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Novel *O*-methyl goniofufurone and 7-*epi*-goniofufurone derivatives: synthesis, *in vitro* cytotoxicity and SAR analysis†

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Novel goniofufurone (**1**) and 7-*epi*-goniofufurone (**2**) derivatives bearing a methoxy group at the C-5 and/or C-7 positions were prepared and their *in vitro* antitumour activity against some human tumour cell lines was evaluated. Some of the analogues displayed powerful antiproliferative effects against the studied tumour cells, but almost all of them were non-cytotoxic toward the normal cells (MRC-5). A SAR study reveals that the introduction of a methoxy group at the C-7 position may increase the antiproliferative effects of the analogues. The most active compounds are 7-*O*-methyl derivatives of goniofufurone (**3**) and 7-*epi*-(+)-goniofufurone (**6**), which exhibited 1177- and 451-fold higher potencies than the leads **1** and **2** toward the MDA-MB 231 cell line. At the same time, compound **3** is almost 1.5-fold more active than the commercial drug doxorubicin (DOX) against the same cell line. Flow cytometry data confirmed that the cytotoxic effects of these analogues are mediated by apoptosis, additionally revealing that these molecules induced changes in the K562 cell cycle distribution.

Introduction

[3.3.0]Furofuranone moieties are frequently found in natural products that show a broad range of biological activities including cytotoxic activity.[1–4] As a consequence of that, the synthesis of [3.3.0]furofuranone derivatives has received considerable attention from synthetic medicinal chemists in the last twenty years.[5] As a part of our continuing interest in the synthesis and antiproliferative evaluation of natural products and analogues with the [3.3.0]furofuranone scaffold,[6–10] we have planned the synthesis of new (+)-goniofufurone and 7-*epi*-goniofufurone derivatives with methoxy groups at the C-5 and/or C-7 positions (**3–10**, Fig. 1). The rationale underlying the preparation of **3** arises from the fact that the parent compound **1** (Fig. 1) has a restricted geometry of the C_5–C_7 segment, due to an intramolecular H-bond formed between the 5-OH and the 7-OH, as established by X-ray analysis.[11] We

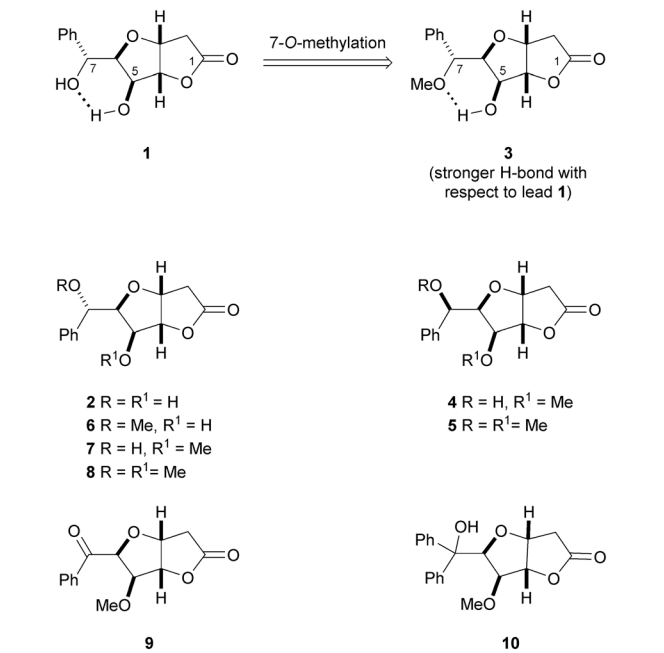

Fig. 1 Structures of (+)-goniofufurone (**1**), 7-*epi*-(+)-goniofufurone (**2**) and the targeted *O*-methyl derivatives **3–10**.

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† Electronic supplementary information (ESI) available: Contains the results of: X-ray crystal structure determination, SAR analysis and flow cytometry. Copies of the ^{1}H and ^{13}C NMR spectra of the final products are also disclosed. CCDC 1843444–1843449. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c8md00431e

assumed that the replacement of the 7-OH group with a 7-OMe group will make the H-bond stronger which will further strengthen the conformational constraint in the C_5–C_7 segment. On the other hand, we have recently demonstrated that readily available (+)-goniofufurone oxetane derivatives,[12,13] which cause much greater structural rigidity, strongly inhibit the growth of certain human neoplastic cell lines.[13] Herein, we report their synthesis starting from D-glucose, as well as their effects on the proliferation of certain human tumour cell lines, including their impact on the K562 cell cycle and their apoptosis inducing properties in the mentioned cell culture.

Results and discussion

Chemistry

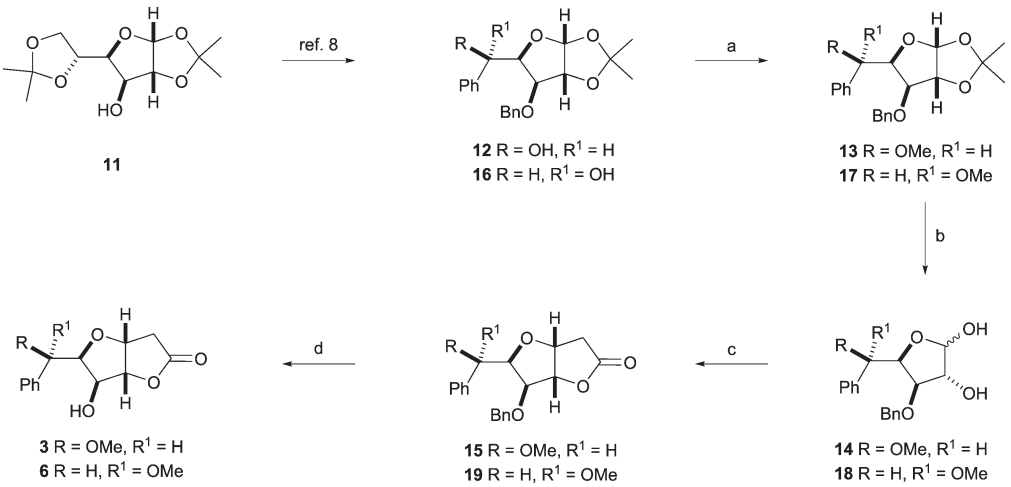
The preparation of 7-*O*-methyl-goniofufurone (**3**) and 7-*O*-methyl-7-*epi*-goniofufurone (**6**) is outlined in Scheme 1. The known[14] benzyl alcohols **12** and **16**, served as convenient starting materials for this part of the work. These compounds (**12** and **16**) have been synthesized earlier in our laboratory starting from **11**.[8] Treatment of compound **12** with MeI and NaH in dry DMF gave the 7-*O*-methyl derivative **13** (94%). Under the same reaction conditions, compound **16** gave the (7*S*)-isomer **17** in 94% yield. Hydrolytic removal of the isopropylidene protecting group in **13** gave the lactol **14** (85%), which upon treatment with Meldrum's acid in the presence of triethylamine gave the expected furanolactone **15** in 51% yield. Under similar reaction conditions, compound **17** gave lactol **18** (99%), which by subsequent cyclocondensation with Meldrum's acid gave the protected lactone **19** (47%). 5-*O*-Benzyl protective groups were removed under the standard reaction conditions (H_2, Pd/C, MeOH) whereby the desired 7-methoxy derivatives **3** and **6** were obtained in 82 and 68% yield, respectively. The stereochemistry of **3** has been unambiguously confirmed by single crystal X-ray diffraction analysis (for the crystal structure of **3**, see the ESI†).

The synthesis of analogues **4** and **7** started from commercially available diacetone-D-glucose **11** (Scheme 2). Treatment of **11** with MeI and NaH in dry DMF gave the known[15] 3-*O*-methyl derivative **20** in 81% yield. When compound **20** was treated with periodic acid in dry ethyl acetate and the crude intermediate **20a** was allowed to react with phenyl magnesium bromide in toluene, the benzyl alcohols **21** and **23** were obtained, with the L-*ido*-derivative **23** as the major product (**23**/**21** ≈ 9 : 1). The stereochemistry of compounds **21** and **23** was confirmed by single crystal X-ray diffraction analysis (for the crystal structures of **21** and **23**, see the ESI†).

