Biochemical Pharmacology, Vol. 46, No. 7, pp. 1273–1283, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

5-BENZYLBARBITURIC ACID DERIVATIVES, POTENT AND SPECIFIC INHIBITORS OF URIDINE PHOSPHORYLASE

FARDOS N. M. NAGUIB,*† DIANE L. LEVESQUE,‡ ENG-CHI WANG,§
RAYMOND P. PANZICA‡§ and MAHMOUD H. EL KOUNI*

*Department of Pharmacology and Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35294, U.S.A.; ‡Departments of Medicinal Chemistry and Chemistry, University of Rhode Island, Kingston, RI 02881, U.S.A.; and §School of Chemistry and Pharmacy, Kaohsiung Medical College, Kaohsiung City 80708, Taiwan, Republic of China

(*Received* 3 *March* 1993; *accepted* 5 *May* 1993)

Abstract—5-Benzylbarbituric acid derivatives were synthesized as a series of new, specific, and potent inhibitors of uridine phosphorylase. Among these, 5-(*m*-benzyloxy)benzyl-1-[(2-hydroxyethoxy)methyl] barbituric acid (5-benzyloxybenzylbarbituric acid acyclonucleoside, BBBA) was found to be the most potent with K_i values of 1.1 ± 0.2 and 2.6 ± 0.3 nM with uridine phosphorylase from human and mouse livers, respectively. BBBA exhibited competitive inhibition with uridine phosphorylase from both human and mouse livers. The 5-benzylbarbituric acid derivatives are specific inhibitors of uridine phosphorylase, as they had no effect on thymidine phosphorylase (EC 2.4.2.4), thymidine kinase (EC 2.7.1.21), uridine-cytidine kinase (EC 2.7.1.48), orotate phosphoribosyltransferase (EC 2.4.2.10), orotidine 5′-monophosphate decarboxylase (EC 4.1.2.23), and dihydrouracil dehydrogenase (EC 1.3.1.2). These compounds are more potent, easier to synthesize, and have better water solubility than their uracil counterparts as inhibitors of uridine phosphorylase. Furthermore, the 5-benzylbarbituric acids were found to be better inhibitors of human uridine phosphorylase than the murine enzyme, whereas the reverse holds true for the 5-benzyluracil derivatives. The 5-benzylbarbituric acid derivatives have potential usefulness in the therapy of cancer and AIDS, as well as other pathological and physiological disorders.

Two distinct pyrimidine nucleoside phosphorylases occur in mammalian cells [1, 2], uridine phosphorylase (UrdPase‖, EC 2.4.2.3) and thymidine phosphorylase (dThdPase, EC 2.4.2.4), which catalyze the general reaction:

Pyrimidine (deoxy)riboside + P_i ↔

Pyrimidine base + (deoxy)ribose-1-P

UrdPase from most mammalian sources primarily cleaves pyrimidine (except cytosine) ribosides but is relatively non-specific, since it also cleaves pyrimidine 2′- and 5′-deoxyribosides [1–14]. dThdPase, on the other hand, seems to be specific for pyrimidine 2′- and 5′-deoxyribosides [3, 4, 9–12, 15–20].

UrdPase plays a crucial role in the chemotherapy of cancer and AIDS. The importance of UrdPase in cancer chemotherapy stems from the fact that UrdPase is the enzyme responsible for the activation and deactivation of several chemotherapeutic analogues, most notably the 5-fluoropyrimidines: 5-fluorouracil (FUra), 5-fluorouridine (FUrd), 5-fluoro-2′-deoxyuridine (FUrd), and 5-fluoro-5′-deoxyuridine (5′-dFUrd) [3, 7, 9–12, 14, 16, 21–23], as many human tumours are apparently devoid of dThdPase activity [1, 3, 11, 15, 24–31]. Moreover, UrdPase, but not dThdPase, exhibits a circadian rhythm in mice [32, 33], which is opposite to that observed for the anticancer efficacy of FdUrd [34]. In addition, host-toxicities of some of these anticancer (e.g. FUra) [35–38], as well as anti-HIV (e.g. 3′-azido-3′-deoxythymidine, AZT) [39, 40] drugs are antagonized by uridine, the availability and concentration of which are controlled by UrdPase [32, 41–45]. The detrimental role played by UrdPase in reducing the effectiveness of various pyrimidine analogues in the chemotherapy of cancer and AIDS has generated a strong interest in developing inhibitors for this enzyme. Such inhibitors would enhance the chemotherapeutic efficacy of these drugs by preventing their degradation and/or host-toxicity.

† Corresponding author: Dr. Fardos N. M. Naguib, Department of Pharmacology, The University of Alabama at Birmingham, Birmingham, AL 35294. Tel. (205) 934-1131; FAX (205) 934-8240.

‖ Abbreviations: AZT, 3′-azido-3′-deoxythymidine; BAU, benzylacyclouridine or 5-benzyl-1-[(2-hydroxyethoxy)methyl]uracil; BB, 5-benzylbarbituric acid; BBAU, 5-benzyloxybenzylacyclouridine or 5-(*m*-benzyloxy)benzyl-1-[(2-hydroxyethoxy)methyl]uracil; BBB, 5-benzyloxybenzylbarbituric acid or 5-(*m*-benzyloxy)benzylbarbituric acid; BBBA, 5-benzyloxybenzylbarbituric acid acyclonucleoside; or 1-[(2-hydroxyethoxy)methyl]-5-(*m*-benzyloxy)benzylbarbituric acid; BBBA acetate, 5-benzyloxybenzylbarbituric acid acyclonucleoside acetate or 1 - [(2 - acetoxyethoxy)methyl] - 5 - (*m* - benzyloxy)-benzylbarbituric acid; AM-BBAU, aminomethyl-BBAU or 5-(*m*-benzyloxy)benzyl-1-[(1-aminomethyl-2-hydroxyethoxy)methyl]uracil; BBU, 5-benzyloxybenzyluracil; BU, 5-benzyluracil; DTT, dithiothreitol; 5′-dFUrd, 5-fluoro-5′-deoxyuridine; dThdPase, thymidine phosphorylase; FdUrd, 5-fluoro-2′-deoxyuridine; FUra, 5-fluorouracil, FUrd, 5-fluorouridine; P_i, orthophosphate; and UrdPase, uridine phosphorylase.

5-Benzylacyclouridines were developed as specific inhibitors of UrdPase [4, 10, 13, 14, 46–50]. They were shown to potentiate the efficacy of FdUrd *in vitro* and *in vivo* [14, 46, 47], to increase the level and duration of uridine in plasma [41–45] and heart-perfusate [51], to enhance the salvage of uridine by various tissues [42–45], and to reduce host-toxicity of FUra [42, 44, 45], FdUrd [52], and AZT [53, 54]. Yet, these inhibitors have less than favorable chemical and pharmacological properties. For example, the potency and/or poor water solubility of the benzylacyclouridines may limit their clinical usefulness. Furthermore, the more potent derivatives, i.e. AM-BBAU (aminomethyl analogue of 5-benzyloxybenzylacyclouridine), were toxic (unpublished observation). In this paper we report the synthesis and testing of certain 5-benzylbarbituric acid derivatives as specific and potent inhibitors of UrdPase. Compared with their respective 5-benzyluracil counterparts, they are far more potent, have better water solubility and are easier to synthesize. A preliminary report has been presented [55].

MATERIALS AND METHODS

Chemicals

[2-^{14}C]Uridine (56 Ci/mol), [2-^{14}C]thymidine (56 Ci/mol), and [6-^{14}C]uracil (56 Ci/mol) were obtained from Moravek Biochemicals, Inc., Brea, CA; [6-^{14}C]orotate (46.9 Ci/mol) was from NEN Research Products, the DuPont Co., Boston, MA; silica gel G/UV$_{254}$ polygram, polyethyleneimine cellulose 300 PEI/UV$_{254}$ and cellulose CEL 300 UV polygram thin-layer chromatography plates were from Brinkmann Instruments, Westbury, NY. The protein assay kit was obtained from Bio-Rad Laboratories, Richmond, CA. 5-Benzyluracil (BU), 5-benzyloxybenzyluracil (BBU), 5-benzylacyclouridine (BAU), and 5-benzyloxybenzylacyclouridine (BBAU) were synthesized and provided by Dr. S. H. Chu, Brown University, Providence, RI. All other chemicals were obtained from the Sigma Chemical Co., St. Louis, MO.

