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Chiral Platinum(II) Metallointercalators with Potent in vitro Cytotoxic Activity

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*Four platinum(II) metallointercalating complexes of 1,10-phenanthroline (phen) with the chiral ancillary ligands trans-*R,R- *and trans-*S,S-*1,2-diaminocyclohexane (*R,R- *and* S,S-*dach, respectively), and* N,N′-*dimethyl-*R,R- *and* N,N′-*dimethyl-*S,S-*1,2-diaminocyclohexane (*Me_2-R,R-*dach and *Me_2-S,S-*dach, respectively) have been synthesised and characterised. The crystal structure of [Pt(Me_2-S,S-dach)(phen)]$(ClO_4)_2 \cdot 1.5\,H_2O$ ($C_{20}H_{26}Cl_2N_4O_{9.5}Pt$) has been determined; orthorhombic, space group* $P2_12_12_1$*(No. 19),* a = *23.194(8),* b = *25.131(9),* c = *8.522(3) Å. In vitro cytotoxic assays (IC_{50}) in the human bladder cancer cell line 5637 and in the murine leukemia L1210 cell line revealed that [Pt(*S,S-dach)-(phen)]$(ClO_4)_2$ *(0.091 and 0.13* μM*, respectively) and [Pt(*R,R-dach)-(phen)]$(ClO_4)_2$ *(0.54 and 1.50* μM*, respectively) were more cytotoxic than cisplatin (0.31 and 0.50* μM*, respectively) and considerably more cytotoxic than their methylated counterparts, [Pt(Me_2-R,R-dach)(phen)]$(ClO_4)_2$ and [Pt(Me_2-S,S-dach)(phen)]$(ClO_4)_2$ (both >* 23 μM*). Chiral discrimination for [Pt(*S,S-dach)(phen)]$(ClO_4)_2$ *over its* R,R-*enantiomer was observed in all 13 cancer cell lines investigated. Moreover, [Pt(*S,S-dach)(phen)]$(ClO_4)_2$ *was more active than cisplatin in all cell lines tested and shows only partial cross-resistance to cisplatin in two cisplatin resistant cell lines.*

Introduction

Cisplatin, *cis*-diamminedichloroplatinum(II) (CDDP), is widely used for the treatment of ovarian and testicular cancers as well as a variety of other solid tumours.[1–3] Many cisplatin analogues with a range of biological properties have been synthesised, but cisplatin still remains the most effective treatment for these particular cancers.[4]

Unfortunately, cisplatin and its analogues can cause side effects such as nephrotoxicity, ototoxicity, and neurotoxicity, and acquired resistance of the drug will lead to a reduction in its overall effectiveness.[5–7] Contributing factors in the development of acquired resistance are increased drug efflux, drug deactivation, alterations in drug target, processing of drug-induced damage, and evasion of cell apoptosis.[5–7] These factors inspire the search for new drugs with different modes of DNA binding,[4] such as intercalation.[8]

The antitumour effect of cisplatin and its analogues is believed to result from their ability to bind coordinatively, to form adducts with DNA, and induce cell apoptosis.[1] For example, the bulky dach ligand of oxaliplatin, *trans-R,R*-1,2-diaminocyclohexaneoxalato platinum(II), fills much of the DNA major groove. The differences in the activity and the absence of cross-resistance between oxaliplatin and cisplatin are thought to arise from subtle differences in the overall DNA cross-links they form.[5–7]

Orally bioavailable Satraplatin (JM216; bis-acetatoammine-dichlorocyclohexylamine platinum(IV)) was developed in an attempt to circumvent tumour resistance and to improve the patients' quality of life. The DNA-binding of the solution products indicates that intrastrand cross-linked adducts similar to cisplatin, form between adjacent guanosine residues.[9]

Recently we published our investigations with a series of platinum(II) metallointercalators,[10] of the type $[Pt(en)(I_L)]Cl_2$ (en = ethylenediamine), containing methylated derivatives of the ligand phen (where I_L = phen, 4-methyl-1,10-phenanthroline, 5-methyl-1,10-phenanthroline, 4,7-dimethyl-1,10-phenanthroline, 5,6-dimethyl-1,10-phenanthroline or 3,4,7,8-tetramethyl-1,10-phenanthroline (3,4,7,8-Me_4-phen)) and determined that the position and number of methyl groups on the phen moiety greatly influenced biological activity and minor groove DNA binding affinity.[11]

Subsequently, we have also varied the ancillary ligand, as its configuration and chirality has been reported to influence the mutagenic and anticancer activity in platinum(II) complexes;[12] moreover, chiral diamminedichloroplatinum(II) complexes show noteworthy enantioselectivity.[13] Chiral platinum(II) complexes

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based on the type $[Pt(A_L)(I_L)]Cl_2$ (where A_L = *R,S*- or *S,R*-1,3-diamino-1,2,2-trimethylcyclopentane (*R,S*-tmcp and *S,R*-tmcp, respectively) and I_L = phen or 3,4,7,8-Me_4-phen) were prepared.[14] Some chiral discrimination was observed in the in vitro cytotoxicity experiments with the complexes having an *S,R*-configuration showing higher biological activity in L1210 cells.[13 14]

In this study we report the preparation of four ancillary dach-based chiral diamines, their incorporation into a metallointercalator and the observed cytotoxicities of the resultant platinum(II) complexes. The most potent complex, [Pt(*S,S*-dach)(phen)]$(ClO_4)_2$, was converted into a water-soluble chloride salt and tested in in vitro cytotoxicity assays.

Results and Discussion

Ancillary Ligand Syntheses

The diamines, *R,R*- and *S,S*-dach, were prepared in significant yields (~70%) using established literature methods.[15,16] Their enantiomeric purity was consistent with published data.[17]

The dimethylation of dach was achieved through reacting the respective dachH_2-tartrate salt with ethyl chloroformate to form a stable diester intermediate that was further cleaved by $LiAlH_4$ to form the desired *N,N'*-dimethylated product. An alternative method of amine methylation using methyl iodide[18] produces an inseparable mixture of mono- and *N,N'*-dimethylated products. The formation of the mono-methylated species was not attempted in the present study.

NMR characterisation of the methylated diamine confirmed the ligand to be a dimethylated species with a single methyl group on each of the two amine nitrogens. Equivalent enantiomeric purity was also observed for Me_2-*R,R*- and Me_2-*S,S*-dach having $[\alpha]_D^{20}$ values of relatively equal magnitude (within experimental error using a Jasco 710 spectrometer) but of opposite sign.[19]

Platinum(II) Complexes

The ^{1}H NMR spectrum of $[PtCl_2(phen)]$ was consistent with published data.[20] A $^{13}C\{^1H\}$ NMR spectrum of the intermediate could not be recorded because of its limited solubility. The CsI background matrix used for the FT-IR characterisation allowed the detection of the δ(N-Pt-N) (258 cm^{-1}) and ν(Pt-Cl) (352 cm^{-1}) vibrational bands present in $[PtCl_2(phen)]$.[21]

The $[Pt(A_L)(phen)]^{2+}$ complexes were obtained by reacting two equivalents of the desired dach-based ancillary ligand (A_L) with $[PtCl_2(phen)]$. This excess of the ancillary ligand was used to compensate for any loss of the diamine reagents because of their volatility. The final products were precipitated with $LiClO_4$ and isolated as microcrystalline powders in relatively high yields (that is, 70–90%). The *R,R*- and *S,S*-enantiomers of each chiral complex gave identical NMR spectra for all characterisation techniques, excluding circular dichroism spectroscopy (as expected).

