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Synthesis and antimalarial activity of metal complexes of cross-bridged tetraazamacrocyclic ligands

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Abstract

Using transition metals such as manganese(II), iron(II), cobalt(II), nickel(II), copper(II), and zinc(II), several new metal complexes of cross-bridged tetraazamacrocyclic chelators namely, cyclen- and cyclam-analogs with benzyl groups, were synthesized and screened for *in vitro* antimalarial activity against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum*. The metal-free chelators tested showed little or no antimalarial activity. All the metal complexes of the dibenzyl cross-bridged cyclam ligand exhibited potent antimalarial activity. The Mn²⁺ complex of this ligand was the most potent with IC₅₀s of 0.127 and 0.157 μM against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) *P. falciparum* strains, respectively. In general, the dibenzyl hydrophobic ligands showed better antimalarial activity compared to the activity of monobenzyl ligands, potentially because of their higher lipophilicity and thus better cell penetration ability. The higher antimalarial activity displayed by the manganese complex for the cyclam ligand in comparison to that of the cyclen, correlates with the larger pocket of cyclam compared to that of cyclen which produces a more stable complex with the Mn²⁺. Few of the Cu²⁺ and Fe²⁺ complexes also showed improvement in activity but Ni²⁺, Co²⁺ and Zn²⁺ complexes did not show any improvement in activity upon the metal-free ligands for anti-malarial development.

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Keywords

Antimalarial agents; metal complexes; tetraazamacrocycles; cross-bridged tetraazamacrocycles; synthesis; biological activity

1. Introduction

Malaria is a major health problem, especially in the developing countries¹ of South Saharan Africa, Southeast Asia and in South America. Today, almost all the existing antimalarial drugs, particularly the 4-aminoquinoline based drugs, such as chloroquine (CQ) that have historically been used to treat the disease effectively, have been rendered less effective due to chemo-resistance problems.² Artemisinin and its derivatives offer promising curative alternatives but due to their thermal instability^{3,4} and high cost of therapy⁵ in the economically disadvantaged regions of the world where the disease is prevalent, access to them is difficult, and their therapeutic use has been severely limited.

Due to the prevalence of *Plasmodium falciparum* strains resistant to chloroquine and other antimalarial drugs, the search for new antimalarial drugs has been of high priority for the control of malaria. Over the past two decades, a number of antimalarial agents, particularly the 4-aminoquinoline-based drugs, have been developed and tested against chloroquine-resistant parasites.^{6,7} Although there has been substantial improvement in the conventional organic synthetic strategies used for the development of antimalarial agents, researchers have sought out ways to develop more innovative approaches in order to develop more efficacious drugs to cure the disease. One of the most promising new approaches involves the use of transition metal ion complexes to produce novel antimalarial drugs.⁸

Metal-based chemotherapies have existed for centuries, but in recent years there has been an increasing interest in the application of transition metal complexes or organometallic complexes in medicine and in other areas of biological sciences.⁹⁻¹² Metal complexes have been used as drugs in a variety of diseases, as exemplified by the continued success of the platinum complex, cis-PtCl₂(NH₃)₂ (cisplatin), as an anticancer drug.¹³⁻¹⁵ This important breakthrough has indeed stimulated a renewed interest in metal complex based chemotherapy. Today, other metal-containing drugs have been developed in a variety of therapeutic areas including malaria. To ensure that effective metal containing antimalarial agents are produced, the present study exploited the metal-drug synergism approach.¹⁶⁻¹⁹

Thus far, several reports have shown that incorporation of transition metal ions into organic pharmacophores offer new opportunities to design unique metal-containing compounds which compliment the molecular diversity created by purely organic scaffolds.^{20,21} These reports show that the incorporation of transition metal ions into rationally designed ligands can result in enhancement of the biological activity.²² There are also several reports of enhancement of the efficacy of existing drugs, e.g. chloroquine, when transition metal ions were coordinated to the parent drug structures.²³ A thorough literature review revealed that several transition metal complexes exhibit high antimalarial activity against chloroquine-sensitive and -resistant strains of *P. falciparum* and, consequently, have become antimalarial drug candidates. It is also well documented that many metal complexes of

chloroquine or other 4-aminoquinoline based antimalarials have activities superior to that of chloroquine, which is one of the most successful drugs currently being used for antimalarial chemotherapy. The consistent enhancement of these drugs when coordinated to metal ions reinforces the fact that metal complexes are important resources for the generation of structural or chemical diversity in the area of antimalarial drug development.^{20,23}

