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Synthesis and biological evaluation of two novel 2′-substituted tiazofurin analogues

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Abstract—Two novel tiazofurin analogues, 2-(2-benzamido-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide **4** and 2-(2-azido-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide **5**, have been synthesized starting from D-glucose and evaluated for their in vitro cytotoxicity against several human leukaemia and solid tumour cell lines.

C-Nucleosides have received much attention in recent years due to their interesting biological activities.[1,2] Among them, tiazofurin (**1**, Fig. 1) has recently been approved as an orphan drug for treatment of chronic myelogenous leukaemia in accelerated phase or blast crisis.[3] It inhibits the enzyme inosine 5′-monophosphate dehydrogenase (IMPDH) inducing shutdown of guanine nucleotide synthesis and causing apoptosis of certain malignant cells.[4] Tiazofurin is intracellularly converted to the NAD analogue, thiazole-4-carboxamide adenine dinucleotide (TAD), which binds to the NAD cofactor-binding site of the enzyme and thus inhibits IMPDH activity.[5,6] Because of its critical role in purine de novo synthesis, IMPDH represents an important target for anticancer and antiviral chemotherapy.[7] Despite the availability of IMPDH inhibitors, lack of specificity remains a problem in their clinical use. A significant efficacy of tiazofurin was achieved in phase II clinical trials. However, hospitalization due to neuro- and cardiovascular toxicities, including their aggressive treatment is required.[8] It is believed that modification of the ribose moiety of tiazofurin may provide an access to analogues with reduced toxicity and enhanced antitumour activity. Accordingly, a number of tiazofurin analogues with a modified sugar moiety have been reported,[9] including a recent preparation of 2-(3-azido- and 3-amino-3-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamides.[10] However, these substances were devoid of any significant biological activity.

We have recently reported on the synthesis and biological evaluation of two novel tiazofurin analogues **2** and **3**.[11] Both *C*-nucleosides **2** and **3** show in vitro cytotoxicity against certain malignant cells, but against normal foetal lung cell line, MRC-5, the fluoro derivative **2** was found to be completely inactive, while the acetamido

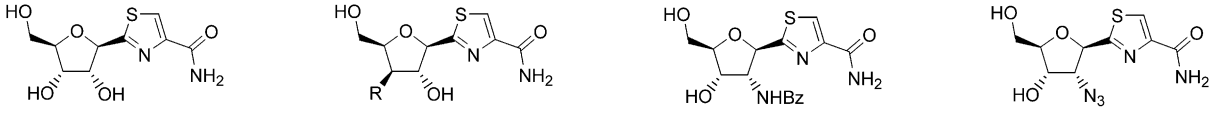

Figure 1. Tiazofurin (**1**) and analogues.

