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Coordination to copper(II) strongly enhances the *in vitro* antimicrobial activity of pyridine-derived *N*(4)-tolyl thiosemicarbazones

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Abstract

Five copper(II) complexes with *N*(4)-*ortho*, *N*(4)-*meta* and *N*(4)-*para*-tolyl thiosemicarbazones derived from 2-formyl and 2-acetylpyridine were obtained and thoroughly characterized. The crystal structure of *N*(4)-*meta*-tolyl-2-acetylpyridine thiosemicarbazone (H2Ac4mT) was determined, as well as that of its copper(II) complex [Cu(2Ac4mT)Cl], which contains an anionic ligand and a chloride in the coordination sphere of the metal. The *in vitro* antimicrobial activities of all thiosemicarbazones and their copper(II) complexes were tested against *Salmonella typhimurium* and *Candida albicans*. Upon coordination a substantial decrease in the minimum inhibitory concentration, from 225 to 1478 μmol L^{-1} for the thiosemicarbazones to 5–30 μmol L^{-1} for the complexes was observe against the growth of *Salmonella typhimurium* and from 0.7–26 to 0.3–7 μmol L^{-1} against the growth of *C. albicans*, suggesting that complexation to copper(II) could be an interesting strategy of dose reduction.

Keywords: Thiosemicarbazones; Copper(II) complexes; Crystal structures; Antimicrobial activity; *Salmonella typhimurium*; *Candida albicans*; Dose reduction

1. Introduction

Thiosemicarbazones present a wide range of pharmacological applications as anticancer, antiviral and antimicrobial agents [1–3]. The biological properties of thiosemicarbazones are often related to metal coordination. Firstly, lipophilicity, which controls the rate of entry into the cell, is modified by coordination. Also, the metal complex can be more active than the free ligand, or the metal complex can be a vehicle for activation of the ligand as the cytotoxic agent [1–3].

The antimicrobial action of thiosemicarbazones against a variety of bacteria and fungi has been investigated. The activity of 2-formyl and 2-acetylpyridine thiosemicarbazones has been demonstrated in clinical isolates of bacteria [1,4]. Significant activity has been found toward gram positive bacilli but poor antibacterial activity has been shown toward gram negative cultures [1,4,5]. In addition, the *in vitro* inhibitory activity of 2-acetylpyridine-derived thiosemicarbazones has been demonstrated against clinically significant bacterial cultures, including isolates with known antibiotic resistance [1,4,5]. A variety of metal complexes of these ligands has also shown to present potent antibacterial activity [1]. Less attention has been given to 2-benzoylpyridine-derived thiosemicarbazones.

Some 2-formyl and 3-formylpyridine thiosemicarbazones and their metal complexes were tested against *Candida albicans* and *Apergillus fumigatus*. In addition, *N*(4)-alkyl and *N*(4)-dialkyl-2-acetylpyridine thiosemicarbazones showed marked inhibitory activity of *A. niger* growth. Moreover, metallocene derivatives of titanium and zirconium with

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thiosemicarbazones derived from 2-acetylpyridine, 2-acetylfuran, 2-acetylthiophene and 2-acetylnaphtalene were tested against a number of pathogenic fungi and bacteria. All the complexes were more active against the studied organisms than the free thiosemicarbazones [1].

It has been demonstrated that the presence of a bulky group at *N*(4) strongly improves the biological activity of thiosemicarbazones [2,3], probably due to an increase in lipophilicity.

In previous works, we started an evaluation of the antimicrobial and cytotoxic activities of pyridine-derived thiosemicarbazones and their metal complexes. *N*(4)-alkyl- and *N*(4)-phenyl-substituted 2-benzoylpyridine thiosemicarbazones have been prepared, as well as their palladium(II), tin(IV) and iron(II) complexes [6–8].

Taking into consideration that copper(II) complexes have found possible medical uses in the treatment of many diseases including microbial infections [2,3,9] we recently prepared *N*(4)-tolyl thiosemicarbazones derived from 2-benzoylpyridine as well as their copper(II) complexes. Both ligands and complexes proved to be active against the growth of *C. albicans* with low values of minimum inhibitory concentration. The activity is improved upon coordination to copper [10].

Salmonella sp. is a *gram*-negative facultative rod-shaped bacterium which causes infections about 12–24 h following ingestion of contaminated food. Strains of *Salmonella* which are resistant to a range of antimicrobials, including first-choice agents for the treatment of humans have emerged, and are threatening to become a serious public health problem [11]. The same occurs with strains of fungi. Among the azole drugs used against infections caused by *C. albicans*, fluconazole and itraconazole exhibit satisfactory safety and efficacy. However, the emergence of clinical resistance during long-term therapy is well known [12].

Resistance results from the use of antimicrobials both in humans and animal husbandry. Multi-drug resistance to critically important antimicrobials is compounding the problems [13]. Therefore, preparation of new antimicrobials with activity in low doses is extremely important.

