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Potent antitumor N-mustard derivatives of 9-anilinoacridine, synthesis and antitumor evaluation

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Abstract—A series of 9-anilinoacridine N-mustard derivatives, in which the alkylating N-mustard residue was linked to the C-3′ or C-4′ position of the anilino ring with an O-ethylene spacer, was synthesized and evaluated for cytotoxicity against human lymphoblastic leukemic cells (CCRF-CEM) in culture. The results showed that all of the new compounds exhibited potent cytotoxicity with IC_{50} values ranging from 0.002 to 0.7 μM, which were as potent or significantly more potent than 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA). Compound **9** did not exhibit cross-resistance against both vinblastine-resistant (CCRF-CEM/VBL) and taxol-resistant (CCRF-CEM/taxol) cells. Additionally, compound **9** demonstrated potent antitumor effect in nude mice bearing human breast carcinoma MX-1 xenografts, resulting in complete tumor remission in two out of three mice at the maximal dose of 1–2 mg/kg (Q3D×7) or 3 mg/kg (Q4D×5) via intravenous injection.

DNA bifunctional alkylating agents containing nitrogen mustard moiety are an important class of anticancer drugs.[1,2] Although the mustard derivatives are capable of cross-linking to DNA doubled strands, the high reactivity of these agents lack the affinity to bind to DNA, resulting in the loss of their antitumor activity. This is perhaps due to either the formation of mono-alkylation via partial hydrolysis or interaction with other cellular components. The formation of the mono-alkylation has proven to produce genotoxicity resulting in less cytotoxicity.[3] The drawback of N-mustard drugs has been improved by linking the alkylating moiety to a DNA affinic carrier such as DNA-intercalating chromophore (e.g., 9-aminoacridine,[4,5] 9-anilinoacridine,[6] anthraquinone,[7] cyclopentanthraquinone[8]) or DNA minor groove binder (such as distamycin A and related analogues)[9] to allow the drug to target the DNA, to offer higher regio- and sequence selectivity, and to minimize the limitations of nitrogen mustards.[10,11]

N-Mustards targeted by intercalating carriers such as acridine (**1**) [5] and anthraquinones (**2**) [7] can achieve 10- to 100-fold improvements in potency and improved antileukemic activities compared with their corresponding untargeted mustards of similar reactivity. Fan et al.[6] synthesized aniline mustard analogues of the DNA-intercalating agent *m*-AMSA (i.e., compounds **3** and **4**) by linking a mustard residue to the anilino ring or acridine chromophore, respectively. These agents, however, were less potent than the parent *m*-AMSA in mice bearing murine leukemia p388. The low potency of **3** and **4** is perhaps due to altering the drug's binding site(s) on the DNA and drug/topoisomerase II (Topo II) interactions. To enhance the interaction between 9-anilinoacridine and Topo II and/or DNA, one can introduce the N-mustard residue to 9-anilinoacridine with a short spacer. Based on this hypothesis, we designed and synthesized N-mustard derivatives of 9-anilinoacridine, in which the N-mustard residue was linked to the anilino ring with a short spacer ($O–C_2$) and located at the C-3′ or C-4′ position (Fig. 1).

3-(9-Acridinylamino)-5-hydroxymethylaniline (AHMA, **5**), was previously demonstrated as a potent topoisomerase II inhibitor and exhibited significant antitumor

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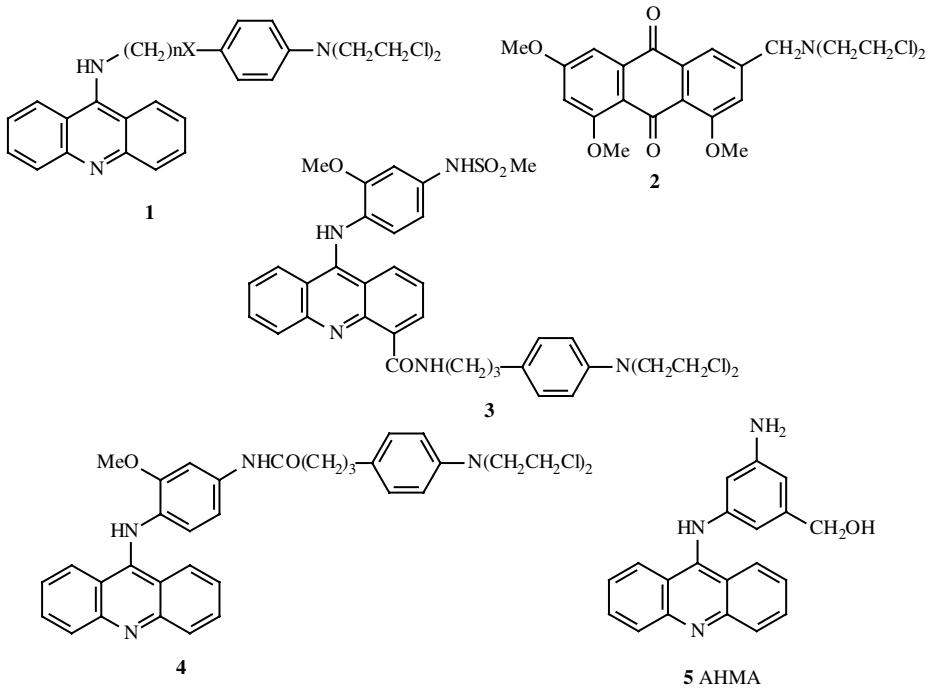
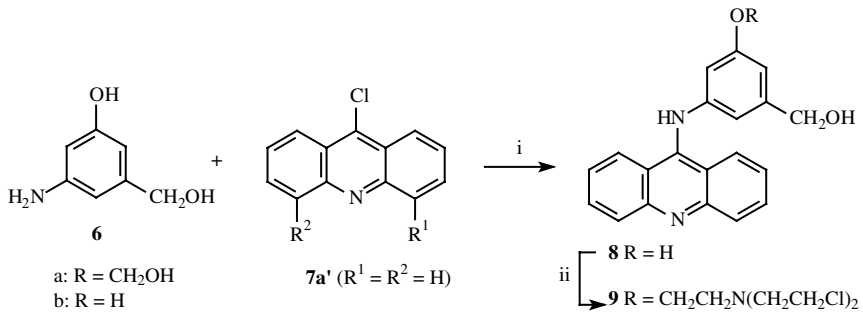


