Dalton Transactions

Accepted Manuscript

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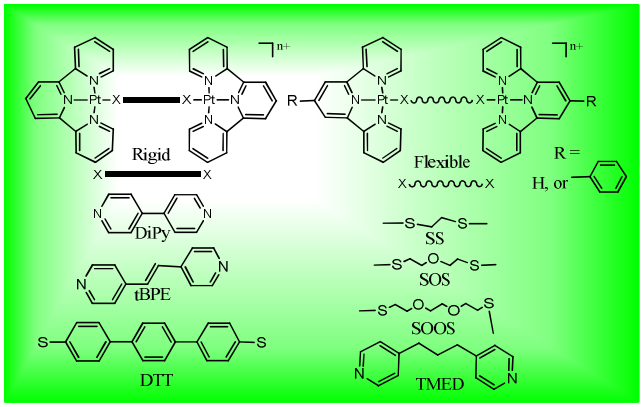

Cite this: DOI: 10.1039/x0xx00000x

The synthesis, characterisation and cytotoxicity of bisintercalating (2,2':6',2''-terpyridine)platinum(II) complexes

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Dinuclear (2,2':6',2''-terpyridine)platinum(II) (PtTerpy) complexes were synthesised by tethering either thiol or pyridine based linkers. All intermediates and resulting complexes were characterised using a combination of ^{1}H and ^{195}Pt NMR, two-dimensional ^{1}H correlation spectroscopy (NOSY/COSY), two-dimensional $^{1}H/^{195}Pt$ heteronuclear multiple bond correlation spectroscopy (HMQC), elemental analysis and electrospray ionisation mass spectrometry (ESI-MS). The cytotoxicity of the complexes were determined against human A2780 ovarian carcinoma cells and its cisplatin-resistant sub-line A2780cis, as well as L1210 murine leukemia cells.

Introduction

Chemotherapy remains an effective non-invasive treatment for many types of cancer. Cisplatin, the most well-known example of a platinum(II)-containing drug, has been the design template for many synthesis efforts to create new chemotherapeutics with increased efficacy. While a myriad of drugs have been identified in the past half century, only a few have gone on to be used for treatment.[1, 2] Many researchers have come to the conclusion that it is highly unlikely that structural analogues of cisplatin will produce clinically suitable alternates, instead, compounds with novel mechanisms of action need to be identified.[3, 4]

It has been hypothesised that development of platinum compounds dissimilar to cisplatin may form different Pt/DNA adducts, to provide a spectrum of clinical activity which is complementary to cisplatin.[5] To this end, different design strategies have been employed in an approach to improve effectiveness of platinum(II) compounds; these include the design of multinuclear platinum(II) complexes as well as complexes that have a different mode of biological interaction.[6] A structure that breaks the cisplatin paradigm by forming novel DNA adducts was BBR3464, a charged trinuclear compound that entered Phase II clinical trials almost a decade ago.[7, 8]

Many organic drugs, including anticancer drugs and antibiotics, are reported to inhibit DNA synthesis by intercalation;[3] platinum(II) complexes that coordinate coplanar aromatic ligands can also bind intercalatively to DNA.[3, 9-11] This was illustrated in X-ray fiber diffraction studies of DNA bound to the monointercalator 2-hydroxyethanethiolato(2,2':6',2''-terpyridine)platinum(II) [Pt(terpy)(HET)]$^{+}$.[11, 12] Monointercalating complexes, such as (2,2':6',2''-terpyridine)platinum(II) [Pt(terpy)]$^{2+}$, can be combined to form bisintercalating complexes whereby the remaining fourth coordinate position is used to produce a bridge linking the two platinum centres.[13, 14] Dinuclear [Pt(terpy)] complexes have been reported to be more cytotoxic than their mononuclear [Pt(terpy)] components.[15] This has been attributed to their increased charge and as a consequence, increased affinity for DNA.[15-17] In addition to increased DNA affinity, these dinuclear [Pt(terpy)] complexes may also demonstrate selectivity and specificity not available to the mononuclear [Pt(terpy)] components. The linker provides the opportunity to include elements that can exploit hydrogen bonding and/or van der Waals forces within the groove of DNA.[15, 17, 18] An increased selectivity and specificity, for differences in specific gene sequences between cancerous cells and normal cells, could be utilised for cancer treatment.[17, 19, 20]

Cytotoxicity studies of *bis*-acridine complexes, linked at the 9-amino position by various alkyl chains, demonstrated that linker length had a significant effect on cytotoxicity. Longer linkers were reported to be more cytotoxic in this case,[21-23] although, this trend has not been observed for all *bis*-intercalators. A small series of dinuclear [Pt(terpy)] complexes, linked by butane-1,4-dithiol, heptane-1,7-dithiol, octane-1,8-dithiol and decane-1,10-dithiol, were prepared, where the length of dithiol used did not appear to be a critical factor in the growth inhibition of L1210 cells.[14, 15] A previous study where the rigidity and length, of hydrophobic or hydrophilic linkers (Fig. 1) was investigated, with results determining that cytotoxicity was dependent on the length and charge density of the linker.[24-26] A correlation between shorter linker lengths and increased cytotoxicity was demonstrated, as well as increased cytotoxicity for linkers that produced complexes with higher charge densities.[24, 25] More recently, investigations on different flexible linkers (Fig. 1) have reported that the cytotoxicity of these dinuclear [Pt(terpy)] were equal to, if not better than, cisplatin in human ovarian cell lines.[26] The results in this instance showed that cytotoxicity improved with increasing linker length,[17, 18] although it is important to note that the flexibility of the chain may also play an important role.

In this study we aim to explore the contradictory nature of the results reported to date, by synthesising 16 dinuclear [Pt(terpy)] complexes as shown in Fig. 2 to compare the effect of varying chain length, flexibility, charge and stabilities on cytotoxicity.

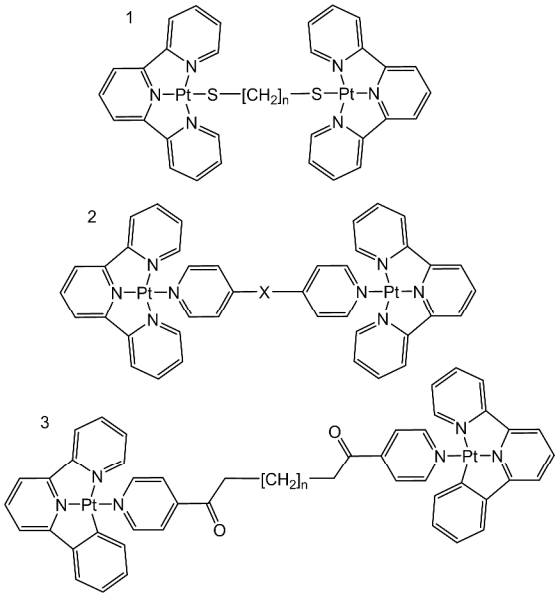

Fig. 1: Previously synthesised complexes: 1) McFadyen et al. where n = 4-10; 2) Lowe et al. where X = trans-CH=CH-, butadiyne, 1,4-diethynylbenzene, 4,4'-diethynylbiphenyl; and 3) Chan et al. where n = 3, 4 and 6.

