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Zinc(II) complexes of 2-acetyl pyridine 1-(4-fluorophenyl)-piperazinyl thiosemicarbazone: Synthesis, spectroscopic study and crystal structures – Potential anticancer drugs

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ABSTRACT

2-Acetyl pyridine thiosemicarbazone containing an 1-(4-fluorophenyl)-piperazinyl ring incorporated at N(4)-position, HAcPipPheF (**1**) and the zinc(II) complexes $[Zn(AcPipPheF)_2]$ (2) and $[Zn(OAc)(AcPipPheF)]_2$ (**3**) have been prepared and structurally characterized by means of vibrational and NMR (^1H and ^{13}C) spectroscopy. The crystal structures of the compounds **1**–**3** have been determined by X-ray crystallography. The metal coordination geometry of $[Zn(AcPipPheF)_2]$ is described as distorted octahedral configuration in a *trans*-N–*cis*-N–*cis*-S configuration. In $[Zn(OAc)(AcPipPheF)]_2$ one of the acetato group exhibits monoatomic bridge and the other bridges in a bidentate manner. The zinc(1) metal ion is coordinated in a distorted octahedral configuration while the metal coordination of Zn(2) is described as distorted square pyramidal. Biomedical studies revealed that, compounds **1**–**3** displayed potent anticancer activity. The antiproliferative activity of **1**–**3** was found to be considerably stronger than that of *cis*-platin. The IC_{50} values range from 26 to 90 nM, against all cell lines tested, while for *cis*-platin the IC_{50} values range from 2 to 17 μM and for the zinc salt, $ZnCl_2$, the IC_{50} values range from 81 to 93 μM. The complex **3** shows the highest activity against all four cancer cell lines and the highest selectivity against K562 and MDA-MB-453 cancer cell lines. The compounds inhibited tumor cell proliferation by arresting the cell cycle progression at the S phase.

1. Introduction

Thiosemicarbazones are versatile molecules not only because of their broad profile in pharmacological activity, but also because can act as ligands in coordination chemistry in different ways. It has been demonstrated in previous publications that thiosemicarbazones afford a diverse variety of compounds with different activities [1–7]. The mechanism of action of TSCs, is due to its ability to inhibit the biosynthesis of DNA, possibly blocking the enzyme ribonucleotide diphosphate reductase; binding to the nitrogen bases of DNA or RNA, hindering or blocking base replication; creation of lesions in DNA strands by oxidative rupture [1].

Zinc the second most prominent trace metal in the human body after iron is essential for growth, development and plays an important role in various biological systems. It is a vital component an essential cofactor, critical for numerous cellular processes and may be a major regulatory ion in the metabolism of cells. Zinc is cytoprotective and suppresses apoptotic pathways. Zinc plays a role in brain, where it has a specific function as a neuromodulator in addition to its other typical cellular functions [7,8]. A structural review of main group metal complexes of semicarbazones and thiosemicarbazones shows that TSCs are very versatile coordination agents with these acceptors [9].

Given the potential biological activity of thiosemicarbazones and the involvement of the Zn(II) in the metabolism of cells and our previous results on Zn(II) TSCs complexes [10–12], we thought it would be of interest to explore this chemistry. In order to widen the scope of investigations on the coordination behaviour of TSCs, towards Zn(II), we carried out systematic studies with the final goal to develop new biologically active pharmaceuticals. With metallopharmaceuticals playing a significant role in therapeutic and diagnostic medicine, the discovery and development of new metallodrugs remain an ever-growing area of research in medicinal inorganic chemistry.

The present paper includes synthesis, spectral characterization of the novel prepared zinc(II) complexes with 2-acetylpyridine N(4)-(4-fluorophenyl)-piperazine thiosemicarbazone, HAcPipPheF,

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1 (Scheme 1) and the crystal structure of the ligand and the complexes [Zn(AcPipPheF)$_2$] (**2**) and [Zn(OAc)(AcPipPheF)]$_2$ (**3**). The compounds **1–3** were tested for their antiproliferative activity *in vitro* against the cells of four human cancer cell lines: HeLa (cervix adenocarcinoma cell line), K562 (chronic myelogenous leukaemia), MDA-MB-361 and MDA-MB-453 (breast cancer cell lines).

