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(*R*)-Goniothalamin: total syntheses and cytotoxic activity against cancer cell lines

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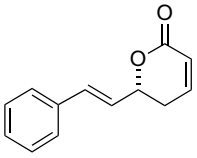
Abstract—The total syntheses of (*R*)-goniothalamin (**1**), a styryl lactone isolated from several *Goniothalamus* species, via catalytic asymmetric allylation of α-benzyloxyacetaldehyde (**2**), followed by ring-closing metathesis and Wittig olefination and via catalytic asymmetric allylation of *trans*-cinnamaldehyde (**12**), followed by ring-closing metathesis are reported. The antiproliferative activities of (*R*)-**1** and its *Z*-isomer **10** as well as of the synthetic dihydropyranone intermediates **7** and **8** against eight different cancer cell lines are also described.

1. Introduction

Phytochemical studies of the genus *Goniothalamus* have resulted in the isolation and characterization of many compounds with a variety of biological activity.[1,2] The styryl lactones have been reported mainly in the genus *Goniothalamus* and the first styryl lactone found within the Annonaceae family was (*R*)-goniothalamin (**1**) (Fig. 1).[1] This natural compound displayed in vitro cytotoxic effect especially by inducing apoptosis on different cancer cell lines [cervical carcinoma (Hela); gastric carcinoma (HGC-27); breast carcinoma (MCF-7, T47D, MDA-MB-231); leukemia (HL-60), ovarian carcinoma (Caov-3)].[2–5] This effect was shown to be selective for cancer cell lines with no significant cytotoxicity toward non-malignant cells.[5] In in vivo models, (*R*)-goniothalamin (**1**) was reported to have tumoricidal and tumoristatic activity on Sprague–Dawley rats with 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumors.[6]



(*R*)-Goniothalamin (**1**)

Figure 1. Structure of (*R*)-goniothalamin (**1**).

Due to the interesting biological activity displayed by (*R*)-goniothalamin (**1**), several successful approaches to this natural product have been described.[7–13] The absolute configuration in the pyran-2-one moiety has generally been secured from chiral starting material, asymmetric allylboration of aldehydes with *B*-allyldiisopinocampehylborane[7,14,15] or through asymmetric reduction using enzymes or microorganisms.[8,11,12,16–20]

The field of chiral catalysis has experienced explosive growth over the last two decades.[21] By now, many of the classical reactions in organic synthesis can be carried out efficiently in asymmetric manner. As one of the fundamental and powerful C–C bond-forming reactions, enantioselective catalytic allylation (ECA) of aldehydes has attracted considerable attention.[22] Particularly, Keck and co-workers have developed a catalytic system[23–25] that has been useful for the total synthesis of natural products such as mucusin,[26] superstolide,[27] centrolobine,[28] and curacin A.[29]

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Maruoka and co-workers have also described a new class of highly reactive and selective titanium complexes for the enantioselective allylation of aldehydes[30,31] that also has been employed in the total syntheses of (*R*)- and (*S*)-argentilactone.[32,33]

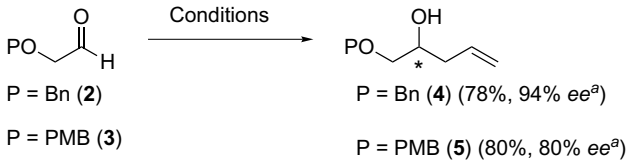
Herein, we report a enantioselective allylation of α-alkoxy aldehydes **2** and **3** and our first generation synthesis of (*R*)-goniothalamin (**1**) featuring ring-closing metathesis[34] reaction and Wittig olefination as the key steps.[8] Additionally, we present a second generation total synthesis of (*R*)-**1** from *trans*-cinnamaldehyde (**12**) featuring enantioselective Maruoka allylation[35] and ring-closing metathesis.[32,34] The antiproliferative activities of (*R*)-goniothalamin (**1**), its *Z*-isomer **10** and 5,6-dihydro-2*H*-pyran-2-ones **7** and **8** against cancer cell lines are also described.

