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5-BENZYLACYCLOURIDINE AND 5-BENZYLOXYBENZYLACYCLOURIDINE, POTENT INHIBITORS OF URIDINE PHOSPHORYLASE*

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Abstract—Various pyrimidine acyclonucleosides (1-(2′-hydroxyethoxymethyl)uracils) are specific inhibitors of uridine phosphorylase [Niedzwicki *et al.*, *Biochem. Pharmac.* **30**, 2097 (1981)]. 5-Benzyluracils have also been shown to inhibit this enzyme [Baker and Kelley, *J. med. Chem.* **13**, 461 (1970); Woodman *et al.*, *Biochem. Pharmac.* **29**, 1059 (1980)]. We have synthesized the acyclonucleoside analogs of 5-benzyluracil (BU) and 5-benzyloxybenzyluracil (BBU). These compounds, 5-benzyl-1-(2′-hydroxyethoxymethyl)uracil (BAU) and 5-(*m*-benzyloxybenzyl)-1-(2′-hydroxyethoxymethyl)uracil (BBAU), are potent inhibitors of uridine phosphorylase. K_i values of 98 and 32 nM were estimated for BAU and BBAU respectively. These compounds are better inhibitors of uridine phosphorylase than BU (K_i = 1575 nM), BBU (K_i = 270 nM), and all other compounds previously tested, and they have no effect on thymidine phosphorylase, uridine-cytidine kinase, or thymidine kinase. Potential chemotherapeutic applications of BAU and BBAU are discussed.

The two known mammalian PyrNPases‡, uridine phosphorylase (UrdPase, EC 2.4.2.3) and thymidine phosphorylase (dThdPase, EC 2.4.2.4), catalyze the reversible phosphorolysis of pyrimidine nucleosides as follows:

$$\text{Pyr(d)R} + \text{P}_i \rightleftharpoons \text{Pyr} + \text{(d)R-1-P}$$

Many pyrimidine nucleosides are substrates for one or both of the PyrNPases, with the notable exceptions of orotidine, 6-azauridine, cytidine, and 2′-deoxycytidine which are not cleaved by either enzyme [1–4]. UrdPase will cleave uridine, 2′-deoxyuridine, thymidine, FUrd, FdUrd, IdUrd, and BrdUrd [4–6]. dThdPase, on the other hand, is more specific, as all of the foregoing 2′-deoxyribonucleosides, but neither uridine nor FUrd, will serve as substrates [6–8].

PyrNPase inhibitors can serve as valuable biochemical tools to elucidate the role of the PyrNPases in the metabolism of pyrimidines and their analogs. Additionally, inhibitors of PyrNPases may be useful as pharmacologic agents by inhibiting either pyrimidine salvage or catabolism and by modulating the metabolism of chemotherapeutic analogs such as FUrd and FdUrd. Unfortunately, the few previously known PyrNPase inhibitors have been found to be of limited value for these applications [9, 10].

Recently we synthesized several pyrimidine acyclonucleosides [11] and found that these compounds are potent inhibitors of UrdPase [12]. BU and BBU were also shown to inhibit this enzyme [13, 14]. We now report the synthesis of BAU and BBAU which have the chemical features of both the pyrimidine acyclonucleosides and the 5-benzyluracils. These new compounds are the most potent inhibitors known for UrdPase. A preliminary report has been presented [15].

MATERIALS AND METHODS

Materials

[2-^{14}C]Thymidine (51.6 mCi/mmole) and [2-^{14}C]-uridine (52.4 mCi/mmole) were obtained from the New England Nuclear Corp., Boston, MA; silica gel G UV254 Polygram TLC plates from Brinkmann, Westbury, NJ; neutral alumina (AG7, 100–200 mesh) and Protein Assay kit from Bio-Rad Laboratories, Richmond, CA; and DEAE-cellulose (DE-23) from Whatman Biochemicals, Maidstone, Kent, U.K. All other chemicals were obtained from either the Sigma Chemical Co., St. Louis, MO, or the Aldrich Chemical Co., Milwaukee, WI.

