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Original article

Synthesis and evaluation of (−)-Massoialactone and analogues as potential anticancer and anti-inflammatory agents

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ABSTRACT

(−)-Massoialactone, an α,β-unsaturated δ-lactone isolated from *Cryptocarya massoia*, and five analogues were synthesized and their antiproliferative and anti-inflammatory activities were evaluated. The lactones were able to mimic the "core" functional group required for the biological activity of their parent natural compounds suggesting that substantially altered analogues may retain their properties.

1. Introduction

The α,β-unsaturated δ-lactone unit is present in several compounds isolated from plants and marine organisms. These compounds exhibit different structural complexities and a broad range of biological activities. Examples are fostriecin, cytostatin, leptomicin B, goniothalamin, and massoialactone (Fig. 1).

Fostriecin and cytostatin are structurally related compounds produced by *Streptomyces pulveraceus* [1] and *Streptomyces* sp. *MJ654-Nf4* [2], respectively. These compounds are described as potent inhibitors of a subset of PPP-family serine/threonine protein phosphatases, being fostriecin a potent inhibitor of PP2/PP4, and cytostatin a potent and selective inhibitor of PP2A [3].

Leptomycin B (LMB) is produced by *Streptomyces* sp. strain ATS 1287 [4], and attracted attention due to its antitumor [5] and antifungal [6] activities. It is a potent inhibitor of the nuclear export of proteins [7] and its mode of action involves the binding to the chromosome maintenance region I (CRM1) exporting through its α,β-unsaturated δ-lactone moiety [8] which leads to selective inhibition of the protein–protein interaction in the ternary CRM1-RAN-cargo protein complex.

Goniothalamin is a styryl lactone isolated from various species of the genus *Goniothalamus* [9], and displays significant cytotoxic and anti-proliferative activities against a variety of cancer cell lines [10]. This lactone also displays other biological activities such as insecticidal [11], larvicidal [12], antifungal [13], antimicrobial [14], and trypanocidal [15].

(−)-Massoailactone, **1** was first isolated from the bark of *Cryptocarya massoia*, and later from other sources [16]. The structural simplicity of (−)-Massoialactone, **1** compared to its α,β-unsaturated δ-lactone analogues shown of Fig. 1 makes this compound a particularly attractive target and many approaches to **1** have been reported [17]. In addition, (−)-Massoialactone is a powerful skin irritant and produces systolic standstill in frog heart muscles [18]. Moreover, it showed good antimicrobial activity against *Staphylococcus aureus*, *B. subtilis* and *E. coli* [19].

The synthesis of relatively simple molecules which could be able to effectively mimic the key elements for the biological activity of a complex natural product such as those depicted in Fig. 1 is a subject of the great interest. As the "*core*" functional group required for the

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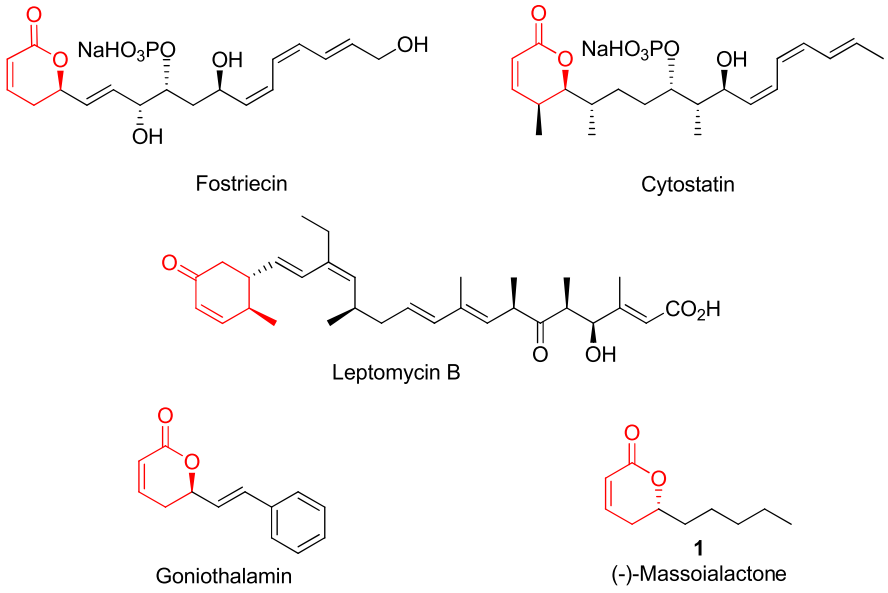


Fig. 1. Examples of natural products containing a α,β-unsaturated δ-lactone unit.

in vitro antiproliferative activity of cytostatin, fostriecin, leptomycin and goniothalamin is the α,β-unsaturated δ-lactone, substantially altered analogues of these natural products may retain their properties.

In this work, we performed molecular docking simulations on (−)-Massoialactone and some analogues in order to gain a better understanding of how these compounds would interact with CRM1, one of the most important molecular targets for the design of new inhibitors that could mimic natural toxins such as LMB. The results of this study provided a new insight for the synthesis of these compounds and the *in vitro* evaluation of their antiproliferative and anti-inflammatory properties.

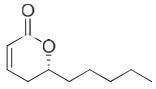
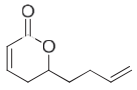
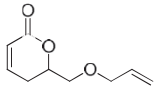
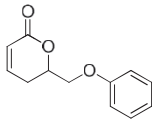
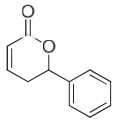
2. Results and discussion

2.1. Docking analysis

Leptomycin B (LMB) efficiently suppresses the nuclear export by inhibiting CRM1, a nuclear export receptor responsible for shuttling a large number of proteins and chemotherapeutic targets [19], playing an essential role in canonical nuclear export signal (NES)-dependent nuclear export, including major tumor suppressor proteins (TSPs).

The crystallographic structure of CRM1-RAN in complex with inhibitors were taken from the Research Collaboratory of Structural Bioinformatics Protein Data Bank (PDB 4HAT) [20]. The ligands and water molecules were extracted from the PDB file. All of the calculations were performed with the package docking program Autodock Tools 4.2 [21]. Initially, the structure was built using the Gauss View 4.1 [22] and optimized using the semi-empiric model AM1 [23] using the atomic charge units (Gasteiger model) [24] were marked and the flexibility of both receptor and ligand were determined using the standard program parameters, in which it was permitted to twist the lactone side chain. The electrostatic energy maps, atom-specific affinity and desolvation were calculated using Autogrid 4.2 with the centre grid at −39.174, 72.955, 29.907; with 19.5 × 15.75 × 15.75 Å dimensions, and 0.375 Å spacing. Fifty populations each with 2.500.000 fits were evaluated using the Generic Algorithm of energy minimization with the Autodock 4.2 program. The predicted binding energies of the envisioned compounds into the CRM1 active site are listed in Table 1.

Table 1
Docking interaction energy of α,β-unsaturated δ-lactones, **1** and **10a**–**e** against CRM1.

	Compound	ΔG (Kcal mol^{-1})
1	 **1**	−6.42
2	 **10a**	−5.61
3	 **10b**	−5.61
4	 **10c**	−7.25
5	 **10d**	−7.72
6	 **10e**	−7.21

From Table 1 it can be seen that the shortening of the alkyl side chain of the lactone resulted in a large difference in the binding energy to CRM1 for (−)-Massoilactone, **1** and **10a** (Table 1, entries 1 and 2), supporting the hypothesis that the side chain plays an important role in the molecular recognition.

The addition of an unsaturated side chain to the lactone proved to have no effect in the binding energy (Table 1, entry 3), however, the presence of an heteroatom in the side chain, greatly enhanced the interaction with the CRM1 active site and the observed binding energy was −7.25 kcal/mol (Table 1, entry 4).

