The Use of Anticancer Drugs in Antiparasitic Chemotherapy

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Abstract: Many similarities exist between cancer cells and parasites. A potentially lucrative starting point for the discovery of novel drugs to combat parasites is to examine available compounds developed against cancer for antiparasitic properties. Here, we review the use of current and promising anticancer agents for treating major human parasitic diseases.

Keywords: Cancer drugs, novel antiparasitic targets, synthetic compounds, "natural" drug-like chemicals.

Novel drugs to combat parasitic diseases of major world importance are urgently needed. Many parasites have developed resistance to available chemotherapeutic agents and many insect vectors have become resistant to insecticides. There are still no proven vaccines for any of these diseases and the multiple life cycle stages and their complex interactions with host immunological and genetic factors will continue to make vaccine development extremely difficult. Even though some control measures such as the use of insecticide-impregnated bednets against malaria infections or vector control for leishmaniasis have been successful, there will always be a need for drugs to treat new infections. One way to circumvent the difficulties associated with antiparasitic drug discovery and the reluctance of pharmaceutical companies to invest in research on tropical diseases is to focus on existing drugs being used to treat other diseases in humans, and test them for antiparasitic properties. The development of drugs of interest as anticancer agents guarantees the availability of data on their pharmacology, toxicology and tolerance in humans, which are essential factors that ultimately influence the overall costs in the development of drugs for parasitic diseases. Some of these approaches based on the use of current or promising cancer drugs will be reviewed in this chapter.

COMMON FEATURES OF CANCER CELLS AND PARASITES

Before examining some means to find alternative, effective and safe drugs for the treatment of parasitic diseases, we would first like to discuss how drug development in parasitology can benefit from cancer drug development research. There are a number of crucial links between cancer cells and parasites. Both share an important feature of living and multiplying in a host organism. Parasites that are well-adapted do not immediately kill their host, nor do cancer cells that cause benign tumours. Tumour cells are characterised by their independence from exogenous growth factors, their resistance to programmed cell death (apoptosis), and their infinite proliferative capacity. Unlimited proliferation and independence of growth factors are also characteristics of many parasites. Although it remains a matter of debate as to whether apoptosis occurs in unicellular parasites at all [1], there is no doubt that intracellular parasites interfere with the programmed cell death machinery of their host cell [2, 3]. In an attempt to escape host immune responses, parasites and cancer cells disseminate in immune compromised tissues. To reach these tissues, they are known to secrete proteolytic enzymes or express them on their surface [4]. However, parasites have found an even better way to escape direct immune attack. They invade host cells and develop within the cell. Some prominent examples of parasites which have been mentioned in the context of uncontrolled proliferation, metastasis, inhibition of host cell apoptosis and their ability to induce certain cancers in the host are discussed below in more detail and should help to illustrate some important links in the fields of parasitology and oncology.

Alveolar echinococcosis in humans caused by *Echinococcus multilocularis* behaves biologically like a malignant tumour of the liver. It was suggested that the unlimited proliferative capacity of the metacestode might be related to the overproduction of a family of proteins termed 14-3-3 [5]. These proteins belong to a ubiquitous family of molecules that participate in protein kinase signalling pathways in all eukaryotic cells. Functioning as phosphoserine/phosphothreonine-binding modules, 14-3-3 proteins participate in phosphorylation-dependent signalling events, including DNA damage checkpoints and prevention of apoptosis. The expression of some 14-3-3 isoforms have been found dysregulated in a number of tumours [6]. In fact, the alignment of the *Echinococcus* 14-3-3 cDNA sequence with known 14-3-3 isoforms from other organisms grouped the parasite sequence into the tumour growth related isoforms [5]. The recent identification of the small GTPases Ras and Raf in *E. multilocularis* [7] and of a gene encoding the epidermal growth factor receptor [8] now provide some interesting tools to further study growth control in this parasitic cestode.

Infections with other parasites are suspected to support the development of certain cancers. Known examples are *Schistosoma* infections and bladder carcinoma or hepatocarcinomas [9]. The underlying mechanisms of these observations are not clear. The same is true for the hypothesis that neurocysticercosis caused by the trematode *Taenia solium* might be a risk factor for human cancer [10].

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It is known that these parasites modulate host immune responses, and this modulation is possibly associated with loss of regulatory mechanisms. Such a loss of function has been implicated in the immunological surveillance against cancer [11]. In view of the many parallels between cancer cells and parasites, it is highly likely that the same mechanisms are in play. The possibility of such a model, however, awaits validation.

In order to survive within the cell, intracellular parasites have to deal with the host cell apoptosis machinery, which represents a very powerful and highly specific defence mechanism of the host. Intracellular protozoan parasites, which have been reported to inhibit the apoptotic programme of the host cell are *Toxoplasma gondii*, *Plasmodium* spp., *Theileria* spp., *Cryptosporidium parvum*, *Trypanosoma cruzi*, and *Leishmania* spp. [2, 3]. Although these parasites differ in their mechanisms of host cell entry and in their final intracellular location, it has been demonstrated that they regulate similar survival pathways in their hosts. In this respect, a number of kinases have been described for their capacity to interrupt the apoptotic programme in parasitised cells. Parasite-dependent phosphorylation of the IκB kinase (IKK) complex of the host cell which results in the activation of NF-κB, a transcription factor that regulates transcription of anti-apoptotic molecules, has been described for several intracellular parasites [2]. Additionally, host cell MAPK pathways [12] as well as STAT/JAK pathways [13] have been found to be under the manipulation of *T. gondii*.

Theileria parasites which infect ruminant leukocytes, are known to extensively control host cell signalling pathways. They not only inhibit apoptosis of their host cells but also simultaneously induce their proliferation [14]. Thus, in many respects, *Theileria*-transformed cells behave like tumour cells. They overcome cellular senescence, produce their own growth factors, and even metastasise and form tumours when injected into nude mice. The major difference to tumour cells is that *Theileria*-induced transformation is entirely reversible. By killing the parasite specifically with a theilericidal drug like buparvaquone, growth of the host cell is arrested and the cured cell undergoes apoptosis within a few days. Whereas the proliferation of *Theileria*-infected cells is mainly due to parasite-dependent activation of the PI3 kinase/protein kinase B (PKB) pathway [15, 16], inhibition of host cell apoptosis is mediated by hijacking and constitutively activating the host cell IKK signalosome [17]. Importantly, inhibition of the NF-κB pathway induces immediate apoptosis of the infected cells without having a direct effect on the parasite.

From these examples, it becomes clear that anticancer drugs may affect parasite survival at two completely different levels. Firstly, they might kill the parasite directly, if the target molecules of parasite and cancer cell are sufficiently similar. In this case, the original cancer drugs may serve as leader compounds and can be modified accordingly to specifically inhibit the parasite homologue. Secondly, to kill intracellular parasites successfully, the drug might also act on a host cell signalling pathway, which is essential for the parasite's survival. The advantage here is that the drug need not be modified, since it is already directed against the target molecule.

