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Synthesis and antitumour activity of new tiazofurin analogues bearing a 2,3-anhydro functionality in the furanose ring

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

[a]*Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 3, 21000 Novi Sad, Serbia*
[b]*Institute of Oncology Sremska Kamenica, Institutski put 4, 21204 Sremska Kamenica, Serbia*

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Abstract—This paper describes a divergent de novo synthesis of 2-(2,3-anhydro-β-D*ribo*furanosyl)thiazole-4-carboxamide (2′,3′-anhydro-tiazofurin) and the corresponding α- and β-homo-C-nucleosides, as well as evaluation of their antitumour activities in vitro.

Of the many classes of nucleoside analogues that have been synthesized for potential clinical applications, one of the most interesting is the class of C-nucleosides.[1] Remarkable among them is tiazofurin (**1**, Fig. 1), a synthetic C-nucleoside that shows antitumour activity in a variety of tumour systems.[2] In the phase II clinical trials, it induced haematological responses in patients with acute myelogenous leukaemia or chronic myeloid leukaemia in blast crisis.[3] The biological activity of tiazofurin derives from a combination of cytotoxicity and maturation-inducing activities.[4] Both effects are attributed to inhibition of inosine 5′-monophosphate dehydrogenase (IMPDH) by the tiazofurin adenine dinucleotide, which induces the shutdown of guanylate synthesis.[5] Despite the remarkable efficacy of **1**, lack of specificity and occasional toxicity remains a problem in its clinical use.[2,6] In order to provide an access to derivatives of reduced toxicity, a number of tiazofurin analogues have been synthesized and evaluated for their antitumour activities.[7] In the course of our recent programme directed towards total syntheses of tiazofurin analogues with modified sugar moieties, we have recently completed the synthesis of a cytotoxic β-D-ribofuranosyl-thiazole via the 2,5-anhydro derivative **4** (Scheme 1).[8] Compound **4** has not only the required β-configuration at the C-1′ position but also the functional groups suitable for introduction of diversity into the nucleoside carbohydrate segment. Therefore, our next endeavour was focused on further modifications of the intermediate **4** for synthesis of the hitherto unknown tiazofurin derivative **2** (2′,3′-anhydro-tiazofurin), as well as its homologue **3** having the 2′,3′-anhydro function in the furanose ring. Synthesis and biological activity of nucleoside analogues with 2′,3′-anhydrofuranosyl sugar moieties has been reported.[9] Some of the results indicate that 2′,3′-anhydro-nucleosides serve as DNA (or RNA) polymerase termination substrates, that might be of use for development of new antitumour agents. Herein, we report on the synthesis of tiazofurin

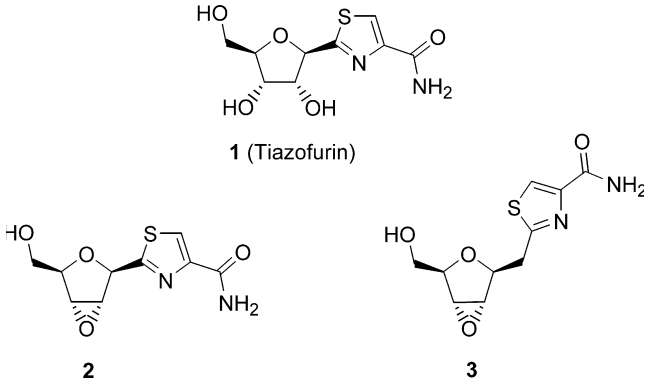

Figure 1. Tiazofurin (**1**) and the targeted analogues **2** and **3**.

