Side-chain Altered Methotrexate Analogs Designed for Improved Membrane Transport (1)

Michael Chaykovsky, Andre Rosowsky, and Edward J. Modest

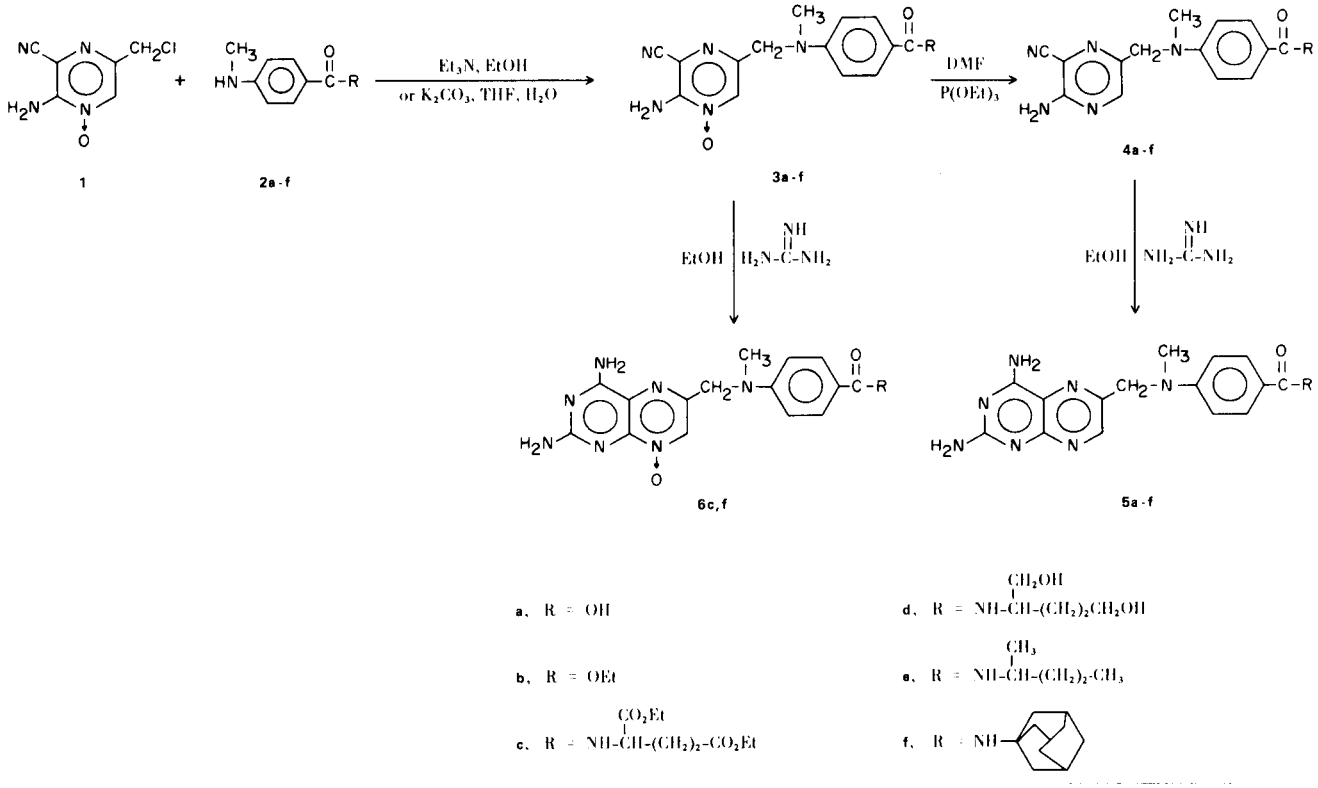
Children's Cancer Research Foundation and the Departments of Biological Chemistry and Pathology, Harvard Medical School, Boston, Massachusetts 02115

Received March 12, 1971

Sir:

