Irreversible Enzyme Inhibitors LXXXII

Candidate Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase VII. Derivatives of 2,4-Diaminopyrimidine I

By B. R. BAKER, GRAHAM D. F. JACKSON, and RICH B. MEYER, JR.

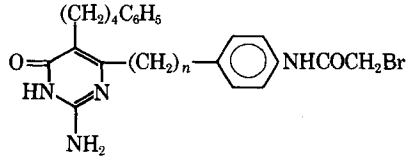
Fusion of α-benzoyl-α-phenylazoacetonitrile (V) with guanidine carbonate gave 2,4-diamino-6-phenyl-5-phenylazopyrimidine (VII) in 52 per cent yield; catalytic reduction of VII to 6-phenyl-2,4,5-triaminopyrimidine (VI) proceeded smoothly. Condensation of the triaminopyrimidine (VI) with *p*-nitrocinnamaldehyde to an anil followed by a two-stage reduction afforded 5-(*p*-aminophenylpropylamino)-2,4-diamino-6-phenylpyrimidine (XI); selective bromoacetylation on the *p*-amino group gave the candidate irreversible inhibitor, 5-(*p*-bromoacetamidophenylpropylamino)-2,4-diamino-6-phenylpyrimidine (XII). When XII was incubated with dihydrofolic reductase in the presence or absence of TPNH, no inactivation occurred; the possible conformational differences in binding to dihydrofolic reductase between XII and other successful active-site-directed irreversible inhibitors are discussed.

TWO TYPES of active-site-directed irreversible inhibitors (1, 2) derived from 2-amino-4-pyrimidinol have been found for dihydrofolic reductase; once the hydrophobic bonding region of dihydrofolic reductase had been discovered and explored (3), irreversible inhibitors could be designed. For example, the 5-phenylbutyl group of I and II is complexed in the hydrophobic region; the 6-side-chain is then projected into a hydrophilic region of the enzyme where covalent bond formation occurs that leads to irreversible inhibition of the enzyme (4). Similarly, the 6-phenyl group of III is complexed in the hydrophobic region which allows the alkylating function on the 5-side-chain to attack a nucleophilic group on the enzyme surface (5).

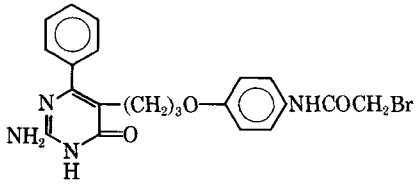
Where 50% of the enzyme was in the form of a reversible complex with the inhibitor, *I*, dihydrofolic reductase was inactivated with a half-life of about 12 min. at 37°. The concentration of the inhibitor, *I*, necessary to form 50% reversible complex (4×10^{-5} *M*) was considered to be too high to be of practical use in an intact animal (4). Therefore, the design of candidate irreversible inhibitors of dihydrofolic reductase that could form 50% reversible complex at a concentration of 10^{-6}–10^{-8} *M* was initiated. Since it is well known that 2,4-diaminopyrimidines are 300 to 3000-fold more effective as inhibitors of dihydrofolic reductase than the corresponding 2-amino-4-pyrimidinols (3, 6–8), this design centered on finding active-site-directed irreversible inhibitors derived from 2,4-diaminopyrimidines; that this design would not necessarily be a simple problem was indicated by the possibility that a 2,4-diaminopyrimidine and the corresponding 2-amino-4-pyrimidinol might not bind to the active site of dihydrofolic reductase in the same rotomeric configuration (7, 9). The first such attempt to find an active-site-directed irreversible inhibitor of dihydrofolic reductase of the 2,4-diaminopyrimidine type—that took into consideration the hydrophobic bonding region—is the subject of this paper; earlier attempts, prior to knowledge of the complications created by the hydrophobic bonding region, have been described (10, 11).

