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One-Pot Synthesis of Novel Antiproliferative 9-Aminoacridines

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Highly efficient one-pot syntheses of antiproliferative 9-aminoacridine (9-AA) derivatives are described. Simple S_NAr and addition/elimination reactions, using readily accessible starting materials, give a fast entry to novel 9-(pyridylamino)-acridines, 9-(pyrimidinylamino)acridines and potential "dual-function" bioreductive 9-(acridinylamino)quinone intercalators. The synthetic routes reported in this work are general and readily applicable, significantly expanding the range and scope of potential 9-AA anticancer candidates.

Introduction

A wide variety of DNA intercalators have found great utility in anticancer therapy, from natural products such as doxorubicin and actinomycin D to synthetic drugs such as mitoxantrone and amsacrine.[1]

Synthetic intercalators based on the 9-aminoacridine (9-AA) core, a structure that appears in many biologically active compounds with antimalarial and anticancer applications, are of particular interest. Aromatic 9-AA derivatives such as quinacrine[1c] intercalate into DNA, and consequently inhibit transcription in parasites. In addition, *N*-alkylated 9-AA analogs have proven to be potent agents against prion diseases in cultured neuroblastoma cells.[1h]

In the field of antitumor DNA-intercalating agents, 9-anilinylacridine (9-AnA) derivatives play an important role due to their antiproliferative properties, mostly as potent DNA topoisomerase II inhibitors.[2] A series of potential topoisomerase II targeting anticancer 9-AnAs, which are designed to avoid biooxidation and possess long durations of drug action, have been reported.[3] Among these substances, 3-(9-acridinylamino)-5-hydroxymethylaniline (AHMA) and its alkylcarbamate derivatives were developed for potential clinical applications.[3a,3c]

It was recently reported that 9-AA is associated with another mechanism of action, as it suppresses PI3K/AKT/mTOR, p53 and NF-kB pathways that are frequently deregulated in tumor cells.[4] The ability to affect simultaneously several biological pathways makes this a candidate for a previously uncharacterized class of bitargeting anticancer drugs. The advantage of 9-AA and similar small molecules as anticancer agents that do not induce DNA damage was recently discussed.[5] These compounds elicit fewer and much less pronounced adverse side effects and present a low risk of secondary cancer development, but are still as potent as DNA-damaging agents in the killing of tumor cells.

In the last two decades a large number of reports regarding synthesis, SAR and anticancer activity have been published, providing a solid foundation for drug design and optimization studies of novel 9-amino- and 9-anilinoacridine-based compounds. However, one of the main obstacles in pursuing these goals is the laborious synthesis of desired 9-AA derivatives, which often involves several steps and difficult conditions.[6] This has prompted us to look for a short and efficient method for the derivatization of the 9-AA scaffold suitable for rapid generation of new compounds for biological evaluation.

Previously, we reported a new and highly efficient one-pot derivatization of 9-AA at the amino position by simple reductive amination (**II**, Scheme 1) and S_NAr reactions (**I**, Scheme 1) using easily accessible starting materials.[7] Here we report on the extension of the one-pot derivatization strategy of 9-AA, which applies a simple addition/elimination (AE) reaction and a new aspect of the S_NAr reaction to give a fast entry to novel (9-acridinylamino)quinone (**V**, **VI**) and 9-(azaarylamino)acridine (**III**, **IV**) derivatives, respectively.

Results and Discussion

A few 9-AAs were previously synthesized from 9-azidoacridines or other "pseudo"-9-chloroacridines.[8] However, no direct one-pot synthesis of 9-AAs from commercially available starting materials has been developed. We recently found that the amino group in 9-AA is nucleophilic enough to take part in efficient nucleophilic aromatic substitution (S_NAr) reactions, yielding 9-anilinylacridines with halo-

