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Synthesis and biological evaluation of platinum–acridine hybrid agents modified with bipyridine non-leaving groups

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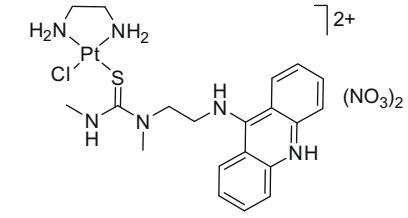
ABSTRACT

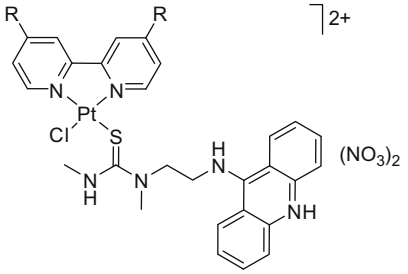
The use of 2,2′-bipyridines (4,4′-R_2-2,2′-bpy; R = H, Me, OMe, CF_3) as non-leaving groups (L–L) in platinum–acridinylthiourea conjugates, [PtCl(L-L)(ACRAMTU)]$(NO_3)_2$, has been investigated. All bpy-substituted complexes (**2**–**5**) show micromolar activity in HL-60 (leukemia) and H460 (lung) cancer cell lines but proved to be significantly less potent than the prototypical compound (**1**) containing aliphatic ethane-1,2-diamine. NMR and mass spectrometry data indicate that bpy accelerates the reaction of platinum with DNA nitrogen, but the resulting adducts are more labile than those formed by the prototype.

There is a continued interest in novel platinum-based agents that have the potential to overcome the limitations associated with current oncology drugs.[1] Platinum–acridine conjugates represented by the prototype, [PtCl(en)(ACRAMTU)]$(NO_3)_2$ ('PT-ACRAMTU', **1**, Fig. 1; en = ethane-1,2-diamine, ACRAMTU = 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea, acridinium cation), hold considerable promise as a new armamentarium against intractable cancer.[2] These non-cross-linking hybrid agents may combat tumor resistance to clinical platinum drugs by inducing structurally unique monofunctional–intercalative adducts with DNA.[3–5] Several structure–activity relationship studies have been performed indicating that efficient platination of DNA is an important determinant of the cytotoxic potential of these agents.[6–8] This has most recently been demonstrated for derivatives of compound **1** containing *N*-[2-(acridin-9-ylamino)ethyl]-*N*-methylpropionamidine, an amidine analogue of thiourea-based ACRAMTU.[9] A simple chemical modification (substitution of a thiourea-S with an amidine-N donor group) resulted in significantly increased DNA binding levels and potent activity in rapidly proliferating chemoresistant[10,11] non-small-cell lung cancer (NSCLC) in vivo.[9]

In a search for novel mechanisms of tuning the reactivity of the metal center we also studied the effect of ligands known to accelerate substitution reactions in Pt(II) complexes through π-backbonding effects. Eldik and co-workers showed that the substitution of aqua ligands by nucleobase nitrogen in diaminediaquaplatinum(II) complexes containing polypyridines, such as 2,2′-bipyridine (bpy), proceeds significantly faster than analogous reactions involving



1 (PT-ACRAMTU)



2: R = H
3: R = OMe
4: R = Me
5: R = CF_3

Figure 1. Structures of platinum–acridine hybrid agents **1**–**5**.

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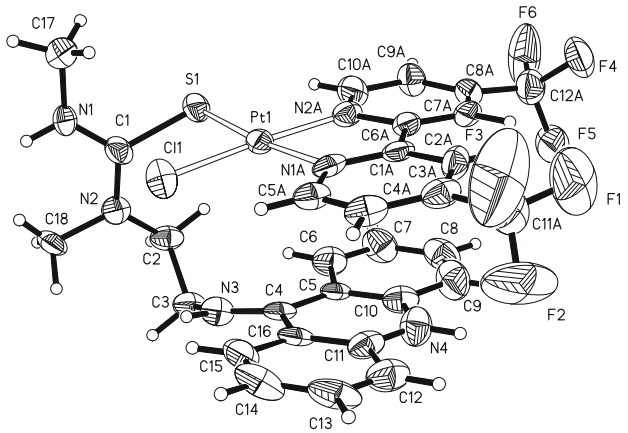