The replacement of an alkyl side chain by an aromatic side chain, also enhanced the interaction with the CRM1 active site, with the calculated binding energy for compound **10e** of −7.21 kcal/mol (Table 1, entry 6). The most stable CRM1/lactone complex was observed for compound **10d**, with the calculated binding energy to CRM1 of −7.72 kcal/mol, which differentiates it significantly from all other studied compounds.

2.2. Chemistry

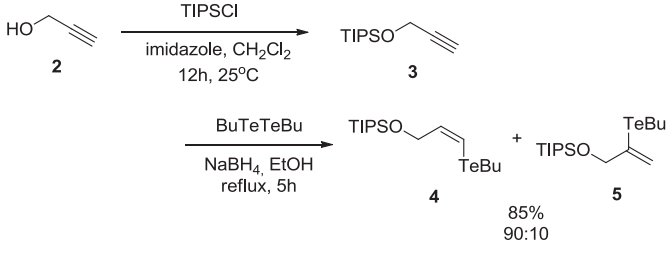
The data obtained from the molecular docking studies prompted us to synthesize the target compounds in order to evaluate their *in vitro* cytotoxicity. Thus, a stereodefined vinyl telluride was used to assembly the *Z* double bond present in (−)-Massoialactone. Thus, propargyl alcohol **2** was converted into its TIPS [25] derivative **3**, and subjected to hydrotelluration conditions to yield the corresponding vinyl tellurides **4** and **5** in a 9:1 ratio [26], being the two regioisomers easily separated by flash column chromatography. The regioisomeric ratio was determined by ^{1}H NMR and confirmed by ^{125}Te NMR [27] and gas chromatography (Scheme 1).

The vinyl telluride **4** was then converted into the corresponding *Z* higher order vinyl cyanocuprate [28] by the reaction with (2-Th)BuCu(CN)Li$_2$ followed by capture with epoxide **6**. Subsequent treatment with TBAF in THF [29] gave the corresponding 1,5-diol **7** in 75% overall yield after purification by chromatographic column. Further oxidation of **7** with BAIB and a catalytic amount of TEMPO following Forsyth protocol [30] gave (−)-Massoialactone, **1** in 70% yield (Scheme 2).

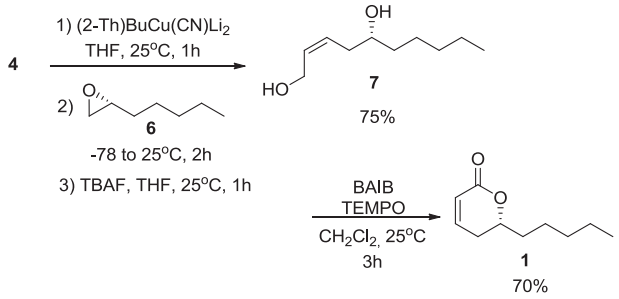
Compared to other previously described methodologies, the synthesis of (−)-Massoialactone **1**, using a vinyl telluride as a precursor showed to be convergent and efficient while the *Z* double bond and the stereocenter present in the natural product were installed in one step. (−)-Massoialactone **1**, was obtained in 52% overall yield after three sequential steps.

The strategy was then applied for the synthesis of compounds **10a**–**e**. Thus, transmetalation of vinyl telluride **4** with (2-Th)BuCu(CN)Li$_2$ followed by the capture of the mixed cuprate with epoxides **8a**–**d** and deprotection gave the corresponding 1,5-diols **9a**–**d** which were oxidized to the corresponding α,β-unsaturated δ-lactones **10a**–**d** in good yields (Scheme 3).

In all cases, the lactones corresponding from the attack to the less-substituted carbon atom in epoxides **8a**–**d** were obtained exclusively. The only exception was observed when styrene oxide, **8e** was used. In this case, a separable mixture of the regioisomeric



Scheme 1. Synthesis of Vinyl Telluride **4**.



Scheme 2. Synthesis of (−)-Massoailactone, **1**.

diols **9e** and **9e′** was obtained in a 1.2 to 1 ratio. Further oxidation of **9e** gave the corresponding lactone **10e** in good yield (Scheme 4).

All the compounds were characterized by ^{1}H and ^{13}C and HRMS. Purity of all compounds was ≥95%.

2.3. Antiproliferative activity

It is known that different cell lines display different sensitivities toward a cytotoxic compound. In this way, (−)-Massoailactone, **1** and lactones **10a**–**e** were submitted to the MTT assay [31] for the evaluation of their cytotoxic effects on MCF-7 (human breast adenocarcinoma), HT-29 (human colon adenocarcinoma), and NCI-H292 (human lung **carcinoma**) cell lines. Some of the most active compounds were also tested on HL-60 (human pro-myelocytic leukemia), K562 (human erythromyeloblastoid leukemia).

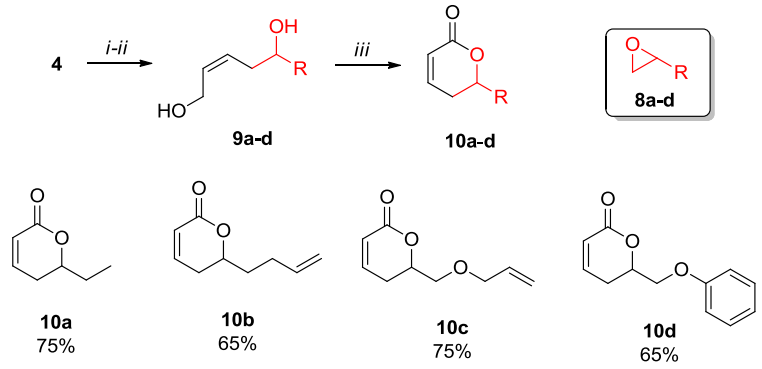
Analogues were first screened at a 25 μg/mL initial concentration and the samples with growth inhibition over 90% were used to determine the full dose response screening and IC$_{50}$ determination. The synthesized compounds were then screened at 25 μg/mL and the samples with growth inhibition over 90% were used to determine the IC$_{50}$ values.

(−)-Massoilactone, **1** displayed moderate cytotoxic activity against all tested cancer cell lines, being more active against NCI-H292 and HL-60 cancer cells lines (Table 2, entry 1). For analogue **10a**, no antiproliferative activity was found demonstrating that the presence of a hydrophobic segment, together with the α,β-unsaturated δ-lactone group, is important for the antiproliferative activity (Table 2, entry 2). This result corroborates with the values obtained from the docking analysis, where analogue **10a** exhibited a higher binding energy to CRM1. This result was previously observed for fostriecin [32].

Analogues **10b** and **10c**, which possess a side chain with a terminal double bond, were also tested, but only **10c** displayed moderate cytotoxicity, confirming the previous docking study, which indicated that the presence of an electronegative atom would increase the potency of the analogue (Table 2, entries 3 and 4). It is also known for cytostatin that the presence of the triene moiety is not essential for the *in vitro* inhibition.

For all tested compounds, the analogues which possess an aromatic ring in the side chain displayed better cytotoxic activities against some of the tested cell lines (Table 2, entries 5 and 6). Compound **10e** exhibited good cytotoxic activity against lung cancer cells (NCI-H292) (Table 2, entry 6). Noteworthy, analogue **10d** displayed a good cytotoxic activity against all tested cell lines (Table 2, entry 5), including human chronic myelogenous (CML) leukemia cell line (K562), which is derived from pleural effusion of a patient in the acute phase of CML and expresses the BCR-ABL protein [33]. This protein confers resistance to apoptosis induction by a number of agents and conditions [34].

From Table 2, it can be observed that the results are in accordance to those obtained from the docking analysis, once analogues **10a** and **10b** exhibited higher binding energies to CRM1 when



Reagents and conditions: (*i*) (2-Th)BuCu(CN)Li_2,THF, 25°C, 1 h; then **8a-d**, -78 to 25°C, 2h; (*ii*) TBAF, THF, 25°C, 3 h; (*iii*) BAIB, TEMPO, CH_2Cl_2, 25°C, 3h.

