A "Golden Age" for the discovery of new antileishmanial agents: Current status of leishmanicidal gold complexes and prospective targets beyond the trypanothione system

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Leishmaniasis is one of the most neglected diseases worldwide and is considered a serious public health issue. The current therapeutic options have several disadvantages that make the search for new therapeutics urgent. Gold compounds are emerging as promising candidates based on encouraging *in vitro* and limited *in vivo* results for several Au^{I} and Au^{III} complexes. The antiparasitic mechanisms of these molecules remain only partially understood. However, a few studies have proposed the trypanothione redox system as a target, similar to the mammalian thioredoxin system, pointed out as the main target for several gold compounds with significant antitumor activity. In this review, we present the current status of the investigation and design of gold compounds directed at treating leishmaniasis. In addition, we explore potential targets in *Leishmania* parasites beyond the trypanothione system, taking into account previous studies and structure modulation performed for gold-based compounds.

1. Background: *Leishmania* and Leishmaniasis

Leishmaniasis is one of the most neglected diseases in the world that is endemic in several tropical and subtropical countries. Alarmingly, in the last decade, the migratory flow of refugees in regional civil conflicts and upheavals caused an increase in the number of cases. The most recent estimates point to about 700 000 to 1 million new cases per year.[1–3]

Classically, the disease is divided in its visceral or cutaneous form, though it has been recognized for many years that clinical variations play a significant role in the burden of the disease.[4,5] The visceral form affects internal organs such as bone marrow, liver, and spleen and is potentially fatal if left untreated.[6] Signals and symptoms of the cutaneous manifestation are mostly restricted to the skin, though, in specific cases, this form can aggravate mucosal spread or uncontrolled body propagation of the lesions.[7,8]

At least 20 species of *Leishmania* are recognized as the causative agents of leishmaniasis in humans[9] that are infected by non-replicating flagellate forms, that is, metacyclic promastigotes, transmitted during the blood meal of sandflies vectors.[10] The following parasitic stage, known as amastigote, is responsible for the clinical outcome, as it infects and replicates within cells that belong to the mononuclear phagocytic system (Figure 1). Amastigote is the relevant stage on which drugs must act when controlling the progression of the disease.[11]

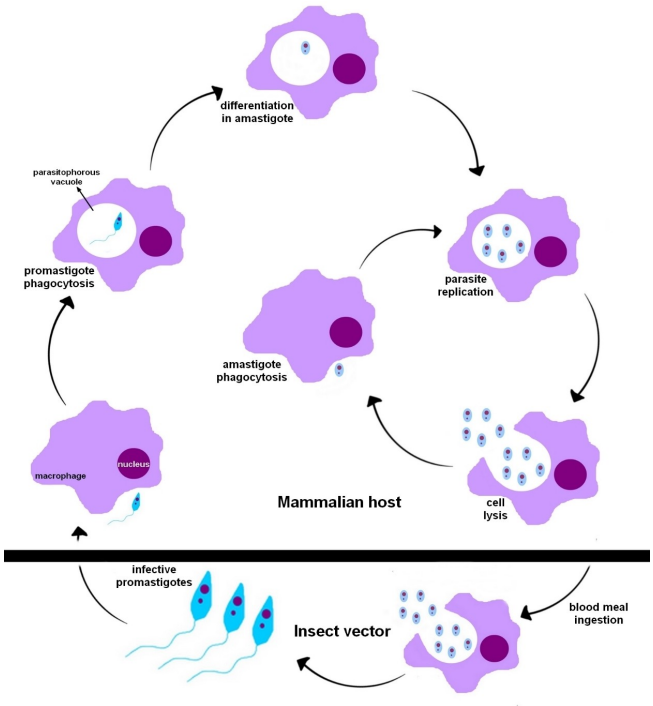

Figure 1. Scheme of the *Leishmania* life cycle. Bottom: promastigotes replicate in the sand fly digestive tract, and infective forms are transmitted to the mammalian host during a blood meal. Top: promastigotes are mainly phagocytosed by macrophages where they differentiate to amastigotes and replicate within parasitophorous vacuoles and maintain the infection in the host. During a new blood meal, the insect vector takes up cells harboring amastigotes and, ■■eventually *'possibly'* ?■■, free amastigotes that will differentiate to promastigotes.

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Unfortunately, the first-choice drug options for leishmaniasis are toxic, require intramuscular or intravenous administration, and may be ineffective in certain conditions. Most recently introduced treatments, such as amphotericin B in its liposomal form, can be quite costly to health systems and are not necessarily effective for all clinical forms of leishmaniasis.[6,11]

Several metal-based compounds were investigated as candidates for the therapy of leishmaniasis. The topic was discussed recently in an extensive review about metal compounds for neglected diseases.[12] Almost all medically relevant transition metals and from principal group (selenium, bismuth and antimony) bearing different ligands were evaluated. From the *in vitro* results we can highlight ferrocenyl derivatives,[13] ternary silver N,N-chelated compounds,[14] bismuth and antimony porphyrins[15] and copper compounds.[16,17] Among them, gold(I) and gold(III) compounds have been attracting increasing attention due to excellent antiparasitic activity. Therefore, this review is focused on a detailed description of gold complexes as antileishmanial agents in the last decade.

2. Current Treatments for Leishmaniasis

The therapeutic arsenal that is mainly employed for treating patients with leishmaniasis is based on the use of pentavalent antimonials (Figure 2), first introduced as urea stibamine in the 1920s by Brahmachari in Indian patients with visceral leishmaniasis.[18] Decades later, two pentavalent antimony compounds were introduced as safer options: sodium stibogluconate (Pentostam®; GlaxoSmithKline) and meglumine antimoniate (Glucantime®; Aventis). Undoubtedly, they have caused an enormous impact in the treatment of leishmaniasis worldwide during the past decades; however, their parenteral administration in long schemes and several side effects, including cardiotoxicity, anemia, kidney failure, nausea, and hematological alterations still represent major drawbacks that affect the success and completion of treatment courses.[11,19,20]

Even after all these years, the mechanisms that explain the leishmanicidal effect induced by pentavalent antimonials are not fully understood.[21] Nevertheless, the hypothesis that Sb^{V} must be reduced to Sb^{III} in a host-dependent system finds experimental evidence when antimonial sensitivity of intracellular amastigotes is compared to axenic amastigotes.[22,23] In parallel, evidence that promastigote's trypanothione reductase is inhibited by trivalent sodium antimony gluconate but not pentavalent antimonials has been presented by Cunningham and Fairlamb in 1995.[24] Yet, antimonials appear to exhibit effects other than interfering with the oxidative stress balance during the infectious process, such as the inhibition of ATP production by modulating fatty acids ß-oxidation and

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Danilo C. Miguel has been teaching parasitology and parasite–host interactions for graduate and undergraduate students for the past 7 years at the University of Campinas. His main interest in basic science is focused on understanding the mechanisms that allow Leishmania to survive in the intracellular environment, that is, macrophages' parasitophorous vacuoles. In his research Laboratory, he also studies the efficacy of several compounds aiming at the discovery of novel/repurposed drugs for leishmaniasis treatment.

