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Review Article

Chemotherapeutic targets for antiparasitic therapy

Philippe Lawton*, Marie-Élisabeth Sarciron, Anne-Françoise Pétavy

Université Claude-Bernard, ISPB-Faculté de Pharmacie, EA-3741, Laboratoire de Parasitologie et Mycologie Médicale, 8 avenue Rockefeller, F-69373 Lyon Cedex 08, France.
**Correspondence: e-mail: lawton@univ-lyon1.fr*

CONTENTS

Abstract

Despite the worldwide importance of parasitic diseases, antiparasitic chemotherapy is faced with spreading resistance and a lack of interest from pharmaceutical companies. In recent years, however, powerful new research techniques, such as high-throughput screening and advanced proteomics, have emerged and allowed the identification of new molecules and potential targets. In addition, some new antiparasitic drugs benefited from antiretroviral and anticancer research. Since parasites share many metabolic routes with their hosts, efficient drugs must reach them without being harmful to the patient, and in this respect, selectivity is a key parameter. In this review, we cover the most important chemotherapeutic targets thus far identified within the complex host-parasite relationships.

Introduction

A number of major human and animal diseases are caused by eukaryotic uni- or pluricellular parasitic organisms. Their complex structure and interactions with their hosts represent a challenge for the yet unsuccessful search for effective vaccines, and chemotherapy still remains the only effective way of combating these infections. Despite the existence of very active drugs, resistance has been spreading among some of the most dangerous parasites, and the appearance of AIDS highlighted the threat caused by opportunistic agents to immunocompromised patients. The high cost of developing new drugs, especially for use against human parasitic diseases in underdeveloped countries or in livestock, explains in part the limited number of new molecules. The low profit/investment ratio does not favor increasing industrial research efforts in this field, which are mainly in the hands of a few academic laboratories. New research tools have allowed the discovery of specific targets and the optimization of the synthesis of molecules of interest in antiparasitic chemotherapy.

Parasites as a major public health concern

Of the many parasites affecting mankind and livestock (Table I), only a few have been thoroughly characterized, often due to technical difficulties including the scarcity of *in vitro* and *in vivo* models. The morbidity and mortality associated with malaria parasites, as well as the availability of animal models and continuous *in vitro* cultivation of *Plasmodium falciparum*, made malaria a top priority. On the other hand, although trypanosomatids are easy to maintain in culture, they cause much fewer cases restricted to certain areas, such as equatorial Africa for the sleeping sickness agent *Trypanosoma brucei* or south America for Chagas' disease due to *Trypanosoma cruzi*. Toxoplasmosis and cryptosporidiosis may become lethal in immunocompromised patients and their importance tremendously increased with the AIDS pandemic. Among diseases caused by metazoan parasites, schistosomiasis and filariasis are helminth infections causing millions of casualties, but research is impaired by the complexity of the parasites and the availability of still very active drugs.

New strategies for drug development

Antiparasitic drugs were traditionally discovered by exploiting traditional medicine or by chemical synthesis. Their mode of action was often unknown, discovered later or deduced from their bacterial target. This is particularly true for anthelmintics such as praziquantel, benzimidazoles (1) and the new cyclooctadepsipeptide class (2). The Vietnam war triggered the development of research on antimalarials (3), but at present, the costs and difficulties of screening molecules active against parasites hinder the classical research strategies (4). New technologies for potential target identification, screen

Table I: Overview of important human parasitic diseases.

	Parasite	Transmission	Disease	Available chemotherapy	Resistance	Population at risk Morbidity/mortality
Protozoans	*Plasmodium* spp.	*Anopheles* mosquitoes	Malaria	+	++	300 million/1 million deaths per year
	Toxoplasma gondii	Food	Toxoplasmosis	+	–	Pregnant women, AIDS patients
	Trypanosoma brucei	Tse-tse fly	Sleeping sickness	+	+	60 million/300-500,000
	Trypanosoma cruzi	Triatominae bugs	Chagas' disease	+	+	100 million/16-18 million
	Leishmania spp.	Sand fly	Leishmaniasis	+	±	350 million/12 million
	Giardia lamblia	Drinking water	Giardiasis	+	–	Children
	Entamoeba histolytica	Drinking water	Amebiasis	+	-	35-50 million/100,000 deaths per year
	Trichomonas vaginalis	Sexually transmitted	Trichomoniasis	+	–	Sexually active persons
Helminths	*Cryptosporidium parvum*	Drinking water	Cryptosporidiosis	–	N/A[§]	Children, AIDS patients
	Schistosoma mansoni	Water	Schistosomiasis	+	±	200 million/120 million
	Brugia malayi, Wuchereria bancrofti	*Culex* mosquitoes	Lymphatic filariasis	+	±	1 billion/120 million

[§]N/A : not applicable.

development and lead optimization are also needed in the field of animal parasitology (5).

Besides the development of analogues of existing agents and the discovery of natural products (6, 7), the use of compounds originally developed for cancer has raised great expectation (8, 9). Although the development of new methods, such as protein crystallography (10, 11), 3D QSAR (12) and combinatorial chemistry (13), may help to design new derivatives of existing lead drugs, the actual trend is to define specific targets in order to synthesize lead compounds (14). This could be aided by the increasing availability of sequenced parasite genomes (15) and understanding of gene expression in parasites (16, 17). Proteomic and gene filtering approaches are increasingly attractive (18-20), but one must keep in mind that both the human immune system and drug use are driving parasite evolution (21). In the present review we will focus on the main feasible targets for chemotherapy.

Specificity and selectivity

Like their vertebrate hosts, parasites are eukaryotes that use the host to fulfill their metabolic needs, concentrating on the vital process of reproduction. The result is the existence of intimate physical relationships and intricate metabolic networks between the parasite and its host. Consequently, an efficient chemotherapeutic strategy should be based on the impairment of one or several parasitic metabolic pathways which the host can bypass with alternate metabolic routes or different or insensitive enzymes. Research has focused on the few parasite-specific targets and on common pathways with differences suggesting potential selectivity (Fig. 1).

Parasite-specific targets

Polyamine and thiol metabolism

Polyamine metabolism in protozoan parasites may be selectively different from that of the host. *Cryptosporidium parvum* has a plant-like polyamine metabolism (22), and the trypanosomatids are metabolized by a unique thiol-redox pathway where glutathione reductase (GR) is replaced by trypanothione reductase (TR), an enzyme using trypanothione, the combination of two molecules of glutathione with spermidine (23-26). Recently, a homologue was found in the human parasite *Entamoeba histolytica* (27), which is involved in the detoxification of H_2O_2, organic hydroperoxides and ribonucleotide reduction (28). Recombinant *Leishmania donovani* TR has been successfully produced and is expected to be useful for testing drug candidates (29).