TREATMENT AND CONTROL OF PARASITES – CURRENT SITUATION

Chemotherapy is still the most cost-effective way of treating parasitic infections. More in depth information on the disease situation is available in the World Health Organisation Tropical Diseases Research homepage (www.who.int/topics/en/). Treatment of malaria is effective with various quinine derivatives (such as quinine sulfate, chloroquine, mefloquine, primaquine and atovaquone) [18]. Quinine has been used to fight malaria long before it was known what caused the disease. Chloroquine is effective, inexpensive and safe and has been the mainstay of malaria chemotherapy for several decades. Chloroquine resistance, particularly in *Plasmodium falciparum* has recently become a major problem in South-East Asia. Resistance to dihydrofolate reductase inhibitors (proguanil, pyrimethamine) and dihydropteroate synthase inhibitors (sulfa drugs) are also becoming more common. Qinghaosu and derivatives (artemesinin, artesunate, arteether) are the more recent additions as antimalarial drugs, with some constraints on their use alone, such as possible toxicity and resistance. They are currently used in combination with other drugs. Control measures against malaria include eradication of infected anopheline mosquitos as well as intensive efforts to develop vaccines but so far, none is available yet for routine use.

The blood stage of African trypanosomiasis can be treated with reasonable success with pentamidine isethionate or suramin, both introduced more than 50 years ago [19]. These drugs have been reported also to be effective in prophylaxis, although they may mask early infection and thus increase the risk of CNS disease. Cases with CNS involvement should be treated with melarsoprol, an organic arsenic compound, in use since 1949. Eflornithine (DFMO) was registered only in 1990. Cure rate is >90 % and has been referred to as the "resurrection drug" for its ability to be effective late in the disease. This is one example of an antitumour agent currently in use against a protozoa-caused disease.

There is no curative therapy available for Chagas disease, as available drugs are either ineffective or highly toxic. Two experimental drugs, benznidazol and nifurtimox have shown to be promising for treating acute disease, but appear unsuitable for chronic cases because of their side effects. Only by reducing vector-host contact can disease transmission be prevented and attempts to develop a vaccine though feasible have not been very successful.

The first-line drugs against different types of leishmaniasis are the organic pentavalent antimonials sodium stibogluconate (Pentostam) and meglumine antimoniate [20]. Antimonials are generally not safe and due to variable antimony composition in the drug formulations and prolonged usage, relapses and resistance are not uncommon. Pentamidine isethionate and antibiotics (such as amphotericin B) serve as alternatives. So far these drugs are administered parentally. Other control measures involve vector control, and to date immunisation has not been effective.

Pyrimethamine combined with sulfadiazine or clindamycin has been the drug of choice for treating

toxoplasmosis, mostly in conjunction with AIDS- and immunosuppression-related infections. Other drugs used include atovaquone, trimetrexate, dapsone, azithromycin and clarithromycin, major disadvantages being their high costs.

Amoebiasis is currently defined as infection with the protozoan parasite *Entamoeba histolytica*. Normally resident in the large bowel, amoebae occasionally penetrate the intestinal mucosa and may disseminate to other organs [21]. For asymptomatic infections, iodoquinol, paromomycin, or diloxanide furoate are recommended. For symptomatic intestinal disease, or extraintestinal infections (e.g., hepatic abscess), the drugs of choice are metronidazole or tinidazole, immediately followed by treatment with iodoquinol, paromomycin, or diloxanide furoate.

The two most important filarial infections of humans are lymphatic filariasis and onchocerciasis. Lymphatic filariasis is caused by *Wuchereria bancrofti, Brugia malayi* and *Brugia timori* which are transmitted by several genera of mosquitoes. Onchocerciasis (river blindness) is caused by *Onchocerca volvulus* and transmitted by blackflies of the genus *Simulium*. Chemotherapeutic approaches to the control and treatment of filarial diseases have traditionally been a difficult topic because control strategies currently rely on drugs that have microfilaricidal activity only. Annual single-dose co-administration of two drugs (ivermectin + diethylcarbamazine (DEC) or albendazole) reduces blood microfilariae (immature worms) by 99% for a full year, but the treatment does not affect macrofilaria (adult worm) [22]. Over recent years, alternative approaches to classical chemotherapy have emerged with the recognition of the *Wolbachia* endosymbionts of filariae as potential drug targets [23]. In onchocerciasis, it has been established that treatment with the antibiotic drug doxycycline at 100 mg per day for six weeks leads to long term sterility of adult female macrofilaria. Doxycycline is also effective at depleting *Wolbachia* from *W. bancrofti* and pilot studies with this antibiotic drug have given promising results.

Theilera infection of ruminants can efficiently be treated with buparvaquone (marketed under the name Butalex) and its derivates [24, 25]. The drug kills specifically the intracellular parasite in the schizont stage by affecting the electron transport in the parasite mitochodrion. So far no development of resistance has been reported against buparvaquones. However, the drug is very expensive and small holders, especially in Eastern Africa where one million animals die of theilerioisis every year [26], cannot afford to buy the drug. Antibiotics like tetracyclins are effective against early stages of *Theileria* infections. Tetracyclins are normally used for the so-called "infection and treatment" method to immunise animals [27, 28]. High doses of *Theileria* sporozoites are adminstered together with long lasting tetracyclins. Animals treated this way are solidly protected from re-infection with minimal clinical reaction. Unfortunately, this vaccination method cannot be used for large-scale treatment in Africa, since it relies on a functional cold chain for maintaining sporozoite stocks.

Coccidiosis is caused by the apicomplexan parasite *Eimeria* spp. and has a major impact in the poultry industry. Anticoccidial compounds have been used prophylactically by the majority of poultry farmers. The most successful anticoccidials have been the polyether ionophores, a family of compounds which has been used for more than 30 years [29]. Not surprisingly, reports of resistance development due to the extended and constant chemotherapeutic pressure exerted by this class of compounds are not uncommon [30]. Since that time no novel anticoccidials of similar efficacy have been introduced into poultry industry, highlighting the need to identify and develop new drugs for the control of coccidiosis.

PROTEIN KINASE INHIBITORS

Key players in parasite growth and survival are protein kinases, of either pathogen or host cell origin. It is therefore not surprising that a major focus of the recent re-emerging interest in drug development against parasites is on this group of proteins. Although protein phosphorylation has been documented to be involved in many processes in the life cycle of parasites, drug development has so far focused more on human kinase families known to be dysregulated in cancer cells, and thus represent potential drug targets. In fact, protein kinases belong to the second largest group of drug targets next to G-protein-coupled receptors, and they account for up to 30 % of the drug discovery programmes in industry [31]. How can drug development against parasitic kinases benefit from cancer research? As previously pointed out, parasite infections are clearly a problem of the developing world and poor marketing prospects have hampered drug discovery research against parasites. However, given that parasitic protein kinases display high amino acid identities (up to 60 %) with their putative mammalian homologues it is likely that many inhibitors developed against human kinases act also on the parasite homologue and represent important lead compounds.

Depending on which amino acid is phosphorylated, kinases are generally divided in tyrosine kinases or serine/threonine kinases. Considering their mode of action, however, kinases have been classified in groups such as TK (tyrosine kinases), TKL (tyrosine kinase-like kinases), STE (homologs of yeast Sterile 7, Sterile 11, Sterile 20 kinases), AGC (PKA, PKG, PKC families), CAMK (calcium/calmodulin-dependent protein kinases), CMGC (CDK, MAPK, GSK3, CLK families) and CK1 (casein kinase 1) [32, 33].

Among the estimated 518 human kinases [32], the family of cyclin-dependent kinases (CDKs) has been most extensively studied because of their essential function in cell proliferation. This has stimulated considerable interest in the development of inhibitors of these enzymes as a means of suppressing tumour growth. As a consequence, potent CDK inhibitors have been successfully developed, some of which are already in human clinical trials [31]. With the identification of several CDKs also in parasites, the next logical step was to test these inhibitors for their capacities to interfere with parasite proliferation. Not uncommonly however, the results often show that some drugs efficiently inhibit isolated or recombinant parasitic CDKs, but exhibit only modest inhibitory activity on parasite growth *in vitro*, or are inactive *in vivo.* In case of intracellular protozoan parasites surrounded by a parasitophorous vacuole membrane (PVM), this might be due to the failure of a drug to cross the three membrane layers (that of the host, the PVM and that of the parasite) in order to reach the target molecule.