Figure 2. Structure of complex **5** in the solid state with non-hydrogen atoms labeled. Nitrate counter ions have been omitted for clarity. Only one of the two independent molecules in the asymmetric unit is shown.

aliphatic amines due to the greater *trans*-labilizing influence of the sp^2 nitrogen donors.[12] These observations prompted us to synthesize a series of PT-ACRAMTU derivatives, [PtCl(4,4′-R_2-2,2′-bpy)(ACRAMTU)]$(NO_3)_2$ (**2**–**5**, Fig. 1) and compare their cell kill potential with that of the en-substituted prototype, **1**. Electron-donating and electron-withdrawing groups (R) were introduced to tune the π-acceptor properties[13] of the bpy moieties.

The desired dicationic conjugates **2**–**5** (Fig. 1) were synthesized[14] from the neutral complexes, [$PtCl_2$(4,4′-R_2-2,2′-bpy)], which were obtained by reacting $K_2[PtCl_4]$ with the appropriate bipyridine derivative.[13,15,16] To generate the platinum–acridine hybrids, **2**–**5**, one chloro ligand in the precursor complexes was abstracted with one equivalent of silver nitrate in DMF, and the monoactivated precursors were then reacted with ACRAMTU (acridinium nitrate salt) as described previously.[17]

A common structural feature in the bpy-substituted complexes appears to be the intramolecular π-stacking at van der Waals contact distance of the acridinium chromophore with the Pt(bpy) moieties. This distinct interaction, which was previously predicted to occur in solution based on chemical shift anomalies observed in ^{1}H NMR spectra and confirmed by X-ray crystallography for complex **2**,[7] has now also been established in the solid state structure of derivative **5** (Fig. 2). A comparison of the solid state structures of **2** and **5** also suggests that the electron-withdrawing trifluoromethyl groups in **5** have no effect on the donor/acceptor properties of bpy nitrogen. While the average Pt–N bond length in complex **2** is 2.022(4) Å,[7] it is 2.018(7) Å in complex **5**. Likewise, no significant differences are observed in the Pt–Cl [2.292(2) Å for **2**,[7] 2.282(2) Å for **5**] and Pt–S [2.292(2) Å for **2**,[7] 2.291(2) Å for **5**] bond lengths. Thus, the solid state structure of **5** provides no indication of enhanced (ground state) *trans*-labilization of the chloro leaving group by 4,4′-$(CF_3)_2$-bpy compared to unmodified bpy, however, a kinetic *trans*-effect cannot be ruled out.

The cytotoxicity data determined for **1**–**5** in human promyelocytic leukemia (HL-60) and non-small cell lung cancer (H460) cell lines using cell proliferation assays[18] are summarized in Table 1. Based on the IC_{50} values, compounds **2**–**5** are considerably less active than PT-ACRAMTU (**1**), and compound **2** containing unsubstituted 2,2′-bipyridine is the most cytotoxic bpy derivative. The bpy complexes perform better in the solid tumor cell line than in the leukemia cell line, however, this cell-line specific effect is less pronounced than that observed for prototype **1** and several of its previously studied analogues. The amidine-modified derivative of **1**, for instance, proved to be 100-fold more cytotoxic in H460 than in HL-60 cells. No major differences in cell kill potential are observed between compounds containing bpy ligands modified

Table 1
Cytotoxicity (IC_{50}, μM[a]) of platinum–acridines **1**–**5** in HL-60 (leukemia) and NCI-H460 (lung) cancer cell lines

