Inorganica Chimica Acta 446 (2016) 54–60

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

DNA binding, topoisomerase inhibition and cytotoxicity of palladium(II) complexes with 1,10-phenanthroline and thioureas

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ARTICLE INFO

Article history:
Received 10 September 2015
Received in revised form 20 January 2016
Accepted 24 February 2016
Available online 3 March 2016

Keywords:
Palladium(II)
Thioureas
Metallointercalator
Cytotoxicity
DNA binding
Topoisomerase inhibitors

ABSTRACT

Metallointercalators represent a promising alternative in cancer chemotherapy. We present herein DNA binding, topoisomerase inhibition and cytotoxic studies on a series of complexes of general formulae $[Pd(phen)(tu^*)_2]^{2+}$ incorporating the intercalator 1,10-phenanthroline and thiourea ligands (L = thiourea **1**, N-methylthiourea **2**, N,N′-dimethylthiourea **3**). DNA-unwinding results showed that the complexes can induce the unwinding of the plasmid DNA. The binding constants (K_b) for the interaction of the complexes with SS-DNA were determined by UV spectroscopy. Competitive experiments with ethidium bromide (EB) were investigated by fluorescence spectroscopy and show that all the complexes were able to displace EB from the DNA–EB complex. The results suggest that they may interact with DNA by intercalation. Compounds were tested against human oral carcinoma cell line (KB), human breast cancer cell line (MCF7) and cisplatin-resistant human breast cancer cell line (MCF7-R) and showed good cytotoxic activity towards MCF7-R. Compounds **2** and **3** were also able to cause topo II inhibition.

1. Introduction

Metal complexes have been used as antitumor agents since the discovery of the biological activity of cisplatin in the late-1960 s [1]. Despite the efforts to develop new metal-based anticancer agents, with the aim to overcome cisplatin resistance and side effects, only carboplatin and oxaliplatin have also received worldwide approval for clinical practice [2]. Since cisplatin and its analogous exhibit similar spectrum of activity, it is generally accepted that these drugs share the same mechanism of action. Their antitumor effect is the result of covalent adducts with DNA that induce cell apoptosis. Thus in order to develop more effective drugs it is necessary to design agents capable to produce damages in DNA by different binding strategies such as electrostatic binding, hydrophobic binding to the minor groove and intercalation [3].

The process by which a molecule interacts with DNA by insertion of its planar moiety between adjacent base pairs of the double helix is denoted intercalation [4], which was first demonstrated by Lerman through his studies into the binding of acridines to DNA [5]. Classical intercalators are organic compounds whose structures are characterized by the presence of a planar aromatic moiety, commonly comprised of fused rings, such as acridines, anthracyclines and phenanthridines [6]. Intercalation can produce deep alterations in the nucleotide secondary structure, such as lengthening, stiffening and unwinding of the DNA helix, with major consequences for DNA replication and transcription [7]. DNA intercalating molecules (e.g. doxorubicin and mitoxantrone) comprise a significant proportion of some of the most potent drugs currently available for the cancer chemotherapy [6]. Besides their ability to intercalate to DNA, these antineoplastic agents also target topoisomerase II and their cytotoxic activity may be mediated by the formation of an intermediate covalent DNA-topoisomerase II complex (termed cleavable complex), resulting in cell death via apoptosis [8].

Although first observed for organic molecules, DNA intercalation is also possible with metal complexes if they possess at least

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http://dx.doi.org/10.1016/j.ica.2016.02.053
0020-1693/

one planar aromatic ligand, such as 1,10-phenanthroline (phen), dipyrido[3,2-a:2′,3′-c]phenazine (dppz) or 2,2′,2″-terpyridine (terpy) [7,9–12]. The incorporation of transition metal centres into such structures can not only be used to impart cationic charge, but also to confer new properties on these molecules, such as magnetism, redox activity, luminescence properties [7,9–12]. Over the last few years, there has been a rapid expansion in the research area of square-planar d^8 metal complexes incorporating the flat "M(N,N)" moiety (N,N = 2,2′-bipyridine, 1,10-phenanthroline; M = Pd, Pt) due to its ability to intercalate DNA [13–15]. According to literature, not only modifications in the intercalator ligand, but also in the ancillary ligand, lead to changes in the binding modes, drug DNA association constants and cytotoxicity [16,17]. Recently, a study on complexes of the type $[Pt(N–N)(L–L)]^{2+}$ (N–N = phenanthroline derivatives, L–L = bidentate ancillary ligand) highlighted the influence of the ancillary ligand, particularly in terms of interactions with non-DNA receptors [18].

