Monocyclic Pteridine Analogues. A Pyrazine Analogue of Methotrexate

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Reaction of aminocyanoacetamide with bromomethylpyruvaldoxime provided 3-amino-6-bromomethyl-2-carbamoylpyrazine 4-oxide (**7**), which was converted to a pyrazine analogue (**3**) of methotrexate by subsequent condensation with diethyl *N*-[4-(methylamino)benzoyl]-L-glutamate, deoxygenation, and hydrolysis. The methotrexate analogue **3** was a poor inhibitor (I_{50} >150 μM) of bacterial or mammalian dihydrofolate reductase.

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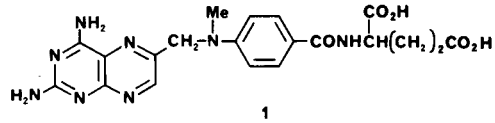
Methotrexate (**1**) is an antineoplastic agent which achieves its cytotoxic effects through potent inhibition of the enzyme dihydrofolate reductase [1-3]. The natural substrate for this enzyme is 7,8-dihydrofolic acid (**2**), and despite the elements of structural similarity in the pteridine portions of **1** and **2**, it now appears that the pteridine rings of these molecules bind to the enzyme in quite different orientations [1,2]. A large number of monocyclic (*e.g.* pyrimidine, triazine) and bicyclic (*e.g.* pteridine, quinazoline) analogues of **1** have been evaluated as dihydrofolate reductase inhibitors [1-3], but potential pyrazine inhibitors appear not to have been studied. In this regard, we envisioned pyrazine **3** as a monocyclic methotrexate analogue with the appropriate *p*-aminobenzoylglutamate side chain and with the potential of presenting the enzyme with orientations of the carbamoyl moiety related either to methotrexate (*cf.* **4a**) or to dihydrofolate (*cf.* **4b**). Accordingly, we synthesized **3** as shown in Scheme I.

In an adaptation of the pyrazine synthesis developed by Taylor [4-6], aminocyanoacetamide (**5**) [7] was reacted in acetic acid solution with bromomethylpyruvaldoxime (**6**) [8] to provide the bromomethylpyrazine *N*-oxide **7**. Condensation of **7** with the diethyl glutamate derivative **8** [9] in dimethylsulfoxide in the presence of potassium carbonate gave the adduct **9**, which was deoxygenated with phosphorus trichloride in tetrahydrofuran to provide the parent pyrazine **10**. Relative to *N*-oxide **9**, pyrazine **10** displayed an upfield shift of 0.17 ppm in the nmr absorbance for the pyrazine ring proton, and exhibited a shift to shorter wavelength of the long wave maximum in the ultraviolet spectrum. Basic hydrolysis of diester **10** then afforded the target diacid **3**.

Although pyrazine **3** possesses many of the structural features, and consequently many of the potential binding sites, of both methotrexate (**1**) and dihydrofolate (**2**), this compound was a poor inhibitor (I_{50} >150 μM) of dihydrofolate reductase from mammalian (rat liver) or bacterial (*E. coli*) sources [10]. These results provide additional support for the critical role of N_1 and the 2-amino group of methotrexate for tight binding to dihydrofolate reductase [1,2].



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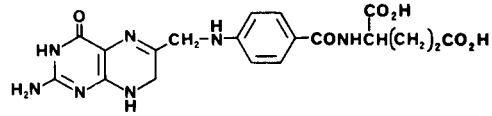

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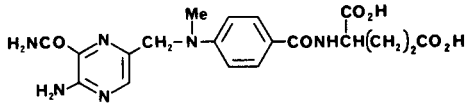

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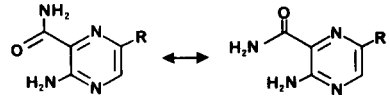


