Inhibition of Uridine Phosphorylase: Synthesis and Structure-Activity Relationships of Aryl-Substituted 5-Benzyluracils and 1-[(2-Hydroxyethoxy)methyl]-5-benzyluracils

G. Faye Orr,[†] David L. Musso,[†] G. Evan Boswell,[†] James L. Kelley,*[,†] Suzanne S. Joyner,[‡] Stephen T. Davis,[‡] and David P. Baccanari[‡]

Divisions of Organic Chemistry and Biochemistry, Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709

Received April 6, 1995[⊗]

A series of 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils were synthesized and tested for inhibition of murine liver uridine phosphorylase (UrdPase). Inhibitors of UrdPase are reported to enhance the chemotherapeutic utility of 5-fluoro-2′-deoxyuridine and 5-fluorouracil and to ameliorate zidovudine-induced anemia in animal models. We prepared a series of 5-aryl-substituted analogues of 5-benzylacyclouridine (BAU), a good inhibitor of UrdPase (IC_{50} of 0.46 μM), to develop a compound with enhanced potency and improved pharmacokinetics. The first phase of structure-activity relationship studies on a series of 32 aryl-substituted 5-benzyluracils found several 5-(3-alkoxybenzyl) analogues of 5-benzyluracil with enhanced potency. The acyclovir side chain, the (2-hydroxyethoxy)methyl group, was substituted on the more potent aryl-substituted 5-benzyluracils. The two most potent compounds, **10y** (3-propoxy) and **10dd** (3-sec-butoxy), were inhibitors of UrdPase with IC_{50}s of 0.047 and 0.027 μM, respectively. Six compounds were tested *in vivo* for effects on steady-state concentrations of circulating uridine in rats. Plasma uridine levels were elevated 3-9-fold by compound levels that ranged from 8 to 50 μM.

Mammalian cells contain two enzymes that catalyze the reversible phosphorolysis of pyrimidine nucleosides: uridine phosphorylase (EC 2.4.2.3) (UrdPase) and thymidine phosphorylase (EC 2.4.2.4).[1] There has been a long-standing interest in the development of inhibitors of these enzymes,[2-6] which are responsible for the degradation of chemotherapeutic agents such as 5-fluoro-2′-deoxyuridine (FUDR).[7] Indeed, the antineoplastic activity of FUDR is potentiated by the UrdPase inhibitors BAU (5-benzylacyclouridine) and BBAU (5-[*m*-(benzyloxy)benzyl]acyclouridine).[8] The therapeutic effectiveness of 5-fluorouracil (5-FU) is enhanced when BAU is administered alone or in combination with uridine. This regimen apparently leads to increased cellular concentrations of uridine and its nucleotides, which rescue normal tissue from the toxic effects of 5-FU.[9] Bone marrow suppression is the most frequent dose-limiting toxicity of zidovudine (Retrovir, AZT), an established treatment for AIDS (acquired immune deficiency syndrome) patients.[10,11] Uridine was reported to prevent and reverse the toxic affects of zidovudine *in vitro*;[12] Calabresi *et al.* later reported that AZT-induced anemia in Balb/c mice was favorably modulated by the UrdPase inhibitor BAU.[13,14]

The first good inhibitors of UrdPase were developed by Baker and Kelley, who discovered a hydrophobic bonding region adjacent to the 5-position of the substrate.[3,15,16] In an extensive study of 5-substituted uracils, 5-benzyluracil (BU) and 5-[3-(benzyloxy)benzyl]-uracil (BBU) were found to bind 75- and 800-fold better, respectively, than the substrate.[15] Niedzwicki *et al.* later improved the inhibitory potency of BU and BBU by substitution of the acyclovir side chain,[17-19] the (2-hydroxyethoxy)methyl group, at the 1-position of BU and BBU.[5] The resultant acyclonucleosides BAU and BBAU have K_i values of 98 and 32 nM, respectively, against the UrdPase from sarcoma S-180 cytosol[5] and of 420 and 170 nM, respectively, against the enzyme from the cytosol of mouse liver.[20] A further enhancement of inhibitory potency was reported for the hydroxymethyl (HM-BBAU, 1′-(hydroxymethyl)-5-[*m*-(benzyloxy)benzyl]acyclouridine) and aminomethyl (AM-BBAU, 1′-(aminomethyl)-5-[*m*-(benzyloxy)benzyl]acyclouridine) analogues of BBAU, which have K_i values of 70 and 20 nM, respectively.[20]

Thus, several potent inhibitors of UrdPase are known, although *in vivo* studies with these agents have been largely limited to work with BAU, an inhibitor of moderate potency.[8,9,13,14,21] Naguib, *et al.* recently reported on the 5-benzylbarbituric acid derivative BBBA, a potent inhibitor (K_i of 2.6 nM) with improved solubility properties.[22] Some of these reports and other factors prompted us to initiate a program to develop inhibitors of UrdPase with enhanced potency and improved pharmacokinetic properties. The initial stages of this program are reported herein.

Chemistry Discussion

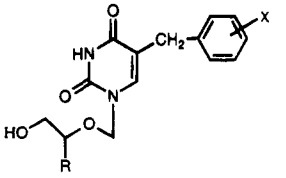
The target 1-(substituted)-5-benzyluracils in Table 1 were prepared in six or seven steps from the appropriate 3-phenyl-2-propenoic acids **1** (Scheme 1). The acids **1** were converted to the ethyl esters **2**, which were reduced by catalytic hydrogenation to provide the 3-phenylpropionates **3**. The intermediate ethers **4** for preparation of **6x-gg** were synthesized by alkylation of ethyl 3-(3-hydroxyphenyl)propionate (**3**, R^2 = 3-OH) with the appropriate alkyl halides.

The 2-thioxo-4-pyrimidinones **6** in Table 2 were prepared from the appropriate ethyl phenylpropionates **3** and **4** in two steps by method D or E. Method D,

[†] Division of Organic Chemistry.
[‡] Division of Biochemistry.
[⊗] Abstract published in *Advance ACS Abstracts*, August 1, 1995.

