Synthesis of Non-nucleoside Analogs of Toyocamycin, Sangivamycin, and Thiosangivamycin: Influence of Various 7-Substituents on Antiviral Activity

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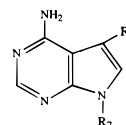
A number of 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidine-5-carbonitrile, -5-carboxamide, and -5-thiocarboxamide derivatives related to the nucleoside antibiotics toyocamycin and sangivamycin were prepared and tested for their activity against human cytomegalovirus (HCMV) and herpes simplex virus type-1 (HSV-1). Treatment of 2-amino-5-bromo-3,4-dicyanopyrrole (**1**) with triethyl orthoformate followed by alkylation *via* the sodium salt method with a variety of alkylating agents furnished the corresponding 1-substituted pyrroles **2a**−**k**. Ring annulation was achieved with methanolic ammonia affording the 7-substituted 4-amino-6-bromopyrrolo[2,3-*d*]pyrimidine-5-carbonitrile derivatives **3a**−**k**. Debromination of **3a**−**k**, *via* catalytic hydrogenation, gave the corresponding 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidine-5-carbonitrile analogs **4a**−**j**,**l**. A selective reduction of 4-amino-6-bromo-7-allylpyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**3k**) in zinc and acetic acid furnished 4-amino-7-allylpyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**4k**). Conventional functional group transformations involving the 5-cyano group of **4** furnished the 5-carboxamide derivatives **5a**−**l** and the 5-thioamide analogs **6a**−**l**. A similar transformation of the aglycone of toyocamycin (**4m**) furnished the corresponding aglycone of thiosangivamycin (**6m**). Several of the new compounds (**4**−**6a**−**e**,**j**−**l**) were evaluated for their ability to inhibit the growth of L1210 murine leukemic cells. Whereas a number of the carboxamide (**5**) and thioamide (**6**) derivatives had modest activity, the corresponding nitrile analogs (**4**) were all inactive. All compounds were tested for activity against HCMV and HSV-1. The non-nucleoside nitrile analogs **4a**−**m** and carboxamide derivatives **5a**−**l** were, with a few exceptions, essentially inactive against HCMV and HSV-1 and relatively nontoxic. In direct contrast, nearly all of the thioamide derivatives **6a**−**l**, including the aglycone of thiosangivamycin (**6m**), were good inhibitors of HCMV and HSV-1. Most were noncytotoxic in their antiviral concentration range. Cytotoxicity which was observed appeared to be a consequence of DNA synthesis inhibition. Several of these compounds, such as **6b**,**e**, were particularly interesting inhibitors of HCMV with IC_{50}'s ranging from 0.1 to 1.3 μM. The antiviral activity of both compounds was well separated from cytotoxicity in KB, HFF, and L1210 cells.

Introduction

Human cytomegalovirus (HCMV) infection is relatively benign in immunocompetent individuals but can be debilitating and often life or sight threatening to immunosuppressed individuals such as organ transplant recipients[1,2] and AIDS patients.[3,4] Moreover, HCMV has been implicated as a potential cofactor in the progression of human immunodeficiency virus (HIV) infection[5,6] and in coronary restenosis.[7] Hence, the ability to control replication of HCMV not only is critical in patients with active HCMV infections but also may be important in individuals afflicted by other life-threatening diseases.

The drugs currently approved by the Food and Drug Administration for the treatment of HCMV are ganciclovir (GCV) and foscarnet (PFA).[8−12] Both drugs, however, suffer from poor oral bioavailability, and their clinical use is limited because of host toxicity.[8,9,13,14] In addition, there have been recent reports that clinical strains of HCMV resistant to both drugs are emerging.[15−17] Hence, there is a continued need to identify compounds to treat HCMV infections which might circumvent the problems associated with the use of GCV and PFA.

Prior work in our laboratory has examined analogs of the pyrrolo[2,3-*d*]pyrimidine nucleosides toyocamycin (**I**), sangivamycin (**II**), and thiosangivamycin (**III**) as potential inhibitors of HCMV.[18] The parent nucleosides possess significant activity against HCMV[19,20] but are highly toxic to mammalian cells.[18,20,21] It is generally



I: R_5= CN, R_7= β-D-Ribofuranose

II: R_5=$CONH_2$, R_7= β-D-Ribofuranose

III: R_5=$CSNH_2$, R_7= β-D-Ribofuranose

IV: R_5=$CSNH_2$, R_7= $CH_2OCH_2CH_2OH$

V: R_5=$CSNH_2$, R_7= $CH_2OCH(CH_2OH)_2$

believed that toxicity in uninfected cells arises because the nucleosides are phosphorylated by cellular adenosine kinase[22] to afford their 5′-monophosphate derivatives which ultimately are incorporated into DNA or RNA.[23−26] Our recent studies with the 5′-deoxy analog

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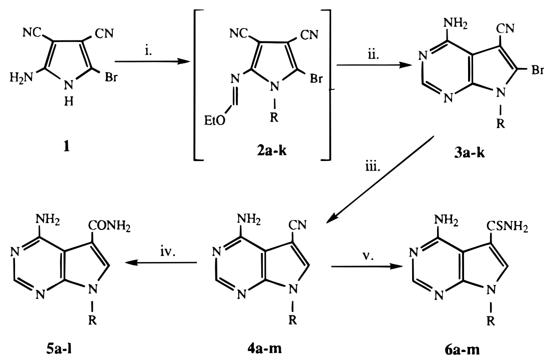
Scheme 1. Synthesis of 7-Substituted 4-Aminopyrrolo[2,3-*d*]pyrimidine Analogs Related to Toyocamycin, Sangivamycin, and Thiosangivamycin[a]