2. Experimental

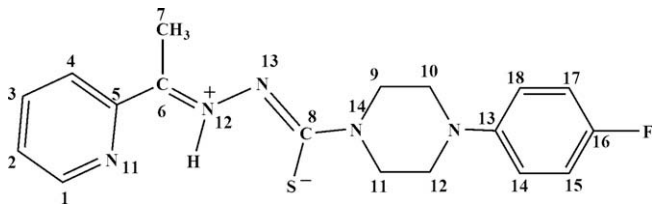
2.1. General and instrumental

All reagents were commercially available (*Aldrich* or Merck) and used as supplied. Solvents were purified according to standard procedures. The MTT (3-(4,5-di-methyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was dissolved (5 mg/mL) in phosphate buffer saline pH 7.2 and filtered (0.22 μm) before use. The RPMI-1640 cell culture medium, fetal calf serum, MTT, ethidium bromide and acridine orange were purchased from Sigma Chemical Company, USA. Melting points were determined in open capillaries and are uncorrected. Infrared and far-infrared spectra were recorded on a Perkin Elmer Spectrum GX Fourier transform spectrophotometer using KBr pellets (4000–400 cm^{-1}) and Nujol mulls dispersed between polyethylene disks (400–40 cm^{-1}). The intensity of reported IR signals is defined as m = medium, mw = medium weak, s = strong, ms = medium strong. NMR spectra were recorded on a Bruker AV-400 spectrometer operating at 400 and 100 MHz for ^{1}H and ^{13}C acquisition, respectively, or on a Bruker AV-250 spectrometer operating at 250.13 and 62.90 MHz for ^{1}H and ^{13}C acquisition respectively. The splitting of proton resonances in the reported ^{1}H NMR spectra is defined as s = singlet, d = doublet, t = triplet, and m = multiplet. The spectra were acquired at room temperature (298 K). The chemical shifts are reported in ppm for ^{1}H and ^{13}C NMR. Samples were dissolved in dimethylsulfoxide-d$_6$ and spectra were obtained at room temperature with the signal of free dimethylsulfoxide-d$_6$ (at 2.50 ppm ^{1}H NMR, 39.5 ppm ^{13}C NMR) as a reference. Mass spectra were recorded on an Agilent LC/MSD Trap SL spectrometer. Elemental analyses, C, H, N and S were performed on a Carlo Erba EA (model 1108).

2.2. Synthesis of the ligand and the complexes

2.2.1. Preparation of 2-acetylpyridine-N4-1-(4-fluorophenyl)piperazinyl thiosemicarbazone, HAcPipPheF; (*1*)

4-Methyl-4-phenyl-3-thiosemicarbazide was prepared according to the method described by Scovill et al. [13]. The crude product, 4-methyl-4-phenyl-3-thiosemicarbazide, was recrystallized from a mixture of EtOH and distilled water (3:1). 1-(4-fluorophenyl)-piperazine (0.5407 g, 3 mM) and 2-acetylpyridine (0.363 mL, 3 mM) were added to a solution of 4-methyl-4-phenyl-3-thiosemicarbazide (0.543 g, 3 mM) in CH_3CN (4 mL). The mixture was stirred and refluxed for 30 min. The resulting yellow precipitate was filtered off, washed with cold CH_3CN and dried *in vacuo* over silica gel. M.p. 155–157 °C. Yield: 56%. IR (cm^{-1}): 3218m, *ν*(NH); 2838m, *ν*(CH); 1584mw, *δ*(NH); 1561m, 1512s, *ν*(C=C) *ν*(C=N); 1416s, 1363s, 1297s, *ν*(NCS); 1223s, (Ar–F); 1088m, *ν*(N–N); 931s, *ν*(CS); 650m, *δ*(py). ^{1}H NMR (DMSO-d$_6$) *δ* (ppm): 9.35 (s, 1H, N(3)H); 8.71 (d, 1H, *J* = 4.4 Hz, C(1)H); 7.49 (t, 1H, *J* = 5.7 Hz, C(2)H); 7.92–7.95 (m, 1H, C(3)H); 7.95–7.97 (m, 1H, C(4)H); 2.57 (s, 3H, C(7)H); 4.14 (t, 4H, C(9,11)H); 3.19 (t, 4H, C(10,12)H); 6.98–7.04 (m, 2H C(14,18)H); 7.05–7.13 (m, 2H, C(15,17)H); ^{13}C NMR *δ* (ppm): 150.25 C(1); 125.71 C(2); 138.48 C(3); 122.34 C(4); 155.28 C(5); 148.48 C(6); 14.10 C(7); 184.37 C(8); 50.02 C(9,11); 49.61 C(10); 48.03 C(12); 148.51 C(13); 118.35 C(14); 116.12 C(15); 159.04 C(16); 116.47 C(17); 118.47 C(18); Mass spectrum, MS (electrospray Ionization, ESI, *m*/*z*): 358 [**1**+H]$^+$, 380 [**1**+Na]$^+$, 326 [**1**−S]$^+$. Elemental analysis are consistent with $C_{18}H_{20}N_5SF$ found: C, 60.3; H, 5.6; N, 19,3; S, 8.9; Calc.: C, 60.5; H, 5.5; N, 19.6; S, 9.0. Suitable crystals for X-ray study were obtained by crystallization from a fresh solution of $CHCl_3/C_6H_6$.



Scheme 1. The numbering scheme for HAcPipPheF, **1**, showing the positively charge N12 and the negatively charged S.

