Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 701–707
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of cytotoxic properties of stilbene-based resveratrol analogs

Dênis Pires de Lima [a,*], Rodrigo Rotta [a], Adilson Beatriz [a], Maria Rita Marques [b], Raquel C. Montenegro [c], Marne C. Vasconcellos [c], Cláudia Pessoa [c], Manoel O. de Moraes [c], Letícia V. Costa-Lotufo [c], Alexandra Christine Helena Frankland Sawaya [d], Marcos Nogueira Eberlin [d]

[a] *Departamento de Química, Universidade Federal de Mato Grosso do Sul, CP 549, 79070-900 Campo Grande, MS, Brazil*
[b] *Departamento de Morfofisiologia, Universidade Federal de Mato Grosso do Sul, CP 549, 79070-900 Campo Grande, MS, Brazil*
[c] *Laboratório de Oncologia Experimental, Universidade Federal do Ceará, Fortaleza, CE, Brazil*
[d] *Laboratório Thomson de Espectrometria de Massas, Instituto de Química, Universidade Estadual de Campinas, UNICAMP, Campinas, SP 13083-970, Brazil*

Received 29 October 2007; received in revised form 2 May 2008; accepted 7 May 2008
Available online 15 May 2008

Abstract

This work deals with the preparation of stilbene-based resveratrol analogs by employing the Perkin reaction, aiming at synthesizing potential antitumor lead compounds and evaluating their pharmacological activities. The proliferation inhibitor test against tumor cell lines identified analogs **9** and **11** as the most active among all synthesized derivatives, presenting IC_{50} in micromolar range for certain cell lines. For study on the embryonic development, compounds **8** and **9** at the lowest tested concentration (41.7 μM) that inhibited sea urchin egg development, but only after third cleavage were used. Both the compounds inhibited 100% of normal development since first cleavage. These data partially corroborated the results obtained with MTT assay using tumor cell lines. None of the tested compounds revealed hemolytic action in assay with mouse erythrocytes.

Keywords: Stilbenes; Resveratrol analogs; Cytotoxic properties; Embryonic development

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources or derived from natural product molecules, especially in cancer therapy. Stilbene derivatives, such as the plant microcomponents resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) and combretastatin A-4 (Fig. 1), exhibit a variety of biological activities including antineoplastic, chemopreventive, antioxidant and antiestrogenic [1–4]. Combretastatin A-4 is the most potent antimitotic agent isolated from *Combretum caffrum* and it interacts with the colchicine site on tubulin [5].

Resveratrol is a polyphenolic compound produced by several species and found especially in *Polygonum* roots, peanuts, seeds, berries and also grapes, and consequently present in human diet or beverages (red wine, for example) [6].

It provides cancer chemopreventive effects for different systems based on its striking inhibition of diverse cellular events associated with tumor initiation, promotion, and progression. It has also been shown that the physiological quantities of resveratrol can modulate multiple cellular pathways relevant for tumorigenesis, among them include phase I and phase II drug-metabolizing enzymes, DNA-synthesis and inflammatory response, as well as cell survival, cell death and cell cycle [7]. A majority of in vitro studies and some in vivo studies show that resveratrol can affect proliferation and survival through the inhibition of cell cycle, stimulation

* Corresponding author. Tel.: +55 67 3345 3578; fax: +55 67 3345 3552.
E-mail address: dlima@nin.ufms.br (D.P. de Lima).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.05.003

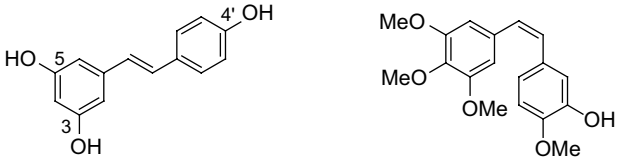


Fig. 1. Structure of bioactive stilbenes.

of differentiation and induction of apoptosis in cancer cell lines [7,8].

In fact, the anticancer potential of resveratrol has been widely studied, but it is still important to contribute for an ample knowledge on the biological effects of related compounds [9]. With this purpose, this work has been aimed at synthesizing selected stilbene-based resveratrol analogs and evaluating the cytotoxic potential of these compounds, thus giving a platform for the correlation between structure and activity using three different cell models: tumor cell lines, sea urchin eggs and mouse erythrocytes.

