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Effect of linkage geometry on biological activity in thiourea- and guanidine-substituted acridines and platinum–acridines

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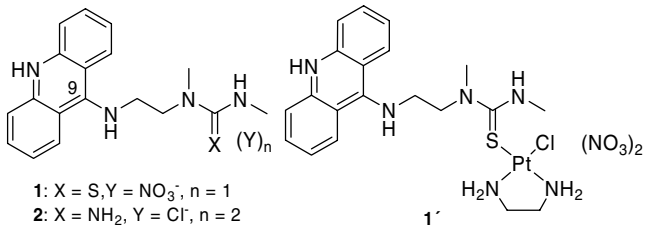
ABSTRACT

Novel thiourea- and guanidine-modified acridine-4-carboxamides (**4**, **7**) and a corresponding platinum–intercalator conjugate (**4′**) have been synthesized and evaluated as cytotoxic agents in human promyelocytic leukemia, HL-60, and a non-small cell lung cancer, NCI-H460. Modification of thiourea sulfur in derivative **4** with a DNA platinating moiety, giving **4′**, resulted in a pronounced cytotoxic enhancement, and the conjugate proved to be the most active of the newly synthesized compounds in NCI-H460 cells. Conjugate **4′** represents a new chemotype with potential applications in the treatment of chemoresistant tumors.

Acridine-based pharmacophores have wide applications in antimicrobial and anticancer therapy.[1] The principal target of these agents is genomic DNA, to which they bind through intercalation. The chemotherapeutic potency of acridines is modulated by their DNA binding properties. Important parameters include global affinity and drug dissociation rates, as well as the geometry of the intercalated complex and its recognition by DNA processing enzymes.[2] All of these factors are highly dependent on the nature and positioning of the residues attached to the planar chromophore. Acridine derivatives carrying side chains in the 4- and 9-position of the heterocyclic base have been studied extensively. These include acridine-4-carboxamides,[3] such as DACA, a topoisomerase-targeted anticancer agent currently being considered as a second-line therapy in refractory non-small cell lung and ovarian cancers,[4] and several classes of 9-anilino- and 9-aminoacridines.[1]

In an effort to combat the notorious resistance of certain tumors to current DNA-targeted therapies, we have developed a new type of hybrid organic–inorganic pharmacophore, whose structure comprises an acridin-9-ylthiourea and a monofunctional platinating moiety.[5,6] The conjugate derived from 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea ('ACRAMTU', compound **1**), PT-ACRAMTU (**1′**) (Fig. 1), and several of its second-generation analogues have demonstrated excellent activity in chemoresistant non-small cell lung cancers[7] and are currently being tested in nude mouse xenograft models. Compound **1** and its analogues modify DNA by a dual mechanism involving intercalation and monofunctional platination of guanine (80%) and adenine (20%) nitrogen.[8–10] N-Donor analogues of ACRAMTU, including acridin-9-yl-guanidine **2** (Fig. 1), have also been synthesized as potential DNA-affinic carrier ligands for platinum.[11] The unique DNA damage profile produced by conjugate **1** is a consequence of its sequence preference and directionality of intercalation, which leads to unprecedented platination of adenine bases at N3 (~10% of adducts) in the DNA minor groove.[12]



1: X = S,Y = NO_3^-, n = 1
2: X = NH_2, Y = Cl^-, n = 2

1′

Figure 1. Structures of 9-aminoacridine derivatives (**1**, **2**) and the hybrid agent, PT-ACRAMTU (**1′**).

