Biochemical Pharmacology, Vol. 36, No. 13, pp. 2195–2201, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

NEW ANALOGUES OF BENZYLACYCLOURIDINES, SPECIFIC AND POTENT INHIBITORS OF URIDINE PHOSPHORYLASE FROM HUMAN AND MOUSE LIVERS*

FARDOS N. M. NAGUIB,† MAHMOUD H. EL KOUNI, SHIH HSI CHU and SUNGMAN CHA
Division of Biology and Medicine, Brown University, Providence, RI 02912, U.S.A.

(*Received* 22 *August* 1986; *accepted* 16 *December* 1986)

Abstract—Kinetic parameters for the phosphorolytic activity of uridine phosphorylase (UrdPase) from human and mouse livers have been determined. The values of these parameters are: $K_{P_i} = 279.0 \pm 66.0\,\mu M$, $K_{Urd} = 242.0 \pm 63.0\,\mu M$ and $V_{max} = 3940 \pm 175$ pmol/min/mg, and $K_{P_i} = 76.0 \pm 7.0\,\mu M$, $K_{Urd} = 143.0 \pm 9.0\,\mu M$ and $V_{max} = 293.0 \pm 5.0$ pmol/min/mg, for human and mouse livers respectively. Benzylacyclouridines, the specific inhibitors of UrdPase, and seventeen newly synthesized derivatives, modified at the pyrimidine ring, the benzyl moiety or the acyclo tail, have been tested for their potency to inhibit UrdPase and thymidine phosphorylase (dThdPase) from both human and mouse livers. None inhibited dThdPase. In contrast, all of the compounds tested inhibited UrdPase. Competitive inhibition was observed in all cases. Several of the new compounds were superior in their inhibition of UrdPase to the parent compounds. The inhibitory potencies of these compounds with UrdPase from human liver roughly paralleled those obtained with UrdPase from mouse liver. The most potent of these compounds was AM-BBAU (aminomethyl-BBAU or 5-(3′-benzyloxybenzyl)-1-[(1′-aminomethyl-2′-hydroxyethoxy)methyl]uracil) with a K_i value of 18 nM with UrdPase from mouse liver. Structure–activity relationships of the binding of these inhibitors of UrdPase are discussed.

Pyrimidine nucleoside phosphorylases play an important role in the metabolism of pyrimidines, as they have the capacity to modulate the so-called salvage pathway, by anabolizing or catabolizing nucleosides and their analogues. In mammalian cells, there are two different pyrimidine nucleoside phosphorylases: uridine phosphorylase (UrdPase‡, EC 2.4.2.3) and thymidine phosphorylase (dThdPase, EC 2.4.2.4) which catalyze the reversible reaction:

$$\mathrm{Pyr(d)Rib} + \mathrm{P_i} \rightleftharpoons \mathrm{Pyr} + \mathrm{(d)Rib\text{-}1\text{-}P}$$

UrdPase primarily cleaves pyrimidine ribosides but is relatively non-specific as it also cleaves pyrimidine 2′- and 5′-deoxyribosides [1–7]. dThdPase, on the other hand, seems to be specific for pyrimidine 2′- and 5′-deoxyribosides [3, 5, 8–12].

Interest in inhibitors of UrdPase stems from the fact that this enzyme is responsible for the degradation of several chemotherapeutic agents, e.g. FUrd, FdUrd, BrdUrd and IdUrd in various tumors [13, 14]. Benzylacyclouridines were developed in this laboratory as potent and specific inhibitors of UrdPase [5, 15, 16]. These compounds were shown to potentiate the efficacy and selective toxicity of FdUrd [16, 17]. In addition, the benzylacyclouridines were shown to increase the levels of plasma uridine as well as the salvage of uridine by various tissues [18, 19] and to protect against 5-fluorouracil (FUra) toxicity [19]. Although it is believed that inhibition of hepatic

---

* This investigation was supported by Grant CH-136 awarded by the American Cancer Society and Grants CA-13943, CA-20892 and CA-39427 awarded by the National Cancer Institute, DHHS.

† Author to whom correspondence should be addressed.

