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Irreversible Enzyme Inhibitors. LXXXIII. (1-3). Candidate Active-site-directed Irreversible Inhibitors of Dihydrofolic Reductase. VIII. Derivatives of 2,4-Diaminopyrimidine. II. (2)

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2,4-Diamino-6-(*p*-aminophenethyl)pyrimidines with a 5-phenylbutyl (XIX) and 5-(*p*-chlorophenyl) (VIII) substituent were synthesized by condensation of the corresponding pyrimidine-6-carboxaldehydes (XVI, X) with the Wittig reagent derived from *p*-nitrobenzyl bromide, followed by catalytic hydrogenation. Selective bromoacetylation of VIII and XIX afforded the candidate active-site-directed irreversible inhibitors of dihydrofolic reductase, namely, 6-(*p*-bromoacetamidophenethyl)-2,4-diaminopyrimidine with a 5-(*p*-chlorophenyl)- (IV) and 5-phenylbutyl- (III) substituents. Although III and IV were excellent reversible inhibitors of dihydrofolic reductase, neither showed any inactivation of the enzyme; in contrast, the corresponding 2-amino-6-(*p*-bromoacetamidophenethyl)-5-phenylbutyl-4-pyrimidinol (II) -- which differs from III only in the 4-substituent (NH_2 vs. OH) -- was an excellent active-site-directed irreversible inhibitor of dihydrofolic reductase, but II was a poor reversible inhibitor. Thus the conformations of II and III are most probably different when complexed to dihydrofolic reductase.

The successful synthesis of 2-amino-5-phenylbutyl-4-pyrimidinols substituted by a *p*-bromoacetamidophenylbutyl group (I) or a *p*-bromoacetamidophenethyl group (II) on the 6-position led to the first demonstration of active-site-directed irreversible inhibition of dihydrofolic reductase (5); the inhibitors inactivated the enzyme with half-lives of 18 and 12 minutes at 37° respectively, and both had K_i's near 3×10^{-5} *M*. The relatively poor reversible inhibitory properties of the compounds rendered them of limited utility for further study on tissue and species specificity (6-8); the main objection was that the low intracellular concentration of inhibitor to be expected in whole animal studies would be insufficient to allow formation of appreciable amounts of reversible enzyme-inhibitor complex, thereby precluding rapid irreversible inactivation of the enzyme (5).

Since the increased basicity of 2,4-diaminopyrimidines considerably increases binding of this type of inhibitor to dihydrofolic reductase (9-12), an obvious consideration was to convert the successful irreversible inhibitor, 2-amino-6-(*p*-bromoacetamidophenethyl)-5-phenylbutyl-4-pyrimidinol (II), to the corresponding 2,4-diaminopyrimidine (III) in order to obtain an irreversible inhibitor with improved reversible inhibitory properties. It is important to note that this premise assumes that the mode of binding of 2,4-diaminopyrimidines is the same as that of 2-amino-4-pyrimidinols and that the 6-(*p*-bromoacetamidophenethyl) substituent would project toward, and alkylate, the same nucleophilic center in the respective enzyme-inhibitor complexes; however, such a premise was not considered likely (10,13).

It was therefore quite possible that 2,4-diaminopyrimidines would not assume a conformation identical with that of 2-amino-4-pyrimidinols in the active-site; such a difference in conformation could cause a juxta-posed alkylating function in a successful irreversible inhibitor such as 2-amino-6-(*p*-bromoacetamidophenethyl)-5-phenylbutyl-4-pyrimidinol (II) to become removed from the enzymic nucleophilic center in the corresponding 2,4-diaminopyrimidine (III)-inhibitor complex, thereby failing to inactivate the enzyme irreversibly.

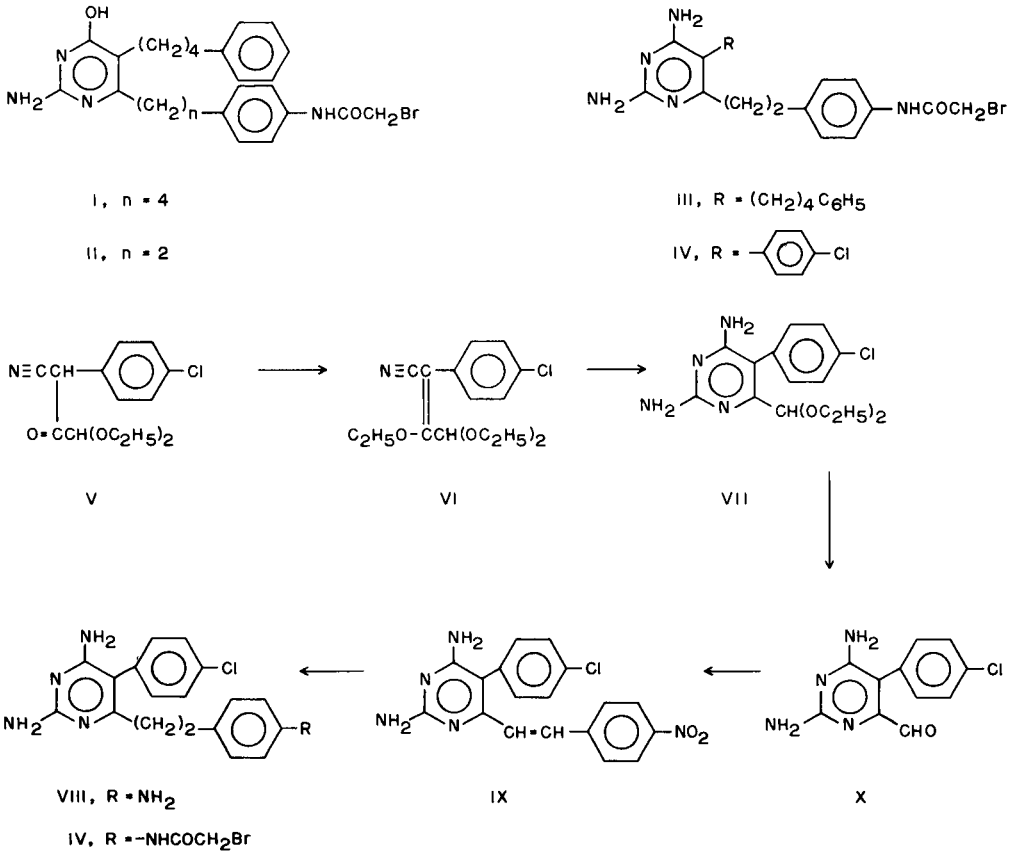
In order to obtain better reversible inhibitors which could possibly inhibit dihydrofolic reductase irreversibly and to resolve the question whether 2,4-diaminopyrimidines and 2-amino-4-pyrimidinols have the same or different conformations in the active-site, 2,4-diamino-6-(*p*-bromoacetamidophenethyl)pyrimidines substituted with phenylbutyl (III) and *p*-chlorophenyl (IV) in the 5-position were synthesized and evaluated as reversible and irreversible inhibitors of the enzyme; the results are the subject of this paper.