DISCUSSION

6-Phenyl-2,4,5-triaminopyrimidine (VI) was selected as the carrier for the covalent forming group, since VI could be readily synthesized in three steps



I, $n = 2$
II, $n = 4$



III

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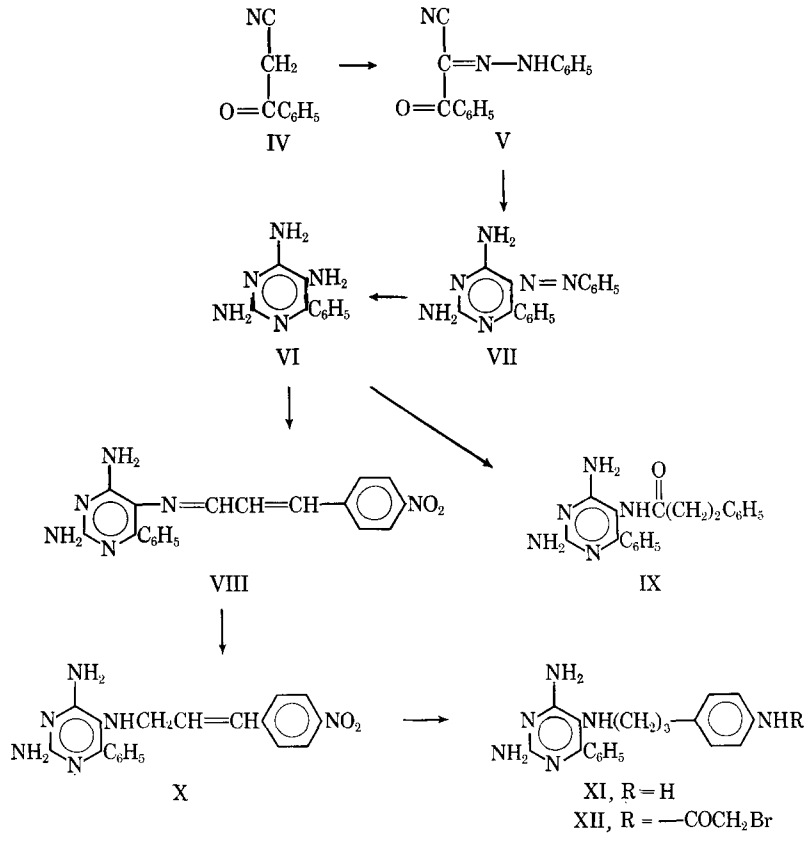


Scheme I

from benzoylacetonitrile (IV) (Scheme I). The latter smoothly coupled with diazotized aniline to the phenylhydrazone (V). When the ketonitrile (V) was fused with an excess of guanidine carbonate at 180°, the phenylazopyrimidine (VII) was obtained in 52% yield. Hydrogenation of VII with a Raney nickel catalyst afforded the desired triaminopyrimidine (VI)

2,4,5-Triamino-6-phenylpyrimidine (VI) was only one-seventh as effective a reversible inhibitor as the corresponding pyrimidine without the 5-amino group, namely, XIII (Table I); since the 5-amino group should not decrease the basicity of the 2,4-diaminopyrimidine (12), the poorer inhibition by VI than XIII may be due to repulsion of the 5-amino group from a hydrophobic area on the enzyme.

Conversion of the triaminopyrimidine (VI) to the 5-phenylpropionyl derivative (IX) with phenylpropionyl chloride resulted in a slight increase in inhibition (Table I); however, the effectiveness of IX was far less than the previously reported (9, 13) 5-anilinopropylpyrimidine (XIV). Thus, a 5-acylamido group on the pyrimidine is not well tolerated, as previously noted in the 2-amino-6-methyl-4-pyrimidinol series (14). Therefore, the triaminopyrimidine (VI) was converted to the 5-(*p*-aminophenylpropyl) derivative (XI), since 2-amino-6-methyl-5-phenylpropylamino-4-pyrimidinol was previously observed to be as good a reversible inhibitor as 2-amino-6-methyl-5-anilinopropyl-4-pyrimidinol (15).

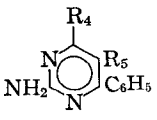
Condensation of the triaminopyrimidine (VI) with *p*-nitrocinnamaldehyde gave the anil (VIII) in 93% yield. Reduction of the anil with sodium borohydride in methanol afforded a 76% yield of X; further reduction of X catalytically afforded the desired 5-(*p*-aminophenylpropyl)pyrimidine (XI). Attempts to reduce VIII directly to XI by catalytic methods were unsuccessful. As anticipated, XI was as good a reversible inhibitor of dihydrofolic reductase as the corresponding 5-anilinopropyl pyrimidine (XIV).

