

Exp. Opin. Ther. Patent. **2005**, *15*, 1241-1257

**Recent Advances in Antitrypanosomal Chemotherapy:
Patent Literature 2002-2004**

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Summary

Sleeping sickness and Chagas' disease (African and American trypanosomiasis, respectively) are protozoan parasitic diseases threatening millions of people in sub-Saharan Africa and Latin America, respectively. Trypanosomiasis are among the most neglected diseases in the world, lacking desperately from financial support for investigation. The current chemotherapy of both diseases is poor and suffers from intolerable side-effects and low efficacy in many cases. A review of the patent literature (2002-early 2005) claiming for molecules with antitrypanosomal activity afforded 36 entries, equally shared between industry and academia. Among the targets validated against trypanosomes, patents dealing with protease inhibitors were the most represented (16 patents). Other targets claimed in the patent literature included membrane architecture (sterol biosynthesis inhibitors, protein farnesyltransferase inhibitors), DNA (DNA binders, tubulin inhibitors) and pyrimidine metabolism (CTP synthetase inhibitors). Natural products were also a great source of trypanocidal lead compounds (9 patents). A few patents claiming for compounds with antitrypanosomal activity but disclosing no specific target were also encountered.

Keywords: *Trypanosoma brucei*, *Trypanosoma cruzi*, Chagas' disease, sleeping sickness, human African trypanosomiasis, chemotherapy, protease inhibitors, sterol biosynthesis inhibitors, DNA binders.

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1. Introduction

Diseases caused by parasitic protozoa are of major impact in the third world. Parasitic protozoa belonging to the order kinetoplastida cause Chagas' disease (American trypanosomiasis), sleeping sickness (Human African Trypanosomiasis or HAT) and leishmaniasis. HAT and Chagas' disease are caused by protozoa of the genus *Trypanosoma* which are transmitted to the mammalian host by biting insect vectors, tsetse fly and triatomine bugs, respectively [1].

Two species of trypanosomes are responsible of the chronic and acute form of HAT respectively, *T. brucei gambiense* and *T. b. rhodesiense*, whereas other species (*T. b. brucei*, *T. congolense* and *T. vivax*) cause animal trypanosomiasis (Nagana) in cattle. HAT is a resurgent disease in sub-Saharan Africa where it had almost disappeared in the 1960's following the systematic screening and treatment of millions of people at risk. Unfortunately, active population screening declined in the latter part of the twentieth century and the disease has re-emerged as a major health problem in recent years [2]. HAT is a daily threat to more than 60 million people in 36 countries of sub-Saharan Africa with prevalence of an estimated 500,000 infected individuals [2]. The disease is characterised by two pathological stages depending on the presence or not of parasites in the cerebrospinal fluid. In stage 1, parasites invade the bloodstream via the lymphatic system following infection of the host by the bite of an infected tsetse fly. This stage can last from a few weeks (rhodesiense sleeping sickness) to several years in the case of *T. b. gambiense*. When parasites invade the central nervous system (CNS), the disease is always fatal if left untreated [3].

Trypanosoma cruzi is responsible of Chagas' disease which is one of the most prevalent infectious diseases infecting between 11-18 million of people in Central and South America. Recent efforts in the control of the vector and systematic screening of blood donors in endemic countries have led to a great decrease in transmission of the

disease over the past decade. The disease is characterised by two distinct stages: an acute phase of parasite growth and invasion of host cells follows the infection. Then, a chronic stage, often asymptomatic, in which the parasite installs inside target organs (gastrointestinal tract, heart) leads in about 15-30 % of patients to irreversible organ damage as late as 10-25 years after infection [4].

Chemotherapeutic drugs exist for both diseases; however none of them is fully satisfactory because of important side-effects and/or a lack of activity against CNS stage of sleeping sickness or against chronic Chagas' disease. Two drugs are licensed for the chemotherapy of Chagas' disease, namely nifurtimox (Lampit™, manufactured by Bayer) and benznidazole (Rochagan™, Radanil™, Roche) which are both nitroheterocyclic compounds active against circulating trypomastigotes (Figure 1). However, these drugs are hardly efficient against the chronic stage of the disease, possibly due to unfavourable pharmacokinetic properties, and their efficacy varies depending on the *T. cruzi* strains [5,6,7].

Only four drugs are licensed for the treatment of HAT (Figure 1): Eflornithine (Ornidyl™; Bayer), suramin (Germanin™, Bayer), pentamidine (Lomidine™, Aventis) and melarsoprol (Mel B, Arsobal™, Aventis) although other drugs such as berenil (usually used against animal trypanosomiasis) or the nitrofurantoin nifurtimox have also proved useful in some limited cases [8,9,10]. These drugs are far from satisfactory because of major side effects, the parenteral mode of administration and unaffordable price for African countries. Moreover, some of those drugs (suramin, pentamidine) are unable to cross the blood-brain barrier (BBB) in sufficient quantity to treat late-stage cases of HAT. The increasing resistance to the arsenical melarsoprol [11,12], which is the main drug used to treat the late stage (when the CNS is affected) of both forms of the disease, has increased the need for alternative drugs. The long term availability of

the existing drugs is also compromised although pharmaceutical industries (i.e. Aventis and Bayer) signed an agreement with WHO in 2001 to guarantee their production for at least five years [1].

Despite many similarities between African and American trypanosomes, there are crucial differences, such as an intracellular existence for *T. cruzi* in the mammalian host whereas *T. brucei* remains exclusively extracellular [1]. These divergences have to be taken into account for drug design since chemotherapeutic agents meant to kill *T. cruzi* intracellular amastigotes will have to cross several cellular membranes to get to their target and drugs targeting the CNS stage of sleeping sickness must be able to cross the BBB.

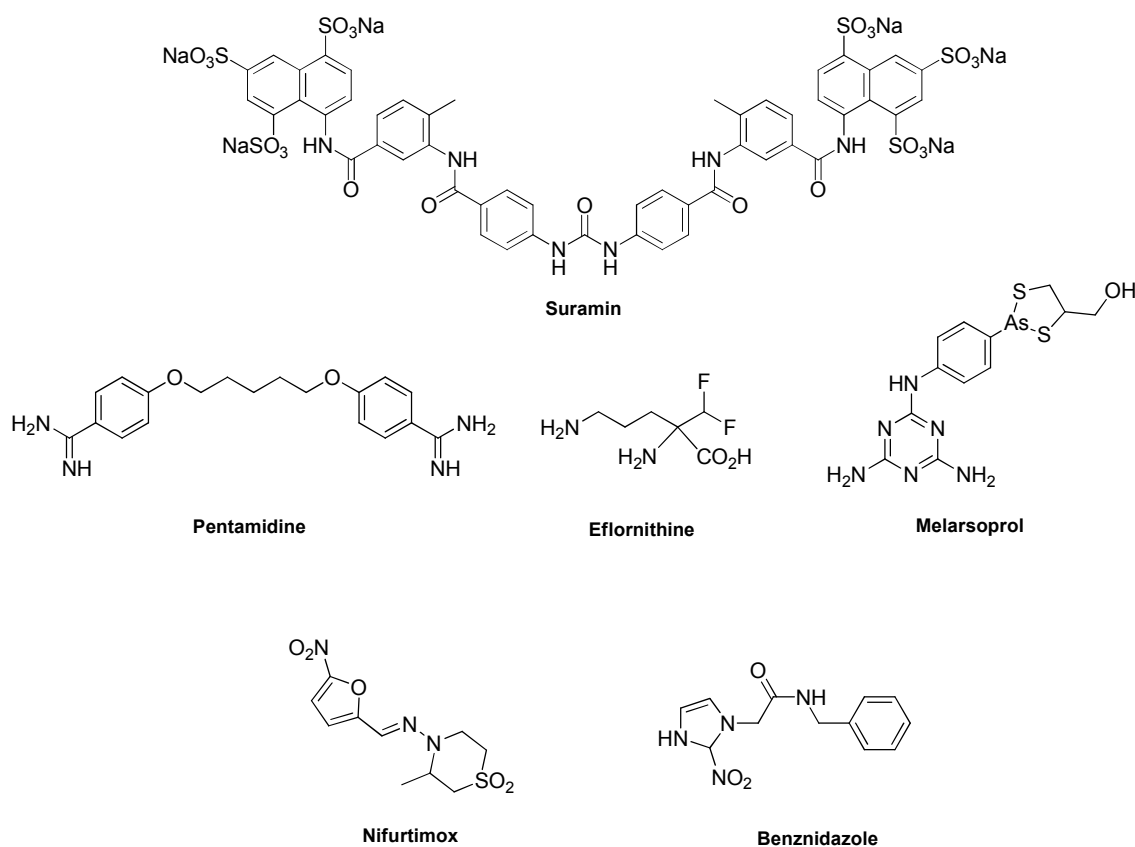


Figure 1. Licensed antitrypanosomal drugs

The rational approach to drug design has been applied to trypanosomes as these parasites have a number of unusual biochemical pathways [13]. Widely studied targets include enzymes of the glycolytic [14,15] and pentose phosphate pathway [16,17,18], trypanothione reductase [19,20], the polyamine pathway [21], glycosylphosphatidylinositol (GPI) biosynthesis and remodelling, and topoisomerases [22,23].

