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Articles

Inhibition of Uridine Phosphorylase. Synthesis and Structure−Activity Relationships of Aryl-Substituted 1-((2-Hydroxyethoxy)methyl)-5-(3-phenoxybenzyl)uracil

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Structure−activity relationship studies on a series of 1-((2-hydroxyethoxy)methyl)-5-(3-(substituted-phenoxy)benzyl)uracils as inhibitors of murine liver uridine phosphorylase have led to compounds with IC_{50}s as low as 1.4 nM. The two most potent compounds, **10j** (3-cyanophenoxy) and **11f** (3-chlorophenoxy) were tested *in vivo* for effects on steady-state concentrations of circulating uridine in mice and rats. Both compounds were substantially more efficacious than BAU (5-benzylacyclouridine) both *in vitro* and *in vivo*.

Uridine phosphorylase (EC 2.4.2.3) (UrdPase) and thymidine phosphorylase (EC 2.4.2.4)[1] are mammalian enzymes that catalyze the reversible phosphorolysis of pyrimidine nucleosides. These phosphorylases, which function as salvage enzymes in pyrimidine metabolism, are responsible for the degradation of chemotherapeutic agents such as 5-fluoro-2′-deoxyuridine (FUDR).[2] Several researchers have studied both the antineoplastic applications[3−5] of UrdPase inhibitors such as BAU (5-benzylacyclouridine) and their use in combination with zidovudine as a means of reducing the severity of bone marrow suppression.[6−10]

Early research on the inhibition of UrdPase led to the development of 5-benzyluracils (BUs) with good inhibitory potency.[11−14] Later work by Niedzwichi, *et al.* provided (2-hydroxyethoxy)methyl derivatives of BUs such as BAU and BBAU (5-(*m*-(benzyloxy)benzyl)-acyclouridine) with enhanced potency against the enzyme from sarcoma S-180 cytosol and the cytosol of mouse liver.[15,16] More recent studies have led to 1′-hydroxymethyl, 1′-aminomethyl, and barbituric acid derivatives with potent inhibitory properties.[16−19]

We recently reported SAR studies of the effect of aryl substituents on the inhibitory potency of BAU.[20] The most potent inhibitors had IC_{50}s ranging from 27 to 70 nM, and several compounds were found to elevate plasma uridine concentrations by 3−9-fold in rats. We now report research on 5-(3-phenoxybenzyl)uracils, which has led to a series of 1-((2-hydroxyethoxy)methyl)-5-(3-(substituted-phenoxy)benzyl)uracils with IC_{50}s as low as 1.4 nM.

Chemistry Discussion

The target inhibitors in Table 1 were prepared in several steps as illustrated in Schemes 1 and 2. The intermediate substituted 3-phenoxyphenyl propionates **5** were prepared by alkylation of the appropriate phenol precursors **1** with 1-bromo-3-fluorobenzene to give the diphenyl ethers **2** (Scheme 1). Reaction of **2** with ethyl acrylate under Heck conditions gave the phenoxyphenyl acrylates **3**, which were reduced to the propionates **5** via catalytic hydrogenation. The parent unsubstituted 3-phenoxyphenyl propionate **5** (R = H) was prepared

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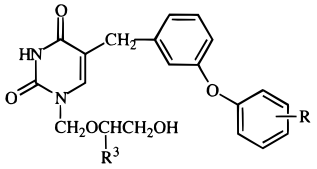
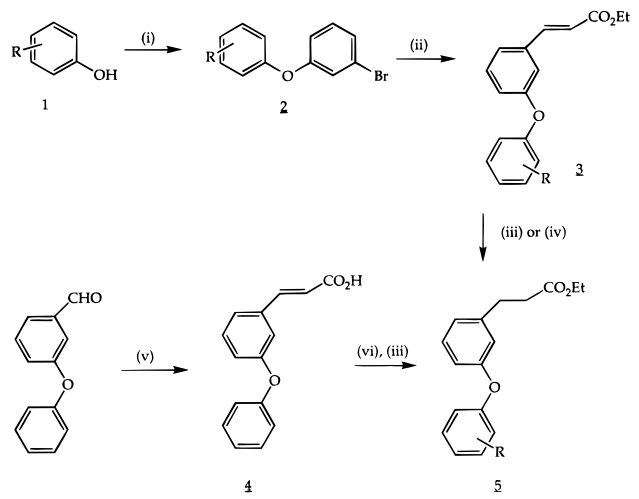
Table 1. Physical Properties of 1-Substituted 5-(3-Phenoxybenzyl)uracils