Methotrexate (4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid: MTX; amethopterin) is a folic acid antagonist of established value as an antitumor agent, especially in the treatment of acute and subacute leukemia (2). However, its clinical usefulness is impaired by the development of drug resistance and by its ineffectiveness against tumors of the central nervous system. Previous studies in these laboratories have involved the design and synthesis of small molecule antifolates as improved antitumor and antimalarial agents (3). We are now engaged in a program aimed at the structural modification of methotrexate in order to prepare more effective analogs with possibly greater ease of transport across biological membranes (4a,b), including the blood-brain barrier. Primary consideration is being given to changes in the highly polar glutamate moiety, since changes at this site should not greatly affect the enzyme inhibitory properties of the 2,4-diaminopyrimido system, common to many folate antagonists (5), but should change the overall lipophilic character and transport properties of the molecule.

Early methods (6) for the synthesis of methotrexate and pteroic acid analogs led to impure products which were very difficult to purify. Recently, alternate synthetic approaches have been developed for folate (7-9) and methotrexate (10) analogs. In the work reported here, we chose to utilize a versatile and efficient syn-



thetic method for the unambiguous preparation of 6-substituted pteridines developed recently by Taylor and coworkers (11). This route involves the use of 2-amino-3-cyano-5-chloromethylpyrazine 1-oxide (**1**) (11), which was condensed with various *p*-(methylamino)benzoyl derivatives **2c-f** to yield the substituted pyrazine 1-oxides **3c-f** (Scheme I). Removal of the *N*-oxide function was carried out by heating to 120° with triethyl phosphite alone or with *N,N*-dimethylformamide as solvent to give pyrazines **4c-f**, which were then allowed to react with guanidine in refluxing ethanol to form the methotrexate analogs **5c-f**. Saponification of **5c** constitutes a new synthesis of methotrexate. Also prepared, starting with the *N*-oxides **3c,f**, were the 8-oxido analog of diethyl methotrexate **6c** and the 8-oxido adamantylamine analog **6f**. The intermediate *p*-(methylamino)benzoyl derivatives **2c, e,f** were synthesized according to the method of Santi (12) by reaction of *N*-tosyl-*p*-(methylamino)benzoyl chloride with the appropriate amine, followed by detosylation. Reduction of the diester **2c** with lithium aluminum hydride in tetrahydrofuran gave the diol **2d**.

Overall yields from **1** of the methotrexate analogs varied from 10-20% for the diesters **5c** and **6c** to 35-40% for **5d** and **5e** to 75-80% for the highly insoluble adamantyl analogs **5f** and **6f**. The relatively low yields for the diesters were due to side reactions of the ester groups with guanidine in the last step of the synthesis (13).

The preparation of **5e** will serve as an example of this synthesis. Reaction of equimolar amounts of **1** (m.p. 140-145° dec.) and **2e** (m.p. 98-99°) with excess potassium carbonate in 1:1 aqueous tetrahydrofuran at 25° for 90 minutes, followed by dilution with water and extraction with methylene chloride, gave a syrup which was chromatographed on silica gel (5% ethanol/benzene) to yield **3e** (63%) as a yellow glassy solid: ir (chloroform): λ 2.80 μ, 2.93, 3.23, 4.48, 6.10, 6.22, 6.50, 6.70. Heating **3e** with excess triethyl phosphite at 120° for 45 minutes and evaporation under vacuum gave **4e** as a yellow solid (72% from ethyl acetate/hexane, m.p. 184-185°): ir (chloroform): λ 2.80 μ, 2.90, 3.33, 4.50, 6.10, 6.22, 6.70.

Anal. Calcd. for $C_{19}H_{24}N_6O$ (352.43): C, 64.75; H, 6.86; N, 23.85. Found: C, 64.61; H, 6.85; N, 23.72.

Condensation of guanidine (1 molar equivalent, prepared from equimolar amounts of sodium ethoxide and guanidine hydrochloride in ethanol) with **4e** (one-half molar equivalent) for one hour at reflux afforded **5e** as a yellow solid (80%, m.p. 264-266° dec.); ir (potassium chloride): λ 3.35 μ, 6.15, 6.22, 6.42, 6.50, 6.63, 6.92; uv λ max (ethanol): 261 nm (ε 25,600), 291 (23,800), 375 (7,830).

