Bioorganic & Medicinal Chemistry Letters 18 (2008) 3856–3859

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of 4-oxo-4,7-dihydrofuro[2,3-*b*]pyridine-5-carboxamides with broad-spectrum human herpesvirus polymerase inhibition

Mark E. Schnute [a,*], Roger J. Brideau [a], Sarah A. Collier [a], Michele M. Cudahy [a], Todd A. Hopkins [a], Mary L. Knechtel [a], Nancee L. Oien [a], Robert S. Sackett [b], Allen Scott [b], Mari L. Stephan [b], Michael W. Wathen [a], Janet L. Wieber [a]

[a] *Global Research and Development, Pfizer Inc., 700 Chesterfield Parkway West, St. Louis, MO 63017, USA*
[b] *Research API, Pfizer Inc., 7000 Portage Road, Kalamazoo, MI 49007, USA*

ARTICLE INFO

Article history:
Received 2 March 2008
Revised 14 June 2008
Accepted 17 June 2008
Available online 20 June 2008

Keywords:
Cytomegalovirus
HCMV
Varicella-zoster
VZV
HSV
EBV

ABSTRACT

A versatile synthesis of 4-oxo-4,7-dihydrofuro[2,3-*b*]pyridine-5-carboxylate esters has been developed which has lead to the identification of a new series of non-nucleoside inhibitors of human herpesvirus polymerases HCMV, HSV-1, EBV, and VZV with high specificity compared to human DNA polymerases.

Infections by viruses of the Herpesvirus family lead to highly debilitating or even life-threatening disease and pose a significant threat to the general and especially the immunocompromised population. Herpes simplex virus (HSV), human cytomegalovirus (HCMV), Epstein–Barr virus (EBV), and varicella-zoster virus (VZV) are the most widespread pathogens of this family. Human cytomegalovirus is associated with clinical symptoms such as pneumonia, retinitis, and graft rejection in the immunocompromised, as well as congenital birth defects in neonates.[1] The existing therapies to treat HCMV infection are hampered by the development of drug resistance and significant drug-associated toxicities which limit their duration of use.[2] Childhood vaccination has reduced the incidence of chickenpox resulting from primary infection by varicella-zoster virus; however, reactivation of latent infection in the form of herpes zoster (shingles) still represents a significant risk to the elderly and those who are immunocompromised.[3] Acyclovir, Famciclovir, and Valacyclovir are prescribed for VZV infection though at high doses due to their modest potency against the virus.[3d] Initial infection by Epstein–Barr virus resulting in infectious mononucleosis can have a profound personal impact, and there is a strong association for the role of the virus in the development of post-transplant lymphoproliferative disease and lymphomas.[1a,4] A well tolerated, broad-spectrum agent against herpesvirus infection is unfortunately not currently available and would constitute a significant break-through in this field.

Recently, we have described two novel series of 4-oxo-4,7-dihydrothieno[2,3-*b*]pyridine-5-carboxamides (**1a** and **1b**) which were broad-spectrum inhibitors of herpesvirus polymerases, Figure 1.[5] One focus of our SAR program was to explore alternative bicyclic ring systems, and of specific interest was furo[2,3-*b*]pyridine based on the bioisosteric replacement of sulfur for oxygen. Initial efforts based on compound **1a** (e.g., **7a**, vide infra) were disappointing due to poor observed antiviral activity as well as the limited scope and scalability of existing syntheses to the

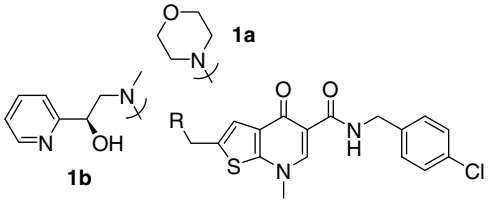


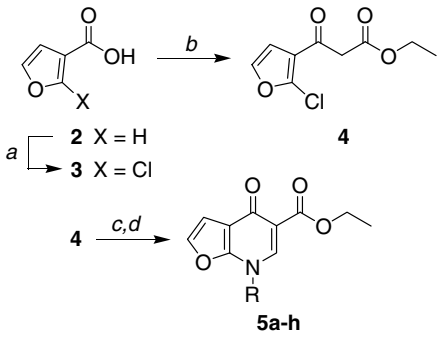
Figure 1. Representative 4-oxo-4,7-dihydrothieno[2,3-*b*]pyridine-based herpesvirus DNA polymerase inhibitors.

