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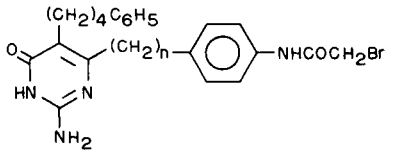
Irreversible Enzyme Inhibitors. LXXXIX. Candidate Active-site-directed Irreversible Inhibitors of Dihydrofolic Reductase. X. Derivatives of 2-Amino-5-benzamidopropyl-4-pyrimidinol (1,2).

B. R. Baker and James K. Coward

Since 2-amino-5-benzamidopropyl-6-methyl-4-pyrimidinol (VII) was a reasonably good reversible inhibitor of dihydrofolic reductase, the benzamido group was substituted with *p*-bromomethyl (XVIIa), *m*-bromomethyl (XVIIIa), and *p*-bromoacetyl (XIXa) groups; these compounds, and the corresponding 6-phenylpyrimidines, were synthesized from the proper 2-amino-5-aminopropyl-4-pyrimidinol (V) by devising methods that were compatible with the high reactivity of the halogen. Compounds XVII-XIX showed inactivation of dihydrofolic reductase; the fact that *p*-nitrobenzyl bromide inactivated the enzyme as rapidly as XVII and XVIII and phenacyl bromide inactivated the enzyme as rapidly as XIX indicated that these inactivations proceeded by a random bimolecular mechanism and not the desired active-site-directed mechanism.

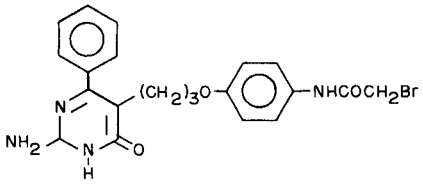
Over thirty candidate active-site-directed irreversible inhibitors for dihydrofolic reductase were synthesized and evaluated (3) before successful ones were found. These irreversible inhibitors (I-III) were successfully designed (4, 5) after the hydrophobic bonding region on dihydrofolic reductase was discovered (6) and its parameters studied (3, 6-13). By allowing the 5-phenylbutyl group of I and II to complex to the hydrophobic region of dihydrofolic reductase, the 6-phenylalkyl group was projected into a hydrophilic region of the enzyme and formed a covalent bond with the enzyme (4); similarly, the 6-phenyl group of III was complexed with the hydrophobic region on dihydrofolic reductase, then the enzyme was alkylated by the 5-side-chain (5). If only one large side-chain was present which carried the alkylating function -- such as the 6-methyl analog of III -- little irreversible inhibition was seen (5) since the 5-side-chain was complexed to the hydrophobic region, an area on the enzyme not apt to have polar groups.

Prior to the discovery of the hydrophobic bonding region on dihydrofolic reductase it was noted that the 5-benzamidopropyl-4-pyrimidinol (VIIa) was as good an inhibitor of dihydrofolic reductase (14) as 2-amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol (15,16). Since candidate irreversible inhibitors derived from VII had certain operational advantages from the synthetic standpoint, a series of such compounds were made and enzymically evaluated. After the discovery that III was an active-site-directed irreversible inhibitor of dihydrofolic reductase, the corresponding 6-phenylpyrimidines (b series, Chart I) were also synthesized and evaluated enzymatically. The results are the subject of this paper.



I, n = 2

II, n = 4



III

The synthesis of the *p*-aminobenzamidopropyl-4-pyrimidinol (XIVa) was previously described (14). The *m*-amino isomer (XIIIa) was synthesized similarly by reaction of the free base of Va in aqueous acetone with *m*-nitrobenzoyl chloride, followed by catalytic reduction of the resultant *m*-nitrobenzamide (IXa). Numerous attempts to convert XIIIa and XIVa to the bromoacetamides, XVa and XVIa, were unsuccessful. The arylamino group reacted sluggishly with bromoacetic anhydride and mixtures were obtained that also contained bromoacetyl groups on the 2-amino group of the pyrimidine; this sluggishness is probably due to the strong electron-withdrawing power of the carboxamido group as previously observed with the sulfonamido group (17).

The *N*-bromoacetyl derivatives, VIa and VIb, could be prepared by selective acylation of the aliphatic amino group of V (17) in aqueous acetone with *p*-nitrophenyl bromoacetate (18).

