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Cytotoxic activity of (*S*)-goniothalamin and analogues against human cancer cells

Ângelo de Fátima,[a] Luciana K. Kohn,[b] João Ernesto de Carvalho[b] and Ronaldo A. Pilli[a,*]

[a]*Instituto de Química, Unicamp, CP 6154, 13084-971, Campinas, São Paulo, Brazil*
[b]*Centro Pluridisciplinar de Pesquisas Química, Biológicas e Agrícolas, Unicamp, CP 6171, 13083-970, Paulínia, São Paulo, Brazil*

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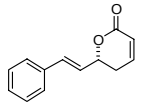
Abstract—(*R*)- and (*S*)-Goniothalamin (**1**) and analogues **2**–**9** were efficiently prepared in high overall yield and enantiomeric purity, and their cytotoxic activities were evaluated against eight human cancer cell lines. A structure–activity relationship study (SAR) allowed us to establish the relevant structural features for the cytotoxic activity of goniothalamin analogues. In addition, we have identified non-natural form of goniothalamin (*S*)-**1** and analogue **5** as the highest and more selective cytotoxic compounds against kidney cancer cell growth (786-0) with IC_{50} = 4 and 5 nM, respectively, and compound **8** (IC_{50} = 4 nM) as the more potent against breast cancer cells with resistance phenotype for adryamycin (NCI.ADR).

1. Introduction

Small exogenous molecules can exert powerful effects on cell functions making them useful for understanding life processes and treating life-threatening diseases. In the center of such scientific endeavor is the molecule which needs to be made. A lead structure may be inspired either from nature (target-oriented approach) or from diversity-oriented synthesis. In both cases, an additional dimension is provided by the chirality of the compounds which may significantly influence the biological activity of such chemical entities.

The styryl-lactones are a group of secondary metabolites commonly isolated from the genus Goniothalamus,[1] and recent studies have demonstrated that these compounds display cytotoxic and antitumor properties.[2–7] Among them, (*R*)-goniothalamin (**1**) (Fig. 1) has shown in vitro cytotoxic effects especially by inducing apoptosis on different cancer cell lines [cervical carcinoma (Hela), gastric carcinoma (HGC-27), breast carcinoma (MCF-7, T47D, and MDA-MB-231), leukemia carcinoma (HL-60), and ovarian carcinoma (Caov-3)].[2,8–12] This cytotoxic activity was observed on cancer cell lines but was shown to be minimal on non-malignant cells. In vivo studies have shown that (*R*)-**1** displays tumoricidal and tumoristatic effects on Sprague–Dawley rats with 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumors.[13] We have previously reported on the cytotoxic activity of (*R*)-goniothalamin (**1**) against eight different human cancer cell lines.[14] Previous studies about the mechanism of action of (*R*)-**1** showed that this compound induces apoptosis in Jurkat T-cells and MCF-7 cells by activation of caspases-3 and -7.[8,11,12] Subsequent studies with HL-60 cells demonstrated that (*R*)-**1**-induced apoptosis occurs via the loss of mitochondrial membrane potential ($\Delta\psi m$) and activation of initiator caspase-9.[9] Chien[11] and Azimahtol[12] showed that (*R*)-**1** was able to modulate the *Bax* expression increasing pro-apoptotic protein (*Bax*) levels without affecting the anti-apoptotic *Bcl-2* expression. It was also demonstrated in vivo that p53 tumor suppressor protein accumulation was more pronounced in rat tumor treated with (*R*)-**1**.[13] In spite of biological activities exhibited by (*R*)-**1**, no studies on the cytotoxic activity of its enantiomer, (*S*)-goniothalamin [(*S*)-**1**] or derivatives have been reported yet.



(*R*)-Goniothalamin [(*R*)-**1**]

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

Keywords: (*R*)- and (*S*)-Goniothalamin; Styryl lactones; Cytotoxic activity; Cancer cell lines.
* Corresponding author. Tel.: +55 19 37883083; fax: +55 19 3788311; e-mail: pilli@iqm.unicamp.br

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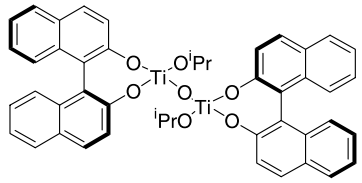


(*S*,*S*)-**A**

Figure 2. The μ-oxo bis (binaphthoxy)(isopropoxy)titanium complexes (*S*,*S*)-**A** developed by Maruoka and co-workers.[25,26]

Several asymmetric syntheses of (*R*)-**1** have been published,[15–21] including the non-natural enantiomer, (*S*)-**1**.[22–24] Recently, we have reported a highly enantioselective synthesis of (*R*)-**1** in three steps, 73% overall yield and 96% enantiomeric excess from *trans*-cinnamaldehyde (**2**).[14,17] This methodology was employed to provide (*S*)-**1** and analogues (*S*)-**2** to (*S*)-**9** to evaluate their cytotoxic activity on various cancer cell lines and to carry out a structure–activity relationship (SAR) study aiming to identify the pharmacophoric groups. Bis(binaphthoxy)(isopropoxy)titanium oxide catalysts (*R*,*R*)- or (*S*,*S*)-**A** (Fig. 2), described by Maruoka and co-workers[25,26] for the enantioselective allylation of cinnamaldehyde, were employed for the total syntheses of (*R*)- and (*S*)-**1**, respectively.[16,24]

2. Results and discussion

Since it is well known that different cell lines display different sensitivities toward a cytotoxic compound, in the present study we have used cell lines of various histological origin {MCF-7 (breast), NCI.ADR (breast expressing the resistance phenotype for adryamycin), NCI.460 (lung, non-small cells), UACC.62 (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 MA, National Cancer Institute/USA]} for the initial evaluation of the cytotoxicity of (*R*)- and (*S*)-goniothalamin [(*R*)- and (*S*)-**1**]. Cell proliferation was determined by spectrophotometric assay using sulforhodamine B as protein-binding dye.[27–29] (*R*)- and (*S*)-**1** were used at 0.25–250 μg/mL and doxorubicin (DOX, at the same concentration range), as positive control (see Section 4 for details). Concentration that elicits 50% inhibition of cell growth (IC_{50}) was determined after 48 h of cell treatment.

Both enantiomers of goniothalamin (**1**) displayed antiproliferative activity in a concentration-dependent way against the cancer cell lines tested (Fig. 3). IC_{50} (μM) for (*R*)- and (*S*)-**1** as well as DOX are summarized in Table 1.

It is noteworthy that goniothalamin (**1**) enantiomers displayed higher potency against 786-0 cell line (kidney tumor) and NCI.ADR (breast expressing the resistance phenotype for adryamycin) than DOX. While for breast resistant cancer cell line (NCI.ADR), (*R*)-**1** showed to be 10 and 20 times more powerful than (*S*)-**1** and DOX, respectively, for kidney cells (786-0), we found (*S*)-**1** (IC_{50} = 4 nM) to be 1, 600-fold more potent than (*R*)-**1**

