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2-Aryl-2-hydroxyethylamine substituted 4-oxo-4,7-dihydrothieno-[2,3-*b*]pyridines as broad-spectrum inhibitors of human herpesvirus polymerases

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Abstract—A novel series of 2-aryl-2-hydroxyethylamine substituted 4-oxo-4,7-dihydrothieno[2,3-*b*]pyridine-5-carboxamides have been identified as potent antivirals against human herpesviruses. These compounds demonstrate broad-spectrum inhibition of the herpesvirus polymerases HCMV, HSV-1, EBV, and VZV with high specificity compared to human DNA polymerases.

Infections by viruses of the Herpesvirus family pose a significant health burden to the general population, leading to diminished quality of life for millions, and are a leading contributor to morbidity in immunocompromised patients. Human cytomegalovirus (HCMV) 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 significant drug-associated toxicities which limit their duration of use. As a consequence and because of the nature of the patient population, emergence of drug resistance is a common concern.[2] The prevalence of other herpesviruses such as herpes simplex virus (HSV), Epstein–Barr virus (EBV), and varicella-zoster virus (VZV) is widespread among the human population.[3] The adoption of routine childhood vaccination against VZV has significantly reduced the incidence of primary disease, chickenpox. However, the impact on corresponding epidemiological outcome and extent of acceptance of vaccination in the elderly toward incidence of reactivation leading to herpes zoster (shingles), a highly debilitating illness characterized by persistent neuropathic pain, remains unclear. Antiviral therapy in the immunocompromised to address complications to VZV infections will likely still be a necessity in the future. Unfortunately, the availability of a well-tolerated, orally bioavailable, broad-spectrum agent to address

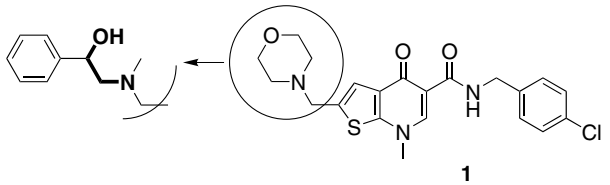

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Figure 1. Optimization of lead 4-oxo-4,7-dihydrothieno[2,3-*b*]pyridine carboxamide **1** by replacement of the morpholine substituent.

Keywords: Cytomegalovirus; HCMV; Varicella-zoster; VZV; HSV.
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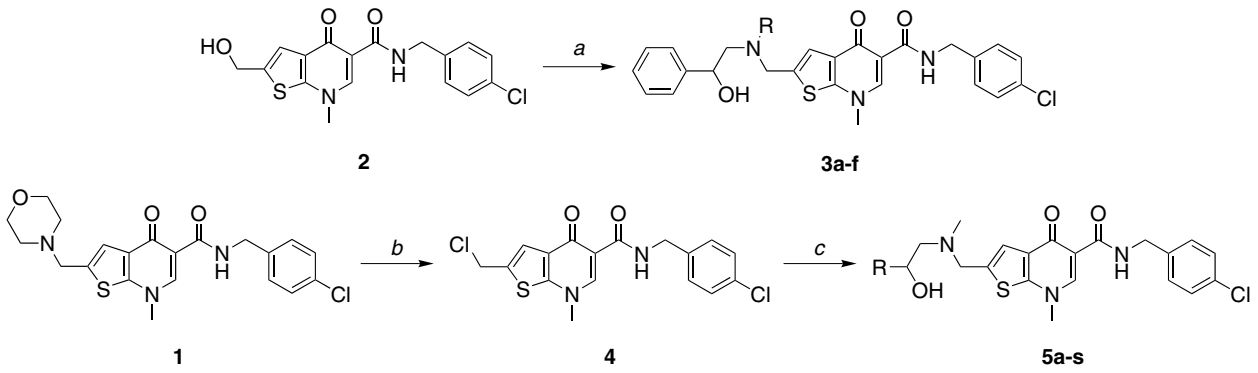


Scheme 1. Synthesis of 2-hydroxyethylamine-substituted 4-oxo-4,7-dihydrothieno[2,3-*b*]pyridines **3a**–**f** and **5a**–**r**. Reagents and condition: (a) MsCl, 2,4,6-collidine, DMAP, DMF; $PhCH(OH)CH_2NHR$ (**6a**–**f**); (b) ethyl chloroformate, $CHCl_3$, reflux; (c) *i*-Pr_2EtN, DMF, $RCH(OH)CH_2N(CH_3)H$ (**7a**–**s**).

herpesvirus infections in the growing immunocompromised population remains an unmet medical need.