0022-2623/95/1838-3850$09.00/0

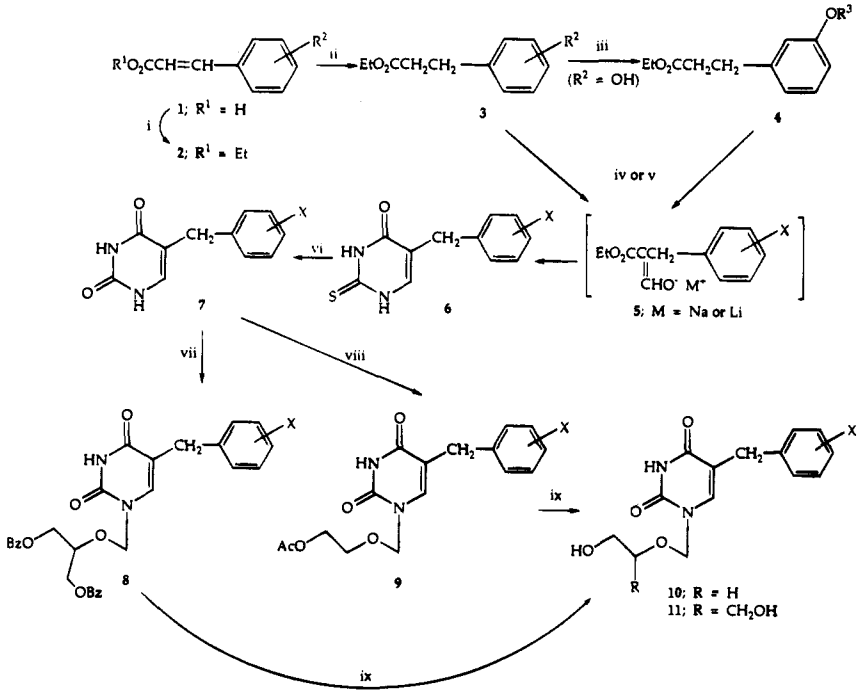
Table 1. Physical Properties of 1-(Substituted)-5-benzyluracils



compd	X	R	method	yield, %[a]	mp, °C	formula[b]	IC_{50}, μM
10a	H	H	G, H	59	144–146[c,d]	$C_{14}H_{16}N_2O_4$	0.46[e]
10i	3-F	H	G, H	44	129–131[f]	$C_{14}H_{15}FN_2O_4$	0.55
10j	3-Cl	H	G, H	73	155–157[c]	$C_{14}H_{15}ClN_2O_4$	0.30
11j	3-Cl	CH_2OH	G, H	60	oil	$C_{15}H_{17}ClN_2O_5 \cdot 0.5H_2O$	0.11
10o	2-F	H	G, H	25	145–147[g]	$C_{14}H_{15}FN_2O_4$	0.87
10t	3,5-F_2	H	G, H	30	138–139[g]	$C_{14}H_{14}F_2N_2O_4$	0.35
10m	3-OCH_3	H					*h*
10x	3-OCH_2CH_3	H	G, H	36	98–99	$C_{16}H_{20}N_2O_5$	0.10
10y	3-$O(CH_2)_2CH_3$	H	G, H	58	85–87[f]	$C_{17}H_{22}N_2O_5 \cdot 0.25H_2O$	0.047
11y	3-$O(CH_2)_2CH_3$	CH_2OH	G, H	20	101–102	$C_{18}H_{24}N_2O_6$	0.06
10z	3-$OCH(CH_3)_2$	H	G, H	40	83–85	$C_{17}H_{22}N_2O_5$	0.16
10aa	3-$OCH_2CH{=}CH_2$	H	G, H	42	89–93[i]	$C_{17}H_{20}N_2O_5$	0.13
10bb	3-$O(CH_2)_2CH_2F$	H	G, H	47	78–82[j]	$C_{17}H_{21}FN_2O_5$	0.068
11bb	3-$O(CH_2)_2CH_2F$	CH_2OH	G, H	41	99–101	$C_{18}H_{23}FN_2O_6$	0.11
10cc	3-$O(CH_2)_3CH_3$	H	G, H	28	80–82	$C_{18}H_{24}N_2O_5$	0.16
10dd	3-$OCH(CH_3)CH_2CH_3$	H	G, H	43	82–86[j]	$C_{18}H_{24}N_2O_5$	0.027
10ee	3-$OCH_2CH(CH_3)_2$	H	G, H	38	95–97	$C_{18}H_{24}N_2O_5$	0.066
10gg	3-$OCH_2C_6H_5$	H	G, H	39	111–112[k]	$C_{21}H_{22}N_2O_5$	0.084[l]

[a] Overall yield from 5-benzyluracils. [b] All compounds were analyzed for C, H, N. [c] Recrystallized from 2-PrOH. [d] Mp 145–146 °C reported in ref 5. [e] IC_{50} of 0.098 μM reported in ref 5. [f] Recrystallized from CH_2Cl_2–hexane. [g] Recrystallized from MeOH–CH_2Cl_2. [h] Apparent K_i of 1.86 μM reported in ref 20 under assay conditions where BAU had a K_i of 3.12 μM. [i] Recrystallized from CH_2Cl_2–Et_2O–hexane. [j] Triturated with hexane. [k] Mp 126–130 °C reported in ref 5. [l] IC_{50} of 0.032 μM reported in ref 5.

Scheme 1[a]

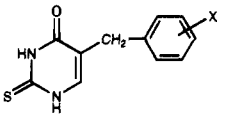


[a] Reagents: (i) EtOH, Et_2O, HCl, reflux; (ii) EtOH, H_2, PtO_2; (iii) Me_2CO, R^3Br, K_2CO_3; (iv) Na, HCO_2Et, Et_2O then thiourea, EtOH, reflux; (v) LDA, THF, HCO_2Et, −60–−78 °C then thiourea, EtOH, reflux; (vi) glacial HOAc, 20% aqueous $ClCH_2CO_2H$, reflux; (vii) BSA, $ClCH_2CH_2Cl$, reflux then $BrCH_2OCH(CH_2OC(O)C_6H_5)_2$; (viii) BSA, $ClCH_2CH_2Cl$, reflux then $BrCH_2OCH_2CH_2OC(O)CH_3$; (ix) MeOH, NH_3, room temperature.

which is based on literature methodology,[15,23,24] involves the formylation of the propionate esters using ethyl formate and sodium metal in diethyl ether followed by condensation with thiourea in refluxing ethanol. Method E involves the treatment of esters **4** with lithium diisopropyl amide,[25,26] prepared *in situ* from *n*-butyllithium and diisopropylamine, to give the lithio salt of the (alkoxyphenyl)propionate esters. Subsequent treatment of the lithio salt with ethyl formate and condensation with thiourea in refluxing ethanol gave the 2-thioxo-4-pyrimidinones in satisfactory yields. Hydrolysis of the 2-thioxo-4-pyrimidinones **6** in refluxing aqueous chloroacetic acid[27] gave 5-benzyluracils **7a–gg** (Table 2).

The acyclonucleosides in Table 1 were prepared from the 5-benzyluracils **7** via the *O*-silyl derivatives,[16] which were prepared using bis(trimethylsilyl)acetamide in