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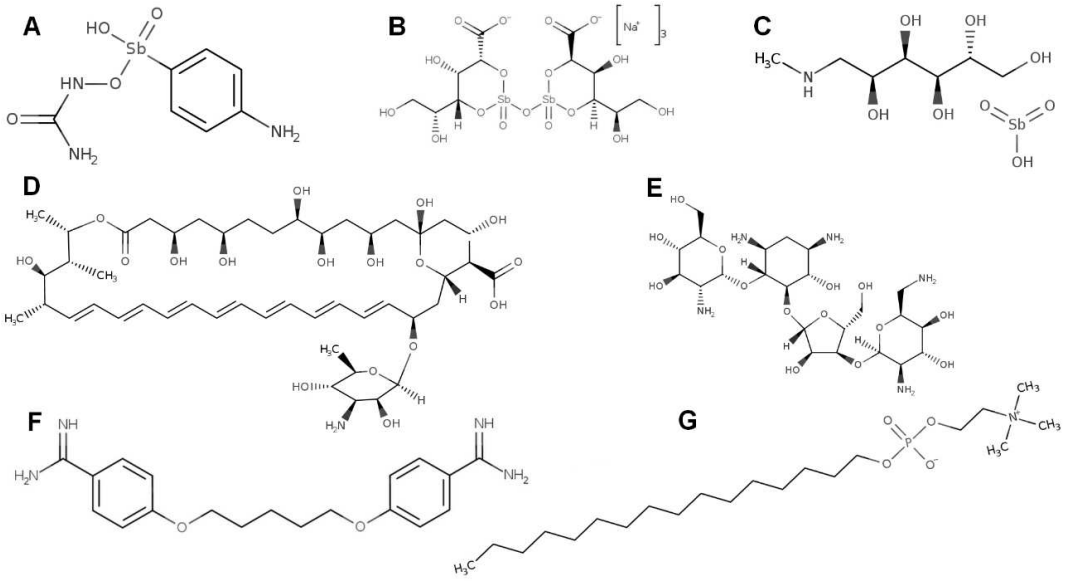

Figure 2. Chemical structures of drugs used to treat *Leishmania* infections. A) urea stibamine, B) sodium stibogluconate, C) meglumine antimoniate, D) amphotericin B, E) paromomycin, F) pentamidine, and G) miltefosine.

glycolysis,[25] and apoptosis-like programmed cell death induction.[26] Also, immunomodulatory effects of sodium antimony gluconate have been recognized by direct interference on both innate and cell-mediated immunity.[27]

For almost 40 years, clinical reports have shown that the administration of antimonials was no longer effective in curing visceral leishmaniasis as in the previous decades. This means that higher doses of antimonials for longer periods have been prescribed, even without showing expected results in Northern India, mainly in the state of Bihar.[28,29] About 50–65% of patients in the Bihar region were refractory to treatment, a fact that was later explained by the detection of antimony-highly resistant *Leishmania donovani* isolates.[30] Isolates obtained from Sudanese patients were also resistant to antimonials in *in vitro* screenings, in agreement with Yardley and collaborators' findings for isolates from Peru.[31,32]

Second choice drugs for leishmaniasis treatment include amphotericin B, paromomycin and pentamidine (Figure 2). Amphotericin deoxycholate requires hospitalization for continuous intravenous infusion and its cumulative toxic effects are intense. The use of liposomal amphotericin B formulations reduces the dose required for the treatment of visceral leishmaniasis, but with prohibitive costs for large-scale treatment in endemic areas.[11,33,34] Regarding amphotericin B mechanism of action, it is well established that this polyene induces the formation of pores at *Leishmania*'s plasma membrane after binding to ergosterol. This interference is capable of altering the ionic balance of the cell leading to its destruction.[35]

Paromomycin is an aminoglycoside antibiotic with leishmanicidal activity firstly observed in the 1960s. Despite its low oral bioavailability, trials in India showed promising activity of the drug in patients refractory to antimonials in the late 90s.[36] Partial efficacy of paromomycin in specific topical formulations or associated with antimonials or antibiotics have been reported.[37–39] The main side effects of paromomycin are described as toxic to the auditory nerve and kidneys. Studies have shown that its activity may be related to the interference with the parasite's mitochondrial function.[11,23,40]

Pentamidine, a broad-spectrum anti-infective agent, is often reserved for cases of therapeutic failure with antimonials and amphotericin B. However, its effectiveness in cutaneous leishmaniasis is questionable as success rates in Latin America seem to vary according to geographic region and parasite species.[41–43] It is worth mentioning that pentamidine application may trigger intense side effects including myalgia, nausea and hypotension.[40] Studies have shown that the leishmanicidal action mechanism of this diamidine appears to be based on the accumulation of the drug in the parasite, allowing its association with the mitochondrial DNA (kDNA), perhaps blocking its replication process, besides disrupting the parasite's mitochondrial membrane potential.[44,45]

Many research groups have focused on finding alternative therapies for the treatment of leishmaniasis for several years. Among them, perhaps the most promising and innovative one was the proposal of using lysophosphatidylcholine derivatives such as hexadecylphosphocholine (miltefosine, Figure 2) against *L. donovani*.[46] In 1997, a clinical trial showed that oral administration of miltefosine was effective in treating Indian patients with visceral leishmaniasis, allowing its approval for clinical use in 2002 in that subcontinent.[47,48] The main side effects are related to gastrointestinal disorders, but the biggest limitations of miltefosine use point to its teratogenic potential and long terminal half-life.[23,40] Unfortunately, unresponsiveness to miltefosine in visceral leishmaniasis cases has been recognized. Pérez-Victoria and collaborators, for example, demonstrated the existence of *L. donovani* strains that are resistant to

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miltefosine and more recently, a field isolate from an Indian patient has been described showing low sensitivity to miltefosine *in vitro*.[49–52] In terms of mechanisms that might explain the leishmanicidal effect of miltefosine, it appears to be primarily related to the action on the parasitic plasma membrane, inhibition of phosphatidylcholine and interference with signal transduction.[23,53]

3. Gold Complexes Current Status

3.1. General activity of gold complexes and rationale

The therapeutic use of gold dates back to ancient times. It culminated with sodium aurothiomalate (Myocrisin) and aurothioglucose (Solganol) approval for rheumatoid arthritis (RA). The painful administration by intramuscular injections is a drawback.[54] Years later, Sutton et al. reported the potent antiarthritic activity of the oral administration of tetra-*o*-acetyl-L-thio-β-D-glucopyranosato(triethylphosphine)gold (auranofin; **1**).[55] These three Au^{I} compounds represent the set of gold drugs clinically used for the RA treatment. After their success, several other therapeutic applications were envisaged and novel gold complexes were developed. Their anticancer properties were the main focus of many researchers in the field of inorganic medicinal chemistry.[56–60]

Regarding leishmaniasis, the first report dates from 1989. Ten patients infected with *L. donovani* who had relapsed after treatment with sodium stibogluconate were treated with Myocrisin.[61] After three months, their bone marrow aspirates were free of Leishman-Donovan bodies and their liver and spleen showed regular sizes. The main side effect reported was platelet dropping, which returned to normal levels after three months. No relapse was observed after one year of follow up. Surprisingly, auranofin, which is orally administered, was never clinically evaluated for the treatment of leishmaniasis. Phase I clinical trial was performed in healthy patients in an NIH effort envisaging an attempt to treat individuals with *Entamoeba histolytica* and *Giardia intestinalis* infections.[62]

After the trial with aurothiomalate, reports on *in vitro* antileishmanial activities in promastigotes of Au^{III}dipyrido[3,2-a:2′,3′-c]phenazine (dppz, $[Au(dppz)_2]Cl_3$), several Au^{III} C<?N cyclometallated and Au^{I} trifenilfosfine-pyridine-2-thiol-N-oxide ($[Au(PPh_3)(mpo)]$) were found until 2007.[63–65] Effective drug concentrations that led to half-maximal response (EC_{50}) varied significantly, from nanomolar to no inhibition at maximum (10 μM), which may underscore relevance structure/activity relationships. However, methods, parameters and *Leishmania* species varied significantly among these reports restraining the comparison and even the results significance (Table 1). Table 1 summarizes fourteen studies reporting the *in vitro* activities of Au^{I} or Au^{III} compounds (Figure 3) against *Leishmania* spp. from 2010 to 2020. The compounds investigated in each case is demonstrated in Figure 3. The selected articles have been found using the PubMed.gov database search tool (https://pubmed.ncbi.nlm.nih.gov) for the descriptors: gold; *Leishmania*; Auranofin; leishmaniasis.

3.2. *In vitro* antileishmanial activity of gold complexes

Figure 3 and Table 1 show the class of gold(I)-N-heterocyclic carbenes (Au^{I}-NHC) as the most studied among the complexes designed for anti-*Leishmania* activity in the last decade. Except by compounds of structure **5**, mostly Au^{I}-NHC bear asymmetric carbenes. The symmetric carbenes **5** showed IC_{50} lower than 10 μM. Due to different methodologies used it is not possible to perform a direct comparison, therefore it suggests symmetric or antisymmetric present similar activity profiles. Paloque et al. designed cationic bis-NHC–N-quinolines gold(I) complexes **4** and evaluated the *Leishmania infantum* promastigote and intracellular amastigotes inhibition.[69] They screened nine derivatives in promastigotes and selected three for amastigote inhibition tests. Complexes inhibited amastigotes in nanomolar concentrations, significantly better than miltefosine, but could not reach the potency and selectivity of amphotericin B. Among them, the N-methyl thiophenyl derivative was better than N-methyl and N-mesityl derivatives. However, it is relevant to stress that this chemotype led to significant toxicity in the J774A.1 cells (macrophage), consequently lowering the selectivity index ($SI = CC_{50}/EC_{50}$, for which CC_{50} represents the half-maximal effective cytotoxic concentration).