Despite many years of efforts in the search of new trypanocidal drugs (mainly by academia institutions), no new chemical entity has been registered for trypanosomiasis since the approval of Eflornithine for the treatment of CNS gambiense sleeping sickness in 1990. Moreover, there is no perspective for the registration of new trypanocidal drugs in the near future and only one compound, the diamidine prodrug DB289 (see section 2.3), is currently being studied in phase II clinical trials for the oral treatment of HAT [9,24,25,26].

A search in the patent literature for the period 2002-early 2005 for the claim of trypanocidal activity against *T. brucei* or *T. cruzi* parasites afforded 36 entries. However, only a small proportion of these patents displayed specific data of biological activity against these parasites. A few patents published in 2002 and claiming anti- *T. cruzi* activity which were previously reviewed by Urbina [27] were not included in this paper. This review is organised in three sections: in the first place, and representing two third of the entries, are the patents referring to specific drug targets such as proteases inhibitors, sterol biosynthesis inhibitors, protein farnesyl transferase inhibitors, DNA modulating agents and pyrimidine metabolism inhibitors. The second section refers to natural product derived chemotherapeutic agents and the third one to single synthetic entities.

2. Drug targets

2.1. Protease inhibitors

Proteases are essential enzymes involved in the regulation of important functions of trypanosome life cycle. Cruzain (or cruzipain), is the major cysteine protease of *T. cruzi* and is expressed in all life cycle stages of the parasite. Rhodesain is a cysteine protease expressed in *T. b. rhodesiense* that regulates replication of the parasite. Various studies have shown that cruzain and rhodesain inhibitors block the parasite life cycle *in vitro* [28,29,30,31] and *in vivo* [32,33] and do not cause toxicity to the host. The crystal structure of cruzain bound to various inhibitors has been solved [34,35,36,37]. Hence, cysteine proteases inhibitors (CPI) represent excellent leads for the design of antitrypanosomal drugs. In addition, cruzain shares a significant homology ($\approx 45\%$) in the amino acid sequence with the mammalian cathepsin L cysteine protease [38,39,40]. As a result, pharmaceutical companies that are interested in the design of CPI for the treatment of various cancers and bone diseases, also claim cruzain and/or rhodesain inhibitory activity in their patents dealing with CPI. The importance of CPI as a drug target is reflected by the number of patents disclosed by various pharmaceutical companies in the last five years: 16 patents for the 2002-2004 period (this review) and 7 patents during the 1999-2002 period [27].

In their search for cathepsin K inhibitors, SmithKline Beecham Corp. published several patents describing the syntheses and use of peptidomimetics based on an azepine [201,202,203,204,205] or thiazepane [206] template. As a whole, about 500 new compounds were prepared and tested as cathepsin K inhibitors and claimed to be useful against different parasitic diseases including trypanosomiasis. However, only two patents [201,204] reported specific biological data. Four compounds had *in vitro* K_i values of 0.3 nM (**1**, Figure 2 and Table 1) to 1.8 nM against human Cathepsin K and

values of 69 to 926 nM against Cathepsin L, Cathepsin S and Cathepsin B when tested in protease catalytic activity assays [201]. The other patent disclosed the inhibition by 4-aminoazepan-3-one derivatives of seven parasitic proteases, including cruzain, rhodesain, falcipain, leishmania B and L and schistosoma B1 and B2 enzymes. Among 222 compounds tested, 43 had a K_i value < 5 nM against cruzain and 79 had a $K_i < 5$ nM against rhodesain. The most potent CPI against rhodesain and cruzain were the 1-(pyridin-2-ylsulfonyl)azepan-3-one derivatives **2** and **3**, respectively (Table 1). Other peptidomimetics useful as CPI were reported by NAEJA Pharmaceuticals Inc. [207]. The preparation of 22 dihydropyrimidine derivatives was illustrated and the *in vitro* rat cathepsin B, L, K and S activity was disclosed (e.g. compound **4**, Figure 2, Table 1). In 2004, Amura Ltd (previous applications were assigned to Incenta Ltd [27]) published an application with the synthesis and cathepsin K activity of over 2000 bicyclic heterocycle compounds. The CP inhibitory activity was determined in four examples and compound **5** showed K_i value > 0.3 μ M against cruzain and < 0.01 μ M against human cathepsin K [208]. Other peptide-based CPI claimed by Dendreon San Diego LLC (exemplified by **6**) were stated to have $IC_{50} < 50$ nM against cruzipain and falcipain. However, no specific biological data was presented [209]. Medivir UK Ltd also published an application with 27 amide-based CPI claimed to be useful for protozoal infections [210]. The *in vitro* cathepsin S, L and K inhibitory activity, and cathepsin K and falcipain proteolytic catalytic activity of 27 compounds (e.g. **7**) were determined but no biological data was presented. Over 450 novel nitrile compounds (e.g. **8**) disclosed by Boehringer Ingelheim Pharmaceuticals Inc were claimed to be useful for the treatment of diseases mediated by cysteine proteases, specifically cathepsin K and S. The best compounds were stated to have IC_{50} values < 50 μ M against cathepsin K and < 10 μ M against cathepsin S, F, L and B although no detailed

results were included [211]. The National Research Council of Canada disclosed interesting propeptide-based non covalent CPIs designed to mimic the reverse binding mode, relative to the substrate, of the propeptides of these enzymes. The compounds were claimed to span both the S' and S subsites of the enzyme active site and a 1.9Å X-ray crystal structure of the complex of cathepsin L with one of the inhibitors confirmed this hypothesis. Ten compounds assayed against cruzain and rhodesain had K_i values from 0.09 (**9**) to 20 μM and from 0.3 (**9**) to 35 μM , respectively [212].

