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Successful kinase bypass with new acyclovir phosphoramidate prodrugs

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ABSTRACT

Novel phosphoramidates of acyclovir have been prepared and evaluated in vitro against acyclovir-sensitive and -resistant herpes simplex virus (HSV) types 1 and 2 and varicella-zoster virus (VZV). Unlike the parent nucleoside these novel phosphate prodrugs retain antiviral potency versus the ACV-resistant virus strain, suggesting an efficient bypass of the viral thymidine kinase.

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Herpes simplex virus infection is often well managed by the use of acyclovir, (ACV, **1**), its prodrug valacyclovir, or related compounds. The widespread use of (**1**) has led to the emergence of HSV strains that are resistant to this drug.

Resistance appears uncommon in immunocompetent patients; Morfin[1] reports a prevalence below 1%. A more recent study in the Netherlands reports a prevalence of 0.27% in this population.[2] However, resistance is significantly more common in immunocompromised patients. Stranska et al.[2] report 7% and Morfin cites 5%. Notably, the proportion of resistant isolates rises to 30% in patients receiving allogeneic bone marrow transplants. Three separate mechanisms of resistance to (**1**) have been considered to occur; a loss of viral thymidine kinase (TK) activity, an altered TK substrate specificity and an alteration of viral DNA polymerase.[3] Given the non-essential nature of the viral TK and the importance of the viral polymerase, it is unsurprising that the great majority of resistant isolates correspond to deletion/inactivation of the TK gene.[4] One approach to manage TK-related resistance is to use agents not requiring HSV TK for activation, such as cidofovir or foscarnet. However, they may carry a risk of increased toxicity. Another approach would be to bypass the dependence of (**1**) on HSV TK by using a suitable phosphate prodrug, or ProTide. Several such methods now exist, such as the cyclosal approach,[5] ester-based methods or SATE[6] and phosphoramidate diesters.[7] Our group has developed an aryloxy phosphoramidate triester approach,[8] which has been recently successfully applied to abacavir for HIV[9] and 4′-substituted nucleosides for Hepatitis C Virus (HCV).[10]

We have previously reported the application of this method to ACV (**1**) and the results indicated that the approach failed.[11] Thus, the ProTide (**2**) derived from (**1**) was found to be poorly active versus HSV2 ($EC_{50} \geqslant 100\ \mu M$) unlike (**1**) itself. Compound (**2**) was roughly equi-active as (**1**) versus VZV and slightly more active versus human cytomegalovirus (HCMV) but there was no clear therapeutic advantage. However we have recently reported a new generation of phosphoramidate protides in which the aryl moiety is a bicyclic system such as 1-naphthyl.[12]

We were keen to explore the application of the naphthyl ProTide methodology to (**1**) for two reasons. Firstly the observation that naphthyl for phenyl can give a potency boost and secondly that these compounds may have a significant lipophilicity enhancement over prior structures. This may be particularly important in the case of (**1**) where the inherent lipophilicity is rather low, and first generation protides may be insufficiently lipophilic for efficient passive diffusion into cells. Indeed, *C*log*P*[13] estimates on (**2**) indicate a figure of ca. −0.8; although significantly more lipophilic than (**1**) this is somewhat lower than what may be viewed as optimal. Thus, in addition to examining naphthyl phosphates we also sought more lipophilic esters than the methyl ester (**2**) previously reported.

Compounds were prepared from (**1**) as shown in Scheme 1.

In order to improve the solubility of ACV we protected the guanine base using *N*,*N*-dimethylformamide dimethyl acetal, to give (**7**). The coupling with the appropriate phosphorochloridate

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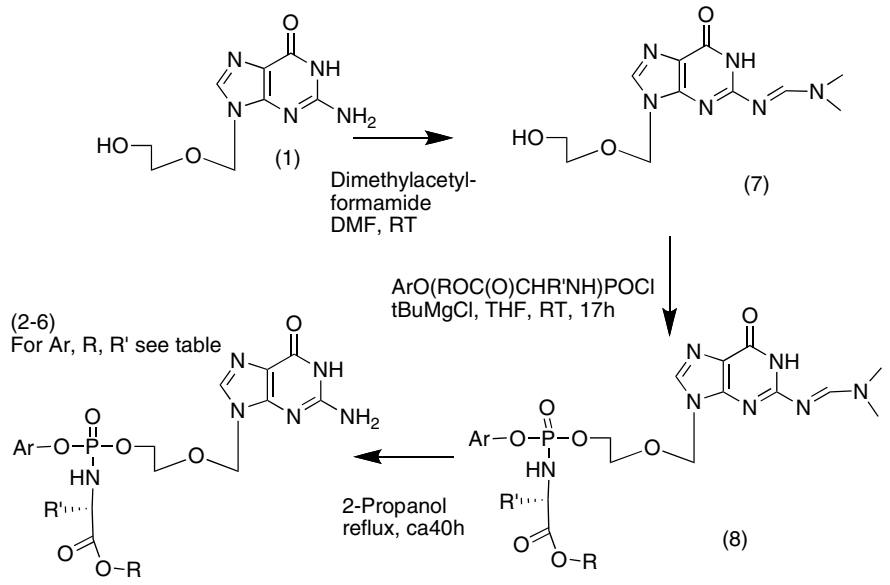

