*John A. Montgomery, James R. Piper, Robert D. Elliott, Eugene C. Roberts, Carroll Temple, Jr., and Y. Fulmer Shealy*

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Received September 22, 1978

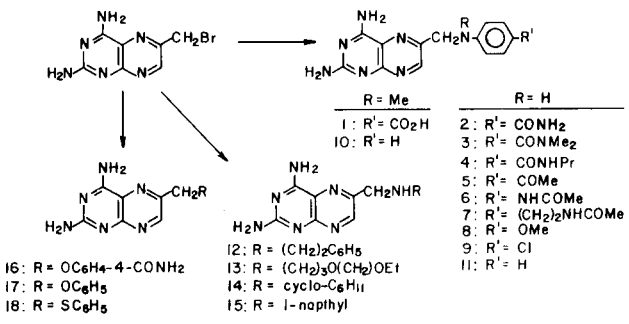
Eighteen derivatives of 2,4-diamino-6-methylpteridine related to methotrexate and aminopterin have been prepared from 6-(bromomethyl)-2,4-pteridinediamine by nucleophilic displacement reactions. None of these compounds showed any antileukemic acitivity.

*J. Heterocyclic Chem.*, **16**, 537 (1979).

The utility of 6-(bromomethyl)-2,4-pteridinediamine hydrobromide for the preparation of methotrexate, aminopterin, and derivatives thereof has been established (1). We have now used this valuable intermediate to prepare a variety of analogs of methotrexate containing an abbreviated side chain in place of *N*-[4-(methylamino)benzoyl]-L-glutamic acid. These analogs are relatively lipophilic and would enter cells primarily by passive diffusion (2). Such agents that are capable of inhibiting dihydrofolic reductase might be useful for the treatment of tumors that do not respond to methotrexate because of an ineffective level of membrane transport (3,4). They might also provide additional therapeutic advantage when used in conjunction with citrovorum factor rescue, a procedure used to increase the therapeutic index of methotrexate (5).

Reaction of 6-(bromomethyl)-2,4-pteridinediamine hydrobromide with a variety of 4-substituted anilines, 1-naphthylamine, two aliphatic amines, two phenoxide ions, and the thiophenoxide ion occurred at room temperature in *N*,*N*-dimethylacetamide. The reaction time varied from three-quarters of a day to six days. The yields in general were good and in many cases the products precipitated, and analytical samples were prepared simply by carefully washing and drying the precipitates. The lower yields obtained in some cases do not reflect the reaction yield but rather product isolation.

Most of these compounds were too insoluble in aqueous media to determine their ability to inhibit dihydrofolic reductase ($I_{50}$ of **1** = 0.88 μM, Mtx = dihydrofolic reductase ($I_{50}$ of **1** = 0.88μM, Mtx = 0.013 μM), but their lack of cytotoxicity and activity against leukemia L1210 would indicate that they are probably not good inhibitors of this enzyme. Two compounds, **5** and **8**, showed some cytotoxicity ($ED_{50}$ vs. KB cells = 9.5 and 2.3 μM, Mtx = 0.015 μM), but were inactive, as were all the other compounds, against L1210 at 80 mg./kg./dose, qd 1-9.




## EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were normally dried *in vacuo* over phosphorus pentoxide at room temperature for 16 hours. Analtech precoated (250 μm) silica gel G (F) plates were used for tlc analyses; the spots were detected by irradiation with a Mineralight. All analytical samples were essentially tlc homogeneous. The uv absorption spectra were determined in 0.1 *N* hydrochloric acid, *p*H 7 buffer, and 0.1 *N* sodium hydroxide with a Cary 17 spectrophotometer. The pmr spectra were determined with a Varian XL-100-15 spectrometer in DMSO-$d_6$ with tetramethylsilane as an internal reference.

### General Reaction Procedure.

A solution of 6-(bromomethyl)-2,4-pteridinediamine hydrobromide and the nucleophile (amine, phenoxide, or thiophenoxide ion) in *N*,*N*-dimethylacetamide (DMA) was stirred at room temperature for the time specified in Table 1. The product was then isolated by one of the procedures described below.

