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Metal- and metalloid-containing drugs for the treatment of trypanosomatid diseases

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1. ABSTRACT

The trypanosomatid-induced diseases are considered as neglected, because the countries where they kill people are not important markets for western big pharmaceutical companies. However, recently some effort has been made to translate the use of already known drugs to neglected infectious disease. Although many metals are essential to life, many disorders affecting metal homeostasis and bioavailability are responsible for several human diseases. Metals can be toxic even at very low concentrations and semimetals are classified as toxic and dangerous for the environment. However, metal- and metalloid-based therapeutic drugs have existed for centuries. Some of them, as antimony and arsenic compounds, are still the first line drugs used for the treatment of leishmaniasis and trypanosomiases in developing countries. Other metal complexes (as those of Ag, Pt, Pd and Au), already present in the market for cancer therapies or to cure bacterial infection or for anti-inflammatory treatments, have been proposed also against the vector-borne infections caused by trypanosomatids. The use of novel approaches based on nanotechnologies, allowing selective targeting, may represent a promising strategy to decrease the toxicity of these drugs.

2. INTRODUCTION

Leishmaniasis, Chagas disease and HAT (Human African Trypanosomiasis) are vector-borne diseases caused by trypanosomatids. These are poverty-related diseases characterised by high morbidity deeply linked to malnutrition, complex humanitarian emergencies and environmental changes that affect vector biology (1). Leishmaniasis is caused by 20 species of *Leishmania* parasites and comprises three major clinical forms, namely visceral leishmaniasis, caused by *Leishmania (L.) donovani and L. infantum*, manifesting itself with fever, low red blood cell counts, enlarged spleen and liver and leads to death if left untreated; cutaneous leishmaniasis (caused by *L. major* and other parasite species), with skin ulcers that can heal spontaneously, often leaving disfiguring scars; mucocutaneous leishmaniasis, causing ulcers of the skin, mouth, and nose (2). Leishmaniasis is widespread in 22 countries in the New World and in 66 countries in the Old World and endemic regions have been spreading further over the last 10 years with a sharp increase in the number of recorded cases. 2 million new cases are considered to occur annually, with an estimated 12 million people presently infected worldwide (3, 4). Chagas disease, also known as American trypanosomiasis, is a potentially

life-threatening illness caused by *Trypanosoma (T.) cruzi* affecting about 8 million people in Latin America, of whom 30–40% either have or will develop cardiomyopathy, digestive megasyndromes, or both (5). Human African Trypanosomiasis (HAT), also known as sleeping sickness, takes two forms, depending on the parasite involved, *T. brucei gambiense* found in western and central Africa, while *T. brucei rhodesiense* is found in eastern and southern Africa. HAT leads to a debilitating and progressive neurological disease that includes sleep disorders, coma and death in all untreated individuals. This killer disease is endemic to several countries, putting millions of people at risk with around 10,000 new cases developing every year (6).

Despite these figures, the therapy of infectious diseases caused by protozoan parasites of the trypanosomatid family is a neglected area of research and drug development. Moreover, the treatments of these diseases are unsatisfactory in terms of safety and efficacy, which sharply contrasts with the therapeutic need in terms of people at risk, number of affected patients, and associated fatalities. This phenomenon is due to the prevalence of these diseases in tropical and subtropical poor countries with little research capacities and that are not attractive markets for the western big pharmaceutical companies. As a consequence, efficacious new drugs have not been developed, and the most used ones are metalloid-based drugs as arsenicals (Melarsoprol), used to treat the second stage of the African trypanosomiasis, and antimony-containing compounds (sodium stibogluconate or meglumine antimoniate) used against Leishmaniasis (3).

The use of metal and metalloid-containing compounds in medicine dates back millennia: as an example, copper was used by Egyptians and gold was utilized by Chinese and Arabic practitioners. Hippocrates (400 BC) employed copper and mercury in the treatment of diseases, while Paracelsus (XVI century) used Sb, As and Mg salts. More recently, $K(Au(CN)_2)$ has been used against tuberculosis, while arsenic-based Salvarsan has been considered to be the mainstay for the treatment of syphilis for the first decades of the XX century (7). However, the potential of metal-based compounds has been fully appreciated only after the discovery of the anticancer activity of cisplatin, which has gone on to revolutionize cancer treatment rendering formerly fatal diseases such as testicular cancer, largely curable (8, 9). At present, metal- and metalloid-containing compounds are in use against several diseases, and are increasingly being studied by modern medicinal chemistry. Metals display unique features, including a wide range of redox states, charges, coordination geometries, thermodynamic and kinetic properties, which can empower the drug efficacy and therefore can be exploited in the design of metal-based compounds alternative to fully organic molecules (10, 11). Many metals are essential to life, and many disorders affecting metal homeostasis and bioavailability are responsible for several human diseases. However, metals can be toxic also at very low concentrations and metalloids are generally classified as poisons. Obviously, as already stated by Paracelsus, the right dose differentiates a poison from a medicine. Thus, the present challenge is to design active formulations for effective drug delivery minimizing the toxicity of metal- and metalloid-based drugs and as a consequence the side effects caused by the use of these drugs (12).

