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Synthesis and antiproliferative activity of new thiazole hybrids with [3.3.0]furofuranone or tetrahydrofuran scaffolds

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Abstract: New thiazole hybrids were synthesized and evaluated for their *in vitro* cytotoxicity against a panel of human malignant cell lines. The key steps in the synthesis of hybrids **3**–**7** involved the initial condensation of appropriate aldononitriles with cysteine ethyl ester hydrochloride, followed by subsequent treatment of resulting thiazolines with diazabicycloundecene to form the thiazole ring. Bioisosteres **8** and **14** have been prepared after the stereoselective addition of 2-(trimethylsilyl)thiazole to the hemiacetals obtained by periodate cleavage of terminal diol functionality in the suitably protected d-glucose derivatives. The obtained analogues showed various antiproliferative activities in the cultures of several tumour cell lines. Hybrid **6** was the most potent in HeLa cells, exhibiting more than 10 and 4 times stronger activity than both leads **1** and **2**, respectively. The most active compound in Raji cells was hybrid **12**, which was nearly 2-fold more potent than the clinical antitumour drug doxorubicin. All analogues were more potent in A549 cells with respect to lead **1**, while compounds **6** and **7** were slightly more active than doxorubicin. Preliminary structure–activity relationship analysis revealed that the presence of a cinnamate group at the C-3 position in analogues of type **7** increases the activity of resulting molecular hybrids.

Keywords: molecular hybridization; pseudo-*C*-nucleosides; goniofufurone; tiazofurin; analogues; antiproliferative activity.

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INTRODUCTION

Molecular hybridization is a strategy of rational drug design based on the combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy, when compared to the parent drugs. In addition, this strategy may provide access to compounds with modified selectivity profiles, different or dual modes of action, and reduced unfavourable side effects.[1–3] Thiazole ring is a pharmacophore nucleus with various pharmaceutical applications. Its derivatives have a wide range of biological activities including anticancer activity.[4,5] We have recently reported on the synthesis of several thiazole bioisosteres of goniofufurone that exhibited *in vitro* antitumour activity against some human tumour cell lines.[6] Goniofufurone (**1**, Fig. 1) is natural styryl lactone with [3.3.0]furofuranone core,[7] which was isolated from the stem bark of tropical plant *Goniothalamus giganteus* (Annonaceae), and showed potent antiproliferative activity against several tumour cell lines.[8] This work describes the synthesis and *in vitro* antitumour screening of several new thiazole hybrids with furofuranone or tetrahydrofuran scaffolds. Compounds **3**–**5** might be considered pseudo-*C*-nucleosides related to tiazofurin (**2**), the oncolytic *C*-nucleoside with potent antileukaemic activity.[9,10] Pseudo-*C*--nucleosides are nucleoside analogues having a C–C bond between C-4 of the carbohydrate moiety and the heterocyclic aglycone.[11] Compound **8** represents a goniofufurone analogue with a thiazole replacing the phenyl ring at the C-7 position.

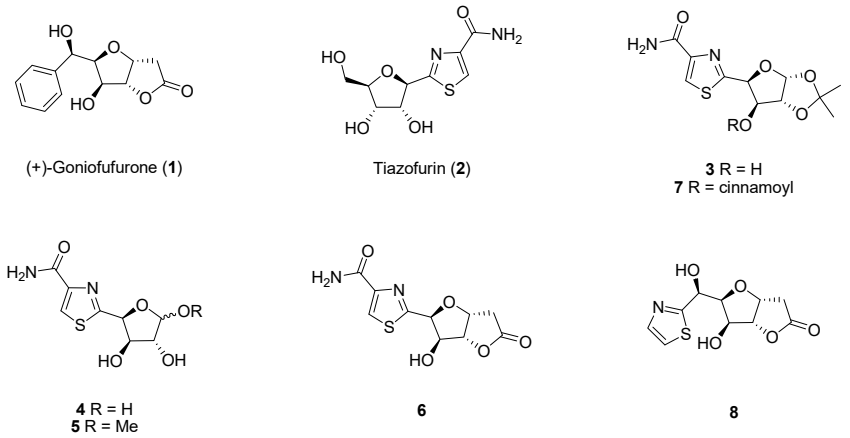


Fig. 1. Structures of (+)-goniofufurone (**1**), tiazofurin (**2**) and the corresponding analogues **3**–**8**.