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‡ Abbreviations: PyrNPase, pyrimidine nucleoside phosphorylase; UrdPase, uridine phosphorylase; dThdPase, thymidine phosphorylase; Pyr(d)R, pyrimidine riboside or pyrimidine-2′-deoxyriboside; P_i, orthophosphate; Pyr, pyrimidine base; (d)R-1-P, α-D-ribose-1-phosphate or α-D-2′-deoxyribose-1-phosphate; BU, 5-benzyluracil; BBU, 5-benzyloxybenzyluracil or 5-{[3-(phenylmethoxy)-phenyl]methyl}uracil; BAU, 5-benzylacyclouridine or 5-benzyl-1-(2′-hydroxyethoxymethyl)uracil; BBAU, 5-*m*-benzyloxybenzylacyclouridine or 5-(*m*-benzyloxybenzyl)-1-(2′-hydroxyethoxymethyl)uracil; acyclothymidine, 5-methyl-1-(2′-hydroxyethoxymethyl)uracil; acyclouridine, 1-(2′-hydroxyethoxymethyl)uracil; FdUrd, 5-fluoro-2′-deoxyuridine; FUrd, 5-fluorouridine; IdUrd, 5-iodo-2′-deoxyuridine; BrdUrd, 5-bromo-2′-deoxyuridine; DTT, dithiothreitol; Urd, uridine; dThd, thymidine; PALA, *N*-(phosphonacetyl)-L-aspartate; and DON, 6-diazo-5-oxo-L-norleucine.

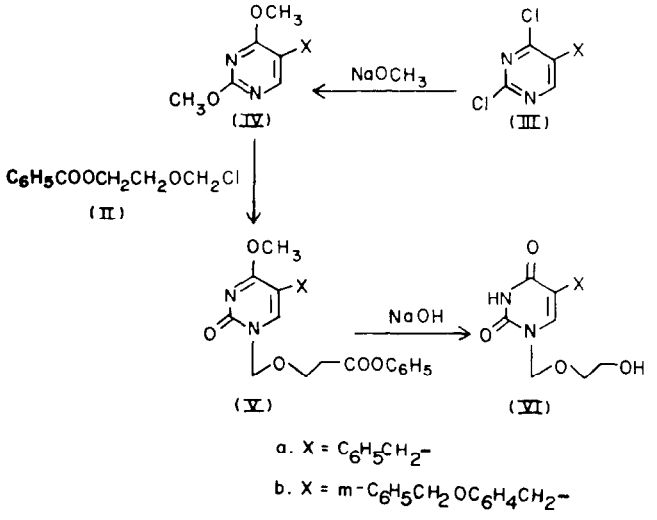

Fig. 1. Synthetic scheme for BAU and BBAU.

Chemical synthesis

The 5-substituted acyclouridines (VI) were prepared from the corresponding uracil derivatives (I) by the method outlined in Fig. 1. 5-Benzyluracil (BU) and 5-benzyloxybenzyluracil (BBU) were synthesized by standard methods [13, 16], chlorinated at the C-2 and C-4 positions with $POCl_3$ in the presence of diethylaniline to give III, converted to the 2,4-dimethoxy derivatives (IV) with sodium methoxide in methanol, and condensed with 2-chloromethoxyethyl benzoate (II) in the presence of anhydrous sodium carbonate to give V [11, 17]. The acyclonucleoside analogs (VI) were then obtained by deblocking at the 2′- and 4-positions of V with 2 N NaOH, neutralizing the solution by stirring with sufficient Dowex 50 (H^+), and evaporating the solvent.

Melting points were determined on a Gallenkamp melting point apparatus and were not corrected. Ultraviolet spectra were determined on a Perkin–Elmer model 402 spectrophotometer and infrared spectra on a Perkin–Elmer model 457 spectrophotometer. NMR spectra were measured on a Varian A-60A spectrometer in DMSO-d_6 or $CDCl_3$ with tetramethylsilane as the internal standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

