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Synthesis, characterization and evaluation of antileishmanial activity of copper(II) with fluorinated α-hydroxycarboxylate ligands

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Abstract In this study, Cu(II) complexes with fluorinated ligands were produced aiming at the development of new, less toxic antileishmanial metallodrugs. Complexes of the general formula CuL_2 (L = lactate, trifluorolactate, 2-hydroxyisobutyrate, trifluoro-2-hydroxyisobutyrate) were synthesized in methanolic medium, purified by crystallization and characterized by elemental analysis and electronic and infrared spectroscopies. In vitro experiments with *Leishmania amazonensis* promastigotes showed that the trifluorolactate derivative more active than its non-fluorinated counterpart. Our results indicate that fluorinated chelators may be interesting to increase metal toxicity and/or open new paths for metallodrug chemotherapy against leishmaniasis.

Keywords Leishmaniasis · Neglected disease · Copper · Fluorine

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Introduction

Leishmaniasis is an infection caused by protozoa of the *Leishmania* genus, which infect macrophages of vertebrate hosts resulting in effects such as skin ulceration, mucous membrane destruction and general infection of viscera. These symptoms define, respectively, the cutaneous, muco-cutaneous or visceral forms of the disease. The leishmaniasis cycle starts when phlebotomine insects (sandfly, genus *Lutzomyia* or *Phlebotomus*) inoculate the parasite in the promastigote phase into the vertebrate host, leading to invasion and/or quick phagocytosis by neutrophils and macrophages. In the macrophages, the surviving parasites multiply as amastigotes which will infect other cells throughout the organism. Host inflammatory and immune responses are ineffective to check the progression of the disease, leading to the progressive development of clinical symptoms in a period of weeks to months. It is estimated that 1–1.5 million new cases of cutaneous leishmaniasis and 0.5 million new cases of visceral leishmaniasis develop annually, with a prevalence of 12 million patients in the world, the vast majority in poor or underdeveloped nations (Murray 2005).

The therapeutic arsenal to treat leishmaniasis is relatively limited, based mostly on antimonial compounds and a few other drugs. Examples of the latter include amphotericin B, introduced in the mid-1980s, pentamidine and, more recently, miltefosine (Croft et al. 2005; Murray 2005). Several other molecules,

such as nerolidol (Arruda 2005), modified chalcones (Boeck 2006) and several 2,4-diamminoquinazolines (Khabnadideh 2005) have been shown to be active in vitro and/or in experimental models of the disease, but none of these have been approved for clinical trials.

Antimony compounds have long been used to treat leishmaniasis, the first of which was emetic tartar (antimony(III) tartrate). However, the high toxicity of antimony (III) results in serious side effects (gastrointestinal intolerance, cardiotoxicity, nephrotoxicity) which have rendered emetic tartar useless for practical purposes (Rath et al. 2003). Since then, trivalent antimonials have been substituted by less toxic pentavalent derivatives such as the sugar complexes sodium stibogluconate (Pentostan®) and meglumine antimoniate (Glucantime®) (Marsden 1985; Berman 1988).

The mechanism of action of pentavalent antimonial compounds is not fully understood. They are probably reduced in vivo by parasite thiols (tripanothione) to the trivalent, more toxic forms, suggesting that the Sb(V) complex acts as a pro-drug. The higher toxicity of the reduced form is probably related to its "softer" character and therefore its preference to bind to sulfur-containing biological substrates responsible for the redox balance of the parasite (e.g., tripanothione). Nucleoside binding and inhibition of DNA repair, followed by induction of apoptosis, is another biochemical route prone to be disturbed by Sb(III) species (Yan et al. 2005). However, Sb compounds are also harmful to the host, leading to serious side effects. Furthermore, resistance to pentavalent antimonials has emerged and it is now widespread in some areas, particularly on the Indian subcontinent (Croft et al. 2006). Therefore, the search for other active metal complexes persists (Fricker et al. 2008).