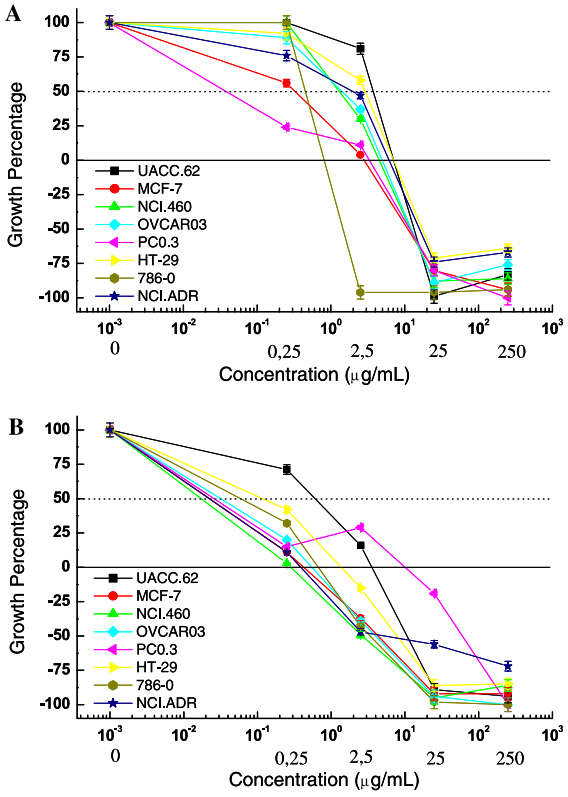


Figure 3. Percentage growth of cancer cells for 48 h with different concentrations (0.25, 2.5, 25, and 250 μg/mL) of (*R*)- or (*S*)-goniothalamin [(*R*)- or (*S*)-**1**]. A, (*S*)-goniothalamin [(*S*)-**1**]; B, (*R*)-goniothalamin [(*R*)-**1**]. Positive values in relation to y axis correspond to cytostatic activity, while the others refer to cytotoxic activity of compounds analyzed. The experiments were done in triplicate as described in Section 4 using a panel of NCI human tumoral cells.

(IC_{50} = 6.4 μM). Additionally, the IC_{50} values for (*S*)-**1** against ovarian (OVCAR03) and prostate (PCO.3) were lower than the ones found for natural goniothalamin [(*R*)-**1**]. Additionally, both enantiomers presented similar IC_{50} values for breast cancer cell line (MCF-7) with natural goniothalamin [(*R*)-**1**] being two times more potent than (*S*)-**1** against lung (NCI.460) and colon (HT-29) cancer cell lines and slightly more active for melanoma (UACC.62).

The extremely high antiproliferative activity (Table 1) and selectivity (Fig. 3A) presented by (*S*)-**1** for 786-0 cell line (IC_{50} 4 nM) prompted us to synthesize analogues **2**–**9** (Fig. 4) to identify the pharmacophoric groups responsible for this activity.

The synthesis of **2** was carried out from (*S*)-**1** by selective reduction of the conjugated double bond of the pyranone ring using Stryker's reagent $[(Ph_3P)CuH]_6$[30–33] in benzene (63% yield, Scheme 1), while catalytic hydrogenation (10% Pd/C, 1 atm of H_2) provided **3** in 79% yield (Scheme 1).

Maruoka's catalyst [(*S*,*S*)-**A**] also performed well in the asymmetric allylation of substituted aromatic and aliphatic aldehydes **11**–**16** thus extending the scope of this methodology (Table 2). As observed in Table 2,

Table 1. IC_{50} values, given in μM, for (*R*)- and (*S*)-goniothalamin [(*R*)- and (*S*)-**1**] and doxorubicin (DOX) necessary for inhibiting tumor cell proliferation[a]

Compound	MCF-7	NCI.ADR	NCI.460	UACC.62	786-0	OVCAR03	PCO.3	HT-29
(*R*)-(**1**)	10.5	2.3	6.4	17.4	6.4	39.0	>100	11.2
(*S*)-(**1**)	9.4	23.5	14.6	27.6	0.004	17.0	24.3	22.5
DOX[b]	3.3	48.7	1.8	9.8	>100	11.7	18.6	5.3

[a] Concentration that elicits inhibition by 50% of cell growth (IC_{50}) was determined from non-linear regression analysis using the GraphPad Prism software ($r^2 > 0.9$).
[b] Doxorubicin (DOX) was the positive control.

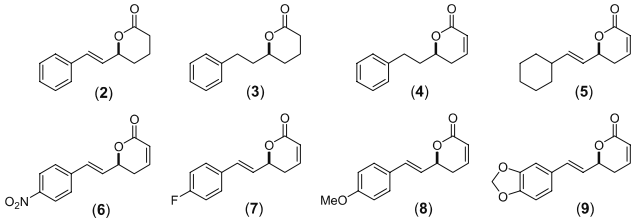
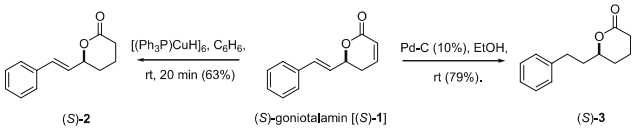


Figure 4. Structures of analogues **2**–**9** of (*S*)-goniothalamin [(*S*)-**1**].

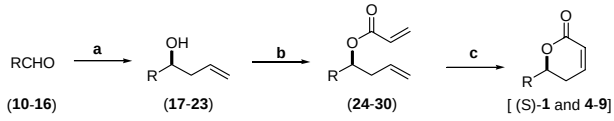


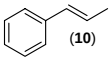
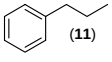
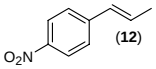
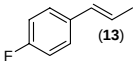
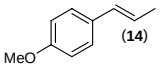
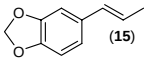
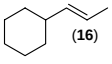
Scheme 1. Syntheses of analogues **2** and **3** from (*S*)-goniothalamin [(*S*)-**1**].

excellent enantiomeric excesses were observed in all cases. While the presence of electron-withdrawing groups in the aromatic ring did not affect either the yield or the enantiomeric excess in the allylation reaction (entries 3 and 4, Table 2), electron-donating groups as in aldehydes **14** and **15** slowed down the reaction rate probably due to additional complexation sites for the catalyst and only provided good yields (61–79%) when 20 mol% of (*S*,*S*)-**A** was employed. However, similar enantioselectivity in the allylation reaction was observed when 10 or 20 mol% (entries 5 and 6, Table 2) of (*S*,*S*)-**A** was used. Aldehyde **16** furnished the corresponding homoallylic alcohol in excellent enantiomeric excess and good yield (entry 7, Table 2).

The next step involved acylation of homoallylic alcohols **17**–**23** with acryloyl chloride and ring closing metathesis reaction catalyzed by Grubbs' ruthenium complex

Table 2. Total syntheses of (*S*)-goniothalamin [(*S*)-**1**] and analogues **4**–**9**



Entry	R	Product (yield, enantiomeric excess)		
		Step a	Step b	Step c
1	 (**10**)	**17** (94%, 96% ee)[b]	**24** (80%)	(*S*)-**1** (98%)
2	 (**11**)	**18** (98%, 99% ee)[c]	**25** (86%)	**4** (99%)
3	 (**12**)	**19** (94%, 95% ee)[c]	**26** (89%)	**6** (77%)
4	 (**13**)	**20** (82%, 96% ee)[c]	**27** (85%)	**7** (88%)
5	 (**14**)	**21** (61%[a], 94% ee)[c]	**28** (84%)	**8** (78%)
6	 (**15**)	**22** (79%[a], 92% ee)[c]	**29** (79%)	**9** (80%)
7	 (**16**)	**23** (64%, 99% ee)[b]	**30** (85%)	**5** (94%)

Reagents and conditions: (a) (*S*,*S*)-**A** [(*S*)-BINOL (10 mol%), $Ti(O^iPr)_4$ (15 mol%), $TiCl_4$ (5 mol%), Ag_2O (10 mol%)], allyltributyltin (1.1 equiv), CH_2Cl_2, −20 °C, 24 h; (b) acryloyl chloride (1.8 equiv), Et_3N (3.6 equiv), CH_2Cl_2, 0 °C; (c) Grubbs' catalyst [$(PCy_3)_2Cl_2Ru{=}CHPh$] (10 mol%), CH_2Cl_2.
[a] 20 mol% of (*S*,*S*)-**A** was used.
[b] The enantiomeric excess was determined by chiral GC analysis (CP-Chirasil-Dex-CB) or [c]^{1}H NMR or ^{19}F NMR analysis of the correspondent Mosher esters derived from alcohols **17**–**23** (see Supplementary Material for details).