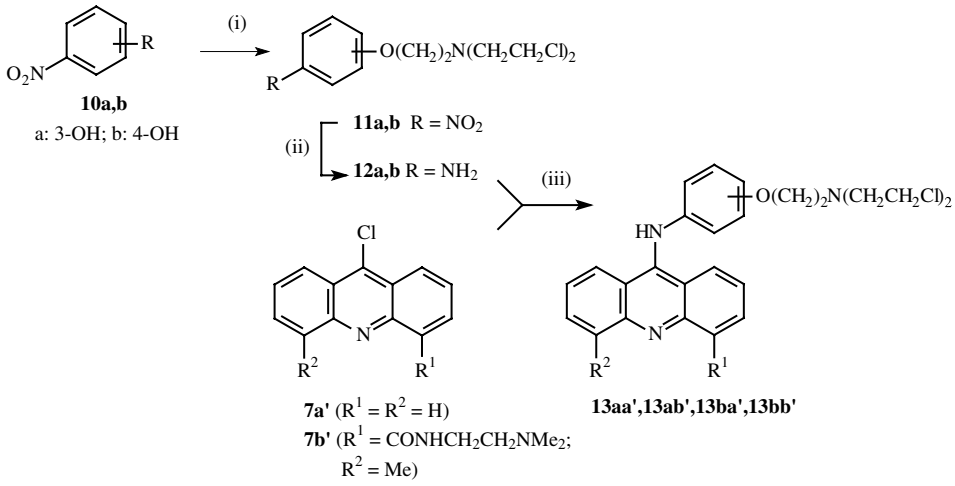
Figure 1. Structures of N-mustard analogues and AHMA.

activity both in vitro and in vivo.[12–14] To introduce the N-mustard moiety to AHMA, we synthesized a bioisosteric isomer of AHMA namely, 3-(9-acridinylamino)-5-hydroxymethylphenol (**8**) (Scheme 1). The known 3-amino-5-hydroxybenzyl alcohol (**6**) was condensed with 9-chloroacridine (**7a′**) to give **8**, which was then converted to potassium salt by treating with 0.2 N KOH in MeOH. The solid salts were then treated with tris(2-chloroethyl)amine hydrochloride in DMF in the presence of K_2CO_3/KF to produce moderate yield (50%) of compound **9**. Alternatively, reacting *m*-nitrophenol (**10a**) or *p*-nitrophenol (**10b**) with tris(2-chloroethyl)amine hydrochloride afforded **11a**,**b**, which were then reduced ($SnCl_2$/concd HCl) to the corresponding aniline derivatives **12a**,**b**. The unstable crude anilines **12a**,**b** were used directly without purification for the condensation with the requisite 9-chloroacridines (**7a′**,**b′**) and the desired 9-anilinoacridine derivatives (**13aa′**, **13ab′**, **13ba′**, and **13bb′**) bearing an O–C_2 spacer between the anilino ring and the N-mustard residue were smoothly obtained in moderate yield (Scheme 2).

The cytotoxicity of the new N-mustard derivatives of 9-anilinoacridine against human lymphoblastic leukemic cells (CCRF-CEM), A549 lung carcinoma, and HCT-116 colon tumor was shown in Table 1 and was compared with AHMA (**5**). This clearly demonstrated that all of the new compounds were significantly more potent than AHMA (**5**). Compound **9** carrying a CH_2OH function with IC_{50} value of 0.007 μM was approximately 107-fold more cytotoxic than AHMA (IC_{50} = 0.753 μM). While compound **13aa′**, lacking the CH_2OH substituent, was approximately 14-fold less active that **9**, thus indicating the CH_2OH function played an important role in the cytotoxicity of AHMA derivatives. It also revealed that introduction of a dimethyaminoethylcarboxamido and a methyl function to the C-4 and C-5 of the acridine chromophore (i.e., compound **13ab′**) further increased the cytotoxicity of the parent compound **13aa′**. Compounds with the alkylating chain at C-4′ (**13ba′**) were about 5-fold more cytotoxic than the corresponding C-3′-substituted one (**13aa′**). The low cytotoxicity of compound



Scheme 1. Reagents and conditions: (i) $CHCl_3$/EtOH/concd HCl (catalytic amount); (ii) K_2CO_3/KF/DMF, 35 °C.