[a] (i) (1) $CH(OEt)_3$, CH_3CN, (2) NaH, RX, CH_3CN; (ii) NH_3/MeOH; (iii) H_2, Pd/C, EtOAc/EtOH or Zn/AcOH (see text); (iv) NH_4OH/H_2O/EtOH, H_2O_2; (v) MeOH, H_2S/NaOMe. **a**, R = $CH_2OCH_2CH_3$; **b**, R = $CH_2O(CH_2)_2OCH_3$; **c**, R = $CH_2OCH_2C_6H_5$; **d**, R = $CH_2C_6H_5$; **e**, R = $CH_2C_6H_4$-4-CH_3; **f**, R = $CH_2C_6H_4$-3-CH_3; **g**, R = $CH_2C_6H_4$-2-CH_3; **h**, R = $CH_2C_6H_4$-4-*t*-Bu; **i**, R = $CH_2C_6H_4$-4-OCH_3; **j**, R = Me; **k**, R = $CH_2CH{=}CH_2$; **l**, R = $CH_2CH_2CH_3$; **m**, R = H.

of **III** showed, however, that this analog also was cytotoxic thereby establishing that phosphorylation of the 5′-position is not necessary to produce cytotoxicity.[27]

In other previous studies of sugar-modified analogs of **I**−**III**,[18,21,28−31] we reported that acyclic analogs of **III** possessed good activity against HCMV coupled with a significant reduction in cytotoxicity;[21,28,29] e.g., 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (**IV**) and 4-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (**V**). In contrast, similar acyclic analogs of **I** and **II** also were nontoxic but inactive against HCMV.[21,29] Together, these results suggested that the structural requirements of the substituent at N-7 (R_7) may have less importance than the thioamide moiety at C-5 (R_5) for activity against HCMV. If this were the case, one might expect that activation of a side chain hydroxyl group may not be essential for biological activity. In fact, recent studies have established that many non−nucleoside analogs have antiviral activity.[32−38] To test this hypothesis, several model compounds were prepared where the substituent at N-7, such as a methyl, allyl, or propyl group, could not be phosphorylated.[20] The data supported the hypothesis and established that the 5-thioamide moiety confers antiviral activity without the requirement for phosphorylation of a side chain hydroxyl group. However, the selectivity of these model compounds was essentially the same when compared to the acyclic analog **IV** or **V**.

In the present study we have expanded this work and now report the synthesis of a diverse class of non-nucleoside 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidine-5-carbonitriles, -5-carboxamides, and -5-thiocarboxamides. 7-Substituents such as arylalkyl and alkyl ether groups have been used to prepare a variety of compounds which along with selected aglycones have been evaluated for cytotoxicity and activity against two herpes viruses.

Chemistry

The synthetic route used to prepare the N-7-substituted non-nucleoside analogs is illustrated in Scheme 1. Compounds **3a**−**k** were prepared from 2-amino-5-bromo-3,4-dicyanopyrrole[39] (**1**). Treatment of **1** with triethyl orthoformate followed by the addition of sodium hydride and 1 equiv of the appropriate alkylating agent gave the intermediates **2a**−**k**. The major advantage of alkylating a pyrrole precursor rather than the pyrrolopyrimidine aglycone is that the site of alkylation is fixed thereby eliminating the possibility of producing an isomeric mixture of alkylated products.[40] Furthermore, we have found this approach[28] to be amenable to the multigram synthesis of the key intermediates **3** and **4**. Reaction of **2a**−**k** with methanolic ammonia afforded the 7-substituted 4-amino-6-bromopyrrolo[2,3-*d*]pyrimidine-5-carbonitriles **3a**−**k**. The site of alkylation at N-7 was confirmed by a comparison of the UV spectra of a representative compound (**3a**) to the corresponding nucleoside, 4-amino-6-bromo-7-(*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile, synthesized by an alternate method.[41]

Various attempts at alkylating the ethoxymethylene derivative of **1** with propyl iodide proved unsuccessful. Since the next step in the reaction scheme involved a catalytic reduction, we felt that the allyl group could substitute at this point since it would probably be coreduced to furnish the debrominated, propyl-substituted derivative **4l**. Indeed **4l** was obtained from **3k** *via* catalytic hydrogenation. Treatment of **3a**−**j**, in a similar fashion, furnished the debrominated derivatives **4a**−**j**. Attempts at selectively reducing the bromo group of **3k** without effecting a reduction of the allyl side chain were examined. Under mild hydrogenation conditions with 5% Pd catalyst for 30 min, two compounds were isolated after silica gel chromatography. While one of these was the fully reduced **4l**, the other was 4-amino-6-bromo-7-propylpyrrolo[2,3-*d*]pyrimidine-5-carbonitrile. These results established that reduction of the allyl side chain of **3k** *via* catalytic hydrogenation occurred *prior* to a reduction of the bromo group. Selective reduction of **3k** was accomplished in zinc and acetic acid affording 4-amino-7-allylpyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**4k**) in a yield of 75%. The IR spectrum of **4k** demonstrated that there had been no modification of the CN since a peak was observed at 2250 cm^{-1}.