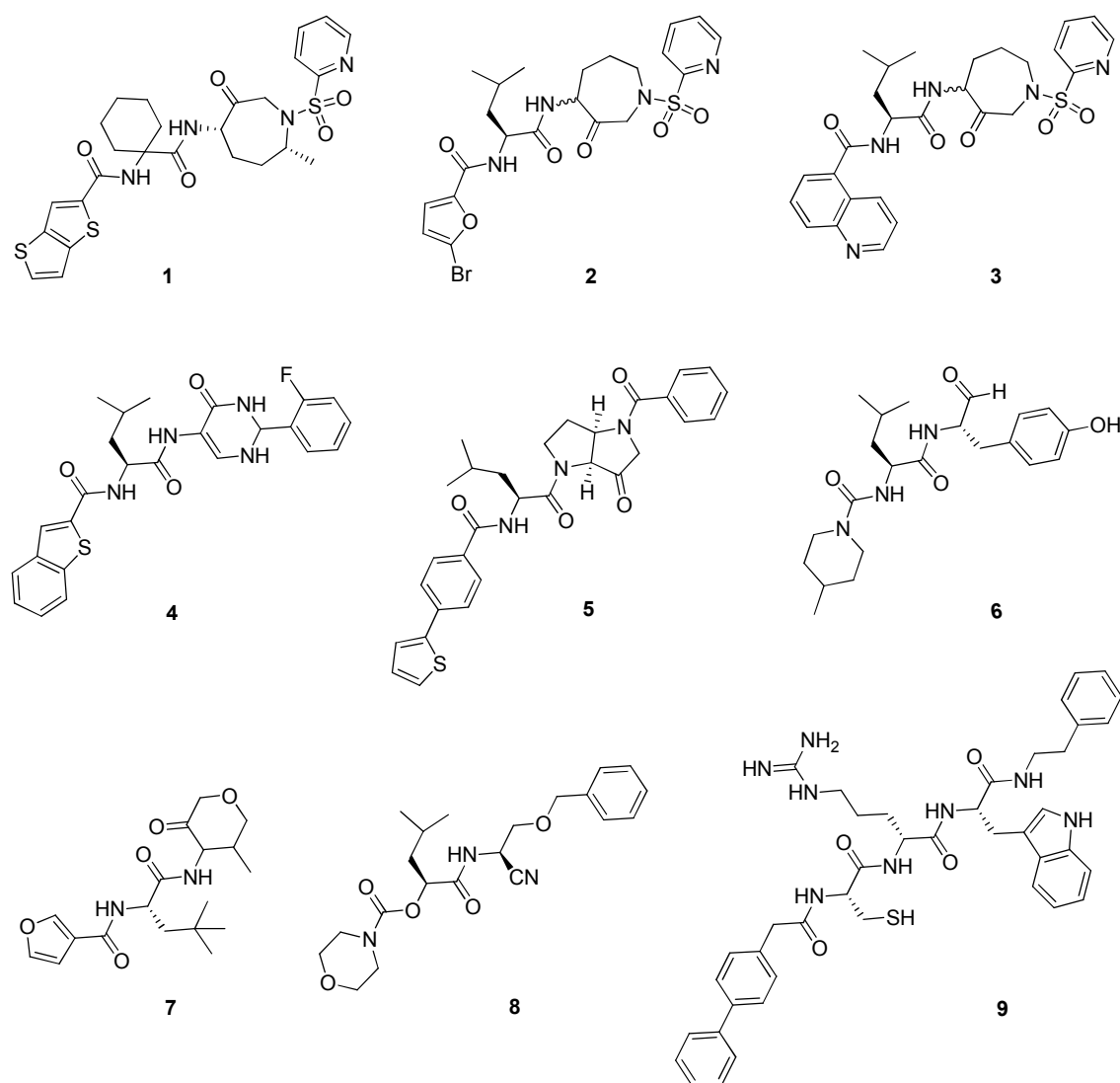


Figure 2. Peptide-based CPIs

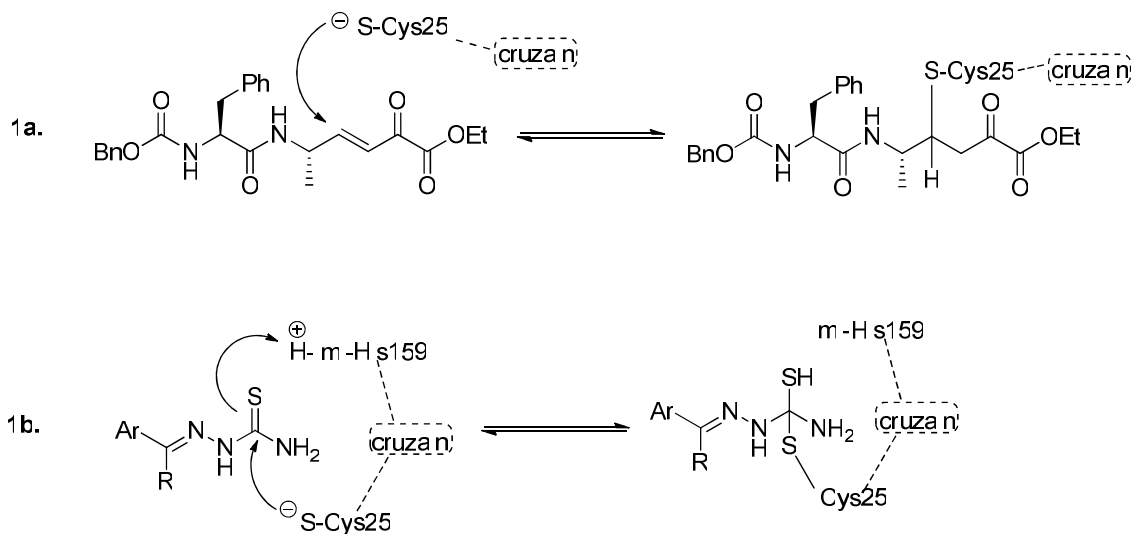
Table 1. Enzymatic activity of CPIs

Compound	Enzyme tested	IC ₅₀ (nM)	K _i (nM)	Reference
1	Human cathepsin K		0.3	[201]
	Human cathepsin L		69	
	Human cathepsin S		175	
	Human cathepsin B		173	
2	Rhodesain		0.93	[204]
	Cruzain		3.3	
	Falcipain		3.1	
3	Rhodesain		2.1	[204]
	Cruzain		1.8	
	Falcipain		4.4	
4	Rat cathepsin K	14		[207]
	Rat cathepsin L	170		
	Rat cathepsin S	37		
	Rat cathepsin B	420		
5	Cruzain		> 300	[208]
	Human cathepsin K		< 10	
	Human cathepsin L		> 3000	
6	cruzipain, falcipain	< 50		[209]
7	cathepsin K, L, S, falcipain 2	ndp ^a	ndp	[210]
8	Human cathepsin K	< 50000		[211]
	Human cathepsin S, L, F, B	< 10000		
9	Cruzain		90	[212]
	Rhodesain		300	
	Human cathepsin B		560	
	Human cathepsin K		1000	
	Human cathepsin L		67	
10	Human cathepsin K		< 50	[213]
11	Human cathepsin K	1.5		[214]
12	Serine peptidase, cysteine protease, metalloprotease, endopeptidase	ndp	ndp	[215]
13	Plasmepsin II	18		[216]

^a no data provided

Apart from the patent literature, new scaffolds of CPI are regularly described. For instance, Y. Choe and colleagues designed a series of α -keto-based peptide inhibitors of cruzain with picomolar potency *in vitro*, some of them showing antitrypanosomal activity in cell cultures. These peptidic covalent reversible inhibitors (Scheme 1a) which represent new scaffolds of CPI, have poor membrane permeability and would need to be optimised in order to display better activity [41]. Another interesting paper reports the

first crystal structure of non-covalent CPI bound to cruzain [37]. The same group also prepared libraries of cyclic ketone-based CPI with nanomolar K_i values [42,43].



Scheme 1. Proposed mechanism for the interaction of reversible covalent CPIs with cruzain: a) α -keto-based peptide inhibitors [from ref 41]; b) thiosemicarbazones [from ref. 45]

Non peptide CPIs were less represented in the patent literature for the 2002-2004 period (Figure 3). An example of unusual structure useful against cruzain and rhodesain was the thiosemicarbazone scaffold described by the team of the University of California [44,45,46]. These compounds were proposed to work as reversible covalent inhibitors of the enzyme (Scheme 1b) [45]. Novartis AG described a series of over 100 novel heteroaryl nitrile derivatives (e.g. **10**) having *in vitro* activity against recombinant human cathepsin K with $K_i < 50$ nM. No compound was specifically claimed and no biological data was provided [213]. Another series of 2-cyano-4-aminopyrimidines CPIs was disclosed by this company in a patent describing the synthesis of over 90 compounds. Compound **11** had an $IC_{50} = 1.5$ nM in the inhibition of cathepsin K [214]. Other atypical CPI were the cyclopalladated bis(diphenylphosphine)-ferrocene compounds disclosed by the State of Sao Paulo Research Foundation in 2004 (e.g. **12**). These derivatives were claimed to inhibit

enzymes of the serine peptidase, cysteine protease, metalloprotease and endopeptidase families and to be useful against the development and metastases of malignant tumors [215]. Finally, Actelion Pharmaceuticals described a series of amino-aza-cycloalkane derivative as inhibitors of aspartic proteases, particularly of the plasmodium falciparum plasmepsin II, useful against protozoal infections. Compound **13**, which is one of 140 derivatives disclosed, inhibited plasmepsin II with $IC_{50} = 18 \text{ nM}$ [216].

