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Full Paper

Copper(II) Complexes with Ligands Derived from 4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one: Synthesis and Biological Activity

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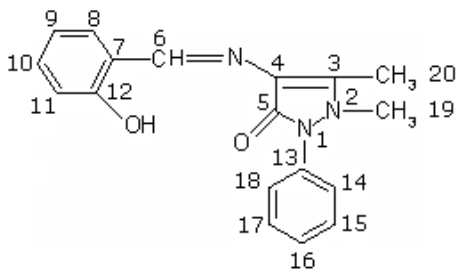
Abstract: The synthesis of Cu(II) complexes derived from Schiff base ligands obtained by the condensation of 2-hydroxybenzaldehyde or terephtalic aldehyde with 4-amino-antipyrine (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) is presented. The newly prepared compounds were characterized by ^{1}H-NMR, UV-VIS, IR and ESR spectroscopy. The determination of the antimicrobial activity of the ligands and of the complexes was carried out on samples of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter boumanii, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida sp.* The qualitative and quantitative antimicrobial activity test results proved that all the prepared complexes are very active, especially against samples of *Ps. aeruginosa, A. Boumanii, E. coli* and *S. aureus.*

Keywords: Schiff bases, antimicrobial activity, copper complexes, aminoantipyrine.

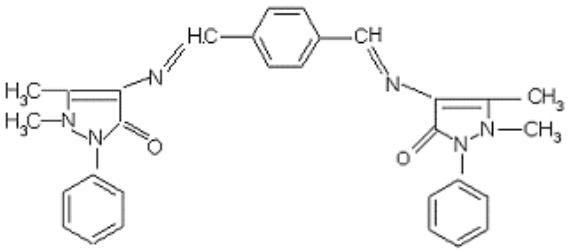
Introduction

The well-known Cu(II) ion forms a series of coordination compounds with well defined structures. It plays an important role in the numerous biological processes that involve electron transfer reactions or the activation of some anti-tumor substances [1]. Copper is an essential micronutrient for feeding and a co-factor of several enzymes involved in oxidative metabolism: β-hydroxylases, quercetinase, ceruloplasmine, cytochromoxidase, mono-aminoxidase, superoxydismutase, ascorbic acid oxidase and tyrosinase. The catalytic role of these enzymes is the result of two processes: a) the reduction of the Cu^{2+} cation to Cu^{+}; b) the fixation of the molecular oxygen [2]. As a cofactor of ceruloplasmin, copper contributes to the oxidation of Fe(II) to the corresponding Fe(III) form. Being related to transferin, the latter may cross the cell membranes [3]. Copper also has functions in erythropoiesis and hemoglobin-genesis, favoring, together with molybdenum, intestinal absorption, sediment mobilization and increases in plasmatic iron levels. Apart from its numerous functions in metabolic processes, copper is also recognized as a part in the immune function [3]. Superoxydismutase, which transforms toxic superoxide radicals in oxygen and peroxide, is dependent on copper and zinc. The Cu^{2+} ion is involved in the expression of genes for the metal-binding proteins [3] and it is also found in copper-protein combinations displaying a pseudotetrahedral symmetry and having effects in bio-systems. Through aminoxidase, copper interferes in the metabolism of the conjunctive tissue, contributing to the trophicity of vascular sides [4-8]. Taking into account the daily necessary quantity of Cu(II) in the organism (2-3 mg/day), its distribution and metabolism in the organism, toxicity, numerous simple or complex combinations of copper are used in the treatment of a variety of diseases, including inflammatory processes, cancer, ulcers, nervous system and heart diseases. This paper presents the synthesis and characterization of Cu(II) complexes with Schiff bases obtained by the condensation of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one with 2-hydroxybenzaldehyde and terephthalic aldehyde, respectively (Figure 1).

Figure 1. Ligand structures.



ASAAP



ATAAP

Furthermore, and taking into consideration the use of copper complexes in the treatment of some diseases, mentioned above, we have tested the antimicrobial activity of the prepared ligands and complexes using strains of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter boumanii, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Candida tropicalis* isolated from different pathological products from patients with infections associated with the use of cardiovascular prosthetic devices. The antimicrobial activity of the Schiff bases of antipyrine and their complexes have been discussed previously [9-16].

