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Antitumour tiazofurin analogues embedded with an amide moiety at the C-2′ position

Mirjana Popsavin [a,*], Miloš Svirčev [a], Ljilja Torović [a], Gordana Bogdanović [b], Vesna Kojić [b], Dimitar Jakimov [b], Saša Spaić [a], Lidija Aleksić [b], Velimir Popsavin [a]

[a] Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
[b] Oncology Institute of Vojvodina, Institutski put 4, 21204 Sremska Kamenica, Serbia

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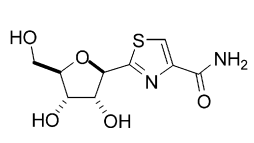
ABSTRACT

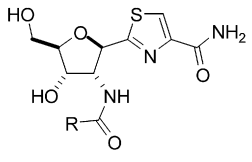
Synthesis of four new tiazofurin analogues has been accomplished starting from D-glucose. The key step of the synthesis was the three-step cascade that enabled an efficient hydrogen sulfide mediated one-pot conversion of 2-azido-3-*O*-acyl-ribofuranosyl cyanides to the corresponding 2-alkylamido ribofuranosyl thiocarboxamides. The resulting key intermediates were first converted to protected tiazofurin derivatives by cyclocondensation with ethyl bromopyruvate, and finally to target C-nucleosides by treatment with ammonia in methanol. In vitro cytotoxicities of tiazofurin analogues against a number of human tumour cell lines were recorded and compared with those observed for the parent molecule (tiazofurin), as well as the commercial antitumour agent doxorubicin (DOX). Analogues **2b**–**d** have shown a potent in vitro cytotoxic activity against human myelogenous leukaemia K562. Among solid tumour cell lines, HT29 was sensitive only to **2d**, while HeLa cells were sensitive to **2a**, **2b** and **2d**. Only analogue **2a** was highly cytotoxic against MCF-7 cells. No tiazofurin analogue exhibits any significant cytotoxicity towards normal foetal lung MRC-5 cells. Downregulation of Bcl-2, activation of caspase-3 and presence of cleavage product of PARP suggest that the cytotoxic effects of tiazofurin analogues **2a**–**d** in K562 might be mediated by apoptosis in a caspase-dependent way. On the contrary, tiazofurin did not induce apoptosis of K562 cells, which suggests a different mechanism of action, most probably through the inhibition of IMPDH. Flow cytometry and Western blot analysis data agreed well with the results of MTT assay, and enabled identification of analogue **2c** as the most promising antitumour agent that preferentially target cancer cells over normal cells and thus represents a new lead for further optimization.

1. Introduction

Among the many classes of nucleoside analogues, C-nucleosides represent popular synthetic targets due to their potential value as therapeutic agents and biochemical probes.[1] Remarkable among them is tiazofurin (**1**, Fig. 1), a synthetic[2] C-nucleoside that shows potent antitumour activity in a variety of tumour systems.[3] Tiazofurin has been extensively studied both in preclinical[4] and clinical studies,[5] and has been approved as an orphan drug for the treatment of patients with acute myeloid leukaemia in blast crisis.[3] It exhibits at least two different mechanisms of action. The first one is the non-competitive inhibition of inosine 5′-monophosphate dehydrogenase, a rate-limiting enzyme for guanylate synthesis,[6] and the second is the induction of apoptosis.[4,7] Despite the remarkable efficacy of tiazofurin, lack of specificity and a significant neurotoxicity[3] limits widespread use of this drug and it is not currently marketed. In the search for new antineoplastic agents with improved therapeutic effects, many tiazofurin derivatives have been synthesized, including a number of those with a modified sugar



Tiazofurin (**1**)



2a R = C_5H_{11}
2b R = C_7H_{15}
2c R = C_9H_{19}
2d R = $C_{11}H_{23}$

Fig. 1. Structures of tiazofurin (**1**) and analogues **2a**–**d**.

* Corresponding author. Tel.: +381 21 485 27 68; fax: +381 21 454 065; e-mail address: mirjana.popsavin@dh.uns.ac.rs (M. Popsavin).

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segment.[8] However, some of these compounds did not show favourable biological properties, although a number of recently synthesized analogues have not been assayed for their antitumour activity. We have recently reported the synthesis of a number of tiazofurin analogues (TAs) that showed increased antitumour activities with respect to the lead compound **1**.[9] Most of these analogues were devoid of any toxicity towards normal mammalian cells. We have also found that some tiazofurin derivatives induced apoptosis in the C6 rat glioma cells.[10] Our previous findings[11] 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, prompted us to study other variations in the structure of the C-2′ portion of the tiazofurin sugar moiety in order to determine the structure–activity relationships associated with this portion of the molecule, as well as to possibly get a better insight into the mechanism of action of TAs in certain tumour cell lines. In particular, we wished to investigate the biological activity of TAs bearing a saturated amide function at the C-2′ position.

Herein, we disclose in detail the synthesis of new TAs **2a**–**d**, with hexan-, octan-, decan- and dodecanamido functions at C-2′, along with their effects on the proliferation of a number of malignant cell lines.[12] In addition, the purpose of the present study was to examine the apoptotic signalling induced by tiazofurin and analogues in the human K562 malignant cell line.

