Synthesis and Preliminary Cytotoxic Activity of Dimethoxyacridines and Dimethoxynitroacridines

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The preparation of a series of dimethoxy and dimethoxynitroacridines and their activity in oxic and hypoxic cells is reported. Anthranilic acids **1**, **4**, **14** were prepared according to the Ullmann condensation. 9-chloroacridines were obtained from anthranilic acids by refluxing in phosphorus oxychloride. The synthesis of two new acridine dimers **9**, **10** is described. Nitration of 9-chloro-2,4-dimethoxyacridine **15** gave 3-nitro isomer **19**. By phenol-mediated coupling reaction from all the 9-chloroacridines, the respective 9-(alkylamino)acridines were obtained. By nitration of **17** a new 2,4-dimethoxy-3,7-dinitroacridine **21** was prepared

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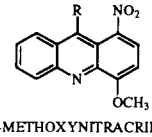
Nitracrine is a nitroacridine DNA-intercalating ligand that has been used clinically as an anticancer drug [1,2]. Nitracrine has potent hypoxia-selective cytotoxicity against tumor cells in culture [3]. This property is of interest, given that hypoxic cells are resistant to ionizing radiation and to most chemotherapeutic agents [4,5]. This hypoxia-selective cytotoxicity justified a renewed evaluation of the therapeutical potential of the acridines.

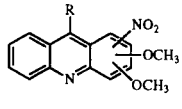
It is known that substituted nitracrines (*e.g.* 4-OMe derivatives) retain the hypoxia selectivity of nitracrine and it may be that these analogues avoid the problem of a rapid loss of bioactivity due to metabolism [6,7,8]. Preparation of new compounds with different substitutions in the aryl ring should be interesting in this activity. We now present some new acridines bearing two electrodonating substituents (methoxy groups), with and without nitro substitution, and preliminary biological results.

NITRACRINE



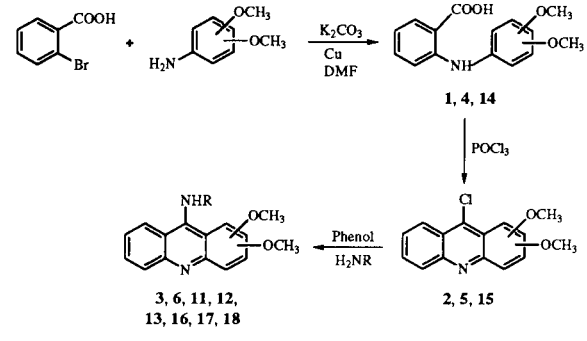
4-METHOXYNITRACRINE

R= -NH(CH$_2$)$_3$N(CH$_3$)$_2$



Dimethoxyphenylanthranilic acids **1**, **4**, **14** were prepared according to the classical Ullmann condensation [7,9] shown in Scheme 1. Thus, 2-bromobenzoic acid and dimethoxyanilines were heated under reflux in the presence of potassium carbonate and copper-bronze as catalyst in DMF. Cyclization of anthranilic acids in boiling phosphorus oxychloride afforded the corresponding 9-chloroacridines **2**, **5**, **15** in variable yields.

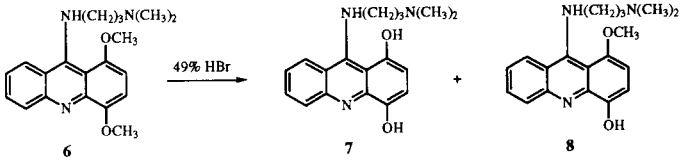
Scheme 1



Compound		R
3	2,3-dimethoxy	$(CH_2)_3N(CH_3)_2$
6	1,4-dimethoxy	$(CH_2)_3N(CH_3)_2$
11	1,4-dimethoxy	$(CH_2)_2N(CH_3)_3$
12	1,4-dimethoxy	CH_2CH_2OH
13	1,4-dimethoxy	$(CH_2)_3$-Imidazolyl
16	2,4-dimethoxy	$(CH_2)_3N(CH_3)_2$
17	2,4-dimethoxy	$(CH_2)_2N(CH_3)_2$
18	2,4-dimethoxy	CH_2CH_2OH

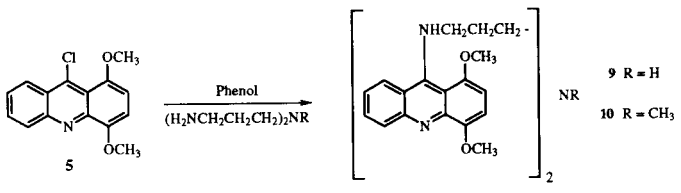
The 9-chloro compounds were obtained as hydrochloride salts. Neutralization in aqueous ammonium hydroxide gave the corresponding free bases. Compounds **3**, **6**, **11**, **12**, **13**, **16**, **17**, **18** were prepared by phenol-mediated coupling of the appropriate amines with 9-chloroacridines **2**, **5**, **15**.

Scheme 2



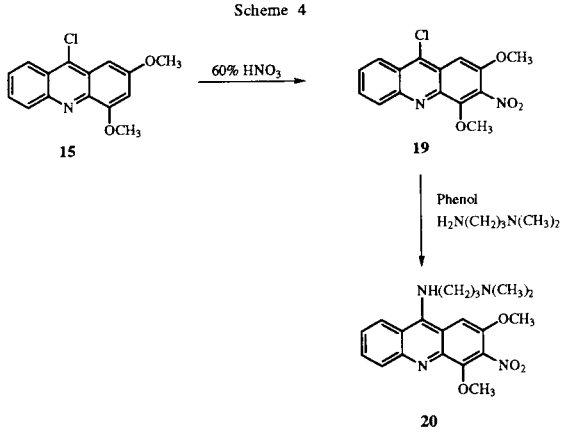
Hydrolysis of the 1,4-dimethoxyacridine **6** in 49% hydrobromic acid afforded 1,4-dihydroxyacridine **7** and 4-hydroxy-1-methoxyacridine **8**.

Scheme 3



9-Chloro-1,4-dimethoxyacridine **5** reacted by phenol-mediated coupling with *N,N*-di-(3-aminopropyl)amines, giving the dimers **9**, **10**.