Chemical synthesis

Heterocycles. The 5-benzylbarbituric acid (BB) analogues were prepared from barbituric acid and the appropriate aldehyde as outlined in Scheme 1. The 5-arylidene intermediates and the target 5-arylalkylbarbiturates were obtained in good yields (*ca.* 90%). Their synthesis followed known methodology [56, 57]. Reduction of the 5-arylidenes was carried out in MeOH with $NaBH_4$ [58]. The known *m*-bromo- [59], *m*-chloro- [59], and *m*-nitro- [57] 5-benzylbarbituric acids (**XVII–XIX**) were prepared for this study as described above.

Acyclonucleosides: 1-[(2-Hydroxyethoxy)methyl]-5-(m-benzyloxy)benzylbarbituric acid or 5-benzyloxybenzylbarbituric acid acyclonucleoside (BBBA, **XXVI***).* BBBA was prepared by two different pathways as depicted in Scheme 2. The first pathway involved alkylation of persilylated barbituric acid (**XX**) with (2-acetoxyethoxy)methyl bromide [60] (**XXI**) in dry acetonitrile (CH_3CN) to provide 1-[(2-acetoxyethoxy)methyl]barbituric acid (**XXII**) in 54% yield after purification by silica gel column chromatography. Deprotection of **XXII** with NaOMe furnished **XXIII** in near-quantitative yield. Either **XXII** or **XIII** could be used in the next step leading to the substituted arylidene, but we selected **XXII** for ease of work-up due to better organic solubility. Reacting **XXII** with **IV** afforded the arylidene **XXIV** (71%) which was then reduced to BBBA acetate (**XXV**, 82%) followed by deprotection to provide BBBA (**XXVI**, 80%). Alternatively, BBBA (**XXVI**) could be prepared from the persilylated derivative (**XXVII**) of **XVI**. Treatment of **XXVII** with **XXI** in anhydrous 1,2-dichloroethane in the presence of a catalytic amount of $AlCl_3$ furnished BBBA acetate (50%) which after deprotection provided BBBA. Satisfactory elemental analyses and physiochemical data were obtained for all compounds. Complete experimental details will be published elsewhere.

Biological testing

Buffers

The following buffers were used: Buffer A [100 mM Tris–Cl, pH 7.5; 2 mM dithiothreitol (DTT); 5 mM $MgCl_2$] and Buffer B [20 mM potassium phosphate, pH 8.0; 1 mM DTT, 1 mM EDTA]. Buffer A was used when the activities of the nucleoside kinases were to be determined. Buffer B was employed when the activities of the other enzymes under study were to be determined.

Animal tissues

Human liver specimens were from donors and obtained through the Alabama Regional Organ Bank. The gall bladder was removed and the liver was sliced, perfused with cold normal saline and stored at −70° until used. Mouse livers were obtained from female Swiss Albino (CD1) mice weighing 20–24 g (Charles River Laboratories, Wellington, MA). Mice were killed by cervical dislocation and the livers removed.

Preparation of extracts

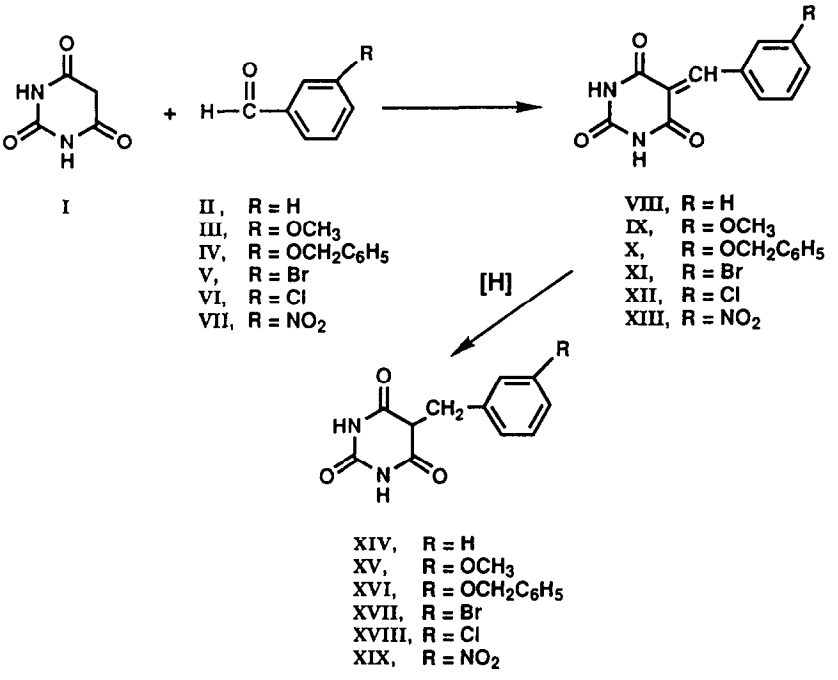
Organs were weighed, minced and homogenized in ice-cold (3:1, v/w) appropriate buffer using a Polytron homogenizer (Brinkman Instruments). The homogenates were centrifuged at 105,000 *g* for 1 hr at 4°. The supernatant fluids (cytosol) were collected and used as the enzyme source.

Enzyme assays

All assays were run at 37° under conditions where enzyme activity was linear with respect to time and enzyme concentration. For each inhibitor five concentrations were used ranging from 50 to 900 μM unless otherwise specified. Reactions were started by the addition of extract and stopped by boiling in a water bath for 2 min followed by freezing. Precipitated proteins were removed by centrifugation. Substrates were separated from products in the supernatant by TLC and the radioactivity in the spots was determined on a percentage basis using a Berthold LB-2821 Automatic TLC-Linear Analyzer.

Pyrimidine nucleoside phosphorylases. Nucleoside cleavage was measured isotopically by following the

Scheme 1



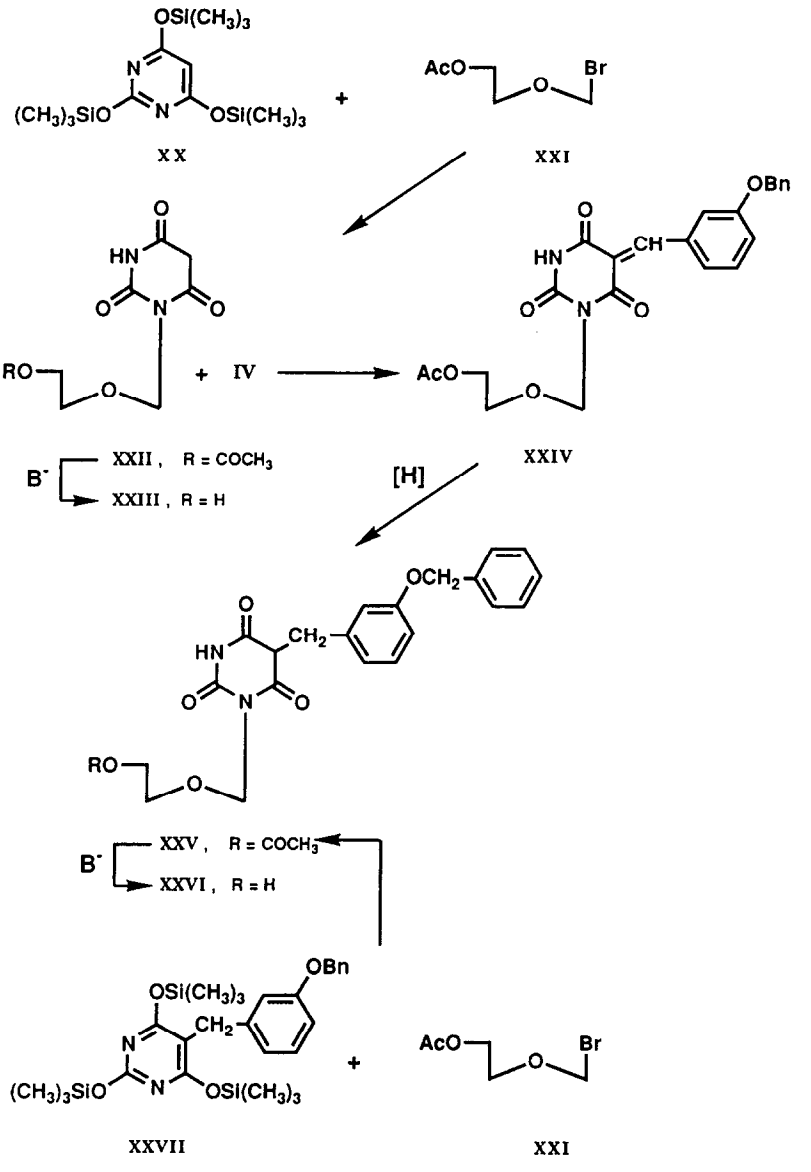
formation of nucleobases from their respective nucleosides as previously described [49]. The reaction mixture contained 20 mM potassium phosphate (pH 8), 1 mM EDTA, 1 mM DTT, 1 mM [2-^{14}C]-uridine or [2-^{14}C]thymidine (6 Ci/mol) and 25 μL cytosol in a final volume of 50 μL. The incubation was terminated after 30 min. Uridine and thymidine were separated from their respective nucleobases on silica gel TLC plates developed with chloroform : methanol : acetic acid (90 : 5 : 5, by vol.). The R_f values were: uridine, 0.07; uracil, 0.43; thymidine, 0.14; and thymine, 0.62.

