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2-Substituted thiazole-4-carboxamide derivatives as tiazofurin mimics: synthesis and in vitro antitumour activity

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ABSTRACT

Tiazofurin analogues bearing a 5-hydroxymethyl-2-methyl-tetrahydrofuro[2,3-*d*][1,3]dioxol-6-ol moiety as a sugar mimic (**2** and **3**), and two novel thiazole-based acyclo-C-nucleosides **4** and **16** have been synthesized in multistep sequences starting from D-xylose (compounds **2** and **3**) or from D-arabinose (compounds **4** and **16**). All synthesized analogues showed potent in vitro antitumour activities against a panel of human tumour cell lines. Flow cytometry data suggest that cytotoxic effects of analogues **2**–**4** and **16** in the culture of K562 cells might be mediated by apoptosis. It was also found that these analogues induced changes in cell cycle distribution of K562 cells. Results of western blot analysis (upregulation of Bax and downregulation of Bcl-2, activation of caspase-3 and the presence of a PARP cleavage product) suggest that tiazofurin mimics (**2**–**4** and **16**) in K562 cells induced apoptosis in a caspase-dependent way.

1. Introduction

The development of practical and efficient routes for the synthesis of drug-like small molecules is of considerable interest for medicinal chemistry and chemical biology.[1] Compounds containing the thiazole moiety are of particular importance for their potential application as antifungal,[2] antimicrobial,[3] antitumour,[4] anti-inflammatory,[5] anticonvulsant[6] and antitubercular[7] agents. The antitumour activity of 2,4-disubstituted thiazole derivatives has been reported and well documented,[8,9] including the pronounced antitumour activity of the thiazole C-nucleoside tiazofurin (**1**, Fig. 1).[10] Tiazofurin is converted in vivo into the active metabolite TAD (thiazole-4-carboxamide adenine dinucleotide), an analogue of NAD that prevents de novo guanine nucleotide synthesis via inhibition of the enzyme inosine monophosphate dehydrogenase (IMPDH, EC 1.1.1.205).[11] Although tiazofurin proved effective in reducing the leukaemic cell burden in acute myelogenous leukaemia patients, it was found to be too toxic for general clinical application.[12] Subsequent discovery of two IMPDH isoforms, of which type II is upregulated in human leukaemia cell lines,[13] has prompted studies towards the design of isoform selective inhibitors[14] and has renewed interest in tiazofurin and its analogues.[15]

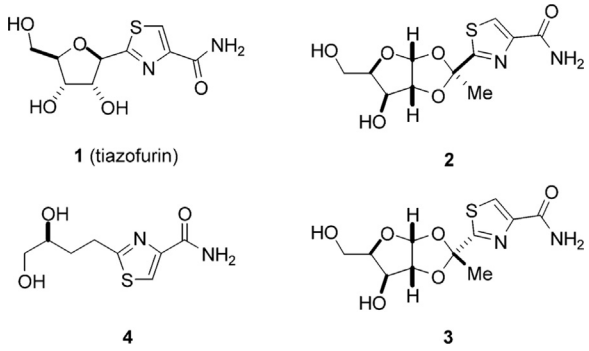

Fig. 1. Tiazofurin (**1**) and the corresponding mimetics **2**, **3** and **4**.

In order to improve therapeutic properties, a number of structural modifications of the tiazofurin sugar moiety have been reported.[16] However, the most of these compounds did not show favourable biological effects, although a number of recently synthesized analogues have not been assayed for their antitumour activity. A series of novel tiazofurin derivatives were recently reported by our group and some of them exhibited a more potent

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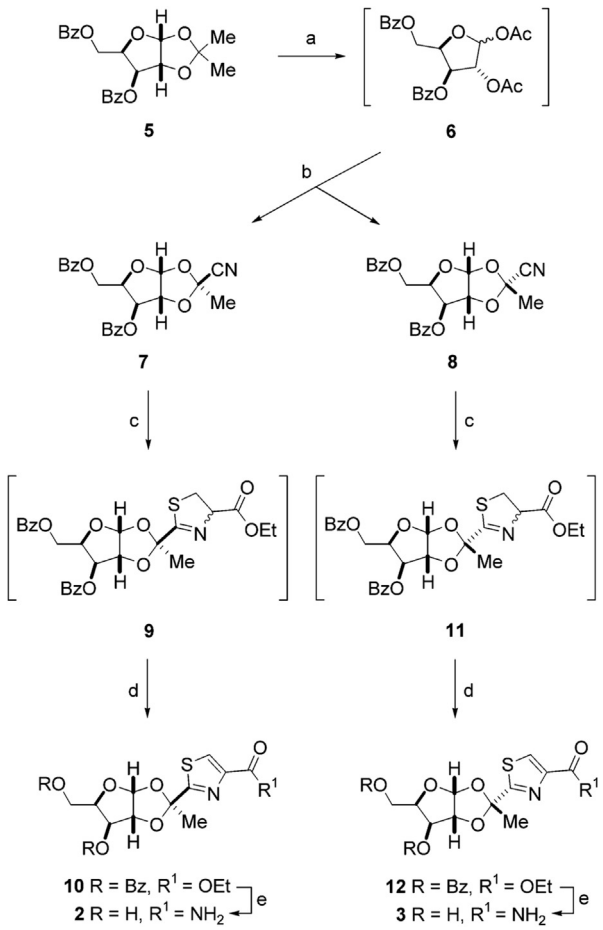
antitumour activity than lead **1**.[17] The majority of these analogues were devoid of any toxicity towards normal human cells. In a recent communication, we reported an approach to tiazofurin analogues **2** and **3** with substituted tetrahydrofurodioxol moiety as a sugar mimic.[18] We herein describe in detail the synthesis of **2** and **3**, along with the extension of our work to a new thiazole-based acyclo-C-nucleoside **4**, which formally represents a ring opened analogue of **1** that lacks the C-4′ hydroxymethyl and the C-2′ hydroxyl group. The effects of the synthesized compounds on the proliferation of selected human tumour cell lines, including their apoptosis-inducing properties in the K562 cell culture, were also studied in this work.

2. Results and discussion

2.1. Chemical synthesis

The preparation of tiazofurin mimics **2** and **3** is summarized in Scheme 1. The sequence started from 3,5-di-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**5**), which is prepared from D-xylose in three synthetic steps.[19] An acetolysis of **5** afforded the mixture of anomeric glycosyl acetates **6** (~100%) in an anomeric ratio of 2:1 in favour of the β-anomer, as established by integration of the anomeric proton signals in ^{1}H NMR spectrum. As the ^{1}H and ^{13}C NMR spectra confirmed a high purity of **6** isolated after the usual workup of the reaction mixture, it was used in further steps without any purification. Exposure of **6** to an excess of cyanotrimethylsilane and boron trifluoride etherate, at room temperature for 2 h, resulted in cyanoethylidene derivatives **7** and **8**, which were obtained in a 7:1 ratio and 64% combined yield. A minor amount of intermediate **6** (26%) was also isolated as the pure α-anomer and fully characterized by physical and spectroscopic data.