More recently, the same group evaluated the neutral derivatives **11**, **12**.[74] However, the methodology was considerably different as they used axenic amastigotes instead of intracellular amastigotes. This change in the protocol may not allow a proper comparison between the cationic bis-*N*-quinoline-NHC–Au^{I} derivatives and the neutral chloride *N*-quinoline-NHC–Au^{I}. When assessing promastigotes sensitivity, neutral complexes were less active than their cationic counterparts. Regarding the EC_{50} in axenic amastigotes and the selectivity index related to J774A.1 CC_{50}, *N*-benzyl-*N*-mesityl NHC (**11**) presented the best activity and SI = 40.

Similar scaffolds containing aliphatic chains instead of quinolines were assessed in different reports. Massai et al. evaluated neutral Au^{I}–NHC (**8**) in *L. infantum* amastigotes. They found EC_{50} in the nanomolar range.[73] Al-Majid et al. not only tested the cationic bis-carbene derivatives ($Au^{I}(NHC)_2$) but also a series of Au^{III} compounds ($Au^{III}Cl_2(NHC)_2$; **7**) finding activity at nanomolar concentrations; EC_{50} = 0.1–0.7 μM for all compounds in promastigotes of *Leishmania major*.[70,72] The lowest EC_{50} was found for Au^{I} derivative. Unfortunately, the protocols and species of Massai and Al-Majid studies are considerably different, making comparisons between neutral $[Au^{I}Cl(NHC)]$ and cationic $[(NHC)_2Au^{I}]^{+}$ difficult.

Recently, Ouji et al. evaluated the inhibitory activity of cationic bis-*N*-triclosan-NHC-gold(I) series **16** against promastigotes and amastigotes of *L. infantum*.[77] In this work, they compared the results with auranofin reported by Sharlow et al.[67] The best SI values were found for miltefosine (~88), auranofin (~38), and triclosan (~12). Their gold compounds had an SI of 0.7 to 2. As the methodologies were the same for **11**, it is possible to assume that triclosan NHC derivatives are less potent than quinoline derivatives.

Considering Au^{III} compounds, **9** presented low EC_{50} for *L. infantum* amastigotes (3.17 μM). The study compared the

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Table 1. Reported *in vitro* EC_{50} for the antileishmanial activity of several gold complexes in the literature from the year 2010 to 2020. Experimental details as parasite strain, stage and the number of cells used are shown. From the series of compounds investigated in each report we selected the lower and higher EC_{50} reported. The structural scaffold investigated in each case is demonstrated in Figure 3.

Compounds (Figure 3)	Strain or cell line	Stage [a]	Cell/vol [b]	Lower EC_{50} (reported code #)	Higher EC_{50} (reported code #)	Ref.
1, 2	*L. infantum* MHOM/TN/80/IPT1	Pro	10^6/mL	9.68 (1)	16.59 (2)	[66]
	L. major MHOM/SU/73/5-ASKH	Pro	10^6/mL	15.66 (1)	17.48 (2)	
1, 2	*L. amazonensis* RAT/BA/72/LV78	Pro	10^7/mL	2.7 (39)	>20 (33, 35)	[67]
	MPRO/BR/72/M1845	intAm	MOI = 30	0.12 (37)	0.70 (4, 8, 32)	
	L. major MHOM/SU/ 74/WR779	intAm	MOI = 10	0.06 (10)	>10 (21, 30)	
	RAW 264.7		2×10^5/mL			
3	*L. amazonensis* IFLA/BR/67/PH8	Pro	–	5.18	>100	[68]
		intAm	–	5.77	>25	
	L. braziliensis MHOM/BR/75/M2903	Pro	–	1.29	>100	
		intAm	–	3.16	>25	
	L. major MHRHO/SU/59/P	Pro	–	4.36	>100	
		intAm	–	5.69	>25	
	murine peritoneal macrophages	-	–	59.68	>150	
	3T3 fibroblasts	-	10^5/mL	64.41	223.57	
4	*L. infantum*	Pro	10^6/mL	0.39 (5)	>100 (1′, 9)	[69]
	MHOM/MA/67/ITMAP-263	intAm	MOI = 10	0.24 (8)	0.96 (6)	
	J774A.1 macrophage cell line	–	5×10^4/mL	0.26 (7)	48.36 (1)	
5	*L. major*	Pro	10^6/mL	3.01 (13)	>100 (5, 7, 8, 10)	[70]
	PC3 prostate cancer cell line	–	10^5/mL	4.69 (13)	>30 (1, 5, 7, 8, 10)	
	HeLa cervical cancer cell line	–	10^5/mL	2.64 (13)	>30 (5, 7, 8, 10)	
	MCF-7 breast cancer cell line	–	10^5/mL	1.56 (11)	35.36 (1)	
	3T3 fibroblasts	–	10^5/mL	1.52 (4)	>30 (5, 7, 8 e 10)	
6	*L. infantum* MHOM/BL/1967/ITMAP263	Pro	10^6/mL	2.73 (8c)	6.85 (7b)	[71]
		inAm	1.5×10^6 MOI = 10	0.97 (6a)	4.25 (5e)	
	L. braziliensis MHOM/BR/1975/M2904	Pro	10^6/mL	4.69 (8c)	10.57 (10c)	
	L. guyanensis MHOM/BR/1975/M4147	Pro	–	2.52 WT (6a)	4.24 WT (10e)	
	WT and SbIII resistant			5.58 SbR (6a)	7.55 SbR (7b)	
	THP-1 monocytic cell line	–	$5x10^5$	4.41 (6a)	11.54 (1a)	
7	*L. major*	Pro	10^6/mL	0.11 (1)	1.62 (7)	[72]
	HeLa cervical cancer cell line	–	10^5/mL	0.08 (9)	1.8 (1)	
	MCF-7 breast cancer cell line	–	10^5/mL	0.17 (9)	~50 (5)	
	3T3 fibroblasts	–	10^5/mL	0.5 (6)	1.9 (5)	
8, 9	*L. infantum* MHOM/MA/67/ITMAP263	inAm	5×10^5	<0.25 (2 e 4)	3.17 (1)	[73]
	murine peritoneal macrophage	–	3×10^4/mL	<0.25 (2)	8.00 (1 e 3)	
10–12	*L. infantum* MHOM/MA/67/ITMAP-263	Pro	10^6/mL	2.52 (12)	57.03 (10)	[74]
		axAm	2×10^6/Ml	0.19 (12)	11.07 (10)	
	J774A.1 macrophage	–	5×10^4/mL	0.66 (14)	32.39 (10)	
13	*L. braziliensis* MHOM/BR/94/H3227	intAm	MOI = 10	2.96 (11)	>10 (7 e 8)	[75]
	THP-1 monocytic cell line	–	5×10^5/well	11.77 (11)	19.44 (10)	
14, 15	*L. infantum* MCAN/BR/2000/BH400	Pro	10^6/mL	0.95 (4)	5.95 (1)	[76]
	MHOM/MA/1967/ITMAP-263	intAm	–	0.54 (3)	1.58 (1)	
	L. braziliensis MHOM/BR/1975/M2904	Pro	10^6/mL	1.7 (3)	6.34 (1)	
	MHOM/BR/1994/H3227	intAm	–	2.29 (2)	5.56 (1)	
	THP-1 monocytic cell line	–	2×10^5/well	3.08 (3)	5.6 (2)	
16	*L. infantum*	Pro	–	5.51 (2a)	63.87 (2b)	[77]
		axAm	–	0.21 (2a)	11.62 (2b)	
	J774A.1 macrophage cell line	–	–	0.21 (2a)	3.76 (2b)	
17–19	*L. amazonensis* IFLA/BR/67/PH8	Pro	4×10^6/well	4.24 (3)	117.3 (4)	[78]
	L. braziliensis MHOM/BR/94/H3227	Pro	4×10^6/well	4.25 (3)	113.2 (4)	
	L. amazonensis and *L. Braziliensis*	intAm	MOI = 2 and 5	2.5 (3)	7.5 (3)	
	BMDM	–	5×10^4/well	7.08 (9)	156.6 (4)	