Dihydrouracil dehydrogenase (EC 1.3.1.2*).* Dihydrouracil dehydrogenase activity was measured by following the formation of dihydrouracil, carbamyl-β-alanine and β-alanine from [6-^{14}C]uracil, as previously described [61]. The reaction mixture contained 20 mM potassium phosphate (pH 8), 1.0 mM EDTA, 2 mM DTT, 5 mM $MgCl_2$, 25 μM [6-^{14}C]uracil (56 Ci/mol), 100 μM NADPH and 25 μL cytosol in a final volume of 50 μL. The incubation was terminated after 15 min. Uracil, dihydrouracil, carbamyl-β-alanine and β-alanine were separated on cellulose TLC plates developed in the top phase of a mixture of *n*-butanol : water : ammonia (90 : 45 : 15, by vol.). The R_f values were: dihydrouracil, 0.46; uracil, 0.23; and carbamyl-β-alanine, 0.09, respectively. Dihydrouracil dehydrogenase activity was determined as the sum of the products dihydrouracil, carbamyl-β-alanine and β-alanine.

Orotate phosphoribosyltransferase (EC 2.4.2.10*) and orotidine 5'-monophosphate decarboxylase (EC* 4.1.2.23*).* Enzyme activity was measured by following OMP, orotidine, UMP, uridine and uracil formation from [6-^{14}C]orotate [62]. The standard assay mixture contained 20 mM potassium phosphate buffer (pH 8.0), 2.5 mM 5-phosphoribosyl-α-1-pyrophosphate, 1 mM EDTA, 5 mM $MgCl_2$, 2 mM DTT, 0.05 mM (56.2 Ci/mol) [6-^{14}C]orotate, and 25 μL cytosol extract, in a final volume of 50 μL. The incubation was terminated after 30 min. A 5-μL aliquot of the supernatant fluid was spotted on prewashed PEI-cellulose plates. The plates were developed first in distilled water to a front of 10 cm. They were then dried and redeveloped in 0.2 M LiCl. The R_f values were: OMP, 0.16; UMP, 0.51; orotate, 0.62; orotidine, 0.77; and uridine and uracil, 0.95. Orotate phosphoribosyltransferase activity was measured as the sum of OMP, orotidine, UMP, and uridine and uracil. Orotidine 5'-monophosphate decarboxylase activity was measured as the sum of UMP, uridine and uracil.

Pyrimidine nucleoside kinases. Thymidine kinase (EC 2.7.1.21) and uridine-cytidine kinase (EC 2.7.1.48) activities were determined isotopically as the sum of nucleotides formed from [2-^{14}C]-thymidine and [2-^{14}C]uridine, respectively [62]. The assay mixture contained 50 mM Tris–Cl (pH 7.4), 125 μM radiolabeled nucleoside (0.4 Ci/mol), 2.5 mM ATP, 5 mM $MgCl_2$, 25 mM NaF, and 30 μL cytosol extract in a final volume of 60 μL. After termination of the reaction and centrifugation, a 10-μL aliquot of the supernatant was spotted on silica gel TLC plates. The plates were developed with

Scheme 2



chloroform : methanol : acetic acid (56 : 24 : 4, by vol.). The R_f values were: thymidine, 0.77; thymidine nucleotides 0.15; uridine, 0.78; and uridine nucleotides, 0.15.

Kinetics studies

Determination and significance of apparent K_i *values.* Using uridine (1 mM) and five different concentrations of the inhibitor ranging from 50 to 900 μM, apparent K_i values were estimated from Dixon plots ($1/v$ vs [I]) [63] of the data by a computer program with least squares fitting. The program was written by Dr. Sungman Cha and modified to fit IBM BASIC by Dr. F. N. M. Naguib. Apparent K_i values are related to K_i values by the following equation:

$$\text{apparent } K_i = \frac{K_{is}(1 + [S]/K_m)}{1 + ([S]/K_m)\,(K_{is}/K_{ii})}$$

where K_{is} and K_{ii} are inhibition constants that would have been estimated from replots of slope and intercept, respectively, of a Lineweaver–Burk plot vs [I]. If a compound is a competitive inhibitor with respect to uridine, $K_{ii} = \infty$ and $K_{is} = K_i$. Therefore,

$$\text{apparent } K_i = K_i\,(1 + [S]/K_m).$$

Thus, for UrdPases from mouse and human livers

Table 1. Apparent K_i values for various 5-benzylbarbituric acid analogues in comparison with those of 5-benzyluracils and 5-benzylcyclouridines for uridine phosphorylase from mouse and human livers

Inhibitor	Apparent K_i* (μM)	
	Mouse	Human
Uracil	2,060 ± 186	863 ± 42
Acyclouridine	113 ± 9.8	68.7 ± 6.9
BU	288 ± 47	275 ± 68
BBU	66.4 ± 11.3	96.4 ± 4.6
BAU	3.12 ± 0.21	3.97 ± 0.30
BBAU	1.25 ± 0.10	1.95 ± 0.23
Barbituric acid (**I**)	19,810 ± 10,680	
Barbituric acid acyclonucleoside (**XXIII**)	880 ± 350	
BB, 5-Benzylbarbituric acid (**XIV**)	158 ± 6.6	
5-(*m*-Nitrobenzyl)barbituric acid (**XIX**)	51.5 ± 5.7	
5-(*m*-Methoxybenzyl)barbituric acid (**XV**)	26.5 ± 0.9	
5-(*m*-Bromobenzyl)barbituric acid (**XVII**)	19.2 ± 1.8	
5-(*m*-Chlorobenzyl)barbituric acid (**XVIII**)	12.4 ± 1.2	2.14 ± 0.26
BBB, 5-(Benzyloxybenzyl)barbituric acid (**XVI**)	11.7 ± 0.5	2.77 ± 0.53
BBBA, BBB acyclonucleoside (**XXVI**)	0.022 ± 0.00	0.010 ± 0.008
BBBA acetate (**XXV**)	7.0 ± 0.3	
5-(*m*-Bromobenzylidene)barbituric acid (**XI**)	2,400 ± 690	
5-(*m*-Chlorobenzylidene)barbituric acid (**XII**)	883 ± 111	
5-(*m*-Nitrobenzylidene)barbituric acid (**XIII**)	321 ± 45	

* Values are means ± standard error of estimation from at least three determinations measured at 20 mM inorganic phosphate, 1.0 mM uridine and different concentrations of inhibitors.

which have K_m values of 143 and 242 μM uridine, respectively [49], the apparent K_i of a competitive inhibitor, measured at a uridine concentration of 1 mM, is approximately 8- and 5-fold higher than their respective K_i values. It should be noted, however, that we have not characterized all of the compounds used in this study with regard to the type of inhibition (competitive, non-competitive or uncompetitive) or whether they are substrates for the enzyme.

