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2-(3-Amino-3-deoxy-β-D-xylofuranosyl)thiazole-4-carboxamide: A new tiazofurin analogue with potent antitumour activity

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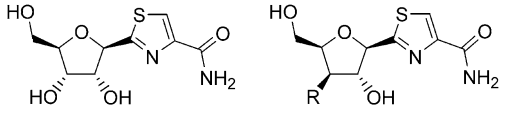
Abstract—A new tiazofurin analogue, 2-(3-amino-3-deoxy-β-D-xylofuranosyl)thiazole-4-carboxamide (**3**), was synthesized starting from D-glucose and evaluated for its in vitro antiproliferative activity against a panel of human tumour cell lines. Compound **3** exhibited the most powerful cytotoxicity against K562 cells, being approximately 100-fold more potent than tiazofurin. This analogue was also active against Jurkat, HT-29 and HeLa malignant cells, with respective IC_{50} values being ca. 2-, 27- and 17-fold lower than those observed for tiazofurin. Remarkably, compound **3** did not exhibit any significant cytotoxicity towards normal foetal lung MRC-5 cell line.

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide; NSC 286193; **1**, Fig. 1), a synthetic nucleoside analogue first described in 1977,[1] is a potent inhibitor of inosine 5′-monophosphate dehydrogenase (IMPDH), a rate-limiting enzyme of the de novo guanine nucleotide synthesis.[2] In 1982 it was reported that **1** represents a high-priority antitumour agent candidate for clinical trials with potential importance for treatment of lung tumours and metastases.[3] Since that time, this compound has been the subject of numerous biological studies[4,5] and it has been recently approved as an orphan-drug for treatment of chronic myelogenous leukaemia in accelerated phase or blast crisis. In phase I/II clinical trials, tiazofurin has shown a significant reduction in leukaemic cell burden in acute myelogenous leukaemia patients.[6] Despite the efficacy achieved in the clinical trials of tiazofurin, lack of specificity and occasional neuro- and cardiovascular toxicity remain a problem in its clinical use.[4a,7] To improve its biological properties, many analogues of **1** have been prepared, including a number of those with variations in the furanose ring.[8] However, none of the reported examples have shown favourable biological effects.[9] As a part of our ongoing project in the synthesis of new C-nucleosides as potential antitumour agents from 2,5-anhydro sugars,[10–14] we have recently reported on the synthesis of several tiazofurin analogues with modified sugar segments that showed increased antitumour activities with respect to the lead compound **1**.[12–14] Notable among them is 2-(3-acetamido-3-deoxy-β-D-xylofuranosyl)thiazole-4-carboxamide (**2**), the first biologically active tiazofurin analogue lacking the ribofuranosyl moiety. Compound **2** showed remarkable antiproliferative activity against K562 cells, being over 40-fold more potent than tiazofurin, but was devoid of any significant cytotoxicity towards the normal MRC-5 cell line.[12] Based upon these findings, but also bearing in mind that certain amino-sugar nucleosides possess antiviral and anticancer activities,[15,16] it was of interest to prepare the D-*xylo*-tiazofurin analogue **3** with a free amino group at the C-3′ position, in order to compare its antiproliferative activity with that observed for both lead compounds **1** and **2**.



1 (Tiazofurin)

