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Substituted 9-aminoacridine-4-carboxamides tethered to platinum(II)diamine complexes: Chemistry, cytotoxicity and DNA sequence selectivity

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ABSTRACT

Three platinum complexes in which substituted (7-OMe, 9-NH_2; 7-F, 9-NH_2; and 7-H, 9-$NH(CH_2)_2OH$) 9-aminoacridine-4-carboxamides were tethered to a platinum(II)diamine moiety were synthesised and characterised at the chemical and biological level. These variants showed a decrease in cytotoxicity, as measured by IC_{50} values in HeLa cells, when compared with the parent 7-H, 9-NH_2 compound. The 7-F and 9-$NH(CH_2)_2OH$ substituents gave rise to a small decrease in cytotoxicity, and the 7-OMe substituent resulted in a larger decrease in cytotoxicity. Their binding to purified pUC19 plasmid DNA was investigated and it was found that the addition of 7-F, 9-$NH(CH_2)_2OH$ and especially the 7-OMe substituents, resulted in reduced DNA binding. This correlated well with the IC_{50} cytotoxicity values. However, the DNA sequence selectivity was unaffected by the addition of these moieties.

1. Introduction

Expanding the spectrum of activity of platinum-based antitumour agents is an important goal which has motivated the continued development of drugs of this type [1]. One approach which has been explored is to develop platinum compounds with an altered pattern of DNA damage to that caused by cisplatin and carboplatin. This strategy has been a significant driving force in the development of bi- and trinuclear complexes exemplified by BBR3464 [2,3]. It has also been a key feature in the development of: (i) platinum–acridinylthiourea conjugates [4,5]; (ii) minor groove targeted multinuclear complexes derived from the 4,4′-dipyrazoylmethane ligand [6]; (iii) complexes in which platinum is tethered to the 5′-terminus of an oligothymidine sequence [7]; (iv) binuclear complexes based on a 1,4-diaminoanthraquinone intercalating chromophore [8,9]; and (v) platinum(II) derivatives of distamycin and netropsin analogues [10,11]. We have also explored this concept using intercalating chromophores such as acridine and 9-anilino-acridine [12,13], phenazine [14] and the phenanthridinium cation [15] and extended this work to include a more comprehensive investigation of 9-aminoacridine carboxamides [16–18]. Of the complexes we have studied to date, those based on the 9-aminoacridine-4-carboxamide platform (compound **1**) are of particular note, showing promising activity against cisplatin resistant cells [16] and exhibiting a different DNA sequence specificity to that of cisplatin [17], shifted away from runs of consecutive guanines (the main binding site for cisplatin). This sequence specificity was shown to be dependent on linker chain length, with the shorter chain length homologues ($n=2$, 3) showing the greatest alteration in DNA sequence specificity, whereas the longer chain length homologues ($n=4$, 5) more closely resembled cisplatin and $PtenCl_2$ in their behaviour [17]. The latter study was the first occasion that an altered sequence specificity was convincingly demonstrated for a cisplatin analogue of the diamine type. We have also shown that the members of the class displayed an altered sequence specificity in intact human cells [18], preferentially targeting GA sequences rather than consecutive G bases. The DNA sequencing results suggest that it is the presence of the 9-amino group in the 9-aminoacridine-4-carboxamide chromophore which results in this positional targeting of platinum. The presence of the intercalator is also expected to rapidly localise compounds similar to **1** on DNA and we have previously shown that these compounds show a marked increase in the rate of DNA platination when compared with cisplatin or $PtenCl_2$ [17].

Since the 9-aminoacridine-4-carboxamide Pt complex (**1**, $n=2$) showed the best activity in earlier studies, we initiated structure/activity studies on substituted 9-aminoacridine complexes, with the ultimate aim of optimizing antitumour activity. We chose to investigate the set of analogues shown in Fig. 1, with an $n=2$ linker