Copper as a metal center of choice appears as an interesting alternative, since its complexes are usually less toxic than their Sb congeners. Also, if the "activation by reduction" mechanism is in fact important, copper has reduced forms (based on Cu^{+}) which are more toxic than the oxidized ones (Lim et al. 2006).

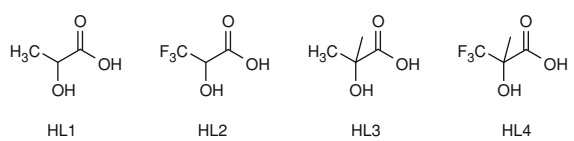
Fluorination confers interesting properties to organic compounds. Fluorine is a small, highly electronegative, atom, with a smaller volume than groups such as CH_3, NH_2 and OH. The C–F bond is highly polar but only slightly polarizable, accounting in some instances for the low aqueous solvation energies and high lipophilicities of some fluorinated molecules in comparison with their non-fluorinated analogs (Kim 1998; Biffinger et al. 2004; Gerebtzoff 2004; Shimizu and Hiyama 2005). Fluorine is already widely present in medicine: currently, there are *ca.* 130 drugs containing at least one F atom in their composition (Bohm 2004). Other benefits of organic fluorination may include increased metabolic stability, increased affinity for a substrate, and altered nucleophilicity. Substitution of an H for an F atom in a drug molecule often improves its bioavailability (Gerebtzoff 2004; Shimizu and Hiyama 2005).

In this study we prepared new fluorinated α-hydroxycarboxylate derivatives of Cu(II) in order to compare the effect of this functionalization on the anti-proliferative activity of the complexes towards *Leishmania*. The α-hydroxycarboxylate motif was chosen as a simplified version of the coordination mode of carbohydrates in antimonial antileishmanial drugs. We observed that bis(trifluorolactato)copper(II) was the most active against promastigotes of *Leishmania amazonensis* promastigotes, suggesting that fluorination may account for increased toxicity in some instances.

Materials and methods

Chemicals: $CuSO_4{\cdot}5H_2O$ (Cromoline, Brazil); methanol, D,L-lactic acid, 2-hydroxypropionic acid (Sigma); trifluorolactic acid, 2-(trifluoromethyl)-2-hydroxypropionic acid (Matrix Scientific) were used without further purification. The structures of the ligands are depicted in Structure 1.

Synthesis: The complexes were formed by the treatment of 0.75 M $CuSO_4$ aqueous solutions with equal volumes of aqueous solutions of the ligands (L) in order to attain 1:2 Cu:L final molar ratios. The mixtures were stirred at room temperature;



Structure 1 Structures of the ligands. HL1 = lactic acid; HL2 = trifluorolactic acid; HL3 = 2-hydroxypropionic acid; HL4 = 2-(trifluoromethyl)-2-hydroxypropionic acid

Table 1 Elemental analysis results for the copper complexes

	%C		%H	
	Calc.	Exp.	Calc.	Exp.
$Cu(L1)_2(H_2O)_2$	25.95	25.61	5.08	5.13
$Cu(L2)_2(H_2O)_5$	16.47	16.12	2.76	2.44
$Cu(L3)_2(H_2O)_2$	31.42	31.72	5.93	5.41
$Cu(L4)_2(H_2O)_2$	23.23	23.30	2.92	2.72

immediately after mixing the reagents the development of different hues of blue was observed, depending on L. The pH was adjusted to 7 with NaOH(aq) in order to ensure total deprotonation of the carboxylic moiety of L. Finally, the solutions were treated with 4×100 ml methanol and a white precipitate was observed (probably NaOH in excess or Na_2SO_4) and discarded. The blue methanolic solutions were concentrated to 20 ml and kept in the refrigerator for 2 days. The crystals were filtered, washed once with cold methanol and oven dried. The elemental analysis results follow in Table 1.

