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Investigating the cytotoxicity of platinum(II) complexes incorporating bidentate pyridyl-1,2,3-triazole "click" ligands

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ABSTRACT

Six platinum(II) complexes of the type $[Pt(P_L)(A_L)]^{2+}$, where P_L is a bidentate pyridyl-1,2,3-triazole "click" ligand and A_L is the *R*,*R* or *S*,*S* isomer of 1.2-diaminocyclohexane, have been synthesised and characterised by several methods including elemental microanalysis, proton NMR spectroscopy and X-ray crystallography. The *in vitro* cytotoxicity of each complex was assessed in eleven cell lines, revealing moderate to good activity for complexes incorporating 2-(1-phenyl-1H-1,2,3-triazol-4-yl)pyridine.

1. Introduction

The study of anticancer metal complexes that can overcome the toxicity and resistance limitations of current agents such as cisplatin continues to be a developing field. Many complexes have been identified with different biological behaviour to cisplatin yet also demonstrate equivalent or higher potential to kill cancerous cells [1–5]. Platinum complexes (PCs) continue to be at the forefront of this field; our group is focused upon compounds of the type $[Pt(P_L)(A_L)]^{2+}$, where P_L is one of several polyaromatic heterocyclic ligands such as 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen) or dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline (dpq), and A_L is one of several chiral diamines such as diaminocyclohexane (dach) [6–8]. These PCs induce cell death in a different way to cisplatin, partly due to non-covalent binding interactions [9,10], and exhibit cytotoxicity at concentrations as low as 7 nM in a variety of cell lines [11]. In a previous report on the potential of ligands with wider polyaromatic surface than our typical archetypes of 1,10-phenanthroline, 2-(2-pyridyl)quinoxaline (2pq) was incorporated as a P_L to synthesise complexes with the potential for novel activity [8]. Unexpectedly, the conformation of the coordinated 2pq ligand resulted in PCs that were unstable in solution and inactive in cancerous cell lines. Here we have successfully synthesised PCs that incorporate atypical P_Ls with no stability issues. The PCs incorporate 2-(1-phenyl-1H-1,2,3-triazol-4-yl)pyridine (phpytri), 2-(1-benzyl-1H-1,2,3-triazol-4-yl)pyridine (bnpytri), or 2-(1-octyl-1H-1,2,3-triazol-4-yl)pyridine (octpytri) as the P_L and 1*S*,2*S*-diaminocyclohexane (*SS*-dach) or 1*R*,2*R*-diaminocyclohexane (*RR*-dach) as the A_L (Fig. 1).

These pyridyl-1,2,3-triazole (R-pytri) ligands have recently emerged as readily functionalised analogues of the common bidentate chelators bpy and phen. The utility of the copper(I) catalysed azide alkyne "click" reaction has allowed for a diverse variety of R-pytri ligands such as the ones in this study [12–14], and the corresponding metal complexes have been synthesised and examined in a range of applications [15–17]. In particular there is a growing interest in using functionalised R-pytri ligands for the development of metal complexes for biomedical purposes. A number of authors have coordinated inert octahedral ions such as Re(I) [18–28], Tc(I) [19,26,27], Ru(II) [29] and Ir(III) [29–31] to functionalised R-pytri ligands and exploited the resulting complexes as infrared, luminescent and radiolabelled bioprobes. Additionally, some of these octahedral R-pytri complexes have displayed respectable cytotoxicity [21,24,25,27,31,32]. Square planar

Abbreviations: 2pq, 2-(2-pyridyl)quinoxaline; bnpytri, 2-(1-benzyl-1H-1,2,3-triazol-4-yl)pyridine; bpy, 2,2′-bipyridine; dach, diaminocyclohexane; DMEM, Dulbecco's modified Eagle's medium; dpq, dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline; HMQC, heteronuclear multiple quantum correlation; ESIMS, electrospray ionisation mass spectrometry; octpytri, 2-(1-octyl-1H-1,2,3-triazol-4-yl)pyridine; PC, platinum complex; phen, 1,10-phenanthronline; phpytri, 2-(1-phenyl-1H-1,2,3-triazol-4-yl)pyridine; *R*-pytri, pyridyl-1,2,3-triazole; *RR*-dach, 1*R*,2*R*-diaminocyclohexane; *SS*-dach, 1*S*,2*S*-diaminocyclohexane.

