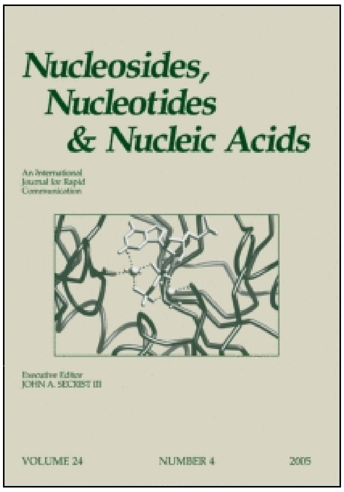

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INHIBITION OF THE HERPES SIMPLEX VIRUS THYMIDINE KINASE BY 5'-SUBSTITUTED THYMIDINE ANALOGUES. COMPARISON OF THE TYPES 1 AND 2 ENZYMES.

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Abstract: A series of 5'-substituted-deoxypyrimidine nucleosides were examined for their ability to inhibit the thymidine kinases of types 1 and 2 herpes simplex virus; structure activity relationships were determined.

A number of nucleosides have been described as antiviral agents effective against herpes simplex virus (HSV) types 1 and 2. These compounds act primarily as inhibitors of the virus DNA polymerase but require specific phosphorylation by the virus thymidine kinase for their initial activation. Studies with virus mutants defective in the production of thymidine kinase have suggested that this enzyme is an important determinant of virus virulence in infected animals, particularly limiting the ability of the virus to replicate in neural tissue,[4,5,6] yet there have been few attempts to develop inhibitors of thymidine kinase as antivirals.[7] 5'-substituted analogues of thymidine have been described as inhibitors of the thymidine kinase of the Walker 256 carcinoma[8,9] and of *Escherichia coli*[10], and a number of analogues of 5'-amino-2',5'-dideoxy-5-iodouridine (AIU) were previously reported as inhibitors of HSV-1 thymidine kinase.[11] We have examined these latter compounds and further analogues of 5'-amino-5'-deoxythymidine (AT) for their ability to inhibit both the HSV-1 and HSV-2 thymidine kinases. We have found certain functionalities at the 5'-position to be preferred for enzyme inhibition and have observed differences between the two enzymes in their sensitivity to these compounds.

MATERIALS AND METHODS

The synthesis of compounds 1-8 and 14-15 has been reported previously.[11,12] Compounds 9-13[13] were synthesised according to the general method described earlier.[12] HSV-1 (strain S3)[14] and HSV-2 (strain 3345)[15] thymidine kinases were prepared from virus-infected BHK cells as described by others[16] and purified by ion exchange chromatography. Briefly, the enzyme was loaded on to a DEAE Sephacel column equilibrated in a buffer containing 20mM Tris HCl, 0.1mM dithiothreitol, 10µg/ml phenylmethylsulphonyl fluoride, 10% glycerol, pH7.5. The column was washed with the same buffer until the E_{280} of the eluate was below 0.01 O.D. units and finally developed with a linear gradient of 0.02M NaCl. Analysis of the pooled fractions containing the thymidine kinase revealed only a single band on polyacrylamide gel electrophoresis; no enzyme activity was detected when an equivalent number of mock-infected cells were treated in a similar manner. The types 1 and 2 enzymes were neutralised by homologous virus-specific antisera. Inhibition assays were run as described previously[11] except that the reaction mix contained buffer (50mM Tris HCl, pH7.5, 0.1mM dithiothreitol, 0.1mg/ml bovine serum albumin), 10mM ATP, 20mM $MgCl_2$, 2µM [2-^{14}C]-thymidine (approximately 55mCi/mmol), enzyme and inhibitor. Each assay was run using compound at 7 concentrations, with 3 replicate determinations at each concentration. The data were analysed by logistic regression[17] using a computer program[18] to determine the best fit to a sigmoid curve and to calculate the 50% inhibitory concentration (IC_{50}) and 95% confidence interval.

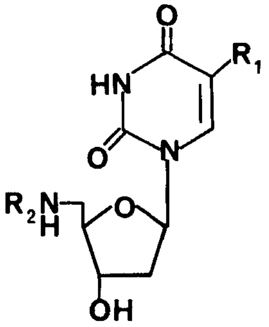
RESULTS AND DISCUSSION

Table 1 shows the inhibitory activity of each of the compounds against the type 1 and type 2 thymidine kinases. All derivatives of AIU (2-8) were more effective against the type 2 than the type 1 enzyme. This may be due in part to the presence of iodine at the 5-position. It has been shown previously that the K_i of 5-iodo-2'-deoxyuridine is approximately 2-fold less for the type 2 enzyme than for the type 1 enzyme.[19] However, the greater activity of these analogues against the type 2 enzyme may not be due entirely to the presence of iodine since optimisation of the 5'-substituent (in 6) resulted in a compound that was approximately 20-fold more inhibitory to the type 2 enzyme than to the type 1 enzyme. At the same time, comparison of 1 and 2 against the type 2 enzyme showed that a more effective compound was obtained when methyl was replaced with iodine at the 5-position of the pyrimidine ring; this is in accord with previous observations.[11]