‡ Abbreviations: BAU, benzylacyclouridine or 5-benzyl-1-[(2′-hydroxyethoxy)methyl]uracil; BBAU, benzyloxybenzylacyclouridine or 5-(3′-benzyloxybenzyl)-1-[(2′-hydroxyethoxy)methyl]uracil; BBBAU, 5-[3′-(4-benzyloxybenzyloxy)benzyl]-1-[(2′-hydroxyethoxy)-methyl]uracil; AM-BAU, aminomethyl-BAU or 5-benzyl-1-[(1′-aminomethyl-2′-hydroxyethoxy)methyl]uracil; AM-BBAU, aminomethyl-BBAU or 5-(3′-benzyloxybenzyl)-1-[(1′-aminomethyl-2′-hydroxyethoxy)methyl]uracil; BAU-P, BAU-phosphate or 5-benzyl-1-[(1′-ethoxy)-methyl]uracil-2′-phosphate; BrdUrd, 5-bromo-2′-deoxyuridine; *C*-methyl-BAU, 5-benzyl-1-[(*C*-(2′-hydroxyethoxy)-*C*-methyl)methyl]uracil; *C*-methyl-BBAU, 5-(3′-benzyloxybenzyl)-1-[(*C*-(2′-hydroxyethoxy)-*C*-methyl)-methyl]uracil; DA-BAU, deoxyamino-BAU or 5-benzyl-1-[(2′-aminoethoxy)methyl]uracil; DA-BBAU, deoxyamino-BBAU or 5-(3′-benzyloxybenzyl)-1-[(2′-aminoethoxy)-methyl]uracil; (d)Rib-1-P, α-D-ribose-1-phosphate; DTT, dithiothreitol; FdUrd, 5-fluoro-2′-deoxyuridine; HM-BAU, hydroxymethyl-BAU or 5-benzyl-1-[(1′-hydroxymethyl-2′-hydroxyethoxy)methyl]uracil; HM-BBAU, hydroxy-methyl-BBAU or 5-(3′-benzyloxybenzyl)-1-[(1′-hydroxy-methyl-2′-hydroxyethoxy)methyl]uracil; IdUrd, 5-iodo-2′-deoxyuridine; *N*3-BB-BAU, 5-benzyl-3-*N*-(4′-benzyl-oxybenzyl)-1-[(2′-hydroxy-ethoxy)methyl]uracil; *m*-hydroxy-BAU, 5-(3′-hydroxybenzyl)-1-[(2′-hydroxy-ethoxy)methyl]uracil; *m*-methoxy-BAU, 5-(3′-methoxy-benzyl)-1-[(2′-hydroxyethoxy)methyl]uracil; *p*-*O*-iso-propyl-BAU, 5-(4′-isopropoxybenzyl)-1-[(2′-hydroxy-ethoxy)methyl]uracil; P_i, orthophosphate; Pyr, pyrimidine base; Pyr(d)Rib, pyrimidine riboside or deoxyriboside; succ-BAU, succinyl-BAU or 5-benzyl-1-[(2′-(3-carboxypropionyloxy)ethoxy)methyl]uracil; succ-BBAU, succinyl-BBAU or 5-(3′-benzyloxybenzyl)-1-[(2′-(3-carboxypropionyloxy)-ethoxy)methyl]uracil; succ-HM-BBAU, succinyl-hydroxymethyl-BBAU or 5-(3′-benzyloxybenzyl)-1-[(2′-(1′,3′-bis-3-carboxypropionyloxy)propoxy)-methyl]-uracil; dThdPase, thymidine phosphorylase; and UrdPase, uridine phosphorylase.

UrdPase plays an important role in these phenomena [20, 21], little information is available on the kinetic parameters of UrdPase from human and mouse livers and their inhibition by benzylacyclouridines.

In the present study, we have established the kinetic parameters and determined the inhibitory potencies of BAU and BBAU for UrdPase from both mouse and human livers. We have also tested seventeen newly synthesized congeners of BAU and BBAU against hepatic UrdPase and dThdPase. AM-BBAU (aminomethyl-BBAU or 5-(3′-benzyloxybenzyl) -1- [(1′-aminomethyl - 2′- hydroxyethoxy)-methyl]uracil) was identified as the most potent specific inhibitor of UrdPase from both species. Structure–activity relationships have been formulated and have revealed the extent and limitations of the type of modifications that may be useful in increasing the binding of benzylocyclouridines to UrdPase.

