and Sb_2O_3 are reacted to form stibocaptate [50].

$$\text{HOOC—(CHSH)}_2\text{—COOH} + \text{Sb(OH)}_3 + \text{NaHCO}_3 \longrightarrow \textbf{15}$$
$$\textbf{50}$$
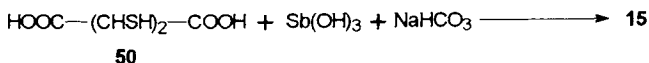
6.14 Stibophen (16)

Reaction of sodium pyrocatechol-1,3-disulphonate (51) with antimony trioxide (Sb_2O_3) in an alkaline solution yields stibophen (16) [51].

$$+ \text{Sb}_2\text{O}_3 + \text{NaOH} \longrightarrow \textbf{16}$$

$$\textbf{51}$$
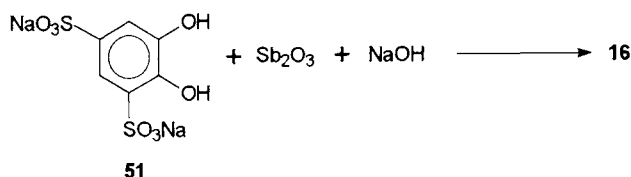
6.15 Metrifonate (18)

A series of dialkyl[2,2,2-trichloro-1-hydroxyethyl]phosphonates of the general formula (18) including metrifonate (R=CH$_3$) have been prepared in 54-74% yields by treating chloral (obtained by distilling chloral hydrate over sulphuric acid) with dialkyl hydrogenphosphite [52,53].

$$(RO)_2P-OH + Cl_3C-CHO \longrightarrow (RO)_2PO-CH(OH)-CCl_3 \quad (18)$$
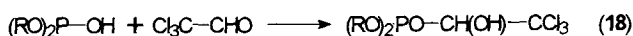
6.16 Fenthion (19)

This has been prepared by the reaction of the sodium salt of 4-methylthio-*m*-cresol (52) with O,O-dimethylthiophosphoryl chloride in acetone at 50°C [54,55].
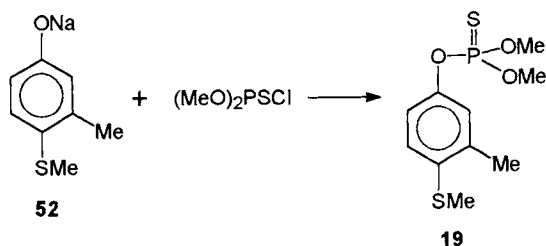
6.17 Haloxon and coumafos (20a,b)

Haloxon (20a) is prepared by treating 3-chloro-4-methyl-7-hydroxycoumarin (53) with O,O-di(2-chloroethyl)phosphoryl chloride [56], while coumafos (20b) is obtained by condensing 53 with O,O-diethylthiophosphoryl chloride in the presence of potassium carbonate and copper powder at 50-100°C [57,58].
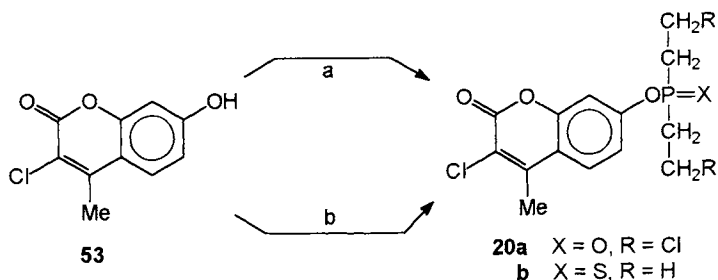
Reagents: (a) (ClCH$_2$CH$_2$)$_2$POCl; (b) (Et$_2$O)$_2$PSCl.

6.18   Crufomate (21)

A solution of 4-*tert*-butyl-2-chlorophenylphosphorodichloride (54) is treated successively with methanol and methylamine at low temperatures to yield crufomate (21) [59].

54
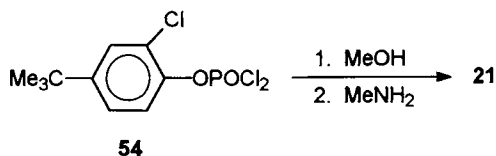
6.19   Dichlorovos (23)

Dichlorovos may be prepared in several ways. Methanol is treated with chloral (CCl_3-CHO) and PCl_3 to get dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphate (55), which is heated with 25% NaOH to yield dichlorovos (23) [60]. Alternatively, chloral alcoholate (56), the precursor of chloral prepared by passing chlorine into cooled ethanol, is allowed to react with $(MeO)_3P$ to form dichlorovos [61]. In another approach, methanol is treated with PCl_3 to get $(MeO)_3P$ (57) [62]. This reaction may also be carried out in the presence of CuCl at - $10^{o}C$ to yield the adduct $(MeO)_3P.CuCl$ (58) [63]. Reaction of 57 or 58 with chloral yields dichlorovos (23) [62,63].
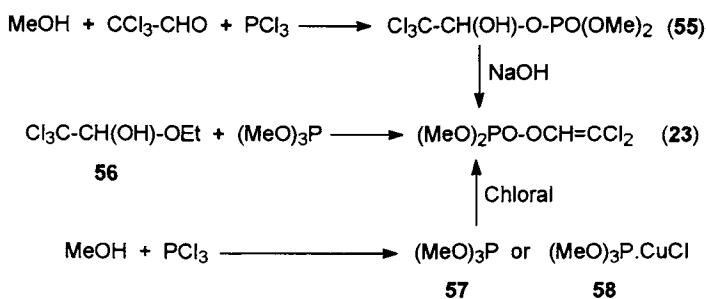
7.   ORGANOMETALLIC AGENTS IN THERAPEUTIC USE

Although quite a good number of compounds containing arsenic, antimony, phosphorus and other metals have been found to display a wide spectrum of activity against protozoal and helminth infections in humans and domestic animals, they find limited use in current therapy because of their high toxicity, low therapeutic indices and availability of better drugs.

7.1 Organometallics in veterinary medicine

7.1.1 Organoarsenicals

A few compounds of this class, namely arsanilic acid (1a) and roxarsone (4) have been used for poultry both as growth promotors and to control coccidiosis due to *E. tenella* and *E. brunetti*. The recommended dose of the above arsenicals is 400 and 50 mg/kg, respectively [64,65]. The precardiac stage of the heartworms, *D. immitis* in ferrets may be treated by Mel B (7a) at an oral dose of 100 mg/kg [66]. A combination of arecoline (59) and acetarsone (60) in a ratio of 1:2, known as nemural, has been used to achieve complete eradication of *E. granulosus* from dogs at a dose of 4 mg/kg [67]. Nemural has been used for over 40 years to treat tapeworm infections in cats, dogs, sheep and fowl. At a dose of 12 mg/kg, it cleared *H. taeniaeformis* from cats, but was not superior to niclosamide [68].
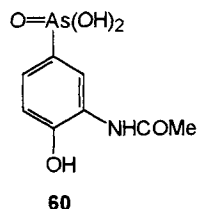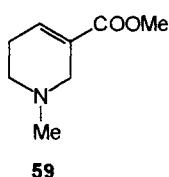
7.1.2 Organoantimonials

Tartar emetic (10) and stibophen (16) have been used to treat *Trypanosoma congolense* and *T. vivax* infections in cattle and *T. evansi* in camel. The usual dose of tartar emetic is 1-1.5 g/animal given intravenously, while stibophen may be administered intramuscularly or subcutaneously at a dose of 3-6 g/100 kg. However, both the drugs are toxic and do not find much use in modern therapy of animal trypanosomiasis [65]. Cattle and sheep infected with *Schistosoma japonicum*, *S. nasalis* and *S. mattheei* may be treated with some organoantimonials like tartar emetic (10), stibocaptate (15) and stibophen (16). These drugs are toxic and require great care of animals during treatment. The usual dose of tartar emetic for treating *S. nasalis* in sheep and cattle is 2 mg/kg daily during 6 days or 3.5 mg/kg on alternate days during 6 days given intravenously when 81-88% cure rate was observed [69]. Similarly for treating *S. mattheei* infection in sheep and cattle, stibophen has been given at a dose of 5-10 mg/kg, intramuscularly for 3-10 days [70,71]. High cures may be achieved against *S. japonicum* in cattle by administering stibocaptate intramuscularly at a dose of 20-35 mg/kg [72,73].

7.1.3 Organophosphates

A number of organophosphates have been used for their antiparasitic activity in domestic animals. Several drugs of this class show a wide spectrum of activity against the gastrointestinal nematodes of cattle, sheep, pigs, horses, cats and dogs [65,74]. A few compounds also find use in the treatment of schistosomiasis in ruminants [73]. The biological profile of a few organophosphate drugs is given below.

(a) **Metrifonate (18):** The drug shows good activity against the gastrointestinal nematodes of cattle and horses. At a dose of 60-80 mg/kg given subcutaneously or as drench, metrifonate exhibits high activity against the adult worms of *Cooperia* and *Haemonchus* spp. However, its activity against *Ostertagia*, *Trichostrongylus* and *Oesophagostomum* spp. and immature nematodes is weak [74]. In horses, a dose of 35 mg/kg of metrifonate is needed to cause above 90% elimination of *Parascaris equorum* and *Oxyuris equi* [74]. At therapeutic doses, the drug may produce toxic effects like transient softening of stool and mild colic; higher doses (60 mg/kg) may cause severe colic lasting for several hours.

Metrifonate also provides satisfactory treatment against *S. mattheei* in sheep, goats and cattle. However, its activity against *S. nasalis* is low. The drug is given orally at doses ranging from 25-120 mg/kg for 3-6 days at 3-4 day intervals [69,71,74-76].

(b) **Dichlorovos (23):** This is the active metabolite of metrifonate and it is more active than the parent drug. Dichlorovos is usually given in the form of a slow release preparation. The drug is incorporated in a polyvinyl chloride (PVC) resin pellet which acts as a slow release vehicle. As the PVC resin passes through the gastrointestinal tract, it slowly releases dichlorovos thereby making the drug better tolerated by the host.

Dichlorovos has been found to possess a high order of activity against the adult worms of *Ascaris suum*, *Hyostrongylus rubidus*, *Trichuris suis* and *Oesophagostomum* spp. in pigs at an oral dose of 10-40 mg/kg. The drug also exhibits outstanding efficacy against *P.equorum*, *S. vulgaris* and *O. equi* in horses when given as PVC resin pellets at a dose of 35 mg/kg [74]. The side effects of the drug include softening of stool, salivation and muscle tremors. Dichlorovos is not recommended to mares during the first 4 months or last month of gestation [65,74].

(c) **Haloxon (20a):** Haloxon has a high activity against *Haemonchus* and *Cooperia* spp. in sheep and cattle at an oral dose of 50 mg/kg. A similar order of activity (above

90%) is also observed against the adult worms of *S. vulgaris*, *P. equorum*, *O.equi* and all the benzimidazole susceptible and resistant forms of small strongyles in horses. The recommended dose of the drug is 60 mg/kg. Haloxon is a safe drug producing no ill effects even upto 3 times the therapeutic dose. Unlike dichlorovos, this drug may be given to pregnant mares [65]. Haloxon has also been used against *S. mattheei* infection in sheep and horses with satisfactory results. Haloxon has also been used against *Capillaria* infection in poultry by administering it at a dose of 25 mg/kg given in food [74].

(d) Coumafos (Coumaphos, 20b): The activity profile of this drug against *Haemonchus* and *Cooperia* spp. in cattle is very similar to that of haloxon. Its effective dose is 15 mg/kg single dose or 2 mg/kg daily for 6 days. The drug may cause mortality of ruminants at therapeutic doses. Atropine can be used as antidote [65,74]. When given in feed at a concentration of 0.004% for 10- 14 days, coumafos showed activity against *Capillaria* infection in poultry [74].

(e) Crufomate (21): Crufomate shows high activity against *Haemonchus* and *Cooperia* spp. in cattle at a single oral dose of 40 mg/kg or 17 mg/kg for 3 days given in feed. Like haloxon and coumafos, it exhibits weak activity against *Ostertagia*, *Trichostrongylus* and *Oesophagostomum* spp. [74].

7.2 Organometallics in human medicine

Systematic investigation of the usefulness of organometallics in the chemotherapy of human parasitic diseases goes back to Paul Ehrlich during early 1900, who established the therapeutic value of organoarsenicals and antimonials in the treatment of filariasis, schistosomiasis, trypanosomiasis and leishmaniasis etc. The organophosphate drugs were added to this list of organometallic drugs after World War II. Although many of the organometallics provide an acceptable clinical response, their longterm usefulness in human medicine is limited by their high toxicity. Consequently a number of these drugs were slowly replaced by more effctive and safer metal-free agents. The clinical profile of a few organometallic drugs is described below.

7.2.1 Activity against filariasis

7.2.1.1 Organoarsenicals

The first member of this class used successfully is phenylarsenoxide (Ph-AsO). This was demonstrated to have *in vitro* and *in vivo* antifilarial activity by Hawking in 1940 [77] and later by Otto and Maren in 1947 [78]. Arsenamide (9) is

another effective antifilarial which kills the microfilariae and adult worms of *W. bancrofti* in humans at an intravenous dose of 1 mg/kg given for 15 days. Although the drug is usually well tolerated, its occasional liver toxicity and low therapeutic index limit its use in routine treatment of lymphatic filariasis in humans [13,79].

Mel-W (**7b**) also shows high activity against the microfilariae and adult worms of *W. bancrofti* in humans at an intramuscular dose of 10 mg/kg given for 3 days. The drug is equally effective in treating *O. volvulus* infection in humans [80]. Mel-W sterilizes the adult females and also kills the microfilariae and adult worms of *O. volvulus* in humans at a dose of 7-10 mg/kg or at a higher dose of 500 mg/adult. Although the drug has been used to treat lymphatic filariasis and onchocerciasis in humans in the Pacific, New Guinea and ex-French West Africa, it could not be accepted in mass therapy due to its toxicity and occasional reports of death of treated patients [80-82].

7.2.1.2 Organoantimonials

MSb-B (**17**) has been used in patients infected with *W. bancrofti*. The drug given orally from 200 mg to 1.2 g/adult for 3-10 days gave high suppression of microfilarial counts and death of adult worm. The side effects of MSb-B were gastrointestinal disturbances, fever, headache and skin rashes [83,84].

7.2.1.3 Organophosphates

Metrifonate (**18**) is an effective drug for onchocerciasis and schistosomiasis. At an oral dose of 10 mg/kg given for 6 days, the drug exhibits high activity against the microfilariae and some action on the adult worms of *O. volvulus* in humans [85,86]. A lower dose of 7.5 mg/kg given at 3-week intervals has been found to kill the microfilariae of *O. volvulus* in patients [87]. The major side effects of metrifonate are vomiting, diarrhea and the Mazzotti reaction [86].

7.2.2 Activity against schistosomiasis

7.2.2.1 Tartar emetic (10)

This is an old drug which has been used to treat human schistosomiasis; with a dose of 4 mg/kg, and a maximum of 700 mg/adult given for 3 days cure rates upto 85% have been achieved.

The sodium derivative of tartar emetic, called sodium antimony tartrate, is a better antimonial which shows high activity (cure rates: 90-100%) against *S. haematobium* at a dose of 7-8 mg/kg. The drug was given to Egyptian farm workers as intra-

venous injections of 33 mg/kg twice a week for 6 weeks when 82% patients were cured showing 90% reduction of S. *haematobium* eggs in urine. However, both these drugs are toxic producing adverse effects on heart and liver [23, 88], and with better drugs available are hardly used now.

7.2.2.2 Antimony sodium gluconate (12)

The trivalent form of this drug, triostam (12a), shows good activity against S. *haematobium* in humans. The usual dose of the drug is 12-17 mg/kg given for 4-6 days; however, a higher dose of 25 mg/kg may also be given to achieve cure rates upto 90%. A better profile of activity may be obtained by using a combination of triostam with leucanthone [88].

7.2.2.3 Stibocaptate (15)

This is one of the most widely used antimonials for treating urinary schistosomiasis (S. *haematobium*) in humans. The drug is administered intramuscularly at a dose of 8-10 mg/kg for 5 days when high cures with nearly 90% reduction in egg counts were obtained. At a higher dose (40-80 mg/kg upto a total of 1.2-2.4 g divided in 5-6 injections) stibocaptate shows 80-90% cures against S. *mansoni* in humans [88]. In China, several patients infected with S. *japonicum* were treated using 30-40 mg/kg of stibocaptate spread over three intramuscular injections [89]. The usual side effects of the drug are vomiting, joint pains, cutaneous eruptions and EKG changes [88].

7.2.2.4 Stibophen (16)

Although this drug has been extensively used to treat S. *mansoni* and S. *haematobium* infections in humans, it is no longer used in clinical therapy due to its high toxicity and unsatisfactory therapeutic response. The usual dose of the drug is 0.5-1.0 mg/kg or 40-60 ml of a 6.3% solution/adult [23,90].

7.2.2.5 Sodium antimony dimethylcystein (15a)

It is a highly effective drug for treating S.*mansoni* and S. *japonicum* infections in humans. The recommended dose of the drug is 5 ml (containing 14.5-14.9% antimony) given intramuscularly once daily for 5 days when 94% cure rates were observed against S.*mansoni*. A similar order of activity was achieved against S. *japonicum* when the drug was given intramuscularly at a dose of 400 mg/day for 5 days [23,88].

7.2.2.6 Metrifonate (18)

This is a broad-spectrum anthelmintic which is given orally to treat *S.haematobium* infections in humans. The recommended dose of the drug is 7.5-10 mg/kg given for 3 doses at a 2 week interval when 81-91% cures were obtained. However, an oral dose of 10 mg/kg, given 1-3 times has been used to treat *S.haematobium* infection in children and adults. This schedule was found to provide about 90% cures with more than 90% reduction of eggs in urine of the patients [3,90-95].

Metrifonate is a well tolerated drug that produces mild gastrointestinal disturbances at therapeutic doses.

8. MODE OF ACTION

8.1 Organo-antimonials and arsenicals

A number of organoantimonial drugs have been shown to interfere with the glycolytic pathways of glycogen and glucose. The antimony compounds inhibit the enzyme phosphofructokinase (PFK) which is essential for the conversion of fructose-6-phosphate into fructose-1,6-diphosphate both in the filariids and schistosomes [96-98]. The inhibition of PFK activity occurs when the antimonials bind with the sulfhydryl group of this enzyme present in both the host and the helminths. However, the parasitic PFK is 80 times more sensitive to the antimony drugs than the mammalian PFK. This leads to the selective toxicity of the drug on the filariids and schistosomes.

It has been suggested that inhibition of PFK activity and subsequent depletion of energy in the parasites may not fully account for the antischistosomal activity of the antimonials [99]. Work carried out in this direction indicates that the antimonials might induce lesions on the fluke's tegument. This tegumental disruption could expose the "hidden" antigens of the parasite making it susceptible to the attack by the host's immune system [100-102].

The antiparasitic activity of arsenicals may also be attributed to their ability to bind with sulfhydryl groups of the proteins/enzymes essential for controlling life supporting processes in the parasites [9].

8.2 Organophosphates

A number of organophosphates such as crufomate, haloxon, metrifonate and dichlorovos have been found to inhibit the acetylcholinesterase (AChE) of nema-

todes at very low concentrations (10^{-3}M) [103]. Since AChE is essential for postsynaptic inactivation of ACh, its inhibition leads to continued depolarisation at the postsynaptic junction causing paralysis of the worms [104-106]. The activity of metrifonate (18) is due to its bioconversion into the active metabolite, dichlorovos (23), which is a better inhibitor of AChE than the parent drug [107,108]. Thus major action of the organophosphates both on the nematodes and flukes is due to their ability to inhibit AChE activity and paralysing the worms [99,104].

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CHAPTER 5

PIPERAZINES

1. INTRODUCTION

The piperazine skeleton, one of the simplest diazacycloalkanes, is of great importance for medicinal chemistry as it provides compounds with a wide-spectrum of biological activities. The importance of piperazines in designing effective drugs for intestinal and tissue-dwelling helminths was not recognised till 1947 when scientists working at the Lederle Laboratories of the American Cyanamid Company discovered the antifilarial activity of 1,4-disubstituted piperazines [1-5]. In fact, the most active compound of the series, 1-carbethoxy-4-methylpiperazine (2) was originally prepared as a structural analogue of the analgesic drug meperidine (1). Compound 2 was found to possess high activity against *Litomosoides carinii* in cotton rats at an intraperitoneal or oral dose of 25-100 mg/kg [2,5,6]. Taking 1-carbethoxy-4-methylpiperazine (2) as a 'lead' molecule, various modifications were carried out at 1- and 4-positions of the piperazine ring which ultimately culminated in the discovery of 1-diethylcarbamoyl-4-methylpiperazine (Diethylcarbamazine, 3, DEC) [2,4] possessing a high order of antifilarial efficacy in man and animals [7]. Soon DEC became the drug of choice for treating lymphatic filariasis, loasis and to some extent onchocerciasis and even after almost five decades of its discovery it is still largely used in clinical practice [8,9].

1 (Meperidine)

2 R = OEt
3 R = NEt$_2$ (DEC)
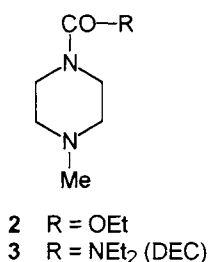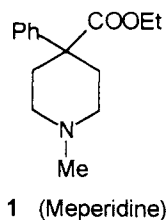
2. SAR IN PIPERAZINES

While the search for newer filaricides derived from piperazine was still on, the basic molecule, piperazine (4), was found to be highly effective against intestinal roundworms, *Ascaris lumbricoides* [10] and pinworms, *Enterobius vermicularis* [11]. It

is interesting to note that piperazine (4) was originally evaluated against rheuma-tism, while its anthelmintic activity was discovered accidently [12].

DEC possesses rapid action on the microfilariae but only slow action on adult worms of only some of the filariids, and does not provide radical cure, and is not equally effective against all forms of human filariasis. Further, the use of the drug is associated with several undesirable side effects making it often unsuitable for mass therapy programmes. These facts prompted search for better antifilarial agents util-izing the characteristics of the molecular frame-work of DEC. Keeping in view a few structural features of DEC such as interatomic distance between the three nitrogen atoms, electronic and steric nature of substituents attached to the nitrogen atom(s) and geometry of the molecule as a whole, the synthesis of a large number of struc-tural congeners of DEC were carried out, which may be broadly divided into three groups 2.1 to 2.3 discussed below [13].

4

5 R = OEt, NEt$_2$, NR$_3$R$_4$
R$_1$ = H, Me, R$_2$ = alkyl.
aryl, alkylcarbonyl

6

7

8a X= CH$_2$
b   X = O
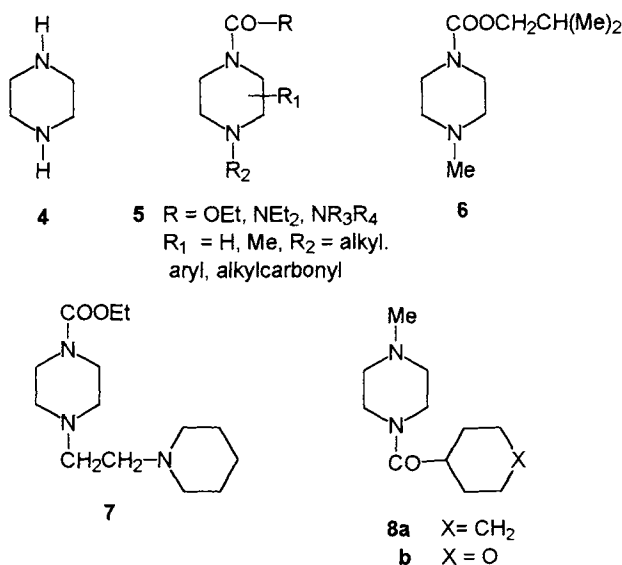
2.1   1,4-Disubstituted piperazines

Following the discovery of DEC, a series of 1,4-disubstituted piperazines (5) were synthesized, many of which showed moderate to good antifilarial activity. The noteworthy active analogues of DEC belonging to this class are 6-8 [13]. When tested against *Litomosoides carinii* infection in cotton rats, 1-isobutoxycarbonyl-4-methylpiperazine (6) caused 91% reduction of microfilariae in blood at an intraperi-toneal dose of 3 mg/kg during 5 days and also killed 100% of the microfilariae and

adult worms of *Dipetalonema viteae* in *Mastomys* at a subcutaneous dose of 50 mg/kg given for 5 days [14]. Similarly 1-carbethoxy-4-(2-piperidin-1-ylethyl)piperazine (7) was found to exhibit high activity against *L. carinii* in cotton rats at a dose of 100 mg/kg [15].

Hoechst Laboratories synthesized 1-cyclohexycarbonyl-4-methylpiperazine (8a, HOE-29691a) possessing antifilarial efficacy very similar to DEC with lower toxicity. Both 8a and 8b exhibited marked microfilaricidal activity against *L. carinii* in *Mastomys* at doses ranging from 60-125 mg/kg for 5 days [16].

A series of 1-carbamoyl-4-methylpiperazines were prepared of which 1-methyl-4-(pyrrolidin-1-yl-carbonyl)piperazine (9) was found to exhibit excellent antifilarial activities. The compound caused more than 92% elimination of microfilariae of *L. carinii* and *D. viteae* in rodents at an oral dose of 3 and 12 mg/kg, respectively. It also showed higher therapeutic indices and lower LD_{50} values than DEC [17].

The fact that demethylation of DEC gives rise to inactive metabolites prompted Wise *et al.* [18] to synthesize some 1-dialkyl-4-diethylcarbamoylpiperazinium salts (10) which would resist a DEC-like demethylation *in vivo*. A few piperazine-N-oxides (11) have also been prepared [19]; however, none of these compounds exhibited antifilarial activity better than DEC.
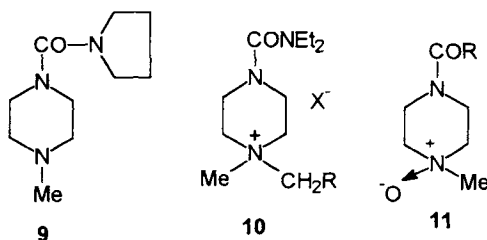
During a detailed SAR study on 1,4-disubstituted piperazines, one of the groups of DEC has been replaced by a heterocycle such as quinoline, isoquinoline and benzimidazole, but in no case a compound with better profile of activity than DEC was obtained. The noteworthy compounds which emerged out of this effort are 12- 15 possessing marked activity against experimental filariasis [20-23]. 2-[2-(4-Hydroxyphenyl)-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole (12, HOE-33258), prepared by Hoechst Laboratories, was found to possess prolonged suppressive effects on the microfilariae of *L. carinii* at a subcutaneous dose of 4 mg/kg for 5 days [20]. Similarly the structural analogues of amodiaquine, 13 and 14 showed

marked activity against *L. carinii* in rodents at a dose of 25-100 mg/kg [21,22]. However, methyl 5(6)-(4-substituted piperazin-1-yl)benzimidazole-2-carbamates (**15a,b**) killed 90-100% of the microfilariae and adult worms of *L. carinii* in cotton rats at an intraperitoneal dose of 30 mg/kg for 5 days [23].

12 (HOE-33258)

13 R = Me, Et
14 R = CONEt$_2$

15a R = NEt$_2$
   b R = 2-Furyl
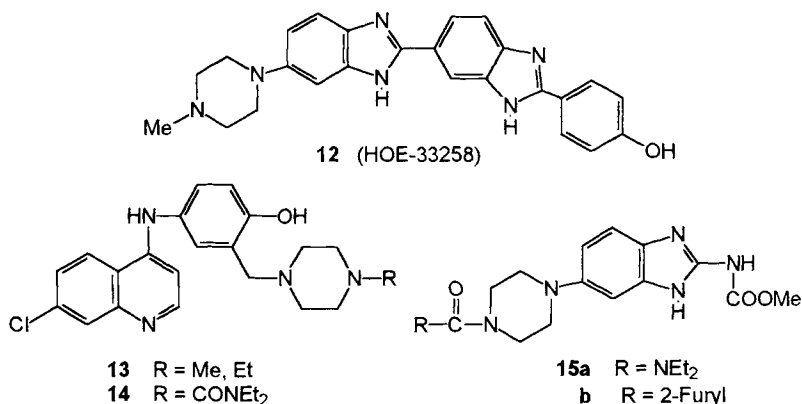
2.2 Non-piperazine analogues of DEC

In an attempt to study the role of the piperazine ring in evoking antifilarial activity, various ring-cleft analogues of DEC have been prepared with the same number of nitrogen atoms and substituents known to provide active 1,4-disubstituted piperazines. Non-piperazine congeners of DEC were obtained by: (a) scissoring the piperazine ring, (b) ring contraction, (c) ring expansion, (d) building other 1,3-diazacycloalkanes by involving two nitrogens of DEC with simultaneous cleavage of the piperazine ring, and (e) scissoring the ring and subsequently closing it in such a way that only one of the nitrogen forms a part of the resulting ring. The compounds thus synthesized were: (a) ethylenediamines (**16**), (b) imidazolidines (**17**), (c) homopiperazines (**18**), hexahydropyrimidin-2-ones (**19,20**), and (e) pyrrolidines (**21,22**) [13, 24-31].

Most of the compounds represented by the molecular skeletons **16-22** either had little or no antifilarial activity except for **18**, which was found to be half as active as DEC. The lack of desired antifilarial activity in **16-22** was explained on the basis of their molecular geometry. None of the above non-piperazine analogues represented the exact interatomic distances and molecular geometry as shown by DEC. This indicated that the interatomic distances between the nitrogen atoms and the spatial disposition of the functional groups in DEC are of importance in governing antifilarial activity.

152

CONEt₂ structures...

16   17   18

19   20   21 R = Me, R₁ = NHCONEt₂
              22 R = CONEt₂, R₁ = NMe₂

R = CH₂CH₂—N(R₁)—Me   R = CH₂—N(Me)—Me
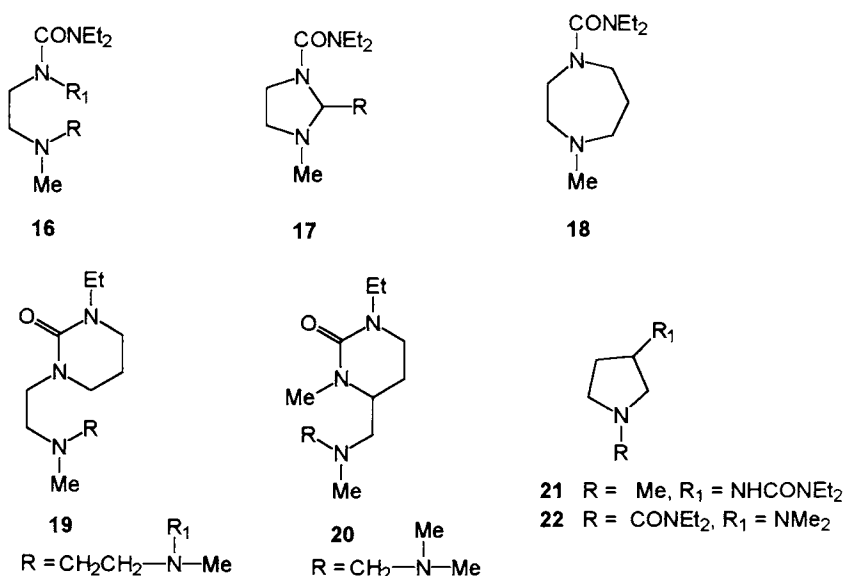
Brookes and coworkers [32] have studied the effect of replacement of the piperazine ring by other diacidic cyclic bases on antifilarial response. Consequently, a series of 2,4'-dipiperidyl, 4,4'-dipiperidyl and 4-aminopiperidines with a diethylcarbamoyl chain were prepared and evaluated for their antifilarial activity. The most effective compounds of this class were 23-26 possessing marked activity against the microfilariae of *L. carinii*, but none was better than DEC.

23   24

25 R = OEt, NEt₂   26 R = OEt, NEt₂
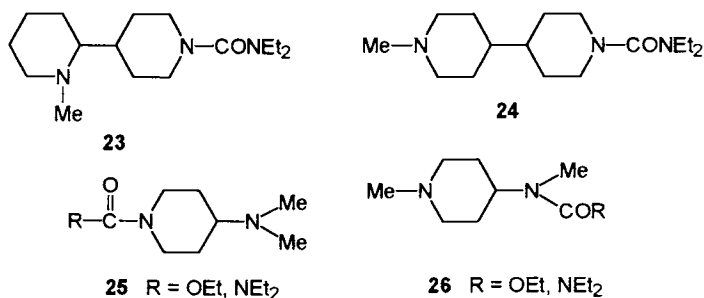
2.3 Condensed piperazine heterocycles

In a further probe to examine the role of the interatomic distances and geometry of the molecule in governing antifilarial activity amongst DEC analogues, it was considered desirable to synthesize some bicyclo-heteroalkanes by incorporating one of the ethyl groups of the diethylcarbamoyl chain of DEC in rigid structures. This structural modification was expected to reduce considerably the free rotation of the

diethylcarbamoyl side chain of DEC without altering the steric and electronic nature of the three nitrogen atoms. Accordingly, 3-ethyl-8-methyl-1,3,8-triazabicyclo[4,4,0]-decan-2-one (27, Centperazine), 6-ethyl-1-methyl-1,4,6-triazabicyclo[4,3,1]decan-5-one (28) were prepared [33-35]. Centperazine (27) was found to be a highly effective microfilaricide at an intraperitoneal dose of 1 mg/kg for 6 days, while 28 had no antifilarial activity. The marked antifilarial activity of 27 has been attributed to its reduced conformational mobility. Compound 28, which did not meet the above structural requirements, was devoid of antifilarial activity. Similarly bicycloalkanes of type 29a,b exhibited no antifilarial activity, presumably because of the difference from the molecular geometry of centperazine [36].
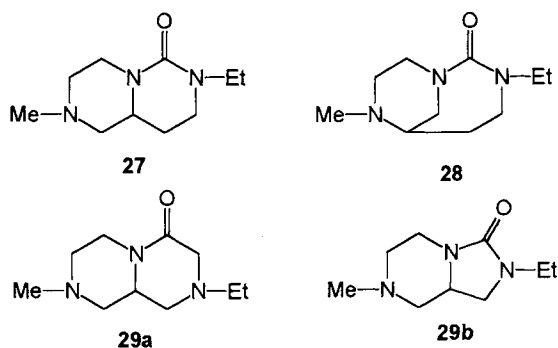
In an expanded study relating to the investigation on the role of bicyclohet-eroalkanes in filarial chemotherapy, some piperazine-heterocycles of type 30-33 were prepared, many of which caused high reduction of microfilarial counts in experimental animals infected with *L. carinii*. However, none was found to be better than DEC [30,37-39] or centperazine.
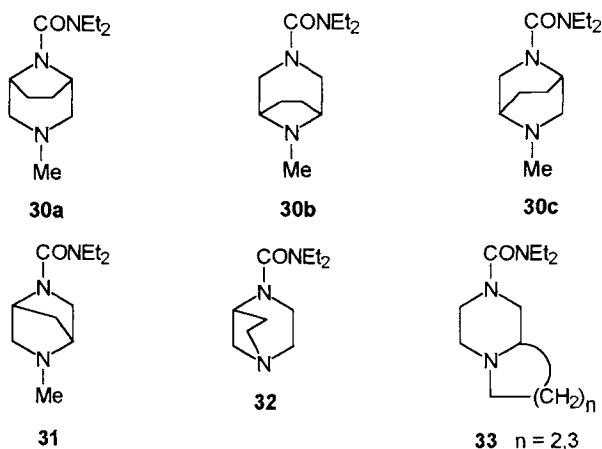
3. SYNTHESIS OF PIPERAZINE ANTHELMINTICS

3.1 Piperazine (4)

Anhydrous piperazine is a white solid which is obtained as leaflets when crystallised from alcohol, m.p. 106°C (b.p. 145-146°C). Piperazine may be prepared by several methods [40]. However, industrially it is best prepared by cyclodehydration of 2-hydroxyethylamine (34) or N-(2-aminoethyl)-2-hydroxyethylamine (35) at high temperatures in the presence of a catalyst like Ra-Ni and halides of Zn, Fe, Al or Mg [41-43]. Catalytic deamination of diethylenetriamine (36) in the presence of Ra-Ni catalyst in an autoclave at 150°C also gives piperazine. Another useful method to prepare piperazine involves reduction of pyrazine (37) by sodium and ethanol [40] (Scheme 1).
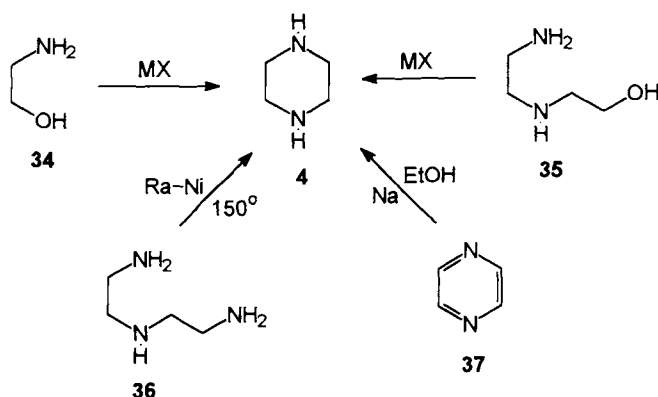
Scheme 1
Reagents: M = Zn, Mg, Al, Fe; X = halogen.

3.2 Diethyl carbamazine (3, DEC) and related 1,4-disubstituted piperazines (Scheme 2)

In most of the methods piperazine (4) is used as the starting material. Reaction of 4 with ethyl chloroformate at pH 3-3.5 gives 1-carbethoxypiperazine (38), which is methylated to form 1-carbethoxy-4-methylpiperazine (39). Hydrolysis of 39 gives 1-methylpiperazine (40), which is treated with N,N-diethylcarbamoyl chloride or phenyl N,N-diethylcarbamate (prepared from Ph_2CO_3 and Et_2NH) to yield 4-diethylcarbamoyl-1-methylpiperazine (3, DEC) [5,45-49]. Another convenient method especially suitable for a large scale preparation of DEC involves the reaction of 4 with N,N-diethylcarbamoyl chloride to give 1-diethylcarbamoylpiperazine (41),

which is subsequently methylated [45]. Alternatively, **40** may be treated with Ph_2CO_3 to yield 1-methyl-4-phenoxycarbonylpiperazine (**42**), which is allowed to react with diethylamine to form DEC [46].

Acylation of **40** with cyclohexanecarbonyl chloride and tetrahydropyran-4-carbonyl chloride affords HOE-28637a (**8a**) and HOE-26961a (**8b**), respectively [50]. Similarly 1-methyl-4-(pyrrolidin-1-ylcarbonyl)piperazine (**9**, CDRI 72-70) has been prepared by action of phosgene on **40** to get N-methylpiperazinecarbonyl chloride (**43**) followed by treatment with pyrrolidine [51] (scheme 2).
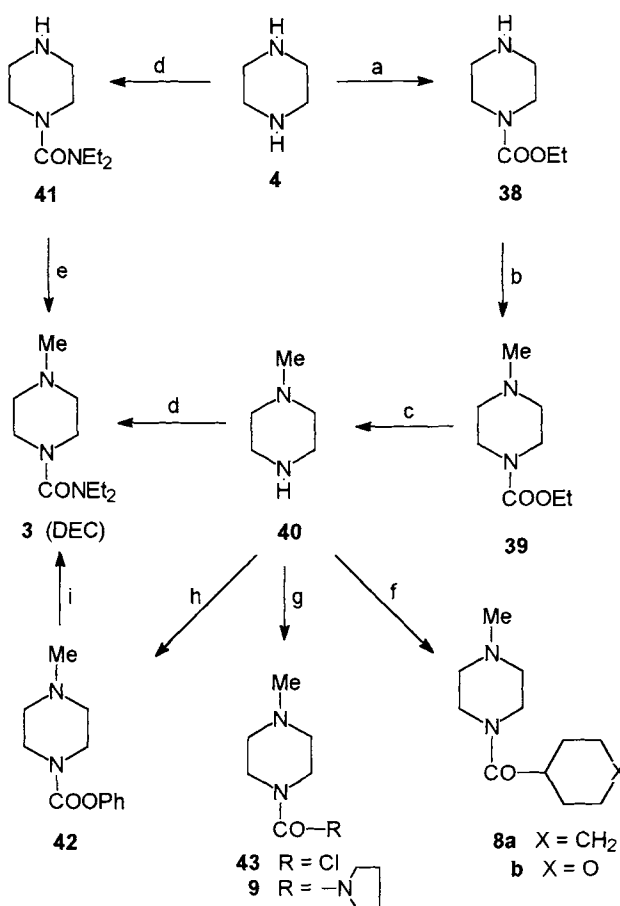
Scheme 2

Reagents: (a) ClCOOEt, MeCOONa at pH 3.0-3.5, (b) MeI,(c) hydrolysis, (d) ClCONEt$_2$, (e) HCOOH, HCHO (f) cyclohexanecarbonyl chloride or tetrahydropyran-4-carbonyl chloride, (g) COCl$_2$; pyrrolidine; (h) Ph$_2$CO$_3$, (i) Et$_2$NH.

3.3 3-Ethyl-8-methyl-1,3,8-triazabicyclo[4,4,0]decan-2-one (27, Centperazine) (Scheme 3)

It has been prepared by several methods [33,34]. The most convenient method to synthesize centperazine in large amounts starts with the Michael addition of N-benzylethylenediamine (44) with diethyl maleate or fumarate (45) followed by ring closure to yield the piperazine ester 46. Reaction of 46 with ethylamine gave the desired amide (47), which is reduced with lithium aluminum hydride to afford 48. Monoethoxycarbonylation of 48 with ethyl chloroformate gave the monocarbamate 49, which undergoes facile ring closure in the presence of NaOEt to form 8-benzyl-3-methyl-1,3,8- triazabicyclo[4,4,0]decan-2-one (50). The latter is also obtained by direct cyclisation of 48 with phosgene. Hydrogenation of 50 in the presence of Pd-C catalyst yielded the debenzylated product 51, which is methylated using the Clarke-Eschweiler method to give centperazine (27) [34] (scheme 3).
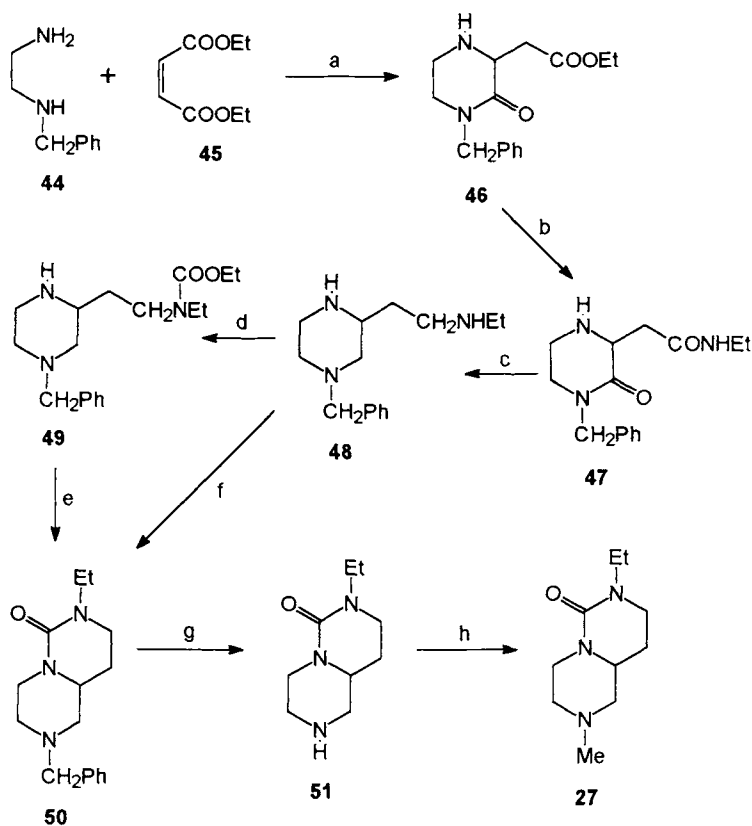
Scheme 3

Reagents: (a) Michael addition, (b) EtNH$_2$, NaOEt, (c) LAH, THF, (d) ClCOOEt, MeCOONa, pH 3-3.5, (e) NaOEt, (f) COCl$_2$, (g) Pd-C, H$_2$, (h) HCOOH, HCHO.

4.   BIOLOGICAL ACTIVITY

Piperazine and DEC are the two important drugs of this class, which have been used to treat various nematode infections in humans and animals [52-57].

4.1   In veterinary medicine

4.1.1   Piperazine (4)

This is an old drug, which still finds use in the treatment of ascarid infections in horses, pigs, cats, dogs and poultry. It is virtually free of any side effect and is well tolerated even by young animals [58].

In horses, piperazine eliminates adult worms of small strongyles and *P. equorumin* in more than 90% of the animals, but is less effective against *S. vulgaris, S. edentatus* and *O.equi*. The recommended dose of the drug is 90 mg (base)/kg administered orally as citrate, phosphate or adipate salts.

Piperazine shows high activity against *A. suum* and *Oesophagostomum* spp. in pigs at a dose of 250-300 mg/kg given in feed or water. The drug is equally effective in eliminating the common ascarids of cats and dogs at a dose of 100-250 mg/kg base administered orally as a salt [56,58].

Piperazine has also been found to possess very high activity against *Ascaridia* spp., but is ineffective against *Heterakis* spp. The usual dose of the drug is 50-100 mg/bird; it may also be administered mixed with feed (0.2-0.4%) or drinking water (0.1-0.2%) [57].

4.1.2   Diethylcarbamazine   (DEC, 3)

Diethylcarbamazine has been found to be active against adult ascarids in cats and dogs. However a combination of DEC (3 mg/kg) and styrylpyridinium chloride (5 mg/kg) gave protection against hookworms, ascarids and heartworms in dogs. DEC has also been used as a prophylactic agent against canine heartworm disease at a dose of 5.5 mg/kg given daily during the mosquito season plus an additional two months [56].

DEC has no action on the microfilaria or adult worms of *L. carinii in vitro,* but exhibits lethal action on the filariids *in vivo*. The drug kills >90% of the microfilariae circulating in the blood in cotton rats, Mongolian jirds and *Mastomys natalensis* infected with *L. carinii* at an intraperitoneal dose of 6 mg/kg given for 5 days [59,60]. However, DEC has little or no action against the microfilariae of *Dipetalonema per-*

stans, Dipetalonema viteae and *Dirofilaria repens* in jirds and dogs [7,16]. The drug has been found to be highly effective against microfilariae of *Onchocerca cervicalis, O. gutturosa* and *O. gibsoni* in horses and cattle at a dose of 20 mg/kg given for 3-5 days [62]. The pre-adult developing stages of *D.immitis* in dogs may be killed at a dose of 55 mg/kg of DEC [61].

The action of DEC on adult filarial worms is species dependent. The drug shows only poor or no activity on the macrofilariae of *L.carinii* and *D. viteae* in rodents and *Dirofilaria immitis* and *D.repens* in dogs. The adult worms of *O. volvulus* are also not susceptible to DEC. However, DEC has been found to kill the adult worms of *Brugia pahangi, B.malayi, W.bancrofti, L. loa, Dipetalonema streptocerca* and *Setaria digitata* in different animals [7,62].

4.2   Piperazine anthelmintics in human medicine

4.2.1   Piperazine (4)

This drug has been in clinical use for over 35 years. Even today it is recommended as a drug of choice for treating roundworm infection in many countries of the world due to its low cost, high activity and practically no toxicity. Till the advent of modern anthelmintics like pyrantel pamoate and mebendazole, piperazine was used as the drug of choice for treating roundworms *(Ascaris lumbricoides)*, and pinworms *(Enterobius vermicularis)* in adults and children [52].

Piperazine is usually given as hexahydrate or citrate, phosphate, adipate or tartrate salts as tablets or syrup. The recommended adult dose of piperazine is 50-75 mg/kg with a maximum of 3.5-4.5 g daily given for 1-2 days when almost 100% elimination of *A.lumbricoides* is obtained. Children under 20 kg receive a maximum of 3 g in single dose [52-56].

Piperazine is also a drug of choice for treating pinworm infections in children and adults. A dose of 2-2.5 g of piperazine given daily for 14 days gives nearly 100% cures, while a dose of 50-75 mg/kg (maximum 0.5-3g) of piperazine citrate given for 7-14 days produces 85-100% elimination of *E. vermicularis* in man with little or no toxicity [52-54,56,57]. Piperazine and its salts show poor activity against whipworms, *Trichuris trichiura* in man [63,64].

Piperazine is one of the safest anthelmintics available today; in some patients it may produce nausea, vomiting, abdominal discomfort and loose stools/diarrhea. Since piperazine has a blocking effect on the myoneural junction, some side effects

related to central nervous system may be seen occasionally. These may include som-
nolence, vertigo, ataxia, incoordination of muscles, speech problems, confused men-
tal state, muscular weakness, myoclonic contractions and epileptic seizures. How-
ever, these symptoms appear usually at high doses of the drug. Piperazine is
contraindicated in patients with poor hepatic and renal functions, epileptic seizures
and other neurological abnormalities, early pregnancy especially in the first trimes-
ter, and those who show a hypersensitivity reaction [52,56,57].

4.2.2 Diethylcarbamazine (DEC, 3)

DEC has been an important drug for filariasis in humans for almost five dec-
ades. The antifilarial profile of DEC against different forms of filariasis in human is
described below:

(i) Lymphatic filariasis: DEC is used to treat lymphatic filariasis due to *W. bancrofti*
and *B. malayi* infections at a dose of 2-3 mg base or 4-6 mg citrate/kg given thrice
daily for 2-3 weeks, preferably after meals. This dose schedule is reported to kill
both the microfilariae and adult worms of *W. bancrofti* and *B. malayi*. If required, a
second course using 2-3 mg/kg (base) of the drug given thrice daily for 10-21 days
may be repeated [7,9,65-68]. Another dose schedule recommended for adult patients
is: day 1, 50 mg oral; day 2, 50 mg, t.i.d.; day 3, 100 mg, t.i.d.; day 4-21, 2 mg/kg,
t.i.d. [55]. However, in Korea, a low dose of 1 mg/kg of DEC citrate given orally for
36 days was found effective for treating *B.malayi* in man. This dose schedule exhib-
ited antifilarial effect comparable to the conventional doses (6 mg/kg daily for 6
days) [69]. Hii and coworkers [70] believe that a dose of 6 mg/kg of DEC citrate
given for 6 days may provide good results against *B. malayi* in man. The drug has
also been used in the prophylaxis of bancroftian filariasis in endemic areas [71-74].

In a comparative trial using DEC and ivermectin, it was observed that DEC,
at a dose of 6 mg/kg given daily for 12 days, caused more damage to adult worms
of *W. bancrofti* with higher reduction in microfilarial density than ivermectin [75].
There had been conflicting reports regarding the macrofilaricidal action of DEC
against *W.bancrofti* and *B. malayi*. This matter has been reviewed by Ottesen [76,77],
who concludes that a full course of treatment with DEC has definite macrofilaricidal
activity.

(ii) DEC in mass therapy: DEC has also been used extensively in the mass therapy
of lymphatic filariasis. Usually a dose of 5 mg/kg of the drug is given once a week

for 5 weeks [78]. Jain and coworkers [78] have found a dose of 9 mg/kg body weight of DEC may be a convenient schedule for mass treatment of *W. bancrofti* infection in man. The drug was given to a large section of the population living in French Oceania, whole of the South Pacific, Malayasia, Tahiti, Ceylon, Brazil, Japan and other parts of the tropics for controlling and preventing bancroftian and malayan filariasis. As a result the microfilarial rate fell down considerably in most of the population undergone DEC treatment [7,70,80]. For example, in East Pahang, mass treatment with DEC caused a fall in *B. malayi* infection from 36% in 1957 to 3% in 1959, which rose to 6% in 1966 [78]. Similarly in American Samoa, the microfilarial rate was 21%. Using community treatment programmes, the rate of infection came down to 3.1% in 1965 and 0.36% in 1967 [81].

Although treatment of the rural population with DEC has been beneficial in controlling lymphatic filariasis in different parts of the tropics, its greater use is limited by logistic difficulties of delivery of health services. To circumvent these problems, it has been suggested to use DEC medicated salt. Medicated salt with 0.1-0.5% DEC has been successfully used in China, Taiwan, Malaya, Brazil, East Africa, India, and some other regions of the tropics to control lymphatic filariasis as also to prevent the population from reinfection [7,78,82-87]. There was a marked reduction in mean microfilarial density. As so far no resistance to DEC has been reported, DEC medicated salt may prove to be a cheap and effective method to control filariasis in endemic areas [7].

(iii) **River blindness:** DEC is the second line of drug for treating *O.volvulus* infection in man. The drug rapidly kills the microfilariae, but has no action on the adult worms. The main problem with DEC treatment is the occurence of a violent Mazzotti reaction as a result of rapid destruction of the microfilariae of *O. volvulus*. Nevertheless, DEC has been used to treat human onchocerciasis with varying success [88-91].

The recommended adult dose of DEC citrate is 50 mg on day 1, 100 mg x 2 on day 2 and thereafter 150-200 mg twice daily for 5-10 days [7,88,92]. Another dose schedule for treating *O.volvulus* infection is 25 mg/day given for 3 days, followed by 50 mg/day for 5 days, 100 mg/day for 3 days and finally 150 mg/day for 12 days. Children receive the drug as follows: 0.5 mg/kg thrice daily for 3 days (max. 25mg/day), then 1 mg/kg, thrice daily for 3-4 days (max. 50 mg/day), then 1.5 mg/kg, thrice daily for 3-4 days (max. 100 mg/day) and finally 2 mg/kg thrice in a day for 14-21 days [55]. This treatment is followed by administration of suramin.

(iv) Topical formulations of DEC: Since river blindness is caused by migration of microfilariae of *O. volvulus* in eye ball, local application of DEC to eyes was first suggested by Lazar *et al.* [93,94]. Later various topical formulations of DEC were used to treat ocular and cutaneous onchocerciasis with varying degree of success. Thus administration of 2 drops of a 3-5% solution of DEC in eyes four times a day for 9-14 days caused marked reduction in microfilarial counts in the eye chamber [95]. Although the treatment was well tolerated, the patient suffered from moderate edema of the eye lids and slight congestion of the conjunctiva. In cases with heavy infections, severe anterior uveitis was observed. Further, the microfilariae soon reappeared after the treatment was stopped. Based on these observations, it was concluded that in patients with a heavy worm load, local application of DEC to eyes was not beneficial. The suppression of microfilarial counts in the eye chamber is temporary and may require attention of an ophthalmologist. Due to these limitations, the topical use of DEC in the mass treatment of ocular onchocerciasis is not recommended [7].

To circumvent the above limitations, Anderson and co-workers [96] suggested the following topical treatment of ocular onchocerciasis: first a low concentration (about 0.2%, 1 drop thrice daily) should be employed for preliminary reduction of microfilariae from periorbital tissues; then a high concentration (about 3%) may be given to eliminate the filariids from the eyeball and finally an intermediate concentration should be instilled to keep the microfilariae away from eyes.

The microfilariae of *O. volvulus* present below the dermis may be eliminated to a great extend by topical application of DEC. Thus an oil-water emulsion containing 2% of DEC was applied on the whole body of patients with *O. volvulus* infection for 6-21 days. There was a high fall of microfilarial counts in the skin after 6 days and itching and pruritus subsided slowly. Later batches of 27 and 93 patients were treated by local application of a lotion containing 1-2% of DEC applied for 7 days [97].

(v) Loasis: DEC is the drug of choice for treating *L. loa* infection in man as it kills both the microfilariae and adult worms [98]. The recommended dose of the drug is 2-5 mg/kg given orally for 2-3 weeks [67,99]. Although the drug is well tolerated care should be taken while treating patients with heavy infections as DEC can provoke ocular problems or meningoencephalitis [55,67].

DEC has also been used in the mass therapy of loasis in Nigeria. The infected persons were given 200 mg of DEC citrate, three times daily for 20 days when microfilarial levels dropped to 2-12% of original counts [100]. The drug may be used as a

chemoprophylactic against filariasis at a dose of 5 mg/kg given twice daily for 3 days [101].

(vi) Adverse effects of DEC: DEC has very low toxicity and its oral LD_{50} in rats is 1380 mg/kg. However, some undesirable side effects may be seen, which are not severe and usually disappear after termination of the therapy. The drug possesses irritant effects on GI mucosa, which leads to nausea and vomiting. Other common side effects of DEC are headache, malaise, lassitude, weakness and joint pains [7,78].

In case of *W. bancrofti* and *B. malayi* infections, chills and fever are the most common side effects which occur as a result of allergic and febrile reactions due to rapid death of microfilariae. The most prominent side effects of DEC observed during treatment of *O. volvulus* is a violent Mazzotti reaction appearing within a few hours after the first dose. The Mazzotti reaction is so specific that it is often used as a diagnostic test for onchocerciasis (administer 50 mg of DEC and observe the pruritic skin within 24 hours). It consists of swelling and edema of the skin around the thighs, buttocks and genitals, intense pruritis, high fever, headache, tachycardia, hypotension, nausea, respiratory distress and tenderness of the inguinal lymph nodes. The severity of the side reactions of DEC are usually proportional to the worm load. Occasionally, irreversible damage of eyes and loss of vision may occur as a result of globule-like lesions appearing in the cornea and limbal area or microhaemorrage of blood vessels in the eyes [7,78].

In case of loasis, the drug may provoke ocular problems and meningoencephalitis, especially in patients with heavy infections [55,99,102]. The occurrence of encephalitis due to the presence of microfilariae of *L.loa* in the central nervous system and cerebrospinal fluid may be fatal. The patient first falls in coma and then dies. A few cases of collapse and death have also been reported during treatment of *W. bancrofti* or *O. volvulus* infections [7]. The use of DEC should be avoided during pregnancy and in patients with renal and cardiac diseases [65].

4.2.3 Centperazine (27)

It has been found to eliminate more than 90% of the microfilariae from the blood circulation of cotton rats infected with *L.carinii* at an intraperitoneal dose of 1 mg/kg given for 6 days [33,34]. Centperazine also shows high microfilaricidal activity against *B. malayi* in gerbils [103]. The drug is well tolerated and possesses high efficacy against *W. bancrofti* in man at an oral dose of 50 mg given thrice daily for 2-3 weeks [104,105].

5. MODE OF ACTION OF PIPERAZINE ANTHELMINTICS

5.1 Piperazine (4)

It is one of the most widely used drugs for treating *A.lumbricoides* and *E.vermicularis* infections in man. Piperazine is known to produce flaccid paralysis of *Ascaris* by blocking the response of the worm to acetylcholine. This anticholinergic action results in neuromuscular blocking at the myoneural junction of the worm. The drug is also responsible for a low production of succinic acid in the nematodes. The paralysed worms are unable to hold their position in the gastrointestinal tract against the peristaltic movement and, therefore, are passed out with the faecal stream [106-108].

It has been suggested that paralysis of *Ascaris* worms is due to the change in the resting potential of muscle cells from -30 to -45 mV caused by piperazine. This change in muscle potential leads to hyperpolarisation resulting in muscular contraction and subsequent paralysis of the worm [109-108]. The hyperpolarising effect of piperazine may also be due to its ability to increase the permeability of the muscle membrane for chloride ions [109-11]. Thus piperazine may act as a weak GABA agonist on extra synaptic GABA receptors located in *Ascaris* muscles.

5.2 Diethylcarbamazine, DEC (3)

The mode of action of DEC has been reviewed [108,112]. Following the discovery of DEC, it was shown by Hawking *et al.* [91] that treatment of cotton rats infected with *L.carinii* with DEC caused disappearance of more than 80% of the microfilariae from blood within 2-3 minutes. These disappeared microfilariae were seen collected in the liver. It was also observed that microfilariae were surrounded by phagocytes and later they were found to be disintegrated. This study suggested that DEC probably acts by "unmasking" or "exposing" the microfilariae in such a way that they are recognised by the host as foreign bodies and destroyed by the immune system of the liver [114,115]. This sensitization of microfilariae caused by DEC resulting in attack by the immune system of the liver may be suppressed by R.E. blockers such as ethyl palmitate [116]. Recently Cesbron *et al.* [117] have shown that the action of DEC is mediated by blood platelets and additional triggering of a filarial excretory antigen (FEA). The action of the drug is antibody dependent. This is supported by the earlier work of Schardein *et al.* [118], who showed that DEC causes removal of the sheath from microfilariae of *L.carinii*. The unsheathed or "naked" microfilariae are then amenable to phagocytosis by Kupffer cells and neutrophils. This has been confirmed by microscopic studies by Gibson *et al.* [114]. These authors showed that the cuticle of

the microfilariae of *O.volvulus*, isolated from patients, was changed after treatment with DEC.

DEC is known to produce morphological changes on the surface of microfilariae. However, this change is not enough to cause death of the parasite [112]. It has also been shown that DEC enhances the specific IgG-mediated cell adherance on *B.malayi* microfilariae, which may be achieved by exposing the "hidden" antigenic determinant sites [119]. Martinez-Paloma [120] have shown that the immunogenic determinants of microfilariae of *Onchocerca* sp. are covered by an acellular cuticle.

DEC also possesses action on the neuromuscular system of the parasites. Thus the killing of the microfilariae of *O.volvulus* by DEC may be a sequel of neuromuscular inhibition and "host reaction" against the parasite [121]. Recently Agrawal *et al.* [122] have shown that DEC is a potent inhibitor of ATPase of *S. cervi in vitro*. Liu and Weller [123] believe that the action of DEC may be due to the ability of the drug to inhibit arachidonic acid biosynthesis in the filariids.

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CHAPTER 6

TETRAHYDROPYRIMIDINES

1.   INTRODUCTION

During the mid 1950s, Pfizer Laboratories developed suitable animal screen-
ing models for human helminth infections such as a triple infection with *Nippos-
trongylus muris, Nematospiroides dubius* and *Hymenolepis nana* in mice [1]. Screening
compounds in these models resulted in identifying 2-(2-thienylmethylthio)imida-
zoline hydrochloride (3), prepared by condensing 2-chloromethylthiophene (1) with
imidazolidine-2-thione (2), exhibiting antiwhipworm activity in mice [2]. However,
when given orally to sheep, 3 was found to have no activity. This was found to be
due to high susceptibility of imidazolines towards hydrolysis forming 2-mercap-
tomethylthiophene (4) and imidazolidin-2-one (5) (Scheme 1) [1,3].
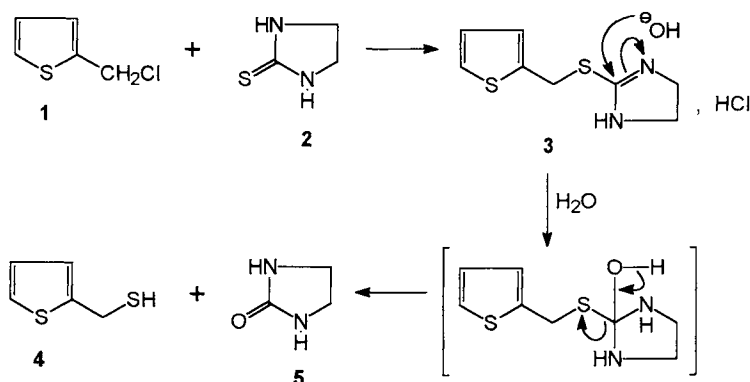
Scheme 1

The structure of 3, however, provided a useful lead to follow. This led to the
synthesis of 2-[2-(2-thienyl)ethyl]-2-imidazoline (8) having activity against different
roundworms in mice and sheep [1]. Compound 8 was synthesized by the reaction
sequence shown in scheme 2. Soon a series of compounds of type 9 [4-6] were pre-
pared of which pyrantel (10a), morantel (10b) and oxantel (11) showed high an-
thelmintic activity.

Scheme 2

Ar = Thienyl, Furanyl, Phenyl etc.
X = Alkyls, Alkenes
R = Alkyl
n = 2, 3

a R = H
b R = Me
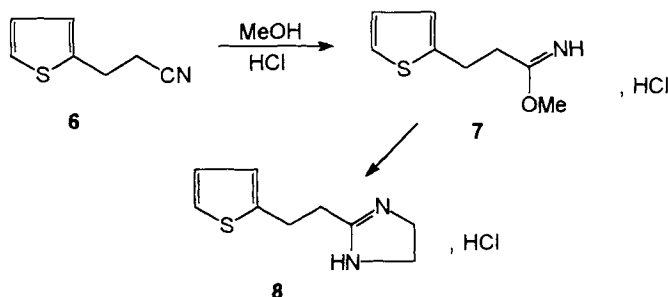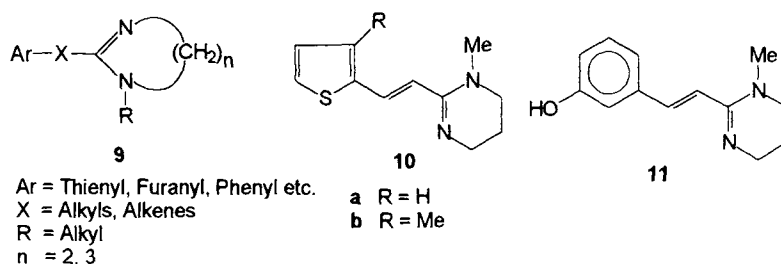
2. SAR IN TETRAHYDROPYRIMIDINES

Following the discovery of pyrantel, a systematic SAR in compounds of type
9 was carried out to determine the effect of various structural changes on biological
activity [5]. It was observed that optimal anthelmintic activity in 9 was obtained
when Ar was 2-thienyl; the activity fell in the order: 2-thienyl >3-thienyl >phenyl >2-
furyl. Similarly, the presence of a tetrahydropyrimidine ring (n=3) was found to con-
fer better activity as compared to the imidazoline ring (n=2). The presence of a
methyl group (R=Me) in tetrahydropyrimidine was found essential for evoking high
anthelmintic response. It was also found that replacement of R by H reduces the ac-
tivity, while introduction of groups larger than methyl (eg. ethyl, propyl etc.) caused
loss of activity. The nature of the linkage (X) joining the 2-thienyl and tetrahy-
dropyrimidine rings in 9 also appeared to play an important role in governing the
activity. Usually a two carbon chain (X=C-C) was responsible for high activity with
the following decreasing order of potency: *trans*-vinylene > ethylene > *cis*-vinylene
[1,3]. It has also been observed that in the aromatic ring (Ar = 2-thienyl), the pres-
ence of substituents on other positions of the ring, except ortho to linkage, caused
loss of activity; morantel (10b), having a methyl group at 3-position (*'ortho'* to
vinylene) exhibited a high order of activity.

In order to establish quantitative structure-activity correlates, the Hansch analysis of compounds represented by structures 12 and 13, was carried out. This resulted in a structure-activity correlation given by the equation below [7].
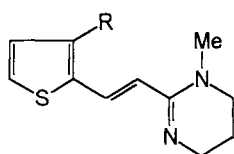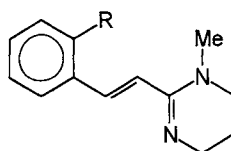
12 R=H, Me, Et, Br   13 R=H, Cl, Br, F, Me, Et, NO_2

$$\log 1/ED_{90} = -1.64\pi^2 + 1.93\pi + 0.66\delta + 0.88 \qquad \text{(Eq.)}$$

$n = 12, \quad r^2 = 0.962, \quad s = 0.117, \quad F_{3,8} = 84.7, \quad p =< 0.0005$

Thus, using the above equation it was possible to account for 96% of the variance in biological activity. This could be done by measuring lipophilicity (π) and by knowing if the particular compound is represented by formula 12 (2-thienyl group; s=1.00) or by 13 (phenyl group; s=0.00) [3].

In addition to the compounds represented by formula 9, 12 or 13, a number of non-cyclic amidines (14), dihydrothiazines and thiazolines (15-17) and 1-(2-arylvinyl) pyridinium salts (18,19) have also been synthesized, many of which exhibit marked anthelmintic activity [8,11]. The SAR in many of these series was found to be in close concordance with the tetrahydropyrimidines.
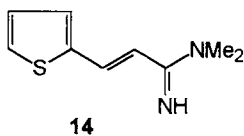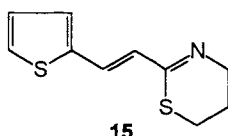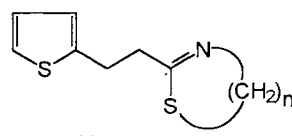
14   15   16
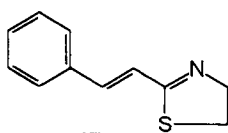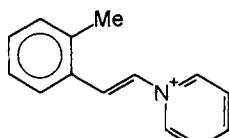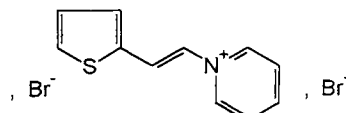
17   18   19

3. SYNTHESIS OF CANDIDATE TETRAHYDROPYRIMIDINE DRUGS

The key intermediate for preparing pyrantel (10a), morantel (10b) and oxantel (11) is 1,4,5,6-tetrahydro-1,2-dimethylpyrimidine (23, R=Me) which may be prepared

starting from acrylonitrile (20). Addition of an alkyl amine to acrylonitrile affords 2-alkylaminopropionitrile (21). Acetylation of the latter with acetic anhydride followed by hydrogenation of the resulting acetyl derivative (22) in the presence of Raney nickel catalyst and alcoholic ammonia yields the 1,4,5,6-tetrahydro-2-methylpyrimid-ines (23) [12]. In an alternate method 23 can be prepared from 2-arylacrylonitrile (24), which is easily obtained by condensation of an aryl aldehyde with cyanoacetic acid followed by decarboxylation. Treatment of 24 with ethanol in the presence of dry HCl yields ethyl-2-arylacrylimidate hydrochloride (25), which is condensed with N-methyl-1,3-diaminopropane to afford 10a,b and 11 [13-17].
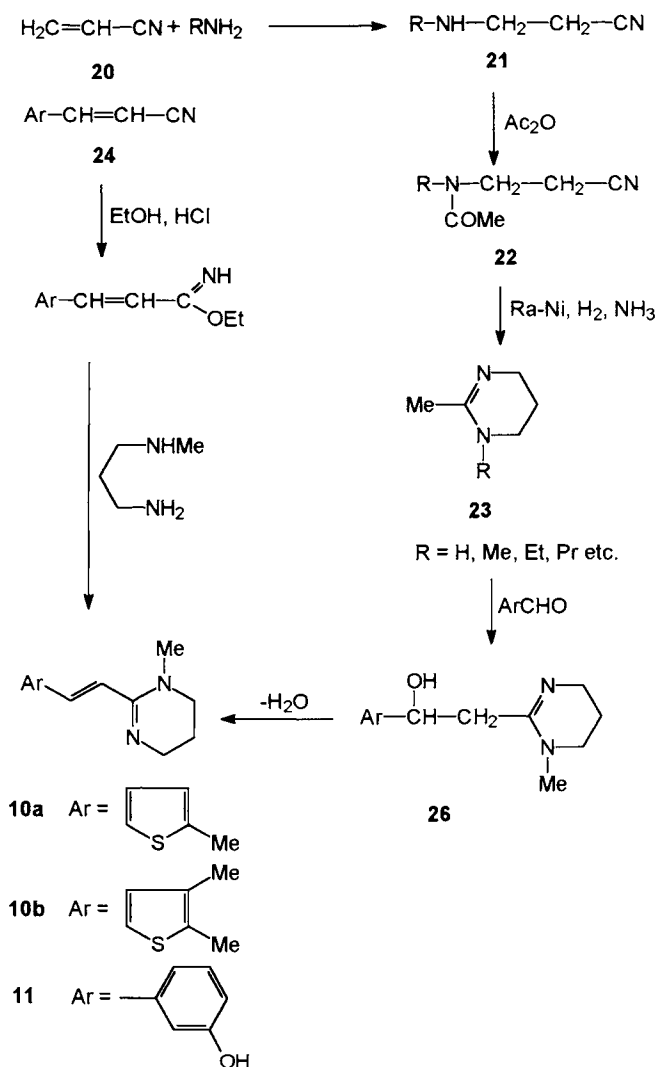
Scheme 3

A simpler large scale method to obtain pyrantel, morantel (**10a,b**) and oxantel (**11**) involves condensation of **23** with an aryl aldehyde in presence of a base. Water is removed by azeotropic distillation or by using a chemical scavenger like methyl/ethyl formate, which reacts with water to push the reaction in the forward direction (Scheme 3). Other methods to prepare pyrantel and its derivatives are also reported [5,6,13-17]. The 1-(2-arylvinyl)pyridium salts (**18,19**), structural congeners of pyrantel/moratel, are prepared by quaternisation of pyridine with the appropriate bromomethylaryl ketones (**27**) to afford **28**. The latter is reduced with sodium borohydride to give the carbinol **29**, which on dehydration leads to the formation of 1-(2-arylvinyl)pyridinium bromides (**18,19**) [8].
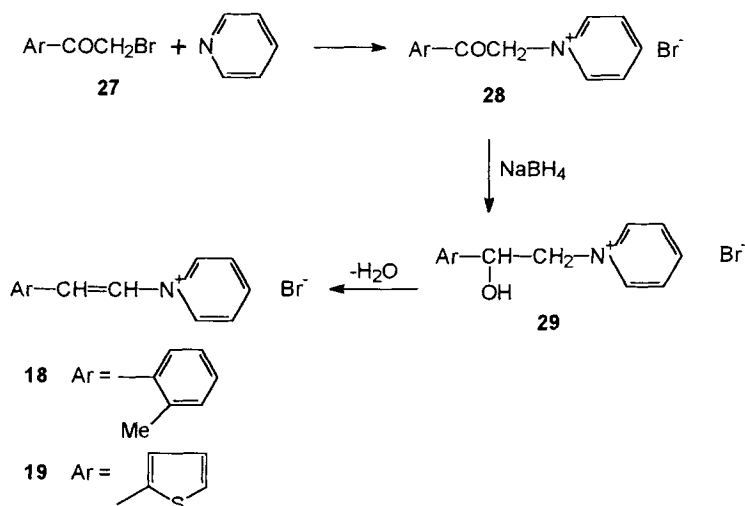
4. BIOLOGICAL ACTIVITY

Of a number of tetrahydropyrimidines synthesized as possible anthelmintic agents, three drugs namely pyrantel (**10a**), morantel (**10b**) and oxantel (**11**) emerged as effective agents for treating intestinal nematode infections in humans and domestic animals [18-25].

4.1 Tetrahydropyrimidines in veterinary medicine

4.1.1 Pyrantel (10a)

It is an effective drug for treating intestinal roundworm infections in cattle, sheep, horses and pigs. The drug is usually given orally as tartrate or pamoate (embonate) salts. Generally, it shows high activity against the adult nematodes of the

gut, but possesses less efficacy against developing forms and poor activity against arrested larvae [18,20].

The recommended dose of pyrantel tartrate for eliminating major intestinal nematodes from cattle and sheep is 12.5-25 mg/kg. In horses, pyrantel pamoate has been found to be highly effective against adult worms of S. vulgaris, P. equorum and small strongyles. It also exhibits good activity against S. edentatus and O. equi. However, the drug is inactive against T. axei, S. westeri, Habronema spp. and D. megastoma. The recommended dose of pyrantel pamoate for horses is 19 mg/kg equivalent to 6.6 mg/kg free base [18,19]. Pyrantel tartrate has been found to prevent the migration and establishment of S. vulgaris infection in equines when given in feed during grazing seasons at a dose of 2.64 mg/kg body weight [26].

Pyrantel tartrate also possesses high efficacy against the adult stages of A. suum, H. rubidus and Oesophagostomum spp. in pigs at a single oral dose of 12.5 mg/kg. However, it has no activity against T. suis and S. ransomi. Nevertheless, pyrantel has been used as a prophylactic agent against ascarids in young pigs [18,19].

The antinematodal activity of pyrantel pamoate has also been established in cats (dose: 20-30 mg/kg) and dogs (dose: 15 mg/kg). The drug has been used to eliminate adult ascarids and hookworms [19]. Recently Ridley and coworkers [27] have found that a dose of 20 mg (base)/kg of pyrantel caused 97-100% elimination of the ascarids (T. cati) and hookworms (A. tubaeformae) in cats.

4.1.2 Morantel (10b)

The activity profile of this drug is almost similar to that of pyrantel except its lower therapeutic dose and higher LD_{50} values. The recommended dose of morantel tartrate for treating gastrointestinal nematode infections in cattle and sheep is 10 mg/kg [18,19,28]. Like pyrantel, the activity of morantel against adult gut nematodes is highest (>90%), less against developing forms (75-90%) and minimal (<50%) against arrested larvae.

Morantel has also been found to be highly effective against immature and mature forms of A. suum in pigs at a dose of 5 mg/kg. In addition, the drug eliminates adult worms of Oesophagostomum and Hyostrongylus spp. [18,19]. The earlier work describing the usefulness of morantel in the treatment/prophylaxis of gastrointestinal nematodes in cattle, sheep, horses and dogs has been reviewed by McFarland [3].

4.1.3 Oxantel (11)

Oxantel tartrate has been found to be effective against *Trichuris suis*, but has little activity against other intestinal nematodes in pigs [29]. However, a combination of pyrantel and oxantel exhibits high activity against ascarids, hookworms and whipworms in cats and dogs at a dose of 100 mg/animal. This drug combination is safe, even in young puppies and pregnant or lactating bitches [19]. In dogs, the hydrochloride salt shows better activity than the pamoate salt [30].

4.2 Tetrahydropyrimidines in human medicine

4.2.1 Pyrantel pamoate (10a)

This is a highly effective drug for treating ascariasis, enterobiasis and hookworm infections in humans. Usually cure rates ranging from 76-100% are achieved against *A. lumbricoides, N. americanus, A. duodenale* and *E. vermicularis* at a single oral dose of 10-20 mg/kg (maximum 1g/patient) [3,21,31]. Recently, Dotsenko et al. [32] have evaluated pyrantel pamoate in 50 patients with intestinal nematodiasis. These workers achieved 100% cure against ascariasis (dose: 5 mg/kg for 1 day), 94.4% cure against enterobiasis (dose: 10 mg/kg for 1 day) and 95.8% cure against hookworms (dose:20 mg/kg for 2 days).

Pyrantel pamoate is a safe drug with no serious side effects. However, a small percentage of treated patients may experience gastrointestinal upset (nausea, vomiting, diarrhea, abdominal pain), headache and dizziness. The drug is not recommended for children below one year and during pregnancy. Care should also be taken while treating patients with impaired liver function [18,21].

4.2.2 Oxantel pamoate (11)

This drug is particularly useful in treating mild to severe infections of the whipworms, *Trichuris trichiura*. Usually a single oral dose equivalent to 10-20 mg/kg, base (2.8 g pamoate salt is equal to 1 g base) is required; the treatment may be repeated after 10-14 days, if necessary. For heavy infections, the drug may be administered once or twice daily for upto 5 days [21]. The cure rates of 53-98% with 90-100% reduction in faecal egg counts have been achieved with a single oral dose of 10-25 mg/kg against *T. trichiura* in humans [25]. Lee *et al.* [33] have used a single oral dose of 10 mg/kg of oxantel for treating light infections of *T. trichiura*, while the same dose was given for 3 days to cure severe infection. In children, a dose of 10 mg/kg given twice a day produced satisfactory response in about 70% of the treated cases. A second treatment produced satisfactory response in remaining 30% cases affected by *T.*

trichiura [30]. Oxantel is an extremely well tolerated drug with no severe toxic manifestations.

Oxantel may also be used to treat ascariasis, enterobiasis, hookworm infections and trichuriasis if given in combination with pyrantel pamoate or levamisole [18,23]. Thus, a combination of pyrantel pamoate (5 mg/kg) and oxantel pamoate (5 mg/kg) given on two consecutive days produced 94% reduction in egg output in a large number of Chinese patients infected with *Ascaris*, whipworms and hookworms [34]. Like pyrantel pamoate, oxantel is also well tolerated producing only mild and transient side effects [18].

5.   MODE OF ACTION

Pyrantel, morantel and oxantel exert their anthelmintic action by paralysing the nematodes [35,36]. This paralytic effect is due to the ability of these drugs to cause a depolarising type neuromuscular block [37]. It has also been shown that pyrantel and its structural congeners are nearly 100 times more potent than acetylcholine in inducing a contraction of *Ascaris* muscles. The only difference between acetylcholine and pyrantel is that the former causes rapid contraction, which is reversible, while the effect of the latter is slow and difficult to reverse [37]. Thus, pyrantel and morantel are potent cholinergic agonists like bephenium and thenium [38,39]. The contraction of muscle strips may be inhibited by cholinergic blocking agents such as piperazine and (+)-tubocurarine [40].

Although the mode of action of morantel is believed to be similar to that of pyrantel, it has been shown that this drug may also interfere with the glucose metabolism of the worms. This is because of the fact that the fumarate-reductase system of *Haemonchus contortus* has been found to be sensitive to morantel tartrate at a high concentration of 2.5×10^{-3} M [41].

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CHAPTER 7

IMIDAZOTHIAZOLES

1. INTRODUCTION

During the search for a novel lead to design broad-spectrum anthelmintics, scientists at the Janssen Pharmaceutica, Belgium, discovered the high nematodicidal activity of the thiazole R-6438 (1) in chicken. Surprisingly, R-6438 was not active in mice and rats, but exhibited high activity against nematodes in sheep. Later it was found that R-6438 was in fact a prodrug, which metabolises in chicken to form R-8141 (2), which is the active metabolite. This compound proved to be a broad-spectrum anthelmintic with a high safety margin and solubility. However, R-8141 was unsuitable for drug development due to its high cost of production and instability in water [1].
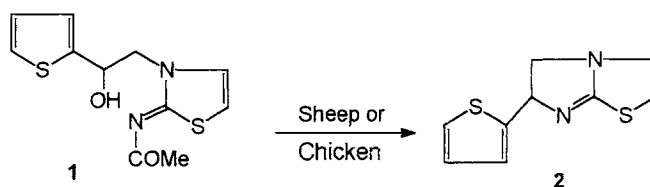
Although compound 2 did not prove to be an ideal drug, it provided a much needed lead to generate better anthelmintics. Accordingly, the Janssen team set out to optimize the activity of this class through a systematic SAR of the imidazothiazoles. Removal of the double bond of the thiazole ring and replacement of the thiophene ring with a phenyl group, yielded DL-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole (3, R-8299), named tetramisole [2], which was cheap and stable, the two limitations of R-8141. Later several 6-arylimidazo[2,1-b]thiazoles were prepared, but none was found to be superior to tetramisole (3) [3], which has been one of the very widely used antihelminths ever since.

Since tetramisole is a racemic mixture, it was resolved into its optical antipodes and their absolute configuration established [4,5]. Most of the anthelmintic activity was found to reside in the laevorotatory isomer called levamisole [6]. The dextro-

rotatory isomer, dexamisole, is practically inactive. In order to achieve maximum turnover of levamisole, the R(+)-isomer (dexamisole) was racemised to the RS-mixture, which can again be resolved into S(-) and R(+)-isomers. In this manner, the inactive isomer can be recycled [4].

S(-)
Levamisole

R(+)
Dexamisole
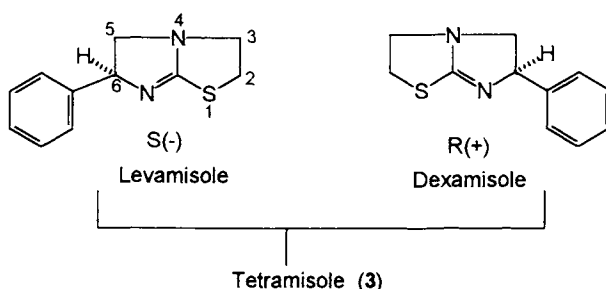
Tetramisole (3)

2. SAR IN IMIDAZOTHIAZOLES

The initial work carried out by Raeymaekers *et al.* [3] was concerned with the synthesis of a number of 6-aryl-2,3,5,6-tetrahydroimidazo[2,1-b]thiazoles (4) of which tetramisole (3) was found to be the most effective. Soon Miller and Bambury [7] pre-

4
Ar = Substituted phenyl, 2-thienyl, 2-furyl

5
R = H, Me

6
R = Alkyl, aryl,
R_1 = H, NO_2

7 R = H, COR_1

8

9

10

11

a R = NO_2
b R = NHCO-*i*Pr
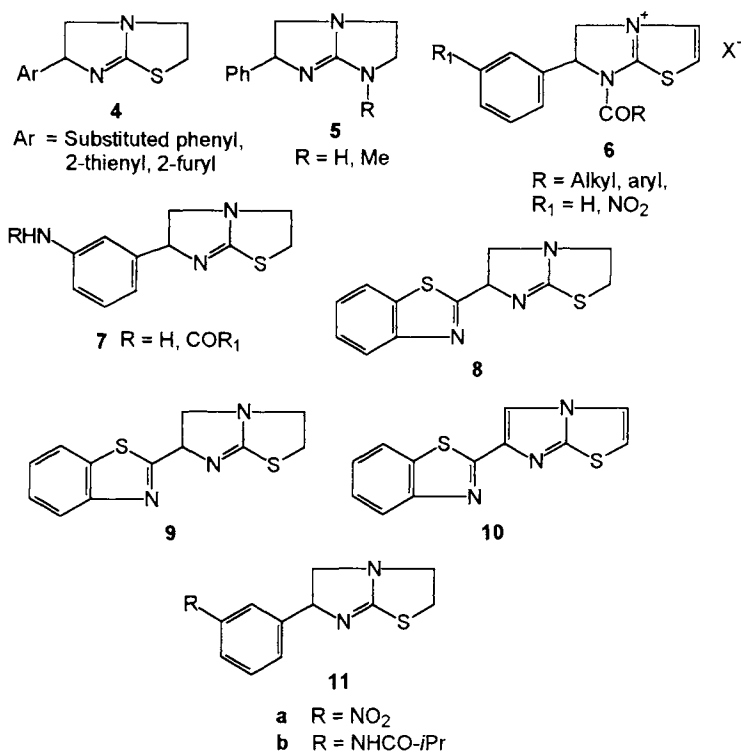
pared 6-phenylimidazo[1,2-a]imidazoles (5) which were found to be inactive against *N. dubius* and *A. lumbricoides* in mice. This study indicated that the imidazothiazole skeleton was crucial for biological activity. Later, Spicer and Hand [8,9] synthesized some imidazothiazolium compounds (6) of which 6 (R=Me, R_1=H) caused 75-99% reduction of *H. contortus* in sheep at an oral dose of 8-10 mg/kg. Some 6-(3-amino/ acylaminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-b]thiazoles (7) have also been prepared and show marked reduction of *N. dubius* in mice at a dose of 4- 12.5 mg/kg [10,11]. In addition few structural analogues (8-10) of tetramisole have been synthesized with no activity against *N. brasiliensis* and *A. ceylanicum* in rodents [12].