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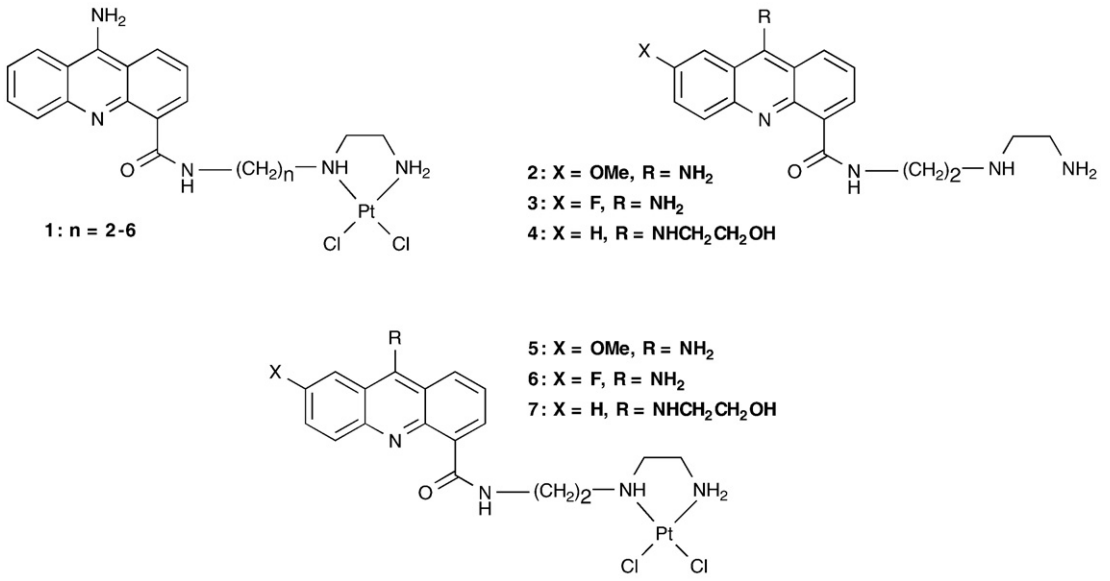


Fig. 1. The structure of the 9-aminoacridine-4-carboxamide Pt complex (**1**) and related compounds used in this study.

chain, since this linker length previously showed the most significant alterations to DNA specificity in the set of parent compounds. We examined the effects of substituents at the 7-position of the acridine ring and alterations to the amino group at the 9-position on the behaviour of these compounds, and report the synthesis, cytotoxicity and sequence specificity of these compounds.

2. Results and discussion

2.1. Chemistry

The acridine ligands **2**–**4** were prepared as shown in Scheme 1. The differentially protected triamine **13** was synthesised by first selectively protecting one of the primary amine groups of diethylenetriamine by reaction with ethyltrifluoroacetate at −78 °C [19] followed by Boc protection of the remaining two amine functional groups. The triamine **13** was reacted selectively with the appropriately substituted 9-chloroacridine-4-carbonyl chloride to give the 9-chloro compounds **8a**, **9a**, and **10a**. These compounds were then activated by *in situ* conversion to the 9-phenoxy compounds which on treatment with ammonia (**8a** and **9a**) or ethanolamine (**10b**) gave the Boc-protected ligand precursors **8b**, **9b**, and **10b** respectively. Deprotection of these was carried out using methanolic HCl to form ligands **2**, **3**, and **4** as their hydrochloride salts. Each of these was characterised by ^{1}H NMR, ESI-MS and elemental analysis.

Preparation of the complexes was carried out by addition of an aqueous solution of K_2PtCl_4 to a solution of the ligand trihydrochlorides in water which had been adjusted to pH 8–9 by the addition of carbonate. The complexes **5** and **6** were obtained in pure form directly from the reaction mixture although in the case of **7** purification was necessary. The ^{195}Pt chemical shift values for the complexes fall at about δ −2350 ppm and are indicative of a platinum coordination to a chelating diamine and two chloride donors in accord with the structures of the complexes. These shift values are similar to those obtained for the previously reported complexes in this series **1** [16] and for Pt(en)Cl_2 (−2345 ppm) [20]. ESI-MS was also used to characterise the platinum complexes **5**–**7**. The complexes were sprayed from H_2O/DMF mixtures and the spectra showed the presence of the $[M+H]^+$, $[M-Cl]^+$ and $[M-HCl_2]^+$ ions, each of which displayed the expected isotopic distribution.

