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New mixed ligand zinc(II) complexes based on the antiepileptic drug sodium valproate and bioactive nitrogen-donor ligands. Synthesis, structure and biological properties

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ABSTRACT

Starting from the precursor [Zinc Valproate complex] (**1**), new mixed ligand zinc(II) complexes of valproic acid and nitrogen-based ligands, formulating as, $[Zn(valp)_2$2,9-dmphen] (**2**), $[Zn_2(valp)_4(quin)_2]$ (**3**), $[Zn(valp)_2(2\text{-ampy})_2]$ (**4**), and $[Zn(valp)_2(2\text{-ampic})_2]$ (**5**) (valp = valproate, 2,9-dmphen = 2,9-dimethyl-1,10-phenanthroline, quin = quinoline, 2-ampy = 2-aminopyridine, 2-ampic = 2-amino-6-picoline) were synthesized and characterized using IR, ^{1}H NMR, $^{13}C\{^1H\}$ NMR and UV–Vis spectrometry. The crystal structures of complexes **2**, **3** and **4** were determined using single-crystal X-ray diffraction. The complexes were also evaluated for their anti-bacterial activity using *in-vitro* agar diffusion method against three Gram-positive (*Micrococcus luteus*, *Staphylococcus aureus*, and *Bacillus subtilis*) and three Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*) species. Complex **2** showed considerable activity against all tested microorganisms and the effect of complexation on the anti-bacterial activity of the parent ligand of **2** was also investigated. The anti-bacterial activity of 2,9-dmphen against Gram-negative bacteria was enhanced upon complexation with zinc valproate. On the other hand, complexes **1** and **3** showed weak inhibition activity against the tested species and complexes **4** and **5** didn't show any activity at all. Two methods were used for testing the inhibition of ferriprotoporphyrinIX biomineralization: a semi-quantitative micro-assay and a previously self-developed quantitative *in-vitro* method. Both were used to study the efficiency of these complexes in inhibiting the formation of the Malaria pigment which considered being the target of many known anti-malarial drugs such as Chloroquine and Amodiaquine. Results showed that the efficiency of complex **2** in preventing the formation of β-Hematin was 80%. The efficiency of Amodiaquine as a standard drug was reported to give 91%.

1. Introduction

Zinc, which occurs naturally as divalent cation Zn(II), is one of the most important metal in biological systems as it plays an essential role in the activity of nearly 300 enzymes that catalyze approximately 50 important cellular biochemical reactions [1–3]. In bacteria, Zinc plays a role in catalysis, protein structure and perhaps as a single molecule [4]. However, at high concentrations Zn(II) shows inhibitory action on the growth of bacterial species like *Escherichia coli*, *Staphylococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *and Proteus aeruginosa* [5,6].

In some cases, the interaction of metal ions (i.e. Zn(II)) with bioactive anti-bacterial organic compounds increases the biological activity of the ligands [7]. The metal oxidation state, the type and number of donor atoms, as well as their relative positions within the ligand are major factors determining the relationship between the structure and activity. In other cases, the interaction of bioactive organic compounds with metals inhibits their activity, e. g. the anti-bacterial activity of cefadroxil is diminished when it binds to Zn(II) complex [8].

Malaria is a mosquito borne parasitic disease of the blood caused by a Protozoan belonging to the genus Plasmodium. It is considered one of the major public health concerns globally where

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about 40% of the world's population is currently at risk for malaria disease [9,10]. Plasmodium species have a complex life cycle. During what is known as the intra-erytrhrocytic stage, active metabolism of hemoglobin takes place in an acidic lysosome-like organelle called the food vacuole that has a pH of about (5.0–5.4) [11–14]. Digestion of hemoglobin by malaria parasite leads to the continuous liberation of free heme (Iron protoporphyrin IX) along with oxygen, causing formation of a ferric form of heme called ferriprotoporphyrin IX which can lead to the killing of the parasites. However, Plasmodium parasite has evolved a unique detoxification method of free heme through its conversion into a non-toxic, inert, insoluble, crystalline and black-brown pigment called hemozoin [15].

Hemozoin is made of dimers of hematin molecules that are joined together by hydrogen bonds to form larger structures. These dimers are formed through an iron–oxygen coordinate bond that links the central ferric iron of one hematin to the carboxylate side group oxygen of another [15–18]. These reciprocal ferric-oxygen bonds are highly unusual and have not been observed in any other porphyrin dimer. Antimalarial drugs are believed to act by inhibiting hemozoin formation in the food vacuole. This prevents the detoxification of the free heme released in this compartment, eventually killing the parasite [15,17].

A crystalline synthetic structure known as β-hematin is believed to be structurally, morphology and spectroscopically identical to purified hemozoin [15,19–23]. β-hematin formation could be accomplished in vitro under specific chemical conditions (acidic pH) through a biocrystallization process [24] making it an outstanding target for in vitro screening of antimalarial compounds.

Chloroquine, a quinoline-ring drug, is widely used for malaria treatment. However, resistance to chloroquine has emerged increasing owing to extensive and uncontrolled use [25,26]. Chloroquine resistance is considered as major universal challenging problem and the need of new effective antimalarial drugs is an urge. In this research both a semi-quantitative [27] and a quantitative screening methods for a new potential antimalarial drugs were used [23,24].

