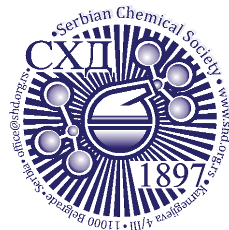

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Synthesis and antiproliferative activity of simplified goniofufurone analogues

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Abstract: Several (+)-goniofufurone analogues with simplified structures were designed, synthesized and evaluated for their *in vitro* antitumour activity, against a panel of human tumour cell lines. Dephenylated compounds **2** and **3** demonstrated remarkable antitumour activities, in the cultures of K562 and Raji cells with IC_{50} values in the range of 3.0–9.3 nM. Each of goniofufurone analogues lacking the tetrahydrofuran ring (**4**, **5** and **6**) strongly inhibited the growth of at least one malignant cell line, with IC_{50} values in the range of 11-30 nM. Brief structure–activity relationship (SAR) analysis showed that the simplified goniofufurone analogues, designed by removing the phenyl group from C-7, or by opening the THF ring, could show stronger antiproliferative effects compared to control molecules. It is noticeable that analogues **2**–**8** are completely inactive with respect to the normal MRC-5 cell line. These findings, together with their potent antitumour activities, provide a suitable basis for the development of new and selective antitumour drugs.

Keywords: structure simplification based drug design; goniofufurone mimics; furanolactones; cytotoxicity; Wittig olefination; oxa-Michael ring-closure; SAR analysis.

INTRODUCTION

The development of practical and efficient routes for the synthesis of natural products and their analogues is of considerable interest for drug design and discovery.[1–3] However, the complex chemical structures of natural products often complicate the general synthesis procedure, SAR investigations and structural optimizations, or result in unfavourable ADMET (absorption, distribution, meta-

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bolism, excretion, toxicity) properties.[4] Therefore, simplifying complex structures without the decrease of biological activity is an effective strategy for improving synthetic accessibility and accelerating the drug development process.[5]

The styryl lactones from the plants of *Goniothalamus* genus are an interesting class of naturally occurring compounds, many of which were found to exhibit impressive biological activities.[6–9] One of the most important among them is (+)-goniofufurone (**1**; Fig. 1), a naturally occurring styryl lactone that have attracted considerable attention since its isolation from the stem bark of *Goniothalamus giganteus* (Annonaceae).[10] Its structure was elucidated by spectroscopic methods, and the relative configurations determined by X-ray crystallography. The absolute configuration of **1** was established independently by Shing[11] and Jäger[12] from the syntheses of its opposite enantiomer, (−)-goniofufurone. Due to its unique structural features and promising antitumour activities,[6–9] the natural product **1**, along with a number of its analogues and derivatives have been the targets of many total syntheses.[13–15] We have also been involved in the synthesis of **1** and related compounds.[16] Our preliminary results on antiproliferative properties of analogues **2** and **3** showed that they exhibit moderate to potent citotoxicity,[17] which led us to prepare a number of analogues for a detailed SAR analysis. More potent analogues can be designed by manipulation of functional groups, by changing the stereochemistry or conformational constraints, and after closing or opening a ring in the natural lead.[3,5] This paper summarizes the application of the structural simplification of (+)-goniofufurone (**1**), in order to elucidate the role of the phenyl group and the tetrahydrofuran ring in antiproliferative activity.

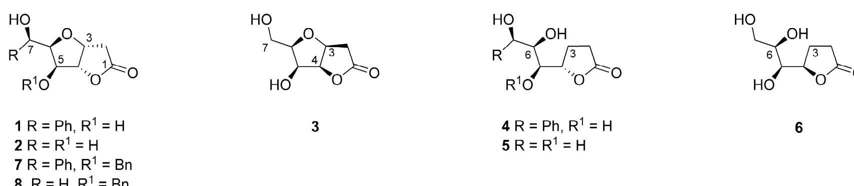

Fig. 1. Chemical structures of (+)-goniofufurone (**1**) and the corresponding analogues (**2**–**8**).

EXPERIMENTAL

General procedures

Melting points were determined on Büchi 510 or on Hot Stage Microscope Nagema PHMK 05 apparatus and were not corrected. Optical rotations were measured on Autopol IV (Rudolph Research) automatic polarimeter. IR spectra were recorded by a FTIR Nexus 670 (Thermo-Nicolet) spectrophotometer. NMR spectra were recorded on a Bruker AC 250 E, or a Bruker Avance III 400 MHz instrument and chemical shifts are expressed in ppm downfield from tetramethylsilane. Low resolution mass spectra were recorded on Finnigan-MAT 8230 (CI) and VG AutoSpec (FAB) mass spectrometers. High-resolution mass spectra were taken on an LTQ OrbitrapXL (Thermo Fisher Scientific Inc., USA) mass spectrometer. TLC was

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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 purities of final products were established by high performance liquid chromatography–high resolution mass spectrometry (HPLC-HRMS), or by elemental microanalysis, and were found to be >95 % pure. The characterization data of synthesized compounds are given in the Supplementary material to this paper.