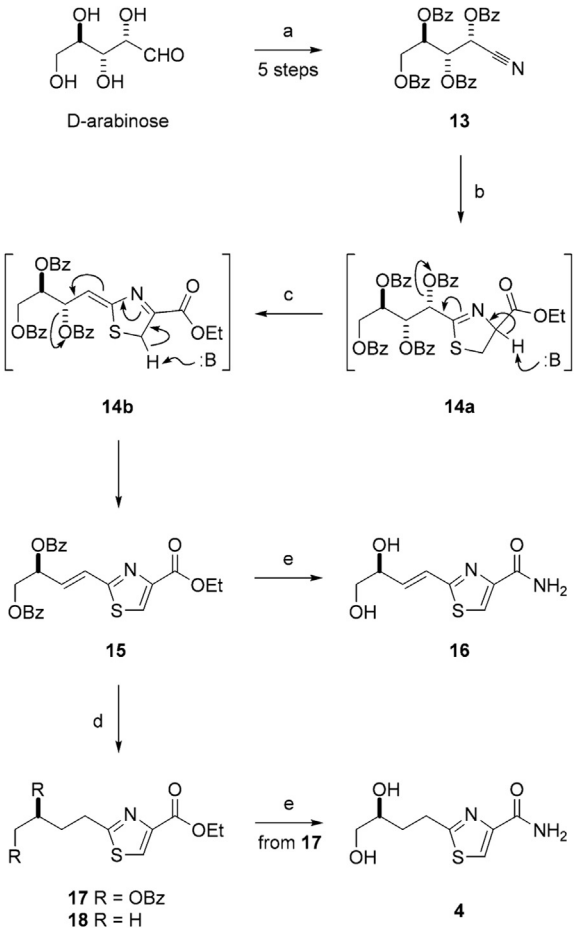


Scheme 1. Reagents and conditions: (a) glacial AcOH, Ac_2O, concd H_2SO_4, rt, 40 h, 100%; (b) 4.5 equiv Me_3SiCN, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 2 h, 56% of **7**, 8% of **8**; (c) cysteine ethyl ester hydrochloride, Et_3N, MeOH, rt, 2 h for **7**, 3.5 h for **8**; (d) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, 5 h, than 4 °C, 4 days for **9**, 54% of **10** (from **7**), 18 h for **8**, 50% of **12** (from **8**); (e) NH_3, MeOH, rt, 7 days for **10**, 89% of **2**, 6 days for **12**, 90% of **3**.

The stereochemistry of cyanoethylidenes **7** and **8** was resolved by NOE differential ^{1}H NMR spectroscopy. It was found that when the methyl group hydrogens of **7** were irradiated, the H-4 peak was enhanced, suggesting that the methyl group and the H-4 has a *syn* orientation that means the nitrile group is *exo*-oriented. This effect was not observed in **8**, thus implying the opposite stereochemistry at the quaternary carbon atom. However, when the methyl group hydrogens of **8** were irradiated, the H-2 proton signal was enhanced, suggesting that the methyl group and the H-2 are *syn*-oriented. Such an arrangement is only possible if the isomer **8** has the nitrile group *endo*-positioned.

Compound **7** was allowed to react with cysteine ethyl ester hydrochloride, in the presence of Et_3N at room temperature, to afford thiazoline **9** as an inseparable mixture of C-4 epimers. The newly formed stereocenter will be destroyed in the next reaction, so no attempts were made to analyze the diastereomeric ratio of this mixture. A clean conversion of the thiazolines **9** was achieved in the presence of DBU and bromotrichloromethane.[20] Thiazole **10** was thus prepared from **7** in a non-optimized yield of 54%, over the last two steps and a single chromatographic separation. Ester aminolysis followed by global deprotection, which were effected by stirring **10** in methanolic ammonia, provided target **2** in 89% yield. The same synthetic methodology was then applied for the preparation of analogue **3** starting from **8**. The corresponding tiazofurin mimics **2** and **3** were thus obtained in 48 and 45% respective yields (calculated to reacted cyanoethylidene derivatives **7** and **8**), over three synthetic steps.

The preparation of thiazole-based acyclo-C-nucleoside **4** is shown in Scheme 2. The sequence commenced from tetra-*O*-benzoyl-D-arabinonitrile **13**, which is readily available from D-arabinose in five steps.[19] Treatment of nitrile **13** with cysteine ethyl ester hydrochloride in the presence of Et_3N, afforded an inseparable mixture of C-4 epimeric thiazolines **14a**. In the first experiments, the epimers **14a** were not purified, but were immediately treated with DBU and $BrCCl_3$ to give the unsaturated thiazole **15**. We are not aware of any precedent for this transformation. Hence, in order to get a better insight into a possible mechanism of this process, the reaction with cysteine ethyl ester was repeated, the resulting mixture of stereoisomers **14a** was purified by flash column chromatography and characterized by ^{1}H and ^{13}C NMR spectra. The stereomeric ratio of 9:1 was determined. Although these isomers could not be separated chromatographically, both spectra of the dominant isomer were fully designated and the resulting data were consistent with structure **14a**. Again, no attempts were made to determine the stereochemistry at C-4 since this stereocenter will be destroyed in the next reaction. It was assumed that the next step of the process involves the elimination of benzoyloxy groups from C-1′ and C-2′ in **14a**, presumably over the tribenzoate **14b** as an intermediate. This assumption suggests that the previous transformation could be realized even in the absence of $BrCCl_3$. Indeed, when the purified **14a** was treated only with DBU, the unsaturated thiazole **15** was obtained in 81% yield. Subsequent treatment of **15** with a saturated solution of ammonia in MeOH gave the corresponding acyclo-C-nucleoside **16** in 87% yield. Catalytic reduction of **15** over 10% Pd/C, gave the expected saturated derivative **17** (54%) accompanied with a minor amount of deoxygenated side-product **18** (31%). Final exposure of **17** to methanolic ammonia provided the target **4** in 71% yield.



Scheme 2. Reagents and conditions: (a) see Ref. 19; (b) cysteine ethyl ester hydrochloride, Et_3N, MeOH, rt, 2 h; (c) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C for 5 h, then 4 °C for 17 days, 47% from **13**; or DBU, CH_2Cl_2, rt for 24 h, 81% from **14a**; (d) H_2, 10% Pd/C, EtOH, rt, 72 h, 54% of **17**, 31% of **18**; (e) NH_3, MeOH, rt, 6 days for **15**, 87% of **16**, 16 days for **17**, 71% of **4**.

2.2. In vitro antitumour activity

The cytotoxic activities of the synthesized tiazofurin mimics **2**, **3** and **4** were evaluated after 72 h by MTT assay against six human cancer cell lines (K562, HL-60, Jurkat, Raji, MCF-7 and HeLa) and one normal human cell line (MRC-5). Unsaturated acyclo-C-nucleoside **16** was also included in the testing for the sake of comparison. Tiazofurin (**1**) and the commercial antitumour agent doxorubicin (DOX) were used as the positive controls in this assay. The results are presented in Table 1.

As shown in Table 1, all synthesized tiazofurin mimics (**2**–**4** and **16**) exhibited diverse cytotoxic activity towards all tumour cell lines under evaluation, but were fully devoid of any toxicity against the normal foetal lung fibroblasts (MRC-5). These results do suggest that analogues **2**, **3**, **4** and **16** are more selective than lead **1**, but this assumption should be verified by additional in vitro experiments with different normal cell lines. Remarkably, all four analogues (**2**, **3**, **4** and **16**) exhibited a potent cytotoxicity against K562 malignant cells, with respective IC_{50} values in the range from 0.02 to 1.02 μM. The most active compound against this cell line was mimic **3**, which was over 90- and 10-fold more potent than both control compounds **1** and DOX, respectively. Compound **2** also inhibited the growth of K562 cell line being over 17- and 2-fold more active than **1** and DOX, respectively. The same compound demonstrated the similar and powerful antiproliferative activity towards HL-60 (IC_{50} 0.05 μM) and HeLa (IC_{50} 0.06 μM) cells being 4- and 64-fold more potent than tiazofurin (**1**), respectively. Analogue **4** demonstrated a low submicromolar activity against Jurkat cells, with an IC_{50} value same as that recorded for tiazofurin (0.04 μM) and similar to that recorded for DOX (0.03 μM) in the same cell line. Molecule **4** demonstrated the same and powerful activity towards MCF-7 cells (IC_{50} 0.04 μM) being 44- and 5-fold more potent than tiazofurin and DOX, respectively. MCF-7 cells were the most sensitive to mimic **16**. This analogue was 90-fold more active than control compound **1** and 10-fold more active than the commercial cytostatic DOX in the same cell line.