Table 2. Physical Properties of 5-Benzyl-1,2-dihydro-2-thioxo-4(3*H*)-pyrimidinones



compd	X	method	yield, %[a]	mp, °C	formula[b]
6a	H	A,B,D	22[c]	208–209[d,e]	$C_{11}H_{10}N_2OS$
6b	4-F	A,B,D	20[c]	212–214[e]	$C_{11}H_9FN_2OS$
6c	4-Cl	A,B,D	35[c]	267–269[e,f]	$C_{11}H_9ClN_2OS$
6d	4-CF_3	A,B,D	53[c]	240–242[e]	$C_{12}H_9F_3N_2OS$
6e	4-CH_3	A,B,D	22[c]	244–245[e,g]	$C_{12}H_{12}N_2OS$
6f	4-OCH_3	A,B,D	28[c]	220–223[e,h]	$C_{12}H_{12}N_2O_2S$
6i	3-F	A[i],B,D	33[c]	224–225[e]	$C_{11}H_9FN_2OS$
6j	3-Cl	A,B,D	31[c]	188–190[e]	$C_{11}H_9ClN_2OS$
6k	3-CF_3	A,B,D	56[c]	213–215[e]	$C_{12}H_9F_3N_2OS$
6l	3-CH_3	A,B,D	15[c]	194–196[e]	$C_{12}H_{12}N_2OS$
6m	3-OCH_3	A,B,D	22[c]	195–198[e,j]	$C_{12}H_{12}N_2O_2S$
6n	3-CN	A,B,D	29[c]	264–266[e]	$C_{12}H_9N_3OS$
6o	2-F	A,B,D	21[c]	204–205[e]	$C_{11}H_9FN_2OS$
6q	2-CH_3	A,B,D	36[c]	248–250[e]	$C_{12}H_{12}N_2OS$
6r	2-OCH_3	A,B,D	15[c]	188–189[e]	$C_{12}H_{12}N_2O_2S$
6s	3,4-F_2	A,B,D	30[c]	229–230[e]	$C_{11}H_8F_2N_2O_2S$
6t	3,5-F_2	A,B,D	41[c]	236–238[e]	$C_{11}H_8F_2N_2O_2S$
6u	3,4-Cl_2	A,B,D	24	214–216[e]	$C_{11}H_8Cl_2N_2OS$
6v	3,4-$(OCH_3)_2$	A,B,D	19[c]	226–229[e]	$C_{13}H_{14}Cl_2O_3S$
6w	3,4-OCH_2O	A,B,D	38[c]	214–215[e]	$C_{12}H_{10}N_2O_3S$
6x	3-OCH_2CH_3	A,B,C,D	11[c]	205–206[e,k]	$C_{13}H_{14}N_2O_2S$
6y	3-$O(CH_2)_2CH_3$	A,B,C,E	38[c]	183–185[e]	$C_{14}H_{16}N_2O_2S$
6z	3-$OCH(CH_3)_2$	A,B,C,E	14[c]		$C_{14}H_{16}N_2O_2S$
6aa	3-$OCH_2CH{=}CH_2$	A,B,C,E	13[c]	183–185[e]	$C_{14}H_{14}N_2O_2S$
6bb	3-$OCH_2CH_2CH_2F$	A,B,C,E	27[c]	166–167[e]	$C_{14}H_{15}FN_2O_2S$
6cc	3-$O(CH_2)_3CH_3$	A,B,C,E	32	168–170[e]	$C_{15}H_{16}N_2O_2S$
6dd	3-$OCH(CH_3)CH_2CH_3$	A,B,C,E	14[c]	127–130	$C_{15}H_{18}N_2O_2S$
6ee	3-$OCH_2CH(CH_3)_2$	A,B,C,E	7	177–179[l]	$C_{15}H_{18}N_2O_2S$
6ff	3-$OCH_2CH_2CH_2CF_3$	A,B,C,E	14[c]	183–184[e]	$C_{15}H_{15}F_3N_2O_2S$
6gg	3-$OCH_2C_6H_5$	A,B,C,E	47[c]	168–169[e,m]	$C_{18}H_{16}N_2O_2S$

[a] Overall yield from 3-phenyl-2-propenoic acid. [b] All compounds were analyzed for C, H, N. [c] Crude yield. [d] Mp 210–211 °C reported in ref 23. [e] Recrystallized from acetonitrile. [f] Mp 266–271 °C reported in ref 24. [g] Mp 240–243 °C reported in ref 24. [h] Mp 222–223 °C reported in ref 24. [i] 3-(3-Fluorophenyl)-2-propenoic acid was prepared from 3-fluorobenzaldehyde and malonic acid as reported by Fuchs, R.; Bloomfield, J. J. *J. Org. Chem.* **1966**, *31*, 3423–3425. [j] Mp 192–194 °C reported in ref 15. [k] Mp 206–208 °C reported in ref 15. [l] Recrystallized from MeOH–water. [m] Mp 169–170 °C reported in ref 15.

refluxing dichloromethane. A 1.2-fold excess of the silylated uracil was reacted with either (2-acetoxyethoxy)methyl bromide[28] or 2-(bromomethoxy)-1,3-propanediyl dibenzoate[29] to provide esters **8** and **9**. The latter were deprotected with methanolic ammonia to give the 1-(substituted)-5-benzyluracils **10** and **11** (Table 1).

Inhibition of Uridine Phosphorylase–Structure–Activity Relationships

The 5-benzyluracils in Tables 1 and 3 were tested for inhibition of UrdPase from murine liver. The IC_{50} is the concentration of inhibitor that results in a 50% decrease in reaction velocity. BU (**7a**) (Table 3) and BAU (**10a**) (Table 1) are good inhibitors of UrdPase with IC_{50}s of 5.7 and 0.46 μM, respectively. Heretofore, little was known about the effect of aryl substituents on the inhibitory potency of these inhibitors. Consequently, the first phase of structure–activity relationship studies focused on the preparation and evaluation of a series of aryl-substituted 5-benzyluracils.

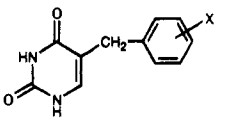
Substitution in the para position with a F (**7b**), Cl (**7c**), CF_3 (**7d**), OCH_3 (**7f**), or CN (**7h**) substituent resulted in a loss of potency (IC_{50}s ranged from 13 to 66 μM). The 4-methyl analogue **7e** and the 4-NO_2 analogue **7g** had potencies comparable to that of **7a** with IC_{50}s of 7.6 and 5.9 μM, respectively. No trend was evident with respect to the lipophilic and electronic properties of these seven substituents.

Substitution in the meta position enhanced potency slightly in some cases; the most potent compounds were the 3-Cl (**7j**) and 3-OCH_3 (**7m**) analogues (IC_{50}s of 2.5 and 3.9 μM, respectively). The 3-CF_3 (**7k**) and 3-CN (**7n**) analogues were less potent (IC_{50}s = 21.4 and 13.2 μM, respectively) suggesting that electron-withdrawing substituents in the 3-position decreased activity. Compounds substituted in the ortho position had lower potency than the parent **7a**, with the exception of the 2-fluoro analogue **7o**, which had an IC_{50} of 4.0 μM. Disubstitution with 3,4-F_2 (**7s**), 3,5-F_2 (**7t**), 3,4-Cl_2 (**7u**), 3,4-$(OCH_3)_2$ (**7v**), and 3,4-(OCH_2O) (**7w**) groups did not improve activity. The IC_{50}s ranged from 7.6 μM for **7t** to 129 μM for **7v**.