2. Results and discussion

2.1. Chemistry

The catalytic system developed by Keck and co-workers is a useful method for enantioselective catalytic allylation (ECA) of the aldehydes.[23–25] However, the influence of the protecting groups of α-alkoxyaldehydes on the efficiency of this catalytic system has not yet been reported and we initially investigated enantiomeric discrimination during the allylation of α-alkoxyaldehydes **2** and **3**, prepared as described in the literature.[36,37]

The enantioselective catalytic allylation of benzyloxyacetaldehyde (**2**) with allyltributyltin, under the influence of chiral (*R*)-BINOL/Ti(O*i*-Pr)$_4$ complex generated in situ in CH_2Cl_2 and in the presence of molecular sieves at −20 °C afforded **4** in 78% yield and 94% ee (Scheme 1) after 60 h. Under the same reaction conditions *p*-methoxybenzyloxyacetaldehyde (**3**) afforded the respective homoallylic alcohol **5** in good yield (80%) and enantiomeric excess (80% ee).

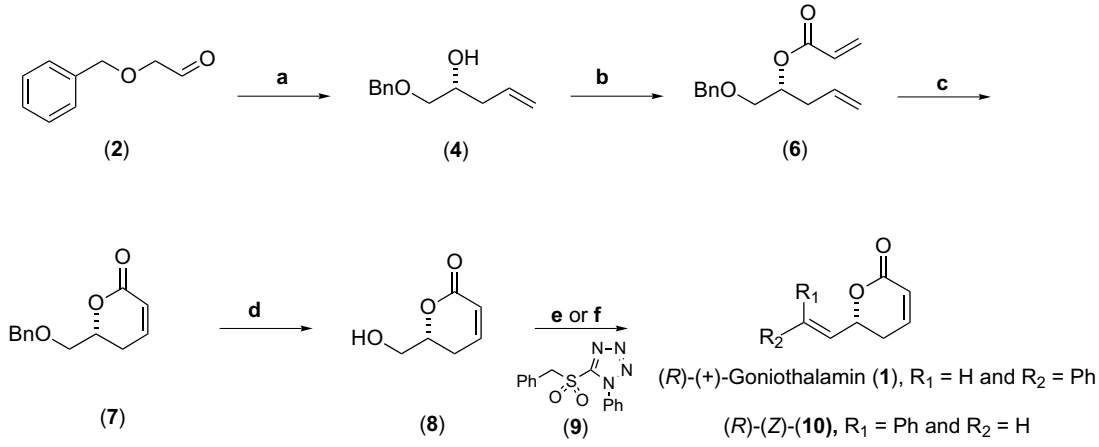


Scheme 1. Conditions: (*R*)-BINOL (10 mol %), Ti(O*i*-Pr)$_4$ (10 mol %), molecular sieves (4 Å), allyltributyltin, −20 °C, 60 h. [a]Enantiomeric excesses were determined by HPLC and CG as described in the experimental section.

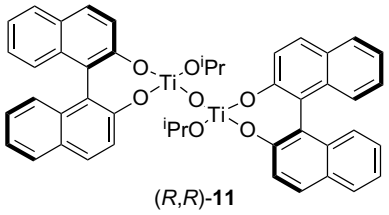
The best results regarding yield and enantioselectivity were observed in the allylation of benzyloxyacetaldehyde **2** and the corresponding homoallylic alcohol was then used in the total synthesis of (*R*)-goniothalamin (**1**).

Acylation of **4** with acryloyl chloride yielded ester **6** (86% yield) which smoothly underwent ring-closing metathesis with Grubbs' catalyst (10 mol %) in refluxing CH_2Cl_2 for 12 h to provide the corresponding α,β-unsaturated δ-lactone **7** in 91% yield from **6** (Scheme 2).[8]