Instrumentation: Infrared spectra were obtained in KBr pellets in a Bomem MB100 instrument. Electronic spectra were acquired in a Tecan Saphire instrument at room temperature.

Partition studies: Distribution of the copper complexes through multilayer lecitin vesicles (MLV) was studied as a means to assess lipophilicity in a biologically relevant model (Engelmann et al. 2007). In 1.5 ml plastic vials, 100 µl of Cu(II) complexes *ca* 30 mM were added to 100 µl of buffered MLV suspension prepared as previously described (Engelmann et al. 2007). The vials were vortexed by 5 min at room temperature, centrifuged and the absorbance of the supernatants was determined at the λ_{max} values of each metal complex (see Table 2). Partition coefficients were determined by the ratio of the absorbance values after and prior the treatment with MLV.

Table 2 Maximum wavelengths (λ_{max}) in different solvents

Compound	H_2O	dmso
$Cu(L1)_2$	762	734
$Cu(L2)_2$	732	700
$Cu(L3)_2$	770	748
$Cu(L4)_2$	760	742

Biological studies: Promastigotes of *L. amazonensis* were grown in Medium 199 supplemented with 10% fetal calf serum (FCS) at 25°C as described previously (Arruda 2005; Souza et al. 2006). Inhibitory concentrations of the copper compounds were determined by seeding 1.3×10^6 promastigotes/ml in the presence of increasing concentrations of the copper complexes (1–1,000 µM), in 96-well culture plates (Corning Life Sciences, Corning, NY) for 48 and 72 h. Quantification of viable cells was assessed by cell counting in Neubauer chambers after 48 and 72 h of incubation with the test solutions. Assays were performed in triplicate, and results are expressed as the mean percent reduction of parasite numbers compared to untreated control wells calculated for at least two independent experiments. The 50% inhibitory concentrations (IC_{50}) were determined from sigmoidal regression of the concentration-response curves using Scientific Graphing and Analysis Software ORIGIN 7.5.

Results and discussion

Fluorination of the ligand induces hypsochromic shifts in the visible spectra of the complexes of *ca*. 10 nm ($Cu(L3)_2 \rightarrow Cu(L4)_2$) and 30 nm ($Cu(L1)_2 \rightarrow Cu(L2)_2$), Fig. 1 and Table 2. This effect is observed both in water and in dimethyl sulfoxide (dmso). These figures correlate with the increased π-acidity of the ligands provided by the CF_3 groups, rendering L2 and L4 with an overall better π-acceptor character than their non-fluorinated analogs and thus increasing the Δ_O values in their complexes (Shriver and Atkins 1999). Positive inductive effects of the methyl groups in L3 and L4 may compensate, to some extent, the electron-removal effect of the CF_3 group, which might explain the smaller shift observed for the $Cu(L3)_2 \rightarrow Cu(L4)_2$ substitution. For any of the complexes, exchanging water for dmso as the solvent leads to a hypsochromic shift of *ca*. 30 nm ($Cu(L1)_2$, $Cu(L2)_2$) or 20 nm ($Cu(L3)_2$, $Cu(L4)_2$). This effect is correlated with the better π-acceptor character of dmso as compared to water. Also, molar absorptivity values of *ca*. 130 M^{-1} cm^{-1} are consistent with *d–d* transitions (Shriver and Atkins 1999).