Scheme 3. Synthesis of α,β-unsaturated δ-lactones **10a**–**d.**

compared to analogues **10d** and **10e**.In the same way, analogues **10a** and **10b** exhibited lower antiproliferative activities when compared to analogues **10d** and **10e**.

The lack of commercial availability of chiral epoxides (**8a**–**e**) used for the synthesis of the α,β-unsaturated δ-lactones makes the synthesis of individual enantiomers difficult. In this way, the binding patterns of both enantiomers of analogue **10d** were independently evaluated by docking analysis.

Thus, for the *R*-**10d** isomer, the approach to CRM1 occurs initially by two intermolecular interactions: between LYS548 and LYS579 with the carbonyl oxygen of lactone and, between LYS579 and the oxygen present in the lactone ring, positioning the double bond 3.65 Å away from CYS539 (Fig. 2(a)). On the other hand, the *S*-**10d** isomer fits into the hydrophobic region with both oxygen atoms positioned toward inside the cavity, with the aromatic ring accommodated in the hydrophobic region of MET556, positioning the double bond 3.66 Å away from CYS539, also resulting in the loss of LYS548 and LYS579 interactions observed for the *R*-isomer. Using this approach it was observed that the energy required to accommodate the *R*-**10d** isomer into the CRM1 cavity is about 0.2 Kcal mol^{-1} higher than the corresponding *S*-**10d** isomer, indicating no enantiomer preference.

It is worth also to note that the lactone ring of LMB makes a covalent bond with CYS539 of CRM1, the same bonding pattern observed in both *R* and *S* enantiomers of **10d**.

Superposition of compound **10d** to LMB reveals a complementary fit into the hydrophobic binding grove of the export receptor as this compound is expected to bind covalently to CRM1 with the side chain mimicking the hydrophobic part of LMB (Fig. 3).

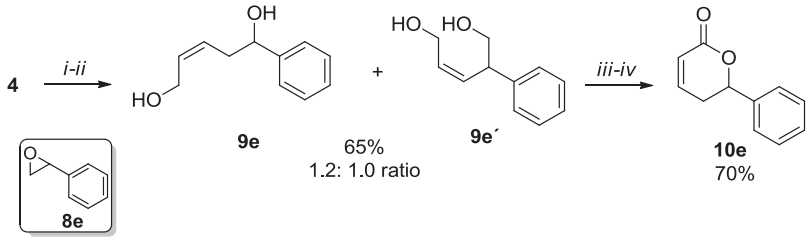
2.4. Anti-inflammatory activity

It is known that bacterial endotoxins, such as lipopolysaccharides (LPS) activate macrophages leading to the production nitric oxide, [35] an important pro-inflammatory mediator associated with the activation of T lymphocytes [36]. LPS promotes activation of Nuclear Factor kappa B (NF-κB) in macrophages, stimulating the production of NO and pro-inflammatory cytokines. Thus, reduction of NO levels can be related to the inhibition of activation of nuclear transcription factor κB, which regulates the expression of inducible nitric oxide synthase (iNOS) and genes related to the production of cytokines [37].

The synthesized lactones **10b**–**d** were then evaluated for *in vitro* anti-inflammatory activity using LPS-stimulated murine peritoneal macrophages. First, the cytotoxicity of lactones to these cells was evaluated in order to avoid the use of cytotoxic concentrations for analysis of anti-inflammatory effect (Table 3).

It was observed that the synthesized α,β-unsaturated δ-lactones affected cell viability in a dose-dependent manner. From Table 3 it can be observed that the cytotoxic effect of lactones **10c** and **10d** at the concentration of 3.1 μg/mL was weak. Conversely, treatment at higher concentrations resulted in a lower percentage of viable cells and thus the lowest concentration was selected for the next assay. Both lactones **10c** and **10d** at a concentration of 3.1 μg/mL were able to reduce the nitric oxide production by macrophages stimulated with LPS (Table 4).

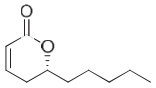
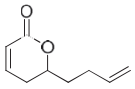
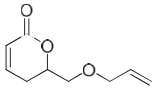
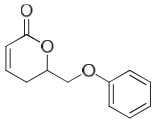
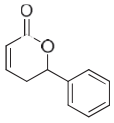
These results indicate that the detected *in vitro* anti-inflammatory activity of **10c** and **10d** is at least in part due to the regulation of NO production.



Reagents and conditions: (*i*) (2-Th)BuCu(CN)Li_2,THF, 25°C, 1 h; then **8e**, -78 to 25°C, 2h; (*ii*) TBAF, THF, 25°C, 3 h; (*iii*) chromatography; (iv) BAIB, TEMPO, CH_2Cl_2, 25°C, 3h.

Scheme 4. Synthesis of α,β-unsaturated δ-lactone **10e**.

Table 2
IC_{50} values for **1** and analogs **10a**–**e**.

		IC_{50}, confidence intervals 95% (μg mL^{-1})				
		MCF-7	NCI-H292	HT-29	Hl-60	K562
1	 **1**	60.0	11.3	22.6	16.0	20.2
2	 **10a**	>100	>100	>100	NT	NT
3	 **10b**	>100	>100	>100	NT	NT
4	 **10c**	44.6	16.6	18.4	25.5	50.0
5	 **10d**	22.5	0.8	4.4	5.4	2.9
6	 **10e**	22.9	9.1	10.9	13.2	8.9
7	DOX	0.34	0.02	NT	0.03	0.26

[a] Doxorubicin (DOX) was the positive control; NT, not tested.

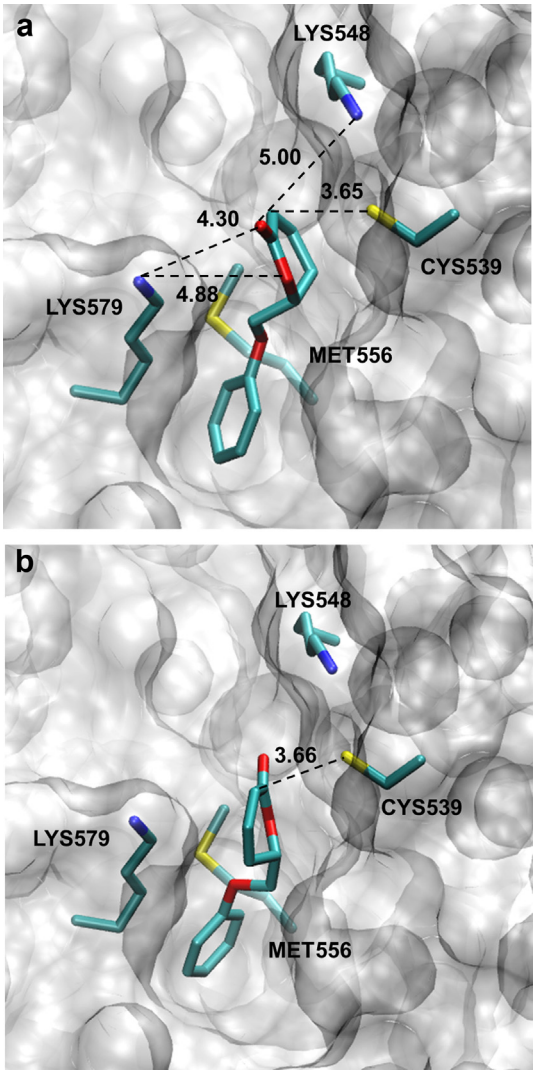


Fig. 2. Lower energy (Kcal mol^{-1}) molecular fit for (a) *R*-**10d** and (b) *S*-**10d** isomers. The numbers indicate the distance (Å) to the aminoacid residues in the fitting process.

