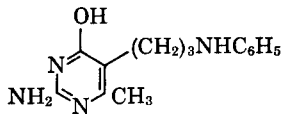
Analogs of Tetrahydrofolic Acid XX

Effects of Modification of the Bridge Between the Pyrimidyl and Phenyl Moieties of 2-Amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol on Inhibition of Dihydrofolic Reductase and Thymidylate Synthetase II

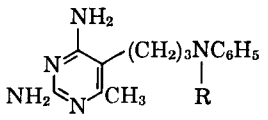
By B. R. BAKER and BENG-THONG HO

Two new bridges between the pyrimidyl and phenyl binding moieties of 2-amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol (I) have been synthesized and enzymatically evaluated. The $-SO_2\cdot N(R)\cdot(CH_2)_3-$ bridge between a *p*-tolyl group and the pyrimidyl moiety gave an inhibitor (X) more effective than I with a $-N(R)\cdot(CH_2)_3-$ bridge, even though the former bridge was one atom longer. The $-CO\cdot N(R)\cdot(CH_2)_3-$ bridge between the pyrimidyl and phenyl moieties gave a compound (XI) that was a more effective inhibitor of dihydrofolic reductase than I, but much less effective against thymidylate synthetase. 2-Amino-5-(*N*-carbophenoxy-3-anilinopropyl)-6-methyl-4-pyrimidinethiol (VIII) showed a cross-over specificity, being at least tenfold more effective on thymidylate synthetase than dihydrofolic reductase.

THE PYRIMIDYL analog (I) of tetrahydrofolic acid has previously been observed to be an inhibitor of folic reductase (2), dihydrofolic reductase (3), and thymidylate synthetase (3), even though I does not have the carboxy-L-glutamate moiety normally present on the substrate. In a later study (4) on the mode of binding of 2,4-diaminopyrimidines to dihydro-



I

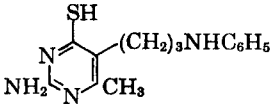


II, R = H

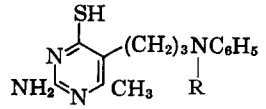
III, R = $-SO_2$⟨⟩CH_3

folic reductase, II was found to bind to dihydrofolic reductase 350 times better than I. The synthesis of II proceeds through the *N*-tosyl derivative (III). Since III was available, it has now been assayed as an inhibitor of dihydrofolic reductase (Table I); III was as good an inhibitor as II. It is indeed pleasantly surprising that dihydrofolic reductase has the tolerance for the extra bulk of the *N*-tosyl group.

The question was then posed whether thymidylate synthetase had a similar bulk tolerance for a group as large as the *N*-tosyl. This answer could not be obtained by assaying III as an inhibitor of thymidylate synthetase due to the lower relative activity of II with this enzyme (3) and the lack of sufficient solubility of III to reach the concentration of III necessary to inhibit the enzyme. Therefore, corresponding derivatives (V–VIII) of the more active (3) 2-amino-4-pyrimidinethiol (IV) were prepared and assayed as inhibitors of both thymidylate synthetase and dihydrofolic reductase.



IV



V, R = $COCH_3$

VI, R = $-SO_2$⟨⟩CH_3

VII, R = $-\overset{O}{\overset{\|}{C}}C_6H_5$

VIII, R = $-\overset{O}{\overset{\|}{C}}OC_6H_5$

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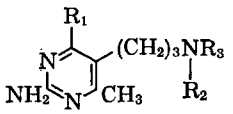
Previous paper: Baker, B. R., and Almaula, P. I., *J. Heterocyclic Chem.*, 1, 263(1965). For Part I on "bridges," see *Reference 1*.

