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Synthesis, Characterisation and Antimicrobial Activity of Dimeric and Monomeric Copper(II)-Schiff base Complexes

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Abstract: *Novel copper(II)-Schiff base derivatives were prepared in 1:1 and 1:2 (M:L) molar ratio reaction in ethanol. These compounds were characterized by infrared, UV-VIS, conductivity measurements, elemental analysis and melting point (decomposition). Dimers of general formula $[Cu(L)(H_2O)]_2.H_2O$ {L = 3-hmp (**1**) or 4-hmp (**3**)} and $[Cu(hmyp)Cl_2]_2$ (**4**) were prepared and a monomer of $[Cu(3\text{-}hmp)_2(H_2O)Cl_2].H_2O$ (**2**). These chemicals are non-electrolytes and have the chloride ions bonded to the metal in terminal, bridging and trans position to the metal, performing geometric patterns of a square planar and square pyramidal arrangements. The UV-VIS of these complexes showed electronic transitions of molar extinction coefficients (ε) between 10^2 and 10^3 L mol^{-1} cm^{-1} related to Metal-to-Ligand Charge Transfer (MLCT) bands and d-d transitions. The bioassay of the ligands and the metal derivatives were screened against Gram-positive (Staphylococcus aureus and Bacillus subtillis), and Gram-negative (Escherichia coli and Salmonella typhimurium) microorganisms. Complex-**4** and the ligands hmyp and 4-hmp showed minimum inhibition concentration (MIC) in the range of 190 to 1020 μM (40 to 350 μg mL^{-1}) against these microorganisms but complex-**1**, **2** and **3** were not active.*

Keywords: *antimicrobial activity, copper(II) complexes, Schiff base, bactericide, coordination compounds*

I. Introduction

Schiff bases are compounds acknowledged as fungicide [1], bactericide [2], antiviral [3] as well as antitumor drugs [4]. Considerable results on biological activity of metal-Schiff base derivatives reveal the potential of these compounds to become commercial drugs in the treatment of human diseases. Transition metal Schiff base complexes are reported to give better antimicrobial results in comparison to free Schiff bases, supporting the coordination compounds as good candidates in the whole of therapeutic treatments [5]. Since the discovery of *cisplatin*, the amount of active compounds based on metals and transition metals have been rising. For instance, copper(II) derivatives of Schiff bases are recognized for the treatment of several illnesses, including cancer therapy [6, 7].

The development of novel and effective drugs with low cost and less side effects are desirable to replace the commercial medicines. It becomes a challenge to researchers in the field to achieve this goal because of the resistance of microorganisms to commercial drugs, imposing limits on the community treatment and therefore increasing security risk to public health.

To continue with our research program on the development of biological active transition metal coordination compounds, three novel Schiff bases (*3-hmp*, *4-hmp* and *hmyp*) and four copper(II) derivatives have been prepared and characterized. These compounds were screened against Gram-negative and Gram-positive microorganisms to evaluate their potential to become antimicrobial drugs.

II. Materials and methods

All chemicals purchased from Sigma-Aldrich, Vetec and FMaia companies were used without prior purification. Elemental analysis was obtained from a Perkin Elmer 200 CHNS Elemental Analyzer. A Conductivity Jenway Meter 4010 was used to gather information on the molar conductivity (ΩM) of the copper(II) complexes in methanol at the concentration of 10^{-3} mol L^{-1}. The infrared spectra were obtained from a Perkin Elmer FT-IR 1000 using Nujol between CsI windows. The UV-VIS spectra were recorded in a spectrophotometer Varian Cary 50, using quartz cuvette in the range of 200 to 800 nm. The minimum inhibitory concentration (MIC) was determined by broth microdilution method using a spectrophotometer Eliza (600 nm) and microplates BioRad model 3550-UV, USA.

2.1. Synthesis of Schiff Bases

Three Schiff bases were prepared by reacting 3-, 4-aminophenol and 2-amino-2-methylpropan-1-ol with 2-hydroxybenzaldehyde. The structures of these Schiff bases are showed in Fig. 1.

(E)-2-((3-hydroxyphenylimino)methyl)phenol (3-hmp)

To a round-bottom flask of 100 mL, 1.00 g (9.16 mmol) of 3-aminophenol was dissolved in ethanol (60 mL). Then, 1.11 g (0.97 mL, 9.09 mmol) of 2-hydroxybenzaldehyde was added into the mixture which was allowed to reflux under stirring for 4 h. Subsequently, half of the solvent was removed under reduced pressure and the flask reserved in a freezer. After cooling, an orange solid separated from the mixture, which was filtered off in air, washed with hexane and stored in desiccators.

