Analogs of Tetrahydrofolic Acid. XVIII.[1,2] On the Mode of Binding of Some 6-Aryl- and 6-Aralkylpyrimidines to Folic Reductase

B. R. BAKER, HOWARD S. SHAPIRO,

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo 14, New York

AND WILLIAM C. WERKHEISER

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo 3, New York

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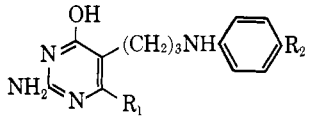
A series of 2-amino-5-anilinopropyl-4-pyrimidinols (X) bearing a *p*-nitrophenyl, *p*-tolyl, or 2-furyl group at the 6-position were synthesized. In addition, a series of 2,4-diaminopyrimidines with a phenylbutyl (XXI) or anilinopropyl group (XVIII) at the 5-position and bearing either a 6-phenyl or 6-benzyl group were prepared. Enzymic evaluation of the effects of these and related compounds on folic reductase showed that the increase in binding of 2-amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol (III) previously observed by single replacement of (a) the anilino group by benzyl, (b) the 6-methyl by 6-phenyl or 6-benzyl, (c) the 4-hydroxyl by 4-amino, or (d) the 4-hydroxyl by 4-mercapto were not necessarily additive if two or more of these changes were made in the same molecule; some cases of additivity in binding were observed with two structural changes.

In previous papers of this series it was reported that (a) the 6-methylpyrimidyl analog of tetrahydrofolic acid (I) was bound more tightly ($K_i = 2 \times 10^{-6}$) to folic reductase than the substrate, folic acid ($K_m = 10 \times 10^{-6}$)[3]; (b) replacement of the 6-methyl with a 6-phenyl group (II)[4] afforded an even better inhibition of folic reductase with $K_i = 9 \times 10^{-8}$; (c) only about 20% of the total free energy of binding to folic reductase was lost when the carboxyl-L-glutamate residue was removed as in III ($K_i = 63 \times 10^{-6}$)[5] and IV ($K_i = 1.8 \times 10^{-6}$)[6]; and (d) the benzylpyrimidine V ($K_i = 4 \times 10^{-6}$)[6] was bound to folic reductase almost as well as the 6-phenylpyrimidine (IV). Since the 6-phenyl and 6-benzyl groups gave more effective binding to folic reductase than the 6-methyl group, we have investigated further the mode of binding of the 6-phenyl (IV) and 6-benzylpyrimidines (V) and wish to report the results in this paper.



I, $R_1 = CH_3$; $R_2 = CONHCHCOOH$ (CH_2CH_2COOH)

II, $R_1 = C_6H_5$; $R_2 = CONHCHCOOH$ (CH_2CH_2COOH)

III, $R_1 = CH_3$; $R_2 = H$

IV, $R_1 = C_6H_5$; $R_2 = H$

V, $R_1 = C_6H_5CH_2$; $R_2 = H$

The first structural change investigated was replacement of the 6-phenyl group in IV by the electron-accepting 6-nitrophenyl group (Xc) and by the electron-donating groups, *p*-tolyl (Xd) and 2-furyl (Xe). Two routes had been previously developed for synthesis of compounds of type X; these routes differ in whether the anilino moiety is introduced last[4] or is introduced first.[6] The nitrophenyl analog was prepared by the first route[4] *via* IXc primarily because the second route was not yet available at the time. The second route[6] *via* XI was used to synthesize the *p*-tolyl (Xd) and 2-furyl (Xe) analogs. That these two routes were fairly good general methods was indicated by the fact that no complications arose in the synthesis of the three compounds. One might have anticipated that the hydrogen bromide used for removal of the N-tosyl group from the furyl analog (XIe) might have attacked the somewhat acid-labile furan ring, but fortunately these fears proved unwarranted.

The second structural change investigated was replacement of the anilinopropyl side chain in X by phenylbutyl to give XIXa and XIXb; since the phenylbutyl derivative of the 6-methylpyrimidine (XIX, $R_1 = CH_3$) was bound more tightly to folic reductase than the anilinopropyl group (X, $R_1 = CH_3$),[7] it was of interest to determine whether an additive tightening of binding were actually possible with compounds such as XIXa and XIXb compared to X ($R_1 = CH_3$). By the second synthetic route[6] *via* XVI, XIXa and XIXb were readily obtained in crystalline form.

Next we wanted to determine if a third factor leading to tighter binding would be additive in a molecule such as XVIIIa or XVIIIb; that is, if the better binding by 6-aryl (or 6-aralkyl) and the better binding by the 4-amino group of molecules of type XVIII[8] or the 4-mercapto group of type XVII[9] were additive. Compounds XVIIIa and XVIIIb were synthesized *via* XII as previously described for the 6-methyl derivative (XVIII, $R_1 = CH_3$).[8] However, the synthesis of the 4-mercapto derivative (XIIIa) showed an interesting difference in reactivity of the halogen of 6-phenylpyrimidine (XIIa) compared to XII where $R_1 = CH_3$.[9] In the latter case, XII ($R_1 = CH_3$) was inert to thiourea in boiling *t*-butyl alcohol unless the 2-amino

(1) This work was supported in part by the U. S. Public Health Service through Grants CA-05867, CA-06624, 2G-555, and CA-05298.

(2) For the previous paper of this series see B. R. Baker and J. H. Jordaan, *J. Med. Chem.*, **8**, 35 (1965).

(3) B. R. Baker and C. E. Morreal, *J. Pharm. Sci.*, **52**, 840 (1963); paper VII of this series.

