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Synthesis and Antitumor Activity of Sulfur-Containing 9-Anilinoacridines

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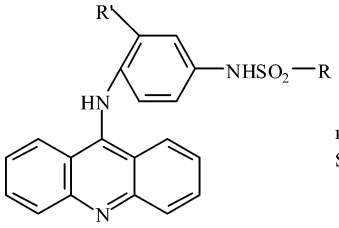
Abstract: A series of sulfur-containing 9-anilinoacridines related to amsacrine were synthesized and evaluated for their anticancer potential. Among the compounds, both diol-containing compounds, **2a** and **3**, were the most cytotoxic of the sulfide series against V-79 cells in vitro (IC_{90} = 2.1 μM and 1.9 μM, respectively). Among the non-alkyl-substituted compounds (**7**−**9**), compounds with electron-donating substitution para to the sulfide (**7** and **9**) were more cytotoxic than the electron-withdrawing nitro-substituted compound **8**. The limited SAR suggested the importance of hydroxyl functionality along with its location for the cytotoxicity in the series. A preliminary anticancer screening against P388 leukemia showed that **2a** is highly active in vivo as well. Topoisomerase II inhibitory activity appeared to be involved in the cytotoxicity of compound **2a**. Sulfoxide compound **2b**, which is 6−7-fold less cytotoxic than its sulfide **2a**, appears to be a potential bioreductive anticancer prodrug on the basis of its bioreductive metabolism findings.

Keywords: Amsacrine; 9-anilinoacridines; V-79 Chinese hamster lung fibroblasts; bioreductive anticancer prodrug; topoisomerase II

Introduction

The primary medicinal interest in intercalating agents has been their potential as anticancer drugs; in 1978, the acridine derivative amsacrine (m-AMSA; Chart 1), a 9-anilinoacridine, became the first synthetic DNA-intercalating agent to be clinically successful.[1] The drug is thought to be held on location through intercalation until a crucial event, such as a change in supercoiling catalyzed by a DNA topoisomerase, occurs. DNA topoisomerase II has been identified as the intracellular target for amsacrine and several other anticancer drugs.[2]

Chart 1



m-AMSA : R = CH_3, R' = OCH_3
SN 12489 : R = p-$NH_2C_6H_4$, R' = H

It has been reported that the presence of a substituent at position 1′ of the anilinoacridine chromophore is required to permit the drug to interfere with the catalytic activity of topoisomerase II.[3] By varying the nature of this side chain

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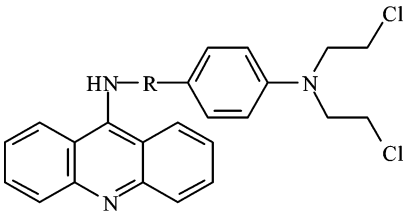
Chart 2



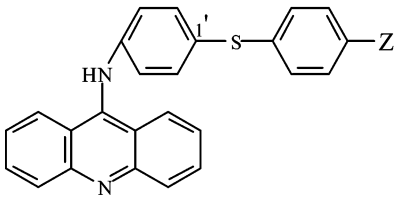
1a: R = -C_6H_4-S-
1b: R = -C_6H_4-SO-
23: R = -$(CH_2)_5$-SO_2-

at position 1′, one can expect to change the capacity of the drug to interfere with topoisomerase II so as to modulate its cytotoxicity. It has been found that certain anilinoacridine derivatives having a sterically demanding substituent at position 1′ remained cytotoxic.[4] For example, SN 12489 (NSC 140701, Chart 1) was found to be as efficient as amsacrine at prompting DNA cleavage by topoisomerase II, and the level of DNA lesions produced by the drug in cells correlated well with its cytotoxicity.[5,6]

It had been previously found in our laboratory that certain sulfoxides were selectively bioreduced under anaerobic conditions in vitro. Subsequently, several types of sulfoxide-containing molecules were designed and explored as potential bioreductive cytotoxins.[7,8] Among the compounds, 4-[*N*,*N*-bis(chloroethyl)]amino-4′-(9-acridinyl)aminodiphenyl sulfoxide, an acridine-substituted diphenyl sulfoxide nitrogen mustard (**1b**, Chart 2), showed an excellent hypoxia selectivity (27-fold), and the putative active drug, the corresponding sulfide (**1a**, Chart 2), was 14 times as cytotoxic as the sulfoxide against V-79 cells. It was, however, unclear whether the acridine moiety provided any contribution to the overall cytotoxicity profile through a possible DNA intercalation, especially in view of the structural similarity of **1a** to amsacrine analogues, for example, SN 12489. Studies on structurally related acridine-containing alkylphenyl sulfone mustard (**23**) demonstrated that topoisomerase II inhibition is responsible for its cytotoxicity but not DNA alkylation.[9]

In this report, a series of nonalkylating analogues of **1a** (Chart 3) were synthesized and their anticancer potential was

Chart 3



	Z
2a :	$N(CH_2CH_2OH)_2$
3 :	$N(CH_2CH_2CH_2OH)_2$
4a :	morpholine
5a :	N-methylpiperazine
6 :	$NHCH_2CH_2OH$
7 :	NH_2
8 :	NO_2
9 :	OH

evaluated through in vitro cytotoxicity. In addition, the possible mechanism of cytotoxicity was evaluated. The corresponding sulfoxides of selected compounds were also synthesized as potential bioreductive prodrugs along with their corresponding sulfones. An in vitro metabolism study of sulfoxide **2b** was also performed to evaluate the bioreductive potential.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Mass spectroscopy was performed by Mass Consortium, San Diego, CA. The following instruments were used: IR, Perkin-Elmer model 281; ^{1}H NMR, Varian EM-360L CW, 60 MHz or Bruker, 400 MHz (TMS as internal standard). Silica gel GF plates (Analtech) were used for TLC (250 μm, 2.5 × 10 cm) and preparative TLC (1000 μm, 20 × 20 cm). Silica gel (40 μm, Baker) was used for flash column chromatography. All chemicals and solvents were reagent grade and were purchased from commercial vendors.

2-{(2-Hydroxyethyl)[4-(4-nitrophenylthio)phenyl]amino}-ethan-1-ol (10a) was prepared according to a previously published procedure.[8]

2-{(2-Hydroxyethyl)[4-(4-nitrophenylsulfinyl)phenyl]-amino}ethan-1-ol (10b). To a solution of **10a** (1.5 g, 4.49 mmol) in trifluoroacetic acid (TFA, 7.11 mL) was added 1.98 mL of CF_3CO_3H (4.49 mmol, prepared by mixing 0.46 mL of 30% H_2O_2 with 1.52 mL of TFA) with stirring and ice-cooling. The blue reaction mixture turned green after 185 min; then the mixture was neutralized with $NaHCO_3$. The resulting mixture was extracted with EtOAc (3 × 50 mL). EtOAc extracts were combined, washed with H_2O (30 mL), and then dried over Na_2SO_4. Orange crystalline **10b** (0.99 g, 63% yield) was obtained after crystallization with EtOAc/hexane: mp 134−136 °C; TLC R_f = 0.43 in CH_2Cl_2/CH_3-OH (9:1); ^{1}H NMR ($CDCl_3$) δ 3.50−3.80 (m, 8H), 4.8 (br s, 2H), 6.75 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 8.9 Hz, 2H), 8.27 (d, J = 8.8 Hz, 2H). Anal. ($C_{16}H_{18}N_2O_5S$) C, H, N, S.

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2-{(2-Hydroxyethyl)[4-(4-nitrophenylsulfonyl)phenyl]-amino}ethan-1-ol (10c). To a solution of **10a** (1.5 g, 4.49 mmol) in trifluoroacetic acid (TFA, 5 mL) was added 2.73 mL of CF_3CO_3H (8.98 mmol, prepared by mixing 0.93 mL of 30% H_2O_2 with 1.8 mL of TFA) with stirring and ice-cooling. The blue reaction mixture turned green after 8 h; then was neutralized by adding 50% $NaHCO_3$ (14.8 mL) solution. The resulting mixture was extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed with H_2O (30 mL) once and then dried over anhydrous Na_2SO_4. After EtOAc was evaporated in vacuo, compound **10b** (0.44 g, 27% yield) was purified by flash column chromatography with EtOAc as the eluting solvent: mp 193−195 °C; TLC R_f = 0.41 in EtOAc; ^{1}H NMR ($CDCl_3$) δ 3.33−3.73 (m, 8H), 4.73 (br s, 2H), 6.83 (d, J = 9.0 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H), 7.93−8.50 (m, 4H).