In the present work, we studied the activity of *N*(4)-*ortho*-, *N*(4)-*meta*- and *N*(4)-*para*-2-formyl (hereafter named H2Fo4oT, H2Fo4mT, H2Fo4pT), 2-acetyl (H2Ac4oT, H2Ac4mT, H2Ac4pT) and 2-benzoylpyridine thiosemicarbazones (H2Bz4oT, H2Bz4mT, H2Bz4pT) (see Fig. 1) and their copper(II) complexes against the growth of *Salmonella typhimurium* and *C. albicans*.

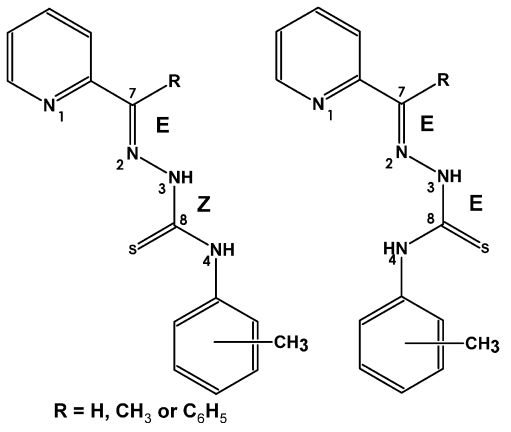

Fig. 1. General structure of *N*(4)-*ortho*-, *N*(4)-*meta*- and *N*(4)-*para*-tolyl 2-formyl 2-acetyl and 2-benzoylpyridine thiosemicarbazones, *E* and *Z* configurations.

2. Experimental

2.1. Apparatus

Partial elemental analyses were performed on a Perkin Elmer CHN 2400 analyzer. Infrared spectra were recorded on a Perkin Elmer FT-IR Spectrum GX spectrometer using CsI/nujol; an YSI model 31 conductivity bridge was employed for molar conductivity measurements. Magnetic susceptibility measurements were carried out on a Johnson Matthey MSB/AUTO balance. Electron paramagnetic resonance (EPR) spectra of samples in frozen DMF solution were obtained in quartz tubes of 3 mm of internal diameter at liquid N_2 temperature (77 K) on a Bruker ESP300E equipment with modulation frequency of 100 kHz operating at 9.5 GHz (X-band). Spectra simulations were performed using the WINSIMFONIA Bruker package. Crystal structures of H2Ac4mT and its copper complex [Cu(2Ac4mT)Cl] (see below) were investigated using single-crystal X-ray diffractometry. Data have been collected at room temperature in a Kappa CCD diffractometer, Mo Kα monochromated radiation, $\lambda = 0.71073$ Å. Crystal data, data collection procedure, structure determination methods and refinement results are summarized in Table 1. The structures were solved by direct methods and refined on F^2 by full-matrix least-squares using the SHELX 97 [14,15] program in a WINGX system [16,17]. Although some of the hydrogen atoms could be identified in a Fourier difference map, in the final model they were geometrically positioned and refined using a riding model. All non-H atoms were refined anisotropically.

2.2. Synthesis of the copper(II) complexes with 2-formyl- and 2-acetylpyridine N(4)-tolyl thiosemicarbazones

The thiosemicarbazones were prepared as described in the literature [18]. The copper(II) complexes were obtained by refluxing an ethanol solution (25 mL) of the desired thiosemicarbazone (3 mmol) with $CuCl_2 \cdot 2H_2O$ (Aldrich) in 1:1 ligand-to-metal molar ratio for 5 h. The resulting solids were filtered then washed with ethanol followed by diethyl ether, and dried *in vacuo*. Crystals of H2Ac4mT were grown by slow diffusion from ethanol solution and crystals of its copper(II) complex from a 1:9 DMSO/acetone solution.

The copper(II) complexes will be hereafter numbered as follows: [Cu(H2Fo4oT)Cl_2] (**1**), [Cu(2Fo4mT)Cl] (**2**),

Table 1
Crystal data and structure refinement for H2Ac4mT and [Cu(2Ac4mT)Cl] (**5**)

Empirical formula	$C_{15}H_{16}N_4S$	$C_{15}H_{15}N_4SCuCl$
Crystal size (mm)	$0.14 \times 0.10 \times 0.04$	$0.13 \times 0.09 \times 0.02$
Molecular weight (g mol^{-1})	284.38	382.36
Crystal system, space group	monoclinic, $P2_1/c$	triclinic, $P\bar{1}$
Lattice parameters		
a(Å)	10.3812(4)	7.8130(3)
b (Å)	5.7074(2)	8.1716(3)
c (Å)	24.606(1)	12.8239(5)
α (°)	90	100.925(3)
β (°)	100.059(2)	100.400(3)
γ (°)	90	97.327(2)
V (Å^3)	1435.5(1)	779.52(5)
Z	4	2
F(000)	600	390
D_{calc} (g cm^{-3})	1.316	1.629
Absorption coefficient (mm^{-1})	0.211	1.706
θ Range for data collection (°)	2.37–25.05	3.31–25.00
Limiting indices	$-12 \leqslant h \leqslant 12$, $-6 \leqslant k \leqslant 6$, $-29 \leqslant l \leqslant 29$	$-9 \leqslant h \geqslant 9$, $-9 \leqslant k \geqslant 9$, $-15 \leqslant l \geqslant 15$
Reflections collected	19929	11681
Reflections unique [R_{int}]	2536 [0.0874]	2750 [0.1036]
Completeness to θ (%)	99.7	99.8
Absorption correction	none	analytical
Parameters/restraints	182/0	199/0
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0488$, $wR_2 = 0.1100$	$R_1 = 0.0441$, $wR_2 = 0.0946$
R indices (all data)	$R_1 = 0.0898$, $wR_2 = 0.1356$	$R_1 = 0.0809$, $wR_2 = 0.1108$
Goodness-of-fit on F^2	1.030	0.995
Largest difference in peak and hole (e Å^{-3})	0.231 and −0.247	0.376 and −0.433