Chemistry.

Demonstration of the general utility of the Wittig reaction for the synthesis of 6-substituted 2-amino-4-hydroxypyrimidines from the corresponding 6-aldehyde and subsequent synthesis of 2-amino-4-hydroxy-6-(*p*-bromoacetamidophenethyl)-5-phenylbutylpyrimidine (II) (14), prompted the application of this route to the synthesis of the desired 2,4-diamino-6-(*p*-bromoacetamidophenethyl)pyrimidines, III and IV.

Claisen condensation of *p*-chlorophenylacetonitrile with ethyl diethoxyacetate proceeded to crystalline V in 55% yield; the spectral characteristics of V indicated that it was enolic in the solid state, since it showed an enolic band at 3.18 and strong enolic C=C at 6.12 μ in the infrared. The compound was partially in the enol form in solution since the ultraviolet spectrum in absolute ethanol showed an inflection at 310 and a strong absorption maximum at 275 mμ; in basic ethanol the spectrum showed maxima at 260 and 310 mμ. Conversion of V to the crude enol ether (VI) by prolonged refluxing with ethyl orthoformate proceeded with extensive darkening of the reaction mixture, indicating decomposition. Condensation of this product with alcoholic guanidine led to the 6-diethoxymethyl pyrimidine (VII) in only 26% yield; VII could only be isolated from the crude product by extraction with 5% acetic acid followed by treatment of the acidic extracts with aqueous sodium hydroxide. Treatment of V with refluxing ethyl orthoacetate (15), however, afforded light yellow VI that gave the desired 6-diethoxymethyl pyrimidine (VII) in 69% overall yield on treatment with alcoholic guanidine; in this case no acid extraction for isolation of VII was required. Hydrolysis of the acetal group of VII with 0.4 *N* aqueous hydrochloric acid proceeded smoothly and a 93% yield of analytically pure pyrimidine-6-carboxaldehyde (X) could be isolated.

Condensation of X with *p*-nitrobenzylidene triphenylphosphorane, produced *in situ* from *p*-nitrobenzyl triphenylphosphonium bromide (16) with potassium *t*-butoxide in *N*,*N*-dimethylformamide, afforded 2,4-diamino-5-(*p*-chlorophenyl)-6-(*p*-nitrostyryl)pyrimidine (IX) in 62% yield. It is surprising that the aldehyde (X), with its electron-donating 2,4-diamino- groups deactivating the 6-carboxaldehyde function toward attack by nucleophiles, reacted so well with the stabilized *p*-nitrobenzylidene phosphorane. It is possible that the

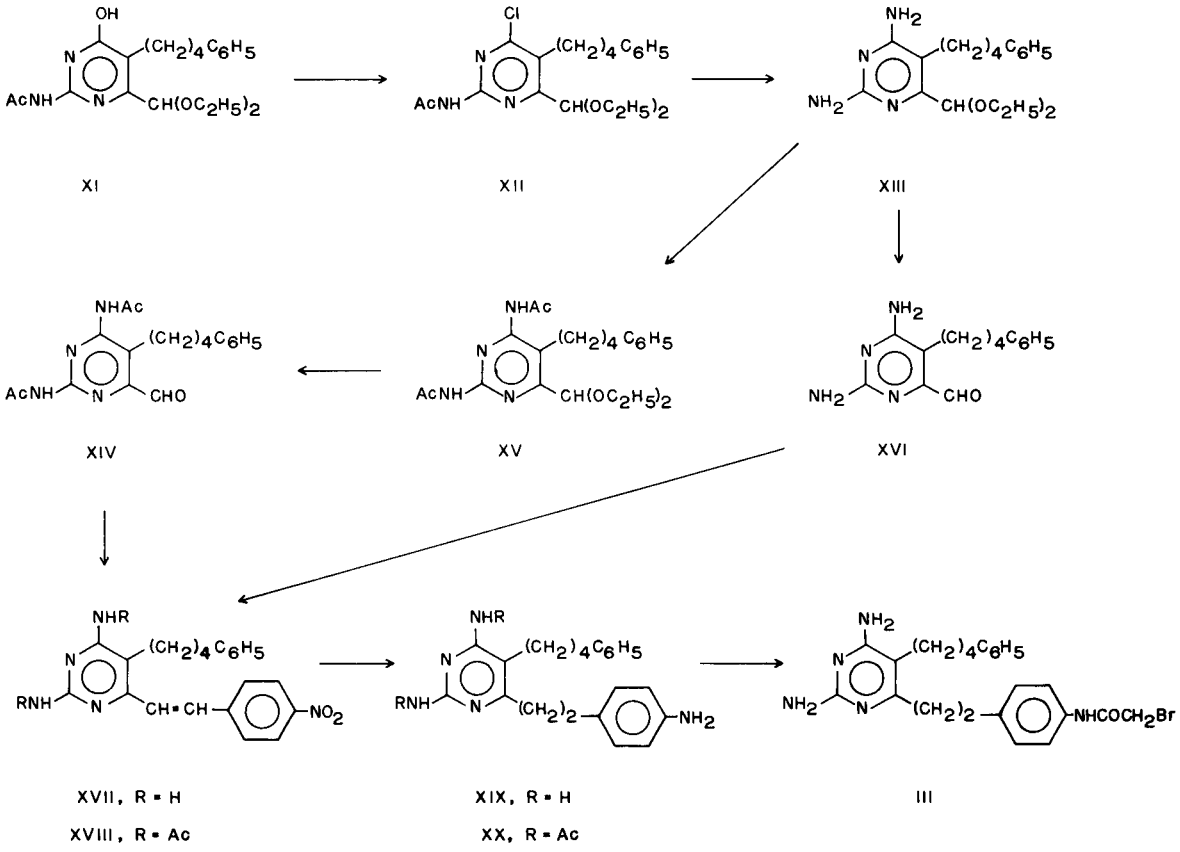


formation of the betaine in the first step of the condensation, which appears to be rate-limiting in the case of stabilized ylides (17) like *p*-nitrobenzylidene phosphorane, is promoted by the polar solvent, *N*,*N*-dimethylformamide. Electron withdrawal by the 5-(*p*-chlorophenyl)substituent, which is conjugated with the 6-carboxaldehyde group, might also increase the susceptibility of this group to nucleophilic attack.