Table 1
In vitro cytotoxicity of tiazofurin (**1**), analogues **2**–**4**, **16** and DOX

Compd	IC_{50}[a] (μM)						
	K562	HL-60	Jurkat	Raji	MCF-7	HeLa	MRC-5
1	1.89	0.19	0.04	5.28	1.78	3.82	0.36
2	0.11	0.05	0.59	2.64	9.21	0.06	>100
3	0.02	0.34	0.64	5.61	11.67	0.64	>100
4	0.95	1.01	0.04	14.02	0.04	2.51	>100
16	1.02	1.11	0.61	4.01	0.02	1.51	>100
DOX	0.25	0.92	0.03	2.98	0.20	0.07	0.10

[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. Coefficients of variation were less than 10%.

2.3. Cell cycle analysis

The cells passes through the series of events (cell cycle) leading to cell division and duplication. Cells that actively pass through the cell cycle are the targets in the therapy of cancer. We studied effects of tiazofurin and its analogues **2**, **3**, **4** and **16** on cell cycle perturbations by staining of DNA with propidium iodide. As shown in Fig. 2 both tiazofurin and analogues increased the S phase and decreased the G2/M phase of K562 cells after 24 h compared to untreated cells. However, longer exposure of K562 cells to analogues resulted in different cell cycle perturbations, which depends

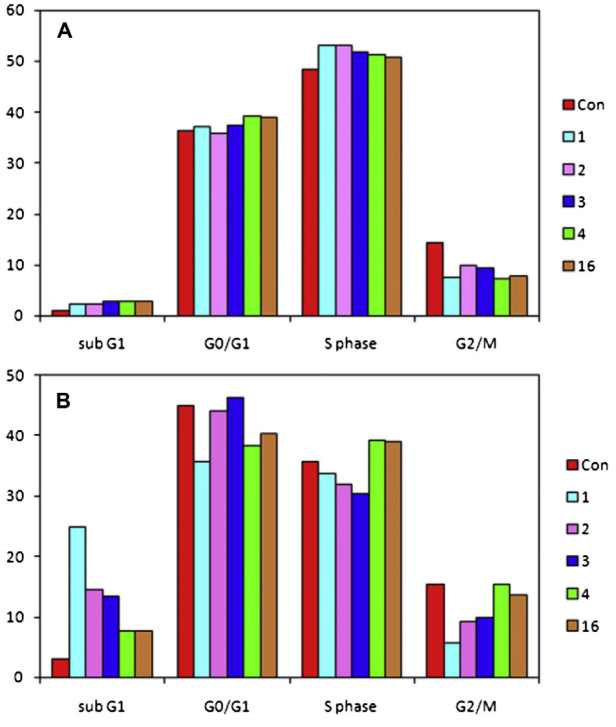


Fig. 2. The effects of tiazofurin (**1**) and the corresponding mimetics **2**, **3**, **4** and **16** on the cell cycle of K562 cells. (A) After 24 h. (B) After 72 h. 'Con' denotes an untreated control.

on type of analogue. All analogues increased the percent of cells in the subG1 phase from 2.5- to 4.5-fold compared to the control. The subG1 peak consisted of cells with hypodiploid DNA content, which is characteristic of cells undergoing apoptosis. Tiazofurin induced the most prominent subG1 peak in K562 cells after 72-h treatment. The obtained results showed that tiazofurin analogues induced changes in cell cycle distribution of K562 cells, which depends on both type of analogue and exposure time.

2.4. Detection of apoptosis

Cell cycle analysis indicated the proapoptotic effect of tiazofurin analogues through the formation of subG1 peak. Therefore we further analyzed apoptotic cell death using double staining with Annexin V-FITC and propidium iodide. Double staining enables detection of cells in the early phase of apoptosis and clearly discriminates truly necrotic cells from the Annexin V positive cells. K562 cell death was evaluated after 24 and 72 h of cells treatment with tiazofurin (**1**) and analogues **2**–**4** and **16**, at their IC_{50} concentrations. The results are summarized in Fig. 3. Apoptotic response presented as a percent of specific apoptosis showed that all tiazofurin analogues after 24 and 72 h induced several-fold more Annexin V positive cells compared to parent compound **1**. Compared to the 24-h treatment, longer cell treatment with analogues **2** and **3**, and **4** and **16**, increased the percentage of Annexin V positive cells 2- and 3-fold, respectively, which is in accordance with results obtained during cell cycle analysis (subG1 peak).

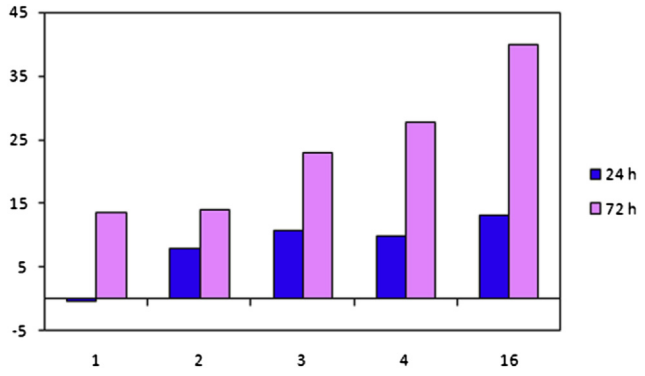


Fig. 3. Percentage of specific apoptosis of K562 cells induced by tiazofurin and analogues **2**–**4** and **16** after 24- and 72-h treatment.

To evaluate the mechanisms underlying the apoptosis, we investigated how tiazofurin analogues (**2**–**4** and **16**) modulated expression of key proteins Bax, Bcl-2, caspase-3 and PARP, involved in apoptotic signalling cascade. Western blot analysis (Fig. 4) revealed that tiazofurin and its mimetics **2**, **3** and **4** decreased Bcl-2 expression after 24-h treatment while analogue **16** increased it, compared to 72-h treatment. All compounds induced a significantly higher expression of proapoptotic protein Bax after 72-h treatment that was in accordance with an increase of specific apoptosis at the same time point. Also, all tiazofurin analogues increased expression of caspase-3 in both time points indicating its involvement in apoptotic process. Activation of caspase-3 is followed by cleavage of downstream targets including PARP. Our experiments clearly showed proteolysis cleavage of PARP in K562 cells by both tiazofurin and all investigated analogues.

Upregulation of Bax and downregulation of Bcl-2, activation of caspase-3, and the presence of PARP cleavage product suggest that cytotoxic effects of tiazofurin analogues in K562 cells might be mediated by caspase-dependent apoptosis. Western blot analysis

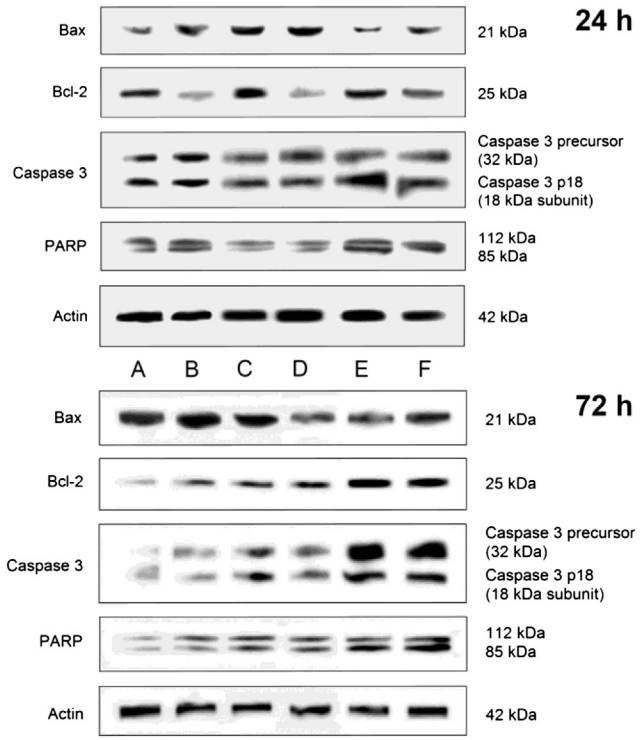


Fig. 4. Western blot analysis of the protein expression of PARP caspase-3, Bcl-2 and Bax after 24- and 72-h treatment: untreated control (A), tiazofurin (B), analogue **16** (C), analogue **4** (D), analogue **2** (E), analogue **3** (F).

and flow cytometry data are in a good agreement with the results of MTT cytotoxicity assay.