Experimental Section

Materials

Reagents were used as received unless otherwise specified. All solvents used were of analytical grade or higher and purchased from Labserv, Merk or Mallinckrodt chemicals. Potassium tetrachloroplatinate (K_2PtCl_4) was purchased from Precious Metals Online. 1,5-cyclooctadiene, 2,2':6',2''-terpyridine, cellite, potassium hexafluorophosphate, 3-mercaptopropionic acid (MPA), 1,2-ethanedithiol, 2-mercaptoethyl ether, sodium perchlorate, 3,6-dioxa-1,8-octanedithiol, 1,2-diaminoethane, 1,4-diaminobutane, 1,6-diaminohexane, 1,12-diaminododecane, 1,4-diaminobenzene, 4,4′-diaminodiphenylmethane, 4,4'-dipyridine, trans-1,2-bis(4-pyridyl)-ethylene, 4,4'-trimethylenedipyridine, *p*-terphenyldithiol, 4-mercaptobenzoiz acid, tetrabutylammonium bromide, silver trifluoromethylsulfonate, *N,N′*-dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt), *N,N,N′,N′*-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), 3,6-diaminoacridine, sulforhodamine B (SRB), trichloroacetic (TCA), tris(hydroxymethyl)aminomethane and 4-bromopyridine.HCl were all purchased from Sigma Aldrich. Silver nitrate, sodium hydroxide, tetrabutylammonium hydroxide (0.8 M in methanol) and DIEA were purchased from Fluka. Deuterated solvents d_6-dimethylsulphoxide (99.9%) anddeuterium oxide (99.9 were purchased from Cambridge Isotope Laboratories. Acetone (≥ 99.5%) was purchased from Biolab. Ultra-pure water was obtained using a Sartorius Arium 611 DI purification system. [Pt(terpy)(MPA)]PF_6 and [Pt(terpy)(2-mercapoethylamine)]ClO_4 were prepared as previously reported.[27, 28]

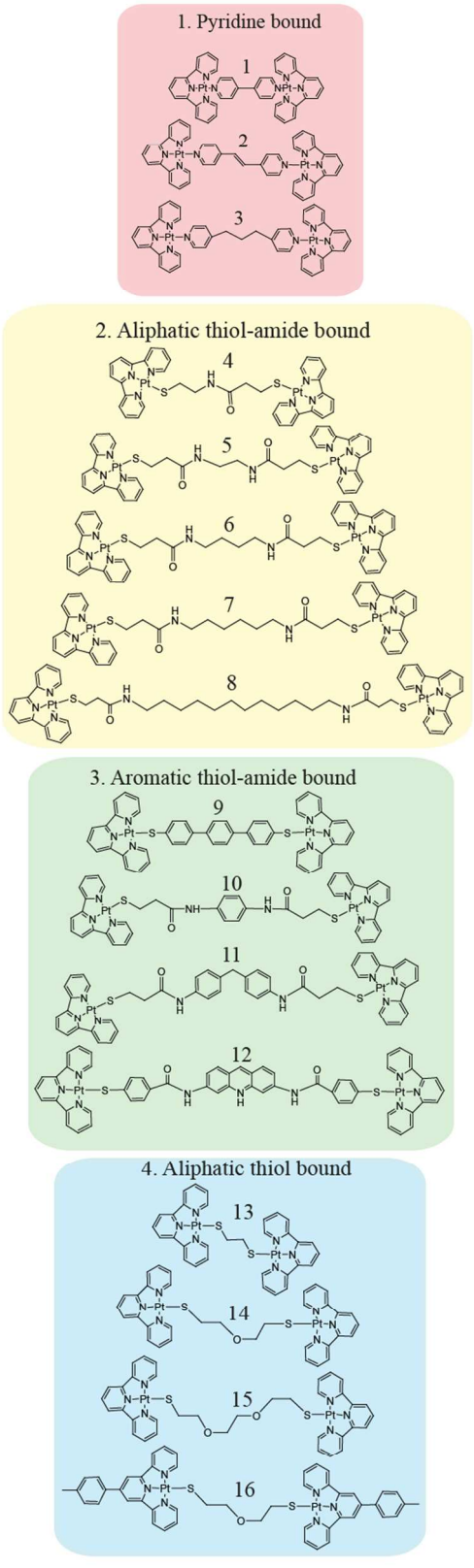

Fig.2: The chemical structures of synthesised dinuclear [Pt(terpy)] complexes. The four linker types of various length: (group 1) pyridine bound; (group 2) aliphatic thiol-amide bound; (group 3) aromatic thiol bound; and (group 4) aliphatic thiol bound complexes.

Instrumentation

^{1}H and ^{195}Pt NMR experiments were performed on a Bruker Avance 400 MHz NMR. A combination of one and two-dimensional NMR spectroscopy was utilized for structural elucidation. Two-dimensional ^{1}H spectroscopy included Nuclear Overhauser effect spectrocopy (NOESY, to observe cross correlations between nuclei that are spatially close), Rotating frame Overhauser effect spectrocopy (ROESY, to observe cross correlations between nuclei taking into account rotational correlation time typically in a range too weak to be observed by NOESY) and correlation spectroscopy (COSY, to observe cross correlations between nuclei that are coupled by closely bound nuclei). Two dimensional ^{1}H-^{195}Pt heteronuclear Multiple Quantum Coherence spectroscopy (HMQC) was used for identification of the protons closest to the platinum. All compounds were dissolved in one of the following: D_2O or d_6-DMSO in a total volume of 600 µL and placed in 5 mm OD NMR tubes. The temperature in the probe was maintained at 25 °C for D_2O or 35 °C for d_6-DMSO. Spectra are referenced to the residual deuterated solvent peak. ^{195}Pt NMR experiments were externally referenced to K_2PtCl_4. The following abbreviations apply to spin multiplicity: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass Spectra were obtained with a Quattro Micro mass spectrometer at the University of Wollongong NSW, Australia. Loop injection was performed using a concentration of 0.1 µM in a 1:1 mixture of H_2O/MeCN at a rate of 10 µL/min. The spectra were scanned in the range of 200-1800 *m/z* using electron spray ionisation with a varying voltage. $N_{2(g)}$ sheath pressure was maintained at 60 psi and capillary temperature was maintained at 200 °C.

Synthesis

The general procedure for preparing pyridine bound dinuclear terpyridineplatinum(II) complexes [{Pt(terpy)}$_2$X], where X = 4,4'-dipyridine, *trans*-1,2-bis(4-pyridyl)-ethylene or 4,4'-trimethylenedipyridine.

[Pt(terpy)(MeCN)](OTf)$_2$ was dissolved in MeCN and separately the desired bispyridine linker was dissolved in acetone and stirred for 5 min. The two solutions were combined and stirred for 10 min under a slightly positive nitrogen atmosphere. The product was precipitated from the solution by the addition of ether:acetone 3:1.

[{Pt(terpy)}$_2$ (4,4'-dipyridine)](OTf)$_4$, (1)

Yield: 0.547 g, 86%. Anal. Calc. for $C_{44}H_{30}F_{12}N_8O_{12}Pt_2S_4$: C: 32.84; H: 1.88; N: 6.96%. Found %: C: 32.42; H: 2.06; N: 6.96%. ESI-MS calc for [Pt(terpy)$^+$ + OTf] *m/z* 577.4, found *m/z* 577.1; calc for [M^{3+} + OTf] *m/z* 387.3, found *m/z* 387.4; calc for [M^{2+} - Pt(terpy)] *m/z* 292.3, found *m/z* 292.6. ^{1}H NMR 400 MHz (D_2O): δ 9.54 (d, 4H, *J* = 6.05 Hz); δ 8.67 (t, 2H, *J* = 7.51); δ 8.56 (m, 16H); δ 8.01 (d, 4H, *J* = 5.34 Hz); δ 7.87 (m, 4H). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (D_2O): δ -2767/9.56; -2767/8.60; -2767/8.03; -2767/7.88 ppm.

