

# Interactions of metal ions with a 2,4-diaminopyrimidine derivative (trimethoprim) Antibacterial studies

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## Abstract

The interaction of copper (II), zinc(II) and cadmium(II) with Trimethoprim (2,4-diamino-5-(3',4',5'-trimethoxybenzyl) pyrimidine) has been studied. The crystal structures of  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$  (**2**) and  $[\text{Cd}(\text{Trim})\text{Cl}_2(\text{CH}_3\text{OH})]_n$  (**4**) are reported. Compound (**2**) exhibits a distorted tetrahedral environment around the metal center and crystallizes in the triclinic space group  $P\bar{1}$  with  $a=10.2397(6)$ ,  $b=10.4500(6)$ ,  $c=16.3336(16)$  Å,  $\alpha=96.141(8)$ ,  $\beta=106.085(5)$ ,  $\gamma=96.551(5)^\circ$  and  $Z=2$ . In complex (**4**), the Cd(II) centers are bridged sequentially by two chlorine ions to form infinite chains and present a six-coordinated environment; the compound crystallizes in the monoclinic  $P2_1/C$  space group with  $a=13.958(5)$ ,  $b=7.532(2)$ ,  $c=18.390(2)$  Å,  $\alpha=90$ ,  $\beta=97.32(5)$ ,  $\gamma=90^\circ$  and  $Z=4$ . In both structures the Trimethoprim acts as a monodentate ligand through the pyrimidinic nitrogen N(1) atom. The characterization of the  $\text{Cu}(\text{Trim})_2(\text{CH}_3\text{O})(\text{ClO}_4)$  complex through EPR and magnetic measurements suggests a binuclear or polynuclear nature, with bridging methoxo groups. The complexes were screened for their activity against several bacteria, showing activity similar to that of trimethoprim. © 2000 Elsevier Science S.A. All rights reserved.

**Keywords:** Antibacterial studies; Metal ions; 2,4-diaminopyrimidine derivative

## 1. Introduction

Compounds containing pyrimidine rings play a significant role in many biological systems [1]. The pyrimidine ring system, present in nucleic acids, several vitamins, coenzymes and antibiotics, provides potential binding sites for metal ions, and any information on their coordinating properties is important as a means of understanding the role of the metal ions in biological systems.

Many compounds of therapeutic importance contain the pyrimidine ring system. So, substituted 2,4-diaminopyrimidines are widely employed as metabolic inhibitors of pathways leading to the synthesis of proteins and nucleic acids. Among these kind of ligands, trimethoprim (Trim=2,4-diamino-5-(3',4',5'-trimethoxybenzyl)

pyrimidine) is a well known biological agent, also employed as a potent metabolic inhibitor of bacterial dihydrofolic acid reductase. This drug is an antibacterial agent highly active against strains resistant to other antibiotics frequently used, e.g.  $\beta$ -lactam antibiotics [2].

Trimethoprim has potential binding sites for metal ions. Several authors have studied the interaction of this ligand with biological metal ions and the coordination of trimethoprim via a  $\text{NH}_2$  nitrogen atom was inferred on the basis of IR and visible measurements [3,4]. However, other authors have shown by X-ray diffraction methods that the coordination site of the Trimethoprim molecule is the  $\text{N}_1$  of the pyrimidine ring [5–9]. On the other hand, more recently, Seekhon et al. [10] have prepared and characterized complexes of trimethoprim with Ag(I), Zn(II), Cd(II), Hg(II) and Ni(II) and the infrared data show that the ligand acts as a monodentate through the 4- $\text{NH}_2$  group.

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In order to enhance the information on the coordination properties of this drug we report here the synthesis and characterization of new metal complexes of trimethoprim and the crystal structures of the complexes  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$  and  $[\text{Cd}(\text{Trim})\text{Cl}_2(\text{CH}_3\text{OH})]_n$ . On the other hand, many drugs modify their pharmacological and toxicological properties when administered in the form of metallic complexes [11], this is the reason for us to investigate the activity of all complexes synthesized against several bacteria strains; we intend to compare it with that of the ligand.

## 2. Experimental

Trimethoprim was provided by Sigma (St. Louis, MO). All reagents used are of analytical grade.

### 2.1. Synthesis of complexes

Synthesis of  $\text{Cu}(\text{Trim})_2(\text{CH}_3\text{O})(\text{ClO}_4)$  (**1**). Two methanolic solutions were mixed, one containing 1.16 g (4 mmol) of trimethoprim and the other one containing 0.37 g (1 mmol) of  $\text{Cu}(\text{ClO}_4)_2 \cdot 2\text{H}_2\text{O}$ . The final volume was 100 ml. The mixture was stirred and heated for 10 min; then a green microcrystalline precipitate of complex was obtained. This product were separated and dried at room temperature.

Synthesis of  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$  (**2**). The complex was prepared as follows: A methanolic solution of  $\text{ZnCl}_2$  (0.50 mmol, 0.07 g) was added to a methanolic solution of trimethoprim (1 mmol, 0.29 g). The final volume was 100 ml. By slow evaporation of the obtained solution at room temperature, almost colorless crystals suitable for X-ray structure determination were formed after 5 days. These crystals were separated and dried at room temperature.