3. Conclusion

In conclusion, two interesting tiazofurin analogues bearing the 5-hydroxymethyl-2-methyl-tetrahydrofuro[2,3-*d*][1,3]dioxol-6-ol moiety as a sugar mimic (**2** and **3**), have been synthesized in eight steps starting from D-xylose. The pivotal steps in the synthesis were the preparation of cyanoethylidene derivatives **7** and **8**, followed by elaboration of the thiazole carboxamide aglycon at the nitrile moiety. In addition, two novel acyclo-C-nucleosides **4** and **16** bearing 2,4-disubstituted thiazole moiety have been prepared starting from D-arabinose. The key steps of the synthesis were the initial condensation of tetra-*O*-benzoyl-D-arabinonitrile **13** with cysteine ethyl ester hydrochloride, followed by the subsequent reaction of resulting C-4 epimeric thiazolines **14a** with DBU and bromotrichloromethane, to give the unsaturated thiazole **15**. Then it was discovered that treatment of **14a** with DBU in the absence of bromotrichloromethane gave the same reaction product **15**, thus implying that this transformation included two successive E_2 processes over the intermediates **14a** and **14b**, as shown in Scheme 2. An in vitro antitumour assay revealed that all of the synthesized compounds showed selective cytotoxic effects on specific cancer cell lines (with IC_{50} values ranging from 0.02 to 14.2 μM), whereas none showed any inhibitory effect on the normal cell line, MRC-5. In contrast, the lead **1** and the commercial drug DOX showed a potent cytotoxic activity towards all cell lines under evaluation, with respective IC_{50} values of 0.36 and 0.10 μM. The most potent antiproliferative activity of synthesized tiazofurin mimics (**2**–**4** and **16**) was observed in the culture of K562 cells (the recorded IC_{50} values were in the range from 0.02 to 1.02 μM). A comparison between the bicyclic tiazofurin mimics (**2** and **3**) and acyclo-C-

nucleosides (**4** and **16**) revealed either similar (e.g., compounds **2** and **16** on Jurkat) or rather different (e.g., **3** and **16** on MCF-7) activity against the same cell line. However, acyclo-C-nucleosides were generally more active. The results obtained by studying the effects of tiazofurin and analogues **2**–**4**, and **16** on cell cycle perturbations showed that synthesized mimics induced changes in cell cycle distribution of K562 cells, which depends on both structure of the analogue and the exposure time. Upregulation of Bax and downregulation of Bcl-2, activation of caspase-3, and the presence of the cleavage product of PARP suggest that cytotoxic effects of tiazofurin mimetics **2**–**4**, and **16** in K562 cells might be mediated by apoptosis in a caspase-dependent way.

4. Experimental section

4.1. General

Melting points were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on P 3002 (Krüss) and Autopol IV (Rudolph Research) polarimeters at 20 °C. ^{1}H (250 MHz) and ^{13}C (62.9 MHz) NMR spectra were recorded on a Bruker AC 250 E instrument and chemical shifts are expressed in parts per million (ppm) downfield from TMS. IR spectra were recorded with an FTIR Nexus 670 spectrophotometer (Thermo-Nicolet). High resolution mass spectra (ESI) of synthesized compounds were acquired on an Agilent Technologies 1200 series instrument equipped with Zorbax Eclipse Plus C18 (100 mm×2.1 mm i.d. 1.8 μm) column and DAD detector (190–450 nm) in combination with a 6210 time-of-flight LC/MS instrument (ESI) in the positive ion mode. Flash column chromatography was performed using Kieselgel 60 (0.040–0.063, E. Merck). Self-made preparative TLC plates were prepared using Kieselgel 60 G (E. Merck) with fluorescent indicator F_{254} as additive. The corresponding bands were scraped and eluted with a convenient solvent. All organic extracts were dried with anhydrous Na_2SO_4. Organic solutions were concentrated in a rotary evaporator under reduced pressure at a bath temperature below 35 °C.

4.2. 3,5-Di-*O*-benzoyl-1,2-*O*-[(1*S*)-1-cyanoethylidene]-α-D-xylofuranose (7) and 3,5-di-*O*-benzoyl-1,2-*O*-[(1*R*)-1-cyanoethylidene]-α-D-xylofuranose (8)

