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De Novo Synthesis of Two New Cytotoxic Tiazofurin Analogues with Modified Sugar Moieties

Mirjana Popsavin,[a,*] Ljilja Torović,[a] Vesna Kojić,[b] Gordana Bogdanović,[b] Saša Spaić[a] and Velimir Popsavin[a]

[a]*Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 3, 21000 Novi Sad, Serbia and Montenegro, Yugoslavia*

[b]*Institute of Oncology Sremska Kamenica, Institutski put 4, 21204 Sremska Kamenica, Serbia & Montenegro, Yugoslavia*

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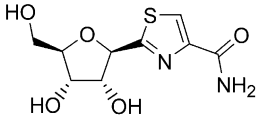
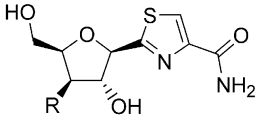
Abstract—A divergent synthesis of two novel tiazofurin analogues, 2-(3-deoxy-3-fluoro-β-D-xylofuranosyl)thiazole-4-carboxamide (**2**) and 2-(3-acetamido-3-deoxy-β-D-xylofuranosyl)thiazole-4-carboxamide (**3**), has been achieved starting from D-glucose. Both nucleoside analogues were evaluated for their in vitro cytotoxicity against several human leukaemia and solid tumour cell lines.

Tiazofurin (**1**) is a thiazole C-nucleoside that exhibits a potent cytotoxic activity against a number of malignant cells.[1] Tiazofurin is currently in phase II/III clinical trials, but it has been recently approved as an orphan-drug for the treatment of chronic myelogenous leukaemia in accelerated phase or blast crisis. The biological activity of tiazofurin is attributed to its ability to reduce intracellular guanine nucleotide pools via inhibition of inosine monophosphate dehydrogenase (IMPDH, EC 1.1.1.205).[2] The inhibition of IMPDH induced by tiazofurin is due to the tiazofurin metabolite, thiazole-4-carboxamide adenine dinucleotide (TAD). This active metabolite inhibits IMPDH activity through competition for the NAD cofactor-binding site of the enzyme.[3] Two IMPDH isoforms (type I and II) were identified, of which type II is up-regulated in human leukaemia cells,[4] as well as in some solid tumour cell lines.[5] These findings were prompted studies towards the design of isoform selective inhibitors,[6] and renewed interest in the synthesis of tiazofurin[7] and its analogues, particularly those with modified heterocyclic aglycons.[8] Structural modifications of the ribofuranosyl moiety have also been reported including preparation of 5′-, 3′-, or 2′-substituted derivatives,[9] xylo- and arabinofuranosyl,[10] pyranosyl,[11] acyclic,[12] carbocyclic[13] and 4′-aza-sugar[14] analogues. However, none of these substances was shown any significant biological activity. In this paper, we wish to report on the synthesis of two novel tiazofurin analogues **2** and **3**, along with their effects on the proliferation of some malignant and normal cell lines.



Tiazofurin (**1**)



2 R = F
3 R = NHAc

Chemistry

The 2,5-anhydro-D-glucose derivative **4** (Scheme 1), earlier prepared in our laboratory starting from D-glucose,[15,16] has been used as a divergent intermediate for the synthesis of both tiazofurin analogues **2** and **3**. Reaction of **4** with triflic anhydride in pyridine and dichloromethane gave the corresponding triflic ester **5**, which was isolated in pure form (TLC, ^{1}H and ^{13}C NMR) after the usual workup and used in the next step without further purification. Subsequent treatment of **5** with tetrabutylammonium fluoride in THF afforded the corresponding 4-fluoro derivative **6**[17] as the only reaction product (67% from **4**). Hydrolytic removal of the dioxolane protective group in **6**, followed by subsequent reaction of the unstable aldehyde **7** with hydroxyl amine hydrochloride and sodium acetate, gave the oximino

*Corresponding author. Tel.: +38-121-350-122; fax: +38-121-454-065; e-mail: popsavin@ih.ns.ac.yu

