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7-Oxo-4,7-dihydrothieno[3,2-*b*]pyridine-6-carboxamides: Synthesis and biological activity of a new class of highly potent inhibitors of human cytomegalovirus DNA polymerase

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Abstract—We report a new class of non-nucleoside antivirals, the 7-oxo-4,7-dihydrothieno[3,2-*b*]pyridine-6-carboxamides, some of which possess remarkable potency versus a broad spectrum of herpesvirus DNA polymerases and excellent selectivity compared to human DNA polymerases. A critical factor in the level of activity is hypothesized to be conformational restriction of the key 2-aryl-2-hydroxyethylamine sidechain by an adjacent methyl group.

Human herpesviruses, including herpes simplex type 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), and human cytomegalovirus (HCMV), are common and usually self-limiting in otherwise healthy individuals. However in the immune-impaired, each of these viruses and the emerging human herpesviruses (HHV-6, -7, and -8) can cause severe disease, and together they remain a significant health threat. The nucleoside antivirals ganciclovir (GCV) and acyclovir (ACV), the nucleotide antiviral cidofovir (CDV), the pyrophosphate analog foscarnet (PFA), and the newly described antisense agent, Formivirsen, have been approved for treatment of certain herpetic infections in patients but are less than ideal due to either their associated toxicity, limited efficacy against only one or two human herpesviruses, or the requirement for non-oral route of delivery.[1–3] Additionally, for certain classes of anti-herpetic drugs, resistance issues may develop during treatment periods. Thus, drug-resistant HSV, VZV, and CMV strains have been identified,[4–6] and cross-resistance of herpesviruses to antiviral drugs has been described.[7]

Nucleoside based antivirals, including GCV, ACV, and famciclovir (FCV), require phosphorylation by a viral-specific thymidine kinase and subsequent cellular enzymes to generate an active form, the nucleoside triphosphate. The triphosphate acts as a substrate for the viral DNA polymerase, competes with the binding of the natural 2′-deoxynucleoside triphosphate, and thereby leads to partial or complete chain termination.[8] We have targeted the herpesvirus DNA polymerase, a proven molecular target, to identify broad-spectrum anti-herpetic agents due to its high degree of homology between nearly all of the eight human herpesviruses.[9] We have previously reported on the anti-herpetic activity in cells and in animals of a novel series of 4-oxo-1,4-dihydroquinoline carboxamides (DHQs) (e.g., **1**, Fig. 1) that demonstrated broad-spectrum inhibition of HCMV, HSV-1, HSV-2, and VZV, as well as activity against EBV and HHV-8 polymerases.[10,11] The DHQs are mixed competitive inhibitors of nucleoside binding and are not cross-resistant to GCV-resistant HCMV or to acyclovir-resistant HSV-1 mutants.[10d] Schnute et al. subsequently demonstrated broad inhibition of human herpesvirus polymerases with a related series of

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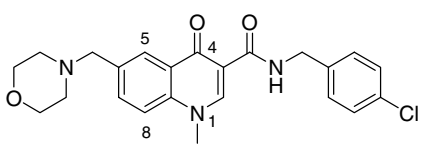
4-oxo-4,7-dihydrothienopyridines (DHTPs), exemplified by **2a**.[12] In contrast to the kinetics determined for the DHQs, this exemplar proved to be a competitive inhibitor of dTTP incorporation into the primer template by HCMV polymerase.

Previous structure–activity relationship (SAR) work had established the critical importance of the 4-chlorobenzyl amide, the pyridone *N*-methyl group, and the aminomethyl sidechain for potent antiviral activity.[10a,12] Recently, Schnute et al. demonstrated that the 2-aryl-2-hydroxyethylamine sidechain (e.g., **2b**) endows the DHTP template with significantly improved activity. This work culminated in the identification of **2c**, which was selected for advanced preclinical development.[13]

