Synthesis and Antitumor Activity of 2,4-Diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine

Eugene M. Grivsky, Shuliang Lee, Carl W. Sigel, David S. Duch, and Charles A. Nichol*

Departments of Organic Chemistry and Medicinal Biochemistry, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709. *Received October 9, 1979*

The synthesis of 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine (BW301U, **7**) by a route that has general applicability to the preparation of many 6-(substituted benzyl)-5-methylpyrido[2,3-*d*]pyrimidines is described. The key intermediate, 2,4-diamino-7,8-dihydro-6-(2,5-dimethoxybenzyl)-5-methyl-7-oxopyrido[2,3-*d*]pyrimidine (**4**), is converted to the 7-chloro compound **5** by treatment with a 1:1 complex of *N*,*N*-dimethylformamide–thionyl chloride, and **5** is hydrogenolyzed with palladium on charcoal in the presence of potassium hydroxide to yield **7**. BW301U is a potent lipid-soluble inhibitor of mammalian dihydrofolate reductase and has significant activity against the Walker 256 carcinosarcoma in rats.

The synthesis[1] and antimicrobial activity[2] of several 6-substituted 2,4-diaminopyrido[2,3-*d*]pyrimidines was reported previously from these laboratories by B. S. Hurlbert and co-workers. The primary focus of that work was to develop compounds which were selective inhibitors of dihydrofolate reductases (DHFR) from microorganisms as opposed to the corresponding mammalian enzyme. Such selectivity could be exploited for the treatment of microbial infections in mammalian species. Initially, compounds within these series that were potent inhibitors of mammalian DHFR were not studied extensively as anticancer drugs, because of the already widespread clinical use of methotrexate (MTX). Subsequently, it became apparent that MTX has limited entry into some cells and tissues because of its polarity and requirement for carrier-mediated transport. Thus, interest arose in pyrimidines and condensed pyrimidines as smaller, more lipid-soluble molecules that cross membranes by passive diffusion. A series of 2,4-diaminopyrimidines were studied in this laboratory, and metoprine [DDMP, 2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine] was chosen for further evaluation.[3]

Metoprine was found to have antitumor activity in man, but its potential was limited by its long physiological half-life, which is about 10 days in man,[3] and its limited efficacy at tolerated doses.[4-9] In addition to folate-related hematological toxicity, other adverse effects included CNS, cutaneous, and gastrointestinal toxicity in patients.[6-9] Similar side effects were reported for triazinate, a diamino-*s*-triazine.[10] Subsequently, both compounds were shown to be potent inhibitors of histamine *N*-methyltransferase, and it is likely that some of their non-folate-related toxicity may be due to elevation of histamine levels.[11] Although the clinical evaluation of metoprine has been limited, these studies have provided support for the concept of developing lipid-soluble DHFR inhibitors as anticancer agents. Thus, attention was focused on some of the condensed pyrimidines and related compounds.

Table I. Cytotoxicity of Antifolates in Cell Culture

compound	cytotoxicity ED_{50}, μM		
	W256	S180	L1210
BW301U	0.008	0.012	0.022
methotrexate	0.020	0.016	0.016
metoprine	0.050	0.050	0.220

The search for selective toxicity among the pyrido[2,3-*d*]pyrimidines eventually led to improvement of the original synthetic scheme and specifically to the preparation of **7** [BW301U, 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine]. BW301U is as effective as MTX as an inhibitor of DHFR purified from human leukemic cells and, in contrast to metoprine, has minimal activity as an inhibitor of histamine metabolism.

Chemistry. Among the various synthetic schemes which have been used for preparing pyridopyrimidines,[12] the most general approach to 5,6-disubstituted 2,4-diaminopyrido[2,3-*d*]pyrimidine derivatives appeared to be through their 7,8-dihydro-7-oxo analogues, readily obtained by condensations with 2,4,6-triaminopyrimidine (Scheme I). Chlorination or thiation of the 7-oxo function, followed by catalytic reduction, should theoretically provide the target compounds.