2.2. In vitro cytotoxicity

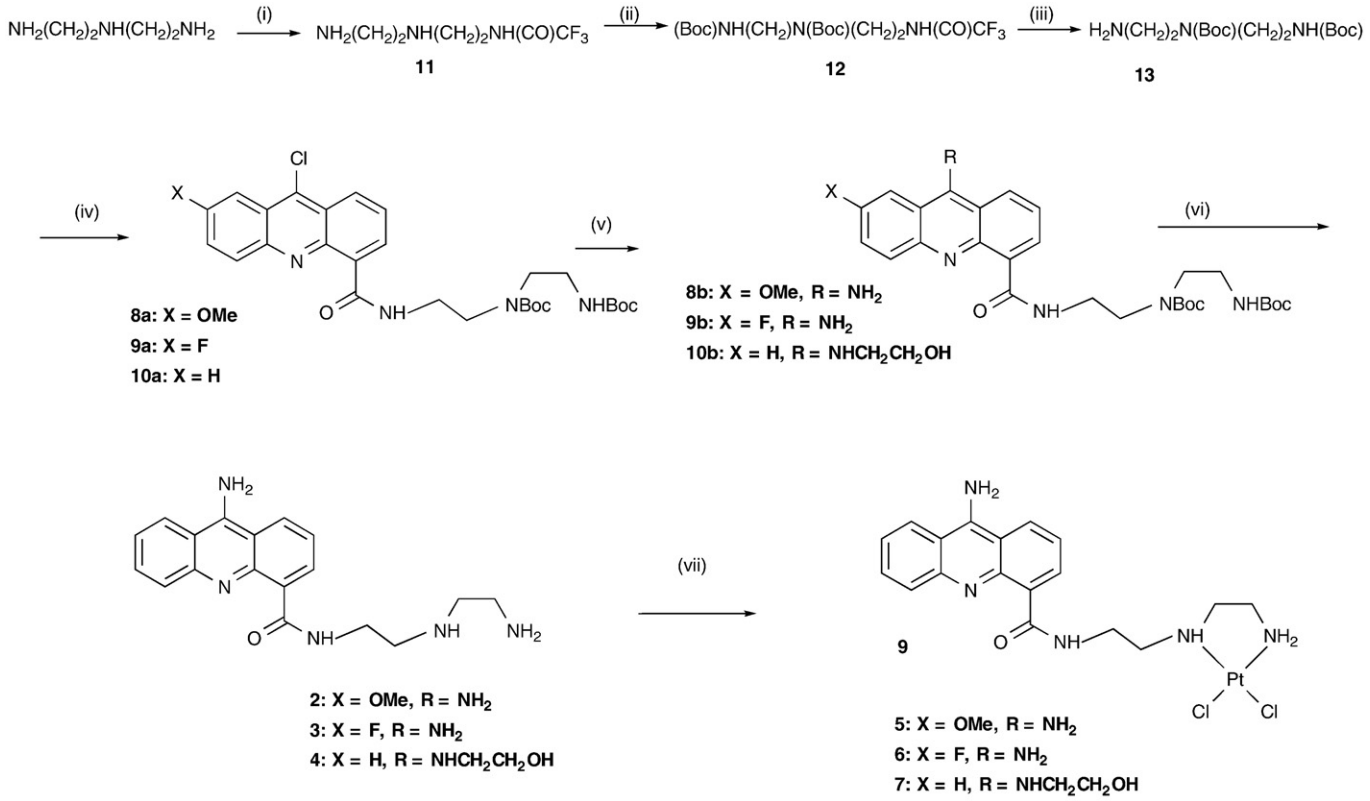
The cytotoxicity of the acridine Pt complexes was compared to that of cisplatin and PtenCl$_2$. The IC_{50} values were determined in the human cell line HeLa using an amylar blue assay [21,22] and are presented in Table 1. Compound **1** ($n=2$) was the most potent in killing HeLa cells with an IC_{50} value of 0.4 μM (Table 1), with **6** and **7** having IC_{50} values between 1.1 and 1.3 μM. Cisplatin, PtenCl$_2$, and compound **5** were the least potent, with IC_{50} values of approximately 10 μM. Thus the addition of substituents to the 9-aminoacridine ring at different positions and of varied nature (fluoro in **6**, 9-NH$(CH_2)_2$OH in **7**, and especially methoxy in **5**) reduced the ability of the compound to kill HeLa cells.