In a recent report, a gold-chloroquine antimalarial agent was obtained by coordinating chloroquine (CQ) to a $[\text{Au}(\text{PPh}_3)]^+$ fragments to give a new compound, $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6$, which is more active than CQ alone *in vitro* against cultures of chloroquine resistant strains of *P. falciparum* and also against *P. berghei* both *in vitro* and *in vivo*.²³ Also, it has been reported that the coordination complexes of gold(I) and thiosemicarbazone exhibited enhanced antiplasmodial activity against two *P. falciparum* strains (D10 and W2) *in vitro* when compared to the activity of the ligand alone.²⁴

Many other enhancements of antimalarial activity by metal complexation of a variety of ligands compared to the antimalarial activity of the free ligands themselves have been extensively documented. For example, a reaction of the chloroquine (CQ) free base with $[\text{Rh}(\text{COD})\text{Cl}]_2$ (COD = 1,5-cyclooctadiene) yielded $\text{Rh}(\text{COD})(\text{CQ})\text{Cl}$, which has comparable *in vitro* antimalarial activity to that of chloroquine diphosphate and reduced parasitemia 1.33 times more than chloroquine *in vivo*, without any sign of acute toxicity observed up to 30 days.^{25,26}

It has been documented that because of the avidity of *Plasmodium* parasites for free iron, one way of using iron in antimalarial drug design is by adding iron to an existing drug such as chloroquine to effectively remove the chloroquine resistance.^{27–29} Several studies have shown that organometallic complex based drug molecules are very promising in overcoming various types of drug resistance and allow improved specificity and drug targeting, thus minimizing side effects associated with chemotherapy.³⁰ For example, ferrocene serves as a reliable organometallic scaffold for the construction of an ordered structure via hydrogen bonding.^{31,32} It is worth noting that as a consequence of this drug development effort, the novel antimalarial drug, ferroquine (FQ), in which ferrocene group is incorporated into chloroquine structure, is being developed at Sanofi-Avantis.³³

In order to circumvent drug resistance caused by the *P. falciparum* parasites, we have employed the metal coordination approach to prepare series of metal complexes using crossbridged and side-bridged tetraazamacrocyclic ligands **L1**, **L2**, and **L3** (Fig. 1). The effect of metal coordination on the ligands was investigated for antimalarial activity.

Even though the synthetic tetraazamacrocyclic compounds, particularly, cyclen and cyclam, and their analogs or their metal complexes have been extensively utilized in applications of a variety of diagnostic and magnetic resonance imaging (MRI) contrast agents,³⁴ their metal based derivatives are still relatively unexplored in antimalarial drug development. A recent report, in which the 7-chloro-4-(1,4,7,10-tetraaza-cyclododec-1-yl)-quinoline- Zn^{2+} complex was found to demonstrate a high antimalarial activity has motivated us to pursue the present investigation.³⁵ We hypothesized that the particularly kinetically inert transition metal complexes of cross-bridged tetraazamacrocycles^{36–49} (Fig. 1, **L1–L3**) would be stable,

potentially active antimalarials against drug-sensitive and –resistant strains of malarial parasites.³⁵ These complexes have several properties that suggested this activity to us: (1) tight-binding metal complexation that will prevent loss of the metal ion even *in vivo*; (2) aromatic functional groups reproducing the aromaticity of chloroquine; and (3) multiple amine functional groups, as also present in chloroquine.

The ligands **L1–L3** in Fig. 1 represent different combinations of aromatic and tetraazamacrocycle units for screening to determine which combination of groups maximizes antimalarial efficacy. Ligands **L1** and **L2** both contain two aromatic groups and only one tetraazamacrocycle. Ligand **L3** contains only one aromatic group and two tetraazamacrocycles. **L3** was also chosen because it has proven to be biologically active against other disease states. Bis-tetraazamacrocycles are potent CXCR4 antagonists, as exemplified by the FDA approved drug Plerixafor. Cross-bridged analogues, particularly in their transition metal complexes, have demonstrated CXCR4 binding efficiency even superior to Plerixafor.^{50–54} Development of this route for biological activity has encouraged us to explore other potential disease states which may benefit from these cross-bridged bis-tetraazamacrocycle complexes. Finally, we also screened six different metal ions in their complexes with each ligand. This approach was taken to allow us to draw a conclusion about which metal ion is most appropriate for inclusion in metal complex antimalarials.

