Molecular Modeling and Synthesis of Inhibitors of Herpes Simplex Virus Type 1 Uracil-DNA Glycosylase

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We recently reported the properties of the first selective inhibitors of herpes simplex virus type 1 (HSV1) uracil-DNA glycosylase (UDG), an enzyme of DNA repair that has been proposed to be required for reactivation of the virus from latency. 6-(4-Octylanilino)uracil (octAU) was the most potent inhibitor among a series of 6-(4-alkylanilino)uracils, acting in the micromolar range and without effect against human UDG. A 28.5-kDa catalytic fragment of HSV1 UDG has been crystallized in the presence of uracil, and the structure was recently solved. We have used the coordinates of this structure in order to study interaction of our inhibitors with the enzyme, and a model of binding between octAU and UDG has been derived. Starting with the optimized model, the activity of several octAU analogues was predicted, and the values compared favorably with experimental results found for the synthetic compounds. Several hydrophilic derivatives were predicted and found to be active as UDG inhibitors. These compounds will be useful to determine if UDG, like the viral thymidine kinase, is required for reactivation of HSV1 from latency in nerve cells.

Introduction

Herpes simplex virus (HSV), following a primary lytic infection in peripheral mucocutaneous tissues, enters nerve terminals and establishes lifelong latent infections in sensory nerve ganglia.[1] During latency, no infectious virus is detectable, but such virus can "reactivate" causing recurrent disease at the original site of infection. Typically for HSV1 diseases, latent infection of the trigeminal ganglia is associated with herpes keratitis and of cervical ganglia with herpes labialis, whereas for HSV2, latent infections of sacral ganglia are associated with genital infections. Several reviews summarize the understanding of the mechanisms underlying herpesvirus latency.[2−4]

Maintenance of the latent state may be mediated by both viral and cellular (host) functions and may last for the life of the host. Reactivation of the virus may be caused by various environmental, immunological, and pharmacological stimuli and involves replication of the virus genome, centrifugal travel of viral DNA or virus particles through the sensory nerve to the original site of infection, and reestablishment of clinical disease. The morbidity of recurrent herpesvirus infections has prompted numerous studies related to treatment or prevention. Currently available antiviral drugs such as arabinosyladenine, acyclovir, and phosphonoacetic acid are effective in the treatment of acute HSV infections, but less so in preventing recurrent infections. For example, recurrent herpes labialis and herpes encephalitis in children and older adults are generally resistant to standard antiviral drugs. Acyclovir prevented in vitro reactivation of HSV1 from latently infected trigeminal ganglia of mice only with continuous presence of the drug, a result also found for arabinosyladenine and phosphonoacetic acid.[5] In human patients, the frequency of HSV recurrences following acyclovir treatment was not different than before treatment.[6]

We have hypothesized that HSV1 uracil-DNA glycosylase (UDG) could be an important enzyme for efficient viral reactivation.[7] UDG contributes to postreplicative DNA repair by removal of uracil residues from DNA, resulting from either cytosine deamination or dUTP incorporation, by cleavage of the *N*-glycosidic bond linking the base to the deoxyribose phosphate backbone. Recent evidence suggests that the viral UDG is required both for virus reactivation from latency and for efficient replication in nerve tissue.[8] The continuous spontaneous deamination of cytosine coupled with the lack of cellular UDG in neurons[9] is consistent with a requirement for the virus-encoded enzyme in the reactivation and replication of HSV in nerve cells. Screening of a large number of uracil derivatives and related compounds against the purified HSV1 and human (HeLa) UDGs in our laboratories revealed some *p*-alkyl-substituted 6-anilinouracils that showed weak activity against the viral enzyme. The results of a synthetic program revealed that 6-anilinouracils with large *n*-alkyl groups in the *para* position of the anilino ring were progressively more potent as inhibitors of the HSV1 enzyme and retained a high degree of selectivity for the viral enzyme.[10] The most potent compound, 6-(4-octylanilino)uracil (octAU, **5**), had $IC_{50} = 8\ \mu M$ against the viral enzyme but $>300\ \mu M$ against the human enzyme. We

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found that compound **5** was competitive with DNA as an inhibitor of HSV1 UDG, in contrast with the reported noncompetitive effect of uracil itself as an inhibitor of human UDG.[11] Uracil likely represents a product inhibitor of UDG, whereas the anilinouracils may bind to the enzyme as analogues of the substrate.

The X-ray structure of HSV1 UDG complexed with uracil has been solved recently.[12] The structure consists of a single domain, with a channeled surface containing a high density of positive charges. Uracil is bound in a pocket in this surface near one end of the protein. Phe 101 forms the floor of the uracil pocket, and Tyr 90 lies perpendicular to and in van der Waals contact with the uracil 5-H. This Tyr residue, the hydrogen bonding of Asn 147 to the 3-NH and 4-oxo groups, and the hydrogen bond from the backbone of Gln 87 explain the uracil specificity of the enzyme. The uracil 1-NH is hydrogen bonded to a water molecule which is at the mouth of the binding pocket. A second complex between HSV1 UDG and $(dT)_3$ shows the hydrophobic sugar and base 5-methyl backbone of the trinucleotide lining a hydrophobic cleft at the mouth of the uracil pocket.[12]

The likelihood that our inhibitors bind to HSV1 UDG in the uracil binding site prompted us to undertake a molecular modeling study with the coordinates of the uracil:UDG structure, kindly provided by Drs. Pearl and Savva. In this paper we describe a plausible model for the binding affinity and selectivity of 6-(4-alkylanilino)-uracils for HSV1 UDG. In addition we report the prediction and activity of analogues of octAU and new hydrophilic inhibitors based on the model.

