Synthesis and Activity of Novel Nitropyrazines for Use as Hypoxic Cell Radiosensitizers

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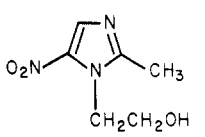
A series of eight novel nitropyrazines has been prepared by oxidation of sulfoximine intermediates. The partition coefficient, one-electron reduction potential, sensitizer enhancement ratio, and chronic and acute aerobic cytotoxicity have been measured for each. Two representatives of this series were tested in the Ames test and were not found to be mutagenic.

The response of mammalian cells to the killing effect of X-rays is critically related to the intracellular concentration of the molecular oxygen.[1-3] As compared to normally oxygenated cells, a threefold increase in radiation dose is required to effect the same amount of cell killing in hypoxic cells ($<10^{-6}$ mol dcm^{-3} of O_2). Evidence indicates that in malignant tissue, hypoxic cells are killed less efficiently by radiation than are aerated cells, and that upon receiving vascular support, these hypoxic cells repopulate the tumor.[4] Hypoxic cell populations, which apparently arise in solid tumors as malignant cell division outpaces microvascular development, are therefore believed to be a major cause of local recurrences following radiation therapy.[5,6]

One approach to overcoming the problem posed by hypoxic cells has been to utilize electron-affinic reagents that mimic oxygen by differentially sensitizing hypoxic cells to the lethal effect of X-rays.[7-9] The suggestion[10] that the property of hypoxic cell radiosensitization may be related to the electron affinity of the sensitizer has led to the identification and development of a structurally diverse group of active compounds. Among these are aliphatic diketones, conjugated ketones, quinones, pyruvic acid derivatives, and nitro aromatics.[11]

The early identification of *p*-nitroacetophenone as a radiosensitizer[12,13] has led to the synthesis of an impressive number of nitro aromatics as potential radiosensitizers, for example, nitrobenzenes,[13,14] nitrofurans,[15] nitroimidazoles,[16,17] and nitropyrroles.[18] So far the most studied compounds have been metronidazole (1) and misonidazole



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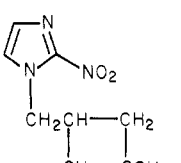

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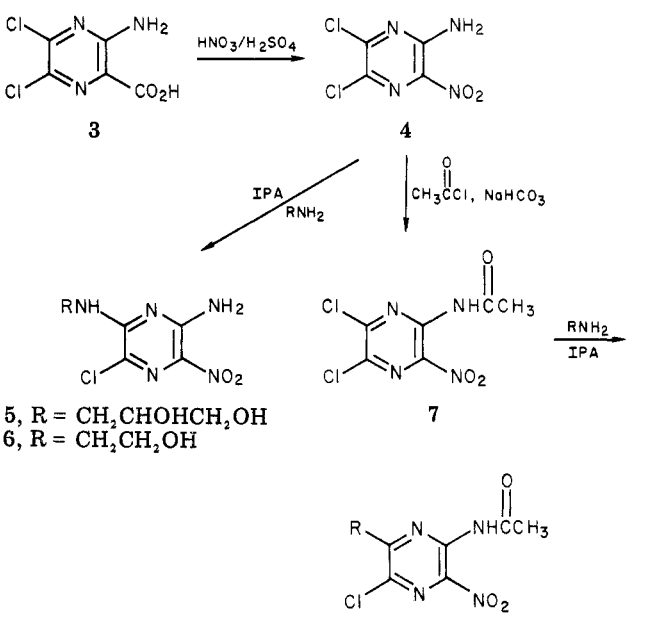
(2), both of which have progressed to phase III clinical trials. However, the dose of misonidazole (2) that can be administered is severely limited because of the appearance of neurotoxicity in man,[19] thus diminishing its usefulness. In addition, both metronidazole (1) and misonidazole (2) have been found to be mutagenic[20] in the Ames test, and metronidazole (1) was carcinogenic in animals.[21] It is noteworthy that numerous other five-membered ring nitroheterocycles that display radiosensitizing activity have also been found to be mutagenic in bacterial[20] and mammalian cell[22] assays.

In an effort to prepare new hypoxic cell radiosensitizers, we have synthesized novel nitropyrazine compounds (Table I). Since it is recognized that one-electron reduction potential is correlated with radiation sensitizating ability[8] and that the partition coefficient is useful in structure–activity

Scheme I



5, R = $CH_2CHOHCH_2OH$
6, R = CH_2CH_2OH

8, R = c-N(CH_2CH_2)$_2$N-CH_3
9, R = NHCH(CH_2OH)$CHOHCH_2OH$
10, R = $NHCH_2CHOHCH_2OH$

† Merck Sharp & Dohme Research Laboratories.
‡ Gray Laboratory.

