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(2R, 4S, 5S)-1-(TETRAHYDRO-4-HYDROXY-5-METHOXY-2-FURANYL)THYMINE: A POTENT SELECTIVE INHIBITOR OF HERPES SIMPLEX THYMIDINE KINASE

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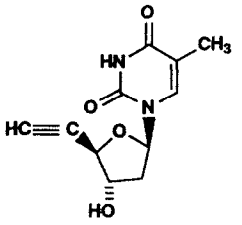
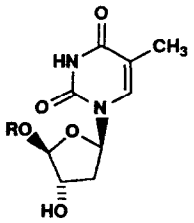
Abstract: A series of thymidine analogues substituted with alkoxy groups on the C-4 position of the furan ring were synthesized. Among these compounds, the methoxy analogue **9** was the most potent inhibitor of herpes simplex virus type 1 thymidine kinase.

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) induce unique virus-specified thymidine kinase (TK) in infected cells. [1-3] While the activity of viral TK does not appear to be critical for virus replication in the cell culture system, studies have suggested that it is important for virus pathogenecity and reactivation of latent virus from neural cells. [4-7] Therefore, the control of herpatic recurrences by inhibiting viral TK would be a potential new approach for treatment of recurrent HSV disease.

In 1987, Cheng reported the 5-ethynyl thymidine analogue **1** as a potent and selective inhibitor of viral TK.[8] Since then, there have been a number of reports describing the selective inhibitory activity of various thymidine kinase inhibitors as potential treatment of HSV latency. [4-7, 9, 10] Among HSV thymidine kinase inhibitors, 5-modified thymidine analogues as exemplified by the 5-ethynyl derivative **1** are substrate analogues of thymidine and competitively inhibit HSV-1 and HSV-2 TKs. Obviously, the lack of the hydroxymethyl functionality at the 5 position prevents them from undergoing phosphorylation. As part of a program to identify new HSV thymidine kinase inhibitors, a new class of thymidine analogues **2** were prepared in which the C-5 hydroxymethyl functionality of thymidine was replaced with various alkoxy groups.



1

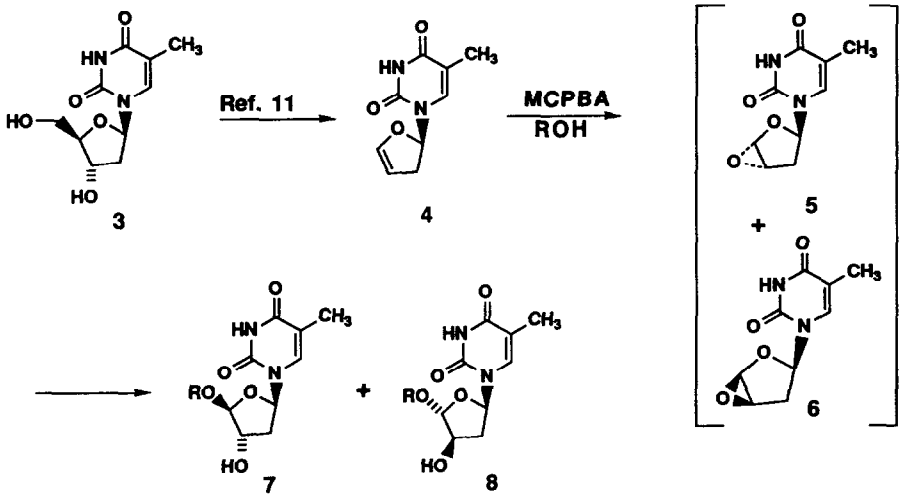


2

We felt that the target structure **2** could arise from the stereoselective oxidative addition of alcohols to the furanoid glycal **4** (Scheme I). The intermediate **4** is in turn derived from thymidine as described by Horwitz and co-workers,[11] making this approach highly convergent. On the basis of our previous stereochemical investigation on the electrophilic addition to the furanoid glycals, [12,13] the oxidative addition of alcohols to the glycal **4** was expected to generate stereoselectively the trans isomer **7** as the major product. However, to our surprise, treatment of **4** with m-chloroperbenzoic acid in the presence of an excess of alcohols provided the desired trans isomers **7** as minor products and the other trans isomers **8** were predominant in the reaction mixture as summarized in Table I. Although there is no good literature precedent, it appears that the initial epoxidation is directed by the hydrogen bonding of m-chloroperbenzoic acid and the ureido moiety of the thymine ring. This directing effect will generate the α epoxide **6** as a major product, from which **8** is produced by the regiospecific epoxide opening with alcohols. Likewise, the trans isomer **7** is derived from the minor epoxides **5**. Many attempts to isolate **5** and **6** were not successful due to their instability toward the column purification on silica gel.

Stereochemical assignment of **7** and **8** by NMR was difficult because both isomers exhibited strong Nuclear Overhauser effects between the C-2 and C-5 protons. Eventually, the structure of **9** was confirmed by a single crystal X-ray study of the 4-p-bromobenzoyl ester of **9** as shown in Figure I. Interestingly, the C-2 proton of each trans isomer **7** appeared consistently as a triplet in their NMR (Table I).