Some classes of drugs acting on human CDKs as well as on parasitic CDKs or other related kinases are discussed below. Oxindoles (indolones), synthesised from 2-alkilanilines, have been shown to potently inhibit human CDKs [34, 35] (Fig. **1**). Comparison of plasmodial and mammalian CDK sequences revealed that there are structural differences within the ATP binding pocket, which is an important consideration for developing specific inhibitors [36, 37], in view of the high homology within the protein kinase family. It has been demonstrated that a number of oxindole-based compounds specifically inhibit the plasmodial CDK Pfmrk [35]. However, of the five compounds tested, none demonstrated significant antimalarial activity. As already pointed out, this could be due to poor bioavailability, the problem being in the compounds having to cross several membranes to reach the target. Clearly, a lot more work is needed to tailor this class of drugs into successful potent inhibitors of the Pfmrk enzyme and of parasite growth.

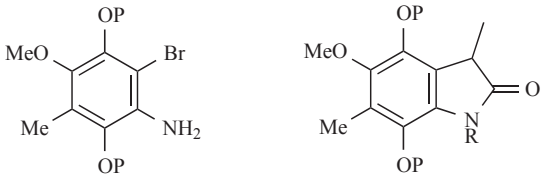
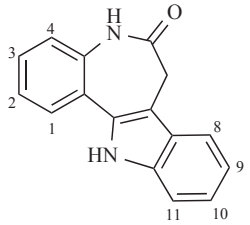


Fig. (1).

Paullones constitute a new family of benzazepinones, originally identified as CDK inhibitors in *in vitro* screening of cancer cell lines [38, 39] (Fig. **2**). Paullone derivates, immobilised on a resin were initially used to purify target kinases. This application led to the identification of a glycogen synthase kinase (GSK3) [40], which is closely related to CDKs, and mitochondrial malate dehydrogenase (MDH). It turned out that paullones are 10-fold more effective against GSK3 than against CDKs. The effect of paullones was also tested on *L. mexicana* [41]. Alsterpaullone, the most active paullone, inhibited at low doses parasite replication in macrophages *in vitro*, and killed the parasites at higher concentrations after several days. Leishmanial mitochondrial MDH was identified as one of the alsterpaullone target kinases. This inhibitor family therefore represents promising lead compounds for antileishmanial drug design. In *Plasmodium* parasites, no mitochondrial MDH was found and the cytoplasmic MDH of the parasite is poorly inhibited by paullones. However, GSK3 or Cdc2-related proteins of *Plasmodium* might still be targeted by these drugs. Therefore, further investigations of the cellular effects of this drug family on *P. falciparum* are expected to produce more promising results.

A screen of several hundred derivates of purine-based CDK inhibitors on *P. falciparum* cultures have led to the identification of purine analogues, like the purvalanols. These are known to inhibit growth of human tumour cell lines [42], but one of them, purvalanol B specifically prevented parasite growth in the low micromolar range [43]. This highly potent CDK inhibitor does not affect human cell lines because of the presence of a carboxyl group, preventing its entry into the cell. How the drug enters the parasite is still not clear, but its transfer *via* purine transporters is considered a possibility [44]. In order to identify the target molecule of purvalanol B in *Plasmodium*, parasite extracts have been loaded onto drug-immobilised affinity columns. Rather unexpectedly, a *Plasmodium* casein kinase 1, and not any of the known CDK-related kinases was purified [41]. Similar approaches using *Leishmania* and *Toxoplasma* cell extracts also resulted in the purification of casein kinase homologues as major binding proteins, confirming that parasite CK 1 kinases and not parasite CDKs were the target molecules [44]. Obviously, major differences exist between the human and parasite CDKs. Here is an impressive illustration of related kinases in higher eukaryotes and parasites having diverse specificities.

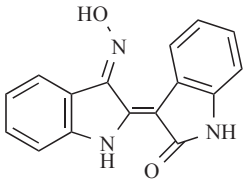


paullone scaffold

Fig. (2).

Nevertheless, *Plasmodium* CDKs represent leading antimalarial drug targets and a three-dimensional in silico pharmacophore model for inhibition of *P. falciparum* CDKs has been set up [45]. The model is composed of four chemical functions localised in space and was first validated with known inhibitors of human CDK2. A screen of the chemical database using this model has so far resulted in sixteen compounds with a predicted inhibitory activity below 25 μM. However, some of the identified substances were only modest inhibitors of the *Plasmodium* cyclin dependent kinase Pfmrk, indicating the limitations of the model, possibly due to its simplicity. The effect of the other identified drugs on parasite growth *in vitro* remains to be investigated. Here, it is worthy to note that CDK2 is not an essential gene in mouse, but rather required for germ cell development [46]. Therefore, CDK2 inhibitors affecting additionally the development of malaria parasites are not expected to cause serious side effects in the host.

Chemical library screen using the CRK3 cyclin-dependent kinase of *L. mexicana* resulted in the identification of diverse chemical classes. The purine-based inhibitors, paullones and staurosporine (a natural product originally isolated from the bacterium *Streptomyces staurosporeus*) have been previously described [47]. The most potent inhibitors of CRK3, however, belonged to the indirubin class (Fig. **3**). Indirubin, a bis-indole is derived from various natural sources, such as *Indigofera indica* by fermentation, oxidation and the presence of light as a by-product of indigo formation. In culture, the drugs caused growth arrest, a change in DNA content, and aberrant cell types, consistent with the intracellular inhibition of a cyclin-dependent kinase and disruption of cell cycle control. Four indirubins displayed antileishmanial activity in the macrophage infection model *in vitro*, and two of them inhibited both promastigote and axenic amastigote growth in culture.



indirubin

Fig. (3).

CDKs belong to the serine/threonine kinases and are involved in the control of cell growth and proliferation. Tyrosine kinases, on the other hand, are mainly responsible for the signalling through membrane receptors. This signalling in mammalian cells and in parasites is very different, as are the receptors. For example, the EGF-R signalling pathway (a focus of cancer research [31]) does not exist in unicellular parasites. Specific inhibitors of tyrosine kinases targetting EGF signalling are therefore not expected to act on parasite kinases. General tyrosine kinase inhibitors, however, have been shown to inhibit development of *Plasmodium* parasites in erythrocytes and thus deserve a brief mention.

TYROSINE KINASE INHIBITORS

In *Plasmodium*-infected erythrocytes, many of the integral membrane proteins undergo reversible tyrosine phosphorylation which is thought to play a role in invasion of the cell by *P. falciparum* [48]. Since tyrosine phosphorylation has also been described for other red-cell phosphorylation processes, the effect of protein kinase inhibitors on the intraerythrocytic development of *Plasmodium* parasites has been determined. Whereas the general tyrosine phosphorylation inhibitor genistein showed only effects in the higher micromolar range on the parasite growth *in vitro*, other inhibitors like R03 and tyrphostin derivates showed activity in the low and medium micromolar range [49]. Since tyrphostine B46 also inhibits *Plasmodium* entry into RBC [50, 51], it is certainly worthwhile investigating how these inhibitors block signalling pathways necessary for invasion or maturation. In this context, it is interesting to note that *P. berghei* infection of hepatocytes induces a strong tyrosine phosphorylation within the PVM and that this phosphorylation can partly be inhibited by genistein. Prolonged treatment of infected HepG2 cells with genistein inhibits parasite development but it cannot be ruled out that genistein, being a relatively unspecific drug, has additional effects on other parasite or host cell molecules (S. Bolte, unpublished observation).