2-{[4-(4-Aminophenylthio)phenyl](2-hydroxyethyl)-amino}ethan-1-ol (11a). Iron powder (19.12 g, 100 mesh) was activated by refluxing it with 4 mL of distilled water and 21 drops of concentrated hydrochloric acid for 30 min with vigorous stirring. Ethanol (33 mL) and **10a** (5.37 g, 16.08 mmol) were added into the mixture at 40−50 °C. The reaction mixture was refluxed and vigorously stirred for 30 min. After compound **10a** disappeared by TLC (hexane/EtOAc, 1:1), 10% NaOH was added to bring the pH of the reaction mixture to 8. The hot reaction mixture was then filtered, and the residue was washed with EtOH (30 mL). After evaporation of EtOH, the residue was diluted with water (30 mL) and extracted with EtOAc (3 × 100 mL). The EtOAc extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was purified on a flash column using EtOAc as eluent and then was recrystallized with EtOAc/hexane to give 2.64 g (54% yield) of **11a** as pale yellow crystals: mp 118.5−121 °C; TLC R_f = 0.41 in EtOAc; ^{1}H NMR ($CDCl_3$) δ 3.37−3.67 (m, 8H), 6.45−6.67 (m, 4H), 6.97−7.20 (m, 4H). Anal. ($C_{16}H_{20}N_2O_2S$) C, H, N, S.

2-{[4-(4-Aminophenylsulfinyl)phenyl](2-hydroxyethyl)-amino}ethan-1-ol (11b) was prepared from **10b** on a 2.86-mmol scale (1.00-g,) according to the procedure for **11a** to give 0.57 g (62% yield) of **11b** after flash column purification using EtOAc/EtOH (9:1) as eluent: mp 67−69 °C; TLC R_f = 0.46 in EtOAc/EtOH (9:1); ^{1}H NMR ($CDCl_3$/CD_3OD, 1:1) δ 3.50−3.83 (m, 8H), 6.58−6.77 (m, 4H), 7.20−7.40 (m, 4H). Anal. ($C_{16}H_{20}N_2O_3S\cdot{}^1/_2H_2O$) C, H, N, S.

2-{[4-(4-Aminophenylsulfonyl)phenyl](2-hydroxyethyl)-amino}ethan-1-ol (11c) was prepared from **10c** on a 1.64-mmol scale (0.6-g) in the procedure for **11a** to give 0.43 g (78% yield) of **11c** after flash column purification using EtOAc/EtOH (9:1) as eluent: mp 193−194.5 °C; TLC R_f = 0.56 in EtOAc/EtOH (9:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 1:1) δ 3.50−3.60 (m, 8H), 6.65−6.79 (m, 4H), 7.45−7.62 (m, 4H).

2-({4-[4-(Acridin-9-ylamino)phenylthio]phenyl}(2-hydroxyethyl)amino)ethan-1-ol hydrochloride (2a) was prepared by dissolving **11a** (0.31 g, 1.03 mmol) and 9-chloroacridine[10] (0.22 g, 1.03 mmol) in *N*-methyl-2-pyrrolidinone (10 mL) with the addition of 1 drop of concentrated HCl. The reaction mixture was stirred for 4 h at room temperature; EtOAc (100 mL) was then added to precipitate out the crude product. The crude product was then mixed with 10 mL of *N*-methyl-2-pyrrolidinone again for 1 h, followed by addition of EtOAc (150 mL) for complete precipitation. The resulting precipitate was collected to give 0.37 g (69% yield) of compound **2a** as a red solid: mp 163−165 °C dec; TLC R_f = 0.76 in EtOAc/MeOH (9.5:0.5); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 2:1) δ 3.60 (s, 8H), 6.77 (d, J = 8.6 Hz, 2H), 7.17−7.57 (m, 8H), 7.90−8.4 (m, 6H). Anal. ($C_{29}H_{27}N_3O_2S\cdot$HCl) C, H, N, S.

2-({4-[4-(Acridin-9-ylamino)phenylsulfinyl]phenyl}(2-hydroxyethyl)amino)ethan-1-ol hydrochloride (2b) was prepared from **11b** (0.48 g, 1.5 mmol) and 9-chloroacridine (0.3 g, 1.4 mmol) according to the procedure for **2a** to give 0.72 g (90% yield) of **2b** as a yellow solid: mp 193−195 °C dec; TLC R_f = 0.37 in EtOAc/EtOH (9:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 2:1) δ 3.52 (s, 8H), 6.79 (d, J = 8.6 Hz, 2H), 7.32−8.31 (m, 14H). Anal. ($C_{29}H_{27}N_3O_3S\cdot$HCl) C, H, N, S.

2-({4-[4-(Acridin-9-ylamino)phenylsulfonyl]phenyl}(2-hydroxyethyl)amino)ethan-1-ol hydrochloride (2c) was prepared from **11c** (0.18 g, 0.55 mmol) and 9-chloroacridine (0.11 g, 0.51 mmol) according to the procedure for **2a** to give 0.22 g (73% yield) of **2c**: mp 248−249.5 °C dec; TLC R_f = 0.75 in EtOAc/EtOH (9:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 2:1) δ 3.52 (s 8H), 6.78 (d, J = 9.0 Hz, 2H), 7.37−8.40 (m, 14H). Anal. ($C_{29}H_{27}N_3O_4S\cdot$HCl) C, H, N, S.

4-{4-[(4-Nitrophenyl)thio]phenyl}morpholine (13a) was prepared according to a published procedure[11] with some modifications. A cooled (0 °C) solution containing **10a** (2.55 g, 7.63 mmol) in 20 mL of anhydrous THF was treated with diethyl azodicarboxylate (2 g, 11.3 mmol) and tributylphosphine (2.35 g, 11.3 mmol) under N_2. The reaction mixture was stirred for 3 h, and the temperature was allowed to return to room temperature. After THF was evaporated in vacuo, the residue was diluted with water (30 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was purified on a flash column using EtOAc/CH_2Cl_2 (1:9) as eluting solvent and then crystallized with EtOAc/hexane to give 1.51 g (63% yield) of **13a** as yellow crystals: mp 170−172 °C; TLC R_f = 0.33 in CH_2Cl_2/hexane (1:1); ^{1}H NMR ($CDCl_3$) δ 3.20 (t, J = 5.0 Hz, 4H), 3.85 (t, J = 4.8 Hz, 4H), 6.82−7.58 (m, 6H), 7.99 (d, J = 9.0 Hz, 2H).

4-{4-[(4-Nitrophenyl)sulfinyl]phenyl}morpholine (13b) was prepared from **13a** on a 4.68-mmol (1.48-g) scale

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according to the procedure for **10b** to give 1.08 g (70% yield) of **13b** after further crystallization with EtOAc/hexane: mp 159.5−161 °C; TLC R_f = 0.23 in EtOAc/CH_2Cl_2 (1:9); ^{1}H NMR ($CDCl_3$/CD_3OD, 1:1) δ 3.22 (t, J = 4.8 Hz, 4H), 3.82 (t, J = 4.8 Hz, 4H), 6.88 (d, J = 8.8 Hz, 2H), 7.43−7.87 (m, 4H), 8.28−8.36 (d, J = 8.8 Hz, 2H).

4-{4-[(4-Nitrophenyl)sulfonyl]phenyl}morpholine (13c). To a solution of **13a** (1.22 g, 3.86 mmol) in 125 mL acetone and 20 mL of water was added a mixture of 38 mL of 30% (wt) of hydrogen peroxide (252 mmol) and 6 mL of 0.3 M ammonium molybdate. The reaction mixture was stirred at room temperature overnight; then the mixture was evaporated in vacuo. The residue was partitioned between EtOAc (40 mL) and water (20 mL). The water layer was extracted with EtOAc (3 × 100 mL). The organic extract was washed with water (20 mL) and was crystallized with EtOAc/hexane to give 0.64 g (48% yield) of **13c** as yellow crystals: mp 201−202.5 °C; TLC *Rf* = 0.44 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$) δ 3.26 (t, J = 4.8 Hz, 4H), 3.81 (t, J = 4.8 Hz, 4H), 6.85 (d, J = 9.0 Hz, 2H), 7.23−8.33 (m, 6H).

