Design, Synthesis and Activity Against Human Cytomegalovirus of Non-Phosphorylatable Analogs of Toyocamycin, Sangivamycin and Thiosangivamycin*

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Abstract: A number of 7-alkyl 4-aminopyrrolo[2,3-d]pyrimidine derivatives related to toyocamycin, sangivamycin and thiosangivamycin have been prepared and tested for their activity against human cytomegalovirus (HCMV). Only the thioamide substituted derivatives demonstrated biological activity.

Human cytomegalovirus (HCMV) infection is relatively benign in healthy individuals but can be debilitating or fatal to immunosuppressed individuals such as transplant recipients[1] and AIDS patients.[2] The drugs currently approved for the treatment of HCMV are ganciclovir (GCV, DHPG)[3] and foscarnet (PFA).[4] The clinical use of these compounds is limited because of host toxicity.[5-6] In addition, there have been recent reports[7-8] that strains of HCMV resistant to both drugs are emerging. Hence, there is a continued need to develop compounds which may circumvent the problems associated with the use of DHPG and PFA to treat HCMV infections.

The naturally occurring pyrrolo[2,3-d]pyrimidine nucleosides toyocamycin (**1**), sangivamycin (**2**) and a structurally related analog, thiosangivamycin (**3**), possess significant activity against HCMV[9-10]
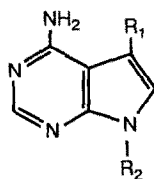

1: R_1 = CN, R_2 = β-D-Ribofuranose
3. R_1 = CSNH$_2$, R_2 = β-D-Ribofuranose

2: R_1 = CONH$_2$, R_2 = β-D-Ribofuranose
4: R_1 = CSNH$_2$, R_2 = CH$_2$OCH$_2$CH$_2$OH

but are highly toxic to mammalian cells.[9-12] Both toyocamycin and sangivamycin are phosphorylated by cellular adenosine kinase which ultimately leads to their toxicity in uninfected cells.[13] In previous studies of sugar modified analogs of **1**, **2** and **3**,[11-12, 14-18] it was reported that acyclic analogs of **3** possesssed good activity against HCMV coupled with a significant reduction in cytotoxicity;[11, 14-15] e.g., 4-amino-7-[(2-

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* Portions of this study were presented at the 204th American Chemical Society National Meeting (Medi # 181), August 1992, Washington D. C.

hydroxyethoxy)methyl]pyrrolo[2,3-d]pyrimidine-5-thiocarboxamide (**4**).[14-15] Interestingly, similar acyclic analogs of **1** and **2** were also non-toxic but inactive against HCMV.[11, 15] This suggested that the structural requirements of the substitutent at N-7 (R_2) may have less importance than the thioamide moiety at C-5 (R_1) for the acyclic thiosangivamycin analogs to have activity against HCMV. If this is indeed the case, one might expect that a side chain hydroxyl group may not be essential for biological activity. In fact, recent studies have established that certain non-nucleoside analogs do have antiviral activity.[19-21] To test our hypothesis, we have prepared several analogs of toyocamycin, sangivamycin and thiosangivamycin where the substituents at N-7 cannot be phosphorylated. In the present report, we describe the synthesis, antiviral activity and cytotoxicity of a number of model compounds.

Scheme I illustrates the synthetic route we used to prepare the non-nucleoside analogs.[22] Compounds **7a** and **7b** were prepared in a similar manner to that reported for **4**[14] from 2-amino-5-bromo-3,4-dicyanopyrrole (**5**)[23] in yields of 20 and 30%, respectively. Treatment of **5**[23] with triethylorthoformate followed by the addition of sodium hydride and the appropriate alkylating agent gave the intermediate **6** which was not isolated but

