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Research paper

Synthesis and in vitro antitumour activity of tiazofurin analogues with nitrogen functionalities at the C-2′ position

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ABSTRACT

Synthesis of three tiazofurin (**1**) isosteres with nitrogen functionalities at the C-2′ position (N_3, NH_2 and $NH_3^+Cl^-$) has been achieved, in multistep sequences, starting from monoacetone D-glucose. A number of potential bioisosteres of **1** bearing acylamido functions at the C-2′ position have also been synthesized from the same sugar precursor. In vitro cytotoxicities of target molecules against a number of human tumour cell lines were recorded and compared with those observed for lead molecule **1**. Some of the synthesized compounds showed potent in vitro antitumour activity, such as 2′-azido derivative **2**, which is the most potent of all molecules under evaluation (IC_{50} 0.004 μM against MCF-7 cells). Flow cytometry data suggest that cytotoxic effects of these compounds in the culture of K562 cells might be mediated by apoptosis, additionally revealing that these molecules induced changes in cell cycle distribution of these cells. Results of Western blot analysis indicate that the synthesized tiazofurin analogues induce apoptosis in a caspase-dependent way.

1. Introduction

Tiazofurin (**1**, Fig. 1), 2-(β-D-ribofuranosyl)thiazole-4-carboxamide, is a well-known *C*-nucleoside with interesting anticancer properties [1,2]. In 2001, the US Food and Drug Administration granted orphan-drug status designation to tiazofurin for the treatment of chronic myelogenous leukaemia in the accelerated phase of blast crisis, despite its high toxicity [3]. It has been found that tiazofurin inhibits inosine 5′-monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme of de novo guanine nucleotide synthesis [1,2]. Subsequent discovery of two IMPDH isoforms, of which type II is upregulated in human leukaemia cell lines [4,5], has directed studies toward the design of isoform selective inhibitors [6] and has renewed interest for the preparation of tiazofurin analogues [7–10]. Inhibitors of IMPDH in patent literature of the last decade were recently reviewed [11], while the potential of IMP dehydrogenase inhibitors for the treatment of cancer was reviewed in 2007 [12]. To improve therapeutic properties of tiazofurin, numerous structural modifications in its sugar residue have been performed [13–24]. However, most of the resulting tiazofurin mimics did not show favourable biological effects, but a number of synthesized analogues still have not been tested for their antitumour activity. In the last ten years, we have prepared a series of new tiazofurin mimics, and some of them have demonstrated stronger antitumour potencies than lead **1** [25–28]. All of these compounds were completely non-toxic to normal human MRC-5 cells, indicating the selectivity of analogues against tumour cells. In continuation of these studies we designed new tiazofurin mimics bearing the nitrogen functionalities at C-2′ position (**2**–**7**). Compounds **2**–**4** may be considered as direct isosteres of **1**, while the analogues **5**–**7** represent *N*-acyl derivatives of **3**. A reason for the preparation of amides **5**–**7** arose from our recent study, which showed that some 2′-acylamido derivatives demonstrate strong antiproliferative activities against a panel of human tumour cell lines [27].

Herein, we disclose full details on the synthesis of **2** and **6** [29], as well as multistep preparations of new tiazofurin mimics **3**–**5** and **7**. The effects of these compounds to the proliferation of certain human tumour cell lines, including their effects on the K562 cell cycle and their apoptosis inducing properties in the same cell

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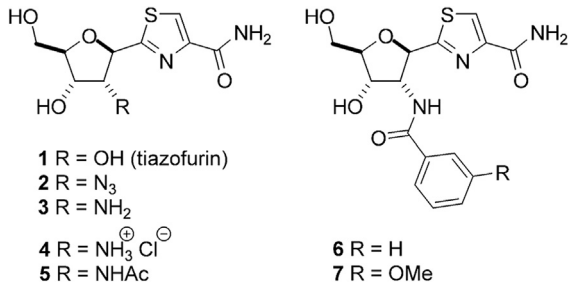


Fig. 1. Chemical structures of tiazofurin (1) and the corresponding analogues **2**–**7**.

culture, have also been studied in this work.

2. Results and discussion

2.1. Chemistry

Synthesis of 2′-azido derivative **2** (Scheme 1), was completed by using the same ten-step sequence previously reported in the preliminary communication [29]. The most critical step of this sequence involves the addition of hydrogen sulphide gas to the nitrile functionality in **10**. However, in addition to the expected reaction with the nitrile functionality H_2S may react with the azide group as well, decreasing the yield of desired thioamide **11**—34%. To avoid the possible low-yielding step we planned an alternative route (Scheme 2) for the construction of the thiazole heterocyclic system by avoiding the use of hydrogen sulphide gas [30].

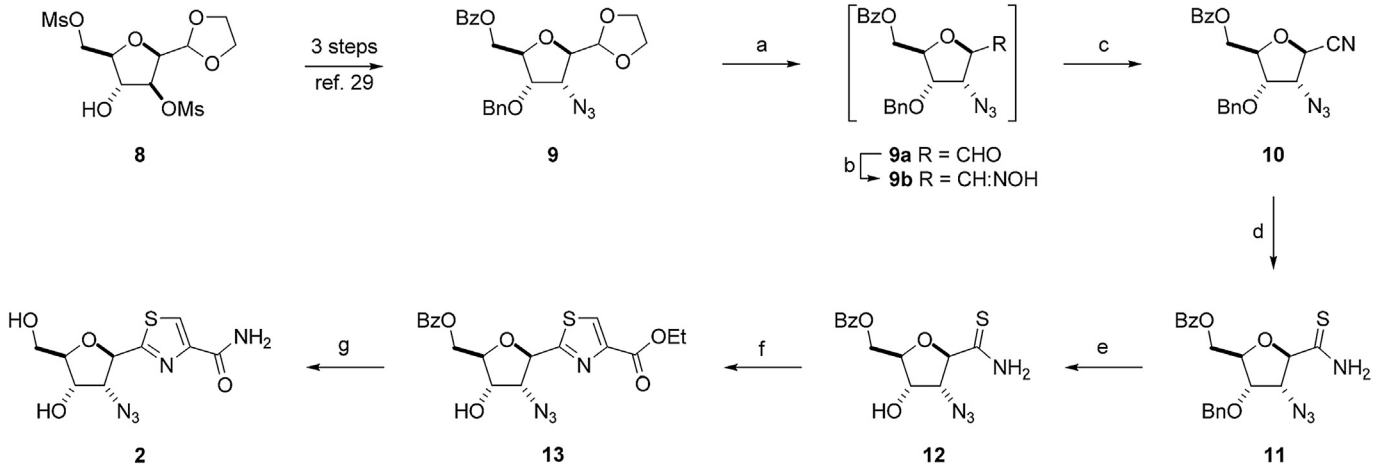
Accordingly, compound **16** was allowed to react with cysteine ethyl ester hydrochloride, in the presence of Et_3N at room temperature, to afford thiazoline **17** as an inseparable mixture of C-4 epimers. As the newly formed stereocenter was to be destroyed during the formation of the aromatic thiazole ring, no attempts were made to analyze the diastereomeric ratio of this mixture. The epimers **17** were subsequently treated with DBU and $BrCCl_3$ to give the expected thiazole **18** in a non-optimized yield of 64%, over the last two steps. Ester aminolysis followed by global deprotection, which was effected by treatment of **18** in methanolic ammonia, provided target **2** in 93% yield. The latter nine-step sequence (Scheme 2) represents a more convenient route for the preparation of analogue **2**, since it provided a considerably higher overall yield (18% from **8**) compared to the six-step route presented in Scheme 1 (2% from **8**).

Compound **2** was converted to desired 2-amino-2-deoxy derivative **3** (65%) after catalytic reduction in EtOH over 10% Pd/C. However, when the catalytic reduction of **2** was carried out under the similar reaction conditions, but in the presence of chloroform [31], the corresponding amine hydrochloride **4** was obtained in 89% yield.

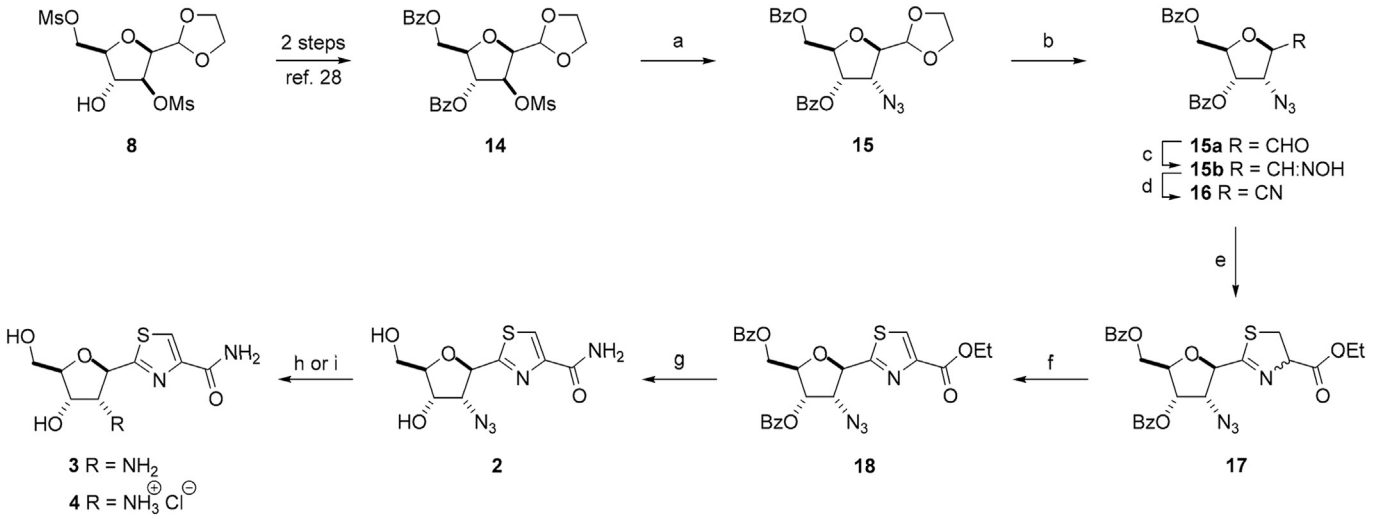
2,5-Anhydro derivative **15** has been conveniently used as a divergent intermediate for the synthesis of 2′-acetamido derivative **5** (Scheme 3). Catalytic hydrogenation of **15** over PtO_2 in a mixture of glacial acetic acid and acetic anhydride gave the corresponding acetamido derivative **19** in 68% yield. Compound **19** was converted to target **5**, by using a six-step sequence similar to that already applied for the conversion of **15** to target **2** (Scheme 2). Thus, hydrolytic removal of the dioxolane protective group in **19** was achieved in a mixture of trifluoroacetic acid and 6 M hydrochloric acid at +4 °C. The resulting crude aldehyde **19a** was treated with hydroxylamine hydrochloride to yield the corresponding oxime **19b** as a mixture of *E*- and *Z*-isomers. An attempted separation of *E*-**19b** and *Z*-**19b** by column chromatography provided only pure *E*-**19b** (44%). As the corresponding *Z*-isomer could not be obtained free of *E*-**19b** the purification step is omitted, and the mixture of *E*- and *Z*-**19b** was converted to the corresponding ribofuranosyl cyanide **20** in an overall yield of 39% (from the last three steps). Exposure of **20** to hydrogen sulphide gas gave the thioamide **21** (86%). The subsequent cyclocondensation of **21** with ethyl bromopyruvate afforded the corresponding thiazole **22** (73%), which was finally converted to target **5** (62%) after treatment with methanolic ammonia.

