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LETTER

Facile synthesis and antitumor activity of novel *N*(9) methylated AHMA analogs†

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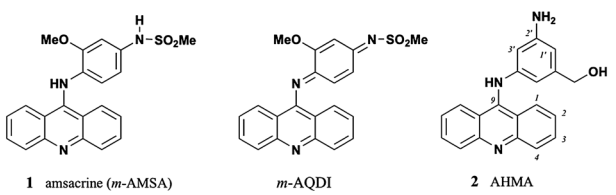
A facile synthesis of novel antitumor *N*(9)-methyl-3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA) derivatives is described. Boc protection of aminobenzoic acids followed by $LiAlH_4$ reduction yielded novel methylaminobenzyl alcohol reactants. Their interaction with 9-chloroacridine provides *N*(9)-methylated AHMA derivatives for biological screening. A preliminary anti-proliferative assay against seven cancer cell lines identified compounds with low μM IC_{50} values.

The discovery of new compounds with antitumor activity has become one of the most important goals in medicinal chemistry. One interesting group of chemotherapeutic agents used in cancer therapy comprises molecules that are DNA intercalators, from natural products such as doxorubicin and actinomycin D to synthetic drugs such as mitoxantrone and amsacrine.[1]

In previous studies on the development of potential 9-anilinoacridine congeners, 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA, **2**, Fig. 1) exhibited both *in vitro* and *in vivo* potent antitumor efficacy.[2,3] AHMA displayed better antitumor efficacy than the parent amsacrine (*m*-AMSA, **1**) in mice bearing mammary and lung carcinomas. Rational drug design for AHMA derivatives was based on the study of the metabolic pathway of *m*-AMSA (**1**), which was easily bio-oxidized to form *m*-AMSA-quinonediimine (*m*-AQDI) and had a short half-life in human plasma.[4,5] Consequently, the substituents (NH_2 and CH_2OH) on the anilino ring of AHMA are located in a *meta*-position to each other, thus preventing oxidation. Therefore, AHMA had a longer half-life in human plasma compared to *m*-AMSA.[6]

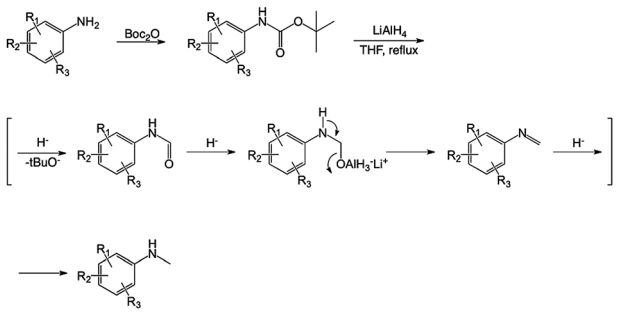


1 amsacrine (*m*-AMSA) *m*-AQDI **2** AHMA

Fig. 1 The structure of amsacrine, its oxidized metabolic product *m*-AQDI, and AHMA.

It was also noticed that 9-anilinoacridines undergo a reversible amine exchange reaction at position nine under near-physiological conditions in water.[7,8] This thermodynamically controlled reaction may have implications in understanding the mode of action of 9-anilinoacridines *in vivo* and in the future design of new drugs that will be based on this scaffold.[9] These findings brought us to hypothesize that methyl substitution at amine nine of AHMA derivatives will influence such exchange reactions or hydrolysis of the aniline moiety, therefore shedding additional light on the SAR of AHMA analogs. Surprisingly, *N*(9) methylated anilinoacridines are hardly known in the literature, most probably due to poor commercial availability of the corresponding *N*(Me) aromatic synthones. Therefore furnishing the synthesis of suitable *N*(Me) arenes was the first task we conducted. Here we report a facile synthesis of novel antitumor *N*(9) methylated AHMA analogs for structure–activity and bio-stability relationship studies. Preliminary anti-cancer activity of these compounds is also reported.

Initially, we prepared the required *N*(Me) arenes from the corresponding aminoarenes, applying an improved two-step synthesis described in the literature.[10–12] It involves Boc protection of the aminobenzoic acids and subsequent reduction of both the acid and the carbamate functional groups with $LiAlH_4$ to *N*-methyl benzyl alcohol. We suggest the following mechanism



Scheme 1 Proposed mechanism for reductive *N*-methylation of Boc-protected anilines.