2.3. The interaction of the complexes with purified plasmid DNA

The interaction of the analogues with purified DNA was investigated using plasmid DNA. Compounds **6** and **7** required a slightly higher concentration of drug to that of **1** ($n=2$) in order to achieve a comparable level of DNA damage, whereas **5** required about a 2-fold higher concentration. The presence of the electron donating methoxy substituent in **5** and electron withdrawing fluoro substituent in **6** affects the charge distribution and the steric outline of the acridine intercalating moiety, and both of these factors could influence the DNA binding of these compounds compared with the unsubstituted parent. Because of the known [23] parallel intercalation mode of compounds of this class, it seems likely that the steric changes are a more important influence on binding, with the larger OMe group having the greater deleterious effect. In contrast, the 9-NH$(CH_2)_2$OH substituent in **7** did not produce a major decrease in the binding of the compound to DNA, possibly because this substituent will lie in the minor groove where its OH group can be accommodated, and may form stabilising H-bond interactions. The decreased DNA binding of **5** (with a methoxy substituent) correlated with the decreased cytotoxicity of the compound.

2.4. The DNA sequence selectivity in purified plasmid DNA

Preliminary experiments to determine the DNA sequence selectivity of these cisplatin analogues in purified plasmid DNA indicated that compounds **5**, **6** and **7** had a very similar profile to that of **1**



Scheme 1. [a]Reagents: (i) CF3CO2Et, −78 °C, MeOH; (ii) (t-BuO)2CO; (iii) NH3(aq), 25%, RT; (iv) **8a**: 9-chloro-7-methoxyacridine-4-carbonyl chloride; **9a**: 9-chloro-7-fluoroacridine-4-carbonyl chloride, **10a**: 9-chloroacridine-4-carbonyl chloride, phenol/NH2CH2CH2OH/120 °C; (v) **8b**: phenol/NH3/120 °C, **9b**: phenol/NH3/120 °C, **10b**: phenol/NH2CH2CH2OH/120 °C, (vi) conc. HCl/MeOH/CH2Cl2; (vii) Na2CO3, K2PtCl4.

($n=2$). In these experiments damage at sites other than consecutive guanines were prominent as previously found for **1** ($n=2$) [17,24–26]. The sequence selectivity of **1** ($n=2$), **5**, **6** and **7** was different to that of cisplatin as the damage sites were shifted away from consecutive guanines. This also indicated that despite a change in the efficiency of damaging DNA, the DNA sequence selectivity of compound **5** was unaffected and similar to that of **1** ($n=2$), **6** and **7**.

The similar DNA sequence specificity of compound **7** (with the 9-NH$(CH_2)_2$OH substitution) compared to compounds **1** ($n=2$), **5**, and **6** was interesting as previously we had shown that the addition of the 9-amino substituent to the acridine was crucial for the alteration in the DNA sequence selectivity compared to cisplatin [17,24]. This indicated that the 9-amino group can be modified to 9-NH$(CH_2)_2$OH without affecting the DNA sequence selectivity of the compound, despite the presence of the extra steric bulk of the latter substituent.

2.5. The DNA sequence selectivity in intact human cells

The interaction of the compounds in intact human cells has also been investigated using the linear amplification assay [24,27,28].

Table 1
The IC_{50} of cisplatin and analogues in HeLa cells.

Compound	Substituent on acridine ring	IC_{50} (μM)
Cisplatin	–	10
$PtenCl_2$	–	11
1 ($n=2$)	None	0.4
5	Methoxy	10
6	Fluoro	1.3
7	9-NH$(CH_2)_2$OH	1.1

Again compound **5** was the least efficient at damaging DNA in intact cells, with **6** and **7** the next most efficient, and **1** ($n=2$) the most efficient. This pattern of damage correlates with the IC_{50} values. Preliminary DNA sequence selectivity experiments indicated that the DNA sequence selectivity profiles of compounds **5**, **6** and **7** were very similar to that of **1** ($n=2$) and different to cisplatin. Again sites that did not consist of consecutive guanines, were found to be prominently damaged as found previously [18,24,27].