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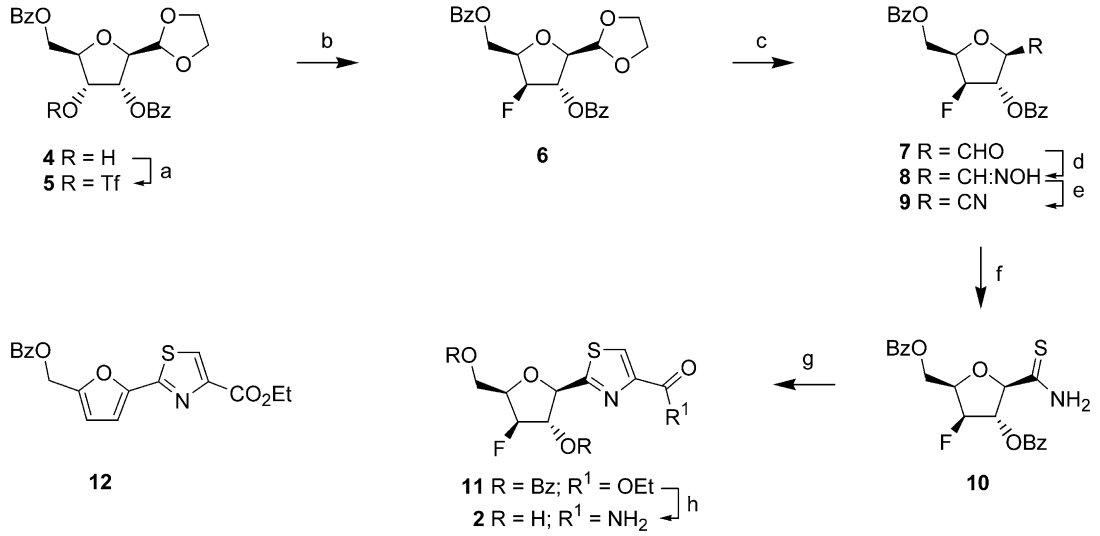
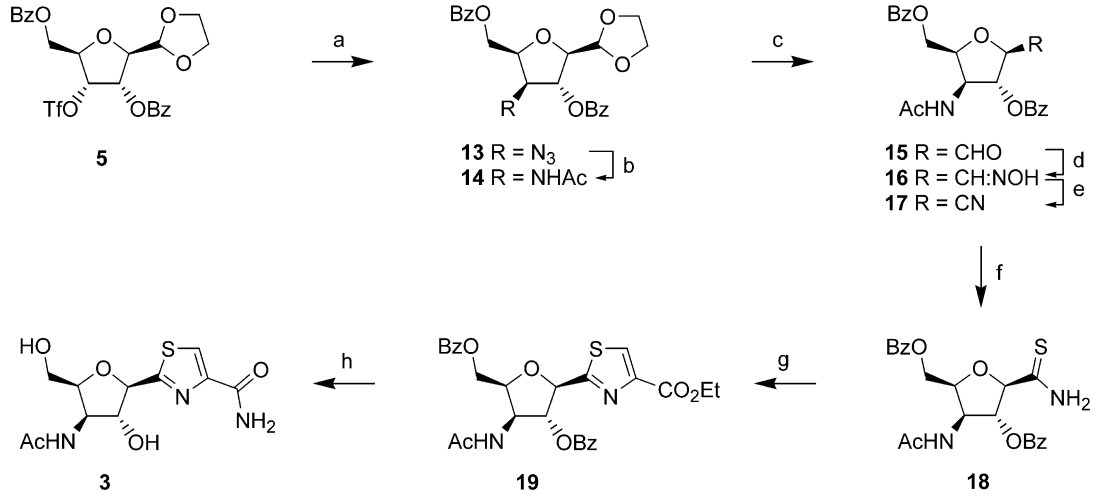


Scheme 1. Reagents and conditions: (a) Tf_2O, Py, CH_2Cl_2, −10 °C, 0.5 h, 0 °C, 0.5 h, rt, 0.5 h, 98%; (b) Bu_4NF, THF, −18 °C, 22 h, 68%; (c) 9:1 TFA–6 M HCl, 4 °C, 6 days; (d) $NH_2OH{\times}HCl$, NaOAc, EtOH, rt, 2 h, 75% from **6**; (e) MsCl, Py, −17 °C, 0.5 h then rt, 2 h, 78%; (f) H_2S, DMAP, EtOH, rt, 8 h, 89%; (g) $BrCH_2COCO_2Et$, EtOH, 80 °C, 50 min, 28.5%; (h) NH_3, MeOH, rt, 9 days, 91%.