Catalytic hydrogenation of the side-chain double bond and nitro-group of IX gave the 6-(*p*-aminophenethyl)pyrimidine VIII in 77% yield of pure material. The bromoacetylation of VIII specifically on the *p*-amino-group was accomplished with bromoacetic anhydride in acetone solution at 0° in the presence of one equivalent of glacial acetic acid (17). Under these conditions the strongly basic diaminopyrimidine system (pK_a about 7) is fully protonated while the aniline amino group of the 6-side chain (pK_a near 5) is not. Since the protonated species will not bromoacylate, a slight excess of bromoacetic anhydride could be employed safely to ensure complete bromoacylation at the desired location (17). The 6-(*p*-bromoacetamidophenethyl)-pyrimidine IV could then be converted to the sulfate salt. The desired product IV was obtained in 48% yield of analytically pure material and was shown to be homogeneous by thin layer chromatography.

In order to use a reaction sequence for the synthesis of 2,4-diamino-6-(*p*-bromoacetamidophenethyl)-5-phenylbutylpyrimidine (III) that may be adapted readily for the synthesis of a variety of 6-substituted pyrimidines of this type the Wittig reaction was again employed. Treatment of 2-acetamido-6-diethoxymethyl-5-phenylbutyl-4-pyrimidinol (XI) (13b) with excess phosphorus oxychloride in benzene (18) afforded the 4-chloro derivative XII in 81% yield of pure material. Reaction of XII with methanolic ammonia in a bomb at 150-160° led to the 2,4-diamino-6-diethoxymethylpyrimidine (XIII) in 75% yield. Hydrolysis of XIII in refluxing 0.3 *N* hydrochloric acid in 20% ethanol followed by an alkaline workup gave the desired 2,4-diaminopyrimidine-6-carboxaldehyde XVI in 78% yield.

Initial attempts to condense the aldehyde (XVI) with *p*-nitrobenzylidene triphenylphosphorane in tetrahydrofuran led to mixtures that showed multiple spots on thin layer chromatograms and a major product could not be isolated. In *N*,*N*-dimethylformamide the condensation of XVI with *p*-nitro-



benzylidene triphenylphosphorane, produced *in situ* by treatment of the corresponding phosphonium bromide (16) with one equivalent of potassium *t*-butoxide, proceeded readily and afforded the desired 2,4-diamino-6-(*p*-nitrostyryl)pyrimidine (XVII) in 44% yield. This result seems to support the suggestion that a polar solvent like *N,N*-dimethylformamide promotes the condensation of stabilized ylides with relatively unreactive aldehydes.

Catalytic reduction of XVII gave the 6-(*p*-aminophenethyl)pyrimidine XIX in 92% yield; XIX was obtained as a gum that crystallized in silky needles from ethanol-water but liquefied when collected. The gum was shown to be pure by thin layer chromatography and was used without further attempts at crystallization.

Since it was anticipated at first that -- due to the electron-donating 2,4-diamino-groups of XVI -- the aldehyde might be of low reactivity toward nucleophiles, XIII was converted to the diacetyl derivative XV and then hydrolyzed in formic acid to the 2,4-diacetamidopyrimidine-6-carboxaldehyde XIV. Condensation of XIV with *p*-nitrobenzylidene triphenylphosphorane, generated *in situ* by treatment of the corresponding phosphonium bromide with potassium *t*-butoxide, in tetrahydrofuran afforded the diacetamido-6-(*p*-nitrostyryl)pyrimidine (XVIII). Although the product showed two spots on thin layer chromatograms the compound gave a satisfactory elemental analysis indicating a mixture of *cis* and *trans* isomers; this mixture could be catalytically reduced in 67% yield to 2,4-diacetamido-6-(*p*-aminophenethyl)-5-phenylbutylpyrimidine (XX). Treatment of XX with 1 *N* sodium hydroxide in 50% ethanol gave the 2,4-diaminopyrimidine XIX; the product was shown to be identical with XIX prepared by hydrogenation of XVII by thin layer chromatography and comparison of spectral properties.

Bromoacetylation of XIX was again performed with bromoacetic anhydride in acetone containing one equivalent of acetic acid (17). The bromo-acetate salt of III precipitated from the reaction mixture and could then be converted to III-sulfate by dissolution of the product in ethanol-dimethylformamide followed by treatment with aqueous sulfuric acid.

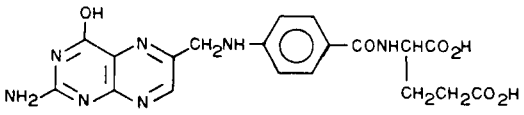
Enzymic Evaluation.

The results on reversible and irreversible inhibition of dihydrofolic reductase with the 6-(*p*-bromoacetamidophenethyl)pyrimidines, III and IV are listed in Table I. Both compounds were excellent reversible inhibitors of the enzyme as anticipated. However, when incubated at a concentration sufficient to convert more than 50% of the enzyme to reversible enzyme-inhibitor complex, E---I, neither bromoacetyl derivative, III or IV, showed any inactivation of dihydrofolic reductase in one hour at 37° in the presence or absence of TPNH; in contrast, 2-amino-6-(*p*-bromoacetamidophenethyl)-5-phenylbutyl-4-pyrimidinol, II, showed 50% inactivation in 12 minutes at 40 μM, a concentration forming 55% of reversible E---I complex (5).