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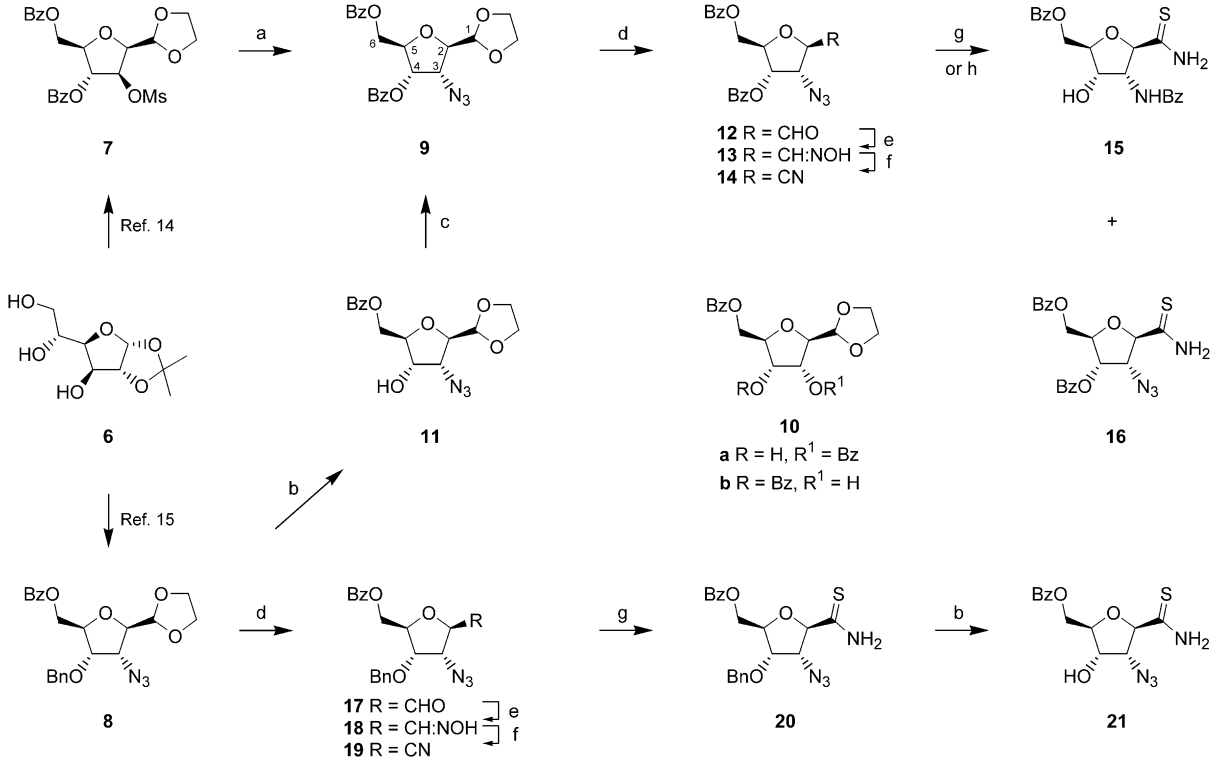
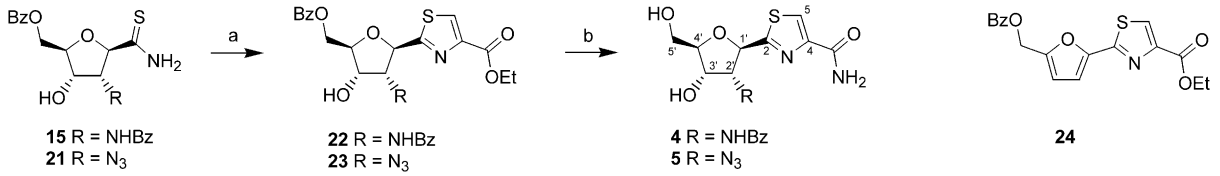
derivative **3** exhibited weak cytotoxicity.[11] Continuing our work in this field, we present herein the synthesis and preliminary biological evaluation of two new tiazofurin analogues **4** and **5** bearing the 2′-benzamido and 2′-azido groups. In view of the interesting biological activities of some 2′-arylamido-[12] or 2′-azido-2′-deoxyribonucleosides,[13] it was of interest to put these groups at the C-2′ position of tiazofurin in order to compare the biological activity of the resulting analogues with that observed for the parent compound **1**.

Our synthetic strategy to the target *C*-nucleosides **4** and **5** was to synthesize the ribofuranosyl thioamides **15** and **21** (Scheme 1) as key intermediates and then to cyclocondense them with ethyl bromopyruvate to form the thiazole ring. Two independent five-step sequences towards the key intermediates **15** and **21** were therefore devised starting from the 2,5-anhydro sugar derivatives **7** and **8**. Both **7** and **8** have been synthesized previously in our laboratory[14,15] starting from commercially available 1,2-*O*-isopropylidene-β-D-glucofuranose **6** via seven[14] and eight[15] synthetic steps, respectively.

