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Irreversible Enzyme Inhibitors 99

Inhibitors of Thymidine Phosphorylase 7. Further Studies on Hydrophobic Bonding with Hydrocarbon Substituents on Acidic Uracils

By B. R. BAKER, MITSUTAKA KAWAZU, and J. D. MCCLURE

Replacement of the 6-methyl group of 6-methyluracil (II) by 6-trifluoromethyl gives a sevenfold increment in binding to thymidine phosphorylase presumably due to the increased acidity of the uracil. Similarly, replacement of the 6-methyl group by 6-methylsulfonyl gave a fifteenfold increment in binding. The binding of 6-methyluracil (II) was increased only 2.5-fold by introduction of a 5-phenylazo group. 6-(Trifluoromethyl)uracil (IV) was further substituted at the 5-position by six different alkyl, aryl, and aralkyl groups; the best inhibitor of this series was 5-phenylbutyl-6-(trifluoromethyl)uracil (XI) which was complexed tenfold better than IV and 67-fold better than 6-methyluracil. Comparison of 5-(phenylazo)uracils substituted with methyl, phenyl, benzyl, or *n*-amyl at the 6-position showed that the *n*-amyl derivative (XVI) gave the best binding; XVI was complexed 23-fold better than 6-methyl-5-(phenylazo)uracil (XII) and 58-fold better than 6-methyluracil.

THYMIDINE PHOSPHORYLASE is an enzyme that catalyzes phosphorolysis of thymidine and related 2′-deoxynucleosides to thymine (1) or the reverse conversion of thymine to thymidine (2), depending upon the stress of genetic or dietary deficiencies upon the cell line (3). The enzyme can also convert the anticancer agent, 5-fluorouracil, to its 2′-deoxyriboside (FUDR) or *vice versa* (3, 4). The chemotherapeutic utility for a tissue-specific inhibitor of thymidine phosphorylase has been previously discussed (5), and initial studies from this laboratory on the mode of binding of inhibitors to this enzyme have been reported (5–10).

It was previously noted that hydrophobic bonding to the enzyme could take place with alkyl or aralkyl groups on uracil at the 1-position (6) or the 5- and 6-positions (7, 8), for example, 6-benzyluracil (XV) was eighteenfold more effective than uracil (7) (Table I). Furthermore, binding of uracils could be made more effective when substituted with electron-withdrawing groups at the 5- or 6-position which increased the acidity of the uracil (9); for example, 5-bromouracil (III) was ninefold more effective than uracil (I). Finally, the best inhibitors were obtained by combining both phenomena, that is, hydrophobic bonding and increased acidity (10); for example, 5-bromo-6-benzyluracil (XVIII) was complexed to the enzyme fortyfold more effectively than the substrate, FUDR, and 150-fold more effectively than uracil (I) (Table I). Therefore, further studies on combinations of hydrophobic groups and electron-withdrawing groups attached to the 5- and 6-positions of uracil were initiated and are the subject of this paper.

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Previous paper: Baker, B. R., and Erickson, E. H., *J. Pharm. Sci.*, **56**, 1075(1967). Previous paper on this enzyme: see *Reference 10*.
The technical assistance of Pepper Caseria with the assays in Table I is acknowledged.

DISCUSSION

It was previously reported (9) that the electron-withdrawing trifluoromethyl group at the 6-position (IV) of uracil gave a sevenfold increment in enzyme

binding over 6-methyluracil (II); similarly, it has now been observed that the electron-withdrawing methylsulfonyl group at the 6-position (V) gave fifteenfold better binding than the 6-methyl group (II). Therefore, a search for hydrophobic bonding by hydrocarbon groups at the 5-position of 6-(trifluoromethyl)uracil was undertaken.

