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Heteroannelated and 7-deoxygenated goniofufurone mimics with antitumour activity: Design, synthesis and preliminary SAR studies

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ABSTRACT

Cytotoxic (+)-goniofufurone mimic such as benzoxepane **2** was preferentially formed after the treatment of 7-*O*-benzoyl-5-*O*-benzyl (+)-goniofufurone derivative **6** with titanium(IV) fluoride. However, the corresponding 7-epimer **5** (derivative of 7-*epi*-goniofufurone) under the similar reaction conditions gave mainly 7-deoxy derivative **7** as a result of an unexpected 1,5-hydride shift. Extension of this methodology to the enantiomer *ent*-**6** provided cytotoxic (−)-goniofufurone mimics *ent*-**2** and *ent*-**7**. Synthesized compounds showed diverse growth inhibitory effects against selected tumour cell lines, but were devoid of any significant toxicity towards the normal foetal lung fibroblasts (MRC-5). A SAR study reveals the structural features of these lactones that are beneficial for their antiproliferative activity, such as presence of an additional oxepane ring, the absolute stereochemistry and the presence of a deoxy function at the C-7 position.

Natural products based drug discovery has become a major strategy in modern pharmaceutical research and development, and roughly half of the currently used drugs are directly or indirectly derived from natural products.[1] The styryl lactones from *Goniothalamus* (Blume) Hook. f. & Thomson (family Annonaceae Juss.) are an interesting class of naturally occurring compounds, many of which were found to exhibit impressive biological activities.[2] In 1990, McLaughlin and co-workers isolated goniofufurone (**1**, Fig. 1), a novel cytotoxic styryl lactone from the Southeast Asian medicinal treelet *Goniothalamus giganteus* Hook. f. & Thomson.[3] Due to its unique and intriguing structure, as well as its promising antitumour activity this natural product along with a number of its analogues has attracted the attention of many synthetic groups.[4,5] We have also been involved in the synthesis of **1** and related compounds.[6,7] Thus, we have recently found that treatment of goniofufurone derivative **5** (Scheme 1) with $TiCl_4$ in dry CH_2Cl_2 gave a mixture of epimeric 7-chloro-7-deoxy goniofufurone derivatives (structures not shown) in 52% combined yield, along with a minor amount of **2** (17%)[8] that was presumably formed by an intramolecular Friedel–Crafts reaction.

As molecule **2** represents a conformationally restricted analogue of **1** it was of interest to prepare it in a more efficient way, and to evaluate its antitumour activity against selected human tumour cell lines. The rationale underlying the preparation of **2** arises from fact that parent compound **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 groups, as established by X-ray analysis.[3] Alternatively, an automated molecular overlay of 3D structures **1** and **2** generated by X-ray diffraction[8] revealed a good structural mach of their furano-furanone segments and styryl moieties (Fig. 2).[9] Synthesis and in vitro antitumour screening of lactones **3** and **4** are also planned as they represent ring-opened analogues of **2**.

Herein, we want to report synthesis of novel goniofufurone mimic **2** along with its ability to inhibit the growth of selected human tumour cell lines. In order to achieve a more efficient synthesis of **2** we have decided to replace the catalyst ($TiCl_4$) for the intramolecular Friedel–Crafts reaction of **5** or **6**[10] with a stronger Lewis acid such as TiF_4 (Scheme 1). However, the treatment of **5** with TiF_4 in dry CH_2Cl_2, for 7 days at −18 °C, unexpectedly gave 7-deoxy derivative **7** (44%) as the major product accompanied with a minor amount of benzoxepane **2** (19%) as a result of an intramolecular Friedel–Crafts alkylation process.[8]

The mechanism of deoxygenation of **5** at C-7 may be explained by the several successive transformations shown in Scheme 1. In the first step TiF_4 coordinates the O-5 and O-7 of **5** to afford **5a** with activated benzylic carbon atom. The C_7–O_7 bond of **5a** is then cleaved with simultaneous transannular 1,5-hydride migration to furnish **5b**. A similar 1,5-hydride shift has recently been observed in the Lewis acid-catalyzed construction of a benzopyran

