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Stereoselective synthesis of (6*S*) and (6*R*)-5,6-dihydro-6-[(2*R*)-2-hydroxy-6-phenylhexyl]-2*H*-pyran-2-one and their cytotoxic activity against cancer cell lines

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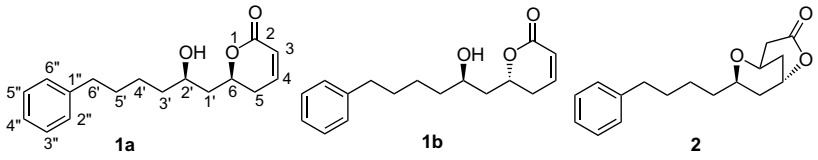
ABSTRACT

Stereoselective total synthesis of α,β-unsaturated lactone (**1a**), isolated from *Ravensara crassifolia*, has been achieved efficiently starting from chiral 2,3-*O*-isopropylidene-D-glyceraldehyde (**3**) followed by asymmetric allylation and ring-closing metathesis. The antiproliferative activities of compounds **1a**, **1b** and the unusual bicyclic compound **2** were evaluated against three-cancer cell lines, THP-1 and U-937 (leukemia) and A-375 (melanoma).

1. Introduction

The family of 5,6-dihydro-α-pyrone derivatives having an alkyl side chain at the C_6 position are important biologically active natural products, which have been isolated from plants. Some of these compounds, such as fostriecin[1–4] and goniothalamin,[5–7] have been found to be anti-cancer agents. Further, (−)-pyronetin[8–10] exhibits immunosuppressive activity, and passifloricin A[11] and strictifolione[12] exhibit antifungal activity.

Compound (6*S*)-5,6-dihydro-6-[(2*R*)-2-hydroxy-6-phenylhexyl]-2*H*-pyran-2-one (**1a**) is an example of this class of natural product, which was isolated from plant *Ravensara crassifolia* by Hostettman and co-workers.[13] This natural pyrone was shown to possess antifungal activity against the phytopathogenic fungus *Cladosporium cucumarinum*. Some of the compounds containing 5,6-dihydro-α-pyrone moiety exhibits cytotoxic activity, such as callystatin A,[14,15] spicigerolide[16] and leptomycin B.[17,18] Inspired by this type of lactones showing anti-cancer activity, we decided to extend the evaluation of the cytotoxic activity of compounds **1a**, **1b** and **2** to the cancer cell lines THP-1 and U-937 (leukemia) and A-375 (melanoma). Several syntheses have been reported for natural compound **1a**.[19–21] No synthesis has been reported for its 6-epimer



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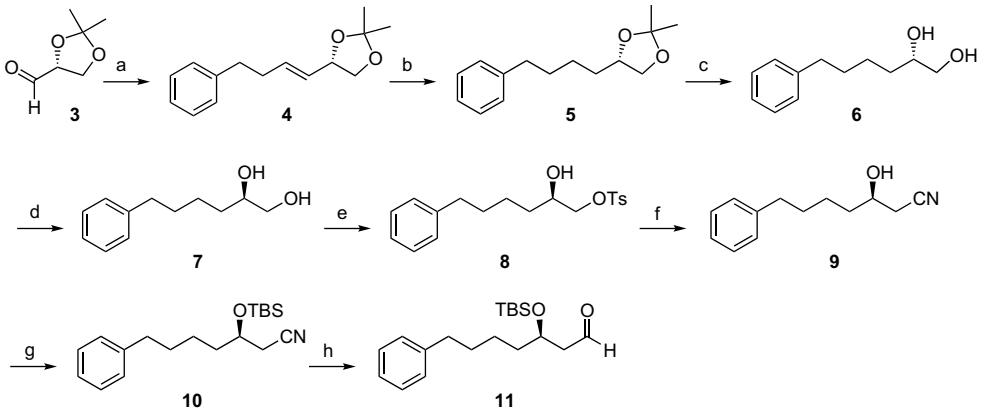
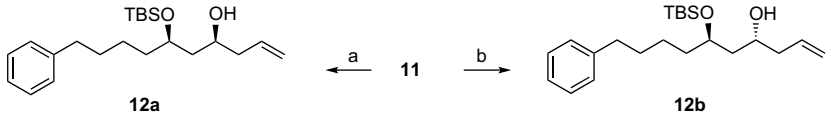
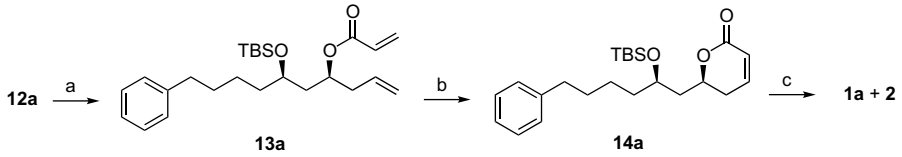