Anal. Calcd. for $C_{20}H_{26}N_8O$ (394.474): C, 60.89; H, 6.64; N, 28.41. Found: C, 60.68; H, 6.49; N, 28.37.

In addition, following the same reaction scheme and starting with the readily available *p*-(methylamino)benzoic acid **2a** or the ethyl ester **2b**, efficient syntheses of 4-amino-4-deoxy-N^{10}-methylpteroic acid **5a** (60%, m.p. > 300° dec.) (6,10) and its ethyl ester **5b** (30%, m.p. 293-296° dec.) were developed. These compounds are potentially useful intermediates for the preparation of other methotrexate analogs.

In preliminary bioassays, several of these compounds show marked antifolate activity in the *Streptococcus faecium* - PGA system: **5a, 5b, 5c, 5e,** and **5f** have ID_{50} values less than 0.01 μg/ml, comparable to methotrexate. Compound **5c** effects a 50% increase in survival of mice with L1210 leukemia when given intraperitoneally at 32 mg/kg/day for four days (14). Additional biological properties of the methotrexate analogs described here, as well as others in preparation, will be reported elsewhere.

REFERENCES

(1) Supported in part by research grant C6516 and research career development award K3-CA-22,151 from the National Cancer Institute, National Institutes of Health, and by research contract DADA-17-71-C-1001 from the U. S. Army Research and Development Command, Office of the Surgeon General. This is publication No. 1098 from the Army Research Program on Malaria.

(2) R. B. Livingston and S. K. Carter, "Single Agents in Cancer Chemotherapy," IFI/Plenum, New York, N. Y., 1970, pp. 130-172.

(3) A. Rosowsky and E. J. Modest, *Ann. N. Y. Acad. Sci.*, 186, 258 (1971).

(4) The subject of methotrexate transport by the L1210 leukemia cell and other cells has been reviewed: (a) I. D. Goldman, *Ann. N. Y. Acad. Sci.*, 186, 400 (1971); (b) F. M. Sirotnak and R. C. Donsbach, *Cancer Res.*, 32, 2120 (1972).

(5) G. H. Hitchings and J. J. Burchall in "Advances in Enzymology", Vol. 27, F. F. Nord, Ed., Interscience Publishers, New York, N. Y., 1965, p. 417.

(6) D. R. Seeger, D. B. Cosulich, J. M. Smith, Jr., and M. E. Hultquist, *J. Am. Chem. Soc.*, 71, 1753 (1949).

(7) J. I. DeGraw, J. P. Marsh, Jr., E. M. Acton, O. P. Crews, C. W. Mosher, A. N. Fujiwara, and L. Goodman, *J. Org. Chem.*, 30, 3404 (1965).

(8) C. W. Mosher, E. M. Acton, O. P. Crews, and L. Goodman, *J. Org. Chem.*, 32, 1452 (1967).

(9) Y. H. Kim, V. Grubliauskas, and O. M. Friedman, *J. Heterocyclic Chem.*, 9, 481 (1972).

(10) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, 35, 1676 (1970).

(11) E. C. Taylor, "Chemistry and Biology of Pteridines, Proceedings of the Fourth International Symposium on Pteridines," Toba, July 1969, International Academic Printing Co., Ltd., Tokyo (1970), p. 79. We thank Professor Taylor, Princeton University, for providing us with experimental details for the preparation of compound **1** advance of his forthcoming publication.

(12) D. V. Santi, *J. Heterocyclic Chem.*, 4, 475 (1967).

(13) Compounds **3c,d,e** and **4c,d** were isolated after purification by column chromatography as viscous glasses or difficultly crystallizable solids and characterized by infrared spectroscopy. All other new compounds reported here were crystalline solids characterized by satisfactory combustion analyses (C, H, N) as well as by spectroscopic means.

(14) The biological data were obtained at The Children's Cancer Research Foundation by Dr. George E. Foley and Associates (*in vitro* assays) and Miss Barbara L. Brown (*in vivo* assays).