The above structural changes of the imidazothiazole skeleton indicate that the presence of a sulphur atom is essential for biological activity. Attempts to introduce further unsaturation in this bicyclic frame-work led to lowering or loss of anthelmintic activity. Presence of an aryl function at 6-position of tetrahydroimidazo[2,1-b]thiazole is also crucial for biological activity. It appears that optimal activity is achieved by introduction of a phenyl or 3-substituted phenyl groups. Consequently, two more anthelmintics, nitramisole (11a) and butamisole (11b) were developed, which exhibit high activity against various gastrointestinal nematodes in domestic animals [13].

3. SYNTHESIS OF TETRAMISOLE AND LEVAMISOLE

3.1 Tetramisole (3)

The initial synthesis of tetramisole (3) involves condensation of 2-aminothiazoline (12) [14] with phenacyl bromide (13) [15] to afford 3-substituted-2-iminothiazoline (14).This product is then converted into tetramisole by the reaction sequences shown in scheme 1 [3,16,17].

Scheme 1

184
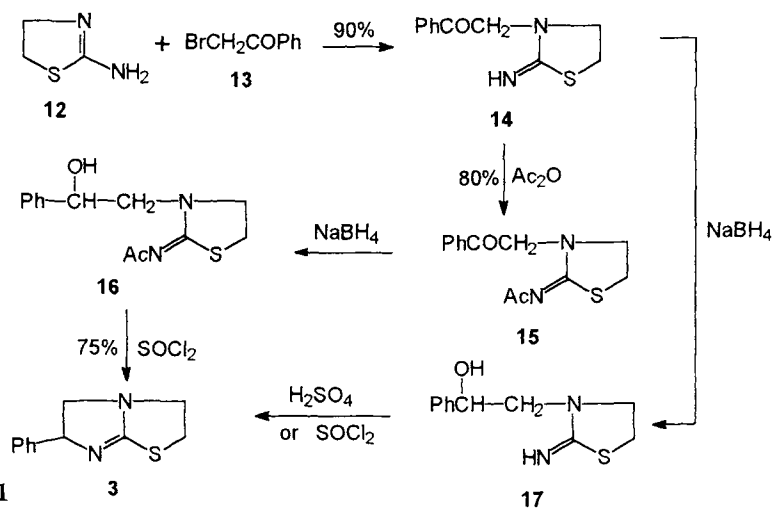
Another method to synthesize tetramizole starts with the reaction of styrene oxide (18) with aziridine to form N-(2-hydroxy-2-phenylethyl)aziridine (19) which is converted into the desired product as shown in scheme 2 [18,19].
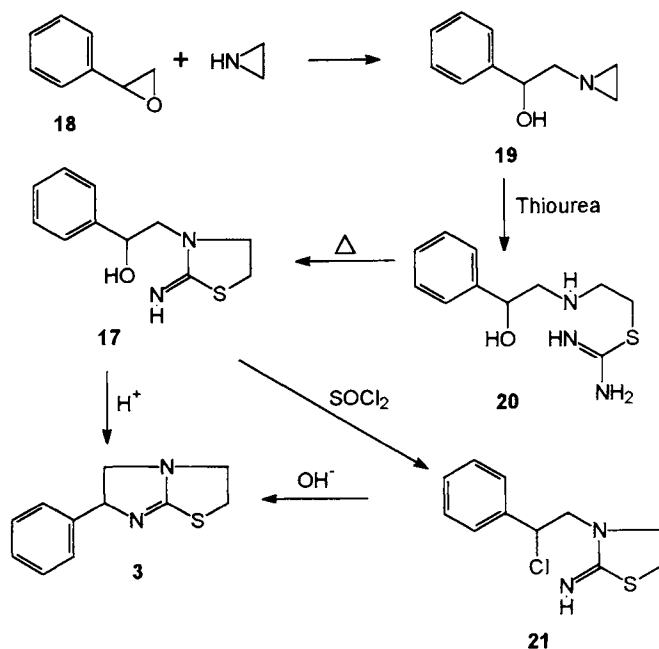
Scheme 2

The ICI laboratories have developed a different approach to synthesize tetramisole, which is outlined in scheme 3 [20,21].

with a minor quantity of
the other regioisomer.
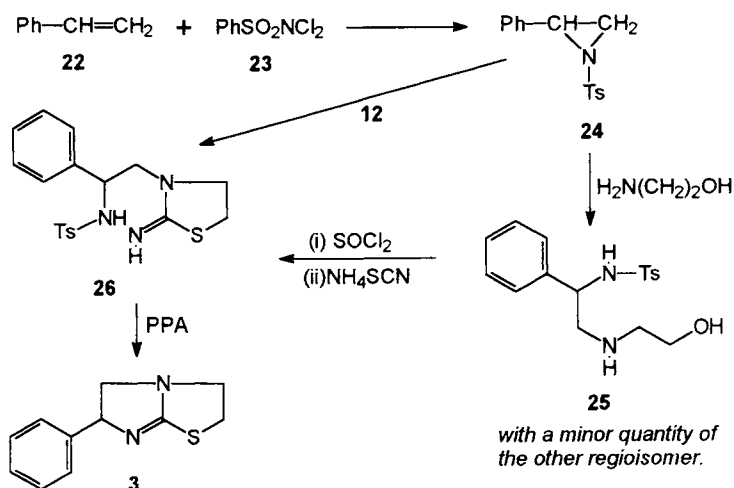
Scheme 3

Tetramisole may also be prepared by cyclisation of thiazoline derivatives (28-30) under different conditions (Scheme 4) [22,23].
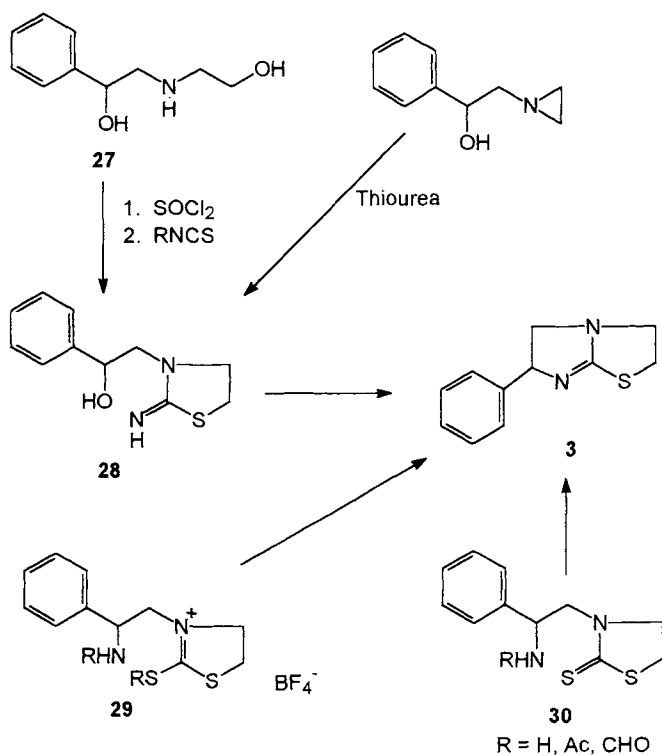
Scheme 4

Another method to prepare tetramisole involves reaction of 2-bromo-1-phenylethylamine (31) with vinyl isothiocyanate in methylene chloride at temperatures below 5°C, followed by treatment with mercuric acetate to form tetramisole (3) [24] (Scheme 5).
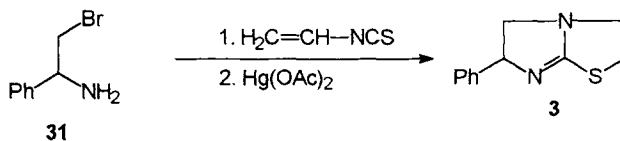
Scheme 5

A few other methods that have been developed to obtain tetramisole are summarised in scheme 6 [25].
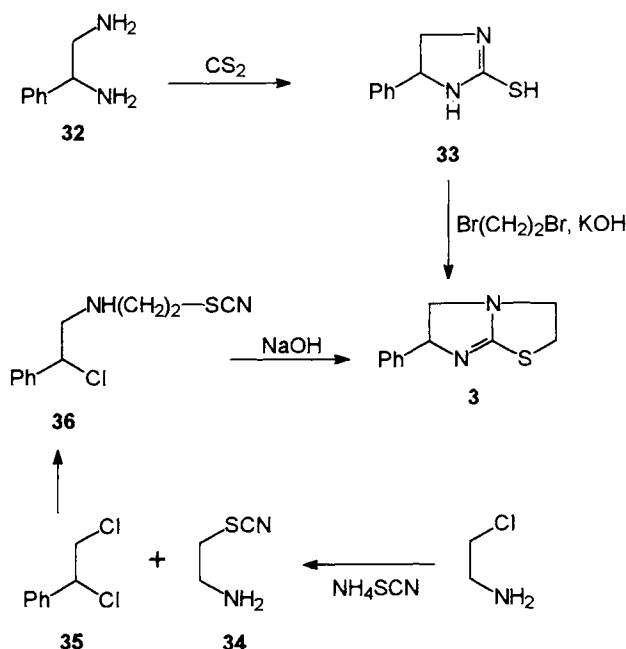
Scheme 6

3.2 Resolution of tetramisole

The resolution of tetramisole into levamisole, L-(-) $[\alpha]_D^{25}$ = -54.7 (H$_2$O), and D-(+) $[\alpha]_D^{25}$ = + 83.0 (H$_2$O) enantiomers has been achieved using d-10-camphorsulphonic acid [4]. The absolute configuration of the isomers was established by carrying out their stereospecific synthesis starting from D(+) and L(-)-1-phenylethylenediamines (32) as shown in scheme 6 [5].

3.3 Chiral synthesis of tetramisole

Several methods to prepare S(-)-tetramisole (levamisole) have been developed. Raghu *et al.* [26-28] have prepared optically active imidazolidinones (39), which were cyclised under different conditions to form levamisole though with rather poor enantiomeric excess (ee = 21-33%) (Scheme 7).

Levamisole has also been prepared by synthesizing optically active N-(2-hydroxy-2-phenylethyl)aziridine (19) which is heated with thiourea in aqueous sulphuric acid at 100°C for 3 hours to give the title compound. Compound (R)-19, in turn, was obtained by reaction of (R)-styrene oxide with aziridine (*cf.* Scheme 2) [29].
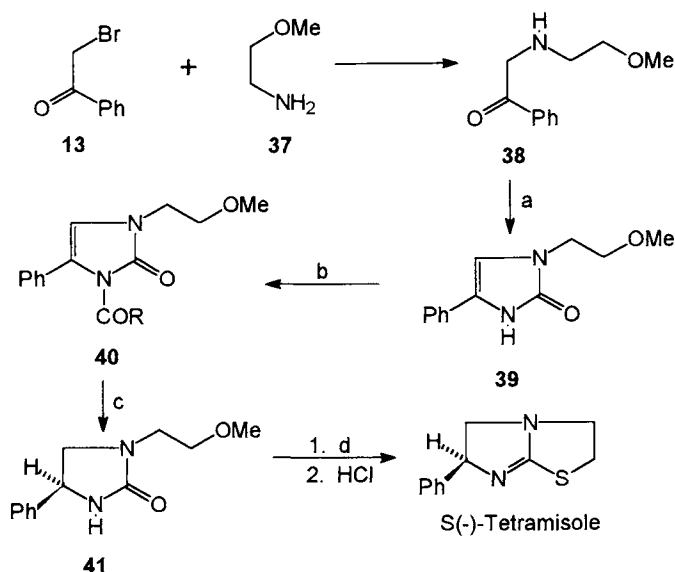
Scheme 7

Reagents: (a) KOCN; (b) RCOCl; (c) hydrogenation in presence of cyclooctadiene-RhCl$_3$ dimer and (+)-2,3-O-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)-butane or (+)-DIOP-Rh (cyclooctadienyl)chloride as catalysts; (d), (i) P$_2$S$_5$, (ii) HCl.

Rama Rao *et al.* [30] have recently reported a chiral synthesis of S(-)-tetramisole starting from the optically active alcohol (44), which was prepared with 97% ee by reducing phenacyl chloride (42) with oxazaborolidines (43) in the presence of BH$_3$. Further reactions leading to S(-)-tetramisole are shown in scheme 8.
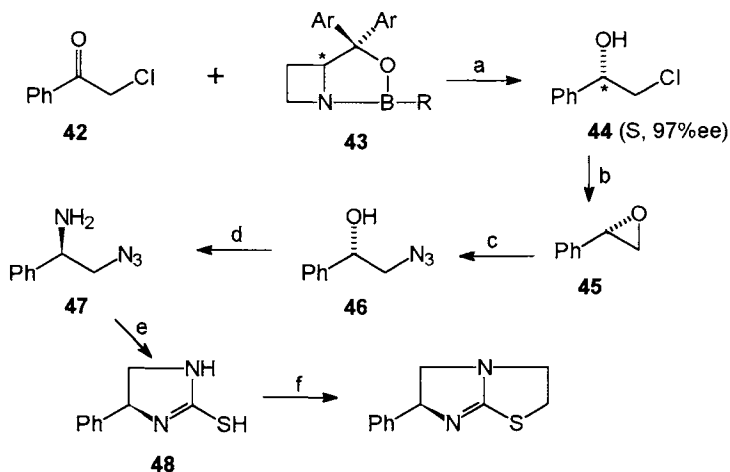
Scheme 8

Reagents: (a), BH$_3$; (b), 2M NaOH-MeOH; (c), NaN$_3$, DMF; (d), (i) DEAD, TPP, (ii) N$_2$H$_4$; (e), (i) Pd-C, H$_2$, (ii) CS$_2$KOH; (f), as in ref. 3 and 5.

4. BIOLOGICAL ACTIVITY

Tetramisole and one of its enantiomers, levamisole, have been used widely to treat intestinal and tissue-dwelling helminths in humans and animals [31-40]. The only other structural congeners of tetramisole, which have found some use against intestinal nematode infections in animals are nitramisole and butamisole (11a,11b) [13,41-44].

4.1 Imidazothiazoles in veterinary medicine

4.1.1 Tetramisole

The initial work carried out by the Janssen group established tetramisole as an effective drug for eliminating immature and adult gastrointestinal nematodes from mice, rats, cats, ducks, pheasants, pigeons, chicks, sheep, cattle, pigs, horses, tigers and monkeys at an oral or parenteral dose of 2.5-40 mg/kg [2]. At a dose of 5-20 mg/kg, given orally, tetramisole was found to be effective against a number of mature and immature gastrointestinal nematodes and the lungworms, *D. viviparus* in sheep and cattle. In dogs, the drug exhibited high activity against *Toxascaris*, *Toxocara* and *Uncinaria* spp. at an oral dose of 20 mg/kg. A dose of 40 mg/kg, administered orally or by injection, was required to remove immature and mature worms of *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria obsignata* in chicks. In all the activity of tetramisole against 56 species of nematodes in 13 animals has been reported by Thienpont *et al.* [2]. The drug is usually well tolerated. No activity was observed against the tapeworms, *Moniezia* spp. and flukes, *Fasciola hepatica* in sheep [43].

Tetramisole has also been found to be highly effective against most of the nematodes except *Trichuris* living in the abomasum and intestine of sheep and goats at a dose of 15 mg/kg. However, a lower dose of 10 mg/kg of the drug was sufficient to eliminate 82-94% of the immature and mature lungworms, *D. filaria* from sheep and goats [42]. The efficacy of tetramisole against various gastrointestinal nematodes in sheep, goats and cattle has been confirmed by various workers [43]. Tetramisole is equally effective against the lungworms, *Dictyocaulus filaria* in cattle [26], *Metastrongylus* sp. in swine, *Cyathosoma* sp. in waterfowl and *Mammomonogamus* sp. in cattle at a dose of 15-40 mg/kg [44].

4.1.2 Levamisole

Levamisole has been found to be highly effective against a wide variety of gastrointestinal nematodes, filariids and lungworms parasitizing cattle, sheep, goats,

pigs, cats, dogs and poultry [36,44]. In cattle, levamisole shows high activity against the mature and immature nematodes of the gastrointestinal tract, and the lung-worms, *Dictyocaulus viviparus* at a dose of 7.5 mg/kg [36,44]. When administered subcutaneously at a dose of 5-10 mg/kg, levamisole exhibited 98-99% activity against *D. viviparus* in calves [45]. The drug is also active against the adult worms of *Parafilaria bovicola* and *Stephanofilaria okinowaensis* in cattle at a dose of 7.5 and 15 mg/kg, respectively, given for two days [46,47].

Levamisole possesses high activity against adult and developing nematodes of the gastrointestinal tract of sheep at a dose of 7.5 mg/kg [48]. The above dose level of the drug is equally effective against *Dictyocaulus filaria* parasitizing the lungs of sheep and goats [49].

Although levamisole is not marketed for use in horses due to its narrow therapeutic index, it exhibits high activity against adult worms of *S. vulgaris*, *P. equorum* and *O. equi*. The recommended dose of the drug is 10 mg/kg (p.o.) or 5 mg/kg (intramuscular). It shows toxic effects like sweating, increased respiration, hyperexcitability and occasional mortality at two times the therapeutic dose [48].

In pigs, levamisole is usually administered subcutaneously at a dose of 5-7.5 mg/kg when more than 90% clearance of adult worms of *A. suum* and *H. rubidus* is achieved. The drug is also effective (75-90%) against adult forms of *T. suis*, *Oeso-phagostomum* spp. and *S. ransomi* in pigs at the above dose regimen. Levamisole pos-sesses high activity against *G. urosubulatus* in wild boars. The drug is well tolerated both by oral and subcutaneous routes of administration. Consequently, it may be used as a drug of choice for treating intestinal nematode infections in pigs [36,48]. Levamisole has also been found to be effective against the lungworms, *Metastrongy-lus* sp. in pigs at an oral dose of 8 mg/kg [49].

For cats and dogs, levamisole is an effective drug for treating ascarids and hookworms parasitizing the gastrointestinal tract, the microfilariae of *D. immitis* and lungworms *Aelurostrongylus obstrusus, Filaroides hirthi* and *F. osleri* at a dose of 5-7.5 mg/kg given subcutaneously for 1-10 days. The drug is safe at recommended doses, but may cause salivation, vomiting, nausea and muscular tremor at higher doses. Le-vamisole is contraindicated in cats and dogs with liver and kidney disorders [36,44,48].

In poultry, levamisole has been found to be effective in treating infestations due to mature and immature stages of *Ascaridia, Capillaria* and *Heterakis* spp. The

usual dose of the drug is 25 mg/kg given in drinking water. It is a safe drug, but there may be a minor reduction in the production of eggs in laying birds [36].

4.1.3 Nitramisole (11a) and butamisole (11b)

Both these drugs have been found to be 98-100% effective against *Trichuris vulpis* in dogs [13]. Further trials with butamisole showed the drug to be active against whipworms and hookworms, but poor activity against ascarids and tapeworms in dogs [41].

4.2 Imidazothiazoles in human medicine

4.2.1 Tetramisole

Tetramisole has been found to be highly effective against *Ascaris lumbricoides* in humans. At a single oral dose of 3-8 mg/kg, the drug has been shown to produce 79-100% cure rates with high reduction in faecal egg counts. The recommended dose of tetramisole for ascariasis is 50-80 mg/child and 100-150 mg/adult [1,50,51]. Tetramisole also yields cures upto 75% against hookworm infections in man at a dose of 2.5-5 mg/kg given daily for 3 days [50-52].

4.2.2 Levamisole

Levamisole possesses better activity than its racemic form tetramisole. At a single oral dose of 2.5-5 mg/kg, the drug has been found to give 100% cures against ascariasis in humans [53]. The recommended dose of levamisole for treating *A. lumbricoides* is 40-80 mg for children and 150 mg for adults [53,54]. It also exhibits high activity against the hookworms, *A. duodenale* and *N. americanus* [55]. A typical treatment using 2.5 mg/kg of levamisole may bring out about 90% cures against ascariasis and 80% cures against hookworm infections. However its activity against enterobiasis, trichuriasis and strongyloidiasis is not very encouraging [31].

Levamisole is a well tolerated drug producing virtually no side effects at therapeutic doses. However, a few cases (about 1% of the treated patients) may show intestinal symptoms (nausea, vomiting, anorexia, abdominal discomfort), headache and dizziness, which are mild and of transient duration [35,56].

5. MODE OF ACTION

In vitro experiments carried out to study the mode of action of levamisole have shown that the drug is immediately and almost completely absorbed through the cuticle of *A. suum* and causes spastic contraction of its muscles. The drug also causes

tonic paralysis of the larval and adult forms of nematodes [57,58]. Further work on the mode of action of levamisole has shown that it acts as a ganglion-stimulant, which later induces a depolarising type neuromuscular transmission that ultimately causes a spastic contraction of the muscles in nematodes [48,59,60].

Both tetramisole and levamisole are also potent inhibitors of fumarate reductase in the mitochondria of nematodes and *F. hepatica*. Since fumarate reductase plays a crucial role in the energy production in helminths, the inhibition of this enzyme would cut-off worm's energy supply and eventually cause its paralysis. The paralysed worm is no longer able to hold its position in the gut against intestinal peristalsis and, therefore, is soon eliminated with the faecal stream [31,58,61,62].

Levamisole has also an immunostimulant activity [63] and its antihelminth activity in part may be mediated through its action on the immune system.

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CHAPTER 8

BENZIMIDAZOLES

1.   INTRODUCTION

Benzimidazole is one of the oldest known nitrogen heterocycles and was first synthesized by Hoebrecker and later by Ladenberg and Wundt during 1872-1878 [1]. However, its therapeutic potential in parasite chemotherapy was recognised only after about 80 years when ICI introduced a combination of 2-phenylbenzimidazole (1, phenzidole) with phenothiazine as a sheep anthelmintic in the early sixties [2]. In 1961, Merck Sharp & Dohme Laboratories discovered 2-(thiazol-4-yl)benzimidazole (2, thiabendazole) as a broad-spectrum anthelmintic [3]. The introduction of thiabendazole in the treatment of helminth infections of humans and domestic animals may be regarded as a landmark providing a major break-through in the design of a new generation of anthelmintics. Today, benzimidazoles are one of the most important class of anthelmintics and have provided many effective drugs for intestinal and tissue-dwelling helminths. The ability of the benzimidazoles to undergo facile electrophilic, nucleophilic and cyclocondensation reactions giving rise to stable compounds, have made it possible to synthesize a large variety of substituted benzimidazoles and benzimidazoheterocycles, many of which have emerged as effective drugs for treating roundworm, tapeworm and fluke infections in humans and domestic animals like cattle, horses, sheep, goats, pigs, cats, dogs and poultry. A few benzimidazoles also find use in the control of various pests [4-6].

Although thiabendazole shows broad-spectrum activity against different helminths in humans and animals, it suffers from the limitation of being readily metabolised to form the inactive 5-hydroxythiabendazole (3, R=OH), with a half-life of only 11 minutes in rats [7]. To prevent this enzymatic hydroxylation of the drug at 5-position, Merck scientists synthesized a variety of 5-substituted thiabendazoles, of which 5-aminothiabendazole (4) and 2-(thiazol-4-yl)-5-isopropylcarbonylaminobenzimidazole (5, cambendazole) showed promising activity. The better anthelmintic activity of 4 and 5 compared to thiabendazole has been attributed to their longer half-life [8,9].

Another milestone in the SAR of benzimidazoles was achieved at Smith Kline and French laboratories where it was found that replacement of the thiazole ring of

thiabendazole by thiocarbamate led to compound **6** with high anthelmintic activity [10]. Further probe in this direction resulted in the synthesis of 2-acylaminobenzimidazoles (**7**), alkyl benzimidazole-2-carbamates (**8**) and 2-benzimidazolylureas (**9**) with marked anthelmintic activity. Optimal activity was observed in methyl 5(6)-substituted benzimidazole-2-carbamates, which ultimately culminated in the discovery of methyl 5-butylbenzimidazole-2-carbamate (**13**, parbendazole) Table 1 [10,11].

2 R = H	**6** X = S, R = OEt
3 R = OH	**7** X = O, R = Alkyl
4 R = NH$_2$	**8** X = O, R = Alkoxy
5 R = NHCOO-*i*Pr	**9** X = O, R = NR$_1$R$_2$
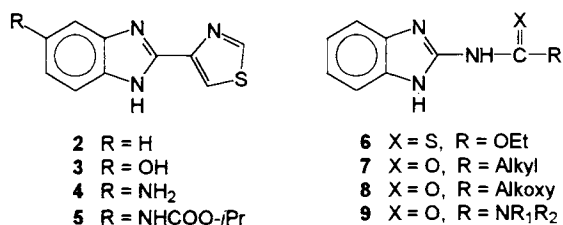
The discovery of parbendazole stimulated a vigorous search for better benzimidazole anthelmintics in different pharmaceutical companies of the world. Soon a number of drugs (**14-22**) were introduced in veterinary and humans medicine. All these benzimidazole anthelmintics may be regarded as the 5(6)-derivatives of carbendazim (**10**) which *per se* is an effective pesticide rather than an anthelmintic [12]. A novel non-carbamate benzimidazole fasciolicide, triclabendazole (**23**) [13] and a benzimidazole-2-carbamate anthelmintic, ricobendazole (**24**) [14] have been introduced recently (Table 1).

A few probenzimidazoles such as benomyl (**12**), thiophanate (**25**), febantel (**26**) and netobimin (**27**) have also been developed, which exhibit anthelmintic activity by virtue of their ability to release the corresponding benzimidazoles as active metabolites in the biophase. Of these, benomyl undergoes hydrolysis to form carbendazim (**10**), while thiophanate, febantel and netobimin (**25-27**) cyclise in the host's body to afford benzimidazole-2-carbamates (**28**) [11,15].

R = COOMe
R$_1$ = H, SPr, SPh
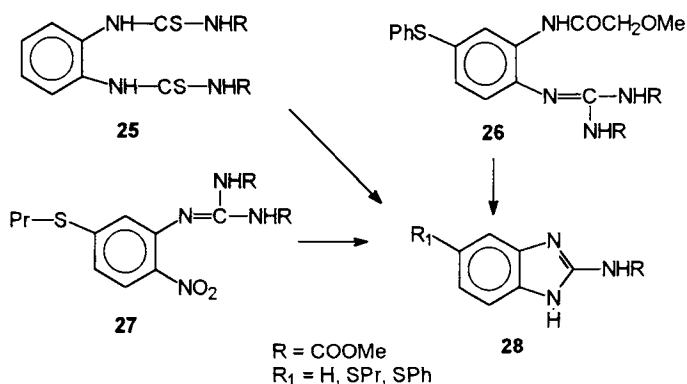
Table 1: Benzimidazole drugs in clinical medicine
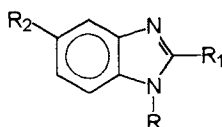
Compd. No.	Generic Name	R	R_1	R_2	Inventor/ Manufacturer
1	Phenzidole	H	C_6H_5	H	ICI
2	Thiabendazole	H	4-thiazolyl	H	MSD
5	Cambendazole	H	4-thiazolyl	NHCOOi-Pr	MSD
10	Carbendazim[a]	H	NHCOOMe	H	duPont
11	Lobendazole	H	NHCOOEt	H	SKF
12	Benomyl	CONHn-Bu	H	H	duPont
13	Parbendazole	H	NHCOOMe	n-Bu	SKF
14	Mebendazole	H	NHCOOMe	COC_6H_5	Janssen
15	Flubendazole	H	NHCOOMe	COC_6H_4F-4	Janssen
16	Cyclobendazole	H	NHCOOMe	COC_3H_5	Janssen
17	Nocodazole[b]	H	NHCOOMe	CO-thien-2-yl	Janssen
18	Fenbendazole	H	NHCOOMe	SC_6H_5	Hoechst
19	Oxfendazole	H	NHCOOMe	SOC_6H_5	Syntex
20	Albendazole	H	NHCOOMe	Sn-Pr	SKF
21	Oxibendazole	H	NHCOOMe	On-Pr	SKF
22	Luxabendazole	H	NHCOOMe	$OSO_2C_6H_4F$-4	Hoechst
23	Triclabendazole[c]	H	SMe	$OC_6H_3Cl_2$-2,4	Ciba-Geigy
24	Ricobendazole	H	NHCOOMe	SOn-Pr	Robert Young

[a]Primarily an antifungal agent; also known as medamine.
[b]Primarily an antitumor agent.
[c]Also contains a 6-Cl.

2. SAR IN BENZIMIDAZOLES

The structural modifications carried out at different positions of the benzimi-dazole nucleus and the resulting structure-activity relationship constituting a volu-minous chapter in medicinal chemistry, is beyond the scope of this section. Only the

salient features of the molecular changes carried out at the four major sites of benzimidazole are discussed below:

Site C: Changes in the pharmacophoric pattern at position 5 (6)

Site B: Changes in the nature of substituents at position 2

Site D: Changes in the molecular natureof the benzimidazole ring

Site A: Changes in the steric bulk of the nitrogen atom at position 1
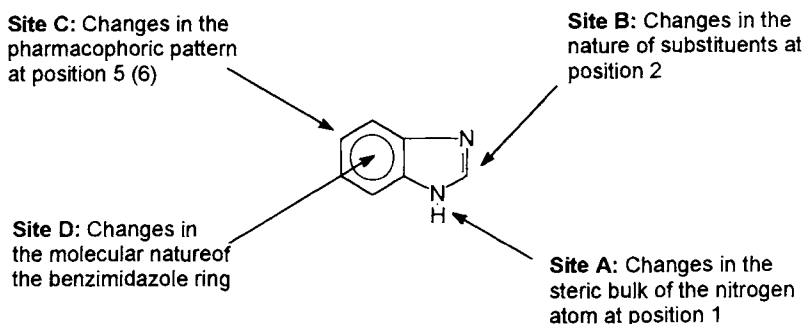
2.1   Modifications at site A

The nucleophilicity, though weak, of the nitrogen at position 3 has been utilized to prepare different 1-alkyl, aryl, aralkyl and acyl benzimidazoles with a view to understand the role of the hydrogen in evoking the anthelmintic response in benzimidazoles. It seems that the presence of a hydrogen atom at 1-position of the benzimidazole is essential for the anthelmintic activity as all 1-substituted benzimidazoles prepared led to lowering or loss of activity [16-30]. The only noteworthy compounds of this class are benomyl (**12**), and benacyl (**29**), which act as probenzimidazoles, releasing carbendazim (**10**) as the active metabolite in the host's body. Of these, benomyl has been found to possess 98-100% activity against *Ascaris suum* and *Trichuris suis* in pigs at an oral dose of 0.2 mg/kg given twice daily in feed [31,32].
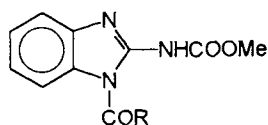
12   R = NH-*n*Bu (benomyl)
29   R = CH$_2$OC$_6$H$_5$ (benacyl)

2.2   Modifications at site B

Since the nature of the substituent present at position 2 of a 5(6)-substituted benzimidazole plays a significant role in determining the anthelmintic profile of the resulting molecule, a variety of structural modifications have been carried out at this site to arrive at a definite structure-activity relationship. Consequently, a series of 2-alkyl and 2-arylbenzimidazoles have been synthesized by condensation of *o*-phenyl-

enediamines with different carboxylic acids, aldehydes, amides, esters and nitriles etc. in the presence of an acid [5]. In general, introduction of an alkyl or aryl group at position 2 of benzimidazoles gives compounds with weak anthelmintic activity. The noteworthy compounds which emerged out of this study are 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (**30a**) and 4,5,6-trichloro-7-(diethylsulfamoyl)-2-trifluoromethylbenzimidazole (**30b**). Of these, **30a** was found to be highly effective against *Ancylostoma caninum, Haemonchus contortus, Ascaris suum* and *Fasciola hepatica* in rats, dogs and sheep at an oral dose of 0.1-5 mg/kg [33], while **30b** eliminated 100% of *F. hepatica* from sheep at oral doses ranging from 100-200 mg/kg [34].

The 2-arylbenzimidazoles exhibit better anthelmintic activity than the 2-alkyl-benzimidazoles. 2-Phenylbenzimidazole (phenzidole, **1**) is an effective drug, which has been used in combination with phenothiazine as a sheep anthelmintic by ICI in the early sixties. Introduction of a chloro or hydroxy group at 2-position of the phenyl ring of phenzidole gives rise to biological activity, though not of very high order. Thus, chlophasol (**31a**) and its hydroxy congener (**31b**) exhibit marked activity against *Nippostrongylus brasiliensis* and *Trichinella spiralis* in mice at a dose of 100 mg/kg given orally for 3 days [35]. In another study, chlophasol was found to be sufficiently effective against trichinosis at a dose of 25 mg/kg, thus warranting its evaluation in humans [36].

Hoechst laboratories synthesized a large variety of 2-phenyl-5-substituted benzimidazoles of which HOE-33258 (**32**) was found to possess high micro- and macrofilaricidal activity against *L. carinii* in cotton rats at a subcutaneous dose of 4-8 mg/kg given for 5 days [37]. Despite the strong antifilarial activity exhibited by **32**, it was not pursued further due to its strong binding with the host's DNA [38].

30a R = Cl
  b R = SO$_2$NEt$_2$

31a R = Cl (Chlophasol)
  b R = OH

32 (HOE - 33258)
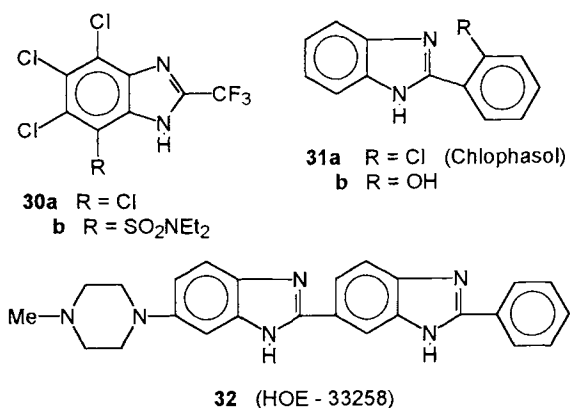
Although benzimidazole-2-carbamates possess broad-spectrum activity against different gastrointestinal helminths, virtually all of them suffer from the limitation of being highly insoluble, due to which these have poor and inconsistent GI drug absorption characteristics, making them weakly active or ineffective against tissue-dwelling helminth disease like filariasis, dracunculiasis, trichinosis, hydatidosis and cysticercosis. In an attempt to improve the solubility of benzimidazole-2-carbamates, a large number of 2-alkyl / arylcarbonylaminobenzimidazoles (33) and 2-benzimidazolylureas (34) have been synthesized [39-43]. Although all the compounds represented by structures 33 and 34 retain the molecular skeleton (disposition of three nitrogen atoms in a cyclic guanidine form) essential for anthelmintic activity, none surpassed the activity of mebendazole (14).
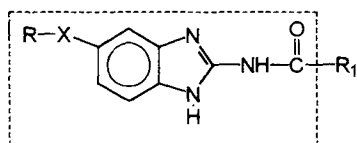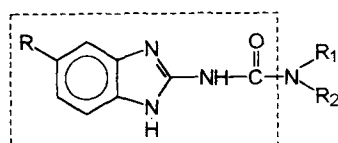
33   **34**

A few compounds belonging to the above two classes of benzimidazoles exhibited interesting activity. Thus, 33, X=CO, R=C_6H_5, R_1=morpholin-1-yl-methyl caused 100% elimination of the hookworms (*A. ceylanicum*) and tapeworms (*H. nana*) from rodents at an oral dose of 250 mg/kg, while 33, X=CO, R=2-acetylaminobenzimidazol-5-yl; R_1=Me killed 74-100% of the microfilariae and adult worms of *L. carinii* and *A. viteae* at a dose of 30-200 mg/kg given intraperitoneally for 5 days to cotton rats and *Mastomys natalensis*, respectively [39]. The latter compound also caused prolonged suppression of microfilariaemia (upto 3 months) in *Mastomys natalensis* infected with the humans filariid, *Brugia malayi*, at a dose of 50 mg/kg, i.p. for 5 days [39]. 2-Acetylamino-5-phenylthiobenzimidazole (33; X=S, R=C_6H_5; R_1=Me) has also been found to kill 100% of the microfilariae and adult worms of *L. carinii* in cotton rats at a dose of 30 mg/kg, i.p. for 5 days [40].

A similar order of antifilarial activity was also obtained by the benzimidazolylurea, (34; R=COC_6H_5; R_1=H; R_2=5-benzoylbenzimidazol-2-yl) at a dose of 50 mg/kg [40]. A better spectrum of antifilarial activity was exhibited by 1-(5-benzoyl benzimidazol-2-yl)-3,3-dimethylurea (34; R=COC_6H_5; R_1=R_2=Me), which killed 100% of the adult filarial worms of *L. carinii* and *B. pahangi* at a dose of 100 mg/kg,

i.p. for 5 days. The other two 2-benzimidazolylureas (**34**, R=COC$_6$H$_5$; R$_1$=H and R$_2$=Ph or C$_6$H$_4$-4-F) showed weak macrofilaricidal activity against *B. pahangi* and *L. carinii* at a dose of 100 mg/kg, s.c. for 5 days [43]. The weak antifilarial activities of the benzimidazolylureas is also attributed to their poor solubility.

These studies indicated that replacement of the methoxycarbonylamino group at 2-position of benzimidazoles by acylamino or urea pharmacophores may help to retain the biological activity, although they are weaker than the benzimidazole-2-carbamates. Thus, it may be concluded that the presence of a methoxycarbonylamino function at 2-position of a 5(6)-substituted benzimidazole is usually essential for optimal anthelmintic activity.

In another approach to alter the solubility characteristics of benzimidazoles the synthesis of a wide variety of 2-alkylthio and 2-arylthiobenzimidazoles (**35**) was carried out by alkylation/ arylation of the corresponding benzimidazole-2-thiones in the presence of a base [5,44-48]. In general, 2-alkyl/arylthio derivatives and their corresponding sulfoxides and sulfones showed poor anthelmintic activity, though **36** and **23** protected rats from *F. hepatica* at a dose of 3 x 10 mg/kg [49-51]. A few benzimidazoles (**35**) having groups like SCOOR, SCONR$_1$R$_2$ and S(CH$_2$)$_n$COOR at 2-position have also been prepared, but these had no antifilarial activity [52,53]. The most fruitful outcome of SAR carried out in this class was the demonstration of promising anthelmintic activity by **23** (triclabendazole), which ultimately emerged as an effective veterinary fasciolicide.

35 R = alkyl, aryl or acyl groups   **36** R = H
                                          23 R = Me
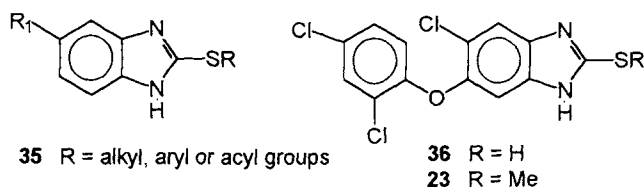
2.3   Modifications at site C

The presence of a pharmacophore at 5(6)-position of 2-substituted benzimidazole is an important factor for determining the biological profile of the compounds of this class. The substituents at this position not only prevent the molecule to undergo facile 5-hydroxylation to form weakly active or inactive metabolites, but also helps in segregating the therapeutic response against enteric and tissue-dwelling helminths. The major structural changes carried out at this site are discussed below.

After it was established that the 2-alkoxycarbonylamino function is an essential requirement for obtaining anthelmintic activity in benzimidazoles, a series of 5(6)-alkyl/arylbenzimidazole-2-carbamates (37) and 5-(2-substituted ethenyl)benzimidazole-2-carbamates (38) were prepared [5,54,55]. The most effective derivative was found to be methyl 5(6)-butylbenzimidazole-2-carbamate (13; parbendazole) [10], which finds wide usage in management of intestinal roundworm infections in domestic animals [14].

37   R = Me, OH, COOH, Ar
      n = O, 2, 3 etc.   38
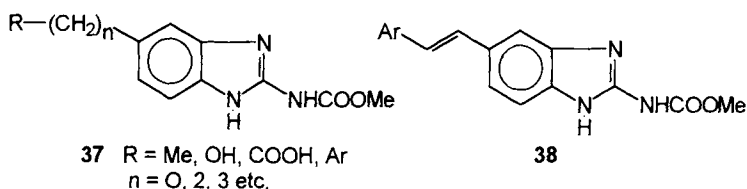
A major break-through in the SAR of benzimidazoles was achieved at Janssen Pharmaceutica, Belgium, where the synthesis of a large number of 5(6)-alkyl, cycloalkyl, phenyl and heteroarylcarbonylbenzimidazole-2-carbamates (39) was carried out [5,56]. Several compounds of this class exhibited potent activity against a number of gastrointestinal and tissue-dwelling helminths, the most effective being methyl 5(6)-benzoylbenzimidazole-2-carbamate (14; mebendazole), its fluoro analogue (15; flubendazole) and methyl 5(6)-cyclopropylcarbonylbenzimidazole-2-carbamate (16; ciclobendazole). These compounds find use in treating different helminth diseases of humans and domestic animals [4,57]. A bisbenzimidazole (40) has recently been reported, which has both micro- and macrofilaricidal activity against L. carinii, A. viteae and B. malayi infections in rodents on oral or parenteral application [58,59].

39   R = Alkyl and aryl   40
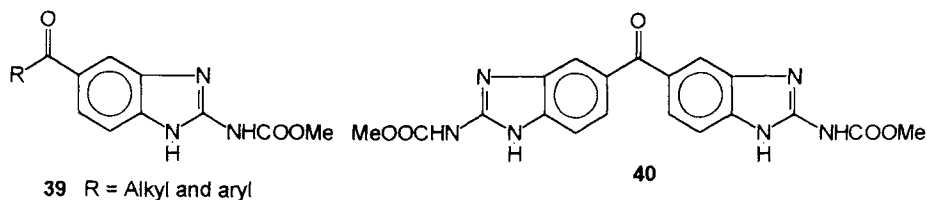
It is also possible to replace the alkyl/arylcarbonyl substituent at 5(6)-position of benzimidazole by various alkoxy groups. Consequently, a series of 5-alkoxycarbonylbenzimidazole-2-carbamates (41) were prepared with promising activity against A. ceylanicum, cystic forms of Taenia hydatigena and microfilariae and adult worms of L. carinii and B. pahangi in experimental animals [60,61]. Substitution of the alkoxy group in 41 by an amino function greatly enhanced the biological profile of

the resultant 5-aminocarbonylbenzimidazole-2-carbamates **42** [60-66]. The compounds thus generated show a broad-spectrum of activity against various intestinal and tissue-dwelling helminths. The effective compounds of this class are **43-46**, of which **43** exhibited nearly 100% macrofilaricidal activity against *B. pahangi* in jirds at a s.c. dose of 25-100 mg/kg [61], while **46** showed broad-spectrum of activity against intestinal helminths and is currently being developed as a human and veterinary anthelmintic in the author's institute [67].

41

42

43

R = Et, *i*Pr, *i*Bu

44 R = Morpholin-1-yl
45 R = 4-Methylpiperidin-1-yl
46 R = 4-(2-Pyridyl)piperazin-1-yl
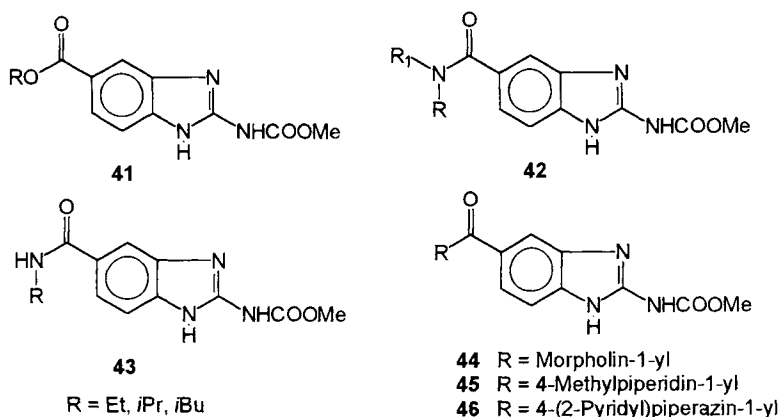
Using the metabolite directed approach, the active metabolites of mebendazole **(14)** and flubendazole **(15)**, namely methyl [5-(α-hydroxy-α-phenylmethyl)-1H-benzimidazol-2-yl]carbamate **(47a)** and methyl [5-(α-hydroxy-α-(4-fluorophenyl)-methyl)-1H-benzimidazole-2-yl]carbamate **(47b)**, respectively, were tested for their antifilarial activity, when **47a,b** were found to kill 100% of the adult worms of *L. carinii, D. viteae* and *B. pahangi* in rodents at a subcutaneous dose of 25-100 mg/kg given for 5 days [66,68]. This observation led to the synthesis of a series of benzimidazolyl alcohols of the type **47c** possessing marked activity against filariids and gastrointesintal helminths; however, none were found to be better than mebendazole [39,69, 70]. *O*-Alkylation of methyl [5-(α-hydroxy-α-phenylmethyl)-1H-benzimidazol-2-yl]carbamate **(47a)** forms a (±) mixture of *O*-alkylated products **48a,b**, which exhibited better antifilarial activity than **47a**. Both the compounds **(48a,b)** showed 100% macrofilaricidal activity against *L. carinii, D. Viteae* and *B. pahangi* at a subcutaneous dose of 25 mg/kg for 5 days [66]. However, complete reduction of the keto group of mebendazole giving rise to methyl 5-benzylbenzimidazole-2-carbamate **(49)** was found to reduce the activity. This compound killed 100% of the *L. carinii* and *B. pahangi* adult worms at a dose of 100 mg/kg (s.c.) for 5 days [66].

47a R = Ph
  b R = C$_6$H$_4$F(p)
  c R = alkyl, aryl

48a R = Me
  b R = Et

49
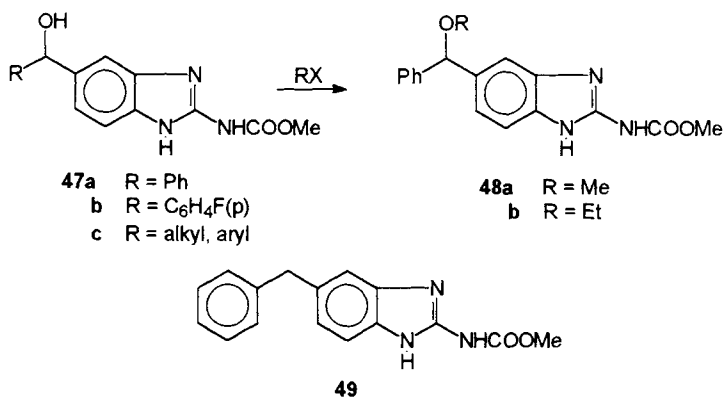
In a further probe to study the nature of the pharmacophore responsible for imparting the improved biological response a variety of 5-alkylthio and 5-arylthiobenzimidazole-2-carbamates (50) have been prepared by cyclisation of the corresponding 4-substituted o-phenylenediamines with 1,3-dicarbomethoxy-S-methylisothiourea or other cyclising reagents [5,71-79]. The corresponding sulfoxides and sulfones (50) have also been prepared [74,75,80-84]. In general, 5-alkyl/arylthiobenzimidazole-2-carbamates and their sulfoxides possess high order of activity. Of these fenbendazole (18), oxfendazole (19), albendazole (20), ricobendazole (24), and the related compounds (51a-d) and 52 have been found to exhibit broad-spectrum anthelmintic activity.

50 X = S, SO, SO$_2$
     R = Alkyl, aryl etc.

51a X = S, R = CH$_2$CH$_2$Cl
  b X = SO, R = CH$_2$CH$_2$Cl
  c C = SO, R = iPr
  d X = SO, R =   -R$_1$
                        R$_2$
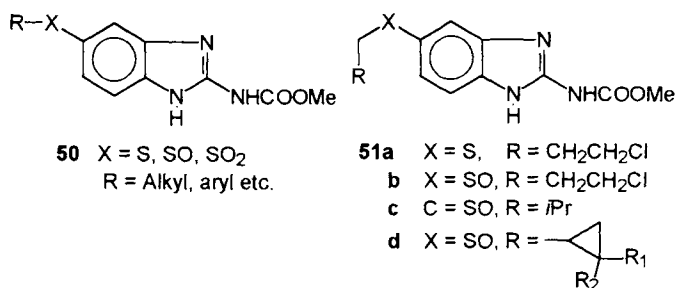
Replacement of sulphur in 5(6)-alkyl/arylthiobenzimidazole-2-carbamates (50) by oxygen gives 5-alkoxy/aryloxybenzimidazole-2-carbamates (53), which have been found to possess weaker activity than the corresponding 5-alkyl/arylthiobenzimidazole-2-carbamates [5,85-88]. The most effective benzimidazoles of this class are oxibendazole (21) and luxabendazole (22), which exhibit high activity against different helminth parasites in domestic animals [57].

52

53 R = Alkyl, aryl
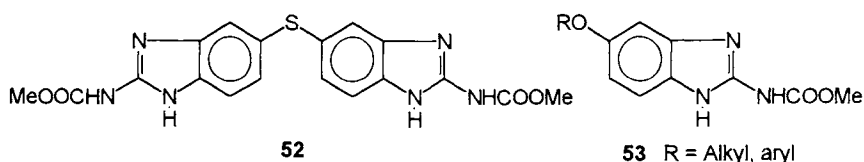
The marked anthelmintic activity associated with 5-aminobenzimidazoles like cambendazole (**5**), prompted the synthesis of various 5(6)-substituted aminobenzimidazole-2-carbamates (**54**). These, however, showed poor anthelmintic activity, except for the benzimidazoles (**55**), which carry a piperazin-1-yl residue at the 5-position [47,66,89-91]. Of these, the 5(6)-(4-substituted piperazin-1-yl)benzimidazoles (**55**) exhibited highly antifilarial activity, the most notable being the Hoechst compound HOE-33258 (**32**) and **55a,b**.

54

55a R = Fur-2-yl
b R = NEt$_2$
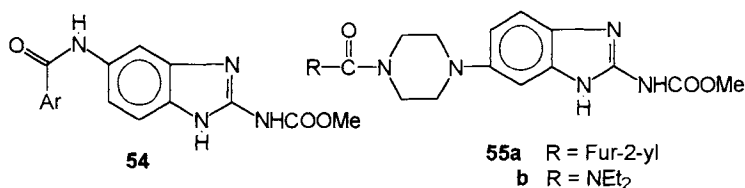
2.4 Modifications at site D

Besides molecular modifications carried out at 1,2 and 5-positions of the benzimidazole nucleus, various changes in the benzimidazole skeleton itself have been carried out keeping in view the planarity of the molecule, spatial disposition of groups attached and interatomic distances between heteroatoms. In this effort, various alkylimidazopyridyl carbamates (**56**) [92], azaindoles (**57-59**) [93], indazoles (**60-61**) [94-95] and imidazo[1,2-a]pyridin-2-carbamates **62a-g** [96,97] were synthesized bearing the same substituents, which are essential for evoking anthelmintic activity in benzimidazoles. However, none of the compounds exhibited activity superior to thiabendazole or mebendazole. The best compound of this class was **59** (Ar=phenyl, thiazolyl), which showed high activity against *Haemonchus contortus* in sheep at a single oral dose of 100 mg/kg, while **62e** and **62g** exhibited broad-spectrum anthelmintic activity. Of these, **62e** was found to be orally effective against a number of helminths in sheep and cattle at a dose of 2.5 mg/kg [98,99]. In a further modification one of the

56

57

58

59 Ar = phenyl, thiazolyl

60

61

62a R = NHCOOEt, R$_1$= thiazolyl
 b R = OPr, R$_1$ = NHCOOMe
 c R = SPr, R$_1$ = NHCOOMe
 d R = SPh, R$_1$ = NHCOOMe
 e R = SOPh, R$_1$ = NHCOOMe
 f R = COPh, R$_1$ = NHCOOMe
 g R = C = CCl$_2$, R$_1$ = NHCOOMe
  |
  Cl

63

64 (CGP-20308)

65 (CGP-20376)

66 (CGI-17658)
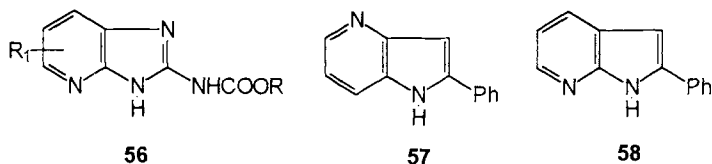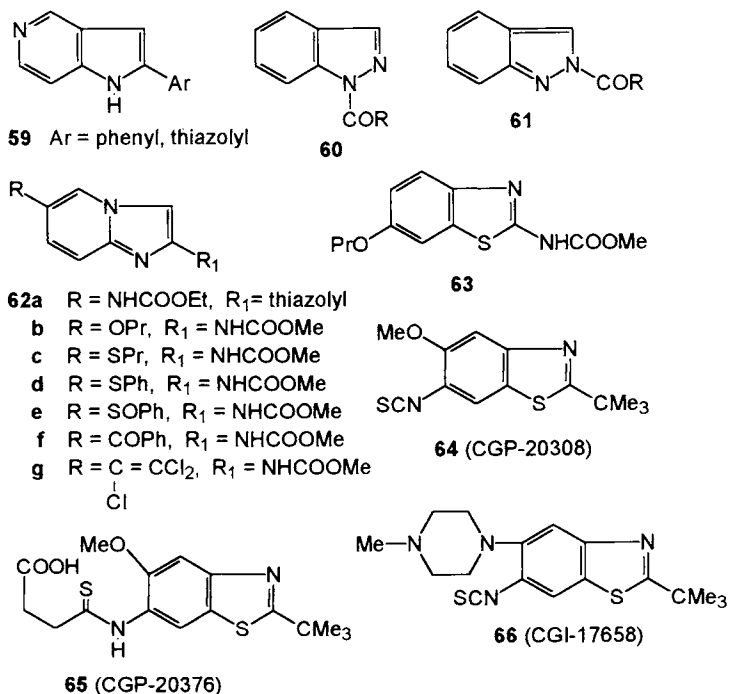
nitrogens of the benzimidazole nucleus was replaced by an oxygen or sulphur atom to give benzoxazoles [99], and benzthiazoles [60,99-103]. However, none exhibited better anthelmintic activity than the corresponding benzimidazoles. The most effective benzthiazoles were tioxidazole (**63**) and the Ciba-Geigy compounds **64-66**. Of these, tioxidazole (**63**) exhibited broad-spectrum activity against gastrointestinal nematodes [104,105], while the benzthiazoles (**64-66**) were found to possess high order of antifilarial activities [106-110].

2.5 SAR in benzimidazoles

From the foregoing discussions on SAR, it may be concluded that it is possible to achieve high anthelmintic activity by a benzimidazole having a hydrogen atom at position 1, a methoxycarbonylamino function at position 2 and an alkyl, aryl, aralkyl or heteroaryl groups attached to 5-position through a CO, CHOH, CONH, S, SO or O bridge [111]. Attempts to change the molecular geometry of the two nitrogen atoms, exchange of one of the nitrogens with oxygen or sulphur, or replacement of the methoxycarbonylamino function by ethoxycarbonylamino, acetylamino, urea, alkyl, aryl, heteroaryl, alkylthio or arylthio groups usually leads to compounds with low or no activity.

The few exceptions to these generalisation are thiabendazole (2), cambenda-zole (5), triclabendazole (23) and a few benzthiazoles (64-66). The demonstration of high order of anthelmintic activity by thiabendazole and cambendazole possessing a 4-thiazolyl group at the 2-position of benzimidazole may be explained by electronic and structural congruence of the thiazolyl pharmacophore with the methoxycar-bonylamino function [112,113]. It is at present not possible to provide any rational explanation for the activity of (23) and (64-66), and may call for synthesis of more ra-tionally designed 2-alkyl/arylthiobenzimidazoles and 2-t-butylbenzthiazoles for SAR studies.

3.   SYNTHESIS OF BENZIMIDAZOLE ANTHELMINTICS

3.1   Thiabendazole (2) and cambendazole (5)

Thiabendazole has been prepared utilizing two major approaches. The first approach consists of building up a 2-benzimidazolyl group at 4-position of thiazole. Accordingly, thiazole-4-carboxylic acid (67a), thiazole-4-carbonyl chloride (67b) or 4-cyanothiazole (69) are allowed to react with aniline, o-nitroaniline or o-phenyle-nediamine to get thiabendazole (2) through the reaction sequence shown in scheme 1 [114-122]. The second approach involves the preparation of 2-(2-hy-droxyethyl)benzimidazole (79) by condensation of o-phenylenediamine with 78 and lactic acid. The 2-substituted benzimidazoles (79), thus obtained, may be converted into thiabendazole (2) as described in scheme 2 [123-128].

Cambendazole (5) has also been prepared in several ways. However, the most convenient method of its synthesis employs 5-nitrothiabendazole (86) as a common intermediate. 86 in turn is prepared either by constructing the heterocyclic ring on a nitroaromatic intermediate or by nitration of thiabendazole. 5-Nitrothiabendazole (86) is then reduced to form 5-aminothiabendazole (87). Acylation of the latter with isopropoxycarbonyl chloride affords 2 [129]. Some other methods of the synthesis of 5 are also outlined in scheme 3 [130-136].

3.2   Mebendazole, flubendazole and ciclobendazole (14-16)

The key intermediates for the synthesis of mebendazole (14), flubendazole (15) and ciclobendazole (16) are 4-benzoyl-2-nitrohalobenzene (94a), 4-(4-fluoroben-zoyl)-2-nitrohalobenzene (94b) and 4-cyclopropylcarbonyl-2-nitrofluorobenzene (94c), respectively, which are prepared by Fridel-Crafts reaction of an acid chloride with the appropriate aromatic compounds. These substituted halobenzenes (94a-c) are converted into 14-16 as shown in scheme 4 [137-139].

71 R = Cl, Br

70

2

67a R = OH
b R = Cl

68

69

72

73

74

75

76

77
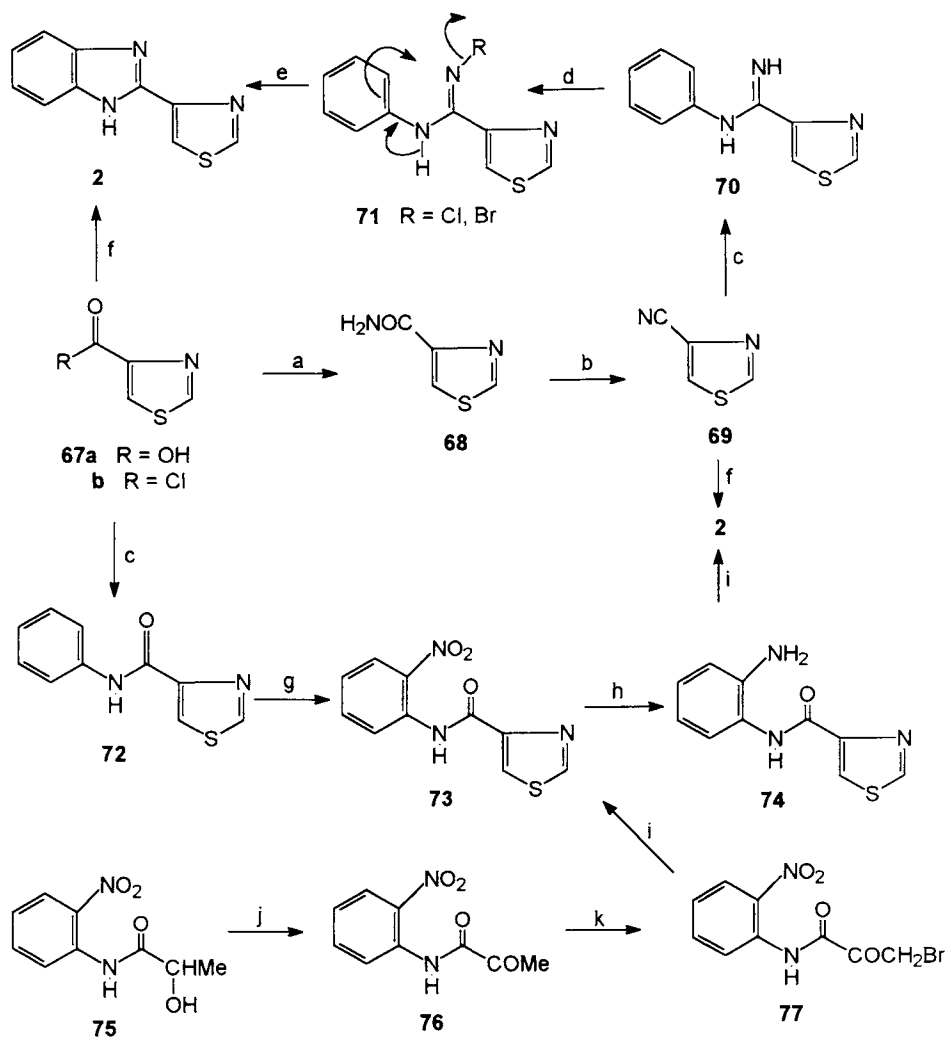
Scheme 1

Reagents: (a) NH_3; (b) P_2O_5; (c) Ph-NH_2; (d) NaOCl or NaOBr or NBS; (e) base; (f) O-phenylendiamine; (g) HNO_3; (h) Ra-Ni, H_2; (i) H^+/HC(S)-NH_2; (j) $K_2Cr_2O_7$, (k) NBS; l, $HCSNH_2$.
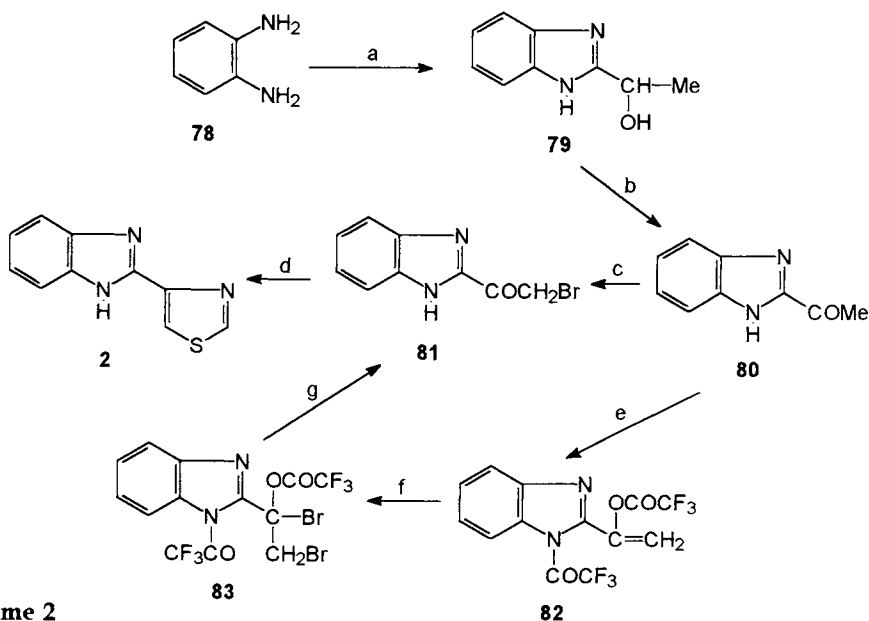
Scheme 2

Reagents: (a) MeCH(OH)COOH; (b) $K_2Cr_2O_7$; (c) Br_2; (d) $HCSNH_2$; (e) $(CF_3CO)_2O$, $PhSO_3H$; (f) Br_2; (g) KOH.
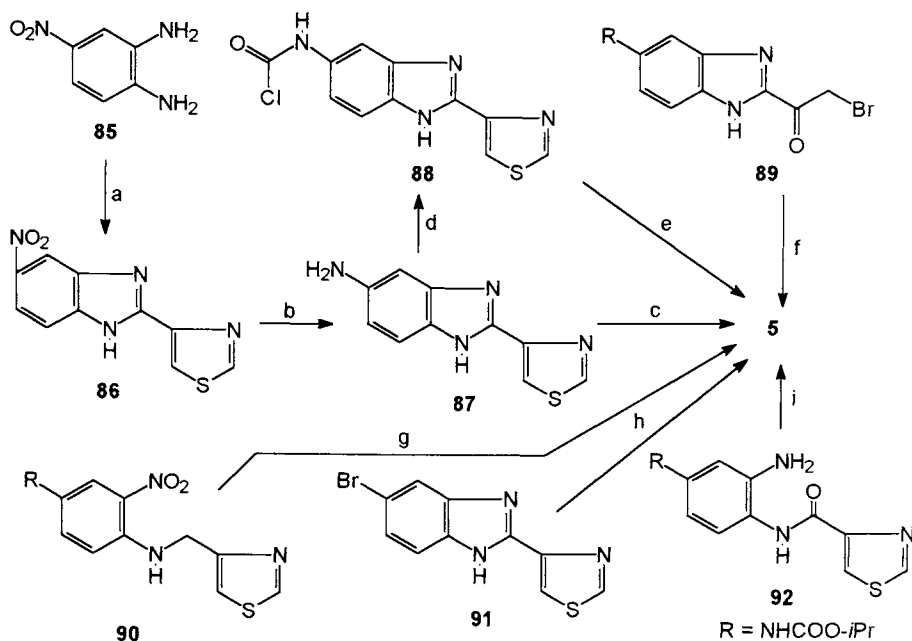
Scheme 3

Reagents: (a) thiazole-4-carboxaldehyde; (b) HNO_3; (c) $Me_2CHOCOCl$; (d) $COCl_2$; (e) Me_2CHOH; (f) $HCSNH_2$; (g) KOH, Fe, AcOH; (h) Cu, Cu_2Cl_2, $Me_2CHOCONH_2$, 175°C; (i) HCl.
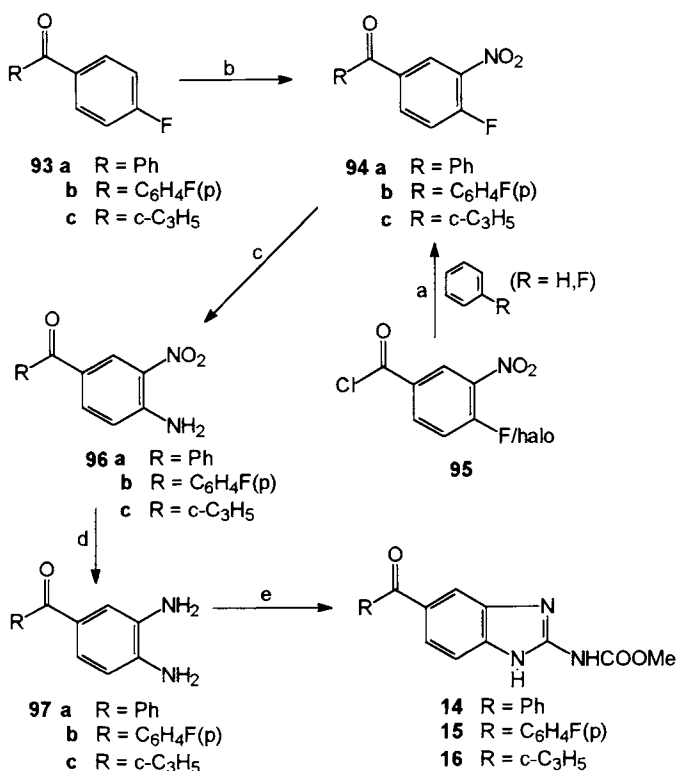
Scheme 4
Reagents: (a) AlCl₃; (b) HNO₃; (c) NH₃, DMSO; (d) Ra-Ni,H₂; (e) MeS-C(=NCOOMe)NHCOOMe or Cl₂C=NCOOMe.

The penultimate intermediate (**97a**) of mebendazole may also be generated from **101a** or **101b**, which, in turn, are prepared starting from 4-(methoxycarbony-lamino)-3-nitrobenzophenone (**99**) and 4-nitrobenzophenone (**100**), respectively [140,141]. Cyclisation of **97a** with methoxycarbonylisothiocyanate in the presence of DCC [52], N-methoxycarbonyl cyanamide [142,143] or N-methoxycarbonyl-O-ethy-lurea [144] yields mebendazole. The latter may also be prepared either by rearrangement of 2-amino-6-benzoyl-1-carbomethoxybenzimidazole (**103**) [145-147], synthesized in two steps starting from **102** [11] (Scheme 5).

3.3 Fenbendazole (18), oxfendazole (19), albendazole (20) and ricobendazole (24)

Base catalysed reaction of 3-chloro-6-nitroaniline (**106a**) and 3-chloro-6-ni-troacetanilide (**106b**) with thiophenol and propanethiol gives 3-substituted thio-6-ni-

211
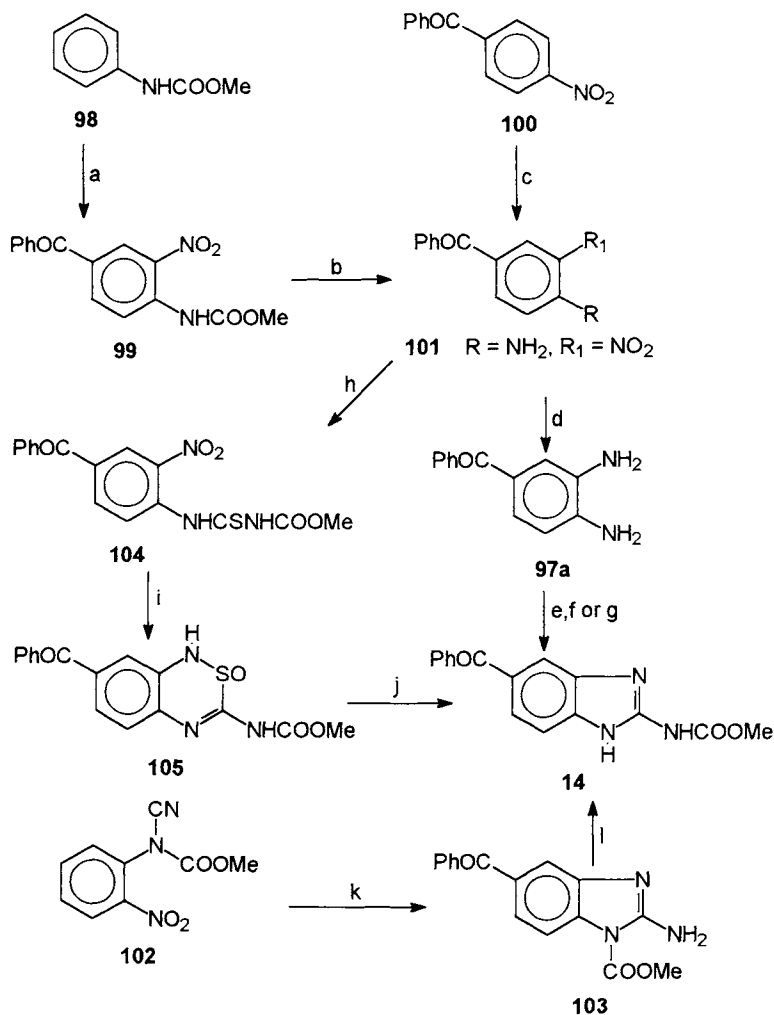
Scheme 5

Reagents: (a) i) PhCOCl, AlCl₃; ii) HNO₃; (b) H⁺; (c) i) Ra-Ni/Pd-C, H₂; ii) Ac₂O; iii) HNO₃; (d) Ra-Ni/Pd-C,H₂; (e) MeOOC-NCS, DCC; (f) NC-NHCOOMe; (g) MeOOCHN-C(OEt)=NH; (h) MeOOC-NCS; (i) Na₂S₂O₄; (j) Ph₃P, CHCl₃; (k) i) PhCOCl, AlCl₃; ii) Ra-Ni, H₂; (l) Pyridine, 100°C.

troanilines (**107a,b**), respectively, which may be converted into fenbendazole (**18**) [148-150], oxfendazole and albendazole (**20**) [151-153] (Scheme 6). Oxidation of **107a** with *m*-chloroperbenzoic acid or peracetic acid gives **108**, which is reduced and cyclised to afford oxfendazole (**19**) [154]. The latter may also be obtained by oxidation of fenbendazole with H₂O₂ (30%) in acetic acid or *m*-chloroperbenzoic acid [155,156].

106a R = H
b R = Ac

a

107a R = H, R$_1$ = Ph or Pr
b R = Ac, R$_1$ = Ph or Pr

d
107b

b

c

110

108

c

111

109a X = S
b X = SO

e

e

18 X = S, R = Ph
19 X = SO, R = Ph
20 X = S, R = Pr
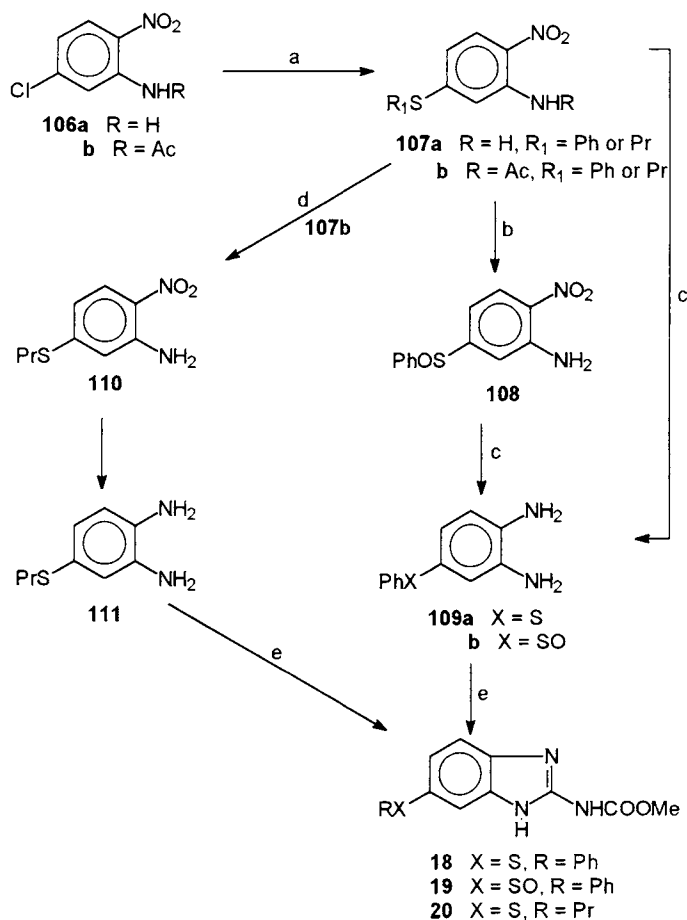
Scheme 6

Reagents: (a) PhSH or PrSH/KOH; (b) ClC$_6$H$_4$CO$_2$OH or CH$_3$CO$_2$OH; (c) Ra- Ni/Pd-C, H$_2$; (d) H$^+$; (e) MeS-C(=NCOOMe)NHCOOMe or other cyclising agents.

Some other methods for synthesizing albendazole (20) have also been reported. Carbendazim (10), prepared by cyclisation of o-phenylenediamine with 1-carbomethoxy/1,3-dicarbomethoxy-S-methylisothiourea, is converted into carbendazim-5-thiol (113) [157] and carbendazim-5-thiocyanate (114) [158], which are then allowed to react with propyl bromide and sodium hydroxide to form albendazole (20). Reaction of carbendazim (10) with Pr-SCl in acetic acid also gives 20 [159]. Albendazole is also obtained either by reaction of 6-nitro-3-thiocyanatoacetanilide (115) [151,152,160,161] or by the action of disulfide 118 with sodium borohydride and propyl bromide [162]. The starting material (117) for the synthesis of 118 is prepared

by treating 3-chloro-6-nitroacetanilide with potassium thiocyanate. Hydrolysis of **117** followed by oxidation and cyclisation of the resulting disulfide affords **119** [162,163] (Scheme 7).
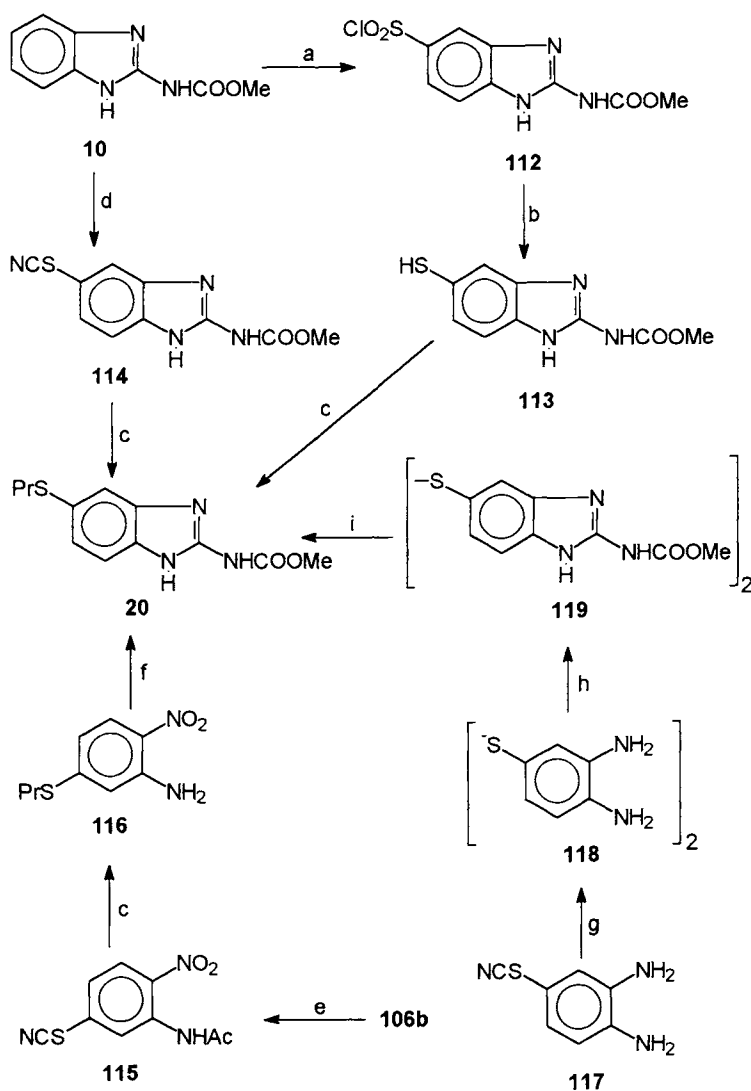
Scheme 7

Reagents: (a) ClSO$_2$OH; (b) Zn,H$_2$SO$_4$; (c) Pr-Br,NaOH; (d) NaSCN,Cl$_2$; (e) KSCN, (f) i) Ra-Ni/Pd-C, H$_2$; ii) MeS-C(=NCOOMe)NHCOOMe; (g) hydrolysis + oxidation; (h) MeS-C(=NCOOMe)NHCO OMe; (i) NaBH$_4$, Pr-Br.

3.4 Parbendazole (13) and oxibendazole (21)

Smith-Kline and French Laboratories [164] have prepared parbendazole by condensation of 4-butyl-*o*-phenylenediamine **(120a)** with 1,3-dicarbomethoxy-S-methylisothiourea. Oxibendazole is prepared starting from 4-propoxyacetanilide **(121)** which in turn is obtained by refluxing a mixture of 4-hydroxyacetanilide and propyl bromide in ethanol in presence of KOH, followed by the steps given in scheme 8 [164].

120a R = Bu   **13** R = Bu
    b R = OPr   **21** R = OPr

122   **121**
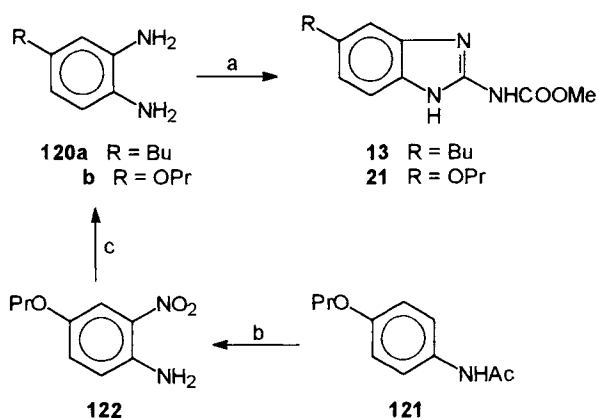
Scheme 8

Reagents: (a) MeS-C(=NCOOMe)NHCOOMe; (b) i) HNO_3-Ac_2O; ii) Claisen's alkali; (c), $SnCl_2$-HCl

3.5 Triclabendazole (23)

Triclabendazole **(23)** is prepared by methylation of 5-chloro-6-(2,3-dichlorophenoxy)benzimidazole-2-thiol **(124)** with methyl iodide. The latter is obtained by cyclisation of 4-chloro-5-(2,3-dichlorophenoxy)-*o*-phenylenediamine **(123)** with carbon disulfide [165].

123   **124** R = H
                 23 R= Me
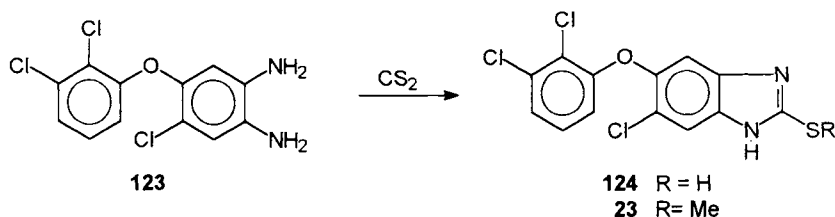
4.   BIOLOGICAL ACTIVITY

Of a large variety of benzimidazoles evaluated for their potential use in veterinary and human medicine, a number of them have been reported to possess a varying degree of activity against a variety of intestinal and tissue-dwelling helminths [4-6,14,57,166-172]. However, this section will be confined to the description of the biological profile of only those drugs which are largely used in veterinary and human medicine.

4.1   Benzimidazoles in veterinary medicine

4.1.1 Activity against roundworms

The benzimidazole anthelmintics have been extensively used to eradicate roundworms (nematodes) parasitizing the gastrointestinal tract, lungs, blood circulation, lymphatic system, body cavity, heart and subcutaneous tissues of cattle, sheep, goats, horses, pigs, cats, dogs and poultry [14,173-176]. The important drugs which have been used to treat domestic animals, are summarised below:

Thiabendazole (2): The drug is given to animals in the form of pastes, drench or in feed to eliminate the immature and mature gastrointestinal nematodes. For cattle a dose of 66-110 mg/kg has been recommended, while lower doses of 44-50 mg/kg of the drug can also be used to eliminate various nematodes parasitizing sheep, pigs and horses. Thiabendazole is equally effective in eliminating ascarids and hookworms from cats and dogs at a dose of 50-60 mg/kg. Higher doses may be needed to remove the lungworms such as *D. arnfieldi* in equines, *F. osteri* in dogs and *Syngamus* sp. in fowls [14,173,177,178].

Cambendazole (5): The activity profile of cambendazole in animals is almost similar to that of thiabendazole except for the therapeutic dose. A dose of 20-25 mg/kg of the drug has been found to cause 50-90% eradication of roundworms from the gastrointestinal tract of cattle, sheep, goats and pigs. However, cambendazole has been shown to be teratogenic, and is not recommended for use in pregnant animals [14,173].

Parbendazole (13): At a dose of 15-30 mg/kg, parbendazole shows high activity against the major gastrointestinal nematodes in sheep, goats, pigs, cattle, poultry, cats and dogs. However, the drug is teratogenic and produces embryotoxicity at a dose of 60 mg/kg in ewes on day 14-24 of pregnancy. It also exhibits 95-100% efficacy against the tapeworms, *Taenia hydatigena* in cats and dogs [4,14]. In a comparative study carried out against gastrointestinal nematodes in goats, albendazole and

parbendazole caused 71 and 85% reduction of the worm load at a dose of 3.8 and 15 mg/kg, respectively [179].

Mebendazole (14): This is the most effective benzimidazole anthelmintic used in veterinary practice. It causes complete removal of intestinal nematodes from sheep and goats at a dose of 10-20 mg/kg. In cattle mebendazole is only registered for tapeworms at a dose of 15 mg/kg [4]. It has also been found to be effective against various nematodes infecting pigs, horses, cats and dogs. In pigs, it shows 100% activity against *Ascaris* at a single oral dose of 1.25 mg/kg, while in horses it exhibits marked activity against *Parascaris equorum*, *Strongyloides* spp. and *Oxyuris equi* at a dose of 8.8 mg/kg. However, the drug is licenced for use in cats and dogs due to its high activity against intestinal nematodes *Toxocara* spp., *Toxascaris* spp., *Trichuris vulpis*, *Uncinaria stenocephala* and *Ancylostoma caninum* at a dose of 6-50 mg/kg or 100 mg/animal given twice a day [4,173]. A dose of 3-4 mg/kg of the drug has been found to show 100% activity against strongylosis in elephants [180]. However, mebendazole is teratogenic and, therefore, finds limited use in pregnant animals [173].