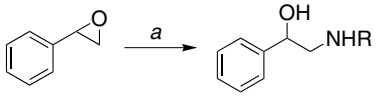
Previously, we have described a novel series of 4-oxo-4,7-dihydrothieno[2,3-*b*]pyridine (DHTP) carboxamides exemplified by **1** which were broad-spectrum inhibitors of herpesvirus polymerases including HCMV, HSV-1, and VZV polymerases, Figure 1.[4] During the course of screening commercially available amine monomers in order to explore the structure–activity relationships surrounding the critical morpholine substituent, an alternative 2-hydroxyethylamine motif was identified. This motif imparted to compounds of the DHTP series differentially improved polymerase inhibition compared to the same analogs in the earlier 4-oxo-1,4-dihydroquinoline carboxamide template.[5] Herein we report optimization of the amine substituent in the thieno[2,3-*b*]pyridine carboxamide series leading to overall increased antiviral potency.

Synthesis of 2-hydroxyethylamine-substituted thieno[2,3-*b*]pyridine carboxamides was accomplished by alkylation of the corresponding aminoethanol with 2-chloromethyl thieno[2,3-*b*]pyridine **4** either discreetly or generated in situ, Scheme 1. Alkylchloride **4** could

Table 1. HCMV polymerase and antiviral inhibition for compounds **3a**–**f**

Compound	R	HCMV Polymerase IC_{50}[a] (nM)	HCMV Antiviral IC_{50}[a,b] (nM)
3a	H	8400	>5000
3b	CH_3	100	180 (±64)
3c	CH_2CH_3	240 (±50)	450 (±150)
3d	CH_2CH_2OH	1580	400 (±200)
3e	$CH(CH_3)_2$	2040	1600
3f	C_3H_5	8900	>5000

[a] Values are means of at least two experiments where standard deviation is given in parentheses, see Table 5 for reference compounds.
[b] Determined by plaque reduction assay (Davis strain).



Scheme 2. Synthesis of 2-hydroxyethylamines **6c**–**f**. Reagents and condition: (a) RNH_2, methanol, reflux (R = **c**, ethyl; **d**, 2-hydroxyethyl; **e**, isopropyl; **f**, cyclopropyl).

Table 2. Configurational preference of stereogenic center in compound **3b** toward herpes polymerase inhibition

Compound	Polymerase IC_{50}[a] (nM)		
	HCMV	HSV-1	VZV
(***S***)-**3b**	100	400	200
(***R***)-**3b**	2550	10,500	5000

[a] Values represent single determinations, see Table 5 for reference compounds.

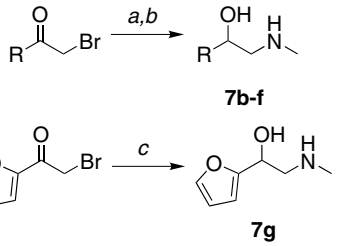
Table 3. Influence of ethylamine C2-substituent on HCMV polymerase and antiviral inhibition for compounds **5a**–**s**