### Isolation Procedures.

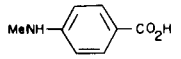
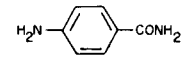
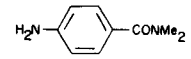
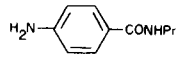
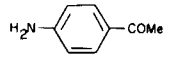
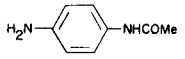
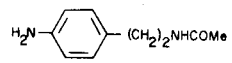
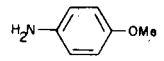
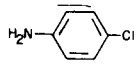
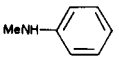
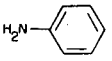
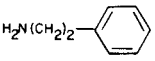
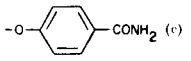
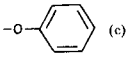
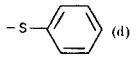
If the product precipitated, it was isolated as the hydrobromide salt by filtration, washing, and drying (A) except for **10**, which was converted to the free base by stirring with 0.5 *M* sodium bicarbonate solution and recrystallized from ethanol, and **18** which was obtained as the free base from the reaction of the bromomethylpteridine with the thiophenoxide ion.

Products that did not precipitate from the reaction mixture were isolated by the addition of or to water to cause precipitation (B), except for **3** and **13** which were obtained by evaporation of the reaction mixture *in vacuo* (C). Both of these procedures incorporated treatment with aqueous sodium hydroxide to give the products as the free base (except in the case of the phenoxides or thiophenoxides). Compound **13** was purified by chromatography on Brinkmann Silica Gel F-254 preparative thin-layer plates (methanol). The product was diluted with hot methanol and triturated with ether.

Analytical data is given in Table II. Although not reported, the uv and pmr spectra of each compound were determined and found to be consistent with the expected structure.

*0022-152X/79/030537-03$02.25*

Table I

| Nucleophile | Ratio nucleophile/ pteridine | Volume of DMA, ml./mmole pteridine | Reaction Time, days | Isolation Procedure (a) | % Yield | Wash Solvents |
|---|---|---|---|---|---|---|
| 1.  | 1.1 | 4 | 4.75 | B | 60 | 0.08 *N* NaOH, (e) $H_2O$ |
| 2.  | 2 | 13.4 | 1 | A | 62 | DMA, EtOH, $Et_2O$ |
| 3.  | 3 | 13.4 | 2 | C | 56 | 1 *N* NaOH, $H_2O$, MeOH (f) |
| 4.  | 3.2 | 6.7 | 3 | A | 74 | $Et_2O$ |
| 5.  | 4 | 6.7 | 1 | A | 68 | DMA, EtOH, $Et_2O$ |
| 6.  | 3 | 6 (b) | 1 | B | 69 | $H_2O$, MeOH-$Et_2O$, $Et_2O$ |
| 7.  | 3 | 13.4 | 2 | A | 35 | $H_2O$, EtOH, $Et_2O$ |
| 8.  | 2 | 3.25 | 3 | A | 34 | DMA, $H_2O$, EtOH (g) |
| 9.  | 3 | 5 | 0.7 | B | 85 | 1 *N* NaOH, (h), $H_2O$, $Et_2O$ |
| 10.  | 31 | 13.4 | 2 | A | 57 | 0.5 *M* $NaHCO_3$, EtOH (g) |
| 11.  | 4 | 6.7 | 1 | A | 38 | $H_2O$, $Me_2CO$, $Et_2O$, $H_2O$ (g) |
| 12.  | 8 | 5 | 0.75 | B | 59 | $Et_2O$ |
| 13. $H_2N(CH_2)_3O(CH_2)_2OEt$ | 4 | 5 | 0.75 | C | 29 | $Et_2O$ (i) |
| 14. $H_2N$-cyclo-$C_6H_{11}$ | 3 | 4 | 1 | B | 46 | $H_2O$, $Et_2O$ (j) |
| 15. $H_2N$-1-napthyl | 4 | 4 | 1 | B | 68 | 0.05 *N* NaOH, $H_2O$, $Et_2O$ |
| 16.  (c) | 2 | 10 | 6 | B | 61 | pet. ether |
| 17.  (c) | 2 | 10 | 6 | B | 70 | pet. ether |
| 18.  (d) | 3.3 | 3.3 | 1 | A | 87 | $Et_2O$, $H_2O$ |

(a) See Tabel II. (b) Hexamethylphosphoramide. (c) Base-potassium *t*-butoxide. (d) Base-potassium carbonate. (e) Precipitated from with 1 *N* hydrochloric acid. (f) Caused only partial conversion to free base. (g) Then recrystallized from. (h) Stirred for 3 hours. (i) After chromatography, see procedure. (j) After precipitation at *p*H 12.

