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Design, synthesis and in vitro evaluation against human cancer cells of 5-methyl-5-styryl-2,5-dihydrofuran-2-ones, a new series of goniothalamin analogues

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ABSTRACT

The present work describes the preparation of a novel series of compounds based on the structure of goniothalamin (**1**), a natural styryl lactone with known cytotoxic and antiproliferative activities against a variety of cancer cell lines. A focused library of 17 goniothalamin analogues displaying the 5-methyl-2,5-dihydrofuran-2-one motif were prepared, and their cytotoxicity evaluated. While the analogues bearing methoxy and/or hydroxy groups on the aromatic moiety usually were at least three times less potent than the lead compound (**1**), *ortho* and *para*-trifluoromethyl analogues **10** and **11** exhibited levels of cytotoxicity similar to goniothalamin (**1**) against most cancer cell lines evaluated. One could suggest that the electronic effect of the trifluoromethyl group activates the inhibitor's electrophilic site via reduction of the electron density of the α,β-unsaturated ester oxygen atom. These results provide new information on the structure activity relationship of these α,β-unsaturated styryl lactones, thereby further focusing the design of novel candidates.

1. Introduction

Natural products are a privileged source of molecular diversity that have shown to exhibit numerous biological activities. It is estimated that half of current drugs used in cancer chemotherapy are—or are based on—natural products. Goniothalamin (GNT, **1**) (Fig. 1) is a naturally occurring styryl lactone originally isolated from various species of the genus *Goniothalamus*,[1] displaying significant cytotoxic and anti-proliferative activities against a variety of cancer cell lines, including kidney, prostate, breast carcinoma, leukemia, lung and liver.[2,3] Other important biological properties such as antimicrobial,[4] antifungal,[5] larvicidal,[6] insecticidal[7] and trypanocidal activities,[8] have also been reported.

Because of the broad spectrum of biological properties, goniothalamin has caught the attention of several research groups, thus triggering a number of studies on the possible mechanism of action for its cytotoxic and antiproliferative activity.[9–12] Recently, in vivo studies performed by our research group with goniothalamin in a solid tumor experimental model in mice confirmed its low acute toxicity and suggested a relationship between anticancer and anti-inflammatory activities, with the anti-inflammatory activity favoring the antiproliferative activity itself.[13]

In order to better understand the mode of action of goniothalamin (**1**) and design more active and selective analogues, several structure–activity relationship (SAR) studies have been conducted.[14–16] These studies established the α,β-unsaturated δ-lactone moiety as the key feature for activity as a result of its ability to act as a Michael acceptor in the presence of nucleophilic biomolecules such as cysteine and glutathione. As demonstrated by Çağır and co-workers, the styryl moiety with *E* double bond geometry could be replaced by 2- and 1-naphthyl group, the latter dramatically enhancing the cytotoxic activity.[17]

Based on these preliminary results, our research group has recently reported the design of three new series of goniothalamin analogues (Fig. 1).[18] The influence of hydroxy and methoxy groups,[19,20] as well as fluorinated substituents[21] on the aromatic ring, such as in **2** was examined; the effect of the electronic nature of the Michael acceptor on the cytotoxic activity was investigated with the synthesis of azagoniothalamin (**3**),[22,23] as well as dihydro-γ-pyrones analogues (**4**).[24] The study concluded on the prevalence of the α,β-unsaturated lactone ring as the pharmacophoric motif for the in vitro antiproliferative activity, with the highest cytotoxicity being observed for the di- and trimethoxy goniothalamin analogues.

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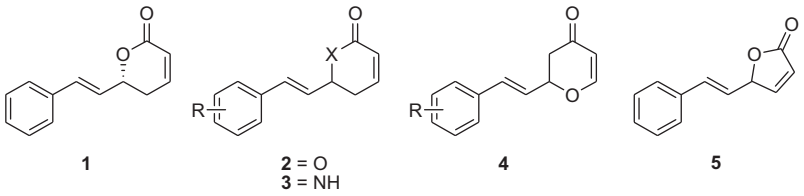


Figure 1. Chemical structure of (*R*)-goniothalamin **1**, and analogues: with modified aromatic moiety (**2**), azagoniothalamin (**3**), dihydro-γ-pyrones (**4**), and homogoniothalamin (**5**, HGNT).

In a continuous effort to enhance the cytotoxic profile of goniothalamin, and to complement previous studies from our research group, we envisioned to prepare analogues with a reduced size of the lactone ring, for example, styryl γ-butyrolactones **5**. In fact, some natural products possess the furan-2(5*H*)-one motif, and are reported to display various biological activities including cytotoxicity.[25] Therefore, the synthesis of goniothalamin analogues such as styryl furan-2(5*H*)-one **5** stands as a promising approach to improve the potency and selectivity of this family of compounds. Although compound **5** has been described in the literature, it appears that there are no other reports of such styryl furanones.[26]

We herein report the design and synthesis of a focused library of goniothalamin analogues displaying the γ-butyrolactone structural motif, and their evaluation in vitro against eight human cancer cell lines: U251 (glioma), UACC-62 (melanoma), MCF-7 (breast), NCI-ADR/RES (multidrug resistant ovary carcinoma), 786-0 (renal), PC-3 (prostate), HT-29 (colon), and K-562 (leukemia).

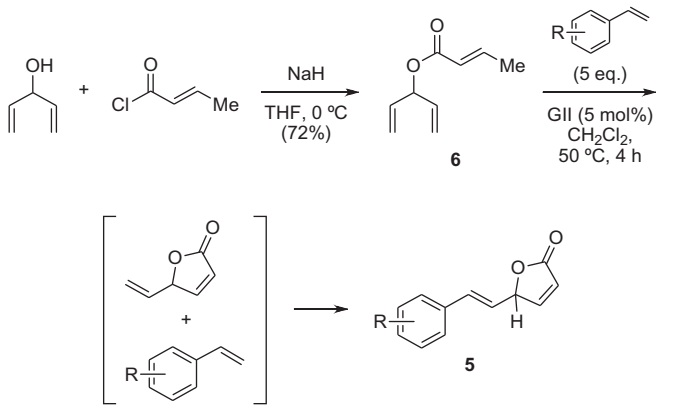
2. Results and discussion

2.1. Chemistry

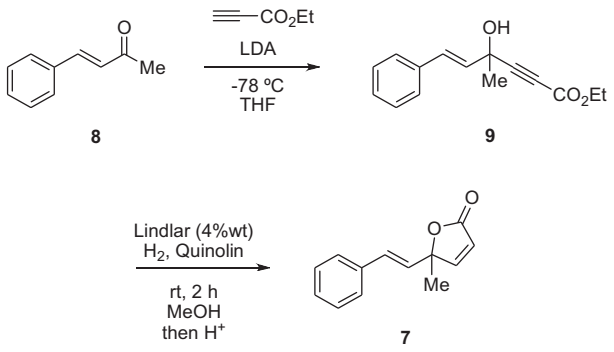
Initial attempts to prepare styryl lactone **5** following the methodology usually applied to the synthesis of goniothalamin analogues in our laboratory failed.[4c] Only mild conditions using a tandem ring-closing/cross-coupling metathesis reaction led to the production of five analogues (Scheme 1).[27] Pentadienyl ester **6** was readily prepared from pentadienyl-3-ol and crotonoyl chloride upon treatment with sodium hydride at room temperature. Reacting a mixture of **6** and excess styrene with Grubbs' second-generation catalyst (GII) under diluted conditions at reflux, delivered the desired styryl butenolide **5** after 4 h, albeit in poor yield. The latter was attributed to the sensitivity of the lactone toward purification conditions, probably due to the proton at C5, which could easily eliminate to produce a highly conjugated system under such conditions.[28] Obtaining analogue lactones with substituted aromatic rings in high purity was also an issue, and therefore, these could not be evaluated in cytotoxic bioassays.[29]

In order to overcome the instability of compound **5**, we thought that replacing this acidic proton with a methyl group, for example, should render the structure more robust. Reassuringly, two antibiotics, OH-3984 K1 and K2, isolated from a *Streptomyces* species and bearing the methyl vinyl furanone unit, as well as a synthetic intermediate are reported in the literature, thus suggesting that this motif is relatively stable.[30,31] Only one synthesis of 5-methyl-5-styrylbutenolide **7** has been reported by Saberi and Thomas.[32] To our knowledge, there are no reports of other syntheses and no biological evaluation of 5-methylated styryl furanones such as **7** in the literature. The synthetic approach for furanones **7** was based on literature precedents for the formation of butenolides, and is exemplified in Scheme 2.[33,34] Commercially available benzylideneacetone **8** was treated with lithium ethylpropiolate formed in situ at low temperature, to generate the tertiary alcohol **9** in excellent yield. The triple bond was subjected to a semi-hydrogenation following a Lindlar protocol, and yielded the desired *Z*-alkene, as verified by ^{1}H NMR. The crude acrylic ester was treated with acidic DOWEX resin to deliver the coveted 5-methylfuranone **7**, in 79% yield over two steps.

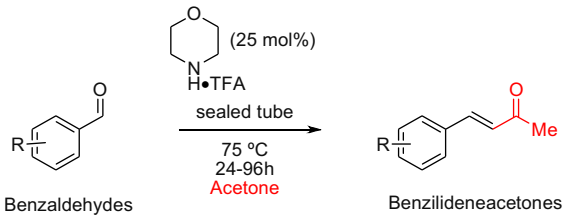
For the preparation of analogues of lactone **7**, we needed access to substituted benzylideneacetones, which are relatively scarce on the market, and we decided to prepare them from readily available benzaldehydes, employing an aldol condensation reaction with acetone. From the plethora of methodologies available in the literature, we chose the recent work of List and colleagues, where a variety of substrates were obtained in excellent yields with simple purifications required.[35,36] List's protocol involved a mixture of benzaldehyde in excess acetone, the latter also acting as the solvent, in the presence of a morpholinium-trifluoroacetate salt (25 mol %) acting as the catalyst (Scheme 3). The whole mixture was heated at reflux in a sealed tube for several hours. Once the reaction was complete, the salt was removed through filtration and impurities separated by chromatography to afford clean benzylideneacetones (**8a–i**). The desired *E*-stereochemistry of the newly created double bonds was confirmed on the basis of the



Scheme 1. Synthesis of styryl lactones using a tandem ring-closing/cross-coupling metathesis reaction.



Scheme 2. Synthesis of 5-methyl-5-styryl butenolide **7**.