2.6. Conclusions

The variation of substituents on the 9-aminoacridine carboxamide platform, (fluoro in compound **5**, 9-NH$(CH_2)_2$OH in **7**, and methoxy in **5**) reduced the cytotoxicity of these compounds as measured by IC_{50} values when compared to the parent compound. The presence of the fluoro and 9-NH$(CH_2)_2$OH substituents gave rise to a small decrease in cytotoxicity, while the methoxy substituent resulted in a large decrease in cytotoxicity. The DNA binding studies produced evidence that the reduction in cytotoxicity (on addition of a substituent), was due to a reduction in interaction with DNA. However, the DNA sequence selectivity was unaffected by the addition of these moieties.

3. Experimental

3.1. Chemistry

^1H and ^{195}Pt NMR spectra were measured on Varian UnityPlus 400 MHz or Unity 300 spectrometers. ^1H chemical shifts were measured relative to the residual hydrogens of bulk solvent (δ 4.85 for D_2O, 7.26 for $CDCl_3$ and 2.49 for DMSO) and quoted relative to TMS. The ^{195}Pt reference was a 0.1 M solution of K_2PtCl_4 in D_2O

(δ −1630). ESI-MS spectra were recorded on a quadrupole ion trap Finnigan-MAT LCQ mass spectrometer equipped with electrospray ionisation. Triethylamine was redistilled from toluene-4-sulfonyl chloride. N,N-Dimethyl formamide and N-methyl pyrrolidinone were dried over 4 Å molecular sieves.

The compounds 9-acridone-4-carboxylic acid, 7-methoxy-9-acridone-4-caboxylic acid and 7-fluoro-9-acridone-4-caboxylic acid were prepared by reported methods [29].

3.2. N-(2-aminoethyl)-N,N′-bis(tert-butoxycarbonyl)ethylenediamine (**13**)

A solution of diethylenetriamine (11.4 g, 111 mmol) in methanol (50 mL) was cooled to −78 °C under nitrogen. Ethyl trifluoroacetate (15.7 g, 111 mmol) in methanol (50 mL) was added dropwise over 10 min and then the mixture was allowed to warm to room temperature over a period of 3 h followed by the addition of $NaHCO_3$ (40 g). A solution of di-*tert*-butyldicarbonate (53.1 g, 243 mmol) in methanol (20 mL) was then added over a 5 min period, the addition being accompanied by some effervescence. The mixture was then sonicated for 20 min and set aside to stir overnight. The solvent was then removed and the residue partitioned between water and ethyl acetate. The organic phase was dried (Na_2SO_4) and the solvent removed to yield a viscous pale yellow oil. (^{1}H NMR confirmed this product as the Boc-protected trifluoroacetamide **12**). This material was taken up in ethanol (100 mL) and water (20 mL) and then potassium carbonate (40 g) was added. The mixture was heated on the steam bath for 6 h then stirred for a further 12 h at room temperature. The solids were then removed by filtration and the solvents removed from the filtrate under reduced pressure. The residue was partitioned between water and ethyl acetate and the organic phase was dried (Na_2SO_4). The solvent was removed under reduced pressure to afford a viscous oil that solidified over several days to form a white waxy solid (26.2 g, 78%). ^{1}H NMR ($CDCl_3$) δ = 1.41 (m, 18H), 2.62 (s, 2H), 2.8–3.4 (m, 8H), 5.1 (bs, 1H); ESI-MS *m/z* (relative intensity), 304.3 (100, $[M+H]^+$), 204.1 (40), 104.1 (85). Anal. Calc. for $C_{14}H_{29}N_3O_4$: C, 55.42; H, 9.63; N, 13.85. Found: C 55.38; H, 9.74; N, 13.80.