2. Results and Discussion

2.1. Chemistry

The ligands **L1**, **L2** and **L3** have all been published previously.⁵⁵ Transition metal complexes of these ligands were generally formed from anhydrous metal salts in nonprotic solvents in an inert atmosphere glove box. Mn^{2+} , Fe^{2+} , and Co^{2+} divalent complexes are often air sensitive and must be protected from oxygen. Additionally, it is known that protection of cross-bridged tetraazamacrocycle ligands from sources of water, or even protic solvents, is helpful in complexation reactions as they are strongly basic and protonation can defeat complexation.

2.1.1. Manganese Complex $[\text{Mn}_2(\text{L3})\text{Cl}_4]$ —This complexation reaction is representative of all of the **L3** complexes. Complexation of the ligand was carried out using the anhydrous metal chloride salt in anhydrous DMF in an inert atmosphere glove box (Scheme 1). The anhydrous MnCl_2 is not very soluble at room temperature in DMF, so the reaction was heated to $\sim 50^\circ\text{C}$ and it proceeded smoothly at this temperature with stirring overnight. This complex is air sensitive, as indicated by formation of a brown solution when the reaction solution was exposed to air.

2.1.2. Copper Complex $[\text{Cu}(\text{L1})(\text{OAc})]\text{PF}_6$ —This complexation is representative of all of the dibenzyl macrocyle complexes of **L1** and **L2**. Complexation of the ligand was carried out using the anhydrous metal acetate salt in anhydrous DMF in an inert atmosphere glove box and proceeded smoothly at room temperature with overnight stirring (Scheme 2). Once the complexation had occurred, the reaction solution was removed from the glove box and concentrated to dryness, which yielded a viscous oil product. In order to produce a more easily handled solid complex, as well as to purify the product, an anion metathesis reaction

with NH_4PF_6 in dry methanol was carried out. With only two *cis* coordination sites available, only one acetate anion was able to coordinate to the metal ion, leaving an uncoordinated acetate anion that could be replaced with PF_6^- . Hexafluorophosphate anions precipitated the complex cation from methanol as a microcrystalline powder.

2.2. Biological Evaluation

2.2.1. *In Vitro* Antimalarial Activity—Both the free ligands and their metal complexes were tested for *in vitro* antimalarial activity against the D6 (chloroquine sensitive) and W2 (chloroquine resistance) strains of *P. falciparum*. The test performed was based on the determination of plasmodial lactate dehydrogenase (LDH) activity. Varying concentrations of the test samples of either the complexes or the free ligands were added to a suspension of red blood cells infected with *P. falciparum* (D6 or W2) strains containing 2% parasitemia and 2% hematocrit in RPMI-1640 medium supplemented with 10% human serum. Inhibition of the uptake of [^3H] hypoxanthine served as a measure of parasite viability^{56,57} and the potencies of all the test compounds against chloroquine resistant strains of *P. falciparum* were also confirmed by [^3H] hypoxanthine incorporation. Artemisinin and chloroquine were used as drug controls and DMSO was included as a vehicle control. The selectivity index (SI) and cytotoxicity to mammalian cells were also determined.

As shown in Table 1, while the Mn^{2+} and Co^{2+} complexes of the dibenzyl cross-bridged cyclam ligand **L1** demonstrated a higher antimalarial activity against the chloroquine resistant (W2) strain of the parasite than the D6 strain, the Fe^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} complexes of the same ligand all showed lower antimalarial activity towards W2 than towards D6. The manganese complex is clearly the most active complex against both strains of the malarial parasites, with nearly an order of magnitude greater SI than some of the other metals. In fact, this manganese complex was the most active of all of the compounds tested.

For the metal complexes of the dibenzyl cross-bridged cyclen ligand **L2**, the Cu^{2+} complex showed a higher antimalarial activity against the chloroquine resistant (W2) strain of the parasite, while the Mn^{2+} complex demonstrated a lower, but comparable antimalarial activity against the parasites. The Fe^{2+} , Co^{2+} , Ni^{2+} , and Zn^{2+} complexes were generally less effective against the chloroquine resistant parasite, and less active overall than either Mn^{2+} or Cu^{2+} . All of the metal complexes showed low to moderate antimalarial activity against the chloroquine sensitive (D6) strain of the parasite.

Bis-macrocyclic ligand **L3** demonstrated an activity towards both malaria strains similar to that of the monocyclic dibenzyl ligands **L1** and **L2**. Interestingly, the ligand made from the combination of two tetraazamacrocycles and one aromatic group (**L3**) exhibits nearly the same activity as the combination of two aromatic groups and one tetraazamacrocyclic, at least for the free ligands themselves. However, a striking difference is seen when comparing the transition metal complexes of **L3**. Only the manganese complex of **L3** was active against either malarial strain. Why the linking of two such macrocycle complexes together greatly reduces the activity is not readily apparent, although the higher positive charge carried by these bimetallic complexes may prevent them from readily crossing the parasite's membranes. It is also worth emphasizing, once again, the superior activity of the manganese complex, compared to the other metal ions. This appears to be a general trend of these

tetraazamacrocyclic complexes, and is one that we hope to test further in future studies on other manganese analogues.