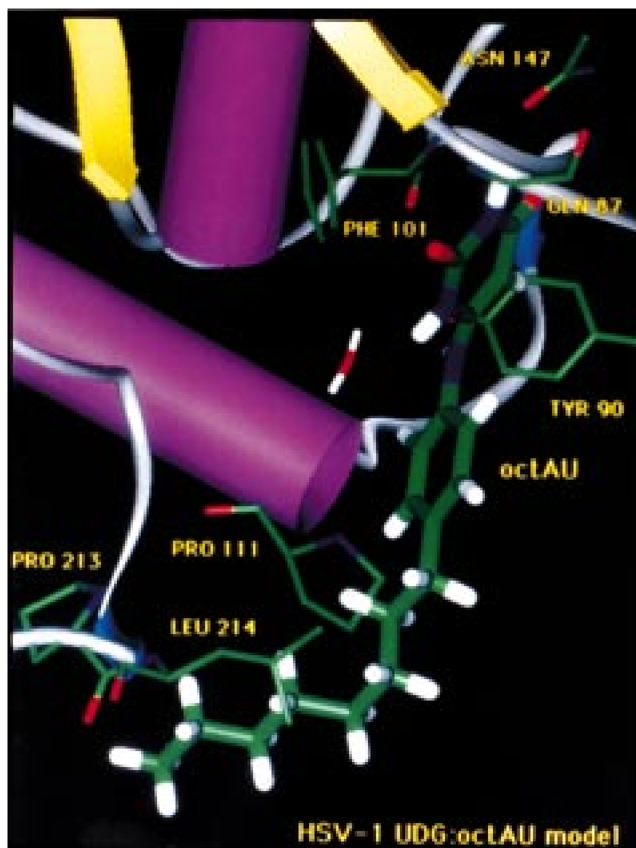


Figure 1. Energy-minimized model of the octAU:HSV1 UDG complex. See text for details.

Modeling and Structural Design

6-(4-Octylanilino)uracil (octAU), **5**, is the best lead inhibitor of HSV1 UDG,[10] although modifications are needed in order to increase the binding affinity and to improve physicochemical properties such as water solubility. For further drug development structural information about octAU:UDG interaction is desirable for rational lead optimization, but attempts to grow octAU: UDG cocrystals failed partly due to the poor water solubility of the inhibitor.[13] As a consequence, we adopted the structure-based rational design approach, starting with computer-assisted prediction of the inhibitor binding mode in the enzyme−inhibitor complex. The calculations and simulations were performed on an Indigo 2 R10000 SGI workstation with software modules in the Biosym/MSI package.

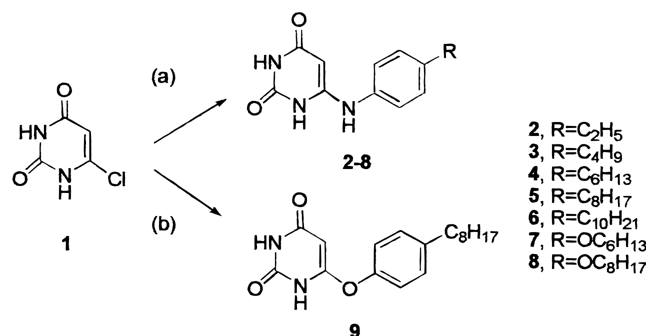
OctAU (**5**) is a highly flexible molecule with 10 freely rotatable single bonds, and it can adopt many low-energy conformations in solution. The real situation in docking the inhibitors to the enzyme becomes more complex if we take into account the flexibility of the enzyme. To make docking feasible, two strategies, the “ligand buildup” and the “in-site combinatorial search”, were applied.[14] The structural model of octAU:UDG resulting from the energy minimization procedure (see Experimental Section) is shown in Figure 1. The model shows that the uracil ring of the inhibitor binds in the same position as free uracil in the UDG crystal structure.[12] The 6-NH bond is approximately perpendicular to the phenyl ring of Tyr 90 at a distance of 3.85 Å, an interaction energetically equivalent to about one-half of a normal hydrogen bond. The second half of the octyl chain lies snugly in a hydrophobic cleft on the surface of the enzyme formed by the side chains of Pro 111, Pro 213, and Leu 214. The hydrophobic side chains of leucines and prolines are typically buried in the interior of proteins, but the appearance of Pro 111, Pro 213, and Leu 214 on the surface of UDG is required by the biological function of the protein. In the case of the human UDG structure,[15] the counterpart of Leu 214 was proposed to insert into the DNA through the minor groove assisting flipping of the nucleotide to be removed, and the Leu−DNA interactions stabilized the extrahelical nucleotide conformation. Comparison between the calculated complex structure in Figure 1 and the crystal structure of HSV1 UDG bound to trithymidylate[12] revealed similar interactions, although the two ligands are very different. By superimposing the backbone atoms of the enzymes (rms = 0.71 Å), we observed a similar orientation of **5** and the first two thymidylates of the trimer. In the crystal structure the first thymine remains at the mouth of the uracil binding pocket because of steric hindrance, and the second thymine base packs against the hydrophobic side chains of Pro 213 and Leu 214, with its attached deoxyribose ring packed against the side chain of Pro 111, the latter believed to be one of the major driving forces for ligand binding. The hydrophobic interactions are quite similar to those found in the modeled octAU:UDG complex (Figure 1). Hydrophobic effects play a pivotal role in many chemical phenomena in aqueous solution,[16] and the association of the hydrophobic *n*-octyl group with the similarly hydrophobic residues on the surface of the protein may play the central role in inhibitor binding in this case. Molecular recognition relies strongly on

Table 1. Molecule–Molecule Interaction Energy Summary for 6-(4-Alkylanilino)uracil:HSV1 UDG Complexes

ligand	total energy (kcal/mol)	van der Waals (kcal/mol)			Coulombic (kcal/mol)	IC_{50}[a] (μM)
		repulsion	dispersion	total		
2	−1.78024	44.2178	69.8937	−25.67595	−16.10429	500
3	−44.44710	44.0705	73.7932	−29.72274	−14.72436	150
4	−46.52708	46.3444	78.1175	−31.77312	−14.75397	30
5	−49.03306	51.4240	85.0409	−33.61688	−15.41618	8

[a] From ref 10.