In this sense, the incorporation of thioureas as ancillary ligands in mixed compounds of the type $[M(phen)(tu^*)_2]^{+2}$ (M = Pd(II), Pt(II); tu = thioureas) has emerged as a promising strategy to design new anticancer metal compounds with intercalating properties. Thiourea type-ligands exchange more slowly and have been employed in order to avoid competitive covalent interactions towards biomolecules [19]. The efforts by Cusumano's group [20–22] on these compounds have provided important information about their solution behavior and non-covalent DNA interactions. In addition, Marverti et al. have also proposed a structure–activity relationship studies with platinum-intercalators of general formulae $[Pt(N–N)(tu^*)_2]^{2+}$ (N–N = phenanthroline derivatives, tu* = thioureas) and observed that changing the ligand environment can tune the cytotoxicity [23].

Despite the extensive work devoted to this class of compounds, studies on the profiles of DNA binding and cytotoxic properties of complexes of general formulae $[Pd(phen)(tu^*)_2]^{2+}$ (Fig. 1) are scarce in the literature. Inspired by the promising anticancer activity of metallointercalators and giving continuity to our research in coordination and biological chemistry of Pd(II) complexes [24–27], we present herein the biological studies on the compounds $[Pd(phen)(tu)_2]Cl_2·2H_2O$ (**1**), $[Pd(phen)(mtu)_2]Cl_2·3H_2O$ (**2**), and $[Pd(phen)(dmtu)_2]Cl_2·3H_2O$ (**3**), tu = thiourea; mtu = N-methylthiourea; dmtu = N,N′-dimethylthiourea. Here, the profiles of DNA binding, topoisomerase inhibition and cytotoxic properties of **1**–**3** have been presented.

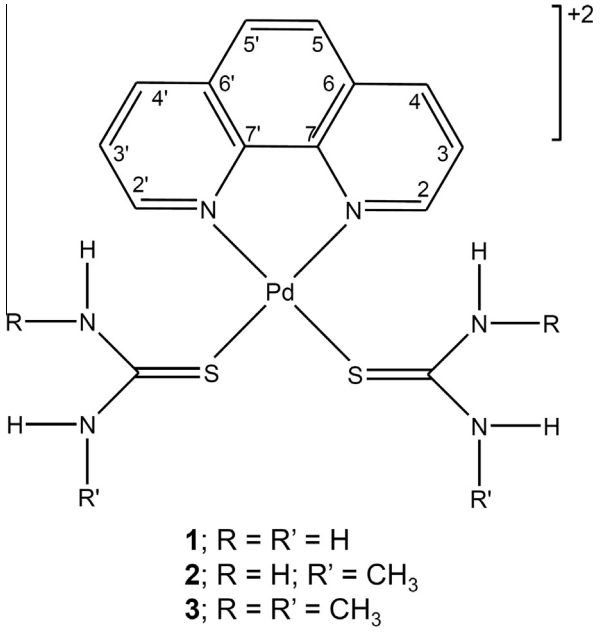

Fig. 1. General structure of the palladium complexes $[Pd(phen)(tu)_2]Cl_2·2H_2O$ (**1**), $[Pd(phen)(mtu)_2]Cl_2·3H_2O$ (**2**), and $[Pd(phen)(dmtu)_2]Cl_2·3H_2O$ (**3**).

2. Materials and methods

2.1. Materials and instrumentation

^1H and $^{13}C\{^1H\}$ NMR spectra were registered at 298 K, in CD_3OD solution, on a Varian model Inova 500 spectrometer operating at 500 and 126 MHz, respectively. Data are reported as chemical shifts (δ) in ppm. Residual solvent signals were used as internal references (^1H, ^{13}C). IR spectra were recorded on a Spectrum 200 from Perkin Elmer, in a range between 4000 and 400 cm^{-1} using KBr pellets. Elemental analyses were performed by the Central Analítica at IQ–University of São Paulo, Brazil. Conductivities were measured with a Digimed-DM-31 conductometer using 1×10^{-3} mol L^{-1} solutions in methanol. Electronic absorption spectra were recorded on a Perkin-Elmer LAMBDA 25 spectrophotometer in a 10 mm quartz cell. Fluorescence spectra were registered on a Varian Cary Eclipse fluorescence spectrophotometer. The sample was excited at 526 nm and its emission spectra were scanned in the range 550–700 nm.