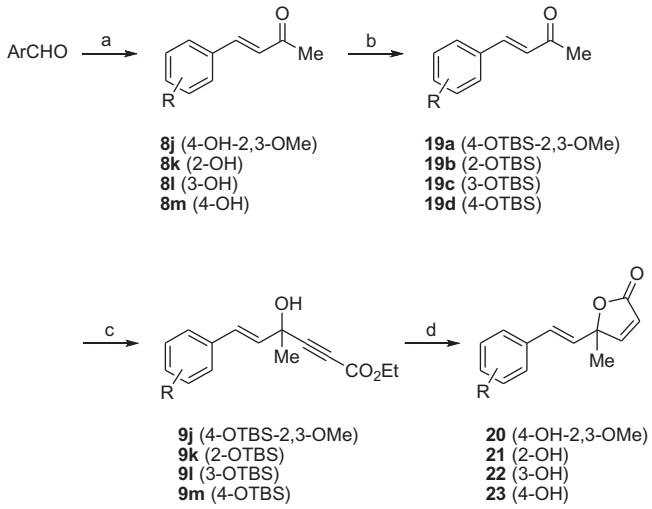
Scheme 3. List's preparation of benzylideneacetones via an ammonium salt catalyzed aldol condensation of substituted benzaldehydes with acetone.

coupling constant values in the vicinity of 16 Hz, as determined by ^{1}H NMR analysis.

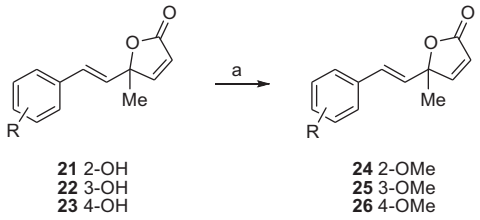
Based on previous work in our research group, we opted for the preparation of mono-, di- and trimethoxylated, as well as fluorinated substrates.[18] Following the reaction sequence shown in Scheme 2, alcohols **9a**–**i** were obtained in good to excellent yields (73–97%). The final Lindlar reduction and cyclization steps delivered the desired substituted MeHGNTs (**10**–**18**) in modest to good yields (55–88%) (Scheme 4).

We also chose to include substrates bearing free hydroxy groups, and it was therefore necessary to protect this functionality prior to the alkyne addition step, the aldol condensation tolerating the presence of unprotected alcohols. The *tert*-butyldimethylsilyl protecting group was selected and it was planned to be removed simultaneously in the last step while enabling the ring closure under acidic conditions, however using hydrogen fluoride (Scheme 5). After obtaining the corresponding benzylideneacetones **8j**–**m** (48–81% yield), their TBS-protected forms (**19a**–**d**) were prepared in excellent yields. The alkyne addition proceeded reasonably well (**9j**–**m**, 61-94%), although both *ortho* and *para*-substituted substrates appeared less reactive. The Lindlar reduction and cyclization went smoothly and the final TBS-group removal using hydrogen fluoride in pyridine gave the desired MeHGNTs (**20**–**23**) in overall good yields (57–84%) (Scheme 5). Additionally, we took advantage of the three monohydroxylated subtrates to convert them into their corresponding methyl ethers using trimethylsilyldiazomethane in the presence of base in moderate yields (42–61%), giving three new monomethoxylated MeGNTs (**24**–**26**) (Scheme 6).

Finally, in order to complete the series of methylated lactones, we decided to prepare the analogous 6-methylgoniothalamin **27**. Although lactone **27** is reported in the literature as a synthetic intermediate,[37] the usual laboratory methodology was applied using benzylideneacetone **8** as starting material, as shown in Scheme 7.



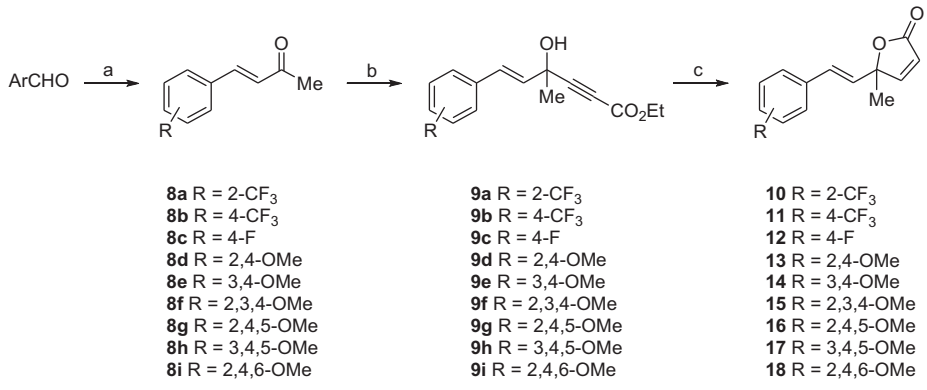
Scheme 5. Reagents and conditions: (a) morpholinium-trifluoroacetate salt (25 mol %), acetone (excess), 75 °C in a sealed tube, 24–48 h (48–81%); (b) imidazole, TBSCl, DMF, rt, 16 h (90–99%); (c) ethylpropiolate lithium salt, THF, −78 °C, 30 min (61–94%); (d) (i) Lindlar catalyst (4% wt), H_2, quinoline, MeOH, rt, 3 h; (ii) HF· pyr, THF, rt, 16 h (57–84%).



Scheme 6. Reagents and conditions: (a) $TMSCHN_2$, iPr_2NEt, MeCN, rt, 16 h (42–61%).

2.2. Biological activities

Considering that different cell lines display different sensitivities toward the same cytotoxic compound, the antiproliferative activity of all the goniothalamin analogues were evaluated in vitro against seven different human cancer cell lines: U251 (glioma), UACC-62 (melanoma), MCF-7 (breast), NCI-ADR/RES (multidrug resistant ovary carcinoma), 786-0 (renal), PC-3 (prostate), HT-29 (colon), and K-562 (leukemia). The antiproliferative activity of each compound was also evaluated in vitro against

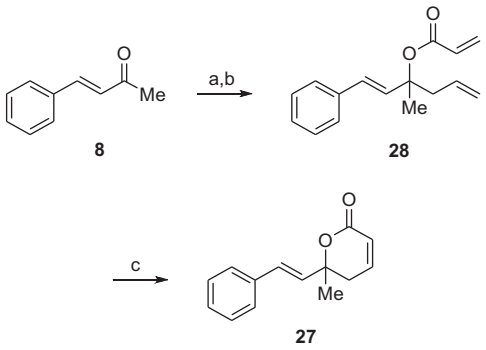


Scheme 4. Reagents and conditions: (a) morpholinium-trifluoroacetate salt (25 mol %), acetone (excess), 75 °C in a sealed tube, 24–96 h (51–89%); (b) ethylpropiolate lithium salt, THF, −78 °C, 30 min (73–97%); (c) (i) Lindlar catalyst (4% wt), H_2, quinoline, MeOH, rt, 3 h; (ii) DOWEX H^+, MeOH, rt, 2 h (55–88%).

Table 1
TGI values, given in μM, for compounds goniothalamin (**1**), compounds **7**, **10**–**18**, **20**–**27** and doxorubicin (DOX), concentration necessary for total inhibition of tumor cell growth[a,b]

TGI	U251	UAAC-62	MCF-7	NCI-ADR/RES	786-0	PC-3	HT-29	K-562	HaCat
DOX	2.1	0.2	4.9	10.3	1.0	2.5	3.6	45.5	0.4
1	9.8	17.4	11.2	14.5	13.9	9.6	10.0	926.8	13.4
7	89.8	97.2	95.3	414.3	170.0	86.9	122.6	>1000	118.8
10	14.1	17.7	13.5	12.2	27.9	17.9	15.8	573.4	12.7
11	15.7	15.5	20.4	9.4	34.0	13.1	12.3	651.8	12.1
12	60.8	55.3	38.5	25.7	101.2	46.5	34.9	>1000	47.5
13	62.8	80.1	43.5	150.7	107.9	63.5	57.5	>1000	53.6
14	192.5	84.1	78.9	110.9	22.91	175.5	927.2	>1000	838.1
15	87.2	71.5	109.5	349.4	132.0	90.0	132.6	>1000	217.1
16	159.6	127.0	144.2	158.2	205.0	183.5	126.7	>1000	179.8
17	124.6	178.5	56.6	123.0	116.4	131.2	390.0	>1000	175.3
18	192.8	207.7	102.4	229.1	458.9	239.0	126.8	>1000	458.0
20	40.7	24.6	45.3	51.6	108.5	130.9	19.1	>1000	64.4
21	70.9	92.5	46.2	78.0	136.2	111.8	31.3	>1000	57.3
22	117.8	108.2	123.6	646.9	196.	98.8	97.4	>1000	88.1
23	106.9	55.3	69.1	111.3	143.3	137.7	122.2	>1000	81.2
24	41.1	31.0	27.0	64.5	35.4	34.2	30.8	860.1	30.8
25	37.6	55.8	25.3	48.6	70.0	31.6	17.0	>1000	5.7
26	127.0	147.3	182.7	>1000	>1000	145.1	92.9	>1000	316.2
27	27.5	37.2	48.0	106.8	68.7	31.7	41.1	>1000	50.7

[a] Concentration that elicits total growth inhibition (TGI) was determined from nonlinear regression analysis using the ORIGIN 7.5® (OriginLab Corporation).
[b] Doxorubicin (DOX) was the positive control.



Scheme 7. Reagents and conditions: (a) allylmagnesium bromide, THF, −78 °C, 30 min (95%); (b) acryloyl chloride, Et_3N, CH_2Cl_2, rt, 20 min (40%); (c) GII (4 mol %), CH_2Cl_2, 45 °C, 4 h (74%).

spontaneously transformed keratinocytes from histologically normal skin (HaCat cells). Doxorubicin was employed as the positive control and goniothalamin (**1**) was included as the reference compound.[38]

Cell growth was determined spectrophotometrically using sulforhodamine B (SRB) as protein-binding dye and analyses were based on the U.S. National Cancer Institute (NCI) 60 human tumor cell line anticancer drug screen (NCI60).[39] Differently from other methods, in the SRB assay measurement of the cell population density at time zero (the time at which drugs are added) is possible, which allows the calculation of the cellular responses for total growth inhibition. The drug concentration resulting in total growth inhibition (TGI) is calculated from $T = T0$, where the amount of protein at the end of drug incubation (T) is equal to the amount at the beginning ($T0$).

Goniothalamin (GNT, **1**) and its analogues were employed at concentrations between 0.25–250 μg/mL and doxorubicin at 0.025–25 μg/mL. Concentration that elicits total growth inhibition (TGI) was determined after 48 h of cell treatment. The initial in vitro screening evaluations were planned to select the best candidates for anticancer drug development in animal models and, eventually, in clinical trials.