SCHEME I



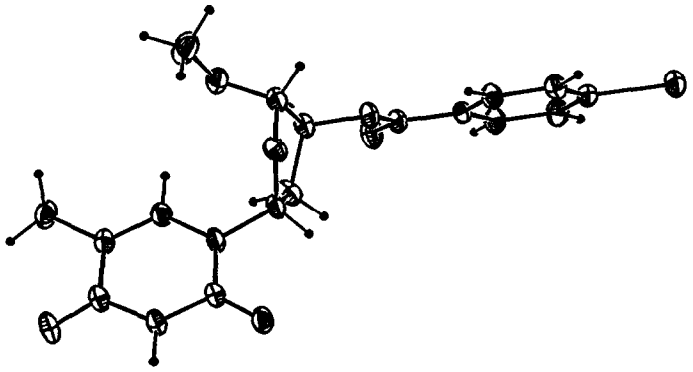
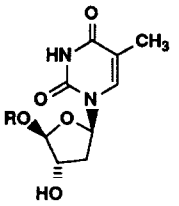
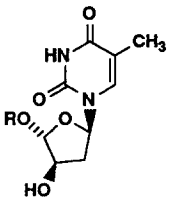


FIGURE I: X-ray crystal structure of 4-p-bromobenzoate of 9

TABLE I



9 R = CH_3
11 R = CH_2CH_3
13 R = $CH_2CH_2CH_3$
15 R = t-Bu
17 R = CH_2Ph
19 R = CH_2CH_2Ph
21 R = $CH_2CH_2CH_2Ph$



10 R = CH_3
12 R = CH_2CH_3
14 R = $CH_2CH_2CH_3$
16 R = t-Bu
18 R = CH_2Ph
20 R = CH_2CH_2Ph
22 R = $CH_2CH_2CH_2Ph$

Cmpd	Yield (%)[a]	^{1}H NMR (δ)[b]		
		H-2	H-4	H-5
9	15	6.43 (t, J=6.6 Hz)	4.12 (bs)	5.41 (d, J=3.9 Hz)
10	55	6.12 (d, J=6.8 Hz)	4.25 (d, J= 4.9 Hz)	5.26 (s)
11	12	6.43 (t, J=6.9 Hz)	4.13 (bs)	5.42 (d, J=3.9 Hz)
12	45	6.13 (dd, J=7.0, 1.3 Hz)	4.29 (d, J=4.8 Hz)	5.25 (s)
13	11	6.52 (t, J=6.9 Hz)	4.26 (d, J=4.1 Hz)	4.95 (s)
14	48	6.29 (dd, J=-8.1,2.5 Hz)	4.20 (d, J=5.4 Hz)	5.13 (s)
15	5	6.34 (t, 6.7 Hz)	3.99 (d, J=3.9 Hz)	5.15 (s)
16	37	6.13 (d, J=6.9 Hz)	4.18 (d, J=4.3 Hz)	5.27 (s)
17	9	6.75 (t, J=6.5 Hz)	4.46 (t, J=4.5 Hz)	5.11 (s)
18	31	6.23 (d, J=7.6 Hz)	4.35 (d, J=4.5 Hz)	5.28 (s)
19	12	6.56 (t, J=6.7 Hz)	4.24 (d, J=4.4 Hz)	4.96 (s)
20	49	6.12 (dd, J=8.0, 2.1 Hz)	4.26 (d, J=5.4 Hz)	5.14 (s)
21	11	6.58 (t, J=6.7 Hz)	4.28 (d, J=4.1 Hz)	4.93 (s)
22	47	6.28 (dd, J=8.3,2.4 Hz)	4.20 (d, J=5.2 Hz)	5.12 (s)

[a] Isolated Yield
[b] $CDCl_3$, 300 MHz

Results of HSV thymdine kinase inhibitory activity by 5-alkoxy thymidine analogues are listed in Table II. In this assay, 5-ethynyl analogue **1** was used as a reference compound. Systematic variations in the R of **2** have shown that for the HSV type 1 thymidine kinase inhibition, the following holds true:

TABLE II: HSV-1 TK Inhibitory Activity of 5-Alkoxy Thymidine Analogues

Compound	IC_{50}[a] (μM)	Ki (μM)	Compound	IC_{50} (μM)
9	5	2.9	**10**	> 50
11	50	ND[b]	**12**	> 50
13	> 50	ND	**14**	> 50
15	> 50	ND	**16**	> 50
17	> 50	ND	**18**	> 50
19	25	26	**20**	> 50
21	10	4.3	**22**	> 50
1	3	2.0		

[a] Concentration causing a 50% inhibition of HSV-1 TK phosphorylation of 1.2 μM dThd[14].

[b] Not determined.

1. Only some derivatives of trans isomer **2**, which possesses the same stereochemical configuration as thymidine at the C-4 and C-5 positions exhibited HSV-1 TK inhibitory activity. All isomers related to **8** appear to be not recognized by HSV-1 TK as substrate analogues of thymidine.

2. Among alkyl derivatives of **7**, the methyl analogue **9** is the most potent inhibitor, thus indicating that increasing alkyl size is detrimental for the HSV-1 TK inhibition. However, addition of the phenyl functionality on the alkyl side chain increased the potency for the HSV-1 TK inhibition (compounds **19** and **21**). This suggests that the binding pocket for the C-5 position is large and the π interaction due to the phenyl group increases the binding affinity toward the HSV-1 TK.

In conclusion, a novel series of 5-alkoxy thymidine analogues has been shown to possess potent HSV-1 TK inhibitory activity. Compounds with this type of activity could play an important role in the treatment of HSV latency.

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14. Affinity-purified HSV-1 (KOS) TK was assayed in 30 mM Tris-HCl pH 7.5, 6 nM $MgCl_2$, 6 mM ATP, 0.5 mM dithiothreitol, 0.075 mg/mL bovine serum albumin, 1.2 μM (^3H) thymidine and 0-50 μM thymidine analogue for 1 h at 37°C; analogues were dissolved in DMSO resulting in a final DMSO concentration of 5%. The K_i we determined for **1** is substantially higher than reported by Nutter et al.[8] and we attribute this discrepancy to different buffer conditions.