EXPERIMENTAL

General procedures

Melting points were determined on a Büchi 510, or a hot stage microscope Nagema PHMK 05 apparatus, and were not corrected. Optical rotations were measured on a Rudolph

Research Analytical automatic polarimeter, Autopol IV. IR spectra were recorded on a FTIR Nexus 670 (Thermo-Nicolet) spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC 250 E (at 250 and 62.5 MHz, respectively) or a Bruker Avance III spectrometer (at 400 and 100 MHz, respectively) employing indicated solvents (*vide infra*) using TMS as the internal standard. Chemical shifts were expressed in ppm (δ) values and coupling constants in Hz (J). High-resolution mass spectra were taken on a Micromass LCT KA111 spectrometer or LTQ Orbitrap XL (Thermo Fisher Scientific Inc.) mass spectrometer. TLC was performed on DC Alufolien Kieselgel 60 F254 (E. Merck). Flash column chromatography was performed using Kieselgel 60 (0.040–0.063, E. Merck). 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. The purity of tested compounds was determined by HRMS and they were found to be > 95 % pure (errors were less than 5 ppm).

Synthetic procedures

*(*E,Z*)-1,2*-O-*Isopropylidene-α-D*-xylo-*pentodialdo-1,4-furanose-5-oxime (**10**).* To a stirred and cooled (0 °C) solution of triol **9** (2.032 g, 9.23 mmol) in a mixture of 2:1 MeOH/H_2O (54 mL) was added $NaIO_4$ (1.795 g, 10.49 mmol) in one portion. After 5 min, the cooling was stopped, and the reaction continues at room temperature for the next 4.5 h. The mixture was filtered through a Celite pad, the adsorbent was washed with MeOH, the filtrate was evaporated, and the residue was suspended in H_2O (10 mL) and extracted with EtOAc (3×50 mL). The extract was dried (Na_2CO_3 and Na_2SO_4), filtered, and evaporated to give the crude aldehyde **9a** (1.13 g) which was dried under a high vacuum overnight.

Suspension of crude aldehyde **9a** (1.631 g), sodium acetate (1.677 g, 20.40 mmol), and hydroxylamine hydrochloride (2.236 g, 32.10 mmol) in EtOH (40.75 mL) was vigorously stirred at room temperature for 24 h. The reaction mixture was evaporated, and the residue was purified by flash column chromatography (1:1 toluene/EtOAc). A mixture of *E*- and *Z*--oximes **10** (1.463 g, 78 % from **9**) was obtained as an amorphous powder, R_f = 0.27 (12:1 $CHCl_3$/ /MeOH). The ratio of isomers (from ^{1}H-NMR): *E*/*Z* = 1:0.8.

3-O-*Acetyl-1,2*-O-*isopropylidene*-α-*D*-xylo-*furanoseurononitrile (**11**).* A solution of compound **10** (1.463 g, 7.20 mmol) in acetic anhydride (29 mL) was stirred at reflux temperature for 1 h, and then evaporated. The residue was purified by flash column chromatography (19:1 toluene/EtOAc) to afford pure **11** (1.502 g, 92 %), as a colourless syrup, $[\alpha]_D$ = = +7.7 (*c* 1.0, $CHCl_3$), R_f = 0.53 (9:1 toluene/EtOAc).

3-O-*Acetyl-1,2*-O-*isopropylidene-4*-C-*(4'-ethoxycarbonylthiazol-2'-yl)*-α-*D*-xylo-*tetrofuranose (**12**).* To a stirred solution of **11** (0.999 g, 4.39 mmol) in absolute ethanol (85 mL) L--cysteine ethyl ester hydrochloride (1.217 g, 6.56 mmol) and anhydrous Et_3N (0.91 mL, 6.55 mmol) were added. The reaction mixture was stirred at room temperature for 3.5 h and then evaporated. The residue was dissolved in CH_2Cl_2 (50 mL), the organic phase was washed with water (15 mL), a saturated solution of $NaHCO_3$ (15 mL), and a saturated solution of NaCl (15 mL) then dried, filtered, and evaporated. A mixture of crude thiazoline derivatives **11a** (1.3279 g) was obtained.

To a solution of crude thiazolines **11a** (1.328 g, 3.70 mmol) in dry CH_2Cl_2 (27 mL) was added DBU (1.11 mL, 7.44 mmol). To the cooled solution (0 °C) was added $BrCCl_3$ (0.31 mL, 3.14 mmol), the reaction mixture was stirred at 0 °C for 2.5 h and then left at 4 °C for another 43 h and then evaporated. The residue was purified on a column of flash silica (9:1 → 4:1 toluene/EtOAc) to give pure product **12** (1.100 g, 87 % based on reacted **11**) as a yellow syrup. Recrystallization from CH_2Cl_2/hexane gave white needles, mp 141 °C, $[\alpha]_D$ = −23.0 (*c* 0.1, $CHCl_3$), R_f = 0.50 (7:3 toluene/EtOAc).

4-C-*(4'-(Carbamoyl)thiazol-2'-yl)-1,2-O-isopropylidene*-α-*D*-xylo-*tetrofuranose (**3**).* A solution of protected thiazole **12** (1.100 g, 3.08 mmol) in saturated methanolic ammonia (25 ml) was kept at room temperature for 7 days. The reaction mixture was then evaporated and purified by flash column chromatography ($CHCl_3$ → 12:1 $CHCl_3$/MeOH), to give pure **3** (0.498 g, 93 %) as a colourless syrup, $[\alpha]_D = -46.5$ (*c* 0.2, MeOH), $R_f = 0.30$ (12:1 $CHCl_3$/MeOH).

