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Synthesis and *in vitro* antitumoral activity of novel *O*,*O*′-di-2-alkyl-(*S*,*S*)-ethylenediamine-*N*,*N*′-di-2-propanoate ligands and corresponding platinum(II/IV) complexes

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Abstract

Syntheses of two novel ligand precursors *O*,*O*′-diisopropyl- (**1a**) and *O*,*O*′-diisobutyl-(*S*,*S*)-ethylenediamine-*N*,*N*′-di-2-propanoate dihydrochloride monohydrate (**1b)** and the corresponding dichloroplatinum(II) (**2a** and **2b**) and tetrachloroplatinum(IV) complexes (**3a** and **3b**) are described here. The substances were characterized by IR, ^{1}H and ^{13}C spectroscopy and elemental analysis. Crystal structures were determined for **1a** and the corresponding platinum(IV) complex, **3a**. *In vitro* antiproliferative activity was determined against tumor cell lines: human adenocarcinoma HeLa, human myelogenous leukemia K562, human malignant melanoma Fem-x, rested and stimulated normal immunocompetent cells (human peripheral blood mononuclear PBMC cells) using KBR test (Kenacid Blue Dye binding test). The $IC_{50}(\mu M)$ values for the most active compound **3a** were: 30.48 ± 2.54; 12.26 ± 2.60; 13.68 ± 3.22; 80.18 ± 24.07 and 71.30 ± 21.70, respectively.

Keywords: Platinum(II) complexes; Platinum(IV) complexes; Antitumoral activity; Edda ligands

1. Introduction

The antitumor activity of cisplatin has been proved over many years in the treatment of various types of cancer [1–6]. Driven by the impressive impact of cisplatin on cancer chemotherapy, great efforts have been made to develop new derivatives with improved pharmacological properties, and cisplatin has become the prototype of a unique class of antineoplastic agents. However, its activity is limited by side effects including nephrotoxicity, emetogenesis and neurotoxicity [7,8]. There is a growing interest in platinum(IV) compounds because of their greater inertness in comparison with Pt(II) complexes. This property may allow oral administration of the drugs, and therefore reduce the toxicities associated with platinum-based chemotherapies [6].

Recently, synthesis and characterization of platinum(II) and platinum(IV) complexes with *N*,*N* bidentate R_2eddp (R_2eddp = *O*,*O*′-dialkyl-ethylenediamine-*N*,*N*′-di-3-propionate; R = *n*-Bu, *n*-Pe; Fig. 1) and halide ligands was reported [9]. Esters were used, as reported earlier, in the hope of

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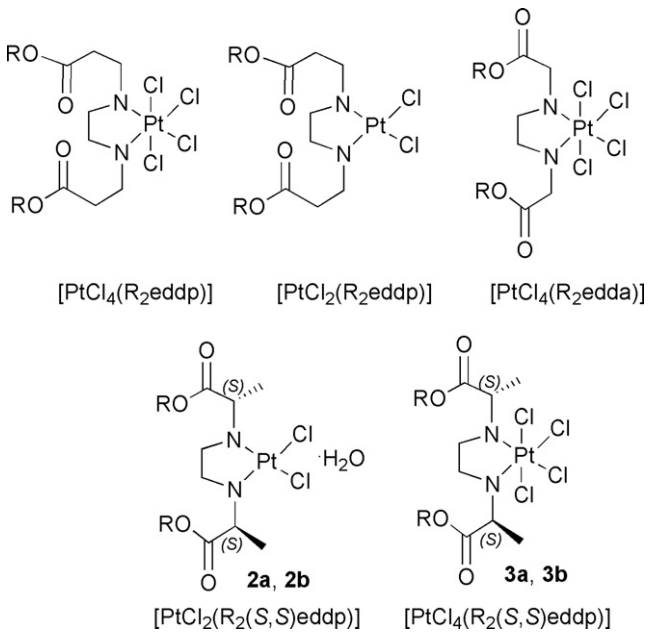

Fig. 1. Complexes of Pt(IV) and Pt(II) with R_2edda-type ligands.

increasing the cytotoxic activity of complexes with *ONNO* tetradentate ligands (*ONNO* = edda^{2-}, ethylenediamine-*N*,*N*′-diacetato; eddp^{2-}, ethylenediamine-*N*,*N*′-di-3-propionato) [10–12]. The *in vitro* cytotoxic evaluation of the investigated complexes in human adenocarcinoma HeLa cells, human myelogenuos leukemia K562 cells and normal immunocompetent cells (PBMC), showed that the cytotoxic action of Pt(IV) complexes with R_2eddp esters, [$PtCl_4(R_2eddp)$] (R = *n*-Bu, *n*-Pe; Fig. 1), is fairly comparable to that of cisplatin [12]. Also, a powerful *in vitro* antitumoral activity of these two compounds was shown on L929 fibrosarcoma and U251 astrocytoma tumor cells [13]. The rate of killing tumor cells with these platinum(IV) complexes was considerably faster in comparison with that of the classical platinum(II)-based drug cisplatin [14]. Furthermore, the complexes of ethylenediamine-*N*,*N*′-diacetato esters [$PtCl_4(R_2edda)$] (R = Me, Et, *n*-Pr, Fig. 1) were tested on human tumor cell lines 1411HP, H12.1 (both testicular germ cell tumors), DLD-1 (colon carcinoma), 518A2 (melanoma), A549 (lung carcinoma) and liposarcoma [15]. The complexes showed mild activity against the tested tumor cell lines.