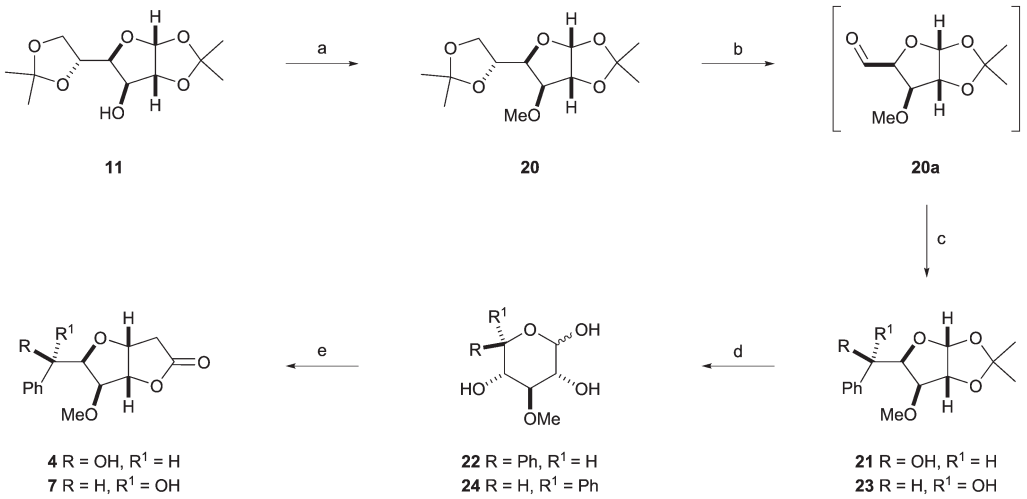
Hydrolytic removal of the isopropylidene protecting group in **21** gave the expected pyranose **22** in an almost quantitative yield (99%). Treatment of lactol **22** with Meldrum's acid in the presence of triethylamine gave the desired 5-*O*-methyl-goniofufurone (**4**) in 41% yield. Under similar reaction conditions, compound **23** was converted to the expected lactol **24** (81%), which was finally converted to **7** (46%) by cyclocondensation with Meldrum's acid.

The synthesis of 5,7-di-*O*-methyl-goniofufurone (**5**) and 5,7-di-*O*-methyl-7-*epi*-goniofufurone (**8**) is outlined in Scheme 3. The preparation started from known[16] benzyl alcohols **25** and **28** that were previously prepared in our laboratory according to a modified procedure.[10] Treatment of compounds **25** and **28** with MeI and NaH in dry DMF gave derivatives **26** and **29** (85% and 90%, respectively). Under the same reaction conditions, compounds **21** and **23** gave the expected methoxy derivatives **26** and **29** in 88% and 93% yields, respectively. Treatment of **26** with aqueous trifluoroacetic acid gave **27** (89%). The reaction of **27** with Meldrum's acid in the presence of triethylamine gave the final product **5** in a 44% yield.

Under similar reaction conditions, compound **29** gave lactol **30** (93%), the condensation of which with Meldrum's acid gave analogue **8** in 51% yield. The stereochemistry of



Scheme 1 Preparation of analogues **3** and **6**: (a) MeI, NaH, DMF, 0 °C → rt, 2 h for **12**, 94% of **13**, 2 h for **16**, 94% of **17**; (b) 90% aq TFA, rt, 0.5 h for **13**, 85% of **14**, 1 h for **17**, 99% of **18**; (c) Meldrum's acid, Et_3N, DMF, 46 °C, 70 h for **14**, 51% of **15**, 68 h for **18**, 47% of **19**; (d) H_2–Pd/C, MeOH, rt, 4 h for **15**, 82% of **3**, 24 h for **19**, 68% of **6**.

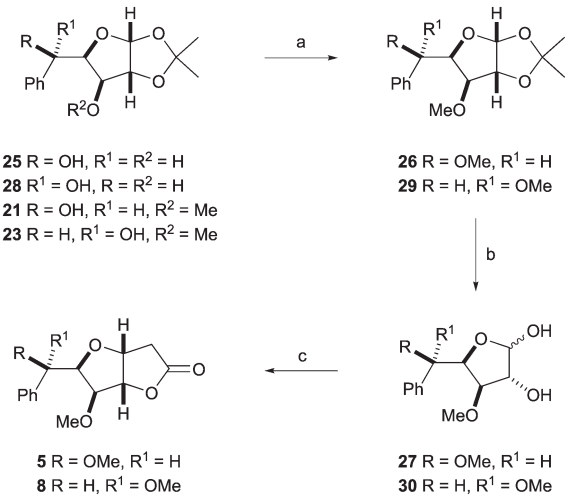


Scheme 2 Preparation of analogues **4** and **7**: (a) MeI, NaH, DMF, 0 °C → rt, 2 h, 81%; (b) H_5IO_6, EtOAc, rt, 1.5 h; (c) PhMgBr/THF, toluene, 0 °C, 5 h, 56% of **23**, 6% of **21**; (d) 90% aq TFA, rt, 0.5 h for **21**, 99% of **22**, 0.5 h for **23**, 81% of **24**; (e) Meldrum's acid, Et_3N, DMF, 46 °C, 74 h for **22**, 41% of **4**, 70 h for **24**, 46% of **7**.

compounds **5** and **8** has been unambiguously confirmed by single crystal X-ray diffraction analysis (for crystal structures of **5** and **8**, see the ESI†).

An attempted preparation of *O*-methyl goniofufurone derivatives by direct alkylation of parent compounds (MeI, NaH, and DMF) gave a complex reaction mixture from which the desired products could not be isolated in pure form.

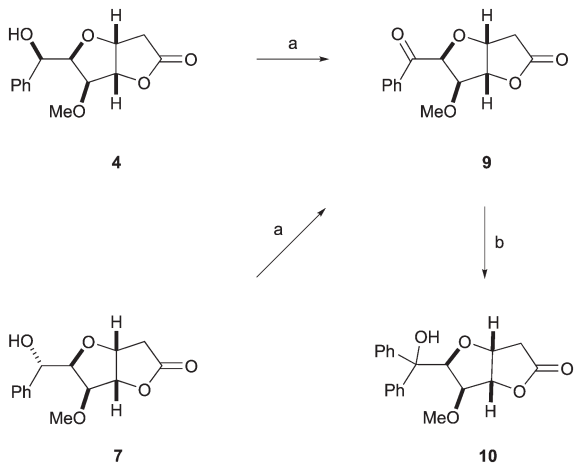
Finally, a simple two step synthesis sequence was also accomplished by oxidation of compounds **4** or **7** with PCC whereby target **9** was obtained in 99% and 91% yields, respectively, (Scheme 4). The Grignard addition of PhMgBr to **9** gave target **10** in 35% yield. The stereochemistry of compound **9** was confirmed by single crystal X-ray diffraction analysis (for the crystal structure of **9**, see the ESI†).



Scheme 3 Synthesis of 5,7-di-*O*-methyl derivatives **5** and **8**: (a) MeI, NaH, DMF, 0 °C → rt, 2 h for **25**, 85% of **26**, 2 h for **21**, 88% of **26**, 2 h for **28**, 90% of **29**, 2 h for **23**, 93% of **29**; (b) 90% aq TFA, rt, 1 h for **26**, 89% of **27**, 1 h for **29**, 93% of **30**; (c) Meldrum's acid, Et_3N, DMF, 46 °C, 71 h for **27**, 44% of **5**, 71 h for **30**, 51% of **8**.

In vitro antitumour activities and SAR

It is well known that many analogues of natural styryl lactones of type **1** and **2** exhibited potent *in vitro* cytotoxic activity against certain human tumour cell lines.[1–4] Hence, the newly synthesized analogues were evaluated for their *in vitro* cytotoxicity against a panel of human malignant cell lines (myelogenous leukaemia, K562, promyelocytic leukaemia, HL-60, T cell leukaemia, Jurkat, Burkitt's lymphoma, Raji, estrogen receptor positive breast adenocarcinoma, MCF-7, estrogen receptor negative breast adenocarcinoma, MDA-MB 231, cervix carcinoma, HeLa, lung adenocarcinoma, A549), and against a single normal cell line, foetal lung fibroblasts (MRC-5). The purpose of the evaluation of cytotoxicity against the normal cells is to check the eventual selectivity of analogues against the malignant cell lines. Cell growth inhibition was evaluated by MTT colorimetric assay,[17] after exposure of cells to the tested compounds for 72 h. (+)-Goniofufurone (**1**),



Scheme 4 Preparation of analogues **9** and **10**: (a) PCC, CH_2Cl_2, reflux, 2.5 h for **4**, 99% of **9**, 2 h for **7**, 91% of **9**; (b) PhMgBr, THF, 0 °C, 1.5 h, 35%.

7-*epi*-(+)-goniofufurone (**2**) and the commercial antitumour agent doxorubicin (DOX) were used as positive controls. The results are presented in Table 1.