[a] Pro = promastigote; intAm = intracellular amastigote; axAm = axenic amastigote. [b] MOI = multiplicity of infection; ratio of infecting agents to a susceptible target.

activity of compound **8** (2.73 μM) to miltefosine (4.13 μM). Other compounds evaluated showed no significant activity, for example the gold(III) dinuclear oxo bridged 6,6′-dimethyl-2,2′-bipyridyne, gold(III) cyclometalated derivative of 6-(1,1-dimethylbenzyl)-2,2′-bipyridine, which features a N,N,C sequence of donor atoms of the tridentate bipyridine ligand and an oxygen atom of a hydroxo ligand coordinated to the gold(III) center in a square planar geometry.[73] The low EC_{50} found for **9** ■■is *or* might be ?■■ related to the compound's ability to dissociate in monomers followed by reduction in the biological medium, releasing two equivalents of gold(I), as demonstrated in an earlier report.[79]

Minori et al. evaluated a series of Au^{I} and Au^{III} complexes as the organometallic bis-trimethylxantine ylidene gold(I) (**17**), C^N cyclometalated Au^{III} series **19**, and the coordination complex N^N Au^{III} **18**.[78] Comparison of their EC_{50} and SI in *Leishmania braziliensis* and *Leishmania amazonensis* promastigotes and amastigotes demonstrated gold(I) complex **17** to be

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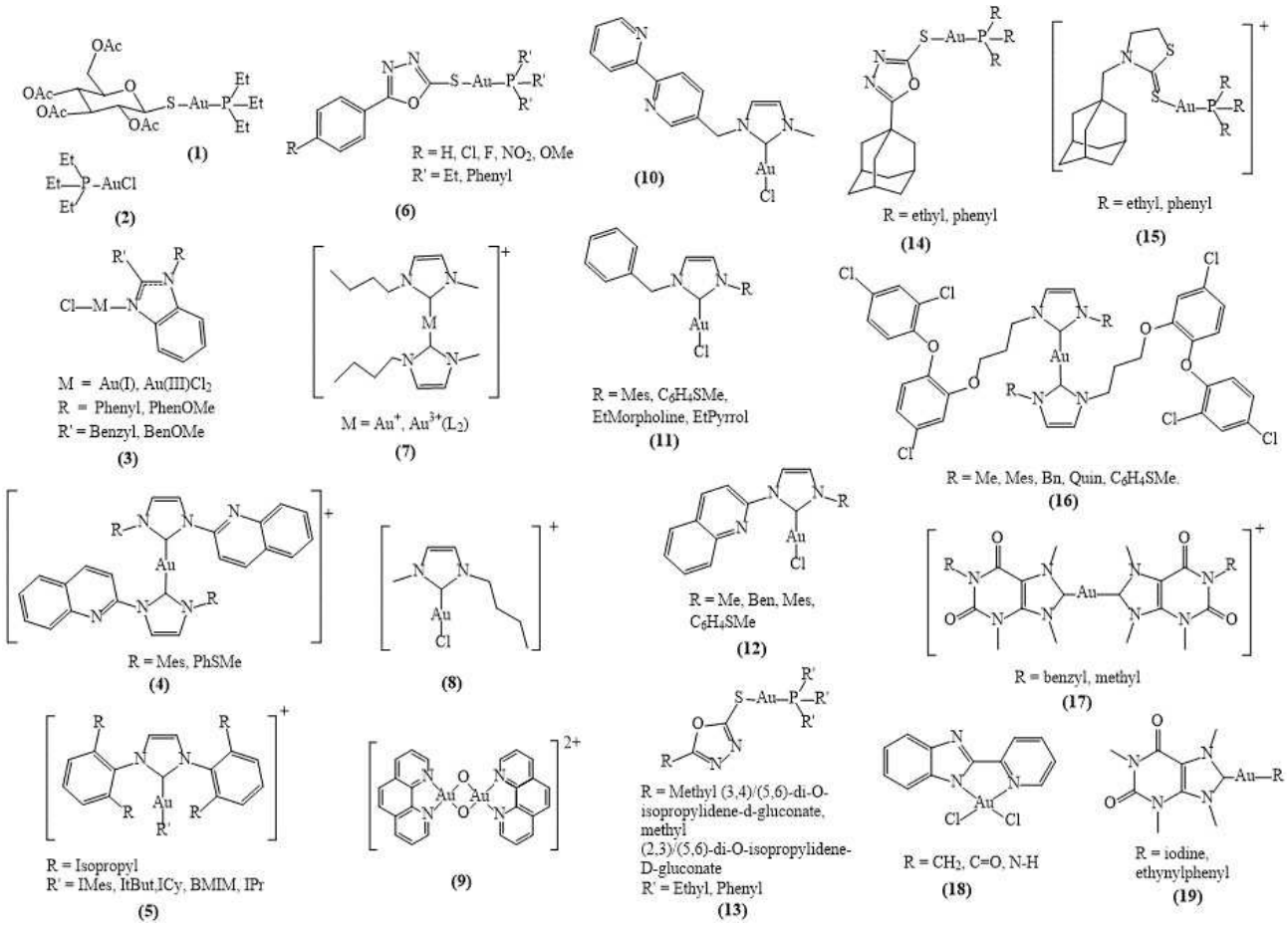

Figure 3. Main general structures of gold complexes with in vitro antileishmanial activity evaluated from 2010 to 2020. Adapted from refs. [66]–[78].

the best candidate. The SI > 23 in promastigotes combined with the pronounced inhibitory activity against intracellular amastigotes makes **17** a promising anti-leishmaniasis candidate. Among Au^{III} compounds, the non-organometallic **18** showed a good decrease of the infection rate and amastigote burden in infected Balb/c mouse primary macrophage.

Differently from the organometallic carbenes, Mota et al. studied a series of methylbenzimidazoles derivatives that N-coordinates to [Au^{I}ClL] and [$Au^{III}Cl_3$L] (**3**) using promastigotes and intracellular amastigotes of three *Leishmania* species.[68] The most active compound in promastigotes was not the best compound contrasted to anti-amastigote screenings, suggesting host-cell activation. Au^{I} complex with benzyl, phenyl-benzimidazoles was the best, except in *L. major*; for which Au^{III} complex with O-benzyl, O-phenyl showed better results, with EC_{50} values close to miltefosine. Also, in this case, K[$AuCl_4$] was not active for all tested concentrations and considering macrophage toxicity, Au^{I}-1-benzyl-*N*-phenyl was a potential scaffold for the design of antileishmanial compounds.

Another group to consider is the triethyl/triphenylphosphines Au^{I} complexes, including auranofin. Ilari and colleagues firstly reported the crystal structure of trypanothione reductase with auranofin.[66] In the same report, the authors evaluated the antiproliferative activity of auranofin and chlorotriethylphosphine gold(I) (**2**) against promastigotes of *L. infantum* and *L. major*. Auranofin EC_{50} values were significantly different between species, while **2** presented similar and less potent activity. The EC_{50} values of **1** and **2** are also higher than 10 μM, which is considered moderate for therapeutic applications. This result contradicts Sharlow et al., which performed HTS of 38 complexes of scaffold R_3P–Au–Cl and auranofin using *L. major* and *L. amazonensis* promastigotes and intracellular amastigotes.[67] Auranofin was the second-best compound being less active and less selective than the R = *t*Bu_2(*p*-($N(CH_3)_2$)Ph). The EC_{50} values in amastigotes were 0.07 μM for *L. major* and 0.27 μM for *L. amazonensis*. Auranofin SI (~80) was higher than paromomycin and stibogluconate but inferior to amphotericin B.