4-[(4-Morpholin-4-ylphenyl)thio]aniline (14a) was prepared from **13a** on a 1.96-mmol (0.62-g) scale in the procedure for **11a** to give 0.25 g (45% yield) of **14a** after flash column purification using EtOAc/CH_2Cl_2 (1:9) as eluent: mp 141−143 °C; TLC R_f = 0.42 in EtOAc/CH_2Cl_2 (1:9); ^{1}H NMR ($CDCl_3$) δ 3.09 (t, J = 5.0 Hz, 4H), 3.83 (t, J = 4.8 Hz, 4H), 6.50−7.25 (m, 8H).

4-[(4-Morpholin-4-ylphenyl)sulfinyl]aniline (14b) was prepared from **13b** on a 2.67-mmol (0.89-g) scale in the procedure for **11a** to give 0.66 g (82% yield) of **14b** after flash column purification using EtOAc as eluent: mp 168−170 °C; TLC R_f = 0.55 in EtOAc; ^{1}H NMR ($CDCl_3$) δ 3.07 (t, J = 4.8 Hz, 4H), 3.77 (t, J = 5.0 Hz, 4H), 6.56−7.48 (m, 8H).

4-[(4-Morpholin-4-ylphenyl)sulfonyl]aniline (14c) was prepared from **13c** on a 1.78-mmol (0.62-g) scale in the procedure for **11a** to give 0.48 g (85% yield) of **13c** after flash column purification using EtOAc/CH_2Cl_2 (1:9) as eluent: mp 176−178 °C; TLC R_f = 0.33 in EtOAc/CH_2Cl_2 (1:9); ^{1}H NMR ($CDCl_3$) δ 3.22 (t, J = 5.0 Hz, 4H), 3.77 (t, J = 5.0 Hz, 4H), 6.54−6.96 (m, 4H), 7.41−7.73 (m, 4H).

***N*-{4-[(4-Morpholin-4-ylphenyl)thio]phenyl}acridin-9-amine hydrochloride (4a)** was prepared from **14a** (0.20 g, 0.71 mmol) and 9-chloroacridine (0.14 g, 0.64 mmol) according to the procedure for **2a** to give 0.32 g (90% yield) of **4a** as an orange solid: mp 295 °C dec; TLC R_f = 0.49 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 2:1) δ 3.04 (t, J = 5.0 Hz, 4H), 3.68 (t, J = 5.0 Hz, 4H), 6.60−7.90 (m, 16H). Anal. ($C_{29}H_{25}N_3OS\cdot HCl\cdot H_2O$) C, H, N, S.

***N*-{4-[(4-Morpholin-4-ylphenyl)sulfinyl]phenyl}acridin-9-amine hydrochloride (4b)** was prepared from **14b** (0.35 g, 1.16 mmol) and 9-chloroacridine (0.25 g, 1.17 mmol) according to the same procedure for **2a** to give 0.56 g (93% yield) of **4b**: mp 232−234 °C dec; TLC R_f = 0.58 in EtOAc; ^{1}H NMR ($CDCl_3$/DMSO-d_6, 2:1) δ 3.23−3.40 (m, 4H), 3.70−3.86 (m, 4H), 7.02 (d, J = 8.6 Hz, 2H), 7.33−8.30 (m, 14H). Anal. ($C_{29}H_{25}O_2N_3S\cdot HCl\cdot {}^1/_2H_2O$) C, H, N, S.

***N*-{4-[(4-Morpholin-4-ylphenyl)sulfonyl]phenyl}acridin-9-amine hydrochloride (4c)** was prepared from **14c** (0.39 g, 1.23 mmol) and 9-chloroacridine (0.26 g, 1.23 mmol) according to the procedure for **2a** to give 0.64 g (98% yield) of **4c**: mp 296−298 °C dec; TLC R_f = 0.76 in EtOAc; ^{1}H NMR ($CDCl_3$/DMSO-d_6, 1:2) δ 3.20−3.43 (m, 4H), 3.65−3.80 (m, 4H), 6.97 (d, J = 9.1 Hz, 2H), 7.33−8.36 (m, 14H). Anal. ($C_{29}H_{25}O_3N_3S\cdot HCl\cdot {}^1/_2H_2O$) C, H, Cl, N, S.

1-Methyl-4-{4-[(4-nitro-phenyl)thio]phenyl}-piperazine (15a) was prepared according to published procedure[12] with some modifications. A mixture of 4-amino-4′-nitrodiphenyl sulfide (3 g, 12.2 mmol), mechlorethamine hydrochloride (2.35 g, 12.2 mmol), and Na_2CO_3 (1.29 g, 24.4 mmol) in 35 mL of EtOH was heated to reflux for 48 h. The solvent was evaporated in vacuo, and the residue was partitioned between H_2O (50 mL) and CH_2Cl_2 (150 mL). The water layer was adjusted to pH 12−13 with 10% NaOH and extracted with CH_2Cl_2 (3 × 100 mL). The combined CH_2Cl_2 extract was washed with water (20 mL) and dried over anhydrous Na_2SO_4. The crude product was decolored with charcoal at room temperature for 30 min. The solvent was evaporated in vacuo, and compound **15a** (2.67 g, 66% yield) was purified by flash column chromatography with CH_2Cl_2/MeOH/TEA (95:5:1) as the eluting solvent: mp 127−128.5 °C; TLC R_f = 0.43 in CH_2Cl_2/MeOH/TEA (95:5:1); ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.58 (t, J = 5.4 Hz, 4H), 3.36 (t, J = 5.4 Hz, 4H), 6.88−7.50 (m, 6H), 8.03 (d, J = 8.8 Hz, 2H).

1-Methyl-4-{4-[(4-nitrophenyl)sulfinyl]phenyl}-piperazine (15b) was prepared from **15a** on a 3.04-mmol (1.00-g) scale in the procedure for **10b** to give 0.43 g (41% yield) of **15b** after recrystallization with EtOAc/hexane: mp 167−170 °C; TLC R_f = 0.29 in CH_2Cl_2/MeOH/TEA (95:5:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 1:1) δ 2.52 (s, 3H), 2.82 (t, J = 4.7 Hz, 4H), 3.45 (t, J = 5.0 Hz, 4H), 6.95 (d, J = 9.1 Hz, 2H), 7.45−7.87 (m, 4H), 8.31 (d, J = 8.8 Hz, 2H).

1-Methyl-4-{4-[(4-nitrophenyl)sulfonyl]phenyl}-piperazine (15c) was prepared according to a published procedure[12] with some modifications. A mixture of 4-chloro-4′-nitrodiphenyl sulfide (4 g, 13.33 mmol), *N*-methylpiperazine (2.8 mL, 25 mmol), and 25 mL of DMF was heated at ca. 120 °C for 24 h. The mixture was poured into 250 mL of water, and the pH value was adjusted to 12−13 by using 10 N NaOH. The solution was extracted with CH_2Cl_2 (3 × 150 mL). The combined CH_2Cl_2 was evaporated in vacuo, and the residue was purified on a flash column with CH_2Cl_2/MeOH/TEA (95:5:1) as eluent to give 2.06 g (43% yield) of **15c**: mp 203−205 °C; TLC R_f = 0.43 in CH_2Cl_2/MeOH/TEA (95:5:1); ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H), 2.50 (t, J = 5.0 Hz, 4H), 3.37 (t, J = 5.0 Hz, 4H), 6.82−8.38 (m, 8H).

(12) Kiritsy, J. A.; Yung, D. K.; Mahony, D. E. Synthesis and quantitative structure−activity relationships of some antibacterial 3-formylrifamycin SV *N*-(4-substituted phenyl)piperazinoacethydrazones. *J. Med. Chem.* **1978**, *21*, 1301−1307.

4-{[4-(4-Methylpiperazin-1-yl)phenyl]thio}aniline (16a) was prepared from **15a** on a 2.94-mmol (0.97-g) scale in the procedure for **11a** to give 0.68 g (78% yield) of **16a** after flash column purification using CH_2Cl_2/MeOH/TEA (95:5:1) as eluent: mp 145−146 °C; TLC $R_f = 0.21$ in CH_2Cl_2/MeOH/TEA (95:5:1); ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H), 2.58 (t, $J = 4.9$ Hz, 4H), 3.22 (t, $J = 4.9$ Hz, 4H), 6.51−7.26 (m, 8H).