According to the results of the cytotoxicity assay, all the malignant cell lines under evaluation were sensitive to all of the synthesized analogues. The normal MCF-7 cell line was not sensitive to compounds **3**, **5**, **6** and **8**. The highest potency in the culture of K562 was recorded after treatment with 5-*O*-methyl-goniofufurone **4** (IC_{50} 5.36 μM). All five goniofufurone analogues (**3**–**5**, **9**, and **10**) demonstrated diverse antiproliferative activities toward HL-60 cells, in contrast to the lead **1**, which was completely inactive against this cell line. All 7-*epi*-goniofufurone derivatives (**6**–**8**) were more potent than the lead **2** against the same cell line. The highest potency in the culture of HL-60 (IC_{50} 1.63 μM) cells was recorded after treatment with 7-*O*-methyl-7-*epi*-(+)-goniofufurone (**6**). The most active compound in the culture of Jurkat cells is the derivative **8** (IC_{50} 1.69 μM). The most active molecule against the Raji cells was the goniofufurone derivative **4** (IC_{50} 8.69 μM), which exhibited a 2-fold higher potency than lead **1**. Analogue **9** was the most potent compound against the MCF-7 cells (IC_{50} 3.44 μM), compared to the natural products **1** and **2**, this analogue exhibited 4.5- and 2.5-fold higher activity against these cells. Two analogues demonstrated submicromolar activities toward MDA-MB 231 cells. These are: 7-*O*-methyl-(+)-goniofufurone **3** (IC_{50} 0.064 μM) and 7-*O*-methyl-7-*epi*-(+)-goniofufurone **6** (IC_{50} 0.13 μM), which exhibited 1177- and 451-fold higher potency than leads **1** and **2**, respectively. Compound **3** was almost 1.5-fold more active than the commercial antitumour agent DOX. The highest potency in the culture of HeLa was recorded after treatment with 7-*O*-methyl-(+)-goniofufurone **3** (IC_{50} 5.64 μM). Compounds **3** and **9** were the most potent against the A549 cell line (IC_{50} 2.64 and 2.58 μM, respectively). These analogues were 13-fold more active than lead **1**, 8-fold more active than lead **2**, and almost 2-fold more active than doxorubicin. Interestingly, only one of eight derivatives (molecule **8**) showed negligible activity against normal MRC-5 cells (IC_{50} 87.61 μM). The other analogues including natural products **1** and **2** were completely inactive toward the normal cell line, MRC-5. In contrast, the commercial drug doxorubicin exhibited potent cytotoxicity (IC_{50} 0.10 μM) against these cells. These results do suggest that these molecules represent selective antitumour agents, but this should be verified by additional *in vitro* experiments with different normal cell lines.

In an attempt to correlate the structures of synthesized goniofufurone analogues with their cytotoxic activities, we first considered the effects of methoxy groups at the C-7 and/or C-5 positions. The natural products **1** and **2** are used as the controls in this SAR analysis. According to the data in Table 1, the introduction of a methoxy group at the C-7 or C-5 position caused the increased cytotoxicity originally displayed by lead **1**, but decreased the antiproliferative potencies originally demonstrated by lead **2**. Also, we wanted to explore the effects of methoxy groups at both C-7 and C-5 positions, and obtained very similar results as in the previous examples. The analogue with two methoxy groups (**5**) was a more potent cytotoxic agent than lead **1**, but the corresponding (7*S*)-epimer (**8**) was a less active compound with respect to that originally shown by the control molecule **2** (for more details see ESI,† Table S2 and Fig. S7).

The next round of modifications undertaken to improve the antiproliferative activities have been performed by introducing a keto group at the C-7 position (compound **9**), as well as a methoxy group at C-5 and the second phenyl group at the C-7 position (compound **10**). The relationships between these structural changes and cytotoxic activities were established by comparing the IC_{50} values of the methoxy analogues **9** and **10** with those recorded for the natural products **1** and **2** that are arbitrarily used as control molecules. As the results shown in Table 1 indicate, in the great majority of the cases, the analogue **9** showed a more potent antiproliferative activity than the control molecules **1** and **2**. Furthermore, insertion of a phenyl group at the C-7 position of the 5-*O*-methoxy derivative (**10**) resulted in the increased activity of the lead **1** against five of the eight studied tumour cell lines. However, compound **10** was a more potent cytotoxic agent

Table 1 *In vitro* cytotoxicities of natural products **1** and **2**, their *O*-methylated derivatives **3**–**10** and DOX

Compounds	IC_{50} (μM),[a] 72 hours								
	K562	HL-60	Jurkat	Raji	MCF-7	MDA-MB 231	HeLa	A549	MRC-5
1	0.41	>100	32.45	18.45	16.59	75.34	8.32	35.21	>100
2	0.028	22.02	18.64	1.25	9.24	58.70	0.89	21.02	>100
3	7.95	2.96	15.64	15.64	>100	0.064	5.64	2.64	>100
4	5.36	25.01	24.85	8.69	4.04	28.64	8.63	23.55	>100
5	14.65	2.21	2.36	12.36	>100	1.36	56.37	26.34	>100
6	11.54	1.63	12.64	21.08	>100	0.13	8.46	5.31	>100
7	8.45	18.95	15.46	12.22	12.65	35.22	11.01	38.25	>100
8	9.24	4.36	1.69	10.47	>100	2.65	89.34	25.05	87.61
9	22.47	11.21	8.97	25.61	3.44	41.02	6.39	2.58	>100
10	28.69	2.36	9.79	18.95	11.36	51.02	12.64	15.08	>100
DOX	0.25	0.92	0.03	2.98	0.20	0.09	0.07	4.91	0.10

[a] IC_{50} is the concentration of the compound required to inhibit the cell growth by 50% compared to an untreated control. The values are means of three independent experiments done in quadruplicates. Coefficients of variation were <10%.

than lead **2** against only four of the eight malignant cell lines (for more details see ESI,† Table S2 and Fig. S7).

Cell cycle analysis

The cell passes through a series of events (cell cycle) leading to cell division and duplication. Cells that actively pass through the cell cycle are the targets in cancer therapy. Effects of (+)-goniofufurone (**1**), 7-*epi*-(+)-goniofufurone (**2**) and analogues **3–10** on the cell cycle changes were further studied. The cell cycle profile of exponentially growing K562 cells treated with the synthesized compounds for 72 h was analyzed by flow cytometry using cells stained with propidium iodide. Untreated cells served as the control. The results are presented in Table 2A (for the graphical presentation see Fig. S10 and S11 in the ESI†).

As the data in Table 2A indicate, treatment of K562 cells with goniofufurone (**1**) and the corresponding analogues (**3–5**, **9** and **10**) slightly change the percentage of cells in the S, G2/M and G0/G1 phases of the cell cycle compared to the control. But only compounds **5** and **10** increased the percentage of cells in the sub-G1 phase (which is suggestive of apoptosis), about 3.5- and 5.5-fold, compared to the control. Analogue **10** induced the most prominent sub-G1 peak in K562 cells after 72-h of cell treatment.

Table 2A further reveals that 7-*epi*-(+)-goniofufurone (**2**) and the corresponding analogues (**6–8**) slightly change the percentage of cells in the G0/G1, S and G2/M phases of the cell cycle compared to the control. However, analogues **6** and **7** increased about 3- and 15.5-fold the percentage of cells in the sub-G1 phase when compared to the untreated control. Analogue **7** induced the most prominent sub-G1 peak in the K562 cells after 72-h of cell treatment.

The obtained results showed that some of the synthesized *O*-methyl derivatives (compounds **5–7** and **10**) induced changes in the cell cycle distribution of the K562 cells.

Detection of apoptosis

The cell cycle analysis indicated the pro-apoptotic effect of the synthesized analogues through the formation of a sub-G1 peak. Therefore, we further analyzed apoptotic cell death using double staining with Annexin V-FITC and propidium iodide. Double staining enables detection of cells in the early phase of apoptosis and clearly discriminates truly necrotic cells from the Annexin V positive cells. The type of cell death induced by goniofufurone (**1**), 7-*epi*-goniofufurone (**2**) and analogues **3–10** in K562 human leukaemia cells was determined by flow cytometry after staining, and the results are shown in Table 2B (for the graphical presentation see Fig. S8 and S9 in the ESI†).

The apoptotic response, which was presented as the percentage of specific apoptosis, shows that all analogues increase the percentage of Annexin V positive cells compared to the parent compound **1**. Compound **10** causes the greatest specific apoptosis (33.41% after 72 h). Also, we can see that all analogues induced several-fold more Annexin V positive cells compared to the parent compound **2**. However, analogue **7** causes the greatest specific apoptosis (55.48% after 72 h). Compound **6** also causes notable specific apoptosis (11.17% after 72 h). These results suggested that the diphenylated analogue **10** and (7*S*) isomers **6** and **7** increased the percentage of Annexin V positive cells which is in accordance with results obtained during the cell cycle analysis (sub-G1 peak, 10.13, 6.03 and 28.67% respectively). The highest percentage of specific necrosis caused analogues **10** and **7** (8.73 and 2.57%, respectively).

A study on the ability of these compounds to modulate the expression of selected apoptosis markers (Bcl-2, Bax, caspase 3 and poly (ADP-ribose) polymerase-PARP) gives us the opportunity to assume the mechanisms of the actions of the synthesized analogues **3–10**. The results are presented in Fig. 2 (for more details see ESI,† Table S3).

