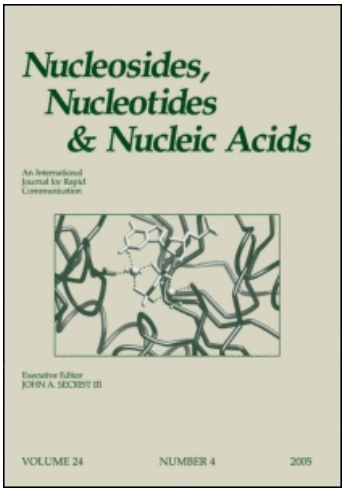

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RIBOSE-MODIFIED MIZORIBINE ANALOGUES: Synthesis and Biological Evaluation

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□ *Synthesis, conformational analysis and antitumor evaluation of 2′- and 3′-C-methyl analogues of mizoribine (bredinine, 4-carbamoyl-1-β-D-ribofuranosylimidazole-5-olate) are reported.*

Keywords Mizoribine analogues; IMPDH inhibitors; Antitumor agents

Mizoribine (bredinine, 4-carbamoyl-1-β-D-ribofuranosylimidazolium-5-olate, MZR), an imidazole nucleoside isolated from *Eupenicillium brefeldianum*,[1] is clinically used as immunosuppressive agent to inhibit rejection reactions after renal transplantation and in the treatment of lupus nephritis and rheumatoid arthritis.[2] Mizoribine also displayed tumor cells growth inhibition[3] and antiviral activity.[4] The biological activities of mizoribine are due to the ability of its metabolite mizoribine 5′-monophosphate (MZR-5′-P) to inhibit IMP dehydrogenase (IMPDH) and guanosine 5′-monophosphate synthetase (GMP-synthetase).[2] Both enzymes are required for the *de novo* biosynthesis of GMP starting from IMP. Thus, MZR-5′-P almost completely inhibits guanine nucleotide synthesis, which may explain one of the significant immunosuppressive roles of mizoribine, inhibitory effects for T cells and B cells proliferation.

Recently, it was ascertained that MZR-5′-P behaves as a transition-state analogue inhibitor able to bind the active site of the enzyme adopting the

Dedicated to the Memory of Dr. John A. Montgomery.

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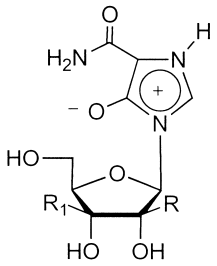
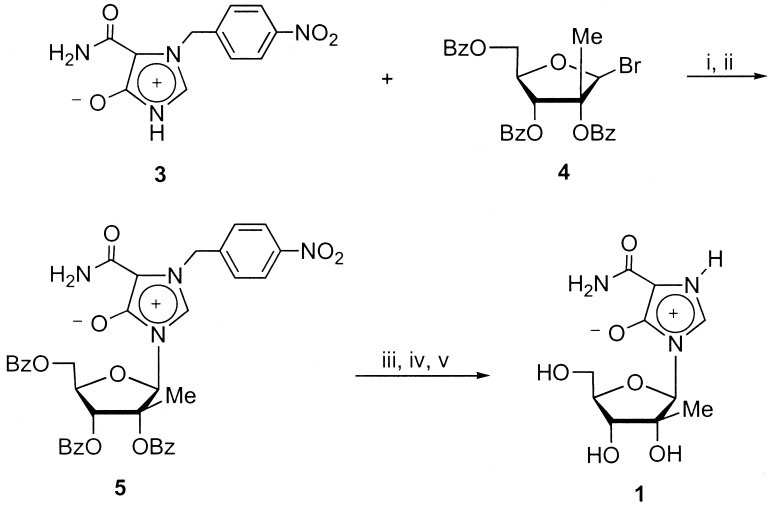


Mizoribine (MZR) R, R_1 = H
2'-Me-MZR (**1**) R = CH_3, R_1 = H
3'-Me-MZR (**2**) R = H, R_1 = CH_3

C3′-*endo* ribosyl pucker as the substrate xantosine monophosphate (XMP).[5] In this paper, we report on the synthesis and the biological evaluation of mizoribine analogues in which the ribose moiety was modified by substitution of the hydrogen atoms in 2′- or 3′-position with a methyl group (2′-Me-MZR and 3′-Me-MZR). These modifications are able to stabilize the ribose conformation into the C3′-*endo* and C2′-*endo* forms, respectively.[6]