Flubendazole (15): Flubendazole shows high efficacy against the major gastrointestinal nematodes of sheep, cattle and horses at a dose of 10 mg/kg. It also possesses excellent activity against the adult roundworms of *Ascaris suum*, *Hyostrongylus rubidus*, *Trichuris suis* and *Oesophagostomum* spp. in pigs at a dose of 4 mg/kg given once for 10 days [4,14,173].

Fenbendazole (18): Like mebendazole, this drug also possesses high activity against the mature, immature and arrested larval stages of a large number of gastrointestinal nematodes of cattle, sheep, pigs and horses. The usual dose of the drug is 5-7.5 mg/kg given in feed or drench. Fenbendazole is licenced for use in cats and dogs as it eliminates the major intestinal nematodes (ascarids, hookworms and whipworms) at a dose of 20 and 50 mg/kg, respectively, given for 2-5 day [4,14,173]. It is also effective in eliminating gastrointestinal nematodes from goats [181]. Deworming of dairy herds, having natural infection of different nematodes, with fenbendazole during lactation and calving periods increased milk production with higher fat content [182].

Fenbendazole has also been recommended for eradicating lungworms (*D. viviparus*, *D. filaria*, *Neostrongylus*, *Mullerius* and *Metastrongylus* spp.) from cattle, sheep, goats, pigs, deer, cats and poultry at a dose of 5-7.5 mg/kg. Higher doses of 20-50 mg/kg may be needed to eliminate *Aelurostrongylus abstrusus* in cats, *Capillaria plica* in dogs and *Syngamus* spp. in poultry [57]

Oxfendazole (19): The activity of this drug against developing and adult forms of important nematodes infecting the gastrointestinal tract and lungs of sheep, cattle and horses is almost similar to that of fenbendazole. The recommended dose of oxfendazole in cattle, sheep and horses is 4.5, 5 and 10 mg/kg, respectively [14,173]. The drug also shows high activity against mature and immature forms of common nematodes of pigs. When given at a dose of 4.5 mg/kg, however, it has been found to be teratogenic and, therefore, is not recommended for pregnant animals [57].

Albendazole (20): This is another effective drug showing high activity against various nematodes parasitizing the gastrointestinal tract or lungs of cattle, sheep, and horses. It is used at a dose of 7.5 mg/kg in cattle and at 5 mg/kg in sheep. A dose of 5-10 mg/kg may also be used to eliminate *Ascaris, Oesophagostomum* and *Trichuris* from pigs [4,14,173]. Albendazole has been found to be highly effective against mature and immature ascarids, trichurids and strongyles in horses at a dose of 5 mg/kg; however, it has been found to be teratogenic in lambs, thereby limiting its use in pregnant animals [57,183].

Luxabendazole (HOE 216V, 22): Luxabendazole possesses 95-100% activity against adult and immature stages of major gastrointestinal nematodes (*H. contortus, Ostertagia* spp., *Trichostrongylus* spp.) and 80-100% activity against less important roundworms infecting sheep when administered at an oral dose of 7.5-10 mg/kg [57]. A 5% suspension of the drug given at a dose of 10 mg/kg has been found to be effective against trichostrongylids in game animals such as mouflon, roe deer, fallow deer and wild boars [184].

Ricobendazole (24): This drug shows above 90% elimination of adult worms and developing larvae of various nematodes in sheep and cattle at a dose of 5 and 7.5 mg/kg, respectively [14].

Other benzimidazoles: 46 (CDRI 82-437) has been found to display potent antifilarial activity as it kills almost 100% of the microfilariae and adult worms of *L. carinii, D. viteae* and *Brugia malayi* in rodents both by oral and parenteral route of administrations. The therapeutic dose of this compound is 10-50 mg/kg (i.p. or s.c.) or 100-200 mg/kg (oral) for 5 days. It also shows high activity against hookworms, *A. ceylanicum* in hamsters at an oral dose of 50 mg/kg [58,59]. Compound **52** (CDRI 81-470) exhibits 100% activity against different nematodes and cestodes in experimental and domestic animals at an oral dose of 2.5-100 mg/kg [67]. The efficacy of

this compound against larval and adult forms of some intestinal nematodes, filariids and tapeworms in experimental animals has also been established at doses ranging from 6.25-50 mg/kg [185,186].

Benzimidazole pro-drugs: Thiophanate, febantel and netobimin have been used to treat various roundworm infections in cattle, sheep and pigs with good success. Thiophanate, a prodrug of ethyl benzimidazole-2-carbamate, was introduced in 1970 as a plant pesticide. Later it was shown to possess 75-90% activity against all the stages of common nematodes invading the gastrointestinal tract of cattle and sheep. The recommended dose of the drugs in cattle, sheep and pigs are 66-132, 50 and 67 mg/kg, respectively [14,57].

Febantel metabolizes to form a mixture of fenbendazole and oxfendazole. Clinical trials carried out with this drug have established its high efficacy against the immature and mature stages of various gastrointestinal nematodes in cattle, sheep, pigs and horses [57, 201]. The usual dose of febantel for cattle, sheep, horses and pigs is 5.6-7.5 mg/kg [14,57].

Netobimin is another important veterinary anthelmintic. Its anthelmintic profile is very similar to that of albendazole, which is due to its biotransformation into albendazole and albendazole-sulfoxide (ricobendazole). The drug may be given as drench or in feed to achieve 90% and above reduction of developing and adult gastrointestinal nematodes such as *T. axei, Ostertagia, Haemonchus, Cooperia* and *Oesophagostomum* spp. in sheep and cattle [14,57]. In horses netobimin was found to be highly effective against *Strongylus* and *Parascaris* at an oral dose of 12 mg/kg and also produced no toxicity [187]. More recently netobimin was shown to reduce faecal egg counts by 44.8% in goats infected with lungworms, *Muellerius capillaris* at a dose of 7.5 mg/kg for 3 days or 10 mg/kg for 2 days [188].

4.1.2 Activity against tapeworms

The benzimidazoles possess weaker activity against animal cestode parasites than the nematodes. Cambendazole (5) was the first which was claimed to be effective against cestodes in sheep. This was later replaced by more effective benzimidazole anthelmintics such as mebendazole (14), fenbendazole (18), oxfendazole (19) and albendazole (20), because of their high activity and low toxicity [176].

Mebendazole (14): The drug has been found to be active against cysts of *Taenia* spp. and *Echinococcus granulosus* in sheep, *Moniezia* spp. in cattle, *Cysticercus tenuicollis*

and *E. granulosus* in pigs. Mebendazole shows high efficacy against *Taenia* spp., *Hydatigera* and *Echinococcus* in cats and dogs at a dose of 100 or 200 mg per animal given twice daily for 5 days [4]. Usually long treatments may be needed to eliminate larval *E. granulosus*, *T. hydatigena* and *T. ovis* from sheep, which may be expensive and preclude the use of mebendazole in animals [176].

Fenbendazole (18): This drug shows high activity against *Moniezia* spp. in sheep (5-25 mg/kg) and calves (10-15 mg/kg) and, therefore, finds wide usage in the treatment of major nematode and a few cestode infections in above animals [176]. At a dose of 100 mg/kg, both fenbendazole and oxfendazole had no lethal effects on 10 week old cysticerci of *T. saginata* in calves [189].

Oxfendazole (19): Oxfendazole exhibits high efficacy against *Moniezia* spp. in lambs at a dose of 5 mg/kg. The drug is well tolerated in sheep and shows no teratogenicity in early pregnancy in heifers [176].

Albendazole (20): The drug possesses broad-spectrum of activity against different nematodes, cestodes and trematodes in sheep and cattle. It removes intestinal tapeworms from sheep at doses ranging from 3.8-7.5 mg/kg. However, a dose of 50 mg/kg is required to eliminate larval *T. saginata* from cattle [176].

Luxabendazole (22): It shows 90% activity against *Moniezia* spp. at a dose of 7.5-10 mg/kg. The drug is a well tolerated with no indication of teratogenicity or mutagenicity in rats and sheep at 250 and 10 times the therapeutic dose, respectively [57].

Recently flubendazole (**15**; dose 40 mg/kg) and oxibendazole (**21**; dose 3.75 mg/kg) have also been shown to be 80% effective against cysts due to *C. cellulosae* in pigs [190].

4.1.3 Activity against flatworms

The activity of benzimidazoles against trematodes is less pronounced as compared to nematodes and cestodes. However, albendazole (**20**) and the newly introduced triclabendazole (**23**) find use in treating liver fluke infections in sheep and cattle [4,5,57,175].

Albendazole (20): The drug shows high activity against *Fasciola* spp. in sheep and cattle at an oral dose of 4.75 and 10 mg/kg, respectively. However, its activity is restricted to fully grown adult flukes only. This makes albendazole unsuitable for treating acute fascioliasis in animals. The drug also exhibits more than 98% activity

against *Dicrocoelium dendriticum* in sheep at a dose of 15-20 mg/kg [175].

Triclabendazole (23): This drug appears to be highly specific to flukes. It shows 97-100% activity against all stages of *Fasciola* spp. at a dose of 10-12 mg/kg in sheep, goats, horses and cattle. The drug is a well tolerated (MTD 200 mg/kg) with a high safety margin (TI 20) and, therefore, holds a great promise as a drug for treating acute fascioliasis and for chemoprophylaxis in ruminants [175,175a].

The usual oral dose of triclabendazole for eliminating mature and immature forms of *F. hepatica* is 5-10 mg/kg for sheep and goats, and 12 mg/kg for cattle [191-195]. The drug also shows marked activity against *Dictyocaulus filaria*, a few gastrointestinal helminths and *F. hepatica* when given in combination with fenbendazole [196,197]. However, triclabendazole shows no activity against *D. dendriticum* or *Paramphistomum* spp. in sheep [177].

Luxabendazole (22): The drug exhibits 95-100% activity against *F. hepatica* and *Dicrocoelium dendriticum* in sheep at a dose of 7.5-10 mg/kg. It is also ovicidal against some nematodes and flukes. A dose of 10 mg/kg of luxabendazole is needed to cause around 93% clearance of benzimidazole resistant *H. contortus* from sheep [57]. Further work on the efficacy of luxabendazole has established the drug to be 97-100% effective against immature and mature gastrointestinal nematodes (*Haemonchus, Ostertagia, Cooperia, Trichostrongylus spp.*) and *Moniezia* in sheep at a dose of 10 mg/kg [197a,b].

4.2   Benzimidazoles in human medicine

The benzimidazoles have emerged as the most versatile group of anthelmintics possessing broad-spectrum activity against a variety of helminths in humans. These drugs show powerful activity against roundworms [4,167,171,198], tapeworms [199], but are less active against flukes [57].

4.2.1 Activity against roundworms

The activity profile of various benzimidazoles against intestinal and tissue-dwelling helminths has been reviewed extensively [4,167,171,198-201].

Thiabendazole (2): It is the drug of choice for treating strongyloidiasis, disseminated strongyloidiasis, visceral larval migrants and creeping eruption. Thiabendazole can also be used successfully to treat *Angiostrongylus costaricensis* and trichostrongylus infections. In case of trichinosis, a combination of thiabendazole with steroids is usually used. The drug is also used as the alternative drug for curing

capillariasis and dracunculiasis. The usual recommended adult dose of thiabendazole is 25 mg/kg, which may be given for 2-8 days depending upon the nature of the infection [198,202].

Cambendazole (5): This drug shows 90-100% activity against intestinal strongyloidiasis in humans at a single oral dose of 5 mg/kg [171,198].

Carbendazim (10): Clinical trials have shown this drug to be effective against ascariasis, trichuriasis, hookworm infections, strongyloidiasis and mixed infections in humans at a dose of 200 mg given thrice daily for 1-3 days when 55-100% cure rates were obtained [203,204].

Mebendazole (14): Mebendazole is the drug of choice for treating *Ascaris lumbricoides, Trichuris trichiura*, hookworm, *E. vermicularis* and *Capillaria philippinensis* infections in children and adults. The recommended dose of the drug for children and adults is 100 mg twice daily for 3 days. However, for capillariasis a dose of 200 mg, twice daily for 20 days may be needed [171,198,202]. Mebendazole shows no activity against the filarial worm, *L. loa* [205], but exhibited appreciable effects against *D. perstans* in humans at a dose of 1 g daily for 21 days [206]. For treating *O. volvulus* infection the patients were first given two priming doses of levamisole (150 mg on two occasions), followed by mebendazole citrate (500 mg) given daily once or twice for 14 days. This treatment caused 80-88% reduction in microfilarial counts and the microfilarial effect remained for 42 weeks [207].

Flubendazole (15): Flubendazole shows high activity against ascariasis, trichuriasis and enterobiasis in humans at a single oral dose of 100-300 mg/patient [198,208,209].

Ciclobendazole (16): This drug shows high activity against ascariasis and hookworm infections. Consequently, it has been used to treat patients with mild helminthiasis at a dose of 200 or 400 mg/day for 3 days. Ciclobendazole was found to be highly effective and well tolerated except for vomiting and diarrhea occurring in only a few cases [210].

Albendazole (20): Albendazole shows a broad-spectrum of activity against *Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis* and hookworm infections in humans. The usual adult dose of the drug is 400 mg per adult [171,209]. Children between 8-24 months of age may be given 200 mg of albendazole in 100 ml suspension in a single dose to achieve 66-100% cure rates against *A. lumbricoides, N. americanus, T. trichiura* and *H. nana* [211]. Albendazole has also been evaluated against

Strongyloides stercoralis in some patients who received 400 mg of the drug for 3 days when nearly 40% cases were found to be cured [212]. The drug has shown great promise in the treatment of cutaneous larval migrants when given an oral dose of 400 mg for 3 days. Using the above dose schedule, 23 patients were cured; all the lesions cleared and the patients became asymptomatic. No recurrence of the disease was observed [213]. Recently Chichino *et al.* [213a] were able to achieve clinical improvement and parasitological cure in one patient with *Capillaria phillipinensis* infection at an oral dose of 200 mg twice daily for 21 days.

4.2.2 Activity against tapeworms

A few benzimidazoles such as mebendazole, flubendazole and albendazole have been used to treat tapeworm infections in humans with varying degree of success [199,214].

Mebendazole (14): Pena-Chavarria and co-workers [215] have used mebendazole to treat *T. solium* infection at a dose of 300 mg twice daily for 3 days. A higher dose of 300-600 mg of the drug has also been used to eradicate taeniasis in Taiwan [216]. Mebendazole shows high activity against human hydatidosis caused by larval forms of *Echinococcus* spp. It has been shown that mebendazole, when given at a dose of 3-5 g per adult daily for several weeks, can cause reduction in cyst size of *E. granulosus* and *E. multicularis* leading to marked clinical improvement in a number of treated patients [199,217]. Even a lower dose of 400 mg/day for only 4 weeks resulted in marked clinical improvement and decrease in the size of the liver in two women suffering from hepatic hydatidosis [218]. However, some workers could not achieve success in treating human hydatidosis by mebendazole [219,220]. Recently, Todorov *et al.* [221a] gave mebendazole at a dose of 50-70 mg/kg daily for 6-24 months to 28 patients suffering from cystic echinococcosis when 8 patients were successfully cured. It is suggested that patients with cystic *E. granulosus* infection of liver, lungs, bone and/or soft tissues may no longer need surgery as they may be well treated by mebendazole at oral doses of 50-60 mg/kg for several months [221b].

Flubendazole (15): The drug was found to be effective against lung echinococcosis in one case, but was not active in 5 patients with liver or other organ localisations at a daily dose of 1.5-4 g [199]. Flubendazole has also been shown to provide clinical improvement in 13 cases with neurocysticercosis caused by cysticerci of *T. solium* at a dose of 20 mg/kg, b.i.d. for 10 days [222].

Albendazole (20): Albendazole has been used to treat hydatid diseases and cysticercosis in patients at a daily adult dose of 800 mg or 15 mg/kg given for 30 days [172,199,202,223- 225]. For treating benign and moderate neurocysticercosis, albendazole (15-30 mg/kg, p.o. daily for 21-30 days) has been used along with the antihistaminic agent dextrochloropheniramine (18 mg/kg, p.o.) when improvement in 60-94% of the treating patients was observed. This drug combination is cheap and may be used as an alternative to praziquantel for rural masses [226]. However, its activity against *T. saginata* in man is not promising as only 50% cure was obtained at a dose of 800 mg for 3 days [226a].

The recommended dose of albendazole for treating *E. granulosus* and *E. multilocularis* is as follows: 800 mg of the drug (12 mg/kg/day for patients below 60 kg weight) should be given in two divided doses of 400 mg each to be taken daily after meals for 28 days. This 28 day treatment may be repeated after a gap of 14 days [227]. For treating cystic echinococcosis, albendazole may be given at a dose of 10 mg/kg daily for 30 days. Four such courses may be repeated at 15 days interval when 43.5% cures could be achieved [221]. The drug (dose: 15 mg/kg/day for 30 days along with steroids) has also been found to be effective against neurocyticercosis in a clinical trial carried out in 100 patients [228].

4.2.3 Activity against Flukes

Mebendazole (14) has been reported to be effective against the liver fluke, *Opisthorchis sp.* in humans at a dose of 30 mg/kg [57,229]. Triclabendazole (23) has been successfully used to treat fascioliasis in humans at a single oral dose of 10-12 mg/kg [230,231a]. The drug is also effective against *Paragonimus* infection in children at a single oral dose of 10 mg/kg [231b].

5. MODE OF ACTION

The work carried out on the mode of action of benzimidazole anthelmintics indicate that drugs of this class may exert their action either by inhibiting the energy metabolism and affecting glucose uptake or by inhibiting the polymerisation of tubulins to microtubules [5,232-235].

5.1 Inhibition of glucose metabolism

Fumarate-reductase, which converts fumarate into succinate in mitochondria, plays an important role in the anaerobic glucose metabolism and eventual energy production in many helminths. Thus, inhibition of this enzyme was considered to

cut-off worm's energy supply leading to its paralysis. Further, fumarate-reductase is unique in the parasites and, therefore, provides an excellent target for chemotherapeutic attack.

Thiabendazole, cambendazole, fenbendazole and oxfendazole have been found to inhibit fumarate-reductase in isolated mitochondria of *A. suum* and *H. contortus*. The above drugs also cause complete inhibition of the oxidation of NADH in the presence of fumarate at low concentrations (10^{-3}M for thiabendazole) in *H. contortus* homogenate, thereby suggesting a common mode of action of various benzimidazole anthelmintics. The inhibition of fumarate-reductase in larvae of *Trichinella spiralis* treated with thiabendazole has also been demonstrated both *in vivo* and *in vitro* systems [236].

5.2 Inhibition of glucose uptake

Mebendazole interferes with glucose uptake in nematodes and cestodes. The uptake of exogenous glucose in *A. suum* and *M. expansa* is inhibited by mebendazole. The inhibition of glucose transport in *A. suum* and *T. spiralis* larvae at 10^{-5}-10^{-6}M concentration of mebendazole has also been demonstrated [233]. The blocking of glucose utilisation or its transport leads to decreased ATP synthesis causing depletion of the energy source in the worm.

Further work on the mode of action of benzimidazoles has shown that drugs of this class interfere with the energy pathway of the helminths by inhibiting both cytoplasmic and mitochondrial malate dehydrogenase (MDH) [237,238]. The cytoplasmic and mitochondrial MDH obtained from *A. suum*, *F. hepatica* and *M. expansa* was inhibited by mebendazole, while albendazole, parbendazole and thiabendazole inhibited the *F. hepatica* enzymes more than the enzyme from *A. suum* [238]. According to McCracken and Stilwell [239], the benzimidazole anthelmintics may act as lipid-soluble proton conductors both in artificial (phospholipid bilayer) and natural (rat-liver mitochondria) membrane systems. These drugs disturb the transmembrane proton gradient severely, leading to a considerable drop in cellular ATP levels. The *in vitro* and *in vivo* results obtained by these authors would indicate that the mode of action of benzimidazole may be, in part, due to a bioenergetic disruption caused by transmembrane proton discharge.

5.3 Inhibition of tubulin polymerisation

Benzimidazole anthelmintics exhibit *in vitro* and *in vivo* binding ability to tubulin, an important component of the cytoskeleton of all living cells, and inhibit its

polymerisation into microtubules. Ultrastructural studies have indicated that mebendazole disrupts cytoplasmic microtubules resulting in degeneration of the cells in adult *A. suum*. Further studies on the binding affinity of mebendazole and fenbendazole with *A. suum* embryonic tubulin showed that the binding affinity of these drugs with nematode tubulin was 250-400 times higher than with bovine brain tubulin. This selective binding of benzimidazoles with parasitic tubulin may explain the lethal action of benzimidazoles on helminth worms. Extrapolation of these results to the ability of benzimidazoles to inhibit egg hatching, larval development, glucose uptake and fumarate reductase activity would indicate that microtubular assembly may be a common factor in many biochemical processes; the tubulin molecule might be associated directly or through conformational changes of an enzyme or protein molecule [233].

The role of cytoskeletal tubulins in the mode of action and mechanism of drug resistance to benzimidazoles has been reviewed by Lacey [111], who also supports the above hypothesis proposing a close relationship between tubulin-microtubules and other sites of the benzimidazole action. It is believed that inhibition of glucose uptake, fumarate-reductase activity or neuromuscular activity are dependent on inhibition of polymerisation of tubulins to microtubules by benzimidazoles. The inhibition of tubulins by benzimidazoles is so pronounced that it is postulated that even the hatching of eggs of *H. controtus* is a microtubule dependent process [240].

Using a molecular modelling approach McCracken and Lipkowitz [112] have found that anthelmintics with a benzimidazole nucleus are more susceptible to electrophilic scavengers. There exists an electronic and structural congruence in the 2-thiazolyl (eg. thiabendazole) and 2-methoxycarbonylamino (eg. mebendazole) groups, thereby suggesting a similar behaviour of both these groups towards transport and binding processes of the drugs to the active sites. Both these groups are coplanar with the benzimidazole skeleton; however, the groups situated at 5-position may be twisted out of plane. Consequently, a highly polar L-shaped cleft has been implicated in binding this class of drugs to the active sites [112,113].

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CHAPTER 9

SALICYLANILIDES

1. INTRODUCTION

The discovery of salicylanilides as anthelmintic agents owes its origin to research for new antiseptics. Following the demonstration of pathogenic effects of microorganisms by Pasteur, Koch and others, Lister was able to show in 1867 the germicidal power of phenol (then called carbolic acid). This observation led to the introduction of several phenol derivatives with marked antibacterial properties as disinfectants, many of which were used for several decades [1,2]. In a further probe to the development of better disinfectants derived from phenols, it was found that compounds containing two phenolic nuclei, linked directly or through a bridge, possessed higher antibacterial activity [2]. Some of these carrying a hydroxy group at 2,2'-positions also exhibited promising activity against liver flukes. The most effective anthelmintics thus discovered were bithionol (1) and hexachlorophene (2). Both these drugs were later used to eradicate tapeworms and liver flukes from humans and domestic animals [3,4].

OH   OH   OH   OH
Cl   S   Cl   Cl   Cl
Cl   Cl   Cl Cl   Cl Cl
        1   2
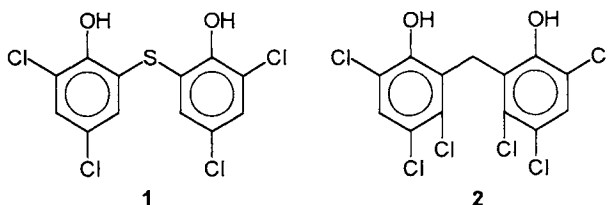
Encouraged by the powerful anthelmintic activity exhibited by bithionol and hexachlorophene, scientists at Farbenfabriken Bayer synthesized a series of hydroxybiphenyls of which 2,2'-dihydroxy-3,3'-dinitro-5,5'-dichlorobiphenyl (niclofolan, 3) emerged as a candidate drug for treating fluke infections in domestic animals and man. The drug has been found to be active against *Paragonimus westermani* and *P. uterobilateralis* at a single oral dose of 2 mg/kg in humans [5], while a dose of 3-6 mg/kg is needed to eliminate *F. hepatica* and *F. gigantica* from sheep and cattle [4].

A careful analysis of the structure-activity results in phenols containing another benzene nucleus linked directly or through a sulfide, sulfoxide or methylene

bridge, revealed that it is essential to have one OH and a chloro group in one of the two benzene rings. The other benzene ring may be joined to the phenol via an amide linkage. It is not necessary for this ring to have a hydroxy function. Utilizing this SAR data, scientists at Farbenfabriken Bayer synthesized a variety of substituted salicylanilides showing potent antihelminthic activity, ultimately culminating in the discovery of 2',5-dichloro-4'-nitrosalicylanilide (niclosamide, yomesan, 4), in 1955 as a potent antithelmintic drug. Both these drugs contain two benzene rings substituted by chloro and nitro groups. In niclofolan, both the aromatic rings are joined directly, while in niclosamide (4) they are held together by an amide moiety.

Niclosamide is a highly effective molluscicide and taenicide, which was marketed in 1960 [6,7]. It soon became the drug of choice for treating virtually all gastrointestinal tapeworm infections in humans and domestic animals [4,5,8-10]. From the medicinal chemistry point of view, the discovery of niclosamide may be regarded as a landmark in the chemotherapy of tapeworms, which provided impetus for the design and synthesis of a new generation of salicylanilide anthelmintics [11].

O_2N OH HO NO_2

Cl   **3**   Cl

OH   Cl

—CONH—   —NO_2

Cl   **4**
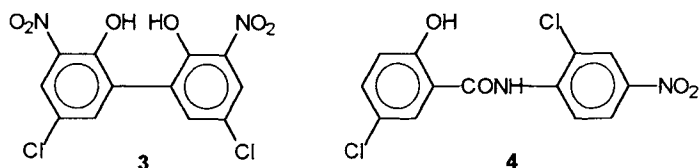
2.   SAR IN SALICYLANILIDES

Although niclosamide itself is the outcome of a systematic SAR carried out on salicylanilide derivatives, its molecular skeleton provided enough scope for further molecular modification. Consequently various leading pharmaceutical companies like Hoechst, ICI, Merck and Parke-Davis set out on a vigorous search to develop better anthelmintics than niclosamide.

The fact that the presence of the hydroxy function originating from the salicylic acid is an essential requirement for cestodicidal and flukicidal activity, led Hoechst scientists to prepare a variety of 2,6-dihydroxybenzoic acid anilides. The most effective compound of the series were 4'-chloro/bromo-6-hydroxysalicylanilides (5a,b). Of these, the bromo analogue, called resorantel (terenol, 5b) was found to be more effective than the 4'-chloro analogue (5a) [12]. Resorantel was later

demonstrated to be a useful veterinary anthelmintic, which causes almost 100% clearance of *M. expansa* and *P. aramphistoma* from ruminants at a dose of 60-65 mg/kg [13-15]. Another compound which emerged from the studies on 2,6-dihydroxybenzoic acid anilides was 3′,5′-di(trifluoromethyl)-2,6-dihydroxy-3-nitrobenzanilide (6), possessing high fasciolicidal activity at a dose of 1.5 mg/kg. However, this compound was found to be toxic and, therefore, it was not taken up for further development [3,12].

OH

—CONH—〈 〉—R

OH

5a R = Cl
b R = Br

O₂N   OH   CF₃

—CONH—

OH   **6**   CF₃
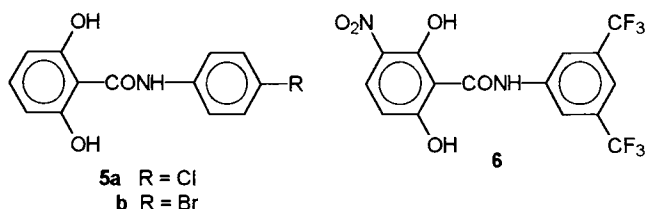
The most fruitful results were obtained by introducing groups of different electronic and steric nature at various positions of both the benzene rings of the salicylanilide molecule [9,11]. The major structural modifications carried out in niclosamide may be broadly divided into four groups, which are discussed below.

2.1   Chloro derivatives of niclosamide

Although niclosamide itself possesses two chloro groups, one each in both the benzene rings, it was considered rational to study the impact of further chloro substitution on the net biological response of the resulting molecules. Accordingly, 3,3′,5,5′,6′-pentachloro-2′-hydroxysalicylanilide (oxyclozanide, 7) was developed in the ICI laboratories, which is widely used to eliminate adult flukes of *Fasciola hepatica* from sheep and cattle at a dose of 80 and 40 mg/kg, respectively [11,16].

Cl   OH   HO   Cl

—CONH—

Cl   Cl   Cl
         7
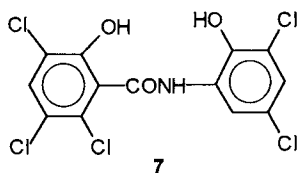
2.2   Bromo derivatives of niclosamide

Introduction of a bromo group in either the acid part or aniline part of the niclosamide molecule is known to evoke a better anthelmintic response. This observation has led to the synthesis of a large variety of bromosalicylanilides [3,11,17-19]. The effective salicylanilides, thus emerged, are brotianide (Bayer 4059, Dirian, 8), hilomid, an equimolar mixture of 3,4′,5-tribromosalicylanilide (tribromsalan, 9a) and

4',5-dibromosalicylanilide (**9b**) and CDRI 77-6 (**10**). Of these, brotianide and hilomid have been shown to be effective against fascioliasis in sheep [11], while CDRI 77-6 exhibited promising antitapeworm activity in experimental animals [18]. Even 3,4,5-tribromosalicylamide (**11**) has been found to eliminate 80-98% of the adult *F. hepatica* worms from goats, sheep and cattle at a single oral dose of 60 mg/kg [20].
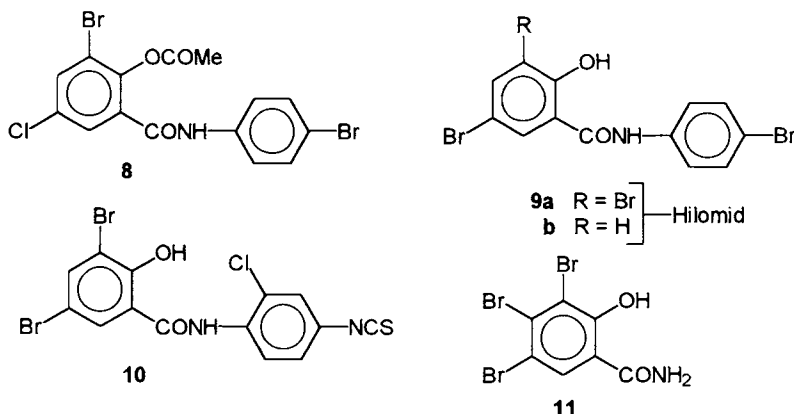
2.3   Iodo derivatives of niclosamide

A better profile of activity may be achieved by introducing an iodo function in the benzene ring of the salicylic acid part of salicylanilides. Therefore, a large variety of 3,5-diiodosalicylanilides were prepared, many of which exhibit high anthelmintic activity [11,21]. The noteworthy salicylanilide, which came out of these studies are rafoxanide (**12a**), salantel (**12b**), clioxanide (**13**) and closantel (**14**). Rafoxanide was selected from more than 200 phenoxysalicylanilides prepared in the Merck Sharp & Dohme laboratories [22]. Clioxanide (**13**) is the acetate ester of 4'-chloro-3,5-diiodosalicylanilide, which was developed jointly by scientists at Parke-
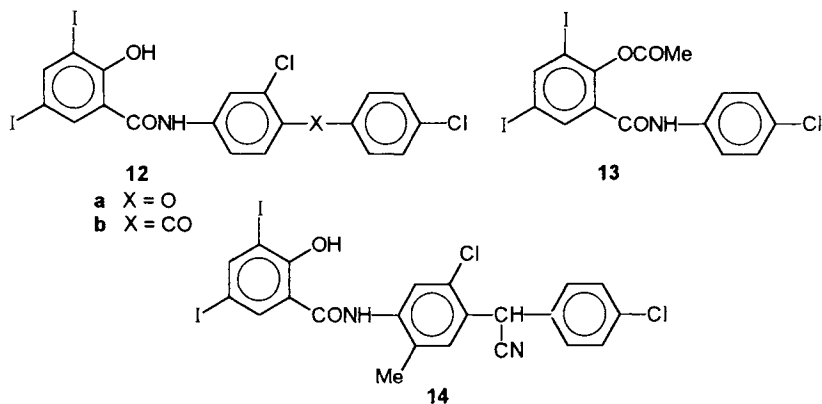
Davis Research laboratories at Michigan and Sydney [23,24]. Closantel (14), having close structural resemblance with rafoxanide, was developed by Janssen Pharmaceutica, Belgium [2,25].

2.4 Other derivatives of niclosamide

In addition to the synthesis of various halosalicylanilides, a large variety of salicylanilides with different groups in both the benzene rings have also been prepared in a search for better drugs for tapeworm and fluke infections [26-29]. Several polyhalogenated-2,2′-diacyloxybenzanilides (15) have been prepared [30]. The noteworthy compound that emerged from these studies is 5-chloro-4′-cyclohexylamino-3′-nitrosalicylanilide (16a), which eliminated all the worms of *H. nana* from mice at an oral dose of 30-50 mg/kg [27]. A similar order of activity against *H. nana* was observed with 4′,5-dichloro-3′-nitrosalicylanilide (16b) and 2′-chloro-4,4′-dinitrosalicylanilide (17) at an oral dose of 250 mg/kg [28].
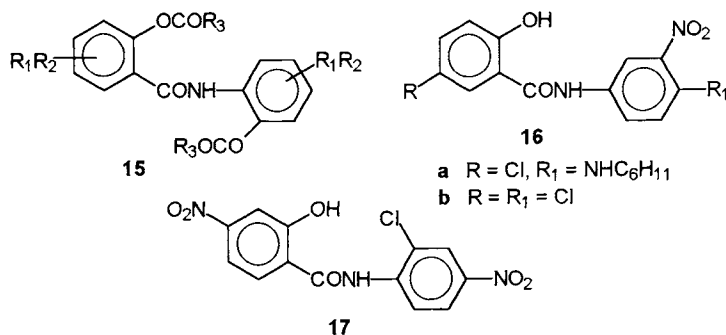
Introduction of an isothiocyanate moiety in the benzene ring of the aniline part of salicylanilide has been found to evoke high order of cestodicidal activity. This effect was more pronounced when the isothiocyanate group was located at 3′- or 4′-positions. Thus, 2′,5-dichloro-4′-isothiocyanatosalicylanilide (18) and 3,5-dibromo-2′-substituted-4′-isothiocyanatosalicylanilides (19) removed 100% of the *H. nana* infection from rats at a dose of 10-30 mg/kg [17,18,28,29,31].
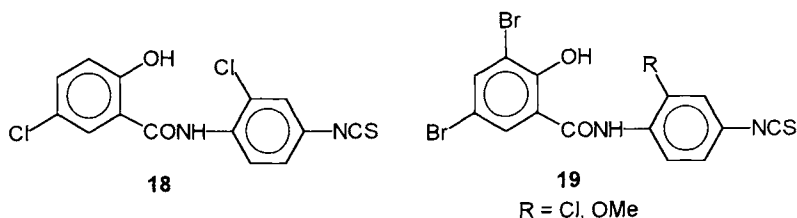
A few 3-nitro-2,6-diacetoxybenzanilides (**20**) have been prepared as structural analogues of clioxanide. Some of these have been found to eliminate *F. hepatica* from sheep [32]. Scientist at Smith Kline & French Labs. prepared various polysubstituted salicylanilides, of which bromoxanide (**21**) showed 92-100% activity against the mature and immature flukes of *F. hepatica* in sheep at a single oral dose of 0.5-1 mg/kg [33]. Similarly, 3-*tert.*-butyl-4′,5-dicyano-6-methyl-2′-bromosalicylanilide (**22**) was found to kill *F. hepatica* in rats at a subcutaneous dose of 0.1 mg/kg. Higher doses (1.5-2.5 mg/kg) of the compound exhibited toxicity and caused paralysis of the treated animals [34].

20   **21**

22   **23**

R = Cl, Br ;   R₁ = H, Cl
R₂ = Cl, Br, NO₂ etc.
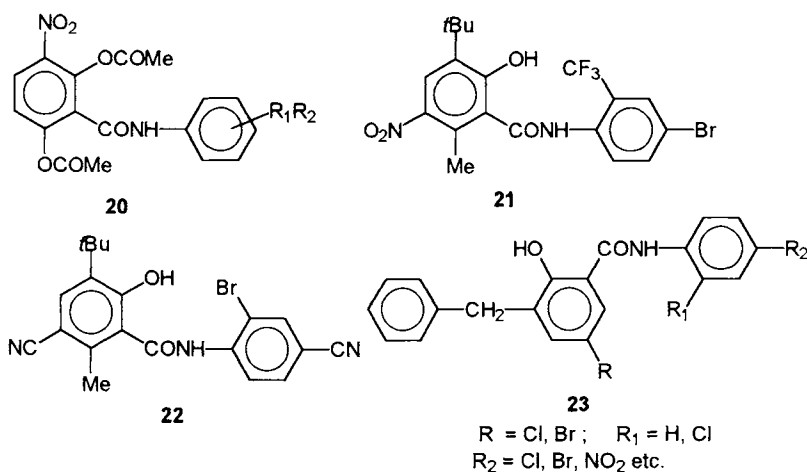
Zikan and coworkers [35-37] have prepared substituted anilides of 3-benzyl-5-bromosalicylic acids (**23**), some of which exhibited high activity against *H. nana* and *Aspicularis tetraptera* in experimental animals.

An additional benzene ring on the acid part of the salicylanilide molecule gives rise to hydroxynaphthanilides with marked anthelmintic activity. Consequently, a number of 1-hydroxy-2-naphthanilides have been prepared [38-40], of which 2′-chloro-1-hydroxy-4′-isothiocyanatonaphthanilide (**24**) was found to kill 100% of *H. nana* in rats at an oral dose of 7.5 mg/kg. The compound also showed high activity against *H. diminuta* in rats and *Taenia* spp. in dogs. Several other

24
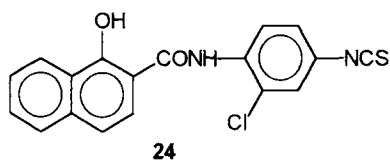
naphthanilides have been synthesized, which cause 100% elimination of *H. nana* infection in rats at oral doses ranging from 130-250 mg/kg [38]. Surprisingly, no appreciable anthelmintic response was exhibited by 2-hydroxy-3-naphthanilides [40,41].

During systematic SAR studies on salicylanilides, various compounds of type 25-26 have been prepared [42-44], of which 25 showed good activity against *H. nana*, while 26 was found to be effective against flukes [42-43].

25

26 R = H, Cl, Br, I; $R_1 = R_2 = $ Cl, Br, I
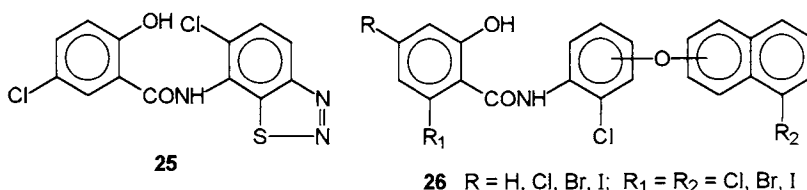
2.5 SAR in salicylanilides

The anthelmintic activity of various salicylanilides and related compounds (27) would suggest the following structure-activity relationship [9,11].

(a) The presence of a hydroxy group at 2-position in ring A is most essential for activity against tapeworms and flukes. This is evident from the fact that removal of this OH (28) or its replacement by OMe (29) causes lowering or loss of anthelmintic activity. Incorporation of OH in a cyclic structure like 1,3-benzoxazine (30) also causes loss of activity [28,29,41].

27

28

29

30 X = O, S
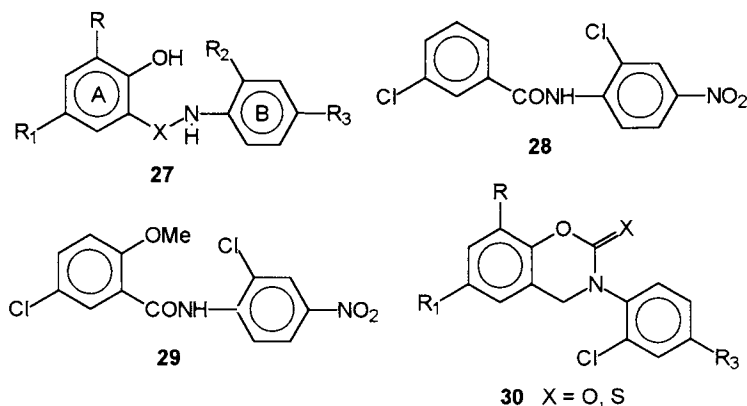
(b) The presence of an amide or thioamide linkage connecting the aromatic rings A and B as in 27 (X = CO or CS) is also important for retaining anthelmintic activity. However, it may be noted that salicylanilides possess better activity than the

thiosalicylanilides. Accordingly, replacement of CONH by an amidine, aminomethyl (27, X = C=NH, CH$_2$) or by an ester group (31) has been shown to diminish the activity [7,45,46].

(c) The presence of halogens at 3,5-positions in ring A and 2,4-positions in ring B plays a crucial role in evoking cestodicidal and flukicidal activities. The best profile of activity is obtained by introducing chlorine at different positions of ring A and B. Replacement of chlorine by bromine or iodine may enhance activity as well as the toxicity of the resulting compounds [9,31]. It may also be noted that introduction of electron withdrawing groups in ring B such as nitro or isothiocyanato, preferably at 4-position, tend to enhance the anthelmintic activity. Likewise electron donating groups such as amino at position 4 of ring B make the molecule poorly active or inactive.

(d) Substitution of rings A or B by the naphthalene nucleus retains the activity, though of lower order. Similarly replacement of ring B by heterocycles such as benzothiadiazole or benzimidazole, gives N-arylsalicylamides with weak or no activity [21,42,47].

The above structure-activity relationship hold well for most of the active salicylanilides and there are only few exceptions to these generalisations. The acetate ester of niclosamide, called aphesal (32a) shows marked anthelmintic activity [48]. This is probably due to the fact that aphesal acts as a pro-drug of niclosamide. Similarly, compound 32b, having no chlorine at 5-position in ring A, shows activity against *H. nana* in mice at an oral dose of 50 mg/kg [49].

31

32
a R = COMe, R$_1$ = Cl
b R = R$_1$ = H
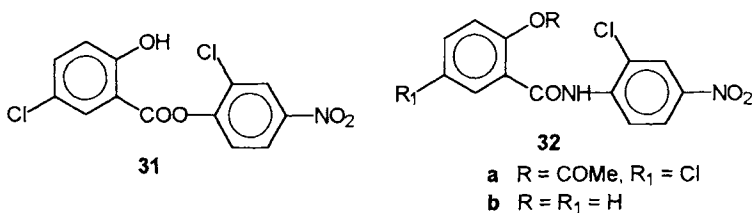
3.   SYNTHESIS OF NICLOSAMIDE AND RELATED DRUGS

The most widely used method to prepare salicylanilides (35) involves condensation of an appropriate salicylic acid (33) with an aniline (34) in the presence of phosphorus trichloride [7,50-54] or phosphorus oxychloride [55] in boiling chlorobenzene, toluene or xylene. In these reactions salicyloyl chloride (36) is generated *in situ*, which then reacts with aniline to form 35. Alternatively salicyloyl chloride

(36), prepared by reacting salicylic acid with phosphorus pentachloride or thionyl chloride, may be reacted with the desired aniline to yield 35 [47,56,57].

The salicylanilides may also be conveniently synthesized by reaction of salicylic acid with aniline in the presence of P_2O_5 [58], tetraalkylpyrophosphite [59,60], $Me_2As(O)OH$ [61], H_3PO_4 or $H_4P_2O_7$ [62] or DCC [45]. In another approach salicylic acid is converted into the phenyl ester (37), which is heated with appropriate aniline at high temperatures to yield the corresponding salicylanilides [63-65].
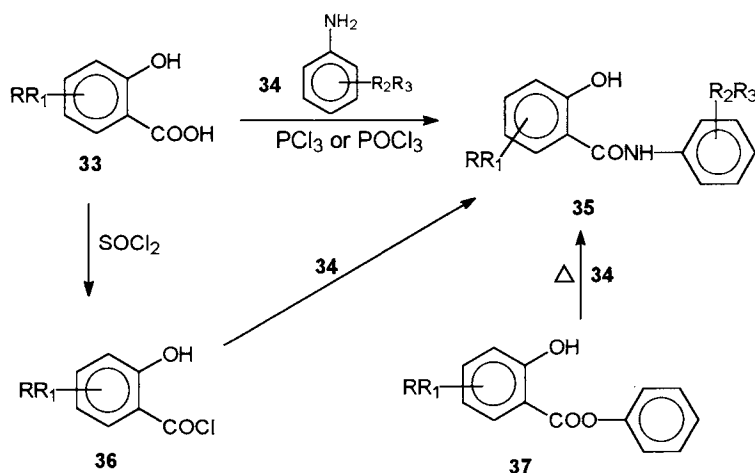
Niclosamide (4) has thus been synthesized by heating a mixture of 5-chlorosalicylic acid (33, R=H, R_1=5-Cl) with 2-chloro-4-nitroaniline (34, R_2=2-Cl, R_3=4-NO_2) in xylene in the presence of PCl_3 [7,52].
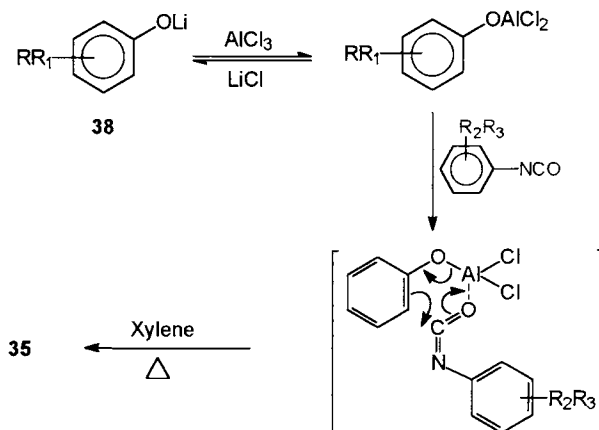
Salicylanilides (35) have also been prepared by *o*-aminocarbonylation of phenols with phenyl isocyanate. Thus, reaction of the lithium salt of phenol (38) with phenyl isocyanate in the presence of aluminum chloride in refluxing xylene affords 15-30% yields of the salicylanilides [66].

4.   BIOLOGICAL ACTIVITY

The salicylanilide drugs have been widely used to treat intestinal tapeworm and liver fluke infections in humans and animals. Their use in the treatment of hydatid diseases, schistosomiasis and nematode infections is limited [11].

4.1   Salicylanilides in veterinary medicine

Niclosamide (4), terenol (5b), oxyclozanide (7), brotianide (8), hilomid (9a,b), rafoxanide (12), clioxanide (13) and closantel (14) are used to eradicate tapeworm and fluke infections from domestic animals [9-11,67-69].

Niclosamide (4): It is a safe and highly effective drug for treating tapeworm infections in cats, dogs, sheep, cattle, horses and poultry. A dose of 100-125 mg/kg of the drug has been found to provide excellent activity against *Taenia ovis, T. hydatigena, T. taeniformis, T. pisiformis* and *M. multiceps* in cats and dogs. Niclosamide also possesses 100% activity against *E. granulosus* in dogs at a dose of 50 mg/kg [69a]. The drug shows high activity against the intestinal tapeworms, *Moniezia expansa, M. benedeni, Thysaniezia giardi* and *Avitellina* spp. in sheep at an oral dose of 50-75 mg/kg; however, its activity against the bile-duct dwelling tapeworms is not satisfactory [9,67].

In horses, higher doses (200-300 mg/kg) of niclosamide may be needed to eliminate the mature and immature stages of *Anophlocephala* spp. However, the drug exhibits excellent efficacy against *Choanotaenia infundibulum, Hymenolepis* spp., and *Dicranotaenia* spp. etc. in chicken and ducks at an oral dose of 50-80 mg/kg [67-69]. A combination of niclosamide and tetramisole, called niclomisol-O, has been found to be highly effective against the intestinal nematodes, *Haemonchus, Oesophagostomum, Bunostomum* and *Trichostrongylus* spp. in sheep [63b].

Niclosamide shows weak activity against *Fasciola* spp., but is nearly 100% effective against immature paramphistomes in sheep at a dose of 50-90 mg/kg. The drug, therefore, may be used to control paramphistome outbreak in sheep flocks [68,69].

Terenol (resorantel, 5b): At a dose of 65 mg/kg, terenol shows high activity against

Moniezia spp. in ruminants [69]. The above dose of the drug was also effective in removing 80-100% of the paramphistomes (*P. cervi*) from sheep, goats and cattle [68,69]. Terenol is effective against *Gastrodiscus aegypticus* infection in horses [69].

Oxyclozanide (7): This is an effective drug for treating *Fasciola hepatica* infection in sheep and cattle. The usual therapeutic dose of oxyclozanide is 13-15 mg/kg for mature flukes; however, repeated doses (15 mg/kg for 3) may be needed to eliminate immature flukes. Thus the drug may be used to control acute fascioliasis in ruminants [68-70].

Brotianide (8): The drug is currently used to treat fascioliasis in sheep. A dose of 7 mg/kg of brotianide shows 91-99% activity against 7-14 weeks old flukes; however, its activity against 6 weeks old flukes is weak (50-90%). It also possesses 85-90% activity against paramphistomes in sheep and cattle [68,69]. The maximum tolerated dose of brotianide is 27 mg/kg in sheep.

Hilomid (9a,b): At a dose of 60 mg/kg, this mixture of bromosalicylanilides (bromsalans) removes 12 week old *F. hepatica* from sheep. For eliminating flukes above 12 weeks of age, a lower dose of 30 mg/kg is sufficient [72-75]. The maximum tolerated dose of hilomid in sheep is only 60 mg/kg [69].

Rafoxanide (12): Rafoxanide is an effective drug for treating *F. hepatica* infection in sheep and cattle. An oral dose of 10-15 mg/kg of this anthelmintic eliminates 91-99% of the mature flukes and 50-90% of the younger flukes in ruminants. However, the above dose level of rafoxanide has been found to be almost 100% effective against immature and mature *F. magna* in cattle. About 92% of the immature paramphistomes may also be removed from sheep and goats at an oral dose of 15 mg/kg of rafoxanide [68,69]. It is effective against the roundworms, *Haemonchus contortus* and *Oestrus ovis* in sheep. The maximum tolerated dose in sheep is 45 mg/kg [68,69].

Clioxanide (13): The drug has been evaluated against mature and immature liver flukes in grazing sheep causing 98-100% reduction of adult worms of *F. hepatica* at a single oral dose of 25 mg/kg. Even a dose range of 10-25 mg/kg of clioxanide was effective in eliminating adult liver flukes. However, a higher dose of 40 mg/kg was necessary for removing the young flukes. The drug was found to be well tolerated as only 0.03% of the animals died when 25,000 sheep were treated. The maximum tolerated dose of clioxanide in sheep is 100 mg/kg [69,76-78]. Clioxanide, therefore, may be used to cure fascioliasis in sheep and cattle with very low toxicity [79].

Closantel (14): It is a highly effective salicylanilide for eliminating liver flukes, *F. hepatica, F. gigantica* and blood-sucking nematodes, *Haemonchus contortus* in sheep, horses and cattle at an oral dose of 7.5-10 mg/kg [80,80a]. However, its activity against adult flukes is more pronounced than the younger ones. Closantel is also effective against *Bunostomum* spp. in ruminants [70] and against *Fascioloides magna* in sheep [81]. The drug has been shown to be highly active against *H. contortus* resistant to either benzimidazoles or levamisole [71]. The usual recommended dose of clioxanide in sheep is 7.5-10 mg/kg. In cattle it is given at a dose of 2.5 mg/kg as injectable solution [69].

4.2 Salicylanilides in human medicine

Although a number of salicylanilide anthelmintics have been developed, most of them find use in the treatment of cestode and trematode infections in domestic animals. Niclosamide (4) is the only drug which has been extensively used to cure cestode infections in human.

Niclosamide (4): The world-wide clinical experience with niclosamide has established that it is a drug of choice for treating *Taenia solium, T. saginata, Diphyllobothrium latum* and *Hymenolepis nana* infections both in adults and children [5,11,69,82-88]. The recommended adult dose of the drug is 2g/adult given as 4 chewable tablets, preferably after a light meal during one day. Children receive the drug according to their age and weight. For example, a child below 2 years is given 0.5 g (1 tablet), while older children (above 2 years weighing 11-34 kg) receive 1 g (2 tablets) of the drug. Heavier children (above 34 kg) may chew 3 tablets (1.5 g) of the drug [84,89].

Usually the above dose schedule gives very high cure rates against all the intestinal tapeworm infections of man. However, better results may be obtained by using a laxative to clean the bowel before initiating the treatment. Lower doses of the drug have also been used, but in that case prolonged treatment is required and the cure rates are not generally higher than 75%. For a *H. nana* infection the treatment should be continued for 5-7 days for complete clearance of the worms.

Niclosamide has also been used successfully to treat less common tapeworm infections by *H. diminuta, D. caninum, Bertiella studeri, Inermicapsifer madagascariensis, Mesocestoides* spp. and *Raillietina celebensis* in humans at a dose of 1-2 g/patient [86,90]. It is a well tolerated drug but may produce minor side effects such as nausea, diarrhea, abdominal discomfort, fever, headache and pruritus etc. in a small percentage of treated patients.

5.   MODE OF ACTION OF SALICYLANILIDES

Niclosamide is a potent uncoupler of the electron-transport associated oxidative phosphorylation in mitochondria *in vivo* and/or *in vitro* [91-94]. Accordingly, the drug has been shown to inhibit the anaerobic incorporation of ^{32}Pi into ATP and the ^{32}Pi-ATP exchange reaction in mitochondria of *H. diminuta* [91]. However, niclosamide has little effect on various enzymes *in vitro* except it stimulates adenosine triphosphatase (ATPase) in isolated mitochondria [95]. Sano and coworkers [96] were able to show that niclosamide exerts *in vitro* a spastic and/or paralytic action on the motility of various preparations including nematodes (*Angiostrongylus cantonensis*), cestodes (*D. caninum*) and trematodes (*F. hepatica* & *S. japonicum*) at concentrations of 3.10^{-9} to 3.10^{-5}M. This spastic and/or paralytic action of niclosamide is reported to occur through a neuropharmacological mechanism involving acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) in *D. caninum*. It has also been suggested that the action of salicylanilides on oxygen uptake and oxidative phosphorylation through ATPases may be explained on the basis of its neuropharmacological effects and release of neurotransmitters.

Further work on the mode of action of niclosamide has shown that the drug also affects the mitochondrial ATP synthesis in the *Ascaris* muscle [97] and inhibits glucose uptake in *Cotugnia diagnophora* [98,99]. The low level of toxicity/side effects of niclosamide is due to its almost absent absorption through the gastrointestinal tract. This protects the host from the uncoupling properties of the drug [94].

The fasciolicidal activity of oxyclozanide (7), rafoxanide (12) and closantel (14) is due to their ability to uncouple the oxidative phosphorylation [70,71,100a]. However, rafoxanide also inhibits *in vitro* the malic and fumarate-reductase activity of *F. hepatica* [100b]. Detailed *in vitro* and *in vivo* experiments carried out with closantel have shown that it acts as a proton ionophore causing uncoupling of the electron-transport mediated phosphorylation. Thus uncoupling appears to be the primary mode of action of this drug against *F. hepatica* [100]. This was evident by the fact that closantel inhibits the malate induced phosphorylation in the mitochondria of *F. hepatica* both *in vitro* and *in vivo*. There was a marked depression in ATP levels and elevation of AMP. The net ATP:ADP ratio fell from 1.2 to 0.77. Since reduction in ATP level is the initial consequence of an uncoupling effect, the observation of lower values of the ATP:ADP ratio may suggest that closantel interferes with the glycolytic path instead of the electron transport in the helminths [101,102].

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CHAPTER 10

NITROARYL COMPOUNDS

1.   INTRODUCTION

The nitroaryls and nitroheterocycles are considered to be medicinally a valuable group of compounds, which have drawn much attention on account of their broad spectrum of activity including antibacterial, antiprotozoan and antihelminthic activity. The biological activities of nitro compounds are attributed to their ability to undergo cytochrome P-450 dependent reduction to form reactive species like Ar-NHOH and $Ar-NH_2$, which interact with DNA and disturb its normal function in different pathogens. This property of nitro compounds is undoubtedly related to their ability to kill a wide variety of microbes and parasites; at the same time it may impart to them mutagenicity and carcinogenicity, and some drugs have had to be withdrawn from the market [1-3] because of this.

As early as 1940's nitrofurans caught attention by virtue of their good antibacterial activity. Exploitation of this lead in helminth chemotherapy showed that nitroheterocycles may be useful in the treatment of various worm infections in different hosts including humans. Consequently, various laboratories set out to search for new anthelmintic drugs derived from nitroheterocycles and nitroaryls, opening one of the fascinating chapters in the chemotherapy of parasitic diseases.

2.   NITROFURANS

The nitrofurans are primarily known for their antibacterial activity; however, a few compounds have been found to possess marked activity against filariasis and schistosomiasis. In experimental filariasis nitrofurazone (1), nitrofurantoin (2), nifurtimox (3), furazolidone (4) and furapyrimidone (5) have been shown to kill both the microfilariae and adult worms of *L. carinii* at parenteral doses ranging from 15-150 mg/kg for 5 days [4-9]. Of these, nifurtimox (3), furazolidone (4) and furapyrimidone (5) have shown good activity against human filariasis.

Following the observation made by Chinese scientists that nitrofurazone (1) possesses prophylactic activity against *S. japonicum* in albino rats [10,11], a large number of nitrofurans were synthesized as potential schistosomicidal agents, of

which F-30066 (furapromidium, 6) and F-30385 (7) emerged as potent anthelminthics [12,13]. Another nitrofuran, SQ-18506 (8), has also been shown to exhibit high activity against gram-negative and gram-positive bacteria, fungi and schistosomes [14-16]. Unfortunately the mutagenic potential of various nitroheterocycles makes this class of compounds suspect as therapeutic agents [17,18].

O_2N — O — $CH=NR$

1 R = NHCONH$_2$

2 R = —N

3 R = —N SO$_2$

4 R = —N

5 R = —N

O_2N — O — $CH=CH—R$

6 R = CONH-iPr
7 R = CONHCH$_2$CH$_2$—N

8 R =
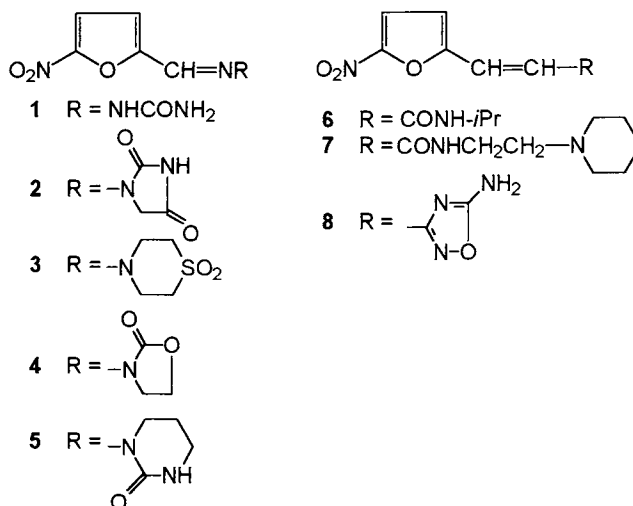
3. NITROTHIOPHENES

A few nitrothiophenes have been shown to possess marked schistosomicidal activity, though weaker than nitrofurans or nitrothiazoles. Of these, compounds 9 and 10 exhibited potent activity. Compound 9, which bears a close resemblance with furapyrimidone, caused 100% elimination of *S. mansoni* from mice at a dose of 250 mg/kg [19], while 10 was found to be active against *S. mansoni*, *S. japonicum* and *S. haematobium* infections in experimental animals and also proved to be less toxic in dogs and cebus monkeys [20a]. Alaimo and Hatton [20b] have prepared a

O_2N — S — $CH=N—N$

9

O_2N — S —

10

R = H, Cl
R$_1$ = Hydroxy alkyl
R$_2$ = H, NH$_2$, alkyl, hydroxy alkyl

10a
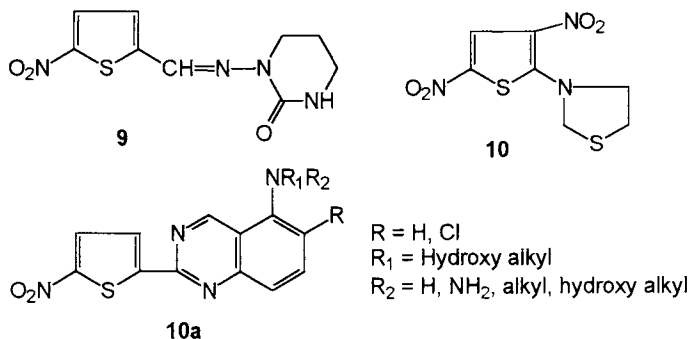
series of 5-nitro-2-substituted thiophenes of which 2-(5-nitro-2-thienyl)-4-dihydroxy-alkylaminoquinazolines (10a) were found to be active against *Ascaris suum, Syphacia obvelata* and *Hymenolepis nana* at a dose of 12.5-300 mg/kg.

4.   NITROIMIDAZOLES

Some 5-nitro-1,2-disubstituted-imidazoles of the type 11 and 12 have been shown to have appreciable *in vitro* and *in vivo* antischistosomal activities, but none find use in clinical practice [21]. However, metronidazole (13) has been used widely to treat guinea worm (*Dracunculus medineusis*) infection in human [22].
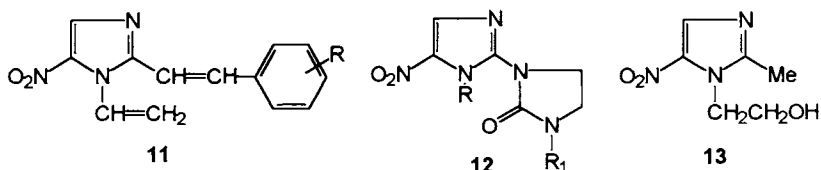
5.   NITROTHIAZOLES

In 1955, Cuckler and coworkers [23] demonstrated the antischistosomal activity of 2-acetamido-5-nitrothiazole (14); this compound was found to be marginally effective in reducing the schistosome population when given at a concentration of 0.1-0.4% in diet. A major breakthrough was achieved when Ciba laboratories undertook an extensive search for potential antibacterial and/or antiparasitic agents derived from nitroheterocycles. In 1964, Lambert *et al.* [24,25] announced the discovery of niridazole (15) as a novel drug for the treatment of schistosomiasis.
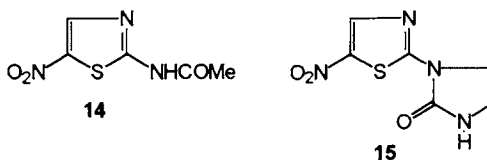
The discovery of niridazole initiated a world-wide search for more effective agents by carrying out structural modifications at the 2- and 5-positions of the thiazole nucleus [26]; however, none was found to be better than the parent drug. The SAR studies indicate that presence of a nitro group at 5-position of thiazole is the most important requirement as replacement of this group by a variety of other electron withdrawing groups led to compounds with poor or no activity [27-29]. An exception to this fact is compound 16, which exhibited high schistosomicidal activity in

mice and monkeys at a dose of 338 and 400 mg/kg, respectively [26,30]. However, various ureas of type **17** and **18** were found to be devoid of antischistosomal activity [27].

16

17

18

Having established that a nitro function is essential for activity, a major thrust was directed towards synthesizing 5-nitro-2-substituted thiazoles. However, activity was usually associated with those 5-nitrothiazoles having substituents linked to the thiazole ring through a nitrogen atom. A few 5-nitro-2-substituted thiazoles which exhibited marked activity against experimental schistosomiasis, are **19a,20-24** [26,31-34], while compounds of type **19b,25** and **26** showed poor or no activity [27,35].

19a X = CH$_2$
b X = O

20 n = 1,2

21

22

23

24

25

26

Henry [36] prepared a series of 5-nitrothiazoles with a C-C rather than C-N bond at their 2-position. Interestingly, compounds **27a** and **27b** were found to cause reversible abnormalities in the reproductive system of female blood flukes [37].

27a   **27b**

A further probe in the SAR of nitrothiazoles led scientists at Parke-Davis laboratories to discover the high order of schistosomicidal activity in 5-nitro-4-thiazolines of type **28-31**. Many of the compounds of this class have been found to be highly effective against *S. mansoni* in mice and monkeys [38-40].
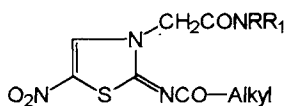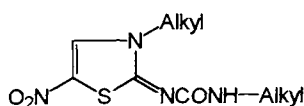
28   **29**
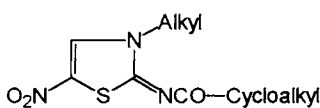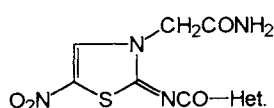
30   **31**

6.   NITROPHENYLS

During a systematic exploitation of the antiparasitic potential of nitroaryls, the synthesis of various substituted nitrobenzenes was carried out, many of which exhibited marked anthelmintic acitivity. The promising candidates of this class are amoscanate (**32a**), nitroscanate (**32b**), amocarzine (CGP-6140, **33**) and nitrodan (**34**). Of these, amoscanate and CGP-6140 have been developed by Ciba for treating schistosomiasis and filariasis, respectively [41-43].
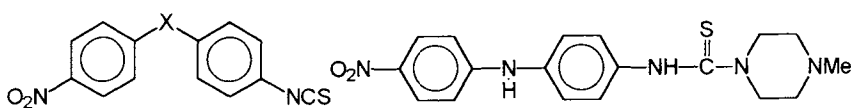
32a X = NH   **33**
b X = O
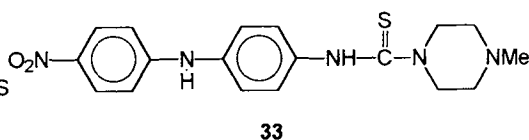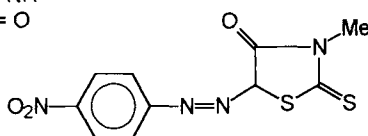
34

7. SYNTHESIS

The synthesis of nitrofuran and nitroimidazole drugs will be discussed in Chapter 17. In this chapter the synthesis of only those nitroaryls will be described, which are of significance in the chemotherapy of helminthiasis.

7.1 Niridazole (15)

The starting material for niridazole is 2-amino-5-nitrothiazole, which is treated with 2-chloroethylisocyanate to form the urea 35, which is cyclised in the presence of a base to yield niridazole [24,44].

35
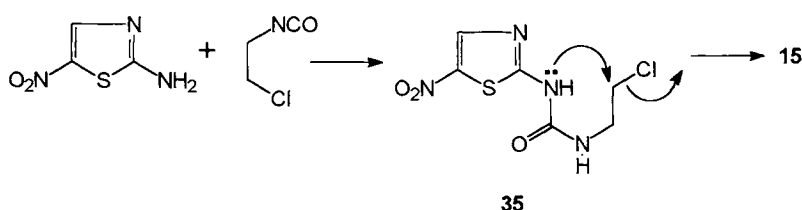
7.2 Amoscanate (32a)

Brenneisen *et al.* [45-49] prepared amoscanate by treating 4-amino-4'-nitrodiphenylamine (36) with thiophosgene (CSCl$_2$), ammonium thiocyanate (NH$_4$SCN), N,N-diethylthiocarbamoyl chloride (Et$_2$N-CS-Cl) or bis (N,N-diethylthiocarbamoyl) disulfide (Et$_2$N-CS-S-S-CS-NEt$_2$) [50]. Other methods to obtain amoscanate (32a) have also been described [51,52].

36

32a

40 X = O,S

38 X = O,S

39
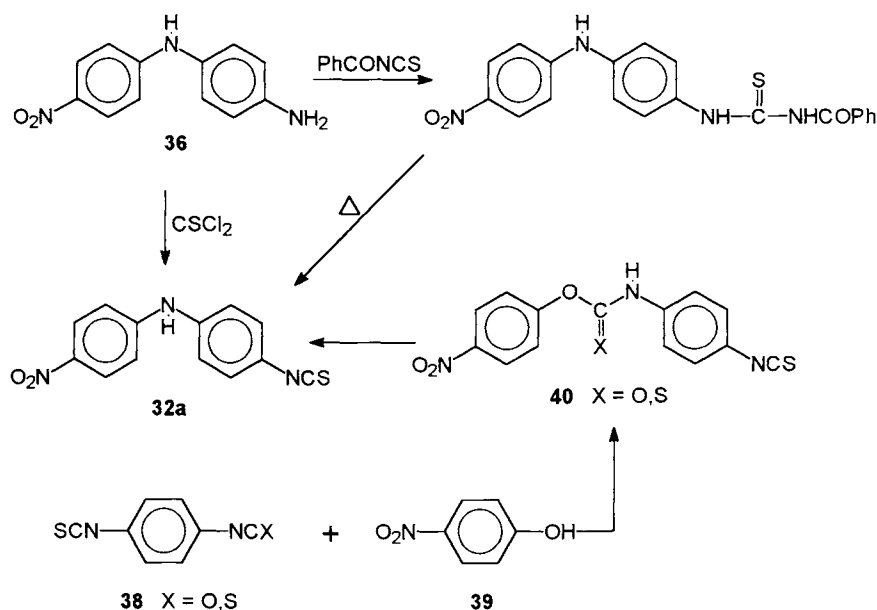
7.3 Nitroscanate (32b)

Several methods are available for preparing nitroscanate [53-56]. A convenient method to obtain nitroscanate (32b) involves reaction of 4-(4-aminophenoxy)nitrobenzene (41) with thiophosgene [53-55].
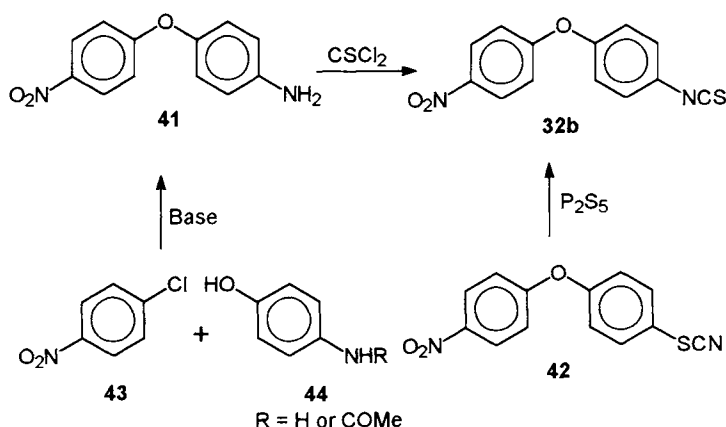
8. BIOLOGICAL ACTIVITY

A number of nitrophenyl and nitroheteroaryl drugs have been found to be effective in treating helminth infestations in humans and domestic animals [57,58].

8.1 Nitroaryls in veterinary medicine

(a) **Niridazole (15):** This drug has been found to be 100% effective against *Schistosoma matthei* in sheep and goats at an oral dose of 100 mg/kg given for 3 days [59].

(b) **Amoscanate (32a):** A single intravenous injection of 1.5-2 mg/kg has been shown by Quin *et al.* [60] to provide a satisfactory response against *Schistosoma japonicum* in cattle and buffaloes.

(c) **Nitroscanate (32b):** This drug was introduced in 1973 by Ciba as an effective agent for the cure of tapeworm and roundworm infections in cats and dogs [57]. The micronised form of the drug has been shown to be highly effective against *Taenia* spp. and *Dipylidium caninum* in dogs at a single oral dose of 50 mg/kg [61-63]. Nitroscanate is also effective against *Echinococcus granulosus*; however, better activity was obtained by using the micronised preparation [61,62]. More recently, nitroscanate has been shown to possess 98-99.8% activity against *D. caninum* in dogs at a single oral dose of 50-56 mg/kg [64,65].

Nitroscanate exhibits 99.6-99.8% activity against the hookworm, *Ancylostoma caninum* in dogs at a single oral dose of 50 mg/kg. However, it has no action against *Trichuris vulpis* [62,64].

(d) **Nitrodan (34):** When given in diet at a concentration of 0.016-0.1%, it removed pinworms, *Syphacia obvelata* from mice, *Ascaridia galli* from chickens and *Toxocara canis, Ancylostoma caninum* and *Uncinaria stenocephala* from dogs [66]. Nitrodan has no activity against *Toxascaris leonina* in dogs [67].

8.2   Nitroaryls in human medicine

Some members of this class have emerged as effective drugs for the treatment of helminth diseases in humans [22,42,43,57,58].

8.2.1   Nitrofurans

Furazolidone (4) has no action on microfilariae and adult worms of *W. bancrofti* in humans upto an oral dose of 12 mg/kg (600 mg/adult) given for 5 days [68]. However, nifurtimox (3) has been found to provide clinical improvement of patients infected with *O. volvulus* at a dose of 15-20 mg/kg for 5 days [69]. The most effective drug derived from nitrofurans is furapyrimidone (5), which has undergone extensive clinical trials against lymphatic filariasis in China. The drug was found to cure patients infected with *W. bancrofti, B. malayi* or *D. perstans* when administered orally at a dose of 15-20 mg/kg given for 6 days. Consequently, two dose schedules, 15-20 mg/kg for 6 days and 20 mg/kg for 7 days have been suggested for the treatment of Malayan and Bancroftian filariasis, respectively. The side effects of furapyrimidone are gastrointestinal irritation, fever, headache and fatigue [70].

8.2.2   Nitrothiazoles and nitroimidazoles

(a) **Niridazole (15):** Niridazole is the most successful compound of this class which has been used to treat schistosomiasis in human. The drug has been found to be highly effective against *S. haematobium* and *S. mansoni*, but is less effective against *S. japonicum* and *S. intercalatum* infections.

Niridazole at an oral dose of 25 mg/kg given for 5-7 days [71-74] or of 30 mg/kg for 4 days [75,76] produced 80-100% cure rates against *S. haematobium* infections in children and adults and was also well tolerated. A higher dose of 50 mg/kg of the drug given three times a day has been shown to be highly effective in treating urinary schistosomiasis in human [77].

The activity of niridazole against *S. mansoni* has been reviewed [74,78,79]. The drug produces 40-100% cure rates at an oral dose of 25-30 mg/kg given for 5-7 days [72]. Higher cure rates (80-100%) may be obtained against *S. mansoni* if the drug is given at a dose of 25-30 mg/kg for 7 days or 35 mg/kg for 5 days [79-81]. A lower dose of 12.5-15 mg/kg has also been used to treat *S. mansoni* infections. This dose level produces lesser side effects [82]. Niridazole shows poor activity against *S. intercalatum*, but is moderately effective against *S. japonicum* in humans [42].

The side effects of the drug are nausea, vomiting, diarrhea, anorexia, abdominal pain and headache, which depend largely on the dose and duration of the treatment. The side effects are usually mild and of short duration, occurring more frequently in adults than in children [73]. The mental and neurological side effects including depression, mania, confusion, convulsions, hallucinations and coma, particularly in patients with severe liver damage [72,83], are more worrying. Rarely the use of niridazole is fatal [84]. The drug is contraindicated in patients with liver, heart and mental diseases or epilepsy. Niridazole is also not advised to persons already on isoniazid therapy [81,85].

(b) Metronidazole (13): This is the drug of choice for the treatment of guinea worm (*Dracunculus medinensis*) infection in humans. The recommended adult dose of the drug is 250 mg, given orally thrice daily for 10 days. Children receive metronidazole at a dose of 25 mg/kg/day (max. 750 mg/day) in three divided doses for 10 days [86]. A better clinical response may be obtained by administering 800 mg of the drug thrice daily for 5-7 days [87-89].

Metronidazole is a well tolerated drug causing expulsion or lysis of the guinea worms. However, the patient may experience nausea, vomiting, weakness, insomnia, headache, dry mouth and metallic taste.

8.2.3 Nitrophenyls

(a) Amoscanate (32a): It is a broad-spectrum anthelmintic possessing high activity against gastrointestinal nematodes, filariids, schistosomes and tapeworms [90]. The drug has been found to be effective in eliminating *A. duodenale* and *N. americanus* from humans at a dose of 100-200 mg/kg [91]. Amoscanate has been evaluated against human schistosomiasis in China using a dose of 7 mg/kg given for 3 days when high activity was observed [81].

(b) Amocarzine (CGP-6140) (33): This is an orally effective drug for treating onchocerciasis in human. Poltera [92,93] has evaluated amocarzine in patients with *O.*

volvulus at an oral dose of 20 mg/kg. The drug exhibited high activity and was also found to be well tolerated. In a pilot study carried out by Guderian *et al.* [94], CGP-6140 was given at a dose of 3 mg/kg, twice daily, postprandial for 3 consecutive days when high micro- and macrofilaricidal activity against *O. volvulus* was observed. The drug killed 65% of the adult female worms, 20% became necrobiotic and 15% were alive.

9. MODE OF ACTION

The mode of action of nitrofurans and nitroimidazoles has been discussed in Chapter 17. The anthelmintic action of a few other nitroaryl drugs are given below.

9.1 Niridazole (15)

The mode of action of this drug is not fully understood. It appears that niridazole inhibits phosphorylase phosphatase with subsequent potentiation of phosphorylase. This may result in depletion of glycogen levels in the schistosomes. Thus, the major action of niridazole seems to be on the glycogen metabolism of the helminths. The drug also causes structural damage to the reproductive system of female schistosomes [95,96].

Another possible mechanism of action of niridazole involves the inhibition of DNA synthesis in schistosomes, which is due to the presence of a 5-nitro function that undergoes enzymatic reduction to form reactive species. It has been suggested that the reactive species of niridazole may bind covalently to the parasite's macromolecules causing a decrease in nonprotein thiol content leading to death of the helminths [2,97,98].

9.2 Amoscanate and nitroscanate (32a,b)

Although the mode of action of amoscanate (32a) is not clearly known, the isothiocyanate group present at one of the phenyl rings may play a significant role in its anthelmintic action. Thus, the drug not only binds irreversibly to amino groups of amino acids and proteins to form thiourea derivatives [99], but may also inhibit the activity of various enzymes catalysed by thiols. The inhibition of the glycolysis as a result of inhibition of PFK may be visualized to be a consequence of interference with thiol activity by amoscanate [96,100]. A further support to this assumption may be drawn from the fact that amoscanate causes reduction in the levels of lactate, succinate and acetate in *H. diminuta*, *B. pahangi* and *L. carinii* [101,102].

It has also been shown that subcurative doses of amoscanate cause tegumental changes in *S. mansoni*. The alteration of tegument includes extensive swelling, constriction and erosion on a large surface area. It has, therefore, been suggested that the drug may be associated in inhibiting the repair and sythesis of tegumental membrane of schistosomes [103,104].

Nitroscanate (**32b**) inhibits ATP synthesis in the liver flukes, *Fasciola hepatica* [105].

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CHAPTER 11

TETRAHYDROQUINOLINES AND ISOQUINOLINES

1. INTRODUCTION

Although the role of tetrahydro(iso)quinolines as a nucleus for building anti-parasitic drugs was established with the discovery of emetine and dehydroemetine, it got highlighted in the begining of the 1970's when two drugs, oxamniquine and praziquantel were introduced in the chemotherapy of human helminth diseases, particularly for schistosomiasis [1]. Oxamniquine was discovered by Pfizer in 1969, while praziquantel was developed jointly by E. Merck and Bayer and was introduced on the market in 1976. The discovery of these two drugs was the outcome of a systematic SAR study of tetrahydroquinolines and tetrahydroisoquinolines, which forms one of the most fascinating chapters in medicinal chemistry.

2. TETRAHYDROQUINOLINES

The story of the development of oxamniquine started from the work of Kikuth *et al.* [2] who introduced lucanthone (miracil D, **1a**), a thioxanthone derivative, in the chemotherapy of schistosomiasis in 1946. In the biophase, the 4-methyl group of lucanthone is converted into the corresponding 4-hydroxymethyl derivative. This led Rosi, Berberian and their colleagues working at Sterling Winthrop to develop hycanthone (**1b**) as a better human schistosomicide than lucanthone [3-6].

In 1956, Mauss *et al.* [7] observed that the *p*-toluidine part of the miracils (lucanthone and hycanthone) plays an important role in evoking antischistomal activity. Consequently, these authors prepared a series of substituted *p*-toluidines of type 2 (called mirasans), which exhibited marked activity against schistosomes [8-11]. This

1a R = H, n = 2
b R = OH, n = 2
(Miracils)

2
(Mirasans)
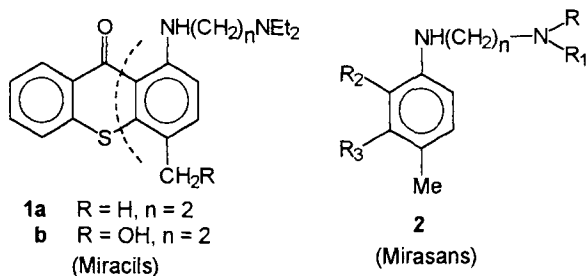
study also pointed out to the fact that the activity resides only in those *p*-toluidines, in which the nitrogen is substitued by an aminoethyl group [12].

Encouraged by the above observations, scientists at Sterling-Winthrop synthesized a large variety of mirasan derivatives [13-16] of which 3-5 exhibited high activity against *S. mansoni*, but none was found to be of clinical importance [12,16].

$R-CH_2$——⟨⟩(Cl)—$NH(CH_2)_2NEt_2$

3a   R = H
b   R = OH

$R-CH_2$——⟨⟩(Cl)—N⟨⟩NH

4a   R = H
b   R = OH

$R-CH_2$——⟨⟩(Cl)—N⟨⟩N—$(CH_2)_6$—O—⟨⟩—$C(CH_3)(CH_3)CH_2Me$

5a   R = H   (Teroxalene)
b   R = OH
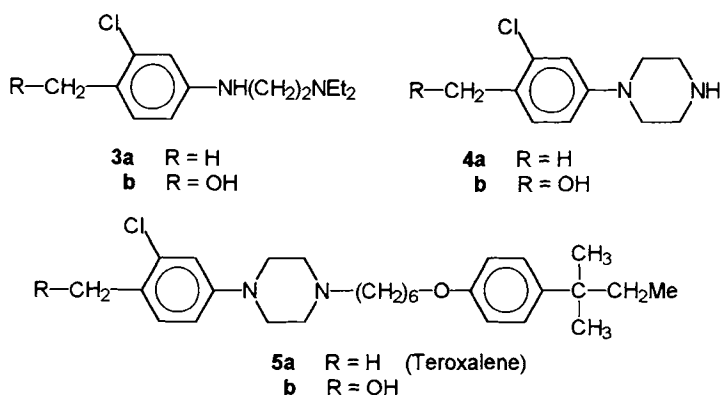
In a further effort to design better antischistosomal agents, it was considered rational to synthesize conformationally rigid analogues of mirasans, where the nitrogen of the *p*-toluidine forms the heteroatom of the quinoline skeleton. A group at Pfizer designed and synthesized the conformationally strained mirasans of type 7 and 8, which may be considered to have been obtained by freezing the free rotation in the manner shown below [17,18].

6

7a   R = H, R_1 = Cl
b   R = Cl, R_1 = H

8a   R = H, R_1 = Cl
b   R = Cl, R_1 = H
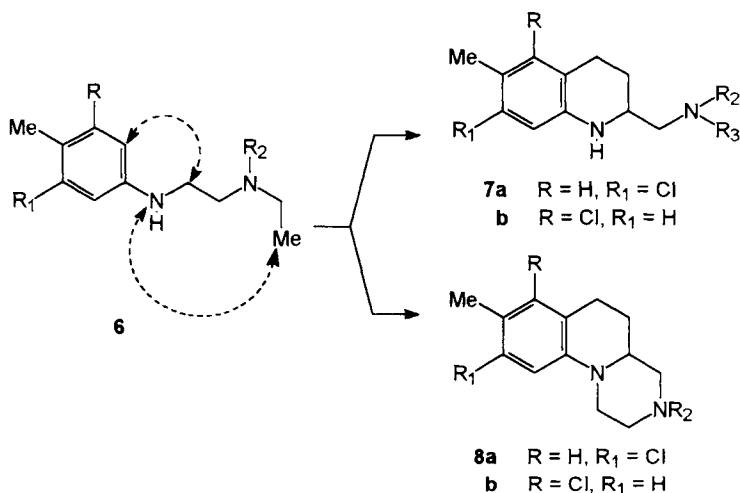
The compounds belonging to class **7a,b** and **8a,b** were evaluated for their schistosomicidal activity against *S. mansoni* in mice and monkeys [19,20]. In general, compounds derived from 2-aminomethyl-1,2,3,4-tetrahydroquinoline (**7a,b**) as well as 2,3,4,4a,5,6-hexahydro-1H-pyrazino[1,2-a]quinoline (**8a,b**) exhibited high schistosomicidal activity in experimental animals; however, promising activity was observed in 2-(N,N-dialkylaminomethyl)-1,2,3,4-tetrahydroquinolines (**9**) [12,17,21].

The SAR studies carried out in **9** indicated that high schistosomicidal activity is obtained when an electron withdrawing group is present at 7-position only. The activity order as consequence of the substitution at 7-position in **9** was NO_2>CN>F>Cl>Br. This is in direct contrast with the mirasan series, where this activity order is reversed (halogen>CN>NO_2). These results may be explained in terms of lipophilicity [17].