The aim of this review is to discuss the use of metal- and metalloid- based drugs against neglected tropical diseases and to discuss the recent studies that allowed the identification of new metal and metalloid based molecules which offer the opportunity to develop new and less toxic drugs against leishmaniasis and trypanosomiases.

3. METALLOID-BASED COMPOUNDS AGAINST NEGLECTED INFECTIOUS DISEASES

3.1. Boron-based compounds

Boron-based drugs are used against a number of infectious diseases: the polyether-macrolide antibiotic, boromycin ($C_{45}H_{74}BNO_{15}$) is active against both gram-positive and gram-negative bacteria and was demonstrated to have a potent anti-HIV activity (13); the boric acid is a weak acid and has antiseptic, antifungal, and antiviral properties (14). Moreover, boron-based drugs have been used against different types of cancer. Indeed, it has been discovered that the chemotherapeutic agent Bortezomib (Molecular formula $C_{19}H_{25}BN_4O_4$) induces apoptosis in tumour cells *in vitro*, including those resistant to conventional chemotherapeutic agents and the first FDA approved therapeutic inhibitor of the 26S proteasome Velcade (bortezomib), is used to treat multiple myeloma and has reached FDA approval also to treat relapsed multiple myeloma (15). Boron-based drugs exhibit attractive properties and activities against a number of protozoans contributing to neglected tropical diseases. Recently, benzoxaboroles have been found to be effective against human African trypanosomiasis, malaria and Chagas disease (16). In particular, the presence of a boron atom in the heterocyclic core structure has been found essential for trypanocidal activity of orally active series of benzoxaborole-6-carboxamides in murine models of human African trypanosomiasis. The target of these class of compounds is still unknown but inhibition studies on *T. brucei* growth show that one of these compounds (SCYX-7158) (Figure 1) possesses the ability to kill efficiently the parasite (IC_{50}=1.1.0±0.2.2 μM) (17). SCYX-7158 (4-fluoro-N-(1-hydroxy-3,3-dimethyl-2,1-benzoxaborol-6-yl)-2-(trifluoromethyl)

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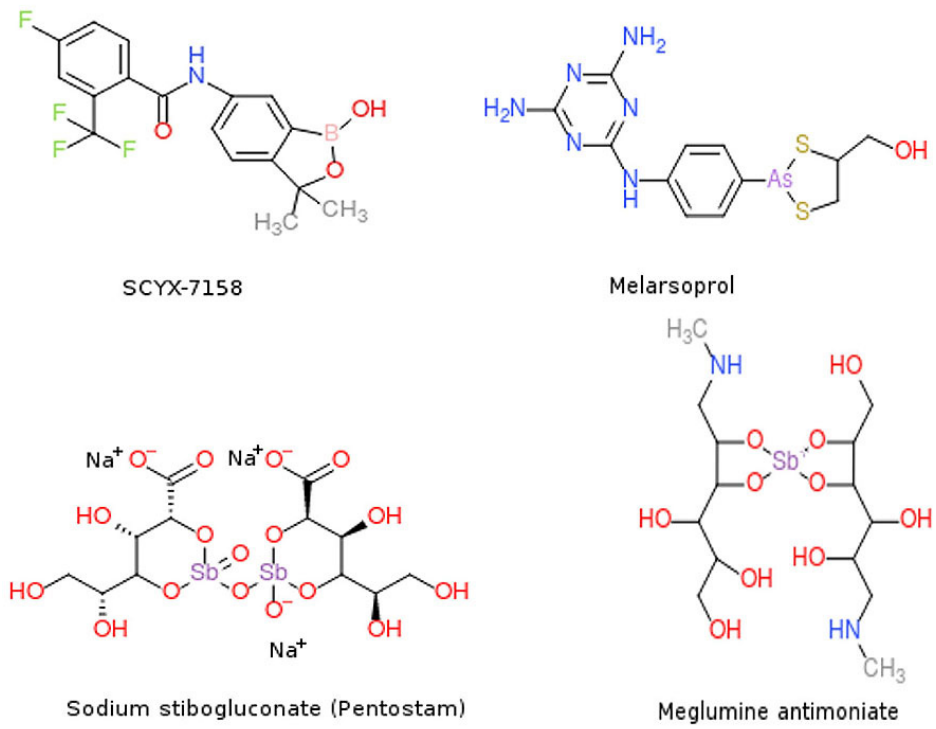

