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Design and synthesis of harzialactone analogues: Promising anticancer agents

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ABSTRACT

New homologues of harzialactone were synthesized using D-glucose as chiral template. Wittig reaction to introduce aromatic moiety in **10** and chemoselective anomeric oxidation of **13** were used as key reactions in our synthesis. Anticancer activity of these target molecules was assessed against five cancer cell lines, P388D1, HL60, COLO-205, Zr-75-1 and HeLa. Both compound **5** and **6**, showed significant activity against colon cancer (COLO-205) and cervical cancer (HeLa) and moderate with others. To the best of our knowledge, this is the first report of harzialactone analogues as potent inhibitors of human colon and cervical cancer.

Various biologically active metabolites have been obtained from marine microorganisms. Numata and co-workers[1] isolated and characterised various secondary metabolites from a fungal strain of *Trichoderma harzianum* OUPS-N115 originally separated from the sponge *Halichondria okadai*. Among these, (+)-harzialactone A **1** (Fig. 1), exhibited cytotoxic activity against the P388 lymphocytic leukaemia test system in cell cultures. Souza et al.[2] isolated and characterised **2**, diastereomer of harzialactone A from the *Eutypa*-like strain FED-3. Due to the potent bioactivity and unique structural feature of harzialactone A, synthesis of it remains a centre of interest of chemists.[3–9] Mereyala et al.[3] developed chiron approach for the synthesis of harzialactone A **1** starting from D-glucose for the first time and assigned correct stereochemistry as (3*R*,5*R*). Further, they also synthesized all its stereoisomers **2**, **3**, **4** from D-glucose.[4] Recently, Chen et al.[8] developed a chemoenzymatic route to harzialactone A and all its stereoisomers using recombinant *F. proliferatum* lactonase (reFPL) starting from phenyl acetone. Sabitha et al.[9] reported the synthesis of harzialactone A and its (3*R*,5*S*)-isomer **4** utilising the chiral-epoxide opening with thioacetal and diastereoselective reductions as key reactions.

Though, the syntheses of stereoisomers of **1** have been reported, their potential as anticancer agents has not yet been assessed. In spite of the significant anticancer activity of harzialactone A, the field of bioevaluation of its other stereoisomers and also the synthesis of homologues is unattended. This need prompted us to synthesize homologues and to estimate their anticancer property. As a part of our continuing interest in the synthesis of bioactive molecules from D-glucose,[10] we planned for the synthesis

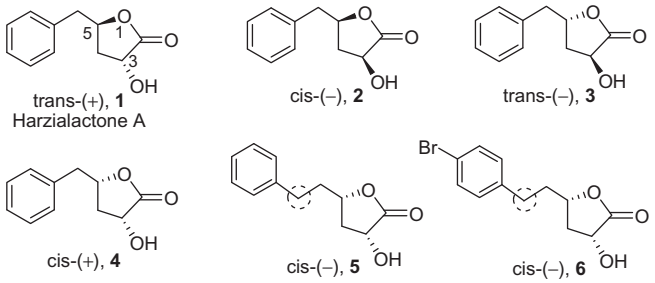

Figure 1. Harzialactone A and its analogues.

of **5** which is homologue of **4** and to evaluate its anticancer activity. Though the racemic compound **5** is known,[11] its bioevaluation was not carried out. During the synthetic course towards **5**, we also obtained bromine substituted compound **6**, whose anticancer activity was also evaluated.