2.2.2. Preparation of bis(2-acetylpyridine-N4-1-(4-fluorophenyl)-piperazinyl thiosemicarbazonato)zinc(II), [Zn(AcPipPheF)$_2$]; (*2*)

A solution of $ZnCl_2$ (0.068 g, 0.5 mM) in 10 mL of EtOH was added to a solution of HAcPipPheF (0.393 g, 1.1 mM) in 15 mL of EtOH. The mixture was stirred and some drops of Et_3N were added. The apparent pH value was 8. The solution was refluxed for 2 h in 80 °C and was left in the fridge overnight. The yellow precipitate was filtered off, washed with cold EtOH and dried *in vacuo* over silica gel. M.p. 275–277 °C. Yield: 48.0%. IR (cm^{-1}): 2828s, *ν*(CH); 1591s, 1548w, 1507s, *ν*(C=C) *ν*(C=N); 1417s, 1366s, 1299s, *ν*(NCS); 1220s, *ν*(Ar–F); 1155s, *ν*(N–N); 928s, 869m, *ν*(CS); 662w, *δ*(py); 433m, 419m, *ν*(Zn–N); 382m, 369m, *ν*(Zn–S); 255m, 239m, *ν*(Zn–N$_{pyr}$) cm^{-1}. ^{1}H NMR (DMSO-d$_6$) *δ* (ppm): 8.65 (d, 1H, C(1)H); 7.33 (t, 1H, *J* = 6.2 Hz, C(2)H); 7.84 (t, 1H, *J* = 4.5 Hz, C(3)H); 7.94 (t, 1H, *J* = 7.5 Hz, C(4)H); 2.66 (s, 3H, C(7)H); 4.10 (t, 4H, C(9,11)H); 3.16 (t, 4H, C(10,12)H); 7.02–7.08 (m, 2H, C(14,18)H); 7.10–7.14 (m, 2H, C(15,17)H); ^{13}C NMR *δ* (ppm): 150.63 C(1); 124.65 C(2); 139.66 C(3); 121.99 C(4); 154.90 C(5); 146.01 C(6); 14.12 C(7); 181.43 C(8); 49.81 C(9,11); 46.85 C(10,12); 148.51 C(13); 118.13 C(14); 116.07 C(15); 158.62 C(16); 115.72 C(17); 118.01 C(18). MS (ESI, *m*/*z*): 779 [**2**+H]$^+$. Elemental analysis are consistent with $ZnC_{36}H_{38}N_{10}S_2F_2$ found: C, 55.3; H, 5.1; N, 17.8; S, 8.0; Zn, 8.10. Calc.: C, 55.6; H, 4.9; N, 18.0; S, 8.2; Zn, 8.40. Suitable crystals for X-ray study were obtained by crystallization from a fresh solution of $EtOH/C_6H_6$.

2.2.3. Preparation of bis(μ-acetato(2-acetylpyridine-N4-1-(4-fluorophenyl)-piperazinyl thiosemicarbazonato)zinc(II), [Zn(HAcPipPheF)(OAc)]; (*3*)

A solution of [Zn(CH$_3$COO)$_2$(H$_2$O)$_2$] (0.241 g, 1.1 mM) in 10 mL of EtOH was added to a solution of HAcPipPheF (0.357 g, 1 mM) in 15 mL of EtOH. The mixture was stirred and some drops of Et_3N were added. The apparent pH value was 8. The solution was refluxed for 2 h and then it was left in the fridge overnight. The yellow precipitate was filtered off, washed with cold EtOH and dried in vacuo over silica gel. Yield: 77.3%. M.p. 280–282 °C. IR (cm^{-1}): 2812s, *ν*(CH); 1580s, 1506s, *ν*(C=C) *ν*(C=N); 1412s, 1366s, 1299s *ν*(NCS); 1217s, *ν*(Ar–F); 1152s, *ν*(N–N); 816s, *ν*(CS); 1655s, 1590s ν_{as}(COO); 1367ms, 1455s, ν_s(COO); 665mw, *δ*(py); 427ms, *ν*(Zn–N); 372ms, *ν*(Zn–S); 250ms, *ν*(Zn–N$_{pyr}$), 376ms, 346ms, 328ms, *ν*(Zn–O) cm^{-1}. ^{1}H NMR (DMSO-d$_6$) *δ* (ppm): 8.49 (d, 1H, *J* = 4.3 Hz, C(1)H); 7.58 (t, 1H, *J* = 6 Hz, C(2)H); 7.86 (t, 1H, *J* = 8.5 Hz, C(3)H); 8.11 (t, 1H, *J* = 7.8 Hz, C(4)H); 2.66 (s, 1H, C(7)H); 4.11 (t, 4H, *J* = 3.7 Hz, C(9,11)H); 3.17 (t, 4H, *J* = 5 Hz, C(10,12)H); 7.04–7.08 (m, 2H, C(14,18)H); 7.10–7.11 (m, 2H, C(15,17)H); 1.79 (s, 3H, OAc); ^{13}C NMR *δ* (ppm): 150.76 C(1); 125.48 C(2); 141.06 C(3); 122.45 C(4); 155.27 C(5); 145.82 C(6); 14.07 C(7); 180.73 C(8); 50.13 C(9,11); 47.09 C(10,12); 148.80 C(13); 118.42 C(14); 116.44 C(15); 158.50 C(16); 116.09 C(17); 118.54 C(18); 23.96, 176.50 CH_3COO^-; MS (ESI, *m*/*z*): 960 [**3**+H]$^+$.