4-C-*(4'-(Carbamoyl)thiazol-2'-yl)-D*-xylo-*tetrofuranose (**4**).* A solution of **3** (0.312 g, 1.09 mmol) in 90 % aq TFA (18 mL) was stirred at 0 °C for 0.5 h and then at room temperature for 4.5 h. The reaction mixture was evaporated by azeotropic distillation with toluene. The remaining oily mixture was treated with EtOAc (2 mL) and saturated $NaHCO_3$ (2 mL) and evaporated again whereby a mixture of anomeric lactols **4** was obtained as a syrup. The residue was purified on a column of flash silica (5:1 → 25:6 → 10:3 $CHCl_3$/MeOH) to give pure product **4** (0.240 g, 89 %) in the form of pale yellow syrup, $R_f = 0.34$ (5:1 $CHCl_3$/MeOH). Anomeric ratio (from ^{1}H-NMR): α/β = 1:1.

Methyl 4-C-*(4'-(carbamoyl)thiazol-2'-yl)-D*-xylo-*tetrofuranoside (**5**)*. A solution of **3** (0.100 g, 0.35 mmol) in 90 % aq TFA (5.80 mL) was stirred at 0 °C for 0.5 h and then at room temperature for 4.5 h. The reaction mixture was evaporated by azeotropic distillation with toluene and methanol. The residue was purified by preparative thin-layer chromatography (2 preparative plates, 5:1 $CHCl_3$/MeOH, eluted with 7:3 EtOAc/^{i}PrOH), whereby a mixture of anomeric glycosides **5** (0.048 g, 52 %) was obtained, in the form of white powder, $R_f = 0.38$ (5:1 $CHCl_3$/MeOH). Anomeric ratio (from ^{1}H-NMR): α/β = 2:1.

3,6-Anhydro-2-deoxy-6-C-*(4'-(carbamoyl)thiazol-2'-yl)-D-ido-hexono-1,4-lactone (**6**)*. A) To a solution of compound **4** (0.202 g, 0.82 mmol) in anhydrous DMF (3.5 mL) was added Meldrum's acid (0.394 g, 2.73 mmol) and dry Et_3N (0.36 mL, 2.583 mmol). The reaction mixture was stirred at 46 °C for 69 h and then evaporated. The crude product was purified by preparative thin-layer chromatography (10 preparative plates, 6:1 $CHCl_3$/MeOH, eluted with 12:1 $CHCl_3$/MeOH), whereby impure **6** was obtained. After additional purification on a column of flash silica (20:1 → 12:1 $CHCl_3$/MeOH) and then by preparative thin-layer chromatography (2 preparative plates, 6:1 $CHCl_3$/MeOH, eluted with 12:1 $CHCl_3$/MeOH), pure product **6** was obtained as a white powder (0.015 g, 7 %), $R_f = 0.22$ (12:1 $CHCl_3$/MeOH). Analytical sample **6** was obtained by crystallization from MeOH in the form of white needles, m.p. 143 °C. B) To a cooled (0 °C) solution of **4** (0.204 g, 0.83 mmol) in dry MeOH (23 mL) was added MCMP (0.7923, 2.37 mmol) and the resulting solution was stirred at room temperature for 1 h. The reaction mixture was evaporated, and the residue was purified on a column of flash silica (20:1 → 12:1 $CHCl_3$/MeOH to give pure **6** as a yellow oil (0.050 g, 22 %), $[\alpha]_D$ = = −9.4 (*c* 0.13, DMSO), $R_f = 0.22$ (12:1 $CHCl_3$/MeOH). Analytical sample **6** was obtained by crystallization from MeOH as colourless needles, m.p. 143 °C.

4-C-*(4'-Carbamoyl)thiazol-2'-yl)-3*-O-*cinnamoyl-1,2*-O-*isopropylidene*-α-*D*-xylo-*tetrofuranose (7)*. To a stirred solution of compound **3** (0.0757 g, 0.2644 mmol) in a mixture of anhydrous MeCN (2 mL) and anhydrous CH_2Cl_2 (14.5 mL) was added cinnamic acid (0.088 g, 0.59 mmol), DCC (0.132 g, 0.64 mmol) and DMAP (0.130 g, 1.07 mmol). After stirring at room temperature for 24 h, the reaction mixture was filtered through a pad of quartz sand, the filtrate concentrated and purified by preparative thin-layer chromatography (5 preparative plates, 12:1 $CHCl_3$/MeOH, eluted with 7:3 EtOAc/^{i}PrOH), to give pure **7** as a white powder (0.103 g, 94 %). Analytical sample **7**, obtained by crystallization from a mixture of MeOH/$^i Pr_2O$ showed mp 180 °C, $[\alpha]_D = -157.1$ (*c* 0.40, $CHCl_3$), $R_f = 0.66$ (12:1 $CHCl_3$/MeOH).

