1,3,4-THIADIAZOLE DERIVATIVES. PART 9¹. SYNTHESIS AND BIOLOGICAL ACTIVITY OF METAL COMPLEXES OF 5-(2-AMINOETHYL)-2-AMINO-1,3,4-THIADIAZOLE

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Abstract: Metal complexes of the title ligand (L) containing Co(II), Ni(II) and Cu(II) were prepared and characterized by elemental analysis, IR, electronic spectroscopy and conductimetry. The new derivatives, possessing the following formulae, $\text{CuL}_2(\text{OH})_2$, NiL_2Cl_2 , and $[\text{Co}_2\text{LCl}_4]_n$ showed *in vitro* antifungal activity against *Aspergillus* and *Candida spp.*

Intoduction

1,3,4-Thiadiazole derivatives possess interesting biological activity probably conferred to them by the strong aromaticity of this ring system,² which leads to great *in vivo* stability and generally, a lack of toxicity for higher vertebrates, including humans. When diverse functional groups that interact with biological receptors are attached to this ring, compounds possessing outstanding properties are obtained. Except for some antibacterial sulfonamides (albucid and globucid), no longer used clinically, but which possessed historical importance,² the most interesting examples are constituted by 5-amino-1,3,4-thiadiazole-derivatives such as the thiol **1**, a compound used as radioprotective agent,³ as well as an investigational antitumor⁴ and gastroprotective⁵ drug; acetazolamide **2**, which was the first non-mercurial diuretic drug,⁶⁻⁹ used clinically thereafter as antiglaucoma,¹⁰ antiepileptic¹¹ or antiulcer drug,¹² together with a large series of its congeners derived from 5-amino-1,3,4-thiadiazole-2-sulfonamide **3**.⁹

1: R=H, Z=SH

2: R=Ac, Z=SO₂NH₂

3: R=H, Z=SO₂NH₂

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These compounds, have also been investigated as complexing agents,¹³⁻¹⁶ in order to obtain substances with a diversified biological activity, conferred to them among others by the presence of the metal ions, except for that of the thiadiazole ligand. Thus, some metal complexes of ligands of type **1-3** have been recently reported as *in vitro* inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1),^{1,14,16} whereas *in vivo* studies showed good antiepileptic action for some Cu(II) and Zn(II) complexes of the sulfonamide type ligands.^{15d} Finally, some 2,5-disubstituted-1,3,4-thiadiazoles as well as their Cu(II) complexes were reported to act as fungitoxic agents.^{16h}

Recently, another group of biologically active 1,3,4-thiadiazoles was reported by us.¹⁷ 5-(2-Aminoethyl)-2-amino-1,3,4-thiadiazole **4** and some of its positively charged pyridinium derivatives showed very good activatory effects on carbonic anhydrase,¹⁷ making such compounds attractive candidates for the development of pharmacological agents for the treatment of CA deficiency syndrome, a rare but dramatic genetic disease which is caused by the lack of isozyme CA II in the human organism.^{18,19}

As **4** possesses an interesting donor atoms system besides its prominent biological activity,^{17,19} a study for preparation and biological assay of some of its metal complexes was initiated in our laboratory. Fungitoxicity has not been investigated previously for amines of type **4**, and since many imidazoles and triazoles such as ketoconazole, fluconazole, clotrimazole, etc possess outstanding fungistatic properties, being widely used clinically,²⁰ it appeared of interest to test thiadiazole **4** and some of its metal complexes for this type of biological activity. In this paper we report the synthesis of Co(II), Ni(II) and Cu(II) complexes of ligand **4**, and their activity as fungistatic agents against *Aspergillus* and *Candida spp.*

Materials and Methods

Infrared spectra were recorded in CsBr pellets with a Nicolet 2DXFT-IR apparatus. Electronic spectra were recorded by the diffuse reflectance technique using MgO as a reference material. Conductometry was done in DMF solution with a Radelkis 1200K/1 apparatus at 25°C. Elemental analyses were done by combustion (C,H,N,S) with an automated Carlo Erba analyser, or by atomic absorption spectroscopy for the metal ions. Ligand **4** was prepared as described in the literature.¹⁷ Metal salts and solvents were analytical grade (from Merck or Aldrich) and were used without further purification.