To a stirred solution of **5** (0.866 g, 2.17 mmol) in glacial AcOH (8.4 mL) were added Ac_2O (2.1 mL) and concentrated H_2SO_4 (0.59 mL). The mixture was stirred at room temperature for 40 h. The mixture was cooled to 0 °C, and quenched by the addition of a saturated solution of aq $NaHCO_3$ (100 mL) in portions, followed by the addition of solid $NaHCO_3$ until pH ~7. The suspension was extracted with CH_2Cl_2 (4×30 mL), the combined organic layers were washed with brine (1×50 mL), dried and evaporated. The residue was dried under high vacuum to afford crude **6**, which was pure enough for the next step of the synthesis. Anomeric ratio (from ^{1}H NMR): β/α ~2:1; R_f=0.15 (19:1 toluene/EtOAc). IR (film): ν_{max} 1759 (C=O, Ac), 1727 (C=O, Bz). ^{1}H NMR ($CDCl_3$): δ 2.07, 2.11, 2.12 (3×s, CH_3 α and β), 4.46 (dd, $J_{5a,5b}$=12.2 Hz, $J_{4,5a}$=4.8 Hz, H-5a α), 4.54 (dd, $J_{5a,5b}$=12.2 Hz, $J_{4,5b}$=4.5 Hz, H-5b α), 4.60 (pseudo d, 2H, $J_{4,5}$=5.8 Hz, H-5 β), 4.82–4.97 (m, H-4 α and β), 5.41 (s, 1H, H-2 β), 5.58 (dd, $J_{1,2}$=4.6 Hz, $J_{2,3}$=6.2 Hz, H-2 α), 5.72 (d, $J_{3,4}$=4.9 Hz, H-3 β), 5.89 (t, J=6.6 Hz, H-3 α), 6.24 (s, H-1 β), 6.54 (d, $J_{1,2}$=4.5 Hz, H-1 α), 7.30–8.13 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$): δ 20.2, 20.2, 20.7, 20.8 (2×Me from 2×Ac α and β), 61.9 (C-5 α), 62.6 (C-5 β), 74.1 (C-3 α), 74.3 (C-3 β), 75.0 (C-2 α), 75.3 (C-4 α), 79.4 (C-2 β), 79.7 (C-4 β), 92.6 (C-1 α), 98.6 (C-1 β), 128.1, 128.2, 128.3, 128.4, 128.6, 129.15, 129.2, 129.4, 129.4, 129.5, 132.95, 133.0, 133.5 (2×Ph α and β), 164.7, 165.4, 165.7 (2×Ph*C*=O α and β), 168.9, 169.1, 169.4 (2×Me*C*=O α and β). To a stirred solution of **6** (0.811 g, 1.83 mmol) in dry CH_2Cl_2 (15 mL) were added $Et_2O{\cdot}BF_3$ (0.02 mL, 0.18 mmol) and Me_3SiCN (1.03 mL, 8.23 mmol). The mixture was stirred at room temperature for 2 h, in an atmosphere of N_2, and then rendered alkaline (to pH ~9) by the addition of a saturated solution of aq $NaHCO_3$ (30 mL). The resulting emulsion was extracted with ether (4×30 mL), the combined organic solutions were washed with brine (1×30 mL), dried and evaporated. The residue was purified by preparative TLC (38 preparative plates, 19:1 toluene/EtOAc, three successive developments, eluted with EtOAc). Pure **7** (0.31 g, 56% calculated to reacted **6**) was isolated as colourless crystals, mp 127 °C (CH_2Cl_2/hexane), $[\alpha]_D^{20}$ −46.2 (*c* 0.2, $CHCl_3$), R_f=0.66 (19:1 toluene/EtOAc, three successive developments). IR (film): ν_{max} 2233 (C≡N), 1726 (C=O, Bz). ^{1}H NMR ($CDCl_3$): δ 1.85 (s, 3H, CH_3), 4.59–4.72 (m, 3H, H-4 and 2×H-5), 4.91 (d, 1H, $J_{1,2}$=4.1 Hz, H-2), 5.64 (d, 1H, $J_{3,4}$=2.6 Hz, H-3), 6.24 (d, 1H, $J_{1,2}$=4.1 Hz, H-1), 7.33–8.07 (m, 10H, 2×Ph). NOE contact: CH_3 and H-4. ^{13}C NMR ($CDCl_3$): δ 24.2 (CH_3), 61.1 (C-5), 75.4 (C-3), 77.8 (C-4), 84.2 (C-2), 100.0 (Cq), 105.5 (C-1), 116.4 (CN), 128.2, 128.3, 128.4, 129.1, 129.5, 129.6, 133.1, 133.7 (2×Ph), 164.8 and 165.8 (2×Ph*C*=O). HRMS (ESI): *m*/*z* 432.1041 (M^++Na), calcd for $C_{22}H_{19}NO_7Na$: 432.1054. Anal. Found: C, 64.65; H, 4.67; N, 3.43. Calcd for $C_{22}H_{19}NO_7$: C, 64.54; H, 4.68; N, 3.42. Pure minor product **8** (0.045 g, 8%, calculated to reacted **6**) was isolated as a colourless oil, $[\alpha]_D^{20}$ −9.1 (*c* 0.4, $CHCl_3$), R_f=0.58 (19:1 toluene/EtOAc, three successive developments). IR (film): ν_{max} 2235 (C≡N), 1725 (C=O, Bz). ^{1}H NMR ($CDCl_3$): δ 1.82 (s, 3H, CH_3), 4.57 (dd, 1H, $J_{5a,5b}$=11.7 Hz, $J_{4,5a}$=4.9 Hz, H-5a), 4.66 (dd, 1H, $J_{5a,5b}$=11.7 Hz, $J_{4,5b}$=6.4 Hz, H-5b), 4.86 (d, 1H, $J_{1,2}$=3.8 Hz, H-2), 5.06–5.23 (m, 1H, H-4), 5.83 (d, 1H, $J_{3,4}$=4.2 Hz, H-3), 6.20 (d, 1H, $J_{1,2}$=3.8 Hz, H-1), 7.38–8.07 (m, 10H, 2×Ph). NOE contact: CH_3 and H-2. ^{13}C NMR ($CDCl_3$): δ 26.9 (CH_3), 62.2 (C-5), 75.6 (C-3), 79.4 (C-4), 86.1 (C-2), 101.6 (Cq), 106.1 (C-1), 117.7 (CN), 128.4, 128.6, 129.6, 129.7, 133.2, 133.3, 133.9 (2×Ph), 165.0 and 165.9 (2×Ph*C*=O). HRMS (ESI): *m*/*z* 432.1049 (M^++Na), calcd for $C_{22}H_{19}NO_7Na$: 432.1054. Unreacted starting compound **6** was recovered as pure α-anomer (0.21 g, 26%), $[\alpha]_D^{20}$ +90.6 (*c* 2.3, $CHCl_3$), R_f=0.27 (19:1 toluene/EtOAc, three successive developments). IR (film): ν_{max} 1759 (C=O, Ac), 1727 (C=O, Bz). ^{1}H NMR ($CDCl_3$): δ 2.09 and 2.11 (2×s, 3H each, 2×CH_3*C*=O), 4.46 (dd, 1H, $J_{5a,5b}$=12.2 Hz, $J_{4,5a}$=4.8 Hz, H-5a), 4.54 (dd, 1H, $J_{5a,5b}$=12.2 Hz, $J_{4,5b}$=4.5 Hz, H-5b), 4.84–4.93 (m, 1H, H-4), 5.59 (dd, 1H, $J_{1,2}$=4.6 Hz, $J_{2,3}$=6.2 Hz, H-2), 5.89 (t, 1H, J=6.6 Hz, H-3), 6.54 (d, 1H, $J_{1,2}$=4.6 Hz, H-1), 7.30–8.01 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$): δ 20.2 and 20.8 (2×*C*H$_3$C=O), 62.0 (C-5), 74.2 (C-3), 75.1 (C-2), 75.4 (C-4), 92.7 (C-1), 128.2, 128.4, 129.4, 129.6, 133.1, 133.6 (2×Ph), 165.5, 165.7 (2×Ph*C*=O), 169.2 and 169.5 (2×CH_3*C*=O). HRMS (ESI): *m*/*z* 465.1153 (M^++Na), calcd for $C_{23}H_{22}O_9Na$: 465.1156.

4.3. General procedure for the synthesis of thiazole derivatives 10 and 12

To a stirred solution of **7** or **8** (1 equiv) in 4:1 mixture of absolute MeOH and dry CH_2Cl_2 (0.03 M) were added L-cysteine ethyl ester hydrochloride (1.5 equiv) and anhydrous Et_3N (1.5 equiv). The mixture was stirred until the starting materials were consumed (TLC, 2 h for **7**, 3.5 h for **8**) and evaporated. The residue was dissolved in CH_2Cl_2 and the organic solution was washed with water, saturated aq $NaHCO_3$ and brine. The organic solution was dried, filtered and evaporated to give crude thiazolines **9** or **11** as inseparable mixtures of stereoisomers. To a stirred solution of crude thiazoline **9** or **11** (1 equiv) in anhydrous CH_2Cl_2 (0.1 M) was added DBU (2 equiv). The solution was cooled to 0 °C and $BrCCl_3$ (1.2 equiv) was added. The reaction mixture was stirred in an atmosphere of N_2, for 5 h at 0 °C and then stored at +4 °C overnight. The mixture was evaporated and the residue was purified by preparative TLC (4:1 toluene/EtOAc, eluted with EtOAc) to give pure thiazoles **10** or **12**.

*4.3.1. 3,5-Di-O-benzoyl-1,2-O-{(1S)-1-[4-(ethoxycarbonyl)-1,3-thiazol-2-yl]ethylidene}-α-D-xylofuranose (**10**).* Yield: 54% (calculated to reacted **7**). Colourless oil, $[\alpha]_D^{20}$ −16.8 (*c* 1.3, $CHCl_3$), R_f=0.22 (19:1 toluene/EtOAc, three successive developments). IR (film): ν_{max} 1725 (C=O, Bz). ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 1.97 (s, 3H, CH_3), 4.35 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 4.61 (d, 2H, $J_{4',5'}$=6.0 Hz, 2×H-5′), 4.73 (d, 1H, $J_{1',2'}$=3.8 Hz, H-2′), 4.82 (td, 1H, $J_{3',4'}$=2.9 Hz, $J_{4',5'}$=5.9 Hz, H-4′), 5.69 (d, 1H, $J_{3',4'}$=2.9 Hz, H-3′), 6.24 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′), 7.31–8.05 (m, 10H, 2×Ph), 8.10 (s, 1H, H-5). NOE contact: CH_3 and H-4′. ^{13}C NMR ($CDCl_3$): δ 14.1 ($CO_2CH_2CH_3$), 25.7 (CH_3), 61.2 ($CO_2CH_2CH_3$), 61.4 (C-5′), 75.9 (C-3′), 77.6 (C-4′), 84.0 (C-2′), 105.7 (C-1′), 109.2 (Cq), 127.8 (C-5), 128.1, 128.4, 128.5, 129.2, 129.5, 133.9, 133.5 (2×Ph), 147.9 (C-4), 160.8 (C-2), 164.7, 165.8 (PhC=O), 171.4 (EtOC=O). HRMS (ESI): *m*/*z* 540.1319 (M^++H), calcd for $C_{27}H_{26}NO_9S$: 540.1323.