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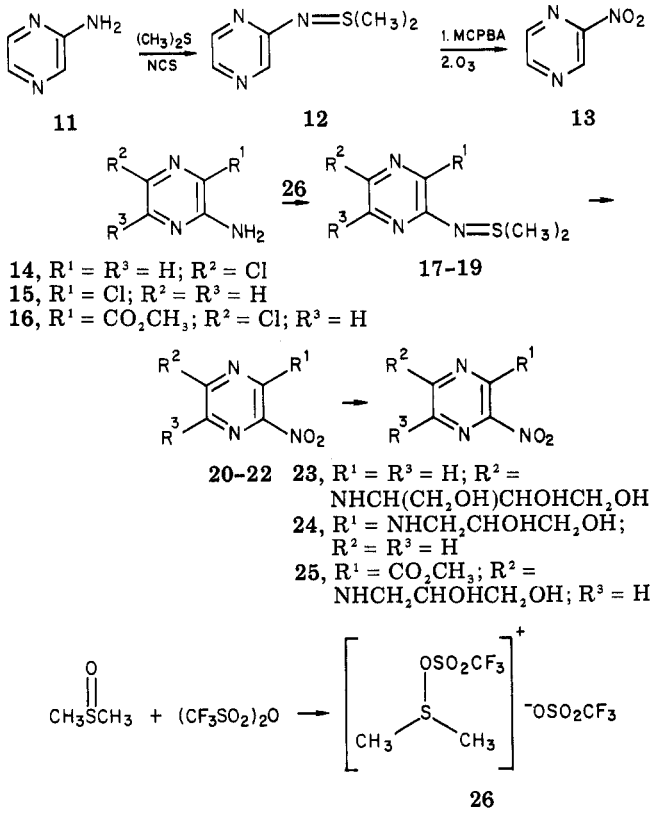
Scheme II



correlations,[23] these physical measurements were made. The radiation sensitizing potential of each of these compounds has been measured as described below, and the mutagenic potential of two of these nitropyrazines was determined in the Ames test.

Chemistry. The synthesis of one series of nitropyrazines used in this study is outlined in Scheme I. Treatment of 5,6-dichloro-3-aminopyrazine-2-carboxylic acid (**3**)[24] with sulfuric acid/nitric acid resulted in a smooth decarboxylation reaction at 0–10 °C with the formation of the desired 5,6-dichloro-3-nitropyrazinamine (**4**). As expected,[25,26] nucleophilic displacement of chloride by amines was facile even at room temperature, resulting in the formation of **5** and **6**. Treatment of **4** with acetyl chloride at reflux in the presence of excess sodium bicarbonate afforded the acetoxy derivative **7** in good yield. Again, displacement of chloride by amines occurred at room temperature to give compounds **8–10**.

The preparation of nitropyrazines **23–25** was somewhat more complicated and is outlined in Scheme II. Recently, Taylor et al. reported the conversion of 2-aminopyrazine (**11**) to 2-nitropyrazine (**13**) via the sulfilimine **12**, generated from **11** with dimethyl sulfide/*N*-chlorosuccinimide complex. We found that although the transformation of **11** to **13** was readily accomplished, the application of this methodology to pyrazines containing an electron-withdrawing functionality was problematic, with little or no reaction of starting material apparent. Other synthetic procedures for formation of sulfilimines were likewise unsuccessful.[28-30] However, we have found that treatment of amino heterocycles **14–16** with dimethyl sulfide ditriflate (**26**),[31] prepared from dimethyl sulfoxide and trifluoromethanesulfonic anhydride at −78 °C, effected clean conversion to the corresponding sulfilimines **17–19**.[40] Oxidation in the normal manner[27] afforded the nitro heterocycles **20–22**, which upon treatment with the required amine gave the desired adducts **23–25**.

Results and Discussion

Radiosensitization Determinations. Asynchronous, logarithmic phase Chinese hamster lung fibroblast-like cells (V79 379A), maintained in suspension culture in Eagle's minimal essential medium modified for spinner culture (Flow Laboratories Ltd.) and supplemented with 7.5% fetal calf serum (fcs, Flow Laboratories, Ltd., or Gibco Europe Ltd.), were used. All sensitizer concentrations were confirmed spectrophotometrically.

Cytotoxicity Measurements. Three measures of cytotoxicity were used. (1) The *acute cytotoxicity* was determined as the upper limit for the concentration of sensitizer that could be used in radiation studies.[8,32] Single cell suspensions in Eagle's MEM + 10% fcs were allowed to attach to glass Petri dishes at 37 °C for 2–3 h. The medium was then removed and replaced with a range of sensitizer concentrations in medium. After 2 h at room temperature in N_2 plus 5% CO_2, the sensitizer was replaced with fresh medium, and the dishes were incubated at 37 °C in air plus 5% CO_2 for 7 days before scoring for colony formation. The concentration of sensitizer required to reduce colony-forming ability to 75% was estimated as a measure of acute toxicity. (2) In order to measure the *chronic aerobic cytotoxicity*, cells were incubated in Petri dishes in the presence of a range of sensitizer concentrations in air plus 5% CO_2 at 37 °C for 7 days before scoring for colony formation. The concentration required to reduce the number of colonies to 50% was estimated as a measure of the chronic aerobic cytotoxicity.[32] (3) The *differential toxicity to hypoxic cells* with respect to oxic cells was measured for one compound by suspending cells in 75 mL of 2 mmol dm^{-3} of sensitizer in Eagle's MEM plus 7.5% fcs at 2×10^5 cells mL^{-1}. In order to obtain hypoxia, N_2 plus 5% CO_2 was passed over the stirred cell suspension; for aerobic studies, air plus 5% CO_2 was used. Samples were taken at regular time intervals, centrifuged, resuspended, counted, diluted, plated, incubated for 7 days at 37 °C, and scored for colony formation.[33]