Synthetic procedures

*3,6-Anhydro-5,7-di-*O*-benzyl-2-deoxy-D-*ido*-heptono-1,4-lactone (**10**) and methyl (*E*)-5,7-di-*O*-benzyl-2,3-dideoxy-D-*xylo*-hept-2-enonate (**11**).* Procedure A: Ph_3P=$CHCO_2Me$ (0.417 g, 1.2 mmol) was added to a solution of **9** (0.332 g, 1.0 mmol) in dry MeOH (10 mL) and the reaction mixture was stirred at room temperature for 24 h. An additional amount of reagent was then added (0.561 g, 1.68 mmol) and the reaction mixture was left at room temperature for another 24 h. The solution was evaporated and the residue purified by flash chromatography (Et_2O) in order to separate the product from Ph_3PO. Subsequent chromatographic purification on a column of flash silica (9:1 hexane/Et_2O) gave pure **10** (0.216 g, 61 %), as a semisolid. Recrystallization from MeOH afforded an analytical sample **10**, m.p.: 90 °C, $[\alpha]_D$ = +8.6° (*c* 1.2, $CHCl_3$), R_f = 0.62 (4:1 hexane/Et_2O). The subsequent elution of the column gave the (*E*)-olefin **11** (0.098 g, 25 %), as a colourless syrup, $[\alpha]_D$ = −188.9° (*c* 1.1, $CHCl_3$), R_f = 0.50 (4:1 Et_2O/hexane). Procedure B: Ph_3P=$CHCO_2Me$ (0.434 g, 1.3 mmol) was added to a solution of **9** (0.355 g, 1.08 mmol) in dry benzene (10 mL) and the reaction mixture was stirred under reflux for 24 h. After workup as described above, pure olefin **11** was obtained as the main product (0.274 g, 66 %), while the lactone **10** (0.091 g, 24 %) was isolated as a minor product under these reaction conditions. ^{1}H- and ^{13}C-NMR spectral data of both **10** and **11** were identical to those previously reported.[17,18]

*3,6-Anhydro-5,7-di-*O*-benzyl-2-deoxy-D-*ido*-heptono-1,4-lactone (**10**) and methyl 3,6-anhydro-5,7-di-*O*-benzyl-2-deoxy-D-*gulo*-heptonoate (**12**).* A solution containing compound **11** (0.182 g, 0.48 mmol) and imidazole (0.034 g, 0.5 mmol) in dry benzene (18 mL) was stirred under reflux for 11 h. The mixture was poured in 10 % aq. NaCl (40 mL) and extracted with CH_2Cl_2 (3×20 mL). The combined extract was dried and evaporated and the residue purified on a column of flash silica (9:1 hexane/Et_2O). Pure lactone **10** (0.085 g, 51 %) was first isolated as a semisolid. Recrystallization from MeOH afforded an analytical sample **10**, m.p.: 90 °C, $[\alpha]_D$ = +8.6° (c 1.2 in $CHCl_3$), R_f = 0.62 (4:1 hexane/Et_2O). The spectral data of thus obtained sample were in full agreement with the data previously reported by us.[17,18] Tetrahydrofuran derivative **12** (0.015 g, 17 %) was next isolated as a colourless oil, $[\alpha]_D$ = −18.7 (*c* 1.0, $CHCl_3$), R_f = 0.50 (4:1 Et_2O/hexane). ^{1}H- and ^{13}C-NMR spectral data were identical to those previously reported by us.[17,18]

*Methyl 3,6-anhydro-5,7-di-*O*-benzyl-2-deoxy-D-*gulo*-heptonoate (**12**) and methyl 5,7-di-*O*-benzyl-2,3-dideoxy-4-oxo-D-*threo*-heptonate (**13**).* A solution of **11** (0.091 g, 0.24 mmol) in 0.1 M NaOMe in MeOH (0.5 mL, 0.05 mmol) was stirred at room temperature for 20 min. The mixture was poured into 10 % aq. NH_4Cl (10 mL) and extracted with EtOAc (3×5 mL). The combined organic solutions were evaporated and the residue purified on a column of flash silica (7:3 Et_2O/hexane). Pure **13** was first isolated as a bright yellow oil (0.029 g, 32 %), $[\alpha]_D$ = −69.9° (*c* 1.4, $CHCl_3$), R_f = 0.71 (4:1 Et_2O/hexane). Spectroscopic data (^{1}H-, ^{13}C-NMR and MS) are in complete agreement with the structure **13** (see Supplementary material). After further elution of the column, the pure product **12** (0.020 g, 22 %) was isolated in the form of

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a colourless syrup, $[\alpha]_D = -18.7°$ (*c* 1.0, $CHCl_3$), $R_f = 0.50$ (4:1 Et_2O/hexane). ^1H- and ^{13}C-NMR spectral data of **12** were identical to those previously reported by us.[17,18]