Table 3. IC_{50} values, given in μM, for analogues **2**–**9** and doxorubicin (DOX) necessary for inhibiting tumor cell proliferation[a]

Compound	MCF-7	NCI.ADR	NCI.460	UACC.62	786-0	OVCAR03	PCO.3	HT-29
2	12.5	0.1	11.0	5.5	19.0	3.5	>100	8.5
3	>100	>100	>100	>100	>100	>100	>100	1.9
4	20.0	6.0	18.3	23.7	20.8	16.3	20.8	28.7
5	4.8	19.4	16.5	2.4	0.005	1.5	15.5	>100
6	16.5	11.2	27.3	13.0	2.2	9.4	26.5	9.4
7	14.4	18.7	53.2	23.8	9.6	26.1	24.3	34.8
8	6.5	0.004	16.9	5.9	7.4	20.8	14.8	28.2
9	40.1	23.7	38.1	26.8	31.9	24.6	21.7	23.7
DOX[b]	3.3	48.7	1.8	9.8	>100	11.7	18.6	5.3

[a] Concentration that elicits inhibition of 50% of cell growth (IC_{50}) was determined from non-linear regression analysis using the GraphPad Prism software ($r^2 > 0.9$).
[b] DOX was employed as positive control.

$PhCH{=}RuCl_2(PCy_3)_2$[34] (Table 2). This approach furnished analogues **4**–**9** in 3 steps, good overall yields (40–83%) and enantiomeric excesses (92–99% ee): (*R*)- or (*S*)-**1** (73%, 96% ee), **4** (83%, 99% ee), **5** (51%, 99% ee), **6** (64%, 95% ee), **7** (61%, 96% ee), **8** (40%, 94% ee), and **9** (50%, 92% ee). At this point, our efforts were directed to evaluate the antiproliferative activity of **2**–**9** against the cancer cell panel described above (see Section 4 for details). IC_{50} values (μM) for analogues **2**–**9** and DOX are summarized in Table 3.

(*S*)-**1** was 4, 750- and 5200-fold more potent in inhibiting proliferation of kidney cancer cells (786-0) than analogues **2** and **4**, respectively (Tables 1 and 3). Remarkably, (*S*)-**5** with a cyclohexyl substituent conserved the same cytotoxic activity as (*S*)-goniothalamin (**1**) against kidney cancer cells (786-0) being active at the nanomolar level (IC_{50} = 5 nM). No antiproliferative activity was found for analogue **3** under our experimental conditions. These results demonstrated that the *endo* and *exo* double bonds in the pyranone ring are essential for the activity of (*S*)-**1** against kidney cancer cell line (786-0). It is noteworthy that analogues (*S*)-**2**, (*S*)-**3**, and (*S*)-**4** lacking either one or both double bonds had much lower activity than (*S*)-**1**. Recently, the importance of the *E*-configuration of the styryl moiet in (*R*)-**1** for its antiproliferative activity was shown[14] and the α,β-unsaturated lactone group has already been implicated in the biological activity of other natural lactones such as cytostatin[35] and fostriecin.[36] This behavior is most probably due to its possible role as a Michael acceptor for nucleophilic amino acid residues (cysteine, lysine, serine or threonine) present in the natural receptors which interact with these compounds.

Interestingly, (*R*)-**2** and (*R*)-**3** were shown to be the main metabolites detected and identified in urine and blood samples of (*R*)-**1**-treated Sprague–Dawley rats thus signaling the double bond reduction in (*R*)-**1** as a potential detoxification route by this species.[37] Moreover, the results obtained from the treatment of kidney cancer cells with pyranones (*S*)-**5** to (*S*)-**9** demonstrated that electron-donating or electron-withdrawing groups in the aromatic ring decreased their potency when compared to (*S*)-**1**. Overall, the findings for kidney cancer cell line (786-0) suggest that aromatic ring or cyclohexyl in (*S*)-**1** and (*S*)-**5**, respectively, likely interacts with a hydrophobic domain of a biomolecule present in the cancer cell since these compounds were shown to be the most active in inhibiting cell proliferation at nanomolar concentrations. From all these results, we have identified the pharmacophoric groups of goniothalamin (**1**) as presented in Figure 5.

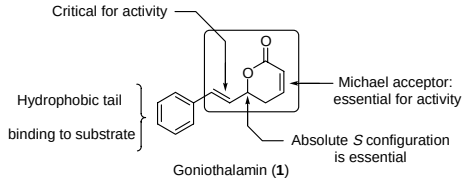


Figure 5. Pharmacophoric groups of goniothalamin (**1**) identified by their cytotoxic activity against kidney cancer cell line (786-0).

Even though the most expressive activity of (*S*)-goniothalamin [(*S*)-**1**] was against kidney cancer cells, very promising results were also obtained for other cancer cell cultures. All analogues evaluated in this study were more potent than DOX against breast expressing the resistance phenotype for adryamycin (NCI.ADR), particularly compound (*S*)-**8** which displayed IC_{50} = 4 nM. For melanoma cell line (UACC.62), (*R*)-**1** was more potent than (*S*)-**1**, although analogues (*S*)-**2**, (*S*)-**5**, and (*S*)-**8** were 3-, 7- and 3-fold more potent, respectively, than (*R*)-**1** (Tables 1 and 3). For breast cancer cells (MCF-7), the substitution of the aromatic group for a cyclohexyl group (compound **5**) or the addition of a 4-OMe group in the aromatic ring [compound (*S*)-**8**] enhanced the potency originally displayed by (*S*)-**1**. Similarly, compounds (*S*)-**5** and (*S*)-**8** were more potent than (*S*)-1 and significantly more than (*R*)-**1** in inhibiting prostate cancer cell (PCO.3) proliferation (Tables 1 and 3). In the case of ovarian cancer cells (OVCAR03), the analogues **2** and **5** were 5- and 7-fold more potent than (*S*)-**1**, respectively, while colon cancer cells (HT-29) were more sensitive to analogue (*S*)-**3** [IC_{50} = 190 nM]. As to the cytotoxic activity against lung cancer cell line (NCI.460), none of the compounds evaluated displayed better activity than (*R*)-**1** (Tables 1 and 3). In our studies, doxorubicin (DOX, an anticancer drug used as a positive control with IC_{50} = 9.8 μM) presented lower activity for melanoma cancer cells (UACC.62) than analogues (*S*)-**2** [IC_{50} = 5.5 μM], (*S*)-**5** [IC_{50} = 2.4 μM], and

(*S*)-8 [IC_{50} = 5.9 μM]. On ovarian cancer cells (OVCAR03), the analogues (*S*)-**2** [IC_{50} = 3.5 μM], (*S*)-**5** [IC_{50} = 1.5 μM], and (*S*)-**6** [IC_{50} = 9.4 μM] were more potent than DOX [IC_{50} = 11.7 μM], while roughly the same values were observed for compounds (*S*)-**5** and (*S*)-**8** for prostate cancer cells (Table 3).

Analogues (*S*)-**2** and (*S*)-**3** presented very low antiproliferative activity, independently of the cancer cell line when compared with (*R*)- or (*S*)-**1**. Since the difference in the kidney cancer cell (786-0) viability was greatest for (*R*)- or (*S*)-**1** and analogue (*S*)-**2**, we have taken phase-contrast microscopic pictures from 786-0 cells treated with (*R*)- or (*S*)-**1** or analogue (*S*)-**2** for 48 h (Fig. 6).

