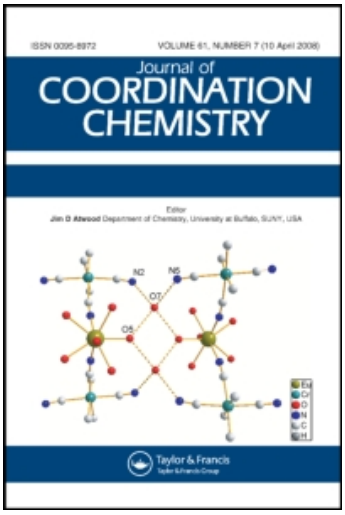

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Zinc(II) complexes of 2-pyridine-derived *N*(4)-*p*-tolyl thiosemicarbazones: study of *in vitro* antibacterial activity

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Reaction of *N*(4)-*p*-tolyl-2-formylpyridine thiosemicarbazone (H2Fo4pT), *N*(4)-*p*-tolyl-2-acetylpyridine thiosemicarbazone (H2Ac4pT), and *N*(4)-*p*-tolyl-2-benzoylpyridine thiosemicarbazone (H2Bz4pT) with $ZnCl_2$ gave [Zn(H2Fo4pT)Cl_2] (**1**), [Zn(H2Ac4pT)Cl_2] (**2**), and [Zn(H2Bz4pT)Cl_2] (**3**). In the first two complexes a tridentate N_{py}–N–S thiosemicarbazone binds to the zinc while in the latter N–S coordination occurs. Upon coordination the antibacterial activity against *Salmonella typhimurium* increases in **1** and **3**.

Keywords: Thiosemicarbazones; Zinc(II) complexes; Antibacterial activity; *Salmonella typhimurium*

1. Introduction

Resistance resulting from indiscriminate use of antibacterial and antifungal drugs both in humans and animals is a serious public health problem [1–3] and preparation of new antimicrobials with activity in low doses is extremely important.

Thiosemicarbazones and their metal complexes have a wide range of pharmacological applications as antitumoral, antiviral, and antimicrobial agents [4]. The antimicrobial action of $\alpha(N)$-heterocyclic thiosemicarbazones and their metal complexes against a variety of fungi and yeasts has been demonstrated [4–7]. In addition, 2-formyl and 2-acetylpyridine-derived thiosemicarbazones proved to be active against clinical isolates of bacteria [4, 8]. Significant activity was found against Gram-positive bacilli, but poor activity was observed against Gram-negative cultures [4, 8, 9]. Furthermore, 2-acetylpyridine thiosemicarbazones showed *in vitro* antibacterial activity against clinically important cultures, including isolates with known antibiotic resistance [4, 8, 9].

The presence of a bulky substituent at *N*(4) in the thiosemicarbazone chain leads to an increase in activity relative to the unsubstituted analogs, probably due to an increase in lipophilicity [10, 11]. Therefore, we recently started an investigation of the pharmacological profile of *N*(4)-tolyl thiosemicarbazones derived from

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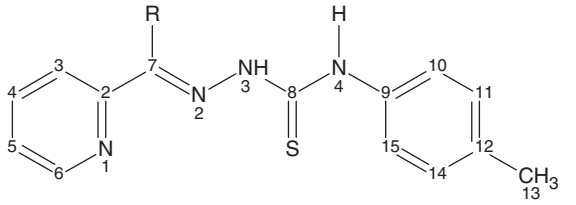

Figure 1. Generic representation for 2-pyridine-derived *N*(4)-*p*-tolyl thiosemicarbazones.

2-formyl-, 2-acetyl-, and 2-benzoylpyridine. We demonstrated that coordination to copper(II) strongly enhances the antibacterial and antifungal effect of these compounds [5, 6]. Modifications of lipophilicity upon complexation could be responsible for the observed results, as well as the rigid structure of the ligand in the complex, which could facilitate its interaction with the biological target.