With regard to the cyclam **L1** analogs, the activity ratio of metal complex: free ligand, with the ligand value normalized to 1.00 as calculated by dividing the IC_{50} (free ligand)/ IC_{50} (metal complex) for the antimalarial activity against chloroquine sensitivity (D6) strains of *P. falciparum* was in the range of 3.25–17.1. For the antimalarial activity against chloroquineresistant (W2) strains of *P. falciparum*, the ratio was in the range of 3.29–34.3. The increasing order of potency of antimalarial activity (W2 and D6) for these complexes is $Co^{2+} < Ni^{2+} < Cu^{2+} < Zn^{2+} < Fe^{2+} < Mn^{2+}$. In contrast, for the cyclen **L2** analogs, the same ratio for the D6 strains of the *P. falciparum*, was in the range of 0.656–9.18 and for the W2 strains of the *P. falciparum*, the ratio was in the range of 0.526–6.44; with the copper complex exhibiting the most potency followed by the manganese complex.

In comparison of these complexes to the positive controls, chloroquine and artemisinin, the cyclam based manganese complex which is the most potent, is 4.24 times less potent than chloroquine and 6.54 times less potent than Artemisinin against the chloroquinesensitive (D6) strains of *P. falciparum*. However, it also shows signs of toxicity as indicated by the Vero IC_{50} value. It is also worth noting that this complex is superior to chloroquine and it is 5.35 times more potent than chloroquine against the chloroquine-resistant (W2) strain of *P. falciparum* although it is 32 times less potent than artemisinin.

These results suggest that the higher antimalarial activity displayed by the manganese complex for the cyclam ligand compared to that of the cyclen may be due to the larger pocket of the cyclam which makes it form more stable complexes with manganese.⁴⁰ The cavity size difference also results in adjustments in the redox properties of the two complexes,⁴⁵ which may also be important, depending on the mechanism of action. We should be able to tune multiple properties of these complexes by adjusting the cavity size and changing the benzyl substituents to other groups. Interestingly, the manganese complex of **L1** exhibits superior antimalarial activity against the chloroquine-resistant strains of *P. falciparum* in comparison to chloroquine.

The fact that all the free ligands tested showed fairly low antimalarial activity, suggests that the metal complexation may have played a critical role in making the complexes exhibit antimalarial activity, rather than the roles played by the free ligand or the metal alone.²² Additionally, the generally low cytotoxicity of these ligands against mammalian cells, also suggests that the ligands are attractive for further development of new metal complex based antimalarial drugs.²²

3. Conclusions

The screening of the antimalarial activity metal complexes of the tetraazamacrocyclic compounds generated by reactions of cyclam or cyclen cross-bridged and side-bridged ligands with the respective transition metal ions of Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} , was carried out. The results revealed that coordination of a transition metal ion to a biologically active molecule may result in an improvement of the activity of the resulting

molecule depending on the identity of the metal ion chosen. As shown in Table 1, this trend is illustrated by the coordination of Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} ions to the dibenzyl cross-bridged cyclam ligand **L1**, which produced complexes with increased potency, whereas only the Co^{2+} complex of the same ligand **L1** did not result in an enhancement of activity compared to the free ligand. This data demonstrates that although the biological activity of a molecule can be enhanced through metal ion coordination, the improvement occurs only when a suitable metal-ligand pairing is made. Interestingly, only Cu^{2+} and Mn^{2+} enhanced the activity of **L2**, emphasizing that a suitable metal-ligand pairing is required. The same metals (Fe^{2+} , Ni^{2+} , Zn^{2+}) which enhance the activity of **L1** do not enhance potency when incorporated into a very similar analogue, **L2**. Apparently, there is a fine balance between the properties of the organic ligand and the metal ion that must be optimized to produce enhancement in biological activity. Finally, the metal complexes generated using ligand **L3**, are mostly inactive. The use of two tetraazamacrocyclic pharmacophores and only one aromatic group does not appear to lead to improved antimalarial activity for the resulting metal complexes, even though the ligand itself has activity similar to **L1** and **L2**, and **L3** complexes are known to have other biological activity.^{50–54} Perhaps the additional positive charge and polarity accompanying the second metal ion are too much to overcome in order to get the drug molecule into the parasite. It should be noted, however, that the Mn^{2+} complex of **L3** does in fact increase its activity. In all three ligand cases, Mn^{2+} improves the activity. This property of Mn^{2+} will be explored further with other tetraazamacrocyclic ligands.