*3,6-Anhydro-2-deoxy-D-*ido*-heptono-1,4-lactone (2).* A solution of **10** (0.484 g, 1.37 mmol) in EtOH (30 mL) was hydrogenated over 10 % Pd/C (0.308 g, 0.29 mmol) for 24 h at room temperature. The mixture was filtered through a Celite pad, the catalyst was washed with EtOH. The combined organic solutions were evaporated and the residue (0.225 g) was purified by flash chromatography (19:1 CH_2Cl_2/MeOH) to afford pure **2** (0.208 g, 87 %) as transparent needles, m.p.: 71–73 °C (EtOAc/MeOH), $[\alpha]_D = +26.8°$ (*c* 1.8, H_2O), lit.[19] m.p.: 72–74 °C, $[\alpha]_D = +28.4°$ (*c* 1.9, H_2O), $R_f = 0.72$ (9:1 CH_2Cl_2/MeOH). The spectroscopic data of compound **2** thus obtained were identical to those previously reported by us.[17]

*2,3-*O*-Isopropylidene-5-*O*-triphenylmethyl-D-lyxofuranose* (**20**). TrCl (0.884 g, 3.64 mmol) was added to a solution of compound **15**[21] (0.389 g, 2.05 mmol) in anhydrous pyridine (5 mL) and the reaction mixture was left at room temperature for 48 h. The solution was poured into 10 % aq HCl and extracted with CH_2Cl_2. The extract was washed with water, dried and evaporated. The residue (1.234 g) was purified by flash column chromatography (9:1 toluene/EtOAc) to give pure compound **20** (0.630 g, 71 %) which crystallizes from MeOH in the form of white needles, m.p.: 173 °C, $[\alpha]_D = -5.2 \rightarrow -7.8°$ (48 h, *c* 0.7, $CHCl_3$), anomeric ratio (from ^1H-NMR): $\alpha/\beta \approx 7:1$ (48 h), $R_f = 0.29$ (9:1 toluene/EtOAc).

*Methyl 3,6-anhydro-2-deoxy-4,5-*O*-isopropylidene-D-*talo- *(**18**) and D-*galacto*-heptanoate (**19**).* Procedure A: To a solution of **15**[21] (0.355 g, 1.87 mmol) in anhydrous CH_3CN (10 mL), was added $Ph_3P{=}CHCO_2Me$ (0.954 g, 2.86 mmol) and the reaction mixture was heated under reflux for 4 h. The solution was then evaporated and the Ph_3PO precipitated by the addition of Et_2O, while standing at 4 °C for 2 h. After filtration and evaporation, the residue was purified on a column of flash silica (3:2 EtOAc/hexane) to give a 1:1.4 mixture of **16** and **17** (0.397 g, 88 %) as determined by ^1H-NMR. The mixture was chromatographically homogeneous material, $R_f = 0.32$ (2:1 EtOAc/hexane). A solution of purified mixture of **16** and **17** (0.391 g, 1.61 mmol) in dry MeOH (20 mL), was added 0.1 M solution of NaOMe u MeOH (1.6 mL, 0.16 mmol) and the reaction mixture was heated under reflux for 48 h. After neutralization with acidic ion exchange resin, IRA-120 and filtration, the solution was evaporated. A mixture of **16** and **17** in the respective ratio of 2:1 (0.350 g, 90 %) was obtained. A 2:1 mixture of **16** and **17** (0.350 g, 1.44 mmol) was treated with TrCl (0.806 g, 3.32 mmol) in anhydrous pyridine (5 mL) at room temperature for 3 days. The mixture was then poured into 10 % aqueous HCl (pH ~ 1) and extracted with CH_2Cl_2. The extract was washed with water (to pH ~ 7), dried and evaporated. Flash column chromatography of the residue (toluene → 19:1 toluene/EtOAc), gave pure **18** (0.069 g, 10 %) and **19** (0.486 g, 69 %). Procedure B: $Ph_3P{=}CHCO_2Me$ (0.550 g, 1.65 mmol) was added to a solution of **20** (0.456 g, 1.06 mmol) in anhydrous CH_3CN (10 mL), and the resulting solution was refluxed for 24 h. A new portion of reagent (0.160 g, 0.48 mmol) was added and heating was continued for additional 24 h. The solution was evaporated, the Ph_3PO precipitated with Et_2O (4 °C), filtered of and the solution again evaporated. The residue was purified on a column of flash silica (toluene → 19:1 toluene/EtOAc), whereby pure product **19** (0.223 g, 43 %) was obtained as a colourless oil. Stereoisomer **18** (0.246 g, 48 %) was also isolated as a solid which crystallizes from MeOH, as white needles. Procedure C: 0.1 M solution of NaOMe in MeOH (0.5 mL, 0.05 mmol) was added to a solution of **18** (0.2464 g, 0.51 mmol) in dry MeOH (15 mL). The mixture was heated under reflux for 48 h, and then neutralized with acidic ion exchange resin IRA-120. The resin was separated by filtration and the filtrate was evaporated. Flash column chromatography of the residue (19:1 toluene/EtOAc) gave **19** (0.151 g, 61 %) as a colourless oil,

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$[\alpha]_D$ = −26.3° (*c* 1.0, $CHCl_3$), R_f = 0.35 (19:1 toluene/EtOAc). A small amount of stereoisomer **18** (0.015 g, 6 %) was also isolated as a solid, which crystallizes from MeOH, in the form of white needles, m.p.: 138 °C, $[\alpha]_D$ = −21.7° (*c* 0.8, $CHCl_3$), R_f = 0.30 (19:1 toluene/ /EtOAc).