* Corresponding author. Tel.: +1 636 247 3662; fax: +1 636 247 5400.
E-mail address: mark.e.schnute@pfizer.com (M.E. Schnute).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.06.060

furo[2,3-*b*]pyridine ring system. The improved potency observed with analogs such as **1b** prompted us to reconsider this series though overcoming the synthetic challenges were paramount. Herein, we report the development of a versatile synthesis of 4-oxo-4,7-dihydrofuro[2,3-*b*]pyridine-5-carboxylate esters and the realization of antiherpetic activity.

Compared to the analogous quinoline and thieno[2,3-*b*]pyridine ring systems, there are relatively few syntheses of the 4-oxo-4,7-dihydrofuro[2,3-*b*]pyridine-5-carboxylate scaffold in the literature. Reported syntheses have utilized the Gould–Jacob cyclization consisting of condensation of a 2-aminofuran with ethoxyethylene malonate followed by thermal ring closure.[6] In fact, 2-aminofuran is not a viable precursor for this chemistry due to its intrinsic instability,[7] and syntheses have only been successful starting from 2-aminofurans bearing strongly electron withdrawing substituents at the 5-position (e.g., CO_2CH_3). For our purposes we needed a route to access the Gould–Jacob product derived from 2-aminofuran and preferably avoiding the high temperature (240 °C) cyclization required for this chemistry. A general and versatile synthesis of quinolone antibacterials has been described, which relies on an intramolecular S_NAryl substitution of an electron deficient fluorobenzene by an enamine to construct the pyridinone ring.[8] A similar approach has been reported to thieno[2,3-*b*]pyridin-4-ones utilizing 2-chlorothiophene derivatives.[9] To our knowledge this approach has not been applied to the synthesis of furo[2,3-*b*]pyridin-4-ones, and we envisioned that such an approach would address the limitations of the existing Gould–Jacob route. Chlorination of the lithium dianion derived from 3-furoic acid (**2**) with hexachloroethane afforded 2-chloro-3-furoic acid (**3**), Scheme 1.[10] The magnesium enolate of ethyl malonate was then acylated with the imidazolide of **3** to provide β-ketoester **4**.[11] The condensation of **4** with triethylorthoformate and acetic anhydride provided the 3-ethoxypropenoate which underwent facile addition of amines (RNH_2, Table 1) to afford the corresponding enamine with elimination of ethanol.[12] Pyridinone ring closure was then affected by the addition of potassium *tert*-butoxide to yield furo[2,3-*b*]pyridin-4-one esters **5a**–**h** in 30–67% yield from compound **3**. The above sequence allows for the introduction of a wide range of functionality to the N7-position in an effective one-pot, three-step procedure.



Scheme 1. Synthesis of 4-oxo-4,7-dihydrofuro[2,3-*b*]pyridine ester **5a**–**h**. Reagents and conditions: (a) LDA, THF, −70 °C; C_2Cl_6, 82%; (b) CDI, THF; potassium ethyl malonate, CH_3CN, $MgCl_2$, Et_3N, 25 °C; (c) $(EtO)_3CH$, Ac_2O, 135 °C; (d) RNH_2, THF, 25 °C; *t*-BuOK, 30–40 °C.

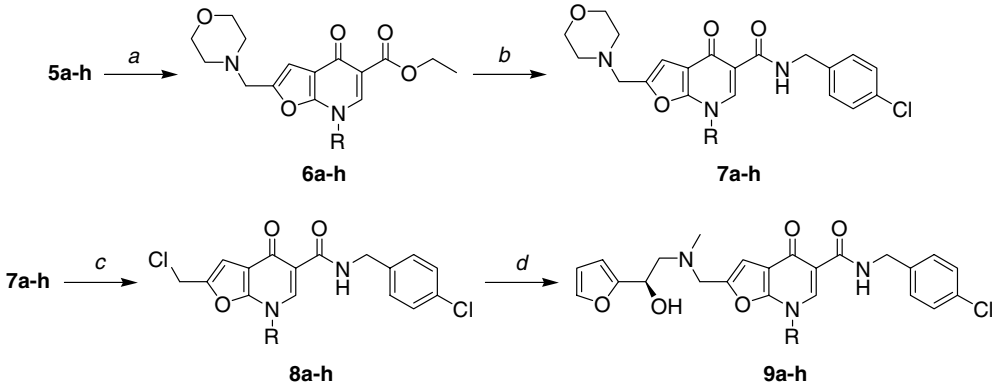
Table 1
HCMV polymerase and antiviral inhibition for compounds **9**