Compound	R	HCMV polymerase IC_{50}[a] (nM)	HCMV antiviral IC_{50}[a,b] (nM)
3b	Phenyl	100	180 (±64)
5a	4-Hydroxyphenyl	100	57 (±11)
5b	4-Fluorophenyl	220 (±40)	200 (±100)
5c	3-Chlorophenyl	203 (±31)	250 (±50)
5d	Pyridin-2-yl	86 (±3)	90 (±10)
5e	Pyridin-3-yl	160 (±20)	60 (±40)
5f	Pyridin-4-yl	200	85 (±15)
5g	2-Furan	77 (±15)	30 (±0)
5h	3-Furan	250 (±10)	400 (±0)
5i	Benzofuran-2-yl	12	150 (±50)
5j	Quinolin-2-yl	65 (±10)	40
5k	2-Thiophene	175 (±25)	250 (±50)
5l	3-Cyanophenyl	66 (±35)	100 (±0)
5m	4-Cyanophenyl	315 (±15)	300 (±100)
5n	3-Methoxyphenyl	87 (±14)	20 (±10)
5o	1,3-Thiazol-2-yl	235 (±35)	200
5p	Pyrimidin-2-yl	100 (±30)	100 (±0)
5q	Pyrazin-2-yl	85 (±25)	65 (±35)
5r	CH_3	3530	1500
5s	$CH(CH_3)_2$	17,500	>5000

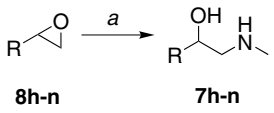
[a] Values are means of at least two experiments where standard deviation is given in parentheses, see Table 5 for reference compounds.
[b] Determined by plaque reduction assay (Davis strain).

be prepared either from the corresponding 2-hydroxymethyl thieno[2,3-*b*]pyridine **2**[4] by reaction with methanesulfonyl chloride in the presence of 2,4,6-collidine and DMAP or in high yield (95%) from 2-morpholinylmethyl thieno[2,3-*b*]pyridine **1**[4] upon treatment with ethyl chloroformate.

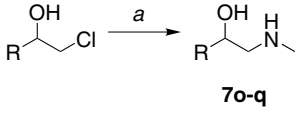
Initial SAR evaluation focused on the role of the hydroxyethylamine nitrogen substituent by reacting *N*-alkyl-substituted 2-hydroxy-2-phenylethylamines (**6a–f**) with **4** generated in situ to afford thieno[2,3-*b*]pyridines **3a–f**, Table 1. Aminoalcohols **6c–f** were prepared from styrene oxide by ring opening with the corresponding primary amine, Scheme 2. HCMV polymerase inhibition and antiviral activity in an HCMV plaque reduction assay was measured for each compound.[6] This survey established that small alkyl substituents (methyl and ethyl) were preferred with a significant potency reduction for the unsubstituted (**3a**) and branched (**3e–f**) analogs. Ring opening of (*R*)- and (*S*)-styrene oxide with methylamine afforded the corresponding scalemic aminoalcohols[7] which subsequently provided (***R***)-**3b** and (***S***)-**3b** of established configurations (>98% ee), Table 2. The configuration of the hydroxy-bearing stereogenic center was found to play a significant role toward polymerase inhibition with the (*S*)-isomer being 25-fold more potent than the (*R*)-isomer for HCMV, HSV, and VZV polymerase. The relevance of substitution at the hydroxy-bearing carbon toward polymerase inhibition was next examined. A series of 2-aryl, 2-heteroaryl, and 2-alkyl-*N*-methyl-2-hydroxyethylamines (**7a–s**) were alkylated with **4** to provide thieno[2,3-*b*]pyridines **5a–s**, Scheme 1 and Table 3. Aminoalcohols **7b–g** were prepared from the corresponding bromoacetyl by reduction to the bromohydrin followed by displacement with methylamine except for furan-2-yl **7g** where the sequence of steps had to be reversed in order to afford satisfactory yields, Scheme 3. Aminoalcohols **7h–n** were readily prepared



Scheme 3. Synthesis of 2-hydroxyethyl-1-methylamines **7b–g**. Reagents and conditions: (a) $NaBH_4$, methanol (R = **b**, 4-fluorophenyl; **c**, 4-chlorophenyl) or $NaBH_4$, methanol; 48% HBr (R = **d**, pyridin-2-yl; **e**, pyridin-3-yl; **f**, pyridin-4-yl); (b) methylamine, methanol, 60 °C; (c) methylamine, methanol, 0 °C; $NaBH_4$.