The 5-methyl furan-2(5*H*)-one analogue **7** proved to be less potent than the 6-methyl analogue of goniothalamin **27** and much less than GNT (**1**) itself, the loss of antiproliferative activity being assigned to the steric hindrance introduced by the methyl group, much more severe in **7** than in the goniothalamin analogue **27** (Table 1). Despite that, potency was recovered by the introduction of a trifluoromethyl group in the *ortho* and *para* positions of the aromatic ring. In fact, compounds **10** and **11** displayed similar TGI values to GNT (**1**) for melanoma (UACC-62) and slightly better cytotoxic activity for ovary resistant to adriamycin cancer cells (NCI-ADR/RES). Derivatives **10** and **11** were as toxic as GNT (**1**) towards spontaneously transformed keratinocytes from histologically normal skin (HaCat cells). The higher toxicity of compounds **10** and **11** could be attributed to the highly electron-withdrawing nature of the trifluoromethyl groups, which could reduce the electron density of the α,β-unsaturated lactone oxygen atom by means of an inductive effect along the styryl moiety. Consequently the reactivity of the Michael acceptor would be increased, leading to higher cytotoxicity. One could also speculate that the lipophilic nature of the trifluoromethyl group could have a privileged interaction with a putative hydrophobic binding site of the biological target of these compounds.On the other hand, the *p*-fluor analogue **12** showed only a minor improvement in cytotoxicity when compared to analogue **7**.[40]

Except for substituents in the *para* position of the aromatic ring, the presence of a methoxy group at *ortho*- and *meta*-positions in the aromatic ring (compounds **24** and **25**, respectively) led to more potent analogues than 5-methyl furan-2(5*H*)-one **7** and the corresponding hydroxy derivatives (**21** and **22**, respectively). Surprisingly, the presence of two and three methoxy groups led to less potent analogues (compounds **13**–**18**). 3,5-Dimethoxy-4-hydroxy derivative **20** was only twice less active than GNT (**1**) against colon cancer cells (HT-29), but five times less toxic to spontaneously transformed keratinocytes from histologically normal skin (HaCat cells).

Analogue **25** displayed lower (MCF-7, NCI-ADR/RES and HT-29) or similar (786-0 and PC-3) TGI values when compared to the 6-methyl analogue of goniothalamin **27**, while the 2-methoxy derivative **24** was shown to be as potent as for UACC-62 and PC-03 and more potent than **27** for MCF-7, NCI-ADR/RES, 786-0 and HT-29 cancer cell lines. Additionally, the 2-methoxy derivative **24** was shown to be three times less toxic to (HaCat cells) than the corresponding 3-methoxy derivative **25**.

3. Conclusions

A synthetic route to styryl furan-2(5*H*)-one **5** has been implemented via a tandem ring-closing/cross-coupling metathesis reaction, but its chemical liability precluded the evaluation of its cytotoxic profile. In order to circumvent this problem, 17 methylated furanone analogues of goniothalamin (MeHGNTs **7**, **10**–**18**, **20**–**26**) and 6-methylgoniothalamin (**27**) were prepared and subjected to bioassays for evaluation of their antiproliferative profile against a panel of seven human cancer cell lines. Although the introduction of a methyl group at the quaternary center of the lactones had a negative effect on the biological activity of 5-methyl furan-2(5*H*)-one (**7**) and the 6-methyl analogue (**27**) of goniothalamin (**1**), *ortho*- and *para*-trifluoromethylated analogues **10** and **11** displayed significant antiproliferative activity, particularly for melanoma (UACC-62) and multidrug resistant ovary carcinoma cells (NCI-ADR/RES), when compared to goniothalamin (**1**). This observation suggests that the trifluoromethyl groups could be responsible for the recovery of potency due to their highly electron-withdrawing and inductive effect via activation of the electrophilic site of the inhibitor. Indeed, analogs bearing hydroxyl or methoxyl substituents generally gave inhibitory values at least three times higher than the reference compound (**1**). These results provide new information on the structure activity relationship of these α,β-unsaturated styryl lactones, thereby further focusing the design of novel candidates.

4. Procedures

Reagents and solvents were commercial grade and were used as supplied, except when specified in the experimental procedure. Grubbs' second generation catalyst (Lot#MKBG2090 V) was purchased from Aldrich. Doxorubicin was purchased from Europharma. Racemic goniothalamin (**1**) was prepared according to previously described methodology. For reactions requiring anhydrous conditions, flame dried glassware and nitrogen atmosphere were used. THF was distilled from calcium hydride and redistilled from sodium/benzophenone, and dichloromethane, diisopropylamine and triethylamine were distilled from calcium hydride immediately prior to use. Reactions were monitored by thin-layer chromatography (TLC) on Silica gel 60 F_{254} aluminium sheets and exposed to UV radiation, followed by treatment with adequate stains and heating. Chromatographic separations were carried out on Merck 60 silica gel (230–400 mesh). Melting points were recorded on an Electrothermal IA9000 Series digital melting point apparatus and are not corrected. IR spectra were registered on a Nicolet Impact 410 (Film) or on a Nicolet iS5/iD3 (ATR), with the observed absorptions expressed in cm^{-1}. ^1H NMR and ^{13}C NMR data were recorded on a Varian Inova (500 MHz for ^1H and 125 MHz for ^{13}C NMR) or Bruker Avance (250 MHz for ^1H and 62.5 MHz for ^{13}C NMR) spectrometer using as internal standard TMS, or the residual non deuterated solvent MeOH. Chemical shifts (δ) were expressed in ppm and multiplicities were reported as singlet (s), doublet (d), double doublet (dd), triplet (t), double triplet (dt), quartet (q), apparent quartet (q (dt)), quintuplet (quint), multiplet (m). Coupling constants (*J*) are expressed in Hertz (Hz). High resolution mass spectra (HRMS) for novel compounds were measured on a Waters XEVO Q-TOF spectrometer (ESI) or on a Waters GCT Premier (EI).

4.1. Experimental procedures

4.1.1. (*E*)-Penta-1,4-dien-3-yl but-2-enoate (6)

To a solution of pentadienyl-3-ol (500 mg, 5.95 mmol) in THF (20 mL) was added sodium hydride (60% dispersion in grease; 416 mg, 10.40 mmol) at 0 °C under nitrogen. After 30 min, crotonoyl chloride (1.11 mL, 10.40 mmol) was added dropwise. After 15 min, the reaction mixture was carefully quenched with a saturated ammonium chloride solution (40 mL) and extracted into ethyl acetate (60 mL). The organic fraction was washed with brine (40 mL), dried over magnesium sulfate and evaporated in vacuo, with the water bath being left at room temperature. The resulting oil was purified by chromatography, eluting with hexane/dichloromethane (7:3) to give the title compound (580 mg, 64%) as a colourless and volatile oil (leaving the compound under high vacuum for a few hours lead to its complete evaporation); δ_H (250 MHz; $CDCl_3$) 7.05 (1H, dq, *J* 15.6, 6.9, H-3′), 5.93–5.74 (4H, m, H-2′, H-2, H-3, H-4), 5.31 (2H, d, *J* 16.9, H-1_{trans}, H-5_{trans}), 5.22 (2H, d, *J* 10.2, H-1_{cis}, H-5_{cis}), 1.89 (3H, dd, *J* 6.9, 1.6, Me); δ_C (62.5 MHz; $CDCl_3$) 165.3 (CO), 145.0 (CH), 135.2 (CH), 122.7 (CH), 117.2 (CH_2), 74.6 (CH), 17.9 (Me).

4.1.2. (*E*)-5-Styrylfuran-2(5*H*)-one (5)

A mixture of triene **6** (1 equiv), styrene (5 equiv) and Grubbs' second generation catalyst (5 mol %) in dichloromethane (50 mM) was heated at reflux for 4 h. Upon cooling, DMSO (50 equiv relative to the catalyst) was added and the mixture was stirred overnight at room temperature. The solvent was removed and the crude residue was purified by chromatography eluting with hexane/ethyl acetate (65:33) to afford the title compound (28 mg, 38%) as an off-white powder: mp 88–100 °C; ν_{max} (film) 2922, 1760, 1601, 1496, 1334, 1132; δ_H (250 MHz; $CDCl_3$) 7.46 (1H, d, *J* 5.6, H-4), 7.36–7.23 (5H, m, ArH), 6.77 (1H, d, *J* 15.8, H-2′), 6.18 (1H, d, *J* 5.6, H-3), 5.99 (1H, dd, *J* 15.8, 7.4, H-1′), 5.62 (1H, d, *J* 7.4, H-5); δ_C (62.5 MHz; $CDCl_3$) 172.8 (CO), 155.0 (CH), 135.3 (C), 135.0 (CH), 128.7 (CH), 126.9 (CH), 126.8 (CH), 122.0 (CH), 121.5 (CH), 83.6 (CH); (found: $[M+H]^+$, 187.0847. $C_{12}H_{10}O_2+H^+$ requires 187.0759).

4.1.3. General procedure for the preparation of benzilideneacetones (8a–m) [36]

A mixture of benzaldehyde (12.0 mmol) and morpholinium trifluoroacetate (3.0 mmol) in acetone (30 mL) was heated at 80 °C in a sealed tube for 24–96 h. After cooling to room temperature, the reaction mixture was diluted with ether (30 mL) and washed with a saturated aqueous sodium hydrogen carbonate solution (30 mL). The organic layer was washed with brine (30 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude was purified by chromatography, eluting with hexane and ethyl acetate (see analytical details).

4.1.3.1. (3*E*)-4-(2-Trifluoromethylphenyl)but-3-en-2-one (8a). Yield: 1.61 g, 63%, as a yellow oil; Rf = 0.33, hexane/ethyl acetate (9:1); ν_{max} (ATR) 1677, 1617, 1487, 1361, 1313, 1290, 116, 1037, 765; δ_H (250 MHz; $CDCl_3$) 7.89 (1H, d, *J* 16.1, H-4), 7.73 (1H, d, *J* 6.8, ArH), 7.72 (1H, d, *J* 6.8, ArH), 7.59 (1H, t, *J* 7.7, ArH), 7.50 (1H, t, *J* 7.7, ArH), 6.65 (1H, d, *J* 16.1, H-3), 2.41 (3H, s, H-1); δ_C (62.5 MHz; $CDCl_3$) 198.0 (C), 138.8 (CH), 133.3 (C), 132.1 (CH), 131.1 (CH), 129.2 (CH), 128.8 (C, q, ^2J 30.2), 127.8 (CH), 126.1 (CH, q, ^3J 5.5), 123.9 (CF_3, q, ^1J 273.9), 27.0 (Me); (Found: $[M+H]^+$, 215.0785. $C_{11}H_9F_3O+H^+$ requires 215.0684).

4.1.3.2. (3*E*)-4-(2,3,4-Trimethoxyphenyl)but-3-en-2-one (8f). Yield: 2.48 g, 88%, as a yellow oil; Rf = 0.33, hexane/ethyl acetate (7:3); ν_{max} (ATR) 2942, 1667, 1590, 1494, 1253, 1094; δ_H (250 MHz; $CDCl_3$) 7.76 (1H, d, *J* 16.4, H-4), 7.31 (1H, d, *J* 8.8, ArH), 6.71 (1H, d, *J* 8.8, ArH), 6.68 (1H, d, *J* 16.4, H-3), 3.94 (3H, s, ArOMe), 3.90 (3H, s, ArOMe), 3.88 (3H, s, ArOMe), 2.38 (3H, s, H-1); δ_C (62.5 MHz; $CDCl_3$) 198.8 (C), 155.7 (C), 153.2 (C), 142.2 (C), 138.3 (CH), 126.3 (CH), 122.8 (CH), 121.3 (C), 107.7 (CH), 61.4

(OMe), 60.8 (OMe), 56.0 (OMe), 27.0 (Me); (found: $[M+H]^+$, 237.1135. $C_{13}H_{16}O_4+H^+$ requires 237.1127).