5-Benzyl-2,4-dichloropyrimidine (IIIa). 5-Benzyluracil (Ia) (3.14 g, 0.015 mole) in 17 ml of freshly distilled phosphorous oxychloride containing 3.03 g (0.020 mole) of freshly distilled diethylaniline was heated for 1 hr between 105 and 110°. Excess $POCl_3$ was flash-evaporated, and a mixture of ether and ice was added to the residue to decompose any remaining $POCl_3$ and dissolve the product. The aqueous layer was extracted with ether, and combined ether layers were washed with 2 N HCl and H_2O and dried over $MgSO_4$. Removal of the solvent followed by vacuum distillation yielded a colorless liquid, 2.75 g (74%), b.p./0.03 mm 120–123°; u.v. (EtOH): λ_{max} 266 nm (3880); i.r. (KBr): 870 cm^{-1} (C—Cl), (carbonyl peaks of uracil at 1667 and 1740 absent); NMR (DMSO-d_6): δ 4.13 (s, 2H, CH_2 at C_5), 7.34 (s, 5H, ArH), 8.85 (s, C_6—H).

Anal. Calc. for $C_{11}H_8N_2Cl_2$: C, 55.26; H, 3.37; N, 11.71; Cl, 29.65. Found: C, 55.37; H, 3.39; N, 11.69; Cl, 29.96.

5-(m-Benzyloxybenzyl)-2,4-dichloropyrimidine (IIIb). 5-Benzyloxybenzyluracil (Ib) (11.0 g, 0.036 mole) in 50 ml of freshly distilled $POCl_3$ and 10.6 g (0.071 mole) of freshly distilled diethylaniline was heated to 105–110° in the same manner as IIIa for 1 hr. The residue crystallized in orange plates from alcohol (77%), m.p. 58–59°; u.v. (EtOH): λ_{max} 266 nm (4990); i.r. (KBr): 870 cm^{-1} (C—Cl) and no carbonyl peaks; NMR ($CDCl_3$): δ 3.96 (s, 2H, CH_2 at C_5), 5.00 (s, 2H, CH_2 of OBzl), 6.72–7.20 (m, 4H, ArH of 5-Bzl), 7.33 (s, 5H, ArH of OBzl), 8.22 (s, C_6—H).

Anal. Calc. for $C_{18}H_{14}N_2Cl_2O$: C, 62.62; H, 4.09; N, 8.11; Cl, 20.54. Found: C, 62.71; H, 4.13; N, 8.14; Cl, 20.40.

5-Benzyl-2,4-dimethoxypyrimidine (IVa). A solution of 2.41 g (0.01 mole) of IIIa in dry methanol was added to 2 g of sodium dissolved in 50 ml of dry methanol and stirred for 40 min. The solvent was flash-evaporated, and the residue was taken up in a mixture of 50 ml of ether and 10 ml of 10% KOH. The ether layer was washed with a second portion of KOH, then H_2O, and then dried over $MgSO_4$. After removal of the solvent, vacuum distillation yielded 1.80 g (78%) of IVa, b.p./0.02 mm 112°; u.v. (EtOH): λ_{max} 266 nm (5900); i.r. (KBr): no peak at 870 cm^{-1} (C—Cl); NMR (DMSO-d_6): δ 3.82 (s, 2H, CH_2 at C_5), 3.92 (s, 6H, OCH_3), 7.29 (s, 5H, ArH), 8.21 (s, C_6–H).

Anal. Calc. for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 68.00; H, 6.19; N, 12.21.

5-(m-Benzyloxybenzyl)-2,4-dimethoxypyrimidine (IVb). To a solution of 3.0 g (0.13 mole) of sodium in 100 ml of MeOH was added 9.38 g (0.027 mole) of IIIb in 120 ml of hot MeOH. After stirring for 1 hr, the mixture was treated in the same manner as for IVa. Recrystallization of the residue from pet-

roleum ether yielded 8 g (85%) of IVb, m.p. 47–48°; u.v. (EtOH): λ_{max} 266 nm (8000); i.r. (KBr): no peak at 870 cm^{-1} (C—Cl) nor carbonyl peaks; NMR ($CDCl_3$): δ 3.76 (s, 2H, CH_2 at C_5), 3.96 (s, 6H, OCH_3), 5.01 (s, 2H of OBzl), 6.60–7.20 (m, 4H, ArH of 5-Bzl), 7.36 (s, 5H, ArH of OBzl), 7.95(s, C_6—H).