Results and Discussion

Synthesis

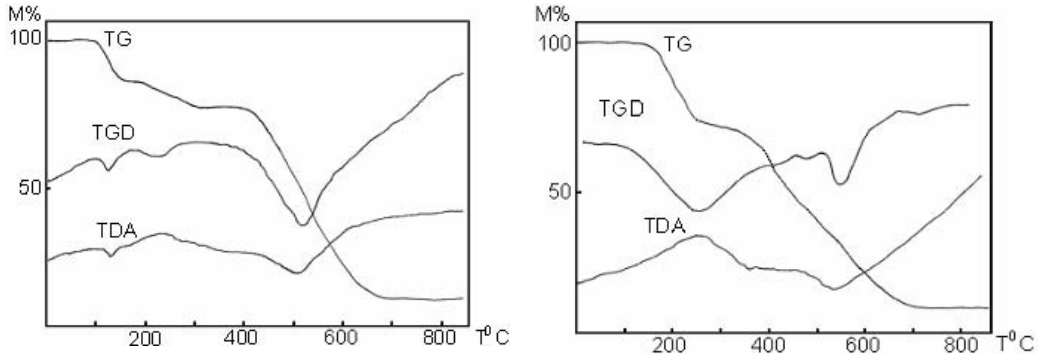
The Schiff base ligands **ASAAP** and **ATAAP** were prepared by the condensation in methanol of one or two molecules of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one with 2-hydroxy-benzaldehyde and terephthalic aldehyde, respectively. The Cu complexes $Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ (**a**) and $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ (**b**) were then prepared by reaction of the appropriate ligands with aqueous solutions of a suitable Cu(II) salt.

Properties

The molar conductivity value (32.7 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$) of the $Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ complex in nitrobenzene indicates that this compound is a 1:1 electrolyte, while the molar conductibility value below 10 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ in nitrobenzene for the $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ complex indicates that the latter is a non-electrolyte.

The complexes were also investigated by thermo-gravimetry (TG). Experimental data for these analyses are presented in Figure 2. The weight loss between 125–158°C for the $Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ complex is attributed to the loss of two water molecules per molecule of complex.

Figure 2. TG, DTG and ATD curves for: $Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ (**a**); $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ (**b**).



(a) (b)

The second step (410-670°C) corresponds to the elimination of one molecule of ligand per molecule of complex. The thermal analysis curve of the $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ complex presents two weight loss steps (196-270°C and 380-694°C). The final residue was analyzed by IR spectroscopy and was identified as CuO and the % Cu corresponded to the calculated one.

IR Spectra

Compared to the IR spectra of the ligands, it was observed that the frequency of the specific band of the $\nu_{C=N}$ bond (1653, 1654 cm^{-1}) is moved towards lower wavenumbers by approx. 13 - 15 cm^{-1} in the spectra of the complexes, which confirms the coordination of the nitrogen atom to the metallic ion. In the IR spectrum of the **ASAAP** ligand, a wide medium intensity band occurs in the 3210-3370 cm^{-1}

range, along with a narrow band of medium intensity (1140 cm^{-1}), assigned to the phenolic –OH groups. In the IR spectrum of complex **(a)** the first band disappears and the second band moves towards lower wavenumbers, indicating coordination of the ligand to the Cu^{2+} ion through the phenolic –OH group oxygen. In this spectrum two narrow intense bands at 897 cm^{-1} and 572 cm^{-1} specific for coordinated water molecules [17] are also seen. For both complexes the specific $\nu_{>C=O}$ band (cyclic keto group present in the pyrazolone ring: 1615, 1596 cm^{-1}) moves towards lower wavenumbers (1589, 1580 cm^{-1}), suggesting the coordination of the ligand to the metallic ion via the >C=O group.

Table 1: IR spectral data for the prepared ligands and complexes.

Compound	$\nu_{C=N}$	ν_{Ar-OH}	$\nu_{>C=O}$	ρ_r	ρ_w	$\nu_{SO_4}^{2-}$			
						ν_1	ν_2	ν_3	ν_4
$C_{18}H_{17}N_3O_2$	1653	3210-3370 1140	1615	-	-	-	-	-	-
$[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ (**a**)	1640	- 1120	1594	897	572	-	-	-	-
$C_{30}H_{28}N_6O_2$	1654	-	1595		-	-	-	-	-
$[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ (**b**)	1639	-	1565		-	995	462	1050 1105 1170	574 610 641

Moreover, in the spectrum of complex (**b**) a characteristic band corresponding to the bidentate coordination of the SO_4^{2-} ion appears. Thus, the ν_1 and ν_2 frequencies specific to a T_d arrangement appear as medium intensity bands, and the ν_3 and ν_4 frequencies each split into three bands, which suggest a low symmetry, probably reduced towards C_{2v} [17].