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* Corresponding author. Tel.: +381 21 485 27 68; fax: +381 21 454 065; e-mail: mpopsavin@ih.ns.ac.yu

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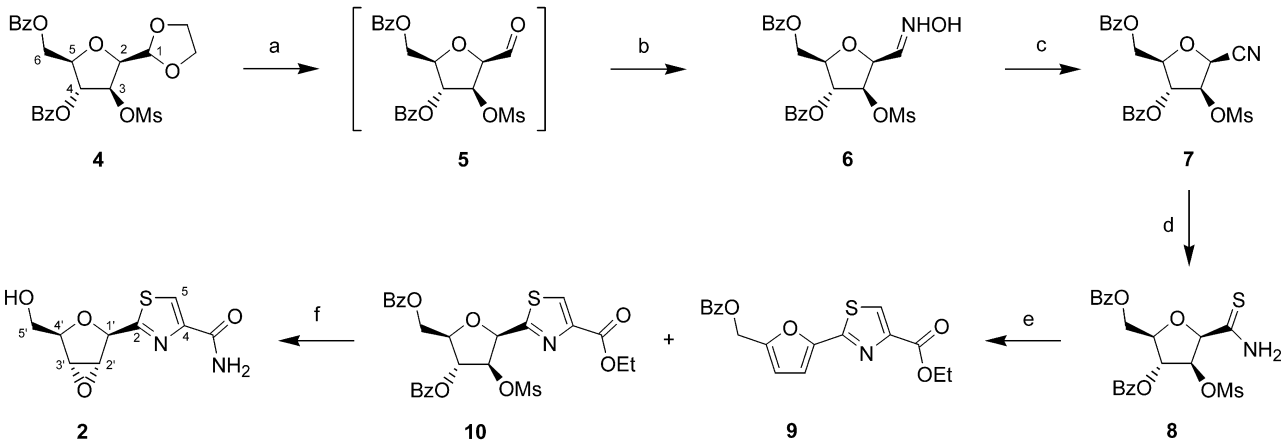


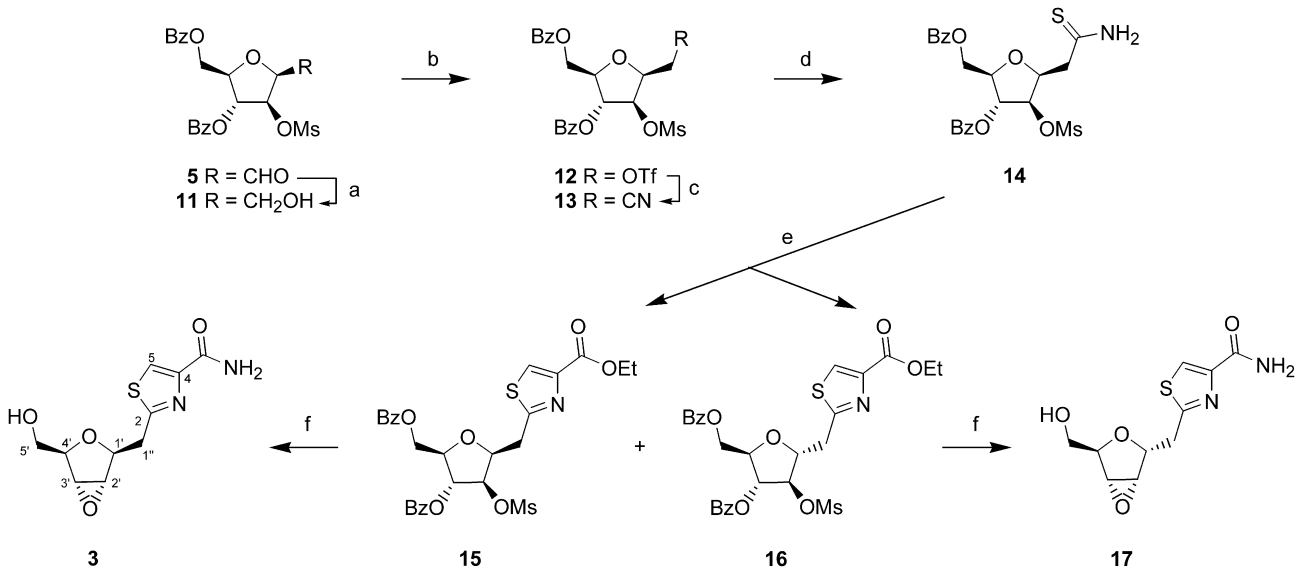
Scheme 1. Reagents and conditions: (a) 4:1, TFA, 6 M HCl, 4 °C, 140 h; (b) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, CH_2Cl_2, rt, 24 h; (c) MsCl, Py, −15 °C, 0.5 h, then rt, 2 h, 60% from **4**; (d) H_2S, Py, rt, 4 h, 90%; (e) $BrCH_2COCO_2Et$, EtOH, 80 °C, 50 min, 43% of **10**, 10% of **9**; (f) NH_3, MeOH, rt, 8 days, 60%.