Difluoromethylornithine (DFMO), an irreversible inhibitor of trypanothione biosynthesis, has been approved for the treatment of African trypanosomiasis, but its cost and mode of administration limit its use (23). A large number of molecules, such as reversible polyamine-, peptide-based and irreversible inhibitors have been reported to selectively inhibit TR, but not human GR (30). Polyamine-peptide norspermidine-based inhibitors displayed an allosteric mode of inhibition of trypanothione reductase (31), and more recently, the macrocyclic alkaloid lunarine and its analogues were shown to be effective (32). The sensitivity of the *T. cruzi* TR to the lead 5-nitroimidazole compounds nifurtimox and benznidazole has been exploited in the design of 5-nitrofuryl derivatives (33), and docking studies were performed to increase the selectivity of this class of inhibitors (34). Phosphinopeptides mimicking the tetrahedral transition state of *T. cruzi* trypanothione synthase were recently reported as potent growth inhibitors (35). Diamine derivatives targeted to disrupt the polyamine metabolism of trypanosomatids are also active *in vitro* against the malaria parasite *P. falciparum* (36) (Fig. 2).

Carbonic anhydrase

At least three isozymes of the α-class were identified in *P. falciparum* and the rodent malarial parasite *Plasmodium berghei* as potential drug targets (37).

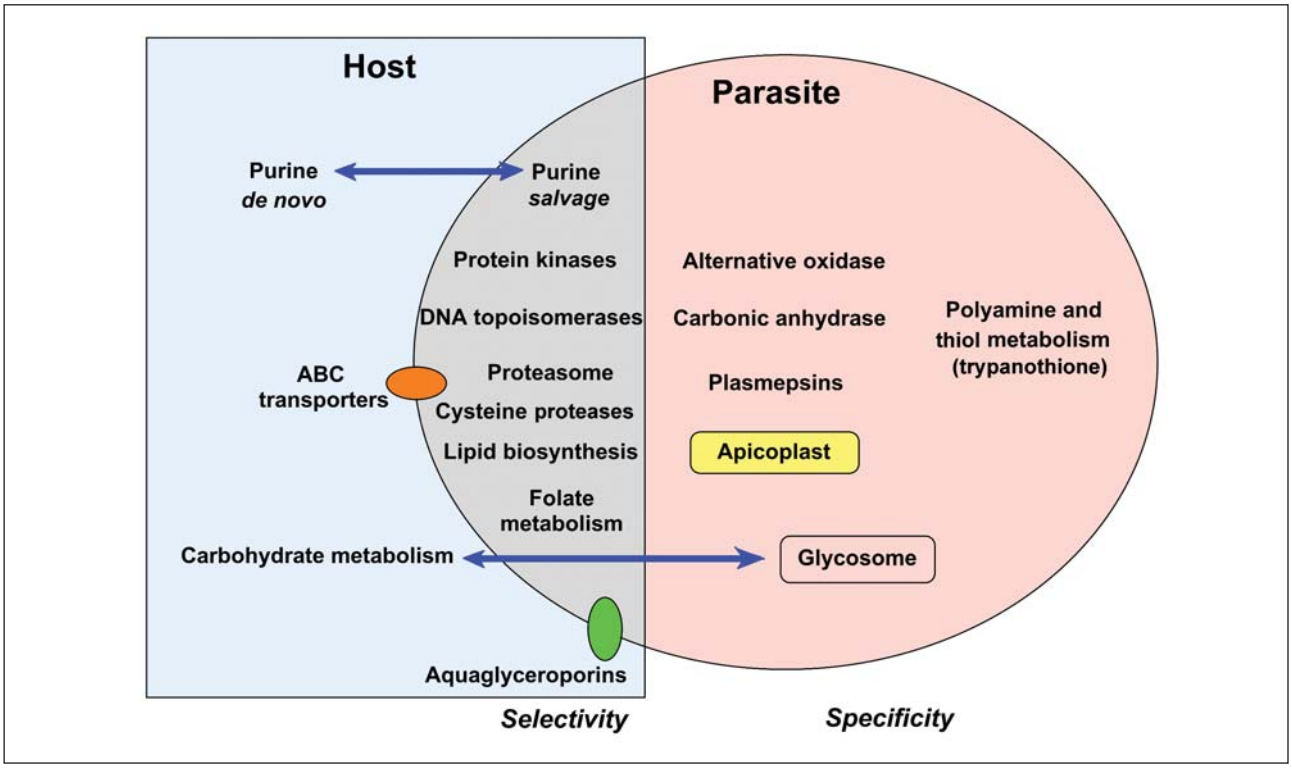

Fig. 1. Chemotherapeutic targets in relation to host-parasite interactions. Parasite-specific pathways or organelles need only be reached by active drugs, whereas common metabolic pathways require inhibitors with good selectivity. Arrows show common metabolic pathways with structural differences (trypanosomal glycosome) or with a pathway missing in parasites (*de novo* purine synthesis).

Aromatic sulfonamides have been produced that inhibit parasite carbonic anhydrase (38).

Alternative oxidase

The adaptation of parasites to the low oxygen tension of the host led to the selection of anaerobic metabolic pathways such as trypanosome alternative oxidase (TAO), a specific enzyme of the respiratory chain that does not exist in the host and is inhibited by ascofuranone (39).

The apicoplast and lipid biosynthesis

Lipid metabolism is an attractive target in malaria due to membrane biogenesis in the developing intraerythrocytic *Plasmodium* (40) and the fact that *Toxoplasma* scavenges lipoic acid from its host (41). In apicomplexan parasites such as *Toxoplasma* and *Plasmodium,* the early steps of lipid synthesis occur in a recently identified specific plastid-like organelle, known as the apicoplast (42, 43). This led to the idea of targeting the apicoplast (44) through the mevalonate-independent pathway of isoprenoid biosynthesis, also known as the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DOXP) pathway (45-49). Type II fatty acid biosynthesis (FAS), the main metabolic route, and the DOXP metabolic pathway are both found in eubacteria and higher plants, thus justifying their interest as biological targets (50, 51). Fosmidomycin, an inhibitor of the DOXP pathway, was reported to be an effective antimalarial (52), and antiplasmodial α-substituted (53) and cyclopropyl analogues (54) were recently synthesized (Fig. 3A).

Although *Cryptosporidium* lacks an apicoplast, parts of its lipid metabolism are susceptible for targeting (55). This organelle also does not exist in trypanosomatids, but the sterol biosynthetic pathway can be used for chemotherapeutic purposes, since it uses ergosterols instead of cholesterol (56-58). The protein prenylation pathway, particularly farnesyl pyrophosphate synthase, is targeted by bisphosphonate-containing drugs in use for the treatment of bone resorption disorders, and analogues active against *T. cruzi* were synthesized (59). The antiproliferative effects against *Toxoplasma gondii* of azasterols both alone and in combination with antifolates were reported by Dantas-Leite (60) (Fig. 3B).

One of the few new antiparasitic drugs recently brought to the market is miltefosine (Fig. 3C), an alkylphospholipid with oral antileishmanial activity (61). Its original target was the lipid biosynthetic pathway of cancer cells, but efficacy against *Leishmania* (62, 63), *T. cruzi* (64), *E. histolytica* (65) and the flagellate *Trichomonas vaginalis* (66) was reported. However, resistance has appeared (67) and there are concerns about its teratogenicity and interactions with other antileishmanials (68). Ring-substituted ether phospholipids were reported to be more potent against *Leishmania* parasites *in vitro* (69) and a thiol analogue of similar efficacy as the parent drug was recently synthesized (70).