2. Results and discussion

2.1. Chemistry and spectroscopic means

Our research group has decided to employ simple methodology to rapidly obtain molecules for screening of cytotoxic properties. Perkin reaction seemed to be very efficient and useful to assemble the correct units for building these types of carbogenic frameworks given that it has been applied to synthesize resveratrol itself [10].

As starting materials we have chosen compounds **1**–**4** (Scheme 1). The Perkin type condensation was employed as the first step for the preparation of compounds **5**–**8**. After decarboxylation of compound **5**, catalyzed by copper chromite in quinoline, compound **9** was formed and treated with methanol and concentrated hydrochloric acid to afford its isomer **10**. Compound **6** was submitted to hydrolysis under basic conditions giving compound **11**.

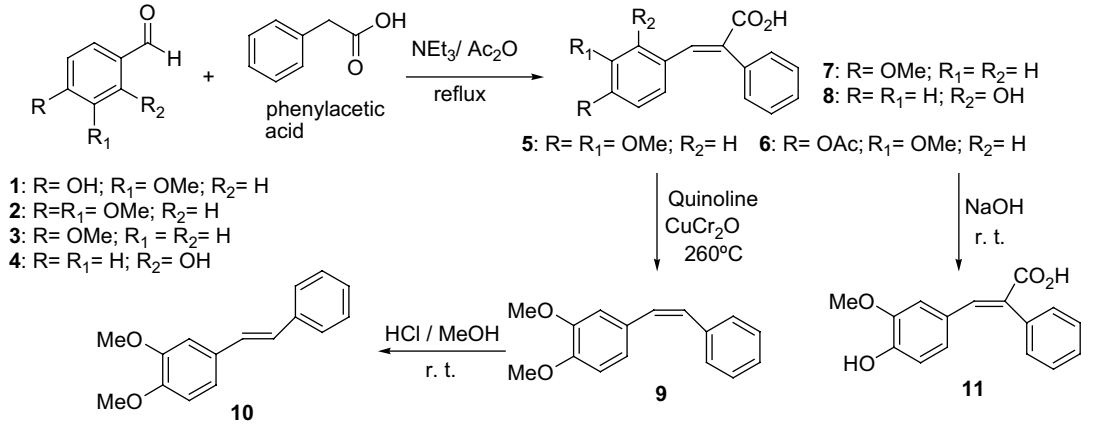
The procedures were performed in conformity with a synthetic study regarding the re-investigation of the Perkin reaction that was recently re-investigated [10]. Structures of these compounds were characterized using IR, HRMS (see Section 4), ^{1}H NMR (Table 1), and ^{13}C NMR (Table 2) analyses alongside comparison with data from literature for similar compounds [10–20].

2.2. Cancer cell assays

The cytotoxicity on tumor cell lines was evaluated by the MTT assay and the results are summarized in Table 3. Resveratrol inhibited the proliferation of all tested cell lines with IC_{50} ranging from 10.74 μM in HL-60 to 62.91 μM in B-16 cells. As previously mentioned, many studies had emphasized the great potential of resveratrol as a potent chemotherapeutic agent leading cancer cells to apoptosis [9,21–23]. Previous data from studies using HL-60 leukemia cell line as a model demonstrated IC_{50} values for resveratrol of the same order of magnitude as the present study (12.1 μM) [23]. Additionally, it was demonstrated that the antileukemic activity is related to the depolarization of mitochondrial membranes and activation of caspase 9, triggering apoptosis [21]. The cytotoxic effects of resveratrol on MDA-MB-435 and MCF-7 included a dramatic G0/G1 arrest, alterations in the expression of Cyclin D1 and Stat3 phosphorylation [22].

To further enhance the cytotoxicity of resveratrol, a few analogs have been synthesized and investigated in the search for an anticancer agent with higher efficacy than resveratrol. Among the analogs obtained in the present study, only compounds **9** and **11** presented a weak to moderate cytotoxicity. Compound **11** inhibited only MDA-MB-435 proliferation with an IC_{50} of 58.8 μM, while compound **9** was active against HL-60, K-562 and MDA-MB-435 cells, with IC_{50} values of 26.71, 26.55 and 25.30 μM, respectively.