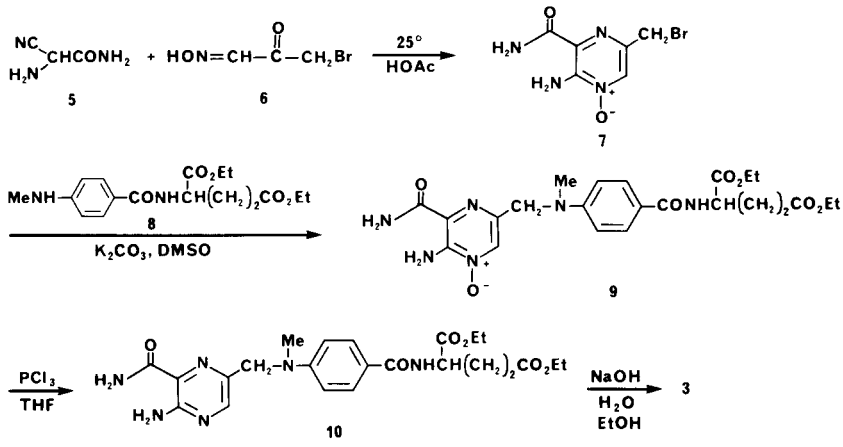
4a 4b

EXPERIMENTAL

Melting points were determined with a Buchi melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. Proton nuclear magnetic resonance (nmr) spectra were determined with Varian XL-100, CFT20, and T-60 spectrometers with tetramethylsilane as internal standard.

3-Amino-6-bromomethyl-2-carbamoylpyrazine 4-Oxide (7).

To a stirred solution of aminocyanoacetamide (**5**, 2.00 g, 20.2 mmoles) [7] in glacial acetic acid (25 ml) was added dropwise under nitrogen a solution of bromopyruvaldoxime (**6**, 3.55 g, 21.4 mmoles) [8] in glacial acetic acid (25 ml) during a period of 20 minutes. After two days, the solvent was removed under reduced pressure and the amber residue was triturated with several portions of ether. The ether layers were discarded and the remaining residue was placed in a Soxhlet thimble and continuously extracted with methylene chloride for two days. The extracts were concentrated under reduced pressure and the residue was recrystallized from acetonitrile to provide **7** (1.58 g, 32%) as a yellow-orange solid, mp



Scheme I

165° dec; nmr (100 MHz, DMSO-d_6): δ 4.60 (s, 2H), 7.8-8.2 (three broad exchangeable bands, 4H total), 8.64 (s, 1H).

Anal. Calcd. for $C_6H_7BrN_4O_2$: C, 29.17; H, 2.86; N, 22.68; Br, 32.34. Found: C, 29.21; H, 2.86; N, 22.66; Br, 32.28.

Diethyl *N*-[4-[[(5-Amino-6-carbamoylpyrazinyl)methyl]methylamino]benzoyl]-L-glutamate *N*-Oxide (**9**).

A mixture of bromomethyl compound **7** (4.90 g, 21.2 mmoles), diethyl *N*-(4-methylaminobenzoyl)-L-glutamate (**8**, 7.14 g, 21.2 mmoles) [9], and potassium carbonate (4.40 g, 31.8 mmoles) in dry dimethylsulfoxide (freshly distilled from calcium hydride) was stirred at ambient temperature for six days and then poured into water (1 liter). The aqueous mixture was exhaustively extracted with chloroform and the combined organic layers were dried (sodium sulfate) and then concentrated under reduced pressure. The viscous amber residue was chromatographed on silica gel (500 g) with chloroform-methanol (500 ml of 99:1, followed by 8 liters of 97:3) to provide the adduct as an oil which upon trituration with ether afforded **9** as a pale yellow solid (4.22 g, 40%) of mp 108-110°; nmr (80 MHz, DMSO-d_6): δ 1.15 and 1.16 (two overlapping triplets, J = 7 Hz, 6H total), 2.07 (m, 2H), 2.39 (br t, 2H, partially obscured by solvent peak), 3.18 (s, 3H), 4.03 and 4.07 (two overlapping q, J = 7 Hz, 4H total), 4.36 (m, 1H), 4.60 (s, 2H), 6.80 ($\frac{1}{2}A_2B_2$ pattern, 2H), 7.6-8.0 (m, 6H), 8.22 (s, 1H), 8.34 (br d, J *ca.* 7 Hz, 1H); uv (methanol): λ max 246 (ε 12,400), 301 (ε 25,900), 378.5 (ε 8600) nm.