Scheme 1[a]



[a] Reagents: (a) 4-substituted aniline, Δ; (b) sodium 4-octylphenolate, 2-methoxyethanol.

noncovalent interactions, for which hydrophobic regions in host and guest are often crucial.

Correlation of Activity with Model-Based Energy Calculations

Table 1 summarizes the intermolecular interaction energies (E_{inter}) between HSV1 UDG and alkylanilinouracils obtained during the ligand buildup procedure described above. The results are separated into van der Waals and Coulombic components. It is clear that a direct relation exists between the total energies and IC_{50} values of the inhibitors, as emphasized by the correlation equation between pIC_{50} [$-\log(IC_{50})$] and total energy for the series of compounds:

$$pIC_{50} = -0.23565E_{inter} - 6.5588$$

$$n = 4,\ R^2 = 0.987$$

Using this equation we predicted activities of novel inhibitors before synthesis, i.e., true predictions rather than post hoc explanations. The new compounds **6**–**9** (Scheme 1) are structurally close to octAU (**5**). The structure of 6-(4-decylanilino)uracil (**6**) was built in the UDG binding site by adding two methylene groups to the alkyl chain of octAU. The binding modes of 6-(4-hexyloxyanilino)uracil (**7**) and 6-(4-octyloxyanilino)uracil (**8**) were generated from **5** and **6**, respectively, by replacing the corresponding atoms and deleting the unnecessary atoms, followed by energy minimization. Compound **9** was derived by isosteric replacement of the 6-NH of **5** by oxygen; this compound was selected to evaluate the possible importance of the 6-NH group in hydrogen bonding to Tyr 90. The above equation was used to predict IC_{50} values from the total interaction energies derived from energy minimization of each inhibitor:UDG model.

Compounds **6**–**9** were synthesized according to Scheme 1 and assayed for their inhibitory potencies against HSV1 UDG. As the results of Table 2 indicate, the predicted IC_{50} values are in good agreement with the experimental ones, being in the same order although lower than experimental IC_{50}'s by 2–3-fold. These differences may result in part from underestimating the dehydration energy of the free ligands. Interestingly, the weakest inhibitor of this series was the 6-phenoxy derivative **9**, which lacks the potential to form a hydrogen bond with Tyr 90. These results serve both to validate the model of inhibitor:enzyme interaction (Figure 1) and to permit prediction of the activity of new inhibitors, at least in a closely related series of molecules.

Derivatization of 6-(4-Alkylanilino)uracils

According to the inhibitor model of Figure 1, substituents at N-3 or C-5 of the uracil ring were predicted to lack inhibitory activity against HSV1 UDG. Indeed, 3-ethyl-6-(4-hexylanilino)uracil and the 5-methyl, i.e., "thymine", analogue 6-(4-hexylanilino)thymine were inactive at 200 μM against the enzyme, although the parent compound **4** had IC_{50} of 30 μM.[10] Substitution of the 3-NH not only blocks the hydrogen bond to Asn 147 but would produce steric clash with this residue. Substitution at C-5 similarly would produce steric clash with the ring of Tyr 90.

One site illustrated on the uracil ring where substituents may be tolerated is N-1. In the X-ray structure,[12] and in the model of Figure 1, a water molecule is hydrogen bonded to 1-NH, and a volume that could be filled with substituents of moderate size is available in the dehydrated enzyme. Accordingly, we prepared several 1-substituted derivatives of 6-aminouracil and 6-(4-alkylanilino)uracil (Scheme 2) to test this hypothesis. 6-Aminouracil itself has been reported to weakly inhibit human UDG,[17] and indeed, a recent X-ray structure of the human enzyme was solved in the presence of this compound.[15] 6-(4-Hexylanilino)uracil (hexAU) was chosen as the platform inhibitor for these syntheses for the simple reason of economy, 4-hexylaniline being considerably less expensive than 4-octylaniline.

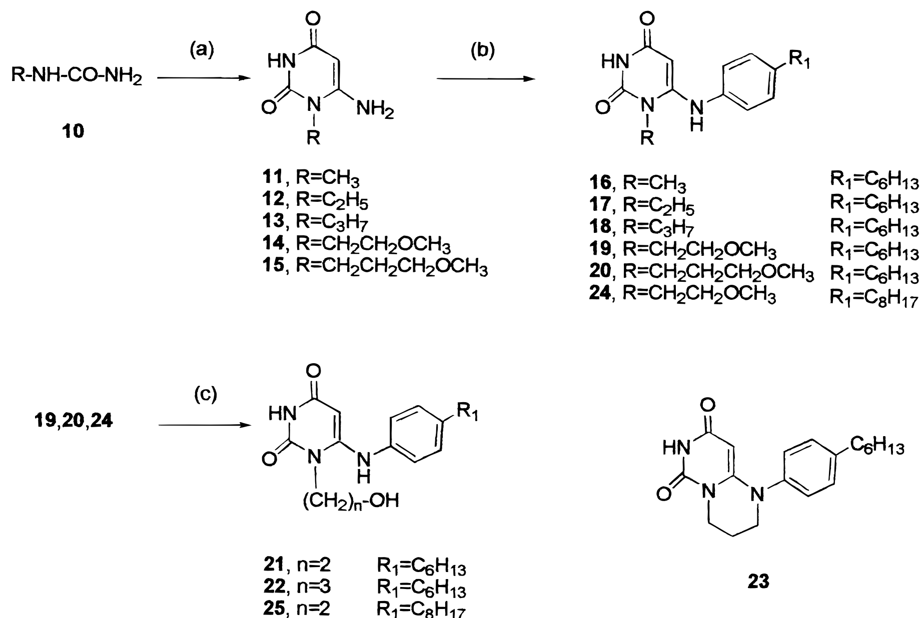
6-Aminouracil was a weak inhibitor of HSV1 UDG with $IC_{50} \sim 1$ mM. 1-Alkyl-6-aminouracils **11**–**13** also weakly inhibited the viral enzyme but were neither more nor less potent than the parent compound (data not shown). The analogous 1-alkyl-hexAU derivatives **16**–**18** were, unfortunately, too insoluble in assay medium to estimate inhibition potencies. Only 10–15% inhibition of HSV1 UDG activity was observed with these compounds at 40 μM.

