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# Synthesis, structural characterization and previous cytotoxicity assay of Zn(II) complex containing 1,10-phenanthroline and 2,2′-bipyridine with valproic acid

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ABSTRACT

A $Zn^{+2}$ complex of the antiepileptic drug valproic acid (VPA) and the *N*-donor ligand 2,2′-bipyridine (complex **3**) was studied and its toxicity against *Artemia salina* was evaluated. Two known compounds (**1** and **2**) were obtained and analyzed for comparison purposes. These compounds were characterized by FTIR, $^{1}H$ and $^{13}C$ NMR, HRMS and complexometric titration. The new zinc complex is constituted by two valproate, one *N*-donor 2,2′-bipy and one hydration water molecule for each metal center ($C_{26}H_{38}N_2O_4Zn$). Toxicity against *A. salina* was also performed, and the new molecule (complex **3**, $LD_{50}$ = 409 μg/mL) showed lower toxicity in comparison with the complex **2** ($LD_{50}$ = 78 μg/mL) and the free ligand 2,2′-bipy ($LD_{50}$ = 143 μg/mL). Moreover, the $LD_{50}$ values were substituent-dependant regarding the *N*-donor ligand, without VPA influence.



## 1. Introduction

Transition metal ions are essentials in cell metabolism concerned in various pathways as catalytic centers in enzymes [1]. The zinc(II) cation is a Lewis acid and its complex chelates participate in numerous cellular redox reactions [2].

The research on metals coordinates with organic molecules used regularly as drugs has increased in the last years [3–5]. In accordance with its features and affinity for biological goals, these organometallic complexes may be more effective than the precursor organic molecules. In a recent example, the ternary copper(II) valproic acid complex increased by more than three thousand times the anticonvulsant activity of valproic acid (VPA) in mouse [6,7].

VPA is the one of the oldest drugs used in seizure, bipolar disorder and migraine headaches therapy [8–10]. However, rare but serious sides effects are observed in the liver because of required VPA high doses [11,12]. While *daily doses* between 10 and 20 mg/kg are often sufficient for *VPA therapy*, its continuous use increases the biotransformation rate, which requires a dose increase up to the toxic limit of 60 mg $kg^{-1}$ $day^{-1}$ [13].

The high affinity of VPA for transition metals results in stable coordination compounds [14,15]. The characteristic of carboxylate bond with metal cation (Lewis acid) is the short length between oxygen atoms and metallic core around 2.6 Å [17], responsible for the cluster chemical stability. The first example of VPA complexation with metals was reported by Hadjikostas et al. [17] using copper(II) and pyridine. Zinc(II) also produces stable complexes with valproate ions and some of them Zn–VPA with aromatic imines afforded molecules with biological activity against microorganisms [18,19].

Investigating and establishing the activity of a new drug alone is not sufficient, the toxic action should also be evaluated for safety concerns. In order to predict toxicity in humans, different models have been performed as, for instance, with brine shrimp – *Artemia salina* Leach – which is a free life micro crustacean from tropical seas [20,21]. This assay is easy, fast, inexpensive and robust to determine acute toxicity in preliminary studies. Aquatic toxicity of $Cu^{+2}$, screening of carbamates insecticides and antiparasitic agents are applicable examples [22–24]. Also, this model was applied to evaluation of the new coordination compounds derived from drug with $Zn^{+2}$ [25].

In this context, the synthesis of new zinc organocomplex using valproic acid, and its toxicity evaluation by the brine shrimp

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model, were the aim of this study. *N*-donor ligands, such 1,10-phenanthroline and 2,2′-bipyridine, were used to obtain stable and soluble coordination compounds. Molecular spectroscopy characterization of ternary valproate zinc(II) complexes and a new complex with *N*-donor 2,2′-bipiridine ligand was done by NMR, HRMS and FTIR.

## 2. Material and methods

### 2.1. Synthesis of the zinc(II) complexes

#### 2.1.1. Chemicals

Sodium valproate 98% was purchased from AK Scientific (Union City, CA, USA). All the other chemicals used in this work, such as the aromatic imines 1,10-phenanthroline 99.5% and 2,2-bipyridine 99.5% zinc salt were purchased from Sigma Aldrich.

#### 2.1.2. Preparation of the zinc valproate complex (**1**)

[Tetrakis-μ-2-propylpentanoatezinc(II)] was prepared by Abu method [18]. An aqueous solution of $ZnCl_2$ (29 mmol) (Scheme 1), was added with continuous stirring to sodium valproate solution (58 mmol) in water at room temperature. A white precipitate was formed immediately. After 24 h, the solid product was washed with pure water, and carried-out after vacuum filtration. Then, complex (**1**) was dried in a desiccator for 6 h at least, over drying agent blue silica-gel indicator. Yield: 88%; MP: 207 °C.

