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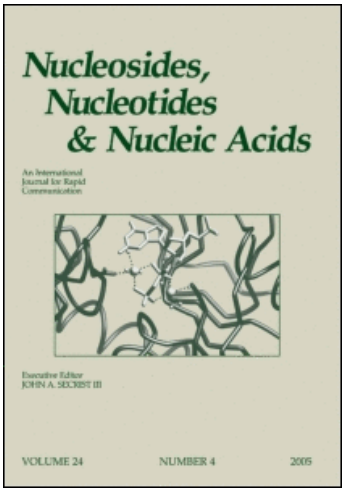

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Relationship Between the Structure of Sangivamycin-Derived Nucleosides and Their Effect on Leukemic Cell Growth and on Protein Kinase A and C Activity

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RELATIONSHIP BETWEEN THE STRUCTURE OF SANGIVAMYCIN-DERIVED NUCLEOSIDES AND THEIR EFFECT ON LEUKEMIC CELL GROWTH AND ON PROTEIN KINASE A AND C ACTIVITY.+

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Abstract

The nucleoside antibiotic sangivamycin (4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carboxamide, (1) is an effective inhibitor of protein kinase A (PKA) and protein kinase C (PKC) but, upon its phosphorylation in intact cells, it gains the ability to affect other targets as well. To retain its selectivity for the protein kinases, a series of nonphosphorylatable sangivamycin derivatives was prepared by replacing the 5'-hydroxyl group with other functions including N_3, F, SO_2NH_2, NO_2, and NH_2, These derivatives were more potent inhibitors of PKA and PKC than were the phosphorylatable compounds, although the latter were more potent inhibitors of leukemic cell growth.

Introduction

Protein kinase C (PKC) as well as protein kinase A (PKA) have been demonstrated to participate in the regulation of cell growth and differentiation[1-3], and agents that can affect these enzymes have the potential for altering these cellular processes. Based on this rationale, we embarked upon the synthesis and evaluation of a series of derivatives of the nucleoside antibiotic sangivamycin (1), which had previously been demonstrated[4] to inhibit PKC and, to a lesser extent, PKA at their catalytic (ATP-dependent) sites. When phosphorylated in intact cells, 1 gains the ability to inhibit additional cellular targets[5] and, by preventing this metabolic transformation, the selectivity of 1 for protein kinases is expected to be enhanced. To achieve this objective, we prepared derivatives of 1 with structural features designed to prevent

+ This contribution is dedicated to the memory of Professor Roland K. Robins, whose deep interest in the chemistry and biology of nucleosides and nucleotides has been a constant inspiration to us.

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phosphorylation, and compared their effect on the growth of ML-1 human myeloblastic leukemia cells and on PKA and PKC activity.

Results and Discussion

Chemical

All compounds examined were prepared in our laboratory, using synthetic procedures developed by others (e.g., compounds **1** and **30**[6], **3**,**11**, and **29**[7], and **5**,7, and **10**[8]), or approaches devised by us. Some of our syntheses have been described previously (e.g., compounds **9**, **12**, **13**, **15**, **21**, **25**, and **26**[9]), others are in the process of being published. A brief outline of the approaches used for preparation of the latter group is given below.

To provide a ready source of toyocamycin (**2**), needed as the starting material for the preparation of some of the target compounds, we developed an improved synthesis[10] involving condensation of silylated 4-amino-6-bromo-5-cyano[2,3-d]pyrimidine with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in the presence of trimethylsilyl trifluoromethylsulfonate[11].

The 4'-thio analog of toyocamycin (**7**) was synthesized by condensation[12] of ,3,5,-tri-O-acetyl-4-thio-D-ribofuranosyl chloride with the chloromercuri derivative of 4-acetamido-6-bromo-5-cyanopyrrolo[2,3-d]pyrimidine, followed by removal of the protecting group and of Br. The 5'-amino derivatives **20** and **34** were prepared by catalytic reduction of the 5'-azides **12** and **14**. 5'-Azido-6-bromotoyocamycin (**19**), 5'-fluorotoycamycin (**16**) and 7-(β-D-allofuranosyl)pyrrolo[2,3-d]pyrimidine (**22**) were synthesized by condensation of the respective 5-substituted protected sugars with the silylated pyrrolo[2,3-d]pyrimidine base, followed by deblocking. Sulfonylation of the 5'-amino group with sulfonyl chlorides gave the sulfonylamino derivatives **31**, **33**, while allylation of 2',3'-isopropylidenesangivamycin yielded **27**. Conversion of the 5-CN group into carboxamide and thiocarboxamide to provide compounds **17**, **18**, **23**, **24**, and **28** was carried out by published methods[8]. Treatment[13] of 5'-aminotoyocamycin (**34**) with thiocarbonyl-bis-imidazole gave **35**.