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(32) (a) Adams, G. E.; Clarke, E. D.; Gray, P.; Jacobs, R. S.; Stratford, I. J.; Wardman, P.; Watts, M. E. *Int. J. Radiat. Biol.* **1979**, *35*, 151. (b) Adams, G. E.; Clarke, E. D.; Jacobs, R. S.; Stratford, I. J.; Wallace, R. G.; Wardman, P.; Watts, M. E. *Biochem. Biophys. Res. Commun.* **1976**, *72*, 824.

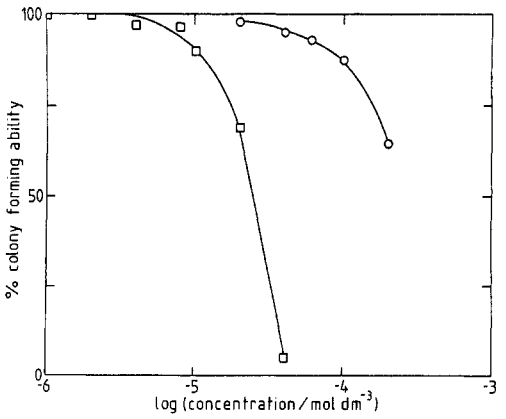

Figure 1. Acute and chronic aerobic cytotoxicity of 8 toward V79 cells as a function of concentration: (○) acute cytotoxicity; (□) chronic aerobic cytotoxicity.

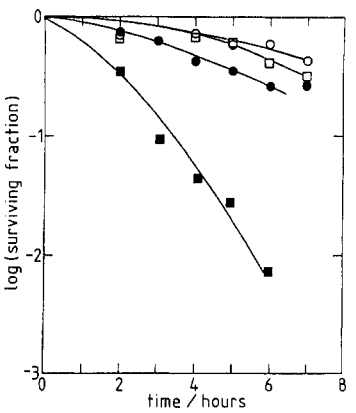

Figure 2. Survival data for V79 cells incubated in **10** at 37 °C in hypoxia or air: (○) air control; (□) nitrogen control; (●) air + 2 mmol dm^{-3} of **10**; (■) nitrogen + 2 mmol dm^{-3} of **10**.

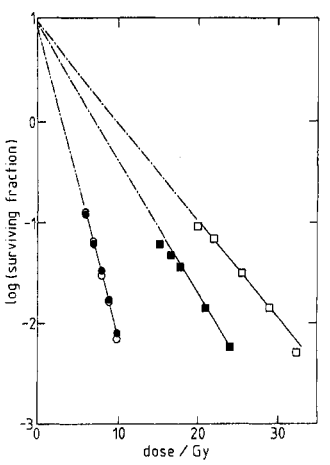

Figure 3. Survival data for X-irradiated V79 cells: (□) nitrogen control; (■) nitrogen + 5 mmol dm^{-3} of **23**; (○) air control; (●) air + 5 mmol dm^{-3} of **23**.

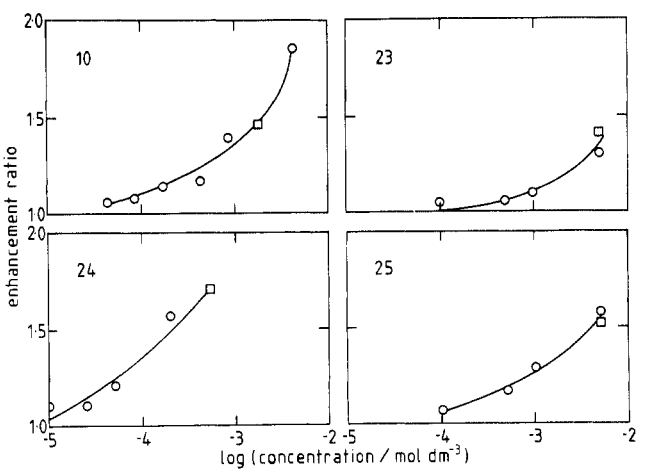

Figure 4. Enhancement ratio as a function of sensitizer concentration for X-irradiated V79 cells. Each curve is labeled with the code of the compound used (see Table I): (□) full survival curves; (○) mean of triplicate measurements at a single radiation dose assuming an unchanged extrapolation number.