Anal. Calc. for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.35; H, 6.02; N, 8.32.

5-Benzyl-1-(2'-benzyloxyethoxymethyl)-2-oxo-4-methoxypyrimidine (Va). To a solution of 4.7 g (0.02 mole) IVa in 50 ml anhydrous methylene chloride was added 4.4 g (0.02 mole) of 2-chloromethoxyethyl benzoate (II) and 3 g of finely powdered anhydrous sodium carbonate. After stirring for 1 day at room temperature, the filtered solvent was removed by spin evaporation, and the residue was washed with petroleum ether by decantation and then stirred with ether to induce crystallization. Recrystallization from EtOH and drying over P_2O_5 yielded 5.5 g of Va (68%), m.p. 92–93°; u.v. (EtOH): λ_{max} 281 nm (5600); i.r. (KBr): 1640–1665, 1720 cm^{-1} (carbonyl); NMR (DMSO-d_6): δ 3.60 (s, 2H, CH_2 at C_5), 3.87 (s, 3H, OCH_3), 3.73–4.06 (m, 2H, $C\underline{H}_2CH_2OBzoyl$), 4.22–4.53 (m, 2H, $CH_2C\underline{H}_2OBzoyl$), 5.29 (s, 2H, CH_2 at N_1), 7.25 (s, 5H, ArH), 7.46–8.11 (m, 6H, ArH of benzoate and C_6—H overlap).

Anal. Calc. for $C_{22}H_{22}N_2O_5$: C, 66.99; H, 5.62; N, 7.10. Found: C, 67.06; H, 5.77; N, 7.15.

5-(m-Benzyloxybenzyl)-1-(2'-benzoyloxyethoxymethyl)-2-oxo-4-methoxypyrimidine (Vb). A solution of IVb (5.9 g, 0.0175 mole) and 3.8 g (0.0177 mole) of II in 75 ml of dry dichloroethylene was stirred with 2.02 g (0.019 mole) of finely pulverized anhydrous sodium carbonate for 23 hr. After filtering and removing the solvent, the residue was triturated with ether to induce crystallization, which yielded 6.3 g (71%) of Vb, m.p. (from EtOH): 93.5–94°; u.v. (EtOH): λ_{max} 280 nm (7000); i.r. (KBr): 1680, 1730 cm^{-1} (carbonyl), none at 870 cm^{-1} (C—Cl) or 3500 cm^{-1} (OH); NMR ($CDCl_3$): δ 3.54 (s, 2H, CH_2 at C_5), 3.93 (s, 5H, $C\underline{H}_2CH_2OBzoyl$ and OCH_3 overlap), 4.40 (m, 2H, $CH_2C\underline{H}_2OBzoyl$), 5.00 (s, 2H, CH_2 of OBzl), 5.20 (s, 2H, CH_2 at N_1), 6.50–7.20 (m, 4H, ArH of 5-Bzl), 7.34 (s, 5H, ArH of OBzl), 7.20–8.15 (m, 6H, ArH of Bzoyl and C_6—H overlap).

Anal. Calc. for $C_{29}H_{28}N_2O_6$: C, 69.59; H, 5.64; N, 5.60. Found: C, 69.63; H, 5.79; N, 5.63.

5-Benzyl-1-(2'-hydroxyethoxymethyl)uracil (BAU, VIa). To a solution of 1.0 g (0.0025 mole) of acyclouridine benzoate (Va) in 15 ml EtOH was added 15 ml of 2 N NaOH, and the mixture was stirred overnight at room temperature. The solution was neutralized with Dowex 50 (H^+) and filtered, and the Dowex was washed thoroughly with aqueous alcohol. After evaporation of combined filtrates, the residue (0.45 g) was washed with ether to remove any remaining benzoic acid, cooled, and scratched. Recrystallization of the product from EtOH yielded 0.43 g (70%), m.p. 145–146°; u.v. (pH 1): λ_{max} 266 nm (9200); (pH 11): λ_{max} 266 nm (5700); i.r. (KBr): 1670, 1720 cm^{-1} (carbonyl), 3400 cm^{-1} (OH); NMR (DMSO-d_6): δ 3.54 (s, 4H, CH_2CH_2OH), 3.57 (s, 2H, CH_2 at C_5), 5.10 (s, 2H, CH_2 at N_1), 7.29 (s, 5H, ArH), 7.66 (s, C_6—H).

