Active Natural Product Scaffolds Against Trypanosomatid Parasites: A Review

Peter E. Cockram† and Terry K. Smith*†

†Biomedical Sciences Research Complex, University of St Andrews, North Haugh, St Andrews, Scotland, KY16 9ST

ABSTRACT

Neglected tropical diseases caused by trypanosomatid parasites are a continuing and escalating problem, which devastate the less economically developed cultures in countries in which they are endemic by impairing both human and animal health. Current drugs for these diseases are regarded as out-of-date, expensive, with unacceptable side-effects and mounting parasite resistance, meaning there is an urgent need for new therapeutics. Natural products have long been a source of potent, structurally diverse bioactive molecules. Herein are reviewed natural products with reported trypanocidal activity, which have been clustered based on core structural similarities, to aid the future discovery of new trypanocidal core motifs with potential routes to synthetically accessible natural product cores suggested.


INTRODUCTION

Trypanosomatid parasites are a group of biochemically related, exclusively parasitic, kinetoplastid eukaryotes, in most cases responsible for life threatening and debilitating Neglected Tropical Diseases (NTD’s), which affect some of the poorest communities in the world. Encompassed within the family are the causative agents of Human African Trypanosomiasis (HAT), namely, two subspecies of Trypanosoma brucei: T. b. gambiense, causing a slow onset, chronic form of the disease in 24 countries throughout West and Central Africa, and T. b. rhodesiense, causing a fast onset, acute form of the disease in 13 countries throughout Eastern and Southern Africa. Furthermore, the related animal-infective species T. vivax, T. congolense and T. brucei brucei, are the most important causative agents of Animal African Trypanosomiasis (AAT), locally named nagana in western Africa. In both HAT and AAT, parasites are transmitted via the bite of the tsetse fly vector (Glossina species). HAT and AAT are endemic exclusively within sub-Saharan Africa where they risk the lives of approximately 65 million people and cause economic loss of $4.5 billion per year.

In addition to HAT, other trypanosomatid parasites are responsible for Chagas' disease (American trypanosomiasis) within South and Central America, and leishmaniasis throughout southern Europe, south-eastern Asia, Northern Africa and the Americas. Chagas' disease is caused by the Trypanosoma cruzi parasite, which is transmitted via the triatomine insect vector native to America, where an estimated 6 to 7 million people are infected with the disease. The disease also shows prevalence in North America, primarily in Mexico, with increasingly significant numbers of infections in the United States. The third human disease caused by trypanosomatid parasites, leishmaniasis, is caused by more than 20 different Leishmania species.
in both the New and Old Worlds, and these include *L. amazonensis*, *L. major*, *L. infantum* and *L. donovani*. The disease is transmitted via the bite of the female phlebotomine sand fly vector, with an estimated 300,000 new infections each year and over 1 billion people living in areas at risk, primarily throughout the tropics and subtropics.\textsuperscript{10}

All three human infective diseases, HAT, Chagas' disease and visceral leishmaniasis are deadly if untreated. HAT infections are characterized as either early or late stage, with parasites in early stage infections being confined to the bloodstream and some intratissue locations, which can progress rapidly to the CNS in late-stage infection. Drugs exist to treat both early- and late-stage infections; however, due to generic flu-like symptoms of early-stage infection, the disease often eludes diagnosis until late-stage infection has been reached, where characteristic symptoms of neurological problems and sleep disturbance are presented.\textsuperscript{6} Most drugs currently approved for the treatment of HAT are antiquated, expensive and often have undesirable side effects, with increasingly reported drug resistance becoming an urgent issue.\textsuperscript{12-14} They must be administered via injection, which increases treatment costs and risks the spread of other diseases endemic in these areas such as hepatitis and the HIV.\textsuperscript{15,16}

Chagas' disease reaches the fatal chronic stage in 30-40\% of patients infected with *T. cruzi* parasites with the disease commonly going undetected until this stage. Often the disease can take many (sometimes as long as 30) years to reach the chronic stage, after which the disease onset is rapid and patients die of heart failure caused by parasite damage to the cardiac system, even with treatment.\textsuperscript{9} Treatments for the disease, such as nifurtimox, have unacceptable side-effects and due to developing resistance are becoming less effective.\textsuperscript{17,18}

The fatal visceral form of *Leishmania* accounts for at least 60,000 deaths per year, with 600 million people living in at-risk areas. The main effective treatments for visceral infections, are
liposomal amphotericin B and meglumine antimonate. Pentavalent antimonials are the standard first-line treatment for both visceral and cutaneous leishmaniasis in most parts of the world, but they require administration by injection, and the emergence of resistance towards them has limited their usefulness. Alternative therapies such as miltefosine, originally an anticancer agent now used in the Indian sub-continent for the treatment of leishmaniasis, and paromomycin, an aminoglycoside with a defined target, see limited use due to their high costs and poor availability. Many of these drugs are only realistically affordable to those in less developed areas through WHO programs, by providing the treatments at up to 90% reduced cost.\textsuperscript{10} As with existing drugs for Chagas' disease and HAT, there is increasingly reported parasite resistance and problematic side effects accompanying these drugs.\textsuperscript{19-21}

A combination of treatment pricing, unacceptable side effects and increasing resistance of current treatments means there is an urgent need for the discovery of new trypanocidal and antileishmanial therapies. Drug development for NTD’s is often overlooked by drug developers, with only 4% of treatments developed between 2000 and 2011 targeting these diseases, even though they account for 11% of the global disease burden.\textsuperscript{22} This is mostly due to the fact that pharmaceutical companies cannot recover the vast cost of drug development and production for such treatments.\textsuperscript{23,24}

One of the major factors impeding the development of new drugs against trypanosomatid diseases is the lack of known effective drug targets. Drug targets must be genetically verified as essential and potential inhibitors must be shown to be chemically “on-target” (with validated direct inhibition or downstream metabolic disruption of a target protein function) to be effective and efficacious for the development of potential treatments. Target determination using known active trypanocidal compounds is one approach that would highlight more vitally needed effective drug
targets and greatly benefit the design of new treatments. The Drugs for Neglected Diseases
initiative’s (DNDi’s) guide for drug screening against kinetoplastid diseases states that ideal lead
compounds against HAT and leishmaniasis must fulfil the following criteria: activity ≤10 μM
against parasite cells; ≥10-fold greater potency against parasites versus mammalian cells; the
biochemical and whole-cell activities must correlate. These criteria highlight the need for new
potent and selective trypanocidal compounds and importantly those which showing chemically
validated, “on-target” activity.

Natural products are secondary metabolites produced by organisms in order to provide an
evolutionary benefit, i.e. an advantage over other competing organisms, often this results in
chemical compounds that are toxic towards the surrounding organisms. These secondary
metabolites can be products of various biosynthetic pathways such as polyketide synthases, the
mevalonate pathway and shikimate pathway. Crossover between separate pathways, as well as
post-synthetic modifications are often involved to furnish the final bioactive natural product, which
ultimately generates a wide variety of complex structural motifs, often with potent biological
activities in various cell types. Due to the nature of their biosynthesis, natural products and their
medicinally used derivatives typically have lower hydrophobicity and higher stereochemical
content than purely synthetic compounds. These structural features are greatly beneficial in both
pharmacokinetic parameters and structural diversity of drugs. As a result, natural products have
been the inspiration for a vast number of current treatments targeting a wide range of diseases,
with 49% of new drugs from 1981-2014 being natural products or structural derivatives thereof. This places them as promising, largely unexplored, candidates for the identification of new
trypanocidal drugs, by utilizing common cores observed in trypanocidal natural products, in order
to harness their activity, and chemically elaborate to give the required pharmacokinetic parameters to be effective new treatments.

Herein is presented a review of published natural products displaying trypanocidal activity. Studies were searched using the National Center for Biotechnology Information’s (NCBI’s) PubMed service alongside CAS SciFinder database services searching for the terms “Trypanosoma, natural product” and “Leishmania, natural product”. Compounds mentioned have been limited to those showing activity in at least one trypanosomatid cell line below 20 μM (within 2-fold activity of DNDi guidelines for lead candidates) as well as conserved structural characteristics shared with other active trypanocidal natural products. The current literature search is primarily confined to compounds with trypanocidal activities reported from 2013-2018, although some older examples have been included to highlight the recurrence of key motifs. While greatly desired, in vivo data for trypanocidal natural products are not widely reported. This is due to the high cost of in vivo screening when compared to testing against whole-cell parasite cultures. Selectivity data (where available) having a SI value >10 has been deemed preferable, but in many cases the selectivity is significantly lower and in some cases favors potency in mammalian cells. Initial selectivity of compounds is not strictly necessary however, as often selectivity can be engineered into drug scaffolds during optimization through various pathways such as differential uptake, metabolism and binding modes.\(^\text{28}\)

Existing reviews highlighting selected potent natural products against trypanosomatid parasites are available. However these often focus on particular sources and do not highlight specific skeletons shared between the wide variety of natural products reported.\(^\text{29-31}\) The primary focus of this review is to identify potential synthetically accessible, active core scaffolds which may be
beneficial in the identification of new targets as well as the development of potent trypanocidal drug compounds.