Since the 3-methoxy analogue **7m** was slightly more potent than the parent **7a**, and Baker *et al.* had reported that the 3-ethoxy analogue **7x** had good activity (IC_{50} = 1.4 μM),[15] we prepared a series of 3-alkoxy analogues **7y–ff**. The IC_{50}s of this series ranged from 0.84 μM for the 3-propoxy analogue **7y** to 8.5 μM for the 3-butoxy analogue **7cc**. The *sec*-butoxy (**7dd**), isobutoxy (**7ee**), and isopropoxy (**7z**) analogues were also more potent than the parent **7a** with IC_{50}s of 1.1, 1.4, and 2.0 μM, respectively, which was suggestive that branching of the 3-alkoxy chain was tolerated. The higher IC_{50} for the 3-butoxy analogue **7cc** indicated that there was limited bulk tolerance.

Since the 1-(2-hydroxyethoxy)methyl substituent on BAU (**10a**) imparts a 12-fold enhancement in inhibitory

Table 3. Physical Properties of 5-Benzyluracils



compd	X	method	yield, %	mp, °C	formula[a]	IC_{50}, μM
7a	H	F	80	292–293[b,c]	$C_{11}H_{10}N_2O_2$	5.7[l]
7b	4-F	F	46	295–296[d]	$C_{11}H_9FN_2O_2$	16.4
7c	4-Cl	F	63	304–305[d]	$C_{11}H_9ClN_2O_2$	13.0
7d	4-CF_3	F	85	284–285[e]	$C_{11}H_9F_3N_2O_2$	16% @ 10
7e	4-CH_3	F	52	304–305[d]	$C_{12}H_{12}N_2O_2$	7.6
7f	4-OCH_3	F	57	285–287[d]	$C_{12}H_{12}N_2O_3$	66.5
7g	4-NO_2	K	27	282–286[d]	$C_{11}H_9N_3O_4$	5.9
7h	4-CN	D,F	17[f]	273–275[g]	$C_{12}H_{19}N_3O_2$	21.1
7i	3-F	F	86	272–273[e]	$C_{11}H_9FN_2O_2$	7.6
7j	3-Cl	F	45	251–253[h]	$C_{12}H_9CLN_2O_2$	2.5
7k	3-CF_3	F	61	228–230[h]	$C_{12}H_9F_3N_2O_2$	21.4
7l	3-CH_3	F	99	265–268[b]	$C_{12}H_{12}FN_2O_2$	8.5
7m	3-OCH_3	F	77	241–244[d,i]	$C_{12}H_{12}N_2O_3$	3.9[m]
7n	3-CN	F	68	260–262[b]	$C_{12}H_9N_3O_2$	13.2
7o	2-F	F	72	287–280[d]	$C_{11}H_9FN_2O_2$	4.0
7p	2-Cl	A,B,D,F	23	282–284[d]	$C_{11}H_9ClN_2O_2$	24.0
7q	2-CH_3	F	59	290–292[e]	$C_{12}H_{12}N_2O_2$	65.0
7r	2-OCH_3	F	69	254–255[d]	$C_{12}H_{12}N_2O_3$	8.2
7s	3,4-F_2	F	89	270–271[e]	$C_{11}H_8F_2O_2$	25.0
7t	3,5-F_2	F	77	303–304[b]	$C_{11}H_8Cl_2N_2O_2$	7.6
7u	3,4-Cl_2	F	58[e]	255–257[e]	$C_{11}H_8Cl_2N_2O_2$	15.5
7v	3,4-$(OCH_3)_2$	F	56[e]	229–232[b]	$C_{13}H_{14}N_2O_2$	129
7w	3,4-OCH_2O	F	66	285–287[d]	$C_{12}H_{10}N_2O_4$	13.6
7x	3-OCH_2CH_3	F	83	244–245[b,j]	$C_{13}H_{14}N_2O_3{\cdot}0.1H_2O$	2.4[n]
7y	3-$O(CH_2)_2CH_3$	F	85	247–249	$C_{14}H_{16}N_2O_3$	0.84
7z	3-$OCH(CH_3)_2$	F	77	218–220[b]	$C_{14}H_{16}N_2O_3$	2.0
7aa	3-$OCH_2CH{=}CH_2$	F	45	241–244[e]	$C_{14}H_{14}N_2O_3$	3.6
7bb	3-$OCH_2CH_2CH_2F$	F	88	240–242[b]	$C_{14}H_{15}FN_2O_3$	1.5
7cc	3-$O(CH_2)_3CH_3$	F	89	242–243	$C_{15}H_{18}N_2O_3$	8.5
7dd	3-$OCH(CH_3)CH_2CH_3$	F	88	213–215[b]	$C_{15}H_{18}N_2O_3$	1.1
7ee	3-$OCH_2CH(CH_3)_2$	F	72	224–215[b]	$C_{15}H_{18}N_2O_3$	1.4
7ff	3-$O(CH_2)_3CF_3$	F	74	213–215[b]	$C_{15}H_{15}F_3N_2O_3$	13
7gg	3-$OCH_2C_6H_5$	F	87	262–263[b,k]	$C_{18}H_{16}N_2O_3$	15% @ 5[o]

[a] All compounds were analyzed for C, H, N. [b] Compound was purified by washing with water and diethyl ether. [c] Mp 294–295 °C reported in ref 24. [d] Recrystallized from AcOH. [e] Recrystallized from AcOH–water. [f] Overall yield from ethyl 3-(4-cyanophenyl)propanoate. [g] Recrystallized from EtOH–MeOH. [i] Mp 243–245 °C reported in ref 15. [j] Mp 245–246 °C reported in ref 15. [k] Mp 266–268 °C reported in ref 15. [l] IC_{50} of 5.3 μM reported in ref 15. [m] IC_{50} of 1.9 μM reported in ref 15. [n] IC_{50} of 1.4 μM reported in ref 15. [o] IC_{50} of 0.5 μM reported in ref 15.

potency with the parent **7a**, this side chain was placed on the more potent aryl-substituted 5-benzyluracils. The most potent 5-benzyluracil, the 3-propoxy analogue **7y**, was alkylated to give **10y**, which had an IC_{50} of 0.047 μM. The N-1-alkylated 3-*sec*-butoxy analogue **10dd** was even more potent with an IC_{50} of 0.027 μM. Other compounds with comparable potency were the 3-(3-fluoropropoxy) analogue **10bb** (IC_{50} = 0.068 μM), the 3-butoxy analogue **10cc** (IC_{50} = 0.16 μM), and the 3-isobutoxy analogue **10ee** (IC_{50} = 0.066 μM).

Three of the 5-benzyluracils **7** were alkylated with the branched acyclic side chain to give **11j,y,bb** (Table 1). The 3-chloro analogue **11j**, the 3-propoxy analogue **11y**, and the 3-(3-fluoropropoxy) analogue **11bb** were more potent than BAU with IC_{50}s of 0.11, 0.06, and 0.11 μM, respectively.