Splitting of the methyl proton peaks observed in the ^{1}H NMR spectra and the two sets of cyclohexyl signals seen in the $^{13}C\{^1H\}$ NMR spectra of the $[Pt(Me_2\text{-dach})(phen)]^{2+}$ enantiomers implies that the H and C atoms of Me_2-dach have become stereochemically nonequivalent upon coordination to the Pt^{II} centre. NMR spectra of the metal complexes upon heating the sample to higher temperatures (up to 60 °C) indicates that the different enantiomers are not interchangeable once the ligand is bound to the platinum (the four methyl resonances remain). Thus, it is possible that the N-methyl groups present in these complexes are axially/axially, axially/equatorially, or equatorially/equatorially oriented, relative to the metal-ligand coordination plane. NMR spectra however, are uninformative. Although there are four methyl resonances, there is only one set of dach resonances and thus differences in the Pt-CH coupling cannot be compared. In an attempt to further examine the structural isomers ^{1}H NOESY experiments using mix times of between 100 and 800 ms were conducted. In all spectra there are no NOE cross-peaks between the methyl resonances indicating separation greater than 5 Å, which is consistent with structural isomers containing the methyl groups in axial/equatorial and equatorial/equatorial arrangements (all other expected intramolecular NOEs are observed at 100 millisecond mix times and higher). Similarly, the NH_2 resonances in $[D_6]$DMSO are equally uninformative. Only two amino resonances are observed, each giving cross-peaks to the methyl resonances in g-COSY and g-DQFCOSY spectra. In all spectra the four methyl resonances are similar or equal to each other in intensity, suggesting an even mixture of structural isomers from the synthesis. However, crystal data analysis has shown that at least one set of the methyl resonances can be accounted for by the methyl groups sitting in a *cis*-arrangement (Figure 1).

FT-IR characterisation of the platinum(II) intercalators showed that the ν(Pt-Cl) vibration (~350 cm^{-1}) was no longer observed due to the replacement of both chloride groups upon substitution with the ancillary ligand. Instead the vibrations characteristic of the dach-based ligand such as ν(C-H) of cyclohexyl methene groups (~2900 cm^{-1}) and ν(N-H) of amine groups (~3300 cm^{-1}) were present in the spectra of the intercalating complexes. In addition, a distinctive broad peak of very strong intensity (~1080 cm^{-1}) was observed and was assigned as a ν(Cl=O) vibration confirming the presence of the perchlorate counter-ion.

Crystallography

The absolute structure of $[Pt(Me_2\text{-}S,S\text{-dach})(phen)](ClO_4)_2 \cdot 1.5\,H_2O$ (Figure 1) was established with the Flack parameter[22–25] refining to 0.000(5). The crystallographic parameters of $[Pt(Me_2\text{-}S,S\text{-dach})(phen)]^{2+}$ are presented in Table 1; further information is available in the Supporting Information.

The complex $[Pt(Me_2\text{-}S,S\text{-dach})(phen)]^{2+}$ was found to have a distorted square-planar geometry around the central metal atom. The N-Pt-N bond angles, Pt-N bond lengths and N-C-C-N torsion angles observed in the complex molecules are summarised in Table 2. The N-Pt-N bond angles of the coordinated phen in the independent molecules **1** and **2** (81.08(14)° and 81.28(15)°, respectively) with those previously reported for the crystal structure of $[Pt(R,R\text{-dach})(phen)](PF_6)_2$[26] (that is, 81.6(2)°) and other analogous complexes[27,28] with bond

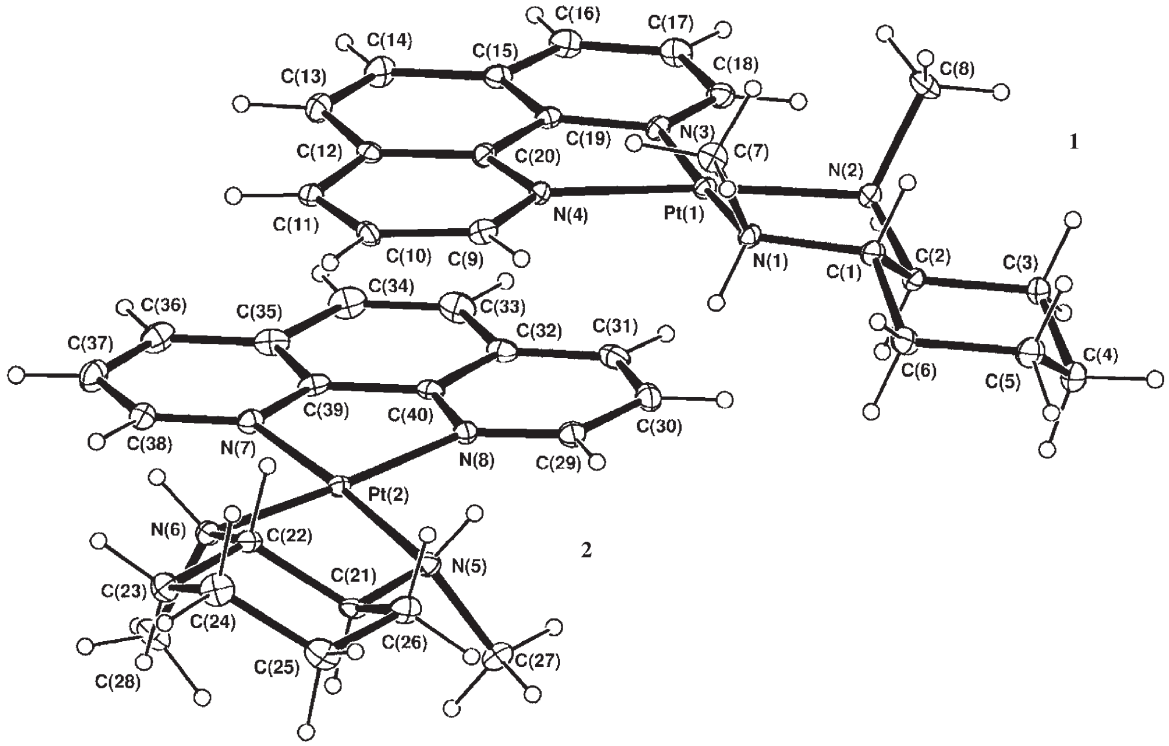


Figure 1. ORTEP representation of the two molecules (**1** and **2**) present in the crystal structure of $[Pt(Me_2\text{-}S,S\text{-dach})(phen)](ClO_4)_2\cdot 1.5\,H_2O$ (ellipsoids are shown at a probability of 20%).

