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A New *C*-Nucleoside Analogue of Tiazofurin: Synthesis and Biological Evaluation of 2-β-D-Ribofuranosylimidazole-4-carboxamide (Imidazofurin)

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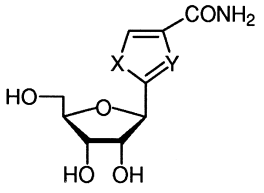
Abstract—2-β-D-Ribofuranosylimidazole-4-carboxamide, an imidazole analogue of the antitumor agent tiazofurin, was synthesized and evaluated for the growth inhibitory activity of human myelogenous leukemia K562 cells. © 2000 Elsevier Science Ltd. All rights reserved.

Inosine 5′-monophosphate dehydrogenase (IMPDH) catalyzes the conversion of IMP to XMP and it is the rate limiting enzyme in de novo guanylate biosynthesis.[1] The activity of this enzyme was shown to be significantly increased in tumor cells and therefore considered to be a potential target for cancer chemotherapy.[2] The inhibition of IMPDH and subsequent reduction in guanine nucleotides interrupts DNA and RNA synthesis in rapidly-dividing tumor cells. Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide) is an oncolytic *C*-nucleoside with potent inhibitory activity against IMPDH.[3] In Phase I/II clinical trials tiazofurin showed a significant reduction in leukemic cell burden in acute myelogenous leukemia patients.[4–6]

Tiazofurin requires metabolic activation to the corresponding nicotinamide adenine dinucleotide (NAD) analogue, thiazole-4-carboxamide adenine dinucleotide (TAD). This NAD analogue inhibits IMPDH activity through competition for the NAD cofactor-binding site of the enzyme.[7]

Several analogues of tiazofurin and TAD have been studied. The selenium analogue, selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide), is more cytotoxic than tiazofurin,[8–10] and its corresponding anabolite, SAD, binds more tightly to IMPDH than TAD does.[11]



Tiazofurin	X = S, Y = N
Selenazofurin	X = Se, Y = N
Oxazofurin	X = O, Y = N
Thiophenfurin	X = S, Y = CH
Selenophenfurin	X = Se, Y = CH
Furanfurin	X = O, Y = CH
Imidazofurin (**1**)	X = NH, Y = N

Structural studies carried out by us and other authors on tiazofurin, selenazofurin and a number of their *C*-nucleoside analogues, have pointed out that configurational and conformational effects are important for IMPDH inhibition.[12–15] In particular, it was found that the active compounds are those with an electrophilic sulfur or selenium in a delocalized environment adjacent to the *C*-glycosidic bond.[15] Compounds in which the sulfur or selenium atom is replaced by a negatively charged oxygen show minimal or no activity. It has been suggested that this requirement may result from either an intermolecular interaction between the sulfur or selenium and a negatively charged side chain in the active site of IMPDH, and/or an intramolecular interaction between the sulfur or selenium and the furanose oxygen (O4′).[12] An intramolecular S/Se–O interaction might be expected to constrain rotation around the *C*-glycosidic bond, both in the parent compounds tiazo- and selenazofurin and in their anabolites TAD and SAD. The specificity of TAD and SAD for the target enzyme would be either enhanced or diminished, depending upon the degree to which the heteroatom–oxygen interaction is maintained by the binding