Scheme 1. Conditions: (a) $[Ph(CH_2)_3Ph_3P]Br$ (1 equiv), *n*-BuLi (1 equiv), THF, 0 °C, 2 h, 90%; (b) H_2, 10% Pd/C, MeOH, rt, 6 h, 96%; (c) 2 M HCl, MeOH, 1 h, rt, 97%; (d) (i) PPh_3 (2 equiv), DIAD (2 equiv), *p*-$NO_2C_6H_4COOH$ (2 equiv), THF, rt, 2 h, 90%; (ii) catalytic Na, MeOH, rt, 1 h, 94%; (e) TsCl (1.1 equiv), $(n\text{-}Bu)_2SnO$ (0.1 equiv), Et_3N (2.5 equiv), 0 °C to rt, 4 h, 79%; (f) KCN (1.5 equiv), rt, 12 h, 91%; (g) TBDMS-Cl (1 equiv), imidazole (2.5 equiv), dry CH_2Cl_2, 98%; (h) DIBAL-H (1 equiv), dry CH_2Cl_2, −78 °C, 0.5 h, 70%.



Scheme 2. Conditions: (a) (−)-Ipc_2BCl, allyl MgBr, −100 °C, 2 h, 76%; (b) (+)-Ipc_2BCl, allyl MgBr, −100 °C, 2 h, 76%.



Scheme 3. Conditions: (a) CH_2=CHCOCl, *i*-Pr_2NEt, 0 °C to rt, 88%; (b) Grubbs' I generation catalyst $[(PCy_3)_2Cl_2Ru$=CHPh] (5 mol %), dry CH_2Cl_2, 55 °C, 12 h, 92%; (c) *p*-TSA (0.1 equiv), MeOH, rt, 90%.

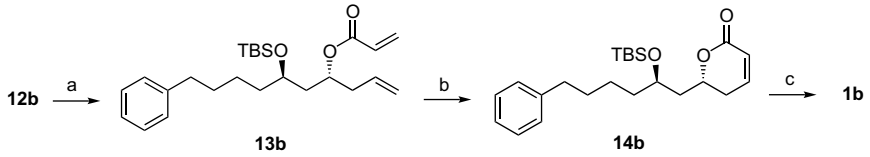
(**1b**). First time we are reporting the synthesis of natural, unnatural isomers and its bicyclic compound **2**.

2. Results and discussion

2.1. Chemistry

Our synthesis began with the chiral aldehyde (**3**), obtained easily from D-mannitol,[22] which was subjected to a Wittig reaction to give compound **4**, in 90% yield. Hydrogenation of compound **4** over 10% Pd/C in methanol gave acetonide (**5**) followed on treating with concd HCl in methanol afforded diol (**6**) in 97% yield. At this stage the asymmetric center of the secondary hydroxyl group in the diol (**6**) was inverted by the Mitsunobu reaction[23,24] to give inverted diol (**7**) in order to match with stereochemistry of the hydroxyl in the natural product (**1a**) at C-2′. The primary hydroxyl group in (**7**) was protected with tosyl chloride using triethyl amine and catalytic amount of Bu_2SnO to produce tosylated compound (**8**) in 79% yield with the minor tosylation at the secondary hydroxyl group of (**7**). The tosylate (**8**) was converted to corresponding nitrile (**9**) in 91% yield by the reaction of KCN in ethanol and H_2O (3:2) at room temperature. The secondary hydroxyl group in the nitrile (**9**) was protected with TBDMS-Cl/imidazole to give tertiary-butyl dimethylsilyl ether (**10**) in 98% yield. The nitrile function was reduced with DIBAL-H in dry dichloromethane at −78 °C to afford the aldehyde (**11**) in 70% yield (Scheme 1).

The introduction of chirality at C-6 carbon is furnished by Brown's asymmetric allylboration.[25,26] The resulting aldehyde (**11**) was subjected to allylboration with (−)-Ipc_2BCl/allyl magnesiumbromide (Ipc=diisopinocampheyl) and (+)-Ipc_2BCl/allyl magnesiumbromide,



Scheme 4. Conditions: same conditions as in Scheme 3.

Table 1
IC_{50} values for antiproliferative activities of compound **1a**, **1b** and **2** against cancer cell lines[a]

Compound	THP-1 (μg/mL)	U-937 (μg/mL)	A-375 (μg/mL)
1a	13.04±1.35	33.63±6.71	21.35±4.32
1b	50.93±0.98	41.47±0.84	38.96±0.49
2	10.41±0.62	32.45±13.12	32.13±0.75
Etoposide[b]	1.27±0.09	10.56±0.70	2.31±0.08

[a] Concentration that elicit inhibition by 50% of the cell growth (IC_{50}), given in μg/mL, were determined from nonlinear regression analysis using the GraphPad Prism software ($r^2>0.9$).
[b] Etoposide was employed as positive control.