compd no.	R	R^3	method	yield, %	mp, °C	formula[a]	IC_{50}, nM
10a	H	H	H, I	38	93−95	$C_{20}H_{20}N_2O_5$	14
11a	H	CH_2OH	H, I	23	139−141	$C_{21}H_{22}N_2O_6$	10
10b	4-F	H	H, I	42	106−109	$C_{20}H_{19}FN_2O_5$	98
11b	4-F	CH_2OH	H, I	15	147−148	$C_{21}H_{21}FN_2O_6$	22
10c	4-Cl	H	H, I	55	109−110	$C_{20}H_{19}ClN_2O_5$	84
10d	4-OCH_3	H	H, I	40	110−112	$C_{21}H_{22}N_2O_6$	210
10e	3-F	H	H, I	28	98−100	$C_{20}H_{19}FN_2O_5$	14
11e	3-F	CH_2OH	H, I	15	122−123	$C_{21}H_{21}FN_2O_6$	8.2
10f	3-Cl	H	H, I	65	118−120	$C_{20}H_{19}ClN_2O_5$	15
11f	3-Cl	CH_2OH	H, I	15	118−119	$C_{21}H_{21}ClN_2O_6$	1.4
10g	3-CF_3	H	H, I	36	103−105	$C_{21}H_{19}F_3N_2O_5$	32
10h	3-CH_3	H	H, I	46	110−111	$C_{21}H_{22}N_2O_5$	13
10i	3-OCH_3	H	H, I	23	98−102	$C_{21}H_{22}N_2O_6$	31
10j	3-CN	H	H, I	40	115−117	$C_{21}H_{19}N_3O_5$	5.9
12	BAU	H	H, I	59	144−146	$C_{14}H_{16}N_2O_4$	460
13	BBAU	H	H, I	39	111−112	$C_{21}H_{22}N_2O_5$	84

[a] All compounds were analyzed for C, H, and N.

Scheme 1[a]



[a] Reagents: (i) NaOMe, MeOH then *m*-bromofluorobenzene, *N*-methylpyrrolidinone, 180 °C; (ii) ethyl acrylate, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Et_3N, MeCN, 125 °C in bomb; (iii) H_2, PtO_2, EtOH, (iv) H_2, 10% Pd−C, EtOH; (v) malonic acid, pyridine, piperidine, 90 °C; (vi) 1.0 N ethereal HCl, EtOH, reflux.

via the acrylic acid **4** from 3-phenoxybenzaldehyde as illustrated in Scheme 1.

The intermediate 2-thioxo-4-pyrimidinones **7** (Scheme 2) were prepared by treating the 3-phenoxyphenyl propionates **5** with potassium *tert*-butoxide, followed by reaction of the potassium salt **6** with ethyl formate and condensation with thiourea[21] to give the 2-thioxo-4-pyrimidinones **7a**−**7j** (Table 3) in 53−84% yields. Hydrolysis of the 2-thioxo-4-pyrimidinones **7** in refluxing aqueous chloroacetic acid[22] provided the 5-(3-phenoxybenzyl)uracils **8a**−**8j** (Table 3) in 61−94% yield.

The 5-benzyluracils **8a**−**8j** were alkylated via the O-silylated intermediates, which were formed using bis(trimethylsilyl)acetamide, with either (2-acetoxyethoxy)methyl bromide[23] or 2-(bromomethoxy)-1,3-propanediyl dibenzoate.[24] The intermediate esters **9** were hydrolyzed with methanolic ammonia to give the 1-substituted-5-(3-phenoxybenzyl)uracils **10** and **11** in satisfactory yields (Table 1).

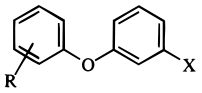
Inhibition of Uridine Phosphorylase: Structure−Activity Relationships. The 1-substituted 5-(3-phenoxybenzyl)uracils in Table 1 were tested for inhibition of UrdPase from murine liver. The IC_{50} is the concentration of inhibitor that results in a 50% decrease in reaction velocity. Our previous work[20] focused on the effects of aryl substituents on the inhibitory potency of the known UrdPase inhibitor BAU. The most active analogs were those with a 3-alkoxy substituent on the aryl moiety with IC_{50}s as low as 27 nm. We subsequently found that the 3-phenoxy analogs **10a** and **11a** are somewhat more potent than the alkoxy compounds with IC_{50}s =14 and 10 nm, respectively. The focus of the work reported herein was to evaluate the effects of substituents on the 3-phenoxy ring on the UrdPase inhibitory potency and on the pharmacokinetics of plasma uridine levels.

Substitution in the para position of the phenoxy ring with a F (**10b**), Cl (**10c**), or OCH_3 (**10d**) substituent, and with the (2-hydroxyethoxy)methyl side chain at N-1, resulted in a loss of potency with IC_{50}s of 98, 84, and 210 nm, respectively. The 4-fluoro analog (**11b**), which contains the branched side chain at N-1, was more potent (IC_{50} = 22 nm) than **10b**.

Derivatives containing a substituent in the meta position [F (**10e**), Cl (**10f**), CF_3 (**10g**), CH_3 (**10h**), and OCH_3 (**10i**)], and the (2-hydroxyethoxy)methyl side chain, gave compounds with IC_{50}s comparable to that of the parent **10a** (IC_{50}s ranging from 32 to 14 nm). The 3-cyano analog **10j** was more potent with an IC_{50} of 5.9 nm. The 3-F (**11e**) and 3-Cl (**11f**) analogs, which contain the branched side chain, were also more potent than the parent **10a** with IC_{50}s of 8.2 and 1.4 nm, respectively.