Biological

The effects these derivatives exert on ML-1 human myeloblastic leukemic cell growth and on PKA and PKC activity is summarized in Tables 1-3. As shown in Table 1, the most potent inhibition of cell growth was provided by sangivamycin and toyocamycin derivatives that carry a hydroxyl group at their 5'-position (FIG.1, R'=OH). The presence of this group potentially allows these agents to become phosphorylated expanding, thereby, the range of cellular targets they can affect[5].

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Table 1. Effect of Sangivamycin Derivatives on Ml-1 Leukemia Cell Growth

Compound	5-Substituent R	5'-Substituent R'	Other Structural Changes	50% Growth Inhibition (μM)
1	$CONH_2$	OH		0.03
2	CN	OH		0 03
3	$CONHNH_2$	OH		0.06
4	$CNHNHNH_2$	OH		0.1
5	H	OH		0.2
6	$CSNH_2$	OH		0.3
7	CN	OH	4'-Thio	0.4
8	CONHNHCHO	OH		0.4
9	$CONH_2$	ONO_2		0.4
10	CNHNHOH	OH		0.5
11	CONHOH	OH		0.8
12	$CONH_2$	N_3		2
13	CN	ONO_2		3
14	CN	N_3		3
15	$CONH_2$	OSO_2NH_2		5
16	CN	F		5
17	$CONH_2$	F		8
18	$CSNH_2$	F		12
19	CN	N_3	6-Br	16
20	$CONH_2$	NH_2		30
21	$CONH_2$	OSO_2CH_3		40
22	CN	$OHCH_2OH$		>100
23	$CONH_2$	$OHCH_2OH$		>100
24	$CONH_2$	OH	1-Iso	>100
25	$CONH_2$	N_3	2',3'-O-C(Me)$_2$	>100
26	$CONH_2$	$O\text{-}SO_2CH_3$	2',3'-O-C(Me)$_2$	>100
27	$CONH_2$	$OCH_2CH{=}CH_2$	2',3'-O-C(Me)$_2$	>100
28	$CSNH_2$	NH_2		>100
29	COOH	OH		>100
30	$CONH_2$	OH	6-Br	>100
31	CN	$NHSO_2CF_3$		>100
32	$CONH_2$	$NHSO_2NH_2$		>100
33	CN	$NHSO_2NH_2$		>100
34	CN	NH_2		>100
35	CN	N=C=S		>100

The effect the compounds exert on ML-1 cell growth was determined by adding graded concentrations of the agents to $3x10^5$ cells/ml of RPMI 1640 medium containing 3% fetal bovine serum. Incubation proceeded at 37°C for 3 days in a 5% CO_2 atmosphere. Cell numbers were counted by hemocytometer. The viability of untreated cell cultures was 98 %.

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Table 2. Effect of Sangivamycin Derivatives on Protein Kinase A Activity

Compound	5-Substituent R	5'-Substituent R'	Other Structural Changes	50% Enzyme Inhibition (μM)
15	$CONH_2$	OSO_2NH_2		0.01
12	$CONH_2$	N_3		0.02
9	$CONH_2$	ONO_2		0.1
13	CN	ONO_2		0.3
17	$CONH_2$	F		1.1
21	$CONH_2$	OSO_2CH_3		1.6
6	$CSNH_2$	OH		2.8
16	CN	F		3.5
18	$CSNH_2$	F		4.5
14	CN	N_3		5.8
25	$CONH_2$	N_3	2',3'-O-C(Me)$_2$	9
23	$CONH_2$	$OHCH_2OH$		10
7	CN	OH	4'-Thio	13
26	$CONH_2$	OSO_2CH_3	2',3'-O-C(Me)$_2$	14
27	$CONH_2$	$OCH_2CH=CH_2$	2',3'-O-C(Me)$_2$	15
2	CN	OH		20
20	$CONH_2$	NH_2		27
35	CN	N=C=S		27
10	CNHNHOH	OH		28
34	CN	NH_2		31
3	$CONHNH_2$	OH		34
8	CONHNHCHO	OH		45
28	$CSNH_2$	NH_2		50
1	$CONH_2$	OH		70
29	COOH	OH		90
4	$CNHNHNH_2$	OH		93
24	$CONH_2$	OH	1-Iso	>100
5	H	OH		>100
30	$CONH_2$	OH	6-Br	>100
31	CN	$NHSO_2CF_3$		>100
32	$CONH_2$	$NHSO_2NH_2$		>100
33	CN	$NHSO_2NH_2$		>100
22	CN	$OHCH_2OH$		>100
11	CONHOH	OH		>100
19	CN	N_3	6-Br	>100

Beef heart PKAwas assayed by measuring the incorporation of ^{32}P from [$\gamma^{32}P$]ATP into histone III-S[12], in a reaction mixture containing 20 mM Tris/HCl, pH 7.5, 10 mM magnesium acetate, 10 μM [$\gamma^{32}P$]ATP (0.5 μCi/nmole), 1μM cyclic AMP and 0.01 u of PKA. After, 3 min at 30° C, the reaction was stopped with ice-cold 25% TCA, the mixture applied to Whatman GF/C filters, washed with 10% TCA and the radioactivity counted in CytoScint.