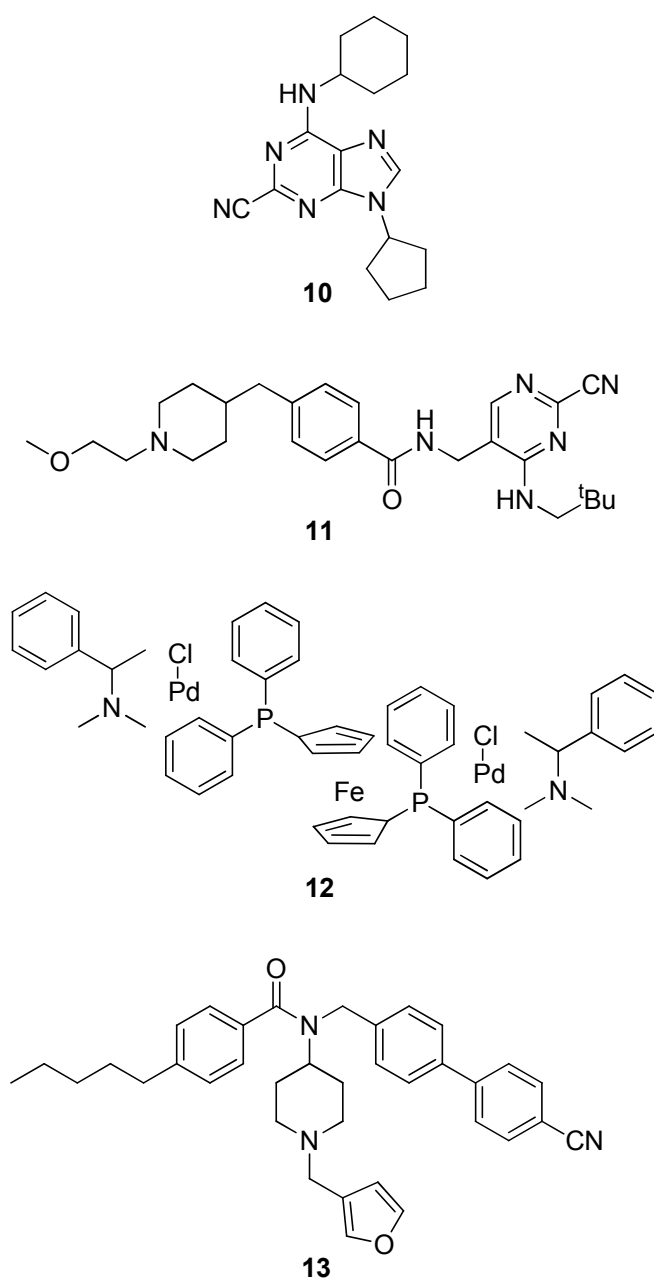


Figure 3. Non-peptide CPIs

2.2. Membrane architecture

2.2.1. Sterol biosynthesis inhibitors

The sterol biosynthetic pathway produces membrane lipids in all eukaryotic organisms. The singularity of this pathway in fungi and kinetoplastid parasites has been successfully exploited as a target for chemotherapy for many years [7,47,48] and several commercial and experimental antifungal azoles, inhibitors of sterol biosynthesis (e.g. ketoconazole, posaconazole, D0870), have been tested successfully against *T. cruzi* [27,49,50]. On the other hand, sterol biosynthetic pathway is unlikely to be a valid target for *T. brucei* bloodstream form trypomastigotes (the relevant form in sleeping sickness) that do not appear to synthesise their own sterols and scavenge them from the human host [51]. Squalene synthase [48,52,53,54,55], sterol 24-methyltransferase [56] or farnesylpyrophosphate synthase [57] are enzymes of the sterol biosynthetic pathway that are showing growing interest as antitrypanosomal targets against *T. cruzi*.

The synthesis of 5-amino-1-benzyl-imidazole derivatives with distinctive anti-bacterial, anti-fungal and antitrypanosomal activity was disclosed by Yale University [217]. These compounds were designed as inhibitors of C-14 α -demethylase. This enzyme is a cytochrome P450 enzyme (P-450_{14DM}) that catalyses the removal of the 14 α methyl carbon (C32) of lanosterol in the presence of molecular oxygen and NADPH. Since 14-methyl sterols cannot function within cell membranes, inhibition of P-450_{14DM} represents a good target for chemotherapy. All of the compounds were tested against *T. cruzi* amastigotes (Tulahuen strain) grown on monolayer of mouse 3T3 fibroblasts and were non toxic to these cells. The SAR study resulting from the analysis of 40 derivatives put into evidence the importance of hydrophobic substitution and *para* phenyl substitution (vs. *ortho* or *meta*) for better activity. EC₅₀ values ranged from the

remarkable 500 pM (**14**, Figure 4) to 1 μ M. However, these compounds had benzoate ester or amide moieties which could potentially be cleaved *in vivo* by esterase and proteases, affording the less potent free benzoic acid derivatives. Further SAR studies showed that the new scaffold containing an aminobiphenyl moiety (**15**: IC₅₀ = 10 nM), although 20-fold less potent than its methyl ester counterpart **14**, was more active *in vivo*. Compound **15**, at twice daily 50 mg/kg doses, caused a suppression of parasitaemia in mouse blood within 45 days. Survival of mice treated with **15** was > 100 days whereas control mice died within 20 days. The authors suggested that the phenylbenzylimidazole moiety dominates the interaction with P-450_{14DM} resulting in inhibition of the enzyme and consequent anti-parasitic activity. However, no data of enzymatic inhibition was reported.

2.2.2. Protein farnesyl transferase inhibitors

Protein prenylation by attachment of polyisoprenoids to specific proteins is involved in signal transduction and anchorage of protein to cell membranes. Protein farnesyl transferase (PFT) inhibitors are potent antitumor agents in experimental animals [58,59] and few molecules have been assayed in phase II clinical trials for the treatment of human malignancies [60]. Different studies have demonstrated the existence of this process in trypanosomatids and validated PFT inhibitors as an antitrypanosomal chemotherapeutic target [13,61]. *T. brucei* and *T. cruzi* PFT have been cloned and their homology to human and rat orthologues compared. This study revealed significant differences between the active site of *T. brucei* PFT and its mammalian counterpart (i.e. rat enzyme), suggesting that selective inhibition of the parasite enzyme was possible [61]. For these reasons, PFT inhibitors, whose medicinal chemistry and pharmacokinetics are well defined already, have potential to become interesting antitrypanosomal molecules.