The preclinical success of compound **1′** and the hypothesis that a relationship exists between its antitumor activity in cisplatin-resistant cell lines and its distinct DNA damage profile have prompted several structure–activity relationship studies. Previously, we have made modifications to the linker that connects the acridine and platinum moieties,[13,14] varied the spectator ligands on the metal center,[15] and have introduced 4-substituted ACRAMTU derivatives as threading intercalators.[16] We have now generated a new set of compounds, in which the thiourea and guanidine groups are incorporated into a 4-carboxamide side

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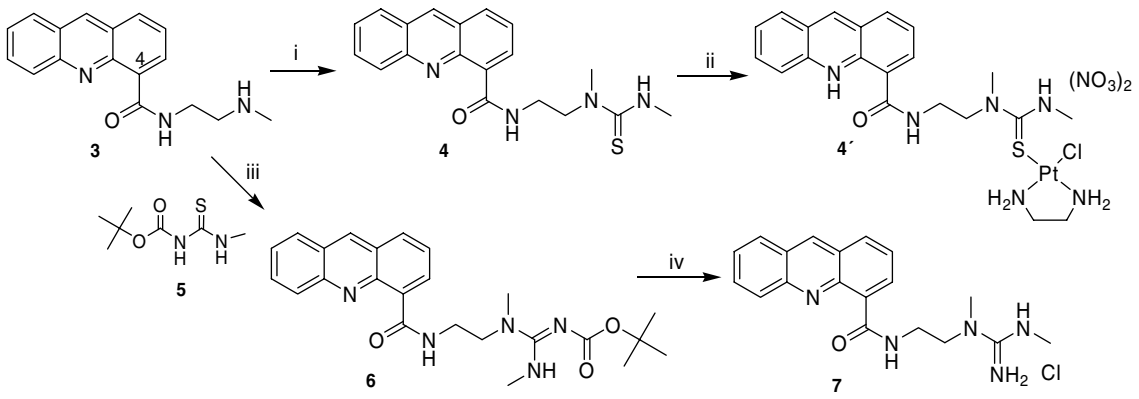

Scheme 1. Synthesis of acridine-4-carboxamides **4** and **7** and platinum conjugate **4′**. Reagents and condition: (i) MeNCS/EtOH, reflux; (ii) 1—[Pt(en)Cl_2]/$AgNO_3$, DMF, r.t., 2—1 M HNO_3; (iii) $HgCl_2$/Et_3N, DMF, 0 °C; (iv) 1. 2 M HCl, r.t., 2. Al_2O_3.

chain. A new platinum-containing hybrid agent was also synthesized and tested along with the platinum-free carriers against HL-60 and NCI-H460 cells.

The new derivatives were synthesized[17] from the common precursor, *N*-(2-(methylamino)ethyl)acridine-4-carboxamide (**3**)[18,19] (Scheme 1). Transformation of the secondary amino group into thiourea was accomplished by reacting **3** with methylisothiocyanate to yield derivative **4** in ~70–80% yield. The corresponding platinum complex, **4′**, was synthesized using previously developed conjugation chemistry involving ligand substitution in the precursor complex, [Pt(en)Cl_2] (en = ethane-1,2-diamine), to form a stable Pt–$S_{thiourea}$ linkage.[6] Complex **4′** was isolated in its fully protonated form as the dinitrate salt. The guanidine analogue, **7**, was generated using guanidylation chemistry developed for the synthesis of compound **2**. The reaction involves condensation of precursor acridine–amine **3** with Boc-activated guanidylating reagent **5** in the presence of $HgCl_2$, followed by removal of the Boc group under mildly acidic conditions.[11] The product was purified on basic alumina to afford **7** as the mono-HCl salt (Scheme 1).

Major differences exist in the protonation states and overall charges of the 4- and 9-substituted acridines. In the 9-amino derivatives, **1** and **2**, acridine is fully protonated at physiological pH (pK_a ~ 9–10),[6,11] leading to + and 2+ overall charges (pK_a > 12 for the guanidinium moiety in **2**).[11] In contrast, a dramatic decrease in acridine basicity is observed for the newly synthesized 4-carboxamides, for which we determined pK_a values in the range 3–4,[17] consistent with data reported previously for this class of compounds.[3] As a consequence, the 4-substituted compounds carry a reduced positive charge and prove to be less soluble in aqueous media than their 9-substituted counterparts. Electroneutral compound **4**, for instance, had to be solubilized in DMSO prior to serial dilution with PBS buffer in cell proliferation assays.