Synthesis of  $\text{Zn}(\text{Trim})(\text{CH}_3\text{COO})_2$  (**3**). A methanol solution of  $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$  (0.50 mmol, 0.11 g) was added to a methanolic solution of trimethoprim (1 mmol, 0.29 g) with continuous stirring. The final volume was 100 ml. A colorless microcrystalline compound was obtained after 3–4 days which was filtered, washed and dried to constant weight.

Synthesis of  $[\text{Cd}(\text{Trim})\text{Cl}_2(\text{CH}_3\text{OH})]_n$  (**4**). The compound was obtained in methanolic medium by mixing with stirring solutions containing 0.30 mmol (0.09 g) of trimethoprim and 0.15 mmol (0.03 g) of  $\text{CdCl}_2 \cdot 2.1/2 \text{H}_2\text{O}$ . The final volume was 50 ml. Immediately, a white precipitate appeared with a few crystals which was filtered. By slow evaporation of the resulting solution at room temperature colorless crystals suitable for X-ray structure determination were formed after a period of 3 months. These crystals were separated by filtration and dried at room temperature.

All compounds gave satisfactory analyses.

### 2.2. Analyses

The content of Cu, Zn and Cd was determined by atomic absorption spectroscopy with a Perkin-Elmer spectrometer model 2380. Elemental chemical analysis for C, H and N was performed in a Perkin-Elmer, model 2400 analyzer. Cl analyses were obtained with an elemental analyzer, Carlo Erba MGO 1106. The anhydrous character was confirmed by thermal analyses with a Shimadzu TGA-50H thermobalance.

### 2.3. Physical measurements

Infrared spectra were carried out in the  $4000\text{--}600 \text{ cm}^{-1}$  range on a Satellite FTIR Mattson spectrophotometer using KBr pellets. Those in the range  $600\text{--}220 \text{ cm}^{-1}$  were taken on a Perkin-Elmer 1750 FTIR spectrophotometer as polyethylene pellets. Raman spectra were recorded on solid samples with a Bruker FT Raman RFS 100/S System in the  $500\text{--}100 \text{ cm}^{-1}$  range. The visible absorption spectra were recorded with a Perkin-Elmer model Lambda 15 spectrophotometer with a reflectance attachment. Electron paramagnetic resonance spectrum of polycrystalline sample was recorded at X-band frequencies with a Bruker ER 200D. Magnetic susceptibility measurements were carried out at  $100\text{--}350 \text{ K}$  with a fully-automatized AZTEC DSM8 pendulum-type susceptometer equipped with a TBT continuous-flow cryostat and a Bruker BE15 electromagnet, operating at 1.8 T. The apparatus was calibrated with  $\text{Hg}[\text{Co}(\text{SCN})_4]$ . Corrections for the diamagnetism were estimated from Pascal's constants. X-ray powder diagrams were performed with a Kristalloflex 810 Siemens diffractometer using  $\text{CuK}\alpha$  radiation ( $\lambda = 1.5405 \text{ \AA}$ ). The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra in  $d^6$ -DMSO were performed in a Bruker AC-250 spectrometer. Cross-polarization magic angle spinning (CP/MAS)  $^{13}\text{C} \{^1\text{H}\}$  NMR spectra were obtained on a Varian Unity 300 spectrometer operating at 7.0 T ( $^{13}\text{C}$  at 75 MHz) using high-power proton decoupling. The conductivity of  $10^{-3} \text{ M}$  solutions was measured at  $25^\circ\text{C}$  on a Conductivity Meter Portland Electronics Ltd. model P335 conductometer (cell constant 1.0).

### 2.4. Antimicrobial tests

#### 2.4.1. Minimum inhibitory concentration (MIC) determination

The MIC of the compounds against 14 bacterial strains obtained from the American Type Culture Collection (ATCC) was performed by the broth microdilution method following the National Committee for Clinical Laboratory Standard (NCCLS) specifications [12]. Briefly: 100  $\mu\text{l}$  of bacteria in the last phase of growth were inoculated in 100  $\mu\text{l}$  of Mueller Hinton Broth (Difco) containing the compounds at a final concentration ranging from 64 to 0.12

μg/ml derived from serial 2-fold dilutions. The final inoculum was approximately  $1 \times 10^5$  viable bacteria/ml and the final volume 200 μl. Inoculated plates were incubated at 35°C for 18–21 h. Readings were made visually after agitation of the plates with a microtiter plate shaker for 5 min. The MIC was defined as the lowest concentration of antimicrobial agent showing complete inhibition of growth. MIC of the reference drug (trimethoprim) were compared with those of the test compounds. Drug- and bacteria-free controls were included. Discrepancies among MIC endpoints of no more than two dilutions were used as criteria of agreement.

### 2.5. X-ray structure determination

Information concerning conditions for crystallographic data collection and structure refinement is summarized in Table 1 for both compounds.

[Zn(Trim)<sub>2</sub>Cl<sub>2</sub>] (2): A colorless prismatic crystal of this compound (0.40×0.20×0.15 mm) was mounted on a glass fiber and used for data collection. Cell constants and an orientation matrix for data collection were obtained by least-squares refinement of the diffraction data from 25 reflections in the range  $19.202 < \theta < 45.133$  in a Enraf Nonius CAD4 automatic diffractometer [13]. Data were collected using CuKα radiation and the ω-scan technique, and corrected for Lorentz and polarization effects [14]. An semi-empirical absorption correction (ψ-Scan) was made [15].