MATERIALS AND METHODS

Chemicals. 5-Benzyl-1-[(2′-hydroxyethoxy)-methyl]uracil (benzylacyclouridine or BAU), 5-(3′-benzyloxybenzyl)- 1- [(2′- hydroxyethoxy)methyl]-uracil (benzyloxybenzylacyclouridine or BBAU), 5-benzyl- 1- [(1′- hydroxymethyl- 2′- hydroxyethoxy)-methyl]uracil (hydroxymethyl-BAU or HM-BAU), 5- (3′-benzyloxybenzyl)- 1- [(1′- hydroxymethyl- 2′-hydroxyethoxy)methyl]uracil (hydroxymethyl-BBAU or HM-BBAU), 5-benzyl-1-[(*C*-(2′-hydroxy-ethoxy)-C-methyl)-methyl]uracil (*C*-methyl-BAU), 5- (3′-benzyloxybenzyl)- 1- [(*C*-(2′-hydroxyethoxy)-*C*-methyl)-methyl]uracil (*C*-methyl-BBAU), 5-benzyl-1-[(1′-hydroxyethoxy)methyl]uracil-2′-phosphate (BAU-P), 5-benzyl-3-*N*-(4′-benzyloxybenzyl)-1-[(2′-hydroxyethoxy)methyl]uracil (*N*3-BB-BAU), 5- [3′- (4- benzyloxybenzyloxy)benzyl]- 1- [(2′- hydroxyethoxy)methyl]uracil (BBBAU), 5-(3′-hydroxybenzyl)-1-[(2′-hydroxyethoxy)methyl]uracil (*m*-hydroxy-BAU), 5-benzyl -1- [(2′-(3-carboxypropionyloxy)ethoxy)methyl]uracil (succinyl-BAU or succ-BAU), 5-(3′-benzyloxybenzyl)-1-[(2′-(3-carboxypropionyloxy)ethoxy)methyl]uracil (succinyl-BBAU or succ-BBAU), 5-(3′-benzyloxybenzyl)-1- [(2′-(1′, 3′-bis-3-carboxypropionyloxy)propoxy)-methyl]uracil (succinyl-hydroxymethyl-BBAU or succHM-BBAU), 5-(3′-methoxybenzyl)-1-[(2′-hydroxyethoxy)methyl]uracil (*m*-methoxy-BAU), 5- (4′-isopropoxybenzyl)- 1- [(2′-hydroxyethoxy)-methyl]uracil (*p*-*O*-isopropyl-BAU), 5-benzyl-1-[(2′-aminoethoxy)methyl]uracil (deoxyamino-BAU or DA-BAU, 5-(3′-benzyloxybenzyl)-1-[(2′-amino ethoxy)methyl]uracil (deoxyamino-BBAU or DA-BBAU), 5-benzyl-1-[(1′-aminomethyl-2′-hydroxy-ethoxy)methyl]uracil(aminomethyl-BAU or AM-BAU) and 5-(3′-benzyloxybenzyl)-1-[(1′-amino-methyl-2′-hydroxyethoxy)methyl]uracil (aminomethyl-BBAU or AM-BBAU) were synthesized as described elsewhere [15, 16, 22, *]. The chemical structures of these compounds are shown in Fig. 1. All other unlabeled pyrimidine compounds, EDTA, 2-mercaptoethanol and DTT were obtained from the Sigma Chemical Co., St. Louis, MO; [2-^{14}C]uridine (50 Ci/mol) and [2-^{14}C]thymidine (50 Ci/mol), from Moravek Biochemicals, Inc., Brea, CA; Silica gel G/UV$_{254}$ polygram TLC plates, from Brinkmann Instruments, Inc., Westbury, NJ; Omnifluor scintillant, from New England Nuclear, Boston, MA; and bovine γ-globulin and dye reagent for protein estimation, from Bio-Rad Laboratories, Richmond, CA.

Animal tissues. Human liver was obtained from autopsies within 8 hr of death; mouse livers were from Swiss Albino (CD1) mice (Charles River Laboratories, Boston, MA). Mice were killed by cervical dislocation, and the livers were removed. Both human and mouse livers were then washed with ice-cold normal saline (0.9%) before any further manipulation.

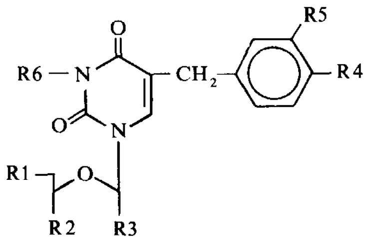
Preparation of extracts. The saline solution was wiped off the organs with tissue paper, and the organs were weighed and then cut into smaller pieces in ice-cold homogenization buffer (3:1, v/w) which contained 20 mM potassium phosphate (pH 8), 1 mM EDTA, and 1 mM mercaptoethanol. The livers were homogenized with a polytron homogenizer (Brinkmann), and the homogenates were centrifuged at 105,000 *g* for 1 hr at 4°. The supernatant fluids (cytosol) were collected and used as the enzyme source.