Compound	R	HCMV polymerase IC_{50}[a] (nM)	HCMV antiviral IC_{50}[a] (nM)
9a	CH_3	76 (±5)	26 (±10)
9b	CH_2CH_3	510	700
9c	C_3H_5	420	1200
9d	$CH_2CH_2CH_3$	1120	
9e	Phenyl	830	
9f	Pyridin-2-yl	140	50 (±10)
9g	CH_2CH_2Ph	690	
9h	$CH_2CH_2N(C_2H_5)_2$	3920	

[a] Values are means of at least two experiments where standard deviation is given in parentheses, see Table 4 for reference compounds.

Following the precedent of Mannich elaboration of the C2-position established in the thienopyridine series[5] and further supported by the increased relative rate of electrophilic aromatic substitution for furan compared to thiophene,[13] a similar strategy in the furopyridine series was explored. Indeed furopyridines **5a**–**h** reacted with 4-methylenemorpholin-4-ium chloride[14] in acetonitrile at 60 °C to provide **6a**–**h** in good yields, Scheme 2. The regiochemistry of addition was confirmed by single crystal X-ray.[15] Subsequent direct transformation to the corresponding 4-chlorobenzylamides **7a**–**h** followed by treatment with ethylchloroformate provided the alkylchlorides **8a**–**h** which were reacted with (*R*)-1-(2-furyl)-2-(methylamino)ethanol[16] to afford compounds **9a**–**h**.

Compounds **9a**–**h** were assayed for their ability to inhibit the incorporation of ^3H-labeled nucleotide into template-primer by



Scheme 2. Synthesis of 4-oxo-4,7-dihydrofuro[2,3-*b*]pyridine amides **9a**–**h**. Reagents and conditions: (a) 4-methylenemorpholin-4-ium chloride, CH_3CN, 60 °C; (b) 4-chlorobenzylamine, ethylene glycol, 130 °C; (c) ethylchloroformate, $CHCl_3$; (d) (*R*)-1-(2-furyl)-2-(methylamino)ethanol, DMF, *i*-Pr_2EtN, 90 °C.

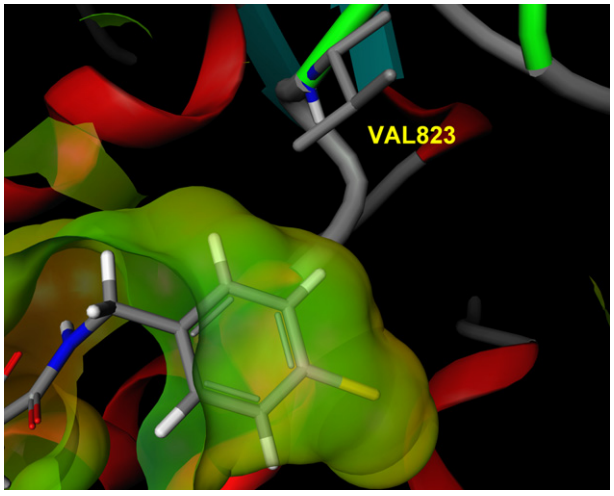
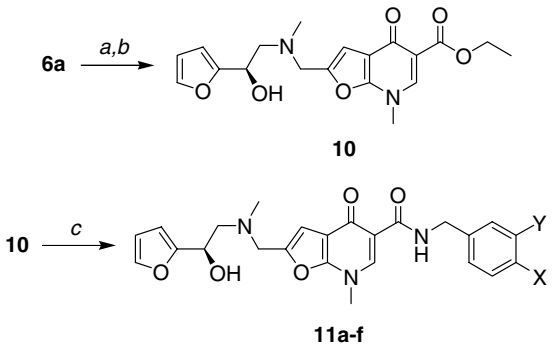


Figure 2. Representation of benzylamide binding pocket based on docking of compound **9a** into crystal structure of HSV-1 DNA polymerase (Ref. 18).