Figure 1. Metalloid-containing compounds used (or under clinical evaluation) against diseases caused by trypanosomatids. The compounds were designed using Chemdoodle (https://web.chemdoodle.com/demos/sketcher/). The picture was generated by Gimp (https://www.gimp.org/).

benzamide) has been identified as an effective, safe and orally active treatment for HAT; a phase I human clinical trial was concluded successfully in 2015 and now the compound is in phase IIb/III of clinical trials in the Democratic Republic of Congo.

The benzoxaborole scaffold was also used to explore the possibility to find new therapies against Chagas disease: compounds based on the 6-amino oxaborole scaffold elaborated vs. HAT show IC_{50} values in the micromolar range. The HAT preclinical candidate most active against *T. cruzi* was found to be SCYX-6759 (IC_{50}=1.1.5 μM) (16).

3.2. Antimony-based compounds

Antimonials have been used against neglected tropical diseases since long time. Indeed, the use of antimony against leishmaniasis and schistosomiasis dates back to 14th century when the alchemist John of Rupescissa used it to treat his patients. The treatment of leishmaniasis with potassium antimony (III) tartrate (tartar-emetic) started in 1913. However, due to the discover of new, more efficacious and less toxic drugs as pentavalent antimonials for the treatment of leishmaniasis and the subsequent larger use of praziquantel against schistosomiasis, the use of trivalent antimonials was phased out in the 1970s.

At the present, the most used drugs against leishmaniasis are still the pentavalent antimonials (i.e. complex of Sb(V) with N-methyl-D-glucamine (meglumine antimoniate or Glucantime®) or sodium gluconate (sodium stibogluconate or Pentostam®) (Figure 1) (18).

Pentavalent antimonials are prodrugs: indeed Sb(V) is reduced to the more effective Sb(III) preferentially in the amastigotes, which have a lower intracellular pH and live at a higher temperature than promastigotes. Recent studies have suggested the participation of parasite-specific enzymes in the process of reduction of Sb(V) to Sb(III), as thiol-dependent reductase (TDR1) and LmACR2 (19, 20).

One of the mechanisms of action of antimonial drugs is the inhibition of trypanothione reductase (TR), the enzyme that keeps reduced the trypanothione molecule ($T(SH)_2$) which allows the parasite to neutralize the ROS (reactive oxygen species) produced by macrophage during the infection. TR is an enzyme essential for the parasite survival inside the human host and for this reason is a good target to find drugs against leishmaniasis. Once reduced to Sb(III), the metalloid is able to inhibit TR with high efficiency (Ki=1.5. ± 0.4. μM). Based on the structural analysis of the TR complex with NADPH and Sb(III) in the reduced state, it was discovered that the

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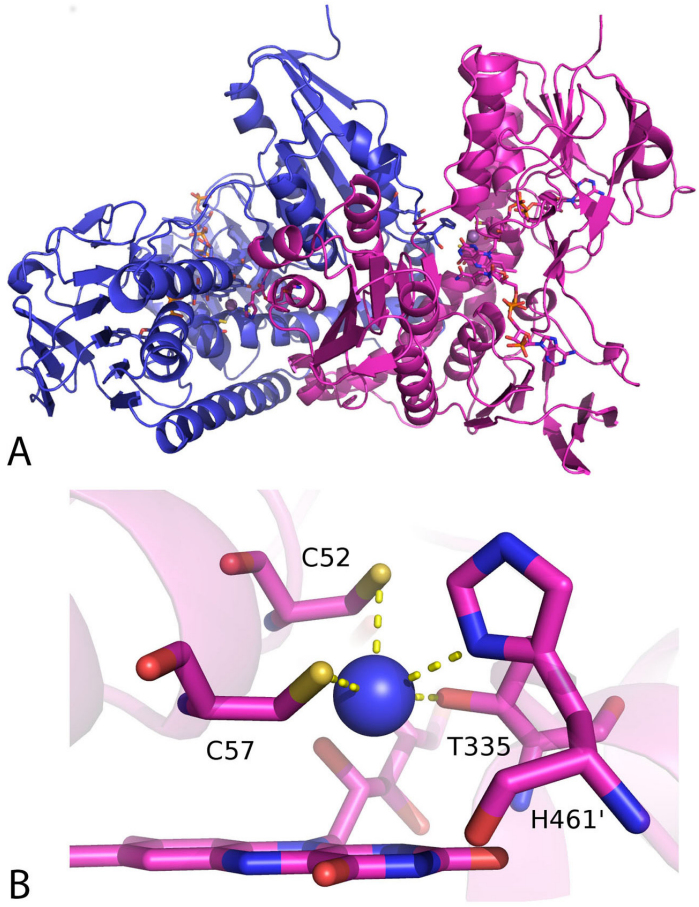