Treatment of XI with bromoacetic anhydride in acetone, containing acetic acid to protonate the diaminopyrimidine system (16), gave the desired candidate active-site-directed irreversible inhibitor (XII); XII could be isolated as a crystalline picrate or as an amorphous hydrobromide.

The candidate irreversible inhibitor (XII) was an excellent reversible inhibitor of dihydrofolic reductase. When XII was incubated with dihydrofolic reductase in the presence of 12 μM TPNH, no inactivation of the enzyme occurred with a concentration of XII sufficient to complex 90% of the available enzyme; similar results were obtained in the absence of TPNH. In contrast, it was previously reported (5) that the corresponding 4-pyrimidinol (III) gave 50% inactivation of the enzyme in 50 min. when a concentration of III was employed that complexed 25% of the available enzyme, but in the absence of TPNH.

These contrasting results between III and XII

TABLE I—INHIBITION OF DIHYDROFOLIC REDUCTASE BY



Compd.	R_4	R_5	Reversible[a] μM Concn. for 50% Inhibition	Reversible[a] Est. $K_i \times 10^6 M$ [c]	Irreversible[b] μM Concn.	Irreversible[b] % $E \cdot \cdot I$ [d]	Irreversible[b] Time, min.	Irreversible[b] % Inactivation
XIII	NH_2	H	160[e]	...	...	...	...	...
VI	NH_2	NH_2	1100	...	...	...	...	...
IX	NH_2	$-NHCO(CH_2)_2C_6H_5$	280	...	...	...	...	...
XIV	NH_2	$-(CH_2)_3NHC_6H_5$	0.88[e]	...	...	...	...	...
XI	NH_2	$-NH(CH_2)_3C_6H_4NH_2$-*p*	0.42	...	...	...	...	...
XII	NH_2	$-NH(CH_2)_3C_6H_4NHCCH_2Br$-*p* (O)	0.33	0.05	0.40	91[f]	120	90[f]
III[g]	OH	$-(CH_2)_3OC_6H_4NHCCH_2Br$-*p* (O)	800	100	40	25	50	50

The technical assistance of Maureen Baker, Susan Lakatos, and Karen Smith is acknowledged. [a] Dihydrofolic reductase was a 45–90% saturated ammonium sulfate fraction from pigeon liver that was prepared and assayed with 6 μM dihydrofolate and 12 μM TPNH at pH 7.4 as previously described (18). [b] Dihydrofolic reductase was incubated with the inhibitor at 37° in 0.05 *M* Tris buffer (pH 7.4) containing no mercaptoethanol and no TPNH as previously described (19) unless otherwise indicated; in each case an enzyme control showed 0–4% inactivation. [c] Estimated from $K_i = I \times K_m/S$, where I = inhibitor concentration giving 50% inhibition (20); this equation is usually valid when $S > 4K_m$ and in this case $S = 6K_m$. [d] Calculated from $[EI] = E_t/(1 + K_i/I)$, where E_t = the total concentration of active enzyme and $[EI]$ = fraction of E_t reversibly complexed by I (1,21). [e] Data from *Reference 9*. [f] The same results were obtained in the presence of 12 μM TPNH in the incubation mixture. [g] Data from *Reference 5*.

clearly show that the terminal bromoacetamido group of the two compounds is positioned differently within the enzyme–inhibitor reversible complex. This different positioning of the bromoacetamido group can be rationalized in at least two different ways: (*a*) the pyrimidines may be complexed in the same way, but the allowable ground-state conformations of the 5-side-chains of III and XII are different, or (*b*) the 2,4-diaminopyrimidine (XII) may be complexed as a rotomer (7) different from the 2-amino-4-pyrimidinol (III), as previously suggested (9). Explanation (*a*) has been eliminated by synthesis of the analog of III where the 4-hydroxyl group has been replaced by 4-amino and both have identical side-chains; this diamino analog of III also failed to show irreversible inhibition of dihydrofolic reductase (17).

EXPERIMENTAL

Melting points were determined in capillary tubes on a Mel-Temp block and those below 230° are corrected. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 137B spectrophotometer. Ultraviolet spectra were determined in 10% ethanol with a Perkin-Elmer 202 spectrophotometer. Thin-layer chromatograms (TLC) were run on silica gel GF and spots were detected by visual examination under ultraviolet light.