Treatment of **7** with sodium azide (DMSO, 108–110 °C) afforded predominantly the 3-azido-3-deoxy derivative **9** (61%) as a result of nucleophilic displacement at C-3 (with Walden inversion). The reaction mixture also contained a minor amount of the regioisomeric 3-*O*- and 4-*O*-benzoyl derivatives **10**, earlier obtained as a major reaction product from the solvolysis of **7** in *N*,*N*-dimethylformamide.[14] The stereochemistry of **9** was elucidated by means of NOE differential ^{1}H NMR spectroscopy. Thus, irradiation at 5.08 ppm (d, 1H, $J_{1,2} = 2.8$ Hz, H-1) gave a strong NOE with H-3 and H-4 thus indicating the close spatial arrangement of these protons and consequently the D-*allo* configuration of product **9**. The structure of **9** was additionally confirmed by its independent synthesis starting from the 4-*O*-benzyl



Scheme 1. Reagents and conditions: (a) NaN_3, DMSP, 108–110 °C, 70 h, 61%; (b) $TiCl_4$, CH_2Cl_2, rt, 1 h, 59% of **11**, 0 °C, 1 h, 67% of **21**; (c) BzCl, Py, rt, 72 h, 84%; (d) 4:1 TFA-6 M HCl, 4 °C, 11 days for **9**, 3 days for **8**; (e) $NH_2OH \times HCl$, NaOAc, EtOH, CH_2Cl_2, rt, 2 h; (f) MsCl, Py, −15 °C, 0.5 h, then 0 °C, 1 h, then rt, 1 h, 59% of **14** (from **9**), **39**% of **19** (from **8**); (g) H_2S, Py, rt, 1.5 h for **14**, 71% of **15**, 25% of **16**, 2 h for **19**, 34% of **20**; (h) H_2S, DMAP, EtOH, rt, 8 h, 82% of **15**.



Scheme 2. Reagents and conditions: (a) $BrCH_2COCO_2Et$, EtOH, reflux, 80 min for **15**, 55% of **22**, 50 min for **21**, 57% of **23**, 22% of **24**; (b) NH_3, MeOH, rt, 7 days for **22**, 56% of **4**, 6 days for **23**, 75% of **5**.

derivative **8**. Debenzylation of **8** with titanium tetrachloride in dry dichloromethane gave a 59% yield of the corresponding azido derivative **11**, which was subsequently converted to **9** (84%) by treatment with benzoyl chloride in pyridine. The IR, ^{1}H and ^{13}C NMR spectra of the product **9** thus obtained displayed the same signals as the material **9** prepared from the 2,5-anhydride **7**.

Hydrolytic removal of the acetal protective group in **9** gave the corresponding aldehyde **12**, which was not purified but was further treated with hydroxylamine hydrochloride and sodium acetate immediately giving the oxime derivatives **13** as an inseparable mixture of the corresponding *E*- and *Z*-isomers. The mixture **13** was used in the next step without purification and was reacted with mesyl chloride in pyridine to give the corresponding nitrile **14** (59% from **9**). Exposure of **14** to hydrogen sulphide gas gave the thioamide **15** as a result of the expected addition of hydrogen sulphide to the nitrile group, followed by subsequent reduction of the azido group to an amino function, as well as a final *O*→*N* acyl migration. Depending on the reaction conditions and reagents used, the major product **15** was accompanied with a variable amount of **16**. Thus when **14** was reacted with hydrogen sulphide in dry pyridine for 1.5 h at room temperature both **15** and **16** were obtained in 71% and 25% yields, respectively. However, on prolonging the reaction time to 8 h, the benzamido derivative **15** was obtained as the only reaction product, but in somewhat lower yield (69%). This result indicates that the addition of hydrogen sulphide to the nitrile function in **14** precedes the reduction of the azido group. Finally, when the reaction was carried out with hydrogen sulphide and *N*,*N*-dimethylaminopyridine in ethanol for 8 h at room temperature, the key intermediate **15** was obtained as the only reaction product in a yield of 82%. To the best of our knowledge, the transformation of **14** to **15** represents the first example of a direct conversion of a 2-azido-3-*O*-acyl-ribofuranosyl cyanide to the corresponding 2-acylamido thiocarboxamide. If this reaction is general it may provide access to a variety of 2-acylamido thiocarboxamides and consequently to novel tiazofurin analogues bearing acylamido functionalities at C-2′.