Table 1. Structural parameters and crystallographic data for $[Pt(Me_2\text{-}S,S\text{-dach})(phen)](ClO_4)_2\cdot 1.5\,H_2O$.

Molecular Formula	$C_{20}H_{26}Cl_2N_4O_{9.50}Pt$
Molecular Weight	740.44
Crystal System	orthorhombic
Space Group	$P2_12_12_1$(No. 19)
A	23.194(8) Å
B	25.131(9) Å
C	8.522(3) Å
V	4967.0(3) Å^3
D_c	1.980 g cm^{-3}
Z	8
Crystal Size (mm)	0.338×0.324×0.020
Crystal Colour	colourless
Crystal Habit	plate
$\lambda(Mo_{K\alpha})$	0.71073 Å
$\mu(Mo_{K\alpha})$	5.925 mm^{-1}
$T(Guassian)_{min,\,max}$	0.155, 0.888
$2\theta_{max}$	56.60°
Hkl range	$-29 \le h \le 29$, $-33 \le k \le 33$, $-11 \le l \le 11$
N	45479
N_{ind}	11437(R_{merge} 0.0404)
N_{obs}	10029($I>2\sigma(I)$)
N_{var}	662
Residuals[a] *R*1(*F*),	0.0244,
*wR*2(F^2)	0.0525
GoF(all)	1.005
Residual Extrema	−0.539, 1.445 e$^-$ Å^{-3}

[a] $R1=\Sigma||F_o|-|F_c||/\Sigma|F_o|$ for $F_o>2\sigma(F_o)$; $wR2=(\Sigma w(F_o^2-F_c^2)^2/\Sigma(wF_c^2)^2)^{1/2}$ all reflections, $w=1/[\sigma^2(F_o^2)+(0.0000P)^2+0.0000P]$ where $P=(F_o^2+2F_c^2)/3$

Table 2. N-Pt-N bond angles and bond lengths, and N-C-C-N torsion angles of $[Pt(Me_2\text{-}S,S\text{-dach})(phen)](ClO_4)_2\cdot 1.5\,H_2O$.

N-Pt-N bond angles			
Atoms	Angle [°]	Atoms	Angle [°]
N(1)-Pt(1)-N(2)	83.59(15)	N(5)-Pt(2)-N(6)	83.56(15)
N(1)-Pt(1)-N(3)	175.51(14)	N(5)-Pt(2)-N(7)	175.00(14)
N(1)-Pt(1)-N(4)	98.09(14)	N(5)-Pt(2)-N(8)	99.06(15)
N(2)-Pt(1)-N(3)	97.64(15)	N(6)-Pt(2)-N(7)	96.23(15)
N(2)-Pt(1)-N(4)	174.63(13)	N(6)-Pt(2)-N(8)	177.07(15)
N(3)-Pt(1)-N(4)	81.08(14)	N(7)-Pt(2)-N(8)	81.28(15)
Pt-N bond lengths			
Atoms	Distance [Å]	Atoms	Distance [Å]
Pt(1)-N(1)	2.055(4)	Pt(2)-N(5)	2.053(4)
Pt(1)-N(2)	2.048(3)	Pt(2)-N(6)	2.036(4)
Pt(1)-N(3)	2.034(4)	Pt(2)-N(7)	2.029(4)
Pt(1)-N(4)	2.038(3)	Pt(2)-N(8)	2.037(3)
N-C-C-N torsion angles			
Atoms	Angle [°]	Atoms	Angle [°]
N(1)-C(1)-C(2)-N(2)	49.9(4)	N(3)-C(19)-C(20)-N(4)	2.6(5)
N(5)-C(21)-C(22)-N(6)	48.0(4)	N(7)-C(39)-C(40)-N(8)	1.2(5)

angles ranging between 80.3° and 81.7°. Likewise, the observed Pt-N(phen) bond lengths (average length 2.04 Å) are also consistent with those found in the analogous $[Pt(diamine)(phen)]^{2+}$ crystal structures; ranging between 2.01 Å and 2.05 Å.[26–28] A slight distortion in the planarity of phen

upon coordination was evident in the N-C-C-N torsion angles of molecules **1** and **2** which were observed as 2.6(5)° and 1.2(5)°, respectively.

In the structure of [Pt(*R*,*R*-dach)(phen)]$^{2+}$[26] and [Pt(Me$_2$-*S*,*S*-dach)(phen)]$^{2+}$ the dach ligand adopted the stable chair configuration upon coordination to the platinum(II) centre. As a result, the five-membered chelate rings of dach in both complex molecules are puckered with a δ-configuration and this is evident by the positive values of the N-C-C-N torsion angles[29] (Table 2) observed in molecules **1** and **2** (that is, 49.9(4)° and 48.0(4)°, respectively). Thus, the stereochemistry of the *trans*-1,2-diaminocyclohexane ligand is confirmed to be *S*,*S*. The methyl substituents on the amine nitrogens of dach are situated in an axial fashion (with respect to the chair) on the same side of the coordination plane. These methyl substituents make the N atoms chiral, thus the conformations of N(1) and N(5) are assigned as *R* and N(2) and N(6) as *S*.

The asymmetric unit contains two independent [Pt(Me$_2$-*S*,*S*-dach)(phen)]$^{2+}$ molecules. However, these two molecules are π-π stacked almost perpendicular to each other along the horizontal plane of each molecule (Figure 2a), with the methyl groups of each molecule facing away from each other (Figure 2b). The intermolecular spacing amid the two molecules of [Pt(Me$_2$-*S*,*S*-dach)(phen)]$^{2+}$ was estimated to be approximately 3.4 Å. This approximation was achieved by calculating the distance between the C15 (molecule **1**) and C32 (molecule **2**), and C18 (molecule **1**) and C31 (molecule **2**), that is, 3.440 Å and 3.301 Å, respectively (Figure 2b). An interplanar spacing of 3.66 Å (measured between the phen ligands) was previously observed for [Pt(*R*,*R*-dach)(phen)](PF$_6$)$_2$ where it was suggested that the spacing had been expanded to accommodate the larger PF_6^- counter-ion.[26]

