Communications to the Editor

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THE DESIGN, SYNTHESIS AND ANTILEUKEMIC ACTIVITY OF 5-ALKYNYL-1-β-D-RIBOFURANOSYLIMIDAZOLE-4-CARBOXAMIDES[1)]

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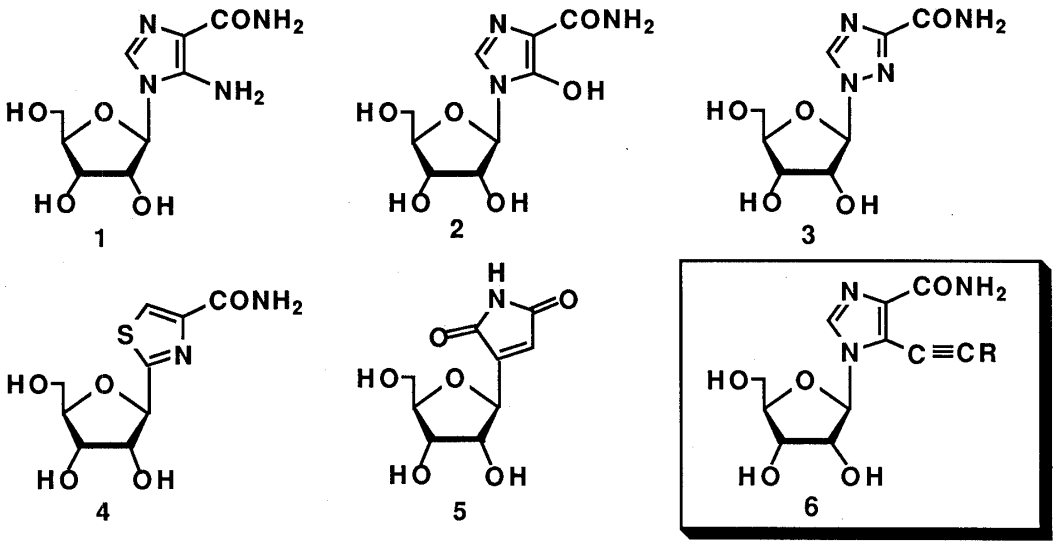
The design, synthesis and antileukemic activity of 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides (**6**) are described. The cross-coupling reaction of 5-iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**8**) with various terminal alkynes in the presence of bis(benzonitrile)palladium dichloride and triethylamine in acetonitrile gave 5-alkynyl derivatives (**9**) in high yields. Coupling of **8** with (trimethylsilyl)acetylene gave the undesired dimer (**10**). Instead of (trimethylsilyl)acetylene, treatment of trimethyl[(tributylstannyl)ethynyl]silane with **8** in the absence of triethylamine produced the desired 5-[2-(trimethylsilyl)ethynyl] derivative (**9f**) in 77% yield. Deblocking of these nucleosides (**9**) gave the target nucleosides (**6a-f**). Among them, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**6f**) is the most potent inhibitor of the growth of murine L1210 cells *in vitro* (IC_{50} = 0.18 μg/ml).

KEYWORDS —— 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide; cross-coupling reaction; palladium catalyst; antimetabolite; nucleoside; trimethyl[(tributylstannyl)ethynyl]silane; antileukemic activity

Inosine 5'-monophosphate (IMP) dehydrogenase catalyzes the conversion of IMP to xanthosine 5'-monophosphate (XMP) and is one of the key rate-controlling enzymes of nucleic acid biosynthesis. Weber has reported that the IMP dehydrogenase activity in hepatoma 3683-F is about 10 to 14 times higher than that found in normal rat liver.[2)] Therefore, IMP dehydrogenase has been suggested as one of the target enzymes for cancer chemotherapy.[3)]

A naturally occurring antibiotic, bredinin (**2**),[4)] and synthetic nucleosides such as ribavirin (**3**)[5)] and tiazofurin (**4**),[6)] which are structurally similar to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA riboside, **1**), are all potent inhibitors of IMP dehydrogenase after activation as their 5'-phosphates or NAD-type analogs. On the other hand, showdomycin (**5**), a naturally occurring maleimide C-nucleoside, acts as an alkylating agent for sulfhydryl, amino acid, and imidazole groups but is not a substrate for nucleoside kinases.[7)] Now we have designed 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides (**6**), which may be expected to be an antimetabolite of purine nucleotide biosynthesis or an alkylating agent of biologically important functional groups.



A most straightforward synthetic route to the target compounds (**6**) is to introduce an alkynyl group in the 5-position of AICA riboside (**1**) by organopalladium chemistry. As our starting material for the palladium-catalyzed cross-coupling reaction with terminal alkynes, the 5-iodo derivative (**8**)[8] was synthesized from 2',3',5'-tri-*O*-acetyl-AICA riboside (**7**) by treatment with isoamyl nitrite in diiodomethane in 55% yield from **1**.

