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First 3,5-diacetyl-1,2,4-triazol derived mono(thiosemicarbazone) and its palladium and platinum complexes: Synthesis, structure and biological properties

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ABSTRACT

Preparation and characterization of 3,5-diacetyl-1,2,4-triazol mono(4-phenylthiosemicarbazone) ligand, H_3L, and its derived palladium(II) and platinum(II) complexes, [Pd(HL)(PPh_3)] and [Pt(HL)(PPh_3)], is described. The molecular structures of the two new metal complexes have been determined by single crystal X-ray diffraction. In both complexes the metal ion is four-coordinated with a [N_2SP] donor environment, *via* one triazole nitrogen atom, the azomethine nitrogen and thione sulfur atoms of the thiosemicarbazone moiety and a phosphorous atom from the triphenylphosphine coligand. The cytotoxicity of the new compounds synthesized was investigated *in vitro* against T-47D, A2780 and A2780*cis*R human cancer cell lines, being H_3L the species with the highest activity. In addition, the DNA binding ability of H_3L with calf thymus DNA was explored by UV–Vis absorption spectroscopy.

1. Introduction

α-N-heterocyclic thiosemicarbazones (N-TSCs) constitute a class of nitrogen-sulfur donor ligands which have attracted considerable attention because of their interesting structural, electronic and wide biological properties. Thus, free N-TSCs as well as their metal complexes, have been intensively investigated for antiviral and antitumor activities [1–3].

The cell growth inhibition ability of N-TSCs has been attributed to their chelating and redox modulating properties and it is shown that the activity strongly depends upon the nature of the N-heterocyclic ring [4–8].

In this sense pyridine-2-carboxaldehyde thiosemicarbazone derivatives have been extensively studied and particularly, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine), di-2-pyridylketone-4,4-dimethyl thiosemicarbazone (Dp44mT) and 2-benzoylpyridine-4,4-dimethylthiosemicarbazone (Bp44mT), Fig. 1, have demonstrated to selectively inhibit tumor growth in a wide range of cancers both *in vitro* and *in vivo* [9–11].

On the other hand, 1,2,4-triazoles are well-known aromatic five-membered heterocycles which, containing three nitrogen atoms, present some important features like aromaticity and tautomerism. Moreover they are very strong N-donors towards d-metal ions and can also be readily deprotonated [12–14].

In the course of our studies we have synthesized a series of 3,5-diacetyl-1,2,4-triazol based bis(thiosemicarbazones), Fig. 2, which have been shown to be versatile polydentate ligands towards Pt(II) and Pd(II) ions since mono-, bi- and tetranuclear compounds have been obtained [15–17]. Moreover some of these compounds have demonstrated good, *in vitro*, antiproliferative activity in various human tumor cell lines [15,18,19].

These results led us to think that it was worthwhile to synthesize 3,5-diacetyl-1,2,4-triazol based mono(thiosemicarbazones) to see the effect of the ketone remaining group on the chelating and biological properties of the derivatives. Bis(thiosemicarbazones) mainly obtained by the condensation of a dialdehyde or a diketone with a thiosemicarbazide derivative, in 1:2 M ratios, are relatively simple to synthesize. However, the synthesis of mono(thiosemicarbazone) ligands derived from a dicarbonyl compounds is difficult to control and mixtures of 1:1 and 1:2 products are usually obtained. Moreover, the separation of these mixtures is complicated by their low solubility [20–22]. It is noteworthy that only three reports on structural properties of mono(thiosemicarbazone)

Abbreviations: CT-DNA, calf thymus DNA; DMSO, dimethyl sulfoxide; FBS, foetal bovine serum; IC_{50}, 50% inhibitory concentration; MeOH, methanol; TCA, trichloroacetic acid; Tris base, tris(hydroxymethyl)aminomethane; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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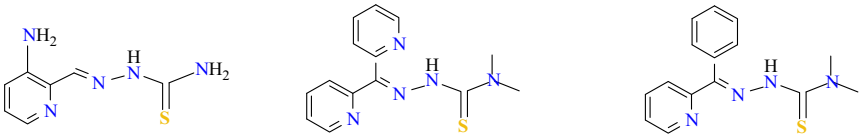

Fig. 1. Chemical structure of pyridine 2-carboxaldehyde thiosemicarbazone derivatives: Triapine (left), Dp44mT (center) and Bp44mT (right).

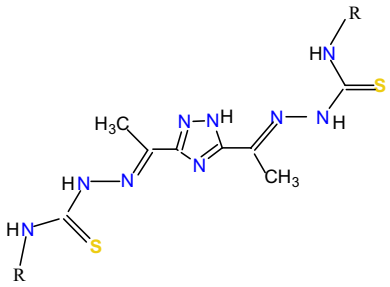

Fig. 2. Chemical structure of 3,5-diacetyl-1,2,4-triazole based bis (thiosemicarbazones).

complexes derived of (N)-heterocyclic diketones have been found in literature [23–25].

Herein, we report the synthesis and chemical characterization of the new 3,5-diacetyl-1,2,4-triazol mono(4-phenylthiosemicarbazone) ligand, H_3L, and its palladium(II) and platinum(II) complexes, [Pd(HL)(PPh_3)] and [Pt(HL)(PPh_3)]. The cytotoxic activity of the new compounds synthesized and cisplatin, assumed as the reference antitumor drug, against three human cancer cell lines: T-47D (breast cancer), A2780 and A2780*cis*R (epithelial ovarian cancer) has been studied. In addition, DNA interaction ability of H_3L was investigated by absorption spectroscopy.

