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Nucleoside Analogues as Highly Potent and Selective Inhibitors of Herpes Simplex Virus Thymidine Kinase

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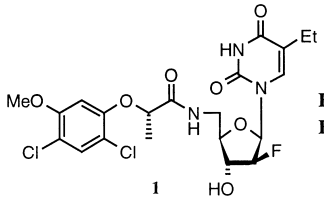
Abstract—A series of carboxamide derivatives of 5′-amino-2′,5′-dideoxy-5-ethyluridine has been prepared as inhibitors of HSV-TK (herpes simplex virus thymidine kinase). The most potent compounds were derived from xanthene, thioxanthene and dihydroanthracene carboxylic acids. The lead compounds show subnanomolar IC_{50} values against HSV TKs.

Introduction

Herpes simplex virus (HSV) is one of the most common viral infections in man.[1] Type 1 (HSV-1) primarily causes orofacial lesions, whereas type 2 (HSV-2) is mainly but not exclusively the causative agent of genital infections, which continue to increase worldwide.[2] Following the primary infection, lesions resolve in around 10 days but unfortunately by this stage the virus has migrated to neuronal tissue, where it resides in a latent state. Upon reactivation, the virus causes a recurrence of the initial infection, which in many patients can be particularly severe resulting in a profound effect on their quality of life. Current therapy for the control of recurrent herpes infections involves the use of antiviral drugs such as Acyclovir, which prevents replication of virus after reactivation has occurred. An alternative strategy would be to prevent the reactivation of latent virus. Although the precise mechanisms involved in reactivation of latent virus are not fully understood, the virally encoded enzyme thymidine kinase (TK) has been implicated.[3] TK catalyses the phosphorylation of thymidine to the monophosphate, which is a precursor for DNA synthesis. HSV TK is not essential for virus replication in rapidly dividing cells where sufficient thymidine triphosphate for viral DNA synthesis is provided by cellular metabolism, whereas it is required in non-dividing nerve cells[4] where little or no cellular DNA synthesis occurs.

TK deficient mutants of HSV do grow in cell culture but are defective in primary neuronal cultures. Furthermore, TK deficient mutants can cause primary infection and establish latency in animal models but do not reactivate from the latent state.[5] These findings have led to considerable interest in the search for potent and selective inhibitors of HSV TK as a novel approach to prevent the reactivation of virus from latency.



HSV-1 TK : IC_{50} = 1.8nM
HSV-2 TK : IC_{50} = 0.19nM

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We and others have previously published[6–17] on the design and biological properties of nucleoside analogues as inhibitors of HSV TK. In our initial studies,[6] we based our design of HSV TK inhibitors on isosteric and isoelectronic analogues of thymidine monophosphate,

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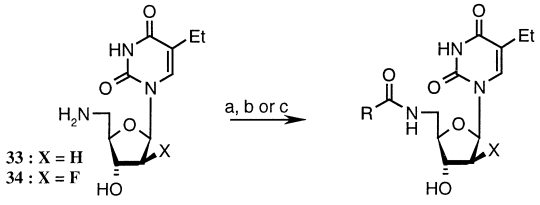
the product of the biochemical process catalysed by TK. Therefore, we synthesised phosphate, phosphonate and sulphonate esters of thymidine as well as a number of amide and sulphonamide derivatives of 5′-amino-5′-deoxythymidine. The most potent of these was 5′-phenylacetamido-5′-deoxythymidine. We then made the corresponding 5-ethyl analogue **2** on the grounds that 2′-deoxy-5-ethyluridine is known to have a much higher affinity for HSV TK than the cellular enzyme.[18] As expected, inhibitors derived from this nucleoside did show high selectivity for the viral enzymes. Furthermore, 2′-deoxy-5-ethyluridines were an order of magnitude more potent than the corresponding thymidine analogues. More recently, we reported[19,20] a preliminary account of further studies which culminated in the synthesis, biochemical properties and activity in animal models of the phenoxyacetamide **1**, a highly potent and very selective inhibitor of HSV TK. We now wish to report additional studies on another series of highly potent inhibitors of HSV TK.

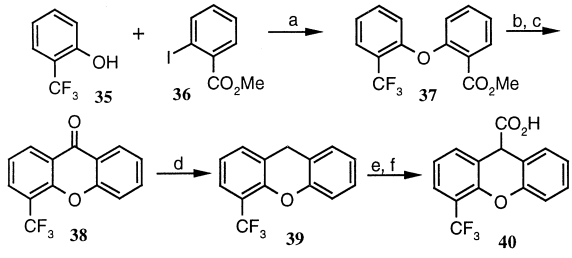
Chemistry

The carboxamides **2**–**17**, **19**, **20**, **22**–**29, 31** and **32** were obtained by acylation of the previously described[19] 5′-amino-2′,5′-dideoxyuridines **33** and **34** by reaction with the appropriate acid chloride under Schotten–Baumann conditions (method A) or through coupling of the acid with the amine in the presence of 1,3-dicyclohexyl carbodiimide (method B) or *N*-ethyl-*N*-(3-dimethylaminopropyl)carbodiimide (method C) as shown in Scheme 1.