As shown in Figure 6A, untreated kidney cancer cells (786-0) exhibited typical growth patterns. After 48 h, significant morphological modifications were observed for 786-0 cancer cells treated with (*R*)- or (*S*)-**1** at 2.5 μg/mL (Figs. 6B and C). Under these conditions, (*R*)-**1** caused decrease of cancer cell growth by 43%, while (*S*)-**1** triggered 100% of death (Fig. 6B, arrows 'a'). At 2.5 μg/mL of (*R*)-**1**, cancer cells came about to present irregular shape with lamellipodia elongation and many detached cells (Fig. 6C, arrows 'b'). The apparent lamellipodia elongation of the (*S*)-**1**-treated cells may have led to the disruption of cell adhesion and consequent cell detachment from the substratum as from their neighbors. Moreover, at 250 μg/mL, the analogue (*S*)-**2** only inhibited the growth of kidney cancer cells (786-0) by 9% (Fig. 6D) and similar morphological features are observed between untreated and treated cells (Figs. 6A and D). Overall, our results show that α,β-unsaturated lactone group is essential for antiproliferative activity observed for (*S*)-**1**.

3. Conclusion

In conclusion, we have applied our previously described approach to (*R*)-goniothalamin (**1**) to the preparation of (*S*)-goniothalamin (**1**) and analogues (*S*)-**2** to (*S*)-**9** in high overall yields and enantiomeric purities. Our studies demonstrated that Maruoka's catalyst (*S*,*S*)-**A** was efficient for the stereoselective addition of allyltributyltin not only to aromatic aldehydes **11**–**15** related to *trans*-cinnamaldehyde but also to aliphatic aldehyde **16**. In addition, we have identified (*S*)-**1** and analogue (*S*)-**5** as the highest and more selective cytotoxic compounds against kidney cancer cell growth (786-0) with IC50 = 4 and 5 nM, respectively, and compound (*S*)-**8** (IC50 = 4 nM) as the more potent against breast cancer cells with resistance phenotype for adryamycin (NCI.ADR). A structure–activity relationship study (SAR) allowed us to establish the relevant structural features for cytotoxic activity of goniothalamin analogues. Studies are underway to prepare and evaluate

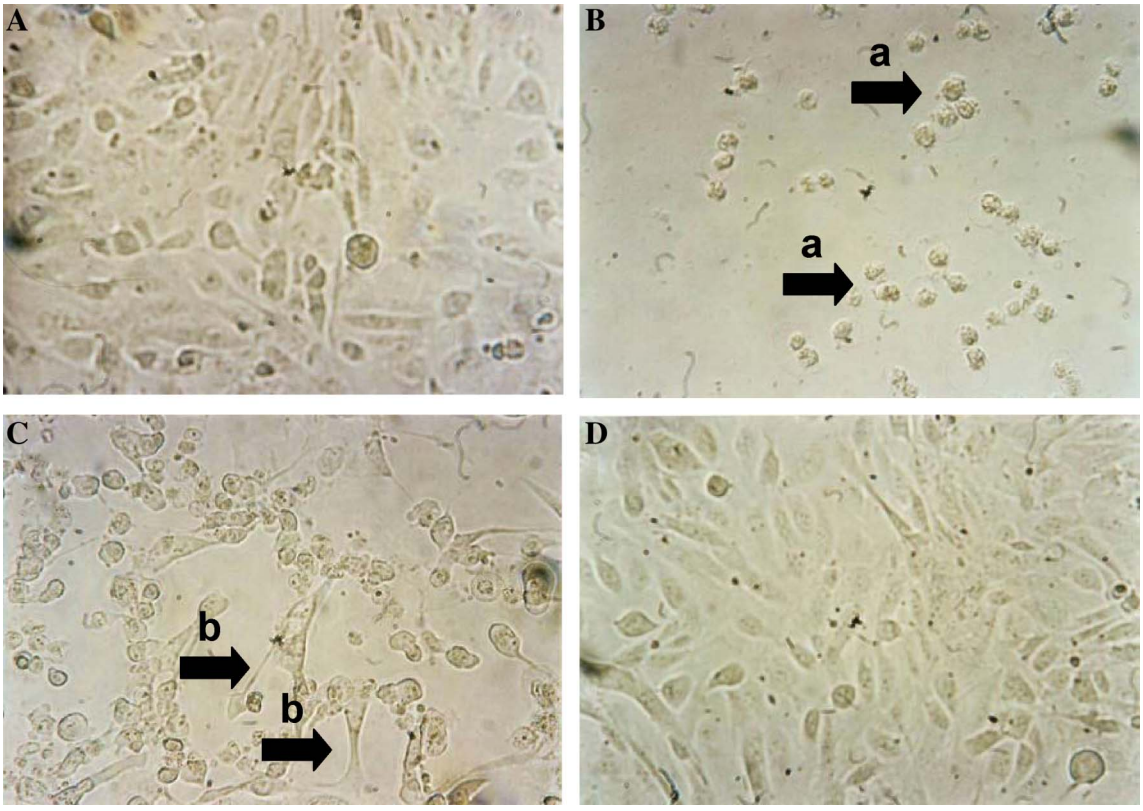


Figure 6. Morphological observation of kidney cancer cell line (786-0) after administration of (*R*)-, (*S*)-goniothalamin [(*R*)- or (*S*)-**1**] or analogue **2**. A, Confluent untreated 786-0 cells at 48 h showed normal morphology. Typical phase-contrast microscopic images of 786-0 cells following treatment with: B, (*S*)-goniothalamin [(*S*)-**1**] (2.5 μg/mL; a, cells died); C, (*R*)-goniothalamin [(*R*)-**1**] (2.5 μg/mL, b, cells became irregularly shaped with elongated lamellipodia), and D, analogue **2** (250 μg/mL). Magnification = original × 200.

the cytotoxic activity of other goniothalamin derivatives as well as to probe its mode of action.

4. Experimental

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. Chromatography separations were performed using 70–230 mesh silica gel. Thin-layer chromatography was carried out on Merck silica plates (0.25 mm layer thickness). IR spectra were obtained on Nicolet Impact 410 FT (film) and ThermoNicolet IR200 spectrometer (neat tablet). ^{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 NMR and $CDCl_3$ for ^{13}C NMR. For ^{1}H NMR, the chemical shifts were followed by multiplicity (s, single; sl, large single; d, doublet; dd, double doublet; ddd, double double doublet; t, triplet; dt, double triplet; q, quartet; dq, double quartet; m, multiplet) and coupling constant J reported in Hertz (Hz). High-solution mass spectra (HRMS) were measured on a VG Autospec-Micromass spectrometer. Chiral GC analyses were performed with a capillary column CP-Chirasil-DEX CB fused silica WCOT (25 m × 0.25 mm × 0.25 μm) on Agilent 6890 series GC system. Optical rotations were measured at 25 °C with Perkin–Elmer 241 or Perkin–Elmer 341 instruments.

4.1.2. General procedure for catalytic asymmetric allylation. To a stirred solution of $TiCl_4$ (0.12 mmol) in CH_2Cl_2 (1.0 mL for each 1.0 mmol of the aldehyde) was added Ti$(O^iPr)_4$ (0.36 mmol) at 0 °C under argon. The solution was allowed to warm to room temperature. After 1 h, recently prepared Ag_2O (0.24 mmol) was added at room temperature, and the whole mixture was stirred for 5 h under exclusion of direct light. The mixture was diluted with CH_2Cl_2 (2.0 mL for each 1.0 mmol of the aldehyde) and treated with (*S*)-BINOL (0.48 mmol) at room temperature for 2 h. After cooling this mixture to −15 °C, it was treated sequentially with aldehyde (2.4 mmol) and allyltributyltin (2.7 mmol). After 24 h, the whole mixture was quenched with satd aqueous $NaHCO_3$ 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 furnished the corresponding homoallylic alcohol.