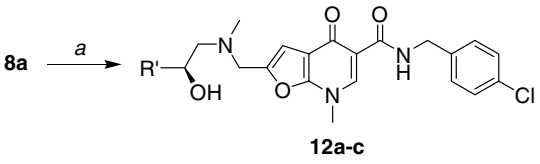
purified HCMV DNA polymerase using a scintillation proximity assay and for antiviral activity in an HCMV plaque reduction assay, Table 1.[17] Polymerase inhibition was noticeably sensitive to the nature of the N7-substituent with methyl (**9a**) and pyrimidin-2-yl (**9f**) being the best suggesting the potential for a steric clash above or below the ring plane in this region. This would be consistent with a binding model in which the furopyridine ring system stacks between the polymerase and the DNA duplex.[18] Overall, methyl substitution (**9a**) imparted the best combination of potency and aqueous solubility (60 μM) which was a marked improvement over the corresponding thienopyridine analog (0.4 μM).[5b]

Further refinements to the benzylamide and aminoalcohol substituent were next explored. Docking of compound **9a** into the previously reported crystal structure of HSV-1 DNA polymerase[18] revealed that the 4-chlorobenzylamide substituent fits into a well-defined pocket partially border by valine 823, Figure 2. We had previously established that mutation of this residue conveys resistance to related quinolone and thienopyridine antivirals suggesting that this pocket plays a critical role in compound binding.[5a,19] A small bulge at the end of the pocket nicely accommodates the chlorine substituent found in **9a**. To determine if chlorine was the optimal substituent, ester **10** was condensed with several 4- and 3,4-substituted benzylamines to provide compounds **11a–f**, Scheme 3. Indeed, the chlorine substituent contributes substantially to the polymerase inhibition of **9a** as compared to benzyl alone **11f** while alternative substituents at the 4-position (Br, F, CH_3, and CF_3) remained inferior to chloride itself, Table 2. Compound **8a** was alkylated with a variety of *N*-methylaminoalcohols, Scheme 4. Most promising were compounds **12a–c** each substituted by a six-membered nitrogen heterocycle, which showed good HCMV polymerase inhibition and antiviral activity, Table 3. Further profiling of compounds **9a** and **12a** indicated that both compounds were broad-spectrum inhibitors of herpesvirus polymerases including HCMV, HSV, and VZV, while not being active against human DNA polymerases (α, γ, and δ), Table 4. Broad-spectrum herpes antiviral activity was also demonstrated with potencies comparable to thienopyridine **1b**. Compound **9a** demonstrated good oral bioavailability in rat (67%) and in dog (76%).[20]

By applying SAR learnings from previous thieno[2,3-*b*]pyridine herpesvirus polymerase inhibitors, furo[2,3-*b*]pyridine analogs have been identified demonstrating comparable potency to their progenitors with improved aqueous solubility. Compound **9a** exhibited broad-spectrum herpes antiviral activity superior to that of available therapies with respect to HCMV, VZV, and EBV. The full optimization of this chemical series was made possible by the development of a practical synthesis of 4-oxo-4,7-dihydrofuro[2,3-*b*]pyridine-5-carboxylate esters.



Scheme 3. Synthesis of amides **11a–f**. Reagents and conditions: (a) ethylchloroformate, $CHCl_3$, 38%; (b) (*R*)-1-(2-furyl)-2-(methylamino)ethanol, DMF, *i*-Pr_2EtN, 90 °C, 72%; (c) benzylamine, ethylene glycol, 130 °C.



Scheme 4. Synthesis of amides **12a–c**. Reagents and conditions: (a) *i*-Pr_2EtN, DMF, R′CH(OH)CH_2NH(CH_3), 90 °C.

Table 2
HCMV polymerase inhibition for compounds **11**

Compound	X	Y	HCMV polymerase IC_{50}[a] (nM)
9a	Cl	H	76 (±5)
11a	Br	H	340 (±80)
11b	F	H	610
11c	F	F	1020
11d	CH_3	H	3140
11e	CF_3	H	6450
11f	H	H	11,400

[a] Values are means of at least two experiments where standard deviation is given in parentheses, see Table 4 for reference compounds.