Scheme 4



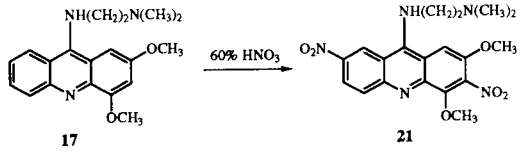
Nitration of 9-chloroacridine **15** in 60% nitric acid at room temperature gave 3-nitro derivative **19**. Strict temperature and time control of the nitration were necessary. Temperatures higher than 25-30° or times longer than 60-70 minutes resulted in appreciable amounts of impure material (Scheme 4).

Substitution of 9-chloroacridine **19** was carried out at 100° in the presence of excess phenol. Replacement of the initially formed phenoxy intermediate, which can not be isolated, at 65-70° gave **20**.

Attempts at nitration of **17** in 60% nitric acid gave the 3,7-dinitro compound **21**, shown in Scheme 5.

Compound **3**, a 2,3-dimethoxy derivative caused a significant toxicity both in oxic and hypoxic cells at a dose of 10 μ*M*. At lower doses, it lost its activity. Compound **6**, a 1,4-dimethoxy acridine was slightly selective at 10 μ*M*. Compounds **11**, **12** and **13** were inactive at 10 μ*M*. Derivatives **16** and **17** were active at 10 and 5 μ*M*, but no selectivity was found in these compounds. The acridine **18**, bearing a 2-hydroxyethylamino lateral chain was less potent than its dimethylaminoalkylamino analogues **16** and **17**.

Scheme 5



EXPERIMENTAL

Melting points were determined using a Mettler FP82+FP80 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3-4 mm Hg, 24 hours at about 60-80°). Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR apparatus, using potassium bromide tablets for solid products and sodium chloride plates for liquid products; the frequencies are expressed in cm^{-1}. The ^1H-nmr spectra were obtained on a Brucker AC-200E (200 MHz) instrument, with tetramethylsilane as the internal reference, at a concentration of about 0.1 g/ml and with dimethyl sulfoxide-d_6 and deuterium oxide as the solvents; the chemical shifts are reported in ppm of tetramethylsilane in δ units. The mass spectra were recorded on a Hewlett-Packard 5988-A instrument at 70 eV.

Thin layer chromatography (tlc) was carried out on silica gel (HF, 254-266, Merck or DSF-s, Cammaga) and the plates were scanned under ultraviolet light at 254 and 366 nm. Column chromatography was carried out with silica gel 60 Merck (70-230 mesh ASTM).

Nitracrine (Positive control) was prepared by phenol-mediated coupling of 3-(*N,N*-dimethylamino)propylamine with 9-chloro-1-nitroacridine, which in turn was conveniently prepared in small amounts by flash chromatography of the mixture of 1- and 3- nitroisomers formed by phosphorus oxychloride cyclization of *N*-(3-nitrophenyl)anthranilic acid [10]. For larger quantities, a more convenient method was fractional crystallization of the 9-(*N*-pyridinium)chlorides [11,12], followed by conversion to 9-chloro derivative.

N-(3,4-Dimethoxyphenyl)anthranilic Acid (**1**).

A mixture of 2-bromobenzoic acid (25.00 g, 124.38 mmoles), 3,4-dimethoxyaniline (22.00 g, 143.79 mmoles), potassium carbonate (10.00 g), copper-bronze (2.00 g) and *N,N*-DMF (400 ml) was stirred and heated under reflux for 15 hours. The resulting suspension was poured over ice-cold excess hydrochloric acid. The precipitate was filtered off and washed with hot water, methanol and diethyl ether. The solid was dried at 100° (phosphorus pentoxide), giving **1** (21.32 g, 63%), mp 165°; ^1H nmr (dimethyl sulfoxide-d_6): δ 3.75 (s, 6 H, 2 OCH_3), 6.85-7.10 (m, 5 H, H_2, H_5, H_2, H_5, H_6), 7.33 (m, 1 H, H_4), 7.86 (m, 1 H, H_6), 9.45 (bs, 1 H, NH), 12.70 (bs, 1 H, COOH); ms: (EI, 70 eV) m/z 273 (M^+, 94%), 240 (97%), 212 (100%), 169 (55%), 141 (24%).

Anal. Calcd. for $C_{15}H_{14}NO_4$: C, 65.93; H, 5.13; N, 5.49. Found: C, 65.45; H, 4.98; N, 5.53.

9-Chloro-2,3-dimethoxyacridine (**2**).

Phosphorus oxychloride (70 ml) was added over dry *N*-(3,4-dimethoxyphenyl)anthranilic acid **1** (5.01 g, 18.35 mmoles) and refluxed for 7 hours. After removal of the excess phosphorous oxychloride, a dark residue was obtained and later dissolved in dichloromethane (60 ml). The suspension was poured over ice-cold water/ammonium hydroxide. Separation and evaporation of the organic layer gave a reddish solid which was chromatographed (chloroform/ethly acetate). 9-chloro-1,2-dimethoxyacridine, Rf (ethyl acetate): 0.7, was eluted by using chloroform/ethly acetate (50/10) (0.11 g, 2%) and **2**, Rf (ethyl acetate): 0.6, was eluted with chloroform/ethly acetate (50/50) (0.40 g, 8%), mp 90°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 4.04 (s, 3 H, OCH_3), 4.06 (s, 3 H, OCH_3), 7.50 (s, 1 H, H_1), 7.52 (s, 1 H, H_4), 7.71 (t, 1 H, H_7, $J_{6,7,8}$ = 7.8 Hz), 7.85 (t, 1 H, H_6, $J_{5,6,7}$ = 7.7 Hz), 8.13 (d, 1 H, H_8, $J_{7,8}$ = 8.3 Hz), 8.35 (d, 1 H, H_5, $J_{5,6}$ = 8.3 Hz); ms: (EI, 70 eV) m/z 273 (M^+, 100%), 230 (33%), 200 (6%), 167 (17%), 152 (22%).

Anal. Calcd. for $C_{15}H_{12}ClNO_2$: C, 65.81; H, 5.12; N, 4.39. Found: C, 65.67; H, 5.06; N, 4.51.

9-[3-(*N,N*-Dimethylamino)propylamino]-2,3-dimethoxyacridine (**3**).