4-{[4-(4-Methylpiperazin-1-yl)phenyl]sulfinyl}aniline (16b) was prepared from **15b** on a 2.06-mmol (0.71-g) scale in the procedure for **11a** to give 0.39 g (60% yield) of **16b** after flash column purification using CH_2Cl_2/MeOH/TEA (95:5:1) as eluent: mp 179−180.5 °C; TLC $R_f = 0.37$ in CH_2Cl_2/MeOH/TEA (95:5:1); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H), 2.52 (t, $J = 5.0$ Hz, 4H), 3.25 (t, $J = 5.0$ Hz, 4H), 6.56−7.50 (m, 8H).

4-{[4-(4-Methylpiperazin-1-yl)phenyl]sulfonyl}aniline (16c) was prepared from **15c** on a 3.00-mmol (1.08-g) scale in the procedure for **11a** to give 0.69 g (69% yield) of **16c** after purification with preparative TLC using CH_2Cl_2/MeOH/TEA (95:5:1) as eluent: mp 188−190 °C dec; TLC $R_f = 0.54$ in CH_2Cl_2/MeOH (9:1); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H), 2.51 (t, $J = 4.8$ Hz, 4H), 3.27 (t, $J = 4.8$ Hz, 4H), 6.69 (d, $J = 8.5$ Hz, 2H), 6.83 (d, $J = 8.5$ Hz, 2H) 7.58 (d, $J = 8.5$ Hz, 2H) 7.67 (d, $J = 8.5$ Hz, 2H).

***N*-(4-{[4-(4-Methylpiperazin-1-yl)phenyl]thio}phenyl)-acridin-9-amine dihydrochloride (5a)** was prepared from **16a** (0.20 g, 0.67 mmol) and 9-chloroacridine (0.14 g, 0.65 mmol) according to the procedure for **2a** to give 0.31 g (83% yield) of **5a** as a red solid: mp 211−213 °C; TLC $R_f = 0.45$ in CH_2Cl_2/MeOH (9:1). Compound **5a** (0.18 g, 0.32 mmol) was then partitioned between CH_2Cl_2 (250 mL) and saturated Na_2CO_3 solution. The water layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined CH_2Cl_2 was dried over anhydrous Na_2SO_4 and evaporated to give 0.10 g (62% yield) of the free base of **5a** for elemental analysis: ^{1}H NMR for the free base form of **5a** ($CDCl_3$) δ 2.33 (s, 3H), 2.60−2.70 (m, 4H), 3.13−3.27 (m, 4H), 6.68−8.04 (m, 16H). Anal. ($C_{30}H_{28}N_4S$) C, H, N, S.

***N*-(4-{[4-(4-Methylpiperazin-1-yl)phenyl]sulfinyl}phenyl)-acridin-9-amine dihydrochloride (5b)** was prepared from **16b** (0.26 g, 0.84 mmol) and 9-chloroacridine (0.19 g, 0.89 mmol) according to the procedure for **2a** to give 0.40 g (85% yield) of **5b** as an orange solid: mp 225−227 °C; TLC $R_f = 0.51$ in CH_2Cl_2/MeOH (9:1); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H), 2.49 (t, $J = 5.0$ Hz, 4H), 3.23 (t, $J = 5.0$ Hz, 4H), 6.74−8.00 (m, 16H). Anal. ($C_{30}H_{28}N_4OS\cdot 2HCl\cdot {}^1/_2H_2O$) C, H, N, S.

***N*-(4-{[4-(4-Methylpiperazin-1-yl)phenyl]sulfonyl}-phenyl)acridin-9-amine dihydrochloride (5c)** was prepared from **16c** (0.21 g, 0.62 mmol) and 9-chloroacridine (0.16 g, 0.73 mmol) according to the procedure for **2a** to give 0.36 g (99% yield) of **5c** as an orange solid: mp 229 °C dec; TLC $R_f = 0.58$ in CH_2Cl_2/MeOH (9:1); ^{1}H NMR for the free base form of **5c** ($CDCl_3$) δ 2.36 (s, 3H) 2.55 (t, $J = 5.2$ Hz, 4H), 3.33 (t, $J = 5.1$ Hz, 4H), 6.78−7.96 (m, 16H). Anal. ($C_{30}H_{28}N_4OS\cdot 2HCl\cdot H_2O$) C, H, N, S.

2-({4-[(4-Nitrophenyl)thio]phenyl}amino)ethanol (17) was prepared from 4-amino-4′-nitrodiphenyl sulfide (10 g, 40 mmol) and a mixture of THF (50 mL), AcOH (100 mL), and 20 mL of 10% (w/v) of ethylene oxide in THF (45.40 mmol) according to the similar procedure for **10a**. The reaction solution was stirred at room temperature overnight. The mixture was then neutralized with saturated Na_2CO_3, and extracted with EtOAc (4 × 150 mL). The EtOAc extract was then dried over anhydrous Na_2SO_4 and evaporated in vacuo, and compound **17** (5.21 g, 44% yield) was purified by flash chromatography using acetone/CH_2Cl_2 (1:9) as eluent: mp 92−94 °C; TLC $R_f = 0.72$ in acetone/CH_2Cl_2 (1:9); ^{1}H NMR ($CDCl_3$) δ 3.37 (t, $J = 5.0$ Hz, 2H), 3.81 (t, $J = 5.0$ Hz, 2H), 6.64−7.45 (m, 6H), 8.05 (d, $J = 9.0$ Hz, 2H).

2-({4-[(4-Aminophenyl)thio]phenyl}amino)ethanol (18) was prepared from **17** on a 4.06-mmol (1.18-g) scale in the procedure for **11a** to give 0.89 g (84% yield) of **18** as a liquid after preparative TLC purification using acetone/CH_2Cl_2 (1:9) as eluent: TLC $R_f = 0.35$ in acetone/CH_2Cl_2 (1:9); ^{1}H NMR ($CDCl_3$) δ 3.22 (t, $J = 5.0$ Hz, 2H), 3.78 (t, $J = 5.0$ Hz, 2H), 6.47−7.23 (m, 8H).

2-[(4-{[4-(Acridin-9-ylamino)phenyl]thio}phenyl)amino]-ethanol hydrochloride (6) was prepared from **18** (0.36 g, 1.39 mmol) and 9-chloroacridine (0.32 g, 0.16 mmol) according to the procedure for **2a** to give 0.24 g (36% yield) of **6**: mp 263−265 °C dec; TLC $R_f = 0.75$ in CH_2Cl_2/MeOH (9:1); ^{1}H NMR ($CDCl_3$) δ 3.13 (t, $J = 5.7$ Hz, 2H), 3.57 (t, $J = 5.7$ Hz, 2H), 6.69 (d, $J = 8.0$ Hz, 2H), 7.11 (d, $J = 8.1$ Hz, 2H), 7.29 (t, $J = 8.9$ Hz, 4H), 7.44 (t, $J = 8.3$, 4H), 7.97 (t, $J = 7.8$ Hz, 2H), 8.08 (d, $J = 7.8$ Hz, 2H), 8.24 (d, $J = 7.8$ Hz, 2H). Anal. ($C_{27}H_{23}N_3OS\cdot HCl$) C, H, N, S.

***N*-{4-[(4-Nitrophenyl)thio]phenyl}acridin-9-amine hydrochloride (8)** was prepared from 9-chloroacridine (0,71 g, 3.32 mmol) and 4-amino-4′-nitrodiphenyl sulfide (0.81 g, 3.27 mmol) according to the procedure for **2a** to give 1.50 g (100% yield) of **8**: mp >290 °C; TLC $R_f = 0.36$ in CH_2Cl_2/MeOH (9.6:0.4); ^{1}H NMR of 8 (DMSO-d_6) δ 7.37 (d, $J = 9.0$ Hz, 2H), 7.56 (m, 4H), 7.68 (d, $J = 8.0$ Hz, 2H), 8.0 (t, $J = 8.0$ Hz, 2H), 8.19−8.22 (m, 4H), 8.35 (d, $J = 8.8$ Hz, 2H). Anal. ($C_{25}H_{17}N_3O_2S\cdot HCl$) C, H, N, S.