[{Pt(terpy)}$_2$ (*trans*-1,2-bis(4-pyridyl)-ethylene)](OTf)$_4$, (2)

Yield 0.556 g, 86%. Anal. Calc. for $C_{46}H_{32}F_{12}N_8O_{12}Pt_2S_4$. Acetone: C: 34.76; H: 2.26; N: 6.62%. Found %: C: 34.56; H: 2.38; N: 6.75%. ESI-MS calc for [Pt(terpy)$^+$ + OTf] *m/z* 577.4, found *m/z* 576.9; calc for [M^{3+} + OTf] *m/z* 396.0, found *m/z* 396.1. ^{1}H NMR 400 MHz (D_2O): δ 9.17 (d, 4H, *J* = 6.03 Hz); δ 8.54 (t, 2H, *J* = 8.02 Hz); δ 8.43 (m, 12H); δ 8.15 (d, 4H, *J* = 6.41 Hz); δ 7.89 (m, 6H); δ 7.73 (m, 4H). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_3-CD_3CN): δ -2752/9.20; -2752/8.55; -2752/8.01; -2752/7.86 ppm.

[{Pt(terpy)}$_2$ (4,4'-trimethylenedipyridine)](OTf)$_4$.H$_2$O, (3)

Yield 0.618 g, 96%. Anal. Calc. for $C_{47}H_{36}F_{12}N_8O_{12}Pt_2S_4$.$H_2O$: C: 33.46; H: 2.39; N: 6.64%. Found %: C: 33.35; H: 2.40; N: 6.60%. ESI-MS calc for [Pt(terpy)$^+$ + OTf] *m/z* 577.4, found *m/z* 576.9; calc for [M^{3+} + OTf] *m/z* 401.3, found *m/z* 401.2. ^{1}H NMR 400 MHz (D_2O): δ 9.01 (d, 4H, *J* = 6.28 Hz); δ 8.51 (t, 2H, *J* = 8.30 Hz); δ 8.40 (m, 12H); δ 7.84 (d, 4H, *J* = 6.21 Hz); δ 7.80 (d, 4H, *J* = 5.72 Hz); δ 7.13 (m, 4H); δ 3.10 (t, 4H, *J* = 7.80 Hz); δ 2.32 (p, 2H, *J* = 7.58 Hz). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_3-CD_3CN): δ -2773/9.03; -2773/7.85 ppm.

The synthesis of [*μ*[3-mercapto-*N*-(2-mercapto-ethyl)-propanamide]]*bis*(2,2':6',2''-terpyridine)platinum(II) (4)

[Pt(terpy)(MPA)]PF_6 (0.171 g; 0.250 mM) was dissolved in DMF (20 mL) with HBTU (0.096 g; 0.2531 mM) and DIEA (128 µL; 0.760 mM) and left to stir for 5 min. To this solution [Pt(terpy)(2-mercapoethylamine)]ClO_4 (0.153 g; 0.250 mM) was added, the reaction flushed with nitrogen and stirred for 4 h at room temperature. The reaction solution was reduced in volume to ~ 5 mL and the product was precipitated as a chloride salt. The solid was collected under vacuum, washed with acetone (100 mL) and diethyl ether (50 mL) and air dried producing a dark purple solid. Yield 0.160 g, 58%. Anal. Calc. for $C_{35}H_{31}F_{12}N_7OP_2Pt_2S_2$: C: 32.09; H: 2.39; N: 7.49%. Found %: C: 32.14; H: 2.68; N: 7.41%. ESI-MS calc for [M2+] m/z 510.0, found m/z 509.7. ^{1}H NMR 400 MHz (D_2O): δ 9.18 (d, 2H, *J* = 5.22 Hz); δ 9.10 (d, 2H, *J* = 5.22 Hz); δ 8.20 (m, 8H); δ 7.92 (t, 6H, *J* = 9.02 Hz); δ 7.80 (t, 2H, *J* = 6.46 Hz); δ 7.73 (t, 2H, *J* = 6.52 Hz); δ 3.36 (t, 2H, J = 6.97 Hz); δ 2.68 (t, 2H, *J* = 6.51 Hz); δ 2.63 (t, 2H, *J* = 7.07 Hz); δ 2.37 (t, 2H, *J* = 6.79 Hz). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3191/9.42; -3191/9.40; -3171/2.72; -3171/2.61.

The general procedure for preparing amide bound dinuclear terpyridineplatinum(II) complexes [{Pt(terpy)}$_2$X] (PF_6)$_2$, where X = 1,2-diaminoethane, 1,4-diaminobutane, 1,6-diaminohexane, 1,12-diaminododecane, 1,4-diaminobenzene, or 4,4′-diaminodiphenylmethane

[Pt(terpy)(MPA)]PF_6 was dissolved in DMF with HBTU (1.1 equivalents) and DIEA (3 equiv.) and left to stir for 5 min. To this solution, the respective diamine linker (0.5 equiv.) was added, the reaction flushed with nitrogen and stirred for 4 h at room temperature. The reaction solution was reduced in volume to ~ 5 mL and purified by a series of salt metathesis.

[{Pt(terpy)(MPA)}$_2$(1,2-diaminoethane)](PF_6)$_2$, (5)

Yield 0.169 g, 50%. Anal. Calc. for $C_{38}H_{36}F_{12}N_8O_2P_2Pt_2S_2$: C: 33.05; H: 2.63; N: 8.11%. Found %: C: 33.00; H: 2.94; N: 8.00%. ESI-MS calc for [M^{2+}] *m/z* 545.5, found *m/z* 545.6. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.31 (d, 4H, *J* = 5.24 Hz); δ 8.58 (m, 10H); δ 8.48 (t, 4H, *J* = 7.66 Hz); δ 7.99 (t, 4H, *J* = 6.59 Hz); δ 7.70 (b, 2H); δ 3.09 (bs, 4H); δ 2.80 (t, 4H, *J* = 7.14 Hz); δ 2.53 (t, 4H, *J* = 7.14 Hz). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3192/9.30; -3192/8.49; -3192/8.00; -3192/2.80 ppm.

[{Pt(terpy)(MPA)}$_2$(1,4-diaminobutane)](PF_6)$_2$.H$_2$O, (6)

Yield 0.330 g, 58%. Anal. Calc. for $C_{40}H_{40}F_{12}N_8O_2P_2Pt_2S_2$.$H_2O$: C: 33.67; H: 2.97; N: 7.85%.

Found %: C: 34.07; H: 3.45; N: 7.59%. ESI-MS calc for $[M^{2+}]$ *m/z* 559.5, found *m/z* 559.3. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.31 (d, 4H, *J* = 5.27 Hz); δ 8.55 (m, 8H); δ 8.48 (t, 6H, *J* = 7.83 Hz); δ 7.97 (t, 4H, *J* = 6.60 Hz); δ 7.75 (t, 2H, *J* = 5.59 Hz); δ 3.02 (bs, 4H); δ 2.73 (t, 4H *J* = 6.68 Hz); δ 2.45 (t, 4H, *J* = 6.68 Hz); δ 1.40 (s, 4H). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3179/9.35; -3179/8.56; -3179/7.99; -3179/2.76 ppm.

