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Metal complexes with high biological activity against chloroquine resistant strain of *Plasmodium falciparum* parasite

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ABSTRACT

Metal complexes of Manganese (II),Iron(II), Cobalt(II), Nickel (II), Copper (II), Zinc (II) and Cadmium (II) containing a sulphur-nitrogen ligand were subjected to biological tests on falcipain-2 (FP-2) and falcipain-3 (FP-3) cysteine protease enzymes from the malaria parasite plasmodium falciparum. They were further tested in vitro against chloroquine resistant strain (W2). Whereas the potency of the five metal complexes was weaker than the control regarding the FP-2 and FP-3, the potency of five metal complexes was found to be exceedingly greater than the control by a magnitude of 130 or more times than the standard drug when tested against the chloroquine resistant strain (W2). Such complexes have the potential to act as lead compounds for drug design.

Key words: Metal complexes, biological activity, *Plasmodium falciparum* parasite.

INTRODUCTION

Malaria annually kills more than one million people world-wide 90% of them in Africa[1].The eradication of malaria continues to be frustrated by the continued drug resistance of the malaria parasite[2-4]. Hence, there is a great need to continue the search for more effective drugs in terms of activity and the cost. Thiosemicarbazones and their corresponding thiosemicarbazides containing 2-acetylpyridine fragment have been found to show biological activity[5] against malaria parasites, trypasomiasis, bacteria and viruses. A good example is chloro [N,N-3-azbicyclo[3,2,2]nonane-3-thiocarbohydrazonato][1-(2-pyridinyl -1-oxide ethylidene) copper(II) complex which has been shown to have biological activity against malaria though less active than the corresponding ligand[4]. On the other hand, the copper and zinc containing thiosemicarbazone-based complexes were found to be more biologically active than the corresponding ligands[6]. Our current findings indicate that the metal complexes containing a sulphur-nitrogen based ligand have moderate potency against falcipain-2 (FP-2) and falcipain-3 (FP-3) cysteine protease enzymes from the malaria parasite plasmodium falciparum while they portray enormous potency against the chloroquine resistant strain (W2) of the parasite. This paper presents the biological results of metal complexes of Manganese (II),Iron(II), Cobalt(II), Nickel (II), Copper (II), Zinc (II) and Cadmium (II)[7].

EXPERIMENTAL

The metal complexes were synthesized and characterized by elemental analysis, proton NMR, Mass spectrometry and Fourier Transform Infrared spectroscopy[7]. The spectroscopic measurements were done at the University of Cape Town and the biological tests were conducted at the University of California, San Francisco.

RESULTS AND DISCUSSION

The results of the biological activities of the metal complexes against malaria parasites are shown in Table 1. The metal complexes were tested against two cysteine protease enzymes falcipain-2 (FP-2) and falcipain-3 (FP-3) as well as the chloroquine-resistant strain from the malaria parasite Plasmodium falciparum. The following activity sequences can be discerned.

Table 1: Biological activities (nM)* of the metal complexes against malaria parasites.

Complex	FP-2	FP-3	W2	S.R (W-2)
Cd	45,620	31,370	14.4	172.4
Zn	13,850	8,462	18.3	135.6
Mn	11,220	10,440	18.8	132.0
Co	27,010	29,720	752.3	3.3
Ni	41,400	41,400	1,734	1.4
Control E64	9.5	56	2,482	1.0
Fe	37,240	49,950	4,984	0.5

* nM = nanomolar, S.R. = Strength Ratio.

FP-2: CONTROL > Mn > Zn > Co > Fe > Ni > Cd
FP-3: CONTROL > Zn > Mn > Co > Cd > Fe
W-2: Cd > Zn > Mn > Co > Ni > CONTROL > Fe

Although the metals were bound to the same ligand, their activities differed dramatically. It is also interesting to note that with the exception of the iron complex, the metal potency was far much greater than the control drug with respect to W-2. This observation is extremely important as malaria resistance against the chloroquine drug is a great challenge today. These metal complexes may act as lead compounds for developing future malaria drugs. The potency of the metal complexes is modest and less than that of the control drug with respect to FP-2 and FP-3 cysteine protease enzymes. Furthermore, the order of potency of zinc and manganese in the above sequences stand out strongly although the potency of cadmium is greatest with respect to W-2.