A mixture of 9-chloro-2,3-dimethoxyacridine **2** (0.92 g, 3.36 mmoles) and phenol (3 g, 31.90 mmoles) was heated at 100°. After cooling to below 50°, 3-(*N,N*-dimethylamino)propylamine (0.35 g, 3.43 mmoles) was added. Then the solution was heated at 70° for 1 hour, cooled and diluted with dichloromethane (150 ml). The organic layer was washed with 2*N* sodium hydroxide (700 ml) and water (150 ml), dried over magnesium sulfate, and filtrated. After removal of the solvent, a reddish oil was obtained and redissolved in methanol (50 ml). The methanolic solution was filtered through charcoal-celite, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (25 ml), methanol (1 ml) and precipitated by the addition of concentrated hydrochloric acid (4 drops) (0.25 g, 15%), mp 165°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.12 (m, 2 H, CH_2), 2.44 (s, 6 H, 2 CH_3), 2.76 (m, 2 H, NCH_2), 4.01 (s, 6 H, 2 OCH_3), 4.12 (m, 2 H, NCH_2), 7.41 (s, 1 H, H_1), 7.49 (t, 1 H, H_7, $J_{6,7,8}$ = 7.2 Hz), 7.86 (s, 1 H, H_4), 7.90-7.97 (m, 2 H, H_5 H_6), 8.45 (d, 1 H, H_8, $J_{7,8}$ = 8.6 Hz), 9.25 (bs, 1 H, NH); ms: (EI, 70 eV) m/z 339 (M^+, 42%), 281 (24%), 239 (40%), 58 (100%).

Anal. Calcd. for $C_{20}H_{25}N_3O_2$•3HCl•3H_2O: C, 47.76; H, 6.77; N, 8.36. Found: C, 48.09; H, 6.77; N, 8.35.

N-(2,5-Dimethoxyphenyl)anthranilic Acid (**4**).

A mixture of 2-bromobenzoic acid (25 g, 124.38 mmoles), 2,5-dimethoxyaniline (22 g, 143.79 mmoles), potassium carbonate (10.00 g), copper-bronze (2.00 g) and *N,N*-DMF (400 ml) was stirred and heated under reflux for 15 hours. The resulting suspension was poured over ice-cold excess hydrochloric acid. The precipitate was filtered off and washed with hot water, methanol and diethyl ether. The solid was dried at 100° (phosphorus pentoxide) giving **4** (18.05 g, 53%), mp 155-157°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.68 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 6.57 (d, 1 H, $H_{4'}$, $J_{3',4'}$ = 8.5 Hz), 6.77 (t, 1 H, H_5, $J_{4,5,6}$ = 7.3 Hz), 6.95 (s, 1 H, $H_{6'}$), 6.97 (d, 1 H, $H_{3'}$, $J_{3',4'}$ = 8.9 Hz), 7.27 (d, 1 H, H_3, $J_{3,4}$ = 8.3 Hz), 7.40 (t, 1 H, H_4, $J_{3,4,5}$ = 7.2 Hz), 7.88 (d, 1 H, H_6, $J_{5,6}$ = 7.7 Hz), 9.61 (s, 1 H, NH), 13.01 (bs, 1H, COOH); ms: (EI, 70 eV) m/z 273 (M^+, 95%), 240 (100%), 212 (98%), 197 (32%), 169 (57%).

Anal. Calcd. for $C_{15}H_{15}NO_4$: C, 65.93; H, 5.49; N, 5.13. Found: C, 65.49; H, 5.59; N, 5.49.

9-Chloro-1,4-dimethoxyacridine (**5**).

Phosphorus oxychloride (40 ml) was added over dry *N*-(2,5-dimethoxyphenyl)anthranilic acid **4** (5.00 g, 18.31 mmoles) and refluxed for 10 hours. After removal of the excess phosphorus oxychloride, a dark residue was obtained and dissolved in dichloromethane (60 ml). The suspension was poured over ice-cold water/ammonium hydroxide. Separation and evaporation of the organic layer gave a yellow solid which was chromatographed (dichloromethane/ethyl acetate) to give **5** (2.27 g, 45%), mp 107°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 4.00 (s, 3 H, OCH_3), 4.11 (s, 3 H, OCH_3), 6.81 (d, 1 H, H_2, $J_{2,3}$ = 8.4 Hz), 6.95 (d, 1 H, H_3, $J_{2,3}$ = 8.4 Hz), 7.64 (t, 1 H, H_7, $J_{6,7,8}$ = 7.9 Hz), 7.80 (t, 1 H, H_6, $J_{5,6,7}$ = 8.0 Hz), 8.34 (d, 1 H, H_8, $J_{7,8}$ = 8.6 Hz), 8.59 (d, 1 H, H_5, $J_{5,6}$ = 8.6 Hz); ms: (EI, 70 eV) m/z 273 (M^+, 100%), 258 (9%), 230 (35%), 152 (23%), 81 (12%).

Anal. Calcd. for $C_{15}H_{12}ClNO_2$: C, 65.81; H, 4.39; N, 5.12. Found: C, 65.73; H, 4.56; N, 5.00.

9-[3-(*N,N*-Dimethylamino)propylamino]-1,4-dimethoxyacridine (**6**).

A mixture of 9-chloro-1,4-dimethoxyacridine **5** (1.50 g, 5.48 mmoles) and phenol (4.50 g, 47.87 mmoles) was heated at 100° for 6.5 hours. After cooling to below 50°, 3(*N,N*-dimethylamino)propylamine (0.57 g, 5.59 mmoles) was added. The solution was then heated at 68-70° for 7 hours, cooled and diluted with dichloromethane (200 ml). The organic layer was washed with 2*N* sodium hydroxide (1 l) and water (200 ml), dried over sodium sulfate and filtrated. After removal of the solvent, a reddish oil was obtained and redissolved in methanol (250 ml). The methanolic solution was filtered through charcoal-celite, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (150 ml) and precipitated by the addition of concentrated hydrochloric acid (4 drops) (0.33 g, 13%), mp 165° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.26 (m, 2 H, CH_2), 2.33 (m, 1 H, CH_2N), 2.78 (s, 6 H, $N(CH_3)_2$), 3.17 (m, 2 H, NCH_2), 4.10 (s, 3 H, OCH_3), 4.14 (s, 3 H, OCH_3), 7.05 (d, 1 H, H_2, $J_{2,3}$ = 8.8 Hz), 7.53 (m, 2 H, H_3 H_7), 7.98 (t, 1 H, H_6 $J_{5,6,7}$ = 8.0 Hz), 8.28 (d, 1 H, H_8, $J_{7,8}$ = 8.2 Hz), 8.48 (m, 1 H, H_5), 10.33 (s, 1 H, NH), 11.25 (bs, 1 H, HCl), 12.56 (bs, 1 H, HCl); ms: (EI, 70 eV) m/z 339 (M^+, 45%), 267 (52%), 239 (61%), 58 (100%).