analogues **2** and **3** along with their effects on the proliferation of selected human tumour cell lines.

The synthesis of **2** started with hydrolytic removal of the dioxolane protection in **4** that was achieved with 4:1 mixture of trifluoroacetic acid and 6 M hydrochloric acid at +4 °C. The resulting unstable aldehyde **5** was not purified, but was rather immediately treated with hydroxylamine hydrochloride to yield the corresponding oxime **6** as a mixture of the corresponding *E*- and *Z*-isomers. The mixture was not separated but was rather further treated with mesyl chloride in pyridine to give the corresponding nitrile **7** in 60% yield (with respect to starting compound **4**). Exposure of **7** to hydrogen sulfide gas gave the thioamide **8** (90%). The Hantzsch reaction of **8** with ethyl bromopyruvate afforded the corresponding thiazole **10** (43%), along with a minor amount of the aromatised product **9** (11%). Treatment of **10** with methanolic ammonia provided the target **2**[10] (60%) as a result of successive aminolysis of ester functions, followed by subsequent epoxide ring closure process. An efficient two-step hemisynthesis of 2′,3′-anhydro-ribavirin (a closely related analogue of **2**) has recently been described starting from ribavirin.[9b] We are looking towards application of this methodology for the preparation of **2** starting from tiazofurin. Regardless of biological activities of both analogues, compound **2** should have greater pharmacological potential due to a profound stability of the C-gycosidic bond in biological environments.

Synthesis of analogue **3** that represents a one-carbon homologue of **2** is outlined in Scheme 2. Compound **4** was hydrolysed under the same reaction conditions as described above, and the resulting crude aldehyde **5** was immediately treated with sodium borohydride in methanol. The corresponding primary alcohol **11** was thus obtained in 64% overall yield. Reaction of **11** with triflic anhydride in pyridine and dichloromethane gave the unstable triflic ester **12**, which was used in the next step immediately after its isolation from the reaction mixture. Treatment of crude **12** with NaCN (DMF, rt), or with KCN in the presence of benzo-15-crown-5 ether (MeCN, 0 °C), gave the heptononitrile **13** as the major reaction product (72–73% from **11**). The nitrile **13** was treated with hydrogen sulfide gas under the conditions similar to those already used for the preparation of **8**. However, the conversion of **13** to **14** required 14 days to be completed, whereby the desired thioamide **14** was obtained in 78% yield. The Hantzsch reaction of **14** with ethyl bromopyruvate in ethanol gave the thiazole **15** (32%), accompanied with a minor amount of the C-1′ epimer **16** (21%). The α-anomer **16** was presumably formed from **15** via a ring opening/ring closure process promoted by HBr, which was formed as a by-product in the Hantzsch reaction. Although the acid-catalysed anomerisation of some α-D-ribofuranosyl-C-nucleosides has been reported,[11] this is the first example of such a conversion involving a β-D-arabinofuranosyl-C-nucleoside. Stereochemistry of **15** and **16** was unambiguously resolved by NOE differential ^1H NMR spectroscopy. Designation of the β-anomer **15** was based upon observation of a NOE at H-1″ when H-5′ was irradiated. This effect was not observed in **16**, presumably the α-anomer. However, this stereoisomer exhibited a strong NOE between H-1″ and H-2′, thus implying a spatial vicinity of these protons. Such an arrangement is only possible if the isomer **16** represents the α-anomer. Both isomers **15** and **16** upon treatment with saturated ammonia in methanol gave the expected homo-C-nucleosides **3**[12] and **17**[13] in 60% and 65% yields, respectively.