Scheme 2. Reagents and conditions: (a) NaN_3, DMSO, THF, rt, 24 h, 50%; (b) PtO_2, Ac_2O, AcOH, rt, 21 h, 56%; (c) 9:1 TFA–6 M HCl, 4 °C, 6 days; (d) $NH_2OH{\times}HCl$, NaOAc, EtOH, rt, 2 h, then +4 °C, 70 h; (e) MsCl, Py, −15 °C, 0.5 h, then rt, 1.5 h, 77% from **14**; (f) H_2S, DMAP, EtOH, rt, 4 h, 81%; (g) $BrCH_2COCO_2Et$, EtOH, 80 °C, 50 min, 37%; (h) NH_3, MeOH, rt, 8 days, 93%.

derivative **8** as a mixture of the corresponding *E*- and *Z*-isomers (75% from **6**). Due to their similar chromatographic properties, the *E*- and *Z*-isomers **8** could not be separated by column chromatography, but were in the mixture further treated with mesyl chloride in pyridine to afford the corresponding nitrile **9**. Treatment of **9** with hydrogen sulphide, in the presence of DMAP as a proton acceptor furnished the corresponding thioamide **10** (69% from **8**). The Hantzsch reaction of **10** with ethyl bromopyruvate gave the expected thiazole **11** (29%), accompanied with a similar amount of the aromatised product **12**. The pure product **11** was isolated by silica gel column chromatography and treated with saturated ammonia in dry methanol to afford the corresponding nucleoside **2**[18] (91%) ready for biological testing.

Synthesis of the tiazofurin analogue **3** bearing the 3′-acetamido group as an isostere is outlined in Scheme 2. The triflic ester **5** reacted with sodium azide in dimethyl sulfoxide and tetrahydrofuran to afford the known[16] azido derivative **13**, with physical and spectral data in excellent agreement with those already reported.[16] Catalytic hydrogenation of **13** over PtO_2 in a mixture of glacial acetic acid and acetic anhydride gave the acetamido derivative **14**,[19] a key intermediate in the planned synthesis of **3**. The intermediate **14** was converted to the final product **3**[20] by using the same six-step sequence already applied for the conversion of **6** into the C-nucleoside **2**. In this way the 2,5-anhydro derivative **14** has been transformed into the target molecule **3** in 21.5% overall yield.

Biological Evaluation

The synthesized tiazofurin analogues **2** and **3** were evaluated for their ability to inhibit the growth of

human myelogenous leukaemia K562, promyelocytic leukaemia HL60, colon adenocarcinoma HT29, breast adenocarcinoma MCF7, and normal foetal lung MRC-5 cells. Tiazofurin (**1**) was used as a reference compound. In vitro cytotoxicity was evaluated by the MTT assay[21] after incubation for 24 h. The results are presented in Table 1.

Compound **3** showed a potent cytotoxic activity against human myelogenous leukaemia K562, which was 44-fold higher than that observed for tiazofurin. Both C-nucleosides **2** and **3** exhibited a potent cytotoxic activity against human colon adenocarcinoma HT29, essentially the same as that exhibited by tiazofurin itself. Moreover, the 3′-fluoro derivative **2** showed a significant cytotoxicity against human promyelocytic leukaemia HL60 cell line, comparable to that of tiazofurin. Both C-nucleoside analogues **2** and **3** were inactive against breast adenocarcinoma MCF7 until a maximum tested concentration of 1000 μM. Compound **2** was shown to be inactive against normal foetal lung MRC-5 cell line, while the acetamido derivative **3** devoid of any significant cytotoxicity towards leukaemia HL60. Finally, compound **2** showed a weak cytotoxicity against K562 cells comparable to that exhibited by compound **3** against MRC-5 cell line. The biological data are somewhat surprising as there is large variation in the results for each cell line. Both **2** and **3** are generally either equipotent with tiazofurin (**1**), or completely inactive, depending on the cell line with the exception of compound **3** which is significantly more potent than tiazofurin (**1**), but only against K562. These data might suggest that compounds **2** and **3** are not acting at the same biological target as tiazofurin (**1**). If the compounds are acting on the same biological target as tiazofurin (IMPDH), then one should observe similar activity to tiazofurin for each cell line. In order to resolve this problem more work should be done including further biological evaluation of **2** and **3** for their ability to inhibit IMPDH activity.