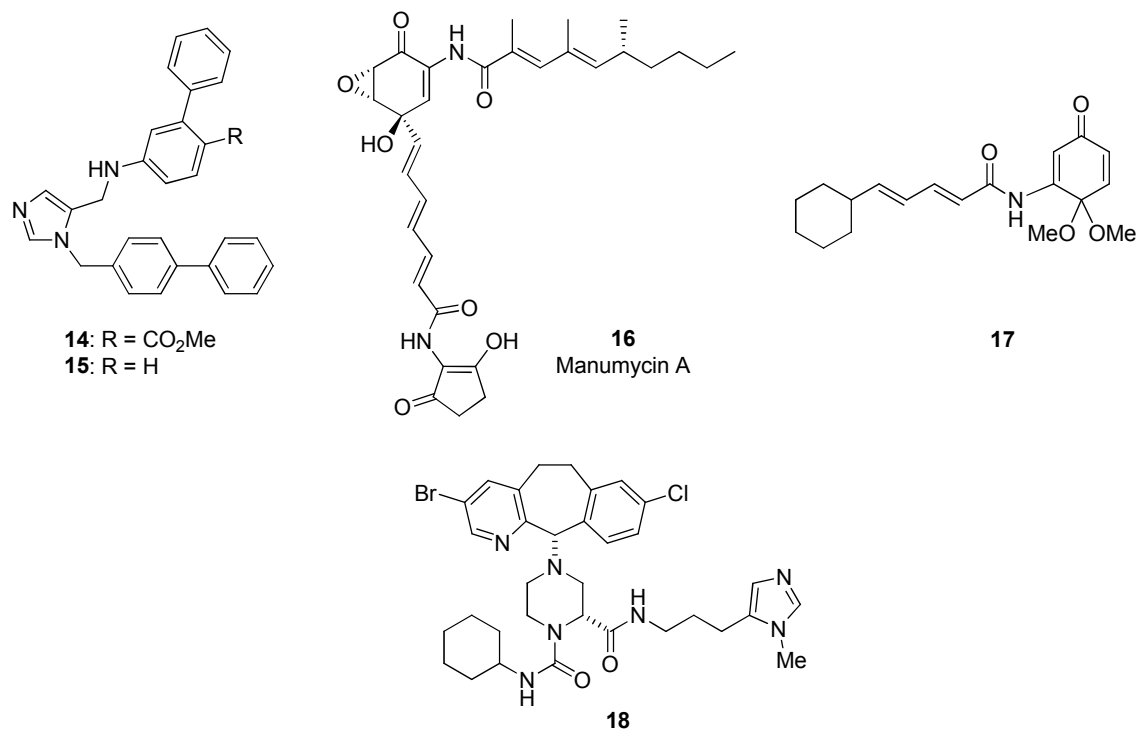


Figure 4. Sterol biosynthesis inhibitors and PFT inhibitors

In 2001, the group of Mark Field from the Imperial College of Sciences, Technology and Medicine (UK) disclosed, for the first time in a patent, the use of PFT inhibitors to treat protozoan parasitic diseases [218]. This patent described the use of Manumycin A (**16**, Figure 4), a Ras farnesyltransferase inhibitor and natural antibiotic extracted from cultures of *Streptomyces sp.*, as well as other synthetic cyclic hexenone compounds as PFT inhibitors active against *T. brucei*. Nine compounds were specifically claimed for use but no synthetic procedure was disclosed. Some evidences suggested that these hydrophobic molecules accumulate to toxic levels into cellular membrane. The most potent compound (**17**, Figure 4) killed > 95 % of bloodstream form *T. brucei* parasites *in vitro* at a concentration < 5.6 μ M (LD₅₀ = 1.5 μ M) whereas Manumycin A (**16**) had a LD₅₀ value of 2.5 μ M. In this series, the presence of the benzoquinone structure resulted in higher toxicity compared to a hydroxyl group.

Schering Corporation disclosed recently 21 specific PFT inhibitors based on a N-(benzo[5,6]cyclohepta[1,2]pyridin-11-yl)piperazine (**18**) or piperidine scaffold for the

treatment of *T. brucei* infection [219]. The compounds were claimed to inhibit *T. brucei* PFT with an IC₅₀ value between 0.0019 μM and 15 μM in a Scintillation Proximity Assay. *In vitro* growth inhibition of *T. brucei* ranged between 0.2 μM to < 10 μM.

2.3. DNA modulating agents (DNA binders, Tubulin inhibitors)

The most promising patent in term of near-future outcome for the treatment of African trypanosomiasis was disclosed by D. Boykin and colleagues in 2004 [220]. This application, which claimed the use of dicationic 2,5-diarylfuran aza-analogues for the treatment of HAT and malaria, refers to a recent paper published by the same group in 2003 [62]. Aromatic diamidines have broad spectrum antimicrobial activity and many of these aromatic dicationic compounds bind to DNA minor groove at AT-rich sites [63,64,65,66,67,68,69,70]. This is the case of drugs such as pentamidine or berenil for instance. The antimicrobial action of diamidines is thought to be the result of inhibition of different DNA dependent enzymes [71,72]. The molecules disclosed by Boykin and colleagues are analogues of DB-289 [2,5-bis(4-amidinophenyl)furan-bis-O-methylamidoxime], an oral diamidoxime prodrug of furamide [2,5-bis(4-amidinophenyl)furan, **19**, Figure 5], which is currently being developed by Immtech as a potential treatment for *Pneumocystis carinii* pneumonia, tuberculosis, sleeping sickness and malaria. DB-289 (**20**, Figure 5) is currently in phase II clinical trial for stage I African trypanosomiasis [9,10,24,25,26,73]. In this series, different aza-derivatives and their respective amidine prodrugs (amidoxime, methoxime, and ethoxime) were synthesised and the compounds tested *in vitro* against *T. b. rhodesiense*. The *in vivo* oral bio-availability in the virulent STIB900 *T. b. rhodesiense* mouse model was also tested. Seven compounds showed IC₅₀ values in the low nanomolar range (IC₅₀ for furamide and pentamidine are 4.5 and 2.2 nM, respectively). Compound **21** and its

methoxime prodrug **22** had IC₅₀ values of 7.0 and 37.1 nM, respectively (Figure 5). Both of these molecules also cured the STIB900 strain of *T. b. rhodesiense* in a mouse model and the methoxime prodrug **22** yielded parasite free mice in the CNS model through day 120. As a whole, the methoxime prodrug was consistently more active than the amidoxime and aza-analogues showed superior oral activity *in vivo* compared to furamide analogues [62]. Compound **22** was claimed to be useful as an oral treatment of CNS stage HAT [220]. A scalable and more economic procedure for the synthesis of DB-289 and related bis-aryl diamidoxime derivatives was disclosed in another patent by the same group. This methodology used 2,5-bis-trialkylstannanes and a one step palladium-catalyzed cross reaction to form the products [221].

“Reversed amidines” and guanidine analogues of furamide were claimed for use as antifungal and antimycobacterial infections. The synthesis, DNA binding affinity [i.e. melting temperature difference: ΔT_m for poly dA·dT and the dodecamer d(CGCGAATTCGCG)₂] and antimicrobial activity of nineteen 2,5-di(4-iminomethylaminophenyl)furan [74], thiophene or pyrrole derivatives was also disclosed [222]. In general, both guanidines and reversed amidines had strong DNA binding affinities (ΔT_m for d(CGCGAATTCGCG)₂ oligomer: from 0 to 15.4 compared to 11.7 for the parent compound **19**), although a terminal pyridyl group lowered such affinity as compared to its phenyl counterpart. The specified compound (**23**, Figure 5) showed MIC value $\leq 1.94 \mu\text{M}$ against *C. albicans* and MIC 80% values of 1.51 to 97 mM against six other microbial species (*Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium solani*, *Rhizopus arrhizus*, *Cryptococcus neoformans*). These compounds were also evaluated against trypanosomes *in vitro*. The (arylimino)aminophenyl furans, especially the (2-pyridylimino)aminophenyl derivatives, were claimed to be effective against *T. b. rhodesiense* in the 0.039 to 0.194 μM range, meaning a 10-fold lower

activity than pentamidine and furamidine in the same assay. On the contrary, these compounds were significantly more potent than pentamidine and furamidine against *T. cruzi*, with an activity comparable to that of benznidazole. However, no specific data was reported in the patent [222]. Detailed biological data on the activity of these dicationic derivatives against *T. cruzi* and *L. donovani* was reported in 2003 [69]. The most active molecules belong to the reversed amidine series, with the most potent compound against *T. cruzi* having an IC₅₀ value of 0.15 μM (**24**, Figure 5) with a selectivity index (SI) of 48.