Elevation of Plasma Uridine Levels. Steady-state concentrations of circulating uridine are maintained by a balance between synthesis and catabolism.[30,31] When catabolism is inhibited, continued biosynthesis results in a time-dependent increase in plasma uridine levels. There are considerable differences in the biochemical effects of BAU in mice and rats. Plasma concentrations of BAU that significantly elevate plasma uridine levels in mice have modest effects in the rat.[32] Therefore, the rat model is a more stringent test for the efficacy of UrdPase inhibitors *in vivo*. When the pharmacodynamics of **10bb** (IC_{50} = 0.068 μM) and BAU (IC_{50} = 0.46 μM) were compared in rats, **10bb** produced a greater elevation of endogenous plasma uridine levels for 3 h (Figure 1A), even though the plasma concentrations of **10bb** were less than or equal to those of BAU over this time period (Figure 1B). The *in vivo* efficacies of **10y,cc,dd,ee** and **11bb** were determined in similar experiments. Plasma uridine concentrations were elevated 3–9-fold by drug levels that varied between 10 and 50 μM, depending upon the inhibitor.

Conclusions

We investigated the effect of 5-aryl substituents on the inhibitory potency of BAU against UrdPase. Selected 5-(3-alkoxybenzyl) analogues were 7–17-fold more potent than BAU as inhibitors of UrdPase, and several compounds enhanced circulating levels of plasma uridine in *in vivo* experiments. This research represents a significant advance in understanding of the structure–activity relationships for inhibition of UrdPase and has led to the design of more potent inhibitors of this therapeutically important enzyme. The latter will be the subject of a subsequent report.

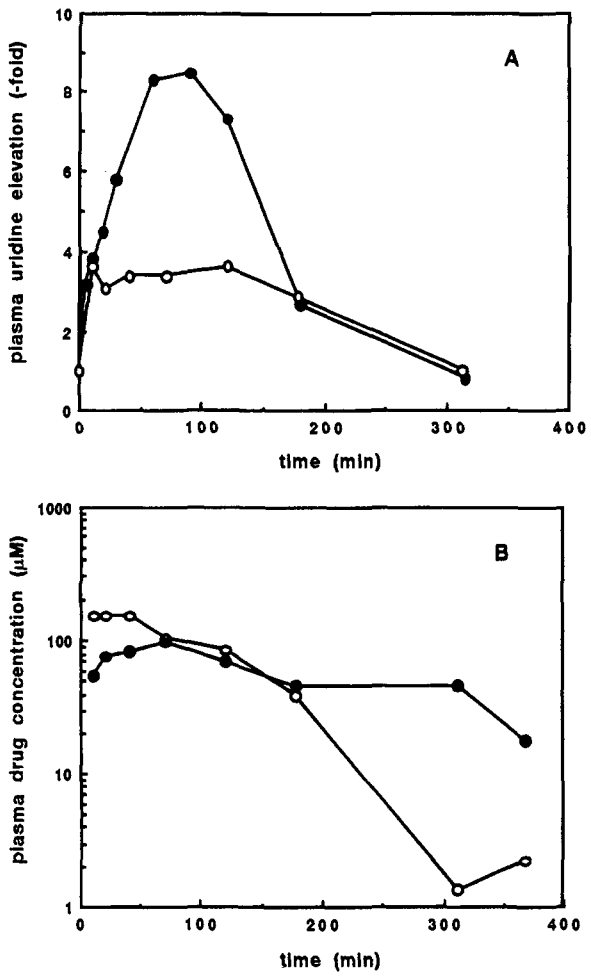


Figure 1. Profiles of uridine, BAU, and **10bb** plasma concentration versus time in rats following oral administration of 90 mg/kg BAU (open symbols) or 100 mg/kg **10bb** (closed symbols). Blood samples were collected at the times indicated and assayed for (A) plasma uridine and (B) plasma drug concentration. Each point is the average from two experiments.

Experimental Section

Melting points were taken in capillary tubes using a Thomas-Hoover Unimelt apparatus and are uncorrected. The NMR spectra were recorded on a Varian XL-100-15-FT, a Varian XL-200, or a Varian XL-300 spectrometer. The UV absorption spectra were measured on a Cary 118 UV–vis spectrophotometer. Mass spectra (~50 eV) were obtained from Oneida Research Services, Whitesboro, NY, using a Finnegan 4500 TFQ mass spectrometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on thin-layer chromatography (TLC). The TLCs were developed on Whatman 200 μm MK6F plates of silica gel with fluorescent indicator. Preparative flash chromatography was performed on silica gel 60 (40–63 μm, E. Merck No. 9385) using the method of Still.[33] Elemental analyses were performed by Atlantic Microlab, Inc.

Method A: Procedure for Preparation of Ethyl 3-Phenyl-2-Propenoates 2. The ethyl propenoates were synthesized as described for (*E*)-ethyl 3-(3-hydroxyphenyl)-2-propenoate (**2**, R^2 = 3-OH) in ref 25.

Method B: Procedure for Preparation of Ethyl 3-Phenylpropanoates 3. The ethyl propanoates were synthesized as described for ethyl 3-(3-hydroxyphenyl)propanoate (**3**, R^2 = 3-OH) in ref 25.

Method C: Procedure for Preparation of (Alkoxyphenyl)propionates 4. The ethers were synthesized as described for ethyl 3-(3-propoxyphenyl)propionate (**4**, R^3 = $(CH_2)_2CH_3$) in ref 25.

Method D: 5-(3-Chlorobenzyl)-1,2-dihydro-2-thioxo-4(3*H*)-pyrimidinone (6j). A mixture of ethyl 3-(3-chlorophenyl)propanoate (32.0 g, 151 mmol) and ethyl formate (12.3 g, 166 mmol) was added dropwise to a solution of sodium (3.5 g, 151 mmol) in diethyl ether (100 mL) stirring under nitrogen. After stirring at ambient temperature for 18 h, the volatiles were removed *in vacuo*. The residue was dissolved in ethanol (75 mL), thiourea (10.4 g, 136 mmol) was added, and the mixture was refluxed for 5 h under nitrogen. The ethanol was evaporated *in vacuo*, and the residue was taken up in cold water (500 mL) and filtered through Celite. The filtrate was washed with dichloromethane, cooled in an ice bath, and acidified with concentrated hydrochloric acid. After stirring for 1 h, the solids were collected by filtration, washed with water, and dried under a vacuum for 3 days to give 20.3 g (53%) of a reddish-brown solid. Recrystallization of 1.5 g from ethanol/water gave 0.86 g of **6j** as a pale yellow solid, mp 187–189 °C; TLC dichloromethane:methanol (19:1). A second recrystallization from acetonitrile gave 0.34 g of analytically pure **6j** as a white solid: mp 188–190 °C; UV (0.1 N sodium hydroxide) λ_{max} 261 nm (ϵ 16 600); NMR (DMSO-d_6) δ 12.45 (s, 1H, NH), 12.25 (s, 1H, NH), 7.38 (s, 1H, H-6), 7.25 (m, 4H, Ar), 3.52 (s, 2H, CH_2Ar); MS *m/e* 253 (M^+). Anal. ($C_{11}H_9N_2OSCl$) C, H, N.

