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Synthesis and antiproliferative activity of two new tiazofurin analogues with 2′-amido functionalities

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Abstract—Two novel tiazofurin analogues **2** and **3** were synthesized starting from D-glucose. The key step of the synthesis was the efficient one-step hydrogen sulfide-mediated conversion of 2-azido-3-*O*-acyl-ribofuranosyl cyanides to the corresponding 2-amido thiocarboxamides. Compounds **2** and **3** were evaluated for their in vitro antiproliferative activity against certain human tumour cell lines. Remarkably, compound **2** was found to be 570-fold more potent than tiazofurin against MCF-7 cells, while compound **3** showed the most powerful cytotoxicity against HT-29 cancer cells, being almost 100-fold more active than tiazofurin.

Tiazofurin (**1**, Fig. 1) is a synthetic[1] *C*-nucleoside with a potent antitumour activity.[2] It is a prodrug as once within the cell it is converted to thiazole adenine dinucleotide (TAD) which blocks the key step in the de novo synthesis of GTP.[3] In phase I/II clinical trials, it induced complete haematological remissions in patients with end-stage acute nonlymphocytic leukaemia, or in myeloblastic crisis of chronic myeloid leukaemia.[4] Despite the remarkable efficacy of tiazofurin, lack of specificity and a significant neurotoxicity remains a problem in its clinical use.[2] In the search for new antineoplastic agents of improved therapeutic effects, many tiazofurin derivatives were synthesized, including a number of those with a modified sugar segment.[5] However, none of these compounds showed favourable biological properties. We have recently reported on the synthesis of several tiazofurin analogues with modified sugar moieties that showed increased antitumour activities with respect to the lead compound **1**.[6,7] The sub-micromolar activity of 2′-benzamido tiazofurin derivative[7] directed our further work in this field to the synthesis and biological evaluation of related molecules bearing different amide functions at C-2′. Herein, we describe the synthesis of two new tiazofurin analogues **2** and **3** with hexan- and dodecanamido functions at C-2′, along with their effects on the proliferation of some malignant cell lines.

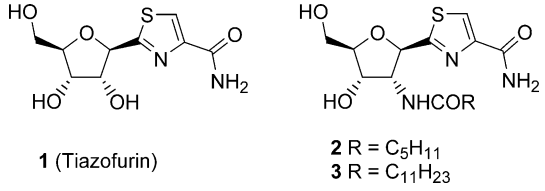

Figure 1. Tiazofurin (**1**) and analogues **2** and **3**.

Our strategy for the synthesis of both targets **2** and **3** assumes previous transformation of D-glucose into the ribofuranosyl thioamides **10** and **16** (Scheme 1), followed by their subsequent cyclocondensation with ethyl bromopyruvate to form the thiazole ring. It was further assumed that the key intermediates **10** and **16** could be accessible from the 2-azido-2-deoxy-D-ribofuranosyl cyanides **9** and **15**, through a one-step H_2S-mediated cascade similar to that recently observed in our laboratory.[7] As the previous conversion included a single aromatic ester derivative, further evaluation of this method in the saturated ester series represents an important task of the present work.

The 2,5-anhydro-D-glucose derivative **4**, readily available from D-glucose,[8] was used as a convenient starting material for the synthesis of both targets **2** and **3**. Com-

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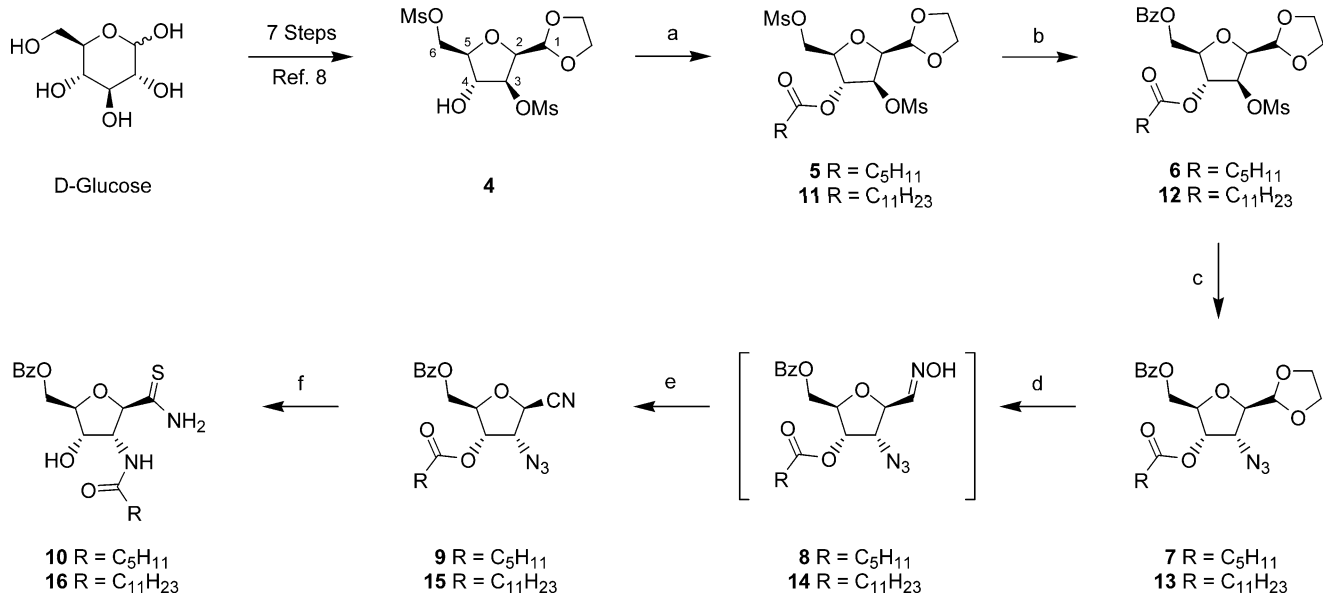