*3,6-Anhydro-2-deoxy-D-*galacto*-heptono-l,4-lactone* (**3**). A solution of compound **19** (0.741 g, 1.52 mmol) in a 2:1 mixture TFA/H_2O (9 mL) was stirred at room temperature for 18 h. The reaction mixture was evaporated and the traces of acid were removed by co-distillation with toluene. The residue was purified by flash column chromatography (EtOAc) to give pure product **3** (0.210 g, 80 %), which crystallized from $CHCl_3$ in the form of white needles, m.p.: 126–127 °C, $[\alpha]_D$ = −98.0° (*c* 0.9, MeOH), R_f = 0.20 (EtOAc).

*Methyl (*E*)-5,7-di-*O*-benzyl-2,3-dideoxy-D-*lyxo*-hept-2-enoate* (**22**). Ph_3P=$CHCO_2Me$ (0.370 g, 1.11 mmol) was added to a solution of **21**[22] (0.286 g, 0.87 mmol) in anhydrous benzene (7 mL) and the resulting mixture was stirred under reflux for 24 h. The solvent was then evaporated and the Ph_3P was removed by flash chromatography (Et_2O). Pure **22** (0.226 g, 68 %) was obtained after crystallization from a mixture toluene/hexane, in the form of colourless, transparent needles, m.p.: 95–96 °C, $[\alpha]_D$ = +14.0° (*c* 1.1, $CHCl_3$), R_f = 0.45 (4:1 Et_2O/ /hexane). The mother liquor was evaporated and purified by column chromatography on flash silica (7:3 Et_2O/hexane), whereby a small amount of pure lactone **10** (0.027 g, 9 %) was obtained.

*2,3-Dideoxy-D-*lyxo*-heptono-1,4-lactone* (**6**). A solution of **22** (0.087 g, 0.23 mmol) in EtOH (3.5 mL) was hydrogenated over 10 % Pd/C (0.054 g) for 24 h, at room temperature and normal pressure of hydrogen. The catalyst was separated by the filtration through a Celite pad, the filtrate was evaporated and the residue was treated with 2:1 TFA/H_2O (3 mL), at room temperature for 20 h. The reaction mixture was evaporated by co-distillation with toluene, and the residue was purified by flash chromatography (47:3 EtOAc/MeOH). Pure product **6** (0.020 g, 50 %) was obtained as a pale yellow syrup, $[\alpha]_D$ = −1.2° (*c* 0.4, $CHCl_3$), R_f = 0.21 (47:3 EtOAc/MeOH).

Compounds **4**, **5** and **8** have been prepared according to procedures previously reported by us.[18,20]

MTT assay. The colorimetric MTT assay was carried out using the reported procedure.[23]

Test cells. The cytotoxicity of the test compounds was assessed against seven human tumour cell lines: K562 (ATCC CCL 243, chronic myeloid leukaemia), HL-60 (ATCC CCL 240, promyelocytic leukaemia), Jurkat (ATCC CCL 1435, T cell leukaemia), Raji (ATCC CCL 86, Burkitt's lymphoma), HT-29 (ATCC HTB38, colorectal adenocarcinoma), MDA- -MB 231 (ATCC HTB-26, ER^- brest adenocarcinoma), and HeLa cells (ATCC CCL2, human cervix adenocarcinoma). Cytotoxicity toward a single normal cell line, MRC-5 (ATCC CCL 171, foetal lung fibroblasts) was also evaluated.

RESULTS AND DISCUSSION

Chemistry

The synthesis of dephenylated goniofufurone analogues **2** and **8** is presented in Scheme 1. Although analogue **2** can be obtained in one step by cyclocondensation of D-xylose with Meldrum's acid,[24] we planned to prepare it *via* a protected furano-lactone **10** in order to explore a possible divergent route that would allow access to monobenzyl derivative **8**. The known[22] and readily available D-xylofuranose lactol derivative **9** was used as a convenient starting material for this part of the work.

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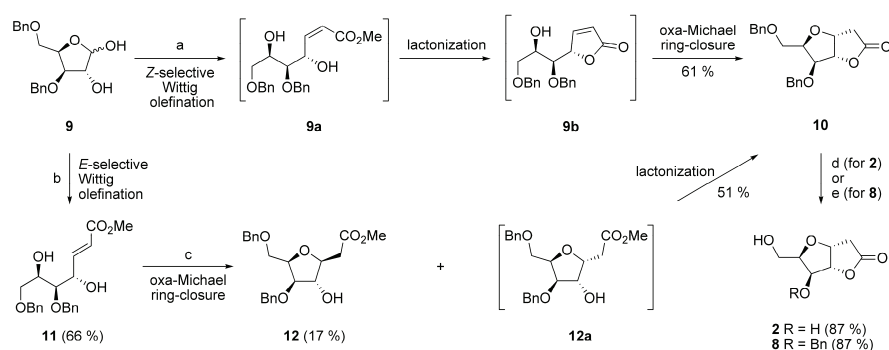
Treatment of **9** with the stabilized ylide, $Ph_3P=CHCO_2Me$, in anhydrous methanol gave the expected furanolactone **10** (61 %), as a product of the initial (*Z*)-selective Wittig olefination[25] followed by γ-lactonization. A minor amount (25 %) of the corresponding (*E*)-enoate **11** (δ_H 6.16 ppm, $J_{2,3}$ = 15.6 Hz, H-2) was also obtained from this reaction. Both **10** and **11** had been prepared earlier in our laboratory, under the (*E*)-selective Wittig conditions ($Ph_3P=CHCO_2Me$, DMF, 70 °C)[25] but in a completely different product ratio (74 % of **11**, 12 % of **10**).[18]