Table II

Analytical Data

| Compound No. | Empirical Formula | Calcd. C | Calcd. H | Calcd. N | Found C | Found H | Found N |
|---|---|---|---|---|---|---|---|
| 1 | $C_{15}H_{15}N_7O_2 \cdot 1.5\ H_2O$ | 51.13 | 5.15 | 27.83 | 51.02 | 5.24 | 27.52 |
| 2 | $C_{14}H_{14}N_8O \cdot HBr \cdot H_2O$ (a) | 41.09 | 4.19 | 27.38 | 41.23 | 3.69 | 27.44 |
| 3 | $C_{16}H_{18}N_8O \cdot 0.52\ HBr \cdot H_2O$ (b) | 48.23 | 5.19 | 28.12 | 48.40 | 5.07 | 28.28 |
| 4 | $C_{17}H_{20}N_8O \cdot HBr$ | 47.12 | 4.88 | 25.86 | 47.10 | 5.18 | 25.55 |
| 5 | $C_{15}H_{15}N_7O \cdot HBr$ | 46.17 | 4.13 | 25.12 | 46.45 | 4.29 | 25.38 |
| 6 | $C_{15}H_{16}N_8O \cdot 1.2\ H_2O$ | 52.08 | 5.36 | 32.39 | 51.99 | 4.94 | 32.38 |
| 7 | $C_{17}H_{20}N_8O \cdot HBr \cdot 2\ H_2O$ | 43.50 | 5.37 | 23.88 | 43.40 | 5.42 | 23.82 |
| 8 | $C_{14}H_{15}N_7O \cdot HBr \cdot 0.1$ MeOH (c) | 44.40 | 4.33 | 25.70 | 44.59 | 4.12 | 25.94 |
| 9 | $C_{13}H_{12}ClN_7$ | 51.75 | 4.01 | 32.49 | 51.65 | 4.15 | 32.78 |
| 10 | $C_{14}H_{15}N_7$ | 59.77 | 5.38 | 34.85 | 59.99 | 5.47 | 34.64 |
| 11 | $C_{13}H_{13}N_7 \cdot HBr$ | 44.84 | 4.05 | 28.16 | 45.09 | 4.16 | 27.96 |
| 12 | $C_{15}H_{17}N_7$ | 61.00 | 5.80 | 33.20 | 60.98 | 5.84 | 33.10 |
| 13 | $C_{14}H_{23}N_7O_2 \cdot 0.2\ H_2O$ | 51.74 | 7.26 | 30.17 | 51.70 | 7.34 | 30.25 |
| 14 | $C_{13}H_{19}N_7 \cdot 0.5\ H_2O$ | 55.30 | 7.14 | 34.73 | 55.18 | 7.14 | 34.88 |
| 15 | $C_{17}H_{15}N_7 \cdot 0.5\ H_2O$ | 62.56 | 4.94 | 30.04 | 62.37 | 4.73 | 29.64 |
| 16 | $C_{14}H_{13}N_7O_2 \cdot 0.5\ H_2O$ | 52.51 | 4.41 | 30.61 | 52.41 | 4.49 | 30.87 |
| 17 | $C_{13}H_{12}N_6O$ | 58.20 | 4.51 | 31.33 | 57.93 | 4.26 | 31.58 |
| 18 | $C_{13}H_{12}N_6S \cdot 0.5\ H_2O$ | 53.23 | 4.47 | 28.65 | 53.10 | 4.09 | 28.79 |

(a) Calcd. Br 19.52, Found 19.73. (b) Calcd. Br 10.43, Found 10.47. (c) Methanol confirmed by pmr.

Acknowledgments.

This investigation was supported by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare, Contract No. N01-CM-43762. The authors are indebted to Dr. W. C. Coburn, Jr., and Mrs. M. C. Thorpe who interpreted NMR data and to other members of the Molecular Spectroscopy Section of Southern Research Institute, who performed most of the microanalytical and spectral determinations.

REFERENCES

(1) J. R. Piper and J. A. Montgomery, *J. Org. Chem.*, **42**, 208 (1977).

(2) F. M. Sirotnak, D. M. Moccio, and D. M. Dorick, *Cancer Treat. Rep.*, **61**, 1325 (1977).

(3) D. Kessel, T. C. Hall, D. Roberts, and I. Wodinsky, *Science*, **150**, 752 (1965).

(4) F. M. Sirotnak and R. C. Donsbach, *Cancer Res.*, **35**, 1737 (1975).

(5) A. Goldin, J. M. Venditti, S. R. Humphreys, D. Dennis, N. Mantel, and S. W. Greenhouse, *ibid.*, **15**, 57 (1955).