respectively, to furnish the required *syn*-1,3-diol (**12a**) and *anti*-1,3-diol (**12b**) in 76% yield (Scheme 2), as a single diastereomer (the minor stereoisomers were lost during chromatographic separation). The relative stereochemistry of 1,3-diol in **13a** and **13b** was established by their conversion to the corresponding acetonide. The *syn* and *anti* relative configuration of the hydroxy groups was confirmed by the analysis of ^{13}C NMR spectrum,[27] which showed signals at δ 30.1 and 19.8 ppm for the two methyl groups and δ 98.4 ppm for the quaternary carbon of the acetonide in *syn*-1,3-diol, while in the *anti*-1,3-diol acetonide having signals at δ 24.52 and 24.54 ppm for the two methyl groups and δ 100.4 ppm for the quaternary carbon. The homoallyl alcohols **12a** and **12b** were transformed independently to lactones **1a** (Scheme 3) and **1b** (Scheme 4), respectively.

The TBDMS protected homoallyl alcohol (**12a**) was reacted with acryloyl chloride and diisopropyl ethylamine at 0 °C in dichloromethane to afford ester (**13a**) in 88% yield for ring-closing metathesis. The ring-closing metathesis was achieved with Grubbs' I generation catalyst[28–30] to afford lactone (**14a**) in 92% yield, which was subjected to deprotection of TBDMS with TBAF in THF to produce the natural product lactone (**1a**) in unsatisfactory yields (30%). The low yield was circumvented by using *p*-toluene sulfonic acid in methanol for the deprotection of TBDMS group to yield compound (**1a**) in 90% yield. It has been observed that if the reaction was run for more than 10 min, it gave the mixture of compounds **1a** and **2** in 70:30 ratio, with an overall yield of 95%. The other diastereomer **12b** was also subjected to the same sequence of reactions to realize the 6-epimer of **1b**; here the side product was not observed.

2.2. Biological activities

Antiproliferative activities of 5,6-dihydro-2*H*-pyran-2-ones **1a**, **1b** and a bicyclic compound **2** were evaluated in the following human cancer cell lines: leukemia (THP-1 and U-937) and melanoma (A-375). Leukemia cell lines (THP-1 and U-937) were cultured in RPMI-1640 and melanoma cell line (A-375) was cultured in DMEM. Chemotherapeutic etoposide was used as positive control.

All the three compounds evaluated displayed antiproliferative activity against the cancer cell lines tested in a concentration-dependent way. The IC_{50} values (μg/ml) for **1a**, **1b**, **2** and etoposide (a positive drug control) are summarized in Table 1. Compound **1a** is more potent on A-375 (melanoma) cancer cell line than **1b** and **2**. While the bicyclic compound **2** was more potent for leukemia cell lines THP-1 and U-937 than **1a** and **1b**. The 6-epimer **1b** is less active than **1a** and **2** on both the cell lines. Overall, these results indicate that natural isomer **1a** is having higher antiproliferative activity on tumour cells than its structure like compound **1b**.

3. Conclusion

In conclusion the Chiron approach synthesis of **1a** and its 6-epimer **1b** was developed and its cytotoxic activity against cancer cell lines was evaluated. The versatility of our synthetic route involves asymmetric allylboration and RCM as key steps. We achieved the biologically active natural product (6*S*)-5,6-dihydro-6-[(2*R*)-2-hydroxy-6-phenylhexyl]-2*H*-pyran-2-one (**1a**) and its 6-epimer (**1b**) in twelve steps with an 88.5% overall yield.

4. Experimental

4.1. Chemistry

4.1.1. General methods

Solvents were dried over standard drying agents and freshly distilled prior to use.[31] The reagents were purchased from Aldrich and Acros, and were used without further purification unless otherwise stated. All moisture-sensitive reactions were carried out under nitrogen. Organic solutions were dried over anhydrous Na_2SO_4 and concentrated below 40 °C in vacuo. All column chromatographic separations were performed using silica gel (Acme's 60–120 mesh). ^1H NMR (200 MHz and 300 MHz) and ^{13}C NMR (50 MHz and 75 MHz) spectra were measured with a Varian Gemini FT-200 MHz and Bruker Avence 300 MHz with tetramethylsilane as internal standard for solutions in deuteriochloroform. *J* values are given in hertz. IR spectra were recorded on a Perkin–Elmer IR-683 spectrophotometer with NaCl optics. Optical rotations were measured with Horiba high sensitive polarimeter SEPA-300 at 25 °C. Mass spectra were recorded on Agilent Technologies 1100 Series (Agilent Chemstations Software).

