(5) G. Ceccarelli, A. Balsamo, P. Crotti, B. Macchia, and F. Macchia, International Symposium on Magnetic Resonance, 5th, Bombay, Jan 1974, Abstract 13A44.
(6) U. M. Teotino, L. Polo Friz, G. Steis, and D. Della Bella, *J. Pharm. Pharmacol.*, **15**, 26 (1963).
(7) L. Almirante and W. Murmann, *J. Med. Chem.*, **9**, 650 (1966).
(8) P. N. Patil, A. Tye, C. May, S. Hetey, and S. Miyagi, *J. Pharmacol. Exp. Ther.*, **163**, 309 (1968).
(9) M. G. Moreira and W. Osswald, *Nature (London)*, **208**, 1006 (1965).
(10) (a) R. P. Ahlquist, *Am. J. Physiol.*, **153**, 568 (1948); (b) P. Pratesi and E. Grana, *Adv. Drug. Res.*, **2**, 127 (1965); (c) E. J. Ariëns, *Ann. N.Y. Acad. Sci.*, **139**, 606 (1967); (d) R. T. Brittain, D. Jack, and A. C. Ritchie, *Adv. Drug. Res.*, **5**, 197 (1970); (e) E. J. Mylecharane and C. Raper, *Eur. J. Pharmacol.*, **21**, 375 (1973); see also the references quoted therein.
(11) In this work, as well as in previous ones,[3,4] morpholine derivatives have been considered to be obtained by cyclizing the corresponding open-chain compounds by substituting the two hydrogen atoms linked, respectively, to the alcoholic oxygen and to the basic nitrogen with a CH_2CH_2 chain; following this criterion, there is correspondence between **5** and **1**, **6**, **2**, and so on. On the other hand, compound **5** could be seen as a derivative of **2** if we cyclize it by substituting the hydrogen of the OH group and one hydrogen of the N-CH_3 group with a CH_2 group. The pharmacological results and, in particular, the good agreement between the changes in values of the biological parameters in the series of the open-chain compounds 1–4 and in the series of the cyclic derivatives 5–8 when the two series are compared following the first point of view (see Table I) speak in favor of this choice.
(12) U. M. Teotino, L. Polo Friz, G. Steis, and D. Della Bella, *Farmaco, Ed. Sci.*, **17**, 252 (1962).
(13) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed, Pergamon Press, Oxford, 1969, p 280–301.
(14) M. Tichý, *Adv. Org. Chem.*, **5**, 115 (1965); G. Drefahal, G. Heublein, and S. Lochner, *J. Prakt. Chem.*, **32**, 87 (1966); G. Bellucci, B. Macchia, F. Macchia, and F. Romoli, *Farmaco, Ed. Sci.*, **26**, 931 (1971).
(15) O. D. Gulati, S. D. Gokhale, H. M. Parikh, B. P. Udwadia, and V. S. R. Krishamurty, *J. Pharmacol. Exp. Ther.*, **166**, 35 (1969).
(16) It can be pointed out at this point that anomalous behaviors in the changes of receptor activity, resulting from the introduction of *N*-alkyl substituents of different bulk, in series of adrenergic agents have also been observed in other cases.[17,18]
(17) J. G. Cannon, J. P. O'Donnell, T. Lee, C. R. Hoppin, J. P. Long, M. Ilhan, B. Costall, and R. J. Naylor, *J. Med. Chem.*, **18**, 1212 (1975).
(18) A. Balsamo, A. Lapucci, B. Macchia, F. Macchia, and R. Ceserani, International Symposium on Medicinal Chemistry, Brighton, Sept 1978, Abstract P42.
(19) C. R. Ganellin, *J. Med. Chem.*, **20**, 579 (1977).
(20) P. Pratesi, E. Grana, and L. Villa, *Farmaco, Ed. Sci.*, **26**, 379 (1971).
(21) B. Belleau, *Ann. N.Y. Acad. Sci.*, **139**, 580 (1967).
(22) P. Pratesi, L. Villa, and E. Grana, *Farmaco, Ed. Sci.*, **30**, 315 (1975).