The first two compounds studied were the 5-(*p*-chlorophenyl) (VII) and 5-phenylbutyl (XI) uracils since they were readily synthesized by deamination of available 2-aminopyrimidines (XIX, XXII) previously prepared in this laboratory (11). The *p*-chlorophenyl substituent of VII give a fourfold increment in binding over 6-(trifluoromethyl)uracil (IV). The 5-phenylbutyl substituent of XI gave a tenfold increment in binding over IV. The intermediate phenylalkyl derivatives (VIII–X) were then synthesized and evaluated; none were as effective as the phenylbutyl substituent (XI). The *n*-amyl substituent at the 5-position (VI) was then investigated; it showed only a twofold increment in binding due to its hydrocarbon interaction with the enzyme. From these studies emerge two problems worthy of pursuit: (*a*) a similar hydrophobic bonding study of 5-substituents on 6-(methylsulfonyl)uracil (V) and (*b*) placement of leaving groups and the benzene ring of VII–XI to convert them to potential active-site-directed irreversible inhibitors (12, 13) of thymidine phosphorylase that could be further modified for isozyme specificity (14).

Since 5-(phenylazo)uracils are readily synthesized by one of two routes and since the phenylazo group should increase acidity of the uracil by its electron-withdrawing power, a series of 5-(phenylazo)uracils with hydrocarbon substituents were investigated. Note that the 5-phenylazo group (XII) on 6-methyluracil (II) gives a threefold increment in binding, which is not as effective an increment as observed with the 5-bromo (III), 6-methylsulfonyl (V), or 6-trifluoromethyl (IV) groups. Replacement of the 6-methyl group of XII by 6-phenyl (XIII) resulted in a loss of binding—a not unexpected result based on the binding ability of 6-phenyluracil previously observed (8). Better hydrophobic interaction was observed with the 6-benzyl (XIV) and 6-(*n*-amyl) (XVI) groups.

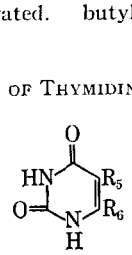
The 6-benzyl group (XIV) on 5-(phenylazo)-uracil gave a fivefold increment in binding due to a hydrophobic interaction; this increment is considerably less than the 37-fold increment between 6-benzyl (XV) and 6-methyluracil (II). Thus it would appear that the large phenylazo group hinders the ability of the benzyl group to assume the proper conformation for optimum binding, as previously observed with 1,5,6-trisubstituted uracils (7). The more flexible *n*-amyl group (XVI) on 5-(phenylazo)uracil gave a 23-fold increment in binding over the 6-methyl group of XII; compared another way, the 5-phenylazo group of XVI gave an eightfold increment in binding compared to the 6-(*n*-amyl)uracil (XVII).

Further studies on placement of a leaving group on the 5-phenyl substituent of XIV and XVI, to convert them to potential active-site-directed irreversible inhibitors (12, 13), would be worthy of pursuit.

CHEMISTRY

Methods—Treatment of the 2-amino-5-phenylbutyl-4-pyrimidinol (XXII) (11) with sodium

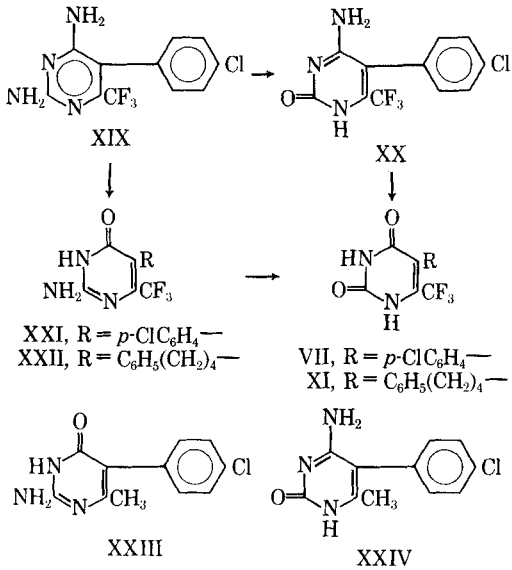
Table I—Inhibition of Thymidine Phosphorylase[a] by