Kang et al. [9] have described that low concentrations of resveratrol dimers and trimers, unlike resveratrol, strongly decreased the MTT-reducing activity by more than 3-fold in HL-60 leukemia cell line. Also, Cai et al. [24] have found that 3,4-dihydroxyl groups are important to enhance



Scheme 1. Synthesis of resveratrol analogs.

Table 1
^{1}H NMR spectral data of synthesized compounds

Compound	^{1}H NMR ($CDCl_3$) δ
5	3.35 (s, 3H), 3.83 (s, 3H), 6.41 (d, 1H, $J = 1.6$ Hz), 6.72 (d, 1H, $J = 8.4$ Hz), 6.86 (dd, 1H, $J = 8.4$, 1.6 Hz), 7.24–7.43 (m, 5H), 7.87 (s, 1H)
6	2.27 (s, 3H), 3.35 (s, 3H), 6.53 (d, 1H, $J = 1.7$ Hz), 6.84 (dd, 1H, $J = 8.2$, 1.7 Hz), 6.91 (d, 1H, $J = 8.2$ Hz), 7.26–7.45 (m, 5H), 7.89 (s, 1H)
7	3.75 (s, 3H), 6.68 (d, 2H, $J = 8.7$ Hz), 7.01 (d, 2H, $J = 8.7$ Hz), 7.23–7.39 (m, 5H), 7.89 (s, 1H)
8	7.13–7.54 (m, 7H), 7.70 (d, $J = 6.5$ Hz, 2H), 7.80 (s, 1H)
9	3.57 (s, 3H), 3.81 (s, 3H), 6.53 (s, 2H), 6.71 (d, 1H, $J = 8.2$ Hz), 6.78 (d, 1H, $J = 1.6$ Hz), 6.82 (dd, 1H, $J = 8.2$, 1.6 Hz), 7.16–7.31 (m, 5H)
10	3.89 (s, 3H), 3.93 (s, 3H), 6.85 (d, 1H, $J = 8.0$ Hz), 6.95 (d, 1H, $J = 16.1$ Hz), 7.05 (d, 1H, $J = 16.1$ Hz), 7.06 (s, 1H), 7.22 (t, 1H, $J = 7.4$ Hz), 7.33 (t, 2H, $J = 7.4$ Hz), 7.48 (d, 2H, $J = 7.4$ Hz)
11	3.28 (s, 3H), 6.26 (d, 1H, $J = 1.5$ Hz), 6.65 (d, 1H, $J = 8.3$ Hz), 6.70 (dd, 1H, $J = 1.5$, 8.3 Hz), 7.13–7.33 (m, 5H), 7.74 (s, 1H)

anticancer activity of *trans*-resveratrol analogs, what corroborates with Murias et al. [23] who demonstrated that hydroxylation of resveratrol at 3, 4′ and 5 positions led to a higher cytotoxicity in HL-60 cells than resveratrol. Moreover, several studies demonstrated that the introduction of methoxy groups into resveratrol, as observed in *trans*-3,4,5,4′-tetramethoxystilbene, enhances its antiproliferative effects against several cancer cell lines and may have different cellular targets than resveratrol, inhibiting microtubule assembly [22].

It is difficult to accurately elucidate a structure–activity relationship that satisfies the disclosed results with respect to reduced activity of the obtained analogs when compared to resveratrol. Gaukroger et al. [25] have pointed out that *anti*-ring configuration of stilbenes shows a remarkable decrease in their inhibitory effects on cancer cell growth when compared to their corresponding *syn* isomers. Initially, this information suggested that the prepared compounds with the exception of compound **10** would present a better activity than resveratrol. Presumably, the absence of a C-4′ hydroxyl group in all compounds might have caused diminution in the cytotoxicity. Nevertheless, compounds **9** and **11** have no great variation in molecular framework from compounds **5**–**8** what places an intriguing question with respect to distinct biological behavior. The activity is not entirely predictable. The additional hydroxyl group in the B ring seemed to disproportionately enhance activity in some cases as in resveratrol or combretastatin A-4. This could also be derived from conformational issues [5] leading to a poor interaction with key amino acid residues in the receptor site, and this in turn results in poor cytotoxicity.