The two independent routes toward the thiazole *C*-nucleoside **6**, bearing the *N*-benzoyl functionality at the C-2′ position, are outlined in Scheme 4.

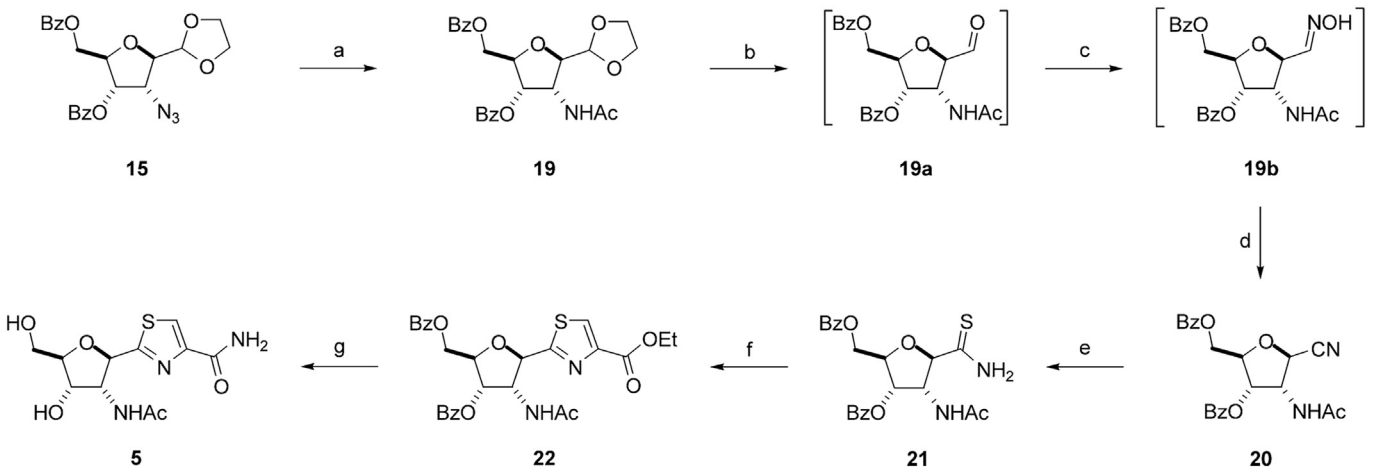
The first route started from 2,5-anhydride **23**, which was converted to the target **6** via the three-step sequence The first route started from 2,5-anhydride **23**, which was converted to the target **6** via the three-step sequence similar to that previously described in the preliminary account [29]. In this way, the glycosyl cyanide **23** was converted into the target *C*-nucleoside **6** in 31% overall yield. The second route for the preparation of **6** started from azido-nitrile **16**, which was converted to the required thioamide **26** through the one-pot H_2S-mediated cascade that we recently used for the preparation of tiazofurin analogues embedded with an amide moiety at the C-2′ position [27]. As expected, exposure of **16** to hydrogen sulphide gas gave the thioamide **26** as a result of the



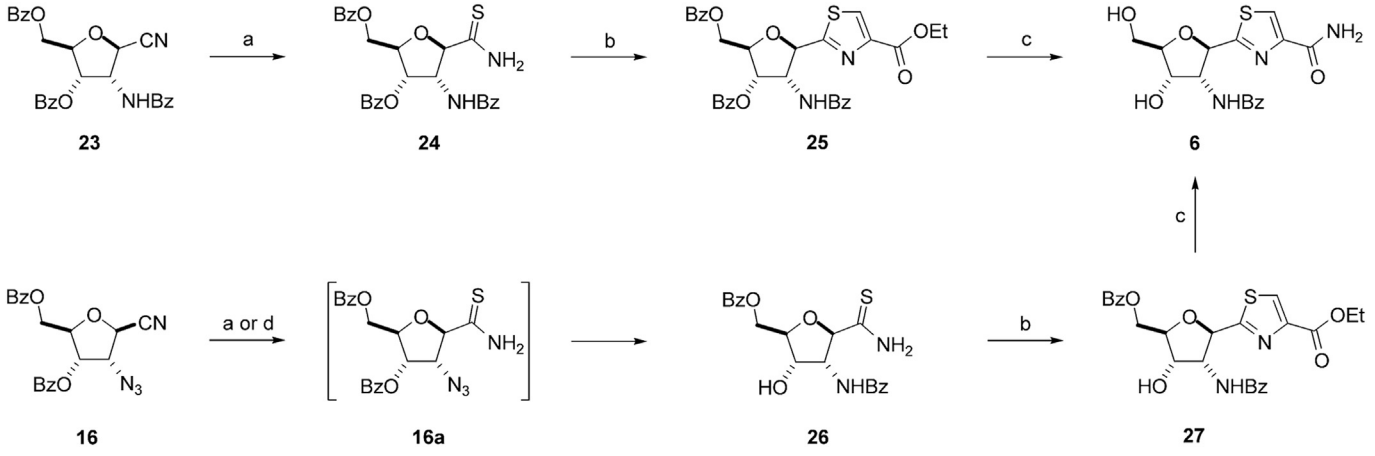
Scheme 1. Reagents and conditions: (a) 4:1 TFA/6 M HCl, 4 °C, 72 h; (b) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, CH_2Cl_2, st, 2 h; (c) MsCl, Py, −15 °C, 0.5 h, then rt, 2 h, 39% from 9; (d) H_2S, Py, rt, 2 h, 34%; (e) $TiCl_4$, CH_2Cl_2, 0 °C, 1 h, 67% (f) $BrCH_2COCO_2Et$, EtOH, 80 °C, 50 min, 57%; (g) NH_3, MeOH, rt, 6 days, 75%.



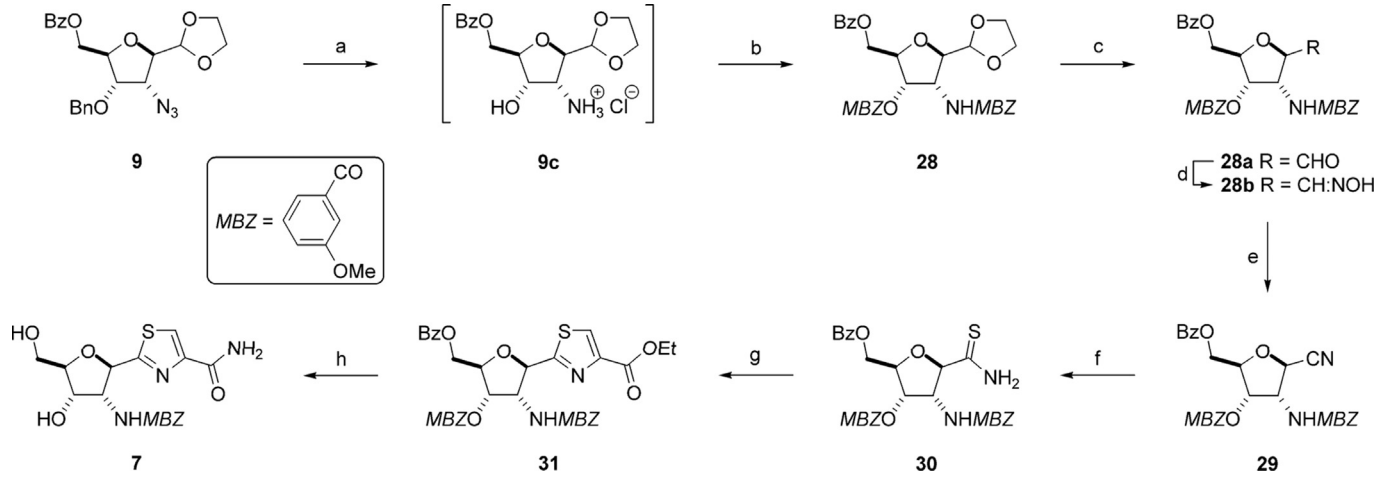
Scheme 2. Reagents and conditions: (a) NaN_3, DMSO, 108–110 °C, 24 h, 61%; (b) 4:1 TFA/6 M HCl, 4 °C, 140 h; (c) $NH_2OH \cdot HCl$, NaOAc, EtOH, CH_2Cl_2, rt, 2 h; (d) MsCl, Py, −15 °C, 0.5 h, then rt, 2 h, 59% from 15; (e) cysteine ethyl ester hydrochloride, Et_3N, MeOH, rt, 2 h, 76%; (f) DBU, $BrCCl_3$, CH_2Cl_2, 0 °C, 5 h → 4 °C, 17 h, 84%; (g) NH_3, MeOH, rt, 7 days, 93% (h) H_2–Pd/C, EtOH, rt, 36 h, 65% of 3; (i) H_2–Pd/C, $CHCl_3$, EtOH, rt, 36 h, 89% of 4.



Scheme 3. Reagents and conditions: (a) H_2, PtO_2, AcOH, Ac_2O, rt, 48 h, 68%; (b) 3:1 TFA/6 M HCl, 4 °C, 10 days; (c) $NH_2OH \cdot HCl$, NaOAc, EtOH, CH_2Cl_2, rt, 2 h; (d) MsCl, Py, −15 °C, 0.5 h, then rt, 2 h, 39% from 19; (e) H_2S, Py, rt, 8 h, 86%; (f) $BrCH_2COCO_2Et$, EtOH, reflux, 50 min, 73%; (g) NH_3, MeOH, rt, 8 days, 62%.



Scheme 4. Reagents and conditions: (a) H_2S, Py, rt, 8 h for 23, 74% of 24, 1.5 h for 16, 71% of 26, 25% of 16a; (b) $BrCH_2COCO_2Et$, EtOH, reflux, 50 min for 24, 78% of 25, 80 min for 26, 55% of 27; (c) NH_3, MeOH, rt, 8 days for 25, 53% of 6, 7 days for 27, 56% of 6; (d) H_2S, DMAP, EtOH, rt, 8 h, 82%.



Scheme 5. Reagents and conditions: (a) H_2, Pd/C, $CHCl_3$, EtOH, rt, 168 h, (b) 3-methoxybenzoyl chloride, Py, rt, 72 h, 63% from 9; (c) 3:1 TFA/6 M HCl, 4 °C, 10 days; (d) $NH_2OH \cdot HCl$, NaOAc, EtOH, CH_2Cl_2, rt, 2.5 h; (e) MsCl, Py, −15 °C, 0.5 h, then rt, 2 h, 50% calculated to reacted 28; (f) H_2S, Py, rt, 8 h, 93%; (g) $BrCH_2COCO_2Et$, EtOH, reflux, 50 min, 60%; (h) NH_3, MeOH, rt, 7 days, 72%.

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 process. Depending on the reaction conditions and reagents used, the major product **26** was accompanied with a variable amount of **16a**. Thus when **16** was reacted with hydrogen sulphide in dry pyridine for 1.5 h at room temperature both **26** and **16a** were obtained in 71% and 25% yields, respectively. However, when the reaction was carried out with hydrogen sulphide and *N*,*N*-dimethylaminopyridine in ethanol (for 8 h at room temperature), the key intermediate **26** was obtained as the only reaction product in a yield of 82%. Moreover, thioamide **26** was treated with ethyl bromopyruvate in refluxing ethanol to give the protected thiazole **27** in 55% yield. Final exposure of **27** to methanolic ammonia provided analogue **6** in 56% yield. This nine-step sequence, which was carried out over the intermediate **16**, provided the target **6** in 8% overall yield with respect to starting 2,5-anhydride **8**. An alternative sequence, realized over **23**, is of same efficiency regarding the total yield (8% from **8**), even though it requires two additional synthetic steps for the completion.