Sponges are an important source for new compounds with biomedical importance. They are the simplest form of multi-cellular animals, though plant-like in appearance, are actually one of the most primitive animals in the sea. To reach the remarkable age of several hundred to several thousand years, sponges have developed many metabolites to control the growth of possible pathogens. These biologically active sponge metabolites are often alkaloids or terpenes. Two alkaloids, homofascaplysin and fascaplysin were shown to potently inhibit *P. falciparum* strains K1 and NF54 [52]. In mammalian cells, both substances have been demonstrated to potently block $p56^{lck}$ tyrosine kinase. Both drugs exhibit cytotoxicity against mammalian cells at a level that would not make these compounds likely antiplasmodial agents, but rather as promising lead structures for advancing drug development.

OTHER KINASE INHIBITORS AND SYNTHETIC SPHINGOLIPID ANALOGUES

The cyclic nucleotide dependent kinase PKG is characterised by the presence of a cyclic GMP-responsive regulatory domain located to the N-terminus of the catalytic domain. PKGs of apicomplexan parasites possess three binding sites [53-55], whereas higher eukaryotes have only two such sites. For the PKG of *Eimeria tenella* this is reflected by functional properties which are distinct from those of mammalian PKGs. The *E. tenella* homologue is activated about 400 times more by cGMP than the mammalian PKG [56]. In work associated with the development of PKG inhibitors, the *Eimeria* PKG was first identified as a target of a tri-substituted pyrrole, called compound 1 [53]. The drug was originally identified by a whole-cell screen on live *Eimeria* parasites. To identify PKG as a target of compound 1, the substance was radioactively labelled and incubated with *Eimeria* extracts and the purified fractions were microsequenced. The drug was later confirmed to inhibit PKG in sub-nanomolar amounts. In contrast, the activity of host cell PKG is about 1000 times less sensitive to the inhibitor. Compound 1 was also demonstrated to prevent the development of several other apicomplexa [55].

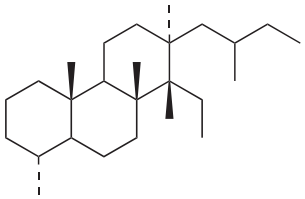
Sphingolipids are essential components of eukaryotic cell membranes. Sphingosine and ceramide are two main members of the sphingolipid family. Originally considered to be structural components of the membrane, more recent data demonstrated that sphingolipids have many additional functions. They are involved in the regulation of membrane fluidity and form membrane rafts implicated in signalling and trafficking in cells. Ceramide can be phosphorylated by a distinct kinase and can be converted into sphingomyelin through transfer of the choline phosphate group from phosphatidylcholine.

It is well documented that ceramide analogues are cytotoxic for tumour cell lines like lymphoma U937 [57] and the human leukemia HL-60 cells [58]. Labaied *et al.* [59a] synthesised a range of ceramide analogues and tested them on *P. falciparum* in comparison with their effect on human embryonic lung cell line (MRC-5). In contrast to what was observed for cancer cells, the results of Labaied *et al.* [59a] suggested that the ceramide analogue AD2646 induced non-apoptotic death of *P. falciparum*. However, the antiplasmodial activity of AD2646 does not correlate with its inhibitory activity on sphingomyelin synthase and consequently does not affect the formation of the tubovesicular network of the parasite. It is believed that the toxic effect of AD2646 is due to effects on raft formation in the parasite membrane and the PVM. Further work is clearly needed to confirm this interesting hypothesis.

INHIBITORS FROM PLANTS

Quassinoids: Plants of the family Simaroubaceae are widely used in traditional medicine for the treatment of cancer, malaria and other diseases [59b]. The antimalarial activity of *Simaba cedron* was reported as early as 1854 and was suggested even then as a substitute for quinine [60].

Further studies carried out in the 1980s on *S. orinocensis*, a native tree found in the Amazon riversides have resulted in the isolation of quassinoids, a subclass of triterpenoids, composed of 19-20 carbon atoms (Fig. **4**). Quassinoids have attracted the attention of researchers because of their antitumour, antimalarial, anti-inflammatory, amoebicidal and herbicidal activities.



quassinoid base

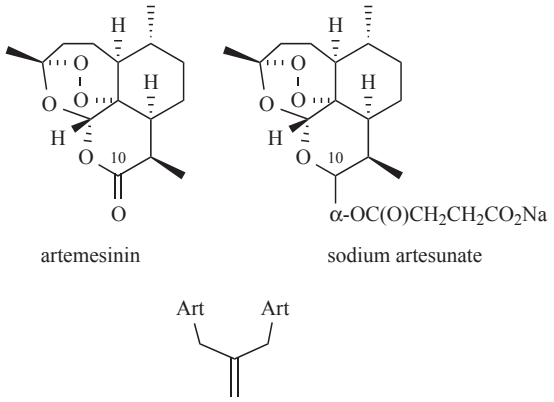
Fig. (4).

Fractionation of an alcohol extract prepared from the root bark and leaves of *S. orinocensis* has resulted in the isolation of a number of quassinoid compounds. Two compounds in particular (named quassinoids 1 and 2) [59b] demonstrated *in vitro* cytotoxic activity against some human cancer cell lines as well as strong *in vitro* antimalarial activity against chloroquine-sensitive and resistant *P. falciparum* clones. While one of the compounds exhibited no antileishmanial activity, the other was found to be more potent than the current antileishmanial agents pentamidine and amphotericin B. The selective antimalarial action of quassinoids may possibly be the result of stronger and more specific binding of the drugs to the parasite ribosomes, and not to the host cell ribosomes. Such a property associated with quassinoids may also explain why other inhibitors of protein synthesis do not demonstrate antimalarial activity.

Other quassinoid-related compounds have also been isolated and extensively characterised from the roots of *Eurycoma longifolia*, a shrub-tree belonging to the family of Simaroubaceae [61]. As expected, amongst them were compounds that exhibited not only strong cytotoxicity toward human breast cancer MCF-7 cell lines, but also potent antimalarial activity against chloroquine-resistant *P. falciparum*. A continued interest in this field to thoroughly study the stereochemistry of different quassinoids will pave the way for designing novel antimalarial agents, in particular to be better prepared to deal with the dire situation of newly emerging chloroquine-resistant parasite strains.

Artemisinin derivates: In the early 1970s, Chinese chemists succeeded in isolating (from the shrub *Artemesia annua*) and elucidating the structure of "qinghaosu" or 1,2,4-trioxane artemesinin, the highly active antimalarial component of the ancient Chinese herbal medicine used for treating fevers [62]. The importance of these findings lies in the fact that this family of antimalarials is not quinoline-based and is therefore effective against multidrug resistant parasites. Sodium artesunate is a succinic acid half-ester of artemisinin that is fast-acting, water-soluble, effective and widely used in areas of the world where malaria is endemic [63]. So far, clinically relevant resistance to such trioxanes have not yet been reported. Active research based on this group of ancient Chinese folk remedy has resulted in the design of several new compounds, with dual medicinal value as both anticancer and antimalarial agents, and which hold promise in being both safer and more effective than the current "gold standard" drug treatments.