α-Benzoyl-α-phenylazoacetonitrile (V)—A solution of 5.6 Gm. (60 mmoles) of aniline in 20 ml. of concentrated hydrochloric acid and 67 ml. of water and was treated at 0° with 4.2 Gm. of sodium nitrite in 8 ml. of water in the usual manner. The solution was carefully neutralized at −5° with a solution of 7.2 Gm. of anhydrous sodium carbonate in 72 ml. of water. To a solution of 6.05 Gm. (41.8 mmoles) of benzoylacetonitrile in 500 ml. of ethanol was added 40 ml. of 50% aqueous sodium acetate and 25 Gm. of solid sodium acetate; to this stirred suspension at 10° was added 150 ml. of above diazonium solution over a period of about 2 min. The product precipitated within a few minutes. The thick suspension was stirred at ambient temperature for 30 min., then cooled to 0° in an ice bath. After addition of 40 ml. of ether, the mixture was stirred an additional 30 min. at 0°, then filtered. The product was washed with 60% aqueous ethanol; yield, 8.28 Gm. (80%) of yellow needles, m.p. 137–138°. Recrystallization from petroleum ether gave yellow crystals, m.p. 137–138°; $\nu_{max.}$ 2200 (C≡N); 1640 (C=O), 1590, 1540 (C=C, C=N); 750, 718, 685, 670 cm.$^{-1}$ (C_6H_5).

Anal.—Calcd. for $C_{15}H_{11}N_3O$: C, 72.3; H, 4.44; N, 16.9. Found: C, 72.5; H, 4.46; N, 16.9.

2,4-Diamino-6-phenyl-5-phenylazopyrimidine (VII)—A well-ground mixture of 2.49 Gm. (10 mmoles) of V and 1.80 Gm. (10 mmoles) of guanidine carbonate was heated in an oil bath; at 130° gas evolution began and the bath was brought to 170–180° over a period of 30 min. with occasional manual stirring. After 30 min. at 170–180°, gas evolution subsided and the mixture solidified. The cooled residue was digested with 30 ml. of boiling petroleum ether (b.p. 60–110°) and the solvent decanted. The residue was extracted with five 50-ml. portions of boiling ethanol. The combined extracts were heated to boiling, then filtered. The filtrate was heated to boiling and water was added to turbidity. After several hours at 0°, the mixture was filtered and the product was washed with 50% aqueous ethanol; yield, 1.51 Gm. (52%) of orange crystals, m.p. 236–237°. Recrystallization of a sample from aqueous ethanol afforded orange needles, m.p. 238–239°; $\nu_{max.}$ 3250 (NH); 1610, 1590, 1520 (NH, N=N, C=C, C=N); 760, 695, 680 cm.$^{-1}$ (C_6H_5);

$\lambda_{max.}$ (pH 1): 238, 285, 357; (pH 7): 230, 280, 379; (pH 13): 280, 378 mμ.

Anal.—Calcd. for $C_{16}H_{14}N_6 \cdot 1/2\ H_2O$: C, 64.2; H, 5.02; N, 28.1. Found: C, 64.6; H, 5.10; N, 28.1.

2,4-Diamino-6-phenylpyrimidine—Fusion of 725 mg. (5 mmoles) of IV with 900 mg. (5 mmoles) of guanidine carbonate was performed as described for the preparation of VII. The cooled melt was extracted with five 20-ml. portions of boiling petroleum ether (b.p. 60–110°). The combined decantates deposited 132 mg. (13%) of white crystals, m.p. 162–163°. This compound was identical with a sample prepared from 6-phenylisocytosine (22, 23).

6-Phenyl-2,4,5-triaminopyrimidine (VI)—A solution of 600 mg. (2 mmoles) of VII in 100 ml. of 2-methoxyethanol was shaken with hydrogen at 2–3 Atm. in the presence of 0.5 Gm. of No. 28 Raney nickel (W. R. Grace Co.) for 3 hr. When 4 mmoles of hydrogen was absorbed, the solution had changed from orange to pale yellow. The mixture was filtered through a pad of diatomaceous earth.[1] The filtrate was spin-evaporated *in vacuo*. Trituration of the residual oil with benzene gave nearly white crystals, m.p. 196–198°; yield, 320 mg. (80%). A portion was recrystallized from ethanol–benzene to give white crystals, m.p. 197–198°; $\nu_{max.}$ 3400, 3300 (NH); 1650–1550 (multiple peaks of NH, C=C, C=N); 760, 695 cm.$^{-1}$ (C_6H_5); $\lambda_{max.}$ (pH 1): 315; (pH 13): 322 mμ.