Based on the findings of this investigation, a significant insight into the design of new transition metal based antimalarial drugs was obtained. These results will support the further development of this promising new class of compounds with potential for the development of new drug(s) for malaria chemotherapy that may contribute to improving future therapeutic potentials. Furthermore, although certainly not an exhaustive survey, certain metal ions which were found to be suitable for the design of the new metal complexes of tetraazamacrocyclic derivatives for malaria chemotherapy have been identified. Depending on the ligand used, manganese(II), copper(II), and iron(II) have shown to be the most suitable metals that can form the basis for rational antimalarial drug design.

4. Experimental Section

4.1. Chemistry

General—All the materials were reagent grade, and used as supplied. The reactions were performed in anhydrous solvents under inert atmosphere unless otherwise indicated. Anhydrous solvents (acetonitrile and DMF) as well as all other reagents were used as received from a commercial source. Elemental analysis was carried out by Quantitative Technologies, Inc. in Whitehouse, NJ. Electrospray MS was obtained using a Shimadzu LCMS-2020 in 1:1 methanol/water mixture. ^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively on Bruker 300 spectrometer with TMS as internal standard.

4.2. Synthesis of ligands

Ligand **L1**, 4,11-dibenzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane and **L2**, 4,10-dibenzyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane were prepared as outlined in reference.⁵⁵ Ligand **L3**, 4-methyl-11-[4-(4-methyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadec-11-ylmethyl)-benzyl]-1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane was prepared according to the literature procedure.⁵⁸

4.3. General synthetic procedure for complexes **M(L)Cl₂** and **[M(L)(OAc)]PF₆**

Reactions of MnCl₂ or FeCl₂ with ligands **L1** and **L2** in a 1:1 ratio under mild conditions in a glove box gave the complexes Mn(L)Cl₂ and Fe(L)Cl₂, respectively.⁴⁵ The complexes were isolated in good yields as air stable white solid Mn(L)Cl₂ and/or pale brown Fe(L)Cl₂. Complexes [M(L)(OAc)]PF₆, where M= Cu, Zn, Ni, and Co, were also synthesized in two steps by first reacting the appropriate metal acetates with the ligands **L1**, **L2**, and **L3** in CH₃CN or DMF at room temperature for 24 h, followed by solvent removal to give an oily product which was subsequently dissolved in methanol and reacted with 5 equivalents of NH₄PF₆ to give the desired complexes.⁵⁸ All these new complexes were characterized by electrospray, MS, and elemental analyses. Complexes Mn(**L1**)Cl₂, Fe(**L1**)Cl₂, Mn(**L2**)Cl₂ and Fe(**L1**)Cl₂ were synthesized^{45,58} and complex [Ni(**L1**)OAc]PF₆ was synthesized according to the literature procedure.⁵⁹

4.3.1. Synthesis of Tetrachloro(4-methyl-11-[4-(4-methyl-1,4,8,11-tetraazabicyclo [6.6.2]hexadec-11-ylmethyl)-benzyl]-1,4,8,11-tetraaza-bicyclo [6.6.2]hexadecane) manganese(II) [Mn₂(L3)Cl₄]—The ligand, **L3** (0.300 g, 0.511 mmol) and anhydrous manganese(II) chloride salt (0.129 g, 1.02 mmol) were added to 20 ml of dry DMF in an inert atmosphere glove box. Upon stirring and heating (~50 °C) both reactants dissolved to give a colorless solution. Overnight stirring at this temperature resulted in the formation of a white precipitate. The reaction was stopped after stirring for ~18 h and cooled to room temperature. Inside the glove box, the white solid product was collected by filtration on a fine glass frit and washed with DMF followed by ether, then dried under vacuum to give the product (0.256 g, 60%). FAB⁺ mass spectral analysis yielded peaks at m/z = 797, consistent with Mn₂LCl₃⁺, and m/z = 379, consistent with Mn₂L₃Cl₂²⁺. Elemental analysis (%) calcd. [Mn₂C₃₄H₆₂N₈Cl₄] (834.599 g/mol): C 48.93, H 7.49, N 13.43; Found C 48.54, H 7.59, N 13.24.