Enzyme assay. The assay mixture contained 20 mM potassium phosphate (pH 8), 1 mM EDTA, 1 mM mercaptoethanol, substrate (uridine or thymidine, 9 mCi/mmol) and 40 μl of enzyme preparation in a final volume of 80 μl. Under these conditions the activity was linear with time and amount of enzyme. Apparent K_i values were estimated from Dixon plots ($1/v$ vs [I]) of the data by a computer program with least squares fitting using thymidine (1 mM) or uridine (1 mM or 0.35 mM) and five different concentrations of the inhibitor. K_i values were estimated from the slopes and intercepts of the double-reciprocal plots by the method of Wilkinson [23] and Cleland [24], using 0.0625, 0.125, 0.250, 0.500 and 1.0 mM uridine for mouse liver and 0.25, 0.5, 1.0, 2.0 and 4.0 mM uridine for human liver enzymes. Incubation was carried out at 37°, for 30 min. The reaction was terminated by immersing the reaction tubes (1-ml Eppendorf tubes) in boiling water for 1 min; the reaction tubes were then frozen for at least 20 min. Proteins were removed by centrifugation, and 5 μl of the supernatant fluid was spotted on silica gel TLC plates, which had been prespotted with 5 μl of a standard mixture of 10 mM each uridine and uracil or thymidine and thymine. The plates were then developed in a mixture of chloroform and methanol (90:10, v/v). Uridine and uracil or thymidine and thymine were identified by u.v. quenching. R_f values for uridine, uracil, thymidine and thymine were 0.09, 0.39, 0.07 and 0.27 respectively. Spots were cut out and counted in 20 ml of Omnifluor-based scintillant.

Protein estimation. Protein concentrations were determined by the method of Bradford [25] as described by the Bio-Rad Laboratories [26], using bovine γ-globulin as a standard.

---

* S. H. Chu, Z. H. Chen, Z. Y. Weng and E. C. Rowe, manuscript in preparation.



Compound	R1	R2	R3	R4	R5	R6
BAU	—OH	—H	—H	—H	—H	—H
succ-BAU	—$OCOCH_2CH_2COOH$	—H	—H	—H	—H	—H
BAU-P	—OPO_3H_2	—H	—H	—H	—H	—H
HM-BAU	—OH	—CH_2OH	—H	—H	—H	—H
AM-BAU	—OH	—CH_2NH_2	—H	—H	—H	—H
DA-BAU	—NH_2	—CH_2NH_2	—H	—H	—H	—H
C-Methyl-BAU	—OH	—H	—CH_3	—H	—H	—H
m-Hydroxy-BAU	—OH	—H	—H	—H	—OH	—H
m-Methoxy-BAU	—OH	—H	—H	—H	—OCH_3	—H
p-*O*-Isopropyl-BAU	—OH	—H	—H	—$OCH(CH_3)_2$	—H	—H
*N*3-BB-BAU	—OH	—H	—H	—H	—H	-*p*-Benzyloxybenzyl
BBAU	—OH	—H	—H	—H	-Oxybenzyl	—H
succ-BBAU	—$OCOCH_2CH_2COOH$	—H	—H	—H	-Oxybenzyl	—H
HM-BBAU	—OH	—CH_2OH	—H	—H	-Oxybenzyl	—H
succ-HM-BBAU	—$OCOCH_2CH_2COOH$	—CH_2OH	—H	—H	-Oxybenzyl	—H
DA-BBAU	—NH_2	—CH_2NH_2	—H	—H	-Oxybenzyl	—H
AM-BBAU	—OH	—CH_2NH_2	—H	—H	-Oxybenzyl	—H
C-Methyl-BBAU	—OH	—H	—CH_3	—H	-Oxybenzyl	—H
BBBAU	—OH	—H	—H	—H	-Oxy(*p*-benzyloxy)benzyl	—H

Fig. 1. Chemical structures of the various benzylacyclouridines.

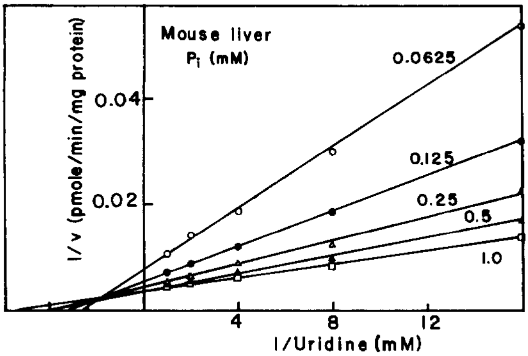


Fig. 2. Plots of l/velocity versus l/[uridine] at various fixed concentrations of phosphate for UrdPase from mouse liver. The assay conditions were as described in Materials and Methods except that the liver was homogenized in 100 mM Tris–HCl (pH 8), containing 2 mM EDTA and 2 mM DTT, and that the cytosolic fluid was dialyzed against 1 liter of the same buffer three times before its use as the enzyme source.

RESULTS

Reaction mechanism and kinetic parameters of UrdPase from human and mouse livers. Figure 2 shows the double-reciprocal plots of varied uridine concentrations at various fixed phosphate concentrations for UrdPase from mouse liver. Similar results were observed with the human liver enzyme, namely the intersecting pattern characteristic of a sequential reaction mechanism [27–29]. The K_m values (extrapolated to the infinite value of the second substrate) of 143 ± 9 μM uridine and 76 ± 7 μM phosphate for UrdPase from mouse liver and 242 ± 63 μM uridine and 279 ± 66 μM phosphate for UrdPase from human liver were estimated from the slopes and intercepts of the double-reciprocal plots by the method of Wilkinson [23] and Cleland [24]. The respective V_{max} values were 293 ± 5 and 3940 ± 175 pmol/min/mg.