The presence of the methyl group at 6-position in **9** was equally important for schistosomicidal activity as the 6-desmethyl and 6-ethyl analogues (**10a,b**) were found to be inactive. In addition, introduction of aromaticity in the piperidine part of **9** (eg. **11**), substitution at 1-position or extension of the chain length at 2-position (**12**) resulted either in lowering or loss of schistosomicidal activity [12].

9

10a R = H
  b R = Et

11

12 X = CH_2CH_2 or -CH(Me)-
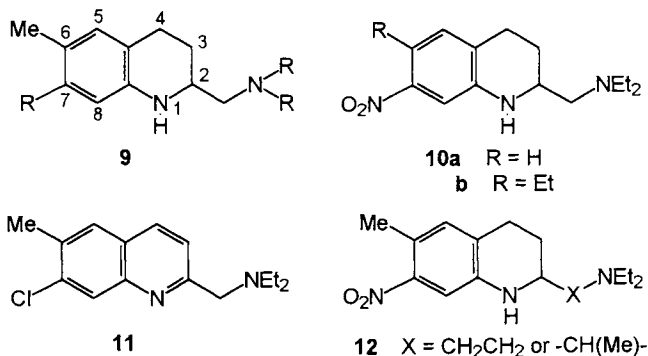
Replacement of the diethylamino group by other secondary amines with an open chain or cyclic structures resulted in decrease in activity. However, introduction of secondary amino functions enhanced the activity; the optimal activity was found in 2-[(isopropyl)aminomethyl]-6-methyl-7-nitro-1,2,3,4-tetrahydroquinoline, called U.K. 3883 (**13**) [12,16]. Exploiting the knowledge that hycanthone is the active metabolite of lucanthone, which may be prepared by microbial oxidation, the Pfizer scientists subjected **13** to microbiological oxidation in the presence of *Aspergillus sclerotiorum* and obtained the hydroxymethyl derivative of UK-3883, which was later known as UK-4271 or oxamniquine (**14**) [22,23]. Oxamniquine proved to be a better

schistosomicide than UK-3883 and subsequently became the drug of choice for the treatment of *S. mansoni* infections in humans [1,24].

13 (UK-3883)   14 (UK-4217)
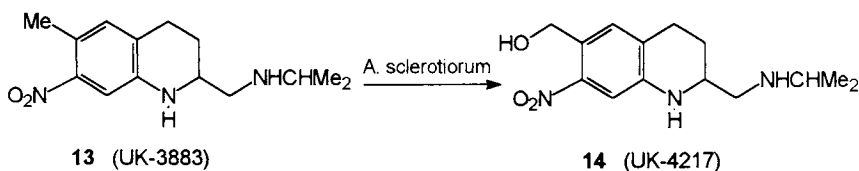
3.   TETRAHYDROISOQUINOLINES

Praziquantel (15) is the outstanding member of this class discovered jointly by E. Merck and Bayer AG, Germany [25]. The drug is known for its excellent activity against blood flukes and tapeworms both in domestic animals and humans [26-30].

The discovery of the anthelmintic activity of the pyrazinoisoquinolines [25,31,32] initiated the synthesis of a variety of substituted pyrazinoisoquinolines. Praziquantel was picked up from more than 400 1,2,3,6,7,11b-hexahydro-4H-pyraz-ino[2,1-a]isoquinolin-4-ones and related compounds, because of its potent and broad spectrum biological activity [26]. The structure activity relationship in the analogues of praziquantel would indicate that positions 2 and 4 are the most critical positions, which govern the cestodicidal as well as antischistosomal activities in the pyrazinoi-soquinolines.

15   16   R = H, NH₂, F   17   X = CH₂, SO₂   18

19   20   21   22
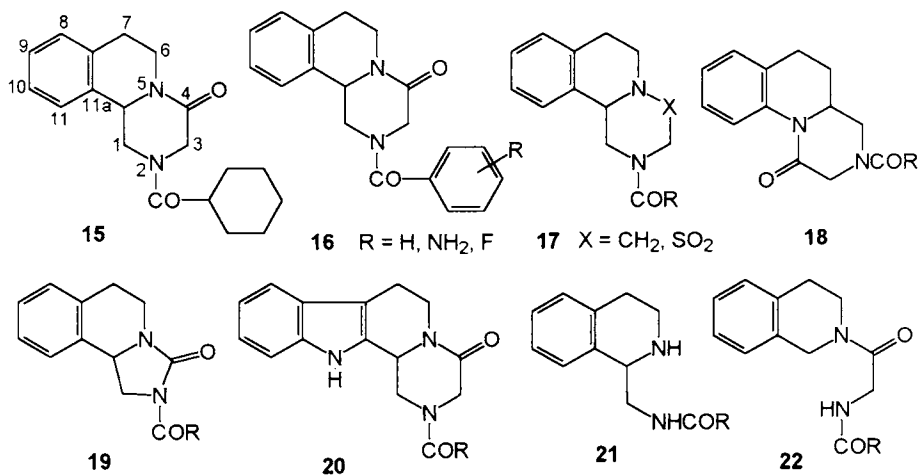
The presence of an acyl or thioacyl group at 2-position is essential for biological activity. Amongst the 2-aroyl derivatives, the 2-benzoyl analogue (16) of praziquantel exhibited very high activity. A further enhancement of activity was achieved by introducing amino (at *m* and *p*) or fluoro (at *o* and *m*) substitution in the benzoyl ring. Among aliphatic acyl groups, the presence of an alkylcarbonyl function was found to lower the activity. Optimal activity was obtained by introducing cycloalkylcarbonyl groups at 2-position. In 2-cycloalkylcarbonyl derivatives activity increased in going from three membered to six membered ring.

The presence of a carbonyl or thiocarbonyl group at 4-position is another important structural requirement as substitution of this group by CH_2 or SO_2 (17) resulted in loss of activity. The SAR results also made it evident that the anthelmintic activity was associated only with the hexahydropyrazino[2,1-a]isoquinoline skeleton. This was obvious from the synthesis of various compounds of type 18-22, none of which exhibited significant anthelmintic activity [26].

Some praziquantel derivatives with substitutions in the benzene ring and other positions of the ring system have also been synthesised. Such structural variations had minor influence on the biological activity. Consequently a number of active analogues were found, but none exceeded the potency of praziquantel [26,33].
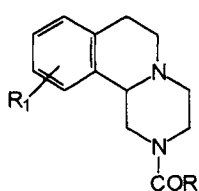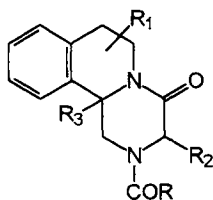
23   R_1 = NH$_2$, Me   24 R_1, R_2, R_3 = H, Me   25
R = Morpholine, piperazine or
benzimidazole
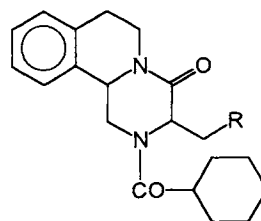
Some pro-drugs of praziquantel (25) have been prepared by a Mannich reaction on praziquantel. A few compounds, thus prepared, showed better activity than the parent drug when tested against *S. mansoni* in mice [34]. Since praziquantel has an asymmetric carbon atom, it has also been resolved into its D- and L-isomers. It has been shown that the anthelmintic activity resides only in laevo-isomer called laevopraziquantel [1,26,35].

4. SYNTHESIS

4.1 Oxamniquine (14)

The synthesis of oxamniquine (14) has been reported by Baxter and Richards [17,18]. The key intermediate is 2-isopropylaminomethyl-6-methylquinoline (31), which may be obtained by three routes as given in scheme 1. Hydrogenation of 31
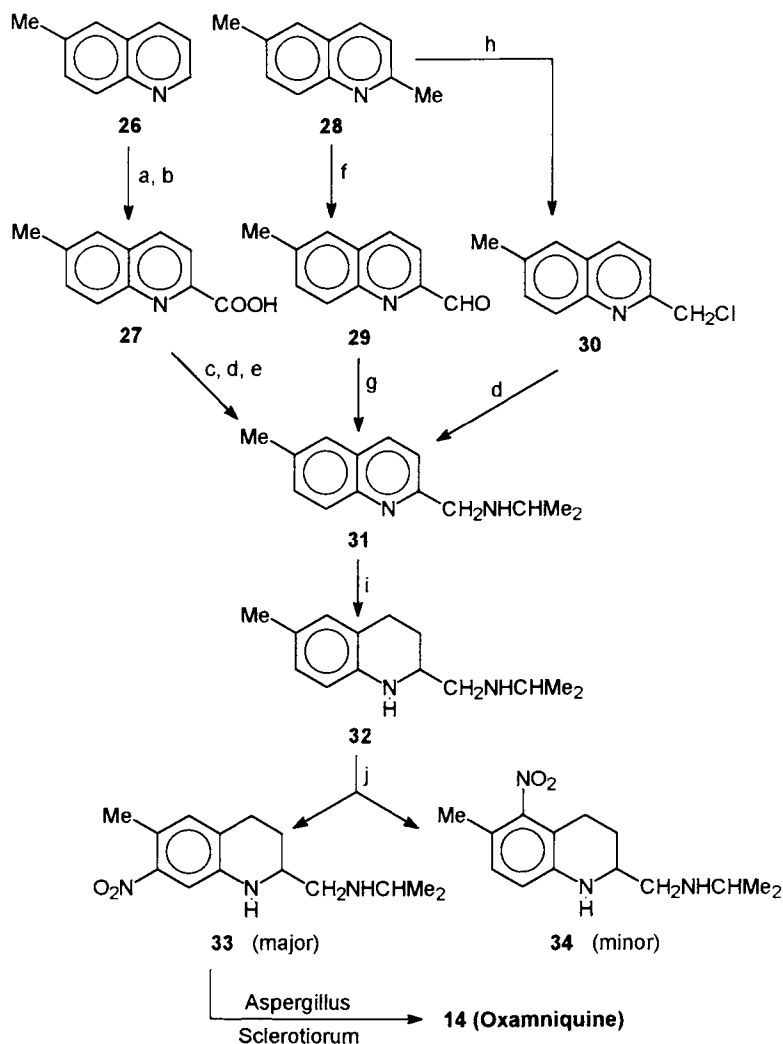
Scheme 1

Reagents: (a) Reissert reaction, (b) HBr in AcOH, (c) PCl₅, PhMe, (d) H₂NCHMe₂, (e) LAH, (f) SeO₂, (g) reductive amination, (h) Cl₂ in CCl₄, Na₂CO₃, (i) Ra-Ni; H₂, (j) HNO₃, H₂SO₄.

over Raney nickel, followed by nitration of the tetrahydroquinoline derivative (32) gave a mixture of 33 (major product) and 34, of which 33 was separated by fractional crystallization. Oxidation of 33 by a fermentation technique using a strain of *Aspergillus sclerotiorum* resulted in its quantitative conversion into oxamniquine [18] (Scheme 1).

4.2 Praziquantel (15)

Seubert *et al.* [25] have reported the first synthesis of praziquantel from the intermediate 37 obtained by the Reissert reaction of isoquinoline and followed by high pressure hydrogenation of the cyano compound 36 [36]. Acylation of 37 followed by base catalysed cyclisation of the diacylated product affords praziquantel [25,37,38] (Scheme 2).
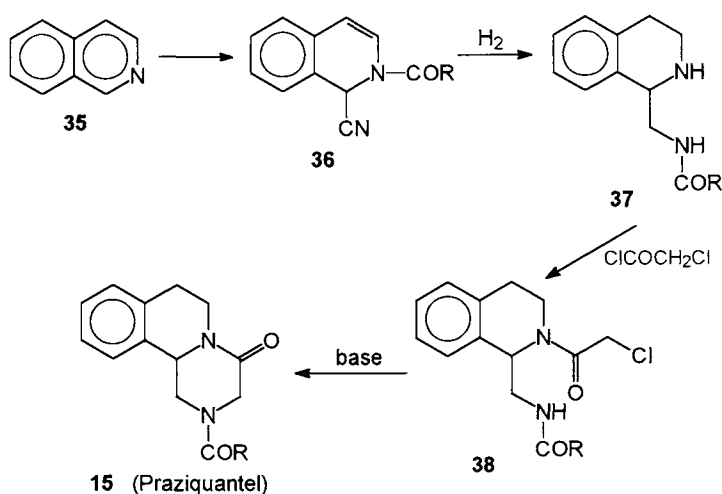
Scheme 2

Frehel and Maffrand [39] have developed two elegant methods of preparing praziquantel, which are outlined in schemes 3 and 4.

Scheme 4 utilizes basically the reaction sequence followed in scheme 3 to prepare 48, which is hydrolysed to give 49. Acylation of the latter with cyclohexanecarbonyl chloride affords praziquantel [39].
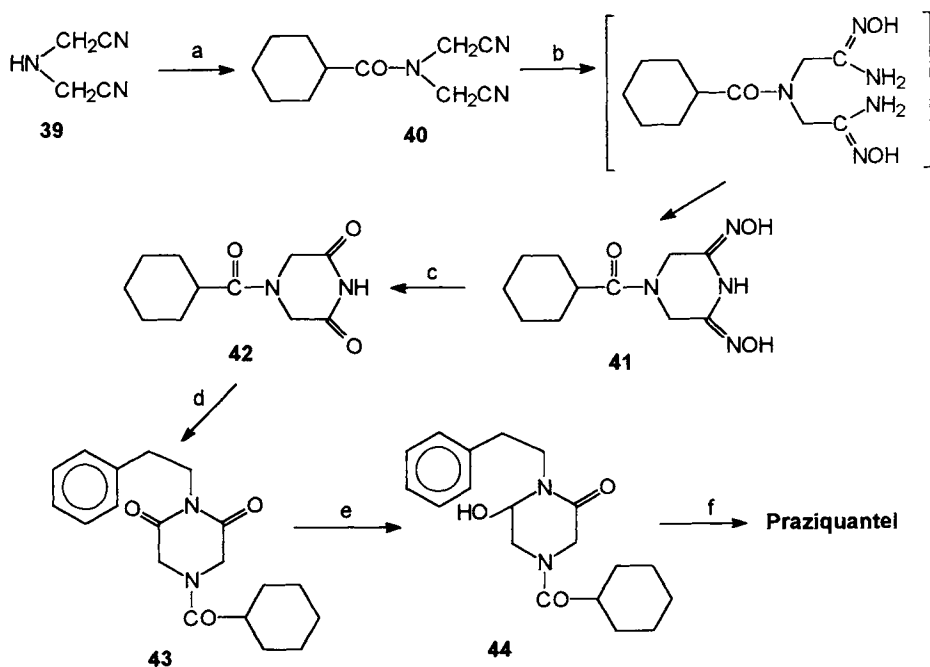
Scheme 3

Reagents: (a) c-C$_6$H$_{11}$COCl, K$_2$CO$_3$, (b) NH$_2$OH, K$_2$CO$_3$, HCl, (c) NaNO$_2$, H$_2$O, (d) MeONa, DMF, PhCH$_2$CH$_2$I, (e) CuCl$_2$, EtOH, NaBH$_4$, (f) 12N HCl.
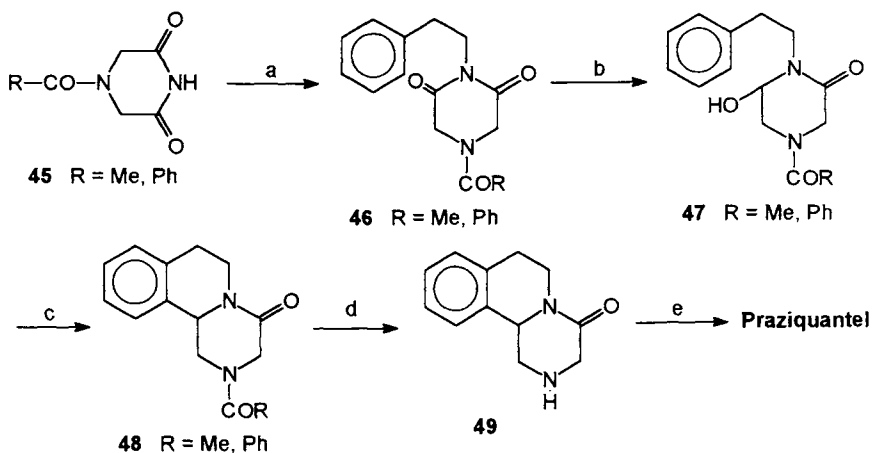
Scheme 4

Reagents: (a) MeONa, DMF, PhCH$_2$CH$_2$CH$_2$I, (b) CuCl$_2$, EtOH, NaBH$_4$, (c) 12N HCl (d) NH$_4$Cl or 70% aqueous H$_3$PO$_4$, reflux, (e) c-C$_6$H$_{11}$COCl, Et$_3$N.

5.   BIOLOGICAL ACTIVITY

The noteworthy drugs of this class are oxamniquine and praziquantel; of these, oxamniquine has been widely used to treat *Schistosomiasis mansoni*, while praziquantel finds use in the chemotherapy of tapeworm and fluke infections both in animals and humans.

5.1   Drugs in veterinary medicine

Praziquantel is a highly effective drug, which may be administered orally or parenterally to eradicate a broad range of tapeworms and flukes from laboratory animals and pets [26,40].

5.1.1 Activity against adult cestodes

Praziquantel causes 95-100% elimination of mature worms of several species of cestodes infecting cats, dogs, goats, sheep, duck, pigeon, rabbits and cattle [26,27].

(a) Dogs: Although oral doses ranging from 0.5-40 mg/kg have been found to produce 100% activity against adult worms of *Mesocestoides corti, Taenia pisiformis, T. ovis, T. hydatigena, Dipylidium caninum, Diphyllobothrium latum, Spirometra erinacei, Echinococcus granulosus* and *E. multilocularis* in dogs [26], usually a dose of 2.5 mg/kg is enough to cause complete removal of *Taenia spp., D. caninum* and *M. corti*. A higher dose of 5 mg/kg is needed to completely remove *E. granulosus* and *E. multilocularis* from dogs [41-46]. A few tapeworms such as *D. latum* and *S. erinacei* appear to be less susceptible to praziquantel as a very high dose (35-40 mg/kg) may be needed to achieve 100% elimination of the parasites [41].

(b) Cats: In cats, praziquantel has been found to exhibit excellent activity against adult tapeworm infections. The drug causes 100% elimination of *Taenia (Hydatigera) taeniaeformis* and *Joyeuxiella pasquali* at a single oral dose of 1 mg/kg. A similar order of activity is obtained against *D. caninum* at a dose of 5 mg/kg. Higher doses ranging from 7.5-25 mg/kg are needed to clear 100% of the adult tapeworms of *Diphyllobothrium erinacei* and *Spirometra spp.* from cats. Keeping in view the outstanding therapeutic profile of praziquantel against cestodes, a dose of 5 mg/kg of this drug has been recommended for deworming cats and dogs infected with tapeworms [26].

(c) Sheep and goats: Praziquantel exhibits 98-100% efficacy against different cestodes parasitizing sheep and goats. A dose of 5 mg/kg of the drug has been found to remove 100% of *Moniezia expansa* from sheep and goats. The drug is

equally effective against *M. benedeni* and *M. automnalia* in sheep at an oral dose of 5 mg/kg. A slightly higher dose (8 mg/kg, p.o.) is needed to kill 100% of *Avitellina centripunctata*, while infestations due to bile duct parasites, *Stilesia spp.* and *T. actinoides* in sheep require a dose of 8-15 mg/kg of praziquantel to achieve 98-100% activity [26,47,48].

(d) Poultry: Praziquantel has been demonstrated to be 100% effective against the adult tapeworms parasitizing geese, ducks, pigeons and chickens at a dose level of 3-20 mg/kg [26,49]. Thus, a dose of 10 mg/kg of the drug provides 100% clearance of *Drepanidotaenia lanceolata, Tschertkovilepis krabbei, T. setigera, Diorchis stefanskii* and *Sobolevicanthus gracilis* from geese, *Dicranotaenia coronula, D. stefanskii, S. gracilis, Frimbaria fasciolaris* and *Microsomacanthus parvula* from ducks, *Raillietina columbae* from pigeons and *Davainea proglottina* from chickens [26]. However, a lower dose of 3 mg/kg is sufficient to eliminate 100% of the tapeworms, *Raillietina cesticellus* and *Choanotaenia infundibulum* from chickens [26]. In ostrich, the drug shows slightly inferior activity; 92% clearance of the cestode, *Houttuynia struthionis* has been reported at a dose of 5 mg/kg [26].

5.1.2 Activity against larval cestodes

The activity of praziquantel against larval cestodes (bladder worms) is less pronounced than against the adult ones. Nevertheless, the marked activity of this drug against larval forms (cysticercii) of different tapeworms in sheep, cattle and pigs may be of great help in interrupting the transmission of cestodiasis in humans.

The activity of praziquantel against cysticercosis in sheep, cattle, pigs and rabbits has been fully established [26,27]. At an oral dose of 50 mg/kg given for 1 or 2 days it has been found to be 100% effective against *Cysticercus ovis* and *C. tenuicollis* in sheep. The drug also kills 100% of *C. bovis* in cattle at a single dose of 100 mg/kg given subcutaneously [26,27]. Pigs infected with *C. tenuicollis* or *C. cellulosae* can be treated by administering praziquantel orally at a dose of 50 mg/kg for several days [26]. More recently Lin and Fan [50] gave praziquantel to pigs at a single oral dose of 100 mg/kg daily for 3-5 days after which all the cysticercii of *T. saginata* were found to be degenerated.

Praziquantel has no appreciable effect on the hydatid cysts caused by larval forms of *E. granulosus* and *E. multilocularis* in sheep [26]. However, Thomas and Gonnert [51] demonstrated that the drug is active against protoscolices of *E. multilocularis*, but could not check the growth of cysts of *Echinococcus* in sheep [52,53].

5.1.3 Schistosomiasis

Praziquantel is a safe and effective drug for treating schistosomiasis in cattle. Bushara *et al.* [54] obtained 98.9% reduction in worm burden in calves experimentally infected with *Schistosoma bovis*. The animals needed only two treatments of praziquantel at a dose of 20 mg/kg.

5.1.4 Activity against flukes

Praziquantel has been successfully used to treat liver fluke (*Dicrocoelium dendriticum*) and pancreatic fluke (*Eurytrema pancreaticum*) infections in sheep at an oral dose of 50-70 mg/kg [55,56]. The drug is equally effective against the pancreatic flukes, *Eurytrema coelmaticum* in cattle and liver flukes, *Platynosomum fastosum* and *P. concinnum* in cats [57-59].

5.2   Drugs in human medicine

5.2.1 Oxamniquine

This drug was introduced in 1975 for the treatment of human schistosomiasis. It shows high activity against *S. mansoni* infection in man, but it has little or no action against *S. haematobium* and *S. japonicum*, respectively. The recommended dose of the drug for adult patients is a single dose of 15 mg/kg where after 70-100% cure rates against *S. mansoni* is achieved [60-66]. Children receive oxaminiquine at a dose of 10 mg/kg given twice (total 20 mg/kg) for one day [66]. In case of East African patients, the dose should be increased to 30 mg/kg for 1 day, while for Egyptians and South African subjects, the dose should be 30 mg/kg for 2 days. In such cases, neuropsychiatric disturbances and seizures may be seen in a few patients [66,67].

Foster [68] has reviewed the clinical experience of oxamniquine in the treatment of *Schistosomiasis mansoni* in children and adults. The author recommends a dose of 15 mg/kg for adults and 20 mg/kg (10 mg/kg in two doses given at 3-8 hours apart) for children. Usually this schedule causes 90% clearance of the worms, though, in some regions such as Egypt, Zimbabwe and South Africa, a total dose of 60 mg/kg (administered as 15 or 20 mg/kg for 3 to 4 days) may be needed. More recently it has been observed that a single oral dose of 20 mg/kg of the drug is effective for treating *S. mansoni* infection in children and adults [69,70]. According to Abdel-Rahim [71], a dose of 40 mg/kg should be used to achieve radical cure of *S. mansoni* infection in human.

Oxamniquine is a well tolerated drug and patients show side effects, which are usually mild and of transient nature like nausea, vomiting, diarrhea, dizziness, headache, abdominal pain and drowsiness. The most serious side effect of the drug is related to CNS disorders, particularly abnormal electroencephalograms (EEG) [68,72,73]. Some patients may develop fever usually ranging from 38-39°C lasting for 2-5 days [72].

5.2.2 Praziquantel

Praziquantel is the drug of choice for treating cestode and trematode infections in humans. The biological profile of this drug against various helminth infections in humans has been extensively reviewed [1,26-30,40,74,75]. The therapeutic uses of this drug have been recorded against the following parasitic diseases.

(a) Intestinal tapeworm infections: Praziquantel is an effective drug for treating *T. solium, T. saginata, D. latum* and *H. nana* infections in man. A single oral dose of 10 mg/kg of the drug has been found to be highly effective (almost 100% cures) against *T. solium* and *T. saginata* [26,29,76-78]. For the treatment of *D. pacificum* and *D. latum* infections, a single oral dose of 10 and 25 mg/kg, respectively, of praziquantel is needed to achieve 95-100% efficacy [26]. However, some workers have found that praziquantel is equipotent to niclosamide against *Diphyllobothrium* infections in humans at a lower dose of 5-10 mg/kg [79,80]. A higher dose of 15-25 mg/kg, given orally once, provides parasitological cures of above 90% of the patients suffering from *H. nana* [26,81]. More recently, Pawlowski [82] has shown that a single oral dose of 2.5 mg/kg of praziquantel is fully effective against *T. solium* in humans.

Praziquantel is a well tolerated and safe drug. However, it may produce some side effects, the most common of which are abdominal pain, nausea, anorexia, diarrhea or loose stools, dizziness, headache, pruritis, skin eruption and fever, which appear shortly after the drug administration [81,83]. These side effects are usually mild and dose related and disappear within 24 hours. Praziquantel has been found to be free of mutagenic, carcinogenic and teratogenic effects [81,83,84].

(b) Cysticercosis: Praziquantel is the drug of choice for the treatment of cysticercosis caused by larval cestodes in man. The therapeutic dose of the drug is 25 mg/kg given orally for 3-6 days. This dose schedule usually destroys the cysticercii lodged in the musculature [77,85,86]. However, for the treatment of neurocysticerosis, a higher dose of 50 mg/kg given in three divided doses for 10-15 days has

been recommended [87,88]. Even a lower dose of 25 or 30 mg/kg of praziquantel has been found to be effective in treating patients with neurocysticercosis [85,89]. While treating patients with neurocysticercosis a varying degree of hyperglycemia may develop in some subjects. This observation was made by Robles [90], who found that this hyperglycemia was of benign character and lasted only during the tenure of drug therapy.

Cerebral cysticercosis has been successfully treated by praziquantel [91,92]. An oral dose of 50 mg/kg was given for 15 days to 26 patients with cysticercosis of the brain parenchyma. There was a marked clinical improvement with decrease in the number of cysts as observed on CT scans [93]. The current status of the diagnosis and management of neurocysticercosis have been reviewed by Del Brutto and Sotelo [94] and Cook [95]. It has been suggested that the state of the cysticerci should be monitored by CT scans before and after the treatment. The assessment of drug therapy can be made by *in vivo* CT scans in conjunction with serial measurements of serum antibody titres against cysticercii antigen [85,86,95].

The treatment of cerebral or neurocysticercosis with praziquantel evokes some inflammatory problems related to the CSF reaction syndrome, which is characterised by headache, meningismus, fever and neurological problems. These side effects may be alleviated by simultaneous use of corticosteroids and praziquantel [87,88,91,96]. Accordingly more and more workers prefer to use corticosteroides, while treating cysticercosis of the brain with praziquantel. This combination protects the patients from the CSF reaction syndrome [97,98].

(c) **Schistosomiasis:** Praziquantel is an excellent drug for the treatment of schistosomiasis in man [26,29,30,32,99-101]. Usually a single oral dose of 40 mg/kg can cause destruction of almost all the schistosomes, namely *S. haematobium, S. mansoni, S. japonicum, S. intercalatum* and *S. mattheii* infecting children and adults. Although the above dose schedule is generally well accepted, some other dose regimens of the drug have also been suggested. Thus a dose of 20 mg/kg given thrice for one day can be used to cure schistosomiasis [66]. Praziquantel may also be given at a dose of 25 mg/kg, twice or 50 mg/kg, once for one day [102-103].

As described earlier, praziquantel is a safe and well tolerated drug with no serious side effects. The typical side effects of the drug are abdominal pain, nausea, diarrhea, headache, skin rash and fever. Rarely praziquantel may cause neuropsychiatric, cardiovascular and dermatological reactions, hepatic changes, delayed fatigue and inability to work. Care should be taken while treating patients with chronic and

complicated heart disease, ascites and impaired renal or hepatic function [102,104].

(d) Liver fluke infection: Praziquantel has been successfully used to cure infestations due to *Chlonorchis sinensis* and *Opisthorchis spp.* in humans. The drug was given to 34 patients with chlonorchiasis at a dose of 25 mg/kg thrice daily for 2 days after which total elimination of the parasite was obtained [105]. A lower dose of 14 mg/kg, given thrice daily for 5 days has also been found to cause complete eradication of the disease [106]. Consequently praziquantel has been recommended for mass treatment of *C. sinensis* in humans at a single oral dose of 40 mg/kg [107].

Praziquantel also shows high efficacy against *Opisthorchis* infection in man. Bunnag and coworkers [108] have summarised the clinical experience with praziquantel in nearly 5000 patients and have concluded that a dose of 25 mg/kg of the drug given twice daily for 1 or 2 days can provide 100% cure against *O. viverrini*. Similar result may be obtained at a single oral dose of 40-50 mg/kg. The effect of praziquantel in the treatment of *O. viverrini* and *O felineus* has been established by several workers [106,109,110]. The drug has also been used in the mass treatment of opisthorchiasis in Nong Wai, Thailand at a single oral dose of 40 mg/kg with high success [111].

(e) Lung fluke infections: Praziquantel is the drug of choice for the treatment of infections due to lung flukes, *Paragonimus spp.* A dose of 25 mg/kg of the drug, given thrice daily for 2 to 3 days was found to be fully effective against *P. westermani* [112-114] and *P. uterobilateralis* [115] in humans. In all the trials the patients reached clinical cures; the drug was usually well tolerated producing only mild side effects. Praziquantel may also prove to be useful in the treatment of *P. heterotremus* infections in humans [112].

(f) Intestinal fluke infections: Praziquantel is the preferred drug for eradicating intestinal flukes. Harinasuta *et al.* [116] have evaluated praziquantel in school children infected with *Fasciolopsis buski* in Thailand. A single oral dose of 15 mg/kg of the drug was found to be 100% effective. The side effects of praziquantel were mild and transient occuring in 21% of the treated children. The most common side effect was sleepiness.

Praziquantel has also been found to be highly effective against *Echinostoma ilocanum* (dose: 25 mg/kg, b.i.d. or 40 mg/kg once) [117], *Metagonimus yokogawai* and *Heterophyes heterophyes* (dose: 25 mg/kg, t.i.d.) [83] in human.

(g) Levopraziquantel in schistosomiasis: Ming-He Wu *et al.* [118] have compared the therapeutic efficacy and side effects of levopraziquantel with the racemic form against *Schistosoma japonicum* in 278 cases. It was found that the levo isomer produced 94% reduction of ova of *S. japonicum* in the stool after a single oral dose of 20 mg/kg as compared to 40 mg/kg needed of the racemic mixture of praziquantel. The levo isomer also caused lesser side effects than the DL-form.

6.　MODE OF ACTION

6.1　Oxamniquine (14)

The exact mode of action of oxamniquine is unknown [83]. The drug exhibits anticholinergic action; its action on neuromuscular transmission has been demonstrated [119]. It has been suggested that oxamniquine (**14**) is converted enzymatically into a phosphate or sulphate ester (**50**), which then dissociates non-enzymatically to form the chemically reactive species **51**. This intermediate then alkylates essential macromolecules of the schistosomes like DNA to give a Drug-DNA complex (**52**) (Scheme 5) [1,120,121]. The antischistosomal activity of oxamniquine is due to its ability to alkylate DNA. It does not intercalate with DNA, because the drug molecule is not planar, an essential structural requirement for intercalation. The lower mutagenic property of oxamniquine may, therefore, be due to the fact that it is not a DNA intercalator [1].
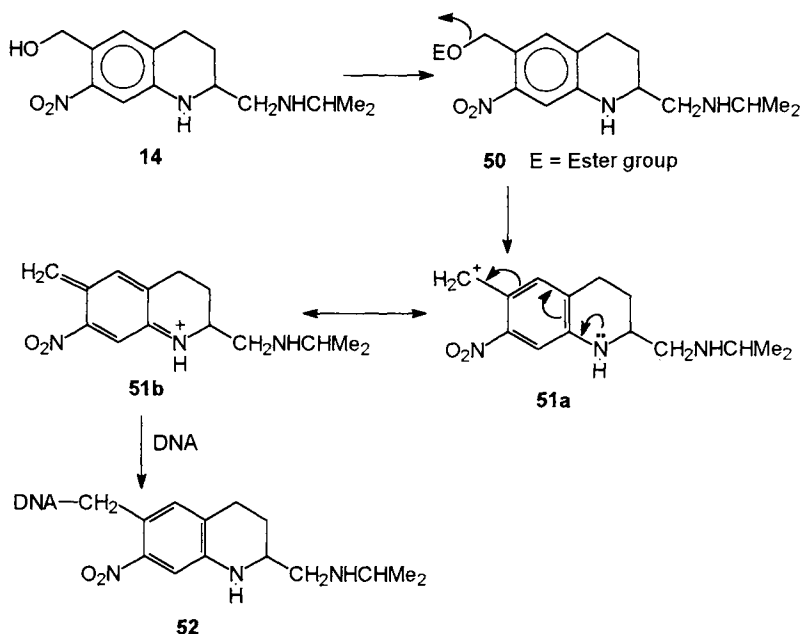
Scheme 5: Possible mode of action of oxamniquine.

6.2 Praziquantel (15)

Although the molecular basis of anthelmintic action of praziquantel is not fully known, the drug has been found to act on the musculature and tegument of the susceptible worms [26]. The major action of the drug may be broadly divided into following two heads:

6.2.1 Action on the worm's musculature

Praziquantel stimulates the movement of *Hymenolepis and Echinococcus spp.* at low concentrations (1 ng/ml). Higher concentrations of the drug causes rapid contraction of the worms. At a concentration of 1-10 µg/ml of praziquantel, the helminths were found to be immobilized and contracted within 10-30 seconds [26,122,123]. The tetanic contraction of the worm's musculature results in its paralysis. Fetterer and coworkers [124] have shown that the drug is capable of inducing spastic and/or paralytic effects in cestodes, trematodes and to some extent in nematodes also.

The spontaneous contraction of the helminthic muscles by praziquantel has been attributed to the drug's ability to change the ion concentration across the muscle membranes. The drug increases the permeability of schistosome or tapeworm cells for Na^+ and Ca^{2+} ions [122, 124,125]. The increase in membrane permeability results in increase in Ca^{2+} ion concentration with concomitant loss of Na^+ and K^+ ions, which triggers the muscles to contract [126]. The drug induced contraction may be alleviated either by lowering the ambient Ca^{2+} ion concentration or by increasing the concentration of Mg^{2+} [122].

6.2.2 Vacuolization of worm's tegument

The increase in cytosolic Ca^{2+} ion concentration has been implicated in causing vacuolization of the teguments of cestodes and trematodes [127-129]. Excessive vacuolization of the syncytical tegument with evaginations or "bleeding" on the surface membranes lead to damage of the parasite's surface. It must be emphasized that sudden vacuolization of the tegument of schistosomes and liver flukes is only a reversible process and therefore, not lethal to the worms [129,130]. Death of the helminths takes place only when the vacuolization causes an irreversible damage to the tegument. It has been suggested that vacuolization and subsequent erosion of the tegument makes the cestodes and trematodes immunologically incapable to escape host defense mechanism and are consequently destroyed by the host [129].

Harnett has correlated the activity of praziquantel with the competent immune status of the host [131]. The role of immune mediated destruction of helminths has been demonstrated by Brindley and Sher [132] and Harnett and Kusel [133].

6.2.3 Other mechanisms

Although the major action of the drug on cestodes and trematodes involves contraction of the tegument muscles followed by vacuolization and surface erosion, it may also include some secondary effects, which include depolarisation of tegument in schistosomes, depletion of glycogen contents and inhibition of glucose uptake by tapeworms and flukes [26].

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CHAPTER 12

MISCELLANEOUS ANTHELMINTICS

1. INTRODUCTION

In addition to the group of compounds discussed in the preceeding chapters, a few other classes of compounds have been found to exhibit promising anthelmintic activity and provide useful leads for exploration. The salient features of these promising compounds are presented in this chapter.

2. QUATERNARY AMMONIUM SALTS

Cyanine dyes are amongst the earliest quaternary ammonium salts, which were evaluated against helminths. The cyanine dyes such as pyrvinium pamoate (1), stilbazium iodide (2), bidimazium iodide (3), and dithiazanine iodide (4) possess a pseudo amidinium system characterised by a quaternary nitrogen and a tertiary nitrogen joined together by a chain of several conjugated carbon atoms. The resonance between these two nitrogen atoms appears to be essential for anthelmintic activity as these may be involved in the formation of charge-transfer (CT) complexes [1].
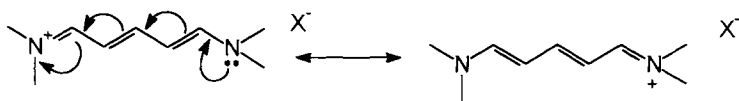
In 1958 Copp and co-workers working at Wellcome Laboratory discovered the potent anthelmintic activity associated with 2-aryloxyethylammonium salts [2], of which bephenium hydroxynaphthoate (5) was found to possess high activity against ascariasis and hookworm infections in humans [3]. Following the discovery of bephenium, a large number of its structural analogues were synthesized [4-10]. Of these thenium (6) and diphezyl (7) exhibited promising activity and have been used to treat canine and feline hookworm infections [11]. Styrylpyridinium halide (8) is also an effective dog antihookworm agent, which has been shown to kill 90% of the worms with a single dose of 5 mg/kg [12].

1

2

3

4

5 R = Ph
6 R = 2-Thienyl
7 R =

8
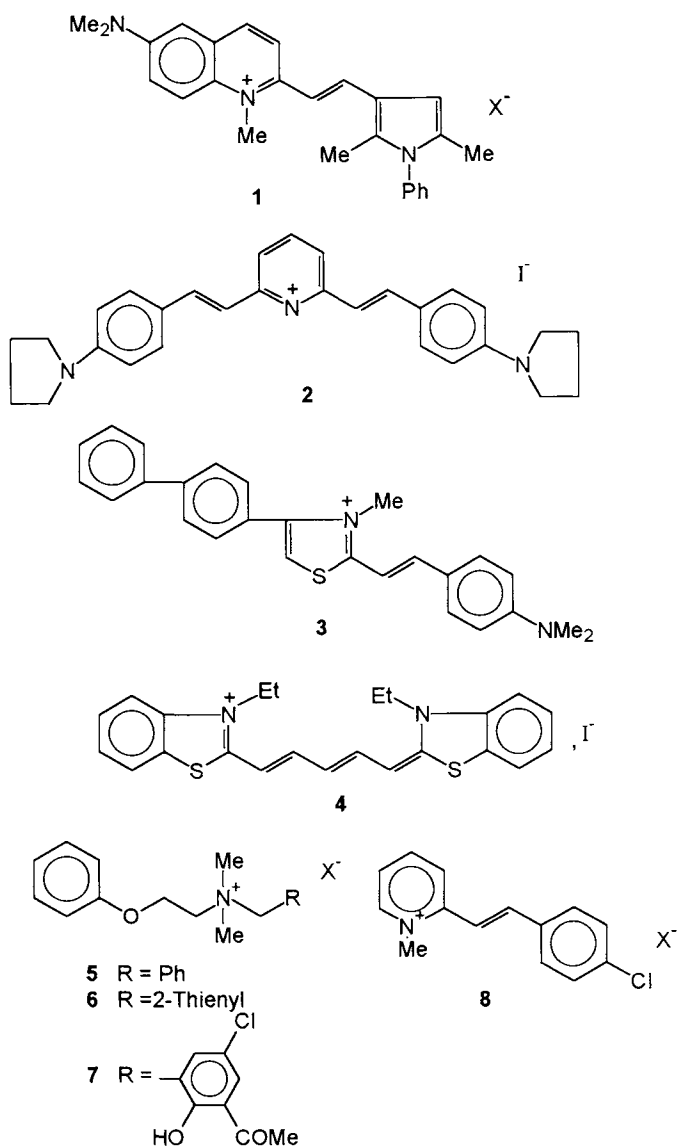
3. SUBSTITUTED PHENOLS

The germicidal property of phenol has been known for long time; its practical use in disinfecting surgical rooms, instruments and ungloved hands was first demonstrated by Joseph Lister in 1867 [13]. Unfortunately, the high toxicity and caustic nature of phenol limited its use as a germicide in clinical practice. The demonstration of high antiinfective activity of cresylic acids (a crude mixture of isomeric cre-

sols from coaltar) with greater safety margin [14] evoked fresh interest in substituted phenols. It was soon realised that introduction of both electron withdrawing (chloro, cyano, nitro etc) and electron donating (alkyl or alkoxy) groups enhanced the antibacterial potency and decreased the toxicity [1,15,16]. In addition, synthesis of biphenols, in which the two phenolic nuclei were joined together either directly or through a bridge, were also undertaken when a number of potent antiinfective agents were developed [17]. In general, halogenated phenols and biphenols have strong antibacterial properties and, therefore, many of them have been used in anti-infective topical formulations and germicidal soaps [17,18]. In 1935, Lamson and his co-workers [19-22] carried out a systematic study on the biological profile of phenols and established their anthelmintic activity. Hexachlorophene was introduced in the late 1950s as a new class of compounds effective against mature *F. hepatica* infection. Today a large number of phenols have been shown to possess activity against different nematodes, cestodes and trematodes [11,23].

The important phenols that have shown marked activity against roundworms are hexylresorcinol (9), 2,4,5-trichlorophenol (10), 2,6-diiodo-4-nitrophenol (disophenol, 11), thymol (12), iodothymol (13) and 4-cyano-2-iodo-6-nitrophenol (nitroxynil,14) and butyphen (15). Of these disophenol (11) and nitroxynil (14) are commonly used as veterinary anthelmintics [11,23].
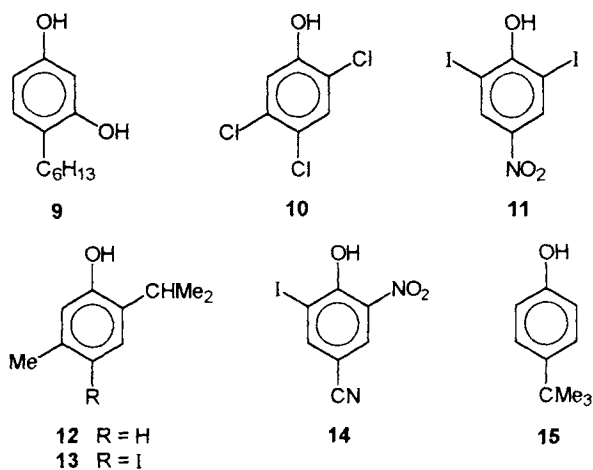
A number of biphenols have been found to exhibit excellent activity against tapeworms and flukes. The noteworthy phenols of this class are dichlorophene (16a), hexachlorophene (16b), trichlorophene (17), bithionol (18a) and bithionol sulphoxide (18b), which have been used to treat tapeworm infections in humans and domestic

animals [23]. A few other halogenated phenols and naphthols such as **19-21** also show high cestodicidal activity [23], while 1-bromo-2-naphthol (**22**) has been used to cure hookworm infections in humans [11,23].

The use of nitroxynil (**14**) and the biphenols (**16a,b** and **18a,b**) along with menichlopholan (**23**) has also been extended to the treatment of liver fluke infections. Accordingly, disophenol (**11**), nitroxynil (**14**), bithionol (**18a**), bithionol sulfoxide (**18b**), niclofolan (menichlopholan, **23**) and bis(3-chloro-2-hydroxy-5-nitrophenyl)sulphide (**24**) have been used to treat *F. hepatica* and *F. gigantica* infections in sheep and cattle [23].
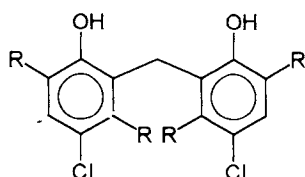
16a R = H
b R = Cl
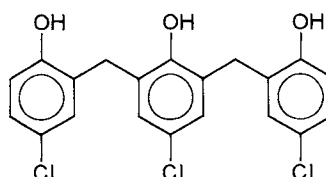
17
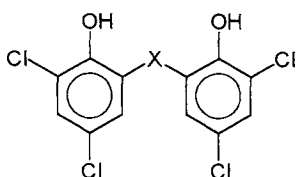
18a X = S
b X = SO
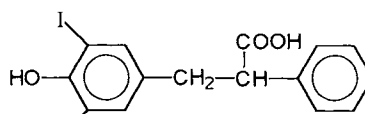
19
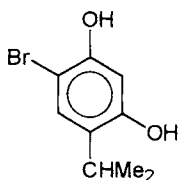
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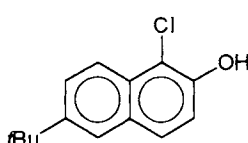
21
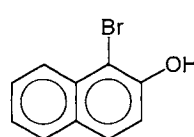
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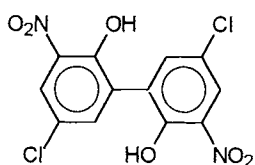
23
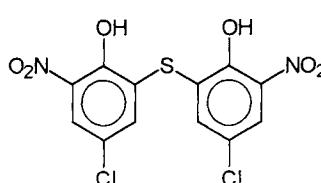
24

4. THE MIRACILS

In an effort to develop an orally effective and metal-free drug for treating schistosomiasis, Kikuth and his colleagues uncovered the potent antischistosomal activity of 1-amino-4-methylxanthones and thioxanthones in 1946 [24]. The most effective compound of this class, called miracil D or lucanthone (25) was selected for detailed biological evaluation. Eventually, lucanthone emerged as the first orally active and metal-free drug for treating *S. mansoni* and *S. haematobium* infections in humans [25].

25
(Lucanthone)

26
(Hycanthone)
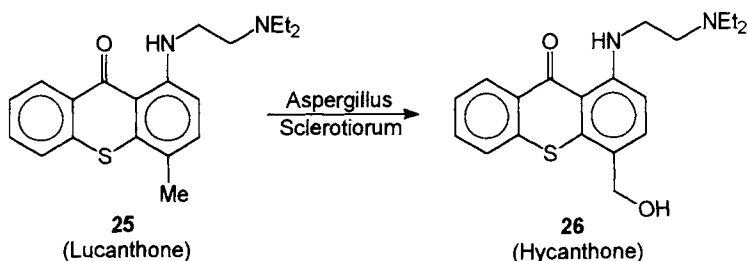
Although lucanthone proved to be a valuable drug for clinical management of schistosomiasis, its use in mass therapy was plagued due to its numerous side effects and occasional fatalities in treated patients [23]. This led to the synthesis of a large variety of substituted xanthones and thioxanthones without much success [26-28]. Even the search for an active metabolite of lucanthone was not rewarding [29,30]. In 1965 Rosi *et al.* [31] discovered hycanthone (26), which was obtained by microbial hydroxylation of the methyl group of lucanthone. Later, hycanthone was shown to be bioactive metabolite of lucanthone. It exhibited better antischistosomal efficacy than the parent drug [32].

5. HALOGENATED HYDROCARBONS

A number of halogenated aliphatic and aromatic hydrocarbons are known to exhibit high activity against different nematodes and trematodes parasitizing domestic animals and humans [23,33]. Carbon tetrachloride (CCl_4) and tetrachloroethylene ($Cl_2C=CCl_2$) are amongst the earliest drugs, which were used to treat hookworm infections in humans [11,34]. However, both these drugs are toxic and, therefore, do not find use in the modern therapy of human helminthiasis.

In veterinary practice many of the halogenated hydrocarbons such as carbon tetrachloride, hexachloroethane (Cl_3C-CCl_3), isomeric difluorotetrachloroethanes ($C_2F_2Cl_4$), namely 1,2-difluorotetrachloroethane ($FCl_2C-CFCl_2$, Freon 112) or 1,1-di-

fluorotetrachloroethane (F$_2$ClC-CCl$_3$, Freon BU) and 1,4-bis(trichloromethyl)benzene (hetol, 27) have been widely used to treat fascioliasis in sheep [23,33]. Recently, the fasciolicidal activity of halogenated sulphanilamides was discovered and the best compound was clorsulon (28). This is a relatively less toxic compound with high activity against *F. hepatica* in sheep and cattle [35].

27   28

6. OTHER ANTHELMINTICS

Following the discovery that certain dyestuffs like trypan blue and trypan red possess trypanosomicidal activity, scientists at Bayer carried out a structure-activity relationships study with related naphthalenes. The most effective compound that emerged in this study, was Bayer 205, called suramin (29) [36,37] synthesized by Dresel and Kothe in 1917 [17]. The effectiveness of suramin in treating acute cases of African sleeping sickness was established during the early 1920s [38]. Later it was found that this drug is equally effective in treating river blindness due to *Onchocerca volvulus* [39]. Since then it has become a primary drug for treating trypanosomiasis and filariasis in humans [40].

29

Oltipraz (30) is a recently discovered drug for schistosomiasis [32]. It shows high activity against *S. mansoni* and *S. haematobium* in humans at a dose of 20-40 mg/kg. The drug has no toxicity on cardiovascular, respiratory or nervous systems and is also free from mutagenic and immunosuppressive properties [18,41].

Other anthelmintics with noteworthy activity are bitoscanate (31), phthalofyne (32), quinacrine (33), bunamidine (34), diamphenetide (35) and hetolin (36). Of

these bitoscanate (31) and quinacrine (33) have been used to treat hookworm and tapeworm infections, respectively, in humans, while others find use in veterinary medicine [23].

30

31

32

33

34

35

36
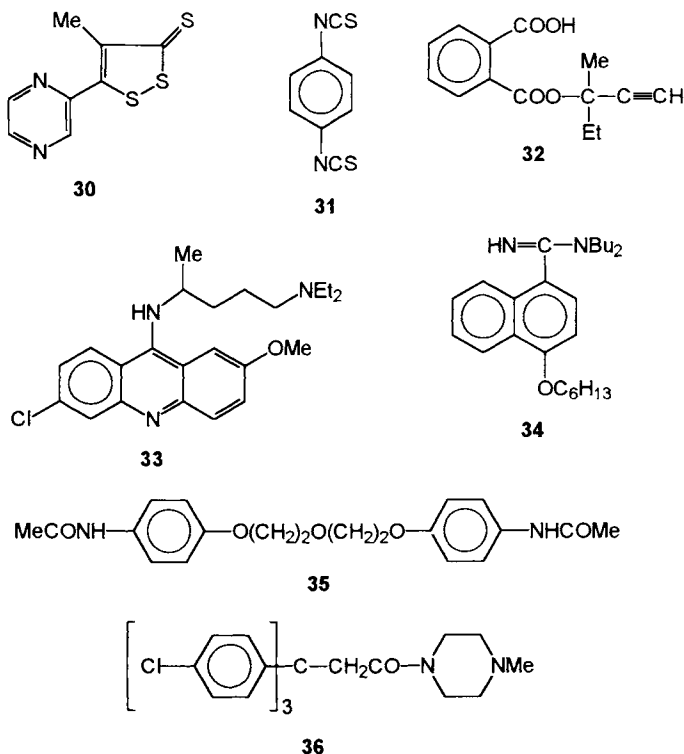
7. SYNTHESIS OF CANDIDATE DRUGS

7.1 Pyrvinium salts (1)

A wide variety of pyrvinium salts (1) have been prepared by treating a quinoline quaternary salt (41) with 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde (40) [42,43]. The latter is obtained by a Paal-Knorr synthesis starting with 2,5-hexanedione (37) and aniline [44,45] (Scheme 1).

7.2 Bephenium salts (5-7)

The starting material for bephenium salts is 2-phenoxyethyl bromide (42), which may be converted into bephenium either in one or two steps as shown in scheme 2 [4-9].
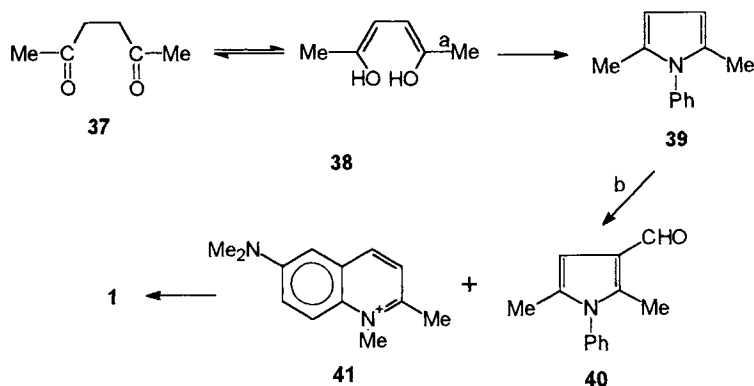
Scheme 1
Reagents: (a) Aniline; (b) POCl$_3$, DMF.
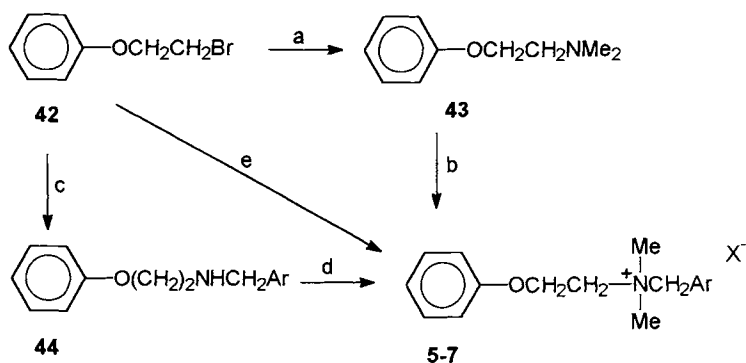
Scheme 2
Reagents: (a) HNMe$_2$, (b) ArCH$_2$X, (c) ArCH$_2$NH$_2$, (d) MeI, (e) PhCH$_2$NMe$_2$.

7.3 Disophenol (11)

This is prepared by iodination of 4-nitrophenol (45) with iodine and potassium iodide in aqueous ammonia [46].
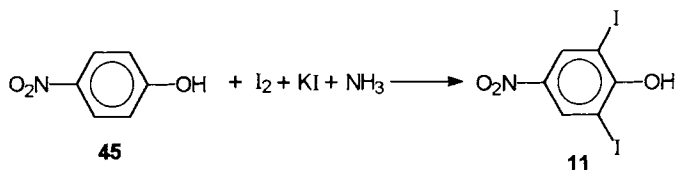
7.4 Nitroxynil (14)

Nitroxynil is obtained either by iodination of 4-cyano-2-nitrophenol (**47**) [47,48] or by nitration of 4-cyano-2-iodophenol (**48**) [49] (Scheme 3).
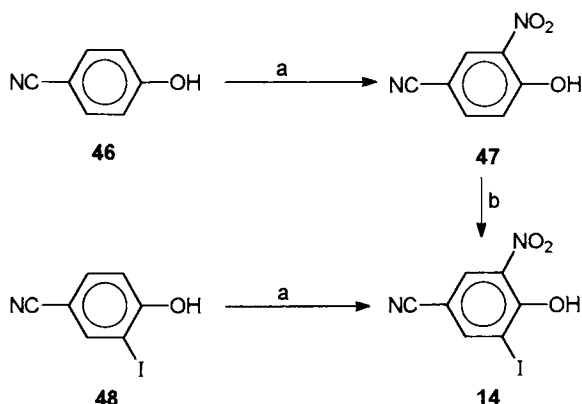
Scheme 3
Reagents: (a) HNO_3, AcOH; (b) KI, KIO_3.

7.5 Dichlorophene and hexachlorophene (16a,b)

Dichlorophene (**16a**) and hexachlorophene (**16b**) are prepared by adding one mole of aqueous HCHO or paraformaldehyde $[(CH_2O)_n]$ to a solution of 4-chlorophenol (**49**) and 2,4,5-trichlorophenol (**50**), respectively, in ethylene dichloride in the presence of sulphuric acid or oleum [50,51].
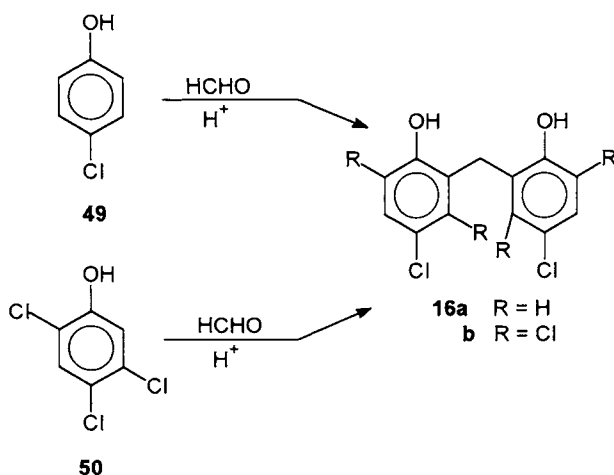
7.6 Bithionol and its sulfoxide (18a,b)

Bithionol (18a) may be prepared by treating 2,4-dichlorophenol (51) with sulphur dichloride [52], which is oxidized with 30% hydrogen peroxide to form bithionol sulphoxide or bitin-S (18b) [53]. Alternatively 18b can be synthesized by reacting 2,4-dichlorophenol (51) with thionyl chloride [52].
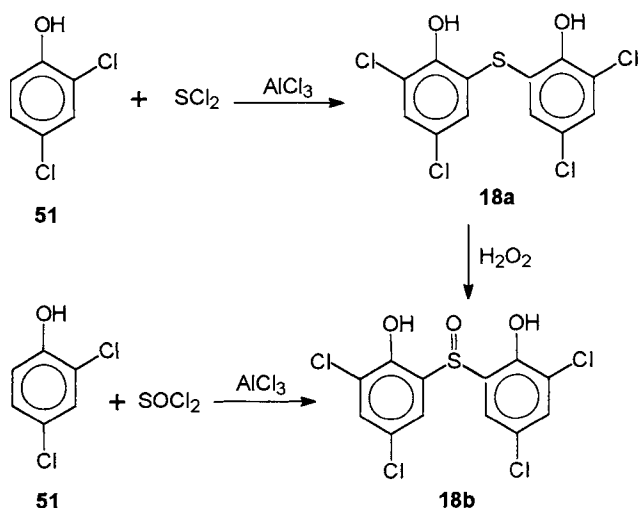
7.7 Lucanthone (25) and hycanthone (26)

The key intermediate for the preparation of lucanthone and hycanthone is 1-chloro-4-methylthioxanthen-9-one (55), which may be prepared in two ways as shown in Scheme 4 [55-57]. Reaction of 55 or a mixture of 54 and 55 with N,N-diethylethylenediamine affords lucanthone (25), which is allowed to undergo microbial oxidation in the presence of *Aspergillus sclerotiorum* to yield hycanthone (26) [31].

Laidlaw *et al.* [57] have developed an elegant method to prepare hycanthone. The key intermediate of this method is 1-chlorothioxanthen-9-one (62), which is obtained by two different routes as shown in Scheme 5.

Compound 62 was conveniently converted into hycanthone (25) by a series of reactions as described in Scheme 6 [57].

7.8 Suramin (29)

The Schotten-Baumann reaction of 1-naphthylamine-4,6,8-trisulphonic acid (71) with 4-methyl-3-nitrobenzoyl chloride (72) gives the corresponding amide (73),

52 + 53 → 54 (Undesired)

55 (Desired)

25 Me

26

59

56 → 57 → 58
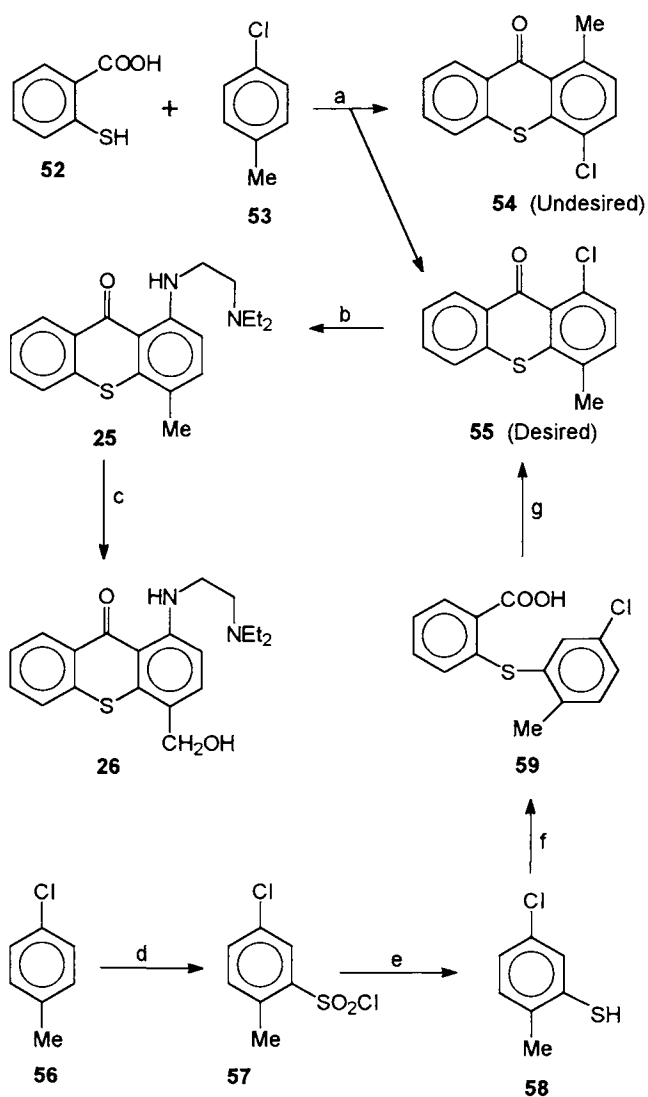
Scheme 4

Reagents: (a) H$^+$, (b) H$_2$NCH$_2$CH$_2$NEt$_2$, (c) microbial oxidation, (d) chlorosulphonic acid, (e) Zn, H$_2$SO$_4$, (f) 2-chlorobenzoic acid (Ulman reaction), (g) H$_2$SO$_4$.

Scheme 5

Reagents: (a) Cu bronze, KI, K_2CO_3, DMF, (b) H_2SO_4, (c) KOt-Bu, DMSO, (d) PPA, H_2O.

which is reduced to the amine **74**. Treatment of **74** with 3-nitrobenzoyl chloride affords the diamide (**75**), which is reduced to **76**. Dimerisation of the latter with phosgene yields suramin (**29**) [58-60] (Scheme 7).

308
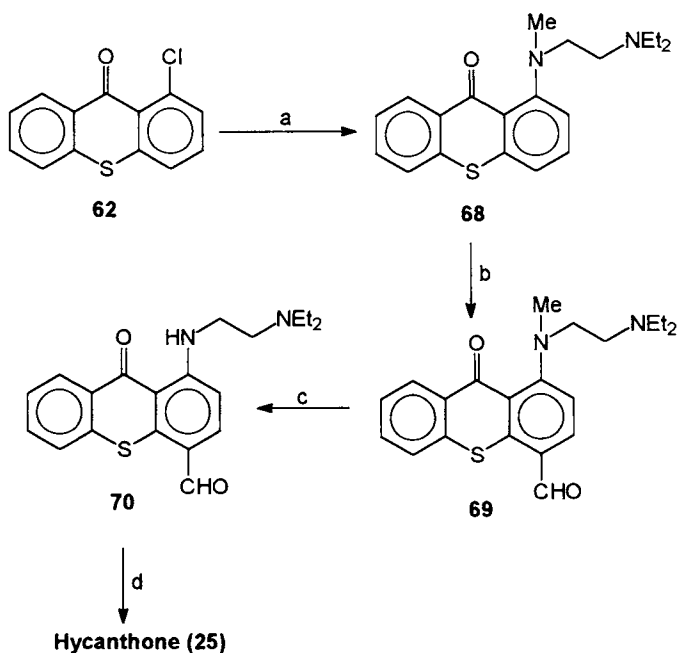
Scheme 6

Reagents: (a) MeNHCH$_2$CH$_2$NEt$_2$, pyridine, (b) DMF, POCl$_3$, (c) Pyridine hydrochloride, 140°C (d) NaBH$_4$, MeOH.
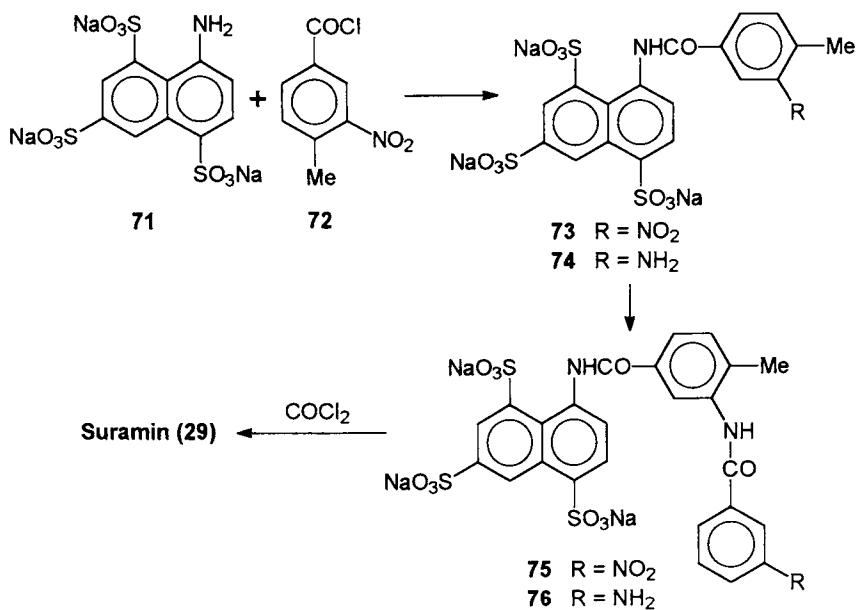
Scheme 7

7.9 Oltipraz (30)

This compound may be prepared conveniently starting from 2-ethoxycarbonylacetylpyrazine (77). Methylation of 77 gives the methylated ester 78, which is heated with P_2S_5 to afford oltipraz [61] (Scheme 8).
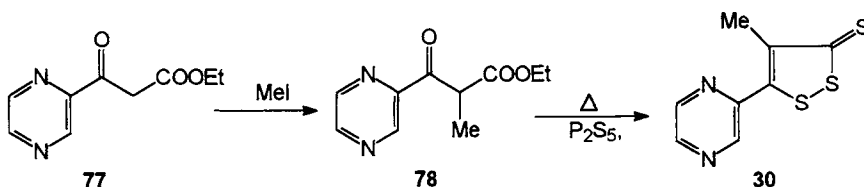
Scheme 8

7.10 Bunamidine (34)

Bunamidine hydrochloride is prepared by condensing 4-hexyloxy-1-naphthonitrile (79) with magnesium di-*n*-butylamide bromide (80). Treatment of the free base with HCl gives bunamidine hydrochloride [62,63,65]. Compound 34 may also be obtained by heating a mixutre of 79 and dibutylamine in presence of anhydrous $AlCl_3$ [62] (Scheme 9).
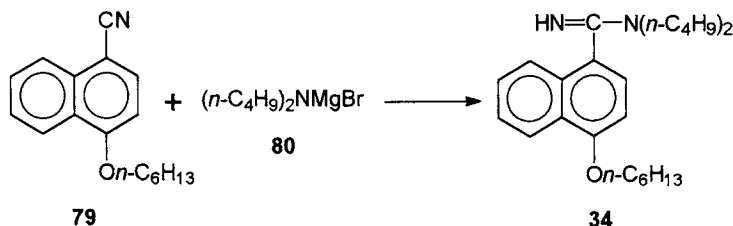
Scheme 9

7.11 Probenzimidazoles

Thiophanate (81) is prepared by treating *o*-phenylenediamine (84) with ethoxycarbonyl isothiocyanate (prepared *in situ* by heating KNCS with ClCOOEt) [64] (Scheme 10).
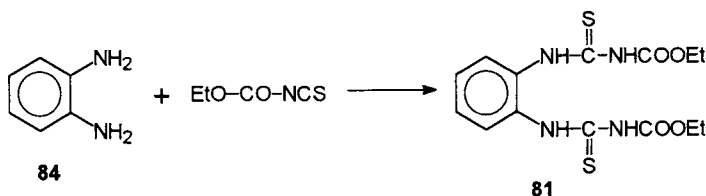
Scheme 10

Netobimin (**84**) has been prepared by treating a solution of N-(methoxycar-bonyl)-S-methyl-N'-[2-nitro-5-(propylthio)phenyl]isothiourea (**83**) with taurine (H$_2$N-CH$_2$CH$_2$SO$_3$H) and NaOH [66-68] (Scheme 11).
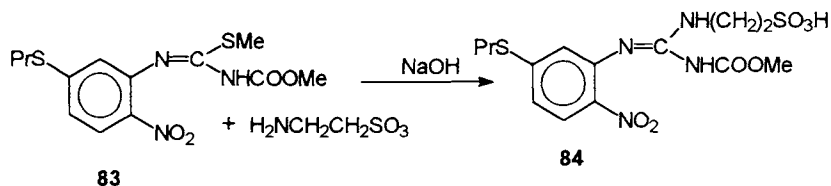
Scheme 11

8. BIOLOGICAL ACTIVITY

Although a number of anthelmintics discussed in this chapter have been demonstrated to be highly effective against different worm infestations in humans and domestic animals, the majority of them are now of historical significance as they have been replaced by more effective and safer drugs.

8.1 Drugs in veterinary medicine

The detailed therapeutic profile of drugs discussed in this chapter and used to treat helminth diseases of domestic animals have been extensively reviewed [12,23,25,26,33,35,69-72]. The present discussion is confined to some of the more commonly used of these drugs.

8.1.1 Bephenium (5) and thenium (6)

Bephenium salts have been used to treat gastrointestinal nematode infections in ruminants, cats and dogs. Bephenium hydroxynaphthoate shows high activity against *Haemonchus, Cooperia, Ostertagia* and *Nematodirus* spp. in cattle and sheep at a single oral dose of 125-225 and 250 mg/kg, respectively [72]. The drug also exhibits 95-100% clearance of hookworms from cats and dogs at the doses ranging from 15-25 mg base/kg [12].

Thenium closylate is an effective drug for the treatment of hookworm infections in cats and dogs. A dose of 200-250 mg/kg of the drug given twice a day has been found to be highly effective against *Ancylostoma caninum* and *Uncinaria stenocephala* in cats and dogs [72]. The recommended dose for treating hookworm infections in young dogs is 62.5 mg base for pups below 2.3 kg weight and 125 mg base

for pups above 2.3 kg. For older dogs weighing above 4.5 kg a dose of 500 mg base/animal has been recommended [12,73]. A combination of thenium with piperazine administered in the morning and the evening for one day produces 98% clearance of the ascarids, *T. canis* in dogs [72].

8.1.2 Disophenol (11)

This drug may be administered orally, subcutaneously or intramuscularly for eliminating hookworm infections from cats, dogs and sheep. Disophenol has a selective action on the adult hookworms, *A. caninum, A. braziliensis, A. tubaeforme* and *U. stenocephala* when given subcutaneously at a dose of 7.5-10 mg/kg [12,33]. The drug is also highly effective against benzimidazole resistant *Haemonchus contortus* in sheep at a subcutaneous dose of 10 mg/kg [74]. Disophenol causes complete clearance of *Spirocerco lupi* in dogs when given in two subcutaneous injections of 7.5-7.7 mg/kg at one week interval [75]. Against fascioliasis in sheep and cattle, disophenol may be used at a subcutaneous dose of 10 mg/kg with >90% efficacy. However, it is not suitable for strategic control of fascioliasis or for management of the outbreak of the acute disease [35].

It is a safe drug and is tolerated well even by young puppies and pregnant bitches. However, repeated application of the drug may give rise to acidosis, circulatory collapse and hyperthermia [33].

8.1.3 Nitroxynil (14)

It is primarily used as a veterinary fasciolicide though it possesses activity against hookworms also. At a subcutaneous dose of 10 mg/kg, nitroxynil shows activity against adult worms of *Haemonchus, Bunostomum* and *Oesophagostomum* in cattle and sheep. It is equally effective against benzimidazole resistant *H. contortus* in sheep [33].

Nitroxynil is currently used for the treatment of *F. hepatica* infection in cattle and sheep. The recommended dose of the drug is 10 mg/kg given subcutaneously to get 90-99% elimination of the adult liver flukes; its activity against immature flukes is low [33,35].

8.1.4 Dichlorophene (16a) and hexachlorophene (16b)

Both these drugs were originally developed as germicides; later their use in the treatment of helminth infections in domestic animals and humans was established. Dichlorophene was introduced in 1946 as a veterinary anthelmintic. At a dose

of 150-200 mg/kg, it eliminates *T.pisiformis* and *D. caninum* from dogs and *Moniezia* sp. from sheep [12,23,33,76]. The drug is also effective against *Raillietina* in chickens at an oral dose of 300 mg/kg [12].

Hexachlorophene is an effective drug for the treatment of *T. hydatigena, M. multiceps, R. cesticillus* and *Echinococcus* infections in dogs and fowls at oral doses ranging from 12-15 mg/kg [23,76]. The drug exhibits high activity against *F. hepatica* and *F. gigantica* in sheep and cattle. The recommended dose of hexachlorophene for treating fascioliasis is 15 mg/kg for sheep and 20 mg/kg for cattle given orally or subcutaneously [23,33,35]. It may cause liver toxicity in sheep and cattle at its maximum tolerated dose of 40 mg/kg [33].

8.1.5 Bithionol (18a) and bithionol sulfoxide (18b)

Bithionol causes complete clearance of intestinal tapeworms, *T. hydatigena, T. ovis, M. multiceps* and *D. caninum* from dogs at a dose of 150-200 mg/kg. It also exhibits 100% efficacy against *Moniezia* and *Anoplocephala* spp. in sheep at a dose of 100 mg/kg. Bithionol eliminates more than 90% of the adult *Fasciola* spp. from sheep and cattle at the oral doses of 75 and 35 mg/kg, respectively. The drug is well tolerated producing no side effects except occasional diarrhea and softening of the stool [12,35,76].

Bithionol sulphoxide (Bitin-S) is the sulphoxide derivative of bithionol possessing high activity against tapeworms and flukes in domestic animals. It eliminates all the worms of *Moniezia* sp. from sheep and *Hymenolepis* sp. from chickens at a dose of 100 and 500 mg/kg, respectively. Against *F. hepatica* infection in ruminants, bithionol sulphoxide shows moderate activity (60-70%) in cattle but high activity (95-100%) in sheep at oral doses of 30-35 mg/kg and 40-75 mg/kg, respectively. Higher doses of bitin-S can eliminate immature paramphistomes from sheep [33,35,76].

8.1.6 Halogenated hydrocarbons

(a) Carbon tetrachloride: This is an old drug which has been used to control *F. hepatica* infections in sheep for nearly 70 years. The routine dose of carbon tetrachloride is 1 ml/animal administered orally in gelatin capsules or mixed with liquid paraffin. This dose is enough for strategic control; for the management of outbreaks of fascioliasis this may be increased to 5 ml/animal. The drug is not recommended for cattle due to severe intoxication after its use. The side effects of carbon tetrachloride are kidney and liver dysfunction and increase in serum glutamic oxaloacetic transaminase (SGOT) levels [12,33]. Some animals may die of carbon tetra chloride poisoning.

The risk of toxicity seen with oral administrations may be reduced by injecting the drug intramuscularly or subcutaneously. However, in such cases necrosis may develop at the site of injection in the animals [33,72].

(b) **Hexachloroethane:** It is safer than carbon tetrachloride and, therefore, can be used to treat fascioliasis in sheep and cattle. It's action on adult flukes is more pronounced than the younger ones. The recommended dose of the drug for cattle and sheep is 200-300 mg/kg; its maximum tolerated dose is 1200 mg/kg. Usually the drug is well tolerated by ruminants, though liver damage and occasional mortality may occur [12,33,72].

(c) **Freon-112:** The drug shows high activity against adult worms of *F. hepatica* in sheep at a dose of 330-660 ml/kg [77].

(d) **Hetol (27):** Hetol is a broad-spectrum veterinary anthelmintic possessing high activity against flukes infecting sheep and cattle. The drug shows high activity against *F. hepatica* and *Dicrocoelium dendriticum* in sheep at a dose of 150 and 300 mg/kg, respectively. A lower dose of 130 mg/kg of the drug is sufficient to achieve nearly 90% clearance of adult worms of *F. hepatica* in cattle. Hetol is well tolerated by animals with no serious side effects [33,35]. The drug is widely used in Russia and CIS (formerly known as U.S.S.R.).

(e) **Chlorsulon (28):** This is a new injectable anthelmintic with high activity against liver flukes in sheep and cattle [78]. The drug appears to be a better flukicide in cattle than in sheep. The older flukes (7-14 weeks) are more susceptible to chlorsulon than the younger ones (4-6 weeks). The drug is administered subcutaneously at a dose of 2 mg/kg to eliminate 90% of adult worms of *F. hepatica* in calves [79]. For eradicating 8 week old flukes, a dose of 4-8 mg/kg of the drug is required to be given subcutaneously. However, an oral dose of 7 mg/kg of chlorsulon was highly effective in eliminating adult flukes from cattle [80]. The recommended dose of this drug for fascioliasis of sheep is 20 mg/kg, p.o.; the maximum tolerated dose being 100 mg/kg [33]. The drug is rapidly metabolised. It is a useful drug for the treatment of meat producing and lactating cattle [33,81].

8.1.7 Bunamidine (34)

This drug was introduced in 1965 by Wellcome. Soon it became a preferred drug for the treatment of *Taenia spp., D. Caninum, Spirometra erinacei* and *E. granulosus* in cats and dogs. Bunamidine is used as hydrochloride and hydroxynaphthoate. For cats and dogs, bunamidine hydrochloride is used at a single oral dose of 50

mg/kg where after high activity against the tapeworms, *T. hydatigena, T. taeniaeformis, S. mansonoides, D. caninum, M. multiceps* and *E. granulosus* in cats and dogs is obtained [12,69,76,82,83]. The drug is well tolerated at therapeutic doses; however, it may cause vomiting and diarrhea. Rarely there may be mortality in the treated animals [33].

Bunamidine hydroxynaphthoate has been used to eliminate *M. expansa* from sheep and goats at doses of 25 and 50 mg/kg, respectively [69,84]. It is also effective against *Raillietina spp.* in chickens [33,76].

8.1.8 Diamphenetide (35)

This is an effective anthelmintic for the treatment and control of fascioliasis and dicrocoeliasis in sheep and cattle. In Russia and CIS (formerly known as U.S.S.R.), it is used under the generic name acemidophene. Diamphenetide shows high activity against young flukes (*F. hepatica*), however, its activity reduces as the flukes grow older. For example, the drug eliminated over 95% of the younger flukes (below 6 weeks old) at a dose of 80-100 mg/kg. On the other hand it caused 85- 95% eradication of older flukes (over 6 weeks old) at a dose of 80-120 mg/kg in sheep [85]. The recommended oral doses of diamphenetide to treat *F. hepatica* in sheep and cattle are 80-120 and 100 mg/kg, respectively [33,35]. The activity of this drug is believed to be due to its deacetylation in the liver to form the active metabolite. Since diamphenetide has a pronounced effect on immature flukes, it can be used for chemoprophylaxis of fascioliasis in sheep [33,35,86].

Diamphenetide is also active against *Dicrocoelium dendriticum* in sheep. At an oral dose of 200 mg/kg, it causes more than 90% clearance of the flukes. However, the therapeutic index in this case falls to 2 as the maximum tolerated dose of the drug is 400 mg/kg [33,35,87,88a].

8.1.9 Probenzimidazoles

(a) **Thiophenate (81):** This is an effective veterinary anthelmintic for the treatment of gastrointestinal roundworms in cattle, sheep and pigs. It shows 90% activity against the adult nematodes. The developing and arrested forms of the enteric nematodes are also very susceptible (75-90% activity) to thiophenate. The recommended dose for eliminating *Haemonchus, Ostertagia, Trichostrongylus, Cooperia, Oesophagostomum* and *Nematodrius spp.* etc. in cattle and sheep is 66-132 and 50 mg/kg, respectively. For the treatment of *Ascaris suum, Oesophagostomum spp. Hyostrongylus*

rubidus and *Trichuris suis* infections in pigs, thiophanate is used at a dose of 67 mg/kg. The drug is given to the animals in feed, in feed block or as drench [33,70,88b,c].

(b) Netobimin (84): This is also a very effective drug for treating gastrointestinal nematode infections in cattle, sheep and pigs. The therapeutic dose of netobimin for eradicating the intestinal roundworms as discussed above is 7.5-20 mg/kg. In addition the drug is also effective against *Moniezia benedeni, F. hepatica* and *D. dentriticum* in sheep. A dose of 15-20 mg/kg of netobimin is required to clear all the helminths such as *Oesophagostomum spp., A. suum* and *T. suis* inhabiting the gastrointestinal tract of pigs [33,88b,c].

8.2   Drugs in human medicine

8.2.1   Bephenium hydroxynaphthoate

This is an effective drug for the treatment of hookworm infections in humans. The drug possesses better activity against *A. duodenale* than against *N. americanus*. The drug is administered orally at a dose of 5 g (2.5g base)/adult and 2.5g (1.25g base)/child [12,23,34,89]. Bephenium hydroxynaphthoate has been used in individual and community treatment of ancylostomiasis with high success; however, it was not possible to eradicate the infection completely [90-92].

It is a bitter tasting drug and induces several side effects, which are related to gastrointestinal upset. The common side effects of bephenium hydroxynaphthoate are nausea, vomiting, diarrhea, abdominal pain, anorexia, dizziness and headache occuring in nearly 60% of the treated cases [89].

8.2.2   Dichlorophene

The drug has been extensively used to treat *T. saginata, T. solium, D. latum* and *H. nana* infections in humans [3,12,34,93]. The recommended dose of dichlorophene is 70 mg/kg, but doses ranging from 60-100 mg/kg (max. 600 mg/adult) have also been used [94]. Since the drug has a mild laxative action, post-treatment purgation is not usually needed. Better results may be obtained by administering the drug for 2-3 days. In Russia and CIS (formerly known as U.S.S.R.), a combination of dichlorophene (1 g) and niclosamide (2 g), called dichlosal, has been used to cure cattle and fish tapeworm infections [94].

The side effects of dichlorophene are nausea, vomiting, diarrhea and other gastrointestinal disturbances. The drug may also give rise to urticaria, lassitude and

jaundice. It is advised to be careful while treating patients with severe heart and liver problems [34,94].

8.2.3 Bithionol

This is an effective drug for treating *T. saginata* and *D. latum* infections. Bithionol is usually given at a dose of 40-60 mg/kg in two divided doses after fasting, followed by a saline purge [34,95]. A single dose of 50-66 mg/kg has also been used. The side effects of the drug are anorexia, dizziness and gastrointestinal discomfort [34].

Bithionol has also been successfully used to cure liver fluke and lung fluke infections in humans [96]. A dose of 30-50 mg/kg given orally on alternate days for 2-3 weeks has been found to eliminate *Fasciola spp.* and *P. westermani* from humans [3,41].

8.2.4 Lucanthone and hycanthone

Lucanthone was introduced as a human schistosomicidal drug in 1946. It is active against *S. haematobium* and *S. mansoni*, but is inactive against *S. japonicum* in humans. The usual dose of the drug for treating *S. haematobium* infection is 20 mg/kg given orally for 3-5 days [12,25]. For the treatment of *Schistosomiasis mansoni*, lucanthone has been administered at a dose of 10 mg/kg, b.i.d. untill a total of 100 mg/kg or 6 g has been consumed. The drug may be given as a single dose of 60 mg/kg. In all the dose schedules about 80% of the treated patients became negative for eggs [12].

Although lucanthone gave satisfactory results in curing human schistosomiasis, it could not be used in mass therapy due to its various side effects like nausea, vomiting, diarrhea, insomnia and occasional haematemesis occuring in some treated patients [12,25].

Hycanthone is a better drug than lucanthone. It exhibits high activity when given orally, but it is more potent if administered parenterally. The usual dose of the drug is 1.5-3.5 mg/kg given orally or intramuscularly for 1-5 days [12]. However, a single intramuscular dose of 3 mg/kg appears to be most suitable for curing *S. mansoni* and *S. haematobium* infections in humans [32]. Hycanthone is inactive against *S. japonicum* in humans. Generally 25-50% of the treated patients experienced various side effects, though of milder intensity. The side effects occurred more frequently in patients receiving the drug orally. The common side effects of hycanthone are nausea, vomiting, headache, anorexia, abdominal pain, pain at the injection site and ver-

tigo [3,12]. Overall it is a well tolerated drug, though rare fatalities may be seen due to liver damage. Hycanthone is contraindicated in patients with renal and hepatic problems, cardiac diseases or acute febrile illness [3].

8.2.5 Hetol

The drug shows varying degree of activity against different flukes infecting humans. For treating *Opisthorchis felineus*, a dose of 60 mg/kg of the drug was given for 5 days after which 75% cure rates were obtained. A dose of 100 mg/kg given on alternate days for 5 or 10 days may be needed to get 80% cure rates against *O. viverrini*. Hetol also exhibits high activity against *Clonorchis sinensis* and *Paragonimus spp.* in humans at different dose regimens ranging from 100-500 mg/kg given for 5-24 days [12]. The activity (<90%) of hetol against paramphistomiasis (dose: 200-300 mg/kg, p.o.) has also been established [12].