Yield of 2.00 g (95 %); Mp (°C): 123.5 - 124.7; Elemental analysis required for $C_{13}H_{11}NO_2$: C, 73.23; H, 5.20; N, 6.57; found: C, 74.40; H, 5.35; N, 6.73. IR (Nujol / CsI): 3320 ν(OH); 1616 ν(C=N); 1591, 1501 ν(C=C); 1288 ν(C-O); 1163 ν(C-N); 844, 755 δ(C-H).

(E)-2-((4-hydroxyphenylimino)methyl)phenol (4-hmp)

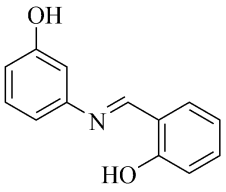
This compound was synthesized following the procedure described for *3-hmp*, using 1.0 g (9.16 mmol) of 4-aminophenol and 1.26 g (1.10 mL, 10.31 mmol) of 2-hydroxybenzaldehyde.

Colour: yellow. Yield of 1.89 g (84 %); Mp (°C): 128.6 - 131.4; Elemental analysis required for $C_{13}H_{11}NO_2$: C, 73.23; H, 5.20; N, 6.57; found: C, 73.73; H, 5.32; N, 6.64. IR (Nujol / CsI): 3266 ν(OH); 1617 ν(C=N); 1572, 1508 ν(C=C);1259 ν(C-O); 1187 ν(C-N); 839, 755 δ(C-H).

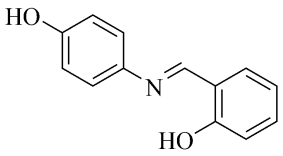
(E)-2-((1-hydroxy-2-methylpropan-2-ylimino)methyl)phenol (hmyp)

To a round-bottom flask of 100 mL, 2.00 g (2.10 mL, 22.4 mmol) of 2-amino-2-methylpropan-1-ol was dissolved in hexane (60 mL). Then, 2.70 g (2.30 mL, 22.10 mmol) of 2-hydroxybenzaldehyde was added into the flask and the mixture allowed to reflux under stirring for 4 h. The resulting yellow mixture was set aside in a freezer for 24 h. After cooling, a green-yellowish material separated from the mixture, which was filtered off in air, washed with hexane and stored in desiccators.

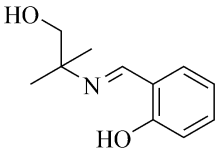
Yield of 3.00 g (64 %); Mp (°C): 60.9 - 61.3; Elemental analysis required for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25; found: C, 69.56; H, 7.44; N, 7.77. IR (Nujol / CsI): 3259 ν(OH); 1631 ν(C=N); 1582, 1502 ν(C=C); 1281 ν(C-O); 1154 ν(C-N); 866, 767 δ(C-H).



(E)-2-((3-hydroxyphenylimino)methyl)phenol (*3-hmp*)



(E)-2-((4-hydroxyphenylimino)methyl)phenol (*4-hmp*)



(E)-2-((1-hydroxy-2-methylpropan-2-ylimino)methyl)phenol (*hmyp*)

Figure 1. Molecular structures of the Schiff bases.

2.2. Synthesis of Copper(II)-Schiff base derivatives

To a round-bottom flask of 100 mL, containing 30 mL of ethanol and 1.0 or 2.0 mmol of the appropriate ligand (*3-hmp*, *4-hmp*, *hmyp*), 1.0 mmol of copper(II) chloride dehydrate was added and the mixture allowed to reflux for 4 h under stirring at 70 °C. Subsequently, the solvent was removed under reduced pressure leaving behind an oil to which chloroform (30 mL) was added and the mixture stirred again for 30 minutes. The solids were filtered off in air washed with hexane and diethyl ether, and stored in desiccators.

$[Cu(3\text{-}hmp)(H_2O)Cl]_2.H_2O$ (**1**). Colour: dark brown. Yield of 0.480 g (88 %); Mp (°C): 251 d. Elemental analysis required for $C_{26}H_{26}N_2O_7Cu_2Cl_2$: C, 46.16; H, 3.87; N, 4.14; found: C, 46.28; H, 3.08; N, 4.03. IR (Nujol / CsI): 3410 ν(H_2O); 3310 ν(O-H); 1607 ν(C=N); 551 ν(Cu-OH_2); 536 ν(Cu-O); 466 ν(Cu-N); 303, 292 ν(Cu-Cl). ΩM (CH_3OH, ohm^{-1} mol^{-1} cm^2): 0.05.