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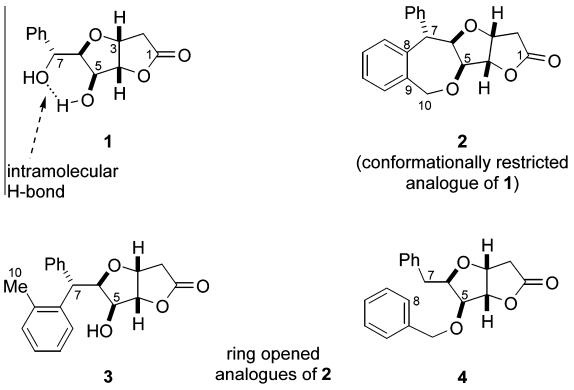


Figure 1. Chemical structure of (+)-goniofufurone (**1**) and analogues **2**–**4**.

skeleton.[11] Final hydrolysis of **5b** gave the deoxygenated product **7** and benzaldehyde.

In an attempt to extend this methodology to a similar substrate, but with opposite stereochemistry at the C-7 position, lactone **6** was treated under the same conditions. Quite unexpectedly, the treatment of **6** with titanium(IV) fluoride in dichloromethane gave oxepane **2** (48%) as a major product, accompanied with a minor amount of 7-deoxy derivative **7** (31%). Accordingly, it appears that (7*R*) stereochemistry of **5** directs the course of the reaction toward preferential formation of 7-deoxygenated derivative **7**, while (7*S*) stereochemistry of **6** favours the preferential formation of benzoxepane **2**. These differences in reactivity could be well rationalized by taking into consideration the stereochemical properties of TiF_4-complexes **5a** and **6a** (Fig. 3).

To promote the desired 1,5-hydride shift, conformers **5a**′ and **6a**″, whose benzylic hydrogens were located close to the electrophilic C-7 atom, should be considered. In the case of **5a**′ and **5a**″, the conformational equilibrium is largely shifted to the twist-boat

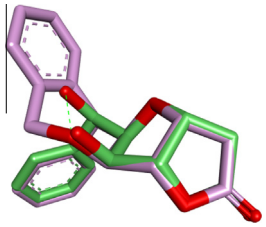
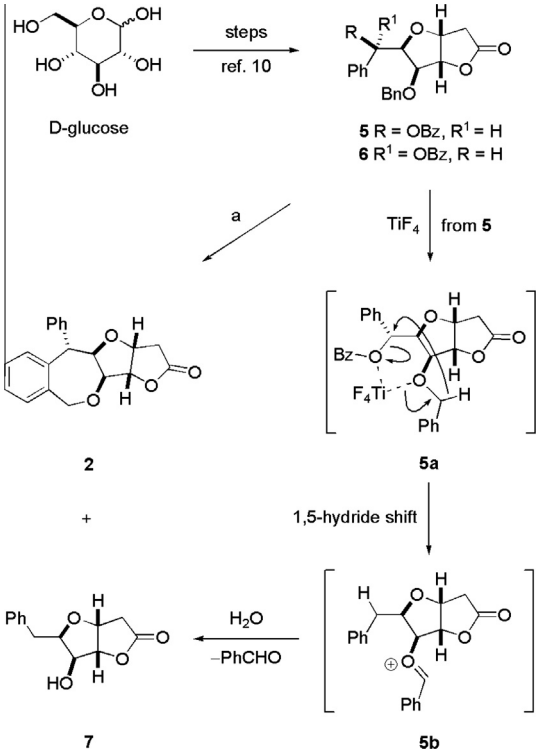


Figure 2. Superimposed structures of **1** (green) and **2** (pink). Green dashed line represents intramolecular H-bond in **1**.

conformer **5a**′ thus favouring a transannular 1,5-hydride shift in the TiF_4-complex of **5**. However, in the case of **6a**′ and **6a**″, the equilibrium is shifted to the chair-like conformer **6a**′ whose benzylic hydrogens are far more distant from the C-7 atom. Such an arrangement disfavoured the transannular 1,5-hydride shift in **6a**. These speculations are confirmed by molecular modelling. Namely, the conformational search[12] performed on both intermediates **5a** and **6a** confirmed a higher stability of conformer **5a**′ (favourable geometry for the hydride shift) over **5a**″ (unfavourable geometry for the hydride shift). A higher stability of **6a**′ (unfavourable geometry for the hydride shift) over **6a**″ (favourable geometry for the hydride shift) was also confirmed. These findings appear to convincingly explain the experimental results.