Synthesis and Biological Activity of 8-Oxadihydropteridines

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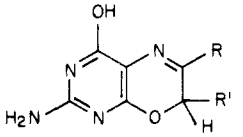
A series of 6-substituted and 6,7-disubstituted pyrimido[4,5-*b*][1,4]oxazines (8-oxadihydropteridines) was synthesized through the condensation of an α-halo ketone and 2,5-diamino-4,6-pyrimidinediol. The resulting 8-oxadihydropteridines were assayed as potential antifolates in a dihydrofolate reductase enzyme system. The 2-amino-4-hydroxyoxadihydropteridines were found to possess greater biological activity than the corresponding 2,4-diamino compounds. The pteroic acid homeostere 2-amino-4-hydroxy-6-phenethyl-8-oxadihydropteridine was the most potent of the compounds tested.

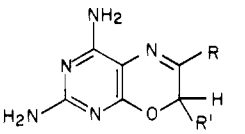
The use of folic acid analogues in the chemotherapy of cancer is well established,[1,2] and alterations in the vitamin structure have produced both classical and nonclassical antimetabolites.[3,4]

Homeosteric[5] substitutions in the pteridine ring system have been primarily limited to carbon–nitrogen interchanges,[6] and their activities have been studied as inhibitors of dihydrofolate reductase,[7] thymidylate synthetase,[8] or growth of microbial systems.[9] A recent report has described the preparation of some pyrimidothiazines which strongly inhibit dihydrofolate reductase.[10] The replacement of nitrogen by oxygen, however, has received little study in this system.[11]

The reactions of 2,5-diamino-4,6-pyrimidinediol hydrochloride with the appropriately substituted α-chloro ketones were effected in refluxing aqueous ethanol with sodium bicarbonate added to maintain a basic medium. The reaction products were the corresponding 6-substituted or 6,7-disubstituted 8-oxadihydropteridines **1** (Table I). Several previously unreported derivatives were synthesized and characterized by elemental analysis and by UV and NMR spectra (Table II). Since the pyrimidine used has a plane of symmetry through the 2 and 5 positions, the structures of the products (**1**) were unequivocal. The analogous 4-amino derivatives were prepared by the method described by Mirza et al. using 2,5,6-triamino-6-pyrimidol.[12]



1, R = alkyl, aryl, or hydrogen
R' = hydrogen, alkyl, or aryl



2, R = CH_3 or C_6H_5
R' = H, CH_3, or C_6H_5

None of the compounds prepared in this study were toxic to the growth of *Streptococcus faecalis* (ATCC 8043) at the limit of their solubilities (ca. 10 μg/mL), whereas amethopterin completely inhibited bacterial growth at a concentration of 1 μg/mL. It is possible that the bicyclic

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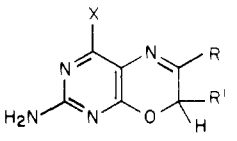
Table I. Biological Activity of the 8-Oxadihydropteridines 1 and 2



compd	X	R	R'	dihydrofolate reductase $[I/S]_{50}$[a]
1a[b]	OH	CH_3	CH_3	377
1b[b]	OH	C_6H_5	C_6H_5	c
1c[b]	OH	C_6H_5	CH_3	c
1d[b]	OH	CH_3	C_6H_5	740[d]
1e[b]	OH	C_6H_5	H	57
1f[b]	OH	CH_3	H	242
1g	OH	$C_6H_5CH_2$-	H	28
1h	OH	$C_6H_5(CH_2)_2$-	H	3
1i	OH	$C_6H_5(CH_2)_3$-	H	7
1j	OH	$C_6H_5(CH_2)_4$-	H	8
1k	OH	$CH_3(CH_2)_4$-	H	11
2a[e]	NH_2	CH_3	CH_3	903
2b[e]	NH_2	C_6H_5	C_6H_5	c
2c[e]	NH_2	C_6H_5	H	168

[a] Methotrexate (1.5×10^{-3} μM) produced 50% inhibition of dihydrofolate reductase in the presence of 3.8 μM dihydrofolic acid under the same conditions. [b] Reference 11. [c] No inhibition was observed at its maximum concentration. [d] The 50% inhibition point could not be reached due to the insolubility of the compound. The $[I/S]_{50}$ value was obtained by extending the V_o/V_I against I curve to the 50% inhibition point. [e] Reference 12.

structure can not penetrate the cell wall in the microbial assay, since the glutamate moiety of folic acid may be necessary for active transport.[13]

In an effort to determine if the oxapteridine ring system has potential physiological activity, the biological activity of the derivatives in the presence of dihydrofolate reductase was determined. Baker and co-workers have evaluated potential antifolate analogues using enzymatic systems such as dihydrofolate reductase.[4]