*4.3.2. 3,5-Di-O-benzoyl-1,2-O-{(1R)-1-[4-(ethoxycarbonyl)-1,3-thiazol-2-yl]ethylidene}-α-D-xylofuranose (**12**).* Yield: 50% (calculated to reacted **8**). Colourless oil, $[\alpha]_D^{20}$ −28.0 (*c* 1.0, $CHCl_3$), R_f=0.41 (4:1 cyclohexane/Me_2CO, four successive developments), R_f=0.51 (4:1 toluene/EtOAc). IR (film): ν_{max} 1725 (C=O, Bz). ^{1}H NMR ($CDCl_3$): δ 1.37 (t, 3H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 1.86 (s, 3H, CH_3), 4.37 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 4.54 (dd, 1H, $J_{5a',5b'}$=5.6 Hz, $J_{4',5a'}$=2.7 Hz, H-5a′), 4.59–4.62 (m, 1H, H-4′), 4.63 (dd, 1H, $J_{5a',5b'}$=5.8 Hz, $J_{4',5b'}$=2.7 Hz, H-5b′), 4.91 (d, 1H, $J_{1',2'}$=1.4 Hz, H-2′), 5.70 (d, 1H, $J_{3',4'}$=2.8 Hz, H-3′), 6.25 (d, 1H, $J_{1',2'}$=2.8 Hz, H-1′), 7.33–8.04 (m, 10H, 2×Ph), 8.15 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$): δ 14.2 ($CO_2CH_2CH_3$), 25.5 (CH_3), 61.3 ($CO_2CH_2CH_3$), 61.7 (C-5′), 75.9 (C-3′), 77.9 (C-4′), 84.4 (C-2′), 105.6 (C-1′), 110.2 (Cq), 128.2, 128.5, 128.6, 129.3, 129.5, 129.6, 133.0, 133.6 (C-5 and 2×Ph), 147.7 (C-4), 161.0 (C-2), 164.9, 165.8 (2×PhC=O), 170.5 (EtOC=O). HRMS (ESI): *m*/*z* 540.1318 (M^++H), calcd for $C_{27}H_{26}NO_9S$: 540.1323.

4.4. General procedure for the preparation of thiazole-4-carboxamide derivatives 2 and 3

A solution of **10** or **12** (1 equiv) in saturated methanolic ammonia (0.03 M) was kept at room temperature (7 days for **10**, 6 days for **12**) and then evaporated. The residue was purified by means of preparative TLC (5:1 $CHCl_3$/MeOH, eluted with 1:1 EtOAc/^{i}PrOH) to afford pure **2** or **3**.

*4.4.1. 1,2-O-[(1S)-1-(4-Carbamoyl-1,3-thiazol-2-yl)ethylidene]-α-D-xylofuranose (**2**).* Yield: 89%. Colourless needles, mp 165 °C (from $^iPr_2O$/MeOH), $[\alpha]_D^{20}$ −13.0 (*c* 0.3, MeOH), R_f=0.44 (5:1 $CHCl_3$/MeOH). IR (KBr): ν_{max} 1672 (C=O, amide I), 1590 (NH, amide II). ^{1}H NMR (methanol-d_4): δ 1.88 (s, 3H, CH_3), 3.78 (dd, 1H, $J_{4',5a'}$=6.6 Hz, $J_{5a',5b'}$=11.7 Hz, H-5a′), 3.86 (dd, 1H, $J_{4',5b'}$=4.9 Hz, $J_{5a',5b'}$=11.6 Hz, H-5b′), 4.23 (d, 1H, $J_{3',4'}$=2.8 Hz, H-3′), 4.24–4.29 (m, 1H, H-4′), 4.48 (d, 1H, $J_{1',2'}$=3.8 Hz, H-2′), 6.09 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′), 8.21 (s, 1H, H-5). NOE contact: CH_3 and H-2′, CH_3 and H-4′. ^{13}C NMR (methanol-d_4): δ 26.2 (CH_3), 60.8 (C-5′), 75.4 (C-3′), 83.2 (C-4′), 87.6 (C-2′), 107.2 (C-1′), 109.9 (Cq), 126.4 (C-5), 151.5 (C-4), 165.4 (C-2), 173.9 ($CONH_2$). HRMS (ESI): *m*/*z* 303.0636 (M^++H), calcd for $C_{11}H_{15}N_2O_6S$: 303.0645.

*4.4.2. 1,2-O-[(1R)-1-(4-Carbamoyl-1,3-thiazol-2-yl)ethylidene]-α-D-xylofuranose (**3**).* Yield: 90%. Colourless oil, $[\alpha]_D^{20}$ +0.01 (*c* 0.45, MeOH), R_f=0.44 (5:1 $CHCl_3$/MeOH). IR (film): ν_{max} 1669 (C=O, amide I), 1589 (NH, amide II). ^{1}H NMR (methanol-d_4): δ 1.78 (s, 3H, CH_3), 3.70 (dd, 1H, $J_{4',5a'}$=6.5 Hz, $J_{5a',5b'}$=11.7 Hz, H-5a′), 3.78 (dd, 1H, $J_{4',5b'}$=5.7 Hz, $J_{5a',5b'}$=11.7 Hz, H-5b′), 4.02–4.11 (m, 1H, H-4′), 4.23 (d, 1H, $J_{3',4'}$=2.9 Hz, H-3′), 4.71 (d, 1H, $J_{1',2'}$=3.7 Hz, H-2′), 6.12 (d, 1H, $J_{1',2'}$=3.7 Hz, H-1′), 8.23 (s, 1H, H-5). ^{13}C NMR (methanol-d_4): δ 26.0 (CH_3), 60.9 (C-5′), 75.3 (C-3′), 83.5 (C-4′), 88.1 (C-2′), 107.1 (C-1′), 110.6 (Cq), 126.7 (C-5), 151.0 (C-4), 165.4 (C-2), 172.9 ($CONH_2$). HRMS (ESI): *m*/*z* 303.0648 (M^++H), calcd for $C_{11}H_{15}N_2O_6S$: 303.0645.

4.5. Ethyl 2-[(1′*E*,3′*S*)-3′,4′-bis(benzoyloxy)but-1′-en-1′-yl]-1,3-thiazole-4-carboxylate (15)

Procedure A. To a stirred solution of **13** (0.210 g, 0.37 mmol) in absolute MeOH (5 mL) was added L-cysteine ethyl ester hydrochloride (0.192 g, 1.04 mmol) followed by Et_3N (0.14 mL, 1.04 mmol). The reaction mixture was stirred for 2 h at room temperature and evaporated. The residue was dissolved in CH_2Cl_2 and the organic solution was washed with water, saturated aq $NaHCO_3$ and brine. The organic layer was dried, filtered and evaporated to give crude **14a** (0.316 g). To a stirred solution of crude **14a** (0.259 g, 0.37 mmol) in anhydrous CH_2Cl_2 (4.3 mL) was added DBU (0.11 mL, 0.74 mmol). The solution was cooled to 0 °C and $BrCCl_3$ (0.04 mL, 0.44 mmol) was added over 10 min. The reaction mixture was stirred in an atmosphere of N_2, for 5 h at 0 °C and then stored at +4 °C for 17 days. The mixture was evaporated and the residue was purified by preparative TLC (9:1 toluene/EtOAc, eluted with EtOAc) to give pure **15** (0.079 g, 47% from two steps) as a colourless oil, $[\alpha]_D^{20}$ −21.9 (*c* 0.8, $CHCl_3$), R_f=0.38 (9:1 toluene/EtOAc).