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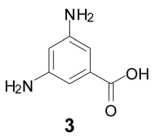
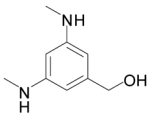
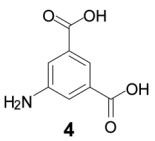
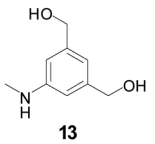
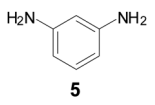
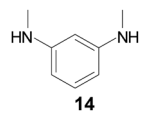
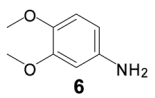
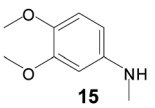
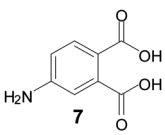
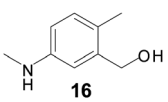
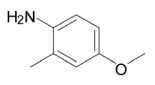
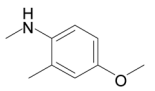
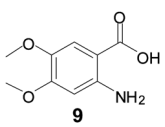
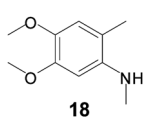
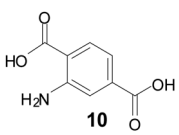
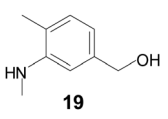
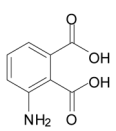
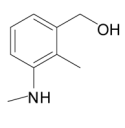
† Electronic supplementary information (ESI) available: ^1H NMR, ^{13}C NMR and HRMS data of all synthetic compounds. See DOI: 10.1039/c2nj40567a

for the conversion of BocNHAr to MeNHAr: *N*-Boc is first reduced to formamide, followed by further reduction to aminal and dehydration to form imine, which is finally reduced to *N*(Me) (Scheme 1).

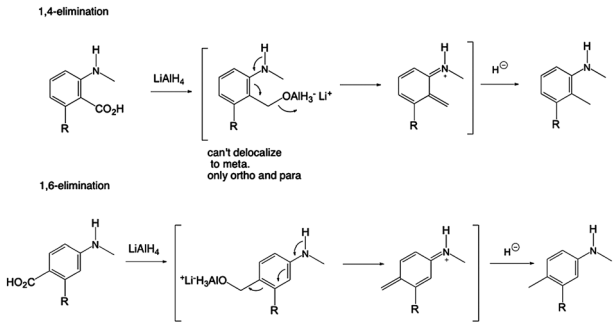
The procedure works smoothly, yielding *N*(Me) arenes **12–20** in good yields (Table 1). Structure determination of **12–20** was confirmed by 2D NMR experiments (see ESI†).

Notably, several building blocks underwent unexpected benzylic OH elimination at *ortho* and *para* positions to the *N*(Me). For example, in compound **7**, only the *meta* positioned carboxyl group was reduced to the corresponding alcohol,

Table 1 Reaction data for reductive *N*-methylation of aminoanilines by Boc protection and $LiAlH_4$ reduction

Entry	Substrate	Product	Yield (%) (for the two steps)
1	 **3**	 **12**	87
2	 **4**	 **13**	86
3	 **5**	 **14**	93[a]
4	 **6**	 **15**	91
5	 **7**	 **16**	75[a]
6	 **8**	 **17**	77
7	 **9**	 **18**	62[a]
8	 **10**	 **19**	70[a]
9	 **11**	 **20**	63[a]

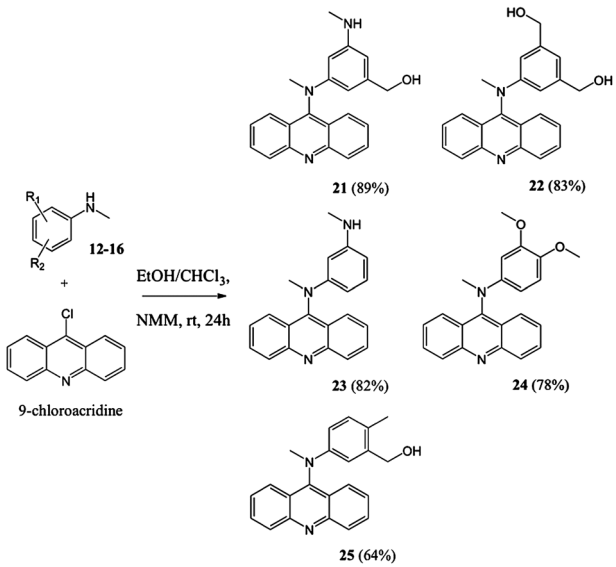
[a] After chromatography.