To summarize, strongly electron withdrawing groups in the meta position of the phenoxy moeity result in more potent analogs, while strongly electron donating groups result in a loss of potency. Furthermore, branch-

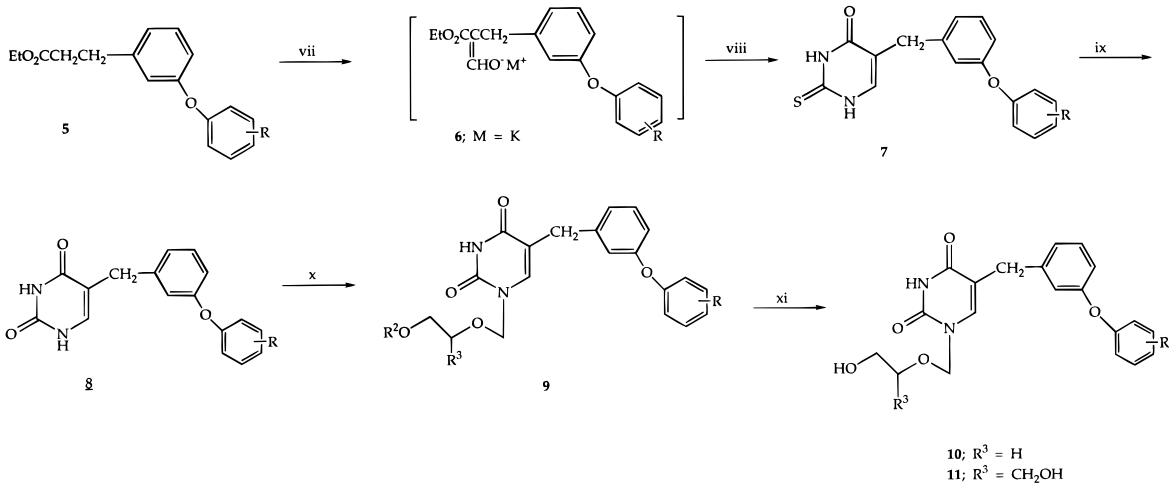
Table 2. Physical Properties of Diphenyl Ethers



compd no.	R	X	method	yield %	mp, °C	formula[a]
2a	4-F	Br	A	47	oil	$C_{12}H_8BrFO$
2b	4-Cl	Br	A	53	oil	$C_{12}H_8BrClO$
2c	4-OCH_3	Br	A	30	oil	$C_{13}H_{11}BrO2$
2d	3-F	Br	A	43	oil	$C_{12}H_8BrFO$
2e	3-Cl	Br	A	63	oil	$C_{12}H_8BrClO$
2f	3-CF_3	Br	A	31	oil	$C_{13}H_8BrF_3O$
2g	3-CH_3	Br	A	17	oil	$C_{13}H_{11}BrO$
2h	3-OCH_3	Br	A	29	oil	$C_{13}H_{11}BrO_2$
2i	3-CN	Br	A	30	oil	$C_{13}H_8BrNO$
2j	2-F	Br	A	27	oil	$C_{12}H_8BrFO$
3a	4-F	CH=$CHCO_2Et$	B	84	oil	$C_{17}H_{15}FO_3$
3b	4-Cl	CH=$CHCO_2Et$	B	67	oil	$C_{17}H_{15}ClO_3$
3c	4-OCH_3	CH=$CHCO_2Et$	B	91	oil	$C_{18}H_{18}O_4$
3d	3-F	CH=$CHCO_2Et$	B	79	oil	$C_{17}H_{15}FO_3$
3e	3-Cl	CH=$CHCO_2Et$	B	79	oil	$C_{17}H_{15}ClO_3$
3f	3-CF_3	CH=$CHCO_2Et$	B	(b)	oil	
3g	3-CH_3	CH=$CHCO_2Et$	B	89	oil	$C_{18}H_{18}O_3$
3h	3-OCH_3	CH=$CHCO_2Et$	B	71	oil	$C_{18}H_{18}O_4$
3i	3-CN	CH=$CHCO_2Et$	B	90	oil	$C_{18}H_{15}NO_3$
3j	2-F	CH=$CHCO_2Et$	B	58	oil	$C_{17}H_{15}FO_3$
4	H	CH=$CHCO_2H$	C	80	111−113[c]	$C_{15}H_{12}O_3$
5a	H	$CH_2CH_2CO_2Et$	D, E	94[b]	oil	
5b	4-F	$CH_2CH_2CO_2Et$	E	90	oil	$C_{17}H_{17}FO_3 \cdot 0.15H_2O$
5c	4-Cl	$CH_2CH_2CO_2Et$	E	88	oil	$C_{17}H_{17}ClO_3$
5d	4-OCH_3	$CH_2CH_2CO_2Et$	E	96	oil	$C_{18}H_{20}O_4$
5e	3-F	$CH_2CH_2CO_2Et$	E	93	oil	$C_{17}H_{17}FO_3$
5f	3-Cl	$CH_2CH_2CO_2Et$	E	87	oil	$C_{17}H_{17}ClO_3$
5g	3-CF_3	$CH_2CH_2CO_2Et$	E	93	oil	$C_{18}H_{17}F_3O_3$
5h	3-CH_3	$CH_2CH_2CO_2Et$	E	99	oil	$C_{18}H_{20}O3 \cdot 0.33H_2O$
5i	3-OCH_3	$CH_2CH_2CO_2Et$	E	91	oil	$C_{18}H_{20}O_4$
5j	3-CN	$CH_2CH_2CO_2Et$	E[d]	70	oil	$C_{18}H_{17}NO_3 \cdot 0.10H_2O$
5k	2-F	$CH_2CH_2CO_2Et$	E	36	oil	$C_{17}H_{17}FO_3$