[Cu(2Fo4pT)Cl] (**3**), [Cu(2Ac4oT)Cl] (**4**), [Cu(2Ac4mT)Cl] (**5**), [Cu(2Ac4pT)Cl] (**6**), [Cu(H2Bz4oT)Cl_2] (**7**), [[Cu(H2-Bz4mT)Cl_2] (**8**), [Cu(H2Bz4pT)Cl_2] (**9**) (see Section 3 for the discussion of the proposed formulations). Complex **1**, previously reported by other authors [18] and complexes **7**–**9**, recently prepared by us [10] are listed in the present work because this is the first investigation on their antibacterial activity.

Chloro(*N*(4)-*m*-tolyl-2-formylpyridine-thiosemicarbazonato)copper(II) [Cu(2Fo4mT)Cl] (**2**): Green solid; *Anal.* Calc. for $CuC_{14}H_{11}N_4SCl$: C, 45.65; H, 3.56; N, 15.20. Found: C, 45.60; H, 3.42; N, 14.94%; FW: 368.34 g mol^{-1}. $UV_{solution}$: λ_{max} (cm^{-1}): 30860 (sh), 27780 (sh), 24210 (4.40), 16304 (2.20). UV_{solid}: λ_{max} (cm^{-1}): 27770, 22568, 19305, 16703. IR (CsI/nujol, cm^{-1}): ν(NH) 3325 ν(C=N) + ν(C=C) 1607–1547, ν(C=S) 760, ρ(py) 624, ν(CuN) 456, ν(CuN_{py}) 343, ν(CuS) 333, ν(CuCl) 311. Molar conductivity (1×10^{-3} mol L^{-1}, dimethylformamide, DMF): 13 Ω^{-1} cm^2 mol^{-1}. Effective magnetic moment = 1.73 (BM). Yield: 84%.

Chloro(N(4)-*p*-tolyl-2-formylpyridine-thiosemicarbazonato)copper(II) [Cu(2Fo4pT)Cl] (**3**): Green solid; *Anal.* Calc. for $CuC_{14}H_{11}N_4SCl$: C, 45.65; H, 3.56; N, 15.20. Found: C, 45.58; H, 3.45; N, 14.99%; FW: 368.34 g mol^{-1}. $UV_{solution}$: λ_{max} (cm^{-1}): 30960 (sh), 27030 (4.40), 16520 (2.28). UV_{solid}: λ_{max} (cm^{-1}): 31230, 27160, 23652, 22543, 16920. IR (CsI/nujol, cm^{-1}): ν(NH) 3332 ν(C=N) + ν(C=C) 1607–1538, ν(C=S) 782, ρ(py) 601, ν(CuN) 416, ν (CuN_{py}) 360, ν(CuS) 330, ν(CuCl) 307. Molar conductivity (1×10^{-3} mol L^{-1} DMF): 15 Ω^{-1} cm^2 mol^{-1}. Effective magnetic moment = 1.76 BM. Yield: 88%.

Chloro(N(4)-*o*-tolyl-2-acetylpyridine-thiosemicarbazonato)copper(II)[Cu(2Ac4oT)Cl] (**4**): Green solid; *Anal.* Calc. for $CuC_{15}H_{13}N_4SCl$: C, 47.12; H, 3.95; N, 14.65. Found: C, 46.92; H, 3.94; N, 14.35%; FW: 382.37 g mol^{-1}. $UV_{solution}$: λ_{max} (cm^{-1}): 32467 (sh), 27624 (4.33), 24213 (4.43), 16393 (2.67). UV_{solid}: λ_{max} (cm^{-1}): 33512, 27770, 22868, 15818. IR (CsI/nujol, cm^{-1}): ν(NH) 3429 ν(C=N) + ν(C=C) 1602–1536, ν(C=S) 763, ρ(py) 604, ν(CuN) 460, ν(CuN_{py}) 346, ν(CuS) 329, ν(CuCl) 308. Molar conductivity (1×10^{-3} mol L^{-1} DMF): 56 Ω^{-1} cm^2 mol^{-1}. Effective magnetic moment = 1.96 BM. Yield: 90%.