Figure 2. X-ray structure of trypanothione reductase (TR) of *Leishmania infantum* in complex with Sb(III), in the reduced state (PDB code 2W0H). A. Overall fold. The two monomers of the homodimer are colored in violet and blue. B. Sb(III) binding site. The Sb(III) ion is depicted as a blue sphere and the residues engaging Sb(III) in a stable complex formation are indicated as sticks. The picture was generated using PyMOL (The PyMOL Molecular Graphics System, Version 1.8. Schrödinger, LLC.)

trivalent Sb(III) ion is able to inhibit the enzyme activity by engaging in a stable complex the residues involved in catalysis, namely the two catalytic cysteines (Cys52 and Cys57), His461' of the two-fold symmetry-related subunit (the residue that together with Glu466' activates the Cys52 similarly to the cysteine proteases) and Thr335 (Figure 2). Binding of Sb(III) is only possible upon enzyme reduction because in the oxidized enzyme the two cysteine residues form a disulfide bridge (21, 22). Even if Sb(V) is less toxic than Sb(III), the pentavalent antimonials display severe side effects (see Table 1). Moreover, acquired resistance to antimonials has become a clinical threat during the last years especially in endemic countries as Bihar (India). To overcome the problem of drug resistance in Bihar the dosage of 10 mg/kg/day used with cure rates >90% in the early 20th century was doubled in the 1970's when treatment failure reached 30% (23). In 2005, when the treatment failure reached 65% in Bihar and 24% in Nepal, the use of antimonial drugs was abandoned in the Indian subcontinent. Drug resistance development is due to different factors, which have created condition for a long and strong selective pressure: *i.* the hyper endemicity, with a cycle of epidemics every 15 years; *ii.* the anthroponotic nature of *L. donovani* transmission; *iii.* the variable dose and quality of antimonial drugs used; *iv.* socioeconomic factors as poverty and malnutrition; *v.* the arsenic contamination in the drinking water, quite common in Bihar (24). The main molecular mechanisms beyond the parasite resistance are: *i.* the intrachromosomal amplification of the H locus, containing among other the gene codifying for MRPA transporter, an ATP binding cassette family member, which binds the Sb(III) thiol complexes and transports them outside the cell (25); *ii.* the abolished functionality of the AQP1 transporter responsible for the Sb(III) influx (26); *iii.* increase of surface N-acetyl-D-galactosamine leading to the overexpression of the macrophage's MRPA and a consequent increased drug efflux (27); *iv.* a higher fitness of the resistant parasites also in the absence of drugs (28).

However, recent studies have shown that some peroxo-vanadate complexes used as antileishmanial agents have Sb(V)-resistance

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Table 1. Metal- and Metalloid-containing drugs used or under clinical trials against neglected tropical diseases

Drugs or lead compound	Metal or semi-metal	Mechanism of action	Actual therapeutic use	Potential therapeutic use against trypanosomatid-induced diseases	Side effects
SCYX-7158	Boron	inhibitor of the 26S proteasome	Under evaluation Phase IIb/III	HAT	Under evaluation Phase IIb/III
Glucantime®	Sb(III)	Inhibitor of TR and in general of dithiol containing enzymes	Visceral Leishmaniasis	Visceral Leishmaniasis	Pancreatic, renal, hepatic and cardiac disorders, reduced appetite, nausea, vomiting, diarrhoea, headache, tiredness, joint pains, muscle aches, dizziness, and anaphylaxis.
Pentostam®	Sb(III)	Inhibitor of TR and in general of dithiol containing enzymes	Visceral Leishmaniasis	Visceral Leishmaniasis	Pancreatic, renal, hepatic and cardiac disorders, reduced appetite, nausea, vomiting, diarrhoea, headache, tiredness, joint pains, muscle aches, dizziness, and anaphylaxis.
Arsobal® (Melarsoprol)	As(III)	Inhibitor of pyruvate kinase, TR, GR and dithiol containing enzymes	HAT	HAT	Brain dysfunction, numbness, kidneys and liver problems.
Auranofin (Ridaura®)	Au (I)	Inhibitor of TR	Rheumatoid arthritis.	Visceral Leishmaniasis, HAT and Chagas disease	Rash, mouth sores, persistent diarrhoea, indigestion, metallic taste, unusual bleeding or bruising, itching, blood in the urine, fainting.
SilvaSorb	Ag(I), Ag(0)	Inhibitor of TR and dithiol containing enzymes	Protecting burns, cuts, scrapes, and other wounds from infections	Cutaneus Leishmaniasis	Breathing difficulty; tightness in the chest; swelling of the mouth, face, lips, or tongue; excessive or sudden bleeding, burning, itching, pain, redness, or swelling of the skin; fever.
SivrSTAT	Ag(I), Ag(0)	Inhibitor of TR and dithiol containing enzymes	Topical anti-infective; it is used to treat Dermatological Disorders	Cutaneus Leishmaniasis	Breathing difficulty; tightness in the chest; swelling of the mouth, face, lips, tongue; excessive or sudden bleeding, burning, itching, pain, redness, or swelling of the skin; fever.