The synthesis of tiazofurin mimic **7** is outlined in Scheme 5. The sequence started with catalytic reduction of the azide group in **9** (10% Pd/C, in the presence of $CHCl_3$) to give the amine hydrochloride **9c** as a product of sequential azide reduction/benzyl ether hydrogenolysis process. Treatment of crude **9c** with 3-methoxybenzoyl chloride in pyridine gave the fully protected product **28** (63% from **9**). Compound **28** was converted to target **7** using the six-step sequence shown in Scheme 5. Final product **7** was obtained in an overall yield of 13% from the last eight steps.

2.2. Antiproliferative activities

Antiproliferative activities of synthesized tiazofurin analogues were evaluated against seven tumour cell lines (myelogenous leukaemia K562, promyelocytic leukaemia HL-60, Jurkat T cells leukaemia, Raji Burkitt's lymphoma, colon adenocarcinoma HT-29, breast adenocarcinoma MCF-7, cervix carcinoma HeLa), and a single human normal cell line (MRC-5). The use of foetal lung fibroblasts (MRC-5) serves to demonstrate the toxicity of synthesized compounds toward normal cells. Cytotoxicity was evaluated by using the standard MTT assay, after exposure of cells to the tested compounds for 72 h. Tiazofurin (**1**) was used as a reference compound in this assay.

As shown in Table 1, of all synthesized compounds, only analogue **6** has a similar profile of cytotoxicity as tiazofurin, although it was completely inactive against HeLa cells, toward which lead **1** showed strong antiproliferative activity (3.82 μM). Analogue **6** exhibited potent to moderate antiproliferative effects against seven of eight investigated cell lines. Particularly strong antiproliferative activities (IC_{50} 0.01–0.03 μM) of analogue **6** were observed in the cultures of the following malignant cells: K562 (over 90-fold more active then lead **1**), Jurkat (a similar activity as **1**), Raji (over 500-fold more active then **1**), and HT-29 (13-fold more active then **1**). The remaining five analogues (**2**–**5** and **7**) showed selective cytotoxicities only against individual cancer cell lines. Thus, analogue **2** exhibited the remarkable antiproliferative activities against K562 (IC_{50} 0.31 μM, 6-fold more potent than **1**) and MCF-7 cells (IC_{50} 4 nM, 445-fold more potent than **1**). Against HeLa

Table 1
In vitro cytotoxicity of tiazofurin (1) and analogues 2–7.

Compounds	IC_{50} (μM)[a]							
	K562	HL-60	Jurkat	Raji	HT-29	MCF-7	HeLa	MRC-5
1	1.89	0.19	0.04	5.28	0.26	1.78	3.82	0.36
2	0.31	>100	>100	>100	>100	0.004	4.08	>100
3	0.51	12.17	>100	2.59	>100	>100	0.15	>100
4	>100	0.02	>100	9.28	>100	>100	>100	>100
5	>100	>100	>100	14.74	>100	3.92	>100	5.26
6	0.02	20.43	0.03	0.01	0.02	19.86	>100	0.05
7	>100	2.69	23.58	6.29	>100	9.54	>100	>100

[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%.

Table 2
Distribution of K562 cells in cell cycle phases (%) after 24- and 72-h cell treatment.

Compound	Sub-G1		G0/G1		S		G2/M	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
control	1.05	3.04	36.28	45.92	48.38	35.73	14.29	15.31
1 (tiazofurin)	2.22	24.99	37.08	35.66	53.16	33.62	7.55	5.74
2	2.93	10.90	42.67	42.24	42.80	35.04	11.60	11.82
3	1.40	13.52	32.21	40.24	46.33	34.08	20.06	12.16
4	2.61	11.13	35.02	44.32	47.22	33.29	15.16	11.26
5	3.71	11.17	43.07	48.09	45.03	31.42	8.21	9.32
6	6.12	11.29	39.08	44.10	49.13	34.11	5.67	10.50
7	4.44	10.84	39.45	43.13	46.71	34.00	9.41	12.04

cells this molecule showed the similar potency as control compound **1**. At the same time, this analogue represents the most active compound synthesized in this work. Analogue **3** was more potent than lead **1** against the following malignant cell lines: K562 (IC_{50} 0.51 μM, almost 4-fold more active than **1**), Raji (IC_{50} 2.59 μM, 2-fold more active than **1**) and HeLa (IC_{50} 0.15 μM, over 25-fold more active than **1**). The corresponding amine hydrochloride **4** however, was more potent than lead **1** only against HL-60 cell line (IC_{50} 0.02 μM, 9.5-fold more active than **1**). This molecule is also active against Raji cells, but shows almost 2-fold weaker cytotoxicity than tiazofurin (**1**). Analogues **5** and **7** gave the poorest results in the biological assay, as neither of these two compounds was more active than tiazofurin (**1**) toward any of the cell lines under evaluation. In the preliminary communication [29] we have reported the results of in vitro antitumour activity of analogues **2** and **6** recorded after 24-h cells treatment. As expected, compound **2** exhibited significantly stronger activities after 72-h cells treatment (Table 1), including a submicromolar activity against K562 cell line (IC_{50} 0.31 μM). However, this molecule was completely inactive after treatment of this cell line for 24 h [29]. Analogue **6** behaves similarly but with one exception: the treatment of HL-60 cells for 72 h (Table 1) results in a lower potency (IC_{50} 20.43 μM) relative to the submicromolar activity recorded after 24-h cells treatment (IC_{50} 0.12 μM) [29]. The synthesized tiazofurin isosteres **2**–**4** are fully non-toxic towards normal human MRC-5 cells while lead compound **1**, as well as analogues **5** and **6** exhibited submicromolar toxicity to the normal cells. The large variations in the results for each cell line suggest that compounds **2**–**7** are not acting at the same biological target as tiazofurin (IMPDH) and points to a different mechanism of action, probably through induction of apoptosis. Despite its strong toxicity toward normal cells, we believe that analogue **6** represents the best candidate for further optimization because of its strong antiproliferative activity against the majority of tumour cell lines under evaluation.

2.3. Cell cycle analysis

The cell cycle profile of exponentially growing K562 cells treated with synthesized compounds for 24 and 72 h was analyzed by flow cytometry in cells stained with propidium iodide. Untreated cells served as a control. The results are presented in Table 2 (for graphic presentation see Fig. S1 in the Supplementary data). As the data in Table 2 indicate, a 24-h treatment of K562 cells with analogues **2**–**7** slightly change the percentage of cells in G0/G1 and S phase of cell cycle compared to control. As it is further seen from Table 2, analogues induce various changes in G2/M phase of cell cycle compared to both tiazofurin and control: analogue **3** significantly increases the percentage of G2M phase cells, while tiazofurin and analogues **5**–**7** decrease it. After the exposure of K562 cells to analogues **2**–**7** during 72 h the increased percentage of cells in G0/G1 phase was detected when compared to tiazofurin, while this percentage is almost the same with respect to control. The percentage of cells in S phase was similar to the values obtained for both tiazofurin and control. All analogues induced a similar percentage of cells in G2/M phase, with the values similar to those obtained after 24-h treatment. However, each of these values was approximately 2-fold higher than the value detected after a 72-h treatment with tiazofurin.

After 72-h cells treatment, the percentage of cells in sub-G1 phase (which is suggestive of apoptosis) was significantly higher than the values recorded after 24-h treatment of cells. After prolonged treatment, each of the synthesized compounds introduced 3–4 times more cells in sub-G1 phase relative to control. Tiazofurin caused significant apoptosis only after 72 h, as observed in our previous study [26].

Based upon these results it may be concluded that synthesized tiazofurin analogues affect the distribution of K562 cells in the cell cycle phases. These changes depend on the nature of the analogue as well as the duration of cells treatment with the tested compounds.

2.4. Detection of apoptosis

As data in Table 2 revealed, all tested compounds increased percentage of K562 cells in sub-G1 phase compared with untreated control. These findings suggested that synthesized analogues may induce apoptosis. Percentage of apoptotic, necrotic and live cells was determined with flow cytometry after double staining of treated cells with Annexin V-FITC/PI and the results are shown in Table 3 (for a graphic presentation see Fig. S2 in the Supplementary data).

Apoptotic response, which was presented as a percentage of specific apoptosis, shows that all analogues increase the percentage of Annexin V-positive cells compared to tiazofurin. Percentage of specific apoptosis increases with prolonging treatment time and after 72 h is 2- to 4-fold higher than the values recorded after 24 h. Compound **6** causes the greatest specific apoptosis in both examined periods (29% after 24 h and 50.41% after 72 h).

The percentage of specific necrosis also increases with increasing incubation time. The highest percentage of specific necrosis causes analogue **5** (24.55% after 72 h), while the other analogues showed slightly lower values (5–10%).

To resolve the mechanisms underlying the apoptosis induced with synthesized tiazofurin analogues (**2**–**7**) we have studied the ability of these compounds to modulate expression of selected apoptosis markers, such as Bcl-2, Bax, caspase 3 and PARP. The results are presented in Fig. 2 (for more details see pages S10 and S11 in the Supplementary data).

Western blot analysis indicates that analogues **3** and **4** after a 24-h treatment decrease the expression of Bcl-2 in comparison to the control and tiazofurin, while analogues **2**, **5**, **6** and **7** slightly increase the total amount of the protein compared to the control. However, this increase is much higher when compared to

Table 3
Percentage of specific apoptosis and necrosis induced with synthesized compounds in the K562 cell culture (24- and 72-h cell treatment).

Compound	Specific apoptosis (%)		Specific necrosis (%)	
	24 h	72 h	24 h	72 h
1 (tiazofurin)	−0.34	13.71	3.76	10.71
2	10.73	38.06	−0.82	5.33
3	17.57	33.39	4.97	7.11
4	10.71	30.26	3.60	6.22
5	12.60	43.60	5.88	24.55
6	29.00	50.41	11.71	6.86
7	11.88	37.07	3.68	10.33

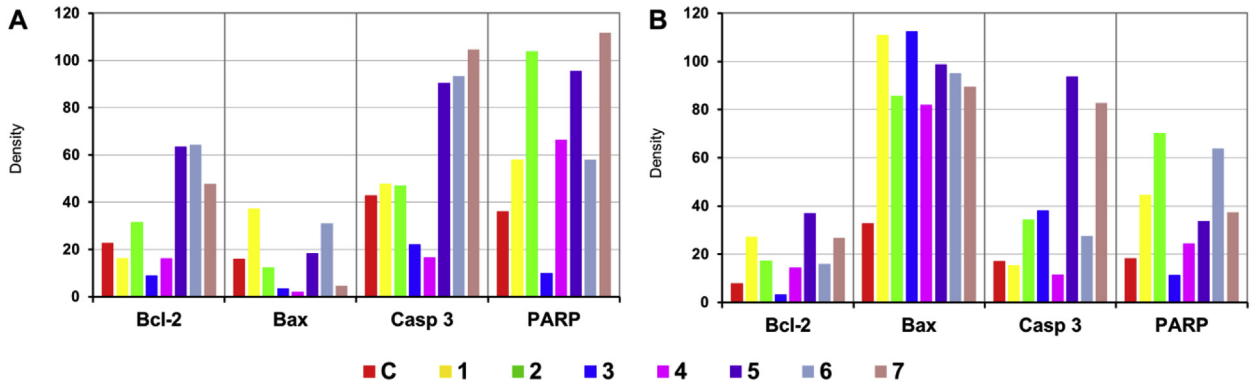


Fig. 2. Results of Western blot analysis after treatment of K562 cells with synthesized compounds for 24 h (A) and 72 h (B).

tiazofurin. After 72-h treatment analogue **3** reduces expression of anti apoptotic Bcl-2 protein with respect to both control and tiazofurin, while analogues **2** and **4** slightly increased the total amount of same protein compared to untreated control only. The greatest decrease of the expression of Bcl-2 protein is caused by the compounds **3** and **4** after both 24 and 72 h of cells treatment.