3,6-Anhydro-2-deoxy-7-C-*(thiazol-2'-yl)-D-glycero-D*-ido-*heptono-1,4-lactone (**8**)*. To a stirred solution of compound **10**[12] (0.145 g, 0.71 mmol) in anhydrous MeCN (15 mL) was

added H_5IO_6 (0.146 g, 0.64 mmol). After stirring at room temperature for 22 h, the reaction mixture was evaporated with silica gel and purified by flash chromatography (9:1 CH_2Cl_2/ /Me_2CO). This gave pure **7a** (0.108 g, 75 %).

To the solution of **7a** (0.069 g, 0.34 mmol) in anhydrous THF (3 mL) 2-TST solution (0.081 mL, 0.51 mmol) in THF (1 mL) is added dropwise. The reaction mixture was stirred at room temperature for 48 h and then evaporated. The residue was dissolved in THF (3 mL) and treated with 1 M tetrabutylammonium fluoride in THF (0.4 mL), while stirring at room temperature for 2 h. The reaction mixture was evaporated, and the oily residue was purified by preparative thin-layer chromatography (10 preparative plates, 17:3 CH_2Cl_2/Me_2CO, second development 4:1 CH_2Cl_2/Me_2CO) to afford pure **8** (0.007 g, 7.5 %) in the form of an oil, $[\alpha]_D$ = = +10.0 (*c* 0.1, $CHCl_3$), R_f = 0.30 (9:1 CH_2Cl_2/Me_2CO, three successive developments).

1,2-O-*Isopropylidene-5*-C-*(thiazol-2'-yl)*-α-*D*-gluco-*pentofuranose (14)*. To a solution of compound **13** (2.166 g, 8.32 mmol) in anhydrous EtOAc (80 mL) was added H_5IO_6 (3.103 g, 13.61 mmol). The reaction mixture was stirred at room temperature for 6 h, then filtered and evaporated, leaving a light-pink reaction mixture. The residue was purified on a column of flash silica (11:9 Et_2O/light petroleum), whereby a mixture of alcohols **13a** was obtained (0.876 g, 56 %) in the form of a colourless syrup, R_f = 0.37 (1:1 Et_2O/light petroleum). IR (film): ν_{max} 3371 cm^{-1} (OH). (+)ESI-HRMS (*m*/*z*): calculated for [$C_{10}H_9O_5$ + NH_4^+] 236.11286, observed 236.11285.

To a solution of purified compound **13a** (0.161 g, 0.74 mmol) in anhydrous CH_2Cl_2 (6 mL) a 2-TST (0.174 g, 1.09 mmol) solution in CH_2Cl_2 (3 mL) was added dropwise at room temperature**.** After stirring at room temperature for 12 h, the solvent was evaporated and to the residue was added THF (10 mL) and tetrabutylammonium fluoride (0.886 mmol in 8.86 mL THF). After stirring at room temperature for 2 h, the reaction mixture was concentrated to a smaller volume, and after the addition of aq. $NaHCO_3$ solution, extracted with EtOAc. The combined extracts were dried and evaporated, and the remaining crude product **14** was purified on a column of flash silica (light petroleum/EtOAc 1:1), whereby pure product **14** (0.048 g, 24 %) was obtained, which crystallized from a mixture of CH_2Cl_2/hexane as white crystals, m.p. 120 °C, $[\alpha]_D$ = −12.5 (*c* 0.2, acetone).

Cytotoxic activity

Test cells. The *in vitro* cytotoxicities of test compounds were evaluated against seven human malignant cell lines: K562 (ATCC CCL-243, chronic myeloid leukaemia), HL-60 (ATCC CCL-240, promyelocytic leukaemia), Jurkat (ATCC CCL-1435, T cells leukaemia), Raji (ATCC CCL-86, Burkitt's lymphoma), MCF-7 (ATCC HTB-22, ER^+ breast adenocarcinoma), HeLa (ATCC CCL2, human cervix adenocarcinoma) and A549 (ATCC HTB-38, lung carcinoma). Cytotoxic activity against one normal human cell line, MRC-5 (ATCC CCL--185, foetal lung fibroblasts), was also estimated.

MTT test. Cytotoxic activity was evaluated by using standard MTT assay,[13] after exposure of cells to the tested compounds for 72 h.