[Cu(*3-hmp*)2(H_2O)Cl_2].H_2O (**2**). Colour: dark brown. Yield of 0.526 g (93 %); Mp (°C): 276 d. Elemental analysis required for $C_{26}H_{26}N_2O_6CuCl_2$: C, 52.31; H, 4.39; N, 4.69; found: C, 53.21; H, 3.77; N, 4.57. IR (Nujol / CsI): 3450 ν(H_2O); 3164 ν(O-H); 1609 ν(C=N); 521 ν(Cu-OH_2); 484 ν(Cu-N); 314 ν(Cu-Cl). ΩM (CH_3OH, ohm^{-1} mol^{-1} cm^2): 0.04.

[Cu(*4-hmp*)(H_2O)Cl]$_2$.H_2O (**3**). Colour: dark brown. Yield of 0.334 g (91 %); Mp (°C): 251 d. Elemental analysis required for $C_{26}H_{26}N_2O_7Cu_2Cl_2$: C, 46.16; H, 3.87; N, 4.14; found: C, 45.50; H, 3.51; N, 5.02; IR (Nujol / CsI): 3430 ν(H_2O); 3168 ν(O-H); 1645 ν(C=N); 518 ν(Cu-OH_2); 484 ν(Cu-O); 456 ν(Cu-N); 310, 294 ν(Cu-Cl). ΩM (CH_3OH, ohm^{-1} mol^{-1} cm^2): 0.04.

[Cu(*hmyp*)Cl_2]$_2$ (**4**). Colour: green. Yield of 0.263 g (46 %); Mp (°C): 155 d. Elemental analysis required for $C_{22}H_{26}N_2O_4Cu_2Cl_4$: C, 40.57; H, 4.02; N, 4.30; found: C, 40.66; H, 5.28; N, 4.34. IR (Nujol / CsI): 3531 ν(O-H)(sharp); 1632 ν(C=N); 452 ν(Cu-N); 336, 309, 297 ν(Cu-Cl). ΩM (CH_3OH, ohm^{-1} mol^{-1} cm^2): 0.06.

2.3. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined by the broth microdilution assays using microplates of 96 wells [8]. The metal standard solution was prepared by dissolving 1.0 mg of the substance test in a mixture of DMSO (250 µL) and sterile water (750 µL). The microorganisms were grown in 3.0 mL of Luria Bertani (LB) medium at 37 °C under stirring until an optical density (OD) range of 0.08 to 0.10 being achieved which contains 1.0 to 2.0 x 10^8 colony-forming unit (CFU) / mL. From the LB medium of each bacteria, 100 µL (5.0 x 10^4 CFU) of that was added to 50 µL of the substance test respectively. Afterwards, the mixture was poured into the wells on the microplates and incubated for 24 h. The MIC was obtained by the use of a spectrometer ELISA at 600 nm. The experiment was finished in duplicate, considering the standard deviation. DMSO was the negative control, and the positive control was *Amoxicillin* and *Norfloxacin*.

III. Results and Discussion

The reactions to prepare the copper(II) Schiff base complexes were carried out in 1:1 and 1:2 (M:L) molar ratio of reactants. Irrespective of the stoichiometry used, complex-**2** is 1:2, and complex-**1**, **3** and **4** are 1:1 concluding products. The molar conductivity of these compounds (ΩM) in methanol showed that these complexes are non-electrolytes.

3.1. Infrared spectroscopy

Typical vibrational bands of the Schiff bases, *3-hmp*, *4-hmp* and *hmyp*, related to the stretching mode of the hydroxyl and the imine bond were in the region of 3280 and 1621 cm^{-1}, respectively [9-11]. Upon coordination, the vibrational band of the imine bond shifted almost 10 cm^{-1} towards low frequency in complex-**1** and **2** and 28 cm^{-1} towards high frequency in complex-**3**. The infrared stretching of the imine bond in complex-**4** did not shift significantly in comparison to the ligand *hmyp*. Frequently, the infrared stretching mode of the imine bond from Schiff bases have a slight shift upon coordination within the range of 10 to 25 cm^{-1} [5, 12].

The difference in infrared shift of the imine bond upon coordination between complex-**1** and **3** reinforce the formation of chelating bonds of the type N-Cu-O having bidentate oxygen-terminal or bidentate oxygen-bridging bonds. For these two coordination modes, the vibrational stretching of the imine moiety depends on the delocalization of electrons within the N-Cu-O chelate rings, affecting the infrared shift. For instance, an infrared stretching of the terminal imine bond from a chelate ring containing oxygen-bridging atoms is expected to shift towards high frequency upon coordination owing to the delocalization of electrons comprising the oxygen-bridging atoms and the two copper(II) ions.

