The samples were dissolved in phosphate buffer 20 mM (pH 7.0) at concentrations 0.1, 0.3, 0.6, and 1.0 μM.

The inhibition effect was monitored for 60 min, and the IC_{50} was derived from the inhibition curves.

The ability of the selected compounds to lower the IOP in vivo was measured in New Zealand rabbits by the method of Bonomi[22] after artificial elevation of the IOP by intravenous injection of a 5% glucose solution in water.

Registry No. 1, 59-66-5; 3, 129504-06-9; 3a, 129504-20-7; 3b, 59133-61-8; 4, 129504-07-0; 4a, 129504-21-8; 5, 140696-97-5; 5a, 140696-98-6; 6, 129504-08-1; 6a, 129504-23-0; 7, 129504-10-5; 7a, 129504-28-5; 8, 129504-15-0; 8a, 129504-38-7; 8b, 129504-39-8; 9, 129504-17-2; 9a, 129524-72-7; 9b, 129504-42-3; 10, 140696-99-7; 11, 129504-11-6; 11a, 129504-30-9; 12, 78851-85-1; 13, 140697-00-3; 14, 138080-12-3; 15, 140697-01-4; 16, 129504-13-8; 16a, 140833-97-2; 17, 140697-02-5; 18, 140697-03-6; 19, 25660-71-3; 20, 14949-00-9; $CH_3OCO(CH_2)_3COCl$, 1490-25-1; $C_2H_5OCO(CH_2)_2COCl$, 14794-31-1; *sec*-$C_4H_9OCO(CH_2)_2COCl$, 140697-04-7; *n*-$C_5H_{11}OCO(CH_2)_2COCl$, 35444-35-0; $C_{12}H_{25}OCO(CH_2)_2COCl$, 41086-58-2; $CH_3OCO(CH_2)_3COCl$, 1501-26-4; $Cl(CH_2)_4COCl$, 1575-61-7; CH_3OCOCH_2COCl, 37517-81-0; $CH_3OCO(CH_2)_4COCl$, 35444-44-1; $CH_3OCO(CH_2)_7COCl$, 56555-02-3; 5-amino-2-mercapto-1,3,4-thiadiazole, 2349-67-9; 5-[(methoxysuccinyl)amino]-4-methyl-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-40-1; 5-[(pentoxysuccinyl)amino]-4-methyl-1,3,4-thiadiazole-2-sulfonyl chloride, 129520-47-4; 5-[(methoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-19-4; 5-[(ethoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-22-9; 5-[(*sec*-butoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 140697-05-8; 5-[(pentoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-24-1; 5-[(dodecoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-29-6; 5-[(methoxyglutaryl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-31-0; 5-[(5-aminovaleryl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 140697-06-9; carbonic anhydrase, 9001-03-0.

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Synthesis of the Acridone Alkaloids Glyfoline and Congeners. Structure–Activity Relationship Studies of Cytotoxic Acridones

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Glyfoline (4, 1,6-dihydroxy-10-methyl-2,3,4,5-tetramethoxyacridin-9-one) and its congeners were synthesized for evaluation of their cytotoxicity. A detailed structure–activity relationships (SAR) of these acridone derivatives were also studied. To study the SAR of glyfoline analogues, substituent(s) at C-1 and C-6 and at the heterocyclic nitrogen of glyfoline nucleus were modified. Nitro- and amino-substituted glyfoline analogues were also synthesized to study the effects of substituent(s) (electron-withdrawing vs electron-donating) on their cytotoxicity. These compounds were synthesized via the Ullmann condensation of anthranilic acids with iodobenzenes or 2-chlorobenzoic acids with aniline derivatives. The SAR studies showed that 1-hydroxy-9-acridones were more active than their 1-OMe derivatives against cell growth of human leukemic HL-60 cells in culture. Replacement of NMe of glyfoline with NH or $N(CH_2)_2NEt_2$ resulted in either total loss or dramatic reduction of cytotoxicity. Glyfoline congeners with nitro function at the A-ring were inactive, while compounds with amino substituent were shown to be cytotoxic in vitro.

Several 9-acridones have been found to exhibit anticancer activity. 9-Acridone derivatives with or without an alkyl side chain attached to the N-position were synthesized and studied for their antitumor activity.[1] Among these, *N*-[2-(dialkylamino)ethyl]-1-nitro-9-acridones (1, Figure 1) were shown to have antitumor activity in the S-180 system in vivo, and these have undergone extensive preclinical testing.[2] It was also reported that the biscationic side-chain-substituted 9-acridone (2, Figure 1) can act as an acceptable chromophore for DNA intercalation.[3]

Recently, the structure–activity relationships of 50 natural acridone alkaloids have been studied for their effects on the inhibition of cell growth and macromolecule biosynthesis of human promyelocytic leukemic HL-60 cells.[4] It was found that 23 out of the 50 alkaloids were more active than acronycine (3, Figure 1), an antineoplastic alkaloid used in clinical trials.[5] For inhibition of cell growth of human leukemic HL-60 cells in vitro, the most potent compound in this series was found to be glyfoline (4, 1,6-dihydroxy-10-methyl-2,3,4,5-tetramethoxyacridin-9-one), with an IC_{50} of 1.1 μM, while acronycine had an IC_{50} of 26.2 μM.

Glyfoline was originally isolated from *Glycosmis citrifolia* (Willd.) (Rutaceae).[6] The structure of this alkaloid,

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§ Seoul National University.

(1) Radzikowski, C.; Wysocka-Skrzela, B.; Jrabowska, M.; Konopa, J.; Kereczek-Marawska, E.; Onoszko, K.; Ledochowski, Z. Search for antitumor compounds, biological studies. Antitumor properties of 17 new acridone derivatives. *Arch. Immunol. Ther. Exp.* 1971, *19*, 219–228.

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(5) Svoboda, G. H.; Poore, G. A.; Simpson, P. J.; Boder, G. B. Alkaloids of Acronychia bauri, isolation of the alkaloids and study of the antitumor and other biological properties of acronycine. *J. Pharm. Sci.* 1966, *55*, 758–768.

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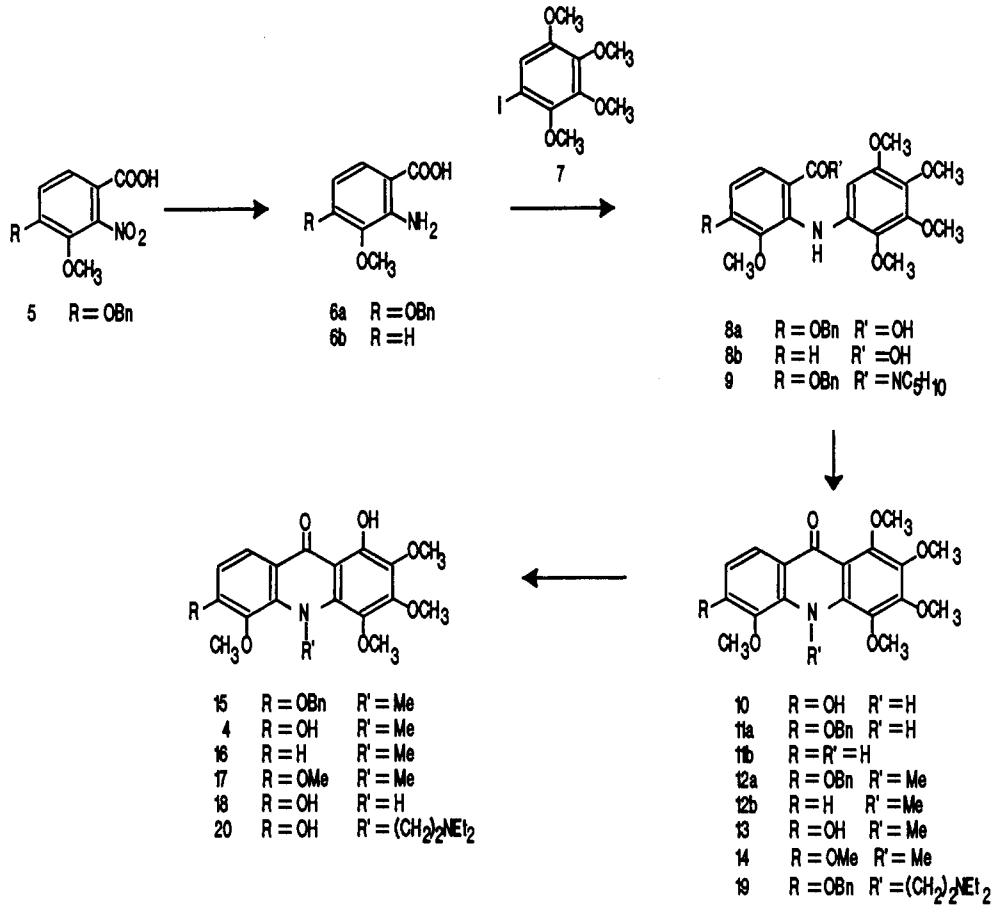
Scheme I



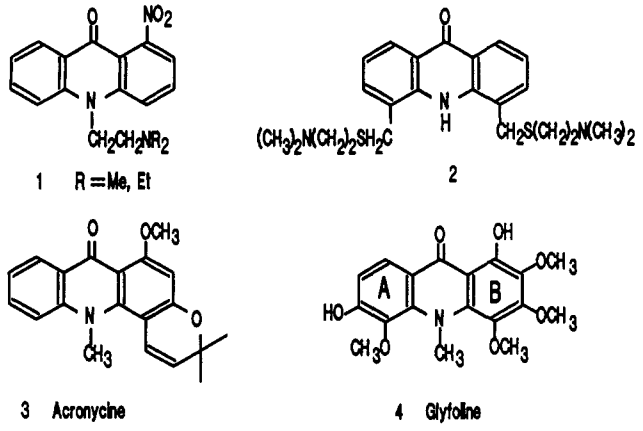


Figure 1.

which was determined originally by spectroscopic analysis,[6] was fully secured by total synthesis.[7] In order to elucidate the structure–activity relationships of glyfoline, we have synthesized several glyfoline congeners and evaluated their cytotoxicity. In this paper, we furnished the experimental details of the synthesis of glyfoline and some of its analogues as well as a study of the SAR relationships in a cell culture system.