TNF-α is largely related to LPS-induced nitric oxide production [38]. Thus, the reduction in NO levels could be attributed to the reduction in the levels of TNF-α. Table 5 shows the data from measurement of cytokine production. Lactone **10d** promoted statistically significant reduction ($p < 0.05$) in the levels of TNF-α released by LPS-stimulated macrophages (Table 5, entry 2). A smaller effect in the production of TNF-α was observed for lactone **10c** (Table 5, entry 1).

The reported anti-inflammatory results may be related to inhibition of activation of NF-κB, which is generally followed by increase in NO, TNF-α and other cytokines levels, however further studies should be conducted to confirm this hypothesis.

3. Conclusion

The binding interactions between (−)-Massoialactone and related α,β-unsaturated δ-lactones to CRM1 were evaluate by molecular docking. The evaluated compounds were then synthesized and tested against five cancer cell lines. Among all derivatives, compound **10d** was the most active against the tested cancer cell lines, and this result is in accordance to molecular docking calculations. Compound **10d** also demonstrated *in vitro* anti-inflammatory activity on LPS-stimulated macrophages by regulating the production of cytokines and NO. From a broader perspective, the synthesized lactones were able to mimic the "core" functional groups required for the biological activity of their parent natural compounds suggesting that substantially altered analogues may retain their properties.

4. Experimental section

^{1}H and ^{13}C NMR data were recorded at 300 and 75 MHz, respectively, using a Varian UNITY PLUS spectrometer. NMR chemical shifts are reported as delta (δ) units in parts per million (ppm) relative to residual $CDCl_3$ Coupling constants (J) were reported in hertz (Hz). ^{125}Te NMR data were obtained at 94.6 MHz using diphenyl ditelluride as an external reference (422.0 ppm). Typical parameters were as follows: acquisition time equal to 0.64 s, pulse of 45°, spectral window of 43.9 kHz; and line broadening equal to 5.0 Hz. Low resolution mass spectra were obtained using a Shimadzu QP-5050A Spectrometer (70 eV) using helium 4.5 as a carrier gas and a DB-5 column (30 m × 0.25 mμ). High resolution mass spectra were obtained by the electro spray ionization time-of-flight (ESI-TOF) mode on a Bruker Micro Toflc Bruker Daltonics mass spectrometer.

Infra-red spectra were recorded using FT/IR spectrometer Bruker IFS 66 and the samples were prepared as thin films on salt plates or as KBr pellets.

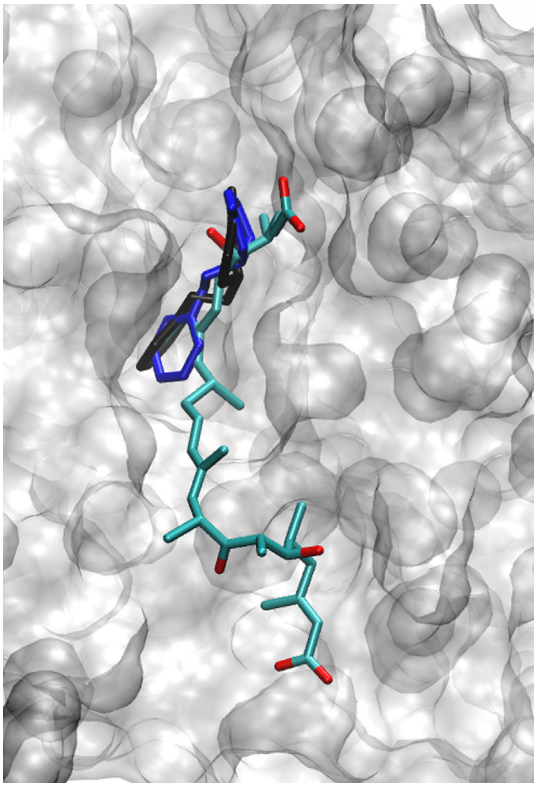


Fig. 3. Lower energy (Kcal mol^{-1}) molecular fit superposition of compound **10d** and Leptomycin B binding to CRM1.

4.1. Preparation of dibutylditelluride (BuTeTeBu)

A 2 L round-bottomed flask was flamed dry and equipped with a 250 mL pressure equalized dropping funnel was charged with tellurium metal (20.1 g, 157 mmol) [dried at 85 °C prior to use] and dry THF (1 L) was cooled to 0 °C. The addition funnel was charged with *n*-butyllithium (180 mmol, 72 mL of a 2.5 M solution in bexanes). The *n*-butyllithium was added dropwise. After the addition was complete, the ice bath was removed and the reaction mixture was stirred at room temperature for 60 min. A saturated solution of ammonium chloride (250 mL) was then slowly added. The reaction was stirred at room temperature for about 3 h while open to the atmosphere (O_2). The organic layer was isolated and the aqueous layer was extracted with EtOAc (2 × 150 mL). The combined organic phases were dried over $MgSO_4$ and filtered through a pad of Celite. Concentration *in vacuo* provided 50.7 g (87%) of dibutylditelluride as a red oil which was used directly without further purification. IR (KBr pellet, cm^{-1}) ν_{max} 2955, 2921, 2868, 1457, 1175; ^1H NMR (300 MHz, $CDCl_3$) δ 3.12 (t, J = 7.80 Hz, 4H, 2 × CH_2), 1.83–1.61 (m, 4H, 2 × CH_2), 1.44–1.33 (m, 4H, 2 × CH_2), 0.90 (t, J = 7.50 Hz, 6H, 2 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.3 (2 × CH_2), 24.2 (2 × CH_2), 13.5 (2 × CH_2), 4.0 (2 × CH_3).

Table 3
Effect of *α,β-unsaturated δ-lactones* **10b–e** on the viability of LPS-stimulated peritoneal macrophages.

Entry		Concentration (μg mL−1)			
		3.1	6.2	12.5	25.0
1	**10b**	57	45.4	19.7	2.5
2	**10c**	100	77.2	51.1	7.7
3	**10d**	93	47.3	0	0
4	**10e**	34.5	30.9	1.0	7.4

The cytotoxicity was evaluated by MTT assay after 24 h incubation of peritoneal macrophages cells with 3.1–25.0 μg/mL **10b–e** and/or LPS. The values represent mean ± SD of three independent experiments (n = 12).

Table 4
Effect of *α,β-unsaturated δ-lactones* ***10c*** *and* ***10d*** on nitric oxide production by murine macrophages stimulated with LPS.

Entry		Concentration (μg mL^{-1})	NO (μM)
1	**10c**	3.1	2.7 ± 0.05*
2	**10d**	3.1	5.2 ± 0.04*
3	LPS	1.0	19.2 ± 0.06*
4	Control	–	2.8 ± 0.13

Data are presented as mean ± standard deviation of three independent experiments. *p < 0.05 compared to control (cells in medium culture only) by ANOVA followed by Newman–Keuls test.

4.2. Preparation of triisopropyl(prop-2-ynyloxy)silane (*3*)

To a round-bottomed flask under argon was added CH_2Cl_2 (30 mL), imidazole (1.70 g, 25 mmol), and propargyl alcohol (0.56 g, 0.58 mL, 10 mmol) the mixture was cooled to 0 °C and TIPSCl (2.30 g, 2.55 mL, 12 mmol) was slowly added. The mixture was stirred for 12 h, diluted with CH_2Cl_2 (20 mL) and quenched with water (20 mL). The organic phase was washed with 3% HCl (10 mL), saturated $NaHCO_3$ (20 mL) and finally water. The organic phase was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. The pure silyl ether was distilled from the residue under reduced pressure (bp 110 °C, 20 mm Hg) to yield 2.0 g (95%) of compound **3**. ^1H NMR (300 MHz, $CDCl_3$) δ 4.35 (d, J = 2.4 Hz, 2H, CH_2), 2.35 (t, J = 2.7 Hz, 1H, CCH), 1.10–1.00 (m, 3H, 3 × CH, 18H, 6 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 81.5 ($CH{\equiv}CCH_2$), 72.3 ($CH{\equiv}CCH_2$), 51.5 (CH_2), 17.5 (6 × CH_3), 12.0 (3 × CH).