Starting from the natural trioxane artemisinin, several 1,2,4-trioxane dimers endowed with high *in vitro* antimalarial, antiproliferative and antitumour activities have been synthesised (Fig. **5**). The first generation C-10 acetal derivatives, even though very simple in their synthesis had the disadvantage of being easily hydrolysed in water [64, 65]. To overcome this problem of instability, Posner and coworkers have subsequently succeeded in converting C-10 acetate directly into C-10 non-acetal trioxane dimers [66]. In parallel, Jung and coworkers disclosed data on similar metabolically more robust dimers [67]. Interestingly, the anticancer activities of some of these compounds have been reported to have comparable activities to taxol against murine P388 cell lines and human breast cancer MCF7 cell lines [67, 68]. Taxol is the new wonder drug, which is currently FDA approved for both advanced ovarian cancer and breast cancer. It was originally extracted from the bark of the Pacific yew tree, and found to exhibit marked antitumour activity against a broad range of activites against rodent tumours as early as 1962. Interest in taxol rekindled after it was shown to possess the unique property of being able to induce the assembly of tubulin into microtubules and to subsequently stabilise them to the extent that mitosis is disrupted [69, 70]. This knowledge made taxol a prototype for a new class of anticancer drugs. Presumably because of extremely high production cost, its effect on parasites has not been extensively explored, with the exception of a piece of work showing it to block replication of *T. cruzi* parasites [71]. It is envisaged that in the years to come, synthetic organic chemistry may provide the basis for cheaper production, thereby encouraging more research in this direction.



artemesinin sodium artesunate

C-10 trioxane dimer

Fig. (5).

In addition to being thermally stable, the new soluble C-10 non-acetal carboxylic acid derivatives had longer half-lifes and potentially lower toxicities [72]. Moreover, strong growth inhibitory but not cytotoxic effects have been demonstrated on several human cancer cell lines. When administered orally to rodents, these compounds were found to be more efficacious as antimalarials than sodium artesunate [73].

An understanding of the chemical mechanism of action and the metabolism of artemisinin has guided the rational

design of new antimalarial trioxanes. The peroxide unit is now known to be essential for high antimalarial potency [73]. Unlike quinoline-based antimalarials (such as chloroquine), which have only one mechanism of action, trioxanes appear to kill malaria parasites by generating more than one type of cytotoxic intermediate. The trioxanes are thus versatile precursors that are iron-induced and capable of producing different highly reactive intermediates (oxy radicals, carbon radicals, high valent iron-oxo species) as well as longer-lived neutral electrophiles (epoxides, aldehydes, dicarbonyl compounds) [74]. By generating different harmful species, the prodrug characteristics have the advantage in making it difficult for the parasites to develop resistance.

Very similar to the trioxane group of compounds described above are 1,2,4,5-tetraoxacyclohexanes (tetraoxanes). A series of such *cis* and *trans* bis-steroidal tetraoxane compounds have now been synthesised and evaluated for both antimalarial and antiproliferative activities [75, 76]. Only submicromolar concentrations are sufficient to kill *P. falciparum* cultures *in vitro*. In antiproliferative screens, some compounds also exhibited pronounced cytotoxicity on cancer cell lines, with initial results revealing an apoptotic nature of cell death induced by the compounds.

PROTEASOME INHIBITORS

The majority of proteins, including those crucial to cell cycle regulation and apoptosis, are degraded *via* the ubiquitin-proteasome pathway, which is made up of the ubiquitin-conjugating system and the multicatalytic proteinase complex of the proteasome [77, 78]. Proteins targeted for degradation are ubiquitin-tagged and transported into the catalytic inner chamber of the proteasome, where the proteins are cleaved into 3 – 25 amino acid long peptides [79]. The discovery that caspases mediated apoptotic-related processes was a first hint of a possible role of proteasome in apoptosis. Studies on this aspect advanced as proteasome inhibitors developed. Of the proteasome inhibitors known to date, the most important ones fall into 5 classes: peptide aldehydes, peptide vinyl sulfones, peptide boronates, peptide epoxyketones and ß-lactones (lactacystin and derivatives) [80].

The discovery that lactacystin induced apoptosis in a human monoblast cell line was the first indication that this class of proteasome inhibitors had antitumour activity [81]. The first *in vivo* demonstration of anticancer activity was through the use of a peptide aldehyde in a murine xenograft model of Burkitt's lymphoma [82]. In addition, peptide aldehyde proteasome inhibitors were found to be more efficient at inducing apoptosis in proliferating, subconfluent endothelial cell cultures than in quiescent confluent cells [83]. This property of being able to induce apoptosis in rapidly dividing cells argues in favour of the use of proteasome inhibitors as an alternative tool to fight fast growing parasites like *Plasmodium* parasites. During different life cycle stages, the parasite undergoes extensive morphological changes and several rounds of replication, during which maximal proteasome activity is required.

So far, most of the work related to the potential use of proteasome inhibitors in antiparasitic therapy has been performed using the natural product lactacystin (Fig. **6**). The first documentation of proteasomes participating in the developmental pathways of protozoan parasites involved the analysis of lactacystin on trypanosomes [84]. Here, it was shown that lactacystin prevented the transfomation of trypomastigotes of *T. cruzi* into amastigotes in incubation medium. Furthermore, the intracellular development of the parasite from amastigotes into trypomastigotes was also blocked in the presence of the inhibitor. To identify the target of lactacystin, two approaches were used. A lactacystin-inhibitable chymotrypsin activity was isolated from crude extracts of the parasite *via* gel filtration and anion exchange chromatography. The target of lactacystin was also identified *in vivo via* immunoprecipitation experiments and shown to be the *T. cruzi* proteasome. In another study, lactacystin also showed potent inhibition of the 20S protease activity purified from bloodstream and procyclic (insect) forms of *T. brucei*. Moreover, it inhibited proliferation of *T. brucei* cells in culture assays, blocking both bloodstream and procyclic forms [85].

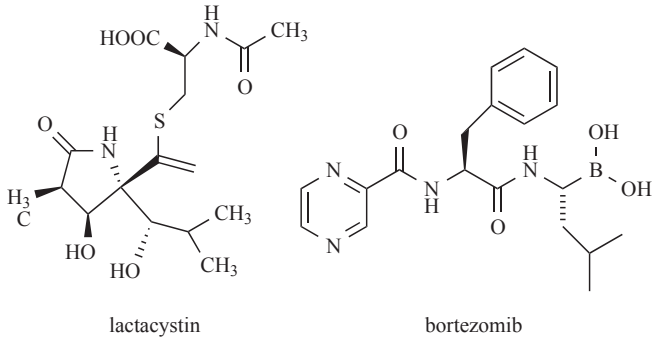


Fig. (6).

Very similar results were obtained with other protozoan parasites. Lactacystin-treated *P. berghei* sporozoites, although still invasive *in vitro*, were prevented from developing normally in exoerythrocytic forms in HepG2 cells [86]. In addition, sporozoites of *P. yoelii* preincubated in lactacystin were found to be at least 10 times less infective in mice. Lactacystin reduced *P. berghei* parasitaemia in rats, however the therapeutic index (measure of drug's safety and efficacy) was low. Even though treatment with higher doses of lactacystin cleared infection, none of the experimental animals survived the regimen of three injections each of 1.3 mg, administered intravenously and 8 h apart.