A different type of candidate irreversible inhibitor was synthesized via α-ethoxy *m*- and *p*-toluic acids (19). The respective acid chlorides were condensed with the two pyrimidylpropylamines (V) in aqueous acetone to give X and XI. The ethoxymethyl group of XI (14) and X was converted to bromomethyl derivative (XVII and XVIII) with 10% anhydrous hydrogen bromide in glacial acetic acid.

p-Acetylbenzoic acid was synthesized by acid hydrolysis of *p*-acetylbenzonitrile (20), which in turn had been prepared from *p*-bromoacetophenone (21). The acid chloride was used to acylate V to give the two *p*-acetobenzamidopropyl-4-pyrimidinols (XII). Selective bromination to XIX was achieved by protonation of the aminopyrimidines (XII) in glacial acetic acid containing 10% anhydrous hydrogen bromide followed by addition of bromine at ambient temperature. In the case of the 6-phenylpyrimidine, XIX, erratic combustion analyses were obtained on the sulfate salt. Therefore XIX was derivatized by reaction with thioacetamide to convert the bromoacetyl group to a 2-methyl-4-thiazolyl moiety (22).

CHART I

a series, R = CH_3

b series, R = C_6H_5

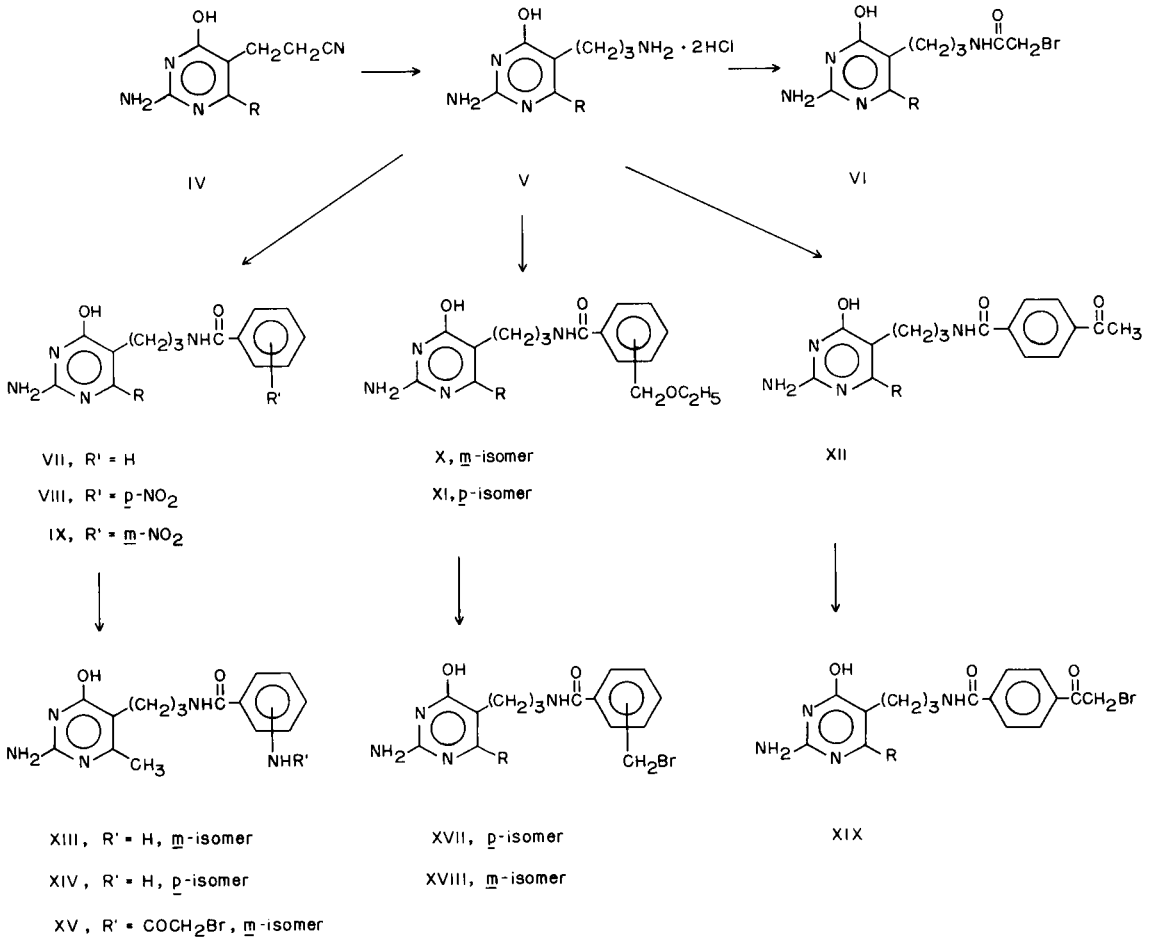
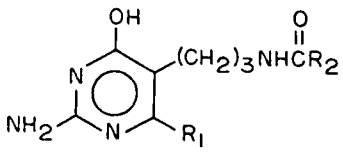