Earlier SAR work also indicated that substitution of the DHQ template **1** in the 8-position afforded analogs with improved potency (unpublished results). Since the DHTP template did not offer the same opportunity for SAR exploration, we considered investigating the isomeric 7-oxo-1,4-dihydrothieno[3,2-b] pyridine (isoDHTP) series, represented by **8**. Synthesis of initial analogs from this class is summarized in Scheme 1.[14] The known bicyclic esters **3**[15] were regioselectively formylated via the corresponding dianions, affording aldehydes **4** in excellent yield. It is worth noting that attempted Vilsmeir formylation of **3** or monoanionic formylation of N-methylated **3** was completely unsuccessful at installing the key C-2 aldehyde group. N-Methylation of **4** followed by borohydride reduction of the carboxaldehyde of **5** provided alcohols **6**. The requisite 4-chlorobenzylamide was installed via simple heating of **6** with the corresponding amine under basic conditions. After reaction with methanesulfonyl chloride, chlorides **7** were obtained, which were used to prepare final analogs **8** by simple alkylation of the requisite sidechain amines. Non-commercial hydroxyethylamines were prepared as previously described.[13]

New compounds were assayed for their ability to inhibit HCMV polymerase and to inhibit plaque formation in human foreskin fibroblast cells infected with HCMV (Table 1).[10b,12] The prototype 2-phenyl-2-hydroxyethylamine isoDHTP analog **8a**, while possessing good activity against HCMV pol, was less potent than the corresponding DHTP **2b**. *To our surprise, however, simple addition of a methyl group at the 3-position* (***8b***) *improved the polymerase* IC_{50} *by a full order of magnitude*, rendering the isoDHTP template more potent than the DHTP template. This remarkable phenomenon proved to be general across a range of other 2-aryl-2-hydroxyethylamine sidechains (**8d**–**8q**, Table 1). Corresponding improvements in plaque reduction IC_{50} of similar or even greater magnitude were also realized.



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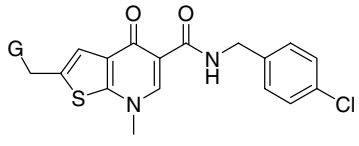
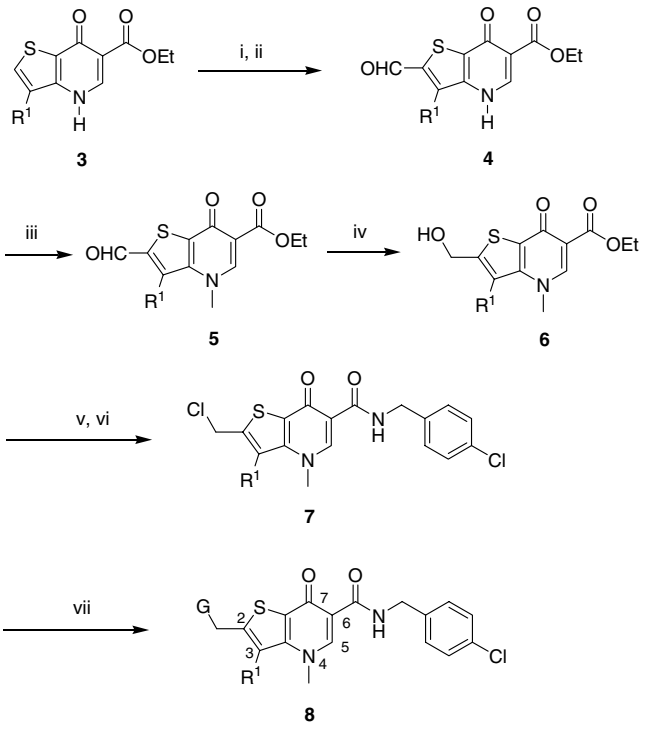


2a: G = morpholinyl (HCMV pol IC_{50} = 1.1 μM)
2b: G = PhCH(OH)CH_2NMe (HCMV pol IC_{50} = 0.10 μM)
2c: G = (R)-2-pyr-CH(OH)CH_2NMe (HCMV pol IC_{50}= 0.06 μM)

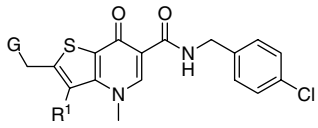
Figure 1. Lead carboxamide CMV pol inhibitors.