4.3. Preparation of (Z)-(3-(butyltellanyl)allyloxy)triiso-propylsilane (*4*)

3 (19.0 g, 90 mmol) and dibutylditelluride (16.8 g, 45 mmol) were dissolved in absolute ethanol (100 mL) at room temperature. Finely powdered sodium borohydride was added in portions to the above solution. Additional sodium borohydride was added as necessary to maintain a yellow color (indicative of the butyltellurolate anion). The solution was heated to reflux for 5 h and cooled to room temperature. The reaction mixture was then poured into a saturated solution of $NaHCO_3$ (200 mL) and diluted with EtOAc (200 mL). The organic layer was isolated and washed with H_2O (500 mL) and brine (500 mL) before drying over $MgSO_4$. The organic phase was filtered and concentrated *in vacuo*. Silica gel chromatography using hexanes provided 30.6 g, (85%) of the title compound as a yellow oil. IR (KBr pellet, cm^{-1}) ν_{max} 2941, 2865, 1462, 1095, 918; ^1H NMR (300 MHz, $CDCl_3$) δ 6.70 (dt, J = 9.9 Hz, 1.5 Hz, 1H, TeCH=CH), 6.40 (dt, J = 9.9 Hz, 5.1 Hz, 1H, TeCH=CH),

Table 5
Effect of *α,β-unsaturated δ-lactones* ***10c*** *and* ***10d*** on TNF-α production by murine macrophages stimulated with LPS.

Entry		Concentration (μg mL^{-1})	TNF-α (pg/mL)
1	**10c**	3.1	210.0 ± 7.7*
2	**10d**	3.1	112.4 ± 9.8*
3	LPS	1.0	274.4 ± 10.6*
4	Control	–	57.5 ± 5.9

Data are presented as mean ± standard deviation of three independent experiments. *p < 0.05 compared to control (cells in medium culture only) by ANOVA followed by Newman–Keuls test.

4.20 (dd, J = 5.1 Hz, 1.5 Hz, 2H, CH=CHCH$_2$), 2.75 (t, J = 7.8 Hz, 2H, CH$_2$), 1.93–1.62 (m, 2H, CH$_2$), 1.45–1.22 (m, 2H, CH$_2$), 1.20–1.05 (m, 3H, 3 × CH, 18H, 6 × CH$_3$), 0.95 (t, J = 7.50 Hz, 3H, CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 138.0 (TeCH=CH), 102.0(TeCH=CH), 65.5 (CH=CHCH$_2$), 34.0 (CH$_2$), 25.0 (CH$_2$), 17.7 (6 × CHCH$_3$), 17.5 (CH$_2$), 13.0 (CH$_3$), 12.0 (3 × CHCH$_3$); ^{125}Te NMR (94.6 MHz, CDCl$_3$) δ 298.9; GCMS (EI, Rel. Int. %) m/z 400 ([M$^+$], 6), 357 (83), 245 (41), 227 (12), 213 (29), 169 (100), 157 (24), 127 (60), 87 (10), 57 (7); HRMS (ESI, MeOH:H$_2$O) calc for $C_{16}H_{34}OSiTe$ [M + H]$^+$, 401.1519, found 400.9330.

4.4. General procedure for the synthesis of 1,5-diols (7), (**9a**–**e**) and (**9e′**) from the reaction of (**4**) with (2-Th)BuCu(CN)Li$_2$ system and epoxides (**6**) or (**8a**–**e**) followed by deprotection

To a flask equipped with a stirring bar and a rubber septum, flame-dried under argon atmosphere was added anhydrous THF (10 mL) and distilled thiophene (1.05 g, 12.5 mmol). The solution was cooled to −78 °C and *n*-butyllithium in hexanes (1.40 M, 9.0 mL, 12.5 mmol) was added dropwise. The resulting light-yellow solution was warmed to −40 °C and kept for 20 min. After that, this solution was transferred via cannula to a CuCN (0.89 g, 10 mmol) suspension in THF cooled at −78 °C. At the end of the addition, the acetone–dry ice bath was exchanged for an ice bath. After 5 min the flask was again placed in a dry ice–acetone bath and *n*-butyllithium in hexanes (1.40 M, 7.10 mL, 10 mmol) was added dropwise. The solution was maintained at this temperature for 15 min. After that, the solution was heated at 0 °C and the vinylic telluride **4** (4.20 g, 10.5 mmol) dissolved in THF (10 mL) was added. After being stirred for 1 h at room temperature, the mixture was cooled to −78 °C and the appropriate epoxide **6** or **8a**–**e** (10 mmol) in THF (10 mL) was added. The reaction mixture was warmed to 0 °C. After 3 h at 0 °C, it was warmed to ambient temperature and stirred for an additional 1 h. The reaction mixture was then cooled to −78 °C and poured on to a solution of saturated aqueous ammonium chloride and concentrated aqueous ammonium hydroxide (9:1). The mixture was stirred for 15 min while the temperature of the system was allowed to rise. After that, the mixture was extracted with EtOAc (2 × 60 mL). The organic layer was washed with brine (2 × 100 mL). The combined organic layers were dried over MgSO$_4$ and concentrated *in vacuo*. The crude product was dissolved in THF (5 mL) and TBAF (10 mL, 1 M solution in THF, 10 mmol) was added dropwise. The reaction was monitored by TLC. The reaction was then quenched by the addition of a saturated solution of NH$_4$Cl (10 mL). The aqueous layer was extracted with EtOAc and the combined organic phases were washed with brine, dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude product were purified by chromatography on silica gel using 50:50 hexanes/EtOAc to yield the corresponding 1,5-diols, **7**, **9a**–**e** and **9e′**.

4.4.1. (R,Z)-dec-2-ene-1,5-diol (**7**)

Isolated as yellow oil, 0.65 g (75%). $[\alpha]_D^{20}$ +9.5 (c 1.00, CHCl$_3$); IR (KBr pellet, cm^{-1}) ν_{max} 3329 (OH), 2921, 2843, 1661, 1472, 1011, 867, 721; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.92–5.82 (m, 1H, CH=CH), 5.65–5.56 (m, 1H, CH=CH), 4.15 (dd, J = 12.0, 7.5 Hz, 1H, CH$_2$CH=CH), 4.00 (dd, J = 12.3, 6.6 Hz, 1H, CH$_2$CH=CH), 3.64–3.60 (m, 1H, CHOH), 2.60 (br. s, 2H, 2 × OH), 2.30–2.20 (m, 2H, CH=CHCH$_2$), 1.50–1.40 (m, 2H, CHOHCH$_2$), 1.35–1.25 (m, 6H, 3 × CH$_2$), 0.90 (t, J = 6.9 Hz, 3H, CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 132.0 (CH=CH), 130.0 (CH=CH), 70.5 (CHOH), 57.0 (CH$_2$CH=CH), 36.6 (CH$_2$), 34.5 (CH$_2$), 32.0 (CH$_2$), 25.2 (CH$_2$), 22.0 (CH$_2$), 13.8 (CH$_3$), GC–MS (EI, Rel. Int. %) m/z 154 (1), 99 (12), 55 (35), 54 (100), 43 (14), 41 (12); HRMS (ESI, MeOH:H$_2$O) calcd for $C_{10}H_{20}O_2Na$ [M + Na]$^+$, 195.1361; found 195.1364.