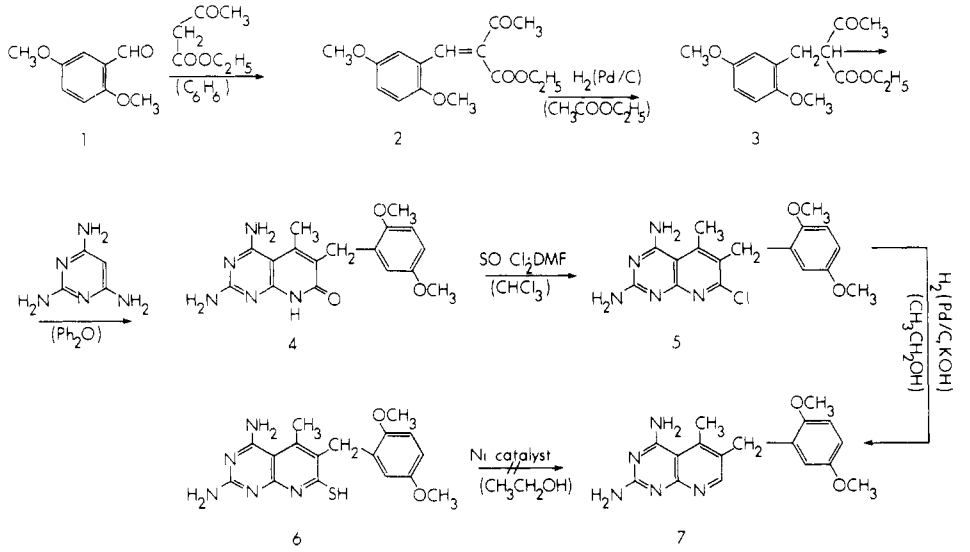
Hurlbert and co-workers[1] found this extremely difficult in practice. The use of phosphorus reagents led to complexes which could not be successfully reduced. The Vilsmeier dimethylformamide–thionyl chloride reagent[13] provided halo derivatives which could be successfully converted to the 7,8-dihydro-7-thiones. Reduction with Raney nickel was accomplished in very low yields, probably because of absorption of the compounds on the huge excess of catalyst.

Here it was found possible to obtain a pure 7-chloro derivative (**5**) by a modification of the Vilsmeier procedure which utilized a 1:1 complex of the two reagents. Hy-

(1) (a) B. S. Hurlbert, K. W. Ledig, P. Stenbuck, B. F. Valenti, and G. H. Hitchings, *J. Med. Chem.*, **11**, 703 (1968); (b) B. S. Hurlbert and B. F. Valenti, *ibid.*, **11**, 708 (1968).
(2) B. S. Hurlbert, R. Ferone, T. A. Herrmann, G. H. Hitchings, M. Barnett, and S. R. M. Bushby, *J. Med. Chem.*, **11**, 711 (1968).
(3) J. C. Cavallito, C. A. Nichol, W. D. Brenckman, Jr., R. L. DeAngelis, D. R. Stickney, W. S. Simmons, and C. W. Sigel, *Drug Metab. Dispos.*, **6**, 329 (1978).
(4) M. L. Murphy, R. R. Ellison, D. A. Karnofsky, and J. H. Burchenal, *J. Clin. Invest.*, **33**, 1388 (1954).
(5) D. R. Stickney, W. S. Simmons, R. L. DeAngelis, R. W. Rundles, and C. A. Nichol, *Proc. Am. Assoc. Cancer Res.*, **14**, 52 (1973).
(6) L. A. Price, J. H. Goldie, and B. T. Hill, *Br. Med. J.*, **2**, 230 (1975).
(7) L. A. Price and B. T. Hill, *Proc. Am. Assoc. Cancer Res.*, **17**, 15 (1976).
(8) D. S. Miller, R. W. Rundles, C. A. Nichol, J. L. Woolley, and C. W. Sigel, *Proc. Am. Soc. Clin. Oncol.*, **17**, 263 (1976).
(9) P. Alberto, R. Peytremann, R. Medenica, and M. Beretta-Piccoli, *Cancer Chemother. Pharmacol.*, 1, 101 (1978).
(10) R. T. Skeel, A. R. Cashmore, W. L. Sawicki, and J. R. Bertino, *Cancer Res.*, **36**, 48 (1976).
(11) D. S. Duch, S. W. Bowers, and C. A. Nichol, *Biochem. Pharmacol.*, **27**, 1507 (1978).
(12) W. T. Irwin and D. G. Wibberley, *Adv. Heterocycl. Chem.*, **10**, 150–171 (1969).
(13) (a) A. Vilsmeier and A. Haack, *Ber. Dtsch. Chem. Ges. B*, **60**, 119 (1927); (b) H. H. Bosshard, R. Mory, M. Schmid, and H. Zollinger, *Helv. Chim. Acta*, **42**, 1653 (1959).