Table 1
In vitro cytotoxicity (IC_{50}, μM[a]) of acridines and platinum–acridines in HL-60 and NCI-H460 cell lines

Compound	Charge[b]	HL-60	NCI-H460
1[c]	+	11.5	9.5
1′[c]	2+	2.8	0.26
2[d]	2+	79.7	10.3
4	0	>100	30.9
4′	+	16.0	2.4
7	+	8.4	>100

[a] Average of at least two experiments based on a colorimetric cell proliferation assay.
[b] Reflects protonation state at physiological pH (see text).
[c] Ref. 16.
[d] Ref. 11.

Compounds **4**, **4′**, and **7** were tested for their cytotoxic effects in a human leukemia (HL-60) and a non-small cell lung cancer (NCI-H460) cell line (Table 1) using a cell proliferation assay.[20] Of the two new acridine-4-carboxamides, only the guanidine derivative **7** showed appreciable cytotoxicity in HL-60 cells, where it proved to be more active, by an order of magnitude, than its 9-substituted congener, **2**. All other 4-substituted derivatives were significantly less active than the 9-substituted derivatives, and both 4- and 9-substituted compounds performed better in the solid tumor cell line. A striking cytotoxic enhancement by >10-fold is observed for the hybrid agent **4′** compared to **4** in the NCI-H460 cell line. A similar trend has been observed for the prototypical 9-substituted pair **1**/**1′** (Table 1).

In the present study, we have generated acridine-4-carboxamide analogues of DNA-targeted thiourea- and guanidine-modified 9-aminoacridines and have introduced one derivative (**4**) as a carrier ligand in a corresponding platinum–intercalator conjugate (**4′**). The results of the cell proliferation assay suggest that the 4-substituted derivatives are less potent than the 9-aminoacridines. Only monobasic compound **7** had an advantage over dibasic compound **2** in HL-60 cells. This observation and the fact that the relative enhancement of activity observed for the conjugates compared to the platinum-free carriers is less pronounced in the leukemia cell line than in the solid tumor suggest that reduced uptake of the more highly charged drugs may limit their activity in HL-60 cells. Derivative **4** was the least active compound in this series, probably due to its poor solubility. Characteristically, the dual intercalating/platinating agents **1′** and **4′** showed the lowest IC_{50} values in H460 cells, which corroborates the view that irreversible DNA adducts formed by the conjugates' metal moieties contribute substantially to the cell kill mechanism.

In addition to drug uptake and bioavailability, differences between the 4- and 9-substituted compounds most likely exist in terms of their DNA affinities (charge) and binding modes. A growing body of evidence from high-resolution structural studies exists, which indicates critical differences in the DNA binding modes of these agents. While in the DNA complexes of both types of acridines the long dimensions of the drug chromophore and the adjacent DNA base pairs are co-aligned, differences exist in the directionality of intercalation. Side chains in the 4-position reside in the major groove, whereas 9-substituents preferentially protrude into the minor groove.[21–23] ACRAMTU's (**1**) distinct groove specificity of intercalation has been implicated as the driving force in the platination of minor groove sites by PT-ACRAMTU (**1′**). Likewise, the preferred geometry of intercalation of derivative **4** can be expected to influence the base and groove preference of platinum–DNA adduct formation by conjugate **4′**. Thus, major differences can be predicted between the DNA damage profiles of this hybrid agent

and that of PT-ACRAMTU (**1′**). It is noteworthy to mention that precedent exists for this groove-specific reactivity in the DNA interactions of analogous dual intercalating/alkylating agents: nitrogen mustard groups attached to the 4-carboxamide residue preferably alkylate guanine-N7 in the major groove, whereas the major target of the analogous 9-anilino-linked mustards is adenine-N3 in the minor groove.[24] We hypothesized that the minor groove adducts formed by PT-ACRAMTU are intercalator-driven and contribute significantly to its cytotoxic effect.[7] Finally, differences may exist in the recognition and repair of the DNA adducts formed by **1′** and **4′**. Future studies on the 4-substituted analogues, therefore, will be concerned with detecting the sites and the rates of formation of the potentially cytotoxic DNA adducts produced by conjugate **4′** and its second-generation derivatives in order to delineate structure–activity relationships in this novel type of pharmacophore. The utility of guanidine derivatives **2** and **7** as carrier ligands in these hybrid agents will also be explored.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.043.