[{Pt(terpy)(MPA)}$_2$(1,6-diaminohexane)](PF$_6$)$_2$.H$_2$O, (7)

Yield 0.062 g, 65%. Anal. Calc. for $C_{42}H_{44}F_{12}N_8O_2P_2Pt_2S_2.H_2O$: C: 34.67; H: 3.19; N: 7.70%. Found %: C: 34.56; H: 3.45; N: 7.66%. ESI-MS calc for $[M^{2+}]$ *m/z* 573.6, found *m/z* 573.5. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.40 (d, 4H, *J* = 5.30 Hz); δ 8.63 (m, 8H); δ 8.53 (t, 6H, *J* = 7.85 Hz); δ 8.01 (t, 4H, *J* = 6.58 Hz); δ 7.73 (t, 2H, *J* = 5.26 Hz); δ 2.97 (q, 4H J_1 = 12.25, J_2 = 6.09 Hz); δ 2.77 (t, 4H *J* = 7.01 Hz); δ 2.49 (t, 4H, *J* = 7.01 Hz); δ 1.34 (bs, 4H); δ 1.25 (bs, 4H). ^{195}Pt NMR 86 MHz (d_6-DMSO): δ -3160.9.30; -3160/2.80 ppm.

[{Pt(terpy)(MPA)}$_2$(1,12-diaminododecane)](PF$_6$)$_2$, (8)

Yield 0.121 g, 54%. Anal. Calc. for $C_{48}H_{56}F_{12}N_8O_2P_2Pt_2S_2$: C: 37.90; H: 3.71; N: 7.37%. Found %: C: 37.54; H: 3.88; N: 7.52%. ESI-MS calc for $[M^{2+}]$ *m/z* 615.6, found *m/z* 615.5. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.33 (d, 4H, *J* = 5.37 Hz); δ 8.64 (m, 12H); δ 8.51 (t, 4H, *J* = 7.64 Hz); δ 7.99 (t, 4H, *J* = 6.23 Hz); δ 7.66 (t, 2H, *J* = 5.37 Hz); δ 2.89 (q, 4H J_1 = 6.65, J_2 = 5.69 Hz); δ 2.75 (t, 4H *J* = 7.32 Hz); δ 2.49 (t, 4H, *J* = 6.62 Hz); 1.23 (m, 15H). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3183/9.38; -3183/8.65; -3183/8.02; -3183/2.78 ppm.

The synthesis of [μ-4,4'-dithiolterphenylbis](2,2':6',2''-terpyridine)platinum(II).3H$_2$O (9)

[Pt(terpy)Cl]Cl.2H$_2$O (0.100 g; 0.188 mM) and 4,4'-dithiolterphenyl (0.028 g; 0.094 mM) were stirred in DMF (20 mL) at room temperature for 8 h to produce a dark purple solution. The solution was reduced to 10 mL and the product was precipitated by the addition of acetone (150 mL), filtered and air dried. The crude 9 was dissolved in H$_2$O (200 mL) and precipitated by the addition of KPF$_6$. The precipitate was washed with acetone (100 mL) and air dried. Yield 0.150 g, 55%. Anal. Calc. for $C_{48}H_{34}Cl_2N_6Pt_2S_2.3H_2O$: C: 45.25; H: 3.16; N: 5.57%. Found %: C: 45.07; H: 3.03; N: 5.70%. ESI-MS calc for $[M^{2+}]$ *m/z* 574.6, found *m/z* 574.7. ^{1}H NMR 400 MHz (D$_2$O): δ 9.20 (d, 4H, *J* = 5.81 Hz); δ 8.80 (m, 5H); δ 8.74 (m, 9H); δ 8.55 (t, 5H, *J* = 8.13 Hz); δ 7.96 (t, 5H, *J* = 6.62 Hz); δ 7.80 (d, 4H, *J* = 7.94 Hz); δ 7.68 (s, 4H); δ 7.48 (d, 4H, *J* = 7.94 Hz). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3148/9.23 ppm.

[{Pt(terpy)(MPA)}$_2$(1,4-diaminobenzene)](PF$_6$)$_2$.H$_2$O, (10)

Yield 0.214 g, 80%. Anal. Calc. for $C_{42}H_{36}F_{12}N_8O_2P_2Pt_2S_2.H_2O$: C: 34.86; H: 2.67; N: 7.74%. Found %: C: 34.41; H: 2.75; N: 7.66%. ESI-MS calc for $[M^{2+}]$ *m/z* 569.5, found *m/z* 569.2. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.60 (s, 2H); δ 9.42 (d, 4H, *J* = 5.67 Hz); δ 8.52 (m, 14H); δ 7.99 (t, 4H, *J* = 6.75 Hz); δ 6.85 (s, 4H); δ 2.93 (t, 2H, *J* = 6.58 Hz); δ 2.74 (t, 2H, *J* = 6.58 Hz). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3172/9.47; -3172/2.97 ppm.

[{Pt(terpy)(MPA)}$_2$(4,4′-diaminodiphenylmethane)](PF$_6$)$_2$ (11)

Yield 0.202 g, 72%. Anal. Calc. for $C_{49}H_{42}F_{12}N_8O_2P_2Pt_2S_2$: C: 38.74; H: 2.79; N: 7.38%. Found %: C: 38.82; H: 2.95; N: 7.22%. ESI-MS calc for $[M^{2+}]$ *m/z* 614.6, found *m/z* 614.3. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.73 (s, 2H); δ 9.37 (d, 4H, *J* = 5.49 Hz); δ 8.44 (m, 14H); δ 7.97 (q, 4H, J_1 = 9.46 J_2 = 4.45 Hz); δ 7.15 (d, 4H, *J* = 8.38 Hz); δ 6.88 (d, 4H, *J* = 8.38 Hz); δ 3.73 (bs, 2H); δ 2.92 (t, 4H *J* = 6.17 Hz); δ 2.71 (t, 4H, *J* = 6.17 Hz). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3166/9.38; -3166/8.42; -3166/7.99; -3166/2.91 ppm.

The synthesis of [{Pt(terpy)}$_2$(1,2-diaminoethane)μ[*N,N*'-(3,6-diaminoacridine)*bis*(4-mercaptobenzoic acid)]]*bis*(2,2':6',2''-terpyridine)platinum(II)(PF$_6$)$_2$ (12)

[Pt(terpy)(Cl)]Cl (0.100 g; 0.187 mM) was dissolved in H$_2$O (20 mL) with a solution of NaOH in water (3 M, 0.15 mL). To this solution 4-mercaptobenzoic acid (MBA, 0.029 g, 0.187 mM) in DMF (15 mL) was combined and stirred for 2 h. [Pt(terpy)(MBA)]Cl (0.052 g; 0.081 mM) was reduced to dryness and dissolved in DMF (50 mL) with DIEA (85 µL, 0.487 mM), DCC(0.018 g; 0.089 mM) and HOBt (0.012 g; 0.089 mM) and stirred for 1 h. To this solution, 3,6-diaminoacridine (0.010 g; 0.04 mM) was added and stirred for 4 h at room temperature. The product was reduced to dryness before being dissolved in H$_2$O (10 mL) and extracted with DCM (3 × 100 mL). The product was collected by lyophilisation as a dark orange solid. Yield 0.031 g, 22%. ESI-MS found *m/z* [Pt(terpy)MBA] 581.1; [Pt(terpy)] 427.1; 3,6-diaminoacridine 210.1. ^{1}H NMR 400 MHz (D$_2$O): δ 9.08 (d, 4H, *J* = 5.88 Hz); δ 8.73 (m, 13H); δ 8.51 (t, 4H, *J* = 7.64 Hz); δ 8.36 (s, 2H); δ 7.91 (t, 4H, *J* = 6.32 Hz); δ 7.66 (d, 4H, *J* = 9.00 Hz); δ 7.58 (q, 8H, J_1 = 8.36 Hz, J_2 = 4.98 Hz); δ 6.91 (dd, 4H, J_1 = 8.80 Hz, J_2 = 2.07 Hz); δ 6.82 (s, 4H); δ 5.80 (s, 4H). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3132/9.08 ppm.