Zinc is an essential nutrient and has many important functions as shown by the great variety of zinc metalloenzymes. Taking into consideration that zinc(II) is practically nontoxic, we now used coordination to zinc(II) of *N*(4)-*p*-tolyl-2-formylpyridine thiosemicarbazone (H2Fo4pT), *N*(4)-*p*-tolyl-2-acetylpyridine thiosemicarbazone (H2Ac4pT), and *N*(4)-*p*-tolyl-2-benzoylpyridine thiosemicarbazone (H2Bz4pT) (figure 1) to evaluate antimicrobial activity.

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 pellets; an YSI model 31 conductivity bridge was employed for molar conductivity measurements. NMR spectra were obtained at room temperature with a Bruker DRX-400 Avance (400 MHz) spectrometer using deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent and tetramethyl silane (TMS) as internal reference.

2.2. *Synthesis of zinc(II) complexes with 2-formyl-, 2-acetyl- and 2-benzoylpyridine N(4)-p-tolyl thiosemicarbazones*

The thiosemicarbazones were prepared as described in the literature [12, 13]. The zinc(II) complexes were obtained by refluxing, for 5 h, an ethanol solution (25 mL) of the desired thiosemicarbazone (3 mmol) with $ZnCl_2$ in 1 : 1 ligand-to-metal molar ratio. The resulting solids were filtered, washed with ethanol followed by diethyl ether, and dried *in vacuo*.

2.2.1. Dichloro[*N*(4)-*p*-tolyl-2-formylpyridine thiosemicarbazone]zinc(II). [Zn(H2Fo4pT)Cl_2] (1). Yellow solid; Anal. Calcd ($ZnC_{14}H_{14}N_4SCl_2$): C, 41.35%; H, 3.47%; N, 13.78%.

Found: C, 41.69%; H, 3.50%; N, 13.85%. FW: 406.67 g mol^{-1}. Molar conductivity (1×10^{-3} mol L^{-1}, DMF): 20 Ω^{-1} cm^2 mol^{-1}. IR [CsI/nujol (cm^{-1})]: 3365, 3201, ν(N–H); 1611, ν(C=N); 1558, 1513, 1476, 1445, ν(C=N + C=C); 817, ν(C=S); 639, ρ(py); 423, ν(Zn–N); 344, ν(Zn–S); 319, 304, ν(Zn–Cl); 273, ν(Zn–N_{py}). ^{1}H NMR (400 MHz, DMSO-d_6) [δ (ppm)]: 12.10 [s, 1H, N(3)–H]; 10.23 [s, 1H, N(4)–H]; 8.64 [d (J = 4.47 Hz), 1H, C(6)–H]; 8.46 [d (J = 5.65 Hz), 1H, C(3)–H]; 8.21 [s, 1H, C(7)–H]; 7.84 [t (J = 7.63 Hz), 1H, C(4)–H]; 7.47 [m, 2H, C(10,15)–H]; 7.41 [m, 1H, C(5)–H]; 7.19 [m, 2H, C(11,14)–H]; 2.31 [s, 3H, C(13)–H]. ^{13}C NMR (400 MHz, DMSO-d_6) [δ (ppm)]: 176.6 [C(8)=S]; 151.9 [C(2)]; 148.2 [C(6)]; 140.9 [C(7)=N]; 138.2 [C(4)]; 136.3 [C(9)]; 134.8 [C(12)]; 128.7 [C(11,14)]; 123.0 [C(10,15)]; 124.6 [C(5)]; 121.6 [C(3)]; 20.5 [C(13)]. Yield 84%.