The insignificant infrared shift of the imine bond in complex-**4**, on the other hand, is an indication that the nitrogen atom is in *trans* position to the Cu-Cl bond. The proximate electronegativity between these two atoms will balance the electron density of the imine bond, reducing the effect on its the infrared shift [13]. Similarly, the small infrared shift of the imine bond in complex-**2** also indicates that the nitrogen atoms are in *trans* positon to the metal centre as a result of a monodentate coordination mode.

The vibrational stretching of the hydroxyl groups were assigned in the region of 3214 cm^{-1} in the spectra of complex-**1**, **2** and **3** which corroborates with the formation of hydrogen bonding [12-14]. In complex-**4**, a narrow weak to medium band at 3521 cm^{-1} is also related to hydrogen bonding within the crystal lattice of this compound. Cesium(I) derivatives of lactose, d-arabinose and l-arabinose also showed hydrogen bonding at 3520 cm^{-1} confirmed by the X-ray structural determination [14].

At the low-frequency, novel bands in the region 530 cm^{-1} were assigned to the wagging vibrational band of coordinated water in complex-**1**, **2** and **3**; complex-**4** did not show bands correlated to this vibrational mode [12, 13, 15, 16]. The Cu-O bond, formed by the loss of hydrochloric acid in complex-**1** and **3**, and the Cu-N bond of all these complexes showed vibrational stretching in the region of 510 cm^{-1} and 472 cm^{-1} respectively [12, 13, 15, 17]. Complex-**1** and **3** also showed two bands associated with the bridging stretching modes of Cu-Cl bonds, contrasting with one band for complex-**2** and three ones for complex-**4** [13, 18]. For the latter two

compounds, these bands supports to the formation of *trans*, terminal and bridging chlorides as showed by the proposed structures in Fig. 2 [13]. However, the Cu-Cl infrared bands of complex-**1** and **3** are also consistent with chlorides at the terminal position of a dimeric structure in which the metals are at the centre of a square pyramidal geometry. Two possible arrangements are conceivable concerning the N-Cu-O bidentate coordination mode as showed in Fig. 2. Both structural arrangements corroborate with the infrared stretching of the Cu-O bond in complex-**1** and **3** because of the slight difference in force constant between the bidentate oxygen-bridging and oxygen-terminal coordination modes of these chelate bonding type.

The literature provide with information on dimeric and monomeric square planar palladium(II) derivatives of N-heterocyclic carbene amidate alkoxide having both N-Pd-O chelating stretching modes. The X-ray structural analysis of these palladium compounds showed that the dimer has shortened metal oxygen-bridging bond length in comparison to the oxygen-terminal bond in the monomer [19]. The structural determination of dimeric square pyramidal copper(II) compounds derivative of N-(salicylidene)-N'-(imidazol-2-ylmethylene)-1,3-propanediamine) also showed elongated metal oxygen-bridging bond length in addition to shortened ones which are associated with the axial or equatorial positon of the oxygen atoms within the structure. The infrared stretching of the coordinated imine moiety of those copper(II)-N-(salicylidene)-derivatives was assigned at 1628 cm^{-1} corroborating with the infrared shift of the imine moiety in complex-**1** and **3** [20]. Consequently, it is reasonable to assume that both complex-**1** and **3** have dimeric structures with oxygen-terminal bonds and bridging chlorides or oxygen-bridging bonds and terminal chlorides as showed in Fig. 2. Complex-**1** most likely has oxygen-terminal bonds and complex-**3** oxygen-bridging bonds, which are in agreement with the infrared shift of the coordinated imine bond and the Cu-O bond stretching from both compounds as well. The formation of dimeric and monomeric structures of complex-**1**, **2**, **3** and **4** in solid state is most likely an outcome of the 1:1 and 1:2 (M:L) stoichiometry among the reactants.