Scheme 2. Reagents and conditions: (i) tris(2-chloroethyl)amine hydrochloride/K_2CO_3/KF/acetone, reflux; (ii) $SnCl_2$/concd HCl, 90 °C; (iii) $CHCl_3$/EtOH/concd HCl (catalytic amount); a series: N-mustard residue substituted at C-3′; b series: N-mustard residue substituted at C-4′; a′ series: $R^1 = R^2 = H$; b′ series: $R^1 = CONHCH_2CH_2NMe_2$, $R^2 = Me$.

Table 1. The cytotoxicity of N-mustard analogues of 9-anilinoacridine against human tumor cell growth in culture[a]

Compound	IC_{50} (μM)				
	CCRF-CEM	CCRF-CEM/VBL	CCRF-CEM/taxol	A549	HCT-116
AHMA **5**	0.753	1.60 [2.1×][b]	0.600 [0.8×]	0.0470	ND[c]
9	0.0070	0.0075 [1.1×]	0.0340 [4.9×]	0.0056	0.0055
13aa′	0.095	ND	ND	ND	ND
13ba′	0.0200	0.066 [3.3×]	0.061 [3.1×]	ND	ND
13ab′	0.0030	ND	ND	ND	ND
13bb′	0.7730	ND	ND	ND	ND
Taxol	0.0015	1.62 [1080×]	0.143 [95.3×]	0.0029	0.0026
Vinblastine	0.0012	0.540 [450×]	0.029 [24.2×]	0.0099	0.0087

[a] XTT assays were used for leukemia cells and SRB assays were used for solid tumor cells. Incubation was 72 h, as described previously.[15]
[b] Numbers in the bracket are folds of resistance of the resistant cells when compared with the IC_{50}'s of the CCRF-CEM parent cells.
[c] ND: not determined.

13bb′ was an exception. Additional compounds are currently being studied in our laboratory to gain a better understanding of their structure–activity relationship.

At present we merely took compound **9** for further antitumor investigation since this agent is structurally closer to AHMA. Table 1 showed the growth inhibition of human lymphoblastic leukemic cells (CCRF-CEM) and its drug-resistant sublines (resistant to vinblastine and taxol, CCRF-CEM/VBL, and CCRF-CEM/taxol, respectively) by compound **9**. The results demonstrated that **9** did not develop cross-resistance to vinblastine or taxol. It suggests that compound **9** is neither a good substrate of *p*-glycoprotein nor mutated tubulin. Table 2 revealed that the therapeutic efficacy of compound **9** [maximal dose: 1–2 mg/kg (Q3D×7) or 3 mg/kg (Q4D×5); intravenous injection] on nude mice ($n = 3$) bearing human breast carcinoma MX-1 xenografts resulted in complete tumor remission in two out of three mice.

The current studies resulted in finding potent antitumor N-mustard derivatives of 9-anilinoacridine, in which the alkylating N-mustard residue is linked to the anilino ring with a short spacer (O–C_2). The significant cytotoxic effects of all new N-mustard derivatives indicated that these agents may possess dual effects: DNA cross-linking and Topo II inhibition. The present study also demonstrated that compound **9** exhibited significant

Table 2. Therapeutic effects and toxicity of compound **9** in nude mice bearing human mammary carcinoma (MX-1) xenografts[a]

	Dose (mg/kg)	Schedule	Average body weight[b] change (g)						Average tumor size (*T*/*C*)				Tumor free	Toxicity (death)
			D11	D14	D17	D20	D23	D26	D17	D20	D23	D26		
Control			29.2	+1.6	+1.5	+1.5	+1.9	—[c]	1.0	1.0	1.0	1.0	0/3[c]	0/3
9	1–2	Q3D×7	28.3	+1.2	−1.4	−3.0	−3.0	−3.9	0.37	0.17	0.14	ND	2/3[d]	0/3

[a] MX-1 tissue 50 mg was implanted S.C. on Day 0. Treatment (iv injection) began on D11 when tumor size were 80 ~ 120 mm^3.
[b] Body weight = total body weight − tumor weight.
[c] Animals were sacrificed when there was excessive tumor burden (e.g., tumor size >3500 mm^3).
[d] Animals were sacrificed on day 32.

cytotoxicity in inhibiting human lymphoblastic leukemic cells (CCRF-CEM) and its drug-resistant sublines. The excellent in vivo therapeutic effect of this agent suggested that other compounds reported herein might also have potent therapeutic activity. Additional in vivo antitumor activity as well as the DNA binding studies are currently carried out in our laboratory and will be reported separately.

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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.2004.06.080.

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