Inhibitory potencies of BAU and BBAU and their congeners for UrdPase and dThdPase from mouse and human livers. Figure 1 shows the chemical structures and the abbreviated names of BAU, BBAU and various congeners studied in the present investigation. None of the tested compounds had any appreciable inhibitory effect on dThdPase, even at a concentration as high as 900 μM. In fact, most of the BAU- and BBAU-derivatives seemed to slightly (<5%) activate dThdPase (data not shown). On the other hand, most of the compounds tested were potent inhibitors of UrdPase. Table 1 shows the apparent K_i values of BAU and BBAU and their congeners for UrdPase from mouse and human livers at a saturating phosphate concentration of 20 mM. All compounds that inhibited mouse liver UrdPase were also inhibitory for the human liver enzyme. Although the apparent K_i values differed for the same compound, the relative inhibitory potencies of these compounds were roughly parallel in the two species. For the same modification, the BBAU-derivatives were better inhibitors than the corresponding BAU-derivatives. BAU-P, AM-BAU, *p*-*O*-isopropyl-BAU, *m*-methoxy-BAU and HM-BAU were more inhibitory than the parent com-

Table 1. Inhibitory potencies of BAU and BBAU and their congeners for uridine phosphorylase from the cytosol of mouse and human livers at a saturating concentration of phosphate (20 mM)

		Apparent K_i* (μM)	
Compound	[Uridine]	Mouse	Human
BAU	1.00	3.12 ± 0.21	3.97 ± 0.30
*N*3-BB-BAU	1.00	2000.00 ± 230.00	11,700.00 ± 2300.00
C-Methyl-BAU	1.00	112.00 ± 12.00	113.00 ± 11.00
DA-BAU	1.00	21.30 ± 0.92	29.70 ± 2.07
succ-BAU	1.00	9.98 ± 0.95	22.60 ± 3.11
	0.35	11.16 ± 0.50	ND†
m-Hydroxy-BAU	1.00	8.90 ± 0.77	27.30 ± 2.28
	0.35	8.76 ± 0.42	ND
BAU-P	1.00	2.79 ± 1.16	3.13 ± 0.26
AM-BAU	1.00	1.95 ± 0.24	8.31 ± 0.60
p-*O*-Isopropyl-BAU	1.00	1.87 ± 0.10	6.45 ± 0.29
m-Methoxy-BAU	1.00	1.86 ± 0.12	3.41 ± 0.30
HM-BAU	1.00	1.63 ± 0.07	2.53 ± 0.33
BBAU	1.00	1.25 ± 0.10	1.95 ± 0.23
	0.35	0.86 ± 0.03	ND
C-Methyl-BBAU	1.00	39.00 ± 2.00	65.00 ± 5.00
DA-BBAU	1.00	3.23 ± 0.14	6.27 ± 0.50
succ-BBAU	1.00	2.50 ± 2.04	4.45 ± 0.36
	0.35	1.03 ± 0.03	ND
succ-HM-BBAU	1.00	2.92 ± 0.05	3.88 ± 0.33
	0.35	1.15 ± 0.03	1.82 ± 0.34
HM-BBAU	1.00	0.32 ± 0.01	0.65 ± 0.07
AM-BBAU	1.00	0.25 ± 0.009	2.74 ± 0.20
BBBAU	1.00	519.00 ± 34.00	340.00 ± 27.80

* Values ± standard error of estimation from at least three determinations.
† Not determined.

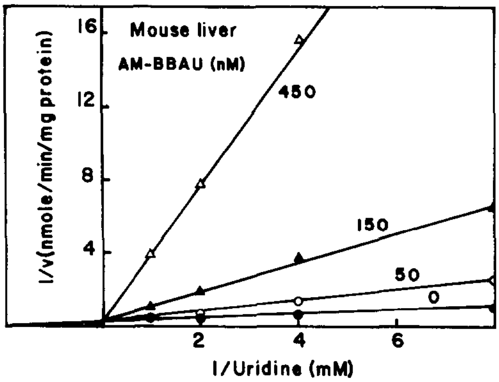


Fig. 3. Inhibition of mouse liver UrdPase by AM-BBAU. Plots of 1/velocity versus 1/[uridine] at various fixed concentrations of AM-BBAU are shown. Each point represents three determinations.

pound BAU. AM-BBAU and HM-BBAU were also more inhibitory than BBAU.