Chloro(*N*(4)-*m*-tolyl-2-acetylpyridine-thiosemicarbazonato)copper(II)[Cu(2Ac4mT)Cl] (**5**): Green solid; *Anal.* Calc. for $CuC_{15}H_{13}N_4SCl$: C, 47.12; H, 3.95; N, 14.65. Found: C, 47.52; H, 3.90; N, 14.29%; FW: 382.37 g mol^{-1}. $UV_{solution}$: λ_{max} (cm^{-1}): 31150 (4.53), 27170 (sh), 24750 (4.41), 16530 (2.47). UV_{solid}: λ_{max} (cm^{-1}): 32062, 27330, 23277, 19810, 16958. IR (CsI/nujol, cm^{-1}): ν(NH) 3320 ν(C=N) + ν(C=C) 1604–1547, ν(C=S) 765, ρ(py) 610, ν(CuN) 449, ν(CuN_{py}) 374, ν(CuS) 337, ν(CuCl) 318. Molar conductivity (1×10^{-3} mol L^{-1} DMF): 55 Ω^{-1} cm^2 mol^{-1}. Effective magnetic moment = 1.85 BM. Yield: 82%.

Chloro(*N*(4)-*p*-tolyl-2-acetylpyridine-thiosemicarbazonato)copper(II)[Cu(2Ac4pT)Cl] (**6**): Green solid; *Anal.* Calc. for $CuC_{15}H_{13}N_4SCl$: C, 47.12; H, 3.95; N, 14.65. Found: C, 47.07; H, 3.97; N, 14.37%; FW: 382.37 g mol^{-1}. $UV_{solution}$: λ_{max} (cm^{-1}): 31250 (4.47), 26600 (sh), 24570 (4.40), 16650 (2.51). UV_{solid}: λ_{max} (cm^{-1}): 31450, 25710, 22830, 17240. IR (CsI/nujol, cm^{-1}): ν(NH) 3322 ν(C=N) + ν(C=C) 1604–1541, ν(C=S) 757, ρ(py) 608, ν(CuN) 446, ν(CuN_{py}) 360, ν(CuS) 341, ν(CuCl) 317. Molar conductivity (1×10^{-3} mol L^{-1}, DMF): 37 Ω^{-1} cm^2 mol^{-1}. Effective magnetic moment = 2.03 BM. Yield: 87%.

2.3. Antibacterial activity

Antibacterial activity was evaluated by minimum inhibitory concentration (MIC) using the macro dilution test [19–21]. *S. typhimurium* ATCC13311 stored in Brain Heart Infusion (BHI) broth was subcultured for testing in the same medium and grown at 37 °C. Then the bacterial cells were suspended, according to the McFarland protocol in saline solution [21], to produce a suspension of about 10^5 CFU mL^{-1} (colony-forming units per mL). Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO), were prepared in test tubes to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 μg mL^{-1}; 100 μL of a 24-h-old-inoculum was added to each tube. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible

growth after 20 h, was determined visually after incubation for 20 h at 37 °C. Tests using chloramphenicol as reference and DMSO as negative control were carried out in parallel. All tests were performed in triplicate with full agreement between results.

As previously, antifungal activity was evaluated by minimum inhibitory concentration (MIC) using the macro dilution test [19–21]. *C. albicans* (ATCC 18804) stored in Sabouraud broth, was subcultured for testing in the same medium and grown at 37 °C. Then the yeast cells were suspended, according to the McFarland protocol in saline solution [21], to produce a suspension of about 10^5 CFU mL^{-1}. Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO), were prepared in test tubes to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.3 and 0.1 μg mL^{-1}. 100 μL of a 24-h-old inoculum was added to each tube. The MIC was determined visually after incubation for 18 h at 37 °C and DMSO was used as negative control. All tests were performed in triplicate.

3. Results and discussion

Microanalyses suggest the formation of [CuLCl] for complexes **2**–**6**, in which the thiosemicarbazones coordinate as anionic ligands. The molar conductivity data reveal that the complexes are non-electrolytes [22], in accordance with the proposed formulations. The values of magnetic moments in the 1.73–2.03 BM range are close to the calculated value of 1.73 BM, characteristic of the presence of one unpaired electron as in copper(II) complexes.

Complexes **1** and **7**–**9** obtained previously [18,10] were formulated as $[Cu(HL)Cl_2]$, in which the thiosemicarbazones coordinate as neutral ligands. Once again the complexes are non-electrolytes, in agreement with the presence of two chlorides in the metal coordination sphere.

3.1. Structural characterization of H2Ac4mT and [Cu(2Ac4mT)Cl] (5)

The structure of H2Ac4mT was refined in the monoclinic system, $P2_1/c$ and that of [Cu(2Ac4mT)Cl] (**5**) in the triclinic system, $P\bar{1}$, both with one molecule in the asymmetric unit. Selected intra-molecular bond distances and angles are given in Table 2; hydrogen bond distances and angles are detailed in Table 3 and angles between planes in Table 4. The asymmetric units are shown in perspective in Figs. 2 and 3.