Complexes **6**, **14**, **15** presented EC_{50} values similar to controls R_3P–Au^{I}–Cl (**2**) against *L. infantum* and *L. braziliensis* intracellular amastigotes This result suggests that oxolidine or thiazolidine does not improve the *in vitro* activity in comparison to Cl.[71,76]

It is important to emphasize that, from the studies listed in Table 1,there is no standardization in several biological parameters. The parasitic species and strains varied substantially,

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probably due to the available strains in the researchers' countries. Regarding all the species capable of causing leishmaniasis worldwide, the number of species tested is limited in these studies. However, works with more than one species tested show little variation in parasite sensitivity to gold complexes. Still, results gathered from these studies can be puzzling concerning direct comparisons, as there is variation in the following parameters: i) number of parasites, ii) tested stage (promastigotes capable of replicating extracellularly and present in the vector insect versus the clinically relevant amastigote stage), iii) concentration ranges, iv) MOI used. Nine out of fourteen studies (~64%) followed the classical investigation protocol testing promastigotes, amastigotes (intracellular and/or axenic forms), and mammalian cells.

In terms of cytotoxicity, we observed a lack of protocol standardization. The choice of lineages and/or primary cells is an intricate factor that may hinder a reliable comparison among SIs. Also, there was a high heterogeneity of macrophage types among reports, including THP-1 (human monocytic cell line), RAW 264.7 (Abelson murine leukemia virus-induced tumor), J774A.1 (murine reticulum cell sarcoma) lineages, and murine peritoneal and bone marrow derived-macrophages. Importantly, some protocols used cell lines that are not even capable of sustaining leishmanial infections, for example, 3T3, HeLa, and MCF-7 cells.

3.3. *In vivo* activity

In vivo studies are still limited in terms of the evaluation of gold compounds against experimental leishmaniasis. Sharlow et al. showed that mice presenting cutaneous leishmaniasis caused by *L. major* that received intraperitoneal injections of **1** at 20 mg/kg/d presented suppression of footpad swelling and lesion sizes comparable to control treatments with liposomal amphotericin B (12.5 mg/kg/d). In a lesion cure model, they verified the complete cure by amphotericin B after 84 days. Although auranofin treatment has promoted a significant reduction in the lesion area, complete healing has not been achieved for the same evaluation period.[67]

Tunes et al. also presented data regarding the activity of complexes containing triethylphosphine gold(I) bound to adamantylthiazoline (AdTEt; **14**) and adamantly-oxo-thiazolidine (AdOEt; **15**) for *in vivo* evaluation using *L. amazonensis* and *L. braziliensis*-infected mice. AdTEt and AdOEt proved to be extremely effective in reducing the size of skin lesions and parasitic load in a murine model, even at low doses (12.5 mg/kg/d, P.O.). Even so, they observed differences in results between species, being *L. amazonensis* more sensitive than *L. braziliensis*. They also observed that the combination of the complexes with miltefosine reduces parasitic load more efficiently, as shown for the association of 12.5 mg/kg/d AdTEt + 15 mg/kg/d miltefosine for 13 days that led to undetected parasitic loads. They were able to demonstrate that the complexes have a significant therapeutic window and low toxicity, even if administered orally without specific formulation to increase safety. Also, miltefosine triggered nephrotoxicity in treated animals, unlike the tested complexes. These data encourage the continuity of studies with gold(I) complexes as promising candidates to treat cutaneous leishmaniasis.[76]

3.4. Mechanisms

The fact that aurothiomalate accumulates in organs rich in mononuclear phagocytes motivated the clinical trial performed in 1989, as *Leishmania* parasites this type of cells.[61]

The action mechanism of gold drugs pertains to intense debate in the literature and is frequently reviewed.[56,57,80–82] It is a consensus that the mechanism is a multitarget; however, it is still elusive if they are independent parallel modes or present a common denominator. For Au^{I}, after ligand substitution reaction with serum albumin, the set of results point to the uptake and storage of metabolites of coordination sphere S–Au^{I}–S in lysosomes, called aurosomes, which seems to function as storage.[83,84] In the case of Au^{III}, the isoelectronic and isogeometric to the Pt^{II} motivated the design of gold based compounds for cancer therapy. Several studies demonstrate In the case of Au^{III}, the isoelectronic and isogeometric to the Pt^{II} motivated the design for cancer therapy and DNA targeting. Several studies demonstrate Au^{III} is easily reduced in the biological media. The use of organometallic, or polydentate ligands produced stable active Au^{III} square planar structure. DNA can be targeted in this case, but other mechanisms are also reported.[85]

Beyond the several investigated human targets to explain the action of gold drugs, the inhibition of the thioredoxin reductase (TrxR) was the most prominent result. This effectiveness of inhibition turned the TrxR into the most studied target of gold compounds in cancer or RA.[86] The chemistry involved is the high affinity of gold for thiol, and mainly selenols. In the case of *Leishmania* parasites, trypanothione system, which is responsible for the parasite redox balance turned into the most investigated target for gold compounds. It is also based in thiol chemistry and, essential for the parasite. However, coordination chemistry offers a mean to direct gold to inhibit other interesting molecular targets in therapy, as demonstrated for cancer. In the next sections we propose a discussion of possible parasite targets based on the knowledge raised in the cancer studies of gold-based candidates. We selected trypanothione (similar to the Trx system of the parasite), cysteine protease, topoisomerase, DNA, aquaporins and zinc fingers. Similarities and contrasts between human targets and Leishmania are discussed.

4. Prospective Targets

4.1. Trypanothione system

The pyridine nucleotide disulfide oxoreductases comprise a family of enzymes that includes glutathione reductase (GR), glutathione peroxidase (Gpx), trypanothione reductase (TR), and the bacterial and mammalian thioredoxin reductase (TrxR),

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among others. The mammalian type TrxR is a selenoprotein highly similar to the glutathione reductase in structure and function.[87,88] However, TrxR carries a C-terminal redox motif involving a selenocysteine (Sec) residue.[89,90] The thioredoxin system is comprised of thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH. Studies revealed several functions for mammalian TrxR as DNA synthesis, defense against oxidative stress, apoptosis, redox signaling, protein disulfide reductase and redox control in mammalian cells.[91] Their expression is altered in many diseases including cancer, diabetes, cardiovascular, neurodegenerative disease, RA, inflammation and some virus infections.[92] However, the overexpression of Trx/TrxR in tumors implied a crucial role in cancer development and progression and they have received support and validation as suitable drug targets for the development of new anticancer agents.[93–97]

The studies about the mechanism of action of gold in RA demonstrated thioredoxin was a significant target. As a soft acid, Au^{I} could selectively bind the active selenocysteine (Sec) in TrxR. Early studies demonstrated that auranofin TrxR K_i of 4 nM contrasts the closely related GR, around 3 μM.[98] An evaluation performed *in vivo* showed that tissue TrxR activity dropped to near zero after injections of aurothioglucose while the activity of Gpx, which also presents an active Sec, did not change significantly.[99]

This high level of inhibition is attributed to the direct coordination of gold ions to the Sec in the second redox-active site of TrxR, which were later evidenced by consistent experimental data.[98,100] For this reason, the Trx system is the most evaluated target to explain the action of gold as an active anticancer compound. Several experts reviewed this topic thoroughly.[56,93,101–103] In general, novel designed complexes do not perform as well as auranofin.[104–112] Some recent data showed better inhibition values for newly designed compounds.[113–118] The reader can also find reports comparing TrxR and GR inhibition. It is an overall trend of gold complexes to inhibit TrxR in much lower concentrations than GR. For this reason, they are considered selective towards TrxR.[104,119–121]

On the side of trypanosomatids, more recent genome sequencing revealed they lack genes for GR and TrxR.[122–124] Trypanosomatids redox balance relies on a low molecular weight bis(glutathionyl) spermidine, known as trypanothione (TSH_2), and trypanothione reductase (TR; Figure 4).[124] The absence of functional redundancy within the parasite redox system and their sensitivity against oxidative stress turn the TSH_2/TR an attractive drug target.[125,126] Moreover, to obtain TR-knockout mutants is challenging and its downregulation impairs infectivity.[127–129]