Scheme 1. Reagents and conditions: (i) 3 equiv LDA, THF, −78 °C, 1 h; (ii) DMF, −78 °C (20 min) → 0 °C, 93% overall; (iii) K_2CO_3, MeI, DMF, 50 °C, 24 h, 81%; (iv) $NaBH(OAc)_3$, 1,2-DCE, HOAc, 0 °C → rt, 4 h, 93%; (v) 7 equiv 4-Cl–$PhCH_2NH_2$, 25% NaOMe/MeOH, 50 °C, 20 h, 88%; (vi) MsCl, DMAP, collidine, DMF, rt, 2 h, 99%; (vii) GH, *i*-Pr_2NEt, DMF, 60 °C, 3 h, 55–80%. Yields are given for R^1 = Me. Detailed experimental procedures can be found in Ref. 14.

Interestingly, the C-3 methyl provided no benefit when the sidechain was morpholine (**8v** vs **8w**), suggesting that the methyl group itself does not augment binding, but perhaps instead effects a beneficial conformational change in the adjacent aryl ethanolamine sidechain. This hypothesis was supported by the observation that the C-3 methyl group was in fact *detrimental* when the sidechain was *N*-methyl phenylalaninol (**8t** vs **8u**). The unique conformational demands presented by the isoDHTP 3-methyl are also apparent in the complete inactivity of **8x**, an analog which bears a benzylmorpholine sidechain that confers reasonably good activity to the DHTP template (HCMV pol IC_{50} = 0.5 μM, unpublished results). Nevertheless, similarities with DHTP SAR are still apparent in the requirement for an *N*-methyl group on the 2-aryl-2-hydroxyethylamine sidechain (**8c**) and the superiority of the (*R*)-enantiomer of **8m** compared to the (*S*)-enantiomer (**8s** vs **8r**).

Table 1. Biological activity of dihydrothieno[3,2-*b*]pyridines **8**

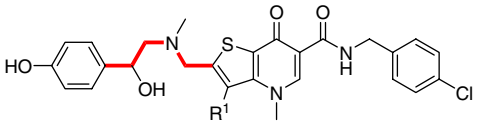


Compound	G[a]	R^1	CMV pol IC_{50}[b] (μM)	Plaque IC_{50}[c] (μM)
8a	Ph–CH(OH)CH_2NMe	H	0.58	nd
8b	Ph–CH(OH)CH_2NMe	Me	0.041	0.04
8c	Ph–CH(OH)CH_2NH	Me	10.6	15
8d	4-HO–Ph–CH(OH)CH_2NMe	H	0.22	0.15
8e	4-HO–Ph–CH(OH)CH_2NMe	Me	0.017	0.0004
8f	3-MeO–Ph–CH(OH)CH_2NMe	H	0.16	0.1
8g	3-MeO–Ph–CH(OH)CH_2NMe	Me	0.02	0.002
8h	3-Indolyl–CH(OH)CH_2NMe	H	0.21	0.5
8i	3-Indolyl–CH(OH)CH_2NMe	Me	0.019	0.03
8j	2-Furyl–CH(OH)CH_2NMe	H	0.18	0.1
8k	2-Furyl–CH(OH)CH_2NMe	Me	0.02	0.004
8l	2-Pyr–CH(OH)CH_2NMe	H	0.25	nd
8m	2-Pyr–CH(OH)CH_2NMe	Me	0.032	0.01
8n	2-Thiazolyl–CH(OH)CH_2NMe	H	0.59	nd
8o	2-Thiazolyl–CH(OH)CH_2NMe	Me	0.072	0.04
8p	2-Pyrazinyl–CH(OH)CH_2NMe	H	0.14	0.15
8q	2-Pyrazinyl–CH(OH)CH_2NMe	Me	0.017	0.06
8r	(*S*)-2-Pyr–CH(OH)CH_2NMe	Me	0.31	0.06
8s	(*R*)-2-Pyr–CH(OH)CH_2NMe	Me	0.026	0.002
8t	$PhCH_2CH(CH_2OH)$NMe	H	0.42	nd
8u	$PhCH_2CH(CH_2OH)$NMe	Me	12.4	>1
8v	Morpholin-4-yl	H	2.3	nd
8w	Morpholin-4-yl	Me	3.0	>5
8x	(*R*)-3-Benzylmorpholin-4-yl	Me	>20	>5

[a] Unless otherwise noted, sidechains are racemic.
[b] Inhibition of human cytomegalovirus DNA polymerase.
[c] Inhibition of plaque formation in human foreskin fibroblasts infected with HCMV. nd, not determined.