**TRYPANOSOMATID CELL TESTING**

Trypanosomatid parasites have complex life cycles, with various morphologies in both insect and mammalian hosts and as a result biological testing can be performed on a variety of parasite forms. The most clinically relevant life cycle stage of each parasite are as follows: trypomastigotes (bloodstream form) of *T. brucei*; intracellular amastigotes of *T. cruzi*; amastigotes of *Leishmania* spp. Due to the significant differences in the environments in which each form of the same parasite lives, they each display equally significant biochemical differences, often meaning data between life cycle stages is not tractable. A report examining this artefact within a high-throughput screen of *Leishmania* intracellular amastigotes and axenic amastigotes is available.\(^{32}\) In vitro testing of intracellular parasites requires compounds to be taken up initially by the mammalian host cell, and then by the parasites encompassed within them; making this more indicative of potential in vivo results than cell culture testing alone. Testing on intracellular parasites, however, is significantly more challenging due to the requirements for parasites to remain virulent against cells, a trait often lost in cultured cell lines. Due to the biochemically related nature of each of the parasite species, it is plausible that a drug target might be conserved across the trypanosomatid parasites, enabling discovery of an active, pan-trypanocidal drug. This is an ideal-world scenario whereas in reality even the subtlest of biochemical differences may mean a potent drug in one parasite species may be rendered inactive in others.
Below (Table 1) is a list of common control drugs and their activities against the relevant parasite species and life-cycle stages, to enable comparison of the discussed natural products with typical positive control values used within the field.
Table 1. Control Drugs Used in Testing of Compounds against the Trypanosomatid Parasites *T. brucei*, *T. cruzi*, and *L. donovani*.

<table>
<thead>
<tr>
<th>Drug</th>
<th><em>T. brucei</em> EC₅₀ (µM)ᵇ</th>
<th><em>T. cruzi</em> EC₅₀ (µM)ᶜ</th>
<th><em>L. donovani</em> EC₅₀ (µM)</th>
<th><em>L. infantum</em> EC₅₀ (µM)</th>
<th>Mammalian EC₅₀ (µM)</th>
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<tr>
<td>pentamidine</td>
<td>0.0012 (<em>Tbr</em>)¹²</td>
<td>0.9¹²</td>
<td>6.4 (P)¹³</td>
<td>8.3 (P)¹³</td>
<td>44.1 (H)³³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.6 (Ax)¹³</td>
<td>2.7 (Am)¹³</td>
<td>6.4 (V)³³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.8 (T)³⁴</td>
</tr>
<tr>
<td>melarsoprol</td>
<td>0.01 (<em>Tbg</em>)¹²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>suramin</td>
<td>0.033 (<em>Tbg</em>)¹³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eflozithine</td>
<td>10.6 (<em>Tbr</em>)³⁴</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>nifurtimox</td>
<td>2.4 (<em>Tbb</em>)³⁵</td>
<td>1.5³⁶</td>
<td>0.024 (P)³⁴</td>
<td>91.2 (H)³⁵</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.004 (Am)³⁴</td>
<td>37.3 (V)³⁴</td>
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<tr>
<td>benznidazole</td>
<td>30.0 (<em>Tbb</em>)¹²</td>
<td>9.3³⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amphotericin B</td>
<td></td>
<td>0.07 (P)³⁴</td>
<td>0.087 (Ax)³⁴</td>
<td>0.04 (P)³⁷</td>
<td>2.0 (T)³⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.07 (Am)³⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miltefosine</td>
<td>0.92³⁶</td>
<td>3.12 (P)³³</td>
<td>12.6 (P)³³</td>
<td>4.67 (T)³⁴</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>2.8 (Ax)³³</td>
<td>4.8 (Am)³³</td>
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<tr>
<td></td>
<td></td>
<td>2.4 (Am)³³</td>
<td></td>
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<tr>
<td>paromomycin</td>
<td>22.7 (<em>Tbr</em>)³⁸</td>
<td>0.005 (P)³⁴</td>
<td>0.007 (Am)³⁴</td>
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</tbody>
</table>

⁴ Abbreviations: *Tbg*, *T. b. gambiense*; *Tbr*, *T. b. rhodesiense*; *Tbb*, *T. b. brucei*; P, promastigote; Ax, axenic; Am, amastigote; H, HeLa; V, Vero; T, THP1.

ᵇ *T. brucei* activities are for bloodstream form parasites.

c *T. cruzi* activities are for epimastigote form parasites.
FLAVONOIDS

One of the most common classes of natural products are the flavonoids, many of which have been reported to show moderate activity across various trypanocidal parasite species. The FDA approved drug flavoxate, used to treat bladder discomfort, is built from a 3-methylflavone scaffold implementing it as a valid platform for drug discovery. Described below are a selection of flavonoids and their derivatives (1-11) shown in Figure 1.
Figure 1. Selected flavonoid natural products 1-11 with reported activity against trypanosomatids.
A comprehensive review on trypanocidal flavonoids is available, and an extensive structural correlation of a set of flavones and their analogues has been investigated by Baldim et al. identifying key SAR characteristics against trypanosomatid parasites. The simplest flavonol (1), a constituent of *Brassica oleracea*, has reported pan-trypanocidal activity with EC$_{50}$ values of 3.0, 2.2, and 33.0 µM against *L. donovani* amastigotes, the *T. b. rhodesiense* bloodstream form and *T. cruzi* intracellular amastigotes, respectively. Compound 1 additionally shows modest selectivity with SI values of 21, 29, and 2 for *L. donovani*, *T. brucei* and *T. cruzi*, respectively, as compared to L6 cells. The closely related flavonol isorhamnetin (2), isolated from a number of sources and maintains antiparasitic activities of 11.2 and 12.0 µM against the *T. brucei* bloodstream form and *L. donovani* amastigotes, respectively, with no activity in *T. cruzi* intracellular amastigotes up to 90 µM. Compound 2 also showed less potency for L6 cells than 1, with SI values of 10.7 and 4.6 for *L. donovani* and *T. brucei*, respectively. Quercetin (3), a constituent of numerous plant sources differing by removal of a single methyl group, displayed a markedly reduced EC$_{50}$ value of 25.8 µM against the *T. b. brucei* bloodstream form, however a higher potency was observed against *L. donovani* amastigotes with an EC$_{50}$ value of 3.3 µM. 3 has also been shown to have some in vivo activity against *L. donovani* amastigotes in mice with 15% inhibition observed using a 30 mg/kg dosing regimen over 5 days. Its glycosylated analogue, 4, isolated from *Vangueria edulis*, bearing an α-linked rhamnose at the 7-hydroxy group, showed slightly improved potency against the *T. b. brucei* bloodstream form with an EC$_{50}$ value of 20.1 µM. The sulfonated myricetin analogue 5, one of a number of flavonoids isolated from the plant *Limonium caspium*, displayed a similar activity of 21.4 µM (EC$_{50}$) against *T. brucei*, whereas the glycosylated variant 6 isolated from the same source showed almost two-fold increased potency with an EC$_{50}$ value of 13.1 µM. Both compounds were also tested against *L. donovani*
promastigotes but no activity was reported, with the life cycle stage and subspecies of T. brucei not reported.\textsuperscript{50} The highly methylated flavonol, 7, the most potent in a series of flavones isolated from the leaves of the plant species Vitex simplicifolia, gave a moderate potency of 12.1 \( \mu \text{M} \) (EC\textsubscript{50}) against the clinically relevant T. b. rhodesiense bloodstream form, with a modest SI of 9.8, as compared to L6 cells. Various flavones, lacking a 3-hydroxy group, have also been shown to have promising potency against trypanosomatid parasites. One relatively simple example, luteolin (8), a common flavone isolated from many plant sources, showed low-micromolar inhibition of L. donovani amastigotes and the T. b. rhodesiense bloodstream form with EC\textsubscript{50} values, in turn, of 2.8 and 14.1 \( \mu \text{M} \). It showed modest selectivity, with SI values of 12 and 3 for L. donovani and T. brucei.\textsuperscript{40} Luteolin (8) and quercetin (3) have been implicated in the induction of topoisomerase II-mediated DNA cleavage within L. donovani promastigotes and have shown promising in vivo activities, with 80\% and 90\% reduction splenic parasite load compared to PBS, based on a 3.5 mg/kg and 14.0 mg/kg dose administered twice per week over four weeks in male golden hamsters.\textsuperscript{51} 7,8-Dihydroxyflavone (9) is a highly potent flavone derivative, showing antileishmanial inhibition of the T. b. rhodesiense bloodstream form having an EC\textsubscript{50} value of 0.27 \( \mu \text{M} \) and a SI value of 116. Interestingly, the methylated variant, 7,8-dimethoxyflavone (10), exhibited a significantly reduced potency with an EC\textsubscript{50} value of 21.6 \( \mu \text{M} \) and SI of 5.\textsuperscript{40} Finally, the isoflavan analogue arizonicanol A (11), isolated with a number of potent 2,5-diphenyl-oxazoles from the roots of the plant species Oxytropis lanata, showed good trypanocidal activity, with an EC\textsubscript{50} of 4.1 \( \mu \text{M} \) against the T. congolense bloodstream form, highlighting the potential for further core scaffold simplification while maintaining potency.\textsuperscript{52}