Compd.	R_5	R_6	m*M* Concn. for 50% Inhibition	$[I/S]_{0.5}$[b]
I	H	H	1.5	3.9[c]
II	H	CH_3	3.2	8.0[d]
III	Br	H	0.18	0.45[d]
IV	H	CF_3	0.47	1.2[d]
V[e]	H	SO_2CH_3	0.21	0.52
VI	*n*-C_5H_{11}—	CF_3	0.21	0.52
VII	*p*-ClC_6H_4—	CF_3	0.13	0.32
VIII	$C_6H_5CH_2$—	CF_3	0.30	0.75
IX	$C_6H_5(CH_2)_2$—	CF_3	0.10	0.25
X	$C_6H_5(CH_2)_3$—	CF_3	0.20	0.50
XI	$C_6H_5(CH_2)_4$—	CF_3	0.049	0.12
XII	$C_6H_5N{=}N$—	CH_3	1.3	3.2
XIII	$C_6H_5N{=}N$—	C_6H_5—	~3.[f]	~8.
XIV	$C_6H_5N{=}N$—	$C_6H_5CH_2$—	0.25	0.63
XV	H	$C_6H_5CH_2$—	0.090	0.22[c]
XVI	$C_6H_5N{=}N$—	*n*-C_5H_{11}—	0.055	0.14
XVII	H	*n*-C_5H_{11}—	0.46	1.1[e]
XVIII	Br	$C_6H_5CH_2$—	0.010	0.025[g]

[a] Thymidine phosphorylase was a 45–90% saturated ammonium sulfate fraction from *E. coli* B that was prepared and assayed with 0.4 m*M* 5-fluoro-2′-deoxyuridine (FUDR) in arsenate–succinate buffer (pH 5.9) diluted with 10% dimethylsulfoxide as previously described (5). [b] Ratio of concentration of inhibitor to 0.4 m*M* FUDR giving 50% inhibition. [c] Data from *Reference 8*. [d] Data from *Reference 9*. [e] The authors thank Professor William Prusoff for a sample of this compound. [f] Estimated from 18% inhibition at 0.5 m*M*, the maximum concentration allowing full light transmission. [g] Data from *Reference 10*.

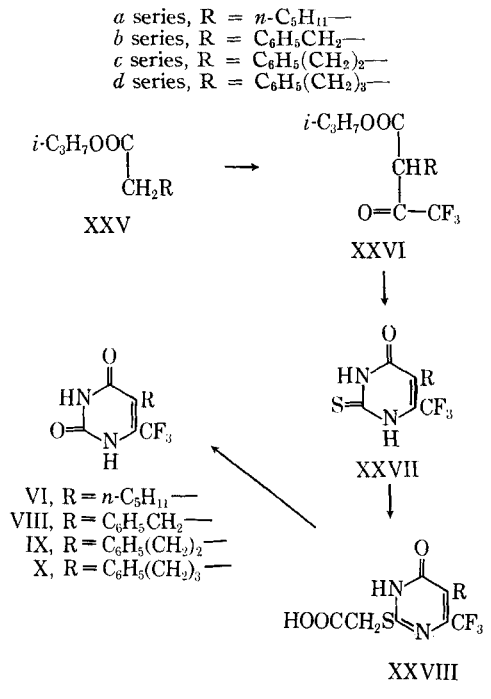
nitrite in glacial acetic acid at room temperature gave a 76% yield of the 5-phenylbutyluracil (XI). Similar treatment of the 5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (XIX) (11) at room temperature did not give the desired uracil derivative (VII), but gave instead an aminopyrimidinol which would have structure XX or XXI. According to Trattner *et al.* (15) treatment of the 6-ethyl analog of XIX with nitrous acid deaminated the 2-amino group; this result was based on comparative ultraviolet data where the 2-amino-4-hydroxypyrimidine showed no shifts between acid and base solution. Later work in this laboratory (16) did not agree with these ultraviolet values—XXIII, synthesized in an unequivocal manner, had an ultraviolet maximum at 15 mμ longer wavelength in alkali than in acid solution, but the 2-hydroxy-4-aminopyrimidine XXIV, also synthesized by an unequivocal method, showed no difference in absorption maximum in acid or base solution. Since the product of deamination of XIX showed a shift of 35 mμ to longer wavelengths in alkali, the 2-amino-4-hydroxypyrimidine structure (XXI) was assigned to this product. These results conflict with those of Trattner *et al.* (15); repetition of their experiments indicated that the product of both hydrochloric acid hydrolysis or nitrous acid deamination was the 2-amino-4-pyrimidinol resulting from attack at the 4-position (17), in agreement with the results of deamination of XIX to XXI. (Scheme I.)



Scheme I

When the reaction of the diaminopyrimidine (XIX) with sodium nitrite was performed at 50° in aqueous acetic–hydrochloric acid, deamination of both amino groups took place to give the desired phenyluracil (VII).