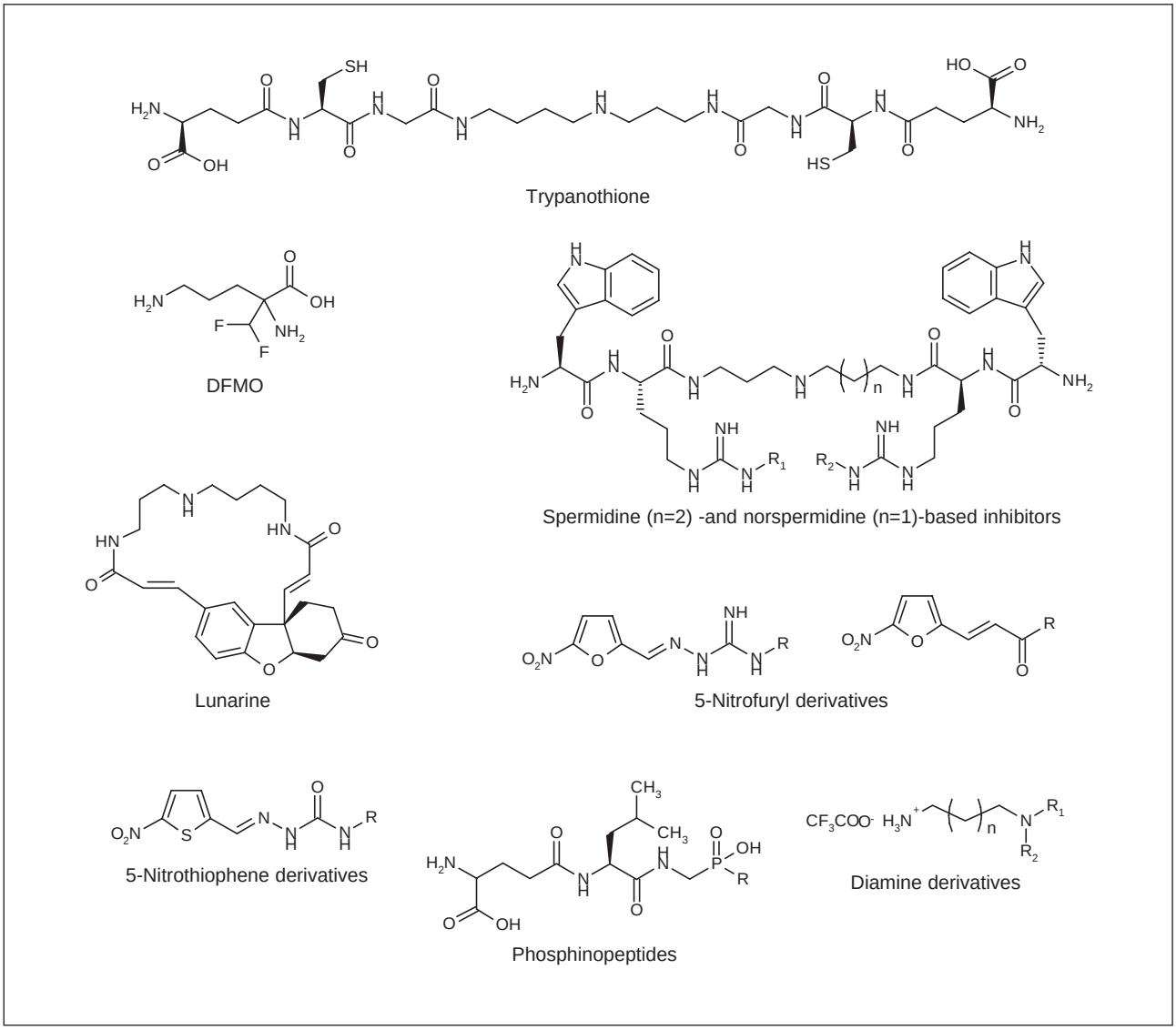

Fig. 2. Structures of trypanothione and some parasitic polyamine metabolism inhibitors.

Carbohydrate metabolism

In amitochondriate protists such as *T. vaginalis* and *Giardia lamblia*, certain enzymes involved in energy metabolism are phylogenetically different and originate from different sources than other eukaryotes (71). Carbohydrate metabolism has been proposed as a drug target in trypanosomatids, particularly because enzymes are compartmentalized in the glycosome, a divergent peroxysome where glycolysis takes place (72). The hexose transporter (73) and the pyruvate kinase from *P. falciparum* have been characterized (74). Enzymes such as *T. brucei* enolase (75) and hexokinase (76) have been described, as well as glyoxalase II from *L. donovani* (77) and lactate dehydrogenase (LDH) from *T. gondii* (78). Recently, three key glycolytic enzymes from *C. parvum* were crystallized (79).

Subversive substrates of 6-phosphogluconate dehydrogenase (6PGDH) from the African trypanosome *Trypanosoma brucei rhodesiense* and related flagellates were designed (80) and bisphosphonates were used to specifically target the *T. cruzi* hexokinase (81). Azole-based compounds identified using a high-throughput enzymatic assay were synthesized and tested as inhibitors of the malarial LDH (82, 83) (Fig. 4).

Proteases

Proteases are involved in many metabolic processes and have been considered potential chemotherapeutic targets for several years. Since the advent of protease inhibitors in antiretroviral chemotherapy, the incidence of opportunistic infections due to *T. gondii* and *Cryptosporidium* spp. has decreased dramatically (84). Some of these inhibitors were also active *in vitro* against two *Leishmania* species (85) and malaria parasites (86, 87).