In conclusion, we have synthesized two novel cytotoxic tiazofurin analogues bearing the 3′-fluoro (**2**), as well as the 3′-acetamido (**3**) function as an isostere. Compound **3** showed a potent in vitro cytotoxicity against K562 leukaemia cells, which was 44-fold higher than that observed for tiazofurin, an approved orphan-drug for treatment of certain human leukaemia. In addition, both **2** and **3** exhibited a potent cytotoxic activity against human colon adenocarcinoma HT29, comparable to that of tiazofurin. To the best of our knowledge compounds **2** and **3** represent the first biologically active tiazofurin analogues lacking the ribofuranosyl moiety, which is thought to be crucial for biological activity. Further work directed towards the synthesis of novel tiazofurin derivatives bearing modified sugar segments is currently underway.

Table 1. In vitro cytotoxicity of compounds **1–3**

Compd	IC_{50}, μM[a]				
	K562	HL-60	HT-29	MCF7	MRC-5
1	4.16	9.32	1.01	8.49	0.85
2	96.60	10.50	0.91	>1000	>1000
3	0.095	1000	1.00	>1000	89.70

[a]IC_{50} is a compound concentration required to inhibit the cell growth by 50% compared to an untreated control.

Acknowledgements

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17. Compound **6**: mp 72.5–73 °C (from CH_2Cl_2–pentane); $[\alpha]_D$ −22.19 (*c* 2.3 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.95–4.09 (m, 4H, 2×CH_2-dioxolane), 4.14 (dd, 1H, $J_{1,2}$ = 5.2, $J_{2,3}$ = 3 Hz, H-2); 4.49 (dddd, 1H, $J_{4,5}$ = 2.7, $J_{5,6a}$ = 5.8, $J_{5,6b}$ = 5.6, $J_{5,F}$ = 28.9 Hz, H-5), 4.64–4.74 (m, 2H, $J_{6a,6b}$ = 11.7, $J_{5,6a}$ = 5.8, $J_{5,6b}$ = 5.6, $J_{6a,F}$ = 1 Hz, 2×H-6), 5.16 (d, 1H, $J_{1,2}$ = 5.2 Hz, H-1), 5.19 (ddd, 1H, $J_{3,4}$ = 0.5, $J_{4,5}$ = 2.7, $J_{4,F}$ = 50.4 Hz, H-4), 5.69 (ddd, 1H, $J_{3,4}$ = 0.5, $J_{3,F}$ = 16.2, $J_{2,3}$ = 3 Hz, H-3), 7.43–8.15 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 61.53 (d, $^3J_{6,F}$ = 10.6 Hz, C-6), 65.42 and 65.52 (2×CH_2-dioxolane), 77.39 (d, $^2J_{3,F}$ = 31.4 Hz, C-3), 79.28 (d, $^2J_{5,F}$ = 19.5 Hz, C-5), 84.22 (C-2), 94.64 (d, $^1J_{4,F}$ = 188.5 Hz, C-4), 102.27 (C-1), 128.33, 128.51, 128.84, 129.56, 129.72, 129.77, 133.16 and 133.66 (2×Ph), 164.96 and 166.14 (2×C=O); CI MS: *m*/*z* 417 ($M^+ + 1$). Anal. found: C, 63.21; H, 5.05; calcd for $C_{22}H_{21}FO_7$: C, 63.46; H, 5.08.