Anal. Calc. for $C_{14}H_{16}N_2O_4$: C, 60.85; H, 5.84; N, 10.14. Found: C, 61.05; H, 6.01; N, 10.35.

5-m-(Benzyloxybenzyl)-1-(2'-hydroxyethoxymethyl)uracil (BBAU, VIb). A solution of 4.00 g (0.008 mole) of Vb in a mixture of 60 ml of 2 N NaOH and 60 ml of MeOH was stirred overnight at room temperature. The solution then treated in the same manner as for VIa and was recrystallized from EtOH. The total yield was 1.25 g (41%), m.p. 126–130°; u.v. (pH 1): λ_{max} 266 nm (10,600); (pH 11): λ_{max} 266 nm (7700); i.r. (KBr): 1670 cm^{-1} (carbonyl), 3470 cm^{-1} (OH); NMR (DMSO-d_6): δ 3.52 (s, 6H, CH_2 at C_5 and CH_2CH_2OH overlap), 4.69 (broad s, 1H, OH), 5.05 (s, 2H, CH_2 of OBzl), 5.08 (s, 2H, CH_2 at N_1), 6.65–7.30 (m, 4H, ArH of 5-Bzl), 7.39 (s, 5H, ArH of OBzl), 7.63 (s, C_6—H).

Anal. Calc. for $C_{21}H_{22}N_2O_5$: C, 65.95; H, 5.80; N, 7.33. Found: C, 65.87; H, 5.99; N, 7.23.

Preparation of cytosol extract for enzyme assay

Sarcoma S-180 cells were carried in the peritoneal cavity of male CD-1 mice (Charles River Laboratories, Wilmington, MA) and were collected in normal saline, washed as described previously [18], and homogenized using a motor-driven Teflon pestle in 3 vol. of 20 mM potassium phosphate buffer (pH 8.0) which contained 1 mM EDTA and 1 mM mercaptoethanol (Buffer A). Livers obtained from freshly killed CD-1 mice were similarly homogenized in Buffer A as above. All homogenates were then centrifuged at 105,000 *g* for 1 hr, and the resulting cytosol fractions were used as the source of PyrNPase activity. S-180 cytosol does not contain dThdPase [12]. Frozen S-180 cytosol was used for determination of kinetic parameters of inhibitory compounds on UrdPase. Mouse liver cytosol contains both UrdPase and dThdPase [6] and was used immediately after preparation for determining the effects of inhibitors on both enzymes separated by DEAE-cellulose chromatography (see below). Tissues to be assayed for pyrimidine nucleoside kinase activity were homogenized in 3 vol. of 0.1 M Tris–HCl buffer (pH 7.5) containing 10% glycerol and 2 mM DTT (Buffer B), and cytosol fractions were obtained by centrifugation as above.

PyrNPase assay

Reaction mixtures contained 20 mM potassium phosphate (pH 8.0), 1 mM EDTA, 1 mM mercaptoethanol, a substrate (uridine or thymidine, 5–6 mCi/mmole, at 0.15 mM), and, when indicated, an appropriate amount of inhibitor. Reactions were started by the addition of 0.5 to 2.0 mg S-180 cytosol protein or 50 μl of DEAE-cellulose eluent, to give a volume in all cases of 140 μl. Assays for uridine and thymidine phosphorolytic activity were carried out at 37° as described previously [12].

Thymidine kinase and uridine-cytidine kinase assay

The assay method used has been described previously in detail [19]. The reaction mixture contained 0.1 M Tris–HCl (pH 7.6), 0.25 mM uridine (0.4 mCi/mmole) or 7.5 μM thymidine (51 mCi/mmole), 2.5 mM ATP, 3 mM $MgCl_2$, 20 mM sodium fluoride, 2 mM DTT, and 15 μl cytosol in a final

volume of 100 μl. Reactions were started by the addition of cytosol and stopped by the addition of 0.2 M EDTA (pH 7.6). Nucleosides were then separated from nucleotides on alumina columns (0.6 g in a Pasteur pipette).