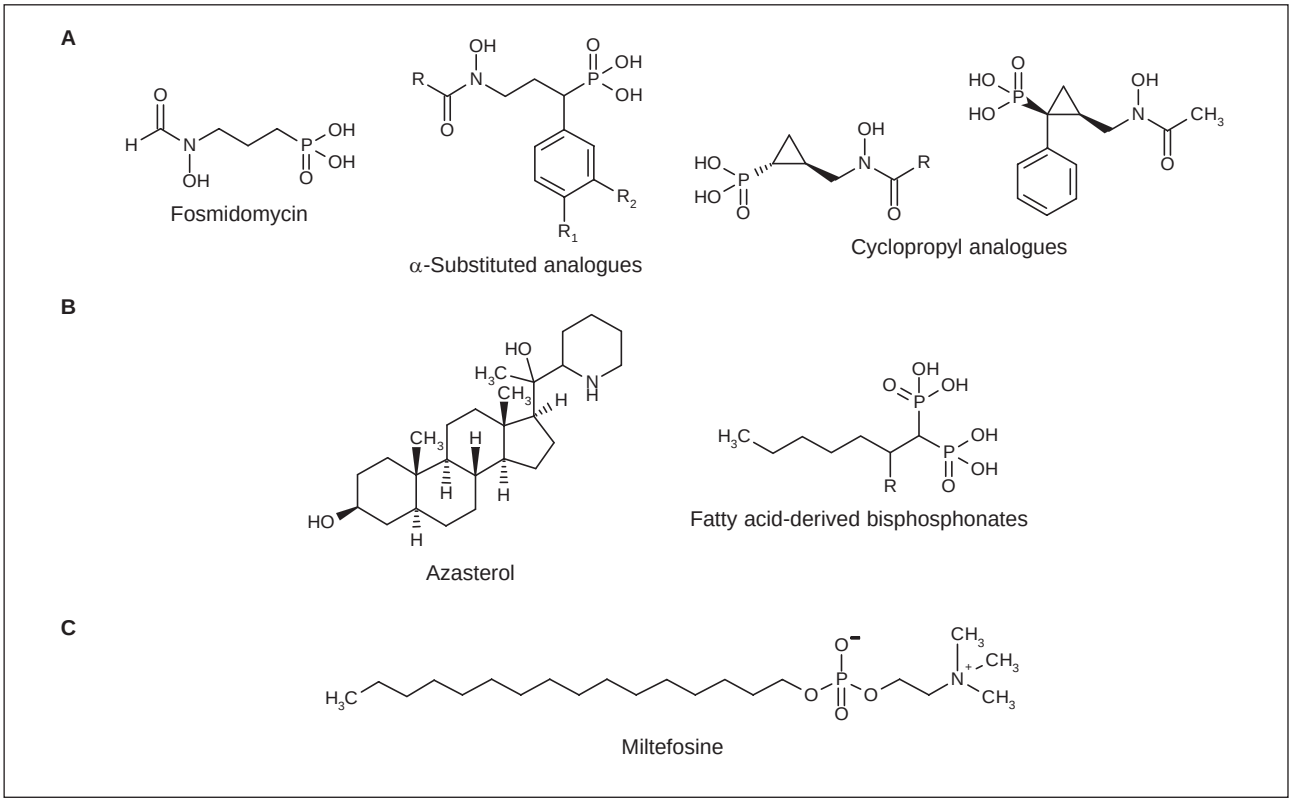

Fig. 3. **A**: Structures of apicoplast nonmevalonate pathway inhibitors**. B**: Ergosterol synthesis and protein prenylation inhibitors. **C**: Alkylphospholipid analogues.

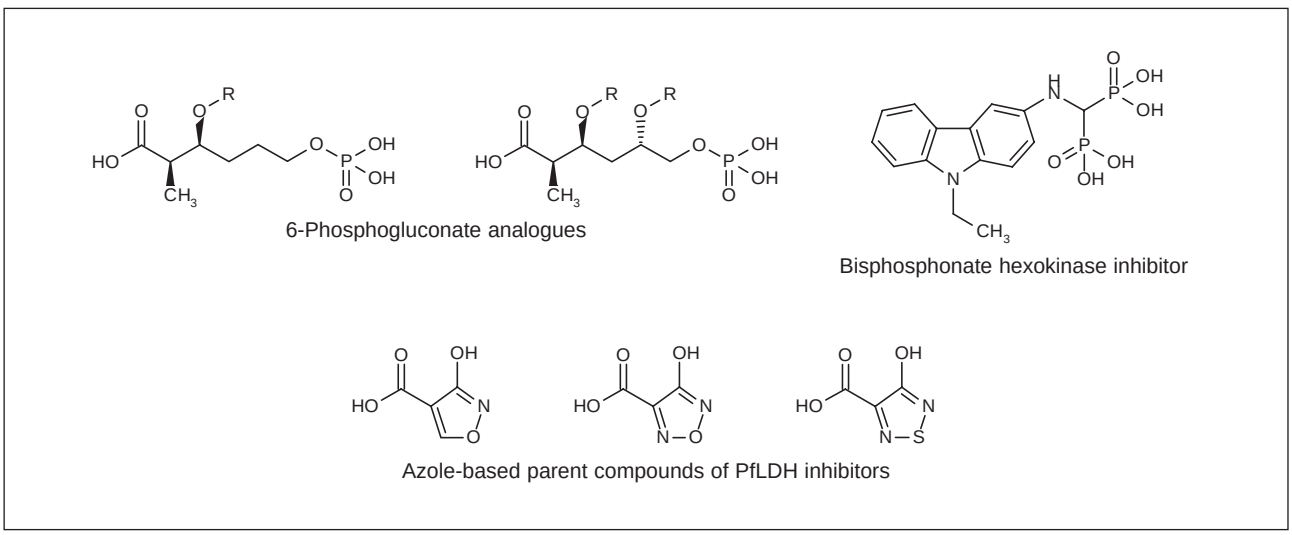

Fig. 4. Structures of inhibitors of parasite carbohydrate metabolism.

Aspartic proteases

The hemoglobin-degrading aspartic proteases from *Plasmodium* spp. are known as plasmepsins and are involved in the early steps of the feeding process of the intraerythrocytic parasites (88). Homology studies revealed that blood-sucking parasites, including schistosomes and hookworms, possess such enzymes and that selective inhibitors could be developed (89), although these enzymes (90) and human aspartic proteases, especially cathepsin D, share significant homology. Plasmepsin PfPM4 crystallization has been achieved with the possibility of targeting all four malarial species (91). The study of knockout parasites showed that they were able to compensate for the loss of an individual plasmepsin and evidenced that drug discovery efforts on these enzymes must focus on the inhibition of two or more members of this family (92) (Table II).

Table II: Aspartic proteases of important parasites.

	Parasite	Protease	Location	Role
Protozoans	*Plasmodium falciparum*	Plasmepsin PfPM1	Food vacuole	Hemoglobin degradation
		Plasmepsin PfPM2	Food vacuole	Hemoglobin degradation
		Histoaspartic protease HAP	Food vacuole	Hemoglobin degradation
		Plasmepsin PfM4	Food vacuole	Hemoglobin degradation
	Plasmodium vivax	Plasmepsin PvM4	Food vacuole	Hemoglobin degradation
	Plasmodium ovale	Plasmepsin PoM4	Food vacuole	Hemoglobin degradation
	Plasmodium malariae	Plasmepsin PmM4	Food vacuole	Hemoglobin degradation
Helminths	*Schistosoma mansoni*	SmCD	Gut of adult stage	Hemoglobin digestion
	Schistosoma japonicum	SjCD	Gut of adult stage	Hemoglobin digestion
	Ancylostoma caninum	Ac-APR-1	Infective larva, adult	Hemoglobinase, serum protein, skin macromolecule degradation
	Necator americanus	Na-APR-1	Infective larva, adult	Hemoglobinase, serum protein, skin macromolecule degradation