Anal.—Calcd. for $C_{10}H_{11}N_5$: C, 59.7; H, 5.51; N, 34.8. Found: C, 60.0; H, 5.38; N, 34.5.

2,4-Diamino-6-phenyl-5-phenylpropionamidopyrimidine (IX) Sulfate—To a stirred solution of 201 mg. (1 mmole) of VI in 10 ml. of reagent pyridine cooled in an ice bath was added 168 mg. (1 mmole) of hydrocinnamoyl chloride. After 30 min. at 0°, the solution was spin-evaporated *in vacuo* and the residue triturated with ethyl acetate. Recrystallization of the solid from 5 ml. of water gave 182 mg. (55%) of VII free base, m.p. 232–233°. A hot solution of 80 mg. of the free base in 4 ml. of water was quickly cooled to about 25°, then 0.2 ml. of 96% sulfuric acid was added with stirring. The sulfate salt was collected; yield, 80 mg. (84% conversion) of white crystals, m.p. 208–213°. Recrystallization from water gave the analytical sample, m.p. 204–206°.

Anal.—Calcd. for $C_{19}H_{19}N_5O \cdot 1/2H_2SO_4 \cdot H_2O$: C, 57.0; H, 5.49; N, 17.5. Found: C, 56.9; H, 5.18; N, 17.5.

2,4-Diamino-5-(p-nitrocinnamalamino)-6-phenylpyrimidine (VIII)—To a solution of 100 mg. (0.50 mmole) of VI in 5 ml. of hot ethanol was added a hot solution of 89 mg. (0.50 mmole) of *p*-nitrocinnamaldehyde in 5 ml. of hot ethanol. After the addition of 2 drops of glacial acetic acid, the mixture was heated 10 min. on a steam bath, then cooled to 0°. The product was collected on a filter and washed with cold ethanol; yield, 168 mg. (93%), m.p. 205–207°. Recrystallization of a portion from 2-methoxyethanol–ethanol gave red crystals, m.p. 206–207°; $\nu_{max.}$ 3500, 3400–3350 (NH); 1610, 1590, 1540 (NH, C=C, C=N); 1500, 1330 (NO_2); 830 (*p*-C_6H_4); 745, 695 cm.$^{-1}$ (C_6H_5).

Anal.—Calcd. for $C_{19}H_{16}N_6O_2$: C, 63.3; H, 4.48; N, 23.3. Found: C, 63.4; H, 4.60; N, 23.4.

[1] Marketed as Celite by the Johns-Manville Corp., New York, N. Y.

2,4-Diamino-5-(p-nitrocinnamylamino)-6-phenylpyrimidine (X)—A hot solution of 540 mg. (1.5 mmoles) of VIII in 250 ml. of methanol was cooled to 25°, then 0.1 ml. of glacial acetic acid was added. Over a period of 30 min., the stirred solution was treated with 650 mg. of sodium borohydride. After a further 30 min., TLC showed only one component was present. The solution was concentrated to about 25 ml. *in vacuo*, then cooled. The product was collected on a filter and washed with cold methanol, then water; yield, 412 mg. (76%), m.p. 214–215°. Recrystallization from ethanol–ethyl acetate gave orange crystals, m.p. 214–215°; $\nu_{max.}$ 3500, 3400 (NH); 1610, 1590, 1555 (NH, C=C, C=N); 1500, 1330 (NO_2); 770, 742, 695 cm.$^{-1}$ (C_6H_5).

Anal.—Calcd. for $C_{19}H_{18}N_6O_2$: C, 63.0; H, 5.01; N, 23.2. Found: C, 63.0; H, 5.20; N, 23.0.

