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

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Abstract : A number of lipophilic platinum(II) complexes of the general structures *cis*-$[Pt(LA)_2Cl_2]$ and $[Pt(LD)Cl_2]$ were synthesised. Long chain amines (LA) and diamines (LD), prepared from lipidic amino acids, were used as ligands. The *in vitro* cytotoxicity of the complexes was evaluated against four cell lines (P388, NSCLC-N6, E39, M96). *cis*-Dichloro-bis(2-aminohexadecanol)platinum(II) was the most active against P388, NSCLC-N6 and E39 (IC_{50}: 11 μg/ml, 25 μg/ ml, 31 μg/ml), while dichloro(1,3-heptadecanediamine)platinum(II) presented the highest activity against M96 (IC_{50}: 13 μg/ml).

cis-Diamminedichloroplatinum(II) (cisplatin, *cis*-DDP) is an anticancer drug widely used to treat a variety of tumours, especially those of the testes, ovaries, head and neck.[1,2] However, the clinical usefulness of *cis*-DDP is limited by its severe side effects[3,4] (nephrotoxicity, nausea and vomiting, ototoxicity, neurotoxicity and myelosuppression), low activity for certain tumours and development of acquired resistance[5]. To overcome these drawbacks a great deal of effort has been focused on the preparation and evaluation of new complexes[2]. A designing strategy, that may produce polyfunctional drugs with synergistic action, includes the use of biomolecules or chemotherapeutic agents as platinum ligands. Based on this strategy we have recently prepared and studied the cytotoxic activity of *cis*-dichloro[bis(aminocoumarin)]platinum(II) complexes[6].

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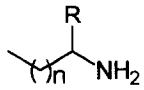
Sphingosine, a breakdown product of sphingolipids, as well as its analogues, have been found to inhibit protein kinase C, an enzyme that has been implicated in cell replication, tumour promotion, oncogenesis and signal transduction[7]. Structure-activity relationship studies showed that a long hydrophobic chain and a free amino group are the structural requirements for the inhibition of protein kinase C[8]. Long chain saturated amines (C12 to C18) and the unsaturated oleyl amine (C18:0) were found to inhibit the growth of *ras*-transformed NIH 3T3 cells (PAP2 cells)[9]. However, such a cell growth inhibition was attributed not only to protein kinase C inhibition but also to other factors[9].

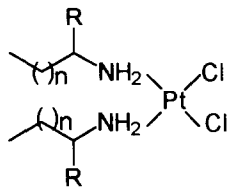
In this paper the synthesis and the *in vitro* cytotoxic activity of lipophilic platinum(II) complexes with long chain amines as ligands are described. The presence of the long chains confers lipophilicity to these complexes that may facilitate transport across the cellular membrane and increase intracellular drug accumulation thus improving drug effectiveness. It has been shown that hydrophobicity parameters for platinum complexes influence the activity[10], while there are indications that transportation may be associated to the problem of resistance to *cis*-DDP[11].



1-5,7,8

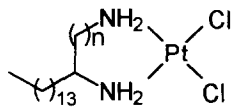
	1	**2**	**3**	**4**	**5**	**7**	**8**
n	12	14	16	13	13	13	13
R	H	H	H	$COOCH_3$	CH_2OH	CH_2NH_2	$CH_2CH_2NH_2$

The long chain amines (LA) tetradecylamine (**1**), hexadecylamine (**2**), octadecylamine (**3**), methyl 2-aminohexadecanoate (**4**) and 2-aminohexadecanol (**5**) were used as ligands for the complexes *cis*-$[Pt(LA)_2Cl_2]$ **6a-e**. The bidentate chelating amines (LD) 1,2-hexadecanediamine (**7**) and 1,3-heptadecanediamine (**8**) were used for the synthesis of chelates $[Pt(LD)Cl_2]$ **9a** (five-membered ring) and **9b** (six-membered ring) respectively.