Electronic spectra.

As seen from the data presented in Table 2, the $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ complex presents a single absorption band at 14600 cm^{-1} corresponding to the d–d transition, indicating the low C_{2V} symmetry of the Cu^{2+} ion [18,19]. The brown colored $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ complex presents two types of d – d type transitions, whose values are characteristic of a deformed tetrahedral symmetry, with the term of the fundamental state d_{xy} [19].

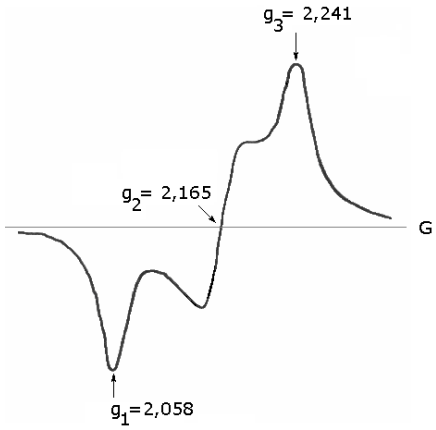
Table 2. Electronic spectra of the synthesized complex combinations.

Compound	**Transitions d-d (cm^{-1})**			**Geometry**
$[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$	$x^2-y^2 \rightarrow$ xz; yz; xy 14600			C_{2v}
$[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$	-	xy→xz; yz 13570	xy→z^2; x^2-y^2 14830	Td deformed

ESR spectra

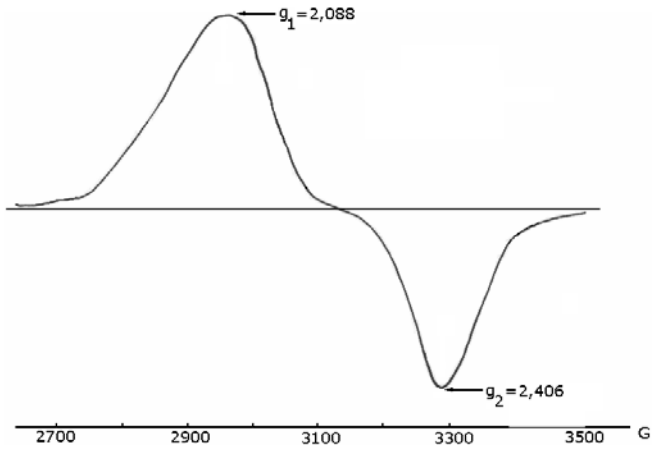
The ESR spectral data concerning the Cu^{2+} ion in tetrahedral symmetry are relatively poor. These present a special interest due to the fact that, in the case of hyperfine interaction, the values of the A constants are considerably lower than the observed ones for a complex of Cu(II) with a O_h or D_{4h} geometry, and the values of the {g} tensor are higher [20-24]. The room temperature ESR spectrum of the complex $[Cu(C_{18}H_{16}N_3O_2)(H_2O)]Cl$, as a powder, is presented in Figure 3.

Figure 3. ESR for $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$, as a powder, at room temperature.



The values of the {g} tensor confirm the C_{2v} symmetry of the Cu^{2+} pentacoordinated ion [24]. For the Cu(II) complex with low symmetry, the fundamental state for the paramagnetic electron is not described by a single *d* function, but there is a mixture of them. The mixture degree of these functions increases as the symmetry decreases [24]. The high values of the {g} tensor confirm a low symmetry. The ESR spectrum for the complex $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ as a powder, at room temperature, is presented in Figure 4.

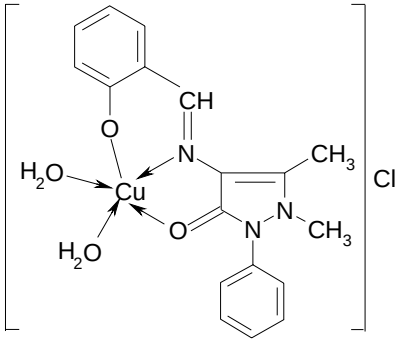
Figure 4. ESR for $[Cu_2(C_{30}H_{28}N_6S_2O_{10})]$, as a powder, at room temperature.