CHEMISTRY

The 2′-*C*-methyl analogue of mizoribine (2′-Me-MZR, **1**) was synthesized as reported in Scheme 1. Glycosylation of silylated derivative of 3-(4-nitrobenzyl)-5-carbamoylimidazolium-4-olate (**3**)[7] with 1-bromo-2,3,5-tri-*O*-benzoyl-2-*C*-methyl-β-D-ribofuranose (**4**) in the presence of HgO and $HgBr_2$ gave the nucleoside derivative **5**. The deprotection of nucleobase

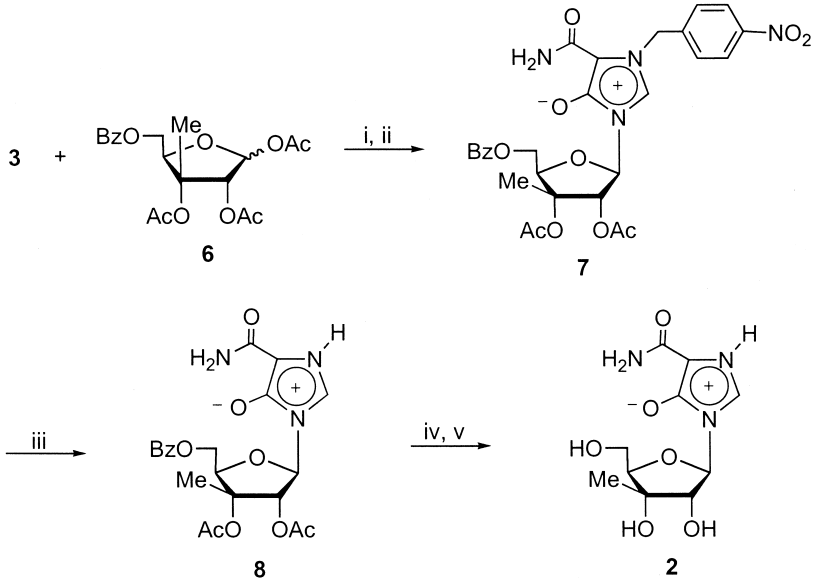


SCHEME 1 i) HMDS, $(NH_4)_2SO_4$ a., xylene a.; ii) HgO, $HgBr_2$, $ClCH_2CH_2Cl$ a.; iii) $HCOONH_4$, 10% Pd/C, MeOH a.; iv) MeONa, MeOH a.; v) Dowex 50W-X8, H^+ form.

of **5** and the debenzoylation gave the desired product 4-carbamoyl-1-(2-*C*-methyl-β-D-ribofuranosyl)imidazolium-5-olate (**1**).

The 3′-*C*-methyl analogue of mizoribine (3′-Me-MZR, **2**) was obtained as reported in Scheme 2. Condensation of **3** with 1,2,3-tri-*O*-acetyl-5-*O*-benzoyl-3-*C*-methyl-D-ribofuranose (**6**)[8] in the presence of TMSOTf and stannic chloride gave the nucleoside **7**. Removal of the nitrobenzyl group and deblocking of **8** under basic conditions gave the desired product 4-carbamoyl-1-(3-*C*-methyl-β-D-ribofuranosyl)imidazolium-5-olate (**2**) in high yield. The same procedure, starting from **3** and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, was followed to obtain mizoribine,[7] which was used as reference product for biological assays. Structural assignments for compounds **1** and **2** were based on elemental analysis and ^{1}H NMR and mass spectrometric data.

Proton nuclear magnetic resonance (^{1}H NMR) data and nuclear Overhauser enhancement (NOE) experiments were employed to determine the predominant conformation of mizoribine and its 2′- and 3′-*C*-methyl analogues in solution. NOE effects in compounds were determined in D_2O. The H-2′ enhancement when H-2 proton in MZR and 3′-Me-MZR was irradiated (2.6 and 4.5%, respectively) supports a spatial arrangement where H-2 of nucleobase and H-2′ are proximate, as would be the case in the *syn* conformers. The irradiation of H-2 proton in 2′-Me-MZR showed the H-3′ enhancement (3.6%) in the ribose ring supporting a spatial arrangement where H-2 and H-3′ protons are proximate, and therefore this nucleoside



SCHEME 2 i) HMDS, $(NH_4)_2SO_4$ a., xylene a.; ii) TMSOTf, $SnCl_4$, $ClCH_2CH_2Cl$ a.; iii) $HCOONH_4$, 10% Pd/C, MeOH a.; iv) MeONa, MeOH a.; v) Dowex 50W-X8, H^+ form.

prefers the *anti* conformation. Further information concerning the solution conformation was obtained by the coupling constant values. The 3′-*C* substitution precludes getting information from the $J_{3'4'}$ value leaving only the $J_{1'2'}$ as a clue to the sugar puckering. It was found that in 3′-Me-MZR the $J_{1'2'}$ value was 8.2 Hz, indicating that this nucleoside is predominantly *S*-puckered (C2′-*endo*).