5-(p-Aminophenylpropylamino)-2,4-diamino-6-phenylpyrimidine (XI)—A hot solution of 362 mg. (1 mmole) of X in 150 ml. of boiling ethanol was stirred with 250 mg. of decolorizing carbon for about 20 min. The filtered solution was shaken with hydrogen at 2–3 Atm. in the presence of 20 mg. of platinum oxide until 4 mmoles of hydrogen had been absorbed. The filtered solution was spin-evaporated *in vacuo*. The residue was crystallized by solution in 2 ml. of ethyl acetate, addition of 15 ml. of benzene, then finally petroleum ether to turbidity; yield, 296 mg., m.p. 72–80°, which showed two spots on TLC. Several recrystallizations from benzene gave 102 mg. (31%) of white crystals, m.p. 91–94°, that were uniform on TLC and had $\nu_{max.}$ 3500, 3300, 3200, 3100 (NH); 1620, 1550 (NH, C=C, C=N); 768, 700 (C_6H_5); no NO_2 near 1500 or 1330 cm.$^{-1}$.

Anal.—Calcd. for $C_{19}H_{22}N_6$: C, 68.2; H, 6.63; N, 25.1. Found: C, 68.4; H, 6.80; N, 24.9.

5-(p-Bromoacetamidophenylpropylamino)-2,4-diamino-6-phenylpyrimidine (XII) Picrate—To a solution of 100 mg. (0.30 mmole) of XI in 2 ml. of acetone and 0.55 ml. of 3.6% aqueous acetic acid (0.66 mmole) at 0° was added 90 mg. (0.34 mmole) of bromoacetic anhydride in 0.5 ml. of acetone. After being stirred for 30 min. in an ice bath, the solution was treated with 0.5 ml. of saturated ethanolic picric acid; a picrate immediately separated. The mixture was warmed to complete solution, then allowed to cool slowly. The picrate was collected and washed with acetone; TLC showed the presence of one major component with two minor impurities. Four recrystallizations from aqueous 2-methoxyethanol and drying at room temperature in high vacuum gave 50 mg. (25%) of yellow crystals, m.p. 150–151° dec. TLC in 1:4 ethanol–chloroform showed one moving spot (viewed at 350 mμ) with the picric acid staying at the origin; the moving spot gave a positive 4-nitrobenzylpyridine test (16) for active halogen. The compound had $\nu_{max.}$ 3330 (NH); 1640 (C=O); 1530, 1420, 1330–1310 (NO_2); 1270, 1075 cm.$^{-1}$ (C—O—C).

Anal.—Calcd. for $C_{27}H_{28}BrN_9O_9 \cdot H_2O$: C, 47.2; H, 4.11; N, 18.4. Found: C, 47.3; H, 4.05; N, 18.1.

An amorphous hydrobromide was also prepared by adding 30% hydrogen bromide in acetic acid, then ether, to the reaction solution. The hydrobromide moved as a single spot on TLC, the spot giving a positive 4-nitrobenzylpyridine test; this hydrobromide was used for enzyme assays.