8.2.6 Suramin

Suramin was till recently the main stay of chemotherapy of human onchocerciasis [3,97,98]. The following dose schedule was used for treating *O. volvulus* infection [99]. For adults, a test dose of 100-200 mg of the drug was administered intravenously. If this dose was tolerated by the patient, 1g of suramin was injected intravenously at weekly intervals for 5 weeks. For children the test dose is 10-20 mg/kg intravenously, followed by a dose of 20 mg/kg administered intravenously every week for 5 doses. It is essential to inject the test dose first to avoid unpredicted toxicity or fatalities associated with the suramin therapy.

Suramin exerts a selective action on the adult female worms of *O. volvulus*, which die within 4-5 weeks of treatment. The adult male worms and microfilariae are killed rather slowly. The drug has no action on *W. bancrofti, L. loa or D. perstans.*

The side effects of suramin are fever, joint pains, photophobia, peripheral neuropathy and allergic reactions. The later phase of drug therapy may cause kidney damage and exfoliative dermatitis. The use of this drug has been recommended only under medical supervision.

9. MODE OF ACTION
9.1 Bephenium and thenium

Both these drugs are cholinergic ganglionic agonists. Thus they mimic the acetylcholine activity by binding to the ACh receptor site and, therefore, foil the action

of AChE [100, 101]. Because of the ability of bephenium and thenium to exert cholinergic properties, they cause contraction of *Ascaris* muscles [100,102,103]. The spastic paralysis of the *A. suum* muscles caused by bephenium can be prevented by adding *d*-tubocurarine, a muscle relaxant, and piperazine [104].

9.2   Dichlorophene

The drug inhibits phosphorylation in *Ascaris* mitochondria (40% inhibition at 5×10^{-5} M concentration) [104]. It also controls incorporation of ^{32}P into ATP in *H. diminuta* both *in vitro* and *in vivo*. Thus its antitapeworm activity may be due to its uncoupling of the electron-transport-linked phosphorylation in mitochondria [104-106].

9.3   Bithionol

Hamajima [107] has studied the effects of bithionol on the glycolytic and oxidative metabolism on adult lung flukes, *Paragonimus westernami*. He believes that the mode of action of bithionol is similar to that of 2,6-dichlorophenol; the phenolic hydroxy groups of the drug may interfere with glycolysis and the tricarboxylic acid (TCA) or Kreb's cycle. It can also act as an uncoupler of the oxidative phosphorylation. The drug causes inhibition of ATP synthesis in helminths [104].

9.4   Lucanthone and hycanthone

The possible mode of action of lucanthone and hycanthone has been reviewed by Archer [32]. Hycanthone is an effective schistosomicide; it is also an antitumor agent in mice and a frameshift mutagen. Hycanthone has been shown to be teratogenic and possibly carcinogenic in mice [32].

The antischistosomal activity of hycanthone is believed to be due to its ability to bind covalently with DNA by a reaction sequence very similar to that described for oxamniquine (Chapter 11). Lucanthone (25) is oxidized in the biophase to form hycanthone (26), which is transformed into the ester (85) in the presence of kinase or sulphotransferase. Non-enzymatic dissociation of 85 gives 86, which alkylates DNA to form the hycanthone-DNA complex (87).

Hillman *et al.* [108,109] have suggested that hycanthone may act by binding irreversibly to the ACh receptor in schistosomes resulting in neuromuscular incoordination and paralysis of the digestive tract of the fluke. The worm later dies of starvation.

25 R = H
26 R = OH

85

OE = OPO$_3^{2-}$, OSO$_3^-$

86b

86a

87
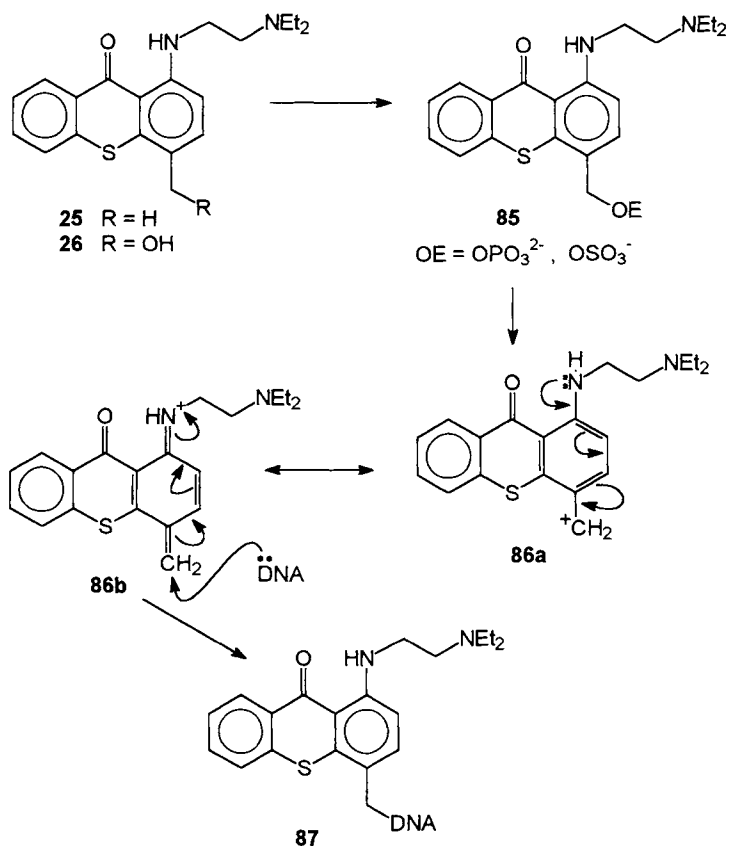
9.5 Suramin

The antifilarial activity of suramin is attributed to its ability to inhibit regulatory enzymes such as protein-kinase or DHFR in the parasites. Presumably it kills *O. volvulus* because the DHFR enzyme of the filariid is more sensitive than of the host [101].

The drug has high tendency to bind with various proteins and, therefore, may interfere with the energy metabolism, calcium transport and phosphorylation-dephosphorylation reactions [110]. Walter and co-workers [111-113] have found that suramin inhibits lactate dehydrogenase, malate dehydrogenase, malic enzyme and protein-kinase. Jaffe [114], showed that it also interferes with the folate metabolism of the helminths.

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CHAPTER 13

BIOCHEMICAL TARGETS FOR
ANTIPROTOZOAL ACTIVITY

1. INTRODUCTION

The parasitic diseases caused by pathogenic protozoans present a serious scene in tropical medicine, especially in terms of their epidemiology, pathology, clinical manifestations and inadequancy of available drugs. A large number of protozoan parasites can invade different parts of the human body such as gastrointestinal tract, blood vessels, musculature, skin, liver, and CNS etc., where they live, reproduce and multiply giving rise to a wide spectrum of clinical complications. They have evolved a number of biochemical mechanisms to evade the host's defence system and to live in symbiosis with the latter. The protozoans have, however, a number of selective biochemical pathways to meet their special energy requirements, DNA, RNA and protein synthesis, requirements of lipids for membrane structure and polyamines to control cell functions. Wherever needed, these parasites may also 'steal' host's biomolecules such as purines for their survival. The protozoans have also well developed antioxidant enzymes to combat oxidative burst of the host during infection. Some of these pathways provide medicinal chemists some vulnerable targets for chemotherapeutic intervention. The present section will, therefore, deal with the biochemistry of various pharmacological processes operating in the parasitic protozoans highlighting the biochemical targets, which may be explored to design new antiprotozoal agents.

2. GLUCOSE METABOLISM

A number of protozoans are known to derive energy by metabolism of glucose, the most notable being kinetoplastid, flagellates such as blood stream forms of African trypanosomes (*Trypanosoma brucei, T. Cruzi*) and a few *Leishmania* spp., whose glucose metabolism has been better studied than of other protozoans. The African trypanosomes largely depend on glycolysis for their energy requirements. Glycolysis in trypanosomes takes place in two parts of the body, namely in glycosomes and mitochondria.

Glucose $\longrightarrow$ Fructose-1,6-diphosphate (FDP)

$OHC—CH—CH_2—OPO_3H_2 \rightleftharpoons HO—CH_2—CO—CH_2—OPO_3H_2$
$\quad\quad\;\;|$
$\quad\quad\;OH$

Dihydroxyacetone phosphate (DHAP)

Glyceraldehyde-3-phosphate (GAP)

a $\quad$ NADH / NAD

$HO—CH_2—CH—CH_2—OPO_3H_2$
$\quad\quad\quad\;|$
$\quad\quad\quad OH$

$HOOC—C=CH_2$
$\quad\quad\;\;|$
$\quad\quad OPO_3H_2$

Glycerol-3-phosphate (G-3-P)

PEP

b $\quad$ ADP / ATP

$CH_3—CO—COOH$

$HO—CH_2—CH—CH_2—OH$
$\quad\quad\quad|$
$\quad\quad\;\;OH$

PYR
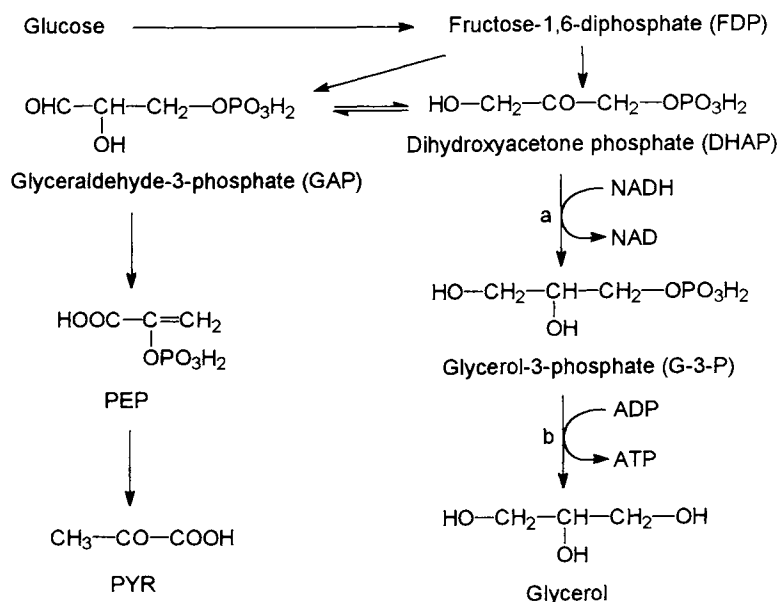
Glycerol

Chart 1: Glucose metabolism in glycosomes of trypanosomes; (a) Glycerolphosphate kinase; (b) Glycerol kinase.

2.1 Glycolysis in glycosomes

Glycosomes are membrane-bound microbody like intracellular organelles, which contain all the enzymes necessary for glycolysis, glycerol metabolism and fixation of CO_2. The glycosomes also possess some enzymes associated with pyrimidine synthesis, purine salvage and ether-lipid biosynthesis [1,2].

The first step of the trypanosomal glycolysis involves the conversion of one molecule of glucose into glucose-6-phosphate, which is transformed to fructose-1,6-diphosphate. The latter is then converted into glyceraldehyde-3-phosphate, PEP and pyruvate (PYR) by a sequence of reactions similar to those described for helminth glycolysis (Chapter 2, Chart 2) [1,3-6].

Fructose-1,6-diphosphate (FDP) may also be converted into dihydroxyacetone phosphate (DHAP), which is also synthesized from glyceraldehyde-3-phosphate (GAP). The dihydroxyacetone phosphate (DHAP), thus obtained, is converted into glycerol-3-phosphate (G-3-P), in the presence of cytosolic NADH. The latter gives away one phosphate radical to ADP to generate ATP and glycerol (Chart 1). Thus in the above process, one mole of glucose is converted into two moles of pyruvate with a net gain of two moles of ATP [1].

2.2 Glycolysis in mitochondria

The conversion of glycerol-3-phosphate (G-3-P) into dihydroxyacetone phosphate (DHAP) in glycosomes generates reducing equivalents, which enter the mitochondria and convert molecular oxygen into water. This respiratory reaction takes place in the presence of the glycerol-3-phosphate oxidase (glycerolphosphate oxidase, GPO) system. It is a complex enzyme system, which is unique to salivarian trypanosomes; this is not found in mammalian cells [7]. Further, GPO is cytochrome free and is bound to the inner membrane of mitochondria (Chart 2).
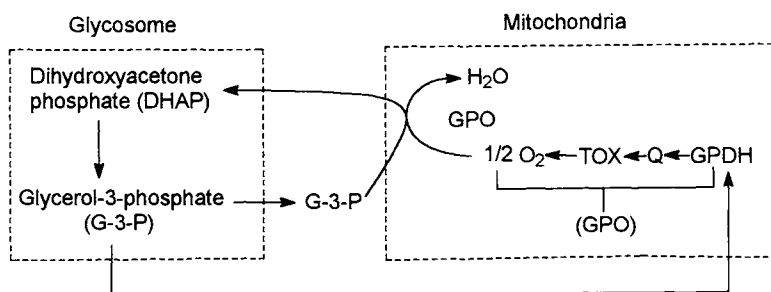
Chart 2: Organisation of the GPO system and respiration in mitochondria in blood forms of trypanosomes (GPDH, mitochondrial glycerol-3-phosphate dehydrogenase; (Q) ubiquinone like electron carrier; TOX, terminal oxidase).

The glucose metabolism in aerotolerant anaerobes like trichomonad flagellates, *Amoeba* and *Giardia* spp. is somewhat different than in trypanosomes. These parasites carry out anaerobic production of energy from glucose, which may be stored in the form of glycogen upto 30% of their dry weight.

The catabolism of glucose in parasitic trichomonads proceeds utilizing classical glycolysis steps leading to the formation of pyruvate and malate in the cytosol. Further degradation of pyruvate takes place in hydrogenosomes, which can produce hydrogen from pyruvate. The pyruvate oxidation in hydrogenosomes is achieved by oxidative decarboxylation via ferredoxin linked enzymatic reactions. The endproducts are acetate and molecular hydrogen. Such metabolism of glucose has been observed in *Trichomonas vaginalis* (Chart 3). It must be noted that unlike trichomonads, *Giardia* spp. lack hydrogenosomes and a ferredoxin-linked hydrogenase. This makes *Giardia lamblia* incapable of producing acetate under anaerobic conditions. Thus, the energy metabolism in this parasite is purely fermentative in nature.
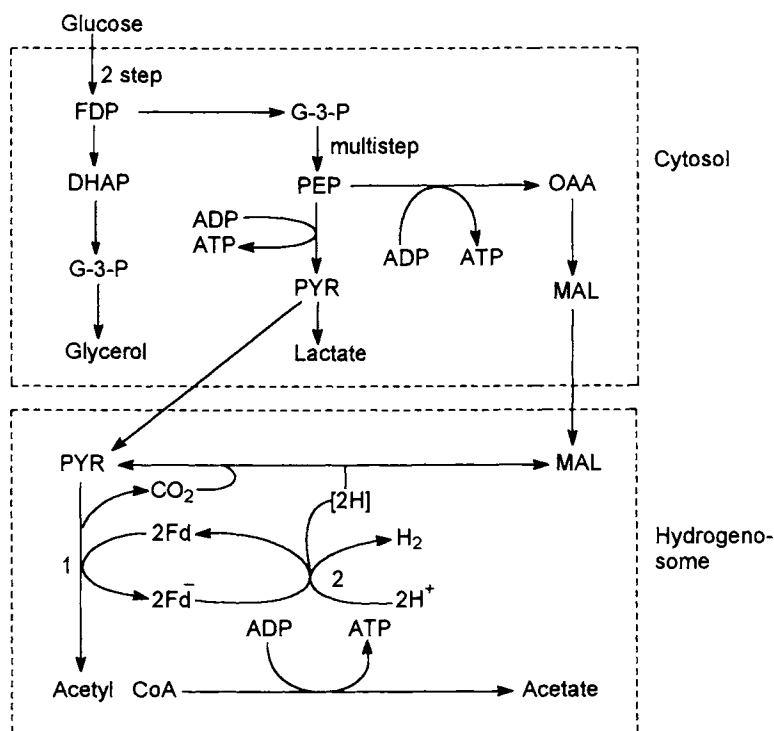
Chart 3: Glucose metabolism in *Trychomonas vaginalis* (for abbreviation of glycolytic products, see chart 2, chapter 2, except 1, pyruvate- ferredoxin oxidore-ductase; 2, hydrogenase; Fd, ferredoxin).

2.3 Biochemical targets in glucose metabolism

The facts that various protozoal parasites generate energy from the catabolism of glucose and that a number of glycolytic enzymes involved in this process are unique to parasites, provide useful targets for inhibition by chemical agents, which could lead to parasite starvation and eventual death. As early as 1969, Flynn and Bowman [8] showed that melarsen oxide causes impairment of the ATP synthesis during the formation of pyruvate from PEP in trypanosomes. This is achieved by inhibiting pyruvate kinase. Arsenicals like melarsen are also known to inhibit *Sn*-glycerophosphate oxidase, while suramin inhibits both *Sn*-glycerophosphate oxidase and *Sn*-glycerophosphate dehydrogenase [9,10]. These two enzymes are involved in mitochondrial respiration of the blood form of African trypanosomes. The combined inhibition of *Sn*-glycerophosphate oxidase and *Sn*-glycerophosphate dehydrogenase prevents reoxidation of NADH to NAD, thereby causing reduction in ATP synthesis.

Another biochemical target for designing effective antiprotozoal agents is provided by glycosomes. It has been observed that African trypanosomes possess a large number of glycosomes, while the same are present in smaller numbers in *Leishmania* and *T. cruzi*. Most of the glycosomal enzymes carry a high positive charge. This creates "hot spots" on the surfurce of glycosomes. Thus design of compounds which would interact with these "hot spots" may lead to inactivation of the glycosome [2], and is another useful target for chemotherapeutic attack.

3. FOLATE METABOLISM

Folic acid or the folate coenzyme [6] is a nutritional factor both for the parasites and the hosts. It exists in two forms, viz. dihydro- and tetrahydrofolic acids [4,5] which act as cofactors involved in the transfer of one carbon units like methyl, hydroxymethyl and formyl. The transfer of a one carbon unit is associated with *de novo* synthesis of purines, pyrimidines and amino acids. Mammals can not synthesize folate and, therefore, depend on preformed dietary folates, which are converted into dihydrofolate by folate reductase. Contrary to this, a number of protozoal parasites like plasmodia, trypanosomes and leishmania can not utilize exogenous folate. Consequently, they carry out a *de novo* biosynthesis of their necessary folate coenzymes [12]. The synthesis of various folates follows a sequence of reactions starting from 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine (1), which is described in Chart 4 [13,14].

Reaction of 1 with ATP in presence of Mg^{2+} and kinase gives 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphate (2, DHP pyrophosphate). The latter is converted into 7,8-dihydropteroate (3, DHP) by its reaction with *p*-aminobenzoic acid (PABA) and DHP synthetase. Addition of L-glutamic acid to DHP in the presence of the enzyme dihydrofolate synthetase (DHF synthetase) yields 7,8-dihydrofolate (4, DHF), which undergoes reduction by the enzyme dihydrofolate reductase to afford 5,6,7,8-tetrahydrofolate (5, THF). DHF may also lose a hydrogen molecule in the presence of DHF dehydrogenase to form folic acid (6).

3.1 Biochemical targets in folate metabolism

The folic acid biosynthesis has been extensively explored to design effective antimalarials. A number of classes of compounds have been developed, which interfere with different steps of the folate synthesis in plasmodia [14]. These antimalarials, which are collectively known as antifolates, may be broadly divided in three groups.
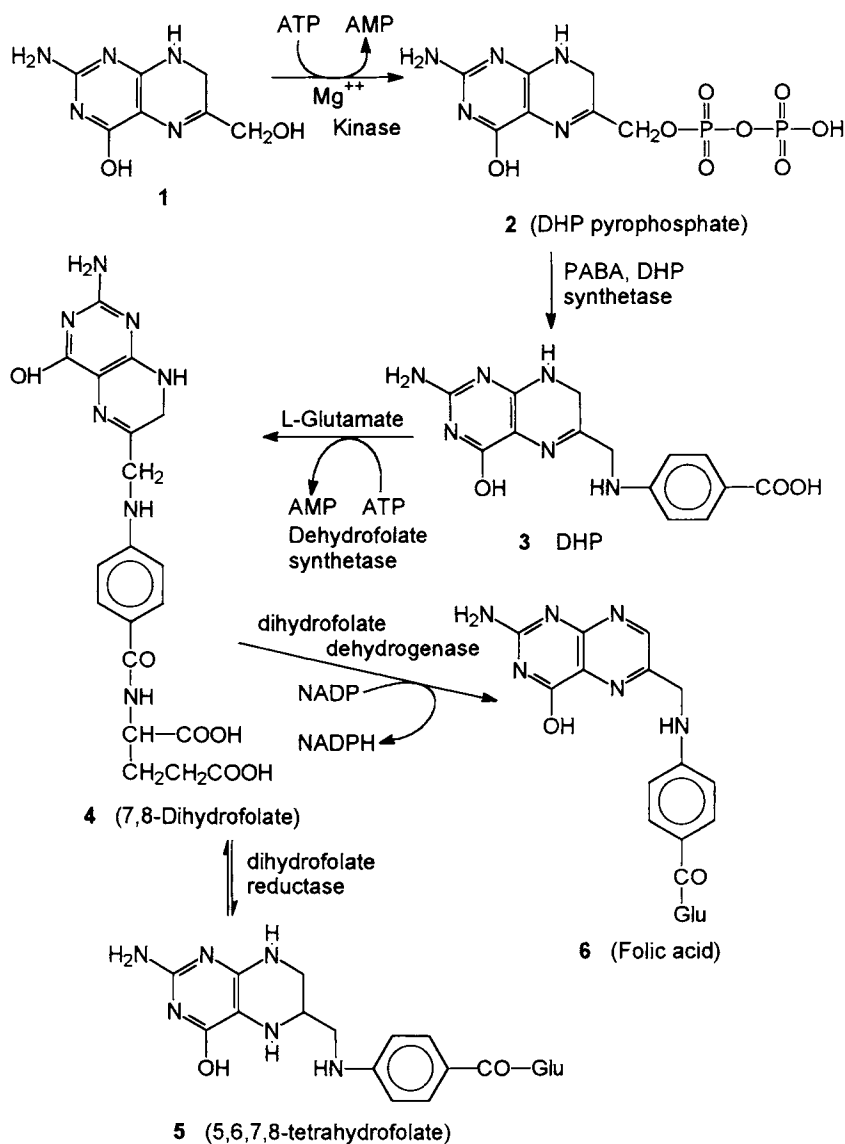
Chart 4: Folate metabolism in protozoans.

(a) PABA antagonists : DHP synthetase inhibitors

Compounds of this class inhibit the synthesis of dihydropteroate (DHP, **3**) from PABA and DHP pyrophosphate (**2**), which is achieved by competing with PABA for the enzyme involved in the conversion of **2** to **3**. Sulphonamides and sul-

phones are the classic examples of inhibitors of the DHP synthesis in bacteria, plasmodia and other parasites.

(b)   DHF reductase inhibitors

Compounds of this class inhibit the reduction of DHF (4) to THF (5) by blocking DHF reductase. Inhibition of the THF synthesis cuts off further synthesis of thymine from uracil. The former is used in the formation of DNA. Compounds belonging to the diaminopyrimidines (trimethoprim and pyrimethamine), biguanides (proguanil, chlorproguanil) and triazines (cycloguanil) are examples of DHF reductase inhibitors. These drugs are ususally given in combination with sulfonamides because of the synergistic effect of these two classes of compounds. Further, inhibition of DHF reductase alone may not be sufficient to prevent complete synthesis of THF. Slow accumulation of unconverted DHF may result in its partial conversion into THF; accumulation of DHF may be prevented by sulfonamides.

(c)   Thymidylate synthetase inhibitors

Thymidylate synthetase is an important enzyme, which is responsible for the reductive methylation of deoxyuridylic acid (dUMP, 7) to deoxythymidylic acid (dTMP, 8). The methylation of the uracil moiety (present in RNA) to 5-methyl uracil (thymine, present in DNA) requires participation of a folic acid coenzyme, N^5, N^{10}-methylenetetrahydrofolate as a methyl donor. The functioning of thymidylate synthetase is coupled with the activity of DHF reductase. That is why this biochemical target is usually referred to as thymidylate synthetase/ DHF reductase.
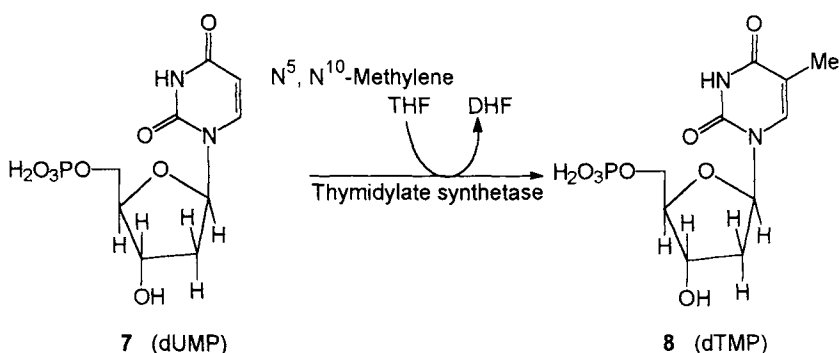
4. NUCLEIC ACID SYNTHESIS

The synthesis of purine and pyrimidine nucleotides needed for the biosynthesis of nucleic acids, and in turn the processes of transcription and translation provide a number of suitable targets for therapeutic intervention. These have been subject of much interest for the discovery of new antiprotozoal drugs as well. Developments in two areas, viz. the purine salvage pathway and the pyrimidine biosynthesis have yielded useful drugs and are discussed.

4.1 Purine salvage pathway

Haemoflagellates are incapable of carrying out *de novo* synthesis of purines and, therefore, completely rely on salvage of preformed purines and purine nucleotides from the host. The purine salvage pathway has been demonstrated in plasmodia, leishmania and other protozoans. Of the two purine bases, adenine and guanine, available in the host, the protozoans prefer to salvage hypoxanthine, which is synthesized extracellularly as follows: during cellular work erythrocytic ATP (10) is successively degraded to ADP, AMP and finally to hypoxanthine (12) [15]. Alternatively, adenosine (9) may be deaminated to form inosine (11), which is later deribosylated to give hypoxanthine [16]. Both these processes occur extracellularly. After hypoxanthine (12) is generated, it is soon taken up by the parasite where its conversion into inosinic acid (inosine 5'-monophosphate, IMP, 13) takes place by the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT). Hypoxanthine may also first be converted into hypoxanthine nucleoside, inosine (11), which then undergoes phosphorylation to form inosinic acid (13). The latter is utilized for the synthesis of RNAs, needed by the parasite to synthesize proteins (Chart 5).

A large number of compounds have been synthesized as antagonists of hypoxanthine-guanine phosphoribosyltransferase (HGPRT), which provides an excellent biochemical target for the design of antiprotozoal agents. Piper and co-workers [17,18] have synthesized several azaheterocycles as potential inhibitors of HGPRT during search for effective antimalarial agents. Following the discovery of allopurinol as an effective antileishmanial agent, a series of structural analogues of pyrazolopyrimidines, inosine, oxypurinol, and thiopurinol have been prepared as HGPRT antagonists [19]. Inhibition of guanine nucleotide formation, deoxynucleoside phosphotransferase, adenosine deaminase, GMP reductase and guanine aminohydrolase may also offer effective targets for chemotherapeutic attack of the nucleotide metablism of parasitic protozoans [19].
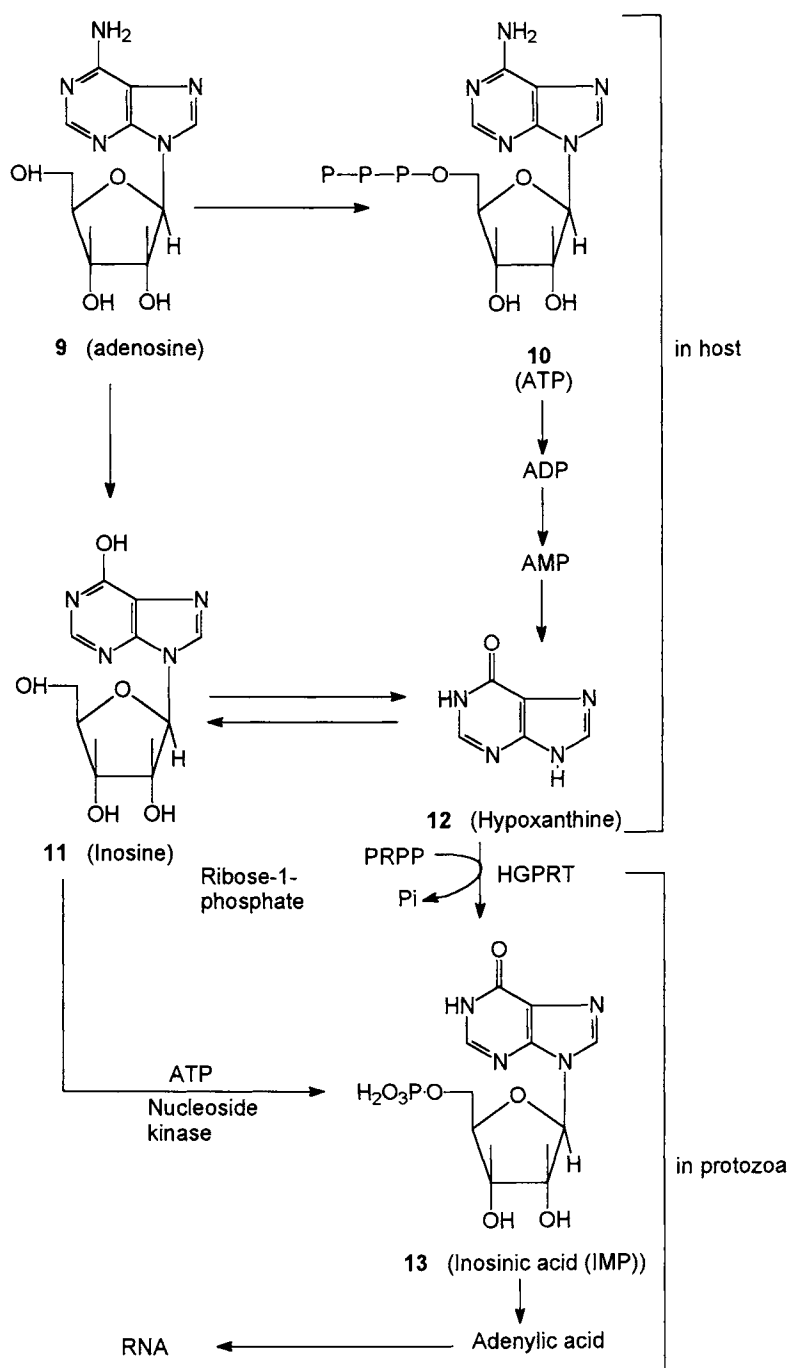
Chart 5: Formation of hypoxanthine in the host and its salvage by protozoans.

4.2   Pyrimidine synthesis

It has been indicated above that for purine requirements the protozoans depend on salvage of preformed purines. Contrary to this, the protozoal parasites have capability for the *de novo* biosynthesis of pyrimidines, which are equally essential for nucleic acid and protein synthesis. The pyrimidine biosynthesis in protozoans is very similar to the pathway mapped for eukaryotes [1,20,21].

The starting materials for the pyrimidine biosynthesis are aspartic acid (14) and carbamoyl phosphoric acid (17); the latter is synthesized alongwith glutamic acid (16) from ATP and glutamine (15) as shown below:

$$H_2N—CO(CH_2)_2—\underset{\underset{NH_2}{|}}{CH}—COOH + ATP \xrightarrow[\text{Carbamoyl phosphate synthase}]{CO_2+H_2O}$$

$$\underset{\textbf{15}}{}$$

$$HOOC—CH_2—CH_2—\underset{\underset{NH_2}{|}}{CH}—COOH + H_2N—CO—O—\underset{\underset{OH}{|}}{\overset{\overset{OH}{|}}{P}}=O + 2ADP + Pi$$

$$\underset{\textbf{16}}{} \qquad\qquad\qquad\qquad \underset{\textbf{17}}{}$$
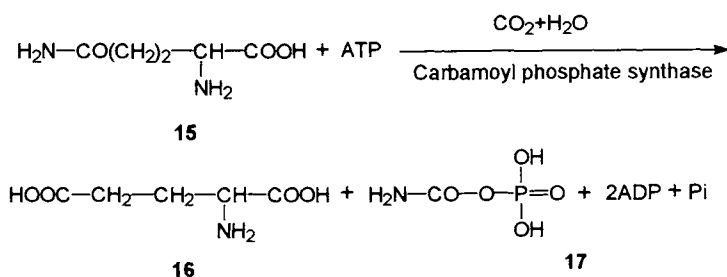
Reaction of aspartic acid (14) with carbamoyl phosphoric acid (17) in the presence of the allosteric enzyme aspartate carbamoyltransferase (aspartate transcarbamoylase) gives N-carbamoyl aspartic acid (18), which is cyclised to L-dihydroorotic acid (19) by dihydroorotase. Oxidation of L-dihydroorotic acid by flavoprotein, orotate reductase gives orotic acid (20), which reacts with 5-phosphoribosyl-1-pyrophosphate (PRPP) in the presence of orotate phosphoribosyl transferase to form orotidine 5′-monophosphate (OMP, 21). Decarboxylation of OMP by orotidine 5′-phosphate decarboxylase yields uridine 5′-monophosphate (UMP, 22), which acts as precursor for the cytidine nucleotides (CTP) (Chart 6).

Although the exact nature of the various enzymes involved in pyrimidine biosynthesis is not fully worked out, it seems that leishmania and trypanosomes possess phosphoribosyl transferase, which is specific for uracil. This makes the protozoal phosphoribosyl transferase distinct from the mammalian orotate phosphoribosyl transferase and, therefore, may be explored in protozoal chemotherapy.

5.   PROTEIN BIOSYNTHESIS

Parasitic protozoans, like other organisms, need all the twenty basic α -amino acids for the synthesis of proteins. Most of these amino acids are either obtained
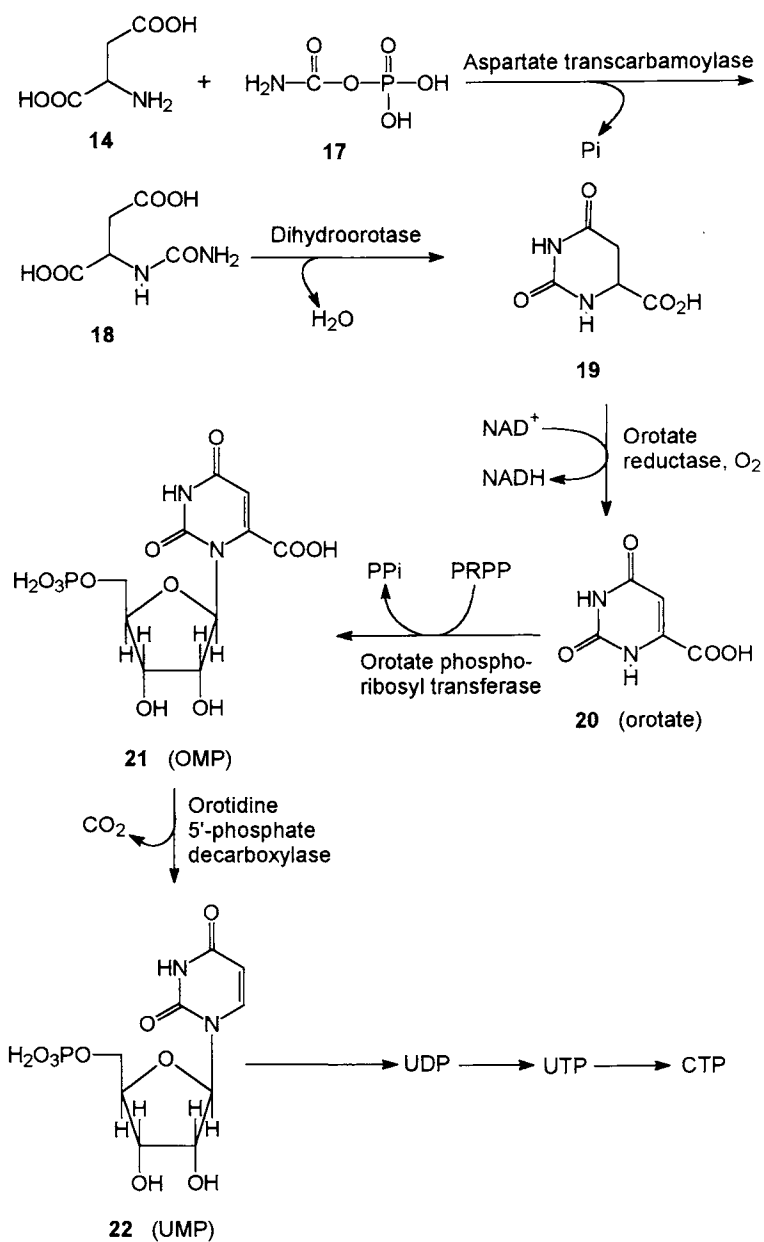
Chart 6: Pyrimidine biosynthesis in eukaryotes.

from diet or by degradation of proteins received from exogenous source. The protozoans possess enzymes like proteinases and peptidases, which hydrolyse the ingested proteins in lysosomes and some specialized vacuoles. Thus, protozoan proteases play a significant role in the amino acid supply to parasites. The protozoans can also carry out *de novo* synthesis of amino acids like other organism by the following pathways.

(a) By transamination

A number of amino acids, like alanine, leucine, tyrosine, aspartic acid, cystein and arginine react with α-ketoacids and transfer their α-amino group to the α-carbon of the α-keto acids. These reactions are catalysed by the enzyme called transaminase or aminotransferase. For example, transfer of the amino group of aspartic acid (**14**) to α-ketoglutaric acid (**23**) gives glutamic acid (**16**) and oxaloacetic acid (**24**).

$$\text{HOOC}-\text{CH}_2-\text{CH(NH}_2)\text{COOH} + \text{HOOC}-(\text{CH}_2)_2-\text{CO}-\text{COOH} \rightleftharpoons$$
$$\quad\quad\quad\quad \textbf{14} \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \textbf{23}$$

$$\text{HOOC}-(\text{CH}_2)_2-\text{CH(NH}_2)-\text{COOH} + \text{HOOC}-\text{CH}_2-\text{CO}-\text{COOH}$$
$$\quad\quad\quad\quad \textbf{16} \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \textbf{24}$$
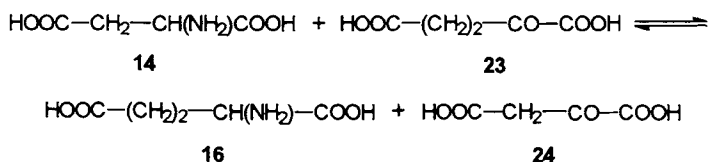
(b) From glutamic acid (16)

Reaction of glutamic acid (**16**) with ammonia and ATP gives glutamine (**15**) in the presence of glutamine synthetase.

$$\text{HOOC}-(\text{CH}_2)_2-\text{CH(NH}_2)-\text{CO}_2\text{H} + \text{NH}_3 + \text{ATP} \rightleftharpoons$$
$$\quad\quad\quad\quad \textbf{16}$$

$$\text{H}_2\text{NCO}-(\text{CH}_2)_2-\text{CH(NH}_2)-\text{COOH} + \text{ADP} + \text{Pi}$$
$$\quad\quad\quad\quad \textbf{15}$$
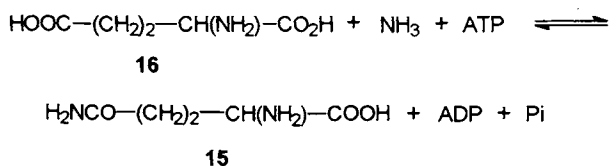
(c) From aspartic acid

As in the case of glutamic acid, aspartic acid (**14**) is converted into asparagine (**25**) in presence of asparagine synthetase.

$$\text{HOOC}-\text{CH}_2-\text{CH(NH}_2)-\text{COOH} + \text{NH}_3 + \text{ATP} \rightleftharpoons$$
$$\quad\quad\quad\quad \textbf{14}$$

$$\text{H}_2\text{NCO}-\text{CH}_2-\text{CH(NH}_2)-\text{COOH} + \text{ADP} + \text{Pi}$$
$$\quad\quad\quad\quad \textbf{25}$$
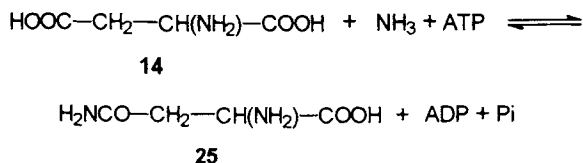
(d)   From 3-phosphoglycerate

3-Phosphoglycerate (26), obtained as an intermediate during glycolysis, is oxidized by phosphoglycerate dehydrogenase to form 3-phosphohydroxypyruvate (27). Transamination reaction between glutamic acid (16) and 3-phosphohydroxypyruvate (27) yields 3-phosphoserine (28), which loses the phosphate group to form serine (29).

NAD   NADH

$HOOC-CH-CH_2OPO_3H_2$ ──a──→ $HOOC-CO-CH_2OPO_3H_2$
       |
       OH   27

       26

                                          b⟨ — Glutamic acid
                                             → α-Ketoglutaric acid

Pi   H2O

$HOOC-CH-CH_2OH$ ──c──→ $HOOC-CH-CH_2OPO_3H_2$
       | |
       NH2   NH2

       29   28

a: Phosphoglycerate dehydrogenase
b: Phosphoserine transminase
c: Phosphoserine phosphatase
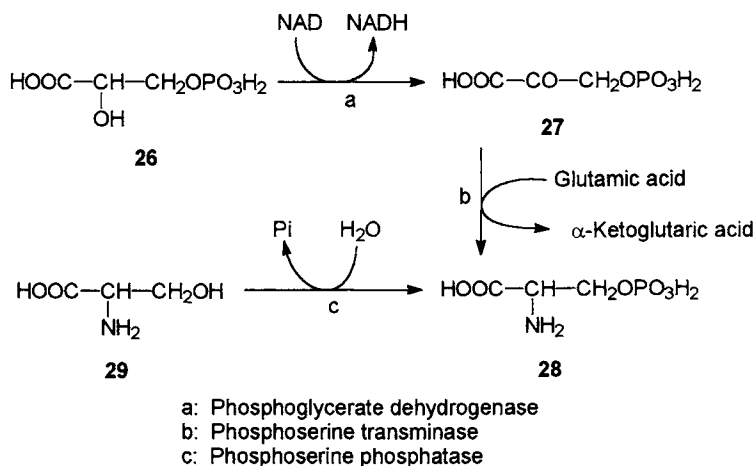
(e)   By oxidative deamination

Glutamic acid (16) may also undergo oxidative deamination in the presence of glutamate dehydrogenase to form α-ketoglutaric acid (23).

$HOOC-(CH_2)_2-CH-NH_2 + NAD^+ (NADP^+) + H_2O \rightleftharpoons$
                 |
                 COOH

       16

$HOOC-(CH_2)_2-CO-COOH + NH_4^+ + NADH (NADPH)$

       23
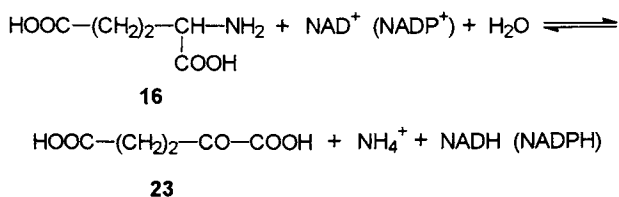
(f)   Interconversion reactions

Although a number of amino acids are synthesized by protozoans utilizing the above reactions, a few amino acids may also be produced by interconversions of certain amino acids. The interconversions of amino acids has been observed in trypanosomes, leishmania and trichomonas. The transformation of some amino acids is discussed below.

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(i) **Interconversion of proline:** Various parasitic protozoans such as *Leishmania* spp., *Trypanosoma brucei* and *T. cruzi* are capable of oxidizing proline (30) to get alanine (31) and other amino acids [22,23].

30 (Proline) → 32 (Glutamic acid)

HOOC—CH$_2$—CH(NH$_2$)—COOH
30 (Aspartic acid)

α-Ketoglutarate

Pyruvic acid

Me—CH(NH$_2$)—COOH
31 (Alanine)
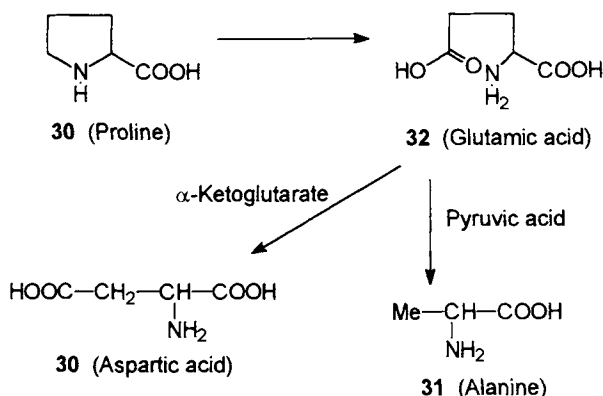
(ii) **Interconversion of threonine:** *Trypanosoma brucei* is also capable of catabolizing threonine (32) to α-amino-β-ketobutyric acid (33) and glycine (34) along with acetyl coenzyme A (35) [24].

CH$_3$—CH(OH)—CH(NH$_2$)—COOH
32 (Threonine)

Threonine dehydrogenase → NH$_3$

CH$_3$—C(=O)—CH(NH$_2$)—COOH
33 (α-Amino-β-ketobutyrate)

CH$_3$—C(=O)—S—CoA
35 (Acetyl CoA)

H$_2$N—CH$_2$—COOH
34 (Glycine)
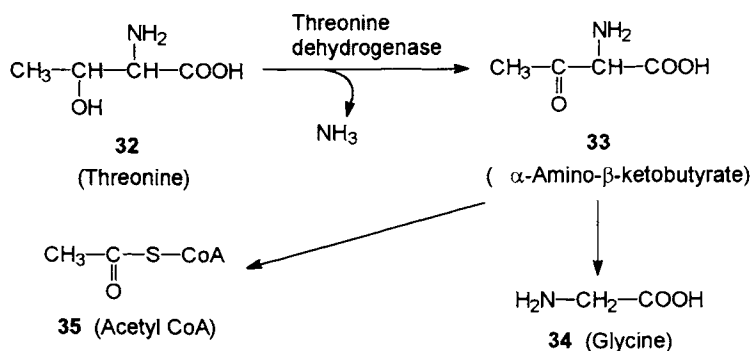
(iii) **Interconversion of arginine:** Arginine is an important amino acid both in higher animals and protozoa. Usually higher animals hydrolyse arginine into ornithine (38) and urea. However, in protozoans like *Trichomonas vaginalis*, arginine (36) is successively converted into citrulline (37), ornithine (38) and carbamoyl phosphate (17) using enzymes that are not found in higher animals [25]. Of these amino acids produced during arginine catabolism, orinithine is particularly important because it is the key substrate to initiate polyamine biosynthesis.

Having procured all the vital amino acids either from external sources or by

de novo synthesis by biosynthetic pathways discussed above, the parasitic protozoans can sucessfully synthesize various proteins required for their survival and growth. Donelson and Turner [26] have studied the synthesis of variant glycoproteins located on the surface of blood stream forms of *Trypanosoma brucei*.

6. LIPID METABOLISM

The synthesis of sterols in protozoa, though not fully worked out, may provide interesting targets for chemotherapeutic attack. Malaria parasites do not synthesize cholesterol *de novo* [27]. The blood forms of African trypanosomes are also incapable of synthesizing fatty acids of their own and depend on the host for supply and uptake [2]. Contrary to this leishmanial parasites synthesize 5-dehydroepisterol and ergosterol *de novo* [28]. The leishmanial cell membrane thus provides a useful chemotherapeutic target due to differences in lipid composition with the mammalian cell membrane. The mammals use cholesterol for construction of their cell wall, while various fungi and leishmania utilize ergosterol for the same [29].

The ergosterol (45) biosynthesis in fungi and leishmania utilizes squalene (41) as starting material, which is obtained from long chain precursors like farnesyl pyrophosphate (39) and presqualene pyrophosphate (40). Epoxidation of squalene in the presence of squalene epoxidase furnishes squalene epoxide (42), which is succes-

sively transformed into lanosterol (**43**), 7-dehydrocholesterol (**44**) and ergosterol (**45**) [21,29] (Fig.7).
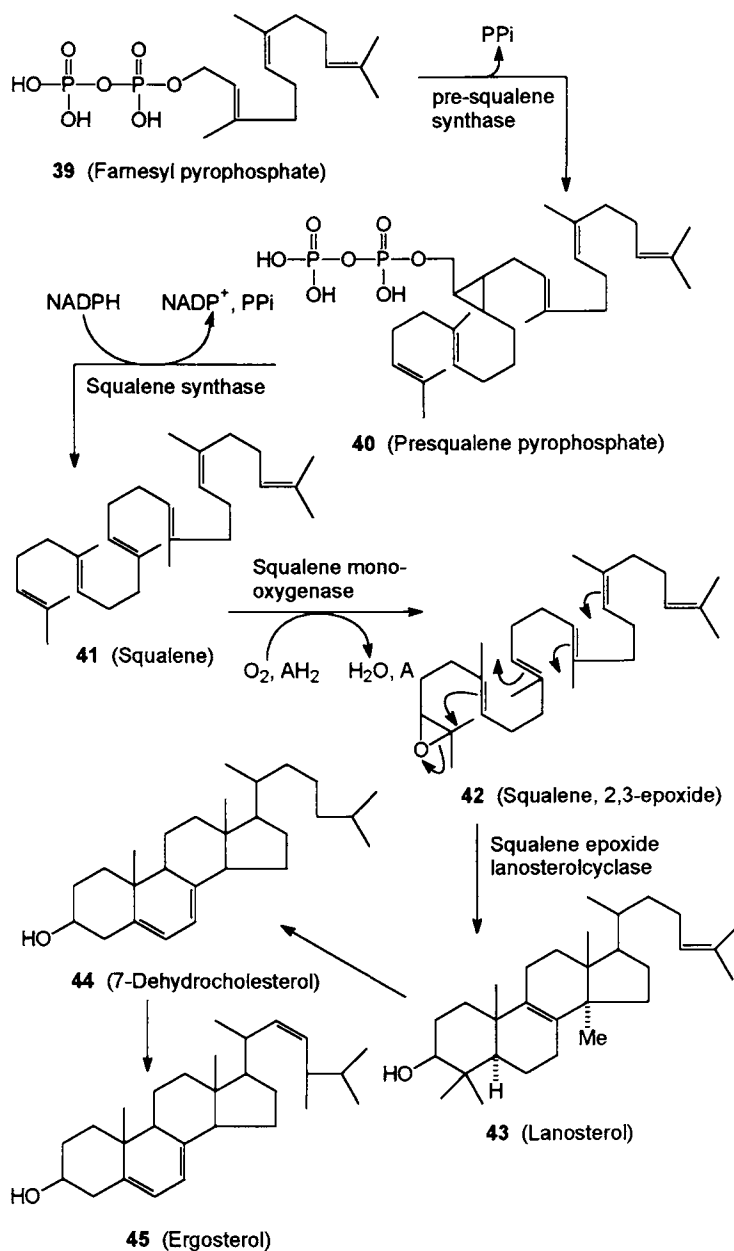
Fig. 7: Biosynthetic pathway to ergosterol in leishmania and fungi.

6.1   Biochemical targets in lipid metabolism

Since ergosterol is used in the formation of the leishmanial cell membrane, inhibition of ergosterol biosynthesis has been considered as a useful target for chemotherapeutic attack. Allylamines (eg. terbinafine) and imidazole antifungals (eg. ketoconazole) have been found to interfere with different steps in the biosynthetic pathway of C_{28} sterols in leishmania and fungi. Allylamines inhibit the microsomal squalene 2,3-epoxidase and, therefore, inhibit the synthesis of squalene epoxide, the precursor of lanosterol. Imidazoles, on other hand, inhibit cytochrome P-450 dependent C-14 demethylation of lanosterol leading to decreased or no synthesis of ergosterol [30].

7.   POLYAMINE BIOSYNTHESIS

Like helminths, some protozoans also require polyamines for their cell growth, membrane stabilization and as cofactors for macromolecular synthesis. Trypanosomes have been found to possess putrescine and spermidine, but not spermine. The precursor of the polyamine biosynthesis in protozoans is ornithine, which is decarboxylated in the presence of ornithine decarboxylase to form putrescine. Other polyamines are formed from putrescine as discussed in chapter 2 (Sec. 5.3).

7.1   Biochemical targets in polyamine synthesis

(a)   Inhibition of ornithine decarboxylase

The rate-limiting enzyme in the polyamine biosynthesis is ornithine decarboxylase, which is associated with the decarboxylation of ornithine to form putrescine. Inhibition of this enzyme has been used to design new antiparasitic and anticancer agents. DL-α-difluoromethylornithine (Eflornithine, DFMO) is the classical example of this class. This drug was originally developed as an anticancer agent. Later its therapeutic value in treating *T. gambiense, T. brucei* and *T. rhodesiense* was established. The FDA (U.S.A.) has approved the use of this drug to treat African trypanosomiasis in humans [31].

DFMO is a potent inhibitor of ornithine decarboxylase causing reversal of the activity of putrescine, spermidine and spermine. Consequently, the drug blocks cell division and related processes [2].

(b)   Inhibition of trypanothione biosynthesis

Trypanothione is a unique low molecular weight peptide containing spermid-

ine and glutathione skeletons in a cyclic structure. Chemically trypanothione is N^1,N^8-bis-(glutathionyl)spermidine (46), which is present in kinetoplastid flagellates [1]. In trypanosomes, it plays an important role in maintaining intracellular thiol redox potentials, in protection of the parasite against oxidative and chemical stress, and in regulation of spermidine contents in the cells. Trypanothione is a spermidine derivative, its synthesis and metabolism is associated with the polyamine biosynthesis in protozoans. The functioning of this unique molecule is regulated by two enzymes, trypanothione peroxidase and trypanothione reductase [2].
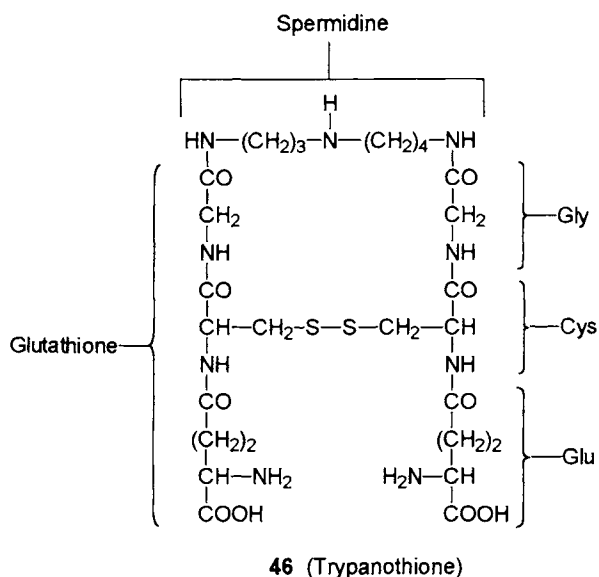
46 (Trypanothione)

Reduction of trypanothione levels in the cells may be achieved in two ways: (a) by inhibition of trypanothione peroxidase/reductase activity, or (b) by interference with the polyamine biosynthesis. Interference with the polyamine synthesis will not only lead to a decreased turnover of various polyamines, but will also bring down trypanothione levels in cells, thereby, exposing the parasites to the toxic effects of peroxides and free radicals generated by the host.

8. ANTIOXIDANT DEFENCE SYSTEM

A number of protozoal parasites such as *Trypanosoma, Plasmodium, Leishmania, Crithidia* and *Toxoplasma* spp. have been shown to contain at least one of the three major anti-oxidant enzymes: superoxide dismutase, catalase and glutathione peroxidase [32]. Thus, many of these parasites are able to quench or scavange the oxidants

like superoxide (O_2^-), hydroxyl radical ($\cdot$OH) and hydrogen peroxide (H_2O_2) generated by the host to kill the parasites. The mechanism of production of anti-oxidants during oxidative burst and oxidant mediated damage to the parasites have been discussed in chapter 2 (Sec. 4.2). The possible biochemical targets, which could be exploited for design of antiprotozoal drugs, are inhibition of catalase or glutathione peroxidase of the parasite.

9.   TUBULIN POLYMERISATION

The polymerisation of α- and β-tubulins to microtubules is an important biochemical event, which is essential for cell division, mitosis and growth in various plants and animals including helminths (Chapter 2, sec. 5.2) and protozoans. A number of parasitic protozoans like trypanosomas and leishmania have been found to contain α- and β-tubulins and microtubular assembly [2,33,34]. The factors [temperature, Mg^{2+} ions and microtubule associate proteins (MAPs)], which are essential for the polymerisation of tubulines to form microtubules in helminths, may be important in protozoa also. Thus, inhibition of microtubules assembly or interference with MAP formation have been recognized as potential targets for antiparasitic drug design [2].

A number of drugs, which are known to bind with tubulin and inhibit its polymerisation to microtubules, have been evaluated in protozoal chemotherapy. The most notable are the alkaloids maytansine, ansamitocin P3, vinblastine and vincristin, which have been shown to be potent inhibitors of tubulin polymerisation in *T. brucei*. However, these are too toxic to be considered as useful trypanocidal agents [2].

10.   REGULATION OF INTERNAL pH

It has been found that leishmania parasites are capable of surviving in a wide range of external pH. For example, *L. donovani*, during its life cycle, is exposed to alkaline pH in the sandfly gut, neutral pH in host blood circulation and acidic pH (4.5-5.0) in lysosomes and macrophages. However, irrespective the external pH, *L. donovani* maintains its internal pH to about 6.5 throughout its life span. Regulation of this constant pH is achieved by the enzyme, H^+-ATPase present in the plasma membrane. This enzyme also appears to be parasite specific and may also be involved in active uptake of glucose and proline thereby helping in the survival and growth of the parasite. Thus, inhibition of H^+-ATPase is likely to disturb the intracellular pH

344

and would also inhibit active transport of glucose and proline. Thus regulation of the internal pH or interference with H^+-ATPase of the parasite is envisaged to form an attractive target for antileishmanial drug design [2].

11. OTHER TARGETS

In addition to the various biochemical targets discussed above, selective inhibition of DNA polymerase and DNA synthesis appear useful targets for antiparasitic drug design [2,35]. A number of diarylamidines, such as pentamidine and berenil, are known to intercalate with kinetoplast DNA and inhibit DNA and RNA synthesis [36,37]. Interference with a nuclear enzyme, poly (ADP-ribose) polymerase, which controls antigenic variations in trypanosomes, is also a useful biochemical target in trypanosomes [2]. Inhibition of proteinkinase by various pyrazolopyrimidines provides a good illustration of the exploitation of this potential target for antileishmanial drug design [38].

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CHAPTER 14

NATURAL PRODUCTS

1. INTRODUCTION

The use of natural products in the treatment and control of human parasitic diseases is referred in the texts of traditional systems of medicine. Ancient medical practioners of traditional remedies in China, India, Egypt, the Middle East and Europe knew several plants having activity against various diseases caused very likely by protozoans, though the etiology and epidemiology of such diseases were not well defined in those days. Many of these remedies are still used, particularly in countries where traditional systems of medicine are a part of the health care systems. In India it is believed that 50-75% of the population uses traditional drugs because of their lower cost, easy access and faith in them [1].

Ch'ang shan is the oldest remedy for malaria known since 200 BC. It was prepared from powdered roots of *Dichroa febrifuga* Lour or *hydrangea* leaves. The active ingredient of the above plant is febrifugine (1) [2,3], whose structure was established by degradation [4-6] and synthesis [7]. Baker and coworkers [8-11] and others [12,13] have prepared a large variety of structural congeners of febrifugine. Unfortunately, neither febrifugine nor any of its derivatives could be used clinically in the treatment of malaria on account of their toxicity [14].

1
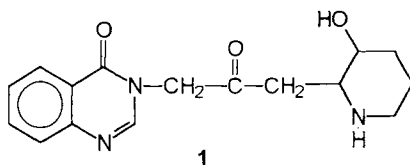
During the seventeenth century the antimalarial activity of cinchona trees growing wild in Peru was discovered. It is believed that Countess del Chinchon, wife of the Spanish Viceroy in Lima, Peru, was cured from severe attacks of malaria by a remedy prepared from the bark of this native tree. Encouraged by the curative effects of the Peruvian bark, the Spanish Viceroy introduced it in his homeland in 1639 for the treatment of ague. The powdered bark was later known as "*los Polvos de la condesa*" Subsequently, the Peruvian bark was widely used to cure "fevers" in

Europe and China. In 1643 this was listed in a medical pamphlet as *Pulvus indicus* by Hermann Van der Heyden from Belgium and was later included in the *London Pharmacopoeia* in 1677 [14,15]. In 1749 Linnaeus named this Peruvial tree as *Cinchona*, which probably symbolized the legend of Countess del Chinchon. The crude powder of this plant was used for over 200 years in different parts of the world as a specific remedy for malaria. Peru was the main supplier of the bark till 1880 and did not allow export of cinchona trees. However, it was possible to smuggle the cinchona seedlings to Java, where it was successfully planted and cultivated. Eventually Java emerged as the main producer of cinchona bark.

Quinine (2) is the major active principle of cinchona, which was isolated by Pelletier and Caventou in 1820 [16]; however, its structure could only be established after 100 years [17]. The total synthesis of quinine was accomplished by Woodward and Doering [18] and others [19] but none of the synthetic methods are economical and, therefore, can not compete with the natural production of quinine; the bark of the cinchona tree is still the only source of the drug. In addition to quinine (2), three more antimalarial components, quinidine (3), cinchonidine (4) and cinchonine (5) are present in the bark.
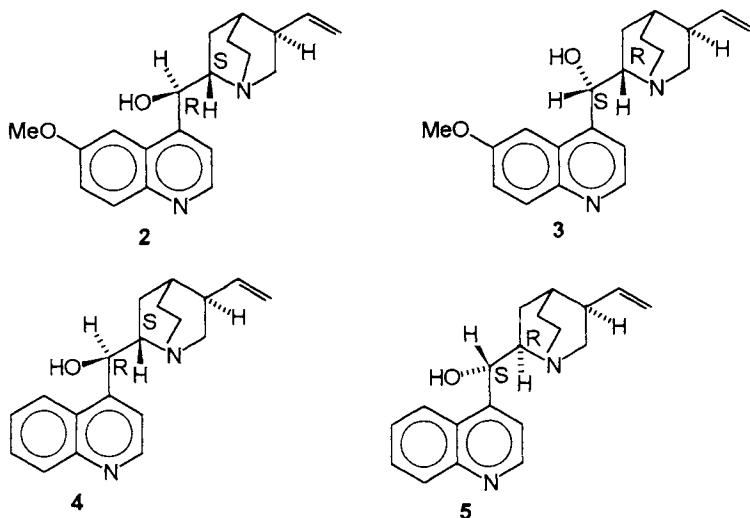
The ipecac alkaloids constitute another group of natural products, which have been used to cure protozoal diseases for centuries. The curative effects of the powdered roots of the herb igecaca had been known to ancient Brazilian Indians [14]. Like cinchona, the powdered roots of this plant became a popular antiparasitic drug after it was used to cure Danphin, the son of Louis XIV. The use of ipecac in human

therapy continued till the second half of the 19th century [14].

The extracts of ipecac, *P. ipecacuanha*, yield several alkaloids of which (-)-emetine (**6**) is the major one. The isolation, structure elucidation, resolution [16-18] and synthesis [19-26] of emetine has been fully worked out.

6
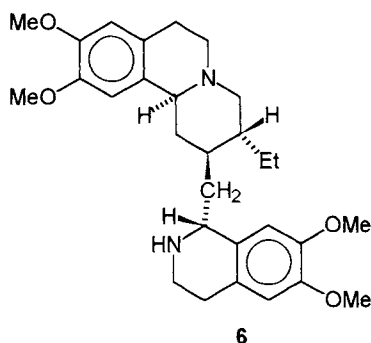
Qinghao (*Artemisia annua* L.) is an important medicinal herb, which has been used in the Chinese traditional system of medicine for many centuries. In 1972, Chinese scientists isolated (+)-artemisinin (**7**, qinghaosu) from the above plant, which is indigenous to China [27-30]. Artemisinin is a sesquiterpene lactone peroxide, whose structure and synthesis have been worked out [27,28]. It has marked activity against both chloroquine-sensitive and resistant strains of *Plasmodium falciparum* and is particularly suitable for treating cerebral malaria as it is very fast acting [29-31].

7

8

9

10
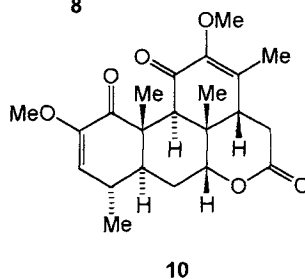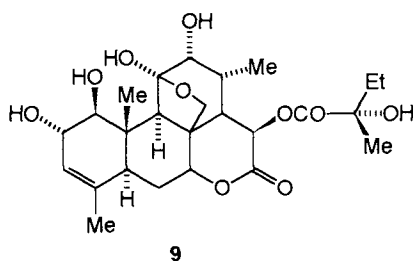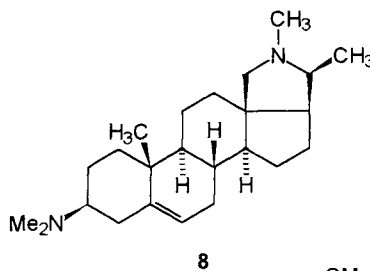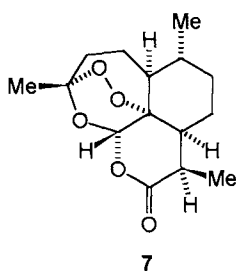
The extracts of the plants of the genus *Holarrhena* have been used in the Ayurvedic medicine for over 1500 years in the Indian subcontinent for the treatment of parasitic infections. An important plant of this class is *H. antidysenterica,* whose bark has been used to treat amoebic dysentery in man. The extract of the bark of this plant, called kurchi, conessi or telicherry bark, contains several alkaloids of which the major one is conessine (8) [32,33]. This alkaloid has been used in the treatment of intestinal and extraintestinal amoebiasis in humans [34,35]. However, neither conessine nor its structural analogues find use in the modern therapy of human amoebiasis [36].

Another medicinally useful plant is Simarouba, whose bark, fruit and seeds have been used earlier in the dysenteric conditions in Asia, Europe, Africa and the Americas. The active constituent of Simarouba is glaucarubin (9), which has been found to possess marked activity against *E. histolytica* in animals [37,38].

Other noteworthy natural products which have been used against amoebiasis in man and/or animals are quassin (10), isolated from the heartwood of *Quassia amara* [36,39] and berberine (11a), which occurs in the plant, *Berberis aristata* Linn [40a].

11a

11b
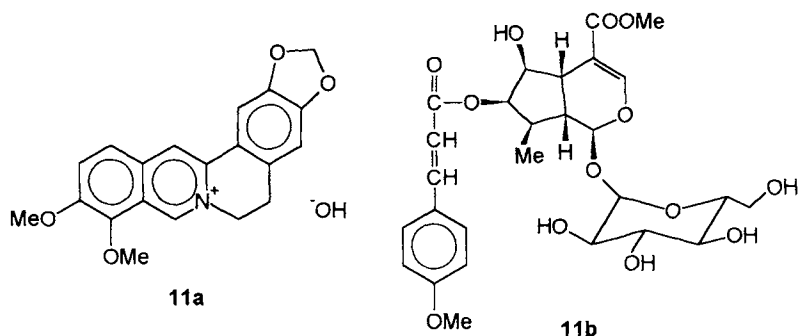
Nyctanthus arbortristis L. (*Oleaceae*) (Hindi: Harsingar) is a garden plant which has been widely used in the traditional remedies and folkloric medicine in India [40b]. The Ayurvedic system of medicine recommends its use in the treatment of fever, rheumatism, intestinal worm infections, animal and snake bites, sores, ulcer, dysentery and cancer [40c]. Recently, Tandon and coworkers [40c-f] have shown that the seeds of *N. arbortristis* possess a high order of *in vitro* and *in vivo* activity against *Leishmania donovani*. The major constituents of the plant are a mixture of iridoid glucosides called arbortristocides A-E, the most effective of which is arbortristocide A (11b). At a dose of 100 μg/ml concentration arbortristocide A caused 64.58 ± 3.60%

inhibition of amastigotes per infected macrophage. When given intraperitoneally (10 mg/kg for 5 days) or orally (100 mg/kg for 5 days) to golden hamsters infected with *L. donovani*, arbortristocide A (**11b**) exhibited 79.68 ± 21.68% and 57% inhibitions, respectively [40e].

A number of antibiotics, known for their activity against bacterial and fungal infections, have also been found to be effective in treating malaria, leishmaniasis and other protozoal diseases in humans and domestic animals [41,42]. Antibiotics, which

12

13 R = H
14 R = B-Me

15

16
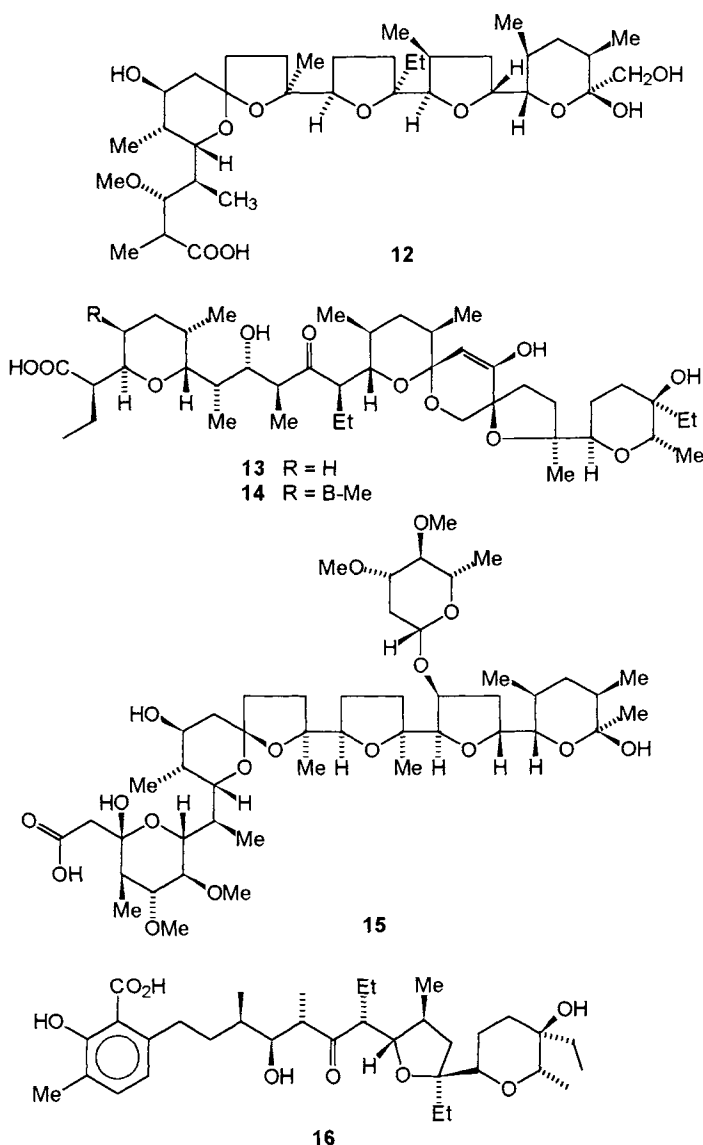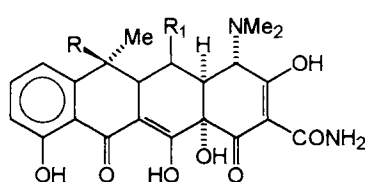
17

a R = OH, R$_1$ = H
b R = H, R$_1$ = OH
c R = R$_1$ = OH
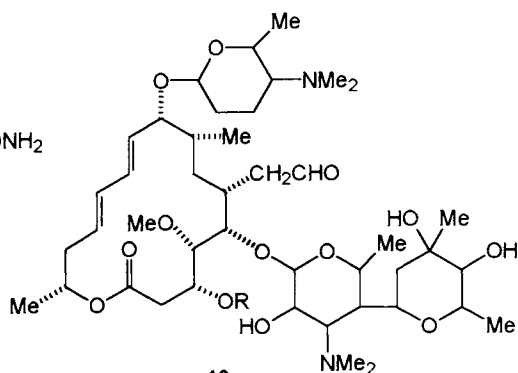
18
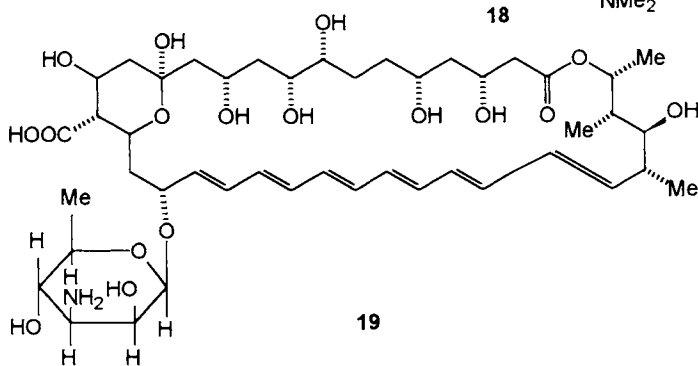
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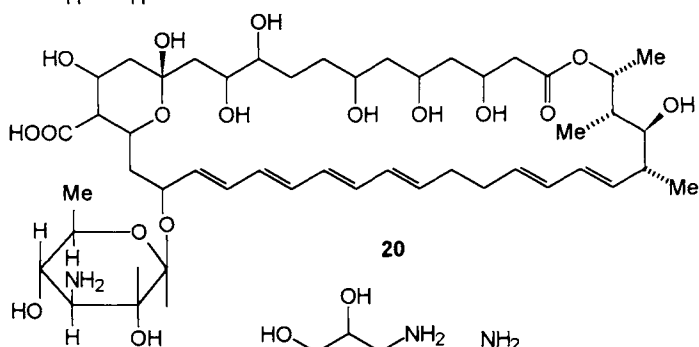
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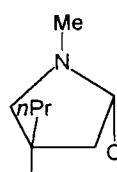
21
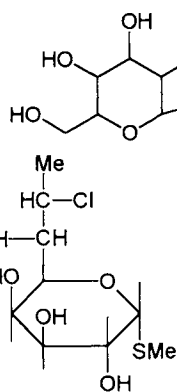
22

have been used against various protozoal infections, include some polyether iono-phores like monensin (12), salinomycin (13), narasin (14), maduramicin (15), lasalo-cid-A (16), which find use in poultry coccidiasis; tetracyclines like tetracycline (17a), doxycline (17b) and oxytetracycline (17c) and glycoside antibodies such as spiramy-cine (18), amphotericin-B (19), nystatin (20), clindamycin (21), and paromomycin (22), which have been used to treat malaria and leishmaniasis [40-45]. Although these antibiotics cause slower clearance of parasitaemia as compared to quinoline drugs and antifolates, they may be especially useful in treating multi-drug resistant *P. falciparum* infections [43].

2.   SAR IN LEAD MOLECULES

Among the various natural products, which have been found to possess an-tiprotozoal activity in humans, quinine (2) and qinghaosu (7) have undergone exten-sive molecular modifications to obtain better antimalarial drugs. The salient results of these studies are described below.

2.1   Quinine

In a broad sense, the quinine molecule may be regarded as an aminoalcohol of type I representing three major sites (A-C) for molecular modifications.

I
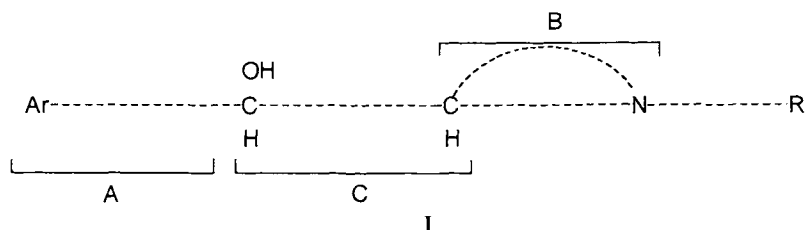
2.1.1 Changes at site A

In a systematic and extensive drug development programme sponsored by US Army Medical Research and Development Command, the quinine molecule was modified in numerous ways leading to the discovery of a number of potent anti-malarials. In the simplest approach, the 6-methoxy-4-quinolinyl residue was re-placed by various other substituted 4-quinolinyls and also by other aromatic resi-dues like 1-naphthyl, 9-phenanthrenyl, 9-anthryl, 12-chrysenyl and 4-pyridyls. However, significant activity was observed only in the following three types of amino alcohols [44].

2.1.1.1 4-Quinolinyl alcohols

The presence of a methoxy group at the 6-position of quinine does not seem to be essential as the naturally occuring desmethoxy derivative, cinchonidine (4) also possesses antimalarial activity [44]. Introduction of various groups in different positions of the quinoline ring in 4 showed marked changes in antimalarial activity. The compounds obtained by attaching halogens at the 6 and 8-positions were found to be usually active even when the quinuclidine was replaced by other amines. A number of 2-phenyl derivatives of quinine were also synthesized utilizing a metabolite directed approach. Metabolism of quinine gives 2-hydroxyquinine having less activity than the parent drug [46,47]. Attempts to prevent oxidation at the 2-position by introducing phenyl groups led to the synthesis of 2-phenyl-4-quinolinylamino alcohols with better antimalarial activites. However, most of the compounds thus generated, showed phototoxicity in laboratory animals [44,48].

Expanded studies in this direction revealed that chlorination of the quinoline and phenyl rings in 2-aryl-4-quinolinyl-2'-piperidinyl methanols may diminish phototoxicity [49]. Further molecular modifications in 2-substituted quinine derivatives showed that replacement of the 2-phenyl group by trifluoromethyl groups could further reduce phototoxicity with retention of antimalarial activity [50]. This observation was systematically exploited at the Walter Reed Army Research Institute culminating in the discovery of mefloquine (23a) [51]. The other effective antimalarials,
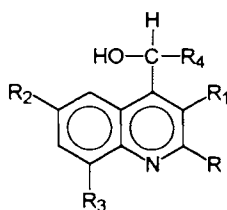
23

a $R = R_3 = CF_3$, $R_1 = R_2 = H$, $R_4 = $ 2- piperidinyl
b $R = Ph$, $R_1 = H$, $R_2 = R_3 = Cl$, $R_4 = $ 2-piperidinyl
c $R = C_6H_3Cl_2$-3,5, $R_1 = H$, $R_2 = R_3 = Cl$, $R_4 = CH_2NBu_2$
d $R = R_3 = CF_3$, $R_1 = R_2 = H$, $R_4 = CH_2CH_2NHt$-Bu
e $R = CF_3$, $R_1 = H$, $R_2 = R_3 = Cl$, $R_4 = $ 2-piperidinyl
f $R = COC_6H_3 (CF_3)_2$-3,5, $R_1 = H$, $R_2 = R_3 = Cl$, $R_4 = CH_2NBu_2$
g $R = OC_6H_4Cl$-4, $R_1 = H$, $R_2 = R_3 = Cl$, $R_4 = $ 2-piperidinyl
h $R = CH = CH$-C_6H_4Cl-4, $R_1 = H$, $R_2 = R_3 = Cl$, $R_4 = CH_2NBu_2$
i $R = C_6H_4Cl$-4, $R_1 = F$, $R_2 = R_3 = Cl$, $R_4 = CH_2NBu_2$
j $R = $ Admantyl, $R_1 = H$, $R_2 = R_3 = Cl$, $R_4 = $ 2-piperidinyl
k $R = R_1 = H$, $R_2 = Br$, $R_3 = Ph$, $R_4 = CH_2NBu_2$
l $R = CH_2NBu_2$, $R_1 = H$, $R_2 = R_3 = Cl$, $R_4 = C_6H_4Cl$-4

which emerged as a result of molecular variations in the quinoline ring and aminoalcohol group in quinine are SN-10275 (23b), WR-30090 (23c), WR-184806 (23d), WR-226253 (23e) and compounds 23f-l [45].

Other interesting compounds of this class are 24 and 25 resembling structurally WR-30090 in many ways, except that these compounds have additional rings fused to the quinoline nucleus [45].

24

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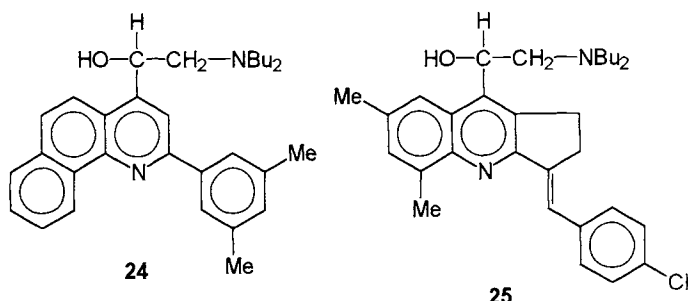
2.1.1.2 9-Phenanthrenylalcohols

Replacement of the quinoline ring in quinine analogues by the phenanthrene moiety gives the corresponding 9-phenanthrenylalcohols, which exhibit high antimalarial activity with little or no phototoxicity [44,52]. Consequently this class of compounds has undergone extensive molecular modifications very similar to that of the 4-quinolinylalcohols. The promising compounds derived from 9-phenanthrenylmethanols were found to be SN-8867 (26a), SN-9160 (26b), WR-33063 (26c), WR-122455 (26d), and WR-171669, (halofantrine 26e), of which 26c-e have been evaluated against *P. falciparum* in man [45,52].

26
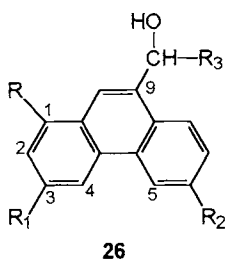
a R = R$_1$ = R$_2$ = H, R$_3$ = CH$_2$N[(CH$_2$)$_8$CH$_3$]$_2$
b R = R$_1$ = H, R$_2$ = Cl, R$_3$ = CH$_2$N[(CH$_2$)$_6$CH$_3$]$_2$
c R = R$_1$ = H, R$_2$ = Br, R$_3$ = CH$_2$N[(CH$_2$)$_6$CH$_3$]$_2$
d R = H, R$_1$ = R$_2$ = CF$_3$, R$_3$ = 2-piperidinyl
e R = R$_1$ = Cl, R$_2$ = CF$_3$, R$_3$ = CH$_2$CH$_2$NBu$_2$

2.1.1.3 4-Pyridylalcohols

This class of compounds may be considered to have been obtained by removing the benzene ring from the quinoline nucleus of quinine. A number of 4-pyridylalcohols have been shown to possess high activity against *P. berghei, P. falciparum* and *P. vivax* in experimental animals. However, promising antimalarial activity was obtained with 2,6-disubstituted-4-pyridylalcohols, of which WR-148946 (**27a**), WR-172435 (**27b**), WR-180409 (enpiroline, **27c**) and **27d** have been studied in detail [45].

27
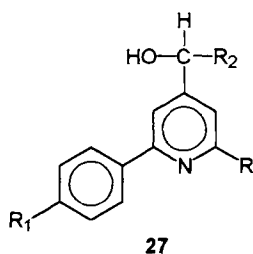
a   R = $C_6H_4CF_3$-4 , R_1 =CF_3, R_2 = CH_2NBu_2
b   R = $C_6H_4CF_3$-4, R_1 = CF_3, R_2 = $CH_2CH_2Bu_2$
c   R = CF_3, R_1 = CF_3, R_2 = 2-piperidinyl
d   R = C_6H_4Cl-4, R_1 = Cl, R_2 = CH_2NHi-Bu

2.1.2 Changes at site B

The quinuclidine part of quinine has three asymmetric carbon atoms at positions 3', 4' and 8', of which C-3' is attached to a vinyl residue. Thus the simplest approach to molecular modification may involve replacement of the quinuclidine by other bases. It is also possible that the vinyl group may be saturated, altered or removed to get compounds with different electronic and steric nature. Replacement of the quinuclidine moiety in quinine by a 2-piperidyl ring gave **28**, which lacks asymmetry at carbons 3' and 4' [53]. Both the racemates of **28** were found to possess slight activity [54], thereby indicating that asymmetry at positions 3' and 4'-may not be essential for antimalarial activity [53,55]. Consequently a series of 4-quinolinylalcohols

28

29 R_3 = H, Et
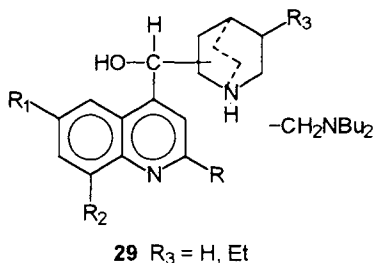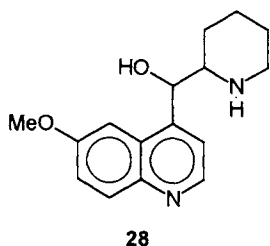
(29) derived from 2-piperidine, 3-piperidine, 3-ethylquinuclidine and dibutylami-nomethane were prepared showing marked activity against *P. berghei* in mouse and *P. lophurae* in ducks [44,51,56-59].

2.1.3 Changes at site C

The stereochemistry of the asymmetric carbons at positions 8' and 9' plays an important role in evoking antimalarial activity in quinine and its analogues. It seems that the juxtaposition of the hydroxy group and the nonaromatic nitrogen at positions 9' and 8', respectively, is associated with antimalarial activity. According to Cheng [60], for antimalarial activity the configuration of the hydroxy and nonaromatic nitrogen should be such that the distance between oxygen and nitrogen is around 3Å. It has also been postulated that the antimalarial activity will decrease if this distance is more than 3Å in the favoured configuration of a 4-quinolinylalcohol [60,61]. These authors also suggest that the activity of the amino alcohols, discussed above, is related to their ability to form a hydrogen bonded cyclic structure of type 30 [44,45].