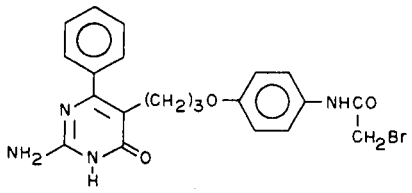
The fact that II was a good irreversible inhibitor of dihydrofolic reductase while III, which differs from II only in having a 4-amino group rather than a 4-hydroxy group, did not irreversibly inactivate the enzyme, gives strong evidence that the mode of binding of 2-amino-4-pyrimidinols is markedly different from that of similarly substituted 2,4-diaminopyrimidines, as previously suggested (5,13).

Evidence that the hydrophobic bonding region (23) of dihydrofolic reductase is in the vicinity of where either the 4- or 8-substituent of folic acid (XXI) resides in the enzyme-folic acid complex has been presented earlier (9,24-26). The success with irreversible inhibitors of the 2-amino-4-pyrimidinol type such as I and II was predicated on the basis of the 5-phenylbutyl group being complexed in the hydrophobic bonding region; this then projected the 6-substituent into a hydrophilic area where alkylation could occur (5). A similar argument was

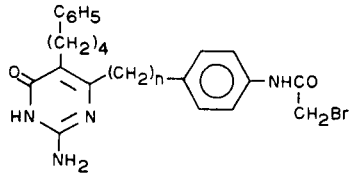
Scheme I



XXI

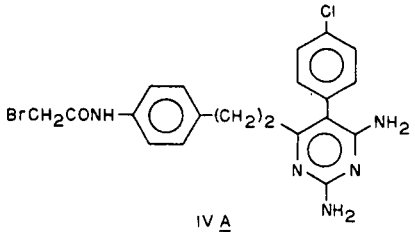


XXII

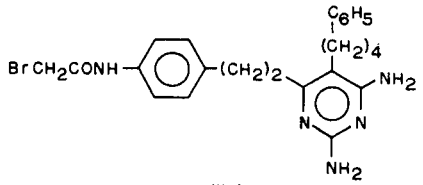


I, n = 4

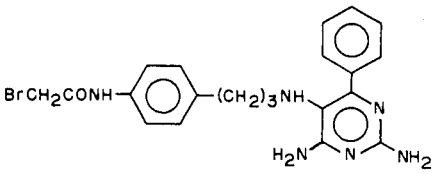
II, n = 2



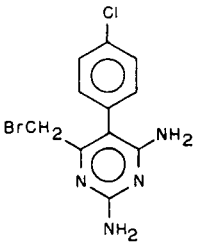
IV A



III A



XXIII



XXIV

advanced for the 6-phenyl-4-pyrimidinol (XXII), where the 6-phenyl group was complexed to the hydrophobic region and the 5-substituent bearing on alkylating function then projected into a hydrophilic area (27).

Since folic acid must have some conformation of its pteridine ring in space when complexed to the enzyme, the pteridine ring is arbitrarily assigned the configuration, XXI (Scheme I); then with respect to the binding of the pteridine ring of XXI, the conformations indicated in XXII, I and II are assigned.

Since the 2,4-diaminopyrimidines must have a different conformation, it is likely that the ring is "flipped" over (9,13) to give conformations IIIA and IVA with respect to folic acid (XXI), when they are complexed to the enzyme.

A similar rationalization for the ineffectiveness of the 2,4-diamino-6-phenylpyrimidine, XXIII, as an irreversible inhibitor (2) is also suggested as shown in conformation XXIII. Similarly, the 6-bromomethyl-2,4-diaminopyrimidine, XXIV may have been ineffective as an irreversible inhibitor (20) due to the bromomethyl group being projected into a non-polar region of the enzyme as indicated in conformation XXIV.

All of the 2,4-diaminopyrimidines depicted in Scheme I were excellent reversible inhibitors compared with the 2-amino-4-pyrimidinols. Attempts to improve reversible binding properties of successful 2-amino-4-pyrimidinol-type irreversible inhibitors simply by converting the 4-hydroxyl-group into a 4-amino group therefore seems to be self-defeating. 2,4-Diamino-type irreversible inhibitors of dihydrofolic reductase will have to be developed on the basis of an intensive and independent study of the mode of reversible binding of this system, as well as a study of the influence of both hydrophobic and other substituents on determination of the conformation that diaminopyrimidines assume in the enzyme-inhibitor complex. Guidelines for the design of inhibitors of this type, based on reversible inhibitory data specifically in the diaminopyrimidine area, are being formulated. Until the first irreversible inhibitor of the 2,4-diaminopyrimidine type is obtained, rationalization of binding data will have to serve only to indicate general areas of study that might be explored to reach the eventual aim of obtaining potent 2,4-diaminopyrimidine type irreversible inhibitors most expeditiously.

EXPERIMENTAL (28)

α-Diethoxyacetyl-*p*-chlorophenylacetonitrile (V).

To a mixture of 14.6 g. (83 mmoles) of ethyl diethoxyacetate and 12.6 g. (83 mmoles) of *p*-chlorophenylacetonitrile stirred at 5° in 50 ml. of benzene was added 3.7 g. (83 mmoles) of a 55.6% dispersion of sodium hydride in mineral oil. Foaming of the mixture was controlled by addition of small portions of benzene when necessary. After initial foaming had subsided, an additional 1.9 g. (41 mmoles) of sodium hydride was added. The mixture was stirred in the ice bath for 30 minutes, then for 30 minutes at room temperature and finally refluxed for 1 hour. The mixture was diluted with 100 ml. of benzene and 50 ml. of solvent was removed by distillation. The solution was cooled, acidified with 15 ml. of glacial acetic acid and diluted with 200 ml. of water. The benzene layer was separated, washed with water, dried over magnesium sulfate, filtered and the solvent removed *in vacuo*. The remaining oil crystallized on standing. The product was washed with 20 ml. of petroleum ether (b.p. 30-60°), then recrystallized from a minimum of ethyl acetate and excess petroleum ether (b.p. 30-60°). Two more recrystallizations gave 13 g. (55%) of colorless crystals, m.p. 71-72°; λ max (1% $NaOC_2H_5$-C_2H_5OH): 260, 309 mμ; λ max 3.18 (enolic OH); 4.52 (C≡N); 6.12 (enol C=C); 7.92, 8.71 (C-O-C); 12.02 μ (*p*-C_6H_4).

Anal. Calcd. for $C_{14}H_{16}ClNO_3$: C, 59.7; H, 5.72; N, 4.97. Found: C, 59.8; H, 5.90; N, 4.93.