(4) B. R. Baker and H. S. Shapiro, *J. Med. Chem.*, **6**, 658 (1963); paper IX of this series.

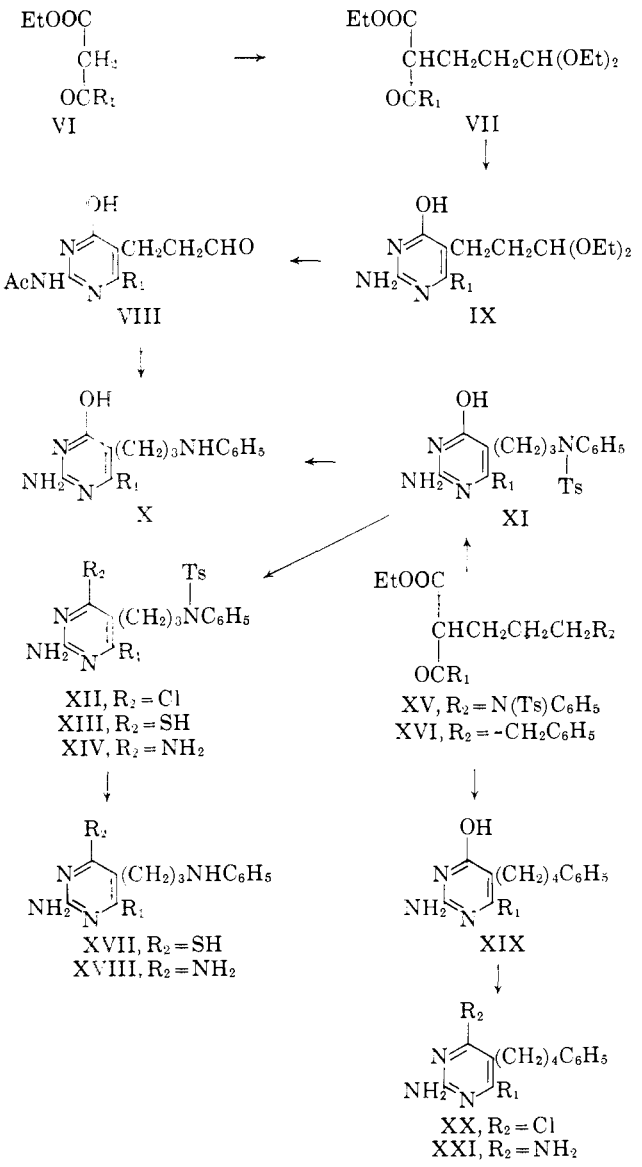
(5) B. R. Baker, D. V. Santi, P. I. Almaula, and W. C. Werkheiser, *ibid.*, **7**, 24 (1964); paper X of this series.

(6) B. R. Baker, D. V. Santi, and H. S. Shapiro, *J. Pharm. Sci.*, **53**, 1317 (1964); paper XIV of this series.

(7) B. R. Baker, B. T. Ho, and G. Chheda, *J. Heterocyclic Chem.*, **1**, 88 (1964); paper XIII of this series.

(8) B. R. Baker and B. T. Ho, *J. Pharm. Sci.*, **53**, 1457 (1964); paper XV of this series.

(9) B. R. Baker, C. E. Morreal, and B. T. Ho, *J. Med. Chem.*, **6**, 658 (1963); paper VIII of this series.



a, $R_1 = C_6H_5$
b, $R_1 = C_6H_5CH_2$
c, $R_1 = 4\text{-}NO_2C_6H_4$
d, $R_1 = 4\text{-}CH_3C_6H_4$
e, R_1 = 2-furyl
Ts = *p*-tolylsulfonyl

group was acetylated[9]; in contrast, XIIa reacted readily with thiourea in boiling *t*-butyl alcohol to give, after treatment with base, the 4-mercaptopyrimidine (XIIIa) in good yield. This difference in reactivity of the halogen of XII certainly indicates that the 6-aryl group can influence the chemistry of 4-position of the pyrimidine ring, the significance of which will be discussed in the enzyme section.

Finally, we tried to put the three factors that enhanced the binding together in one molecule such as XXIa and XXIb; if all these factors were additive, one might obtain a compound with an estimated K_i of about 5×10^{-11}, the order of aminopterin and amethopterin. Compounds XXIa and XXIb were readily synthesized *via* the 4-chloropyrimidines XXa and XXb.

Experimental

Melting points were taken in capillary tubes in a Mel-Temp block; those below 230° are corrected. Infrared (in KBr disk) and ultraviolet spectra (10% ethanol) were determined on Perkin-Elmer spectrophotometers, Models 137B and 202, respectively; thin layer chromatograms (t.l.c.) were run on silica gel G (Brinkmann) and spots were detected in iodine vapor; all analytically pure compounds described in this paper gave a single spot on t.l.c., unless otherwise indicated.

2-Amino-4-hydroxy-6-(*p*-nitrophenyl)-5-pyrimidylpropionaldehyde Diethyl Acetal (IXc).—Reaction of ethyl *p*-nitrobenzoylacetate with acrolein, conversion to the acetal (VIIc),[4] and condensation with guanidine carbonate in dimethyl sulfoxide as described for the preparation of IXa[4] gave a 25% over-all yield of IXc. Recrystallization from aqueous ethanol, then ethyl acetate–petroleum ether afforded nearly white crystals: m.p. 161–163°; λ_{max} 2.95 (NH), 5.94, 6.00–6.08, 6.25, 6.39 (NH, C=N, C=C, C=O), 6.50, 7.44 (NO_2), 9.45 μ (ether C–O–C); $\lambda_{max}^{pH\ 6}$ 225 mμ (ε 16,100), 255 (15,450), 300 (infl) (9500); $\lambda_{max}^{pH\ 7}$ 275 mμ (ε 14,800); $\lambda_{max}^{pH\ 13}$ 275 mμ (ε 16,100).