30
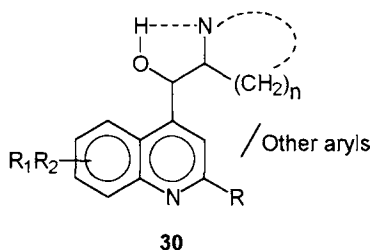
2.2   Artemisinine (Qinghaosu)

A summary of the chemical transformations carried out and resulting SAR studies of artemisinine analogues is presented in scheme 1. The work carried out on structure-activity relationship has clearly established that the peroxide moiety is indispensable for antimalarial activity, which is evident from the fact that conversion of qinghaosu (7) into deoxyqinghaosu (31) retaining an epoxide skeleton, is devoid of antimalarial activity [29]. Contrary to this, dihydroqinghaosu (32) with the peroxide linkage intact, exhibits strong antimalarial activity. Dehydraton of 32 gives an ethylenic compound (33) with no antimalarial activity. These observations pointed out that the presence of a peroxide linkage at C_3-C_{12} position and a hydroxy or keto function at C_{10} is essential for antimalarial activity. Consequently dihydroqinghaosu (32) was converted into a series of ethers (34), esters (35a) and carbonates (35b) by
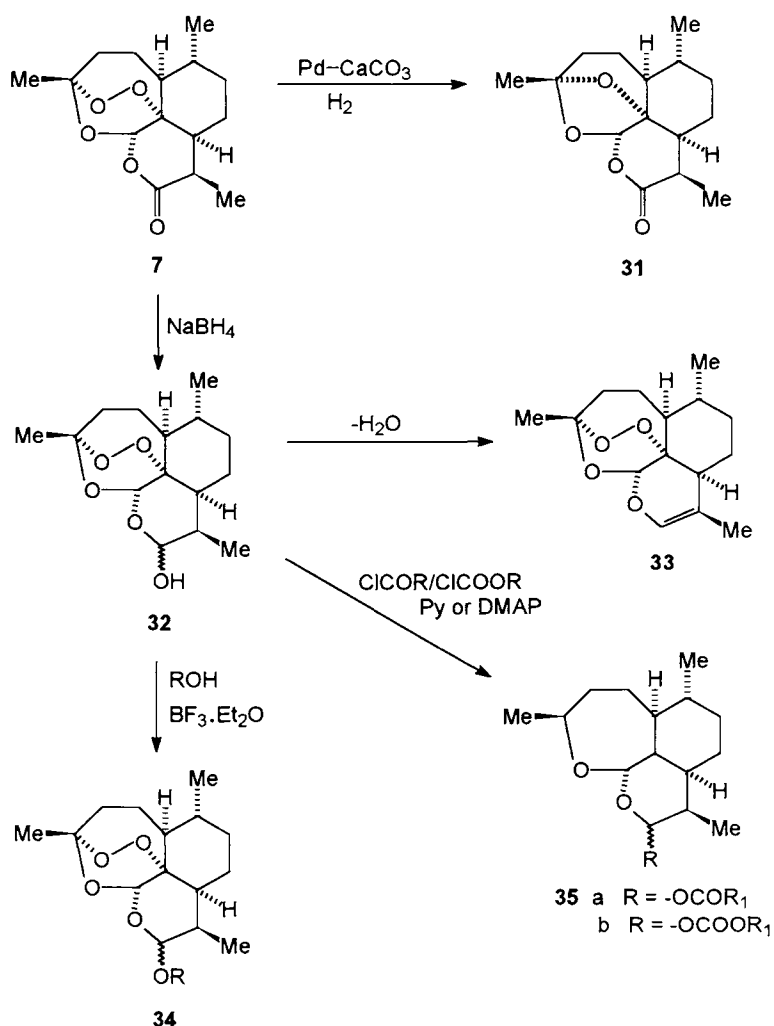
Scheme 1

treating **32** with ROH, ClCOR and ClCOOR, respectively [29]. All these compounds exhibited significant antimalarial activity and the order of their activity may be arranged in the following way: qinghaosu (**7**) < dihydroqinghaosu (**32**) < ethers (**34**) < carboxylic acid esters (**35a**) < carbonates (**35b**) [29]. Many of the analogues of dihydroqinghaosu (**32**) exhibited better activity than qinghaosu and are under extended biological and clinical evaluation. These are artemether (**36a**), arteether (**36b**), the water soluble derivative sodium artesunate (**36c**) and artenilic acid (**36d**) [62]. Recently, dextro-deoxoartemisinin (**37**) was prepared from artemisinin and found to exhibit superior activity [63]. These compounds have gametocytocidal activity also and, therefore, may find use in preventing transmission of malaria [64].

36

a R = Me
b R = Et
c R = COCH₂CH₂COONa

d R =CH₂—⟨benzene ring⟩—COOH

37
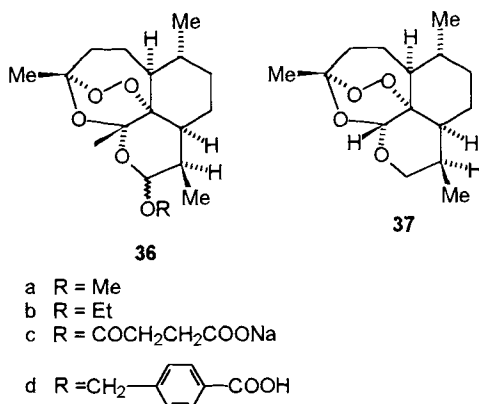
Qinghaosu is a sesquiterpene 1,2,4-trioxane simulating the peroxide linkage, which is an important structural requirement for antimalarial activity. Further, qinghaosu and its synthetic derivatives suffer from various limitations such as high rate of recrudescence, poor oral absorption, short half-life and embryo-toxicity [65]. These facts led to an intense search for better antimalarials derived from simple 1,2,4-trioxanes (38). This class of compounds have been extensively studied by Jefford *et al.* [66-69] and others [70-75]; some of them show marked *in vitro* and *in vivo* antimalarial activities [62,65].

38

39
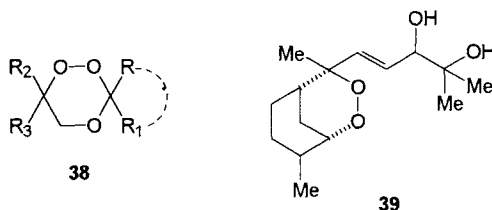
A further support to the antimalarial potential of peroxides may be derived from yingzhaosu A. (39), a sesquiterpene peroxide isolated from *Artabotrys uncinatus* [76]. The structure-activity relationship in the analogues of yingzhaosu has also been studied. The promising members of this class are Ro-40-6772, Ro-41-3823 and Ro-42-1611, which are under detailed biological evaluations [62].

3. SYNTHESIS

3.1 Quinine (2)

Apart from the classical papers describing the total synthesis of quinine by Woodward and Doering [77,78], several ingenious routes to the total synthesis of
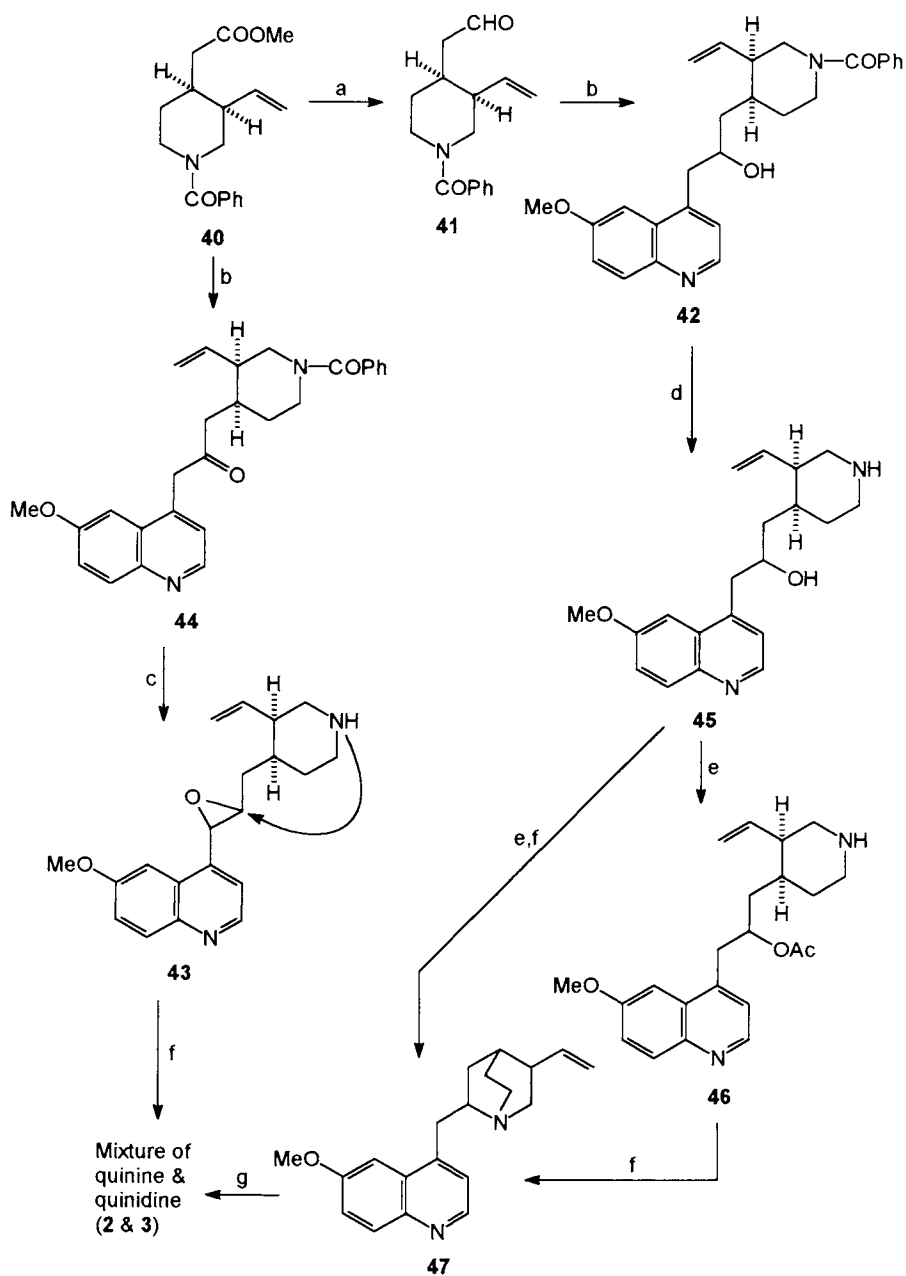
Scheme 2

Reagents : (a) Diisobutyl aluminum hydride; (b) 4-lithiomethyl-6-methoxyquinoline; (c) NBS, hv and DIBAL; (d) DIBAL, Tol.; (e) BF₃.Et₂O, AcOH; (f) heat; (g) O₂, DMSO, *t*-BuOH, *t*-BuOK.

quinine and related cinchona alkaloids have been developed [79-85]. Most of the synthetic strategies involve condensation of the N-acetyl/benzoylmeroquinine methyl ester (40) with 4-substituted quinolines [80,82,83] to give the corresponding 4-substituted-6-methoxyquinolines (42,44), which are subsequently converted into desoxyquinine and desoxyquinidine (47). Base catalysed hydroxylation of the epi-meric mixture of desoxyquinine and quinidine yields mixtures of quinine and quinidine (2 and 3) (Scheme 2).

3.2 Mefloquine (23a)

Ohnmacht *et al.* [86] described the first synthesis of mefloquine, which was later resolved into (+)-and (-)-mefloquine by Carroll and Blackwell [87]. The synthetic route to mefloquine starting from 2-trifluoromethylaniline (48) is outlined in scheme 3.
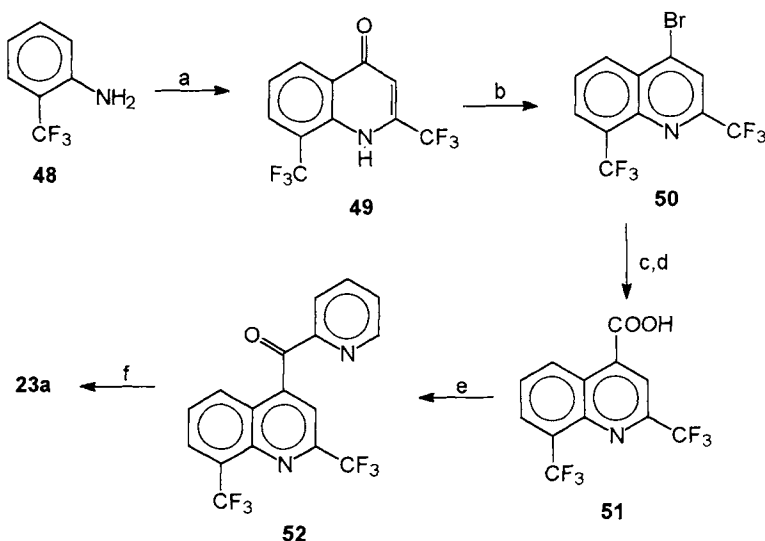
Scheme 3
Reagents: (a) Ethyl trifluoroacetylacetate; (b) POBr₃; (c) BuLi; (d) CO₂; (e) Pyridyl-Li, -60°C; (f) Pt, H₂.

3.3 Emetine (6) and dehydroemetine (71a)

The synthesis of emetine has been reported by several workers [19-26,90,91]. A few of the more effecient methods to synthesize (±)-emetine are described in schemes 4-6.
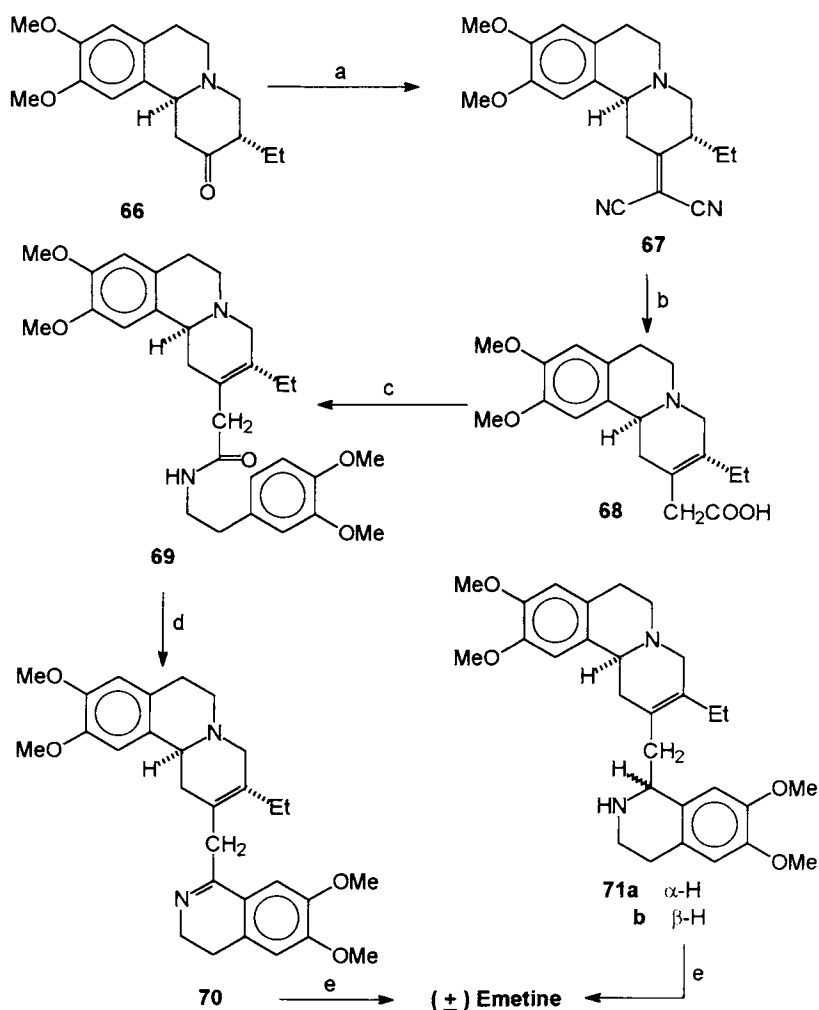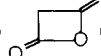
Scheme 4

Reagents: (a) Malononitrile, ammonium acetate, (b) 20% HCl, (c) 2-(3,4-dimethoxy-phenyl)ethylamine, DCC, (d) POCl$_3$, (e) PtO$_2$, H$_2$.

Brossi *et al.* [19,90] working at the Hoffmann-La Roche Laboratories in Basel, and Clark *et al.* [91] working at Glaxo Labs. in U.K. described the earliest practical synthesis of racemic 2-dihydroemetine and emetine. Brossi *et al.* obtained racemic 2-dehydroemetine (**71a**) and 2-dehydroisoemetine (**71b**) starting from 2-oxo-3-ethyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b-H-benzo[a]isoquinoline (**66**) (Scheme 4). The synthesis by the Glaxo group is based on recognition of the elements of symmetry in the emetine molecule; they built the emetine structure starting from 1,3-bis-

363
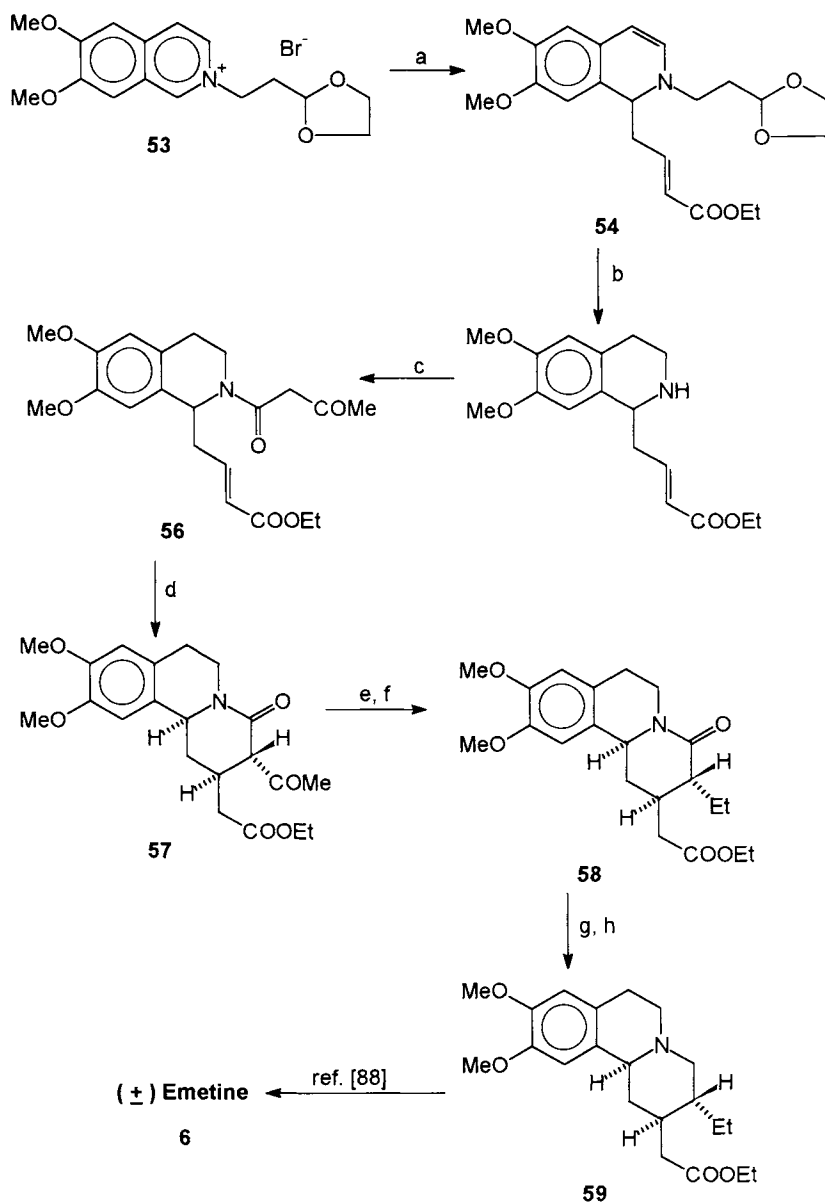
Scheme 5

Reagents: (a) Ethyl bromocrotonate, Zn, (b) 20% oxalic acid, (c) (d) NaOEt, (e) ethanedithiol, (f) Ra-Ni, H_2, (g) Et_2OBF_3, (h) $NaBH_4$.

MeO
MeO
N
CH₂R

60 a R = H
b R = Li

a →

MeO
MeO
N
O
(furan ring: O)

61

b ↓

MeO
MeO
N
O
H
H
O
9
10

62a (9,10-didehydro)

62b

(9,10-dihydro)

← c

d ↓

MeO
MeO
N
O
R

63a R = OH

→ **63b** (R = OMs) → **63c** (R = H)

e ↓

MeO
MeO
N
O
H
H
Et
H
N
OMe
OMe

65a 3α-H (43%)
b 3β-H (11%)

(±) **Emetine** ← ref. [89]
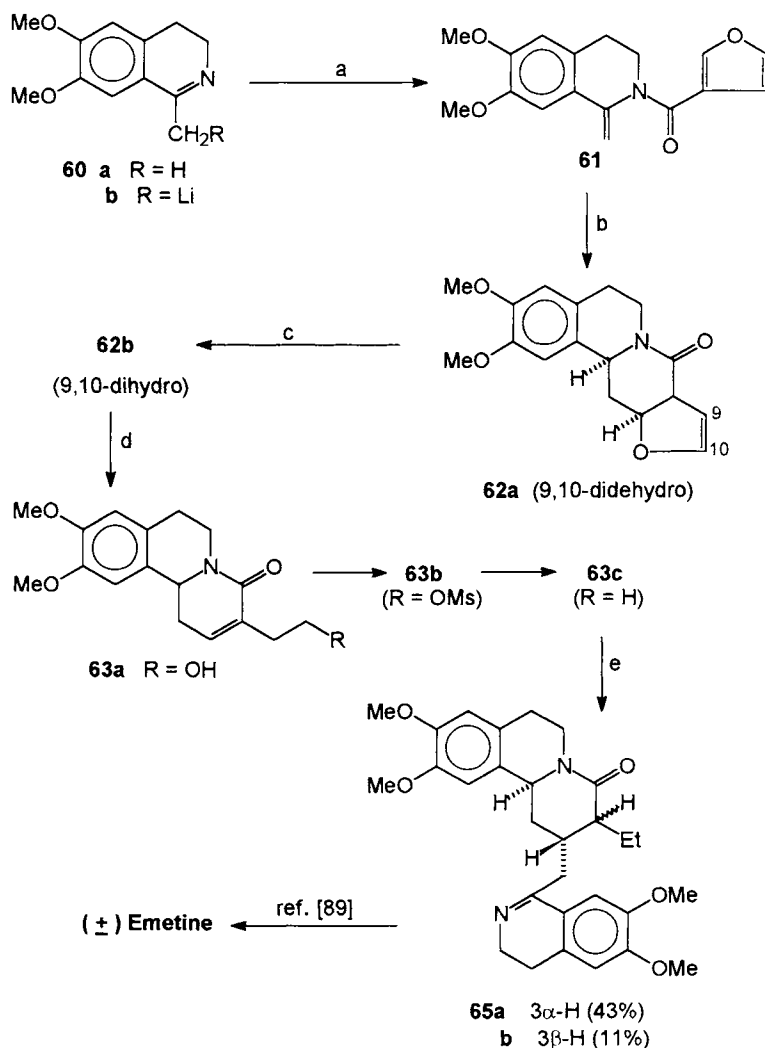
Scheme 6

Reagents: (a) Furan-3-carbonyl chloride, **60a**, (b) light, (c) H₂, (d) LDA, -78°C, (e) **60b**.

(6,7-dimethoxy-1,2,3,4-tetrahydro-1-isoquinolyl)acetone by condensing 3,4-dihydroi-soquinoline with acetone dicarboxylic ester. Dehydroemetine, in view of its lower toxicity than emetine is preferred for clinical use in hepatic amoebiasis.

Hirai *et al.* [25] utilize the iminium salt (**53**) as the starting material, which may be obtained in 98% yield by reaction of 6,7-dimethoxyisoquinoline and 2-(2-bro-moethyl)-1,3-dioxolane. Compound **53** was converted into **59** which was transformed into (±) emetine by the method of Battersby and Turner [88]. (Scheme 5).

The synthesis by Naito *et al.* [26] makes use of 1-methyl-3,4-dihydroisoquinoline (**60a**) or its lithium salt (**60b**) to prepare Michael acceptors (**63a-c**). Michael reaction of **63c** with **60b** gives a mixture of two products **65a,b**, of which **65a** has been isolated and converted into (±)-emetine by Kametani *et al.* [89] (Scheme 6).

3.4 Artemisinine (Qinghaosu, 7)

The key intermediate for the synthesis of artemisinine is the acid **80**, which has been used to build-up the peroxide linkage resulting in the formation of **7**. Schmid and Hofheinz [92] obtained **80** starting from (-)-isopulegol (**72**) (Scheme 7), while the Chinese scientists have synthesized it starting from R(+)-citronellal (**81**) (Scheme 8) [93].

The acid **80**, thus formed, is converted to artemisinine as described in scheme 9 [92,93].
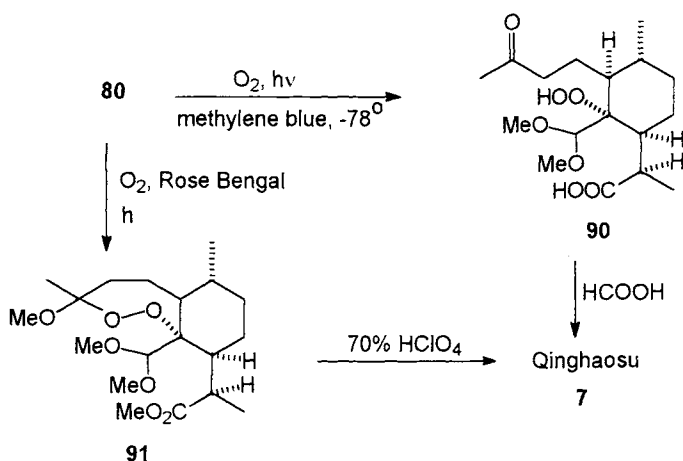
Scheme 9

Recently Avery *et al.* [94] have developed a stereoselective total synthesis of (+)-artemisinine starting from (R)-(+)-pulegone (**92**). Elaboration of **92** gives the known sulphoxide **93**, which was allowed to undergo dianion alkylation and desulphurisation to yield *trans*-2,3-disubstituted-cyclohexanone (**95**). Homologation of the latter afforded the aldehyde **96** in two steps. This product was then converted into the silyl acetate (**97**), which underwent Tandem Claisen ester-enolate rearrangement to give the vinylsilane **98**. Ozonolysis and cyclisation of **98** provided **7** (Scheme 10).

A short and practical synthesis of qinghaosu and deoxyqinghaosu (**102**) from arteannuic acid (**101**) has been developed by Ye and Wu [99] (Scheme 11).

366
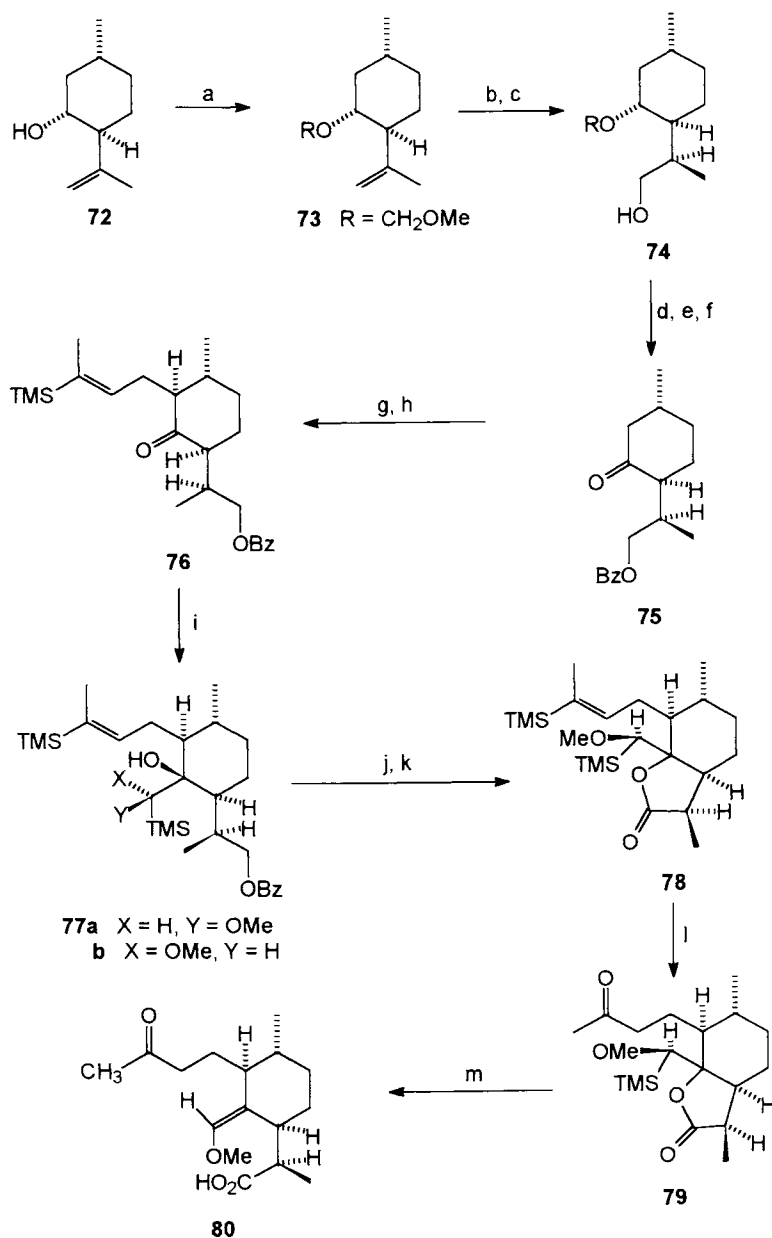
Scheme 7

Reagents: (a) MeOCH₂Cl, DMA, (b) B₂H₆, (c) H₂O₂, (d) PhCH₂Cl, KH, (e) HCl, (f) PCC, (g) LDA, (h) ICH₂CH=C(Me)TMS, (i) MeOCH(Li)TMS, -78°C, (j) Li, NH₃, (k) PCC, (l) MCPBA, TFA, (m) Bu₄NF.
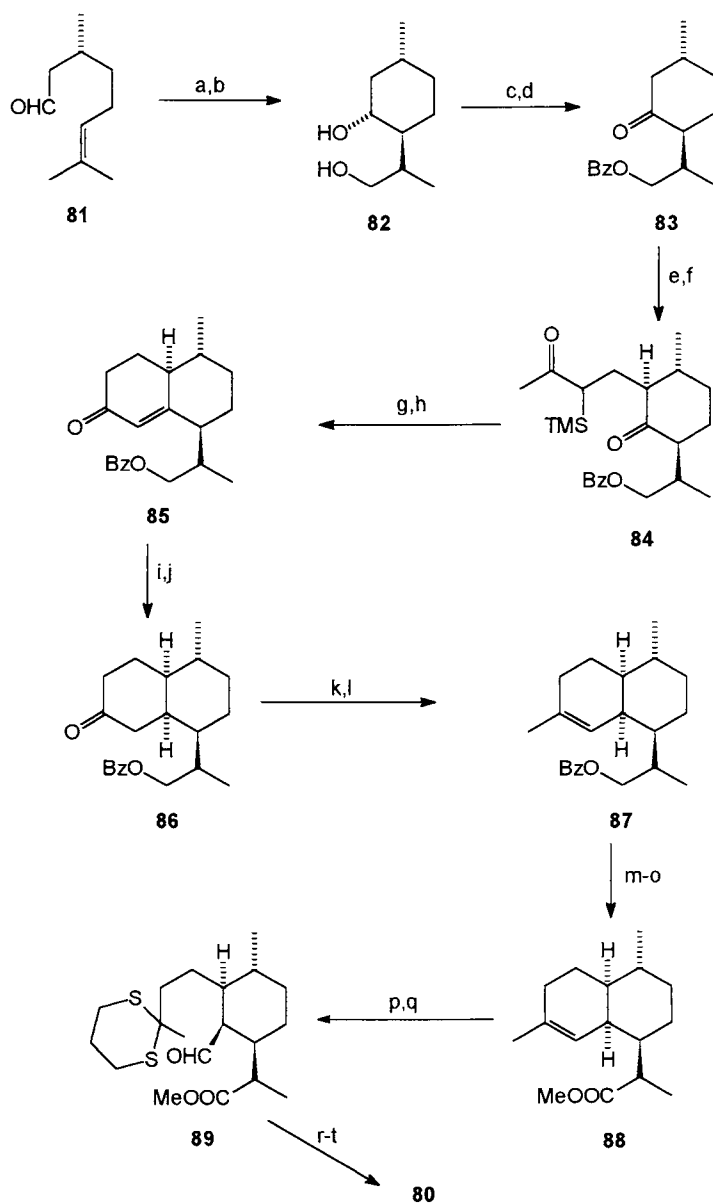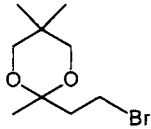
Scheme 8

Reagents: (a) ZnBr₂, (b) B₂H₆.H₂O₂, (c) PhCH₂Cl, (d) Jone's oxidation, (e) LDA, (f) H₂C=C(TMS)COMe, (g) Ba(OH)₂, (h) oxalic acid, (i) NaBH₄-Py, (j) Jone's oxidation, (k) MeMgI, (l)TsOH, (m) Na-NH₃, (n) Jone's oxidation, (o) CH₂N₂, (p) O₃, (q) ketalisation (r) TsOH, CH(OMe)₃, (s) xylene, heat, (t) HgCl₂.
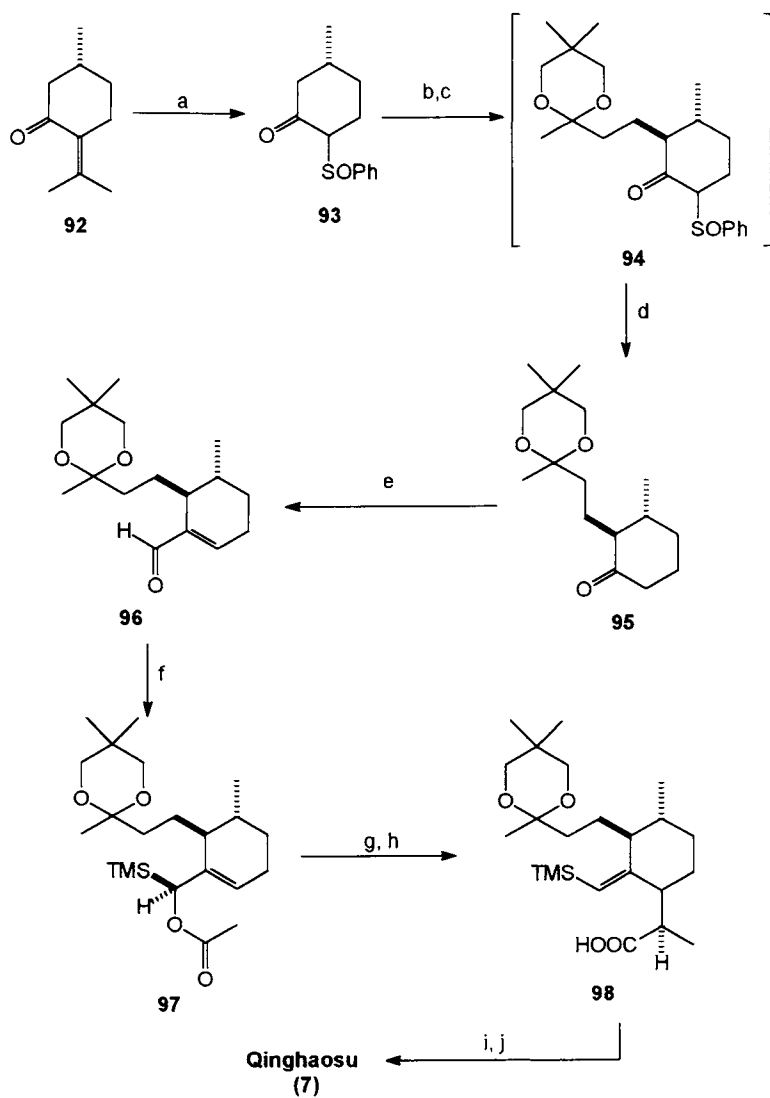
Scheme 10

Reagents: (a) 3 steps, (b) LDA, HMPT, (c) (d) Al-Hg amalgam, (e) homologation, (f) tris(trimethylsilyl)aluminum etherate, -78°; (g) LDEA, THF; (h) LDA, MeI; (i) O_3/O_2, -78°, (j) H_2SO_4
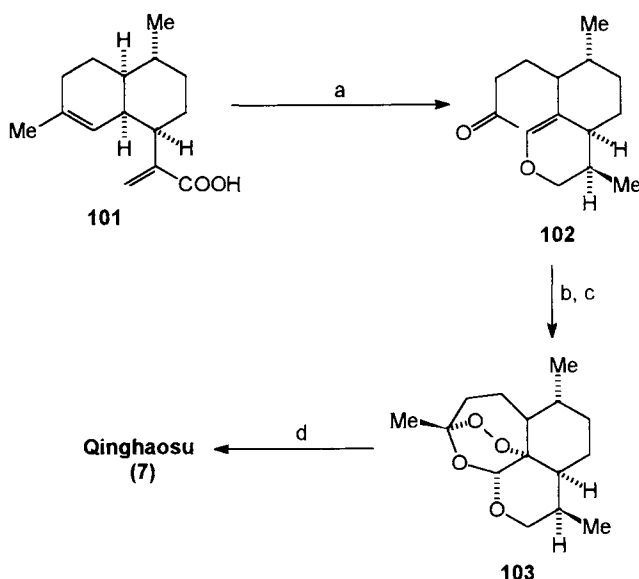
Scheme 11

Reagents: (a) 6 steps, (b) O$_2$, methylene blue, -70 to -78°C, (c) TfOTMS, (d) RuCl$_3$-NaIO$_4$ / MeCN, H$_2$O, CCl$_4$.

3.5 Synthesis of artemisinin analogues

Dihydroqinghaosu (**32**) (Scheme 1) is a key intermediate in the synthesis of artemisinin analogues / derivatives, which have shown antimalarial activity. Following the Chinese scientists's work, Lin *et al.* [95] synthesized a series of water soluble derivatives of artemisinin of which artenilic acid (**36d**) was found to exhibit activity and better stability than artemisinin or artesunate. Brossi *et al.* [96] reported the synthesis of arteether (**36b**), which after preclinical studies is now in clinical evaluation in India [96a]. Deoxyqinghaosu described in scheme 11 has been reported to have high antimalarial activity. Synthesis of (+)-12-butyldeoxoartemisinin (**99**) and some tricyclic analogues (**100**) of artemisinin has recently been achieved [97,98].

a R = H, R$_1$ = Me
b R = R$_1$ = Me

370
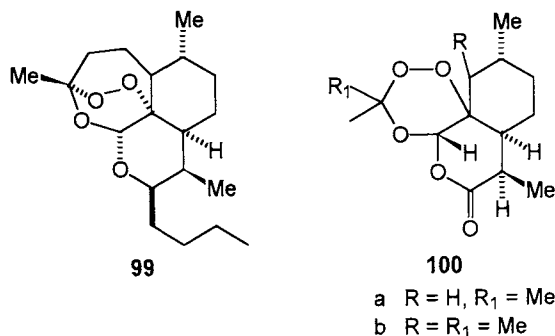
3.6   Antibiotics

A number of antibiotics have been reported to possess antiprotozoal activities in humans and domestic animals. These include monensin (**12**) [100,101], salinomycin (**13**) [102,103], narasin (**14**) [104], maduramicin, (**15**) [105] lasalocid-A (**16**) [106], tetracycline (**17a**) [107,108], doxycycline (**17b**) [109], oxytetracycline (**17c**) [110], spiramycin (**18**) [111], amphotericin-B (**19**) [112], nystatin (**20**) [113], clindamycin (**21**) [114] and paromomycin (**22**) [115].

4.   BIOLOGICAL ACTIVITY

4.1   Quinine

Quinine is an effective blood schizontocidal agent which even today remains as the preferred drug for treating chloroquine-resistant malaria in man. The drug shows high activity against the asexual stages of all the four species of *Plasmodium* infecting man. It may be given orally or intravenously depending upon the condition of the patient. For the emergency treatment of severe malaria, 600 mg of quinine in 300 ml of normal saline is injected intravenously over a period of 2-4 hours. The dose may be repeated with a maximum of 2g of the drug in 24 hours. Once the emergency is over, quinine or chloroquine may be given orally. Usually an oral dose of 200-300 mg of quinine hydrochloride or sulphate has been recommended for adults, which is given four times a day for 5-10 days. The drug may also be given at a dose of 10 mg/kg at 8 hourly intervals [62,116-118].

The side effects of quinine are headache, nausea, abdominal pain, tinnitus, visual disturbances, haemolytic anaemia, hypoglycemia, hypotension and arrhythmias. Intravenous injections, if given too rapidly, may cause sudden heart block, ventricular fibrillation and death [52]. These facts when taken in conjunction with the prolonged course of treatment needed and poor drug compliance call for medical supervision of the patient during therapy.

4.2   Quinidine

The drug, though used as an antiarrhythmic drug (cardiac depressant), also shows antimalarial activity including against *P. falciparum* in humans. Quinidine has been found to produce higher cure rate and less toxicity than quinine when given as oral tablets or slow-release preparations [119-121]. A combination of quinine, quinidine and cinchonine (1:1:1), given at a dose of 12 mg/kg has been reported to cure chloroquine-resistant falciparum malaria [122].

Although quinidine is an effective antimalarial, on account of its higher cost and possibility to cause cardiac problems, it is used only when quinine is not available [62,123].

4.3   Mefloquine

Mefloquine is a long-acting blood schizontocide with high efficacy against malarial parasites resistant to quinine, chloroquine and sulphonamide-pyrimethamine combinations [124-129]. A single oral dose of the drug of 15 mg/kg (with a maximum of 750-1000 mg/adult) produces cure rates above 90%. Detailed clinical trials carried out in Zimbabwe, Thailand, Burma, Brazil and Europe have established mefloquine as an effective drug both for prophylaxis and treatment of vivax and falciparum malaria [125-130].

Mefloquine is reasonably well toterated. However, some patients may experience nausea, vomiting, diarrhea, abdominal pain, loss of appetite and dizzines. The prophylactic or therapeutic doses of mefloquine can occasionally give rise to serious neurological and psychiatric side effects [62,131-133].

A synergistic drug combination consisting of mefloquine, sulfadoxine and pyrimethamine (Fansimef) has been used to treat falciparum malaria to delay or avoid development of mefloquine resistance and also to get better clinical response [45,134-136]. The usual adult dose of the combination is 500 mg mefloquine, 1000 mg sulfadoxine and 50 mg pyrimethamine. Recent clinical trials carried out have shown that fansimef may give rise to severe cutaneous reactions in a very small percentage (12 in 79000 therapeutic doses) of treated cases [137]. Further it has been observed that the above triple drug combination is as effective as mefloquine alone. In view of the fact that fansimef does not provide any therapeutic advantage over mefloquine alone and is associated with an additional risk (severe cutaneous reaction), it is no longer recommended either for treatment or prophylaxis of malaria [62].

4.4   Halofantrine (26e) and enpiroline (27c)

Halofantrine is a potent blood schizontocidal agent with high activity against chloroquine-resistant and chloroquine-sensitive *P. falciparum*. The drug has been used at a dose of 1g/adult given daily for 3 days; no phototoxicity or gastrointestinal problems were observed [138,139].

Halofantrine was registered for treatment of human malaria in 1988 in France

and some French speaking countries in Africa. The drug is available either as 250 mg halofantrine hydrochloride tablets or a syrup containing 100 mg of the hydrochloride per 5 ml. The recommended adult dose of the drug is two 250 mg tablets given three times in a day at 6 hour intervals. Children receive halfantrine at a dose of 8 mg/kg body weight given thrice a day 6 hours apart [62]. This dose schedule (500 mg at 6 hours interval for 3 doses) has been found to give 88-100% cures against *P. falciparum* [140,141]. However, Shank and coworkers [142] believe that a single day therapy with 1500 mg of halofantrine may be adequate for curing falciparum malaria. The drug should, therefore, be taken after meals so that it is better absorbed. The common side effects of halofantrine are nausea, vomiting, diarrhea, abdominal pain, cough, dizziness, headache and pruritus [62].

Enpiroline is an effective drug which is readily absorbed and distributed throughout the body. A single dose of 10 mg/kg or 750 mg of the drug has been found to be curative; however, further work regarding development of this compound as an antimalarial has been discontinued by the U.S. Army [62,143] for reasons not fully described.

4.5   Artemisinin

This is the most fast-acting blood schizontocidal drug available today possessing high activity against both *vivax* and *falciparum* malaria in humans. In 1987 it was approved for marketing in China. The most striking feature of qinghaosu is its very fast action and thus its ability to control and cure cerebral *falciparum* malaria [29-31,45,144,145].

The drug is given orally or as intramuscular injections in oil solution or oil/water suspension. The usual adult dose of qinghaosu is 2.5-3.2 g (total) given in divided doses for 3 consecutive days. When administered parenterally a total dose of 0.5-0.8 g (oil solution), 0.8-1.2 g (oil suspension) or 1.2 g (water suspension) is given in divided doses for 3 days. The above dose schedule provides clinical cure of patients suffering from vivax or falciparum malaria. For vivax malaria, the decline in fever was observed in 20-30 hrs and disappearance of parasitaemia occured in 30-40 hrs. There was 10-30% recrudescence occuring within a month. In the case of falciparum malaria, including those infected with chloroquine resistant strains, the time required for decline of fever and disappearance of parasitaemia was 30-40 and 30-50 hours, respectively. The drug produced no serious side effects and therefore is considered safe, even in patients with heart and liver complications and renal diseases caused by pregnancy [29].

The drug has also been found to be useful in treating cerebral malaria due to chloroquine-resistant falciparum malaria. After qinghaosu therapy, patients recovered from coma within 21.5-30.8 hours, the fever subsided in 34.1-56.7 hours, while the parasites were cleared from blood within 33.3-64.5 hours [29].

A suppository formulation of artemisinin developed in China, has been found to be highly effective in treating falciparum malaria. In a field trial conducted during 1982-1984, this formulation was given to 416 patients infected with *P. falciparum* afer which 355 cases were cured. The fever subsided in 15-39 hours and the parasitaemia was cleared off in 36-53 hours. A total of 2.8 g of the drug was administered over 3 days, supplemented with other drugs on the 4th day [62]. Arnold *et al.* [146] have also used artemisinin suppositories to treat acute falciparum malaria in adult patients. The patients received 600 mg of the drug at zero and after 4 hours followed by 400 mg after 24, 32, 48 and 56 hours. There was a rapid clearance of parasitaemia (50% clearance in 11.3 hours and complete clearance in 41.8 hours) in 32 patients.

4.6   Artemether

This drug is administered by intramuscular route as an oil solution because it is much more soluble in oil than in water. The total dose administered over a 3 day period is 0.24-0.64 g. A better dose schedule of artemether is a total of 600 mg given in 3 days [29]. Myint *et al.* [147,148] have used artemether to cure cerebral malaria due to *P. falciparum*. The drug was given at an intramuscular dose of 200 mg initially, followed by 100 mg at 12 hourly intervals for 2 days (total dose 600 mg) whereafter all the patients were cured. However, 39.1% of the treated patients recrudesced on day 28. Thus it has been suggested that artemether is an useful drug for treating complicated falciparum malaria because of its more rapid clinical and parasitological response than quinine.

4.7   Artesunate

Sodium artesunate is a water soluble derivative of qinghaosu. It is given by intramuscular or intravenous injections at a total dose of 400 mg spread over 3 days. The drug exhibits a fast onset of action. The patients recovered from coma with in 12 hours and were cured of malaria. Unfortunately the rate of recrudescence is very high [29]. Recently a 5-day course of oral artesunate given at a dose of 1200 and 600 and intramuscular artemether (480 mg) proved to be 90-100% effective against multidrug-resistant falciparum malaria in Thailand [149a]. It is possible to cure falciparum malaria in man using a sequential treatment with artesunate (600

mg over 5 days) followed by mefloquine 750 mg and 500 mg, six hours apart [149b].

5. MODE OF ACTION

5.1 Quinine

The exact mode of action of quinine is still unclear [150a]. The DNA-binding theory proposed by Hofheinz and Merkli in 1984 [150b] is slowly loosing favour. According to the drug-DNA binding concept, the quinoline ring of the drug is believed to intercalate between the base pairs of the double stranded DNA and forms a charge-transfer complex. The alcoholic group at the 4-position forms a hydrogen bond with one of the DNA bases. The quinuclidine portion of the drug projects into one of the grooves of DNA, while its tertiary aliphatic nitrogen undergoes protonation followed by formation of an ionic linkage with the negatively charged phosphate group of the deoxyribose phosphate backbone of the DNA double helix. This three point attachment of quinine with DNA of the plasmodia leads to blockade of the DNA function [150c].

The DNA-binding hypothesis has also been proposed for the action of acridines, 4-aminoquinolines (eg. Chloroquine), 8-aminoquinolines (Primaquine) and quinolinemethanols (quinine, mefloquine), which has recently been reviewed [150-152].

5.2 Artemisinin (qinghaosu) and its analogues

Qinghaosu does not inhibit carbohydrate metabolism of the plasmodia, though it markedly affects protein and nucleic acid synthesis [29]. Qinghaosu, dihydroqinghaosu and artemether inhibit the uptake of ^{3}H-isoleucine by human erythrocytes infected with *P. falciparum* at a concentration of 5-50 μmol/litre. The above compounds have also been shown to inhibit the uptake of [^{3}H]-hypoxanthine. Thus, protein synthesis was envisaged to be the primary site of attack by qinghaosu and its derivatives, which is possibly due to the oxidative damage to the protein synthesis machinary [153-155]. Later it was shown that qinghaosu and artesunate are involved in increased oxidant stress on the infected red blood cells [155]. Artesunate has further been demonstrated to inhibit cytochrome oxidase in *P. berghei* at high concentrations [156]. The activity of artemisinin may, therefore, be mediated through generation of activated oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals [155, 157-159].

Although the antimalarial activity of artemisinin has been attributed to its

ability to generate reactive oxygen species, the basis of selective toxicity of this drug to *Plasmodium* parasites is not clearly understood. Meshnick *et al.* [160] have demonstrated the role of intracellular hemin in the antimalarial action of artemisinin. These authors suggested that the antimalarial activity of artemisinin may be mediated by a reaction with intraparasitic hemin with subsequent formation of free radicals [157-160]. This was based on the fact that malaria parasites are rich in hemin (called hemozoin), which is derived from the digestion of host haemoglobin, possibly without breaking down hemin.

Zhang and coworkers [161] experimentally established the role of hemin in catalysing the reductive decomposition of artemisinin and dehydroartemisinin leading to cleavage of the oxygen-oxygen bond. The role of intraparasitic a hemin (hemozoin) in catalysing the decomposition of artemisinin through formation of a hemin-artemesinin adduct [He-Fe(III)-Artemisinin] and subsequent antimalarial action was supported by Peters and coworkers [162], who demonstrated that artemisinin was more than 50 times less effective against a chloroquin-resistant *P. berghei* strain lacking hemozoin. Further, it has been shown that no hemin-artemisinin adducts are formed when uninfected red cells are incubated with radiolabelled artemisinin [160]. This suggests that artemisinin does not react with haemoglobin-bound hemin. This observation, therefore, explains the selective toxicity of artemisinin for malaria parasite [161].

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CHAPTER 15

ORGANOMETALLICS

1.   INTRODUCTION

The historical development of organo-arsenicals, -antimonials and -phos-phates has been dealt in Chapter 4. This chapter will be confined to a discussion of their therapeutic value in the management of protozoal infections; organometallic drugs continue to be used, though to a limited extent in the treatment of leishmani-asis and trypanosomiasis.

2.   BIOLOGICAL ACTIVITY

Unlike helminth infections where a variety of drugs derived from arsenic, anti-mony, phosphorus, tin and lead have been used to eradicate intestinal and extrain-testinal parasites, only organo-arsenicals and -antimonials find use in the treatment of protozoal diseases [1-10].

2.1   Activity against leishmaniasis (see Chpater 4)

The introduction of tartar emetic (1) in 1912 for the treatment of mucocutane-ous leishmaniasis heralded a new era in the treatment of the disease, which was fol-lowed by the investigation of a number of other antimonials for clinical efficacy in leishmaniasis. This led to the discovery of a number of fivevalent antimony com-pounds, that have laboratory and clinical efficacy and are better tolerated than tar-tar emetic. These included sodium stibogluconate (2), meglumine antimoniate (3), derivatives of stibanilic acid (stibamine, 4), stibacetin (5), ethyl stibamine (a mixture of 4, 5, antimonic acid and Et_2NH in a molar ratio of 1:2:1:3 with 41-44% of fiveva-lent antimony content) and urea stibamine (6) have been used to treat kala-azar, cu-taneous and mucocutaneous leishmaniasis in man.

(a) Tartar emetic (1): This drug is now rarely used in the therapy of leishmaniasis, as drugs which are much better tolerated are available [10].

(b) Sodium stibogluconate (2): The fivevalent form of this drug, pentostam, is the drug of choice for treating different forms of leishmaniasis. Pentostam is available as a 33% solution (equivalent to 10% Sb^{V+}) [11] or 100 mg Sb^{V+}/ml [8], which should be stored in a refrigerator and administered intramuscularly or intravenously.

The recommended adult and pediatric dose of pentostam for treating visceral leishmaniasis is 20 mg/kg given daily (maximum 800 mg/ daily) for 20 days. The treatment may be repeated, if neccessary, after a gap of 10 days [12]. Usually this dose schedule provides high cure rates. However, a lower dose of 10 mg/kg given for 30 days has also been used, and found to exhibit almost 100% cures in children and adults suffering from visceral leishmaniasis [13a,b]. It is advised to give a full course of pentostam treatment to avoid risk of relapse of visceral leishmaniasis, which eventually leads to post *kala-azar* dermal leishmaniasis. This has been clearly demonstrated by Thakur [14], who used 20 mg/kg of pentostam to treat an epidemic in Bihar (India) and found that only 0.5% of the 603 treated patients exhibited relapse of the disease. Thakur [15b] has recently reviewed the current status of the treatment of *kala-azar* in India. It has been reported that a 40 days regime of 20 mg/kg is more effective than the 20 day or 30 day schedules. However, in such cases where the treatment is extended beyond 30 days, the incidence of cardiac toxicity is increased due to a cumulative dose pressure of the drug. Zijlstra *et al.* [15a] have evaluated sodium stibogluconate in 280 children and 413 adults in Sudan. A dose of 10 mg/kg for 30 days was found to be highly effective (almost 100% cures), but there were 12% deaths and 4% relapses. A dose of 20 mg/kg for 15 days of the drug was equally effective. At this dose level, children showed 3% deaths and 7% relapses, while in adults there was 8% deaths and 5% relapses.
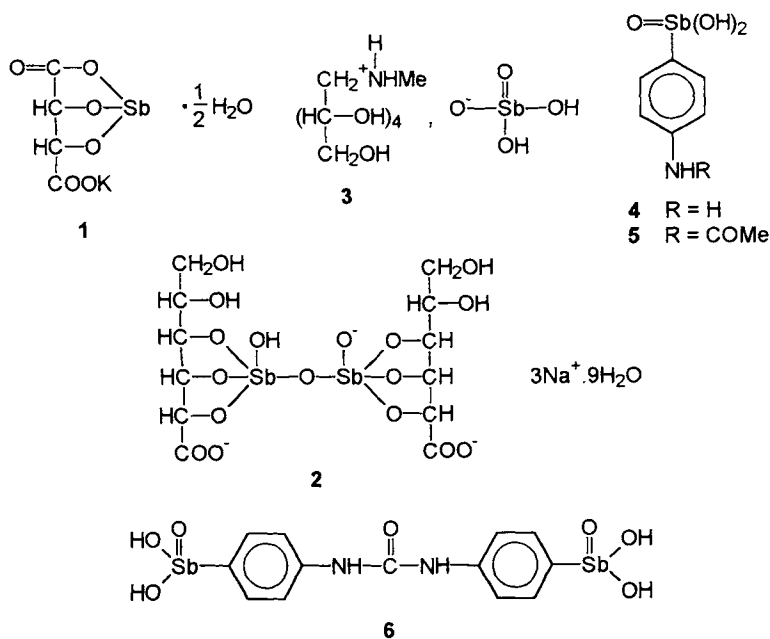
For treating mucocutaneous leishmaniasis, a dose of 20 mg/kg of pentostam is administered intramuscularly or intravenously for 20 days (maximum 800 mg/daily). The treatment may be repeated if required [12].

Pentostam is also used to treat cutaneous leishmaniasis at a dose of 10 mg/kg daily (maximum 600 mg/day) injected intravenously or intramuscularly for 6-10 days [11,12]. A dose of 8 mg/kg or 600 mg of Sb^{V+}/ day/person given for 10 days has been found to cure patients suffering from L. *chagasi, L. mexicana spp., L. mexicana amazonensis* and other species of the L. *mexicana* complex [16]. For treating simple cutaneous lesions caused by L. *aetropica* a dose of 18-20 mg/kg of pentostam given twice daily for 30 days was found to be successful [17a]. Recently, Sanez *et al.* [17b] treated some Panamanians suffering from mucosal leishmaniasis due to L.*brasiliensis panamensis*. The patients received pentostam at a dose of 20 mg Sb^{V+}/kg intravenously daily for 28 days after which 77% patients were cured.

(c) **Meglumine antimoniate (glucantime) (3):** This is another preferred antimonial drug commonly used for treating cutaneous and mucocutaneous leishmaniasis. The drug has also been used to treat visceral leishmaniasis and post-kala-azar dermal leishmaniasis [3,10]. Glucantime is available as a solution containing 85 mg/ml of Sb; it is injected either by intramuscular or intravenous routes. There is some controversy about the curative dosage schedule of this drug. In Brazil an adult dose of 17-28 mg/kg administered intramuscularly daily for 10-20 days is recommended for patients suffering from cutaneous and mucocutaneous leishmaniasis caused by L. *braziliensis braziliensis* [8,13b,18,19]. A second course of therapy may be repeated after 15 days interval [8]. Sampaio [20] evaluated three dose regimens of glucantime: (a) 28 mg/kg for 10 days, repeated thrice 15 days apart, (b) 10 mg/kg daily for 30 days, and (c) 20 mg/kg daily for 30 days and observed the last dose schedule to be the most effective in curing mucocutaneous leishmaniasis in humans. Marsden *et al.* [21] found that the drug may be given at a dose of 20 mg/kg daily for 85 days to achieve successful treatment of leishmaniasis.

(d) **Stibamine (4) and its derivatives:** Stibamine (4), stibacetin (5) and ethyl stibamine have been used in the management of kala-azar in humans; however, all of them were found to be highly toxic. Thus, despite their high activity in curing kala-azar, they could not be accepted in clinical practice [10].

(e) **Urea stibamine (6):** Based on the testing of a variety of antimonials, Brahmachari introduced urea stibamine as a clinically useful drug in 1920, which was successfully used in an epidemic of leishmaniasis sweeping the eastern part of

India in the early 1920s [22-28]. The effective dose of the drug is 170 mg/adult given intravenously daily for 6 days [27]. A better regimen of urea stibamine is: 50 mg/adult on day 1; 100 mg/adult on day 2; 150 mg/adult on day 3 and 200 mg/adult to be given on day 4 and subsequent days [28]. As urea stibamine is a complex with a non-defined structure derived from urea and p-aminophenyl stibome acid, it is very little used in clinical practice today.

2.2   Limitations and side effects of antimonials

Although antimonial compounds have been used for the treatment of human leishmaniasis since 1911, these have several shortcomings and are far from being ideal drugs [10,29,30]. The antimonials are not active orally and long courses of intravenous or intramuscular treatments are required to cure the disease. Moreover, most of the antimonials have non-defined structures; their activity and toxicity may vary from batch to batch. The therapeutic regimens also vary for infections received in different geographical regions [4]. The lack of consistency in activity of antimonials may be due to the variations in percentage of fivevalent antimony (Sb^{V+}) in different formulations/preparations.

The half-life of Sb^{V+} in blood after administering pentostam or glucantime is only 2 hours and most of it (81-97%) is excreted within 6-8 hours [8,31,32]. Despite the fast excretion of antimonials, there is slight accumulation of Sb^{V+} in tissues. The usual side effects of antimonials are nausea, vomiting, headache, anorexia, muscle pain, joint stiffness and bradycardia. The patients may also experience weakness, fever, epigastric discomfort, heartburn, itching, dizziness, insomnia, nervousness and myocardial damage. Rarely the drugs may also cause liver and renal damage, haemolytic anaemia, shock and abnormal ECG (inverted T wave on ECG). Sudden death of treated patients may take place on rare occassions as a result of severe dysrhythmias such as ventricular fibrillations [8,12,20,33].

The biochemical parameters of the patients are also affected by antimonial therapy. Marsden et al. [21] observed evidences of arthritis and raised transaminases with tender hepatomegaly when a long course (20 mg/kg of Sb^{V+} for 85 days) was given to a nonresponding patient with mucosal leishmaniasis. The renal toxicity of Sb^{V+} also influences the antidiuretic hormone (ADH) function and cell respiration [8]. A transient increase in the levels of alanine aminotransferase, lactate dehydrogenase, aspartate aminotransferase, triglycerides, creatine phosphokinase and alkaline phosphatase has also been observed in patients receiving pentostam at a dose of 10 mg/kg for 10 days [8].

The antimonials are contraindicated in patients with pneumonia, myocarditis, hepatitis, nephritis and pregnant women and breast-feeding mothers [34].

2.3 Activity against African trypanosomiasis

A number of organoarsenicals have been found to be highly effective in treating late stage of the disease with the involvement of the central nervous system. The drugs, which have been used successfully against African trypanosomiasis (African sleeping sickness) caused by *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*, are tryparsamide (7), melarsoprol (Mel B, 8a), melarsonyl potassium (Mel W, 8b) and melarsen sodium (9) [2,4-6, 8-10, 35,36].

(a) **Tryparsamide (7)**: This is the second line drug for treating African trypanosomiasis in man. For treating the late disease due to *T.b. gambiense* tryparsamide is given intravenously at a dose of 2-3 g/adult/week in 10-12 doses. However, for treating advanced cases of *T.b. rhodesiensis* infections involving the CNS, tryparsamide is given in combination with suramin [9]. Another dose schedule involves one injection (i.v.) of 30 mg/kg of tryparsamide given every 5 days for 12 injections. If required, the treatment may be repeated after a month [12].

The frequent side effects of the drug are nausea and vomiting. However, the drug may also be responsible for occasional optic atrophy, blindness, loss of appetite, diarrhea, renal damage, exfoliative dermatitis, allergic reactions and even death [9,12].

(b) **Melarsoprol (Mel B, 8a)**: This is the drug of choice for treating the meningoencephalitis stage of Gambian and Rhodesian trypanosomiasis. The recommended adult dose of Mel B is 2-3.6 mg/kg given intravenously daily for 3 days. After a week the drug is given at a dose of 3.6 mg/kg (i.v.) daily for 3 days. This
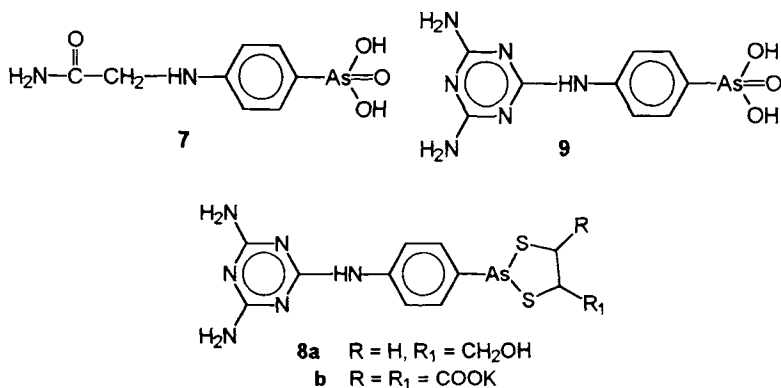
dose schedule is repeated again after a gap of 10-21 days [12]. For children, an initial dose of 0.36 mg/kg is administered intravenously and then the dose is gradually increased to a maximum of 3.6 mg/kg at an interval of 1-5 days for a total of 9-10 injections. The total dose of the drug in children in a month should not exceed 18-25 mg/kg [12].

Mel B is less toxic than tryparsamide. However, its use may be associated with renal and myocardial damage, albuminuria, hypertension and colic. Other side effects of the drug are jaundice, diarrhea and conjunctival infections [9,12]. About 1% of the treated patients may develop encephalopathy [37]. The drug is also known to cause headache, tremor, fever, convulsion, coma and death. It is contraindicated during epidemics of influenza and G-6-PD deficiency [36,38].

(c) **Melarsonyl potassium (Mel W, 8b)**: It is more toxic and less active than Mel B. The only advantage with this drug is that unlike Mel B, it may be administered intramuscularly or subcutaneously. Thus it may be useful in treating children where intravenous administration is not possible [39,40]. The drug may be given at a dose of 3-4 mg/kg (maximum 200 mg) daily for 3-4 days. The therapy can be repeated after 2 weeks, if required [41].

Melarsen sodium (9): This is administered intravenously at a dose of 20 mg/kg given for 8-12 doses separated 5-7 days apart. The toxicity profile of the drug is very much similar to that of tryparsamide [41].

3.   MODE OF ACTION

The fivevalent antimonials *per se* do not exert toxic effects on leishmania and trypanosomes. After entering the human body, the fivevalent antimonials are reduced to the threevalent form [42]. This threevalent form reacts with the sulphhydryl groups of the enzymes/proteins essential for parasites and inactivate them [34,43].

The organoantimonials and arsenicals are also known to interfere with the glycolytic pathways of the protozoans leading to decreased energy production. For example, in *Leishmania*, antimonials seem to inhibit the initial steps of the glycolysis and also interfere with certain enzymes of the Krebs cycles. The resultant effect is a decrease in the energy production by the parasites [44]. On the other hand, arsenicals such as melarsen oxide inhibits pyruvate kinase and prevents the synthesis of ATP in trypanosomes [45]. Arsenicals have also been found to inhibit another glycolytic enzyme, *Sn*-glycerol phosphate oxidase, found only in trypanosomes, which

is essential for generating NAD from NADH [46]. The death of the trypanosomes may be the consequence of the inhibition of pyruvate kinase and Sn-glycerol-3-phosphate oxidase [47].

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CHAPTER 16

QUINOLINES

1. INTRODUCTION

The discovery of quinine may be regarded as a landmark in the chemotherapy of malaria. This drug, isolated from the cinchona tree, not only remained as the mainstay of clinical management of human malaria till 1932, but also provided a lead to design better quinoline antimalarials. When World War I (1914-1918) broke, the supply of quinine in Germany fell down. This compelled Germans to search for a synthetic substitute of this natural product. The pioneering work of Schulemann and his colleagues in Germany led to the discovery of pamaquine (Plasmochin) as the first clinically acceptable (but toxic) synthetic antimalarial in 1925. Till the beginning of World War II (1939-1945), pamaquine was the only antirelapse drug known; however, its use in clinical practice could not last long because of its toxicity, thus, once more the need to develop a better antimalarial was felt world wide. Consequently a systematic SAR study was carried out concerning the quinoline nucleus in France, Britain, United States and the Soviet Union during 1941-45 resulting in the discovery of several quinoline antimalarials, many of which still find use in the treatment of human malaria [1-3]. Today, quinoline is recognised as one of the most extensively studied group of heterocycles because of its great potentiality to yield compounds with high activity against *Plasmodium, Entamoeba, Leishmania* and *Babesia* spp. A majority of drugs have been developed as a consequence of structural modifications primarily at 4- and 8-positions of quinoline nucleus, which are discussed below.

2. 4-AMINOQUINOLINES

The development of 4-aminoquinoline antimalarials owes its origin to World War II when the Allies were deprived of the supply of quinine (1) as a consequence of the Japanese occupation of Java, where cinchona was grown widely to produce quinine. To overcome this problem, a synthetic alternative, quinacrine (2) was developed, which was extensively used during the war to treat malaria in man. Nevertheless, the need for a more effective and safer antimalarial was pressing. Examination of the structural frame-work of quinine (1) and quinacrine (2) revealed that the common feature in the two drugs is the presence of a 4-substituted quino-

line skeleton which was probably responsible for evoking the antimalarial response [1,2].

The above rationale was amply exploited by scientists working in Germany and the Soviet Union resulting in the development of two drugs, chloroquine (Resochin, 3) and Sontoquine (Sontochin, 4) [4,5]. Of these, sontoquine was used by French troops in North Africa [6]. However, the supplies of this drug with German research data were captured by United States soldiers. This evoked further studies in in the USA where a large number of 4-aminoquinolines were synthesized and screened emerging chloroquine (3) as the most effective and least toxic drug [2,7-10].
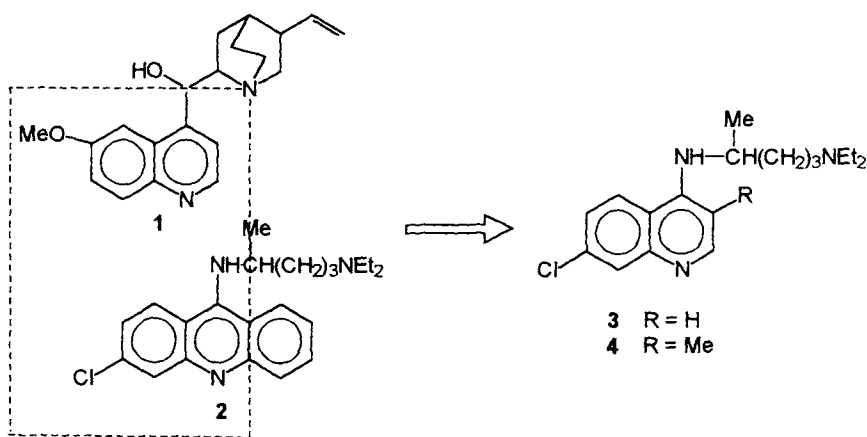
In a further effort to develop better antimalarials by changing the substitution at the 4-amino function of chloroquine led to the discovery of hydroxychloroquine (5) with high antimalarial activity [11,12]. The search for newer 4-aminoquinoline drugs received a new dimension when Burckhalter et al. [13] discovered the antimalarial activity with some α-dialkylamino-o-cresols of the type 6 and 7. Consequently these authors prepared a large variety of Mannich bases attached to the 7-chloroquinolin-4-
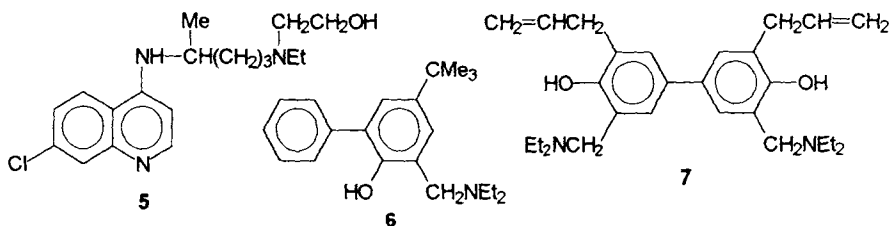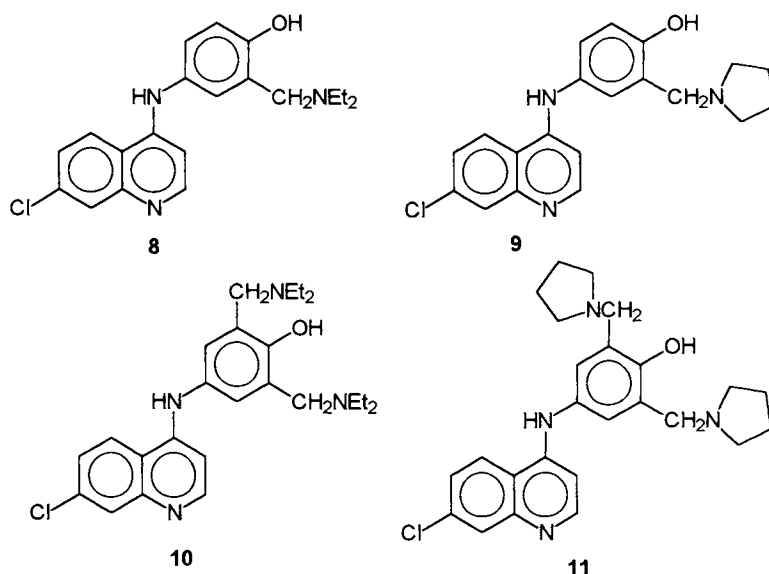
yl function through a nitrogen bridge [13]. The most effective drugs thus discovered are amodiaquine (8), amopyroquine (9), cycloquine (10) and bispyroquine (11), all exhibiting high activity against the asexual blood stages of all the species of *Plasmodium*, except chloroquine-resistant *P. falciparum* in human [14-16].

The SAR studies in 7-chloro-4-substituted aminoquinolines have been carried out by Bass *et al.* [17]. These authors used regression analysis of a large variety of 4-aminoquinolines and observed that their findings are in close resemblance with the model proposed by Hahn and Co-workers [18]. In a further probe to study the role of the 4-substituted amino function in evoking the antimalarial activity in quinolines, a number of 7-chloro-4-substituted quinolines (12) were prepared, but none exhibited noteworthy antimalarial activity [19-23]. Some bisquinolines of type 13-15 have also been synthesized, of which 14 and 15 have been shown to exhibit potent antimalarial activity [23-25].

14 (12278-RP)

15
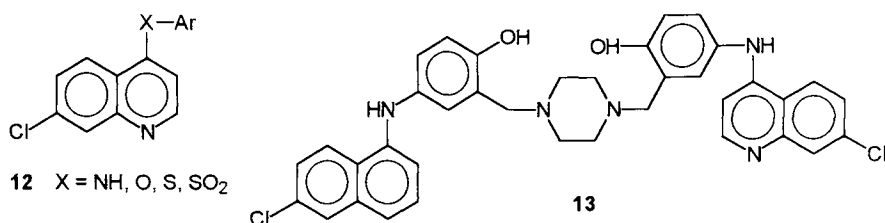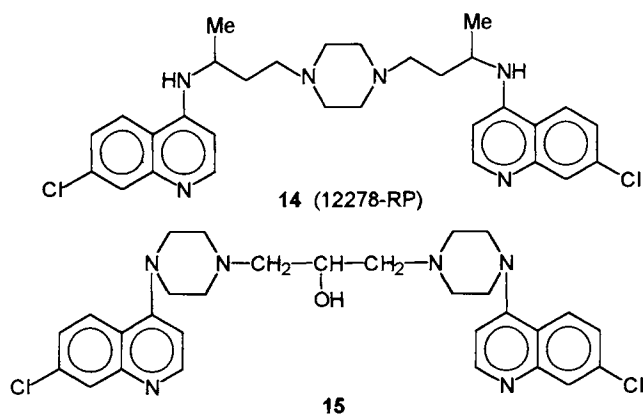
A number of other 7-chloro-4-substituted quinolines (**16-20**) have also been synthesized as structural analogues of chloroquine [26]. Of these 14153-RP (**17**), 12494-RP (**18**) and tripiperaquine (M-1020, **20**) showed promising activity against *Plasmodium* spp. in laboratory animals [26].

16   X = SO₂, CO

17

18

19

20
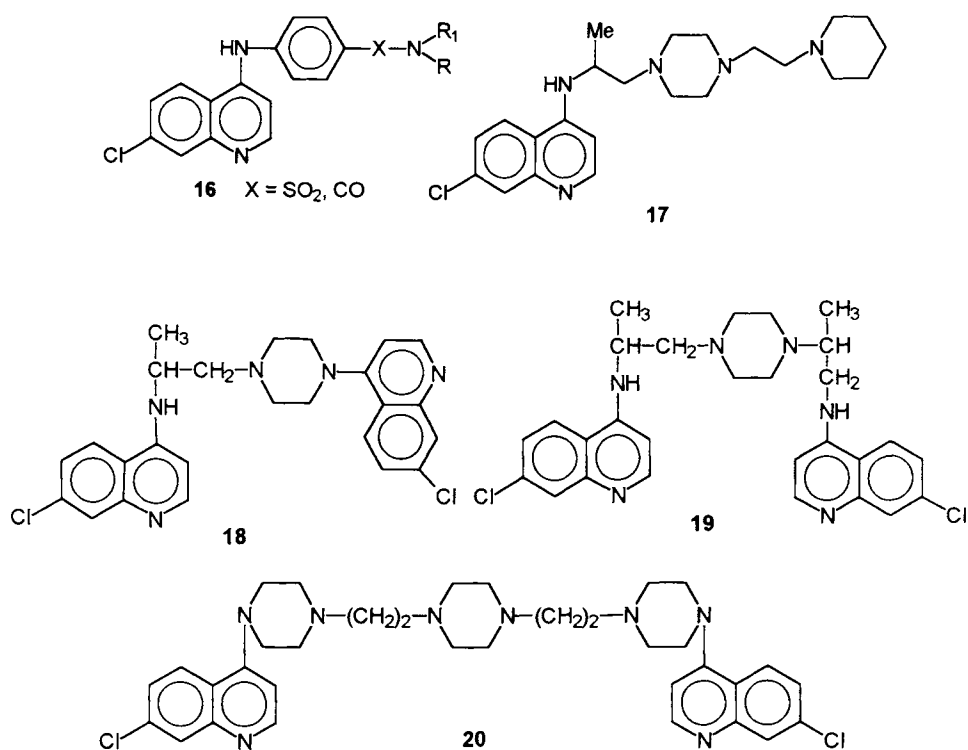
A variety of structural congeners of amodiaquine have also been synthesized, of which compounds of type **21-25** exhibited a varying degree of antimalarial activity [27-31]. The most interesting compounds of this class are **24** and **25**, of which **25** exhibited both curative and prophylactic effects in mice. The compound showed curative action at a dose of 5-100 mg/kg, while a dose of 100 or 250 mg/kg, given by subcutaneous routes, was needed to protect the animals against infection for a minimum of two weeks [26]. Interestingly, amodiaquine and a few of its structural analogues have also been found to possess antifilarial activity [32].

21a R = Me, Et
b R = CONEt$_2$

22a R = H
b R = —N

23

24 R = NEt$_2$, R$_1$, R$_2$ = -(CH$_2$)$_4$-
25 R = NHBu, R$_1$ = C$_6$H$_4$Cl(p), R$_2$ = H
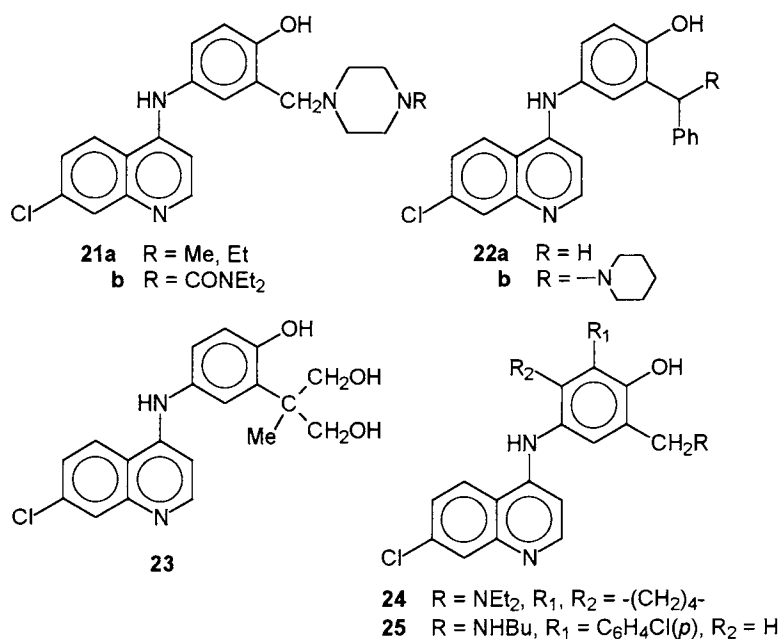
3. 6-AMINOQUINOLINES

Nickel and coworkers [33,35-37] and Temple, Jr. *et al.* [34] have prepared a series of 5,8-dimethoxy-6-aminoquinolines to delineate a structure-antimalarial correlation in this class of compounds. The most notable compounds are **26** and **27**, which were found to possess marked activity against rodent malaria, though weaker than primaquine [26]. A number of compounds of this class exhibited activity against *P. Vinckei* in mice; however, the optimal activity was shown to be associated with Ni-147/36 (**26**, R=OMe) [26].

26 R = H, Me, OMe

27
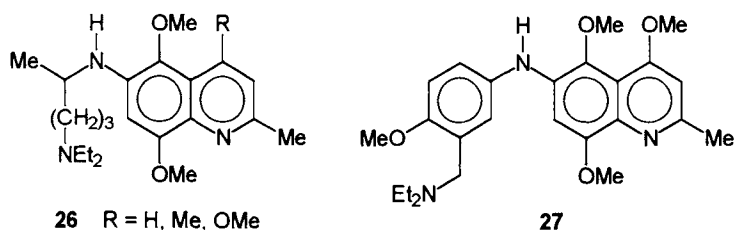
4. 8-AMINOQUINOLINES

As indicated earlier, the shortage of quinine in Germany during World-War I stimulated German scientists for searching newer chemotherapeutic tools to combat malaria. At that time it was known that methylene blue possesses appreciable antimalarial activity [38,39]. Structural modification in this molecule revealed that introduction of a diethylaminoethyl chain in place of one of the methyl groups of methylene blue (28) yields a compound (29) with slight improvement in biological activity [3]. A further exploitation of this observation led to the synthesis of various N,N-dialkylaminoalkylamines carrying a quinoline nucleus attached to the primary amino function [40-42]. The first active compound thus emerged was plasmocid (30), which was found to be active against *P. relictum* in canaries [42]. The demonstration of marked antimalarial activity of 30 initiated a vigorous search for better 8-aminoquinoline drugs. In 1925, pamaquine (plasmochin, 31) was discovered as a clinically acceptable drug [41]. However, this was found too toxic and, therfore, soon abandoned [43].

28

29

30 (Plasmocid)
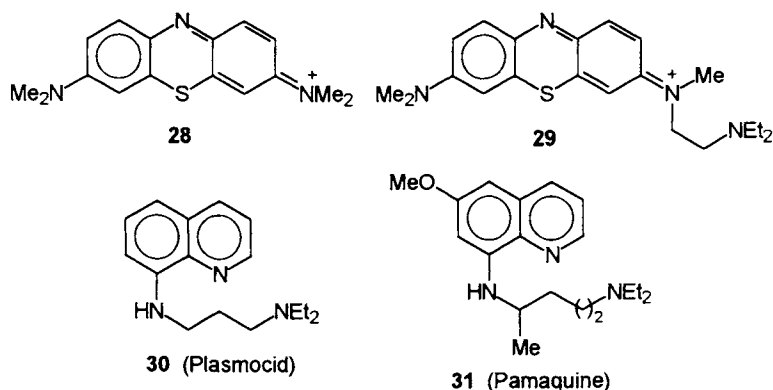
31 (Pamaquine)

Although pamaquine did not last long in clinical practice, it definitely opened a new window in drug design for malaria. Consequently thousands of 6-methoxy-8-

substituted aminoquinolines were synthesized in the United States and the Soviet Union that ultimately led to the discovery of a number of effective antimalarials. The most effective antimalarial drugs discovered are pentaquine (32), isopentaquine (33), SN-3883 (34), primaquine (35) and quinocide (36) [26].
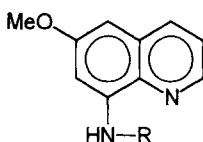
32 R = -(CH$_2$)$_5$NHCHMe$_2$ 35 R = -CH(Me)-(CH$_2$)$_3$-NH$_2$
33 R = -CH(Me)-(CH$_2$)$_3$NHCHMe$_2$ 36 R = -(CH$_2$)$_3$-CH(Me)-NH$_2$
34 R = -(CH$_2$)$_4$-NH$_2$

Primaquine (35) is the most effective drug amongst the 8-aminoquinolines, which has been widely used as an antirelapse drug of choice. Quinocide (36), a closely related congener of primaquine, is equipotent to primaquine, but is somewhat more toxic. The drug has been used in Eastern Europe and Russia. A combination of plasmocid or rhodoquine (30) and pamaquine or praequine (31) called 710F has been used in France under the trade name Rhodopraequine [2].

4.1   SAR in 8-aminoquinolines

As discussed above, primaquine is the best drug amongst the 8-aminoquino-line antimalarials. It is used as a drug for achieving a radical cure of malaria world-wide. In addition to the radical curative activity, primaquine also possesses causal prophylactic, gametocytocidal and sprontocidal activities. Despite the broad-spectrum of the antimalarial activity, primaquine may not be considered as an ideal anti-malarial. This is because of the fact that the drug exhibits poor blood schizontocidal activity and is associated with a number of serious side effects such as haemolysis (particularly in patients with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency), methaemoglobinemia and gastrointestinal disturbances. It is because of these limitations of primaquine, the need for a safer and more effective antimalarial has been always advocated. Consequently the 8-aminoquinoline molecule has undergone extensive molecular modifications with a view to establish a conclusive SAR as also to evolve a better antimalarial. This subject has been dealt in excellent articles by Bhat *et al.* [44] and Nodiff and his colleagues [45].