Procedure B. To a stirred solution of **13** (0.544 g, 0.97 mmol) in a mixture of absolute MeOH (13 mL) and anhydrous CH_2Cl_2 (2 mL) was added L-cysteine ethyl ester hydrochloride (0.499 g, 2.69 mmol) and dry Et_3N (0.37 mL, 2.65 mmol). The mixture was stirred for 2 h at room temperature and evaporated. The residue was dissolved in CH_2Cl_2 and the organic solution was washed with water, saturated aq $NaHCO_3$ and brine. The organic layer was dried, filtered and evaporated to give crude **14a** (0.629 g). Flash column chromatography (19:1 toluene/EtOAc) of the residue gave purified mixture of thiazoline **14a** (0.303 g, 45%) as a colourless oil. ^{1}H NMR ($CDCl_3$): δ 1.28 (t, 5.9H, *J*=7.2 Hz, $CO_2CH_2CH_3$), 3.25–3.64 (m, 2.6H, 2×H-5), 4.03–4.30 (q, 2H, $CO_2CH_2CH_3$), 4.55 (dd, 1H, $J_{3',4'a}$=5.8 Hz, $J_{4'a,4'b}$=12.8 Hz, H-4′a), 4.86 (dd, 1H, $J_{3',4'b}$=3.1 Hz, $J_{4'a,4'b}$=12.5 Hz, H-4′b), 5.02 (t, 1H, $J_{4,5b}$=10.1 Hz, H-4), 5.95 (m, 1H, H-3′), 6.24–6.44 (m, 2H, H-1′ and H-2′), 7.17 (d, 1H, $J_{1',2'}$=16.2 Hz, H-1′), 7.31–8.20 (m, 20H, 4×Ph). ^{13}C NMR ($CDCl_3$): δ 13.9 ($CO_2CH_2CH_3$), 34.3 and 34.5 (C-5), 61.6 ($CO_2CH_2CH_3$), 62.3 (C-4′), 68.9 and 69.1 (C-3′), 71.4 and 71.6 (C-1′ and C-2′), 128.2, 128.4, 128.6, 128.8, 128.9, 129.2, 129.55, 129.6, 129.7, 129.8, 132.9, 133.2, 133.4, 133.5 (4×Ph), 164.6, 164.7, 164.95, 165.0, 165.8, 169.8, 171.8 (4×PhC=O and $CO_2CH_2CH_3$). To a stirred solution of purified thiazoline **14a** (0.133 g, 0.19 mmol) in anhydrous CH_2Cl_2 (2.23 mL) was added DBU (0.056 mL, 0.38 mmol). The mixture was stirred at room temperature for 24 h, and then evaporated. The residue was purified by flash column chromatography (19:1 toluene/EtOAc), to afford pure **15** (0.070 g, 81%) as a colourless oil, $[\alpha]_D^{20}$ −21.9 (*c* 0.8, $CHCl_3$), R_f=0.38 (9:1 toluene/EtOAc). IR (film): ν_{max} 1722 (PhC=O). ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3H, *J*=7.2 Hz, $CO_2CH_2CH_3$), 4.33 (q, 2H, $CO_2CH_2CH_3$), 3.73 (dd, 1H, $J_{3',4'a}$=6.6 Hz, $J_{4'a,4'b}$=12.0 Hz, H-4′a), 3.68 (dd, 1H, $J_{3',4'b}$=3.7 Hz, $J_{4'a,4'b}$=12.0 Hz, H-4′b), 6.00–6.14 (m, 1H, H-3′), 6.99 (dd, 1H, $J_{2',3'}$=6.4 Hz, $J_{1',2'}$=16.0 Hz, H-2′), 7.17 (d, 1H, $J_{1',2'}$=16.2 Hz, H-1′), 7.40–8.16 (m, 10H, 2×Ph), 8.33 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$): δ 14.2 ($CO_2CH_2CH_3$), 61.5 ($CO_2CH_2CH_3$), 64.7 (C-4′), 71.0 (C-3′), 126.0 (C-1′), 127.0 (C-5), 128.3, 128.4, 129.2, 129.3, 129.6, 129.7, 129.8, 131.5, 133.2, 133.4 (2×Ph), 131.5 (C-2′), 147.6 (C-4), 161.0 (C-2), 165.2, 165.7, 166.0 (2×PhCO and CO_2Et). HRMS (ESI): *m*/*z* 452.1161 (M^++H), calcd for $C_{24}H_{22}NO_6S$: 452.1162.

4.6. 2-[(1′*E*,3′*S*)-3′,4′-Dihydroxybut-1′-en-1′-yl]-1,3-thiazole-4-carboxamide (16)

A solution of **15** (0.068 g, 0.15 mmol) in saturated methanolic ammonia (5 mL) was kept at room temperature for 6 days, and then evaporated. The residue was purified by preparative TLC (4:1

EtOAc/^{i}PrOH, eluted with 1:1 EtOAc/^{i}PrOH) to afford pure **16** (0.028 g, 87%) as a pale yellow oil, $[\alpha]_D^{20}$ −3.82 (*c* 1.02, CH_3OH), R_f=0.40 (4:1 EtOAc/^{i}PrOH). ^{1}H NMR (methanol-d_4): δ 3.62 (dd, 1H, $J_{3',4'a}$=6.3 Hz, $J_{4'a,4'b}$=11.2 Hz, H-4′a), 3.68 (dd, 1H, $J_{3',4'b}$=5.2 Hz, $J_{4'a,4'b}$=11.2 Hz, H-4′b), 4.35–4.41 (m, 1H, H-3′), 6.80 (dd, 1H, $J_{2',3'}$=4.9 Hz, $J_{1',2'}$=16.2 Hz, H-2′), 6.96 (d, 1H, $J_{1',2'}$=16.2 Hz, H-1′), 8.14 (s, 1H, H-5). ^{13}C NMR (methanol-d_4): δ 66.7 (C-3′), 73.1 (C-4′), 124.3 (C-1′), 124.8 (C-5), 139.5 (C-2′), 151.0 (C-4), 165.6 (C-2), 168.3 (*C*ONH_2). HRMS (ESI): *m*/*z* 237.0317 (M^++Na), calcd for $C_8H_{10}N_2NaO_3S$: 237.0304.

4.7. Ethyl 2-[(3′*S*)-3′,4′-bis(benzoyloxy)butyl]-1,3-thiazole-4-carboxylate (17) and ethyl 2-butyl-1,3-thiazole-4-carboxylate (18)

To a stirred solution of **15** (0.135 g, 0.3 mmol) in 96% EtOH (7 mL) was added 10% Pd/C (0.065 g). The suspension was hydrogenated at room temperature and normal pressure of H_2 for 72 h, then filtered through a Celite pad, washed with EtOH and evaporated. The residue was purified by preparative TLC (9:1 toluene/EtOAc, eluted with 1:1 CH_2Cl_2/EtOAc) to give pure **17** (0.073 g, 54%) as a colourless oil, $[\alpha]_D^{20}$ −24.2 (*c* 0.4, $CHCl_3$), R_f=0.29 (9:1 toluene/EtOAc). ^{1}H NMR ($CDCl_3$): δ 1.38 (t, 3H, *J*=7.2 Hz, $CO_2CH_2CH_3$), 2.28–2.47 (m, 2H, CH_2-2′), 3.12–3.38 (m, 2H, CH_2-1′), 4.40 (q, 2H, *J*=7.2 Hz, $CO_2CH_2CH_3$), 4.51 (dd, 1H, $J_{3',4'a}$=6.0 Hz, $J_{4'a,4'b}$=12.0 Hz, H-4′a), 4.61 (dd, 1H, $J_{3',4'b}$=3.7 Hz, $J_{4'a,4'b}$=12.0 Hz, H-4′b), 5.59 (m, 1H, H-3′), 7.35–8.08 (m, 10H, 2×Ph), 8.03 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$): δ 14.3 ($CO_2CH_2CH_3$), 29.4 (C-1′), 31.0 (C-2′), 61.4 ($CO_2CH_2CH_3$), 65.2 (C-4′), 71.1 (C-3′), 127.0 (C-5), 128.4, 129.5, 129.5, 129.6, 133.1, 133.2 (2×Ph), 145.0 (C-4), 161.2 (C-2), 165.9, 166.1, 170.0 (3×PhC=O and CO_2Et). HRMS (ESI): *m*/*z* 454.1320 (M^++H), calcd for $C_{24}H_{24}NO_6S$: 454.1319. The side-product **18** (0.02 g, 31%) was also isolated as a colourless syrup, R_f=0.40 (9:1 toluene/EtOAc). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H, $(CH_2)_3CH_3$), 1.34–1.53 (m, 2H, CH_2 from Bu), 1.40 (t, 3H, *J*=7.2 Hz, $CO_2CH_2CH_3$), 1.70–1.89 (m, 2H, CH_2 from Bu), 3.07 (t, 2H, $CH_2(CH_2)_2CH_3$), 4.43 (q, 2H, *J*=7.2 Hz, $CO_2CH_2CH_3$), 8.05 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$): δ 13.6 [$(CH_2)_3CH_3$], 14.3 ($CO_2CH_2CH_3$), 22.2, 32.1, 33.2 (3×CH_2 from Bu), 61.3 ($CO_2CH_2CH_3$), 126.7 (C-5), 146.6 (C-4), 161.4 (C-2), 172.5 ($CO_2CH_2CH_3$).