Radiation Studies. The method was as described by Adams et al.[8] The cells were irradiated, attached to glass Petri dishes, at room temperature with 250 kVp 15-mA X-rays filtered with 0.5 mm Cu and 0.8 mm Al at a dose rate of 3.26 gray min^{-1}. Three dishes for each survival point were irradiated simultaneously. After colony scoring, the surviving fraction for each sensitizer concentration was calculated and plotted logarithmically as a function of radiation dose (gray). Full survival curves at a single concentration of each compound were obtained by irradiating five sets of triplicate dishes with increasing X-ray doses. The ratio of the final, linear portions of the log survival vs. radiation dose gave enhancement ratios (ER). These survival curves showed purely dose-modifying sensitization, that is, no change in the extrapolation number. This is determined by extrapolating the exponential portion of the curve back to zero dose and measuring the intercept on the ordinate plotted on the logarithmic scale.[34] Accordingly, enhancement ratios at other concentrations were calculated by dividing the dose required to give a surviving fraction of 10^{-2} in the absence of sensitizer by the dose required to give a surviving fraction of 10^{-2} in the presence of sensitizer. These ER are then plotted as a function of log concentration, thus enabling comparisons to be easily made between all the compounds studied. A full survival curve for one compound irradiated in air plus 5% CO_2 was obtained.

Figure 1 shows data for the effect of drug concentration on cell colony forming ability for **8** for both acute and chronic exposure. The results for this compound, together with those for the other compounds studied, are given in Table I. For three of the compounds it was not possible to measure the acute cytotoxicity because of solubility limitations. Two compounds were not acutely cytotoxic at 5 mmol dm^{-3}, the maximum concentration tested. In Figure 2 are shown survival data for cells suspended in hypoxic or aerobic 2 mmol dm^{-3} of **10**. Hypoxia alone had little effect on colony-forming ability. However, the presence of 2 mmol dm^{-3} of **10** under hypoxia greatly reduced survival. After an initial shoulder region, survival decreased rapidly with increasing time. These results contrast with those seen for aerobic cells in the presence of 2 mmol dm^{-3} of **10** when survival was only slightly reduced by the presence of the drug.

Some typical radical data for cells irradiated in air or hypoxia are shown in Figure 3. In hypoxia, 5 mmol dm^{-3} of **23** gives an ER of 1.5. For aerobic cells irradiated in the presence of 5 mmol dm^{-3} of **23**, no sensitization other than that caused by the presence of air alone was observed. This lack of sensitization of aerobic cells by **23** is typical of nitro aromatic and nitro heterocyclic compounds.[8,35-37]

(33) Stratford, I. J.; Adams, G. E. *Br. J. Cancer* **1977**, *35*, 307.
(34) Alper, T.; Gillies, N. E.; Elkind, M. M. *Nature (London)* **1960**, *186*, 1062.
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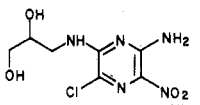
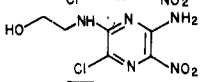
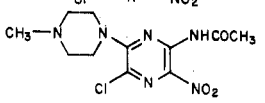
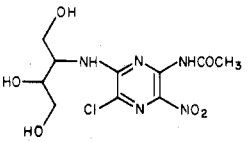
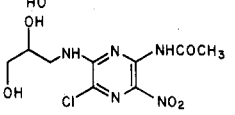
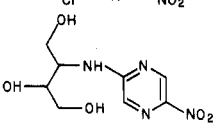
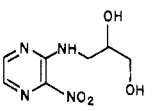
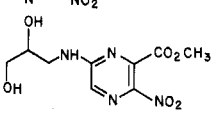
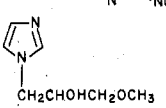
Table I. Physical Properties and Radiosensitizing Effects of Nitropyrazines

no.	structure	$E_{1/2}$,[a] V	partition coefficient[b]	SER[c]	chronic aerobic cytotoxicity,[d] mol/dm^{-3}	acute toxicity,[e] mol/dm^{-3}
5		−1.01	0.6	1.25	5.8×10^{-4}	$>4 \times 10^{-3}$ (limited by solubility)
6		−1.02	1.32	1.22	2.6×10^{-4}	$>3 \times 10^{-3}$ (limited by solubility)
8		−0.78	3.18	1.5 @ 5×10^{-5}	2.5×10^{-5}	1.6×10^{-4}
9		−0.81	0.22	1.35	4.0×10^{-4}	$>3.3 \times 10^{-3}$ (limited by solubility)
10		−0.75	1.01	1.30	9.6×10^{-5}	4×10^{-3}
23		−0.96	0.13	1.10	6.5×10^{-4}	$>5 \times 10^{-3}$
24		−0.83	0.32	1.71	2.7×10^{-5}	7×10^{-4}
25		−0.78	0.79	1.30	$>5 \times 10^{-4}$	$>5 \times 10^{-3}$
2[f]		−0.97	0.48	1.60	1.3×10^{-3}	3×10^{-2}

[a] Determined by Y. C. Lee in 0.1 M tetrabutylammonium perchlorate/dimethylformamide with a dropping mercury electrode vs. saturated calomel electrode. [b] Measured as *C* (octanol)/*C* (pH 7 buffer) at 22 °C. [c] Sensitizer enhancement ratio at 10^{-3} mol/dm^{-3}. [d] Concentration for 50% survival after 7 days exposure in air at 37 °C. [e] Concentration for 75% survival after 2-h exposure in nitrogen at 22 °C. [f] Misonidazole.