Inspection of infrared spectra (Fig. 2) revealed characteristic frequencies of the organic moiety of the complexes (Table 3) which allowed us to confirm their

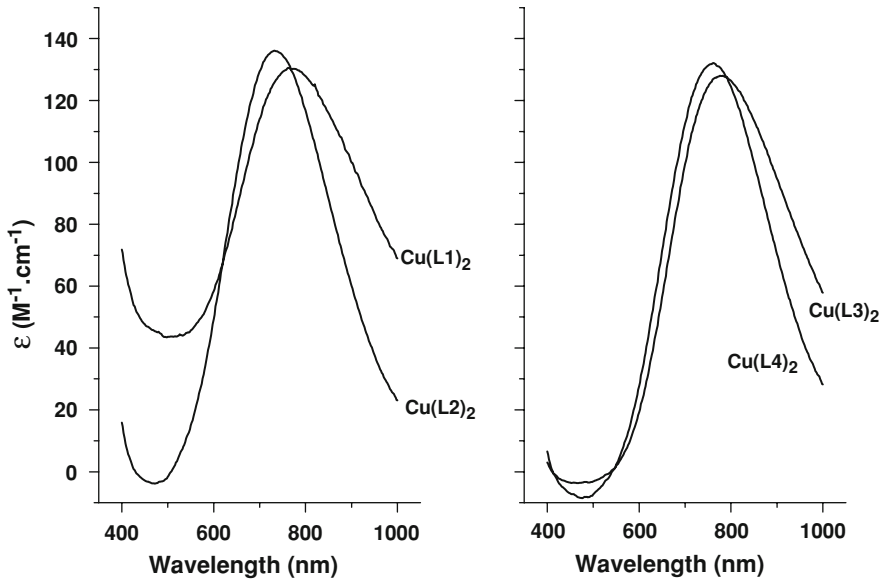


Fig. 1 Visible electronic spectra of *ca* 10 mM aqueous solutions of the complexes

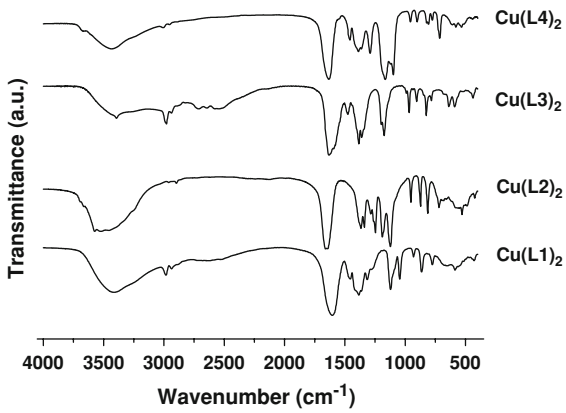


Fig. 2 Infrared spectra in KBr pellets of the copper complexes

coordination modes. Both ν_{as}(COO) and ν_s(COO) vibrations are coherent with deprotonated carboxylates (theoretical ranges of 1,550–1,650 and 1,400 cm^{-1}, respectively (Goulden 1960; Silverstein et al. 2005)), with Δ(COO) values of 200–300 cm^{-1} indicating monodentate carboxylate binding to the metal center (Deacon and Philips 1980). Frequencies at *ca*. 3,500 cm^{-1} are attributable to ν(OH) at the α-carbon (Silverstein et al. 2005), which remained comparatively unchanged in comparison to the respective parent α-hydroxycarboxylic acids (data not shown), indicating that this oxygen remains protonated during coordination. Frequencies of around 1,000–1,260 cm^{-1} may be attributed to ν(CO) of the alpha carbon—OH stretch. Bands at 1,100–1,350 and 700–750 cm^{-1} appear only in the fluorinated complexes and are indicative of ν(CF) stretching frequencies (Silverstein et al. 2005).

α-hydroxycarboxylic acids behave as bidentade ligands toward Cu(II) (Dillon and Rossotti 1966), producing neutral 1:2 complexes with lactate (Bobtelsky and Bar-Gadda 1953; Mohanty and Patnaik 1984) and other compounds (Goulden 1960; Forrest et al. 1966; Prout et al. 1968; Buravchuk et al. 1974). A pH adjustment to neutral is required to keep the carboxylic moiety of the ligands deprotonated, however, in all instances the α-OH groups coordinate to the metal without deprotonation. The Jahn-Teller effect stabilizes Cu(II) ions in a tetragonally distorted