Inhibitors that exhibited an apparent K_i value equal to or lower than 2 μM were subjected to further kinetic studies, to determine the mechanism of inhibition and the K_i value. In all cases, competitive inhibition was observed. Figure 3 shows the double-reciprocal plots for AM-BBAU with UrdPase from mouse liver. The data were also analyzed by a computer program which uses the Wilkinson/Cleland procedure for the estimation of kinetic parameters [23, 24]. Table 2 summarizes the K_i values estimated for the more potent inhibitors of mouse and human liver enzymes. The most potent was the aminomethyl-derivative of BBAU, AM-BBAU, with a K_i value of 18.1 ± 1.4 nM with mouse liver enzyme. The K_i value for AM-BBAU was approximately 10-fold less than that of the parent compound BBAU with UrdPase from mouse liver (170 nM). Table 2 also includes the K_i values for BAU-P, DA-BBAU and succ-BBAU. Although these compounds had a higher apparent K_i value than 2 μM (see Table 1), they may be of practical value in chemotherapy because of their higher solubility in water relative to the parent compounds BAU and BBAU.

Table 2. Inhibition constants of BAU, BBAU and some of their more potent congeners for uridine phosphorylase from the cytosol of mouse and human livers

Compound	K_i* (μM)	
	Mouse	Human
BAU	0.42 ± 0.04	1.19 ± 0.20
BAU-P	2.13 ± 0.36	0.90 ± 0.13
HM-BAU	0.34 ± 0.08	0.33 ± 0.12
AM-BAU	0.09 ± 0.004	ND†
BBAU	0.17 ± 0.00	0.22 ± 0.02
succ-BBAU	0.38 ± 0.01	2.09 ± 0.47
DA-BBAU	0.36 ± 0.04	ND
HM-BBAU	0.07 ± 0.01	0.07 ± 0.01
AM-BBAU	0.02 ± 0.001	ND

Assay conditions are described under Materials and Methods.

* Values ± standard error of estimation from at least three determinations.

† Not determined.

DISCUSSION

The liver is believed to be a main site of pyrimidine metabolism, and UrdPase is a key regulatory enzyme in this metabolism and the maintenance of plasma uridine levels [20, 21]. For the first time, the kinetic parameters of hepatic UrdPase from human and mouse, a most commonly used laboratory animal, have been determined. The K_m values for the phosphorolytic activity of UrdPase from human (242 μM) and mouse (143 μM) livers obtained in the present study are comparable with those estimated for UrdPase purified from rat liver (240 μM) [30] and Novikoff rat hepatoma cells (231 μM) [31].

Benzylacyclouridines, the inhibitors of UrdPase, have been shown to potentiate the efficacy and selective toxicity of FdUrd [16, 17], to increase the levels of plasma uridine as well as the salvage of uridine by various tissues [18, 19], and to protect against FUra toxicity [19]. In addition to their potential usefulness in cancer chemotherapy, benzylacyclouridines can also be used to characterize the structure of the binding site of UrdPase. In the present study, we have evaluated the potency of benzylacyclouridines along with seventeen newly synthesized analogues to inhibit human and mouse hepatic UrdPase and dThdPase. None showed inhibition with dThdPase. In fact, these compounds slightly activated this enzyme. In contrast, with the exception of *N*3-BB-BAU, BBBAU and *C*-methyl-BAU, all remaining compounds were potent inhibitors of UrdPase. Several of the new compounds were more potent in their inhibition of UrdPase than the parent compounds, BAU or BBAU. AM-BBAU was the most potent of all the compounds tested with a K_i value of 18 nM, followed by HM-BBAU (K_i = 70 nM) and then AM-BAU (K_i = 92 nM). AM-BAU has also been found to be a potent inhibitor of UrdPase from sarcoma 180 [32, 33]. HM-BBAU, like BBAU, is poorly soluble in water. BAU-P, DA-BBAU and succ-BBAU, on the other hand, are readily soluble in water. Therefore, BAU-P, DA-BBAU and succ-BBAU were subjected to detailed kinetic analysis in spite of the fact that their apparent K_i values were larger than 2 μM.

UrdPase from both mouse and human liver enzymes was inhibited to similar extents by the same inhibitor. The K_i values of BAU, BBAU and AM-BAU for mouse and human enzymes (Table 2) were four to five times larger than those for UrdPase from sarcoma 180 [5, 15, 32, 33]. Whether this variability stems from differences between normal and neoplastic mouse tissues or between the liver and a hematopoietic tissue is an open question. The fact remains, however, that BAU and BBAU are potent inhibitors of UrdPase from human and mouse livers.

The inhibitors listed in Fig. 1 can be divided into three general categories: derivatives that arose from modifications of the uracil ring, derivatives that arose from modifications of the 5-benzyl moiety, and derivatives that arose from modifications of the acyclo tail.