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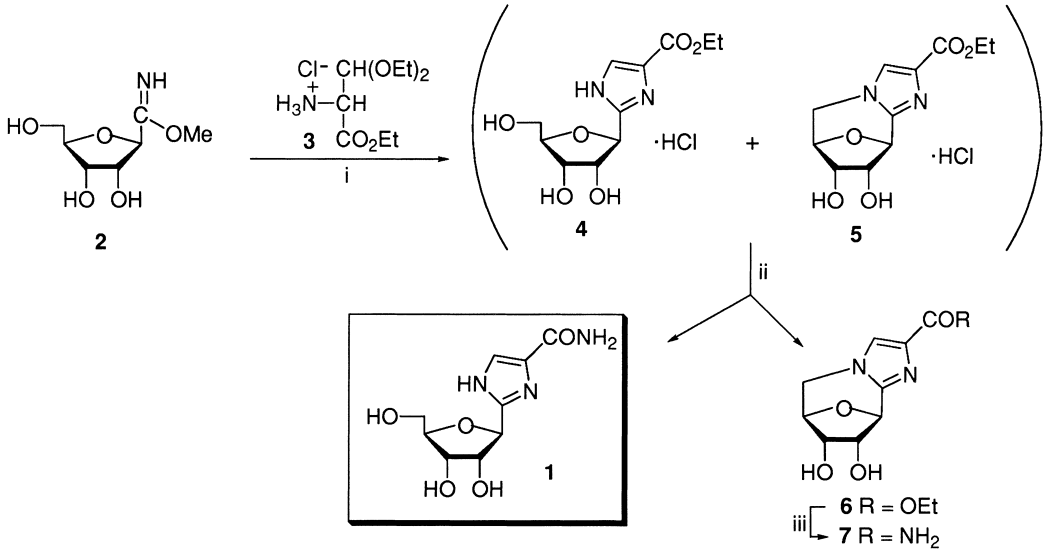
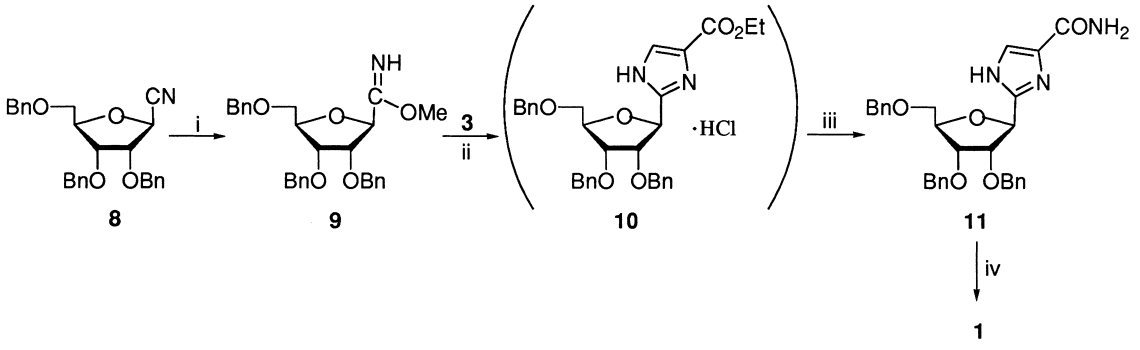
enzyme.[16] Further support for the electrostatic hypothesis is provided by the inactive tiazo- and selenazofurin analogue oxazofurin (2-β-D-ribofuranosyloxazole-4-carboxamide).[17] In this *C*-nucleoside the positive sulfur or selenium heteroatom is replaced by a more electronegative oxygen. Ab initio computations suggest that the positive charge in the region of the former sulfur or selenium position is replaced by a negative charge. Thus, this analogue is expected to demonstrate a *C*-glycosidic angle higher than those observed in the thiazole and selenazole nucleosides, a prediction confirmed in the crystal structure of oxazofurin.[18] These findings were further confirmed by the synthesis of thiophenfurin and furanfurin, two *C*-nucleoside isosteres of tiazofurin, in which the thiazole ring was replaced by a thiophene and furan heterocycle, respectively.[13] While thiophenfurin was found active as an antitumor agent both in vitro and in vivo, furanfurin proved to be inactive. In 1997, Makara and Keserű have questioned the electrostatic hypothesis and suggested that the flexibility of the *C*-glycosydic bond in this type of *C*-nucleosides is ultimately determined by steric interactions of the heteroatoms with the C2′-H and O4′ of the ribose.[19] Application of this theory led these authors to design 2-β-D-ribofuranosylimidazole-4-carboxamide (imidazofurin, **1**) as an analogue that exhibits an almost identical behavior to tiazofurin in ab initio computations. This hypothesis prompted us to synthesize imidazofurin and to test its antitumor activity.

Chemistry

Imidazofurin was synthesized through two slightly different approaches. The first approach is summarized in Scheme 1. The reaction of β-D-ribofuranosyl-1-carboximidic acid methyl ester (**2**)[20] with ethyl 2-amino-3,3-diethoxypropionate hydrochloride (**3**)[21] in anhydrous methanol gave a mixture of ethyl 2-β-D-ribofuranosylimidazole-4-carboxylate (**4**) and by-product **5**, a 1,5′-cycloimidazole *C*-nucleoside, as hydrochlorides. Treatment of this mixture with methanolic ammonia gave a mixture of imidazofurin[22] (**1**, 30.6%) and product **6**[23] (5%) which were separated by column chromatography using $CHCl_3$–MeOH (85:15) as eluent. Compound **6** was converted into the amide **7** by reaction with 30% aqueous ammonia. In order to avoid the formation of **5** we developed the method reported in Scheme 2. The reaction of 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl cyanide (**8**)[24] with sodium methoxide in methanol gave the methyl imidate **9** which, by reaction with **3** in methanol, followed by treatment of intermediate **10** with methanolic ammonia, was converted into the amide **11**. Debenzylation of **11** with ammonium

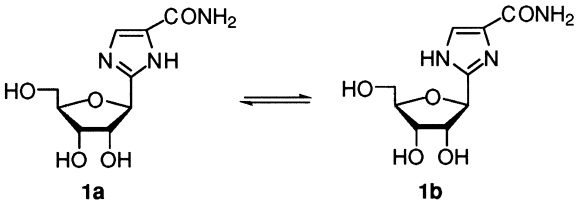


Scheme 1. (i) CH_3OH (27 h, rt); (ii) NH_3/CH_3OH (6 h, rt); (iii) 30% NH_4OH (48 h, 60 °C).