The ER measured from full survival curves and from triplicate measurements made at single radiation doses for all the compounds studied are shown in Figures 4 and 5. All show increasing ER with increasing sensitizer concentration. The shapes of the curves are similar to those previously reported.[8,36]

Except for **8** and **24**, the enhancement ratios for the nitropyrazines studied were somewhat lower than that of misonidazole and were fairly well correlated with their $E_{1/2}$ values. Both compound **8**, with an SER of 1.5 at a concentration of 5×10^{-5} mol/dm^{-3}, and compound **24**, with an enhancement ratio of 1.71 at a concentration of 5×10^{-4} mol/dm^{-3}, were much more active than expected on the basis of reduction potential. The reason for this heightened activity in **24** is not known; however, since **23** and **25** fail to behave in this manner, the ortho disposition of the displayed functionality is apparently of importance.

Finally, compounds **5** and **6** were not found to be mutagenic in the standard plate-incorporation Ames test.[38]

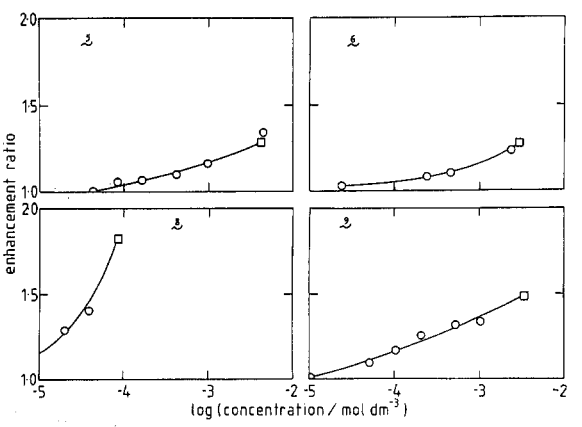


Figure 5. Enhancement ratio as a function of sensitizer concentration for X-irradiated V79 cells. Each curve is labeled with the code of the compound used (see Table I): (□) full survival curves; (○) mean of triplicate measurements at a single radiation dose assuming an unchanged extrapolation number.

This result holds promise that other nitro heterocyclic radiosensitizers might be designed that would lack the mutagenic/carcinogenic liabilities of the nitroimidazoles.[39]

(36) Adams, G. E.; Clarke, E. D.; Flockhart, I. R.; Jacobs, R. S.; Sehmi, D. S.; Stratford, I. J.; Wardman, P.; Watts, M. E.; Parrick, J.; Wallace, R. G.; Smithen, C. E. *Int. J. Radiat. Biol.* **1979**, *35*, 133.

(37) Smithen, C. E.; Clarke, E. D.; Dale, J. A.; Jacobs, R. S.; Wardman, P.; Watts, M. E.; Woodcock, M. "Radiation Sensitizers: Their Use in the Clinical Management of Cancer"; Brady, L. W., Ed.; Masson: New York, 1980.

(38) The performance and evaluation of these procedures were supervised by Drs. M. Hite and M. O. Bradley of the Safety Assessment Department of these laboratories.

Experimental Section

Melting points were determined in air with a Thomas-Hoover apparatus using a capillary tube and are uncorrected. Proton NMR spectra were obtained on a Varian T-60A spectrometer. The elemental analyses and physical measurements were carried out by Dr. W. C. Randall, Y. C. Lee, J. P. Moreau, and K. Streeter. The mass spectral analyses were carried out by Dr. H. Ramjit and his staff using an LKB-9000 S instrument at 70 eV.

5,6-Dichloro-3-nitropyrazinamine (4). To 176 mL of concentrated sulfuric acid was added 20.8 g (0.1 mol) of 5,6-dichloro-3-aminopyrazine-2-carboxylic acid (**3**), and after stirring for 5–10 min, most of the acid had dissolved. This was then cooled to <5 °C, and a solution of 6.3 mL of concentrated nitric acid in 6.3 mL of concentrated sulfuric acid was added dropwise over 30 min with the temperature maintained at <5 °C. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred for 4 h. Evolution of carbon dioxide was apparent during the addition and continued for much of the stirring period. The reaction mixture was then quenched with good agitation into 1-L of ice, and the resulting yellow solid was collected by filtration. This yellow solid was dissolved in ethyl acetate, washed with 3 × 250 mL of saturated sodium carbonate, and dried over anhydrous sodium sulfate. The ethyl acetate solution was then passed through a silica gel column (500 g, 70–230 mesh), which was eluted with ethyl acetate. Fractions were collected until the impurity on analytical TLC (2% methanol/chloroform elution) appeared. Removal of solvents in vacuo afforded 9.6 g (46%) of **4** as a yellow solid: mp 169–170 °C; MS, *m/e* 209 (M^+). Anal. ($C_4H_2Cl_2N_4O_2$) C, H, N, Cl.

3-[(6-Amino-3-chloro-5-nitropyrazin-2-yl)amino]-1,2-propanediol (5). To 3.0 g (0.014 mol) of **4** in 20 mL of 2-propanol at room temperature was added 1.3 g (0.014 mol) of 3-amino-1,2-propanediol and 1.45 g (0.014 mol) of triethylamine. This mixture was stirred for 18 h. The yellow product was then filtered off, washed twice with chloroform, and recrystallized from acetonitrile to give a yellow solid: mp 202–203 °C; ^1H NMR (Me_2SO-d_6) δ 3.40 (4 H, br s); MS, *m/e* 263 (M^+). Anal. ($C_7H_{10}N_5ClO_4$) C, H, N.