The semi-quantitative western blot analysis indicates that the compounds **5**, **8**, **9** and **10** and natural products **1** and **2** increased the expression of the anti-apoptotic Bcl-2 protein when compared to the control, while the compounds **3**, **4**, **6** and **7** reduced the expression of the same protein. On the other hand, analogues **4**, **5**, **8**, **9**, and **10** and the lead compound **1** increased expression of the pro-apoptotic Bax

Table 2 (A) Influence of the synthesized compound on the K562 cell cycle; (B) percentage of specific apoptosis and necrosis induced after treatment with the synthesized compounds

	(A) Distribution of K562 cells in the cell cycle phases (%)				(B) Type of cell death	
Compd	Sub G1	G0/G1	S	G2/M	Specific apoptosis (%)	Specific necrosis (%)
Control	1.84	47.51	26.52	24.13	—	—
1	2.16	52.47	21.03	24.34	−0.36	−0.31
3	2.40	52.09	20.61	24.89	−0.27	−0.50
4	1.82	47.34	26.52	24.32	2.69	−0.54
5	6.36	44.86	20.48	28.30	4.32	0.11
9	1.33	45.46	23.68	29.53	2.10	−0.59
10	10.13	43.35	17.77	28.75	33.41	8.73
Control	1.84	47.51	26.52	24.13	—	—
2	2.73	51.46	22.11	23.71	−0.22	−0.33
6	6.03	53.39	19.68	20.90	11.17	0.55
7	28.67	35.04	15.08	21.21	55.48	2.57
8	2.40	48.59	22.66	26.35	1.70	−0.44

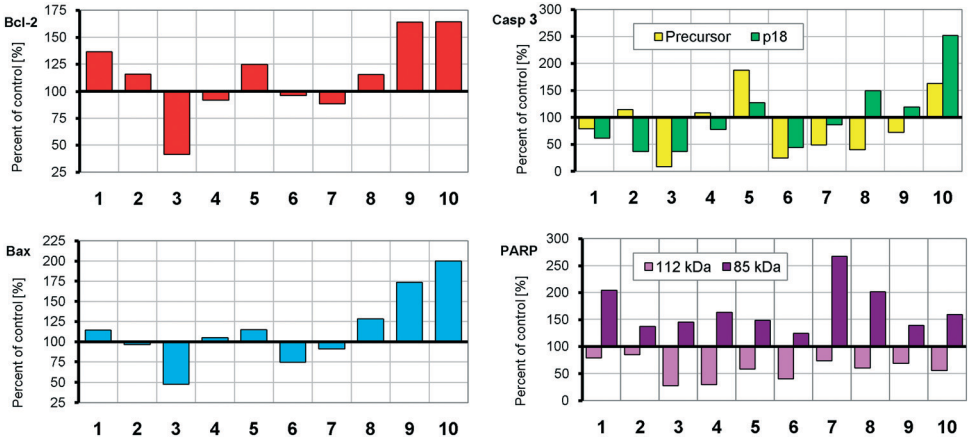


Fig. 2 Results of the western blot analysis after treatment of K562 cells with lead compounds **1** and **2** and synthesized compounds (**3**–**10**) for 72 h.

protein, while analogues **3**, **6** and **7** and the lead compound **2** decreased expression of this protein. Since the equilibrium of Bax and Bcl-2 can lead to the induction of apoptosis, we can assume that the synthesized analogues do not cause apoptosis based on these groups of proteins.

However, the expression levels of caspase 3 (precursor and active subunit) and the catalytic fragment of PARP in the K562 cells exposed to compounds **1**–**10** were measured in order to determine whether apoptosis was associated with the activation of caspase. The western blot analysis revealed overexpression of both caspase-3 units and the cleaved catalytic PARP fragment (85 kD) in K562 cells after treatment with analogues **5** and **10** while all other synthesized analogues influenced only the PARP cleavage. With the exception of compounds **8** and **9**, the analogues increased the expression of the active subunit of caspase 3 (p18) and PARP cleavage. The most expressed level of the cleavage of the PARP unit (85 kD) was recorded in K562 cells after treatment with analogue **7**, whereby this compound caused the greatest specific apoptosis (55.48% after 72 h). These results allowed us to hypothesize that all of the synthesized analogues and natural products **1** and **2** caused apoptosis in the K562 cells in a caspase-dependent manner.

All these biological results can be helpful for the further development of new antitumour agents derived from (+)-goniofufurone and 7-*epi*-(+)-goniofufurone and from the related styryl lactones.

Experimental

General experimental procedures

Melting points were determined on a Hot Stage Microscope Nagema PHMK 05 and were not corrected. Optical rotations were measured on an Autopol IV (Rudolph Research) polarimeter at room temperature. NMR spectra were recorded on a Bruker AC 250 E or a Bruker Avance III 400 MHz instrument and the chemical shifts are expressed in ppm downfield from TMS. IR spectra were recorded with an FTIR Nexus 670 spectrophotometer (Thermo-Nicolet). The band positions are given in cm^{-1}. High resolution mass spectra (ESI) of the synthesized compounds were acquired on an Agilent Technologies 1200 series instrument equipped with a Zorbax Eclipse Plus C18 (100 mm × 2.1 mm, i.d. 1.8 μm) column and a DAD detector (190–450 nm) in combination with a 6210 time-of-flight LC/MS instrument (ESI) in the positive ion mode. Flash column chromatography was performed using Kieselgel 60 (0.040–0.063, E. Merck). Preparative TLC was performed on hand-made plates, 20 × 20 cm in size with an ≈1 mm layer thickness. Kieselgel 60 G (E. Merck) with a fluorescent indicator F254 as an additive was used as the stationary phase. The corresponding bands were scraped and eluted with EtOAc. All organic extracts were dried with anhydrous Na_2SO_4. The organic solutions were concentrated in a rotary evaporator under diminished pressure at a bath temperature below 35 °C. The purities of the final products were established by HRMS and were found to be >95% pure.

General procedure for the preparation of *O*-methyl derivatives 13, 17, 20, 26 and 29

To a cooled (0 °C) and stirred solution of **12**, **16**, **11**, **21**, **23**, **25** or **28** (1 equiv.) in dry DMF (0.05 M) was added NaH (3.0–5.0 equiv.) and MeI (3.0–3.3 equiv.). The mixture was stirred at 0 °C for 0.5 h and then at room temperature for 1.5 h. The mixture was evaporated and extracted with EtOAc. The combined extracts were dried and evaporated. The residues were purified by flash column chromatography (9 : 1 light petroleum/Et_2O for **13**, 4 : 1 light petroleum/Et_2O for **17**, 7 : 3 light petroleum/Et_2O for **20**, 17 : 3 light petroleum/Et_2O for **26**, and 3 : 1 light petroleum/Et_2O for **29**) to afford pure products **13**, **17**, **20**, **26** or **29**.

3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*O*-methyl-5-*C*-phenyl-α-D-*gluco*-pentofuranose (13). Yield 94%. Colourless syrup, $[\alpha]_D$ = −65.6 (*c* 0.5, $CHCl_3$); R_f = 0.45 (4 : 1 light petroleum/Et_2O). IR (film): ν_{max} 3018 and 2936 (CH), 1216 (C–O–C). ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 and 1.46 (2 × s, 3 H each, Me_2C), 3.21 (s, 3 H, OMe), 4.23 (d, 1 H, $J_{3,4}$ = 2.0 Hz, H-3), 4.31 (dd, 1 H, $J_{3,4}$ = 2.0, $J_{4,5}$ = 9.2 Hz, H-4), 4.50 (d, 1 H, $J_{4,5}$ = 9.2 Hz, H-5), 4.66

(d, 1 H, $J_{1,2}$ = 3.5 Hz, H-2), 4.71 and 4.80 (2 × d, 2 H, J_{gem} = 11.7 Hz, CH_2Ph), 5.91 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1), 7.31–7.59 (m, 10 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.3 and 26.7 (Me_2C), 56.1 (OMe), 72.4 (CH_2Ph), 79.8 (C-5), 81.7 (C-3), 82.3 (C-2), 82.8 (C-4), 105.0 (C-1), 111.5 (Me_2C), 127.7, 127.8, 127.84, 128.0, 128.3, 128.4, 137.8 and 139.1 (Ph). HRMS (ESI): *m*/*z* 409.1407 (M^+ + K), calcd. for $C_{22}H_{26}KO_5$: 409.1412.