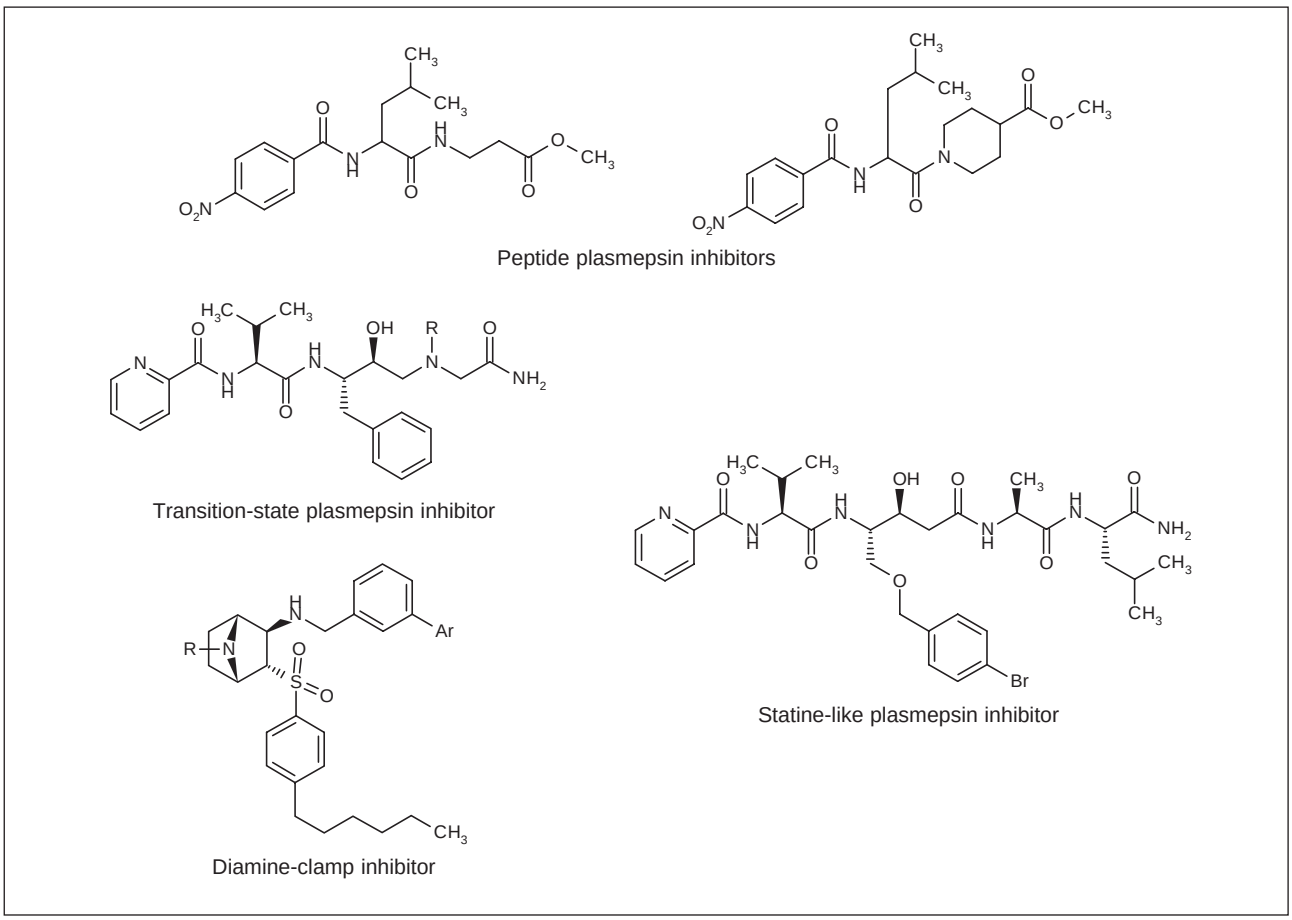

Fig. 5. Inhibitors of *Plasmodium* aspartic proteases.

It is not yet clear which plasmepsins are essential and the currently known aspartic protease inhibitors are chemically too complicated and therefore too expensive for use. A recent study of the active site of plasmepsins showed that it has high conformational flexibility, which renders docking studies for drug design computationally expensive (93). Efficient molecules should possess good selectivity and be small enough to penetrate into the food vacuole. Peptide (94), transition state-like (95), statine-like (96) or reverse statine-type isostere inhibitors of plasmepsins I and II have been synthesized (97), albeit with modest activity (98). The use of a combinatorial optimization protocol allowed the production of highly selective inhibitors (99), and recently, macrocyclic inhibitors of plasmepsins I, II and IV were produced with no activity against human cathepsin D (100). Diamine-clamp inhibitors are also promising broad-spectrum antimalarials (101). Although the selective activity of the HIV inhibitors is a promising sign, caution must be exercised, since HIV protease and plasmepsins belong to two differ-

ent groups of enzymes, the latter being much more closely related to human cathepsin D, indicating that potent inhibitors should be tested for their selectivity relative to cathepsin D (102) (Fig. 5).

Cysteine proteases

A number of parasite cysteine proteases have been described in the literature that are also potential targets for specific inhibition (Table III). Their importance is often related to developmental events such as the release of infective parasites and cell invasion, or to pathophysiological and digestive processes (103). The most extensively studied malarial cysteine proteases are the falcipains, which are involved in hemoglobin degradation and have homologues in other malarial species (104).

It has been reported that cysteine proteases are natural targets for nitric oxide (NO) and NO donor block replication of *Plasmodium, Trypanosoma* and *Leishmania* (105). Octapeptide inhibitors have been synthesized which are active against *Leishmania mexicana* (106). Peptidomimetic compounds, such as aziridine 2,3-dicarboxylates, were recently synthesized as trypanocidal and antileishmanial agents (107) and were active on promastigotes and decreased the infection rates of macrophages (108). Peptidyl vinyl sulfones were reported as promising inhibitors of the malarial falcipains (109). Thiosemicarbazones were screened against the three parasite cysteine proteases cruzain, rhodesain and falcipain-2 (110), and virtual screening was used against these proteases (111, 112). Although cross-resistance to known inhibitors of falcipain-2 is not expected, the selection of resistant *Plasmodium* strains resulted in increased overall expression of falcipain genes and the transport of inhibitors was altered (113) (Fig. 6A).

Proteasomes

Proteasomes, large nonlysosomal multiprotease complexes, are found in eukaryotes and are involved in the differentiation and replication of parasites of medical importance (114, 115). Potent and specific nonpeptide and peptide-derived inhibitors of the 20S proteasome have been developed recently as anticancer agents.

The bloodstream forms of the African sleeping sickness agent *T. brucei* are sensitive to peptidyl proteasome inhibitors (116) and to the highly selective α',β'-epoxyketone peptide proteasome inhibitors epoxomicin and YU-101 (117). The intra- and exoerythrocytic development of malaria parasites is also impaired by the dipeptidyl boronic acid proteasome inhibitor MLN-273, a bortezomib (Velcade®) analogue (118) (Fig. 6B).

Folate metabolism

Rapidly dividing cells such as cancer cells and protozoans require folate derivatives used in the synthesis of DNA. Protozoan parasites possess a bifunctional DHFR-TS and the combination of the dihydropteroate synthase (DHPS) inhibitor sulfadoxine and the dihydrofolate synthase (DHFR) inhibitor pyrimethamine has been a leading antimalarial chemotherapy since the 1940s. However, *P. falciparum* resistance due to point mutations in DHPS and DHFR-TS is spreading (119, 120).

Analogues of the third-generation antifolate WR-99210 are remarkably effective even against highly pyrimethamine-resistant *Plasmodium* parasites (121). 2,4-Diaminopteridine-based compounds were effective as aminopterine and methotrexate prodrugs against *P. falciparum* (122). 5-Benzyl-2,4-diaminopyrimidine derivatives produced by target-guided synthesis proved to be effective against *P. falciparum* DHFR (123), and potent and selective inhibitors (124) were tested against the DHFR of opportunistic pathogens, including *T. gondii*. Phenoxypropoxybiguanide analogues of the prodrug proguanil were shown to be converted in the liver to active metabolites (125) (Fig. 7). Expression levels of DHFR-TS are upregulated in the presence of inhibitors, a short-term defense mechanism counteracting the deleterious effects of the drugs (126). However, resistance can

Table III: Importance of cysteine proteases in parasite pathophysiology (for details, see Ref. 103).