Determination of K_i *values.* K_i values were estimated from the slopes and intercepts of the double-reciprocal plots by the methods of Wilkinson [64] and Cleland [65], using uridine concentrations ranging from 30 to 700 μM.

Protein determination

Protein concentrations were determined spectrophotometrically by the method of Bradford [66] using bovine γ-globulin as a standard.

RESULTS

Inhibition constants and mechanisms of the 5-benzylbarbituric acid derivatives as inhibitors of UrdPase

Table 1 shows the apparent K_i values of various 5-benzylbarbituric acid derivatives, compared with those of the 5-benzyluracils (BU and BBU) and the 5-benzylacyclouridines (BAU and BBAU). Addition of a benzyl group to the 5-position of barbituric acid (5-benzylbarbituric acid, BB, **XIV**) increased the binding of barbituric acid to mouse liver UrdPase by 125-fold. Addition of a nitro, methoxy, bromo, chloro, or benzyloxy group at the *meta* position of the benzyl group of BB further improved the binding of barbituric acid to UrdPase, by 385-, 745-, 1000-, 1600- and 1700-fold, respectively, with 5-benzyloxybenzylbarbituric acid (BBB, **XVI**) being the most potent inhibitor.

The binding of 5-benzyl- (BB, **XIV**) and 5-benzyloxybenzyl- (BBB, **XVI**) barbituric acids to mouse liver UrdPase was better than that obtained by their uracil counterparts (Table 1). BB had an apparent K_i value (158 μM) 1.8-fold better than that of BU (288 μM). Similarly, the apparent K_i value of BBB (11.7 μM) was 6-fold superior to that of BBU (66.4 μM). These results encouraged us to examine the effect of attaching the (2-hydroxymethoxy)methyl chain, i.e. the "acyclo" tail, to BBB (**XVI**) applying the same rationale that was used to enhance the binding of uracil, BU and BBU to UrdPase [3, 4, 10].

Addition of the (2-hydroxyethoxy)methyl "acyclo" tail enhanced the binding of barbituric acid (**I**) and BBB (**XVI**) to UrdPase from mouse liver by over 22- and 500-fold, respectively. The acyclo derivative of BBB, 5-benzyloxybenzylbarbituric acid acyclonucleoside (BBBA, **XXVI**), had an apparent K_i value of 0.022 μM (Table 1). This apparent K_i was 57-fold better than that of BBAU (1.25 μM) and 140-fold better than that of BAU (3.12 μM) with UrdPase from mouse liver.

BBB and BBBA were also superior inhibitors of human UrdPase to their uracil counterparts, BBU and BBAU (Table 1). BBB inhibited human UrdPase 35-fold better than BBU. The acyclonucleoside, BBBA (apparent K_i value = 10 nM), was a 195- and 397-fold better inhibitor than BBAU (apparent K_i =

Table 2. Inhibitory potencies of 5-benzylbenzylbarbituric acid acyclonucleoside (BBBA) and 5-benzylacyclouridines (BAU and BBAU) for uridine phosphorylase from mouse and human livers

Inhibitor	K_i* (nM)	
	Mouse	Human
BAU	420 ± 40	1200 ± 200
BBAU	170 ± 0	220 ± 20
BBBA (XXVI)	2.6 ± 0.3	1.1 ± 0.2

* Values are means ± standard error of estimation measured at 20 mM inorganic phosphate, 30–700 μM uridine and inhibitor concentrations ranging from 50 to 900 nM. The number of determinations for these values is found in Fig. 1.

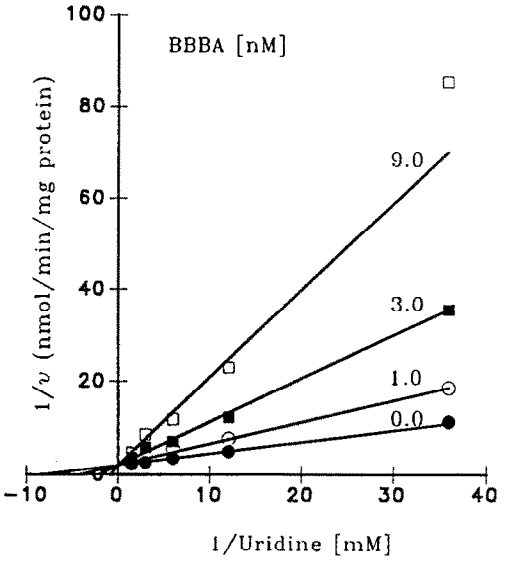


Fig. 1. Inhibition of human liver UrdPase by BBBA. Plots of 1/velocity versus 1/[uridine] at various fixed concentrations of BBBA. Each point represents at least three determinations.

1950 nM) and BAU (apparent K_i = 3970 nM), respectively. Furthermore, the inhibitory effects of BBB and BBBA were greater on human liver UrdPase than on mouse liver UrdPase. The apparent K_i of BBB (2.77 μM) with the human UrdPase was 4-fold better than that obtained with mouse liver UrdPase (11.7 μM). Similarly, BBBA (**XXVI**) had a 2-fold better apparent K_i (10 nM) with human UrdPase than with murine UrdPase (22 nM). This trend is the opposite of that found for the 5-benzyluracil derivatives, BBU, BAU and BBAU, which are better inhibitors of murine UrdPase than the human enzyme (Tables 1 and 2, and [13, 49]).

BBBA, the most potent inhibitor identified, was subjected to further kinetic studies to determine the mechanism of inhibition and the K_i value, and was also compared with BAU and BBAU. All three inhibitors, BBBA, BBAU and BAU, showed competitive inhibition with UrdPase from mouse and human livers. Figure 1 shows the Lineweaver–Burk plot of BBBA with human liver UrdPase.

BBBA had K_i values of 2.6 and 1.1 nM for UrdPase from mouse and human livers, respectively (Table 2). These K_i values were 65- and 200-fold better than those obtained with BBAU and over 160- and 1000-fold better than those obtained with BAU using mouse and human UrdPases, respectively. These results confirm that BBBA is a superior inhibitor to BAU and BBAU. They also demonstrate that, in contrast to BAU and BBAU, BBBA is a better inhibitor of the human UrdPase than the murine enzyme.

Inhibitor specificity

To check the specificity of the 5-benzylbarbituric acid analogues, these compounds were tested against various enzymes that could utilize the substrate (uridine or thymidine) or products (uracil or thymine) of UrdPase. Under our experimental conditions, the 5-benzylbarbituric acid analogues were neither inhibitors nor substrates for thymidine phosphorylase, uridine-cytidine kinase, thymidine kinase, dihydrouracil dehydrogenase, orotate phosphoribosyltransferase or orotidine 5′-monophosphate decarboxylase, as there was no significant effect on their activity in the presence of these compounds. These experiments indicate that the 5-benzylbarbituric acids are specific inhibitors of UrdPase.

Water solubility of 5-benzylbarbituric acid analogues

Table 3 shows the solubility of various 5-benzylbarbituric acid analogues when compared with those of BAU and BBAU. These compounds were at least 3-fold more soluble in water than either BAU or BBAU, with BBBA being 10-fold more soluble.

Lack of toxicity of BBBA

The toxicity of the most potent inhibitor among the various 5-benzylbarbituric acid analogues, BBBA, was tested in mice (CD1, Charles River Laboratories, Wellington, MA). Mice were injected i.p. with BBBA (50 mg/kg/day for 5 consecutive days) and monitored for 2 weeks. The mice did not show any mortality, and the mean body weight did not differ significantly from that of the controls (data not shown). Similar results were observed when BBBA (50 mg/kg/day) was injected i.v. into the tail vein of the mice.