2.2.2. Dichloro[*N*(4)-*p*-tolyl-2-acetylpyridine thiosemicarbazone]zinc(II). [Zn(H2Ac4pT)Cl$_2$] (2). Yellow solid; Anal. Calcd ($ZnC_{15}H_{16}N_4SCl_2$): C, 42.82%; H, 3.83%; N, 13.32%. Found: C, 42.79%; H, 3.80%; N, 13.35%. FW: 420.70 g mol^{-1}. Molar conductivity (1×10^{-3} mol L^{-1}, DMF): 17 Ω^{-1} cm^2 mol^{-1}. IR [CsI/nujol (cm^{-1})]: 3289, 3213, ν(N–H); 1618, ν(C=N); 1549, 1513, 1479, 1442, ν(C=N + C=C); 818, ν(C=S); 643, ρ(py); 423, ν(Zn–N); 350, ν(Zn–S); 314, 294, ν(Zn–Cl); 275, ν(Zn–N_{py}). ^{1}H NMR (400 MHz, DMSO-d_6) [δ (ppm)]: 10.89 [s, 1H, N(3)–H]; 10.29 [s, 1H, N(4)–H]; 8.71 [d (J = 4.47 Hz), 1H, C(6)–H]; 8.49 [d (J = 5.60 Hz), 1H, C(3)–H]; 8.16 [t (J = 7.65 Hz), 1H, C(4)–H]; 7.66 [m, 2H, C(10,15)–H]; 7.40 [m, 1H, C(5)–H]; 7.19 [m, 2H, C(11,14)–H]; 2.50 [s, 3H, C(16)–H]; 2.31 [s, 3H, C(13)–H]. ^{13}C NMR (400 MHz, DMSO-d_6) [δ (ppm)]: 177.4 [C(8)=S]; 148.7 [C(2)]; 147.9 [C(7)=N]; 146.4 [C(6)]; 140.7 [C(4)]; 136.3 [C(9)–N]; 135.1 [C(12)]; 128.8 [C(11,14)]; 126.1 [C(10,15)]; 125.0 [C(5)]; 122.9 [C(3)]; 20.5 [C(13)]; 12.8 [C(13)]. Yield 79%.

2.2.3. Dichloro[*N*(4)-*p*-tolyl-2-benzoylpyridine thiosemicarbazone]zinc(II). [Zn(H2Bz4pT)Cl$_2$] (3). Yellow solid; Anal. Calcd ($ZnC_{20}H_{18}N_4SCl_2$): C, 49.76%; H, 3.76%; N, 11.61%. Found: C, 49.82%; H, 3.79%; N, 11.65%. FW: 482.76 g mol^{-1}. Molar conductivity (1×10^{-3} mol L^{-1}, DMF): 23 Ω^{-1} cm^2 mol^{-1}. IR [CsI/nujol (cm^{-1})]: 3288, 3207, ν(N–H); 1628, ν(C=N); 1562, 1515, 1471, 1444, ν(C=N + C=C); 828, ν(C=S); 595, ρ(py); 412, ν(Zn–N); 347, ν(Zn–S); 300, ν(Zn–Cl). ^{1}H NMR (400 MHz, DMSO-d_6) [δ (ppm)]: 12.99 [s, 1H, N(3)–H]; 10.65 [s, 1H, N(4)–H]; 8.89 [d (J = 4.40 Hz), 1H, C(6)–H]; 8.07 [t (J = 7.63 Hz), 1H, C(4)–H]; 7.72 [t (J = 5.60 Hz), 1H, C(3)–H]; 7.66 [m, 1H, C(19)–H]; 7.65 [m, 2H, C(10,15)–H]; 7.50 [m, 2H, C(18,20)–H]; 7.47 [m, 2H, C(17,21)–H]; 7.44 [m, 1H, C(5)–H]; 7.18 [m, 2H, C(11,14)–H]; 2.31 [s, 3H, C(13)-H]. ^{13}C NMR (400 MHz, DMSO-d_6) [δ (ppm)]: 176.6 [C(8)=S]; 151.2 [C(2)]; 148.7 [C(6)]; 143.5 [C(7)=N]; 138.5 [C(4)]; 136.7 [C(16)]; 136.3 [C(9)–N]; 134.8 [C(12)]; 129.4 [C(19)]; 129.1 [C(17,21)]; 128.6 [C(11,14)]; 128.4 [C(18,20)]; 126.3 [C(3)]; 125.2 [C(10,15)]; 123.9 [C(5)]; 20.6 [C(13)]. Yield 80%.

