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Neglected Tropical Diseases: Multi-Target-Directed Ligands in the Search for Novel Lead Candidates against *Trypanosoma* and *Leishmania*

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1. Introduction

"We have never had such a sophisticated arsenal of technologies for treating diseases, yet the gap in health outcomes keep getting wider. This is unacceptable." This claim was made by the Director-General of the World Health Organization (WHO[a]), Margaret Chan, in 2007. Since then, a special Outlook dedicated to neglected tropical diseases (NTDs) has been published by *Nature*.[1] Nowadays, NTDs affect more than one billion people (one-sixth of the world's population),[2] and in aggregate, NTDs cause almost 550 000 deaths annually.[3] Surprisingly, the NTDs drug discovery pipeline is still almost dry. Low returns of investments have discouraged drug companies from investing in research projects devoted to the discovery of novel drug candidates for NTDs.[4] At an academic level, where publications rather than direct contribution to practical social good are currently rewarded, the translational gap in drug discovery research has greatly widened. Moreover, academic institutions are sometimes naive about what it takes to develop a drug, and much basic research is therefore unusable.[5] However, over the past decade, the translational gap has started to close in the field of NTDs because of the emergence of a series of public−private partnerships whose main objective has been to fill the gap between basic scientific research, which is usually publicly funded, and clinical development, which is usually funded by pharmaceutical companies.[6,7]

Among NTDs, there is a group of diseases, whose etiological agents belong to the trypanosomatid family of the kinetoplastida order, that are responsible for infections concentrated in the poorest, mainly rural areas of the planet and that are grouped under the name of "most neglected diseases".[8] In particular, *Trypanosoma* is responsible for Chagas disease in South America and sleeping sickness in Africa[9] while *Leishmania* is responsible for cutaneous and visceral infections, endemic in 88 countries in the Horn of Africa, South Asia, and Latin America (Figure 1 and Table 1).[10] Because of their occurrence in low-income and middle-income countries, trypanosomiases and leishmaniasis do not have high visibility in Western societies, although Chagas disease, sleeping sickness, and visceral leishmaniasis are the three NTDs with the highest rates of death.[11] For these reasons, the WHO characterizes them as the most challenging of NTDs. Their name is also related to the fact that they are often neglected when health agendas and budgets are set at the level of research and development, at both academic and industrial levels.[12]

Despite the difference in epidemiology and visibility, all these diseases share a similar history of strategies for their treatment and control. Vaccine development has been an imperative for decades, but today it seems unlikely because of the extreme degree of antigenic variation exhibited by these parasites and because of the lack of resources for translational work and large vaccine trials.[13,14] Therefore, chemotherapy remains the only treatment option for controlling infection once acquired, but none of the different chemotherapeutic strategies used in the past has proven consistently successful.[15] If we exclude the recent encouraging results obtained with miltefosine and paramomycin, the treatment modalities for trypanosomatid infections mostly rely on drugs that date back over 50 years and that suffer from poor efficacy, high toxicity, and increasing resistance. This gloomy outlook has changed during the past decade as a result of important advances in anti-trypanosomatid drug discovery.

Advances in the research field have accelerated with improved in vitro cultivation methods,[16] enhanced genetic accessibility, completed genome sequences for key trypanosomatid species,[17] and increased prominence of these diseases on the agendas of well-resourced public figures and foundations. Despite this, trypanosomiases and leishmaniases remain incurable and even fatal, representing, especially for the academic pharmaceutical community, an urgent issue. There are several current medicinal chemistry strategies and initiatives for tackling this problem.

The objective of this Perspective is to report on possible innovative drug discovery approaches to the development of drug candidates for NTDs. We propose the multitarget drug design strategy as a means to overcome the challenges

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[a] Abbreviations: ACT, artemisinin-based combination therapy; ADMET, adsorption, distribution, metabolism, excretion, and toxicity; CDK, cyclin-dependent kinase; CP, cruzipain; CRK, Cdc2-related kinases; DHFR-TS, dihydrofolate reductase-thymidylate synthase; ETC, electron transport chain; GSK-3, glycogen synthase kinase-3; HAPT1, high-affinity pentamidine transporter 1; HAT, human African trypanosomiasis; HDAC, histone deacetylases; kDNA, kinetoplast DNA; MTDL, multi-target-directed ligand; NTD, neglected tropical disease; NO, nitric oxide; PTR1, pteridine reductase-1; RNAi, RNA interference; SE, squalene epoxidase; SI, selectivity index; TAO, *Trypanosoma* alternative oxidase; TDR, Special Programme for Research and Training in Tropical Diseases; TR, trypanothione reductase; WHO, World Health Organization.

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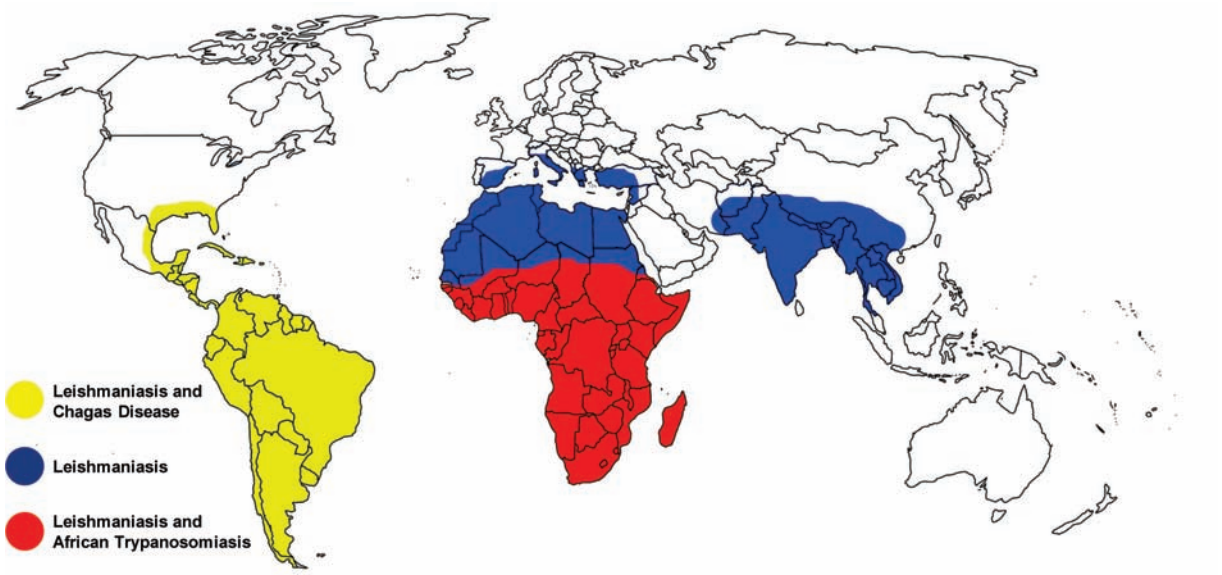

Figure 1. Geographic distribution of leishmaniasis, Chagas disease, and human African trypanosomiasis. Chagas occurs mainly in Central and South America. Sleeping sickness is prevalent in Africa. Leishamaniases are endemic in about 90 countries. The map was adapted from the WHO Web site.[2]

Table 1. Burden of Protozoal Neglected Tropical Diseases along with Major Characteristics and Currently Available Drugs[11]

disease	global prevalence	population at risk	regions of highest prevalence	deaths	clinical manifestations and associated disabilities	primary drugs	weakness of current drugs
Chagas disease	8−9 million	25 million	Latin America and Caribbean	14 000	cardiomyopathy, megacolon, megaesophagus	Nifurtimox (**4**), benznidazole (**5**)	poor efficacy and toxicity
human African trypanosomiasis	300 000	60 million	sub-Saharan Africa	48 000	sleeping sickness	Suramin (**32**), pentamidine (**33**), melarsoprol (**34**), eflornithine (**35**)	drug toxicity and drug resistance
leishmaniasis	12 million	350 million	India, South Asia, sub-Saharan Africa, Latin America, Caribbean, and Mediterranean area	51 000	cutaneous and mucocutaneous disease, kala-azar	Antimonials (**39**, **40**), amphotericin B (**41**), pentamidine (**33**), miltefosine (**42**), paromomycin (**43**)	drug toxicity and drug resistance

highlighted above. We will illustrate examples of how this has been explored to design innovative lead candidates against trypanosomiases and leishmaniases. Before this, we will present a brief overview of the most validated and novel parasitic targets for developing new chemical entities against these NTDs.

1.1. Drug Targets. Trypanosomatids are relatively early branching eukaryotic organisms, and their cell organization differs considerably from that of mammals. Therefore, biochemical pathways present in trypanosomatids and absent from their hosts should provide excellent targets for rational drug design (Figure 2).[18] Several of these pathways are common to all pathogenic trypanosomatids, so a broad-spectrum drug, clinically useful against all trypanosomatid diseases, is in principle possible. However, this is yet to be achieved, perhaps because of the very different biology of the parasites once inside their hosts. African trypanosomes live in the bloodstream and cerebrospinal fluid, South American trypanosomes live in the cytosol of a variety of cell types, and *Leishmania* species live within phagolysosomes of macrophages.

The route to drug target identification has usually been through comparative genetic and biochemical studies. Genes

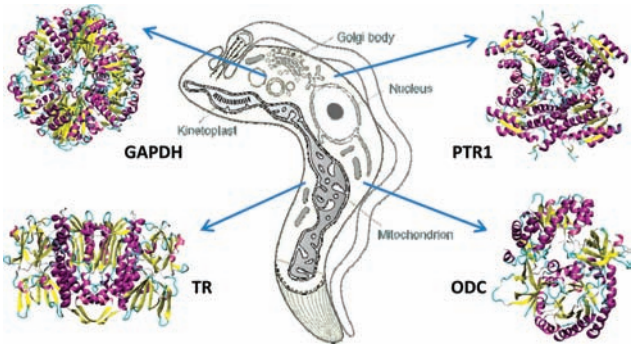

Figure 2. Schematic representation of the trypanosome cell along with the three-dimensional X-ray structure of several possible target proteins for anti-trypanosomatid drug discovery. These targets are either unique for the parasite or quite different from the corresponding human enzymes.

and proteins identified in parasites and known to be absent from, or strikingly different in, the mammalian host were considered ideal targets. Thus, carbohydrate metabolism (i.e., glycolysis) of trypanosomatids has been considered an excellent target owing to the unusual compartmentation

of the glycolytic enzymes in the glycosomes.[19] Likewise, the discovery that an unusual low-molecular-weight thiol, N^1, N^8-bisglutathionyl spermidine (or trypanothione), rather than glutathione, is the major redox reactive metabolite in trypanosomatids has elevated this molecule, and the enzymes involved in its metabolism (e.g., trypanothione reductase, TR), to paradigms of antiparasite drug targets.[20]

Target validation is a key step of any rational drug design program. As in other fields of pharmaceutical research, the validation of a given target involves the gene knockout approach of investigating whether a specific protein is or is not essential for the parasite's survival and therefore a suitable drug target. In particular, the high efficiency of targeted gene disruption in *Trypanosoma* and *Leishmania* has made gene knockout procedures particularly straightforward in this field. However, a "real" target validation can be achieved only when the target-hitting molecule is actually exploited as a drug to treat the disease and, more importantly, when it works.

A major breakthrough in identifying new drug targets came with the recent publication of the "Tritryp" (*Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania major*) genome,[21−23] which revealed that each genome contains 8300−12000 protein-coding genes, of which approximately 6500 are common to all three genomes. It has led to the postgenomic era for trypanosomatid drug discovery. Moreover, the sequence of the genomes of two other species of *Leishmania* (*L. infantum* and *L. braziliensis*) was reported in 2007.[24] This vast amount of new information makes possible a more comprehensive and accurate identification and validation of novel drug targets. To further exploit the "Tritryp" genomes, the Special Programme for Research and Training in Tropical Diseases (TDR) has recently developed the TDR targets database, which aims to facilitate the identification and prioritization of candidate drug targets for these and other pathogens.[25]

In the following section, we report a brief overview of the biochemical pathways and targets for *Trypanosoma* and *Leishmania* drug discovery. The following description should not be considered a systematic classification of all trypanosomatid drug targets. However, we highlight some of the most promising pathways in the search for potential antitrypanosomatid drug candidates. In this regard, we also mention some examples of validated and innovative target proteins.

1.1.1. Electron Transfer Chain and Alternative Oxidase. Trypanosomatids have developed a variety of physiological functions necessary for their survival within the specialized environment of the host. Using metabolic systems that are very different from those of the host, they can adapt to low oxygen tension present within the host animals. Most parasites do not use the oxygen available within the host to generate ATP but rather employ their own system's anaerobic metabolic pathways. The enzymes in these parasite-specific pathways are potential targets for chemotherapy.

The mitochondrion of trypanosomatids can be considered a valuable and potential organelle in which to seek potential parasite-specific targets. This is because of its unique structure and function when compared to the natural host habitat. Indeed, the respiratory systems of parasitic protozoa typically show great diversity in electron pathways when compared to their host animals. These peculiar aspects of electron transport chain (ETC) complexes and their related enzymes represent promising targets for chemotherapy.[26]

A cytochrome-independent *Trypanosoma* alternative oxidase (in the literature, referred to as TAO or AOX) of *T. brucei* is the terminal oxidase of the respiratory chain of long slender bloodstream forms of the African trypanosomes and is a leading drug target.[27] Since TAO does not exist in the host, it has been proposed as an innovative target for antitrypanosomatid drug discovery, and some attempts in this respect have been reported in the literature.[28]

1.1.2. Fatty Acid Biosynthesis. It has recently been reported that *T. brucei* has adapted microsomal elongases for bulk fatty acid synthesis, with some evidence that *L. major* and *T. cruzi* utilize the same enzyme family for fatty acid synthesis.[29] Whereas the *T. brucei* genome encodes four elongases, *L. major* and *T. cruzi* genomes encode 13 and 5 enzymes, respectively. As with *T. brucei*, the genomes of these related parasites also encode a type II fatty acid synthase but not a type I. However, type II fatty acid biosynthesis, which occurs at the mitochondrial level, has been shown to produce fatty acids at a very low level. Therefore, nearly all of the fatty acids synthesized in *T. brucei* are produced by the elongase pathway. This mechanism has been reported as a third type of fatty acid biosynthesis,[29] and it could represent an innovative pathway in the search for novel drug targets. Indeed, both trypanosomatid mitochondrial type II fatty acid biosynthesis and elongase pathways are apparently essential for parasite survival and could therefore serve as potential drug targets.[30]

Miltefosine, an alkylphospholipid with oral antileishmanial activity, was originally discovered as an anticancer compound able to interfere with the lipid biosynthesis in tumor cell lines. However, activity against the same pathways in *Leishmania*[31] and *T. cruzi*[32] brought the drug to the market as antileishmanial compound. Furthermore, protein prenylation pathways, particularly farnesyl pyrophosphate synthase, can be targeted by compounds potentially useful for the treatment of NTDs.[33]

1.1.3. Kinetoplast DNA Replication Machinery. In the specialized region of unique trypanosomatid mitochondria known as kinetoplast, kinetoplastids show a DNA known as kinetoplast DNA (kDNA), which, unlike all other DNA in nature, comprises a giant network of interlocked DNA rings with a rather unique topology.[34] This is also known as mitochondrial genome. The replication of the kDNA network is quite complex, and the discovery of new proteins involved in this process is currently the best approach for illuminating the replication mechanism. kDNA is unique in its structure and function, making its replication machinery a valuable source for novel anti-trypanosomatid drug targets.[35] As a matter of fact, topoisomerases play key functions in the replication and organization of kDNA and are therefore considered potential targets for antiparasite drug discovery. Researchers have recently reported, as potential novel anti-trypanosomatid drug candidates, topoisomerase II inhibitors, such as nalidixic acid, novobiocin, and etoposide, on the ultrastructure of trypanosomatids that present distinct kDNA arrangements.[36] In this respect, recent evidence for a new and unexpected function for topoisomerase II of *T. brucei*[37] makes this enzyme an even more intriguing target for anti-trypanosoma lead discovery.

1.1.4. Histone Deacetylases. Reversible protein acetylation has been established as a modification of major regulatory significance. In particular, histone acetylation regulates

access to genetic information in eukaryotes. For example, class I and class II histone deacetylases (HDAC) are regulatory components of corepressor complexes involved in cell cycle progression and differentiation. It has recently been demonstrated that in *T. brucei* there are four genes encoding histone deacetylase orthologues that have been identified, cloned, and characterized.[38] The predicted deacetylases, HDAC1−4, have been shown to share significant similarity with mammalian and yeast class I (HDAC1 and HDAC2) and class II (HDAC3 and HDAC4) histone deacetylases. Moreover, all except HDAC2 have the critical residues predicted to be required for deacetylase activity. In gene-targeting experiments, HDAC1 and HDAC3 appear to be essential whereas HDAC2 and HDAC4 are not required for viability. Researchers have therefore provided genetic validation of HDAC1 and HDAC3 as potential chemotherapy targets and demonstrated that *T. brucei* expresses at least three probable histone deacetylases with distinct functions.

Further evidence in this respect can be found in a recent paper, where a series of histone deacetylase inhibitors was tested against *L. donovani* promastigotes, showing an interesting in vitro profile. A possible correlation between HDAC inhibition and antileishmanial activity has therefore been proposed.[39]

1.1.5. Purine and Pyrimidine Metabolism and Salvage. Purines and pyrimidines are indispensable to all life, performing many vital functions for cells. ATP serves as the universal currency of cellular energy. cAMP and cGMP are key second messenger molecules. Purine and pyrimidine nucleotides are precursors for activated forms of both carbohydrates and lipids. Nucleotide derivatives of vitamins are essential cofactors in metabolic processes. Finally, nucleoside triphosphates are the immediate precursors for DNA and RNA synthesis. Unlike their mammalian and insect hosts, trypanosomatids lack the metabolic machinery to make purine nucleotides de novo and must rely on their host for preformed purines.[40] The obligatory nature of purine salvage offers, therefore, a plethora of potential targets for drug discovery, and the pathway has consequently been the focus of considerable scientific efforts. Some differences can be found in *Leishmania* that is prototrophic for pyrimidines and that also expresses a small complement of pyrimidine salvage enzymes. Because the pyrimidine nucleotide biosynthetic pathways of *Leishmania* and humans are similar, pyrimidine metabolism in *Leishmania* has generally been considered less amenable to therapeutic manipulation than the purine salvage pathway. However, evidence garnered from a variety of parasitic protozoa suggests that the selective inhibition of pyrimidine biosynthetic enzymes offers a rational therapeutic paradigm.