Anal. Calcd. for $C_{23}H_{30}N_6O_7$: C, 54.97; H, 6.02; N, 16.73. Found: C, 54.71; H, 6.05; N, 16.64.

Diethyl *N*-[4-[[(5-Amino-6-carbamoylpyrazinyl)methyl]methylamino]benzoyl]-L-glutamate (**10**).

To a cool (0°) solution of *N*-oxide **9** (1.21 g, 2.41 mmoles) in dry tetrahydrofuran (120 ml, freshly distilled from lithium aluminum hydride) was added phosphorus trichloride (10 ml). The mixture was stirred at 0° for two hours and then at room temperature for five hours, whereupon the mixture was poured onto ice-water slush and extracted with chloroform. The extracts were dried (sodium sulfate) and then concentrated under reduced pressure. The residue was applied to four preparative layer plates (2 mm × 20 cm × 20 cm; Merck) which were developed thrice with ethyl acetate. The band with Rf 0.45 was scraped from the plates and the product eluted with ethanol. The solvent was removed under reduced pressure and the foamy residue was thoroughly extracted with ether. Concentration of the extracts gave pyrazine **10** as an off-white solid (0.353 g, 30%), mp 106-108°; nmr (80 MHz, DMSO-d_6): δ 1.15 and 1.17 (two overlapping triplets, J = 7 Hz, 6H total), 2.05 (m, 2H), *ca.* 2.4 (br t, 2H, partially obscured by solvent peak), 3.13 (s, 3H), 4.00 and 4.05 (two overlapping q, J = 7 Hz, 4H total), 4.33 (m, 1H), 4.57 (s, 2H), 6.76 ($\frac{1}{2}A_2B_2$ pattern, 2H), 7.38 (br s, 2H), 7.67 ($\frac{1}{2}A_2B_2$ pattern, 2H) overlapping *ca.* 7.60 (br, 2H), 8.05 (s, 1H), 8.22 (br d, J *ca.* 8 Hz, 1H); uv (methanol): λ max 249.5 (ε 16,700), 304.5 (ε 29,500), 361 (ε 8400) nm.

Anal. Calcd. for $C_{23}H_{30}N_6O_6$: C, 56.78; H, 6.22; N, 17.28. Found: C, 56.76; H, 6.23; N, 17.26.

N-[4-[[(5-Amino-6-carbamoylpyrazinyl)methyl]methylamino]benzoyl]-L-glutamic Acid (**3**).

A solution of diester **10** (0.500 g, 1.02 mmoles) in aqueous sodium hydroxide (0.1*N*, 25 ml, 2.5 mmoles) and ethanol (15 ml) was stirred at room temperature for five days. The solution was brought to p*H* 4 with aqueous hydrochloric acid (0.1*N*) and then cooled in an ice bath. The resulting precipitate was collected and dried under vacuum at 50° to provide **3** as a dark yellow solid (0.292 g, 66%) of mp 190-192° dec; ^{1}H nmr (60 MHz, DMSO-d_6): δ 1.8-2.5 (m, 4H), 3.17 (s, 3H), 4.40 (v br d of t, 1H), 4.63 (s, 2H), 6.83 ($\frac{1}{2}A_2B_2$ pattern, 2H), 7.33-7.90 (m, 6H), 8.10 (s, 1H) overlapping *ca.* 8.15 (br, 1H); the carboxylic acid protons did not appear as discrete signals; uv (0.1*N* sodium hydroxide): λ max 252 (ε 15,500), 305 (ε 23,600), 356 (ε 6700) nm.

Anal. Calcd. for $C_{19}H_{22}N_6O_6$: C, 53.02; H, 5.15; N, 19.53. Found: C, 52.92; H, 5.20; N, 19.46.

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