At the beginning of the 2000s, studies suggested that TSH_2/TR was involved in the reduction and also the mechanism of action of Sb^{V}-based drugs.[130] Complexation of Sb^{III} by TSH_2 was observed by mass spectrometry.[131] In 2009, the crystal structure of TR treated with Sb^{III} showed the ion coordination to the Cys in the redox-active site and kinetic measurements confirmed its inhibition.[132] In 2012, Au^{I} also showed to coordinate to the active site and inhibit *L. infantum* TR. Ilari et al. reported the crystal structure of TR–auranofin binding (**1**, **2**).[66] Gold(I) binds linearly the Cys52 and Cys57, while the thiosugar was found to interact with His461, Glu466 and Glu467, placed in the inner cavity.[66] Colloti et al. screened Au^{I} and Au^{III} complexes to the TR inhibition constant (K_i), but they did not perform activity evaluations on parasite species. The K_i values varied from 40 to 25 000 nM. Auranofin showed $K_i = 155$ nM and, in general, Au^{III} performed better as TR inhibitors than Au^{I} complexes.[133]

From the fourteen articles from 2010 to date that evaluate the anti-leishmaniasis activity of gold complexes, one also

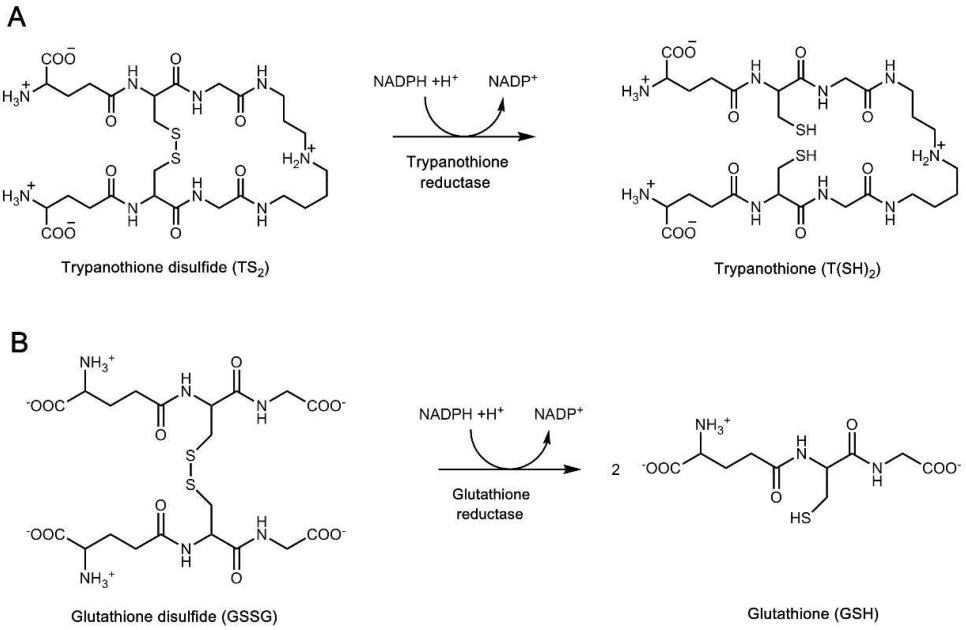

Figure 4. Schematic redox reactions for the A) trypanothione and B) glutathione systems.

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evaluated TR inhibition. Tunes et al. reported the inhibition of recombinant *Trypanosoma cruzi* TR by a series of gold(I) phosphine complexes of general coordination L–Au–PR_3 (**2**, **14**, **15**).[76] The IC_{50} (7.00–1.07 μM) is dependent on the nature of R and independent of the nature of L. The triethylphosphine complex presents lower inhibition concentrations than the triphenylphosphine. The inhibition of TR correlates with the anti-amastigote activity, thus suggesting TR as a target.

Selectivity is a fundamental parameter for a drug to avoid off-target interaction. TR is an attractive and validated target; though, substantial differences between parasites and host homologs are crucial to minimizing side-effects. Considering gold compounds, studies on the isolated TR showed promising inhibition values; nonetheless, the compounds might also present higher inhibition of TrxR (not evaluated in comparison). Given that GR is the closest human homolog and most gold compounds inhibit TrxR over GR preferentially, how could they be designed to be TR specific and avoid off-target TrxR or GR?

There are significant structural differences between TR and GR that might be key for the drug design to target the parasite protein. TSH_2 is the substrate of TR and it is bulkier than GSSG, the substrate of GR. Also, TSH_2 is positively charged in physiological pH, while GSSH is negatively charged. Consequently, TR has a wider, negatively charged active site, while GR is the opposite.[134–137] The selectivity of TR by TSH_2 is due to an interaction between spermidine moiety and Glu18, Trp21, Ser109, Tyr110, and Met114 placed at the entrance of the TSH_2 binding (catalytic) site. This site is known as the mepacrine binding site (MBS) and is replaced by Arg37, Arg38, and Arg347 in GR. Most of the designed TR organic inhibitors bind to the MBS.[137] Bulky, delocalized and hydrophobic organic compounds as acridine, modification of 3,4-dihydroquinazolines, diarylpirroles can bind to MBS.

A few ligands bind the inner cavity, close to the Cys52 and Cys57, which is rather inaccessible. Gold compounds act in the TSH_2 binding site by coordinating with Cys52 and Cys57. However, auranofin demonstrated that gold binds the cysteines and the thiosugar are retained in this inner cavity interacting with His461, Glu466 and Glu467.[66] This finding suggests a double mechanism of inhibition that could be used for the design of novel compounds. Other metals as Sb^{III} and Ag^{I} were co-crystallized with TR and also bind Cys52 and Cys57.[132,138]

4.2. Cysteine protease

Cysteine protease (CP) is a protease family that contains a cysteine as the nucleophile in the catalytic triad. They are mainly present in the lysosomes and act as proteolytic enzymes. Some cysteine proteases emerged as drug targets due to their relevance and overexpression in the disease progression.[139] Cathepsin B and cathepsin K were both considered relevant auranofin targets in the treatment of RA. Cathepsin K is the most potent collagenase. It is secreted by osteoclasts to resorb collagen by the bones and it is considered an essential target for bony disease therapies. Its overexpression is detected in RA and auranofin inhibition of cathepsin K could be one of the mechanisms.[140,141] Cathepsin B is overexpressed in several diseases, but it has a particular role in cancer progression.[142,143] It is used as a biomarker of cancer metastasis. Auranofin inhibits cathepsin B in low concentrations, K_i 144–180 μM. On the other hand, Fricker et al. developed Au^{III} cyclometallated complexes that inhibit Cathepsin B in the range 0.16–1.36 μM. In this work, we can highlight the compound diacetate(2-((2dimethylamino)-methyl)phenyl)Au^{III}.

Cysteine proteases are expressed during the whole life cycle of trypanosomatids and are essential to nutrition, reproduction, invasion and bypassing of the host immune response and consequently are central in the progression of the disease-associated. They were validated as targets for Chagas disease and African trypanosomiasis.[144] Cruzain, the specific *T. cruzi* cysteine protease, rhodasain, and Tb cathepsin B, specifically from *Trypanosoma brucei*, falcipain 1, 2, and 3 from *Plasmodium*, have been widely explored in drug discovery efforts.[145,146] However, only a vinyl sulfone based inhibitor of cruzain (K1117) is moving to clinical trials for Chagas disease therapy so far. This subject was recently reviewed.[147]

Leishmania species express a broad range of CPs. The best-characterized ones are cathepsin like CPA, CPB and CPC.[148,149] They are stage regulated, but most of them present their highest levels in the mammalian amastigote. Consistently they play central roles in the interaction parasite host. CPA knock-outs in *L. infantum* show lower *in vivo* infectivity, and *Leishmania mexicana* CPB knockdown led to impaired macrophage infectivity and delayed lesion progression. Some CPC inhibitors proved to be effective in the treatment of experimental cutaneous and visceral leishmaniasis.[150,151]

Fricker et al. compared the ability of cyclometallated Au^{III} complexes to inhibit a human liver cathepsin B, a recombinant *L. major* CPB, and cruzain. The best compound diacetate(2-((2-dimethylamino)methyl)phenyl)Au^{III} showed IC_{50} of 1.29, 0.7, and 1.7 μM for human cathepsin B, cruzain, and CPB, respectively, showing no relevant selectivity.[65]

More recently, Massai et al. evaluated the inhibition of *L. mexicana* recombinant mature CPB by Au^{I} and Au^{III} complexes **8** and **9**.[73] They compared to human cathepsin B and L. The IC_{50} of Au^{III} complex ranged from 5 to 8 μM while [AuCl(NHC)] IC_{50} was around 11 μM. In that case, Au^{III} compounds inhibited human and *Leishmania* cathepsin in the same range, while Au^{I} showed to be more potent to human cathepsin.