Compounds **2**, **3** and **17** were evaluated for their in vitro cytotoxicity towards human myelogenous leukaemia K562, promyelocytic leukaemia HL-60, human T-cell leukaemia (Jurkat), human Burkitt's lymphoma (Raji), human colon adenocarcinoma HT-29, estrogen receptor positive breast adenocarcinoma MCF-7 cell line, as well as normal foetal lung fibroblasts (MRC-5). Cytotoxic activity was determined by using the standard MTT assay, after exposure of cells to the tested compounds for 48 h. Tiazofurin (**1**) was used as a reference compound. The results are presented in Table 1.



Scheme 2. Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, 40 min, then rt, 40 min, 64% from **4**; (b) Tf_2O, Py, CH_2Cl_2, −10 °C, 0.5 h, then rt, 0.5 h; (c) NaCN, DMF, rt, 1.5 h, 73%; (d) H_2S, Py, rt, 14 days, 78%; (e) $BrCH_2COCO_2Et$, EtOH, 80 °C, 50 min, 32% of **15**, 21% of **16**; (f) NH_3, MeOH, rt, 8 days, 60% of **3**, 65% of **17**.

Table 1. In vitro cytotoxicity of **1**, **2**, **3** and **17**

Compound	IC_{50}[a] (μM)						
	K562	HL-60	Jurkat	Raji	HT-29	MCF-7	MRC-5
Tiazofurin (**1**)	2.98	1.84	0.51	16.06	0.89	6.39	0.49
2	0.19	2.08	4.84	0.54	>100	0.96	>100
3	0.49	3.50	2.18	0.07	0.09	9.09	>100
17	0.09	>100	11.01	>100	0.53	10.01	>100

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

Remarkably, all three analogues **2**, **3** and **17** exhibit sub-micromolar cytotoxicity against K562 malignant cells, with IC_{50} values ranging from 0.09 to 0.49 μM. The most active compound against these cells is the α-homo-C-nucleoside **17**, being 33-fold more cytotoxic than tiazofurin, which was recently approved as an orphan drug for treatment of the corresponding malignant disease. Compounds **2** and **3** also efficiently inhibited the growth of K562 cells, with respective IC_{50} values being 16- and 6-fold lower than those observed for the reference compound **1**. According to the data shown in Table 1, analogues **2** and **3**, as well as tiazofurin itself, showed similar and potent antitumour activities towards the HL-60 cells. However, the analogue **17** was found to be completely inactive against this cell line. Tiazofurin remains the most potent compound towards the Jurkat T cells and was over 9-fold more potent than the 2′,3′-anhydro derivative **2**. The homo-C-nucleosides **3** and **17**showed a 4- and 22-fold lower cytotoxicity in the same cell line when compared to tiazofurin, respectively. Compound **2** showed a potent antiproliferative activity towards the Raji cells being almost 30-fold more active than tiazofurin. The β-homo-C-nucleoside **3** exhibited much more pronounced cytotoxicity against this cell line, being approximately 230-fold more active with respect to the reference compound **1**. In contrast, the α-homo-C-nucleoside **17** was completely inactive against this cell line. Compound **2** is devoid of any cytotoxicity against HT-29 cells, while both homo-C-nucleosides **3** and **17** exhibited almost 10- and 2-fold stronger cytotoxicity in the same cell line when compared to **1**, respectively. The analogues **3** and **17** were found to be somewhat less active than the parent compound **1** against the breast adenocarcinoma MCF-7. However, the 2′,3′-anhydro derivative **2** exhibited a stronger cytotoxicity towards this cell line, being approximately 7-fold more active with respect to tiazofurin itself. Remarkably, all newly synthesized tiazofurin analogues **2**, **3** and **17** were found to be completely inactive against the normal MRC-5 cells. These results do suggest that compounds **2**, **3** and **17** are more selective than tiazofurin, but this should be verified by additional in vitro experiments with different normal cell lines. The difference in cytotoxic activity indicates that analogues **2**, **3** and **17** are not acting at the same biological target (IMPDH) as tiazofurin itself. It is possible that these analogues act as specific alkylating agents, and that the observed cytotoxicities originated from an irreversible covalent binding ('suicide inhibition'). Furthermore, the difference in cytotoxicity of compounds **2**, **3** and **17** (including their inactivity against the normal MRC-5 cell line) may well be explained by a specificity difference in the hosts' DNA polymerases or nucleoside kinases, but further studies will be needed to address this hypothesis.