An example of a modification of the uracil ring is *N*3-BB-BAU. Attaching a benzyloxybenzyl group to *N*3 decreased the binding to UrdPase some 700-fold relative to BAU. This observation is in agreement with the suggestion of Niedzwicki *et al.* [5], that an undissociated *N*3 hydrogen is required for binding the enzyme, as concluded from their observations on the weak binding of 3-methyluracil and 3-oxauracil [5].

m-Hydroxy-BAU, *m*-methoxy-BAU, *p*-*O*-isopropyl-BAU, BBAU and BBBAU are examples of modifications of the 5-benzyl moiety. The hydroxyl group of *m*-hydroxy-BAU decreased the hydrophobicity of the 5-benzyl group. As a result, the binding of *m*-hydroxy-BAU to UrdPase was weakened. Addition of a hydrophobic group (i.e. methyl or isopropyl) to the 5-benzyl moiety improved the binding of *m*-methoxy-BAU and *p*-*O*-isopropyl-BAU over BAU. This is in agreement with the suggestion that a hydrophobic pocket exists on UrdPase, adjacent to the binding site of the 5-position of the pyrimidine ring [5, 34]. However, addition of the large oxy(*p*-benzyloxy)benzyl group to the 5-benzyl moiety of BAU decreased dramatically the binding of BBBAU. This is most likely due to steric hindrance or to size limitation of the hydrophobic pocket on UrdPase.

Examples of modifications on the acyclo tail are represented by *C*-methyl-BAU,*C*-methyl-BBAU, succ-BAU, succ-BBAU, BAU-P, HM-BAU, HM-BBAU, succ-HM-BBAU, DA-BAU, DA-BBAU, AM-BAU, and AM-BBAU. Substituting a methyl group for a hydroxyl at the R3 position (Fig. 1) decreased the binding of *C*-methyl-BAU and *C*-methyl-BBAU (Table 1). In contrast, replacing the hydroxy group on R1 or R2 with amino, phosphate, hydroxymethyl or succinate as in AM-BAU (or AM-BBAU), DA-BAU (or DA-BBAU), BAU-P, HM-BAU (or HM-BBAU), succ-BAU (or succ-BBAU) and succ-HM-BBAU, respectively, did not affect significantly the binding of these compounds to UrdPase (Tables 1 and 2). It has been suggested [5, 16] that the terminal hydroxyl on the acyclo tail of acyclopyrimidines (R1 or R2 in Fig. 1) is essential for hydrogen bonding to UrdPase, presumably at the same site that binds the 3′-hydroxyl group of uridine (for review see Ref. 5). Substituents that lack a group capable of hydrogen bonding to the enzyme at this particular position (e.g. Cl, NO_2, but not NH_2) do not inhibit UrdPase [35]. All of the present modifications in the acyclo tail, —NH_2, —OPO_3H_2 and —$OCOCH_2CH_2COOH$, like OH, are capable of forming a hydrogen bond, hence the lack of any significant effect of such substitutions on binding UrdPase. It is interesting that AM-BAU, AM-BBAU, HM-BAU and HM-BBAU yielded the best binding capacities. The significant increase in binding of AM-BBAU (10-fold) and HM-BBAU (3-fold) over the parent compound, BBAU, may be due to the availability of a second terminal group capable of hydrogen bonding on the acyclo tail of AM-BBAU and HM-BBAU. It is to be noted that the K_i value of HM-BBAU may represent an over-estimation in view of the poor solubility of this compound relative to AM-BBAU.

Finally, it is possible that the active inhibitor in succ-BAU, succ-BBAU, and succ-HM-BBAU is the BAU, BBAU or HM-BBAU moiety, arising from the cleavage of the succinate moiety by cytosolic esterase(s). A similar argument can be made for BAU-P.

In conclusion, modifications in BAU and BBAU have yielded superior specific inhibitors of UrdPase from human and mouse livers. BAU, BBAU and their congeners are also specific inhibitors of UrdPase from the bacteria *Escherichia coli* [36, 37] and the parasite *Schistosoma mansoni* (F. N. M. Naguib and M. H. el Kouni, unpublished results). Although the apparent K_i values differed from species to species, all the compounds that inhibited mouse or human liver enzymes also inhibited the bacterial and trematode enzymes. These results suggest that the topology of the active site of UrdPase is somewhat conserved in these diverse species.

Acknowledgements—We thank Dr. S. A. Nasr for providing the human liver autopsy specimens and Dr. Z. H. Chen for his help in the preparation of the compounds.