Table 3
HCMV polymerase and antiviral inhibition for compounds **12**

Compound	R′	HCMV polymerase IC_{50} (nM)[a]	HCMV antiviral IC_{50}[a] (nM)
9a	2-Furyl	76 (±5)	26 (±10)
12a	Pyridin-2-yl	220 (±80)	170 (±20)
12b	Pyrimidin-2-yl	170	360 (±5)
12c	Pyrazin-2-yl	220	28 (±15)

[a] Values are means of at least two experiments where standard deviation is given in parentheses, see Table 4 for reference compounds.

Table 4
Broad-spectrum activity of **9a** and **12a** compared to that of 4-oxo-4,7-dihydrothieno[2,3-*b*]pyridine **1b**, and established therapies

Compound	Polymerase IC_{50}[a] (nM)				Antiviral IC_{50}[a,b] (nM)				CC_{50}[c]
	HCMV	HSV-1	VZV	α/γ/δ	HCMV	HSV-1	VZV	EBV	(μM)
1b	61 (±1)	76	21	>20,000	100	3000	2	200	>100
9a	76 (±5)	220	78	>20,000	26 (±10)	4300	20	400	>100
12a	220 (±80)	350	100	>20,000	170 (±20)	5400	90	nd	>100
Ganciclovir					1300	nd	nd	nd	>100
Acyclovir					>20,000	2100	8100	6900	>100
Foscarnet	2,500	nd	nd	<280 (γ)					
Aphidicolin	487 (±74)	438 (±136)	473 (±90)	2600 (α)					
AZT-TP[d]	22,100	3300	5800	2300 (δ)					

[a] Values are means of at least two experiments where standard deviation is given in parentheses (nd = not determined).
[b] Determined by plaque reduction assay (HCMV, Davis strain; HSV-1, KOS strain; VZV, Webster strain).
[c] CC_{50}, 50% cellular cytotoxicity in HFF cells derived from single determination, Ref. 21.
[d] AZT-TP = zidovudine triphosphate.

Acknowledgments

The authors thank Garold Bryant for X-ray structural determinations, Francis Schwende, Karen Wilkinson, and Bob Rush for pharmacokinetic analysis, Kevin Stafanski for solubility measurements, and Paul Herrington, William Perrault, William McGhee, and Thomas Beauchamp for providing chiral aminoalcohols.