2 R = NHAc
3 R = NH_2

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

Keywords: 2,5-Anhydro sugars; C-Nucleosides; Antitumour agents; Epoxide ring opening; Tiazofurin analogues; Thiazoles.
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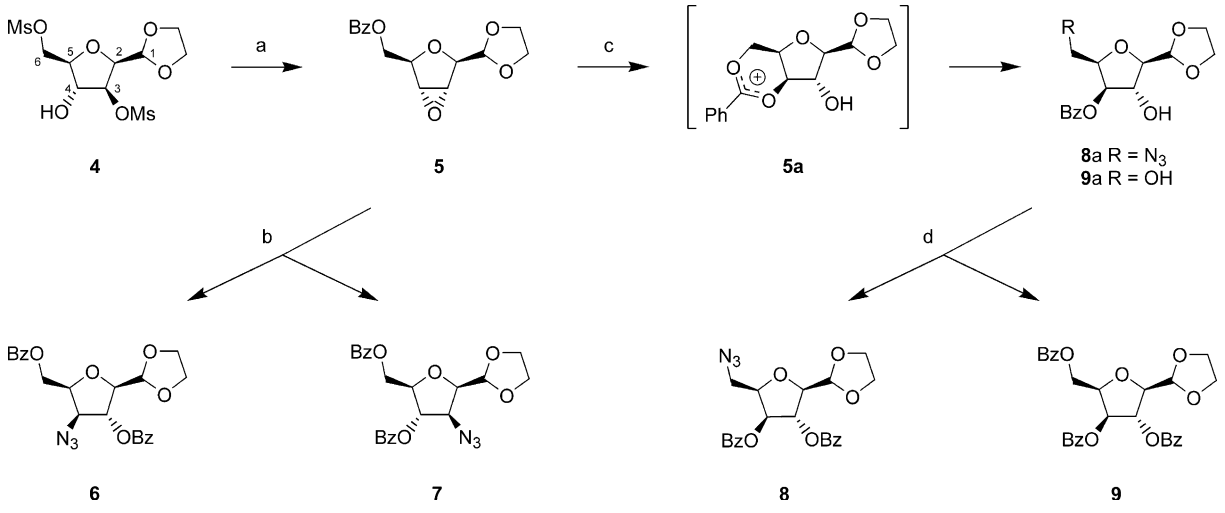
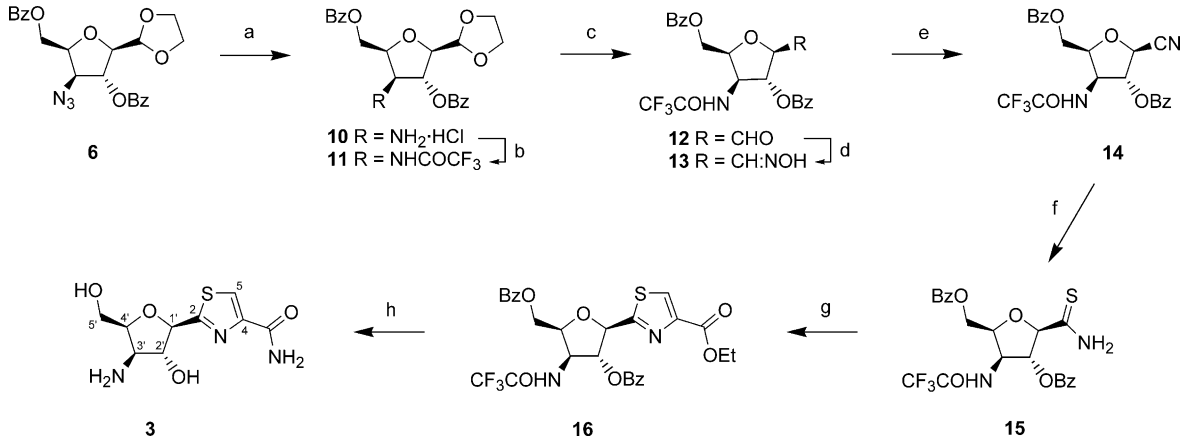
The synthesis of **3** is presented in Schemes 1 and 2. The 2,5-anhydro-D-glucose derivative **4**, readily available from D-glucose,[17] was used as a starting material in this work. Treatment of **4** with potassium benzoate in *N*,*N*-dimethylformamide afforded the epoxide **5**, through a sequential 3,4-anhydro ring closure—primary mesyloxy group displacement process. Reaction of **5** with sodium azide in dimethyl sulfoxide yielded a mixture of the corresponding 3- and 4-azido derivatives (not shown in the reaction scheme), which could not be separated by chromatographic methods due to their close structural similarity. Therefore, the crude mixture of regioisomers was subsequently treated with benzoyl chloride in pyridine to produce the corresponding *O*-benzoyl derivatives **6** and **7**, which were readily separated by column chromatography. Eluted first was the 4-azido-4-deoxy derivative **6** (23% from **5**), with physical and spectral data in full agreement with those reported in our previous paper.[11] Eluted second was the minor product **7**, which was isolated in 16% overall yield with respect to the epoxide **5**. The intermediate **6** was previously prepared starting from **4** in 21.5% overall yield in five synthetic steps.[11]