Compd	L–L	HL-60	NCI-H460
1[b]	En	2.9 ± 1.8	0.26 ± 0.1
2[c]	Bpy	5.0 ± 0.2	1.6 ± 0.2
3	4,4′-$(OMe)_2$-bpy	15.0 ± 0.5	4.8 ± 0.6
4	4,4′-$(Me)_2$-bpy	12.9 ± 1.1	4.7 ± 0.7
5	4,4′-$(CF_3)_2$-bpy	10.1 ± 0.9	3.5 ± 0.2

[a] Average of a minimum of three experiments in a colorimetric cell proliferation assay.
[b] Ref. 8.
[c] Ref. 7.

with electron-donating 4,4′ substituents (in **3** and **4**) or electron-withdrawing groups (in **5**).

One possible explanation for the significantly reduced activity of compounds **2**–**5** compared to that of PT-ACRAMTU (**1**) may lie in the relative lability of the DNA adducts they form. In DNA polymerase inhibition assays, complex **2** produced distinct stop sites in sequences of consecutive guanine (G) bases, reminiscent of cisplatin's DNA damage profile.[7] This observation is consistent with some degree of covalent modification of the template DNA by platinum (intercalation alone is unable to stall Taq DNA polymerase). Attempts in the current study to isolate and characterize G adducts in enzymatic digests of calf thymus DNA treated with complex **2** using a bioanalytical assay[19] developed for PT-ACRAMTU (**1**) were unsuccessful. The only platinum-containing species detectable by in-line reverse-phase liquid chromatography–mass spectrometry (LC–MS) is the fragment $[Pt(bpy)]^{2+}$ ($[M-H]^+$, m/z 350) associated with aqua and hydroxo ligands. These observations indicate loss of both the chloro and ACRAMTU ligands due to the *trans*-labilizing influence of bpy. However, from the LC–MS data it is unclear if both groups are displaced in reactions of complex **2** with DNA or during the biochemical work-up (digestion, separation) following drug incubation.

To probe the nucleobase binding potential of platinum, model reactions between complex **2** and 2′-deoxyguanosine (dG) were monitored by arrayed ^{1}H NMR experiments[20] (1 mM Pt complex/2 mM dG; D_2O, 37 °C, 15 mM sodium phosphate, pH* 7.0). The data confirm that complex **2**, indeed, reacts with dG with a half-life of approximately 200 min. For comparison, PT-ACRAMTU (**1**) reacts with a half-life of approximately 300 min under the same conditions.[21] This moderate acceleration of guanine binding with **2** is surprising, as analogous studies on diaminediaquaplatinum(II) complexes have shown a sixfold increase in reaction rate upon replacing en with bpy.[12] No free ACRAMTU ligand is observed in the reaction mixture after incubation for 24 h, consistent with selective substitution of chloride by guanine nitrogen. This observation suggests that the loss of the intercalator in **2** observed in the biochemical assays does most likely not occur at the DNA level. Electrospray mass spectra acquired in positive-ion mode of the reaction mixture are dominated by the fragment $[Pt(bpy)ACRAMTU]^{3+}$ ($[M-2H]^+$, m/z 675) and show only traces of the intact adduct $[Pt(bpy)ACRAMTU(dG)]^{3+}$ ($[M-2H]^+$, m/z 942). These results seem to confirm the relative lability and thermal reversibility of the Pt–guanine bond *trans* to the bpy ligand.

Several structural and reactivity features at the DNA level may contribute to the relatively low cytotoxicity of **2**–**5**. On the basis of the chemical and biochemical studies performed on complex **2**, the DNA adducts formed by the bpy-modified complexes can be expected to be more labile than those formed by analogous complexes containing aliphatic amines as non-leaving groups. Such adducts may not be recognized by DNA-processing enzymes. The inability to form strong, permanent bonds with DNA, among other factors, including altered complex uptake, distribution, and

detoxification, may render **2–5** only moderately cytotoxic agents. On the other hand, the geometry of the complexes may adversely affect the DNA interactions of the bpy complexes. The folding of the acridine chromophore on top of the platinum moiety observed in these agents may prevent efficient nucleophilic attack of the metal by DNA nitrogen, which may explain the slower than expected dG binding kinetics. Furthermore, self-stacking may compete with intercalation of acridine into double-stranded DNA, which has been identified as a prerequisite for submicromolar activity in this class of agents.[17] Finally, the 2–3-fold decrease in activity resulting from the addition of the 4/4′ substituents to the bpy scaffold in **3–5** may be caused by a clash between the non-leaving group and the DNA helix as a consequence of the additional steric bulk.