Some chemotherapeutics induce apoptosis that exerts a significant expression of Bax. However, in our experiments, tiazofurin analogues did not effect the expression of Bax protein after 24 h. Expression of Bax protein is increased after 72-h treatment with all of the synthesized compounds, as well as tiazofurin. These results are in agreement with increase of the specific apoptosis and also with higher percent of cells in sub-G1 phase of cell cycle, after treatment of cells for 72 h.

Expression levels of caspase 3 precursor and the corresponding active subunit in cells treated with tiazofurin and analogues, were determined in order to verify whether these compounds induce apoptosis through a caspase-dependent way. It was confirmed that treatment of K562 cells with all of the synthesized analogues increases expression of the precursor of caspase 3, as well as the corresponding active moiety. It was also found that the expression level of active subunit of caspase 3 depends on the nature of the analogue, as well as the time of treatment. After 24 h, most of the analogues (except **3** and **4**) including lead **1** enhance the expression of the caspase 3, clearly indicating its participation in the apoptotic process.

Activation of caspase 3 leads to cleavage of different "downstream" proteins, including poly (ADP-ribose) polymerase (PARP), which is specifically cleaved on a 24 kDa DNA-binding domain to give a catalytic fragment of 89 kDa [32,33]. PARP participates in repairing damaged DNA chains during the cell cycle and represent one of the biochemical hallmarks of apoptosis. Western blot analysis confirmed the proteolytic cleavage of PARP upon treatment of K562 cells with all synthesized analogues. In comparison to other analogues, molecule **3** causes the weakest cleavage of PARP.

3. Conclusions

Six tiazofurin mimics (**2**–**7**) bearing the nitrogen functionalities at the C-2′ position have been synthesized in multistep sequences starting from monoacetone D-glucose. The synthetic strategy to targets **2**–**7** assumed an initial preparation of the corresponding ribofuranosyl thioamides as key intermediates followed by their subsequent cyclo-condensation with ethyl bromopyruvate to form the thiazole ring. An alternative and more efficient procedure for the construction of the thiazole ring by avoiding the use of hydrogen sulphide gas has also been developed. It comprises of cysteine cyclocondensation with ribofuranosyl cyanide **16**, followed by subsequent treatment of the resulting thiazoline **17** with DBU and $BrCCl_3$. Final exposure of protected *C*-nucleosides to methanolic ammonia provided targets **2**–**7** in good yields.

Synthesized compounds were evaluated for their antiproliferative activity against a panel of human malignant cell lines. 2′-Azido derivative **2** showed the highest potency toward MCF-7 cells (IC_{50} 0.004 μM) being the most active compound under evaluation. Analogue **6** has shown potent to moderate antiproliferative effects against seven of eight investigated cell lines. Remarkable antiproliferative activities of this analogue were recorded in the following cell cultures: K562 (over 90-fold more active then lead **1**), Jurkat (a similar activity as **1**), Raji (over 500-fold more active then **1**), and HT-29 (13-fold more active then **1**). It should be also emphasized that both analogues **2** and **6** exhibit stronger antitumour potencies after cells treatment for 72 h unlike the preliminary published results recorded after the cells treatment for 24 h [29]. Analogues **3** and **4** also exhibited potent activities, but only against individual cancer cell lines. The cell cycle analysis reveals that following treatment of cells with synthesized compounds, there was an increase in the sub-G1 peak indicating that the mechanism of action of these compounds probably involves apoptosis. The flow-cytometry further confirmed a significant percentage of specific apoptosis that was detected after treatment with majority of analogues for both exposure times. Western blot analysis of apoptosis markers (Bcl-2, Bax, caspase 3, PARP) suggested that all analogues induced apoptosis in K562 cells in caspase-dependent way.

4. Experimental section

4.1. General experimental procedures

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. ^1H (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) and band positions (ν_{max}) are given in cm^{-1}. Low resolution mass spectra were recorded on Finnigan-MAT 8230 (CI), VG AutoSpec (FAB) spectrometers and on an Agilent Technologies HPLC/MS 3Q system, series 1200/6410 (ESI). High resolution mass spectra (ESI) of synthesized compounds were acquired on an Agilent technologies 6210 TOF LC/MS instrument (LC series 1200). Column chromatography was performed on Kieselgel 60 (<0.063 mm, E. Merck). Flash column chromatography was performed using Kieselgel 60 (0.040–0.063, E. Merck). Preparative TLC

was performed on hand-made plates, 20 × 20 cm size with ~1 mm layer thickness. Kieselgel 60 G (E. Merck) with fluorescent indicator F254 as additive was used as a stationary phase. The corresponding bands were scraped and eluted with 1:1 EtOAc/^{i}PrOH. 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. General procedure A: Synthesis of ribofuranosyl cyanides

A solution of acetal **9**, **15**, **19** or **28** (1 equiv) in a 4:1 mixture of TFA/6 M HCl (~0.25 M) was kept at +4 °C until the starting materials were consumed (TLC, 70 h for **9**, 140 h for **15**, 240 h for **19** and **28**). The mixture was concentrated to a third of the initial volume and poured into saturated aq $NaHCO_3$. The aqueous solution was rendered alkaline with solid $NaHCO_3$ to pH 8–9 and extracted with CH_2Cl_2. The combined extracts were washed successively with saturated aq $NaHCO_3$ and water, dried and evaporated. The remaining crude aldehyde (**9a**, **15a**, **19a**, or **28a**) was immediately dissolved in a mixture of EtOH/CH_2Cl_2 (~0.25 M) and treated with $NH_2OH\cdot HCl$ (~2 equiv) and NaOAc (~3 equiv) while stirring at room temperature until the starting materials were consumed (TLC, 2 h for **9a**, **15a** and **19a**; 2.5 h for **28a**). The mixture was evaporated and the residue distributed between water and CH_2Cl_2. The organic layer was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic solutions were washed with water, dried and evaporated to afford crude oxime (**9b**, **15b**, **19b** or **28b**) as a mixture of the corresponding *E*- and *Z*-isomers. To a cooled (−15 °C) and stirred solution of **9b**, **15b**, **19b** or **28b** in anhydrous pyridine (~0.8 M) was added dropwise during 0.5 h a cold solution of MsCl (~5 equiv) in dry pyridine (4.6 M). The mixture was first stirred at 0 °C for 0.5 h, then at room temperature for 2 h and then poured into a 1:1 mixture of ice and concentrated HCl (pH ~2). The emulsion was extracted with CH_2Cl_2, the combined extracts were washed with water, saturated aq $NaHCO_3$ and again with water. The extract was dried and evaporated, and the residue was purified on a column of silica gel.

4.3. General procedure B: Synthesis of ribofuranosyl thioamides

Through a solution of ribofuranosyl cyanides (1 mmol) in anhydrous pyridine (0.1–0.2 M) was passed H_2S gas at room temperature until the starting materials were consumed (TLC, 2 h for **10**, 8 h for **20**, **23** and **29**). The solvent was evaporated in vacuum and the residue was purified on a column of silica gel, or by crystallization.

4.4. General procedure C: Hantzsch thiazole synthesis

To a solution of thioamide **12**, **21**, **24**, **26** or **30** (1 equiv) in absolute EtOH (0.1 M) was added ethyl bromopyruvate (1.5–2 equiv) and the mixture was stirred under reflux until the starting materials were consumed (TLC, 50 min for **12**, **21**, **24** and **30**, 80 min for **26**). The solvent was evaporated in vacuum and the residue was purified on a column of silica gel.

4.5. General procedure D: Ester amonolysis

A solution of protected thiazole **13**, **18**, **22**, **25**, **27** or **31** (1 mmol) in methanolic ammonia (0.05 M) was kept at room temperature for 7 days, then evaporated and the residue was purified on a column of silica gel or by preparative TLC.

4.6. 2,5-Anhydro-3-azido-6-O-benzoyl-4-O-benzyl-3-deoxy-D-allononitrile (*10*)

Prepared from **9** by General procedure A. Purified by column chromatography (4:1 light petroleum/Me_2CO). Yield 40%. Colourless syrup, $[\alpha]_D^{20}$ +17.2 (*c* 1.0, $CHCl_3$). IR (film): ν_{max} 2280, 2100, 1730. ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.29 (dd, 1H, $J_{2,3}$ = 3.2, $J_{3,4}$ = 4.6 Hz, H-3), 4.33–4.47 (m, 3H, H-4, H-5 and H-6a), 4.52 (dd, 1H, $J_{5,6b}$ = 2.8, $J_{6a,6b}$ = 11.9 Hz, H-6b), 4.63 and 4.75 (2 × d, 2H, J_{gem} = 11.6 Hz, CH_2Ph), 4.65 (d, 1H, $J_{2,3}$ = 3.2, H-2), 8.09–7.29 (m, 10H, 2 × Ph). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 62.5 (C-6), 64.1 (C-3), 69.0 (C-2), 73.5 (*C*H_2Ph), 78.4 (C-4), 80.1 (C-5), 116.3 (C-1), 128.1, 128.4, 128.6, 128. 7, 129.2, 129.7, 133.3, 136.0 (2 × Ph), 166.0 (Ph*C* = O). LRMS (CI): *m*/*z* 379 (M^++H).

4.7. 2,5-Anhydro-3-azido-6-O-benzoyl-4-O-benzyl-3-deoxy-D-allonothioamide (*11*)

Prepared from **10** by General procedure B. Purified by column chromatography (17:1 toluene/EtOAc). Yield 34%. Colourless syrup, $[\alpha]_D^{20}$ +106.3 (*c* 1.1, $CHCl_3$). IR (film): ν_{max} 2110, 1720, 1536, 1315, 1112. ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.02 (dd, 1H, $J_{3,4}$ = 3.8, $J_{4,5}$ = 8.2 Hz, H-4), 4.42 (m, 1H, $J_{5,6a}$ = 2.7, $J_{5,6b}$ = 4.9 Hz, H-5), 4.48–4.59 (m, 3H, PhCH_a, 2 × H-6), 4.67–4.74 (m, 2H, H-2 and H-3), 4.78 (d, 1H, J_{gem} = 11.6 Hz, PhCH_b), 7.22–7.93 (m, 10H, 2 × Ph), 8.12 and 8.53 (2 × bs, 2H, NH_2). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 63.1 (C-6), 65.9 (C-3), 72.9 (*C*H_2Ph), 76.6 (C-4), 79.4 (C-5), 87.0 (C-2), 127.9, 128.3, 128.46, 128.5, 129.0, 129.4, 133.4, 136.2 (2 × Ph), 166.4 (Ph*C* = O), 203.1 (C═S). LRMS (CI): *m*/*z* 413 (M^++H).

4.8. 2,5-Anhydro-3-azido-6-O-benzoyl-3-deoxy-D-allonothioamide (*12*)

To a cooled (0 °C) and stirred solution of **11** (0.038 g, 0.09 mmol) in dry CH_2Cl_2 (2 mL) was added $TiCl_4$ (0.02 mL, 0.18 mmol) in anhydrous CH_2Cl_2 (0.3 mL). The mixture was first stirred at 0 °C for 40 min, then poured into a mixture of ice and water and the resulting suspension was extracted with CH_2Cl_2 (4 × 2 mL). The combined extracts were washed with saturated aq $NaHCO_3$ (1 mL) and with water (1 mL), dried and evaporated. The residue was purified by column chromatography (8:1 → 4:1 toluene/EtOAc) to give pure **12** (0.020 g, 67%) as a colourless oil, $[\alpha]_D^{20}$ +46.8 (*c* 1.0, $CHCl_3$). IR (film): ν_{max} 3350, 2110, 1500, 1310, 1100. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.74 (bs, 1H, OH), 4.20 (dd, 1H, $J_{4,5}$ = 7.0, $J_{3,4}$ = 4.9 Hz, H-4), 4.27 (td, 1H, $J_{5,6a}$ = 2.4, $J_{5,6b}$ = 4.9 Hz, H-5), 4.50–4.60 (m, 2H, H-3 and H-6a), 4.66 (dd, 1H, $J_{5,6b}$ = 4.9, $J_{6a,6b}$ = 13.6 Hz, H-6b), 4.74 (d, 1H, $J_{2,3}$ = 3.4 Hz, H-2), 7.41–8.09 (m, 5H, Ph), 7.76 and 8.50 (2 × bs, 2H, NH_2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 63.6 (C-6), 68.7 (C-3), 71.1 (C-4), 81.7 (C-5), 86.8 (C-2), 128.7, 129.1, 129.6, 133.8 (Ph), 166.9 (Ph*C* = O), 203.5 (C═S). LRMS (CI): *m*/*z* 323 (M^++H).