7-Deoxy derivative **7** that was isolated as the major product after treatment of **5** with TiF_4 was recently prepared in our laboratory and evaluated for its antiproliferative activity against several tumour cell lines.[7] As a consequence of the in vitro antitumour activity of this product, we were especially keen to prepare and evaluate the opposite enantiomer of **7** (structure *ent*-**7**, Scheme 2), since it is well known that enantiomers of certain biologically active molecules may show improved potencies,[13,14] or even different biological activities altogether.[15]



Scheme 1. Reagents and conditions: (a) TiF_4, CH_2Cl_2, −18 °C, 7 days for **5**, 14 days for **6**, 19% of **2**, 44% of **7** (from **5**), 48% of **2**, 31% of **7** (from **6**).

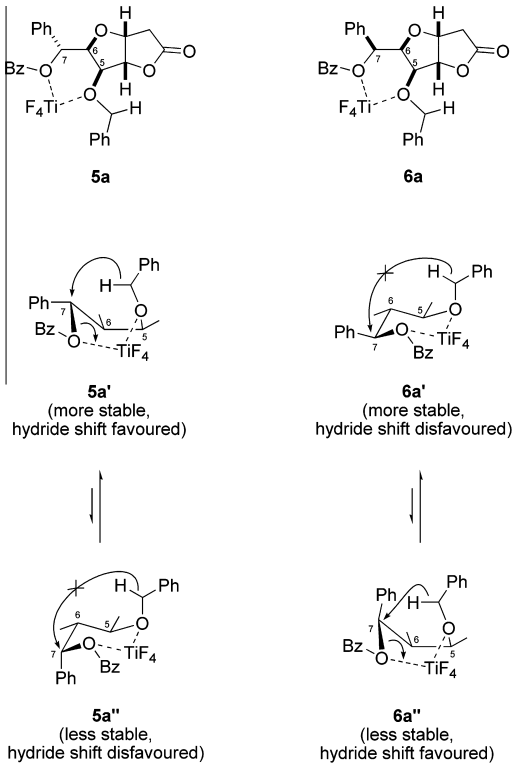
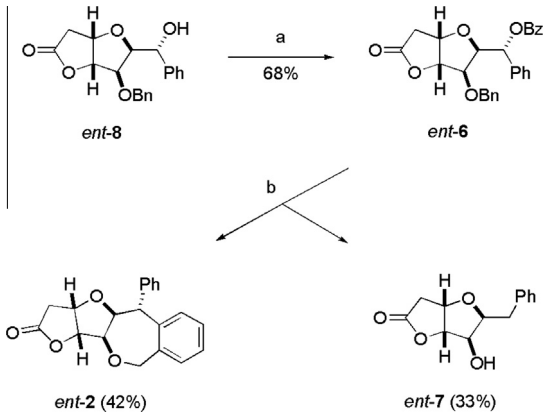


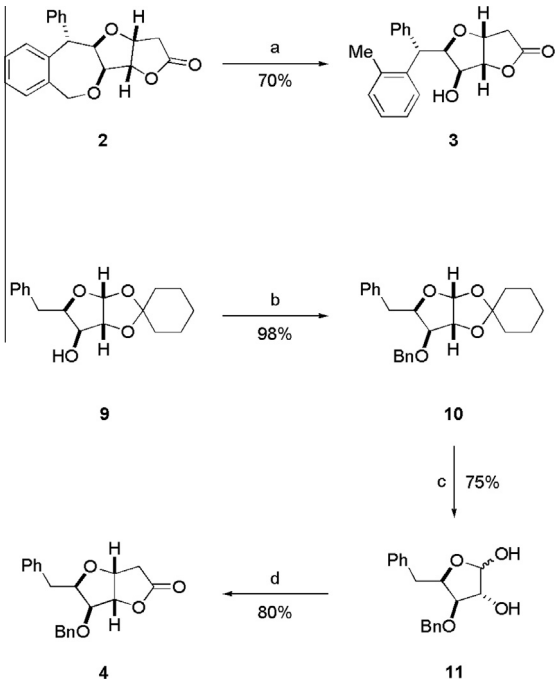
Figure 3. Stereochemical properties of titanium(IV) complexes **5a** and **6a**.