Flavon-3-ol natural products have been shown to inhibit T. brucei pteridine reductase 1 (TbPTR1), with compounds 1 and 2 showing low micromolar IC\textsubscript{50} values of 12.8 and 13.9 \( \mu \text{M} \)
respectively. PTR1 has been genetically validated as essential in *T. brucei* and plays a key role for in vivo virulence. With their synthetic accessibility, which is discussed later in this review, flavonoids are placed as having a potentially strong core structure for the development of future lead compounds, and have a strong resource of SAR studies, and examples demonstrating in vivo activity and on-target effects against a potential new drug target in *T. brucei*. Their generally modest potency, however, requires vast improvement to attain desirable activity levels for a lead drug candidate.

**XANTHONES**

The structurally related class of xanthones is characterized by a core structure containing a tricyclic core of two substituted phenyl rings with a central pyrone motif. An extensive review examining clinical trials of xanthones is available, which describes their physiochemical and pharmacokinetic properties in order to aid the development of lead xanthone-like drug candidates. The FDA-approved amlexanox, an orally available drug used in the treatment of mouth ulcers, bears a structurally related chromeno-pyridinone core, highlighting xanthone-like cores as having good pharmacokinetic parameters. A number of trypanocidal xanthones have been reported, some structurally interesting examples (12-16) are shown below (Figure 2).
Sterigmatocystin (12), a pentacyclic natural product isolated from a fungal strain from the Microthyriaceae order, showed high potency with an EC$_{50}$ of 0.13 µM against *T. cruzi* amastigotes, but along with a strong, undesirable potency for Vero cells with an SI of 0.46.\textsuperscript{55} The prenylated xanthone $\alpha$-mangostin 13 isolated from the pericarp of *Garcinia mangostana* fruits showed impressive pan-trypanocidal activity, with EC$_{50}$ values of 7.9, 8.9, and 8.0 µM respectively for the *T. brucei* bloodstream form, *T. cruzi* intracellular amastigotes and *L. infantum* amastigotes. However, $\alpha$-mangostin showed a similar potency for MRC-5 leading to SI values of 0.95, 0.84 and 0.93, respectively.\textsuperscript{56} The chlorinated xanthone 14 isolated from the fungus *Chaetomium* sp. showed a high potency against *T. cruzi* intracellular amastigotes and *L. donovani* promastigotes, with EC$_{50}$ values of 3.8 and 7.9 µM, respectively, and good selectivities of 31 for *T. cruzi* and 6.8 for *L. donovani*. Its activity against *T. b. rhodesiense* bloodstream form was considerably less...
potent, with an EC$_{50}$ of 109 µM.$^{39}$ The related structures 15 and 16 isolated along with 14 showed potencies against the *T. b. rhodesiense* bloodstream form in having EC$_{50}$ values of 13.3 and 19 µM, respectively, with 15 showing no activity in *T. cruzi* intracellular amastigotes up to 27 µM, and 16 having an EC$_{50}$ of 19.8 µM. Compounds 15 and 16 showed moderate activities against *L. donovani* promastigotes with EC$_{50}$ values of 14.3 and 9.6 µM, respectively.$^{57}$

The pan-trypanocidal nature of many reported xanthones places them as potential interest for further evaluation against multiple trypanosomatid cell lines, with overall activities slightly higher than those seen in the 3-flavonols. Combined with the vast knowledge accumulated by several xanthones progressing through clinical trials for other diseases, this makes them a favourable target for further development.

**QUINONES**

Numerous trypanocidal quinones have been reported, with a postulated mode of action of naphthoquinones generating reactive oxygen species and inhibiting mitochondrial function within the parasites.$^{58,59}$ Unlike higher eukaryotes such as mammalian cells, which contain thousands of mitochondria, trypanosomatid parasites remarkably contain only a single mitochondrion.$^{60}$ This means that they are particularly susceptible to disruption of mitochondrial function, thus this is proposed as a potential drug target and putative mechanism toward a selective mode of action.$^{61}$ Mitomycin, a FDA-approved quinone natural product, is used as an anticancer therapy, and exemplifies the promising pharmacokinetic qualities of this scaffold.$^{39}$ Structurally interesting quinone examples (17-21) have been identified and are discussed below (Figure 3).
Komaroviquinone (17), a potent inhibitor of *T. cruzi* trypomastigotes, was isolated from the plant species *Dracocephalum komarovii*, and has an interesting tetracyclic core structure including a 1,4-oxygen bridged cyclohept-5,6-ene gave an EC\textsubscript{50} value of 0.25 µM. An extensive synthetic SAR study of komaroviquinone-derived analogues has been completed producing potent trypanosomatid inhibitors, of which four displayed submicromolar activities. Another tetracyclic natural product, the abietane-derived product 18 isolated from the plant species *Salvia cuspidata*, which also shows activity against *T. cruzi* epimastigotes, although with markedly reduced potency.
(EC$_{50}$ of 16.6 μM). Three synthetic analogues were prepared from 18, extending from the free 12-hydroxy motif, the most potent of which being a trimethylsilyl ether showing a more promising EC$_{50}$ value of 5.4 μM. All of the examples showed no mammalian activity up to 15 μM in Vero cells, although this is a low maximum threshold level.$^{64}$ Even the simple anthraquinone damnacanthal (19) showed moderate activity against T. cruzi intracellular amastigotes with an EC$_{50}$ value of 39.5 μM but having a low selectivity with a SI value of 0.79.$^{65}$ Ascidiathiazone A (20), a structurally related tricyclic thiazoquinone isolated from the tunicate Aplidium sp., showed low micromolar activity against T. b. rhodesiense bloodstream form, with an EC$_{50}$ value of 3.1 μM, and a high SI of 50 versus L6 cells. This compound exhibited no activity against T. cruzi intracellular amastigotes and L. donovani amastigotes up to 250 μM. Several synthetic thiazoquinone analogues were prepared, with one key example showing sub-micromolar activity against T. b. rhodesiense bloodstream form along with increased selectivity, and another showing modest pan-trypanocidal activities. In vivo studies were carried out investigating the antimalarial activity of 20. A furan ring-based analogue was tested in vivo for activity against P. berghei, and gave 85% reduction in parasitaemia for mice using ip injection and 45% reduction on oral administration, thereby highlighting the good oral bioavailability of these scaffolds.$^{66}$ The bis-glycosylated benzanthraquinone actinosporin A (21), a constituent of the bacterium Actinokineospora sp., also demonstrated moderate activity against T. b. brucei bloodstream form, with an EC$_{50}$ value of 15.5 μM with a good SI of >13. A compound family member of 21, actinosporin B (22), was found to be inactive against the T. b. brucei bloodstream form in the same study, and both compounds were reported to show no activity against L. major (the life cycle stage was not reported).$^{67}$
Quinones are a reasonably promising class of structurally related trypanocidal compounds, with a suggested mode of action inhibiting mitochondrial function; a validated drug target. Due to the structural diversity of many of the natural products reported, firm SARs are challenging to conclude, although there have been promising correlations found in isolated examples. Related thiazoquinones have been shown to have good oral bioavailability through in vivo testing against plasmodial parasites.