DISCUSSION

In the present study we prepared from barbituric acid a series of new, highly specific and potent inhibitors of UrdPase. Among these, 5-benzyloxybenzylbarbituric acid acyclonucleoside (BBBA, **XXVI**) has been identified as the most potent inhibitor of UrdPase with K_i values of 1.1 ± 0.2 and 2.6 ± 0.3 nM with UrdPase from human and mouse livers, respectively.

Although, barbituric acid (**I**) is a very poor

Table 3. Water solubility of 5-benzylbarbituric acid analogues and 5-benzylacyclouridines

Inhibitor	Solubility* (mM)
BAU	1
BBAU	<1
Barbituric acid (**I**)	>3
BB (5-Benzylbarbituric acid, **XIV**)	>3
5-(*m*-Methoxy)BB (**XV**)	>3
BBB (5-Benzyloxybenzylbarbituric acid, **XVI**)	>3
BBBA (BBB acyclonucleoside, **XXVI**)	>10

* In water at room temperature.

inhibitor of UrdPase (Table 1 and [10]), addition of a benzyl or a *meta*-substituted benzyl group to the *C5* of the pyrimidine ring yielded extremely powerful inhibitors of UrdPase. Additional attachment of a (2-hydroxyethoxy)methyl "acyclo" tail to *N1* further increased the potency of these inhibitors (Table 1). The data presented here together with results from previous studies [3, 4, 10, 13, 49, 50] allow us to postulate a mechanism(s) for the stronger binding of the 5-benzylbarbituric acid derivatives to the active site of UrdPase relative to their unsubstituted counterparts, barbituric acid (**I**) and its acyclonucleoside (**XXIII**). The fact that the most potent inhibitor among the *N1*-unsubstituted 5-benzylbarbituric acid derivatives was BBB (**XVI**) is consistent with the notion that increased hydrophobicity at the 5-position of the pyrimidine ring enhances binding of pyrimidines to UrdPase by interacting with a hydrophobic pocket on the enzyme adjacent to the 5-position of the pyrimidine ring [3, 4, 10, 13, 49, 50]. The poor binding of the 5-benzylidene derivatives of barbituric acids (**XI, XII, XIII**) as compared with their 5-benzyl counterparts (**XVII, XVIII, XIX**) (Table 1) could be explained by the relative rigidity of the 5-benzylidene group. The presence of the double bond between the *C5* of barbituric acid and the benzyl methene would restrict the movement of the phenyl ring around the double bond and hinder its proper orientation to fit adequately into the hydrophobic pocket of the enzyme. The lack of proper orientation of substituents at *C5* of the pyrimidine ring is probably also the main reason underlying the reported poor binding of the "classical" 5-disubstituted barbituric acid analogues (e.g. phenobarbital and pentobarbital) [10, 67]. The presence of two hydrophobic groups (e.g. phenyl and ethyl) at *C5* would cause a steric hindrance at the hydrophobic pocket of the enzyme and reduce binding.

In addition to hydrophobicity, ionization of 5-benzylbarbituric acid derivatives may also play a role in enhancing the binding of these compounds to UrdPase. Niedzwicki *et al.* [10] have shown that addition of an electron-withdrawing group (e.g. nitro, bromo, chloro) at the 5-position of uracil enhances the binding of these analogues to UrdPase. Since such electron withdrawing groups enhance the acidity of these analogues, it was postulated that the dissociated anionic forms bind more strongly to UrdPase than the uncharged species [10]. Under our assay conditions (pH 8), barbituric acid and its 5-substituted derivatives are most likely present as monoanions [10]. Addition of a benzyl group at the 5-position increases the acidity of 5-benzylbarbituric acid ($pK_a = 3.03$) over barbituric acid ($pK_a = 4.01$) by approximately 10-fold [59]. This acidity is further increased by addition of a nitro, bromo or chloro group at the *meta*-position of the benzyl group of 5-benzylbarbituric acid [59].

However, the decrease in pK_a values and increased hydrophobicity alone cannot explain the better binding of 5-benzylbarbituric acid (**XIV**) and its derivatives (**XVI, XXVI**) to UrdPase when compared with their uracil counterparts, particularly in view of the fact that the 5-unsubstituted barbituric acids (**I, XXIII**) bind weaker than their uracil analogues (Table 1). We postulate that the addition of a benzyl group at *C5* of barbituric acid, may favor a tautomeric form of 5-benzylbarbituric acid derivatives with better binding capacity than their uracil analogues. In contrast to uracils, and, for that matter, 5-benzylidenebarbituric acid derivatives, as well as the "classical" 5-disubstituted barbiturates, where the monoanions are formed by the loss of a proton from *N1* or *N3* [10], evidence presently available suggests that the anions of 5-benzyl derivatives of barbituric acid (e.g. BBB, **XVI**), and presumably their acyclo analogues (e.g. BBBA, **XXVI**), are formed by the loss of a proton from the *C5* position of the pyrimidine ring with subsequent enolization of either the 4- or 6-position oxo-group [10, 68]. The charged oxygen probably enhances the binding of a preferred tautomer of the 5-benzyl derivatives of barbituric acid to UrdPase. Furthermore, the resulting negative charge localized on the exocyclic oxygen at *C4* or *C6* of the ionized 5-benzylbarbituric acid derivatives or their acyclonucleosides may also be involved in orienting and/or stabilizing the *C5* benzyl group, and certain *meta*-substituents attached to the benzyl moiety, in a conformation better suited to fit into the adjacent hydrophobic pocket on the enzyme. Clearly, a better understanding of the binding of the 5-benzyl derivatives of barbituric acid to UrdPase must wait for more conclusive studies on the tautomeric equilibrium, ionization, and structural conformation of these compounds.

The enhanced binding of BBBA over BBB is probably due to the involvement of the hydroxyl group of the (2-hydroxyethoxy)methyl "acyclo" tail in hydrogen bonding to the second binding site on UrdPase. Similar multisubstrate binding was postulated for several acyclouridines [3, 4, 10, 49]. Therefore, the potency of BBBA can be increased further by additional chemical modification of the "acyclo" tail. This would include the addition of a terminal hydroxymethyl of an aminomethyl group to the "acyclo" tail, i.e. replacement of the (2-hydroxyethoxy)methyl "acyclo" tail with a (1,3-dihydroxy-2-propoxy)methyl or a (1-aminomethyl-2-hydroxyethoxy)methyl side chain. Such modifications increase the potency of BAU and BBAU by approximately 2.5- and 8.5-fold, respectively [49]. We are currently in the process of synthesizing and testing such derivatives.

BBBA has an excellent potential in various aspects of chemotherapy. The usefulness of UrdPase inhibitors has already been established in the field of experimental chemotherapy of cancer and AIDS. We have previously developed the 5-benzylacyclouridines as specific inhibitors of UrdPase [4, 10, 14, 46–50]. These inhibitors were shown to enhance the efficacy of FdUrd against human tumors *in vitro* and *in vivo* [14, 46, 47]. Furthermore, the benzylacyclouridines were shown to elevate the concentration and prolong the half-life of uridine in the plasma [41–45], as well as increase the salvage of uridine by various tissues [42, 44, 45]. Therefore, these inhibitors were used to protect against or rescue from host-toxicity of anticancer (i.e. FUra) [42, 44, 45] and anti-HIV (i.e. AZT) [52, 53] drugs, the toxicities of which were shown to be antagonized by administration of exogenous uridine [35–40]. The new acyclonucleoside BBBA is likely to share the various chemotherapeutic characteristics of the older inhibitors. BBBA, however, has certain advantages over the 5-benzylacyclouridines: (1) more potent; (2) cheaper and easier to make in large quantities; and (3) much more soluble in water. The last property is important in practical chemotherapy since the benzylacyclouridines are insoluble in water at the concentrations and volumes required to be administered to patients or experimental animals. Furthermore, the benzylbarbituric acid derivatives are, in general, better inhibitors of human UrdPase than the murine enzyme, whereas the reverse is true for the 5-benzyluracil derivatives. This is the first time an inhibitor of UrdPase is shown to be a better inhibitor of the human enzyme than its murine counterpart (Tables 1 and 2 [13, 49]).