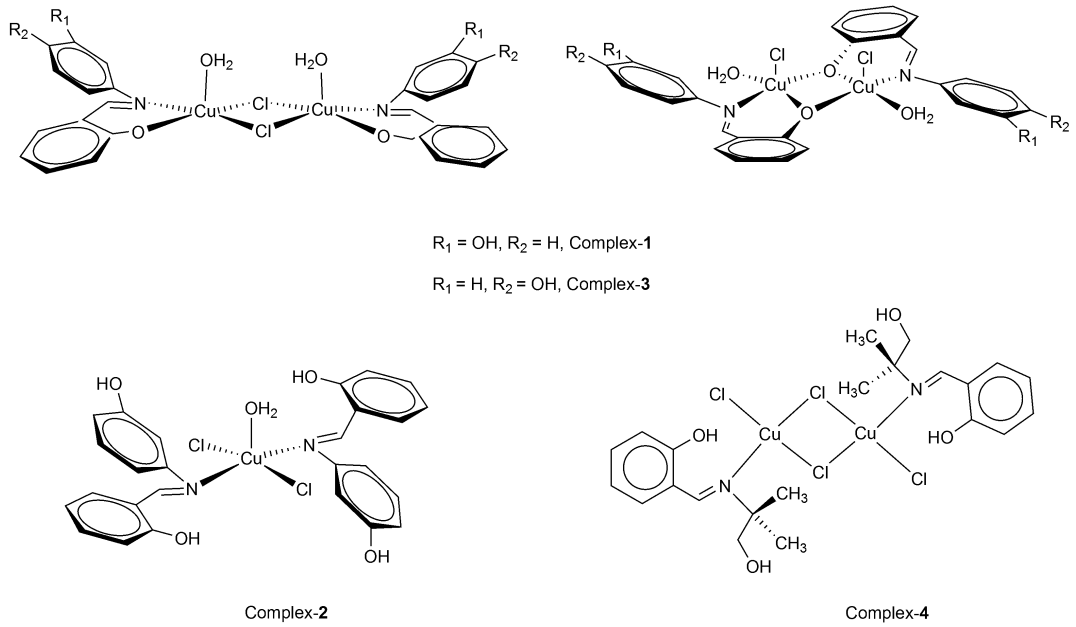
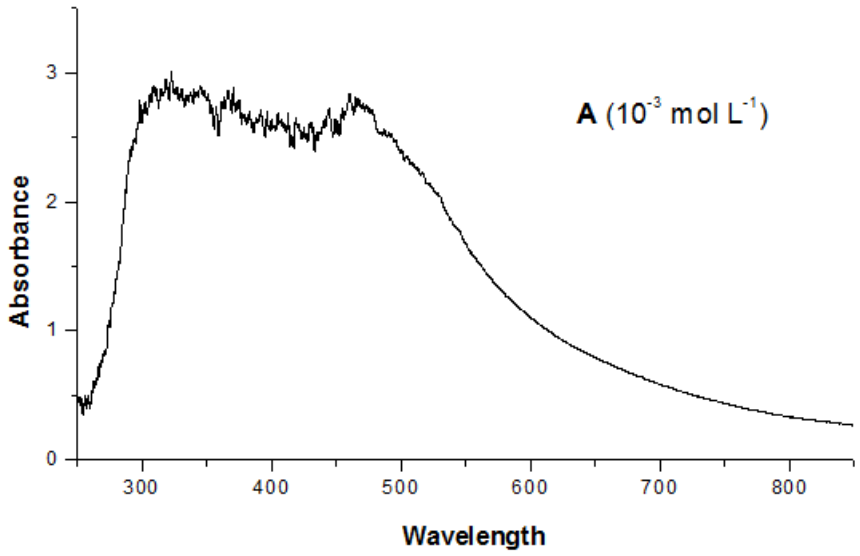


Figure 2. Proposed structures of the copper(II)-Schiff base complexes in solid state.

3.2. Electronic spectra

The electronic spectra of the Schiff bases, *3-* and *4-hmp*, in methanol and DMSO showed electronic transitions of the type $n \rightarrow \pi$ and $\pi \rightarrow \pi^*$ in the range of 267 to 348 nm. The ligand *hmyp* showed four bands in the range of 253 to 399 nm related to the former electronic transitions. These bands shifted upon coordination and have been observed in the spectra of the copper(II) derivatives within the range of wavelengths of the corresponding ligands as showed in Fig. 3.