2-[(6-Amino-3-chloro-5-nitropyrazinyl)amino]ethanol (6). To 0.29 g (0.0048 mol) ethanolamine in 20 mL of 2-propanol at room temperature was added 0.48 g (0.0048 mol) of triethylamine and 1.0 g (0.0048 mol) of **4**, and the resulting mixture was stirred for 4 h. The yellow solid was collected by filtration and recrystallized from acetonitrile to give 0.8 g (72%) of **6** as a yellow solid: mp 196–197 °C; ^1H NMR (Me_2SO-d_6) δ 3.48 (4 H, s); MS, *m/e* 233 (M^+). Anal. ($C_6H_8ClN_5O_3$) C, H, N.

***N*-(5,6-Dichloro-3-nitropyrazinyl)acetamide (7).** To 1.0 g (0.0048 mol) of 5,6-dichloro-3-nitropyrazinamine (**4**) in 15 mL of acetyl chloride under nitrogen at room temperature was added 0.84 g (0.01 mol) of anhydrous sodium bicarbonate. This mixture was stirred and heated at reflux for 4 days. Excess acetyl chloride was removed on the rotary evaporator, and the residue was flash chromatographed on silica gel (230–400 mesh) with chloroform elution to give 0.7 g (58%) of **7** as a white solid: mp 129–130 °C; R_f 0.6 (silica gel; 2% methanol/chloroform); ^1H NMR ($CDCl_3$) δ 2.42 (3 H, s), 9.80 (1 H, br); MS, *m/e* 251 (M^+). Anal. ($C_6H_4Cl_2N_4O_3$) C, H, N.

***N*-[5-Chloro-6-(4-methyl-1-piperazinyl)-3-nitropyrazinyl]acetamide (8).** To 0.255 g (0.001 mol) of **7** in 5 mL of 2-propanol at room temperature was added 0.1 g (0.011 mol) of *N*-methylpiperazine and 0.1 g (0.001 mol) of triethylamine. This mixture was stirred for 1 h, and the solid was filtered off, washed with chloroform/*n*-butyl chloride (1:10), and air-dried: mp 189–190 °C; ^1H NMR (Me_2SO-d_6) δ 2.10 (3 H, s), 2.22 (3 H, s), 2.42 (4 H, t), 3.73 (4 H, t); MS, *m/e* 330 (M^+). Anal. ($C_{11}H_{15}ClN_6O_3$) C, H, N.

***N*-[5-Chloro-3-nitro-6-[(1,3,4-trihydroxybutyl)amino]pyrazinyl]acetamide (9).** To 0.83 g (0.003 mol) of **7** in 20 mL of 2-propanol at room temperature were added 0.42 g (0.0035 mol) of 2-amino-1,3,4-butanetriol and 0.35 g (0.003 mol) of triethylamine. This mixture was stirred at room temperature for 6 h. The solvent was then removed on the rotary evaporator, and the oily residue was flash chromatographed on silica gel (230–400 mesh) with 8% methanol/methylene chloride elution. The product had R_f 0.4 and was obtained as a yellow solid (68%): mp 139–141 °C; ^1H NMR (Me_2SO-d_6) δ 2.18 (3 H, s), 3.20 (6 H, br s); MS, *m/e* 335 (M^+). Anal. ($C_{10}H_{14}ClN_5O_6$) C, H, N.

***N*-[5-Chloro-3-nitro-6-[(2,3-dihydroxypropyl)amino]pyrazinyl]acetamide (10).** To 5.0 g (0.0198 mol) of **7** in 50 mL of 2-propanol at room temperature were added 1.8 g (0.0198 mol) of 3-amino-1,2-propanediol and 2.0 g (0.0198 mol) of triethylamine. After the mixture was stirred at room temperature for 16 h, the solvent was removed on the rotary evaporator, and the residue was flash chromatographed on silica gel (230–400 mesh) with 5% methanol/chloroform elution. The product had R_f 0.5 and was obtained as a yellow solid (72%): mp 130–131 °C; ^1H NMR (Me_2SO-d_6) δ 2.21 (3 H, s), 3.50 (5 H, br t), 4.83 (1 H, d); MS, *m/e* 305 (M^+). Anal. ($C_9H_{12}ClN_5O_5$) C, H, N.

***S,S*-Dimethyl-*N*-(3-chloropyrazin-2-yl)sulfilimine (18).** To a mechanically stirred solution of 3.9 g (0.05 mol) of dimethyl sulfoxide in 30 mL of dry methylene chloride at −78 °C under nitrogen was added 13.1 g (0.046 mol) of trifluoromethanesulfonic anhydride dropwise at −78 °C to afford a white precipitate. To this was added a solution of 5.0 g (0.039 mol) of 2-amino-3-chloropyrazine (**15**)[41] in 30 mL of methylene chloride/15 mL dimethyl sulfoxide, and the resulting orange solution was stirred at −78 °C for 2 h and at −55 °C for 1 h. The reaction mixture was quenched with 50 mL of a 5% aqueous sodium hydroxide solution and stirred at −5 °C for 5 min. The reaction mixture was diluted with 200 mL of methylene chloride, and the phases were separated. The aqueous phase was extracted with 250 mL of methylene chloride, and the combined organic phases were washed with three 75-mL portions of water and dried over anhydrous sodium sulfate. The solvent was removed on the rotary evaporator to give 5.8 g (79%) of **18** as yellow crystals: mp 106–108 °C; ^1H NMR ($CDCl_3$) δ 2.85 (6 H, s), 7.57 (1 H, d), 7.78 (1 H, d); MS, *m/e* 189 (M^+). Anal. ($C_6H_8ClN_3S$) C, H, N.