Valproic acid (Fig. 1) is a broad spectrum anti-epileptic drug which is effective against all seizure types and is increasingly used in the treatment of other diseases, including bipolar disorder, migraine, and neuropathic pain [28]. In addition, valproic acid was shown to enhance the effect of chemotherapy on EBV-positive tumors, and to possess a multitude of anti-tumor properties *in-vitro* and in clinically relevant animal models [29,30]. Esiobu and Hoosein [31] found that sodium valproate is selectively potent against yeast strains and *Mycobacterium smegmati*. Moreover, synthesis, characterization and biological activity of mixed ligands metal complexes of valproate with different nitrogen based ligands have been studied for copper [32–38], rhodium [39] and platinum [40].

Four bioactive nitrogen base compounds were chosen in the present work: 2,9-dimethyl-1,10-phenanthroline, (2,9-dmphen); quinoline, (quin); 2-aminopyridine, (2-ampy) and 2-amino-6-picoline, (2-ampic). These ligands and their derivatives, as well as many of their complexes, are exhibiting anti-bacterial [41–43], anti-microbial [44–50], anti-fungal [51], anti-viral [52,53] and anti-tumor activities [54–57] which depend on the nature of the ligand and the type of the metal ion.

Zinc complexes of aliphatic carboxylate such as formate, acetate, propionate and butyrate with nitrogen based ligands have been synthesized and screened as bio-active compounds [58–64]. In the present work, we describe the structure and biological activity of mixed ligand zinc valproate complexes with four nitrogen based ligands. The crystal structure, spectroscopic properties, antibacterial and anti-malarial activity of: [Zn(valproate)$_2$2,9-dimethyl-1,10-phenanthroline] (**2**), [Zn$_2$(valproate)$_4$(quinoline)$_2$] (**3**), [Zn(valproate)$_2$(2-aminopyridine)$_2$] (**4**), and [Zn(valproate)$_2$(2-amino-6-picoline)$_2$] (**5**) is reported.

2. Results and discussion

2.1. Synthesis of zinc complexes

Water insoluble white solid, [zinc valproate complex] (**1**), was obtained via the reaction of 1:2 M ratio of $ZnCl_2$ with sodium valproate in water (Scheme 1). A series of novel mixed ligand zinc complexes were prepared by adding N-donor ligands to complex **1**, as shown in Scheme 2. An appropriate molar ratio of the reactants were mixed in methanol and stirred for several hours. The obtained complexes were soluble in methanol and separated from methanol by evaporation. The physical properties of **1**–**5** are summarized in Table S1 (Appendix A. Supplementary Materials).

2.2. Crystallographic studies

2.2.1. X-ray crystal structure determination of [Zn(valp)$_2$2,9-dmphen] (**2**)

The atomic numbering scheme and atom connectivity for complex **2** are shown in Fig. 2 and selected bond lengths and angles are reported in Table 1. Complex **2** crystallizes in *P2*(1)/c space group with four molecules of **2** in the unit cell. The structure consists of monomeric units in which Zn(II) exhibits a highly distorted octahedral geometry in the $ZnN_2O_2 + O_2$ chromospheres. Zn(II) is covalently bonded to two valproate ligands and one bidentate 2,9-dmphen ligand. The mean Zn–N bond distance (2.097 Å) is similar to the same bond in other zinc complexes of 2,9-dmphen ligand; although these complexes exhibit more symmetrical Zn–N distances [66–69].

Each of the valproate groups bond in an asymmetric coordination mode with one of the two valproate oxygens is tightly bonded to Zn (II) {Zn1–O1 = 1.942(2), Zn1–O2 = 2.560; and Zn1–O3 = 2.135(5), Zn1–O4 = 2.324 Å} with the calculated difference between these distances $\Delta_1O = 0.618$ and $\Delta_2O = 0.189$ Å for the two valproate groups, respectively. These distances correspond to Zn–O distance for aliphatic carboxylates [70]. The difference in Zn–O bond distances between the two tightly binding oxygens {Zn1–O1 and Zn1–O3 is significantly large (0.193 Å). This difference is smaller in zinc acetate and zinc isovaleroate complexes {e.g. 0.065 Å in zinc isovaleroate complex} [71–74]. The analysis of C15–O1 and C15–O2 bond distances, 1.260(4) and 1.193(4) Å, respectively, showed that the covalent character of the Zn–O linkage is large, *ca.* 22.8% on the basis of the theory by Hocking and Hambley

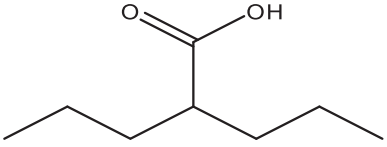
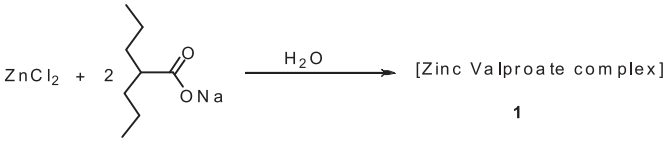


Fig. 1. Structure of valproic acid.



Scheme 1. Synthesis of complex **1**.