Scheme 2 Proposed mechanism for reductive deoxygenation of *N*(Me) methyleneoxy anilines.

while the *para* CO_2H was further reduced to produce the methyl substitution, yielding compound **16**. Such a phenomenon was also observed in **9**, **10** and **11**, yielding dehydrated products **18**, **19** and **20**, respectively, as opposed to aminobenzoic acids **3** and **4**, bearing carboxylic groups at *meta* positions to the amine group. Based on the above results we assume that such dehydration reactions occur through 1,4-elimination (for *ortho*) and similar 1,6-elimination (for *para*) of the intermediate aluminum complex, followed by further reduction. Such a mechanism could not be realized in the *meta* hydroxymethyl anilines, preserving it from reductive dehydration (Scheme 2). The extra elimination–reduction reactions and the formation of a reactive amino quinone methide intermediate may explain the somewhat lower yields in the reduction reactions involving the *ortho* and *para* amino carboxylic acids.

Next, we proceeded to the synthesis of the *N*(9)Me AHMA derivatives from the corresponding *N*-methylated arenes. The other component, 9-chloroacridine, was prepared by microwave assisted synthesis.[13] Finally, the reaction of aminoarenes **12–16** with 9-chloroacridine under mild basic conditions (NMM) in a chloroform–ethanol mixture at room temperature afforded new *N*(9)-Me AHMA derivatives in good yields (Scheme 3). Unfortunately, aminoarenes **17–20** did not afford the corresponding 9-anilinoacridines. Only the starting materials were recovered under various reaction conditions. This might be



Scheme 3 Synthesis of AHMA analogs.

Table 2 Cell growth inhibition assay of the synthetic AHMA derivatives against a non-tumorigenic breast epithelial cell line and seven cancer cell lines[a]

AHMA derivatives	MFA-10A	H 1299	MCF7 MDR	MCF7 MITO	OVCAR8	NCI ADR	MDA-MD-131	HT29
AHMA	11.2	5.3	1.8	NA	NA	1.5	0.6	1.7
21	23.7	4.5	1.79	NA	NA	2.5	0.9	1.6
22	NA	NA	NA	NA	NA	5.5	0.9	NA
23	30.1	NA	NA	NA	NA	4.1	1.9	8.8
24	NA	**0.7**	NA	NA	**1.5**	2.3	1.2	NA
25	NA	5.8	NA	**2.9**	3.1	2.7	0.7	1.2

[a] Cell growth inhibition assay after 48 h incubation. Values reported are IC_{50} (μM) (n = 3, all SE were between 0.02 and 0.06 μM). Boldfaced values mark combinations that exhibit a significantly higher cytotoxic effect than that of AHMA. Cell lines are as follows: MDA-MD-131 (renal cancer), OVCAR8 (ovarian cancer), NCI-ADR (ovarian cancer associated with multidrug resistance (MDR)), MCF-7mito (mitoxantrone selected breast cancer associated with MDR), HT29 (colon carcinoma), H1299 (lung carcinoma), and MFA-10A (noncancerous epithelial cells). NA – IC_{50} values above 40 μM; no effect was observed with the vehicle (DMSO) alone.

due to steric hindrance caused by the presence of a methyl group in an *ortho* position to the aromatic *N*(Me).

Preliminary anti-proliferative tests of the synthetic compounds identified low micromolar leads against MDA-MD-131 (renal cancer), OVCAR8 (ovarian cancer), NCI-ADR (ovarian cancer associated with MDR phenomena), MCF-7mito (mitoxantrone selected breast cancer associated with MDR phenomena), HT29 (colon carcinoma) and almost insensitive to chemotherapy H1299 (lung carcinoma). Some promising compounds exhibited enhanced cytotoxic effect as compared with AHMA, while showing very moderate cytotoxic effect on the non-tumorigenic breast epithelial MFA10A cell line (Table 2). Interestingly, significant differences in the sensitivity to the tested compounds were observed between these cell lines. Of special interest is compound **24**, exhibiting the best activity against the H1299 cell line (almost an order of magnitude better than AHMA and the other tested compounds), and against the ovarian cancer OVCAR8 cell line (against which AHMA exhibits no activity). In addition, compound **24** exhibited no cytotoxicity against the non-malignant MFA-10A cell line. These results are very promising since the H1299 lung carcinoma cell line is very resistant to other chemotherapies, and ovarian cancer is one of the deadliest cancers in women, with a 5 year survival rate of only 47%.[14] Thus, compound **24** is an attractive target for further development of anticancer drugs.