Table 3 Selected infrared frequencies (cm^{-1}) for the copper complexes in KBr pellets

Compound	ν_{as}(COO)	ν_s(COO)	Δ(COO)	ν(αOH)	ν(CO)	ν(CF)
$Cu(L1)_2$	1,609	1,391	218	3,447	1,124	
$Cu(L2)_2$	1,666	1,371	295	3,570	1,126	1,248, 723
$Cu(L3)_2$	1,647	1,387	260	3,402	1,176	
$Cu(L4)_2$	1,643	1,393	250	3,472	1,173	1,296, 716

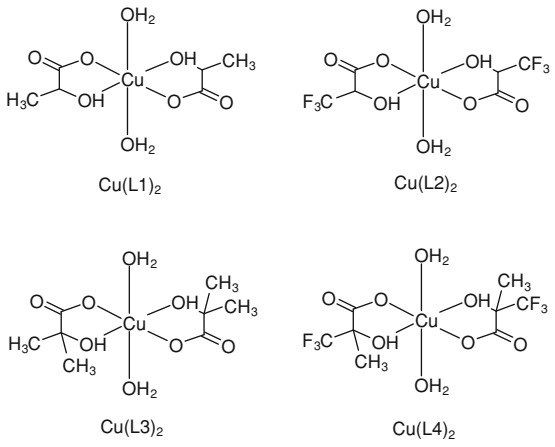


Fig. 3 Proposed structures for the Cu(II) complexes in this study

octahedral environment, with two solvent molecules in the axial positions and two bidentate ligands *trans* to each other in the plane. α-hydrocarboxylates coordinate through one oxygen of the carboxylate group and through the hydroxyl oxygen at the alpha carbon, which remains protonated (Bolard 1965; Carballo et al. 2001). Our infrared data indicate that our complexes fit this structural motif, and their proposed structures are shown in Fig. 3.

All the complexes are fairly soluble in water, with solubilities of *ca.* 1 g ml^{-1} ($Cu(L1)_2$, $Cu(L3)_2$, $Cu(L4)_2$,) or 0.2 g ml^{-1} ($Cu(L2)_2$) at 25°C. Despite considerable water solubility, copper trifluorolactate (L2) was the most hydrophobic compound when challenged to multilayer lecitin vesicles (MLV; Table 4), forming metal complexes of amphiphilic character as observed in other metal fluorocarboxylates (Esposito et al. 1999).

Table 4 IC_{50} values (μM ± SD; $n = 3$) of the copper complexes after 48 and 72 h of treatment on promastigotes of *L. amazonensis* and partition coefficients (*P*) in multilayer lecitin vesicles

Compound	48 h	72 h	*P*
$CuSO_4$	218.4 ± 40.0	106.5 ± 11.1	–
$Cu(L1)_2$	242.9 ± 32.1	292.7 ± 11.4	0.055
$Cu(L2)_2$	257.7 ± 42.5	166.7 ± 18.3	0.152
$Cu(L3)_2$	339.6 ± 41.0	187.4 ± 21.5	0.040
$Cu(L4)_2$	412.4 ± 94.7	272.9 ± 21.3	0.082

The biological activities of the Cu(II) complexes and $CuSO_4$ were tested in promastigotes of *L. amazonensis* (Fig. 4) and cytotoxicity was also determined against a mammalian cell line (HeLa). The parent α-hydrocarboxylic acids were also tested but displayed virtually no activity (data not shown).