H2Ac4mT adopts the *EE* configuration in relation to the C7–N2 and C8–N3 bonds (see Fig. 1). In complex **5** the ligand adopts the *EZ*, configuration, to match the sterical requirements of coordination through the N_{py}–N–S chelating system. The C8–N3 bond distance goes from 1.359(3) Å in the free base to 1.311(5) Å in the complex, due to deprotonation at N3 and formation of new predominantly double bond. The C8–S bond changes from 1.671(3) Å in H2Ac4mT to 1.749(4) Å in complex **5** in agreement with deprotonation at N3 and formation of a thiolate bond. Significant modifications have also been observed in the bond angles upon coordination (see Table 2). For example, the N2–N3–C8 bond angle changes from 118.5(2)° in the free base to 111.6(3)° in the complex, and N3–C8–S goes from 120.3(2)° to 125.2(3)° (see Table 2).

The bond distances and angles in **5** are comparable to those related in the literature for copper(II) complexes containing anionic thiosemicarbazones [23].

In H2Ac4mT N3-H forms an intermolecular hydrogen bond with a sulfur of an adjacent molecule, N3–H···S1 ($-x$, $-y+1$, $-z+1$). In [Cu(2Ac4mT)Cl] (**5**) N4 is hydrogen bonded to a thiolate sulfur of a second adjacent molecule forming dimers (see Fig. 4); N4–H···S ($-x+1$, $-y+1$, $-z+2$), d(D–H) = 2.68 Å, ∠DHA = 173.4° and d(D···A) 3.539(3) Å (Table 3).

The C7–N2–N3–C8S1–N4 skeleton is approximately planar in the structures of both H2Ac4mT and complex **5**, with mean deviation from planarity of 0.0591 Å in the free thiosemicarbazone and 0.0132 Å in **5**.

The angle between the pyridine plane and the thiosemicarbazone moiety is 17.2° in H2Ac4mT and 2.6° in complex **5**. Similarly, the angle between the thiosemicarbazone chain and the tolyl ring is 65.4° in H2Ac4mT and 3.2° in **5**, due to the almost planar structure of the complex (see Table 4).

Table 2
Selected bond distances and angles for H2Ac4mT and [Cu(2Ac4mT)Cl] (**5**)

Atoms	H2Ac4mT (Å)	**5** (Å)	Atoms	H2Ac4mT (°)	**5** (°)
S1–C8	1.671(3)	1.749(4)	N2–N3–C8	118.5(2)	111.6(3)
N2–C7	1.289(3)	1.299(5)	N3–C8–S1	120.3(2)	125.2(3)
N2–N3	1.381(3)	1.380(4)	N4–C8–N3	114.8(2)	119.3(4)
N3–C8	1.359(3)	1.311(5)	N4–C8–S1	124.9(2)	115.3(3)
N4–C8	1.339(3)	1.353(5)	C7–N2–N3	119.0(2)	118.3 (3)
N4–C9	1.429(3)	1.419(5)	C8–N4–C9	125.7(2)	131.2(3)
Cu–N1		2.012(3)	N2–Cu–N1		80.5(1)
Cu–N2		1.960(3)	N2–Cu–S1		84.12(9)
Cu–S1		2.253(1)	N2–Cu–Cl1		175.2(1)
Cu–Cl1		2.211(1)	N1–Cu–Cl1		97.8(1)
			N1–Cu–S1		164.6(1)
			Cl1–Cu–S1		97.56(4)

Table 3
Hydrogen bonds parameters for H2Ac4mT and [Cu(2Ac4mT)Cl] (**5**)

Compound	D–H···A	*d* (D–H)	*d*(H···A)	DHA	*d*(D···A)
H2Ac4mT	N3–H···S1 [$-x, -y+1, -z+1$]	0.86	2.78	174.7	3.642(2)
[Cu(2Ac4mT)Cl]	N4–H···S1 [$-x+1, -y+1, -z+2$]	0.86	2.68	173.4	3.539(3)

Table 4
Angles between planes in the structures of H2Ac4mT and [Cu(2Ac4mT)Cl] (**5**)

Atoms defining the plane	Deviation from the plane (Å)		Angle between planes (°)	
	HAc4mT	**5**	H2Ac4mT	**5**
N1–C2–C3–C4–C5–C6	0.0021	0.0016	17.2(2)	2.6(2)
C7–N2–N3–C8–S1–N4	0.0591	0.0132		
C7–N2–N3–C8–S1–N4	0.0591	0.0132	65.39(8)	3.2(2)
C9–C10–C11–C12–C13–C14	0.0031	0.0068		

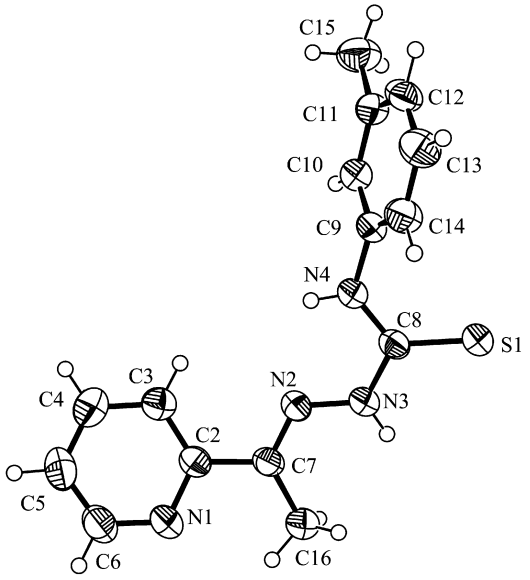

Fig. 2. Molecular plot of H2Ac4mT showing the labeling scheme of the non-H atoms with displacement ellipsoids at the 50% probability level.