4.1.4. General procedure for the preparation of TBS-protected phenols (19a–d)

A mixture of phenol (1.0 equiv), imidazole (1.5 equiv) and *tert*-butyldimethylsilyl chloride (1.3 equiv) in dry DMF (0.2 M) was stirred for 4 h at room temperature under a nitrogen atmosphere. The reaction mixture was then diluted with an equal amount of ether and washed three times with brine. The organic layer was dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude was purified by chromatography, eluting with hexane and ethyl acetate (see analytical details).

4.1.4.1. (3*E*)-4-{4-[(*tert*-Butyldimethylsilyl)oxy]-3,5-dimethoxyphenyl}but-3-en-2-one (19a). Yield: 0.68 g, 99%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (9:1); ν_{max} (ATR) 2994, 2856, 1685, 1577, 1456, 1346, 1281, 1178, 908; δ_H (250 MHz; $CDCl_3$) 7.43 (1H, d, *J* 16.2, H-4), 6.75 (2H, s, ArH), 6.60 (1H, d, *J* 16.2, H-3), 3.83 (6H, s, 2× ArOMe), 2.37 (3H, s, H-1), 1.01 (9H, s, Si^{t}Bu), 0.15 (6H, s, $SiMe_2$); δ_C (62.5 MHz; $CDCl_3$) 198.2 (C), 151.8 (C), 144.0 (CH), 137.2 (C), 126.9 (C), 125.4 (CH), 105.5 (CH), 55.7 (OMe), 27.2 (Me), 25.7 (Me), 18.7 (C), −4.6 (Me); (found: $[M+H]^+$, 337.1873. $C_{18}H_{28}O_4Si+H^+$ requires 337.1835).

4.1.4.2. (3*E*)-4-{2-[(*tert*-Butyldimethylsilyl)oxy]phenyl}but-3-en-2-one (19b). Yield: 0.68 g, 99%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (9:1); ν_{max} (ATR) 2956, 2859, 1670, 1599, 1482, 1253, 919, 836; δ_H (250 MHz; $CDCl_3$) 7.94 (1H, d, *J* 16.7, H-4), 7.57 (1H, d, *J* 8.2, ArH), 7.27 (1H, dd, *J* 9.7, 7.2, ArH), 6.97 (1H, dd, *J* 7.5, 7.4, ArH), 6.85 (1H, d, *J* 8.2, ArH), 6.65 (1H, d, *J* 16.7, H-3), 2.37 (3H, s, H-1), 1.05 (9H, s, Si^{t}Bu), 0.25 (6H, s, $SiMe_2$); δ_C (62.5 MHz; $CDCl_3$) 202.1 (C), 158.6 (C), 141.8 (CH), 133.2 (CH), 129.9 (CH), 127.6 (CH), 122.7 (C), 121.0 (CH), 117.2 (CH), 26.6 (Me), 25.7 (Me), 18.3 (C), −4.3 (Me); (found: $[M+H]^+$, 277.1649. $C_{16}H_{24}O_2Si+H^+$ requires 277.1624).

4.1.4.3. (3*E*)-4-{3-[(*tert*-Butyldimethylsilyl)oxy]phenyl}but-3-en-2-one (19c). Yield: 0.68 g, 99%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (3:2); ν_{max} (ATR) 2956, 2859, 1671, 1597, 1480, 1255, 979, 840; δ_H (250 MHz; $CDCl_3$) 7.46 (1H, d, *J* 16.3, H-4), 7.26 (1H, dd, *J* 8.0, 7.7, ArH), 7.15 (1H, d, *J* 7.7, ArH), 7.02 (1H, s, ArH), 6.89 (1H, d, *J* 8.0, ArH), 6.68 (1H, d, *J* 16.3, H-3), 2.39 (3H, s, H-1), 1.01 (9H, s, Si^{t}Bu), 0.22 (6H, s, $SiMe_2$); δ_C (62.5 MHz; $CDCl_3$) 198.3 (C), 156.1 (C), 143.3 (CH), 135.8 (C), 129.9 (CH), 127.2 (CH), 122.3 (CH), 121.5 (CH), 119.4 (CH), 27.4 (Me), 25.6 (Me), 18.1 (C), −4.5 (Me); (found: $[M+H]^+$, 277.1649. $C_{16}H_{24}O_2Si+H^+$ requires 277.1624).

4.1.4.4. (3*E*)-4-{4-[(*tert*-Butyldimethylsilyl)oxy]phenyl}but-3-en-2-one (19d). Yield: 0.68 g, 99%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (9:1); ν_{max} (ATR) 2929, 2858, 1681, 1596, 1510, 1270, 1180, 910, 838; δ_H (250 MHz; $CDCl_3$) 7.47 (1H, d, *J* 16.4, H-4), 7.44 (2H, d, *J* 8.6, ArH), 6.85 (2H, d, *J* 8.6, ArH), 6.60 (1H, d, *J* 16.4, H-3), 2.35 (3H, s, H-1), 0.99 (9H, s, Si^{t}Bu), 0.22 (6H, s, $SiMe_2$); δ_C (62.5 MHz; $CDCl_3$) 198.3 (C), 158.1 (C), 143.3 (CH), 129.9 (CH), 127.6 (C), 125.2 (CH), 120.6 (CH), 27.3 (Me), 25.6 (Me), 18.2 (C), −4.4 (Me); (found: $[M+H]^+$, 277.1649. $C_{16}H_{24}O_2Si+H^+$ requires 277.1624).

4.1.5. General procedure for the preparation of ethyl phenylhex-5-en-2-ynoates (9a-m)

To a freshly prepared solution of lithium diisopropylamine in THF (0.5 M; 1.8 equiv) at −78 °C under nitrogen atmosphere, was added ethyl propiolate (1.8 equiv) dropwise. After 10 min, a solution of the ketone in THF (0.5 M; 1.0 equiv) at −78 °C was added via cannula. After 30 min, a saturated aqueous ammonium chloride solution was slowly poured onto the reaction mixture and the latter was extracted into ether twice. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude was purified by chromatography, eluting with hexane and ethyl acetate (see analytical details).

4.1.5.1. Ethyl (5*E*)-4-hydroxy-4-methyl-6-phenylhex-5-en-2-ynoate (9). Yield: 810 mg, 97%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (4:1); ν_{max} (ATR) 3406, 2984, 2239, 1712, 1590, 1246, 968; δ_H (250 MHz; $CDCl_3$) 7.42-7.26 (5H, m, ArH), 6.85 (1H, d, *J* 16.0, H-6), 6.27 (1H, d, *J* 16.0, H-5), 4.26 (2H, q, *J* 7.1, CH_2(Et)), 2.66 (1H, s, OH-4), 1.71 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 153.4 (C), 135.7 (C), 131.3 (CH), 130.0 (CH), 128.6 (CH), 128.2 (CH), 126.8 (CH), 88.3 (C), 76.5 (C), 67.8 (C), 62.2 (CH_2), 29.7 (Me), 13.9 (Me); (found: $[M-OH]^+$, 227.1094. $[C_{15}H_{16}O_3-OH]^+$ requires 227.1072).

4.1.5.2. Ethyl (5*E*)-4-hydroxy-4-methyl-6-[2-(trifluoromethyl)-phenyl]hex-5-en-2-ynoate (9a). Yield: 341 mg, 78%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (85:15); ν_{max} (ATR) 3419, 2987, 2241, 1713, 1576, 1314, 1248, 1161, 1122, 1034; δ_H (250 MHz; $CDCl_3$) 7.64 (1H, d, *J* 7.8, ArH), 7.61 (1H, d, *J* 7.8, ArH), 7.51 (1H, t, *J* 7.6, ArH), 7.38 (1H, t, *J* 7.6, ArH), 7.26 (1H, dq, *J* 15.7, 2.1, H-6), 6.26 (1H, d, *J* 15.7, H-5), 4.26 (2H, q, *J* 7.1, CH_2(Et)), 2.85 (1H, s, OH-4), 1.72 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 153.4 (C), 135.6 (CH), 135.0 (C), 131.9 (CH), 127.82 (C, q, ^2J 30.0), 127.8 (CH), 127.6 (CH), 126.2 (CH), 125.8 (CH, q, ^3J 5.7), 124.1 (CF_3, q, ^1J 273.9), 87.8 (C), 76.6 (C), 67.7 (C), 62.2 (CH_2), 29.5 (Me), 13.9 (Me); (found: $[M-OH]^+$, 295.0956. $[C_{16}H_{15}F_3O_3-OH]^+$ requires 295.0946).

4.1.5.3. Ethyl (5*E*)-4-hydroxy-4-methyl-6-[4-(trifluoromethyl)-phenyl]hex-5-en-2-ynoate (9b). Yield: 332 mg, 76%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (85:15); ν_{max} (ATR) 3420, 2991, 2241, 1714, 1617, 1325, 1249, 1123, 1067; δ_H (250 MHz; $CDCl_3$) 7.58 (2H, d, *J* 8.3, ArH), 7.49 (2H, d, *J* 8.3, ArH), 6.89 (1H, d, *J* 16.0, H-6), 6.36 (1H, d, *J* 16.0, H-5), 4.27 (2H, q, *J* 7.1, CH_2(Et)), 2.82 (1H, s, OH-4), 1.72 (3H, s, Me-4), 1.33 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 153.4 (C), 139.3 (C), 133.8 (CH), 129.9 (C, q, ^2J 32.5), 128.7 (CH), 127.0 (CH), 125.8 (CH, q, ^3J 3.6), 124.1 (CF_3, q, ^1J 273.5), 87.9 (C), 76.7 (C), 67.7 (C), 62.3 (CH_2), 29.7 (Me), 13.9 (Me); (found: $[M-OH]^+$, 295.0956. $[C_{16}H_{15}F_3O_3-OH]^+$ requires 295.0946).