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Table 3. Effect of Sangivamycin Derivatives on Protein Kinase C Activity

Compound	5-Substituent R	5'-Substituent R'	Other Structural Changes	50% Enzyme Inhibition (μM)
12	$CONH_2$	N_3		0.5
18	$CSNH_2$	F		1.3
15	$CONH_2$	OSO_2NH_2		1.4
9	$CONH_2$	ONO_2		2.7
17	$CONH_2$	F		4.8
6	$CSNH_2$	OH		5.0
20	$CONH_2$	NH_2		6.5
1	$CONH_2$	OH		12
13	CN	ONO_2		21
16	CN	F		22
3	$CONHNH_2$	OH		22
23	$CONH_2$	$OHCH_2OH$		22
10	CNHNHOH	OH		24
26	$CONH_2$	$O\text{-}SO_2CH_3$	2',3'-O-C(Me)$_2$	30
14	CN	N_3		32
21	$CONH_2$	$OSO_2\text{-}CH_3$		38
35	CN	N=C=S		40
8	CONHNHCHO	OH		43
24	$CONH_2$	OH	1-Iso	50
28	$CSNH_2$	NH_2		50
25	$CONH_2$	N_3	2',3'-O-C(Me)$_2$	72
11	CONHOH	OH		85
27	$CONH_2$	$OCH_2CH{=}CH_2$	2',3'-O-C(Me)$_2$	90
2	CN	OH		90
29	COOH	OH		>100
5	H	OH		>100
4	$CNHNHNH_2$	OH		>100
19	CN	N_3	6-Br	>100
30	$CONH_2$	OH	6-Br	>100
31	CN	$NHSO_2CF_3$		>100
32	$CONH_2$	$NHSO_2NH_2$		>100
33	CN	$NHSO_2NH_2$		>100
34	CN	NH_2		>100
7	CN	OH	4'-Thio	>100
22	CN	$OHCH_2OH$		>100

Rat brain PKC activity was determined by the procedures given in Table 2, except that c-AMP and PKA were replaced by 0.5 mM calcium chloride, 12 μg/mL phosphatidylserine, 12 μg/mL 1,2 dioleoylglycerol and 0.01 u of PKC.

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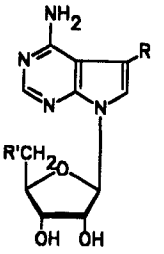

FIG. 1

The growth inhibitory activity of the compounds is not only influenced by their susceptibility to phosphorylation, but also by the type of substituent attached at position 5 (R, FIG. 1). In the presence of the 5'-OH group, the contribution the 5-substituents make to growth inhibition decreased in the order: $CONH_2$ = CN > $CONHNH_2$ > $CNHNHNH_2$ > H > $CSNH_2$ > CONHNHCHO > CNHNOH > CONHOH. It remains to be determined whether these groups exert their differential activity by influencing the extent of phosphorylation the sangivamycin derivatives undergo, or whether they modify the activity of the compounds following phosphorylation. Whatever the case, the presence of a carboxyl group at position 5 or of bromine at position 6 (compounds **29** and **30**) caused a marked decrease in activity.

Replacing the 5'-OH moiety with other groups including O-NO_2, N_3, O-SO_2NH_2, F, NH_2 and O-SO_2CH_3 resulted in less potent growth-inhibiton, but, as shown in Tables 2 and 3 increased the inhibitory activity of the compounds against PKA and PKC. Derivatives of **1** carrying O-SO_2NH_2, O-NO_2, F or N_3 at the 5'-position were more potent inhibitors of the kinases than were those bearing NH_2, NCS, $NHSO_2NH_2$ groups. The activity of the compounds is also influenced by the 5-substituent, the $CONH_2$ providing for more effective inhibitors than does CN or $CSNH_2$.

In conclusion, sangivamycin derivatives designed to resist phosphorylation were found to be more effective inhibitors of PKA and PKC than were phosphorylatable derivatives, which were more effective inhibitors of leukemic cell growth.

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