Crystal structure determination

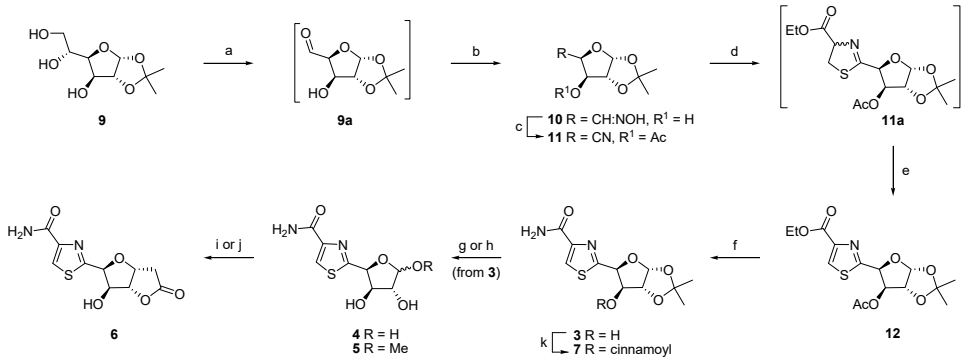
Diffraction experiments were performed on an Oxford Diffraction Gemini S diffractometer. Crystal structures were solved and refined as reported previously[14]. All hydrogen atoms are introduced in idealized positions are refined using a riding model. Pertinent crystallographic and refinement data are listed in Table S-III of the Supplementary material to this paper. CCDC 2218113 and CCDC 2218112 contain supplementary crystallographic data for

this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk/structures.

RESULTS AND DISCUSSION

Chemistry

Synthesis of compounds **3**–**7** is shown in Scheme 1 and commenced from the commercially available monoacetone-D-glucose (**9**).

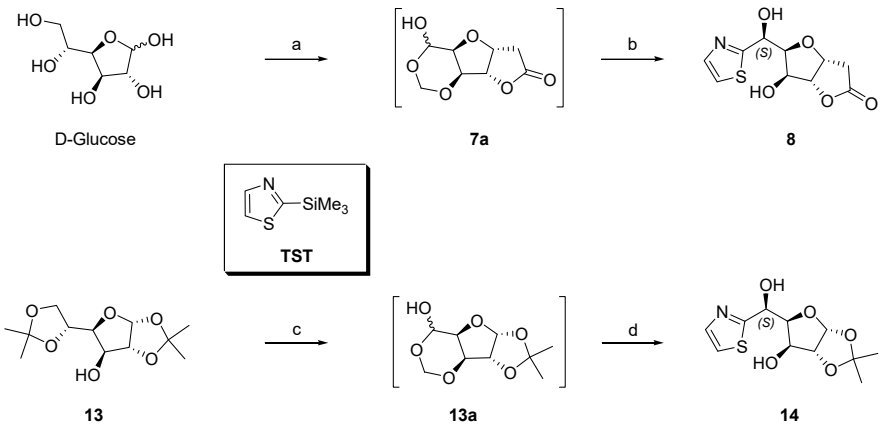


Scheme 1. a) $NaIO_4$, MeOH/H_2O, rt, 4.5 h; b) $NH_2OH{\times}HCl$, NaOAc, EtOH, rt, 24 h, 78 % from **9**; c) Ac_2O, reflux, 1 h, 92 %; d) L-cysteine ethyl ester hydrochloride, Et_3N, EtOH, CH_2Cl_2, rt, 3.5 h; e) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, 2.5 h, then 4 °C, 43 h, 87 % from **11**; f) saturated NH_3, MeOH, rt, 7 days, 93 %; g) 90 % aq TFA, 0 °C, 0.5 h, then rt, 4.5 h, 89 %; h) 90% aq TFA, MeOH, 0 °C, 0.5 h, then rt, 4.5 h, 52 %; i) Meldrum's acid, Et_3N, DMF, 46 °C, 69 h, 7 %; j) MCMP, MeOH, rt, 1 h, 22 %; k) cinnamic acid, DCC, DMAP, MeCN, CH_2Cl_2, rt, 24 h, 94 %.

Terminal diol cleavage in **9** was achieved with sodium periodate in aqueous MeOH to afford the unstable aldehyde **9a**. The resulting aldehyde **9a** was not purified but was rather immediately treated with hydroxylamine hydrochloride to yield the expected oxime **10** as a mixture of the corresponding *E*- and *Z*-isomers. The mixture was not separated but was further treated with refluxing acetic anhydride to give the corresponding nitrile **11** in 92 % yield. Nitrile **11** was allowed to react with ethyl ester of cysteine hydrochloride, in the presence of triethylamine at room temperature, to afford thiazoline **11a** as an inseparable mixture of C-4 epimers. The crude mixture was not separated but was immediately oxidized with bromotrichloromethane and DBU to give the thiazole **12** in an overall yield of 87 % from two steps. Treatment of **12** with methanolic ammonia provided the amide **3** (93 %) as a result of successive ester ammonolysis and *O*-deacetylation at the C-3 position. Hydrolytic removal of the isopropylidene protective group in **3** gave the expected lactol **4**, which upon treatment with Meldrum's acid in the presence of triethylamine gave a low yield (7 %) of target **6**. A better yield of **6** (22 %) was obtained by using the *Z*-selective Wittig olefination

of **4** with a stabilized C_2-ylide ($Ph_3P{=}CHCO_2Me$, MCMP).[15] Apart from spectroscopic methods, the structure of compound **6** was confirmed by X-ray analysis (see Supplementary material for details). Finally, compound **3** was esterified with cinnamic acid, under the Steglich conditions,[16] to afford the corresponding 3-*O*--cinnamoyl derivative **7** in 94 % yield. The reason for the preparation of cinnamate **7** lies in the fact that a significant number of cinnamic acid hybrids show antitumour activity.[17–19]

The preparation of bioisostere **8** is shown in Scheme 2. D-Glucose was first converted to the protected aldehyde **7a** using the procedure recently developed in our laboratory[20] (see the Supplementary Material for details).