The carboxamides **5a**−**l** were synthesized from the appropriate compound **4** in yields ranging from 55% to 93% using 30% hydrogen peroxide in aqueous base. For this step, absolute ethanol was typically added to aid in dissolving the nitrile derivatives. The thiosangivamycin analogs **6a**−**l** were prepared in modest yields by a reaction of the appropriate nitrile **4** with sodium hydrogen sulfide generated *in situ* from methanolic sodium methoxide and hydrogen sulfide at 95 °C in a pressure bottle. Treatment, in a similar manner, of the aglycone of toyocamycin[42] (**4m**) furnished the aglycone of thiosangivamycin (**6m**) in 56% yield. IR and UV spectra of a representative compound (**6a**) confirmed the successful transformation of the nitrile to the thioamide. The IR spectrum of **6a** demonstrated the absence of a CN peak, and the UV spectrum of **6a** was essentially identical with that of thiosangivamycin.[43] Synthetic and physical data for compounds **3**−**6** are recorded in Table 1.

Biological Results and Discussion

In Vitro **Antiproliferative Testing.** A number of the non-nucleoside pyrrolopyrimidines were evaluated

Table 1. Synthetic and Physical Data of the 5,7-Disubstituted 4-Aminopyrrolo[2,3-*d*]pyrimidines Prepared for This Study

compd	method of prep[a]	yield (%)	mp (°C)	formula (C,H,N)	NMR data (δ, DMSO) H-2	NMR data (δ, DMSO) H-6
3a	A	37	222–223	$C_{10}H_{10}BrN_5O$	8.23	
3b	A	30	159–160	$C_{11}H_{12}BrN_5O_2$	8.23	
3c	A	54	205–208	$C_{15}H_{12}BrN_5O\cdot H_2O$	8.25	
3d	A	43	259–262	$C_{14}H_{10}BrN_5$	8.23	
3e	A	51	253–255	$C_{15}H_{12}BrN_5$	8.23	
3f	A	29	200–201	$C_{15}H_{12}BrN_5$	8.23	
3g	A	24	294–295	$C_{15}H_{12}BrN_5$	8.18	
3h	A	9	275–276	$C_{18}H_{18}BrN_5$	8.23	
3i	A	37	264 dec	$C_{15}H_{12}BrN_5O$	8.24	
3j	A	42	275–280	$C_8H_6BrN_5$	8.13	
3k	A	23	243–245	$C_{10}H_8BrN_5$	8.20	
4a	B	73	175–176	$C_{10}H_{11}N_5O$	8.34	8.24
4b	B	75	169–171	$C_{11}H_{13}N_5O_2$	8.34	8.24
4c	B	67	170–172	$C_{15}H_{13}N_5O$	8.37	8.26
4d	B	56	218–219	$C_{14}H_{11}N_5$	8.31	8.22
4e	B	81	244–245	$C_{15}H_{13}N_5$	8.29	8.22
4f	B	69	238–239	$C_{15}H_{13}N_5$	8.30	8.26
4g	B	41	256–259	$C_{15}H_{13}N_5\cdot 0.5H_2O$	8.21	8.16
4h	B	22	242–244	$C_{18}H_{19}N_5$	8.32	8.23
4i	B	20	220–221	$C_{15}H_{13}N_5O$	8.29	8.22
4j	B	51	275–276	$C_8H_7N_5$	8.21	8.16
4k	*b*	75	188–189	$C_{10}H_9N_5\cdot 0.25H_2O$	8.21	8.18
4l	B	60	178–180	$C_{10}H_{11}N_5$	8.24	8.20
5a	C	71	222–223	$C_{10}H_{13}N_5O_2$	8.10	8.07
5b	C	86	184–186	$C_{11}H_{15}N_5O_3$	8.11	8.07
5c	C	93	231–233	$C_{15}H_{15}N_5O_2\cdot 0.25H_2O$	8.13	8.12
5d	C	70	255–256	$C_{14}H_{13}N_5O$	8.18	8.10
5e	C	82	273–276	$C_{15}H_{15}N_5O$	8.09	7.97
5f	C	84	285–287	$C_{15}H_{15}N_5O\cdot 0.75H_2O$	8.11	7.99
5g	C	66	265–267	$C_{15}H_{15}N_5O\cdot 0.25H_2O$	8.09	7.91
5h	C	73	165–167	$C_{18}H_{21}N_5O\cdot 0.25H_2O$	8.09	7.99
5i	C	55	259–260	$C_{15}H_{15}N_5O_2\cdot 0.25H_2O$	8.10	7.96
5j	C	62	300–302	$C_8H_9N_5O$	8.07	7.91
5k	C	79	244–245	$C_{10}H_{11}N_5O$	8.07	7.94
5l	C	92	236–239	$C_{10}H_{13}N_5O$	8.06	7.97
6a	D	93	207–209	$C_{10}H_{13}N_5OS\cdot 0.25H_2O$	8.14	7.85
6b	D	64	150–152	$C_{11}H_{15}N_5O_2S$	8.14	7.85
6c	D	80	180–182	$C_{15}H_{15}N_5OS$	8.17	7.91
6d	D	78	192–195	$C_{14}H_{13}N_5S$	8.13	7.93
6e	D	87	225–227	$C_{15}H_{15}N_5S$	8.13	7.83
6f	D	70	225–226	$C_{15}H_{15}N_5S\cdot 0.5H_2O$	8.14	7.85
6g	D	88	248–250	$C_{15}H_{15}N_5S$	8.13	7.72
6h	D	53	145–147	$C_{18}H_{21}N_5S$	8.13	7.85
6i	D	63	222–224	$C_{15}H_{15}N_5OS\cdot 0.5H_2O$	8.13	7.82
6j	D	44	250–252	$C_8H_9N_5S$	8.11	7.75
6k	D	59	201–202	$C_{10}H_{11}N_5S$	8.11	7.76
6l	D	70	186–189	$C_{10}H_{13}N_5S$	8.10	7.80
6m	D	56	>340	$C_7H_7N_5S$	8.06	7.71