In the purine salvage pathway, the most extensively studied targets are the following enzymes: (i) nucleoside hydrolase, (ii) purine nucleoside phosphorylase, (iii) inositol monophosphate dehydrogenase, and (iv) methylthioadenosine phosphorylase, which is a border enzyme between the purine salvage and the polyamine biosynthesis pathways.

1.1.6. Kinetoplastid Protein Transporter. Kinetoplastid protozoa express hundreds of membrane transport proteins that allow them to take up nutrients, establish ion gradients, efflux metabolites, translocate compounds from one intracellular compartment to another, and take up or export drugs. The combination of molecular cloning, genetic approaches, and the completed "Tritryp" genome has allowed detailed functional analysis of various transporters and predictions about the likely functions of others. Thus, many opportunities now exist to define the biological and pharmacological properties of parasite transporters, whose genes were often difficult to identify in the pregenomic era. A subset of these transporters is essential for parasite viability. They could therefore serve as targets for novel drug therapies if researchers can identify compounds that interfere with their uptake functions. In this respect, we should mention the different classes of *Leishmania* ABC transporters that extrude antimonials, azoles, and folates, resulting in drug-resistant phenotypes.[41]

Conversely, other permeases provide routes for uptake of selectively cytotoxic compounds and can thus be useful for the delivery of drugs.[18] Drug resistance may develop in strains where such drug uptake transporters are nonfunctional or in parasites that overexpress other permeases that export a drug. Transporters for glucose and for purines are being studied in molecular detail as promising drug targets against *Leishmania*.[42]

Aquaporins are another membrane protein family that deserves attention as potential anti-trypanosomatid drug targets. They transport water across membrane, and their role in drug uptake in *Leishmania*, *T. brucei*, and *T. cruzi* was recently described.[43]

1.1.7. Carbohydrate Biosynthesis. Carbohydrate biosynthesis of trypanosomatids has been regarded as a biochemical pathway in the search for selective parasite-specific targets. Carbohydrate metabolism enzymes are compartmentalized in the glycosome, a divergent peroxysome where glycolysis takes place.[19] Enzymes such as *T. brucei* enolase and hexokinase have been envisaged as possible proteins to address anti-trypanosoma lead identification. In this pathway, further possible molecular targets should be mentioned, such as pyruvate kinase, phosphofructokinase (whose three-dimensional structure was recently solved by X-ray crystallography[44]), fructose-1,6-biphosphate aldolase, phosphoglycerate kinase, glucose-6-phosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase.

1.1.8. Polyamine Biosynthesis, Transport, and Metabolism. The *T. brucei* enzyme ornithine decarboxylase (ODC) is a well-recognized target for the discovery of novel drug candidates against African sleeping sickness. ODC is involved in the early stage of polyamine biosynthesis, but its inhibition at this step can be bypassed in the presence of a high concentration of exogenous polyamines. Notably, within mammalian bloodstream, polyamine concentration is relatively low, and in such conditions, blocking ODC is lethal for the parasite. Trypanosomatid polyamines are metabolized by a unique thiol redox pathway, where the glutathione reductase is replaced by the unique TR enzyme.[45] TR and trypanothione biosynthesis inhibitors should be regarded as possible anti-trypanosomatid lead candidates. Concerning TR inhibitors, interested readers can find recent and comprehensive surveys in refs 46 and 47.

1.1.9. Cell Cycle. Cell cycle progression has been seen as one of the most interesting pathways for several classes of chemotherapy agent. Trypanosomatid cell cycle progression might also be investigated as a possible source for new drug targets.

The "TriTryp" genome sequences has greatly impacted trypanosomatid cell cycle research, leading to faster functional analyses particularly in *T. brucei*, where RNA interference (RNAi) is possible, and allowing the description of the trypanosomatid kinomes.[48] Orthologues of

many conserved protein kinases, such as cyclin-dependent kinases (CDKs), mitogen-activated protein kinases, Aurora and polo-like kinases, are present in *T. brucei*, although their functions are often divergent[49] and may also differ in different life cycle stages. Conversely, no receptor-linked tyrosine kinases were found; tyrosine phosphorylation is likely carried out by dual specificity protein kinases.

Recently, the expression levels of four Cdc2-related kinases (CRK1, -2, -4, and -6) in the procyclic form of *T. brucei* were knocked down using the RNAi technique. A double knockdown of CRK1 and CRK2 resulted in arrested cell growth in the G1 phase accompanied by an apparent cessation of nuclear DNA synthesis.[50]

In *Leishmania*, cdc2-related protein kinases have recently attracted attention, since as homologues of CDKs, these enzymes are thought to be essential for cell cycle progression. Gene deletion experiments have shown that *L. mexicana* CRK1 and CRK3 are essential for the parasite.[51]

In addition, tubulin of trypanosomatids[52] has recently been demonstrated to be a possible target for anti-trypanosomatid drug discovery. It has been shown that classical ligands, binding trypanosome tubulin, are able to inhibit the parasite growth in vitro. The authors have shown that the inhibition of trypanosome growth was univocally due to the interactions of the molecules with tubulin and therefore have pointed to this protein as a practical anti-trypanosomatid drug target.[53]

Among protein kinases, special attention has recently been paid to the glycogen synthase kinase, which is a validated target in the research fields of Alzheimer's and diabetes. A recent publication reports genetic and chemical validation data that support the hypothesis that glycogen synthase kinase-3 (GSK-3) might be a drug target for the *T. brucei* parasite.[54] This finding is particularly stimulating from a medicinal chemistry perspective, since all the available information related to GSK-3 inhibitors can be directly exploited in anti-trypanosoma lead discovery. The same applies to some of the above-reported proteins, which are well-known and validated drug targets for "less-neglected" diseases. However, major issues related to selectivity should be carefully taken into account when considering common (mammal and trypanosomatid) targets.

1.1.10. Folate Biosynthesis. Dihydrofolate reductase−thymidylate synthase (DHFR-TS) enzymatic activities are present on a single polypeptide encoded by a single gene. In *L. major*, it is has been shown that such enzymatic activity is fundamental for the parasite's survival. As with polyamine biosynthesis inhibition, this kind of bifunctional enzyme is essential only when exogenous substrates are not provided.[55,56] The finding that the null mutant does not survive in vivo is the definitive demonstration that DHFR-TS is a valid target for drug discovery.[55,56] From a multifunctional compound perspective, this DHFR-TS enzymatic machinery is very intriguing. Indeed, mutants lacking TS activity have univocally established that TS is essential.[57] In contrast, the occurrence in *Leishmania* of an alternative pteridine reductase1 (PTR1) raises the possibility that DHFR might not be an essential activity. However, studies have reported that PTR1 may not be able to fully satisfy the *Leishmania* requirement for reduced pteridines.[58] Therefore, an ideal modulator of DHFR-TS enzymatic machinery should be able to modulate both activities, although directed against a single target protein. Moreover, a suitable antifolate compound should also be endowed with a certain affinity toward PTR1, since it has been established that this enzyme plays a significant role in reducing the sensitivity of *Leishmania* to certain antifolates.[59]

1.1.11. Proteases. Proteases are responsible for many biochemical processes. For several years, they have been regarded as potential therapeutic targets for many different diseases. Pharmaceutical research in the proteases field has thus been very active in recent decades. Despite recent studies reporting on the antileishmanial activity of certain HIV protease inhibitors,[60] cysteine proteases have been regarded as possible innovative target proteins for anti-trypanosomatid drug discovery. It has been reported that nitric oxide (NO) can block cysteine protease activity and NO donors can block replication of *Plasmodium*, *Trypanosoma*, and *Leishmania*.[61] Furthermore, a library of octapeptide inhibitors was recently obtained by solid-phase synthesis. These molecules were then tested against recombinant cysteine protease B from *L. mexicana* that showed inhibitory activity in the micromolar and nanomolar ranges. Tested using whole-cell assays, these compounds were able to affect parasite survival.[62]

Further studies were recently carried out by Schirmeister and co-workers, who synthesized a series of aziridine-2,3-dicarboxylate-based cysteine protease inhibitors. Tested using whole-cell assays, these compounds displayed trypanocidal activity equipotent to that of the drug eflornithine.[63] Furthermore, Schirmeister and co-workers have shown that some of these aziridine-2,3-dicarboxylates were also active and selective against *L. major* promastigotes with low toxicity against host cells.[63] Notably, the vinyl sulfone cysteine protease inhibitor *N*-methyl-Pip-F-homoF-vinyl sulfonyl phenyl has shown potential in experimental models of infection with *T. cruzi* and is in late-stage preclinical development.[64]

1.1.12. Proteasomes. Proteasomes are one of the cellular complexes controlling protein degradation from archaebacteria to mammalian cells. In 1997, Mutomba et al. carried out a clear-cut experiment that pointed to *T. brucei* proteasome as a candidate target for the discovery of trypanocidal drug candidates.[65] In particular, they showed that lactacystin, a specific inhibitor of proteasome activity, was able to inhibit the activity of 20S proteasomes purified from both bloodstream and procyclic forms of *T. brucei*. When tested using whole-cell assays, lactacystin was able to interfere with parasite proliferation, blocking both bloodstream and procyclic forms. More recent studies have confirmed that proteasome inhibitors are possible antitrypanosomal lead candidates.[66,67]

A comment is here required on the plethora of targets mentioned above. Clearly, we have not covered all of the validated and innovative proteins currently investigated as possible anti-trypanosomatid targets. However, it is our opinion that, from the above, a clear picture emerges of how the "Tritryp" project and extensive biological and biochemical studies have brought to the attention of the medicinal chemistry community a vast variety of possible targets for NTDs. However, even in the postgenomic era, the pipeline for NTDs novel drugs is almost dry, and research efforts and novel ideas in the field are urgently required. In the following section, we draw attention to one possible innovative strategy that addresses the drawbacks and pitfalls of drug discovery, in general, and in the identification of NTDs leads, in particular.

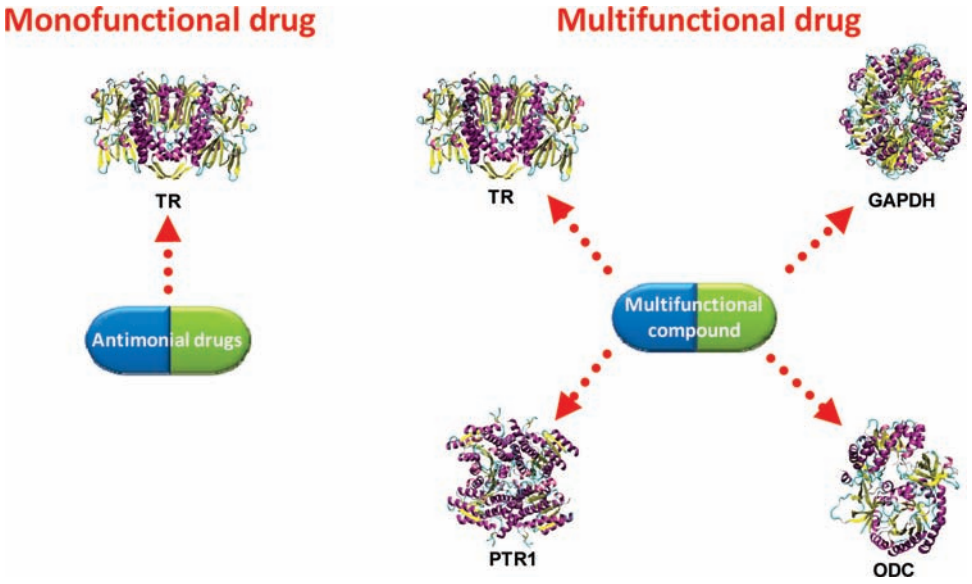

Figure 3. Monofunctional compounds are able to modulate a single vital molecular target of the parasite. For instance this is the case of antimonial drugs, which have been recently demonstrated to inhibit the TR enzyme.[87] Alternatively, multifunctional compounds can be conceived as molecules with improved efficacy because they are able to simultaneously modulate more than a single vital parasitic target.

2. Rational Base of Multifunctional Compounds against NTDs

Notwithstanding a renewed interest in developing drugs for NTDs, novel and effective drugs to tackle diseases caused by trypanosomatids have yet to arrive. Mechanisms of partnering with industry and philanthropic organizations have been established to overcome this obstacle and to open up and build on scientific opportunities for improved chemotherapy in the future.[68] As reported above, both potential and validated molecular targets are nowadays available.[17] However, the emergence of drug resistance is a critical issue. In this respect, after increasing unresponsiveness to most monotherapeutic regimens, scientists are moving to extend the combination therapy strategy to trypanosomatid diseases.[69] This strategy has already proven successful in the treatment of other important parasitic infections, such as malaria and tuberculosis, where it achieves maximum efficacy by exploiting synergy and minimizing individual toxicity.[70]

More generally, drug combinations have proven to be an essential feature of antimicrobial treatment due to a number of important considerations: (i) they increase activity through the use of compounds with synergistic or additive activity; (ii) they thwart drug resistance; (iii) they decrease required doses, reducing both cost and the chances of toxic side effects; (iv) they increase the spectrum of activity.[69]

2.1. Can Multifunctional Compounds Offer a Way To Fight Drug Resistance? The use of combinations to combat resistance has been well-deployed in antimalarials. Artemisinin-based combination therapy (ACT) has been advocated as the therapy of choice for handling widespread drug resistance in *P. falciparum*, at the same time preventing recrudescence due to artemisinin monotherapy.[71] Using a drug combination shortens the treatment course. It has also been shown to be well-tolerated and to effectively protect each individual drug from resistance.[72] For instance, with resistance due to point mutations, it has been estimated that symptomatic individuals harbor up to about 1012 parasites. If a target enzyme has a mutation rate of 10^{-7}, the chance of developing resistance to a single agent is high, but the likelihood of developing resistance to two compounds with different targets is very low.[73]

Combination therapy in trypanosomatid infections is now emerging. In 2001, Bryceson first hypothesized this approach as the way to increase treatment efficacy, prevent the development of drug resistance, reduce treatment duration, and eventually decrease the overall treatment cost.[74]

In addition to the use of combination therapy, there are other possible ways to achieve polypharmacology (Figure 3).[75] Usually, a combination regimen (also known as drug cocktail) is composed of two or three different drugs that combine different therapeutic mechanisms. But this approach might present disadvantages in terms of meeting patient compliance.[76] An alternative strategy might be the use of "single-pill combinations" (or "fixed-dose combinations"), which incorporate different drugs into the same formulation. The single-pill therapy would be superior to a multipill regimen in the achievement of adherence, as a means of simplifying a patient's treatment, reducing pill burden, and synchronizing therapies.[77] Finally, a third approach is now emerging based on the assumption that a single compound may be able to hit multiple targets.[76] This approach is based on the multifunctional strategy, i.e., the design of single chemical entities able to simultaneously modulate multiple targets. Recently, we have suggested that the multifactorial nature of Alzheimer's disease provides the logical foundation for the development of the innovative drug design strategy centered on what we have dubbed multi-target-directed ligands (MTDLs).[78] It has been widely recognized that drugs hitting a single target may be inadequate for the treatment of diseases like neurological disorders and also for somatic diabetes, cardiovascular diseases, and cancer,[79] which involve multiple pathogenic factors.[80,81]

More importantly, in cancer,[79] AIDS, and parasitic infections,[82−85] MTDLs have an added value with respect to the single-target agents: the onset of drug resistance, a common problem in chemotherapy, might be significantly delayed by inhibiting multiple targets simultaneously.

As a general rule, chemotherapeutic agents that target single proteins are susceptible to high-level resistance resulting

from single-step mutation in the target protein. Conversely, drugs that have a low likelihood of the development of high-level endogenous resistance are those that interact with multiple molecular targets, the structures of which are determined by multiple genes. This favors the development of multitarget over single-target compounds. The relationship of the multitarget nature of successful antibacterials and their lowered potential for target-based resistance has been highlighted in a recent review article.[86]

2.2. Single Pill and Multipill Drug Combinations vs MTDLs. Clearly, therapy with a single drug that has multiple biological properties would have inherent advantages over the single pill or the multipill combinations. It would obviate the challenge of administering multiple single-drug entities, which could have different bioavailability, pharmacokinetics, and metabolism.[76,78] Indeed, if a single molecular species can show a complex ADMET profile, a combination approach might be untenable. In addition, the risk of possible drug−drug interactions would be avoided and the therapeutic regimen greatly simplified in relation to drug cocktails.

There is, therefore, a strong indication that the development of compounds able to hit multiple targets might provide new avenues for the treatment of major NTDs, for which an effective cure is urgently needed. This approach has been discussed in recent articles, which were mostly concerned with neurodegenerative diseases. It has also been suggested that the multitarget approach might overcome the pitfalls that have hampered the development of effective treatment for NTDs: the drug's potency and efficacy might be increased and regimens simplified, thereby potentially reducing resistance and promoting patient adherence.[88] With regard to parasitic diseases, the MTDL approach has even more inherent advantages compared to drug combination. As recently highlighted by Hastings and Watkins,[89] mismatched combinations not only fail to affect the spread of resistance but might even jeopardize the efficacy of components against which resistance has not yet emerged. Although the design of multifunctional compounds can be very complicated as multiple target proteins, often in different cellular compartments have to be reached and hit, some other steps of drug development (e.g., pharmacokinetic and ADME studies) are in principle no different from those of any other single molecule. Therefore, MTDLs can even be less expensive than drug combinations. Another favorable issue is that the risk of possible drug−drug interactions of polytherapy is considerably reduced. NTDs occur predominantly in vulnerable populations, such as pregnant women, nursing mothers, or very young children, all of whom are at significant risk for drug toxicity.[90]

Even more important are concerns related to patient compliance and adherence issues. In malaria, the deployment of ACT has seen a simultaneous increase in the assessment of compliance to these new treatments. Noncompliance increases the risk of therapeutic failures, and subtherapeutic concentrations, following incomplete treatment, could lead to a major risk of drug resistance.[91] The benefits of combination regimens will be negated if the recommendations are too complex for compliance by caregivers as well as by the drug supply management of the program.[92] An antiparasitic MTDL might fulfill the goal of simplifying dosage regimens whenever possible without compromising efficacy.