4.4.2. (Z)-Hept-2-ene-1,5-diol (**9a**)

Isolated as a yellow oil, 0.91 g; (70%); IR (KBr pellet, cm^{-1}) ν_{max} 3329 (OH), 3019, 2963, 2932, 2877, 1461, 1113, 1013; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.75–5.70 (m, 1H, CH=C*H*), 5.60–5.50 (m, 1H, C*H*=CH), 4.10 (dd, J = 12.3, 7.2 Hz, 1H, C*H*$_2$CH=CH), 4.00 (dd, J = 12.3, 7.2 Hz, 1H, C*H*$_2$CH=CH), 3.90 (br. s., 2H, 2 × OH), 3.50–3.45 (m, 1H, CHOH), 2.20 (t, J = 7.5 Hz, 2H, CH=CHC*H*$_2$), 1.40 (qui, J = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 0.90 (t, J = 7.5 Hz, 3H, CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 131.0 (*C*H=CH), 129.0 (CH=*C*H), 72.0 (CHOH), 57.0 (*C*H$_2$CH=CH), 34.5 (CH=CH*C*H$_2$), 30.0 (*C*H$_2$CH$_3$), 10.0 (CH$_2$*C*H$_3$); GCMS (EI, Rel. Int. %) m/z 130 ([M$^+$], 1), 83 (8), 57 (33), 54 (100), 41 (18); HRMS (ESI, MeOH:H$_2$O) calcd for $C_7H_{14}O_2Na$ [M + Na]$^+$, 153.0892, found 153.0899.

4.4.3. (Z)-Nona-2,8-diene-1,5-diol (**9b**)

Isolated as a yellow oil, 1.11 g; (71%); IR (KBr pellet, cm^{-1}) ν_{max} 3329 (OH), 2933, 1640, 1436, 1076, 1007, 911, 861; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.95–5.70 (m, 1H, CH=C*H*, 1H, C*H*=CH$_2$), 5.65–5.50 (m, 1H, C*H*=CH), 5.00–4.95 (m, 2H, CH=C*H*$_2$), 4.15 (dd, J = 12.3, 7.2 Hz, 1H, C*H*$_2$CH=CH), 4.00 (dd, J = 12.3, 6.6 Hz, 1H, C*H*$_2$CH=CH), 3.68–3.60 (m, 1H, CHOH), 3.25 (br. S, 2H, 2 × OH), 2.30–2.00 (m, 2H, CH=CHC*H*$_2$, 2H, C*H*$_2$CH=CH$_2$), 1.60–1.50 (m, 2H, C*H*$_2$CH$_2$CH = CH$_2$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 138.0 (*C*H=CH$_2$), 131.0 (*C*H=CH), 129.5 (CH=*C*H), 115.0 (CH=*C*H$_2$), 70.0 (CHOH), 57.5 (*C*H$_2$CH=CH), 36.0 (CHOH*C*H$_2$), 35.0 (CHOHCH$_2$*C*H$_2$), 30.0 (CH=CH*C*H$_2$), GCMS (EI, Rel. Int. %) m/z 138 (1), 83 (14), 79 (6), 71 (10), 67 (14), 54 (100), 41 (37); HRMS (ESI, MeOH:H$_2$O) calcd for $C_9H_{16}O_2Na$ [M + Na]$^+$, 179.1048; found 179.1051.

4.4.4. (Z)-6-(allyloxy)hex-2-ene-1,5-diol (**9c**)

Isolated as a yellow oil, 1.25 g; (73%); IR (KBr pellet, cm^{-1}) ν_{max} 3355 (OH), 2865, 1647, 1423, 1087, 1006, 930, 668; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.90–5.72 (m, 1H, C*H*=CH, 1H, C*H*=CH$_2$), 5.60–5.50 (m, 1H, CH=C*H*), 5.25 (ddd, J = 17.0, 3.0, 1.5 Hz, 1H, CH=C*H*$_2$), 5.15 (ddd, J = 10.8, 3.0, 1.5 Hz, 1H, CH=C*H*$_2$), 4.13 (dd, J = 12.3, 7.5 Hz, 1H, C*H*$_2$CH=CH), 4.00 (dd, J = 12.3, 7.5 Hz, 1H, C*H*$_2$CH=CH), 3.98 (ddd, J = 7.2, 3.0, 1.5 Hz, 2H, C*H*$_2$CH=CH$_2$), 3.80–3.78 (m, 1H, C*H*OH), 3.41–3.30 (m, 2H, CHOHC*H*$_2$, 2H, 2 × OH), 2.35–2.20 (m, 2H, C*H*$_2$CH=CH); ^{13}C NMR (75 MHz, CDCl$_3$) δ 134.0 (*C*H=CH$_2$), 131.5 (*C*H=CH), 128.8 (CH=*C*H), 117.5 (CH=*C*H$_2$), 73.8 (CHOH*C*H$_2$), 72.0 (*C*H$_2$CH=CH$_2$), 69.0 (CHOH), 57.5 (*C*H$_2$CH=CH), 31.0 (CH=CH*C*H$_2$); GCMS (EI, Rel. Int. %) m/z 136 (1), 101 (5), 83 (31), 55 (56), 54 (48), 41 (100); HRMS (ESI, MeOH:H$_2$O) calcd for $C_9H_{16}O_3Na$ [M + Na]$^+$,195.0997; found 195.0998.

4.4.5. (Z)-6-Phenoxyhex-2-ene-1,5-diol (**9d**)

Isolated as a colorless oil; 1.45 g; (70%); IR (KBr pellet, cm^{-1}) ν_{max} 3330 (OH), 2926, 2875, 1598, 1495,1292, 1244, 1078, 754; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.35–7.25 (m, 2H, 2 × Ar–CH), 7.00–6.90 (m, 3H, 3 × Ar–CH), 5.95–5.82 (m, 1H, C*H*=CH), 5.72–5.60 (m, 1H, CH=C*H*), 4.24–4.20 (m, 1H, C*H*OH), 4.15–4.01 (m, 2H, CHOHC*H*$_2$), 4.00–3.88 (m, 2H, C*H*$_2$CH=CH), 3.10 (br. s., 2H, 2 × OH), 2.55–2.35 (m, 2H, CH=CHC*H*$_2$); ^{13}C NMR δ (75 MHz, CDCl$_3$) δ 158.5 (C^q-Ar), 131.6 (*C*H=CH), 129.5 (CH=*C*H), 128.0 (2 × CH–Ar), 121.0 (CH–Ar), 114.5 (2 × CH–Ar), 71.0 (CHOH*C*H$_2$), 69.0 (CHOH), 57.5 (*C*H$_2$CH=CH), 31.0 (CH=CH*C*H$_2$); GC/MS (EI, Rel. Int. %) m/z 208 ([M$^+$], 3), 154, 136 (20), 119 (12), 108 (35), 94 (100), 77 (45), 65 (18), 55 (44), 51 (19), 43 (38), 41 (26); HRMS (ESI, MeOH:H$_2$O) calcd for $C_{12}H_{16}O_3Na$ [M + Na]$^+$, 231.0997; found, 231.0991.

4.4.6. (Z)-5-Phenylpent-2-ene-1,5-diol (**9e**)

Isolated as a yellow oil, 0.62 g; (35%); IR (KBr pellet, cm^{-1}) ν_{max} 3344 (OH), 3062, 2923, 1712, 1493, 1026, 760, 733, 700; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.30–7.25 (m, 5H, 5 × Ar–CH), 5.77 (m, 1H, C*H*=CH), 5.55 (m, 1H, CH=C*H*), 4.65 (dd, J = 8.1, 4.5 Hz, 1H, C*H*OH),

4.00 (dd, J = 12.3, 7.5 Hz, 1H, CH_2CH=CH), 3.90 (dd, J = 12.3, 6.6 Hz, 1H, CH_2CH=CH), 3.00 (br. s., 2H, 2 × OH), 2.60–2.55 (m, 1H, CH=CHCH_2), 2.45–2.40 (m, 1H, CH=CHCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.0 (C^q-Ar), 131.5 (*C*H=CH), 129.0 (CH=*C*H), 128.5 (2 × CH–Ar), 127.5 (CH–Ar), 125.8 (2 × CH–Ar), 72.8 (CHOH), 57.5 (*C*H_2CH=CH), 37.0 (CH=CH *C*H_2); GCMS (EI, Rel. Int. %) *m/z* 178 ([M^+], 1), 107 (76), 106 (13), 105 (100), 79 (91), 78 (12), 77 (66), 54 (93), 51 (20); HRMS (ESI, MeOH:H_2O) calcd for $C_{11}H_{14}O_2Na$ [M + Na]$^+$ 201.0892; found 201.0896.