Anal. Calcd. for $C_{20}H_{25}N_3O_2$•2HCl•1.5H_2O: C, 54.67; H, 6.83; N, 9.57. Found: C, 54.50; H, 6.99; N, 9.28.

9-[3-(*N,N*-Dimethylamino)propylamino]-1,4-dihydroxyacridine (**7**).

A mixture of **6** (free base) (0.20 g, 6.00 mmoles) and 49% hydrobromic acid (1.5 ml) was refluxed (126°) for 4 hours. The melt was cooled and diluted with acetone (6 ml). The mixture was refluxed for 1 hour. The precipitated solid was filtered off and washed with hot acetone and diethyl ether (86 mg, 30%), mp 300°; ^{1}H nmr (deuterium oxide): δ 2.38 (m, 2 H, CH_2), 3.12 (s, 6 H, 2 CH_3), 3.44 (m, 2 H, NCH_2), 4.07 (t, 2 H, CH_2N, J = 4.1 Hz), 6.58 (d, 1 H, H_2, $J_{2,3}$ = 7.0 Hz), 7.06 (d, 1 H, H_3, $J_{2,3}$ = 7.2 Hz), 7.44 (t, 1 H, H_7, $J_{6,7,8}$ = 5.8 Hz), 7.62 (d, 1 H, H_5, $J_{5,6}$ = 6.0 Hz), 7.88 (t, 1 H, H_6, $J_{5,6,7}$ = 5.8 Hz), 8.11 (d, 1 H, H_8, $J_{7,8}$ = 6.8 Hz); ms: (EI, 70 eV) m/z 312 (M^++1, 100%), 296 (38%), 241 (20%), 212 (35%), 103 (32%), 58 (29%).

Anal. Calcd. for $C_{18}H_{21}N_3O_2$•2 HBr•H_2O: C, 43.99; H, 5.09; N, 8.55. Found: C, 43.80; H, 5.10; N, 8.32.

9-[3-(*N,N*-Dimethylamino)propylamino]-4-hydroxy-1-methoxyacridine (**8**).

9-[3-(*N,N*-Dimethylamino)propylamino]-1,4-dimethoxyacridine **6** (0.40 g, 1.18 mmoles) and 49% hydrobromic acid (1.5 ml) were refluxed (126°) for 1.5 hours. A saturated aqueous solution

of sodium carbonate was added and the organic compounds were extracted with dichloromethane. After removal of the solvent, a yellow oil was obtained and chromatographed (ethyl acetate/methanol 1/1) which gave yellow needles, 0.15 g (39%), mp >300°; ^{1}H nmr (deuterium oxide): δ 2.38 (m, 2 H, CH_2), 3.12 (s, 6 H, 2 CH_3), 3.40 (m, 2 H, NCH_2), 4.10 (m, 2 H, CH_2N), 4.12 (s, 3 H, CH_3), 6.76 (d, 1 H, H_2, $J_{2,3}$ = 7.1 Hz), 7.17 (d, 1 H, H_3, $J_{2,3}$ = 7.2 Hz), 7.52 (t, 1 H, H_7, $J_{6,7,8}$ = 5.9 Hz), 7.71 (d, 1 H, H_5, $J_{5,6}$ = 6.4 Hz), 7.95 (t, 1 H, H_6, $J_{5,6,7}$ = 5.8 Hz), 8.17 (d, 1 H, H_8, $J_{7,8}$ = 6.9 Hz); ms: (EI, 70 eV) m/z 326 (M^++1, 100%), 310 (15%), 296 (11%), 241 (18%), 103 (25%), 58 (41%).

Anal. Calcd. for $C_{19}H_{23}N_3O_2$•2.5HCl•0.7H_2O: C, 53.16; H, 6.27; N, 9.79. Found: C, 53.11; H, 6.07; N, 9.63.

N,N-Di-[3-(9-(1,4-dimethoxyacridinyl)amino)propyl]amine (**9**).

9-Chloro-1,4-dimethoxyacridine **5** (1.56 g, 5.70 mmoles), triethylamine (0.60 g, 5.90 mmoles), di-*N,N*-(3-aminopropyl)amine (0.40 g, 2.90 mmoles) and ethanol (25 ml) were heated under reflux for 8 hours. After removal of the solvent, the resulting orange oil was chromatographed (dichloromethane/methanol 9/1), giving the acridine-dimer. The compound was dissolved in methanol (8 ml) at room temperature and two drops of concentrated hydrochloric acid were added. Ethyl acetate was added until turbidity, affording **9** as yellow crystals, 1.22 g (54%), mp 224-226°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.37 (m, 4 H, 2 CH_2), 3.04 (m, 4 H, 2 CH_2N), 4.10 (s, 6 H, 2 OCH_3), 4.12 (s, 6 H, 2 OCH_3), 4.24 (m, 4 H, 2 NCH_2), 6.99 (d, 2 H, 2 H_2, $J_{2,3}$ = 8.1 Hz) 7.46 (d, 2 H, 2 H_3, $J_{2,3}$ = 7.8 Hz), 7.50 (t, 2 H, 2 H_7, $J_{6,7,8}$ = 7.9 Hz), 7.92 (t, 2 H, 2 H_6, $J_{6,5}$ = 7.4 Hz), 8.24 (d, 2 H, 2 H_5, $J_{5,6}$ = 7.8 Hz), 8.44 (d, 2 H, 2 H_8, $J_{7,8}$ = 7.6 Hz), 10.06 (bs, 2 H, 2 NH), 10.36 (s, 2 H, 2 NH), 12.48 (s, 2 H, 2 HCl); ms: (FAB, 70 eV) m/z 606 (M^++1, 100 %).