modifying ability in experimental infection with Sb(V)-resistant *L. donovani* isolates in murine model. These findings suggested the use of vanadium compounds in combination with Sb(V) in the treatment of Sb(V) resistant cases of visceral leishmaniasis (23). To overcome the drug resistance phenomenon and increase the effectiveness of the therapy, in March 2010, the World Health Organization Expert Committee on the Control of Leishmaniases recommended the sodium stibogluconate-paromomycin combination as first-line treatment for visceral leishmaniasis in East Africa. This kind of treatment is already being used in countries such as Sudan and South Sudan (29, 30).

3.3. Arsenic-based compounds

Arsenic exists in two oxidation states, As(III) and As(V). It is considered to be a paradox since it behaves as a poisoning and carcinogenic substance but it is also a therapeutic agent used by early physicians, such as Hippocrates and Paracelsus, and still used against some diseases. The first arsenic-based drug was Salvarsan (arsphenamine), that was put on the market in 1909 to cure syphilis, a sexually transmitted disease caused by the bacterium *Treponema pallidum*. It was the first important antisyphilitic agent, but it was phased out in the 1930s by better arsenical compounds (neoarsphenamine), and then by modern antibiotics.

Arsenic compounds are still used against sleeping sickness (African trypanosomiasis). The first drug used against late-stage Gambian (or West African) sleeping sickness caused by *T. brucei rhodesiense* is Melarsoprol, an organoarsenic compound, introduced in 1949 for its ability to cross the blood-brain barrier which make it particularly effective (31). It was initially administered as a very poorly tolerated non-aqueous solution. More soluble formulations of Melarsoprol (Melarsoprol with hydroxypropyl-β-cyclodextrin and randomly-methylated-β-cyclodextrin) have been employed as oral treatments for CNS-stage human African trypanosomiasis, delivering considerable improvements over current chemotherapy (32). The main target of this drug is the pyruvate kinase, an enzyme used from the parasite to produce ATP, but it is well known that As(III) may bind and inactivate up to 200 enzymes by binding to closely spaced protein thiols, forming stable cyclic dithioarsinite complexes in which both sulfur atoms are bound to arsenic. The formation of cyclic dithioarsinite complexes is mostly

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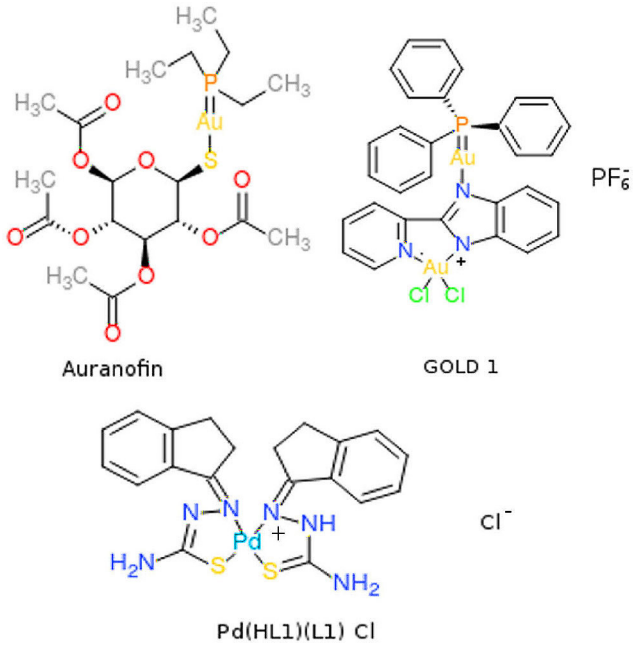

Figure 3. Metal-containing compounds of potential use against diseases caused by trypanosomatids. The compounds were designed using Chemdoodle (https://web.chemdoodle.com/demos/sketcher/). The picture was generated by Gimp (https://www.gimp.org/).

responsible for arsenic cytotoxicity. For this reason, Melasoprol is very toxic and causes brain dysfunction, numbness, and kidneys and liver problems. Even if the less toxic eflornithine should be usually preferred, in the very poor regions of the world where the disease is common, Melarsoprol is still the most used drug since it is provided for free by the World Health Organization.