4.1.2. (*S*)-2,2-Dimethyl-4-[(*E*)-4-phenylbut-1-enyl]-1,3-dioxolane (***4***)

To a suspension of $[Ph_3P(CH_2)_3Ph]Br$ (9.2 g, 20 mmol) in THF (50 mL) was added *n*-BuLi (1.6 M in hexane, 12.5 mL, 20 mmol) at 0 °C and stirred for 15 min. Then a solution of ketal (**3**) (2.6 g, 20 mmol) in THF (10 mL) was added dropwise and the mixture was allowed to stir for an additional 0.5 h at room temperature. After completion of the reaction, the reaction was quenched with saturated NH_4Cl solution (40 mL) and extracted into diethyl ether (3×50 mL). The ether solution was washed with brine and dried with anhydrous Na_2SO_4. After removal of solvent, the crude product was purified on a flash column of silica gel (eluent PE/EtOAc, 98:2) to afford the pure compound **4** (4.17 g, 90%) as a mixture of geometric isomers (both are separated). Liquid, IR (KBr): ν=2956, 2926, 2860, 1635, 1457, 1374, 1217, 1063, 980 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.30–7.14 (m, 5H), 5.70–5.61 (m, 1H), 5.40 (t, 1H, *J*=8.49 Hz), 4.73–4.66 (m, 1H), 3.76 (dd, 1H, *J*=6.04 and 8.1 Hz), 3.34 (t, 1H, *J*=7.9 Hz), 2.81–2.70 (m, 1H), 2.68–2.56 (m, 1H), 2.53–2.30 (m, 2H), 1.39 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.6, 134.5, 128.3, 128.2, 127.4, 125.8, 109.4, 75.0, 66.8, 35.5, 29.6, 26.9, 25.2. LCMS: *m*/*z* 255 $[M+Na]^+$. $[\alpha]_D^{25}$ +40.8 (*c* 1, $CHCl_3$).

4.1.3. (*S*)-2,2-Dimethyl-4-(4-phenylbutyl)-1,3-dioxolane (***5***)

To a solution of compound **4** (3.9 g, 16.8 mmol) in methanol (15 mL) was added 10% Pd/C (300 mg) and stirred under hydrogen for 6 h at room temperature. After completion of the reaction, the catalyst was removed by filtration, solvent evaporated and crude product was purified on a flash column of silica gel (eluent PE/EtOAc, 50:1) to afford compound **5** (3.78 g, 96%) as colourless oil. Liquid, IR (KBr): ν=3061, 3026, 2985, 2934, 2860, 1603, 1496, 1216, 1061 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.24–7.08 (m, 5H), 4.04–3.92 (m, 2H), 3.42 (t, 1H, *J*=6.7 Hz), 2.60 (t, 2H, *J*=6.7 Hz), 1.72–1.44 (m, 6H), 1.36 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.2, 128.2, 128.1, 125.6, 108.4, 75.8, 69.3, 35.6, 33.3, 31.3, 26.8, 25.6, 25.3. LCMS: *m*/*z* 257 $[M+Na]^+$. $[\alpha]_D^{25}$ +5.8 (*c* 1.2, $CHCl_3$).

4.1.4. (*S*)-6-Phenylhexane-1,2-diol (***6***)

To a solution of compound **5** (3.6 g, 15.4 mmol) in methanol (20 mL) was added HCl (2 M, 10 mL) and the mixture was stirred for

1 h at room temperature. After completion of the reaction, the contents were neutralized with solid K_2CO_3 and removed the methanol, diluted with water (30 mL) and extracted into ethyl acetate three times (3×50 mL). The combined organic layers was dried over anhydrous sodiumsulphate and the solvent was removed under vacuum to obtain the crude product (**6**), which was purified on a flash column of silica gel (eluent PE/EtOAc, 1:1) to afford a colourless oil (2.89 g, 97%).

IR (KBr): ν=3395, 3016, 2983, 2929, 1637, 1608, 1486, 1453 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.24–7.08 (m, 5H), 3.66–3.56 (m, 2H), 3.7 (t, 1H, J=7.55 Hz), 3.30 (br s, 2H), 2.59 (t, 2H, J=7.55 Hz), 1.64–1.56 (m, 2H), 1.50–1.32 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.4, 128.4, 128.3, 125.7, 72.2, 66.7, 35.8, 33.0, 31.4, 25.2. LCMS: m/z 194.2 [M^+]. $[\alpha]_D^{25}$ −28.2 (c 1.02, $CHCl_3$).

4.1.5. (R)-6-Phenyl hexane-1,2-diol (7)