Because goals of this work were to prepare more potent and more water-soluble UDG inhibitors, we synthesized several derivatives of hexAU (**4**) with hydrophilic 1-substituents. The reaction sequence (Scheme 2) resulted first in the 1-(methoxyalkyl) compounds **19** and **20** which were converted to the 1-(hydroxyalkyl)

Table 2. Molecule–Molecule Interaction Energies and Calculated and Experimental IC_{50} Values of Novel UDG Inhibitors

ligand	total energy (kcal/mol)	van der Waals (kcal/mol)			Coulombic (kcal/mol)	IC_{50}[a] (μM)	
		repulsion	dispersion	total		pred	exptl
6	−48.70344	50.1479	84.2068	−34.05885	−14.64459	12	35
7	−48.13001	50.6867	83.5828	−32.89606	−15.23395	16	30
8	−49.00301	51.3359	85.4334	−34.09749	−14.90552	10	25
9	−45.01171	53.5577	87.9681	−34.41046	−10.60125	89	150

Scheme 2[a]



[a] Reagents: (a) NC-CH_2-COOEt, Na, EtOH; (b) 4-alkylaniline, 4-alkylaniline hydrochloride, Δ; (c) TMSI/$CHCl_3$.

Table 3. Effect of Compounds on HSV1 UDG

compd	1-substituent	IC_{50} (μM)[a]
	1-Substituted-hexAU	
4	H	30
16	CH_3	*b*
17	C_2H_5	*b*
18	C_3H_7	*b*
19	$CH_2CH_2OCH_3$	>100
20	$CH_2CH_2CH_2OCH_3$	>100
21	CH_2CH_2OH	20
22	$CH_2CH_2CH_2OH$	90
	1-Substituted-octAU	
5	H	8
24	$CH_2CH_2OCH_3$	15
25	CH_2CH_2OH	10

[a] IC_{50} is the concentration of compound that caused half-maximal inhibition of [^{3}H]uracil release from [^{3}H]dUMP-containing DNA. Assays were performed as previously described[10] in the presence of varying concentrations of test compounds. Control assays contained the same concentration of the diluent, dimethyl sulfoxide. [b] Because of insolubility, no estimates of IC_{50} could be made.

compounds **21** and **22**. The 1-(2-hydroxyethyl) derivative of hexAU, **21**, was slightly more potent than **4**, although the 1-(3-hydroxypropyl) derivative **22** was 3-fold weaker (Table 3). The corresponding methoxyalkyl compounds were weaker still (Table 3). Although the introduction of hydrophilic 1-substituents did not result in *increased* potency of the platform inhibitor, the compounds had apparent increased water solubility and maintained, at least for the small 2-hydroxyethyl group, similar inhibitory activity. Encouraged by the results with hexAU we synthesized and assayed the corresponding hydrophilic octAU derivative **25** (Table 3). Indeed, 1-(2-hydroxyethyl)octAU (**25**) was as potent an inhibitor as **5**, and the 1-(2-methoxyethyl) compound **24** was nearly as potent.

These results provide further support for the model of inhibitor:UDG binding presented above and validate the strategy for developing more water-soluble derivatives for further study. In particular, the potential use of a compound such as **25** to test the role of HSV1 UDG expression in viral reactivation becomes more feasible, given the likely improved absorption and distribution of this derivative in appropriate animal models. The same strategy applied to a different family of inhibitors of HSV1 thymidine kinase (TK),[18] another viral enzyme implicated in the reactivation process, did result in demonstration of efficacy, i.e., reduction in the frequency of HSV1 recurrences in two animal models.[19,20] We anticipate that, like the case of TK inhibitors, suitable water-soluble UDG inhibitors may also be applied to determine if viral UDG can be exploited as a target to reduce or prevent recurrent herpes simplex infections in animals. In addition, several compounds predicted by the use of the LUDI program to have substantially increased affinity to HSV1 UDG are being synthesized.

Synthetic Chemistry

Reactions of substituted anilines with a 6-chlorouracil in 2-methoxyethanol as solvent[10,21] or without solvent[22] are used routinely to prepare 6-anilinouracils in good to moderate yields (Scheme 1). The former method was used to prepare 3-ethyl-6-(4-hexylanilino)uracil and 6-(4-decylanilino)uracil, **6**, while the fusion method was used in the synthesis of 6-(4-hexyloxyanilino)uracil, **7**,

and 6-(4-octyloxylanilino)uracil, **8**. 6-(4-Octylphenoxy)uracil, **9**, was prepared by reacting 6-chlorouracil with sodium 4-octylphenolate in 2-methoxyethanol (Scheme 1). 6-(4-Hexylanilino)thymine was obtained from the reaction between 6-chlorothymine and the aniline.

1-Substituted-6-anilinouracils. *N*-Substituted ureas **10** were cyclized with ethyl cyanoacetate in ethanol in the presence of sodium ethoxide, to give exclusively 1-substituted-6-aminouracils. The known 1-alkyl-6-aminouracils[23,24] and new 1-(methoxyalkyl)-6-aminouracils were made by this method.