The need for a better drug became evident by the demonstration of the development of resistance of *P. falciparum* against chloroquine in 1960. In 1963, the U.S. Army Medical Research and Development Command initiated a massive research

programme for safe and more effective antimalarial drugs, which was coordinated through the Walter Reed Army Institute of Research in Washington, D.C. This effort was further strenghtened by the Special Programme for Research and Training in Tropical Diseases (TDR) sponsored by UNDP/WORLD BANK/WHO and U.S. Agency for International Development. Efforts to improve the biological profile of primaquine have also been carried out in the People's Republic of China and India [44,45]. The different structural changes made in the primaquine molecule is discussed below.

4.1.1 Modifications at the 8-amino function

The development of drugs from plasmocid (30) to quinocide (36) clearly demonstrates that the presence of an alkylamino function with a 4 or 5 carbon chain (straight or branched) attached to the aminogroup at the 8-position of 6-methoxyquinoline is essential for antimalarial activity. The terminal amino may be free or substituted by alkyl groups. A further structural variation of the 8-amino substitution has resulted in the synthesis of a large number of 6-methoxy-8-(alkylamino)aminoquinolines (37) with varying degrees of antimalarial activities [46-60]. A detailed description of such modifications may be seen in the review by Bhat *et al.* [44].

In an endeavour to discover compounds with better activity and lesser toxicity than primaquine, two chemical approaches have been followed to develop a prodrug of primaquine. The first approach involves introduction of small peptide units linked covalently to the terminal amino group of the side chain [61]. Alternatively, substituents like sulphonyl, aminoacyl, glucopyranosyl, galactopyranosyl, mannopyranosyl and cyclic/open chain enaminone groups may be attached to the terminal amine of the side chain. The second approach is concerned with the modification of the side chain attached to the amino group of 8-amino-6-methoxyquinoline. Thus the synthesis of variety of 6-methoxy-8-(substituted) aminoquinolines was carried out, of which N^1-(3-acetyl-4,5-dihydro-2-furanyl-N^4-(6-methoxy-8-quinolinyl)-1,4-pen-
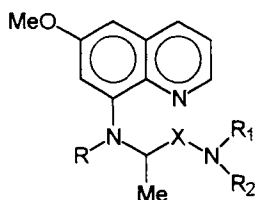
37 R = H, Ph, Me
R$_1$ = H, alkyls
R$_2$ = H, alkyls, CONH$_2$ etc.
X = alkyls (linear or branched)
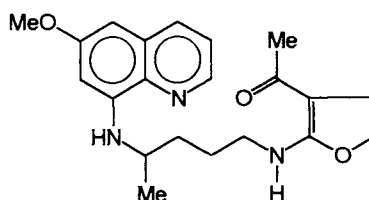
38 (CDRI 80/53)

tanediamine (**38**, CDRI 80/53) was found to possess promising activity and, therefore, was chosen for drug development [44,62-67].

Compound 80/53 (**38**) exhibits radical curative and prophylactic activities against *Plasmodium cyanomolgi* infection in rhesus monkeys [63,64]. The maximum tolerated dose of this compound is 450 mg/kg, given intraperitoneally to mice. Compound 80/53 has shown to be safe with no teratogenic action in toxicological studies in rats and monkeys. It is also safer than primaquine because it produces nearly three times less methhaemoglobinaemia than the parent drug. Phase I trials carried on humans have shown the drug to be well tolerated [67].

4.1.2 Modifications at position 2

It is presumed that 8-aminoquinolines undergo metabolic conversion to form the labile 5,6-quinones, which could be stabilized by substitution at 2-position of the quinoline ring [68]. This led to the synthesis of 2-substituted analogues (**39**) of primaquine [69-73]. A number of compounds, thus prepared, exhibited activity in the Rane's blood schizontocidal screen (*P. berghei* in mice) [68]. Of these the most effective compound was found to be 2-benzyloxy and 2-benzylthio derivatives (**40**) of primaquine. Detailed biological studies indicated that 2-benzyloxyprimaquine (**41**) is the best compound of this series, which showed radical curative activity against *P. cyanomolgi* in rhesus monkeys. In Rane's test this compound exhibited no toxicity upto a dose of 640 mg/kg as compared to 160 mg/kg, which is the toxic dose for primaquine [45]. Another active member of this class is **42**, which was equipotent to primaquine in producing radical cure against cyanomolgi malaria in monkeys [45].
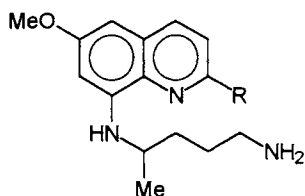
39 R = alkyl, alkoxy, halo, alkenyl, aryloxy, thioaryloxy etc.
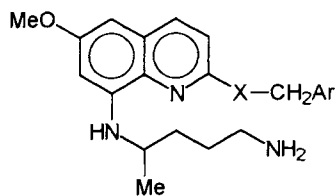
40 X = O, S
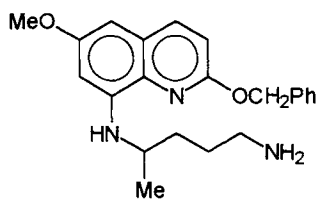
41
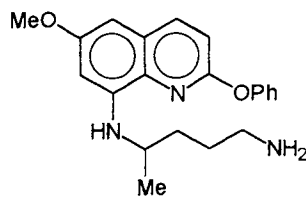
42

4.1.3 Modifications at position 3

Detailed work regarding SAR on 3-substituted primaquines is lacking. 3-Methylprimaquine (**44**) exhibits powerful causal prophylactic activity in mice. It also shows high tissue schizontocidal efficacy; however, its blood schizontocidal effect is insignificant. 3-Methylprimaquine (**44**) is more toxic than its corresponding 2-methyl analogue and primaquine; the toxic dosages of 3-methylprimaquine, 2-methylprimaquine (**43**) and primaquine being 80, 320 and 160 mg/kg, respectively [72,74].
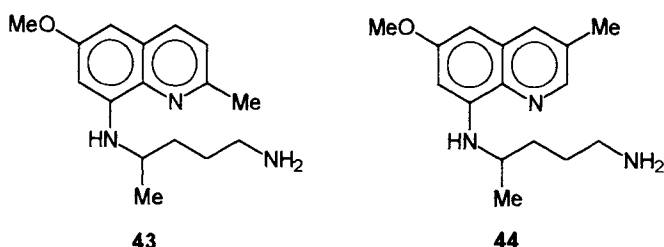
4.1.4 Modification at position 4

4-Methylprimaquine (**45**) is the first member of this class which was synthesized by Elderfield *et al.* in 1955 [54]. This compound was found to possess less toxicity and better radical curative and blood schizontocidal activity than primaquine in mice [72]. However, 4-methylprimaquine could not be taken up for drug development because of its high toxicity found in dogs and monkeys during detailed studies [75]. Attempts to improve the biological profiles of **45** led to the synthesis of a series of 4-substituted primaquines [76,77], of which **46-49** exhibited blood schizontocidal activities [44,45].
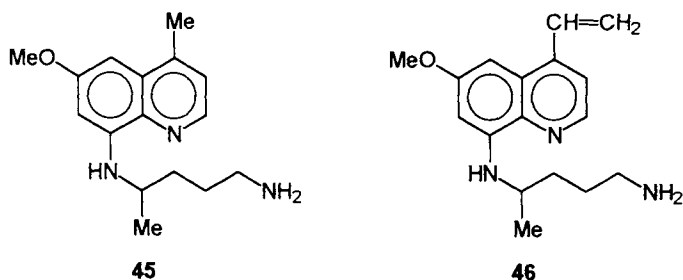
At a dose of 80 mg/kg, the vinyl (**46**) and ethyl (**47**) derivatives of primaquine exhibited weaker blood schizontocidal activity than primaquine. However, **47** was non-toxic at the doses ranging from 20-640 mg/kg and also produced 100% curative

effects at 320 mg/kg. Compound **47** has also been found to be equipotent to primaquine against *P. cynomolgi* in rhesus monkeys at a dose level of 1 mg/kg [44]. Similarly, 4-(4-chlorophenoxy)primaquine (**49**) exhibited 60 and 100% activities in the Rane screen at a dose of 160 and 320-640 mg/kg, respectively [45].

47

48a R = F
b R = OMe

49
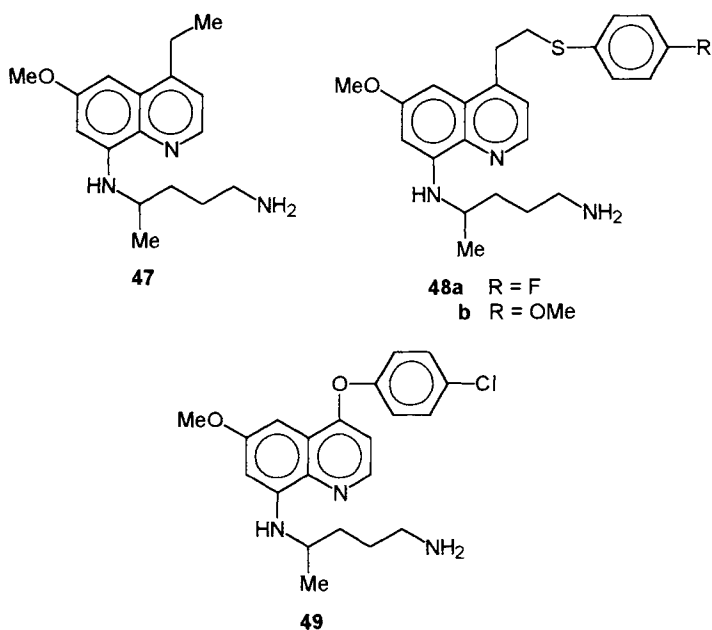
4.1.5 Modifications at position 5

The introduction of an aryloxy substitution at 5-position of primaquine has been found to greatly influence the net antimalarial response of the resulting molecules. The first example of this class is 5-(4-methoxyphenoxy)primaquine (**50**), which had a therapeutic index of 177 as compared to 30 for primaquine and 57 for pentaquine [45]. Encouraged by the marked therapeutic profile of **50** Nodiff and his colleagues [78-80] synthesized a variety of 5-phenoxy-, 5-thiophenoxy-, and 5-anilino-primaquine (**51**). A number of other 5-substituted primaquines (**51**) have also been prepared by other workers [81-85].

In general the 5-aryloxy analogues (**51**, X=0) of primaquine exhibited moderate to good activity in radical curative tests. The corresponding arylthio and arylamino derivatives (**51**, X=S,NH) were found to be nontoxic with poor or no activity upto a dose level of 640 mg/kg in the blood schizontocidal screen [45]. In the radical curative test, 5-aryloxy or 5-arylthio derivatives of primaquine with a halogen exhib-

ited optimal activity. Thus the 4-fluoro (**52**) and 3-trifluoromethyl (**53**) analogues in the aryloxy series were completely curative at a dose of 320 and 640 mg/kg, while in the phenylthio series the 4-chloro- and 3-trifluoromethyl derivatives (**54a** and **54b**) exhibited curative effects at a dose of 1 mg/kg. The anilino compound (**51**, X=NH, R=CF$_3$) corresponding to **54b** shows no activity even at a dose of 10 mg/kg [45].

50

51   X = O, S, NH

52   R = 4-F
53   R = 3-CF$_3$

54a   R = 4-Cl
54b   R = 3-CF$_3$
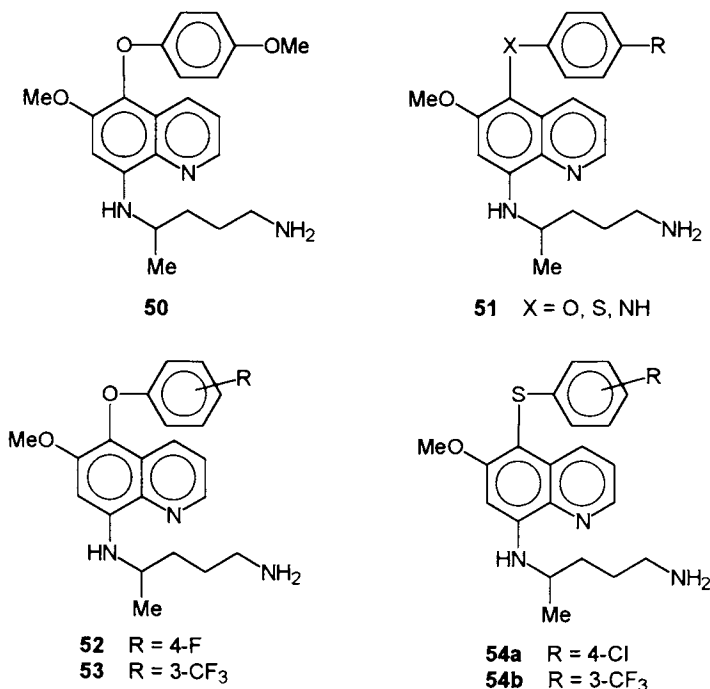
A number of 5-alkoxyprimaquines have also been synthesized, many of which showed promising antimalarial activity [72,86]. 5-Methoxyprimaquine (**55**, R=Me) was equipotent to primaquine in radical curative activity. Elongation of the alkyl chain upto ten carbon atoms retained the high order of activity. The decyl derivative (**55**, R=n-C$_{10}$H$_{21}$) was found to possess radical curative activity at a dose of

55
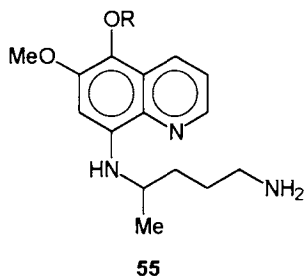
10 mg/kg, while other 5-alkoxy derivatives (**55** R=Et, CH_2CF_3, n-C_3H_7, n-C_4H_9, n-C_5H_{11}, n-C_6H_{13}, n-C_8H_{17}) were curative at 1 mg/kg. Of these, the 5-propoxy, butoxy and pentoxy analogues of primaquine were even better than the parent drug as all of them exhibited curative effects at a dose of 0.316 mg/kg [45].

4.1.6 Modifications at positions 2 and 4

A few derivatives of primaquine carrying substituents at its 2- and 4-positions have been synthesized, but none showed promising therapeutic response [87]. Interestingly, compounds **56a-c** were found to be less toxic than primaquine; at the same time these were less active too than primaquine in the blood schizontocidal screen. However, primaquine and **56a** possess equal activity as tissue schizontocides [45].
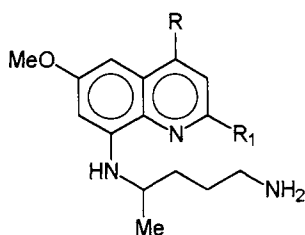
56a R = Me, R_1 = Et
   b R = Me, R_1 = CH=CH$_2$
   c R = Et, R_1 = Me

4.1.7 Modifications at positions 2 and 5

Chen *et al.* [72] have prepared some 2,5-disubstituted derivatives of primaquine. The most notable activity was displayed by 2-methyl-5-(n-hexyloxy- and n-hexylthio)primaquines (**57a,b**). Both these compounds exhibited blood schizontocidal activity at a dose of 20 and 160 mg/kg, respectively [45]. Another series of 2,5-disubstituted primaquines, represented by the general formula **58**, have been

57a X = O
   b X = S

58
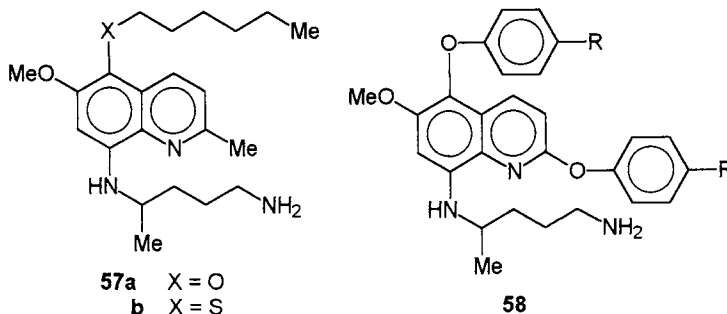
prepared [81,88], of which **58** (R=4-F) was found to be effective against sporozoite-induced infection of *P. yoelii* in mice [45].

4.1.8 Modifications at positions 4 and 5

The radical curative activity of 4-methylprimaquine and 5-phenoxypri-maquine has been exploited by Nodiff, LaMontagne and their coworkers, who found that introduction of a 4-methyl group in 5-aryloxy/alkoxyprimaquines causes dramatic enhancement in antimalarial activity [44,45]. This observation led to the synthesis of a large variety of 5-aryloxy/alkoxy derivatives of primaquine carrying different substituents at their 3- and 4-positions [72,76,86,89-95]. Most of the 4-methyl-5-aryloxyprimaquines exhibited less toxicity and better blood schizontocidal and radical curative activities [45]. Similarly 4-methyl-5-alkoxyprimaquines were found to possess high order of blood schizontocidal properties. Nodiff *et al.* [45] have summarised the structure-activity correlations in various 4-methyl-5-alkoxyprimaquine derivatives with respect to their blood schizontocidal and radical curative properties.

4,5-Disubstituted primaquine analogues exhibited broader spectrum of antimalarial activities; compounds **59-61** exhibited both blood schizontocidal (*P. berghi* in mice) and radical curative (*P.cynomolgi* in rhesus monkeys) activities and were also less toxic [26,44,45].

59 R = C_6H_{13} (WR-242511)
60 R = $C_6H_4CF_3(m)$ (WR-225448)

61 (WR-238605)
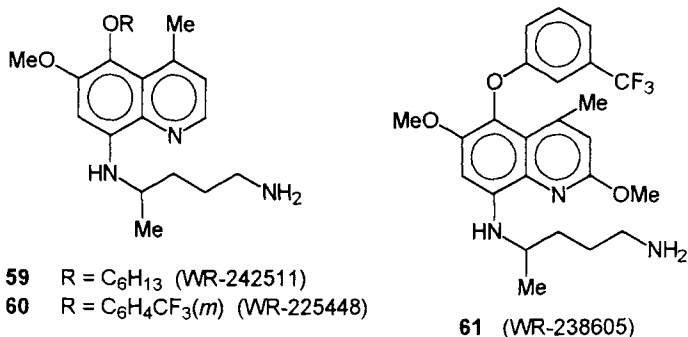
WR-242511 (**59**) has been found to be curative at a dose of 2.5 mg/kg in the Rane screen. It also cured 5 of 5 monkeys treated at a dose of 0.1 mg/kg. Unfortunately WR-242511 produces worrisome methaemoglobinemia in dogs (48.1% as compared to 25.3% of WR-225448 and 16% for WR-238605) [96]. Nevertheless, this compound is considered as a candidate antimalarial because of its excellent radical curative and blood schizontocidal activities at dose levels of 0.1 and 20 mg/kg, respectively. WR-242511 is at present undergoing detailed preclinical studies [45].

WR-225448 (60) has been selected for preclinical biological studies because of its powerful antimalarial activity. At a dose of 1 mg/kg given daily for 7 days, the compound was 100% curative against *P. cynomolgi* in monkeys; it was suppressive at a dose of 0.0316 mg/kg. Both primaquine and WR-225448 were found to be equipotent against *P. cynomolgi* in rhesus monkeys at a dose of 3.5 mg/kg. However, when given in combination with chloroquine, the curative dose dropped to 0.875 mg/kg for WR-225448 (60) and increased to 14 mg/kg for primaquine [45]. In the toxicological studies compound 60 was found to be more toxic than primaquine. This compound exibited haemototoxicity and also produced higher methaemaglobin levels than primaquine in dogs [96].

WR-238605 (61), which is the 2-methoxy derivative of compound 60, has been shown to possess better blood schizontocidal and radical curative activities. It also produced less methaemoglubinemia (16% as compared to 25.3% of WR-225448) [97]. This compound also showed slightly better activity than WR-225448 against *P. vivax* in Aotus monkeys at a dose of 1 mg/kg for 3 days [45].

A few structural congeners of primaquine have shown activity against leishmaniasis in laboratory animals. The 8-aminoquinolines that exhibit antileishmanial activity are 45 and 62-65, some of which were found to be better than antimonials or pentamidine [98]. Of these 62 was found to be most active and possesses 400-700 times higher activity than meglumine antimoniate against *L. donovani* in hamsters [98]. This compound shows high activity against visceral leishmaniasis in rodents and dogs but is less effective against cutaneous leishmaniasis in experimental animals [99]. WR-6026 has been found to be well tolerated upto a single dose of 60 mg and is currently under clinical trials [100].

62 R = NEt$_2$ (WR-6026)
63 R = NHCHMe$_2$
64 R = —N⟨ ⟩N—CH$_2$–CHMe with OH
65
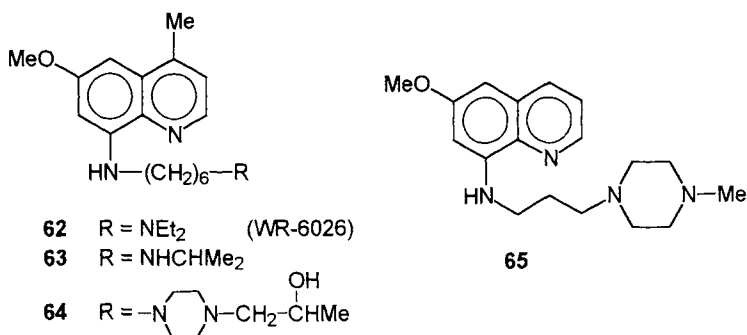
4.1.9 Other modifications

In addition to the above modifications in the primaquine molecule, various other structural changes were made in the quinoline heterocycles itself. Carrol *et al.* [101] have prepared a number of 1,2-dihydroprimaquines (66) and 1,2,3,4-tetrahydroprimaquines. Evaluating these compounds in mice and monkeys established that partial or complete reduction of the pyridine ring of primaquine leads to lowering or loss of antimalarial activity. However, one compound of this class, viz. 1-diethylaminoethyl-1,2,3,4-tetrahydro-8-butylamino-6-methoxyquinoline (67) was found to possess strong prophylactic activity against *P. berghei* (mouse) and *P. cynomolgi* (rhesus monkey) infections [26].

It is also possible to replace the pyridine part of primaquine by benzene or furan rings. The compounds (68,69) thus generated, were found to possess weak or no antimalarial activity [102,103]. Some acridine analogues of primaquine have also

66

67

68   R = H, Br

69

70   R = H, Me
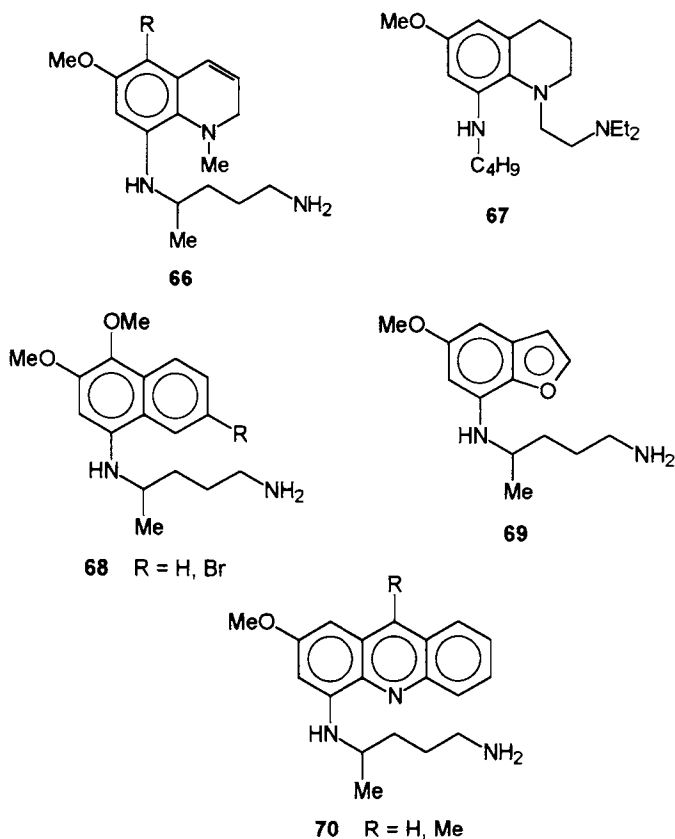
been synthesized, of which **70** showed antimalarial activity though far weaker than primaquine [104].

5.   QUINOLINE-4-METHANOLS

Quinine (**1**) is the oldest example of this class which has been used as a "lead molecule" to design quinoline-4-methanols with improved antimalarial activity. The most successful outcome of this effort had been the discovery of mefloquine (**71**) as an antimalarial. A discussion on the development of mefloquine and related quinoline-4-methanols is given in chapter 14 (sec. 2.1.1.1).
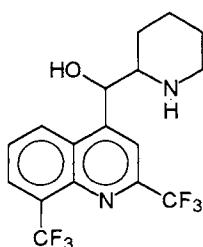
71 (Mefloquine)

6.   HYDROXYQUINOLINES

Quinoline derivatives are amongst the earliest drugs used to treat amoebiasis. However, their value as antiseptic agents [eg. oxyquinoline (**72**) and benzoxyquine (**73**)] was established long before their amoebicidal properties were discovered. In fact, a random screening programme of halogenated quinolin-8-ols for search of antiseptic agents led to the discovery of sodium 7-iodo-8-quinolinol-5-sulphonate (**74**, chiniofon) [1,105-107]. Substitution of the sulphonate moiety in chiniofon (**74**) by halogens resulted in the synthesis of 5-chloro-7-iodo-8-hydroxyquinoline (**75**, clioquinol,vioform), 5,7-diiodo-8-hydroxyquinoline (**76**, embequine, diiodohydroxyquinoline), and broxyquinoline (**77**) [1,105,108,109]. Later, these halogenated 8-hydroxyquinolines were found to possess strong amoebicidal properties [110-113]. The

72 R = H   **74**   **75**
73 R = COPh

76   77   78
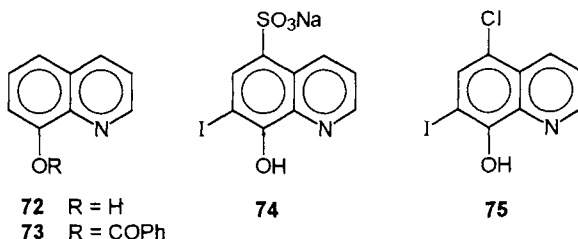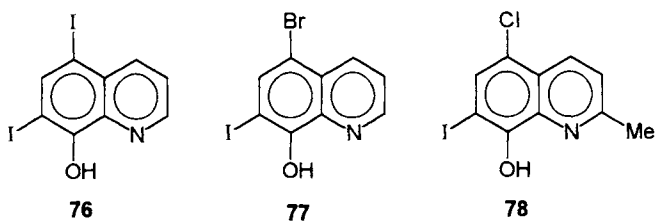
2-methyl derivative of **75** called chloroquinaldol (**78**) has also been found to be effective against intestinal amoebiasis in human [114,115]. However, the three iodinated 8-hydroxyquinolines namely chiniofon (**74**), vioform (**75**) and embequin (**76**) have been extensively used to treat intestinal amoebiasis in human [116].

A Mannich reaction with 5-halo-8-hydroxyquinolines has led to a series of 7-aminomethyl-5-halo-8-hydroxyquinolines with potent *in vitro* and *in vivo* activity against *E. histolytica* [116]. The most effective compound was found to be 7-(N,N-diethylaminomethyl)-5-chloro-8-hydroxyquinoline (**79**) [117], which exhibited activity against intestinal amoebiasis in man [112].

Demonstration of high amoebicidal activity associated with **79** established the fact that introduction of an aminomethyl chain at 7-position of 8-hydroxyquinolines provides compounds with potent activity against *E. histolytica*. Consequently a wide range of Mannich bases derived from 8-hydroxyquinolines were synthesized by Helin and Vander Werf [118] and Burckhalter *et al.* [119,120] emerging clamoxyquin (**80**) as a powerful drug for treating various forms of amoebisis in animals and man [120-124].

Two other hydroxyquinoline drugs that need to be mentioned here are decoquinate (**81**) and buquinolate (**82**), which find use in the treatment of coccidiosis in poultry [125, 126]. Of these, decoquinate is a highly effective and well tolerated

79   80

81   82
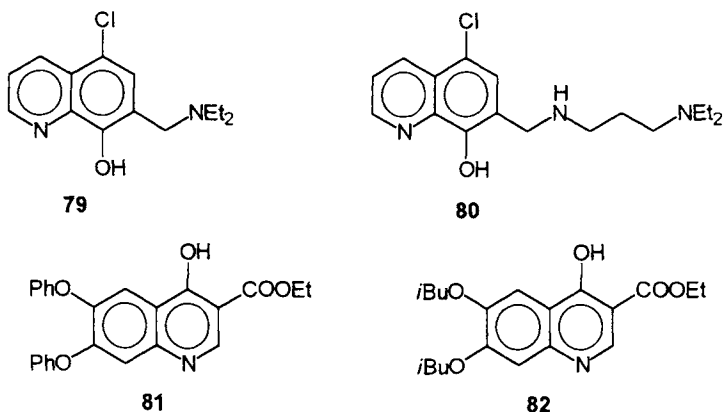
drug that can be used to prevent coccidiosis (*Eimeria* spp.) in chickens at a dose of 30 mg/kg [127].

7.   SYNTHESIS OF DRUGS

7.1   Chloroquine (3) and amodiaquine

The key intermediate for synthesizing chloroquine, amodiquine and other 4-aminoquinoline drugs is 4,7-dichloroquinoline (**91**), which can be prepared by reacting *m*-chloroaniline (**83**) with diethyl oxaloacetate (EtO-CO-CH$_2$-CO-COOEt) or ethoxymethylene malonic ester [EtO-CH=C(COOEt)$_2$] as shown in scheme 1 [8,128-133].
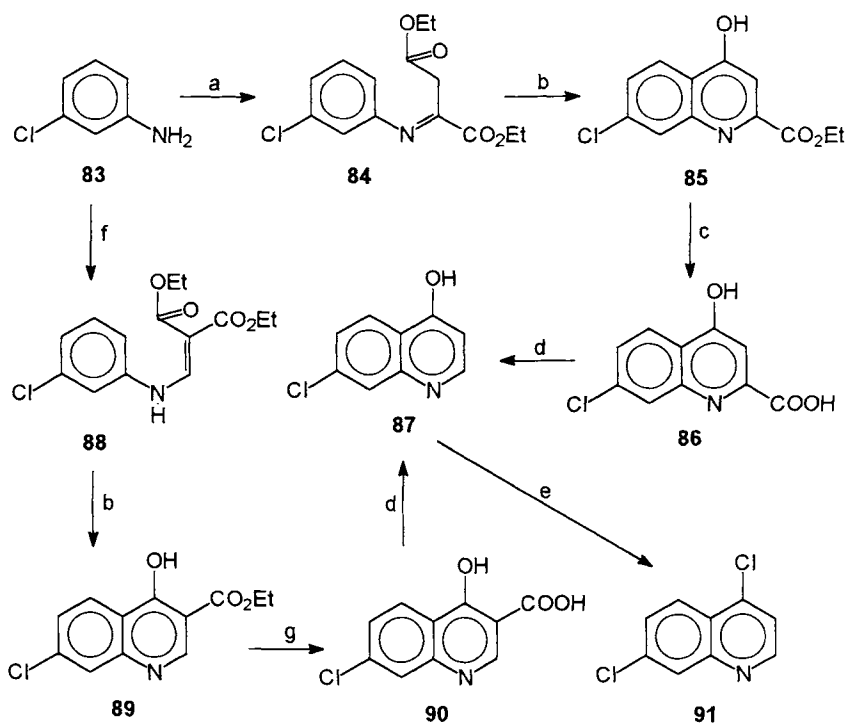
Scheme 1

Reagents: (a) EtOCO-CH$_2$CO-COOEt, (b) heat and separation of isomers (c) NaOH, heat, (d) heat (250°C), (e) POCl$_3$, (f) EtOCH=C(COOEt)$_2$, (g) NaOH, heat, HCl

The synthesis of various 4-aminoquinoline antimalarials may be achieved by nucleophilic reaction of **91** with desired amines. Scheme 2 outlines the preparation of chloroquine (**3**) and amodiaquine (**8**) starting from 4,7-dichloroquinoline (**91**) [134-136].
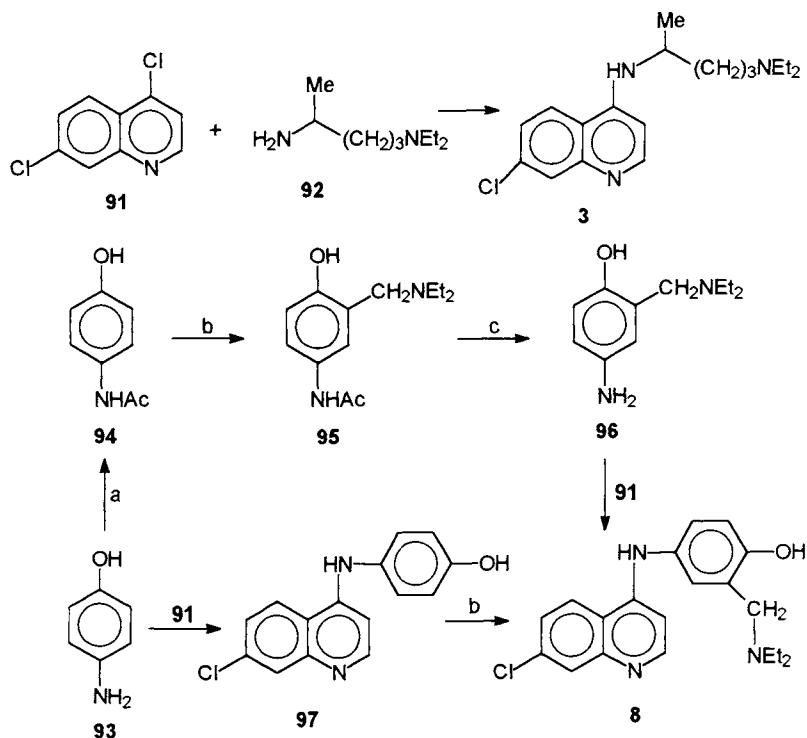
Scheme 2
Reagents: (a) Ac$_2$O; (b) HCHO, NHEt$_2$, (c) HCl.
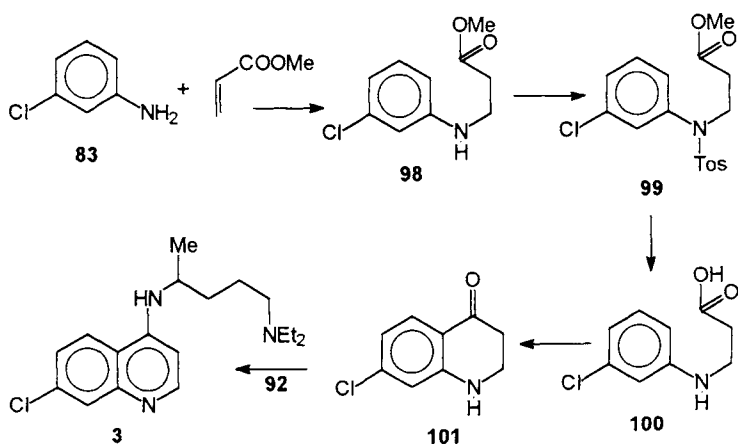
Scheme 3

Another method to prepare chloroquine (3) involves reaction of 83 with methyl acrylate to get via 98 and 99 the adduct 100, which is converted into 7-chloro-1,2,3,4-tetrahydroquinoline-4-one (103). Reaction of 103 with novaldiamine (92) under dehydrogenating conditions gives chloroquine in about 25% overall yield [133] (Scheme 3).

7.2   Primaquine (35)

Elderfield and coworkers [137,138] synthesized primaquine starting from 8-amino-6-methoxyquinoline (104) by the reaction sequence outlined in Scheme 4. The required 8-amino-6-methoxyquinoline can be obtained by Skraup's reaction on 4-methoxy-2-nitroaniline (106) to get 107, which is reduced to yield 104 [139].
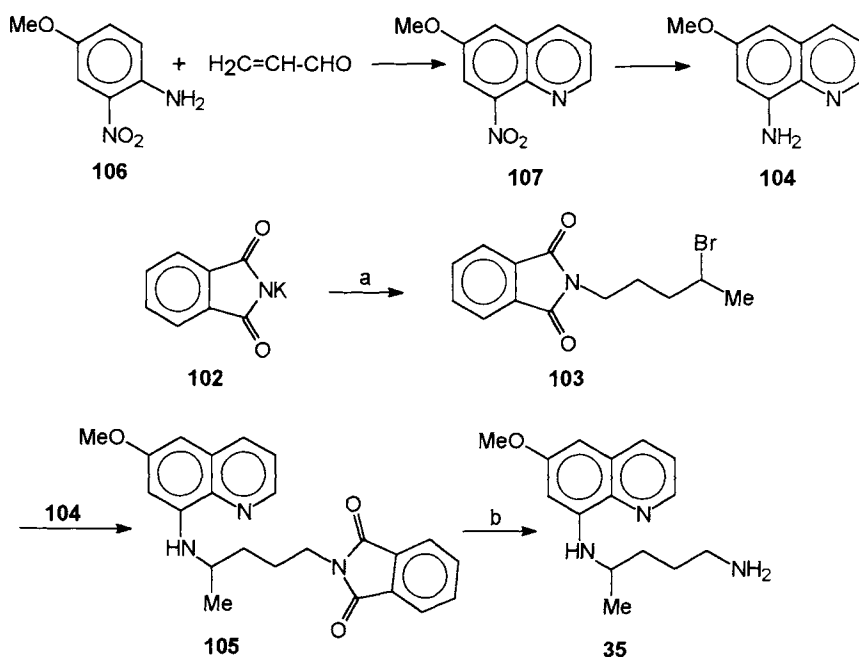
Scheme 4
Reagents: (a) 1,4-dibromopentane, (b) H_2N-NH_2.

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CHAPTER 17

NITROHETEROCYCLES

1. INTRODUCTION

During early the 1940s Dodd and Stillman [1,2] discovered the antibacterial and antitrypanosomal activity of nitrofurans. In 1948 Bartz introduced chloramphenicol, a nitro group containing antibiotic with high antimicrobial activity [3]. In the early 1950s antitrichomonal activity of azomycin was reported [4,5]. These findings focussed attention on nitroheterocycles in the chemotherapy of microbial diseases. Consequently a large variety of nitro compounds derived from furans, imidazoles and other hetererocycles were tested, which led to the discovery of several effective drugs, many of which find extensive use in the chemotherapy of microbial and protozoal diseases of humans and animals [6,7].

2. NITROFURANS

Following the discovery of nitrofurans as a potent class of antimicrobial agents, nitrofurazone (1) was evaluated against trypanosomiasis. This compound was found to be highly effective against *Trypanosoma equiperdum, T. gambiense* and *T. rhodesiensis* in rodents [1,2,8-10]. Nitrofurazone emerged as a drug for treating relapse cases of African sleeping sickness resistant to suramin, pentamidine or melarsprol [11,12]. Several other structural analogues of nitrofurazone were tested against trypanosomiasis, histomoniasis and amoebiasis. Some important nitrofurans which exhibited promising antiprotozoal activities are 2-7. Of these 2-4 were active against trypanosomes, 5 and 6 exhibited efficacy against *Histomonas* spp., while 7 had amoebicidal activity. A number of other 5-nitrofurans such as 8-12 have also been found to possess a varying degree of antiprotozoal efficacy [7,8].

1 R = NHCONH₂   (Nitrofurazone)
2 R = NHCOOCH₂CH₂OH   (Furaltadone)

3 R =   (Nitrofurantoin)

4 R =   SO₂   (Nifurtimox)

5 R =   (Furazolidone)

6 R = -NH-CO- (nitrophenyl) (Nifursol)

7 R = -NH-CO- (phenyl) -OH (Ercefuryl)

8 R =

9 R =

10 R = NHCOMe

11 (SQ 18506)   **12**
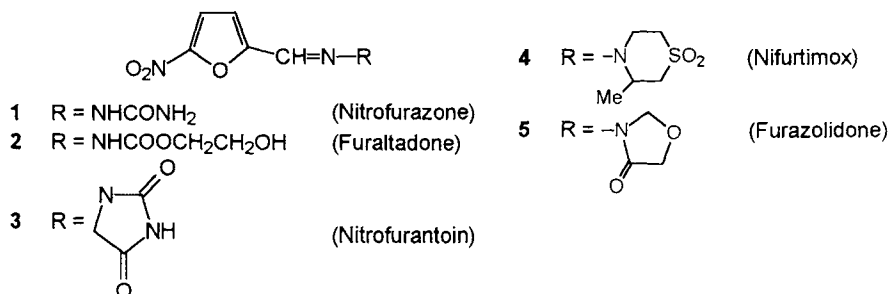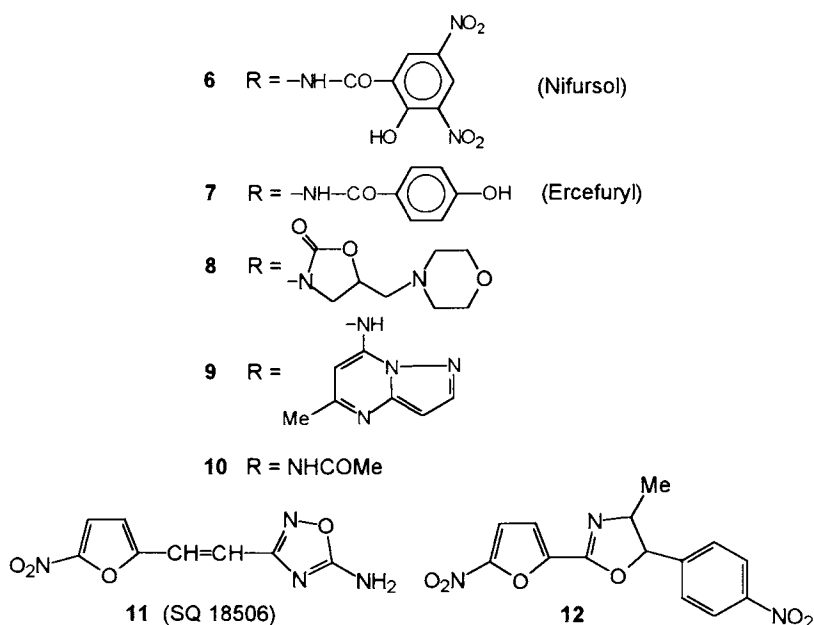
3. NITROTHIAZOLES

Like nitrofurans, the compounds of this class have been found to possess a wide-spectrum of antiparasitic activity. The first compound which exhibited potent antitrichomonal activity is entramin (**13**) [13]. Acylation of entramin gave a number of active compounds; the most effective compound was 2-acetylamino-5-nitrothiazole (acinitrazole, **14**). This compound exhibited high *in vitro* and *in vivo* activity against *Trichomonas vaginalis* and *T. foetus* in animals, but did not prove to be of value in clinical trials [13-16]. Acinitrazole has, however, been found to be effective against intestinal amoebiasis in rats and dogs [13].

Further molecular modification of acinitrazole revealed that compounds of type **15** possess high trichomonacidal activity. The order of activity increased from S<O<N. [17]. Consequently a series of other nitrothiazoles were evaluated for their antitrichomonal activity, emerging niridazole (**16**) and the open chain urea derivative (**17**) as effective antiprotozoal agents. Niridazole is a potent antischistosomal and antiamoebic agent [6,8], while **17** showed interesting activity against hepatic amoebiasis in rodents; however, toxicity precluded its development as an antiamoebic drug [19].

13 R = H
14 R = COMe

15 n = 2, 3
    X = S, O, NH

16

17
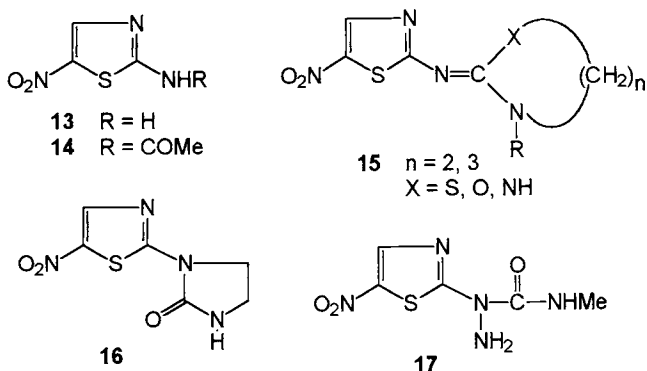
4.   NITROIMIDAZOLES

This is the most extensively studied class of nitroheterocycles, which has revolutionized the treatment of trichomoniasis, amoebiasis and giardiasis in humans [20]. The design of nitroimidazole drugs may be broadly grouped in two heads.

4.1   2-Nitroimidazoles

The simplest member of this class is azomycin (18), a naturally occuring nitroimidazole antibiotic, which was found to be active against *Trichomonas vaginalis* [4,5]. Attempts to improve the antitrichomonal activity of azomycin led to the synthesis of a large number of substituted 2-nitroimidazoles. However, none of these derivatives achieved clinical status [20]; the 2-nitroimidazoles, which exhibited promising antitrichomonal activity, are 19-21.
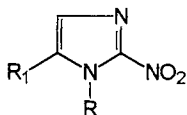
18 R = R_1 = H (Azomycin)
19 R = Me, R_1 = *i*-Pr
20 R = $CH_2CH(OH)CH_2OMe$, R_1 = H (Misonidazole)
21 R = $CH_2CONHCH_2Ph$, R_1 = H (Radanil, Benznidazole)

Compound 19 was found to be 5 times more potent than metronidazole (22), but was dropped due to its high toxicity [20,21]. Similarly misonidazole was equipotent to metronidazole against *Trichomonas* spp. and *E. histolytica* [22]. Radanil, on the other hand, exhibited promising trypanosomicidal activity, particularly against Chaga's disease [20].

4.2   5-Nitroimidazoles

Extensive SAR studies on 2-nitroimidazoles did not result in getting a clinically acceptable drug, but focussed attention to investigate the potential of other nitroimidazoles. Consequently, Rhone-Poulenc set out to carry out structural modifications in 5-nitroimidazoles, which are more readily accesible than 2-nitroimidazoles. By the end of the 1950s, the discovery of metronidazole (22) was announced [23,24], which is now the drug of choice for treating amoebiasis, giardiasis and trichomoniasis in humans [25-27].

The discovery of metronidazole stimulated a vigorous search for better nitroimidazole drugs in different laboratories over the world, and led to a new generation of antiprotozoal drugs. Variation of the alkyl chain at 1-position of 2-methyl-5-nitroimidazole provided a number of compounds with high order of antiamoebic and antitrichomonal activities. Some of these are used in clinical practice and include benzoylmetronidazole (23), tinidazole (24), ornidazole (25), bamnidazole (26), carnidazole (27), panidazole (28), secnidazole (29) and dimetridazole (30) [20].
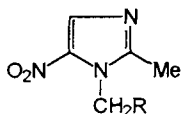
22   (Metronidazole), R = CH_2OH
23   (Benzoylmetronidazole), R = CH_2OCOPh
24   (Tinidazole), R = CH_2SO_2Et
25   (Ornidazole), R = $CH(OH)CH_2Cl$
26   (Bamnidazole), R = CH_2OCONH_2
27   (Carnidazole), R = $CH_2NHCS\text{-}OMe$
28   (Panidazole), R = CH_2-4-pyridyl
29   (Secnidazole), R = $CH(OH)Me$
30   (Dimetridazole), R = H

Variations in the 2-position of 2-substituted-1-methyl-5-nitroimidazoles also provided compounds with promising activity, which include ipronidazole (31), ronidazole (32), azanidazole (33), fexinidazole (34) satranidazole (35), pirinidazole (36) and chloronidazole (37) [20,28].

The demonstration of high antiprotozoal activity in chloronidazole (37) [29] and nimorazole (38) [20] indicated that an alkyl group at the 2-position of 5-nitro-1-substituted imidazoles is not essential for biological activity, though the large majority of antiprotozoal drugs of this class carry a substituent at 2-position, which seems to modulate their activity. Some of the later drugs in this group include MCA-ni-

troimidazole (**39**), MF-nitroimidazole (**40**), flunidazole (**41**), moxnidazole (**42**) and HOE-316 (**43**) [20].
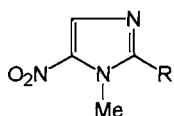
| **31** (Ipronidazole) | : | R = CHMe₂ |
| **32** (Ronidazole) | : | R = CH₂OCONH₂ |

33 (Azanidazole) : R = CH=CH—[pyrimidinyl]—NH₂

34 (Fexinidazole) : R = CH₂O—[phenyl]—SOMe

35 (Satranidazole) : R = —N[imidazolidinone]—SO₂Me

36 (Pirinidazole) : R = CH₂S-2-pyridyl
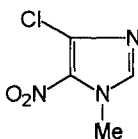
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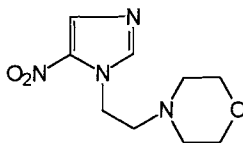
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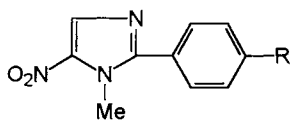
39 R = CONH₂
40 R = F
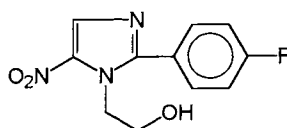
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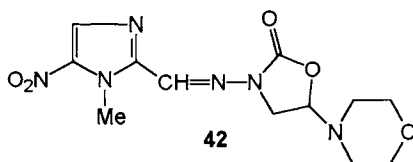
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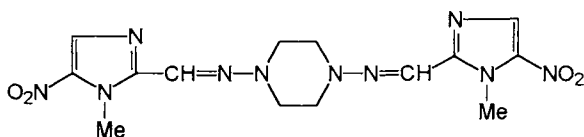
43

5. SYNTHESIS

5.1 Nitrofurans

The key intermediate for the synthesis of various nitrofuran drugs is 5-nitro-furfural (**45**, isolated as diacetate), which is obtained by careful nitration of furfural (**44**) with a nitric acid-acetic anhydride mixture at low temperatures [30]. Reaction of **45** with various nucleophiles gives nitrofuran drugs of the general formula **46**.
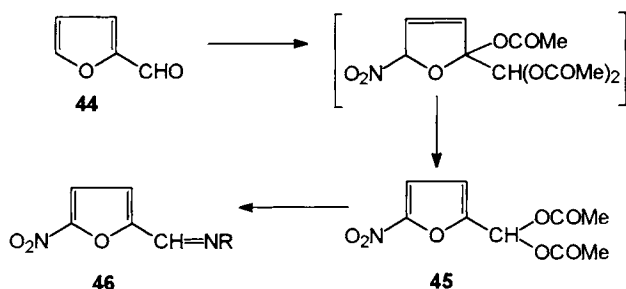
(a) **Nitrofurazone (1):** This is prepared by reaction of **45** with semicarbazide (H$_2$NNHCONH$_2$) hydrochloride [31]. A general method to prepare nitrofurazone and other similar nitrofuran drugs involves the reaction of semicarbazones of type **48** with 2-oximino-5-nitrofuran (**47**) [32].
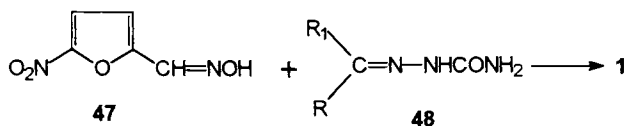
(b) **Nitrofurantoin:** This is prepared by reacting **45** with 1-aminohydantoin (**49**) which, in turn, is obtained starting from chloroacetic acid or ethyl chloroacetate [33-36].
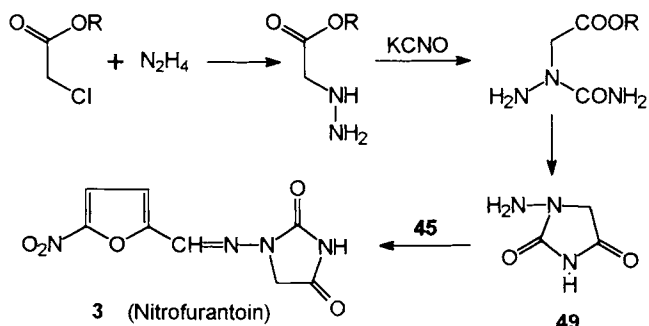
(c) Nifurtimox (4): Nifurtimox is synthesized by heating a mixture of **45** and 4-amino-3-methyltetrahydro-1,4-thiazine-1,1-dioxide (**51**) in acetic acid. The latter is obtained from 2-mercaptoethanol and propylene oxide (**50**) [32,37].
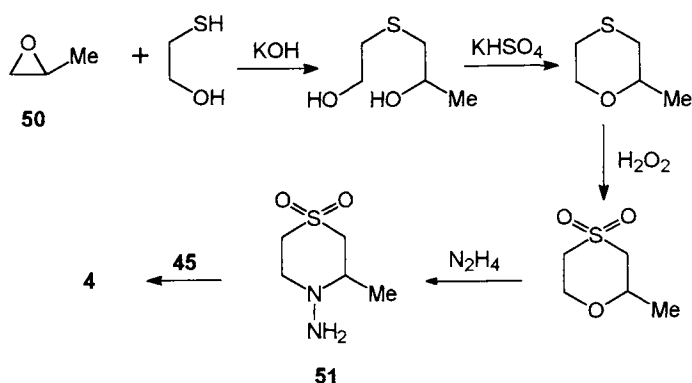
(d) Furazolidone (5): Reaction of **45** with 3-amino-2-oxazolidone (**52**) or 3-benzylideneamino-2-oxazolidone (**53**) affords furazolidone (**5**) [38-43]. A more convenient method to obtain **5** involves treatment of **47** with **53** [44].
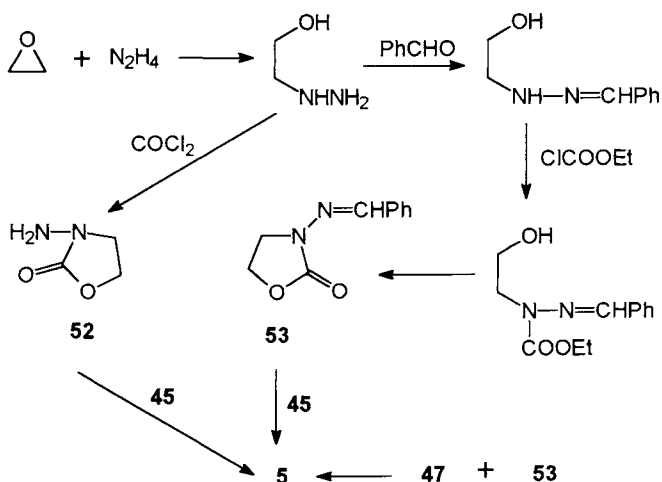
5.2 Nitroimidazoles

The key intermediate for preparing various 1-substituted-2-methyl-5-nitroimidazole drugs like metronidazole (**22**), tinidazole (**24**), ornidazole (**25**) etc. is 2-methyl-5-nitroimidazole (**55**), which is conveniently prepared by nitration of 2-methylimidazole (**54**) [45]. Nucleophilic reaction with various substrates in presence of acids

leads to the formation of 1-substituted-2-methyl-5-nitroimidazoles, which is illustrated by the following examples.

(a) Metronidazole (22): Alkylation of 2-methyl-nitromidazole (55) with ethylenechlorohydrin in the presence of a strong base such as sodium ethoxide affords metronidazole [46]. Reaction of 55 with ethylene oxide in formic acid also gives 22 [47] (Scheme 1).

(b) Tinidazole (24): Treatment of metronidazole (22) with thionyl chloride gives the corresponding chloro compound (56), which is condensed with ethanethiol in the presence of KOH to form 1-(2-ethylthio)ethyl-2-methyl-5-nitroimidazole (57) [48]. The latter may also be prepared by direct alkylation of 55 with 2-bromoethyl ethyl sulphide [45]. Oxidation of 57 with 50% H_2O_2 or sodium hypochlorite yields tinidazole [45,48] (Scheme 1).
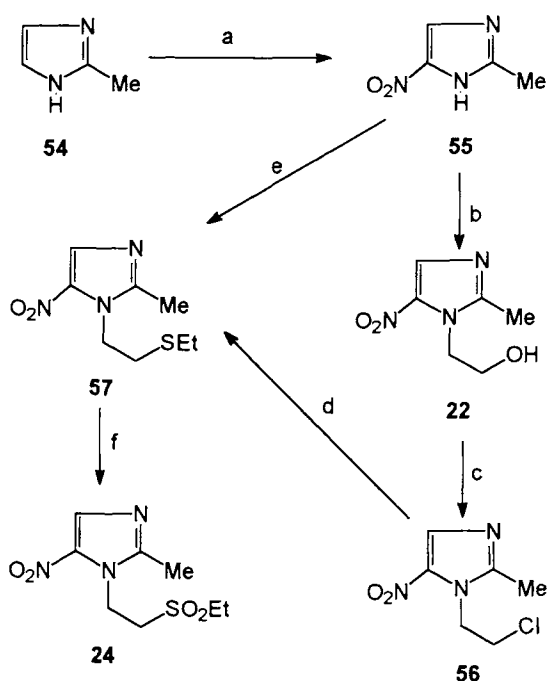
Scheme 1

Reagents : (a) HNO_3, H_2SO_4; (b) $ClCH_2CH_2OH$ or ethylene oxide; (c) $SOCl_2$; (d) EtSH, (e) $BrCH_2CH_2SEt$, (f) H_2O_2 or NaOCl.

(c) Ornidazole (25) and carnidazole (27): Reaction of 2-methyl-5-nitroimidazole (55) with epichlorohydrin in the presence of an acid gives ornidazole (25) [49]. Compound 55 is also allowed to react with N-benzoylaziridine in the presence of boron

trifluoride etherate and acetic acid to form **58a**, which is hydrolysed with 48% HBr to yield 1-(2-aminoethyl)-2-methyl-5-nitroimidazole (**56b**). Treatment of **58b** with O,S-dimethyl dithiocarbonate gives carnidazole (**27**) [50,50a].
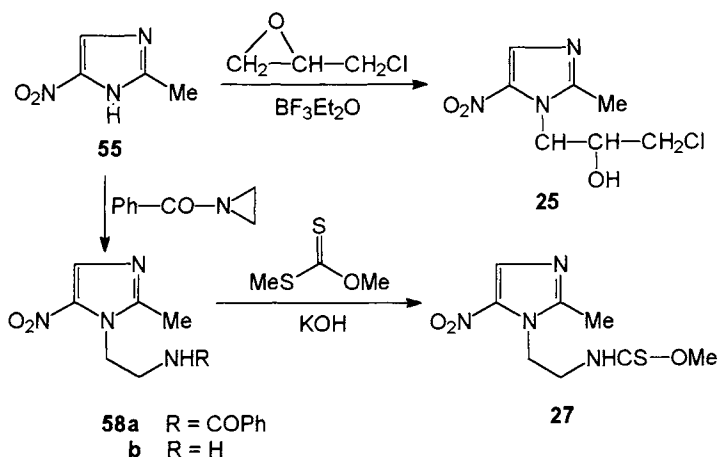
(d) Nimorazole (38): This drug is prepared by treating the sodium salt of 5-nitroimidazole (**59**) with N-(2-chloroethyl)morpholine (**60**). Alternatively 1-(2-*p*-toluenesulphonyloxyethyl)-5-nitroimidazole (**61**) is reacted with morpholine to form nimorazole (**38**) [51-52].
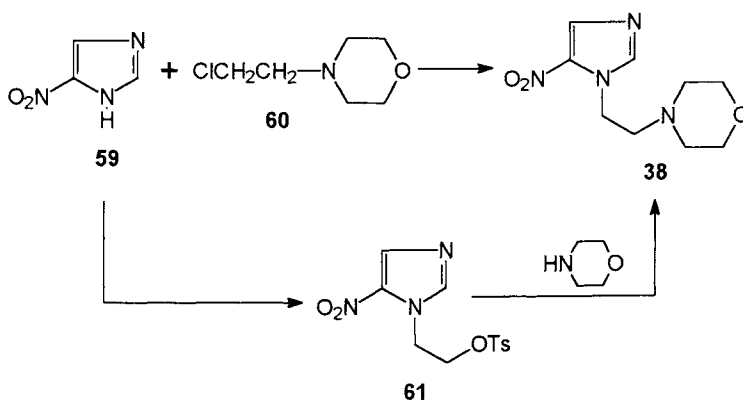
6.   BIOLOGICAL ACTIVITY

6.1   Nitroheterocycles in veterinary medicine

The nitroheterocycles find only limited use in the chemotherapy of protozoal diseases of domestic animals. A few nitrofurans such as nitrofurazone (**1**) and fura-

zolidone (5) exhibit coccidiostatic action and, therefore, may be used to control coc-cidiosis in poultry [53]. Nifursol (6) has also been used to treat blackhead disease (*Histomonas*) in turkeys [54]. Among the nitroimidazoles dimetridazole (30), iproni-dazole (31) and ronidazole (32) are used in the prevention of turkey histomoniasis [20,54].

6.2   Nitroheterocycles in human medicine

6.2.1 Nitrofurans

Nifurtimox (4) and furazolidone (5) exhibit high activity against human try-panosomiasis and giardiasis, respectively, and are used in the treatment of protozoal infections in human.

(a) **Nifurtimox (4):** Nifurtimox is the drug of choice for treating chaga's disease (*Trypanosoma cruzi*) in man. The recommended dose of the drug for adults is 8-10 mg/kg given orally after meals daily for 2-4 months [55-57]. Children between 1-10 years receive nifurtimox at a dose of 15-20 mg/kg given daily for 3 months. Older children (11-16 years) are given the drug at a dose of 12.5-15 mg/kg for 3 months [55]. The drug appears to be highly effective both against the acute and chronic stages of Chaga's disease, though there is some difference in efficacy in patients be-longing to different geographical regions [56]. Recently, nifurtimox (30 mg/kg/day for 30 days) was given to 30 patients with arsenicals resistant *Trypanosoma b. gambi-ense* sleeping sickness and appreciable activity was observed [58b].

Nifurtimox produces several side effects; children tolerate the drug better than adults. The drug may cause gastric upset (nausea, vomiting, diarrhea), weigth loss, weakness, sleep disorders, memory loss, dermatitis and polyneuritis. Occasion-ally, convulsions, fever and joint pains may also be observed. Higher doses (15-20 mg/kg) may be associated with peripheral neuritis and psychosis. Like other nitro-furans, nifurtimox is known to cause haemolytic anaemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency [55,58a].

(b) **Furazolidone (5):** It is the second line of drugs for the treatment of giardiasis in man. Since the drug is available as a suspension, it is particularly suitable for treat-ing children due to ease in administration [59-61]. The usual dose of the drug is 100 mg four times a day for adults and 1.2-2.0 mg/kg four times a day for children given for 7-10 days after which 80-89% cure rates may be achieved [62,63].

Furazolidone therapy is associated with several side effects, the most fre-quent being nausea and vomiting. Allergic reactions, headache, hypoglycemia, fever

and hypotension may also occur occasionally. The drug may also cause haemolytic anaemia in G6PD deficient subjects and neonates, discolouration of urine, a disulfiram-like reaction of alcohol and polyneuritis [55,59].

6.2.2 Nitroimidazoles

Compounds of this class constitute the front-line therapy for many protozoal diseases of man. In fact, emergence of drugs like metronidazole and tinidazole has revolutionised the treatment of intestinal protozoal infections [20,27,64-66]. The therapeutic profile of a few nitroimidazole drugs is given below.

(a) **Metronidazole (22):** This is a highly effective drug for the treatment of various disease conditions caused by *Entamoeba histolytica, Giardia lamblia* and *Balantidium coli*, both in adults and children. It is also effective against trichomoniasis and anaerobic bacterial infections [20].

Metronidazole is the drug of choice for treating all forms of amoebiasis (intestinal amoebiasis, amoebic dysentery and amoebic liver abscess) due to its marked action on both luminal and tissue forms of *E. histolytica* [20,59]. The recommended dose of the drug for amoebic dysentery and liver abscess is 750 mg given orally three times daily for 10 days. For children, the recommeded dose of metronidazole is 30-35 mg/kg/day given orally in 3 doses for 10 days [55]. The drug may also be administered parenterally for treating amoebic liver abscess and fulminating amoebic dysentery [67]. Metronidazole has been used to treat 150 patients with pleuropulmonary amoebiasis with the result that 145 patients were cured and there was no sign of recurrence [68].

For the treatment of giardiasis, metronidazole is the second line drug. The recommeded adult dose of the drug is 250 mg given orally thrice daily for 5 days. Children receive metronidazole at a dose of 5 mg/kg p.o., thrice daily for 5 days [55]. A 3 day course using 2g/day has been found to be highly effective [69]; however, a single dose therapy (1.6-2.4 g metronidazole given orally) has yielded less acceptable results [70].

Metronidazole has also been used to treat intestinal infection due to *Balantidium coli* in some patients with an oral dose of 0.5-1.25 g/day given in three divided doses for 5-10 days [71].

Trichomonas vaginitis can be successfully treated by metronidazole, which has been in clinical practice as the first-line drug for over 3 decades. The recommended adult dose of the drug is 2 g once or 250 mg given orally thrice daily for 7 days. The

pediatric dose of the drug is 15 mg/kg/daily in three doses for 7 days [55]. The 3-day regimen using 500 mg of the drug twice daily may also be used to treat vaginal trichomoniasis [72]. It is also advised to treat sexual partners separately [55]. A single dose therapy using 2 g of the drug given orally has been found to yield 84-97% cure rates [73,74].

Metronidazole has been shown to be of great value in the management of anaerobic bacterial infections [20,27,75,76]. The role of this drug in the prophylaxis and treatment of various anaerobic bacterial infections, which may develop following appendectomy, elective colonic surgery, colo-rectal surgery and hysterectomy [20,77,78]. Mebendazole is equally useful in cases of endocarditis, osteomyelitis, lung abscess, empyema, peritonitis, septicemia and pelvic infections [79].

Metronidazole is a well tolerated drug producing no serious toxic effects. However, its use may be frequently associated with nausea, headache, dry mouth and metallic taste. In addition, vomiting, diarrhea, weakness, stomatitis, dark urine, urethral burning and a disulfiram-like reaction to alcohol may be also be occasionally seen. Rarely the drug may cause ataxia, dizziness, encephalopathy and neutropenia and peripheral neuropathy [20,55,80]. Metronidazole has been found to be mutagenic in bacteria and carcinogenic in rodents. It's use should therefore be avoided in pregnant women, especially during the first trimester [20,53,81].

7. MODE OF ACTION
7.1 Nitrofurans

The nitrofuran drugs like nifurtimox and furazolidone are known for their therapeutic value in the treatment of trypanosomiasis, giardiasis and urinary tract infections in humans. The activity/toxicity of nitrofurans is due to their ability to form free radicals that react with molecular oxygen to form superoxide anion, hydrogen peroxide and hydroxyl radical. The generation of these reactive oxygen species (ROS) such as O_2^-, H_2O_2 and $^{\cdot}OH$ induced by nifurtimox in $Trypanosoma\ cruzi$ has been demonstrated by Docampo and Stoppani [82]. The reduction of the nitro group of nitrofurans is catalysed by a flavin containing nitroreductase such as NADH or NADPH. The nitroaryl anion free radical, thus formed, spontaneously reacts with O_2 to form superoxide anion radical (O_2^-) and gives back the nitrofuran [83]. Dismutation of O_2^- in presence of SOD gives rise to H_2O_2, which interacts with O_2^- to form the hydroxyl radical ($^{\cdot}OH$) (Scheme 2) [84,85].

The reduction of nitrofurans leading to the generation of ROS takes place

O_2N —furan— $CH=NR$ $\overset{\bullet}{O_2^-}$ SOD

Catalase

$H_2O_2 \xrightarrow{\;\;X\;\;} H_2O$

NADH

$\overset{\bullet}{O_2N}$ —furan— $CH=NR$ O_2^- $\overset{\bullet}{O}H + \overset{\bullet}{O}H$
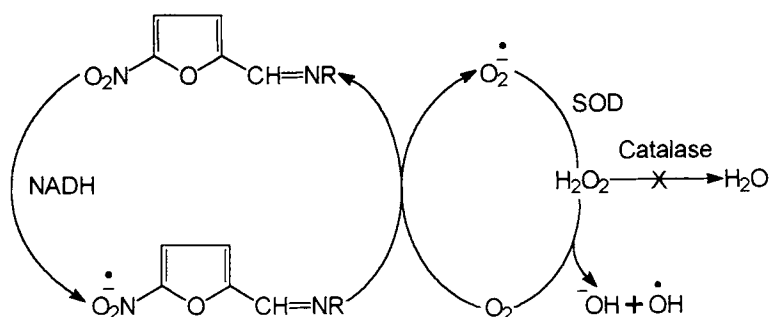
Scheme 2

more rapidly in bacterial and protozoal cells than in the mammalian cells. This may explain the selective toxicity of nitrofurans to bacteria and trypanosomes. All the generated ROS are toxic, because they react with cellular macromolecules leading to peroxidation of lipids and DNA, membrane injury, enzyme inactivation and mutagenesis [86-89]. The trypanosomes (T. cruzi) possess SOD, but lack catalase and glutathione peroxidase and are also poor in reduced glutathione contents. This makes them unfit to scavange the generated ROS resulting in death of the cells [90]. Similarly, the mutagenicity of nitrofurans increases in bacteria because they lack normal DNA excision repair mechanisms [84]. The ROS generated by nitrofuran drugs such as nifurtimox may also damage tissues as a result of lipid peroxidation and ultrastructural changes [91].

7.2 Nitroimidazoles

The mode of action of nitroimidazoles appears to be similar to that of nitrofurans. Metronidazole, an important representative of this class, is an effective agent against E. histolytica, G.lamblia and T. vaginalis. The drug itself is relatively nontoxic; however, reduction of its nitro group gives rise to various short-living intermediates, which damage the cells. The reduction of the nitro group in nitroimidazoles takes place both in the mammals and microorganisms. In mammals the flavoproteins catalysed by nitro reductase cause reduction of the nitro group, while in bacteria electron-transport proteins such as ferridoxin or flavodoxin type components catalysed by iron-sulphur complexes bring out reduction of nitroimidazoles [92]. Such reductive reactions are observed in anaerobic bacteria and certain protozoans with anaerobic metabolism. In trichomonads, reduction of metronidazole takes place in hydrogenosomes, where pyruvate is converted into acetyl-CoA. In G. lamblia and E.

histolytica the reduction may take place in the cytosol. In all the cases, the reduced nitroimidazoles interact with DNA, RNA and cellular proteins resulting in damage or death of the cells [85,93-95] (Chart 1).

O_2N ... Me ... OH

Ferredoxin ... $O_2^{\cdot -}$ ... O_2

$R =$ ... Me ... OH

$\cdot O_2N$ ... Me ... OH $\longrightarrow$ R—NO $\longrightarrow$ R—NO$\cdot$ $\longrightarrow$ R—NH—OH ROS

Interaction with intracellular molecules

Cell Damage and death
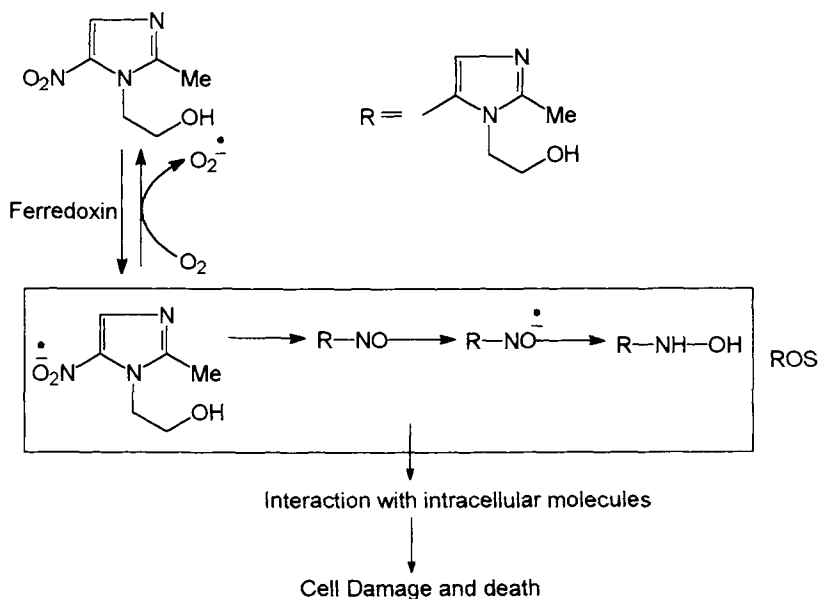
Chart 1: Metronidazole reduction and mode of action.

The interaction of reduced metronidazole species with DNA of microorganisms causes inhibition of DNA and RNA synthesis. The DNA double helix is destroyed and the strand is broken. Both the antiprotozoal activity and mutagenicity of metronidazole has been attributed to the ability of its nitro group to get reduced to form short living toxic intermediates [84,92,96,97].

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CHAPTER 18

ANTIFOLATES

1. INTRODUCTION

The folate pathway is one in which selective chemotherapeutic intervention has been very successful, and a number of drugs acting through this pathway are in current use. This is because of the fact that mammals receive FA from their diet and convert it into dihydrofolic acid (DHFA) and tetrahydrofolic acid (THFA), which give rise to folate cofactors. The bacteria and protozoans, on the other hand can not effectively utilize FA to get their requirements of DHFA and THFA. Consequently, these organisms synthesize DHFA *de novo* (for details, see Chapter 13). Further the affinity of the enzymes involved from different sources (mammalian, bacterial and protozoan) for different classes of inhibitors is quite different, which has resulted in the development of drugs with selective action.

The key enzymes involved in the biosynthesis of DHFA and THFA are dihydropteroate synthetase, dihydrofolate synthetase and dihydrofolate reductase [1-3]. Drugs that block the synthesis of dihydropteroic acid are known as dihydropteroate synthetase inhibitors (PABA antagonists) and those which control the reduction of DHFA to THFA are called dihydrofolate reductase (DHFR) inhibitors. Collectively these drugs are known as antifolates.

2. PABA ANTAGONISTS

A number of sulphonamides and sulphones interrupt the synthesis of dihydropteroic acid by inhibiting the activity of the enzyme dihydropteroate synthetase or by antagonising the action of *p*-aminobenzoic acid (PABA), a metabolite essential for its biosynthesis.

2.1 Sulphonamides

Sulphanilamide (3), the parent member of this class, was synthesized by Gelmo [4] in 1908 and later by Heinrich Hoerlein of Bayer, who was awarded the German Patent 226,239 on May 18, 1909 [5], long before its antimicrobial activity was discovered. Klarer and Mietzsch working at I.G. Farbenindustrie in Germany synthesized a variety of azo dyes as continuation of Ehrlich's interest in dyes as antimalari-

als and included those containing a sulphonamide radical, such as prontosil (1) [6]. Domagk, also at I.G. Farbenindustrie, showed in 1932 that prontosil protected mice and rabbits against streptococcal infections. Trefouel, Nitti and Bovet at the Pasteur Institute in Paris proposed that the antibacterial activity of prontosil was due to its biotransformation into sulphanilamide (3), and showed that 3 was as effective as prontosil in protecting infected mice [8]. Another major advance in the field of sulphonamides [1] came with the observation by Woods [2] of the competitive reversal of the action of sulphonamide by PABA. This led Fildes to propose his classical theory of the antimetabolite [1], and that the mode of action of this class of drugs is the consequence of their antagonism with PABA in the FA metabolism of the bacteria.

$$H_2N-C_6H_3(NH_2)-N=N-C_6H_4-SO_2NH_2 \xrightarrow{\text{Reductase}}$$

1

$$H_2N-C_6H_3(NH_2)-NH_2 \quad + \quad \overset{4}{N}H_2-C_6H_4-\overset{1}{S}O_2NH_2$$

2   **3**
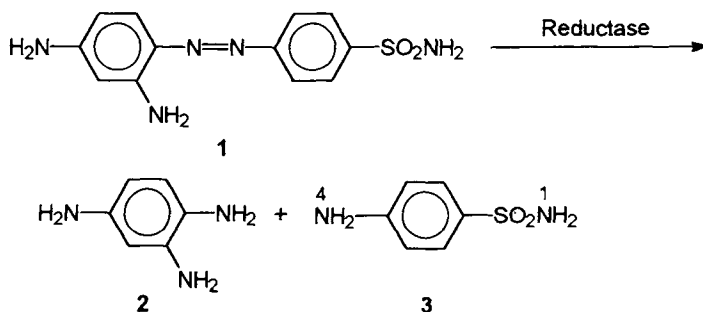
These discoveries had a great impact, not only on the development of sulphonamides as antimicrobials, but also on the development of chemotherapy in general. Recognising the potential of sulphonamides, many laboratories over the world launched research programs for the synthesis of analogs and derivatives of sulphanilamide; those substituted at N-1 had a greatly improved activity and attracted most attention. New sulphonamides were introduced in quick succession. Today, there exists a battery of sulphonamide drugs, which are extensively used in the treatment of microbial infections in humans and domestic animals [9-11]. Besides the antibacterial properties, a number of sulphonamides have also been found to possess high antiprotozoal activity. The important sulphonamides, which have been used in the treatment of *Plasmodium, Eimeria, Pneumocystis, Toxoplasma* and *Isospora* infections in animals and humans, are listed below. Most of these are characterised by a long half-life, viz. >20 hours.

The structure-activity relationships (SAR) of the substituted sulphanilamides has been the subject of many studies. The following generalisations on SAR were arrived at quite early in the development of sulphonamides and still hold: is quite well understood [13].

(a) The amino and sulphonyl radicals should be in 1,4-position for optimal activity. The only exception to this SAR is metachloridine (13) which showed better activity than *p*-aminobenzenesulphonamides against avian malaria [12,14].

(b) Replacement of the amino group by nitro, hydroxy or methyl or incorporating it in a cyclic frame-work caused either lowering or loss of activity.

(c) Replacement of the benzene ring by other ring systems, or the introduction of additional substituents on it, decreases or abolishes the activity.

(d) Substitution of the sulphonamide nitrogen (N^1) by alkyl, acyl or aryl groups usually reduces the toxicity as well as the activity. However, the presence of N^1- heterocyclic substituents was found to enhance the activity, reduce toxicity and greatly modify pharmacokinetic properties (4-12).

H_2N—⟨benzene⟩—SO_2—NHR

4 R = (Sulfadiazine)

5 R = (Sulfapyrazine)

6 R = (Sulfadimethoxine)

7 R = —OMe (Sulfamethoxypyridazine)

8 R = (Sulfadoxine)

9 R = (Sulfalene)

10 R = (Sulfaquinoxaline)

11 R = (Sulfamethoxazole)

12 R = (Sulfamethyldiazine)

H_2N—⟨benzene⟩—SO_2—NH—⟨pyrimidine⟩—Cl

13
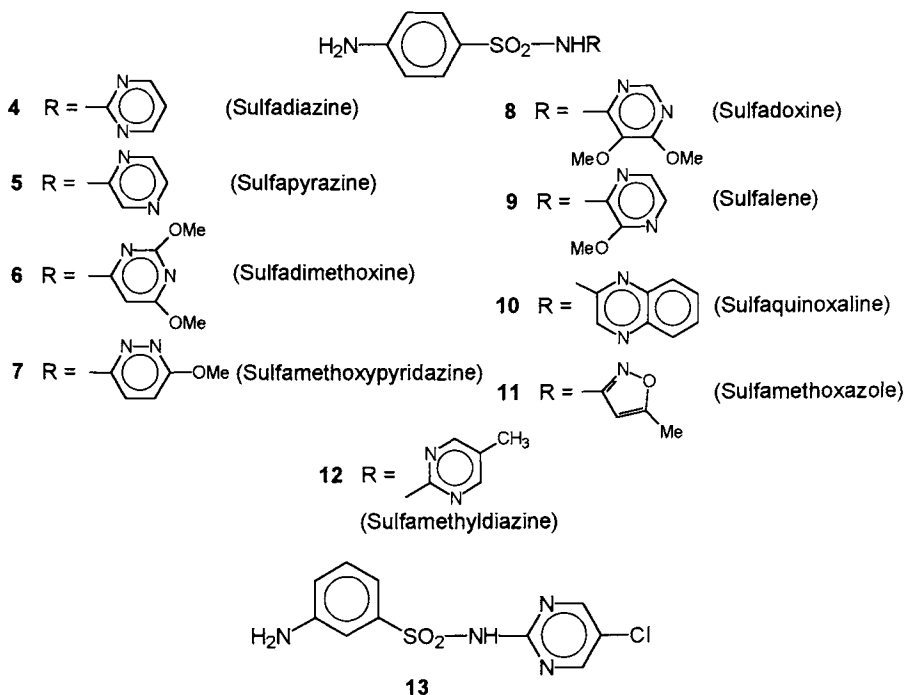
A number of the sulphonamides have been evaluated against human malaria including chloroquin-resistant *P. falciparum*. Of these sulfadoxine (8) and sulphalene (9) were found to possess better antimalarial activity than the other sulpha drugs. Although sulphonamides have been found to be of value in the chemotherapy of

falciparum malaria, their use as monotherapy is not advisable due to the easy development of drug resistance. Consequently, the sulphonamides are usually given in combination with various DHFR inhibitors (such as pyrimethamine, trimethoprim, proguanil, cycloguanil discussed below) for the prophylaxis and treatment of falciparum malaria. The choice of the individual drugs in the combination used is based on the best pharmacokinetic fit [15,16].

2.2 Sulphones

4,4'-Diaminodiphenylsulphone, dapsone or DDS (14) (and its didextrose sulphonate, promin 17) was evaluated for its antimycobacterial and antibacterial activities owing to its close structural similarity with sulphanilamides and had shown to be effective in experimental tuberculosis in 1940 [17]. Although dapsone and promin proved disappointing in the therapy of human tuberculosis, the clinical usefulness of dapsone in the treatment of human leprosy was soon established [18,19]. This observation led to the synthesis and testing of a large number of structural congeners and derivatives of DDS [20-32].

14   R = H (Dapsone)
15   R = CHO (Formyldapsone)
16   R = COMe (Acedapsone)
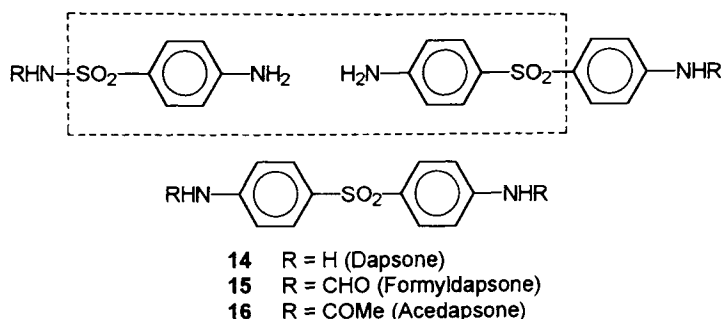
The antimalarial activity of sulphones and sulphonamides was reported by Coggeshall *et al.* as early as 1937 [33], though, somewhat more later than chloroquin.

Later as a result of the investigation of Archibold and Ross [33a] and others, it has been shown that dapsone (and its repository forms) could clear the blood from trophozoites of different plasmodium spp. The sensitivity of the different species of parasites varied; *P. falciparum* is very sensitive, while *P. vivax* is less so. Their action, as with sulphonamides, is mainly against the blood stages with marginal activity against primary (pre-erythrocytic) tissue forms and no activity against sexual and latent tissue forms. It has also been shown that dapsone potentiated the action of pyrimethamine, and a combination of the two markedly delayed the development of

resistance. These results prompted the synthesis and study of a variety of structural analogues of DDS [20-31], of which compounds **18-24** showed promising antimalarial activities.

NaSO$_3$—HC—NH—⟨benzene⟩—SO$_2$—⟨benzene⟩—NH—CH—SO$_3$Na

HOCH$_2$—(CHOH)$_4$ (CHOH)$_4$CH$_2$OH

17

R

R$_1$—⟨benzene⟩—SO$_2$—⟨benzene⟩—R$_2$
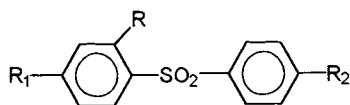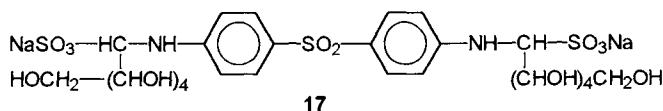
18	R = H, R$_1$ = NO$_2$, R$_2$ = NHCHO
19	R = H, R$_1$ = R$_2$ = N (COMe)$_2$
20	R = H, R$_1$ = NHEt, R$_2$ = NH$_2$
21	R = H, R$_1$ = NHBu, R$_2$ = NH$_2$
22	R = OMe, R$_1$ = NHPr, R$_2$ = NH$_2$
23	R = OMe, R$_1$ = NHBu, R$_2$ = NH$_2$
24	R = H, R$_1$ = R$_2$ = NMe$_2$

Although a number of these sulphones (**15-24**) exhibit curative and/or prophylactic activities against experimental malaria and experimental mycobacterial infections, none offered any distinct advantage over DDS. Even after over 50 years of its use in clinical practice DDS or its repository forms, DFDDS (**15**) and DADDS (**16**) are the main sulphones in clinical use. The therapeutic effects of DFDDS and DADDS have been attributed to their metabolic conversion in to DDS [34,35].

3. DHFR INHIBITORS

A number of compounds derived from biguanides, triazines and diaminopyrimidines have been shown to be potent inhibitors of dihydrofolate reductase (DHFR) and, therefore, occupy an important position in the chemotherapy of human malaria and bacterial infections.

3.1 Biguanides and triazines

During World War II there was a great demand for an effective antimalarial, which could be used to protect the Allied troops. Consequently a large variety of synthetic compounds were tested against avian malaria. Those found effectives were tested in human subjects in federal prisons and military hospitals [5]. This effort resulted in the discovery of sulpha drugs with marked antimalarial activity. The most

promising activity was observed in sulphapyrimidines, which were later used in combination with other drugs.