Anal. Calcd. for $C_{36}H_{39}N_5O_4$•3HCl•3.5H_2O: C, 55.56; H, 6.30; N, 9.00. Found: C, 55.23; H, 6.16; N, 9.06.

N,N-Di-[3-(9-(1,4-dimethoxyacridinyl)amino)propyl]-*N*-methylamine (**10**).

A mixture of **5** (2.00 g, 7.31 mmoles), triethylamine (0.74 g, 7.33 mmoles), *N,N*-di-(3-aminopropyl)-*N*-methylamine and ethanol (25 ml) was heated under reflux for 6 hours. Reaction was carried on by tlc. After removal of the solvent, the resulting orange oil was chromatographed (dichloromethane/methanol 9/1). The dimer was dissolved in methanol (10 ml) and two drops of concentrated hydrochloric acid were added. Ethyl acetate (10 ml) was added until turbidity and the mixture was heated in a steam-bath giving **10** as yellow crystals (1.20 g, 39%), mp 255° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.38 (m, 4 H, 2 CH_2), 2.78 (d, 3 H, NCH_3), 3.19 (m, 4 H, 2 CH_2N), 4.08 (s, 6 H, 2 OCH_3), 4.11 (s, 6 H, 2 OCH_3), 4.18 (m, 4 H, 2 NCH_2), 7.00 (d, 2 H, 2 H_2, $J_{2,3}$ = 8.3 Hz), 7.44 (d, 2 H 2 H_3, $J_{2,3}$ = 8.0 Hz), 7.48 (t, 2 H, 2 H_7, $J_{6,7,8}$ = 7.7 Hz), 7.94 (t, 2 H, 2 H_6, $J_{6,5}$ = 7.4 Hz), 8.25 (d, 2 H, 2 H_5, $J_{5,6}$ = 7.6 Hz), 8.45 (d, 2 H, 2 H_8, $J_{7,8}$ = 7.6 Hz), 10.29 (bs, 2 H, 2 NH), 11.60 (bs, 1 H, NH), 12.50 (s, 2 H, 2 HCl); ms: (FAB, 70 eV) m/z 620 (M^++1, 100%).

Anal. Calcd. for $C_{37}H_{41}N_5O_4$•3.5HCl•5H_2O: C, 53.06; H, 6.51; N, 8.36. Found: C, 53.25; H, 6.61; N, 8.32.

9-[2-(*N,N*-Dimethylamino)ethylamino]-1,4-dimethoxyacridine (**11**).

A mixture of 9-chloro-1,4-dimethoxyacridine **5** (1.50 g, 5.48 mmoles) and phenol (4.50 g, 47.87 mmoles) was heated at 100° for 5 hours. After cooling to below 50°, 2-(*N,N*-dimethylamino)ethylamine (0.49 g, 5.57 mmoles) was added. The solution was then heated at 65-67° for 6 hours, cooled and diluted with dichloromethane (200 ml). The organic layer was washed with 2*N* sodium hydroxide (1 l) and water (200 ml), dried over sodium sulfate, and filtrated. After removal of the solvent, a reddish oil was obtained and redissolved in methanol (250 ml). The methanolic solution was filtered through charcoal-celite, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (150 ml) and precipitated by the addition of concentrated hydrochloric acid (5 drops), (0.72 g, 30%), mp 152-153°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.78 (s, 3 H, NCH_3), 2.80 (s, 3 H, NCH_3), 3.50 (m, 2 H, CH_2N), 4.06 (s, 3 H, OCH_3), 4.09 (s, 3 H, OCH_3), 4.43 (m, 2 H, NCH_2), 6.98 (d, 1 H, H_2, $J_{2,3}$ = 8.7 Hz), 7.49 (m, 2 H, H_3 H_7), 7.96 (t, 1 H, H_6, $J_{5,6,7}$ = 8.2 Hz), 8.27 (d, 1 H, H_5, $J_{5,6}$ = 8.4 Hz), 8.42 (m, 1 H, H_8), 10.33 (s, 1 H, NH), 11.57 (bs, 1 H, HCl), 12.85 (bs, 1 H, HCl); ms: (EI, 70 eV) m/z 325 (M^+, 22%), 267 (28%), 58 (100%).

Anal. Calcd. for $C_{19}H_{23}N_3O_2$•2HCl•2.5H_2O: C, 51.47; H, 6.77; N, 9.48. Found: C, 51.89; H, 6.65; N, 9.02.

9-[2-Hydroxyethylamino]-1,4-dimethoxyacridine (**12**).

A mixture of 9-chloro-1,4-dimethoxyacridine **5** (2.00 g, 7.31 mmoles) and phenol (6.00 g, 63.83 mmoles) was heated at 100° for 5 hours. After cooling to below 50°, 2-aminoethanol (0.45 g, 7.38 mmoles) was added. The solution was then heated at 70° for 5 hours, cooled, and diluted with dichloromethane (300 ml). The organic layer was washed with 2*N* sodium hydroxide (1 l) and water (250 ml), dried over sodium sulfate and filtrated. After removal of the solvent, a yellow oil was obtained and redissolved in methanol (200 ml). The methanolic solution was filtered through charcoal-celite, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (150 ml) and precipitated by the addition of concentrated hydrochloric acid (4 drops) (253 mg, 10%), mp 155°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.61 (m, 2 H, CH_2OH), 3.77 (m, 2 H, NCH_2), 3.87 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 5.01 (bs, 1 H, OH), 6.64 (d, 1 H, H_2, $J_{2,3}$ = 8.5 Hz), 6.90 (t, 1 H, H_7, $J_{6,7,8}$ = 8.3 Hz), 7.22 (t, 1 H, H_6, $J_{5,6,7}$ = 7.8 Hz), 7.60 (d, 1 H, H_3, $J_{2,3}$ = 7.2 Hz), 7.81 (d, 1 H, H_8, $J_{7,8}$ = 8.6 Hz), 8.16 (d, 1 H, H_5, $J_{5,6}$ = 8.3 Hz), 8.80 (bs, 1 H, NH); ms: (EI, 70 eV) m/z 298 (M^+, 15%), 283 (16%), 267 (22%), 239 (100%).