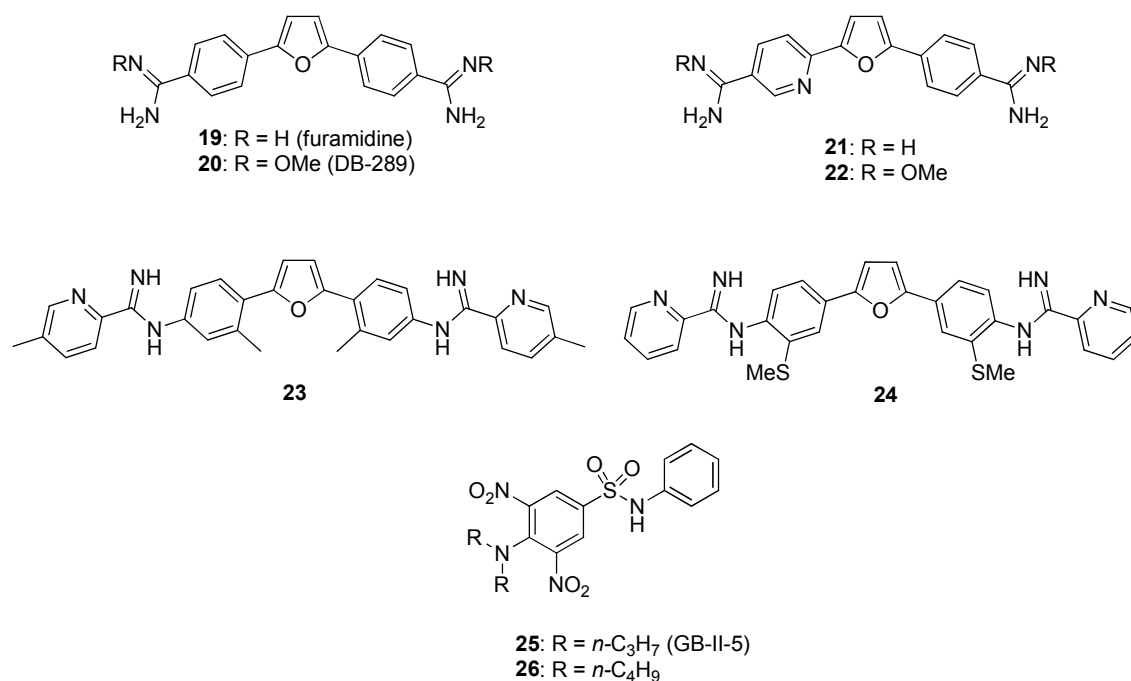


Figure 5. DNA modulating agents

Kinetoplastid tubulin has been proposed as a potential target against *Leishmania* and trypanosome species and started being exploited for the development of new drugs few years ago [75,76,77]. The synthesis, antimicrotubule activity and *in vitro* cytotoxicity of analogues of the antimitotic dinitroaniline sulfonamide herbicide compound, oryzalin (3,5-dinitro-*N*⁴, *N*⁴-di-*n*-propylsulfanilamide), was recently

described by Bhattacharya and co-workers [223,78,79,]. Six compounds were claimed among which the most potent analogue, *N*¹-phenyl-3,5-dinitro-*N*⁴, *N*⁴-di-*n*-propylsulfanilamide (GB-II-5, **25**, Figure 5), was a selective antimitotic agent against *L. donovani* and *T. brucei* tubulin. This molecule displayed also potent and selective *in vitro* inhibitory activity against *T. b. brucei* variant 221 (IC₅₀ = 0.41 μM) and *T. b. brucei* Lab 110 EATRO (IC₅₀ = 0.58 μM) [76]. The recent preparation of thirty new analogues of GB-II-5 with variations at the N1 and N4 positions allowed the discovery of a more potent derivative, *N*¹-phenyl-3,5-dinitro-*N*⁴,*N*⁴-di-*n*-butylsulfanilamide (**26**), which displayed antimitotic activity in cultured *T. b. brucei* (IC₅₀ = 6.9 μM, accumulation of parasites in the G₂M cell cycle phases). In general, the most active compounds against purified leishmanial tubulin were also the most efficient against *L. donovani* and *T. b. brucei* *in vitro* [79]. Despite their good *in vitro* activity, these compounds failed to cure mice infected with *T. b. brucei* STIB795 parasites at 4×20 mg/kg ip dose. Thus, issues regarding pharmacokinetics and possible toxicity due to nitroaromatic groups need to be addressed in order to fully assess the potential of these molecules.

2.4. Purines and pyrimidine metabolism

Purine and pyrimidine nucleotides are essential bricks for the synthesis of DNA and RNA. Trypanosomes, unlike their mammal hosts, lack of *de novo* purine biosynthesis pathway. Hence, they are dependent on purine salvage from their host medium in order to get these building blocks essential for their survival [80]. An important enzyme of the purine salvage pathway that has been used as a potential target for chemotherapy is hypoxanthine-guanine phosphoribosyl transferase (HGPRT) [13]. The potential of allopurinol (4-hydroxypyrazol-[3,4-D]pyrimidine) and derivatives to

act as a “subversive substrate” of this enzyme was discovered by Marr and colleagues [81,82]. More attention has been paid recently to the potential of pyrimidine metabolism as chemotherapeutic target in parasites. Trypanosomes are able to synthesise uridine 5'-triphosphate nucleotide (UTP) *de novo* and by salvage of uracil. The enzyme dUTPase (deoxyuridine 5'-triphosphate nucleotide-hydrolase), which catalyses hydrolysis of dUTP to dUMP and pyrophosphate, is essential for the biosynthesis of dTTP (from UMP via thymidylate synthase) and for maintaining the integrity of genomic DNA [83]. Trypanosomal dUTPases possess a distinct tridimensional structure and have been proposed as potential targets for chemotherapy [83,84]. Recently, a series of triphenyl deoxyuridine derivatives selective for plasmodium dUTPase and with *in vitro* antimalarial activity were described [85].

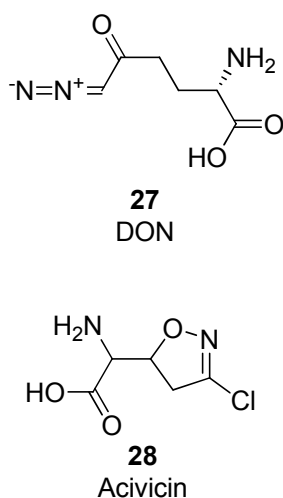


Figure 6. CTP synthetase inhibitors

CTP synthetase is another enzyme of the pyrimidine biosynthesis pathway that had not been studied in *T. brucei* until the Hofer and co-workers 2001 paper was published [86]. This study showed that the low levels of CTP found in *T. brucei* were the result of slow *de novo* synthesis and the lack of salvage pathways, making the inhibition of this enzyme a potential target for chemotherapy. The use of CTP

synthetase inhibitors such as 6-diazo-5-oxo-L-norleucine (DON: **27**, Figure 6) and α -amino-3-chloro-4,5-dihydro-5-isoxazolacetic acid (acivicin: **28**) provoked a reduction of CTP levels and blocked the growth of *T. b. brucei* *in vitro* and *in vivo*. DON also inhibited the growth of bloodstream forms *T. b. rhodesiense* *in vitro* ($IC_{50} = 0.36 \mu M$). The *in vivo* assays with both compounds showed a relapse of trypanosomes after the treatment was stopped, making the authors suggest that these molecules only block the proliferation of the parasites and that an immune response may be required to eradicate the parasites. A patent related to this invention was disclosed by these authors [224]. They claimed the use of a combination of a CTP synthetase inhibitor, such as a glutamine analogue, and a nucleobase (hypoxanthine, adenine or guanine) able to alleviate the toxic effects thereof *in vivo*, for the treatment of malaria, leishmaniasis, Chagas' disease and sleeping sickness. The combination of DON (**27**) and hypoxanthine was preferred.

3. Natural product derived chemotherapeutic agents

Natural products derived from plants and animals continue being the major source of medicines for man and the basis of many pharmaceutical drugs. In the area of tropical medicine in particular, widely used antiprotozoal drugs derive from natural products. Quinine, an alkaloid extracted from the cinchona trees (Rubiaceae) found in South America and the East Indies, and artemisinin, a sesquiterpene lactone from *Artemisia annua* (Asteraceae) have been used by traditional medicine to treat malaria. Many synthetic derivatives of these natural compounds were developed over the decades and allowed the discovery of new antiprotozoal drugs with better drug-like profiles. In the recent literature, nine patents claiming for use of natural extracts or

synthetic natural product derivatives against Chagas' disease [225,226,227,228,229,230] and/or sleeping sickness [230,231,232] were found.