TABLE I.—INHIBITION OF DIHYDROFOLIC REDUCTASE AND THYMIDYLATE SYNTHETASE BY



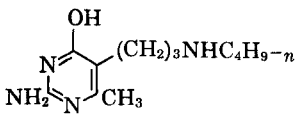
				Dihydrofolic Reductase[a]				Thymidylate Synthetase[b]				
Compd.	R_1	R_2	R_3	μM Concn. FAH_2[c]	mM Concn. Inhibitor	% Inhibition	Inhibitor: Substrate Ratio[d]	μM Concn. M-FAH_4[e]	mM Concn. Inhibitor	% Inhibition	Inhibitor: Substrate Ratio[f]	Synthetase: Reductase Ratio[g]
II[h]	NH_2	C_6H_5	H	6	0.0022	50	0.37	25.7	0.80	50	63	170
III	NH_2	C_6H_5	Ts[i]	6	0.0019[j]	50	0.32					
IV[h]	SH	C_6H_5	H	3	0.022	50	7.3	25.7	0.080	38	11	1.5
V[k]	SH	C_6H_5	$-COCH_3$	6	0.020	50	3.3	25.7	0.040	50	3.9	1.2
VII	SH	C_6H_5	$-COC_6H_5$	6	0.050[j]	50	8.3	25.7	0.075[l]	46	7.0	0.84
VIII	SH	C_6H_5	$-COOC_6H_5$	6	0.080[j]	0	>53	25.7	0.065[l]	50	5.1	<0.096
								51.4	0.13[l]	50	5.1	
I[h]	OH	C_6H_5	H	6	0.60[j]	43	130	25.7	0.62[l]	50	50	0.39
								51.4	1.2[l]	50	50	
IX[k]	OH	C_4H_9-*n*	H	6	6.0	0	>4000	25.7	4.5	50	350	<0.087
X	OH	C_4H_9-*n*	Ts[i]	6	0.19[j]	50	32	51.4	0.15[l]	26	17	0.50
								25.7	0.20[l]	50	15	
XI	OH	C_4H_9-*n*	$-COC_6H_5$	6	0.19[j]	50	32	51.4	1.0[l]	0	>150	>4.7

The technical assistance of Miss Rita Zielinski and Miss Karen Smith with these enzyme assays is gratefully acknowledged.
[a] Dihydrofolic reductase from pigeon liver was prepared and assayed with 6 μM dihydrofolate (unless otherwise indicated) and 12 μM TPNH in 0.05 *M* Tris buffer at pH 7.4 as previously described (3). [b] Thymidylate synthetase from *E. coli* B was prepared and assayed with 80 μM 2′-deoxyuridylate, either 25.7 or 51.4 μM *dl*-tetrahydrofolate, magnesium chloride, and formaldehyde in 0.05 *M* Tris buffer at pH 7.4 as previously described (3), except that the cells were broken by passage of a suspension of *E. coli* B (1 Gm./1 ml. of buffer) through a French press. [c] FAH_2 = dihydrofolate. [d] Estimated ratio of concentrations of inhibitor to dihydrofolate required for 50% inhibition. [e] M-FAH_4 = 5,10-methylene-*dl*-tetrahydrofolate. [f] Estimated ratio of concentrations of inhibitor to the active isomer, 5,10-methylene-*l*-tetrahydrofolate required for 50% inhibition. [g] Ratio of inhibitor:substrate for 50% inhibition of thymidylate synthetase to inhibitor:substrate for 50% inhibition of dihydrofolic reductase. [h] Enzyme data from *Reference 3*. [i] Ts = *p*-tolylsulfonyl. [j] Enzyme assay with 10% *N,N*-dimethylformamide present. [k] Enzyme data from *Reference 5*. [l] Enzyme assay with 5% 2-methoxyethanol present.

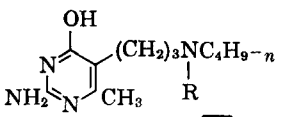
DISCUSSION

The *N*-acetyl-4-pyrimidinethiol (V) had been assayed previously (5) and found to be somewhat more active than IV on both enzymes (Table I). Attempts to synthesize the *N*-tosyl derivative (VI) were unsuccessful, but the *N*-benzoyl (VII) and *N*-carbophenoxy (VIII) were successfully prepared from IV. The *N*-benzoyl group of VII was well tolerated by both enzymes; in fact, VII was as active as IV on both enzymes. Although the *N*-carbophenoxy derivative (VIII) was an even more effective inhibitor of thymidylate synthetase than the parent (IV), effectiveness against dihydrofolic reductase was greatly decreased (Table I). Thus, VIII showed at least a tenfold better inhibition of thymidylate synthetase than dihydrofolic reductase.