18. Selected data for **2** (syrup): $[\alpha]_D$ −35.91 (c 1.04 in MeOH); ^{1}H NMR (methanol-d_4): δ 3.91 (pseudo d, 2H, $J_{4',5'}$ = 6.4 Hz, 2×H-5′), 4.38 (dtd, 1H, $J_{4',F}$ = 30.7, $J_{3',4'}$ = 2.4, $J_{4',5'}$ = 6.4 Hz, H-4′), 4.57 (ddd, 1H, $J_{1',2'}$ = 1.2, $J_{2',3'}$ = 0.6, $J_{2',F}$ = 13.9 Hz, H-2′), 4.97 (ddd, 1H, $J_{2',3'}$ = 0.6, $J_{3',4'}$ = 2.4, $J_{3',F}$ = 50.7 Hz, H-3′), 5.13 (d, 1H, $J_{1',2'}$ Hz, = 1.2, H-1′), 8.15 (s, 1H, H-5); ^{13}C NMR (methanol-d_4): δ 60.21 (d, $^3J_{5',F}$ = 10.7 Hz, C-5′), 81.72 (d, $^2J_{4',F}$ = 27 Hz, C-4′), 84.04 (d, $^2J_{2',F}$ = 19.4 Hz, C-2′), 86.56 (C-1′), 97.86 (d, $^1J_{3',F}$ = 184.8 Hz, C-3′), 125.62 (C-5), 150.88 (C-4), 165.72 (C-2), 173.73 (C=O); CI MS: *m*/*z* 263 ($M^+ + 1$).
19. Selected data for **14** (syrup): $[\alpha]_D$ +24.93 (*c* 4.35 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.04 (s, 3H, NHAc), 4.01–4.20 (m, 4H, 2×CH_2-dioxolane), 4.31 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 1.2 Hz, H-2), 4.44–4.63 (m, 3H, $J_{4,5}$ = 3.4 Hz, H-5 and 2×H-6), 4.88 (ddd, 1H, $J_{3,4}$ = 0.6, $J_{4,5}$ = 3.4, $J_{4,NH}$ = 9.5 Hz, H-4), 5.31 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1), 6.89 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NH), 7.39–8.17 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 23.24 (MeCO), 54.85 (C-4), 62.92 (C-6), 65.63 and 65.67 (2×CH_2-dioxolane), 78.57 (C-3), 79.07 (C-5), 84.02 (C-2), 102.08 (C-1), 128.30, 128.45, 128.96, 129.72, 133.03 and 133.56 (2×Ph), 165.38 and 166.22 (2×PhC=O), 169.25 (MeCO); HR MS: *m*/*z* 478.1472 (M^+ + Na); calcd for $C_{24}H_{25}NO_8Na$: 478.1478.
20. Selected data for **3** (syrup): $[\alpha]_D$ −3.93 (*c* 1.3 in MeOH); ^{1}H NMR (methanol-d_4): δ 1.97 (s, 3H, MeCO), 3.68 (dd, 1H, $J_{5'a,5'b}$ = 12.3, $J_{4',5'a}$ = 4.6 Hz, H-5′a), 3.79 (dd, 1H, $J_{5'a,5'b}$ = 12.3, $J_{4',5'b}$ = 3.5 Hz, H-5′b), 4.36 (t, 1H, $J_{1',2'}$ = 5.8, $J_{2',3'}$ = 5.7 Hz, H-2′), 4.40 (ddd, 1H, $J_{4',5'a}$ = 4.6, $J_{4',5'b}$ = 3.5, $J_{3',4'}$ = 6.6 Hz, H-4′), 4.47 (dd, 1H, $J_{2',3'}$ = 5.7, $J_{3',4'}$ = 6.6 Hz, H-3′), 4.98 (d, 1H, $J_{1',2'}$ = 5.8 Hz, H-1′), 8.23 (s, 1H, H-5); ^{13}C NMR (methanol-d_4): δ 22.52 (MeCO), 59.96 (C-3′), 61.88 (C-5′), 81.22 (C-4′), 81.81 (C-2′), 83.83 (C-1′), 125.86 (C-5), 150.80 (C-4), 165.66 (C-2), 172.60 ($CONH_2$), 173.75 (MeCO). Anal. Found: C, 44.02; H, 5.18; N, 13.67; S, 10.43; calcd for $C_{11}H_{15}N_3O_5S$: C, 43.85; H, 5.02; N, 13.95; S, 10.64.
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