3.3. N-[2-[(2-aminoethyl)amino]ethyl]-9-[(2-hydroxyethyl)amino] acridine-4-carboxamide (**4**)

Acridone-4-carboxylic acid (2.39 g, 10 mmol) was converted to 9-chloroacridine-4-carbonyl chloride by a reported procedure [30]. The acid chloride was dissolved into a mixture of CH_2Cl_2 (50 mL) and Et_3N (5 mL), then a solution of compound **13** (2.79 g, 10 mmol) in CH_2Cl_2 (20 mL) was added and the mixture was stirred at ambient temperature overnight. The volatiles were removed under reduced pressure and the residue of crude 9-chloroacridine-4-carboxamide was suspended in N-methyl pyrrolidinone (5 mL) containing phenol (1.412 g, 15 mmol). Heating the rapidly stirred mixture was commenced and ethanolamine (0.92 g, 15 mmol) was added once 80 °C was reached. The mixture was heated between 80 and 100 °C for 3 h, and then let cool. It was then partitioned between EtOAc and 1 M aq. NaOH. The organic phase was dried (Na_2SO_4) and evaporated to yield an impure compound **10b** as a brown oil. Chromatography on SiO_2 eluting with EtOAc removed the mobile impurities and Me_2CO eluted the desired component as a bright yellow band. The solvent was removed to yield compound **10b** (2.85 g). The Boc groups were removed by stirring this material in methanolic HCl (44 mL) for 3 h. (The HCl solution was generated by adding acetyl chloride (4 mL) to ice cold MeOH (40 mL) and then allowed the solution to stand for 10 min). Following solvent removal at reduced pressure, the residue was recrystallised from MeOH/EtOH. The hydrochloride salt obtained in this way is a hygroscopic amorphous yellow solid that becomes gummy upon exposure to atmospheric moisture but solidifies again upon standing in a sealed vial over a few days (1.53 g, 31%). ^{1}H NMR (D_2O) δ = 8.57 (d, 1H, J = 8.5 Hz), 8.37 (d, 1H, J = 8.7 Hz), 8.31 (d, 1H, J = 7.5 Hz), 7.97 (t, 1H, J = 7.8 Hz), 7.79 (d, 1H, J = 8.6 Hz), 7.58 (t, 2H, J = 8.0 Hz), 4.28 (t, 2H, J = 4.9 Hz, CH_2–O), 4.08 (t, 2H, J = 4.9 Hz, Acr–N–CH_2–), 3.91 (t, 2H, J = 5.5 Hz, CONH–CH_2), 3.58 − 3.46 (m, 6H, CH_2–N). ESI-MS (H_2O) *m/z* (relative intensity), 368.2 (70, $[M+H]^+$); 308.2 (100, $[M+H–NHCH_2CH_2OH]^+$).

Anal. Calc. for $C_{20}H_{25}N_5O_2.3HCl.0.75H_2O$ requires: C, 48.63; H, 6.27; N, 13.83; Cl, 21.00. Found: C, 48.74; H, 6.24; N, 13.49; Cl, 20.80.

3.4. (N-[2-[(2-aminoethyl)amino]ethyl]-9-[(2-hydroxyethyl)amino] acridine-4-carboxamide)dichloroplatinum(II) (**7**)

A solution of K_2PtCl_4 (87 mg, 0.21 mmol) in H_2O (1 mL) was filtered into a stirred solution of compound **4** (100 mg, 0.21 mmol) in H_2O (1.5 mL) that had been brought to pH 8 with aqueous Na_2CO_3 solution (1 M). An orange amorphous solid separated almost immediately. The suspension was stirred rapidly at ambient temperature overnight. LiCl (89 mg, 2.1 mmol) was then added and the mixture again brought to pH 8 with aqueous Na_2CO_3 (1 M). After another day stirring at ambient temperature the solid was collected, washed with water and dried at 50 °C. This solid was then dissolved in hot (90 °C) DMF (3 mL) and the solution was filtered into aqueous LiCl (33 mL, 1 M). The precipitated solid was collected by centrifugation, the supernatant liquid decanted off and the solid washed with water (2 × 10 mL) and then dried for 4 h under reduced pressure (44 mg, 29%). ^{195}Pt NMR (NMP) δ −2351.4. ESI-MS *m/z* (relative intensity), 634.3 (25, $[M+H]^+$), 594.4 (10, $[M-Cl]^+$), 560.3 (100, $[M-HCl_2]^+$). Anal. Calc for $C_{20}H_{25}N_5O_2PtCl_2.HCl.3H_2O$ requires: C, 33.3; H, 4.5; N, 9.7. Found: C, 33.3; H, 4.4; N, 9.8.