Scheme 4. Synthesis of 2-hydroxyethyl-1-methylamines **7h–n**. Reagents and conditions: (a) methylamine, methanol, rt (**i**, benzofuran-2-yl; **k**, 2-thiophene) or 60–100 °C (**h**, 3-furan; **j**, quinolin-2-yl; **l**, 3-cyanophenyl; **m**, 4-cyanophenyl;**n**, 3-methoxyphenyl).

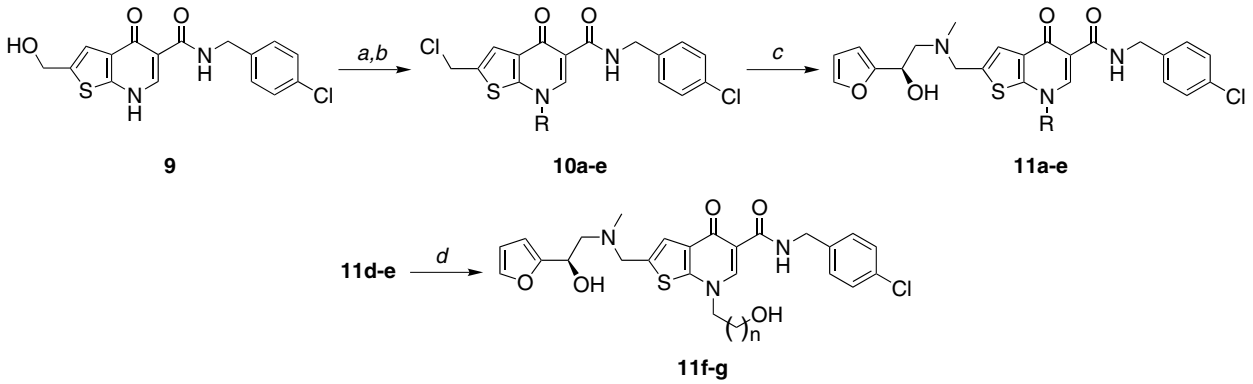


Scheme 5. Synthesis of 2-hydroxyethyl-1-methylamines **7o–q**. Reagents and condition: (a) methylamine, methanol, NaI, 60 °C (**o**, 1,3-thiazol-2-yl; **p**, pyrimidin-2-yl; **q**, pyrazin-2-yl).

Table 4. Influence of N7-substituent on HCMV polymerase and antiviral inhibition for compounds (***R***)-**5g**, **11a–c**, **11f**, and **11g**

Compound	R	HCMV polymerase IC_{50}[a] (nM)	HCMV antiviral IC_{50}[a,b] (nM)
(***R***)-**5g**	CH_3	35 (±6)	7 (±2)
11a	CH_2CH_3	200 (±20)	150 (±50)
11b	$CH_2CH_2CH_3$	180 (±20)	35 (±5)
11c	$CH_2CH_2OCH_3$	350	250 (±50)
11f	CH_2CH_2OH	240 (±40)	250 (±50)
11g	$CH_2CH_2CH_2OH$	280 (±81)	300

[a] Values are means of at least two experiments where standard deviation is given in parentheses, see Table 5 for reference compounds.
[b] Determined by plaque reduction assay (Davis strain).



Scheme 6. Synthesis of 7-substituted 4-oxo-4,7-dihydrothieno[2,3-*b*]pyridines **11a–c**, **11f**, and **11g**. Reagents and conditions: (a) RX (**a**, iodoethane; **b**, iodopropane; **c**, 2-bromoethyl methyl ether), K_2CO_3, DMF, rt or RX (**d**, $THPOCH_2CH_2I$; **e**, $THPOCH_2CH_2CH_2I$), Cs_2CO_3, DMF, 60–100 °C; (b) MsCl, 2,4,6-collidine, DMAP, DMF; (c) *i*-Pr_2EtN, DMF, (***R***)-**7g**; (d) 65% perchloric acid, THF (**f**, $n = 1$; **g**, $n = 2$).