At this point, our efforts were directed to the selective removal of the benzyl group in **7**. While no reaction was observed with lithium naphthalenide,[38,39] lithium *tert*-butyldiphenyl (LiDBB)[40] or tin(IV) chloride,[41] hydrogenolysis with Pd(OH)/C (1 atm of H_2)[42] promoted the reduction of the α,β-unsaturated bond. Low yield was obtained using titanium(IV) chloride,[43,44] chromium(II) chloride/lithium iodide,[45] and boron tribromide.[46] In our hands, utilization of ferric chloride[47] in CH_2Cl_2 at room temperature achieved debenzylation of **7** to give **8** in 88% yield, which was converted to (*R*)-**1** according to the route described by Tsubuki and co-workers.[9] Accordingly, Wittig olefination of the corresponding aldehyde (prepared from **8** by Swern oxidation and employed in the next step without further purification) with a solution of benzylidenetriphenylphosphorane (prepared by treatment of the corresponding triphenyl phosphonium chloride with



Scheme 2. Conditions: (a) (*R*)-BINOL (10 mol %), Ti(O*i*-Pr)$_4$ (10 mol %), molecular sieves (4 Å), allyltributyltin, −20 °C, 60 h (78%; 94% e); (b) acryloyl chloride, Et_3N, CH_2Cl_2, 0 °C (86%); (c) Grubbs' catalyst [$(PCy_3)_2Cl_2$ Ru=CHPh], CH_2Cl_2 (91%); (d) $FeCl_3$, CH_2Cl_2 (88%); (e) $(COCl)_2$, CH_2Cl_2, DMSO, Et_3N, −65 °C, 30 min; then Ph_3P=CHPh, THF (53%, two steps); (f) $(COCl)_2$, CH_2Cl_2, DMSO, Et_3N, −65 °C, 30 min; then solution of the **9**, KHMDS, THF, −78 °C (<20%).



(*R*,*R*)-**11**

Figure 2. The μ-oxo bis(binaphthoxy)(isopropoxy)titanium complex (*R*,*R*)-**11** developed for Maruoka and co-workers.[50]

n-BuLi in THF) afforded, after column chromatography on silica gel, a 1:3 molar ratio of (*R*)-goniothalamin (**1**) (13% yield) and its corresponding *Z*-isomer [(*Z*)-**10**, 40% yield]. Attempts to employ the Julia–Kociensky protocol to selectively install the desired *E* double bond configuration provided only (+)-goniothalamin (**1**), albeit in low yields (<20%).[48,49]

The hurdle associated with the correct stereochemistry of the exocyclic double bond was solved by using the methodology developed by Maruoka and co-workers for the allylation of aldehydes.[30,31,50] The μ-oxo bis(binaphthoxy)(isopropoxy)titanium complex (*R*,*R*)-**11** (Fig. 2) displays excellent enantioselectivity for the addition of allyltributyltin to aldehydes, including *trans*-cinnamaldehyde.[31] The efficiency of this catalyst is proposed to be due to simultaneous coordination and double activation ability of the bidentate Ti(IV) catalyst (*R*,*R*)-**11**.[50]

In the event, catalytic asymmetric allylation of *trans*-cinnamaldehyde (**12**) with allyltributyltin, under the influence of chiral (*R*,*R*)-**11** complex (CH_2Cl_2, −20 °C, 24 h) afforded **13** in 78% yield and 96% ee. High yield was obtained when freshly prepared Ag_2O[51] was used (94% yield and 94% ee) without loss of enantiomeric excess. Enantiomeric excess was determined by chiral GC (column Chirasil-DEX CB). The conversion of homoallylic alcohol **13** to acrylate **14** (acryloyl chloride, Et_3N, cat. DMAP in CH_2Cl_2 at −23 °C) was achieved in 80% yield. When compared to other catalytic asymmetric allylation protocols, the one developed by Maruoka and co-workers provided higher level of enantioselection in the addition of allyltributyltin to *trans*-cinnamaldehyde. Ring-closing metathesis[34] of **14** with 10 mol % Grubbs' catalyst in refluxing CH_2Cl_2 for 12 h (98%) furnished goniothalamin (**1**) in 73% of overall yield from cinnamaldehyde (**12**) (Scheme 3).