***S,S*-Dimethyl-*N*-(5-chloropyrazin-2-yl)sulfilimine (17)** was prepared from 2-amino-5-chloropyrazin (**14**)[42] as described above for **18** to give **17** in 88% yield as a tan solid: mp 119–120 °C; ^1H NMR ($CDCl_3$) δ 2.74 (6 H, s), 7.76 (2 H, s); MS, *m/e* 189 (M^+). Anal. ($C_6H_8ClN_3S$) C, H, N.

***S,S*-Dimethyl-*N*-(5-chloro-3-carbomethoxypyrazin-2-yl)sulfilimine (19)** was prepared from methyl 6-chloro-3-amino-2-pyrazinoate (**16**)[43] as described above for **18**. Crude **19** was recrystallized from methylene chloride/hexane to give pure **19** in 90% yield as yellow crystals: mp 167–169 °C; ^1H NMR ($CDCl_3$) δ 2.77 (6 H, s), 3.88 (3 H, s), 7.86 (1 H, s); MS, *m/e* 247 (M^+). Anal. ($C_8H_{10}ClN_3O_2S$) C, H, N.

3-Chloro-2-nitropyrazine (21). To 7.9 g (0.46 mol, 80–90%) of *m*-chloroperbenzoic acid in 70 mL of methylene chloride cooled to −5 °C and stirred mechanically was added a solution of 5.36 g (0.028 mol) of **18** in 30 mL of methylene chloride dropwise at such a rate that the temperature did not exceed 0 °C. The reaction mixture was stirred at 0 °C for 40 min, and then 3 mL of dimethyl sulfide was added with stirring for an additional 10 min. The cold reaction mixture was filtered quickly to afford a clear, green solution of the nitroso derivative. This solution was cooled to 0 °C, and ozone was bubbled through until the solution was nearly colorless. The resulting suspension was extracted with 2 × 50 mL of saturated sodium bicarbonate, and the organic phase was dried over anhydrous sodium sulfate. The solvent was removed on the rotary evaporator to give a pungent yellow oil, which was flash chromatographed on silica gel (230–400 mesh) with chloroform elution, yielding 2.5 g (56%) of **21** as a yellow oil: R_f 0.6 ($CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.60 (1 H, s), 8.83 (1 H, s); MS, *m/e* 159 (M^+). Anal. ($C_4H_2ClN_3O_2$) C, H, N, Cl.

2-Chloro-5-nitropyrazine (20) was prepared from **17** as described above for **21** to give crude **20** as a yellow solid. This was purified by flash chromatography on silica gel with chloroform elution to give pure **20** as yellow crystals in 60% yield: mp 88–90

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°C; ^{1}H NMR ($CDCl_3$) δ 8.68 (1 H, d), 9.38 (1 H, d); MS, *m/e* 189 (M^+). Anal. ($C_4H_2ClN_3O_2$) C, H, N.

Methyl 6-chloro-3-nitropyrazinoate (22) was prepared from **19** as described above for **21**. Crude **22** was purified by flash chromatography on silica gel with 2% methanol/chloroform to afford a yellow oil in 51% yield: ^{1}H NMR ($CDCl_3$) δ 4.08 (3 H, s), 8.75 (1 H, s); MS, *m/e* 217 (M^+).

5-[(1,3,4-Trihydroxybutyl)amino]-2-nitropyrazine (23). To 0.5 g (0.003 mol) of **20** in 10 mL of 2-propanol were added 0.36 g (0.003 mol) of 2-amino-1,3,4-butanetriol and 0.3 g (0.003 mol) of triethylamine in 10 mL of acetonitrile. After the mixture was stirred at room temperature for 18 h, the solvent was removed on the rotary evaporator, and the residue was flash chromatographed on silica gel (230–400 mesh) with 20% methanol/chloroform elution. The product (R_f 0.4) was an oil and was triturated with methanol/ether to give a yellow solid: mp 133–135 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.33 (5 H, br s), 3.51 (1 H, m), 8.02 (1 H, s), 8.28 (1 H, d), 8.95 (1 H, s); MS, *m/e* 244 (M^+). Anal. ($C_8H_{12}N_4O_5$) C, H, N.