The procedure for preparation of **6** developed in the present work resulted in somewhat lower overall yield (15.4% from **4**), but it was carried out through a three-step sequence that provided a faster access to the intermediate.

In the next step, we have focused on the ring opening of epoxide **5** by $Me_3SiN_3/BF_3{\cdot}OEt_2$. This reagent system sometimes gives a different regioselectivity compared to NaN_3 in DMF, as observed recently in the ring opening reaction of benzyl 2-acetamido-3,4-anhydro-2,6-dideoxy-α-L-talopyranoside.[18] However, it has been shown that the reaction of **5** with $Me_3SiN_3/BF_3{\cdot}OEt_2$ in CH_2Cl_2 (rt, 24 h) was not so straightforward and afforded a mixture of **8a** and **9a**. The crude reaction mixture was not purified but was treated with benzoyl chloride in pyridine to give the 6-azido derivative **8** (34%) as the major product, accompanied by the tri-*O*-benzoyl derivative **9** (16%). Both rearranged products **8a** and **9a** were formed by the migration of the benzoyloxy group via a regioselective cleavage of oxirane ring at the C-4 position. The stereoselectivity in the case of the formation of 2,5-anhydro-D-gulose systems **8a** and



Scheme 1. Reagents and conditions: (a) KOBz, DMF, 100 °C, 8 h, 67%; (b) i—NaN_3, DMSO, 108–112 °C, 26 h, ii—BzCl, Py, rt, 24 h, 23% of **6**, 16% of **7**; (c) Me_3SiN_3, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 24 h; (d) BzCl, Py, rt, 24 h, 34% of **8**, 16% of **9**.



Scheme 2. Reagents and conditions: (a) H_2, 10% Pd/C, $CHCl_3$ (cat.), EtOH, rt, 24 h; (b) TFAA, Py, CH_2Cl_2, −10 °C, 0.5 h, 4 °C, 72 h, 62% from **6**; (c) 4:1 TFA–6 M HCl, 4 °C, 72 h; (d) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, rt, 2 h, 33% from **11**; (e) MsCl, Py, −18 °C, 144 h, then rt 1 h, 59%; (f) H_2S, Py, rt, 4 h, 99%; (g) $BrCH_2COCO_2Et$, EtOH, 80 °C, 50 min, 32%; (h) NH_3, MeOH, rt, 8 days, 69%.

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

Compounds	IC_{50}[a] (μM)				
	K562	Jurkat	HT-29	HeLa	MRC-5
1	5.29 (0.25)	0.14 (0.006)	1.01 (0.002)	4.76 (0.054)	0.85 (0.01)
2	0.095[b] (0.003)	0.45 (0.01)	1.0[b] (0.02)	>100 (1.56)	89.70[b] (0.29)
3	0.052 (0.001)	0.06 (0.002)	0.037 (0.002)	0.28 (0.01)	>100 (2.61)

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control. Values are means of three independent experiments, standard deviation is given in parentheses.
[b] Results taken from Ref. 11.

9a arises from the trapping of the cyclic oxonium ion **5a**, derived from neighbouring group participation of the 6-benzoyloxy group in **5**, by azide anion or water.

