Irreversible Enzyme Inhibitors. LXXVI.[1,2] Inhibitors of Thymidine Phosphorylase. II.[2] Hydrophobic Bonding by 1-Substituted Uracils

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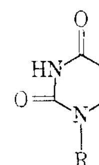
The ninefold increment in binding of 1-(*n*-butyl)uracil to thymidine phosphorylase over that of 1-methyluracil was most probably due to hydrophobic bonding; binding was changed 1.5-fold or less, compared to *n*-butyl, with the *n*-amyl, isoamyl, cyclopentyl, or isohexyl groups. However, better binding was observed with some 1-aralkyluracils, 1-benzyluracil and 1-phenylamyluracil being complexed 35- and 100-fold better, respectively, than 1-methyluracil. 1-Phenethyl-, 1-phenylpropyl-, and 1-phenylbutyluracils were complexed less effectively than 1-phenylamyluracil, but still better than 1-butyluracil. Substitution of polar groups such as carboxyl, carboxamido, or acetamido on the terminal phenyl of some of the 1-aralkyluracils led to a decrease in binding in each case, indicating that the phenyl binding to thymidine phosphorylase occurred with a hydrophobic region of the enzyme.

In the previous paper of this series,[2] the mode of binding of the ribofuranose moiety of thymidine to thymidine phosphorylase was studied; the 5′-hydroxyl of thymidine did not contribute to binding, but the 3′-hydroxyl group was a binding point. A series of 1-(ω-hydroxyalkyl)uracils were also investigated to determine if the ribofuranose binding could be simulated by a simpler moiety. Although the four compounds from hydroxyethyl (II) through hydroxypentyl (III) were complexed better than uracil with a 1-methyl group, the nearly identical binding by the four hydroxyalkyl moieties indicated that the hydrocarbon part of these moieties was complexed hydrophobically to the enzyme rather than the hydroxyl group being bound in a donor–acceptor complex.[2] That hydrophobic bonding could occur was substantiated by the ninefold better binding of 1-butyluracil than 1-methyluracil[2] (Table I). In this paper is described an extension of this observation by synthesis and enzymic evaluation of additional 1-alkyl- and 1-aralkyluracils.[4]

Higher alkyl groups including *n*-amyl (V), isoamyl (VI), cyclopentyl (VII), and isohexyl (VIII) were complexed about the same as the *n*-butyluracil (IV) (Table I). Although about 1.5-fold better binding by *n*-amyl (V) than *n*-butyl (IV) was observed, higher unbranched homologs were not investigated. Note that the isoamyl group of VI and the cyclopentyl group of VII give little change in binding to thymidine phosphorylase compared to *n*-butyl (IV). These results contrast sharply with those observed on hydrophobic bonding to dihydrofolic reductase[5] where the isoamyl group gave 6–10-fold increased binding[6] and the cyclopentyl gave an 11-fold decrease in binding.[7] Since the increment in binding between methyl and IV–VII is about 1.3 kcal/mole, these results could be explained by hydrophobic bonding by only the C-2 methylene groups of IV–VII; that is, hydrophobic bonding occurs through about two carbons but little past, since the maximum energy of hydrophobic bonding and van der Waals forces for a methylene group can be as large as 1.3 kcal/mole.[5,8,9] Thus the remaining carbons of IV–VII past two carbons may not be in contact with enzyme since there appears to be little conformational