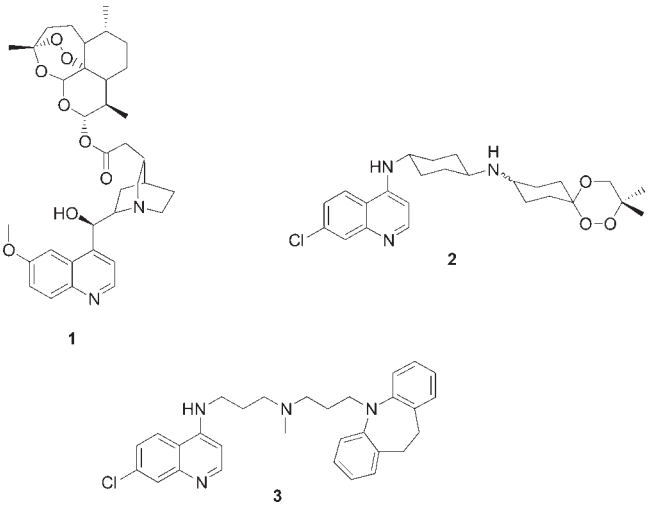

Figure 4. Examples of multifunctional compounds for the treatment of malaria.

2.3. MTDLs in Parasitic Infectious Diseases. All these considerations, especially those related to economical factors, strongly indicate that the development of MTDLs might open up new avenues for the cure of diseases caused by trypanosomatids. The approach is still in its infancy for these infections. However, for malaria treatment, this idea has been advanced[82−85] and several multifunctional drug and lead candidates have been developed by academia and industry in the past couple of years, supported by the recent advances in the knowledge about the action and resistance mechanisms involved. Examples of such drugs, classically obtained by combining two pharmacophores into a new chemical entity and therefore defined by the authors "drug hybrids", were recently highlighted in the *Chemical & Engineering News* journal (Figure 4).[93]

On the basis of the synergy between fast-acting artemisinin and slow-acting quinine, Walsh and co-workers designed artemisin−quinine hybrid **1** with a lower IC_{50} than either drug alone or both drugs together, in two different drug-sensitive and drug-resistant strains of the *P. falciparum* parasite.[94] Earlier, Meunier, who can be considered a pioneer in the field of antimalaria hybrids, developed trioxaquines (trioxane−quinoline), a new class of compounds that associates the properties of the two antimalarial drugs artemisinin and chloroquine.[95] One of these molecules, PA1103/SAR116242 (**2**), is active at nanomolar concentrations on sensitive and resistant strains of *P. falciparum* and also on multidrug-resistant strains obtained from fresh patient isolates in Gabon.[96] This molecule is very efficient by oral route with a complete cure of mice infected with chloroquine-sensitive or chloroquine-resistant strains of plasmodia and in humanized mice infected with *P. falciparum*. Combined with a good drug profile (preliminary absorption, metabolism, and safety parameters), these data were particularly promising for the selection of this trioxaquine for development as a drug candidate from among 120 other active compounds.[96] As a result of the collaboration with Sanofi-Aventis, market availability could become a reality in 2013. The potential for overcoming *P. falciparum* resistance by exploiting an MTDL design strategy was demonstrated by Peyton[97] by connecting the quinoline moiety of chloroquine to a dihydrodibenzoazepine "reversal agent", known to block the action of a transporter that removes chloroquine

from the parasite. This resulted in a hybrid compound (**3**) that has demonstrated potent growth inhibition of *P. falciparum* in culture and promising results in vivo.[97] In other approaches, similarly aimed at reversing resistance, the tetraoxane skeleton and 4-aminoquinoline,[98] the antiplasmodial agents chloroquine and astemizole,[99] and 4-aminoquinoline- and clotrimazole-based pharmacophores[100] were conjugated into a single molecule. The application of MTDL drug candidates in the field of HIV[101] and bacterial infection[102] treatment was also reviewed recently.

In the last section of this Perspective, we first report on the three selected trypanosomatid NTDs, namely, Chagas disease, human African trypanosomiasis (HAT), and leishmaniasis. We describe their geographic distribution and mode of transmission, along with the currently available chemotherapeutics. Then, we describe in sequence recent examples of small molecules displaying more than one activity against them. Notably, the deliberate aim of creating an MTDL has not always been explicitly stated. Instead, the classic molecular hybridization strategy[103] was exploited, leading to molecules endowed with the ability to hit multiple targets.

3. Chagas Disease

3.1. General Considerations, Transmission, and Geographic Distribution. Chagas disease is caused by the protozoan parasite *T. cruzi*.[104] There are various strains of *T. cruzi* in terms of epidemiology, pathogeny, response to treatment, biochemistry, or immunogeny that have been classified into two major *T. cruzi* groups defined as I and II.[105] Both *T. cruzi* I and *T. cruzi* II are associated with cardiac lesions in human infections, but it seems that only *T. cruzi* II is also associated with digestive tract lesions.

Humans are accidental hosts for *T. cruzi* and are infected at night through contact with the feces of blood-sucking triatominae bugs. Of the more than 100 recognized triatomine species, only about 10 are widespread colonizers of human dwellings. Some have become completely adapted to dwellings and are highly anthropophilic, such as *Triatoma infestans* in the southern cone countries (responsible for 85% of cases) and *Rhodnius prolixus*, the second most important Chagas vector,[106] in many countries in Central America. Often, the bugs defecate on the host while feeding, and the infected fecal droplets may be inadvertently passed to the mucosa of eye, nose, or mouth. Parasite transmission through intact skin probably does not occur. The probability of human infection through contact with an infected triatomine is about 1 in 1000.[107] Blood transfusion and organ transplantation are other possible mechanisms of infection transmission, and the third and most relevant way is the congenital transmission in children by chagasic mothers.

Chagas disease attacks people living in remote rural areas that lack diagnostic facilities and good health records or statistics, and therefore, sufficient epidemiological information about its magnitude has not always been accessible. Available epidemiological information shows that the disease represents a major public health problem in South America (Figure 1), affecting at least 8−9 million people with more than 25 million at risk of infection.[108] The incidence has been calculated to be about 300 000 new cases per year in the absence of control interventions, and about 14 000 deaths due to Chagas disease occur every year (Table 1).[109] With migration, many infected individuals have moved from the rural zone to cities and to other countries, and a few hundred-thousand chagasic individuals are estimated to live today in the U.S., Europe, and Asia.

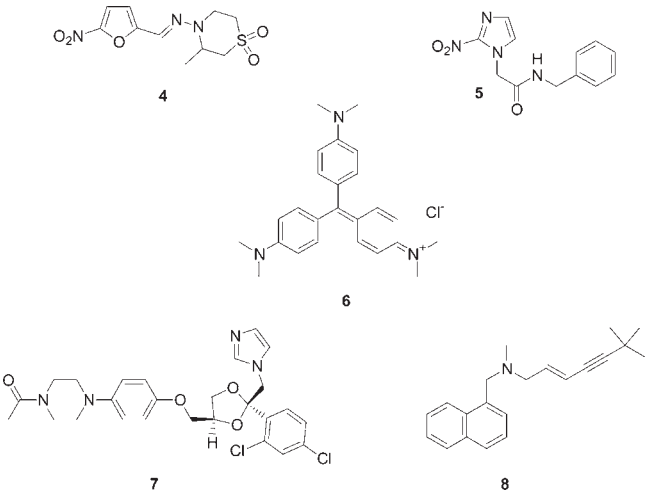

Figure 5. Chemical structures of nifurtimox (**4**) and benznidazole (**5**), the two drugs available for the treatment of Chagas disease. Gentian violet (**6**) and two anti-Chagas drug candidates ketoconazole (**7**) and terbinafine (**8**) are also shown.

3.2. Currently Available Drugs. The currently available drugs for treating trypanosomatid infections were predominantly developed at the beginning of the past century, as the tropical pharmacopeia was driven by colonial requirements or, in other cases, first developed for other pharmacological indications. Therefore, although helpful during the acute stages, they are often ineffective for chronic administration and produce severe side effects due to their high toxicity.

The currently available chemotherapy for Chagas disease is based on two agents introduced in the market in the 1970s: nifurtimox (**4**, Lampit) and benznidazole (**5**, Rochagan or Radanil), belonging to the class of nitroaromatic compounds (Figure 5). They show efficacy limited to the disease's acute phase and to only some pathogen strains. Moreover, the serious side effects, such as anorexia, vomit, and diarrhea pose controversy for their use for chronic patients.[15,110,111] Benznidazole efficacy and tolerance are inversely related to the age of the patient, while its side effects are more frequent in elderly patients.[112] In addition, medication is expensive with, for example, nifurtimox regimen requiring 10 mg/kg in three or four doses per day over a 60- to 120-day period. Because of these problems, the recommended course of treatment is often not completed, resulting in considerable scope for the development of resistance.[113]

Despite more than 40 years of research, the mechanism(s) of action and resistance of these nitroheterocyclic derivatives has remained elusive. It has long been recognized that they function as prodrugs and must be activated within the parasite to have trypanocidal effects. More recently it has been advanced that the two-electron reductions of the nitro group is mediated by a NADH-dependent, mitochondrially localized, type I nitroreductase,[114] leading to moieties that promote DNA damage.[115,116] Down-regulation of this enzyme might be responsible for resistance emergence.[114]

Gentian violet (**6**, Figure 5) is used as a trypanocidal agent in vitro to prevent transfusion-associated Chagas disease.

Other compounds have been studied as anti-Chagas drugs, for example, the commercially available antifungal agents ketoconazole (**7**, Figure 5) and terbinafine

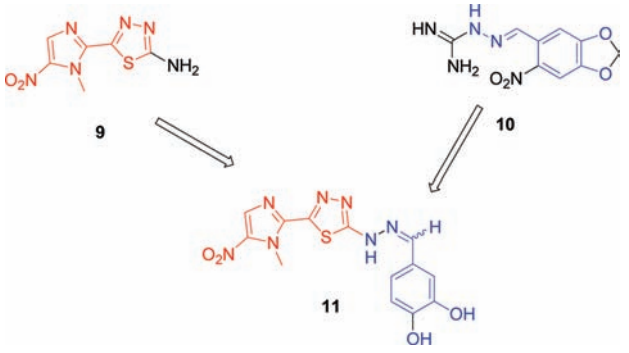

Figure 6. Design strategy leading to the hybrid derivative brazilizone A (**11**). The molecule was obtained by combining the structural features (in red) of **9** with those (in blue) of **10**.

(**8**, Figure 5), both acting as sterol-membrane biosynthesis inhibitors.[117,118]

3.3. Multi-Target-Directed Ligands. The first report of antichagasic compounds designed by conjugating two fragments into a single molecule deals with a series of megazol (**9**) and guanylhydrazone (**10**) hybrids (Figure 6).[119] Megazol is a 5-nitroimidazole compound endowed with a potent trypanocidal activity, despite a concomitant serious toxicity associated with the presence of the nitro group. Hydrazones have been demonstrated to have trypanocidal activity, with **10** being able to lyse trypomastigote forms of *T. cruzi* with IC_{50}/(24 h) = 17.1 μM. The design concept for these compounds primarily explored the introduction of the arylhydrazone moiety onto the heterocyclic framework of **9** (see Figure 6), aimed at conferring to the new molecules a radical scavenger activity, which could reduce the oxidative stress induced by formation of toxic nitro radical species. The authors were also interested in additional activities, as some hydrazone derivatives have shown a trypanocidal profile likely due to interaction with cruzipain (CP) and *T. cruzi* TR.

The hybrid derivative named brazilizone A (**11**, Figure 6, IC_{50} = 5.3 μM) was the most active compound against trypomastigote forms of *T. cruzi*, being 2-fold more potent than the prototype megazol (**9**, IC_{50} = 9.9 μM) in the same whole-parasite-based assay.[119] Although the authors have not investigated **11**'s mechanism of action at a molecular level, it is highly conceivable that it acts through the interference in multiple steps of the oxidative metabolism of *T. cruzi*. Moreover, to further support the therapeutic potential of **11**, theoretical calculations have shown that it has a potential for good in vivo absorption, since it satisfies Lipinski's rule of five without violations.[120]

The reported inhibitory activity of thiosemicarbazone (**12**) and *N*-amidinohydrazone (**13**) derivatives toward CP and TR was the starting point for recent interesting hybrids designed by Cerecetto and González,[121] by combining these moieties with a benzofuroxan pharmacophore (**14**) (Figure 7).[122] Two series of compounds were generated and tested for their antiproliferative effects against two strains of *T. cruzi* and for their inhibitory activity against CP and TR. Thiosemicarbazone **15** was the most promising: against the Tulahuen 2 strain, it displayed an IC_{50} value of 15 μM, not dissimilar to that of reference compounds **4** and **5**, coupled with a slight toxicity against mammals cells (selectivity index SI = 27).

Unfortunately, the investigation of the inhibitory profile of **15** against TR and CP target enzymes did not allow the authors to fully confirm the design rationale. The negligible

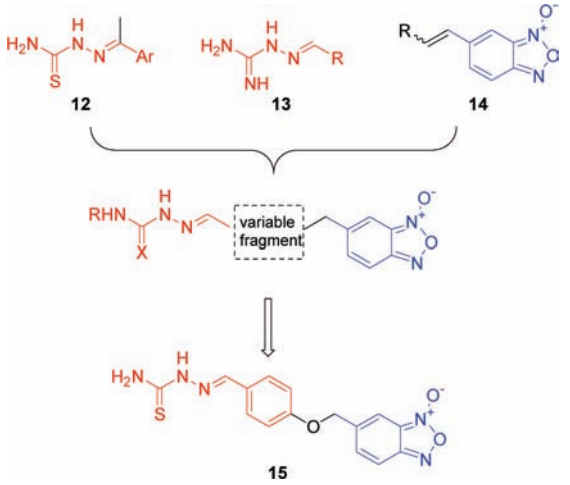

Figure 7. Design strategy leading to the thiosemicarbazone **15** by combining the structural features of **12** and **13** (in red) with the benzofuroxan pharmacophore (in blue) of **14**.

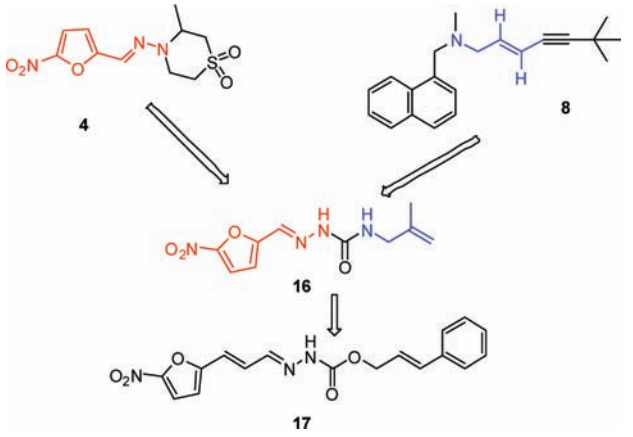

Figure 8. Design strategy leading to the dual ligands **16** and **17** by combining the nitrofurane moiety (in red) of **4** with the heteroallyl group (in blue) of **8**.

activity displayed instead made them postulate that **15**'s main mode of action is the production of oxidative stress, as confirmed by purposely addressed ESR studies. However, from an MTDL perspective, even an IC_{50} value of 43 μM (as that of **15**) is interesting, especially since no lead optimization studies have been performed by the authors. At the earliest stages of an MTDL's discovery, even if only weak activity is observed for a given target, this may provide a useful baseline for increasing that activity by incorporating additional structural elements and/or exploiting in silico studies.[76]

In another embodiment,[123] the same group pursued the multitarget approach for the discovery of dual ligands derived from the drug **4** and terbinafine (**8**), the promising anti *T. cruzi* agent acting by reducing the parasite's endogenous membrane sterol levels (Figure 8). The ergosterol biosynthesis pathway is a validated target for drug therapy against *T. cruzi*, as this parasite requires specific 24-alkyl sterols for viability and proliferation in all stages of its life cycle and cannot use the supply of cholesterol from its mammalian hosts. Squalene epoxidase (SE), an essential enzyme of this pathway, can be inhibited by the allylamine **8**. The design strategy was aimed at combining, in a single

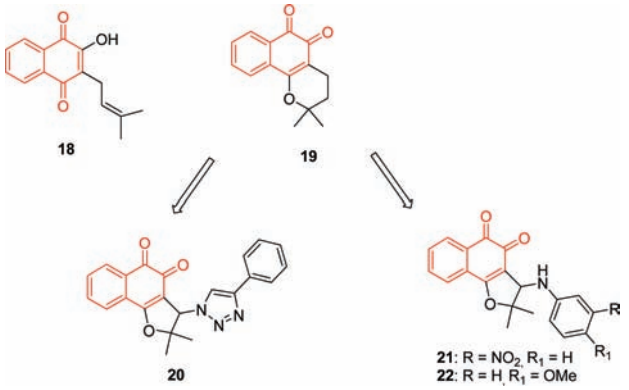

Figure 9. Design strategy leading to the potential MTDLs **20**−**22** starting from the natural products **18** and **19**. The common structural features are colored in red.

molecule, the nitrofuran moiety of **4** (with the potential for the generation of free radicals and redox cycling) with the heteroallyl group of **8**, which could inhibit the parasite's SE. The resulting heteroallyl-containing 5-nitrofuranes were active against *T. cruzi* proliferative stages, with a biological profile in most cases superior to those of **4** and **8**. In particular, derivatives **16** and **17** showed high potency against the intracellular amastigote form (low micromolar to submicromolar IC_{50} values), with appreciable SIs. More interestingly, the in vitro biological investigation confirmed for both derivatives a dual mechanism of action, producing oxidative stress and inhibiting membrane sterol biosynthesis at the level of SE.

