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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.

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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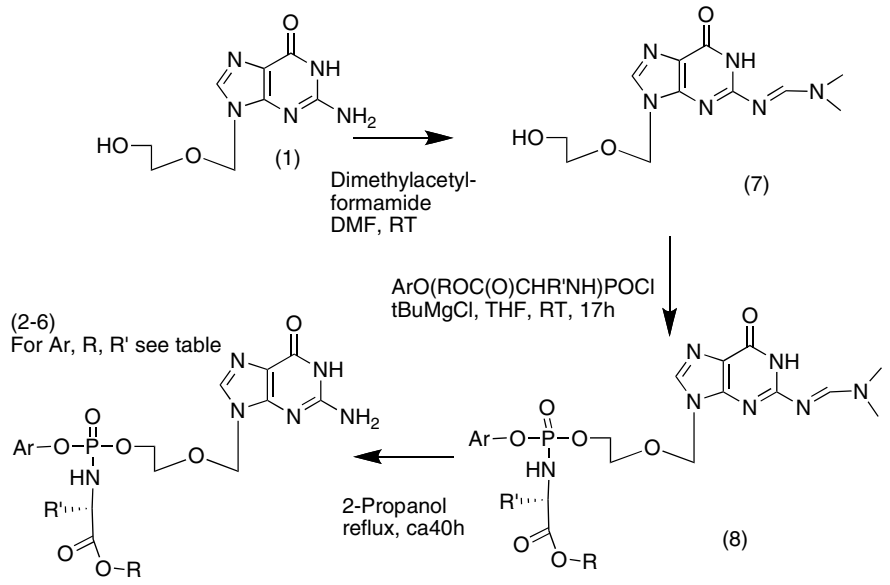

Scheme 1.

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.

Table 2
Anti-HSV-1 and -2 activity of ProTides

Compound	Ar	R	Amino acid	EC_{50}[a]/μM			MCC [b] (μM)
				HSV-1 (Kos)	HSV-2 (G)	HSV-1 TK-Kos ACV^R	
1	—	—	—	0.4	0.2	50	>250
2	Ph	Me	Ala	20	15	80	>100
3	1-Nap	Bn	Ala	2	1.4	11	>100
4	Ph	Bn	Ala	0.9	1.4	8	>100
5	1-Nap	Me	Ala	16	10.4	80	>100
6	Ph	Bn	Phe	17	8	>100	>100

[a] EC_{50}, 50% effective concentration that inhibits virus-induced cytopathicity by 50%, in HEL cells.
[b] MCC, minimal cytotoxic concentration that causes a microscopically visible alteration of cell morphology.

Table 3
Anti-VZV activity of ProTides

Compound	EC_{50}[a] (μM)				MCC[b] (μM)	CC_{50}[c] (μM)
	OKA TK$^+$	YS TK$^+$	07/1TK$^-$	YS/RTK$^-$		
1	2.5	2.9	61	43	>500	1350
2	19	20	24	16	>100	162
3	7.2	3.3	6.9	ND	>50	20
4	0.72	1.0	1.8	0.59	>50	>100
5	7.6	10.9	22	6.1	>50	>100
6	5.2	6.6	8.4	10.0	>50	86.9

[a] EC_{50}, 50% effective concentration that inhibits virus-induced cytopathicity by 50%, in HEL cells.
[b] MCC, minimal cytotoxic concentration that causes a microscopically visible alteration of cell morphology.
[c] CC_{50}, 50% cytostatic concentration that inhibits cell proliferation by 50%.

viruses and appear to represent successful thymidine kinase bypass. Notably the ProTides do not in general suffer from significant cytotoxicity.

The phenyl benzyl alanine compound (**4**) appears a particularly promising lead for further development.

These data strongly support the notion that ProTide derivatives are successful in the intracellular delivery of the monophosphate of ACV and bypass the dependence of the nucleoside analogue on (viral) thymidine kinase. Interestingly, the naphthyl phosphate is not a pre-requisite for activity, and some phenyl phosphates display good potency, particularly in kinase-deficient cells. Modification of the ester moiety or the aryl, or both, seems to be beneficial to tune the ProTide for optimal activity. Although the overall lipophilicity may be an important feature for activity, it is not the only determinant.

We are exploring further the limits of the technology on acyclic nucleoside analogues and the opportunities that by-passing the viral kinase may bring.