In this work, we have performed the total synthesis of (*R*)-goniothalamin (**1**), which was obtained by two different routes: catalytic allylation methodology developed by Keck and co-workers (seven steps, 94% ee and 6% overall yield), and the catalytic allylation methodology developed by Maruoka and co-workers (three steps, 96% ee and 73% overall yield). These approaches illustrate the utility of the enantioselective catalytic allylation to provide rapid access to synthetically useful α,β-unsaturated δ-lactone and nicely highlights the usefulness of the latter approach.

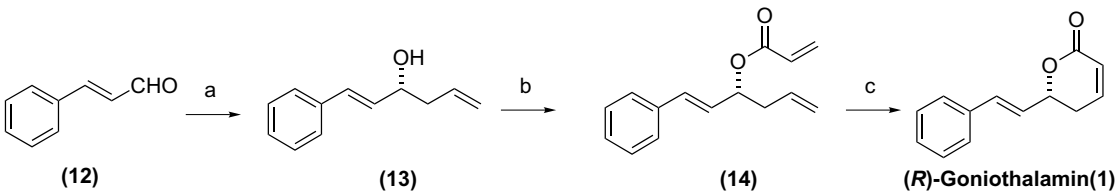
2.2. Biological activities

Antiproliferative activities of (*R*)-goniothalamin (**1**) and 5,6-dihydro-2*H*-pyran-2-ones **7**, **8**, and **10** against cancer cell lines were evaluated in the following human cancer cell lines: MCF-7 (breast), NCI-ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung, non-small cells), UACC62 (melanoma), 786-0 (kidney), OVCAR03 (ovarian), PCO 3 (prostate), and HT-29 (colon), which were grown in vitro (cell lines were kindly provided by Frederick Cancer Research & Development Center, National Cancer Institute, Frederick, MA, USA). Chemotherapic doxorubicin (DOX) was used as positive control.

All compounds evaluated displayed antiproliferative activity against the cancer cells line tested in a concentration-dependent way. The IC_{50} values (μM) for **1**, **7**, **8**, **10**, and doxorubicin (DOX) (a positive drug control) are summarized in Table 1. In fact, (*R*)-goniothalamin (**1**) was more potent for a large number of cancer cell lines tested [NCI-ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung, non-small cells), UACC62 (melanoma), 786–0 (kidney), and HT-29 (colon)] while compound **10** was more potent for prostate (PCO 3) and **7** for breast (MCF-7) and ovarian (OVCAR03) cancer cell lines than **1**.

In the case of cell lines NCI-ADR and 786-0, (*R*)-goniothalamin (**1**) presents higher potency than doxorubicin (DOX). It is noteworthy that the (*E*)-configuration of the double bound in (*R*)-goniothalamin (**1**) is essential for its potency. In general, compound **10** displayed lower potency, except for prostate cancer cell line (PCO 3).

Interestingly, **7** was more potent in inhibiting the cancer cell growth than **8** for all cell lines studied. Thus, it is



(12) **(13)** **(14)** **(*R*)-Goniothalamin(1)**

Scheme 3. Conditions: (a) (*R*,*R*)-**11** [(*R*)-BINOL (10 mol %), Ti(O*i*-Pr)$_4$ (15 mol %), $TiCl_4$ (5 mol %), Ag_2O (10 mol %)], allyltributyltin (1.1 equiv), CH_2Cl_2, −20 °C, 24 h (94%; 94% ee); (b) acryloyl chloride (1.8 equiv), Et_3N (3.6 equiv), CH_2Cl_2, 0 °C (80%); (c) Grubbs' catalyst [$(PCy_3)_2Cl_2Ru{=}CHPh$] (10 mol %), CH_2Cl_2 (98%).

Table 1. IC_{50} values for antiproliferative activities of (*R*)-goniothalamin (**1**), **7**, **8**, and **10** against cancer cell lines[a]

Compound	MCF-7	NCI ADR	NCI 460	UACC62	786-0	OVCAR03	PCO 3	HT 29
1	10.5	2.3	6.4	17.4	6.4	39.0	>100	11.2
7	8.2	13.3	21.0	36.6	20.6	19.2	61.7	50.1
8	51.9	38.2	34.3	>100	>100	67.5	>100	>100
10	35.5	31.5	35.2	40.0	38.0	28.0	26.0	29.0
DOX[b]	3.3	48.7	1.8	9.8	>100	11.7	18.6	5.3

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

likely that the efficiency of **7** is due to the presence of the benzyl group in this molecule. Overall, these results indicate that (*R*)-goniothalamin (**1**) brings together structural characteristics that allow it to exert a higher antiproliferative activity on tumor cells than its structure-like compounds **7**, **8**, and **10**.