The ability of the *N*-benzoyl derivative (VII) to inhibit both enzymes posed the question concerning whether the benzoyl group or the anilino group of VII was binding to the enyme. The question was answered by reaction of the relatively ineffective butylaminopyrimidine (IX) (5) with *p*-tolylsulfonyl chloride or benzoyl chloride to give X and XI, respectively. In this case, comparison of the inhibitory powers of X and XI with that of the anilino-pyrimidinol (I) was made (Table I). The *N*-tosyl derivative (X) was a considerably better inhibitor of both thymidylate synthetase and dihydrofolic reductase than was the anilino derivative (I). Thus, the *N*-sulfamylpropyl group represents a new bridge between the pyrimidyl and



IX



X, R = $-SO_2-C_6H_4-CH_3$

XI, R = $-COC_6H_5$

phenyl moieties which gives effective inhibition, even though the bridge contains one more atom then the aminopropyl bridge of I.

The *N*-benzoyl derivative (XI) showed a specificity between the two enzymes; XI was as effective an inhibitor as the *N*-tosyl derivative (X) against dihydrofolic reductase, but XI showed no activity against thymidylate synthetase at the highest concentration measurable. Several useful conclusions can be drawn by comparison of the data in Table I.

(*a*) Both enzymes can tolerate the extra bulk of an *N*-benzoyl group on the anilino moiety of IV. (*b*) The bridge length between the pyrimidyl and phenyl moieties of I can be increased from the 4-

atom aminopropyl bridge to the 5-atom sulfaminopropyl group of X with retention of binding to both enzymes; binding is still retained on dihydrofolic reductase with the —$CONHCH_2CH_2CH_2$— bridge. (*c*) The anilino phenyl—and not the benzoyl phenyl—of VII binds to thymidylate synthetase since XI without an anilino phenyl group has no detectable binding to the enzyme. (*d*) It cannot be deduced from the data in Table I whether the anilino phenyl or the benzoyl phenyl of VII binds to dihydrofolic reductase, since I, IV, and XI all bind to this enzyme. (*e*) The *N*-carbophenoxy group of VIII is poorly tolerated by dihydrofolic reductase, but is well tolerated by thymidylate synthetase. (*f*) Comparison of (*c*) and (*e*) shows a cross-over in enzyme specificity; that is, VIII is a good inhibitor of thymidylate synthetase and not dihydrofolic reductase. In contrast, the reverse is true with XI.

The fact that the *N*-benzoyl group of XI cannot bind effectively to thymidylate synthetase, whereas the *N*-tosyl group of X can bind effectively, is not simple to rationalize. It is obviously not a steric problem since the sulfonyl group is bigger than the carbonyl group. A remote possibility was that the sulfonyl group was binding to thymidylate synthetase through a polarized form, even though this explanation was not likely—since the phenyl group of I can bind to this enzyme—an aliphatic sulfonyl derivative related to structure X was synthesized and did not bind to the enzyme (6). Most of the evidence available points to the possibility that the phenyl ring of I binds as an electron acceptor in a charge–transfer complex with dihydrofolic reductase (5, 7), whereas the phenyl ring of I binds as an electron donor in a charge–transfer complex with thymidylate synthetase (5, 8). Thus, it may be possible that the *N*-tosyl of X can complex as an electron acceptor or an electron donor since the tosyl moiety contains both the electron-withdrawing sulfonamide group and the electron-donating methyl group. In contrast, the *N*-benzoyl of XI should bind well as an electron-acceptor, but poorly as an electron donor.

Further work is being pursued to resolve this enigma. These new bridges should have certain operational advantages in synthesis of potential active-site-directed irreversible inhibitors (9) and are currently being investigated, particularly since the bulk of the *N*-butyl is tolerated by the two enzymes.

EXPERIMENTAL

Melting points were taken on a Fisher-Johns apparatus and those below 230° are corrected. Infrared and ultraviolet spectra were determined on Perkin-Elmer recording spectrophotometers 137B and 202, respectively. Thin-layer chromatograms (TLC) were run with Brinkmann Silica Gel G and spots were detected by iodine vapor.

2 - Amino - 5 - (N - benzoyl - 3 - anilinopropyl) - 6-methyl-4-pyrimidinethiol (VII).—A solution of 100 mg. (0.364 mmole) of IV (10) in 2 ml. of warm reagent pyridine was cooled to 0°, then treated with 76 mg. (0.546 mmole) of benzoyl chloride with magnetic stirring. The mixture was removed from the ice bath and magnetically stirred for 1 hr. at ambient temperature, protected from moisture. After dilution with 5 ml. of water, the mixture was extracted with chloroform (4 × 5 ml.). Dried with magnesium sulfate, the combined extracts were spin evaporated *in vacuo*, leaving 71 mg. (52%) of product, m.p. 201–202°. Recrystallization of 65 mg. from ethyl acetate–petroleum ether, b.p. 30–60°, gave 48 mg. of analytical sample, m.p. 202–202.5°. $\nu_{max.}^{KBr}$ 3400, 3150 (NH); 1645, 1620, 1560 $cm.^{-1}$ (amide C=O, NH, C=C, C=N); $\lambda_{max.}^{pH\ 1}$ 340 (ϵ 15,400), 255 mμ (sh, ϵ 10,800); $\lambda_{max.}^{pH\ 7}$ 350 (ϵ 16,200), inflection centering at 265 mμ (ϵ 10,000); $\lambda_{max.}^{pH\ 13}$ 265 (ϵ 9800), 319 mμ (ϵ 13,000).