Retrosynthetic analysis of homologues **5** and **6** indicates (Scheme 1) that it could be derived from anomers **13** by chemoselective oxidation of the anomeric hydroxyl group of the lactol and this lactol could be obtained by acetonide deprotection of the 3-deoxy-gulose derivative **12**. Compound **12** can be derived by double bond reduction of styrene derivative **11** which in turn could be obtained by Wittig olefination of in situ generated ylide from benzyl triphenylphosphonium bromide with aldehyde **10**. This aldehyde can be synthesized from D-glucose according to known procedures with slight modifications.[4,12]

Our synthesis started with D-glucose (Scheme 2) which was converted into 3-deoxy-D-gulose derivative **8** according to known procedures.[12] Cleavage of 5,6-acetonide protection was selectively

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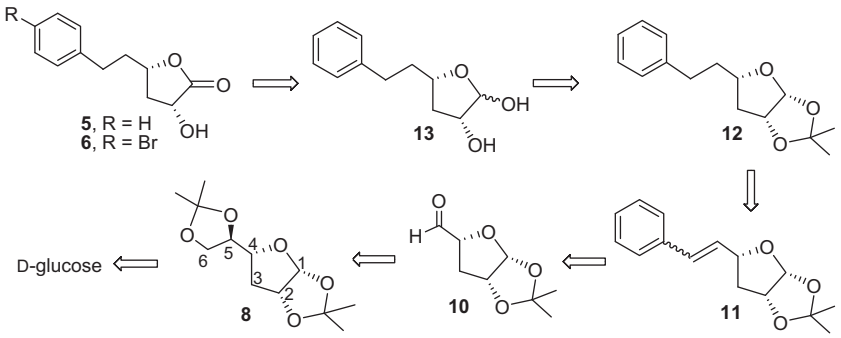
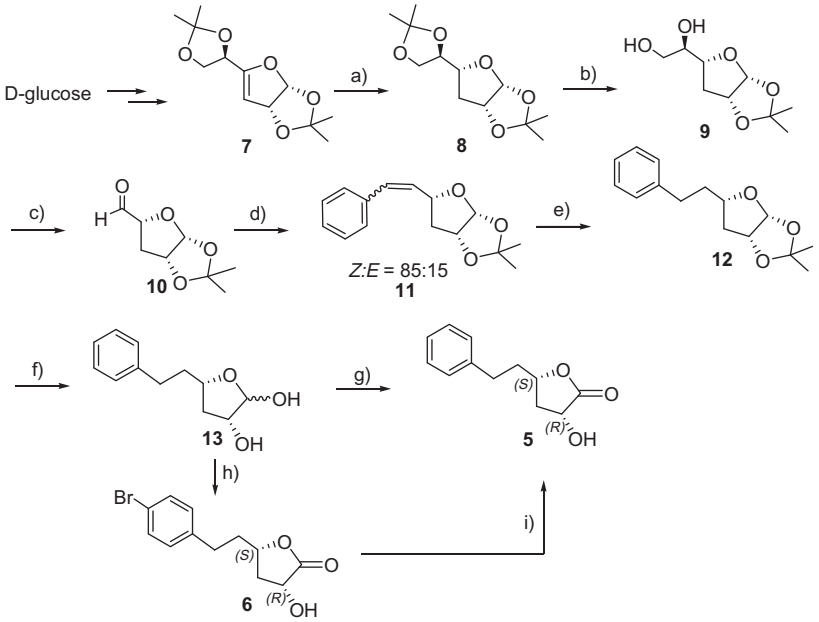


Scheme 1. Retrosynthetic analysis.



Scheme 2. Synthesis of (−)-**5** and (−)-**6**. Reagents and conditions: (a) H2, Pd/C, AcOEt, rt, 2 h, 94%; (b) 30% $HClO_4$, THF, 0 °C, <5 min, 88%; (c) $NaIO_4$, satd $NaHCO_3$, CH_2Cl_2, 0 °C to rt, 2.5 h, 87%; (d) $Ph_3P^+CH_2PhBr^-$, *n*-BuLi, THF, −5 °C to rt, 12 h, (*Z*:*E* = 85:15), 71%; (e) H_2, Pd/C, AcOEt, rt, 2 h, 96%; (f) cat. H_2SO_4, THF/H_2O (4:1), 65 °C, 2.5 h, 85%; (g) Ag_2CO_3–Celite, toluene, reflux, 3 h, 79%; (h) Br_2, $BaCO_3$, dioxane/H_2O (2:1), (in dark), rt, 1 h, 94%; (i) H_2, Pd/C, AcOEt, 260 psi, rt, 24 h, 96%.