0022-2623/80/1823-0327$01.00/0

Scheme I



drogenolysis of **5** was accomplished with palladium on charcoal catalyst in the presence of KOH to obtain the desired pyridopyrimidine **7** in reasonably good yield. This proved to be a successful route for obtaining a large number of related derivatives, to be reported in a future publication.[14]

Biological Results and Discussion. BW301U was tested as an inhibitor of DHFR purified from human chronic granulocytic leukemic cells using a Sepharose–MTX affinity column.[15] The enzyme was assayed as described previously.[16] It had activity comparable to MTX and was more potent than metoprine; IC_{50} values for BW301U, MTX, and metoprine were 5, 7, and 95 nM, respectively. Likewise, in cell cultures of S-180, W-256, and L1210, BW301U was at least as potent as MTX and much more potent than metoprine in preventing cell growth (Table I).

The results presented in Figure 1 show that BW301U had a marked antitumor effect against the Walker 256 carcinosarcoma. At 17 days after implantation of tumors, the mean tumor volume for the group treated with 25 mg/kg daily was 29% of control, and for the group treated with 15 mg/kg twice daily was 10% of control. Compared to untreated tumor-bearing animals, the appearance of palpable tumors in the treated groups was significantly delayed or prevented by drug treatment. Seventeen days after implantation, 7 of 20 rats treated with 25 mg/kg daily and 4 of 20 rats treated with 15 mg/kg twice daily had no palpable tumors. In the group treated with 25 mg/kg, 6 of 20 animals were considered cured, since no tumors developed by 80 days after tumor implantation. Using change in body weight as an index of toxicity, there was no sign of significant drug toxicity.

In another study, Walker 256 tumors were implanted subcutaneously and drug treatment was delayed until 10 days after implantation. Drug treatment consisted of 30 mg/kg on days 10–13 and 50 mg/kg on day 20. Tumors in rats that received no treatment grew rapidly, whereas those in rats which received drug showed a decrease in mean tumor volume during the period of observation.

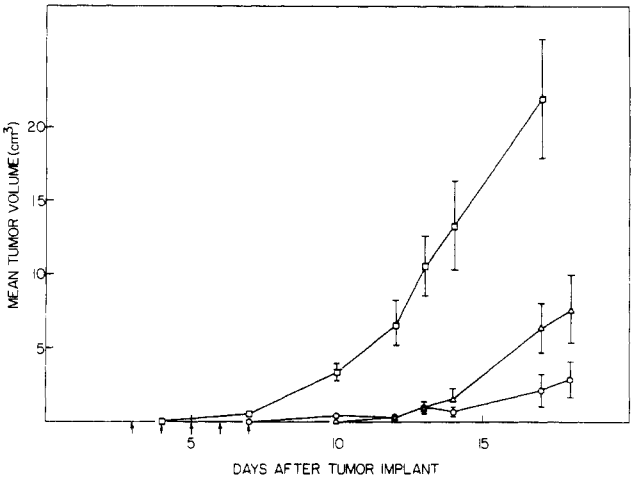


Figure 1. Antitumor effect of BW301U on Walker 256 carcinosarcoma: (□–□) tumor control; (△–△) 25 mg/kg daily; (○–○) 15 mg/kg twice daily.

Twenty-three days after implantation the mean tumor volume for the treated group was 3% of the control group.

Thus, BW301U is a potent inhibitor of mammalian DHFR and inhibits the growth of several cell lines in vitro at least as well as MTX. Also, the compound has significant antitumor activity in vivo against the Walker 256 carcinosarcoma in rats. BW301U has a degree of lipophilicity [log *P* (octanol/water) = 1.74] which may facilitate passage across cell membranes and thereby act on MTX-resistant cells or forms of cancer unresponsive to MTX. In addition, this compound has minimal activity as an inhibitor of histamine metabolism, which should reduce the risk of histamine-related side effects.[17]

Experimental Section

Melting points were determined in open glass capillary tubes with a Büchi melting point apparatus and are uncorrected. Satisfactory elemental analyses (C, H, N, and Cl within ±0.4% of the theoretical values) were obtained from the Microanalytical Laboratories of Burroughs Wellcome Co., R.T.P., NC, or Atlanta Microlab, Inc., Atlanta, GA. UV spectra were recorded on a Beckman DU spectrophotometer and IR (impressed KBr disks or films) on a Beckman IR-8 infrared spectrometer. NMR spectra were recorded on a Varian TA-60 instrument using tetra-