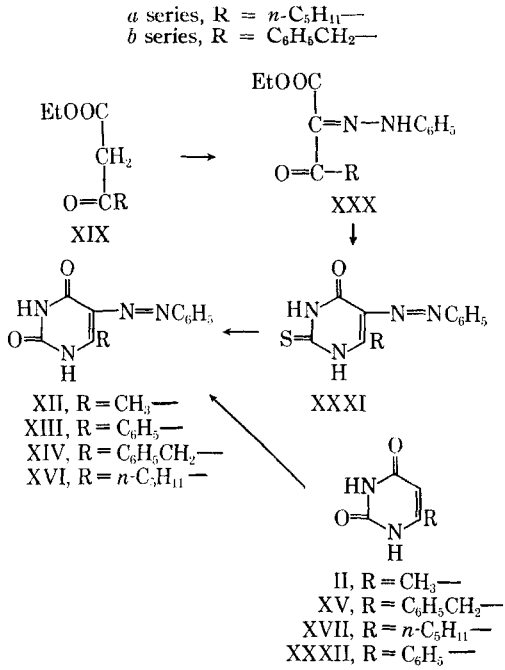
The remaining 5-substituted-6-(trifluoromethyl)-uracils (VI, VIII–X) (Scheme II) were synthesized by a variant of the earlier synthesis (11). The appropriate isopropyl esters (XXV) were condensed with ethyl trifluoroacetate in the presence of sodium hydride; the resultant β-keto esters (XXVI) were then condensed with thiourea to give the 2-mercapto-4-pyrimidinols (XXVII), rather than condensed with guanidine (11). Treatment of XXVII with boiling 5% aqueous chloroacetic acid converted the mercapto group to the carboxymethylthio group (XXVIII), but the thio group did not hydrolyze in the reaction mixture to give the desired uracils (VI, VIII–X), as previously observed with other 5- or 6-substituted-2-thiouracils (8). However, the thioglycolate residue was readily hydrolyzed with aqueous hydrochloric acid. Apparently, the 6-trifluoromethyl group of XXVIII deactivated attack by acid. If it is logically assumed that the pyrimidine ring of XXVIII must be protonated before attack by water occurs, then an acid stronger than 5% aqueous chloroacetic acid would have to be used for protonation since the 6-trifluoromethyl group decreases the basicity of the pyrimidine by about 5 pKa units compared to 6-methyl (11).



Scheme II

6-Methyl-5-(phenylazo)uracil (XII) has been previously synthesized (18) by coupling 6-methyluracil (II) with phenyldiazonium chloride; this reaction was successfully repeated to give a 48% yield of pure material. The 6-phenyl analog (XIII) was also synthesized in this manner in 31% yield of pure material; XIII has been prepared previously by an alternate method (19). The 6-benzyl analog (XIV) was prepared similarly from 6-benzyluracil (XV) in 63% yield. (Scheme III.)

The identical 5-phenylazo-6-benzyluracil (XIV) was also synthesized by an alternate route previously used (19) for the 6-phenyl derivative (XIII) which gave a structurally unequivocal product. Ethyl γ-phenylacetoacetate (XIX*b*) was coupled with phenyldiazonium chloride in aqueous alcohol buffered with sodium acetate; the resultant phenyl-



Scheme III

azo keto ester (XXX*b*) was condensed with thiourea to give the 2-*thio*uracil derivative (XXXI*b*) in good over-all yield. Since hydrolysis of the 2-mercapto group of XXXI*b* with aqueous chloroacetic acid to XIV was unsuccessful, the group was removed by oxidative cleavage with hydrogen peroxide (19) to give XIV in 58% yield that was identical with XIV prepared *via* XV. The remaining 6-(*n*-amyl)-5-phenylazouracil (XVI) was synthesized *via* XXXI*a* since the route starting with 6-(*n*-amyl)uracil (XVII) gave a mixture which was difficult to purify.

EXPERIMENTAL

Melting points were taken in capillary tubes on a Mel-Temp block and those below 230° are corrected. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 137B or 337 spectrophotometer. Ultraviolet spectra were determined in 10% alcohol with a Perkin-Elmer 202 spectrophotometer. Thin-layer chromatograms (TLC) were run on Brinkmann Silica Gel GF, and spots were detected by visual examination under ultraviolet light.