***N*-{4-[(4-Aminophenyl)thio]phenyl}acridin-9-amine dihydrochloride (7)** was prepared from **8** on a 1.19-mmol (0.55-g) scale in the procedure for **10a** to give 0.46 g (82% yield) of **7** after flash column purification using CH_2Cl_2/MeOH (9.5:0.5) as eluent: mp 289 °C dec; TLC $R_f = 0.21$ in CH_2Cl_2/MeOH (9.5:0.5); ^{1}H NMR of 7 (DMSO-d_6) δ 7.20 (d, $J = 8.0$ Hz, 2H), 7.35 (d, $J = 8.0$ Hz, 2H), 7.38−7.43 (m, 4H), 7.50 (t, $J = 7.9$ Hz, 2H), 8.01 (t, $J = 7.8$ Hz, 2H), 8.16 (d, $J = 8.0$ Hz, 2H), 8.31 (d, $J = 8.5$ Hz, 2H). Anal. ($C_{25}H_{19}N_3S\cdot 2HCl\cdot H_2O$) C, H, N, S; MS *m*/*z* 394 (MH^+).

4-[(4-Nitrophenyl)thio]phenol (19). To a solution of 4-amino-4′-nitrodiphenyl sulfide (5 g, 20.3 mmol) and acetone (30 mL) was added 14 mL of hot 35% sulfuric acid while the temperature was kept under 5 °C. A solution of 1.63 g of sodium nitrite in 4.47 mL of ice water was then added dropwise, and 0.15 g of urea in 15.23 g ice water

was added 5 min later. A boiling solution containing 5.5 mL of sulfuric acid and 5 mL of water was then added into the reaction mixture. The mixture was refluxed for 5 h. The resulting solution was then extracted with EtOAc (3 × 100 mL) and washed with $NaHCO_3$ and brine successively. The EtOAC extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified on a flash column with EtOAc/hexane (1:3) as the eluting solvent to give 2.52 g (51% yield) of **19**: mp 150−151 °C; TLC R_f = 0.42 in EtOAc/hexane (1:3); ^{1}H NMR ($CDCl_3$) δ 6.85 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 8.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 7.91 (d, J = 8.5 Hz, 2H) 9.33 (s, 1H).

4-[(4-Aminophenyl)thio]phenol (20) was prepared from **19** on a 3.44-mmol (0.85-g) scale in the procedure for **11a** to give 0.55 g (74% yield) of **20** after preparative TLC purification using EtOAc/hexane (1:2) as eluent: mp 147−149 °C; TLC *Rf* = 0.66 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$) δ 3.94 (s, 2H), 6.53 (d, J = 8.1 Hz, 2H), 6.68 (d, J = 8.5 Hz, 2H), 7.13−7.17 (m, 4H), 8.80 (s, 1H).

4-{[4-(Acridin-9-ylamino)phenyl]thio}phenol hydrochloride (9) was prepared from **20** (0.12 g, 0.48 mmol) and 9-chloroacridine (0.11 g, 0.51 mmol) according to the procedure for **2a** to give 0.44 g (83% yield) of **9**: mp 279−281.5 °C; TLC R_f = 0.40 in CH_2Cl_2/MeOH (9.5:0.5); ^{1}H NMR (DMSO-d_6) δ 10.04 (s, 1H, OH), 6.90 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H), 7.39−7.35 (m, 4H), 7.46 (t, J = 7.5 Hz, 2H), 7.99 (t, J = 7.8 Hz, 2H), 8.12 (d, J = 8.5 Hz, 2H), 8.27 (d, J = 8.5 Hz, 2H). Anal. ($C_{25}H_{18}N_2OS$·HCl) C, H, N, S.

3-((3-Hydroxypropyl){4-[(4-nitrophenyl)thio]phenyl}-amino)propan-1-ol (21). To a solution of 4-amino-4′-nitrodiphenyl sulfide (2.5 g, 10 mmol) and 3-iodopropanol (3.89 g, 20 mmol) in dry THF (10 mL) was added a mixture of tetrabutylammonium bromide (0.16 g, 0.5 mmol) and 5.5 g of potassium carbonate. After being refluxed for 15 h, the mixture was evaporated in vacuo. The residue was partitioned between CH_2Cl_2 (60 mL) and water (30 mL). The water layer was extracted with CH_2Cl_2 (3 × 100 mL). The CH_2Cl_2 extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo. Compound **21** (0.6 g, 17% yield) was purified by flash column chromatography using EtOAc as eluting solvent: TLC R_f = 0.59 in EtOAc/MeOH (10:1); ^{1}H NMR ($CDCl_3$) δ 1.88−1.95 (m, 4H), 3.54 (t, J = 7.3 Hz, 4H), 3.78 (t, J = 6.0 Hz, 4H), 6.80 (d, J = 9.0 Hz, 2H), 7.11 (d, J = 9.2 Hz, 2H), 7.38 (d, J = 9.0 Hz, 2H), 8.05 (d, J = 9.0 Hz, 2H).

3-({4-[(4-Aminophenyl)thio]phenyl}(3-hydroxypropyl)-amino)propan-1-ol (22) was prepared from **21** on a 1.08-mmol (0.39-g,) scale in the procedure for **11a** to give 0.28 g (80% yield) of a pale yellow liquid of **22** after preparative TLC purification using EtOAc/MeOH (95:5) as eluent: TLC R_f = 0.38 in EtOAc/MeOH (95:5); ^{1}H NMR ($CDCl_3$) δ, 1.80−1.86 (m, 4H), 3.43 (t, J = 7.1 Hz, 4H), 3.72 (t, J = 5.9 Hz, 4H), 6.62 (d, J = 8.1 Hz, 2H), 6.69 (d, J = 8.9 Hz, 2H), 7.18 (d, J = 8.6 Hz, 2H) 7.23 (d, J = 8.9 Hz, 2H).

3-({4-[4-(Acridin-9-ylamino)phenylthio]phenyl}(3-hydroxypropyl)amino)propan-1-ol hydrochloride (3) was prepared from **22** (0.28 g, 0.85 mmol) and 9-chloroacridine (0.18 g, 0.85 mmol) by the procedure for **2a** to give 0.72 g (90% yield) of **3** as a yellow solid: mp 149−152 °C; TLC R_f = 0.37 in EtOAc/EtOH (9:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 2:1) δ 1.65−1.70 (m, 4H), 3.40 (t, J = 7.1 Hz, 4H), 3.45 (t, J = 6.0 Hz, 4H), 6.88 (s, br, 2H), 7.18 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 8.2 Hz, 4H), 7.48 (t, J = 7.3 Hz, 2H), 7.99−8.05 (m, 4H), 8.23 (d, J = 8.7 Hz, 2H). Anal. ($C_{31}H_{31}N_3O_2S$·HCl·H_2O) C, H, N, S; MS *m*/*z* 510 (MH^+), 508 ($[M - H]^-$).

In Vitro Clonogenic Cytotoxicity Assay. The published procedure of Fracasso and Sartorelli[13] with some modification was used. The Chinese hamster lung transformed V-79 cells were used for in vitro cytotoxicity evaluations. A confluent dish of the V-79 cells was washed with 5 mL of sterile HBSS twice and followed by treatment with 0.25% trypsin (Gibco labs) for 5 min. Care was made to verify that all cells were floating in the solution. After centrifugation, the precipitated cells were resuspended with medium and collected. The cells were counted by trypan blue dye exclusion with a bright-line hemocytometer (Fisher Inc). In each Petri dish, 500000 cells were seeded in 10 mL of minimum essential medium supplemented with 10% heat inactivated fetal bovine serum, penicillin G sodium, streptomycin sulfate, amphotericin, nonessential amino acids, and vitamins (Gibco, Grand Island, NY) and then incubated for 24 h in a 95% air/5% CO_2 humidified atmosphere. The medium was then removed, and cells were fed with 10 mL of fresh medium.