(14) E. M. Grivsky and S. Lee, unpublished results.
(15) D. S. Duch, C. W. Sigel, S. W. Bowers, M. P. Edelstein, J. C. Cavallito, R. G. Foss, and C. A. Nichol, Proceedings of the 11th International Congress of Chemotherapy, Boston, Oct 1979, in press.
(16) D. S. Duch, D. D. Bigner, S. W. Bowers, and C. A. Nichol, *Cancer Res.*, **39**, 487 (1979).
(17) D. S. Duch, M. P. Edelstein, and C. A. Nichol, *Pharmacologist*, **21**, 266 (1979).

methylsilane as the internal standard. UV, IR, and NMR spectra on all intermediates and the final product were consistent with the proposed structure and are not included. TLC was performed on silica gel sheets with a fluorescent indicator. Compounds were visualized using a UV lamp and migrated as single spots. Octanol–water partition coefficients were determined by the method of Leo, Hansch, and Elkins.[18]

Ethyl α-Acetyl-β-(2,5-dimethoxyphenyl)acrylate (2). A mixture of 2,5-dimethoxybenzaldehyde (100 g, 0.6 mol), freshly distilled ethyl acetoacetate [84.5 g, 0.65 mol, bp 73 °C (15 mm)], anhydrous benzene (200 mL), piperidine (6 mL), and glacial acetic acid (12 mL) was refluxed in the presence of a Dean–Stark trap until almost the theoretical amount of water had been collected (~3 h). The reaction mixture was cooled, benzene (300 mL) was added, and the solution was washed successively with water (100 mL), cold 0.1 N hydrochloric acid (200 mL), 5% aqueous sodium bicarbonate (200 mL), and acetic acid–water (1:99, 100 mL). After drying over anhydrous magnesium sulfate, the benzene solution was filtered and concentrated by distillation of the solvent in vacuo. The residue was distilled, yielding 104.0 g (64%) of **2**, bp 169–170 °C (0.3 mmHg). Recrystallization from ethanol–pentane afforded an analytical sample, mp 72–73 °C. Anal. ($C_{15}H_{18}O_5$) C, H.

Ethyl α-Acetyl-β-(2,5-dimethoxyphenyl)propionate (3). The acrylate **2** (38.0 g, 0.15 mol) was dissolved in ethyl acetate (150 mL) and hydrogenated (20–40 psi of H_2) in a Parr low-pressure apparatus using 5% palladium on charcoal catalyst (1.5 g) until approximately the theoretical quantity of hydrogen was absorbed (1–2 h). The catalyst was removed by filtration and the solvent concentrated by distillation in vacuo. The reaction product was purified by vacuum distillation to afford 38.4 g (89%) of **3**, bp 146–148 °C (0.3 mmHg). Anal. ($C_{15}H_{21}O_5$) C, H.

2,4-Diamino-7,8-dihydro-6-(2,5-dimethoxybenzyl)-5-methyl-7-oxopyrido[2,3-*d*]pyrimidine (4). In a flask equipped with a Dean–Stark trap, a mixture of the propionate **3** (21.2 g, 0.079 mol), 2,4,6-triaminopyrimidine (100 g, 0.08 mol), and diphenyl ether (100 mL) was heated rapidly with vigorous stirring to 190 °C and maintained at 195–230 °C until no additional water–ethanol mixture was distilled (~1.5 h). The reaction mixture was cooled to room temperature, methanol (100 mL) was added, and the crude product was collected by filtration. The product was suspended in boiling water (500 mL) and filtered, and the solid was washed with hot water (500 mL) followed by methanol (100 mL) and pentane (50 mL) and then dried to give 17.0 g (62%) of the 7-oxo compound **4** as a yellow brownish solid, mp 325–326 °C. Anal. ($C_{17}H_{19}N_5O_3$) C, H, N.