3.1. Quinoline derivatives

Naphtylisoquinoline alkaloids isolated from African plants (Ancistrocladaceae and Dioncophyllaceae families) have shown promising antiprotozoal activity, especially against *Plasmodium* species (e.g. dioncophylline C), *T. b. brucei* and *T. b. rhodesiense* (e.g. dioncophyllines A, B and E) [87,88,89]. University of Mainz disclosed the preparation of a series of 3,3-dimethyl-isoquinoline compounds derived from these naturally occurring naphtylisoquinolines but lacking of the asymmetric substituents in C-1 and C-3 of the ring (**29**, Figure 7). These modifications made the synthesis of analogues much easier. The *in vitro* activity on *T. cruzi* infected L6-cells of four compounds was presented with IC₅₀ values ranging from 10.6 (**29**) to 50.3 μM. The specified compound [3,3-dimethyl-6-methoxy-8-hydroxy-3,4-dihydroisoquinoline, **29**] also displayed selectivity (SI > 40) towards the parasite with cytotoxicity on L6-cells > 439 μM [226]. These compounds were also claimed to be potentially useful for the treatment of neurodegenerative diseases based on their glutamate receptor antagonistic activity. The same team described a series of chiral 1-phenyl-2-aminomethyl-naphtalene derivatives for the same indications [225]. The activity of seven compounds was disclosed, being trifluoromethanesulfonate derivative **30** the most interesting one in terms of activity (IC₅₀ = 1.9 μM) and selectivity (SI > 36).

Antidesmone (**31**) [(S)-4,8-dioxo-3-methoxy-2-methyl-5-*n*-octyl-1,4,5,6,7,8-hexahydroquinoline], is an isoquinoline alkaloid extracted from several species of *Antidesma* and *Hyeronima* (Euphorbiaceae) [90,91]. The *in vitro* activity against *T. cruzi*, *T. brucei*, *L. donovani* and *P. falciparum* of antidesmone as well as nine

tetrahydroisoquinoline and 1,4,5,6,7,8-hexahydroisoquinoline derivatives was claimed by the Institut Für Pflanzenbiochemie [Note: the C-2 stereochemical configuration of these derivatives is the opposite to the stereochemistry of the natural product] [227]. IC₅₀ values against *T. cruzi* were high in general and ranged from 0.02 (compound **32**, SI > 8000) to 31.6 μM. In comparison, antidesmone (**31**) and benznidazole had IC₅₀ values of 0.113 (SI > 1500) and 2.29 μM (SI > 1500), respectively. On the contrary, trypanocidal activity of antidesmone on *T. b. rhodesiense* and *T. b. evansi* was low (IC₅₀ = 14.3 and 6.1 μM, respectively) compared to that of melarsoprol (0.0032 and 0.0042 μM, respectively).

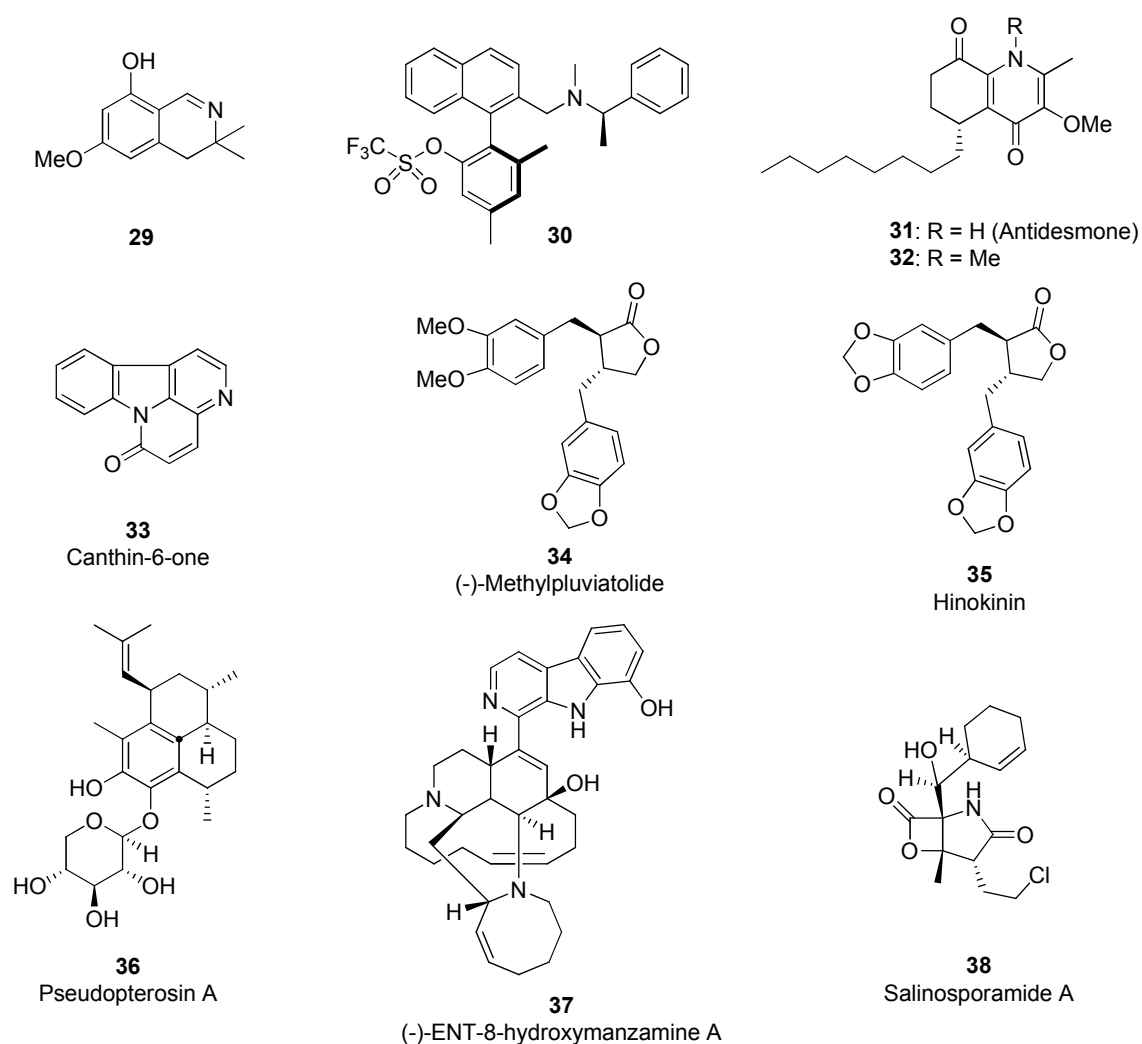


Figure 7. Natural product derivatives

3.2. Miscellaneous

In a 2004 patent, the Institut de Recherche pour le Développement disclosed the use of canthin-6-one (**33**), extracted from a *Zanthoxylum chiloperone* of the *angustifolium* variety, for the treatment of *T. cruzi* infections [229]. Canthin-6-one had been previously tested against *P. falciparum* [92] or *Leishmania* spp. [93] but its trypanocidal activity had never been reported. This application shows that this natural product is a highly effective trypanocide in the mouse model of Chagas' disease. Canthin-6-one was assayed in chronic and acute mouse models by oral and subcutaneous administration. In the model of acute *T. cruzi* infection, in a 15 days treatment at an oral dose of 5 mg/kg/day (n = 7), canthin-6-one was more effective than benznidazole (50 mg/kg/day po, n = 8) at day 15 post-treatment and, contrary to benznidazole, the parasites were eradicated at day 68. In the chronic mouse model, in a 20 days treatment at 5mg/kg/day po, canthin-6-one was again more effective than benznidazole (50 mg/kg/day) after a 79 days post-treatment follow-up. The mice treated with canthin-6-one (n = 8) were parasite free and protected from death [229].

Lignans obtained from leaves of *Zanthoxylum naranjillo* or *Piper cubeba* such as (-)-cubebin, (-)-methylpluviatolide [94], and semi-synthetic dibenzylbutyrolactonic derivatives were claimed as useful for the treatment and prophylaxis of Chagas' disease [228]. The process to obtain these compounds was disclosed and the *in vitro* anti-Chagas disease evaluation of seven compounds was reported (Figure 7). In an assay with blood trypomastigotes of *T. cruzi* IC₅₀ values ranged from 2.54 (**35**), 3.5 (**34**) to 274 μM (cubebin). Three compounds which displayed 100% inhibitory activity *in vitro* at 135 μM [O-acetyl cubebin, (-)-methylpluviatolide and (-)-O-(*N,N*-dimethylaminoethyl) cubebin] were claimed to show chemo prophylactic ability into healthy mice. In a recent study, (-)-hinokinin (**35**) was shown to have also a potent

activity ($IC_{50} = 0.7 \mu M$) *in vitro* against free amastigote forms of *Trypanosoma cruzi* [95].