Scheme 2. a) See Supplementary material and/or Ref.[20]; b) (*i*) TST, THF, rt, 48 h, (*ii*) TBAF, THF, rt, 2 h, 7.5 %; c) Ref.[21]; d) (*i*) TST, CH_2Cl_2, rt, 12 h, (*ii*) TBAF, THF, rt, 2 h, 24 %.

The addition of 2-(trimethylsilyl)thiazole (TST) to hemiacetal **7a** (Scheme 2) in THF using the adopted procedure developed by Dondoni *et al.*[22] occurred with high diastereofacial selectivity affording, after desilylation with tetrabutylammonium fluoride, a low yield of thiazole **8** (7.5 %). This two-step transformation involves the initial unmasking of hemiacetal function with the subsequent addition of reagents to the liberated aldehyde group. Given that compound **8** showed relatively weak antiproliferative activity against tumour cells, the yield of this reaction was not optimized. To resolve the stereochemistry at the C-7 position in product **8**, the above-described addition reaction was repeated with the known[23] hemiacetal derivative **13a**. The corresponding thiazole derivative **14** was obtained in a yield of 24 %. The stereochemistry of **14** was unambiguously established by X-ray crystallographic analysis (see Supplementary material for details). Based on this result, as well as the observations of Dondoni *et al.*,[22] we concluded that the newly introduced stereocenter of product **8** has (7*S*)-stereochemistry.

Antiproliferative activity

Table I shows *in vitro* cytotoxicities of synthesized compounds against a panel of human cell lines (K562, HL-60, Jurkat, Raji, MCF-7, HeLa, A549 and MRC-5), using the standard MTT assay. Apart from the final products (**5**–**8**), intermediates, **3**, **4** and **12** were also included in the assay since they can be considered pseudo-*C*-nucleosides related to tiazofurin.

TABLE I. *In vitro* cytotoxicity (IC_{50}* / μM; values are means of three independent experiments. Coefficients of variation were less than 10 %) of (+)-goniofufurone (**1**), tiazofurin (**2**), DOX and analogues **3**–**8** and **12** after 72 h

Compound	Cell line							
	K562	HL-60	Jurkat	Raji	MCF-7	HeLa	A549	MRC-5
(+)-Goniofufurone (**1**)	0.41	201.32	32.45	18.45	16.59	8.32	35.21	>100
Tiazofurin (**2**)	2.06	0.67	0.09	5.28	2.03	3.26	5.92	0.36
3	21.01	7.64	7.09	15.64	10.52	4.36	18.21	>100
4	2.55	8.51	11.36	14.32	8.65	8.31	24.64	>100
5	17.50	7.79	11.36	7.63	18.36	8.64	5.46	>100
6	1.63	1.02	18.52	9.02	2.61	0.75	4.64	97.12
7	3.54	12.63	4.32	12.64	10.02	1.25	3.45	>100
8	3.05	3.54	25.02	25.41	7.62	9.06	11.59	>100
12	3.47	9.10	7.52	1.58	15.20	3.70	10.35	>100
DOX	0.25	0.92	0.03	2.98	0.20	0.07	4.91	0.10

The results in Table I show that five compounds exhibited micromolar activity in the culture of K562 cells, although only compound **6** was more potent than tiazofurin (**2**). Almost all synthesized compounds were more active than **1** against MCF-7 and HL-60 cells, with lactone **6** being the most potent. It is noteworthy that analogue **6** exhibited a prominent potency (IC_{50} = 1.02 μM) against promyelocytic leukaemia cells (HL-60) with activity similar to DOX. Among all synthesized molecules, which showed moderate activity in Jurkat and Raji cell cultures where they were more active than **1** (except **8** against Raji cells), the isopropylidene derivative **12** stands out, which was almost twice as active as DOX and 3 times as active as tiazofurin (**2**) against Raji cells. Against alveolar basal adenocarcinoma cells (A549), all compounds were more active than **1**, while two compounds (**6** and **7**) were slightly more active than DOX. Molecules **6** and **7** showed higher potency than both leads **1** and **2** against HeLa cells of which compound **6** showed submicromolar activity (IC_{50} = 0.75 μM), the best activity recorded in this assay. Like natural product **1**, none of the synthesized analogues were active against normal MRC-5 cells, in contrast to tiazofurin (**2**) and DOX, which showed high potencies against these cells in the submicromolar range.