Anal. Calcd. for $C_{17}H_{18}N_2O_3$•0.5H_2O: C, 66.45; H, 6.19; N, 9.12. Found: C, 66.05; H, 5.86; N, 8.90.

9-[3-(Imidazolylpropyl)amino]-1,4-dimethoxyacridine (**13**).

A mixture of 9-chloro-1,4-dimethoxyacridine **5** (0.76 g, 2.78 mmoles) and phenol (4.00 g, 42.55 mmoles) was heated at 100° for 1.5 hours. After cooling to below 50°, 1-(3-aminopropyl)imidazol (0.35 g, 2.80 mmoles) was added. The solution was then heated at 70° for 1.5 hours, cooled and diluted with dichloromethane (200 ml). The organic layer was washed with 2*N* sodium hydroxide (800 ml) and water (150 ml), dried over sodium sulfate and filtrated. After removal of the solvent, a yellow solid was obtained and redissolved in methanol (200 ml). The methanolic solution was filtered through charcoal-celite, and the solvent was removed under reduced pressure to give a yellow solid **13** (0.80 g, 80%), mp 155°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.14 (t, 2 H, CH_2, J = 6.6), 3.23 (m, 2 H, CH_2), 3.72 (m, 2 H, CH_2), 3.90 (s, 3 H, OCH_3), 4.01 (s, 3 H, OCH_3), 6.71 (d, 1 H, H_2, $J_{2,3}$ = 8.2 Hz), 6.84 (m, 1 H, H_3), 6.95 (d, 1 H, H imidazole), 7.08 (m, 1 H, H imidazole), 7.26 (t, 1 H, H_7, $J_{6,7,8}$ = 7.6 Hz), 7.50 (s, 1 H, $H_{2'}$), 7.63 (t, 1 H, H_6, $J_{5,6,7}$ = 7.1 Hz), 7.86 (d, 1 H, H_8, $J_{7,8}$ = 8.6 Hz), 8.08 (d, 1 H, H_5,

$J_{5,6}$ = 8.4 Hz), 9.53 (s, 1 H, NH); ms: (EI, 70 eV) m/z 362 (M^+, 17%), 267 (45%), 253 (100%), 237 (18%).

Anal. Calcd. for $C_{21}H_{22}N_4O_2 \cdot H_2O$: C, 66.32; H, 6.32; N, 14.74. Found: C, 66.41; H, 6.28; N, 14.45.

N-(2,4-Dimethoxyphenyl)anthranilic Acid (**14**).

A mixture of 2-bromobenzoic acid (24.97 g, 124.00 mmoles), 2,4-dimethoxyaniline (38.00 g, 248.00 mmoles), potassium carbonate (26.66 g), copper-bronze (0.50 g) and *N,N*-DMF (167 ml) was stirred and heated under reflux for 12 hours. The resulting suspension was poured over ice-cold excess hydrochloric acid (200.00 g). The precipitate was filtered off and washed with hot water, methanol and diethyl ether. The solid was dried at 100° (phosphorous pentoxide), giving **14** (25.56 g, 75%), mp 158-162°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.34 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 6.55 (d, 1 H, $H_{5'}$, $J_{5',6'}$ = 8.4 Hz), 6.68 (m, 2 H, $H_{3'}$, H_4), 6.81 (d, 1 H, $H_{6'}$, $J_{5',6'}$ = 8.3 Hz), 7.22 (d, 1 H, H_3, $J_{3,4}$ = 8.8 Hz), 7.31 (t, 1 H, H_5, $J_{4,5,6}$ = 7.6 Hz), 7.84 (d, 1 H, H_6, $J_{5,6}$ = 7.6 Hz), 9.24 (s, 1 H, NH), 12.85 (bs, 1 H, COOH); ms: (EI, 70 eV) m/z 273 (M^+, 100%), 258 (5%), 256 (5%).

Anal. Calcd. for $C_{15}H_{15}NO_4$: C, 65.93; H, 5.50; N, 5.13. Found: C, 65.66; H, 5.50; N, 5.50.

9-Chloro-2,4-dimethoxyacridine Hydrochloride (**15**).

Phosphorus oxychloride (20 ml) was added over dry *N*-(2,4-dimethoxyphenyl)anthranilic acid **14** (4.12 g, 15.00 mmoles) and refluxed for 6 hours. The mixture was allowed to cool overnight and the orange crystals which appeared were filtered off and washed with dry acetone, affording **15** (3.66 g, 56%), mp 170-172°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.97 (s, 3 H, OCH_3), 4.03 (s, 3 H, OCH_3), 6.88 (s, 1 H, H_3), 7.04 (s, 1 H, H_1), 7.78 (m, 2 H, H_6 H_7), 8.16 (d, 1 H, H_5), 8.20 (d, 1 H, H_8); ms: (EI, 70 eV) m/z 273 (M^+, 97%), 244 (100%), 242 (70%).

Anal. Calcd. for $C_{15}H_{12}ClNO_2 \cdot 3HCl \cdot 3H_2O$: C, 41.19; H, 4.80; N, 3.20. Found: C, 41.06; H, 4.90; N, 3.08.

9-[3-(*N,N*-Dimethylamino)propylamino]-2,4-dimethoxyacridine (**16**).