3. Conclusion

The total syntheses of (*R*)-goniothalamin (**1**) were developed and its cytotoxic against cancer cell lines was evaluated. The use of the methodology developed by Maruoka and collaborators coupled with ring-closing metathesis reaction provided an efficient approach to (*R*)-goniothalamin (**1**). Our results demonstrate that the *E*-configuration of the styryl group present in (*R*)-goniothalamin (**1**) is essential for its antiproliferative activity. The versatility of our synthetic route allows its application to the synthesis of goniothalamin analogues to be screened against cancer cell lines in order to improve their cytotoxic profile.

4. Experimental section

4.1. Chemistry

4.1.1. General procedures. Reagents and solvents are commercial grade and were used as supplied, except dichloromethane and triethylamine which were distilled from calcium hydride. Chromatographic separations were performed using 70–230 mesh silica gel. Thin-layer chromatography was carried out on Macherey–Nagel precoated silica plates (0.25 mm layer thickness). IR spectra were obtained on Nicolet Impact 410 FT (film or KBr). ^{1}H NMR and ^{13}C NMR data were recorded on a Varian Gemini 2000 (7.0 T) or Varian Inova 500 (11.7 T) spectrometer. Chemical shifts are reported in δ [ppm relative to $(CH_3)_4Si$] for ^{1}H- and $CDCl_3$ for ^{13}C NMR. For ^{1}H NMR, the chemical shifts were followed by multiplicity (s, singlet; d, doublet; dd, double dublet; ddd, double double dublet; t, triplet; q, quartet; m, multiplet) and coupling constant *J* reported in Hertz (Hz). High-resolution mass spectra (HRMS) were measured on a VG Autospec-Micromass spectrometer. Chiral GC analyses were performed with capillary column CP-Chirasil-DEX CB fused silica WCOT (25 m × 0.25 mm × 0.25 μM) on Agilent 6890 series GC system. HPLC analyses were performed in Hewlett–Packard HP1050 equipment. Optical rotations were measured at 25 °C with Perkin–Elmer 241 instrument. For the preparation of (*R*)-1-(benzyloxy)-4-penten-2-ol (**4**), (*R*)-1-(benzyloxy)-4-penten-2-ol acryloyl ester (**6**), (*R*)-6-(benzyloxymethyl)-5,6-dihydro-2-pyranone (**7**), (*R*)-6-hydroxymethyl-5,6-dihydro-2-pyranone (**8**), and (6*R*,7*Z*)-6-styryl-5,6-dihydro-2-pyranone (**10**) (Scheme 2) see Ref. 8.