Scheme 1. Reagents and conditions: (a) $C_5H_{11}COCl$, Py, CH_2Cl_2, 0 °C → rt, 24 h, 87% of **5**, 22 h, 99% of **11**; (b) KOBz, DMF, 100 °C, 12 h, 86% of **6**, 8 h, 77% of **12**; (c) NaN_3, DMSO, 110 °C, 24 h, 47% of **7**, 70 h, 44% of **13**; (d) i—4:1 TFA/6 M HCl, 4 °C, 5 days for **7**, 8 days for **13**, ii—$NH_2OH{\cdot}HCl$, NaOAc, EtOH, rt, 4 h; (e) MsCl, Py, −15 °C → rt, 2 h, 46% of **9**, 36% of **15**; (f) H_2S, Py, rt, 12 h, 99% of **10**, or H_2S, DMAP, EtOH, rt, 8 h, 92% of **16**.

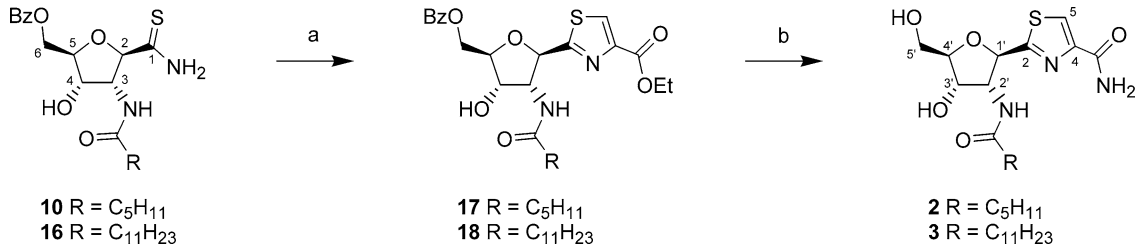
pound **4** readily reacted with hexanoyl chloride under the standard acylation conditions to afford the corresponding 4-*O*-hexanoyl derivative **5** in 87% yield. Reaction of **5** with potassium benzoate in DMF gave the corresponding 6-*O*-benzoyl derivative **6** (86%), which was subsequently treated with sodium azide in dimethylsulfoxide to afford the 3-azido derivative **7** in 47% yield. Hydrolytic removal of the dioxolane protective group in **7** was achieved in a mixture of trifluoroacetic acid and 6 M hydrochloric acid at +4 °C. The resulting unstable aldehyde (not shown in the scheme) was not purified, but was rather immediately treated with hydroxylamine hydrochloride to yield the corresponding oxime **8** (68%) as a mixture of the corresponding *E*- and *Z*-isomers. The mixture was not separated (except for the characterisation purpose), but was rather further treated with mesyl chloride to give a 68% yield of the corresponding nitrile **9**. Moreover, **9** was treated with hydrogen sulfide in pyridine,[7] to provide an almost quantitative yield of the desired thioamide **10**,[9] a key intermediate in the synthesis of analogue **2**.

After the synthesis of thioamide **10**, we were also interested in preparation of its six-carbon homologue **16**, a key intermediate in the synthesis of dodecan-amido derivative **3**. By applying essentially the same multistep sequence as that used for the preparation of **9**, compound **4** was converted to the nitrile **15**. The conversion of **13** to **15** was carried out without purification of both aldehydo and oximino intermediates to afford the nitrile **15** in 36% overall yield (three steps). Although the nitrile **15** can be converted to the thioamide **16** under the reaction conditions similar to those already used for the conversion of **9** to **10** (H_2S, Py, rt), at this point we wanted to explore alternative reagent system for the preparation of **16** (H_2S/DMAP in EtOH), which was also successfully applied in the aromatic ester series.[7] Accordingly, the nitrile **15** was treated with hydrogen sulfide gas and 4-dimethylaminopyridine in ethanol, whereby the desired thioamide **16**[10] was obtained in 92% yield.