6a-e

6	**a**	**b**	**c**	**d**	**e**
n	12	14	16	13	13
R	H	H	H	$COOCH_3$	CH_2OH



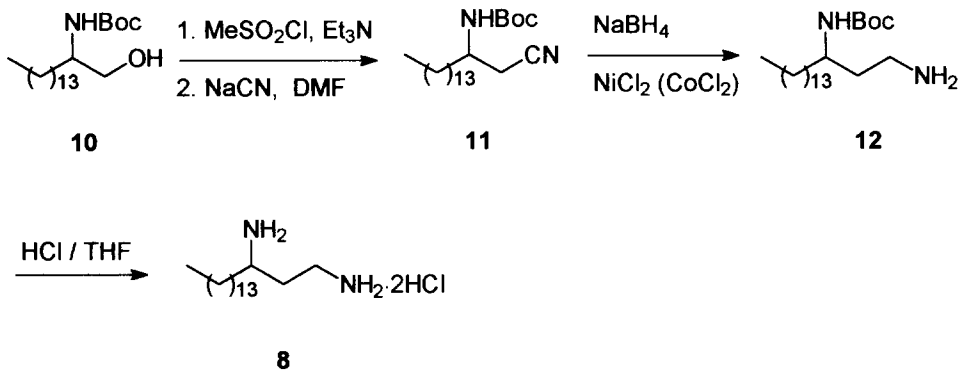
9a,b

9	**a**	**b**
n	1	2

Chemistry The lipidic α-amino acids are non-natural α-amino acids with long aliphatic side chains and together with their derivatives represent a class of biologically interesting compounds[12]. 2-Aminohexadecanoic acid, a representative example of lipidic amino acids, was chosen as starting material for the amines **4**, **5**, **7** and **8**. Methyl 2-aminohexadecanoate was prepared by esterification of the free acid[13] and the sphingosine analogue 2-aminohexadecanol by the reduction of 2-(*tert*-butoxycarbonylamino)hexadecanoic acid and subsequent deprotection[14].

2-(*tert*-Butoxycarbonylamino)hexadecanol (**10**) was used for the preparation of the diamines **7** and **8**. Compound **7** was prepared following reactions sequence described in literature[14]. Compound **8** was prepared as described in Scheme 1. The hydroxyl group of **10** was activated as the mesylate and the methanesufonate was converted directly into the nitrile **11**[15] by treatment with sodium cyanide in *N,N*-dimethylformamide at 60 °C. Selective reduction of **11** with sodium borohydride-transition metal system ($NiCl_2$ or $CoCl_2$) led to N^3-monoprotected diamine **12**. Free 1,3-heptadecanediamine **8**[16] was obtained by treatment of **12** with HCl in tetrahydrofuran.

Scheme 1



The complexes *cis*-$[Pt(LA)_2Cl_2]$ **6a-e** were prepared by the following general method: A mixture of lipidic amine (0.4 mmmol) and K_2PtCl_4 (0.2 mmol) in water (25 ml) containing 10 drops of 0.1 N HCl was stirred for 24-48 hours at room temperature until the colour of the precipitate became yellow[17]. The yellow precipitate was filtered, washed with ice-cold 0.1 N HCl (5 ml), water and dried over P_2O_5 under vacuum. Yield 90-95 %. For the synthesis of complexes $[Pt(LD)Cl_2]$ **9a,b** 0.2 mmol of diamine were used and acetone (5 ml) was added to the reaction mixture. The organic solvent was removed before the precipitation of the yellow product.

All platinum complexes were characterised by elemental analysis, IR and ^1H NMR spectroscopy[18]. Elemental analysis data clearly established that the ratio ligand to metal atom was 2:1 for complexes **6a-e** and 1:1 for complexes **9a,b**. 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 (ν_{NH} 3270-3100cm^{-1} and δ_{NH} 1590-1580cm^{-1}), due to Pt(II)-NH_2 coordination. 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[19]. In the ^{1}H NMR spectra of the complexes the protons attached to carbon atoms near the binding site appeared at different chemical shifts compared to the free ligand. Thus, the methylene protons (CH_2NH_2) of **6a-c** were shifted upfield by 0.2-1.0 ppm. The methylene protons of **9a,b** were also shifted upfield by 0.3-1.1 ppm, while their methine proton ($CHNH_2$) was shifted upfield by 0.3 ppm.

All complexes were higly soluble in chloroform and other organic solvents. The molar conductances of the complexes in *N,N*-dimethylformamide (DMF) solutions showed that they were non-electrolytes[20], as expected[21].

Cytotoxicity Assays Experiments were performed in 96-wells microtiter plates ($2x10^5$ cells/ml). Cell growth was estimated by a colorimetric assay based on the conversion of 3-(4,5-dimethyl-2-thiazolyl)2,5-diphenyl-tetrazolium (MTT) to a blue formazan product using live mitochondria[22]. Optical density at 570 nm, corresponding to the solubilized formazan, was read for each well on a Titertek Multiskan MKII. Eight determinations were performed for each concentration. Control growth was estimated by 16 determinations.

Results and Discussion The platinum(II) complexes **6a-e** and **9a,b** were tested for their cytotoxicity against four cell lines (leukemia P388[23], lung NSCLC-N6[24], renal E39, melanoma M96) by MTT assay[22]. This is a rapid colorimetric assay for cellular growth and survival, which determines the mitochondrial cell activity after treatment of cells with varying doses of the compounds tested.

