

Communications to the Editor

[Chem. Pharm. Bull.]
[36(7)2730—2733(1988)]

THE DESIGN, SYNTHESIS AND ANTILEUKEMIC ACTIVITY OF 5-
ALKYNYL-1- β -D-RIBOFURANOSYLIMIDAZOLE-4-CARBOXAMIDES¹⁾

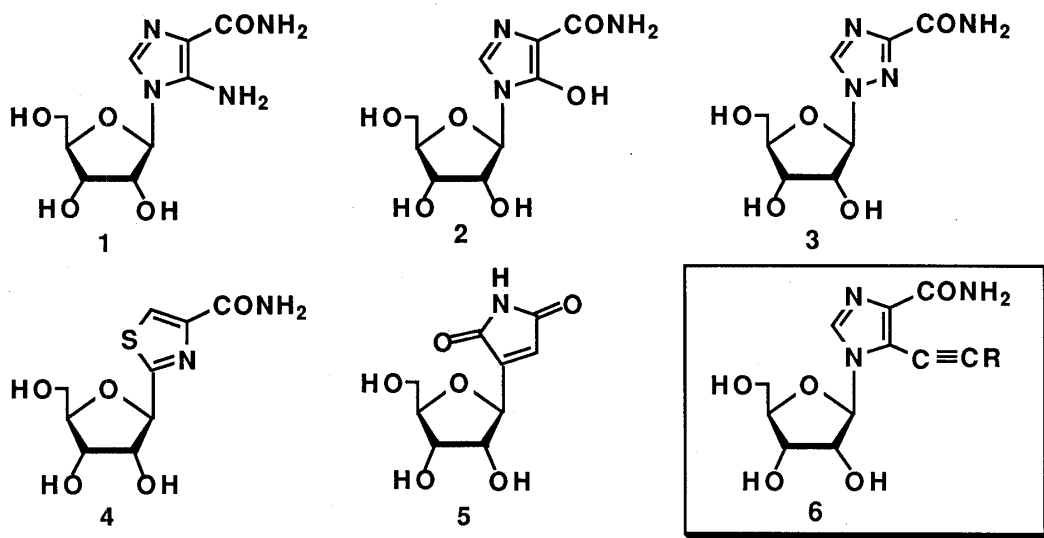
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The design, synthesis and antileukemic activity of 5-alkynyl-1- β -D-ribofuranosylimidazole-4-carboxamides (**6**) are described. The cross-coupling reaction of 5-iodo-1-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)imidazole-4-carboxamide (**8**) with 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* (IC₅₀ = 0.18 μ g/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]silane¹¹ 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 $\text{Et}_3\text{N}/\text{MeOH}$ gave the target nucleosides (6a-f) in good yields (Table I).

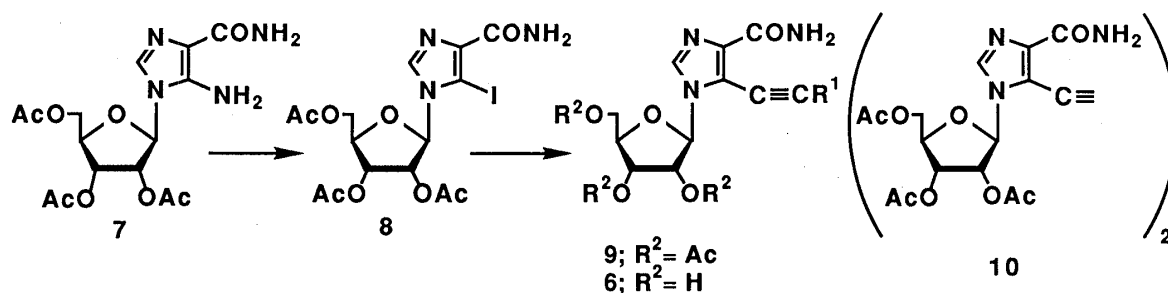


Table I. Synthesis of 5-Alkynyl-1- β -D-ribofuranosylimidazole-4-carboxamides

	R	Yield(%)		R	Yield(%)	mp(°C)
9 a	CH ₂ OH	9 4	6 a	CH ₂ OH	8 6	1 4 8 - 9
9 b	Ph	7 9	6 b	Ph	7 5	1 6 8 - 9
9 c	CH ₂ CH ₂ OH	9 3	6 c	CH ₂ CH ₂ OH	7 9	1 5 6 - 8
9 d	(CH ₂) ₂ CH ₃	7 5	6 d	(CH ₂) ₂ CH ₃	8 0	1 7 2 - 4
9 e	(CH ₂) ₃ CH ₃	7 3	6 e	(CH ₂) ₃ CH ₃	7 8	foam
9 f	Si(CH ₃) ₃	7 7	6 f	H	8 7	1 8 2 - 5

The antileukemic activities of **6a-f** were tested for their ability to inhibit the growth of murine L1210 cells *in vitro*.¹²⁾ The IC₅₀ (μ g/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 μ g/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	6 f	6 a	6 c	6 d	6 b
R	H	CH ₂ OH	CH ₂ CH ₂ OH	(CH ₂) ₂ CH ₃	Ph
IC ₅₀ (μ g/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 activity¹⁴⁾ 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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(Received May 9, 1988)