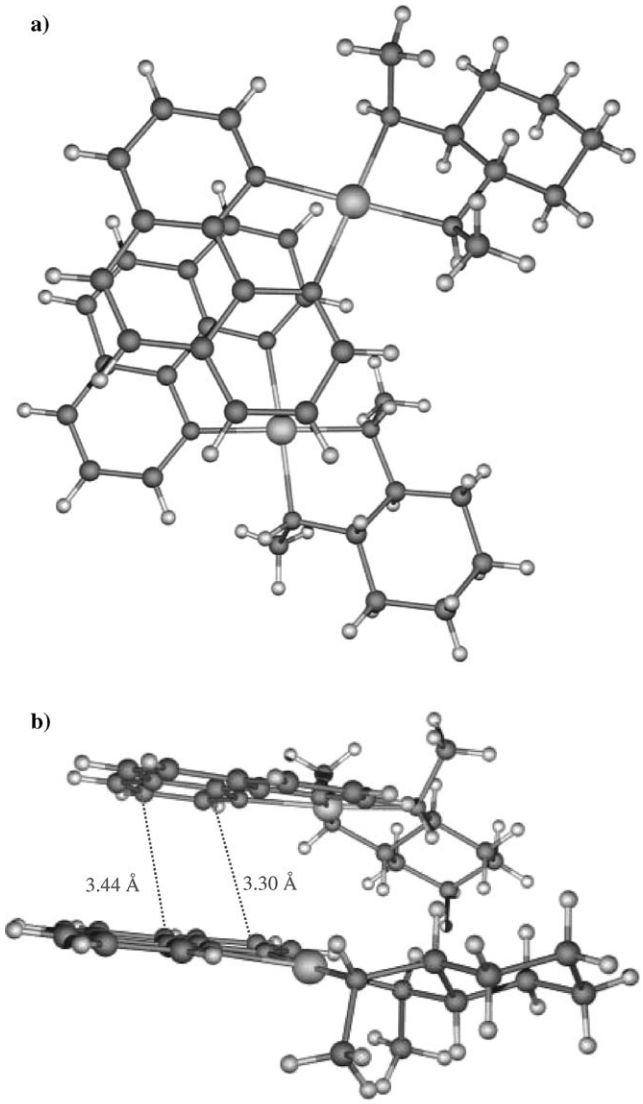


Figure 2. Characteristic π-π stacking of the two [Pt(Me$_2$-*S*,*S*-dach)(phen)]$^{2+}$ molecules within the unit cell: a) top view and b) side view.

Cytotoxicity and Structure–Activity Relationships

The results of the in vitro cytotoxicity assays of the Pt^{II} intercalators tested in the 13 tumour cell lines are summarised in Table 3. Of the four perchlorate complexes tested in the cell lines, [Pt(*R*,*R*-dach)(phen)]$^{2+}$ and [Pt(*S*,*S*-dach)(phen)]$^{2+}$ were observed to have similar or higher cytotoxicity compared to cisplatin. The cytotoxicity displayed by [Pt(*R*,*R*-dach)(phen)]$^{2+}$ and [Pt(*S*,*S*-dach)(phen)]$^{2+}$ was also markedly higher than that observed for the achiral analogue [Pt(en)(phen)]$^{2+}$, suggesting that replacement of the ancillary ligand en with a bulkier diamine such as dach (in the resolved forms) significantly lowered the IC_{50} values by a factor of approximately 150. However, increasing the bulkiness of the dach ancillary ligand by methylating the amine N atoms did not further enhance the level of cytotoxicity. Rather, this had a negative impact on cytotoxic effectiveness of the complexes as was observed by the significantly higher IC_{50} values of [Pt(Me$_2$-*R*,*R*-dach)(phen)]$^{2+}$ and [Pt(Me$_2$-*S*,*S*-dach)(phen)]$^{2+}$ (23.6 ± 8.95 and 55.6 ± 14.0 μM, respectively, in the 5637 cell line).

Similar results were observed for the activity of the perchlorate salt complexes in the murine leukaemia L1210 (cisplatin-sensitive) and L1210/CDDP (cisplatin-resistant) cell lines (Table 3). The complexes [Pt(*R*,*R*-dach)(phen)]$^{2+}$ and [Pt(*S*,*S*-dach)(phen)]$^{2+}$ had comparable (or higher) cytotoxicity compared to cisplatin, and up to 50 times more activity than [Pt(en)(phen)]$^{2+}$. Again, the methylated complexes [Pt(Me$_2$-*R*,*R*-dach)(phen)]$^{2+}$ and [Pt(Me$_2$-*S*,*S*-dach)(phen)]$^{2+}$ had low activity in the L1210 and L1210/CDDP cell lines, and would require concentrations greater than 40 μM to inhibit cell growth by 50%. It is important to note that the IC_{50} of [Pt(en)(phen)]$^{2+}$ in the L1210 cell line was initially reported as 2 μM; however, no positive control such as cisplatin was included in the study.[30] In a recent study, [Pt(en)(phen)]Cl$_2$·2H$_2$O was reported to have an IC_{50} of 9.7 ± 0.4 μM.[11]

To further compare the cytotoxicity of the Pt^{II} complexes, resistance factors (RF) were calculated by dividing the IC_{50} values for the L1210/CDDP cell line with those for the L1210 cell line (Table 3), where possible. The higher the RF value for a particular complex, the lower the cytotoxicity of that complex against L1210/CDDP compared with the L1210 cell line. Based on RF values, the activity of cisplatin in the L1210/CDDP cell line was

Table 3. Combined in vitro cytotoxicities (IC_{50}) of $[Pt(A_L)(phen)](ClO_4)_2$ for specified cell lines.[a]

	A_L $IC_{50} \pm SD$[b] [μM]					
Cell Line	*R*,*R*-dach	*S*,*S*-dach	Me_2-*R*,*R*-dach	Me_2-*S*,*S*-dach	en	CDDP[d]
5637	0.54 ± 0.44	0.091 ± 0.008	23.6 ± 8.95	55.6 ± 14.0	23.7 ± 1.30	0.31 ± 0.02
5637/CDDP		0.19 ± 0.07				1.15 ± 0.19
RF[c]		2.1				3.7
L1210	1.50 ± 0.14	0.13 ± 0.00	> 40	> 40	9.65 ± 0.49	0.50
L1210/CDDP	4.40 ± 0.14	0.28 ± 0.09	> 40	> 40	–	6.90
RF[c]	2.93	2.15				13.8
L1210[e]		0.19 ± 0.01[e]				0.50
L1210/CDDP		0.20 ± 0.04[e]				6.90
RF[c]		1.05				13.8
A-427	1.19 ± 0.85	0.11 ± 0.05	> 40	> 40	15.1 ± 10.6	3.95 ± 0.33
RT-112	0.51 ± 0.08	0.10 ± 0.01	25.4 ± 6.1	> 40	> 40	1.47 ± 0.39
RT-4	2.95 ± 0.79	0.88 ± 0.64		> 40	> 40	3.42 ± 1.64
KYSE-70	1.75 ± 0.44	0.14 ± 0.02	> 40	> 40	> 40	1.18 ± 0.37
SISO	0.34 ± 0.19	0.14 ± 0.04	28.5 ± 2.4	36.8 ± 8.8	9.47 ± 8.15	0.19 ± 0.02
MCF-7	0.25 ± 0.14	0.15 ± 0.08	14.5 ± 9.2	> 40	21.8 ± 8.2	1.43 ± 0.43
2008	3.20 ± 0.14	0.41 ± 0.06				0.60
C13*5	7.15 ± 2.90	0.72 ± 0.23				10.0
SKOV-7	3.45 ± 0.21	0.37 ± 0.08				8.00

[a] Values with standard deviations (SD) are averages of at least three independent determinations; values without SD are averages of two determinations. [b] Where provided. [c] RF (resistance factor) = IC_{50}(L1210/CDDP)/IC_{50}(L1210) or IC_{50}(5637/CDDP)/IC_{50}(5637). [d] Purchased from Aldrich and used without further purification. [e] $[Pt(S,S\text{-dach})(phen)]Cl_2 \cdot 1.5H_2O \cdot 0.5\,HCl$.

found to be 13.8 times lower than in L1210. Notably smaller RF values were calculated for $[Pt(R,R\text{-dach})(phen)](ClO_4)_2$ and $[Pt(S,S\text{-dach})(phen)](ClO_4)_2$ (2.93 and 2.15, respectively). The difference in the RF values for these two complexes suggest that the mechanism of resistance may have been overcome to a greater extent by the *S*,*S*-enantiomer. Similar cytotoxicity was displayed for $[Pt(S,S\text{-dach})(phen)]Cl_2 \cdot 1.5H_2O \cdot 0.5\,HCl$[31] in the L1210 and L1210/CDDP cell lines ($IC_{50} = 0.19 \pm 0.01$ and 0.20 ± 0.04 μM, respectively; RF = 1.05) compared with its perchlorate analogue.