The 2,5-anhydride **8** was used as a convenient starting compound for the preparation of **21**, a key intermediate in a planned synthesis of the 2′-azido-substituted tiazofurin analogue **5**. Compound **8** was converted to the glycosyl cyanide **19** using the same three-step sequence already applied for the conversion of **9** into the nitrile **14**. In this way, the dioxolane derivative **8** was converted into the glycosyl cyanide **19** in 39% overall yield. Treatment of **19** with hydrogen sulphide gas in dry pyridine for 2 h at room temperature gave a moderate yield of the desired thioamide **20** (34%). All attempts to improve the yield of **20** by varying the reaction conditions were unsuccessful. The relatively low yield of the reaction might be the result of a competitive H_2S-mediated reduction process that involved further conversion of **20** to the corresponding 2-amino-2-deoxy derivative (not shown in the Scheme). Indeed, apart from the isolated product **20**, TLC of the reaction mixture showed the presence of several additional components of higher polarity, with one of them presumably being the amino derivative. However, none of these by-products could be isolated in pure form due to their similar chromatographic properties. Moreover, *O*-debenzylation of **20** with titanium tetrachloride gave the corresponding azido derivative **21** in 67% yield.

Having obtained the key intermediates **15** and **21**, we next focused on their further transformations in order to elaborate the thiazole-4-carboxamide heterocylic system using a modified Hantzsch thiazole synthesis.[16] Accordingly, treatment of **15** with ethyl bromopyruvate in refluxing ethanol produced the corresponding thiazole **22** in 55% yield. The cyclocondensation of **21** under the same reaction conditions gave the required thiazole **23** (57%), but with a minor amount of the known[17] furan **24** (22%). Finally, exposure of **22** and **23** to methanolic ammonia provided the tiazofurin analogues **4**[18] and **5**[19] in 56% and 75% yields, respectively (Scheme 2).

The newly synthesized tiazofurin analogues **4** and **5** were preliminarily evaluated for their antiproliferative activity against human myelogenous leukaemia K562, promyelocytic leukaemia HL60, colon adenocarcinoma HT29, estrogen receptor positive breast adenocarcinoma MCF7, cervix carcinoma HeLa and normal foetal lung MRC-5 cells. Tiazofurin **1** was used as a reference compound. In vitro cytotoxicity was evaluated after 24 h cell treatment by the MTT assay.[20] The results are presented in Table 1.

Compound **4** was inactive against the MCF7 and HeLa cells, but showed significant cytotoxic activity against K562, HL-60, HT-29 and MRC-5 cell lines. The activity of this compound towards K562 and HT-29 cells was comparable to that observed for tiazofurin. However the analogue **4** was found to be significantly more active against the HL-60 and MRC-5 cell lines, being almost 80- and 8-fold more potent than tiazofurin **1**, respectively. Conversely, the *C*-nucleoside **5** was completely inactive against K562, HL-60, HT-29 and MRC-5 cells, but exhibited strong cytotoxicity against the MCF7 cell line, being approximately 200-fold higher than observed for the reference compound **1**. This analogue was also active against HeLa cells, but was more than 3-fold less active with respect to tiazofurin **1**. These results indicate that the incorporation of 2′-nitrogen functionalities into the tiazofurin sugar moiety may result in an improvement of the lead compounds cytotoxity against some neoplastic cells, and therefore is a process, which may

Table 1. In vitro cytotoxicity of compounds **1**, **4** and **5**

Compds	IC50, μM[a]					
	K562	HL-60	HT-29	MCF7	HeLa	MRC-5
1	5.29	9.32	1.01	7.98	4.76	0.85
4	9.84	0.12	0.93	>100	>100	0.11
5	>100	>100	>100	0.04	16.43	>100

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

be of use in the search for new anticancer agents derived from the parent molecule **1**.