To a stirred solution of PPh_3 (7.29 g, 27 mmol), diol (**6**) (2.7 g, 13.91 mmol) and *p*-nitrobenzoic acid (4.67 g, 27 mmol) in THF (30 mL) was added DIAD (95%, 2.02 mL, 27 mmol) dropwise at 0 °C. The reaction mixture was stirred for 2 h at room temperature. After the completion of reaction, the mixture was diluted with water (20 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layer was washed with $NaHCO_3$ and brine solutions, and dried with anhydrous Na_2SO_4. Evaporation of the solvent gave a crude product, which was purified by flash chromatography on a column of silica gel (eluent PE/EtOAc, 9:1), to afford (*R*)-diester as a light yellow viscous liquid (6.16 g, 90%). IR (KBr): ν=3028, 2978, 1734, 1715, 1528, 1336, 1278 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.31–8.24 (m, 4H), 8.20–8.12 (m, 4H), 7.28–7.15 (m, 5H), 5.55 (m, 1H), 4.65 (dd, 1H, J=3.2 and 12.0 Hz), 4.52 (dd, 1H, J=6.9 and 12.08 Hz), 2.63 (t, 2H, J=7.5 Hz), 1.92–1.80 (m, 2H), 1.78–1.67 (m, 2H), 1.58–1.49 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.2, 164.1, 150.5, 141.8, 135.1, 134.8, 130.6, 128.2, 125.7, 123.5, 73.0, 66.2, 35.4, 30.8, 30.5, 24.5. LCMS: m/z 492.3 [M^+].

Further, the diester (5.9 g, 12 mmol) was taken in methanol (50 mL) and added sodium (60 mg, 2.4 mmol) and the reaction mixture stirred for 1 h. After completion of the reaction, solid NH_4Cl (0.5 g) was added to the reaction mixture. After removal of methanol under vacuum, the residue was dissolved in EtOAc (25 mL), washed with brine and dried over anhydrous Na_2SO_4. The crude product was purified by flash column chromatography on a silica gel (eluent PE/EtOAc, 1:1), to afford the desired diol (**7**) (2.18 g, 94%) as a colourless oil. Its spectroscopic data were identical to those of compound **5**, except that the optical rotation value is opposite: $[\alpha]_D^{25}$ +27.8 (c 1.3, $CHCl_3$).

4.1.6. (R)-2-Hydroxy-6-phenylhexyl-4-methyl benzene sulfonate (8)

To an ice-cold solution of diol (**7**) (2 g, 10.3 mmol), catalytic amount of dibutyl tin oxide (5 mg) and triethyl amine (3.16 mL, 22.68 mmol) in dichloromethane (30 mL) was added. Then a solution of tosyl chloride (1.96 g, 10.3 mmol) in dichloromethane (10 mL) was added dropwise and stirred the reaction for 4 h at room temperature. After completion of reaction, the mixture was diluted with water and extracted into dichloromethane (3×50 mL). The organic layer was washed with brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get the crude residue, which was purified on a silica gel column, eluting with PE/EtOAc (7:3) to afford compound **8** as a viscous liquid (2.83 g, 79%). IR (KBr): ν=3442, 3062, 2933, 2858, 1636, 1454, 1357, 1212, 1176, 699 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.77 (d, 2H, J=8.3 Hz), 7.33 (d, 2H, J=8.3 Hz), 7.24–7.06 (m, 5H), 4.03–3.98 (m, 1H), 3.86–3.78 (m, 2H), 2.57 (t, 2H, J=7.5 Hz), 2.45 (s, 3H), 2.1 (br s, 1H), 1.65–1.55 (m, 2H), 1.50–1.36 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.0, 142.2, 142.0, 132.6, 129.9, 129.8, 128.3, 128.2, 127.9, 127.8, 125.6, 73.9, 69.3, 35.6, 32.4, 31.2, 24.8, 21.6. LCMS: m/z 371.2 [M^+].

4.1.7. (R)-3-Hydroxy-7-phenylheptane nitrile (9)

The compound tosylate (**8**) (2.7 g, 7.76 mmol) was dissolved in 30 mL of 60% aq ethanol, cooled to 0 °C, and then added KCN (0.756 g, 11.63 mmol). The reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, ethanol was removed under vacuum and diluted with water (20 mL), extracted into EtOAc and the organic phase was washed with brine and dried over anhydrous Na_2SO_4. After removal of solvent, the crude residue was purified on a flash column of silica gel, eluting with PE/EtOAc (7:3), to afford compound **9** (1.43 g, 91%) as colourless oil.

IR (KBr): ν=3446, 3061, 3026, 2934, 2252, 1634, 1495 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.25–7.08 (m, 5H), 3.88–3.81 (m, 1H), 2.81 (br s, 1H), 2.60 (t, 2H, J=7.5 Hz), 2.41 (dd, 2H, J=4.5 and 8.3 Hz), 1.69–1.29 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.1, 128.2, 128.1, 125.6, 117.0, 69.3, 36.1, 35.5, 30.9, 25.8, 24.7. LCMS: m/z 203.2 [M^+]. $[\alpha]_D^{25}$ +18.3 (c 1.8, $CHCl_3$).

4.1.8. (R)-3-(tert-Butyl dimethyl silanyloxy)-7-phenyl-heptane nitrile (10)

To a stirred solution of cyano compound (**9**) (1.4 g, 6.89 mmol) and imidazole (1.172 g, 17.2 mmol) in dichloromethane (20 mL) at 0 °C was added tertiary-butyl dimethylsilyl chloride (0.955 g, 6.89 mmol) dropwise. After the completion of reaction, the mixture was diluted with water (15 mL) and extracted with dichloromethane (3×25 mL). The organic layer was washed with brine solution (10 mL) and dried over anhydrous Na_2SO_4. The solvent removed under vacuum to furnish the crude residue, which was purified by flash chromatography on silica gel eluting with PE/EtOAc (9:1) to afford compound **10** (2.04 g, 98%) as a colourless oil.