In studies involving *Entamoeba invadens*, the specific conversion of the disease-causing trophozoite stage into the infectious cyst stage was blocked in the presence of lactacystin. The amoeba target of lactacysin was purified using similar biochemical techniques applied for the *T. cruzi* proteasome chymotrypsin [87]. Two-dimensional PAGE fractionation demonstrated that the lactacystin-inhibited column-purified material displayed features typical of eukaryotic 20S proteasome complexes, containing major species with molecular masses between 25 to 35 kDa and isoelectric points of 4.5 to 8.5.

Even though lactacystin has been useful in studying the effects of proteasome inhibition in both cancer cell lines as well as in parasites, its low therapeutic index precludes clinical usefulness. In an attempt to find more potent antiplasmodial drugs, a number of lactacystin analogues was synthesised and tested [86]. The finding however, that most

lactacystin analogues do not discriminate between the mammalian and the parasite proteasome means the search for drugs that are more selective for the parasite proteasome has to continue. To date, only the peptide boronates appear to possess properties suitable for clinical development [80, 88]. Compared to the other classes of proteasome inhibitors, peptide boronates are metabolically more stable and show higher potency and greater specificity. Of the many boron-containing compounds screened for anticancer activity using the National Cancer Institute panel of cell lines, the most successful was the compound called PS-341, now designated bortezomib (Fig. **6**) [89]. Bortezomib was selected for intensive study because it is potent, inhibiting the 20S core chymotrypsin at nM concentrations. It is active against a broad range of cancer cell types, including lung, colon, central nervous system, melanoma, ovarian, renal, prostate and breast. Because different proteins are degraded by the proteasome and multiple cellular processes are affected by proteasome inhibition, the activity of bortezomib in different cancers probably involves a variety of molecular mechanisms. Bortezomib induces apoptosis, but the molecular mechanism is still unclear, although a shift in the balance between pro- and antiapoptotic signals appears to be associated with proteasome inhibition [80].

Bortezomib is the first drug of its kind to enter phase I and II trials in solid tumours, in hematologic malignancies (in addition to relapsed and refractory multiple myeloma, also leukaemia and non-Hodgkin's lymphoma) [80, 88]. Preclinical and clinical testing have now validated the hypothesis that the proteasome is a viable therapeutic target, and the most encouraging outcome of these studies is that it is currently in use for the treatment of cancer, and has taken only a little more than ten years to move from hypothesis to clinical use.

The first of these small molecule anticancer drugs, related to bortezomib called MLN-273 was recently tested on parasites. MLN-273 was chosen based on its longer half-life and therefore thought to be a better therapeutic for infectious disease indications [90]. Recent findings that MLN-273 also acts on mycobacterial proteasomes have lent support for its further development in the treatment of diseases outside of oncology [91]. MLN-273 blocks *P. falciparum* erythrocytic development at the ring stage and also prevents *P. berghei* exoerythrocytic forms to develop into schizonts [92]. In both species, condensation of the nuclei and shrinkage of the parasite cytoplasm were observed, leading to the speculation that the drug affected cell cycle progression and stage transformation *via* inhibition of the proteasome. Notably, only nanomolar concentrations of MLN-273 are required to retard parasite development, in contrast to micromolar ranges of lactacystin to achieve the same killing effect. Neither erythrocyte nor HepG2 host cells were affected by low drug concentrations. The effects of the drug *in vivo* remain to be investigated.

COMPETITIVE INHIBITORS OF PYROPHOSPHATES

Bisphosphonates are stable nonhydrolysable pyrophosphate analogues (P-O-P) in which the oxygen is replaced by a carbon (P-C-P) with various side chains (R^1 or R^2) (Fig. **7**). The fact that bisphosphonates are FDA approved cancer drugs makes them another attractive group of drugs for the potential treatment of tropical diseases. Many bisphosphonate drugs are currently in clinical use for the treatment and prevention of osteoporosis, Paget's disease, hypercalcemia due to malignancy, tumour metastases in bone [93]. Several of these inhibit the process of bone resorption by binding bone mineral. In particular, some bisphosphonates (such as clodronate) inhibit enzymes in signal transduction pathways (e.g. vacuolar H+-ATPase or protein tyrosine phosphatase) of osteoclast cells, which are large multinucleated cells involved in bone repair and responsible for breaking down old and fatigued bone. Other more potent nitrogen-containing bisphosphonates or aminobisphosphonates (such as pamidronate, alendronate, ibandronate and risedronate) have also been reported to have profound effects inducing apoptosis in osteoclasts.

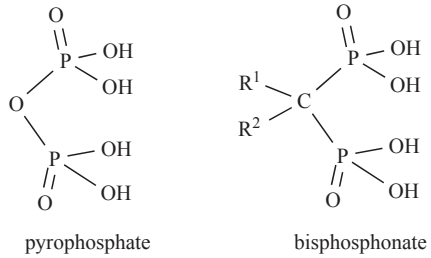


Fig. (7).

Some of these compounds were also active against several apicomplexan and trypanosomatid parasites [94-96]. For example, using the drug pamidronate in an experimental model of cutaneous leishmaniasis, Balb/c mice were found to be radically cured of their lesions. *L. mexicana amazonensis* parasites disappeared totally from lesion sites. Indeed, parasites were no longer detectable, despite the use of a variety of well-established detection methods, such as histopathological analysis, parasite cultivation and PCR amplification for kinetoplast DNA in necropsy material [97]. The compound pamidronate, like alendronate or risedronate with a side chain carrying an aromatic ring also exhibited activity *in vitro* and *in vivo* against *T. cruzi*, with no toxicity to the cells [94]. Intracellular forms (amastigotes) of the parasite were killed, but no correlation was found between growth inhibition and inhibition of vacuolar pyrophosphate, one presumed target of bisphosphonates [94]. Inhibition of *T. gondii* intracellular proliferation *in vitro* was also seen with pamidronate and risedronate, but again no correlation was found between growth inhibition and inhibition of the parasite vacuolar pyrophosphate [95]. Nonetheless, with some of the N-containing bisphosphonate drugs tested, their effects were shown to be protozoan-specific. Since the drugs accumulate preferentially in the parasites, the inhibitory activities are probably related to the fact that pyrophosphate-rich metabolic pathways as well as calcium- and pyrophosphate-rich compartments called acidocalcisomes exist in these cells [98].

The action of bisphosphonates on *E. histolytica in vitro* was shown in early work by Eubank and Reeves [99]. Because bisphosphonates are competitive inhibitors of PP_i, the *E. histolytica* PP_i-dependent phosphofructokinase was proposed as a target for bisphosphonate-based chemotherapy of amoebiasis, in particular since the enzyme is thought to play a critical role in energy metabolism and is different

from the ATP-dependent counterpart of its human host. However, even though a number of bisphosphonates were found to have inhibitory effects on the amoebic enzyme, there was no clear correlation between amoeba killing and inhibition of its PP_i-PFK [100], results which would suggest that PFK is not the target of the drug.

Work on different nitrogen and non-nitrogen containing bisphosphonates now seems to suggest the site of action to be in the mevalonate pathway, in which the enzyme farnesyl pyrophosphate synthase (FPPS) is inhibited and protein prenylation is prevented from taking place. The detrimental effects are seen through their capacities to block the post-translational transfer of prenyl groups (C15 farnesyl or C20 geranylgeranyl) to a family of crucial GTP-binding proteins, protein prenylation being essential for their membrane localisation and biological functions. The genes encoding protein farnesyl transferases (PFT), responsible for attaching the farnesyl group to proteins have been identified in *P. falciparum*, *T. brucei* and *L. major* [101]. In the framework of the present review, it is noteworthy that several hundred PFT inhibitors have been developed, some of which have entered phase II clinical trials for the treatment of human malignancies. Further studies identifying PFT inhibitors to be more toxic to *P. falciparum* and *T. brucei* than to mammalian cells is a nice illustration of the piggy-back approach for the rapid development of antiparasitic agents.