A mixture of 9-chloro-2,4-dimethoxyacridine **15** (1.00 g, 2.29 mmoles) and phenol (2.70 g, 28.72 mmoles) was heated at 100° for 30 minutes. After cooling to below 50°, 3-(*N,N*-dimethylamino)propylamine (0.40 g, 3.92 mmoles) was added. The solution was then heated at 65° for 6.5 hours, cooled and diluted with dichloromethane (75 ml). The organic layer was washed with 2*N* sodium hydroxide (80 ml) and water (150 ml), dried over sodium sulfate, and filtrated. After removal of the solvent, a reddish oil was obtained and redissolved in methanol (30 ml). The methanolic solution was filtered through charcoal-celite, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (15 ml) and methanol (1 ml) and precipitated by the addition of concentrated hydrochloric acid (4 drops) (0.60 g, 58%), mp 229-231°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.39 (t, 2 H, CH_2), 2.75 (s, 6 H, $N(CH_3)_2$), 3.18 (bs, 2 H, CH_2N), 4.01 (s, 3 H, OCH_3), 4.12 (s, 3 H, OCH_3), 4.21 (bs, 2 H, NCH_2), 7.21 (s, 1 H, H_3), 7.51 (t, 1 H, H_7, $J_{6,7,8}$ = 7.6 Hz), 7.76 (s, 1 H, H_1), 7.93 (t, 1 H, H_6, $J_{5,6,7}$ = 7.6 Hz), 8.28 (d, 1 H, H_5, $J_{5,6}$ = 8.6 Hz), 8.52 (d, 1 H, H_8, $J_{7,8}$ = 8.7 Hz), 10.01 (s, 1 H, NH), 10.87 (s, 1 H, HCl), 12.91 (s, 1 H, HCl); ms: (EI, 70 eV) m/z 339 (M^+, 52%), 281 (40%), 267 (31%), 253 (65%).

Anal. Calcd. for $C_{20}H_{25}N_3O_2 \cdot 2HCl \cdot 2H_2O$: C, 53.57; H, 6.92; N, 9.37. Found: C, 53.60; H, 7.02; N, 9.01.

9-[2-(*N,N*-Dimethylamino)ethylamino]-2,4-dimethoxyacridine (**17**).

A mixture of 9-chloro-2,4-dimethoxyacridine **15** (4.00 g, 9.15 mmoles) and phenol (10 g, 106.2 mmoles) was heated at 100° for 1 hour. After cooling to below 50°, 2-(*N,N*-dimethylamino)ethylamine (1.15 g, 11.25 mmoles) was added. The solution was then heated at 65° for 6 hours, cooled and diluted with dichloromethane (75 ml). The organic layer was washed with 2*N* sodium hydroxide (80 ml) and water (150 ml), dried over sodium sulfate, and filtrated. After removal of the solvent, a reddish oil was obtained and redissolved in methanol (30 ml). The methanolic solution was filtered through charcoal-celite, and the solvent was removed under reduced pressure to give **17** (1.67 g, 56%), mp 135-138°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.20 (s, 6 H, $N(CH_3)_2$) 2.57 (bs, 2 H, CH_2), 3.74 (t, 2 H, CH_2, J = 6.0), 3.93 (s, 3 H, OCH_3), 3.97 (s, 3 H, OCH_3), 6.33 (s, 1 H, NH), 6.77 (s, 1 H, H_3), 7.11 (s, 1 H, H_1), 7.41 (t, 1 H, H_6, $J_{5,6,7}$ = 7.5 Hz), 7.62 (t, 1 H, H_7, $J_{6,7,8}$ = 7.5 Hz), 7.96 (d, 1 H, H_5, $J_{5,6}$ = 8.6 Hz), 8.27 (d, 1 H, H_8, $J_{7,8}$ = 8.6 Hz); ms: (EI, 70 eV) m/z 325 (M^+, 32%), 253 (5%), 58 (100%).

Anal. Calcd. for $C_{19}H_{23}N_3O_2$: C, 70.15; H, 7.07; N, 12.92. Found: C, 70.60; H, 7.05; N, 12.60.

9-(2-Hydroxyethyl)amino-2,4-dimethoxyacridine (**18**).

A mixture of 9-chloro-2,4-dimethoxyacridine **15** (0.50 g, 1.06 mmoles) and excess phenol was heated at 100° for 1 hour. After cooling to below 50°, 2-aminoethanol (0.15 g, 2.45 mmoles) was added and the mixture was heated at 75° for 6 hours. Dichloromethane (20 ml) was added and the organic layer was washed with 2*N* sodium hydroxide (4 x 100 ml) and water (2 x 100 ml). After removal of the solvent, the residue was dissolved in methanol and filtered through charcoal-celite. The solvent was eliminated *in vacuo* and the resulting oil was chromatographed (chloroform/methanol 1/1), giving an orange solid (0.11 g, 24%), mp 246°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.34 (bs, 2 H, NCH_2), 3.87 (d, 2 H, CH_2), 3.97 (s, 3 H, OCH_3), 4.10 (s, 3 H, OCH_3), 5.21 (s, 1 H, OH), 7.16 (s, 1 H, H_3), 7.49 (t, 1 H, H_6), 7.58 (s, 1 H, H_1), 7.87 (t, 1 H, H_7), 8.20 (d, 1 H, H_5, $J_{5,6}$ = 8.5 Hz), 8.58 (d, 1 H, H_8, $J_{7,8}$ = 8.5 Hz); ms: (EI, 70 eV) m/z 298 (M^+, 80%), 267 (100%).

Anal. Calcd. for $C_{17}H_{18}N_2O_3 \cdot 2HCl \cdot 2H_2O$: C, 50.12; H, 5.90; N, 6.88. Found: C, 49.75; H, 5.44; N, 6.77.

9-Chloro-2,4-dimethoxy-3-nitroacridine (**19**).

A mixture of 9-chloro-2,4-dimethoxyacridine **15** (free base, 1.78 g, 6.51 mmoles) and 60% nitric acid (20 ml) was set aside at room temperature for 1 hour. The solution was then added over a saturated aqueous solution of sodium carbonate (200 ml) to *p*H = 7.. Two different compounds precipitated; one of them, yellow, was extracted with dichloromethane and the other one, red, was not soluble in water or in dichloromethane. The organic layer was dried over sodium sulfate and after removal of the solvent, **19** was obtained (0.61 g, 33%), mp 251-255°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 3.97 (s, 3 H, OCH_3), 4.15 (s, 3 H, OCH_3), 7.26 (t, 1 H, H_6), 7.34 (s, 1 H, H_1), 7.70 (t, 1 H, H_7, $J_{6,7,8}$ = 7.6 Hz), 8.10 (d, 1 H, H_5, $J_{5,6}$ = 8.0 Hz), 8.20 (d, 1 H, H_8, $J_{7,8}$ = 8.6 Hz); ms: (EI, 70 eV) m/z 318 (M^+, 47%), 283 (93%), 252 (100%).