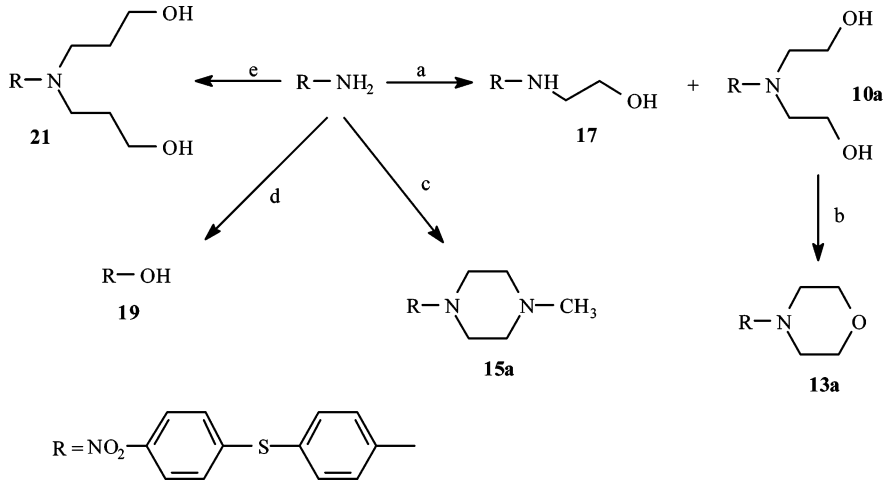
The testing drugs were first dissolved in DMF as stock solution; then the stock solutions were further diluted with DMF to appropriate concentrations before being added into the cultured cells. After 3 h of drug exposure, the cells were washed twice with 5 mL of sterile HBSS and treated with 0.25% trypsin (Gibco Labs) for 5 min. Cells were collected by centrifugation, resuspended in fresh medium, and counted by trypan blue dye exclusion. After an appropriate dilution, 500 cells were plated in triplicate in 10 mL of medium. After 6−8 days of incubation to allow colony formation, colonies were rinsed with 0.9% saline twice, fixed with ethanol, stained with crystal violet, and counted. Results were reported as the number of colonies that survived chemical treatment per number of colonies in the solvent-treated control. The LC_{90} values were determined by semilogarithmically plotting the drug concentration versus cell viability as determined by the number of colonies that survived the treatments.

Topoisomerase II Inhibitory Study. The topoisomerase II inhibitory assay was performed using the topoisomerase II drug screening kit (TopoGen, Inc., Columbus, OH). The pRYG DNA was used as DNA substrate in this assay.

The final reaction volume was 20 μL, which included 2 μL of cleavage buffer (30 mM Tris-HCl, pH7.6, 3 mM ATP,

(13) Fracasso, P. M.; Sartorelli, A. C. Cytotoxicity and DNA lesions produced by mitomycin C and porfiromycin in hypoxic and aerobic EMT6 and Chinese hamster ovary cells. *Cancer Res.* **1986**, *46*, 3939−3944.

Scheme 1 [a]



[a] Reagents: (a) ethylene oxide, AcOH; (b) tributylphosphine, diethyl azodicarboxylate; (c) mechlorethamine hydrochloride, Na_2CO_3; (d) HONO, H^+, reflux; (e) 3-iodopropanol, tetrabutylammonium bromide.

15 mM mercaptoethanol, 8 mM $MgCl_2$, and 60 mM NaCl), 1 μL of supercoiled pRYG DNA (0.25 μg/μL), with or without 2 μL of drug solution and varying amounts of enzyme, which was added last. The optimal amount of topoisomerase II (2 units/μL) needed to cause DNA relaxation was predetermined by incubating different amounts of topoisomerase II (1−4 μL) with DNA substrate (1 μL). The incubations carried out with and without 10% DMSO were both used as negative control in each assay, and amsacrine was used to serve as positive control.

The incubations were carried out at 37 °C for 30 min; then 16 μL of each reaction mixture was loaded onto agarose gel after addition of 2 μL of loading buffer (0.25% bromophenol blue, 50% glycine) into the mixture. Samples were electrophoresed on a 1% agarose gel containing 0.5 μg/mL EtBr at 90 V for 2 h in 1× TAE buffer (Tris-Acetate-EDTA buffer, Sigma) also containing 0.5 μg/mL EtBr. The 10× TAE buffer contains 0.4 M Tris acetate (pH 8.3) and 0.01 M EDTA. After destaining for 30 min in water, agarose gels were photographed (Polaroid, 667 film, 0.5 s, *f*/11) under UV illumination by a UV transilluminator (Ultra-Lum, Inc).

In Vitro Bioreduction Study. The rat S-9 fraction was prepared essentially as previously reported.[7] The final incubation volume was 3 mL, which consists of 50 μL (5 μmol) of the tested drug in DMF, 50 μL (5 μmol) of cofactor in DMF, and 2 mL of S-9 fraction. Cofactors used were benzaldehyde (0.1 M, in DMF), 2-hydroxypyramidine (0.1 M, in DMF), NADH, or NADPH. Phosphate buffer (12.5 mM, pH 7.4) was used to make up the final volume. A parallel incubation without cofactors served as control. A hypoxia condition was induced by gently purging nitrogen gas through the S-9 fraction and phosphate buffer mixture for 10 min in vials sealed with rubber septa and fitted with two needles. Drug and cofactors were then added by injection through the septa without breaking the hypoxia. All incubations were carried out for 30 min at 37 °C.

The incubations were terminated with 3 mL of MeOH, and then the precipitate was separated by centrifugation at 3000 rpm for 15 min. Appropriate aliquots of the supernatant were spotted on TLC plates and eluted with EtOAc/EtOH (9:1). A Uniscan densitometer (Analtech, Newark, DE) was used to quantify the metabolite on TLC plates.

In Vivo Antitumor Assay. The in vivo testing was performed by Medicinal Research Center, CKD Corp., Seoul, Korea. Briefly, 10^6 P388 leukemia cells were intraperitoneally implanted into male BFD1 mice on day 1; the drug treatment schedule was five daily treatments (day1 through day 5). The drug, compound **2a**, was dissolved in 10% DMSO in distilled water with a total dose of 1000 mg/kg. Groups of 6 animals were used; and there were control groups which consisted of 8 animals.

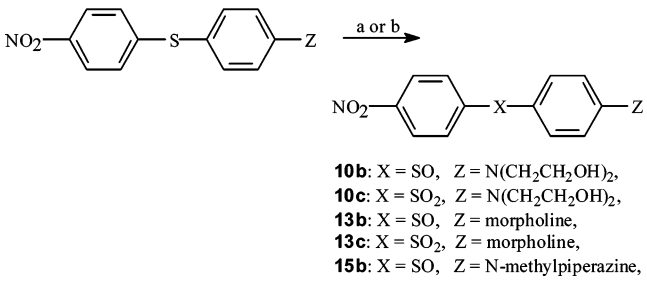
Results

Chemistry. The monohydroxyethylated 2-({4-[(4-nitrophenyl)thio]phenyl}amino)ethanol (**17**) and dihydroxyethylated 2-{(2-hydroxyethyl)[4-(4-nitrophenylthio)phenyl]amino}-ethan-1-ol (**10a**) were prepared by hydroxylation of 4-amino-4′-nitrodiphenyl sulfide with ethylene oxide (Scheme 1).[8,9] The morpholine-containing compound **13a** was synthesized by the cyclization of **10a** through Mitsunobu reaction (Scheme 1).[11] The *N*-methylpiperazine-containing compound **15a** was prepared through a ring closure method by reacting 4-amino-4′-nitrodiphenyl sulfide with mechlorethamine hydrochloride (Scheme 1).[12] The anilino group of 4-amino-4′-nitrodiphenyl sulfide was converted into the phenolic compound **19** through the formation of a diazonium salt (Scheme 1).[14,15] The dihydroxypropylated compound **21** was synthesized by the alkylation of 4-amino-4′-nitrodiphenyl sulfide with 3-iodopropanol (Scheme 1).[16] The sulfoxides (**10b**, **13b**,

(14) Cohen, T.; Fietz, A. G.; Miser, J. R. A Simple preparation of phenols from Diazonium ions via the generation and oxidation of aryl radicals by copper salts. *J. Org. Chem.* **1977**, *42*, 2053−2058.

(15) Ungnade, H. E.; Orwoll, E. F. 3-Bromo-4-hydroxytoluene. *Organic Syntheses*; Horning, E. C., Ed.; Wiley: New York, 1955; Collect. Vol. III, pp 130−131.

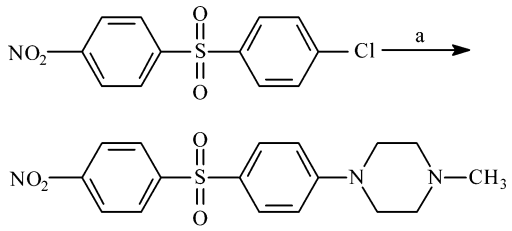
Scheme 2 [a]



10b: X = SO, Z = $N(CH_2CH_2OH)_2$,
10c: X = SO_2, Z = $N(CH_2CH_2OH)_2$,
13b: X = SO, Z = morpholine,
13c: X = SO_2, Z = morpholine,
15b: X = SO, Z = N-methylpiperazine,

[a] Reagents: (a) H_2O_2, TFA; (b) H_2O_2, $(NH_4)_2MoO_4$.