**INDOLE ALKALOIDS**

Indole alkaloids are a class of natural products typically biosynthesized from tryptophan, and they contain an indole moiety furnished with either isoprenyl-derived units or other alkaloid moieties. Indole is a key nucleus of many bioactive molecules and natural products and drugs, with many indole-containing drugs used in the current market for a wide range of diseases from delavirdine as an anti-HIV medicine to binedaline as an antidepressant. A key review on the pharmaceutical uses of indoles is available, highlighting the importance of indole-based ring systems in existing drugs and pharmaceutical development. A number of trypanocidal indole alkaloids (23-30, Figure 4) have been collated and are discussed below.
Figure 4. Selected indole alkaloids and derivatives (23-32) with reported activity against trypanosomatid parasites

One of the most common moieties observed in indole alkaloids is the β-carboline ring system. Opacaline A (23), a brominated β-carboline natural product isolated from the ascidian *Pseudodistoma opacum*, showed moderate trypanocidal activity with an EC$_{50}$ value of 30 μM against the *T. b. rhodesiense* bloodstream form and an SI of 2.6 when compared to L6 cells. Interestingly, the synthetic de-bromo analogue 24 showed increased potency against *T. b. rhodesiense* bloodstream with an EC$_{50}$ of 12 μM and a modest SI of 7 in comparison to L6 cells. Both carbolines 23 and 24 exhibited little or no activity versus *T. cruzi* intracellular amastigotes or *L. donovani* amastigotes below 100 μM. Another 6-brominated tryptophan-derived natural product, iotrochamide A (25), isolated from the sponge *Iotrochota* sp., showed potent trypanocidal...
activity with an EC$_{50}$ value of 4.7 μM against the *T. b. brucei* bloodstream form and with a good SI of >15. The tyrosine-derived analogue iotrochamide B, isolated together with 25, also showed similar potency and selectivity, suggesting the eastern fragment of these molecules are crucial for activity.\textsuperscript{70} Neocryptolepine (26), the methylated indoloisoquinoline isolated from the plant species *Cryptolepis sanguinolenta*, showed high to moderate potency against a wide range of trypanosomal cells, with EC$_{50}$ values of 2.2, 2.0, and 49.5 μM against *T. b. rhodesiense*, *T. cruzi*, and *L. donovani* amastigotes, respectively (the trypanosome life cycle stages were not reported).\textsuperscript{71} Modest selectivity was observed with SI values of 2.9, 3.3 and 0.13 for *T. b. rhodesiense*, *T. cruzi*, and *L. donovani* amastigotes, respectively. Extensive SAR analysis around the neocryptolepine scaffold has been investigated\textsuperscript{71,72} with numerous analogues being synthetically prepared, of which many displayed submicromolar activities against *T. b. rhodesiense*. The isomeric compound cryptolepine (27) isolated from *Cryptolepis sanguinolenta*,\textsuperscript{73} showed improved potency against both the *T. brucei* bloodstream form and *L. donovani* promastigotes, having EC$_{50}$ values of 0.3 and 1.6 μM respectively. Compound 27, however, also showed higher mammalian cytotoxicity with SI values of 29.3 for *T. brucei* and 0.7 for *L. donovani* promastigotes as compared to macrophage cells. Similarly to 26, various ring substitutions of cryptolepine have been investigated, with a key example being 2,7-dibromocryptolepine (28) that showed a considerable potency of 0.0029 μM against *T. brucei* bloodstream form and 0.5 μM against *L. donovani* promastigotes, with exceptional SI values of 2083 and 18, respectively. Furthermore, cryptolepine analogues have shown promising in vivo activity against *T. brucei* in mice with 28 exhibiting similar suppression of parasitaemia by oral, im and iv administration.\textsuperscript{74,75} Fascaplysin (29), a β-carboline alkaloid isolated from the marine sponge *Hyrtios*, contains a similar, almost planar structure with only a single sp$^2$ carbon center. This compound gave selective, submicromolar, trypanocidal activity with
an EC$_{50}$ value of 0.63 µM against *T. b. rhodesiense* (life cycle stage not reported) and a SI value of 16 when compared to L6 cells. A structurally related amino-quinoline class of compounds named huprines, derived from the naturally occurring huperzines isolated from *Huperzia* spp., are known inhibitors of acetylcholinesterase and were developed as a treatment for Alzheimer’s disease. Huprine Y (30) was determined as the most potent in a series of related natural and synthetic compounds when tested against the *T. brucei* bloodstream form, with an EC$_{50}$ of 0.6 µM and a good SI value of 13 versus L6 cells. Investigation of synthetic bis-huprines has also been undertaken, with examples retaining similar potency, however, with increased selectivity.

The manzamines are a family of over 100 complex β-carboline-containing natural products isolated from a variety of marine sponges, which as a family have shown broad bioactivity and importantly antileishmanial activity. A comprehensive review on the manzamine alkaloid family and their antileishmanial activity is available. One key example, manzamine A (31), was first isolated from a sponge of the genus *Haliclona* and in addition to its known anticancer activity, possesses potent antileishmanial activity with an EC$_{50}$ of 1.6 µM against *L. donovani* promastigotes. This alkaloid displayed significant cytotoxic activity against Vero cells, however, and gave a SI of just 1.3. Another key example, the structurally related compound manzamine X (32), first isolated from a *Xestospongia* sp. Sponge, showed modest antileishmanial activity with an EC$_{50}$ value of 9.8 µM against *L. donovani* promastigotes, although with no discernible cytotoxicity towards Vero cells.

Diketopiperazines (DKPs) are a common motif within natural products, originating from the cyclization of two amino-acid partners during biosynthesis. Tadalafil, marketed under the name Cialis, is an FDA approved, orally administered treatment for erectile dysfunction, which contains an indole-linked DKP motif and highlights the usefulness of this core as a drug platform.
trypanocidal indole-linked DKPs have been reported, and some examples (33-36, Figure 5) are discussed below.

**Figure 5.** Selected indole-containing DKP natural products (33-36) with reported activity against trypanosomatids

12,13-Dihydroxyfumitremorgin C (33), one of a number of polycyclic DKPs isolated from *Aspergillus fumigatus*, displayed good antitrypanosomal activity, with an EC$_{50}$ value of 6.4 μM against the *T. b. brucei* bloodstream form and having an SI of 15 when compared to Jurkat cells.$^{84}$ The structurally related spirocyclic 6-methoxyspirotrypostatin B (34) and dehydrobis(methylthio)gliotoxin (35), isolated from the same organism showed similar whole-cell activity with EC$_{50}$ values of 5.7 and 8.5 μM, respectively, against the *T. b. brucei* bloodstream form. Both exhibited modest respective SI values of 3.7 and 16.6 when compared to Jurkat cells.
Compounds 33 and 35 were shown to inhibit *T. b. rhodesiense* cysteine protease with IC$_{50}$ values of 10.9 and 19.5 µM, respectively. Compound 34 showed no enzymatic inhibition up to 50 µM.$^{84}$ Although non-essential, cysteine protease has been suggested to play a role in the migration of the parasites across the blood-brain barrier, making it a target worthy of further investigation.$^{85}$ Verticillin B (36) a complex indole-containing DKP with an interesting disulfide-bridging functionality, isolated from the fungus *Nectria inventa*, showed extremely potent activity against *T. b. brucei*, with an EC$_{50}$ value of 0.007 µM. This compound did, however, exhibit considerable cytotoxicity with an EC$_{50}$ of <0.6 µM against Jurkat cells. A number of other disulfide-bridged DKPs were also isolated in the same study and displayed equally high levels of trypanocidal potency, but also high cytotoxicity demonstrating this as a highly potent but generally cytotoxic motif.$^{84}$

A wide range of structurally diverse, trypanocidal, indole-containing natural products has been reported in the recent literature. Examples such as the cryptolepine, neocryptolepine and related quinone huprine scaffolds have been subjected to SAR studies, highlighting key trends for both trypanocidal and leishmanicidal activity. Coupled with the promising in vivo activity of cryptolepine analogues and ongoing development of huprines as potential Alzheimer’s disease treatments, there is a solid foundation for the further development of such core structures into active antiparasitic compounds. Additionally, indole-containing DKP scaffolds have been shown to be modest inhibitors of *T. b. rhodesiense* cysteine protease, which is influential in the progression of this parasitic disease to the later CNS stage, which may be exploited to prolong the lifetime of first-stage treatments.

**SESQUITERPENE DERIVATIVES**
Terpene-derived natural products encompass a wide range of skeletal motifs, based upon the number of isoprene units encompassed within the molecule and further structural modifications. Numerous trypanocidal terpenoids have been reported, with many sharing a common 7,5 or 6,6-membered, fused ring system, and a number of examples [37-43, Figure 6 and 44-49 Figure 7] are discussed below.