[a] All compounds were analyzed for C and H. [b] This compound was used without purification. [c] Recrystallized from MeCN−water. [d] The 3-cyano analog, **3i**, was reduced using 10% palladium−carbon instead of platinum oxide.

Scheme 2[a]

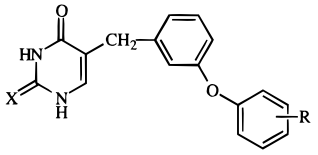


[a] Reagents: (vii) potassium *tert*-butoxide in THF, HCO_2Et, Et_2O; (viii) thiourea, 2-PrOH, reflux; (ix) glacial HOAc, 20% aqueous $ClCH_2CO_2H$, reflux; (x) BSA, $ClCH_2CH_2Cl$, reflux then $BrCH_2OCH(R^3)CH_2OR^2$; (xi) MeOH, NH_3, room temperature.

ing of the acyclic side chain (R_3 = $CH_2OCH(CH_2OH)$-CH_2OH in Table 1) also increases potency. The IC_{50}s of **10j** and **11f** represent 4.5-fold and 19-fold increases in potency, respectively, as compared to our best inhibitors reported previously.[20]

Elevation of Plasma Uridine Levels. Plasma uridine concentration is maintained at a steady-state level *via* a balance between synthesis and catabolism.[25,26] When catabolism is inhibited, continued biosynthesis results in a time-dependent increase in plasma uridine levels. The pharmacodynamic effects of **10j** (IC_{50} = 5.9 nM) and **11f** (IC_{50} = 1.4 nM) were compared to those of BAU (IC_{50} = 460 nM) in BDF1 mice (Figure 1A). Following treatment with 30 mg/kg of **10j**, plasma uridine levels increased over 6 h and achieved a maximum concentration of 28 μM, a 10-fold elevation

Table 3. Physical Properties of 5-(3-Phenoxybenzyl)-2-thiouracils and 5-(3-Phenoxybenzyl)uracils



compd no.	R	X	method	yield, %	mp, °C	formula[a]
7a	H	S	F	72	195−197	$C_{17}H_{14}N_2O_2S$
8a	H	O	G	72	281−283	$C_{17}H_{14}N_2O_3$
7b	4-F	S	F	76	199−201	$C_{17}H_{13}FN_2O_2S$
8b	4-F	O	G	92	260−263	$C_{17}H_{13}FN_2O_3$
7c	4-Cl	S	F	80	187−189	$C_{17}H_{13}ClN_2O_2S$
8c	4-Cl	O	G	88	255−257	$C_{17}H_{13}ClN_2O_3$
7d	4-OCH_3	S	F	63	179−181	$C_{18}H_{16}N_2O_3S$
8d	4-OCH_3	O	G	93	238−240	$C_{18}H_{16}N_2O_4$
7e	3-F	S	F	53	169−171	$C_{17}H_{13}FN_2O_2S$
8e	3-F	O	G	94	243−245	$C_{17}H_{13}FN_2O_3$
7f	3-Cl	S	F	54	163−165	$C_{17}H_{13}ClN_2O_2S$
8f	3-Cl	O	G	88	209−211	$C_{17}H_{13}ClN_2O_3$
7g	3-CF_3	S	F	84	149−151	$C_{18}H_{13}F_3N_2O_2S$
8g	3-CF_3	O	G	61	203−205	$C_{18}H_{13}F_3N_2O_3$
7h	3-CH_3	S	F	62	161−163	$C_{18}H_{16}N_2O_2S$
8h	3-CH_3	O	G	85	229−230	$C_{18}H_{16}N_2O_3$
7i	3-OCH_3	S	F	79	165−168	$C_{18}H_{16}N_2O_3S$
8i	3-OCH_3	O	G	78	188−190	$C_{18}H_{16}N_2O_4$
7j	2-F	S	F	58	210−213	$C_{17}H_{13}FN_2O_2S$
8j	2-F	O	G	90	266−268	$C_{17}H_{13}FN_2O_3$

[a] All compounds were analyzed for C, H, and N.

over the predose value. Compound **11f** produced 20 μM uridine (peak at 2 h postdose), whereas BAU elevated plasma uridine to 11 μM (peak at 1 h postdose). The enhanced effects of **10j** and **11f** compared to BAU were evident within the first 2 h after dosing, at a time when the plasma drug concentrations were similar (Figure 1B). Thus both compounds were more potent than BAU *in vivo*. In addition, **10j** and **11f** exhibited biphasic elimination kinetics, and the somewhat more pronounced pharmacodynamic effect of **10j** may be due to its higher plasma levels at the latter time points.