In conclusion, we have synthesized two novel tiazofurin analogues **4** and **5**, which have shown potent cytotoxic activity against some human leukaemia and solid tumour cell lines, whereupon the 2′-azido derivative **5** did not exhibit any significant cytotoxicity towards normal foetal lung MRC-5 cells. In addition, this approach provided a new and convenient one-step procedure for efficient H_2S-mediated conversion of 2-azido-2-deoxy-3,6-di-*O*-benzoyl-β-D-ribofuranosyl cyanide **14** to the corresponding 2-benzamido thiocarboxamide derivative **15**, thus enabling a facile access to a key intermediate for preparation of the corresponding tiazofurin analogue **4**. Further work on the scope and limitations of this reaction is currently underway and the results will be reported elsewhere.

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18. Compound **4**: mp 225 °C (from MeOH-^{i}Pr$_2$O); $[\alpha]_D^{23}$ − 96.2 (*c* 1.05, MeOH); ν_{max} 3455 (OH), 1641 (C=O); ^{1}H NMR (methanol-d_4): δ 3.78 (pseudo d, 2H, $J_{4',5'}$ = 4.5 Hz, 2 × H-5′), 4.17 (td, 1H, $J_{3',4'}$ = 2.6, $J_{4',5'}$ = 4.5 Hz, H-4′), 4.36 (dd, 1H, $J_{2',3'}$ = 5.5, $J_{3',4'}$ = 2.6 Hz, H-3′), 4.71 (dd, 1 H, $J_{1',2'}$ = 8.8, $J_{2',3'}$ = 5.5 Hz, H-2′), 5.30 (d, 1 H, $J_{1',2'}$ = 8.8 Hz, H-1′), 7.40–7.89 (m, 5H, Ph), 8.20 (s, 1H, H-5); ^{13}C NMR (DMSO-d_6): δ 59.2 (C-2′), 62.2 (C-5′), 71.4 (C-3′), 78.6 (C-1′), 87.7 (C-4′), 125.1 (C-5), 127.8, 128.6, 131.8 and 134.4 (Ph), 150.0 (C-4), 162.8 (C-2), 167.1 (PhC=O), 171.2 (*C*ONH$_2$); HR MS (ES+): *m/z* 364.0959 (M$^+$+H); calcd for $C_{16}H_{18}N_3O_5S$: 364.0967.
19. Compound **5**: mp 160 °C (from MeOH-^{i}Pr$_2$O); $[\alpha]_D^{23}$ − 48.2 (*c* 0.85 in MeOH); ν_{max} 3441 (OH), 2119 (N$_3$), 1683 (C=O); ^{1}H NMR (methanol-d_4): δ 3.69 (dd, 1H, $J_{4',5'a}$ = 4.6, $J_{5'a,5'b}$ = 12.1 Hz, H-5′a), 3.79 (dd, 1H, $J_{4',5'b}$ = 3.3, $J_{5'a,5'b}$ = 12.1 Hz, H-5′b), 4.05 (ddd, 1H, $J_{3',4'}$ = 5.1, $J_{4',5'a}$ = 4.6, $J_{4',5'b}$ = 3.3 Hz, H-4′), 4.17 (t, 1H, $J_{1',2'}$ = $J_{2',3'}$ = 5.5 Hz, H-2′), 4.33 (dd, 1H, $J_{2',3'}$ = 5.5, $J_{3',4'}$ = 5.1Hz, H-3′), 5.16 (d, 1H, $J_{1',2'}$ = 5.5 Hz, H-1′), 8.22 (s, 1H, H-5); ^{13}C NMR (methanol-d_4): δ 63.0 (C-5′), 69.3 (C-2′), 73.7 (C-3′), 81.4 (C-1′), 87.0 (C-4′), 126.2 (C-5), 150.9 (C-4), 165.5 (C-2), 172.6 (*C*ONH$_2$); CI MS: *m/z* 286 (M$^+$+H). Anal. Found: C, 37.84; H, 3.95; N, 24.89; S, 10.78; calcd for $C_9H_{11}N_5O_4S$: C, 37.89; H, 3.89; N, 24.55; S, 11.24.
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