7-Chloro-2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine (5). To a solution of *N,N*-dimethylformamide (17.5 mL, 0.24 mol) in dry chloroform (100 mL), carefully cooled in an ice bath, a solution of thionyl chloride (28.6 g, 0.24 mol) in dry chloroform was added dropwise so that the temperature remained below 5 °C. When the exothermic reaction resulting from formation of the dimethylformamide–thionyl chloride complex (1:1) subsided, the 7-oxo compound **4** (8.2 g, 0.024 mol) was added over a period of 10–15 min. The reaction mixture was gradually allowed to reach room temperature and then heated under gentle reflux for 3 h. The reaction mixture was cooled and treated with ethanolic base (80 mL) while maintaining the temperature at 25–30 °C by cooling. The brownish solid thus formed was collected by filtration, slurried in 50% aqueous ammonium hydroxide (200 mL), filtered, washed with water (150–200 mL), and dried to afford 3.4 g (40%) of the 7-chloro compound **5**, mp 192–200 °C dec. Recrystallization from aqueous ethanol afforded an analytical sample, mp 193–196 °C dec. Anal. ($C_{17}H_{18}ClN_5O_2 \cdot 0.25H_2O$) C, H, N, Cl.

2,4-Diamino-6-(2,5-dimethoxybenzyl)-5 methylpyrido[2,3-*d*]pyrimidine (7). The pure 7-chloro compound **5** (0.3 g, 0.8 mmol) was dissolved in absolute ethanol (200 mL), and potassium hydroxide (0.2 g) and 5% palladium on charcoal catalyst (0.2 g) were added. Hydrogenolysis was conducted in a Parr low-pressure apparatus with the hydrogen pressure between 35 and 40 psi. The reduction required about 36 h. The reaction mixture was filtered to remove the catalyst, and the solvent was eliminated by evaporation under reduced pressure. The residue was dissolved in a small amount of ethanol, and water was added to produce 0.1 g (38%) of **7** as a yellow powder, mp 252–254 °C. An analytical sample from a similar preparation was recrystallized from aqueous ethanol–hydrochloric acid as the hydrochloride salt, mp 283–286 °C. Anal. ($C_{17}H_{19}N_5O_2 \cdot HCl \cdot 0.5H_2O$) C, H, N, Cl.

Cytotoxicity in Cell Culture. To determine the cytotoxicity of the antifolates in cell culture, 1×10^5 cells were seeded in 35-mm Petri dishes (W-256, S-180) or 15-mL culture tubes (L1210) using Dulbecco's modified Eagle medium containing 10% fetal calf serum. Drugs were added 6 h later. Following a growth period of 72 h, monolayers of W256 and S180 were trypsinized and cell counts determined using a Coulter counter. Results are expressed as ED_{50}, the concentration of drug required for 50% inhibition of cell growth.

Antitumor Activity. Walker 256 carcinosarcoma was implanted subcutaneously in the right flank of male Sprague–Dawley rats. Drug treatment commenced on the 3rd day following implantation of tumors and continued for 5 successive days. Tumor-bearing animals received BW301U at a dose of either 25 mg/kg daily or 15 mg/kg twice daily. Drug was administered intraperitoneally as a suspension in methylcellulose. Tumor volumes and body weights were monitored daily.

Acknowledgment. The authors thank Dr. David A. Yeowell and his associates of the Chemical Development Laboratories for helpful suggestions regarding the synthesis of the pyridopyrimidines and S. W. Bowers and M. P. Edelstein, Department of Medicinal Biochemistry, for their technical assistance. Thanks are due Dr. Stuart B. Hurlbert and Dr. David A. Brent, Organic Chemistry Department, for NMR and mass spectral data, respectively.

(18) A. Leo, C. Hansch, and D. Elkins, *Chem. Res.*, 71, 525 (1971).

Synthesis and Biological Activity of Some 15-Oxaestranes

Perry Rosen,* Alfred Boris, and Gloria Oliva

Chemical Research Division, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received October 1, 1979*

The estrogenic activity of orally administered 15-oxaestrone was evaluated by the uterotropic assay in rats and was found to be 12 times greater than that of estrone. In addition, several analogues of 15-oxaestrone were prepared and their estrogenic potency was determined.

The preparation of 15-oxaestrone[1] (**1**) has been described previously. We now report the synthesis of various analogues derived from **1**, as well as the estrogenic activity found for these compounds.

Chemistry. Various derivatives of the natural hormone estrone have been prepared in an attempt to obtain compounds which would possess greater activity than the parent compound when administered orally. One such derivative, 17α-ethynylestradiol, has been shown in hu-

(1) P. Rosen and G. Oliva, *J. Org. Chem.*, 38, 3040 (1973).

0022-2623/80/1823-0329$01.00/0