The perturbation of plasma uridine by BAU is species-dependent, and the rat is a stringent test for the efficacy of UrdPase inhibitors *in vivo* .[27] When **10j** or **11f** (90 mg/kg, po) was administered to rats, plasma uridine levels were increased 4−8-fold for 4 h (Figure 2A). The elevation in plasma uridine levels produced by **10j** and **11f** in rats persisted for at least 1.3 times longer than the elevation produced by one of our best inhibitors reported previously.[20] In contrast, the same dose of BAU elevated plasma uridine 2−4-fold (peak at 30 min postdose), and plasma uridine was back to control level within 5 h. The enhanced pharmacodynamic effects of **10j** and **11f** were produced by plasma drug concentrations that were 2−10-fold less than BAU for the first 3 h.

Conclusions

A systematic study of the effect of aryl substituents on potency has led to 5-(3-phenoxybenzyl)uracils **10j** and **11f**, which have IC_{50}s of 5.9 and 1.4 nM, respectively, for inhibition of UrdPase. Compound **10j** exhibited the greater effect on circulating uridine levels in BDFI mice. This compound was substantially more active than BAU at equivalent doses and elevated circulating uridine levels for a longer period of time. Compound **10j** is a good candidate for antineoplastic studies in combination with chemotherapeutic agents such as 5-fluorouracil and 5-fluoro-2′-deoxyuridine.

Experimental Section

Melting points were taken in capillary tubes using a Thomas-Hoover Unimelt and are uncorrected. The NMR spectra were recorded on a Varian XL-100-15-FT or a Varian FT-80A spectrometer. The UV absorption spectra were measured on a Cary 118 UV−vis spectrophotometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on thin-layer chromatography (TLC). The TLC's were developed on Whatman 200 m MK6F plates of silica gel with fluorescent indicator. Preparative flash chromotography as performed on silica gel 60 (40-63 mm, E. Merck No. 9385) using the method of Still et al.[28] Elemental analyses were performed by Atlantic Microlab, Inc.

Method A. 3-Bromo-3'-fluorodiphenyl Ether (2d). A solution of 3-fluorophenol (8.25 g, 74 mmol) and 25% sodium methoxide in methanol (18.5 mL, 81 mmol) was stirred for 3 h at ambient temperature. The solution was concentrated *in vacuo* to a tan solid. The phenoxide was dissolved in *N*-methyl-2-pyrrolidinone (50 mL) and heated in a 180 °C oil bath. 1-Bromo-3-fluorobenzene (12.95 g, 74 mmol) was added, and the mixture was stirred for 24 h at 180 °C. After being cooled to room temperature, the reaction mixture was diluted with water (100 mL) and extracted with dichloromethane (2 × 100 mL). The organic layer was concentrated to dryness *in vacuo*, redissolved in diethyl ether (100 mL), washed with water (100 mL) and a saturated aqueous sodium bicarbonate solution (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to a brown oil (12.5 g). The oil was chromatographed on silica gel 60, eluting with hexane. The fractions containing only 3-bromo-3′-fluorodiphenyl ether were combined and concentrated *in vacuo* to give 8.42 g (43%) of **2d** as a clear, colorless oil; NMR (DMSO-d_6): δ 7.14-6.87 (m, 4H, Ar), 7.53−7.29 (m, 4H, Ar).

Method B. Ethyl 3-(3-(3-Fluorophenoxy)phenyl)acrylate (3d). A mixture of **2d** (5.30 g, 19.8 mmol), ethyl acrylate (2.48 g, 24.8 mmol), triethylamine (2.51 g, 24.8 mmol), palladium acetate (44.5 mg, 0.2 mmol), tri-*o*-tolylphosphine (244 mg, 0.8 mmol), and acetonitrile (20 mL) were added to a heavy