4.1.3. Homoallylic alcohol 17. Viscous colorless liquid. IR (film): 3381, 3078, 3026, 2978, 2927, 2870, 1641, 1493, 1446, 1030, 966, 916, 692 cm^{-1}. ^{1}H 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 and 6.2 Hz), 5.92–5.84 (m, 1H), 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, 134.0, 131.5, 130.3 (2C), 128.5, 127.6, 126.4 (2C), 118.4, 71.6, 41.9. $[\alpha]_D^{25}$ = +22.2 (*c* 2.0, $CHCl_3$) for *R* isomer and $[\alpha]_D^{25}$ = −22.4 (*c* 2.0, $CHCl_3$) for *S* isomer. HRMS (EI) *m*/*z* calculated for $M^{+\cdot}$ = 174.10447; found: 174.10496. The enantiomeric purity of **17** was determined to be 96% by chiral GC analysis (CP-Chirasil-Dex-CB) and comparing with racemic standard (see page S5 of Supporting Information for details).

4.1.4. Homoallylic alcohol 18. White solid. Mp: 37.8–38.6 °C IR (neat tablet): 3559, 3360, 3063, 3026, 2930, 2860, 1648, 1595, 1495, 1453, 1048, 992, 915, 701 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30–7.15 (m, 5H), 5.88–5.74 (m, 1H), 5.16–5.11 (m, 1H), 3.72–3.62 (m, 1H), 2.85–2.63 (m, 2H), 2.36–2.13 (m, 2H), 1.85–1.61 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.8, 134.4, 128.3 (2C), 128.2 (2C), 125.7, 118.1, 69.9, 42.1, 38.5, 32.1. $[\alpha]_D^{25}$ = +23.5 (*c* 2.2, $CHCl_3$). HRMS (EI) *m*/*z* calculated for $M^{+\cdot}$ = 176.12012; found: 176.11543. The enantiomeric purity of **18** was determined to be 99% by chiral GC analysis (CP-Chirasil-Dex-CB) and comparing with racemic standard (see page S6 of Supporting Information for details).

4.1.5. Homoallylic alcohol 19. Viscous yellow liquid. IR (film): 3320, 3206, 3073, 2976, 2933, 2906, 1641, 1594, 1513, 1338, 1109, 1086, 973, 916, 871, 746 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (d, 2H, J = 8.9 Hz), 7.52 (d, 2H, J = 8.9 Hz), 6.72 (d, 1H, J = 16.1 Hz), 6.44 (dd, 1H, J = 16.1 and 5.5 Hz), 5.93–5.79 (m, 1H), 5.25–5.20 (m, 2H), 4.45–4.41 (m, 1H), 2.54–2.34 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.9, 143.2, 136.4, 133.4 (2C), 127.9, 126.9, 123.9 (2C), 119.1, 70.9, 41.8. $[\alpha]_D^{25}$ = −45.0 (*c* 1.0, $CHCl_3$). HRMS (EI) *m*/*z* calculated for $M^{+\cdot}$ = 219.08954; found: 219.08669. The enantiomeric purity of **19** was determined to be 95% by ^{1}H NMR analysis after their conversion to Mosher ester and comparing with racemic standard (see page S7 of Supporting Information for details).

4.1.6. Homoallylic alcohol 20. Viscous colorless liquid. IR (film): 3363, 3076, 2978, 2927, 2860, 1641, 1601, 1508, 1230, 1157, 968, 918, 850, 816 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35–7.28 (m, 2H), 7.01–6.95 (m, 2H), 6.55 (d, 1H, J = 15.9 Hz), 6.15 (dd, 1H, J = 15.9 and 6.4 Hz), 5.91–5.77 (m, 1H), 5.20–5.14 (m, 2H), 4.36–4.29 (m, 1H), 2.47–2.31 (m, 2H), 2.05 (sl, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.2 (d, J = 246.6 Hz), 133.8, 132.7, 131.1, 129.1, 127.8 (d, 2C, J = 7.9 Hz), 118.5, 115.4 (d, 2C, J = 21.9 Hz), 71.6, 42.1. $[\alpha]_D^{25}$ = −29.5 (*c* 1.2, $CHCl_3$). HRMS (EI) *m*/*z* calculated for $M^{+\cdot}$ = 192.09504; found: 192.09303. The enantiomeric purity of **20** was determined to be 96% by ^{1}H NMR analysis after their conversion to Mosher ester and comparing with racemic standard (see page S8 of Supporting Information for details).

4.1.7. Homoallylic alcohol 21. Viscous colorless liquid. IR (film): 3400, 3074, 2929, 2910, 2835, 1639, 1606, 1510, 1300, 1248, 1174, 1034, 968, 814 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.25 (m, 2H), 6.87–6.82 (m, 2H), 6.53 (d, 1H, J = 15.9 Hz), 6.10 (dd, 1H, J = 15.9 and 6.6 Hz), 5.92–5.78 (m, 1H), 5.20–5.15 (m, 2H), 4.28–4.38 (m, 1H), 3.80 (s, 3H), 2.48–2.31 (m, 2H), 1.78 (d, 1H, J = 3.7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.3, 134.1, 130.0, 129.4 (2C), 129.3, 127.6, 118.4,

114.0 (2C), 71.9, 55.3, 42.1. $[\alpha]_D^{25} = -29.8$ (*c* 1.6, $CHCl_3$). HRMS (EI) *m/z* calculated for $M^{+\cdot} = 204.11503$; found: 204.11298. The enantiomeric purity of **21** was determined to be 94% by ^{19}F NMR analysis after their conversion to Mosher ester and comparing with racemic standard (see page S9 of Supporting Information for details).

4.1.8. Homoallylic alcohol 22. Viscous colorless liquid. IR (film): 3556, 3384, 3074, 2978, 2777, 1847, 1639, 1606, 1504, 1489, 1444, 1245, 1190, 1039, 928, 800 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.12–6.93 (m, 3H), 6.71 (d, 1H, $J = 15.7$ Hz), 6.25 (dd, 1H, $J = 15.7$ and 6.2 Hz), 6.15 (s, 2H), 6.12–5.98 (m, 1H), 5.41–5.34 (m, 2H), 4.52 (q, 1H, $J = 6.2$ Hz), 2.66–2.56 (m, 2H), 2.05 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.9, 147.1, 134.0, 131.0, 130.0, 129.7, 121.1, 118.2, 108.2, 105.7, 101.0, 71.7, 42.0. $[\alpha]_D^{25} = -33.1$ (*c* 1.11, $CHCl_3$). HRMS (EI) *m/z* calculated for $M^{+\cdot} = 218.09427$; found: 218.09430. The enantiomeric purity of **22** was determined to be 92% by ^{19}F NMR analysis after their conversion to Mosher ester and comparing with racemic standard (see page S10 of Supporting Information for details).

4.1.9. Homoallylic alcohol 23. Viscous colorless liquid. IR (film): 3348, 3075, 2923, 2850, 1641, 1447, 1029, 967, 911 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 5.87–5.73 (m, 1H), 5.62 (dd, 1H, $J = 15.4$ and 6.6 Hz), 5.43 (ddd, 1H, $J = 15.4$, 6.6, and 1.1 Hz); 5.17–5.10 (m, 2H), 4.14–4.08 (m, 1H), 2.32–2.22 (m, 2H), 1.97–1.91 (m, 2H), 1.73–1.63 (m, 5H), 1.34–0.87 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.9, 134.4, 129.5, 117.9, 71.9, 42.0, 40.2, 38.8 (2C), 26.1, 26.0 (2C). $[\alpha]_D^{25} = -12.0$ (*c* 1.5, $CHCl_3$). HRMS (EI) *m/z* calculated for $M^{+\cdot} = 180.15141$; found: 180.15515. The enantiomeric purity of **23** was determined to be 99% by ^1H NMR analysis after their conversion to Mosher ester and comparing with racemic standard (see page S11 of Supporting Information for details).