It was impossible to establish the pucker conformation of the sugar of 2′-Me-MZR on the basis of $J_{3'4}$ owing the overlapping of H-3′ and H-4′ signals. However, it is well known that 2′-*C*-methyl-ribonucleosides are predominantly *N*-puckered (C3′-*endo*).[6]

BIOLOGICAL EVALUATION

Mizoribine and its 2′- and 3′-*C*-methyl derivatives **1** and **2** were evaluated for their ability to inhibit the replication of human myelogenous leukemia K562. K562 cells (1×10^5 cells/ml) were cultured in 0.1 ml volume in 96 well plates. Twenty-four hours later, cells were incubated with various concentrations of agents in 3 ml volume in triplicates for 72 h and the cell proliferation was determined by using MTS assay. Surprisingly, mizoribine and its analogues **1** and **2** proved to be inactive against this type of tumor cells up to 100 μM concentration. The inactivity of these nucleosides might be due to their inability to be converted into the corresponding 5′-monophosphate in K562 cells. Further experiments are underway in order to check this hypothesis.

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REFERENCES

1. Mizuno, K.; Tsujino, M.; Takada, M.; Hayashi, M.; Atsumi, K.; Asano, K.; Matsuda, T. Studies on bredinine: I. Isolation, characterization and biological properties. J. Antibiot. **1974**, 27, 775–782.
2. Ishikawa, H. Mizoribine and mycophenolate mofetil. Curr. Med. Chem. **1999**, 6, 575–597.
3. (a) Shuto, S.; Itoh, H.; Endo, E.; Fukukawa, K.; Tsujino, M.; Ueda, T. Synthesis of bredinin 5′-alkylphosphates involving photochemical manipulation of the imidazole moiety, and their antitumor activities. Chem. Pharm. Bull. **1987**, 35, 3523–3526. (b) Yoshida, N.; Nakamura, M.; Fukui, M.; Morisada, S.; Ogino, S.; Inaba, M.; Tsukagoshi, S.: Sakurai, Y. Optimal treatment schedule and antitumor spectrum of 4-carbamoylimidazolium 5-olate (SM-108) in murine tumors. Cancer Res. **1983**, 43, 5851–5856.

4. Kosugi, Y.; Saito, Y.; Mori, S.; Watanabe, J.; Baba M.; Shigeta, S. Antiviral activities of mizoribine and other inosine monophosphate dehydrogenase inhibitors against several ortho- and paramyxoviruses. Antiviral Chem. Chemother. **1994**, 5, 366–371.
5. Gan, L.; Seyedsayamdost, M.R.; Shuto, S.; Matsuda, A.; Petsko, G.A.; Hedstrom, L. The immunosuppressive agent mizoribine monophosphate forms a transition state analogue complex with inosine monophosphate dehydrogenase. Biochemistry **2003**, 42, 857–863.
6. Franchetti, P.; Cappellacci, L.; Marchetti, S.; Trincavelli, L.; Martini, C.; Mazzoni, M.R.; Lucacchini, A.; Grifantini, M. 2′-*C*-Methyl analogues of selective adenosine receptor agonists: Synthesis and binding studies. J. Med. Chem. **1998**, 41, 1708–1715.
7. Tarumi, Y.; Takebayaschi, Y.; Atsumi, T. Studies on imidazole derivatives and related compounds. **4.** Synthesis of *O*-Glycosides of 4-Carbamoylimidazolium-5-olate. J. Heterocyclic Chem. **1984**, 21, 849–854.
8. Franchetti, P.; Cappellacci, L.; Pasqualini, M.; Petrelli, R.; Vita, P.; Jayaram, H.N.; Horvath, Z.; Szekeres, T.; Grifantini, M. Antitumor activity of *C*-methyl-β-D-ribofuranosyladenine nucleoside ribonucleotide reductase inhibitors. J. Med. Chem. **2005**, 48, 4983–4989.