4.4.7. (Z)-4-Phenylpent-2-ene-1,5-diol (**9e'**)

Isolated as a yellow oil, 0.53 g; (30%); IR (KBr pellet, cm^{-1}) ν_{max} 3330 (OH), 2955, 2924, 2870, 1492, 1453, 1037, 699; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.30 (m, 2H, 2 × Ar–CH), 7.20–7.28 (m, 3H, 3 × Ar–CH), 5.98–5.90 (m, 1H, C*H*=CH), 5.85–5.78 (m, 1H, CH=C*H*), 4.35 (ddd, J = 12.4, 7.4, 1.4 Hz, 1H, CH_2CH=CH), 4.10 (ddd, J = 12.4, 6.3, 0.8 Hz, 1H, CH_2CH=CH), 3.95–3.80 (m, 2H, CH_2OH), 3.75–3.68 (m, 1H, CH), 2.15 (br. s., 2H, 2 × OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.8 (C^q-Ar), 133.5 (*C*H=CH), 131.0 (CH=*C*H), 129.0 (2 × CH–Ar), 127.5 (2 × CH–Ar), 127.0 (CH–Ar), 66.5 (CH_2), 58.0 (*C*H_2CH=CH), 46.5 (CH), GCMS (EI, Rel. Int. %) *m/z* 169 (1), 130 (100), 129 (75), 128 (29), 115 (32), 91 (70), 77 (18), 51 (16), 41 (21); HRMS (ESI, MeOH:H_2O) calcd for $C_{11}H_{14}O_2Na$ [M + Na]$^+$, 201.0892; found 201.0883.

4.5. General procedure for the synthesis of α,β-unsaturated δ-lactones (**1**) and (**10a**–**e**) from the reaction of 1,5-diols (**7**) and (**9a**–**e**) with TEMPO/BAIB

To a stirred solution of the appropriate diol**7** or **9a**–**e** (2.5 mmol, 1 equiv) in CH_2Cl_2 (30 mL) was added bis-acetoxyiodobenzene (BAIB) (2.5 g, 7.7 mmol, 3 equiv) and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) (0.08 g, 20 mol%) at room temperature. After stirring for 3 h, the reaction mixture was quenched with a saturated solution of $Na_2S_2O_3$ (10 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The combined organic extracts were washed with saturated solutions of $NaHCO_3$ (10 mL), NH_4Cl (10 mL) and brine (2 × 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel using 100:0 to 90:10 (hexanes/EtOAc) to yield the lactones **1** and **10a**–**e**.

4.5.1. (−)-Massoialactone (**1**)

Isolated as a colorless oil, 0.28 g, (70%). $[\alpha]_D^{20}$ − 115.6 (c 1.00, $CHCl_3$); IR (thin film, cm^{-1}) ν_{max} 2927, 2849, 1718, 1381, 1257, 1041, 812, 665; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (dd, J = 9.6, 4.4 Hz, 1H, CH=CHCH_2), 5.95 (d, J = 9.6 Hz, 1H, CH=CHCH_2), 4.40–4.30 (m, 1H, CH), 2.30–2.25 (m, 2H, CH=CHCH_2), 1.75–1.20 (m, 8 H, 4 × CH_2), 0.85 (t, J = 6 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0 (C=O), 144.8 (CH=CH), 121.0 (CH=CH), 77.5 (CH_2CHO), 34.5 (CH=CHCH_2), 31.5 (CH_2), 30.0 (CH_2), 24.5 (CH_2), 22.0 (CH_2), 13.5 (CH_2); GC–MS (EI, Rel. Int. %) *m/z* 169 ([M + 1], 2), 154 (41), 137 (23), 126 (62), 109 (42), 97 (37), 95 (20), 55 (28), 43 (89), 42 (29), 41 (83), 40 (34); HRMS (ESI, MeOH:H_2O) calcd for $C_{10}H_{16}O_2Na$ [M + Na]$^+$, 191.1048; found 191.1045.

4.5.2. 6-Ethyl-5,6-dihydro-2H-pyran-2-one (**10a**)

Isolated as a colorless oil, 0.24 g, (75%); IR (thin film, cm^{-1}) ν_{max} 1714 (C=O), 1251, 1036, 865; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (ddd, J = 9.6, 5.1, 3.3 Hz, 1H, CH=C*H*CH$_2$), 6.00 (dt, J = 9.6, 1.5 Hz, 1H, C*H*=CHCH$_2$), 4.40–4.30 (m, 1H, CH), 2.35–2.30 (m, 2H, CH=CHCH_2), 1.85–1.65 (m, 2H, CH_2), 1.00 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5 (C=O), 145.0 (CH=*C*H), 121.5 (*C*H=CH), 79.0 (CH_2CHO), 30.0 (CH_2), 28.0 (CH_2), 9.0 (CH_3); GCMS (EI, Rel. Int. %) *m/z* 126 ([M^+], 2), 97 (72), 69 (28), 68 (100), 41 (33), 40 (21); HRMS (ESI, MeOH:H_2O) calcd for $C_7H_{10}O_2Na$ [M + Na]$^+$, 149.0579; found 149.0230.

4.5.3. 6-(But-3-enyl)-5,6-dihydro-2H-pyran-2-one (**10b**)

Isolated as a colorless oil, 0.25 g, (65%); IR (thin film, cm^{-1}) ν_{max} 1716 (C=O), 1388, 1065, 1039, 996, 955, 864, 817; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (ddd, J = 9.6, 5.1, 3.9 Hz, 1H, CH=C*H*CH$_2$), 6.00 (dt, J = 9.6, 1.8 Hz, 1H, C*H*=CHCH$_2$), 5.85–5.72 (m, 1H, C*H*=CH$_2$), 5.10–5.00 (m, 2H, CH=C*H*$_2$), 4.50–4.40 (m, 1H, CH), 2.35–2.20 (m, 4H, 2 × CH_2);^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5 (C=O), 145.0 (CH=*C*H), 137.0 (*C*H=CH$_2$), 121.5 (*C*H=CH), 115.8 (CH=*C*H$_2$), 77.0 (CH_2CHO), 34.0 (*C*H_2CHO), 29.5 (CH_2), 28.8 (CH_2); GCMS (EI, Rel. Int. %) *m/z* 152 ([M^+], 1), 110 (17), 97 (85), 69 (53), 68 (100), 67 (52), 55 (34), 42 (20), 41 (79), 40 (34); HRMS (ESI, MeOH:H_2O) calcd for $C_9H_{12}O_2Na$ [M + Na]$^+$, 175.0735; found 175.0729.