[a] See the Experimental Section for methods A–D. [b] See the Experimental Section for the preparation of **4k**.

as potential antitumor agents by determining their effect on the growth of L1210 murine leukemic cells *in vitro*. As shown in Table 2, none of the nitrile derivatives (**4**) caused any significant inhibition. In contrast, several of the carboxamide (**5**) and thioamide (**6**) derivatives had modest inhibitory activity. Specifically, the benzyl-substituted carboxamide analogs **5d**,**e** gave IC_{50} values of 16 and 20 μM, respectively. The corresponding thioamide derivatives **6d**,**e** were less active. The ether derivative **5c** and propyl analog **5l** had slight growth inhibitory effects with IC_{50}'s equal to 70 μM. Of the thiocarboxamide derivatives, the 7-methyl (**6j**)- and 7-allyl (**6k**)-substituted analogs were the most active with IC_{50}'s of ca. 40 μM. Interestingly, the propyl derivative **6l** was inactive. Although a number of the carboxamide- and thiocarboxamide-substituted analogs had modest antiproliferative effects, all of the compounds examined were significantly less active than the corresponding ribonucleosides which had IC_{50} values in L1210 cells ranging from 3–4 nM for toyocamycin (**I**) and sangivamycin (**II**) to 23 nM for thiosangivamycin (**III**).[44]

***In Vitro* Antiviral Activity.** Compounds **4a–m**, **5a–l**, and **6a–m** were evaluated for activity against HCMV and herpes simplex virus type-1 (HSV-1) replication. Cytotoxicity and inhibition of cell growth by each compound were determined by several techniques in both growing and stationary cells. The results are presented in Table 3. Antiviral and cytotoxicity data for the ribosyl-, (hydroxyethoxy)methyl-, and (dihydroxypropoxy)methyl-substituted pyrrolo[2,3-*d*]pyrimidines have been presented before[20,21,29] and are included here for comparison. The data confirm that the major factor required for the antiviral activity of these 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidines is the thioamide moiety at the 5-position (R_5) and that the hydroxyl group on the side chain of **IV** or **V** is not necessarily required for the compound to have activity against either HSV-1 or HCMV. Like the acyclic

Table 2. Cell Growth Inhibitory Activity of Several 7-Substituted 4-Aminopyrrolo[2,3-*d*]pyrimidines against L1210 Murine Leukemic Cells *in Vitro*

compd	initial screen:[a] growth rate, % of control	IC_{50} (μM)[b]
4a	76	
4b	88	
4c	83	
4d	60	
4e	64	
4j	87	
4k	75	
4l	74	
5a	91	
5b	92	
5c	0	70
5d	0	16
5e	2	20
5j	89	
5k	66	
5l	0	73
6a	111	
6b	88	
6c	52	100
6d	0	70
6e	92	>10[c]
6j	0	38
6k	0	38
6l	79	

[a] The effect of each compound at 100 μM on the growth rate of L1210 cells. [b] IC_{50} is the concentration required to decrease the growth rate to one-half of the control rate. [c] Highest concentration tested.

analogs,[21,29] the non-nucleoside analogs of toyocamycin (**4a**−**m**) and sangivamycin (**5a**−**l**) were, with a few exceptions, nontoxic to cells and relatively inactive against HCMV and HSV-1. The 2-methylbenzyl derivatives **4g** and **5g** were notable exceptions with IC_{50}'s in the HCMV plaque reduction assay of 3.4 and 15 μM, respectively. There was relatively little cytotoxicity in the antiviral dose range for each compound in the two cell lines examined. Compound **4g**, however, was less potent in an HCMV enzyme-linked immunosorbent assay (ELISA; IC_{50} = 32 μM). Although the *tert*-butylbenzyl-substituted analogs **4h** and **5h** as well as the 4-methoxybenzyl-substituted carboxamide derivative **5i** were slightly active against HCMV, they were also more toxic than **4g** and **5g** to uninfected cells. The aglycone of toyocamycin (**4m**) was also active against HCMV with an IC_{50} = 3.9 μM; however, like a number of the benzyl-substituted analogs with activity, the antiviral activity for **4m** was not well separated from the toxicity in uninfected cells.