Activated amino groups in pyrimidines are readily displaced by nucleophiles, including both aliphatic and aromatic amines.[25] For oxoamino pyrimidines, e.g., cytosine and 1,3-dimethyl-6-aminouracil, Whitehead and Traverso found that heating with amines to 160−170 °C without solvent required an equivalent of the amine hydrochloride to give the substitution product.[25] This reaction was used to prepare 1-alkyl-6-(4-hexylanilino)uracils **16**−**18** in good yields (Scheme 2). The synthesis of the target hydrophilic compound 1-(2-hydroxyethyl)-6-(4-hexylanilino)uracil, **21**, was attempted by this fusion reaction starting from 1-(2-hydroxyethyl)-6-aminouracil. This was not successful, because considerable decomposition occurred under the high temperatures required for this reaction (results not shown). Therefore, the methoxy-protected 6-aminouracils **14** and **15** were successfully constructed by fusion reactions to give the corresponding 1-(2-methoxyethyl)- and 1-(3-methoxypropyl)-6-(4-hexylanilino)uracils, **19** and **20,** respectively. Demethylation of the ethers with trimethylsilyl iodide (TMSI)[26] afforded the expected 1-(2-hydroxyethyl)- and 1-(3-hydroxypropyl)-6-(4-hexylanilino)uracils, **21** and **22**, respectively. However, demethylation of **20** gave a second product whose NMR spectrum and elemental analysis suggest a cyclized compound, tentatively identified as N^1,N^6-propylene-6-(4-hexylanilino)uracil, **23**. Indeed, prolonged treatment of **20** with TMSI gave exclusively compound **23** (see Experimental Section). A similar sequence was used to prepare the analogous 1-(2-methoxyethyl)- and 1-(2-hydroxyethyl)-6-(4-octylanilino)uracils, **24** and **25**, respectively, in good yields.

Conclusions

Three comparisons will serve to illustrate the plausibility of the model of Figure 1. First, the model predicts that 3-alkyl and 5-alkyl inhibitor derivatives would lack activity: indeed, 3-ethyl-hexAU and 5-methyl-hexAU (the thymine analogue) did not inhibit HSV1 UDG at 200 μM. Second, a phenoxy ether replacing the anilino NH would be expected to have reduced activity by eliminating a potentially directed hydrogen bond to Tyr 90. In fact, 6-(4-octylphenoxy)uracil, **9**, was predicted to be weaker and was found to be a 19-fold weaker inhibitor (IC_{50} = 150 μM) than **5** (8 μM). Energy calculations for the UDG:**9** complex revealed a reduction in total interaction energy of 4.0 kcal/mol relative to **5**, possibly due in part to the loss of the 6-NH/Tyr 90 hydrogen bond.

Finally, 1-substitution in the uracil ring would be expected to be tolerated in the inhibitor:UDG complex. In fact, certain 1-substituents could enhance activity by introducing suitable interactions in the nearby space. Significantly, the 1-(hydroxyalkyl) derivatives **21**, **22**, and **25** were equipotent with the 1-H compounds as inhibitors of HSV1 UDG. In addition, the increased water solubility of these compounds is likely a desirable property for their further study as HSV UDG inhibitors in vitro and as drugs to prevent recurrent HSV infections in vivo. We will continue to use ligand buildup and in-site combinatorial search strategies with the model of Figure 1 to aid in the design of inhibitors with increased potencies for these purposes.

Experimental Section

Melting points were measured using a Mel-temp hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz using a Varian Unity 300 spectrometer. ^{1}H chemical shifts (δ, ppm) are reported with Me_4Si (δ = 0.00 ppm) as internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, and m = multiplet. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer, and results were within ±0.4% of the theoretical values. All reactions were monitored by TLC on 0.25-mm Merck silica gel (60 F254) visualized by UV light (λ = 264 or 365 nm). Flash chromatography was performed using silica gel (60−200 μm, Merck). Substituted ureas **10a** and **10b** were synthesized as described.[23,24] Compounds **2**−**5** were prepared from 6-chlorouracil as previously reported.[10] 3-Ethyl-6-(4-hexylanilino)uracil was prepared from 3-ethyl-6-chlorouracil by the same method and was isolated in 38% yield (from toluene), mp 235 °C. Anal. ($C_{18}H_{25}N_3O_2$) C, H, N. 6-(4-Hexylanilino)thymine was prepared similarly by reaction of 6-chlorothymine with the aniline and was isolated in 35% yield (from toluene), mp 247−248 °C. Anal. ($C_{17}H_{23}N_3O_2$) C, H, N.

6-(4-Decylanilino)uracil, 6. A mixture of 6-chlorouracil (146.5 mg, 1.0 mmol) and 4-decylaniline (467 mg, 2.0 mmol) was placed in a pear-shaped flask and heated at 200 °C for 2 h under nitrogen. The greenish reaction mixture was washed with 15 mL of chloroform and then crystallized from AcOH/H_2O, giving 310 mg (90%) of white crystals, mp 292−293 °C. ^{1}H NMR (DMSO-d_6): δ 0.88 (t, 3H, CH_3), 1.25−1.42 (m, 14H, 7×CH_2), 1.61 (m, 2H, $ArCH_2\mathit{CH_2}$), 2.55 (t, 2H, $ArCH_2$), 4.60 (s, 1H, 5-H), 7.15 (2d, 4H, C_6H_4), 8.14 (s, 1H, 6-NH), 10.13 (s, 1H, 1-NH), 10.43 (s, 1H, 3-NH). Anal. ($C_{20}H_{29}N_3O_2$) C, H, N.

6-(4-Hexyloxyanilino)uracil, 7. A mixture of 6-chlorouracil (146.5 mg, 1.0 mmol) and 4-(hexyloxy)aniline (386.6 mg, 2.0 mmol) was heated at 200 °C in an oil bath for 3 h under nitrogen. The cooled mixture was washed with 25 mL of 1:1 EtOH/H_2O and then crystallized from AcOH/H_2O giving 300 mg (95%) of purple crystals, mp 320 °C. ^{1}H NMR (DMSO-d_6): δ 0.89 (t, 3H, CH_3), 1.32 (m, 4H, $CH_3\mathit{CH_2CH_2}$), 1.42 (m, 2H, $OCH_2CH_2\mathit{CH_2}$), 1.70 (m, 2H, $OCH_2\mathit{CH_2}$), 3.95 (t, 2H, OCH_2), 4.41 (s, 1H, 5-H), 6.92 and 7.10 (2d, 4H, C_6H_4), 7.96 (s, 1H, 6-NH), 10.13 (s, 1H, 1-NH), 10.37 (s, 1H, 3-NH). Anal. ($C_{16}H_{21}N_3O_3$) C, H, N.