4.1.5.4. Ethyl (5*E*)-4-hydroxy-4-methyl-6-(4-fluorophenyl)hex-5-en-2-ynoate (9c). Yield: 466 mg, 97%, as a dark orange oil; Rf = 0.33, hexane/ethyl acetate (4:1); ν_{max} (ATR) 2986, 2240, 1712, 1602, 1510, 1368, 1247, 1159, 817; δ_H (250 MHz; $CDCl_3$) 7.37 (2H, dd, *J* 8.4, 8.4, ArH), 7.02 (2H, dd, *J* 8.6, 8.6, ArH), 6.82 (1H, d, *J* 15.9, H-6), 6.19 (1H, d, *J* 15.9, H-5), 4.26 (2H, q, *J* 7.1, CH_2(Et)), 2.67 (1H, s, OH-4), 1.70 (3H, s, Me-4), 1.33 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 162.7 (CF, d, ^1J 247.8), 153.5 (C), 131.9 (C, d, ^4J 3.2), 131.0 (CH), 128.9 (CH), 128.5 (CH, ^3J 8.2), 115.6 (CH, ^2J 21.7), 88.2 (C), 76.5 (C), 67.8 (C), 62.2 (CH_2), 29.8 (Me), 14.0 (Me); (found: $[M-OH]^+$, 245.0929. $[C_{15}H_{15}FO_3-OH]^+$ requires 245.0978).

4.1.5.5. Ethyl (5*E*)-4-hydroxy-4-methyl-6-(2,4-dimethoxyphenyl)-hex-5-en-2-ynoate (9d). Yield: 406 mg, 92%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (7:3); ν_{max} (ATR) 3452, 2939, 2238, 1710, 1609, 1504, 1245, 1209, 1159, 1030; δ_H (250 MHz; $CDCl_3$) 7.32 (1H, d, *J* 8.2, ArH), 7.05 (1H, d, *J* 16.0, H-6), 6.47 (1H, dd, *J* 8.2, 2.4, ArH), 6.46 (1H, d, *J* 2.4, ArH), 6.24 (1H, d, *J* 16.0, H-

5), 4.25 (2H, q, *J* 7.1, CH_2(Et)), 3.83 (3H, s, ArOMe), 3.81 (3H, s, ArOMe), 2.44 (1H, s, OH-4), 1.70 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 160.9 (C), 158.3 (C), 153.3 (C), 129.8 (CH), 128.4 (CH), 125.0 (CH), 117.7 (C), 104.8 (CH), 98.4 (CH), 88.9 (C), 76.3 (C), 68.2 (C), 62.1 (CH_2), 55.4 (OMe), 55.3 (OMe), 29.7 (Me), 14.0 (Me); (found: $[M-OH]^+$, 287.1246. $[C_{17}H_{20}O_6-OH]^+$ requires 287.1283).

4.1.5.6. Ethyl (5*E*)-4-hydroxy-4-methyl-6-(3,4-dimethoxyphenyl)-hex-5-en-2-ynoate (9e). Yield: 563 mg, 80%, as a dark orange oil; Rf = 0.33, hexane/ethyl acetate (65:35); ν_{max} (ATR) 3481, 2931, 2237, 1709, 1515, 1247, 1139, 1025; δ_H (250 MHz; $CDCl_3$) 6.95 (1H, d, *J* 8.6, ArH), 6.94 (1H, s, ArH), 6.82 (1H, d, *J* 8.6, ArH), 6.79 (1H, d, *J* 15.9, H-6), 6.15 (1H, d, *J* 15.9, H-5), 4.27 (2H, q, *J* 7.1, CH_2(-Et)), 3.90 (3H, s, ArOMe), 3.88 (3H, s, ArOMe), 2.59 (1H, s, OH-4), 1.71 (3H, s, Me-4), 1.33 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 153.5 (C), 149.3 (C), 149.0 (C), 129.9 (CH), 129.3 (CH), 128.7 (C), 120.2 (CH), 111.1 (CH), 109.0 (CH), 88.5 (C), 76.5 (C), 67.9 (C), 62.2 (CH_2), 55.9 (OMe), 55.8 (OMe), 29.8 (Me), 14.0 (Me); (found: $[M-OH]^+$, 287.1293. $[C_{17}H_{20}O_5-OH]^+$ requires 287.1283).

4.1.5.7. Ethyl (5*E*)-4-hydroxy-4-methyl-6-(2,3,4-trimethoxy-phenyl)hex-5-en-2-ynoate (9f). Yield: 360 mg, 85%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (7:3); ν_{max} (ATR) 3450, 2985, 2238, 1712, 1597, 1495, 1437, 1244, 1095; δ_H (250 MHz; $CDCl_3$) 7.14 (1H, d, *J* 8.7, ArH), 7.05 (1H, d, *J* 16.1, H-6), 6.66 (1H, d, *J* 8.7, ArH), 6.24 (1H, d, *J* 16.1, H-5), 4.26 (2H, q, *J* 7.1, CH_2(Et)), 3.874 (3H, s, ArOMe), 3.869 (6H, s, ArOMe), 2.55 (1H, s, OH-4), 1.71 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 153.7 (C), 153.5 (C), 151.9 (C), 142.3 (C), 130.7 (CH), 124.6 (CH), 122.7 (C), 121.5 (CH), 107.6 (CH), 88.6 (C), 76.4 (C), 68.1 (C), 62.1 (CH_2), 61.1 (OMe), 60.8 (OMe), 56.0 (OMe), 29.7 (Me), 13.9 (Me); (found: $[M-OH]^+$, 317.1368. $[C_{18}H_{22}O_6-OH]^+$ requires 317.1389).

4.1.5.8. Ethyl (5*E*)-4-hydroxy-4-methyl-6-(2,4,5-trimethoxy-phenyl)hex-5-en-2-ynoate (9g). Yield: 387 mg, 91%, as a red oil; Rf = 0.33, hexane/ethyl acetate (3:2); ν_{max} (ATR) 3470, 2937, 2213, 1708, 1609, 1511, 1465, 1244, 1032; δ_H (250 MHz; $CDCl_3$) 7.09 (1H, d, *J* 15.9, H-6), 6.94 (1H, s, ArH), 6.50 (1H, s, ArH), 6.21 (1H, d, *J* 15.9, H-5), 4.26 (2H, q, *J* 7.1, CH_2(Et)), 3.90 (3H, s, ArOMe), 3.86 (3H, s, ArOMe), 3.83 (3H, s, ArOMe), 2.55 (1H, s, OH-4), 1.72 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 153.5 (C), 151.9 (C), 150.0 (C), 143.2 (C), 129.6 (CH), 124.6 (CH), 116.3 (C), 110.3 (CH), 97.5 (CH), 88.9 (C), 76.2 (C), 68.1 (C), 62.1 (CH_2), 56.5 (OMe), 56.0 (OMe), 29.7 (Me), 14.0 (Me); (found: $[M-OH]^+$, 317.1368. $[C_{18}H_{22}O_6-OH]^+$ requires 317.1389).

4.1.5.9. Ethyl (5*E*)-4-hydroxy-4-methyl-6-(3,4,5-trimethoxy-phenyl)hex-5-en-2-ynoate (9h). Yield: 178 mg, 42%, as light orange oil; Rf = 0.33, hexane/ethyl acetate (3:2); ν_{max} (ATR) 3457, 2942, 2238, 1711, 1584, 1508, 1419, 1242, 1127; δ_H (250 MHz; $CDCl_3$) 6.77 (1H, d, *J* 15.9, H-6), 6.62 (2H, s, ArH), 6.19 (1H, d, *J* 15.9, H-5), 4.27 (2H, q, *J* 7.1, CH_2(Et)), 3.88 (6H, s, ArOMe), 3.85 (3H, s, ArOMe), 2.59 (1H, s, OH-4), 1.71 (3H, s, Me-4), 1.33 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 153.5 (C), 153.3 (C), 138.3 (C), 131.4 (C), 130.7 (CH), 130.0 (CH), 104.0 (CH), 88.3 (C), 76.5 (C), 67.8 (C), 62.2 (CH_2), 60.9 (OMe), 56.1 (OMe), 29.8 (Me), 14.0 (Me); (found: $[M-OH]^+$, 317.1368. $[C_{18}H_{22}O_6-OH]^+$ requires 317.1389).

4.1.5.10. Ethyl (5*E*)-4-hydroxy-4-methyl-6-(2,4,6-trimethoxy-phenyl)hex-5-en-2-ynoate (9i). Yield: 311 mg, 73%, as a dark orange oil; Rf = 0.33, hexane/ethyl acetate (65:35); ν_{max} (ATR) 2939, 2210, 1707, 1605, 1456, 1245, 1205, 1119, 736; δ_H (250 MHz; $CDCl_3$) 7.15 (1H, d, *J* 16.2, H-6), 6.65 (1H, d, *J* 16.2, H-5), 6.12 (2H, s, ArH), 4.25 (2H, q, *J* 7.1, CH_2(Et)), 3.83 (6H, s, ArOMe), 3.82 (3H, s, ArOMe), 2.41 (1H, s, OH-4), 1.70 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)); δ_C (62.5 MHz; $CDCl_3$) 160.7 (C), 159.6 (CH), 153.7 (C), 132.0 (CH), 120.4 (CH), 106.2 (C), 90.5 (CH), 89.4 (C), 76.3 (C), 68.9 (C), 62.0 (CH2), 55.6 (OMe), 55.2 (OMe), 29.7 (Me), 14.0 (Me); (found: $[M-OH]^+$, 317.1343. $[C_{18}H_{22}O_6-OH]$+ requires 317.1389).

4.1.5.11. Ethyl (5*E*)-6-{4-[(*tert*-Butyldimethylsilyl)oxy]-3,5-dimethoxyphenyl]-4-hydroxy-4-methyl-hex-5-en-2-ynoate (9j). Yield: 528 mg, 80%, as an off-white solid; Rf = 0.33, hexane/ethyl acetate (4:1); mp 139-140 °C; ν_{max} (ATR) 3433, 2930, 2239, 1697, 1584, 1510, 1240, 1110, 892, 785; δ_H (250 MHz; $CDCl_3$) 6.75 (1H, d, *J* 15.9, H-6), 6.60 (2H, s, ArH), 6.14 (1H, d, *J* 15.9, H-5), 4.26 (2H, q, *J* 7.1, CH_2(Et)), 3.81 (6H, s, ArOMe), 2.50 (1H, s, OH-4), 1.70 (3H, s, Me-4), 1.33 (3H, t, *J* 7.1, CH_3(Et)), 1.01 (9H, s, Si^tBu), 0.13 (6H, s, $SiMe_2$); δ_C (62.5 MHz; $CDCl_3$) 153.5 (C), 151.7 (C), 134.9 (C), 130.4 (C), 129.4 (CH), 128.4 (C), 104.0 (CH), 88.5 (C), 76.5 (C), 67.9 (C), 62.2 (CH_2), 55.7 (OMe), 29.8 (Me), 25.7 (Me), 18.7 (C), 14.0 (Me), −4.7 (Me); (found: $[M-OH]^+$, 417.2172. $[C_{23}H_{34}O_6Si-OH]^+$ requires 417.2097).