Table 5. Broad-spectrum activity of (***R***)-**5d** and (***R***)-**5q** compared to morpholinyl-substituted DHTP **1** and established therapies

Compound	Polymerase IC_{50}[a] (nM)				Antiviral IC_{50}[a,b] (nM)			CC_{50}[c] (μM)
	HCMV	HSV-1	VZV	α/γ/δ	HCMV	HSV-1	VZV	
1	1100 (±400)	1200	770	>40,000	800 (±300)	5530 (±160)	770 (±180)	>100
(***R***)-**5d**	61 (±1)	76	21	>40,000	100	3000	2	>100
(***R***)-**5q**	37 (±3)	76 (±9)	19 (±1)	>40,000	15 (±5)	1600	7	>100
Ganciclovir					1300 (±300)	nd	nd	>100
Acyclovir					>20,000	2100	8100	>100
Foscarnet	2500	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. 14.
[d] AZT-TP, zidovudine triphosphate.

through ring opening of the corresponding oxiranes,[8] Scheme 4. Lastly, aminoalcohols **7o–q** were prepared from the corresponding chlorohydrins[9,10] by reaction with methylamine, Scheme 5. The resulting phenyl and heteroaromatic substituted thieno[2,3-*b*]pyridines all showed strong HCMV polymerase inhibition and corresponding HCMV antiviral activity in contrast to the corresponding alkyl derivatives (**5r** and **5s**) suggesting the aromatic ring plays an important role in the enhanced activity of these analogs. Several analogs demonstrated HCMV polymerase and antiviral IC_{50} values below 100 nM including substitution by pyridin-2-yl (**5d**), 2-furan (**5g**), and pyrazin-2-yl (**5q**). Analogs **5d** and **5q** demonstrated good aqueous solubility (9 and 72 μM, respectively); however, furan derivative **5g** unfortunately showed poor solubility (0.4 μM). From previous work in the series,[4] polymerase inhibition was tolerant of substitution in the 7-position of the thieno[2,3-*b*]pyridine ring system, and we felt it may be possible to modify the compound's physical properties by functionalization in this region. Alcohol **9**[4] was alkylated by a series of alkyl and alkyl ether halides and subsequently converted to the corresponding chloride **10**, Scheme 6. The resulting chlorides were reacted with chiral 2-furyl aminoalcohol (***R***)-**7g** (>96% ee)[9,11] to afford thieno[2,3-*b*]pyridines **11a–e**, Table 4. THP-ether derivatives **11d** and **11e** were deprotected to provide alcohols **11f** and **11g**, respectively. Resolution by chiral chromatography afforded (***R***)-**5g**.[12] By incorporating a hydroxyl group into the N7-substituent, compounds **11f** and **11g** showed improved aqueous solubility (22 and 72 μM, respectively) over the alkyl derivatives for which solubility remained poor. Unfortunately, for these analogs as well as alkyl derivatives **11a–c**, HCMV polymerase and antiviral potency was reduced compared to the sterically smaller methyl substituent.

The more potent stereoisomer of the pyridin-2-yl and pyrazin-2-yl derivatives was similarly prepared by reaction of **4** with the corresponding scalemic (*R*)-aminoalcohols[9] to provide (***R***)-**5d**[13] and (***R***)-**5q** (>98% ee), respectively. Both compounds were found to broad-spectrum inhibitors of herpesvirus polymerases including HCMV, HSV, and VZV while not being active against human DNA polymerases (α, γ, and δ), Table 5. Broad-spectrum herpes antiviral activity was also demonstrated with (***R***)-**5d** and (***R***)-**5q** being substantially more potent than **1** against HCMV and VZV. Both compounds also demonstrated good oral bioavailability in rat (45% and 21%, respectively) and in dog (76% and 66%, respectively).[15] Compound (***R***)-**5d** was also found to inhibit Epstein–Barr virus (EBV) polymerase (IC_{50} = 70 nM)[16] and demonstrate EBV antiviral activity in a cell culture assay (IC_{50} = 200 nM).[17] As seen previously with compound **1**,[4] HSV-1 mutants containing a V823A mutation in their polymerase gene were significantly less sensitive to inhibition by (***R***)-**5d** than wild-type virus.