The complexes $[Pt(R,R\text{-dach})(phen)]^{2+}$ and $[Pt(S,S\text{-dach})(phen)]^{2+}$ were further tested in 11 additional cell lines: A-427 (human lung cancer), RT-112, 5637, and RT-4 (human bladder cancer), 5637/CDDP (human bladder cancer with acquired resistance), KYSR-70 (human esophagus cancer), SISO (human cervical cancer), MCF-7 (human breast cancer), 2008 (human ovarian carcinoma), C13*5 (human ovarian carcinoma cell line with acquired resistance to cisplatin), and SKOV-3 (human ovarian carcinoma cell line with intrinsic resistance to cisplatin) (Table 3). For the A-427, KYSE-70, 2008, C13*5, and SKOV-7 cell lines, $[Pt(S,S\text{-dach})(phen)]^{2+}$ was approximately ten times more active than its *R*,*R*-enantiomer. Although exhibiting a slightly lower IC_{50} compared with cisplatin in 2008 cells, $[Pt(S,S\text{-dach})(phen)]^{2+}$ showed remarkable cytotoxicity in the cisplatin-resistant cell lines C13*5 and SKOV-3, by having greater than ten times the activity of cisplatin. Similarly, $[Pt(R,R\text{-dach})(phen)]^{2+}$ was also more active than cisplatin in the cisplatin-resistant cell lines. The improved activity displayed by $[Pt(R,R\text{-dach})(phen)]^{2+}$ and $[Pt(S,S\text{-dach})(phen)]^{2+}$ in the cisplatin-resistant cell lines (including L1210/CDDP and 5637/CDDP) are able to overcome cisplatin resistance mechanisms in some cancer cell lines.

In the 13 cell lines tested using the perchlorate salt complexes, $[Pt(S,S\text{-dach})(phen)]^{2+}$ had significantly higher cytotoxicity, up to ten times greater than its respective enantiomer, $[Pt(R,R\text{-dach})(phen)]^{2+}$. The complex $[Pt(S,S\text{-dach})(phen)]^{2+}$ displayed a higher degree of cytotoxicity in all cell lines compared with the observed activity of cisplatin, whereas $[Pt(R,R\text{-dach})(phen)]^{2+}$ had improved cytotoxicity compared with cisplatin in the cisplatin-resistant cell lines only (L1210/CDDP, C13*5, and SKOV-3). The methylated-dach complexes, $[Pt(Me_2\text{-}R,R\text{-dach})(phen)]^{2+}$ and $[Pt(Me_2\text{-}S,S\text{-dach})(phen)]^{2+}$, were observed to be largely inactive against the cancer cells. The IC_{50} values observed in the 5637 and MCF-7 cell lines suggested that the *R*,*R*-enantiomer was more active; however, nonspecific results in the L1210 and L1210/CDDP cell lines did not support this. It is likely that, upon intercalation, the methyl groups would be situated in close proximity to an adjacent DNA base pair. Unfavourable steric interactions between one (or both) of the methyl groups and the adjacent base pair may exist and inhibit adequate penetration of the intercalating moiety into the DNA, thus resulting in decreased cytotoxicity. Subsequent testing of these complexes in the L1210 and L1210/CDDP cell lines at concentrations greater than 40 μM would confirm if the *R*,*R*-enantiomer was more active; however, the activity displayed by these complexes thus far does not warrant their further investigation.

It is clear from the in vitro testing discussed above that the choice of the ancillary ligand and chirality plays a major role in affecting the biological activity of the complex against cancer cells. From these results it is possible to suggest a ranking of cytotoxicity for the ancillary ligands examined: that is, *S*,*S*-dach > *R*,*R*-dach ≫ en ≫ Me_2-*R*,*R*-dach = Me_2-*S*,*S*-dach.

Chiral discrimination has been reported for oxaliplatin where the *R*,*R*- form is more active than the S,S. The adducts that oxaliplatin forms with DNA adducts direct the dach ligand towards the major groove of DNA which is thought to prevent DNA transcription and replication.[32] The complexes reported here demonstrate opposite chiral discrimination where the *S*,*S*- form is more active than the *R*,*R*. These compounds intercalate from the minor groove and so position the dach in the minor groove of DNA. It may be that *S*,*S*-dach has the same effect in the DNA minor groove as the *R*,*R*-dach in the major groove, or it could be that the *S*,*S*-dach ligand acts by some other mechanism (for example, assists in drug transport).[33] Preliminary ^1H NMR binding studies with the oligonucleotide, $d(GTCGAC)_2$

have shown that these complexes all intercalate into double stranded DNA, with the ancillary ligand located in the DNA minor groove.[11] In all cases examined, intercalation increased the length of the DNA and induced other subtle changes in the secondary structure of the helix. Preliminary genome-wide mutational analysis in *Saccharomyces cerevisiae* have shown that both the Golgi apparatus and metal transporting proteins appear to play a role in the activity of these types of metal complexes, either as simultaneous targets with DNA, or in drug transport to the nucleus.[34] Preliminary platinum uptake studies show that these metal complexes are taken up into the cell readily, and thus cell surface binding can be excluded as a mechanism of action.

It was somewhat unexpected that the dicationic Pt^{II} complexes are such potent inhibitors of cancer cell growth because they lack a good leaving group(s) (for example, chloride). Cisplatin solvates with water to generate a electrophilic Pt^{II} species that coordinates to purine bases of DNA.[3] The new complexes, which are tetra-amines and stable to substitution reactions under biological conditions, are thought to act through intercalation of double stranded DNA,[11,14] although the exact mechanism of these drugs is not known. Thus, the different mechanism of action of the complexes presumably overcomes cisplatin resistance (Table 3).

Conclusions

Four square-planar metallointercalators of the type $[Pt(A_L)(phen)](ClO_4)_2 \cdot x H_2O$ were successfully synthesised by firstly preparing chiral dach-based ancillary ligands and coordinating them to the intermediate $[PtCl_2(phen)]$. The complexes were characterised using a variety of techniques to ensure their chemical and enantiomeric purity. An X-ray crystal structure of $[Pt(Me_2\text{-}S,S\text{-dach})(phen)](ClO_4)_2 \cdot 1.5\,H_2O$ was solved in the space group $P2_12_12_1$(No. 19), and the unit cell was found to contain two crystallographically independent complex molecules.