Scheme 3 [a]



15c

[a] Reagent: (a) *N*-methylpiperazine.

and **15b**) and sulfone **10c** were prepared by oxidizing the corresponding sulfides with hydrogen peroxide in trifluoroacetic acid (TFA) (Scheme 2).[8,17] The sulfone compound **13c** was prepared by oxidizing its sulfide **13a** with hydrogen peroxide using ammonium molybdate as catalyst (Scheme 2).[8,18] Compound **15c** was prepared by reacting 4-chloro-4′-nitrodiphenyl sulfone with *N*-methylpiperazine (Scheme 3).[12]

The amino compounds (**7**, **11a**−**c**, **14a**−**c**, **16a**−**c**, **18**, **20**, and **22**) were synthesized from the corresponding nitro compounds by reduction with Fe/HCl (Scheme 4).[8] The acridine-containing compounds (**2a**−**c**, **3**, **4a**−**c**, **5a**−**c**, **6**, **8**, and **9**) were synthesized by condensation of their precursors (**11a**−**c**, **22**, **14a**−**c**, **16a**−**c**, **18**, and **20**) with 9-chloroacridine, respectively (Scheme 4).[8,19]

Cytotoxicity of Target Compounds. The comparative cytotoxicities of compounds **1a** and **2a** and amsacrine were determined by clonogenic assay with V-79 cells (Table 1). Compound **2a** was considerably more cytotoxic (LC_{90} = 2.1 μM) than **1a** as well as amsacrine. The unexpected potent cytotoxicity of **2a** warranted further studies. Therefore, comounds **3**−**9** were synthesized for conducting the limited SAR study of compound **2a** regarding its cytotoxicity.

It is known that aldehyde oxidase could reduce diaryl sulfoxides to their corresponding sulfides under anaerobic conditions; the sulfoxide and sulfone derivatives (**2b**, **4b**, **5b**, **2c**, **4c**, and **5c**) of compounds **2a**, **4a**, and **5a** were therefore synthesized and their cytotoxicities were evaluated, as shown in Table 1, with the expectation of finding potential sulfoxide-containing bioreductive anticancer agents.

Inhibition of Topoisomerase II by 2a. The mechanism by which amsacrine and other DNA-intercalating agents stimulate the production of DNA breaks by topoisomerase II appears to be the primary mechanism of cytotoxicity of this class of drugs.[20] On the basis of the structural similarity between **2a** and amsacrine, **2a** was also evaluated for its topoisomerase II inhibitory activity. The relaxation ability of topoisomerase II was inhibited by **2a** to different degrees at concentrations of 10, 100, and 500 μM. As in lane 4 of Figure 1, the relaxation activity of topooismerase II was almost completely inhibited by 500 μM of compound **2a**.

In Vivo Antitumor Activity of 2a. A preliminary in vivo anticancer screening test showed that **2a** was highly effective with ILS of 175 and 2/6 40-day long-term survival against P388 leukemia bearing BFD1 mice as shown in Table 2.

In Vitro Bioreduction of 2b. As stated earlier, compound **2a** was highly cytotoxic in vitro against the V-79 cell line and active in vivo against P388 bearing mice. Its metabolic precursor, **2b** (6−7-fold less cytotoxic than **2a**), was then considered as a candidate bioreductively activated anticancer prodrug. The metabolism profile of compound **2b** was studied and is summarized in Table 3.

Discussion

In an attempt to answer whether the acridine moiety contributed to the overall cytotoxicity profile of **1a** through a possible intercalating/topoisomerase inhibitory capability, compound **2a**, in which both chloroethyl moieties were replaced by a diol functionality, was synthesized. Since the substitution of mustard with bis(2-hydroxyethyl)amino moiety is the only structural difference between **1a** and **2a**, the hydroxyl groups in the bis(2-hydroxyethyl)amino moiety were suspected to be important for the cytotoxicity of **2a**. To test the importance of the bis(2-hydroxyethyl)amino moiety, **4a** and **5a** were prepared, in which the bis(2-hydroxyethyl)amino moiety was replaced with morpholine and methylpiperazine, respectively. The monohydroxyl group containing compound **6** was prepared to test if both the hydroxyl groups are required. The diol-containing analogue, compound **3**, was prepared to test the influences of the distance between the hydroxyl group and the anilino nitrogen as well. On the basis of the structural similarity between compound **2a** and SN 12489, compounds **7**−**9** were designed as the sulfide analogues of SN 12489 instead of sulfonamide.

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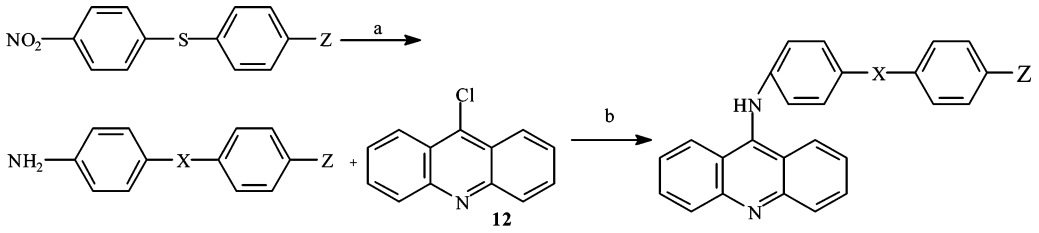
Scheme 4 [a]

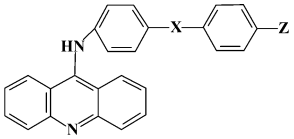


	X	Z
11a :	-S-	$N(CH_2CH_2OH)_2$
11b :	-SO-	$N(CH_2CH_2OH)_2$
11c :	$-SO_2-$	$N(CH_2CH_2OH)_2$
14a :	-S-	Morpholine
14b :	-SO-	Morpholine
14c :	$-SO_2-$	Morpholine
16a :	-S-	N-methylpiperazine
16b :	-SO-	N-methylpiperazine
16c :	$-SO_2-$	N-methylpiperazine
18 :	-S-	$NHCH_2CH_2OH$
20 :	-S-	OH
22 :	-S-	$N(CH_2CH_2CH_2OH)_2$
7 :	-S-,	9-NH-acridine

	X	Z
2a :	-S-	$N(CH_2CH_2OH)_2$
2b :	-SO-	$N(CH_2CH_2OH)_2$
2c :	$-SO_2-$	$N(CH_2CH_2OH)_2$
4a :	-S-	Morpholine
4b :	-SO-	Morpholine
4c :	$-SO_2-$	Morpholine
5a :	-S-	N-methylpiperazine
5b :	-SO-	N-methylpiperazine
5c :	$-SO_2-$	N-methylpiperazine
6 :	-S-	$NHCH_2CH_2OH$
9 :	-S-	OH
3 :	-S-	$N(CH_2CH_2CH_2OH)_2$
8 :	-S-	NO_2

[a] Reagents: (a) Fe, HCl; (b) HCl.

Table 1. In Vitro Cytotoxicity against V-79 Cells by Synthesized Compounds and Amsacrine



compd	X	Z	cytotoxicity LC_{90} (V-79),[a] μM
amsacrine			47.5 ± 14.5
1a	S	$N(CH_2CH_2Cl)_2$	>3000
2a	S	$N(CH_2CH_2OH)_2$	2.1 ± 0.7
2b	SO	$N(CH_2CH_2OH)_2$	15.4 ± 2.5
2c	SO_2	$N(CH_2CH_2OH)_2$	4.8 ± 0.3
3	S	$N(CH_2CH_2\ CH_2OH)_2$	1.9 ± 0.4
4a	S	morpholine	24.9 ± 6.3
4b	SO	morpholine	8.4 ± 2.4
4c	SO_2	morpholine	7.0 ± 1.9
5a	S	*N*-methylpiperazine	9.4 ± 1.5
5b	SO	*N*-methylpiperazine	3.9 ± 0.4
5c	SO_2	*N*-methylpiperazine	3.5 ± 0.7
6	S	NH CH_2CH_2OH	10.4 ± 1.5
7	S	NH_2	46.4 ± 21.7
8	S	NO_2	588 ± 247
9	S	OH	118 ± 38

[a] IC90: The drug concentration required to reduce cell survival to 10% of controls using V-79 cells in the clonogenic assay; mean ± SD was from three experiments.