2-[(2,3-Dihydroxypropyl)amino]-3-nitropyrazine (24). To a solution of 1.5 g (0.0094 mol) of **21** and 1.01 g (0.01 mol) of triethylamine in 25 mL of 2-propanol was added 0.91 g (0.01 mol) of 3-amino-1,2-propanediol. After stirring at room temperature, the crude reaction mixture showed a single major yellow spot in TLC (R_f 0.5) with 10% methanol/chloroform. The solvent was removed on the rotary evaporator, and the residue was purified by flash chromatography on silica gel with 8% methanol/chloroform to afford 1.7 g (83%) of **24** as a yellow solid: mp 100–102 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.50 (5 H, m), 4.65 (1 H, t), 5.03 (1 H, d), 7.85 (1 H, d), 8.33 (1 H, t), 8.58 (1 H, d) MS, *m/e* 214 (M^+). Anal. ($C_7H_{10}N_4O_4$) C, H, N.

6-[(2,3-Dihydroxypropyl)amino]-3-nitropyrazinoate (25). To 2.0 g (0.008 mol) of **22** in 60 mL of 2-propanol at room temperature were added 0.72 g (0.008 mol) of 3-amino-1,2-propanediol and 0.8 g (0.008 mol) of triethylamine. After the mixture was stirred for 16 h, the solvent was removed on the rotary evaporator, and the residue was flash chromatographed on silica gel (230–400 mesh) with 10% methanol/chloroform elution. The product had R_f 0.4 and was isolated as a yellow solid (1.3 g, 60%): mp 107–109 °C; ^{1}H NMR (CD_3OD) δ 3.57 (5 H, m), 3.92 (3 H, s), 4.70 (2 H, br s), 7.87 (1 H, s); MS, *m/e* 272 (M^+). Anal. ($C_9H_{12}N_4O_6$) C, H, N.

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Registry No. **3**, 4853-52-5; **4**, 87155-51-9; **5**, 88793-46-8; **6**, 86845-62-7; **7**, 87155-52-0; **8**, 89083-22-7; **9**, 89690-74-4; **10**, 89083-18-1; **14**, 33332-29-5; **15**, 6863-73-6; **16**, 1458-03-3; **17**, 87885-44-7; **18**, 86536-74-5; **19**, 86536-76-7; **20**, 87885-45-8; **21**, 87885-43-6; **22**, 89690-75-5; **23**, 89690-76-6; **24**, 87885-48-1; **25**, 87885-53-8; **26**, 55153-71-4; $NH_2CH_2CH(OH)CH_2OH$, 616-30-8; $NH_2(CH_2)_2OH$, 141-43-5; *N*-methylpiperazine, 109-01-3; 2-amino-1,3,4-butanetriol, 83168-64-3.

Pyrido[3′,2′:4,5]thieno[3,2-*d*]-N-triazines: A New Series of Orally Active Antiallergic Agents

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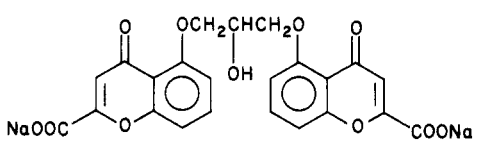
A new series of orally active mediator release inhibitors, pyrido[3′,2′:4,5]thieno[3,2-*d*]-*N*-triazines, was synthesized and evaluated for antiallergic activity. Several products showed high activity as inhibitors or wheal information in the rat passive cutaneous anaphylaxis screen and as inhibitors of histamine release from passively sensitized rat mast cells. Many compounds were orally active in the PCA test. The most potent compound, 7-phenylpyrido-[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazin-4(3*H*)-one (**10**) with an I_{50} value of 0.05 μM, was 60 times more potent than disodium cromoglycate (DSCG) in the RMC assay.

Cromolyn sodium (DSCG, I) is a well-established drug that inhibits release of the mediators of anaphylaxis[1] and thus provides a prophylactic treatment of asthma. The main disadvantage of DSCG is that it is not orally effective and thus must be used as an insufflated powder. A desirable compound should show DSCG-like capacity to inhibit allergy-induced mast cell and be orally effective. Since the discovery of DSCG,[1] there have been intensive efforts in numerous laboratories to find orally active DSCG-like antiallergic agents. Such agents should certainly have an important place in the therapy of asthma and other allergic disorders. Most of the orally active compounds reported are carboxylic acids or derivatives



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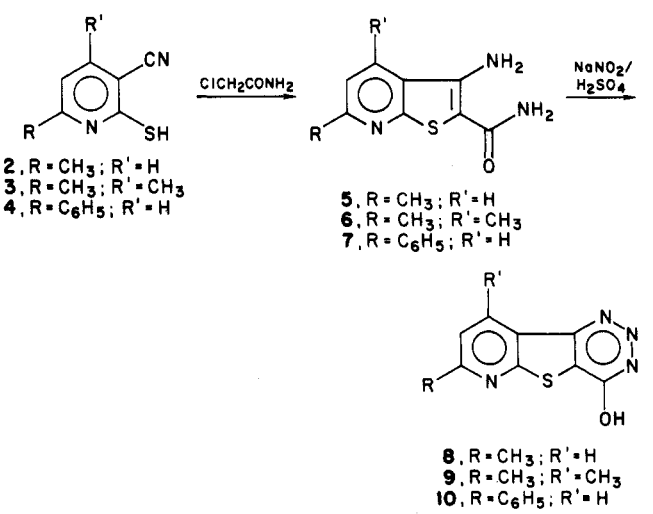
Scheme I



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