2-(*p*-Chlorophenyl)-3,4,4-triethoxycrotononitrile (VI).

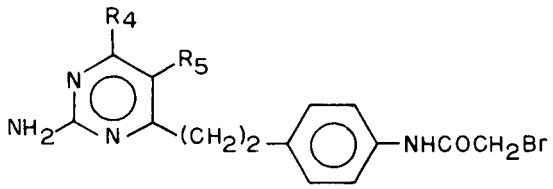
A solution of 10.6 g. (37.6 mmoles) of V in 50 ml. of ethyl orthoacetate was concentrated to 40 ml. *in vacuo* and then refluxed for 2 hours. The mixture was evaporated to dryness *in vacuo*, then traces of ethyl orthoacetate removed at 2 mm. The oily product was used without further purification; λ max (1% $NaOC_2H_5$-C_2H_5OH or C_2H_5OH): 279 mμ; λ max (film): 4.52 (CN); 6.27 (C=C); 9.0-10.0 (broad, C-O-C); 12.02 μ (*p*-C_6H_4). This product was not purified, but was used directly in the next step.

5-(*p*-Chlorophenyl)-2,4-diamino-6-(diethoxymethyl)pyrimidine (VII).

The crude enol ether VI obtained from 10.6 g. (37.6 mmoles) of V was dissolved in a filtered solution of guanidine obtained from 3.6 g. (37.6 mmoles) of guanidine hydrochloride and 2.3 g. (42 mmoles) of sodium methoxide in 100 ml. of ethanol. The mixture was refluxed for 4 hours concentrated to 50 ml. *in vacuo* and diluted with 100 ml. of hot water. The mixture was cooled and the crystalline precipitate collected. Three recrystallizations from ethanol-water gave 8.7 g. (69% overall) of white crystals, m.p. 190-191°; λ max (*p*H 1): 279

TABLE I

Inhibition of Dihydrofolic Reductase By



Compound	R_4	R_5	Reversible Inhibition (a) μM conc. for 50% Inhibition	Estimated (c) $K_i \times 10^8 M$	Irreversible Inhibition (b) μM conc.	% E---I (d)	Time mins.	% Inactivation
IV	NH_2	$-C_6H_4Cl(p)$	0.53	9	0.25	73	60	0 (e)
III	NH_2	$-(CH_2)_4C_6H_5$	0.04	0.7	0.01	60	60	0 (e)
II (f)	OH	$(CH_2)_4C_6H_5$	180	3000	40	55	12	50

The technical assistance of Maureen Baker and Barbara Baine is acknowledged. (a) The dihydrofolic reductase was a 45-90% ammonium sulfate fraction from pigeon liver that was prepared and assayed with 6 μM dihydrofolate and 12 μM TPNH in 0.05 M Tris buffer (*p*H 7.4) containing 10 mM 2-mercaptoethanol, as previously described (19). (b) Dihydrofolic reductase was incubated with the inhibitor at 37° in 0.05 M Tris buffer (*p*H 7.4) containing no mercaptoethanol and no TPNH; in each case an enzyme control was run that showed 0-4% inactivation (20). (c) Estimated from $K_i = I \times K_m/S$, where I = inhibitor concentration giving 50% inhibition (7,21); this equation is usually valid when $S > 4\ K_m$ and in this case, $S = 6\ K_m$. (d) Calculated from $[EI] = [E_t]/[1 + \frac{K_i}{I}]$, where $[E_t]$ = the concentration of total active enzyme and [EI] = fraction of the total enzyme, E_t, reversibly complexed by I (21,22). (e) The same results were obtained with 12 μM TPNH in the incubation mixture. (f) Data from reference 5.

mμ; (*p*H 13); 297 mμ; λ max 2.85, 2.96, 3.17 (NH); 6.03, 6.11, 6.21, 6.39, 6.48 (NH, C=N, C=C); 9.00, 9.22, 9.50 (C-O-C); 11.98 μ (p-C_6H_4).

Anal. Calcd. for $C_{15}H_{19}ClN_4O_2$: C, 55.8; H, 5.93; N, 17.4. Found: C, 55.9; H, 6.12; N, 17.1.

5-(*p*-Chlorophenyl)-2,4-diaminopyrimidine-6-carboxaldehyde (X).

A mixture of 5 g. (15.5 mmoles) of VII and 80 ml. of 0.4 N aqueous hydrochloric acid was refluxed for 1 hour, treated with decolorizing charcoal, filtered and the filtrate cooled in an ice bath. The *p*H of the filtrate was adjusted to 9 with cold 1 N sodium hydroxide and the yellow precipitate collected. The product was suspended in 100 ml. of water, collected on a filter, washed with water and dried on a steam-bath; yield 3.6 g. (93%) of yellow powder; m.p. 156° (softens). The product showed one spot on t.l.c. in 9:1 chloroform-ethanol and had λ max (*p*H 1): 279 mμ; (*p*H 7): 295 mμ; (*p*H 13): 293 mμ; λ max 2.86, 2.95, 3.10 (NH); 5.86 (C=O); 6.2-6.5, 6.96 (C=N, C=C, NH); 12.06 μ (p-C_6H_4).

Anal. Calcd. for $C_{11}H_9ClN_4O$: C, 53.1; H, 3.65; N, 22.5. Found: C, 53.4; H, 3.76; N, 22.3.

5-(*p*-Chlorophenyl)-2,4-diamino-6-(*p*-nitrostyryl)pyrimidine (IX).

To a stirred mixture of 500 mg. (2 mmoles) of X, 959 mg. of *p*-nitrobenzyl triphenylphosphonium bromide (16), and 15 ml. of N,N-dimethylformamide at room temperature was added 224 mg. (2 mmoles) of solid potassium *t*-butoxide. The resulting red solution was stirred at ambient temperature for 1 hour and then at 60° for 16 hours protected from moisture. The mixture, containing a yellow precipitate, was diluted with 20 ml. of water, then the precipitate was collected on a filter. The product was recrystallized from acetone-water to give 455 mg. (62%) of bright yellow crystals; m.p. 289-290°; λ max (*p*H 1): 359 mμ; (*p*H 7): 356 mμ; (*p*H 13): 380 mμ; λ max 2.84, 2.88, 2.98, 3.11 (NH); 6.10, 6.23, 6.49, 6.62, 6.85, 6.97 (C=N, C=C, NH, NO_2); 7.48 (NO_2); 11.88 μ (p-C_6H_4).