Anal. Calcd. for $C_{17}H_{22}N_4O_5$: C, 56.3; H, 6.09; N, 15.4. Found: C, 56.1; H, 6.22; N, 15.2.

2-Acetamido-4-hydroxy-6-(*p*-nitrophenyl)-5-pyrimidylpropionaldehyde Diethyl Acetal.—Acetylation of 200 mg. (0.55 mmole) of IXc with pyridine and acetic anhydride as described for the preparation of the 6-phenyl analog[4] gave, after recrystallization from toluene, 152 mg. (68%) of pure product: m.p. 164–168°; λ_{max} 5.90 (amide C=O), 6.05–6.10, 6.18, 6.45 (pyrimidine), 6.50, 7.44 (NO_2), 9.45 μ (ether C–O–C); $\lambda_{max}^{pH\ 1,7}$ 250 mμ (ε 17,400), 276 (18,800); $\lambda_{max}^{pH\ 13}$ 275 mμ (ε 18,400).

Anal. Calcd. for $C_{19}H_{24}N_4O_6$: C, 56.5; H, 5.95; N, 13.9. Found: C, 56.5; H, 6.00; N, 13.9.

2-Acetamido-4-hydroxy-6-(*p*-nitrophenyl)-5-pyrimidylpropionaldehyde (VIIIc).—A mixture of 7.35 g. (18.2 mmoles) of the preceding acetal and 700 ml. of water was refluxed with stirring for 2 hr. during which time solution occurred and the product separated. After being cooled to 0°, the mixture was filtered and the product was washed with water; yield 5.17 g. (86%) of yellow crystals; m.p. 203–206° dec.; λ_{max} 3.10 (NH), 3.67 (aldehyde CH), 5.85 (aldehyde C=O), 5.90 (amide C=O), 6.58, 7.44 (NO_2), no ether C–O–C near 9.5 μ; $\lambda_{max}^{pH\ 1,7}$ 248 mμ (ε 17,200), 276 (19,500); $\lambda_{max}^{pH\ 13}$ 275 mμ (ε 18,300).

Anal. Calcd. for $C_{15}H_{18}N_4O_5$: C, 54.5; H, 4.24; N, 16.9; O, 24.3. Found: C, 54.6; H, 4.35; N, 16.6; O, 24.1.

2-Amino-5-(3-anilinopropyl)-6-(*p*-nitrophenyl)-4-pyrimidinol (Xc).—Reductive condensation of VIIIc with aniline, as previously described for the preparation of Xa,[6] gave an 86% yield of Xc, m.p. 207–218°. Recrystallization from aqueous ethanol gave 1.55 g. (69%) of nearly white crystals: m.p. 216–218°; λ_{max} 2.9–3.0 (NH), 6.00–6.26 (NH, C=C, C=N), 6.65, 7.45 μ (NO_2); $\lambda_{max}^{pH\ 6}$ 225 mμ (ε 15,800), 256 (14,900), 300 (infl) (8600); $\lambda_{max}^{pH\ 7,13}$ 243 mμ (ε 20,600), 277 (15,100).

Anal. Calcd. for $C_{19}H_{19}N_5O_3$: C, 62.6; H, 5.22; N, 19.2; O, 13.2. Found: C, 62.4; H, 5.36; N, 19.3; O, 13.5.

2-Amino-6-*p*-tolyl-5-[N-(*p*-tolylsulfonyl)anilinopropyl]-4-pyrimidinol (XId).—Alkylation of ethyl *p*-toluylacetate[10] with N-(3-bromopropyl)-*p*-tolysulfonanilide, as described for the preparation of XIa,[6] gave XId in 35% over-all yield, m.p. 203–204°.

Anal. Calcd. for $C_{27}H_{28}N_4O_3S$: C, 66.5; H, 5.75; N, 11.5. Found: C, 66.3; H, 5.90; N, 11.2

Similarly, **2-amino-6-(α-furyl)-5-[N-(*p*-tolylsulfonyl)anilinopropyl]-4-pyrimidinol (XIe)** was prepared in 15% over-all yield from ethyl α-furoylacetate.[11] This compound had m.p. 228–230° dec; $\lambda_{max}^{pH\ 1}$ 231 mμ (ε 21,500), 316 (15,300); $\lambda_{max}^{pH\ 7}$ 234 mμ (ε 24,100), 280 (13,000), 321 (9800); $\lambda_{max}^{pH\ 13}$ 236 mμ (infl), 270 (infl), 310 (ε 7900).

Anal. Calcd. for $C_{25}H_{24}N_4S$: C, 62.2; H, 5.19; N, 12.1. Found: C, 62.5; H, 5.47; N, 12.0.

2-Amino-5-(3-anilinopropyl)-6-(α-furyl)-4-pyrimidinol (Xe).—To a solution of 41 mg. of phenol in 0.4 ml. of 30% HBr in acetic acid was added 100 mg. (0.216 mmole) of XIc. After 1 hr. the mixture was diluted with 25 ml. of ether. The hydrobromide salt of Xe was collected on a filter and washed with ether. The crude salt was dissolved in about 35% ethanol, then adjusted to pH 8 with 10% aqueous NaOH. After several hours at 0°, the mixture was filtered, and the product was washed with water. Recrystallization from aqueous ethanol gave 52 mg. (78%) of nearly white crystals: m.p. 211–214°; λ_{max} 2.90 (NH), 6.01, 6.25,

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(11) J. E. Zanetti and C. O. Beckman, *J. Am. Chem. Soc.*, **50**, 1438 (1928).

