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SYNTHESIS OF *C*-OXETANOSYL-THIAZOLE AND ITS CARBOCYCLIC ANALOG NUCLEOSIDES AS POTENTIAL CHEMOTHERAPEUTIC AGENTS

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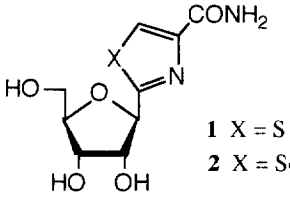
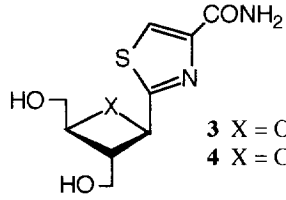
Abstract: Novel *C*-nucleosides (2'*S*, 3'*S*)-2-[2', 3'-(bishydroxymethyl)-oxetan-1'-yl]-1,3-thiazole-4-carboxamide **3** and its carbocyclic analog **4** have been synthesized. Compound **4** enhanced NGF-induced differentiation on PC12 cells at 100 μg/ml, and moreover, **4** alone also induced morphological differentiation significantly at 10 and 100 μg/ml.

Inhibitors of inosine monophosphate dehydrogenase (IMPD, IMP:NAD^+ oxidoreductase, EC 1.1.1.205), a rate-limiting enzyme in guanine nucleotide biosynthesis, have been shown to possess the ability to induce differentiation in neoplastic cells,[1] to inhibit the G protein-mediated cellular signaling mechanism,[2] and to downregulate oncogene activity.[3] Amongst the synthetic *C*-nucleosides, tiazofurin (NSC-286193, Tiazole™) **1**, 2-(β-D-ribofuranosyl)-1,3-thiazole-4-carboxamide[4] and selenazofurin **2** (NSC-340847)[5] are potent inhibitors of this enzyme.[6] In sensitive cells, both compounds are anabolized to thiazole-4-carboxamide adenine dinucleotide (TAD) and selenazole-4-carboxamide adenine dinucleotide (SAD), respectively, analogs of the coenzyme nicotinamide adenine dinucleotide (NAD), and these dinucleotides act as an inhibitor of the target enzyme IMPD.[7] Tiazofurin **1** has demonstrated potent antitumor activity against several tumor cell lines *in vivo* and the clinical trials of **1**, alone or in combination with other agents such as paclitaxel and ribavirin, are underway in patients with myelogenous leukemia, ovarian cancer, and breast cancer.[8] In continuation of our studies on the preparation and antiviral and antitumor evaluations of analogs of the unusual antiviral antibiotic nucleoside oxetanocin-A, we set ourselves the targets of preparing (2'*S*, 3'*S*)-2-[2', 3'-(bishydroxymethyl)-oxetan-1'-yl]-1,3-thiazole-4-carboxamide **3** and its carbocyclic analog **4** aiming at IMPD. In this report we would like to describe the synthesis and the biological activities of **3** and **4**.



1 X = S Tiazofurin
2 X = Se Selenazofurin

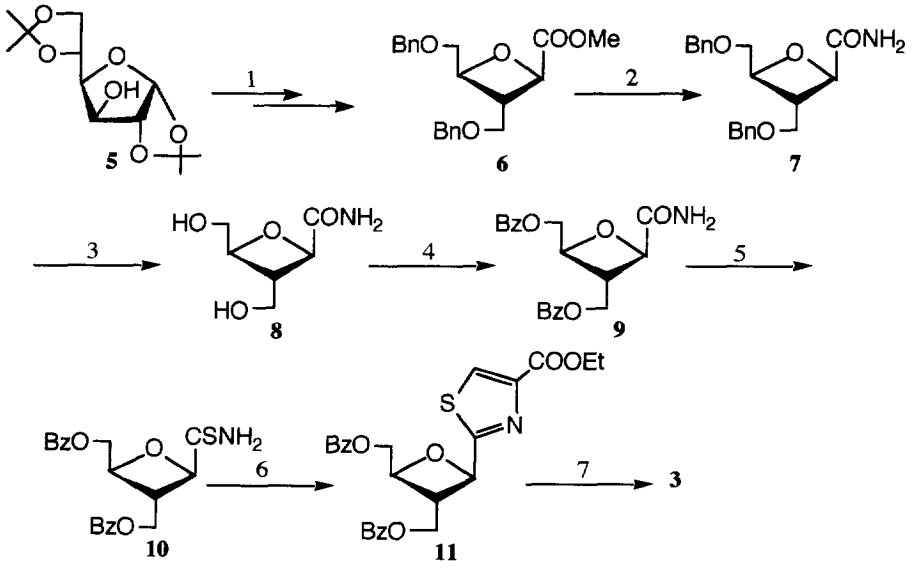


3 X = O
4 X = CH_2

Computational studies: Recently, Goldstein and co-worker suggested that the presence of an attractive interaction between the thiazole sulfur and furanose oxygen O_1' would have important implications for drug