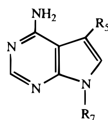
In contrast to the toyocamycin and sangivamycin non-nucleoside derivatives, the non-nucleoside thiosangivamycin analogs **6a**−**m** were potent inhibitors of HCMV and HSV-1 (Table 3). Similar results were obtained for a number of key compounds (**6a**−**e**,**i**) in the HCMV ELISA. In most cases, the potency of **6a**−**m** against HCMV was greater than that observed for **IV** or **V**, but the toxicity in uninfected cells also was greater. The separation of cytotoxicity from antiviral activity, though, was greater for **6a**−**m** when compared to that of thiosangivamycin (**III**). Although GCV was less active than the thioamide-substituted analogs in both the plaque reduction assay and ELISA, it was also less toxic in HFF and KB cells. Compounds **6a**−**c**, containing an acyclic ether side chain, were the least toxic of the thioamide-substituted derivatives studied. More extensive examination of the cytotoxicity of **6a**−**c** by incorporation of labeled precursors into protein, RNA, and DNA of CEM cells substantiated the cytotoxicity data in Table 3. IC_{50}'s for incorporation of [^{3}H]Leu, [^{3}H]Urd, and [^{3}H]dThd were >200 μM for the three compounds except that the IC_{50} for inhibition of [^{3}H]dThd incorporation by **6a** was 27 μM. This suggests that the weak effect on KB cell growth (Table 3) could result from inhibition of cellular DNA synthesis. Of the three compounds, 4-amino-7-[(2-methoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (**6b**) had good activity against HCMV in both the plaque reduction assay and the ELISA, and this activity was well separated from toxicity to uninfected cells as shown in Table 3. Also, IC_{50}'s for incorporation of labeled precursors were ≥400 μM for effects on protein, RNA, and DNA synthesis in CEM cells. **6b** and the other non-phosphorylatable compounds also were as active but considerably less cytotoxic than the 5′-deoxyribosyl analog of thiosangivamycin (**III**) which is both active and cytotoxic.[27] Thus failure to be phosphorylated at the 5′-position is not sufficient to ensure lack of cytotoxicity. Furthermore, **6b** was more potent and less toxic than the acyclic analogs **IV** and **V** thereby emphasizing that an acylic group alone is not sufficient to reduce or eliminate cytotoxicity.

Although in most cases the N-7 benzyl-substituted 5-thioamide derivatives **6d**−**i** were more potent inhibitors of HCMV than **6a**−**c**, they were invariably more toxic than the ether-substituted analogs. Nonetheless the selectivity ratios for a number of these analogs are quite good. For example, the ratio of cytotoxicity to activity against HCMV for the unsubstituted benzyl derivative **6d** is ca. 100 compared to 140 for the ether analog **6b** (Table 3). **6d** also had only minor effects on incorporation of [^{3}H]Leu, [^{3}H]Urd, and [^{3}H]dThd (IC_{50}'s = 130−195 μM) in CEM cells providing additional evidence for low cytotoxicity. The best separation between activity and toxicity was observed with **6e** (ratio > 650; see Table 3). IC_{50}'s for incorporation of labeled precursors were >400 μM for [^{3}H]Leu and [^{3}H]Urd and equal to 67 μM for [^{3}H]dThd. These results are consistent with those in Table 3 and suggest the observed cytotoxicity could be from effects on DNA synthesis. There was essentially no difference in antiviral activity between a substituted or unsubstituted phenyl ring. For example, the 4-methoxybenzyl derivative **6i** and the methyl-substituted derivatives **6e**−**g** were as potent as the unsubstituted benzyl analog **6d**. Changing the site of substitution on the phenyl ring had similar inconclusive results; eg., **6e**−**g** were equally active whether the CH_3 group was substituted at the para, meta, or ortho position. Interestingly, the aglycone **6m** had a similar biological profile as the alkyl-substituted analogs **6j**−**l**. The antiviral activity of the latter compounds was not well separated from their toxicity to uninfected cells.

Taken together, the results from the 5-thioamide-substituted analogs demonstrate that although the substituent at N-7 is less important than the requirement for a thioamide moiety at C-5 for activity against HCMV, the N-7 substituent does act to modulate the toxicity of the compounds.

These results which establish that only the 5-thioamide analogs **6a**−**m** are active against HCMV and HSV-1 are interesting in light of our other recent

Table 3. Antiviral Activity and Cytotoxicity of 5,7-Disubstituted 4-Aminopyrrolo[2,3-*d*]pyrimidines