In conclusion, the bpy ligands introduced as non-leaving groups appear to accelerate ligand substitution but may produce unfavorable electronic and steric effects that prevent efficient target binding of the new complexes and compromise the stability of their DNA adducts.

Acknowledgments

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[PtCl(4,4′-$(MeO)_2$-2,2′-bpy)(ACRAMTU)]$(NO_3)_2\cdot H_2O$ (**3**$\cdot H_2O$). Yield 0.154 g (42%). ^{1}H NMR (D_2O): δ 8.48 (1H, d, J = 6.3), 8.03 (2H, d, J = 8.7), 7.98 (1H, d, J = 6.6), 7.76 (2H, t, J = 7.8), 7.45 (2H, d, J = 8.4), 7.26 (2H, t, J = 7.2), 7.00 (1H, s), 6.82 (1H, s), 6.81 (2H, d, J = 8.4), 6.68 (1H, d, J = 5.1), 4.42 (2H, m), 4.00 (3H, s), 3.88 (3H, s), 3.66 (2H, m, J = 6.9), 3.22 (3H, s), 3.21 (3H, s). ES-MS (MeOH, +ve mode) m/z: 896.23 $[M-H]^+$. Anal. Calcd $C_{30}H_{35}ClN_8O_9PtS$: C, 39.42; H, 3.86; N, 12.27. Found: C, 38.96; H, 3.87; N, 12.01.
[PtCl(4,4′-Me_2-2,2′-bpy)(ACRAMTU)]$(NO_3)_2\cdot 2H_2O$ (**4**$\cdot 2H_2O$). Yield 0.214 g (46%). ^{1}H NMR (D_2O): δ 8.45 (1H, d, J = 5.5), 8.00 (2H, d, J = 9.0), 7.95 (1H, d, J = 5.5), 7.74 (2H, t, J = 7.5), 7.39 (2H, d, J = 8.0), 7.38 (1H, s), 7.22 (3H, br s, t, overl.), 7.08 (1H, d, J = 5.5), 6.94 (1H, d, J = 5.5), 4.71 (2H, br m), 4.56 (2H, br m), 3.25 (3H, s), 3.22 (3H, s), 2.41 (3H, s), 2.25 (1H, 3). ES-MS (MeOH, +ve mode) m/z: 863.16 $[M-H]^+$. Anal. Calcd $C_{30}H_{37}ClN_8O_8PtS$: C, 40.04; H, 4.15; N, 12.27. Found: C, 40.25; H, 4.17; N, 12.08.
[PtCl(4,4′-$(CF_3)_2$-2,2′-bpy)(ACRAMTU)]$(NO_3)_2$ (**5**). Yield 0.200 g (62%). ^{1}H NMR (MeOH-d_4): δ 9.45 (1H, d, J = 6.0, Pt satellites), 9.29 (1H, d, J = 6.0, Pt satellites), 9.04 (2H, d, J = 8.4), 8.10 (1H, d, J = 1.2), 8.08 (1H, d, J = 1.5), 7.85 (2H, t, J = 7.5), 7.62 (2H, d, J = 8.4), 7.49 (2H, t, J = 7.5), 4.70 (4H, br m), 3.59 (3H, s), 3.29 (3H, s). ^{19}F NMR (MeOH): δ 64.54 (3F, s), 64.61 (3F, s). ES-MS (MeOH, +ve mode) m/z: 971.1 $[M-H]^+$. (Combustion analyses of single-crystalline material of **5** gave unsatisfactory results).
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