Dose-response profiles for the parasite obtained at different time intervals indicated maximum activity against the parasites at 72 h (Table 4). There was no statistically significant differences ($P > 0.05$) in the IC50 values of the copper complexes and $CuSO_4$, indicating that the complexes keep the same biocidal activity of the "free" ion. Along with the fact that the α-hydroxycarboxylic acids displayed no activity, it seems clear that the chelator role in these complexes is thus to ferry the toxic metal center in a stable way to its target. Chelation allows for better targeting and protection against possible side reactions, making it possible, in some instances, for the complex to be more active than either the metal or ligands alone, as demonstrated for a mixture of Cu(II) and lactic acid against *Escherichia coli* or a number of different *Salmonella* strains in different growth media (Ibrahim et al. 2008). This is very important for systemic metallodrug chemotherapy, which can not rely on the simple administration of metal salts due to the possibility of a multitude of side reactions. Specifically, Cu(II) has a very high affinity binding site in the abundant plasma protein albumin (Harford and Sarkar 1997), therefore copper chelation is important to protect the metal ion and decrease administered doses. Our results indicate that we were successful in

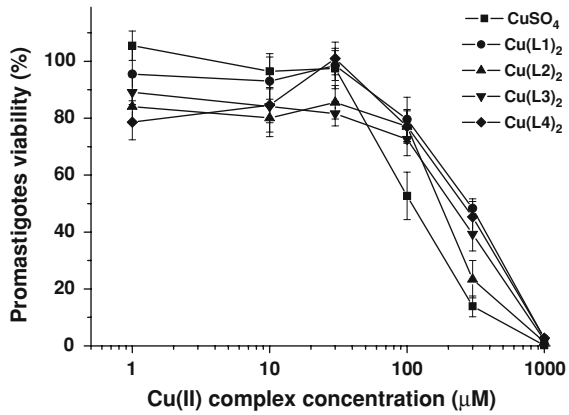


Fig. 4 Dose-response curves after 72 h for promastigotes of *L. amazonensis* incubated with the copper compounds

producing biologically active copper complexes which retain the biological toxic activity of the copper ion at the same time that may allow for enhanced stability towards undesired side reactions involving the metal center and non-targets.

Despite the similarity of the antileishmanial activities (Table 4; Fig. 4), hydrophobic copper trifluorolactate was more toxic than its non-fluorinated analog copper lactate, probably due to enhanced parasite membrane permeation of the former compound. CF_3 and CH_3 possess opposite inductive effects, and the insertion of both groups at the same time (as in $Cu(L4)_2$) seems to reduce the therapeutic value of the final complex. All the copper species induced a maximum of *ca*. 65% toxicity toward mammalian (HeLa) cells at their highest concentrations, allowing us to estimate their IC_{50} values at higher than 300 μM, i.e., two to three times higher than the values for the promastigotes (data not shown). Further studies in infected animals are needed in order to assess whether this difference is therapeutically useful.

Other metallodrugs have been developed for antileishmanial therapy. Platinum bound to sterol hydrazones in concentrations of *ca*. 10 μM decreased the proliferation of *L. mexicana* promastigotes by around 70% (Visbal et al. 2008). Parasitic cysteine proteases have been successfully inhibited by a plethora of complexes of Au(III), Pd(II) and Re(V) compounds, with IC_{50} values ranging from 0.009—1.4 μM (Fricker et al. 2008). DNA intercalators based on Cu(II) (Navarro et al. 2003a, b) or Au(I) (Navarro et al. 2007) are interesting antileishmanial drugs; $[Au(dppz)_2]Cl_3$ showed a marked antiproliferative activity against *L. mexicana*, with 17 nM LD26 after 48 h. Here, we showed that fluorination of Cu(II) complexes may increase their activity (provided that inductive effects are taken into account), and probably increase the activity of other complexes as well.

Conclusions

We showed in this work that Cu(II) complexes with α-hydrocarboxylates retain the toxic activity of copper ions towards *L. amazonensis*. The strategy of ligand fluorination allowed us to develop a lipophilic complex (bis(trifluorolactato)copper(II)) whose antileishmanial activity against *L. amazonensis* was higher than the activity of its non-fluorinated analog, indicating that ligand fluorination may prove to be an alternative to the rational design of new, more active antileishmanial metallodrugs.