Chemistry

The chemical synthesis of glyfoline was described previously as shown in Scheme I.[7] Ullmann reaction of 4-(benzyloxy)-3-methoxyanthranilic acid (**6a**) with 1-iodo-2,3,4,5-tetramethoxybenzene (**7**) gave diphenylamine carboxylic acid **8a**, which was then converted into glyfoline in four steps. The details of the synthesis are reported in the Experimental Section. It should be noted here that the methoxy group at C-1 of the permethylated intermediate **12a** was selectively hydrolyzed with concentrated hydrochloric acid in refluxing methanol to give 1-hydroxy-9-acridone (**15**), which was then converted smoothly into the desired compound, glyfoline (**4**), in good yield by catalytic hydrogenolysis. Selective de-O-methylation of 1-methoxy-9-acridone can also be achieved by treatment with a Lewis acid such as boron tribromide (BBr_3). The latter method was utilized in the synthesis of glyfoline, which was obtained in 54% yield from **12a**.

In our previous report,[4] we demonstrated that 1-hydroxy-9-acridones bearing a substituent at C-4 and several substituents on rings A and B possessed significant cytotoxic activity. It was also found that simple 1-hydroxy-9-acridones with substituents only on the B-ring (i.e., normelicopicine, **21**, 1-hydroxy-10-methyl-2,3,4-trimethoxyacridin-9-one) are generally inactive.[4,11] These

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(7) Su, T-L.; Dziewiszek, K.; Wu, T-S. Synthesis of glyfoline, a constituent of Glycosmis citrifolia (Willd.) Lindl. and a potential anticancer agent. *Tetrahedron Lett.* **1991**, *32*, 1541–1544.
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(9) Crow, W. D. Oxidative demethylation of melicopicine. *Aust. J. Sci.* **1949**, *2A*, 264–271.
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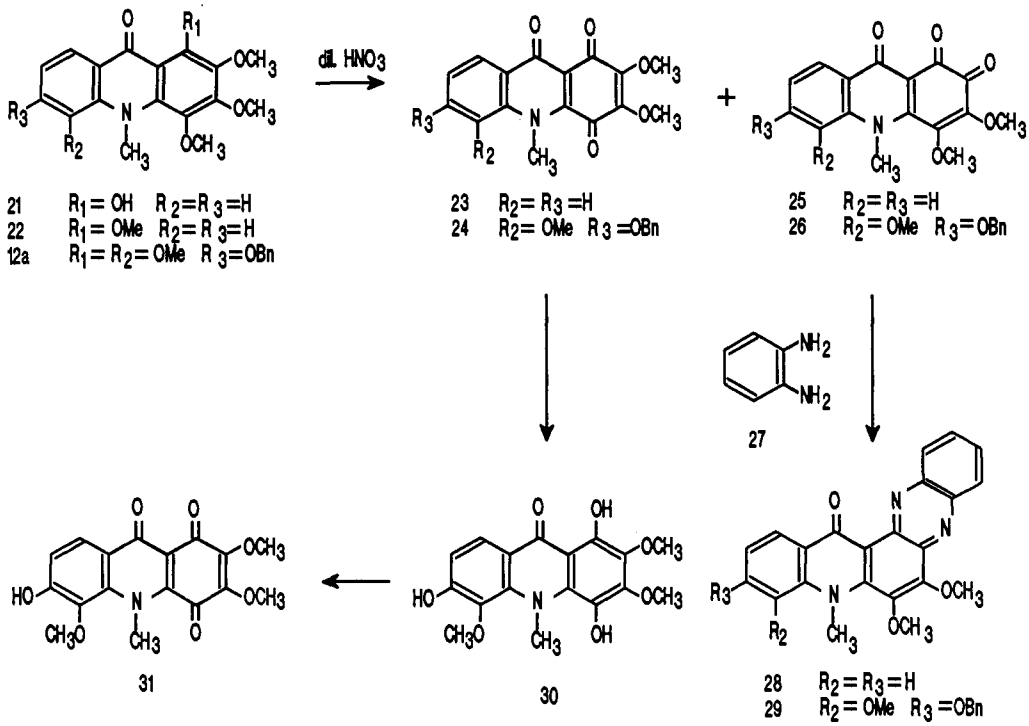
(11) Budesinsky, Z.; Lederer, P.; Roubinek, F.; Srab, A.; Vavrina, J. Alkoxy-2,4-quinazolinediamines. *Collect. Czech. Chem. Commun.* **1976**, *41*, 3405–3414.

Scheme II



findings suggest that formation of an intramolecular hydrogen bond between 1-OH and the peri carbonyl function, and substituent(s) at the A-ring of normelicopicine nucleus (H or OMe) and at the heterocyclic nitrogen (NH or NMe) appear to play an important role in cytotoxicity. We have modified the substituents at C-1 and C-6 of glyfoline molecule. The glyfoline analogues, 1-*O*-methyl- (**13**), 1,6-di-*O*-methyl- (**14**), 6-*O*-methyl- (**17**), and 6-deoxy-1-*O*-methyl- (**12b**) and 6-deoxyglyfoline (**16**) (Scheme I), were synthesized for comparison of their cytotoxicity. Compound **12a** was debenzylated by catalytic hydrogenolysis to give 1-*O*-methylglyfoline (**13**), which was then treated with MeI/K_2CO_3 to form 1,6-dimethoxyglyfoline (**14**) in good yield. Partial de-O-methylation of **14** by treatment with concentrated HCl/MeOH afforded 6-*O*-methylglyfoline (**17**).

By following a similar procedure for the synthesis of glyfoline, 6-deoxy-1-*O*-methylglyfoline (**12b**, 10-methyl-1,2,3,4,5-pentamethoxyacridin-9-one) and its 1-OH derivative (**16**) were prepared (Scheme I). Ullmann condensation of 3-methoxyanthranilic acid (**6b**) with iodobenzene (**7**) afforded the diphenylamine carboxylic acid **8b**, which was cyclized with polyphosphoric acid to give the 9-acridone **11b** in 62% yield. N-Methylation of **11b** afforded 5-methoxymelicopicine (**12b**), which was then converted into its 1-OH derivative **16** by treatment with concentrated HCl in MeOH.

We have also modified the substituent at the heterocyclic nitrogen by introducing hydrogen or a (diethylamino)ethyl side chain (Scheme I). N-Desmethylglyfoline (**18**) was previously prepared from **10** by treatment with BBr_3, while *N*-[2-(diethylamino)ethyl]glyfoline (**20**) was synthesized from **11a**. Treatment of **11a** with an excess of 2-(diethylamino)ethyl chloride in dimethylformamide (DMF) in the presence of sodium hydride overnight gave **19** (32%), which was then converted into **20** via partial de-O-methylation and debenzylation.

In order to study the role of substituent(s) (electron-withdrawing vs electron-donating) on the acridone nucleus, we have synthesized glyfoline congeners in which only the substituent(s) on the A-ring was modified without altering the B-ring. Derivatives of glyfoline with a nitro or amino substituent at the A-ring of the molecule were synthesized.

Direct nitration of 9-acridones would be the most convenient way to introduce a nitro function to acridone nucleus. However, it was reported that 9-acridone gave the quinone derivatives upon oxidation with diluted nitric acid (Scheme II).[13] Both melicopicine (**22**) and normelicopicine (**21**) upon nitric acid oxidation yield the same mixture of two isomeric quinones, acridine-1,4,9-trione (**23**) and acridine-1,2,9-trione (**25**), without forming a nitro-substituted product. We also found that compound **12a** was oxidized to form the two para- and ortho-quinones (**24** and **25**, respectively), which were separated by chromatography. The bright red quinones (**23**[13] and **24**) did not react with o-phenylenediamine (**27**), but their dark red isomers (**25**[13] and **26**) gave quinophenazines (**28** and **29**, respectively), and are therefore the ortho-quinones. Compound **24** was converted into 1-hydroxy-10-methyl-2,3,5-trimethoxyacridine-1,4,9-trione (**31**) via catalytic hydrogenation (Pd/C, H_2) and oxidation (*m*-chloroperbenzoic acid).