Baiocco and coworkers have shown that As_2O_3 is able to inhibit also the trypanothione reductase of *Leishmania infantum* in the micromolar range but with an inhibition constant one order of magnitude higher that antimonials (K_i = 14 ± 4 μM) (21).

4. METAL-BASED COMPOUNDS TARGETING NEGLECTED INFECTIOUS DISEASES

4.1. Gold-containing compounds

Gold(I) complexes such as auranofin (1-thio-β-D glucopyranosato-(triethylphosphine) gold 2,3,4,6-tetraacetate) (Figure 3), gold sodium thiomalate, gold thioglucose and others have been used for decades against rheumatoid arthritis. These gold-thiol drugs act on multiple targets since they inhibit many enzymes as the serine esterases, elastase, cathepsin G, the first component of complement (C1), and lysosomal hydrolytic enzymes, such as acid phosphatases and ß-glucuronidase. Moreover, these compounds can reduce all classes of immunoglobulin and serum rheumatoid factors, and hamper lymphoblastogenesis *in vitro* by directly inhibiting mononuclear phagocytes (33). The ability of gold to bind to cysteine residues makes this metal a good candidate to be used to inhibit parasite enzymes. On this basis, Fricker *et al.* have developed various Au(III) compounds able to inhibit the cysteines proteases to find new lead compounds against trypanosomiases and leishmaniasis (34).

The ability of auranofin, a gold complex used as antirheumatic agent, to inactivate parasite detoxification enzymes has been recently investigated. Angelucci and coworkers solved the X-ray structure of the seleno-protein thioredoxin-glutathione reductase (TGR), a key enzyme in the pathway of *Schistosoma mansoni* for detoxification of reactive oxygen species, incubated with auranofin (35, 36). The structure shows that Au(I) binds to the catalytic cysteines (Cys154 and Cys159) over the FAD binding site thereby inhibiting TGR activity.

The ability to bind the cysteines makes auranofin able to inhibit also *Leishmania infantum* TR. Indeed, auranofin is able to inactivate the enzyme with high efficiency (Ki=0.155±0.35 μM) thereby killing the parasite in the promastigote stage (IC_{50}=9.68 ± 1.02 μM). The low-resolution crystal structure of auranofin–TR complex allowed the comprehension of the molecular basis of this inhibition (37). As expected, the gold ion was found to be tightly bound to the two catalytic cysteines (Cys52 and Cys57) in the active site of the enzyme, thereby hampering hydride transfer from the protein to trypanothione (Figure 4A).

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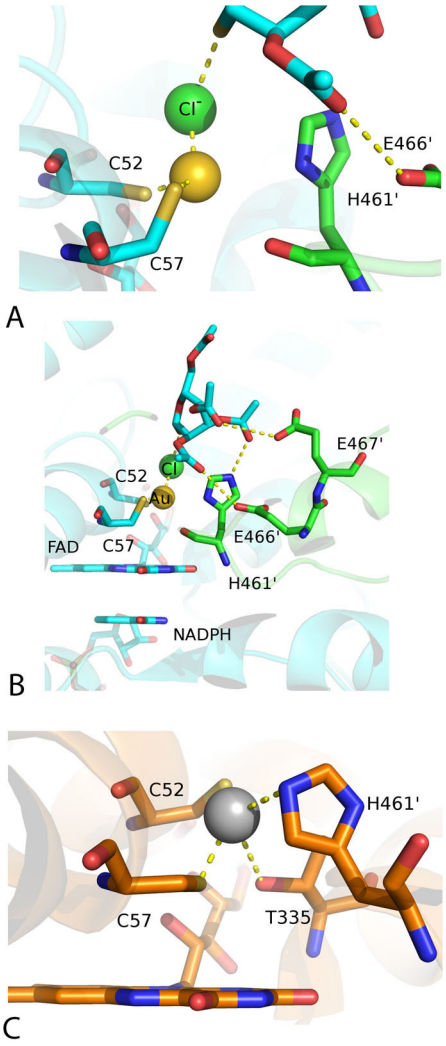