**Figure 6.** Selected structurally related sesquiterpene and iridoid natural products (37-43) with reported activity against trypanosomatids

Psilostachyin (37), a sesquiterpene lactone isolated from the plant species *Ambrosia tenuifolia*, displayed potent inhibition of *T. cruzi*, with EC$_{50}$ values of 4.3 and 12.5 μM against the epimastigote and trypomastigote forms, respectively, and *L. mexicana* promastigotes with an EC$_{50}$ value of 0.42 μM. The SI values against L6 cells were 21 and 34 for *T. cruzi* epimastigotes and
trypomastigotes and 214 for *L. mexicana*.\(^8\) The related natural product family member, psilostachyin C (38), isolated from *Ambrosia sabra* showed more potent activity against *T. cruzi* cells with EC\(_{50}\) values of 2.3 and 3.4 \(\mu M\) against epimastigote and intracellular amastigotes as well as 4.5 and 5.7 \(\mu M\) for *L. mexicana* and *L. amazonensis* promastigotes.\(^8\) Compounds 37 and 38 have both been found to display in vivo activity against *T. cruzi* in mice. Of these, compound 37 gave an approximately 1.5-fold reduction in parasitaemia over acute infection (up to 30 days), and 100\% survival rate up to 50 days.\(^8\) In line, 38 gave a two-fold reduction in parasitaemia over acute infection and a 20\% survival rate over the first 30 days.\(^8\) Both compounds out-performed the current treatment benznidazole in these studies, in which all mice treated with this drug died by day 35 for the psilostachyin (37) study and 30 for the psilostachyin C (38) study. In both studies, the sesquiterpenes and benznidazole were given by ip administration as a 5-day treatment of 1 mg/kg per day. The modes of action of 37 and 38 have been explored in *T. cruzi* and are reported to induce trypanosome death by independent modes of action, with the suggested mechanism action of 37 being heme interaction and of 38 being inhibition of sterol synthesis.\(^8\) This could explain the significant differences of in vivo activity between these two structurally similar compounds. The related lactone vernolepin (39), the most potent of four sesquiterpene lactones isolated from the plant *Veronia* sp., showed a very high activity and a good selectivity against the *T. brucei* bloodstream form, with an EC\(_{50}\) of 0.19 \(\mu M\) and SI of 14.5 as compared to L6 cells.\(^8\) A guaianolide (40) isolated from the plant *Tanacetum parthenium*, which bears a 5-7-5 fused tricyclic core structure, gave a moderate trypamocidal activity against both the *T. cruzi* epimastigote and trypomastigote forms with EC\(_{50}\) values of 18.1 and 5.7 \(\mu M\), respectively, but an EC\(_{50}\) value of only 67 \(\mu M\) against intracellular amastigotes. Compound 40 showed fairly low cytotoxicity with good SI values of 5.2, 16 and 1.4 against the epimastigote, trypomastigote and intracellular amastigote
forms, respectively, when compared to LLCMK2 cells. Some synergistic effects were observed between this guaianolide and benznidazole with epimastigotes, and investigations into phenotypic effects on parasite cells have suggested that trypanocidal action occurs through multiple mechanisms.\textsuperscript{90} The carboxylic acid \textit{41}, isolated from the plant \textit{Eupatorium perfoliatum}, which bears the same 5-7-5 core structure, showed no inhibition against either \textit{T. cruzi} or \textit{L. donovani}. It was, however, determined as a moderate inhibitor of the \textit{T. b. rhodesiense} bloodstream form growth, with an EC\textsubscript{50} of 20.7 \textmu M and a SI of 11 when compared to L6 cells. The related compound \textit{42}, a suspected dimeric product of \textit{41}, and isolated from the same source, displayed an increased potency against the \textit{T. b. rhodesiense} bloodstream form with an EC\textsubscript{50} of 4.5 \textmu M, and additionally demonstrated modest potency against both \textit{T. cruzi} intracellular amastigotes and \textit{L. donovani} amastigotes, with EC\textsubscript{50} values of 30.3 and 10.2 \textmu M, respectively. The selectivity of \textit{42} was poor, with SI values of 2.6, <1 and 1.6 for the \textit{T. b. rhodesiense} bloodstream form, \textit{T. cruzi} intracellular amastigotes, and \textit{L. donovani} amastigotes, respectively.\textsuperscript{91} Molucidin (\textit{43}), the parent compound of a series of iridoids isolated from the tropical plant species \textit{Morinda lucida}, which despite being skeletally distinctive from compounds \textit{37-42}, also contains a highly oxygenated, fused polycyclic core and showed submicromolar potency against \textit{T. b. brucei} cells with an EC\textsubscript{50} value of 1.2 \textmu M, with a modest SI of 11.2 as compared to HF-19 cells.\textsuperscript{92} Phenotypic investigation into trypanosome death by treatment with molucidin analogues has shown flagellar deformation as well as cell cycle alteration as possible modes of action. In the same study, the free acid of \textit{43} was tested for in vivo activity, giving complete clearance of parasitemia in mice for 20 days post-infection based on five consecutive daily doses of 30 mg/kg. Molducin, however showed significant toxicity and caused death of the mice at day 7.\textsuperscript{93}
Figure 7. Further selected sesquiterpene natural products and synthetic derivatives (44-49) with reported activity against trypanosomatids

Nobilin (44), a natural product isolated from Anthemis nobilis, displays a 5-10 fused core structure related to the 5-7-5 core observed for other trypanocidal sesquiterpenes. It exhibited potent trypanocidal activity with an EC₅₀ value of 3.2 μM against the T. b. rhodesiense bloodstream form with a modest SI of 2.1 when compared to L6 cells. A number of synthetic transannular cyclization products of nobilin have also been investigated, revealing several antitrypanosomal compounds, the most potent of which, 45, showed near ten-fold increased activity over nobilin, with an EC₅₀ of 0.46 μM and a similar SI of 2.4. A related natural product deoxyelephantopin (46), was identified through a bioassay-guided isolation from the tropical plant species Elephantopus scaber, which contains an additional lactone ring, forming a 5-10-5 fused ring system. This compound displayed a very high potency against the T. b rhodesiense bloodstream form, with an EC₅₀ value of 0.07 μM, and a high SI of 65 as compared to L6 cells. A number of elephantopin derivatives have been reported previously, displaying potent antileishmanial activities, with the parent compound and others having EC₅₀ values of <0.28 μM against L. major
promastigotes. It has been reported that the $\alpha$-methylene-$\gamma$-lactone moiety is a key factor in mediating the anti-trypanosomal activity of sesquiterpene derivatives.\textsuperscript{96} This is well-known to act as a strong Michael acceptor, leading to inactivation of the trypanothione cycle, a key aspect in regulating peroxide levels within trypanosomatids, which therefore induces oxidative stress.\textsuperscript{95,97}

4-Acetoxydolastane (47), a natural product isolated from the marine alga Canistrocarpus cervicornis bearing a 6-7-5 fused cyclic core, displayed potent antileishmanial activity with EC$_{50}$ values of 5.5 and 18 $\mu$M, respectively, against L. amazonensis promastigote and intracellular amastigote forms. Additionally, 47 exhibited low cytotoxicity, with a selectivity index of 93 versus J774G8 macrophages. Investigation of its mechanism of action revealed induction of ultrastructural changes, including mitochondrial damage, suggesting interference with the mitochondrial membrane potential.\textsuperscript{98} Shagene A (48), a terpenoid isolated from an undescribed coral genus also displayed inhibitory activity against Leishmania, with an EC$_{50}$ of 5 $\mu$M against L. donovani intracellular amastigotes, with no observed cytotoxicity, and a SI value of >70.\textsuperscript{99} Interestingly, an almost identical keto variant, shagene B (49), displayed no trypanocidal nor cytotoxic activity, a drastic effect from minimal structural alteration, alluding to the importance of the methoxy group substituent.\textsuperscript{99}

The terpenoid lactones are a quite diverse class of natural products, although many of those active against trypanosomatid parasites are somewhat closely related in structure. Seemingly key to their activity is the presence of an exocyclic methylene substituent at the $\alpha$-position of five-membered lactone ring. Additionally, 5-10 type fused ring systems seem to be common bioactive cores, with several examples displaying submicromolar activities. Mode of action studies have been carried out on some key structures, suggesting interference with trypanosome oxidant regulation and the resultant induction of oxidative stress. Along with promising in vivo results for
one reported compound, this overall class should provide additional promising leads for the further development of new trypanocidal and leishmanicidal compounds.

**GUANIDINE ALKALOIDS**

Guanidine alkaloids are relatively common natural products, and many have been reported to show trypanocidal activity, with several of those extracted from marine sources appearing to be particularly potent. A number of guanidine alkaloids of interest (50-54, Figure 8) are discussed below.