IR (KBr): ν=3062, 3027, 2933, 2858, 2250, 1635, 1496, 1464, 1255 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.08–7.25 (m, 5H), 3.92–3.83 (m, 1H), 2.60 (t, 2H, J=7.5 Hz), 2.38 (d, 2H, J=5.2 Hz), 1.72–1.50 (m, 4H), 1.45–122 (m, 2H), 0.881 (s, 9H), 0.09 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.1, 128.27, 128.24, 128.21, 125.6, 117.6, 68.1, 36.7, 35.6, 31.1, 26.0, 25.63, 25.60, 25.5, 24.4, 17.8, −4.76, −4.79. LCMS: m/z 340 [M+Na]$^+$. $[\alpha]_D^{25}$ +6.2 (c 1.4, $CHCl_3$).

4.1.9. (R)-3-(tert-Butyl dimethyl silanyloxy)-7-phenyl-heptanal (11)

To a stirred solution of compound **10** (2 g, 6.6 mmol) in dry dichloromethane (25 mL) was added DIBAL-H (5.154 mL, 20 wt % in solution) slowly for 15 min at −78 °C and the reaction mixture was stirred for half an hour at −78 °C. After completion of reaction, the reaction mixture was quenched with saturated sodium potassium tartrate solution (15 mL) and was stirred vigorously at room temperature for additional 1 h and the contents were extracted into dichloromethane (3×25 mL). The combined organic layer was washed with brine solution (30 mL), dried over anhydrous Na_2SO_4 and the solvent removed under vacuum to give a crude product, which was purified by flash chromatography on a silica gel column eluting with PE/EtOAc (50:2) to afford the aldehyde (**11**) (1.478 g, 70%) as a colourless oil. IR (KBr): ν=3027, 2926, 2858, 1725, 1634, 1452 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.24–7.10 (m, 5H), 4.12 (m, 1H), 2.58 (t, 2H, J=7.5 Hz), 2.51–2.42 (m, 2H), 1.65–1.21 (m, 6H), 0.86 (s, 9H), .008 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 202.5, 142.5, 128.6, 128.5, 125.9, 68.3, 51.1, 37.6, 35.8, 31.3, 26.1, 25.9, 25.5, 24.7, 17.9, −4.7, −4.4. LCMS: m/z 320 [M^+].

4.1.10. (4S,6R)-6-(tert-Butyl dimethyl silanyloxy)-10-phenyldec-1-en-4-ol (12a)

To a stirred solution of (−)-Ipc_2BCl (900 mg, 2.81 mmol) in dry ether (15 mL), allyl magnesium bromide (1 M solution in Et_2O, 2 mL, 2 mmol) was added dropwise under nitrogen atmosphere at −78 °C. After finishing the addition, the RB flask replaced from dry ice–acetone bath to ice bath and the mixture was stirred for 1 h. The solution was allowed to stand, whereby precipitation of

magnesium chloride took place. The supernatant solution was carefully transferred to another flask through a cannula. After cooling this flask at −100 °C, a solution of the aldehyde (**11**) in dry Et_2O (5 mL) was added dropwise through a syringe. The resulting solution was further stirred at −100 °C for 2 h. The reaction mixture was quenched by the addition of phosphate pH 7 buffer solution (10 mL), MeOH (10 mL) and 30% H_2O_2 (5 mL). After stirring for 30 min, the mixture was poured into saturated aq $NaHCO_3$ and extracted into Et_2O (3×25 mL), the combined organic layers were dried over anhydrous magnesium sulfate and concentrated. The crude residue was purified by silica gel column chromatography eluted with normal hexane to 1:9 EA/PE to afford the corresponding homoallyl alcohol (**12a**) (515 mg, 76%) as colourless oil. IR (KBr): ν=3437, 2936, 2853, 2363, 1636, 1454, 1052 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.33–7.12 (m, 5H), 5.95–5.73 (m, 1H), 5.13 (d, 2H, J=15.42 Hz), 4.01–3.88 (m, 1H), 3.85–3.70 (m, 1H), 2.90 (br s, 1H), 2.65 (t, 2H, J=7.3 Hz), 2.23 (t, 2H, J=6.6 Hz), 1.75–1.28 (m, 8H), 0.94 (s, 9H), 0.15 (s, 3H), 0.124 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.6, 135.2, 128.5, 128.4, 125.9, 117.6, 73.1, 70.4, 42.4, 42.3, 38.0, 36.1, 31.8, 26.1, 24.5, 18.1, −3.8, −4.4. LCMS: m/z 363 $[M+1]^+$. $[\alpha]_D^{25}$ −6.9 (c 0.65, $CHCl_3$).