The structure was solved by Patterson methods [16] and subsequent difference Fourier maps, and refined on  $F^2$  by a full-matrix least-squares procedure using anisotropic displacement parameters [17]. All hydrogen atoms were located in their calculated positions (C–H 0.93–0.97 Å), and were refined using a riding model. Atomic scattering

factors from ‘International Tables for X-ray Crystallography’ [18]. Molecular graphics from ZORTEP [19].

[Cd(Trim)Cl<sub>2</sub>(CH<sub>3</sub>OH)]<sub>n</sub> (4): Analysis on single crystals of C<sub>15</sub>H<sub>22</sub>CdCl<sub>2</sub>N<sub>4</sub>O<sub>4</sub> (approximate size 0.15×0.20×0.20 mm) were carried out with an Enraf-Nonius CAD-4 single crystal diffractometer. The unit cell dimensions were measured from the angular setting of 25 reflections with  $\Theta$  between 15° and 25°.

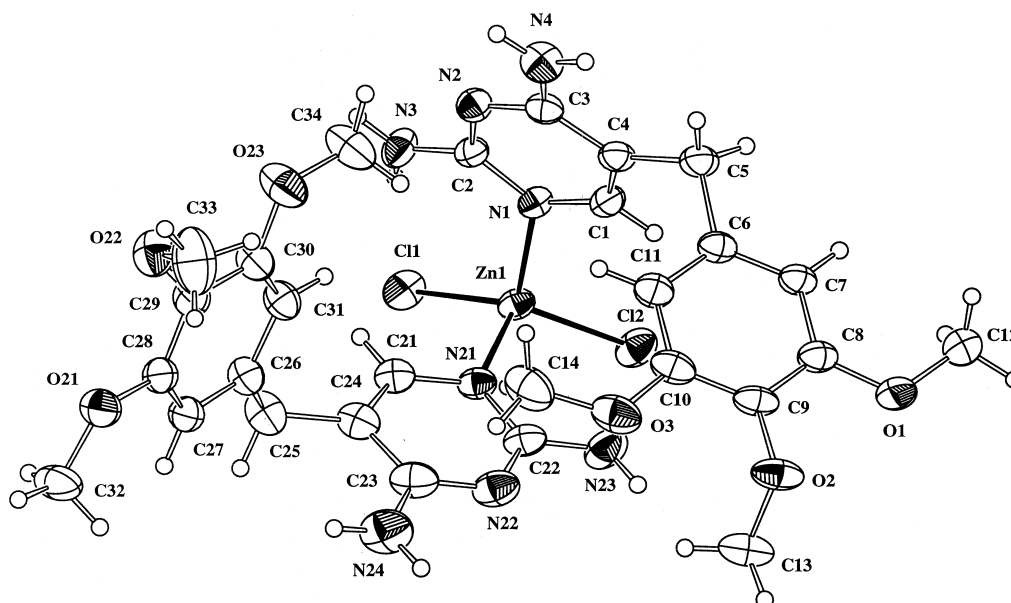
The reflections were measured in the  $hkl$  range (0, 0, –21) to (16, 8, 21) between limits:  $1^\circ < \Theta < 25^\circ$ . The ω-2Θ scan technique and a variable scan rate with a maximum scan time of 60 s per reflection were used. The intensity of the primary beam was checked throughout the data collection by monitoring three standard reflections every 3600 s. The final drift correction factors were in the range of 0.98 and 1.02. Profile analysis was performed on all reflections [20,21] a semiempirical absorption correction, ψ-scan based, was performed reflections [22]. In total, there were 3019 reflections, of which 2295 had  $F_o > 4\sigma F_o$ . Lorentz and polarization corrections were applied and the data were reduced to  $F_o^2$  values. The structure was solved by the Patterson method using the program SHELXS-86 [23] running on an IBM PENTIUM II 300 computer. Isotropic least-squares refinement was performed by means of the program SHELXL-93 [24]. Hydrogen atoms were placed in calculated positions.

During the final stages of the refinement the positional parameters and the anisotropical thermal parameters of the non-hydrogen atoms were refined. The hydrogen atoms were refined with a common thermal parameter. The final conventional agreement factors were  $R_1 = 0.064$  and  $wR_2 = 0.172$ . The maximum shift of the e.s.d ratio in the last full matrix least-squares cycle was 0.001. The final difference Fourier map showed no peaks higher than 2.52e. Å<sup>–3</sup> and not deeper than –1.10 e. Å<sup>–3</sup>. Atomic scattering factors

Table 1

Summary of crystal data and collection of intensities of the compounds [Zn(Trim)<sub>2</sub>Cl<sub>2</sub>] (2) and [Cd(Trim)Cl<sub>2</sub>(CH<sub>3</sub>OH)]<sub>n</sub> (4)