We were intrigued by the possibility that the 3-methyl group might actually be restricting the rotational freedom of the 2-aryl-2-hydroxyethyl sidechain into a favorable conformation for binding, which would constitute an interesting example of non-covalent conformational restriction.[16] To support this hypothesis, the 3D structures of a pair of analogs differing only in the presence or absence of the C-3 methyl group (**8d** and **8e**) were modeled. The graphical modeling program MOSAIC[17] was used to build a preliminary structural model for each compound using structural templates in MOSAIC derived from analysis of crystal structure data contained in the Cambridge Crystal Structure Database[18] (CCSD). Each of these structures was then minimized using the structural potential energy minimization and conformational search program BATCHMIN.[17] An MM2* potential function[19] filtered by a generalized Born solvation model[20] was employed for the energy evaluations and minimization. The conformational flexibility of the C-2 side chain of each compound was explored using the systematic pseudo-Monte-Carlo search feature of BATCHMIN to find minimum energy rotational isomers. The five rotatable bonds of the side chain (colored red in Table 2) were searched considering 10,000 rotational poses and retaining those conformations calculated to be within 5 kcal/mol of the lowest energy conformation. The flexible amide sidechain was omitted from these calculations to reduce the complexity of the calculation and computation time required. The number of conformations obtained for each search is given in Table 2.

Table 2. Activity versus calculated sidechain conformational mobility



Compound	R^1	CMV pol IC_{50}[a] (μM)	No. conf.[b]
8d	H	0.22	30
8e	Me	0.02	6

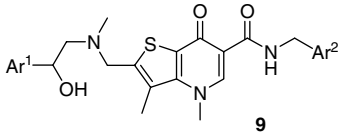
[a] Inhibition of human cytomegalovirus polymerase.
[b] Number of rotational isomers (red bonds) within 5 kcal/mol of the lowest energy conformation.

The computational data in Table 2 support our hypothesis that the superior activity is associated with attenuated conformational freedom of the amine sidechain. **8e**, possessing the C-3 methyl group, has only six low energy conformations available to it. Removal of the C-3 methyl group (**8d**) allows the C-2 sidechain to adopt a full 30 conformations within the same energy range, and at the same time reduces the polymerase inhibitory activity by an order of magnitude. Distribution of **8d** into a greater number of low energy conformations would be expected to increase the entropy of binding, explaining the overall decrease in activity.

Unfortunately the superior activity of the isoDHTP series was associated with very poor aqueous solubility. Among the analogs in Table 1, one of the most soluble was **8m** (0.7 μg/mL). Efforts to improve the solubility are summarized in Table 3. Other heterocycles were examined with limited success, the best being imidazole (**9d**) at 2.6 μg/mL. Changing the benzyl amide phenyl to pyridine (**9a**) dramatically improved solubility, but at an unacceptable cost to activity. Polar substituents on the sidechain phenyl also improved solubility, but increases beyond 2.7 μg/mL (**9h**) were not possible without losing too much activity (**9i** and **9k**). Replacing the chlorine on the benzyl amide with fluorine effected approximately a 10-fold improvement in solubility (**9e** vs **9f**) but again this costs a penalty with regard to activity. Ultimately the best balance of activity and solubility was realized with pyrimidine analog **9n**, although it is also worth noting the excellent activity and improved solubility of the 3-hydroxy-6-pyridyl analog **9j**.

Selected isoDHTP analogs were evaluated in a rat pharmacokinetic model, and complete antiviral and selected PK data for the two possessing the best bioavailabilities (**8s** and **9n**) are presented in Table 4. Data for benchmark antivirals and the DHTP preclinical candidate **2c** are included for comparison. An examination of the two pyridyl analogs **8s** and **2c** reveals the superior antiviral activity of the isoDHTP template; however, the isoDHTP analog suffers from inferior solubility and a greater propensity to inhibit the hERG channel. hERG blockage is considered to be a significant cardiovascular risk factor when selecting compounds for advanced development.[21] The fluoro isoDHTP analog **9n**, although possessing somewhat less impressive antiviral activity than **8s**, has much better solubility and a markedly improved hERG profile. It is also noteworthy that the HSV-1 plaque reduction IC_{50} of **9n** is nearly 10-fold lower than that of the DHTP development candidate, perhaps due at least in part to its superior solubility.