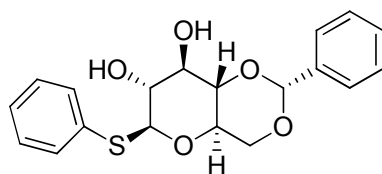
University of California disclosed a method for the isolation and purification of marine pseudopterosin compounds from *Symbiodinium* spp. symbionts, preferably obtained from the coral *Pseudopterogorgia elisabethae* [230]. This method has the advantage of using a non-animal source for the production of these molecules. Pseudopterosin A (**36**) is one of the 18 pseudopterosin compounds specifically claimed for preparation and for use as chemotherapy. However, no biological data was reported for that application. Another class of natural products derived from marine sponges, manzamine alkaloids, were claimed for use in the treatment of drug-resistant infections including malaria and sleeping sickness [232]. Several semi-synthetic derivatives prepared from manzamine A were described. Manzamine A and (-)-ENT-8-hydroxymanzamine A (**37**) had IC_{50} values in the range 0.008 to 0.014 μM against *P. falciparum* and SI values of 36 and 40, respectively. Both molecules also exhibited *in vivo* activity against *P. berghei* greater than chloroquine or artemisinin [96]. No biological data regarding trypanocidal activity was disclosed. A recent patent from Nereus Pharmaceuticals Inc. [233] claimed the use of [3.2.0] heterocyclic compounds analogues of the marine natural product salinosporamide A as proteasome and NF- κ B inhibitors for the treatment of neoplasm, inflammation and microbial infection. Salinosporamide A (**38**) had a mean GI_{50} value of 10 nM against 60 tumor cell lines and also inhibited proteasome activity with an IC_{50} value of 11.8 nM (NF- κ B activity of HEP293 cells). No biological data of antitrypanosomal activity was disclosed. This claim is based on the well-known potential of proteasome inhibitors against trypanosomes *in vitro* [28,97].

4. Single synthetic entities

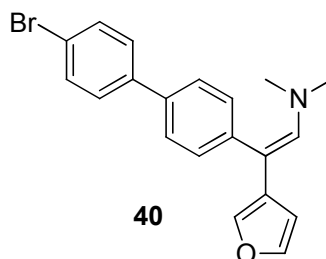
This section includes patents describing the synthesis and screening of compounds against several pathogenous agents, including protozoa. No specific targets were claimed to explain the trypanocidal activity of these molecules.

Bicyclic carbohydrates for the treatment of parasitic diseases such as leishmaniasis and trypanosomiasis were disclosed by Kemin Pharma Europe [234]. Three compounds were specifically claimed. Their synthesis and *in vitro* activity against *T. b. rhodesiense*, *T. cruzi*, *P. falciparum* and *L. donovani* was disclosed. One compound (**39**, Figure 8) was active against *T. b. rhodesiense* trypomastigotes ($IC_{50} = 0.187 \mu\text{M}$ vs. $0.21 \mu\text{M}$ for suramin) and displayed selectivity vs. human MRC-5 cells ($SI > 171$). The other two compounds had IC_{50} values of 1.01 and $< 0.98 \mu\text{M}$ against *L. donovani* amastigote infected macrophages, respectively.

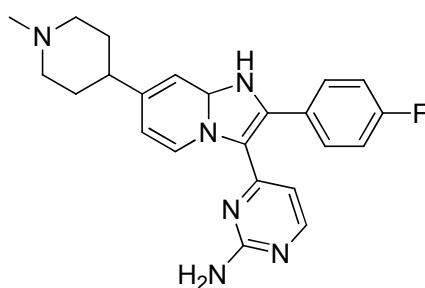
The synthesis and *in vitro* *T. cruzi* activity of a series of 4-bromophenyl derivatives was disclosed by the Universidade Estadual de Campinas in Brazil [235]. The furanyl derivative (**40**) showed interesting trypanocidal activity against *T. cruzi* ($IC_{50} = 9.5 \mu\text{M}$). Merck & CO Inc. disclosed a series of novel imidazo[1,2-a]pyridines (**41**) and N-oxides derivatives claimed to be useful for the treatment and prevention of protozoan infections, particularly African trypanosomiasis, Chagas' disease, malaria and toxoplasmosis [236]. Representative synthesis for the final compounds was given in 8 examples but no relevant biological data were presented.



39



40



41

Figure 8. Single synthetic entities

5. Expert opinion

Trypanosomiases affect millions of people in Africa and South America and kill around 60,000 people annually. The economic burden for affected countries is huge and represent 1 598 000 and 649 000 DALYs (Disability Adjusted life-years) for sleeping sickness and Chagas' disease, respectively [98]. Nevertheless, they are among the most neglected diseases in the world. The drugs available for the treatment of these diseases are far from satisfactory (e.g. low effectiveness, toxicity, high price, mode of administration, increasing treatment failures) and there is currently no drug able to cure chronic Chagas' disease. These diseases are totally neglected by pharmaceutical companies because of the low income of affected countries and the low financial return expected. For these reasons, the search for new antitrypanosomal drugs relies almost

exclusively on public research funded by governments and not-for-profit organisations, or on public-private partnerships in a few cases [99]. If we refer to the patents reported in this review, it seems, at first sight, that antitrypanosomal research is shared between industry (18 patents) and academia (18 patents). However, a deeper look at these trends reveals the true fact: most industry patents reporting possible trypanocidal activity are targeting other human diseases (e.g. cysteine protease inhibitors for the treatment of various cancers and bone diseases) and most patents claiming specifically antitrypanosomal activity (as the main claim) belong to the academia. Hence, industry patents lack, in general, of specific biological data (only found in 8 patents) supporting their claims for antitrypanosomal chemotherapeutic potential. Thus, it appears that pharmaceutical companies have libraries of synthetic compounds patented as possible antitrypanosomal agents but not tested as such, probably because of the low financial return expected. If academia could get the financial resources and industry agreement to run the biological screening of these compounds against trypanosomes (e.g. in a WHO-TDR granted screening centre), new antitrypanosomal lead compounds would probably emerge from such collaboration. There is a real need for academia-industry partnership in order to get enough financial backing for projects aimed at the discovery of new chemotherapeutic drugs against these diseases.

Thanks to financial backing from the Gates Foundation and the Drug for Neglected Diseases (DNDi) initiative from Médecins sans Frontières (MSF), one drug, the diamidine prodrug DB289, is currently in the bottleneck for the oral treatment of first stage sleeping sickness. This drug, which successfully finished a phase IIa clinical trial in first-stage *T. b. gambiense* sleeping sickness patients, is also being investigated in preclinical studies on an African green monkey model for second-stage disease [73]. DB289 and its aza-analogues [220] are at the moment the most promising

antitrypanosomal drugs in development for sleeping sickness. No other new drugs are currently in clinical trial for HAT or Chagas' disease. Hence, a pragmatic approach for the rapid development of new antitrypanosomal chemotherapy would be the clinical assessment of drug combination with existing trypanocides.

Despite being among the most studied parasites from a biological point of view, trypanosomes remain a challenge for chemotherapy. The growing knowledge of the parasite biochemistry has opened the way to new chemotherapeutic approaches based on newly validated biochemical targets [100]. A number of patents have appeared which dealt with new targets and disclosed interesting *in vitro* activity against *T. cruzi* (e.g. CPIs, sterol biosynthesis inhibitors) or *T. brucei* (e.g. PFT inhibitors, tubulin inhibitors, CTP synthetase inhibitors). However, only a few exciting *in vivo* results were reported among which the rationally designed C14-demethylase inhibitors (**15**) of Yale University, with an excellent potential against *T. cruzi* [217]. As usually happens in medicinal chemistry, natural products furnished another source of interesting lead compounds (**30** and **32**) with excellent *in vitro* activity and also exciting *in vivo* cures (**32**) of an acute and chronic mouse model of *T. cruzi* infection [227,229]. In this sense, screening of natural products and other in-house libraries of synthetic compounds against trypanosomes should allow to discover new trypanocidal lead compounds [101].

Acknowledgments

The technical assistance of Dr Sabela Fernández Pérez during the writing of this manuscript is gratefully acknowledged.

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