TABLE I

Reversible Inhibition of Dihydrofolic Reductase by



No.	R_1	R_2	mM Conc. Inhibitor	Percent Inhibition (a)	Estimated (b) $[I/S]_{0.5}$	Estimated (c) K_i (mM)
VIa	CH_3	$-CH_2Br$	10 (d,e)	39	3000	3.0
VIb	C_6H_5	$-CH_2Br$	4.0 (d)	23	1300	1.3
VIIa	CH_3	C_6H_5	0.48	50	80 (f)	0.80
Xa	CH_3	m-$C_6H_4CH_2OC_2H_5$	0.95	50	160	0.16
XVIIa	CH_3	p-$C_6H_4CH_2Br$	0.40	50	65	0.065
XVIIb	C_6H_5	p-$C_6H_4CH_2Br$	0.50 (d)	26	230	0.23
XVIIIa	CH_3	m-$C_6H_4CH_2Br$	0.50	50	83	0.083
XVIIIb	C_6H_5	m-$C_6H_4CH_2Br$	1.1	50	180	0.18
XIXa	CH_3	p-$C_6H_4COCH_2Br$	0.083	50	14	0.014
XIXb	C_6H_5	p-$C_6H_4COCH_2Br$	0.50 (d)	30	200	0.20

(a) The technical assistance of Barbara Baine, Maureen Baker, Ann Jaqua and Gail Salomen with the enzyme assays in Table I and II is acknowledged. The dihydrofolic reductase was a 45-90% ammonium sulfate fraction from pigeon liver that was isolated and assayed with 6 μM dihydrofolate and 12 μM TPNH in 10% N,N-dimethylformamide at pH 7.4 as previously described (15). (b) The ratio of concentration of inhibitor to 6 μM dihydrofolate giving 50% inhibition. (c) Estimated from $K_i = K_m \times [I/S]_{0.5}$ and $K_m = 1 \times 10^{-6}$ M; this equation is usually valid when the substrate concentration is greater than four times K_m (24,27). (d) Maximum solubility. (e) See reference (17) for preparation. (f) Data previously reported (14).

RESULTS

The results of reversible inhibition of dihydrofolic reductase by the candidate inhibitors are listed in Table I. Substitution on the benzamido group of the 6-methyl-4-pyrimidinol series (VIIa) had little effect on reversible binding except in the case of the p-bromoacetyl derivative (XIXa); the latter was a 6-fold better reversible inhibitor than the parent VIIa. In contrast, the p-bromoacetyl group (XIXb) had little effect on the binding of the benzamido group in the 6-phenyl-4-pyrimidinol series.

The 5-bromoacetamidopropyl-6-methyl-4-pyrimidinol (VIa) was a weak reversible inhibitor as expected from the earlier observed weak inhibition by 2-amino-5-acetamidopropyl-6-methyl-4-pyrimidinol (14). The 6-phenyl analog (VIb) was 2-3 fold more effective than VIIa as a reversible inhibitor, as previously noted with some 6-phenyl-5-anilinopropylpyrimidines (12).