The synthesis of [μ[1,2-ethanedithiol]]*bis*(2,2':6',2''-terpyridine)platinum(II)(PF$_6$).3H$_2$O (13)

1,2-Ethanedithiol (11.3 µL; 0.068 mM) was added to MeCN (10 mL) and a saturated NaOH solution in H$_2$O was added drop-wise until a pH of 8 was achieved. The 1,2-ethanedithiol was stirred for 5 min under a nitrogen atmosphere before the addition of [Pt(terpy)(MeCN)](OTf)$_2$ (0.103 g; 0.135 mM) dissolved in MeCN (50 mL). The reaction was allowed to stir under a nitrogen atmosphere for 2 h before being precipitated as a PF$_6$ salt and washed with H$_2$O (20 mL). Yield 0.080 g, 48%. Anal. Calc. for $C_{32}H_{26}F_{12}N_6O_2P_2Pt_2S_2.3H_2O$: C: 29.73; H: 2.49; N: 6.50. Found %: C: 29.81; H: 2.27; N: 5.69. ESI-MS calc *m/z* 474.1, found *m/z* [Pt(terpy)] 467.9 427.1; terpyridine 210.1. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.26 (d, 2H, *J* = 5.37 Hz); δ 8.56 (t, 1H, *J* = 8.01); δ 8.46 (t, 3H, *J* = 8.01 Hz); δ 8.25 (m, 5H); δ 7.93 (t, 2H, *J* = 6.58); δ 3.20 (br, 3H); ^{195}Pt/^{1}H HMQC NMR 86 MHz (d_6-DMSO): δ -2961/9.37 ppm.

The synthesis of [μ[2-(2-mercapto-ethoxy)-ethanethiol]]*bis*(2,2':6',2''-terpyridine)platinum(II)(PF$_6$)$_2$ (14)

[Pt(terpy)Cl]Cl (0.200 g; 0.380 mM) was dissolved in water (25 mL) and stirred under nitrogen. Separately, 2-mercaptoethyl ether (24 µL; 0.190 mM) and tetrabutylammonium hydroxide (0.8 M solution in methanol, 465 µL) were combined in ethanol (4 mL) with nitrogen bubbled through for 5 min. The product, **14** was transferred to the [Pt(terpy)Cl]Cl solution under a strict

nitrogen atmosphere. The reaction was stirred under nitrogen for 20 min, producing a dark purple solution. The solution was filtered and the solvent reduced to ~5 mL. The solution was cooled in an ice bath, producing a dark purple precipitate. The solid was collected under vacuum and washed with acetone (100 mL), diethyl ether (200 mL) and then air dried. Yield 0.180 g, 82%. Anal. Calc. for $C_{34}H_{36}F_{12}N_6O_4P_2Pt_2S_2$: C: 30.55; H: 2.71; N: 6.29%. Found %: C: 30.30; H: 2.65; N: 5.76%. ESI-MS calc. for $[M^{2+}]$ *m/z* 496.5, found *m/z* 496.1. ^{1}H NMR 400 MHz (d_6-DMSO): δ 8.79 (d, 4H, *J* = 5.25 Hz); δ 8.44 (m, 6H); δ 7.89 (t, 8H, *J* = 6.53 Hz); δ 7.57 (t, 4H, *J* = 6.53 Hz); δ 3.58 (t, 4H *J* = 5.78 Hz); δ 2.40 (t, 4H, *J* = 5.78 Hz). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3120/8.81; -3120/2.42 ppm.

The synthesis of [*μ*[2-[2-(2-mercapto-ethoxy)-ethoxy]-ethanethiol]]*bis*(2,2':6',2''-terpyridine)platinum(II)(PF_6)$_2$ (15)

[Pt(terpy)Cl]Cl (0.410 g; 0.780 mM) was dissolved in water (25 mL) and stirred under nitrogen. Separately, 3,6-dioxa-1,8-octanedithiol (62 μL; 0.390 mM) and tetrabutylammonium hydroxide (0.8 M solution in methanol, 1000 μL) were combined in ethanol (4 mL) with nitrogen bubbled through for 5 min. The solution was transferred to the [Pt(terpy)Cl]Cl solution under a strict nitrogen atmosphere. The reaction was stirred under nitrogen for 15 min producing a dark purple solution. The solution was filtered and acetone (100 mL) added, producing a purple precipitate. The solid was collected under vacuum, washed with acetone (100 mL) and then air dried. The crude complex was purified by column chromatography using an aluminium oxide column. The complex was dissolved in a minimum of water (2 mL), loaded onto the column and then eluted with 15% water/acetonitrile mix. The purple complex separated from an orange band attributed to unreacted [Pt(terpy)Cl]Cl. The purple band was collected and the solution reduced to ~10 mL under reduced pressure. The purple complex was precipitated with acetone (200 mL) and was collected under vacuum and washed with acetone (100 mL), diethyl ether (100 mL) and air dried. Yield 0.240 g, 56%. Anal. Calc. for $C_{36}H_{34}F_{12}N_6O_2P_2Pt_2S_2.H_2O$: C: 32.15; H: 2.70; N: 6.25%. Found %: C: 32.23; H: 2.65; N: 6.53%. ESI-MS calc for $[M^{2+}]$ *m/z* 518.5, found *m/z* 518.1. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.41 (d, 4H, *J* = 5.47 Hz); δ 8.61 (m, 6H); δ 8.49 (m, 8H); δ 7.99 (t, 4H, *J* = 6.74 Hz); δ 3.73 (t, 4H *J* = 6.93 Hz); δ 3.51 (s, 4H); δ 2.69 (t, 4H, *J* = 6.90 Hz). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3187/9.43; -3187/8.63; -3187/8.02; -3187/2.72 ppm.

Synthesis of [*μ*[2-(2-mercapto-ethoxy)-ethanethiol]] *bis*(4'-(4-methylphenyl)-2,2':6',2''-terpyridine)platinum(II)(PF_6).3H_2O (16)

[Pt(4'MPterpy)Cl]Cl (0.134 g; 0.227 mM), and silver nitrate ($AgNO_3$) (0.773 g; 0.454 mM) were combined in ACN:H_2O (50:50, 20 mL) and the reaction stirred at room temp in the absence of light for 24 h. The solution was filtered through celite, to remove AgCl, to reveal the orange filtrate of [Pt(4'MPterpy NO_3)]NO_3. Tetrabutylammonium hydroxide (0.402 g; 0.681 mM) and 2-mercaptoethyl ether (14 μL; 0.11 mM) were added to the filtrate and stirred at 60 °C for 12 h. KPF_6 was added to the resulting brown/yellow suspension, the solution was filtered, removing the brown precipitate, and leaving a yellow filtrate. The filtrate was evaporated to dryness and washed with H_2O (100 mL). Yield 0.068 g, 41%. Anal. Calc. for $C_{48}H_{42}F_{12}N_6O_2P_2Pt_2S_2.3H_2O$: C: 38.00; H: 3.19; N: 5.54%. Found %: C: 37.61; H: 2.53; N: 5.49%. ESI-MS calc for $[M^+ + PF_6]$ *m/z* 1317.18, found *m/z* 1317.54; $[M^+]$ *m/z* 1172.22, found *m/z* 1172.49; [Pt(4'MPterpy)] *m/z* 518.11, found *m/z* 518.18. ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.37 (d, 4H, *J* = 5.33 Hz); δ 8.76 (s, 4H); δ 8.67 (d, 4H, *J* = 7.53 Hz); δ 8.38 (t, 4H, *J* = 7.32 Hz); δ 7.92 (d, 4H, *J* = 7.82 Hz); δ 7.86 (t, 4H, *J* = 6.51 Hz); δ 7.36 (d, 4H, *J* = 7.38 Hz); δ 3.73 (t, 4H, *J* = 5.04 Hz); δ 2.70 (t, 4H, *J* = 4.60 Hz); δ 2.44 (s, 6H). ^{195}Pt/^{1}H HMQC NMR 86/400 MHz (d_6-DMSO): δ -3120/9.41; -3120/2.75 ppm.