2.3. Antimitotic activity

The antimitotic activity was determined as the ability to inhibit sea urchin egg development. The sea urchin egg development has some peculiarities, making it possible to suggest how the test substances acted. The sea urchin cell cycle is highly abbreviated, essentially cycling from S (synthesis) to M (mitosis) and the S with no G1 phase and a relatively short G2 phase [26]. The inhibition of the first cleavage in these cells is related to DNA and/or protein synthesis or microtubule assembling, once RNA synthesis is very slow or absent after fertilization [27]. At this time, the rapid increase in the rate of protein synthesis is largely due to the recruitment of material mRNA into polysomes [28]. However, when the substance interacts with microtubule assembly, clear spots indicating nucleus duplication can be observed in the cytoplasm. In the present work (Table 4), resveratrol at 43.8 μM inhibited the sea urchin egg development since the first cleavage showing that this compound may be interacting with DNA and/or protein synthesis. In addition, compounds **8** and **9** at the lowest tested concentration (41.7 μM) also

Table 2
^{13}C NMR spectral data of synthesized compounds

Compound	^{13}C NMR ($CDCl_3$) δ
5	55.1 (CH_3), 55.8 (CH_3), 110.4 (CH), 112.3 (CH), 126.1 (CH), 127.1 (C), 127.9 (CH), 128.9 (2CH), 129.0 (C), 130.0 (2CH), 136.0 (C), 142.4 (CH), 148.2 (C), 150.4 (C), 172.5 (C)
6	20.6 (CH_3), 55.2 (CH_3), 113.7 (CH), 122.6 (CH), 124.9 (CH), 125.9 (C), 128.1 (CH), 128.9 (2CH), 129.8 (2CH), 132.9 (C), 135.4 (C), 140.7 (CH), 141.6 (C), 150.5 (C), 168.7 (C), 172.1 (C)
7	55.2 (CH_3), 113.8 (2CH), 126.9 (C), 127.9 (CH), 128.8 (2CH), 129.1 (C), 129.8 (2CH), 132.7 (2CH), 135.8 (C), 142.1 (CH), 160.6 (C), 173.0 (C)
8	116.0 (CH), 119.6 (C), 124.1 (CH), 127.5 (CH), 128.1 (2CH), 128.1 (2CH), 128.3 (C), 128.4 (CH), 131.4 (CH), 134.7 (C), 139.8 (CH), 153.4 (C), 160.6 (C)
9	54.7 (CH_3), 55.1 (CH_3), 110.4 (CH), 111.3 (CH), 121.4 (CH), 126.4 (CH), 127.7 (2CH), 128.3 (2CH), 128.4 (CH), 129.3 (C), 129.5 (C), 137.2 (C), 147.8 (C), 147.8 (C)
10	55.9 (CH_3), 56.0 (CH_3), 108.8 (CH), 111.2 (CH), 119.9 (CH), 126.3 (2CH), 126.8 (CH), 127.3 (CH), 128.5 (CH), 128.7 (2CH), 130.5 (C), 137.5 (C), 148.9 (C), 149.1 (C)
11	55.2 (CH_3), 111.9 (CH), 112.9 (CH), 126.6 (C), 126.8 (CH), 127.9 (CH), 128.6 (C), 128.9 (2CH), 130 (2CH), 136.1 (C), 142.5 (CH), 145.9 (C), 147.3 (C), 172.0 (C)

Table 3
Inhibitory effect on cultured cell growth of resveratrol and its analogs on tumor cell lines

Compound	B-16	HCT-8	HL-60	CEM	K-562	MBA-MD-435	PC3
Doxorubicin	0.03 (0.05)	0.04 (0.07)	0.02 (0.03)	0.02 (0.03)	0.14 (0.24)	0.47 (0.81)	0.45 (0.77)
	0.02–0.04	0.03–0.05	0.01–0.02	0.01–0.02	0.14–0.21	0.34–0.65	0.38–0.68
Resveratrol	14.35 (62.91)	5.82 (25.51)	2.45 (10.74)	5.91 (25.91)	9.62 (42.18)	13.81 (60.55)	3.51 (15.39)
	11.97–17.21	5.14–6.59	1.95–3.09	4.39–7.97	3.48–2.65	10.44–18.25	2.99–2.45
5	>25 (88.0)	>25 (88.0)	>25 (88.0)	>25 (88.0)	>25 (88.0)	>25 (88.0)	>25 (88.0)
6	>25 (80.1)	>25 (80.1)	>25 (80.1)	>25 (80.1)	>25 (80.1)	>25 (80.1)	>25 (80.1)
7	>25 (98.4)	>25 (98.4)	>25 (98.4)	>25 (98.4)	>25 (98.4)	>25 (98.4)	>25 (98.4)
8	>25 (104)	>25 (104)	>25 (104)	>25 (104)	>25 (104)	>25 (104)	>25 (104)
9	>25 (104)	>25 (104)	6.42(26.71)	>25 (104)	6.38 (26.55)	6.08 (25.30)	>25 (104)
					3.13–13.01	2.22–16.70	
10	>25 (104)	>25 (104)	>25 (104)	>25 (104)	>25 (104)	>25 (104)	>25 (104)
11	>25 (82.8)	>25 (82.8)	>25 (82.8)	>25 (82.8)	>25 (82.8)	17.76 (58.8)	>25 (82.8)
						16.36–19.30	