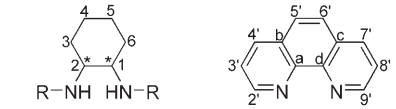
The therapeutic potential of the four metallointercalators was also examined using in vitro cytotoxicity experiments. The perchlorate salt complexes were tested in a number of cisplatin-sensitive and resistant cell lines to assess biological activity and to determine enantioselectivity and structure–activity relationships. In each cell line tested, $[Pt(S,S\text{-dach})(phen)]^{2+}$ was observed to be the most biologically active agent, exhibiting greater cytotoxicity compared with its enantiomer, $[Pt(R,R\text{-dach})(phen)]^{2+}$, and cisplatin. Substitution of methyl groups on the dach amine nitrogens dramatically decreased the observed cytotoxicity, compared to its nonmethylated analogue. From this it was evident that the ancillary ligand plays a significant role in affecting the biological activity of these complexes. The water-soluble complex $[Pt(S,S\text{-dach})(phen)]Cl_2 \cdot 1.5\,H_2O \cdot 0.5\,HCl$ was found to have cytotoxicity comparable or better to its perchlorate analogue.

The results of this study have led to the development of additional chiral platinum(II) metallointercalators which are currently being investigated for their biological activity in cancer cell lines and their affinity for binding to DNA.

Experimental Section

Materials: Reagent grade solvents and chemicals were used without purification unless otherwise noted. *Trans-R,R-* and *trans-S,S-*1,2-diaminocyclohexane (*R,R-* and *S,S-*dach) were synthesised using literature procedures.[15,16] IRA-400(Cl) Amberlite ion exchange resin (AR) used to convert perchlorate salt complexes to chloride salts was washed thoroughly before use with water, 1 M HCl, and finally water to remove any residual impurities. Reference compound $[Pt(en)(phen)]Cl_2 \cdot 2H_2O$ was donated by Mr C. Brodie (University of Western Sydney) and was prepared according to a literature method.[30]

NMR assignments: The numbering schemes used for the assignment of the H and C atoms present in the dach-based and phen ligands, and their respective Pt^{II} complexes are shown.



R = H, Me, or COOEt

N,N′-Bis(ethoxycarbonyl)-1*R*,2*R*- and *N,N′*-bis(ethoxycarbonyl)-1*S*,2*S*-diaminocyclohexane ($(EtOCO)_2$-*R,R*- and $(EtOCO)_2$-*S,S*-dach): The intermediates were synthesised using a procedure from previous works.[13,35]

$(EtOCO)_2$-*R,R*-dach: *R,R*-dachH_2(+)-tartrate (23.2 g, 0.09 mol); EtOCOCl (19.5 mL, 0.19 mol). Yield: 17.1 g, 73%; white flocculent solid; ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 1.25$ (m, 10H; C*H*$_3$, C*H*$_2$ (H4, H5)), 1.88 (m, 4H; C*H*$_2$ (H3, H6)), 3.35 (br s, 2H; C*H* (H1, H2)), 4.10 (q, ^3J(H,H) = 7 Hz; 4H; OC*H*$_2$), 4.95 ppm (br s, 2H; N*H*).

$(EtOCO)_2$-*S,S*-dach: *S,S*-dachH_2(−)-tartrate (12.5 g, 0.05 mol), EtOCOCl (10.5 mL, 0.10 mol). Yield: 11.0 g, 85%; white flocculent solid. The ^{1}H NMR spectrum was identical to that reported for $(EtOCO)_2$-*R,R*-dach.

N,N′-Dimethyl-(1*R*,2*R*)- and *N,N′*-dimethyl-(1*S*,2*S*)-diaminocyclohexane (Me_2-*R,R*- and Me_2-*S,S*-dach): The free diamines were prepared from the ester intermediates using literature methods.[13,34]

Me_2-*R,R*-dach: $(EtOCO)_2$-*R,R*-dach (12.9 g, 0.05 mol). Yield: 3.89 g, 55%; white crystalline solid; $[\alpha]_D^{20} = -119.8°$ ($c = 0.8$ in MeOH); ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 1.09$ (m, 4H; C*H*$_2$ (H4, H5)), 1.77 (m, 4H; C*H*$_2$ (H3, H6)), 2.10 (m, 4H; N*H*, C*H* (H1, H2)), 2.40 ppm (s, 6H; C*H*$_3$); ^{13}C{^{1}H} NMR (50 MHz, $CDCl_3$, 25 °C, TMS): $\delta =$ 25.2 (*C*H$_2$ (C4, C5), 31.1 (*C*H$_2$ (C3, C6), 33.9 (*C*H$_3$), 63.2 ppm (*C*H (C1, C2).

Me_2-*S,S*-dach: $(EtOCO)_2$-*S,S*-dach (10.0 g, 0.04 mol). Yield: 4.50 g, 79%; white crystalline solid; $[\alpha]_D^{20} = +128.6°$ ($c = 1.1$ in MeOH). NMR characterisations were identical to those reported for Me_2-*R,R*-dach.

Dichloro-1,10-phenanthrolineplatinum(II), $[PtCl_2(phen)]$ (platinum intermediate): The synthetic procedure used was from previously published works,[36,37] using the general procedure.[38] Yield: 0.41 g, 90%; ^{1}H NMR (200 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 8.19$ (dd, ^3J(H3′/H8′,H4′/H7′ or H2′/H9′) = 8 Hz, 2H; C*H* (H3′, H8′)), 8.29 (s, 2H; C*H* (H5′, H6′)), 9.05 (dd, ^3J(H4′/H7′,H3′/H8′) = 8 Hz, ^4J(H4′/H7′,H2′/H9′) = 1 Hz, 2H; C*H* (H4′, H7′)), 9.80 ppm (dd, ^3J(H2′/H9′,H3′/H8′) = 6 Hz, ^4J(H2′/H9′,H4′/H7′) = 1 Hz, 2H; C*H* (H2′, H9′)); IR (CsI): $\tilde{\nu} = 3078$, 1438, 848, 703, 352, 258 cm^{-1}; ESI–MS: *m/z* (%): 469 (60) $[Na^+ + M]^+$.

$[Pt(A_L)(phen)](ClO_4)_2 \cdot x H_2O$ (A_L = *R,R*-dach, *S,S*-dach, Me_2-*R,R*-dach, or Me_2-*S,S*-dach): $[PtCl_2(phen)]$ (~0.50 mmol) was suspended in H_2O (100 mL) and a solution of the diamine, A_L (~1.0 mmol), in

H_2O (10 mL) was added. The reaction mixture was refluxed for 24 h, or until the solution had turned clear. The clear yellow solution was then cooled to room temperature and filtered through a Sartorius Minisart micro-filter (0.45 μm). The volume of the solution was reduced to 50 mL under vacuum and the solution was refiltered through a micro-filter (0.45 μm). The complex was precipitated using a small amount of a saturated $LiClO_4$ solution.[39] The product was then collected by suction filtration through a small sintered-glass filter. The microcrystalline solid was washed with ice cold H_2O (2×5 mL), EtOH (5 mL), and Et_2O (5 mL), and air dried. The product was further dried overnight in vacuo over silica. The filtrate was filtered again through a micro-filter (0.45 μm) and put aside for slow crystal formation.