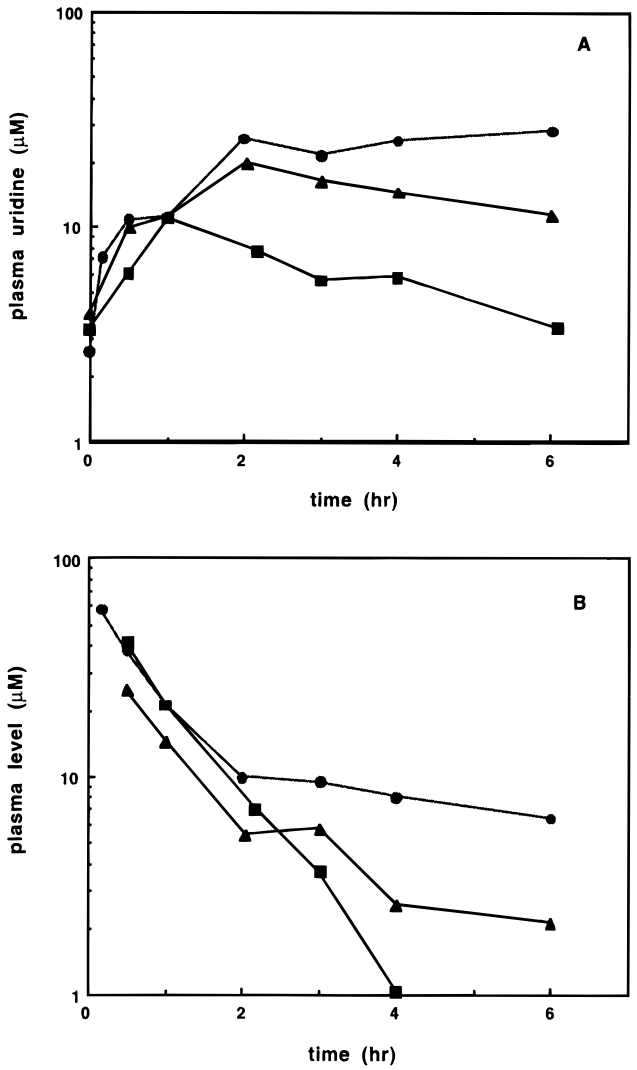


Figure 1. Plasma profiles of uridine and UrdPase inhibitors in mice following intraperitoneal administration of 30 mg/kg BAU (squares), **10i** (circles), or **11f** (triangles). Blood samples were collected at the times indicated and assayed for (A) plasma uridine and (B) plasma drug concentration. Each point is the average value from three mice.

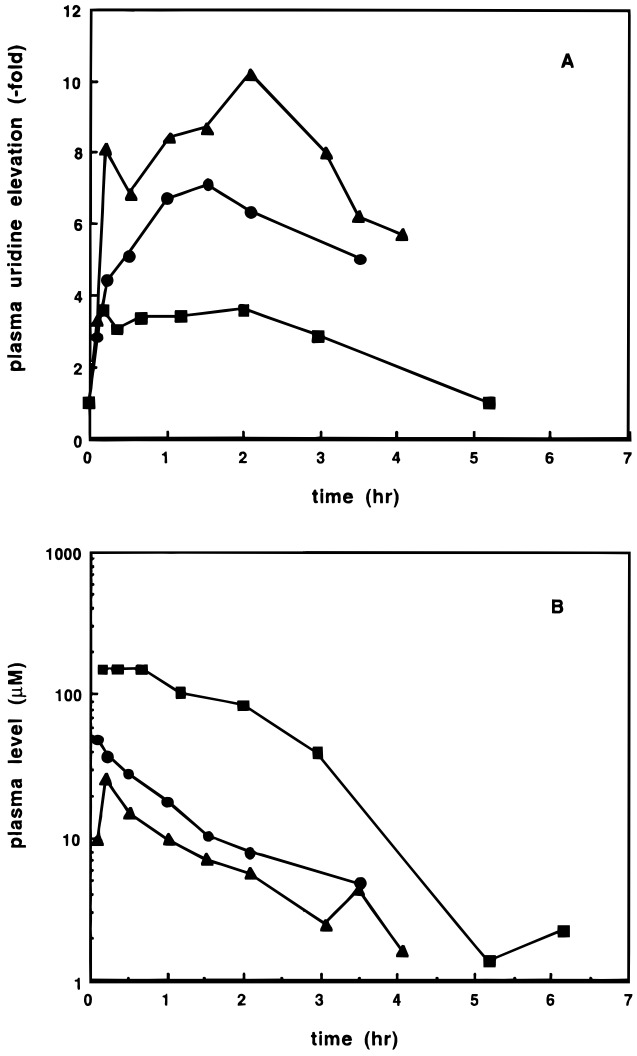


Figure 2. Plasma profiles of uridine and UrdPase inhibitors in rats following oral administration of 90 mg/kg BAU (squares), **10i** (circles), or **11f** (triangles). Blood samples were collected at the times indicated and assayed for (A) plasma uridine and (B) plasma drug concentration. Points are the average value from two to three rats. Control plasma uridine concentration ranged from 0.6 to 1.1 mM in the various experiments.

glass-walled bottle, flushed with nitrogen, sealed, and heated in a 100 °C oil bath for 5 h. Cold 0.1 N hydrochloric acid (40 mL) was added to the cooled reaction mixture. The lower layer was collected and concentrated *in vacuo* to a dark-green oil and chromatographed on silica gel 60 with 0−3% ethyl acetate/hexanes as eluent. The fractions containing only ethyl 3-(3-(3-fluorophenoxy)phenyl)acrylate were combined and concentrated *in vacuo* to give 4.48 g (79%) of **3d** as a clear, colorless oil: NMR (DMSO-d_6): δ 7.66 (d, 1H, J = 16 Hz, =CH), 7.60−7.31 (m, 4H, Ar), 7.15-6.83 (m, 4H, Ar), 6.68 (d, 1H, J = 16 Hz, =CH), 4.20 (q, 2H, J = 7 Hz, CH_2), 1.26 (t, 3H, J = 7 Hz, CH_3).