2 - Amino - 5 - (p - chlorophenyl) - 6 - (trifluoromethyl) - 4 - pyrimidinol (XXI)—*Method C*—To a stirred solution of 58 mg. (2 mmoles) of XIX (11) in 20 ml. of glacial acetic acid cooled in an ice bath was added a solution of 560 mg. (8 mmoles) of $NaNO_2$ in 20 ml. of water at such a rate that the temperature was 2–5°. The solution was then allowed to stand at ambient temperature for about 18 hr. during which time the product separated. The product was collected on a filter and recrystallized from 50% aqueous acetic acid; yield, 300 mg. (52%) of slightly yellow plates, m.p. 326–328°; $\nu_{max.}$ 3470, 3300, 3180 (NH); 1650–1600, 1550 (NH, C=O, C=C, C=N); 1020 (CF_3); 840 cm.$^{-1}$ (p-C_6H_4). See Table II for additional data.

5 - (p - Chlorophenyl) - 6 - (trifluoromethyl)-uracil (VII)—To a stirred solution of 350 mg. (1.2 mmoles) of XIX (11) in 10 ml. of glacial acetic acid and 5 ml. of 10% aqueous hydrochloric acid maintained at 50° was added dropwise a solution of 480 mg. (7 mmoles) of $NaNO_2$ in 10 ml. of water. After standing 3 days at ambient temperature, the mixture was filtered and the product was recrystallized from aqueous ethanol; yield, 120 mg. (34%) of white plates, m.p. 250–251°; $\nu_{max.}$ 3180, 3130, 3030 (NH); 1710, 1680 (uracil C=O); 1600 (C=C); 1000 (CF_3); 830 cm.$^{-1}$ (p-C_6H_4). See Table II for additional data.

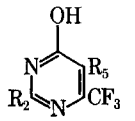
5 - Substituted - 6 - (trifluoromethyl) - 2 - thiouracils (XXVII)—*Method A*—The appropriate α-substituted isopropyl γ,γ,γ-trifluoroacetoacetate (XXVI) was prepared by condensation of XXV with ethyl trifluoroacetate as previously described for XXVI, R = $C_6H_5(CH_2)_4$ (11). A mixture of 30 mmoles of XXVI, 50 ml. of *tert*-butyl alcohol, 30 mmoles of thiourea, and 30 mmoles of solid sodium methoxide was refluxed with magnetic stirring for about 18 hr., then spin evaporated *in vacuo*. The residue was dissolved in water, the solution was clarified by filtration, then acidified with acetic acid. The product was collected on a filter, washed with water, then recrystallized from aqueous ethanol. See Table II for additional data.

5 - Substituted - 6 - (trifluoromethyl)uracils—*Method B*—A mixture of 3 mmoles of XXVII and 20 ml. of 5% aqueous chloroacetic acid was refluxed for 5 hr.; the intermediate (XXVIII) which could be isolated at this point, was further hydrolyzed by addition of 10 ml. of 10% aqueous hydrochloric acid and by reflux for another 5 hr. The mixture was cooled, the product was collected on a filter, washed with water, then crystallized from aqueous ethanol. See Table II for additional data.

6 - Substituted - 5 - (phenylazo)uracils—*Method D*—A solution of 0.93 Gm. (10 mmoles) of aniline in 5.0 ml. of 12 *N* aqueous HCl was diazotized at −5 to 0° in the usual fashion with a solution of 0.69 Gm. (10 mmoles) of $NaNO_2$ in 5 ml. of water. This diazonium solution was added to a stirred and recooled solution of 10 mmoles of XXXII or XVII in 150 ml. of 2 *N* aqueous NaOH at such a rate that the temperature was 0–5° (in some cases, such as with XXXII, it was necessary to add about 50 ml. more of water to dissolve the uracil prior to addition). After being stirred for an additional 3 hr. in the ice bath, the solution was acidified with acetic acid. The product was collected on a filter and washed thoroughly with water, then recrystallized from aqueous dioxane. See Table III for additional data.