4.1.10. General procedure for acylation. To a solution of homoallylic alcohol (2.0 mmol), obtained as described above, in CH_2Cl_2 (1.0 mL/1.0 mmol of the homoallylic alcohol) and cooled to 0 °C were added acryloyl chloride (3.5 mmol) and Et_3N (7.0 mmol). The mixture was warmed to room temperature and stirred for 2–4 h. The resulting mixture was filtered through a short pad of Celite, poured into water, and then the product was extracted with CH_2Cl_2. Solvent evaporation under reduced pressure and purification of this residue by column chromatography on silica gel furnished the corresponding acrylic esters.

4.1.11. Acrylic ester 24. Viscous yellow liquid. IR (film): 3080, 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, $J = 16.6$ and 1.5 Hz), 6.19 (ddd, 2H, $J = 17.5$, 11.9, and 8.8 Hz), 5.86 (dd, 1H, $J = 10.4$ and 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 (2C), 127.9, 126.8 (2C), 126.5, 118.1, 73.9, 39.0. $[\alpha]_D^{25} = +82.8$ (it c 1.2, $CHCl_3$) for *R* isomer and $[\alpha]_D^{25} = -80.7$ (*c* 1.2, $CHCl_3$) for *S* isomer. HRMS (EI) *m/z* calculated for $M^{+\cdot} = 228.11503$; found: 228.11981.

4.1.12. Acrylic ester 25. Viscous colorless liquid. IR (film): 3078, 3026, 2951, 2860, 1940, 1722, 1651, 1469, 1406, 1269, 1196, 1047, 985, 808, 700 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.29–7.14 (m, 5H), 6.40 (dd, 1H, $J = 17.2$ and 1.5 Hz), 6.13 (dd, 1H, $J = 17.2$ and 10.2 Hz), 5.82 (dd, 1H, $J = 10.2$ and 1.5 Hz), 5.78–5.69 (m, 1H), 5.11–5.00 (m, 3H), 2.73–2.55 (m, 2H), 2.38 (t, 2H, $J = 7.0$ Hz), 1.99–1.87 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.8, 141.4, 133.3, 130.5, 128.7, 128.4 (2C), 128.3 (2C), 125.9, 117.9, 73.0, 38.6, 35.3, 31.6. $[\alpha]_D^{25} = +10$ (*c* 2.2, $CHCl_3$). HRESI-MS = 231.13851 *m/z* calculated for $[M+H]^{+\cdot} = 231.1385$; found: 231.1385.

4.1.13. Acrylic ester 26. Yellow solid. Mp: 53.3–54.0 °C. IR (neat tablet): 3079, 2919, 2850, 1721, 1597, 1516, 1405, 1342, 1265, 1186, 1110, 1044, 970, 919, 863, 808, 746 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ. 8.18 (d, 2H, $J = 8.8$ Hz), 7.51 (d, 2H, $J = 8.8$ Hz), 6.69 (d, 1H, $J = 15.9$ Hz), 6.46 (dd, 1H, $J = 17.4$ and 1.5 Hz), 6.36 (dd, 1H, $J = 15.9$ and 6.6 Hz), 6.17 (dd, 1H, $J = 17.4$ and 10.4 Hz), 5.89 (dd, 1H, $J = 10.4$ and 1.5 Hz), 5.85–5.73 (m, 1H), 5.59 (ddd, 1H, $J = 12.8$, 6.6, and 0.9 Hz), 5.20–5.13 (m, 2H), 2.56 (td, 2H, $J = 6.6$ and 0.9 Hz).^{13}C NMR (75 MHz, $CDCl_3$): δ 165.0, 147.0, 142.5, 132.3, 131.8, 131.1, 130. 1 (2C), 128.2, 127.0, 123.8 (2C), 118.5, 73.2, 38.9. $[\alpha]_D^{25} = -70.6$ (*c* 0.92, $CHCl_3$). HRMS (EI) *m/z* calculated for $M^{+\cdot} = 273.10011$; found: 273.09830.

4.1.14. Acrylic ester 27. Viscous colorless liquid. IR (film): 3078, 3039, 2981, 2941, 1722, 1601, 1510, 1404, 1267, 1230, 1190, 1043, 966, 810 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ. 7.36–7.32 (m, 2H), 7.02–6.97 (m, 2H), 6.60 (d, 1H, $J = 16.6$ Hz), 6.43 (dd, 1H, $J = 16.6$ and 1.4 Hz), 6.17–6.07 (m, 2H), 5.85–5.75 (m, 2H), 5.56–5.52 (m, 1H), 5.16–5.09 (m, 2H), 2.57–2.48 (m, 2H).^{13}C NMR (125 MHz, $CDCl_3$): δ 165.3, 162.5 (d, $J = 247.1$ Hz), 132.9, 132.6 (d, $J = 3.1$ Hz), 131.6, 130.8, 128.6, 128.1 (d, 2C, $J = 8.3$ Hz), 126.6 (d, $J = 2.1$ Hz), 118.2, 115.4 (d, 2C, $J = 21.8$ Hz), 73.8, 39.0. $[\alpha]_D^{25} = -66.0$ (*c* 2.03, $CHCl_3$). HRMS (EI) *m/z* calculated for $M^{+\cdot} = 246.10561$; found: 246.10285.

4.1.15. Acrylic ester 28. Viscous colorless liquid. IR (film): 3076, 3035, 3003, 2955, 2910, 2837, 1720, 1606, 1512, 1404, 1252, 1190, 1036, 966, 808 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.32–7.29 (m, 2H), 7.25–6.81 (m, 2H), 6.58 (d, 1H, $J = 15.7$ Hz), 6.42 (dd, 1H, $J = 17.2$ e 1.5 Hz), 6.15 (dd, 1H, $J = 17.2$ and 10.2 Hz), 6.04 (dd, 1H, $J = 15.7$ and 7.3 Hz), 5.86–5.72 (m, 2H), 5.53 (q, 1H, $J = 6.6$ Hz), 5.16–5.06 (m, 2H), 3.79 (s, 3H), 2.54–2.49 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.2, 159.3, 133.0, 132.3, 130.5, 128.9 (2C), 128.6, 127.7, 124.6, 117.9, 113.9 (2C), 74.2, 55.3, 39.2. $[\alpha]_D^{25} = -88.5$ (*c* 1.15, $CHCl_3$). HRMS (EI) *m/z* calculated for $M^{+\cdot} = 258.12559$; found: 258.12450.

4.1.16. Acrylic ester 29. Viscous colorless liquid. IR (film): 3076, 2979, 2900, 2779, 1720, 1653, 1504, 1489, 1446, 1404, 1252, 1188, 1039, 964, 930, 808 cm^{-1}. ^1H

NMR (300 MHz, $CDCl_3$): δ 6.92–6.74 (m, 3H), 6.56 (d, 1H, J = 16.1 Hz), 6.46–6.39 (m, 1H), 6.19–6.09 (m, 1H), 6.02 (dd, 1H, J = 16.1 and 6.6 Hz), 5.95 (s, 2H), 5.87–5.65 (m, 2H), 5.53 (q, 1H, J = 6.6 Hz), 5.17–5.04 (m, 2H), 2.73–2.47 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.4, 147.9, 147.5, 133.0, 132.5, 130.7, 130.6, 128.6, 125.1, 121.5, 118.1, 108.2, 105.7, 101.0, 74.0, 39.1. $[\alpha]_D^{25} = -74.8$ (c 0.65, $CHCl_3$). HRMS (EI) m/z calculated for $M^{+\cdot}$ = 272.10486; found: 272.10490.