4.3.2. Synthesis of Acetato(4,11-dibenzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane)copper(II)hexafluorophosphate[Cu(L1)(OAc)]PF₆—Ligand **L1**, (0.406 g, 1.00 mmol) and anhydrous copper(II) acetate salt (0.182 g, 1.00 mmol) were added to 25 ml of dry DMF in an inert atmosphere glove box. The reaction was stirred at room temperature for 18 h. The crude blue-green [Cu(**L1**)(OAc)] [(OAc)] solution was removed from the glove box, filtered to remove any trace solids, and evaporated to dryness, giving a blue-green oil. The crude product was dissolved in 10 ml of dry methanol, to which was added dropwise, a 5 ml dry methanol solution of 5 equivalents (0.815 g, 5.00 mmol) of NH₄PF₆. A blue-green powder of the [Cu(**L1**)(OAc)]PF₆ salt precipitated immediately, and was stored overnight in a freezer at -5 °C to complete the precipitation. The solid was collected on a fine glass frit, washed with cold methanol and

ether, and dried under vacuum to give the final product (0.573 g, 83%). Elemental analysis (%) calcd. $[\text{CuC}_{26}\text{H}_{38}\text{N}_4(\text{C}_2\text{H}_3\text{O}_2)]\text{PF}_6 \cdot \text{H}_2\text{O}$ (692.185 g/mol): C 48.59, H 6.26, N 8.09; Found C 48.65, H 6.18, N 8.21. MS (ES) m/z 528.3 and 530.3 $[\text{CuL}(\text{OAc})]^+$.

4.4. Biological evaluation

4.4.1. *In vitro* antimalarial activity—The *in vitro* antimalarial activity was measured by a colorimetric assay that determines the parasitic lactate dehydrogenase (pLDH) activity. The assay was performed in a 96-well microplate using two *P. falciparum* clones, [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. For the assay, a suspension of red blood cells infected with *P. falciparum* (D6 or W2) strains (200 μL , with 2% parasitemia and 2% hematocrit in RPMI-1640 medium supplemented with 10% human serum and 60 $\mu\text{g}/\text{mL}$ amikacin) was added to the wells of a 96-well plate containing 10 μL of test samples at various concentrations. The plate was flushed with a gas mixture of 90% N_2 , 5% O_2 , and 5% CO_2 in a modular incubation chamber (Billups-Rothenberg, 4464 M) and incubated at 37 $^\circ\text{C}$ for 72 h. Plasmodial LDH activity was determined by using Malstat reagent (Flow Inc., Portland, OR) as described earlier.^{56,57} The IC_{50} values were computed from the dose-response curves generated by plotting percent growth against test concentrations. DMSO (0.25%), artemisinin, and chloroquine were included in each assay as vehicle and drug controls, respectively. The selectivity indices (SI) were determined by measuring the cytotoxicity of samples toward mammalian cells (Vero; monkey kidney fibroblast).

4.4.2. Cytotoxicity assay—The *in vitro* cytotoxic activity was determined against noncancerous Vero cell lines (monkey kidney fibroblast), obtained from the American Type Culture Collection (ATCC, Rockville, MD). The assay was performed in 96-well tissue culture treated microplates. Cells (25 000 cells/well) were seeded in the wells of the plate and incubated for 24 h. Samples were added and plates were again incubated for 48 h. The number of viable cells was determined using Neutral Red according to a modification of the procedure of Borenfreund et al.^{60,61}