Synthesis of complexes 5-7

All complexes reported in this paper have been synthesised according to the following general procedure: methanolic solutions containing the metal chloride (CuCl₂ 2H₂O, CoCl₂ 6H₂O, NiCl₂ 6H₂O) and the heterocyclic diamine, **4**, (L.2HCl),¹⁷ were mixed with stirring in molar ratios of 1:1 and 1:2, respectively. The dihydrochloride of amine **4** was used in synthesis, due to its good methanol solubility.¹⁷ The resulting mixture was treated with a stoichiometric amount of triethylamine (in order to neutralize the HCl of the dihydrochloride) and the obtained mixture was heated for 60 min. on a water bath. The complexes precipitated from these solutions, were filtered, washed with diethylether and dried in vacuum. The following coordination compounds were synthesised and characterised: CuL₂(OH)₂ (green blue) **5**, NiL₂Cl₂ (blue) **6**, and Co₂LCl₄ (red brown) **7**. In the above formulas L stands for the corresponding diamino ligand. It is to note that, even when working in molar ratio MCl_x: L of 1:1 or 1:2, only the above mentioned compounds were obtained.

Copper (II) complex 5: non-electrolyte, IR (CsBr), cm⁻¹, 470, 617, 763, 927, 1069, 1127, 1348, 1456, 1482, 1512, 1600, 2361, 2952. UV-VIS (MgO), cm⁻¹ × 10⁻³: 7.0, 11.0, 15.7, 23.1, 26.3. Analysis: found, %Cu, 16.35; %C, 25.17; %H, 4.46, %N, 29.24, %S, 16.48; CuC₈H₁₈N₈S₂O₂; requires %Cu, 16.58; %C, 24.87; %H, 4.66, %N, 29.02, %S, 16.58.

Nickel (II) complex 6: non-electrolyte, IR (CsBr), cm⁻¹, 440, 624, 862, 941, 1127, 1162, 1283, 1351, 1422, 1510, 1605, 2368, 2952, UV-VIS (MgO), cm⁻¹ × 10⁻³, 10.0, 17.5, 20.1, 25.3. Analysis: found, %Ni, 14.01; %C, 23.17; %H, 3.66, %N, 26.24, %S, 15.48; %Cl, 16.75, NiC₈H₁₆N₈S₂Cl₂; requires %Ni, 14.11; %C, 22.96; %H, 3.82, %N, 26.79, %S, 15.31; %Cl, 16.98.

Cobalt (II) complex 7: electrolyte, IR (CsBr), cm⁻¹, 316, 450, 526, 580, 859, 971, 1127, 1157, 1340, 1419, 1532, 1605, 2361, 2952, UV-VIS (MgO), cm⁻¹ × 10⁻³, 9.1, 11.2, 14.3, 18.8, 21.5, 26.3. Analysis: found, %Co, 27.89; %C, 12.17; %H, 1.66, %N, 14.14, %S, 7.48; %Cl, 34.88, Co₂C₄H₈N₄SCl₄; requires %Co, 29.20; %C, 11.88; %H, 1.98, %N, 13.86, %S, 7.92; %Cl, 35.15.

Assay of fungistatic activity of compounds 4-7

The fungistatic activities of derivatives 4-7 were determined by a modification of the growth method²¹ recently reported by us,²² utilizing two *Aspergillus* and one *Candida spp.* The fungi were cultivated in agar plates at 25°C, in the absence and in the presence of compounds 4-7, in concentrations ranges of 10^{-3} - 10^{-7} M (solutions in DMSO). No significant fungistatic effects were observed at concentrations of 10^{-6} and 10^{-7} M of the new compounds, except for the Cu(II) derivative 5 (the most active in the series), which showed interesting inhibitory activity in micromolar concentrations too. Percentual inhibition of growth was calculated with the following formula:^{21,22}

$$\% \text{ inhibition} = 100 \times (\text{Dc}-\text{Di})/\text{Dc}$$

where Dc represents the average diameter of the fungi colony in the control plate after 48 hours, whereas Di the same parameter in the presence of tested compound.