The spectra of complex-**1**, **2** and **3** have revealed high intensity overlapped broad bands in the range of 260 to 500 nm in methanol and in DMSO. These electronic transitions fall within the visible region of the spectra as showed in Fig. 3, characteristic of Metal-to-ligand Charge Transfer (MLCT) bands. The spectrum of complex-**4** also showed high intensity broad bands below 450 nm and a band of low intensity in both solvents in the region of 668 nm. The extinction coefficients (ε) of the estimated lowest energy band of complex-**1**, **2** and **3** were in the range of 10^2 to 10^3 L mol^{-1} cm^{-1} that corresponds to the transfer charge bands involving complexes having unsaturated ligands [21].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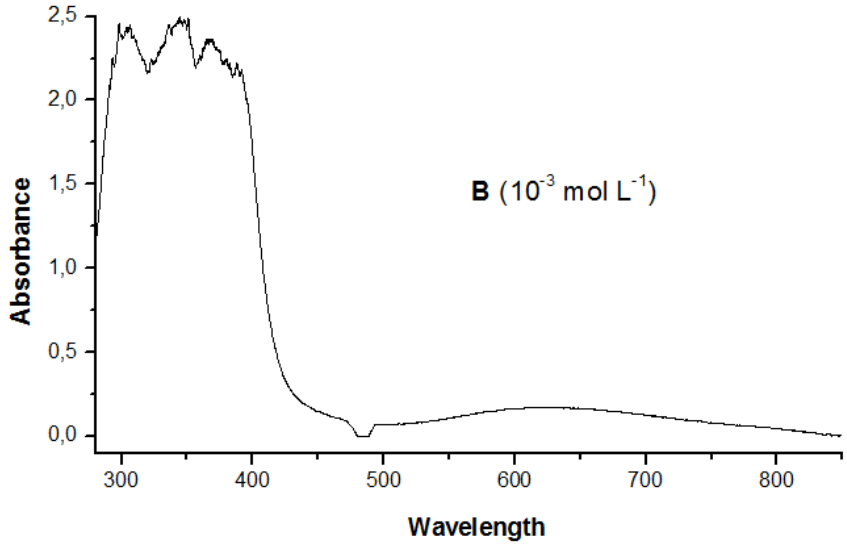
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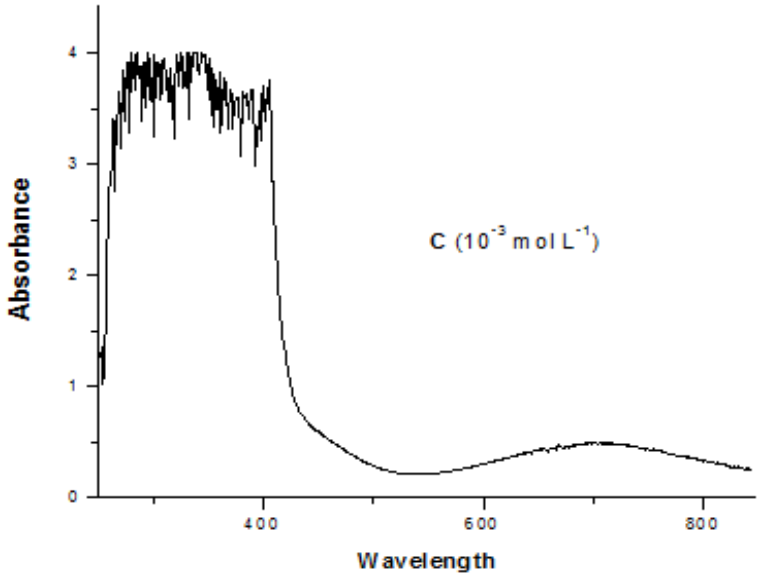
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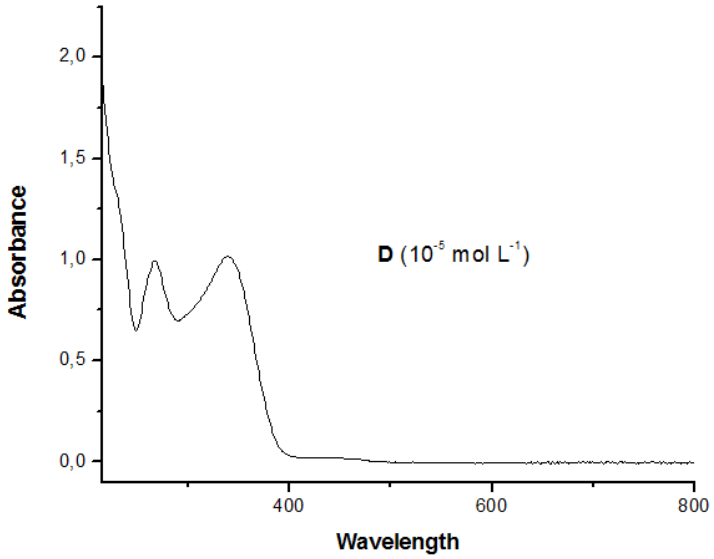

Figure 3. Spectra of complex-**1** (A), complex-**4** (B and C), and *3-hmp* (D); A, B and D in methanol and C in DMSO. The spectra show MLCT, *d-d*, n → π, π → π* transitions and saturation of the detector in A, B and C below 500 nm.