6.70 (NH, C=C, C=N), 13.4, 14.4 μ (C_6H_5); $\lambda_{max}^{pH\ 1}$ 235 mμ (ϵ 9300), 315 (16,800); $\lambda_{max}^{pH\ 7}$ 240 mμ (ϵ 20,000), 285 (14,200), 320 (11,300); $\lambda_{max}^{pH\ 13}$ 310 mμ (ϵ 9200).

Anal. Calcd. for $C_{17}H_{18}N_4O_2$: C, 65.9; H, 5.81; N, 18.0. Found: C, 65.6; H, 5.69; N, 17.7.

Similarly, **2-amino-5-(3-anilinopropyl)-6-*p*-tolyl-4-pyrimidinol (Xd)** was made from XId, except the reaction time was 6 hr.; the yield after recrystallization from aqueous ethanol was 299 mg. (89%); m.p. 177–178°; $\lambda_{max}^{pH\ 1}$ 230 mμ (ϵ 13,600), 280 (9900); $\lambda_{max}^{pH\ 7}$ 242 mμ (ϵ 23,400), 295 (8800); $\lambda_{max}^{pH\ 13}$ 290 mμ (ϵ 8300).

Anal. Calcd. $C_{20}H_{22}N_4O\cdot 0.5H_2O$: C, 69.8; H, 6.78; N, 16.3; O, 6.80. Found: C, 69.4; H, 6.45; N, 16.7; O, 6.57.

2-Amino-6-phenyl-5-(4-phenylbutyl)-4-pyrimidinol (XIXa).—Alkylation of ethyl benzoylacetate with 4-phenylbutyl bromide to give XVIa followed by condensation with guanidine carbonate as previously described for the preparation of XIX (R_1 = CH_3)[7] gave, after recrystallization from hot 95% ethanol, a 27% over-all yield of product; m.p. 256–259°; λ_{max} 2.97 (NH), 6.02, 6.10, 6.40, 6.45, 6.70 (NH, C=N, C=C), 14.25 μ (C_6H_5); $\lambda_{max}^{pH\ 1}$ 233 mμ (ϵ 11,600), 278 (7600); $\lambda_{max}^{pH\ 7}$ 233 mμ (ϵ 14,300), 300 (6500); $\lambda_{max}^{pH\ 13}$ 290 mμ (ϵ 5100).

Anal. Calcd. for $C_{20}H_{21}N_3O$: C, 75.2; H, 6.59; N, 13.2. Found: C, 75.2; H, 6.68; N, 13.2.

Similarly, by alkylation of ethyl γ-phenylacetoacetate to XVIb followed by condensation with guanidine gave a 21% over-all yield of **2-amino-6-benzyl-5-(4-phenylbutyl)-4-pyrimidinol (XIXb)**; m.p. 240–243°; $\lambda_{max}^{pH\ 1}$ 228 mμ (ϵ 13,200) 266, (8900); $\lambda_{max}^{pH\ 7}$ 228 mμ (ϵ 11,800), 295 (7300); $\lambda_{max}^{pH\ 13}$ 283 mμ (ϵ 7200).

Anal. Calcd. for $C_{21}H_{23}N_3O$: C, 75.9; H, 6.91; N, 12.6. Found: C, 76.0; H, 7.10; N, 12.6.

2-Amino-4-chloro-6-phenyl-5-[N-(*p*-tolylsulfonyl)-3-anilinopropyl]pyrimidine (XIIa).—A solution of 2.29 g. (4.84 mmoles) of XIa[6] in 10 ml. of phosphorus oxychloride was heated for 1 hr. under reflux in an oil bath, preheated and maintained at 110°. The cooled reaction mixture was poured into 100 g. of ice and 30 ml. of acetone; the gummy product, which soon solidified, was collected on a filter and washed with water. The moist product was dissolved in a mixture of 35 ml. of acetone, 20 ml. of 95% ethanol, and 25 ml. of water; the solution was neutralized to pH 7 with 10% aqueous NaOH, then spin evaporated *in vacuo* to the cloud point, diluted with 20 ml. of water, and cooled in an ice bath. The separated product was recrystallized from aqueous ethanol to give 1.58 g. (66%) of product, m.p. 185–189°, that was suitable for further transformations. In a pilot run the analytical sample had m.p. 187–189°; $\lambda_{max}^{pH\ 1}$ 237 mμ (ϵ 29,800), 322 (7700); $\lambda_{max}^{pH\ 7,13}$ 237 mμ (ϵ 33,400), 315 (6400).

Anal. Calcd. for $C_{26}H_{25}ClN_4O_2S$: C, 63.3; H, 5.09; N, 11.4. Found: C, 63.5; H, 5.12; N, 11.1.

2-Amino-6-benzyl-4-chloro-5-[N-(*p*-tolylsulfonyl)-3-anilinopropyl]pyrimidine (XIIb).—A solution of 488 mg. (1 mmole) of XIb in 2.5 ml. of phosphorus oxychloride was heated under reflux for 1 hr. in an oil bath, preheated and maintained at 110°. The cooled reaction mixture was poured into 25 g. of ice and 20 ml. of petroleum ether (b.p. 30–60°). An oil separated that soon solidified; the product was collected on a filter and washed with water. The crude, moist product was suspended in 30 ml. of water and neutralized to pH 7 with 5% aqueous $NaHCO_3$. The product was collected on a filter and recrystallized from aqueous ethanol to give 257 mg. (51%) of white crystals: m.p. 123–126°; $\lambda_{max}^{pH\ 1,7}$ 236 mμ (ϵ 26,300), 309 (4200); $\lambda_{max}^{pH\ 13}$ 305 mμ (ϵ 4600).