Scheme 2. (i) CH_3ONa/CH_3OH (5 h, rt); (ii) CH_3OH (24 h, rt); (iii) NH_3/CH_3OH (2 h, rt); (iv) Pd/C, $HCOONH_4$, CH_3OH (1.5 h, reflux).

formate and Pd/C (10%) in methanol afforded imidazofurin in 35% yield. All compounds were characterized by mass spectrometry, ^{1}H NMR spectroscopy, and elemental analysis. Two tautomeric forms of imidazofurin (**1a** and **1b**) were observed in solution by ^{1}H NMR in DMSO-d_6 as well as in D_2O in the ratio 2:1.



1a **1b**

The structure of compound **6** was confirmed by nuclear Overhauser enhancement (NOE) effects. In fact, when H5′ protons were irradiated a NOE effect was observed on H5, indicating that these protons are proximate.

Biological Evaluation

Imidazofurin was evaluated for its ability to inhibit the growth of human myelogenous leukemia K562 cells. Tumor cell proliferation was evaluated by incubating the cells continuously with either the compound or saline for 48 h.[14] Thiophenfurin was used as a reference compound. Contrary to what Makara and Keserű have predicted,[19] imidazofurin proved to be nontoxic to cell growth (no growth inhibition observed at 100 μM) as compared with thiophenfurin (IC_{50} = 4.6 μM). The poor activity of imidazofurin might be due to its inability to be phosphorylated by cellular kinases and nucleotidases, inability to be converted to the dinucleotide analogue of NAD, or failure of the dinucleotide to bind to the target. We believe that the presence of imidazole annular prototropic tautomerism could destabilize the conformation of imidazofurin suitable to bind the enzyme. Makara and Keserű carried out their ab initio computations taking into consideration only the tautomeric form **1b**. Thus, the contribution of tautomer **1a** was neglected.

Acknowledgements

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22. Selected data for **1**: TLC ($CHCl_3$–MeOH, 8:2): R_f 0.12; ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.52 (m, 1H, H5′b); 3.62 (m, 1H, H5′a); 3.75 (m, 1H, H4′); 3.90 (m, 2H, H2′ and H3′); 4.35 (two overlapping d, J = 5.5 Hz, 1H, H1′); 4.90 (m, 1H, OH); 5.15 (m, 1H, OH); 5.25 (pseudo d, 1H, OH); 7.10, 7.39 (2 s, 1H, H5), 7.32, 7.58 (2 br s, 2H, NH_2); 10.55, 10.70 (2 br s, 1H, NH). Positive ion electrospray MS (M + H) = 244.1. Anal. calcd for $C_9H_{13}N_3O_5$: C, 44.45; H, 5.39; N, 17.28. Found: C, 44.32; H, 5.13; N, 17.15.
23. Selected data for **6**: TLC ($CHCl_3$–MeOH, 9:1): R_f 0.56; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.25 (t, J = 7.0 Hz, 3H, CH_3); 3.49 (dd, J = 4.3, 11.9 Hz, 1H, H5′b); 3.63 (dd, J = 3.7, 11.9 Hz, 1H, H5′a); 3.81 (pseudo q, J = 4.2 Hz, 1H, H4′); 3.95 (pseudo q, J = 5.5 Hz, 1H, H3′); 4.15 (pseudo q, J = 5.0 Hz, 1H, H2′); 4.22 (q, J = 7.2 Hz, 2H, CH_2CH_3); 4.69 (d, J = 5.5 Hz, 1H, H1′); 4.98 (d, J = 5.3 Hz, 1H, OH3′); 5.22 (d, J = 5.6 Hz, 1H, OH2′); 7.80 (br s, 1H, H5). Positive ion electrospray MS (M + H) = 226.0. Anal. calcd for $C_{11}H_{14}N_2O_5$: C, 51.97; H, 5.55; N, 11.02. Found: C, 51.92; H, 5.85; N, 10.98.
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