Another remarkable example of an MTDL against *T. cruzi*, based on the combination of two molecular scaffolds, took advantage of the independent biological activities displayed by [1,2,3]triazoles and naphtoquinones.[124] In folk medicine, especially among native Amerindian populations, plants containing naphtoquinones are widely used to treat many diseases, including parasitic infections. In this respect, laphacol (**18**) and β-laphacone (**19**), extracted from the hearthwood of Brazilian trees of the genus *Tabebuia*, have been widely exploited as a prototypes in medicinal chemistry programs aimed at developing anti-Chagas lead candidates (Figure 9).[125] The cyclic reduction−oxidation of the quinone moiety is at the basis of their biological mechanism of action, which includes involvement in the electron transport and oxidative-phosphorylation processes. In particular, it has been demonstrated that treatment of *T. cruzi* with **19** led to alterations of chromatin distribution, mitochondrion, and plasma membrane, which are suggestive of an apoptosis-like process.[126] In addition, triazoles exert multiple biological activities, as antiplatelet agents, anticonvulsants, antiinflammatory, antiallergic, antiviral, and antimicrobial agents and antifungals share a triazole nucleus.[127] Despite this rational basis, the authors did not fully explore the multifunctional profile of the promising naphtoquinoidal triazoles synthesized. Rather, they seemed more interested in investigating the chemical reactivity of natural quinones. However, they could identify the phenyl substituted triazole **20**, which has emerged as an interesting MTDL against Chagas disease. Against trypomastigotes forms of *T. cruzi*, this derivative is significantly more active than benznidazole, as revealed by comparing the respective IC_{50}/day (17.3 μM vs 103.6 μM). A detailed investigation

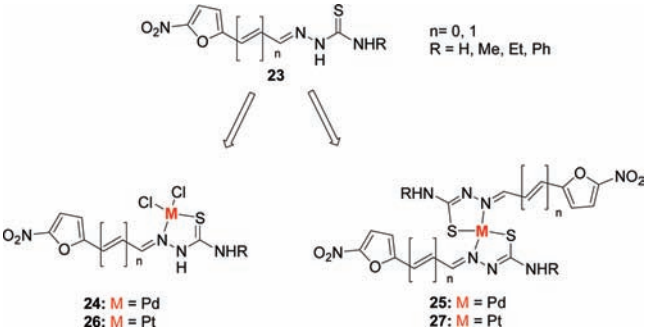

Figure 10. Palladium-based (**24** and **25**) and platinum-based complexes (**26** and **27**) of 3-(5-nitrofuryl)acroleine thiosemicarbazones of general formula **23**, designed following the metal complexation approach.

to disclose the multiple mechanisms of action of **20**, as well its profile against the emergence of resistance, would be highly desirable. It is worth noting that other substituted arylaminonaphthofuranquinones were developed by the same group following a similar molecular hybridization concept.[128] Studies of these compounds against Y strain trypomastigote forms of *T. cruzi* revealed that **21** and **22** were more active than **5**, possibly opening new perspectives for the development of novel MTDLs.

In the anti-Chagas MTDLs scenario, the complexes obtained by combining ligands bearing antitrypanosomal activity and pharmacologically active metals represent an interesting opportunity for enhancing efficacy and/or improving safety relative to single-target drugs. The development of metal complexes of clotrimazole and ketoconazole by Sánchez-Delgado et al., showing synergistic effects in anti-trypanosome therapy, has been well-regarded as the leading work in the field.[129] Gambino and colleagues recognized the multitarget potential of these derivatives and recently reported on two series of platinum-based complexes (**26** and **27**) of 3-(5-nitrofuryl)acroleine thiosemicarbazones of general formula **23**, which seem to have considerable promise in the treatment of Chagas disease.[130,131] In this peculiar application of the MTDL strategy, which the authors termed "metal complexation approach", the metal complexes act through dual or even multiple mechanisms of action, by combining the pharmacological properties of the ligand and the metal. Through this approach they have previously developed different series of metal derivatives of aromatic amine *N*-oxides[132] and 5-nitrofuryl containing thiosemicarbazones **23**[133] and characterized their mechanism of action. In particular, the palladium-5-nitrofuryl-containing thiosemicarbazones complexes **24** and **25** retained the mechanism of action of the thiosemicarbazone ligands. For their trypanocidal activity, they relied mainly on the production of toxic radical species resulting from the enzymatic reduction of the nitro moiety and subsequent redox cycling.[133] Moreover, they strongly interacted with DNA and inhibited TR in an irreversible fashion.[133] Notably and probably as a consequence of this multifunctional profile, these ligands displayed higher in vitro activity against *T. cruzi* than **4**.

As a further step, they decided rationally to develop the corresponding series of platinum complexes (with general formulas **26** and **27**, Figure 10), based on the consideration that several platinum compounds exert anti *T. cruzi* activity by binding DNA and, in addition, by inhibiting TR. The

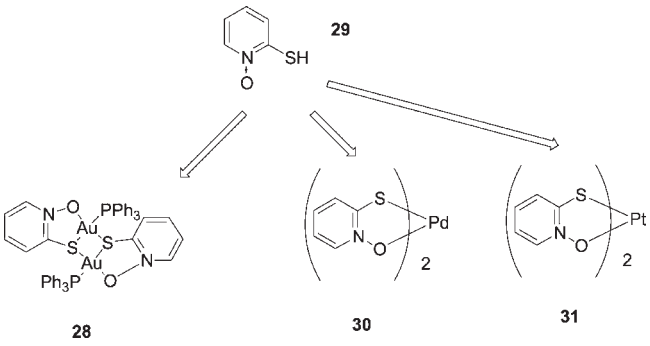

Figure 11. Gold(I) triphenylphosphine-based (**28**), palladium-based (**30**), and platinum-based complexes (**31**) of bioactive coligand pyridine-2-thiol *N*-oxide (**29**), designed following the metal complexation approach.

complexes were evaluated for their activities against epimastigotes of Tulahuen 2 and Dm28c strains. Most of the platinum complexes were active against both strains, with many of them showing IC_{50} values of the same order as **4** and the corresponding free ligands. Furthermore, their capacity to produce free radicals was evaluated by ESR experiments in the parasite and by respiration measurements, together with their DNA interaction ability. Although no conclusive SAR data can be drawn, results confirmed that some of the compounds could act as dual inhibitors in the parasite, through production of toxic free radicals and interaction with DNA.[130,131]

In a more recent application, the same authors have reported on the synthesis and biological evaluation of a novel coordination compound (**28**) of the ligand pyridine-2-thiol *N*-oxide **29** and gold(I) triphenylphosphine (Figure 11). Previously, they have characterized and evaluated in vitro the correspondent palladium (**30**) and platinum (**31**) complexes for their potential against Chagas disease, revealing that the trypanocidal action of the complexes could mainly rely on the inhibition of the enzyme NADH-fumarate reductase, a kinetoplastid parasite-specific enzyme absent in the mammalian host.[132]

The compound showed growth inhibitory effect on *T. cruzi* epimastigotes in culture ($IC_{50} = 0.09\ \mu M$), being even more active than the reference drug **4** ($IC_{50} = 6\ \mu M$). In addition, at a concentration of 1 μM, **28** induced in vitro a potent leishmanicidal effect against promastigotes of *L. mexicana*, while on *L. braziliensis* only a leishmanistatic effect was observed at the same concentration, analogous with the profile exhibited by lead compound **29**. DNA interaction studies showed that this macromolecule was not the main molecular target for **28**. Instead, the significant potentiation of the antiproliferative effect against both *Leishmania* species and *T. cruzi* could be linked to the inhibition of NADH fumarate reductase. Nevertheless, it cannot be excluded that modulation of other additional targets may help explain the increased activity observed. This is because it has been shown that gold(I)-monophosphine complexes act on mitochondria leading to apoptosis. This would further expand the MTDL profile of these complexes. Furthermore, owing to a low cytotoxicity on mammalian macrophages, the new gold complex showed a selective promising antiparasite activity, which warrants their further in vivo investigation in experimental models of leishmaniasis and Chagas disease.[131]

4. Human African Trypanosomiasis

4.1. General Considerations, Transmission, and Geographic Distribution. HAT, or sleeping sickness, was largely controlled in the 1960s, but a lack of human and financial resources put into combating the disease, and years of conflict in the most affected countries have hampered efforts to monitor and control the disease.[134] As a result, sleeping sickness re-emerged in the 1980s. There are two forms of HAT caused by two morphologically identical parasites: *Trypanosoma brucei gambiense* HAT is primarily a human chronic disease, endemic in west and central African countries; *Trypanosoma brucei rhodesiense* HAT has a huge animal reservoir and is primarily zoonotic. It causes acute illness in people in eastern and southern African countries.

HAT is transmitted to humans through the bites of infected tsetse flies. Cattle are the main reservoir for *T. b. rhodesiense*, in contrast to sleeping sickness caused by *T. b. gambiense* in west Africa where there appears to be no epidemiologically significant animal reservoir. Both forms of HAT show two distinct clinical stages. The first, which is often undiagnosed, corresponds to the multiplication of trypanosomes in the blood and lymphatic system. Then the parasites cross the blood−brain barrier and the disease progresses to the second stage, which is characterized by neurological symptoms and, without treatment, evolves toward body wasting, somnolence, coma, and death. Onset and fatal pathology of both subspecies are associated with a febrile illness followed by meningoencephalitis. The infection's progression with the two subspecies is markedly different. Disease resulting from *T. b. rhodesiense* infection has a rapid onset resulting in a fatal condition, with more than 80% fatality within the first 6 months of infection.[135] Infection with *T. b. gambiense* produces a chronic condition with long symptom-free periods, which may last several years.

The two occur in sub-Saharan Africa and are geographically separated more or less along the line of the Great Rift Valley.[136] *T. b. gambiense* occurs in western and central Africa, and *T. b. rhodesiense* infections occur in the east of the continent. The only country with known foci of infection for both parasites is Uganda; *T. b. gambiense* in the northwest and *T. b. rhodesiense* in the south, until recently limited to districts close to the shores of Lake Victoria. Angola, the Democratic Republic of Congo, and Sudan are the most affected, and prevalence has also increased in the Central African Republic. Today 60 million people are exposed to HAT. A total of 36 000 cases were reported to the WHO in 1998, but only 3−4 million people are under surveillance, and it is estimated that 300 000 people are currently infected. An estimation of 48 000 annual deaths for sleeping sickness has been reported.[109]

4.2. Currently Available Drugs. Drugs to treat sleeping sickness are mostly antiquated, scarce, and highly toxic and encounter parasite resistance.[137] Among the four licensed drugs, two are used against stage 1 disease, suramin (**32**) and pentamidine (**33**), whereas against stage 2 disease (neurological phase), melarsoprol (**34**) (active against *T. b. gambiense* and *T. b. rhodesiense*) and eflornithine (**35**) (only useful against *T. b. gambiense*) can be used (Figure 12).[138,139] In some cases, nifurtimox, not registered for HAT, is used compassionately after melarsoprol relapse.

Suramin (**32**, Bayer 205, Antrypol, Belganyl, 309 F, Fourneau 309, Germanin, Moranyl, Naganin, Naganol,

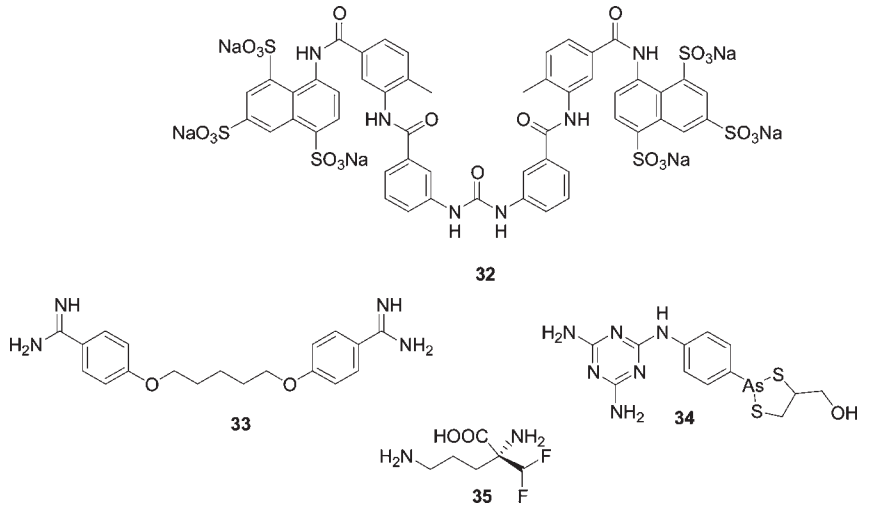

Figure 12. Chemical structures of the four drugs (**32**−**35**) available for sleeping sickness treatment.

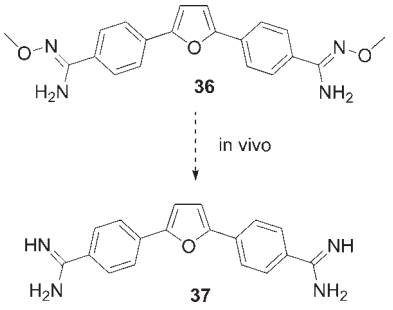

Figure 13. Chemical structures of furamidine (**37**) and its orally bioavailable prodrug pafuramidine (**36**).

Naphuride), which was developed in 1920, is a polysulphonated naphthyl urea that is strongly negatively charged at physiological pH. This is the reason that this medication is only effective for the treatment of the early stages of HAT, as it does not cross the blood−brain barrier. Because of this polyanionic structure, **32** modulates several enzymatic targets. It inhibits endocytosis of some molecules and the binding of low-density lipoproteins to specific receptors and causes changes in the phenotypic expression of surface antigens and a redistribution of cell surface negative charges.[140,141] Which of these various activities is most related to the trypanocidal activity is still uncertain, but it was suggested that deprivation of the parasite from cholesterol and phospholipids by inhibition of the LDL uptake is the main contributor to the mechanism of action.[142] Pentamidine (**33**), a diamidine first synthesized in the late 1930s, is currently produced by Sanofi-Aventis as Pentacarinat but is donated, free of charge, to the WHO for distribution. Pentamidine uptake into trypanosomes has been characterized in detail with the involvement of P2 aminopurine permease, the high-affinity pentamidine transporter 1 (HAPT1) and the low-affinity pentamidine transporter 1. However, its definitive mode of action is still unclear.[143] The parasite mitochondrion appears to be a target for pentamidine mechanisms of action and resistance. As a dication, pentamidine interacts electrostatically with cellular polyanions. It binds DNA, including the unique kDNA molecules. Melarsoprol (**34**, Arsobal, Mel B, Melarsen Oxide-BAL) was introduced for late-stage sleeping sickness in 1947[144] to replace earlier arsenical compounds, which had become

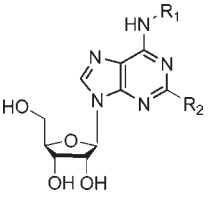

Figure 14. General structure of adenosine analogues designed by Gelb, Hol, and co-workers as potential multifunctional lead candidates: R_1 = H, alkyl, aminoalkyl, hydroxyalkyl, arylalkylamino; R_2 = H, NH_2, alkyl, aminoalkyl, hydroxyalkyl, arylalkylamino.

ineffective because of the emergence of drug resistance. For the past 50 years, treatment failures with **34** were not a major problem. But in recent years, these have increased dramatically in some important foci of *T. b. gambiense* sleeping sickness such as southern Sudan, Democratic Republic of Congo, Uganda, and Angola.[145,146]

The only new option in the therapy of HAT is represented by eflornithine (**35**, Ornidyl, Figure 12), an analogue of ornithine, introduced in therapy in 1981, which acts as inhibitor of trypanosomal ornithine decarboxilase, interfering with the polyamine's metabolic pathway. The major drawback of this drug is that it is expensive and only effective against *T. b. gambiense*.[147,148]

The first serious new drug candidate, brought to phase III clinical trials by the Consortium for Parasitic Drug Development, is pafuramidine (**36**, DB289),[149] an *O*-methyl amidoxime prodrug, which is converted in vivo into the diamidine furamidine (**37**, DB75) (Figure 13). If approved, it will be the first oral drug ever discovered for HAT.[150] In fact, because of the lack of oral drugs to treat this disease, patients currently have to be hospitalized for 10−30 days to enable treatment with parenterally administered drugs.

Pafuramidine is also in a pivotal phase III trial for pneumocystis pneumonia, a fungal infection that affects people living with HIV/AIDS.[151] It is also being developed for malaria. However, at the moment, there are no new drugs for the treatment of late-stage disease. Combinations of eflornithine with melarsoprol or nifurtimox have been the focus of recent clinical studies.[152] In particular, the eflornithine−nifurtimox combination is currently in phase III clinical trial for the treatment of the neurological phase of HAT.

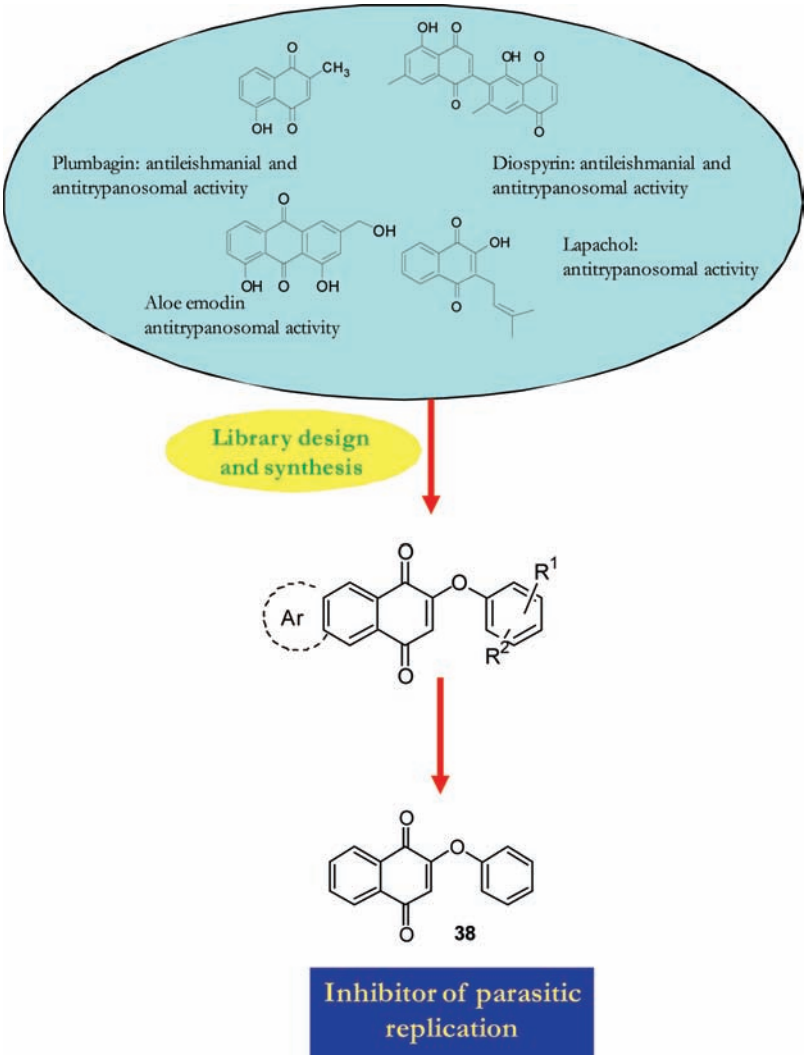

Figure 15. Design strategy of a library of potential MTDLs (exemplified by **38**), inspired by the antiparasitic activity of several quinone-based natural products.