Scheme 1. Reagents: (a) RCOCl, NaOH, H_2O (method A); (b) RCO_2H, DCCI, HOBT, DMF (method B); (c) RCO_2H, EDC, HOBT, DMF (method C).

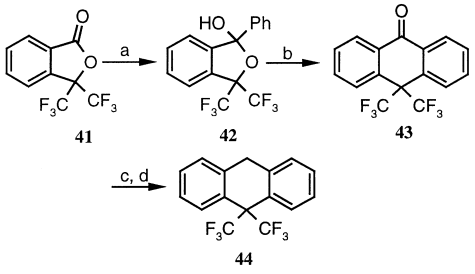
New carboxylic acids were synthesised through lithiation and carboxylation of suitably substituted xanthenes, thioxanthenes or 9,10-dihydroanthracenes.



Scheme 2. Reagents: (a) CuO, DMA; (b) NaOH; (c) H_2SO_4; (d) BH_3·THF; (e) LDA, THF; (f) CO_2.

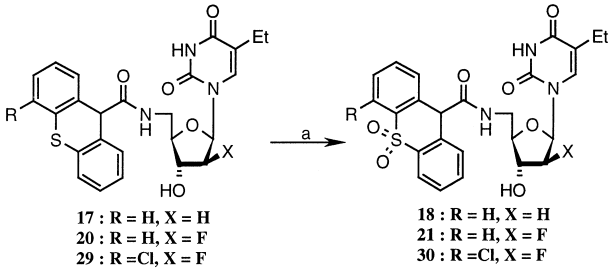
If these were not commercially available, they were prepared by published procedures[21–23] or by a general route illustrated in the synthesis of the 4-trifluoromethylxanthene carboxylic acid[24] **40** (Scheme 2). Thus, Ullmann coupling of the trifluoromethylphenol **35** with methyl 2-iodobenzoate **36** gave the diphenyl ether **37**. Hydrolysis of the ester and cyclisation to the xanthone **38** was followed by reduction to the xanthene **39**, which was then lithiated and carboxylated. Analogous strategies were applied to the synthesis of 4,5-dichloroxanthene-9-carboxylic acid and 4,5-dimethylxanthene-9-carboxylic acid. The 10,10-bis(trifluoromethyl)-9-10-dihydro-9-anthracene carboxylic acid was prepared from the lactone[25] **41** through reaction with phenyl magnesium bromide and cyclisation of the product **42** to the anthrone **43**.

In this case, reduction of the anthrone **43** with diborane proceeded only as far as the anthrol, catalytic hydrogenation of which gave the desired dihydroanthracene **44** (Scheme 3).



Scheme 3. Reagents: (a) PhMgBr, Et_2O; (b) PPA; (c) BH_3, THF; (d) H_2, Pd–C.

The sulphones **18**, **21** and **30** were obtained by oxidation of the corresponding thioxanthene derivatives with *m*-chloroperbenzoic acid as shown in Scheme 4.



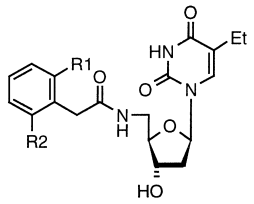
Scheme 4. Reagents: (a) MCPBA, DCM.

Results and Discussion

The compounds described herein were tested in assays following previously described procedures.[19]

In the phenylacetamide series, the effect of introducing a substituent, particularly in the *ortho*-position, resulted in increased potency (Table 1). Whilst the 2-fluorophenylacetamide **3** was only slightly more potent than **2**, a diverse range of other 2-substituted phenylacetamides **4**–**8** showed higher and rather similar potencies suggesting that the principle influence of the 2-substituent was steric in nature. We reasoned that a 2,6-disubstitution pattern would restrict the rotational freedom of the aryl ring and could enhance potency further.

Table 1. IC_{50} values[a] of phenylacetamides **3**–**11**

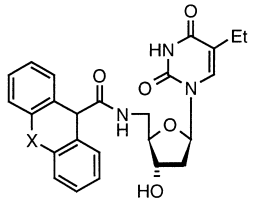


Compound	R^1	R^2	IC_{50} (nM)	
			HSV-1 TK	HSV-2 TK
2	H	H	1000	300
3	F	H	700	140
4	Cl	H	100	30
5	Me	H	200	60
6	CF_3	H	200	90
7	MeO	H	300	70
8	Ph	H	240	90
9	Cl	Cl	1.0	3.3
10	Me	Me	40	8.4

[a]Values are means of three determinations.