Our first approach to the synthesis of nitro-substituted normelicopicine (**47a,b**, Scheme IV) was to prepare 6(or 7-)-nitro-1,2,3-trimethoxyacridin-9-one (**35a,b**, **37**, or **38**, Scheme III), which could then be converted into 4-hydroxy derivative (**39**) via formylation, Baeyer-Villiger oxidation, and hydrolysis by the procedure developed by Hannan et al.,[14] who synthesized 4-hydroxy-1,5-dimethoxynaphthalene from 1,5-dimethoxynaphthalene. 4-Nitro- and 5-nitrodiphenylamine carboxylic acids (**34a** and **34b**) were prepared in 26% and 15% yield, respectively, by

(12) Chou, T-C.; Otter, G. M.; Schmid, F. A.; Su, T-L. Antitumor activity of glyfoline in vitro and in vivo. *Proc. Am. Assoc. Cancer Res.* **1991**, *32*, 403.

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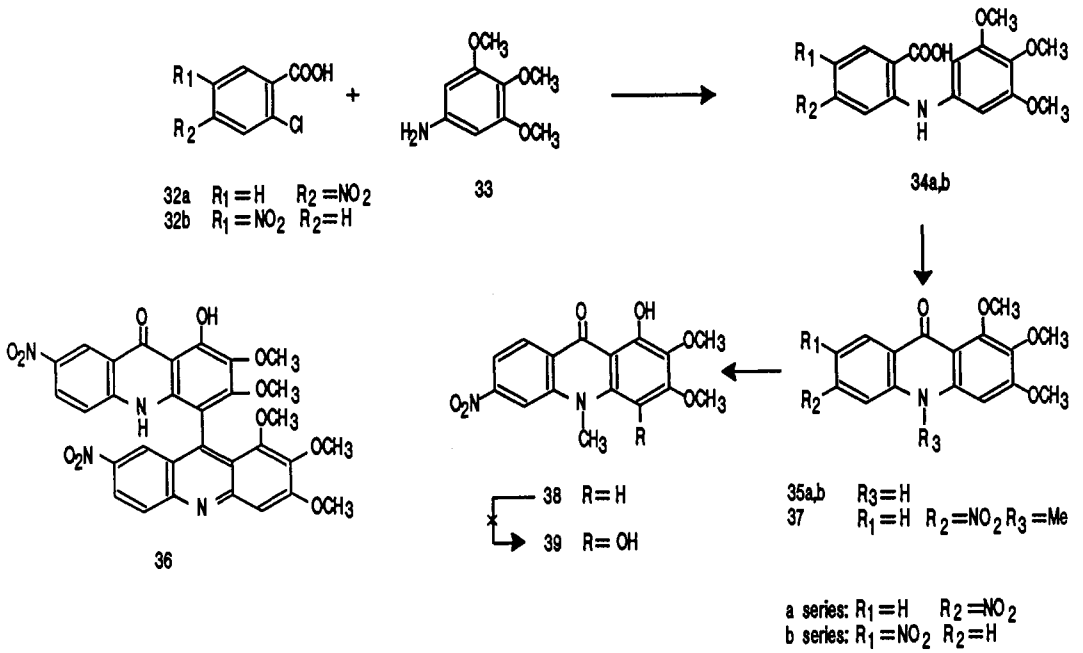
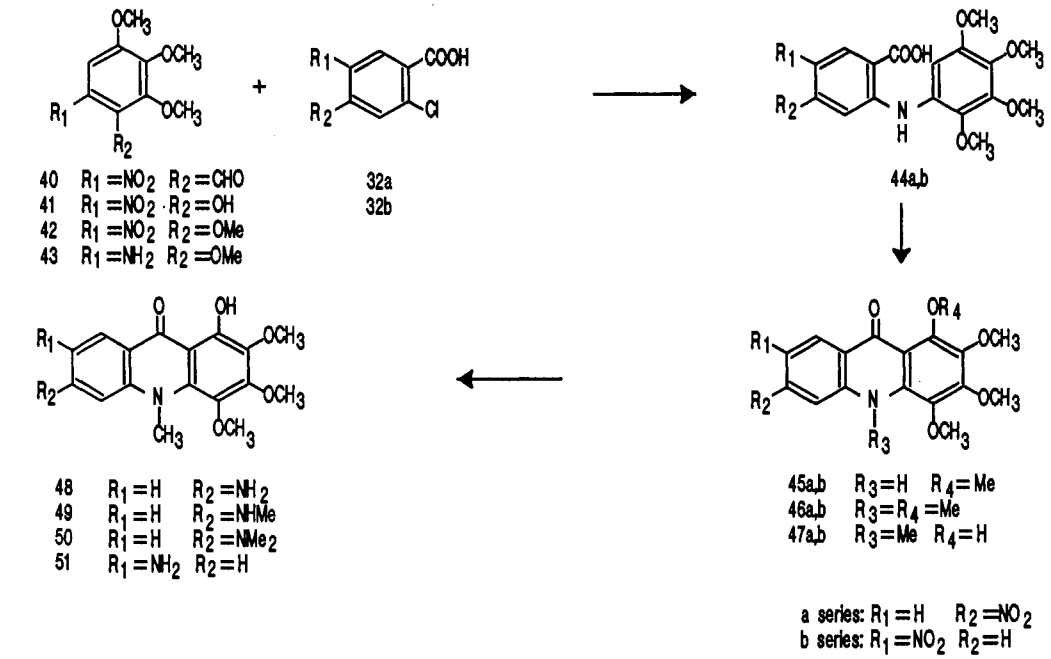
(14) Hughes, G. K.; Neill, K. K.; Ritchi, E. The synthesis of melicopicine and some trimethoxy-10-methylacridones. *Aust. J. Sci. Res.* **1954**, *3A*, 497–503.

Scheme III



Scheme IV



Ullmann condensation of the corresponding 2-chloronitrobenzoic acids (**32a** and **32b**) with trimethoxyaniline (**33**). 6-Nitro-9-acridone (**35a**) was smoothly synthesized in 90% yield from 4-nitrodiphenylamine carboxylic acid **34a** by treatment with polyphosphoric acid at 110 °C for 2.5 h. Under the same conditions, the 5-nitro derivative **34b**, however, was cyclized to form a mixture of 7-nitroacridone (**35b**) and its dimer (**36**). The nitro function of **34a** is located at the para position to the carboxylic acid group and also to the meta position of the amino function. The carboxylic acid function of **34a** would then be more susceptible to electrophilic attack in comparison with that of **34b** and, thus, prefer intramolecular acylation to form **35a** exclusively. In the case of **34b**, both intra- and intermolecular acylation had occurred, leading to a mixture of 7-nitroacridone **35b** and dimer **36**, respectively. N-Methylation of **35a** afforded **37**, which was further converted into its 1-hydroxy-9-acridone **38**. However, attempts to convert **35a**, **37**, or **38** to their 4-hydroxy derivatives **39** failed, since formylation at C-4 of the acridones did not occur.

To synthesize nitro-substituted normelicopicines (**47a,b**), we prepared 2,3,4,5-tetramethoxyaniline (**43**), which then was allowed to react with the nitrobenzoic acids (**32a,b**) to furnish the desired acridones. Thus, the known compound **40**[15] (Scheme IV) was converted into 2-hydroxy-3,4,5-trimethoxynitrobenzene (**41**) in good yield via Baeyer-Villiger oxidation (*m*-ClPBA) and hydrolysis (NaOH). Compound **41** was O-methylated by treatment with diazomethane, and the product **42** was reduced to yield 2,3,4,5-tetramethoxyaniline (**43**). Ullmann condensation of nitrobenzenes (**32a** and **32b**) and aniline **43** afforded diphenylamine carboxylic acids (**44a** and **44b**), which were cyclized to form nitro-9-acridones (**45a** and **45b**,

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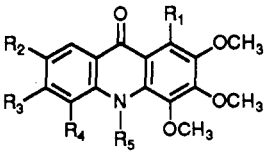
Table I. Structure and Effects of Glyfoline Congeners



compd	R_1	R_2	R_3	R_4	R_5	cell growth inhibn HL-60: IC_{50} (μM)
glyfoline (4)	OH	H	OH	OMe	Me	2.2
13	OMe	H	OH	OMe	Me	91.9
17	OH	H	OMe	OMe	Me	3.4
14	OMe	H	OMe	OMe	Me	23.3
16	OH	H	H	OMe	Me	12.8
13	OMe	H	H	OMe	Me	23.7
18	OH	H	OH	OMe	H	>100
20	OH	H	OH	OMe	$(CH_2)_2NEt_2$	62.2
47a	OH	H	NO_2	H	Me	>100
48	OH	H	NH_2	H	Me	11.6
49	OH	H	NHMe	H	Me	10.6
50	OH	H	NMe_2	H	Me	9.4
47b	OH	NO_2	H	H	Me	>100
51	OH	NH_2	H	H	Me	44.4

respectively). Compounds **45a,b** were N-methylated to give **46a,b**, which were then partially de-O-methylated to yield 1-hydroxy-9-acridones (**47a,b**). The nitro function of **47a,b** was reduced to the corresponding aminoacridones (**48** and **51**, respectively). Treatment of the 6-aminonormelicopicine (**48**) with excess MeI in refluxing acetone in the presence of K_2CO_3 overnight led to the formation of a mixture of *N*-monomethyl (**49**) and *N,N*-dimethyl (**50**) acridones, which were separated by chromatography.