References and notes

1. (a) Fishman, J. A. *N. Engl. J. Med.* **2007**, *357*, 2601; (b) Steininger, C.; Puchhammer-Stöckl, E.; Popow-Kraupp, T. *J. Clin. Virol.* **2006**, *37*, 1; (c) Gaytant, M. A.; Steegers, E. A. P.; Semmekrot, B. A.; Merkus, H. M. M. W.; Galama, J. M. D. *Obstet. Gynecol. Surv.* **2002**, *57*, 245.
2. (a) Gilbert, C.; Boivin, G. *Antimicrob. Agents Chemother.* **2005**, *49*, 873; (b) Chakrabarty, A.; Pang, K. R.; Wu, J. J.; Narvaez, J.; Rauser, M.; Huang, D. B.; Beutner, K. R.; Tyring, S. K. *Expert Opin. Emerg. Drugs* **2004**, *9*, 237; For prospective therapies see: (c) Biron, K. K. In *New Concepts of Antiviral Therapy*; Bogner, E., Holzenburg, A., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp 309–336.
3. (a) Heininger, U.; Seward, J. F. *Lancet* **2006**, *368*, 1365; (b) Guenther, L. C. *Exper. Rev. Dermatol.* **2006**, *1*, 607–618; (c) Kimberlin, D. W.; Whitley, R. J. *N. Engl. J. Med.* **2007**, *356*, 1338; (d) Gnann, J. W., Jr.; Whitley, R. J. *N. Engl. J. Med.* **2002**, *347*, 340.
4. Thorley-Lawson, D. A.; Gross, A. *N. Engl. J. Med.* **2004**, *350*, 1328.
5. (a) Schnute, M. E.; Cudahy, M. M.; Brideau, R. J.; Homa, F. L.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Pitts, T. W.; Poorman, R. A.; Wathen, M. W.; Wieber, J. L. *J. Med. Chem.* **2005**, *48*, 5794; (b) Schnute, M. E.; Anderson, D. J.; Brideau, R. J.; Ciske, F. L.; Collier, S. A.; Cudahy, M. M.; Eggen, M.; Genin, M. J.; Hopkins, T. A.; Judge, T. M.; Kim, E. J.; Knechtel, M. L.; Nair, S. K.; Nieman, J. A.; Oien, N. L.; Scott, A.; Tanis, S. P.; Vaillancourt, V. A.; Wathen, M. W.; Wieber, J. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3349.
6. (a) Snyder, H. R., Jr.; Ebetino, F. F. *J. Heterocycl. Chem.* **1966**, *3*, 202; (b) Bhupathy, M.; Conlon, D. A.; Wells, K. M.; Nelson, J. R.; Reider, P. J.; Rossen, K.; Sager, J. W.; Volante, R. P.; Dorsey, B. D.; Hoffman, J. M., Jr.; Joseph, S. A.; McDaniel, S. L. *J. Heterocycl. Chem.* **1995**, *32*, 1283.
7. Ramsden, C. A.; Milata, V. *Adv. Heterocycl. Chem.* **2006**, *92*, 1.
8. Clay, R. J.; Collom, T. A.; Karrick, G. L.; Wemple, J. *Synthesis* **1993**, 290.
9. El-Abadelah, M. M.; Sabri, S. S.; Al-Ashqar, H. A. *Heterocycles* **1997**, *45*, 255.
10. *2-Chloro-3-furoic acid* (**3**). A solution of diisopropylamine (713 mL, 5.08 mol) in dry THF (5.1 L) was cooled to less than −50 °C. A solution of 2.5 M *n*-butyl lithium in hexanes (2033 mL, 5.08 mol) was added over 40 min maintaining the temperature below −50 °C. A solution of 3-furoic acid (285 g, 2.54 mol) in dry THF (1742 mL) was added maintaining the temperature below −70 °C. The solution was stirred at −70 °C for 40 min and then a solution of hexachloroethane (662 g, 2.8 mol) in dry THF (1017 mL) was added maintaining the temperature below −70 °C. The solution was stirred for 3 h at −70 °C and then was allowed to warm to room temperature overnight. The reaction mixture was quenched with water (10.9 L). The aqueous layer was extracted with MTBE (5.8 L) and then acidified with 2 N HCl to a pH 1–2. The product was extracted into ethyl acetate (2 × 3.8 L), and then the solvent was removed in vacuo to provide a solid. Recrystallization from water (7.5 L) provided 233 g (82%) of **3** as pale tan crystals on cooling to 0–5 °C overnight. Mp 135.3–137.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.06 (br s, 1H), 7.75 (d, J = 2.3 Hz, 1H), 6.80 (d, J = 2.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 162.42, 143.38, 140.12, 113.78, 112.65; MS (ESI+) m/z 147 (M+H). Anal. Calcd for $C_5H_3ClO_3$: C, 40.98; H, 2.06. Found: C, 40.83; H, 1.90.