Anal. Calcd. for $C_{18}H_{14}ClN_5O_2$: C, 58.8; H, 3.84; N, 19.0. Found: C, 58.7; H, 3.84; N, 18.9.

6-(*p*-Aminophenethyl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (VIII).

A suspension of 0.95 g. (2.58 mmoles) of IX in 100 ml. 2-methoxyethanol was hydrogenated in the presence of 150 mg. of platinum oxide at 2-3 atmospheres for 26 hours when reduction was complete. The mixture was filtered through a Celite pad and the filtrate evaporated *in vacuo*. The remaining colorless oil was dissolved in ethanol, hot water was added to slight turbidity and the mixture treated with charcoal and filtered. Water was added to the filtrate to turbidity. The crystalline product was collected, after the mixture was cooled, and recrystallized from ethanol-water; yield 680 mg. (77%) of colorless crystals; m.p. 96-97°; λ max (*p*H 1): 276 mμ; (*p*H 7): 283 mμ; (*p*H 13): 289 mμ; λ max 2.83, 2.90, 2.96, 3.11 (NH), 6.05, 6.18, 6.26, 6.40, 6.61, 6.98 (C=N, C=C, NH); 12.00, 12.22 μ (p-C_6H_4).

Anal. Calcd. for $C_{18}H_{18}ClN_5$: C, 63.6; H, 5.34; N, 20.6. Found: C, 63.4; H, 5.50; N, 20.4.

6-(*p*-Bromoacetamidophenethyl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (IV) Sulfate.

To a solution of 113 mg. (0.33 mmoles) of VIII in 0.5 ml. of acetone was added 0.3 ml. of a 10% solution of glacial acetic acid in acetone. The mixture was cooled to 0° in an ice bath and a solution of 94 mg. (0.36 mmole) of bromoacetic anhydride in 0.3 ml. acetone added to the suspension. The resulting clear solution was left at 0° for 30 minutes, filtered and 0.5 ml. of cold 2 N aqueous sulfuric acid added to the filtrate. The mixture was diluted with 5 ml. of cold water, and the white precipitate collected on a filter. The product was washed with 10 ml. of water and dried *in vacuo*; yield, 80 mg. (48%), m.p. 191-194°. The product showed

one spot on t.l.c. using chloroform-methanol (4:1). The spot gave a blue color for active halogen with *p*-nitrobenzylpyridine (17, 20). In 10% $CH_3OC_2H_4OH$ the compound had λ max (*p*H 1): 271 mμ (inflection); (*p*H 13) 250, 284 mμ (inflection); λ max 3.03, 3.15 (NH); 5.97, 6.06, 6.10, 6.23, 6.48, 6.54, 6.64 (C=O, C=N, NH, C=C); 9.17 ($SO_4^=$); 12.05, 12.33 μ (*p*-C_6H_4).

Anal. Calcd. for $C_{20}H_{19}BrClN_5O \cdot 0.5\ H_2SO_4$: C, 47.1; H, 3.95; N, 13.7. Found: C, 47.4; H, 4.22; N, 13.5.

2-Acetamido-4-chloro-6-diethoxymethyl-5-phenylbutylpyrimidine (XII).

A solution of 4.66 g. (12 mmoles) of XI (14b) in 100 ml. of benzene was concentrated to about 50 ml. *in vacuo*. To the cooled stirred solution was added 2.5 ml. (27 mmoles) of phosphorus oxychloride and the mixture stirred at ambient temperature for 30 minutes in a flask equipped with a reflux condenser and a drying tube. The mixture was refluxed for 10 minutes, then rapidly cooled in an ice bath and poured into a vigorously stirred ice cold solution of 30 g. sodium acetate trihydrate in 200 ml. of water (18). The mixture was stirred for 5 minutes, then the layers were separated. The aqueous phase was extracted with three 25 ml. portions of benzene and the combined benzene extracts were washed once with water. The solution was dried over magnesium sulfate and then spin-evaporated *in vacuo*. The remaining oil crystallized on addition of 10 ml. of petroleum ether (b.p. 30-60°). The solid was collected and recrystallized from ethyl acetate-petroleum ether (b.p. 30-60°). Two more recrystallizations gave 3.93 g. (81%) of colorless crystals; m.p. 93-94°. The product showed one spot on t.l.c. in 9:1 chloroform-ethanol; λ max (*p*H 7): 244, 284 mμ; (*p*H 1): 244, 285 mμ; λ max 3.15 (NH); 5.98 (C=O); 6.40, 6.72, 7.05, 7.11 (NH, C=C, C=N); 9.50 (C-O-C); 13.35, 14.32 μ (C_6H_5).

Anal. Calcd. for $C_{21}H_{28}ClN_3O_3$: C, 62.1; H, 6.95; N, 10.4. Found: C, 62.4; H, 6.78; N, 10.6.

2,4-Diamino-6-diethoxymethyl-5-phenylbutylpyrimidine (XIII).

To 3.0 g. (7.4 mmoles) of vacuum dried XII in a 100 ml. Parr bomb at 0° was added 40 ml. of methanol saturated with ammonia at 0-5°. The bomb was sealed and heated in an oil bath at 150-160° for 24 hours. The mixture was cooled and spin-evaporated *in vacuo*. The residue was dissolved in 50 ml. of 10% aqueous acetic acid, decolorized with charcoal, filtered and the *p*H of the cooled filtrate adjusted to 9-10 with 1 *N* sodium hydroxide. The precipitate was collected and recrystallized from ethyl acetate-petroleum ether (b.p. 60-110°); yield 1.9 g. (75%) of white needles, m.p. 116-117°. The product showed one spot on t.l.c. in 4:1 chloroform-ethanol; λ max (*p*H 7 and 13): 297 mμ; (*p*H 1): 281 mμ; λ max 2.88, 2.97, 3.10 (NH); 6.05, 6.12, 6.31, 6.40, 6.71, 6.97 (C=N, NH, C=C); 9.46 (C-O-C); 13.51, 14.34 μ (C_6H_5).

Anal. Calcd. for $C_{19}H_{28}N_4O_2$: C, 66.3; H, 8.19; N, 16.3. Found: C, 66.1; H, 8.37; N, 16.4.