binding and activity, i. e. the observed S···O_1' distance from the crystallographic and computational studies of tiazofurin is 2.96 Å and is less than the sum of the sulfur and oxygen van der Waals radii (3.3 Å).[9] In the case of **3**, the corresponding S···O_1' distance was calculated to be 4.11 Å on the basis of the most stable conformer and the weighted mean distance was also estimated to be 3.78 Å based on relative population of each conformer because of a low energy barrier (ca. 1.8 kcal/mol).[10]

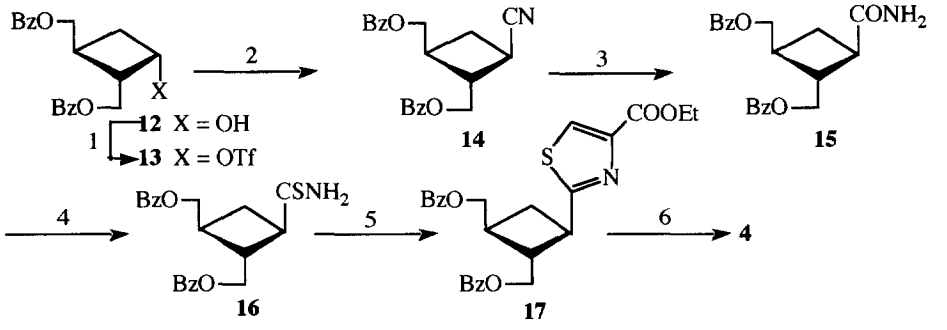
Chemistry: As shown in Scheme 1, the synthesis of the optically active oxetanosyl-thiazole **3** began with the chiral oxetane-ester **6** prepared from diacetone D-glucose **5** in 15 steps.[11,12] Treatment of **6** thus obtained with NH_3 in MeOH gave the amide **7** in 89 % yield. Initial attempts to convert amide group of **7** into thioamide group with P_2S_5 and Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide] under various conditions were unsuccessful just affording the complex compounds. Alternatively, hydrogenolysis of **7** over 10 % Pd on carbon to the diol **8** followed by benzoylation provided the dibenzoate **9** in 81 % yield in two steps. Compound **9** was readily reacted with Lawesson's reagent to afford the thioamide **10** in 48 % yield. Then, reaction of **10** with ethyl bromopyruvate in EtOH gave smoothly the thiazole **11** in 68 % yield. Finally, amination of compound **11** in saturated methanolic ammonia afforded the target compound **3** in 94 % yield.[15]



Scheme 1 *Reagents and Conditions*: 1) 15 steps. See ref.11 and 12; 2) NH_3, MeOH, rt, 15 h; 3) H_2, 10 % Pd on Carbon, MeOH, rt, 15 h; 4) PhCOCl, pyridine, rt, 15 h; 5) Lawesson's reagent (1.0 eq), pyridine (0.1 eq), dioxane, 65 °C, 2 h; 6) $BrCH_2COCOOEt$, EtOH, reflux, 2 h; 7) NH_3, MeOH, sealed tube, rt, 15 h.

Synthesis of the optically active carbocyclic-thiazole **4** started from the known chiral cyclobutyl alcohol **12**[13] as shown in Scheme 2. Reaction of **12** with trifluoromethanesulphonic anhydride and pyridine at -40 °C yielded the triflate **13**, which was used to the next reaction without purification because of its instability. Replacement reaction of compound **13** with LiCN in DMF-HMPA at -40 °C gave the desirable nitrile **14** in 55 % yield in two

steps.[14] Hydrolysis of the cyano group of **14** into the amide **15** was successfully accomplished by the treatment with 98 % formic acid saturated with HCl gas in 97 % yield. Compound **15** was reacted with Lawesson's reagent to afford the thioamide **16** in 77 % yield, which reacted with ethyl bromopyruvate in EtOH to give the carbocyclic-thiazole **17** in 85 % yield. After amination of compound **17** in saturated methanolic ammonia, the target compound **4** could be obtained in 96 % yield.[16]



Scheme 2 *Reagents and Conditions*: 1) Tf_2O, pyridine, -40 °C, 2 h ; 2) LiCN, DMF, HMPA, -40 °C, 1 h; 3) HCl gas, 98 % HCOOH, rt, 4 h; 4) Lawesson's reagent (1.0 eq), pyridine (0.1 eq), dioxane, 65 °C, 2 h; 5) $BrCH_2COCOOEt$, EtOH, reflux, 2 h; 6) NH_3, MeOH, sealed tube, rt, 15 h.