carried out using 30% $HClO_4$ to afford diol **9** in 88% yield with recovery of some starting material. This diol on oxidative cleavage using sodium *meta*-periodate afforded aldehyde **10** which was found to be relatively unstable and used as such for further reaction. Aldehyde **10** was subjected to the Wittig olefination with in situ generated benzylidenetriphenylphosphonium ylide from benzyl triphenylphosphonium bromide to afford styrene derivative **11** as a diastereomeric mixture[13] of *Z*:*E* in the ratio of 85:15 as evident from ^{1}H NMR spectrum in 71% yield. This olefinic mixture was then hydrogenated by using 10% Pd/C in ethyl acetate which afforded compound **12** in 96% yield. Cleavage of 1,2-isopropylidene functionality at 65 °C using cat. H_2SO_4 afforded a mixture of hemiacetal **13** in good yield. In the final step, selective anomeric oxidation was carried out by using Ag_2CO_3/Celite[14] which afforded target molecule **5** as a white crystalline solid in 79% yield. Appearance of carbonyl frequency at 1773 cm^{-1} in IR along with peak at 3436 for hydroxyl group and signal at 177.7 ppm in ^{13}C NMR confirmed the formation of α-hydroxy-γ-lactone. In order to improve the yield of target compound, we tried another method[15] for anomeric oxidation with Br_2/$BaCO_3$. This bromine mediated oxidation worked smoothly with 2.5 equiv of bromine and 1.1 equiv of $BaCO_3$ within 1 h to give the required lactone, but in this reaction we also observed electrophilic bromine substitution in phenyl ring exclusively at *para* position to give **6** in 94% yield. Targeting compound **5** from **6**, ring de-bromination of **6** was achieved under hydrogenation condition using 10% Pd/C in ethyl acetate at 260 psi afforded compound **5** in 96% yield and with overall 90% yield from **13**.

The cytotoxicity of compounds **5** and **6** was tested in vitro against five cancer cell lines, P388D1 (murine lymphocytic leukaemia), HL60 (human promyelocytic leukaemia),[16] COLO-205 (human colon cancer), Zr-75-1 (human epithelial breast cancer),[17] HeLa (human epithelial cervical cancer)[18] by MTT colorimetric assay using Mitomycin C as a standard.[19] The assay results were then used to evaluate the corresponding percent inhibition. These synthetic compounds exhibited interesting in vitro anticancer activity. As shown in Table 1, compound **5** (at 100 μg/mL) was found to be very potent inhibitor of COLO-205 and HeLa. It showed moderate inhibition against Zr-75-1 as well as HL-60. Compound **5** exhibited reduced inhibitory activity against P388D1 cell lines as compared to other cell lines. Compound **6** (at 100 μg/mL) showed very high activity against COLO-205 (Table 2). It also inhibited HeLa to a significant level even at lower concentration which was comparable with Mitomycin C. P388D1 and HL60 were inhibited by **6** to a moderate level while it exhibited poor inhibition against Zr-75-1. Thus, substitution of bromine at *para* position in phenyl ring as in compound **6** increased its anticancer activity against four of the cancer cell lines at 100 μg/mL and also showed

Table 1
In vitro anticancer activity of compound **5** on P388D1, COLO-205, Zr-75-1, HL60 and HeLa