6-(4-Octyloxyanilino)uracil, 8. This product was prepared by the same procedure described above except that 4-(octyloxy)aniline (7.0 mmol) was employed. Crystallization from AcOH/H_2O gave gray crystals (95%), mp 318 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H, CH_3), 1.20−1.45 (m, 10H, 5×CH_2), 1.65 (m, 2H, $OCH_2\mathit{CH_2}$), 3.95 (t, 2H, OCH_2), 4.41 (s, 1H, 5-H), 6.80 and 7.07 (2d, 4H, C_6H_4), 8.02 (s, 1H, 6-NH), 10.95 (s, 1H, 1-NH), 12.80 (s, 1H, 3-NH). Anal. ($C_{18}H_{25}N_3O_3$) C, H, N.

6-(4-Octylphenoxy)uracil, 9. A mixture of 6-chlorouracil (146.5 mg, 1.0 mmol) and sodium 4-octylphenolate (206 mg, 1.0 mmol) was heated to 125 °C in an oil bath for 2 h under nitrogen. The reaction mixture was washed with 10 mL of EtOH and then crystallized from AcOH/H_2O, giving 90 mg (30%) of white crystals, mp 212−215 °C. ^{1}H NMR (DMSO-d_6): δ 0.89 (t, 3H, CH_3), 1.30 (m, 10H, 5×CH_2), 1.60 (m, 2H, $ArCH_2\mathit{CH_2}$), 2.60 (t, 2H, $ArCH_2$), 4.19 (s, 1H, 5-H), 7.18 and 7.30 (dd, 4H, C_6H_4), 10.95 (s, 1H, 1-NH), 11.87 (s, 1H, 3-NH). Anal. ($C_{18}H_{24}N_2O_3$) C, H, N.

1-Alkyl-6-aminouracils 11−**13.** Sodium ethoxide was prepared from sodium (200 mmol) and 150 mL of "superdry"

EtOH. The corresponding alkylurea (100 mmol) and ethyl cyanoacetate (100 mmol) were added, and the mixture was refluxed for 6 h. The mixture was concentrated to one-half volume and chilled on ice for 1 h. The product was collected by filtration. Recrystallization from H_2O (pH 7.0) gave over 90% yield of 1-alkyl-6-aminouracil.

1-Methyl-6-aminouracil, 11. Yield 95%; mp 310−312 °C. ^{1}H NMR (DMSO-d_6): δ 3.17 (s, 3H, CH_3), 4.54 (s, 1H, 5-H), 6.75 (s, 2H, NH_2), 10.29 (s, 1H, 3-NH). Anal. ($C_5H_7N_3O_2$) C, H, N.

1-Ethyl-6-aminouracil, 12. Yield 95%; mp 297−298 °C. ^{1}H NMR (DMSO-d_6): δ 1.08 (t, 3H, CH_3), 3.77 (q, 2H, CH_2), 4.52 (s, 1H, 5-H), 6.79 (s, 2H, NH_2), 10.29 (s, 1H, 3-NH). Anal. ($C_6H_9N_3O_2$) C, H, N.

1-Propyl-6-aminouracil, 13. Yield 96%; mp 279−281 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H, CH_3), 1.51 (m, 2H, CH_2), 3.67 (t, 2H, CH_2), 4.51 (s, 1H, 5-H), 6.78 (s, 2H, NH_2), 10.30 (s, 1H, 3-NH). Anal. ($C_7H_{11}N_3O_2$) C, H, N.

1-Alkyl-6-(4-hexylanilino)uracils 16−18. A few drops of 4-hexylaniline were added to wet a mixture of 1-alkyl-6-aminouracil (10 mmol) and 4-hexylaniline hydrochloride (10 mmol). The mixture was heated at 165 °C for 6 h in an oil bath under nitrogen. The reaction mixture was washed with 20 mL of 1:1 EtOH/H_2O and filtered, and the solid crystallized from AcOH/H_2O.

1-Methyl-6-(4-hexylanilino)uracil, 16. Yield 85%; mp 268 °C. ^{1}H NMR (DMSO-d_6): δ 0.89 (t, 3H, CH_3), 1.31 (m, 4H, CH_3*CH_2CH_2*), 1.61 (m, 4H, $ArCH_2$*CH_2CH_2*), 2.64 (t, 2H, $ArCH_2$), 3.37 (s, 3H, CH_3), 4.43 (s, 1H, 5-H), 7.17 and 7.28 (dd, 4H, C_6H_4), 8.41 (s, 1H, NH), 10.61 (s, 1H, 3-NH). Anal. ($C_{17}H_{23}N_3O_2$) C, H, N.

1-Ethyl-6-(4-hexylanilino)uracil, 17. Yield 95%; mp 222−225 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H, CH_3), 1.18 (t, 3H, CH_3), 1.28 (m, 4H, CH_3*CH_2CH_2*), 1.57 (m, 4H, Ar*CH_2CH_2*), 2.59 (t, 2H, $ArCH_2$), 3.99 (q, 2H, CH_2), 4.33 (s, 1H, 5-H), 7.14 and 7.27 (dd, 4H, C_6H_4), 8.43 (s, 1H, NH), 10.60 (s, 1H, 3-NH). Anal. ($C_{18}H_{25}N_3O_2$) C, H, N.