Method C. (*E*)-3-(3-Phenoxyphenyl)-2-propenoic Acid (4). A solution of 3-phenoxybenzaldehyde (25.0 g, 126 mmol), malonic acid (26.2 g, 252 mmol), and piperidine (2.0 mL, 20 mmol) in pyridine (50 mL) was stirred in an oil bath heated at 90 °C for 18 h. After being cooled to ambient temperature, the solution was poured into cold water (1 L). The pH of the aqueous mixture was adjusted to pH 2 with concentrated hydrochloric acid. The solids which formed were collected by suction filtration, washed with water, and recrystallized from acetonitrile/water to give 24.18 g (80%) of **4** as a white solid: mp 111−113 °C; TLC, methanol: dichloromethane (1:19), one spot with R_f = 0.25; NMR ($CDCl_3$) δ 7.71 (d, 1H, J = 16 Hz, ArC*H*), 7.40-7.00 (m, 9H, ArH), 6.38 (d, 1H, J = 16 Hz, C*H*CO_2).

Method D. (*E*)-Ethyl 3-(3-Phenoxyphenyl)-2-propenoate (5a). A solution of **4** (14.51 g, 60.4 mmol) and 1.0 M ethereal hydrochloric acid (40 mL) in absolute ethanol (150 mL) was refluxed with stirring under nitrogen for 24 h. The ethanol was removed *in vacuo* and the residue taken up in ethyl acetate (150 mL) and washed with a saturated aqueous sodium bicarbonate solution (2 × 75 mL). The washes were back-extracted with ethyl acetate (50 mL) and the combined extracts washed with brine, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated *in vacuo* to give 15.31 g (94%) of **5a** as a yellow oil, which was used without further purification: NMR (DMSO-d_6) δ 7.62 (d, 1H, ArCH), 7.12 (m, 9H, Ar), 6.36 (d, 1H, $CHCO_2$), 4.24 (q, 2H, CH_2),1.32 (t, 3H, CH_3).

Method E. Ethyl 3-(3-(3-Fluorophenoxy)phenyl)propionate (5e). To a solution of **3d** (6.33 g, 22 mmol) and absolute ethanol (100 mL) was added platinum oxide hydrate (100 mg, 0.44 mmol). The mixture was shaken on a Parr apparatus under hydrogen atmosphere (30 psi) for 6 h. The mixture was filtered to remove the catalyst and concentrated *in vacuo* to give 5.91 g (93%) of **5e** as a clear, colorless oil: NMR (DMSO-d_6): δ 7.48−7.30 (m, 2H, Ar), 7.10−6.78 (m, 6H, Ar), 4.03 (q, 2H, J = 7.0 Hz, CH_3CH_2O), 2.87 (t, 2H, J = 7.6 Hz, CH_2), 2.63 (t, 2H, J = 7.4 Hz, CH_2Ar), 1.14 (t, 3H, J = 7.0 Hz, CH_3CH_2O).

Method F. 1,2-Dihydro-5-(3-(3-fluorophenoxy)benzyl)-2-thioxo-4(3*H*)-pyrimidinone (7e). A solution of **5e** (5.51 g, 19.0 mmol) and ethyl formate (3.07 g, 41.4 mmol) in diethyl ether (35 mL) was added dropwise with stirring to a solution of potassium *tert*-butoxide (1 M in THF, 47.0 mL, 47.0 mmol) in diethyl ether (125 mL) cooled in an ice bath under nitrogen. The solution was stirred at ambient temperature for 18 h, and the solvent was removed *in vacuo*. The residue was dissolved in 2-propanol (50 mL), thiourea (2.89 g, 38.0 mmol) was added, and the mixture was refluxed under nitrogen for 5 h. The solvent was removed *in vacuo*, and the solid residue was washed with cold diethyl ether and dissolved in cold water, and the pH was adjusted to 4 with glacial acetic acid. The beige 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 3.29 g (53%) of **7e**, mp 170−172 °C dec. Recrystallization of 0.40 g from methanol gave 0.20 g of an analytically pure sample: NMR (DMSO-d_6) δ 12.45 (s, 1H, NH), 12.25 (s, 1H, NH), 7.11 (m, 9H, Ar and H-6), 3.55 (s, 2H, CH_2Ar); MS: *m/e* 329 (M^+).