DEAE-cellulose chromatography

DEAE-cellulose was equilibrated with 20 mM potassium phosphate (pH 8.0), 1 mM EDTA, and 1 mM mercaptoethanol (Buffer A), and packed into columns (2.5 × 5 cm). Three milliliters of mouse liver cytosol were applied to the columns. Each column was washed with 400 ml of Buffer A, and then a linear gradient to 1 M sodium chloride was applied over a volume of 100 ml. Fractions of 10 ml were collected, and 50-μl aliquots were assayed for uridine (0.15 mM) and thymidine (0.15 mM) phosphorolytic activities in the presence and absence of inhibitor using the radioisotopic technique described above, except that reactions were allowed to proceed for 60 min rather than 20 min.

Protein determination

Protein concentrations were determined by the Bio-Rad Laboratories assay procedure using bovine γ-globulin as a standard.

RESULTS

Inhibition constants and mechanism

BU, BBU, BAU, and BBAU were screened for inhibition of uridine (0.15 mM) cleavage by UrdPase from S-180 cytosol (K_m for uridine, 0.1 mM). Kinetic studies were then performed to determine the inhibition mechanism and K_i values (Table 1), using enzyme from the same source. In all cases, competitive inhibition was observed. A double-reciprocal plot for BBAU is presented in Fig. 2.

Inhibitor specificity

Since the two PyrNPases are known to occur in mouse liver, we separated these enzymes using DEAE-cellulose chromatography as described by Krenitsky *et al.* [6] and Niedzwicki *et al.* [12]. As can be seen in Fig. 3, mouse liver dThdPase was bound weakly to the DEAE-cellulose and eluted from the column by washing with Buffer A, whereas UrdPase was retained until a linear gradient of sodium chloride was applied. BBAU (4 μM, Fig. 3) had no effect on thymidine cleavage by dThdPase, whereas the cleavage of both uridine and thymidine by UrdPase was totally inhibited by this compound.

Table 1. Inhibitory potencies of 5-benzyluracils and 5-benzylacyclouridines for uridine phosphorylase from S-180 cytosol

Compound	K_i (nM)
BU	1575 ± 520
BAU	98 ± 10
BBU	270 ± 152
BBAU	32 ± 7

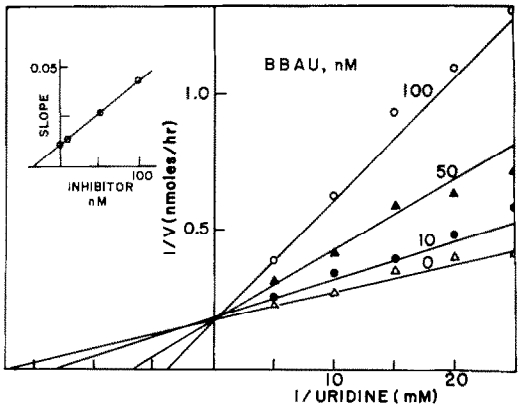

Fig. 2. Double-reciprocal plot for inhibition of UrdPase by BBAU. Key: (△) 0 nM BBAU; (●) 10 nM BBAU; (▲) 50 nM BBAU; and (○) 100 nM BBAU.

Similar DEAE-cellulose chromatograms were obtained with BU (50 μM), BBU (0.5 μM) and BAU (100 μM). These results demonstrated that BU, BBU, BAU, and BBAU are specific inhibitors of UrdPase and have no effect on dThdPase.

BAU (0.1 mm) and BBAU (8 μM) had no significant effect on the phosphorylation of thymidine or uridine by S-180 cytosol, indicating that these compounds did not inhibit either thymidine kinase (EC 2.7.1.75) or uridine-cytidine kinase (EC 2.7.1.48).