Anal. Calcd. for $C_{15}H_{11}ClN_2O_4$: C, 56.51; H, 3.45; N, 8.79. Found: C, 56.78; H, 3.48; N, 8.53.

9-[3-(*N,N*-Dimethylamino)propylamino]-2,4-dimethoxy-3-nitroacridine (**20**).

9-Chloro-2,4-dimethoxy-3-nitroacridine **19** (1.18 g, 4.30

mmoles) and phenol (5.51 g, 58.50 mmoles) were stirred and heated at 100° for 30 minutes. The dark solution was cooled to 50° and *N,N*-dimethylaminopropylamine (0.45 g, 4.40 mmoles) was added. The solution was heated at 68° for 4 hours. Chloroform was added (100 ml) and the organic layer was washed with 2*N* sodium hydroxide (4 x 100 ml) and water. After removal of the solvent, a dark compound was obtained and dissolved in methanol (20 ml). The solution was passed through charcoal-celite and the solvent was removed. A yellow solid was obtained and recrystallized from methanol (0.06 g, 4%), mp 162-164°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 1.63 (bt, 2 H, CH_2), 2.10 (s, 6 H, $N(CH_3)_2$), 2.29 (t, 2 H, CH_2, J = 6.9 Hz), 3.33 (s, 1 H, NH), 3.88 (t, 2 H, CH_2), 4.06 (s, 3 H, OCH_3), 4.21 (s, 3 H, OCH_3), 7.01 (t, 1 H, H_6, $J_{5,6,7}$ = 7.9 Hz), 7.05 (s, 1 H, H_1), 7.40 (t, 1 H, H_7, $J_{6,7,8}$ = 7.3 Hz), 7.61 (d, 1 H, H_5, $J_{5,6}$ = 8.4 Hz), 7.75 (d, 1 H, H_8, $J_{7,8}$ = 8.4 Hz), 9.88 (s, 1 H, NH); ms: (EI, 70 eV) m/z 384 (M^+, 8%), 298 (3%), 58 (100%).

Anal. Calcd. for $C_{20}H_{24}N_4O_4 \cdot 1.2CH_3OH$: C, 60.22; H, 6.81; N, 13.25. Found: C, 59.90; H, 6.40; N, 12.84.

9-[3-(*N,N*-Dimethylamino)ethylamino]-2,4-dimethoxy-3,7-dinitroacridine (**21**).

9-[3-(*N,N*-Dimethylamino)ethylamino]-2,4-dimethoxyacridine **17** (0.50 g, 1.54 mmoles) and 60% nitric acid (15 ml) were allowed to stand at room temperature for 65 minutes. The mixture was neutralized with a saturated aqueous solution of sodium carbonate. Extraction with dichloromethane (25 x 3 ml) gave, after removal of the solvent, an oil, which was chromatographed (chloroform/ethyl acetate 1/1), giving **21** as an orange solid (0.08 g, 11%), mp 208-209°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 2.73 (s, 6 H, $N(CH_3)_2$), 3.34 (s, 4 H, 2 CH_2), 3.90 (s, 3 H, OCH_3), 4.10 (s, 3 H; OCH_3), 7.17 (s, 1 H, H_1), 7.82 (d, 1 H, H_5, $J_{5,6}$ = 9.3 Hz), 8.32 (d, 1 H, H_6, $J_{5,6}$ = 8.8 Hz), 8.60 (s, 1 H, H_8), 11.00 (s, 1 H, NH); ms: (EI, 70 eV) m/z 415 (M+, 3%), 58 (100%).

Anal. Calcd. for $C_{19}H_{21}N_5O_6 \cdot 3.5H_2O$: C, 47.70; H, 5.85; N, 14.64. Found: C, 47.88; H, 5.47; N, 14.28.

Biological Methods.

In vitro selective cytotoxicity in hypoxia was evaluated by a clonogenic assay after treating V79 suspension cultures, gassed with air or nitrogen, for 2h.

Cells.

V79 cells (Chinese hamster lung fibroblasts) [10] were obtained from ECACC (European Collection of Animal Cell Cultures), and maintained in logarithmic-phase growth as subconfluent monolayers by trypsinization and subculture to 1-$2 \cdot 10^4$ cells/cm^2 twice weekly. The growth medium was EMEM containing 10% v/v foetal bovine serum (FBS) and Penicillin/Streptomycin 100 U/100 μg/ml.

Aerobic and Hypoxic Cytotoxicity.

Suspension Cultures.

Monolayers of V79 cells in exponential growth were trypsinized and suspension cultures were set up in 50 ml erlenmeyers: $2 \cdot 10^4$ cells/ml in 30 ml of EMEM containing 10% v/v FBS and HEPES 10 m*M*. The erlenmeyers were tightly closed with rubber caps which were perforated with two needles of 19G•40 mm to provide gas inlet and outlet. Erlenmeyers were submerged and stirred in a water bath at 37°, where they were gassed with humidified air or nitrogen.

Treatment.

Drug solutions were prepared just before the assay was carried out. Stock solutions, 150-fold more concentrated, were prepared in pure dimethylsulfoxide (DMSO). Thirty minutes after starting to gas the suspension cultures, 0.2 ml of the stock solution was added to the 30 ml of total medium. In every assay there was an erlenmeyer with 0.2 ml of DMSO (Negative control) and another with Nitracrine 0.06 μ*M* (Positive control). For screening, treatment lasted two hours during which gassing was continous.

Cloning.

After treatment, cells were centrifuged and resuspended in plating medium (EMEM suplemented with 15% v/v FBS and Penicillin/Streptomycin 100 U/100 μg/ml. The cell density was determined with a Hemocytometer and 10^2-10^5 cells were plated in 30 mm 6-well plates to give a final volume of 2 ml/well. Plates were incubated at 37° in 5% carbon dioxide for 7 days and were stained with aqueous crystal violet. Colonies with more than 64 cells were counted. The plating efficiency (PE) was calculated by dividing the number of clones by the number of cells seeded. The surviving fraction (SF) is the percentage of PE of treated cultures with respect to the control.

Screening Assays.

Compounds were tested at 10 μ*M* in duplicate flasks in both aerobic and hypoxic conditions. Compounds active at 10 μ*M* were tested at 5 and 1 μ*M*.

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