Therefore, BBBA can be useful as a "protective" and/or "rescuing" agent against drug toxicity that can be rescued or prevented by uridine or similar nucleosides. In this respect, BBBA can substitute, totally or partially, for the "uridine rescue regimens" in treating cancer, viral, and other diseases. BBBA alone or in combination with low concentrations of uridine can maintain an adequate uridine concentration for a long enough time to rescue selectively the normal or uninfected cells from drug toxicity, without having to suffer from the toxic side-effects (e.g. phlebitis, pyrogenic reactions, changes in body temperature and diarrhea) observed with the high concentration of uridine required to achieve the rescuing or protective effects in the absence of the inhibitor (43, 53, 69–72]. The use of BBBA in manipulating the uridine pool is not limited to the treatment of cancer or AIDS but can also be extended to treat other pathological and physiological disorders, where administration of uridine has been shown to be useful. Such disorders are quite numerous and include CNS disorders (e.g. cerebrovascular disorders and convulsions) [73–84], sleep promotion [85], muscle performance [86, 87], liver diseases [88–90], and cardiac damage [91–96]. The potential use of BBBA and other UrdPase inhibitors in treating such disorders has not yet been recognized in contemporary experimental or applied chemotherapy.

In conclusion, we have synthesized the 5-benzylbarbituric acids as specific and superior inhibitors of UrdPase to those previously known. These compounds could have a wide application in the therapy of cancer and AIDS, as well as other pathological and physiological disorders.

Acknowledgements—The authors are grateful to Dr. Sungman Cha for his help and encouragement, Dr. John G. Niedzwicki for many fruitful discussions and suggestions on the possible mechanisms of the binding of barbiturates to UrdPase, and Dr. Osama Ashour and Mr. Ben Striks for excellent technical assistance. This investigation was supported in part by Grant CA 13148 awarded by the National Cancer Institute, DHHS.

REFERENCES

1. Krenitsky TA, Barclay M and Jacquez JA, Specificity of mouse uridine phosphorylase. Chromatography, purification and properties. *J Biol Chem* **293**: 805–812, 1964.
2. Krenitsky TA, Mellors JW and Barclay RK, Pyrimidine nucleosidases, their classification and relationship to uric acid ribonucleoside phosphorylase. *J Biol Chem* **240**: 1281–1286, 1965.
3. Niedzwicki JG, el Kouni MH, Chu SH and Cha S, Pyrimidine acyclonucleosides, inhibitors of uridine phosphorylase. *Biochem Pharmacol* **30**: 2097–2101, 1981.
4. Niedzwicki JG, Chu SH, el Kouni MH, Rowe EC and Cha S, 5-Benzylacyclouridine and 5-benzyloxybenzylacyclouridine, potent inhibitors of uridine phosphorylase. *Biochem Pharmacol* **31**: 1857–1861, 1982.
5. Pontis H, Degerstedt G and Reichard P, Uridine and deoxyuridine phosphorylase from Ehrlich ascites tumor. *Biochim Biophys Acta* **51**: 138–147, 1961.
6. Kraut A and Yamada EW, Cytoplasmic uridine phosphorylase of rat liver: Characterization and kinetics. *J Biol Chem* **246**: 2021–2030, 1971.
7. Ishitsuka H, Miwa M, Takemoto K, Fukuoka K, Itoga A and Maruyama HB, Role of uridine phosphorylase for antitumor activity of 5'-deoxy-5-fluorouridine. *Gann* **71**: 112–123, 1980.
8. Miwa M, Nakamura J and Ishitsuka H, A simple and convenient assay method for the phosphorolysis of 5'-deoxy-5-fluorouridine. *Gann* **72**: 965–967, 1981.
9. Kono A, Hara Y, Sugata S, Karube Y, Matsushima Y and Ishitsuka H, Activation of 5'-deoxy-5-fluorouridine by thymidine phosphorylase in human tumors. *Chem Pharm Bull (Tokyo)* **31**: 175–178, 1983.
10. Niedzwicki JG, el Kouni MH, Chu SH and Cha S, Structure–activity relationship of ligands of the