Discussion

The structure–activity relationships of glyfoline congeners are shown in Table I. These compounds were evaluated for their ability to inhibit human leukemic HL-60 cell growth in culture. The results show that 1-hydroxy-9-acridones are more active than their corresponding 1-O-methylated derivatives (i.e., **4** vs **13**, **17** vs **14**, and **16** vs **13**), indicating that the presence of the intramolecular hydrogen bond in 1-hydroxy-9-acridones played an important role for their cytotoxicity. 6-*O*-Methylglyfoline (**17**) was shown to be as potent as glyfoline with an IC_{50} of 3.4 μM. Removal of 6-OH of glyfoline (5-methoxynormelicopicine, **16**) decreased the cell growth inhibitory activity. Replacement of the methyl function at the heterocyclic nitrogen with hydrogen (*N*-desmethylglyfoline, **18**) or (diethylamino)ethyl side chain (**20**) resulted in either total loss or dramatic reduction of activity, respectively. These results demonstrated that 1-OH, 6-OH, and *N*-Me functions were essential to preserve the cytotoxicity of glyfoline.

The IC_{50} values for 1-hydroxy-9-acridones with an electron-withdrawing or electron-donating function were also shown in Table I. Normelicopicine was previously reported to have no cytotoxic activity. We found that both 6- and 7-nitronormelicopicine derivatives (**47a** and **47b**) were also inactive against HL-60 cell growth. Their corresponding amino counterparts (**48** and **51**, respectively), however, demonstrated cytotoxicity with IC_{50} values of 9.4 and 44.4 μM, respectively. The cytotoxicity of 1-hydroxy-9-acridones was enhanced by electron-donating function(s) (especially at the para position of the carbonyl function) on the aromatic rings. Moreover, the cytotoxicity of normelicopicine analogues was porportionally enhanced following the order of increasing inductive strength of the substituent (NMe_2 > NHMe > NH_2; cf. **48**, **49**, and **50**), although the activity was not greatly increased. None of the glyfoline congeners synthesized appeared to be as active as glyfoline. The SAR studies may, however, lead to the development of more potent analogues. The in vivo antitumor activity of glyfoline has been studied.[12] It was demonstrated that this agent exhibited significant antitumor activity against several solid tumors in mice.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Column chromatography was performed on silica gel G60 (70–230 mesh, ASTM, Merck). Thin-layer chromatography was performed on Analtech Uniplates with short-wavelength UV light for visualization. Elementary analyses were performed by MHW Laboratories, Phoenix, AZ. ^1H NMR data were recorded on a JEOL-FX90Q spectrometer with Me_4Si as the internal standard. Chemical shifts were reported in ppm (δ), and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), dt (double triplet), br s (broad singlet). Values reported for coupling constants are first order.

4-(Benzyloxy)-3-methoxyanthanilic Acid (6a). To a vigorously stirring suspension of 4-(benzyloxy)-3-methoxy-2-nitrobenzoic acid (**5**)[13] (36.45 g, 0.12 mol) in H_2O (600 mL) containing $NaOAc{\cdot}3H_2O$ (16.32 g) was added $Na_2S_2O_4$ (107 g, 0.615 mol) portionwise and the mixture was heated for 4 h at 70 °C. After cooling, the precipitate was collected by filtration and washed with H_2O. The product was crystallized from EtOH to give **6a**: 22.4 g (68.3%); mp 167–8 °C; ^1H NMR ($CDCl_3$) δ 3.69 (3 H, s, OMe), 5.15 (2 H, s, CH_2), 6.40 (1 H, d, $J_{5,6}$ = 9.33 Hz H-5), 7.32–7.49 (5 H, m, Ar-H), 7.47 (1 H, d, $J_{5,6}$ = 9.33 Hz, H-6). Anal. ($C_{15}H_{15}NO_4$) C, H, N.

2-Hydroxy-1-nitro-3,4,5-trimethoxybenzene (41). A mixture of 2-nitro-4,5,6-trimethoxybenzaldehyde[15] (**40**, 19.3 g, 0.08 mol) and *m*-chloroperbenzoic acid (20.7 g, 0.12 mol) in $CHCl_3$ (350 mL) was heated under reflux for 5 h. After cooling, the reaction mixture was washed with 10% $NaHCO_3$ solution until no more *m*-chlorobenzoic acid could be detected by TLC. The solvent was removed by evaporation in vacuo, and the residue was treated with a 10% KOH solution (KOH, 5.0 g, 0.089 mol in 50 mL of H_2O) and MeOH (150 mL) at room temperature under N_2. The mixture was evaporated in vacuo and the solid residue was dissolved in H_2O (300 mL) and then acidified with 6 N HCl. The precipitated product was collected by filtration, washed well with H_2O, and dried to give (after recrystallization from 2-propanol) 13.1 g of **41**: mp 72–3 °C; ^1H NMR ($CDCl_3$) δ 3.87, 3.94, and 4.09 (each 3 H, s, 3 OMe), 7.32 (1 H, s, H-6), 10.78 (1 H, exchangeable, OH). Anal. ($C_9H_{11}NO_6$) C, H, N.

1-Nitro-2,3,4,5-tetramethoxybenzene (42). Compound **41** (4.58 g, 0.02 mol) was treated with CH_2N_2 (prepared from 1-methyl-3-nitro-1-nitrosoguanidine, 5.88 g, 0.04 mol) in ether (300

mL) for 4 h at room temperature. The reaction mixture was evaporated in vacuo to dryness, and the residue was chromatographed on a silica gel column (3 × 40 cm) using n-C_6H_{14}/EtOAc (9:1) as the eluent. Compound **42** was obtained as a syrup: 3.45 g (71%); ^{1}H NMR ($CDCl_3$) δ 3.80, 3.94, 3.96, and 3.98 (each 3 H, s, 4 OMe), 7.18 (1 H, s, H-6). Anal. ($C_{10}H_{13}NO_6$) C, H, N.

2,3,4,5-Tetramethoxyaniline (43). A solution of **42** (1.22 g, 5 mmol) in EtOH (50 mL) was catalytically hydrogenated over 10% Pd/C (0.5 g) at 40 psi for 1 h. After removal of the catalyst by filtration, the filtrate was evaporated in vacuo to dryness to give crude **43**. The pure product, 0.931 g (87%), was obtained by chromatography over a silica gel column (3 × 40 cm, n-C_6H_{14}/EtOAc, 4:1 v/v): mp 99–100 °C; ^{1}H NMR ($CDCl_3$) δ 3.65 (2 H, br s, NH_2), 3.78, 3.79, 3.80, and 3.94 (each 3 H, s, 4 OMe), 6.09 (1 H, s, H-6). Anal. ($C_{10}H_{15}NO_4$) C, H, N.

4-(Benzyloxy)-3-methoxy-*N*-(2,3,4,5-tetramethoxyphenyl)anthranilic Acid (8a). A mixture of **6a** (8.199 g, 0.03 mol), 1-iodo-2,3,4,5-tetramethoxybenzene[14] (**7**, 11.026 g, 0.033 mol), powdered Cu (0.48 g) Cu_2O (0.48 g), and K_2CO_3 (5.17 g) in diglyme (60 mL) was heated under reflux for 5 h. The solvent was removed by distillation in vacuo, and the residue was triturated with 1% NaOH aqueous solution (300 mL). After filtration, the alkaline solution was washed with ether (50 mL × 4) and acidified with 6 N HCl, and then extracted with EtOAc (100 mL × 6). The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated to dryness in vacuo. The residue was chromatographed over a silica gel column (5 × 40 cm) using $CHCl_3$/MeOH (50:1 v/v) as the eluent. The main fraction was collected to give, after recrystallization from n-C_6H_{14}/EtOH, **8a**: 8.69 g (60%); mp 138–9 °C; ^{1}H NMR ($CDCl_3$) δ 3.54, 3.65, 3.82, 3.94, and 3.98 (each 3 H, s, 5 OMe), 5.20 (2 H, s, CH_2), 5.99 (1 H, s, H-6′), 6.89 (1 H, d, J = 9.0 Hz, H-5), 7.38 (5 H, m, Ph), 7.94 (1 H, d, J = 9.0 Hz, H-6). Anal. ($C_{25}H_{27}NO_8$) C, H, N.

By following the same procedure, the following diphenylamine carboxylic acids were synthesized.

3-Methoxy-*N*-(2,3,4,5-tetramethoxyphenyl)anthranilic acid (8b) was obtained by condensation of 3-methoxyanthranilic acid (**6b**, 3.34 g, 20 mmol) with 2,3,4,5-tetramethoxyiodobenzene (**7**, 9.72 g, 30 mmol): yield 3.85 g (53%); mp 146–7 °C; ^{1}H NMR (DMSO-d_6) δ 3.58, 3.67, 3.75, 3.77, and 3.84 (each 3 H, s, 5 OMe), 5.82 (1 H, s, H-6′), 7.04 (1 H, t, J = 7.68, J = 7.96 Hz, H-5), 7.28 (1 H, dd, J = 1.92, J = 7.96 Hz, H-4), 7.51 (1 H, dd, J = 1.92, J = 7.68 Hz, H-6), 8.55 (1 H, br s, NH). Anal. ($C_{18}H_{21}NO_7$) C, H, N.