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

In an attempt to determine the structural features important to the activity of this series of compounds, we compared the activities of: a) compound **7** with a cinnamoyl ester group at the C-3 position, with **3** (which has an OH group at C-3); b) the activity of lactol **4**, with free OH groups at C-1 and C-2, with the activity of compounds **6** and **3** with a lactone or isopropylidene ring; c) the activity of lactol **4** with the activity of the methyl glycoside **5**; d) the activity of the C-7 thiazole hybrid **8** with the natural product **1** having a phenyl group at the C-7 position (Fig. S-1 of the Supplementary material). The results of this brief SAR analysis showed that the presence of the cinnamoyl group at C-3 is beneficial for the activity of this type of compound; the absence of OH groups at C-1 and C-2 and the structural architecture of a five-membered lactone or isopropylidene ring (the analysis also showed that pseudo-*C*-nucleoside **5** is more active against 50 % of the tested cell lines) and that the introduction of a thiazole ring at the C-7 position of the natural product **1** instead of the phenyl ring, increases the activity against four of seven cell lines.

*Crystal structure of pseudo-*C*-nucleoside **6***

The molecular structure of **6** is depicted in Fig. 2. Absolute configuration of all stereocenters is determined both from resonant scattering effects, and findings are in line with assumed absolute configurations of stereocenters whose stereochemistries remain unchanged during the synthetic route.

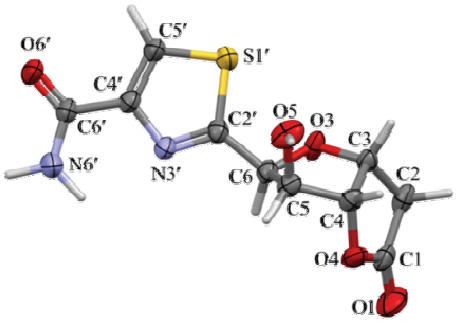


Fig. 2. Molecular structure of **6** (CCDC 2218113) with the atom numbering scheme.

From the structural point of view, **6** is the first structurally characterized compound that bears a furofuranone ring core coupled to a thiazole ring. The search of the CSD[24] resulted in only ten structures that contain a thiazole ring coupled to the C1′ atom of a tetrahydrofuran ring substructure depicted in Fig. 3a. All these structures can be regarded as tiazofurin analogues. Since **6** can also be regarded as a tiazofurin analogue, where a furanose ring is fused to a lactone ring, it is of interest to compare the furanose ring conformation in **6** and these tiazofurin analogues. For this purpose, atom numbering nomenclature established for furanose rings in nucleotides is used,[25,26] as shown in Fig. 3a.

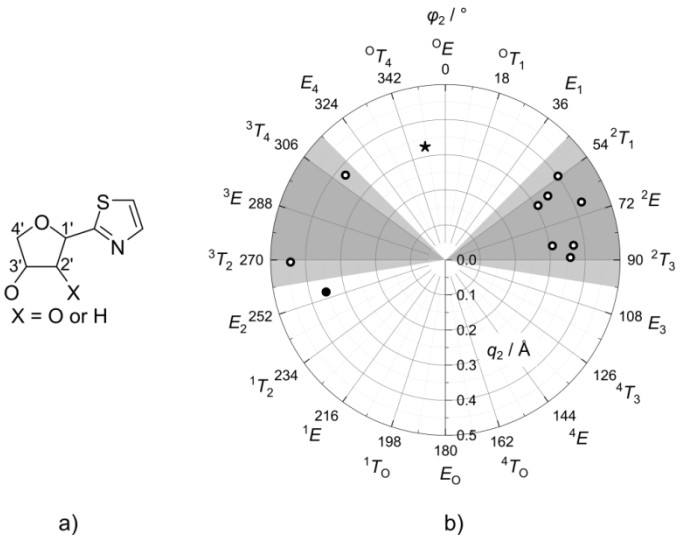


Fig. 3. a) Substructure fragment used in CSD search. Substituents at C4′ were unspecified. b) Pseudorotational circle for furanose ring conformations found in CSD hits. Conformation of **6** is indicated by a filled circle, and that of YIHCAT with a star. Preferred conformational ranges are shaded.

Conformations of the furanose rings are analysed *via* Cremer–Pople formalism.[27] It is found that in seven structures furanose rings adopt conformations that spread in pseudorotation regions demarcated by $^{2}T_1$ and $^{2}T_3$ conformations, while for two structures the range is enclosed between $^{3}T_2$ and $^{3}T_4$ conformations. These conformational ranges have been established as preferred for ribose and deoxyribose in nucleosides.[28,29] Structure YIHCAT,[30] with the furanose ring in conformation between $^{O}T_4$ and ^{O}E is the only outlier. The conformation of the furanose ring in the **6** is very close to E_2, which is also an aberration. What separates YIHCAT and **6** from other structures is the presence of fused rings – isopropylidene in YIHCAT and furofuranone in **6**, which may explain their different conformations. Notably, for all investigated structures, including **6**, ring puckering amplitude falls in the range from 0.30 to 0.45 Å. A graphical representation of ring conformation space for the investigated structures is given in Fig. 3b, while details are summarized in Table S-III of the Supplementary material.

