Sulfoxides as leaving groups. Effect of sterically hindered aliphatic sulfoxides on the antitumor activity of chloro(substituted sulfoxide)(1,1-diaminomethylcyclohexane)platinum(II) nitrate

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Abstract

The preparation and spectral properties of a series of complexes of general formula [PtCl(R′R″SO)(1,1-diaminomethylcyclohexane)]NO_3 (R′R″SO = substituted sulfoxide) is reported. The complexes were studied for cytotoxicity and antitumor efficacy where high activity was seen for n-propyl- and n-butylsulfoxide derivatives. The results confirm previous reports that the biological activity of this series is dependent on the nature of the sulfur ligand and extends the type of useful sulfoxides to sterically hindered aliphatic ligands.

Introduction

The series $[PtCl(R'R''SO)(diamine)]^+$ (diamine = bidentate amine such as 1,2-diaminocyclohexane (dach) or 1,1-diaminomethylcyclohexane (damch) and R′R″SO = substituted sulfoxide) represents a unique series of antitumor active cationic complexes [1]. The charge on the complex and the presence of the Pt–S bond defy the empirical structure–activity relationships originally set down for platinum complexes [2]. In our previous report on this series we used phenyl substitution either directly on the S atom or in an S–CH_2R (R = Ph) group to study the effect of lability and chirality of the sulfoxide group on the antitumor activity. The antitumor activity is clearly dependent on the leaving group ability of the sulfoxide and we wished to examine this point further for a wider range of sulfoxides. To facilitate the characterization of the compounds we used as diamine 1,1-diaminomethylcyclohexane (damch), thus avoiding the production of diastereomers from the combination of an optically active *trans*-1,2-diaminocyclohexane ligand and an asymmetric sulfoxide (R′ ≠ R″). This paper reports on the preparation, characterization and biological activity of $[PtCl(R'R''SO)(damch)]^+$ with sterically hindered sulfoxide ligands.

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Experimental

IR spectra were obtained as KBr discs on Nicolet FT6000 series and Perkin-Elmer 1430 spectrophotometers. NMR spectra were run on Bruker 250 and 270 MHz spectrometers. ^{195}Pt NMR spectra were run in either d_4-MeOH or D_2O with reference to a Na_2PtCl_6 solution in D_2O as external reference. ^{1}H chemical shifts of the complexes (spectra run in either d_4-MeOH or D_2O) are relative to TSS. Elemental analyses were by Robertson Laboratories, Madison, NJ.

The sulfoxides were commercially available and were used without further purification. The ligand 1,1-diaminomethylcyclohexane was prepared according to a procedure involving transformation of the diethyl ester of cyclohexane-1,1-dicarboxylic acid to the free acid followed by functionalization to the carboxamide and conversion to the diamine.

Cyclohexane-1,1-dicarboxylic acid diethyl ester [3]

Sodium ethyl malonate was prepared by dissolving sodium metal (40 g, 1.73 mol) in 1 l of ice-cold anhydrous ethyl alcohol and adding ethyl malonate (132.03 ml, 0.87 mol). The sodium ethyl malonate was added dropwise to 1,5-dibromopentane (200 g, 0.87 mol) in a flask surrounded by a water bath at 80 °C. The solution was heated under reflux for 16 h at which time it was neutral to litmus paper. The greater part of the alcohol was removed to give a small volume of solution. Water was added to the residue and a yellow oil separated. The aqueous solution was extracted with chloroform,

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and the extracts were combined with the oil, reduced in volume, dried with calcium chloride and distilled at 16 mm Hg of pressure. Between 135–145 °C, the main colorless fraction of the diethyl ester was collected, yield 30%, 60 g. ^{1}H NMR ($CDCl_3$, δ ppm): 4.19–4.16 (q, 4 H), 1.56–1.43 (br m, 6H), 1.24 (t, 6H) and 1.07 (br m, 4H).