The result of studies of these compounds as irreversible inhibitors are listed in Table II. A few words about the kinetics of irreversible inhibition are in order (23,24). If inactivation of an enzyme proceeds by the active-site-directed mechanism, the equation is represented by 1) and the rate of enzyme inactivation by 2).

$$E + I\text{-}X \rightleftharpoons E\cdots I\text{-}X \xrightarrow{k_3} E\cdots I + X^- \quad 1)$$

$$-\frac{dE}{dt} = k_3 [E\cdots I\text{-}X] \quad 2)$$

The rate of formation (k_1) of an enzyme-inhibitor reversible complex, $E\cdots I\text{-}X$, is a rapid diffusion process on the order of nonaseconds; the equilibrium value is measured by the binding constant, $K_i = k_2/k_1$. The inactivation rate is a relatively slower process on the order of minutes. Therefore the rate of inactivation of the enzyme is dependent upon k_3 and the concentration of the reversible complex as expressed in 2). The amount of reversible complex is in turn dependent upon K_i and the inhibitor concentration, as expressed in Table II, footnote (b).

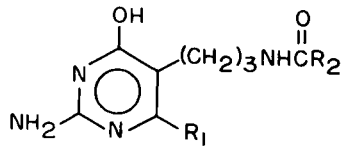
The active-site-directed irreversible inhibition, as expressed in 1) and 2) must be differentiated from random bimolecular inactivation, which is shown in 3), and its rate expression 4):

$$E + I\text{-}X \xrightarrow{k} E\,I + X^- \quad 3)$$

$$-\frac{dE}{dt} = k [E][I\text{-}X] \quad 4)$$

TABLE II

Irreversible Inhibition of Dihydrofolic Reductase by



No.	Set	R_1	R_2	Estimated (a) K_i (m*M*)	m*M* Conc. Inhibitor	Percent E···I (b)	Time (min.)	Percent Inactivation (c)
XVIIa	A	CH_3	p-$C_6H_4CH_2Br$	0.065	0.080	55	60	near 0
XVIIIa	B	CH_3	m-$C_6H_4CH_2Br$	0.083	0.18	68	60	37
XVIIIb	B	C_6H_5	m-$C_6H_4CH_2Br$	0.18	0.18	50	60	38
XVIIb	C	C_6H_5	p-$C_6H_4CH_2Br$	0.23	0.18	44	60	38
XVIIIb	C	C_6H_5	m-$C_6H_4CH_2Br$	0.18	0.18	50	60	37
XVIIb	D	C_6H_5	p-$C_6H_4CH_2Br$	0.23	0.18	44	60	42
XX	D	p-$NO_2C_6H_4CH_2Br$		poor ?	0.18	?	60	55
XIXa	E	CH_3	p-$C_6H_4COCH_2Br$	0.014	0.014	50	60	7
XIXb	F	C_6H_5	p-$C_6H_4COCH_2Br$	0.20	0.20	50	60	83
XIXb	G	C_6H_5	p-$C_6H_4COCH_2Br$	0.20	0.20	50	20	56
XXI	G	Phenyl	Bromide	poor ?	0.20	?	20	53
XIXb	H	C_6H_5	p-$C_6H_4COCH_2Br$	0.20	0.040	17	60	61
XXI	H	Phenyl	Bromide	poor ?	0.040	?	60	58
VIa	I	CH_3	$-CH_2Br$ (d)	3.0	4.0	57	60	0
VIb	J	C_6H_5	$-CH_2Br$	1.3	1.0	43	60	0

(a) From Table I. (b) Percent of enzyme complexed reversibly with inhibitor as calculated from $[EI] = [E_t]/(1 + K_i/I)$ where EI = fraction of enzyme reversibly complexed, E_t = total enzyme, and I = concentration of inhibitor (23,24). (c) Dihydrofolic reductase from pigeon liver was incubated at 37° in 10% *N*,*N*-dimethylformamide at *p*H 7.4 in the absence of TPNH as previously described (28); in each case an enzyme control was run that showed only 0-5% thermal inactivation. (d) See reference (17) for preparation.