Relative to the sugar moiety, the aglycone fragment of the nucleoside can adopt two main orientations about the glycosyl C1′–N link called *syn* and *anti*.[25,26] In analogy to that, for *C*-nucleosides such as tiazofurin and its derivatives, a torsion angle χ (O–C1′–C–S) can be defined to assess thiazole ring orientation. It is found that the thiazole ring orients in such a way that sugar O and thiazole S atoms are in *syn* orientation, with a restricted range of $|\chi|$ (0–60°), and the peak of the distribution at *ca.* 30° (see Fig. S-3 of the Supplementary material). The cor-

responding torsion angle for **6** amounts to −24.3(2)°, indicating that the mutual disposition of the studied rings in **6** is in line with the literature data.

Two intermolecular hydrogen bonds were found in the crystal structure of **6**. Hydroxyl O5–H5 group is bonded to carboxamide oxygen O6′ of the neighbouring molecule. Interestingly, only one of the carboxamide hydrogen atoms is involved in hydrogen bonding, with carbonyl oxygen O1 of the lactone ring being the hydrogen bond acceptor. Details of hydrogen bonding are listed in Table S-V of the Supplementary material.

CONCLUSION

In conclusion, seven new thiazole hybrids with furofuranone or tetrahydrofuran scaffolds have been synthesized and evaluated for their *in vitro* cytotoxicity against a panel of human malignant cell lines (K562, HL-60, Jurkat, Raji, MCF-7, HeLa and A549), as well as toward a single normal cell line (MRC-5). The key steps in the synthesis of pseudo-*C*-nucleosides **3**–**7** and **12** involved the initial cyclocondensation of the corresponding aldononitriles with cysteine ethyl ester hydrochloride, followed by subsequent treatment of the resulting C-4′ epimeric thiazolines with DBU to form the thiazole ring. Goniofufurone bioisosteres **8** and **14** have been prepared by stereoselective addition of 2-(trimethylsilyl)thiazole to partially protected hemiacetals, obtained by periodate cleavage of the terminal diol function in the appropriate D-glucose derivatives. The synthesized analogues showed moderate to strong antiproliferative activity in cultures of several malignant cell lines. The strongest activity was shown by hybrid **6** (HeLa cells, IC_{50} 0.75 μM) which was more than 10 or 4 times more active than both control compounds **1** and **2**, respectively. The most active compound in Raji cell culture was hybrid **12**, which was nearly two times more potent than the commercial antitumour drug doxorubicin (DOX). Lung adenocarcinoma cells (A549) were the most sensitive against the synthesized compounds. All were more active than lead **1**, while two compounds (**6** and **7**) were slightly more active than DOX. A brief SAR study revealed that the presence of the cinnamoyl group at C-3 may enhance the activity of this type of analogues.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12157, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА И АНТИПРОЛИФЕРАТИВНА АКТИВНОСТ НОВИХ ТИАЗОЛНИХ ХИБРИДА СА [3.3.0]ФУРОФУРАНОНСКИМ ИЛИ ТЕТРАХИДРОФУРАНСКИМ СКЕЛЕТОМ

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Синтетизовани су нови тиазолни хибриди и одређена је њихова *in vitro* цитотоксичност према панелу хуманих малигних ћелијских линија. Кључни кораци у синтези хибрида **3**–**7** подразумевали су иницијалну кондензацију одговарајућих алдононитрила са хидрохлоридом етилестра цистеина, након чега је уследио третман резултујућих тиазолина са DBU при чему је формиран тиазолни прстен. Биоизостере **8** и **14** су добијене након стереоселективне адиције 2-(триметилсилил)тиазола на хемиацетале добијене перјодатним раскидањем терминалне диолне функције погодних деривата D-глукозе. Добијени тиазолни аналози су показали различите антипролиферативне активности у културама појединих туморских ћелијских линија. Најјачу активност према HeLa ћелијама показао је хибрид **6**, који је био више од десет, односно четири пута активнији од контролних молекула **1** и **2**, редом. Најактивније једињење према Raji ћелијама био је хибрид **12**, који је скоро два пута активнији од клиничког антитуморског лека доксорубицина. Сви аналози су били активнији према A549 ћелијама у односу на контролу **1**, док су једињења **6** и **7** била нешто активнија од доксорубицина. Прелиминарна SAR анализа је открила да присуство цинаматне групе на положају C-3, у аналозима типа **7**, повећава активност резултујућих хибрида.

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