TABLE I

HYDROPHOBIC BONDING TO THYMIDINE PHOSPHORYLASE[a] BY



Compd	R	mM concn	% inhib	Estd[b] ([I]/[S])0.5
I	CH_3	50	50	200[c]
II	$HOCH_2CH_2$	12	30	70[c]
III	$HO(CH_2)_5$	12	30	70[c]
IV	*n*-C_4H_9	9.1	50	22[c]
V	*n*-C_5H_{11}	5.5	50	14
VI	*i*-C_5H_{11}	8.0	50	20
VII	Cyclopentyl	7.3	50	19
VIII	*i*-C_6H_{13}	6.0	50	15
IX	$C_6H_5CH_2$	2.3	50	5.7
X	$C_6H_5(CH_2)_2$	2.5	50	6.3
XI[d]	$C_6H_5(CH_2)_3$	5.4	50	13
XII	$C_6H_5(CH_2)_4$	1.6	50	4.0
XIII	$C_6H_5(CH_2)_5$	0.86	50	2.1
XIV[e]	$CH_2C_6H_4COOH$-*p*	6	0	>60[f]
XV[e]	$CH_2C_6H_4CONH_2$-*p*	3	33	15
XVI[d]	$CH_2C_6H_4NHCOCH_3$-*p*	1.5[g]	0	>15[f]
XVII[d]	$(CH_2)_3C_6H_4COOH$-*p*	4	21	60
XVIII	$CH_2C_6H_4NHCOCH_2Br$-*m*	2[g]	20	16
XIX	$CH_2C_6H_4NHCOCH_2Br$-*o*	11	50	27

[a] Thymidine phosphorylase was a 45–90% ammonium sulfate fraction from *E. coli* B that was prepared and assayed with 0.4 m*M* 2′-deoxy-5-fluorouridine (FUDR) in succinate–arsenate buffer (pH 5.9) in the presence of 10% DMSO as previously described.[2] The technical assistance of Barbara Baine, Maureen Baker, Pepper Caseria, and Gail Salomon is acknowledged. [b] The ratio of concentration of inhibitor to 0.4 m*M* FUDR giving 50% inhibition. [c] From ref 2. [d] For preparation see B. R. Baker and T. J. Schwan, *J. Med. Chem.*, **9**, 73 (1966). [e] For preparation see B. R. Baker and G. B. Chheda, *J. Pharm. Sci.*, **54**, 25 (1965). [f] Since 20% inhibition is readily detectable, the concentration for 50% inhibition is at least four times greater than the concentration measured. [g] Maximum concentration allowing readable optical density change.

(1) This work was generously supported by grants CA-05845 and CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series, see B. R. Baker, *J. Med. Chem.*, **10**, 297 (1967).

(3) On leave from Tanabe Seiyaku Co., Ltd., Tokyo, Japan.

(4) The chemotherapeutic utility for inhibitors of thymidine phosphorylase has been discussed.[2]

(5) For a review see 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.

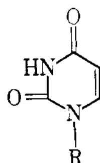
(6) B. R. Baker, B.-T. Ho, and D. V. Santi, *J. Pharm. Sci.*, **54**, 1415 (1965).

(7) B. R. Baker and G. J. Lourens, *J. Heterocyclic Chem.*, **2**, 344 (1965).

(8) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

(9) B. Belleau and G. Lacasse, *J. Med. Chem.*, **7**, 768 (1964).

TABLE II
PHYSICAL CONSTANTS OF 1-SUBSTITUTED URACILS PREPARED BY REACTION OF RX WITH URACIL



Compd[a]	R	X	% yield[b]	Mp, °C	Calcd, % C	H	N	Found, % C	H	N
V	n-C_5H_{11}	Br	29	73–74[c]	59.3	7.74	15.4	59.5	7.86	15.5
VI	i-C_5H_{11}	Br	38	148–149[c]	59.3	7.74	15.4	59.2	7.66	15.6
VII	Cyclopentyl	Br	35	173–174[c]	59.3	7.74	15.4	59.7	6.68	15.4
VIII	i-C_6H_{13}	Br	21	114–115[d]	61.2	8.28	14.3	61.4	8.24	14.5
IX	$C_6H_5CH_2$	Cl	54	173–174[e,f]						
X	$C_6H_5(CH_2)_2$	Br	43	156–158[g]	66.7	5.59	13.0	66.9	5.68	12.9
XII	$C_6H_5(CH_2)_4$	Cl[h]	41	117–118[g]	68.8	6.60	11.5	69.0	6.81	11.2
XIII	$C_6H_5(CH_2)_5$	Cl[h]	45[i]	117–119[g]	69.7	7.02	10.8	69.9	7.16	10.6
XX	m-$NO_2C_6H_4$-CH_2	Cl	69[j-l]	261–263	53.4	3.67	17.0	53.2	3.82	17.1
XXI	o-$NO_2C_6H_4$-CH_2	Cl[m]	53[j-l]	255–257	53.4	3.67	17.0	53.6	3.80	16.7