2.2. Synthesis of the complexes

The starting complexes $[PdCl_2(CH_3CN)_2]$ and $[Pd(phen)Cl_2]$ were prepared according to literature procedures [28,29]. The complexes $[Pd(phen)(tu)_2]Cl_2$ (**1**) and $[Pd(phen)(mtu)_2]Cl_2$ (**2**) were prepared as previously described by Rotondo et al. [21].

2.2.1. $[Pd(phen)(tu)_2]Cl_2·2H_2O$ (*1*)

Dark orange solid. Elemental *Anal.* Calc. for $C_{14}H_{16}Cl_2N_6PdS_2·2H_2O$: C, 30.81; H, 3.69; N, 15.40. Found: C, 30.21; H, 3.73; N, 15.37%. ^1H NMR (500 MHz, CD_3OD): δ (ppm) 9.46 [dd, *J*(3, 9), 2H, H-2]; 8.96 [dd, *J*(3, 14), 2H, H-4]; 8.29 [s, 2H, H-5]; 8.16 [dd, *J*(9, 14), 2H, H-3]. ^{13}C NMR (125 MHz, CD_3OD): δ (ppm) 125.9 [C-3]; 127.8 [C-5]; 131.3 [C-6]; 140.6 [C-4]; 147.2 [C-7]; 150.0 [C-2]; 177.4 [C=S]. IR (KBr, cm^{-1}): 3269, 3102 (νNH); 1617 (δNH); 1514, 1424 (νCC_{ring}); 854, 784, 716 (γCH_{ring}); 671 (νCS). UV–Vis (in buffer 10 mM tris–HCl, 10 mM NaCl, pH 7.2): $\varepsilon_{268\ nm}$ = 22 278 M^{-1} cm^{-1}. Λ_M = 213 Ω^{-1} cm^2 mol^{-1} in methanol. Yield: 96%.

2.2.2. $[Pd(phen)(mtu)_2]Cl_2·3H_2O$ (*2*)

Red brown solid. Elemental *Anal.* Calc. for $C_{16}H_{20}Cl_2N_6PdS_2·3H_2O$: C, 32.47; H, 4.43; N, 14.20. Found: C, 32.47; H, 4.19; N, 14.48%. ^1H NMR (500 MHz, CD_3OD): δ (ppm) 9.45 [dd, *J*(2, 9), 2H, H-2]; 8.96 [dd, *J*(2, 14), 2H, H-4]; 8.29 [s, 2H, H-5]; 8.15 [dd, *J*(9, 14), 2H, H-2]; 2.92 [s, 6H, CH_3]. ^{13}C NMR (125 MHz, CD_3OD): δ (ppm) 29.6 [CH_3]; 125.9 [C-3]; 127.8 [C-5]; 131.3 [C-6]; 140.6 [C-4]; 147.2 [C-7]; 149.9 [C-2]. IR (KBr, cm^{-1}): 3280, 3144, 3071 (νNH); 1638 (δNH); 1577 (νCN); 1515, 1424 (νCC_{ring}); 850, 770, 716 (γCH_{ring}); 633 (νCS). UV–Vis (in buffer 10 mM tris–HCl, 10 mM NaCl, pH 7.2): $\varepsilon_{271\ nm}$ = 23 820 M^{-1} cm^{-1}. Λ_M = 197 Ω^{-1} cm^2 mol^{-1} in methanol. Yield: 85%.

2.2.3. $[Pd(phen)(dmtu)_2]Cl_2·3H_2O$ (*3*)

An aqueous suspension of $[Pd(phen)Cl_2]$ (0.2 mmol) in 40 mL of water (pH 6.5) was heated under reflux and then a solution containing N,N′-dimethylthiourea (0.4 mmol) in 5.0 mL of water was added, affording a red-brownish solution. The reaction mixture was stirred for 1 h under reflux. The obtained solution was filtered to eliminate any excess of $[Pd(phen)Cl_2]$. After solvent evaporation, at room temperature, a crystalline solid was obtained.