However, the lowest energy band in the UV-VIS region of complex-**4** is most likely due to *d-d* electronic transitions at the metal centre. Square planar and square pyramidal copper(II) derivatives of Schiff bases, triazanone 2,6-diones and mixed ligands are reported to present *d-d* transitions in the range of 600 to 770 nm and Ligand-to-Metal Charge Transfer (LMCT) bands in the range of 220 to 440 nm. The extinction coefficient of these square planar and square pyramidal copper(II) compounds vary from 71 to 478 L mol^{-1} cm^{-1}. The ε value of complex-**4** is within this range in both solvents, which reinforces a structural arrangement of a dimeric square planar geometry in solution [16, 17, 22-24].

The structural arrangements of these copper(II) complexes in solid state are probably preserved in methanol and in DMSO as revealed by the UV-VIS spectrum of complex-**4** in both solvents. In addition, the non-electrolyte properties of them in methanol support the preservation of the original structures in solution, regardless of the coordinating property of DMSO [17]. The most significant UV-VIS bands of the Schiff bases and the corresponding complexes are showed in Table 1.

Table 1. UV-VIS bands of the Schiff bases and the copper(II) derivatives in Methanol

Compound	Transitions Energy (cm^{-1}) {nm, ε}‡				
3-hmp	37,453 (267; 15,434)	29,498 (339; 15,722)			
[Cu(*3-hmp*)(H_2O)Cl]$_2$·H_2O (**1**)	*Overlapped bands below 500 nm*				21,598 (463; 2,309)#
[Cu(*3-hmp*)$_2$(H_2O)Cl_2]·H_2O (**2**)	*Overlapped bands below 500 nm*				21,276 (470; 2,140)#
4-hmp	37,037 (270; 12,812)	28,735 (348; 23,808)			
[Cu(*4-hmp*)(H_2O)Cl]$_2$·H_2O (**3**)	*Overlapped bands below 500 nm*				15,748 (635; 2,000)#
hmyp	39,525 (253; 3,583)	36,101 (277; 2,624)	31,746 (315; 997)	25,062 (399; 1,408)	
[Cu(*hmyp*)Cl_2]$_2$ (**4**)	*Overlapped bands below 500 nm*				15,974 (632; 145) [a]
	Overlapped bands below 500 nm				14,204 (704; 150)†,[a]

† DMSO - dimethyl sulfoxide; ‡{wavelength; extinction coefficient (L mol^{-1} cm^{-1})}; # estimated lowest energy band due to saturation of the detector; [a] *d-d* lowest energy transition.

3.3. Antimicrobial activity

The potential of these copper(II) complexes to become antimicrobial drugs was tested for activity against *Staphylococcus aureus* (ATCC 33591) and *Bacillus subtillis* (ATCC 23858) (Gram-positive), and *Escherichia coli* (ATCC 29214) and *Salmonella typhimurium* (ATCC 14028) (Gram-negative) microorganisms. The *3-hmp* was inactive against all of these microorganisms. The *4-hmp* was only active against *S. aureus* and *B. subtillis* similarly to *Amoxicilli*n as showed in Table 2. Considering that both compounds, *4-hmp* and *Amoxicillin*, have a hydroxyl group at the *para* position of the phenyl ring, Fig. 1 and 4, this group is hypothetically a good candidate for being the chemical site in the biochemical interaction with the Gram-positive microorganisms.

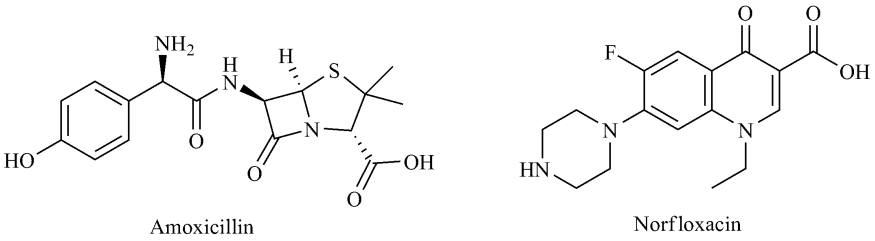

Figure 4. Structure of the commercial drugs

The *hmyp* was active as *Norfloxacin* against *E. coli*, but the former compound was practically 1000 times less active in comparison to this commercial drug, giving activity against this microorganism only. Since these compounds have no common structural moieties, it becomes difficult to envisage a possible structural correlation between them for the biochemical interaction with the *E. coli*.