4-Nitro-*N*-(3,4,5-trimethoxyphenyl)anthranilic acid (34a) was prepared by the condensation of 2-chloro-4-nitrobenzoic acid (**32a**, 20.1 g, 0.2 mol) and 3,4,5-trimethoxyaniline (**33**, 18.3 g, 0.2 mol): yield 13.8 g (40%); mp 272–3 °C; ^{1}H NMR (DMSO-d_6) δ 3.69 (3 H, s, OMe), 3.78 (6 H, s, 2 OMe), 6.68 (2 H, s, H-2′ and H-6′), 7.46 (1 H, dd, J = 2.20, J = 8.78 Hz, H-5), 7.86 (1 H, d, J = 2.20 Hz, H-3), 8.09 (1 H, d, J = 8.78 Hz, H-6). Anal. ($C_{16}H_{16}N_2O_7$) C, H, N.

5-Nitro-*N*-(3,4,5-trimethoxyphenyl)anthranilic acid (34b) was synthesized by condensation of 2-chloro-5-nitrobenzoic acid (**32b**, 2.02 g, 10 mmol) and aniline (**33**, 1.83 g, 10 mmol): yield 0.528 g (15%), mp 281–2 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.69 (3 H, s, OMe), 3.79 (6 H, s, 2 OMe), 6.69 (2 H, s, H-2′ and H-6′), 7.16 (1 H, d, J = 9.33 Hz, H-3), 8.15 (1 H, dd, J = 2.47, J = 9.33 Hz, H-4), 8.69 (1 H, d, J = 2.47 Hz, H-6). Anal. ($C_{16}H_{16}N_2O_7$) C, H, N.

4-Nitro-*N*-(2,3,4,5-tetramethoxyphenyl)anthranilic acid (44a) was prepared by condensation of 2-chloro-4-nitrobenzoic acid (**32a**, 3.03 g, 15 mmol) and aniline (**43**, 2.13 g, 10 mmol): yield 1.31 g (26%); mp 230–1 °C; ^{1}H NMR ($CDCl_3$) δ 3.79, 3.84, 3.93, and 3.99 (each 3 H, s, 4 OMe), 6.68 (1 H, s, H-6′), 7.49 (1 H, dd, J = 2.20, J = 8.78 Hz, H-5), 7.79 (1 H, d, J = 2.20 Hz, H-3), 8.19 (1 H, d, J = 8.78 Hz, H-6), 9.42 (1 H, br s, NH). Anal. ($C_{17}H_{18}N_2O_8$) C, H, N.

5-Nitro-*N*-(2,3,4,5-tetramethoxyphenyl)anthranilic acid (44b) was synthesized by reaction of 2-chloro-5-nitrobenzoic acid (**32b**, 3.03 g, 15 mmol) with aniline (**43**, 2.13 g, 10 mmol): yield 1.18 g (20%), mp 217–9 °C; ^{1}H NMR ($CDCl_3$) δ 3.79, 3.84, 3.94, and 3.99 (each 3 H, s, 4 OMe), 6.63 (1 H, s, H-6′), 7.00 (1 H, d, J = 9.61 Hz, H-3), 8.17 (1 H, dd, J = 2.47, J = 9.61 Hz, H-4), 8.98 (1 H, d, J = 2.47 Hz, H-6), 9.86 (1 H, br s, NH). Anal. ($C_{17}H_{18}N_2O_8$) C, H, N.

6-Hydroxy-1,2,3,4,5-pentamethoxyacridin-9-one (10). Compound **8a** (960 mg, 2 mmol) was treated with polyphosphoric acid (5 g) in an oil bath (90 °C) for 1 h with stirring. After cooling, the mixture was triturated with ice (20 g), and extracted with $CHCl_3$ (20 mL × 3). The combined extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed over a silica gel column (3 × 30 cm) using $CHCl_3$/MeOH (50:1 v/v) as the eluent. Compound **10** was obtained, after recrystallization from EtOH: 178 mg (25%); mp 215–6 °C; ^{1}H NMR ($CDCl_3$) δ 3.94, 4.02, 4.05, 4.07, and 4.09 (each 3 H, s, 5 OMe), 7.08 (1 H, d, J = 9.0 Hz, H-7), 8.00 (1 H, d, J = 9.0 Hz, H-8), 8.82 (2 H, br, NH and OH). Anal. ($C_{18}H_{19}NO_7$) C, H, N.

In a similar manner, the following compounds were prepared.

1,2,3,4,5-Pentamethoxyacridin-9-one (11b) was prepared from **8b** (1.21 g, 3.3 mmol): yield 0.710 g (62%), mp 126–7 °C; ^{1}H NMR (DMSO-d_6) δ 3.82, 3.84, 3.99, 4.02, and 4.03 (each 3 H, s, 5 OMe), 7.16 (1 H, t, J = 7.41, J = 7.68 Hz, H-7), 7.32 (1 H, dd, J = 1.92, J = 7.68 Hz, H-6), 7.72 (1 H, dd, J = 1.92, J = 7.41 Hz, H-8), 8.81 (1 H, br s, NH). Anal. ($C_{18}H_{19}NO_6$) C, H, N.

6-Nitro-1,2,3-trimethoxyacridin-9-one (35a) was prepared from **34a** (8.71 g, 25 mmol): yield 7.41 g (90%); mp 309–11 °C; ^{1}H NMR (DMSO-d_6) δ 3.75, 3.83, and 3.95 (each 3 H, s, 3 OMe), 6.19 (1 H, s, H-4), 7.57 (1 H, dd, J = 1.98, J = 8.81 Hz, H-7), 8.21 (1 H, J = 1.98 Hz, H-5), 8.36 (1 H, d, J = 8.81 Hz, H-8). Anal. ($C_{16}H_{14}N_2O_6$) C, H, N.

7-Nitro-1,2,3-trimethoxyacridin-9-one (35b) and 1-Hydroxy-7-nitro-4-(7-nitro-1,2,3-trimethoxyacridin-9-yl)-2,3-dimethoxyacridin-9-one (36). Compound **34b** (348 mg, 1.0 mmol) was treated with polyphosphoric acid as described above. The TLC (SiO_2, $CHCl_3$/MeOH, 10:1 v/v) showed that two products were formed (R_f = 0.52 and R_f = 0.42). After workup, the reaction mixture was chromatographed over a silica gel column (2 × 40 cm) using $CHCl_3$/MeOH (50:1 v/v) as the eluent. Product (**35b**, R_f 0.52) was eluted first from the column followed by the dimer **36**. After recrystallization from EtOH, **35b** (71.0 mg, 21%) had mp 315–6 °C: ^{1}H NMR (DMSO-d_6) δ 3.77, 3.84, and 3.93 (each 3 H, s, 3 OMe), 6.68 (1 H, s, H-4), 7.39 (1 H, d, J = 9.06 Hz, H-5), 8.25 (1 H, dd, J = 2.75, J = 9.06 Hz, H-6), 8.80 (1 H, d, J = 2.75 Hz, H-8). Anal. ($C_{16}H_{14}N_2O_6$) C, H, N. Compound **36** (83.0 mg, 13%): mp 273–4 °C; ^{1}H NMR (DMSO-d_6) δ 3.54, 3.76, 3.85, 3.87, and 3.96 (each 3 H, s, 5 OMe), 6.98 (1 H, s, H-4′), 7.66 (1 H, d, J = 9.16 Hz, H-5), 7.88 (1 H, d, J = 9.30 Hz, H-5′), 8.35 (1 H, dd, J = 2.71, J = 9.30 Hz, H-6), 8.48 (1 H, dd, J = 2.54, J = 9.16 Hz, H-6′), 8.93 (1 H, d, J = 2.71 Hz, H-8), 8.97 (1 H, d, J = 2.54 Hz, H-8′), 10.24 (1 H, s, NH), 12.34 (1 H, s, 1-OH). Anal. ($C_{31}H_{24}N_4O_{11}$) C, H, N.

6-Nitro-1,2,3,4-tetramethoxyacridin-9-one (45a) was prepared from **44a** (1.10 g, 2.9 mmol): yield 1.03 g (98%); mp 225–6 °C; ^{1}H NMR ($CDCl_3$) δ 3.96, 4.01, 4.08, and 4.11 (each 3 H, s, 4 OMe), 7.97 (1 H, dd, J = 2.20, J = 8.78 Hz, H-7), 8.22 (1 H, d, J = 2.20 Hz, H-5), 8.55 (1 H, d, J = 8.76 Hz, H-8). Anal. ($C_{17}H_{16}N_2O_7 \cdot H_2O$) C, H, N.