SCHEME I

a R = Me   b. R = CH₂CH=CH₂   c R = CH₂CH₂CH₃
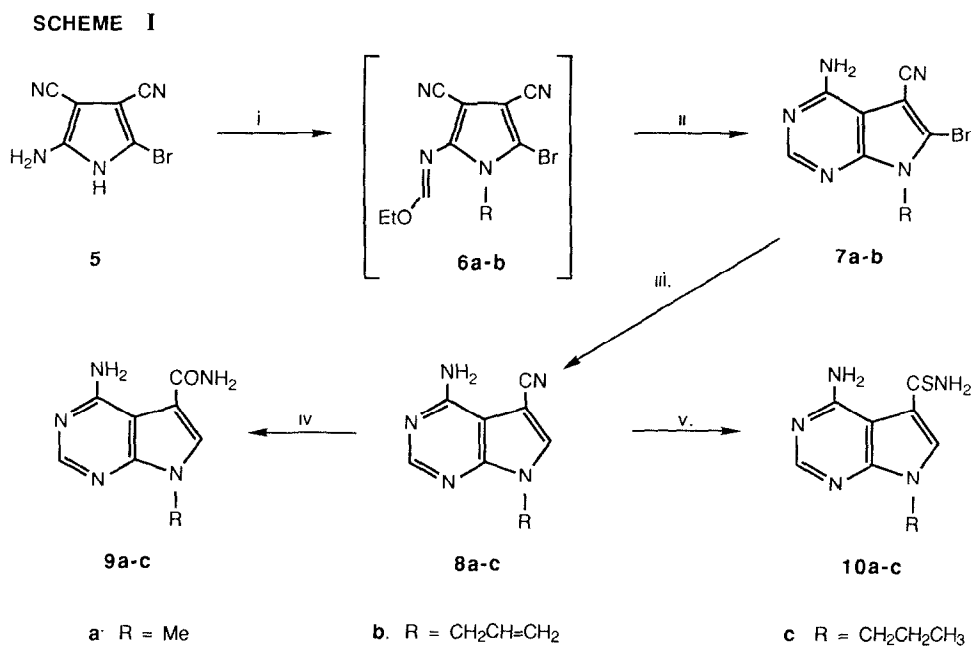
i. 1) CH(OEt)₃, CH₃CN; 2) NaH, RX, CH₃CN; ii. NH₃/MeOH;
iii. H₂, Pd/C, EtOAc/EtOH or Zn/AcOH (see text); iv NH₄OH/H₂O/EtOH, H₂O₂,
v MeOH, H₂S/NaOMe

reacted directly with methanolic ammonia to afford the 7-substituted 4-amino-6-bromopyrrolo[2,3-d]-pyrimidine-5-carbonitriles (**7a-b**). The toyocamycin analogs **8a** and **8c** were obtained in 50-70% yields from **7a** and **7b**, respectively, *via* catalytic hydrogenation while a selective reduction of **7b** in zinc and acetic acid afforded 4-amino-7-(allyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (**8b**) in a yield of 75%. The carboxamides (**9a-c**) were synthesized from the appropriate compound **8** in 75-90% using 30% hydrogen peroxide in aqueous

Table I: Antiviral activity and cytotoxicity of several 5,7-disubstituted 4-aminopyrrolo[2,3-d]-pyrimidines.

