Published in final edited form as:

Bioorg Med Chem Lett. 2009 July 1; 19(13): 3423–3425. doi:10.1016/j.bmcl.2009.05.046.

Synthesis and biological evaluation of platinum–acridine hybrid agents modified with bipyridine nonleaving groups

Alexander R. Kheradi^a, Gilda Saluta^b, Gregory L. Kucera^b, Cynthia S. Day^a, and Ulrich Bierbach^a

^aDepartment of Chemistry, Wake Forest University, Winston-Salem, NC 27109, USA

^bComprehensive Cancer Center, Wake Forest University Health Sciences, Winston-Salem, NC 27157, USA

Abstract

The use of 2,2'-bipyridines (4,4'-R₂-2,2'-bpy; R = H, Me, OMe, CF₃) as nonleaving groups (L-L) in platinum–acridinylthiourea conjugates, [PtCl(L-L)(ACRAMTU)](NO₃)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)](NO₃)₂ ("PTACRAMTU", **1**; Figure 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 coworkers 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 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'-R₂-2,2'-bpy)(ACRAMTU)](NO₃)₂ (**2–5**; Figure 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 properties¹³ of the bpy moieties.

The desired dicationic conjugates **2–5** (Figure 1) were synthesized¹⁴ from the neutral complexes, $[\text{PtCl}_2(4,4'\text{-R}_2\text{-2,2'-bpy})]$, which were obtained by reacting $\text{K}_2[\text{PtCl}_4]$ 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 ^1H 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** (Figure 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 transeffect 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 assays¹⁸ 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 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 PTACRAMTU (**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 assay¹⁹ 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 $[\text{Pt}(\text{bpy})]^{2+}$ ($[\text{M}-\text{H}]^+$, 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 ^1H NMR experiments²⁰ (1 mM Pt

complex/2 mM dG; D₂O, 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 6-fold 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 nonleaving 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 nonleaving group and the DNA helix as a consequence of the additional steric bulk.

In conclusion, the bpy ligands introduced as nonleaving 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

We thank Lauren C. Eiter and Dr. Rajsekhar Guddneppanavar (Department of Chemistry, WFU) for technical assistance. A.R.K. was supported by the Undergraduate Summer Research Fellowship Program of WFU. This work was funded by a grant from the National Institutes of Health (CA101880).

References and notes

1. Kelland L. *Nat Rev Cancer* 2007;7:573. [PubMed: 17625587]
2. Guddneppanavar R, Bierbach U. *Anticancer Agents Med. Chem* 2007;7:125. [PubMed: 17266509]
3. Baruah H, Bierbach U. *J. Biol. Inorg. Chem* 2004;9:335. [PubMed: 15024635]
4. Baruah H, Rector CL, Monnier SM, Bierbach U. *Biochem. Pharmacol* 2002;64:191. [PubMed: 12123739]
5. Baruah H, Wright MW, Bierbach U. *Biochemistry* 2005;44:6059. [PubMed: 15835895]
6. Ackley MC, Barry CG, Mounce AM, Farmer MC, Springer BE, Day CS, Wright MW, Berners-Price SJ, Hess SM, Bierbach U. *J. Biol. Inorg. Chem* 2004;9:453. [PubMed: 15067524]
7. Guddneppanavar R, Choudhury JR, Kheradi AR, Steen BD, Saluta G, Kucera GL, Day CS, Bierbach U. *J. Med. Chem* 2007;50:2259. [PubMed: 17408248]