[Pt(*R*,*R*-dach)(phen)](ClO$_4$)$_2$: [$PtCl_2$(phen)] (0.22 g, 0.50 mmol); *R*,*R*-dach (0.12 g, 1.09 mol). Yield: 0.29 g, 85%; Colour: cream (microcrystalline solid); ^{1}H NMR (200 MHz, [D_6]DMSO, 25 °C, TMS): δ = 1.25 (m, 2H; CH_2 (H4 and/or H5)), 1.47 (m, 2H; CH_2 (H3 and/or H6)), 1.65 (m, 2H; CH_2 (H4 and/or H5)), 2.11 (m, 2H; CH_2 (H3 and/or H6)), 2.59 (m, 2H; C*H* (H1, H2)), 6.55 (m, 2H; NH_2), 7.15 (m, 2H; NH_2), 8.28 (dd, ^3J(H3′/H8′,H2′/H9′ or H4′/H7′) = 8 Hz) 2H; C*H* (H3′, H8′)), 8.37 (s, 2H; C*H* (H5′, H6′)), 9.15 (d, ^3J(H4′/H7′,H3′/H8′) = 8 Hz, 2H; C*H* (H4′, H7′)), 9.20 ppm (m, 2H; C*H* (H2′, H9′)); ^{13}C{^{1}H} NMR (50 MHz, [D_6]DMSO, 25 °C, TMS): δ = 24.2 (CH_2 (C4, C5)), 32.1 (CH_2 (C3, C6)), 60.9 (C*H* (C1, C2)), 125.3 (C*H* (C5′, C6′)), 126.5 (C*H* (C3′, C8′)), 129.7 (*C* (Cb, Cc)), 139.9 (C*H* (C4′, C7′)), 145.7 (*C* (Ca, Cd)), 150.5 ppm (C*H* (C2′, C9′)); IR (CsI): $\tilde{\nu}$ = 3060, 2948, 1440, 1082, 866, 716, 634, 258 cm^{-1}; CD (DMSO): λ_{max} ($\Delta\varepsilon$) = 322 (+0.52), 343 nm (−1.51 mol^{-1} m^3 cm^{-1}); ESI–MS: *m/z* (%): 245 (100) [*M*]$^{2+}$, 488 (12) [*M*−H]$^+$, 589 (27) [*M*+ClO_4^-]$^+$; elemental analysis calcd (%) for $C_{18}H_{22}N_4Cl_2O_8Pt$ (688.396): C 31.4, H 3.22, N 8.14; found: C 31.1, H 3.19, N 7.88.

[Pt(*S*,*S*-dach)(phen)](ClO$_4$)$_2$: [$PtCl_2$(phen)] (0.22 g, 0.49 mmol); *S*,*S*-dach (0.12 g, 1.09 mmol). Yield: 0.30 g, 90%; Colour: cream (microcrystalline solid); CD (DMSO): λ_{max} ($\Delta\varepsilon$) = 322 (−0.48), 343 nm (−1.39 mol^{-1} m^3 cm^{-1}); ESI–MS: *m/z* (%): 245 (100) [*M*]$^{2+}$, 488 (8) [*M*−H]$^+$, 589 (13) [*M*+ClO_4^-]$^+$; elemental analysis calcd (%) for $C_{18}H_{22}N_4Cl_2O_8Pt$ (688.396): C 31.4, H 3.22, N 8.14; found: C 31.1, H 3.20, N 7.93. All other characterisations were identical to those reported for [Pt(*R*,*R*-dach)(phen)](ClO$_4$)$_2$.

[Pt(Me$_2$-*R*,*R*-dach)(phen)](ClO$_4$)$_2$: [$PtCl_2$(phen)] (0.22 g, 0.50 mmol); Me$_2$-*R*,*R*-dach (0.14 g, 1.00 mmol). Yield: 0.26 g, 73%; Colour: yellow (microcrystalline solid); ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C, TMS): δ = 1.25 (m, 2H; CH_2 (H4 and/or H5)), 1.50 (m, 1H; CH_2 (H3 or H6)), 1.70 (m, 4H; CH_2 (H3 and/or H6, H4 and/or H5)), 1.95 (m, 1H; CH_2 (H3 or H6), 2.90 (s, 3H; CH_3), 3.05 (s, 3H; CH_3), 3.15 (br s, 2H; C*H* (H1, H2)), 7.30 (m, 1H; N*H*), 7.80 (m, 1H; N*H*), 8.30 (m, 2H; C*H* (H3′, H8′)), 8.40 (s, 2H; C*H* (H5′, H6′)), 9.15 (m, 2H; C*H* (H4′, H7′)), 9.30 ppm (q, ^3J(H2′/H9′,H3′/H8′) = 5 Hz, 2H; C*H* (H2′, H9′)); ^{13}C{^{1}H} NMR (100 MHz, [D6]DMSO, 25 °C, TMS): δ = 24.7 (CH_2 (C4 or C5)), 25.7 (CH_2 (C4 or C5)), 28.2 (CH_2 (C3 or C6)), 29.2 (CH_2 (C3 or C6)), 35.8 (CH_3), 43.7 (CH_3), 66.0 (CH_2 (C1 or C2)), 69.0 (CH (C1 or C2)), 126.1 (CH (C5′, C6′)), 127.8 (CH (C3′, C8′)), 130.0 (*C* (Cb, Cc)), 140.5 (*C*H (C4′, C7′)), 146.1 (*C* (Ca, Cd)), 151.1 ppm (*C*H (C2′, C9′)); IR (CsI): $\tilde{\nu}$ = 3197, 3053, 2900, 1455, 1437, 1093, 842, 711 cm^{-1}; CD (DMSO): λ_{max} ($\Delta\varepsilon$) = 324 (+1.05), 344 (−1.24), 382 nm (−0.08 mol^{-1} m^3 cm^{-1}); ESI–MS: *m/z* (%): 516 (100) [*M*-H]$^+$; elemental analysis calcd (%) for $C_{20}H_{26}N_4Cl_2O_8Pt{\cdot}1.5H_2O$ (743.472): C 32.3, H 3.89, N 7.58; found: C 32.1, H 3.79, N 7.38.