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References and notes

1. Morfin, F.; Thouvenot, D. *J. Clin. Virol.* **2003**, *26*, 29.
2. Stranska, R.; Schuurman, R.; Nienhuis, E.; Goedegebuure, I. W.; Polman, M.; Weel, J. F.; Wertheim-Van Dillen, P. M.; Berkhout, R. J. M.; VanLoon, A. M. *J. Clin. Virol.* **2005**, *32*, 7.
3. Larder, B. A.; Cheng, Y.-C. ; Darby, G. *J. Gen. Virol.* **1983**, *64*, 523.
4. Nugier, F.; Colin, J. N.; Aymard, M.; Larylois, M. *J. Med. Virol.* **1992**, *36*, 2.
5. Meier, C.; Balzarini, J. *Antiviral Res.* **2006**, *71*, 282.
6. Peyrottes, S.; Egron, D.; Lefebure, I.; Gosselin, G.; Imbach, J. L. *MiniRev. Med. Chem.* **2004**, *4*, 395.
7. Drontle, D. P.; Wagner, C. R. *MiniRev. Med. Chem.* **2004**, *4*, 409.
8. Cahard, D.; McGuigan, C.; Balzarini, J. *MiniRev. Med. Chem.* **2004**, *4*, 371.
9. McGuigan, C.; Harris, S. A.; Daluge, S. M.; Gudmundsson, K. S.; McLean, Ed. W.; Burnette, T. C.; Marr, H.; Hazen, R.; Condreay, L. D.; Johnson, L.; Clercq, E.; De Balzarini, J. *J. Med. Chem.* **2005**, *48*, 3504.
10. Perrone, P.; Luoni, G. M.; Kelleher, M.-R.; Daverio, F.; Angell, A.; Mulready, S.; Congiatu, C.; Rajyaguru, S.; Martin, J. A.; Léêue, V.; Le Pogam, S.; Najera, I.; Klumpp, K.; Smith, D. B.; McGuigan, C. *J. Med. Chem.* **2007**, *50*, 1840.
11. McGuigan, C.; Slater, M. J.; Parry, N. R.; Perry, A.; Harris, S. *BioOrg. Med. Chem. Lett.* **2000**, *10*, 645.
12. Congiatu, C.; McGuigan, C.; Jiang, W. G.; Davies, G.; Mason, M. D. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 485.
13. Clog*P* calculations based on ChemDraw Ultra 9.0.
14. Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373.
15. *Procedures for the preparation of* (**3**): Synthesis of N^2-DMFacyclovir-[1-naphthyl(benzoxy-L-alaninyl)]phosphate (**8**). To a stirring suspension/solution of N^2-DMF acyclovir (**7**) (0.30 g, 1.08 mmol) in anhydrous THF (10 mL) was added, ^{t}BuMgCl (1.0 M THF solution, 2.16 mL, 2.16 mmol), dropwise under an argon atmosphere. After 30 min, 1-naphthyl(benzoxy-L-alaninyl)-phosphorochloridate (1.31 g, 3.25 mmol, 2.00 mol/eq) was added dropwise in dry THF (10 ml) and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography eluting with DCM/(95:5), to give a colourless solid (17%, 0.12 g).^{31}P NMR (MeOD, 202 MHz): δ 4.18, 3.92.^{1}H NMR (MeOD, 500 MHz): δ 8.47, 8.46 (1H, 2s, NC*H*N(CH$_3$)$_2$), 8.01–7.98 (1H, m, H-8 Naph), 7.78–7.74 (2H, m, H-8, H-6 Naph), 7.56, 7.55 (1H, m, H-2 Naph), 7.41–7.12 (9H, m, Naph, OCH$_2$*Ph*), 5.37–5.36 (2H, 2s, H-1′), 5.00–4.93 (2H, m, OC*H*$_2$Ph), 4.14–4.06 (2H, m, H-4′), 3.96–3.88 (1H, m, C*H*CH$_3$), 3.88–3.59 (2H, m, H-3′), 2.95–2.93 (6H, m, N(C*H*$_3$)$_2$), 1.20–1.17 (3H, m, CHC*H*$_3$). Synthesis of acyclovir-[1-naphthyl(benzoxy-L-alaninyl)]phosphate (**3**). A solution of the protected protide (0.10 g, 0.16 mmol) in 2-propanol (5 mL) was stirred under reflux for 2 days. The solvent was then removed under reduced pressure and the residue was purified by column chromatography eluting with DCM/MeOH = 96:4. The product was purified by preparative TLC (gradient elution of DCM/MeOH = 99:1, then 98:2, then 96:4) to give a colourless solid (35%, 0.032 g). ^{31}P NMR (MeOD, 202 MHz): δ 4.13, 3.96. ^{1}H NMR (MeOD, 500 MHz): δ *8.01–7.99* (1H, m, H-8 Naph), 7.77–7.75 (1H, m, H-6 Naph), 7.67, 7.64 (1H, 2s, H-8), 7.58–7.13 (10H, m, Naph, OCH$_2$*Ph*), 5.28, 5.25 (2H, 2s, H-1′), 4.99–4.94 (2H, m, OC*H*$_2$Ph), 4.12–4.06 (2H, m, H-4′), 3.97–3.93 (1H, m, C*H*CH$_3$), 3.64–3.59 (2H, m, H-3′), 1.24–1.20 (3H, m, CHC*H*$_3$). ^{13}C NMR (MeOD, 125 MHz): δ 20.32 (d, CH$_3$, J_{C-P} = 7.63) 20.43 (d, CH$_3$, J_{C-P} = 6.61), 51.76, 51.81 (2s, *C*HCH$_3$), 67.20 (d, C-4′, J_{C-P} = 5.58), 67.28 (d, C-4′, J_{C-P} = 4.91) 67.95, 67.98 (2s, OCH$_2$Ph), 69.34 (d, C-3′, J_{C-P} = 7.72), 69.40 (d, C-3′, J_{C-P} = 8.14),73.65 (C-1′), 116.26, 116.29, 116.35, 122.69, 122.80, 125.92, 126.51, 127.20, 127.42, 127.46, 127.74, 128.81, 128.83, 129.27, 129.33, 129.52, 129.57 (C-5, C-2 Naph, C-3 Naph, C-4 Naph, C-5 Naph, C-6 Naph, C-7 Naph, C-8 Naph, C-8a Naph, OCH$_2$*Ph*), 136.26, 137.23 (C-4a Naph, 'ipso' OCH$_2$*Ph*), 139.69 (C-8), 147.98, 148.04 ('ipso' Naph, C-4), 152.44 (C-2), 159.39 (C-6), 174.61, 174.88 (*C*OOCH$_2$Ph). EI MS = 615.17 (M+Na).
16. Biological methods: Table 1. Vero cells (ECACC #84113001) were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine

serum, 300 μg/ml L-glutamine, 105 IU/ml penicillin and 100 μg/ml streptomycin and grown in 24-well plates to density. Appropriate wells were preincubated for 30 minutes with drug (prediluted in DMEM without additives), and kept growing in the appropriate amount of drug over the course of the experiment. HSV 2 strain (100 pfu) HG32 (ECACC # 158) and HSV 2 ACR (ECACC # 513) were inoculated per well, adsorbed for 45 min and then overlayed with 1.2% Avicel RC-591 (Camida Ltd.) suspended in DMEM. After 3 days the overlay was removed and the cells stained with crystal violet, the plates were scanned and the plaques counted. All assays were run in quadruplicate on each plate and plaques were counted as averages of four assays. EC50 was expressed as averages with standard deviation of 3 experiments.Tables 2 and 3. The antiviral assays were based on inhibition of virus-induced cytopathicity in human embryonic lung (HEL) fibroblasts [herpes simplex virus type 1 (HSV-1) (KOS and KOS-R) and herpes simplex virus type 2 (HSV-2) (G)]. Confluent HEL cell cultures in 96-well microtiter plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After 1-hour virus adsorption period, residual virus was removed, and the cells were incubated in the presence of serial dilutions of the test compounds. Viral cytopathicity was recorded within 48 h. For varicella-zoster virus [VZV (wild-type Oka and YS and TK^- deficient 07/1 and YS-R)], the inhibition of plaque formation was recorded. Confluent HEL cells in 96-well microtiter plates were infected with 20 plaque forming units (PFU)/ well. After 2-hours incubation, residual virus was removed and the cells were incubated in the presence of the compounds. Virus plaque formation was recorded after 5 days.[17].

17. Matrosovich, M.; Matrosovich, T.; Garten, W.; Klenk, H. D. *Virol. J.* **2006**, *3*, 63.