4.3. DNA

Historically, the isoelectronic and isostructural nature of Au^{III} complexes with Pt^{II} made them attractive for the design of anticancer drugs. They could present similar mechanisms in pharmaceutical activity, targeting DNA. However, given the high reduction potential of gold and fast ligand exchange reactions, reduction to Au^{I} and Au^{0} in the physiological conditions caused the mechanism to be very complex and the compounds significantly toxic.[152] Subsequently, ligands were designed to stabilize the Au^{III} center, for example, porphyrins, dithiocarbamate, organometallic cyclometalated and others.

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Although the square planar d^8 Au^{III} structures developed showed to be stable at physiological conditions, enzymes and proteins, especially the highly thiolated or selenium-based residues are still preferred targets over DNA.[93,153,154] Therefore, DNA may represent a primary target by ligand design, as demonstrated by some authors.[155–159]

The first mechanistic study evaluating gold compounds for leishmaniasis treatment explored the isolated biomolecules inhibition to identify possible targets. Navarro et al. investigated the interaction of Au^{III}-dipyrido[3,2-a:2′,3′-c]phenazine (dppz, $[Au^{III}(dppz)_2]Cl_3$) with DNA due to the ability dppz has to intercalate DNA.[63] The results based on spectrophotometrically, viscosity and electrophoretic mobility of DNA demonstrate interaction and suggest an intercalative mode for the gold complex. The nanomolar inhibition concentration of promastigotes forms of *L. mexicana* was suggested to be caused by DNA interaction.

As DNA is not the primary target of gold compounds, a few reports explored this target for gold-based antiparasitic compounds. Kinetoplastids contain a network of circular DNA inside a large mitochondrion that contains many copies of the mitochondrial genome called kinetoplast, also known as kDNA. It contains DNA maxicircles (20–40 kb) and minicircles (0.5–10 kb).[160] Several DNA binding compounds demonstrated antikinetoplastid activity by interfering in the kinetoplast function. Pentamidine is an important example. Diamines derivatives enter parasite cells rapidly and appear first in the kinetoplast. After some time, some derivatives are also found in the nucleus. They appear to selectively target AT sequences and change selectively the DNA minicircles, causing synergistic destruction of the catenated kinetoplast network and cell death.[161,162] Acridines derivatives also showed similar effects.[163,164] Design of gold complexes to target specifically these kDNA regions may be a good drug design strategy. Structures that can interact with non-canonical kDNA structures through noncovalent interactions (**17**), can promote interesting interactions with the parasite kinetoplast.[119]

4.4. Topoisomerase I

Human topoisomerase IB (TopIB) is a validated target for anticancer drugs.[165,166] It is responsible for reducing the DNA superhelical stress caused by the separation of DNA strands. It is a key enzyme for replication and repair. It is overexpressed in several types of cancer. The camptothecin derivatives, topotecan, irinotecan and belotecan are used alone or in combinatory regimens for cancer treatment.[167] They act by intercalating DNA bases once the cleavage produced by TopI occurred, blocking its religation and promoting apoptosis. Most of the inhibitors, as camptothecin, present a planar structure formed by fused heterocycles. Several Au^{III}-macrocyclic[168] and Au^{III}-complexes[169–171] showed to inhibit TopIB and less importantly some Au^{I} complexes.[172,173] For Au^{III} complexes, polydentate ligands containing planar aromatic moieties show low micromolar inhibition of TopIB.[168,174] In the case of Au^{I} organometallic ligands containing planar moieties formed of fused rings demonstrate the ability to inhibit the action of TopIB.[173] Most of the reports do not compare the inhibition of TopIB with other targets, as TrxR, except by Sâmia et al., which found the complex Au^{III}-3-(4-bromophenyl)-1-pyridin-2-ylprop-2-en-1-one thiosemicarbazone inhibits the TopIB in concentrations of the order of 1.5 μM while inhibition of TrxR was significant in concentrations higher than 10 μM.[170]

Members of the *Trypanosomatidae* family present a whole set of topoisomerases divided into two subcategories: nuclear TopIB and TopIIA, involved in DNA replication and repair, and mitochondrial TopIA and TopII, closely linked to kDNA replication.[175] From a structural point of view, TopIB is the most interesting as a drug target. Unlike all homologous enzymes, the trypanosomatid TopIB is the only Top whose two functional motifs are split out in two proteins, assembled in the presence of DNA, forming a heterodimer to recover activity.[176,177] The two subunits have different lengths and are known as the large and small subunit, respectively. The large subunit N-terminal domain does not match the human sequence, while the C-terminal aligns with the human TopIB core domain. In the small domain, the N-terminal aligns very well with the human Top while the C-terminal does not.

Camptothecin derivatives, especially gimatecan, showed to inhibit *L. infantum* parasites by inhibiting TopIB.[178] Naturally occurring flavones showed to block religation of *L. donovani* TopIB to DNA by stabilizing the complex.[179] *Leishmania* TopIB was sensitive to fatty acids[180] and indenosilquinolines.[181,182] None of them inhibited the assembly of the small and large subunits. It would be an interesting strategy for anti-*Leishmania* drug development. Further knowledge of the interaction interface of the two subunits is needed to provide a rational design for this type of inhibitor.[183]

4.5. Aquaporins

Water metabolism and cellular transport of water are essential for human, animal, vegetable and microbial life. The existence of water-specific transport membrane proteins has been predicted for decades.[184,185]

Later, the characterization of a family of water and solute-permeable membrane proteins, known as aquaporins (AQP), showed its essentiality to all domains of life. Twelve types of AQP are present in mammalians, divided into three groups according to their primary structure and permeability: i) *orthodox or classical* AQPs, considered water selective; ii) *aquaglyceroporins*, permeable to glycerol and other small solutes in addition to water (e.g., urea, hydrogen peroxide, ammonia, metalloids); and iii) *superaquaporins*, with lower sequence homology to the other mammalian AQPs and unique subcellular localization, whose specificity has been hard to establish.

Most studies on *Leishmania* AQP were performed in *L. major*[186] and *L. donovani*.[187] Five AQP genes have been identified.[188] Its localization and permeability profile (water, glycerol, urea, methylglyoxal) are compatible with functions in

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osmo-protection, for example, during transmission and metabolism.[186,188]

In general, it is quite striking that all characterized plasma membrane residing parasite AQPs exhibit biochemical properties of the mammalian aquaglyceroporin subfamily and a broad permeability profile, which includes, besides water, relevant metabolites, such as glycerol, urea, ammonia, and various carbonyl compounds.[189] The biochemical and physiological data hint at several crucial functions of parasite AQPs: i) Alleviation of osmotic stress, e.g. during the passage of the salt-laden kidneys or during transmission between the insect and the human host; ii) Uptake of glycerol from the host blood serum as a precursor for glycerolipid synthesis, enabling rapid parasite growth by extension of the lipid plasma membrane; iii) Release of nitrogen waste, i.e. urea and ammonia, and of aldehyde metabolites, that is, methylglyoxal, preventing self-intoxication of the parasite; iv) New data suggest that the cell motility of amoeba may depend on AQP water permeability; v) Drug resistance mechanisms have been directly linked to AQPs in *Leishmania* and *Trypanosoma*.[189]

Overall, due to the numerous possible physiological roles of AQPs in parasite growth and development, the identification of selective inhibitors is likely to lead to the discovery of drugs with novel mechanisms of action. Transgenic parasites expressing AQP specific domains have propelled our knowledge on the role of AQP, such as Gene et al.'s findings showing that *L. infantum* promastigotes overexpressing Li-BH3AQP (a specific amino acid stretch at the C terminus of a described aquaporin) were more resistant to hypotonic stress or nutrient deprivation when compared with wild-type parasites.[190] A few differences in parasite isoforms are present, despite the high similarity with the human isoforms, which can be exploited in the design of selective inhibitors. Casini et al. were the first group to investigate the possibilities to design gold-based AQPs inhibitors. They reported the potent and selective inhibition of human isoform AQP3 by a water-soluble gold(III) compound, $[Au(phen)Cl_2]Cl$ (phen = 1,10-phenanthroline, auphen). The compound auphen, inhibited glycerol transport in human red blood cells (hRBC), with an IC_{50} of 0.8 μM, while having no inhibitory effect on water permeability mediated by the water channel AQP1.[191] The compound effects were confirmed in transfected PC12 cell lines with overexpression of either AQP1 or AQP3. In silico studies demonstrated the selective blockage of AQ3 versus AQ1, due to the accessibility of Cys40 in AQ3, which binds to Au^{III}.[192–194]

Another interesting compound is the dioxo-bridged dinuclear gold(III) complexes with 2,9-dimethyl-1,10-phenantroline (**9**) could dissociate into monomers $[Au^{III}(phen)L]^{n+}$ in biological medium and be a candidate for the inhibition of aquaporins.[73,79] Although only human isoforms were assessed, it is worth investigating the potential of gold-based compounds for parasite AQ isoforms.