REFERENCES

(1) Baker, B. R., "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active Site," John Wiley & Sons, Inc., New York, N.Y., 1967.
(2) Baker, B. R., *J. Pharm. Sci.*, **53**, 347(1964).
(3) *Reference 1*, chap. X.
(4) Baker, B. R., and Jordaan, J. H., *J. Pharm. Sci.*, **55**, 1417(1966).
(5) Baker, B. R., and Shapiro, H. S., *ibid.*, **55**, 1422(1966).
(6) Baker, B. R., and Jordaan, J. H., *J. Heterocyclic Chem.*, **2**, 162(1965).
(7) Baker, B. R., and Jordaan, J. H., *J. Pharm. Sci.*, **54**, 1740(1965).
(8) Bertino, J. R., Booth, V. A., Bieber, A. L., Cashmore, A., and Sartorelli, A. C., *J. Biol. Chem.*, **239**, 479(1964).
(9) Baker, B. R., and Shapiro, H. S., *J. Pharm. Sci.*, **55**, 308(1966).
(10) Baker, B. R., and Santi, D. V., *ibid.*, **56**, 380(1967).
(11) Baker, B. R., and Ho, B.-T., *ibid.*, **56**, 28(1967).
(12) Brown, D. J., "The Pyrimidines," John Wiley & Sons, Inc., New York, N.Y., 1962, pp. 473, 476.
(13) Baker, B. R., Shapiro, H. S., and Werkheiser, W. C., *J. Med. Chem.*, **8**, 283(1965).
(14) Baker, B. R., and Sachdev, K., *J. Pharm. Sci.*, **53**, 1020(1964).
(15) Baker, B. R., and Santi, D. V., *ibid.*, **54**, 1252(1965).
(16) Baker, B. R., Santi, D. V., Coward, J. K., Shapiro, H. S., and Jordaan, J. H., *J. Heterocyclic Chem.*, **3**, 425(1966).
(17) Baker, B. R., and Meyer, R. B., Jr., *J. Pharm. Sci.*, **56**, 570(1967).
(18) Baker, B. R., Ho, B.-T., and Neilson, T., *J. Heterocyclic Chem.*, **1**, 79(1964).
(19) Baker, B. R., and Jordaan, J. H., *ibid.*, **2**, 21(1965).
(20) Baker, B. R., and Patel, R. P., *J. Pharm. Sci.*, **53**, 714(1964).
(21) Baker, B. R., Lee, W. W., and Tong, E., *J. Theoret. Biol.*, **3**, 459(1962).
(22) Russell, P. B., *J. Chem. Soc.*, **1954**, 2951.
(23) Baker, B. R., and Ho, B.-T., *J. Heterocyclic Chem.*, **2**, 340(1965).

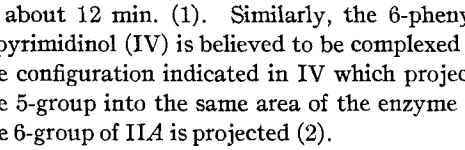
Irreversible Enzyme Inhibitors LXXXIV

Candidate Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase IX. Derivatives of 2,4-Diaminopyrimidine III

By B. R. BAKER and RICH B. MEYER, JR.

The candidate active-site-directed irreversible inhibitor, 5-(*p*-bromoacetamidophenoxypropyl)-2,4-diamino-6-phenylpyrimidine (VI) has been synthesized *via* the key intermediate, 2-amino-5-(*p*-nitrophenoxypropyl)-6-phenyl-4-pyrimidinol (XI*c*). Although VI was a better reversible inhibitor than the corresponding 4-pyrimidinol, 2-amino-5-(*p*-bromoacetamidophenoxypropyl)-6-phenyl-4-pyrimidinol (IV), VI failed to inactivate dihydrofolic reductase when incubated in the presence or absence of TPNH. The fact that IV in the absence of TPNH could inactivate the enzyme, but VI at the same concentration showed no inactivation of the enzyme, clearly demonstrated that IV and VI had different conformations when complexed to dihydrofolic reductase. Evidence for the possible binding conformations of IV and VI is presented.

THE SUCCESS of the 5-phenylbutyl-4-pyrimidinol (II) (1) and the 6-phenyl-4-pyrimidinol (IV) (2) as active-site-directed irreversible inhibitors (3, 4) of dihydrofolic reductase was predicated on a knowledge of (*a*) the strength and location of the hydrophobic bonding region on the enzyme (5–10), and (*b*) the probability that one of several possible rotomers of the pyrimidine ring could be complexed in the active-site (7) which was determined by the position of the hydrophobic group on the pyrimidine carrier (10). The substrate, dihydrofolate (I), must have some given conformation of the pteridine ring when complexed to the enzyme, which is arbitrarily depicted as indicated in I; other rotomeric conformations of the pyrimidines to be discussed below are in relation to the conformation depicted in I. Thus, the active-site-directed irreversible inhibitor of dihydrofolic reductase derived from a 4-pyrimidinol with a hydrophobic 5-phenylbutyl group (II) probably has its hydrophobic group projected vertically as indicated; the 4-pyrimidinol (II) is believed to complex in configuration II*A* which projected the 6-group into a hydrophilic region where covalent bond formation to the enzyme rapidly occurred within the enzyme-inhibitor complex with a half-life of about 12 min. (1). Similarly, the 6-phenyl-4-pyrimidinol (IV) is believed to be complexed in the configuration indicated in IV which projects the 5-group into the same area of the enzyme as the 6-group of II*A* is projected (2).



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