	Parasite	Name	Role
Protozoans	*Plasmodium falciparum*	Falcipains 1, 2, 2', 3	Hemoglobin hydrolysis, replication, host-parasite interactions
	Trypanosoma cruzi	Cruzipain	Growth, development, host-parasite interactions, evasion of immune response
	Trypanosoma brucei	Brucipain	Growth, development, host-parasite interactions, evasion of immune response
	Trypanosoma brucei rhodesiense	Rhodesain	Growth, development, host-parasite interactions, evasion of immune response
	Leishmania spp.		Growth, development, host-parasite interactions
	Giardia lamblia		Excystation, evasion of immune response
	Entamoeba histolytica		Nutrition, extracellular matrix degradation, invasion
Helminths	*Schistosoma* spp.		Host-parasite interactions, growth, hemoglobinase, evasion of immune response
	Brugia spp.		Growth and development
	Fasciola spp.		Host-parasite interactions, growth, hemoglobinase, evasion of immune response
	Ascaris spp.		Hemoglobinase, growth and development
	Necator americanus		Hemoglobinase, evasion of immune response

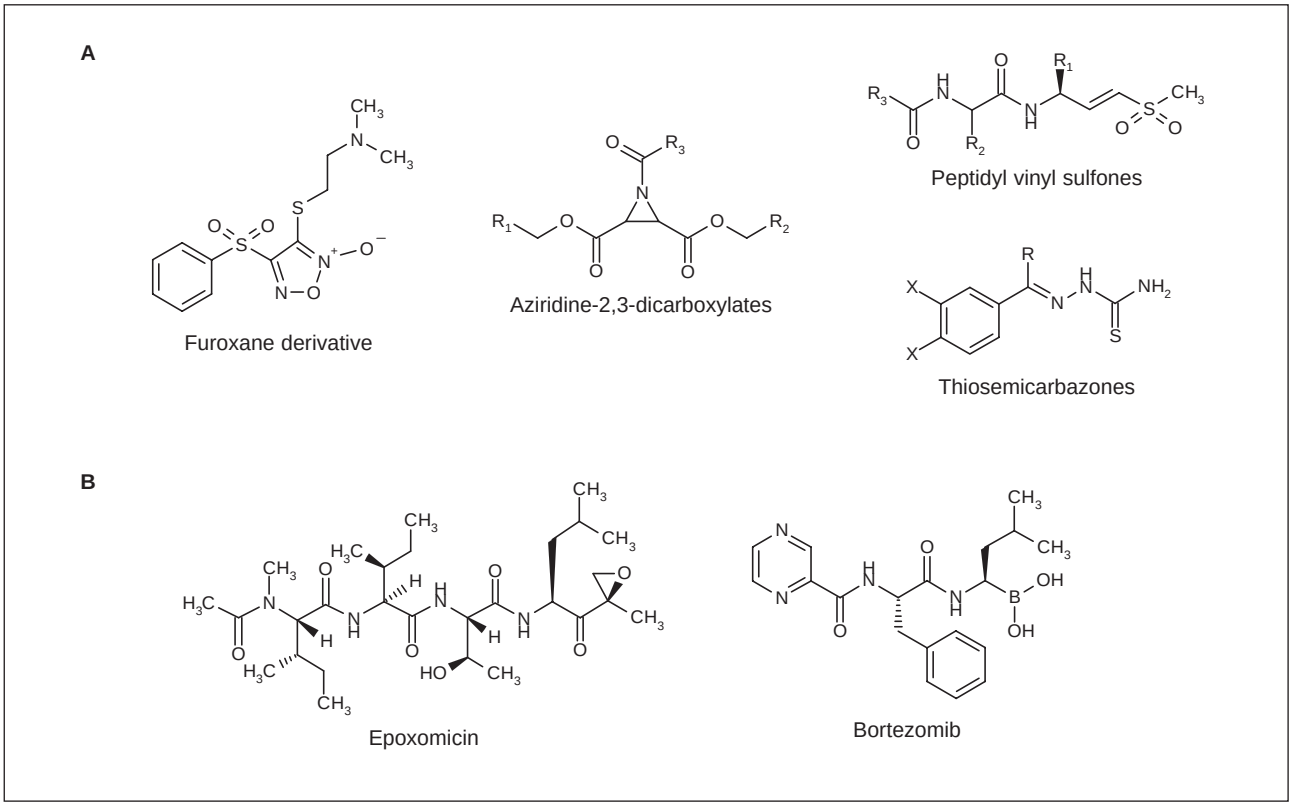

Fig. 6**. A**: Inhibitors of parasite cysteine proteases. **B**: Proteasome inhibitors.

not be solely attributed to genetic processes and switching to other metabolic pathways should be addressed (127).

Aquaporins

Aquaporins are membrane proteins that transport water across membranes; similar transporters known as aquaglyceroporins can be permeated by water, glycerol and some small solutes. They have been described in the parasite nematode *Toxocara canis* (128) and in the protozoans *P. falciparum* and *T. gondii*, where similarity with plant homologues was evidenced. Their role in drug uptake was also evidenced in *Leishmania*, *T. brucei* and *T. cruzi* (129), where swelling of acidocalcisomes mediated by an aquaporin is involved in osmotic regulation (130). These new permeability pathways are regarded as targets with potential for the development of antimalarials (131). Recently, dihydroxyacetone and methylglyoxal, permeants of the *P. falciparum* aquaglyceroporin, were reported as inhibitors of parasite proliferation (132).

Ubiquitous targets

Protein kinases

Protein kinases are key effectors in the growth of both parasites and the host, and are also involved in cancer pathogenesis, a process with many common features (9). In particular, cyclin-dependent kinases (CDKs) are targets in parasites (133, 134). The protein kinases from *P. falciparum* were phylogenetically analyzed and were found to be highly divergent from their mammalian counterparts (135). They are also putative targets in trypanosomatids (136).

Oxindole-based compounds are inhibitors of CDKs and were reported as potent antimalarials (137). Imidazopyridines were recently synthesized as potent inhibitors of the coccidian pathogen *Eimeria tenella* cGMP-dependent protein kinase (138) and were active against *Toxoplasma* (139), and a PfPKG inhibitor was reported to be active on erythrocytic stages of *P. falciparum* and in a murine model (140). Staurosporine (a serine/threonine kinase inhibitor), genistein (a tyrosine kinase inhibitor) and wortmannin (a phosphatidylinositol 3-kinase inhibitor) were active against the agent *T. cruzi* (141). As a novel approach for identifying anticoccidial agents, the inhibitory activity of isoflavone analogues (dihydroxyisoflavone and trihydroxydeoxybenzoin derivatives) on epidermal growth factor receptor (EGFR) protein tyrosine kinase (PTK) was investigated and they showed efficacy against three coccidian protists (142) (Fig. 8A).