4.9. Ethyl 2-(2-azido-5-O-benzoyl-2-deoxy-β-D-ribofuranosyl) thiazole-4-carboxylate (*13*)

Prepared Prepared from **12** by General procedure C. Purified by column chromatography (8:1 → 4:1 toluene/EtOAc). Yield 57%. Colourless syrup, $[\alpha]_D^{20}$ −2.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.39 (t, 3H, *J* = 7.0 Hz, $CO_2CH_2CH_3$), 3.08 (bs, 1H, OH), 4.17–4.50 (m, 5H, $CO_2CH_2CH_3$, H-2′, H-3′ and H-4′), 4.54 (dd, 1H, $J_{4',5'a}$ = 3.9, $J_{5'a,5'b}$ = 12.9 Hz, H-5′a), 4.70 (dd, 1H, $J_{5'a,5'b}$ = 12.9, $J_{4',5'b}$ = 2.9 Hz, H-5′b), 5.29 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 7.40–8.07 (m, 5H, Ph), 8.08 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 14.2 ($CO_2CH_2CH_3$), 61.6 ($CO_2CH_2CH_3$), 63.6 (C-5′), 67.9 (C-2′), 72.3 (C-3′), 80.8 (C-1′), 81.7 (C-4′), 128.1 (C-5), 128.4, 129.6, 129.7, 133.3 (Ph),

147.5 (C-4), 161.2 (C-2), 166.4 (PhC = O), 171.0 ($CO_2CH_2CH_3$). HRMS (ESI+): *m/e* 419.1032 (M^++H). Calcd for $C_{18}H_{19}N_4O_6S$: 419.1025.

4.10. 2,5-Anhydro-3-azido-4,6-di-O-benzoyl-3-deoxy-D-allose ethylene acetal (**15**)

To a solution of **14** [34] (4.66 g, 9.46 mmol) in DMSO (80 mL) was added NaN_3 (5.34 g, 82.22 mmol), and the resulting suspension was stirred at 108–110 °C for 24 h. The mixture was poured in water (200 mL) and extracted with 1:1 benzene-hexane (4 × 80 mL). The extract was washed with water (100 mL), dried and evaporated. The remaining crude mixture was purified by flash column chromatography (17:3 cyclohexane/Me_2CO) to afford pure **15** (1.95 g, 47%) as a colourless oil, $[\alpha]_D^{20}$ −31.7 (*c* 1.0, $CHCl_3$). Less pure fractions were additionally purified by preparative TLC (17:3 cyclohexane/Me_2CO) to give an additional amount of pure product **15** (0.570 g, total yield 61%). IR (KBr): ν_{max} 2100, 1730. ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.88–4.10 (m, 4H, 2 × CH_2-dioxolane), 4.22 (dd, 1H, $J_{1,2}$ = 2.8, $J_{2,3}$ = 5.3 Hz, H-2), 4.34 (t, 1H, *J* = 5.7 Hz, H-3), 4.46–4.55 (m, 2H, H-5 and H-6a), 4.62 (dd, 1H, $J_{6a,6b}$ = 13.3, $J_{5,6b}$ = 5.5 Hz, H-6b), 5.08 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1), 5.55 (t, 1H, *J* = 5.6 Hz, H-4), 7.35–8.13 (m, 10H, 2 × Ph). NOE contact: H-1 and H-3. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 60.8 (C-3), 63.8 (C-6), 65.4 and 65.6 (2 × CH_2-dioxolane), 74.3 (C-4), 79.5 (C-5), 82.2 (C-2), 102.2 (C-1), 128.3, 128.5, 128.7, 129.6, 129.8, 133.1, 133.6 (2 × Ph), 165.7 and 166.1 (2 × PhC = O). LRMS (FAB): *m/z* 462 (M^++Na). Anal. Found: C, 60.10; H, 4.85; N, 9.80. Calcd for $C_{22}H_{21}N_3O_7$: C, 60.14; H, 4.78; N, 9.57.

4.11. 2,5-Anhydro-3-azido-4,6-di-O-benzoyl-3-deoxy-D-allononitrile (**16**)

Prepared from **15** (via **15a** and **15b**) by General procedure A. Purified by column chromatography (19:1 toluene/EtOAc). Yield 59%. Colourless syrup, $[\alpha]_D^{20}$ −48.9 (*c* 1.0, $CHCl_3$). IR (film): ν_{max} 2280, 2100, 1730. ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.56–4.68 (m, 3H, H-5 and 2 × H-6), 4.70 (d, 1H, $J_{2,3}$ = 4.9 Hz, H-2), 4.75 (t, 1H, *J* = 4.9 Hz, H-3), 5.74 (t, 1H, *J* = 4.9 Hz, H-4), 7.40–8.14 (m, 10H, 2 × Ph). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 62.9 (C-6), 64.7 (C-3), 69.2 (C-2), 73.2 (C-4), 80.7 (C-5), 116.0 (C-1), 128.1, 128.4, 128.9, 129.1, 129.6, 129.9, 133.3, 134.0 (2 × Ph), 165.3 and 165.91 (2 × PhC = O). LRMS (FAB): *m/z* 415 (M^++Na). HRMS (ESI+): *m/z* 415.1028 (M^++Na). Calcd for $C_{20}H_{16}N_4O_5Na$: 415.1018.

4.12. Ethyl 2-(2-azido-3,5-di-O-benzoyl-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxylate (**18**)

To a stirred solution of **16** (0.904 g, 2.30 mmol) in dry MeOH (10 mL) was added L-cysteine ethyl ester hydrochloride (0.640 g, 3.45 mmol) followed by Et_3N (0.45 mL, 3.22 mmol). The reaction mixture was stirred for 2 h at room temperature and evaporated. The residue was purified by flash column chromatography (19:1 → 9:1 toluene/EtOAc) to afford thiazoline **17** (0.924 g, 76%) as a mixture of C-4 epimers. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.27 (t, 3H, *J* = 7.1 Hz, $CO_2CH_2CH_3$), 3.27–3.61 (m, 2H, 2 × H-5), 4.22 (q, 2H, $CO_2CH_2CH_3$), 4.43–4.71 (m, 4H, H-2′, H-4′, 2 × H-5′), 4.92 (d, 1H, $J_{1',2'}$ = 6.2 Hz, H-1′), 5.07–5.18 (2 × t, 0.51 and 0.37H, $J_{4,5}$ = 8.8 Hz, H-4), 5.61 (t, 1H, *J* = 5.0 Hz, H-3′), 7.34–8.12 (m, 10H, 2 × Ph). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 13.8 ($CO_2CH_2CH_3$), 34.2 and 34.3 (2 × H-5), 61.6 ($CO_2CH_2CH_3$), 63.3 and 63.4 (C-5′), 64.3 and 64.5 (C-2′), 73.8 (C-3′), 80.5 (C-4), 80.7 and 80.8 (C-1′ and C-4′), 128.1, 128.4, 128.7, 129.2, 129.4, 129.4, 129.6, 132.9, 133.4 (2 × Ph), 165.2, 165.7, 169.77, 169.8, 173.5, 174.1 (PhC = O, $CO_2CH_2CH_3$ and C-2).

To a stirred solution of **17** (0.924 g, 1.76 mmol) in anhydrous CH_2Cl_2 (18 mL) was added DBU (0.53 mL, 3.50 mmol) while stirring. The solution was cooled to 0 °C and $BrCCl_3$ (0.21 mL, 2.10 mmol) was added during 15 min. The reaction mixture was stirred for 5 h at 0 °C and then stored for 17 h at +4 °C. The mixture was evaporated and the residue was purified by flash column chromatography (4:1 cyclohexane/Me_2CO) to give pure **18** (0.775 g, 84%) as a colourless oil, $[\alpha]_D^{20}$ −65.6 (*c* 1.8, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.41 (t, 3H, *J* = 7.0 Hz, $CO_2CH_2CH_3$), 4.43 (q, 2H, $CO_2CH_2CH_3$), 4.58 (dd, 1H, $J_{4',5'a}$ = 3.7, $J_{5'a,5'b}$ = 12.0 Hz, H-5′a), 4.61–4.75 (m, 2H, H-2′ and H-4′), 4.78 (dd, 1H, $J_{4',5'b}$ = 3.3, $J_{5'a,5'b}$ = 12.0 Hz, H-5′b), 5.41 (d, 1H, $J_{1',2'}$ = 5.1 Hz, H-1′), 5.64 (t, 1H, *J* = 5.6 Hz, H-3′), 7.38–8.10 (m, 10H, 2 × Ph), 8.08 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 14.2 ($CO_2CH_2CH_3$), 61.5 ($CO_2CH_2CH_3$), 63.3 (C-5′), 66.2 (C-2′), 73.6 (C-3′), 80.3 (C-1′), 81.1 (C-4′), 128.1 (C-5), 128.4, 128.6, 128.9, 129.3, 129.6, 129.9, 133.2, 133.8 (2 × Ph), 147.7 (C-4), 161.0 (C-2), 165.5 and 165.9 (2 × PhC = O), 169.7 ($CO_2CH_2CH_3$). HRMS (ESI+): *m/e* 523.1284 (M^++H). Calcd for $C_{25}H_{23}N_4O_7S$: 523.1287.

4.13. 2,5-Anhydro-3-acetamido-4,6-di-O-benzoyl-3-deoxy-D-allose ethylene acetal (**19**)

A solution of **15** (0.90 g, 2.05 mmol) in glacial AcOH (4.5 mL) and Ac_2O (4.5 mL), was hydrogenated over PtO_2 (0.011 g) at normal pressure of hydrogen, at rt for 48 h. The suspension was filtered and the catalyst washed with MeOH (5 mL). The filtrate was concentrated and residual AcOH removed by co-distillation with a 1:1 mixture of toluene/MeOH (6 × 10 mL). Column chromatography on silica gel (7:3 → 3:7 toluene/EtOAc) gave pure **19** (0.637 g, 68%) as a colourless solid. Recrystallization from a mixture of CH_2Cl_2/hexane gave colourless needles, mp 190.5–191.5 °C, $[\alpha]_D^{20}$ −75.6 (*c* 1.2, $CHCl_3$). IR (KBr): ν_{max} 3300, 1730, 1660, 1570, 1380, 1290. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.97 (s, 3H, CH_3CO), 3.81–4.05 (m, 4H, 2 × CH_2-dioxolane), 4.02 (dd, 1H, $J_{1,2}$ = 2.9, $J_{2,3}$ = 9.0 Hz, H-2), 4.51 (m, 2H, H-5 and H-6a), 4.60 (dd, 1H, $J_{6a,6b}$ = 12.7, $J_{5,6b}$ 5.4 Hz, H-6b), 4.99 (td, 1H, $J_{3,NH}$ = 8.4, $J_{3,4}$ = 5.8 Hz, H-3), 5.10 (d, 1H, $J_{1,2}$ = 2.9 Hz, H-1), 5.62 (dd, 1H, $J_{4,5}$ = 1.7, $J_{3,4}$ = 5.8 Hz, H-4), 5.86 (d, 1H, $J_{3,NH}$ = 8.4 Hz, AcN*H*), 7.39–8.12 (m, 10H, 2 × Ph). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 23.1 (CH_3CO), 50.9 (C-3), 64.2 (C-6), 65.4 and 65.7 (2 × CH_2-dioxolane), 75.2 (C-4), 81.3 (C-2), 81.8 (C-5), 102.6 (C-1), 128.4, 128.6, 129.2, 129.55, 129.6, 129.8, 133.1, 133.6 (2 × Ph), 165.3 and 166.2 (2 × PhC = O), 169.8 (CH_3CO). LRMS (CI): *m/z* 456 (M^++H). Anal. Found: C, 62.15; H, 5.64; N, 3.07. Calcd for $C_{24}H_{25}NO_8 \cdot 0.5H_2O$: C, 62.06; H, 5.64; N, 3.01.