[a] All compounds had infrared spectra compatible with their assigned structures; all had λ_{max} 265–271 mμ in 0.1 N NaOH, characteristic of 1-substituted uracils. [3-Alkyluracils show about a 30-mμ shift to longer wavelength in alkali, but 1-alkyluracils do not; *cf.* D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).] All compounds were uniform on tlc in benzene–ethyl acetate (5:1) unless another solvent was indicated. [b] Reactions were run with a 3:1 ratio of uracil to alkyl halide in DMSO at 80–90° as previously described by Baker and Chheda (footnote e, Table I) for 3–4 hr unless otherwise indicated. Yields are for analytically pure product; no attempt was made to obtain optimum conditions. [c] Recrystallized from $CHCl_3$–ether–petroleum ether (bp 30–60°). [d] Recrystallized from $CHCl_3$–petroleum ether. [e] Recrystallized from $CHCl_3$–ethyl acetate. [f] C. C. Cheng and L. R. Lewis, *J. Heterocyclic Chem.*, **1**, 260 (1964), have recorded mp 173–174° for this compound prepared by a different route. [g] Recrystallized from ethyl acetate. [h] Reaction run with 1:1 ratio of NaI:RX. [i] Reaction time 5 hr. [j] The crude product crystallized on addition of water; it was leached with 30 ml of boiling water/mmole of starting uracil to remove uracil, then recrystallized. [k] Recrystallized from dimethylformamide. [l] Uniform on tlc in ethyl acetate–benzene (2:3). [m] Reaction time was 2 hr.

restriction on binding, a sharp contrast to the conformational restrictions to hydrophobic bonding to dihydrofolic reductase.[5–7]

Since 1-benzyluracil (IX) was complexed to thymidine phosphorylase about fourfold tighter than 1-butyluracil (IV), the higher aralkyl uracils were synthesized and investigated as inhibitors (Table I). Phenethyl- (X) and phenylbutyluracils (XII) were complexed about equally to 1-benzyluracil (IX), but the phenylpropyluracil (XI) was complexed only about one-half as well as 1-benzyluracil (IX); in contrast, 1-phenylamyluracil (XIII) was complexed about threefold better than 1-benzyluracil (IX) and about sixfold better than 1-phenylpropyluracil (XI). Thus there are some conformational restrictions on the ability of a terminal phenyl group of a phenylalkyl moiety to bind to thymidine phosphorylase.

That the phenyl group of 1-benzyluracil (IX) was most probably complexed to a hydrocarbon area on the enzyme was confirmed by assay of 1-benzyluracils having a polar substituent on the phenyl ring, namely XIV–XVI. Substitution of the highly polar p-carboxylate group (as in XIV) led to a greater than tenfold loss in binding. When the polarity was decreased by conversion to the less polar carboxamide ($\pi = -1.5$)[10] (as in XV), the amount of repulsion from the hydrocarbon area was reduced.[11] Similarly, the polar acetamido group ($\pi = -0.97$)[10] (as in XVI) gave a greater than threefold loss in binding. In the same manner, insertion of a p-carboxy group (as in XVII) on 1-phenylpropyluracil (XI) gave nearly a fivefold loss in binding. The best compound listed in Table I is 1-(5-phenylamyl)uracil (XIII) which complexes about 100-fold more strongly than 1-methyluracil (I); since the phenylamyl group is probably complexed to a hydrophobic region, the 100-fold increment in binding compared to 1-methyluracil represents about 2.6 kcal/mole in free energy of binding by the phenylbutyl part of the phenylamyl group.