4.1.17. Acrylic ester 30. Viscous colorless liquid. IR (film): 2925, 2852, 1724, 1637, 1448, 1403, 1294, 1265, 1187, 1043, 968, 916, 808 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.39 (dd, 1H, J = 17.4 and 1.6 Hz), 6.11 (dd, 1H, J = 17.4 and 10.3 Hz), 5.80 (dd, 1H, J = 10.3 and 1.5 Hz), 5.77–5.65 (m, 2H), 5.43–5.30 (m, 2H), 5.12–5.04 (m, 2H), 2.49–2.34 (m, 2H), 2.00–1.91 (m, 1H), 1.73–1.63 (m, 5H), 1.44–1.00 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.2, 140.1, 133.3, 130.2, 128.8, 124.9, 117. 6, 74.2, 40.3, 39.2, 32.6 (2C), 26.2, 26.0 (2C). $[\alpha]_D^{25} = -36.0$ (c 1.6, $CHCl_3$). HRMS (EI) m/z calculated for $M^{+\cdot}$ = 234.16198; found: 234.15814.

4.1.18. General procedure for ring closing metathesis. To a stirred solution of Grubbs' catalyst ($[P(C_6H_{11})_3]_2RuCl_2$ CHPh, 10 mol%) in dichloromethane (1.0 mL/1.0 mmol of the acrylic ester) at 55–60 °C was added the acrylic ester (0.7 mmol), obtained as described above, dissolved in dichloromethane (65 mL). The resulting mixture was heated for 12–18 h. After this period, the mixture was cooled at room temperature and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to give the analogues of goniothalamin.

4.1.19. (*S*)-goniothalamin [(*S*)-1]. White solid. Mp: 81–82 °C, (Ref. 38: 85 °C). IR (neat tablet): 3024, 2924, 2854, 1720, 1381, 1246, 1018, 968, 814, 748, 694 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41–7.25 (m, 5H), 6.92 (dt, 1H, J = 9.5 and 4.0 Hz), 6.72 (d, 1H, J = 15.9 Hz), 6.27 (dd, 1H, J = 15.9 and 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 163.5, 144.5, 135.5, 132.8, 128.5 (2C), 128.1 (2C), 126.4, 125.5, 121.4, 77.8, 29. 8 $[\alpha]_D^{25} = -170$ (c 1.7, $CHCl_3$); Ref. 21: $[\alpha]_D^{25} = -178.5$, (c 0.2, $CHCl_3$). HRMS (EI) m/z calculated for $M^{+\cdot}$ = 200.08373; found: 200.07891.

4.1.20. Analogue 4. White solid. Mp: 45.2–45.8 °C. IR (neat tablet): 3060, 3026, 2937, 2866, 1716, 1495, 1454, 1387, 1248, 1146, 1036, 958, 816, 752, 702 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.16 (m, 5H), 6.88–6.26 (m, 1H), 6.01 (dt, 1H, J = 9.9 and 1.8 Hz), 4.45–4.35 (m, 1H), 2.93–2.71 (m, 2H), 2.35–2.30 (m, 2H), 2.19–2.06 (m, 1H), 1.99–1.87 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.3, 144.9, 140.7, 128.4 (2C), 128.3 (2C), 126.0, 121.3, 76.5, 36.4, 30.8, 29.3. $[\alpha]_D^{25} = -50$ (c 1.5, $CHCl_3$). HRMS (EI) m/z calculated for $M^{+\cdot}$ = 202.09938; found: 202.09229.

4.1.21. Analogue 5. White solid. Mp: 45.0–46.1 °C. IR (neat tablet): 2925, 2852, 1708, 1675, 1448, 1384, 1247, 1160, 1054, 1022, 977, 829 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ. 6.88 (dt, 1H, J = 9.7 and 4.4 Hz), 6.04 (dt, 1H, J = 9.7 and 1.8 Hz), 5.79 (dd, 1H, J = 15.4 and 6.8 Hz), 5.53 (ddd, 1H, J = 15.4, 6.8, and 1.5 Hz), 4.86 (q, 1H, J = 7.3 Hz), 2.45–2.40 (m, 2H), 2.00–1.95 (m, 1H), 1.74–1.62 (m, 5H), 1.35–1.01 (m, 5H). ^{13} C NMR (75 MHz, DCl_3): δ 164.0, 144.6, 141.1, 124.1, 121.5, 78.5, 40.3, 32.5, 32.4, 30.0, 26.1, 25.9 (2C). $[\alpha]_D^{25} = -53$ (c 1.24, $CHCl_3$). HRMS (EI) m/z calculated for $M^{+\cdot}$ = 206.13068; found: 206.13208.

4.1.22. Analogue 6. Yellow solid. Mp: 125.8–127.6 °C. IR (neat tablet): 3062, 3034, 1719, 1593, 1510, 1383, 1336, 1254, 1106, 1085, 971, 823, 742 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ. 8.21 (d, 2H, J = 8.8 Hz), 7.54 (d, 2H, J = 8.8 Hz), 6.95 (ddd, 1H, J = 8.4, 5.3, and 3.2 Hz), 6.84 (d, 1H, J = 16.1 Hz), 6.44 (dd, 1H, J = 16.1 and 5.4 Hz), 6.13 (ddd, 1H, J = 3.2, 1.8, and 1.4 Hz), 5.20–5.13 (m, 1H), 2.66–2.55 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.3, 147.4, 144.3, 142.1, 130.5 (2C), 130.2 (2C), 127.2, 124.0, 121.7, 76.9, 29.6. $[\alpha]_D^{25} = -205.0$ (c 1.0, $CHCl_3$). HRMS (EI) m/z calculated for $M_{+\cdot}$ = 245.06881; found: 245.06891.

4.1.23. Analogue 7. White solid. Mp: 125.4–126.5 °C. IR (neat tablet): 3066, 2958, 2925, 1702, 1598, 1508, 1428, 1384, 1249, 1228, 1056, 1010, 966, 860, 815 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ. 7.39–7.35 (m, 2H), 7.07–6.99 (m, 2H), 6.96–6.90 (m, 1H), 6.70 (d, 1H, J = 15.9 Hz), 6.20 (dd, 1H, J = 15.9 and 6.2 Hz), 6.10 (dt, 1H, J = 9.9 and 1.8 Hz), 5.13–5.06 (m, 1H), 2.57–2.52 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.6, 162.6 (d, J = 247.8 Hz); 144.3, 131.8, 128.2 (d, 2C, J = 8.5 Hz), 128.1, 125.3, 121.6, 115.6 (d, 2C, J = 20.7 Hz), 77.8, 29.9. $[\alpha]_D^{25} = -158.0$ (c 1.0, $CHCl_3$). HRMS (EI) m/z calculated for $M^{+\cdot}$ = 218.07431; found: 218.07497.

4.1.24. Analogue 8. White solid. Mp: 102.5–102.8 °C. IR (neat tablet): 2962, 2933, 2838, 1708, 1604, 1511, 1245, 1178, 1024, 968, 848, 809 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35–7.30 (m, 2H), 6.94–6.84 (m, 3H), 6.65 (d, 1H, J = 15.9 Hz), 6.14 (dd, 1H, J = 15.9 and 6.6 Hz), 6.08 (dt, 1H, J = 9.5 and 1.8 Hz), 5.11–5.03 (m, 1H), 3.81 (s, 3H), 2.55–2.51 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.8, 159.6, 144.4, 132.7, 128.4 (2C), 127.8, 123.3, 121.6, 114.0 (2C), 78.2, 55.3, 30.0. $[\alpha]_D^{25} = -133.7$ (c 1.02, $CHCl_3$). HRMS (EI) m/z calculated for $M^{+\cdot}$ = 230.09430; found: 230.09428.