1-Propyl-6-(4-hexylanilino)uracil, 18. Yield 96%; mp 226 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H, CH_3), 0.90 (t, 3H, CH_3), 1.27 (m, 4H, CH_3*CH_2CH_2*), 1.55−1.63 (m, 6H, $ArCH_2$*CH_2CH_2* and NCH_2*CH_2*), 2.58 (t, 2H, $ArCH_2$), 3.89 (q, 2H, NCH_2), 4.31 (s, 1H, 5-H), 7.15 and 7.24 (dd, 4H, C_6H_4), 8.41 (s, 1H, NH), 10.60 (s, 1H, 3-NH). Anal. ($C_{19}H_{27}N_3O_2$) C, H, N.

1-(2-Methoxyethyl)-6-aminouracil, 14. Sodium methoxide was prepared from sodium (4.0 g, 174 mmol) and 100 mL of anhydrous MeOH. (2-Methoxyethyl)urea (6.0 g, 50.8 mmol) and ethyl cyanoacetate (5.75 g, 50.8 mmol) were added, and the mixture was refluxed for 6 h. The mixture was allowed to cool, and 2 N HCl was added until the solution was weakly acidic. The inorganic salts were removed by filtration on a short column of silica gel. The solvent was removed in vacuo, and 7.7 g of **14** (82% yield) was obtained as a gray solid. Crystallization from EtOH/H_2O gave white crystals, mp 236−238 °C. ^{1}H NMR (DMSO-d_6): δ 3.26 (s, 3H, OCH_3), 3.46 (t, 2H, OCH_2), 3.95 (t, 2H, NCH_2), 4.55 (s, 1H, 5-H), 6.66 (s, 2H, NH_2), 10.35 (s, 1H, NH). Anal. ($C_7H_{11}N_3O_3$) C, H, N.

1-(3-Methoxypropyl)-6-aminouracil, 15. This compound was prepared by the same procedure as above, yield 86%. Crystallization from EtOH gave white crystals, mp 209.5−211 °C. ^{1}H NMR (DMSO-d_6): δ 1.72 (m, 2H, CH_2), 3.21 (s, 3H, OCH_3), 3.36 (t, 2H, OCH_2), 3.77 (t, 2H, NCH_2), 4.52 (s, 1H, 5-H), 6.73 (s, 2H, NH_2), 10.30 (s, 1H, NH). Anal. ($C_8H_{13}N_3O_3$) C, H, N.

1-(2-Methoxyethyl)-6-(4-hexylanilino)uracil, 19. A mixture of **14** (0.2 g, 1.1 mmol), 4-hexylaniline hydrochloride (0.26 g, 1.2 mmol), and a few drops of 4-hexylaniline was heated at 160 °C for 40 min. After cooling to room temperature, the product was chromatographed on silica gel with $CHCl_3$/MeOH (97:3−95:5) as eluent, to give 0.32 g (86% yield) of **19**. Crystallization from EtOH/H_2O gave white crystals, mp 188−190 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, 3H, CH_3), 1.29 (m, 6H, 3×CH_2), 1.60 (m, 2H, CH_2), 2.60 (t, 2H, $ArCH_2$), 3.34 (s, 3H, OCH_3), 3.59 (t, 2H, OCH_2), 4.16 (t, 2H, NCH_2), 4.47 (s, 1H, 5-H), 7.17 (dd, 4H, C_6H_4), 8.26 (s, 1H, NH), 10.68 (s, 1H, 3-NH). Anal. ($C_{19}H_{27}N_3O_3$) C, H, N.

1-(3-Methoxypropyl)-6-(4-hexylanilino)uracil, 20. This compound was prepared by the same procedure as above, yield 85%. Crystallization from EtOH/H_2O gave white crystals, mp 112−113 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (m, 3H, CH_3), 1.28 (m, 6H, 3×CH_2), 1.57 (m, 2H, CH_2), 1.85 (m, 2H, CH_2), 2.58 (t, 2H, $ArCH_2$), 3.23 (s, 3H, OCH_3), 3.40 (t, 2H, OCH_2), 4.00 (t, 2H, NCH_2), 4.36 (s, 1H, 5-H), 7.19 (dd, 4 H, C_6H_4), 8.36 (s, 1H, NH), 10.61 (s, 1H, 3-NH). Anal. ($C_{20}H_{29}N_3O_3$) C, H, N.

Demethylation of 21 and 22. Trimethylsilyl iodide (3 equiv) was added to a stirred solution of **21** or **22** (0.5 mmol) in dry $CHCl_3$ (15 mL). The reaction mixture was stirred at room temperature until disappearance of the starting material (TLC, about 4 h). Methanol (10 mL) and 0.5 g of sodium sulfite were then added to the brown-purple solution. After stirring at room temperature for 30 min, the mixture was filtered and the solvent was removed.

1-(2-Hydroxyethyl)-6-(4-hexylanilino)uracil, 21, was purified by chromatography on silica gel with $CHCl_3$/MeOH (90:10) as eluent, to give 146 mg (88% yield). Crystallization from EtOH/H_2O gave white crystals, mp 245−247 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (m, 3H, CH_3), 1.28 (m, 6H, 3×CH_2), 1.57 (m, 2H, CH_2), 2.57 (t, 2H, $ArCH_2$), 3.67 (t, 2H, OCH_2), 4.05 (t, 2H, NCH_2), 4.55 (s, 1H, 5-H), 5.62 (s, 1H, OH), 7.15 (dd, 4H, C_6H_4), 8.50 (s, 1H, NH), 10.70 (s, 1H, 3-NH). Anal. ($C_{18}H_{25}N_3O_3$) C, H, N.

1-(3-Hydroxypropyl)-6-(4-hexylanilino)uracil, 22, was purified by chromatography on silica gel with $CHCl_3$/MeOH (97:3−90:10) as eluent, to give 32 mg (30% recovery) of starting material, 56 mg (54% yield) of **22**, and 8 mg (8% yield) of compound **23** (see below). Crystallization of **22** from EtOH/H_2O gave white crystals, mp 173−175 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (m, 3H, CH_3), 1.28 (m, 6H, 3×CH_2), 1.57 (m, 2H, CH_2), 1.79 (m, 2H, CH_2), 2.58 (t, 2H, $ArCH_2$), 3.50 (t, 2H, OCH_2), 3.98 (t, 2H, NCH_2), 4.41 (s, 1H, 5-H), 5.00 (s, 1H, OH), 7.19 (dd, 4H, C_6H_4), 8.52 (s, 1H, NH), 10.65 (s, 1H, 3-NH). Anal. ($C_{19}H_{27}N_3O_3$) C, H, N.