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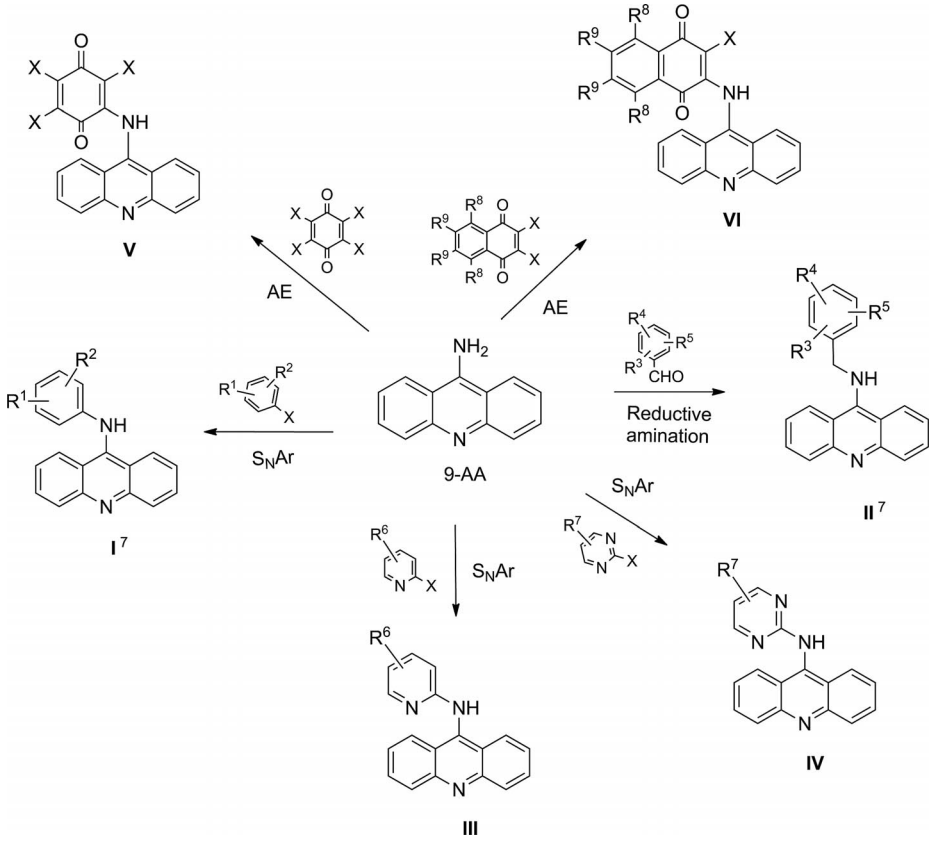


Scheme 1. General approaches for the one-pot derivatization of 9-aminoacridines.

benzenes bearing electron-withdrawing (EW) groups at various positions on the reacting aryl ring.[7] The starting electrophilic halobenzenes with one or two strong electron-withdrawing groups reacted smoothly with 9-AA to give substituted 9-anilinylacridines in good yields. Recently, we decided to examine the influence of other types of substitution and structural factors on the one-pot formation of 9-AnAs. First, aza substitution in the electrophilic aryl reagents,[9] as in chloropyridines and chloropyrimidines, was employed, leading to novel *N*-pyridyl- and *N*-pyrimidinyl-9-AA derivatives (**III** and **IV**, Scheme 1). Secondly, haloquinone electrophiles were used in an AE reaction with 9-AA, affording novel 9-(acridinylamino)quinones (**V** and **VI** Scheme 1), the structural chimeras bearing both reductive (quinone)[10] and intercalating (9-AA) moieties. Two general one-pot reaction conditions were established. Route (a) involves refluxing in EtOH overnight, whereas Route (b) calls for the use of Cs_2CO_3 in DMF at 90 °C for a period of 12 h. These conditions are optimized for the preparation of the desired products in moderate to good yields. Moreover, simple workup procedures further contribute to the synthetic accessibility enabled by both routes.

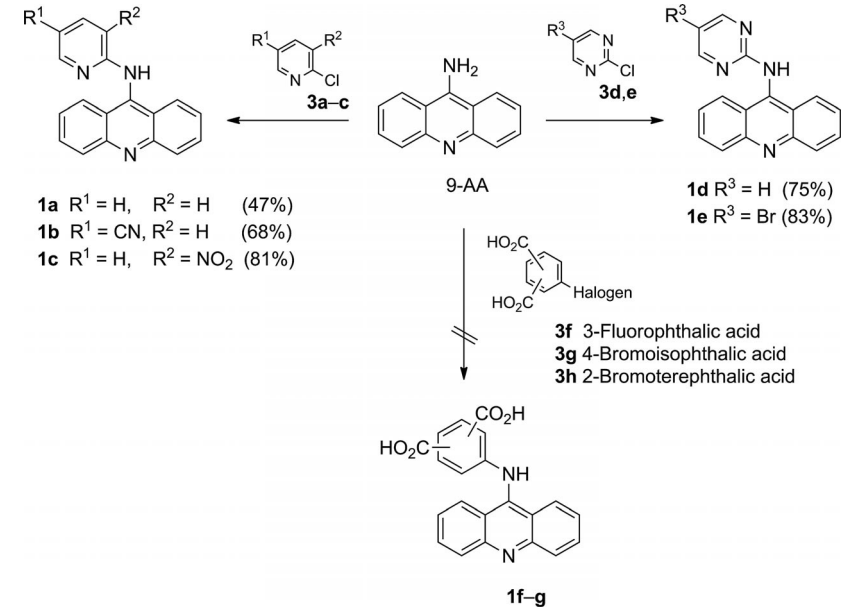
Synthesis of 9-(Azaarylamino)acridine Derivatives 1a–e by Using S_NAr Reactions

We treated representative chloropyridines and pyrimidines **3a**–**e** with 9-AA in the presence of 1 equiv. of Cs_2CO_3 in DMF at 90 °C to afford substituted 9-(pyridylamino)-acridines **1a**–**c** and 9-(pyrimidinylamino)acridines **1d**,**e** in reasonable to good yields (47–83%, Scheme 2).[11] This method, namely Cs_2CO_3 in DMF at 90 °C [Route (b)], is an efficient means of preparing substituted 9-AA derivatives. It significantly simplifies the formation of aryl-[12] and aza-aryl-substituted 9-AAs with EW groups, which are not accessible by using the standard "reverse" approach, which calls for nucleophilic substitution of the deactivated aminopyridines or aminopyrimidines with 9-chloroacridines.[12,13]