Sample (μg/ml) **5**	% Inhibition on various cell lines				
	P388D1	COLO-205	Zr-75-1	HL60	HeLa
100	45.06 ± 1.06	69.29 ± 0.60[a]	50.66 ± 0.76	49.03 ± 0.74	62.42 ± 0.46[a]
80	40.00 ± 0.64	65.35 ± 0.45 [a]	43.61 ± 0.25	43.22 ± 2.23	40.97 ± 0.20
60	38.27 ± 3.21	35.95 ± 0.47	37.88 ± 0.23	40.00 ± 0.37	38.98 ± 0.26
40	37.65 ± 3.56	25.45 ± 0.76	37.44 ± 1.27	38.70 ± 0.02	33.97 ± 0.32
20	35.18 ± 1.06	22.83 ± 0.30	36.12 ± 0.76	36.77 ± 1.12	31.31 ± 0.27
10	31.48 ± 1.42	16.79 ± 0.31	35.68 ± 1.53	30.96 ± 0.37	31.27 ± 0.29
5	27.78 ± 0.71	09.18 ± 0.90	29.51 ± 0.18	28.38 ± 0.74	23.44 ± 0.32
Mitomycin[b]	55.39 ± 0.06	56.27 ± 0.71	51.26 ± 0.39	53.21 ± 0.46	56.48 ± 0.12

The data is indicated as the mean ± SEM ($n = 3$).
[a] Denoting more significant value ($P < 0.05$).
[b] Concentration of mitomycin used was 5 μg/ml.

Table 2
In vitro anticancer activity of compound **6** on P388D1, COLO-205, Zr-75-1, HL60 and HeLa

Sample (μg/ml) **6**	% Inhibition on various cell lines				
	P388D1	COLO-205	Zr-75-1	HL60	HeLa
100	52.46 ± 0.71	75.85 ± 0.75[a]	42.29 ± 1.02	52.9 ± 0.74	69.14 ± 0.06[a]
80	40.74 ± 0.35	72.17 ± 0.00 [a]	38.32 ± 0.50	47.74 ± 0.37	62.51 ± 0.08
60	40.12 ± 0.73	43.30 ± 0.45	37.00 ± 0.53	32.58 ± 0.00	60.58 ± 0.12
40	34.56 ± 1.08	40.94 ± 0.30	36.56 ± 0.56	39.35 ± 0.36	57.48 ± 0.13
20	31.48 ± 2.14	29.39 ± 0.32	35.24 ± 0.00	24.51 ± 0.74	54.40 ± 0.06
10	25.92 ± 1.78	26.24 ± 1.06	33.03 ± 0.25	22.58 ± 0.00	52.51 ± 0.23
5	10.49 ± 0.61	23.09 ± 0.31	20.95 ± 0.27	20.64 ± 0.73	52.34 ± 0.19
Mitomycin[b]	55.39 ± 0.06	56.27 ± 0.71	51.26 ± 0.39	53.21 ± 0.46	56.48 ± 0.12

The data is indicated as the mean ± SEM ($n = 3$).
[a] Denoting more significant value ($P < 0.05$).
[b] Concentration of mitomycin used was 5 μg/ml.

significant activity against COLO-205 and HeLa even at lower concentration (5 μg/mL) when compared to parent compound **5**. By contrast, compound **5** was found to be more potent against Zr-75-1 as compared to **6** at both 5 and 100 μg/mL. At lower concentration (5 μg/mL), compound **5** was found to be more active against P388D1 and HL60 than compound **6**.

In conclusion, we have synthesized two new homologues of harzialactone by chiron approach starting from D-glucose in 10 steps with overall yields of 20% and 23% for compounds **5** and **6** respectively. $Br_2/BaCO_3$ mediated reaction apart from being chemoselective also resulted in bromination of phenyl ring exclusively at *para* position. Thus compound **5** and **6**, were studied for their anticancer activity against five cancer cell lines which indicated their specificity towards the COLO-205 and HeLa at a concentration of 100 μg/mL unlike the harzialactone A **1** which is reported as a cytotoxic against P388 cell line.[1] Further work is in progress for the synthesis of other analogues and to evaluate their anticancer activity that might represent possible leads in drug discovery processes.

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Supplementary data

Supplementary data (general experimental methods, inhibition assay methods, experimental details and ^1H and ^{13}C NMR spectra of new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.100.

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