4.6. Zinc fingers

Zinc finger (ZF) domains are protein motifs increasingly recognized for their relevance and importance in diseases. They contain a structural zinc ion coordinated to cysteines and histidines in a tetrahedral geometry. ZFs are primarily classified based on the identity of the zinc coordinating residues and named Cys_2His_2 (CCHH), Cys_3His (CCHC, CCCH), and Cys_4 (CCCC) types. As the largest transcription factor family in the human genome, they are involved in several cellular functions like transcription, apoptosis, DNA repair and RNA packaging.[158] Progressively, researchers find straight evidence for their role in cancer progression, immune regulation, cytokine production and other inflammatory mediators.[195] ZF motifs have been reported in several virus proteins and have been shown to play critical roles in their respective multiplication sites. The high conservation of these domain sequences in different viral strains turns them into attractive targets for antiviral therapies.[196,197]

In kinetoplastids, gene expression regulation occurs mostly post-transcriptionally.[198] Consequently, RNA-binding proteins play a critical role in the regulation of several functions as differentiation from the bloodstream to procyclic forms, modulation of mRNA in bloodstream forms,[199,200] development in vector (flies), differential regulation of metabolism and nuclear export of mRNA,[201] cell cycle and rRNA processing[202] and other housekeeping functions as protection from heat-shock stress.[203]

In general, CCCH type ZF proteins are RNA-binding proteins with regulatory functions at all mRNA metabolism stages. A systematic listing of CCCH proteins in kinetoplastids was made available by *in silico* screening and a set of CCCH domains was identified, being 48 in *T. brucei*, 51 in *T. cruzi* and 54 in *L. major*, excluding redundancy.[204] Interestingly, putative Mex67 orthologue and a *Leishmania*-specific 3′-exoribonuclease have a CCCH motif that is not found in their counterparts in other eukaryotes. The vast majority of the CCCH ZF proteins are unique to kinetoplastids or a subgroup within.[204]

ZF studies revealed that zinc displacement or replacement for other metals decrease or disrupt the ability of the domain to bind DNA/RNA. It represents a possible mechanism of toxicity of metals, as Pb^{II}, and the implication in these domains in the metallodrugs interactions. The discovery opened the doors for the design of metal complexes that target ZF domains.[205,206] As Cys-rich proteins, ZFs are excellent targets for Au^{III}/Au^{I} compounds. For example, the organometallic cyclometalated compound $[Au(pyb\text{-}H)Cl_2]$ (pyb = 2-benzylpyridine, Au–C^N) inhibited the anticancer drug target PARP-1 *in vitro* at nM level, which is an order of magnitude more potent than that of the FDA-approved drug Olaparib.[207] This class of compounds is also able to arylate cysteine residues.[208,209] Furthermore, Abbehausen and co-workers have exploited the potential of damaging the antiretroviral molecular target nucleocapsid protein 7 from HIV-1 by disruption of its two CCHC ZF domains via gold binding.[210] Mechanistic studies show the possibility of a specific inhibitor design for HIV-1 NCp7.[211] More recently, it was found that the $[Au^{I}PCy_3L]$ compounds (Cy = cyclohexyl)

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inhibit the NC-SL2 DNA interaction due to the steric hindrance of the long-lived [(PCy_3)Au–NCp7].[212]

Another remarkable aspect of parasite ZF is their relation to the activity of the widely used Sb-based compounds. Sb^{III} displaces zinc from CCHC NCp7-HIV-1 zinc finger model peptide,[213] and the antimony-containing compound, NSC 13778, was found to effectively inhibit the nucleic acid chaperone activity of the viral nucleocapsid NCp7 protein of HIV.[214] Competition experiments using CCHC and CCCH *Leishmania* zinc finger model peptides demonstrated that Sb^{III} displace the Zn^{II} ion preferentially from CCCH models than CCHC models.[215]

One of the main challenges of ZF targeting is to achieve the desired selectivity.[205,216] Further knowledge of the structure and function of kinetoplastid ZF is needed; however, the sequencing and comprehension of structural differences between host and parasite domains are central for the antiparasitic inhibitors' design.

5. Conclusions and Perspectives

Investigation of gold complexes as antileishmanial agents has emerged in the last decade. Considering the gold-based drugs' immunomodulation activity in RA, the thioredoxin/thioredoxin reductase inhibition and the redox imbalance promoted by gold-based compounds, it makes perfect sense to explore their effectiveness against *Leishmania* parasites. The set of results, mostly *in vitro*, suggests that Au^{I} compounds are more potent than Au^{III}. Among Au^{I} complexes, N-heterocyclic carbenes are the most explored ligands, varying mostly in the N-group. Neutral [$Au^{I}Cl$(NHC)] and cationic [Au^{I}(NHC)$_2$]$^+$ had their performance varying according to the groups attached. Phosphines pose as a second important class of ligands in Au^{I} complexes and the numbers suggest they are significantly potent, in some cases surpassing the *in vitro* activities of [Au^{I}(NHC)], although the direct comparison is available only in a few reports. Auranofin also appears to be potent, being a promising option for repurposing studies. As models and protocols vary significantly among the articles, the results' comparison is quite difficult, which makes a structure/activity definition limited.

In vivo evaluations using murine models were performed in two independent works for auranofin (**1**) and adamantyl derivatives of triethylphosphines **14** and **15**. Despite the fact that protocols, *Leishmania* species and administration routes were very different, the studies independently showed significant and promising results.

Trypanothione/trypanothione reductase is the most studied target for gold, based on the known selective inhibition of the human thioredoxin system. Very few studies on cathepsin were performed. Other prospective targets for gold were described in this review. Recently, membrane disruption experiments on gold compounds **17**–**19** showed their ability to promote this effect. Though trypanothione is an attractive target for gold, distinct sets of enzymes and metabolic pathways should be explored to design selective leishmanicidal drugs.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: gold complexes · *Leishmania* · leishmaniaisis · targets

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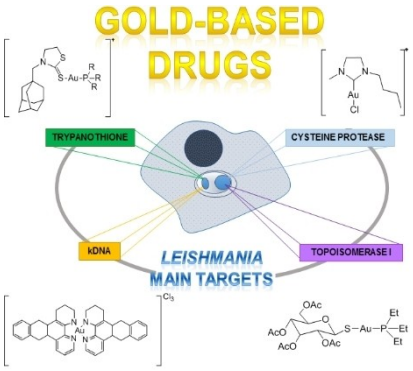
REVIEWS

The study of gold-based compounds as therapeutic agents for *Leishmania* has emerged in the last decade. The promising results demonstrate that gold compounds are alternatives for therapy of this neglected disease. In this review, the studies are comprehensively organized to show recent advances in this field. Prospective targets that could inspire investigators to design selective antileishmaniasis candidates are also described.



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A "Golden Age" for the discovery of new antileishmanial agents: Current status of leishmanicidal gold complexes and prospective targets beyond the trypanothione system

A Golden Age for the discovery of new antileishmanial agents: Current status of leishmanicidal gold complexes and prospective targets beyond the trypanothione system (Abbehausen @unicampoficial)

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