![Image of guanidine alkaloids](image)

**Figure 8.** Selected guanidine alkaloid natural products (50-56) with reported activity against trypanosomatids
The relatively simple molecule monalidine A (50), one of several guanidine alkaloids isolated from the marine sponge Monanchora arbuscular, showed potent activity against both T. cruzi trypomastigotes and L. infantum promastigotes with EC_{50} values of 8 and 2 μM, respectively, along with moderate SI values of 3 and 13, respectively, using LLC-MK2 cells. In the same study, the more complex dimer batzelladine L (51), isolated from the same source, showed improved activity against T. cruzi promastigotes, with an EC_{50} value of 2 μM, and retained antileishmanial activity (EC_{50} of 2 μM) and having SI of values of 11 for both parasite species.\textsuperscript{100} Synthetically prepared agelasine D (52), first isolated from the sponge Agelas nakamura,\textsuperscript{101} while not containing a guanidine moiety, displays a close chemical analogue in its adenine core, which has been suggested to aid its uptake via P2 transporters. It exhibited potent pan-trypanocidal activity against the T. b. brucei bloodstream form, T. cruzi intracellular amastigotes, and L. infantum amastigotes, with EC_{50} values, in turn, of 1.8, 9.0, and 3.0 μM, and SI values of 5, 2 and 6.7, respectively, as compared to MRC-5 cells. Investigation of several agelasine D structural analogues highlighted this core structure as having potential for trypanocidal and leishmanicidal activity, with many compounds surpassing the potency of the parent natural product. One key example (53) showed potent activity with EC_{50} values of 0.5, <0.25, and 2 μM for the T. b. brucei bloodstream form, T. cruzi intracellular amastigotes, and L. infantum amastigotes, respectively. However, significant cytotoxicity was also shown, with corresponding SI values of 1.8, <3.6 and 0.45 as compared to MRC-5 cells.\textsuperscript{102}

The simple 2-amino-imidazole-containing fragment-like natural product 54, one in a series of imidazolium compounds isolated from Agelas oroides, also showed modest pan-trypanocidal activity against the T. b. rhodesiense bloodstream form, T. cruzi intracellular amastigotes and L. donovani amastigotes, with reported EC_{50} values of 17, 18, and 95 μM, respectively. However, it
exhibited high cytotoxicity in L6 cells, with SI values, in turn, of 3, 3 and 0.05. Compound 54, along with other imidazolium compounds in the series, was revealed as an enoyl reductase inhibitor in the related protozoan species Plasmodium falciparum, suggesting this as a potential mechanism of action, although this has not been described in trypanosomatids. 103 Sceptrin (55), a bromo-imidazole natural product containing an interesting tetrasubstituted cyclobutane core, was originally isolated from Agelas sceptrum. 104 It showed modest potency against the T. b. rhodesiense bloodstream form, with an EC₅₀ value of 15.7 μM, in addition to low toxicity in L6 cells with an SI value of >10. This substance, however, showed little activity against T. cruzi intracellular amastigotes and L. donovani amastigotes (EC₅₀ values of 96 μM and 83 μM, respectively). 105 Dibromopalau’amine (56), a complex dibrominated polycyclic natural product extracted from the sponge Anixella verrucosa 106 bears similar amino-imidazolium moieties to 55. It proved to be a submicromolar inhibitor of the T. b. rhodesiense bloodstream form, with an EC₅₀ value of 0.8 μM, and demonstrated potent inhibition of L. donovani amastigotes (EC₅₀ value of 1.9 μM) but low activity for T. cruzi intracellular amastigotes (EC₅₀ value of 120 μM). This compound showed modest selectivity for both T. b. rhodesiense and L. donovani, with SI values of 10 and 4 respectively. 105

Examples of guanidine-derived and related alkaloids have been shown to be potent inhibitors of individual trypanosomatid species and are modest pan-trypanocidal agents. Although existing SAR and in vivo studies are limited in this class of alkaloids, preliminary studies have shown some success. The adenosine-derived natural product agelasine D (52) showed low micromolar inhibition of all three parasite species investigated, and a follow-up study of synthetic analogues has yielded submicromolar inhibitors of T. brucei and T. cruzi. Despite the unfortunate
accompanying cytotoxicity thus far shown by these analogues, their scaffold presents a good basis for further development of trypanocidal agents.

**ENDOPEROXIDES**

The endoperoxide motif exemplifies the diverse three-dimensional structural nature of bioactive natural products. Its functionality is rarely seen in synthetic drug compounds, yet is a recurrent feature in reported highly trypanocidal natural products, many of which are isolated from marine animals. Examples of some endoperoxide-containing natural products (57-64) are shown in Figure 9. Interestingly FDA-approved drugs artemether and arteether, and the natural product from which they are derived, artemisinin, all contain an endoperoxide functionality and are all used as frontline treatments for malaria, a related neglected tropical disease caused by *Plasmodium falciparum*.\(^{39,107}\) This highlights the moiety as a feasible active core in the treatment of other NTD’s such as those caused by the trypanosomatids.
Manadoperoxide B (57), an endoperoxide isolated from the sponge *Plakortis cf. lita* showed submicromolar potency against the *T. b. rhodesiense* bloodstream form with an EC₅₀ value of 0.0088 μM. Compound 57 additionally showed very high selectivity with an SI value of >3000 as compared to HMEC cells. The hydrolyzed variant, manadoperoxidic acid (58), also isolated from the *Plakortis cf. lita* sponge, showed somewhat reduced activity with an EC₅₀ value of 5.7 μM and only moderate selectivity (SI value of 3.8 compared with L6 cells). 12-Isomanadoperoxide B (59), the Z-isomer of manadoperoxide B (57), isolated from *Plakortis cf. lita*, showed a slight
reduction in potency but still exhibited submicromolar inhibition of the *T. b. rhodesiense* bloodstream form with an EC$_{50}$ value of 0.032 μM and a SI value of >350 when compared to L6 cells. The structurally related plakortide P (60), isolated from the sponge *Plakortis angulospiculatus* showed modest potency against *T. cruzi* trypomastigotes with an EC$_{50}$ value of 6.3 μM. The compound, however, exhibited poor selectivity when compared to the other structurally related endoperoxides discussed, with an SI value of 7. 11,12-Dehydro-13-oxo-plakortide Q (61) and manadoperoxide I (62), isolated from the sponges *Plakortis angulospiculatus* and *Plakortis cf. lita* respectively, have shown the tolerance of varying appendages to the endoperoxide core. Compounds 61 and 62 have reported activities against the *T. b. brucei* and *T. b. rhodesiense* bloodstream form parasites with respective EC$_{50}$ values of 0.049 and 0.17 μM, and SI values of 105 as compared to HEK-293 cells for 61 and >160 as compared to HMEC cells for 62. Isolation and testing of a range of manadoperoxides are described by Chianese et al. 108 Ergosterol peroxide (63), a sterol isolated from *Pleurotus ostreatus* and *Trametes versicolor*, showed trypanocidal and leishmanicidal activities with EC$_{50}$ values of 16.8, 13.9, and 4.0 μM against *T. cruzi* epimastigotes, *L. amazonensis* promastigotes, and *L. amazonensis* amastigotes, respectively. Sterol 63 showed only modest selectivity, with SI values of >5, 3, and 11 for *T. cruzi*, *L. amazonensis* promastigotes, and *L. amazonensis* amastigotes, respectively. 112,113 Ergosterol is the dominant de novo synthesized sterol in trypanosomatids, and has been implemented in signaling for the regulation of *T. brucei* growth, potentially alluding to the trypanocidal activity of 63. 114 Plakortide E (64), isolated from the sponge *Plakortis halichondroides*, possesses a five-membered endoperoxide core as opposed to the six-membered core displayed in compounds 57-63. Compound 64 showed activity against *T. brucei* with an EC$_{50}$ value of 5 μM and an SI of >20 compared to J744.1 macrophages, but no activity against *L. major*
promastigote cells. Plakortide E (64) has been shown to be a non-competitive, reversible inhibitor of the trypanosomatid cysteine protease, rhodesain, in addition to similar cysteine proteases from other species.\textsuperscript{115} This suggests a similar mode of action for other endoperoxides, although further investigation into structure-activity relationships is required.

Endoperoxides comprise a range of extremely potent trypanocidal compounds, with many examples showing submicromolar activity. Manadoperoxides, in particular, have been revealed as being very potent, with some key SAR factors and structural tolerances already revealed. While seemingly complex, these core structures can be readily accessed synthetically, as discussed later in this review. Insights into their suspected mode of action, via the inhibition of cysteine proteases, requires further investigation as well as analysis of their in vivo performance against parasitic infections.