The sulphonamide derivatives of AT (9-15) showed a pattern of inhibition similar to the amides with greater activity against the type 2 than the type 1 enzyme. A comparison of 8 with 2 showed a measurable gain in inhibitory activity was obtained against the type 2 enzyme when the amide function was replaced with a sulphonamide. However, the increase in activity was small and further comparisons are required to determine whether the sulphonamide function is generally preferred to the amide.

Further differences between the two enzymes were apparent from comparisons of the inhibitory activity of various 5'-amido and 5'-sulphamido substituents and preferred functions for the inhibition of the type 2 enzyme were identified. The increasing lipophilicity of the 5'-substituent (2-7) correlated approximately with the increasing inhibitory activity against both enzyme types.

TABLE 1. Inhibition of HSV-1 and HSV-2 thymidine kinases by various derivatives of AIU and AT.



Compound	R1	R2	IC_{50} ± error* - μM	
			HSV-1	HSV-2
1	CH_3	CH_3CO	>200	≃200
2	I	CH_3CO	>200	78±13
3	I	C_2H_5CO	159	21±6
4	I	$(CH_3)_2CHCO$	55±9	7±2
5	I	$(CH_3)_2CHCH_2CO$	68±18	8±1
6	I	$CH_3(CH_2)_3CO$	38±15	2±0.4
7	I	C_6H_5CO	85±14	16±2
8	I	CH_3SO_2	>200	35±10
9	CH_3	$p\text{-}CH_3C_6H_4SO_2$	>200	88±20
10	CH_3	$p\text{-}CH_3OC_6H_4SO_2$	>200	116±12
11	CH_3	$p\text{-}NO_2C_6H_4SO_2$	127±39	21±2
12	CH_3	$p\text{-}BrC_6H_4SO_2$	171±29	13±2
13	CH_3	CF_3SO_2	>200	>200
14	CH_3	$HOSO_2(CH_2)_3SO_2$	>200	>200
15	CH_3	$p\text{-}HOSO_2NHC_6H_4SO_2$	>200	>200

* 95% confidence interval

The somewhat lower than expected activity of the isovaleryl (5) and benzoyl (7) derivatives is suggestive of a spatial requirement for optimum activity although this cannot be defined on the basis of the available data. Compound 6 was the most effective inhibitor of the HSV-2 thymidine kinase (IC_{50} = 2μM) and showed the greatest selectivity for this type enzyme (approximately 20-fold). The relative activities of the aryl sulphonamides (9-12) suggested that the electron withdrawing substituents (i.e, NO_2 and Br of 11 and 12) were preferred to the electron releasing ones (9 and 10). Those compounds that had an acidic 5'-substituent (14 and 15) had no activity against either enzyme type. The trifluoromethyl derivative (13) was also found to be inactive.

Although there is extensive homology between the amino acid sequences of the HSV-1 and -2 thymidine kinases,[20,21] differences between the two enzymes, both antigenic[16] and biochemical, have been described. Previous studies have shown that the two enzyme types differ markedly in the extent to which they will accept thymidine analogues as alternative substrates when 5-methyl is replaced with larger groups, such as in the case of (E)-5-(2-bromovinyl)-2'-deoxyuridine whose affinity for the type 1 enzyme is 10-fold greater than for the type 2 enzyme.[22] Similarly, 5'-ethyl-5'-deoxythymidine, an inhibitor of HSV thymidine kinase, was more active against the type 1 than the type 2 enzyme.[7] Several of the compounds described here were effective inhibitors of the thymidine kinases of both virus types and a trend towards the selective inhibition of the type 2 enzyme was observed.

The results presented here provide the basis for the design of more potent inhibitors of the HSV thymidine kinase. There are few reports of the synthesis and testing of compounds of this type yet studies such as these may yield valuable information on the structure of the enzyme active site. In addition, the use of thymidine kinase inhibitors of the type described here may provide a new means to study the role of the virus enzyme in determining virulence in infected animals. Finally, it is possible that compounds of this type may be useful in the management of herpes virus infections in man. Nutter et al.[7] showed that the treatment of virus-infected cells in culture with 5'-ethyl-5'-deoxythymidine resulted in a substantial reduction in the size of the intracellular thymidine triphosphate pool. Thymidine kinase inhibitors may be particularly effective in suppressing the reactivation of latent virus in the resting, non-dividing cells of the spinal ganglia where the virus enzyme may have an essential role, increasing the thymidine triphosphate concentration to the point where virus replication can occur.

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