7-Nitro-1,2,3,4-tetramethoxyacridin-9-one (45b) was prepared from **44b** (207 mg, 0.55 mmol): yield 71.0 mg (36%); mp 239–41 °C; ^{1}H NMR ($CDCl_3$) δ 3.96, 4.00, 4.08, and 4.11 (each 3 H, s, 4 OMe), 7.35 (1 H, d, J = 9.05 Hz, H-5), 8.40 (1 H, dd, J = 2.75, J = 9.05 Hz, H-6), 8.68 (1 H, br s, NH), 9.28 (1 H, d, J = 2.75 Hz, H-8). Anal. ($C_{17}H_{16}N_2O_7$) C, H, N.

6-(Benzyloxy)-1,2,3,4,5-pentamethoxyacridin-9-one (11a). By modification of a procedure developed by Cain et al.,[15] compound **11a** was synthesized. To a solution of **8a** (14.9 g, 31 mmol) in dry benzene (100 mL) containing pyridine (2.5 mL) was added $SOCl_2$ (5.11 g, 43 mmol) in one portion at 0 °C. After stirring for 10 min, the mixture was evaporated to dryness in vacuo. The residue was dissolved in $CHCl_3$ (300 mL), and piperidine (8.52 g, 0.1 mol) and Et_3N (10.12 g, 0.1 mol) were successively added to the solution. The reaction mixture was stirred at room temperature for 20 min, and then evaporated in vacuo to dryness. The residue was dissolved in EtOAc (500 mL), washed successively with 1 N HCl, 10% $NaHCO_3$, and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was then treated with $POCl_3$ (15 mL) at 100 °C for 30 min, and evaporated to dryness. The residue was triturated with ice–water (100 mL) and then heated at 60 °C for 30 min. After cooling, the mixture was extracted with EtOAc (300 mL × 3). The combined extracts were filtered, washed

with H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed over a silica gel column (5 × 50 cm) using n-C_6H_{14}/$CHCl_3$/EtOAc (3:2:1 v/v/v) as the eluent. After recrystallization from n-C_6H_{14}/EtOH, compound **11a** (6.23 g, 45%) was obtained: mp 130–1 °C; ^{1}H NMR ($CDCl_3$) δ 3.94, 4.02, 4.05, 4.07, and 4.09 (each 3 H, s, 5 OMe), 7.08 (1 H, d, J = 9.0 Hz, H-7), 8.00 (1 H, d, J = 9.0 Hz, H-8), 8.82 (1 H, br s, NH). Anal. ($C_{25}H_{25}NO_7$) C, H, N.

6-(Benzyloxy)-10-methyl-1,2,3,4,5-pentamethoxyacridin-9-one (12a). A mixture of **11a** (4.97 g, 11 mmol), MeI (6.25 g, 44 mmol), and K_2CO_3 (6.07 g, 44 mmol) in acetone (100 mL) was heated under reflux for 8 h. Additional MeI (6.25 g) and K_2CO_3 (6.07 g) were added to the reaction mixture, and heating was continued for another 8 h. The mixture was filtered and the filter cake was washed well with acetone. The combined filtrate and washings were evaporated to dryness in vacuo. The residue was dissolved in $CHCl_3$ (200 mL), washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The solid residue was recrystallized from EtOH to give **12a**: 4.91 g (96%); mp 127–8 °C; ^{1}H NMR ($CDCl_3$) δ 3.70, 3.87, 3.91, 3.93, 3.98, and 4.10 (each 3 H, s, 5 OMe, 1 NMe), 5.24 (2 H, s, CH_2), 6.96 (1 H, d, J = 9.05 Hz, H-7), 7.32–7.48 (5 H, m, Ph), 8.01 (1 H, d, J = 9.05 Hz, H-8). Anal. ($C_{25}H_{27}NO_7$) C, H, N.

In a similar manner, the following compounds were synthesized.

10-Methyl-1,2,3,4,5-pentamethoxyacridin-9-one (12b) was prepared from **11b** (530 mg, 1.5 mmol): yield 511 mg (93%); mp 115–6 °C; ^{1}H NMR ($CDCl_3$) δ 3.56 (3 H, s, NMe), 3.83 (6 H, s, 2 OMe), 3.81, 3.97, and 4.00 (each 3 H, s, 3 OMe), 7.23 (1 H, t, J = 6.86, J = 7.96, H-7), 7.36 (1 H, dd, J = 2.47, J = 7.96 Hz, H-6), 7.64 (1 H, dd, J = 2.47, J = 6.86 Hz, H-8). Anal. ($C_{19}H_{21}NO_6$) C, H, N.

10-Methyl-1,2,3,4,5,6-hexamethoxyacridin-9-one (14) was prepared from **10** (361 mg, 1.0 mmol): yield 320 mg (82%); mp 111–2 °C; ^{1}H NMR ($CDCl_3$) δ 3.69 (3 H, s, NMe), 3.94 and 4.10 (each 3 H, s, 2 OMe), 3.88 and 3.99 (each 6 H, s, 4 OMe), 6.92 (1 H, d, J = 9.05 Hz, H-7), 8.05 (1 H, d, J = 9.05 Hz, H-8). Anal. ($C_{20}H_{23}NO_7$) C, H, N.

10-Methyl-6-nitro-1,2,3-trimethoxyacridin-9-one (37) was prepared from **35a** (4.35 g, 13.2 mmol): yield 3.71 g (82%); mp 235–7 °C; ^{1}H NMR (DMSO-d_6) δ 3.77, 3.83, 3.90, and 4.03 (each 3 H, 3 OMe, 1 NMe), 6.92 (1 H, s, H-4), 7.91 (1 H, dd, J = 1.92, J = 8.78 Hz, H-7), 8.36 (1 H, s, J = 8.78 Hz, H-8), 8.43 (1 H, d, J = 1.92 Hz, H-5). Anal. ($C_{17}H_{16}N_2O_6$) C, H, N.

10-Methyl-6-nitro-1,2,3,4-tetramethoxyacridin-9-one (46a) was prepared from **45a** (720 mg, 2.0 mmol): yield 643 mg (98%); mp 126–7 °C; ^{1}H NMR ($CDCl_3$) δ 3.81 (3 H, s, NMe), 3.96, 3.98, 4.01, and 4.13 (each 3 H, s, 4 OMe), 7.98 (1 H, dd, J = 1.92, J = 8.65 Hz, H-7), 8.31 (1 H, d, J = 1.92 Hz, H-6), 8.49 (1 H, d, J = 8.65 Hz, H-8). Anal. ($C_{17}H_{16}N_2O_7$) C, H, N.

10-Methyl-7-nitro-1,2,3,4-tetramethoxyacridin-9-one (46b) was prepared from **45b** (250 mg, 0.70 mmol): yield 211 mg (81%); mp 200–1 °C; ^{1}H NMR ($CDCl_3$) δ 3.83 (3 H, s, NMe), 3.97 and 3.13 (each 3 H, s, 2 OMe), 4.00 (6 H, s, 2 OMe), 7.45 (1 H, d, J = 9.33 Hz, H-5), 8.41 (1 H, dd, J = 2.75, J = 9.33 Hz, H-6), 9.17 (1 H, d, J = 9.33 Hz, H-8). Anal. ($C_{17}H_{16}N_2O_7$) C, H, N.

6-(Benzyloxy)-1-hydroxy-10-methyl-2,3,4,5-tetramethoxyacridin-9-one (15). A mixture of **12a** (4.90 g, 10.5 mmol) in MeOH (100 mL) containing concentrated HCl (15 mL) was heated at reflux for 12 h. After cooling, the orange precipitate was collected by filtration, and recrystallized from EtOH to give **15**: 4.09 g (86%); mp 131–2 °C; ^{1}H NMR (acetone-d_6) δ 3.79, 3.83, 3.87, 3.92, and 4.09 (each 3 H, s, 4 OMe, 1 NMe), 5.38 (2 H, s, CH_2), 7.25 (1 H, d, J = 9.05 Hz, H-7), 7.34–7.45 (5 H, m, Ph), 8.01 (1 H, d, J = 9.05 Hz, H-8), 14.07 (1 H, s, 1-OH). Anal. ($C_{25}H_{25}NO_7$) C, H, N.

By following the same procedure, the following compounds were prepared.

1-Hydroxy-10-methyl-2,3,4,5-tetramethoxyacridin-9-one (16) was prepared from **12b** (290 mg, 0.80 mmol): yield 200 mg (72%); mp 130–1 °C; ^{1}H NMR (DMSO-d_6) δ 3.69, 3.76, 3.83, 4.00, and 4.04 (each 3 H, s, 4 OMe, 1 NMe), 7.30 (1 H, t, J = 7.41, J = 7.96 Hz, H-7), 7.46 (1 H, dd, J = 2.19, J = 7.96 Hz, H-6), 7.78 (1 H, dd, J = 1.96, J = 7.41 Hz, H-8), 13.97 (1 H, s, 1-OH). Anal. ($C_{18}H_{19}NO_6 \cdot {}^3/_4H_2O$) C, H, N.