Scheme 1. Reagents and conditions: a) $Ph_3P=CHCO_2Me$, MeOH, rt, 48 h; b) $Ph_3P=CHCO_2Me$, C_6H_6, reflux, 24 h; c) imidazole, C_6H_6, reflux, 11 h; d) H_2, 10 % Pd/C, EtOH, rt, 24 h; e) H_2, 10 % Pd/C (0.1 equiv of Pd), abs. EtOH, rt, 105 min.

In this paper, we examined alternative conditions for the (*E*)-selective Wittig olefination ($Ph_3P=CHCO_2Me$, C_6H_6, under reflux), whereby the (*E*)-enoate **11** was obtained in 66 % yield. We supposed that **11** could be used to verify that conversion of **9** to **10** involves initial lactonization, followed by subsequent oxa-Michael cyclization. If the sequence included the oxa-Michael cyclization that precedes lactonization, we would find at least traces of the *β-C*-furanoside **12** in the reaction mixture, which from steric reasons cannot lactonize. In an alternative sequence, in which Michael's ring closure precedes lactonization, (*E*)-enoate **11** was treated with imidazole in hot benzene for 11 h. Under these reaction conditions, the lactone **10** was obtained in 51 % yield, along with a minor amount of the *β-C*-glycoside **12** (17 %) thus providing an indirect proof for the mechanism of conversion of **9** to **10**. By the catalytic reduction of **10** over 10 % Pd/C (0.1 M eq. of Pd), for 105 min at room temperature, the benzyl group was selectively removed from the primary position to give the required alcohol **8** in 87 % yield. When the catalytic hydrogenolysis of **10** was performed with twice the amount of catalyst (0.2 M eq. Pd), with an extension of the reaction time to 24 h, the known[17] analogue **2** (87 %) was obtained, with physical constants and spectral data in good agreement with the reported values.[17]

In an attempt to convert enone **11** to lactone **10** in an alternative manner, compound **11** was treated with NaOMe in MeOH at room temperature (Scheme

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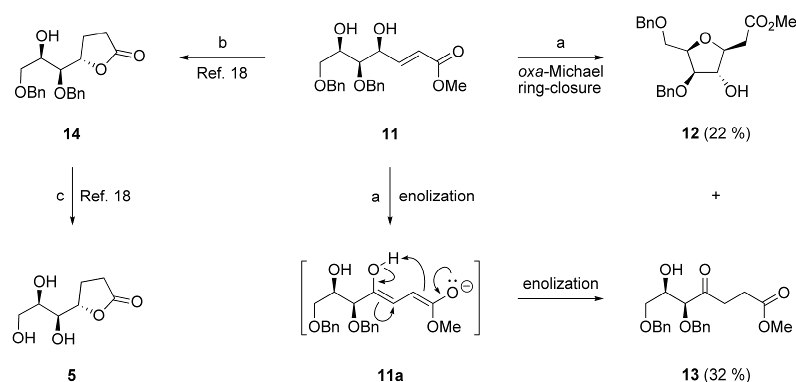
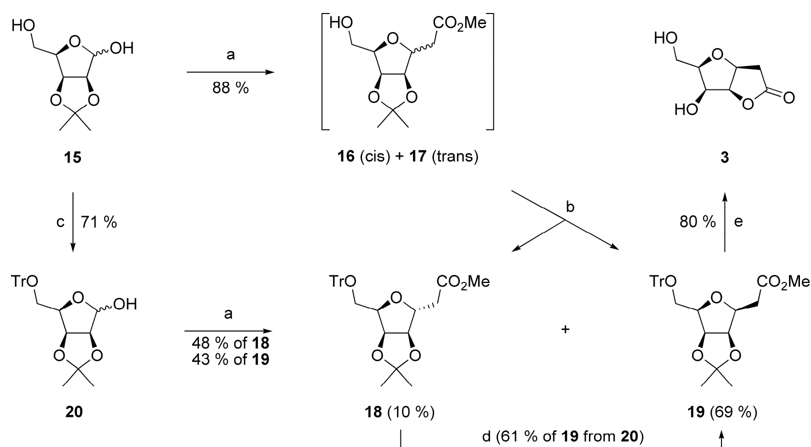
2). Quite unexpectedly, under these reaction conditions, γ-ketoester **13** was preferentially formed (32 %) as a result of enolization. The tetrahydrofuran derivative **12** was also isolated (22 %) as a product of the oxa-Michael cyclization process.