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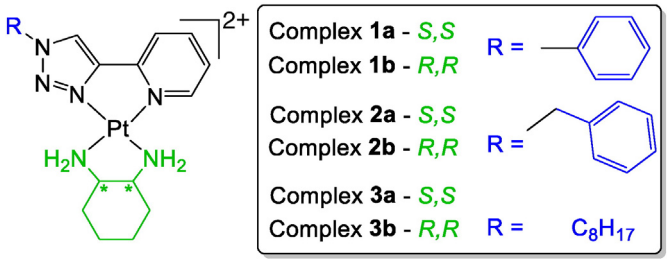

Fig. 1. General structure of complexes **1–3**. Counter-ions have been omitted for clarity, and * indicates a stereocentre, either *S* or *R*.

palladium and platinum chloride R-pytri complexes have also been reported to display moderate anticancer activity [33–35]. Some of us have examined the use of dimetallic R-pytri complexes as anti-bacterial [36,37] and anti-fungal [38] agents with modest success. Additionally, derivatives of the R-pytri ligands alone have demonstrated potent inhibition of NMRPTase [39] and macrophage migration inhibitory factor [40]. Herein, we show that combining the "click" ligands bnpytri, phpytri and octpytri with *SS*-dach and *RR*-dach ligands resulted in a series of new PCs with moderate anticancer activity.

2. Experimental section

2.1. Materials

The ligands phpytri, bnpytri and octpytri [41], and $[Pt(A_L)Cl_2]$ [6], where A_L is *SS*-dach or *RR*-dach, were prepared using previously reported methods. All purchased reagents were used as received and all solvents used were of analytical grade or higher. Methanol and acetone were obtained from Honeywell, while ethanol was obtained from Chem Supply. Sep-Pak® (20 cc, 2 g) columns were obtained from Waters. Deuterated solvents D_2O and DMSO-d_6 were obtained from Cambridge Isotope Laboratories.

2.2. Physical measurements

Characteristic NMR spectra were obtained using a 400 MHz Bruker Avance nuclear magnetic resonance spectrometer. All spectra were referenced internally to the solvent (either D_2O or DMSO-d_6) and obtained at room temperature. ^1H spectra were obtained using a spectral width of 15 ppm and 128 accumulations. ^1H-^{195}Pt heteronuclear multiple quantum correlation (HMQC) spectra were obtained using a spectral width of 2500 ppm and 256 data points for the ^{195}Pt nucleus (F1 dimension), and a spectral width of 12 ppm and 2048 data points for the ^1H nucleus (F2 dimension). The following abbreviations apply to spin multiplicity: s (singlet), bs (broad singlet), d (doublet), t (triplet), and m (multiplet). The chemical shift (parts per million) of each resonance was quoted as an approximate midpoint of its multiplicity.

Electronic spectra were obtained on a Cary 1E spectrophotometer at a wavelength range of 200–350 nm, using a 10 mm quartz cell. All spectra were recorded at room temperature and were automatically corrected for solvent baseline.

Circular dichroism spectra were recorded using a Jasco-810 spectropolarimeter at room temperature. The instrument was left to equilibrate for 30 min prior to use. Spectra were obtained in a 10 mm quartz cell, and were measured from 200 to 400 nm with a data pitch of 1 nm, bandwidth of 1 nm and response time of 1 s. For each spectrum, 40 accumulations were collected and a water baseline was subtracted.