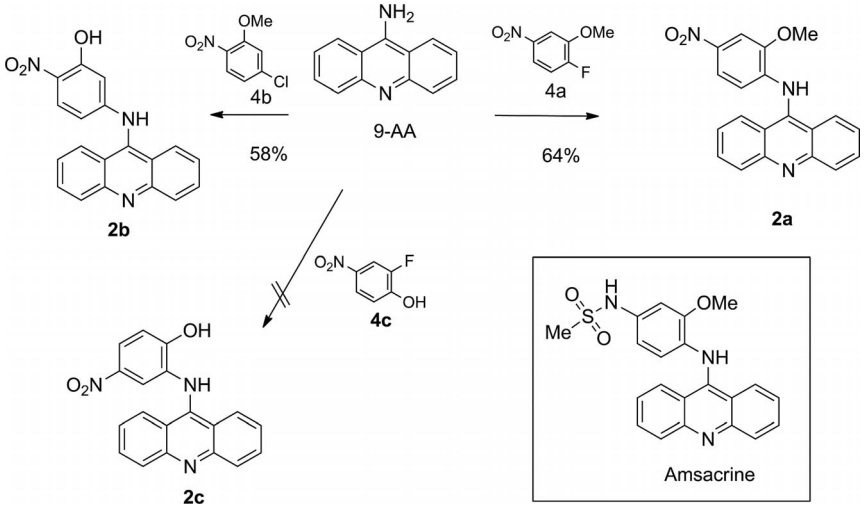
An additional advantage of the one-pot S_NAr reaction with 9-AAs is the commercial availability of appropriately substituted 2-pyridyl and 2-pyrimidinyl halide reagents. We also attempted the preparation of dicarboxyl compounds **1f**–**h** by treating 9-AA with 3-fluorophthalic, 4-bromoisophthalic and 2-bromoterephthalic acids **3f**–**h**, respectively (Scheme 2). Unfortunately, only starting materials were isolated, probably due to insufficient electrophilicity of the halobenzoic acids used. This suggested the necessity of harsher Ullmann conditions.[14]

Synthesis of 9-(Arylamino)acridine Derivatives 2a,b by Using S_NAr Reactions

The above successful results encouraged us to examine the influence of the unfavorable electron-donating (ED) methoxy group on the haloaryl reagent for the preparation of 9-AnAs. Electron-donating groups frequently appear on



Scheme 2. Synthesis of 9-AA derivatives **1** by S_NAr reactions. Reagents and conditions: Cs_2CO_3, DMF, 90 °C, 12 h.



Scheme 3. Synthesis of 9-aminoacridine derivatives **2** by S_NAr reactions. Reagents and conditions: Cs_2CO_3, DMF, 90 °C, 12 h.

9-AnA-based drugs.[1,2] Of particular interest was 1-fluoro-2-methoxy-4-nitrobenzene (**4a**), since the resulting S_NAr product **2a** could be considered an intermediate in the synthesis of amsacrine (Scheme 3). The nitro group could be reduced to the amine and the resulting amine then mesylated to afford amsacrine. To our satisfaction, the reaction of 9-AA with **4a** under standard conditions [Route (b)] yielded compound **2a** in 64% yield, indicating a non-interruptive character of the ED methoxy group at the *ortho* position with the powerful EW nitro group at the *para* position.

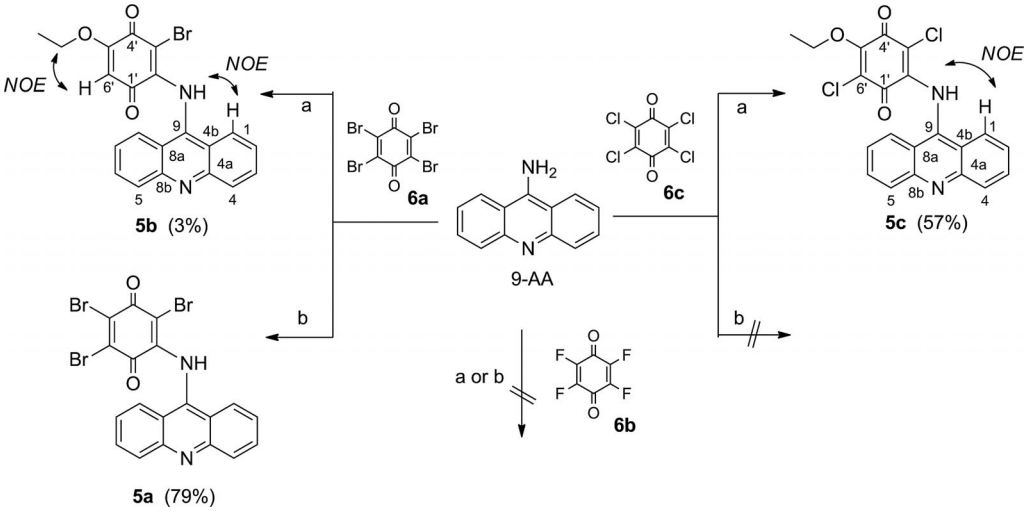
Next, we examined the impact of different substitutional combinations of nitro and methoxy or hydroxy groups. When **4b** (methoxy group in *meta* position) was treated with 9-AA under the same reaction conditions, **2a** was obtained in 58% yield. Production of **2b** may be the result of an unexpected alkali demethylation, most likely assisted by the adjacent nitro group.[15] All attempts towards a reaction of 2-fluoro-4-nitrophenol (**4c**), bearing a free hydroxy group, with 9-AA under standard conditions [Route (b)] led to a complex mixture of unidentified products. We reasoned that the formation of a phenolate anion by Cs_2CO_3 may interfere with the formation of the desired products. Furthermore, substitution of an F atom by the S_NAr reaction requires an activating NO_2 substitution in *para* or *ortho* position, whereas the corresponding substitution of **4c** is in *meta* position.