To expand our investigations we synthesized and characterized new chiral branched-chain ester hydrochlorides (ligand precursors: **1a** and **1b**) and the corresponding platinum(II) (**2a** and **2b**) and platinum(IV) (**3a** and **3b**) complexes. All compounds were tested against HeLa, K562, Fem-x and normal PBMC with the aim of assessing their selectivity of their antitumor action.

2. Experimental

2.1. Material and methods

All reagents were of analytical grade. The ligand (*S*,*S*)-ethylenediamine-*N*,*N*′-di-2-propanoic acid hydrochloride, (*S*,*S*)-H_2eddp·HCl, was prepared as previously reported [16]. $K_2[PtCl_4]$ and $K_2[PtCl_6]$ were obtained by Merck and used without further purification.

Infrared spectra were recorded on a Perkin–Elmer FTIR 31725-X spectrophotometer using the KBr pellet technique (4000–400 cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 500 NMR spectrometer in DMSO-d_6. Elemental analyses for C, H and N were done on a Vario III CHNOS Elemental Analyzer, Elemental Analysensysteme GmbH.

2.2. Synthesis

2.2.1. Synthesis of ligand dihydrochlorides, *1a* and *1b*

These esters were prepared by using the esterification reaction previously described [17,18]. Thionyl chloride (4,0 ml, 55 mmol) was introduced into a flask containing 50 ml of ice cooled isopropyl or isobutyl alcohol (anhydrous conditions) during 1 h. After that 2.00 g (7.21 mmol) of (*S*,*S*)-ethylenediamine-*N*,*N*′-di-2-propanoic acid hydrochloride, (*S*,*S*)-H_2eddp·HCl, was added to the flask and the suspension was refluxed 16 hours. The mixture was filtered and the filtrate was left for a few days at 4 °C, yielding the white crystalline product. An additional quantity of the ester was extracted from the filter cake on washing with hot ethanol several times. (Numbering Scheme see Fig. 2).

1a: Yield 0.96 g, 37%. Anal. Calcd. for **1a**, $C_{14}H_{30}Cl_2N_2O_4$ (%): C, 44.33; H, 8.50; N, 7.38. Found (%): C, 44.40; H, 8.42; N, 7.56. ^{1}H NMR [500 MHz, DMSO-d_6]: δ 1.25 (d, $^3J_{H,H}$ = 4.15, 12H, C$^{6,7}H_3$), 1.47 (d, $^3J_{H,H}$ = 7.06, 6H, C$^4H_3$), 3.40 (m, 4H, C$^1H_2$), 4.16 (m, 2H, C^2H), 5.01 (m, 2H, C^5H), 10.0 (m, 4H, NH_2^+). ^{13}C NMR [125 MHz, DMSO-d_6]: δ 14.1 (C^4), 21.2 (C^{6,7}), 40.5 (C^1), 54.6 (C^2), 69.9 (C^5), 168.2 (C^3). IR [cm^{-1}]: ν 3448 (w), 2982 (m), 2732 (m), 2655 (m), 2598 (m), 2416 (m), 1734 (s), 1558 (m), 1241 (s), 1156 (m), 804 (w), 431 (w).

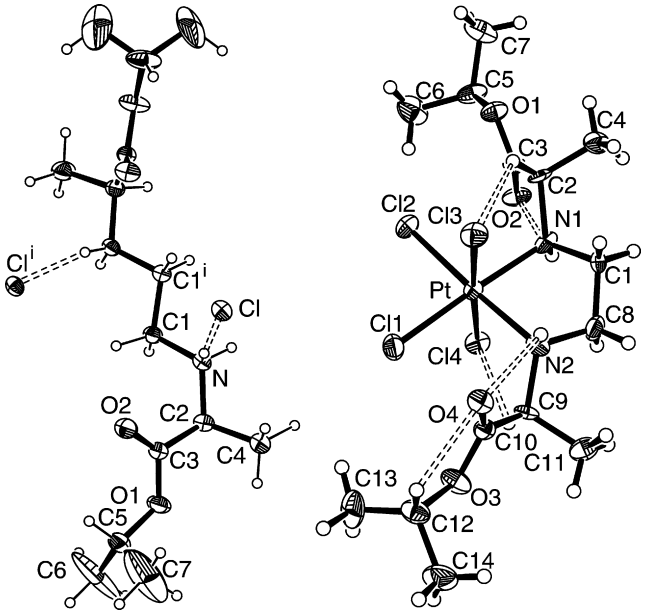

Fig. 2. ORTEP plot of **1a** (left) and **3a** (right).