4.1.5.12. Ethyl (5*E*)-6-{2-[(*tert*-Butyldimethylsilyl)oxy]phenyl}-4-hydroxy-4-methylhex-5-en-2-ynoate (9k). Yield: 544 mg, 61%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (9:1); ν_{max} (ATR) 3450, 2931, 2239, 1714, 1598, 1485, 1250, 1028, 919, 878; δ_H (250 MHz; $CDCl_3$) 7.44 (1H, d, *J* 7.6, ArH), 7.21 (1H, d, *J* 16.0, H-6), 7.15 (1H, t, *J* 8.0, ArH), 6.92 (1H, t, *J* 7.6, ArH), 6.80 (1H, d, *J* 8.0, ArH), 6.22 (1H, d, *J* 16.0, H-5), 4.25 (2H, q, *J* 7.1, CH_2(Et)), 2.42 (1H, s, OH-4), 1.70 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)), 1.02 (9H, s, Si^tBu), 0.22 (6H, s, $SiMe_2$); δ_C (62.5 MHz; $CDCl_3$) 153.4 (C), 153.3 (C), 131.1 (CH), 129.1 (CH), 127.1 (C), 126.7 (CH), 125.7 (CH), 121.4 (CH), 119.6 (CH), 88.4 (C), 76.6 (C), 68.3 (C), 62.1 (CH_2), 29.7 (Me), 25.7 (Me), 18.2 (C), 14.0 (Me), −4.2 (Me); (found: $[M-OH]^+$, 357.1881. $[C_{21}H_{30}O_4Si-OH]^+$ requires 357.1886).

4.1.5.13. Ethyl (5*E*)-6-{3-[(*tert*-Butyldimethylsilyl)oxy]phenyl}-4-hydroxy-4-methylhex-5-en-2-ynoate(9l). Yield: 812 mg, 94%, as a yellow oil; Rf = 0.33, hexane/ethyl acetate (95:5); ν_{max} (ATR) 3408, 2956, 2239, 1715, 1580, 1486, 1253, 1173, 974, 855; δ_H (250 MHz; $CDCl_3$) 7.18 (1H, at, *J* 7.8, ArH), 7.00 (1H, d, *J* 7.6, ArH), 6.87 (1H, s, ArH), 6.79 (1H, d, *J* 15.9, H-6), 6.76-6.74 (1H, m, ArH), 6.22 (1H, d, *J* 15.9, H-5), 4.26 (2H, q, *J* 7.1, CH_2(Et)), 2.57 (1H, s, OH-4), 1.70 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)), 0.99 (9H, s, Si^tBu), 0.20 (6H, s, $SiMe_2$); δ_C (62.5 MHz; $CDCl_3$) 155.9 (C), 153.5 (C), 137.2 (C), 131.3 (CH), 130.0 (CH), 129.5 (CH), 120.00 (CH), 119.96 (CH), 118.5 (CH), 88.3 (C), 76.5 (C), 67.8 (C), 62.2 (CH_2), 29.7 (Me), 25.7 (Me), 18.2 (C), 14.0 (Me), −4.4 (Me); (found: $[M-OH]^+$, 357.1881. $[C_{21}H_{30}O_4Si-OH]^+$ requires 357.1886).

4.1.5.14. Ethyl (5*E*)-6-{4-[(*tert*-Butyldimethylsilyl)oxy]phenyl}-4-hydroxy-4-methylhex-5-en-2-ynoate (9m). Yield: 619 mg, 69%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (9:1); ν_{max} (ATR) 3440, 2931, 2240, 1714, 1604, 1509, 1253, 912, 840; δ_H (250 MHz; $CDCl_3$) 7.28 (2H, d, *J* 8.6, ArH), 6.80 (2H, d, *J* 8.6, ArH), 6.78 (1H, d, *J* 15.9, H-6), 6.13 (1H, d, *J* 15.9, H-5), 4.26 (2H, q, *J* 7.1, CH_2(Et)), 2.54 (1H, s, OH-4), 1.69 (3H, s, Me-4), 1.32 (3H, t, *J* 7.1, CH_3(Et)), 0.98 (9H, s, Si^tBu), 0.20 (6H, s, $SiMe_2$); δ_C (62.5 MHz; $CDCl_3$) 155.9 (C), 153.5 (C), 129.7 (CH), 129.3 (CH), 129.0 (C), 128.0 (CH), 120.3 (CH), 88.5 (C), 76.4 (C), 67.9 (C), 62.2 (CH_2), 29.8 (Me), 25.6 (Me), 18.2 (C), 14.0 (Me), −4.4 (Me); (found: $[M-OH]^+$, 357.1881. $[C_{21}H_{30}O_4Si-OH]^+$ requires 357.1886).

4.1.6. General procedure for the preparation of 5-methyl-5-[(*E*)-2-phenylethenyl]-2,5-dihydrofuran-2-ones (7, 10-18, 20-26)

A mixture of alkyne (1.0 equiv), quinoline (1.0 equiv) and Lindlar's catalyst (4 wt %) in methanol (0.1 M) was stirred for 3 h at room temperature under a hydrogen atmosphere. The reaction

mixture was then filtered through Celite® and the filter cake washed several times with ethyl acetate. The filtrate was washed with an equal amount a hydrochloric acid solution (1 M), brine, dried over magnesium sulfate, filtered and evaporated under reduced pressure. If necessary, the crude was taken up in a mixture of methanol and dichloromethane (1:1) and stirred for another 2 h at room temperature. The reaction mixture was filtered and evaporated under reduced pressure. The crude was purified by chromatography, eluting with hexane and ethyl acetate (see analytical details).

4.1.6.1. 5-Methyl-5-[(*E*)-2-phenylethenyl]-2,5-dihydrofuran-2-one (7). Yield: 0.51 g, 79%, as a light orange solid; Rf = 0.33, hexane/ethyl acetate (7:3); mp 90-91 °C; ν_{max} (ATR) 2982, 1754, 1602, 1449, 1233, 1104, 943, 751; δ_H (250 MHz; $CDCl_3$) 7.46 (1H, d, *J* 5.5, H-4), 7.37-7.26 (5H, m, ArH), 6.65 (1H, d, *J* 15.9, H-2′), 6.18 (1H, d, *J* 16.4, H-1′), 6.05 (1H, d, *J* 5.5, H-3), 1.67 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.3 (C), 159.3 (CH), 135.5 (C), 130.9 (CH), 128.6 (CH), 128.4 (CH), 126.8 (CH), 126.6 (CH), 119.8 (CH), 87.8 (C), 24.1 (Me); (found: $[M+H]^+$, 201.0914. $C_{13}H_{12}O_2+H^+$ requires 201.0910).

4.1.6.2. 5-Methyl-5-[(*E*)-4-(2-trifluoromethylphenyl)ethenyl]-2,5-dihydrofuran-2-one (10). Yield: 225 mg, 90%, as a light yellow oil; Rf = 0.33, hexane/ethyl acetate (4:1); ν_{max} (ATR) 1759, 1605, 1314, 1276, 1161, 1036, 916, 813, 766; δ_H (250 MHz; $CDCl_3$) 7.64 (1H, d, *J* 7.9, ArH), 7.55 (1H, at, *J* 7.9, ArH), 7.51 (1H, d, *J* 7.5, ArH), 7.48 (1H, d, *J* 5.6, H-4), 7.39 (1H, at, *J* 7.5, ArH), 7.01 (1H, dq, *J* 16.0, 2.1, H-2′), 6.15 (1H, d, *J* 16.0, H-1′), 6.10 (1H, d, *J* 5.6, H-3), 1.70 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.0 (C), 158.8 (CH), 134.8 (C), 132.0 (CH), 131.6 (CH), 128.0 (CH), 127.7 (C, q, ^2J 30.0), 127.6 (CH), 127.5 (CH), 125.8 (CH, q, ^3J 5.6), 124.1 (CF_3, q, ^1J 273.5), 120.4 (CH), 87.6 (C), 23.8 (Me); (found: $[M+H]^+$, 269.0797. $C_{14}H_{11}F_3O_2+H^+$ requires 269.0789).

4.1.6.3. 5-Methyl-5-[(*E*)-2-(4-trifluoromethylphenyl)ethenyl]-2,5-dihydrofuran-2-one (11). Yield: 222 mg, 88%, as a light yellow oil; Rf = 0.33, hexane/ethyl acetate (4:1); ν_{max} (ATR) 2933, 1757, 1617, 1324, 1234, 1104, 1067, 944, 816; δ_H (250 MHz; $CDCl_3$) 7.58 (2H, d, *J* 8.3, ArH), 7.49 (1H, d, *J* 5.6, H-4), 6.46 (2H, d, *J* 8.3, ArH), 6.71 (1H, d, *J* 16.1, H-2′), 6.29 (1H, d, *J* 16.1, H-1′), 6.08 (1H, d, *J* 5.6, H-3), 1.69 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.1 (C), 158.9 (CH), 139.1 (C), 130.1 (C, q, ^2J 32.6), 129.5 (CH), 129.4 (CH), 126.8 (CH), 125.6 (CH, q, ^3J 3.8), 124.0 (CF_3, q, ^1J 272.0), 120.0 (CH), 87.5 (C), 24.1 (Me); (found: $[M+H]^+$, 269.0797. $C_{14}H_{11}O_3F_2+H^+$ requires 269.0789).

4.1.6.4. 5-Methyl-5-[(*E*)-4-(4-fluorophenyl)ethenyl]-2,5-dihydrofuran-2-one (12). Yield: 299 mg, 82%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (7:3); ν_{max} (ATR) 2984, 1756, 1602, 1510, 1231, 1104, 944, 819; δ_H (250 MHz; $CDCl_3$) 7.47 (1H, d, *J* 5.5, H-4), 7.34 (1H, d, *J* 8.6, ArH), 7.33 (1H, d, *J* 8.6, ArH), 7.01 (2H, at, *J* 8.6, ArH), 6.62 (1H, d, *J* 16.1, H-2′), 6.10 (1H, d, *J* 16.1, H-1′), 6.06 (1H, d, *J* 5.5, H-3), 1.67 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.2 (C), 162.2 (CF, d, ^1J 248.2), 159.2 (CH), 131.8 (C, d, ^4J 3.3), 129.7 (CH), 128.3 (CH, d, ^3J 8.1), 126.6 (CH), 119.8 (CH), 115.6 (CH, d, ^2J 21.7), 87.7 (C), 24.1 (Me); (found: $[M+H]^+$, 219.0791. $C_{13}H_{11}FO_2+H^+$ requires 219.0821).