			IC_{50} (μM)[a,b]				
			antiviral activity			cytotoxicity	
	substituent		HCMV		HSV-1	HFF	KB
compd	R_5	R_7	plaque	ELISA	ELISA	visual	growth
4a	CN	$CH_2OCH_2CH_3$	>100		>100	>100	>100
4b	CN	$CH_2O(CH_2)_2OCH_3$	90		>100	>100	>100
4c	CN	$CH_2OCH_2C_6H_5$	>100		>100	>100	>100
4d	CN	$CH_2C_6H_5$	>100		90	>100	>100
4e	CN	$CH_2C_6H_4$-4-CH_3	>100		>100	>100	>100
4f	CN	$CH_2C_6H_4$-3-CH_3	>100		>100	>100	>100
4g	CN	$CH_2C_6H_4$-2-CH_3	3.4	32	>100	>100	>100
4h	CN	$CH_2C_6H_4$-4-*t*-Bu	32		>100	>32	3
4i	CN	$CH_2C_6H_4$-4-OCH_3	>100		>100	>100	>100
4j[c]	CN	CH_3	>100	>100	>100	>100	>100
4k[c]	CN	$CH_2CH{=}CH_2$	>100		>100	>100	>100
4l[c]	CN	$CH_2CH_2CH_3$	36		>100	>100	>100
4m	CN	H	3.9		>100	10	18
5a	$CONH_2$	$CH_2OCH_2CH_3$	>100	>100	>100	>100	>100
5b	$CONH_2$	$CH_2O(CH_2)_2OCH_3$	>100		>100	>100	>100
5c	$CONH_2$	$CH_2OCH_2C_6H_5$	>100		>100	>100	>100
5d	$CONH_2$	$CH_2C_6H_5$	>100		33	>32	75
5e	$CONH_2$	$CH_2C_6H_4$-4-CH_3	>100		>100	>100	>100
5f	$CONH_2$	$CH_2C_6H_4$-3-CH_3	>100		>100	>100	>100
5g	$CONH_2$	$CH_2C_6H_4$-2-CH_3	15		>100	>100	>100
5h	$CONH_2$	$CH_2C_6H_4$-4-*t*-Bu	28		50	100	0.9
5i	$CONH_2$	$CH_2C_6H_4$-4-OCH_3	13		35	>32	47
5j[c]	$CONH_2$	CH_3	>100		>100	>100	>100
5k[c]	$CONH_2$	$CH_2CH{=}CH_2$	>100		>100	>100	100
5l[c]	$CONH_2$	$CH_2CH_2CH_3$	>100	>100	>100	>100	100
6a	$CSNH_2$	$CH_2OCH_2CH_3$	2.7	7	40	66	68
6b	$CSNH_2$	$CH_2O(CH_2)_2OCH_3$	1.0	1.9	85	>100	183
6c	$CSNH_2$	$CH_2OCH_2C_6H_5$	2.6	1.8	10	>100	100
6d	$CSNH_2$	$CH_2C_6H_5$	0.36	0.33	7.4	>32	32
6e	$CSNH_2$	$CH_2C_6H_4$-4-CH_3	0.1	0.15	3.5	100	65
6f	$CSNH_2$	$CH_2C_6H_4$-3-CH_3	0.25		15	66	30
6g	$CSNH_2$	$CH_2C_6H_4$-2-CH_3	0.1		0.9	66	>17
6h	$CSNH_2$	$CH_2C_6H_4$-4-*t*-Bu	1.5		15	>100	7
6i	$CSNH_2$	$CH_2C_6H_4$-4-OCH_3	0.5	0.5	4	66	28
6j[c]	$CSNH_2$	CH_3	1.2		2.8		15
6k[c]	$CSNH_2$	$CH_2CH{=}CH_2$	2.1		4.5		19
6l[c]	$CSNH_2$	$CH_2CH_2CH_3$	1.2		12	25	17
6m	$CSNH_2$	H	1.6		35	>32	25
ganciclovir (GCV)			7.4	22	3.5	>100	>100
acyclovir (ACV)			86		1.9	>100	>100
I[d]	CN	β-D-ribofuranose	0.05			<0.01	0.03
II[d]	$CONH_2$	β-D-ribofuranose	0.03			<0.01	0.08
III[c]	$CSNH_2$	β-D-ribofuranose	0.2			0.2	0.03
IV[d]	$CSNH_2$	$CH_2OCH_2CH_2OH$	8.8		21	>100	115
V[e]	$CSNH_2$	$CH_2OCH(CH_2OH)_2$	6.2		>100	>100	>100

[a] Results are the average of two or more experiments with most assays except for HSV-1 ELISA's which were done once. [b] A greater than sign (>) indicates IC_{50} not reached at highest concentration tested. Data also reported in [c] ref 20, [d] ref 21, and [e] ref 29.

observations.[44] We have found that 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamides, such as **6b**,**d**, are converted to their corresponding 5-carbonitrile derivatives *in vitro*. Although we did not examine all of the thioamides in the present study for this phenomenon, we expect they would undergo this conversion in cell culture media. Hence, the biological data for compounds **6a**–**m** may be the result of a mixture of 5-thioamide and 5-carbonitrile derivatives. Because the nitrile analogs **4a**–**m** are inactive (except for **4g**), the activity of **6a**–**m** must be greater than we observed if conversion from the 5-thioamide to the 5-nitrile occurs. Clearly, the 5-thioamide pyrrolo[2,3-*d*]pyrimidines **6a**–**m** have completely different biological properties when compared to the 5-nitrile analogs **4a**–**m**.

In summary, the design, synthesis, and antiviral evaluation of several non-nucleoside pyrrolo[2,3-*d*]pyrimidines were performed. The antiviral activity and cytotoxicity of several compounds reported herein are significant improvements over the biological properties of the initial model compounds.[20] The data reported in the present study have unequivocally established that the 5-thioamide moiety can provide selective antiviral activity without the requirement for phosphorylation of a side chain hydroxyl group.

Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was run on

silica gel 60F-254 plates (Analtech, Inc.). Detection of components on TLC was made by UV light absorption at 254 nm. Ultraviolet (UV) spectra were recorded on a Kontron-Uvikon 860 spectrophotometer. Infrared (IR) spectra were taken on a Perkin-Elmer 1310 infrared spectrophotometer. Nuclear magnetic resonance (^1H NMR) spectra were determined at 200, 300, or 360 MHz with a Bruker WP 200/300/360 SY spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent (DMSO-d_6). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4% of the theoretical values. E. Merck silica gel (230−400 mesh) was used for gravity or flash column chromatography. All evaporations were carried out on a rotary evaporator under reduced pressure (water aspirator) with the bath temperature at 37 °C. Acetonitrile was dried over activated molecular sieves (4 Å).