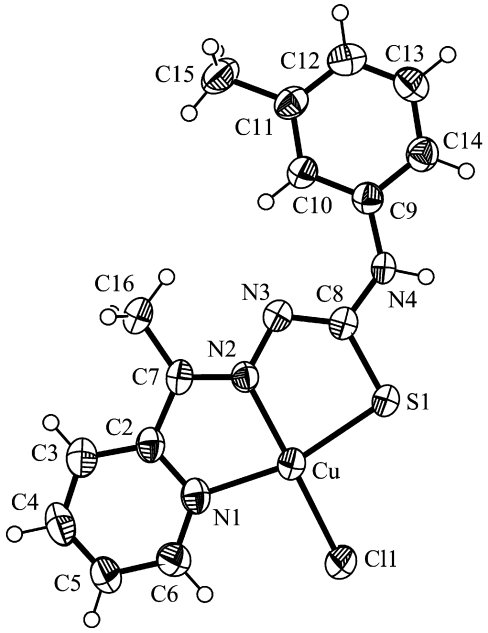

Fig. 3. Molecular plot of [Cu(2Ac4mT)Cl] (**5**) showing the labeling scheme of the non-H atoms with displacement ellipsoids at the 50% probability level.

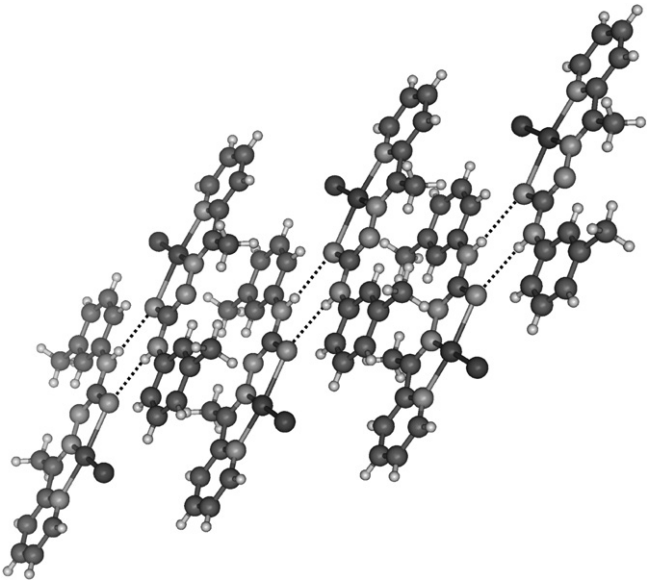

Fig. 4. Molecular packing of [Cu(2Ac4mT)Cl] (complex **5**) showing the presence of dimers.

3.2. Infrared spectra

The ν(C=C) + ν(C=N) composed mode observed in the 1608–1564 cm^{-1} in the spectra of the thiosemicarbazones shifts 1607–1538 cm^{-1} in the spectra of the complexes, indicating coordination of the azomethine nitrogen N2 [6–8,10].

The ν(C=S) absorption at 870–821 cm^{-1} in the spectra of the uncomplexed thiosemicarbazones is observed at 782–757 cm^{-1} in those of the complexes, in accordance with coordination through a thiolate sulfur. The 88–64 cm^{-1} shift observed upon complexation is compatible with coordination of an anionic thiosemicarbazone in all cases [6–9]. The pyridine in-plane deformation mode at 639–595 cm^{-1} in the spectra of the ligands shifts to 624–601 cm^{-1} in those of the complexes, suggesting coordination of the heteroaromatic nitrogen [6–8,10]. In addition, new absorptions at 460–416 cm^{-1} and 374–343 cm^{-1} have been attributed to ν(Cu–N) and ν(Cu–N_{py}), respectively,

and bands in the 341–329 cm^{-1} range have been assigned to ν(Cu–S). An absorption at 318–308 cm^{-1} has been attributed to ν(Cu–Cl) [23,24].

The infrared data for the complexes indicate coordination of the thiosemicarbazones through the N_{py}–N–S chelating system.