4.1.2. Preparation of (*R*)-1-(*p*-methoxybenzyloxy)-4-penten-2-ol (5). To a stirred solution of (*R*)-BINOL (75 mg, 0.26 mmol) in dichloromethane (2.6 mL) were added 4 Å molecular sieves (powdered and activated by storing in an oven at 120 °C for several days). To the resulting suspension was added 1 M titanium tetraisopropoxide in dichloromethane (0.34 mL, 0.26 mmol) at room temperature. The orange-reddish suspension was heated at reflux for 1 h when the color of the reaction mixture changed to red-brownish. Then it was cooled to rt and a soln. of *p*-methoxybenzyloxyacetaldehyde (**3**) (478 mg, 2.64 mmol) in dichloromethane (0.4 mL) was added. After 5 min of stirring at room temperature, the mixture was cooled to −78 °C and allyltri-*n*-butyltin (0.98 mL, 3.12 mmol) was added. The resulting reaction was at −20 °C for 60 h without stirring when it was quenched with satd. aq $NaHCO_3$ soln. (20 mL) and diluted with dichloromethane (20 mL). This mixture was stirred at room temperature for 2 h and then filtered through a pad of Celite. The organic layer was separated and aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic phases were dried over magnesium sulfate and evaporated under reduced pressure and the residual product purified by column chromatography [initially, hexane was used to elute recovered allyltributylstannane, then 4:2 hexane/acetone (v/v) was used as eluent to isolate the desired product] to give (*R*)-1-(*p*-methoxybenzyloxy)-4-penten-2-ol (**5**) (469 mg, 80%, and 80% ee). Enantiomeric excess (ee %) was determined by HPLC analysis on a chiral column (Welk-01) using hexane/isopropanol (95:5, v/v) with a flow rate of 1.0 mL/min (λ_{max} 274 nm). IR (film): 3442, 3074, 3001, 2933, 2908, 2860, 2837, 1612, 1514, 1464, 1302, 1248, 1093, 1036, 997, 916, 820 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (d, 1H, *J* = 8.4 Hz), 6.87 (d, 1H,*J* = 8.4 Hz), 5.87–5.73 (m, 1H), 5.12–5.05 (m, 2H), 4.48 (s, 2H), 3.86–3.78 (m, 4H), 3.46 (dd, 1H, *J* = 9.5; 3.3 Hz), 3.33 (dd, 1H, *J* = 9.5; 7.3 Hz), 2.48 (sl, 1H), 2.23 (t, 2H, *J* = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.0, 134.1, 129.9, 129.2, 117.4, 113.7, 73.5, 72.9, 69.6, 55.2, 37.9. HRMS (EI) *m/z* calculated

for $M^{+\cdot}$ = 222.12560; found: 222.12026. For (*R*)-**5** $[\alpha]_D^{25}$ +1.7 (*c* 4.0, $CHCl_3$).

4.1.3. Preparation of sulfone 9.[52] To a solution of 1-phenyl-1*H*-tetrazole-5-thiol (500 mg, 2.80 mmol) in dry THF (10 mL), Et_3N (0.48 mL, 3.4 mmol) was added, and the mixture was stirred at room temperature. After 40 min, benzyl bromide (0.40 mL, 3.4 mmol) was added and the reaction refluxed for 6 h, then diluted with water (20 mL), and extracted with Et_2O (3 × 20 mL). The combined organic layers were dried and evaporated at reduced pressure to give the crude thioether. MCPBA (77% w/w) (2.2 g, 9.8 mmol) was added in small portions to a solution of the crude thioether in CH_2Cl_2 (19 mL) at 0 °C, and the mixture was stirred at room temperature for 24 h. The reaction mixture was washed with $NaHSO_3$ (50 mL), and saturated $NaHCO_3$ solution (3 × 50 mL). The organic layer was dried, and the solvent removed by evaporation. The residue was submitted to flash-chromatography (hexane/AcOEt, 2:1) to afford compound **9** (753 mg, 89%) as a light yellow solid (mp: 102–104 °C) after trituration with hexanes. IR (film): 3064, 2968, 2914, 1593, 1495, 1458, 1348, 1155, 1128, 883, 766, 692 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.55–7.25 (m, 10H), 4.92 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.8, 132.7, 131.6, 131.3, 129.8, 129.3, 129.1, 125.2, 124.7, 62.3.

