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SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF AMINOCOUMARIN PLATINUM(II) COMPLEXES

George Kokotos,* Vassiliki Theodorou, Chryssa Tzougraki

Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistiniopolis, Athens 15771, Greece

Dieter L.D. Deforce, and Elfreide G. Van den Eeckhout

Laboratory for Pharmaceutical Biotechnology, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium

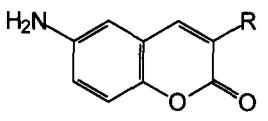
Abstract : A number of *cis*-dichloro[bis(aminocoumarin)]platinum(II) complexes have been synthesized and evaluated for their *in vitro* cytotoxicity against Caco-2T cells. The complex with 7-amino-4-trifluoromethylcoumarin as ligand has been found to be the most active (IC_{50} 10 μg/ml) in this study.

cis-Diaminedichloroplatinum(II) (cisplatin)[1] is one of the most effective anticancer agents, clinically used alone or in combination with other anticancer agents (e.g. doxorubicin, 5-fluorouracil etc), for the treatment of human solid tumors such as genito-urinary and gynecologic tumors as well as head, neck and lung tumors[2]. Since the clinical usefulness of cisplatin is limited by drawbacks as toxicity, low activity for certain tumors and development of acquired resistance, thousands of analogues have been prepared and screened in experimental tumor models. However, only a few of them appear to be promising. An efficient strategy, that may produce polyfunctional drugs with synergistic action, includes the use of bioactive molecules as platinum ligands.

Coumarin, a naturally occuring plant constituent, has been used in the trearment of cancer[3] and oedemas[4], while coumarin derivatives present interesting biological properties. 7-Hydroxycoumarin is a prodrug for coumarin[5] and has been investigated in clinical trials for its effectiveness in cancer treatment[6]. The antitumorinogenic properties of 6-aminocoumarin have been illustrated by a variety of *in vitro* and *in vivo* assays[7]. It is believed that 6-aminocoumarin acts through the competitive inhibition of poly(ADP-ribose) polymerase[7].

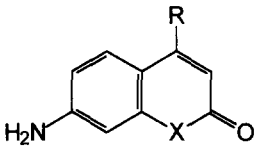
In this paper the synthesis of aminocoumarin platinum(II) complexes and their *in vitro* cytotoxic activity are described. The heterocyclic amines 6-aminocoumarin (**1a**), 3-acetamido-6-aminocoumarin (**1b**), 7-amino-4-methylcoumarin (**2a**), 7-amino-4-trifluoromethylcoumarin (**2b**) and 7-amino-4-methyl-quinolin-2-one (**2c**) have been used as ligands.

*fax : +301 7249101, e-mail: gkokotos@atlas.uoa.gr



1 a,b

1	R
a	H
b	$NHCOCH_3$



2 a-c

2	R	X
a	CH_3	O
b	CF_3	O
c	CH_3	NH

Chemistry Compounds **1a,b** were prepared by reduction of their corresponding nitrocoumarins by the $NaBH_4$ - Pd/C method[8]. Compounds **2a,b** were prepared as described in literature[9,10]. Compound **2c** was prepared by direct condensation of 1,3-phenylenediamine with ethyl acetoacetate, as previously described[11], modifying the isolation procedure.

The complexes *cis*-[Pt(aminocoumarin)$_2$Cl$_2$] **3a-e** were prepared by the following general method : A mixture of aminocoumarin (0.2 mmol) and K_2PtCl_4 (0.1 mmol) in water (10ml) containing 5-6 drops of 0.01N HCl was heated at 40 ^{0}C under stirring for 4-5 hours[12]. Precipitation of a yellow powder was occured and increased gradually as the reaction proceeded. The precipitate was filtered, washed with cold water, acetone and ether and dried over P_2O_5 under vacuum. Yield 60-70%.

3 a-e

3	Aminocoumarin
a	6-aminocoumarin
b	3-acetamido-6-aminocoumarin
c	7-amino-4-methylcoumarin
d	7-amino-4-trifluoromethylcoumarin
e	7-amino-4-methyl-quinolin-2-one

All platinum complexes were characterized by elemental analysis, IR and ^{1}H NMR spectroscopy[13]. Elemental analysis data clearly established that the ratio ligand to metal atom was 2:1. The binding site proposed for aminocoumarins and derivatives was the amino group at the 6- or 7- position. The amino group participation in binding with Pt (II) was confirmed by the examination of the νNH_2 and the δNH_2 frequencies in IR spectra, which were shifted to lower frequencies, due to Pt(II)-NH_2 coordination, as expected. The complexes also showed two medium intensity bands (310-330 cm^{-1}), which were assigned to the two ν(Pt-Cl) motions expected for a *cis* configuration[14]. In the NMR spectra of the complexes the aromatic protons near the binding site were shifted downfield by 0.5 ppm compared to the free ligand.