***N*^{1},*N*^{6}-Propylene-6-(4-hexylanilino)uracil, 23**. Trimethylsilyl iodide (0.2 mL, 1.41 mmol) was added to a stirred solution of **20** (108 mg, 0.3 mmol) in $CHCl_3$ (10 mL). The reaction mixture was stirred at room temperature until disappearance of the starting material **20** (about 14 h). Methanol (10 mL) and 0.5 g of sodium sulfite were then added to the brown-purple solution. After stirring at room temperature for 30 min, the mixture was filtered and the solvent was removed. The residue was purified by chromatography on silica gel with $CHCl_3$/MeOH (97:3) as eluent, to give 81 mg (82%) of **23**. Crystallization from EtOH/H_2O gave white crystals, mp >220 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (m, 3H, CH_3), 1.29 (m, 6H, 3×CH_2), 1.58 (m, 2H, CH_2), 2.10 (m, 2H, CH_2), 2.61 (t, 2H, $ArCH_2$), 3.55 (t, 2H, OCH_2), 3.83 (m, 3H, NCH_2 and 5-H), 7.29 (dd, 4H, C_6H_4), 10.51 (s, 1H, 3-NH). Anal. ($C_{19}H_{25}N_3O_2$) C, H, N.

1-(2-Methoxyethyl)-6-(4-octylanilino)uracil, 24. A mixture of **14** (0.6 g, 3.24 mmol), 4-octylaniline hydrochloride (0.78 g, 3.24 mmol), and 4-octylaniline (0.74 mL, 3.24 mmol) was heated at 160 °C for 40 min under nitrogen. After cooling to room temperature, the residue was chromatographed on silica gel with $CHCl_3$/MeOH (97:3) as eluent, to give 1.02 g (85% yield) of **24**. Crystallization from EtOH/H_2O gave white crystals, mp 189−193 °C. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, CH_3), 1.30 (m, 10H, 5×CH_2), 1.58 (m, 2H, CH_2), 2.60 (t, 2H, $ArCH_2$), 3.38 (s, 3H, OCH_3), 3.60 (t, 2H, OCH_2), 4.15 (t, 2H, NCH_2), 4.49 (s, 1H, 5-H), 7.18 (dd, 4H, C_6H_4), 8.25 (s, 1H, NH), 10.68 (s, 1H, 3-NH). Anal. ($C_{21}H_{31}N_3O_3$) C, H, N.

1-(2-Hydroxyethyl)-6-(4-octylanilino)uracil, 25. Demethylation was done as described for **21** and **22**. The residue was purified by chromatography on silica gel with $CHCl_3$/MeOH (90:10) as eluent, to give 79% yield of **25**. Recrystallization from EtOH/H_2O gave white crystals, mp 241−245 °C. ^{1}H NMR (DMSO-d_6): δ 0.90 (m, 3H, CH_3), 1.30 (m, 10H, 5×CH_2), 1.57 (m, 2H, CH_2), 2.60 (t, 2H, $ArCH_2$), 3.68 (t, 2H, OCH_2), 4.05 (t, 2H, NCH_2), 4.55 (s, 1H, 5-H), 5.62 (s, 1 H, OH),

7.15 (dd, 4H, C_6H_4), 8.50 (s, 1H, NH), 10.66 (s, 1H, 3-NH). Anal. ($C_{20}H_{29}N_3O_3$) C, H, N.

Molecular Modeling. The calculations and simulations were performed on an Indigo 2 R10000 SGI workstation, by using the software modules Discover, Builder, Biopolymer, Dock, and LUDI in the Biosym/MSI package. The structure of the HSV1 UDG complex with uracil[12] consists of 1928 heavy atoms. Hydrogen atoms were added automatically using the Builder module with the value of pH set to the physiological value of 7.4. The resulting system had a net charge of −7.0. To remove any artifacts introduced by adding explicit hydrogen atoms, the structure was subjected to steepest descents (sd) minimization for 500 iterations with all backbone atoms tethered. After removal of all the water molecules except two at the active site, which are believed to be related to enzymatic function, the structure was further minimized for 1000 steps using the sd algorithm to relax any remaining hot spots. The backbone atoms of the resulting structure had a root-mean-square (rms) deviation of 0.20 Å from the crystal coordinates.

The 6-anilinouracil (AU) molecule was built up in the protein by attaching an anilino group to the uracil C-6. If the uracil is assumed to stay in its original position, the AU molecule has only two degrees of freedom, representing a suitable case for in-site combinatorial search. By rotating the two dihedral angles around C-6/N (ϕ) and N/C-1′ (in the phenyl ring) (ψ) while monitoring the change of the intermolecular interaction energy, the local conformational space was efficiently sampled. Favorable interactions were achieved only when ϕ was about 150°. Since the variation of the angle ψ will not affect the position of C-4′ in the phenyl ring, where the octyl group is attached, ψ was arbitrarily set to 150°. The *n*-octyl group was then added in two carbon units, with energy minimization after each addition. For each minimization procedure, those amino acids 12 Å away from the binding site were fixed, and the remaining amino acids and the uracil ring were tethered to their original positions by a template force of 100.0 kcal/Å for 100 cycles. The template force was gradually released in another 100 steps, followed by 200 cycles of sd minimization without any external forces. The rms deviation was only 0.28 Å upon superimposing the backbone atoms of the enzyme before and after docking with compound **5**. The final structure was fully minimized using the sd method until the maximum derivative was less than 0.5 kcal/Å and by the method of conjugate gradients until the maximum derivative was less than 0.01 kcal/Å.

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