Catalytic reduction of **6** over 10% Pd/C, according to the procedure developed by Secrist and Logue,[19] gave the corresponding amine hydrochloride **10**. Treatment of crude **10** with trifluoroacetic anhydride in pyridine gave the expected trifluoroacetamido derivative **11** in 62% yield. Hydrolytic removal of the dioxolane protection in **11** was achieved with a mixture of trifluoroacetic acid and 6 M hydrochloric acid at +4 °C. The resulting unstable aldehyde **12** was not purified, but was rather immediately treated with hydroxylamine hydrochloride to yield the corresponding oxime **13** as a 5:1 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 in pyridine to give a 59% yield of the corresponding nitrile **14**. Treatment of **14** with hydrogen sulfide in dry pyridine gave a quantitative yield of the corresponding thioamide **15**. The final conversion of the intermediate **15** into the target C-nucleoside **3** was achieved by using a modified Hantzsch thiazole synthesis.[20] Accordingly, treatment of **15** with ethyl bromopyruvate in refluxing ethanol gave the required thiazole **16**, which upon final exposure to the saturated solution of ammonia in dry methanol provided the target tiazofurin analogue **3**[21] in 69% yield.

The newly synthesized tiazofurin analogue **3** was evaluated for its antiproliferative activity against human myelogenous leukaemia K562, Jurkat T cell leukaemia, colon adenocarcinoma HT-29, cervix carcinoma HeLa and normal foetal lung fibroblasts, MRC-5. In vitro cytotoxicity was evaluated after 24-h cell treatment by the MTT assay.[22] The results, including the data for the reference compounds, tiazofurin (**1**) and the known[12] analogue **2**, are presented in Table 1.

Remarkably, the analogue **3** exhibits sub-micromolar cytotoxicity against all malignant cells, with IC_{50} values ranging from 0.052 to 0.28 μM. Additionally, this compound is significantly more active than the corresponding acetamido derivative **2**, as well as tiazofurin itself, but was devoid of any cytotoxicity against the normal foetal lung fibroblasts MRC-5. The most pronounced antiproliferative activity of compound **3** was found to be against the K562 cells, being ca. 100-fold more potent than tiazofurin. Moreover, compound **3** was approximately 2-fold more active than the acetamido derivative **2** against the K562 cell line. Tiazofurin was the most potent compound towards the human Jurkat T cell line and was over 3-fold more potent than the acetamido derivative **2**. Analogue **3** demonstrated a 2- and 7-fold greater cytotoxicity in the same cell line when compared to **1** and **2**, respectively. As reported in our previous paper, the 3′-acetamido derivative **2** as well as tiazofurin showed equal and potent antiproliferative activities towards the colon adenocarcinoma HT-29 cells.[12] The title compound **3** exhibited even more pronounced cytotoxicity against this cell line, being approximately 27-fold more active with respect to both parent compounds **1** and **2**. The analogue **3** also inhibited the growth of HeLa cells being 17-fold more efficient with respect to tiazofurin, while the 3′-acetamido derivative **2** was completely inactive against this malignant cell line. These results agree well with our previous findings[12] that the introduction of 3′-functionalities into the tiazofurin sugar moiety, in spite of the changes to its original stereochemistry, may provide an access to analogues of improved antiproliferative effects towards selected neoplastic cells.

In conclusion, a new tiazofurin analogue, 2-(3-amino-3-deoxy-β-D-xylofuranosyl)thiazole-4-carboxamide (**3**), was synthesized starting from D-glucose and evaluated for its in vitro antiproliferative activity against a panel of human tumour cell lines. Compound **3** exhibited the most powerful cytotoxicity against K562 cells, being approximately 100-fold more potent than tiazofurin. To the best of our knowledge, none of tiazofurin analogues were hitherto reported to exhibit such a potent antileukaemic activity. This analogue was also active against Jurkat, HT-29 and HeLa malignant cells, with respective IC_{50} values being ca. 2-, 27- and 17-fold lower than those observed for tiazofurin, but did not exhibit any cytotoxicity towards normal foetal lung MRC-5 cells. Based upon these results, we believe that the analogue **3** may serve as an important lead in the synthesis of more potent and selective antitumour agents derived from the parent compound **1**.

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