4.5.4. 6-(Allyloxymethyl)-5,6-dihydro-2H-pyran-2-one (**10c**)

Isolated as a colorless oil, 0.31 g, (75%); IR (thin film, cm^{-1}) ν_{max} 3524, 3079, 2914, 2867, 1722 (C=O), 1423, 1249, 130, 1051, 848, 663; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (ddd, J = 9.6, 6.0, 2.7 Hz, 1H, CH=C*H*CH$_2$), 5.98 (ddd, J = 9.6, 2.4, 0.9 Hz, 1H, C*H*=CHCH$_2$), 5.90–5.80 (m, 1H, C*H*=CH$_2$), 5.28–5.15 (m, 2H, CH=C*H*$_2$), 4.60–4.50 (m, 1H, CH), 4.00 (dt, J = 5.7, 1.2 Hz, 2H, C*H*$_2$CH=CH$_2$), 3.60 (d, J = 4.5 Hz, 2H, CH_2), 2.60–2.30 (m, 1H, CH=CHCH_2), 2.30–2.45 (m, 1H, CH=CHCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.8 (C=O), 145.0 (CH=*C*H), 134.0 (*C*H=CH$_2$), 121.0 (*C*H=CH), 117.5 (CH=*C*H$_2$), 76.5 (CHOCH_2OCH_2), 72.5 (*C*H_2CHCH_2), 70.8 (CH_2CHO), 26.0 (CH=CHCH_2); GCMS (EI, Rel. Int. %) *m/z* 169 ([M + 1], 1), 154 (46), 126 (68), 97 (34), 81 (13), 69 (74), 55 (30), 43 (90), 41 (86), 40 (37); HRMS (ESI, MeOH:H_2O) calcd for $C_9H_{12}O_3Na$ [M + Na]$^+$, 191.0684; found 191.0680.

4.5.5. 6-(Phenoxymethyl)-5,6-dihydro-2H-pyran-2-one (**10d**)

Isolated as a white solid, 0.33 g, (65%); m.p 80–82 °C; IR (KBr pellet, cm^{-1}) ν_{max} 1721 (C=O), 1599, 1495, 1386, 1238, 1087, 1044, 812, 756; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29–7.14 (m, 2H, 2 × Ar–CH), 6.95–6.80 (m, 3H, 3 × Ar–CH, 1H, CH=C*H*CH$_2$), 6.00 (ddd, J = 9.6, 3.9, 2.2 Hz, 1H, C*H*=CHCH$_2$), 4.78–4.68 (m, 1H, CH), 4.15–4.00 (m, 2H, CH_2), 2.65–2.40 (m, 2H, CH=CHCH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5 (C=O), 158.0 (C^q-Ar), 145.0 (CH=*C*H), 129.5 (2 × CH–Ar), 121.5 (*C*H=CH), 121.0 (CH–Ar), 114.4 (CH–Ar), 75.5 (CH_2CHO), 68.0 (CH_2O–Ar), 26.0 (CH_2), GCMS (EI, Rel. Int. %) *m/z* 204 ([M^+], 43), 111 (28), 110 (36), 107 (24), 97 (100), 94 (33), 83 (15), 81 (16), 79 (17), 77 (67), 69 (56), 55 (25), 43 (70), 41 (59); HRMS (ESI, MeOH:H_2O) calcd for $C_{12}H_{12}O_3Na$ [M + Na]$^+$, 227.0684; found 227.0672.

4.5.6. 6-Phenyl-5,6-dihydro-2H-pyran-2-one (**10e**)

Isolated as a colorless oil, 0.22 g, (50%); IR (thin film, cm^{-1}) ν_{max} 1722 (C=O), 1454, 1382, 1246, 1061, 1022, 816, 760, 699; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.35 (m, 5H, 5 × Ar–CH), 7.00 (ddd, J = 10.0, 5.6, 3.2 Hz, 1H, CH=C*H*CH$_2$), 6.15 (ddd, J = 10.0, 2.4, 1.2 Hz, 1H, C*H*=CHCH$_2$), 5.45 (dd, J = 10.8, 5.2 Hz, 1H, CH), 2.65–2.60 (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0 (C=O), 145.0 (CH=*C*H), 138.5 (C^q-Ar), 128.8 (2 × CH–Ar), 128.5 (2 × Ar–CH), 126.0 (Ar–CH), 121.8 (*C*H=CH), 79.0 (CH_2CHO), 31.5 (CH_2); GCMS (EI, Rel. Int. %) *m/z* 174 ([M^+], 17), 128 (5), 105 (9), 77 (16), 68 (100), 51 (14); HRMS (ESI, MeOH:H_2O) calcd for $C_{11}H_{10}O_2Na$ [M + Na]$^+$, 197.0579; found 197.0575.

4.6. Antiproliferative activity

The antiproliferative activities of (−)-Massoialactone, **1** and α,β-unsaturated δ-lactones **10a**–**e** were evaluated in the following human cancer cells lines: HL-60 (pro-myelocytic leukemia), K562

(chronic myelogenous leukemia), HT-29 (colon carcinoma), NCI-H292 (lung carcinoma) and MCF-7 (breast carcinoma) obtained from Rio de Janeiro Cell Bank (RJ-Brazil). All cancer cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin at 37 °C with 5% CO_2. The cytotoxicity of all compounds was tested using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide (MTT) (Sigma Aldrich Co., St. Louis, MO/USA) reduction assay. For all experiments, tumor cells were plated in 96-well plates (10^5 cells/mL for adherent cells or 3×10^5 cells/mL for leukemias). Tested Compounds (0.1–25 μg/mL) dissolved in DMSO 0.1% were added to each well and incubated for 72 h. Control groups received the same amount of DMSO. After 69 h of treatment 25 μL of MTT (5 mg/mL) was added, 3 h later, the MTT formazan product was dissolved in 100 μL of DMSO, and absorbance was measured at 595 nm in plate spectrophotometer. The IC_{50} values and their 95% confidence intervals for two different experiments were obtained by nonlinear regression using Graphpad Prism version 5.0 for Windows (GraphPad Software, San Diego, California USA).

4.7. Anti-inflammatory activity

4.7.1. Effect of α,β-unsaturated δ-lactones **10b–e** on viability of LPS-stimulated peritoneal macrophages

Balb/c mice received by intraperitoneal route 2.5 mL 3% sodium thioglycollate. After 72 h, the animals were euthanized in a CO_2 chamber and peritoneal exudate was collected by washing the cavity with cold sterile PBS (5 mL). The viability of cells was assessed by trypan blue exclusion test and cell suspension was cultured (5×10^6 cells/mL) in microplates containing RPMI-1640 medium supplemented with 10% FBS, antibiotic solution (1000 UI/mL penicillin and 100 mg/L streptomycin), 200 mM L-glutamine and incubated for 2 h at 37 °C with a 5% CO_2 atmosphere. After that, non-adherent cells were discarded and the **10b–e** compounds (6.25–50 μg/mL) were added to macrophages adhered on the plate in presence of LPS (1 μg/mL). The controls included native cells and medium alone. After incubation at 37 °C and 5% CO_2 for 24 h, the supernatant was collected for further analysis of the nitrite and cytokine production and the cell viability was assessed by MTT assay. For the cytotoxicity evaluation, 20 μL MTT solutions (5 mg/mL) (Sigma Chemical Company, St. Louis, MO, USA) were added to each well for 4 h. The resulting formazan crystals were dissolved in DMSO. The spectrophotometric absorbance was measured at 550 nm wavelength using a microplate reader. The cytotoxicity as percentage of cell death was calculated by the formula: (1 − [absorbance of experimental wells/absorbance of control wells]) × 100%. Each concentration was tested in quadruplicate. Three independent experiments were performed and to assess the *in vitro* anti-inflammatory activity only concentrations at which the percentage of viable cells was more than 90% were chosen (compounds **10b** and **10c**) [39].

4.7.2. Analysis of nitrite and measurement of cytokines

The nitrite present in the supernatant of macrophages culture and in the pleural exudate was used as an indicator of NO production using the Griess reaction. Briefly, the samples (50 μL) were mixed with an equal volume of Griess reagent in a 96-well microtiter plate and incubated at room temperature for 10 min. The absorbance was read at 540 nm using a microplate reader and the nitrite concentrations were determined by comparison with a standard curve of sodium nitrite. Results were expressed as μM. The concentrations of TNF-α was measured using sandwich ELISA kits specific for mice (eBioscience, San Diego, California, USA) according to the manufacturer's instruction. Results were expressed at pg/mL.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.02.013. These data include MOL files and InChiKeys of the most important compounds described in this article.

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