4.14. 2,5-Anhydro-3-acetamido-4,6-di-O-benzoyl-3-deoxy-D-allononitrile (**20**)

Prepared from **19** (via **19a** and **19b**) by General procedure A. Purified by column chromatography (3:2 toluene/EtOAc). Yield 39%. Colourless needles, mp 156–157 °C (CH_2Cl_2/hexane), $[\alpha]_D^{20}$ −66.0 (*c* 1.4, $CHCl_3$). IR (KBr): ν_{max} 3300, 1730, 1660, 1540, 1275. ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.00 (s, 3H, CH_3CO), 4.59 (bs, 3H, H-5 and 2 × H-6), 4.77 (d, 1H, $J_{2,3}$ = 8.1 Hz, H-2), 5.28 (dt, 1H, $J_{3,NH}$ = 8.2, $J_{3,4}$ = 6.1 Hz, H-3), 5.61 (dd, 1H, $J_{3,4}$ = 6.1, $J_{4,5}$ 2.0 Hz, H-4), 6.23 (d, 1H, $J_{3,NH}$ = 8.2 Hz, AcN*H*), 7.38–8.18 (m, 10H, 2 × Ph). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 22.9 (CH_3CO), 54.9 (C-3), 63.6 (C-6), 68.9 (C-2), 73.5 (C-4), 82.7 (C-5), 116.6 (C-1), 128.4, 128.6, 128.8, 129.0, 129.7, 129.8, 133.5, 134.1 (2 × Ph), 165.1, 166.2 (2 × PhC = O), 170.4 (CH_3CO). LRMS (CI): *m/z* 407 (M^+−H). Anal. Found: C, 64.77; H, 4.78; N, 6.72. Calcd for $C_{22}H_{20}N_2O_6$: C, 64.70; H, 4.94; N, 6.86.

4.15. 2,5-Anhydro-3-acetamido-4,6-di-O-benzoyl-3-deoxy-D-allonothioamide (**21**)

Prepared from **20** by General procedure B. Purified by crystallization. Yield 86%. Colourless needles, mp 190 °C (Me_2CO),

$[\alpha]_D^{20}$ −111.5 (*c* 1.1, $CHCl_3$). IR (KBr): ν_{max} 1724, 1655, 1541, 1273. ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.78 (s, 3H, C*H*$_3$CO), 4.45–4.60 (bs, 3H, H-5 and 2 × H-6), 4.65 (d, 1H, $J_{2,3}$ = 8.5 Hz, H-2), 4.74 (m, 1H, H-3), 5.40 (dd, 1H, $J_{3,4}$ = 5.2, $J_{4,5}$ = 2.7 Hz, H-4), 7.41–8.11 (m, 10H, 2 × Ph), 8.54 (d, 1H, $J_{3,NH}$ = 7.9 Hz, AcN*H*), 9.00 and 9.86 (2 × bs, 2H, CSN*H*$_2$). ^{13}C NMR (62.5 MHz, DMSO-d_6): δ 22.7 (*C*H$_3$CO), 56.2 (C-3), 65.0 (C-6), 74.2 (C-4), 81.2 (C-5), 85.1 (C-2), 129.1, 129.2, 129.4, 129.6, 129.8, 130.0, 134.0, 134.2 (2 × Ph), 165.6 and 166.3 (2 × Ph*C* = O), 170.3 (CH$_3$*C*O), 202.5 (C═S). LRMS (FAB): *m*/*z* 443.1 (M^++H). HRMS (ESI+): *m*/*z* 465.1078 (M^++Na). Calcd for $C_{22}H_{22}N_2O_6SNa$: 465.1096.

4.16. Ethyl 2-(2-acetamido-3,5-di-O-benzoyl-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxylate (22)

Prepared from **21** by General procedure C. Purified by column chromatography (7:3 → 7:4 CH_2Cl_2/EtOAc). Yield 73%. Colourless syrup, $[\alpha]_D^{20}$ −40.2 (*c* 1.0, $CHCl_3$). IR (film): ν_{max} 1732, 1539, 1372, 1268. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.35 (t, 3H, *J* = 7.0 Hz, $CO_2CH_2CH_3$), 1.95 (s, 3H, C*H*$_3$CO), 4.56–4.69 (m, 2H, H-4′ and H-5′a), 4.82 (dd, 1H, $J_{4',5'b}$ = 4.0, $J_{5'a,5'b}$ = 12.5 Hz, H-5′b), 4.94 (ddd, 1H, $J_{1',2'}$ = 10.1, $J_{2',NH}$ = 8.9, $J_{2',3'}$ = 5.5 Hz, H-2′), 5.45 (d, 1H, $J_{1',2'}$ = 10.1 Hz, H-1′), 5.73 (d, 1H, $J_{2',3'}$ = 5.5 Hz, H-3′), 6.69 (d, 1H, $J_{2',NH}$ = 8.9 Hz, AcN*H*), 7.35–8.21 (m, 10H, 2 × Ph), 8.07 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 14.2 ($CO_2CH_2CH_3$), 22.9 (*C*H$_3$CO), 57.8 (C-2′), 61.4 ($CO_2CH_2CH_3$), 75.1 (C-3′), 79.5 (C-1′), 83.1 (C-4′), 128.55, 128.6, 129.0, 129.3, 129.6, 129.8, 133.3, 133.7 (2 × Ph), 128.4 (C-5), 146.7 (C-4), 160.9 (C-2), 165.4 and 166.2 (2 × Ph*C* = O), 170.0 and 170.6 (CH$_3$*C*O and *C*O$_2$CH$_2$CH$_3$). LRMS (CI): *m*/*z* 539 (M^++H). HRMS (ESI+): *m*/*e* 539.1492 (M^++H). Calcd for $C_{27}H_{27}N_2O_8S$: 539.1488.

4.17. 2,5-Anhydro-3-benzamido-4,6-di-O-benzoyl-3-deoxy-D-allonothioamide (24)

Prepared from **23** by General procedure B. Purified by crystallization. Yield 74%. Colourless needles, mp 162–163 °C (MeOH), $[\alpha]_D^{20}$ −130.9 (*c* 1.2, $CHCl_3$). IR (KBr): ν_{max} 1722, 1654, 1528, 1316, 1273, 1110. ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.44 (m, 1H, H-3), 4.61 (t, 1H, *J* = 4.1 Hz, H-5), 4.80 (pseudo d, 2H, 2 × H-6), 5.00 (d, 1H, $J_{2,3}$ = 10.4 Hz, H-2), 5.94 (bd, 1H, $J_{3,4}$ = 5.1 Hz, H-4), 7.21 (d, 1H, $J_{3,NH}$ = 4.9 Hz, PhCON*H*), 7.33–8.18 (m, 15H, 3 × Ph), 7.88 and 8.45 (2 × bs, 2H, CSN*H*$_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 57.4 (C-3), 64.3 (C-6), 74.4 (C-4), 83.6 (C-5), 84.5 (C-2), 127.1, 128.3, 128.5, 128.52, 128.6, 129.0, 129.1, 129.6, 129.8, 131.8, 133.5, 133.6 (3 × Ph), 165.6, 167.1 and 167.6 (2 × Ph*C* = O and Ph*C*ONH), 202.4 (C═S). LRMS (CI): *m*/*z* 505 (M^++H). Anal. Found: C, 61.69; H, 5.43; N, 5.05; S, 5.98. Calcd for $C_{27}H_{24}N_2O_6S \cdot 1.5\ CH_3OH$: C, 61.94; H, 5.47; N, 5.07; S, 5.80.

4.18. Ethyl 2-(2-benzamido-3,5-di-O-benzoyl-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxylate (25)

Prepared from **24** by General procedure C. Purified by column chromatography (4:1 toluene/EtOAc). Yield 78%. Colourless syrup, $[\alpha]_D^{20}$ −102.6 (*c* 1.2, $CHCl_3$). IR (KBr): ν_{max} 1724, 1665, 1532, 1270. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.34 (t, 3H, *J* = 7.0 Hz, $CO_2CH_2CH_3$), 4.36 (q, 2H, $CO_2CH_2CH_3$), 4.65–4.75 (m, 2H, $J_{4',5a'}$ = 3.3 Hz, H-4′ and H-5a′), 4.90 (dd, 1H, $J_{4',5'b}$ = 4.2, $J_{5'a,5'b}$ = 13.0 Hz, H-5′b), 5.06 (ddd, 1H, $J_{1',2'}$ = 10.1, $J_{2',3'}$ = 5.3, $J_{2',NH}$ = 8.4 Hz, H-2′), 5.66 (d, 1H, $J_{1',2'}$ = 10.1 Hz, H-1′), 5.87 (bd, 1H, $J_{2',3'}$ = 5.3, $J_{3',4'}$ = 0.6 Hz, H-3′), 7.00 (d, 1H, $J_{2',NH}$ = 8.4 Hz, PhCON*H*), 7.32–8.26 (m, 15H, 3 × Ph), 8.14 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 14.3 ($CO_2CH_2CH_3$), 58.6 (C-2′), 61.4 ($CO_2CH_2CH_3$), 64.2 (C-5′), 75.2 (C-3′), 79.4 (C-1′), 83.3 (C-4′), 128.3 (C-5), 128.5, 128.6, 128.7, 129.0, 129.4, 129.6, 129.8, 131.8, 133.4, 133.5, 133.8 (3 × Ph), 146.8 (C-4), 161.0 (C-2), 165.4 and 166.3 (2 × Ph*C* = O), 167.8 (*C*O$_2$CH$_2$CH$_3$), 169.8 (Ph*C*ONH). LRMS (CI): *m*/*z* 601 (M^++H). HRMS (ESI+): *m*/*e* 601.1653 (M^++H). Calcd for $C_{32}H_{29}N_2O_8S$: 601.1645.

4.19. 2,5-Anhydro-3-benzamido-4,6-di-O-benzoyl-3-deoxy-D-allonothioamide (26)

Procedure A. Through a solution of **16** (0.065 g, 0.16 mmol) in anhydrous pyridine (2 mL) was passed H_2S gas at room temperature for 1.5 h. The mixture was evaporated and the residue was purified on a column of silica gel (8:1 toluene/EtOAc). Eluted first was the minor product **16a** (0.018 g, 25%) isolated as a colourless oil, $[\alpha]_D^{20}$ −34.4 (*c* 1.2, $CHCl_3$). IR (film): ν_{max} 2116, 1716, 1522, 1315, 1111. ^{1}H NMR (250 MHz, DMSO-d_6): δ 4.56–4.75 (m, 4H, H-3, H-5 and 2 × H-6), 4.84 (d, 1H, $J_{2,3}$ = 4.4 Hz, H-2), 5.46 (t, 1H, *J* = 5.9 Hz, H-4), 7.41–8.15 (m, 10H, 2 × Ph), 7.76 and 8.50 (2 × bs, 2H, $CSNH_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 63.6 (C-6), 66.5 (C-3), 72.7 (C-4), 80.3 (C-5), 86.8 (C-2), 128.5, 128.6, 128.7, 129.0, 129.6, 130.0, 133.7, 133.9 (2 × Ph), 165.6 and 166.7 (2 × Ph*C* = O), 202.6 (C═S). LRMS (CI): *m*/*z* 427 (M^++H).