General Method A for the Synthesis of 7-Substituted 4-Amino-6-bromopyrrolo[2,3-*d*]pyrimidine-5-carbonitrile Derivatives 3a−k: 4-Amino-6-bromo-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (3a). A mixture of 2-amino-5-bromo-3,4-dicyanopyrrole[39] (**1**; 30.0 g, 141 mmol) and freshly distilled triethyl orthoformate (42.77 g, 282 mmol) in dry acetonitrile (500 mL) under argon was heated at reflux for 2.5 h and cooled to room temperature, and the solvent evaporated *in vacuo*. The resulting residue was coevaporated with toluene (4 × 50 mL) and evaporated *in vacuo* until a dry powder was obtained. The crude powder was dissolved in dry acetonitrile (250 mL) and then treated with sodium hydride (80%, w/w, 6.36 g, 212 mmol) at room temperature. The mixture was stirred for 0.5 h; then 1 equiv of chloromethyl ethyl ether (13.3 g, 141 mmol) was added. The alkylation was followed closely by monitoring the reaction with TLC. The reaction mixture was stirred at room temperature for 30 min and then filtered. The filtrate was evaporated, and the resulting oil (**2a**) was transferred to a pressure bottle containing a saturated solution of NH_3 in MeOH (150 mL). The bottle was sealed and the solution stirred for 24 h at room temperature. The resulting suspension was cooled at 4 °C for 16 h and then filtered and dried for 16 h at 60 °C to yield 14.93 g (37%) of **3a**. This compound was used without further purification. A sample was recrystallized from H_2O containing a small amount of MeOH to yield pure **3a**: mp 222−223 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 282 (19.1), (MeOH) 284 (22.1), (pH 11) 284 (19.1); ^1H NMR (DMSO-d_6) δ 8.23 (1H, s, H-2), 7.00 (2H, br s, NH_2), 5.58 (2H, s, NCH_2), 3.46−3.52 (2H, q, OCH_2), 1.03−1.07 (3H, t, CH_3). Anal. ($C_{10}H_{10}BrN_5O$) C, H, N.

General Method B for the Synthesis of 7-Substituted 4-Aminopyrrolo[2,3-*d*]pyrimidine-5-carbonitrile Derivatives 4a−j,l: 4-Amino-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4a). To a mixture containing EtOAc/EtOH (600 mL, 2:1, v:v) was added **3a** (14.9 g, 50.0 mmol) with 10% Pd/C (1.49 g, 10% by wt) and 1 N NaOH (50 mL, 50.0 mmol). The mixture was hydrogenated at 50 psi at room temperature and the reaction closely monitored by TLC. After 30 min the mixture was filtered and washed with hot EtOAc (2 × 25 mL) and the filtrate evaporated to dryness. The resulting solid was suspended in H_2O/MeOH (450 mL, 3:1, v:v) and heated to boiling. To this solution was added decolorizing charcoal (1.5 g) which was filtered over Celite, and the filtrate was cooled for 16 h at 4 °C. The resulting solid was collected by filtration and dried for 16 h at 60 °C to yield pure **4a** (7.97 g, 73%): mp 175−176 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 233 (22.2), 272 (18.1), (MeOH) 278 (19.3), (pH 11) 277 (21.1); ^1H NMR (DMSO-d_6) δ 8.34 (1H, s, H-2), 8.24 (1H, s, H-6), 6.87 (2H, s, NH_2), 5.53 (2H, s, NCH_2), 3.42-3.52 (2H, q, OCH_2), 1.01−1.08 (3H, t, CH_3). Anal. ($C_{10}H_{11}N_5O$) C, H, N.

4-Amino-7-allylpyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4k). Compound **3k** (100 mg, 0.4 mmol) was dissolved in AcOH (99.7%, 20 mL), and Zn dust (209 mg, 3.2 mmol) was added in one portion. The reaction mixture was stirred for 1.5 h at room temperature and filtered and the filtrate evaporated to dryness. The resulting solid was triturated with toluene (2 × 10 mL) and dissolved in hot EtOAc (50 mL). This solution was filtered and the filtrate adsorbed onto 500 mg of silica gel and applied to a column prepacked with silica. Compound **4k** was eluted from the column with $CHCl_3$/MeOH (98:2, v:v) to give 60 mg (75%) of an off-yellow solid which was recrystallized from H_2O/MeOH: mp 188−189 °C; IR (KBr) ν 2250 (CN) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.21 (1H, s, H-2), 8.18 (1H, s, H-6), 6.82 (2H, br s, NH_2), 5.90−6.15 (1H, m, CH), 5.15−5.20 and 4.95−5.03 (1H each, dd, $=CH_2$), 4.78−4.81 (2H, d, NCH_2). Anal. ($C_{10}H_9N_5 \cdot 0.25H_2O$) C, H, N.

General Method C for the Synthesis of 7-Substituted 4-Aminopyrrolo[2,3-*d*]pyrimidine-5-carboxamide Derivatives 5a−l: 4-Amino-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (5a). Compound **4a** (200 mg, 0.9 mmol) was added to 10 mL of distilled water and dissolved with NH_4OH (10 mL, 38%) (or 1:1:1 H_2O/NH_4OH/EtOH, v:v). Hydrogen peroxide (2 mL, 30%) was added to the solution, and the reaction mixture was stirred at room temperature for 1 h, at which time no starting material was present as determined by TLC. The solution was evaporated *in vacuo*; the residue was triturated with EtOH (2 × 10 mL) and recrystallized from H_2O/EtOH to yield 153 mg (71%) of **5a**: mp 222−223 °C; ^1H NMR (DMSO-d_6) δ 8.10 (1H, s, H-2), 8.07 (1H, s, H-6), 7.94 (2H, br s, NH_2), 7.36 (2H, br s, $CONH_2$), 5.51 (2H, s, NCH_2), 3.42−3.48 (2H, q, OCH_2), 1.04−1.08 (3H, t, CH_3). Anal. ($C_{10}H_{13}N_5O_2$) C, H, N.