**MACROCYCLES**

Macrocycles are a diverse branch of natural products, encompassing cyclic secondary metabolites with rings comprising of 12 or more atoms. Various trypanocidal macrocycles have been reported, with selected examples (65-71) shown in Figure 10.
Cembrane-type diterpenes are a class of macrocyclic natural products typically comprising a 14-membered cycle often fused with furanyl rings or bridged at various ring positions. A key collection of antiprotozoal cembrane diterpenes is available, with important examples of the family being laevigatol B (65) and laevigatol A (66). Laevigatol B (65), isolated from the coral *Lobophytum crassum* and *Lobophytum laevigatum* showed trypanocidal activity against the *T. b. brucei* bloodstream form with an EC$_{50}$ value of 5.3 µM, and SI of >20 as when comparing to the HEK293T and HepG2 cell lines. Additionally, 65 showed weak activity against *T. cruzi*
intracellular amastigotes, with a 15.2% reduction in cell viability at 50 μM.\textsuperscript{118} Interestingly, laevigatol A (66), the epoxidated analogue of 65 exhibited no reported activity against \textit{T. b. brucei} up to 20 μM.\textsuperscript{116} Lobocrasol A, 67, a structurally related macrocycle isolated from \textit{Lobophytum crassum}\textsuperscript{117} displayed potent leishmanicidal activity and moderate trypanocidal activity with EC\textsubscript{50} values of 0.18 and 10.0 μM against \textit{L. donovani} amastigotes and the \textit{T. b. rhodesiense} bloodstream form with SI values of 310 and 6, respectively, as compared to L6 cells. Hypothemycin (68), a 14-membered, macrolactone featuring a fused phenolic substituent isolated from the fungi \textit{Hypomyces trichothecoides},\textsuperscript{119,120} \textit{Coriolus versicolor},\textsuperscript{121} and \textit{Aigialus parvus}\textsuperscript{122} showed potent activity against the \textit{T. brucei} bloodstream form with an EC\textsubscript{50} value of ~0.17 μM. In vivo investigation of its activity showed a 33% cure rate of mice infected with \textit{T. brucei}, based on a 10 mg/kg dose administered once daily for 7 days. At doses higher than 10 mg/kg, however, significant signs of toxicity were observed in the mice, likely due to the known covalent inhibition of CDX kinases by hypothemycin.\textsuperscript{123,124} Further to this known activity, the trypanocidal mechanism of 68 was investigated, revealing covalent inhibition of \textit{TbCLK1} kinase with a recorded IC\textsubscript{50} value of 0.15 μM.\textsuperscript{124} No selectivity data have been reported, and the competing activity in human kinases could be problematic in further development.

Bastimolide A (69), a 40-membered macrolide isolated from the marine cyanobacterium \textit{Okeania hirsute}, showed multi-trypanocidal activity against both \textit{T. cruzi} intracellular amastigotes and \textit{L. donovani} amastigotes with EC\textsubscript{50} values of 6.5 and 3.0 μM, respectively. However, compound 69 showed somewhat low selectivity with SI values of 2 and 1 for \textit{T. cruzi} and \textit{L. donovani}, respectively, as compared to Vero cells.\textsuperscript{125} Lobosamides A and B (70 and 71) are 26-membered polyene macrolactams isolated from the actinobacterium \textit{Micromonospora} species, varying only by a single alkene geometry. Lactam 70 showed submicromolar potency against \textit{T.
*b. brucei* with an EC\textsubscript{50} value of 0.8 µM, whereas 71 exhibited an almost 10-fold reduced activity of 6.1 µM. Both had low toxicity to T98G mammalian cells, with SI values of 83 and 11 for compounds 70 and 71, respectively. Other macrolactams isolated together with Lobosomides A and B showed no trypanocidal activity despite their structural similarities.\textsuperscript{126}

Macrocyclic natural products have contributed several low and submicromolar trypanocidal compounds, some with high selectivity indices versus mammalian cells. Larger macrocycles, such as bastimolide, present a significant challenge in their synthesis and investigation of SAR, due to their large size and high flexibility. Smaller macrocycles such as the cembrane-type diterpenes are more appropriate for such studies, as many structurally related examples are known, some with reported trypanocidal activities. This foundation of knowledge can help build SAR correlations for further investigations into the activity of this core structure. However their synthesis remains challenging and requires intelligent route planning to achieve systematic SAR analyses.

**CYCLIC PEPTIDES**

A wide range of cyclic peptides are known, with varying reported biological activities, ranging from beneficial antibiotics to potent neurotoxins. Cyclic peptides vary in ring size, comprised primarily of amino-acid building blocks, which are cyclized either through the peptide backbone or by secondary linkages through side chains. Various trypanocidal natural cyclic peptides have been reported, a number of which (72-77) are shown in Figure 11. It should be noted that many of the trypanocidal cyclic peptides reported show accompanying antiplasmodial activity.
**Figure 11.** Selected natural cyclic peptides (72-77) with reported activity against trypanosomatid parasites

Venturamides A and B (72 and 73), two cyclic hexapeptides isolated from the cyanobacterium *Oscillatoria* species, display an interesting mixed oxazole and thiazole containing core, with a singular alanine/threonine variation differentiating the two. Both showed moderate
antitrypanosomal activity with EC\textsubscript{50} values against \textit{T. cruzi} intracellular amastigotes of 14.6 and 15.8 \(\mu\text{M}\) for compounds 72 and 73, respectively, but showed no detectable leishmanicidal activity against \textit{L. donovani} amastigotes up to 20 \(\mu\text{M}\). They both exhibited low cytotoxicity with moderate SI values 6 and 3.5 for 72 and 73, respectively, for \textit{T. cruzi} amastigotes when compared to Vero cells.\textsuperscript{127} The structurally related hexapeptide aerucyclamide C (74), the most potent in a family of cyclic peptides isolated from the cyanobacteria \textit{Microcystis aeruginosa}, similarly contains both oxazole and thiazole motifs, with the addition of a methyl-oxazoline replacing one thiazoles moiety. It showed moderate trypanocidal activity against the \textit{T. b. rhodesiense} bloodstream form, with an EC\textsubscript{50} value of 9.2 \(\mu\text{M}\), and good selectivity with an SI value of 12 compared to L6 cells.\textsuperscript{128} The parent compound family member, aerucyclamide A (75), also isolated from \textit{Microcystis aeruginosa},\textsuperscript{129} showed almost two-fold reduced trypanocidal activity, with an EC\textsubscript{50} value of 15.9 \(\mu\text{M}\), but maintained moderate selectivity with an SI of 8.\textsuperscript{128}

The large peptide tachyplesin I (76), isolated from the crab species \textit{Tachypleus tridentatus},\textsuperscript{130} comprises a 17 amino acid chain bicyclic structure, cyclized through two pairs of cysteine-cysteine disulfide bridges. It displayed good leishmanicidal activity with an EC\textsubscript{50} value of 9.3 \(\mu\text{M}\) against \textit{L. braziliensis} promastigote cells and moderate trypanocidal activities with EC\textsubscript{50} values of 9.3 and 47.9 \(\mu\text{M}\) for \textit{T. cruzi} trypomastigote and epimastigote cells, respectively.\textsuperscript{131} These activities surpass the previously known antifungal and antibacterial activities of 76,\textsuperscript{130} but remain only modest in comparison to other trypanocidal natural products. Valinomycin (76), a known antibiotic and potassium (K\textsuperscript{+}) ionophore originally isolated from \textit{Streptomyces fulvissimus},\textsuperscript{132} consists of a 12-membered depsipeptide core, consisting of D- and L-valine, D-\(\alpha\)-hydroxyisovaleric acid and L-lactic acid moieties. Compound 77 showed potent antitrypanosomal and antileishmanial properties, with EC\textsubscript{50} values of 0.0032 \(\mu\text{M}\) and <0.11 \(\mu\text{M}\) for the \textit{T. b. brucei} bloodstream form
and *L. major* promastigotes, respectively, with very high selectivity (SI values of 3500 and >100 for *T. b. brucei* and *L. major*, when compared to 293T cells).\textsuperscript{133}

Despite the potent trypanocidal and leishmanicidal activity of cyclic peptides, they present a challenging structural core for further development. Peptide chemical synthesis is challenging and tedious, with very poor atom economies on regularly applied routes. Desirable promiscuous enzymatic processes are needed to produce the wide variety of cyclic peptides required for a systematic SAR study are not yet available. Few trypanocidal cyclic peptides have been investigated with the focus of elucidating their mechanisms of action, a key factor that must first be tackled in order to develop them further as trypanocidal agents.