DISCUSSION

Our design of BAU and BBAU as inhibitors of UrdPase was based on the expectation that appropriate substitution of both the 1 and 5 positions of uracil would produce compounds that bind more tightly to the enzyme than the substitution of each position individually. Thymine and acyclothymidine are better inhibitors of UrdPase than uracil [20] and acyclouridine [12], respectively, and it has been speculated that a hydrophobic "pocket" exists on UrdPase adjacent to the binding site of the 5-position of the pyrimidine ring [12, 13]. This region of the

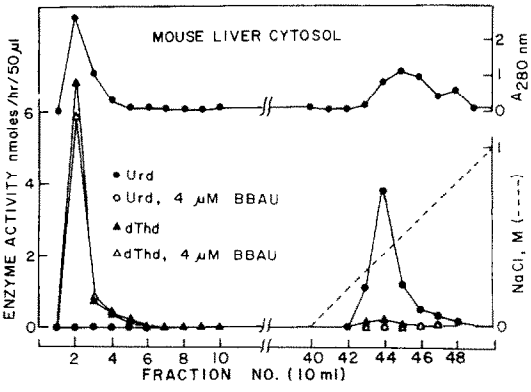

Fig. 3. DEAE-cellulose chromatograph of mouse liver cytosol. Key: (●) 0.15 mM uridine; (○) 0.15 mM uridine with 4 μM BBAU; (▲) 0.15 mM thymidine; and (△) 0.15 mM thymidine with 4 μM BBAU.

enzyme appears to bind various 5-benzyluracils most tightly [13, 14]. We have shown that a new class of 1-substituted pyrimidine analogs, the pyrimidine acyclonucleosides, are potent competitive inhibitors of UrdPase and have speculated that the terminal hydroxyl group of the 2′-hydroxyethoxymethyl side chain of these compounds might participate in hydrogen bonding to the active site of UrdPase at the same site that presumably interacts with the 3′-hydroxyl group of uridine [12]. In the present study, we found that the contributions of C-1 and C-5 substitutions to the binding force were nearly additive. The attachment of an O-benzyl group to BU to make BBU contributed $\Delta G°'$ (at 37°, pH 8.0) of −1.09 kcal (5.8-fold decrease in K_i); and the addition of the 2′-hydroxyethoxymethyl group, −1.7 kcal (16-fold decrease in K_i); while the addition of both groups contributed −2.4 kcal (49-fold decrease in K_i). If the effects of the two modifications were strictly additive, a contribution of approximately −2.8 kcal would have been obtained. These findings are consistent with the presence of the two binding sites postulated above for UrdPase.

One of the principal interests in the development of UrdPase inhibitors has been the possible use of these compounds to potentiate the cytotoxicity of pyrimidine nucleoside analogs (e.g. FdUrd and FUrd) which may be cleaved to less active bases by UrdPase [5]. However, attempts to potentiate FdUrd toxicity in cell culture by the co-administration of BU and BBU have not been successful [10]. Possible reasons for this lack of efficacy *in vivo* may be that these compounds are either insufficiently potent or poorly transported into cells. Additionally, the low water-solubility of BU and BBU may limit the usefulness of these two compounds. We have found that BAU and BBAU are both more potent and more water soluble than BU and BBU, respectively, by an order of magnitude, and, indeed, our preliminary studies indicate that BAU does potentiate the effects of FUrd and FdUrd in cultured L5178Y cells (unpublished results).

Another possible chemotherapeutic application of potent UrdPase inhibitors might involve the salvage of uracil and uridine. Regenerating rat liver [1, 21] and many neoplastic tissues [21–26] have been shown to salvage uracil to uridine and nucleotides by way of UrdPase whereas these conversions are minimal in normal rat liver [1, 21, 22, 26] and various other non-malignant tissues [27–30]. Thus, it is possible that, in those tumors which depend heavily on uracil salvage for nucleic acid synthesis, the inhibition of UrdPase by administration of BAU and BBAU could produce selective cytotoxic or antiproliferative effects. On the other hand, a significant portion of the pyrimidine requirements of various tissues (e.g. 30% in the liver) may be met by circulating uridine and cytidine [31]. It would be interesting, therefore, to find whether or not UrdPase inhibitors have differential effects on normal and tumor tissues, especially when co-administered with such inhibitors of the biosynthesis *de novo* of uridine-5′-monophosphate as PALA, DON, or 6-azauridine.

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