1-Hydroxy-10-methyl-2,3,4,5,6-pentamethoxyacridin-9-one (17) was prepared from **14** (260 mg, 0.67 mmol): yield 240 mg (96%); mp 178–9 °C; ^{1}H NMR (DMSO-d_6) δ 3.69, 3.75, 3.80, 3.81, 3.97, and 4.04 (each 3 H, s, 5 OMe, 1 NMe), 7.22 (1 H, d, J = 9.06 Hz, H-7), 7.98 (1 H, d, J = 9.06 Hz, H-8), 14.03 (1 H, s, 1-OH). Anal. ($C_{19}H_{21}NO_7$) C, H, N.

2,3-Dimethoxy-1-hydroxy-10-methyl-6-nitroacridin-9-one (38) was prepared from **37** (344 mg, 1.0 mmol): yield 281 mg (85%); mp 239–41 °C; ^{1}H NMR (DMSO-d_6) δ 3.73, 3.88, and 3.99 (each 3 H, s, 2 OMe, 1 NMe), 6.57 (1 H, s, H-4), 7.91 (1 H, dd, J = 1.92, J = 8.78 Hz, H-7), 8.31 (1 H, d, J = 8.78 Hz, H-8), 8.46 (1 H, d, J = 1.92 Hz, H-5). Anal. ($C_{16}H_{14}N_2O_6$) C, H, N.

1-Hydroxy-10-methyl-6-nitro-2,3,4-trimethoxyacridin-9-one (47a) was prepared from **46a** (542 mg, 1.45 mmol): yield 420 mg (81%); mp 147–8 °C; ^{1}H NMR (DMSO-d_6) δ 3.71 and 3.83 (each 3 H, s, NMe, OMe), 4.07 (6 H, s, 2 OMe), 8.00 (1 H, dd, J = 1.92, J = 8.78 Hz, H-7), 8.38 (1 H, d, J = 8.78 Hz, H-8), 8.48 (1 H, d, J = 1.92 Hz, H-5). Anal. ($C_{17}H_{16}N_2O_7$) C, H, N.

1-Hydroxy-10-methyl-7-nitro-2,3,4-trimethoxyacridin-9-one (47b) was prepared from **46b** (170 mg, 0.50 mmol): yield 160 mg (98%); mp 223–4 °C; ^{1}H NMR ($CDCl_3$) δ 3.79, 3.98, 4.08, and 4.17 (each 3 H, 3 OMe, 1 NMe), 7.53 (1 H, d, J = 9.40 Hz, H-5), 8.50 (1 H, dd, J = 2.78, J = 9.40 Hz, H-6), 9.24 (1 H, d, J = 2.78 Hz, H-8), 13.97 (1 H, s, 1-OH). Anal. ($C_{17}H_{16}N_2O_7$) C, H, N.

1,6-Dihydroxy-10-methyl-2,3,4,5-tetramethoxyacridin-9-one (Glyfoline, 4). Method 1. A mixture of **15** (4.0 g, 8.86 mmol) and 10% Pd/C (1.0 g) in EtOH/dioxane (3:1, 200 mL) was hydrogenated at 45 psi for 2 h. The mixture was filtered through a pad of Celite, and the filter cake was washed well with $CHCl_3$. The combined filtrate and washings were evaporated to dryness in vacuo, and the solid residue was recrystallized from MeOH to give pure **4**: 2.85 g (89%); mp 214–5 °C (lit.[6] mp 215–7 °C); ^{1}H NMR (DMSO-d_6) δ 3.69, 3.75, 3.78, 3.80, and 4.02 (each 3 H, s, 4 OMe, 1 NMe), 6.94 (1 H, d, J = 9.0 Hz, H-7), 7.84 (1 H, d, J = 9.0 Hz, H-8), 10.45 (1 H, br s, 6-OH), 14.18 (1 H, s, 1-OH). Anal. ($C_{18}H_{19}NO_7$) C, H, N.

Method 2. To a solution of **12a** (453 mg, 1 mmol) in dry CH_2Cl_2 (30 mL) was added BBr_3 (249 mg, 1 mmol) at −78 °C and stirred for 0.5 h. The reaction mixture was then allowed to warm to −20 °C within 10 min and then poured into ice–water (30 mL). The mixture was neutralized with 10% $NaHCO_3$ aqueous solution and extracted with $CHCl_3$ (20 mL × 4). The combined extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed over a silica gel column (2 × 30 cm) using $CHCl_3$/MeOH (50:1 v/v) as the eluent. The product **4** was obtained (195 mg, 54%), after recrystallization from MeOH, and was identical to the product prepared by the method 1.

In a similar manner, compound **18** was synthesized.

1,6-Dihydroxy-2,3,4,5-tetramethoxyacridin-9-one (18) was prepared from **10** (46.6 mg, 0.20 mmol): yield 44.0 mg (38%); mp 235–6 °C; ^{1}H NMR (DMSO-d_6) δ 3.81, 3.92, 3.96, and 4.03 (each 3 H, s, 4 OMe), 6.91 (1 H, d, J = 9.05 Hz, H-7), 7.85 (1 H, d, J = 9.05 Hz, H-8), 8.94 (1 H, br s, NH), 10.73 (1 H, br, 6-OH), 13.88 (1 H, s, 1-OH). Anal. ($C_{17}H_{17}NO_7$) C, H, N.

6-(Benzyloxy)-10-[2-(diethylamino)ethyl]-1,2,3,4,5-pentamethoxyacridin-9-one (19). To a suspension of NaH (60% oil suspension, 207 mg, 5.1 mmol) and **11a** (451 mg, 1.0 mmol) in dry DMF (5 mL) was added 2-(diethylamino)ethyl chloride hydrochloride (430 mg, 2.5 mmol). The reaction mixture was heated at 110 °C for 22 h and the solvent was removed in vacuo by distillation. The residue was triturated with $CHCl_3$ (50 mL), washed with H_2O, dried (Na_2SO_4), and evaporated to dryness in vacuo. The residue was chromatographed on a silica gel column (3 × 60 cm) using $CHCl_3$ as the eluent, which eluted unreacted **11a**. The column was then washed with $CHCl_3$/MeOH (100:1 v/v) to elute **19**, which was obtained as yellow crystals, 172 mg (32%) with mp 133–4 °C, after concentration of the solvent and recrystallization of the residue from hexane/EtOH: ^{1}H NMR ($CDCl_3$) δ 0.51 (6 H, t, J = 7.14 Hz, 2 CH_2CH_3), 2.04 (4 H, q, J = 7.14 Hz, 2 CH_2CH_3), 2.16 (2 H, t, J = 6.03 Hz, CH_2), 4.48 (2 H, t, J = 6.03 Hz, CH_2), 5.24 (2 H, s, $PhCH_2$), 6.95 (1 H, J = 9.05 Hz, H-7), 7.31–7.49 (5 H, m, Ph), 7.95 (1 H, d, J = 9.05 Hz, H-8). Anal. ($C_{31}H_{38}N_2O_7$) C, H, N.

10-[2-(Diethylamino)ethyl]-1,6-dihydroxy-2,3,4,5-tetramethoxyacridin-9-one (20). A mixture of **19** (150 mg, 0.27 mmol) in EtOH (10 mL) containing concentrated HCl (3 mL) was heated at reflux for 20 h. The solvent was removed in vacuo, the residue was dissolved in $CHCl_3$ (30 mL), washed with NH_4OH, dried

(Na_2SO_4), and evaporated in vacuo to dryness. The residue was dissolved in EtOH (15 mL) and then treated with 10% Pd/C (30 mg)/H_2 at 45 psi for 2 h. After removal of catalyst, the filtrate was evaporated in vacuo to dryness. The product (**20**), 58.0 mg (48%) with mp 118–9 °C, was obtained after chromatography (silica gel column, $CHCl_3$/MeOH, 100:1) and recrystallization from hexane/EtOH: ^{1}H NMR (DMSO-d_6) δ 0.36 (6 H, t, J = 7.14 Hz, 2 CH_2CH_3), 1.93 (4 H, q, J = 7.14, 2 CH_2CH_3), 2.14 (2 H, q, J = 6.03 Hz, CH_2), 3.76 and 4.00 (each 3 H, s, 2 OMe), 3.79 (6 H, s, 2 OMe), 4.57 (2 H, t, J = 6.03 Hz, CH_2), 6.93 (1 H, d, J = 8.78 Hz, H-7), 7.83 (1 H, d, J = 8.78 Hz, H-8), 10.41 (1 H, br, 6-OH), 14.05 (1 H, s, 1-OH). Anal. ($C_{23}H_{30}N_2O_7$) C, H, N.