Scheme 2. Reagents and conditions: (a) BzCl, Py, 0 °C → rt, 18 h; (b) TiF_4, CH_2Cl_2, −18 °C, 14 days.

The known[13] 5-*O*-benzyl derivative *ent*-**8** served as a convenient starting material for this part of the work. Alcohol *ent*-**8** readily reacted with benzoyl chloride under standard conditions to give the corresponding 7-*O*-benzoyl derivative *ent*-**6** in 68% yield. Treatment of *ent*-**6** with TiF_4 under the same reaction conditions as that applied for the transformation of **6**, furnished the expected products *ent*-**2** and *ent*-**7** in 42 and 33% respective yields. The physical and spectroscopic data of thus obtained samples *ent*-**2**, *ent*-**6** and *ent*-**7** were identical to those previously reported for the opposite enantiomers (**2**, **6** and **7**).[7,8]

Preparation of ring-opened analogues **3** and **4** is outlined in Scheme 3. Compound **3** was prepared directly from **2** by catalytic hydrogenolysis over 10% Pd/C as a catalyst. The reaction was completed for 8 days, at room temperature and normal pressure of hydrogen, to afford a good yield of **3** (70%). Analogue **4** however, was prepared by a three-step sequence starting from secondary alcohol **9** that was easily accessible from D-xylose.[7] Treatment of



Scheme 3. Reagents and conditions: (a) H_2, Pd/C, MeOH, rt, 8 days; (b) BnBr, NaH, DMF, 0 °C → rt, 1.5 h; (c) 70% aq AcOH, reflux, 3 h; (d) Meldrum's acid, Et_3N, 46 °C, 49 h.

9 with benzyl bromide in anhydrous DMF, in the presence of sodium hydride as a catalyst, gave the expected 5-*O*-benzyl derivative **10** in 98% yield. Hydrolytic removal of the cyclohexylidene protective group in **10** with aqueous acetic acid afforded the expected lactol **11** (75%), which upon treatment with Meldrum's acid in anhydrous DMF, in the presence of triethylamine, afforded **4** in 80% yield. Both newly synthesized analogues **3** and **4** were fully characterized by the corresponding physical and spectral data.

The biological activities of the synthesized derivatives **2**, *ent*-**2**, **3**, **4**, **7** and *ent*-**7** were evaluated by an in vitro cytotoxicity test carried out with a panel of five human tumour cell lines (myelogenous leukaemia K562, promyelocytic leukaemia HL-60, Jurkat T cells leukaemia, Raji Burkitt's lymphoma, cervix carcinoma HeLa) and one normal cell line (foetal lung fibroblasts MRC-5). Cell growth inhibition was evaluated after 72-h cells treatment by using the MTT test. (+)-Goniofufurone (**1**) and the commercial antitumour agent doxorubicin (DOX) were used as the positive controls in this assay.

As shown in Table 1, a number of goniofufurone mimics exhibit potent in vitro anticancer activities, with IC_{50} values in the low micromolar range. Moreover, all of them including the lead **1** were completely inactive toward normal MRC-5 cells, in contrast to the commercial drug DOX that showed a potent cytotoxic activity toward these cells (0.10 μM). Additionally, styryl lactones **1** and **2** show selective cytotoxicities toward certain cancer cell lines, whereas analogues *ent*-**2**, **3**, **4**, **7** and *ent*-**7** are broadly toxic against all tumour cell lines under evaluation. The most potent antiproliferative activity of lactones **4** and *ent*-**7** were recorded in the K562 (0.14 and 0.02 μM) and HL-60 cells (0.56 and 0.17 μM), being notably more active than the commercial antitumour agent doxorubicin against these cell lines. Analogue **2** demonstrated a submicromolar activity against HL-60 cells with IC_{50} value (0.78 μM), similar to that recorded for DOX (0.92 μM) in the same cell line. The highest potency of **2** was recorded in Jurkat cell line (0.074 μM), although this analogue exhibited 2.5-fold lower activity than DOX (0.03 μM). Compounds **2** and **7** showed the most potent antiproliferative activity against Raji cells, with respective IC_{50} values being 6.5- and 3-fold lower than that recorded for the commercial cytostatic doxorubicin in the same cell line. Raji cells were the most sensitive to *ent*-**7**. This analogue was almost 100-fold more active in Raji cells than control compound (DOX). Additionally, analogue *ent*-**7** appeared to be the most potent cell-growth inhibitor synthesized in this work with IC_{50} values in the range 0.02–1.21 μM. Lactone *ent*-**7** also exhibited the most potent growth inhibitory activity against HeLa cell line (1.21 μM), although it demonstrated 17-fold lower activity than DOX (0.07 μM) in the same cell culture.