Biological activities: Induction of morphological differentiation by compound 4 in PC12 cells

The signal transduction through NGF receptor to induce differentiation in rat pheochromocytoma PC12 cells is known to include c-Ras function, since microinjection of anti-Ras inhibits NGF-induced differentiation in PC12 cells.[17] We examined the effects of compounds **1**, **3**, and **4** on the differentiation of PC12 cells either in combination with NGF or alone. As shown in Fig. 1, **1** inhibited NGF-induced differentiation at 1-100 μg/ml, as expected. Compound **3** showed no effect even at 100 μg/ml either with NGF or alone. Unexpectedly, compound **4** enhanced NGF-induced differentiation at 100 μg/ml, and moreover, **4** alone also induced morphological differentiation significantly at 10 and 100 μg/ml. Both **3** and **4** did not inhibit IMPD (data not shown), and the mechanism of differentiation induced by **4** remains to study.[18]

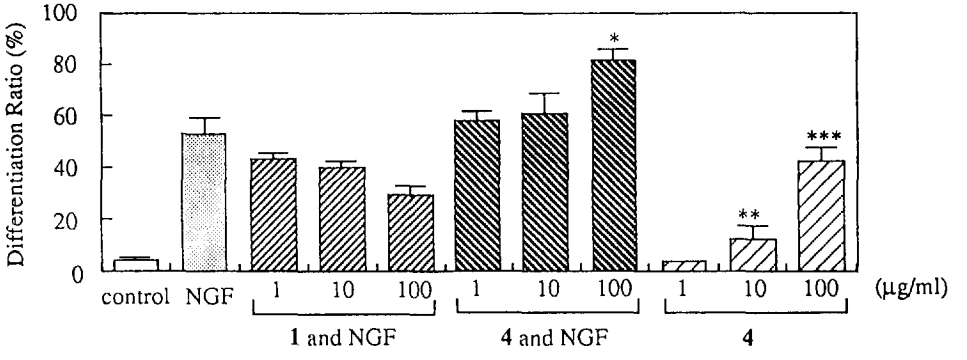


Fig. 1 Induction of morphological differentiation by **4** in PC12h cells. The cells were incubated for 48 hours in the medium containing 0.2 % semifetal bovine serum with chemicals and/or 50 ng/ml of NGF. The cells having neurites longer than 1.5 times of cell diameter were scored positive.

*P < 0.01 against NGF alone, **P < 0.05 against none, ***P < 0.001 against none.

In summary, we have developed the synthesis of novel *C*-nucleosides (2'*S*, 3'*S*)-2-[2', 3'-(bishydroxymethyl)-oxetan-1'-yl]-1,3-thiazole-4-carboxamide **3** and its carbocyclic analog **4**. Although both compounds did not inhibit inosine monophosphate dehydrogenase, compound **4** enhanced NGF-induced differentiation on PC12 cells, and moreover, **4** alone also induced significant morphological differentiation. Compound **4** should be further pursued for its therapeutic potential as a new differentiation inducer.

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References and Notes:

1. Goldstein, B. M.; Leary, J. F.; Farley, B. A.; Marquez, V. E.; Rowley, P. T. *Blood* **1991**, *78*, 593-598.
2. Parandoosh, Z.; Rubalcava, B.; Matsumoto, S. S.; Jolley, W. B.; Robins, R. K. *Life Sci.* **1990**, *46*, 315-320.
3. Kharbanda, S. M.; Sherman, M. L.; Springgs, D. R.; Kufe, D. W. *Cancer Res.* **1988**, *48*, 5965-5968.
4. Srivastava, P. C.; Pickerling, M. V.; Allen, L. B.; Streeter, D. G.; Campbell, M. T.; Witkowski, J. T.; Sidwell, R. W.; Robins, R. K. *J. Med. Chem.* **1977**, *20*, 256-262.
5. Srivastava, P. C.; Robins, R. K. *J. Med. Chem.* **1983**, *26*, 445-448.
6. Jayaram, H. N.; Smith, A. L.; Glazer, R. I.; Johns, D. G.; Cooney, D. A. *Biochem. Pharmacol.* **1982**, *31*, 3839-3845. Jayaram, H. N.; Ahluwaria, G. S.; Dion, R. L.; Gebeyehu, G.; Marquez, V. E.; Kelley, J. A.; Robins, R. K.; Cooney, D. A.; Johns, D. G. *Biochem. Pharmacol.* **1983**, *32*, 2633-2636.
7. Kuttan, R.; Robins, R. K.; Saunders, P. P. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 862-868. Cooney, D. A.; Jayaram, H. N.; Gebeyehu, G.; Betts, C. R.; Kelley, J. A.; Marquez, V. E.; Johns, D. G. *Biochem. Pharmacol.* **1982**, *31*, 2133-2136. Li, H.; Hallows, W. H.; Punzi, J. S.; Marquez, V. E.; Carrell, H. L.; Pankiewicz, K. W.; Watanabe, K. A.; Goldstein, B. M. *Biochemistry* **1994**, *33*, 23-32.
8. Yeh, Y. A.; Olah, E.; Wendel, J. J.; Sledge, G. W. Jr.; Weber, G. *Proc. Amer. Assoc. Cancer Res.* **1994**, *35*: Abst. 1978.
9. Burling, F. T.; Goldstein, B. M. *J. Am. Chem. Soc.* **1992**, *114*, 2313-2320.
10. This calculation was carried out using the same program (MOPAC-MNDO) as cited in the reference 9.
11. Witty, D. R.; Fleet, G. W. J.; Choi, S.; Vogt, K.; Wilson, F. X.; Wang, Y.; Storer, R.; Myers, P. L.; Wallis, C. J. *Tetrahedron Lett.* **1990**, *47*, 6927-6930.
12. Izawa, T.; Nakayama, K.; Nishiyama, S.; Yamamura, S.; Kato, K.; Takita, T. *J. Chem. Soc., Perkin Trans. 1.* **1992**, 3003-3004.
13. Ichikawa, Y.; Narita, A.; Shiozawa, A.; Hayashi, Y.; Narasaka, K. *J. Chem. Soc., Chem. Commun.* **1989**, 1919-1921. Ahmad, S. *Tetrahedron Lett.* **1991**, *32*, 6997-7000.
14. Several attempts of the substitution reaction of the stable mesylate of the alcohol **14** with cyanide ion under various conditions were unsuccessful.
15. Selected spectroscopic data for **3**: colorless foam, $[\alpha]^{21}_D$ +45° (*c* 0.64, H_2O); λmax (H_2O) 238 nm (ε 10,100); ^1H NMR (400 MHz, D_2O) δ 3.07 (1H, m), 3.65 (2H, d, J=3.9Hz), 3.73 (1H, dd J=6.4 and 12.0Hz), 3.77 (1H, dd, J=6.4 and 12.0Hz), 4.69 (1H, ddd, J=6.4, 6.4 and 6.6Hz), 5.64 (1H, d, J=6.4Hz) and 8.11 (1H, s); ^{13}C NMR (100.5 MHz, D_2O) δ 45.9, 61.1, 64.3, 79.1, 83.2, 127.5, 149.0, 166.3 and 173.8 ; HRMS m/z 245.0594 calcd for $C_9H_{13}N_2O_4S$ (M^++1) , found 245.0583.
16. Selected spectroscopic data for **4**: colorless foam, $[\alpha]^{21}_D$ -43° (*c* 0.78, EtOH); λmax (EtOH) 237 nm (ε 7,900); ^1H NMR (400 MHz, CD_3OD) δ 2.08 (1H, q, J=9.0Hz), 2.33 (1H, m), 2.45 (1H, q, J=9.0Hz), 2.55 (1H, m), 3.52-3.61 (3H, complex), 3.63 (1H, dd, J=6.0 and 14.0Hz), 3.67 (1H, dd, J=6.0 and 14.0Hz) and 8.08 (1H, s); ^{13}C NMR (100.5 MHz, CD_3OD) δ 29.7, 37.7, 38.1, 49.8, 64.4, 65.7, 124.8, 150.5, 165.8 and 176.1; HRMS m/z 242.0724 calcd for $C_{10}H_{14}N_2O_3S$, found 242.0715.
17. Hagag, N.; Halegoura, S.; Viola, M. *Nature* **1986**, *319*, 680.
18. Evaluation of compounds **3** and **4** against HSV-1 and HSV-2 in Vero cells by a plaque reduction assay at concentrations up to 100 μg/ml revealed these compounds to be devoid of antiviral activity and cytotoxicity.

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