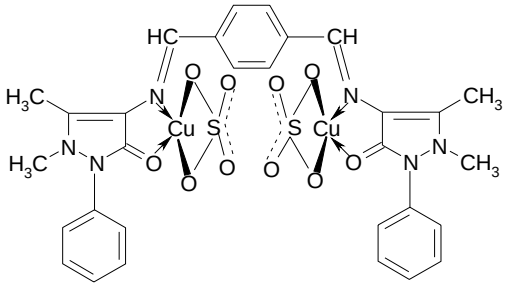
The {g} tensor values, $g_1 = 2.088$ and $g_2 = 2.406$, confirm the T_d symmetry [20-23]. As noted, the spectrum does not present interactions that would indicate a hyperfine structure. The lack of hyperfine structure or the presence of very small hyperfine splitting for the Cu^{2+} ion in T_d or lower symmetry were explained by C.A. Bates *et al.*, by mixing the $4p_z$ orbital of the metallic ion with the $3d_{xy}$ orbital which defines the fundamental state [20]. The values of the electronic transitions, of the {g} tensor,

and IR spectral data lead to the conclusion that the Cu^{2+} ion of the $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ complex is pentacoordinated with a C_{2v} symmetry (Figure 5a) [24], while in the $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ complex the Cu^{2+} ion has a deformed tetrahedral geometry (Figure 5b) [19].

Figure 5. Structures of the complexes $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ (**a**) and $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ (**b**).



(**a**)

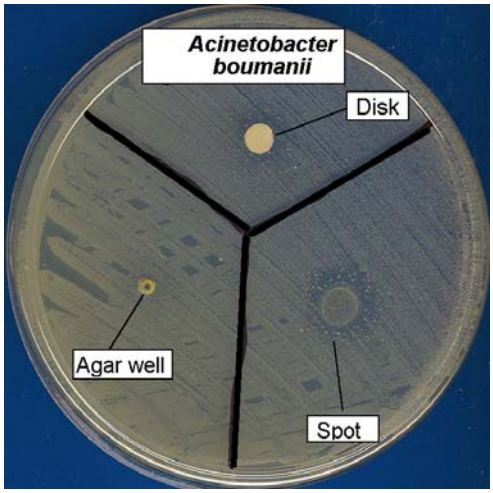


(**b**)

Antimicrobial activity assays

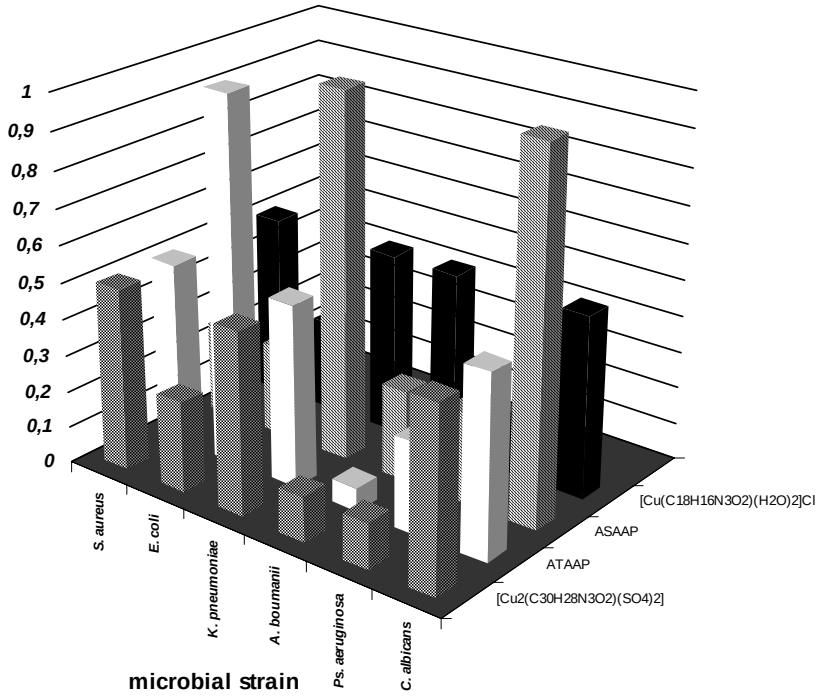
The antimicrobial activity of the complexes and ligands was screened by adapted qualitative, diffusimetric methods (i.e. distribution of the tested solutions on filter paper disks, in agar wells or in spots on solid media that have been inoculated with test microbial strains) and quantitative methods based on serial two-fold dilutions of the tested compounds in order to establish the corresponding Minimal Inhibitory Concentrations (MIC). Five bacterial strains, i.e. *Escherichia coli, Klebsiella pneumoniae, Acinetobacter boumanii, Pseudomonas aeruginosa, Staphylococcus aureus* and two fungal strains, i.e. *Candida albicans* and *Candida tropicalis*, freshly isolated from different clinical sources from patients with infections associated with the use of cardiovascular prosthetic devices and identified by conventional methods were cultivated on solid media and incubated at 37°C for 24 hrs prior to testing.