Method G. Preparation of 5-(3-(3-Fluorophenoxy)-benzyl)uracil (8e). A suspension of **7e** (2.70 g, 8.2 mmol) in glacial acetic acid (40 mL) and 20% aqueous chloroacetic acid (40 mL) was refluxed with stirring for 6 h. After being cooled to ambient temperature and then in an ice bath, the mixture was filtered and the solids were washed with water and ether and dried in a vacuum oven at 80 °C for 18 h to give 2.34 g (91%) of **8e** as an off-white solid, mp 243−245 °C. Recrystallization of 0.400 g from acetic acid−water gave 0.161 g of an analytically pure sample: NMR (DMSO-d_6): δ 11.10 (s, 1H, NH), 10.75 (s, 1H, NH), 7.10 (m, 9H, Ar and H-6), 3.50 (s, 2H, CH_2Ar); MS *m/e* 313 (M^+).

Method H. Preparation of 1-((2-Acetoxyethoxy)methyl)-5-(3-(3-fluorophenoxy)benzyl)uracil (9e). Bis(trimethylsilyl)acetamide (1.38 mL, 5.6 mmol) was added to a stirred suspension of **8e** (1.00 g, 3.2 mmol) in dichloroethane (35 mL) under nitrogen. The mixture was refluxed with stirring for 1 h, the heat was removed, and the solution which formed was cooled in an ice bath. A solution of (2-acetoxyethoxy)methyl bromide (0.55 g, 2.8 mmol) in acetonitrile (4 mL) was added to the cooled solution, and the resulting solution was allowed to warm to ambient temperature and stirred under nitrogen for 18 h. The solvents were removed *in vacuo*, and the residue was dissolved in dichloromethane (75 mL) and washed with water (3 × 25 mL) and brine. The solvents 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:2-propanol (100:2), and the fractions containing product were combined. The solvents were removed *in vacuo* to give 0.58 g (48%) of **9e** as a clear oil: NMR (DMSO-d_6) δ 11.42 (s, 1H, NH), 7.69 (s, 1H, H-6), 7.11 (m, 8H, Ar), 5.08 (s, 2H, NCH_2O), 4.07 (t, 2H, OCH_2), 3.68 (t, 2H, CH_2CH_2O), 3.54 (s, 2H, CH_2Ar), 1.96 (s, 3H, CH_3); MS *m/e* 429.

Method I. Preparation of 1-((2-Hydroxyethoxy)methyl)-5-(3-(3-fluorophenoxy)benzyl)uracil (10e). A solution of 0.53 g (1.2 mmol) of **9e** in methanol (75 mL) saturated with ammonia gas was stirred in a stoppered flask for 24 h at ambient temperature. The methanol was removed *in vacuo*, and the residue was recrystallized from 2-propanol:hexane and dried in a vacuum oven at 80 °C to give 0.282 g (74%) of **10e** as a white solid: mp 98−100 °C; NMR (DMSO-d_6) δ 11.40 (s, 1H, NH), 7.69 (s, 1H, H-6), 7.11 (m, 8H, ArH), 5.08 (s, 2H, NCH_2O), 4.68 (s, 1H, OH), 3.54 (s, 2H, CH_2Ar), 3.49 (s, 4H, $(CH_2)_2$) MS *m/e* 387.

Enzyme Assays. Inhibition of UrdPase from mouse liver was quantitated using a radiochemical assay previously described.[27] Briefly, 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 ± inhibitor. After 30 min at 37 °C, the assay was terminated by heating in a 100 °C water bath for 1 min, and uridine and uracil were separated via TLC. 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 conducted in mice and rats. UrdPase inhibitors were solubilized in saline by adjusting to pH 9 with 0.1 N NaOH and administered in a volume of 10 mL/kg. Mice (groups of 18−21 BDF1 females) were dosed ip, and blood was collected from three mice at each time point. Whole blood was obtained by cardiac puncture of CO_2-anesthetized mice with a syringe containing 50 μL of 5% EDTA, and plasma was isolated by centrifugation (3000*g* for 10 min). 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. Rats (three/group) were dosed po, and whole blood samples (0.35 mL) were removed from the cannula using a 1 cm^3 syringe containing 50 μL of 5% EDTA. This volume of blood was replaced by the injection of 0.35 mL of saline at each time point. The ability to take sequential blood samples from the same rat resulted in a standard error of approximately 10% between replicates compared to 35% in the mouse studies where the pharmacokinetic curves were constructed from single blood samples taken from individual mice. Plasma was frozen at −20 °C and stored for HPLC analyses.

HPLC Analyses. Plasma concentrations of uridine and the UrdPase inhibitors were determined using reverse-phase HPLC.[27] Briefly, 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. An isocratic elution (1 mL/min) in 50 mM ammonium acetate buffer, pH 4.8, 0.5% acetonitrile 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 effluent was monitored by UV absorption at 265 nm. Uridine eluted as a distinct peak under the HPLC conditions used for the analysis of each inhibitor. Control (t = 0) plasma uridine concentrations in BDF1 mice and CD rats were 3.3 ± 1.4 μM (n = 9) and 0.8 ± 0.3 μM (n = 29), respectively.

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