It is also well known that a change in molecular structure may influence its biological activity dramatically. The biological activity may either remain the same, decrease, increase or disappear completely. This has been observed in thiosemicarbazones and thiosemicarbazides in the malaria studies[2,4-5, 8-9]. For instance, the 2-acetylpyridine moiety in thiosemicarbazones has been found to be crucial in promoting the biological activity against malaria parasites and Trypanosoma rhodesiense and so was the presence of the sulphur atom[8-12]. The modifications at the pyridine nitrogen and/or the terminal nitrogen (N^4) of the thiosemicarbazone chain also affected the biological activity against malaria, trypanosomiasis, and Herpes Simplex Virus[4,13-14].

The molecular geometry is also crucial in determining the biological activity in metal complexes. This is illustrated by cis-$[PtCl_2(NH_3)_2]$ (Cisplatin) is biologically active and used as a drug against cancer whereas the trans isomer is biologically inactive against cancer[15].

Dissociative mechanism of the Cl ligands was advanced to explain the anti-tumour activity in cis-$[PtCl_2(NH_3)_2]$ complex[15]. In this mechanism one of the Cl ligand is replaced by water to form $[Cl(H_3N)_2Pt(OH_2)]^+$ complex. Then the platinum aquo complex reacts further with a DNA 'molecule' of the cancerous cell to form the new complex $[Cl(H_3N)_2Pt(DNA)]^+$ and in so doing terminates or minimizes the cancerous growth[15]. The DNA molecule binds the platinum metal via the guanine moiety. Green and Berg also observed that the retroviral nucleocapsid from the Rauscher murine leukemia binds to metal ions, in particular, it has a higher affinity[16] for Co^{2+} and Zn^{2+}. In this case the nucleocapsid behaves as a 'ligand' for the metal ions. It is also very interesting to note that complexation mechanism has been advanced to explain the antimalarial activity of chloroquine[17]. It does this by binding the heme fragments and thereby preventing the crucial polymerization process of the parasite. This ultimately leads to the death of the parasite. In this case the chloroquine molecule acts as a ligand to bind the biological heme fragment. Circular dichroism studies of [MLCl] (M = Pd, Pt, L = methyl-3-[2-pyridylmethylene]hydrazinecarbodithioate ion) with DNA also indicate that an adduct is formed between the two moieties[18]. Biological activities of certain thiosemicarbazone ligand complexes of

Cu(II), Ni(II) and Fe(III) were found to be less active against malaria parasites than the corresponding ligands[4].On the other hand, it was observed that metal complexes of pyridoxal semicarbazones, thiosemicarbazones and isothiosemicarbazones were more biologically active than the corresponding ligands[6]. It is quite clear from our work that keeping the ligand constant and varying the central metal atom, affects the biological activity of the complex.

The activity of the malaria parasite is explained as follows. The malaria parasite decomposes a human haemoglobin to produce free heme fragments and peptides in its food vacuole[17-19]. The proteins are utilized by the parasite for its growth and replication[17-19]. The heme acts as a parasite waste and is thus toxic to the parasite. Its toxicity is thought to occur by the heme lysing the membranes and producing reactive oxygen intermediates (ROI) and interfering with other biochemical processes. The parasite neutralizes the toxicity of the heme by converting it into a hemazoin polymer also known as the malarial pigment through a process called biocrystallization[17-19]. The action of chloroquine drug is its interference with these processes. Chloroquine enters the food vacuole of the parasite due to its enabling environment. This environment includes the parasite transporters that assist in the uptake of chloroquine, the existence of a specific parasite receptor for binding chloroquine and acidity of the food vacuole that promotes the protonation of the chloroquine nitrogen atoms. A postulated mechanism by which this activity occurs is through the formation of a complex with the heme and hence preventing it from forming a non-poisonous hemozoin[17-19] The complex formed between the heme and chloroquine is poisonous to the parasite. This results into the death of the parasite.

However, the malaria parasite has the ability to develop drug resistance. Several possible mechanisms for drug resistance have been put forward[17-19]. These include mutations in the target DNA gene, increase in the production of the target, decrease in the drug accumulation in the food vacuole, and drug inactivation. As it is now well known, the malaria parasite has developed resistance against chloroquine[20].

The high biological activity of the metal complexes could probably involve further complexation with the malaria parasite or complexing with the heme fragments resulting into chemical species which are poisonous to the malaria parasite. Clearly more work is needed to establish more plausible mechanism regarding the activities of the metal complexes.

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