**FACILITATING DEVELOPMENT THROUGH ACCESSIBILITY**

The discovery of new drug targets for trypanocidal agents is vital to the development of new and improved therapeutics that are more appropriate for modern medicinal use than those currently administered. To facilitate this process, therapeutic scaffolds must be easily synthesized and chemically modified to allow their further furnishing with a wide variety of moieties and functionalities. This enables the generation of SAR correlations, in addition to the tailoring of pharmacokinetic characteristics such as logP, oral bioavailability, protein binding and metabolic stability. One key derivatization step is to allow the conjugation of biochemical tags, which may be used to aid target identification, localization and uptake studies of potential therapeutics. An extensive review of the methods available for the use of various approaches to identify drug modes of action has been published by our group.\textsuperscript{134} Key applicable techniques include the use of photoaffinity labeling chemistry to covalently link modified lead compounds to their target, enabling subsequent target identification by mass spectrometry or selective isolation using an installed chemical tag.
For these techniques, isolation of natural products from their natural sources is often not a viable option. This is because yields of many isolated natural compounds such as artemisinin, paclitaxel, and various antibiotics are normally low and may be from limited natural resources. Chemical modification of existing natural products is limited by existing functional groups and is often challenging. Synthetically accessible natural product cores that retain the trypanocidal activity of parent natural products are beneficial in this aspect, as they can be designed to contain chemical handles for further modification. Unlike natural products themselves, however, cores can also be designed with synthetic tractability in mind, enabling rapid synthesis for SAR and target identification studies.

We have suggested routes by which common trypanocidal cores highlighted in this study might be accessed, enabling the synthesis of furnished cores to further examine modes of action. These routes are shown in Figure 12.
Figure 12. Common trypanocidal natural product cores and suggested synthetic routes by which they may be accessed through preceded chemistry.

Flavone and flavonol syntheses are well-known chemical routes, where a variety of \( o \)-hydroxyacetophenones may undergo an aldol condensation with a corresponding benzaldehyde to form 2-hydroxychalcone derivatives. Chalcones can be either cyclized with molecular iodine to form flavones, or via the Algar-Flynn-Oyamada reaction with hydrogen peroxide to give the corresponding flavonol. This approach has previously been used by Lam et al. using a range of
substituted benzaldehydes and acetophenones in the combinatorial generation of a library of various flavones and flavonol derivatives.\textsuperscript{135}

The formation of $\beta$-carboline ring systems can be accessed via an acid-catalyzed Pictet-Spengler condensation of tryptophan with various aldehydes, giving the corresponding tetrahydro-$\beta$-carboline unit. This reaction has a wide substrate scope, where additional substitution on the indole moiety and various aldehydes are tolerated. In some cases, the aldehyde component may be replaced with a ketone, giving the corresponding 1,1-disubstituted $\beta$-carboline unit.\textsuperscript{136} Further oxidation of the monosubstituted tetrahydro-$\beta$-carboline ring system can be performed using hypervalent iodine reagents to give fully conjugated variants. Decarboxylative ring oxidation occurs where the naked carboxylic acid moiety originating from tryptophan is present during the oxidation reaction, although it is prevented via simple esterification or further derivatization at this position.\textsuperscript{137}

1,2-Dioxane derivatives can be accessed through the modification of various $\alpha,\beta$-unsaturated aldehydes through initial modification via Ragoussis’ modified Knoevenagel condensation to form the corresponding skipped diene.\textsuperscript{138} Dienes can then be converted to the 4,5-unsaturated 1,2-dioxane systems through reaction with photo-generated singlet oxygen species.\textsuperscript{139} Various aldehydes can be utilized in the Knoevenagel condensation, or the diene could be installed via Wittig reaction of phosphonium ylides, giving a very wide scope of possible substitutions. Further functionalization of the alkene of the 4,5-dehydro-1,2-dioxane core, such as reduction with Adam’s catalyst (PtO$_2$/H$_2$,\textsuperscript{140} or dihydroxylation with OsO$_4$,\textsuperscript{141} are additional options for molecular derivatization. A recent study by Taglialetela-Scafati et al. investigated a range of 1,2-dioxane derivatives accessed via similar routes for their antiplasmodial activities.\textsuperscript{142} The stability
of the endoperoxide group to further functional group interconversions is surprising. During the total synthesis of plakortide E by Sun et al., early installation of the 1,2-dioxolane motif was followed by exposure to various harsh chemical reagents including borohydride reduction, \( n \)-butyllithium, and tin hydride reagents, with reactions proceeding with moderate to high yields.\(^{143}\)

**EXAMPLES OF APPLYING TRYPANOCIDAL NATURAL PRODUCT CORES**

The concept of applying active natural product cores in the development of trypanocidal compounds, as well as aiding identification of novel targets has already been applied. Two examples are shown in Figure 13. In the case of convolutamine I (78), an antitrypanosomal alkaloid from the bryozoan *Amithia tortusa*,\(^ {144}\) a potency and pharmacokinetic-based SAR study examined analogues of 78. Through the study, a new lead, 79, was developed that showed increased potency with an EC\(_{50}\) value of 0.5 \( \mu \)M, as compared to the isolated natural product EC\(_{50}\) value of 1.1 \( \mu \)M, and exhibited a significantly improved pharmacokinetic profile.\(^ {145}\)
Figure 13. Examples utilizing natural product cores to improve synthetic accessibility, drug-likeness and selectivity while maintaining trypanocidal activity

Chamuvarinin (80), an acetogenin isolated from the plant species *Uvaria chamae*, \(^{146}\) which bears a tri-heterocyclic core, was the subject of an extensive SAR study, with the use of a synthetically accessible core analogous to that of the natural product. In this study, compounds maintaining the low micromolar activity of the natural product (EC\(_{50}\) of 1.3 \(\mu\)M vs. the *T. b. brucei* bloodstream form and a SI value of 2 vs. HeLa cells)\(^{147}\) while significantly improving synthetic accessibility and importantly selectivity towards mammalian cells were synthesized. Compound 81, one such example, exhibits pan-trypanocidal activity, with EC\(_{50}\) values of 5.1, 12.7, and 15.3 \(\mu\)M for *T. b. brucei*, *T. cruzi* epimastigotes, and *L. major* promastigotes, respectively, with SI values of 100, 39 and 33 as compared to Vero cells.\(^{148}\)
MODIFICATION OF NATURAL PRODUCT CORES

Natural products often do not follow the Lipinski “rule of 5”, however, many are already in use as potent drugs for a variety of diseases.149,150 This should be noted in the further development of key natural product cores, where factors such as logP, plasma protein binding and solubility should be monitored, however strict adherence to Lipinski rules is not absolutely necessary.

For natural products where simple or reliable chemical synthesis methods are not viable, for example, in the case of small cyclic peptides and macrocycles, heterologous expression in laboratory-culturable systems is a valid alternative. In such systems, it is preferable that enzymes along the biosynthetic pathway show some promiscuity, allowing the introduction of modified substrates furnishing the desired products with chemically modifiable handles. This approach has already been shown in the modification of a range of natural products,151 as well as biologically active cyclic peptides.152,153

CONCLUSIONS

Natural products historically are an important source of biologically active structures for the development of treatments for a wide range of human and animal diseases. The natural product structures described in this review show that certain natural product scaffolds hold great potential for the pursuit of potent trypanocidal compounds, with many compounds having low or submicromolar activities against some, or even all three kinetoplastid species, while still maintaining favorable selectivity. Some key scaffolds uphold the strong reputation of favorable bioavailability of natural products through their promising in vivo activities, but this number is relatively small. A priority of further research should be to investigate further those promising leads that have not yet been tested in vivo. Furthermore, the recurrence of certain core moieties throughout the large number of trypanocidal compounds examined shows several active compound
skeletons, some with desirable three-dimensional characteristics often lacking in purely synthetic libraries, which should be investigated further in the development of urgently required antiparasitic therapies, and also be key players in the discovery of novel drug targets. Chemical tractability of these scaffolds will allow easy and rapid structure activity relationships to be explored, as well as informed positioning of fluorescent and/or affinity tags, to aid uptake, mode of action studies and target identification studies.

**AUTHOR INFORMATION**

** Corresponding author

*Phone: +44 (0)1334 463412. Fax: +44 (0)1334 462595. E-mail: tks1@st-andrews.ac.uk.

†These authors contributed equally.

**ORCID**


Peter E. Cockram: 0000-0002-6476-9199

**Notes**

The authors declare no competing financial interest

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