Positive-mode electrospray ionisation mass spectrometry (ESIMS) experiments were performed using a Waters TQ-MS triple quadrupole mass spectrometer fitted with an ESI source. Spectra were recorded in positive ion mode from analyte solutions injected (10 μL) into 0.1% formic acid in 50% aqueous methanol flowing at 0.1 mL min^{-1}. A capillary voltage of 3.0 kV, cone voltage of 30 V, desolvation temperature of 300 °C and desolvation flow rate (nitrogen) of 500 L h^{-1} were employed. Spectra were collected over 1 min with an *m/z* range of 100–1000.

Microelemental analysis (C, H and N) was performed at the Chemical Analysis Facility, Department of Chemistry and Biomolecular Sciences, Macquarie University. An Elemental Analyser, Model PE2400 CHNS/O produced by PerkinElmer, USA, was used.

2.3. Synthesis of $[Pt(P_L)(A_L)]^{2+}$

Using the previously established method [7], the complex $[Pt(A_L)Cl_2]$ (1 equiv), where A_L is either *SS*-dach or *RR*-dach, was suspended in water with the P_L (1.1 equiv) and refluxed for 24 h, resulting in a clear solution. The solution volume was reduced to approximately 3 mL and filtered. A Sep-Pak® (20 cc, 2 g) column was activated with methanol (10 mL) followed by water (20 mL). The complex solution was loaded onto the column and eluted with water. The fractions containing product were combined, reduced under pressure and lyophilised to produce a solid white product. Yield and characterisation data are presented in Table 1, while NMR chemical shifts are presented in Table 2.

2.4. X-ray crystallography

Crystals of complex **2a** were obtained *via* slow diffusion of acetone into a concentrated solution of **2a** in water. Single Crystal data for **2a** was collected at 100 K on the MX1 beamline at the Australian Synchrotron with an energy equivalent to Mo-Kα radiation (17.4 keV, λ =

Table 1
Summary of the characterisation data of complexes **1–3**.

No.	Molecular formula	Yield (%)	ESI-MS (*m/z*) $[M-H]^+$ Calc.(found)	Microanalysis Calc.(found)			UV/λ_{max} (nm) (ε/$mol^{-1}\cdot dm^3$ cm^{-1})	CD/λ_{max} (nm) (CD/mdeg. $L\cdot mol^{-1}$)
				C	H	N	$\times 10^2$	$\times 10^{-1}$
1a	$[Pt(phpytri)(SS\text{-dach})]Cl_2\cdot 4H_2O$	75	530.2 (530.2)	33.83 (33.96)	4.78 (4.74)	12.46 (12.48)	260 (205), 235 (245)	325 (5), 283 (−11), 246 (−11), 220 (15)
1b	$[Pt(phpytri)(RR\text{-dach})]Cl_2\cdot 4H_2O$	79	530.2 (530.4)	33.83 (34.06)	4.78 (4.82)	12.46 (12.57)	259 (200), 235 (240)	327 (−5), 279 (16), 245 (17), 215 (−11)
2a	$[Pt(bnpytri)(SS\text{-dach})]Cl_2\cdot 3.5H_2O$	76	544.2 (544.4)	35.35 (35.19)	4.90 (4.86)	12.37 (12.21)	292 (95), 230 (260)	323 (6), 288 (−7), 244 (−17), 219 (17),
2b	$[Pt(bnpytri)(RR\text{-dach})]Cl_2\cdot 3.5H_2O$	68	544.2 (544.3)	35.35 (35.15)	4.90 (4.89)	12.37 (12.39)	292 (90), 230 (250)	323 (−6), 288 (11), 244 (18), 222 (−18)
3a	$[Pt(octpytri)(SS\text{-dach})]Cl_2\cdot 4H_2O$	68	567.2 (567.1)	35.49 (35.79)	6.24 (6.14)	11.83 (11.85)	292 (85), 230 (220)	328 (4), 290 (−9), 246 (−16), 224 (14)
3b	$[Pt(octpytri)(RR\text{-dach})]Cl_2\cdot 4.5H_2O$	69	567.2 (567.2)	35.05 (35.16)	6.30 (6.39)	11.68 (11.64)	292 (85), 230 (225)	324 (−4), 291 (13), 245 (20), 222 (−11)

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