The last H_2S-mediated conversion of 2-azido-3-*O*-acyl-2-deoxy-D-ribofuranosyl cyanides **9** and **15** into the corresponding thioamides **10** and **16** deserves some additional comments. This cascade process is presumably comprised of an initial addition of hydrogen sulfide to the nitrile group,[11] followed by the azide reduction and spontaneous *O*,*N*-acyl rearrangement. This sequence already gave good results in the aromatic ester series,[7] but the present work reveals that this method is even more efficient when applied to the saturated ester derivatives. The transformation is likely to be a general method that provides an access to a variety of 2-amido-ribofuranosyl-thiocarboxamides, which may be further converted to an array of tiazofurin analogues with different amido functions at the C-2′ position.

With the requisite intermediates **10** and **16** in hands, we next focused on their transformation to the target *C*-nucleosides **2** and **3**, by using a modified Hantzsch thiazole synthesis,[12] as outlined in Scheme 2. Treatment of both **10** and **16** with ethyl bromopyruvate in refluxing ethanol gave the required thiazoles **17** and **18** in 54% and 56% yield, respectively. Final exposure of **17** and **18** to methanolic ammonia provided the tiazofurin analogues **2**[13] and **3**[14] in 66% and 80% yield, respectively.

The newly synthesized tiazofurin analogues **2** and **3** were evaluated for their antiproliferative activity against human myelogenous leukaemia K562, promyelocytic leukaemia HL-60, colon adenocarcinoma HT-29, estrogen receptor positive breast adenocarcinoma MCF7, cervix carcinoma HeLa and normal foetal lung fibroblasts MRC-5. In vitro cytotoxicity was evaluated after 24-h



Scheme 2. Reagents and conditions: (a) $BrCH_2COCO_2Et$, EtOH, 80 °C, 50 min, 54% of **17**, 56% of **18**; (b) NH_3, MeOH, rt, 7 days, 66% of **2**, 80% of **3**.

cell treatment by using the MTT assay.[15] The results, including the data for the reference compound tiazofurin (**1**), are presented in Table 1.

Compound **2** was inactive against the K562, HL-60 and HT-29 malignant cell lines, but showed a powerful cytotoxic activity against MCF-7 cells being 570-fold more potent than the reference compound **1**. This analogue was also active against HeLa cells, but it was almost 2-fold less potent with respect to the parent molecule **1**. However, compound **3** exhibited a significant cytotoxicity against HL-60, HT-29 and HeLa cell lines, but devoid of any activity towards the MCF-7 neoplastic cells. This analogue showed the most pronounced cytotoxicity against the HT-29 cells, being almost 100-fold more potent than tiazofurin. Compound **3** was approximately 2- and 1.5-fold more active than tiazofurin against HL-60 and HeLa cells, respectively. It also showed a moderate antiproliferative activity against K562 cells, but it was more than 6-fold less active than the reference compound **1**. Remarkably, both analogues were devoid of any cytotoxicity against the normal foetal lung fibroblasts MRC-5. These results do suggest that **2** and **3** are more selective than tiazofurin, but such a generalization should be supported by additional in vitro experiments with a larger number of cell lines.

Due to such a powerful cytotoxicity against certain malignant cell lines, and their nontoxicity towards the normal MRC-5 cells, the analogues **2** and **3** may serve as important leads in the synthesis of more potent and selective anticancer agents. However, the difference in their cytotoxic activity is large and suggests that compounds **2** and **3** are not acting at the same biological target (IMPDH) as tiazofurin.[2,3] In order to explain the biological activity, structure–activity relationship should be performed that would include the synthesis and a more detailed biological evaluation of a series of 2′-amido tiazofurin analogues bearing different R groups in their amide moiety. Such a study is currently underway in our laboratory the results will be reported elsewhere.