REFERENCES

1. H. Pontis, G. Degerstedt and P. Reichard, *Biochim. biophys. Acta* **51**, 138 (1961).
2. G. D. Birnie, H. Kroeger and C. Heidelberger, *Biochemistry* **2**, 566 (1936).
3. T. A. Krenitsky, M. Barclay and J. A. Jacquez, *J. biol. Chem.* **239**, 805 (1964).
4. T. A. Krenitsky, J. W. Mellors and R. K. Barclay, *J. biol. Chem.* **240**, 1281 (1965).
5. J. G. Niedzwicki, M. H. el Kouni, S. H. Chu and S. Cha, *Biochem. Pharmac.* **32**, 399 (1983).
6. H. Ishitsuka, M. Miwa, K. Takemoto, K. Fukuoka, A. Itoga and H. B. Maruyama, *Gann* **71**, 112 (1980).
7. M. Miwa, J. Nakamura and H. Ishitsuka, *Gann* **72**, 965 (1981).
8. M. Zimmerman and J. Seidenberg, *J. biol. Chem.* **239**, 2619 (1964).
9. C. Nakayama, Y. Wataya, R. B. Meyer and D. V Santi, *J. med. Chem.* **23**, 962 (1980).
10. A. Kono, Y. Hara, S. Sugata, Y. Karube, Y. Matsushima and H. Ishitsuka, *Chem. pharm. Bull. Tokyo* **31**, 175 (1983).
11. M. H. Iltzsch, M. E. el Kouni and S. Cha, *Proc. Am. Ass. Cancer Res.* **26**, 14 (1985).
12. D. Meynial, M. C. Malet-Martino, A. Lopez and R. Martino, *J. Pharm. Pharmac.* **38**, 426 (1985).
13. C. Heidelberger, L. Greisbach, O. Cruz, R. J. Schnitzer and E. Grunberg, *Proc. Soc. exp. Biol. Med.* **97**, 470 (1958).
14. K. L. Mukherjee, J. Boohar, D. Wentland, F. J. Ansfield and C. Heidelberger, *Cancer Res.* **23**, 49 (1963).
15. J. G. Niedzwicki, S. S. Chu, M. E. el Kouni, E. C. Rowe and S. Cha, *Biochem. Pharmac.* **31**, 1857 (1982).
16. S. H. Chu, Z. H. Chen, E. C. Rowe, F. N. M. Naguib, M. H. el Kouni and Y. M. Chu, *Nucleosides Nucleotides* **3**, 303 (1984).
17. M. Y. Chu, F. N. M. Naguib, M. H. Iltzsch, M. H. el Kouni, S. H. Chu, S. Cha and P. Calabresi, *Cancer Res.* **44**, 1852 (1984).
18. A. Monks, O. Ayers and R. L. Cysyk, *Biochem. Pharmac.* **32**, 2003 (1983).
19. J. W. Darnowski and R. E. Handschumacher, *Cancer Res.* **45**, 5364 (1985).
20. T. Gasser, J. D. Moyer and R. E. Handschumacher, *Science* **213**, 777 (1981).
21. A. Monks and R. L. Cysik, *Am. J. Physiol.* **11**, R465 (1982).

22. S. H. Chu, Z. H. Chen, Z. Y. Weng, E. C. Rowe, E. Chu and M. Y. W. Chu, *J. heterocyclic Chem.* **23**, 1651 (1986).
23. G. N. Wilkinson, *Biochem. J.* **80**, 321 (1961).
24. W. W. Cleland, *Adv. Enzymol.* **29**, 1 (1967).
25. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
26. Bio-Rad Laboratories, *Bulletin 1069.* Bio-Rad Laboratories, Richmond, CA (1979).
27. W. W. Cleland, *Biochim. biophys. Acta* **67**, 104 (1963).
28. W. W. Cleland, *Biochem. biophys. Acta* **67**, 173 (1963).
29. W. W. Cleland, *Biochim. biophys. Acta* **67**, 188 (1963).
30. A. Kraut and E. W. Yamada, *J. biol. Chem.* **246**, 2021 (1971).
31. R. S. McIvor, R. M. Wohlhueter and P. G. Plagemann, *J. cell. Physiol.* **122**, 397 (1985).
32. S. A. Siegel and T-S. Lin, *Biochem. Pharmac.* **34**, 1121 (1985).
33. T-S. Lin and M-C. Liu, *J. med. Chem.* **28**, 971 (1985).
34. B. R. Baker and J. L. Kelly, *J. med. Chem.* **13**, 461 (1970).
35. J. G. Niedziwicki, M. H. el Kouni, S. H. Chu and S. Cha, *Biochem. Pharmac.* **30**, 2097 (1981).
36. D. Shugar, *Pure appl. Chem.* **57**, 423 (1985).
37. K. S. Park, M. H. el Kouni, T. A. Krenitsky, S. H. Chu and S. Cha, *Biochem. Pharmac.* **35**, 3853 (1986).