The relative biological activities of the 8-oxadihydropteridines as inhibitors of dihydrofolate reductase are summarized in Table I. Several of the new derivatives did inhibit the enzyme and it was observed that the 2-amino-4-hydroxy-8-oxadihydropteridines are more active than the corresponding 2,4-diamino analogues. For example, compare the inhibition indices of **1a** (377) with **2a** (903) and of **1e** (57) with **2c** (168), and the phenethyl analogue **1h** was the most potent of the compounds tested ($[I/S]_{50}$ = 3). This presumably is the result of the

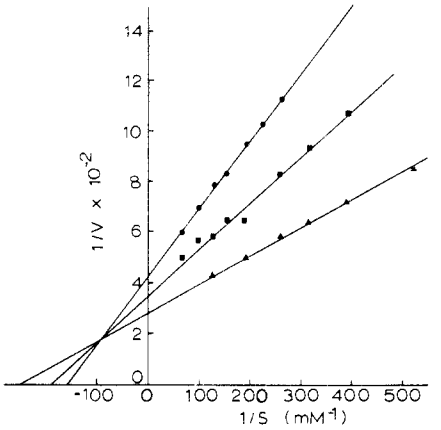


Figure 1. Lineweaver–Burk reciprocal plot of the effect of 2-amino-4-hydroxy-6-(β-phenylethyl)-8-oxadihydropteridine (**1h**) on dihydrofolate reductase. Reaction velocities (V) were determined in the presence of dihydrofolic acid (S), using 15 (▲), 31 (■), and 62 μM (●) inhibitor. V is expressed in optical density change per minute.

phenethyl moiety being homeosteric with the corresponding *N*-methyleneaniline side chain of pteroic acid. Substitutions at the 7 position of these analogues resulted in a decrease in activity which may be caused by a steric interference with the binding of the analogue to an enzyme site. A Lineweaver–Burk reciprocal plot using the 6-phenylethyl analogue (Figure 1) showed that for three different inhibitor concentrations the lines did not intersect on either the x or y axis, which suggests that the 8-oxadihydropteridines function as mixed-type inhibitors. The inhibitory effect of amethopterin on chicken liver dihydrofolate reductase has been reported to be noncompetitive.[15]

On the basis of these data, the 8-oxadihydropteridine ring system appears to be sufficiently similar to the pteridine nucleus to warrant further investigation as a source of potential antimetabolites.

Experimental Section

Melting points were determined in a capillary melting point apparatus and are uncorrected. UV spectra were determined with a Beckman DB-GT grating spectrophotometer and NMR spectra on a Jeol-PS-100 high-resolution NMR spectrophotometer at 100 MHz, using tetramethylsilane as internal standard. Microanalyses were performed by Heterocyclic Chemical Co., Harrisonville, Mo., and were within ±0.4% of the theoretical value unless otherwise indicated. Pigeon liver acetone powder was obtained from Nutritional Biochemicals Corp., and dihydrofolic acid (sealed under nitrogen) was purchased from Sigma Chemical Co.

α-Chloro Ketones. These compounds were prepared according to a general procedure for the synthesis of chloromethyl ketones.[16]

Table II. Physical and Spectral Data for 6-Substituted 2-Amino-4-hydroxy-7*H*-pyrimido[4,5-*b*][1,4]oxazines 1g–1k

compd	substit at C-6	mp (dec), °C	yield, % (anal.)	UV: λ_{max}, nm; log ε		NMR, δ[a]			
				2 N HCl	2 N NaOH	C-9 CH_2	$(CH_2)_n$	7-H, s, 2 H	NH_2, C_6H_5, m, 7 H
1g	$C_6H_5CH_2$	239–242	62 (C, H, N)	257, 3.99 349, 3.73	276, 3.98 326, 3.91	4.23 (s, 2 H)		5.46	7.47
1h	$C_6H_5(CH_2)_2$	280–284	66 (C, H, N)	264, 4.06 352, 3.88	273, 3.65 320, 3.64	3.23 (m, 4 H)[b]		5.43	7.38
1i	$C_6H_5(CH_2)_3$	250–253	61 (C, H, N)	266, 3.94 351, 3.71	276, 4.04 323, 4.00	2.87 (m, 4 H)[b]	2.19 (m, 2 H)	5.29	7.29
1j	$C_6H_5(CH_2)_4$	259–263	65 (C, H, N)	262, 3.85 347, 3.89	276, 3.89 323, 3.89	2.63 (m, 4 H)[b]	1.17 (m, 4 H)	5.20	7.02
1k	$CH_3(CH_2)_4$	272–275	70 (C, H, N)	264, 3.96 350, 3.72	277, 4.02 323, 4.00	3.01 (t, 2 H)	1.88 (m, 2 H)[c]	5.70	7.82

[a] Solvent used was CF_3COOH. Abbreviations used are: s, singlet; d, doublet; m, multiplet. [b] Signal includes 2 H of benzylic methylene. [c] Signal was assigned to C-10 methylene and seven others overlapped in a broad band.