During the course of the cross-coupling reactions of **8** with propargyl alcohol to 5-(3-hydroxy-1-propynyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**9a**, Table I), we had a satisfactory result (94% yield) when bis(benzonitrile)palladium dichloride was used as a catalyst without adding cuprous iodide. Sonogashira et al. found that addition of cuprous iodide as a co-catalyst to this type of system facilitated the cross-coupling reaction, although the role of copper was not well understood.[9] However, in our case, the addition of a catalytic amount of cuprous iodide resulted in a lower yield of **9a** (19% after 20 h). Under optimized conditions, a series of 5-alkynyl derivatives (**9b-e**)[10] were obtained in high yields (Table I). In the cross-coupling of **8** with (trimethylsilyl)acetylene, however, the reaction proceeded rather slowly to give a dimer (**10**) in 46% yield as an isolable nucleosidic product. To circumvent the undesired dimer formation, trimethyl[(tributylstannyl)ethynyl]silane[11] was used in the absence of triethylamine. Thus, the desired 5-[2-(trimethylsilyl)ethynyl] derivative (**9f**) was obtained in 77% yield accompanied by a small amount of 5-ethynyl derivative. Deblocking of these compounds (**6**) by NH_3/MeOH or Et_3N/MeOH gave the target nucleosides (**6a-f**) in good yields (Table I).

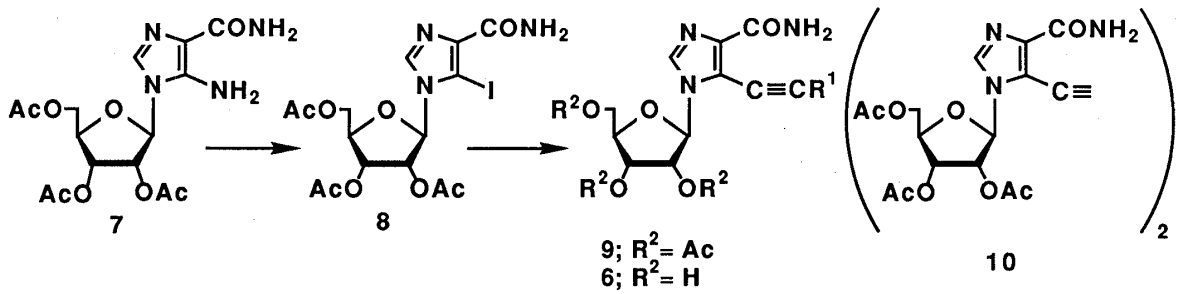


Table I. Synthesis of 5-Alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides

	R	Yield(%)		R	Yield(%)	mp(°C)
9a	CH_2OH	94	6a	CH_2OH	86	148-9
9b	Ph	79	6b	Ph	75	168-9
9c	CH_2CH_2OH	93	6c	CH_2CH_2OH	79	156-8
9d	$(CH_2)_2CH_3$	75	6d	$(CH_2)_2CH_3$	80	172-4
9e	$(CH_2)_3CH_3$	73	6e	$(CH_2)_3CH_3$	78	foam
9f	$Si(CH_3)_3$	77	6f	H	87	182-5

The antileukemic activities of **6a-f** were tested for their ability to inhibit the growth of murine L1210 cells *in vitro*.[12] The IC_{50} (μg/ml) values (the concentration required for 50% inhibition of cell growth) for these compounds are summarized in Table II. Among these, the most potent inhibitor of the cell growth was 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**6f**). An increase of the chain length of the R substituent (**6a, c** and **d**) resulted in a reduction in the inhibitory activity. The increase in the size of the R substituent (R = Ph, **6b**) also reduced the activity. Since the 5-vinyl derivative did not show any activity up to 10 μg/ml concentration (data not shown), the acetylenic group at the 5-position of the imidazole ring seems to be essential for the antileukemic activity.

Table II. Inhibitory Effects of 5-Alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides (**6a-f**) on the Growth of L1210 Cells *in Vitro*

Compound	6f	6a	6c	6d	6b
R	H	CH_2OH	CH_2CH_2OH	$(CH_2)_2CH_3$	Ph
IC_{50}(μg/ml)	0.18	0.70	1.28	2.29	20.6

Preliminary studies on the mechanism of the cytotoxic action of **6f** were performed using mouse FM3A cells. It has been reported that the intracellular imbalance of deoxyribonucleoside triphosphate pools causes cell death.[13] The imbalance of intracellular ribo- and deoxyribonucleoside triphosphate pools was induced by adding **6f**. Great decreases in GTP and dGTP levels were detected in 6 to 24 h. These results should be correlated with the inhibition of IMP dehydrogenase activity[14] which would eventually result in the decrease of GMP from XMP. Further investigations of the mechanism of action of **6f**, especially its alkylating ability, together with *in vivo* studies, will be reported elsewhere.

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