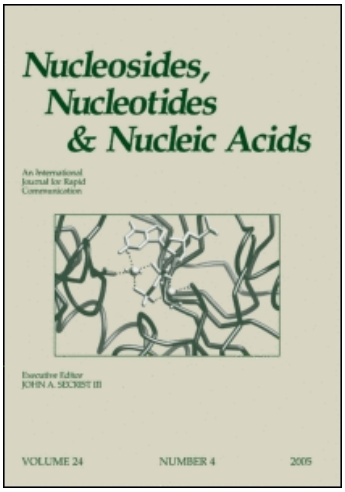


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Molecular Modeling of Novel HSV1 Uracil-DNA Glycosylase Inhibitors

M. Pregnolato[a]; D. Ubiali[a]; A. Verri[b]; F. Focher[b]; S. Spadari[b]; H. Sun[c]; C. Zhi[c]; G. E. Wright[c]
[a] Dipartimento di Chimica Farmaceutica, Università degli Studi, Pavia, Italy [b] Istituto di Genetica Biochimica ed Evoluzionistica, CNR, Pavia, Italy [c] Department of Pharmacology and Molecular Toxicology, University of Massachusetts Medical Center, Worcester, MA

To cite this Article Pregnolato, M. , Ubiali, D. , Verri, A. , Focher, F. , Spadari, S. , Sun, H. , Zhi, C. and Wright, G. E.(1999) 'Synthesis and Molecular Modeling of Novel HSV1 Uracil-DNA Glycosylase Inhibitors', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 709 — 711
To link to this Article: DOI: 10.1080/15257779908041549
URL: http://dx.doi.org/10.1080/15257779908041549

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 709–711 (1999)

SYNTHESIS AND MOLECULAR MODELING OF NOVEL HSV1 URACIL-DNA GLYCOSYLASE INHIBITORS

M. Pregnolato,[1*] D. Ubiali,[1] A. Verri,[2] F. Focher,[2] S. Spadari,[2]
H. Sun,[3] C. Zhi,[3] G.E. Wright[3]

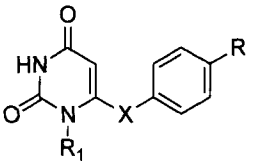
[1] *Dipartimento di Chimica Farmaceutica, Università degli Studi, I-27100 Pavia, Italy.*
[2] *Istituto di Genetica Biochimica ed Evoluzionistica, CNR, I-27100 Pavia, Italy.*
[3] *Department of Pharmacology and Molecular Toxicology, University of Massachusetts Medical Center, Worcester, MA 10655.*

ABSTRACT: In a recent paper the first selective inhibitors of HSV1 uracil-DNA glycosylase (UDG) acting in the micromolar range have been reported [1]. A 28.5 kDa catalytic fragment of HSV1 UDG has been crystallized in the presence of uracil, and the structure was recently solved[2]. Starting with the optimized model of binding between 6-(4'-*n*-octylanilino)uracil (octAU) and UDG some new derivatives have been predicted to be active. *In vitro* studies with the novel synthetized compounds confirm the plausibility of the model and define the structure features for UDG inhibitors.

Recent evidence suggests that the Herpes Simplex Virus type 1 (HSV1) uracil-DNA glycosylase (UDG) is required both for virus reactivation from latency and for efficient replication in nerve tissue. Drugs acting on viral targets involved in virus DNA synthesis, such as UDG, may have clinical benefits in herpes encephalitis as well as other HSV1 associated diseases. The most potent compound, 6-(4'-*n*-octylanilino)uracil (octAU) **1** (Figure 1), had IC_{50} = 8 μM against the viral enzyme but >300 μM against the human enzyme[1].