Table 3. Biological activity and solubility of dihydrothieno[3,2-*b*]pyridines[a]



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Compound	Ar^1	Ar^2	CMV pol IC_{50}[b] (μM)	Plaque IC_{50}[c] (μM)	Aq soln (μg/mL)
8m	2-Pyridyl	4-Cl–Ph	0.032	0.01	0.7
9a	2-Pyridyl	4-Cl-3-pyr	1.2	nd	30
9b	2-Pyrazinyl	4-Cl–Ph	0.017	0.06	0.25
9c	3-Pyridazinyl	4-Cl–Ph	0.20	0.015	1.9
9d	2-Imidazolyl	4-Cl–Ph	0.25	0.45	2.6
9e	3-Pyrazolyl	4-Cl–Ph	0.08	0.035	0.4
9f	3-Pyrazolyl	4-F–Ph	0.51	0.8	6.8
9g	4-NH_2SO_2Ph	4-Cl–Ph	0.10	0.5	0.11
9h	3-AcNHPh	4-Cl–Ph	0.093	0.02	2.7
9i	4-Me_2NCH_2Ph	4-Cl–Ph	1.8	nd	19
9j	3-OH–Pyridin-6-yl	4-Cl–Ph	0.0097	0.0004	5.0
9k	4-HOOC–Ph–	4-Cl–Ph	0.030	4.2	>117
9l	2-Pyrimidinyl	4-Cl–Ph	0.037	0.015	1.3
9m	(*R*)-2-Pyrimidinyl	4-Cl–Ph	0.015	0.004	1.4
9n	(*R*)-2-Pyrimidinyl	4-F–Ph	0.067	0.05	19

[a] Unless otherwise noted, sidechains are racemic.
[b] Inhibition of human cytomegalovirus DNA polymerase.
[c] Inhibition of plaque formation in human foreskin fibroblasts infected with HCMV. nd, not determined.

Table 4. Broad-spectrum antiviral, PK, and safety data of isoDHTP analogs versus DHTP **2c** and established therapies

Compound	Polymerase IC_{50}[a] (μM)				Antiviral IC_{50}[b] (μM)			Aq soln (μg/mL)	CL (L/h/kg)[c]	*F*%[d]	hERG IC_{20} (μM)
	HCMV	HSV-1	VZV	Human DNA-pol	HCMV	HSV-1	VZV				
2c	0.061	0.076	0.021	>20 (α)	0.10	3.0	0.002	10	2.8	45	0.5
8s	0.027	0.023	0.0058	>20 (α)	0.002	1.2	0.001	0.3	1.4	30	0.1
9n	0.067	0.13	0.054	>20 (α)	0.05	0.41	0.02	19	2.1	29	2.0
Acyclovir					>20	2.1	8.1				
Ganciclovir					1.3	nd	nd				
Foscarnet	2.5	nd	nd	<0.28 (γ)							
Aphidicolin	0.4	0.5	0.6	2.6 (α)							
AZT-TP	22.1	3.3	5.8	2.3 (δ)							

[a] Inhibition of human cytomegalovirus DNA polymerase.
[b] Inhibition of plaque formation in human foreskin fibroblasts infected with HCMV.
[c] Dosed iv (5 mg/kg) in rats.
[d] Dosed po (15 mg/kg) in rats. nd, not determined.

We have established that the DHTP template can be modified to accommodate substitution in the same region as C-8 of the DHQ template with only minimal diminution in activity. Furthermore we observed that simple methyl substitution at C-3 of the new isoDHTP template effects a dramatic boost in potency, which may be due to conformational restriction of the key 2-aryl-2-hydroxyethylamine sidechain, an hypothesis that is supported by both SAR and modeling. A compound was identified from this class (**9n**) with antiviral activity nearly equivalent to the DHTP development candidate **2c**, but which also possesses improved solubility and reduced hERG liability. Additional SAR studies on C-3 and N-4 substitution will be reported in due course.

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