DNA topoisomerases

Type I and type II DNA topoisomerases from protozoan parasites are involved in complex DNA topology and substantial differences exist with their mammalian coun-

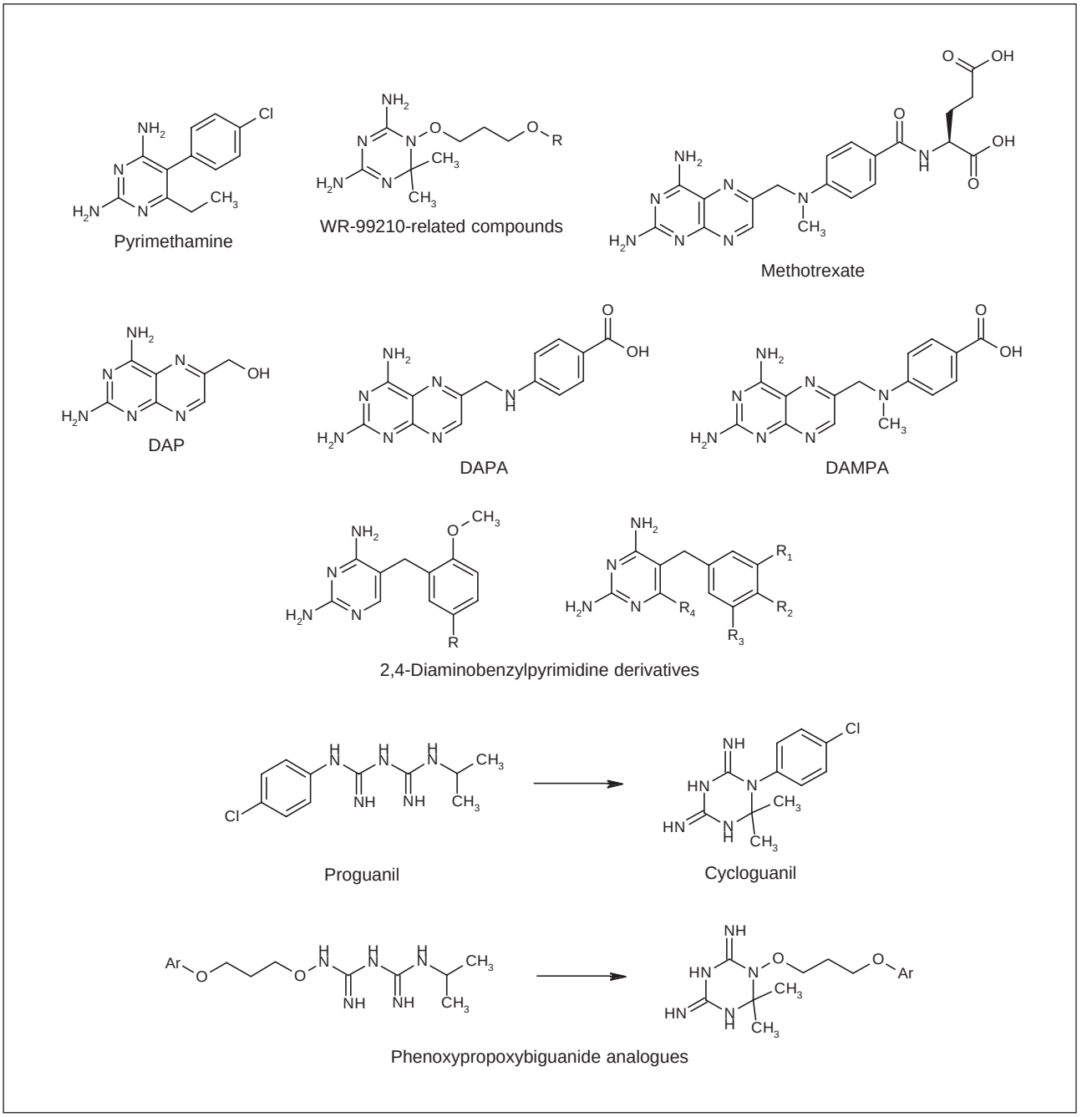

Fig. 7. Inhibitors of folate metabolism. DAP: 2,4-diamino-6-hydroxymethylpteridine; DAPA: 2,4-diaminopteroic acid; DAMPA: 2,4-diamino-N^{10}-methylpteroic acid.

terparts. Their identification as promising drug targets is based on the clinical success of camptothecin derivatives as anticancer agents, and DNA topoisomerase I is regarded as a promising target in parasitic protists (143, 144). The antileishmanial pentamidine is at least partly targeted to the parasite topoisomerase I (145).

The bisnaphthoquinone derivative of plant origin, diospyrin, was reported to be an inhibitor of *Leishmania* topoisomerase I (146). Naturally occurring flavones are potent inhibitors of recombinant *L. donovani* topoisomerase I (147). 9-Anilinoacridines are potent inhibitors of plasmodial DNA topoisomerase II and also target hematin, derived from hemoglobin (148). Fluoroquinolones, inhibitors of DNA topoisomerase II, were active against *T. brucei* (149) and *Leishmania panamensis* (150), and there are indications that the apicomplexan plastids could be a target (151). The adult stages of filarial nematodes insensitive to current drugs were killed by quinolones in an *in vivo* model (152). Compounds of the coumarin class were evaluated against *Brugia malayi,* a