4.1.25. Analogue 9. White solid. Mp: 86.2–87.5 °C. IR (neat tablet): 3072, 2962, 2915, 1704, 1650, 1504, 1444, 1376, 1243, 1097, 1037, 1008, 968, 933, 809 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18–6.80 (m, 5H); 6.33–6.26 (m, 2H); 6.16 (s, 2H); 5.26 (dq, 1H, 13 J = 7.3 and 1.1 Hz); 2.90–2.65 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.8, 148.1, 147.8, 144.5, 132.9, 130.1, 123.8, 121.7, 121.6, 108.3, 105.8, 101.2, 78.0, 29.9. $[\alpha]_D^{25} = -141.7$ (c 0.93, $CHCl_3$). HRMS (EI) m/z calculated for $M^{+\cdot}$ = 244.07356; found: 244.07358.

4.1.26. Synthesis of analogue (*S*)-2 from (*S*)-1. To a solution of (*S*)-goniothalamin [(*S*)-**1**] (0.060 g 0.30 mmol) in degassed benzene (17 mL) under argon atmosphere was

added Stryker's reagent, {[$(Ph_3)CuH]_6$, 0.178 g, 0.090 mmol}. After 20 min, the mixture was exposed to air for 2 h, quenched with satd aqueous $NaHCO_3$, 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 (hexanes/ethyl acetate, 7:3) furnished (*S*)-**2** (0.039 g, 0.19 mmol) in 63% yield. White solid. Mp: 74.6–76.0 °C. IV (neat tablet): 3024, 2951, 2879, 1736, 1493, 1331, 1236, 1186, 1034, 968, 928, 748, 694 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.23 (m, 5H), 6.67 (d, 1H, $J = 15.8$ Hz), 6.20 (dd, 1H, $J = 15.8$ and 6.0 Hz), 5.02–4.96 (m, 1H), 2.69–2.47 (m, 2H), 2.11–1.69 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9, 135.9, 132.1, 128.6 (2C), 128.1, 127.0, 126.6 (2C), 80.3, 29.6, 28.5, 18.3. $[\alpha]_D^{25} = -5.5$ (*c* 1.45, $CHCl_3$). HRMS (EI) *m*/*z* calculated for $M^{+\cdot} = 202.09938$; found: 202.09840.

4.1.27. Synthesis (*S*)-3 from (*S*)-1. To a solution of (*S*)-goniothalamin [(*S*)-**1**] (0.050 g, 0.25 mmol) in 2.0 mL of ethanol under H_2 atmosphere (1 atm) was added 10% Pd/C (5 mg). After 2 h, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/ethyl acetate, 7:3) to give (*S*)-**3** in 87% yield (0.036 g, 0.18 mmol). Viscous colorless liquid. IR (film): 3024, 2930, 2858, 1728, 1495, 1452, 1388, 1240, 1178, 1047, 930, 748 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.31–7.18 (m, 5H), 4.30–4.22 (m, 1H), 2.91–2.69 (m, 2H), 2.64–2.40 (m, 2H), 2.09–1.76 (m, 5H), 1.64–1.52 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.8, 141.0, 128.4 (4C), 126.0, 79.3, 37.4, 31.0, 29.4, 27.8, 18.4. $[\alpha]_D^{25} = +49$ (*c* 1.0, $CHCl_3$). HRMS (EI) *m*/*z* calculated for $M^{+\cdot} = 204.11503$; found: 204.11466.

4.2. Biological assay

4.2.1. Cell lines. The human tumoral MCF-7 (breast), NCI.ADR (breast expressing the resistance phenotype for adryamycin), NCI.460 (lung, non-small cells), UACC.62 (melanoma), 786-0 (kidney), OVCAR03 (ovarian), PCO.3 (prostate), and HT-29 (colon) were obtained from the Frederick MA, National Cancer Institute/USA.

4.2.2. Cell culture. The cells were maintained in RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO, USA), supplemented with 10% fetal calf serum (FCS: Nutricell Nutrientes Celulares, Campinas, SP, Brazil) at 37 °C with 5% CO_2. The medium was changed every 2 days until the cells reached confluence, at which point they were subcultured.

4.2.3. Antiproliferative assay. The adherent cell lines were detached from the culture flasks by adding 0.5 mL trypsin solution 0.025% (Nutricell). The trypsin was inactivated by adding 5 mL of 5% FCS in RPMI-1640 medium. Single-cell suspensions were obtained by a gentle pipetting acton. After counting, the cells were diluted to appropriate seeding densities and transferred to 96-well microtiter plates (Nunc Brand Products) in a fixed volume of 100 μM per well. The seeding densities were as follows: 6.5×10^4 (MCF-7), 5×10^4 (NCI.ADR, 786-0, HT-29), 4×10^4 (NCI.460, PCO.3), 3×10^4 (UACC.62), and 7×10^4 (OVCAR03) cells/mL. This initial cell concentration was determined from individual growth curves and ensured that the cells would be in the logarithmic growth throughout the experiment. The microtiter plates containing cells were pre-incubated for 24 h at 37 °C to allow stabilizations prior to addition (100 μL) of goniothalamin (**1**) and analogues (**2**–**9**) as well as doxorubicin (DOX). The plates were incubated with the test substance for 48 h at 37 °C and 5% CO_2.[28,29]

4.2.4. Solubilization and dilution of test substance. Goniothalamin (**1**) and analogues (**2**–**9**) were tested at four concentrations (0.25, 2.5, 25, and 250 μg/mL), each in triplicate wells. The substances tested were initially solubilized in dimethylsufoxide (DMSO) (Sigma) at 400× the final maximum test concentration. The stock solution was diluted with complete medium containing 50 μg gentamicin/mL (Schering-Plough).

4.2.5. Sulforhodamine B (SRB) assay. Sulforhodamine B (SRB) is an aminoxanthine with a bright pink color that has two sulfonic groups. Since it is an anionic dye in weak acid solution, it is capable of bonding to basic groups of proteins in cells fixed with trichloroacetic acid. Therefore, this non-clonogenic methodology permits a highly sensitive protein with a straight relationship to cell culture. The SRB assay was done as described by Skehan et al.[29] Briefly, the cells were fixed with 50% TCA (sigma) at 4 °C (50 μL/well, final concentration 10%) for 1 h. The supernatant was then discarded and the plates were washed five times with tap water. The cells were stained for 30 min with 0.4% SRB in 1% acetic acid (50 μL/well) (Sigma) and subsequently washed 4× with 1% acetic acid to remove unbound dye. The plates were air-dried and protein-bound dye was solubilized with 150 μL (100 mM) of Trizma buffer (Sigma). The resulting optical density was read in a multiwell plate reader at 540 nm. For cells grown in suspension (e.g., leukemia), the same method was used, but the TCA concentration was 80% to fix the cells to the plate bottom.

4.2.6. Data calculations. Absorbances were calculated using the Excell® program (Microsoft Office package) and the mean ± SE of the mean were calculated for 4 wells in triplicate samplings. The background absorbances were subtracted from the appropriate control and drug-blank measurements. To assess the effect of goniothalamin (**1**) and analogues (**2**–**9**) on cell growth, three measurements were obtained at time zero (T_0) values for all cells at the beginning of incubation, and control (C) and test (T) values at the end of incubation without and with the test substance, respectively. For T value $\leqslant T_0$ (cytostatic effect), the calculation was $100 \times [(T - T_0)/C - T_0]$. While for $T < T_0$ (cytocidal effect), the calculation was $100 \times [(T - T_0)/T_0]$. The IC_{50} values (test substance concentration eliciting 50% inhibition) were determined by non-linear regression analysis. These results presented here refer to a representative experiment since all assays were run in triplicate and the average standard error was always <5%.

Supplementary Material

Determination of enantiomeric purity and spectra of alcohols homoallylic **17**–**23**, and spectra of goniothalamin (**1**) and analogues **2**–**9** cited in this article are available free via internet at http://www.sciencedirect.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.08.036.

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