Anal. Calcd. for $C_{27}H_{27}ClN_4O_2S$: C, 64.1; H, 5.33; N, 11.1. Found: C, 63.8; H, 5.46; N, 10.8.

2-Amino-6-phenyl-5-[N-(*p*-tolylsulfonyl)-3-anilinopropyl]-4-pyrimidinethiol (XIIIa).—A mixture of 200 mg. (0.407 mmole) of XIIa, 31.6 mg. (0.417 mmole) of thiourea, and 3 ml. of *t*-butyl alcohol was refluxed with stirring for 2 hr. Then 5 ml. of 10% NaOH was added and the mixture was refluxed an additional 10 min. The mixture was adjusted to about pH 6 with 3 *N* HCl. The product was collected on a filter and washed with water. Recrystallization from aqueous ethanol gave 157 mg. (79%) of the analytical sample: m.p. 230–240° dec; λ_{max} 2.95 (NH), 6.05, 6.15, 6.30, 6.45 (NH, C=N, C=C), 7.47, 8.62 (SO_2), 14.34 μ (C_6H_5); $\lambda_{max}^{pH\ 1}$ 348 mμ (ϵ 13,200); $\lambda_{max}^{pH\ 7}$ 365 mμ (ϵ 13,100); $\lambda_{max}^{pH\ 13}$ 330 mμ (ϵ 10,800).

Anal. Calcd. for $C_{26}H_{26}N_4O_2S_2$: C, 63.9; H, 5.32; N, 11.4. Found: C, 63.8; H, 5.35; N, 11.3.

2-Amino-5-(3-anilinopropyl)-6-phenyl-4-pyrimidinethiol (XVIIa).—In a solution of 600 mg. (6.36 mmoles) of phenol in 1.2 ml. of 30% HBr in acetic acid was dissolved 300 mg. (0.715 mmole) of XIIIa. After 1 hr. at room temperature, the solution was diluted with 30 ml. of reagent ether. The yellow solid was collected on a filter and washed with ether. The crude product was stirred with 20 ml. of 5% NaOH, then filtered from some insoluble disulfide of XVIIa. Acidification of the filtrate gave 137 mg. (66%) of solid; t.l.c. showed the presence of a minor component that was not removed by recrystallization. The remainder of the material was dissolved in the minimum amount of hot ethanol and streaked across a 20 × 20 cm. thin layer plate coated with 20 g. of silica gel G. The plate was developed with 3:1 benzene–methanol. The faster moving product (XVIIa) was located under an ultraviolet lamp; this area was scraped off the plate and eluted with 20 ml. of hot methanol. The methanol extract was further clarified by filtration through a Celite pad. The solvent was removed *in vacuo* and the residual 64 mg. (31%) of product was recrystallized from aqueous ethanol to give 44 mg. (21%) of pure product; m.p. 170–173°; λ_{max} 2.90 (NH), 6.03, 6.23, 6.43, 6.65 (NH, C=N, C=C), 13.35, 14.30, 14.43 μ (C_6H_5); $\lambda_{max}^{pH\ 1}$ 265 mμ (ϵ 7700), 350 (12,400); $\lambda_{max}^{pH\ 7}$ 245 mμ (ϵ 19,500), 363 (13,200); $\lambda_{max}^{pH\ 13}$ 328 mμ (ϵ 11,100).

Anal. Calcd. for $C_{19}H_{20}N_4S$: C, 67.9; H, 5.95; N, 16.6; S, 9.52. Found: C, 67.8; H, 5.87; N, 16.5; S, 9.70.

2-Amino-4-chloro-6-phenyl-5-(4-phenylbutyl)pyrimidine (XXa).—A solution of 319 mg. (1 mmole) of XIXa in 25. ml. of phosphorus oxychloride was converted to XXa as described for XIIb. To the moist solid was added 20 ml. of acetone and 10 ml. of water; the mixture was neutralized to pH 7 with 5% aqueous $NaHCO_3$. The mixture was spin evaporated *in vacuo* to about one-fourth volume. The white emulsion was extracted with chloroform and dried with $MgSO_4$, and the combined chloroform extracts were spin evaporated *in vacuo*. Recrystallization of the residue from aqueous ethanol gave 156 mg. (47%) of white crystals: m.p. 88–89°; λ_{max} 2.85 (NH), 6.16, 6.40, 6.60, 6.70 (NH, C=N, C=C), 13.0, 14.34 μ (C_6H_5); $\lambda_{max}^{pH\ 1,7}$ 238 mμ (ϵ 19,300), 315 (5600); $\lambda_{max}^{pH\ 13}$ 238 mμ (ϵ 21,100), 315 (5900).

Anal. Calcd. for $C_{20}H_{20}ClN_3$: C, 71.1; H, 5.93; N, 12.4. Found: C, 70.9; H, 5.92; N, 12.3.