The same above procedure was used except for the use of (+)-Ipc_2BCl instead of (−)-Ipc_2BCl to afford (4*R*,6*R*)-6-(*tert*-butyl dimethyl silanyloxy)-10-phenyldec-1-en-4-ol **12b**. For (4*R*,6*R*)-**12b**: $[\alpha]_D^{25}$ −5.2 (c 0.8, $CHCl_3$).

4.1.11. (4S,6R)-6-(tert-Butyl dimethyl silanyloxy)-10-phenyl dec-1-en-4-yl acrylate (*13a*)

To a stirred solution of compound **12a** (150 mg, 0.414 mmol) in dichloromethane (10 mL) were added acryloyl chloride (67 μL, .828 mmol) and *i*-Pr_2NEt (138 μL, 1.656 mmol) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was diluted with water (5 mL) and extracted into dichloromethane (3×10 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum to get the crude residue, which was purified by silica gel column chromatography (PE/EtOAc, 1:9) to afford the pure product (**13a**) (152 mg, 88%) as colourless oil. IR (KBr): ν=3075, 2927, 2858, 1725, 1635, 1408, 1257, 1190, 1052 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.25–7.11 (m, 5H), 6.39 (dd, 1H, J=1.5 and 17.3 Hz), 6.09 (dd, 1H, J=9.8 and 16.6 Hz), 5.85 (m, 1H), 5.78–5.66 (m, 1H), 5.09 (d, 2H, J=13.5 Hz), 5.08–5.02 (m, 1H), 3.77–3.64 (m, 1H), 2.65–2.59 (t, 2H, J=7.5 Hz), 2.42–2.32 (m, 2H), 1.71–1.28 (m, 8H), 0.89 (s, 9H), 0.05 (s, 3H), 0.021 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6, 142.5, 133.3, 130.4, 130.3, 128.8, 128.7, 128.3, 128.2, 125.6, 117.8, 71.2, 68.7, 41.1, 40.7, 37.6, 35.9, 31.6, 25.8, 24.2, 17.9, −4.5, −4.7. LCMS: m/z 416 $[M^+]$. $[\alpha]_D^{25}$ +4.3 (c 1, $CHCl_3$). The same above procedure was used except for the use of (4*R*,6*R*)-6-(*tert*-butyl dimethyl silanyloxy)-10-phenyldec-1-en-4-ol (**12b**), to afford the (4*R*,6*R*)-6-(*tert*-butyl dimethyl silanyloxy)-10-phenyl dec-1-en-4-yl acrylate **13b**. For (4*R*,6*R*)-**13b**: $[\alpha]_D^{25}$ +5.2 (c .85, $CHCl_3$).

4.1.12. (6S)-5,6-Dihydro-6-[(2R)-2-(tert-butyl dimethyl silanyloxy)-6-phenylhexyl]-2H-pyran-2-one (*14a*)

To a stirred solution of bis(tricyclohexyl phosphine)benzylidine ruthenium(IV) dichloride (Grubbs catalyst, 13 mg, 5 mol %) in dichloromethane (50 mL) at 55 °C was added compound **13a** (125 mg, 0.3 mmol) dissolved in dichloromethane (25 mL). The resulting mixture was heated for 12 h. After completion of the reaction, the contents were cooled and solvent was removed under reduced pressure to yield crude product, which was purified on silica gel column eluting with (PE/EtOAc, 7:3) to afford the pure compound (**14a**) (102 mg, 92%). IR (KBr): ν=2935, 1722, 1634, 1392, 772 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.29–7.13 (m, 5H), 6.90–6.82 (m, 1H), 6.02 (d, 1H, J=9.8 Hz), 4.59–4.50 (m, 1H), 3.93–3.85 (m, 1H), 2.60 (t, 2H, J=7.5 Hz), 2.38–2.31 (m, 2H), 2.01–1.96 (m, 1H), 1.65–1.29 (m, 6H), 0.85 (s, 9H), 0.03 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.2, 144.9, 142.3, 128.2, 128.1, 125.3, 121.3, 75.1, 68.4, 41.8, 36.3, 35.7, 31.4, 29.7, 25.7, 24.6, 17.8, −4.4, −4.6. LCMS: m/z 411 $[M+Na]^+$. $[\alpha]_D^{25}$ −9.6 (c, .45, $CHCl_3$). The same above procedure was followed except for the use of (4*R*,6*R*)-6-(*tert*-butyl dimethyl silanyloxy)-10-phenyl dec-1-en-4-yl acrylate (**13b**), to afford the (6*R*)-5,6-dihydro-6-[(2*R*)-2-(*tert*-butyl dimethyl silanyloxy)-6-phenylhexyl]-2*H*-pyran-2-one (**14b**). For (6*R*,2*R*)-**14b**: $[\alpha]_D^{25}$ −4.2 (c 1.2, $CHCl_3$).