Method E: 1,2-Dihydro-5-(3-propoxybenzyl)-2-thioxo-4(3*H*)-pyrimidinone (6y). A solution of ethyl 3-(3-propoxyphenyl)propionate (2.00 g, 8.5 mmol) in tetrahydrofuran (3 mL) was added to a solution of lithium diisopropylamine (this salt was prepared from diisopropylamine (1.42 mL, 10.0 mmol) and *n*-butyllithium (5 mL of a 2 M pentane solution, 10.0 mmol)) in tetrahydrofuran (7 mL), cooled to −78 °C under nitrogen. The solution was stirred for 1.5 h while the temperature was allowed to rise to −60 °C. The solution was cooled to −78 °C, ethyl formate (0.8 mL, 10.0 mmol) was added, and stirring was continued for 2.5 h while the solution warmed to −30 °C. After recooling to −78 °C, thiourea (0.76 g, 10.0 mmol) was added in one portion, and the resulting suspension was allowed to warm to ambient temperature. Ethanol (15 mL) was added, and the solution was refluxed under nitrogen for 18 h. The ethanol was removed *in vacuo*, and the residue was partitioned between dichloromethane:water (75:125). The layers were separated, and the aqueous layer was washed with additional dichloromethane (2 × 100 mL). The organic washes were combined and back-extracted with 0.5 N sodium hydroxide. The combined aqueous layers were cooled in an ice bath, and the pH was adjusted to 4 with concentrated hydrochloric acid. The light brown precipitate was collected on a filter, washed several times with water and diethyl ether, and dried under a vacuum at ambient temperature for 18 h to give 1.24 g (53%) of **6y**, mp 183–185 °C (lit.[25] mp 184–185 °C). Recrystallization from acetonitrile gave 0.53 g of an analytically pure sample: TLC dichloromethane:methanol (19:1); UV (0.1 N hydrochloric acid + 10% methanol) λ_{max} 278 (ϵ 19 600), (pH 7 buffer + 10% methanol) 275 (ϵ 18 100), (0.1 N sodium hydroxide + 10% methanol) 261 (ϵ 17 200), sh 306 nm (ϵ 9400); NMR (DMSO-d_6) δ 12.45 (br s, 1H, NH), 12.1 (br s, 1H, NH), 7.26 (s, 1H, H-6), 6.95 (m, 4H, Ar), 3.86 (t, 2H, J = 6.5 Hz, OCH_2), 3.48 (s, 2H, CH_2Ar), 1.69 (dq, 2H, J = 6.5, 7.4 Hz, CCH_2C), 0.95 (t, 3H, J = 7.4 Hz, CH_3); MS *m/e* 277 (M^+). Anal. ($C_{14}H_{16}N_2O_2S$) C, H, N.

Method F: 5-(3-Propoxybenzyl)uracil (7y). A suspension of **6y** (0.350 g, 1.3 mmol) in glacial acetic acid (5 mL) and 20% aqueous chloroacetic acid (5 mL) was refluxed with stirring for 18 h. After cooling to ambient temperature and then in an ice bath, the mixture was filtered, and the solids were washed with water and diethyl ether and dried in a vacuum oven at 80 °C for 18 h to give 0.28 g (85%) of **7y** as an off-white solid: mp 247–249 °C; TLC dichloromethane:methanol (19:1); UV (0.1 N hydrochloric acid + 10% methanol) λ_{max} 266 (ϵ 10 300), (pH 7 buffer + 10% methanol) 266 (ϵ 10 400), (0.1 N sodium hydroxide + 10% methanol) 290 nm (ϵ 8000); NMR (DMSO-d_6) δ 11.07 (s, 1H, NH), 10.71 (s, 1H, NH), 7.25 (s, 1H, H-6), 6.98 (m, 4H, Ar), 3.88 (t, 2H, J = 6.5 Hz, OCH_2), 3.45 (s, 2H, CH_2Ar), 1.71 (dq, 2H, J = 6.5, 7.4 Hz, CCH_2C), 0.97 (t, 3H, J = 7.4 Hz, CH_3); MS *m/e* 261 (M^+). Anal. ($C_{14}H_6N_2O_3$) C, H, N.

Method G: 1-[(2-Acetoxyethoxy)methyl]-5-(3-propoxybenzyl)uracil (9y). Bis(trimethylsilyl)acetamide (0.85 mL, 3.4 mmol) was added to a stirred suspension of **7y** (0.50 g, 1.9 mmol) in 1,2-dichloroethane (20 mL) under nitrogen. The mixture was refluxed with stirring for 35 min and the resultant solution cooled in an ice bath. A solution of (2-acetoxyethoxy)methyl bromide (0.327 g, 1.7 mmol) in acetonitrile (3 mL) was added to the cooled solution, and the resultant solution was allowed to warm to ambient temperature and stirred under nitrogen for 18 h. The volatiles were removed *in vacuo*, and the residual oil was introduced onto a column of silica gel 60 wetted with dichloromethane. The column was eluted with dichloromethane:methanol (30:1), and the fractions containing product were combined. The solvents were removed *in vacuo* to give 0.63 g (83%) of **9y** as a light yellow oil: TLC dichloromethane:methanol (19:1); UV (0.1 N hydrochloric acid + 10% methanol) λ_{max} 266 (ϵ 10 100), (pH 7 buffer + 10% methanol) 266 (ϵ 9500), (0.1 N sodium hydroxide + 10% methanol) 267 nm (ϵ 7200); NMR ($CDCl_3$) δ 8.77 (s, 1H, NH), 6.90 (s, 1H, H-6), 6.98 (m, 4H, Ar), 5.11 (s, 2H, NCH_2O), 4.17 (t, 2H, J = 4.7 Hz, OCH_2CH_2), 3.89 (t, 2H, J = 6.5 Hz, $OCH_2CH_2CH_3$), 3.75 (t, 2H, J = 4.7 Hz, CH_2CH_2O), 3.61 (s, 2H, CH_2Ar), 2.04 (s, 3H, $CH_3C(O)$), 1.79 (dq, 2H, J = 6.5, 7.4 Hz, CCH_2C), 1.02 (t, 3H, J = 7.4 Hz, CH_2CH_3); MS *m/e* 377. Anal. ($C_{19}H_{24}N_2O_6 \cdot 0.5H_2O$) C, H, N.

Method H: 1-[(2-Hydroxyethoxy)methyl]-5-(3-propoxybenzyl)uracil (10y). A solution of 0.30 g (0.8 mmol) of **9y** in methanol (250 mL) saturated with ammonia gas was stirred in a stoppered flask for 18 h at ambient temperature. The methanol was removed *in vacuo*, and the residue was recrystallized from dichloromethane/hexane/1 drop of water to give 0.19 g (70%) of **10y** as a white solid: mp 85–87 °C; UV (0.1 N hydrochloric acid + 10% methanol) λ_{max} 266 (ϵ 9800), (0.1 N sodium hydroxide + 10% methanol) 265 nm (ϵ 10 900); NMR (DMSO-d_6) d 7.62 (s, 1H, H-6), 6.94 (m, 4H, ArH), 5.06 (s, 2H, NCH_2O), 4.68 (s, 1H, OH), 3.86 (t, 2H, J = 6.5 Hz, OCH_2), 3.47 (s, 6H, CH_2Ar, $(CH_2)_2$), 1.71 (dq, 2H, J = 6.5, 7.4 Hz, CCH_2C), 0.94 (t, 3H, J = 7.4 Hz, CH_3); MS *m/e* 335. Anal. ($C_{17}H_{22}N_2O_5 \cdot 0.25H_2O$) C, H, N.