Scheme 2. Reagents and conditions: a) NaOMe, MeOH, rt, 20 min; b) H_2, PtO_2, AcOH, rt, 51 h; c) H_2, 5 % Pd/C, rt, 20 h.

Finally, compound **11** was converted to target **5** (in 44 % overall yield), using the known[18] two-step sequence shown on the left-hand side of Scheme 2. Since this synthetic sequence is described in in our earlier work,[18] it will not be further discussed here.

The synthesis of furofuranone **3** is shown in Scheme 3. The sequence started from D-lyxofuranose derivative **15**, which is readily available from D-lyxose in one step.[21] We have planned two independent routes to the key intermediate **19**.



Scheme 3. Reagents and conditions: a) $Ph_3P=CHCO_2Me$, MeCN, reflux, 4 h for **15**, 48 h for **20**; b) *i*, NaOMe, MeOH, reflux, 48 h, *ii*, TrCl, Py, rt, 72 h; c) TrCl, Py, rt, 48 h; d) NaOMe, MeOH, reflux, 48 h; e) 2:1 TFA/H_2O, rt, 18 h.

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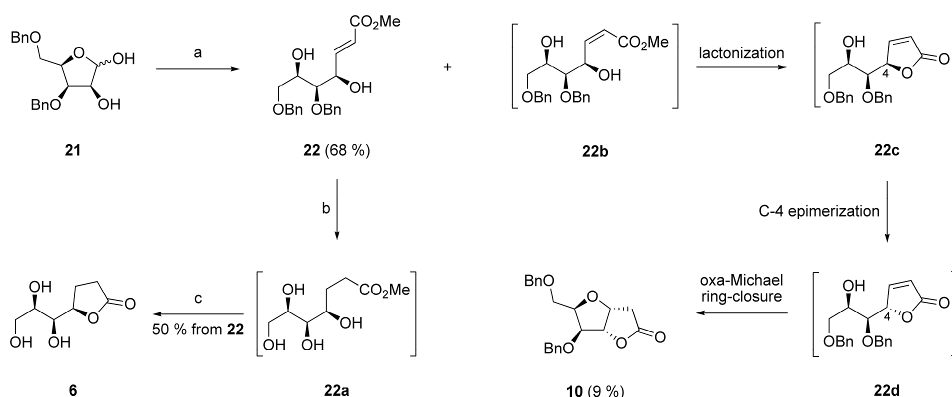
The first one started with the reaction of **15** with $Ph_3P=CHCO_2Me$, in boiling MeCN, whereupon an inseparable mixture of the corresponding *α*- and *β*-*C*-furanoside (**16** + **17**) was obtained as a result of the initial Wittig olefination of **15**, followed by the subsequent oxa-Michael cyclization. This mixture further reacted with TrCl in dry pyridine to give stereoisomeric triphenylmethyl ethers **18** and **19** which were successfully separated by flash chromatography. The treatment of purified **18** with NaOMe in MeOH resulted in equilibration, *via* a ring-opening and ring-closure mechanism, similar to that observed in *α*- and *β*-*C*-ribofuranoside derivatives.[26] This procedure gave a 7:1 mixture of **19** and **18**, with the *β*-*C*-glycoside **19** as the major product. The overall yield of **19** according to this route was 55 % with respect to starting compound **15**.

The second synthetic route started with the selective protection of primary hydroxyl group of **15** using trityl chloride in pyridine (Scheme 3) to give the corresponding 5-*O*-trityl ether **20** in 71 % yield. Lactol **20** readily reacted with the stabilized ylide $Ph_3P=CHCO_2Me$, in dry acetonitrile, to give the approximately equal amounts of the expected *C*-glycosides **18** and **19**, which were readily separated by flash column chromatography. The isomerisation of *α*-*C*-glycoside **18** (NaOMe, MeOH, under reflux) provided an additional amount of *β*-isomer, so that the required stereoisomer **19** was obtained in a total yield of 64 % relative to starting compound **15**. The hydrolytic removal of the isopropylidene protective group in **19** with aqueous trifluoroacetic acid occurred with the related lactonization and de-*O*-tritylation, in order to give the target molecule **3** in 80 % yield.

The synthesis of *γ*-lactone **6**, an analogue of **3,** which lacks a tetrahydrofuran ring, is shown in Scheme 4. Partially protected D-lyxose derivative **21**, which is readily available from D-xylose in several synthetic steps,[22] served as a convenient starting compound for this part of the work. Reaction of **21** with $Ph_3P=CHCO_2Me$, in boiling benzene, gave two reaction products: the main one



Scheme 4. Reagents and conditions: a) $Ph_3P=CHCO_2Me$, C_6H_6, reflux, 24 h; b) H_2, 10 % Pd/C, EtOH, rt, 24 h; c) 2:1 TFA/H_2O, rt, 20 h.