4.8. 2-[(3′*S*)-3′,4′-Dihydroxybutyl]-1,3-thiazole-4-carboxamide (4)

A solution of **17** (0.035 g, 0.08 mmol) in saturated methanolic ammonia (5 mL) was left at room temperature for 16 days and then evaporated. After purification by preparative TLC (5:1 $CHCl_3$/MeOH, eluted with 1:1 EtOAc/^{i}PrOH) pure **4** (0.012 g, 71%) was isolated as an amorphous solid, $[\alpha]_D^{20}$ −46.25 (*c* 0.2, CH_3OH), R_f=0.37 (5:1 $CHCl_3$/MeOH). ^{1}H NMR (methanol-d_4): δ 1.78–1.97 and 1.99–2.18 (2×m, 2H, 2×H-2′), 3.01–3.31 (m, 2H, 2×H-1′), 3.50 (d, 2H, $J_{3',4'}$=5.5 Hz, 2×H-4′), 3.62–3.74 (m, 1H, $J_{3',4'}$=5.5 Hz, H-3′), 8.07 (s, 1H, H-5). ^{13}C NMR (methanol-d_4): δ 30.26 and 34.3 (C-2′ and C-1′), 67.1 (C-4′), 72.1 (C-3′), 125.0 (C-5), 150.4 (C-4), 165.7 (C-2), 173.1 (*C*ONH_2). HRMS (ESI): *m*/*z* 217.0634 (M^++H), calcd for $C_8H_{13}N_2O_3S$: 217.0641.

4.9. Biological materials

Rhodamine B, RPMI 1640 medium, foetal calf serum, 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide, propidium iodide and RNase A, were purchased from Sigma (St. Louis, MO, USA). Penicillin and streptomycin were purchased from ICN Galenika (Belgrade, Serbia). Annexin V-FLUOS apoptosis detection kit was purchased from BD Biosciences Pharmingen (Belgium). Proteins were detected by Western blotting using the following monoclonal: the antibodies against human Bcl-2 and caspase-3 were obtained from R&D Systems (Minneapolis, MN). Anti- poly (ADP-ribose) polymerase (PARP) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced chemiluminescence (ECL Plus) kit and Hyperfilm were purchased from Amersham Biosciences (Arlington Heights, IL). All other chemicals used in the experiments were commercial products of reagent grade. Stock solution (10 mM) was prepared in DMSO and diluted to various concentrations with serum-free culture medium.

4.10. Cell lines

Human chronic myelogenous leukaemia (K562), human promyelocytic leukaemia (HL-60), human T cell leukaemia (Jurkat) and Burkitt's lymphoma (Raji) were grown in RPMI 1640, while breast adenocarcinoma (MCF-7), cervix carcinoma (HeLa) malignant cells and normal foetal lung fibroblasts (MRC-5) were grown in DMEM medium. Both media were supplemented with 10% of foetal calf serum (FTS, NIVNS) and antibiotics (100 IU/mL of penicillin and 100 μg/mg of streptomycin). Cell lines were cultured in flasks (Costar, 25 mL) at 37 °C in the atmosphere of 100% humidity and 5% of CO_2 (Heraeus). Exponentially growing viable cells were used throughout the assays.

4.11. Cells treatment

The cells were seeded in six-well plates at a concentration of 5×10^5 cells/well. Cells were treated for 24 and 72 h with tiazofurin (**1**) and mimics (**2**, **3**, **4** and **16**) at their IC_{50}/24 and 72 h concentrations. Untreated cells were used as control. Viable cells of treated and control samples were used for apoptosis detection and Western blot analysis. Viability was determined using trypan blue dye-exclusion assay.

4.12. MTT assay

Cells were harvested, counted by trypan blue and plated into 96-well microtiter plates (Costar) at optimal seeding density of 5×10^3 cells per well to assure logarithmic growth rate throughout the assay period. Viable cells were placed in a volume of 90 μL per well, and preincubated in complete medium at 37 °C for 24 h to allow cell stabilization prior to the addition of substances. Tested substances, at 10-fold the required final concentration, were added (10 μL/well) to all wells except to the control ones and microplates were incubated for 72 h. The wells containing cells without tested substances were used as control. Three hours before the end of incubation period MTT solution (10 μL) was added to all wells. MTT was dissolved in medium at 5 mg/mL and filtered to sterilize and remove a small amount of insoluble residue present in some batches of MTT. Acidified 2-propanol (100 μL of 0.04 M HCl in 2-propanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals of formazan. The samples were left for a few minutes at room temperature, to ensure that all crystals were dissolved and the plates were read on a plate reader (Multiscan MCC340, Labsystems) at 540 and 690 nm. The wells without cells containing complete medium and MTT acted as blank.

4.13. Flow cytometry

4.13.1. Cell cycle analysis. After treatment K562 cells were washed in cold PBS, fixed and incubated for 30 min in 70% ethanol on ice, centrifuged and incubated with 500 μL RNase A (100 units/mL) and 500 μL propidium iodide (400 μg/mL) for 30 min at 37 °C. Cell cycle was analyzed by FACS Calibur E440 (Becton Dickinson) flow

cytometer and Cell Quest software. Results were presented as percent of cell cycle phases.

4.13.2. Apoptosis. Apoptosis of K562 cells was evaluated with an Annexin V-FITC detection kit. Cells from each sample were collected (800 rpm/5 min, Megafuge 1.0R, Heraeus, Thermo Fisher Scientific) and pellet was re-suspended in 1 mL of phosphate buffer (PBS, pH 7.2). K562 cells were washed twice with cold PBS and then re-suspended in binding buffer to reach the concentration of 1×10^6 cells/mL. The cell suspension (100 μL) was transferred to 5 mL culture tubes and mixed with Annexin V (5 μL) and propidium iodide (5 μL). The cells were gently vortexed and incubated for 15 min at 25 °C. After incubation, 400 μL of binding buffer was added to each tube and suspension was analyzed after 1 h on FACS Calibur E440 (Becton Dickinson) flow cytometer. Results were presented as percent of Annexin V positive gated cells. Percentage of specific apoptosis was calculated according to Bender et al.[21]

4.14. Western blot

For the Western blot, 50 μg of proteins per sample were separated by electrophoresis and electro-transferred to a PVDF membrane Hybond-P and then blotted with primary antibodies Bcl-2, caspase-3 and PARP. Proteins were detected by an enhanced chemiluminescence (ECL Plus) kit that includes peroxidase-labelled donkey anti-rabbit and sheep anti-mouse secondary antibodies. Blots were developed with an ECL Plus detection system and recorded on the Amersham Hyperfilm.

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Supplementary data

Modified experimental procedures for preparation of starting compounds, alternative presentations of flow cytometry and western blot data obtained after treatment of K562 cells with tiazofurin and analogues, copies of ^{1}H and ^{13}C NMR spectra of final products. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.02.035. These data include MOL files and InChiKeys of the most important compounds described in this article.

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