3.3. Electronic spectra

In the electronic spectra of the thiosemicarbazones (DMSO) the π–π* absorptions at ca. 37 000 cm^{-1} are not significantly modified upon complexation. The absorption at 31 153–30 395 cm^{-1} in the spectra of the free bases was attributed to n–π* transitions associated to the azomethine and thioamide [6–8,10] functions which overlap under the same envelope. In the spectra of complexes three absorptions were observed at 31 250–30 860 cm^{-1}, 27 780–26 600 cm^{-1} and 24 750–24 210 cm^{-1}. The first two were assigned to n–π* transitions of the azomethine and thioamide functions, and the latter to N–Cu^{II} and S–Cu^{II} ligand-to-metal charge transfer transitions [10,24]. Ligand field transitions were found at 16 650–16 304 cm^{-1} [10,24]. The electronic spectra of the solids are not significantly different, particularly in the d–d region.

3.4. ESR spectra

Fig. 5 shows the experimental EPR spectra in frozen DMF solution, liquid nitrogen (77 K), for the studied complexes. The Hamiltonian parameters obtained from the spectra, together with the d–d ligand field transitions, are resumed in Table 5.

The g values are in agreement with the relation $g_{\parallel} > g_{\perp} > 2$, indicating the presence of $d_{x^2-y^2}$ ground states. The $g_{\parallel}/A_{\parallel}$ ratio can be used as a convenient empirical index of distortion from square-planar structure. This value ranges from ca. 105 to 135 cm for square-planar structure, and this quotient increases on the introduction of tetrahedral distortion to the chromospheres [25]. The values of the $g_{\parallel}/A_{\parallel}$ ratio (Table 5) indicate nearly square-planar environments for complexes **2**–**6**, some with small distortions, which is in good agreement with the X-ray determined structure for complex **5**. The relatively low g values of the studied compounds are characteristic of an N enriched environment around the Cu(II) ions [26].

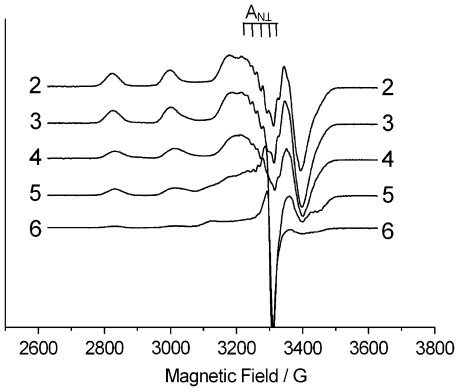

Fig. 5. X-band EPR spectra of [Cu(2Fo4mT)Cl] (**2**), [Cu(2Fo4pT)Cl] (**3**), [Cu(2Ac4oT)Cl] (**4**), [Cu(2Ac4mT)Cl] (**5**) and [Cu(2Ac4pT)Cl] (**6**) in DMF at 77 K (Inset: five perpendicular super-hyperfine interaction lines – $A_{N\perp} \approx 16 \times 10^{-4}$ cm^{-1} – of two ^{14}N ($I = 1$) atoms with the Cu(II) ions from each complex).

An interesting feature of the spectra is the appearance of nitrogen super-hyperfine lines (five lines) in the $g_{\perp}$ region, which generally mirror the number of nitrogen donors ($2nI + 1$, where $I = 1$ for nitrogen and n is the number of nitrogen atoms in the same chemical environment). The five lines correspond to two nitrogen donor atoms in the equatorial positions. The $A_{N\perp}$ values, around 16×10^{-4} cm^{-1} are in agreement with the imine/amine N coordination to the Cu(II) ions [27].

3.5. Antimicrobial activity

Table 6 lists the minimum inhibitory concentration (MIC) of the thiosemicarbazones and their copper(II) complexes against the growth of *S. typhimurium* and Table 7 lists the MIC against *C. albicans*. Included in this section are values previously obtained for 2-benzoylpyridine *N*(4)-tolyl thiosemicarbazones and their copper(II) complexes [10].

For the antibacterial action, MIC values in the 225–1478 μmol L^{-1} range were found for the thiosemicarbazones. Upon coordination, a substantial decrease in MIC was observed, with values in the 5–30 μmol L^{-1} range. Among the thiosemicarbazones, the lowest values of MIC were found for the 2-acetylpyridine derivatives, but in the complexes the lowest values were found for those with the 2-benzoylpyridine-derived thiosemicarbazones (**7**–**9**). The most significant decrease upon coordination occurred for these same complexes (Table 6). Interestingly, in the latter a neutral ligand is attached to the metal center, along with two chloride ions, forming species with coordination number five and with geometries which are different from that of complexes **2**–**6**.

To our knowledge this is one of the few cases of thiosemicarbazones' activity against *gram* negative bacilli, which indicates new possibilities of applications for this class of compounds.

Much lower values of MIC were obtained for the thiosemicarbazones against *C. albicans* (0.5–26 μmol L^{-1}) (Table 7), indicating selectivity. Upon coordination a decrease of the MIC values was observed as well, although not as significant as in the case of the antibacterial action, probably because the free thiosemicarbazones were already very active.