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17. Synthesis and characterization of compounds: *N-(2-(1,3-dimethylthioureido) ethyl)acridine-4-carboxamide,* ***4***. A solution of methylisothiocyanate (0.33 g, 4.55 mmol) in 10 mL of absolute ethanol was added dropwise within 10 min to a solution of 1.0 g (3.58 mmol) of **3** in 50 mL of absolute ethanol. The mixture was heated at reflux for 6 h and passed, while hot, through a Celite Pad. Ethanol was removed in vacuum and the residue was recrystallized from hot methanol to afford microcrystalline **4**. Yield: 0.98 g (77%); mp 198 °C; ^{1}H NMR (DMSO-d_6): δ 11.43 (1H, t, J = 5.5 Hz), 9.33 (1H, s), 8.73 (1H, d, J = 7.1 Hz), 8.39 (1H, d, J = 8.4 Hz), 8.32 (1H, d, J = 8.8 Hz), 8.24 (1H, d, J = 8.4 Hz), 7.97 (1H, t, J = 6.8 Hz), 7.76 (1H, t, J = 8.1 Hz), 7.71 (1H, t, J = 8.0 Hz), 7.58 (1H, NH, b), 4.09 (2H, t, J = 6.4 Hz), 3.79 (2H, q, J = 6.1 Hz), 3.18 (3H, s), 2.91 (3H, d, J = 4.1 Hz); ^{13}C-{H} NMR (DMSO-d_6): δ 181.9, 165.2, 147.0, 145.4, 138.5, 134.5, 132.9, 131.7, 128.7, 128.4, 128.1, 126.5, 126.4, 125.6, 125.2, 51.7, 38.2, 37.2, 32.6. Anal. ($C_{19}H_{20}N_4OS$) C, H, N, S: Calcd 64.75, 5.72, 15.90, 9.10. Found: 64.42, 5.63, 15.86, 9.02; pK_a < 3 (UV, 50% MeOH).
[PtCl(en)($C_{19}H_{21}N_4OS$)](NO_3)$_2$, ***4′***. The nitrate salt of **4** was formed by adding 1.4 mL of 1 M HNO_3 to 0.5 g of **4** in methanol. The solvent was removed completely under vacuum and the residue redissolved in 10 mL of anhydrous DMF. A mixture of 0.46 g (1.41 mmol) of [$PtCl_2$(en)] and 0.23 g (1.41 mmol) of $AgNO_3$ in 10 mL of anhydrous DMF was stirred at room temperature in the dark for 14 h. Precipitated AgCl was filtered off through a Celite pad. The solution containing **4** was combined with the filtrate, and the mixture was stirred for 4 h in the dark. DMF was removed in a vacuum at 30 °C yielding a yellow oily residue, which was dissolved in 1.0 L of dry methanol. Activated carbon was added, and the solution was stirred for 15 min. Carbon was filtered off, and the solution was concentrated to a final volume of 100 mL. Crude **4′** was obtained as a bright yellow solid after the solution was stored at °20 °C for 24 h. The crude batch was recrystallized from hot methanol. The solution was stored in the refrigerator for 48 h to afford **4′** as a microcrystalline yellow solid, which was dried at 60 °C. Yield: 650 mg (60%); mp 185 °C. ^{1}H NMR (MeOH-d_4): δ 9.53 (1H, s), 8.61 (1H, d, J = 7.3 Hz), 8.37 (1H, d, J = 8.5 Hz), 8.27 (1H, d, J = 8.7 Hz), 8.20 (1H, d, J = 8.5 Hz), 8.00 (1H, t, J = 8.4 Hz), 7.70 (2H, m), 5.28 (2H, s), 5.05 (2H, s), 4.19 (2H, t, J = 5.9 Hz), 3.82 (2H, t, J = 6.0 Hz), 3.12 (3H, s), 2.38 (4H, s); ^{13}C-{H} NMR (DMF-d_7): δ 176.6, 167.0, 147.8, 146.2, 140.23, 135.8, 134.1, 133.0, 129.3, 129.2, 128.3, 127.6, 127.5, 126.9, 126.0, 54.4, 50.5, 48.5, 40.2, 38.3, 34.5; ^{195}Pt NMR (DMF-d_7, ext. Na_2PtCl_6): δ −2859. Anal. ($C_{21}H_{29}ClN_8O_7PtS{\cdot}H_2O$) C, H, N: Calcd 32.08, 3.97, 14.25. Found: 32.09, 3.86, 14.03. ESI-MS (MeOH, +ve mode) *m/z*: 642.2 [M]$^+$.
tert-Butyl-((2-(acridine-4-carboxamido)ethyl)-(methyl)amino)(methylamino) methylenecarbamate, ***6***. A mixture of 159 mg (0.92 mmol) of **5**, 256 mg of **3** (0.92 mmol), and 260 μL of triethylamine was prepared in 7 mL of dry DMF. The solution was cooled to 0 °C and 249 mg (0.92 mmol) of $HgCl_2$ was added. The yellow slurry was stirred for 1 h at 0 °C and for another 4 h at room temperature until the mixture turned black. DMF was removed in a vacuum, and the residue was dissolved in ethyl acetate, filtered through Celite, and dried over Na_2SO_4. After the solvent was removed, the crude product was purified by flash chromatography on an alumina column using methanol/ethyl acetate (2:3) as eluent. Yield: 280 mg (70%). ^{1}H NMR (MeOH-d_4): δ 9.11 (1H, s), 8.78 (1H, d), 8.30 (2H, m), 8.14 (1H, d), 7.92 (1H, t), 7.67 (2H, m), 3.84 (2H, t), 3.75 (2H, m), 3.09 (3H, s), 2.82 (3H, s), 1.41 (9H).
((2-(Acridine-4-carboxamido)ethyl)(methyl)amino)-(methylamino)methaniminium chloride, **7**. To remove the Boc group, 200 mg (0.46 mmol) of **6** was dissolved in 2.0 M HCl. The mixture was stirred at room temperature overnight. Acid was removed using a rotary evaporator, and the residue was redissolved in ethanol. The product was precipitated with diethyl ether purified by flash chromatography on an alumina column using methanol/ethyl acetate (1:1) as eluent. Yield: 100 mg (59%); mp 182 °C. ^{1}H NMR (D_2O): δ 8.25 (1H, s), 8.11 (1H, d, J = 7.1 Hz), 7.71 (2H, one triplet and one doublet overlap), 7.66 (1H, d, J = 8.1 Hz), 7.49 (1H, t, J = 7.9 Hz), 7.44 (1H, d, J = 8.4 Hz), 7.27 (1H, t, J = 7.3 Hz), 3.47 (2H, t, J = 6.0 Hz), 3.38 (2H, t, J = 6.5 Hz), 2.92 (3H, s), 2.39 (3H, s); ^{13}C-{H} NMR (D_2O): δ 168.3, 156.8, 146.6, 144.5, 138.5, 134.8, 134.3, 132.3, 128.7, 128.0, 126.8, 126.0, 125.6, 124.9, 124.7, 49.7, 36.7, 36.4, 28.2; ESI-MS (MeOH, +ve mode) *m/z*: 336.4 [M]$^+$. Anal.($C_{19}H_{22}ClN_5O{\cdot}0.5H_2O{\cdot}0.5EtOH$) C, H, N: Calcd 59.47, 6.49, 17.33. Found: 59.04, 6.54, 16.90; pK_{a1} = 3.45, pK_{a2} > 11 (^{1}H NMR).
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