Table 1. Cytotoxic Activity of Lipophilic Platinum Complexes

	IC_{50} (μg/ml)[a]			
Compound	P388	NSCLC-N6	E39	M96
6a	inactive	36	45	48
6b	58	42	89	92
6c	46	38	43	58
6d	46	36	42	39
6e	11	25	31	26
7	0.1	1.1	5.3	7.8
9a	39	35	48	16
9b	inactive	36	86	13

[a] Mean values of 8 experiments. SD < 12 % of the mean value.

The IC_{50} values exhibited by the complexes are summarised in Table 1. Compound **6e**, with 2-aminohexadecanol as ligand, proved to be the most active complex in this study against P388, NSCLC-N6 and E39 (IC_{50}: 11 μg/ml, 25 μg/ml, 31 μg/ml respectively). It was also the most active among the complexes **6a-e** against M96. Complex **6d**, with methyl 2-aminohexadecanoate as ligand, exhibited higher activity against all cell lines studied than complexes **6a-c** containing linear long chain amines as ligands. It seems that the presence of the substituent ($COOCH_3$ or CH_2OH) at the carbon atom bearing the amino group increases the cytotoxic activity of the complexes.

The chelates **9a** and **9b** were more potent than the other complexes in the case of M96 cell line (IC_{50}: 16 μg/ml and 13 μg/ml respectively). Complex **9a** presented similar activity with **9b** against M96 and NSCLC-N6 but significantly higher activity than **9b** against P388 and E39. Surprisingly, the most interesting results were obtained when the free ligand of **9a** was tested. As is shown in Table 1 the compound 1,2-hexadecanediamine (**7**) showed the greatest activity against all the cell lines tested. Based on these promising data, a project concerning the synthesis and study of lipidic diamine analogues is in progress.

References and Notes

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15. Compound **11**: m.p. 55-56 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.87 (t, 3H, *J*=7 Hz, CH_3), 1.20-1.40 (m, 24H, 12xCH_2), 1.44 [s, 9H, $C(CH_3)_3$], 1.59 (m, 2H, CH_2CHCH_2CN), 2.51 (dd, 1H, *J*=4 Hz, *J*=17 Hz, $CHHCN$), 2.75 (dd, 1H, *J*=5 Hz, *J*=17 Hz, $CHHCN$), 3,78 (m, 1H, α-CH), 4.60 (d, 1H, *J*=8 Hz, OCONH). FAB MS: m/e 367 ($M+H^+$, 10 %), 311 (100), 267 (25), 226 (34). Analysis for $C_{22}H_{42}N_2O_2$ (366.59): Calc C 72.08, H 11.55, N 7.64 %; Found C 71.91, H 11.51, N 7.32 %.

16. Compound **8**: ^{1}H NMR (200 MHz, CD_3OD) δ ppm: 0.84 (t, 3H, *J*=7 Hz, CH_3), 1.00-1.50 (m, 24H, 12xCH_2), 1.62 [m, 2H, $CH_2(CH_2)_{12}CH_3$], 2.00 (m, 2H, $CH_2CH_2NH_3^+$), 3.08 (m, 2H, $CH_2NH_3^+$), 3.35 (m, 1H, α-CH). FAB MS: m/e 271 ($M-2HCl+H^+$, 100 %), 254 (8), 226 (11). Analysis for $C_{17}H_{38}N_2$ 2HCl 0.5H_2O (352.43): Calc C 57.94, H 11.73, N 7.95 %; Found C 57.91, H 11.77, N 7.93 %.

17. The flask has to be kept under aluminium foil. Heating up to 40 °C accelerates the reaction. However, increase of temperature has to be avoided in the case of 2-aminohexadecanol.

18. For example: Compound **6b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.88 (m, 6H, 2xCH_3), 1.18-1.43 (m, 52H, 26xCH_2), 1.60 (b, 4H, 2xNH_2), 1.70-1.80 (m, 6H, 2x$CH_2CH_2NH_2$, 2x$CHHNH_2$), 2.78 (m, 2H, 2x$CHHNH_2$) Analysis for $C_{32}H_{70}N_2Cl_2Pt$ (748.91): Calc C 51.32, H 9.42, N 3.74 %; Found C 51.48, H 9.49, N 3.61 %. Compound **9a**: ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.88 (t, 3H, *J*=7 Hz, CH_3), 1.12-1.40 (m, 24H, 12xCH_2), 1.45-1.62 [m, 6H, $CH_2CHCH_2NH_2$, 2xNH_2), 2.10 (m, 1H, $CHHNH_2$), 2.58 (m, 1H, $CHHNH_2$), 3.00 (m,1H, α-CH). Analysis for $C_{16}H_{36}N_2Cl_2Pt$ H_2O (540.48): Calc C 35.56, H 7.09, N 5.18 %; Found C 35.82, H 6.70, N 5.08 %.

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20. Compound **1d** : Λ_M($ohm^{-1}cm^2mole^{-1}$) ~ 22 (DMF).

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