In conclusion, we introduce a useful method for the efficient synthesis of medicinally important *N*(9)Me AHMA derivatives. Efficient two-step preparation of *N*(Me) aromatic synthones, followed by simple coupling with 9-chloroacridine, afforded novel AHMA analogs that can provide valuable information on the bio-mechanistic issues associated with *N*(9) substitution.

The new compounds were tested as anti-proliferative agents against seven cancer cell lines. Some of these compounds exhibited significant anti-tumor activity. In three cases, the activity was an order of magnitude or more better than that of AHMA. Detailed biological tests and mechanistic studies are in progress and will be published in due course.

Experimental

General procedure for the synthesis of 12–20 *via* Boc protection and reduction

To a solution of a substituted aniline (33 mmol) in methanol (30 mL) was added a 10% solution of triethylamine in methanol (33 mL). The solution was stirred vigorously, and then *t*-butoxycarbonyl anhydride (28.8 g, 132 mmol) was added in portions. The mixture was stirred at room temperature for 24 h. The solution was concentrated, and the residue was partitioned between ethyl acetate and 1 M potassium hydrogen sulfate. The organic layer was separated, washed with water, and dried over magnesium sulfate before the removal of ethyl acetate.

Lithium aluminum hydride (4.9 g, 129 mmol) was suspended in THF (200 mL) at 0 °C. The protected aniline (32 mmol) was added slowly in small portions. The reaction mixture was heated at reflux overnight and then cooled to room temperature. Saturated K_2CO_3 was added slowly. Filtration and evaporation of the solvent gave a colorless oil. Compounds that did not precipitate were purified by flash column chromatography on silica gel 60 (20% EtOAc in petroleum ether) to yield pure products.

Data for **12** (2.36 g, 87% yield): ^{1}H NMR (300 MHz, $CDCl_3$): 6.02 (d, 2H, J = 1.5 Hz), 5.81 (br s, 1H), 4.53 (s, 2H), 2.82 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 151.0, 143.4, 101.3, 95.9, 66.1, 31.1; HRMS (CI, m/z) calcd for $C_9H_{14}N_2O$ (MH^+) 166.1106, found 166.116.

General procedure for the synthesis of 21–25

A solution of 9-chloroacridine (3.12 g, 14.6 mmol) in $CHCl_3$ (5 mL) was added dropwise to a mixture of the corresponding aniline (14.6 mmol) and 4-methylmorpholine (3.21 mL, 29.2 mmol) in EtOH (20 mL) at an ice bath temperature. After stirring for 1 h, the temperature was raised to room temperature and the mixture was stirred further for 24 h. The precipitated orange product was collected by filtration, washed with EtOH and dried. Compounds that did not precipitate were purified by flash column chromatography on silica gel 60 (5% MeOH in $CHCl_3$) to yield pure products.

Data for **21** (2 g, 89% yield): ^{1}H NMR (400 MHz, DMSO-d_6): 8.18 (d, 2H, J = 8.7 Hz), 7.81 (dd, 2H, J = 7.3 Hz), 7.63 (d, 2H, J = 8.7 Hz), 7.49 (dd, 2H, J = 7.3 Hz), 6.35 (s, 1H), 5.91 (s, 1H), 5.70 (br s, 1H), 4.67 (m, 1H), 3.71 (br s, 1H), 3.60 (d, 2H, J = 4.8 Hz), 2.83 (d, 3H, J = 4.8 Hz), 2.48 (d, 3H, J = 4.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): 158.9, 158.4, 155.8, 147.4, 146.7, 142.0, 141.1, 127.7, 127.3, 126.4, 121.5, 103.3, 96.6, 61.0, 32.7, 29.9; HRMS (CI, m/z) calcd for $C_{22}H_{21}N_3O$ (MH^+) 343.1685, found 343.1690.

Abbreviations

AHMA, 3-(9-acridinylamino)-5-hydroxymethylaniline; *m*-AMSA, amsacrine; *m*-AQDI, *m*-AMSA-quinonediimine; Boc, benzyloxycarbonyl; MDR, multidrug resistance; NMM, *N*-methylmorpholine; SAR, structure–activity relationship.

Acknowledgements

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