In a previous attempt to correlate the structures of some bioactive lactones with their cytotoxic activities, we have observed that

Table 1
Antiproliferative activities of **1**, **2**–**4**, **7**, *ent*-**2**, *ent*-**7** and DOX

Compds	IC_{50}[a] (μM)					
	K562	HL-60	Jurkat	Raji	HeLa	MRC-5
1	0.41	>100	32.45	18.45	8.32	>100
2	1.43	0.78	0.074	0.46	>100	>100
ent-2	1.21	11.69	18.45	15.88	6.56	>100
3	1.56	11.67	21.36	5.67	11.40	>100
4	0.14	0.56	1.01	9.48	4.32	>100
7	0.51	43.81	2.52	1.03	4.42	>100
ent-7	0.02	0.17	0.12	0.03	1.21	>100
DOX	0.25	0.92	0.03	2.98	0.07	0.10

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

heteroannelation of natural leads may increase the cytotoxicities of the resulting conformationally restricted goniofufurone mimics.[13] It was also found that the opposite enantiomers of certain bioactive styryl lactones may exhibit more potent cytotoxicity with respect to the parent compounds.[13,14a] These findings have prompted us to perform a preliminary SAR study[16] of the analogues **2**, *ent*-**2**, **3**, **4**, **7** and *ent*-**7**. The first structural feature considered in the SAR study was the influence of heteroannelation of leads **1** and *ent*-**1**. The results obtained from the treatment of cancer cells with **2** demonstrated that the introduction of a new benzoxepane ring increases the potency originally displayed by lead **1** against three of five tested malignant cell lines. This effect is even more pronounced if the structure of *ent*-**1** is extended with a benzoxepane ring (analogue *ent*-**2**). These findings are in agreement with data shown in Table 1, which revealed that the opening of seven-member ring by disconnection of C_{10}–O bond decreases the activities of resulting (+)-goniofufurone mimic **3** against the majority of tumour cell lines under evaluation. However, the formal cleavage of C_7–C_8 bond of the benzoxepane ring in **2**, increases the potency originally displayed by **2**, against three of five tested malignant cell lines. The next structural element considered was the absolute stereochemistry of analogues. A comparison of IC_{50} values for (+)- and (−)- enantiomers reveals that *ent*-**2** had more profound effect only against HeLa cells, while the analogue **2** was completely inactive against this cell line. However, (−)-enantiomer *ent*-**7** demonstrated a superior cytotoxicity against all cell lines under evaluation being 4- to 258-fold more potent than (+)-enantiomer **7**. Finally, the most pronounced antiproliferative activities were observed with deoxygenated analogues **4**, **7** and *ent*-**7**. These findings indicated that introduction of a deoxy function at the C-7 position increases the antiproliferative activity of the analogues against majority of tumour cell lines under evaluation.

To summarize, 7-*O*-benzoylated styryl lactones such as **5** or **6**, upon treatment with titanium(IV) fluoride in dichloromethane preferentially gave the cytotoxic 7-deoxy derivative **7** or benzoxepane **2**, respectively. On the contrary to both titanium(IV) chloride and titanium(IV) bromide that were found to catalyse the displacement of the C-7 benzylic ester function in the same lactones **5** or **6** with the corresponding halogenide anions,[8] titanium(IV) fluoride served as a selective catalyst for an intramolecular Friedel–Crafts alkylation process (conversion of **6** and *ent*-**6** to **2** and *ent*-**2**), or for a C-7 deoxygenation through a novel 1,5-hydride shift (conversion of **5** to **7**). All synthesized compounds showed diverse growth inhibitory effects against the tested malignant cells, but were devoid of any significant cytotoxicity toward the normal foetal lung fibroblasts (MRC-5). A SAR study reveals that the following structural features are beneficial for the antiproliferative activity of synthesized lactones: the presence of an additional oxepane ring, the absolute stereochemistry, as well as the presence of a deoxy function at the C-7 position.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.069.

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