2,4-Diamino-5-phenylbutylpyrimidine-6-carboxaldehyde (XVI).

To a solution of 3.45 g. (10 mmoles) of XIII in 20 ml. of ethanol was added 80 ml. of 0.4 *N* hydrochloric acid. The mixture was refluxed for 1 hour, decolorized with carbon, then the *p*H of the cold filtrate was adjusted to 9-10 with 5% aqueous sodium hydroxide. The precipitate was collected, redissolved in a mixture of 20 ml. of ethanol and 80 ml. of 10% acetic acid, decolorized and reprecipitated with 5% sodium hydroxide. The product was collected, washed profusely with water, dried in air at room temperature, then finally dried at room temperature under 1 mm.; yield 2.1 g. (78%), m.p. indefinite; λ max (*p*H 1): 282 mμ; (*p*H 7): 298 mμ; (*p*H 13): 295 mμ; λ max 2.90, 2.96 (NH); 5.85 (C=O); 6.19, 6.33, 6.39, 6.67, 6.85 (C=C, C=N, NH), 13.37, 14.25 μ (C_6H_5).

Anal. Calcd. for $C_{15}H_{16}N_4O$: C, 66.7; H, 6.71; N, 20.7. Found: C, 66.4; H, 6.95; N, 20.5.

2,4-Diamino-5-phenylbutyl-6-(*p*-nitrostyryl)pyrimidine (XVII).

To a mixture of 811 mg. (3 mmoles) of XVI and 1.44 g. (3 mmoles) of *p*-nitrobenzyl triphenylphosphonium bromide (16) stirred in 20 ml. of *N*,*N*-dimethylformamide at room temperature was added 336 mg. (3 mmoles) of potassium *t*-butoxide. The red solution, protected from moisture, was stirred at ambient temperature for 12 hours, then stirred at 80° for 6 hours. The mixture was diluted with 80 ml. of water and the gummy precipitate collected. The gum solidified on standing at room temperature for 1 hour. The product was triturated with 10 ml. of methanol and collected on a filter. Three recrystallizations from 2-methoxyethanol-water gave 515 mg. (44%) of bright yellow crystals, m.p. 201-203°. The product showed one spot on t.l.c. in 5:1 chloroform-ethanol. An analytical sample was obtained on recrystallization from 2-methoxyethanol as bright yellow needles, m.p. 203-204°; λ max (*p*H 1): 299 (inflection), 357 mμ; (*p*H 7): 306 (inflection), 335 mμ; (*p*H 13): 306 (inflection), 327 mμ; λ max 2.85, 2.88, 2.90, 2.96, 3.03 (NH); 6.10, 6.19, 6.25, 6.67 (NH, C=N, C=C); 6.45, 7.44 (NO_2); 11.84 (*p*-C_6H_4); 13.27, 13.48, 14.05, 14.40 μ (C_6H_5).

Anal. Calcd. for $C_{22}H_{23}N_5O_2$: C, 67.9; H, 5.95; N, 18.0. Found: C, 67.6; H, 6.03; N, 17.9.

2,4-Diacetamido-6-diethoxymethyl-5-phenylbutylpyrimidine (XV).

A solution of 1.4 g. (4 mmoles) of XIII in a mixture of 5 ml. pyridine and 5 ml. acetic anhydride was heated on a steam-bath for 3 hours. The mixture was evaporated to dryness *in vacuo* and traces of pyridine were removed by evaporating the residue with three 10 ml. portions of toluene. The remaining oil was dissolved in 5 ml. of ethyl acetate and diluted with 20 ml. of petroleum ether (b.p. 30-60°). The crystals that were deposited on cooling were collected and recrystallized from ethyl acetate-petroleum ether (b.p. 30-60°); yield, 700 mg. (41%), m.p. 128-129°; λ max (*p*H 7); 243, 286 mμ; (*p*H 13): 292 mμ; (*p*H 1): 291 mμ; λ max 2.90, 3.06, 3.15 (NH); 5.80, 5.87 (C=O); 6.23, 6.32, 6.59, 7.02 (C=N, C=C, NH); 9.42 (C-O-C); 13.42, 14.40 μ (C_6H_5).

Anal. Calcd. for $C_{23}H_{32}N_4O_4$: C, 64.5; H, 7.53; N, 13.1. Found: C, 64.3; H, 7.46; N, 12.9.

2,4-Diacetamido-5-phenylbutylpyrimidine-6-carboxaldehyde (XIV).

A solution of 500 mg. (1.17 mmoles) of XV in 5 ml. of 97% formic acid was heated on a steam-bath for 45 minutes. The mixture was evaporated to dryness *in vacuo* and the process repeated with four 10 ml. portions of ethyl acetate. The remaining solid was dissolved in acetone, treated with Darco and filtered. To the hot filtrate was added petroleum ether (b.p. 30-60°) to turbidity; the crystals were collected after cooling. The product was recrystallized from acetone-petroleum ether (b.p. 30-60°); yield, 330 mg. (80%); m.p. 156° (softens), 162-163° (liquefies); λ max 2.95, 3.08 (NH); 5.80, 5.98 (C=O); 6.03, 6.10, 6.31, 6.37, 6.70, 6.85 (C=N, C=C, NH); 13.45, 14.38 μ (C_6H_5).

Anal. Calcd. for $C_{19}H_{22}N_4O_3$: C, 64.4; H, 6.26; N, 15.8. Found: C, 64.5; H, 6.43; N, 16.0.

2,4-Diacetamido(*p*-nitrostyryl)-5-phenylbutylpyrimidine (XVIII).

To a mixture of 500 mg. (1.41 mmoles) of XIV and 675 mg. (1.41 mmoles) of *p*-nitrobenzyl triphenylphosphonium bromide (16) stirred at 0° in 10 ml. of tetrahydrofuran was added 158 mg. (1.41 mmoles) of solid potassium *t*-butoxide. The mixture was stirred at ambient temperature for 1 hour, then refluxed for 12 hours protected from moisture. The mixture was diluted with 40 ml. of water and the gummy yellow precipitate collected. The product was dissolved in 10 ml. of acetone, 20 ml. of petroleum ether (b.p. 30-60°) was added, then the yellow solid was collected after cooling. The product was recrystallized from *N*,*N*-dimethylformamide-water; yield 230 mg. (34%) of bright yellow crystals; m.p. 270-272°. The product showed two spots on t.l.c. in 5:1 chloroform-ethanol; λ max 2.88, 3.03, 3.10, 3.16 (NH); 6.06, 6.14, 6.67, 7.67 (C=N, C=C, NH); 6.35, 7.53 (NO_2); 10.44 (*trans* C=C); 11.90 (*p*-C_6H_4); 13.62, 14.40 μ (C_6H_5).