Results and Discussion

Due to the complicated donor system of the ligand 4, at least four different more common complexation modes are probable, depicted as I-IV in the formulas bellow. The most favourable one for mononuclear complexes would be II or IV, due to the stability of six membered cycles formed in these cases, while I and III should lead to less stable four membered rings. From IR data discussed shortly, we ruled out coordination of types III or IV, as among others, no M-S bands were observed in the IR spectra. From the stereochemical point of view it is also hardly possible a coordination of type IV. Thus, as for histamine and histidine complexes, the most probable coordination mode is probably II, involving the aminoethyl NH_2 group and the endocyclic nitrogen N-4 of the heterocyclic ring.²³

In the IR spectra of complexes 5-7 the large stretching vibrations of the ammonium bands from the IR spectrum of 4 (as dihydrochloride salt),¹⁷ around 3000 cm^{-1} , are absent, due to the involvement of the free NH_2 groups in coordinating the metal ions. Characteristic vibrations of the ligand (δ_{NH_2} at 1630 cm^{-1} , $\nu_{\text{C=N}}$ at 1564 cm^{-1} in the spectrum of 4) are also present in the spectra of complexes 5-7, but shifted towards lower wavenumbers as compared to the spectrum of 4. Thus the thiazolothiazine band appears at $1510 - 1532 \text{ cm}^{-1}$ ($\nu_{\text{C=N}}$) in the spectrum of these complexes. The shift with $25\text{-}35 \text{ cm}^{-1}$ towards lower wavenumbers of the NH_2 and C=N vibrations in the IR spectra of the complexes is indicative that the coordination of type II or I proposed above might describe the behaviour of 4 in its metal complexes. The coordination by two types of nitrogen atoms is also supported by the existence of large bands in the range $200\text{-}600 \text{ cm}^{-1}$ in the spectra of complexes 5-7, presumably due to M-N vibrations. A band centred at 470 cm^{-1} was observed for the Cu(II) complex 5, at 440 cm^{-1} for the Ni(II) complex 6 and several peaks for the Co(II) complex 8, at 316 , 450 and 526 cm^{-1} .²⁴

Supplementary stereochemical information for the new complexes 5-7 was obtained from the absorption electronic spectra of the three compounds.

In the electronic spectrum of the Cu(II) derivative 5, two large bands were observed: i) the first one is located in the range $5\text{-}18 \times 10^3 \text{ cm}^{-1}$ and offers information about d-d- transitions. By deconvolution using the Gauss method,²⁵ the following transition bands were identified, at 7×10^3 , 11×10^3 , and $15.7 \times 10^3 \text{ cm}^{-1}$, respectively. The lowest energy, according to the $d_{x^2-y^2} \Rightarrow d_z^2$, transitions, is characteristic for the copper (II)

ion in a (pseudo)-octahedral configuration.²⁵ The band appeared at a slightly higher energy due to the involvement of the 4s orbital in the coordination sphere. ii) The second transition observed in the range $18\text{--}25 \times 10^3 \text{ cm}^{-1}$ can be attributed to charge transfer bands.²⁵

The diffuse reflectance spectrum of the nickel complex **6** is characteristic for a (pseudo)-octahedral arrangement with the following transition bands: ν_1 : $10 \times 10^3 \text{ cm}^{-1}$ (${}^3T_{2g} \leftarrow {}^3A_{2g}$), ν_2 : $17.5 \times 10^3 \text{ cm}^{-1}$ (${}^3T_{1g} (F) \leftarrow {}^3A_{2g}$), ν_3 : at higher energies (charge transfer energy). After deconvolution the first band (ν_1) was splitted in two components and the second band (ν_2) in three components, this supplementary division being probably due to the low symmetry of the ligand field and to the non-equivalence of the donor atoms.^{25,26}