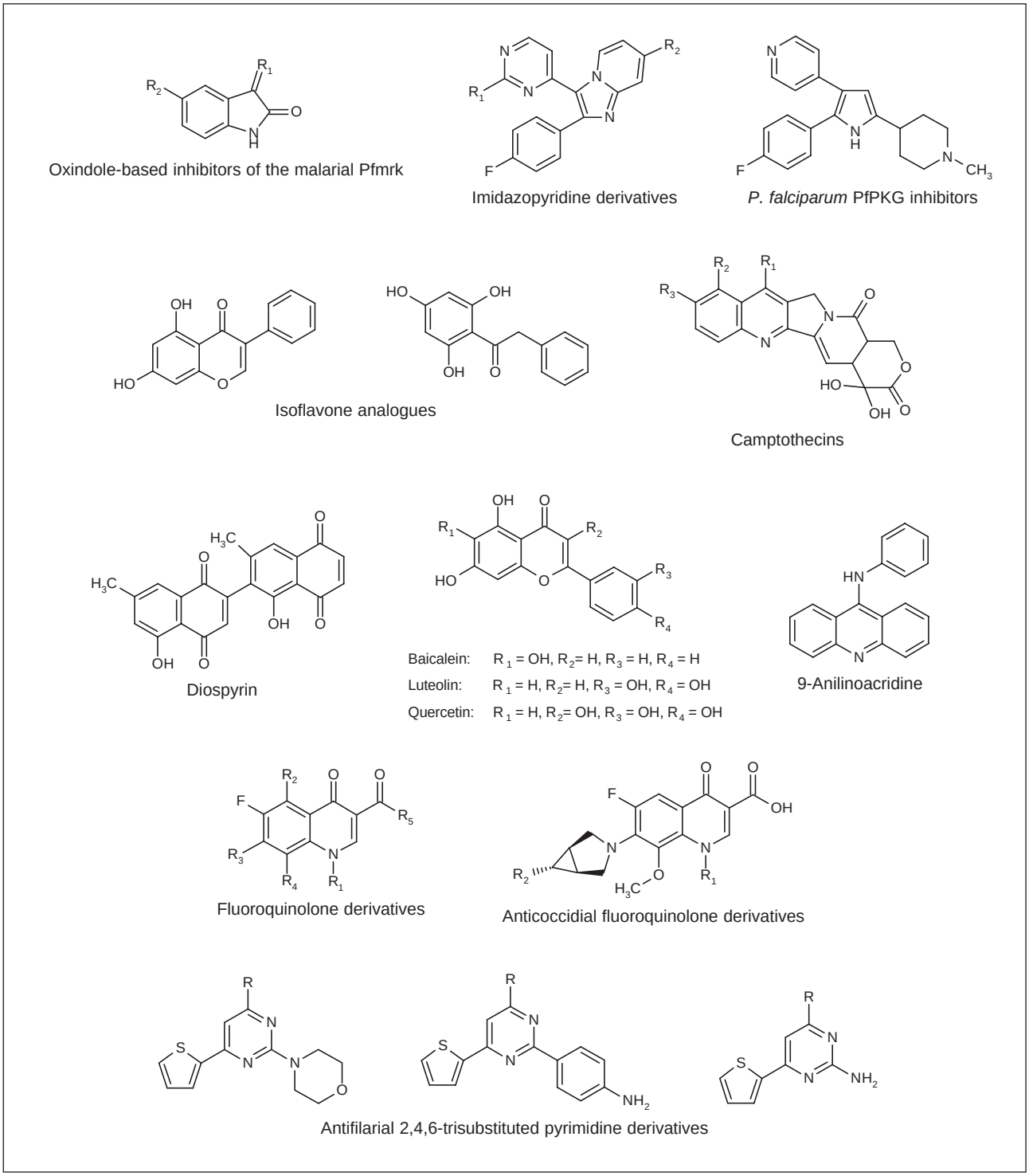

Fig. 8. **A**: Protein kinase inhibitors with efficacy against protozoan parasites. **B**: Parasite DNA topoisomerase inhibitors.

filarial nematode, and inhibited DNA topoisomerase II activity in the filarial parasite, alone or in combination with the existing drugs DEC and ivermectin (153). Trisubstituted pyrimidine derivatives were better topoisomerase II inhibitors than the standard antifilarial drug DEC (154) (Fig. 8B).

Purine salvage

Nearly all parasites of medical importance are obligate purine auxotrophs, and there are marked differences in salvage pathways and preferred substrates among the parasites (155). Despite intricate pathways, only a few

enzymes involved in purine metabolism are drug target candidates (156). The trypanosomatid nucleoside *N*-hydrolase (NH), purine nucleoside phosphorylase (PNP) (157, 158), IMP dehydrogenase (IMPDH) (159) and 5'-deoxy-5'-methylthioadenosine phosphorylase (MTAP), an enzyme at the border of polyamine biosynthesis and purine salvage, are the most extensively studied. Transporters are also susceptible to inhibition in *Leishmania* and *Plasmodium* (160).

Allopurinol was the first purine analogue to reach the clinical stage, but its efficacy is not optimal (161). The NH transition-state inhibitors immucillins (162) are promising antiparasitic subversive substrates and 6-benzylthioinosine analogues are promising antitoxoplasmic drugs (163, 164). A deoxyadenosine-derived subversive substrate of *T. vaginalis* PNP was investigated (165) and tricyclic purine antimetabolites interacting with transporters from *T. brucei* were recently tested (166). N^6-Adenosine derivatives have displayed antimalarial activity (167). The P2 transporter can also translocate melamine and the coupling of the melamine moiety to selected nitro heterocycles was effective against pathogenic trypanosomatids (168) (Fig. 9).

ABC transporters

Drug efflux in resistant cancer cells is mediated by ATP-binding cassette (ABC) transporters, also known as P-glycoproteins (P-gps). Homologues have been found in parasites and are involved in resistance to chemotherapy (169), and it may be possible to reverse parasite resistance by blocking these transporters. It has been hypothesized that these efflux pumps could be responsible for the natural resistance of *Cryptosporidium* spp. to treatment. Also, in *Leishmania,* different classes of ABC transporters extrude antimonials, azoles and folates, resulting in drug-resistant phenotypes (170). The expression of three genes coding for the ABC transporter MRPA (PGPA) was increased in an antimony-resistant *Leishmania infantum* strain (171).

Flavonoids are inhibitors of the *Leishmania tropica* multidrug transporter (172) and isoflavonoids have recently been described as efficient anticryptosporidial agents (173).

Conclusions

Great progress has been made in the understanding of parasite biology and parasite-host relationships. Research in antiviral and anticancer chemotherapy has provided a wealth of information that may sometimes be adapted to parasites and which has also resulted in the discovery of a number of interesting chemotherapeutic targets and many promising specific inhibitors. The future seems bright, at both the chemical and the biological level: high-throughput screening, combinational chemistry, parallel synthesis, genome sequencing, target identification and recombinant gene technology are now being used. However, little progress has been made in antiparasitic drug testing, which is still a tedious procedure. We should also be aware that these new methods will not eliminate the selective pressure and subsequent parasite adaptation resulting in drug resistance. Despite the fine-tuning of promising candidates, selectivity is a vital issue to be addressed before they reach the preclinical and clinical stages.

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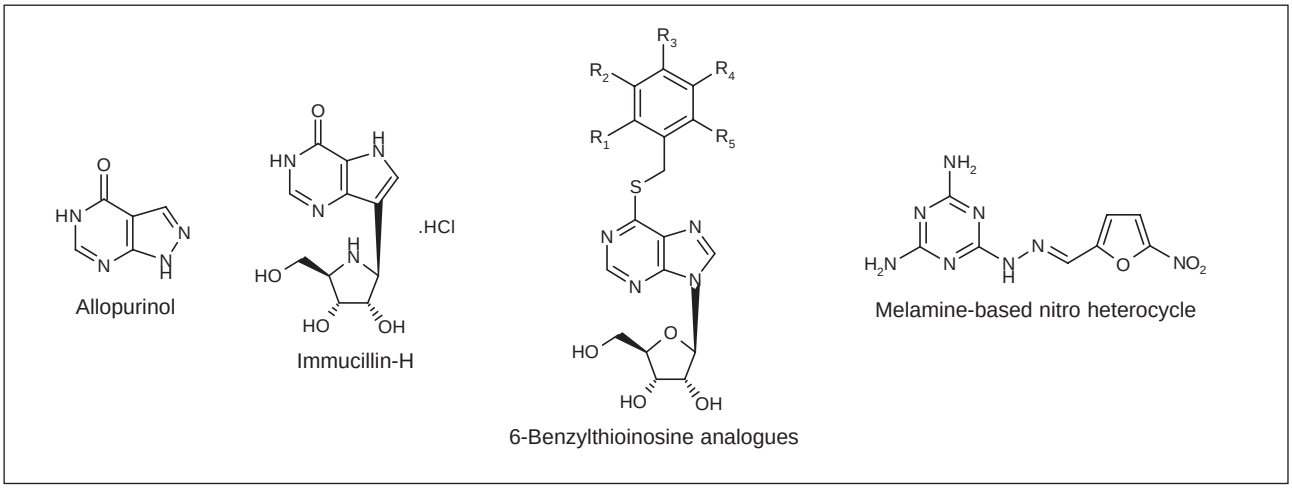

Fig. 9. Subversive substrates of parasite purine salvage and transport.

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