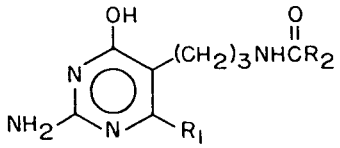
Note that 3) is directly dependent upon inhibitor concentration, whereas 1) is dependent upon the amount of complex. The amount of complex in 1) is in turn dependent upon K_i, but process 3) is not dependent upon K_i. Therefore, one can distinguish the active-site-directed inactivation of 2) from the random bimolecular inactivation by changing I in I-X so that the I does not form a complex. For example, the bromomethylbenzamido-4-pyrimidinol (XVIIb), which inactivates dihydrofolic reductase, should be compared with *p*-nitrobenzyl bromide (XX) which presumably does not form a reversible complex with the enzyme (see Table II, set D). Note that XX inactivates the enzyme even more rapidly than XVIIb; therefore, the latter is inactivating by the bimolecular mechanism 3), else XX should have been much less effective (23,24). Therefore all of the benzyl bromides (XVII) in Table II that show inactivation of the enzyme most probably do so by the random bimolecular process 3).

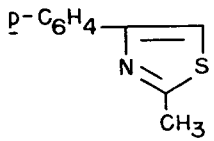
The second class of irreversible inhibitors studied were the pyrimidyl phenacyl bromides (XIX); these should be compared with phenacyl bromide (XXI) (Table II, Sets G and H). Note that phenacyl bromide (XXI) gives the same rate of inactivation as XIXb; therefore the pyrimidyl phenacyl bromides (XIX) inactivate by the random bimolecular mechanism 3) (25).

Although the 5-bromoacetamidopropyl-4-pyrimidinols, VI, were poor reversible inhibitors of the enzyme (Table I), they were investigated as irreversible inhibitors at concentrations sufficiently high to convert about 50% of the enzyme to the reversible complex of equation 1) and 2); no inactivation occurred (Table II, Sets I and J). That bimolecular inactivation by mechanism 3) was not seen is probably not due to the lower chemical reactivity of the halogen of VIa and VIb. Compounds XVIIa and XVIIIa were 120 and 76-fold, respectively, more reactive with 4-(*p*-nitrobenzyl)pyridine than the standard TPCK = L-1-chloro-4-phenyl-3-tosylamido-2-butanone (28); since 4-(bromoacetamido)salicylic acid was 28 times as reactive as TPCK it can be estimated that VIa and VIb would be on the order of 4-10

TABLE III

Physical Constants and Methods of Preparation of



No. (a)	R_1	R_2	Method	% Yield	M.p. °C	Analyses Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
VIb	C_6H_5	$-CH_2Br$	A	85	215-220	(b)					
VIIIb	C_6H_5	$-C_6H_4NO_2$-*p*	B	96	289-291 (c)	61.1	4.87	17.8	60.9	5.01	17.5
IXa	CH_3	$-C_6H_4NO_2$-*m*	B	75 (c)	138-140 (c)	54.4	5.17	21.1	54.0	5.37	21.0
IXb	C_6H_5	$-C_6H_4NO_2$-*m*	B	62 (c)	273-274 (c)	61.1	4.87	17.8	61.2	5.05	17.6
Xa	CH_3	$-C_6H_4CH_2OC_2H_5$-*m*	B	48	223-225 (d)	62.8	7.02		62.6	6.85	
Xb	C_6H_5	$-C_6H_4CH_2OC_2H_5$-*m*	B	55	237-239 (e)	68.0	6.45	13.8	68.0	6.31	13.6
XIb	C_6H_5	$-C_6H_4CH_2OC_2H_5$-*p*	B	85	280-285 (e)	68.0	6.45	13.8	67.6	6.51	13.8
XIIa	CH_3	$-C_6H_4COCH_3$-*p*	B	82	258-261 (e)	59.0 (r)	6.40	16.2	59.5	6.36	16.3
XIIb	C_6H_5	$-C_6H_4COCH_3$-*p*	B	87	273-274 (e)	67.7	5.68	14.4	67.4	5.66	14.2
XIII	CH_3	$-C_6H_4NH_2$-*m*	C	69	252-254 (d)	57.2 (f)	6.57	22.3	57.2	6.76	22.2
XVIIa	CH_3	$-C_6H_4CH_2Br$-*p*	D (g)	77 (h)	243-245 (i)	41.8 (h)	4.39	12.2	41.6	4.51	12.3 (j)
XVIIb	C_6H_5	$-C_6H_4CH_2Br$-*p*	E	88 (k)	166-167 (l)	49.0 (k)	4.41	15.5 (m)	48.7	4.70	15.1 (m)
XVIIIa	CH_3	$-C_6H_4CH_2Br$-*m*	D	88 (h)	250-252 (i)	41.8 (h)	4.39	12.2	41.5	4.34	12.0 (n)
XVIIIb	C_6H_5	$-C_6H_4CH_2Br$-*m*	E	95 (k)	140-142 (l)	49.0 (k)	4.41	15.5 (m)	49.1	4.70	15.1 (m)
XIXa	CH_3	$-C_6H_4COCH_2Br$-*p*	F	99 (h)	230-232	41.8 (h)	4.13	32.7 (m)	41.6	3.99	32.5 (m)
XIXb	C_6H_5	$-C_6H_4COCH_2Br$-*p*	F (o)	60 (p)	155-165						
XXII	C_6H_5		G (q)	63	278-280 (e)	64.8	5.00	15.8	64.9	5.08	15.5