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References

Arruda D (2005) Antileishmanial activity of the terpene nerolidol. Antimicrob Agents Chemother 49:1679–1687

Berman JD (1988) Chemotherapy for leishmaniasis–biochemical mechanisms, clinical efficacy, and future strategies. Revi Infect Dis 10:560–586

Biffinger JC, Kim HW, DiMagno SG (2004) The polar hydrophobicity of fluorinated compounds. Chembiochem 5:622–627

Bobtelsky M, Bar-Gadda I (1953) Complexes of copper with alkali salts of phthalic, malonic, succinic, maleic, salicylic, and lactic acids-constitution and properties. Bull Soc Chimique France 27:6–279

Boeck P (2006) Synthesis of chalcone analogues with increased antileishmanial activity. Bioorg Med Chem 14: 1538–1545

Bohm HJ (2004) Fluorine in medicinal chemistry. Chembiochem 5:637–643

Bolard J (1965) Infrared spectra of complex salts of hydroxyacids. I. Cobalt(II)-, nickel(II)- and copper(II) salts. J Chimie Physique Physico-Chimie Biologique 62:900–907

Buravchuk YP, Sokolov VP, Kogan VA, Osipov OA, Parimski A (1974) Coordination-compounds of Divalent Cobalt, Nickel and Copper with Higher Alpha-Hydroxycarboxylic Acids. Zh Obshchei Khimii 44:1626–1630

Carballo R, Castineiras A, Covelo B, Vazquez-Lopez EM (2001) Coordination polymers of copper(II) based on mixed N- and O-donor ligands: the crystal structures of CuL2(4, 4′-bipy)(n) (L = lactate or 2-methyllactate). Polyhedron 20:899–904

Croft S, Barret M, Urbina J (2005) Chemotherapy of trypanosomiases and leishmaniosis. Trends Parasitol 21: 508–512

Croft SL, Sundar S, Fairlamb AH (2006) Drug resistance in leishmaniasis. Clin Microbiol Rev 19:111–126

Deacon GB, Philips RJ (1980) Relationships between the carbon-oxygen stretching frequencies of carboxylato complexes and the type of carboxylate coordination. Coord Chem Rev 33:227–250

Dillon KB, Rossotti FJ (1966) Location of binding sites for Cu(II) in carboxylate ligands by differential proton relaxation. Chem Comm 21:768–769

Engelmann FM, Mayer I, Gabrielli DS, Toma HE, Kowaltowski AJ, Araki K, Baptista MS (2007) Interaction of cationic meso-porphyrins with liposomes, mitochondria and erythrocytes. J Bioenerg Biomembr 39:175–185

Esposito BP, Faljoni-Alario A, de Menezes JFS, de Brito HF, Najjar R (1999) A circular dichroism and fluorescence quenching study of the interactions between rhodium(II)

complexes and human serum albumin. J Inorg Biochem 75:55–61

Forrest JG, Prout CK, Rossotti FJ (1966) Crystal structures of copper(II) alpha-hydroxy- and alpha-alkoxy-carboxylates. Chem Comm 65:8–659

Fricker SP, Mosi RM, Cameron BR, Baird I, Zhu YB, Anastassov V, Cox J, Doyle PS, Hansell E, Lau G, Langille J, Olsen M, Qin L, Skerlj R, Wong RSY, Santucci Z, McKerrow JH (2008) Metal compounds for the treatment of parasitic diseases. J Inorg Biochem 102:1839–1845

Gerebtzoff G (2004) Halogenation of drugs enhances membrane binding and permeation. Chembiochem 5:214–231

Goulden JDS (1960) Infrared spectra of lactates in aqueous solution. Spectrochim Acta 16:715–720

Harford C, Sarkar B (1997) Amino terminal Cu(II)- and Ni(II)-binding (ATCUN) motif of proteins and peptides: metal binding, DNA cleavage, and other properties. Acc Chem Res 30:123–130