Cytotoxocity Assays The Caco-2T was derived from a Caco-2 culture transfected with an activated c-HA-ras oncogene[15]. The Caco-2 cell line was derived from a human colon cancer. The cells were cultured in the supplemented DMEM (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 0.05% (w/v) L-glutamine, 250 UI/ml penicillin, 100 μg/ml streptomycin and 10 μg/ml bovine insulin) and maintained in 25-cm^2 plastic culture flasks. Solutions of the complexes (1mg/ml in 15% DMSO, 85% supplemented DMEM) were further diluted by supplemented DMEM and DMSO (final DMSO concentration 3%) and were used immediately after their preparation.

Assay for Cell Viability : 100 μl of a cell suspension containing 500,000 cells/ml was transferred to 88 wells of a 96-well microtiter plate. The cells were incubated for 4 hours at 37 ^{0}C, 10% CO_2 (humidified incubator National Appliance Co, Portland, OR). Then 100μl of the solution of the compound tested were added (8 wells for each concentration). The plate was sealed with Micropore tape and further incubated for 4 days at 37 ^{0}C in the humified incubator gassed with air containing 10% CO_2. 100 μl medium was removed from each well, mixed with 100 μl of a solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium (MTT) (1mg/ml) in phosphate-buffered saline and incubated for 2 hours. After having removed most of the medium, 200 μl DMSO was added to the wells followed by incubation for 45 min. After solubilization of the formazan crystals, the content of the microtiter plates was homogenized with the multichannel pipettor before reading the optical densities (OD) at 490 nm in the Ceres UV 900 C plate reader (Bio-Tek Instruments).

Table 1. Cytostaticity of Aminocoumarin Platinum(II) Complexes Against Caco-2T Cells *in Vitro*[a]

Compound	IC_{80}(μg/ml)[b]	IC_{50}(μg/ml)[b]
3a	5 ± 2.5	25 ± 10
3b	100 ± 20	-
3c	30 ± 10	80 ± 20
3d	5 ± 2.5	10 ± 5
3e	30 ± 10	100 ± 20

[a]Tested by MTT assay. [b]Mean values of 8 experiments.

Results and Discussion The platinum (II) complexes **3a-e** prepared were tested for their cytotoxicity and cytostaticity against Caco-2T cells by MTT assay[16]. This assay is a cell-survival test which determines the mitochondrial cell activity after treatment of cells with varying doses of the components. It is based on the enzymatic reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium salt (MTT salt).

The IC_{80} and IC_{50} values exhibited by the complexes are summarized in Table 1. Complexes **3a** and **3d** were potent cytotoxic and cytostatic components (IC_{50} 25 μg/ml and 10 μg/ml respectively). Complexes **3c** and **3e** presented weak cytotoxic and cytostatic activity, while **3b** was inactive even at a concentration of 100 μg/ml.

Compound **3d**, where the amino group was at the 7- position, proved to be the most active compound in this study. Comparing the activity of compounds **3c**, **3d** and **3e** it was concluded that : a) there was no substantial difference when the oxygen atom of the coumarin ring was replaced by NH (conversion of the coumarin ring into 2-quinolinone ring), and b) the replacement of the methyl group by trifluoromethyl significantly increased the activity. It has to be noticed that the presence of the acetamido group at the 3-position of the coumarin ring abolished the cytotoxic activity.

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12. For the preparation of **3d** the mixture was heated at 70 ^{0}C for 7 hours.
13. For example: Compound **3c**: ^{1}H NMR (200 MHz, DMSO-d_6) δ ppm: 2.50 (s, 6H, 2xCH_3), 6.50 (m, 2H, 2x3-H), 7.23-7.85 (m, 6H, 2x8-H, 2x6-H, 2x5-H). Analysis for $C_{20}H_{18}Cl_2N_2O_4Pt$ (616.36): Calc. C 38.97, H 2.94, N 4.54; Found C 38.96, H 2.79, N 4.28.
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