Alkylbisphosphonates, in particular are active in blocking *E. histolytica* growth and are also potent inhibitors of FPPS activity. The mode of action is presumably explained by the lipophilic nature of the alkyl side chains in enhancing membrane transport, and targeting FPPS [102]. Examples of active compounds of this type include simple *n*-alkyl-1-hydroxy-1,1-bisphosphonates with alkyl side chains of 9 or 10 carbons, ones with branched, phenylalkyl or arylalkyl side chains. Additionally, a number of 1-hydroxy-1,1-bisphosphonates derived from fatty acids were also shown to be potent and competitive inhibitors of *T. cruzi* FPPS activity with IC_{50} values in the low micromolar range (< 10). The efficacy of the drugs on the enzyme also correlated well with the effectiveness of the drug as antiparasitic agents [103]. Interestingly, these inhibitors were also active, even in the nanomolar range on the *T. brucei* FPPS activity. On the other hand, there were aniline derivatives (containing either phenoxylalkyl, biphenyl or phenyl-di-*tert*-butyl side chains) which were highly active (with IC_{50} values ~ 4 – 9 μM) against *E. histolytica*, but none of them were potent FPPS inhibitors. For some of these bisphosphonates, it appears that neither the FPPS nor the PP_i-PFK is the targeted enzyme in *E. histolytica* parasites.

Several bisphosphonate compounds have also been investigated for *in vitro* inhibition of *P. falciparum* growth. Like the complex trend observed for *E. histolytica* parasites, compounds that exhibited measurable antiplasmodial activity (IC_{50} <200 μM) were also of different structures. Many of the active compounds belonged to the simple *n*-alkylbisphosphonates, where the general pattern of activity increased with chain length for the shorter chain compounds and activity decreased for compounds with the very long side chains. There were also similarities in the structures of the most active species. In both organisms, compounds with long hydrophobic side chains had higher activities, suggestive of an important role in membrane transport. The most active compounds against *Plasmodia* belonged to the more hydrophobic analogues with large and/or uncharged side chains as well as the simple alkyl bisphosphonates, rather than to the nitrogen-containing species. Such an observation is in line with the reasoning that lipophilic bisphosphonates (containing n-alkyl side chains) are taken up more efficiently than those bisphosphonates with a charged nitrogen atom in the side chain, thereby making them more suitable for future inhibitor design.

Despite the fact that the mechanism of action is still an open question, some of the potentially interesting bisphosphonates were examined in a hamster model of *E. histolytica*-induced liver abscess formation. The choice of compounds was made on the basis of their low IC_{50} values but high therapeutic indices. These included some simple alkyl bisphosphonates and other more complex aniline derivatives. *In vitro* and *in vivo* findings did not readily correlate with each other, but nevertheless, some promising initial *in vivo* results for reduction in liver abscess formation have emerged, so that useful drug leads are now available for the development of antiamoebic drugs. Equally encouraging results have been reported with *P. berghei*, whereby the pattern of activity of some of the drugs *in vivo* (reflected as percentage reduction of parasitaemia) was found to parallel their *in vitro* activity.

INHIBITORS OF POLYAMINE SYNTHESIS

The targeting of polyamine metabolism which has served as a powerful tool in antitumor chemotherapy is also proving useful as an intervention method to fight human parasites. It is long known that the cellular requirements for polyamines are high when cells are rapidly dividing, such as in tumour cells and parasitic organisms. Like in the development of anticancer agents, the working hypothesis to test the potential of this pathway in antiparasitic chemotherapy is based on the depletion of cellular polyamine levels as a strategy to inhibit parasite proliferation. Different approaches that have been taken include the prevention of polyamine biosynthesis through inhibition of enzymes in the pathway, the blockade of the uptake of exogenous polyamines or the manipulation of polyamine concentrations in the cell using polyamine analogues. When analogues are taken up by the cell, they replace the natural polyamines. They also lead to a decrease in polyamine synthesis and an increase in polyamine catabolism and export. The cell is unable to divide and consequently dies. In humans, polyamines are synthesised from the amino acid ornithine. The three commonly occurring polyamines required for cell growth and division are putrescine, spermidine and spermine (Fig. **8**), and are made in three consecutive steps.

The enzyme ornithine decarboxylase (ODC), the initial enzyme of polyamine synthesis, converts ornithine into putrescine, while S-adenosylmethionine decarboxylase (AdoMetDC) generates the substrate used by spermidine and spermine synthases to form spermidine and spermine, respectively [104, 105]. Interestingly, while the host enzymes are encoded by two different genes, the genome of *P. falciparum* has an open reading frame encoding a bifunctional ODC/AdoMetDC protein [106-108]. Distinct regulatory features between the monofunctional host

enzymes and the bifunctional parasite enzyme can therefore be exploited in the design of new chemotherapeutic agents against malaria.

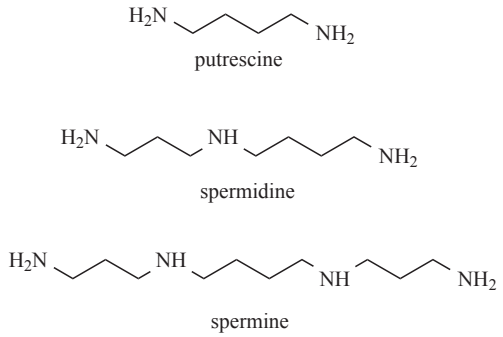


Fig. (8).

A number of classical ODC/AdoMetDC inhibitors, such as α-difluoromethylornithine (DFMO), methylglyoxal bis(guanylhydrazone) (MGBG) and MDL73811 have been assessed for their capacities to interfere with polyamine synthesis in *Plasmodium* species. Even though DFMO inhibited sporozoite formation in the insect vector and prevented further development of liver stages, it only showed limited effects on the erythrocytic stages *in vivo.* The AdoMetDC inhibitor MDL73811 was potent against *P. falciparum* in culture, but was found to be inactive against *P. berghei* in infected mice, probably because of rapid clearance of the drug. Inhibition of polyamine metabolism as a means of antitrypanosomal chemotherapy has also been successful using DFMO and MDL 73811 [109]. MGBG used alone at 25 mg/kg for 3 days was not curative for a laboratory strain of *T. brucei brucei* [110]. A combination of DFMO and MGBG showed antagonistic effects.

In view of these drawbacks, a new generation of inhibitors of ODC and AdoMetDC with structures related to 3-aminooxy-1-aminopropane (APA) and bis(guanylhydrazones) (CGP derivatives), respectively, have now emerged (Fig. **9**). Several of these compounds have been reported to be more successful in blocking cell proliferation of tumour cells and parasites than their progenitors. The ODC inhibitors APA and the APA derivatives CGP 526A and CGP 54169A were 500 to 1000 fold more effective than the classical inhibitor DFMO in inhibiting growth of *P. falciparum* as determined by tritiated hypoxanthine incorporation [111]. Addition of exogenous putrescine to the culture medium completely abrogated their inhibitory effects, which is taken as evidence that the effects of the drugs on parasite growth were the result of putrescine synthesis inhibition.