Cytotoxicity

The cytotoxicity of dinuclear [Pt(terpy)] complexes against L1210 cells was undertaken at the Peter McCallum Cancer Institute, using a published method.[7, 8] The platinum complexes were dissolved in distilled water or DMSO (final concentration <1%) and then diluted to the required concentrations. Growth inhibition results for L1210 cells are reported after 48 h continuous exposure to the metal complex.

For growth inhibition tests with A2780 and A2780*cisR*, cells were seeded in 96 well plates at 1500 cells per well in order to ensure the cells being tested were in the exponential growth phase. The cells were allowed to grow for a further 24 h before the addition of freshly prepared stocks of platinum complexes and subsequently incubated for 72 h. The A2780 and A2780*cisR* were assessed as an average of three independent experiments using the sulforhodamine B (SRB) colorimetric assay.[29, 30] Following exposure to platinum complexes, the cells were fixed for 1 h in cold trichloroacetic (100 μL, 12.5%, TCA) acid at 4 °C. TCA fixed plates were washed with water (4 × 200 μL) and stained by the addition of SRB (100 μL of 0.4% wt/vol) in acetic acid (1%) for 30 min. At the completion of the staining period, the plates were washed 4 times with 1% acetic acid to remove any unbound dye and the plates air dried. The stained cells were solubilised with tris(hydroxymethyl)aminomethane solution (200 μL of 10 mM, pH 10.5, Tris base) and the subsequent absorbance measured in a microtiter plate reader (Bio-rad Microplate reader 680) at 515 nm. The percentage growth inhibition was calculated from a plot between the absorbance ratios of treated to untreated cells against a linear drug concentration.

Results and Discussion

Synthesis

The primary synthon used to make these dinuclear [Pt(terpy)] complexes, chloroterpyridineplatinum(II) chloride ([Pt(terpy)(Cl)]Cl.2H_2O), was obtained from a two-step process using established methods.[31, 32] The complexes, 2-mercapoethylamine(2,2':6',2''-terpyridine)platinum(II)[Pt(terpy)(2-mercapoethylamine)]$^+$ and 3-mercaptopropionic (2,2':6',2''-terpyridine)platinum(II) [Pt(terpy)(MPA)]$^+$, were obtained by adaptation from published methods and were utilised to assemble amide bound dinuclear complexes.[27, 28] Purification of thiol-bound dinuclear complexes were achieved by a series of salt metatheses unless otherwise stated.

Characterisation was confirmed by a range of NMR techniques. Multinuclear ^{1}H/^{195}Pt and multidimensional 1D/2D NMR techniques provided structural and conformational elucidation as well as information on impurities. ^{195}Pt chemical shifts are highly sensitive to variations in platinum oxidation state and ligand substitution.[33, 34] Typically, the ^{195}Pt chemical shifts for this class of compounds are between -2600 and -3400 ppm. The sensitive nucleus has an additive relationship of

chemical shift containing various ligands, with each ligand causing large shifts as observed in the characterisation of these complexes. Identification of the protons closest to the platinum can be achieved utilising ^{1}H-^{195}Pt HMQC NMR. ^{1}H-^{195}Pt NMR is effectively utilized to confirm successful coordination. Further confirmation was achieved with elemental analysis and mass spectrometry (MS) to ensure that the complexes were of a sufficient purity for biological studies. The elemental analysis results were generally in agreement with the calculated values, although the aliphatic thiol bound complexes showed slight variation, possibly due to the purification process of salt conversions that are NMR and MS inactive. The slight variation found in the elemental analysis for these complexes would not affect the trend observed for the IC_{50}'s which varied to a greater extent.

The NMR characterisation process used for all dinuclear [Pt(terpy)] complexes is illustrated for **4**, with the atomic numbering used to describe the characterisation shown in Fig. 3 Characterisation of **4** was achieved using ^{1}H and ^{195}Pt NMR spectra. Due to the asymmetry of the dinuclear complex, two sets of terpy peaks were observed in the 1D ^{1}H spectra (Fig. 3) which were characterised using 2D ^{1}H COSY and ROESY spectra, illustrated in Fig. 4 and Fig. 5 respectively.

Initially the aliphatic linker was characterised in order to determine the orientation of the aromatic peaks. In d_6-DMSO the amide peak was visible at 6.80 ppm and provided a COSY cross correlation to the triplet at 3.36 ppm integrating for two protons, assigned to the H8 proton. The ROESY spectrum also provided a cross correlation to the triplet, integrating for two protons at 2.37 ppm, was assigned as the H10 proton. Further correlations in the COSY and ROESY spectra between the H8 and H7 proton at 2.68 ppm and from the H7 to the H6 of the aromatic terpy were observed. Correlation between the H10 and the triplet assigned to the H11 protons at 2.63 ppm were also observed, as well as correlations between the H11 and H6a protons. Conformation of both the H6/H6a and H7/H11 protons was achieved by ^{1}H-^{195}Pt HMQC, as seen in Fig. 6, with four peaks observed at δ -3191/9.42; -3191/9.40; -3171/2.72 and -3171/2.61 ppm. The cross peaks at -3191 and -3171 ppm in the platinum spectrum were confirmed to be from the H6 and H7 protons respectively, due to the downfield shift from the nitrogen of the peptide linkage. Overlapping chemical shifts of the H3'/3 with the H3'a/3a protons, as well as the H4'/4 with the H4'a/H4a, produced two large multiplets. The remaining aromatic resonances were characterised by the observed cross correlation peaks between H6, H5 and H4 protons, as well as those between the H6a, H5a and H4a protons (Fig. 5).

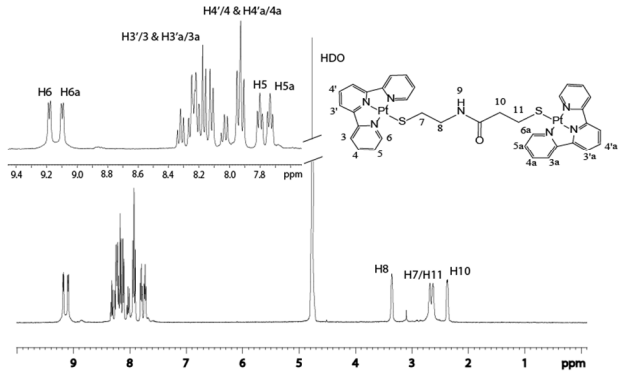

Fig. 3: The ^{1}H spectrum of **4** in D_2O. Inset: the chemical structure and numbering of $[\{Pt(terpy)\}_2(\mathbf{4})]^{2+}$ as well as the expansion of the aromatic region between 7.6 and 9.4 ppm.