Method I: 5-(4-Nitrobenzyl)uracil (7g). 5-Benzyluracil (3.0 g, 15.0 mmol) was added to a stirred mixture of concentrated sulfuric acid (15 mL) and 70% aqueous nitric acid (15 mL) over a 10-min period while the temperature was maintained at 25 °C using a water bath. When dissolution was complete, the solution was poured into ice water (300 mL). The precipitate was collected by filtration, washed with water and acetone, and dried under vacuum to give 3.07 g (83%) of crude product. Two recrystallizations from acetic acid gave analytically pure **7g**: mp 282–286 °C dec; NMR (Me_2SO-d_6) δ 11.13 (s, 1H, NH), 10.84 (s, 1H, NH), 7.83 (m, 5H, H-6, Ar), 3.66 (s, 2H, CH_2Ar); MS *me/* 248 (M^+). Anal. ($C_{11}H_9N_3O_4$) C, H, N.

Enzyme Assays. Inhibition of UrdPase from mouse liver was quantitated using a radiochemical assay.[32] The conversion of [2-^{14}C]uridine to [2-^{14}C]uracil was determined in 20 mM potassium phosphate buffer (pH 8.0), 1 mM EDTA, 170 μM [2-^{14}C]uridine (specific activity 7.1 μCi/μmol), 1 mM dithiothreitol, and enzyme with and without the inhibitor. After 30 min at 37 °C, the assay was terminated by heating in a 100 °C water bath for 1 min. TLC (Machery-Nagel; polygram sil G/UV254 plates developed in chloroform:methanol:acetic acid (90:5:5)) was used to separate uridine (R_f = 0.1) and uracil (R_f = 0.4). The amount of enzyme used in the assay was chosen to catalyze 10% conversion of uridine to uracil in the uninhibited reaction.

Animal Dosing and Blood Collection. Pharmacokinetic studies were performed on male CD rats implanted with a jugular vein cannula. The animals were placed in individual metabolic cages and fed chow and water overnight. Food was removed 6 h before the start of the experiment. UrdPase inhibitors were either suspended in 1% methyl cellulose or solubilized in saline (by adjusting to pH 8.5 with 0.1 N NaOH) and administered via gavage in a volume of 10 mL/kg. Whole blood samples (0.35 mL) were removed from the cannula using a 1 cc syringe containing 50 μL of 5% EDTA. Plasma was frozen at −20 °C and stored for HPLC analyses.

HPLC Analyses. Plasma concentrations of the UrdPase inhibitors were determined using reverse-phase HPLC.[32] Protein was removed from the samples by ultrafiltration using the Centrifree micropartition system (Amicon Division, W. R. Grace and Co., Beverly, MA) or by acetonitrile extraction. HPLC was performed on a reverse-phase Microsorb C18 column (250 mm × 4.6 mm i.d.; Rainin Instrument Co., Woburn, MA) with a Dynamax axial compression guard column.[32] An isocratic elution (1 mL/min) in 50 mM ammonium acetate buffer, pH 4.8, 0.5% acetronitrile was followed by a linear gradient to 36% acetonitrile in the same mobile phase. The exact time of each segment was dependent upon the elution properties of the UrdPase inhibitor being analyzed. The eluent was monitored by UV absorption at 265 nm. Uridine was eluted as a distinct peak under the HPLC conditions used for the analysis of each inhibitor. Control (t = 0) plasma uridine concentrations were 0.8 ± 0.3 μM (n = 29) in CD rats.

Acknowledgment. The authors acknowledge the assistance of R. Tansik and H. Roberts with the UrdPase inhibition assays. We thank A. Ragouzeos for some of the NMR spectra, D. Staton, S. Paris, L. Cotterman, and R. Carmichael for assistance in preparation of the manuscript, J. S. Downey and R. A. Sterlen for assistance in preparation of the synthetic intermediates, and M. Almond for helpful advice.

References

(1) (a) Krenitsky, T. A.; Barclay, M.; Jacquez, J. A. Specificity of Mouse Uridine Phosphorylase. Chromatography, Purification, and Properties. *J. Biol. Chem.* **1964**, *239*, 805–812. (b) Krenitsky, T. A.; Mellors, J. W.; Barclay, R. K. Pyrimidine Nucleosidases. Their Classification and Relationship to Uric Acid Ribonucleoside Phosphorylase. *J. Biol. Chem.* **1965**, *240*, 1281–1286.

(2) Baker, B. R. Irreversible Enzyme Inhibitors. LXXV. Inhibitors of Thymidine Phosphorylase. I. Mode of Ribofuranose Binding. *J. Med. Chem.* **1967**, *10*, 297–301.

(3) Baker, B. R.; Kelley, J. L. Irreversible Enzyme Inhibitors. CLXIX. Inhibition of FUDR Phosphorylase from Walker 256 Rat Tumor by 6-Substituted Uracils. *J. Med. Chem.* **1970**, *13*, 456–458.

(4) Baker, B. R.; Kelley, J. L. Irreversible Enzyme Inhibitors. 188. Inhibition of Mammalian Thymidine Phosphorylase. *J. Med. Chem.* **1971**, *14*, 812–816.

(5) Niedzwicki, J. G.; Chu, S. H.; El Kouni, M. H.; Rowe, E. C.; Cha, S. 5-Benzylacyclouridine and 5-Benzyloxybenzylacyclouridine, Potent Inhibitors of Uridine Phosphorylase. *Biochem. Pharmacol.* **1982**, *31*, 1857–1861.

(6) For a comprehensive review of phosphorylase ligands, see: Niedzwicki, J. G.; El Kouni, M. H.; Chu, S. H.; Cha, S. Structure-Activity Relationship of Ligands of the Pyrimidine Nucleoside Phosphorylases. *Biochem. Pharmacol.* **1983**, *32*, 399–415.

(7) el Kouni, M. H.; el Kouni, M. M.; Naguib, F. N. M. Differences in Activities and Substrate Specificity of Human and Murine Pyrimidine Nucleoside Phosphorylases: Implications for Chemotherapy with 5-Fluoropyrimidines. *Cancer Res.* **1993**, *53*, 3687–3693.

(8) Chu, M. Y. W.; Naguib, F. N. M.; Iltzsch, M. H.; el Kouni, M. H.; Chu, S. H.; Cha, S.; Calabresi, P. Potentiation of 5-Fluoro-2′-Deoxyuridine Antineoplastic Activity by the Uridine Phosphorylase Inhibitors Benzylacyclouridine and Benzyloxybenzylacyclouridine. *Cancer Res.* **1984**, *44*, 1852–1856.

(9) Darnowski, J. W.; Handschumacher, R. E. Tissue-specific Enhancement of Uridine Utilization and 5-Fluorouracil Therapy in Mice by Benzylacyclouridine. *Cancer Res.* **1985**, *45*, 5364–5368.