Cyclohexane-1,1-dicarboxylic acid [4]

A solution of the diethyl ester of cyclohexane-1,1-dicarboxylic acid (60 g, 0.26 mol) in 140 ml of ethyl alcohol was heated under reflux for 16 h with potassium hydroxide (60 g, 1.07 mol) in 100 g of water. The reaction was evaporated to dryness and the residue triturated with ether to remove neutral impurities and acidified with dilute sulfuric acid (750 ml, 1 N). The cyclohexane-1,1-dicarboxylic acid was extracted with ether and the resulting solution filtered. The solution was evaporated to dryness and the diacid was recrystallized as small prisms from a benzene–ether solution, yield 96%, 43.19 g. Melting point: 178–180 °C. ^{1}H NMR (d_6-DMSO, δ ppm): 12.59 (br s, 2H), 1.81 (br s, 4H), 1.42 (br s, 4H).

Cyclohexane-1,1-dicarboxamide

A solution of cyclohexane-1,1-dicarboxylic acid (40 g, 0.24 mol) in 120 ml of thionyl chloride was heated under reflux with stirring for 4 h. Upon completion, approximately 2/3 of the thionyl chloride was distilled off. The residual solution was added dropwise to cold concentrated ammonium hydroxide under the hood! The resultant white precipitate was suction filtered, washed with water until neutral and dried in an oven at 37 °C overnight, yield 83%, 33.22 g. Melting point: 253–257 °C. ^{1}H NMR (d_6-DMSO, δ ppm): 6.99–6.91 (d, 4H), 1.81 (br s, 4H), 1.39 (br m, 6H).

1,1-Diaminomethylcyclohexane

Cyclohexane-1,1-dicarboxamide (13.94 g, 0.08 mol) was added in portions to a cooled (5 °C) slurry of 24.94 g of lithium aluminum hydride in 300 ml of freshly distilled *N*-ethyl-morpholine. The solution was allowed to warm to room temperature before being heated under reflux for 36 h at which time it was cooled (5 °C) and dropwise quenched with 20% potassium hydroxide in water while stirring. The quenched solution was then heated under reflux for 2 h, cooled, and the liquid filtered from the aluminum salts. The salts were again extracted with 300 ml of *N*-ethyl-morpholine under reflux for 2 h. The liquid was filtered from the salts and the combined fractions reduced in volume on an evaporator. The cloudy residue was taken up in diethyl ether, dried with sodium sulfate and concentrated under vacuum. The residue was distilled through a 15 cm Vigreux column at 3 mm Hg and the fraction boiling at 82–83 °C was collected. Yield 67%, 7.80 g, colorless dense oil. ^{1}H NMR ($CDCl_3$, δ ppm): 2.61 (s, 4H), 1.42 (s, 6 H), 1.28 (br s, 4 H), 1.15 (br, 4 H). Mass spectrum 125.2 $M^+ - NH_3$ and 143.2 $M^+ + 1$.

1,1-Diaminomethylcyclohexane dihydrochloride

The dihydrochloride was prepared by bubbling HCl gas into a benzene solution of the diamine. ^{13}C NMR (D_2O, δ ppm): 46.38, 37.48, 32.40, 27.37, 22.94 (lit. [5] 44.5, 35.9, 30.8, 25.8, 21.3). ^{1}H NMR (D_2O, δ ppm): 3.13 (s 4 H), 1.52 (br s, 10 H). ^{1}H NMR (d_6-DMSO, δ ppm): 3.34 (br s, 6 H), 2.5 (s, 4H), 1.40 (br, 10H).

Preparation of complexes

The starting material [$PtCl_2$(damch)] was prepared by the literature method [5]. The sulfoxide complexes [PtCl(R′R″SO)(damch)]$^+NO_3$ were also prepared by the previously published procedure [1]. The only modifications were that, in the case of the less reactive sulfoxides, stirring of the solution was continued until no traces of the yellow diamine were seen. Evaporation is best done without heating to avoid decomposition. All complexes gave elemental analyses consistent with their formulation, see 'Supplementary material'.