Synthesis of 9-(Acridinylamino)quinone Derivatives 5a–f by AE Reactions

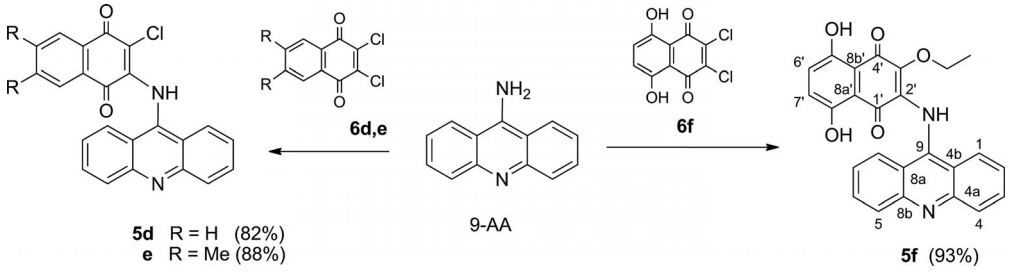
In the context of exploring the rapid and simple derivatization of the 9-AA scaffold, we applied AE reactions of 9-

AA with multisubstituted haloquinones (products **V** and **VI**, Scheme 1). Halobenzoquinones and halonaphthoquinones are widely used as anticancer agents with bioreductive properties.[10] Members of this class of compounds can be selectively activated to cytotoxic species by reduction. The selective bioactivation may be due to elevated levels of some reductases in certain tumors, or to hypoxia. Significant precedence has shown that bioreductively activated drugs are more toxic to acidic hypoxic cells than they are to well-oxygenated ones.[16]

Thus, we decided to combine a quinone anticancer moiety with a DNA intercalator, 9-AA, to obtain potential bifunctional compounds with very interesting biological activities. Although, a number of bifunctional bioreductive structures have been published,[17] the use of DNA intercalators as an active component of such compounds has not yet been reported. The rationale driving the idea was to use the intercalator moiety to locate the bifunctional molecule between the DNA strands followed by active species generation by reductive metabolism of the quinone under aerobic conditions, which would then lead to damage to the cancer cells exceeding that possible using either the quinone or intercalator independent of each other. We based our work on several published syntheses calling for the straightforward amination of haloquinones under mild conditions.[18] Successful one-pot syntheses of "dual" agents **5a**–**c** and **5d**–**f** in moderate to good yields are presented in Schemes 4 and 5, respectively. To demonstrate the synthetic potential of this reaction with 9-AA, we employed two classes of representative quinones: halobenzoquinones **6a**–**c** (Scheme 4) and halonaphthoquinones **6d**–**f** (Scheme 5), yielding the respective bifunctional quinone-9AAs **5a**–**f** under mild conditions. Interestingly, during the course of our work, we noticed that different reaction conditions lead to different products. When 9-AA was treated with equimolar amounts of tetrachlorobenzoquinone (**6c**) in refluxing EtOH overnight, the corresponding quinone-9-AA product **5c** was obtained in 57% yield. This product possesses one ethoxy group as the result of an additional AE reaction with EtOH. The same reaction, with analogous tetrabromobenzoquinone (**6a**), led to the formation of a single product **5b** in very low yield (3%). Mass spectrometry clearly showed the presence of only one bromine atom, and ^{1}H NMR spectroscopy confirmed the presence of one quinone hydrogen atom [δ = 5.93 (s) ppm]. The chemical structures of **5b**,**c** were fully assigned on the basis of NOE, HMBC, HMQC experiments. The mechanism of the reduction remains unclear to us. On the other hand, when using aprotic conditions (1 equiv. of Cs_2CO_3 in DMF at 90 °C for 12 h) only **6a** underwent a classical AE reaction, leading to tribromoquinone **5a** in 79% yield after purification. The use of tetrachloroquinone **6c** did not afford any recognizable products



Scheme 4. Synthesis of 9-aminoacridine derivatives **5a**–**c** by using AE reactions. Reagents and conditions: (a) EtOH, reflux, overnight; (b) Cs_2CO_3, DMF, 90 °C, 12 h.