Figure 6. Appearance of the qualitative screening test showing the activity of $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ by the three adapted agar diffusion methods.



The qualitative screening results demonstrated that the three examined diffusion methods all exhibited different sensitivities in detecting the antimicrobial potential of the tested compunds. The most efficient one for the different bacterial strains proved to be the spot method, as exemplified in Figure 6. The quantitative assay results (Figure 7) showed that the tested compounds exhibited variable MICs and selective antimicrobial activity, depending on the microbial strains. All tested compounds proved to be active on *Ps. aeruginosa*, well known for its high constitutive and acquired resistance rates. The Schiff base **ASAAP** and the complex $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ exhibited high bactericidal activity towards *E. coli* and *A. Boumanii*, while the Schiff base ligands **ASAAP** and **ATAAP** and the $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ complex also showed good activity against *S. aureus*, *Ps. aeruginosa* and *E. coli*, proving their potential usefulness as broad spectrum antimicrobial agents.

Figure 7. The graphic representation of the MIC values (mg/mL) of the tested compounds towards different bacterial strains.



Conclusions

The IR, electronic transition and {g} tensor value data lead to the conclusion that the Cu^{2+} ion in the complex$[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ is pentacoordinated with a C_{2v} symmetry (Figure 5a), whereas in the complex $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ the Cu^{2+} ion has a deformed tetrahedral geometry (Figure 5b). The sensitivity spectrum of the microbial strains towards the ligands and the corresponding complexes was determined by qualitative and quantitative methods and the following conclusions were reached:

a) the qualitative anti-microbial activity screening results of the tested compounds proved that the most efficient test method was the spot method.

b) the quantitative anti-microbial activity test results proved that both the ligands and the complex combinations have specific anti-microbial activity, depending on the microbial species tested.

Experimental

General

The reagents used in this work were commercial products (Merck and Chimopar Bucuresti). Electronic spectra were recorded using a Jasco V-550 spectrophotometer, in diffuse reflectance mode, using MgO dilution matrices. IR spectra (KBr pellets) were recorded in the 4000-400 cm^{-1} region with a BioRad FTS 135 spectrophotometer. ESR spectra were recorded on a ART-6 model IFA-Bucuresti type spectrophotometer, equipped with a field modulation unit at 100kHz. The measurements were done in the X band, on micro-crystalline powder at room temperature using DPPH as standard. The ^{1}H-NMR spectra were recorded using a Bruker DRX 400 spectrometer. Chemical elemental analyses were done with a Carlo-Erba LA-118 microdosimeter (for C, N) and an AAS-1N Carl-Zeiss-Jena spectrometer [Cu(II)], respectively. Chlorine was determined by gravimetric analysis. The complexes were studied by thermo-gravimetry (TG) in a static nitrogen atmosphere, with a sample heating rate of 10°C/min., using a DuPont 2000 ATG thermobalance. Molar conductances of the complexes were measured in nitrobenzene at room temperature using a Consort type C-533 conductivity instrument.

Synthesis of the Schiff base 1-phenyl-2,3-dimethyl-4-(N-salicylidene)-3-pyrazolin-5-one (**ASAAP**)

A solution of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (0.203 g, 1 mmol) in methanol (20 mL) was added to a solution of *o*-hydroxybenzaldehyde (0.15 mL, 1 mmol) in methanol (10 mL). The mixture obtained was refluxed for an hour, then stirred for 3 hrs at room temperature and left at the same temperature for a day. The resulting intense yellow colored precipitate was filtered, washed with methanol and dried. Elemental analysis: Calc. C%, 70.35; N%, 13.66; Found. C%, 71.16; N%, 13.21. IR: 1653 cm^{-1}(azomethine group) [8,9]; ^{1}H-NMR spectra [$CDCl_3$, δ (ppm), *J* (Hz)]: δ = 2.41 (s, 3H, -CH_3); 3.16 (s, 3H,-CH_3); 6.90 (m, 1H, H-Ar); 6.95 (d, 1H, H-Ar); 7.24-7.56 (m, 7H, H-Ar); 9.84 (s, H, -N=CH-); δ>7 (H-OH).