Insertion of a bromoacetamido group on the phenyl moiety of 1-benzyluracil (IX), as in XVIII and XIX (Table I), gave the candidate active-site-directed irreversible inhibitors[5,12] of thymidine phosphorylase. This relatively polar bromoacetamido group gave a threefold loss in reversible complexing in the *meta* position and of a fivefold loss in the *ortho* position. The rate of inactivation by the active-site-directed mechanism is dependent upon the concentration of reversible enzyme–inhibitor complex;[5,13] therefore, the concentration necessary for 50% reversible complexing with the enzyme was considered too high (6–11 mM) to be useful *in vivo* and XVIII and XIX were not investigated as irreversible inhibitors. Since much better reversible inhibitors than those in Table I have been found,[14] the use of a 1-aralkyl group for a carrier of the covalent-

(10) The Hansch π constant is a measure of relative polarity on a log scale, a negative value being more polar than hydrogen; *cf.* T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

(11) Although the un-ionized carboxyl has $\pi = 0.28$,[10] the ionized carboxyl is much more polar. The ionization of p-toluic acid is apparently influenced by its environment such as solvent. H. L. Goering, T. Rubin, and M. S. Newman, *J. Am. Chem. Soc.*, **76**, 787 (1954), have reported that p-toluic acid has $pK_a = 4.8$ in 20% dioxane and $pK_a = 5.9$ in 50% ethanol. The solvent effects of DMSO, the effect of a possible interaction of the two rings on the strength of the carboxyl of XIV, and the influence of the environment of the enzyme are unknown; although the first two effects could be measured, the third is difficult, if not impossible, to measure at the present time. Thus at the pH 5.9 of the assay, the carboxyl group of XIV could be 50% ionized if its $pK_a = 5.9$ or 95% ionized if its pK_a is 4.8.

(12) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964).

(13) B. R. Baker, W. W. Lee, and T. Tong, *J. Theoret. Biol.*, **3**, 459 (1962).

(14) B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 316 (1967); paper LXXX of this series.

forming group for irreversible inhibition[5,12] was not investigated further.

The required 1-substituted uracils in Table I were synthesized by alkylation of uracil in DMSO in the presence of potassium carbonate[7] (Table II). In the case of XVIII and XIX, the appropriate nitrobenzyl chloride was used, followed by reduction, then bromoacetylation.[15]

Experimental Section[16]

1-(*o*-Aminobenzyl)uracil.—A solution of 1.20 g (5 mmoles) of XXI (Table II) in 40 ml of 1% aqueous NaOH was shaken with hydrogen at 2–3 atm in the presence of 1 ml of Raney nickel for 1 hr when reduction was complete. The filtered solution was acidified to pH 5, then the separated product was collected on a filter and washed with water. Recrystallization from ethanol gave 0.68 g (54%) of pure product as yellow prisms: mp 185–187°; ν_{max} 3350 (NH), 1700–1600 (multiple, broad uracil bands), 745 cm^{-1} (*o*-C_6H_4); λ_{max} (EtOH), 235 mμ (ε 8500), 268 mμ (ε 9500); (pH 13), 266 mμ (ε 7200). The material moved as one spot on tlc in ethyl acetate–benzene (2:3).

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

(16) 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 17B spectrophotometer. Ultraviolet spectra were determined in 10% ethanol with a Perkin-Elmer 202 spectrophotometer unless otherwise indicated. Thin layer chromatograms (tlc) were run on Brinkmann silica gel GF and spots were detected by visual examination under ultraviolet light.

Anal. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.8; H, 5.10; N, 19.3. Found: C, 60.7; H, 4.92; N, 19.1.

1-(*m*-Aminobenzyl)uracil was prepared similarly from XX in 43% yield after recrystallization from ethanol; mp 170–171°; λ_{max} (EtOH), 245, 268, mμ; (pH 13), 245, 266 mμ.

Anal. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.8; H, 5.10; N, 19.3. Found: C, 60.9; H, 5.25; N, 19.1.

The compound moved as a single spot on tlc in ethyl acetate–benzene (2:3).