4.3. Multi-Target-Directed Ligands. In 2000, Gelb, Hol, and co-workers[153] reported on a series of adenosine analogues as potential multifunctional compounds for the treatment of HAT. Evaluating the X-ray structures of *T. brucei* phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, and glycerol-3-phosphate dehydrogenase in complex with their adenosyl-bearing substrates, they designed adenosine analogues (Figure 14) aimed at simultaneously binding all three target proteins. Such glycolytic enzymes are well-known drug targets in anti-HAT drug discovery, as they also can ensure a certain degree of selectivity with respect to the corresponding mammalian enzymes. Despite the sound design strategy, the new compounds were only able to inhibit in the micromolar range glycosomal phosphoglycerate kinase. Actually, none of the new compounds inhibited *T. brucei* glyceraldehyde-3-phosphate dehydrogenase and glycerol-3-phosphate dehydrogenase.[153]

Natural quinone lapachol (**18**) inspired another series of potential MTDLs, designed through a combinatorial approach.[154] In the generation of small focused compound libraries, natural product-derived and -inspired collection concepts are particularly attractive. This is because they recognize natural product fragments as evolutionary selected and biologically prevalidated starting points in the chemical space to be used for compound collection development.[155] The quality of collections and the likelihood of generating hits may be particularly high if links already exist between such compound classes and the biological phenomena monitored in the respective cellular screens.[155] In terms of meeting the criteria described above for generating a library of anti-trypanosomatid compounds, the quinone unit was selected as a core structure for combinatorial derivatization. As already mentioned, napthoquinones and other related quinone compounds constitute one of the major natural product classes that have significant activity against *Leishmania* and *Trypanosoma*.[156] Lapachol is a representative example. Through a parallel approach (see Figure 15 for design strategy) 16 anthra- and naphtho-quinones, which incorporated in the 2-position a selection of aromatic groups mimicking the structural elements of the general biocide triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol), were synthesized.

From this small compound collection, several molecules were active against trypanosomes and *Leishmania* at low concentration. In particular, **38** showed an IC_{50} value of 80 nM against *T. b. rhodesiense* cells and a SI of 74, which is very close to the specifications required by WHO/TDR, pointing to **38** as a promising anti-trypanosomatid hit. A

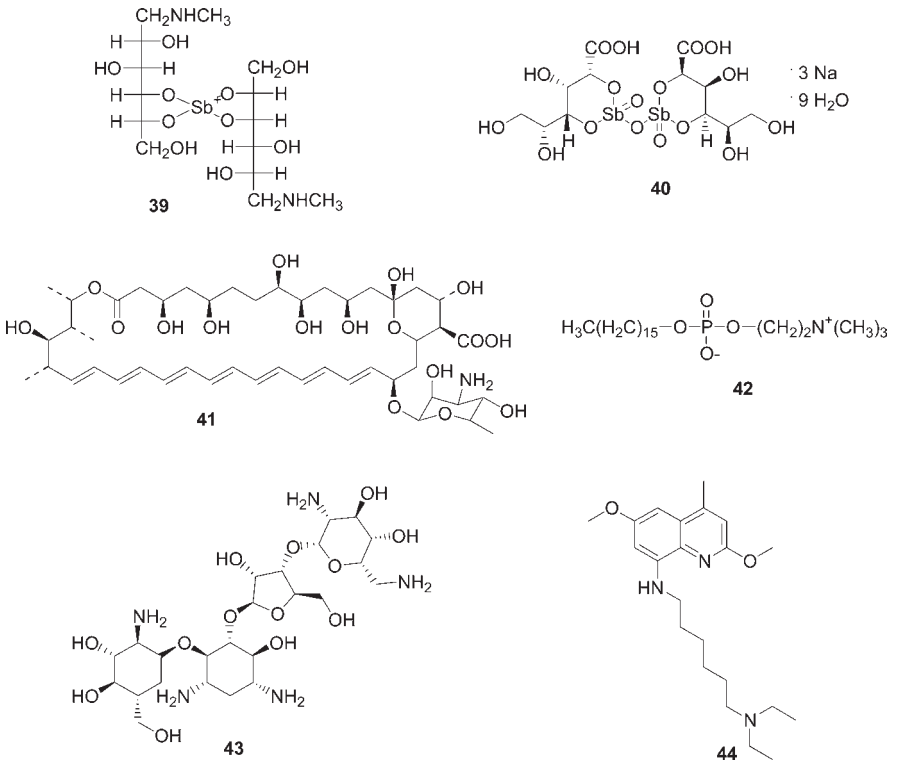

Figure 16. Chemical structures of the drugs (**39**−**43**) and the drug candidate sitamaquine (**44**) available for leishmaniasis treatment.

chemical proteomics approach will allow researchers to elucidate the targets involved in the trypanocidal activity of **38**. As in the previous case, a multitarget profile for this compound is easily conceivable, since quinones, like many other natural products, serve plants as potent defense chemicals with an intrinsic pleiotropic bioactivity.[157] It is worth noting that, in addition to their target-related mechanism, the general free-radical-generation mechanism of quinones (on the basis of their general cytotoxicity) may be exploited to prevent resistance development.[154]

5. Leishmaniasis

5.1. General Considerations, Transmission, and Geographic Distribution. Leishmaniases affect almost 12 million people in nearly 90 countries, representing a worldwide public health problem. More than 350 million people are currently at risk, and 2 million new cases are registered each year. An estimated 51 000 annual deaths for leishmaniases have been reported.[109] Leishmaniases are caused by the obligate intracellular protozoan parasite of the genus *Leishmania* spp. and constitute a cluster of diseases with diverse clinical manifestations, from cutaneous lesions to visceral leishmaniasis.[158] Broadly, the manifestations include three main groups of disorders: visceral leishmaniasis (kala-azar), cutaneous leishmaniasis, and mucocutaneous leishmaniasis. Visceral leishmaniasis, diffuse cutaneous leishmaniasis, and mucocutaneous leishmaniasis are severe forms of leishmaniasis resulting from the host's inability to control the infection, whereas spontaneous healing often occurs in cutaneous leishmaniasis because of the appropriate immune response. *Leishmania* infection is often asymptomatic, but latent parasites reactivate in immunosuppressed conditions and undergo uncontrolled multiplication, manifesting clinically as visceral leishmaniasis, which is fatal if untreated. Key features in the clinical presentation of kala-azar are prolonged fever, hepatosplenomegaly, and weight loss. Post-kala-azar dermal leishmaniasis is a complication of visceral leishmaniasis.[159] It is characterized by a macular, maculopapular, and nodular rash in a patient who has recovered from kala-azar and who is otherwise well.

Leishmaniases are transmitted to human beings through the bite of the female sandfly, which inoculates promastigotes into the skin of the host. In humans, these are taken by macrophages or dendritic cells and transformed into aflagellar amastigotes. The future course of infection depends upon the strain of *Leishmania* and the type of immune response mounted by the host. In India, visceral leishmaniasis is considered to be anthroponotic with people as the only known reservoir. In other areas, the picture is less clear and transmission may be anthroponotic as well as zoonotic, with rodents and canines as candidate reservoirs.

There are various forms of leishmaniasis depending on the species of etiologic agents involved. Cutaneous and mucocutaneous leishmaniasis are caused by *L. major* and *L. tropica*, while in south America, *L. mexicana* may cause localized lesions that usually self-heal, resulting in lifelong immunity. *L. brazilianensis*, *L. panamensis*, and *L. guyanensis* initially cause cutaneous lesions that may then metastasize leading to mucocutaneous lacerations that are often resistant to treatment or cure. Concerning visceral diseases, the species mainly responsible are *L. donovani*, *L. infantum*, and in the Americas, *L. chagasi*. These species cause chronic disseminating visceral disease in the liver and spleen, which can become fatal unless treated with chemotherapy.

As mentioned, leishmaniases occur worldwide although most cases occur on the Indian subcontinent (India, Nepal, and Bangladesh) and in east Africa (Sudan, Ethiopia, and Kenya), where *L. donovani* is the etiologic agent. Other endemic areas for visceral leishmaniasis include countries in the Mediterranean basin, such as Italy and Spain, where

L. infantum is the species involved, and the New World, where the identical *L. chagasi* circulates. In both areas, canines are the reservoir hosts.

5.2. Currently Available Drugs. Treatment of leishmaniasis is still complicated, since different causative species and various clinical manifestations exist.[69,160] Nowadays there are nearly 25 compounds and formulations that show antileishmanial effects, but only a few are classified as antileishmanial drugs for humans and most of them are parenteral.[161] First-line treatments are pentavalent antimonials meglumine antimoniate (**39**, Glucantime) and sodium stibogluconate (**40**, Pentostam), compounds discovered more than 50 years ago, which present severe, undesirable side effects (Figure 16). They have to be administered in low doses, and hence, drug resistance has appeared so rapidly[162] that in India they can no longer be used.[163] They are organic complexes of Sb^V, with improved solubility and uptake properties. Pentavalent antimony itself is inert; it is reduced in vivo to the more toxic trivalent form, either spontaneously or by glutathione *S*-transferase. Recently, the involvement of antimonite reductase has also been proposed. The crystal structures of oxidized TR from *L. infantum* and of the complex of reduced TR with NADPH and Sb(III), reported in a recent paper, disclose for the first time the TR-mediated mechanism of action of antimonial drugs against the parasite.[87]

The second-line drugs are pentamidine (**33**) and amphotericine B (**41**), which suffer from the same toxicity concerns. To overcome nephrotoxicity of this latter antifungal compound, different colloidal and lipid formulations have been successfully prepared.[164] But for those countries where cost is the main concern, they are too expensive and therefore unsuitable. Amphotericine B liposomal formulation has revealed a milestone in leishmaniasis treatment, due to its safety and the high administrable dosage. However, despite the drastic price reduction in May 2007, the cost of U.S. $300 for an average course is still prohibitive for VL-endemic countries. Recently, miltefosine (**42**), an alkylphosphocholine derivative developed as an anticancer drug, has been registered in India for oral treatment of VL.[165] However, it is contraindicated in women of childbearing age and shows severe gastrointestinal side effects.[166] Currently, the efficacy of liposomal amphotericin B in combination therapies is being evaluated and has displayed 90% cure rates in combination with oral miltefosine for the visceral disease form.[167] However, some cutaneous leishmaniases are refractory and other drug treatments have 50% cure rates.

Paromomycin (**43**) is an aminoglycoside antibiotic registered in India in August 2006 for leishmaniasis. The results of clinical trials showed excellent efficacy and safety. No nephrotoxicity was observed, reversible high-tone ototoxicity was found in 2% of patients, and 1.8% of patients showed a significant increase (> 5-fold) in hepatic transaminases. Mild injection pain was reported by over 50% of patients. Other advantages of **43** include the fact that it is active against a wide variety of pathogens, including bacteria, and its low cost (U.S. $5−0 per treatment).[168]

Sitamaquine, a 8-aminoquinoline (**44**, WR6026, Figure 16) is a new antileishmanial oral drug currently in phase IIb clinical trials by GlaxoSmithKline.[169] Although its mechanism of action is still unknown, it has shown acceptably good efficacy. It was well-tolerated in the most recent phase II study, but there are still some unanswered questions concerning its potential toxicities.[170,171]

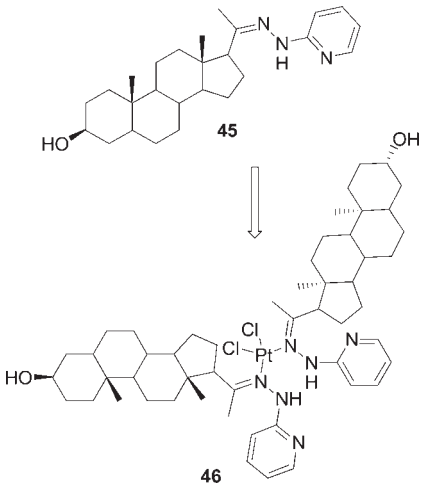

Figure 17. Platinum complex (**46**) of the sterol hydrazone ligand (**45**).

Also in leishmaniasis combined therapies are now emerging, and different clinical assays are on going to establish the best combinations possible according to geographical areas.

5.3. Multi-Target-Directed Ligands. If we exclude an early example of a thiosemicarbazone and 3-carboxy-β-carbinolines hybrid,[172] for whom the multifunctional profile was not elucidated, and recent examples of dimeric acridine derivatives,[173] the only case of rationally designed MTDLs we could find in the literature builds on the metal−drug synergism paradigm.[174] Navarro and colleagues pointed out that in addition to the synergism in mechanism of actions already described, another favorable issue of this approach is the possible stabilization of the drug. This feature might lead to a longer residence time of the drug in the body, allowing it to reach the biological targets more efficiently, and may also result in a decrease in toxicity.[174] With this in mind, they combined the sterol hydrazone ligand (**45**, Figure 17) to platinum. Steroid compounds have been shown to inhibit sterol methyl transferase enzyme and consequentely *Leishmania* growth, by altering lipid composition of the parasite's mitochondrial inner membrane.[175] Conversely, certain platinum complexes, such as (2,2′:6′2″-terpyridine)platinum-(II), have produced remarkable leishmanicidal activity against amastigote forms of *L. donovani*, exploiting the intercalative DNA properties of the terpyridine ligand along with the covalent binding ability of the Pt(II) center.[176]

Therefore, the new platinum−sterol hydrazone complex **46** might exert a synergistic mechanism of action by combining inhibition of the sterol biosynthesis pathway and dual interaction with the DNA of the parasite. When tested against *L. mexicana* promastigotes, **46** produced a higher leishmanistatic activity than **45** (71% growth inhibition vs 39%, respectively), associated with motility loss and swelling of parasites, vacuolation, and formation of parasite clusters. Studies for both the sterol profile and the interaction with DNA are in progress and may confirm the designed multiple mechanism of action.

6. Perspectives

Can system complexity arising from parasites' resistance be addressed with MTDLs? Drug-resistance is usually triggered by the appearance of one or more mutations on the gene

encoding for drug target proteins. Alternatively, the appearance of resistance may be more complex, as it can be related to mutations in food vacuole membrane proteins, which may lower the concentration of the drug at the target.

Combination therapy has been shown to be a possible strategy for both preventing and overcoming chemotherapy-induced resistance. The rationale for using drugs in combination is well-established in the treatment of tuberculosis, infection with HIV, and cancer. The probability of a parasite developing resistance simultaneously toward two drugs with unrelated modes of action has been elaborately described by White in 1999.[177] Resistance has been one of the major challenges in efforts to tackle malaria in sub-Saharan Africa. Several effective combination regimens that retain therapeutic and prophylactic efficacy in the face of resistance have been tested, and nowadays, we note that there have been no reports of clinical resistance to the artemisinin-based combinations in antimalarial therapies. However, combination therapies could suffer from major drawbacks related to pharmacokinetics and pharmacodynamics.

Following the same rationale, we suggest that multifunctional compounds represent an innovative approach to addressing chemotherapy-induced drug resistance. A single compound endowed with a multifunctional profile is able to simultaneously modulate the activity of two or more biological counterparts. As with combination therapy, the probability of simultaneous mutations in both genes encoding for both target proteins is greatly reduced. Moreover, the use of a single drug that has multiple biological properties would have inherent advantages over combination therapies. Indeed, it would obviate the challenge of administering multiple single-drug entities, which could have different bioavailability, pharmacokinetics, and metabolism. Moreover, in terms of pharmacokinetic and ADMET optimization, the clinical development of a drug able to hit multiple targets should not, in principle, be different from the development of any other single lead molecule. It thus offers a much more simple approach than the development of new combination therapies. In addition, the risk of possible drug−drug interactions would be avoided and the therapeutic regimen greatly simplified when compared to drug cocktails. All these considerations are of particular relevance because they can dramatically influence the costs of drug development, which is a major concern in the development of drug candidates for tropical neglected diseases.

A systematic and rational approach to the discovery of novel multifunctional drug candidates has not yet been reported in the literature. In our opinion, it would be a step forward in the rational design of balanced multifunctional compounds if we could gain a superior understanding of the cellular mechanisms at which the drug candidate is aimed.[178] Systems biology has revealed that cells are composed of complex, networked systems with redundant, convergent, and divergent signaling pathways.[179] In contrast to the reductionist paradigm, systems biology considers the description of the system, that is, the complex interactions between the molecular constituents of living cells.[180] A union between systems biology and medicinal chemistry would certainly help overcome the limits of the one-molecule−one-target approach. In target-centric drug discovery strategies, potential leads are identified and optimized for activity against specific molecular targets. In contrast, approaches based on systems biology are aimed at the generation of leads that potentially affect interconnected pathways. A step in this direction was reported in 2007 by Hornberg et al.[5] In this paper, the authors have reported on a metabolic control analysis that could improve effectiveness and selectivity of drugs for parasitic diseases. They developed a mathematical model to identify a target enzyme with a high flux control coefficient in the parasite and a low flux control coefficient in the host cells. This target should at best ensure efficacy and selectivity. Although the model was not explicitly exploited in the design of multifunctional compounds, slight modifications of the model can be designed to select the two targets, which ensure high effectiveness and selectivity, and at the same time greatly reduce the risk of resistance insurgence (P. Michels, personal communication). Such systems biology models allow us to directly measure disease-relevant cellular responses and represent a practical means of following drug-discovery-based pathways rather than targets.