Similarly, **2-amino-6-benzyl-4-chloro-5-(4-phenylbutyl)pyrimidine (XXb)** was prepared from XIXb except that the product was collected by filtration rather than chloroform extraction. Recrystallization from aqueous 2-ethoxyethanol gave 270 mg. (51%) of white crystals: m.p. 92–93°; λ_{max} 2.85, 2.95 (NH), 6.02, 6.11, 6.32, 6.53 (NH, C=N, C=C), 14.0, 14.35 μ (C_6H_5); $\lambda_{max}^{pH\ 1,7}$ 236 mμ (ϵ 15,600), 305 (4400); $\lambda_{max}^{pH\ 13}$ 235 mμ (ϵ 16,300), 308 (4600).

Anal. Calcd. for $C_{21}H_{22}ClN_3$: C, 71.7; H, 6.25; N, 11.9. Found: C, 71.7; H, 6.21; N, 11.9.

6-Benzyl-2,4-diamino-5-[N-(*p*-tolylsulfonyl)anilinopropyl]pyrimidine (XIVb).—A mixture of 1.12 g. (2 mmoles) of XIIb and 40 ml. of methanol saturated with ammonia at 0° was heated in a steel bomb at 150° for 24 hr. The cooled mixture was clarified with Norit, then concentrated to about one-third volume *in vacuo*. The solution was made strongly alkaline with 10% NaOH, warmed on a steam bath, then diluted with water to turbidity. After being chilled, the solution deposited 0.68 g. (71%) of product which was collected on a filter and washed with water. Recrystallization from aqueous alcohol afforded 495 mg. (51%) of crystals: m.p. 186–188°; λ_{max} 2.85, 2.93 (NH), 6.15, 6.25, 6.40, 6.70 (NH, pyrimidine, C=C), 7.53, 8.65 (SO_2N), 12.15, 13.0, 13.5, 14.1 μ (aromatic CH); $\lambda_{max}^{pH\ 1}$ 276 mμ (ϵ 8450); $\lambda_{max}^{pH\ 7}$ 280 mμ (ϵ 8650); $\lambda_{max}^{pH\ 13}$ 293 mμ (ϵ 9850).

Anal. Calcd. for $C_{27}H_{29}N_5O_2S$: C, 66.5; H, 5.95; N, 14.4. Found: C, 66.6; H, 6.09; N, 14.2.

Similarly, reaction of XXb with methanolic ammonia gave 105 mg. (46%) of pure **6-benzyl-2,4-diamino-5-(4-phenylbutyl)pyrimidine (XXIb)**: m.p. 128–131°; λ_{max} 2.85, 3.00 (NH), 6.02, 6.10, 6.40 (NH, pyrimidine, C=C), 13.50, 14.15, 14.45 μ (phenyl CH); $\lambda_{max}^{pH\ 1}$ 280 mμ (ϵ 8300); $\lambda_{max}^{pH\ 7}$ 287 mμ (ϵ 8300); $\lambda_{max}^{pH\ 13}$ 292 mμ (ϵ 7900).

Anal. Calcd. for $C_{21}H_{24}N_4$: C, 75.9; H, 7.22; N, 16.9. Found: C, 75.8; 7.08; N, 16.9.

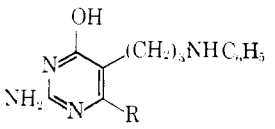
2,4-Diamino-6-phenyl-5-(4-phenylbutyl)pyrimidine (XXIa).—By reaction of 335 mg. (1 mmole) of XXa with methanolic ammonia at 150° for 43 hr. as described for the preparation of XIVb was obtained 138 mg. (44%) of analytically pure product: m.p. 158–161°; λ_{max} 2.85, 2.95 (NH), 6.06, 6.20, 6.45 (NH, pyrimidine, C=C), 13.55; 14.30 μ (aromatic CH); $\lambda_{max}^{pH\ 1}$ 288 mμ (ϵ 10,700), $\lambda_{max}^{pH\ 7}$ 290 mμ (ϵ 8500); $\lambda_{max}^{pH\ 13}$ 298 mμ (ϵ 7500).

Anal. Calcd. for $C_{20}H_{22}N_4$: C, 75.4; H, 6.92; N, 17.6. Found: C, 75.6; H, 6.86; N, 17.5.

2,4-Diamino-6-phenyl-5-[N-(*p*-tolylsulfonyl)anilinopropyl]-pyrimidine (XIVa) Hydrochloride.—A mixture of 200 mg.

TABLE I

COMPARATIVE EFFECTS OF SUBSTITUENTS AT THE 6-POSITION ON BINDING TO FOLIC REDUCTASE



Compd.	R	$K_i \times 10^9$	$\frac{K_i \text{ of 6-Me}}{K_i \text{ of 6-R}}$
III	CH_3	63[a]	
Xa	C_6H_5	1.8[b]	35
Xb	$CH_2C_6H_5$	4.0[b]	16
Xc	p-$C_6H_4NO_2$	20	3.2
Xd	p-$C_6H_4CH_3$	2.0	32
Xe	2-Furyl	2.5	25

[a] Previously reported in ref. 5. [b] Previously reported in ref. 6.

(0.407 mmole) of XIIa and 15 ml. of methanol saturated with ammonia at 0° was heated in a steel bomb at 150° for 48 hr. The cooled solution was clarified with Norit, then spin evaporated to residue *in vacuo*. Two precipitations from chloroform with petroleum ether (b.p. 60–110°) gave 108 mg. (53%) of amorphous product: m.p. 130–150°; λ_{max} 2.8, 2.9 (NH), 6.0, 6.1, 6.22, 6.4, 6.69 (NH, pyrimidine, C=C), 7.43, 8.60 (SO_2N), 12.23, 13.0, 14.3 μ (aromatic CH); $\lambda_{max}^{pH\ 1}$ 232 mμ (ϵ 25,900), 288 (7300); $\lambda_{max}^{pH\ 7}$ 233 mμ (ϵ 26,800), 293 (6800); $\lambda_{max}^{pH\ 13}$ 300 mμ (ϵ 6800).