2. Results and discussion

2.1. Chemical synthesis

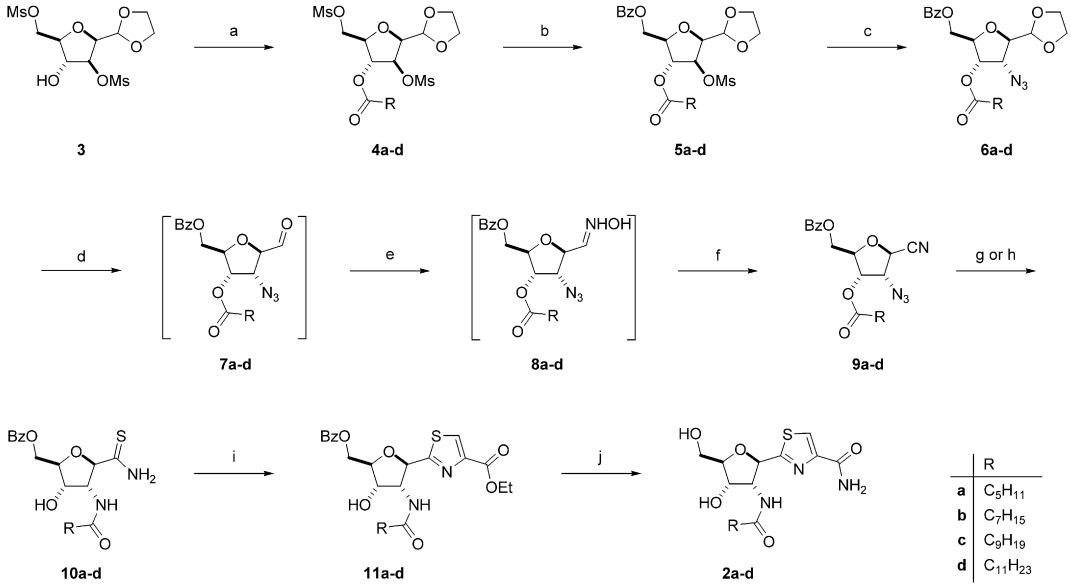
Our strategy for the synthesis of target C-nucleosides of type **2** is based on initial multi-step chemical transformations of D-glucose to the ribofuranosyl-thioamides **10a**–**d** (Scheme 1), followed by their subsequent cyclocondensation with ethyl bromopyruvate to form the thiazole ring. The 2,5-anhydro-D-glucose derivative **3**, readily available from D-glucose[13] was used as a convenient starting material in this work. The synthesis commenced with a three-step sequence for the conversion of **3** to the key azido intermediates **6a**–**d**.

Thus, by use of the appropriate acyl chlorides, the corresponding 4-*O*-acyl derivatives **4a**–**d** were first prepared from the common precursor **3**, as shown in Scheme 1 and Table S1 in the Supplementary data. The corresponding 4-*O*-alcanoyl derivatives **4a**–**d** were thus obtained in 87, 93, 69 and 99% respective yields. Regioselective displacement of the primary mesyloxy groups of **4a**–**d** with potassium benzoate in DMF then produced the 6-*O*-benzoyl derivatives **5a**–**d**, in 86, 70, 86 and 77% yields, respectively. Subsequent treatment of **5a**–**d** with sodium azide in DMSO proceeded with configurational inversion at C-3 to afford moderate yields of **6a**–**d**. Presumably the lower yields in these reactions are due to a number of side processes, that may give elimination and/or neighbouring group participation products, as observed in our preceding work.[14] However, in the present work neither of the side-products could be isolated in pure form, due to their similar chromatographic properties.

In the next stage of the synthesis the protected 2,5-anhydro-3-azido-3-deoxy-derivatives **6a**–**d** were converted to the corresponding glycosyl cyanides **9a**–**d**. Hydrolytic removal of the dioxolane protective group in **6a**–**d** was achieved in a mixture of trifluoroacetic acid and 6 M hydrochloric acid at +4 °C. The resulting unstable aldehydes **7a**–**d** were not purified, but were immediately treated with hydroxylamine hydrochloride to yield the corresponding oximes **8a**–**d** as mixtures of the corresponding *E*- and *Z*-isomers. An attempted separation of *E*-**6a** and *Z*-**6a** by flash column chromatography provided only pure *E*-**6a** (35%), while the corresponding *Z*-isomer could not be obtained free of *E*-**6a**. At this point we decided to avoid the purification step of the remaining oximino derivatives **8b**–**d**. Accordingly, the mixtures of *E*- and *Z*-isomers **8b**–**d** were not separated but were further treated with mesyl chloride in pyridine. The expected ribofuranosyl cyanides **9a**–**d** were thus obtained in respective overall yields of 46, 57, 47 and 36% (from three synthetic steps).

It was assumed that the 2-azido-2-deoxy-D-ribofuranosyl cyanides **9a**–**d** may be converted to the corresponding thioamides through the one-step H_2S-mediated cascade that was recently used for the conversion of 2-azido ribofuranosyl cyanide **9e** to the ribofuranosyl thioamide **10e**[11] (Scheme 2).

This efficient one-pot process is comprised of an initial addition of hydrogen sulfide to the nitrile group, followed by the azide reduction and spontaneous *O*,*N*-shift of the acyl group. Depending on the reaction conditions and reagents used, the major product **10e** was accompanied with a variable amount of 2-azido ribofuranosyl



Scheme 1. Reagents and conditions: (a) RCOCl, Py, CH_2Cl_2, rt; (b) KOBz, DMF, 100 °C; (c) NaN_3, DMSO, 110–112 °C; (d) 6 M HCl, TFA, +4 °C; (e) NaOAc, $HONH_2\times HCl$, EtOH, CH_2Cl_2, rt; (f) MsCl, Py, 0 °C to rt; (g) H_2S, Py, rt; (h) H_2S, DMAP, EtOH, rt; (i) $BrCH_2COCO_2Et$, EtOH, reflux; (j) NH_3, MeOH, rt.