From the observations above, it is clear that, as with drug combination, multifunctional compounds for parasitic diseases call for innovative strategies to properly address their discovery. Furthermore, systems biology studies applied to parasitic cells can open up new avenues for applying similar models to more complex living organisms. For instance, understanding the pathways of programmed cell death and apoptosis in *Trypanosoma* could hopefully provide the basis for investigating a more comprehensive topic such as the origin of apoptosis through four billion years of evolution.[181]

7. Conclusions

NTDs affect more than one billion people worldwide but have been largely neglected for drug development because they affect poor people in poor regions of the world. Currently available drugs for NTDs worsen the scenario, since they are decades old (back to 1920) and have many limitations, including high toxicity and the emergence of drug resistance. The NTDs drug discovery pipeline is almost dry, and efforts to reinvigorate the identification of novel lead candidates for these diseases are largely driven by support from major philanthropies. The parasites responsible for these diseases have a fascinating biology, and many potential biochemical targets are now apparent. Actually, recent genome projects have brought to the attention of the drug discovery community a plethora of novel drug targets. Therefore, NTDs present unique challenges to drug development that are being addressed by new public−private partnerships of scientists from academia and industry, and innovative approaches to drug design and discovery could be advanced.

In this Perspective, besides having reported on some novel and validated molecular targets for NTDs drug discovery, we have briefly mentioned the currently available drugs and we have introduced the multifunctional drug design strategy that can open up a new avenue in the field of NTDs. It is opinion of the present authors that MTDLs can be a convenient alternative to drug combinations, which are currently regarded as one of the best strategies to face drug resistance, a major concern in NTDs drug discovery and development. At the same extent, we are convinced that molecules able to simultaneously modulate more than a single vital parasitic protein can be a way to confront drug resistance. Nowadays, a few efforts have been devoted in this direction, and some examples have here been reported. Thereby, we are confident that medicinal chemists working in the field of NTDs can find here a useful overview about possible exploitations of the MTDL strategy in the field of neglected diseases.

We conclude by suggesting that MTDLs can be regarded as a new technology in NTDs drug discovery. Although it is clear that many health issues in developing countries will not be solved by new technologies alone, these will still be important for reducing poverty and its consequences. Thus, political institutions should find the right way of uniting industry, investors, academic, and governmental organizations to intensify efforts toward the practical exploitation of new technologies against infectious NTDs.

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Biographies

Andrea Cavalli received his Ph.D. in Pharmaceutical Sciences in 1999 from the University of Bologna (Italy) under the supervision of Maurizio Recanatini. He was then a Postdoctoral Fellow in Biophysics at the International School for Advanced Studies (SISSA/ISAS) of Trieste (Italy), where he worked with Vincent Torre and Paolo Carloni. In 2001−2002, he was Visiting Scientist at the Swiss Federal Institute of Technology (ETH) of Zurich (Switzerland), working with Gerd Folkers and Leonardo Scapozza. At present, he is Assistant Professor at the Department of Pharmaceutical Sciences of the University of Bologna and Senior Scientist at the Department of Drug Discovery and Development of the IIT, where he supervises the Structure-Based Drug Design Group. In 2003, he was awarded the Farmindustria Prize for Pharmaceutical Research.

Maria Laura Bolognesi graduated from the University of Bologna with a degree in Medicinal Chemistry in1990 and then moved to the Sigma Tau Industries, Rome, as Research Scientist at the Department of Chemical Research. In 1996, she received her Ph.D. in Pharmaceutical Sciences from the University of Bologna under the direction of Prof. Carlo Melchiorre. After postdoctoral studies at University of Minnesota in Prof. Philip S. Portogheses's laboratory, she returned to the University of Bologna where she has been appointed first Assistant Professor in 1998 and then Associate Professor of Medicinal Chemistry in 2005. In 2009, she was invited as Visiting Professor at the Universidad Complutense of Madrid, working with Prof. Jose Carlos Menendez.

References

(1) O'Connell, D. Neglected diseases. *Nature* **2007**, 449–157.
(2) http://www.who.int/neglected_diseases/en/.
(3) Hotez, P. J.; Molyneux, D. H.; Fenwick, A.; Ottesen, E.; Ehrlich Sachs, S.; Sachs, J. D. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. *PLoS Med.* **2006**, *3*, No. e102.
(4) Pink, R.; Hudson, A.; Mouries, M. A.; Bendig, M. Opportunities and challenges in antiparasitic drug discovery. *Nat. Rev. Drug Discovery* **2005**, *4*, 727–740.
(5) Hornberg, J. J.; Bruggeman, F. J.; Bakker, B. M.; Westerhoff, H. V. Metabolic Control Analysis To Identify Optimal Drug Targets. In Progress in Drug Research; Boshoff, H. I., Barry, C. E., III, Eds.; Birkhauser: Basel, Switzerland, 2007; Vol. 64, pp 172−189.
(6) Nwaka, S.; Ridley, R. G. Virtual drug discovery and development for neglected diseases through public−private partnerships. *Nat. Rev. Drug Discovery* **2003**, *2*, 919–928.
(7) Herrling, P. L. Financing R&D for neglected diseases. *Nat. Rev. Drug Discovery* **2009**, *8*, 91.
(8) Butler, D. Lost in translation. *Nature* **2007**, *449*, 158–159.
(9) Fevre, E. M.; Wissmann, B. V.; Welburn, S. C.; Lutumba, P. The burden of human African trypanosomiasis. *PLoS Neglected Trop. Dis* **2008**, *2*, No. e333.
(10) Bern, C.; Maguire, J. H.; Alvar, J. Complexities of assessing the disease burden attributable to leishmaniasis. *PLoS Neglected Trop. Dis* **2008**, *2*, No. e313.
(11) Hotez, P. J.; Molyneux, D. H.; Fenwick, A.; Kumaresan, J.; Sachs, S. E.; Sachs, J. D.; Savioli, L. Control of neglected tropical diseases. *N. Engl. J. Med.* **2007**, *357*, 1018–1027.
(12) Renslo, A. R.; McKerrow, J. H. Drug discovery and development for neglected parasitic diseases. *Nat. Chem. Biol.* **2006**, *2*, 701–710.
(13) Miyahira, Y. *Trypanosoma cruzi* infection from the view of CD8+ T cell immunity—an infection model for developing T cell vaccine. *Parasitol. Int.* **2008**, *57*, 38–48.
(14) Kinoshita, T. Designing sleeping sickness control. *ACS Chem. Biol.* **2008**, *3*, 601–603.
(15) Paulino, M.; Iribarne, F.; Dubin, M.; Aguilera-Morales, S.; Tapia, O.; Stoppani, A. O. The chemotherapy of Chagas' disease: an overview. *Mini-Rev. Med. Chem* **2005**, *5*, 499–519.
(16) Muskavitch, M. A.; Barteneva, N.; Gubbels, M. J. Chemogenomics and parasitology: small molecules and cell-based assays to study infectious processes. *Comb. Chem. High Throughput Screening* **2008**, *11*, 624–646.
(17) Stuart, K.; Brun, R.; Croft, S.; Fairlamb, A.; Gurtler, R. E.; McKerrow, J.; Reed, S.; Tarleton, R. Kinetoplastids: related protozoan pathogens, different diseases. *J. Clin. Invest.* **2008**, *118*, 1301–1310.
(18) Barrett, M. P.; Mottram, J. C.; Coombs, G. H. Recent advances in identifying and validating drug targets in trypanosomes and leishmanias. *Trends Microbiol.* **1999**, *7*, 82–88.
(19) Michels, P. A.; Bringaud, F.; Herman, M.; Hannaert, V. Metabolic functions of glycosomes in trypanosomatids. *Biochim. Biophys. Acta* **2006**, *1763*, 1463–1477.
(20) Fairlamb, A. H.; Cerami, A. Metabolism and functions of trypanothione in the kinetoplastida. *Annu. Rev. Microbiol.* **1992**, *46*, 695–729.
(21) Berriman, M.; Ghedin, E.; Hertz-Fowler, C.; Blandin, G.; Renauld, H.; Bartholomeu, D. C.; Lennard, N. J.; Caler, E.; Hamlin, N. E.; Haas, B.; Bohme, U.; Hannick, L.; Aslett, M. A.; Shallom, J.; Marcello, L.; Hou, L.; Wickstead, B.; Alsmark, U. C.; Arrowsmith, C.; Atkin, R. J.; Barron, A. J.; Bringaud, F.; Brooks, K.; Carrington, M.; Cherevach, I.; Chillingworth, T. J.; Churcher, C.; Clark, L. N.; Corton, C. H.; Cronin, A.; Davies, R. M.; Doggett, J.; Djikeng, A.; Feldblyum, T.; Field, M. C.; Fraser, A.; Goodhead, I.; Hance, Z.; Harper, D.; Harris, B. R.; Hauser, H.; Hostetler, J.; Ivens, A.; Jagels, K.; Johnson, D.; Johnson, J.; Jones, K.; Kerhornou, A. X.; Koo, H.; Larke, N.; Landfear, S.; Larkin, C.; Leech, V.; Line, A.; Lord, A.; Macleod, A.; Mooney, P. J.; Moule, S.; Martin, D. M.; Morgan, G. W.; Mungall, K.; Norbertczak, H.; Ormond, D.; Pai, G.; Peacock, C. S.; Peterson, J.; Quail, M. A.; Rabbinowitsch, E.; Rajandream, M. A.; Reitter, C.; Salzberg, S. L.; Sanders, M.; Schobel, S.; Sharp, S.; Simmonds, M.; Simpson, A. J.; Tallon, L.; Turner, C. M.; Tait, A.; Tivey, A. R.; Van Aken, S.; Walker, D.; Wanless, D.; Wang, S.; White, B.; White, O.; Whitehead, S.; Woodward, J.; Wortman, J.; Adams, M. D.; Embley, T. M.; Gull, K.; Ullu, E.; Barry, J. D.; Fairlamb, A. H.; Opperdoes, F.; Barrell, B. G.; Donelson, J. E.; Hall, N.; Fraser, C. M.; Melville, S. E.; El-Sayed, N. M. The genome of the African trypanosome *Trypanosoma brucei*. *Science* **2005**, *309*, 416–422.
(22) El-Sayed, N. M.; Myler, P. J.; Bartholomeu, D. C.; Nilsson, D.; Aggarwal, G.; Tran, A. N.; Ghedin, E.; Worthey, E. A.; Delcher, A. L.; Blandin, G.; Westenberger, S. J.; Caler, E.; Cerqueira, G. C.; Branche, C.; Haas, B.; Anupama, A.; Arner, E.; Aslund, L.; Attipoe, P.; Bontempi, E.; Bringaud, F.; Burton, P.; Cadag, E.; Campbell, D. A.; Carrington, M.; Crabtree, J.; Darban, H.; da Silveira, J. F.; de Jong, P.; Edwards, K.; Englund, P. T.; Fazelina, G.; Feldblyum, T.; Ferella, M.; Frasch, A. C.; Gull, K.; Horn, D.; Hou, L.; Huang, Y.; Kindlund, E.; Klingbeil, M.; Kluge, S.; Koo, H.; Lacerda, D.; Levin, M. J.; Lorenzi, H.; Louie, T.; Machado, C. R.; McCulloch, R.; McKenna, A.; Mizuno, Y.; Mottram, J. C.; Nelson, S.; Ochaya, S.; Osoegawa, K.; Pai, G.; Parsons, M.; Pentony, M.; Pettersson, U.; Pop, M.; Ramirez, J. L.; Rinta, J.; Robertson, L.; Salzberg, S. L.; Sánchez, D. O.; Seyler, A.; Sharma, R.; Shetty, J.; Simpson, A. J.; Sisk, E.; Tammi, M. T.; Tarleton, R.; Teixeira, S.; Van Aken, S.; Vogt, C.; Ward, P. N.; Wickstead, B.; Wortman, J.; White, O.; Fraser, C. M.; Stuart, K. D.; Andersson, B. The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. *Science* **2005**, *309*, 409–415.
(23) Ivens, A. C.; Peacock, C. S.; Worthey, E. A.; Murphy, L.; Aggarwal, G.; Berriman, M.; Sisk, E.; Rajandream, M. A.; Adlem, E.; Aert, R.; Anupama, A.; Apostolou, Z.; Attipoe, P.; Bason, N.; Bauser, C.; Beck, A.; Beverley, S. M.; Bianchettin, G.; Borzym, K.; Bothe, G.; Bruschi, C. V.; Collins, M.; Cadag, E.; Ciarloni, L.; Clayton, C.; Coulson, R. M.; Cronin, A.; Cruz, A. K.; Davies, R. M.; De Gaudenzi, J.; Dobson, D. E.; Duesterhoeft, A.; Fazelina, G.; Fosker, N.; Frasch, A. C.; Fraser, A.; Fuchs, M.; Gabel, C.; Goble, A.; Goffeau, A.; Harris, D.; Hertz-Fowler, C.; Hilbert, H.; Horn, D.; Huang, Y.; Klages, S.; Knights, A.; Kube, M.; Larke, N.; Litvin, L.; Lord, A.; Louie, T.; Marra, M.; Masuy, D.; Matthews, K.; Michaeli, S.; Mottram, J. C.; Muller-Auer, S.; Munden, H.; Nelson, S.; Norbertczak, H.; Oliver, K.; O'Neil, S.; Pentony, M.; Pohl, T. M.; Price, C.; Purnelle, B.; Quail, M. A.; Rabbinowitsch, E.; Reinhardt, R.; Rieger, M.; Rinta, J.; Robben, J.; Robertson, L.;

Ruiz, J. C.; Rutter, S.; Saunders, D.; Schafer, M.; Schein, J.; Schwartz, D. C.; Seeger, K.; Seyler, A.; Sharp, S.; Shin, H.; Sivam, D.; Squares, R.; Squares, S.; Tosato, V.; Vogt, C.; Volckaert, G.; Wambutt, R.; Warren, T.; Wedler, H.; Woodward, J.; Zhou, S.; Zimmermann, W.; Smith, D. F.; Blackwell, J. M.; Stuart, K. D.; Barrell, B.; Myler, P. J. The genome of the kinetoplastid parasite, *Leishmania major*. *Science* **2005**, *309*, 436–442.

(24) Peacock, C. S.; Seeger, K.; Harris, D.; Murphy, L.; Ruiz, J. C.; Quail, M. A.; Peters, N.; Adlem, E.; Tivey, A.; Aslett, M.; Kerhornou, A.; Ivens, A.; Fraser, A.; Rajandream, M. A.; Carver, T.; Norbertczak, H.; Chillingworth, T.; Hance, Z.; Jagels, K.; Moule, S.; Ormond, D.; Rutter, S.; Squares, R.; Whitehead, S.; Rabbinowitsch, E.; Arrowsmith, C.; White, B.; Thurston, S.; Bringaud, F.; Baldauf, S. L.; Faulconbridge, A.; Jeffares, D.; Depledge, D. P.; Oyola, S. O.; Hilley, J. D.; Brito, L. O.; Tosi, L. R. O.; Barrell, B.; Cruz, A. K.; Mottram, J. C.; Smith, D. F.; Berriman, M. Comparative genomic analysis of three Leishmania species that cause diverse human disease. *Nat. Genet*. **2007**, *39*, 839–847.

(25) Aguero, F.; Al-Lazikani, B.; Aslett, M.; Berriman, M.; Buckner, F. S.; Campbell, R. K.; Carmona, S.; Carruthers, I. M.; Chan, A. W.; Chen, F.; Crowther, G. J.; Doyle, M. A.; Hertz-Fowler, C.; Hopkins, A. L.; McAllister, G.; Nwaka, S.; Overington, J. P.; Pain, A.; Paolini, G. V.; Pieper, U.; Ralph, S. A.; Riechers, A.; Roos, D. S.; Sali, A.; Shanmugam, D.; Suzuki, T.; Van Voorhis, W. C.; Verlinde, C. L. Genomic-scale prioritization of drug targets: the TDR targets database. *Nat. Rev. Drug Discovery* **2008**, *7*, 900–907.

(26) Sen, N.; Majumder, H. K. Mitochondrion of protozoan parasite emerges as potent therapeutic target: exciting drugs are on the horizon. *Curr. Pharm. Des*. **2008**, *14*, 839–846.

(27) Chaudhuri, M.; Ott, R. D.; Hill, G. C. Trypanosome alternative oxidase: from molecule to function. *Trends Parasitol*. **2006**, *22*, 484–491.

(28) Nihei, C.; Fukai, Y.; Kita, K. Trypanosome alternative oxidase as a target of chemotherapy. *Biochim. Biophys. Acta* **2002**, *1587*, 234–239.

(29) Lee, S. H.; Stephens, J. L.; Paul, K. S.; Englund, P. T. Fatty acid synthesis by elongases in trypanosomes. *Cell* **2006**, *126*, 691–699.

(30) Lee, S. H.; Stephens, J. L.; Englund, P. T. A fatty-acid synthesis mechanism specialized for parasitism. *Nat. Rev. Microbiol*. **2007**, *5*, 287–297.

(31) Soto, J.; Soto, P. Miltefosine: oral treatment of leishmaniasis. *Expert Rev. Anti-Infect. Ther*. **2006**, *4*, 177–185.

(32) Santa-Rita, R. M.; Santos Barbosa, H.; Meirelles, M. N.; de Castro, S. L. Effect of the alkyl-lysophospholipids on the proliferation and differentiation of *Trypanosoma cruzi*. *Acta Trop*. **2000**, *75*, 219–228.

(33) Gelb, M. H.; Brunsveld, L.; Hrycyna, C. A.; Michaelis, S.; Tamanoi, F.; Van Voorhis, W. C.; Waldmann, H. Therapeutic intervention based on protein prenylation and associated modifications. *Nat. Chem. Biol*. **2006**, *2*, 518–528.

(34) Liu, B.; Liu, Y.; Motyka, S. A.; Agbo, E. E.; Englund, P. T. Fellowship of the rings: the replication of kinetoplast DNA. *Trends Parasitol*. **2005**, *21*, 363–369.

(35) Sela, D.; Milman, N.; Kapeller, I.; Zick, A.; Bezalel, R.; Yaffe, N.; Shlomai, J. Unique characteristics of the kinetoplast DNA replication machinery provide potential drug targets in trypanosomatids. *Adv. Exp. Med. Biol*. **2008**, *625*, 9–21.