3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*O*-methyl-5-*C*-phenyl-β-L-*ido*-pentofuranose (17). Yield 94%. Colourless syrup, $[\alpha]_D$ = −12.8 (*c* 0.5, $CHCl_3$); R_f = 0.28 (7 : 3 light petroleum/Et_2O). IR (film): ν_{max} 3031 (CH) and 2987, 1075 and 1036 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 and 1.54 (2 × s, 3 H each, Me_2C), 3.24 (s, 3 H, OMe), 3.37 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-3), 4.09 and 4.41 (2 × d, 2 H, J_{gem} = 11.3 Hz, CH_2Ph), 4.47 (dd, 1 H, $J_{3,4}$ = 3.0, $J_{4,5}$ = 8.8 Hz, H-4), 4.53 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-2), 4.55 (d, 1 H, $J_{4,5}$ = 8.8 Hz, H-5), 6.06 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 7.29–7.50 (m, 10 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.1 and 26.6 (Me_2C), 56.3 (OMe), 71.4 (CH_2Ph), 81.1 (C-2), 81.9 (C-5), 82.0 (C-3), 83.6 (C-4), 105.3 (C-1), 111.5 (Me_2C), 127.3, 127.6, 127.8, 128.1, 128.2, 137.1 and 137.8 (Ph). HRMS (ESI): *m*/*z* 393.1658 (M^+ + Na), calcd. for $C_{22}H_{26}NaO_5$: 393.1673; *m*/*z* 409.1399 (M^+ + K), calcd. for $C_{22}H_{26}KO_5$: 409.1412.

1,2 : 5,6-Di-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (20). Yield 81%. Colourless syrup, $[\alpha]_D$ = −37.0 (*c* 0.5, EtOH), lit[15] $[\alpha]_D$ = −33.0 (*c* 0.4, EtOH); R_f = 0.63 (1 : 1 light petroleum/Et_2O). IR (film): ν_{max} 2987 and 2937 (CH), 1083 and 1020 (C–O–C). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.21, 1.25, 1.32 and 1.38 (4 × s, 3 H each, 2 × Me_2C), 3.34 (s, 3 H, OMe), 3.66 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-3), 3.82–4.03 (m, 3 H, 2 × H-6 and H-4), 4.18 (m, 1 H, H-5), 4.45 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-2), 5.75 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 25.1, 26.0 and 26.6 (2 × Me_2C), 57.9 (OMe), 66.9 (C-6), 72.2 (C-5), 80.8 (C-4), 81.7 (C-2), 83.5 (C-3), 104.9 (C-1), 108.7 and 111.4 (2 × Me_2C). HRMS (ESI): *m*/*z* 275.1487 (M^+ + H), calcd. for $C_{13}H_{23}O_6$: 275.1489; *m*/*z* 292.1755 (M^+ + NH_4), calcd. for $C_{13}H_{26}NO_6$: 292.1755.

1,2-*O*-Isopropylidene-3,5-di-*O*-methyl-5-*C*-phenyl-α-D-*gluco*-pentofuranose (26). Yield 85% (from **25**), 88% (from **21**). Colourless prisms, mp 54–55 °C (CH_2Cl_2/hexane), $[\alpha]_D$ = −98.0 (*c* 0.5, $CHCl_3$); R_f = 0.33 (4 : 1 light petroleum/Et_2O). IR (KBr): ν_{max} 2933 and 2918 (CH), 1089 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 and 1.44 (2 × s, 3 H each, Me_2C), 3.25 and 3.54 (2 × s, 3 H each, 2 × OMe), 3.93 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-3), 4.24 (dd, 1 H, $J_{3,4}$ = 2.9, $J_{4,5}$ = 9.2 Hz, H-4), 4.40 (d, 1 H, $J_{4,5}$ = 9.2 Hz, H-5), 4.59 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-2), 5.86 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 7.29–7.43 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.2 and 26.7 (Me_2C), 56.2 and 58.2 (2 × OMe), 79.7 (C-5), 81.7 (C-2), 82.6 (C-4), 83.7 (C-3), 104.9 (C-1), 111.4 (Me_2C), 127.6, 127.9, 128.2 and 139.1 (Ph). HRMS (ESI): *m*/*z* 333.1090 (M^+ + K), calcd. for $C_{16}H_{22}KO_5$: 333.1099.

1,2-*O*-Isopropylidene-3,5-di-*O*-methyl-5-*C*-phenyl-β-L-*ido*-pentofuranose (29). Yield 90% (from **28**), 93% (from **23**). Colourless syrup, $[\alpha]_D$ = +66.6 (*c* 0.5, $CHCl_3$); R_f = 0.29 (7 : 3 light petroleum/Et_2O). IR (film): ν_{max} 3018 and 2935 (CH), 1217 and 1081 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 and 1.50 (2 × s, 3 H each, Me_2C), 3.14 (s, 3 H, C_3–OMe), 3.25 (s, 3 H, C_5–OMe), 3.93 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-3), 4.34 (dd, 1 H, $J_{3,4}$ = 2.9, $J_{4,5}$ = 8.6 Hz, H-4), 4.45–4.50 (m, 2 H, $J_{1,2}$ = 4.0, $J_{4,5}$ = 8.8 Hz, H-2 and H-5), 6.01 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 7.30–7.45 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.2 and 26.7 (2 × Me_2C), 56.6 (C_5–OMe), 57.1 (C_3–OMe), 80.7 (C-2), 82.1 (C-5), 83.6 (C-4), 83.8 (C-3), 105.5 (C-1), 111.7 (Me_2C), 127.8, 128.2, 128.3 and 138.1 (Ph). HRMS (ESI): *m*/*z* 312.1793 (M^+ + NH_4), calcd. for $C_{16}H_{26}NO_5$: 312.1806; *m*/*z* 333.1096 (M^+ + K), calcd. for $C_{16}H_{22}KO_5$: 333.1099.

1,2-*O*-Isopropylidene-3-*O*-methyl-5-*C*-phenyl-α-D-*gluco*- (21) and β-L-*ido*-pentofuranose (23)

To a solution of compound **20** (3.40 g, 12.39 mmol) in dry EtOAc (340 mL) was added H_5IO_6 (3.54 g, 15.55 mmol). The mixture was stirred at room temperature for 1.5 h, then filtered and evaporated to afford crude aldehyde **20a**. To a stirred and cooled (0 °C) solution of **20a** (2.38 g) in dry toluene (80 mL) was added a solution of PhMgBr (16 mL, 32.00 mmol) in dry THF. The mixture was stirred for 5 h at 0 °C, in an atmosphere of nitrogen, then poured into 10% aq NH_4Cl (150 mL) and extracted with EtOAc (2 × 100 mL). The combined extracts were washed with 10% aq NaCl (100 mL), the organic phase was dried and evaporated and the residue purified by flash column chromatography (1 : 1 light petroleum/Et_2O). Eluted first was the pure minor product **21** (0.20 g, 6%) isolated as a colourless oil. Crystallization from CH_2Cl_2/hexane gave colourless prisms, mp 71–73 °C, $[\alpha]_D$ = −43.7 (*c* 0.5, $CHCl_3$), R_f = 0.33 (1 : 1 light petroleum/Et_2O). IR (KBr): ν_{max} 3508 (OH). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.31 and 1.46 (2 × s, 3 H each, Me_2C), 3.41 (s, 3 H, OMe), 3.62 (bs, 1 H, OH), 3.71 (d, 1 H, $J_{3,4}$ = 3.2 Hz, H-3), 4.28 (dd, 1 H, $J_{3,4}$ = 3.2, $J_{4,5}$ = 5.7 Hz, H-4), 4.56 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-2), 5.09 (d, 1 H, $J_{4,5}$ = 5.6 Hz, H-5), 6.01 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 7.20–7.50 (m, 5 H, Ph). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 26.1 and 26.7 (Me_2C), 57.5 (OMe), 72.3 (C-5), 81.0 (C-2), 82.3 (C-4), 85.1 (C-3), 105.1 (C-1), 111.5 (Me_2C), 126.0, 127.6, 128.4 and 141.4 (Ph). HRMS (ESI): *m*/*z* 298.1646 (M^+ + NH_4), calcd. for $C_{15}H_{24}NO_5$: 298.1649; *m*/*z* 561.2679 (2 M^+ + H), calcd. for $C_{30}H_{41}O_{10}$: 561.2694.

Eluted second was the pure major product **23** (1.95 g, 56%) isolated as a colourless oil. Crystallization from CH_2Cl_2/hexane gave colourless prisms, mp 97–98 °C, $[\alpha]_D$ = −70.6 (*c* 0.5, $CHCl_3$), R_f = 0.25 (1 : 1 light petroleum/Et_2O). IR (KBr): ν_{max} 3528 (OH). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.29 and 1.46 (2 × s, 3 H each, Me_2C), 2.88 (bs, 1 H, OH), 3.25 (s, 3 H, OMe), 3.25 (d, 1 H, H-3), 4.23 (dd, 1 H, $J_{3,4}$ = 3.2, $J_{4,5}$ = 7.9 Hz, H-4), 4.53 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-2), 5.00 (d, 1 H, $J_{4,5}$ = 7.9 Hz, H-5), 5.97 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 7.20–7.55 (m, 5 H, Ph). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 26.1 and 26.6 (Me_2C), 57.2 (OMe), 72.2 (C-5), 81.4 (C-2), 83.7 (C-3), 84.6 (C-4), 105.0 (C-1), 111.7 (Me_2C), 126.9, 128.0, 128.2 and 139.7 (Ph). HRMS (ESI): *m*/*z* 298.1648 (M^+ + NH_4), calcd. for $C_{15}H_{24}NO_5$: 298.1649.