Upon coordination, the free *para* and *ortho* hydroxyl groups from *3*- and *4-hmp* did not have any influence on the antimicrobial activity because its copper(II) derivatives, complex-**1**, **2** and **3**, were inactive against the Gram-negative and Gram-positive microorganisms. Nevertheless, copper(II) chloride was active against *S. aureus* and *B. subtillis*, which led to postulate that the coordination of *3*- and *4-hmp* to the metal ion deactivate the copper(II) derivatives towards the antimicrobial activity. The absence of a synergistic effect between the copper(II) ion and both *3*- and *4-hmp* is most likely one of the reasons for these complexes not being active against the microorganisms tested. Complex-**4** was only active against *S. aureus* and *B. subtilli*s.. For this complex, a synergistic effect between the metal ion and the ligand appears to be crucial for the antimicrobial activity but against *E. coli* and *S. typhimurium*, complex-**4** was inactive and copper(II) chloride as well. In this situation, the synergistic effect in complex-**4** was not significant to the antimicrobial activity owing probably to an "intrinsic resistance" in connection to the extra membrane cell wall of the Gram-negative bacteria [9].

Table 2. Antimicrobial data* of the copper(II)-Schiff base complexes.

Compound	*S. aureus*	*B. subtillis*	*E. coli*	*S. typhimurium*
4-hmp	195 (42)	391 (83)	*na*	*na*
hmyp	*na*	*na*	1726 (333)	*na*
[Cu(*hmyp*)Cl_2]$_2$ (**4**)	508 (165)	1017 (332)	*na*	*na*
Amoxicillin	14.2	0.4	*na*	*na*
Norfloxacin	16.2	4.1	1.8	16.2
$CuCl_2 \cdot 2H_2O$	1951	1951	*na*	*na*

*MIC - μM (μg mL^{-1}); *na* – not active

The geometrical arrangements of coordination compounds may also be important to antimicrobial activity. For instance, octahedral copper(II) derivatives of 1,2,4-triazole Schiff bases showed considerable antibacterial activity in comparison to the corresponding free ligands. The MIC of these octahedral copper(II) compounds was in the range of 25 to 100 μg mL^{-1} against *S. aureus* and *E. coli* [12]. Monomeric and dimeric square pyramidal in addition to square planar copper(II) derivatives of substituted imidazole showed IC_{50} in the region of 43 μM on U937 Leukemia cell line but these compounds did not show any chemotherapeutic potential on other Leukemia and Melanoma cell lines [22]. Square pyramidal copper(II) derivatives of 2,6-diacetylpyridine bis(benzenesulfonohydrazide) Schiff base also showed antimicrobial activity against *S. aureus* (MRSA 0701-4, MRSA 1, MRSA 0307-28, MRSA 0302-4, MRSA 0704-3). Although the free 2,6-

diacetylpyridine- Schiff base were not active, the MIC of the corresponding square pyramidal copper(II) derivatives were in the range of 1250 to 5000 μg mL^{-1} [16]. The bioassay datum of complex-**4** revealed MIC values at 165 μg mL^{-1} against *S. aureus* (ATCC 33591) and 332 μg mL^{-1} for *B. subtillis*, which is equivalent to the free *hmyp* ligand for *E. coli*. The MIC of the *4-hmp* ligand was below 100 μg mL^{-1} for *S. aureus* (ATCC 33591) and *B. subtillis* (ATCC 23858), showing a better result in comparison to complex-**4**. Nevertheless, this complex reveal MIC values of antimicrobial activity comparable to other copper(II) Schiff base derivatives.

The electronic properties of the copper(II)-Schiff base compounds in this work may also give an insight into the bioassay results. The electronic spectra of complex-**1**, **2** and **3** (see Table 1) showed MLCT bands which are regarded as an internal redox process in compounds presenting such electronic properties [21]. Consequently, it is conceivable that this chemical property might be a contributing factor to the polarization of these compounds to get across the cell wall of the microorganisms. A common chemical aspect between copper(II)-1,2,4-triazole- or copper(II)-2,6-diacetylpyridine- Schiff base derivatives and complex-**4** is that all of them exhibited *d-d* electronic transitions. This chemical property reinforces that an internal redox process in complex-**1**, **2** and **3**, by the MLCT bands, in addition to the lack of a synergistic effect is hypothetically the source of these copper(II) compounds not being effective against the Gram-positive and Gram-negative microorganisms.

IV. Conclusion

Although the mechanism of action is still unknown, a synergistic effect in complex-**4** was significant to achieve antimicrobial activity against the microorganisms tested. It is assumed that upon coordination of *3*- and *4-hmp*, the biochemical interaction of the corresponding copper(II) derivatives with the microorganisms is deactivated by an internal redox process. Based on the MIC values of complex-**4**, *4-hmp* and *hmyp* ligands, these compounds might eventually be useful as part of the chemical composition of medicines for human illnesses treatment.

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