(a) All compounds gave infrared and ultraviolet compatible with their assigned structure and moved as single spots on TLC. (b) See experimental for characterization. (c) Recrystallized from 50% aqueous dioxane. (d) Recrystallized from aqueous ethanol. (e) Recrystallized from aqueous 2-methoxyethanol. (f) With 3/4 mole of water of crystallization. (g) See reference (14) for the starting material (XIa). (h) Hydrobromide salt. (i) Recrystallized from glacial acetic acid. (j) Bromine: Calcd.: 34.7. Found: 34.6. (k) Contains 3/4 mole sulfuric acid. (l) Recrystallized from 2-methoxyethanol by addition of the solution to 1 *N* aqueous sulfuric acid. (m) Bromine analysis. (n) Bromine: Calcd.: 34.7. Found: 34.4. (o) Reaction run by method F, but product isolated by method E. (p) Sulfate salt that gave erratic combustion results. The product was characterized by conversion to XXII by method G. (q) By reaction of XIXb with thioacetamide in 2-methoxyethanol at 90° for 3 hours, then dilution with water. (r) Monohydrate.

times less reactive than the benzyl bromides, XVIIa and XVIIIa. Therefore some additional factor plays a part in the difference in bimolecular inactivation of the enzyme by XVIIa and XVIIIa versus VIa and VIb.

EXPERIMENTAL

Methods.

Melting points were taken in capillary tubes on a Mel-temp block and are uncorrected. Infrared spectra were taken in potassium bromide pellets with a Perkin-Elmer 137B or 337 spectrophotometer. Ultraviolet spectra were determined in 10% ethanol with a Perkin-Elmer 202 spectrophotometer. Thin layer chromatograms (TLC) were run on Brinkmann silica gel and spots were detected by visual examination under ultraviolet light. The presence of active halogen was detected with *p*-nitrobenzylpyridine as previously described (17).

2-Amino-4-hydroxy-6-phenyl-5-pyrimidylpropylamine Dihydrochloride (Vb).

A mixture of 4.8 g. (20 mmoles) of IVb (26), 250 mg. of platinum oxide catalyst, 200 ml. of 85% aqueous ethanol, and 3.4 ml. of 12 *N* aqueous hydrochloric acid was shaken with hydrogen at 2-3 atmospheres until 60 mmoles had been absorbed, which required about 4 hours; during this time, IVb dissolved. The catalyst was removed by filtration and the filtrate spin-evaporated *in vacuo*. The glassy residue was dissolved in a minimum amount of water, then several volumes of acetone were added; yield, 5.07 g. (80%) of material suitable for further transformation. An analytical sample was obtained by a similar recrystallization as white crystals, m.p. 189-196° dec.

Anal. Calcd. for $C_{13}H_{18}Cl_2N_4O$: C, 49.2; H, 5.71; N, 17.7. Found: C, 49.0; H, 5.88; N, 17.6.

2-Amino-5-bromoacetamidopropyl-6-phenyl-4-pyrimidinol (VIb) (Method A).