1-Chloro-5-phenyl-2-pentanone: bp 127–132 °C (7 Torr), solidified and melted at 26–29 °C; n^{30}_D 1.5211; NMR (Me_2SO-d_6) δ 1.84 (q, 2 H, $-COCH_2CH_2CH_2C_6H_5$), 2.41 (m, 4 H, $-COCH_2CH_2CH_2C_6H_5$), 3.88 (s, 2 H, $-COCH_2Cl$), 7.20 (s, 5 H, $-C_6H_5$). Anal. ($C_{11}H_{13}C_{10}$) C, H. 1-Chloro-6-phenyl-2-hexanone: bp 111–116 °C (3 Torr); n^{20}_D 1.5230; NMR (Me_2SO-d_6) δ 1.47 (m, 4 H, $-COCH_2CH_2CH_2CH_2C_6H_5$), 2.40 (m, 4 H, $-COCH_2CH_2CH_2CH_2C_6H_5$), 3.85 (s, 2 H, $-COCH_2Cl$), 7.13 (s, 5 H, $-C_6H_5$). Anal. ($C_{12}H_{15}ClO$) C, H. 1-Chloro-3-phenyl-2-propanone,[16] 1-chloro-4-phenyl-2-butanone,[16,17] and 1-chloro-3-heptanone[18] physical constants were in agreement with literature values.

7*H*-Pyrimido[4,5-*b*][1,4]oxazines (8-Oxadihydropteridines) (1). The 2-amino-4-hydroxy-6-ω-phenylalkyl derivatives **1g–1j** and the 6-pentyl derivative **1k** were synthesized by the same general procedures. A suspension of 2.06 g (0.01 mol) of 2,5-diamino-4,6-pyrimidinediol[11] hydrochloride sesquihydrate in 500 mL of 1:1 ethanol–water was heated under reflux, and an appropriate α-halo ketone (0.02 mol) dissolved in 25 mL of ethanol was added dropwise. After 10–15 min, 25 mL of aqueous sodium bicarbonate (1.68 g, 0.02 mol) was added, heating was continued for 6 h, and the mixture was cooled overnight. The precipitated 8-oxadihydropteridine was separated by filtration, washed, and dried under reduced pressure. Physical constants and spectral data of all new compounds are summarized in Table II.

The 2,4-diamino derivatives **2** were synthesized from 2,4,5-triamino-6-pyrimidinol using a previously described procedure.[12]

Dihydrofolate Reductase Assay. The enzyme was obtained as previously described,[14] except that centrifugation in an International refrigerated centrifuge Model B-20 at 10000*g* and 2 °C for 0.5–1 h was used to separate precipitated protein instead of filtration through Celite. After a threefold dilution, 50 μL of enzyme solution gave an optical density change of 0.0062 unit/min.

For a typical assay, 50 μL of dihydrofolate reductase solution and 75 (reference cuvette) or 100 μL (sample cuvette) of 0.744 mM TPNH were mixed with sufficient buffer (0.05 M Tris, pH 7.4; 10 mM mercaptoethanol; and 1 mM EDTA) to make a total volume of 3 (reference cuvette) or 2.95 mL (sample cuvette). The enzymatic reaction was initiated by the addition of 50 μL of 0.186 mM dihydrofolic acid solution to the sample cuvette, and the decrease in absorbancy at 340 nm was recorded.[19] Solutions of the analogues were prepared by dissolving them in 25% *N,N*-dimethylformamide or 0.002 N sodium hydroxide and adjusting the pH to 7.4 if necessary. A maximum of 1 mL of inhibitor solution was used in the assay.