Anal. Calcd. for $C_{26}H_{27}N_5O_2S \cdot HCl$: C, 61.5; H, 5.52; N, 13.8. Found: C, 61.5; H, 5.78; N, 13.7.

powder that was uniform on t.l.c. and had m.p. 50–65°; λ_{max} 2.85, 2.95 (NH), 6.05, 6.10, 6.15, 6.25, 6.40, 6.70 (NH, pyrimidine, C=C), 13.25, 14.14, 14.4 μ (phenyl CH); $\lambda_{max}^{pH\ 1}$ 280 mμ (ϵ 7500); $\lambda_{max}^{pH\ 7}$ 240 mμ (ϵ 22,400), 292 (ϵ 9600); $\lambda_{max}^{pH\ 13}$ 242 mμ (ϵ 16,500), 293 (6950).

Anal. Calcd. for $C_{20}H_{23}N_5$: C, 72.1; H, 6.91; N, 21.1. Found: C, 72.1; H, 6.99; N, 21.0.

Enzyme Assays.—Folic reductase from the liver of mature Holtzman rats was prepared and assayed by the break down of tetrahydrofolate to *p*-aminobenzoyl-L-glutamate and determination of the latter was by the Bratton-Marshall method as previously described.[12,13] Apparent dissociation constants (K_i) were determined by the reciprocal plot method and all compounds showed "competitive" kinetics. Results are recorded in Tables I and II; folic acid had $K_m = 10 \times 10^{-9}$.

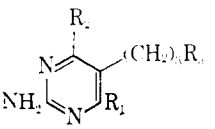
Enzyme Results and Discussion

In an earlier paper of this series,[6] it was reported that replacement of the 6-methyl group of 2-amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol (III) (Table I) by 6-phenyl (Xa) or 6-benzyl (Xb) led to more favorable binding to folic reductase; similar enhancement of binding was observed with the 6-phenyl analog (II).[4] In order to study the mode of this increase in binding, the analogs Xc, Xd, and Xe were synthesized.

An elevenfold decrease in binding was observed with the 6-*p*-nitrophenyl analog (Xc) compared to the 6-phenyl analog (Xa); that this decrease in binding was

TABLE II

EFFECT OF BINDING OF THE 6-SUBSTITUENT TO FOLIC REDUCTASE WHEN THE 4-POSITION OR SIDE CHAIN IS VARIED



Group	Compd.	R_1	R_2	R_3	$K_i \times 10^9$	$\frac{K_i \text{ of 6-Me}}{K_i \text{ of 6-R}}$
	III	CH_3	OH	NHC_6H_5	63[a]	
A	Xa	C_6H_5	OH	NHC_6H_5	1.8[b]	35
	Xb	$CH_2C_6H_5$	OH	NHC_6H_5	4.0[b]	16
	XXII	CH_3	OH	$CH_2C_6H_5$	17	
B	XIXa	C_6H_5	OH	$CH_2C_6H_5$	0.54	32
	XIXb	$CH_2C_6H_5$	OH	$CH_2C_6H_5$	10	1.7
	XXIII	CH_3	NH_2	NHC_6H_5	0.024[c]	
C	XVIIIa	C_6H_5	NH_2	NHC_6H_5	0.023	1.0
	XVIIIb	$CH_2C_6H_5$	NH_2	NHC_6H_5	0.30	0.080
D	XXIa	C_6H_5	NH_2	$CH_2C_6H_5$	0.041	
	XXIb	$CH_2C_6H_5$	NH_2	$CH_2C_6H_5$	0.029	
E	XXIV	CH_3	SH	NHC_6H_5	4.5[d]	
	XVIIa	C_6H_5	SH	NHC_6H_5	0.8	0.46

[a] Previously reported in ref. 5. [b] Previously reported in ref. 6. [c] Previously reported in ref. 8. [d] Previously reported in ref. 9.

5-(3-Anilinopropyl)-2,4-diamino-6-phenylpyrimidine (XVIIIa).—A mixture of 300 mg. (0.597 mmole) of XIVa, 113 mg. (1.2 mmoles) of phenol, and 3 ml. of 30% HBr in acetic acid was magnetically stirred at ambient temperature for 10 hr. protected from moisture. The reaction mixture was poured into about 30 ml. of anhydrous ether. The hydrobromide salt of XVIIIa was collected on a filter, washed with ether, and dissolved in 20 ml. of water. The solution was made strongly alkaline with 10% aqueous NaOH, then the product (153 mg.) was collected on a filter and washed with water. Two recrystallizations from aqueous ethanol gave 111 mg. (59%) of crystals: m.p. 105–108°; λ_{max} 2.75 (H_2O), 2.85, 2.91, (NH), 6.0, 6.17, 6.21, 6.36, 6.65 (NH, pyrimidine, C=C), 13.32, 14.23, 14.46 (phenyl CH); $\lambda_{max}^{pH\ 1}$ 286 mμ (ϵ 8300); $\lambda_{max}^{pH\ 7}$ 296 mμ (ϵ 8300); $\lambda_{max}^{pH\ 13}$ 298 mμ (ϵ 7700).

Anal. Calcd. for $C_{19}H_{21}N_5 \cdot H_2O$: C, 67.6; H, 6.82; N, 20.8. Found: C, 67.7; H, 6.84; N, 20.5.