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was the (*E*)-unsaturated ester **22**, which was formed as a consequence of the expected Wittig (*E*)-selective olefination process. A minor amount of furanolactone **10** was unexpectedly obtained in this reaction, presumably as a product of a three-step sequence, comprised of the initial *γ*-lactonization of the minor (*Z*)-olefin **22b**, followed by the successive C-4 epimerization, and the final oxa-Michael cyclization process. A catalytic reduction of **22** over 10 % Pd/C resulted in a simultaneous reduction of the double bond and the hydrogenolytic removal of the benzyl groups. Intermediate **22a** was not purified, but was in non-purified form further treated with aqueous TFA, whereby the target lactone **6** was obtained in 50 % overall yield from the last two steps.

In vitro *antiproliferative activity and SAR analysis*

The biological activities of synthesized compounds **2**–**8** were evaluated by an *in vitro* cytotoxicity test against a panel of seven human malignant cell lines, including human myelogenous leukaemia (K562), human promyelocytic leukaemia (HL-60), T cell leukaemia (Jurkat), Burkitt's lymphoma (Raji), colorectal adenocarcinoma (HT-29), ER^{-} breast adenocarcinoma (MDA-MB 231) and cervix carcinoma (HeLa). Cytotoxicity was also evaluated against one normal human cell line, MRC-5. The purpose of this test was to determine whether the synthesized compounds showed the selectivity against tumour cells. Cell growth inhibition was evaluated using the standard MTT assay[23] after the exposure of cells to the test compounds for 72 h*. (+)-Goniofufurone (**1**) and the commercial antitumour agent doxorubicin (DOX) were used as positive controls. The results are presented in Table I, which displays that the natural product **1** and the corresponding analogues show variable activities towards the tested cells ranging from nanomolar IC_{50} values to complete inactivity for individual cells. Such different activities indicate that the synthesized lactones do not act on the same molecular target. However, much more work is needed to confirm this assumption. This could be the subject of our future work.

It is to be noted that five analogues (compounds **2**–**4**, **7** and **8**) showed submicromolar cytotoxicities against K562 malignant cells, with IC_{50} values ranging from 0.003 to 0.54 μM. The remaining two analogues (compounds **5** and **6**) showed a strong cytotoxicity, with IC_{50} values in the micromolar range (4.21 and 3.54 μM, respectively). The most active compounds against these cells are the furanolactones **2** and **3**, which exhibited a powerful cytotoxicity (IC_{50} of 3.0 and 5.1 nM, respectively). Accordingly, compound **2** was over 130-fold more active than natural product **1** and over 80-fold more potent than the commercial antitumor agent DOX. Compound **3** showed slightly lower, but still noticeable cyto-

* Antiproliferative activities of analogues **2** and **3** against three malignant and one normal cell line were reported in the preliminary communication, but after 24-h of cells treatment (see reference[17]).

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toxicity (80-fold higher potency than lead **1** and 49-fold higher activity than DOX). Also, analogue **2** showed a notable antiproliferative activity in the culture of HeLa cells (IC_{50} = 0.01 μM), showing over 800-fold higher activity than the natural lead **1** and 6.5-fold higher potency than DOX. Compound **3** however, exhibited a significant activity against Raji cells (IC_{50} = 9.3 nM) being over 1980-fold more active than the natural product **1**, and over 320-fold more potent than the commercial antitumor drug DOX. The demonstrated antitumour properties of lactones **2** and **3**, as well as their complete inactivity against the normal MRC-5 cells, make these analogues suitable leads for further development of new antitumour drugs.

TABLE I. *In vitro* cytotoxicity (+)-goniofufurone (**1**), DOX and analogues **2**–**8** after 72 h

Compound	IC_{50} / μM[a]							
	K562	HL-60	Jurkat	Raji	HT-29	MDA-MB 231	HeLa	MRC-5
1	0.41	>100	32.45	18.45	0.59	75.34	8.32	>100
2	0.003	5.56	3.73	>100	>100	75.31	0.01	>100
3	0.0051	>100	>100	0.0093	0.056	0.11	>100	>100
4	0.54[b]	0.09 [b]	2.23[b]	2.21[b]	>100	>100	2.34[b]	>100
5	4.21	0.02	>100	>100	94.35	0.011	>100	>100
6	3.54	>100	11.84	89.64	0.12	>100	4.10	>100
7	0.12	20.62	9.45	56.37	12.45	67.50	0.03	>100
8	0.065	0.09	1.02	11.39	>100	>100	5.92	>100
DOX	0.25	0.92	0.03	2.98	0.15	0.09	0.065	0.10

[a]IC_{50} is the concentration of compound required to inhibit the cell growth by 50 % compared to an untreated control. Values are means of three independent experiments. Coefficients of variation were less than 10 %; [b]taken from reference[27]

Analogue **3** proved to be the most potent antiproliferative agent in the HT-29 cell culture (IC_{50} = 0.056 μM). It showed over 10-fold stronger activity than the natural product **1** and almost 3-fold higher activity then DOX in the culture of these cells.

The most active compound in HeLa cell culture is analogue **2** (IC_{50} = 0.01 μM) being over 830- and 6.5-fold more active than the lead **1** and DOX, respectively.