The replacement of the morpholinyl-substituent in herpes antivirals of the thieno[2,3-*b*]pyridine series such as **1** with a 2-aryl-2-hydroxyethylamine moiety has afforded orally bioavailable compounds with significantly improved viral polymerase inhibition. Compounds (***R***)-**5d** and (***R***)-**5q** exhibit antiviral activity comparable (HSV-1) or superior (HCMV and VZV) to that of **1** and established therapies. The application of the 2-aryl-2-hydroxyethylamine motif to core ring systems other than thieno[2,3-*b*]pyridines offers the hope of further improved antiviral potency within this novel class of broad-spectrum agents.

Acknowledgments

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6. Compounds were assayed for their ability to inhibit the incorporation of ^{3}H-labeled nucleotide into template-primer by purified viral DNA polymerase (HCMV, HSV-1, VZV) and human DNA polymerases (alpha, gamma, and delta) using a scintillation proximity assay. Assay conditions and methods to purify the corresponding DNA polymerases have been previously described in Ref. 4. Antiviral activities against HCMV, VZV, and HSV-1 herpesviruses were determined using plaque reduction assays utilizing conditions described in Ref. 4. HCMV (Davis strain) and VZV (Webster strain) were grown on human foreskin fibroblast (HFF) cells. HSV-1 (KOS strain) was grown on African green monkey kidney cells (Vero).
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11. Although (***S***)-**3b** and (***R***)-**7g** have different configurational notations, this is due to a change in the Cahn-Ingold-Prelog priority assignment for the aromatic moiety (phenyl vs 2-furan) while the spatial orientation of the hydroxyl group with respect to the aromatic moiety is the same in both molecules.
12. Configuration was subsequently confirmed by reacting (***R***)-**7g** with **4** to afford an authentic sample.
13. The configuration for the active enantiomer of **5d** was confirmed by single crystal X-ray determination of the corresponding 4-bromobenzoate ester. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 636553.
14. 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.
15. 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.
16. The EBV polymerase gene was isolated from pSVEBV (obtained from D. Coen, Harvard Univ.) and inserted into pGEM-3zf (Promega Corp.). Similarly, the sequence for BMRF-1 accessory protein was isolated from plasmid p128.5 (obtained from W. Sugden, Univ. Wisconsin) and inserted into pGEM-3zf at the EcoRI and XbaI sites. The T7 quick-coupled transcription/translation system (Promega Corp.) was used to produce active EBV polymerase and BMRF-1 protein from pGEM + EBVpol and pGEM + BMRF-1 DNA. The polymerase assay employed 2.5 μL each of EBV polymerase and BMRF-1 in 45 μL of buffer comprised of 50 mM Tris, pH 8, 70 mM NH_4SO_4, 10 mM $MgCl_2$, 300 mM dCTP/dTTP/dGTP, 0.5 μCi ^{32}P-dATP (3000 Ci/mmol), and 100 μg/mL activated calf thymus DNA. Compounds were added prior to enzyme addition at a final DMSO concentration of 1 %. Reaction mixtures were incubated at 37 °C for 30 min and then the reaction was stopped by adding the reaction volume to 2 mL of 5% TCA. Precipitated DNA was harvested onto 2.4 cm GFC glass filters. Filters were washed twice with 5% TCA and once with 95% ethanol. Incorporation of ^{32}P-dATP was measured by scintillation counter.
17. EBV antiviral activity was measured in cell culture employing EBV lytic replication. GG68 cells (obtained from W. Sugden, Univ. Wisconsin) were induced with 40 ng/mL TPA and 4 mM *n*-butyric acid. Compounds were added at a final DMSO concentration of 0.5%. After 72 h at 37 °C, cells were lysed and DNA was harvested. Purified DNA extracts were separated by Southern blot analysis, quantitated using Imagequant software, and IC_{50} values determined by an Excel program. Acyclovir EBV antiviral IC_{50} = 6900 nM.