3.5. 9-amino-N-[2-[(N,N′-bis(tert-butoxycarbonyl)ethylenediamino)]-ethyl]-7-methoxyacridine-4-carboxamide (**8b**)

7-Methoxy-9-acridone-4-caboxylic acid (1.30 g, 4.83 mmol) was heated in thionyl chloride (20 mL) containing one drop of DMF for 1 h. Excess thionyl chloride was removed under reduced pressure and the residue mixed with toluene, followed by removal of volatiles under reduced pressure. The residue was suspended with stirring in CH_2Cl_2 (20 mL) and to this was added compound **13** (1.21 g, 4.0 mmol) dissolved in triethylamine (10 mL) and DCM (10 mL) with a darkening in color. The mixture was stirred overnight at room temperature and then DCM (50 mL) was added. The mixture was washed with 10% $NaHCO_3$ solution, then dried ($MgSO_4$) and the solvent removed under reduced pressure. To the residue was added benzene (10 mL) and phenol (20 g) and the mixture was heated with stirring to 90 °C, with most of the benzene being allowed to boil off. The mixture was then cooled to 45 °C and ammonia gas was bubbled through the stirred mixture which was then heated to 110 °C for 10 min followed by further addition of ammonia gas. The mixture was then cooled to room temperature and CH_2Cl_2 added. After washing twice with 10% NaOH solution the organic phase was dried ($MgSO_4$) then volatiles removed under reduced pressure. The residue was subjected to silica chromatography (EtOAc) to afford compound **8b** as a yellow powder (1.26 g, 57%). ^{1}H NMR (d_6-DMSO) δ = 1.28 (m, 18H), 3.02 (m, 2H), 3.21 (m, 2H), 3.37 (m, 2H), 3.58 (m, 2H), 3.84 (s, 3H), 6.25 (bs, exch), 6.60 (bs, exch), 7.35 (m, 2H), 7.55 (s, 1H), 7.70 (m, 1H), 8. 41 (d, 1H, J = 5.2 Hz), 8.48 (d, 1H, J = 2.0 Hz).

3.6. 9-amino-N-[2-[(2-aminoethyl)amino]ethyl]-7-methoxyacridine-4-carboxamide (**2**)

Acetyl chloride (5 mL) was added dropwise to stirred methanol (30 mL) at room temperature with the evolution of heat. Compound **8b** (0.45 g) was dissolved in methanolic HCl (35 mL) (prepared by adding acetyl chloride (5 mL) to methanol 30 mL) and stirred for 3 h

at room temperature at which time TLC indicated the absence of starting material. Volatiles were removed under reduced pressure and diethyl ether (20 mL) was added. The resulting suspension was sonicated, and the solid collected by centrifugation. The product was dried under reduced pressure to afford **2** as the trihydrochloride salt which was a hygroscopic orange powder (0.35 g, 86%). ^{1}H NMR (D_2O) δ = 3.38–3.55 (m, 6H), 3.77 (s, 3H), 3.83 (t, 2H, J = 6.4 Hz), 7.01 (s, 1H), 7.41 (d, 1H, J = 9.2 Hz), 7.44 (d, 1H, J = 9.2 Hz), 7.50 (t, 1H, J = 7.6 Hz), 8.21 (d, 1H, J = 8.4 Hz), 8.25 (d, 1H, J = 7.2 Hz). ESI-MS *m/z* (relative intensity), 354.4 (95, $[M+H]^+$), 303.3 (44). Anal. Calc. for $C_{19}H_{26}N_5O_2$.3HCl.MeOH requires: C, 48.54; H, 6.11; Cl, 21.49; N, 14.15. Found: C, 48.74; H, 6.24; Cl, 20.89; N, 13.79.

3.7. (9-amino-N-[2-[(2-aminoethyl)amino]ethyl]-7-methoxyacridine-4-carboxamide)dichloroplatinum(II) (5)