(36) Cavalcanti, D. P.; Fragoso, S. P.; Goldenberg, S.; de Souza, W.; Motta, M. C. The effect of topoisomerase II inhibitors on the kinetoplast ultrastructure. *Parasitol. Res*. **2004**, *94*, 439–448.

(37) Lindsay, M. E.; Gluenz, E.; Gull, K.; Englund, P. T. A new function of *Trypanosoma brucei* mitochondrial topoisomerase II is to maintain kinetoplast DNA network topology. *Mol. Microbiol*. **2008**, *70*, 1465–1476.

(38) Ingram, A. K.; Horn, D. Histone deacetylases in *Trypanosoma brucei*: two are essential and another is required for normal cell cycle progression. *Mol. Microbiol*. **2002**, *45*, 89–97.

(39) Mai, A.; Cerbara, I.; Valente, S.; Massa, S.; Walker, L. A.; Tekwani, B. L. Antimalarial and antileishmanial activities of aroyl-pyrrolyl-hydroxyamides, a new class of histone deacetylase inhibitors. *Antimicrob. Agents Chemother*. **2004**, *48*, 1435–1436.

(40) el Kouni, M. H. Potential chemotherapeutic targets in the purine metabolism of parasites. *Pharmacol. Ther*. **2003**, *99*, 283–309.

(41) Leandro, C.; Campino, L. Leishmaniasis: efflux pumps and chemoresistance. *Int. J. Antimicrob. Agents* **2003**, *22*, 352–357.

(42) Landfear, S. M. Drugs and transporters in kinetoplastid protozoa. *Adv. Exp. Med. Biol*. **2008**, *625*, 22–32.

(43) Beitz, E. Aquaporins from pathogenic protozoan parasites: structure, function and potential for chemotherapy. *Biol. Cell* **2005**, *97*, 373–383.

(44) McNae, I. W.; Martinez-Oyanedel, J.; Keillor, J. W.; Michels, P. A.; Fothergill-Gilmore, L. A.; Walkinshaw, M. D. The crystal structure of ATP-bound phosphofructokinase from *Trypanosoma brucei* reveals conformational transitions different from those of other phosphofructokinases. *J. Mol. Biol*. **2009**, *385*, 1519–1533.

(45) Muller, S.; Liebau, E.; Walter, R. D.; Krauth-Siegel, R. L. Thiol-based redox metabolism of protozoan parasites. *Trends Parasitol*. **2003**, *19*, 320–328.

(46) Krauth-Siegel, R. L.; Bauer, H.; Schirmer, R. H. Dithiol proteins as guardians of the intracellular redox milieu in parasites: old and new drug targets in trypanosomes and malaria-causing plasmodia. *Angew. Chem., Int. Ed*. **2005**, *44*, 690–715.

(47) Krauth-Siegel, R. L.; Comini, M. A. Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochim. Biophys. Acta* **2008**, *1780*, 1236–1248.

(48) Boerma, M.; van der Wees, C. G.; Vrieling, H.; Svensson, J. P.; Wondergem, J.; van der Laarse, A.; Mullenders, L. H.; van Zeeland, A. A. Microarray analysis of gene expression profiles of cardiac myocytes and fibroblasts after mechanical stress, ionising or ultraviolet radiation. *BMC Genomics* **2005**, *6*, 6.

(49) Hammarton, T. C.; Clark, J.; Douglas, F.; Boshart, M.; Mottram, J. C. Stage-specific differences in cell cycle control in *Trypanosoma brucei* revealed by RNA interference of a mitotic cyclin. *J. Biol. Chem*. **2003**, *278*, 22877–22886.

(50) Tu, X.; Wang, C. C. Coupling of posterior cytoskeletal morphogenesis to the G1/S transition in the *Trypanosoma brucei* cell cycle. *Mol. Biol. Cell* **2005**, *16*, 97–105.

(51) Mottram, J. C.; McCready, B. P.; Brown, K. G.; Grant, K. M. Gene disruptions indicate an essential function for the LmmCRK1 cdc2-related kinase of *Leishmania mexicana*. *Mol. Microbiol*. **1996**, *22*, 573–583.

(52) Morgan, R. E.; Werbovetz, K. A. Selective lead compounds against kinetoplastid tubulin. *Adv. Exp. Med. Biol*. **2008**, *625*, 33–47.

(53) Ochola, D. O.; Prichard, R. K.; Lubega, G. W. Classical ligands bind tubulin of trypanosomes and inhibit their growth in vitro. *J. Parasitol*. **2002**, *88*, 600–604.

(54) Ojo, K. K.; Gillespie, J. R.; Riechers, A. J.; Napuli, A. J.; Verlinde, C. L.; Buckner, F. S.; Gelb, M. H.; Domostoj, M. M.; Wells, S. J.; Scheer, A.; Wells, T. N.; Van Voorhis, W. C. Glycogen synthase kinase 3 is a potential drug target for African trypanosomiasis therapy. *Antimicrob. Agents Chemother*. **2008**, *52*, 3710–3717.

(55) Cruz, A.; Beverley, S. M. Gene replacement in parasitic protozoa. *Nature* **1990**, *348*, 171–173.

(56) Cruz, A.; Coburn, C. M.; Beverley, S. M. Double targeted gene replacement for creating null mutants. *Proc. Natl. Acad. Sci. U.S. A*. **1991**, *88*, 7170–7174.

(57) Gueiros-Filho, F. J.; Beverley, S. M. Selection against the dihydrofolate reductase-thymidylate synthase (DHFR-TS) locus as a probe of genetic alterations in *Leishmania major*. *Mol. Cell. Biol*. **1996**, *16*, 5655–5663.

(58) Nare, B.; Hardy, L. W.; Beverley, S. M. The roles of pteridine reductase 1 and dihydrofolate reductase−thymidylate synthase in pteridine metabolism in the protozoan parasite *Leishmania major*. *J. Biol. Chem*. **1997**, *272*, 13883–13891.

(59) Hardy, L. W.; Matthews, W.; Nare, B.; Beverley, S. M. Biochemical and genetic tests for inhibitors of Leishmania pteridine pathways. *Exp. Parasitol*. **1997**, *87*, 157–169.

(60) Savoia, D.; Allice, T.; Tovo, P. A. Antileishmanial activity of HIV protease inhibitors. *Int. J. Antimicrob. Agents* **2005**, *26*, 92–94.

(61) Ascenzi, P.; Bocedi, A.; Gentile, M.; Visca, P.; Gradoni, L. Inactivation of parasite cysteine proteinases by the NO-donor 4-(phenylsulfonyl)-3-((2-(dimethylamino)ethyl)thio)-furoxan oxalate. *Biochim. Biophys. Acta* **2004**, *1703*, 69–77.

(62) St Hilaire, P. M.; Alves, L. C.; Herrera, F.; Renil, M.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Juliano, M. A.; Juliano, L.; Arevalo, J.; Meldal, M. Solid-phase library synthesis, screening, and selection of tight-binding reduced peptide bond inhibitors of a recombinant *Leishmania mexicana* cysteine protease B. *J. Med. Chem*. **2002**, *45*, 1971–1982.

(63) Ponte-Sucre, A.; Vicik, R.; Schultheis, M.; Schirmeister, T.; Moll, H. Aziridine-2,3-dicarboxylates, peptidomimetic cysteine protease inhibitors with antileishmanial activity. *Antimicrob. Agents Chemother*. **2006**, *50*, 2439–2447.

(64) McKerrow, J. H.; Rosenthal, P. J.; Swenerton, R.; Doyle, P. Development of protease inhibitors for protozoan infections. *Curr. Opin. Infect. Dis*. **2008**, *21*, 668–672.

(65) Mutomba, M. C.; To, W. Y.; Hyun, W. C.; Wang, C. C. Inhibition of proteasome activity blocks cell cycle progression at specific phase boundaries in African trypanosomes. *Mol. Biochem. Parasitol*. **1997**, *90*, 491–504.

(66) Nkemngu, N. J.; Rosenkranz, V.; Wink, M.; Steverding, D. Antitrypanosomal activities of proteasome inhibitors. *Antimicrob. Agents Chemother*. **2002**, *46*, 2038–2040.
(67) Glenn, R. J.; Pemberton, A. J.; Royle, H. J.; Spackman, R. W.; Smith, E.; Rivett, A. J.; Steverding, D. Trypanocidal effect of alpha′,beta′-epoxyketones indicates that trypanosomes are particularly sensitive to inhibitors of proteasome trypsin-like activity. *Int. J. Antimicrob. Agents* **2004**, *24*, 286–289.
(68) Caffrey, C. R.; Steverding, D. Recent initiatives and strategies to developing new drugs for tropical parasitic diseases. *Exp. Opin. Drug Discovery* **2008**, *3*, 173–186.
(69) Croft, S. L.; Sundar, S.; Fairlamb, A. H. Drug resistance in leishmaniasis. *Clin. Microbiol. Rev*. **2006**, *19*, 111–126.
(70) Martinelli, A.; Moreira, R.; Ravo, P. V. Malaria combination therapies: advantages and shortcomings. *Mini-Rev. Med. Chem* **2008**, *8*, 201–212.
(71) Kremsner, P. G.; Krishna, S. Antimalarial combinations. *Lancet* **2004**, *364*, 285–294.
(72) Smithuis, F.; Kyaw, M. K.; Phe, O.; Aye, K. Z.; Htet, L.; Barends, M.; Lindegardh, N.; Singtoroj, T.; Ashley, E.; Lwin, S.; Stepniewska, K.; White, N. J. Efficacy and effectiveness of dihydroartemisinin−piperaquine versus artesunate−mefloquine in falciparum malaria: an open-label randomised comparison. *Lancet* **2006**, *367*, 2075–2085.
(73) White, N. J.; Pongtavornpinyo, W. The de novo selection of drug-resistant malaria parasites. *Proc. Biol. Sci* **2003**, *270*, 545–554.
(74) Bryceson, A. A policy for leishmaniasis with respect to the prevention and control of drug resistance. *Trop. Med. Int. Health* **2001**, *6*, 928–934.
(75) Zimmermann, G. R.; Lehar, J.; Keith, C. T. Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discovery Today* **2007**, *12*, 34–42.
(76) Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem*. **2005**, *48*, 6523–6543.
(77) Shwe, T.; Lwin, M.; Aung, S. Influence of blister packaging on the efficacy of artesunate + mefloquine over artesunate alone in community-based treatment of non-severe falciparum malaria in Myanmar. *Bull. W. H. O*. **1998**, *76*, 35–41.
(78) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem*. **2008**, *51*, 347–372.
(79) Petrelli, A.; Giordano, S. From single- to multi-target drugs in cancer therapy: when aspecificity becomes an advantage. *Curr. Med. Chem*. **2008**, *15*, 422–432.
(80) Millan, M. J. Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol. Ther*. **2006**, *110*, 135–370.
(81) Morphy, R.; Kay, C.; Rankovic, Z. From magic bullets to designed multiple ligands. *Drug Discovery Today* **2004**, *9*, 641–651.
(82) Chibale, K. Towards broadspectrum antiprotozoal agents. *ARKIVOC* **2002**, *ix*, 93–98.
(83) Jenwitheesuk, E.; Horst, J. A.; Rivas, K. L.; Van Voorhis, W. C.; Samudrala, R. Novel paradigms for drug discovery: computational multitarget screening. *Trends Pharmacol. Sci*. **2008**, *29*, 62–71.
(84) Jenwitheesuk, E.; Samudrala, R. Identification of potential multitarget antimalarial drugs. *JAMA, J. Am. Med. Assoc*. **2005**, *294*, 1490–1491.
(85) Biot, C.; Chibale, K. Novel approaches to antimalarial drug discovery. *Infect. Disord. Drug Targets* **2006**, *6*, 173–204.
(86) Silver, L. L. Multi-targeting by monotherapeutic antibacterials. *Nat. Rev. Drug Discovery* **2007**, *6*, 41–55.
(87) Baiocco, P.; Colotti, G.; Franceschini, S.; Ilari, A. Molecular basis of antimony treatment in leishmaniasis. *J. Med. Chem*. **2009**, *52*, 2603–2612.
(88) Bolognesi, M. L.; Cavalli, A. Trypanothione reductase inhibitors as lead candidates to treat trypanosomatid diseases: recent advances and outlooks. *Curr. Trends Med. Chem* **2006**, *4*, 33–45.
(89) Hastings, I. M.; Watkins, W. M. Tolerance is the key to understanding antimalarial drug resistance. *Trends Parasitol*. **2006**, *22*, 71–77.
(90) Reddy, M.; Gill, S. S.; Kalkar, S. R.; Wu, W.; Anderson, P. J.; Rochon, P. A. Oral drug therapy for multiple neglected tropical diseases: a systematic review. *JAMA, J. Am. Med. Assoc*. **2007**, *298*, 1911–1924.
(91) Sarrassat, S.; Sakho, M.; Le Hesran, J. Y. Biological measure of compliance to artesunate plus amodiaquine association: interest in a mono-desethyl-amodiaquine blood assay?. *Acta Trop*. **2009**, *110*, 1–6.
(92) Barnes, K. I.; Watkins, W. M.; White, N. J. Antimalarial dosing regimens and drug resistance. *Trends Parasitol*. **2008**, *24*, 127–134.
(93) Arnaud, C. H. Drug hybrids enter the fray. *Chem. Eng. News* **2007**, *85*, 46–48.
(94) Walsh, J. J.; Coughlan, D.; Heneghan, N.; Gaynor, C.; Bell, A. A novel artemisinin−quinine hybrid with potent antimalarial activity. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 3599–3602.
(95) Meunier, B. Hybrid molecules with a dual mode of action: dream or reality? *Acc. Chem. Res*. **2008**, *41*, 69–77.
(96) Cosledan, F.; Fraisse, L.; Pellet, A.; Guillou, F.; Mordmuller, B.; Kremsner, P. G.; Moreno, A.; Mazier, D.; Maffrand, J. P.; Meunier, B. Selection of a trioxaquine as an antimalarial drug candidate. *Proc. Natl. Acad. Sci. U.S.A*. **2008**, *105*, 17579–17584.
(97) Burgess, S. J.; Selzer, A.; Kelly, J. X.; Smilkstein, M. J.; Riscoe, M. K.; Peyton, D. H. A chloroquine-like molecule designed to reverse resistance in *Plasmodium falciparum*. *J. Med. Chem*. **2006**, *49*, 5623–5625.
(98) Opsenica, I.; Opsenica, D.; Lanteri, C. A.; Anova, L.; Milhous, W. K.; Smith, K. S.; Solaja, B. A. New chimeric antimalarials with 4-aminoquinoline moiety linked to a tetraoxane skeleton. *J. Med. Chem*. **2008**, *51*, 6216–6219.
(99) Musonda, C. C.; Whitlock, G. A.; Witty, M. J.; Brun, R.; Kaiser, M. Chloroquine−astemizole hybrids with potent in vitro and in vivo antiplasmodial activity. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 481–484.
(100) Gemma, S.; Campiani, G.; Butini, S.; Joshi, B. P.; Kukreja, G.; Coccone, S. S.; Bernetti, M.; Persico, M.; Nacci, V.; Fiorini, I.; Novellino, E.; Taramelli, D.; Basilico, N.; Parapini, S.; Yardley, V.; Croft, S.; Keller-Maerki, S.; Rottmann, M.; Brun, R.; Coletta, M.; Marini, S.; Guiso, G.; Caccia, S.; Fattorusso, C. Combining 4-aminoquinoline- and clotrimazole-based pharmacophores toward innovative and potent hybrid antimalarials. *J. Med. Chem*. **2009**, *52*, 502–513.
(101) Muhanji, C. I.; Hunter, R. Current developments in the synthesis and biological activity of HIV-1 double-drug inhibitors. *Curr. Med. Chem*. **2007**, *14*, 1207–1220.
(102) Bremner, J. B.; Ambrus, J. I.; Samosorn, S. Dual action-based approaches to antibacterial agents. *Curr. Med. Chem*. **2007**, *14*, 1459–1477.
(103) Viegas-Junior, C.; Danuello, A.; da Silva Bolzani, V.; Barreiro, E. J.; Fraga, C. A. Molecular hybridization: a useful tool in the design of new drug prototypes. *Curr. Med. Chem*. **2007**, *14*, 1829–1852.
(104) Prata, A. Clinical and epidemiological aspects of Chagas disease. *Lancet Infect. Dis*. **2001**, *1*, 92–100.
(105) Recommendations from a satellite meeting. *Mem. Inst. Oswaldo Cruz* **1999**, *94* (Suppl. 1), 429−432.
(106) Schofield, C. J.; Dujardin, J. P. Chagas disease vector control in Central America. *Parasitol. Today* **1997**, *13*, 141–144.
(107) Rabinovich, J. E.; Wisnivesky-Colli, C.; Solarz, N. D.; Gurtler, R. E. Probability of transmission of Chagas disease by *Triatoma infestans* (Hemiptera: Reduviidae) in an endemic area of Santiago del Estero, Argentina. *Bull. W. H. O*. **1990**, *68*, 737–746.
(108) www.who.int/tdr.
(109) Gelb, M. H.; Hol, W. G. Parasitology. Drugs to combat tropical protozoan parasites. *Science* **2002**, *297*, 343–344.
(110) Urbina, J. A.; Docampo, R. Specific chemotherapy of Chagas disease: controversies and advances. *Trends Parasitol*. **2003**, *19*, 495–501.
(111) Schofield, C. J.; Jannin, J.; Salvatella, R. The future of Chagas disease control. *Trends Parasitol*. **2006**, *22*, 583–588.
(112) Viotti, R.; Vigliano, C.; Lococo, B.; Alvarez, M. G.; Petti, M.; Bertocchi, G.; Armenti, A. Side effects of benznidazole as treatment in chronic Chagas disease: fears and realities. *Expert Rev. Anti-Infect. Ther* **2009**, *7*, 157–163.
(113) Jannin, J.; Villa, L. An overview of Chagas disease treatment. *Mem. Inst. Oswaldo Cruz* **2007**, *102* (Suppl. 1), 95–97.
(114) Wilkinson, S. R.; Taylor, M. C.; Horn, D.; Kelly, J. M.; Cheeseman, I. A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes. *Proc. Natl. Acad. Sci. U.S.A*. **2008**, *105*, 5022–5027.
(115) McCalla, D. R.; Reuvers, A.; Kaiser, C. Breakage of bacterial DNA by nitrofuran derivatives. *Cancer Res*. **1971**, *31*, 2184–2188.
(116) Streeter, A. J.; Hoener, B. A. Evidence for the involvement of a nitrenium ion in the covalent binding of nitrofurazone to DNA. *Pharm. Res*. **1988**, *5*, 434–436.
(117) Lazardi, K.; Urbina, J. A.; de Souza, W. Ultrastructural alterations induced by two ergosterol biosynthesis inhibitors, ketoconazole and terbinafine, on epimastigotes and amastigotes of *Trypanosoma* (*Schizotrypanum*) *cruzi*. *Antimicrob. Agents Chemother*. **1990**, *34*, 2097–2105.
(118) Maldonado, R. A.; Molina, J.; Payares, G.; Urbina, J. A. Experimental chemotherapy with combinations of ergosterol