General procedure for the cyclocondensation with Meldrum's acid (preparation of 15, 19, 4, 7, 5 and 8)

The starting compound **13**, **17**, **21**, **23**, **26** or **29** (1 equiv.) was dissolved in 90% TFA (0.1 M) and the resulting solution was

stirred at room temperature until the starting material was consumed (TLC, 0.5 h for **13**, **21** and **23**, 1 h for **17**, **26** and **29**). The mixture was concentrated by co-distillation with toluene, and the residue was purified by flash column chromatography (7 : 3 light petroleum/Et_2O for **14**, 4 : 1 Et_2O/light petroleum for **18** and **27**, 4 : 1 EtOAc/toluene for **22**, 3 : 2 toluene/EtOAc for **24**, and Et_2O for **30**). To a stirred solution of compound **14**, **18**, **22**, **24**, **27** or **30** (1 equiv.) in dry DMF (0.07 M) was added Meldrum's acid (3.0–3.6 equiv.) and dry Et_3N (3.0–3.1 equiv.). The mixture was stirred at 44–46 °C until the starting material was consumed (TLC, 70 h for **14** and **24**, 68 h for **18**, 74 h for **22**, 71 h for **27** and **30**). The residue was evaporated and purified by flash column chromatography (3 : 2 light petroleum/Et_2O for **15**, 1 : 1 light petroleum/Et_2O for **5**, 7 : 3 toluene/EtOAc for **4**, 4 : 1 Et_2O/light petroleum for **7**, and 7 : 3 Et_2O/light petroleum for **19** and **8**).

3,6-Anhydro-5-*O*-benzyl-2-deoxy-7-*O*-methyl-7-*C*-phenyl-D-*glycero*-D-*ido*-heptono-1,4-lactone (15). Yield 51%. Colourless needles, mp 172–173 °C (CH_2Cl_2/hexane), $[\alpha]_D$ = −10.0 (*c* 0.5, $CHCl_3$); R_f = 0.39 (3 : 2 Et_2O/light petroleum). IR (KBr): ν_{max} 1780 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.51 (bd, 1 H, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.61 (dd, 1 H, $J_{2b,3}$ = 5.7, $J_{2a,2b}$ = 18.8 Hz, H-2b), 3.19 (s, 3 H, OMe), 4.14 (dd, 1 H, $J_{5,6}$ = 3.2, $J_{6,7}$ = 8.9 Hz, H-6), 4.44 (d, 1 H, $J_{5,6}$ = 3.2 Hz, H-5), 4.46 (d, 1 H, $J_{6,7}$ = 8.9 Hz, H-7), 4.79 (s, 2 H, CH_2Ph), 4.91–4.94 (m, 2 H, H-3 and H-4), 7.31–7.59 (m, 10 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.9 (C-2), 56.15 (OMe), 73.3 (CH_2Ph), 77.1 (C-3), 78.0 (C-7), 81.0 (C-5), 83.4 (C-6), 85.2 (C-4), 127.8, 128.1, 128.2, 128.3, 128.5, 128.6, 137.4, 139.0 (Ph) and 175.50 (C-1). HRMS (ESI): *m*/*z* 377.1349 (M^+ + Na), calcd. for $C_{21}H_{22}NaO_5$: 377.1359; *m*/*z* 393.1088 (M^+ + K), calcd. for $C_{21}H_{22}KO_5$: 393.1099.

3,6-Anhydro-5-*O*-benzyl-2-deoxy-7-*O*-methyl-7-*C*-phenyl-L-*glycero*-D-*ido*-heptono-1,4-lactone (19). Yield 47%. Colourless syrup, $[\alpha]_D$ = +92.0 (*c* 0.5, $CHCl_3$); R_f = 0.24 (49 : 1 CH_2Cl_2/EtOAc). IR (film): ν_{max} 1789 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.73 (dd, 1 H, $J_{2a,3}$ = 6.3, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.84 (d, 1 H, $J_{2a,2b}$ = 18.8 Hz, H-2b), 3.23 (s, 3 H, OMe), 4.65 (d, 1 H, $J_{5,6}$ =3.5 Hz, H-5), 4.29 and 4.40 (2 × d, 2 H, J_{gem} = 11.5 Hz, CH_2Ph), 4.32 (dd, 1 H, $J_{5,6}$ = 3.6, $J_{6,7}$ = 7.9 Hz, H-6), 4.46 (d, 1 H, $J_{6,7}$ = 7.9 Hz, H-7), 4.84 (d, 1 H, $J_{3,4}$ = 4.6 Hz, H-4), 5.12 (t, 1 H, $J_{2a,3}$ =5.5, $J_{3,4}$ = 5.2 Hz, H-3), 7.23–7.50 (m, 10 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.0 (C-2), 56.5 (OMe), 72.6 (CH_2Ph), 77.6 (C-3), 81.8 (C-5), 82.2 (C-7), 84.2 (C-6), 84.4 (C-4), 127.6, 127.8, 128.1, 128.4, 128.5, 128.6, 136.9, 137.6 (Ph) and 175.3 (C-1). HRMS (ESI): *m*/*z* 372.1813 (M^+ + NH_4), calcd. for $C_{21}H_{26}NO_5$: 372.1806; *m*/*z* 377.1361 (M^+ + Na), calcd. for $C_{21}H_{22}NaO_5$: 377.1359; *m*/*z* 393.1102 (M^+ + K), calcd. for $C_{21}H_{22}KO_5$: 393.1099.

3,6-Anhydro-2-deoxy-5-*O*-methyl-7-*C*-phenyl-D-*glycero*-D-*ido*-heptono-1,4-lactone (4). Yield 41%. Colourless syrup, $[\alpha]_D$ = +5.5 (*c* 0.5, $CHCl_3$); R_f = 0.23 (7 : 3 Et_2O/light petroleum). IR (film): ν_{max} 3482 (OH), 1786 (C═O). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.63 (bd, 1 H, $J_{2a,2b}$ = 18.3 Hz, H-2a), 2.69 (dd, 1 H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 5.6 Hz, H-2b), 3.20 (bs, 1 H, OH), 3.48 (s, 3 H, OMe), 4.01 (d, 1 H, $J_{5,6}$ = 3.6 Hz, H-5), 4.61 (d, 1 H, $J_{5,6}$ = 3.6, $J_{6,7}$ = 7.0 Hz, H-6), 4.88–5.01 (m, 3 H, $J_{3,4}$ = 4.6 Hz, H-4, H-7 and H-3), 7.26–7.49 (m, 5 H, Ph). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 35.7 (C-2), 58.3 (OMe), 71.8 (C-7), 77.0 (C-3), 83.0 (C-6), 83.8 (C-5), 84.0 (C-4), 126.1, 127.8, 128.3, 141.0 (Ph) and 175.3 (C-1). HRMS (ESI): *m*/*z* 282.1334 (M^+ + NH_4), calcd. for $C_{14}H_{20}NO_5$: 282.1336.

3,6-Anhydro-2-deoxy-5-*O*-methyl-7-*C*-phenyl-L-*glycero*-D-*ido*-heptono-1,4-lactone (7). Yield 46%. Colourless needles, mp 144–146 °C (CH_2Cl_2/hexane), $[\alpha]_D$ = +122.4 (*c* 0.5, $CHCl_3$); R_f = 0.36 (Et_2O). IR (KBr): ν_{max} 3450 (OH), 1783 (C═O). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.63 (d, 1 H, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.72 (dd, 1 H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 4.9 Hz, H-2b), 2.99 (bs, 1 H, OH), 3.29 (s, 3 H, OMe), 3.52 (d, 1 H, $J_{5,6}$ = 3.7 Hz, H-5), 4.61 (d, 1 H, $J_{5,6}$ = 3.8, $J_{6,7}$ = 7.1 Hz, H-6), 4.87 (d, 1 H, $J_{3,4}$ = 4.6 Hz, H-4), 4.94 (d, 1 H, $J_{6,7}$ = 7.1 Hz, H-7), 5.00 (m, 1 H, H-3), 7.20–7.44 (m, 5 H, Ph). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 35.8 (C-2), 57.9 (OMe), 72.5 (C-7), 77.2 (C-3), 83.3 (C-5), 84.3 (C-4), 84.7 (C-6), 126.7, 128.0, 128.2, 139.7 (Ph) and 175.3 (C-1). HRMS (ESI): *m*/*z* 282.1343 (M^+ + NH_4), calcd. for $C_{14}H_{20}NO_5$: 282.1336.