Anal. Calcd. for $C_{28}H_{27}N_5O_4$: C, 66.0; H, 5.74; N, 14.8. Found: C, 66.2; H, 5.80; N, 15.1.

6-(*p*-Aminophenethyl)-2,4-diacetamido-5-phenylbutylpyrimidine (XX).

A suspension of 600 mg. (1.26 mmoles) of XVIII in 100 ml. ethyl acetate was shaken with hydrogen during 24 hours at 2-3 atmospheres in the presence of 100 mg. platinum oxide. The mixture was filtered through a Celite pad and the filtrate evaporated *in vacuo*. Since t.l.c. showed the reduction was incomplete, the remaining yellow oil was dissolved in 100 ml. ethanol, treated with decolorizing carbon, and again hydrogenated during 24 hours with an additional 100 mg. of Adam's catalyst. The mixture was filtered through Celite and the filtrate evaporated *in vacuo*. The remaining nearly white solid was dissolved in hot ethanol, the solution decolorized, then diluted with hot water to turbidity. Partial concentration of the solution *in vacuo* caused the product to crystallize. Recrystallization of the precipitate from ethanol-water gave 430 mg. (67%) of white crystals, m.p. 155-158° (dec.); λ max (*p*H 1): 229, 271 mμ; (*p*H 7): 230, 281 mμ; (*p*H 13): 237, 285 mμ; λ max 2.91, 2.95, 2.99, 3.13 (NH); 6.06, 6.17, 6.31, 6.45, 6.66 (C=O, C=N, C=C); 12.14 (*p*-C_6H_4); 13.60, 14.29 μ (C_6H_5).

Anal. Calcd. for $C_{26}H_{31}N_5O_2$: C, 70.1; H, 7.01; N, 15.7. Found: C, 70.2; H, 7.03; N, 15.9.

6-(*p*-Aminophenethyl)-2,4-diamino-5-phenylbutylpyrimidine (XIX).

(a) A suspension of 390 mg. (1 mmole) of XVII in 100 ml. of

absolute ethanol was shaken with hydrogen at 2-3 atmospheres in the presence of 50 mg. of platinum oxide during 24 hours. The mixture was filtered through a Celite pad, then evaporated *in vacuo*. The light yellow oil was redissolved in ethanol, decolorized with charcoal, and the solvent evaporated to dryness; yield 330 mg. (92%) of colorless oil. The product showed one spot on t.l.c. in 5:1 chloroform-ethanol and was used without further purification; λ max (*p*H 1): 281 mμ (*p*H 7): 287 mμ; (*p*H 13): 292 mμ; λ max (film): 2.90, 3.01, 3.15 (NH); 6.23, 6.43, 6.66 (C=N, C=C); 7.04 (NH); 12.12 (*p*-C_6H_4); 13.33, 14.25 μ (C_6H_5).

(b) A mixture of 200 mg. (0.45 mmole) of XX, 5 ml. of absolute ethanol and 5 ml. of 2 *N* aqueous sodium hydroxide was refluxed for 2 hours. The mixture was cooled, acidified with 2 ml. of glacial acetic acid, then diluted with 20 ml. of water. The solution was decolorized with Darco and the *p*H of the cooled solution adjusted to 10 with 2 *N* sodium hydroxide. The crystalline precipitate liquefied when collected on a filter. The product was dissolved in acetone and evaporated to dryness *in vacuo*; yield 140 mg. (88%) of colorless oil. The product showed one spot on t.l.c. when run in admixture with XIX obtained by hydrogenation of XVII as in (a).

6-(*p*-Bromoacetamidophenethyl)-2,4-diamino-5-phenylbutylpyrimidine (III) Sulfate.

To a solution of 90 mg. (0.25 mmole) of XIX in 0.5 ml. of acetone was added a solution of 15 mg. (0.25 mmole) of glacial acetic acid in 0.25 ml. of acetone. The solution was cooled in an ice bath and a cold solution of 71.5 mg. (0.275 mole) of bromoacetic anhydride in 0.25 ml. of acetone added. The mixture was kept at 0° with occasional stirring; after 15 minutes, a white crystalline product started precipitating. After 1 hour at 0° the solid was dissolved by addition of 2 ml. of *N,N*-dimethylformamide. The solution was poured into 10 ml. of ice cold 1 *N* sulfuric acid. The mixture was diluted with 10 ml. of ice water and the crystalline precipitate collected. The product was dissolved in 9:1 ethanol-*N,N*-dimethylformamide, decolorized with charcoal at 60°, then 0.1 *N* aqueous sulfuric acid added to the cooled filtrate until no more precipitate formed. The mixture was diluted with 5 ml. of water, then the product was collected on a filter and dried *in vacuo*; yield 78 mg. (59%), m.p. 120° (softens), 127-129° (liquefies). The product showed one spot on t.l.c. in 4:1 chloroform-ethanol that gave a blue color with 4-(*p*-nitrobenzyl)pyridine spray reagent (17,20); λ max (*p*H 1): 268 mμ (inflection); (*p*H 13): 286 mμ; λ max 3.03, 3.15, 3.23 (NH); 6.06, 6.10, 6.23, 6.46, 6.56, 6.65 (C=O, C=N, NH); 9.02 (SO_4); 12.05 (*p*-C_6H_4); 13.46, 14.29 μ (C_6H_5).

Anal. Calcd. for $C_{24}H_{28}BrN_5O \cdot 0.5\ H_2SO_4$: C, 54.2; H, 5.50; N, 13.2. Found: C, 53.9; H, 5.61; N, 12.9.

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(3) The previously published papers on Nonclassical Antimetabolites and on Analogs of Tetrahydrofolic Acid have been combined into one series, since they have the common objective of the design of active-site-directed irreversible enzyme inhibitors. A collected list of references on Irreversible Enzyme Inhibitors will be sent on request.

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