biosynthesis inhibitors in murine models of Chagas' disease. *Antimicrob. Agents Chemother*. **1993**, *37*, 1353–1359.
(119) Carvalho, S. A.; da Silva, E. F.; Santa-Rita, R. M.; de Castro, S. L.; Fraga, C. A. Synthesis and antitrypanosomal profile of new functionalized 1,3,4-thiadiazole-2-arylhydrazone derivatives, designed as non-mutagenic megazol analogues. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 5967–5970.
(120) Carvalho, S. A.; Lopes, F. A.; Salomao, K.; Romeiro, N. C.; Wardell, S. M.; de Castro, S. L.; da Silva, E. F.; Fraga, C. A. Studies toward the structural optimization of new brazilizone-related trypanocidal 1,3,4-thiadiazole-2-arylhydrazone derivatives. *Bioorg. Med. Chem*. **2008**, *16*, 413–421.
(121) Porcal, W.; Hernandez, P.; Boiani, L.; Boiani, M.; Ferreira, A.; Chidichimo, A.; Cazzulo, J. J.; Olea-Azar, C.; González, M.; Cerecetto, H. New trypanocidal hybrid compounds from the association of hydrazone moieties and benzofuroxan heterocycle. *Bioorg. Med. Chem*. **2008**, *16*, 6995–7004.
(122) Cerecetto, H.; Porcal, W. Pharmacological properties of furoxans and benzofuroxans: recent developments. *Mini-Rev. Med. Chem* **2005**, *5*, 57–71.
(123) Gerpe, A.; Odreman-Nunez, I.; Draper, P.; Boiani, L.; Urbina, J. A.; González, M.; Cerecetto, H. Heteroallyl-containing 5-nitrofuranes as new anti-*Trypanosoma cruzi* agents with a dual mechanism of action. *Bioorg. Med. Chem*. **2008**, *16*, 569–577.
(124) da Silva, E. N., ; Menna-Barreto, R. F.; Pinto Mdo, C.; Silva, R. S.; Teixeira, D. V.; de Souza, M. C.; De Simone, C. A.; De Castro, S. L.; Ferreira, V. F.; Pinto, A. V. Naphthoquinoidal [1,2,3]-triazole, a new structural moiety active against *Trypanosoma cruzi*. *Eur. J. Med. Chem*. **2008**, *43*, 1774–1780.
(125) Pinto, A. V.; Menna-Barreto, R. F. S.; De Castro, S. L. Naphthoquinones Isolated from Tabebuia: A Review about the Synthesis of Heterocyclic Derivatives, Screening against *Trypanosoma cruzi* and Correlation Structure−Trypanocidal Activity. In Recent Progress in Medicinal Plants; Govil, J. V., Ed.; Studium Press: Houston, TX, 2007; Vol. 16, pp 112−127.
(126) Menna-Barreto, R. F.; Salomao, K.; Dantas, A. P.; Santa-Rita, R. M.; Soares, M. J.; Barbosa, H. S.; de Castro, S. L. Different cell death pathways induced by drugs in *Trypanosoma cruzi*: an ultrastructural study. *Micron* **2009**, *40*, 157–168.
(127) Aperis, G.; Mylonakis, E. Newer triazole antifungal agents: pharmacology, spectrum, clinical efficacy and limitations. *Expert Opin. Invest. Drugs* **2006**, *15*, 579–602.
(128) da Silva Junior, E. N.; de Souza, M. C.; Fernandes, M. C.; Menna-Barreto, R. F.; Pinto Mdo, C.; de Assis Lopes, F.; de Simone, C. A.; Andrade, C. K.; Pinto, A. V.; Ferreira, V. F.; de Castro, S. L. Synthesis and anti-*Trypanosoma cruzi* activity of derivatives from nor-lapachones and lapachones. *Bioorg. Med. Chem*. **2008**, *16*, 5030–5038.
(129) Sánchez-Delgado, R. A.; Anzellotti, A. Metal complexes as chemotherapeutic agents against tropical diseases: trypanosomiasis, malaria and leishmaniasis. *Mini-Rev. Med. Chem* **2004**, *4*, 23–30.
(130) Vieites, M.; Otero, L.; Santos, D.; Toloza, J.; Figueroa, R.; Norambuena, E.; Olea-Azar, C.; Aguirre, G.; Cerecetto, H.; González, M.; Morello, A.; Maya, J. D.; Garat, B.; Gambino, D. Platinum(II) metal complexes as potential anti-*Trypanosoma cruzi* agents. *J. Inorg. Biochem*. **2008**, *102*, 1033–1043.
(131) Vieites, M.; Otero, L.; Santos, D.; Olea-Azar, C.; Norambuena, E.; Aguirre, G.; Cerecetto, H.; González, M.; Kemmerling, U.; Morello, A.; Diego Maya, J.; Gambino, D. Platinum-based complexes of bioactive 3-(5-nitrofuryl)acroleine thiosemicarbazones showing anti-*Trypanosoma cruzi* activity. *J. Inorg. Biochem*. **2009**, *103*, 411–418.
(132) Vieites, M.; Smircich, P.; Parajon-Costa, B.; Rodríguez, J.; Galaz, V.; Olea-Azar, C.; Otero, L.; Aguirre, G.; Cerecetto, H.; González, M.; Gomez-Barrio, A.; Garat, B.; Gambino, D. Potent in vitro anti-*Trypanosoma cruzi* activity of pyridine-2-thiol *N*-oxide metal complexes having an inhibitory effect on parasite-specific fumarate reductase. *J. Biol. Inorg. Chem*. **2008**, *13*, 723–735.
(133) Otero, L.; Vieites, M.; Boiani, L.; Denicola, A.; Rigol, C.; Opazo, L.; Olea-Azar, C.; Maya, J. D.; Morello, A.; Krauth-Siegel, R. L.; Piro, O. E.; Castellano, E.; González, M.; Gambino, D.; Cerecetto, H. Novel antitrypanosomal agents based on palladium nitrofurylthiosemicarbazone complexes: DNA and redox metabolism as potential therapeutic targets. *J. Med. Chem*. **2006**, *49*, 3322–3331.
(134) Legros, D.; Ollivier, G.; Gastellu-Etchegorry, M.; Paquet, C.; Burri, C.; Jannin, J.; Buscher, P. Treatment of human African trypanosomiasis—present situation and needs for research and development. *Lancet Infect. Dis*. **2002**, *2*, 437–440.
(135) Odiit, M.; Kansiime, F.; Enyaru, J. C. Duration of symptoms and case fatality of sleeping sickness caused by *Trypanosoma brucei rhodesiense* in Tororo, Uganda. *East Afr. Med. J* **1997**, *74*, 792–795.
(136) Welburn, S. C.; Fevre, E. M.; Coleman, P. G.; Odiit, M.; Maudlin, I. Sleeping sickness: a tale of two diseases. *Trends Parasitol*. **2001**, *17*, 19–24.
(137) Delespaux, V.; de Koning, H. P. Drugs and drug resistance in African trypanosomiasis. *Drug Resist. Updates* **2007**, *10*, 30–50.
(138) D'Silva, C. Human African trypanosomiasis: future prospects for chemotherapy. *Drugs Future* **2002**, *32*, 149–160.
(139) Barrett, M. P.; Boykin, D. W.; Brun, R.; Tidwell, R. R. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *Br. J. Pharmacol*. **2007**, *152*, 1155–1171.
(140) Pepin, J.; Milord, F. The treatment of human African trypanosomiasis. *Adv. Parasitol*. **1994**, *33*, 1–47.
(141) Wang, C. C. Molecular mechanisms and therapeutic approaches to the treatment of African trypanosomiasis. *Annu. Rev. Pharmacol. Toxicol*. **1995**, *35*, 93–127.
(142) Vansterkenburg, E. L.; Coppens, I.; Wilting, J.; Bos, O. J.; Fischer, M. J.; Janssen, L. H.; Opperdoes, F. R. The uptake of the trypanocidal drug suramin in combination with low-density lipoproteins by *Trypanosoma brucei* and its possible mode of action. *Acta Trop*. **1993**, *54*, 237–250.
(143) Werbovetz, K. Diamidines as antitrypanosomal, antileishmanial and antimalarial agents. *Curr. Opin. Invest. Drugs* **2006**, *7*, 147–157.
(144) Friedheim, E. A. Mel B in the treatment of human trypanosomiasis. *Am. J. Trop. Med. Hyg*. **1949**, *29*, 173–180.
(145) Brun, R.; Schumacher, R.; Schmid, C.; Kunz, C.; Burri, C. The phenomenon of treatment failures in human African trypanosomiasis. *Trop. Med. Int. Health* **2001**, *6*, 906–914.
(146) Balasegaram, M.; Harris, S.; Checchi, F.; Hamel, C.; Karunakara, U. Treatment outcomes and risk factors for relapse in patients with early-stage human African trypanosomiasis (HAT) in the Republic of the Congo. *Bull. W. H. O*. **2006**, *84*, 777–782.
(147) Fairlamb, A. H. Chemotherapy of human African trypanosomiasis: current and future prospects. *Trends Parasitol*. **2003**, *19*, 488–494.
(148) Nok, A. J. Effective measures for controlling trypanosomiasis. *Expert Opin. Pharmacother*. **2005**, *6*, 2645–2653.
(149) Ansede, J. H.; Anbazhagan, M.; Brun, R.; Easterbrook, J. D.; Hall, J. E.; Boykin, D. W. *O*-Alkoxyamidine prodrugs of furamidine: in vitro transport and microsomal metabolism as indicators of in vivo efficacy in a mouse model of *Trypanosoma brucei rhodesiense* infection. *J. Med. Chem*. **2004**, *47*, 4335–4338.
(150) Mdachi, R. E.; Thuita, J. K.; Kagira, J. M.; Ngotho, J. M.; Murilla, G. A.; Ndung'u, J. M.; Tidwell, R. R.; Hall, J. E.; Brun, R. Efficacy of the novel diamidine compound 2,5-bis(4-amidinophenyl)-furan-bis-*O*-methlylamidoxime (Pafuramidine, DB289) against *Trypanosoma brucei rhodesiense* infection in vervet monkeys after oral administration. *Antimicrob. Agents Chemother*. **2009**, *53*, 953–957.
(151) Chen, D.; Marsh, R.; Aberg, J. A. Pafuramidine for *Pneumocystis jiroveci* pneumonia in HIV-infected individuals. *Expert Rev. Anti-Infect. Ther*. **2007**, *5*, 921–928.
(152) Croft, S. L. Kinetoplastida: new therapeutic strategies. *Parasite* **2008**, *15*, 522–527.
(153) Bressi, J. C.; Choe, J.; Hough, M. T.; Buckner, F. S.; Van Voorhis, W. C.; Verlinde, C. L.; Hol, W. G.; Gelb, M. H. Adenosine analogues as inhibitors of *Trypanosoma brucei* phosphoglycerate kinase: elucidation of a novel binding mode for a 2-amino-N(6)-substituted adenosine. *J. Med. Chem*. **2000**, *43*, 4135–4150.
(154) Bolognesi, M. L.; Lizzi, F.; Perozzo, R.; Brun, R.; Cavalli, A. Synthesis of a small library of 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-anthraquinone derivatives bearing anti-trypanosomal and anti-leishmanial activity. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 2272–2276.
(155) Breinbauer, R.; Vetter, I. R.; Waldmann, H. From protein domains to drug candidates—natural products as guiding principles in the design and synthesis of compound libraries. *Angew. Chem., Int. Ed*. **2002**, *41*, 2879–2890.
(156) Kayser, O.; Kiderlen, A. F.; Croft, S. L. Natural products as antiparasitic drugs. *Parasitol. Res*. **2003**, *90* (Suppl. 2), S55–S62.
(157) Wink, M. Ecological Roles of Alkaloids. In Modern Alkaloids: Structure, Isolation, Synthesis and Biology; Fattorusso, E., Taglialatela-Scafati, O., Eds.; Wiley-VCH: Weinheim, Germany, 2008; pp 1−24.
(158) Sundar, S.; Rai, M. Advances in the treatment of leishmaniasis. *Curr. Opin. Infect. Dis*. **2002**, *15*, 593–598.

(159) Zijlstra, E. E.; Musa, A. M.; Khalil, E. A.; el-Hassan, I. M.; el-Hassan, A. M. Post-kala-azar dermal leishmaniasis. *Lancet Infect. Dis.* **2003**, *3*, 87–98.
(160) Murray, H. W.; Berman, J. D.; Davies, C. R.; Saravia, N. G. Advances in leishmaniasis. *Lancet* **2005**, *366*, 1561–1577.
(161) Le Pape, P. Development of new antileishmanial drugs—current knowledge and future prospects. *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 708–718.
(162) Polonio, T.; Efferth, T. Leishmaniasis: drug resistance and natural products (review). *Int. J. Mol. Med.* **2008**, *22*, 277–286.
(163) Maltezou, H. C. Visceral leishmaniasis: advances in treatment. *Recent Pat. Anti-Infect. Drug Discovery* **2008**, *3*, 192–198.
(164) Golenser, J.; Domb, A. New formulations and derivatives of amphotericin B for treatment of leishmaniasis. *Mini-Rev. Med. Chem.* **2006**, *6*, 153–162.
(165) Berman, J. J. Treatment of leishmaniasis with miltefosine: 2008 status. *Expert Opin. Drug. Metab. Toxicol.* **2008**, *4*, 1209–1216.
(166) Berman, J. D. Development of miltefosine for the leishmaniases. *Mini-Rev. Med. Chem.* **2006**, *6*, 145–151.
(167) Sundar, S.; Rai, M.; Chakravarty, J.; Agarwal, D.; Agrawal, N.; Vaillant, M.; Olliaro, P.; Murray, H. W. New treatment approach in Indian visceral leishmaniasis: single-dose liposomal amphotericin B followed by short-course oral miltefosine. *Clin. Infect. Dis.* **2008**, *47*, 1000–1006.
(168) Chappuis, F.; Sundar, S.; Hailu, A.; Ghalib, H.; Rijal, S.; Peeling, R. W.; Alvar, J.; Boelaert, M. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control?. *Nat. Rev. Microbiol.* **2007**, *5*, 873–882.
(169) Yeates, C. (GlaxoSmithKline/Walter Reed Army Institute) Sitamaquine. *Curr. Opin. Invest. Drugs* **2002**, *3*, 1446−1452
(170) Jha, T. K.; Sundar, S.; Thakur, C. P.; Felton, J. M.; Sabin, A. J.; Horton, J. A phase II dose-ranging study of sitamaquine for the treatment of visceral leishmaniasis in India. *Am. J. Trop. Med. Hyg.* **2005**, *73*, 1005–1011.
(171) Wasunna, M. K.; Rashid, J. R.; Mbui, J.; Kirigi, G.; Kinoti, D.; Lodenyo, H.; Felton, J. M.; Sabin, A. J.; Albert, M. J.; Horton, J. A phase II dose-increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. *Am. J. Trop. Med. Hyg.* **2005**, *73*, 871–876.
(172) Dodd, R. H.; Ouannes, C.; Robert-Gero, M.; Potier, P. Hybrid molecules: growth inhibition of *Leishmania donovani* promastigotes by thiosemicarbazones of 3-carboxy-beta-carbolines. *J. Med. Chem.* **1989**, *32*, 1272–1276.
(173) Di Giorgio, C.; Delmas, F.; Filloux, N.; Robin, M.; Seferian, L.; Azas, N.; Gasquet, M.; Costa, M.; Timon-David, P.; Galy, J. P. In vitro activities of 7-substituted 9-chloro and 9-amino-2-methoxyacridines and their bis- and tetra-acridine complexes against *Leishmania infantum*. *Antimicrob. Agents Chemother.* **2003**, *47*, 174–180.
(174) Visbal, G.; Marchan, E.; Maldonado, A.; Simoni, Z.; Navarro, M. Synthesis and characterization of platinum−sterol hydrazone complexes with biological activity against *Leishmania* (*L.*) *mexicana*. *J. Inorg. Biochem.* **2008**, *102*, 547–554.
(175) Rodrigues, J. C.; Bernardes, C. F.; Visbal, G.; Urbina, J. A.; Vercesi, A. E.; de Souza, W. Sterol methenyl transferase inhibitors alter the ultrastructure and function of the *Leishmania amazonensis* mitochondrion leading to potent growth inhibition. *Protist* **2007**, *158*, 447–456.
(176) Lowe, G.; Droz, A. S.; Vilaivan, T.; Weaver, G. W.; Tweedale, L.; Pratt, J. M.; Rock, P.; Yardley, V.; Croft, S. L. Cytotoxicity of (2,2′:6′,2″-terpyridine)platinum(II) complexes to *Leishmania donovani*, *Trypanosoma cruzi*, and *Trypanosoma brucei*. *J. Med. Chem.* **1999**, *42*, 999–1006.
(177) White, N. J. Delaying antimalarial drug resistance with combination chemotherapy. *Parassitologia* **1999**, *41*, 301–308.
(178) Stark, H. Turning from monogamy to strategic promiscuity. *Drug Discovery Today* **2004**, *9*, 736–737.
(179) Hopkins, A. L. Network pharmacology: the next paradigm in drug discovery. *Nat. Chem. Biol.* **2008**, *4*, 682–690.
(180) Barabasi, A. L.; Oltvai, Z. N. Network biology: understanding the cell's functional organization. *Nat. Rev. Genet.* **2004**, *5*, 101–113.
(181) Ameisen, J. C. On the origin, evolution, and nature of programmed cell death: a timeline of four billion years. *Cell Death Differ.* **2002**, *9*, 367–393.