To a magnetically stirred solution of 317 mg. (1 mmole) of Vb in 0.67 ml. of 3 *N* aqueous sodium hydroxide and 0.50 ml. of water was added 3.5 ml. of acetone followed by 260 mg. (1 mmole) of *p*-nitrophenyl bromoacetate (18). An immediate yellow color developed, then within 5 minutes the product began to separate. After 45 minutes, the mixture was filtered and the product washed with water. The product was leached with hot ethyl acetate to remove any residual *p*-nitrophenol leaving 311 mg. (85%) of a nearly white solid, m.p. 215-220° dec.; the solid showed one spot on TLC that gave a positive *p*-nitrobenzylpyridine test for active halogen (17). No suitable solvent for recrystallization could be found. Since the product gave erratic combustion results, it was characterized by reaction with pyridine as follows:

2-Amino-5-[3-(α-pyridiniumacetamido)propyl]-6-phenyl-4-pyrimidinol Bromide.

A mixture of 50 mg. (0.14 mole) of VIb and 25 ml. of reagent pyridine was stirred for about 20 hours at ambient temperature. During this time, the product separated. The product was collected on a filter, washed with benzene, then recrystallized from 80% aqueous ethanol; yield, 27 mg. (43%) of white crystals, m.p. 192-193°.

Anal. Calcd. for $C_{20}H_{22}BrN_5O_2 \cdot H_2O$: C, 51.9; H, 5.23; N, 15.2. Found: C, 51.5; H, 5.42; N, 15.0.

2-Amino-5-(*p*-nitrobenzamidopropyl)-6-phenyl-4-pyrimidinol (VIIIb) (Method B).

To a solution of 634 mg. (2 mmoles) of Vb in 1.33 ml. of 3 *N* aqueous sodium hydroxide and 1.0 ml. of water containing 318 mg. (3 mmoles) of anhydrous sodium carbonate was added 7 ml. of acetone. After the addition of 557 mg. (3 mmoles) of *p*-nitrobenzoyl chloride, the mixture was stirred at ambient temperature for 5 hours, during which time the product separated. The product was collected on a filter and washed with water; yield, 754 mg. (96%), m.p. 285-287° dec. Recrystallization from 50% aqueous dioxane gave yellow crystals, m.p. 289-291° dec. See Table III for analytical data.

2-Amino-5-(*m*-aminobenzamidopropyl)-6-methyl-4-pyrimidinol (XIII) (Method C).

A mixture of 315 mg. (1 mmole) of IXa, 100 ml. of 85% aqueous ethanol, 0.17 ml. (2 mmoles) of 12 *N* aqueous hydrochloric acid, and 50 mg. of 5% palladium-charcoal was shaken with hydrogen at 2-3 atmospheres until 3 mmoles were absorbed (about 20 hours). The filtered solution was spin-evaporated *in vacuo*. The residue was dissolved in hot, dilute aqueous ammonia. The resulting solution was spin-evaporated *in vacuo* to remove excess ammonia, then the product began to separate. The mixture was cooled overnight at 5°. The product was collected on a filter and washed with water; yield, 207 mg. (69%), m.p. 250-253°. Recrystallization from aqueous ethanol gave white crystals, m.p. 252-254°. See Table III for additional data.

2-Amino-5-(*p*-bromomethylbenzamidopropyl)-6-methyl-4-pyrimidinol (XVIIa) Hydrobromide (Method D).

A mixture of 344 mg. (1 mmole) of XIa and 10 ml. of glacial acetic acid containing 10% hydrogen bromide was refluxed for 2 hours during which time the starting material dissolved and the product separated. The mixture was cooled, then filtered. The product was recrystallized from glacial acetic acid; yield, 350 mg. (77%) of white crystals, m.p. 243-245°. See Table III for analytical data.

2-Amino-5-(*p*-bromomethylbenzamidopropyl)-6-phenyl-4-pyrimidinol (XVIIb) Sulfate (Method E).

To a solution of 203 mg. (0.5 mmole) of XIb in 5 ml. of glacial acetic acid containing 10% anhydrous hydrogen bromide was refluxed for 4 hours. Since the product did not separate on cooling, the solution was spin-evaporated *in vacuo*. The glassy residue was dissolved in about 2 ml. of ethanol, then the solution was added to 10 ml. of 1 *N* aqueous sulfuric acid. After standing overnight at 5°, the mixture was filtered; yield, 208 mg. (88%), m.p. 150-157°. Recrystallization by solution in 2-methoxyethanol, then dilution with several volumes of 1 *N* aqueous sulfuric acid gave white crystals, m.p. 166-167°. See Table III for analytical data.