[Pt(Me$_2$-*S*,*S*-dach)(phen)](ClO$_4$)$_2$: [$PtCl_2$(phen)] (0.22 g, 0.50 mmol); Me$_2$-*S*,*S*-dach (0.15 g, 1.03 mmol). Yield: 0.26 g, 72%; Colour: yellow (microcrystalline solid); CD (DMSO): λ_{max} ($\Delta\varepsilon$) = 324 (−1.08), 344 (+1.28), 382 nm (+0.08 mol^{-1} m^3 cm^{-1}); elemental analysis calcd (%) for $C_{20}H_{26}N_4Cl_2O_8Pt{\cdot}1.5H_2O$ (743.472): C 32.3, H 3.89, N 7.58; found: C 31.7, H 3.79, N 7.38. All other characterisations were identical to those reported for [Pt(Me$_2$-*R*,*R*-dach)(phen)](ClO$_4$)$_2$.

Preparation of [Pt(*S*,*S*-dach)(phen)]Cl$_2$·1.5 H$_2$O·0.5 HCl: The platinum(II) complex [Pt(*S*,*S*-dach)(phen)](ClO$_4$)$_2$ was suspended in H_2O (50 mL) with IRA-400(Cl) Amberlite ion exchange resin (1 g). The mixture was stirred continuously at room temperature until the complex had fully dissolved. The solution was filtered by suction filtration through a sintered-glass filter, followed by filtration through a micro-filter (0.45 μm). The resultant solution was freeze-dried to produce a flocculent pale yellow powder. ^{1}H NMR (200 MHz, D_2O, 25 °C, [D_6]DMSO): δ = 1.33 (m, 2H; CH_2 (H4 and/or H5)), 1.56 (m, 2H; CH_2 (H3 and/or H6)), 1.76 (m, 2H; CH_2 (H4 and/or H5)), 2.31 (m, 2H, CH_2 (H3 and/or H6)), 2.82 (m, 2H; C*H* (H1, H2)), 8.08 (dd, ^3J(H3′/H8′,H2′/H9′ or H4′/H7′) = 6 Hz, 2H; C*H* (H3′, H8′)), 8.19 (s, 2H; C*H* (H5′, H6′)), 8.93 (d, ^3J(H3′/H8′,H4′/H7′) = 8 Hz, 2H; C*H* (H4′, H7′)), 8.99 ppm (d, ^3J(H2′/H9′,H3′/H8′) = 5 Hz, 2H; C*H* (H2′, H9′)); ^{13}C{^{1}H} NMR (50 MHz, D_2O, 25 °C, [D_6]DMSO): δ = 24.5 (CH_2 (C4, C5), 32.8 (CH_2 (C3, C6), 62.3 (C*H* (C1, C2), 126.9 (C*H* (C5′, C6′), 128.5 (C*H* (C3′, C8′), 131.3 (*C* (Cb, Cc), 141.4 (C*H* (C4′, C7′), 147.7 (*C* (Ca, Cd)), 151.5 ppm (C*H* (C2′, C9′)); IR (CsI): $\tilde{\nu}$ = 3320, 3047, 2926, 1520, 1438, 1034, 875, 719, 263 cm^{-1}; UV-Vis (H_2O): λ_{max} (ε) = 227 (38960), 277 (33907), 326 (2550, sh), 341 (1939), 358 nm (1598 M^{-1} cm^{-1}); CD (H_2O): λ_{max} ($\Delta\varepsilon$) = 309 (−1.06), 333 nm (+0.66 mol^{-1} m^3 cm^{-1}); ESI–MS: *m/z* (%): 245 (100) [*M*]$^{2+}$, 489 (30) [*M*−H]$^+$; elemental analysis calcd for $C_{18}H_{22}N_4Cl_2Pt{\cdot}1.5H_2O{\cdot}0.5HCl$ (605.649): C 35.7, H 4.24, N 9.25; found: C 35.5, H 4.26, N 9.08.[40]

Crystal growth and X-ray structure determination of [Pt(Me$_2$-*S*,*S*-dach)(phen)](ClO$_4$)$_2$·1.5 H$_2$O: The complex [Pt(Me$_2$-*S*,*S*-dach)(phen)](ClO$_4$)$_2$ was recrystallised from hot H_2O and the solution was set aside for slow evaporation. Over a period of six months, large clusters of suitable single pale yellow crystals formed. Single crystal X-ray diffraction data of a colourless plate-like crystal (0.34×0.32×0.02 mm) was collected on a Bruker AXS SMART 1000 CCD diffractometer equipped with graphite monochromated Mo-K_α (λ = 0.70173) radiation. The data integration and reduction were undertaken with SAINT and XPREP,[41] and subsequent computations were carried out using teXsan[42] and WinGX[43] graphical user interfaces. A Gaussian absorption correction was applied to the data,[41,44] as was a subsequent empirical correction determined with SADABS.[45,46] The structure was solved in the space group $P2_12_12_1$(No. 19) by direct methods with SIR97,[47] and extended and refined with SHELXL-97.[48] The asymmetric unit contains two crystallographically independent complex molecules (**1** and **2**), four perchlorate counter-ions, and three water molecules. The nonhydrogen atoms were modelled with anisotropic displacement parameters and a riding atom model was used for hydrogen atoms. An ORTEP[49,50] depiction of the two complex molecules is provided in Figure 1. Crystallographic data are summarised in Table 1. The CCDC reference number for this structure is 618961.

In vitro cytotoxicity studies: Solutions of the perchlorate complexes were prepared by suspending the samples in DMSO with thorough mixing for complete dissolution. The compounds were tested for in vitro cell growth inhibition by using a well established crystal violet microtiter assay.[51] The following cell lines were tested with this method: the human bladder carcinoma cell lines RT-112, RT-4, and 5637, as well as the cisplatin resistant 5637 subline (5637/CDDP); the human lung cancer A-427; the human oesophagus cancer line KYSE-70; the human cervical cancer line SISO; and the MCF-7 human breast cancer. Cells were exposed to test substance for 96 h at five concentrations (twofold serial dilution) within the range of the expected IC_{50} values. IC_{50} values are the average of 3–4 independent determinations. In vitro cytotoxicity studies were also conducted at the Andrew Durant Drug Testing Facility of the Peter MacCallum Cancer Institute in Melbourne. For

the murine leukaemia L1210 and L1210/CDDP (cisplatin-resistant) cell lines, compounds were tested using a standard cytotoxicity assay which utilises Coulter counting to obtain IC_{50} values.[52] The maximum complex concentration used for testing was 40 μM and cells were exposed to the complexes for 48 h. For the human ovarian carcinoma 2008, C13*5 (acquired cisplatin-resistant), and SKOV-3 (intrinsically cisplatin-resistant) cell lines, the complexes were tested using an SRB assay.[53] Cells were exposed to each complex for a period of 72 h. For each assay, IC_{50} values were calculated and averaged from the dose response curves for the compound tested. The clinical agent, cisplatin, was tested as a positive control with each cell line. Comparison testing of [Pt(en)(phen)]Cl_2·2 H_2O was conducted in the 5637 and L1210 cell lines. An aqueous solution of the chloride complex [Pt(*S*,*S*-dach)(phen)]Cl_2·1.5 H_2O·0.5 HCl was also examined in the L1210 and L1210/CDDP cell lines (as described previously).

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Keywords: chirality · cytotoxicity · diaminocyclohexane · metallointercalator · platinum

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