11. *Ethyl 3-(2-chloro-3-furyl)-3-oxopropanoate* (**4**). A solution of **3** (228.0 g, 1.56 mol) in dry THF (373 mL) was added over 30 min to a slurry of *N,N′*-carbonyldiimidazole (278.3 g, 1.72 mol) in dry THF (2280 mL). The slurry became a solution as the mixture was stirred over 2 h. In a separate reaction vessel, a slurry of potassium ethyl malonate (530.5 g, 3.12 mol) and dry acetonitrile (4560 mL) was cooled to 10–15 °C. Triethylamine (435 mL, 3.12 mol) was then added. Solid anhydrous $MgCl_2$ (370 g, 3.89 mol) was added portion-wise maintaining the reaction temperature below 25 °C. The resulting slurry was stirred at room temperature for 2.5 h, and then the previously prepared imidazolide solution was added via cannula maintaining the temperature below 25 °C. The slurry was stirred overnight at room temperature. The solvent was removed in vacuo and the residue was treated with 0.5 N HCl (9.3 L) and toluene (4.6 L). The aqueous layer was extracted with toluene (1.9 L) and the combined organic layers were washed with saturated aqueous sodium bicarbonate (1.9 L). The organic layer was concentrated to provide 317.9 g of **4** as a brown oil which could be used as is in subsequent steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm (major isomer) 7.80 (d, J = 2.3 Hz, 1H), 7.01 (d, J = 2.3 Hz, 1H), 4.09 (q, J = 7.1 Hz, 2H), 3.95 (s, 2H), 1.15 (t, J = 7.1 Hz, 3H); (minor isomer) 12.35 (s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.00 (d, J = 2.3 Hz, 1H), 5.69 (s, 1H), 4.18 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.0 Hz, 3H).
12. *General procedure for preparation of esters* **5a–h**. A mixture of **4** (10.0 g, 46.2 mmol), triethylorthoformate (15.4 mL, 92.4 mmol), and acetic anhydride (15.3 mL, 161.7 mmol) was heated to 135 °C with removal of ethyl acetate distillate with a Dean–Stark trap. After 3 h, volatiles were removed at 40 Torr (100 °C) and then at 0.2 Torr (65 °C, 1 h) to afford a black oil. The resulting oil was dissolved in THF (50 mL) and a corresponding amine (50.8 mmol) was added while cooling in an ice bath. The mixture was allowed to stir at room temperature for 20 h. A solution of potassium *tert*-butoxide (1.0 M in THF, 50.8 mL, 50.8 mmol) was then added maintaining the internal temperature below 0 °C. The mixture was allowed to warm to room temperature and was then held at 30–40 °C for 1 h. The mixture was diluted with ethyl acetate (400 mL) and satd aq ammonium chloride (200 mL). The organic layer was washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The product was purified by column chromatography. Yield from **3**: **5a**, 67%; **5b**, 50%; **5c**, 38%; **5d**, 30%; **5e**, 34%; **5f**, 37%; **5g**, 52%; **5h**, 44%.
13. (a) Heaney, H.; Papageorgiou, G.; Wilkins, R. F. *Tetrahedron Lett.* **1988**, *29*, 2377; (b) Heaney, H.; Papageorgiou, G.; Wilkins, R. F. *Tetrahedron* **1997**, *53*, 2941.
14. Dimmock, J. R.; Erciyas, E.; Bigam, G. E.; Kirkpatrick, D. L.; Duke, M. M. *Eur. J. Med. Chem.* **1989**, *24*, 379.
15. X-ray determination employed the 4-bromobenzoate ester of **9a**. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 679175.
16. Tanis, S. P.; Evans, B. R.; Nieman, J. A.; Parker, T. T.; Taylor, W. D.; Heasley, S. E.; Herrinton, P. M.; Perrault, W. R.; Hohler, R. A.; Dolak, L. A.; Hester, M. R.; Seest, E. P. *Tetrahedron: Asymmetry* **2006**, *17*, 2154.
17. Assay methods have been previously decribed in Ref. 5 to assess inhibition of compounds against viral DNA polymerases (HCMV, HSV-1, and VZV) and human DNA polymerases (α, γ, and δ). Antiviral activities against herpesviruses were determined using plaque reduction assays (HCMV, VZV, and HSV-1) or lytic replication (EBV) as described in Ref. 5.
18. Liu, S.; Knafels, J. D.; Chang, J. S.; Waszak, G. A.; Baldwin, E. T.; Deibel, M. R., Jr.; Thomsen, D. R.; Homa, F. L.; Wells, P. A.; Tory, M. C.; Poorman, R. A.; Gao, H.; Qiu, X.; Seddon, A. P. *J. Biol. Chem.* **2006**, *281*, 18193.
19. Thomsen, D. R.; Oien, N. L.; Hopkins, T. A.; Knechtel, M. L.; Brideau, R. J.; Wathen, M. W.; Homa, F. L. *J. Virol.* **2003**, *77*, 1868.
20. Compounds were dosed IV at 5 mg/kg and PO at 15 mg/kg using a vehicle of 80/20 PEG400/0.01 M methanesulfonic acid.
21. Toxicity of compounds to non-infected mammalian cells was determined using replicating HFF cells seeded as subconfluent monolayers and treated with compound for 3 days. Cell viability determinations were performed using both microscopic evaluation and a quantitative neutral red dye uptake assay as previously described. Lowik, C. W. G. M.; Alblas, M. J.; van de Ruit, M.; Papapoulos, S. E.; van der Pliijm, G. *Anal. Biochem.* **1993**, *213*, 426.