Empirical formula	C <sub>28</sub> H <sub>36</sub> Cl <sub>2</sub> N <sub>8</sub> O <sub>6</sub> Zn	C <sub>15</sub> H <sub>22</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>4</sub> Cd
Formula weight	716.92	473.62
Temperature	293(2) K	293(2) K
Wavelength	1.54184 Å	0.71073 Å
Crystal system	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P2_1/C$
Unit cell dimensions	$a = 10.2397(6)$ Å $\alpha = 96.141(8)^\circ$ $b = 10.4500(6)$ Å $\beta = 106.085(5)^\circ$ $c = 16.3336(16)$ Å $\gamma = 96.551(5)^\circ$	$a = 13.958(5)$ Å $\alpha = 90^\circ$ $b = 7.532(2)$ Å $\beta = 97.32(5)^\circ$ $c = 18.39(2)$ Å $\gamma = 90^\circ$
Volume	1650.7(2) Å <sup>3</sup>	1918(2) Å <sup>3</sup>
Z	2	4
Density (calculated)	1.442 Mg/m <sup>3</sup>	1.640 Mg/m <sup>3</sup>
Absorption coefficient	2.971 mm <sup>–1</sup>	1.436 mm <sup>–1</sup>
$F(000)$	744	944
$\theta$ range	2.85–75.91°	1.47–24.97°
Reflections collected	7281	3508
Independent reflections	6889 [ $R(\text{int}) = 0.0097$ ]	3362 [ $R(\text{int}) = 0.0229$ ]
Refinement method	Full-matrix least-squares on $F^2$	Full-matrix least-squares on $F^2$
Goodness-of-fit on $F^2$	1.043	1.032
Final $R$ [ $I > 2\sigma(I)$ ]	$R_1 = 0.0429$ , $wR_2 = 0.1206$	$R_1 = 0.0467$ , $wR_2 = 0.1321$

Fig. 1. ZORTEP drawing of  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$ .

were taken from the International Tables for X-ray Crystallography [25]. The molecular plots were produced by program ORTEP [26].

### 3. Results and discussion

#### 3.1. Description of the structure of $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$ (**2**)

The ZORTEP [19] plot of complex with labeling schemes is shown in Fig. 1. The selected bond lengths and angles in the complex are listed in Table 2.

The structure consists of neutral monomeric  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$  units linked by hydrogen-bond interactions. The complex exhibits a four-coordinate motif with a distorted tetrahedral environment around the metal center. The Zn(II) ion is coordinated to two chlorine and two pyrimidinic nitrogen atoms (N(1)) from the two trimethoprim ligands resulting in a  $\text{ZnN}_2\text{Cl}_2$  chromophore. The amino nitrogen atoms are not involved in the coordination to the metal.

The Zn–Cl distances are longer than the Zn–N(1) distances and are both in agreement with those found for essentially tetrahedral  $\text{ZnCl}_2\text{L}_2$  molecules involving nitro-

Table 2

Selected bond lengths (Å) and angles (°) for the metal coordination sphere<sup>a</sup>

$[\text{Zn}(\text{Trim})_2\text{Cl}_2]$			
Zn(1)–N(1)	2.039(2)	Zn(1)–N(21)	2.048(2)
Zn(1)–Cl(2)	2.2331(8)	Zn(1)–Cl(1)	2.2668(8)
N(1)–Zn(1)–N(21)	97.03(9)	N(1)–Zn(1)–Cl(2)	112.43(6)
N(21)–Zn(1)–Cl(2)	112.39(7)	N(1)–Zn(1)–Cl(1)	113.45(6)
N(21)–Zn(1)–Cl(1)	106.14(7)	Cl(2)–Zn(1)–Cl(1)	113.99(3)
$[\text{Cd}(\text{Trim})\text{Cl}_2(\text{CH}_3\text{OH})]_n$			
Cd(1)–N(1)	2.353(4)	Cd(1)–O(4)	2.393(5)
Cd(1)–Cl(2)	2.602(1)	Cd(1)–Cl(1) <sup>b</sup>	2.603(1)
Cd(1)–Cl(2) <sup>c</sup>	2.630(1)	Cd(1)–Cl(1)	2.646(1)
Cl(1)–Cd(1) <sup>b</sup>	2.603(1)	Cl(2)–Cd(1) <sup>c</sup>	2.630(1)
N(1)–Cd(1)–O(4)	172.6(1)	N(1)–Cd(1)–Cl(2)	94.5(1)
O(4)–Cd(1)–Cl(2)	89.5(1)	N(1)–Cd(1)–Cl(1) <sup>b</sup>	98.1(1)
O(4)–Cd(1)–Cl(1) <sup>b</sup>	87.9(1)	Cl(2)–Cd(1)–Cl(1) <sup>b</sup>	92.1(1)
N(1)–Cd(1)–Cl(2) <sup>c</sup>	92.9(1)	O(4)–Cd(1)–Cl(2) <sup>c</sup>	80.9(1)
Cl(2)–Cd(1)–Cl(2) <sup>c</sup>	88.4(1)	Cl(1) <sup>b</sup> –Cd(1)–Cl(2) <sup>c</sup>	168.83(3)
N(1)–Cd(1)–Cl(1)	87.02(9)	O(4)–Cd(1)–Cl(1)	89.0(1)
Cl(2)–Cd(1)–Cl(1)	178.41(3)	Cl(1) <sup>b</sup> –Cd(1)–Cl(1)	87.22(5)
Cl(2) <sup>c</sup> –Cd(1)–Cl(1)	92.03(5)	Cd(1) <sup>b</sup> –Cl(1)–Cd(1)	92.78(5)
Cd(1)–Cl(2)–Cd(1) <sup>c</sup>	91.64(5)		

<sup>a</sup> Symmetry transformations used to generate equivalent atoms: <sup>b</sup>  $-x, -y, -z+1$ ; <sup>c</sup>  $-x, -y+1, -z+1$ .