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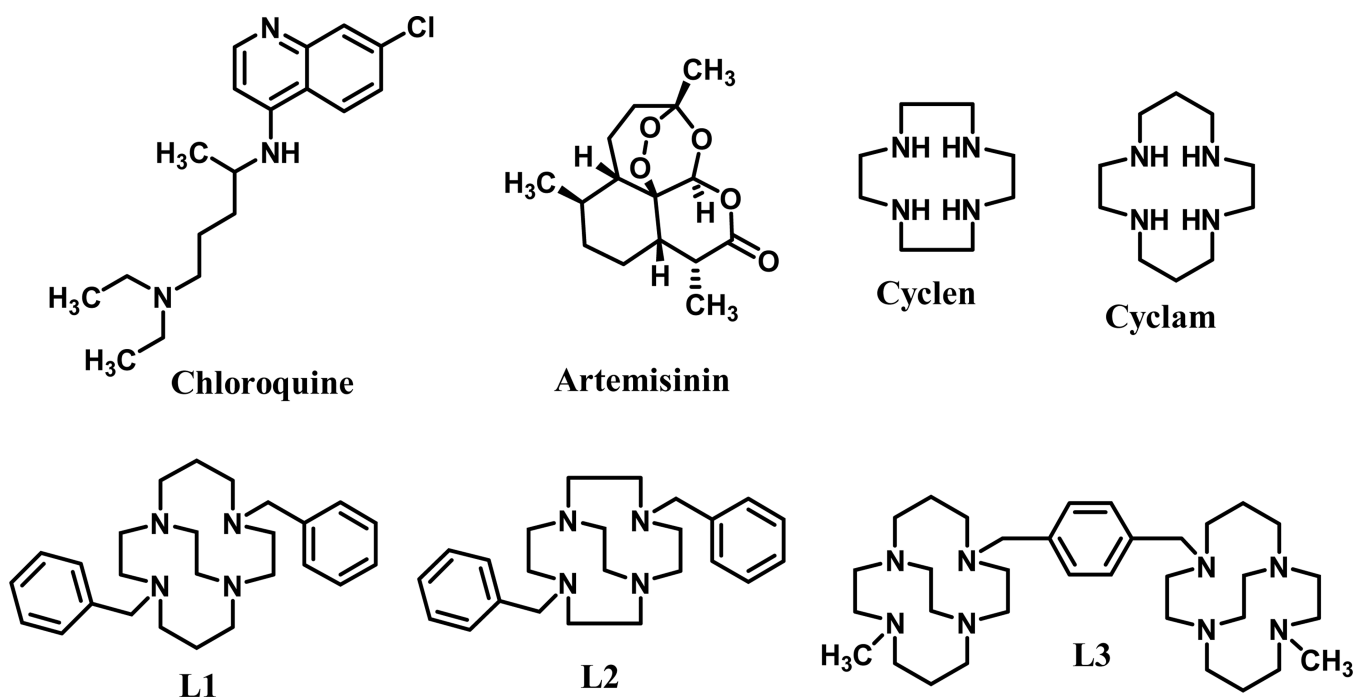
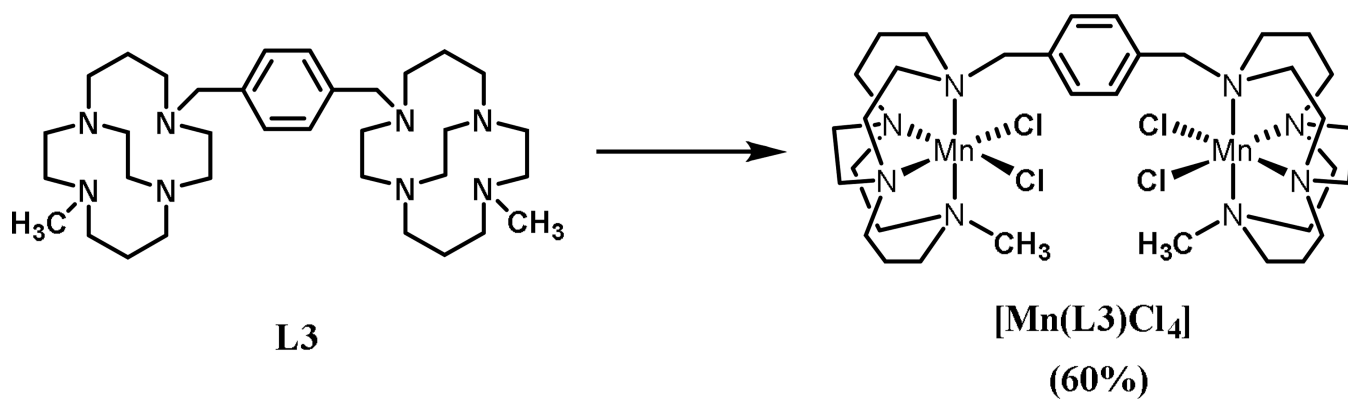
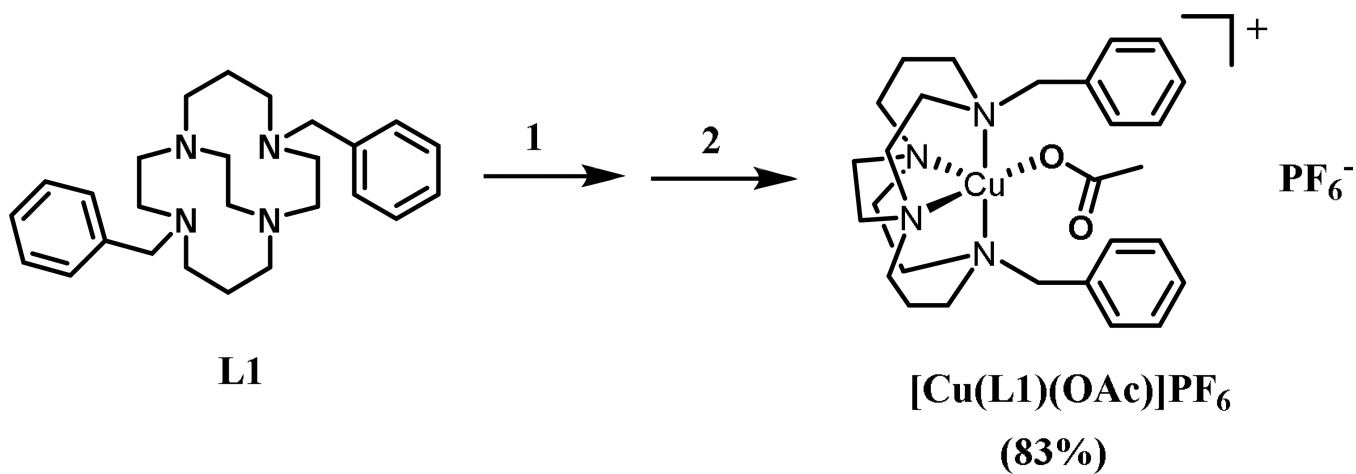