2-Amino-5-(*p*-bromoacetobenzamidopropyl)-6-methyl-4-pyrimidinol (XIXa) Hydrobromide (Method F).

To a solution of 164 mg. (0.5 mmole) of XIIa in 4 ml. of glacial acetic acid containing 10% anhydrous hydrogen bromide was added 0.5 ml. of 1 *M* bromine in glacial acetic acid. After 20 hours the solution was poured into 50 ml. of ethyl acetate containing 46 mg. (0.5 mmole) of phenol as a bromine scavenger. The product was collected on a filter and washed with ethyl acetate to give a quantitative yield, m.p. 227-228°. Recrystallization by solution in 2-methoxyethanol containing a few drops of 10% anhydrous bromide in acetic acid followed by addition of ethyl acetate gave 162 mg. (62%) of white crystals, m.p. 230-232°. See Table III for analytical data.

REFERENCES

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(2) For paper LXXXVIII see B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, in press; for paper IX on irreversible inhibitors of dihydrofolic reductase see B. R. Baker and R. B. Meyer, Jr., *J. Pharm. Sci.*, 56, 570 (1967).

(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site", John Wiley and Sons, Inc., New York, N. Y., 1967.

(4) B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, 55, 1417 (1966); paper LXVII of this series.

(5) B. R. Baker and H. S. Shapiro, *ibid.*, 55, 1422 (1966); paper LXVIII of this series.

(6) B. R. Baker, B.-T. Ho, and D. V. Santi, *ibid.*, 54, 1415 (1965).

(7) B. R. Baker and J. H. Jordaan, *ibid.*, 54, 1470 (1965).

(8) B. R. Baker and B.-T. Ho, *J. Heterocyclic Chem.*, 2, 335 (1965).

(9) B. R. Baker and G. J. Lourens, *ibid.*, 2, 344 (1965).

(10) B. R. Baker, T. J. Schwan, J. Novotny, and B.-T. Ho, *J. Pharm. Sci.*, 55, 295 (1966).

(11) B. R. Baker, B.-T. Ho, J. K. Coward, and D. V. Santi, *ibid.*, 55, 302 (1966).

(12) B. R. Baker and H. S. Shapiro, *ibid.*, 55, 308 (1966).

(13) B. R. Baker and B.-T. Ho, *ibid.*, 55, 470 (1966).

(14) B. R. Baker and J. K. Coward, *ibid.*, 54, 714 (1966).

(15) B. R. Baker, B.-T. Ho, and T. Neilson, *J. Heterocyclic Chem.*, 1, 79 (1964).

(16) B. R. Baker, D. V. Santi, P. I. Almaula, and W. C. Werkheiser, *J. Med. Chem.*, 7, 24 (1964).

(17) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *J. Heterocyclic Chem.*, 3, 425 (1966).

(18) D. W. Russell, *Biochem. J.*, 78, 696 (1961).

(19) Y. S. Zalkind, *J. Russ. Phys. Chem.*, 46, 508; *Chem. Abstr.*, 8, 3187 (1914).

(20) W. K. Detweiler and E. D. Amstutz, *J. Am. Chem. Soc.*, 72, 2882 (1950).

(21) L. Friedman and H. Shechter, *J. Org. Chem.*, 26, 2522 (1961).
(22a) A. Hantzch, *Ber.*, 21, 942 (1888); *Ann. Chem.*, 250, 257 (1889); (b) R. H. Wiley, D. C. England, and L. C. Behr, *Org. Reactions*, 6, 367 (1951).
(23) B. R. Baker, W. W. Lee, and E. Tong, *J. Theoret. Biol.*, 3, 459 (1962).
(24) See reference (3), chapter VIII.
(25) For discussion of the chemotherapeutic reasons that the active-site-directed mechanism 1) is necessary for enzyme specificity see references 3, 23 and B. R. Baker, *J. Pharm. Sci.*, 53, 347 (1964).
(26) B. R. Baker and P. I. Almaula, *J. Heterocyclic Chem.*, 1, 263 (1964).
(27) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, 53, 714 (1964).
(28) B. R. Baker and J. H. Jordaan, *J. Heterocyclic Chem.*, 2, 21 (1965).

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