Table 3  
Hydrogen bonds for [Zn(Trim)<sub>2</sub>Cl<sub>2</sub>] (Å) and (°)<sup>a</sup>

D–H...A	d(D–H)	d(H...A)	d(D...A)	<(DHA)
N(3)–H(3A)...O(21) <sup>b</sup>	0.86	2.07	2.926(3)	172.5
N(3)–H(3A)...O(22) <sup>b</sup>	0.86	2.63	3.092(3)	114.8
N(3)–H(3B)...Cl(1)	0.86	2.41	3.242(2)	161.9
N(4)–H(4A)...Cl(2) <sup>c</sup>	0.86	2.98	3.662(3)	137.4
N(4)–H(4B)...O(2) <sup>d</sup>	0.86	2.18	2.906(3)	142.7
N(23)–H(23A)...O(1) <sup>e</sup>	0.86	2.25	3.012(3)	148.1
N(23)–H(23B)...Cl(2)	0.86	2.45	3.270(3)	159.2
N(24)–H(24A)...Cl(1) <sup>f</sup>	0.86	2.98	3.801(3)	160.5

<sup>a</sup> Symmetry transformations used to generate equivalent atoms: <sup>b</sup>  $-x, -y, -z+1$ ; <sup>c</sup>  $x, y-1, z$ ; <sup>d</sup>  $-x-1, -y, -z$ ; <sup>e</sup>  $-x-1, -y+1, -z$ ; <sup>f</sup>  $x-1, y, z$ .

gen-donor ligands [27]. The relevant angles vary from 97 to 120°; these values differ from those expected for a regular tetrahedron.

Hydrogen bonds with symmetry codes are given in Table 3. The N(3) and N(23) nitrogen atoms participate in two intramolecular hydrogen bonds with Cl(1) and Cl(2), respectively. Furthermore, a hydrogen bonding network is observed, which involves the chlorine ions, the amino nitrogen atoms and the oxygen atoms of OCH<sub>3</sub> groups.

### 3.2. Description of the structure of [Cd(Trim)Cl<sub>2</sub>(CH<sub>3</sub>OH)]<sub>n</sub> (4)

Fig. 2 shows an ORTEP [26] drawing of the complex

with the atomic numbering and thermal ellipsoids. Selected bond distances and angles are given in Table 2.

The Cd(II) centers in [Cd(Trim)Cl<sub>2</sub>(CH<sub>3</sub>OH)]<sub>n</sub> are bridged sequentially by two chlorine ions to form infinite chains. The packing occurs through a network of hydrogen bond interactions; possible N–H...Cl and N–H...O hydrogen bonds were identified from the N...Cl and N...O distances in the neighborhood of 3.1 to 3.3 Å. The Cd(II) ion is hexacoordinated within each mononuclear unit. The metal environment is formed by one oxygen of the CH<sub>3</sub>OH molecule, one nitrogen pyrimidinic (N(1)) of trimethoprim ligand and the four chlorine ions which act as a bridge. The observed value for the Cd–Cd (3.821 Å) distance strongly suggests that no direct cadmium–cadmium bond interaction is present in this structure [28].