$$\begin{array}{c} NH_2 \quad R_1 \\ N \diagdown \quad \diagup \\ \diagup \diagdown N \diagdown N \\ \quad | \\ \quad R_2 \end{array}$$
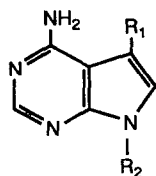
Compound	Substituent		50% Inhibitory Concentration[a] (µM)		
	R_1	R_2	HCMV	Growth[b,c]	SI
1[d]	CN	β-D-Ribofuranose	0.05	0.03[e]	
f	CN	$CH_2OCH_2CH_2OH$	>100	>100[g]	
d	CN	$CH_2OCH(CH_2OH)_2$	>100	>100[g]	
8a	CN	CH_3	>100	>100	
8b	CN	$CH_2CH=CH_2$	>100	>100	
8c	CN	$CH_2CH_2CH_3$	36	>100	
2[d]	$CONH_2$	β-D-Ribofuranose	0.03	0.08[e]	
f	$CONH_2$	$CH_2OCH_2CH_2OH$	>100	>100[g]	
d	$CONH_2$	$CH_2OCH(CH_2OH)_2$	>100	>100[g]	
9a	$CONH_2$	CH_3	>100	>100	
9b	$CONH_2$	$CH_2CH=CH_2$	>100	90	
9c	$CONH_2$	$CH_2CH_2CH_3$	>100	90	
3	$CSNH_2$	β-D-Ribofuranose	0.19 ± 0.2	0.03	< 1
4f, h	$CSNH_2$	$CH_2OCH_2CH_2OH$	8.8 ± 5.8	115	13
d	$CSNH_2$	$CH_2OCH(CH_2OH)_2$	6.2 ± 5.1	>100[g]	
10a	$CSNH_2$	CH_3	1.2 ± 0.8	14.7 ± 6.0	12
10b	$CSNH_2$	$CH_2CH=CH_2$	2.1 ± 1.9	19.0 ± 2.8	9
10c	$CSNH_2$	$CH_2CH_2CH_3$	1.2 ± 0.7	17.0 ± 4.2	14
Ganciclovir (DHPG)			8.4[i]	>100	

[a]Results are the mean of three or more experiments ± SD. [b]Unless otherwise noted, cytotoxicity tests were performed in KB cells as described in Ref. 24. [c]">" indicates IC_{50} concentration not reached at highest concentration tested. [d]Ref. 11. [e]Cytotoxicity determined in L1210 cells. [f]Ref. 15. [g]Visual cytotoxicity in HFF cells. [h]Ref. 14. [i]Average of > 50 experiments. SI: selectivity index-CC_{50}/IC_{50}.

base, and the thiosangivamycin analogs (**10 a-c**) were also prepared from the appropriate nitrile (**8**) in methanolic sodium sulfide in a sealed vessel at 95° C in 80-95% yields.

Compounds **8a-c**, **9a-c** and **10a-c** were evaluated for activity against HCMV by a plaque reduction assay[9] in human foreskin fibroblasts (HFF cells), and the cytotoxicity of each compound in uninfected cells was determined by examining the effects on the growth of KB cells.[24] The results are presented in Table I. Antiviral and cytoxicity data for the ribosyl, hydroxyethoxymethyl and dihydroxypropoxymethyl substituted pyrrolopyrimidines are presented 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_1), and that the hydroxyl group of **3** or **4** is not necessarily required for the compound to have activity against HCMV. As with the acyclic analogs,[11,15] the non-nucleoside analogs of toyocamycin (**8a-c**) and sangivamycin (**9a-c**) were non-toxic and relatively inactive against HCMV. In general, the thioamides were more toxic than the carboxamides which, in turn, were more toxic than the corresponding nitriles. Although the potency of compounds **10a-c** against HCMV is greater than that of **4**, the toxicity in uninfected cells also was greater. Therefore, the selectivity index for analogs **10a-c** remain essentially the same when compared to that of the parent compound **4** (therapeutic ratios of 9-14 vs 13, respectively). The separation of cytotoxicity from antiviral activity, though, is greater for compounds **10a-c** when compared to that of thiosangivamycin (**3**), indicating viral selectivity.

In summary, the design, synthesis and antiviral evaluation of several non-nucleoside pyrrolo-[2,3-d]pyrimidines was examined. This study was initiated to investigate the possibility that a side chain hydroxyl group of analogs with known activity against HCMV, such as compounds **3** or **4**, may not be required for biological activity. The data herein suggest that the thioamide moiety provides antiviral activity without the requirement for phosphorylation of a side chain hydroxyl group. Since the activity of these compounds is mediated *via* a unique mechanism, biological studies to elucidate the mechanism of action are underway. We are expanding our studies with this class of compounds by making several selective alkyl and aryl substitutions at the 7-position of the base as well as examining modifications at other sites on the heterocycle with the initial goal of increasing the therapeutic index.

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