This proved to be the case when the 2,6-dichloro and 2,6-dimethyl analogues **9** and **10** were found to be an order of magnitude more potent than monosubstituted compounds. However, these compounds were still significantly less potent than the phenoxyacetamide **1**. In an attempt to further define the lipophilic sites responsible for binding of the aryl residue of our inhibitors to the enzymes we were intrigued by the possibility of designing conformationally restricted analogues of the 2-phenyl-phenylacetamide **8**. Thus, introduction of a direct bond between the two phenyl substituents in structure **8** and the α-carbon atom resulted in the fluorenyl structure **11** (Table 2).

Table 2. IC_{50} values[a] of compounds **12**–**19**

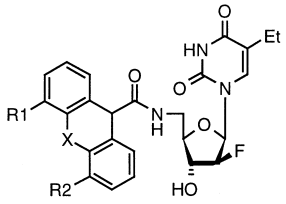


Compound	X	Ring system	IC_{50} (nM)	
			HSV-1 TK	HSV-2 TK
11	Direct bond	Fluorene	28.0	5.2
12	CH_2	Dihydroxanthene	7.0	1.2
13	$(CH_2)_2$	Dibenzosuberene	260.0	44.0
14	CH	Anthracene	1650.0	420.0
15	O	Xanthene	2.9	0.13
16	NMe	NMe-acridine	23.0	2.6
17	S	Thioxanthene	11.5	1.4
18	SO_2	Thioxanthene dioxide	0.95	0.13

[a]Values are means of three determinations.

This compound proved to be significantly more potent than **8** and therefore additional tricyclic derivatives were examined. The dihydroanthracene **12** was somewhat more potent than **11**, whereas the dibenzosuberene derivative **13**, which has a greater degree of conformational mobility was considerably less potent. Interestingly, the planar anthracene carboxamide **14** was a relatively weak inhibitor, whilst the heterocycles **15**–**18** were markedly more potent. In our earlier studies on inhibitors of HSV TK we noted that the introduction of a 2′-fluorine substituent in the arabino configuration in 5′-amino-2′,3′-dideoxy-5-ethyluridine carboxamides resulted in a modest increase in potency and good oral bioavailability. Therefore, we studied 2′-fluoro analogues of the most potent tricyclic carboxamides **15**, **17** and **18**. As expected, these inhibitors **19**–**21** showed at least equivalent potency to the parent compounds (Table 3).

Table 3. IC_{50} values[a] of 2′-fluoroarabinofuranosides



Compound	R^1	R^2	X	IC_{50} (nM)	
				HSV-1 TK	HSV-2 TK
19	H	H	O	2.1	0.11
20	H	H	S	8.5	0.70
21	H	H	SO_2	0.93	0.11
22	Cl	H	O	0.85	0.14
23	Me	H	O	1.6	0.19
24	CF_3	H	O	1.0	0.14
25	MeO	H	O	1.7	0.21
26	Ph	H	O	0.66	0.14
27	Cl	Cl	O	0.30	0.10
28	Me	Me	O	2.2	0.43
29	Cl	H	S	1.5	0.21
30	Cl	H	SO_2	0.27	0.09
31	H	H	$C(Me)_2$	1.5	0.28
32	H	H	$C(CF_3)_2$	0.19	0.11

[a]Values are means of three determinations.

We then examined the effect of substituents in the heterocyclic tricyclic residues. A variety of substituents, particularly in the 4-position resulted in the highly potent inhibitors **22**–**26**, **29** and **30**. By introducing a single substituent into the tricyclic moiety, these compounds suffered the disadvantage of being asymmetric. For synthetic convenience these carboxamides were prepared using racemic carboxylic acids and the resulting products were evaluated as 1:1 mixtures of diastereoisomers. The stereochemical issue was overcome through the use of symmetrically disubstituted carboxylic acids. This is illustrated with the 4,5-dichloro- and 4,5-dimethylxanthene carboxamides **27** and **28**. Compounds in Table 3 are amongst the most potent HSV TK inhibitor reported thus far, especially **21**, **22**, **26**, **27**, **30** and the symmetrical 10,10-disubstituted-dihydroanthracene carboxamide **32**, all of which are subnanomolar against both enzymes.

Having shown that these compounds were potent inhibitors of HSV TK, we wished to demonstrate their

selectivity for the viral enzymes. Therefore, a representative selection of the most potent inhibitors was evaluated against cytosolic TK from both HeLa and Vero cells. As had been found with our previously published inhibitors, no inhibition of cellular TK was measured at a concentration of 10 μM.

In conclusion, the xanthene, thioxanthene and dihydroanthracene carboxamides described herein represent a new class of highly potent and selective inhibitors of HSV TK. A number of these compounds have shown activity in murine models of HSV infection when dosed intraperitoneally but were significantly less effective when given by the oral route. The reason for these differences has yet to be resolved.

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