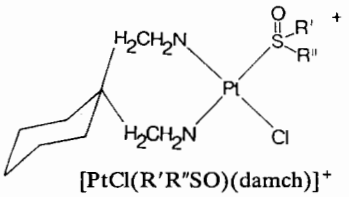
Biological assays

The *in vitro* and *in vivo* biological activities in L1210 cell lines were assessed using standard assays [6]. Briefly, for the *in vitro* activity, L1210 cells grown in RPMI-1640 medium supplemented with either 10% horse serum (L1210/0) or 10% fetal bovine serum (L1210/R or L1210/dach) were exposed to varying concentrations of complex dissolved in H_2O for 72 h. Final cell concentrations were measured using a Coulter particle counter and ID_{50} values (drug concentrations required to kill cell growth by 50%) were calculated for each complex. For *in vivo* studies BDF_1 mice were inoculated i.p. with 10^6 L1210/0 cells (day 0) and treated i.p. with the dose of test complex on days 1,5,9. Mean survival times of treated mice and control tumor-bearing mice were calculated and *%T/C* determined by *%T/C* = MST(treated) + MST(control). Long-term survivors (defined as alive on day 60) were not included in *%T/C* calculations. Studies with P388 leukemia were performed in a similar manner. B16 melanoma is routinely maintained in our laboratories by serial peritoneal passage in BDF_1 mice [7]. Animals received a tumor innoculum of 50000 cells injected subcutaneously between the scapulae on day 0, and drug treatment was initiated the following day by the intraperitoneal route. Animals were observed at least every other day, tumor growth was noted, and individual animals were sacrificed when a tumor exceeded 1.0 cm in diameter. Animals were observed for at least 30 days, and animals not demonstrating tumor growth during this period were

disregarded from the data analysis; in no case was more than one animal from a group of six disregarded on the basis of this criterion.

Results and discussion

The general structure is



[PtCl(R′R″SO)(damch)]$^+$

The complexes were prepared by the previously published procedure [1]. The damch ligand was prepared by a new procedure outlined in 'Experimental', which in our hands is easier than the published method [5]. The structure of the sulfoxides were chosen to systematically change the structures of the previously studied ligands. Thus, **I** (R′ = n-Bu, R″ = Ph) and **II** (R′ = n-Pr, R″ = Ph) are derivatives of MePhSO. To examine the influence of the Ph group two derivatives containing naphthyl groups were examined **III** (R′ = Et, R″ = 2-Np) and **IV** (R′ = Me, R″ = 1-CH_2Np). In **III** the naphthyl group is directly bound to the S while in **IV** the ligand may be considered an analog of the previously studied methylbenzylsulfoxide (MeBzSO). To examine steric effects in the absence of the phenyl group substituted aliphatic sulfoxides **V** (R′ = R″ = n-Pr), **VI** (R′ = R″ = n-Bu), **VII** (R′ = Me, R″ = sec-Bu) and **VIII** (R′ = Me, R″ = t-Bu) were studied.

Spectroscopic characterization data are collected in Table 1. The strongest peak in the 1100–1200 cm^{-1} region of the IR spectrum is assigned to ν(SO). No attempts were made to distinguish ν(Pt–S) from ν(Pt–Cl) [8]. All compounds show ^{195}Pt NMR chemical shifts in the −3250 to −3300 ppm region, confirming the PtN_2ClS coordination sphere. The ^{1}H NMR spectra are most informative with respect to the structures of the complexes. The $-NH_2CH_2$ protons of the damch ligands are inequivalent (*trans* S, *trans* Cl) and in favorable cases give rise to two distinct peaks in the 2.4–2.8 ppm region, see Table 1. As with Me_2SO, the S–CH_3 or S–CH_2R protons are shifted to low field by approximately 1 ppm upon complexation. For asymmetric sulfoxide ligands **I–III** the diastereotopic S–CH_2 protons of **I** (n-Bu), **II** (n-Pr) and **III** (Et) moieties give rise to two broad but distinct resonances. For the phenyl or naphthyl groups the low-field resonances also correspond to the protons *ortho* to the S–C bond (integration). The phenyl protons are in general shifted 0.5 ppm in comparison to the free ligand. In the case of **III**, molecular models show that the C3(H) proton undergoes a weak axial interaction with the Pt atom. This is reflected in the large downfield shift of this proton to 8.68 ppm [9]. In **IV**, the CH_2–protons are also diastereotopic and the doublet of doublets is shifted from 4.53 to 5.35 ppm upon complexation. The S–CH_2 protons of **V** and **VI** are inequivalent and give rise to a broad doublet. In **VII** the unique –CH– proton is low field to the S–CH_3 set (2.62 ppm) – the broad resonance slightly downfield to S–CH_3 upon complexation is therefore assigned to the unique CH proton. Complexation in fact results in dispersion of the absorptions due to the protons quite nicely and the two C–CH_3 sets are distinguishable as is the doublet due to the unique CH_2 proton. In **VIII**, molecular models show that the C–CH_3 protons of the tert-butyl groups are also inequivalent and give rise to a number of sharp singlets in the 0.8–1.5 ppm region. The lowest field signal (1.51 ppm) may also be due to a weak axial interaction with the Pt atom.