Scheme 5. Synthesis of 9-AA derivatives **5d**–**f** by AE reactions. Reagents and conditions: EtOH, reflux, overnight.

under the same conditions. Unfortunately, tetrafluoroquinone **6b** yielded only mixtures of unidentified products under both conditions [Routes (a) and (b)].

Moving forward, we decided to use chloronaphthoquinone reactants in AE reactions to attach the larger derivatized naphthoquinone moiety to the 9-AA scaffold. Naphthoquinones can bear additional amino, hydroxy and methoxy groups that, together with the quinonic ketone, are able to enhance biologically important chelating properties, possibly leading to the formation of more powerful DNA-damaging reactive species.[19] First, we treated 9-AA with commercially available naphthoquinones[20] **6d**,**e** using Route (a) conditions, which led smoothly to monosubstitution by 9-AA (Scheme 5) and formation of the dark gray crude chloronaphthoquinone products **5d**,**e**. Simple workup followed by flash-chromatography purification (silica gel; EtOAc/petroleum ether, 1:2) gave the corresponding products in 82% and 88% yields, respectively. The existence of one Cl atom in **5d**,**e** was confirmed by mass spectrometry. We then treated 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone[21] (**6f**) with 9-AA in refluxing EtOH [Route (a)] to afford reddish crude product **5f** in 93% yield. This product was of sufficient purity that no further purification was necessary. The spectral analysis of **5f** revealed it to be a result of a double AE reaction. One AE reaction entailed 9-AA as the nucleophile, whereas the second AE reaction involved EtOH as the nucleophile (see ^{1}H NMR and mass spectra of **5f** in the Supporting Information). This observation parallels those involving quinone derivatives **5b**,**c** (Scheme 4) but contrasts those involving naphthoquinone analogs **5d**,**e** (Scheme 5), which underwent only one substitution reaction. The assignment of a double AE reaction was supported by the observed low-field shift in the ^{1}H NMR spectrum for the signals of the nonsymmetrical phenolic protons at δ = 12.10 and 11.82 ppm, and the typical high-field shift for the signal of the ethoxy methylene group attached to a double bond [δ = 4.58 (q, J = 6.5 Hz, 2 H) ppm; see full assignment in Supporting Information].

Preliminary antiproliferative tests of the synthesized compounds revealed submicromolar leads against MDMMD-A31 (renal cancer), OVCAR8 (ovarian cancer), NCI-ADR (associated with MDR ovarian cancer), MCF-7mito (mitoxantrone selected and associated with MDR breast cancer), HT29 (colon carcinoma) and H1299, and almost completely insensitive to chemotherapy lung carcinoma. Some of the tested compounds showed greatly enhanced cytoxicity relative to amsacrine. Interestingly, large differences in the sensitivity to the tested compounds were observed among these cell lines, and more detailed biological results will be published in due course.

In summary, the one-pot synthetic methodologies described in this paper can efficiently yield novel medicinally important 9-(arylamino)acridines by S_NAr reactions and potential "dual-function" bioreductive intercalators, 9-(acridinylamino)quinones, by AE reactions using easily accessible starting materials. The synthetic methodology reported in this work is general and readily applicable, significantly expanding the scope of potential 9-AA anticancer compounds. Attempts to facilitate the reactions by the use of microwave-assisted chemistry are also in progress.

Conclusions

We have developed new methods for the efficient one-pot derivatization of the medicinally important 9-AA scaffold. Surprisingly, the S_NAr reaction between 9-AA and aryl halides does not require the strong electron-withdrawing NO_2 group on the aryl halide reagent. 2-Methoxy- and 3-methoxy-4-nitroaryl halides, which bear the ED methoxy group, also undergo this reaction to give products structurally related to amsacrine in good yields. Additionally, a simple AE reaction between haloquinones and 9-AA represents a fast entry to many potential "bitargeting" 9-(acridinylamino)-quinone intercalators. The synthetic routes described in this paper provide a rapid access to novel 9-AA derivatives, which are being further evaluated for their biological properties.

Experimental Section

General: Analytical HPLC was performed on a 250 × 4.2 mm LiChroprep RP-18 column from Merck, with a 1 mL/min flow and detection at 214 nm. The eluents were triply distilled water and HPLC-grade CH_3CN containing 0.1% TFA or MeOH. The concentration of all the samples was 0.5%. Mass spectra were measured in the positive and negative modes with a quadruple mass spectrometer equipped with an electrospray ionization source and cross-flow inlet. ^{1}H and ^{13}C NMR spectra were recorded at 700 or 300 and 75 MHz, respectively, in [D_6]DMSO, unless otherwise indicated. Assignments in the final products were supported by 2D COSY, TOCSY, NOESY, ROESY, HMBC, and HMQC spectroscopy. All chemical shifts are reported with respect to TMS. SPE (solid-phase extraction) was performed with LiChrospher 60 RP-18 columns purchased from Agilent Technologies. Chromatography was carried out by standard flash chromatography and TLC on silica gel (Merck 7735).