8. Guddneppanavar R, Saluta G, Kucera GL, Bierbach U. J. Med. Chem 2006;49:3204. [PubMed: 16722638]
9. Ma Z, Choudhury JR, Wright MW, Day CS, Saluta G, Kucera GL, Bierbach U. J. Med. Chem 2008;51:7574. [PubMed: 19012390]
10. Seve P, Dumontet C. Curr. Med. Chem. Anticancer Agents 2005;5:73. [PubMed: 15720263]
11. Gray J, Simon G, Bepler G. Expert Rev. Anticancer Ther 2007;7:545. [PubMed: 17428174]
12. Summa N, Schiessl W, Puchta R, van Eikema Holmes N, van Eldik R. Inorg. Chem 2006;45:2948. [PubMed: 16562950]
13. McInnes EJJ, Farley RD, Rowlands CC, Welch AJ, Rovatti L, Yellowlees LJ. J. Chem. Soc. Dalton Trans 1999;4203
14. Synthesis and product characterization: The following compounds were prepared according to previously described procedures: ACRAMTU, hydronitrate salt (ref. 17), **1** (ref. 17), **2** (ref. 7), and 4,4'-(CF₃)₂-2,2'-bpy (ref. 15). All other chemical were commercially available and used as supplied. Complexes **3–5** were synthesized according to the published procedure for compound **2** (ref. 7). Crystallographic data (excluding structure factors) for compound **5** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 731112. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or deposit@ccdc.cam.ac.uk]. [*PtCl(4,4'-(MeO)₂-2,2'-bpy)(ACRAMTU)(NO₃)₂ · H₂O (3 · H₂O)*]. Yield 0.154 g (42%). ¹H NMR (D₂O): δ 8.48 (1 H, d, *J* = 6.3), 8.03 (2 H, d, *J* = 8.7), 7.98 (1 H, d, *J* = 6.6), 7.76 (2 H, t, *J* = 7.8), 7.45 (2 H, d, *J* = 8.4), 7.26 (2 H, t, *J* = 7.2), 7.00 (1 H, s), 6.82 (1 H, s), 6.81 (2 H, d, *J* = 8.4), 6.68 (1 H, d, *J* = 5.1), 4.42 (2 H, m), 4.00 (3 H, s), 3.88 (3 H, s), 3.66 (2 H, m, *J* = 6.9), 3.22 (3 H, s), 3.21 (3 H, s). ES-MS (MeOH, +ve mode) *m/z*: 896.23 [M-H]⁺. Anal. (C₃₀H₃₅ClN₈O₉PtS) calcd 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)(NO₃)₂ · 2H₂O (4 · 2H₂O)*]. Yield 0.214 g (46%). ¹H NMR (D₂O): δ 8.45 (1 H, d, *J* = 5.5), 8.00 (2 H, d, *J* = 9.0), 7.95 (1 H, d, *J* = 5.5), 7.74 (2 H, t, *J* = 7.5), 7.39 (2 H, d, *J* = 8.0), 7.38 (1 H, s), 7.22 (3H, br. s, t, overl.), 7.08 (1 H, d, *J* = 5.5), 6.94 (1 H, d, *J* = 5.5), 4.71 (2 H, br. m), 4.56 (2 H, br. m), 3.25 (3 H, s), 3.22 (3 H, s), 2.41 (3 H, s), 2.25 (1 H, 3). ES-MS (MeOH, +ve mode) *m/z*: 863.16 [M-H]⁺. Anal. (C₃₀H₃₇ClN₈O₈PtS) calcd C: 40.04%, H: 4.15%, N 12.27%; found C: 40.25%, H: 4.17%, N: 12.08%. [*PtCl(4,4'-(CF₃)₂-2,2'-bpy)(ACRAMTU)(NO₃)₂ (5)*]. Yield 0.200 g (62%). ¹H NMR (MeOH-*d*₄): δ 9.45 (1 H, d, *J* = 6.0, Pt satellites), 9.29 (1 H, d, *J* = 6.0, Pt satellites), 9.04 (2 H, d, *J* = 8.4), 8.10 (1 H, d, *J* = 1.2), 8.08 (1 H, d, *J* = 1.5), 7.85 (2 H, t, *J* = 7.5), 7.62 (2 H, d, *J* = 8.4), 7.49 (2 H, t, *J* = 7.5), 4.70 (4 H, br. m), 3.59 (3 H, s), 3.29 (3 H, s). ¹⁹F NMR (MeOH): δ 64.54 (3 F, s), 64.61 (3 F, s). ES-MS (MeOH, +ve mode) *m/z*: 971.1 [M-H]⁺. (Combustion analyses of single-crystalline material of **5** gave unsatisfactory results.)
15. Furue M, Maruyama K, Oguni T, Naiki M, Kamachi M. Inorg. Chem 1992;31:3792.
16. Egan TJ, Koch KR, Swan PL, Clarkson C, Van Schalkwyk DA, Smith PJ. J. Med. Chem 2004;47:2926. [PubMed: 15139771]
17. Martins ET, Baruah H, Kramarczyk J, Saluta G, Day CS, Kucera GL, Bierbach U. J. Med. Chem 2001;44:4492. [PubMed: 11728195]
18. The cytotoxicity studies were carried out using the Celltiter 96 Aqueous Non-Radioactive Cell Proliferation Assay kit. HL-60 leukemia cells and H460 lung cancer cells were kept in a humidified atmosphere of 5 % CO₂ at 37 °C. Cells were plated on 96-well plates with a total 700 cells/well for H460 and 27500 cells/well for HL-60 cells. Stock solutions of the complexes were prepared in phosphate-buffered saline (PBS). The cells growing in log phase were incubated with appropriate serial dilutions of the drugs in duplicate for 72 h. To each well was added 20 μL of MTS/PMS solution, and the mixtures were allowed to equilibrate for 3 h. The absorbance at 490 nm was recorded using a plate recorder (none of the derivatives tested absorbs at this wavelength). The reported IC₅₀ data were calculated from non-linear curve fits using a sigmoidal dose-response equation and are averages of three experiments.
19. Barry CG, Baruah H, Bierbach U. J. Am. Chem. Soc 2003;125:9629. [PubMed: 12904029]
20. The 1-D ¹H kinetics experiments were carried out as a standard Bruker arrayed 2-D experiment using a variable delay list. All NMR spectra in arrayed experiments were collected on a Bruker 500 DRX spectrometer equipped with a 5-mm triple-resonance broadband inverse probe and a variable temperature unit. Reactions were performed at 37 °C in 5-mm NMR tubes containing 1 mM complex

2 and 2 mM dG (15 mM phosphate buffer, D₂O, pH* 6.8). Acquisition began ca. 15 min after mixing of the reactants. Incremented 1-D spectra were processed exactly the same, and suitable signals were integrated. All data were processed with XWINNMR 3.6 (Bruker, Ettlingen, Germany).

21. Guddneppanavar R, Wright MW, Tomsey AK, Bierbach U. J. Inorg. Biochem 2006;100:972. [PubMed: 16488016]

1 (PT-ACRAMTU)

- 2: R = H
3: R = OMe
4: R = Me
5: R = CF₃

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

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.

Table 1

Cytotoxicity (IC₅₀, 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.