While the search of new antimalarials was still on, Schoenhoefer [36] proposed that the antimalarial activity of aminoquinolines was due to their ability to exist in tautomeric forms. This observation and the fact that sulfapyrimidines possess antimalarial activity was exploited by Curd and Rose working at Imperial Chemical Industries in the UK. These workers selected 4-aminopyrimidines (25) for molecular modifications on two counts: (a) such a skeleton is present in various antimalarial sulphonamides, and (b) they exist in the tautomeric structures similar to 4-aminoquinoline antimalarials [37-40].

25   **25**
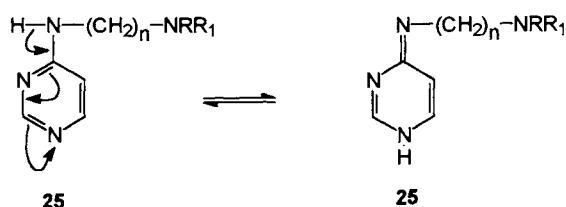
Another structural requirement considered for antimalarial activity of acridines and quinolines was the planarity of the heterocycles. Simulation of all these facts led Curd and Rose to synthesize 2-(4-chlorophenylamino)pyrimidines of the type 26 and 27 [37,39,40]. Since both these structure contained a guanidino function, though in a cyclic structure, the characteristic tautomeric structures were possible. In a further effort to modify the structure of the pyrimidines, the synthesis of the biguanide (28) was carried out, which may be considered to have been obtained by scissoring of the pyrimidine ring of 26 [41]. Though this compound (28) was inactive, replacement of the N,N-diethylaminoethyl chain by simple alkyl groups gave highly potent antimalarial biguanides. The most effective compounds were found to be proguanil (29) and chloroproguanil (30) [42-46]. Detailed biological studies indicated that these biguanides (eg. 29 and 30) are *per se* inactive *in vitro*. However, when administered in rabbits or humans, they undergo metabolic cyclisation in the liver to form 1-aryl-4,6-diamino-2,2-dimethyl-1,2-dihydro-*s*-triazine (31a,b) as the active metabolite [47-50]. The active metabolite of proguanil is cycloguanil (31b) (Fig. 1). Cycloguanil is a commercially available drug, which has been used extensively alone or in combination with other antimalarials for the treatment of human malaria [51].
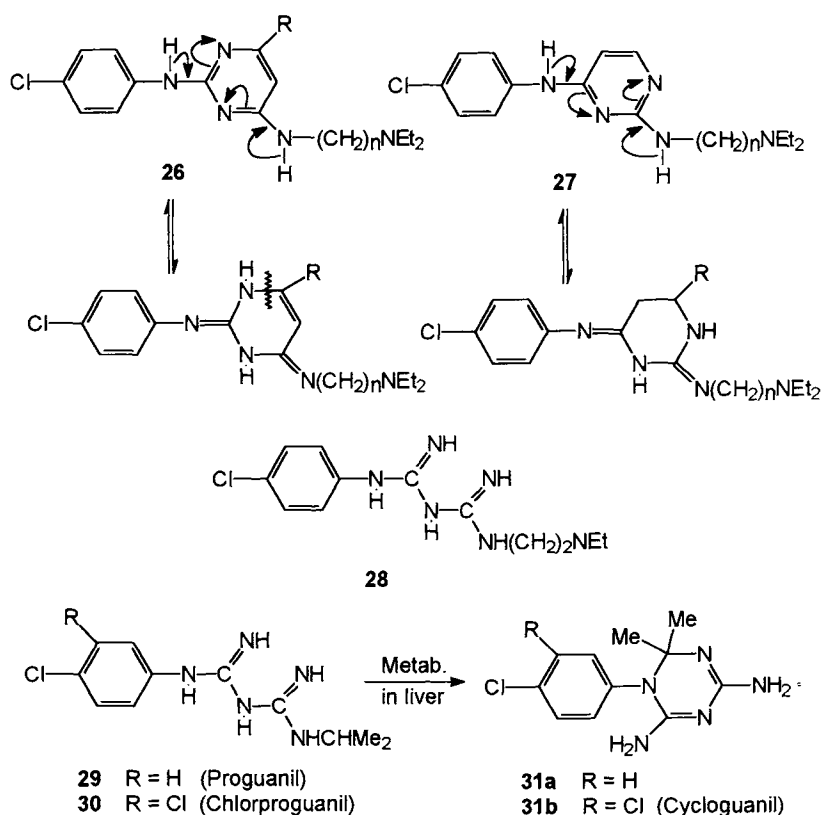
Fig. 1: Molecular changes leading to the discovery of cycloguanil.

The history of another class of DHFR inhibitors, the 5-aryldiaminopyrimidines, is equally interesting. A team of scientists working at Wellcome Research laboratories in the United States was studying the effect of 2,4-diaminopyrimidines on the growth of *Lactobacillus casei*. They found that 2,4-diamino-5-(4-chlorophenoxy)pyrimidine (**32**) could inhibit the growth of *Lactobacillus casei in vitro* [52]. The antimicrobial activity of **32** was attributed to its ability to antagonize FA synthesis. Since FA synthesis is also inhibited by proguanil and cycloguanil, it was considered rational to explore the antimalarial potential of these 2,4-diaminopyrimidines, which also bear a close structural resemblance with the antimalarial guanides **29-31**. Consequently a large variety of structural congeners of **32** were synthesized and screened

for antimalarial activity; the optimal activity in the 2,4-diaminopyrimidines was observed when the 5-phenoxy group of **32** was replaced by phenyl or benzyl groups [53-57]. The most effective compounds thus discovered are pyrimethamine (**33**) and trimethoprim (**34**) [54,58].

29   **32**

33   **34**
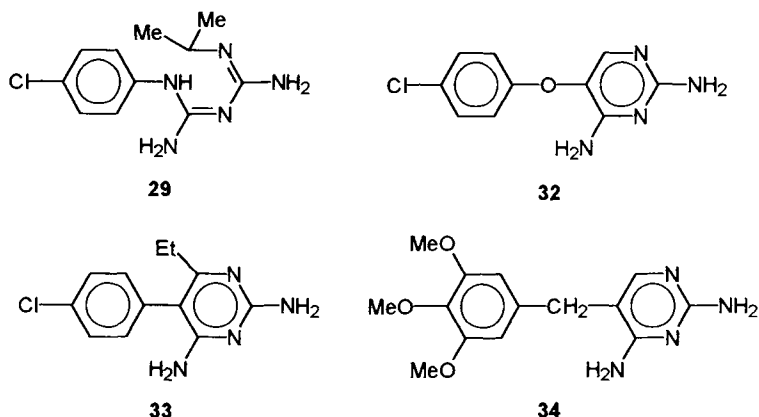
A most interesting and useful development concerning DHR inhibitors was the selectivity of inhibition observed between different classes of compounds against dihydrofolate reductases from mammals, protozoa and bacteria, which was found to be due to marked differences in binding affinity to the enzyme; methotrexate binds very tightly to all reductases tested and is lethal to any cell it can enter, while trimethoprim and pyrimethamine have selectively strong affinity for bacterial and plasmodial reductases, respectively. This helped to rationalise the clinical use of DHFR inhibitors alone or in combination with sulphonamides and sulphones; while trimethoprim is used mainly for bacterial infections, pyrimethamine is used for protozoal infections [58a].

The use of antifolates in the treatment of coccidiosis in domestic animals has also been established. It has been found that compounds derived from 2,4-diamino-pyrimidines and 2,2-dialkyl-1-aryl-4,6-diamino-1,2-dihydro-s-triazines exhibit promising anticoccidial activity. The noteworthy compounds of this class were found to be pyrimethamine (**33**), diaverdine (**35**) and ormetoprim (**36**) [59]. The metabolites **31a,b** of proguanil and chlorproguanil also exhibit marked activity against coccidiosis [60].

Clociguanil (WR-38839) (**37**) and WR-99210 (**38**) are DHFR inhibitors possessing a molecular frame-work closely related to cycloguanil. Both these compounds
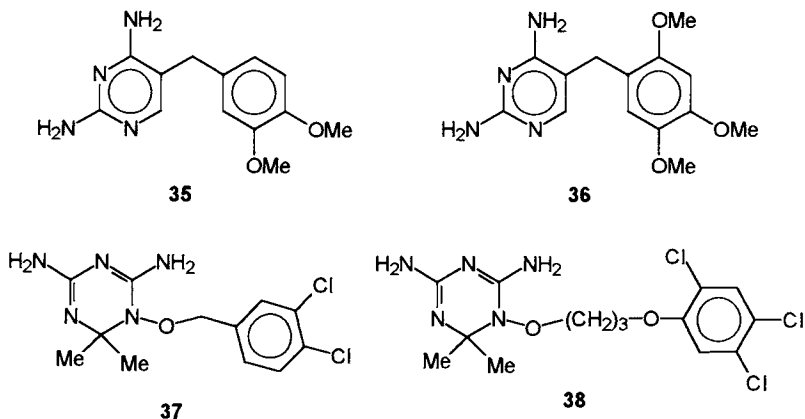
have been found to be effective against pyrimethamine-sensitive or resistant strains of *P. berghei*. Clociguanil and WR-99210 exhibited curative effects against the Palo Alto strain of *P. falciparum* in owl monkeys at an oral dose of 2.5 and 10 mg/kg, respectively, given for 7 days [61]. When tested in man, alone or in combination with sulpha drugs [62,63] clociguanil gave promising results. Thus a mixture of clociguanil (0.28 g) and sulphadiazine (2 g) prevented parasitaemia for 60 days against chloroquine resistance *P. falciparum*. Similarly a combination of clociguanil (300 mg) and sulphadimethoxine (3 mg/kg) cleared parasitaemia in children suffering from acute falciparum malaria [15].

4.   SYNTHESIS

4.1   Sulphonamides

The chemistry of sulphonamides has been dealt exhaustively by Lednicer and Mitscher [64] and by Korger [65]. Generally the sulpha drugs are prepared by two alternative routes (Scheme 1). The first method uses acetanilide (39) as the starting material, which is chlorosulphonated to get 4-acetamidobenzenesulphonyl chloride (40). Reaction of the latter with the appropriate arylamine affords the intermediate 41, which is hydrolysed with an acid or base to form the sulphonamides. Alternatively 4-nitrobenzenesulphonyl chloride (43) may be used to synthesize the sulpha drugs (as shown in Scheme 1). One can also use benzenesulphenyl chloride (45) as the starting material to synthesize the desired sulphonamides [65].

Some illustrative examples of the synthesis of arylamines needed to prepare different sulphonamides are given below:
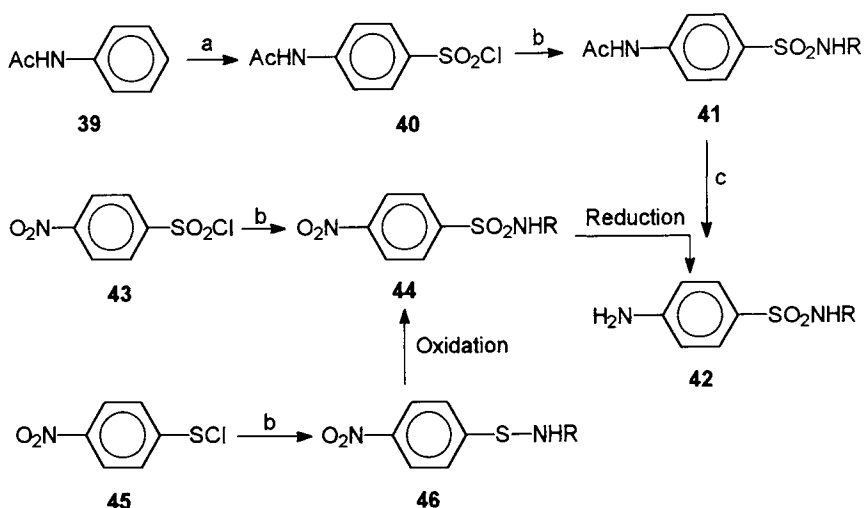
Scheme 1
Reagents: (a) ClSO₃H, (b) RNH₂, (c) acid/base.

(a) Sulphadiazine (4): This is prepared by treating 4-acetamidobenzenesulphonyl chloride (40) with 2-aminopyrimidine (51), followed by hydrolysis. The latter is obtained as shown in scheme 2 [64,66].
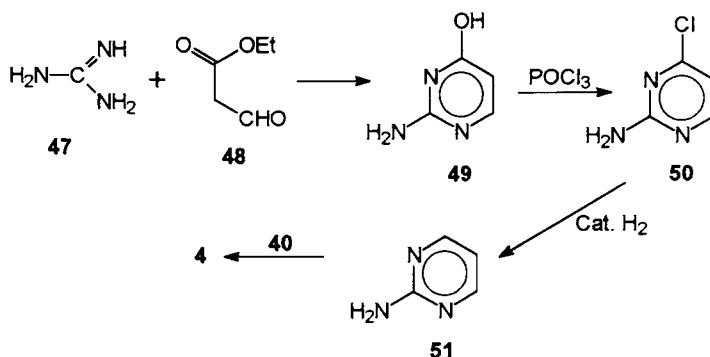
Scheme 2

(b) Sulphadimethoxine (6): Barbituric acid (52) is converted into the trichloro compound 53 by treatment with POCl₃. Reaction of 53 with two equivalents of sodium methoxide gives 54, which is converted in to 6 by the usual way [64] (Scheme 3).
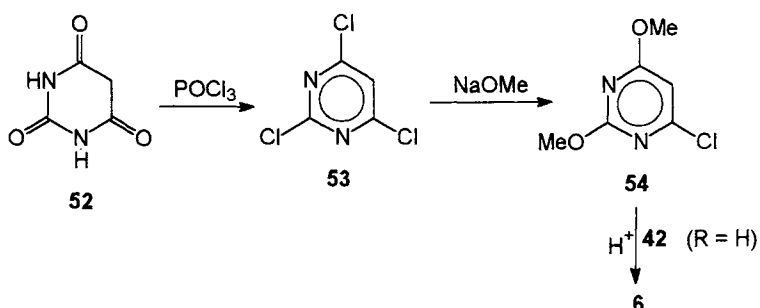
Scheme 3

(c) **Sulphamethoxypyridazine (7):** Halogenation of the adduct, obtained from hydrazine and maleic anhydride, gives 3,6-dichloropyridazine (55), which can be converted into 7 as shown below in scheme 4.
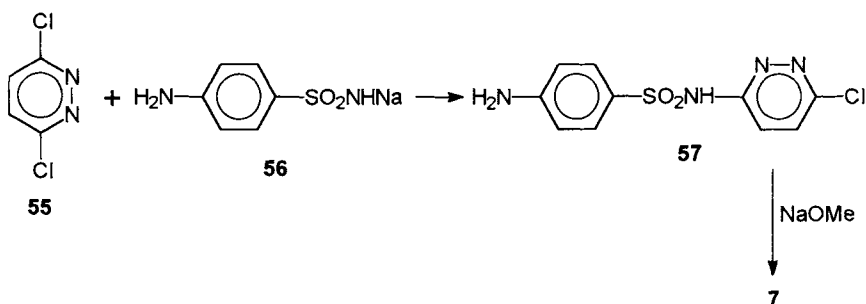
Scheme 4

(d) **Sulphadoxine (8):** The synthetic methods to obtain this drug have been reviewed [67] being outlined in scheme 5.
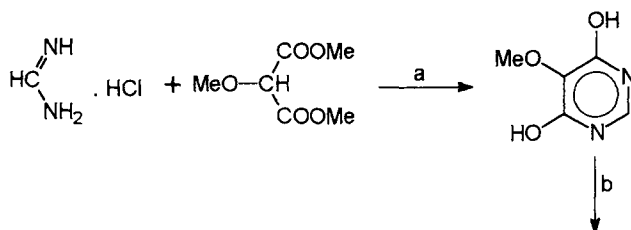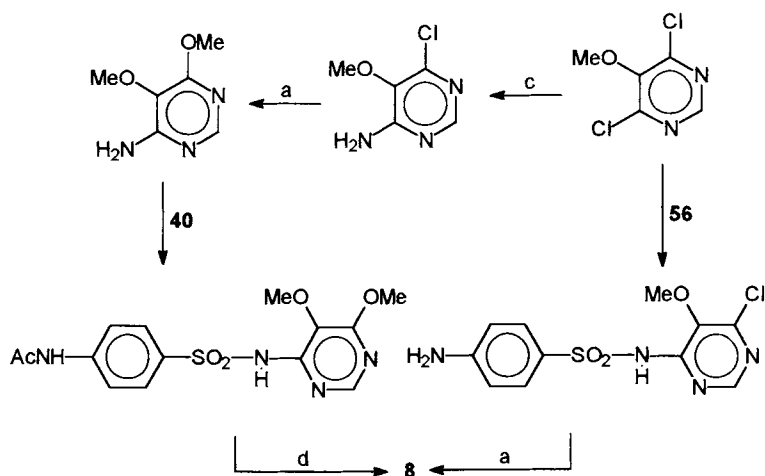
Scheme 5
Reagents: (a) NaOMe, (b) POCl₃, PhNMe₂, (c) liq. NH₃, (d) 2N NaOH.

(e) Sulphamethoxazole (11): It can be conveniently prepared starting from 3-amino-5-methylisoxazole as described in scheme 6 [68].
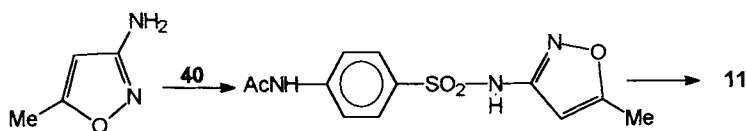
Scheme 6

4.2 Pyrimethamine

A number of methods have been described for the synthesis of pyrimethamine. Essentially all of them build the pyrimidine ring from open chain precursors and use different reactions for the cyclisation [69]. The method used by Russel and Hitchings [70], discovers of pyrimethamine, is described below (Scheme 7).
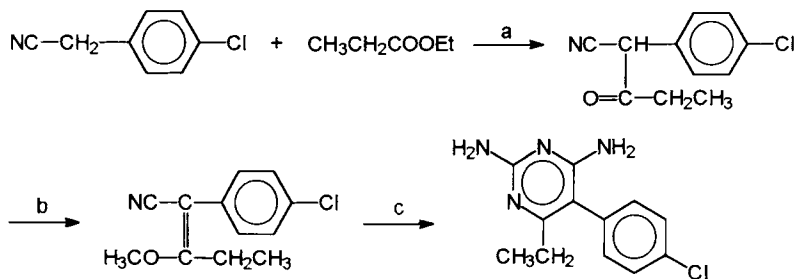
33 (Pyrimethamine)

Scheme 7
Reagents: (a) NaOCH₃, (b) via hemiketal prepared by treatment with (CH₃O)₂SO₂, (c) HN=C(NH₂)₂.

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CHAPTER 19

BISAMIDINES

1.   INTRODUCTION

The introduction of amidines in the chemotherapy of protozoal diseases was based on the study of the biochemistry of protozoans. In 1925, Schern [1] observed that the motility of trypanosomes depends on extracellular supply of glucose. Later, Poindexter found that the multiplication of trypanosomes may be reduced by injecting insulin in the animal hosts [2]. This led von Joncso' and von Jancs'o [3] to evaluate the blood sugar lowering (hypoglycemic) agent, 1,10-bisguanidinodecane (1, synthalin) against *T. brucei* in experimented animals; in this *in vivo* test the drug was found to be active. Following this, a major break through regarding the activity of synthalin was achieved by Lourie and Yorke [4], who demonstrated that the drug was highly trypanosomicidal *in vitro* even in the presence of glucose. This observation clearly indicated the fact that the activity of synthalin was not due to its hypoglycemic properties, instead it had direct action on trypanosomes.

$$HN{=}\overset{\underset{\displaystyle NH_2}{|}}{C}{-}NH{-}(CH_2)_{10}{-}NH{-}\overset{\underset{\displaystyle NH_2}{|}}{C}{=}NH$$
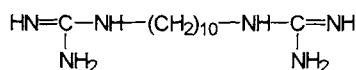
1 (Synthalin)

The discovery of the trypanosomicidal efficacy of synthalin triggered the synthesis and biological evaluation of various diamidines (2), dithioureas (3) and diguanidines (4), whose two functional groups were linked through a long alkyl chain [5,6]. These compounds exhibited strong *in vitro* activity, but when tested against *T. rhodesiense* in mice, only moderate activity was observed in 2 and 4 (n=10-14) and 3 (n=6).

$$HN{=}\overset{\underset{\displaystyle NH_2}{|}}{C}{-}(CH_2)_{n}{-}\overset{\underset{\displaystyle NH_2}{|}}{C}{=}NH$$

2

$$S{=}\overset{\underset{\displaystyle NH_2}{|}}{C}{-}NH{-}(CH_2)_{n}{-}NH{-}\overset{\underset{\displaystyle NH_2}{|}}{C}{=}S \qquad HN{=}\overset{\underset{\displaystyle NH_2}{|}}{C}{-}NH{-}(CH_2)_{n}{-}NH{-}\overset{\underset{\displaystyle NH_2}{|}}{C}{=}NH$$
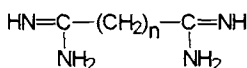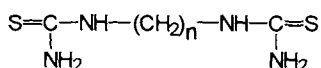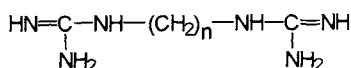
3   4

A new horizon in the design of therapeutically useful diamidines was discovered when the alkyl chain in **2** was replaced by $C_6H_5-X-C_6H_5$. The aromatic diamidines (**5a-f**), thus obtained, were found to exhibit varying degrees of antiprotozoal activities [7-9]. The first active member of this series was **5a**, which was active when tested against *T. equiperdum* and *T. rhodesiense* in mice [10]. Later other effective aromatic diamidines were discovered, of which the most notable are pentamidine (**5b**), propamidine (**5c**), stilbamidine (**5d**), dimethyl stilbamidine (**5e**) and phenamidine (**5f**). One compound of this series, pentamidine, shows high trypanosomicidal and leishmanicidal activities and, therefore, finds use in clinical practice, especially as diisethionate salt (Pentam 300) [11,12]. Pentamidine has been used in the mass therapy of Gambian sleeping sickness [13] and is also the preferred drug for the treatment of visceral leishmaniasis in humans [11,12,14,15].

$$HN=C-\bigcirc-X-\bigcirc-C=NH$$
$$NH_2 \qquad\qquad NH_2$$
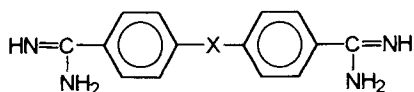
5

a	X = -CH$_2$-	
b	X = -O(CH$_2$)$_5$O-	(Pentamidine)
c	X = -O(CH$_2$)$_3$O-	(Propamidine)
d	X = -CH=CH-	(Stilbamidine)
e	X = -C(Me) = C (Me)-	(Dimethylstilbamidine)
f	X = -O-	(Phenamidine)

Another compound stilbamidine (**5d**) has been found to be highly effective against *Leishmania donovani* in experimental animals and humans [11,12,14]. Although this drug proved to be quite useful in the treatment of visceral leishmaniasis [11], its use in clinical medicine was limited due to its high toxicity. The toxicity of this drug was attributed to its facile photochemical dimerisation to form *cis, trans, cis, trans*-1,2,3,4-tetra(4-guanylphenyl)cyclobutane (**6**) [16]. This limitation was overcome by synthesizing 2-hydroxystilbamidine (**7**), which was found to be stable to light and was also less toxic than stilbamidine [16]. 2-Hydroxystilbamidine (**7**) has been used to treat visceral and American cutaneous leishmaniasis in humans with good success [11].

Further work on the design of better antiprotozoals derived from aromatic diamidines has culminated in the synthesis of a series of novel compounds, of which diminazene (**8**) and HOE-668 (**9**) exhibited promising antiprotozoal activity. [17-19]. Both these compounds were found to be highly effective against *L. donovani* in hamsters. Diminazene (berenil) has been widely used to treat early stages of African try-

5d

6 R =—C=NH
           |
           NH₂

7 (Hydroxystibamidine)

8 (Berenil)

9 (HOE-668)
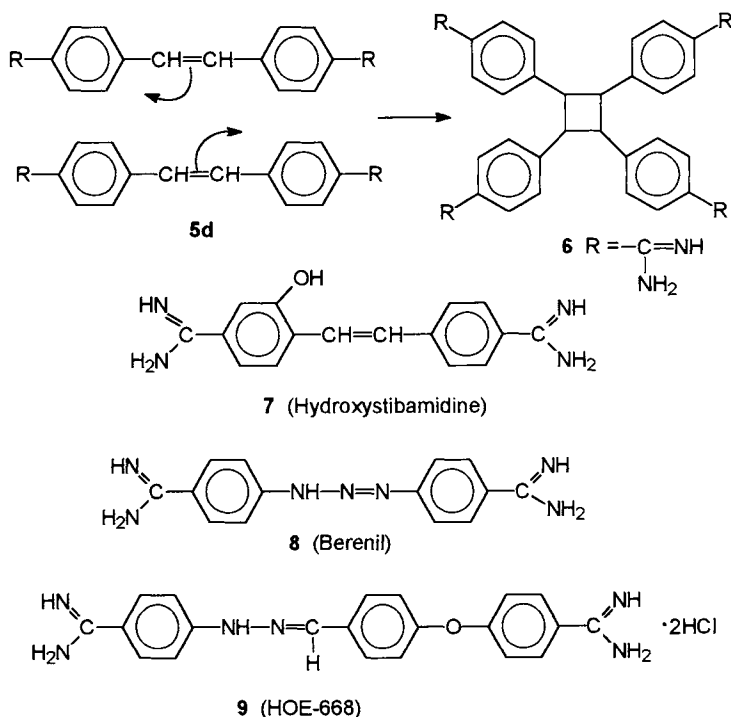
panosomiasis [11]. The drug has also been found effective against *T. vivax* and *T. congolense* infections in cattle [20,21].

The antiprotozoal activity of aryldiamidines is believed to be due to their ability to bind to the parasite DNA [22,23]. The binding of diamidines with DNA is mediated through the electrostatic interactions between negative phosphate groups on the DNA helix and the positive centre of protonated amidines [24]. The molecular models of diarylamidines indicate that such compounds may be broadly divided in two classes; one class in which two amidine groups are separated by 12 Å and the other where the interamidine distance is about 20 Å. These values correspond well with the distances between major and minor groups of DNA [25]. On the basis of these findings, the synthesis of a large number of aromatic diamidines (**10-13**) was carried out displaying the characteristic distance of 12-20 Å between the two amidine functions [26-28]. All these compounds have been found to cause 71-93% inhibition of amastigotes in the macrophages in the spleen of hamsters infected with *L. donovani* at an intraperitoneal dose of 2.5 mg/kg [26-28].
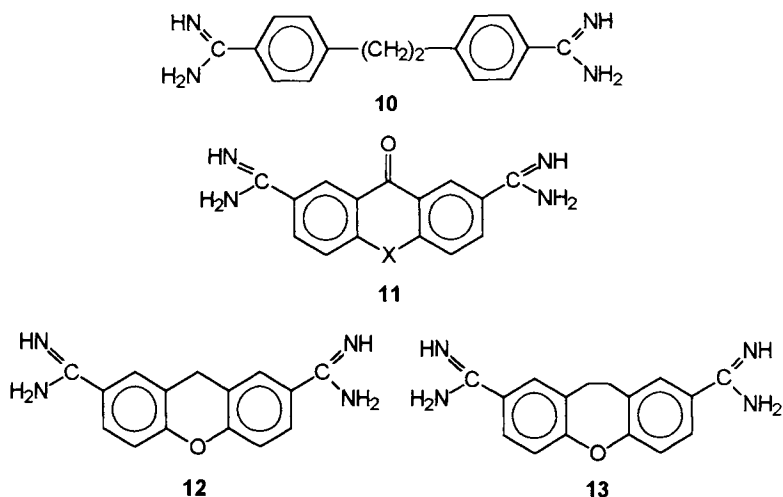
It is also possible to link the two amidino functions through a heterocyclic ring. Accordingly a variety of heterocycles carrying 4-guanylphenyl groups have been synthesized [11]. Dann and coworkers [29-32] have carried out a detailed SAR study on diamidines derived from some benzoheterocycles. Among the several heterocyclic diamidines prepared, 2-(4-guanylphenyl)-6-guanylbenzothiophene (14a) and the corresponding indene analogue (14b) exhibited better activity than pentamidine (5b) or berenil (8) against *T.b. gambiense*. Similarly the indole and benzofuran derivatives (14c and 15) were found to possess high trypanosomicidal activity against *T.b. congoliense* and *T.b. rhodesience* respectively [32-34].
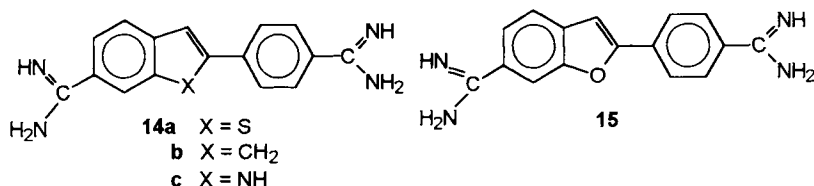
A series of aromatic diamidines wherein the two 4-guanylphenyl groups are attached at 2- and 5-positions of furan (16), thiophene (17), pyrrole (18), oxazole (19), thiazole (20) and thiadiazole (21) have been synthesized as possible antiparasitic agents [23,35-38]. The most effective compound was found to be 2,5-bis(4-guanylphenyl)-1,3-oxazole (19) and 2,5-bis(4-guanylphenyl)-1,3,4-thiadiazole (21), which exhibited high trypanosomicidal activity. The cure rates of these two compounds against *T.b. rhodesiense* in mice was comparable to pentamidine, stilbamidine and hydroxystilbamidine at a dose of 3 mg/kg [38].
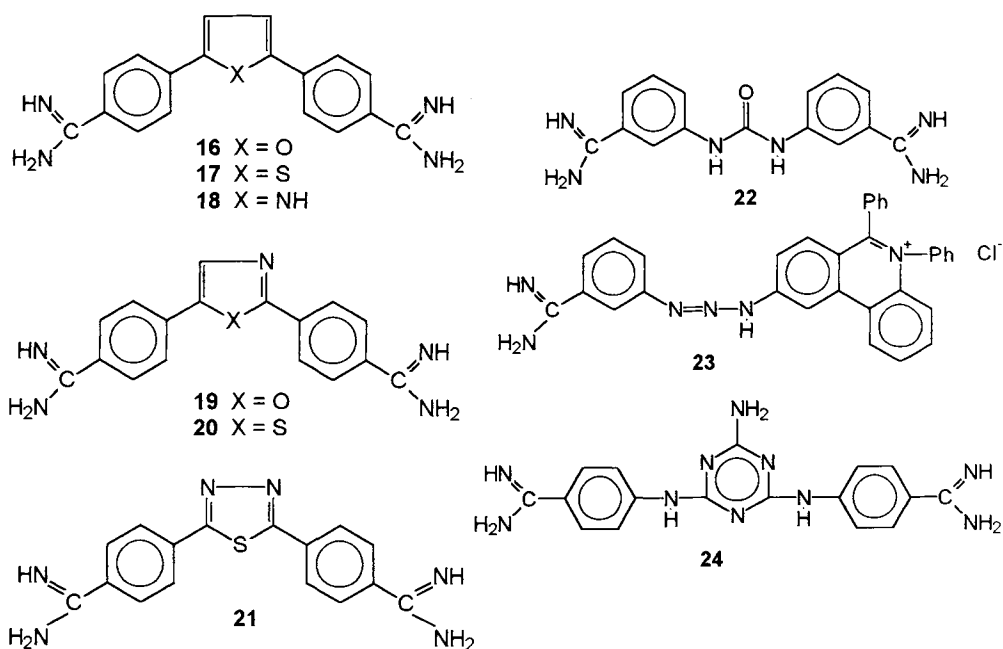
The other phenylamidines prepared to generate effective antiprotozoals include amicarbalide (**22**) [39], isometamidium chloride (**23**) [40] and the triazino compound (**24**) [41]. Of these, amicarbalide (**22**) and isometamidium chloride (**23**) are effective veterinary drugs for treating babesiosis and trypanosomiasis in domestic animals [42], while **24** exhibit trypanosomicidal activity against *T. congoliense* [10].

2. SYNTHESIS

2.1 Pentamidine

Pentamidine has been synthesized by Qshley et al. [43]. A convenient route to obtain pentamidine isethionate from 4-hydroxybenzonitrile is outlined in scheme 1.
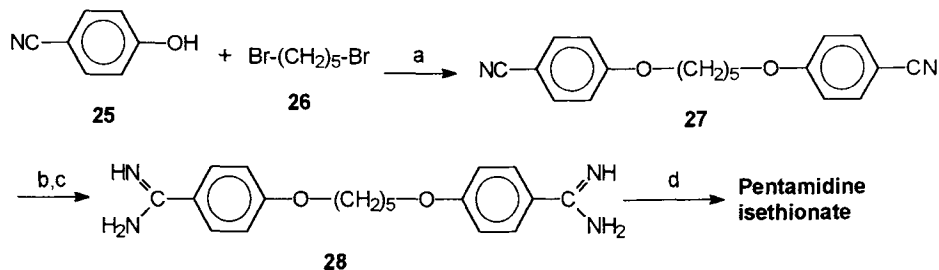
Scheme 1

Reagents: (a) K_2CO_3, (b) HCl, EtOH, (c) NH_3, EtOH, (d) Isethionic acid.

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CHAPTER 20

HALOACETAMIDES

1. INTRODUCTION

In 1954, Dennis and Berberian [1] reported that certain 2-phenylthiazolidin-4-one-1,1-dioxides (1) exhibit significant amoebicidal activity against *E. criceti* in hamsters. The most effective compound of this class was 2-(3,4-dichlorophenyl)thiazolidin-4-one-1,1-dioxide (**1**, R=H, R_1=3-Cl, R_2=4-Cl) [1,2]. This observation led Surrrey and coworkers [3-8] to synthesize a wide variety of benzylacetamides of type **2**, which may be regarded to have been obtained by removing the SO_2 group from **1**.
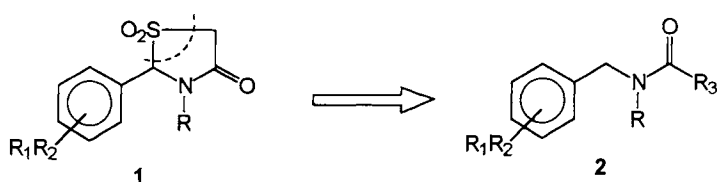
Although Surrey and his colleagues carried out broad range of substitutional variations in **2**, the antiamoebic activity was found primarily in those benzylacetamides in which R is alkyl, hydroxyalkyl, acyloxyalkyl, cyanoalkyl, carbamoylalkyl or alkoxyalkyl, R_1, R_2 are 2,4-dichloro, 4-butoxy or 4-isopropyl residues, and R_3 is dichloromethyl. The most promising compound thus obtained was chlorbetamide (**3**) possessing excellent activity against experimental amoebiasis [1,9]. The molecule of chlorbetamide has a close structural resemblance with the antibiotic, chloramphenicol (**4**), which *per se* is a derivative of dichloroacetamide.

Both chlorbetamide (**3**) and chloramphenicol (**4**) possess *in vitro* activity against *E. histolytica*. This fact when taken in conjunction with the high order of amoebicidal activity of chlorbetamide in experimental animals and humans [9-11]
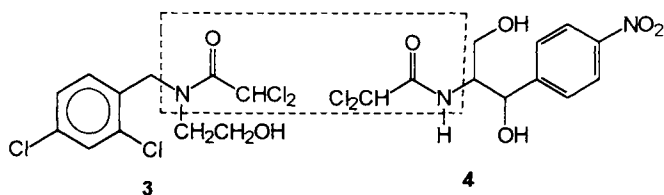
unequivocally established the association of the N-benzyldichloroacetamide residue with antiamoebic activity. Consequently molecules incorporating an N-benzyldichloroacetamide moiety have been subjected to detailed SAR studies and the general conclusions which have emerged, are presented below [12].

2. SAR IN DICHLOROACETAMIDES

Substitution of the $CHCl_2$ group by CH_2Cl or CH_3 in 3 gave N-benzyl compounds (5), which exhibit inferior activity compared to the corresponding dichloroacetamide derivatives [12]. Though replacement of the two chlorine atoms at 3- and 4-positions of phenyl ring by a 4-methylsulphonyl group in chlorbetamide yielded 6, which showed four fold higher activity against *E. histolytica* in rats, it was found to be of no value in clinical trials carried out in humans [13,14].

5 R = CH_2Cl, Me   **6**
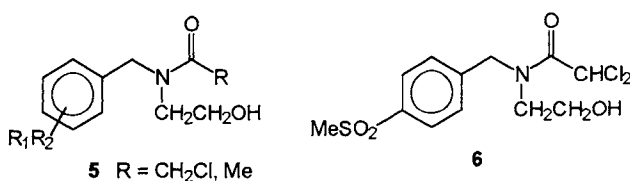
A number of N-(4-substituted benzyl)acetamides of the general formula 7 were synthesized, which showed promising antiamoebic activity. The most effective compounds of this series were 8 and 9 of which chlorphenoxamide, clefamide (9), was found to be useful in treating intestinal amoebiasis in human [15-18]. Replacement of the OH group in clefamide with OEt gives etofamide (10), which exhibited a high order of amoebicidal efficacy [19].

7

8 X = S, R = H
9 X = O, R = H (Clefamide)
10 X = O, R = Et (Etofamide)
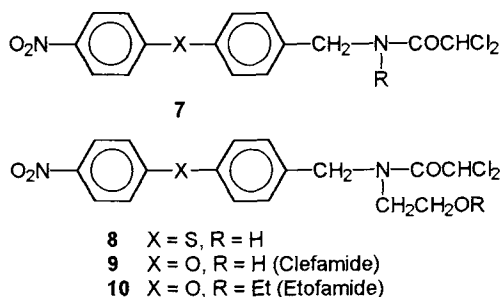
Further structural modifications of chlorbetamide and chlorphenoxamide molecules resulted in a series of mono- and disubstituted dichloroacetamides [20-27],

of which compounds 11 and 12 emerged as most effective antiamoebic agents. In general, the bisacetamides (12) exhibited good antiamoebic activity; they also showed antispermatogenic properties [28,29]. Attempts to seggregate these two biological properties led to the decamethylenediamine analog 12 (n=10) with selective antiamoebic activity [12].

11   **12**
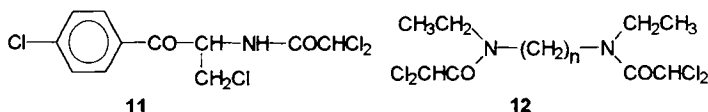
Although the tetrasubstituted diaminoalkanes (12) did not provide clinically acceptable antiamoebic drugs, their activity served as a useful lead. Thus, replacement of the polymethylene chain of 12 by an aromatic ring gave 1,4-di-(disubstituted aminomethyl)benzenes. The best compound was found to be teclozan or falmonox (13) [23], which emerged as an effective drug for treating acute and chronic forms of intestinal amoebiasis in man [30-32]. Like chlorphenoxamide, this drug has also been used as a chemoprophylactic agent against amoebiasis [33].

13
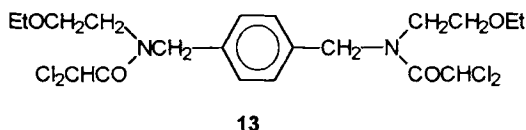
Bristow, Woolfe and their colleagues [34,35] reported a new series of analogs of chlorbutamide by replacing the hydroxyethyl group by an alkyl and benzyl by phenyl moieties. The most effective member of this class was N-(4-hydroxyphenyl)-N-methyl dichloroacetamide called diloxanide or entamide (14). Although diloxanide (14) was found to be highly effective against intestinal amoebiasis, it could not be used in cases with acute amoebic dysentery and systemic amoebiasis [18]. To circumvent this limitation, some soluble derivatives and esters have been prepared [12,36]. Of these diloxanide furoate (15) was found to be 10 times more active *in vitro* and 2-4 times more active *in vivo* than diloxanide. Further work established diloxanide furoate as amoebicide superior to diloxanide and is one of the commonly used drugs for the treatment of acute amoebiasis [18, 37-39].

14   **15**
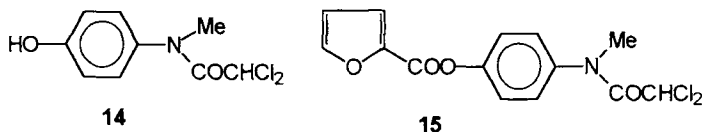
The structure of diloxanide furoate has been further modified by incorporating its nitrogen in a cyclic structure resulting in the active compound quinfamide (16) [19]. Elslager and coworkers [40] have replaced the phenyl ring of chlorbetamide by various heterocycles; the most promising compound in this series was N-(2-hydroxyethyl)-N-(4-pyridylmethyl)dichloroacetamide (17). These compounds are not in clinical use.

16　　　　**17**
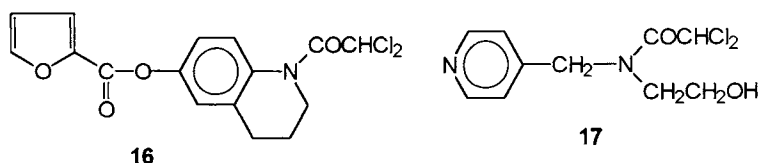
3. SYNTHESIS
3.1 Diloxanide (14)

Bristow and Oxlay [41] have developed an easy method to synthesize 2,2-dichloro-N-(4-hydroxyphenyl)-N-methylacetamide (14) (Scheme 1).

19

18　　　　**14**
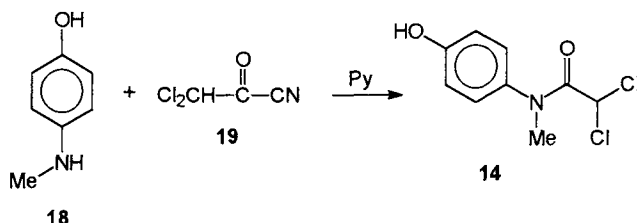
Scheme 1

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CHAPTER 21

MISCELLANEOUS ANTIPROTOZOALS

1. INTRODUCTION

Most of the important antiprotozoal drugs in clinical or veterinary use today have been described in the chapters 15-20 according to the class to which they belong. In addition there are a number of compounds/drugs, which may not be in widespread clinical use due to one reason or the other, but have sufficient activity to provide useful leads for further exploration. These are discussed in this chapter.

2. UREAS AND THIOUREAS

Afridol violet (1) is probably the earliest urea derivative which was developed as a trypanosomicidal agent [1] through systematic exploitation of Ehrlich's observation that certain dyes could stain malaria parasites in histological sections. Although afridol violet could not be introduced in the chemotherapy of human trypanosomiasis, it provided a definite lead warranting search for better urea derivatives. Consequently scientists at Bayer laboratories in Germany synthesized 2, a colourless analogue of trypan red with marked antitrypanosomal properties [2]. Further molecular modifications in 2 led to the discovery of suramin (3) as an effective drug for treating early stage of sleeping sickness due to *T. gambiense* and *T. rhodesiense* in man [3,4]. Later this drug was also found to be valuable in the treatment of onchocerciasis (river blindness) caused by *Onchocerca volvulus* [5].
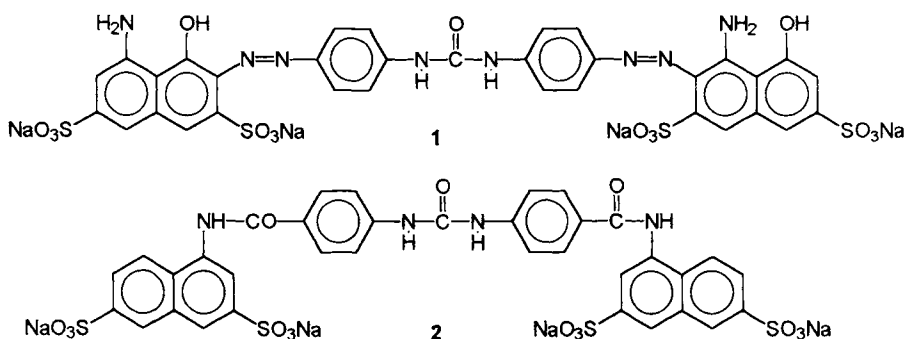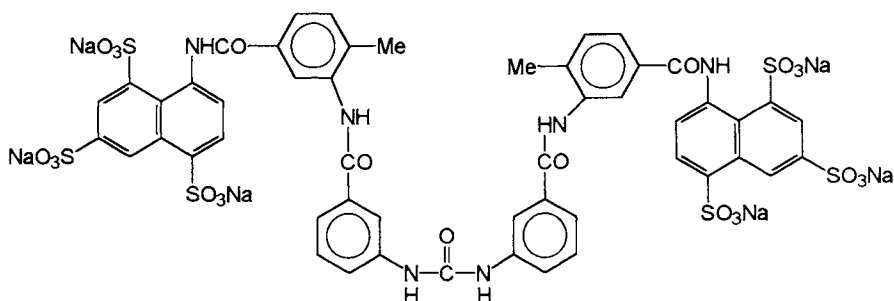
3 (Suramin)

The entire molecular disposition of suramin does not appear to be essential for activity. It has been reported that the N,N'-diphenylurea derivatives retain the biological activity to a large extent [2,6]. Consequently a number of N,N'-diarylureas were evaluated for their antiprotozoal activity and as a result amicarbanilide (**4**), imidocarb (**5**), nicarbazine [a complex formed from N,N'-bis(4-nitrophenyl)urea (**6**) and 4,6-dimethyl-2-hydroxyprimidine (**7**)] and quinuronium sulphate (**8**) were found to exhibit high activity against babesiasis in domestic animals [2,7].
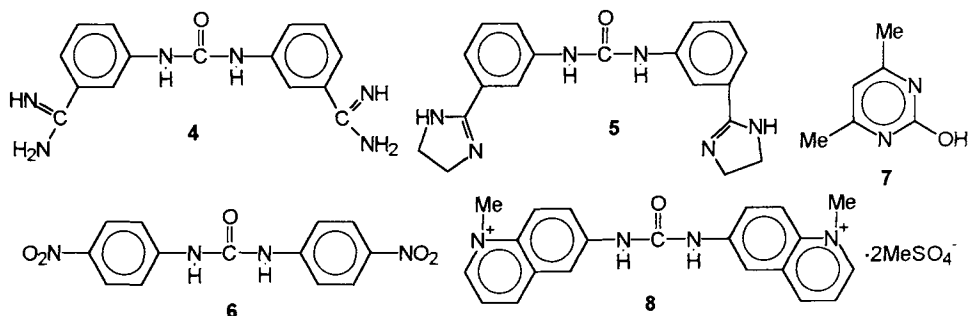
Gloxazone (**8a**), a bishydrazinocarbothioamide, which may be regarded to have been derived from thiourea, has been used to treat acute anaplasmosis in domestic animals [7].

$$H_2N-CS-NH-N=C-CH=N-NH-CS-NH_2$$
$$\overset{|}{CH_2CH_2OEt}$$
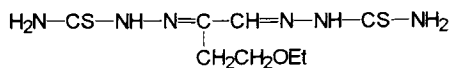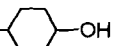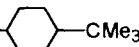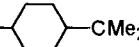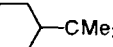
8a (Gloxazone)

3. NAPHTHOQUINONES

The importance of naphthoquinones, particularly 2-hydroxy-3-alkyl-1,4-naphthoquinones, in chemotherapy of malaria came to light when a naphthoquinone, hydrolapachol (9), was found to possess activity against *P. lophurae* in ducks [8]. This observation prompted Fieser and coworkers [9-13] to synthesize a series of substituted naphthoquinones, of which lapinone (10) and menoctone (11) emerged as promising antimalarials.

When tested in humans, lapinone was found to be suppressive and curative against *P. vivax* after intravenous administration [12,14]. Although this drug exhibited good activity in human malaria, it was not pursued further because it had to be given parenterally and also better drugs like primaquine and chloroquine became available in clinical practice. However, when chloroquine-resistance became a problem in 1964, the interest in naphthoquinones as antimalarials revived. Consequently, Berberian and Slighter [15] tested a variety of 2-hydroxy-3-alkylnaphthoquinones against *P. berghei* in mice with the result that menoctone (11) was again picked up with causal prophylactic activity against *P. berghei* in mice [16]. However, menoctone did not show any gametocidal, sporontocidal or causal prophylactic activity against. *P. falciparum* in humans at an oral dose of 0.4 to 0.5 g daily for 3 days. The inactivity of menoctone when administered orally has been attributed to its poor absorption from the gastrointestinal tract and its strong binding with plasma proteins [8].
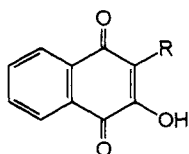
9 R = -$CH_2CH_2CHMe_2$ (Hydrolapchol)
10 R = -$(CH_2)_8$-$C(OH)(n$-$C_5H_{11})_2$ (Lapinone)
11 R = -$(CH_2)_8$—⬡   (Menoctone)
12 R = —⬡   (Parvaquone)
13 R = -$(CH_2)_3$—⬡   (1080C)
14 R = -$(CH_2)_3$—⬡—OH   (1081C)
15 R = —⬡—CMe_3 (BW 58C)
16 R = —⬡—CMe_2Et (BW 568C)
17 R = -CH_2—⬡—CMe_3 (BW 720C)

A number of 2-hydroxy-3-substituted naphthoquinones have since been synthesized of which parvaquone (12), 1080C (13) 1081C (14), BW 58C (15), BW 568C (16) and BW 720C (17) were found to exhibit potent and broad-spectrum antiprotozoal activity in experimental animals [17].

Menoctone (10) and parvaquone (11) exhibited high activity against *Theileria parva* in cattle. Similarly, BW 58C (15) was found to be 650 and 5600 times more effective than chloroquine and menoctone, respectively, against *P. falciparum in vitro*. The compound is also highly active against *P. yoelli* in mice and against chicken coccidia, *Eimeria tenella*. Replacement of one of the methyl groups in BW 58C by an ethyl group gives BW 568C (16), which shows better activity than monensin against chicken coccidia, *E. tenella* and, therefore, is currently used as the drug of choice for this infection. BW 720C (17), the higher homologue of BW 58C, is a better drug for treating *T. parva* infection in cattle. The veterinary therapeutic dose of this antiprotozoal (17) is 2.5 mg/kg as compared to 20 mg/kg of parvaquone (11) [18].

The fact that naphthoquinones are potent inhibitors of respiration in parasitized RBCs, characterised by an abnormal content of coenzyme Q (ubiquinone, 18) [16], a variety of heterocyclic quinones have been tested for their antimalarial activity [19-26]. Of these compounds 19-21 exhibited promising prophylactic activity. At a dose of 10 mg/kg given subcutaneously, 20 gave complete protection to ducks against sporozoite-induced *P. gallinaceum* infection [27], while 21 suppressed parasitaemia in monkeys infected with *P. cynomolgi* at an intravenous dose of 3.16 mg/kg [8].

18

19 R = $-(CH_2)_8$—⬡
20 R = S-$C_{17}H_{35}$
21 R = S-$C_{18}H_{37}$
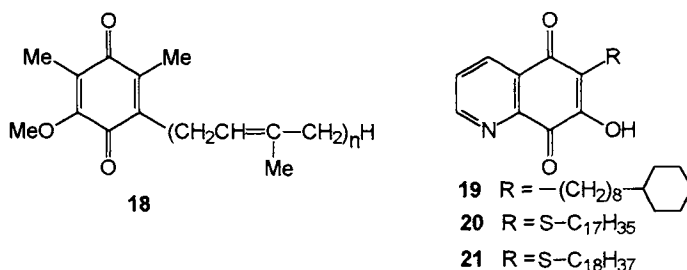
4.   ACRIDINES

The discovery of pamaquine, developed by replacing one of the methyl groups of methylene blue by a dialkylaminoalkyl chain, during the 1920s by Schoenhoefer, Schuleman and Roehl [28] was a landmark in the design of drugs for malaria. Although pamaquine itself could not be used clinically due to high toxicity, it pro-

vided an important lead to develop better antimalarials. By replacing the quinoline nucleus with acridine, Kikuth and his colleagues discovered a new blood schizonto-cidal drug, quinacrine (Atebrin, 22) in 1932 [29,30]. The marked antimalarial activity exhibited by quinacrine initiated a worldwide search for more effective acridine anti-malarials. Consequently, a large variety of 9-aminoacridines were synthesized in the USA, United Kingdom, Russia & CIS (formerly known as USSR) [31-35]. The note-worthy compounds that emerged were 23-30, which exhibited high order of anti-malarial activity, but none was better than quinacrine (22) [8,16,36].

Evaluation of quinacrine (mepacrine, 22), acrichin N5 (23), acrichin N8 (24), acranil (25), aminoquinacrine (26), desmethoxyquinacrine-N-oxide (27), azacrine (28), pyronaridine (29) and dabequine (30) against *Plasmodium* infections in experi-mental animals and humans provided encouraging results. Although des-methoxyquinacrine-N-oxide and azacrine exhibited an activity profile very similar to quinacrine, none offered any distinct advantage over quinacrine [8,16].
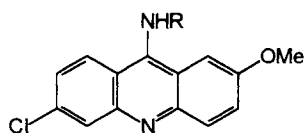
22 R = CH(Me)-(CH₂)₃NEt₂ (Quinacrine)
23 R = -(CH₂)₃NEt₂ (Acrichin N5)
24 R = -(CH₂)₄NEt₂ (Acrichin N8)
25 R = CH₂CH(OH)CH₂NEt₂ (Acranil)

26
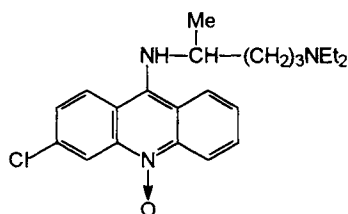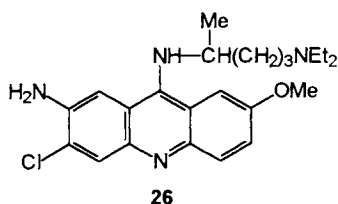
27 (Desmethoxy quinacrine -N-oxide)

28 (Azacrine)
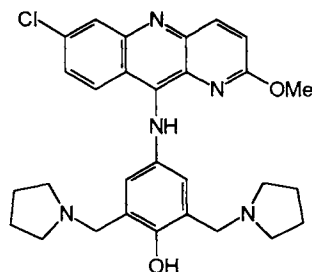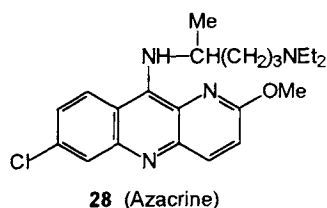
29 (Pyronaridine)

30 (Dabequine)

Some hexahydroderivatives of quinacrine have also been synthesized of which floxacrine (HOE-991, 31) exhibited causal prophylactic and blood schizontocidal activiy against drug- resistant strains of *P. berghei, P. falciparum* and *P. vivax* in experimental animals [37,38]. The imino analogue (32) of floxacrine has been found to possess even better activity than 31 [8, 39, 40]. However, due to toxicity floxacrine and its analogues could not be used clinically.

31 (Floxacrine)   32

5.   QUATERNARY AMMONIUM SALTS

A number of thiamine antagonists have been found to exhibit anticoccidial activity. Amprolium (33) is the first member of this class, which selectively inhibits uptake of thiamine by the parasites [41,42]. The marked activity exhibited by amprolium against *Eimeria* led to the evaluation of various amprolium analogues for their anticoccidial activity. The ethyl derivative (34) of amprolium was found to be active. Replacement of the pyridine ring by thiazole resulted in the discovery of several effective anticoccidial agents, the most notable of which are beclotiamine (36), dimethalium (37) and methylsulphinylethylthiamine (38). These are basically structural congeners of vitamin B_1 (thiamine, 35) [2,43].

33 R = *n*Pr
34 R = Et

35 R = CH_2CH_2OH
36 R = CH_2CH_2Cl
37 R = Me
38 R = CH_2CH_2SOMe

A few quaternary salts have been found to be valuable in the treatment of tryponosomiasis. These include metamidium (39), homidium (40), pyrithidium (41) and quinapyramine (42) [44].

39 **40**

41 **42**

6. FUSED PYRIMIDINES

In view of their structural similarity to quinolines and pyrimidines quinazolines have been extensively explored for antiprotozoal activities. A large number of 2,4-diamino-6-substituted quinazolines (**43**) and 2,4-diamino-7-(arylmethylamino)-pyrido[3,2-d]pyrimidines (**44**) were found to exhibit potent activity against *P. berghei*, *P. gallinaceum* and *P. falciparum* in experimental animals [8]. One compound of this class, WR-158122 (**43**, R=2-naphthalenesulphonyl) has been evaluated in humans infected with the Uganda I strain of *P. falciparum*; the compound caused only temporary elimination of parasitaemia and recrudescence was observed just after 9-16 days post- treatment [8].

43 **44**
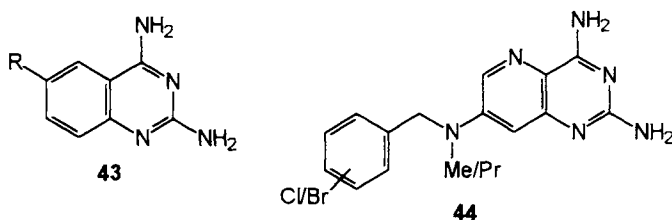
The most noteworthy compounds of this class are febrifugine (**45**) and halofuginone (**46**). Febrifugine is a plant product (See Chapter 14), which shows 50-100 times better activity than quinine against *P. lophurae* and *P. cynomolgi* in ducks and monkeys, respectively. Although this drug showed activity against *P. vivax* in humans, it could not be used in clinical practice due to its toxic effects such as nausea, vomiting and hepatotoxicity [8]. The halo derivative of febrifugine called halofuginone (**46**) finds use in the treatment of coccidiosis in chickens [45].
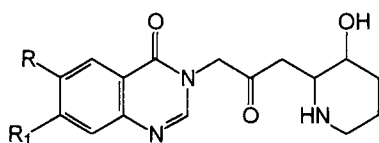
45 R = R$_1$ = H (Febrifugine)
46 R = Cl, R$_1$ = Br (Halofuginone)

A number of purines have also been found to exhibit promising antiprotozoal activity, which include sinefungin (**47**), arprinocid (**48**) and allopurinol (**49**). Of these, sinefungin is a natural product isolated from *Streptomyces griseolus* at Lilly Research Laboratory in 1971 [46]. This has been used to treat trypanosomiasis in domestic animals [47]. Arprinocid (**48**) is an effective anticoccidial drug used in poultry [45]. Allopurinol (**49**) possesses xanthine oxidase inhibitory activity and is an established drug for gout. The antileishmanial activity of allopurinol was discovered in 1974. Since then it has been extensively studied for its potential use in treating leishmaniasis in human. Allopurinol (**49**) has been found to interfere with the purine salvage pathway of the parasite leading to abnormal RNA synthesis, which eventually leads to death of the parasite [48]. Its clinical usefulness as antiprotozoal agent is uncertain.
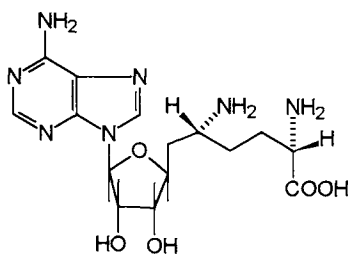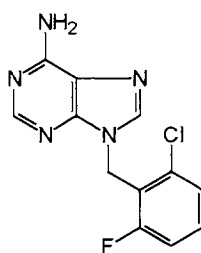
47 (Sinefungin)   **48** (Arprinocid)   **49** (Allopurinol)
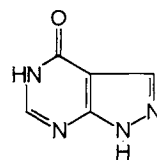
7.   QUINOLONES

A number of alkyl esters of 4-oxodihydroquinoline-3-carboxylic acid have been found to possess high order of anticoccidial properties. The first member of this class is mequinalate (**50**) [49], which was followed by the discovery of buquinolate (**51**) [50], methyl benzoquate (**52**) [51], decoquinate (**53**) [52], cyproquinate (**54**) [53] and amquinolate (**55**) [54], all showing high activity against *Eimeria spp*. SAR studies on 4-quinolones indicated that introduction of alkyl or alkoxy substitution in the benzene ring retains the activity, while replacement of the ester group by the acid function causes loss of activity [2,55]. Although the quinolone drugs exhibit a high order of anticoccidial activity and low toxicity, *Eimeria spp*. rapidly develop resistance against them [56].
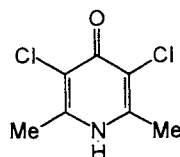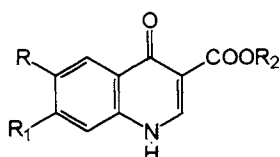
50 R = R₁ = O*i*-Pr, R₂ = Me   (Mequinalate)

50 $R = R_1 = Oi\text{-}Pr$, $R_2 = Me$ (Mequinalate)
51 $R = R_1 = i\text{-}Bu$, $R_2 = Et$ (Buquinolate)
52 $R = Bu$, $R_1 = OCH_2Ph$, $R_2 = Me$ (Methyl benzoquate)
53 $R = OC_{10}H_{21}$, $R_1 = OEt$, $R_2 = Et$ (Decoquinate)
54 $R = R_1 = OCH_2c\text{-}C_3H_5$, $R_2 = Et$ (Cyproquinate)
55 $R = Pr$, $R_1 = NEt_2$, $R_2 = Me$ (Amquinolate)

56 (Clopidol)

Clopidol (**56**), a closely related analogue of 4-quinolone drugs, has been found to exhibit marked activity against *E. acervulina* in chickens [45].

8.   BENZENE DERIVATIVES

A few polysubstituted benzenes have been evaluated for their possible use in protozoal chemotherapy. RC-12 (**57**) is a pyrocatechol derivative, which was initially found to possess promising antimalarial activity. However, in clinical trials against *P. vivax* it failed to protect volunteers exposed to infected mosquitoes [16]. Another compound ethyl 4-acetamido-2-ethoxybenzoate (ethopabate, **58**) has been found to exhibit anticoccidial activity [45,57]. Also some nitrobenzamides have been shown to have anticoccidial properties. The important drugs of this class are zoalene (**59**) [58], nitromide (**60**) [59] and alkomide (**61**) [45,60]. The detailed SAR on nitrobenzamides has been studied [59,61].
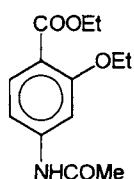
57 (RC-12)   **58** (ethopabate)
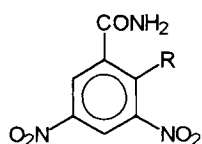
59 R = Me (zoalene)
60 R = H (nitromide)

Benflumetol (**62**) is a new antimalarial synthesized by Chinese workers in the 1970s. Since 1987 it is being used in China for treating falciparum malaria [62].

61

62
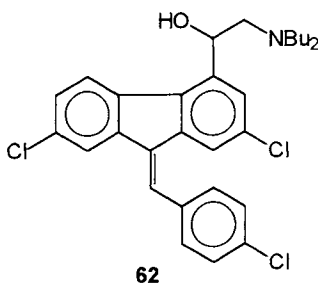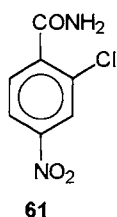
9. DIAMINOALKANES

Ornithine decarboxylase is an important enzyme involved in the polyamine biosynthesis of all living cells. This enzyme causes decarboxylation of ornithine to form putrescine, which is subsequently converted into spermidine and spermine. A number of 1,4-diaminobutane derivatives such as α-difluoromethylornithine (DFMO, **63**), α-monofluromethylornithine (MFMO, **64**), Δ-MFMO (**65**), Δ-MFMO methyl ester (**66**), α-difluoromethylputrescine (DFMP, **67**), α-monofluoromethylpu-trescine (MFMP, **68**) and (R)-α-ethynyl-ε-methylputrescine (MAP, **69**) have been shown to be potent inhibitors of ornithine decarboxylase, the most important of which is DFMO (eflornithine or ornidyl, **63**) [63]. This compound has been found to be highly effective in treating African tryponosomiasis in human [5,64].

63 (DFMO)

64 (MFMO)

65 (Δ-MFMO)

66 (Δ-MFMO methyl ester)

67 (DFMP)

68 (MFMP)

69 (MAP)
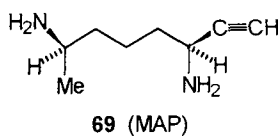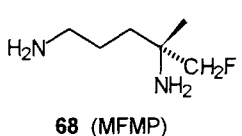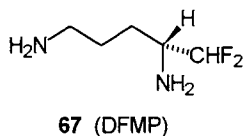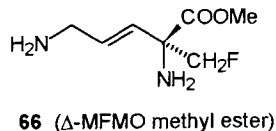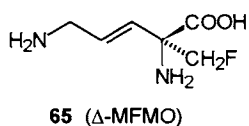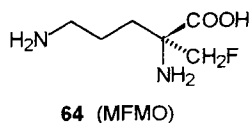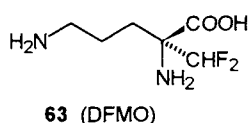
10. SYNTHESIS

10.1 Amicarbanilide (4)

The key intermediate for this drug is 3,3'-dicyanocarbanilide (71), which is obtained by condensing 3-aminobenzonitrile (70) with phosgene. Pinner reaction on 71 yielded diguanylcarbanilide (4), which is isolated as its di-isethionate salt [65,66].
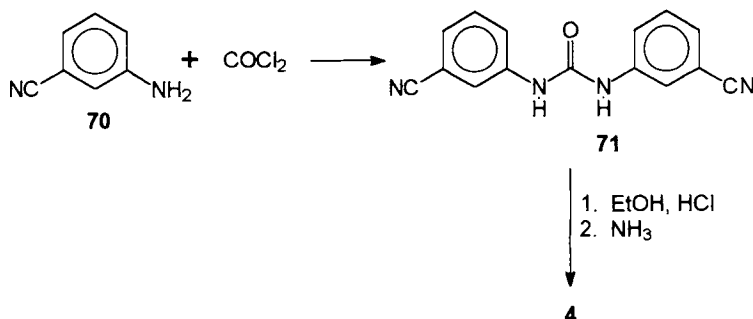
10.2 Quinuronium sulphate (8)

This can be easily prepared by reacting 6-aminoquinoline (72) with phosgene. The resulting urea derivative 73 is quaternised with dimethyl sulphate in nitrobenzene to afford 8 [67].
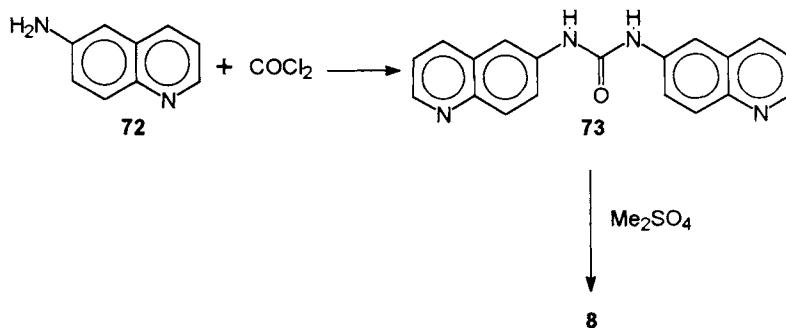
10.3 Lapinone (10)

Lapinone has been synthesized by Fieser et al. [68,69]. A convenient route to obtain 10 starting form α-naphthol (74) is outlined in scheme 1.

Scheme 1
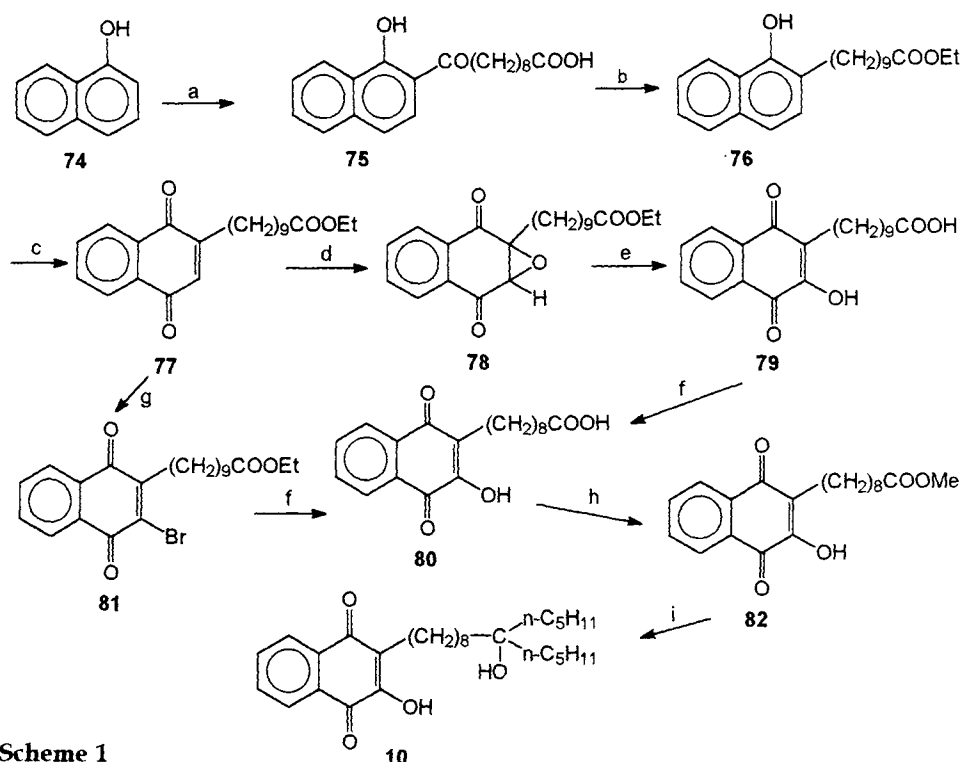
Reagents: (a) HOOC-$(CH_2)_8$-COOH, $ZnCl_2$, (b) Zn, HCl, EtOH, (c)CrO_3, (d) H_2O_2, (e) H_2SO_4, (f) Hooker oxidation, (g) Br_2, MeCOONa, (h) MeOH, $BF_3.Et_2O$, (i) Grignard reaction using n-amyl bromide.

10.4 Menoctone (11)

Fieser and coworkers [70] have developed an elegant method to synthesize 2-hydroxy-3-(cyclohexylalkyl)-1,4-naphthoquinones. Menoctone (11) may be prepared starting form 5-phenylvaleric acid (83) by the reaction sequence given in scheme 2.
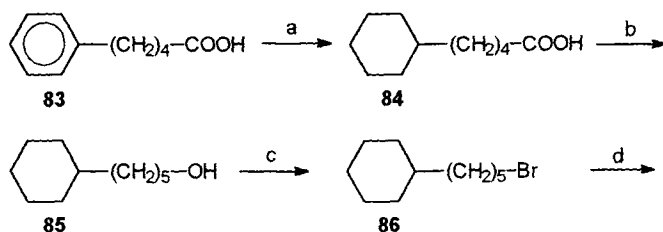
Contd..
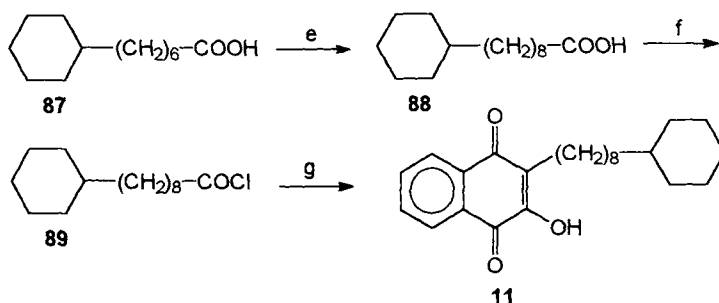
Scheme 2

Reagents: (a) Pt, H₂, (b) LAH, (c) HBr-H₂SO₄, (d) i) Malonic ester, ii) hydrolysis, iii) decarboxylation, (e) i) LAH, ii) HBr, iii) malonic ester, iv) hydrolysis, v) decarboxylation, (f) SOCl₂ (g) Silbert and Swern Procedure:H₂O₂, 2-hydroxy-1,4-naphthoquinone.

10.5 Quinacrine (22) and azacrine (28)

Quinacrine may be easily prepared starting form 2,4-dichlorobenzoic acid (**90**). Ullmann reaction of **90** with *p*-anisidine (**91**) gives the desired diphenylamine **92**, which is cyclised to form the 9-chloroacridine derivative (**93**). Condensation of the latter with 1-diethylamino-4-aminopentane affords quinacrine (**22**) [71]. A similar sequence of reaction starting from **90** and 5-amino-2-methoxypyridine (**94**) gives azocrine (**28**) [72] (Scheme 3).
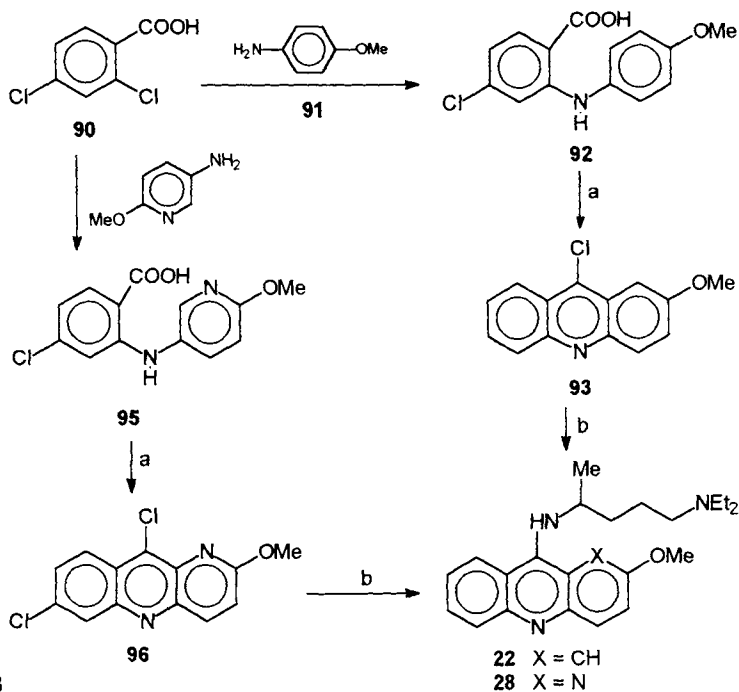
Scheme 3

Reagents: (a) POCl₃, (b) H₂N-CH(Me)-(CH₂)₃NEt₂.

10.6 Sinefungin (47)

Starting from the phosphonate (99), Geze *et al.* [75] have synthesized sinefungin as described in scheme 4.
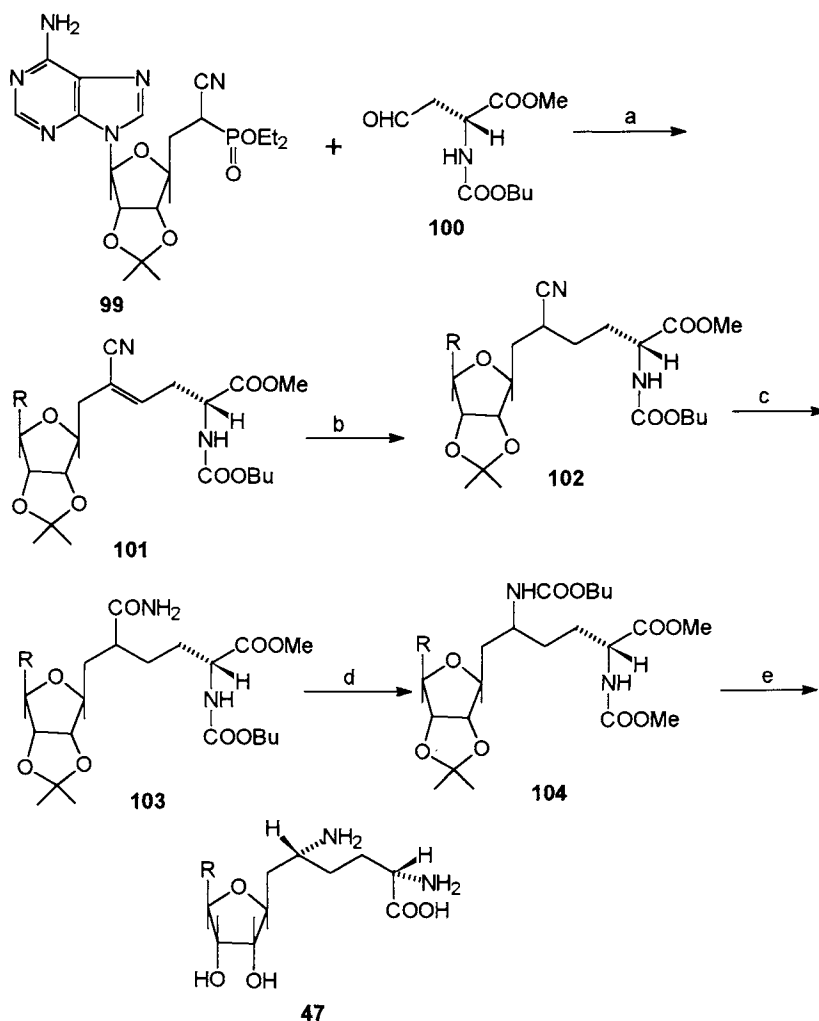
Scheme 4

Reagents: (a) Mg(OMe)$_2$, (b) Mg, MeOH, (c) MeOH, H$_2$O$_2$ (d) DMF, Pyridine, (CF$_3$CO)$_2$O, (BuOCO)$_2$O, (e) K$_2$CO$_3$, CF$_3$COOH.

10.7 Quaternary ammonium salts

The synthesis of various 1-(2-alkyl-4-amino-5-pyrimidinylmethyl)alkylpyridinium salts and analogous 3-thiazolium compounds has been described by Rogers *et al.* [73]. Typically, amprolium (33) and beclotiamine (36) may be obtained by reaction of 2-alkyl-4-amino-5-pyrimidinylmethyl halide (97,98) [74] with the appropriate pyridine and thiazole derivatives, respectively, in acetonitrile [73].
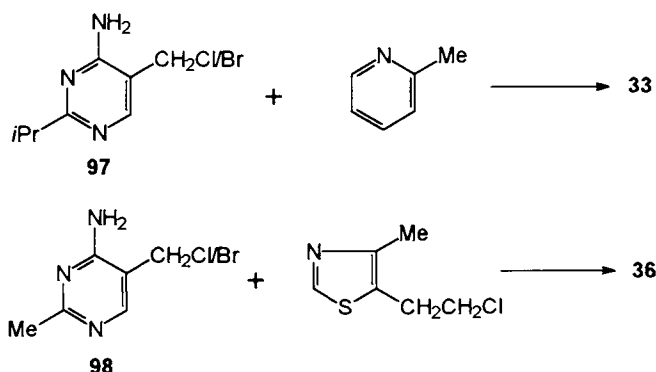
10.8 Aprinocid (48)

Treatment of 4,5,6-triaminopyrimidine (105) with thionyl chloride affords 7-amino-1,2,5-thiadiazolo[3,4,-d]pyrimidine (106), which can be smoothly transformed into aprinocid (48) through the reaction sequence given in scheme 5 [76].
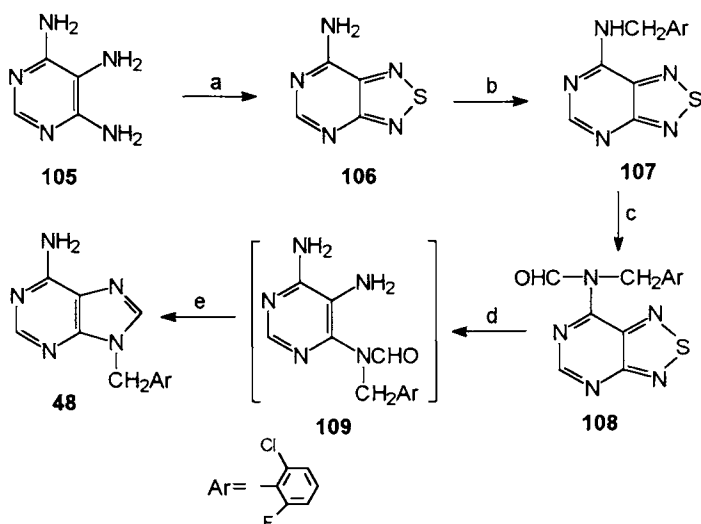
Scheme 5
Reagents: (a) SOCl$_2$; (b) 2-chloro-6-fluorobenzylamine, (c) HCOOH, Ac$_2$O, (d) Ra-Ni, (e) Δ.

10.9 Allopurinol (49)

The key intermediates for preparing allopurinol are 3-amino-4-cyanopyrazole (111) or 3-amino-4-carbethoxypyrazole (114), which may be prepared by reacting ethoxymethylenemalononitrile (110) and ethyl ethoxymethylenecyanoacetate (113), respectively, with hydrazine [77,78]. These may be smoothly converted in to allopurinol as described in scheme 6.
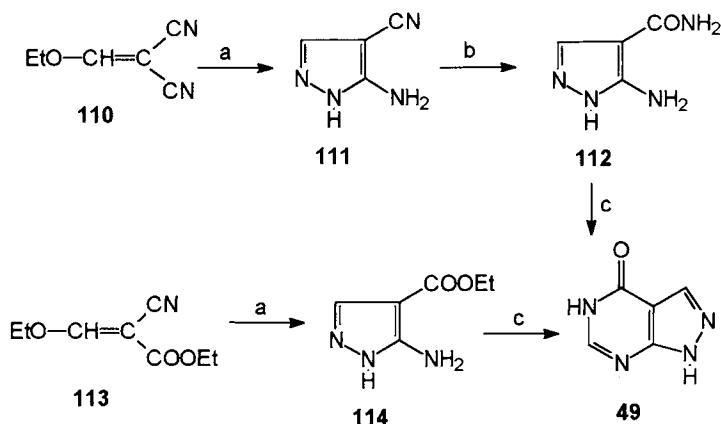
Scheme 6
Reagents: (a) H_2N-NH_2, (b) H_2SO_4, (c) $HCONH_2$.

10.10 Buquinolate (51)

The Norwich Pharmacal Co. has developed the synthesis of buquinolate starting from catechol (115), which is outlined in scheme 7 [79].
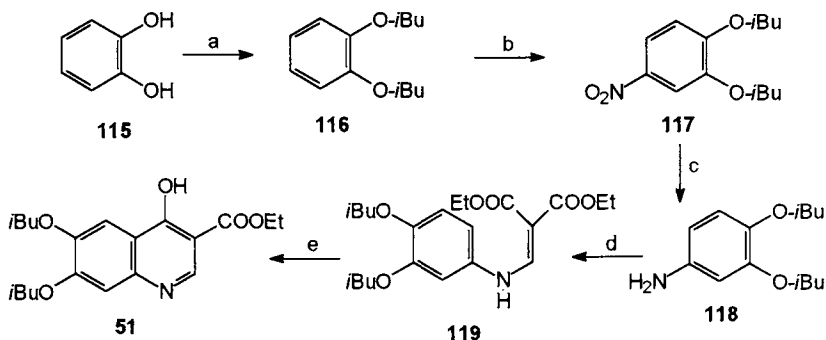
Scheme 7
Reagents: (a) NaOH, i-BuBr; (b) Conc.HNO_3; (c) Pd-C, H_2; (d) EtO-CH=C(COOEt)$_2$; (e) heat.

10.11 Cyproquinate (54)

Mizzoni *et al.* [53] have described the synthesis of cyproquinate (54) and related anticoccidial agents. The method is very similar to that of buquinolate (51) given in scheme 7. Catechol (115) is alkylated with chloro- or bromomethylcyclopropane to give 120, which is converted in to 54 following the reaction sequence outlined in scheme 8.
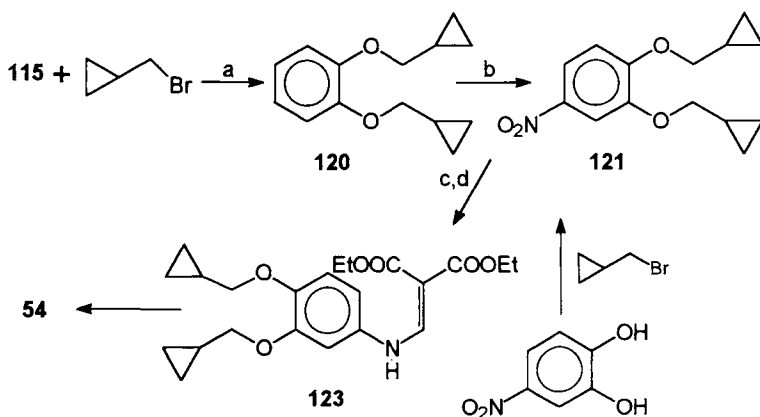
Scheme 8

Reagents: (a) NaH, DMF, (b) HNO₃; (c) PtO₂, H₂; (d) EtOH=C(COOEt)₂; (e) Dowtherm, 25°C.

10.12 Eflornithine (DFMO, Ornidyl, 63)

This is prepared by the reaction of N^2,N^5-dibenzylidenelysine methyl ester (124) with chlorodifluoromethane ($HCClF_2$) in the presence of lithium diisopropylamide. The resulting ester is hydrolysed with HCl to get DL-DFMO, which is isolated as its monohydrochloride, monohydrate [80].
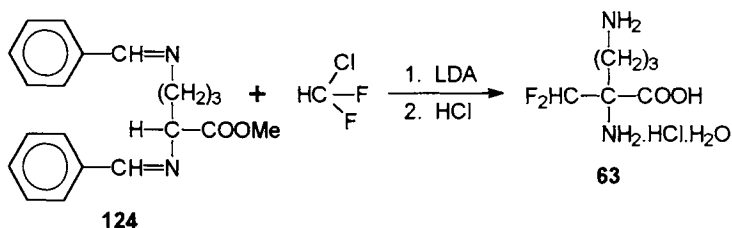
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