Similarly, **5-(3-anilinopropyl)-2,4-diamino-6-benzylpyrimidine (XVIIIb)** was obtained in 72% yield from XIVb as an amorphous

unlikely to be steric was shown by the nearly equal binding of the 6-*p*-totyl (Xa) and the 6-phenyl (Xa) analogs. Therefore the poorer binding by the 6-*p*-nitrophenyl analog is most probably due to the electron-withdrawing effect of the *p*-nitro group. It follows conversely that an electron-rich group such as the 2-furyl (Xe) might increase binding; however, since this was not the case, it is probable that the maximum electronic effect on binding has already been attained by the 6-phenyl group and cannot be further increased by the more electron-rich furyl group. Since the 6-benzyl group of Xb cannot effect the mode of binding of the pyrimidine ring due to the insulation between the two

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conjugated systems by the methylene group, the benzyl group of Xb binds directly to the enzyme in some fashion such as a charge-transfer complex[6-8] or less likely by hydrophobic binding.[14,15]

Whether the 6-phenyl or 6-benzyl groups of X aand Xb bind in the same fashion still cannot be resolved at this point; that is, the 6-phenyl might influence pyrimidine binding and the 6-benzyl might bind directly. Nevertheless, the positive results remain that the 6-phenyl, 6-benzyl, or 6-furyl groups of Xa, Xb, and Xe increase binding compared to a 6-methyl group, and an electron-withdrawing nitro group as in Xc decreases binding.

It was previously observed that replacement of the 4-hydroxyl group of 2-amino-5-(3-anilinopropyl)-4-pyrimidinol (III) by amino (XXIII) gave a 2600 enhancement in binding[8] and by 4-mercapto (XXIV) a 14-fold enhancement.[7] In another paper of this series it was noted that replacement of the anilinopropyl group of III by a 4-phenylbutyl (XXII) also led to an enhancement of binding.[7] In Table II, a comparison of the effects of these three substitutions on binding of the 6-phenyl and 6-benzyl groups of Xa and Xb is made.

The 35-fold enhancement of 6-phenyl (Xa) over 6-methyl (Xb) in group A (Table II) with an anilinopropyl group is maintained in group B when the phenylbutyl side chain is present (XXII *vs.* XIXa). In contrast this increment is almost completely lost in the 6-benzyl series (XIXb *vs.* XXII); thus the phenylbutyl side chain causes a decrease in the binding of the 6-benzyl group or *vice versa*, or both.

A further enhancement in binding by the 6-phenyl or 6-benzyl moieties in the 4-amino series (groups C and D) is not observed; of interest is the fact that 6-benzyl group causes a decrease in binding compared to the 6-methyl group with the anilinopropyl side chain (group C), but had no detrimental effect with the phenylbutyl series (group D). Similarly, a further enhancement in binding by the 6-phenyl group in the 4-mercapto series (group E) was not observed; in fact, the 6-phenyl (XVIIa) was about one-half as effective as a 6-methyl (XXIV). This lack of additivity with the 6-phenyl moiety in groups C, D, and E (Table II) could be attributed to the fact that the 4-amino and 4-mercapto groups on the pyrimidine already have the most favorable tautomeric form and can no longer be influenced by the phenyl ring as in group A; however, such an explanation for the 6-benzyl moiety is untenable. One possible explanation is compatable with both the 6-benzyl and 6-phenyl systems; when the 4-amino and 4-mercapto groups are bound to folic reductase, a conformational change results in the enzyme which no longer allows binding of these aryl moieties. If this explanation is correct, it should be possible to construct irreversible inhibitors that will detect this enzymic conformational change by utilization of the bridge principle of specificity[16]; currently a search for such types of irreversible inhibitors is underway.

Furthermore, even though the three ways of increasing the binding of III are not additive, placement of a phenyl or benzyl group at the 6-position of III does not decrease binding (except in the case of XVIIIb which is not a serious loss); thus it should be feasible to synthesize a variety of potential irreversible inhibitors with covalent bond forming groups built off the 6-position in molecules related to XVIII, XXI, or XXIII.

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Amino Acid Analogs of Tryptamine Antagonists of Serotonin

FREDERICK LEONARD[1] AND WALTER TSCHANNEN

Department of Medicinal Chemistry, Geigy Research Laboratories, Ardsley, New York

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Amino acid analogs of two tryptamine antagonists of serotonin were prepared for biological evaluation. The 1-benzyltryptophans prepared in this study, like 1-benzyl-5-methoxy-2-methyltryptamine (BAS), produce sedation in mice while a nonbenzylated tryptophan caused stimulation.

Serotonin[2] (5-hydroxytryptamine) and the serotonin antagonist, 1-benzyl-5-methoxy-2-methyltryptamine[3] (BAS), do not readily cross the blood–brain barrier. The biological precursor of serotonin, 5-hydroxytryptophan, however, is transported into the brain where it is decarboxylated to serotonin.[2] It was conjectured, therefore, that amino acid analogs of BAS might possess interesting biological properties. Several tryptophan derivatives (**1a–1c**) were consequently prepared in these laboratories to explore this possibility.[4]

Two methods were considered for the preparation of the desired amino acids, the Warner–Moe[5] tryptophan

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(5) D. T. Warner and O. A. Moe, *J. Am. Chem. Soc.*, **70**, 2764 (1948), prepared the key tryptophan intermediate, diethyl acetamido(3-indolylmethyl)malonate (b) by Fisher cyclization of the phenylhydrazone of diethyl acetamido(β-formylethyl)malonate (a) and converted it to DL-tryptophan by previously described procedures.