pyrimidine nucleoside phosphorylases. *Biochem Pharmacol* **32**: 399–415, 1983.
11. Woodman PW, Sarrif AM and Heidelberger C, Specificity of pyrimidine nucleoside phosphorylases and the phosphorolysis of 5-fluoro-2′-deoxyuridine. *Cancer Res* **40**: 507–511, 1980.
12. Veres Z, Szabolcs A, Szinai I, Denes G and Jeney A, Enzymatic cleavage of 5-substituted-2′-deoxyuridines by pyrimidine nucleoside phosphorylases. *Biochem Pharmacol* **35**: 1057–1059, 1986.
13. el Kouni MH, Naguib FNM, Chu SH, Cha S, Ueda T, Gosselin G, Imbach J-L, Shealy F and Otter BA, Effect of the *N*-glycosidic bond conformation and modifications in the pentose moiety on the binding of nucleoside ligands to uridine phosphorylase. *Mol Pharmacol* **34**: 104–110, 1988.
14. Chu MYB, Naguib FNM, Iltzsch MH, el Kouni MH, Chu SH, Cha S and Calabresi P, Potentiation of 5-fluoro-2′-deoxyuridine antineoplastic activity by the uridine phosphorylase inhibitors benzylacyclouridine and benzyloxybenzylacyclouridine. *Cancer Res* **44**: 1852–1856, 1984.
15. Zimmerman M and Seidenberg JI, Thymidine phosphorylase and nucleoside deoxyribosyltransferase in normal and malignant tissues. *J Biol Chem* **239**: 2618–2621, 1964.
16. Nakayama C, Wataya Y, Meyer RB, Santi DV, Saneyoshi M and Ueda T, Thymidine phosphorylase. Substrate specificity for 5-substituted 2′-deoxyuridines. *J Med Chem* **23**: 962–964, 1980.
17. Desgranges C, Razaka G, Rabaud M and Bricaud H, Catabolism of thymidine in human blood platelet: Purification and characterization of thymidine phosphorylase. *Biochim Biophys Acta* **654**: 211–213, 1981.
18. Kono A, Hara Y, Sugata S, Matsushima Y and Ueda T, Substrate specificity of a thymidine phosphorylase in human liver tumor. *Chem Pharm Bull (Tokyo)* **32**: 1919–1921, 1984.
19. Iltzsch MH, el Kouni MH and Cha S, Kinetic studies of thymidine phosphorylase from mouse liver. *Proc Am Assoc Cancer Res* **26**: 14, 1985.
20. Meynial D, Malet-Martino MC, Lopez A and Martino R, Phosphorolysis of 5′-deoxy-5-fluorouridine in human plasma, serum and blood platelets. *J Pharm Pharmacol* **38**: 426–431, 1985.
21. Heidelberger C, Greisbach L, Cruz O, Schnitzer RJ and Grundberg E, Fluorinated pyrimidines VI. Effects of 5-fluorouridine and 5-fluoro-2′-deoxyuridine on transplanted tumors. *Proc Soc Exp Biol Med* **97**: 470–475, 1958.
22. Mukherjee KL, Boohar J, Wentland D, Ansfield FJ and Heidelberger C, Studies on fluorinated pyrimidines. XVI. Metabolism of 5-fluorouracil-2-C^{14} and 5-fluoro-2′-deoxyuridine-2-C^{14} in cancer patients. *Cancer Res* **23**: 49–66, 1963.
23. Birnie GD, Kroeger H and Heidelberger C, Studies of fluorinated pyrimidines. XVIII. The degradation of 5-fluoro-2′-deoxyuridine and related compounds by nucleoside phosphorylase. *Biochemistry* **2**: 566–572, 1963.
24. Marsh JC and Perry S, Thymidine catabolism by normal and leukemic human leukocytes. *J Clin Invest* **43**: 267–278, 1964.
25. Zimmerman M, Selective inhibition by deoxyglucosyl thymine of thymidine phosphorylase not catalyzing deoxyribosyl transfer. *Biochem Biophys Res Commun* **16**: 600–603, 1964.
26. Lazarus H, Barell EF, Oppenheim S and Krishan A, Divergent properties of two human lymphocytic cell lines isolated from a single specimen of peripheral blood. *In Vitro* **5**: 303–310, 1974.
27. Fox RM, Piddington SK, Tripp EH, Dudman NP and Tattersall MHN, Thymidine sensitivity of cultured leukemic lymphocytes. *Lancet* **II**: 391–393, 1979.
28. Piper AA and Fox RM, Biochemical basis for the differential sensitivity of human T- and B-lymphocyte lines to 5-fluorouracil. *Cancer Res* **42**: 3753–3760, 1982.
29. Vertongen F, Fondu P, Van den Heule B, Cauchie C and Mandelbaum IM, Thymidine kinase and thymidine phosphorylase activities in various types of leukaemia and lymphoma. *Tumour Biol* **5**: 303–311, 1984.
30. Vezzoni P, Giardini R, Lombardi L, Rilke F, Lucchini R, Vezzoni M and Clerici L, Multienzymatic analysis of human malignant lymphomas. Correlation of enzymatic data with pathologic and ultrastructural findings in Burkitt's and lymphoblastic lymphomas. *Cancer* **54**:489–499, 1984.
31. Naguib FNM, Niedzwicki JG, Iltzsch MH, Weimann MC, el Kouni MH and Cha S, Effects of *N,N*-dimethylformamide and sodium butyrate on enzymes of pyrimidine metabolism in cultured human tumor cells. *Leuk Res* **11**: 855–861, 1987.
32. el Kouni MH, Naguib FNM, Park KS, Cha S, Darnowski JW and Soong S-j, Circadian rhythm of hepatic uridine phosphorylase activity and plasma concentration of uridine in mice. *Biochem Pharmacol* **40**: 2479–2485, 1990.
33. Naguib FNM, Soong S-j and el Kouni MH, Circadian rhythm of orotate phosphoribosyltransferase, pyrimidine nucleoside phosphorylases and dihydrouracil dehydrogenase in mouse liver: Possible relevance to chemotherapy with 5-fluoropyrimidines. *Biochem Pharmacol* **45**: 667–673, 1993.
34. von Roemeling R and Hrushesky WJM, Determination of the therapeutic index of floxuridine by its circadian infusion pattern. *J Natl Cancer Inst* **82**: 386–393, 1990.
35. Martin DS, Stolfi RL, Sawyer RC, Spiegeleman S and Young CW, High-dose 5-fluorouracil with delayed uridine "rescue" in mice. *Cancer Res* **42**: 3964–3970, 1982.
36. Klubes P, Cerna I and Meldon MA, Uridine rescue from lethal toxicity of 5-fluorouracil in mice. *Cancer Chemother Pharmacol* **8**: 17–21, 1982.
37. Klubes P and Cerna I, Use of uridine rescue to enhance antitumor selectivity of 5-fluorouracil. *Cancer Res* **43**: 3182–3186, 1983.
38. van Groeningen CJ, Peters GJ, Leyva A, Laurensse E and Pinedo HM, Reversal of 5-fluorouracil-induced myelosuppression by prolonged administration of high-dose uridine. *J Natl Cancer Inst* **81**: 157–162, 1989.
39. Sommadossi JP, Carlisle R, Schinazi RF and Zhou Z, Uridine reverses the toxicity of 3′-azido-3′-deoxythymidine in normal human granulocyte-macrophage progenitor cells *in vitro* without impairment of antiretroviral activity. *Antimicrob Agents Chemother* **32**: 997–1001, 1988.
40. Sommadossi JP, Zhu Z, Carlisle R, Xie MY, Weidner DA and el Kouni MH, Novel pharmacologic approaches for the treatment of AIDS and potential use of uridine phosphorylase inhibitors. In: *Advances in Chemotherapy of AIDS* (Eds. Diasio RB and Sommadossi JP), pp. 63–73. Pergamon Press, New York, 1990.
41. Monks A, Ayers O and Cysyk RL, Effect of 5-benzylacyclouridine, a potent inhibitor of uridine phosphorylase, on the metabolism of circulating uridine by the isolated rat liver. *Biochem Pharmacol* **32**: 2003–2009, 1983.
42. Darnowski JW and Handschumacher RE, Tissue-specific enhancement of uridine utilization and 5-fluorouracil therapy in mice by benzylacyclouridine. *Cancer Res* **45**: 5364–5368, 1985.
43. Peters GJ, van Groeningen CJ, Laurenssee EJ, Lankelma J, Leyva A and Pinedo HM, Uridine-induced hypothermia in mice and rats in relation to