4.1.4. Preparation of (*R*)-goniothalamin (1) via Julia–Kociensky olefination. To a stirred solution of oxalyl chloride (150 μL, 1.77 mmol) in dichloromethane (1.5 mL) was added a solution of dimethyl sulfoxide (180 μL, 2.34 mmol) in dichloromethane (1.5 mL) at −65 °C under argon atmosphere. After 15 min, (*R*)-6-hydroxymethyl-5,6-dihydro-2-pyranone (**8**) (150 mg, 1.17 mmol) was added and stirring was continued for 30 min. Triethylamine (0.81 mL, 5.85 mmol) was added and the mixture was stirred for further 15 min at the same temperature. In another flask, sulfone **9** (527 mg, 1.75 mmol) was dissolved in 1.0 mL of THF and 1.6 mL of 0.75 M KHMDS soln. in THF at 0 °C was added. The solution was stirred at rt for 20 min and cooled to −78 °C when the residue obtained from the Swern oxidation of **8** in 1.0 mL of THF was added. The mixture was stirred at −78 °C for 3 and 8 h at rt. The reaction mixture was quenched with 10 mL of Et_2O and 10 mL of satd. aq $NaHCO_3$. The organic layer was separated and aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and the solvent was removed under reduced pressure. The residual product was purified by column chromatography (15% ethyl acetate in hexane, v/v, as eluent) to afford (*R*)-goniothalamin (**1**) as a white solid (0.042 g, 18% yield). Mp 81–82 °C, {lit.[53], mp 85 °C}; IR (film): 3055, 3024, 2924, 1720, 1246, 814, 698 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.41–7.25 (m, 5H), 6.92 (dt, 1H, *J* 9.5; 4.0 Hz), 6.72 (d, 1H, *J* 15.9 Hz), 6.27 (dd, 1H, *J* 15.9; 6.2 Hz), 6.08 (d, 1H, *J* 9.5 Hz), 5.10 (q, 1H, *J* 6.9 Hz), 2.56–2.52 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.6, 144.5, 135.5, 132.8, 128.5, 128.1, 126.5, 125.5, 121.4, 77.8, 29.8. HRMS (EI) *m*/*z* calculated for $M^{+\cdot}$ = 200.08373; found: 200.07891. $[\alpha]_D^{25}$ +169 (*c* 1.7, $CHCl_3$), {lit.[53], $[\alpha]_D^{25}$ +170.3 (*c* 1.38, $CHCl_3$)}.

4.1.5. Preparation of (*R*)-1-phenyl-5-hexen-3-ol (13). To a stirred solution of $TiCl_4$ (13 μL, 0.12 mmol) in CH_2Cl_2 (2.4 mL) was added $Ti(O\textit{i}\text{-}Pr)_4$ (110 μL, 0.36 mmol) at 0 °C under argon. The solution was allowed to warm to room temperature. After 1 h, recently prepared silver(I) oxide[51] (56 mg, 0.24 mmol) was added at rt, and the whole mixture was stirred 5 h under exclusion of direct light. The mixture was diluted with CH_2Cl_2 (4 mL), and treated with (*R*)-BINOL (138 mg, 0.48 mmol) at rt for 2 h. After cooling this mixture to −15 °C, it was treated sequentially with *trans*-cinnamaldehyde (**12**) (318 mg, 2.41 mmol) and allyltributyltin (871 mg, 2.65 mmol). The whole mixture was allowed to warm to 0 °C and stirred for 24 h. The reaction mixture was quenched with satd. $NaHCO_3$, and extracted with ether. The organic extracts were dried over $MgSO_4$. Evaporation of solvents and purification of this residue by column chromatography on silica gel (hexane/ethyl acetate, 9:1) furnished (*R*)-1-phenyl-5-hexen-3-ol (**13**) in 94% yield (394 mg). The enantiomeric purity of **13** was determined to be 94% ee by chiral GC analysis after their conversion to **14** (column: CP-Chiralsil-DEX CB fused silica WCOT, 25 m × 0.25 mm × 0.25 μm; conditions—initial temperature/time: 60 °C/1 min, rate: 0.5 °C/min, final temperature/time: 180 °C/70 min, H_2 as the carrier gas and FID detector) and comparing with racemic standard. IR (film): 3356, 3078, 2956, 2933, 2860, 2227, 1641, 1462, 1433, 1379, 1331, 1144, 1036, 995, 914 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.41–7.24 (m, 5H), 6.63 (d, 1H, *J* 16.0 Hz), 6.27 (dd, 1H, *J* 16.0; 6.2 Hz), 5.92–5.84 (m, 2H), 5.23–5.17 (m, 2H), 4.40–4.36 (m, 1H), 2.49–2.37 (m, 2H), 2.00 (sl, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.6, 133.9, 131.5, 130.3, 128.5, 127.6, 126.4, 118.4, 71.6, 41.9. HRMS (EI) *m*/*z* calculated for $M^{+\cdot}$ = 174.10447; found: 174.10496. $[\alpha]_D^{25}$ +22.2 (*c* 2.0, $CHCl_3$).