On complexation modifications of lipophilicity can occur [1,2]. Also, the rigid structure of the ligand in the complex could facilitate its interaction with the biological target, so that the metal complex may be a vehicle for activation of the ligand as the antimicrobial agent. Since the copper salt presents antimicrobial action as well, a syner-

Table 5
Ligand field transitions in the electronic spectrum of frozen DMF solution, at liquid N_2 temperature (77 K), parameters for the copper(II) complexes of *N*(4)-tolyl 2-formyl and *N*(4)-tolyl 2-acetylpyridine thiosemicarbazones

Compound	$g_{\parallel}$	$A_{\parallel}$[a]	$g_{\parallel}/A_{\parallel}$	$g_{\perp}$	$A_{\perp}$[a]	$A_{N\perp}$[a]	ΔE(d–d)[b]
[Cu(2Fo4mT)Cl] (**2**)	2.1850	177	123	2.0435	22	17	16703
[Cu(2Fo4pT)Cl] (**3**)	2.1830	177.5	122	2.0435	22	17	16920
[Cu(2Ac4oT)Cl] (**4**)	2.1745	182	119	2.0480	15	16	15818
[Cu(2Ac4mT)Cl] (**5**)	2.1740	162	134	2.0460	22	16	16958
[Cu(2Ac4pT)Cl] (**6**)	2.1745	184	118	2.0460	20	16	17240

[a] A values in 10^{-4} cm^{-1} units.
[b] ΔE(d–d) transitions, in cm^{-1} units, of the solid compounds.

Table 6
Minimum inhibitory concentration (MIC) against *Salmonella typhimurium* ATCC13311 for *N*(4)-tolyl 2-formyl, *N*(4)-tolyl 2-acetyl and *N*(4)-tolyl 2-benzoylpyridine thiosemicarbazones and their copper(II) complexes

Compounds	MIC (μmol L^{-1})	Complexes	MIC (μmol L^{-1})
H2Fo4oT	959	[Cu(HL1)Cl_2] (**1**)	30
H2Fo4mT	959	[Cu(2Fo4mT)Cl] (**2**)	24
H2Fo4pT	966	[Cu(2Fo4pT)Cl] (**3**)	20
H2Ac4oT	890	[Cu(2Ac4oT)Cl] (**4**)	21
H2Ac4mT	225	[Cu(2Ac4mT)Cl] (**5**)	15
H2Ac4pT	450	[Cu(2Ac4pT)Cl] (**6**)	13
H2Bz4oT	1478	[Cu(H2Bz4oT)Cl_2] (**7**)	12
H2Bz4mT	985	[Cu(H2Bz4mT)Cl_2] (**8**)	5
H2Bz4pT	985	[Cu(H2Bz4pT)Cl_2] (**9**)	8
Cloramphenicol	12	$CuCl_2 \cdot 2H_2O$	2663

Table 7
Minimum inhibitory concentration (MIC) against *Candida albicans* ATCC 18804 for *N*(4)-tolyl 2-formyl, *N*(4)-tolyl 2-acetyl and *N*(4)-tolyl 2-benzoylpyridine thiosemicarbazones and their copper(II) complexes

Compounds	MIC (μmol L^{-1})	Complexes	MIC (μmol L^{-1})
H2Fo4oT	3	[Cu(HL1)Cl_2] (**1**)	4
H2Fo4mT	3	[Cu(2Fo4mT)Cl] (**2**)	3
H2Fo4pT	3	[Cu(2Fo4pT)Cl] (**3**)	2
H2Ac4oT	14	[Cu(2Ac4oT)Cl] (**4**)	7
H2Ac4mT	5	[Cu(2Ac4mT)Cl] (**5**)	4
H2Ac4pT	26	[Cu(2Ac4pT)Cl] (**6**)	4
H2Bz4oT	6	[Cu(H2Bz4oT)Cl_2] (**7**)	2
H2Bz4mT	23	[Cu(H2Bz4mT)Cl_2] (**8**)	0.5
H2Bz4pT	0.7	[Cu(H2Bz4pT)Cl_2] (**9**)	0.3
Amphotericin B	4.4	$CuCl \cdot 2H_2O$	375

gistic effect of both ligand and metal acting together is also possible.

It is interesting to compare the values of MIC found for the studied complexes (5–30 μmol L^{-1} in the case of the antibacterial action and 0.3–7 μmol L^{-1} in the case of the antifungal action) with the values observed for the clinically used drugs chloramphenicol (12 μmol L^{-1}) and amphotericin (4.4 μmol L^{-1}). As already mentioned, resistance to used drugs has increased due to the increased and indiscriminate use of antibiotics and antifungal agents in the treatment of humans and animals [13].

These results suggest that coordination of the studied thiosemicarbazones with copper(II) could be an interesting strategy for preparing new antimicrobial agents which could be used against clinically significant bacteria and fungi cultures presenting resistance. In the case of the antifungal action, the free *N*(4)-tolyl thiosemicarbazones could be promising drug candidates, as well as their copper(II) complexes.

Structure–activity relationship analyses are presently underway in our laboratory as well as an investigation on the mechanisms of action of the studied compounds.

4. Supplementary material

CCDC 632461 and 632460 contain the supplementary crystallographic data for H2Ac4mT and [Cu(2Ac4mT)Cl] (**5**). These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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