6-(Benzyloxy)-2,3-dimethoxy-10-methylacridine-1,4,9-trione (24) and 6-(Benzyloxy)-3,4-dimethoxy-10-methylacridine-1,2,9-trione (26). Compound **12a** (465 mg, 1.0 mmol) was added to 17% HNO_3 (20 mL) over a period of 20 min at 0 °C with vigorous stirring. The mixture was then allowed to stir at room temperature for additional 1 h (the color of the reaction mixture turned from orange into red). The reaction was monitored by TLC (SiO_2, $CHCl_3$/MeOH, 10:1), which indicated that two products were formed. EtOAc (20 mL) was added directly to the reaction mixture. The aqueous layer was separated and extracted with EtOAc (20 mL × 3). The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated in vacuo to dryness. The residue was chromatographed over a silica gel column (2 × 40 cm) using $CHCl_3$ as the eluent. Compound **24** (R_f = 0.53) was eluted ($CHCl_3$/MeOH, 250:1) first from the column followed by **26** (R_f = 0.43). Compound **24** was obtained as red crystals (130 mg, 30%): mp 185–6 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 3.86 (6 H, s, 2 Me), 3.90 and 3.98 (each 3 H, s, 2 Me), 5.30 (2 H, s, CH_2), 7.41 (1 H, 8.78 Hz, H-7), 7.38–7.65 (5 H, m, Ph), 7.85 (1 H, d, J = 8.78 Hz, H-8). Anal. ($C_{24}H_{21}NO_7$) C, H, N. After recrystallization from EtOH, compound **26** was obtained as dark red crystals (93.0 mg, 25%): mp 207–8 °C; ^{1}H NMR (DMSO-d_6) δ 3.76 (3 H, s, Me), 3.89 (6 H, s, 2 Me), 4.10 (3 H, s, Me), 5.29 (2 H, s, CH_2), 7.37 (1 H, d, J = 9.05 Hz, H-7), 7.43–7.7.48 (5 H, m, Ph), 7.80 (1 H, d, J = 9.05, H-8). Anal. ($C_{24}H_{21}NO_7$) C, H, N.

3-(Benzyloxy)-5-methyl-4,6,7-trimethoxyquino[3,2-*a*]phenazin-14(5*H*)-one (29). 1,2-Phenylenediamine (**27**, 8.0 mg, 0.074 mmol) was added into a solution of **26** (27 mg, 0.062 mmol) in EtOH/dioxane (5:1, 8 mL) at room temperature. After stirring for 10 min, the solvent was removed in vacuo, and the dark-red residue was recrystallized from EtOH to yield **29** (17.0 mg, 54%): mp 240–2 °C; ^{1}H NMR (DMSO-d_6) δ 3.85, 3.94, 4.08, and 4.32 (each 3 H, s, 3 OMe, 1 NMe), 5.33 (2 H, s, CH_2), 7.32–7.56 (5 H, m, Ph), 7.45 (1 H, d, J = 8.78 Hz, H-2), 7.88–8.00 (2 H, m, Ar-H), 7.95 (1 H, d, J = 8.78 Hz, H-1), 8.19–8.31 (2 H, m, Ar-H). Anal. ($C_{20}H_{25}N_3O_5$) C, H, N.

10-Methyl-1,4,6-trihydroxy-2,3,5-trimethoxyacridin-9-one (30). A mixture of **24** (130 mg, 0.3 mmol) and 10% Pd/C (50 mg) in EtOH/dioxane (5:1, 30 mL) was hydrogenated at 40 psi for 1.5 h. After removal of the catalyst, the filtrate was evaporated in vacuo to dryness. The residue was recrystallized from EtOH to give **30**: 53.0 mg (51%); mp 205–6 °C; ^{1}H NMR (DMSO-d_6) δ 3.71, 3.79, 3.83, and 3.93 (each 3 H, s, 3 OMe, 1 NMe), 6.91 (1 H, d, J = 9.33 Hz, H-7), 7.82 (1 H, d, J = 9.33 Hz, H-8), 8.87 (1 H, br, OH), 10.50 (1 H, br, OH), 13.84 (1 H, br s, 1-OH). Anal. ($C_{17}H_{17}NO_7$) C, H, N.

6-Hydroxy-10-methyl-2,3,5-trimethoxyacridine-1,4,9-trione (31). To a solution of **30** (35 mg, 0.1 mmol) in MeOH (6 mL) was added *m*-chloroperbenzoic acid (20 mg) and the mixture stirred for 20 min at room temperature. The solvent was removed in vacuo and the solid residue was recrystallized from MeOH to yield **31**: 22.0 mg (60%); mp 240–1 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.80, 3.83, 3.90, and 3.98 (each 3 H, s, 3 OMe, 1 NMe), 7.70 (1 H, d, J = 9.05 Hz, H-7), 7.73 (1 H, d, J = 9.05 Hz, H-8). Anal. ($C_{17}H_{16}NO_7$) C, H, N.

6-Amino-1-hydroxy-10-methyl-2,3,4-trimethoxyacridin-9-one (48). A mixture of **47a** (216 mg, 0.60 mmol) and 10% Pd/C (50 mg) in EtOH/dioxane (3:1, 50 mL) was hydrogenated at 40 psi for 1 h. The mixture was filtered through a pad of Celite, and the filter cake was washed successively with EtOH and $CHCl_3$. The combined filtrate and washings were evaporated in vacuo to dryness. The residue was chromatographed on a silica gel (2 × 15 cm) using $CHCl_3$ as the eluent. The product **48** (153 mg, 77%) was obtained after recrystallization from EtOH: mp 169–70 °C; ^{1}H NMR (DMSO-d_6) δ 3.66, 3.79, 3.84, and 3.99 (each 3 H, s, 3 OMe, 1 NMe), 6.45 (2 H, br s, NH_2), 6.54 (1 H, d, J = 1.65 Hz, H-5), 6.70 (1 H, dd, J = 1.65, J = 8.50 Hz, H-7), 7.90 (1 H, d, J = 8.50 Hz, H-8), 15.27 (1 H, s, 1-OH). Anal. ($C_{17}H_{18}N_2O_5 \cdot H_2O$) C, H, N.

Compound **51** was prepared in a similar manner.

7-Amino-1-hydroxy-10-methyl-2,3,4-trimethoxyacridin-9-one (51) was prepared from **47b** (50.0 mg, 0.14 mmol): yield 33 mg (92%); mp 168–70 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 3.73, 3.80, 3.95, and 4.02 (each 3 H, s, 3 OMe, 1 NMe), 3.80 (2 H, br, NH_2), 7.17 (1 H, dd, J = 2.87, J = 9.05 Hz, H-6), 7.38 (1 H, d, J = 2.87 Hz, H-5), 7.62 (1 H, d, J = 9.05 Hz, H-8), 14.00 (1 H, s, 1-OH). Anal. ($C_{17}H_{18}N_2O_5 \cdot {}^1/_2H_2O$) C, H, N.

1-Hydroxy-10-methyl-6-(methylamino)-2,3,4-trimethoxyacridin-9-one (49) and 6-(Dimethylamino)-1-hydroxy-10-methyl-2,3,4-trimethoxyacridin-9-one (50). A mixture of **48** (66 mg, 0.2 mmol), MeI (0.2 mL), and K_2CO_3 (0.5 mg) in acetone (5 mL) was heated at reflux for 3 h. An additional MeI (0.4 mL) was added to the reaction mixture and continuously heated overnight. TLC (SiO_2, toluene/EtOAc 2:1) showed that two products were formed. The solvent was removed in vacuo and the residue was dissolved in EtOAc (40 mL), washed with H_2O, dried (Na_2SO_4), and evaporated in vacuo to dryness. The residue was chromatographed over a silica gel column (2 × 30 cm) using toluene/EtOAc (3:1 v/v) as the eluent. Compound **50** (R_f = 0.36) was eluted first from the column followed by **49** (R_f = 0.26). After recrystallization from ether, compound **49** (13.0 mg, 20%) was obtained: mp 141–2 °C; ^{1}H NMR ($CDCl_3$) δ 3.19 (3 H, s, NMe), 3.93 (3 H, s, NMe), 4.16 (6 H, s, 2 OMe), 4.33 (3 H, s, OMe), 6.48 (1 H, d, J = 2.20 Hz, H-5), 6.73 (1 H, dd, J = 2.20, J = 8.78 Hz, H-7), 8.33 (1 H, s, J = 8.78 Hz, H-8), 14.98 (1 H, s, 1-OH). Anal. ($C_{18}H_{20}N_2O_5 \cdot {}^3/_2H_2O$) C, H, N.

Compound **50** (13.0 mg, 20%): mp 173–4 °C (Et_2O); ^{1}H NMR ($CDCl_3$) δ 3.15 (6 H, s, 2 NMe), 3.72 (3 H, s, NMe), 3.95 (6 H, s, 2 OMe), 4.12 (3 H, s, OMe), 6.29 (1 H, d, J = 2.20 Hz, H-5), 6.70 (1 H, dd, J = 2.20, J = 9.05 Hz, H-7), 8.18 (1 H, d, J = 9.05, H-8), 14.96 (1 H, s, 1-OH). Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

Biological Studies on 9-Acridone Derivatives. HL-60 cells (1.5 × 10^5/mL) were incubated in RPMI 1640 media (GIBCO, Grand Island, NY) at 37 °C in humidified 5% CO_2 for 72 h. Viable cells were determined using trypan blue exclusion and counted in a hemocytometer. The fractional inhibition for each compound concentration (0.0025–0.05 mg/mL in 0.1% dimethyl sulfoxide, DMSO) was analyzed with the median-effect plot using a computer program.[16] The median-effect concentration (IC_{50}) was determined automatically for three to five dose–effect levels. Cell growth in the absence of acridones and in the presence of DMSO was used as a control. DMSO alone inhibited cell growth 3.9 ± 1.5% during the 72-h incubation period.

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