4.1.6. Preparation of (*R*)-1-phenyl-5-hexen-3-ol acrylolyl ester (14). To (*R*)-1-phenyl-5-hexen-3-ol (**13**) (340 mg, 1.95 mmol) dissolved in CH_2Cl_2 (2.0 mL) and cooled to 0 °C, were added acryloyl chloride (300 μL, 3.51 mmol) and Et_3N (990 μL, 7.04 mmol). The mixture was warmed to rt and stirred for 2 h. The resulting mixture was filtered through a short pad of Celite, poured into water, and the product was extracted with CH_2Cl_2. Solvent evaporation under reduced pressure and purification of this residue by column chromatography on silica gel (hexane/ethyl acetate, 9:1) furnished (*R*)-1-phenyl-5-hexen-3-ol acrylolyl ester (**14**) in 80% yield (356 mg). IR (film): 3083, 3060, 3026, 2979, 2939, 2848, 1722, 1637, 1495, 1404, 1265, 1188, 1043, 964, 918, 750, 692 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.41–7.25 (m, 5H), 6.66 (d, 1H, *J* 16.6 Hz), 6.46 (dd, 1H, *J* 16.6; 1.5 Hz), 6.19 (ddd, 2H, *J* 17.5; 11.9; 8.8 Hz), 5.86 (dd, 1H, *J* 10.4; 1.5 Hz), 5.85–5.78 (m, 1H), 5.61–5.56 (m, 1H), 5.19–5.11 (m, 2H), 2.61–2.51 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.3, 136.2, 132.9, 132.7, 130.7, 128.6, 128.5, 127.9, 126.8, 126.5, 118.1, 73.9, 39.0. HRMS (EI) *m*/*z* calculated for

$M^{+\cdot}$ = 228.11503; found: 228.11981. $[\alpha]_D^{25}$ −81.8 (*c* 1.2, $CHCl_3$).

4.1.7. (*R*)-Goniothalamin (1). To a stirred solution of Grubbs' catalyst (66 mg, 10 mol %) in dichloromethane (5 mL) at 55–60 °C was added (*R*)-1-phenyl-5-hexen-3-ol acrylolyl ester (**14**) (150 mg, 0.66 mmol) dissolved in dichloromethane (65 mL). The resulting mixture was heated for 12 h. After this period, the mixture was cooled at rt and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (30% ethyl acetate in hexane, v/v) to give (*R*)-goniothalamin (58 mg, 98%). The spectroscopic data were identical to those described above [see preparation of (*R*)-goniothalamin (**1**) featuring Julia–Kocienski olefination].

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, cell lines of different histological origin were used in the present study. Human tumor cell lines UACC62 (melanoma), MCF-7 (breast), NCI 460 (lung, non-small cells), OVCAR03 (ovarian), PCO 3 (prostate), HT-29 (colon), 786-0 (renal) and NCI-ADR (breast expressing phenotype multiple drugs resistance) were kindly provided by National Cancer Institute (NCI). Stock cultures were grown in a medium containing 5 mL of RPMI 1640 (GIBCO BRL, Life Technologies) and supplemented with 5% of fetal bovine serum. Gentamicine (50μg/mL) was added to the experimental cultures. Cells in 96-well plates (100 μL cells/well) were exposed to various concentrations of samples in DMSO (0.25, 2.5, 25, and 250 μg/mL) at 37 °C, 5% of CO_2 in air for 48 h. The final concentration of DMSO did not affect the cell viability. Then, a 50% solution of trichloroacetic acid was added and after incubation for 30 min at 4 °C, washing and drying, the cell proliferation was determined by spectrophotometric quantification (540 nm) of the cellular protein content using sulforhodamine B assay described by Skehan and co-workers.[54]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.02.007.

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