1-(*m*-Bromoacetamidobenzyl)uracil (XVIII).—To a solution of 434 mg (2 mmoles) of 1-(*m*-aminobenzyl)uracil in 3 ml of DMSO at about 50° was added 7 ml of $CHCl_3$ followed by 540 mg (2.1 mmoles) of bromoacetic anhydride. The solution was refluxed for 30 min during which time the product separated. After 2 hr at room temperature, the mixture was filtered and the product was washed ($CHCl_3$); yield 520 mg (77%) of pure product as light pink crystals, mp 248–250°; the product was homogeneous on tlc in ethyl acetate–benzene (2:3) and gave a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen.[15] The compound had ν_{max} 3350 (NH), 1700–1680 (broad), 1560 (C=O, C=C, NH), 770 cm^{-1} (*m*-C_6H_4); λ_{max} (EtOH), 265 mμ; (pH 14), 264 mμ.

Anal. Calcd for $C_{13}H_{12}BrN_3O_3$: C, 46.0 H, 3.86; N, 12.3; Br, 23.6. Found: C, 46.3; H, 3.79; N, 12.4; Br, 23.5.

1-(*o*-Bromoacetamido)uracil (XIX) was prepared as described for XVIII; after recrystallization from DMSO, the yield of product, mp 226–227°, was 56%; ν_{max} 3300 (NH), 1710, 1690, 1650 (C=O, C=C, NH), 769 cm^{-1} (*o*-C_6H_4); λ_{max} (EtOH), 264 mμ; (pH 13), 263 mμ.

Anal. Calcd for $C_{13}H_{12}BrN_3O_3$: C, 46.0; H, 3.86; N, 12.3; Br, 23.6. Found: C, 46.0; H, 3.62; N, 12.2; Br, 23.4.

The compound moved as a single spot on tlc in ethyl acetate–benzene (2:3) and gave a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen.[15]

Irreversible Enzyme Inhibitors. LXXVII.[1,2] Inhibitors of Thymidine Phosphorylase. III.[3] Hydrophobic Bonding by 1-Substituted Uracils Containing Additional Substituents at the 5 and 6 Positions

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1-Phenylpropyluracils substituted by alkyl, aryl, or aralkyl groups at the 5 or 6 position were studied as inhibitors of thymidine phosphorylase. In addition to the 15-fold increment in binding by 1-phenylpropyluracil compared to 1-methyluracil, further bonding by the 5-allyl, 5-phenyl, 6-propyl, 6-pentyl, 6-phenyl, and 6-benzyl substituents were noted. Up to a sixfold further increment in binding could be obtained that was most probably due to hydrophobic bonding.

The mode of binding of the ribofuranose moiety of thymidine to thymidine phosphorylase has been presented in an earlier paper of this series.[3] The ribofuranose moiety could be replaced by the 1-phenylamyl group with less than a twofold loss in binding;[2] since it was probable that the phenylamyl group, as well as its lower phenylalkyl homologs, were complexed to a hydrophobic region on the enzyme, it is likely that this hydrophobic region is in a different direction with respect to the 1 position of uracil than the area of the enzyme that complexes the 3′-hydroxyl or thymidine.[3] Therefore, a study was made to determine if additional hydrophobic bonding could occur with 5 or 6 substituents or both on a 1-aralkyluracil; the results are the subject of this paper.[4]

1-Phenylpropyluracil (I) was arbitrarily chosen as a base line for most of this study. That a hydrophobic region might be near the 5 position of uracil when I was complexed to the enzyme was indicated by the nearly twofold loss in binding obtained by substitution with the polar 5-hydroxymethyl group (IV) (Table I); this loss was regained by substitution with the less polar ethoxymethyl group of V, but additional hydrophobic bonding was not obtained with the 5-isoamyloxy group of VII. These results were confirmed in the 1-(*n*-butyl)uracil (II) series where the 5-ethoxymethyl derivative (VI) gave the same binding as II.

By substituting a 5-phenyl (IX) or 5-allyl (VIII) group on 1-phenylpropyluracil (I) only about a twofold

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(2) For the previous paper of this series, see B. R. Baker and M. Kawazu, *J. Med. Chem.*, **10**, 302 (1967).

(3) B. R. Baker, *ibid.*, **10**, 297 (1967); paper LXXV of this series.

(4) The chemotherapeutic utility for inhibitors of thymidine phosphorylase has been previously discussed.[3]