4.1.13. (6S)-5,6-Dihydro-6-[(2R)-2-hydroxy-6-phenylhexyl]-2H-pyran-2-one (*1a*)

To a stirred solution of compound **13** (50 mg) in methanol catalytic amount of *p*-toluene sulfonic acid (5 mol %) was added and stirred for 10 min. Then the reaction mixture was treated with solid $NaHCO_3$. The methanol was removed and extracted into ethyl acetate (3×10 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure to yield crude product, which was purified on silica gel column eluting with PE/EtOAc (6:4) to afford the pure product **14** (31 mg, 90%) as a pale yellow solid. Mp=34–36 °C. IR (KBr): ν=3445, 2928, 2848, 1694, 1487, 1396, 1262 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.30–7.13 (m, 5H), 6.93–6.84 (m, 1H), 6.01 (dd, 1H, J=1.45 and 9.46 Hz), 4.81–4.66 (m, 1H), 4.06–3.92 (m, 1H), 2.62 (t, 2H, J=7.5 Hz), 2.39–2.30 (m, 2H), 1.95–1.81 (m, 1H), 1.71–1.58 (m, 3H), 1.51–1.41 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.4, 145.3, 142.3, 128.3, 128.2, 125.6, 121.3, 74.9, 67.1, 42.25, 37.8, 35.8, 31.3, 29.9, 25.1. LCMS: m/z 275 $[M+1]^+$. $[\alpha]_D^{25}$ −63.8 (c 0.5, $CHCl_3$). The same above procedure was followed except for the use of (6*R*)-5,6-dihydro-6-[(2*R*)-2-(*tert*-butyl dimethyl silanyloxy)-6-phenylhexyl]-2*H*-pyran-2-one (**14b**), to afford the (4*R*,6*R*)-**1b** as gummy syrup. For (6*R*,2*R*)-**1b**: $[\alpha]_D^{25}$ −18.4 (c 0.5, $CHCl_3$).

4.1.14. Spectroscopic data for compound (*2*) [(1R,7R)-7-(4-phenylbutyl)-2,6-dioxa-bicyclo[3.3.1]nonan-3-one]

It was obtained as a colourless solid, mp 36–38 °C. IR (KBr): ν=2928, 2856, 2102, 1734, 1494, 1454, 1073 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.30–7.15 (m, 5H), 4.92–4.88 (m, 1H), 4.38–4.32 (m, 1H), 3.78–3.64 (m, 1H), 2.86–2.78 (m, 2H), 2.58 (t, 2H, J=7.5 Hz), 2.04–188 (m, 3H), 1.75–1.28 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 142.3, 128.27, 128.2, 125.6, 73.0, 65.7, 65.5, 36.8, 36.4, 35.7, 35.6, 31.2, 29.7, 24.7. LCMS: m/z 297.4 $[M+Na]^+$. $[\alpha]_D^{25}$ −16.8 (c 0.5, $CHCl_3$).

4.2. Biological activities

4.2.1. Biological assay

Since it is known that different cell lines display different sensitivities towards a cytotoxic compound, the use of more than one cell line is therefore considered necessary in the detection of cytotoxic compounds. Bearing this in mind different cell lines leukemia (THP-1 and U-937) and melanoma (A-375) were used in the present study. Human tumour cell lines were kindly provided by National Center for Cellular Sciences (NCCS), Pune, India. Leukemia cell lines were cultured in RPMI-1640 and melanoma cell line was cultured in DMEM. Both the media were supplemented with 10% heat-inactivated FCS, 1 mmol $NaHCO_3$, 2 mmol L-glutamine and penicillin–streptomycin in a humidified atmosphere of 95%, 5% CO_2 at 37 °C. In all experiments, THP-1, U-937 and A-375 cells were seeded at a final density of 2×10^4 cells/well in 96 well microtiter plates. The cells were treated with different test concentrations (10, 20, 40, 60, 80 and 100 μg/mL) of compounds **1a**, **1b** and **2** and their cytotoxicities were compared with the activity of a positive control, etoposide at identical conditions with five replicates each. Cytotoxicity was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, according to the Mosmann method[32] (1983). Briefly, the cells (2×10^4) were seeded

in each well containing 0.1 ml of RPMI medium or DMEM in 96 well plates. After 24 h, different test concentrations were added and cell viability was assessed after 2 days, by adding 10 μL per well of MTT (5 mg/mL; stock solution, Sigma). The plates were incubated at 37 °C for additional 4 h. The medium was discarded and the formazan blue, which formed in the cells, was dissolved with 100 μL dimethylsulfoxide. The rate of colour production was measured at 570 nm in a spectrophotometer. All experiments were conducted under the standard laboratory illumination. The percent inhibition of cell viability was determined with reference to the control values (without test compound). The data was subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} concentrations were calculated using the respective regression analysis.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.01.101.

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