Ibrahim SA, Yang H, Seo CW (2008) Antimicrobial activity of lactic acid and copper on growth of *Salmonella* and *Escherichia coli* O157:H7 in laboratory medium and carrot juice. Food Chem 109:137–143

Khabnadideh S (2005) Design, synthesis and evaluation of 2, 4-diaminoquinazolines as inhibitors of trypanosomal and leishmanial dihydrofolate reductase. Bioorg Med Chem 13:2637–2649

Kim H (1998) Structure and transport properties of a novel, heavily fluorinated carbohydrate analogue. J Am Chem Soc 120:9082–9083

Lim CY, Yoo YH, Sidharthan M, Ma CW, Bang IC, Kim JM, Lee KS, Park NS, Shin HW (2006) Effects of copper (I) oxide on growth and biochemical compositions of two marine microalgae. J Environ Biol 27:461–466

Marsden PD (1985) Clinical presentations of Leishmania braziliensis braziliensis. Parasitol Today 1:129–133

Mohanty NK, Patnaik RK (1984) Lactate complexes of cadmium(II), zinc(II), cobalt(II), nickel(II) and copper(II). J Inst Chem (India) 56:91–94

Murray H (2005) Advances in Leishmaniasis. Lancet 366: 1561–1577

Navarro M, Cisneros-Fajardo EJ, Fernandez-Mestre M, Arrieche D, Marchan E (2003a) Synthesis, characterization, DNA binding study and biological activity against *Leishmania mexicana* of Cu(dppz)(2)BF_4. J Inorg Biochem 97:364–369

Navarro M, Cisneros-Fajardo EJ, Sierralta A, Fernandez-Mestre M, Silva P, Arrieche D, Marchan E (2003b) Design of copper DNA intercalators with leishmanicidal activity. J Biol Inorg Chem 8:401–408

Navarro M, Hernandez C, Colmenares I, Hernandez P, Fernandez M, Sierraalta A, Marchan E (2007) Synthesis and characterization of Au(dppz)(2)Cl_3. DNA interaction studies and biological activity against Leishmania (L) mexicana. J Inorg Biochem 101:111–116

Prout CK, Armstron Ra, Carruthe JR, Forrest JG, Murrayru P, Rossotti FJ (1968) Structure and stability of carboxylate complexes. 1.Crystal and molecular structures of copper(2) glycollate, DL-lactate, 2-hydroxy-2-methylpropionate, methoxyacetate and phenoxyacetate. J Chem Soc A 279:1–2813

Rath S, Trivelin LA, Imbrunito TR, Tomazela DM, de Jesus MN, Marzal PC, Junior HFD, Tempone AG (2003) Antimonials employed in the treatment of leishmaniaisis: the state of the art. Quim Nova 26:550–555

Shimizu M, Hiyama T (2005) Modern synthetic methods for fluorine-substituted target molecules. Angew Chem Int Ed 44:214–231

Shriver D, Atkins P (1999) Inorganic chemistry. Oxford University Press, Oxford

Silverstein RM, Webster FX, Kiemle D (2005) Spectrometric identification of organic compounds. Wiley, New York

Souza GFP, Yokoyama-Yasunaka JKU, Seabra AB, Miguel DC, Oliveira MG, Uliana SRB (2006) Leishmanicidal activity of primary S-nitrosothiols against Leishmania major and *Leishmania amazonensis*: Implications for the treatment of cutaneous leishmaniasis. Nitric Oxide 15: 209–216

Visbal G, Marchan E, Maldonado A, Simoni Z, Navarro M (2008) Synthesis and characterization of platinum-sterol hydrazone complexes with biological activity against *Leishmania* (L.) *mexicana*. J Inorg Biochem 102:547–554

Yan S, Jin L, Sun H (2005) 51-Sb antimony in medicine. In: Gielen M, Tiekink ERT (eds) Metallotherapeutic drugs and metal-based diagnostic agents. Wiley, New York, pp 441–461