2.3. *Antimicrobial activity*

Antibacterial activity was evaluated by minimum inhibitory concentration (MIC) using the macro dilution test [14–16]. *Salmonella typhimurium* (ATCC 13311) 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 [16] to produce a suspension of about 10^5 CFU mL^{-1} (colony-forming units per milliliter). 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 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.

3. Results and discussion

Microanalyses suggest formation of $[Zn(HL)Cl_2]$ complexes **1**–**3** in which the thiosemicarbazones coordinate as neutral ligands (HL). The molar conductivity data reveal that the complexes are nonelectrolytes [17], indicating two chlorides in the metal coordination sphere.

3.1. *Infrared spectra*

Vibrations attributed to ν(C=N) at 1587–1597 cm^{-1} in the spectra of the free thiosemicarbazones shift to 1611–1628 cm^{-1} in spectra of the complexes, in agreement with coordination via the azomethine nitrogen [5, 6]. The ν(C=S) in the 849–869 cm^{-1} range in spectra of the free bases shifts to 818–828 cm^{-1} in the complexes, indicating coordination of the sulfur [5, 6]. In-plane deformation of the pyridine ring at 595–622 cm^{-1} in spectra of the uncomplexed thiosemicarbazones shifts to 639–657 cm^{-1} in **1** and **2**, suggesting coordination of the hetero-aromatic nitrogen [5, 6]. In **3**, no change was observed relative to the free base, indicating that the hetero-aromatic nitrogen is not coordinated.

New absorptions at 412–423 cm^{-1}, 344–350 cm^{-1}, and 294–319 cm^{-1} were attributed to ν(Zn–N), ν(Zn–S), and ν(Zn–Cl), respectively [18]. For **1** and **2**, bands at 273–275 cm^{-1} were assigned to $\nu(Zn–N_{py})$, indicating coordination of the pyridine nitrogen.

3.2. *NMR spectra*

The ^1H and ^{13}C NMR assignments for the zinc(II) complexes are reported in section 2.2. The ^1H resonances were assigned based on chemical shifts, multiplicities and coupling constants. The carbon type (C, CH) was determined by using DEPT135 experiments. Assignments of protonated carbons were made by 2-D heteronuclear-correlated experiments (HMQC) using delay values, which correspond to ^1J(C, H).

In the ^1H NMR spectra of H2Fo4pT and H2Ac4pT only one signal of N(3)–H was found at δ 11.99 and 10.61, respectively, suggesting the presence of the *E* configurational isomer, in which N(3)–H is hydrogen bonded to the solvent (DMSO-d_6) [7]. Only one signal was observed for all hydrogens and carbons.

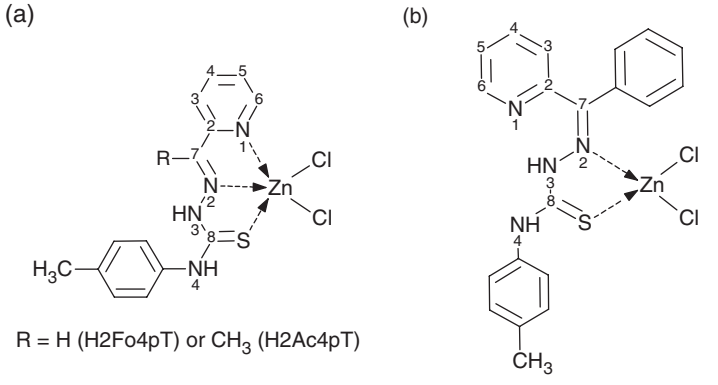

Figure 2. Coordination geometries of (a) **1** and **2** and (b) **3**.