4.1.6.5. 5-Methyl-5-[(*E*)-2-(2,4-dimethoxyphenyl)ethenyl]-2,5-dihydrofuran-2-one (13). Yield: 224 mg, 70%, as a light yellow oil; Rf = 0.33, hexane/ethyl acetate (7:3); ν_{max} (ATR) 2938, 1750, 1608, 1505, 1288, 1209, 1160, 1103, 941, 819; δ_H (250 MHz; $CDCl_3$) 7.46 (1H, d, *J* 5.6, H-4), 7.30 (1H, d, *J* 8.3, ArH), 6.84 (1H, d, *J* 16.3, H-2′), 6.45 (1H, d, *J* 8.3, ArH), 6.43 (1H, s, ArH), 6.11 (1H, d, *J* 16.3, H-1′), 6.03 (1H, d, *J* 5.6, H-3), 3.82 (3H, s, ArOMe), 3.81 (3H, s, ArOMe), 1.67 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.5 (C), 161.0 (C), 159.7 (CH), 158.1 (C), 128.1 (CH), 126.0 (CH), 125.1 (CH), 119.7 (CH), 117.5 (C), 104.9 (CH), 98.4 (CH), 88.5 (C), 55.3 (2× OMe), 24.1 (Me); (found: $[M+H]^+$, 261.1238. $C_{15}H_{16}O_4+H^+$ requires 261.1127).

4.1.6.6. 5-Methyl-5-[(*E*)-2-(3,4-dimethoxyphenyl)ethenyl]-2,5-dihydrofuran-2-one (14). Yield: 215 mg, 72%, as a white solid; Rf = 0.33, hexane/ethyl acetate (1:1); mp 113-114 °C; ν_{max} (ATR) 2938, 1747, 1601, 1515, 1268, 1106, 1026, 946, 820; δ_H (250 MHz; $CDCl_3$) 7.47 (1H, d, *J* 5.6, H-4), 6.93-6.91 (2H, m, ArH), 6.82 (1H, d, *J* 8.7, ArH), 6.58 (1H, d, *J* 16.1, H-2′), 6.05 (1H, d, *J* 16.1, H-2′), 6.05 (1H, d, *J* 5.6, H-3), 3.89 (3H, s, ArOMe), 3.88 (3H, s, ArOMe), 1.68 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.3 (C), 159.4 (CH), 149.3 (C), 149.0 (C), 130.7 (CH), 128.5 (C), 124.7 (CH), 120.0 (CH), 119.6 (CH), 111.0 (CH), 108.7 (CH), 87.9 (C), 55.8 (OMe), 55.7 (OMe), 24.0 (Me); (found: $[M+H]^+$, 261.1138. $C_{15}H_{16}O_4+H^+$ requires 261.1127).

4.1.6.7. 5-Methyl-5-[(*E*)-2-(2,3,4-trimethoxyphenyl)ethenyl]-2,5-dihydrofuran-2-one (15). Yield: 154 mg, 55%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (3:2); ν_{max} (ATR) 2939, 1751, 1597, 1495, 1464, 1295, 1095, 942, 820; δ_H (250 MHz; $CDCl_3$) 7.47 (1H, d, *J* 5.5, H-4), 7.11 (1H, d, *J* 8.7, ArH), 6.82 (1H, d, *J* 16.3, H-2′), 6.66 (1H, d, *J* 8.7, ArH), 6.14 (1H, d, *J* 16.3, H-1′), 6.06 (1H, d, *J* 5.5, H-3), 3.86 (9H, s, 3× ArOMe), 1.68 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.4 (C), 159.5 (CH), 153.8 (C), 151.8 (C), 142.2 (C), 126.2 (CH), 125.7 (CH), 122.5 (C), 121.3 (CH), 119.9 (CH), 107.6 (CH), 88.2 (C), 61.1 (OMe), 60.8 (OMe), 56.0 (OMe), 24.0 (Me); (found: $[M+H]^+$, 291.1220. $C_{16}H_{18}O_5+H^+$ requires 291.1233).

4.1.6.8. 5-Methyl-5-[(*E*)-2-(2,4,5-trimethoxyphenyl)ethenyl]-2,5-dihydrofuran-2-one (16). Yield: 164 mg, 55%, as an orange oil; Rf = 0.33, hexane/ethyl acetate (3:2); ν_{max} (ATR) 2936, 1749, 1609, 1511, 1465, 1208, 1103, 1031, 941, 819; δ_H (250 MHz; $CDCl_3$) 7.47 (1H, d, *J* 5.5, H-4), 6.91 (1H, s, ArH), 6.89 (1H, d, *J* 16.3, H-2′), 6.49 (1H, s, ArH), 6.09 (1H, d, *J* 16.3, H-1′), 6.05 (1H, d, *J* 5.5, H-3), 3.90 (3H, s, ArOMe), 3.85 (3H, s, ArOMe), 3.83 (3H, s, ArOMe), 1.69 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.5 (C), 159.7 (CH), 151.8 (C), 150.2 (C), 143.2 (C), 125.8 (CH), 125.0 (CH), 119.8 (CH), 116.1 (C), 110.0 (CH), 97.3 (CH), 88.4 (C), 56.5 (OMe), 56.3 (OMe), 56.0 (OMe), 24.0 (Me); (found: $[M+H]^+$, 291.1239. $C_{16}H_{18}O_5+H^+$ requires 291.1233).

4.1.6.9. 5-Methyl-5-[(*E*)-2-(3,4,5-trimethoxyphenyl)ethenyl]-2,5-dihydrofuran-2-one (17). Yield: 64 mg, 50%, as a yellow solid; Rf = 0.33, hexane/ethyl acetate (3:2); mp 125-126 °C; ν_{max} (ATR) 2970, 1739, 1581, 1510, 1421, 1247, 1105, 936, 815,; δ_H (250 MHz; $CDCl_3$) 7.48 (1H, d, *J* 5.5, H-4), 6.59 (3H, s+d, *J* 16.0, ArH, H-2′), 6.10 (1H, d, *J* 16.0, H-1′), 6.07 (1H, d, *J* 5.5, H-3), 3.87 (6H, s, 2× ArOMe), 3.85 (3H, s, ArOMe), 1.69 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.3 (C), 159.3 (C), 153.3 (C), 138.3 (C), 131.2 (C), 130.9 (CH), 126.2 (CH), 119.8 (CH), 103.7 (CH), 87.8 (C), 60.8 (OMe), 56.0 (OMe), 24.0 (Me); (found: $[M+H]^+$, 291.1212. $C_{16}H_{18}O_5+H^+$ requires 291.1233).

4.1.6.10. 5-Methyl-5-[(*E*)-2-(2,4,6-trimethoxyphenyl)ethenyl]-2,5-dihydrofuran-2-one (18). Yield: 190 mg, 79%, as a light yellow oil; Rf = 0.33, hexane/ethyl acetate (3:2); ν_{max} (ATR) 2939, 1749, 1604, 1456, 1205, 1156, 1119, 941, 816; δ_H (250 MHz; $CDCl_3$) 7.47 (1H, d, *J* 5.5, H-4), 6.88 (1H, d, *J* 16.5, H-2′), 6.54 (1H, d, *J* 16.5, H-1′), 6.11 (2H, s, ArH), 6.01 (1H, d, *J* 5.5, H-3), 3.82 (6H, s, 2× ArOMe), 3.81 (3H, s, ArOMe), 1.66 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.8 (C), 160.8 (C), 160.2 (CH), 159.6 (C), 127.5 (CH), 121.6 (CH), 119.4 (CH), 106.1 (C), 90.5 (CH), 89.4 (C), 55.6 (OMe), 55.2 (OMe), 24.2 (Me); (found: $[M+H]^+$, 291.1259. $C_{16}H_{18}O_5+H^+$ requires 291.1233).

4.1.7. General procedure for the preparation of hydroxylated 5-methyl-5-[(*E*)-2-phenylethenyl]-2,5-dihydrofuran-2-ones (20-23)

A mixture of alkyne (1.0 equiv), quinoline (1.0 equiv) and Lindlar's catalyst (4 wt %) in methanol (0.1 M) was stirred for 3 h at room temperature under a hydrogen atmosphere. The reaction mixture was then filtered through Celite® and the filter cake washed several times with ethyl acetate. The filtrate was washed with an equal amount a hydrochloric acid solution (1 M), brine, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude was then taken up in THF (50 mM) under a nitrogen atmosphere and a solution of HF· pyridine (70%; 50 equiv) was added dropwise. After stirring for 16 h at room temperature, the reaction mixture was poured onto ice water and diluted with ether. This mixture was then washed twice with a saturated aqueous sodium hydrogen carbonate solution (!!!vigorous reaction!!!). The organic later was washed with brine, dried over magnesium sulfate, filtered and evaporated under reduces pressure. The crude was purified by chromatography, eluting with hexane and ethyl acetate (see analytical details).

4.1.7.1. 5-[(*E*)-2-(4-Hydroxy-3,5-dimethoxyphenyl)ethenyl]-5-methyl-2,5-dihydrofuran-2-one (20). Yield: 177 mg, 57%, as a light orange powder; Rf = 0.33, hexane/ethyl acetate (3:2); mp 150-151 °C; ν_{max} (ATR) 3446, 2960, 1743, 1607, 1515, 1214, 1105, 971, 829; δ_H (250 MHz; $CDCl_3$) 7.47 (1H, d, *J* 5.6, H-4), 6.60 (2H, s, ArH), 6.55 (1H, d, *J* 16.2, H-2′), 6.06 (1H, d, *J* 5.6, H-3), 6.02 (1H, d, *J* 16.2, H-1′), 5.69 (1H, bs, ArOH) 3.89 (6H, s, 2× ArOMe), 1.68 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.3 (C), 159.4 (CH), 147.1 (C), 135.2 (C), 131.1 (CH), 127.0 (C), 124.7 (CH), 119.7 (CH), 103.5 (CH), 87.9 (C), 56.2 (OMe), 24.0 (Me); (found: $[M+H]^+$, 277.1103. $C_{15}H_{16}O_5+H^+$ requires 277.1076).

4.1.7.2. 5-[(*E*)-2-(2-Hydroxyphenyl)ethenyl]-5-methyl-2,5-dihydrofuran-2-one (21). Yield: 246 mg, 77%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (3:2); ν_{max} (ATR) 3341, 2978, 1731, 1603, 1457, 1254, 1103, 947, 819, 754; δ_H (250 MHz; $CDCl_3$) 7.49 (1H, d, *J* 5.5, H-4), 7.32 (1H, d, *J* 7.6, ArH), 7.13 (1H, at, *J* 8.4, ArH), 6.92 (1H, d, *J* 16.1, H-2′), 6.89-6.81 (2H, m, ArH), 6.58 (1H, bs, ArOH), 6.25 (1H, d, *J* 16.1, H-1′), 6.04 (1H, d, *J* 5.5, H-3), 1.67 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 173.3 (C), 160.1 (CH), 153.8 (C), 129.4 (CH), 127.5 (CH), 127.2 (CH), 126.4 (CH), 122.7 (C), 120.6 (CH), 119.7 (CH), 116.1 (CH), 88.9 (C), 24.0 (Me); (found: $[M+H]^+$, 217.0933. $C_{13}H_{12}O_3+H^+$ requires 217.0865).