3,6-Anhydro-2-deoxy-5,7-di-*O*-methyl-7-*C*-phenyl-D-*glycero*-D-*ido*-heptono-1,4-lactone (5). Yield 44%. Colourless needles, mp 96–97 °C (CH_2Cl_2/hexane), $[\alpha]_D$ = −57.0 (*c* 0.5, $CHCl_3$); R_f = 0.23 (CH_2Cl_2). IR (KBr): ν_{max} 1784 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.47 (bd, 1 H, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.61 (dd, 1 H, $J_{2a,2b}$ = 18.9, $J_{2b,3}$ = 6.4 Hz, H-2b), 3.21 (s, 3 H, C_7–OMe), 3.58 (s, 3 H, C_5–OMe), 4.07 (dd, 1 H, $J_{5,6}$ = 3.2, $J_{6,7}$ = 8.9 Hz, H-6), 4.14 (d, 1 H, $J_{5,6}$ = 3.0 Hz, H-5), 4.35 (d, 1 H, $J_{6,7}$ = 8.9 Hz, H-7), 4.87 (bt, 1 H, *J* = 5.6, *J* = 5.1 Hz, H-3), 4.93 (d, 1 H, $J_{3,4}$ = 4.5 Hz, H-4), 7.28–7.41 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.0 (C-2), 56.1 (C_7–OMe), 58.8 (C_5–OMe), 76.8 (C-3), 79.8 (C-7), 82.8 (C-5), 83.1 (C-6), 84.5 (C-4), 127.6, 128.1, 128.3 and 139.0 (Ph) and 175.4 (C-1). HRMS (ESI): *m*/*z* 317.0777 (M^+ + K), calcd. for $C_{15}H_{18}KO_5$: 317.0786.

3,6-Anhydro-2-deoxy-5,7-di-*O*-methyl-7-*C*-phenyl-L-*glycero*-D-*ido*-heptono-1,4-lactone (8). Yield 51%. Colourless prisms, mp 119–120 °C (CH_2Cl_2/hexane), $[\alpha]_D$ = +206.8 (*c* 0.5, $CHCl_3$); R_f = 0.14 (24 : 1 CH_2Cl_2/EtOAc). IR (KBr): ν_{max} 1787 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.74 (dd, 1 H, $J_{2a,3}$ = 6.1, $J_{2a,2b}$ = 18.9 Hz, H-2a), 2.82 (d, 1 H, $J_{2a,2b}$ = 18.8 Hz, H-2b), 3.22 and 3.23 (2 × s, 6 H, 2 × OMe), 3.31 (d, 1 H, $J_{5,6}$ = 2.9 Hz, H-5), 4.23 (dd, 1 H, $J_{5,6}$ = 3.2, $J_{6,7}$ = 8.2 Hz, H-6), 4.46 (d, 1 H, $J_{6,7}$ = 8.2 Hz, H-7), 4.86 (d, 1 H, $J_{3,4}$ = 4.6 Hz, H-4), 5.08 (t, 1 H, *J* = 5.3 Hz, H-3), 7.31–7.44 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.0 (C-2), 56.6 and 57.8 (2 × OMe), 77.6 (C-3), 82.2 (C-7), 83.1 (C-5), 83.7 (C-4), 84.2 (C-6), 127.8, 128.5, 128.55, 137.7 (Ph) and 175.39 (C-1). HRMS (ESI): *m*/*z* 301.1038 (M^+ + Na), calcd. for $C_{15}H_{18}NaO_5$: 301.1046; *m*/*z* 317.0774 (M^+ + K), calcd. for $C_{15}H_{18}KO_5$: 317.0786.

General procedure for benzyl deprotection (compounds 3 and 6)

A solution of **15** or **19** (1 equiv.) in MeOH (0.01 M) was hydrogenated over 10% Pd/C (0.01 or 0.02 g; the catalyst contained 50% of water) for 4 h (for **15**) or 24 h (for **19**) at room temperature. The mixture was filtered through a Celite pad and the catalyst was washed with MeOH. The combined organic solutions were evaporated and the residues were purified by flash chromatography (4 : 1 Et_2O/light petroleum for **3**; 9 : 1 Et_2O/light petroleum for **6**) to afford pure **3** and **6**.

3,6-Anhydro-2-deoxy-7-*O*-methyl-7-*C*-phenyl-D-*glycero*-D-*ido*-heptono-1,4-lactone (3). Yield 82%. Colourless needles, mp 123–124 °C (CH_2Cl_2/hexane), $[\alpha]_D$ = +6.4 (*c* 0.5, $CHCl_3$); R_f = 0.32 (4 : 1 Et_2O/light petroleum). IR (KBr): ν_{max} 3421 (OH), 1784 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (d, 1 H, $J_{2a,2b}$ = 18.7 Hz, H-2a), 2.74 (dd, 1 H, $J_{2a,2b}$ = 18.8, $J_{2b,3}$ = 5.9 Hz, H-2b), 3.35 (s, 3 H, OMe), 3.98 (dd, 1 H, $J_{5,6}$ = 2.7, $J_{6,7}$ = 4.3 Hz, H-6), 4.42 (d, 1 H, $J_{5,6}$ = 2.4 Hz, H-5), 4.71 (d, 1 H, $J_{6,7}$ = 4.3 Hz, H-7), 4.88 (d, 1 H, $J_{3,4}$ = 4.2 Hz, H-4), 5.10 (m, 1 H, H-3), 7.34–7.46 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.0 (C-2), 57.9 (OMe), 74.1 (C-5), 77.0 (C-3), 83.0 (C-7), 83.5 (C-6), 87.4 (C-4), 126.9, 128.7, 128.73, 128.9, 129.0, 137.0 (Ph) and 175.4 (C-1). HRMS (ESI): *m*/*z* 282.1332 (M^+ + NH_4), calcd. for $C_{14}H_{20}NO_5$: 282.1336; *m*/*z* 287.0899 (M^+ + Na), calcd. for $C_{14}H_{16}NaO_5$: 287.0890.

3,6-Anhydro-2-deoxy-7-*O*-methyl-7-*C*-phenyl-L-*glycero*-D-*ido*-heptono-1,4-lactone (6). Yield 68%. Colourless powder, mp 118 °C (CH_2Cl_2/hexane), $[\alpha]_D$ = +187.0 (*c* 0.5, $CHCl_3$); R_f = 0.17 (Et_2O). IR (KBr): ν_{max} 3413 (OH), 1782 (C═O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (dd, 1 H, $J_{2a,3}$ = 2.9, $J_{2a,2b}$ = 18.8 Hz, H-2a), 2.76 (dd, 1 H, $J_{2a,2b}$ = 18.8, $J_{2b,3}$ = 4.5 Hz, H-2b), 3.28 (s, 3 H, OMe), 3.98 (dd, 1 H, $J_{6,7}$ = 5.2 Hz, H-6), 4.22 (bs, 1 H, H-5), 4.57 (d, 1 H, $J_{6,7}$ = 5.1 Hz, H-7), 4.86 (d, 1 H, $J_{3,4}$ = 4.6 Hz, H-4), 5.11 (m, 1 H, H-3), 7.34–7.43 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.9 (C-2), 56.6 (OMe), 75.1 (C-5), 77.0 (C-3), 82.4 (C-7), 83.0 (C-6), 87.5 (C-4), 127.6, 128.7, 137.2 (Ph), and 175.4 (C-1). HRMS (ESI): *m*/*z* 282.1329 (M^+ + NH_4), calcd. for $C_{14}H_{20}NO_5$: 282.1336; *m*/*z* 287.0893 (M^+ + Na), calcd. for $C_{14}H_{16}NaO_5$: 287.0890; *m*/*z* 303.0630 (M^+ + K), calcd. for $C_{14}H_{16}KO_5$: 303.0629.