Scheme 1.

was performed using *tert*-butyl magnesium chloride as a hydroxyl activator, to give blocked compounds of type (**8**).[14] The DMF-protecting group was then removed by refluxing in 2-propanol (40–72 h). Owing to the chirality of the phosphorus, all of these compounds have been isolated and tested as a mixture of two diastereoisomers. Their structures have been demonstrated by NMR (^{31}P, ^{1}H and ^{13}C), mass spectroscopy and elemental analysis.[15]

The target compounds were first evaluated by plaque assay for their ability to inhibit the replication of ACV-sensitive and ACV-resistant HSV2 in Vero cells (Table 1).[16]

As can be seen from Table 1, while the previously reported phenyl methylalanine phosphate (**2**) is poorly active, being ca 7-fold less active than (**1**), in marked contrast to (**1**) it does retain full potency versus the resistant strain. This implies that (**2**) does function as a monophosphate prodrug as intended, but with low efficiency, particularly in the nucleoside-sensitive assay. By comparison, the naphthyl phosphate (**3**) is roughly equi-active with (**1**) versus TK-competent virus and notably retains full potency versus ACV-resistant HSV-2. The simplest explanation of this is that the ACV-resistant HSV-2 mutant is TK deficient and that (**3**) is TK-independent, strongly implying a successful thymidine kinase bypass. Notably, ClogP calculations on (**3**) indicate a significant enhancement over (**1**) to a figure of ca. 2 which may be regarded as near optimal. Indeed the 'mixed' compounds (**4**) and (**5**) have lower *C*log*P* values and are less active in the HSV-2 TK$^+$ assay, but retain good activity in the HSV-2 TK$^-$ assay. In a further assay in HEL cells we evaluated the samples against both TK$^+$ and TK$^-$ HSV-1 and HSV-2 with data shown in Table 2.

Similar data emerge here, with (**3**) being particularly active and retaining significant activity in the TK$^-$assay. In this case compound (**4**) shows a similar profile, while (**5**) is less active. This implies that the ester moiety (benzyl in (**3**) and (**4**), versus methyl in (**2**) and (**5**)) is more important than the aryl moiety. Notably, the amino acid-modified compound (**6**) which has Phe in place of Ala, is poorly active in this assay, particularly versus the resistant virus. This is despite what might be regarded as a near-optimal lipophilicity for (**6**) (Table 1) and points to the importance of the amino acid moiety for activity.

One concern with bypassing the HSV-TK might be of enhanced cytotoxicity and loss of antiviral selectivity. However, the MCC data on this series (Table 2) do not reveal a significant toxicity. If, as appears likely, the viral TK is being bypassed, there must still be some element of viral specificity at another stage, most likely at the polymerase level.

In another assay we examined this family of prodrugs against kinase-competent and kinase deficient VZV (in HEL cells) with data shown in Table 3. As noted in this table, unlike ACV (**1**) several of the agents retain good potency in the TK$^-$ VZV assays, notably compound (**4**) which essentially retains full potency. Interestingly (**4**) is also non-toxic while (**3**) does have some toxicity here.

Thus, compound (**4**) emerges as particularly active in a range of assays. It retains full activity versus all resistant viral strains, HSV-1, -2 and VZV, being low or sub-μM in most cases, and non-toxic.

In conclusion, we report the successful application of the ProTide approach to acyclovir. The naphthyl and phenyl benzyl alanine ProTides are fully active in vitro against ACV—resistant

Table 1
Anti-HSV-2 activity of ProTides

Compound	Ar	R	Amino acid	*C*log*P*	EC_{50}[a] (μM)	
					HSV2-HG32 (ECACC 158)	HSV2-ACVR (ECACC 513)
1	—	—	—	−2.42	6±1.3	>100
2	Ph	Me	Ala	−0.82	43.1±10.6	18.7±7.2
3	1-Nap	Bn	Ala	2.06	9.8±2.2	14.5±6.6
4	Ph	Bn	Ala	0.89	20.3±6.6	15.5±7.4
5	1-Nap	Me	Ala	0.35	40.4±13.4	15±6.2
6	Ph	Bn	Phe	2.31	20.3±6.6	33.2±3.9

[a] Values are means of three experiments, with standard deviations given, in Vero cells.

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