Biological activity

Data for the biological activity are collected in Tables 2–4. All complexes show activity in both cisplatin sensitive and resistant cell lines. The activity and resistance factor is dependent on the nature of the sulfoxide. The effect of steric hindrance is similar to that previously observed. If we consider **I–IV** we note that the presence of the aryl group directly bound to S exerts a greater influence than when separated by a –CH_2 group (**IV**, R″ = 1-CH_2Np). The variations in R′ and R″ however make no major difference in comparison to the values obtained for the Me(*p*-TolSO) ligand [1]. However, the water solubilities of **III** and **IV** are significantly reduced by the extra aromatic ring. The more interesting result is that sterically hindered aliphatic sulfoxides **V–VIII** also give a highly active set of complexes. For comparison the 'parent' [PtCl(Me_2SO)(damch)]$^+$ ususally gives an ID_{50} of 3–4 μM in this test.

The *in vivo* activity of selected complexes was then examined (Table 3). Despite the high cytotoxicity the *in vitro* complex **VIII** (R′ = Me, R″ = t-Bu) was inactive. High antitumor activity was however observed for the n-Pr and n-Bu derivatives **V** and **VI**. Again, the activity observed is significantly better than the parent Me_2SO derivative. The properties of the n-Bu complex were further examined in P388 leukemia and B16 melanoma (Table 4) where good activity was also observed. In one case (B16, 12.5×3 dose schedule) one long term survivor was observed. In all three cell lines the activity of [PtCl(n-Bu_2SO)(damch)]$^+$ is at least equivalent to cisplatin as control [1, 7, 10].

A plausible mechanism of action of this series is direct displacement of sulfoxide by DNA [11, 12].

TABLE 1. Spectral data for [PtCl(R'R''SO)(damch)]NO_3[a]

Complex	R'	R''	IR(cm^{-1})[b] ν (SO)	^1H NMR (δ, ppm)[c]		
				R'	R''	$-NH_2CH_2$[d]
I	n-Bu	Ph	1135	3.94, 3, 74(m, S–CH_2) 1.9, 2.05(m) 1.60(q) 0.93(t)	8.10 7.74	2.6
II	n-Pr	Ph	1140 1160	3.95, 3.75(m, S–CH_2) 1.9, 2.1(m), 1.16(t)	8.10 7.52	2.6
III	Et	2-Np	1121	4.01, 3.71(m, S–CH_2) 1.49	8.68 8.14 7.71	2.56 2.51
IV	Me	1-CH_2Np	1125	3.50(S–CH_3)	8.5(d), 8.05 7.9, 7.6 5.4(S–CH_2)	2.45(d) 2.52(d)
V	n-Pr	n-Pr	1130	3.45, 3.51 (S–CH_2) 2.1(m), 1.2(t)		2.6
VI	n-Bu	n-Bu	1120	3.60, 3.45(S–CH_2) 2.18, 2.05(m), 1.65(q) 0.95(t)		2.6
VII	Me	sec-Bu	1115	3.43(S–CH_3)	3.45(S–CH_2) 2.0(m), 1.45(m)	2.61
VIII	Me	t-Bu	1120	3.30(S–CH_3)	1.51 0.90 0.88	2.75 2.80

[a]Me = methyl, Et = ethyl, Bu = butyl, Pr = propyl, Ph = phenyl, Np = 2-Naphthyl. [b]IR as KBr discs. [c]Complexes **I**, **II**, **V**, **VI**, **VII** in D_2O; **III**, **IV** in d_4-MeOH; **VIII** in d_6-acetone. [d]The protons of the cyclohexane ring give rise to a multiplet at 1.1–1.4 ppm.