2. Experimental

2.1. Measurements

Elemental analyses were performed on a LECO CHNS-932 microanalyzer. Fast atom bombardment (FAB) mass spectrum (MS) was performed on a VG AutoSpec spectrometer and electrospray ionization (ESI) mass spectra were carried out on a QSTAR mass spectrometer. Nuclear Magnetic Resonance (NMR) spectra were recorded on a BRUKER AMX-300 spectrometer. All cited physical measurements were obtained out by the Servicio Interdepartamental de Investigación (SIdI) of the Universidad Autónoma de Madrid.

Melting points were determined with a Stuart Scientific SMP3 apparatus. The pH measurements were carried out with a Crison BASIC 20+ pH-meter equipped with a combined Crison glass electrode. Infrared spectra were recorded on a Jasco FT/IR-410 spectrophotometer. Electronic spectra were recorded on a Thermo Scientific Evolution 260 Bio UV–Vis spectrophotometer.

2.2. Materials

Solvents were purified and dried according to standard procedures. Ultrapure Milli Q water was used for all biological experiments. 4-Phenylthiosemicarbazide, lithium hydroxide and dichloridobis(triphenylphosphine)palladium(II) were commercially available. 3,5-Diacetyl-1,2,4-triazole was prepared as described in bibliography [26] and disodium salt of CT-DNA was purchased from Sigma–Aldrich.

2.3. Synthesis of compounds

2.3.1. 3,5-diacetyl-1,2,4-triazol mono(4-phenylthiosemicarbazone) ligand, H_3L

A methanolic solution (20 mL) of 4-phenylthiosemicarbazide (0.20 g, 1 mmol) was added dropwise with constant stirring to a water solution (20 mL) of 0.153 g (1 mmol) of 3,5-diacetyl-1,2,4-triazole, acidified with 2 drops of concentrated HCl. The mixture was stirred at 0 °C for 1 h and then the white solid formed was filtered, washed with cold methanol and diethyl ether and dried in vacuo. Yield (76%), mp 188 °C. Elemental analysis found, C, 51.68; H, 4.94; N, 27.10; S 10.56; $C_{13}H_{14}N_6OS$ requires C, 51.66; H, 4.63, N, 27.81; S 10.60%. MS (FAB^+ with mNBA: nitrobenzyl alcohol matrix): m/z 303 for $[C_{13}H_{14}N_6OS+H]^+$. IR (KBr pellet): ν/cm^{-1} 3325 (s, NH-triazol), 3269 and 3155 (s, 2NH and 4NH), 1690 (s, CO), 1594 (m, CN), 793 (w, CS-thioamide IV band). ^1H NMR (300.14 MHz, DMSO-d6): δ (ppm) 15.06 (s, NH-triazol, 1H), 11.25 and 10.36 (s, NH, 1H), 7.66–7.09 (m, aromatic-thiosemicarbazide, 4H); 2.59 and 2.44 (s, CH_3, 3H). UV/Vis (DMSO): λ/nm 230, 313.

2.3.2. Palladium(II) and platinum(II) complexes

0.33 mmol of the corresponding $MCl_2(PPh_3)_2$ metallic salt (0.23 g for M = Pd and 0.33 g for M = Pt) was added to an ethanolic solution (20 mL) of 3,5-diacetyl-1,2,4-triazol mono(4-phenylthiosemicarbazone) ligand (0.33 mmol, 0.10 g) and $LiOH{\cdot}H_2O$ (0.66 mmol, 0.028 g). The reaction mixture was stirred for 2 h at room temperature and then, the solid formed was filtered, washed with ethanol and diethyl ether and finally dried *in vacuo*.

2.3.3. [Pd(HL)(PPh_3)]

Yield (60%), mp 196 °C. Elemental analysis found, C, 55.55; H, 4.30; N, 12.80; S, 5.00; $C_{31}H_{27}N_6SOPPd$ requires C, 55.65; H, 4.05; N, 12.60; S, 4.80%. MS (ESI+ with MeOH + HCOOH 0.1%): m/z 669 for $[C_{31}H_{27}N_6SOPPd+H]^+$. IR (KBr pellets): ν/cm^{-1} 3166 (s, NH); 1696 (s, CO); 1599 (s, CN); 1097 (s, PC). ^1H NMR (300.14 MHz, DMSO-d6): δ (ppm) 10.05 (s, NH, 1H); 7.70–7.01 (m, aromatic protons); 2.51 and 2.50 (s, CH_3, 3H). UV/Vis (DMSO): λ/nm 260, 320, 366, 455. Crystallization in CH_3CN allowed us to isolate single crystals, which were studied by X-ray diffraction techniques.

2.3.4. [Pt(HL)(PPh_3)]

Yield (76%), mp 240 °C. Elemental analysis found, C, 49.00; H, 3.80; N, 11.95; S, 4.05; $C_{31}H_{27}N_6SOPPt$ requires C, 49.15; H, 3.55; N, 11.10; S, 4.20%. MS (ESI+ with MeOH + HCOOH 0.1%): m/z 758 for $[C_{31}H_{27}N_6SOPPt+H]^+$. IR (KBr pellets): ν/cm^{-1} 3136 (s, NH); 1693 (s, CO); 1602 (s, CN); 1097 (s, PC). ^1H NMR (300.14 MHz, DMSO-d6): δ (ppm) 10.06 (s, NH, 1H); 7.68–7.01 (m, aromatic protons); 2.51 and 2.50 (s, CH_3, 3H). UV/Vis (DMSO): λ/nm 261, 338, 403, 444. Crystallization in DMSO allowed us to isolate single crystals, which were studied by X-ray diffraction techniques.

2.4. Crystallography

Data were collected on a Bruker Kappa Apex II diffractometer. A summary of the crystal data, experimental details and refinement results is listed in Table 1. The software package SHELXTL was used for space group determination, structure solution, and refinement