(10) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; Nusinoff-Lehrman, S.; the AZT Collaborative Working Group. The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex. *N. Engl. J. Med.* **1987**, *317*, 192–197.

(11) Yarchoan, R.; Weinhold, K. J.; Lyerly, H. K.; Gelmann, E.; Blum, R. M.; Shearer, G. M.; Mitsuya, H.; Collins, J. M.; Myers, C. E.; Klecker, R. W.; Markham, P. D.; Durack, D. T.; Lehrman, S. N.; Barry, D. W.; Fischl, M. A.; Gallo, R. C.; Bolognesi, D. P.; Broder, S. Administration of 3′-Azido-3′-deoxythymidine, an Inhibitor of HTLV-III/LAV Replication, to Patients with AIDS or AIDS-Related Complex. *Lancet* **1986**, 575–580.

(12) Sommadossi, J.-P.; Carlisle, R.; Schinaze, R. F.; 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.* **1988**, *32*, 997–1001.
(13) Calabresi, P.; Falcone, A.; St. Clair, M. H.; Wiemann, M. C.; Chu, S. H.; Darnowski, J. W. Benzylacyclouridine Reverses Azidothymidine-Induced Marrow-Suppression without Impairment of Anti-HIV Activity. *Blood* **1990**, *76*, 2210–2215.
(14) Falcone, A.; Darnowski, J. W.; Ruprecht, R. M.; Chu, S. H.; Brunetti, I.; Calabresi, P. Differential Effect of Benzylacyclouridine on the Toxic and Therapeutic Effects of Azidothymidine in Mice. *Blood* **1990**, *76*, 2216–2221.
(15) Baker, B. R.; Kelley, J. L. Irreversible Enzyme Inhibitors. CLXXI. Inhibition of FUDR Phosphorylase from Walker 256 Rat Tumor by 5-Substituted Uracils. *J. Med. Chem.* **1970**, *13*, 461–467.
(16) Kelley, J. L.; Baker, B. R. Irreversible Enzyme Inhibitors. 202. Candidate Active-Site-Directed Irreversible Inhibitors of FUDR Phosphorylase from Walker 256 Rat Tumor Derived from 1-Benzyl-5-(3-ethoxybenzyl)uracil. *J. Med. Chem.* **1982**, *25*, 600–603.
(17) Schaeffer, H. J.; Beauchamp, L.; de Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. 9-(2-Hydroxyethoxymethyl)guanine Activity Against Viruses of the Herpes Group. *Nature* **1978**, *272*, 583–585.
(18) Elion, G. B.; Furman, P. A.; Fyfe, J. A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H. J. Selectivity of Action of an Antiherpetic Agent, 9-(2-Hydroxyethoxymethyl)guanine. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5716–5720.
(19) Kelley, J. L. In *Topics in Medicinal Chemistry-Fourth SCI-RSC Medicinal Chemistry Symposium*; Leeming, P. R., Ed.; Royal Society of Chemistry: London, U.K., 1988; pp 189–212.
(20) Naguib, F. N.; el Kouni, M. H.; Chu, S. H.; Cha, S. New Analogues of Benzylacyclouridines, Specific and Potent Inhibitors of Uridine Phosphorylase from Human and Mouse Livers. *Biochem. Pharmacol.* **1987**, *36*, 2195–2201.
(21) Martin, D. S.; Stolfi, R. L.; Sawyer, R. C. Use of Oral Uridine as a Substitute for Parental Uridine Rescue of 5-Fluorouracil Therapy, with and without the Uridine Phosphorylase Inhibitor 5-Benzylacyclouridine. *Cancer Chemother. Pharmacol.* **1989**, *24*, 9–14.
(22) Naguib, F. N.; Levesque, D. L.; Wang, E.-C.; Panzica, R. P.; el Kouni, M. H. 5-Benzylbarbituric Acid Derivatives, Potent and Specific Inhibitors of Uridine Phosphorylase. *Biochem. Pharmacol.* **1993**, *46*, 1273–1283.
(23) Johnson, T. B.; Ambelang, J. C. Researches on Pyrimidines. CLIX. Synthesis of 6-Benzyl and 5-Benzyluracils. *J. Am. Chem. Soc.* **1938**, *60*, 2941–2944.
(24) Falco, E. A.; Russell, P. B.; Hitchings, G. H. The Preparation of Some Substituted 2-Thiouracils and 2,4-Dimercaptopyrimidines. *J. Am. Chem. Soc.* **1951**, *73*, 4466–4468.
(25) Orr, G. F.; Musso, D. L. Improved Synthesis of 5-Benzyl-2-Thiouracils, *Synth. Commun.*, in press.
(26) Basnak, I.; Farkas, J. Synthesis of Uracils Substituted in the Position 5 or 5,6 with Alkyl or Cycloalkyl Groups and Their UV Spectra. *Collect. Czech. Chem. Commun.* **1979**, *44*, 2426–2437.
(27) Johnson, T. B.; Hemingway, E. H. Researches on Pyrimidines. CLIX. Synthesis of 4-Phenylcytosine. *J. Am. Chem. Soc.* **1915**, *37*, 378–383.
(28) Robins, M. J.; Hatfield, P. W. Nucleic Acid Related Compounds 37. Convenient and High-Yield Synthesis of N-[(2-hydroxyethoxy)methyl]heterocycles as "Acyclic Nucleoside" Analogues. *Can. J. Chem.* **1982**, *60*, 547–549.
(29) Beauchamp, L. M.; Serling, B. L.; Kelsey, J. E.; Biron, K. K.; Collins, P.; Selway, J.; Lin, J.-C.; Schaeffer, H. J. Effect of Acyclic Pyrimidines Related to 9-[(1,3-Dihydroxy-2-propoxy)methyl]-guanine on Herpesviruses. *J. Med. Chem.* **1988**, *31*, 144–149.
(30) Gasser, T.; Moyer, J. D.; Handschumacher, R. E. Novel Single-Pass Exchange of Circulating Uridine in Rat Liver. *Science* **1981**, *213*, 777–779.
(31) Monks, A.; Cysyk, R. L. Uridine Regulation By the Isolated Rat Liver: Perfusion With an Artificial Oxygen Carrier. Am. *J. Physiol.* **1982**, *242*, 465–470.
(32) Davis, S. T.; Joyner, S. S.; Chandrasurin, P.; Baccanari, D. P. Species-Dependent Differences in the Biochemical Effects and Metabolism of 5-Benzylacyclouridine. *Biochem. Pharmacol.* **1993**, *45*, 173–181.
(33) Still, W. C.; Kahn, M.; Mutra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(34) Stodola, F. H. Base-Catalyzed Preparation of Methyl and Ethyl Esters of Carboxylic Acids. *J. Org. Chem.* **1964**, *29*, 2490–2491.
(35) Bruce, J. M.; Creed, D.; Dawes, H. Light-induced and Related Reactions of Quinones. Part VIII. Some (2-Hydroxyalkyl)-, Phenethyl-, (2-Ethoxycarbonylethyl)-, and Styryl-1,4-benzoquinones. *J. Chem. Soc. C* **1971**, 3749–3756.

JM950258D