4.1.7.3. 5-[(*E*)-2-(3-Hydroxyphenyl)ethenyl]-5-methyl-2,5-dihydrofuran-2-one (22). Yield: 358 mg, 82%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (1:1); ν_{max} (ATR) 3353, 1732, 1584, 1450, 1236, 1105, 948, 819, 779 δ_H (250 MHz; $CDCl_3$) 7.46 (1H, d, *J* 5.6, H-4), 7.17 (1H, at, *J* 8.0, ArH), 6.89-6.87 (2H, m, ArH), 6.80 (1H, dd, *J* 8.0, 1.4, ArH), 6.56 (1H, d, *J* 16.1, H-2′), 6.11 (1H, d, *J* 16.1, H-1′), 6.05 (1H, d, *J* 5.6, H-3), 1.64 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 173.2 (C), 159.9 (CH), 156.3 (C), 137.0 (C), 131.0 (CH), 129.8 (CH), 126.6 (CH), 119.7 (CH), 119.1 (CH), 115.7 CH), 113.3 (CH), 88.5 (C), 24.0 (Me), (OH not observed); (found: $[M+H]^+$, 217.0933. $C_{13}H_{12}O_3+H^+$ requires 217.0865).

4.1.7.4. 5-[(*E*)-2-(4-Hydroxyphenyl)ethenyl]-5-methyl-2,5-dihydrofuran-2-one (23). Yield: 277 mg, 82%, as a beige solid; Rf = 0.33, hexane/ethyl acetate (3:2); mp 105-106 °C; ν_{max} (ATR) 3354, 2983, 1729, 1609, 1515, 1266, 1101, 947, 820, 736; δ_H (250 MHz; $CDCl_3$) 7.48 (1H, d, *J* 5.6, H-4), 7.20 (2H, d, *J* 8.5, ArH), 6.80 (2H, d, *J* 8.5, ArH), 6.72 (1H, bs, ArOH), 6.53 (1H, d, *J* 16.1, H-2′), 6.06 (1H, d, *J* 5.6, H-3), 5.98 (1H, d, *J* 16.1, H-1′), 1.66 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 173.3 (C), 160.2 (CH), 156.6 (C), 130.9 (CH), 128.1 (CH), 127.8 (C), 123.8 (CH), 119.6 (CH), 115.7 (CH), 88.7 (C), 24.0 (Me); (found: $[M+H]^+$, 217.0933. $C_{13}H_{12}O_3+H^+$ requires 217.0865).

4.1.8. General procedure for the methylation of phenols (24-26)

To a solution of phenol (1.0 equiv) and diisopropylethylamine (1.5 equiv) in acetonitrile and methanol (9:1) (0.25 M) was added trimethylsilyl diazomethane (1.3 equiv) at room temperature. The reaction mixture was stirred for 16 h upon which time an equal amount of saturated aqueous ammonium chloride solution and ether were poured. The organic layer was washed with brine, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude was purified by chromatography, eluting with hexane and ethyl acetate (see analytical details).

4.1.8.1. 5-[(*E*)-2-(2-Methoxyphenyl)ethenyl]-5-methyl-2,5-dihydrofuran-2-one (24). Yield: 65 mg, 61%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (4:1); ν_{max} (ATR) 2925, 1755, 1602, 1464, 1246, 1102, 943, 818, 753; δ_H (250 MHz; $CDCl_3$) 7.47 (1H, d, *J* 5.6, H-4), 7.37 (1H, d, *J* 7.6, ArH), 7.25 (1H, at, *J* 7.4, ArH), 6.95 (1H, d, *J* 16.6, H-2′), 6.94 (1H, t, *J* 7.5, ArH), 6.88 (1H, d, *J* 8.3, ArH), 6.22 (1H, d, *J* 16.3, H-1′), 6.03 (1H, d, *J* 5.6, H-3), 3.83 (3H, s, ArOMe), 1.67 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.4 (C), 159.5 (CH), 156.9 (C), 129.4 (CH), 127.3 (CH), 127.1 (CH), 126.1 (CH), 124.4 (C), 120.5 (CH), 119.7 (CH), 110.8 (CH), 88.2 (C), 55.3 (OMe), 24.0 (Me); (found: $[M+H]^+$, 231.1091. $C_{14}H_{14}O_3+H^+$ requires 231.1021).

4.1.8.2. 5-[(*E*)-2-(3-Methoxyphenyl)ethenyl]-5-methyl-2,5-dihydrofuran-2-one (25). Yield: 45 mg, 42%, as a colourless oil; Rf = 0.33, hexane/ethyl acetate (4:1); ν_{max} (ATR) 2982, 1757, 1581, 1489, 1232, 1104, 943, 818, 778; δ_H (250 MHz; $CDCl_3$) 7.46 (1H, d, *J* 5.6, H-4), 7.24 (1H, at, *J* 7.8, ArH), 6.96 (1H, d, *J* 7.7, ArH), 6.89 (1H, s, ArH), 6.83 (1H, dd, *J* 8.2, 1.6, ArH), 6.53 (1H, d, *J* 16.1, H-2′), 6.17 (1H, d, *J* 16.1, H-1′), 6.05 (1H, d, *J* 5.6, H-3), 3.80 (3H, s, ArOMe), 1.67 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.2 (C), 159.8 (C), 159.2 (CH), 136.9 (C), 130.8 (CH), 129.6 (CH), 127.1 (CH), 119.8 (CH), 119.2 (CH), 114.1 (CH), 111.8 (CH), 87.8 (C), 55.2 (OMe), 24.1 (Me); (found: $[M+H]^+$, 231.1091. $C_{14}H_{14}O_3+H^+$ requires 231.1021).

4.1.8.3. 5-[(*E*)-2-(4-Methoxyphenyl)ethenyl]-5-methyl-2,5-dihydrofuran-2-one (26). Yield: 40 mg, 52%, as a white solid; Rf = 0.33, hexane/ethyl acetate (7:3); mp 118-120 °C; ν_{max} (ATR) 2925, 1732, 1605, 1512, 1257, 1116, 1030, 835; δ_H (250 MHz; $CDCl_3$) 7.45 (1H, d, *J* 5.6, H-4), 7.30 (2H, d, *J* 8.6, ArH), 6.85 (2H, d, *J* 8.6, ArH), 6.58 (1H, d, *J* 16.1, H-2′), 6.04 (1H, d, *J* 5.6, H-3), 6.03 (1H, d, *J* 16.1, H-1′), 3.80 (3H, s, ArOMe), 1.66 (3H, s, Me); δ_C (62.5 MHz; $CDCl_3$) 172.4 (C), 159.8 (C), 159.5 (CH), 130.5 (CH), 128.3 (C), 127.9 (CH), 124.5 (CH), 119.7 (CH), 114.1 (CH), 88.0 (C), 55.2 (OMe), 24.1 (Me); (found: $[M+H]^+$, 231.1091. $C_{14}H_{14}O_3+H^+$ requires 231.1021).

4.1.9. (1*E*)-3-Methyl-1-phenylhexa-1,5-dien-3-yl prop-2-enoate (28)

To a solution of benzilideneacetone **8** (2.0 g, 13.6 mmol) in anhydrous THF (27 mL), was added a solution of allylmagnesium bromide (1.0 M; 33 mL, 16.32 mmol) at 0 °C under nitrogen atmosphere. After 30 min, a saturated aqueous ammonium chloride solution (50 mL) was poured and this mixture was extracted into ethyl acetate (2 × 100 mL). The organic layer was washed with brine (2 × 100 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure. To a solution of the resulting crude allylic alcohol (1.0 g, 5.31 mmol) in anhydrous THF (18 mL) at −78 °C was added triethylamine (1.64 mL, 11.68 mmol), followed by acryloyl chloride (0.90 mL,

11.16 mmol) at 0 °C under a nitrogen atmosphere. After 45 min, a saturated aqueous ammonium chloride solution (40 mL) was poured and this mixture was extracted into dichloromethane (50 mL). The organic layer was washed with brine (30 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure. The crude oil was purified by chromatography, eluting with hexane and ethyl acetate (4:1), to afford the title compound (0.52 g, 40%) as a colourless oil; ν_{max} (ATR) 2979, 1711, 1417, 1362, 1222, 973; δ_H (400 MHz; $CDCl_3$) 7.40-7.22 (5H, m, ArH), 6.56 (1H, d, *J* 16.3, H-1), 6.43 (1H, d, *J* 16.3, H-2), 6.35 (1H, dd, *J* 17.3, 1.5, H-3′$_{trans}$), 6.09 (1H, dd, *J* 17.3, 10.4, H-2′), 5.81 (1H, ddt, *J* 16.9, 10.3, 7.3, H-5), 5.77 (1H, dd, *J* 10.4, 1.5, H-3$_{cis}$), 5.12 (1H, d, *J* 17.1, H-6$_{trans}$), 5.11 (1H, d, *J* 10.0, H-6$_{cis}$), 2.75 (2H, 2× dd, *J* 13.0, 7.3, H-4), 1.68 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 164.9 (C), 136.6 (C), 132.75 (CH), 132.73 (CH), 129.87 (CH_2), 129.75 (CH), 128.9 (CH), 128.5 (CH), 127.6 (CH), 126.5 (CH), 118.7 (CH_2), 82.3 (C), 44.4 (CH_2), 23.9 (Me); (found: $[M+K]^+$, 281.1388. $C_{16}H_{18}O_2+K^+$ requires 281.0938).

4.2. In vitro antiproliferative assay

4.2.1. Cell lines

Human tumor cell lines U251 (glioma), MCF-7 (breast), HT-29 (colon), 786-0 (kidney), UACC (melanoma), PC-3 (prostate), K-562 (leukemia) and NCI-ADR/RES (multidrug resistant ovary carcinoma) were obtained from National Cancer Institute at Frederick MA-USA.

4.2.2. Cell culture

Stock cultures were grown in medium containing 5 mL RPMI 1640 (GIBCO BRL) supplemented with 5% fetal bovine serum (FBS, GIBCO) at 37 °C with 5% CO_2. Penicillin: streptomicyne (1000 μg/L:1000 U/L, 1 mL/L) were added to the experimental cultures.

4.2.3. Antiproliferative assay

Cells in 96 well plates (100 μL cells/well) were exposed to goniothalamin and its analogues in concentrations 0.25, 2.5, 25 and 250 μg/mL in DMSO (Merck)/RPMI at 37 °C, 5% of CO_2 in air for 48 h. Doxorubicin was used as positive control (0.025, 0.25, 2.5 and 25 μg/mL). Final DMSO concentration did not affect cell viability. Afterwards cells were fixed with 50% trichloroacetic acid (Merck) and cell growth determined by spectrophotometric quantification (540 nm) of cellular protein content using sulforhodamine B assay.[39a] Using the concentration-response curve for each cell line, the TGI (concentration that produces total growth inhibition or cytostatic effect) were determined through nonlinear regression analysis (Table 1) using software ORIGIN 8.0® (OriginLab Corporation).

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