Synthesis of the Schiff base bis(1-phenyl-2,3-dimethyl-3-pyrazolin-5-one-4-imino) terephthalic aldehyde (**ATAAP**)

A solution of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (0.253 g, 1.25 mmol) in methanol (15 mL) was added to a solution of terephthalic aldehyde (0.067 g, 0.5 mmol) in methanol (15 mL). The resulting yellow solution was refluxed for 30 minutes and then left at room temperature for approx. 6 hrs. The intense yellow precipitate formed was filtered, washed with methanol and dried. Elemental analysis: Calc. C%, 71.42; N%, 16.66; Found. C%, 72.11; N%, 16.21; IR: 1654 cm^{-1} (azomethine group) [8,9]. ^{1}H-NMR spectra [$CDCl_3$, δ (ppm), *J* (Hz)]: δ= 9.61 (s, 2H, -N=CH-); 7.88 (s, 4H, Ar-H 1,4- disubstituted); 7.57-7.37 (m,10H, Ar-H); 3.21 (s, 6H, N-CH_3); ≈2.5 (s, 6H, -CH_3).

Synthesis of the complex $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$ (**a**)

A methanol solution (15 mL) of **ASAAP** (0.307 g, 1 mmol) was added to $CuCl_2 \cdot 2H_2O$ (0.170 g, 1 mmol) dissolved in distilled water (10 mL). This solution was refluxed for 2 hrs and left at room

temperature for three days. A brown-red precipitate was formed, which was filtered, washed with ethanol and dried. The elemental analysis results (calc.: C%, 48.81; N%, 9.49; Cu%, 14.46; Cl%, 8.04; found: C%, 49.33; N%, 9.09; Cu%, 14.25; Cl%, 7.83) confirm the molecular formula $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]Cl$.

Synthesis of the complex $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$ (**b**)

A DMF solution (15 mL) of **ATAAP** (0.252g, 0.5 mmol) was added to $CuSO_4 \cdot 5H_2O$ (0.250 g, 1 mmol) dissolved in distilled water (15 mL). The resulting green solution was refluxed for two hours, during which time the green color turned brown. This solution was left at room temperature for four days. A brown precipitate was formed, which was filtered, washed with ethanol and dried. The elemental analysis data (calc.: C%, 43.69; N%, 10.19; Cu%, 15.53; found: C% 44.12; N%, 16.11; Cu%, 15.24) confirms the molecular formula $[Cu_2(C_{30}H_{28}N_6O_2)(SO_4)_2]$.

Biological assays

The fresh cultures obtained from clinical isolates were suspended in sterile saline and adjusted to a standard density of 0.5 MacFarland. The microbial suspensions were plated on solid Mueller Hinton medium and solutions of the test compounds (10 µL) prepared in DMF (1 mg/mL) were added on filter paper disks, in agar wells or in spots. Concomitantly, the disks were impregnated with the same concentration of gentamycin, which was used as reference standard for reporting the antibiotic sensitivity. The plates were incubated at 37°C for 24 hrs. During incubation, the tested compounds diffused around the test area creating a concentration gradient. The antimicrobial activity was recorded as any area of microbial growth inhibition that occurred in the diffusion area. The quantitative antimicrobial activity assays wer performed by the two-fold serial microdilution method in liquid medium (nutrient broth for bacterial and liquid YPG for fungal strains). Serial two-fold dilutions of a stock solution of test compound in DMF (from 1000 to 62.5 µg/mL) were performed in 60 multi-well plates, in a total volume of 200 µL medium and standard microbial suspension (50 µL) was added in each well. After 18-24 hours, the plates are examined visually for evidence of bacterial growth. Results are recorded as minimum inhibitory concentrations (MIC) at the highest dilution (lowest concentration) of the tested compound that completely inhibited microbial growth.

Acknowledgements

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Sample availability: Samples of the ligands **ASAAP** and **ATAAP** and the complexes (**a)** and (**b**) are available from MDPI.