General Procedure for the Synthesis by Using AE Reactions [Route (a)]: 9-AA (0.194 g, 1 mmol) and haloquinone (1 mmol) were refluxed in EtOH (15 mL) overnight. While heating, the color of the reaction mixture in most cases changed to dark red or gray. After completion of the reaction (determined by TLC monitoring), the mixture was cooled and concentrated to give a crude red or gray solid. The products were purified by flash column chromatography on silica gel 60 to yield the corresponding products.

General Procedure for the Synthesis by Using S_NAr Reactions [Route (b)]: 9-AA (0.194 g, 1 mmol), aryl halide or haloquinone (1 mmol), and Cs_2CO_3 (0.161 g, 0.5 mmol) were heated in dry DMF (5 mL) at 90 °C for 12 h. While heating, the color of the reaction mixture in most cases changed to dark red. After completion of the reaction (determined by TLC monitoring), the mixture was cooled and poured into water. The resulting precipitate was collected by filtration, washed several times with water, and dried to give a crude red or orange solid. The products were purified by flash column chromatography on silica gel 60 to yield the corresponding products.

9-(2-Pyridylamino)acridine (1a): Synthesized according to Route (b) from **3a** (0.113 g). Orange solid after chromatography ($CHCl_3$);

0.13 g, 47% yield; R_f = 0.65 (EtOAc). ^{1}H NMR (300 MHz, [D$_6$]-DMSO): δ = 9.12 (br. s, 1 H, NH), 8.23 (d, J = 6.8 Hz, 2 H), 8.04–7.89 (m, 3 H), 7.76–7.51 (m, 3 H), 7.38–7.27 (m, 2 H), 6.74–6.68 (m, 2 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 157.1, 150.6, 148.2, 141.5, 130.3, 129.0, 125.2, 122.6, 121.0, 119.6, 113.2, 109.5 ppm. HRMS (CI): calcd. for $C_{18}H_{14}N_3$ [M + H]$^+$ 272.118; found 272.086.

9-(5-Cyano-2-pyridylamino)acridine (1b): Synthesized according to Route (b) from **3b** (0.137 g). Orange solid after chromatography ($CHCl_3$); 0.18 g, 68% yield; R_f = 0.60 (EtOAc). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.85 (br. s, 1 H, NH), 8.34–8.11 (m, 3 H), 8.10–7.97 (m, 2 H), 7.82–7.57 (m, 2 H), 7.39–7.18 (m, 4 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 160.2, 151.3, 149.4, 143.5, 141.7, 130.0, 127.1, 125.2, 121.0, 118.2, 116.1, 114.5, 103.9 ppm. IR (KBr): $\tilde{\nu}$ = 2250, 1570, 1430, 1105 cm^{-1}. HRMS (CI): calcd. for $C_{19}H_{13}N_4$ [M + H]$^+$ 297.1134; found 297.1114.

9-(3-Nitro-2-pyridylamino)acridine (1c): Synthesized according to Route (b) from **3c** (0.158 g). Reddish solid after chromatography ($CHCl_3$); 0.25 g, 81% yield; R_f = 0.50 (EtOAc). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.52 (br. s, 1 H, NH), 8.60–8.41 (m, 2 H), 8.22–8.03 (m, 2 H), 7.87–7.41 (m, 5 H), 7.19–7.04 (m, 2 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 154.3, 152.0, 150.4, 143.2, 136.6, 134.3, 130.2, 128.9, 127.7, 120.8, 115.3, 113.5 ppm. HRMS (CI): calcd. for $C_{18}H_{13}N_4O_2$ [M + H]$^+$ 317.103; found 317.120.

9-(2-Pyrimidinylamino)acridine (1d): Synthesized according to Route (b) from **3d** (0.114 g). Orange solid after chromatography ($CHCl_3$); 0.19 g, 75% yield; R_f = 0.40 (EtOAc). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.80 (br. s, 1 H, NH), 8.52 (d, J = 6.7 Hz, 2 H), 8.28 (d, J = 6.8 Hz, 2 H), 8.12–7.98 (m, 4 H), 7.64–7.38 (m, 2 H), 7.08 (t, J = 6.7 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.3, 156.1, 150.0, 148.3, 144.4, 135.7, 130.3, 129.8, 124.2, 121.7, 117.1 ppm. HRMS (CI): calcd. for $C_{17}H_{13}N_4$ [M + H]$^+$ 273.113; found 273.122.

9-(5-Bromo-2-pyrimidinylamino)acridine (1e): Synthesized according to Route (b) from **3e** (0.194 g). Orange solid after chromatography ($CHCl_3$); 0.24 g, 83% yield; R_f = 0.70 (MeOH/$CHCl_3$, 2:98). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.64 (br. s, 1 H, NH), 8.58 (s, 2 H), 8.24 (d, J = 6.8 Hz, 2 H), 8.10–7.92 (m, 4 H), 7.60–7.33 (m, 2 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 154.7, 151.2, 143.4, 142.9, 133.7, 131.1, 127.2, 120.0, 119.8, 116.1, 114.4 ppm. HRMS (EI): calcd. for $C_{17}H_{11}BrN_4$ [M + H]$^+$ 350.016, 352.014; found 350.020 (46%), 352.021 (43%).