In conclusion, two novel tiazofurin derivatives bearing a hexan- (**2**), or a dodecanamido function (**3**) in the C-2′ position, were synthesized starting from D-glucose. The key intermediates for this approach, that is, 2-amido-D-ribofuranosyl thiocarboxamides **10** and **16**, were easily accessible from the 2-azido-2-deoxy-D-ribofuranosyl cyanides **9** and **15**, through the one-step H_2S-mediated cascade, comprised of an initial addition of hydrogen sulfide to the nitrile group, followed by the azide reduction and spontaneous *O*,*N*-acyl rearrangement. The sequence represents a general and particularly versatile approach to a variety of 2-amido-D-ribofuranosyl thiocarboxamides, which can be easily converted to the corresponding ribofuranosyl thiazole-4-carboxamides, as exemplified by the conversion of **10** and **16** to **2** and **3**, respectively. Analogues **2** and **3** have shown a potent cytotoxic activity against some human leukaemia and solid tumour cell lines, but did not exhibit any significant cytotoxicity towards normal foetal lung MRC-5 cells. These results, along with our previous findings,[7] confirmed that the introduction of 2′-nitrogen functionalities into the tiazofurin sugar moiety may provide analogues of improved antiproliferative effects against some neoplastic cells, and therefore, it may be of use in the search for new anticancer agents derived from the lead compound **1**.

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

Compound	IC_{50}[a] (μM)					
	K562	HL 60	HT-29	MCF-7	HeLa	MRC-5
1	5.29	9.32	1.01	7.98	4.76	0.85
2	>100	>100	>100	0.014	8.75	>100
3	35.62	4.82	0.011	>100	3.22	>100

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

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10. Selected data for **16**: mp 110.5–111 °C (from CH_2Cl_2–hexane), $[\alpha]_D^{25}$ +15.36 (*c* 1.34, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 3H, J = 6.7 Hz, Me), 1.24 (m, 16H, 8× CH_2), 1.60 (m, 2H, CH_2), 2.24 (dd, 2H, J = 7.3, J = 8.2 Hz, CH_2CO), 4.06 (ddd, 1H, $J_{2,3}$ = 9.0, $J_{3,4}$ = 5.2, $J_{3,NH}$ = 5.5 Hz, H-3), 4.13 (br s, 1H, exchangeable with D_2O, OH), 4.36 (m, 1H, H-5), 4.41–4.61 (m, 3H, 2× H-6 and H-4), 4.65 (d, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 6.85 (br s, 1H, after treatment with D_2O changed to d, $J_{3,NH}$ = 5.5 Hz, CONH), 7.36–8.04 (m, 5H, Ph), 8.21 and 8.47 (2× br s, 1H each, $CSNH_2$); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.00 (Me), 22.56, 25.43, 29.20, 29.22, 29.41, 29.50, 29.52, 29.5 and 31.78 (9× CH_2), 36.59 (CH_2CO), 58.99 (C-3), 64.76 (C-6), 71.90 (C-4), 84.05 (C-5), 84.63 (C-2), 128.50, 129.17, 129.61 and 133.43 (Ph), 166.87 (Ph*C*=O), 174.74 ($C_{11}H_{23}C$=O), 203.65 (C=S). CI MS: *m*/*z* 479 (M^++H).
11. After shorter reaction time, the main reaction product was accompanied with the thiocarboxamide bearing an unchanged azido group at C-2. This proves that addition of hydrogen sulfide to the nitrile group precedes reduction of the azido function.
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14. Selected data for **3**: mp 133.5–134.5 °C (from MeOH–^{i}Pr_2O), $[\alpha]_D^{25}$ −17.03 (*c* 0.88, MeOH). ^{1}H NMR (250 MHz, methanol-d_4): δ 0.87 (t, 3H, J = 7.0 Hz, Me), 1.18–1.62 (m, 18H, 9× CH_2), 2.25 (t, 2H, J = 7.3 Hz, CH_2CO), 3.74 (d, 2H, $J_{4',5'}$ = 4.5 Hz, 2× H-5′), 4.12 (m, 1H, H-4′), 4.26 (dd, 1H, $J_{2',3'}$ = 5.1, $J_{3',4'}$ = 2.2 Hz, H-3′), 4.52 (dd, 1H, $J_{1',2'}$ = 8.7, $J_{2',3'}$ = 5.1 Hz, H-2′), 5.09 (d, 1H, $J_{1',2'}$ = 8.7 Hz, H-1′), 8.21 (s, 1H, H-5); ^{13}C NMR (62.9 MHz, methanol-d_4): δ 14.41 (Me), 23.61, 26.86, 30.10, 30.32, 30.46, 30.59 and 32.93 (9× CH_2), 36.94 (CH_2CO), 59.92 (C-2′), 63.42 (C-5′), 72.65 (C-3′), 80.66 (C-1′), 88.74 (C-4′), 126.27 (C-5), 150.36 (C-4), 165.59 (C-2), 172.17 and 176.69 (2× C=O); CI MS: *m*/*z* 442 (M^++H); Anal. Calcd for $C_{21}H_{35}N_3O_5S$: C, 57.12; H, 7.99, N, 9.52; S, 7.26. Found: C, 56.92; H, 7.77, N, 9.86; S, 6.90.
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