3,6-Anhydro-2-deoxy-5-*O*-methyl-7-*C*-phenyl-D-*ido*-hept-7-ulosono-1,4-lactone (9). To a solution of compound **4** or **7** (1 equiv.) in dry CH_2Cl_2 (0.01 M) was added PCC (2.6 equiv.). The mixture was stirred at reflux for 2.5 h (for **4**) or 2 h (for **7**) then concentrated, and the residues were purified by flash column chromatography 4 : 1 Et_2O/light petroleum. Pure **9** (yields 99% from **4**, 91% from **7**) was isolated as a colourless solid. Recrystallization from CH_2Cl_2/hexane gave colourless needles, mp 135–136 °C, $[\alpha]_D$ = +27.4 (*c* 0.5, $CHCl_3$); R_f = 0.30 (4 : 1 Et_2O/light petroleum). IR (KBr): ν_{max} 1779 (C_1═O, lactone), 1698 (C_7═O). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.63 (d, 1 H, $J_{2a,2b}$ = 18.4 Hz, H-2a), 2.72 (dd, 1 H, $J_{2a,2b}$ = 18.6, $J_{2b,3}$ = 4.5 Hz, H-2b), 3.12 (s, 3 H, OMe), 4.43 (d, 1 H, $J_{5,6}$ = 5.1 Hz, H-5), 4.94 (d, 1 H, $J_{3,4}$ = 4.0 Hz, H-4), 5.19 (m, 1 H, *J* = 4.3 Hz, H-3), 5.56 (d, 1 H, $J_{5,6}$ = 5.1 Hz, H-6), 7.32–7.92 (m, 5 H, Ph). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 35.9 (C-2), 58.5 (OMe), 78.2 (C-3), 83.2 (C-6), 84.6 (C-4), 85.3 (C-5), 127.6, 128.5, 133.1, 135.6 (Ph), 174.7 (C-1) and 194.4 (C-7). HRMS (ESI): *m*/*z* 263.0909 (M^+ + H), calcd. for $C_{14}H_{15}O_5$: 263.0914; *m*/*z* 280.1175 (M^+ + NH_4), calcd. for $C_{14}H_{18}NO_5$: 280.1180; *m*/*z* 285.0729 (M^+ + Na), calcd. for $C_{14}H_{14}NaO_5$: 285.0733; *m*/*z* 301.0476 (M^+ + K), calcd. for $C_{14}H_{14}KO_5$: 301.0473.

5-*O*-Methyl-7-*C*-phenyl-goniofufurone (10). To a stirred and cooled (0 °C) solution of **9** (0.11 g, 0.42 mmol) in dry THF (1.9 mL) was added a solution of PhMgBr (0.46 mL, 0.46 mmol) in dry THF. The mixture was stirred for 1.5 h at 0 °C, in an atmosphere of nitrogen, then poured into 10% aq NH_4Cl (15 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The combined extracts were washed with 10% aq NaCl (15 mL), the organic phase was dried and evaporated and the residue purified by two flash column chromatography (first 7 : 3 Et_2O/light petroleum; second 19 : 1 CH_2Cl_2/EtOAc). Eluted first was the pure product **10** (0.05 g, 35%) isolated as a colourless solid. Crystallization from Et_2O/hexane gave colourless cloudy crystals, mp 122–125 °C, $[\alpha]_D$ = −18.6 (*c* 1.0, $CHCl_3$), R_f = 0.35 (24 : 1 CH_2Cl_2/EtOAc). IR (KBr): ν_{max} 3424 (OH), 1738 (C═O). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.78 (d, 2 H, $J_{2,3}$ = 3.4 Hz, H-2), 3.13 (s, 3 H, OMe), 3.80 (d, 1 H, $J_{5,6}$ = 3.5 Hz, H-5), 4.70 (s, 1 H, exchangeable with D_2O, OH), 4.84 (d, 1 H, $J_{3,4}$ = 4.4 Hz, H-4), 5.04 (d, 1 H, $J_{5,6}$ = 3.5 Hz, H-6), 5.12 (m, 1 H, H-3), 7.14–7.56 (m, 10 H, Ph). NOE contact: H-4 and H-5. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 36.0 (C-2), 58.4 (OMe), 77.8 (C-3), 78.2 (C-7), 82.7 (C-6), 83.6 (C-4), 85.1 (C-5), 125.0, 126.0, 126.9, 126.96, 128.0, 128.2, 144.3, 145.5 (Ph) and 175.2 (C-1). HRMS (ESI): *m*/*z* 323.1274 (MH^+ − H_2O), calcd. for $C_{20}H_{18}O_4$: 323.1278; *m*/*z* 363.1200 (M^+ + Na), calcd. for $C_{20}H_{20}NaO_5$: 363.1203. Further elution gave the pure unreacted starting compound **9** (0.04 g, 35%).

X-ray crystal structure analysis

Diffraction experiments were performed on an Oxford Diffraction Gemini S diffractometer. The crystal structures were solved using SHELXT,[18] or SIR92,[19] and refined with SHELXL.[20] The structures are shown in the ESI.† The crystallographic data have been deposited at the Cambridge Crystallographic Data Centre. CCDC numbers: 1843444 (**5**), 1843445 (**23**), 1843446 (**3**), 1843447 (**21**), 1843448 (**9**) and 1843449 (**8**).

Biological evaluations

Chemicals and cell lines. Rhodamine B, RPMI 1640 medium, foetal calf serum, propidium iodide 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide and RNase A were purchased from Sigma (St. Louis, MO, USA). Penicillin and streptomycin were purchased from Galenika AD (Belgrade, Serbia). The Annexin V FLUOS apoptosis detection kit was purchased from BD Biosciences Pharmingen (Belgium). All other chemicals used in the experiments were commercial products of reagent grade. The stock solution (10 mM) was prepared in DMSO and diluted to various concentrations with serum-free culture medium. Human chronic myelogenous leukaemia (K562, ATCC CCL 243), promyelocytic leukaemia (HL-60, ATCC CCL 240), human T cell leukaemia (Jurkat, ATCC TIB 152) and Burkitt's lymphoma (Raji, ATCC CCL 86) were grown in RPMI 1640, while ER^+ breast adenocarcinoma (MCF-7, ATCC HTB 22), ER^- breast adenocarcinoma (MDA-MB 231, ATCC HTB 26), cervix carcinoma (HeLa, ATCC CCL 2), lung adenocarcinoma epithelial (A549, ATCC CCL-185) malignant cells, and normal foetal lung fibroblasts (MRC-5, ATCC CCL 171) were grown in DMEM medium. Both media were supplemented with 10% of foetal calf serum (FTS, NIVNS) and antibiotics (100 IU mL^{-1} of penicillin and 100 mg

mg^{-1} of streptomycin). The cell lines were cultured in flasks (Costar, 25 mL) at 37 °C in an atmosphere of 100% humidity and 5% of CO_2 (Heraeus). Exponentially growing viable cells were used throughout the assays. All of the cell lines were obtained from the ATCC (American Type Culture Collection).

Cell treatment. The cells were seeded in six-well plates at a concentration of 5×10^5 cells per well. The cells were treated for 72 h with goniofufurone (**1**), 7-*epi*-goniofufurone (**2**) and the analogues (**3**–**10**) at their IC_{50} concentrations. Untreated cells were used as the positive control. Viable cells of the treated and control samples were used for apoptosis detection. Viability was determined using the trypan blue dye-exclusion assay.

MTT assay. The colorimetric MTT assay was carried out following the literature procedure.[17]

Cell cycle analysis. The cell cycle analysis was carried out following a previously reported procedure.[21]

Detection of apoptosis. Apoptosis of K562 cells was evaluated according to a previously reported procedure.[21]

Western blot. Western blot was carried out as reported previously.[21]

Conclusions

In conclusion, eight new analogues of (+)-goniofufurone and 7-*epi*-(+)-goniofufurone bearing methoxy groups at C-7 and/or C-5 were synthesized and evaluated for their antiproliferative activity against a panel of human malignant cell lines. All synthesized analogues showed a stronger activity than lead **1** against at least two tumour cell lines, while seven analogues are more active than lead **2** toward at least two malignant cell lines. Two analogues were more active than DOX, while a single analogue shows similar activity to DOX against at least two tumour cell lines. The 7-*O*-methoxy derivative **3** showed the highest potency toward MDA-MB 231 cells (IC_{50} 0.064 μM) being the most active compound under evaluation. None of the analogues showed any significant cytotoxicity toward normal MRC-5 cells. In contrast, the commercial drug DOX showed a potent cytotoxic activity with an IC_{50} value of 0.10 μM.

The preliminary SAR analysis suggested the following structural requirements for the cytotoxic effects of the synthesized compounds: (A) introduction of methoxy groups at the C-7 and/or C-5 positions caused the increased cytotoxicity originally displayed by lead **1**, but decreased the antiproliferative potency originally demonstrated by lead **2**; (B) the keto derivative **9** with a methoxy group at C-5, showed a more potent antiproliferative activity than the control molecules **1** and **2**; (C) insertion of an additional phenyl group at the C-7 position of the 5-*O*-methoxy derivative (compound **10**) resulted in an increase in the cytotoxic activity with respect to lead **1**, but decreased the activity with respect to **2**.

The cell cycle analysis reveals that the treatment of cells with some of the synthesized analogues increases the intensity of the sub-G1 peak of K562 cells indicating that the mechanism of action of these compounds involves apoptosis. Flow cytometry further confirmed that a significant percentage of specific apoptosis was detected after treatment with some of the analogues. Compounds **6**, **7** and **10** caused the greatest specific apoptosis (11.17, 55.48, and 33.41%, respectively).

X-ray analysis of the methylated goniofufurone analogues did not confirm the existence of intramolecular H-bonds in their molecules. This suggests that the formation of a quasi-ring is unlikely (at least in solid state). However, there are numerous other reasons that could render the mono- or dialkylated derivatives more or less active such as a change in lipophilicity, increased or decreased uptake, *etc.*

Conflicts of interest

There are no conflicts to declare.

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