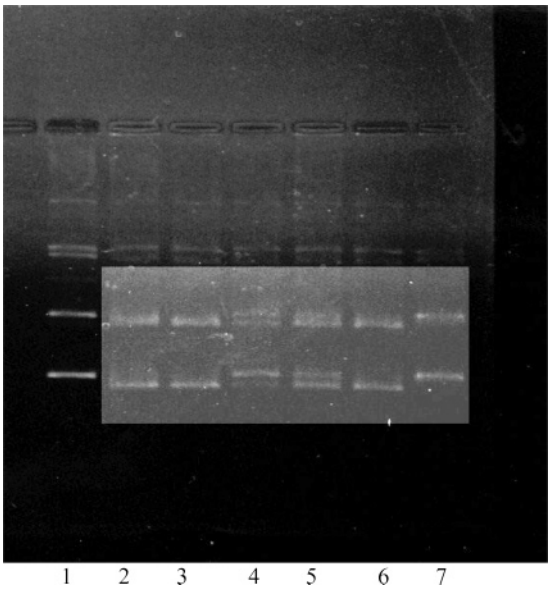


Figure 1. Topoisomerase II inhibitory assay of compound **2a**. Reactions indicated above lanes 2−7 were carried out using 6 units of enzyme and 0.25 μg of supercoiled pRYG DNA each. The reactions were incubated for 30 min at 37 °C. Lane 1 is the standard supercoiled DNA. Lanes 2 and 3 were incubated with or without 10% DMSO. Lanes 4−6 contained **2a** at concentrations of 500, 100, and 10 μM, respectively. Lane 7 contained amsacrine, 100 μM.

In this study, V-79 Chinese hamster lung fibroblasts were chosen to evaluate the cytotoxicity of all the synthesized compounds. V-79 cells formed into tightly packed spheroids have been widely used in the evaluation of drug penetration in solid tumors, and radiosensitizing agents and cytotoxic drugs specifically on hypoxia cells.[1,21]

Among compounds **7**−**9**, the electron-donating substituted **7** and **9** were more cytotoxic than the electron-withdrawing nitro-substituted **8**. This possibly suggested the involvement of hydrogen bonding, a dipole−dipole electronic effect in drug−receptor interaction, or electronic effects transmitted to the acridine ring, varying its pK_a and DNA binding ability.[22]

All of the *N*-alkyl-substituted aniline analogues (**2a**, **3**, **4a**, **5a**, and **6**) were more cytotoxic than the unsubstituted aniline compound **7**. The higher homologue of **2a**, **3**, was as potent as **2a**. Both **2a** and **3** were more cytotoxic than the

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Table 2. Preliminary in Vivo Anticancer Screening Test of **2a**

treatment	total dose (mg/kg)	MST (day)[a]	ILS[b]	40-day survivals
untreated		12		0
vehicle control		12		0
lucanthone[c]	1000	22	83.3	0
2a	1000	33	175	2

[a] Mean of the survival time of the treatment group. [b] The percentage increase in lifespan of drug-treated, tumor-bearing animals compared to nontreated, tumor-bearing controls. [c] A known DNA intercalating agent used as positive control.

Table 3. Bioreduction of Compound **2b** with Rat S-9 Fractions

	bioreductive activity[a] (μM **2a** formation/g of protein per h)	
incubation conditions	aerobic	anaerobic
drug + boiled S-9	nd[b]	nd
drug + S-9	nd	nd
drug + 2-hydroxypyrimidine + S-9	nd	38.14 ± 11.95
drug + benzaldehyde + S-9	nd	35.40 ± 14.95
drug + benzaldehyde + menadione + S-9	nd	nd
drug + NADH + S-9	nd	nd
drug + NADPH + S-9	nd	nd

[a] Compound **2a** was incubated with boiled rat S-9 fraction, and a standard curve was constructed and was used to estimate the formation of **2a**. [b] Nondetectable.

morpholine-containing **4a** and *N*-methylpiperazine-containing **5a**, and **5a** was more cytotoxic than **4a**. Compound **6**, containing a monohydroxyl group, was less cytotoxic than compounds **2a** and **3**. It is interesting to note that **6** is still considerably more cytotoxic than **9**. The above results may suggest the importance of the hydroxyl group as well as the distance between the hydroxyl group and the phenyl ring for the cytotoxicity of the series.

The diaryl sulfoxides were found to be better substrates for aldehyde oxidase compared to alkylaryl sulfoxides in the presence of suitable electron donors such as benzaldehyde, acetaldehyde, and 2-hydroxypymidine.[8] As one of the requirements for bioreductive activity, potential sulfoxide-containing bioreductive agents should be less cytotoxic than the corresponding metabolites, the sulfides. Of all the diaryl sulfoxide-containing compounds in this study, compound **2b** was the only compound which is less cytotoxic than the corresponding sulfide; **2b** was 6−7-fold less cytotoxic than **2a**. All of the sulfone-containing compounds (**2c**, **4c**, and **5c**) were quite potent, and their cytotoxicities were similar. This might indicate that the sulfonyl groups may also contribute to their cytotoxicities. For sulfide-containing as well as sulfoxide-containing compounds (**2a**, **2b**, **4a**, **4b**, **5a**, and **5b**), the Z substituent appears to play an important role in providing cytotoxicity. However, the sulfone-containing compounds (**2c**, **4c**, and **5c**) may have a possibly different drug−receptor interaction through the influences of the sulfonyl group. The push−pull mechanism for the 1′ sulfonamide group of amsacrine has been proposed to explain the action of amsacrine to topoisomerase II. The sulfonamide oxygen of amsacrine may act as a Lewis base binding to a Lewis acid binding site on topoisomerase II.[23] The oxygens of the sulfonyl group might also serve a role similar to that of the sulfonamide oxygens.

Amsacrine was considered to bind to DNA by intercalation of the acridine (the DNA-binding domain) between the base pairs, with the aniline ring (the protein-binding domain) protruding into the minor groove, where it is capable of making contacts with topoisomerase II.[1] The topoisomerase II used in the assay is the human 170 kDa isoform. It has been known that the levels of the 170 kDa enzyme are higher in actively growing cells than in stationary-phase cells, and highest during the S phase.[24] Our results indicated that compound **2a** is a topoisomerase II inhibitor. Although compound **2a** appears to be a less potent inhibitor than amsacrine, its cytotoxic potency suggests that low levels of cleavable complexes induced by **2a** may be unusually toxic like the triazoloacridone C-1325.[25] The inhibition preference among topoisomerase II isoforms and stability of the induced cleavable complexes by drugs could also be important factors contributing to their anticancer activity.[26]

Benzaldehyde and 2-hydroxypyrimidine are known electron donors for the aldehyde oxidase, and menadione is a known inhibitor for aldehyde oxidase. Our results showed that compound **2b** was converted into **2a** only in the presence of benzaldehyde and 2-hydroxypyrimidine, and this reduction was inhibited in the presence of menadione. There was no reduction observed when the NADH or NADPH was coincubated with compound **2b** and S-9 fraction. These results suggested that compound **2b** is a good substrate for aldehyde oxidase, and NADH- or NADPH-dependent enzymes are not involved in the bioreduction of compound **2b**. One of the advantages of the sulfoxide-containing amsacrine

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analogues is that they are not likely to be metabolized into quinone derivatives as is observed for amsacrine. Quinoneimine derivatives of amsacrine were reported to be responsible for the side effects of amsacrine through free-radical-mediated genotoxicity.[27−29]

In conclusion, the above results suggested the importance of hydroxyl functionality along with its location for the cytotoxicity of the series. Both diol-containing compounds, **2a** and **3**, were the most cytotoxic of the sulfide series against V-79 cells in vitro (IC_{90} = 2.1 μM, 1.9 μM, respectively). Among the non-alkyl-substituted compounds (**7**−**9**), compounds with electron-donating substitution para to the sulfide (**7** and **9**) were more cytotoxic than the electron-withdrawing nitro-substituted compound **8**. A preliminary anticancer screening against P388 leukemia showed that **2a** is highly active in vivo as well. Topoisomerase II inhibitory activity appeared to be involved in the cytotoxicity of compound **2a**. Sulfoxide compound **2b**, which is 6−7-fold less cytotoxic than its sulfide **2a**, appears to be a potential bioreductive anticancer prodrug on the basis of its bioreductive metabolism findings.

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Supporting Information Available: Table of elemental analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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