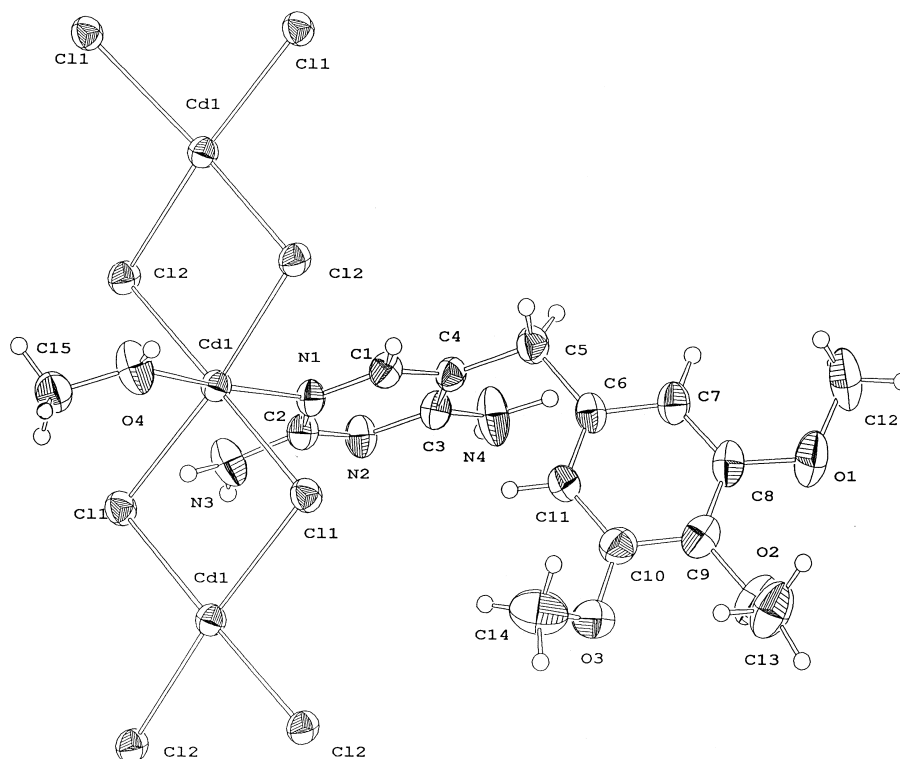


Fig. 2. ORTEP drawing of [Cd(Trim)Cl<sub>2</sub>(CH<sub>3</sub>OH)]<sub>n</sub>.

The distortion of the octahedron is not severe; so, the Cd–Cl distances range from 2.602(1) to 2.6446(1) Å and are longer than the Cd–O (2.393(5) Å) and Cd–N (2.3543(4) Å) ones, and the relevant angles lie between 80.9 and 98.1°. These distances and angles are similar to those observed in compounds of related ligands [28].

### 3.3. General physicochemical characteristics of the complexes

The Cu(II) complex is soluble in DMF and the Zn(II) complexes (**2** and **3**) in DMSO. The low values for molar conductivity in dimethylsulfoxide of the compounds **2** ( $\Lambda_{\text{mol}}=4.7 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ ) and **3** ( $\Lambda_{\text{mol}}=2.0 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ ) correspond to non-electrolyte behavior. Moreover, the  $\Lambda_{\text{mol}}$  value in dimethylformamide of the compound **1** is  $114.3 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ ; this value correspond to a 2:1 type of electrolyte [29].

IR bands of the compounds have been assigned based on data available in the literature. So, the  $\nu_{\text{as.}}(\text{N–H})$  and  $\nu_{\text{s.}}(\text{N–H})$  modes of the pyrimidine- $\text{NH}_2$  groups in the free ligand (trimethoprim) are assigned to the strong and sharp bands at 3470 and 3318  $\text{cm}^{-1}$ , respectively, which are affected by the presence of hydrogen bonds [30]. In all the complexes the bands  $\nu(\text{N–H})$ , due to asymmetric and symmetric vibrations, are present in the region 3475–3320  $\text{cm}^{-1}$ ; these bands don't shift significantly with respect to those of the ligand. In the infrared spectrum of  $[\text{Cd}(\text{Trim})\text{Cl}_2(\text{CH}_3\text{OH})]_n$  compound, the bands at 3474 and 3438  $\text{cm}^{-1}$  are assigned to the N–H asymmetric stretching vibration and the O–H stretching vibration of coordinated methanol, respectively [31]. In spite of the complexity of the vibrational spectrum of trimethoprim in the 1700–1200  $\text{cm}^{-1}$  region, the bands corresponding to  $\nu_{\text{as.}}(\text{CO}_2)$  and  $\nu_{\text{s.}}(\text{CO}_2)$  from the acetate group are identified at 1594 and 1422  $\text{cm}^{-1}$  [32] in the IR spectrum of the acetate compound. In the IR spectrum of the  $\text{Cu}(\text{Trim})_2(\text{CH}_3\text{O})(\text{ClO}_4)$  compound, the  $\nu_3$  vibration of the perchlorate ion appears as a broad band at 1122  $\text{cm}^{-1}$ , implying that the ion is not coordinated to the copper(II) ion [33].

In the low frequency range, 600–220  $\text{cm}^{-1}$ , the spectrum of the Cd(II) complex exhibit a band centered at 233  $\text{cm}^{-1}$  assigned to the  $\nu(\text{Cd–N})$  stretching vibration [27]. In the  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$  complex IR two bands at 306 and 284  $\text{cm}^{-1}$  are observed, attributable to  $(\text{Zn–Cl})_{\text{terminal}}$  stretching [34]. As for the  $\text{Zn}(\text{Trim})(\text{CH}_3\text{COO})_2$  complex, its 600–220  $\text{cm}^{-1}$  spectrum shows a new band at 392  $\text{cm}^{-1}$  which can be assigned to  $\nu(\text{Zn–O})$  stretching vibration of the acetate group [34]. It seem possible to identify in the Raman spectra [33] of the  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$  and  $\text{Zn}(\text{Trim})(\text{CH}_3\text{COO})_2$  compounds bands at 209 and 194  $\text{cm}^{-1}$ , respectively, assignable to  $\nu(\text{Zn–N})$ ; according to the bridging nature of the chloride ion, the  $\nu(\text{Cd–Cl})$  vibration is observed at 190  $\text{cm}^{-1}$  for the cadmium

compound [27,34]. The Raman spectrum of  $\text{Cu}(\text{Trim})_2(\text{CH}_3\text{O})(\text{ClO}_4)$  compound shows two bands at 463 and 279  $\text{cm}^{-1}$  assigned to the  $\nu(\text{Cu–N})$  and  $\nu(\text{Cu–O})$  modes, respectively [8,34]. These assignments suggest that the coordination of the ligand probably occurs through the pyrimidine nitrogen atom.