TABLE 2. Cytotoxicity in L1210 leukemias of [PtCl(R'R''SO)(damch)]$^+NO_3$

Complex	R'	R''	L1210/0 (ID_{50}, μM)	L1210/R (ID_{50}, μM)	Resistance factor[a]
I	n-Bu	Ph	0.81	4.54	5.60
II	n-Pr	Ph	0.45	1.74	3.87
III	Et	2-Np	0.23	5.79	25.17
IV	Me	1-CH_2Np	1.17	5.63	4.81
V[b]	n-Pr	n-Pr	0.67	2.60	3.88
VI[b]	n-Bu	n-Bu	0.40	2.26	5.90
VII[b]	Me	sec-Bu	1.19	2.61	2.19
VIII[b]	Me	tert-Bu	0.09	1.15	12.78
cis-DDP			0.13	10.60	81.54

[a]Resistance factor is ID_{50}(L1210/R)/L1210/0. [b]Averages of at least 3 experiments.

TABLE 3. Antitumor activity of [PtCl(R'R''SO)(damch)]NO_3 in L1210 Leukemia

Complex	Dose[a] (mg/kg)	Solvent[b]	%T/C
III	50×3	S	134
	25×3	S	131
IV	50×3	S	136
	25×3	S	127
V	25×3	S	185
	12.5×3	S	162
	6.25×3	S	112
VI	25×3	S	260
VII	100×3	S	138
	50×3	S	131
	25×3	S	123
[PtCl(Me_2SO)damch]$^+$	100×3	S	131[c]

[a]Compounds administered at days 1, 5, 9; see 'Experimental'. [b]S = saline. [c]See ref. 1.

$$[PtCl(R'R''SO)(diam)] \longrightarrow$$

$$[Pt(R'R''SO)(diam)DNA] \longrightarrow [Pt(diam)DNA]$$

The rate of displacement of R'R''SO by DNA bases is also dependent on the steric hindrance around the S atom; MePhSO is displaced much more readily than Me_2SO [12]. In comparison to the simple [$PtCl_2$(amine)$_2$], platinum–sulfur complexes may also affect properties such as tissue binding, metabolism through reaction with endogenous sulfur nucleophiles and nephrotoxicity. These factors will also be dependent on the nature of the sulfoxide ligand. This work confirms our previous observations that alteration of the sulfoxide ligand in [PtCl(R'R''SO)(diam)]$^+$ may give enhanced

TABLE 4. Antitumor activity of $[PtCl(n\text{-}Bu_2SO)(damch)]NO_3$ in P388 and B16 cells

Dose[c] (mg/kg)	Solvent[d]	%T/C
P388 Leukemia[a]		
25×3	H_2O	195
25×3	S	189
12.5×3	S	146
6.25×3	S	167
B16 Melanoma[b]		
25×3	S	188
12.5×3	S	136 (1/6)
6.25×3	S	154

[a]For comparison *cis*-DDP gives a %T/C of 184 at 5×3 schedule [7]. [b]For comparison *cis*-DDP gives a %T/C of 170 at 5×3 schedule [10]. [c]Compounds administered at days 1, 5, 9; see 'Experimental'. [d]S = saline.

antitumor activity relative to Me_2SO. The studies presented here expand the range of useful sterically hindered sulfoxides to simple aliphatic groups. In particular the $(n\text{-}Bu)_2SO$ and $(n\text{-}Pr)_2SO$ complexes are highly active. Thus a major factor in increasing antitumor activity is lability and the structure of the sulfoxide *per se* (e.g. presence of planar Ph group) is not a prerequisite. The results also suggest that an appropriate sulfoxide can be designed to maximize the differences between the Pt–S complexes and $[PtCl_2(amine)_2]$.

Supplementary material

Tables of elemental analyses (C, H, N) and HPLC purity for all new complexes of structure $[PtCl(R'R''SO)(damch)]NO_3$ are available from the authors on request.

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