Ethyl α - Phenylazo - γ - phenylacetoacetate (XXXb)—To a stirred solution of 4.12 Gm. (20 mmoles) of XXIX*b* (20) in 72 ml. of ethanol cooled in an ice bath was added a solution of 10.7 Gm. (0.13 mole) of anhydrous sodium acetate in 25 ml. of water; water was then added dropwise until the separated sodium acetate dissolved. Then the diazonium solution from 1.86 Gm. (20 mmoles) of aniline (see under *Method D*) was added dropwise with vigorous stirring, maintaining the temperature at 0–5°. After the addition of 25 ml. more of water, the mixture was stirred in the ice bath for another hour. The product was collected on a filter and washed with cold 50% alcohol; yield, 5.4 Gm.

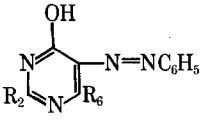
TABLE II—PHYSICAL CONSTANTS OF



Compd.[a]	R_2	R_5	Method	Yield, %	M.p., °C.	Anal. Calcd.	Anal. Found	λmax. (mμ) pH 6	λmax. (mμ) pH 13
VI	OH	*n*-C_5H_{11}—	*B*	80	157–158	C, 48.0 H, 5.24 N, 11.2	C, 47.9 H, 5.27 N, 11.3	280	303
VII	OH	*p*-ClC_6H_4—		34	250–251	C, 45.6 H, 2.08 N, 9.68	C, 45.5 H, 2.00 N, 9.42	272[c]	302
VIII	OH	$C_6H_5CH_2$—	*B*	40	207–208	C, 53.4 H, 3.36 N, 10.4	C, 53.5 H, 3.33 N, 10.2	270	304
IX	OH	$C_6H_5(CH_2)_2$—	*B*	47	233–234	C, 54.9 H, 3.89 N, 9.85	C, 54.7 H, 3.99 N, 10.1	270	304
X	OH	$C_6H_5(CH_2)_3$—	*B*	53	148–149	C, 56.3 H, 4.39 N, 9.39	C, 56.3 H, 4.40 N, 9.23	273	305
XI	OH	$C_6H_5(CH_2)_4$—	*C*	76	123–124	C, 57.7 H, 4.84 N, 8.98	C, 57.6 H, 4.91 N, 9.15	270	303
XXI	NH_2	*p*-ClC_6H_4—	*C*	52	326–328	C, 45.5 H, 2.77 N, 14.2	C, 45.7 H, 2.61 N, 14.2	280[c]	315
XXVII*a*	SH	*n*-C_5H_{11}—	*A*	49	168–169	C, 45.4 H, 4.93 N, 10.6	C, 45.4 H, 4.96 N, 10.5	263 330	263 308
XXVII*b*	SH	$C_6H_5CH_2$—	*A*	25	205–206	C, 50.3 H, 3.16 N, 9.79	C, 50.0 H, 2.99 N, 9.69	270 315	265 308
XXVII*c*	SH	$C_6H_5(CH_2)_2$—	*A*	10[d]	204–205	C, 52.0 H, 3.66 N, 9.33	C, 52.0 H, 3.74 N, 9.41	262 328	262 323
XXVII*d*	SH	$C_6H_5(CH_2)_3$—	*A*	6[d]	147–149	C, 53.5 H, 4.17 N, 8.93	C, 55.3 H, 4.39 N, 8.65	273 315	263 320

[a] All compounds had infrared spectra in agreement with their assigned structures and moved as a single spot on TLC. [b] See under *Experimental*. [c] At pH 1. [d] Over-all yield from XXV.

TABLE III—PHYSICAL CONSTANTS OF



Compd.[a]	R_2	R_6	Method	Yield, %	M.p., °C.	Anal. Calcd.	Anal. Found	λmax. (mμ) pH 6	λmax. (mμ) pH 13
XII	OH	CH_3—	*D*	48	256–257[b]			338	355
XIII	OH	C_6H_5—	*D*	31[d]	230–233[c,d]			277 348	279 368
XIV	OH	$C_6H_5CH_2$—	*D*	63	221–223	C, 65.8[e] H, 4.93 N, 17.1	C, 65.8 H, 4.92 N, 17.2	288 343	295 364
XIV	OH	$C_6H_5CH_2$—	*F*	58	221–223			288 343	295 364
XVI	OH	*n*-C_5H_{11}—	*F*	52	148–149	C, 60.4[f] H, 6.48 N, 18.9	C, 60.3 H, 6.52 N, 18.8	258 338	290 358
XXXI*a*	SH	*n*-C_5H_{11}—	*E*	96	169–170	C, 58.5[g] H, 6.05 N, 18.1	C, 58.6 H, 6.06 N, 18.1	290 370	395
XXXI*b*	SH	$C_6H_5CH_2$—	*E*	60	189–190	C, 63.4 H, 4.35 N, 17.4	C, 63.2 H, 4.27 N, 17.1	287 380	395