Eluted second was the major product **26** (0.047 g, 71%) isolated as a colourless solid, mp 188 °C (EtOH), $[\alpha]_D^{20}$ −91.9 (*c* 1.0, Me_2CO).

Procedure B: Through a solution of **16** (0.516 g, 1.32 mmol) and DMAP (0.005 g, 0.04 mmol) in absolute EtOH (7 mL) was passed H_2S gas at room temperature for 8 h. The mixture was evaporated and the residue was purified on a column of silica gel (5:1 → 7:3 toluene/EtOAc, 1:1 EtOAc/^{i}PrOH), to afford pure **26** (0.434 g, 82%) as a colourless solid. Recrystallization from EtOH gave colourless crystals, mp 188 °C, $[\alpha]_D^{20}$ −91.9 (*c* 1.0, Me_2CO). IR (KBr): ν_{max} 3375, 1720, 1655, 1535, 1530, 1300, 1275, 1140. ^{1}H NMR (250 MHz, DMSO-d_6): δ 4.17 (m, 1H, $J_{3,4}$ = 4.3, $J_{4,5}$ = 3.7 Hz, H-4), 4.27 (m, 1H, H-5), 4.50 (pseudo d, 2H, $J_{5,6}$ = 4.3 Hz, 2 × H-6), 4.64 (m, 1H, H-3), 4.70 (d, 1H, $J_{2,3}$ = 7.9 Hz, H-2), 5.61 (d, 1H, $J_{4,OH}$ = 4.9 Hz, OH), 7.42–8.10 (m, 10H, 2 × Ph), 8.40 (d, 1H, $J_{3,NH}$ = 7.9 Hz, PhCON*H*), 9.01 and 9.87 (2 × bs, 2H, $CSNH_2$). ^{13}C NMR (62.5 MHz, DMSO-d_6): δ 58.2 (C-3), 65.4 (C-6), 71.2 (C-4), 83.0 (C-5), 85.1 (C-2), 127.8, 128.3, 129.0, 129.5, 129.6, 131.4, 133.6, 134.7 (2 × Ph), 166.0 and 166.4 (Ph*C* = O and Ph*C*ONH), 203.5 (C═S). LRMS (CI): *m*/*z* 401 (M^++H). Anal. Found: C, 59.80; H, 5.23; N, 7.02; S, 8.01. Calcd for $C_{20}H_{20}N_2O_5S$: C, 59.99; H, 5.03; N, 7.00; S, 8.00.

4.20. Ethyl 2-(2-benzamido-5-O-benzoyl-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxylate (27)

Prepared from **26** by General procedure C. Purified by column chromatography (7:3 → 3:2 → 1:1 toluene/EtOAc). Yield 55%. Colourless syrup, $[\alpha]_D^{20}$ −35.8 (*c* 1.0, $CHCl_3$). IR (film): ν_{max} 3450, 1735, 1670, 1530, 1290. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.26 (t, 3H, *J* = 7.0 Hz, $CO_2CH_2CH_3$), 4.23 (q, 2H, $CO_2CH_2CH_3$), 4.41–4.82 (m, 6H, H-2′, H-3′, H-4′, 2 × H-5′ and OH), 5.49 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1′), 7.14–8.15 (m, 11H, 2 × Ph and PhCON*H*), 8.03 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 14.1 ($CO_2CH_2CH_3$), 60.5 (C-2′), 61.5 ($CO_2CH_2CH_3$), 64.6 (C-5′), 72.5 (C-3′), 79.5 (C-1′), 84.4 (C-4′), 127.2, 128.2, 128.4, 129.5, 129.7, 131.6, 133.2, 133.4 (2 × Ph), 128.2 (C-5), 146.5 (C-4), 161.2 (C-2), 166.4 (PhCO_2), 168.6 and 171.7 (*C*O$_2$CH$_2$CH$_3$ and Ph*C*ONH). LRMS (CI): *m*/*z* 496 (M^+). HRMS (ESI+): *m*/*e* 519.1208 (M^++Na). Calcd for $C_{25}H_{24}N_2NaO_7S$: 519.1202.

4.21. 2,5-Anhydro-6-O-benzoyl-3-deoxy-3-(3-methoxybenzamido)-4-O-(3-methoxybenzoyl)-D-allose ethylene acetal (28)

A solution of **9** [35] (2.05 g, 4.82 mmol) in a mixture of absolute EtOH (70 mL) and $CHCl_3$ (3.45 mL) was hydrogenated over 10% Pd/C

(4.57 g) at 1 atm, for 168 h at room temperature. The mixture was filtered and the catalyst washed with EtOH. The filtrate and washings were combined and evaporated to give crude **9c** as a colourless solid. Crude **9c** was dissolved in anhydrous pyridine (38 mL) and 3-methoxybenzoyl chloride (2.85 mL, 20.92 mmol) was added to the solution. The mixture was left at room temperature for 72 h, then poured onto ice and acidified with 6 M HCl (pH 3–4), and the resulting suspension was extracted with CH_2Cl_2 (4 × 50 mL). The extract was washed with water (20 mL), saturated aq $NaHCO_3$ (20 mL) and again with water (20 mL), dried and evaporated to afford a colourless solid. Recrystallization from MeOH gave pure **28** (1.758 g, 63%) as white crystals, mp 159–160 °C, $[\alpha]_D^{20}$ −56.2 (*c* 1.0, $CHCl_3$). IR (KBr): ν_{max} 3330, 1730, 1640, 1540, 1280. ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.76 and 3.79 (2 × s, 3H each, 2 × OC*H*$_3$), 3.83–4.10 (m, 4H, 2 × CH_2-dioxolane), 4.21 (dd, 1H, $J_{1,2}$ = 2.7, $J_{2,3}$ = 8.7 Hz, H-2), 4.50–4.65 (m, 2H, H-5 and H-6a), 4.66 (dd, 1H, $J_{5,6b}$ = 5.3, $J_{6a,6b}$ = 12.7 Hz, H-6b), 5.12 (m, 1H, H-3), 5.19 (d, 1H, $J_{1,2}$ = 2.7 Hz, H-1), 5.73 (dd, 1H, $J_{3,4}$ = 6.4, $J_{4,5}$ = 1.0 Hz, H-4), 6.43 (d, 1H, $J_{3,NH}$ = 8.2 Hz, MeOC$_6$H$_4$CON*H*), 6.94–8.17 (m, 13H, Ph and 2 × MeOC$_6$*H*$_4$). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 51.4 (C-3), 55.3 and 55.4 (2 × O*C*H$_3$), 64.2 (C-6), 65.5 and 65.8 (2 × CH_2-dioxolane), 75.3 (C-4), 81.6 (C-2), 81.9 (C-5), 102.6 (C-1), 112.1, 114.2, 118.1, 118.6, 119.9, 121.8, 128.4, 129.6, 129.8, 130.4, 133.1, 135.4, 159.6, 159.8 (Ph and 2 × MeOC$_6$H$_4$), 165.2, 166.2 and 167.0 (3 × C=O). LRMS (CI): *m/z* 578 (M^++H). Anal. Found: C, 64.43; H, 5.35; N, 3.11. Calcd for $C_{31}H_{31}NO_{10}$: C, 64.46; H, 5.41; N, 2.42.

4.22. 2,5-Anhydro-6-O-benzoyl-3-deoxy-3-(3-methoxybenzamido)-4-O-(3-methoxybenzoyl)-D-allononitrile (29)

Prepared from **28** (via **28a** and **28b**) by General procedure A. Purified by column chromatography (17:3 toluene/EtOAc). Yield 50%. Colourless needles, mp 146.5–147.5 °C (MeOH), $[\alpha]_D^{20}$ −83.2 (*c* 1.2, $CHCl_3$). IR (KBr): ν_{max} 3400, 1730, 1640, 1530, 1275. ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.76 and 3.80 (2 × s, 3H each, 2 × OCH_3), 4.64 (bs, 3H, H-5 and 2 × H-6), 4.90 (d, 1H, $J_{2,3}$ = 7.6 Hz, H-2), 5.41 (m, 1H, $J_{3,4}$ = 6.0 Hz, H-3), 5.70 (dd, 1H, $J_{3,4}$ = 6.0, $J_{4,5}$ = 2.4 Hz, H-4), 6.76 (d, 1H, $J_{3,NH}$ = 7.9 Hz, MeOC$_6$H$_4$CON*H*), 6.96–8.22 (m, 13H, Ph and 2 × MeOC$_6$*H*$_4$). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 55.3 and 55.4 (2 × O*C*H$_3$), 55.5 (C-3), 63.6 (C-6), 69.3 (C-2), 73.7 (C-4), 82.6 (C-5), 116.6 (C-1), 112.2, 114.4, 116.6, 118.7, 120.3, 121.8, 128.6, 129.1, 129.6, 129.7, 129.8, 133.4, 134.2, 159.7, 159.8 (Ph and 2 × MeOC$_6$H$_4$), 165.0, 166.2 and 167.4 (3 × C=O). LRMS (CI): *m/z* 530 (M^++H). Anal. Found: C, 65.86; H, 5.21; N, 5.45. Calcd for $C_{29}H_{26}N_2O_8$: C, 65.65; H, 4.94; N, 5.28.

4.23. 2,5-Anhydro-6-O-benzoyl-3-deoxy-3-(3-methoxybenzamido)-4-O-(3-methoxybenzoyl)-D- allonothioamide (30)

Prepared from **29** by General procedure B. Purified by crystallization. Yield 93%. Colourless needles, mp 149–150 °C (CH_2Cl_2/hexane), $[\alpha]_D^{20}$ −128.0 (*c* 1.1, $CHCl_3$). IR (KBr): ν_{max} 1721, 1644, 1532, 1317, 1275, 1106. ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.75 and 3.78 (2 × s, 3H each, 2 × OCH_3), 4.38 (m, 1H, H-3), 4.60 (t, 1H, *J* = 3.6 Hz, H-5), 4.79 (pseudo d, 2H, $J_{5,6}$ = 3.6 Hz, 2 × H-6), 4.96 (d, 1H, $J_{2,3}$ = 10.4 Hz, H-2), 5.92 (d, 1H, $J_{3,4}$ = 5.2 Hz, H-4), 7.17 (d, 1H, $J_{3,NH}$ = 4.9 Hz, PhCON*H*), 7.76 and 8.46 (2 × bs, 2H, CSNH_2), 6.98–8.20 (m, 13H, Ph and 2 × MeOC$_6$*H*$_4$). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 55.6 (OCH_3), 57.8 (C-3), 64.6 (C-6), 74.8 (C-4), 83.9 (C-5), 84.8 (C-2), 112.4, 114.5, 118.6, 119.3, 120.3, 122.3, 129.0, 129.4, 129.9, 130.1, 130.7, 134.0, 135.2159.9, 160.0 (Ph and 2 × MeOC$_6$H$_4$), 165.8, 167.4 and 167.7 (3 × C=O), 202.8 (C=S). LRMS (CI): *m/z* 565 (M^++H). Anal. Found: C, 61.47; H, 5.09; N, 5.10; S, 5.79. Calcd for $C_{29}H_{28}N_2O_8S$: C, 61.69; H, 5.00; N, 4.96; S, 5.68.