In summary, three novel tiazofurin derivatives, 2′,3′-anhydro-tiazofurin (**2**) and the corresponding β-(**3**) and α-(**17**) homo-C-nucleosides, have been synthesized and evaluated for their in vitro antitumour activity against a number of human neoplastic cell lines. Compound **2** exhibited the most pronounced cytotoxic activity against Raji cells, being almost 30-fold more potent than the reference compound, tiazofurin (**1**). Compound **3** showed even more potent cytotoxicity towards these cells, being approximately 230-fold more active with respect to the reference compound **1**. The most powerful antitumour activity of compound **17** was recorded in the K562 cell line, being 33-fold more active than tiazofurin. Moreover, none of the synthesized analogues showed any significant cytotoxicity towards the normal foetal lung fibroblasts. Finally, to the best of our knowledge compounds **2**, **3** and **17** represent the first biologically active tiazofurin analogues bearing a 2,3-anhydro ribofuranosyl moiety, while the analogues **3** and **17** are the first homo-C-tiazofurin derivatives that demonstrate antiproliferative activity.

Acknowledgment

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10. Selected data for **2**: mp 120–121 °C (from MeOH), $[\alpha]_D^{25}$ +32.8 (*c* 1.2, MeOH). ^{1}H NMR (250 MHz, methanol-d_4): δ 3.36 (dd, 1H, $J_{5'a,5'b}$ = 11.4 Hz, $J_{4',5'a}$ = 6.5 Hz, H-5′a), 3.45 (dd, 1H, $J_{4',5'b}$ = 5.5 Hz, H-5′b), 3.75 (d, 1H, $J_{2',3'}$ = 2.8 Hz, H-3′), 4.05 (dd, 1H, H-4′), 4.11 (d, 1H, H-2′), 5.10 (s, 1H, H-1′), 8.21 (s, 1H, H-5); NOE contact: H-1′ and H-4′; ^{13}C NMR (62.9 MHz, methanol-d_4): δ 59.89 (C-3′), 61.02 (C-2′), 63.30 (C-5′), 78.95 (C-1′), 82.12 (C-4′), 126.06 (C-5), 151.19 (C-4), 165.50 (C-2), 172.08 ($CONH_2$); CI MS: *m*/*z* 243 (MH^+).
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13. Selected spectral data for **17**: mp 200 °C (from MeOH–$CHCl_3$), $[\alpha]_D^{25}$ +4.0 (*c* 0.5, DMSO). ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.15 (dd, 1H, $J_{1''a,1''b} = 14.9$ Hz, $J_{1',1''a} = 7.3$ Hz, H-1″a), 3.28 (dd, 1H, $J_{1',1''b} = 5.7$ Hz, H-1″b), 3.45 (m, 2H, 2 × H-5′), 3.79 (d, 1H, $J_{2',3'} = 3.0$ Hz, H-3′), 3.96 (d, 1H, H-2′), 3.99 (t, 1H, H-4′), 4.37 (dd, 1H, H-1′), 4.94 (br t, 1H, exchangeable with D_2O, OH), 7.52 and 7.71 (2× br s, 2H, exchangeable with D_2O, NH_2), 8.10 (s, 1H, H-5); NOE contact: H-5′ and H-3′; ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 34.59 (C-1″), 57.67 (C-2′), 58.04 (C-3′), 61.51 (C-5′), 76.32 (C-1′), 79.08 (C-4′), 124.73 (C-5), 149.72 (C-4), 162.77 (C-2), 167.25 ($CONH_2$); CI MS: *m*/*z* 257 (MH^+).