Figure 4. Complexes between TR and metal-containing compounds. A. TR-Au(I) complex. Blow-up of the gold binding site. The gold ion is represented as a yellow sphere, whereas the chloride ion is represented as a green sphere. The residues close to the gold ions are represented as sticks. B. TR-auranofin complex. Zoom out of the gold binding site. The thio-sugar moiety of auranofin (3,4,5-triacetyloxy-6-(acetyloxymethyl) oxane-2-thiolate) is represented as sticks. The residues interacting with auranofin are indicated. C. TR-Ag complex. Blow-up of the silver binding site. The silver atom is represented as grey sphere. The residues coordinating silver are represented as sticks.

Differently from the TGR structure where the geometry of the Cys-gold-Cys array is linear, in TR the gold ion displays a planar-trigonal coordination, where the ligands are the two catalytic cysteine residues and a chloride ion. The thiosugar of auranofin also contributes to TR inhibition by occupying the trypanothione binding site within TR (Figure 4B). The X-ray structure of TR in complex with auranofin proved that Au(I) binds with high affinity to the TR active site, thereby causing pronounced TR inhibition, and prompted Colotti and coworkers (38) to consider more systematically TR inhibition by a group of structurally diverse gold-containing compounds. They measured the K_i of different Au(I) and Au(III) compounds and evaluated their toxicity, identifying the most appropriate candidates for further evaluation. Among the tested compounds, the most potent TR inhibitor is the (Cl_2Au(III)(Pbi)Au(I)(PPh_3))(PF_6) (Pbi= 2-(2'-pyridyl)benzimidazole) compound (GOLD I in Figure 3) which displays an apparent K_i value of 22±11 nM, much lower than that of Sb(III) (1.5 μM). This compound is possibly the best candidate for further evaluation, since it displays also a low toxicity compared to other gold compounds, similar to that measured for auranofin which is a drug still present in the market (IC_{50}=0.6 μM vs. 0.5 μM, measured against human ovarian carcinoma cell line).

4.2. Silver containing compounds

Silver has antibacterial action at very low concentrations, since it reacts very easily with protein carboxylates, hydroxyls, and thiols. Thus, silver compounds are used as antibacterial agents; SilvaSorb®, ionic silver in an amorphous hydrogel

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base (Medline Industries, Inc.) and SilvrSTAT®, formed by metallic silver nanoparticles (Ag(0)) coated with tetrasilver tetroxide, are used as antimicrobial wound gels to prevent bacterial infections due to Gram-negative Bacteria (Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*).

In 2006, Navarro and co-workers have discovered that silver polypyridyl complexes are biologically active against *Leishmania mexicana*, since they can tightly bind DNA (39).

Ferritin-based encapsulated silver nanoparticles are effective against *Leishmania infantum*, with an IC_{50} against promastigote stage of 2.18 ± 0.33 μM and an IC_{50} evaluated against amastigote in murine macrophages from Balb/c mice of 1.76 ± 0.24 μM. These values are lower than the IC_{50} of antimonial drugs in free and encapsulated forms, which range from 30 to 900 μM (40). The ability to kill the parasite is due to the inhibitory effect of the metal in both main oxidative states Ag(0) and Ag(I), to inhibit TR activity. The X-ray structure of the TR in complex with Ag shows that the metal engages the two catalytic cysteines (Cys52 and Cys57) and the His461' of the enzyme in the formation of a stable complex, similar to that observed with Sb(III): therefore the metal strongly inhibits TR activity (the inhibition constant calculated for Ag(0) and Ag(I) are 500±200 nM and 50±10 nM, respectively) (Figure 4C) (40). The effectiveness of silver nanoparticles against *Leishmania* parasites is higher than that of antimony *in vitro*. Although silver (as other metals, including antimony) displays toxicity, since it binds to the thiols of many proteins (including those of the human host), and selectivity issues could be raised, the use of a novel approach, based on protein-encapsulated metals, may represent a promising strategy for the treatment of cutaneous leishmaniasis.

4.3. Platinum-, Palladium- and Ruthenium-containing compounds

Platinum is used in compounds effective against cancer. Cisplatin, i.e., *cis*-diamminedichloroplatinum(II), a square planar compound containing Pt(II) complexed by anionic ligands, has been the first metal-based agent used to treat cancer, and is currently used either by itself or in combination with other drugs for treating lung, ovarian, bladder, testicular, head and neck, esophageal, colon, gastric, breast, prostate cancer and melanoma, being particularly effective against testicular cancer (41, 42). Toxicity and resistance issues have prompted the design and clinical approval of second and third generation of cisplatin analogues with lower toxicity profiles, namely carboplatin and oxaliplatin (12).