4.24. Ethyl 2-[5-O-benzoyl-2-deoxy-2-(3-methoxybenzamido)-3-O-(3-methoxybenzoyl)-β-D-ribofuranosyl]thiazole-4-carboxylate (31)

Prepared from **30** by General procedure C. Purified by column chromatography (4:1 toluene/EtOAc). Yield 60%. Colourless syrup, $[\alpha]_D^{20}$ −99.9 (c 1.2, $CHCl_3$). IR (film): ν_{max} 1724, 1666, 1532, 1273. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.33 (t, 3H, *J* = 7.1 Hz, $CO_2CH_2CH_3$), 3.75 and 3.82 (2 × s, 3H each, 2 × OCH_3), 4.40 (q, 2H, $CO_2CH_2CH_3$), 4.77 (m, 2H, $J_{4',5'a}$ = 3.3 Hz, H-4′ and H-5′a), 4.96 (dd, 1H, $J_{4',5'b}$ = 4.3, $J_{5'a,5'b}$ = 13.0 Hz, H-5′b), 5.08 (ddd, 1H, $J_{1',2'}$ = 10.1, $J_{2',3'}$ = 5.4, $J_{2',NH}$ = 9.1 Hz, H-2′), 5.65 (d, 1H, $J_{1',2'}$ = 10.1 Hz, H-1′), 5.91 (dd, 1H, $J_{2',3'}$ = 5.4, $J_{3',4'}$ = 1 Hz, H-3′), 7.12 (d, 1H, $J_{2',NH}$ = 9.1 Hz, PhCON*H*), 7.01–8.30 (m, 13H, Ph and 2 × MeOC$_6$H$_4$), 8.19 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 14.2 ($CO_2CH_2CH_3$), 55.2 and 55.4 (2 × OCH_3), 58.6 (C-2′), 61.4 ($CO_2CH_2CH_3$), 64.2 (C-5′), 75.2 (C-3′), 79.4 (C-1′), 83.2 (C-4′), 112.0, 112.1, 114.1, 118.5, 119.0, 120.2, 121.9, 128.4, 129.3, 129.5, 129.8, 130.2, 133.3, 134.8159.67, 159.7 (Ph and 2 × MeOC$_6$H$_4$), 128.0 (C-5), 146.8 (C-4), 160.9 (C-2), 165.3, 166.2 and 167.7 (3 × C=O), 169.8 ($CO_2CH_2CH_3$). LRMS (FAB): *m/z* 683 (M^++Na). HRMS (ESI+): *m/e* 683.1663 (M^++Na). Calcd for $C_{34}H_{32}N_2O_{10}SNa$: 683.1675.

4.25. 2-(2-Azido-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (2)

Prepared from **13** by General procedure D. Purified by preparative TLC (5:1 $CHCl_3$/MeOH). Yield 75% (from **13**), 93% (from **18**). Colourless needles, mp 160 °C (MeOH/^{i}Pr$_2$O), $[\alpha]_D^{20}$ −48.2 (*c* 0.8, CH_3OH). For spectral data see ref. 29.

4.26. 2-(2-Amino-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (3)

To a stirred solution of **2** (0.133 g, 0.47 mmol) in absolute EtOH (14 mL) was added 10% Pd/C (0.043 g). The suspension was hydrogenated at room temperature and normal pressure of H_2 for 24 h. The suspension was filtered through a celite pad, washed with EtOH and evaporated to a colourless powder. Recrystallization from a mixture MeOH/^{i}Pr$_2$O afforded pure **3** (0.079 g, 65%), as colourless needles, mp 164 °C, $[\alpha]_D^{20}$ −10.4 (*c* 0.5, H_2O). IR (film): ν_{max} 3433, 3365, 3298, 3200, 1688, 1598, 1241. ^{1}H NMR (250 MHz, methanol-d_4): δ 3.36 (m, 1H, H-2′), 3.71 (d, 2H, $J_{4',5'}$ = 4.7 Hz, H-5′), 4.08–4.21 (m, 2H, H-3′ and H-4′), 5.42 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1′), 8.22 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, methanol-d_4): δ 61.9 (C-2′), 63.3 (C-5′), 74.1 (C-3′), 83.0 (C-1′), 88.3 (C-4′), 126.3 (C-5), 150.1 (C-4), 166.1 (C-2), 173.5 ($CONH_2$). LRMS (CI): *m/z* 260 (M^++H). Anal. Found: C, 41.80; H, 5.46; N, 15.64; S, 11.95. Calcd for $C_9H_{13}N_3O_4S$: C, 41.69; H, 5.05; N, 16.21; S, 12.37.

4.27. 2-(2-Amino-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide amine hydrochloride (4)

A solution of **2** (0.076 g, 0.267 mmol) in a mixture of dry EtOH (7 mL) and $CHCl_3$ (0.7 mL) was hydrogenated over 10% Pd/C (0.025 g) for 24 h at room temperature. The mixture was filtered through a celite pad and the catalyst washed with EtOH. The filtrate and washings were combined and evaporated to give crude **4** (0.070 g, 89%) as a colourless solid. Recrystallization from a mixture MeOH/^{i}Pr$_2$O afforded pure **4**, as colourless needles, mp 223 °C, $[\alpha]_D^{20}$ −78.8 (*c* 0.9, H_2O). IR (film): ν_{max} 3440, 2924, 1670, 1460. ^{1}H NMR (250 MHz, D_2O): δ 3.74 (dd, 1H, $J_{4',5'a}$ = 4.9, $J_{5'a,5'b}$ = 12.5 Hz, H-5′a), 3.83 (dd, 1H, $J_{5'a,5'b}$ = 12.5, $J_{4',5'b}$ = 3.4 Hz, H-5′b), 4.06 (dd, 1H, $J_{1',2'}$ = 7.4, $J_{2',3'}$ = 5.8 Hz, H-2′), 4.24 (m, 1H, $J_{3'4'}$ = 3.8 Hz, H-4′), 4.53 (dd, 1H, $J_{2',3'}$ = 5.8, $J_{3'4'}$ = 3.8 Hz, H-3′), 5.42 (d, 1H, $J_{1',2'}$ = 7.4 Hz, H-

1′), 8.25 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, D_2O): δ 60.0 (C-2′), 64.0 (C-5′), 72.6 (C-3′), 80.9 (C-1′), 89.5 (C-4′), 129.6 (C-5), 151.0 (C-4), 168.0 (C-2), 171.5 (*C*ONH$_2$). LRMS (CI): *m*/*z* 296 (M$^+$+H). Anal. Found: C, 34.48; H, 4.77; N, 13.13; S, 10.35. Calcd for $C_9H_{14}ClN_3O_4S\cdot H_2O$: C, 34.45; H, 5.14; N, 13.39; S, 10.22.

4.28. 2-(2-Acetamido-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (**5**)

Prepared from **22** by General procedure D. Purified by column chromatography (4:1 → 7:3 → 3:2 → 1:1 → 1:2 EtOAc/^{i}PrOH). Yield 62%. Colourless syrup, $[\alpha]_D^{20}$ −11.6 (*c* 1.1, CH_3OH). IR (film): ν_{max} 3304, 1667, 1550, 1377, 1295. ^{1}H NMR (250 MHz, methanol-d_4): δ 2.00 (s, 3H, C*H*$_3$CO), 3.73 (pseudo d, 2H, $J_{4',5'}$ = 4.5 Hz, 2 × H-5′), 4.11 (m, 1H, $J_{3',4'}$ = 2.8 Hz, H-4′), 4.24 (dd, 1H, $J_{2',3'}$ = 5.4, $J_{3',4'}$ = 2.8 Hz, H-3′), 4.49 (dd, 1H, $J_{1',2'}$ = 8.4, $J_{2',3'}$ = 5.4 Hz, H-2′), 5.09 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1′), 8.21 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, methanol-d_4): δ 22.65 (*C*H$_3$CO), 57.81 (C-2′), 63.49 (C-5′), 72.57 (C-3′). 80.93 (C-1′), 88.62 (C-4′), 125.96 (C-5), 150.59 (C-4), 165.60 (C-2), 172.56 (CH$_3$*C*O), 173.58 (*C*ONH$_2$). LRMS (CI): *m*/*z* 302 (M$^+$+H). HRMS (ESI+): *m*/*e* 302.0816 (M$^+$+H). Calcd for $C_{11}H_{16}N_3O_5S$: 302.0811.

4.29. 2-(2-Benzamido-2-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (**6**)

Prepared from **25** (or from **27**) by General procedure D. Purified by column chromatography (9:1 → 4:1 → 7:3 EtOAc/^{i}PrOH). Yield 53% (from **25**), 56% (from **27**). Colourless crystals, mp 225 °C (MeOH/^{i}Pr$_2$O), $[\alpha]_D^{20}$ −96.2 (*c* 1.0, CH_3OH). For spectral data see ref. 29.

4.30. 2-[2-Deoxy-2-(3-methoxybenzamido)-β-D-ribofuranosyl]thiazole-4-carboxamide (**7**)

Prepared from **31** by General procedure D. Purified by column chromatography (9:1 → 7:3 EtOAc/^{i}PrOH). Yield 72%. Colourless needles, mp 135.5–136.5 °C (MeOH/^{i}Pr$_2$O), $[\alpha]_D^{20}$ −81.6 (*c* 1.0, ^{i}PrOH). IR (film): ν_{max} 3334, 1668, 1539, 1291. ^{1}H NMR (250 MHz, methanol-d_4): δ 3.80 (pseudo d, 2H, $J_{4',5'}$ = 4.4 Hz, 2 × H-5′), 3.84 (s, 3H, OCH_3), 4.20 (m, 1H, H-4′), 4.39 (dd, 1H, $J_{2',3'}$ = 5.5, $J_{3',4'}$ = 2.5 Hz, H-3′), 4.73 (dd, 1H, $J_{1',2'}$ = 8.7, $J_{2',3'}$ = 5.5 Hz, H-2′), 5.30 (d, 1H, $J_{1',2'}$ = 8.7 Hz, H, H-1′), 7.06–7.51 (m, 4H, MeO*C*$_6$*H*$_4$), 8.22 (s, 1H, H-5). ^{13}C NMR (62.5 MHz, methanol-d_4): δ 55.9 (OCH_3), 60.7 (C-2′), 63.5 (C-5′), 72.8 (C-3′), 80.6 (C-1′), 88.9 (C-4′), 113.8, 118.7, 120.6, 130.6, 136.8, 150.6 (MeO*C*$_6$H$_4$), 126.0 (C-5), 142.3 (C-4), 161.2 (C-2), 170.3 (MeO*C*$_6$H$_4$*C*O), 172.4 (*C*ONH$_2$). LRMS (CI): *m*/*z* 394 (M$^+$+H). Anal. Found: C, 51.61; H, 5.18; N, 10.31; S, 8.00. Calcd for $C_{17}H_{19}N_3O_6S$: C, 51.90; H, 4.87; N, 10.68; S, 8.15.

4.31. MTT assay

The colorimetric MTT assay was carried out following the reported procedure [36].

4.32. Cell cycle analysis

K562 Cell cycle analysis was performed on FACS Calibur E440 (Becton Dickinson) flow cytometer following the reported procedure [27].

4.33. Detection of apoptosis

Apoptosis of K562 cells was evaluated with an FACS Calibur E440 (Becton Dickinson) flow cytometer according to the reported procedure [27]. Results were presented as percent of Annexin V positive gated cells. Percentage of specific apoptosis was calculated according to Bender et al. [37].

4.34. Western blot

Western blot was carried out following the reported procedure [27].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.01.037.

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