Anal.—Calcd. for $C_{21}H_{22}N_4OS$: C, 66.6; H, 5.86; N, 14.8. Found: C, 66.4; H, 5.89; N, 14.6.

An attempt to run the benzoylation in aqueous sodium hydroxide gave poor results.

2 - Amino - 5 - (N - carbophenoxy - 3 - anilinopropyl) - 6 - methyl - 4 - pyrimidinethiol (VIII).—Reaction of 100 mg. (0.364 mmole) of IV (10) in 3 ml. of reagent pyridine with 100 mg. (0.639 mmole) of phenyl chloroformate was performed as described for the preparation of VII. When the reaction mixture was poured into 20 ml. of water, an oil separated. When the mixture was stirred with 5 ml. of benzene, the oil solidified. The product was collected on a filter and washed with benzene; yield, 118 mg. (82%), m.p. 189–190°. In a pilot run the product was recrystallized from benzene to give light yellow crystals, m.p. 189–190°. $\nu_{max.}^{KBr}$ 3500, 3400, 3100 (NH); 1730, 1700 (C=O); 1650, 1600, 1550 $cm.^{-1}$ (NH, pyrimidine); $\lambda_{max.}^{pH\ 1}$ 341 (ϵ 15,300) sh at 253 mμ; $\lambda_{max.}^{EtOH}$ 267 (ϵ 5800), 357 mμ (ϵ 15,700).

Anal.—Calcd. for $C_{21}H_{22}N_4O_2S$: C, 63.9; H, 5.62; N, 14.2. Found: C, 63.7; H, 5.59; N, 13.9.

2 - Amino - 5 - [N - (p - tolylsulfonyl) - 3 - (n - butylamino)propyl] - 6 - methyl - 4 - pyrimidinol (X).—To a solution of 233 mg. (0.75 mmole) of IX dihydrochloride (5) in 7.5 ml. of 1 *N* aqueous sodium hydroxide was added a solution of 213 mg. (1.12 mmoles) of *p*-tolylsulfonyl chloride in 5 ml. of chloroform. The mixture was magnetically stirred for 15 hr. in a stoppered flask, then adjusted to about pH 8 with glacial acetic acid. The separated aqueous layer was extracted with chloroform (5 × 3 ml.); the combined chloroform extracts were dried with magnesium sulfate, then spin-evaporated *in vacuo*. Trituration of the residue with petroleum ether, b.p. 30–60°, gave 188 mg. (64%) of crude product, m.p. 120–123°. Recrystallization from aqueous ethanol gave 113 mg. (42%) of white crystals, m.p. 142–145°. A second recrystallization gave the analytical sample with the same melting point. $\nu_{max.}^{KBr}$ 3400, 3200 (NH); 1660, 1640, 1600 (NH, C=C, C=N); 1340, 1160 $cm.^{-1}$ (—SO_2—); $\lambda_{max.}^{pH\ 1}$ 232 (ϵ 20,500), sh at 265 mμ (ϵ 9240); $\lambda_{max.}^{pH\ 7}$ 231 (ϵ 20,000), 275 (sh, ϵ 5800), 294 mμ (ϵ 5400); $\lambda_{max.}^{pH\ 13}$ 280 mμ (ϵ 7000).

Anal.—Calcd. for $C_{19}H_{28}N_4O_3S$: C, 58.1; H, 7.19; N, 14.3. Found: C, 58.0; H, 7.23; N, 14.1.