General Method D for the Synthesis of 7-Substituted 4-Aminopyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide Derivatives 6a−m: 4-Amino-7-(ethoxymethyl)pyrrolo-[2,3-*d*]pyrimidine-5-thiocarboxamide (6a). Dry H_2S was passed through a solution of sodium methoxide (97 mg, 1.8 mmol) in dry methanol (20 mL) for 0.5 h. The nitrile **4a** (200 mg, 0.9 mmol) was added in one portion, and the mixture was stirred in a sealed pressure tube at 95 °C for 2 h. The resulting solution was allowed to cool to room temperature and then adjusted to pH 7 with 1 N HCl. The solvent was rotary evaporated to dryness, and the resulting compound was recrystallized from H_2O containing a small amount of EtOH to yield 210 mg (93%) of **6a**: mp 207−209 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 241 (15.4), 294 (11.8), (MeOH) 242 (12.9), 289 (10.8), (pH 11) 242 (9.8), 279 (11.4); IR (KBr) ν no cyano absorption; ^1H NMR (DMSO-d_6) δ 9.46 and 9.65 (1H each, s, $CSNH_2$), 8.14 (1H, s, H-2), 7.92 (2H, br s, NH_2), 7.85 (1H, s, H-6), 5.51 (2H, s, NCH_2), 3.46−3.52 (2H, q, OCH_2), 1.05−1.09 (3H, t, CH_3). Anal. ($C_{10}H_{13}N_5OS \cdot 0.25H_2O$) C, H, N.

***In Vitro* Antiproliferative Studies.** The *in vitro* cytotoxicity against L1210 was determined in an L1210 cell growth assay described previously.[45] L1210 murine leukemic cells were grown in Fischer's medium supplemented with 10% heat inactivated (56 °C, 30 min) horse serum and subcultured by serial dilution. Growth rates were calculated from determinations of the number of cells at 0, 24, 48, 72, and 96 h in the presence of selected concentrations of the test compound. The IC_{50} was defined as the concentration required to decrease the growth rate to 50% of the untreated control cells. Growth rate was calculated as the slope of a semilogarithmic plot of cell number against time for the treated culture as a percent of the control.

Antiviral Evaluation: (a) Cells and Viruses. Human foreskin fibroblast (HFF) cells and MRC-5 cells, a human embryonic lung cell line, were grown in minimal essential medium (MEM) with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum (FBS). KB cells, an established human cell line derived from an epidermoid oral carcinoma, were grown in MEM with Hank's salts [MEM(H)] supplemented with 10% calf serum (CS). These cell lines were subcultured according to conventional procedures as described previously.[19] All cell lines were screened periodically for mycoplasma contamination and were negative. A plaque-purified isolate, P_o, of the Towne strain of HCMV was used in all experiments and was a gift of Dr. Mark Stinksi, University of Iowa. The S-148 strain of HSV-1 was provided by Dr. T. W. Schafer of Schering Corp. Stock preparations of HCMV and HSV-1 were prepared as described elsewhere.[19]

(b) Assays for Antiviral Activity. HCMV plaque-reduction assays were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above for

titration of HCMV, with the exception that the virus inoculum (0.2 mL) contained ca. 100 plaque-forming units (PFU) of HCMV and the compounds to be assayed were dissolved in the overlay medium. HSV-1 ELISA assays were performed using a procedure described by Prichard and Shipman.[46]

HCMV was also assayed using an ELISA in MRC-5 cells by the modification of a procedure previously described to assay HSV-1.[46] MRC-5 cells were incubated at 37 °C overnight and the cells infected with HCMV (moi = 0.002 PFU/cell). Following a 1 h adsorption, up to 10 concentrations of drugs were applied in triplicate. After a 6.5 day incubation, cells were fixed with 95% EtOH. The ELISA was performed in the wells containing the infected cell sheets. Wells were blocked and then treated with a 1:400 dilution of monoclonal mouse antibody to HCMV. After 1 h, a 1:1000 dilution of peroxidase-conjugated rabbit anti-mouse antibody was added to each well, the wells were incubated for 2 h, and plates were developed and read at 450/570 nm in a microplate kinetics reader. Background was subtracted using control wells.

(c) Cytotoxicity Assays. Two basic tests for cellular toxicity were employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the plaque-reduction assay described above. Drug-induced cytopathology was estimated at 35-fold magnification and scored on a zero to four basis on the day of staining for plaque counting. Cytotoxicity in exponentially growing KB cells was determined by a staining method previously described.[47] For selected compounds, cytotoxicity also was measured by determining incorporation of labeled precursors into protein, RNA, and DNA of uninfected cells. Exponentially growing CEM cells were incubated with 1 μCi/mL [^{3}H]Leu, [^{3}H]Urd, or [^{3}H]dThd, and the amount of incorporation into acid-precipitable material was determined as described previously.[19]

(d) Data Analysis. Dose−response relationships were used to quantify drug effects. These were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. The 50% inhibitory (IC_{50}) concentrations were calculated from the regression lines. Ganciclovir (GCV) and acyclovir (ACV) were used as positive controls in HCMV and HSV-1 assays, respectively.

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