The bicyclic analogue of MGBG, CGP 40215A is a potent inhibitor of *P. falciparum* AdoMetDC activity (K_i value of 1 μM) and an *in vivo* plasmodial effect (IC_{50} of 3 μM). The effect however was only slightly abolished by supplementation of spermidine, it therefore appears to act on targets distinct from the polyamine biosynthetic pathway of the parasite. CGP 40215A was also active and strongly synergistic with DFMO against a model CNS infection. In addition, the compound was able to cure infections by 19 clinical isolates of *T. brucei* subspecies as well as a *T. congolense* isolate, several of which exhibited resistance to DFMO as well as standard trypanocides [112]. Therefore this compound is an excellent candidate to fight human and veterinary trypanosomiasis, and thus deserves to be further studied.

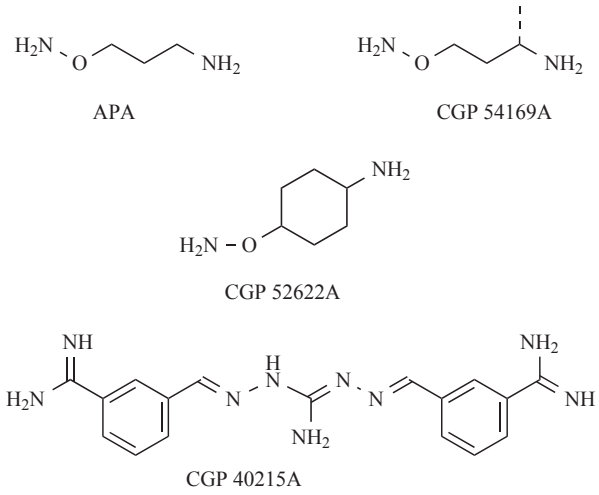


Fig. (9).

Different features between the *Onchocerca volvulus* AdoMetDC enzyme and that of the host allows the specific inhibition of the nematode enzyme to be exploited. The effects of several new and previously synthesised tetramines (with a 3-3-3 or 3-3-4 carbon backbone) that carry at least one terminal amino moiety stimulated the nematode enzyme efficiently, but had no or low stimulatory effect on the host enzyme [113]. Another group of tetramines with a 3-7-3 backbone (bis(benzyl)polyamine), previously shown to have a filaricidal effect *in vitro* [114] and another with a 3-8-3 backbone inhibit the uptake of putrescine, spermidine and spermine by *Brugia pahangi*. The analogues when tested with putrescine-stimulated AdoMetDC led to reduction of enzyme activity, with the nematode enzyme more sensitive than the human enzyme.

An increasingly successful method is to use polyamine analogues to manipulate natural intracellular polyamine concentrations. Early work has shown that bis(benzyl)polyamine analogues, as substrates for purified polyamine oxidase have both antiplasmodial [115] and antileishmanial properties in experimental models [116]. The major polyamine analogues available to date are analogues of spermidine or spermine [117]. The best characterised spermine analogue called IPENSpm was shown to induce apoptosis in human leukaemia cells. The presence of peroxide and other lethal hydroxyl radical byproducts resulting from the interaction of the analogue with the enzyme presumably leads to apoptosis [118]. The mechanisms by which IPENSpm induces cell death and alters polyamine synthesis is a promising new lead in drug discovery for the treatment of human cancers, and possibly also parasite infections. A thorough understanding of the precise mode of action of the drug will provide a solid platform from which novel analogues can be designed and tailored for specific uses.

ALKYLLYSOPHOSPHOLIPID INHIBITORS

A group of anticancer alkyllysophospholipids (ALPs), edelfosine, miltefosine, ilmofosine and SRI 62-834 (Fig. **10**), with activity against *L. donovani*, *T. cruzi* and *T. brucei* has

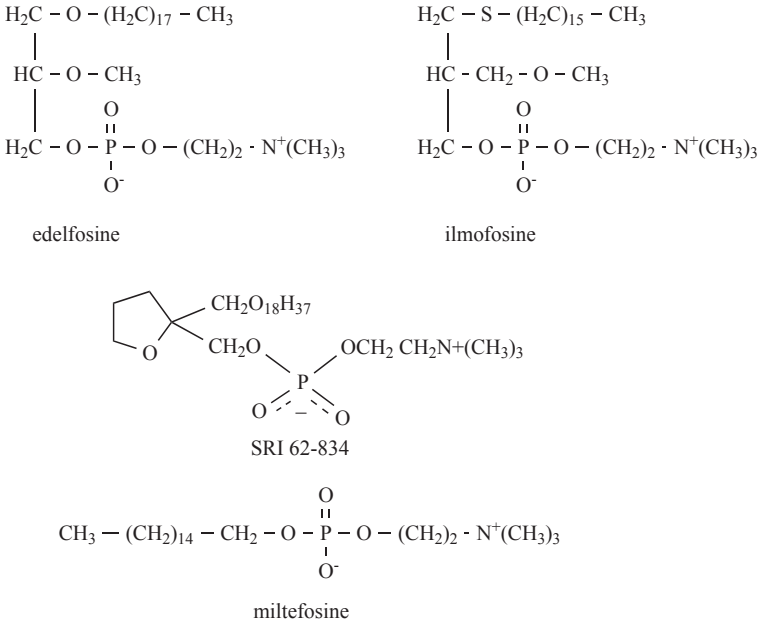


Fig. (10).

been identified [119]. All ALP analogues were significantly active against intracellular *L. donovani* amastigotes *in vitro* as well as against intracellular *T. cruzi* amastigotes in macrophages. Even though all four ALPs have *in vivo* activities against tumour cells [120], in the BALB/c mouse models, only ilmofosine and miltefosine showed promising activity against *L. donovani* and *T. cruzi*. The difference between the anticancer and antiparasitic properties of the ALPs is not clear, but could be explained in terms of variation in pharmacological properties in the mouse model. The anticancer activity of ALPs is believed to affect phospholipid biosynthesis, protein kinase C, phospholipase C, cell invasion and macrophage activation [120].

More recent work on the lysophospholipid analogues demonstrated that edelfosine, miltefosine and ilmofosine presented potent antiproliferative activity for promastigotes and intracellular amastigotes of *L. amazonensis* [121]. In both forms, edelfosine was able to induce extensive mitochondrial damage, multinucleation and in promastigotes the drug also caused plasma membrane alterations, formation of autophagic structures and membranous arrangements inside the flagella pocket. More conclusive data from ultrastructural and flow cytometry studies currently point to the parasite mitochondrion as the target of edelfosine. ALPs thus represent novel antiprotozoal drugs with potential for the treatment of leishmaniasis and South American trypanosomiases.

CONCLUDING REMARKS

Drug development in cancer research has certainly paved the way for entirely new and innovative antiparasitic approaches. A myriad of promising candidate drugs has been tested on diverse parasites and this review has attempted to demonstrate some of the efforts made in this endeavour but the list is far from complete. Renewed interest in parasitic diseases among the scientific community is likely to lead to more rapid progress in the field. The prospects in infectious disease therapy are therefore not as dull as it was 10-20 years ago. Despite this encouraging progress in drug development against parasites, real success stories are still rare. This might in part be due to missing links between the different branches of related research areas like parasitology, biochemistry and pharmacology. Obviously, more multidisciplinary projects are needed to combine forces and to develop effective antiparasitic drugs from the many identified lead compounds. Supported by the complete sequencing of the genomes of all major human and animal parasites, new pathways will be identified and currently unforeseen therapeutic indications expected to be discovered. Let us hope that pharmaceutical companies, despite low profit perspectives, will regain their interest and become involved again in the process and help to make the drugs affordable for the people living in developing countries.

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