The electronic spectrum of the Co(II) complex **7** possibly contains two overlapped bands of two chromophores in different stereochemistries. The presence of an intense band at $15 \times 10^3 \text{ cm}^{-1}$ confirms the tetrahedral stereochemistry, while other bands at 11.2×10^3 , 16.3×10^3 , and $21.5 \times 10^3 \text{ cm}^{-1}$, respectively, are characteristic for a (pseudo)-octahedral configuration of the Co(II) ion.²⁷ It is difficult to formulate this compound as *octahedral cation: tetrahedral anion* complex, (due to its brown-red color characteristic of polynuclear complexes of Co(II))²⁵ as the presence of the tetrahedral anion $[\text{CoCl}_4]^{2-}$ would imprint an intense blue colour to it.^{25,27} Thus, the polynuclear structure shown below is proposed, whereas the other two mononuclear complexes were formulated as containing octahedral Cu(II) and Ni(II) ions.

The fungistatic activities of derivatives **4–7** against two *Aspergillus* species and *Candida albicans* are presented in Table I. Clotrimazole **8**, a topically effective fungistatic agent with clinical applications was used as a standard in these assays.^{20,28}

As seen from the data of Table I, the thiadiazole **4** is much less effective as fungitoxic agent as compared to clotrimazole, an imidazole derivative with a potent such activity, against all three fungi species investigated. The metal complexes **5–7** already show an increased fungitoxic activity (at the concentration of $10 \mu\text{M}$, for which the data of Table I stand). At $0.1\text{--}1 \mu\text{M}$ concentrations no such activity was observed for the complexes, except for the Cu(II) derivative **5** (data not shown). At $10 \mu\text{M}$, significant inhibitions of growth were detected for **5**, which is almost as effective as clotrimazole against *A. niger*, slightly more effective against *A. flavum*, and much less effective against *Candida*. The other two complexes showed a modest activity, which is anyhow greater against the *Aspergillus* spp. as compared to *Candida*. The metal ion inducing best fungitoxic activity was Cu(II), followed by Co(II) and Ni(II), which possessed comparative efficiency.

As for the mechanism of action of these derivatives, probably it is similar to that of the imidazole- and triazole derivatives used clinically, i.e., the inhibition of sterol 14- α -demethylase, a microsomal cytochrome P-450 dependent enzyme system.²⁹ These compounds thus impair the biosynthesis of ergosterol for the cytoplasmic membrane and lead to the accumulation of 14- α -methyl sterols which may disrupt the close packing of acyl chains of phospholipids, impairing the function of membrane-bound enzymes and inhibiting growth.^{29,30}

Table I: Inhibition of growth with derivatives **4-7** and clotrimazole **8** (at concentrations of 10 μ M) of *Aspergillus* and *Candida spp.* after 48 hours, at 25°C.

Compound	% Inhibition ^a		
	<i>Aspergillus niger</i>	<i>Aspergillus flavum</i>	<i>Candida albicans</i>
4	22.5 $\pm$ 3.6	14.1 $\pm$ 2.7	5.2 $\pm$ 1.4
5	78.9 $\pm$ 5.4	83.6 $\pm$ 4.2	42.1 $\pm$ 2.8
6	43.7 $\pm$ 3.0	29.5 $\pm$ 4.1	13.1 $\pm$ 3.5
7	44.9 $\pm$ 3.7	31.6 $\pm$ 2.8	10.3 $\pm$ 4.0
8	88.5 $\pm$ 5.2	79.0 $\pm$ 4.2	66.7 $\pm$ 5.5

^aMean $\pm$ standard error from 10 plates.

Acknowledgments. This research was financed in part from the EU grant ERB CIPDCT 940051.

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**Received: September 13, 1996 - Accepted: September 17, 1996 -
Received in revised camera-ready format: September 24, 1996**