9-(2-Methoxy-4-nitrophenylamino)acridine (2a): Synthesized according to Route (b) from **4a** (0.171 g). Orange solid after chromatography (5% MeOH/$CHCl_3$); 0.21 g, 64% yield; R_f = 0.60 (MeOH/$CHCl_3$, 5:95). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.37 (d, J = 6.8 Hz, 2 H), 8.08 (d, J = 6.8 Hz, 2 H), 7.90–7.76 (m, 2 H), 7.70–7.65 (m, 1 H), 7.53–7.49 (m, 2 H), 7.30–7.20 (m, 2 H), 6.25 (d, J = 6.5 Hz, 1 H, NH), 4.18 (s, 3 H, OMe) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.3, 145.7, 143.1, 134.4, 131.0, 128.3, 127.2, 126.0, 124.8, 124.0, 122.4, 121.7, 120.2, 56.5 ppm. HRMS (CI): calcd. for $C_{20}H_{16}N_3O_3$ [M + H]$^+$ 346.119; found 346.177.

9-(3-Hydroxy-4-nitrophenylamino)acridine (2b): Synthesized according to Route (b) from **4b** (0.187 g). Orange solid after chromatography (10% MeOH/$CHCl_3$); 0.19 g, 58% yield; R_f = 0.50 (MeOH/$CHCl_3$, 1:9). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.08 (br. s, 1 H, OH), 8.38–8.27 (m, 3 H), 8.02 (d, J = 6.8 Hz, 2 H), 7.88 (t, J = 6.8 Hz, 2 H), 7.61 (t, J = 6.8 Hz, 2 H), 6.83 (d, J = 6.8 Hz, 2 H), 6.24 (br. s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.6, 135.2, 130.3, 127.7, 125.2, 124.6, 123.2, 121.8, 119.5, 118.2 ppm. HRMS (CI): calcd. for $C_{19}H_{14}N_3O_3$ [M + H]$^+$ 332.103; found 332.106.

2-(Acridin-9-ylamino)-3,5,6-tribromo-1,4-benzoquinone (5a): Synthesized according to Route (b) from **6a** (0.424 g). Dark green solid after chromatography ($CHCl_3$); 0.32 g, 79% yield; R_f = 0.80 ($CHCl_3$). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.07 (d, J = 6.8 Hz, 2 H), 7.81 (t, J = 6.8 Hz, 2 H), 7.54 (t, J = 6.8 Hz, 2 H), 6.92 (d, J = 6.8 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 188.3, 181.9, 173.1, 168.4, 144.0, 140.8, 133.2, 131.7, 129.2, 126.5, 122.3, 121.6, 118.9 ppm. HRMS (EI): calcd. for $C_{19}H_{10}Br_3N_2O_2$ [M]$^+$ 534.829; found 533.802 (35%), 535.807 (100%), 537.811 (98%), 539.798 (28%).

2-(Acridin-9-ylamino)-3-bromo-5-ethoxy-1,4-benzoquinone (5b): Synthesized according to Route (a) from **6a** (0.424 g). Greenish solid after chromatography ($CHCl_3$); 0.01 g, 3% yield; R_f = 0.60 ($CHCl_3$). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.28 (br. s, 1 H, NH), 7.96 (d, J = 7.2 Hz, 2H-4,5), 7.75 (t, J = 7.2 Hz, 2H-2,7), 7.60 (d, J = 7.2 Hz, 2H-1,8), 7.29 (t, J = 7.2 Hz, 2H-3,6), 5.92 (s, 1 6′-H), 4.06 (q, J = 6.2 Hz, OCH_2), 1.36 (t, J = 6.2 Hz, CH_3) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 178.05 (s, C-1′, C=O), 172.77 (s, C-4′, C=O), 160.16 (s, C-5′), 157.27 (s, C-9), 152.79 (s, C-2′), 139.59 (s, 2 C-4a,8b), 133.40 (d, 2 C-2,7), 126.43 (d, 2 C-4,5), 122.51 (d, 2 C-3,6), 117.42 (d, 2 C-1,8), 116.45 (s, 2 C-4b,8a), 104.91 (s, C-3′), 103.90 (s, C-6′), 65.56 (t, OCH_2), 13.74 (q, CH_3) ppm. HRMS (ES): calcd. for $C_{21}H_{14}BrN_2O_3$ [M – H]$^-$ 421.018, 423.016; found 421.284 (89%), 423.281 (100%).