plasma and tissue levels of uridine and its metabolites. *Cancer Chemother Pharmacol* **20**: 101–108, 1987.
44. Martin DS, Stolfi RL and Sawyer C, Use of oral uridine as a substitute for parenteral uridine rescue of 5-fluorouracil therapy, with and without the uridine phosphorylase inhibitor 5-benzylacyclouridine. *Cancer Chemother Pharmacol* **24**: 9–14, 1989.
45. Darnowski JW and Handschumacher RE, Enhancement of fluorouracil therapy by the manipulation of tissue uridine pools. *Pharmacol Ther* **41**: 381–392, 1989.
46. Chu SH, Chen ZH, Rowe EC, Naguib FNM, el Kouni MH and Chu MY, Synthesis and biological activity of hydroxymethyl analogs of 5-benzylacyclouridine and 5-benzyloxybenzylacyclouridine. *Nucleosides Nucleotides* **3**: 303–311, 1984.
47. Chu SH, Weng ZY, Chen ZH, Rowe EC, Chu E, Naguib FNM, el Kouni MH, Cha S and Chu MY, Synthesis of 5-benzyl and 5-benzyloxybenzyl 2,2′-anhydrouridines and related nucleoside analogs as inhibitors of uridine phosphorylase. *Nucleosides Nucleotides* **7**: 91–102, 1988.
48. Park KS, el Kouni MH, Krenitsky TA, Chu SH and Cha S, Inhibition of uridine phosphorylase from *Escherichia coli* by benzylacyclouridines. *Biochem Pharmacol* **35**: 3853–5855, 1986.
49. Naguib FNM, el Kouni MH, Chu SH and Cha S, New analogues of benzylacyclouridines, specific and potent inhibitors of uridine phosphorylase from human and mouse livers. *Biochem Pharmacol* **36**: 2195–2201, 1987.
50. el Kouni MH, Naguib FNM, Niedzwicki JG, Iltzsch MH and Cha S, Uridine phosphorylase from *Schistosome mansoni*. *J Biol Chem* **263**: 6081–6086, 1988.
51. Chua BHL, Watson P, Kleinhams B and Morgan HE, Effect of 5-benzyloxybenzylacyclouridine on the labeling of UTP and RNA in perfused rat heart. *Fed Proc* **44**: 1773, 1985.
52. Chu S-H, Wiemann MC, Chu MY and Calabresi P, Modulation of FUdR toxicity by two benzyl-acyclouridine analogs. *Prog Am Assoc Cancer Res* **28**: 410, 1987.
53. Calabresi P, Falcone A, St. Clair MH, Weimann MC, Chu SH and Darnowski JW, Benzylacyclouridine reverses azidothymidine-induced marrow suppression without impairment of anti-human immunodeficiency virus activity. *Blood* **76**: 2210–2215, 1990.
54. Falcone A, Darnowski JW, Ruprecht RM, Chu SH, Burnetti I and Calabaresi P, Differential effect of benzylacyclouridine on the toxic and therapeutic effects of azidothymidine in mice. *Blood* **76**: 2216–2221, 1990.
55. Naguib FNM, Levesque DL, Wang E-c, Panzica RP and el Kouni MH, 5-Benzylbarbiturates, potent and specific inhibitors of uridine phosphorylase (UrdPase). *Pharmacologist* **34**: 412, 1992.
56. Speer JH and Dabovich TC, Alloxan monohydrate. *Org Syn* **21**: 5–8, 1941.
57. Sekiya M and Yanaihara C, Barbituric acid derivatives. Reduction of 5-arylmethylenebarbituric acid and some analogous barbituric acid derivatives. *Chem Pharm Bull (Tokyo)* **17**: 747–751, 1969.
58. Tanaka K, Chen X, Kimura T and Yoneda F, 5-Arylidene 1,3-dimethylbarbituric acid derivatives, mild organic oxidation for allylic and benzylic alcohols. *Chem Pharm Bull (Tokyo)* **36**: 60–69, 1988.
59. Tate J, Tinnerman WN, Jurveics V, Jesky H and Biehl ER, Preparation of 5-substituted benzylbarbituric acids and investigation of the effect of the benzyl and substituted benzyl groups of the acidity of barbituric acid. *J Heterocycl Chem* **23**: 9–11, 1986.
60. Robins MJ and Hatfield PW, Nucleic acid related compounds. 37. Convenient and high-yield syntheses of *N*-[(2-hydroxyethoxy)methyl-heterocycles as "acyclic nucleoside" analogues. *Can J Chem* **60**: 547–553, 1982.
61. Naguib FNM, el Kouni MH and Cha S, Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* **45**: 5405–5412, 1985.
62. el Kouni MH and Naguib FNM, Pyrimidine salvage pathways in adult *Schistosoma mansoni*. *Int J Parasitol* **20**: 37–44, 1990.
63. Dixon M and Webb EC, *Enzymes*. Academic Press, New York, 1979.
64. Wilkinson GN, Statistical estimations in enzyme kinetics. *Biochem J* **80**: 324–332, 1961.
65. Cleland WW, The statistical analysis of enzyme kinetic data. *Adv Enzymol* **29**: 1–32, 1967.
66. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
67. Potvin BW, Stern HJ, May SR, Lam GF and Krooth RS, Inhibition by barbituric acid and its derivatives of the enzymes in rat brain which participate in the synthesis of pyrimidine ribotides. *Biochem Pharmacol* **27**: 655–665, 1978.
68. de Meester P, Jovanovic MV, Chu SSC and Biehl ER, Investigation of the structure and ^{13}C NMR spectrum of 5-phenylbarbituric acid. *J Heterocycl Chem* **23**: 337–341, 1986.
69. Cradock JC, Vishnuvajjala BR, Chin TF, Hochstein HD and Ackerman SK, Uridine-induced hyperthermia in the rabbit. *J Pharm Pharmacol* **38**: 226–229, 1986.
70. van Groeningen CJ, Leyva A, Kraal I, Peters GJ and Pinedo HM, Clinical and pharmacokinetic studies of prolonged administration of high-dose uridine intended for rescue from 5-FU toxicity. *Cancer Treat Rep* **70**: 745–750, 1986.
71. Peters GJ, van Groeningen CJ, Laurensse EJ, Lankelma J, Kraal I, Leyva A, Lankelma J and Pinedo HM, Effect of pyrimidine nucleosides on body temperatures of man and rabbit in relation to pharmacokinetic data. *Pharm Res* **4**: 113–119, 1987.
72. van Groeningen CJ, Peters GJ, Nadal JC, Laurensse E and Pinedo HM, Clinical and pharmacologic study of orally administered uridine. *J Natl Cancer Inst* **83**: 437–441, 1991.
73. Geiger A and Yamasaki S, Cytidine and uridine requirement of the brain. *J Neurochem* **1**: 93–100, 1956.
74. Bonavita V, Monaco P and Tripi E, Analisi degli effetti centrali di nucleotidi purinici e pirimidinici e loro derivati III. Esperimenti farmacologici. *Acta Neurol (Napoli)* **19**: 215–222, 1964.
75. Sanguineti I and Zerbi D, L'impiego di alcuni nucleosidi non vasoattivi (citidina e uridina) nella terapia delle vasculopatie cerebrali. *Minerva Med* **56**: 3352–3361, 1965.
76. Monticone GF, Bergamasco B and Congnazzo A, Sull'impiego terapeutico dei nucleosidi citidina ed uridina in alcune affezioni neurologiche. *Minerva Med* **57**: 4348–4352, 1966.
77. Bonavita V and Zito M, Analisi dell'azione di nucleosidi pirimidinici sull'elettroencefallogramma umano attivato con megimide. *Riv Neurol* **38**: 317–319, 1968.
78. Jann G and Delzanno GB, Azione della citidina ed uridina nel trattamento delle sindromi neuropsichiche da encefalopatie di varia origine. *Minerva Med* **60**: 2092–2108, 1969.
79. Sepe O, Efficacia dell'assoiazione citidina-uridina nelle affezioni vascolari dell'encefalo. *Minerva Med* **61**: 5934–5941, 1970.
80. Roberts CA, Anticonvulsant effects of uridine: Comparative analysis of metrazol and penicillin induced foci. *Brain Res* **55**: 291–308, 1973.
81. Roberts CA, Kreisman NR and Waltman M, Uridine

anticonvulsant effects: Selective control of nucleoside incorporation in experimental epilepsy. *Epilepsia* **15**: 479–500, 1974.
82. Dwivedi C and Harbison RD, Anticonvulsant activities of Δ-8 and Δ-9 tetrahydrocannabinol and uridine. *Toxicol Appl Pharmacol* **31**: 452–458, 1975.
83. Guarneri P, Guarneri R, Mocciaro C and Piccoli F, Interaction of uridine with GABA binding sites in cerebellar membranes of the rat. *Neurochem Res* **8**: 1537–1545, 1983.
84. Guarneri P, Guarner R, Mocciaro C and Piccoli F, Interaction of uridine with GABA-mediated inhibitory transmission: studies *in vivo* and *in vitro*. *Epilepsia* **26**: 666–671, 1985.
85. Inoué S, *Biology of Sleep Substances*, pp. 71–95. CRC Press, Boca Raton, 1989.
86. Kypson J and Hait G, Effects of uridine and inosine on glucose metabolism in skeletal muscle and activated lipolysis in adipose tissue. *J Pharmacol Exp Ther* **199**: 565–574, 1976.
87. Kypson J and Hait G, Metabolic effects of inosine and uridine in rabbit hearts and rat skeletal muscles. *Biochem Pharmacol* **26**: 1585–1591, 1977.
88. Elrick H, Hlad CJ Jr and Arai Y, Influence of certain nucleosides on glucose metabolism in man. *Metabolism* **11**: 46–55, 1962.
89. Bushma MI, Parkhovchenko EI and Peschanskii VS, Effects of cytidine and uridine on the regeneration of the liver in rats poisoned with carbon tetrachloride. *Bull Exp Biol Med* **88**: 722–723, 1980.
90. Songu E, Haugaard ES, Wildley G and Haugaard N, The relationship between uracil nucleotide concentrations and glycogen synthesis in hepatocytes from fed and fasted rats. *Metabolism* **30**: 119–122, 1980.
91. Buckley NM, Tsuboi KK and Zeic NJ, Effects of nucleosides on acute left ventricular failure in isolated dog heart. *Circ Res* **7**: 847–857, 1959.
92. Kypson J, Hait G and Mathew R, Effects of uridine on the performance and the metabolism of oxygenated and hypoxic rabbit hearts. *J Mol Cell Cardiol* **10**: 545–565, 1978.
93. Aussedat J, Verdetti J, Grably S and Rossi A, Nucleotides uridyliques et glycogene cardiaque: Effect de l'administration d'uridine et de ribose chez le rat. *J Physiol (Paris)* **78**: 331–336, 1982.
94. Aussedat J, Effect of uridine supply on glycogen resynthesis after ischaemia in the isolated perfused rat heart. *Cardiovasc Res* **17**: 145–151, 1983.
95. Aussedat J, Uridine incorporation in normal and ischaemic perfused rat heart. *Mol Physiol* **6**: 247–256, 1984.
96. Meerson FZ and Dosmagabetova RS, The use of glucose and uridine to control contractility and extensibility disturbances in the nonischemized compartments of the heart in myocardial infarction. *Kardiologiya* **25**: 91–94, 1985.