Compound **2** (200 mg, 0.432 mmol) was dissolved in water (10 mL) and the pH was adjusted to 8–9 with 1 M aqueous Na_2CO_3. A solution of K_2PtCl_4 (179 mg, 0.432 mmol) in water (4 mL) was added and the mixture was stirred in the dark for 24 h at room temperature. A solution of 5% aqueous KCl (30 mL) was then added, and the mixture was stirred for a further 2 h. The resulting solid was collected and washed several times with distilled water. After drying at 80 °C this material was ground to a fine powder, washed several times with distilled water and dried to give **2** as an orange powder (214 mg, 80%). ^{195}Pt NMR (DMF) δ −2353.2. ESI-MS *m/z* (relative intensity), 619.3 ($[M+H]^+$, 22), 682.4 ($[M-Cl]^+$, 45), 645.1 ($[M-HCl_2]^+$, 12). Anal. Calc. for $C_{19}H_{23}Cl_2N_5O_2Pt$ requires C, 36.84; H, 3.74; Cl, 11.45; N, 11.31. Found: C, 36.67; H, 3.69, Cl, 11.51; N, 11.27.

*3.8. 9-amino-N-[2-[(N,N′-bis(tert-butoxycarbonyl)ethylenediamino)-ethyl]-7-fluoroacridine-4-carboxamide (**9b**)*

This was prepared in a similar way to compound **8b** but in this case by reaction between 7-fluoro-9-acridone-4-caboxylic and compound **13**. ^{1}H NMR (DMSO) δ = 1.2–1.4 (m, 18H), 3.10 (m, 2H), 3.35 (m, 2H), 3.54 (m, 2H), 3.71 (m, 2H), 6.82 (bs, exch), 7.49 (t, 1H, J = 7.1 Hz), 7.72 (m, 1H), 8. 01 (m, exch), 8.28 (dd, 1H, J = 1.8,7.1 Hz), 8.60 (d, 1H, J = 7.1 Hz), 8.66 (d, 1H, J = 4.4 Hz). ESI-MS *m/z* (relative intensity), 542.2 ($[M+H]^+$, 100). Anal. Calc. for $C_{28}H_{36}FN_5O_5$ requires: C, 62.09; H, 6.70; N, 12.93. Found: C, 62.07; H, 6.84; N, 12.86.

*3.9. 9-amino-N-[2-[(2-aminoethyl)amino]ethyl]-7-fluoroacridine-4-carboxamide (**3**)*

This was prepared in a similar way to **2** but in this case by reaction between compound **9b** (0.45 g) and HCl in methanol (0.33 g, 83%). ^{1}H NMR (D_2O) δ 3.25–3.35 (m, 6H), 3.70 (t, 2H, J = 5.6 Hz), 7.36 (t, 1H, J = 7.2 Hz), 7.56 (m, 3H), 8.10 (d, 1H, J = 8.4 Hz), 8.13 (d, 1H, J = 7.2 Hz). Anal. Calc. for $C_{18}H_{20}FN_5O$.3HCl.MeOH requires: C, 47.27; H, 5.64; N, 14.51; Cl, 22.03. Found: C, 46.95; H, 5.31; N, 14.53; Cl, 21.85.

*3.10. (9-amino-N-[2-[(2-aminoethyl)amino]ethyl]-7-fluoroacridine-4-carboxamide)dichloroplatinum(II) (**6**)*

This was prepared in a similar way to **5** but in this case by reaction between compound **3** (200 mg) and K_2PtCl_4 in water (209 mg, 78%). ^{195}Pt NMR (NMP) δ −2351.4. ESI-MS *m/z* (relative intensity), 608.3 (100, $[M+H]^+$), 571.3 (15, $[M-Cl]^+$), 536 (12, $[M-HCl_2]^+$). Anal. Calc. for $C_{18}H_{20}Cl_2FN_5OPt$.HCl requires: C, 33.58; H, 3.29; Cl, 16.52; N, 10.88. Found: C, 33.51; H, 3.34; N, 10.92.

3.11. Cytotoxicity assays

The IC_{50} values were determined in the human cell line HeLa using an amylar blue assay as previously described [21,22]. Briefly this involved seeding of 4×10^6 HeLa cells per well, growth for 24 h, addition of amylar blue, further growth for 48 h, and measurement of fluorescence at 590 nm after excitation at 544 nm. At least three independent experiments were performed for each IC_{50} value. Solvent only controls were also employed in each experiment.

3.12. Sequence selectivity studies

The DNA sequence specificity of the Pt complexes was determined as previously described for purified pUC19 plasmid DNA [17,24–26] and intact human cells [24,27,28]. The DNA sequence specificity studies were performed on at least three occasions.

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