2-(Acridin-9-ylamino)-3,6-dichloro-5-ethoxy-1,4-benzoquinone (5c): Synthesized according to Route (a) from **6c** (0.246 g). Greenish solid after chromatography ($CHCl_3$); 0.27 g, 57% yield; R_f = 0.70 ($CHCl_3$). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.40 (br. s, 1 H, NH), 8.01 (d, J = 6.8 Hz, 2 H-4,5), 7.78 (t, J = 6.8 Hz, 2 H-2,7), 7.63 (d, J = 7.2 Hz, 2 H-1,8), 7.30 (t, J = 7.2 Hz, 2 H-3,6), 4.63 (q, J = 5.8 Hz, OCH_2), 1.36 (t, J = 6.2 Hz, CH_3) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.91 [s, C-1′(4′), C=O], 172.69 [s, C-4′(1′), C=O], 158.16 (s, C-9), 156.51 (s, C-5′), 149.43 (s, C-2′), 139.55 (s, 2 C-4a,8b), 133.59 (d, 2 C-2,7), 126.57 (d, 2 C-4,5), 122.65 (d, 2 C-3,6), 119.67 (s, C-3′), 117.54 (d, 2 C-1,8), 116.39 (s, 2 C-4b,8a), 112.12 (s, C-6′), 70.70 (t, OCH_2), 15.88 (q, CH_3) ppm. HRMS (ES): calcd. for $C_{21}H_{15}Cl_2N_2O_3$ [M]$^-$ 413.045; found 413.270 (48%), 415.276 (31%), 417.273 (7%).

2-(Acridin-9-ylamino)-3-chloro-1,4-naphthoquinone (5d): Synthesized according to Route (a) from **6d** (0.226 g). Dark gray solid after chromatography ($CHCl_3$); 0.34 g, 82% yield; R_f = 0.75 ($CHCl_3$). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.20–8.03 (m, 3 H), 7.90–780 (m, 3 H), 7.72–7.65 (m, 2 H), 7.60–7.57 (m, 2 H), 7.23 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 181.3, 181.0, 164.1, 157.2, 148.4, 146.2, 138.9, 135.5, 132.8, 133.0, 132.5, 130.1, 129.3, 127.3, 125.7, 120.8, 118.2 ppm. HRMS (ES): calcd. for $C_{23}H_{14}ClN_2O_2$ [M + H]$^+$ 385.074, 387.072; found 385.136 (100%), 387.1410 (36%).

2-(Acridin-9-ylamino)-3-chloro-6,7-dimethyl-1,4-naphthoquinone (5e): Synthesized according to Route (a) from **6e** (0.254 g). Dark gray solid after chromatography ($CHCl_3$); 0.38 g, 88% yield; R_f = 0.80 ($CHCl_3$). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.17–8.00 (m, 3 H), 7.86–7.80 (m, 2 H), 7.77–7.60 (m, 3 H), 7.26 (t, J = 6.9 Hz, 2 H), 2.46 (s, 3 H, Me), 2.53 (s, 3 H, Me) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 181.1, 180.6, 164.0, 157.2, 148.5, 147.3, 137.8, 134.3, 133.7, 133.4, 131.9, 131.1, 129.0, 128.2, 126.7, 121.8, 117.5, 19.2, 18.7 ppm. HRMS (ES): calcd. for $C_{25}H_{18}ClN_2O_2$ [M + H]$^+$ 413.105, 415.103; found 413.110 (98%), 415.111 (34%).

2-(Acridin-9-ylamino)-3-ethoxy-5,8-dihydroxy-1,4-naphthoquinone (5f): Synthesized according to Route (a) from **6f** (0.256 g). Dark

red solid; 0.41 g, 93% yield; R_f = 0.30 (MeOH/$CHCl_3$, 1:99). ^1H NMR (300 MHz, [D_6]DMSO): δ = 12.10 (s, 1 H, OH), 11.82 (s, 1 H, OH), 10.16 (br. s, 2 H, NH_2^+), 8.72 (d, J = 7.0 Hz, 2H-1,8) 8.02–7.95 (m, 4H-3,4,5,6), 7.59 (t, J = 7.0 Hz, 2H-2,7), 7.40–7.38 (m, 2H-6′,7′), 4.58 (q, J = 6.2 Hz, OCH_2), 1.37 (t, J = 6.2 Hz, CH_3) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 181.56 (s, 2-C=O), 157.72 (s, C-9), 157.20 (s, C-3′), 156.88 (s, C-5′), 156.16 (s, C-8′), 139.25 (s, 2C-4a,8b), 135.48 (d, 2C-3,6), 129.52 (d, C-7′), 129.10 (d, C-6′), 127.38 (s, C-2′), 124.72 (d, 2 C-1,8), 123.73 (d, 2 C-2,7), 118.67 (d, 2 C-4,5), 111.44 (s, 2 C-4b,8a), 127.38 (s, C-2′), 111.24 (s, C-8b′), 110.46 (s, C-8a′), 70.67 (t, OCH_2), 15.83 (q, CH_3) ppm. HRMS (ES): calcd. for $C_{25}H_{19}N_2O_5$ $[M + H]^+$ 427.129; found 427.134 (38%), 425.131 $[M - H]^-$ (100%).

Supporting Information (see footnote on the first page of this article): NMR and mass spectra.

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