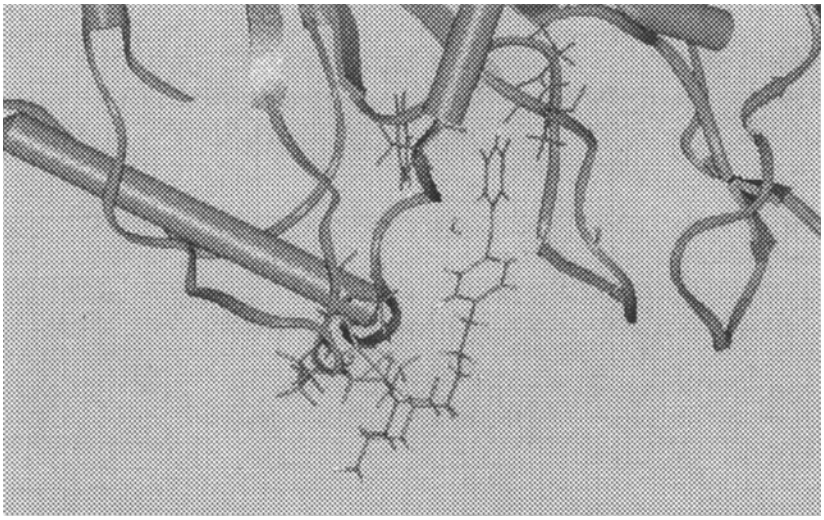
OctAU **1** is a good lead inhibitor of HSV1 UDG although modifications are needed in order to increase the binding affinity and to improve physicochemical properties such as water solubility. We adopted the structure-based rational design approach, starting with the computer-assisted prediction of the inhibitor binding in the enzyme-inhibitor complex.

Figure 1



	X	R	R_1
1:	NH	C_8H_{17}-*n*	H
2:	NH	$C_{10}H_{21}$-*n*	H
3:	NH	OC_6H_{13}-*n*	H
4:	NH	OC_8H_{17}-*n*	H
5:	O	C_8H_{17}-*n*	H

Figure 2



The structural model of octAU:UDG resulting from the energy minimization procedure is shown in Figure 2. In this model the uracil ring of the inhibitor binds in the same position as free uracil in the UDG crystal structure. The 6-NH bond is approximately perpendicular to the phenyl ring of Tyr 90, with a distance of 3.85 Å, an interaction energetically equivalent to about half of a normal hydrogen bond. The second half of the octyl chain lies snugly in the 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 its biological function. Leu 214 proposed to insert into the DNA through the minor groove, in the human UDG structure, assisting flipping of the nucleotide to be removed, and the Leu-DNA interactions stabilized the extrahelical nucleotide conformation. Thus the association of the hydrophobic *n*-octyl group with the similarly hydrophobic residues on the surface of

Figure 3

$$pIC_{50} = -0.23565E_{inter} - 6.5588 \quad (R^2 = 0.987)$$

the protein plays a central role in inhibitor binding. The intermolecular interaction energies (E_{inter}) between HSV1 UDG and alkylanilinouracils have been calculated. The results were plotted against observed pIC_{50} [-log(IC_{50})] and a good correlation was observed for the training set (Figure 3). Using the equation, we are able to make predictions of activities of novel inhibitors prior to synthesis.

The synthetized inhibitors were all structurally related to alkylAUs. Table 1 shows that the predicted IC_{50} values are in good agreement with the experimental IC_{50} obtained *in vitro*.

Table 1. Inhibitor-enzyme interaction energy, and calculated and experimental IC_{50} values.

Ligand	Total Energy (kcal/mole)	Van der Waals' (kcal/mole) repulsion - dispersion = total			Coulombic (kcal/mole)	IC_{50}c* (μM)	IC_{50}e* (μM)
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

*IC_{50}c is the calculated value, and IC_{50}e is the experimental value.

The model predicts that 3-alkyl and 5-alkyl inhibitor derivatives would lack activity: indeed, 3-ethyl-hexylAU and 5-methyl-hexylAU, i.e. the thymine analog, did not inhibit UDG at 500 mM. Furthermore a phenoxy ether replacing the anilino NH should reduce activity. The synthetized 6-(4'-octylphenoxy)uracil **5** was a 19-fold weaker inhibitor (IC_{50} = 150 mM) than **1**. Finally, several 1-substituted derivatives (R_1= alkyl, alkyloxy) have been synthetized and then tested as inhibitors in "*in vitro*" assays. The inhibition data obtained are in complete agreement with the model which can be used, indeed, for the design of more active compounds with better physicochemical properties. All experimental data will follow.

REFERENCES

[1]Focher, F.; Verri, A.; Spadari, S.; Manservigi, R.; Gambino, J.; Wright, G.E. *Biochem. J.* **1993**, *292*, 883-889.

[2]Savva, R.; McAuley-Hecht, K.; Brown, T.; Pearl, L. *Nature*, **1995**, *373*, 487-493.