$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectral data of zinc compounds in  $d^6$ -DMSO show that all the signals of the free ligand [35] are present in the  $^1\text{H}$  NMR spectrum of the complexes but the protons of the two amino groups of trimethoprim exhibit characteristic dowfield shifts by 0.5–0.8 ppm in both complexes, suggesting a deshielding effect via intramolecular or intermolecular hydrogen bonding [36,37]. Besides, the  $\text{H}_6$  signal pyrimidine proton is slightly shifted to higher field values [3] probably due to the coordination of ring N atom to the metal ion. Furthermore, in the  $^1\text{H}$  NMR spectrum of the acetate complex a new signal at 1.77 ppm appears, which can be assigned to the  $\text{CH}_3\text{COO}$  group [38].

On the other hand,  $^{13}\text{C}$  NMR signal of  $\text{C}_6$  (at 155.91 ppm in the ligand) is shifted to high field values in both compounds (at 152.41 ppm for compound **2** and 152.08 ppm for compound **3**); likewise, the coordination of the trimethoprim, to the metal ion causes a deshielding of the  $\text{C}_5$  atom in the pyrimidine ring [27] (at 105.96 ppm in the ligand and 107.40 and 107.17 ppm in the compounds **2** and **3**, respectively). The signal of  $\text{CH}_3$  of acetate group appears at 23.03 ppm [38]. The  $^{13}\text{C}$  NMR solid-state spectra of the ligand and its zinc complexes are shown in Fig. 3. All the signals of the RMN spectra in  $d^6$ -DMSO are present in the solid state spectra. Moreover, we can appreciate in the spectra of the complexes that some signals of carbon atoms are slightly splitted as a consequence of the fact that the ligands are coordinated to Zn(II) in different ways in each asymmetric unit. Since atoms within those molecules are not identical, the chemical shifts may be different as well, causing extra lines to appear in the solid-state spectra [39].

The electronic reflectance spectrum of  $\text{Cu}(\text{Trim})_2(\text{CH}_3\text{O})(\text{ClO}_4)$  consists of one asymmetric broad band centered around 17544  $\text{cm}^{-1}$ . This spectrum suggests an asymmetric planar or tetragonal arrangement of the ligand atoms around the cupric ion [4,40]. The d-d electronic spectrum of the complex in DMF shows a band at 16 000  $\text{cm}^{-1}$ ; from this value it is possible to deduce that the complex could exhibit a coordination geometry with a less planar character [8,40].

The copper (II) complex has a magnetic moment of 1.06 B.M. per copper at room temperature, which is lower than the spin-only value for one electron. This can be explained by assuming a metal-metal interaction due to spin-exchange [41], and this can only be achieved in a dimeric or polymeric structure. In spite of this, the polycrystalline EPR spectrum of the complex shows no signal at this temperature; apparently, relaxation phenomena hamper the observation. Variable-temperature solid-state magnetic sus-