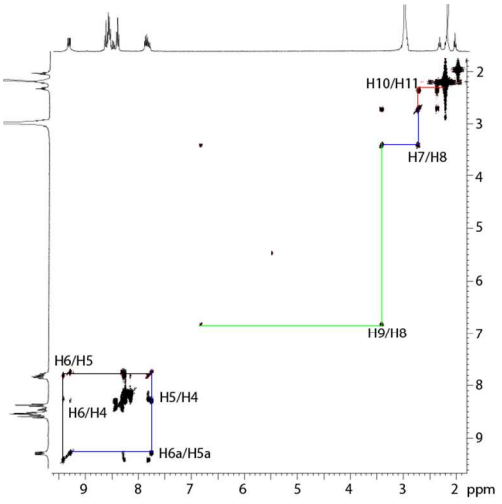

Fig. 4: The COSY spectrum of **4** in d_6-DMSO with lines of connectivity highlighting cross correlations.

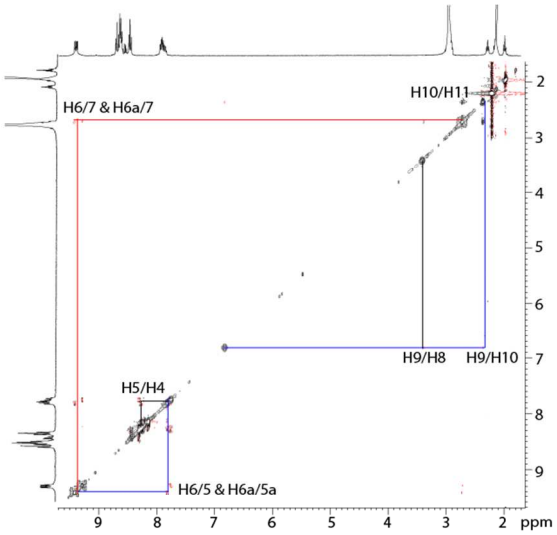

Fig. 5: The ROESY spectrum of 4 in d_6-DMSO with lines of connectivity highlighting cross correlations.

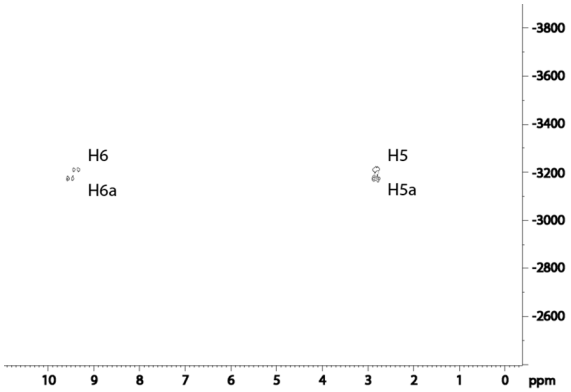

Fig. 6: The ^{1}H-^{195}Pt HMQC spectrum of **4** in d_6-DMSO.

Cytotoxicity

For analysis and comparison, dinuclear [Pt(terpy)] complexes were divided into four different types based on the linker: (group 1) highly charged pyridine bound complexes, with limited flexibility; (group 2) highly flexible aliphatic thiol-amide bound complexes with large variations in linker length; (group 3) aromatic thiol bound complexes with variations in aromaticity, produced intermediate flexibility to the aliphatic thiol-amide bound complexes; and (group 4) aliphatic thiol bound complexes of various length.

A comparison of the cytotoxicity of the four classes, in addition to cisplatin and $[Pt(terpy)(X)]^+$ complexes (where X = 2-mercapoethylamine or glutathione, for expected degradation products) was expected to offer the most insight. L1210 cells were used to provide an initial indication of the effectiveness of the dinuclear [Pt(terpy)] complexes. Subsequently, cytotoxicity was also assessed against human ovarian cancer cell lines A2780 and A2780*cisR* in order to compare cytotoxicity results of previously published Pt(terpy) based dinuclear complexes.[26] The results reported in Table 1 indicate that although the complexes are not as cytotoxic as cisplatin in L1210 or A2780 cells, they demonstrated promising IC_{50} values against the A2780*cisR* cell line.

In general, there did not appear to be a correlation between the linker length of dinuclear [Pt(terpy)] complexes and cytotoxicity. It was observed that the rigid and highly charged pyridine linked complexes (Group 1) have poor cytotoxicity against L1210 cells independent of chain length or flexibility, with cytotoxicity at least 10 fold less than that of cisplatin for all three complexes. Variable cytotoxicity values were observed for this group against A2780 cells, with 1 (2.7 ± 1.3 μM) comparable to cisplatin (1.0 μM), while the longer 2 and 3 proved to be ineffective. However against the A2780*cisR* cell line, the pyridine bound complexes, 1 and 3 (5.6 ± 0.4 and 1.9 ± 0.1 μM respectively), proved highly effective, with improved cytotoxicity relative to cisplatin (9.0 μM), while 2 remained ineffective (>30 μM). No structural activity relationship for pyridine linked complexes was evident, based on the cytotoxicity assays. Covalent binding to DNA would be expected to remain consistent for the dinuclear [Pt(terpy)] complexes, particularly with similar platinum bound 4-coordinate groups, irrespective of linker length.[35, 36] On the contrary, the observed cellular activity did not relate to complexes with similar 4-coordinate groups, providing indirect evidence for activity through a DNA intercalation or a protein binding mechanism.

Flexible aliphatic thiol-amide coupled complexes (group 2) showed poor cytotoxicity relative to cisplatin in L1210 cell lines, although cytotoxicity increased with increasing linker length. The exception was complex **7** which may be due to poor water solubility of the complex. The increasing cytotoxicity in the L1210 cell line may be explained by increased 'DNA stapling', a binding mechanism where longer flexible dinuclear [Pt(terpy)] complexes span a longer distance between DNA base pairs to form an intercalating 'staple', as described by McFayden *et al.*[14, 37] This trend in cytotoxicity, however was not observed in either the A2780 or A2780*cisR* cell lines. Slightly less cytotoxicity, compared with cisplatin, was observed for A2780 cells, while improved cytotoxicity was observed for A2780*cisR* cells.