Figure 1.
Structures of ligands used for the preparation of complexes and standard drugs (chloroquine and artemisinin) used as controls.

**Scheme 1.**

Synthetic procedure of metal complex **[Mn(L3)Cl₄]**. Reagents and conditions: MnCl₂, anhydrous DMF, 50 °C, 18 h, 60%.

**Scheme 2.**

Synthetic procedure for **[Cu(L1)(OAc)]PF₆**. Reagents and conditions: (1) **Cu(OAc)₂**, anhydrous DMF, rt, 18 h. (2) **NH₄PF₆**, MeOH.

Table 1

In Vitro Antimalarial Activities of Ligands and Complexes Against D6 and W2 strains of *Plasmodium falciparum*.

Compound	D6 IC ₅₀ (μ M)	D6 SI	W2 IC ₅₀ (μ M)	W2 SI	VERO IC ₅₀ ng/mL
L1	2.679	>4.4	4.358	>2.7	NC
Mn(L1)Cl ₂	0.157	38.45	0.127	47.793	3222.8
Fe(L1)Cl ₂	0.324	>27.6	0.426	>21	NC
[Co(L1)(OAc)]PF ₆	NA	-	5.162	>1.4	NC
[Ni(L1)(OAc)]PF ₆	0.825	>8.6	1.326	>5.4	NC
[Cu(L1)(OAc)]PF ₆	0.423	>16.7	0.809	>8.7	NC
[Zn(L1)(OAc)]PF ₆	0.342	>20.6	0.593	>11.9	NC
L2	1.350	>9.3	2.008	>6.3	NC
Mn(L2)Cl ₂	0.265	>35.6	0.545	>17.3	NC
Fe(L2)Cl ₂	1.384	>6.8	2.139	>4.4	NC
[Co(L2)(OAc)]PF ₆	NA	-	NA	-	NC
[Ni(L2)(OAc)]PF ₆	1.110	>6.7	1.538	>4.8	NC
[Cu(L2)(OAc)]PF ₆	0.147	>50.3	0.312	>23.6	NC
[Zn(L2)(OAc)]PF ₆	2.057	>3.6	3.814	>1.9	NC
L3	1.278	>6.4	1.414	>5.8	NC
[Mn ₂ (L3)Cl ₄]	0.957	>6	0.959	>5.9	NC
Fe(L3) ₂ Cl ₄	NA	-	NA	-	NC
[Co ₂ (L3)(OAc) ₂](PF ₆) ₂	NA	-	NA	-	NC
Ni ₂ (L3)(OAc) ₂](PF ₆) ₂	NA	-	NA	-	NC
[Cu ₂ (L3)(OAc) ₂](PF ₆) ₂	NA	-	NA	-	NC
[Zn ₂ (L3)(OAc) ₂](PF ₆) ₂	NA	-	NA	-	NC
Artemisinin (ART)	0.024	>>35.5	0.004	>211.4	NC
Chloroquine (CQ)	0.037	20.4	0.679	>1.1	NC

NC, no cytotoxicity up to about 10 μ M; NA, no activity; SI, selectivity index (IC₅₀ for Vero cells/IC₅₀ for *P. falciparum*)