2 - Amino - 5 - [N - benzoyl - 3 - (n - butyl - amino)propyl] - 6 - methyl - 4 - pyrimidinol (XI).—Treatment of 117 mg. (0.36 mmole) of IX dihydrochloride (5) in 3.5 ml. of 1 *N* aqueous sodium hydroxide with 79 mg. (0.56 mmole) of benzoyl chloride in 1.5 ml. of chloroform, as described

for the preparation of X, gave 90 mg. (73%) of amorphous white solid, m.p. 102–108°. This material showed a single spot on TLC with 12 or 25% methanol in benzene. $\nu_{max.}^{KBr}$ 3500, 3400, 3350, 3150–3100 (NH, OH); 1670–1610, 1540 cm.$^{-1}$ (amide C=O, NH, pyrimidine); $\lambda_{max.}^{pH\ 1}$ 266 mμ (ε 7300); $\lambda_{max.}^{pH\ 13}$ 280 mμ (ε 6900); $\lambda_{max.}^{EtOH}$ 293 mμ (ε 7900).

Anal.—Calcd. for $C_{19}H_{26}N_4O_2$: C, 66.6; H, 7.65; N, 16.4. Found: C, 66.3; H, 7.70; N, 16.6.

To a solution of 225 mg. of a different preparation of amorphous XI in 2 ml. of 95% ethanol was added a solution of 229 mg. of picric acid in 4 ml. of ethanol. The crystalline picrate was collected and washed with alcohol; yield, 193 mg. (53% based on IX), m.p. 170–173°. Recrystallization from 50% ethanol gave yellow crystals, m.p. 171–172°.

Anal.—Calcd. for $C_{19}H_{26}O_2 \cdot C_6H_3N_3O_7$: C, 52.5; H, 5.11; N, 17.2. Found: C, 52.7; H, 5.40; N, 17.0.

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Synthesis and Pharmacological Properties of Some Fluorine-Containing Amide Derivatives

By MARCUS W. JORDIN, WILLIAM D. EASTERLY, JR., THOMAS E. WINNINGHAM, and WALTER C. HUBBARD

Eight fluorine-containing compounds, seven of which are derivatives of bromal or dichloroacetaldehyde, were synthesized and their physical constants determined. These compounds have been screened for anticancer, antispasmodic, tranquilizing, and blood pressure effects. Acute toxicity studies have also been carried out.

VARIOUS amide derivatives of chloral, bromal, trichlorobutyraldehyde, and dichloroacetaldehyde (1–9) have been reported in the literature. Work with these compounds has shown their potentialities as fungicides (9) and sedative-hypnotics (10). Other than this, however, little has been reported in regard to the pharmacological activities exhibited by these compounds. In view of this fact, eight fluorinated amide derivatives have been synthesized and subjected to certain pharmacological screens.

Interest in these particular compounds stemmed from the fact that some of our anticancer drugs today are amide derivatives, an example is the phosphoramides. The fluorinated amide derivatives were chosen for study since certain fluorine-containing antimetabolites, such as 5-fluorouracil and 5-fluorodeoxyuridine, have found a place in cancer chemotherapy. Interest, too, was prompted by the fact that some amide derivatives, such as 3,4,5-trimethoxycinnamide and 2-ethyl-3-methylvaleramide, are being used as tranquilizers today.

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EXPERIMENTAL

Materials

The intermediates used in this investigation were obtained through ordinary commercial sources. The fluoroacetamide, 4-fluorobenzoic acid, 3-aminobenzotrifluoride, and 4-fluorophenylacetic acid were purchased from Aldrich Chemical Co., Inc. The trifluoroacetamide and 2,4-dichlorobenzoyl chloride were obtained from the Matheson, Coleman, and Bell Division of the Matheson Co. The aldehydes used, bromal and dichloroacetaldehyde, were obtained from Eastman Kodak Co. and the Westvaco Chemical Co., respectively.

Synthesis

The amide derivatives of bromal and dichloroacetaldehyde were prepared by reacting the desired amide with the appropriate aldehyde in equimolar portions according to procedures previously reported (7, 8). The procedure (8) was modified for the preparation of the trifluoroacetamide derivative of bromal in that the condensation was carried out in a vacuum oven (Labline Duo-Vac, model 3620) at 20° and −20 lb. pressure rather than in a constant-temperature bath. The general reaction is shown in Scheme I, with R representing either a fluorinated alkyl or fluorinated aryl carbon chain.

The one compound reported in this paper that is neither a derivative of bromal or dichloroacetaldehyde, the *N*-(3-trifluoromethylphenyl)-2,4-dichlorobenzamide, was prepared by reacting 2,4-