The analogue **8** exhibited the strongest cytotoxicity in K562 and HL-60 cell cultures (IC_{50} of 0.065 and 0.09 μM, respectively). In the K562 cell culture, it showed almost 6 times the potency of the lead **1**, as well as 3.6 times the activity of DOX. In cell culture HL-60 (where the lead **1** is inactive), the analogue **8** showed 10 times stronger activity than DOX. The same activity towards these cells was shown by the analogue **4** (IC_{50} = 0.09 μM, 10-fold more potent than DOX).

All synthesized analogues (**2**–**8**) as well as the lead **1**, were completely inactive against normal MRC-5 cells. On the contrary, the commercial antitumour agent DOX exhibited a potent cytotoxicity against this cell line (IC_{50} = 0.10 μM). These

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results do suggest that the natural product **1** and the analogues **2**–**8** are more selective anticancer agents than DOX, but such a conclusion should be supported by the additional *in vitro* experiments with a larger number of normal cell lines.

We performed a brief SAR analysis in order to identify structural features beneficial for potencies of analogues (such as the presence phenyl and/or tetrahydrofuran ring, and the influence of stereochemistry at the C-3 and/or C-4 positions).

As shown in Table I, the removal of phenyl ring from the C-7 position in **1** increase the activities of the resulting analogues **2**, **4** and **6**, against three to four cell lines. In a slightly smaller number of malignant cell lines the control compounds retained stronger potencies. (For a graphical presentation see, Fig. S-1a of the Supplementary material). The analogues designed by opening of the THF ring (**4**–**6**) showed essentially the same cytotoxic effects. Similar effects on the antiproliferative activity were observed when the stereochemistry was changed at C-3 and C-4. (Fig. S-1b and c of the Supplementary material). As it can be seen from Table I (and from Fig. S-1 of the Supplementary material), the results of the SAR analysis are relatively inconsistent. In order to draw more reliable conclusions, a larger number of analogues should be prepared, and the SAR analysis repeated. This will be the subject of our further work.

CONCLUSION

In conclusion, several novel (+)-goniofufurone analogues were designed and synthesized starting from D-xylose or D-lyxose. The synthesized molecules were evaluated for their *in vitro* antitumour activity against a panel of human malignant cell lines. The strongest potency, in the region of low, nanomolar IC_{50} values, was shown by the following compounds: dephenylated analogues **2** (IC_{50} = 3.0 nM, K562) and **3** (IC_{50} = 5.1 nM, K562 and 9.3 nM, Raji). In addition, each of the synthesized compounds showed submicromolar activity (IC_{50} 0.01–0.54 μM) against at least one tumour cell line under evaluation.

Preliminary SAR analysis showed that the dephenylated goniofufurone analogues (designed by removing the aromatic ring from C-7), as well as the analogues with open tetrahydrofuran ring (designed by disconnecting the C_3–O_6 bond), could show improved antiproliferative activity. Changing the stereochemistry at C-4 in the analogue **5** may increase cytotoxicity, while changing the configuration at both C-3 and C-4 in the bicyclic analogue **2** causes a decrease in cytotoxicity in most of the cells tested.

We believe that the potent antitumour activities of the synthesized analogues **2**–**8** toward the malignant cells, as well as their complete inactivity against normal MRC-5 cell line, make them suitable leads for further development of more potent and selective antitumour agents.

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SUPPLEMENTARY MATERIAL

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

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И З В О Д

СИНТЕЗА И АНТИПРОЛИФЕРАТИВНА АКТИВНОСТ ПОЈЕДНОСТАВЉЕНИХ АНАЛОГА ГОНИОФУФУРОНА

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Више нових аналога (+)-гониофуфурона је дизајнирано и синтетизовано полазећи из D-ксилозе, односно из D-ликсозе. Испитана је способност аналога да инхибирају раст одабраних хуманих туморских ћелијских линија. Најактивнији молекули, који показују ниску наномоларну цитотоксичност, су дефениловани аналози **2** (IC_{50} = 3,0 nM, K562) и **3** (IC_{50} = 5,1 nM, K562 и 9,3 nM, Raji). Коначно, свако од добијених једињења показало је суб-микромоларну активност (IC_{50} 0,01–0,54 μM) према најмање једној од седам испитиваних малигних ћелијских линија. Прелиминарном SAR анализом је утврђено да дефениловани аналози гониофуфурона (дизајнирани уклањањем ароматичног прстена са C-7), а такође и аналози са отвореним тетрахидрофуранским прстеном (синтетисани раскидањем C_3–C_6 везе) могу показати побољшану антипролиферативну активност. Такође, промена стереохемије на C-4 у молекулу 5 може повећати цитотоксичност аналога, док промена конфигурације на C-3 и C-4 у бицикличном аналогу 2 смањује активност према већини испитиваних ћелија. Снажна антитуморска активност испољена према малигним ћелијама и потпуна неактивност синтетизованих аналога **2–8** према нормалним ћелијама MRC-5, представљају погодну основу за даљи развој нових, потентнијих и селективнијих антитуморских агенаса.

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