For H2Bz4pT two signals were observed for all hydrogens, including two isomers in DMSO-d_6. The signal at δ 10.48 was assigned to the *E* isomer and that at δ 13.03 to the *Z* isomer [7, 19, 20]. In the latter, N(3)–H is hydrogen bonded to the pyridine nitrogen. In the ^{13}C NMR of H2Bz4pT all signals were duplicated, also indicating the existence of two isomers in solution.

In the ^{1}H NMR spectra of the three zinc(II) complexes the presence of a signal for N(3)–H was observed, indicating that the thiosemicarbazone remains protonated after coordination. In **1** and **2** the signal for N(3)–H was at δ 12.10 and 10.89, respectively, suggesting that the ligand is in the *E* configuration. In **3** only one signal of N(3)–H was found at δ 12.99, indicating the presence of the *Z* isomer [7, 19, 20] and that the heteroaromatic nitrogen is not involved in coordination.

Upon formation of **1** and **2** the signals of the pyridine carbons and the signal of C(7)=N shift, in accord with coordination of the imine nitrogen and the heteroaromatic nitrogen. The C(8)=S signal stays practically unaffected after complexation, probably due to the d^{10} configuration of zinc(II), which precludes electron density transfer from sulfur to the metal.

In the spectrum of **3** only one signal was observed for all carbons. Upon coordination and formation of **3** most of the signals remain unchanged and are characteristic of the thiosemicarbazone in the *Z* configuration, suggesting that coordination does not involve the heteroaromatic nitrogen, as indicated previously by the ^{1}H NMR spectrum. The existence of only one signal for all hydrogens and carbons unequivocally confirms coordination. Therefore, the NMR data suggest coordination of the thiosemicarbazone through the N_{py}–N–S chelating system in **1** and **2** and through the N–S system in **3** (figure 2).

3.3. *Antimicrobial activity*

Table 1 lists the minimum inhibitory concentration (MIC) against the growth of *S. typhimurium* for the thiosemicarbazones and their zinc(II) complexes, together with values obtained previously for copper(II) complexes of the same ligands [5, 6].

Upon coordination to zinc(II) the values of MIC against *S. typhimurium* decrease in the cases of **1** and **3**. Comparison with results obtained for copper(II) complexes with

Table 1. MIC against *S. typhimurium* ATCC 13311 for *N*(4)-*p*-tolyl 2-formyl, *N*(4)-*p*-tolyl 2-acetyl, and *N*(4)-*p*-tolyl 2-benzoylpyridine thiosemicarbazones, their zinc(II) and copper(II) complexes, $ZnCl_2$, $CuCl_2$, and cloramphenicol.

Compounds	MIC $\mu mol\,L^{-1}$	Zinc complexes	MIC $\mu mol\,L^{-1}$	Copper complexes [5, 6]	MIC $\mu mol\,L^{-1}$
H2Fo4pT	946	$[Zn(H2Fo4pT)Cl_2]$ (**1**)	323	[Cu(2Fo4pT)Cl]*	22
H2Ac4pT	450	$[Zn(H2Ac4pT)Cl_2]$ (**2**)	609	[Cu(2Ac4pT)Cl]*	5
H2Bz4pT	1478	$[Zn(H2Bz4pT)Cl_2]$ (**3**)	547	$[Cu(H2Bz4pT)Cl_2]$	8
Chloramphenicol	12	$ZnCl_2$	945	$CuCl_2{\cdot}2H_2O$	1475

*2Fo4pT and 2Ac4pT stand for the anionic ligands formed upon deprotonation at N(3)–H.

the same ligands [5, 6] reveals that coordination to copper(II) is much more effective in enhancing antimicrobial activity. Since the structures of the copper(II) and zinc(II) complexes are not the same, comparison between complexes with the same ligand and the two different metal centers are not possible, suggesting that the ability of copper(II) to undergo redox processes could be related to the greater activity of its complexes. Although the zinc(II) complexes did not prove to be as active as the copper(II) complexes against *S. typhimurium*, the antibacterial activity of the thiosemicarbazones underwent a threefold increase upon coordination to zinc(II) in **1** and **3**.

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