[a] All compounds had infrared spectra in agreement with their assigned structures and moved as a single spot on TLC. [b] Lit. (18) m.p. 253–255°. [c] Lit. (19) m.p. 218–225°. [d] Recrystallized from 80% ethanol, then ethyl acetate. [e] Calcd. with 0.25 mole dioxane. [f] Calcd. with 2/3 mole water. [g] Calcd. with 1/3 mole water.

(87%), m.p. 58–59°. Recrystallization from aqueous ethanol gave yellow crystals, m.p. 58–59°; $\lambda_{max.}$ (EtOH): 242, 362 mμ; (pH 13): 242, 370 mμ.

Anal.—Calcd. for $C_{18}H_{18}N_2O_3$: C, 69.7; H, 5.81; N, 9.03. Found: C, 69.5; H, 5.84; N, 8.91.

Similarly, XXX*a* was obtained as an oil that was isolated by ether extraction in 37% yield.

6 - Benzyl - 5 - phenylazo - 2 - thiouracil (XXXIb)—*Method E*—A mixture of 3.10 Gm. (10 mmoles) of XXX*b*, 1.10 Gm. (14 mmoles) of thiourea, and 1.08 Gm. (20 mmoles) of solid sodium methoxide in 20 ml. of methanol was refluxed with magnetic stirring for about 18 hr., then processed as under *Method A*. Recrystallization from ethanol gave 1.9 Gm. (60%) of yellow crystals, m.p. 189–190°. See Table III for additional data.

6 - Benzyl - 5 - (phenylazo)uracil (XIV)—*Method F*—To a stirred solution of 1.61 Gm. (5 mmoles) of XXXI*b* in 20 ml. of 2 *N* aqueous NaOH cooled in an ice bath was added dropwise 20 mmoles of dilute aqueous hydrogen peroxide; the deep red solution became bright yellow during the addition. The solution was then stirred for 2 hr. with the ice bath removed; occasionally the solution was cooled to keep the temperature at 27–30°. The solution was acidified with aqueous HCl, then the product was collected on a filter. After being washed with water and ice cold alcohol, the product was recrystallized from aqueous dioxane; yield, 0.90 Gm. (58%) of red needles. See Table III for additional data.

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Irreversible Enzyme Inhibitors 100

Inhibitors of Thymidine Phosphorylase 8. 6-(*p*-Bromoacetamidobenzyl)uracil, an Active-Site-Directed Irreversible Inhibitor

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Four candidate active-site-directed irreversible inhibitors for thymidine phosphorylase have been synthesized from 6-benzyluracil (I). Of these four, only 6-(*p*-bromoacetamidobenzyl)uracil (III) inactivated the enzyme; since neither iodoacetamide nor the *m*-isomer (VI) of III inactivated the enzyme under the conditions used for III, the inactivation by III most probably proceeds by a neighboring group reaction within the enzyme–inhibitor complex—the so-called active-site-directed mechanism.

PREVIOUS PAPERS in this series have described the mode of uracil binding (1, 2) to thymidine phosphorylase. Hydrophobic bonding to this enzyme by uracil derivatives has also been detected (2–6) and the mode of binding has been summarized (7). An effective inhibitor of this enzyme was 6-benzyluracil (I) (5), which was complexed 4.5-times better than the substrate, 5-fluoro-2′deoxyuridine; increasing the acidity of the uracil system by introduction of a 5-bromo group (II) gave a further ninefold increment in binding (6), II being complexed fortyfold better than the substrate. Therefore, 6-benzyluracil (I) and its 5-bromo derivative (II) were considered logical structures to be further modified for con-

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