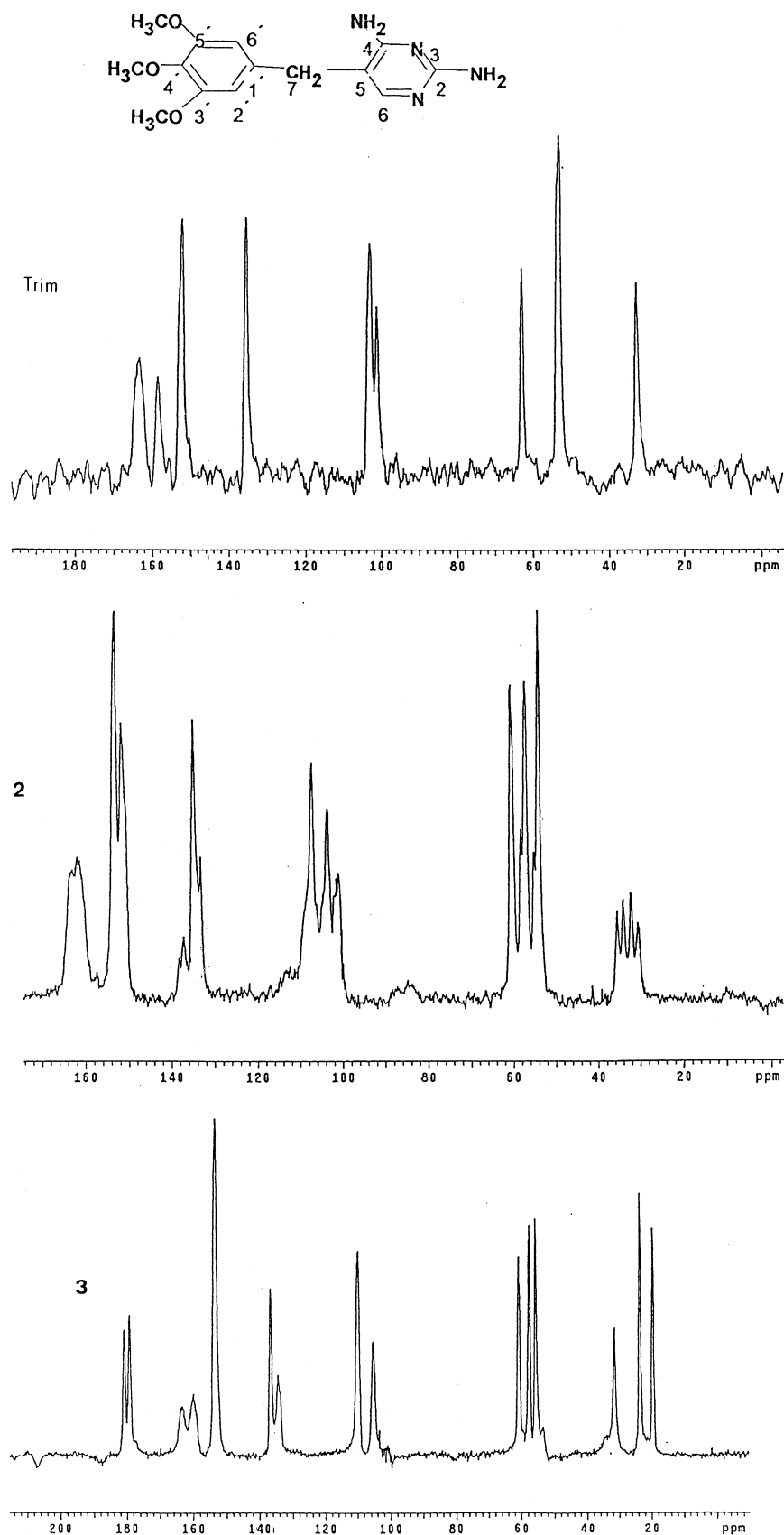


Fig. 3.  $^{13}\text{C}$  NMR spectra in the solid state of trimethoprim (Trim) and their complexes  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$  (2) and  $[\text{Cd}(\text{Trim})\text{Cl}_2(\text{CH}_3\text{OH})]_n$  (3).

Table 4

Minimal inhibitory concentration (MIC,  $\mu\text{g/ml}$ ) of the drugs for the assayed bacteria

Microorganism	Compounds				
	Trim	HTrim( $\text{NO}_3$ )	1	2	3
<i>E. coli</i> ATCC 25922	0.5	0.5	0.5	1	1
<i>E. aerogenes</i> ATCC 13048	0.25	0.5	0.5	1	1
<i>E. cloacae</i> ATCC 13047	0.5	1	2	2	2
<i>E. cloacae</i> ATCC 23355	1	1	2	2	2
<i>K. pneumoniae</i> ATCC 13883	1	1	2	4	2
<i>S. marcescens</i> ATCC 8100	4	2	8	4	8
<i>C. freundii</i> ATCC 8090	0.5	0.5	1	1	1
<i>S. flexneri</i> ATCC 12022	0.5	0.5	0.25	0.5	1
<i>P. bulgaris</i> ATCC 13315	16	32	32	16	32
<i>P. morganii</i> NCTC 235	2	2	1	4	4
<i>P. aeruginosa</i> ATCC 9721	32	32	32	64	32
<i>P. aeruginosa</i> ATCC 27853	4	32	8	4	8
<i>A. calcoaceticus</i> ATCC 19606	32	8	16	16	16
<i>S. aerus</i> ATCC 25923	4	4	4	8	8

ceptibility studies have been performed on a powdered sample in the 100–350 K range. The molar magnetic susceptibility per copper atom,  $\chi_M$ , continuously decreases as the temperature is lowered, from a value of effective magnetic moment of approximately zero, indicating the presence of an antiferromagnetic exchange interaction between the Cu(II) ions. A rough estimation of  $J$  value in the 100–350 K temperature range was obtained by using Bleaney–Bowers equation; this value can be estimated to be ca.  $-600 \text{ cm}^{-1}$ .

In conclusion, on the basis of all these measurements, no definite molecular formulation for the Cu(II) compound can be inferred. Magnetic moment and EPR measurements support the hypothesis of a binuclear or polynuclear nature, with bridging methoxo groups. We are pursuing the study of these systems in an attempt to obtain suitable crystals for X-ray structural analysis.

### 3.4. Antibacterial studies

Table 4 shows the results of the antibacterial activity of the compounds. According to the agreement criteria, the activity of the four compounds is similar to that of the reference drug (Trimethoprim) but with some differences: compounds  $\text{Cu}(\text{Trim})_2(\text{CH}_3\text{O})(\text{ClO}_4)$ ,  $[\text{Zn}(\text{Trim})_2\text{Cl}_2]$  and  $\text{Zn}(\text{Trim})(\text{CH}_3\text{COO})_2$  show a tendency towards MIC values from two to four times higher than that of the ligand, in most of the cases, and for the *A. calcoaceticus* ATCC 19606, they exhibit a tendency which is half of that of drug.

A question which remains unsolved is whether it is the ligand or M-complex the species which is biologically active against the bacteria. More studies are required to obtain a conclusion about the action mechanism.

## 4. Supplementary material

Atomic coordinates, anisotropic thermal parameters, H-atom parameters and lists of structure amplitudes are available from the authors on request.

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