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References and Notes

(1) J. R. Bertino, Ed., *Ann. N.Y. Acad. Sci.*, **186**, 1–519 (1971).
(2) W. R. Shapiro, B. M. Mehta, and D. J. Hutchison, Eds., *Cancer Treat. Rep.*, **61**, 505–756 (1977).
(3) B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez, and E. Tong, *J. Med. Pharm. Chem.*, **2**, 633 (1960).
(4) B. R. Baker, "Design of Active-Site Directed Irreversible Enzyme Inhibitors", Wiley, New York, 1967, pp 192–284.
(5) Defined as two or more chemical moieties which are similar in mass or shape and valence bond angles. C. G. Skinner and D. R. Sargent, *J. Agr. Food Chem.*, **21**, 1057 (1973).
(6) J. A. Montgomery, T. P. Johnston, and Y. R. Shealy in "Medicinal Chemistry", Vol. I, 3rd ed, A. Burger, Ed., Wiley-Interscience, New York, 1970, pp 714–719.
(7) F. M. Huennekens, R. B. Dunlap, J. H. Freisheim, L. E. Gundersen, N. G. L. Harding, S. A. Levison, and G. P. Mell, *Ann. N.Y. Acad. Sci.*, **186**, 85 (1971).
(8) R. B. Dunlap, N. G. L. Harding, and F. M. Huennekens, *Ann. N.Y. Acad. Sci.*, **186**, 85 (1971).
(9) (a) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood, and H. Vanderwerff, *J. Biol. Chem.*, **183**, 1 (1950); (b) G. H. Hitchings, *Ann. N.Y. Acad. Sci.*, **186**, 444 (1971).
(10) M. N. Preobrazhenskaya in "Methods of Development of New Anti-cancer Drugs", J. F. Saunders and S. K. Carter, Eds., U.S. Government Printing Office, Washington, D.C., 1977, pp 195–205.
(11) D. L. Dunn and C. G. Skinner, *J. Org. Chem.*, **40**, 3713 (1975).
(12) J. Mirza, W. Pfleiderer, A. D. Brewer, A. Stuart and H. C. S. Wood, *J. Chem. Soc. C*, 437 (1970).
(13) L. T. Weinstock, D. E. O'Brien, and C. C. Cheng, *J. Med. Chem.*, **11**, 1238 (1968).
(14) B. R. Baker, B. T. Ho, and T. Neilson, *J. Heterocycl. Chem.*, **1**, 79 (1964).
(15) M. J. Osborn, M. Freeman, and F. M. Huennekens, *Proc. Soc. Exp. Biol. Med.*, **97**, 429 (1958).
(16) W. E. McPhee and E. Klingsberg, "Organic Syntheses", Collect. Vol. III, 1955, p 119.
(17) H. R. Henze and C. B. Holden, *J. Am. Chem. Soc.*, **63**, 1943 (1941).
(18) J. F. Bunnett and D. S. Tarbell, *J. Am. Chem. Soc.*, **67**, 1944 (1945).
(19) C. K. Mathews and F. M. Huennekens, *J. Biol. Chem.*, **238**, 3436 (1963).

Synthesis and Antibacterial Activity of 2-Oxocephalosporins

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The first reported synthesis of 2-oxocephalosporin derivatives has been achieved via ozonolysis of the corresponding 2-methylenecephalosporins. The new cephalosporin derivatives showed some antibacterial activity against Gram-positive bacteria, but the 2-oxo analogue of cephalothin was much less active than cephalothin itself.

In a search for unique and potent cephalosporin antibiotics, we have prepared a series of 2-oxocephalosporins. By total synthsis, Woodward and co-workers prepared a 2-oxocepham[1] which lacks the crucial Δ^3 double bond.[2] We anticipated that the 2-keto group in conjugation with the Δ^3 double bond would modify the reactivity of the β-lactam carbonyl and thereby enhance the antibacterial activity of this class of cephalosporin derivatives.[3]

Chemistry. The 2-oxocephalosporins were prepared by a brief treatment of 2-methylenecephalosporins[4] with ozone at low temperatures as outlined in Scheme I. Prolonged reaction times resulted in ozonolysis of the Δ^3 double bond. Cleavage of the phenoxyacetyl group of compound **2a** was accomplished by the procedure of Peter and Bikel[5] to afford the key intermediate **4**, from which new 2-oxocephem derivatives could be synthesized as exemplified by the preparation of compound **6**. 2-Oxocephalosporin derivatives were found to be quite stable under acidic conditions. For example, the treatment of the 2-oxocephalosporin diphenylmethyl ester **2a** with trifluoroacetic acid and anisole produced the free acid **3a** in high yield. However, 2-oxocephalosporins are quite labile under basic

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