More rigid aromatic thiol coupled complexes (Group 3) demonstrated higher cytotoxicity values across all cell lines as the linker length was increased. The short and highly rigid **9** linked complex showed poor overall cytotoxicity, while the GI_{50} could not be determined confidently due to poor aqueous solubility. The incorporation of a central aromatic ring in the linker produced the complex **10** and although flexible, the complex also exhibited poor cytotoxicity. The addition of two central aromatic rings to the linker for **11**, decreased the water

Table 1: **The cytotoxicity of dinuclear [Pt(terpy)] complexes against the L1210, A2780 and A2780*cisR* cell lines, determined by the average of three independent experiments, with population standard deviation.**

Compound	GI_{50} (μM) L1210	GI_{50} (μM) A2780	GI_{50} (μM) A2780*cisR*	Resistance factor
Cisplatin	0.5	1.0	9.0	9.0
[Pt(terpy)(2-mercapoethylamine)]ClO_4	31 ± 3.0	15 ± 0.1	15 ± 0.0	1.0
[Pt(terpy)(glutathione)]Cl	13.6 ± 0.6	14 ± 2.1	>30 ± n/a	>2.1
1	44.3 ± 8.0	2.7 ± 1.3	5.6 ± 0.4	2.1
2	>60 ± n/a	30 ± 3.3	>30 ± n/a	>1.0
3	46.3 ± 5.2	18 ± 4.1	1.9 ± 0.1	0.1
4	14.7 ± 2.1	4.0 ± 0.8	5.3 ± 0.5	1.3
5	12.3 ± 2.5	5.6 ± 0.5	7.0 ± 0.8	1.3
6	7.0 ± 0.6	2.9 ± 0.7	4.3 ± 0.3	1.5
7	11.6 ± 2.2	2.8 ± 0.9	4.0 ± 0.4	1.4
8	4.2 ± 0.6	6.0 ± 3.0	4.0 ± 0.5	0.7
9	>10 ± n/a	>10 ± n/a	>10 ± n/a	-
10	>40 ± n/a	14.8 ± 4.4	>15 ± n/a	>1.0
11	10.3 ± 0.4	8.9 ± 1.0	10 ± 0.0	1.1
12	2.9 ± 0.1	7.7 ± 0.9	4.9 ± 0.3	0.6
13	2.7 ± 0.4	0.5 ± 0.0	0.9 ± 0.1	1.8
14	7.5 ± 0.4	2.0 ± 0.4	4.0 ± 0.7	2.0
15	6.9 ± 1.2	1.9 ± 0.2	3.3 ± 0.5	1.7
16	0.6 ± 0.2	1.0 ± 0.0	2.5 ± 0.1	2.5

Four different types based on the linker: (group 1) pyridine bound complexes (highlighted in red) produced highly charged complexes with limited flexibility; (group 2) aliphatic thiol-amide bound complexes (highlighted in yellow) produced highly flexible complexes with large variations in linker length; (group 3) aromatic thiol bound complexes (highlighted in green) with variations in aromaticity to produce intermediate flexibility; and (group 4) aliphatic thiol bound complexes (highlighted in blue) of various length.

solubility and with it cytotoxicity in L1210 and A2780 cells but it only produced comparable results, with cisplatin, against the A2780*cisR* cell line. The aliphatic thiol-amide complexes (Group 2) were more effective than the aromatic complexes against the A2780 cell line, although still not as effective as cisplatin.

The linker incorporating diamminoacridine and *p*-mercaptobenzoic acid produced the highly water soluble **12**. This complex was the most cytotoxic of the aromatic thiol bound complexes across all cell lines, with better cytotoxicity than cisplatin in A2780*cisR* cells. Flexible aliphatic thiol linked complexes (Group 2) displayed similar cytotoxic results for the complexes **14** (L1210 7.5 ± 0.4 μM, A2780 2.0 ± 0.4 μM, A2780*cisR* 4.0 ± 0.7 μM) and **15** (L1210 6.9 ± 1.2 μM, A2780 1.9 ± 0.2 μM, A2780*cisR* 3.3 ± 0.5 μM) across the cell lines. Surprisingly, the short, relatively unstable and highly soluble **13** complex was the most cytotoxic complex against the ovarian cell carcinomas, with a 2 and 10-fold increase over cisplatin against A2780 (0.5 ± 0.0 μM) and A2780*cisR* (0.9 ± 0.1 μM) cell lines, respectively. The addition of a weak electron donating 4-methylphenyl substitution on the terpy group of **16** improved the cytotoxicity 2-fold for both human ovarian lines and ~11-times for the L1210 cell line, despite limitations imparted by decreased solubility. The decreased rate of ligand substitution and increased stability was expected to increase the potential to intercalate over platination of DNA.[26]

Across the groups, the pyridine bound dinuclear [Pt(terpy)] complexes (Group 1) were the least effective against L1210 cells, while the thiol bound complexes (Group 4) were the most effective. Against human ovarian A2780 cells, the pyridine complexes produced varied activity and resistance factors. Aromatic thiol bound complexes (Group 3) displayed poor activity, while similar results between A2780 and A2780*cisR* cell lines indicated some effectiveness against with cisplatin resistant cell lines. The aliphatic thiol-amide complexes (Group 2) were more effective than the aromatic complexes against the A2780 cell line, although still not as effective as cisplatin.

Against the cisplatin resistant A2780*cisR* cells the dinuclear [Pt(terpy)] complexes proved to be comparable to cisplatin with no cross-resistance observed. Against A2780*cisR* cells a 2-fold increase in activity, compared to cisplatin, was observed, with the exception of the **2** and the aromatic thiol bound complexes that displayed a lower cytotoxicity than cisplatin. Compared to the mononuclear [Pt(terpy)(2-mercapoethylamine)] and [Pt(terpy)(GSH)] complexes, the dinuclear [Pt(terpy)] complexes displayed increased cytotoxicity against the cell lines tested. While dinuclear [Pt(terpy)] complexes could be expected to have an increase in toxicity ~2-fold, compared to the mononuclear, the observed cytotoxicities were much higher, indicating a more effective mode of action facilitated by the linker. A nine fold resistance factor was determined for cisplatin against A2780/A2780*cisR* cells, while the resistance of dinuclear [Pt(terpy)] complexes was observed to be lower for all complexes. Similar cytotoxicity results were obtained between A2780 and A2780*cisR* cells lines, with the exception of **1** and the aliphatic thiol bound complexes (group 4) which showed a 2 fold decrease in activity for the A2780*cisR* cell line. The lack of cross-resistance observed indicates the potential for development of dinuclear [Pt(terpy)] based complexes in the design of future chemotherapeutics.

Conclusions

This study has shown that the 16 newly synthesised and characterised dinuclear [Pt(terpy)] complexes demonstrate varying cytotoxicity between differing linker lengths. In general, cytotoxicity was not consistent within different linker types. The difficulty in determining structural-activity relationships for dinuclear [Pt(terpy)] complexes is evident by the variation in cytotoxicity observed for complexes with subtle structural differences, as well as similar cytotoxicity observed for complexes with large structural variation. It is possible that the complexes investigated here elicit their cytotoxic effects by binding to biologically significant proteins, with DNA not being the primary target.

Against L1210 cells cisplatin was more cytotoxic than the synthesised dinuclear [Pt(terpy)] complexes. Against A2780 cells cisplatin and dinuclear [Pt(terpy)] complexes synthesised demonstrated comparable cytotoxicity. Against A2780cisR cells, dinuclear [Pt(terpy)] complexes were determined to produce cytotoxicity values 2-fold less than cisplatin, with the exception of the **2** and the aromatic thiol bound complexes. A2780 and A2780cisR showed a lack of observed cross resistance, indicating the potential for further investigation as anticancer agents with unique mechanisms of action.

Previous reports have concluded that linker length, change density and flexibility contribute to the cytotoxicity of the complexes and in general we concur, however the results demonstrated here indicate that while both the intercalating moiety and the linker are critical considerations in the design of dinuclear [Pt(terpy)] complexes, there is no clear structural-activity relationship based on linker length, change density and flexibility evident.

Acknowledgements

Dr Benjamin. W. J. Harper was supported by an Australian Postgraduate Award from the University of Western Sydney. We wish to acknowledge the University of Western Sydney for funding through internal research grants and International Research Initiative Scheme and the School of Science and Health for support through a New Research Project Development Grant. Aspects of this research were supported by Australian Government, through the International Science Linkages program. Dr Simon Myers is thanked for providing access to the PC2 facility used for cell line testing.

Notes and references

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