A good correlation was found between antitumor and trypanostatic properties of platinum derivatives: cisplatin was used against *T. brucei* (43) and *T. cruzi*, where it gave different responses in different strains, depending on the level of expression of components of the DNA mismatch repair pathway (44).

Platinum and palladium have been also used to produce complexes combining ligands bearing anti-trypanosomal activity and pharmacologically active metals. This strategy takes advantage of the medicinal chemistry emerging drug discovery paradigm of developing agents that could modulate multiple targets simultaneously with the aim of enhancing efficacy or improving safety relative to drugs that address only a single target. Cis-platinum(II) pentamidine complexes are effective against *T. brucei* (45).

Platinum and palladium complexes with bioactive thiosemicarbazones show an elevated activity against *T. cruzi* in the epimastigote stage, with a five-fold activity increase with respect to the free ligand. These compounds act with a dual mode of action, targeting both reductive metabolism (with intra-parasite free radical production) and DNA (46, 47). Organoruthenium complexes with ketoconazole, clotrimazole or benznidazole and nitric oxide are more effective than the compounds used alone against the parasite, but are non-toxic against human or murine cells (48–50).

Besides their ability to intercalate in the DNA, Pt(II) and Pd(II) complexes can react with cysteine and histidine residues of proteins. (2,2′:6′,2″-terpyridine)-Pt(II) complexes, active *in vitro* against *L. donovani*, *T. cruzi*, and *T. brucei* (51), are effective irreversible inhibitors of *T. cruzi* TR, and most of them are only weak reversible ligands of human GR (52).

In addition, cyclometallate complexes of gold(III) and palladium(II) are potent inhibitors of parasitic cysteine proteases, and in particular of cathepsin B (53, 54).

5. CONCLUSIONS AND PERSPECTIVES

Despite millions of people are affected by Leishmaniasis, Chagas disease and HAT, these diseases only receive limited attention from authorities and private companies since they spread in poor tropical and subtropical countries. The arsenal of drugs available to control these diseases is limited and comprises also metal and metalloid containing drugs.

Chemotherapy to treat Leishmaniasis currently relies on four main drugs: pentavalent antimonials, miltefosine, amphotericin B and paromomycin (55); Chagas disease is treated with benznidazole and nifurtimox (http://www.who.int/mediacentre/factsheets/fs340/en/); only four drugs

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are registered for the treatment of human African trypanosomiasis: pentamidine, suramin, melarsoprol and eflornithine, whereas a fifth drug, nifurtimox, can be used in combination under special authorizations (http://www.who.int/trypanosomiasis_african/diagnosis/en/). All the cited drugs are toxic, develop resistance and some of them display cost problem in endemic poor countries. For these reasons there is an urgent need to find new, less toxic and more affordable drugs.

Metals and metalloids range from the essential to the highly toxic. Metal- and metalloid-containing drugs are used as chemotherapeutic agents to combat infectious diseases caused by pathogenic parasites as well as cancer, including acute promyelocytic leukemia. Moreover, the enhancement of activity of a therapeutic agent through metal complexation is known from decades, and metal-drug synergism can be employed to identify powerful molecules with good therapeutic indices (56). Finally, metal- and metalloid-containing drugs display often a cost lower than that of the other drugs. As an example, in the very poor regions of the world where HAT is common, Melarsoprol is still the most used drug since it is provided for free by the World Health Organization instead of pentamidine, suramine, eflornithine which are the recommended drug in the HAT early stages in the western countries.

The major problem with using the metals and metalloids containing drug remains their toxicity related to their ability to bind and/or interact with many targets including host proteins. However, a good number of new metal- or metalloid-containing compounds active against the parasites and with low toxicity against mammalian cells have been recently identified. Moreover, the use of the nanotechnologies furnishes a powerful tool to design nano-carriers able to target the drugs directly to the parasite. Thus, the major challenges of the present and the future research in this field are to increase the efficacy of the metal and metalloid containing compounds, to lower their toxicity in order to identify new, cheaper more efficacious and safe treatments of the neglected diseases caused by trypanosomatids.

6. ACKNOWLEDGMENTS

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Abbreviations: TR: Trypanothione reductase; HAT: Human African Trypanosomiasis; *L.*: *Leishmania*; *T.*: *Trypanosoma*; GR: Glutathione reductase; TGR: Thioredoxin-glutathione reductase

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