#### 2.1.3. Synthesis of [bis(2-propil-pentanoate)(1,10-phenanthroline) zinc(II)] (**2**) and [bis(2-propil-pentanoate)(2,2-bipyridine)zinc(II)] (**3**)

To a solution of compound **1** (5.7 mmol) in DMF (10 mL) was added a solution of 2,2′-bipy (11.4 mmol) [or 1,10-phen·$H_2O$ (11.4 mmol)] in DMF (10 mL) under vigorous stirring for 20 min. After 24 h, a white solid was formed, which was carried-out by filtration and dried under vacuum for 12 h. Compound **2**: Yield: 90%; MP: 223 °C. Compound **3**: Yield: 59%; MP: 150 °C.

### 2.2. Zinc II assay

Zinc(II) percentages in compounds **1**, **2** and **3** were determined by volumetric method, based on Raba's modified method [26]. Zn(II) determinations were performed by volumetric titration with the 0.01 M EDTA standardized solution in triplicate.

### 2.3. FTIR analysis

Dried products were mixed with spectroscopic grade KBr (Merck) (1 mg/100 mg) [27]. Infrared spectra of the complexes were obtained of the complexes were obtained in KBr disks on a Spectrum 400 (Perkin Elmer) spectrometer. The spectra were obtaining in the range 4000.0–400.0 $cm^{-1}$, resolution of 1.0 $cm^{-1}$ and 32 scans. Spectral data were treated in IR Tutor software (Columbia University, USA).

### 2.4. $^1H$ and $^{13}C$ NMR analysis

The samples (5 mg for $^1H$ and 15 mg for $^{13}C$) were dissolved in 0.5 ml of deuterated chloroform and analyzed in accordance with method for zinc(II) complexes with modification [28]. The NMR spectra were recorder on a Varian Inova 300 spectrometer (299.96 MHz for $^1H$ and 75.43 MHz for $^{13}C$) at the room temperature. $^1H$ NMR: spectral width 4.799 Hz, number of points 16.384. $^{13}C$NMR: spectral width 4.799 Hz, number of points 9.832. The chemical shifts expressed in parts per million (ppm) were referenced to internal solvent $CDCl_3$ (7.26 ppm for $^1H$ and 77.00 for $^{13}C$ NMR spectra). Spectral data (Fig. 2) were analyzed with MestReNova software, version 6.0.2-5475.

### 2.5. Stability assay

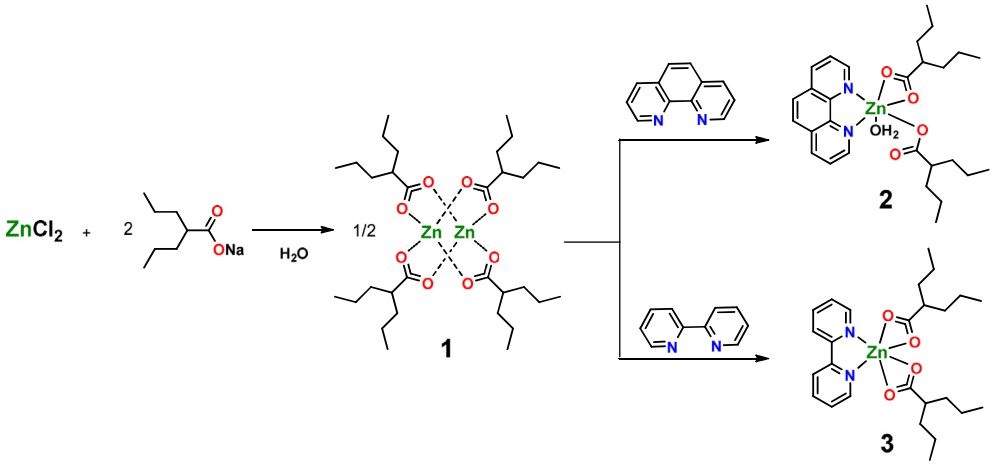
The compound **3** stability was performed by $^1H$ and $^{13}C$NMR. A complex saturated solution in $D_2O$ was analyzed and after 24 h. All conditions of the previous NMR experiment were kept except the chemical shifts of $^1H$NMR spectra, which was referenced to internal solvent $D_2O$ and expressed in parts per million (ppm).

### 2.6. Mass analysis

The products were dissolved in 50% (v/v) mixture of chromatographic grade acetonitrile (Tedia, Fairfield, OH, USA) 50% (v/v) and

**Table 1**
Total Zn(II) content of for all compounds.

| Compound | Experimental Zn(II) (%) | Theoretical Zn(II) (%) | Error (%) |
|---|---|---|---|
| **1** | 18.19 | 18.59 | −2.15 |
| **2** | 11.29 | 11.89 | −5.04 |
| **3** | 12.48 | 12.87 | −2.70 |




**Scheme 1.** Synthesis of complexes **1**, **2** and **3**.