Data are presented as IC_{50} μg/mL (μM) values and their 95% confidence interval (CI, 95%) obtained by nonlinear regression. Doxorubicin was used as a positive control.

inhibited sea urchin egg development, but only after third cleavage. At 41.7 μM, both the compounds inhibited 100% of normal development since first cleavage. These data partially corroborated the results obtained with MTT assay using tumor cell lines, where compound **9** was the most active among all synthesized derivatives. On the other hand, compound **11** was poorly active in this model, indicating that the structural requirements for cytotoxic action will depend upon the model used.

This is the first report on resveratrol's effects on sea urchin eggs. This model has been used for decades to detect cytotoxic, teratogenic and antineoplastic activities of new compounds [29–31]. It is worthwhile mentioning that stilbene effects on sea urchin eggs are linked to antioxidant properties. Tamoxifen – the selective estrogen receptor modulator used in the treatment of breast cancer – effects on sea urchin eggs included early embryonic mortality to exposed embryos and to offspring exposed eggs, developmental defects to the offspring of exposed sperm, decrease in sperm fertilization success and cytogenetic effects in the offspring of exposed sperm or eggs [32].

Table 4
Percentage of the inhibition of the cell proliferation by the resveratrol and its analogs on the embryos of the sea urchin *Lytechinus variegatus* on the 1st and 3rd cleavage and blastulae stages

Compound	Concentration μg/mL (μM)	1st Cleavage (mean ± SEM)	3rd Cleavage (mean ± SEM)	Blastulae (mean ± SEM)
Doxorubicin	10 (18.4)	100 ± (0)	100 ± (0)	100 ± (0)
	100 (184)	100 ± (0)	100 ± (0)	100 ± (0)
Resveratrol	10 (43.8)	97.9 ± 1.1	100 ± 0	100 ± 0
5	10 (35.2)	4.3 ± (1.5)	0 ± (0)	0.7 ± (0.7)
	100 (352)	3 ± (2.1)	0 ± (0)	0.7 ± (0.7)
6	10 (32.0)	0 ± (0)	2.3 ± (1.2)	5 ± (2.5)
	100 (320)	100 ± (0)	100 ± (0)	100 ± (0)
7	10 (39.4)	0 ± (0)	3.6 ± (3.2)	0.3 ± (0.3)
	100 (394)	0 ± (0)	9.3 ± (5.5)	68.7 ± (3)
8	10 (41.7)	12.3 ± 3.6	96.3 ± 1.6	97.3 ± 2.7
	100 (417)	44.5 ± 1.6	100 ± 0	95.4 ± 2
9	10 (41.7)	0 ± (0)	100 ± (0)	100 ± (0)
	100 (417)	100 ± (0)	100 ± (0)	100 ± (0)
10	10 (41.7)	0 ± (0)	6 ± (2.3)	83 ± (5.5)
	100 (417)	0 ± (0)	100 ± (0)	100 ± (0)
11	10 (37.0)	0 ± (0)	7.6 ± (3.8)	5.3 ± (2.7)
	100 (370)	0 ± (0)	7.3 ± (1.6)	99.7 ± (0.3)

The inhibition was obtained considering negative control as 100% of cell proliferation. Doxorubicin was used as a positive control.

2.4. Hemolytic activity

In order to verify whether the observed cytotoxicity is related to membrane disruption, resveratrol analogs were tested for their ability to induce lysis of mouse erythrocytes. The erythrocyte membrane is a dynamic structure that can show significant changes on interaction with drugs [33]. However, these substances show no lytic activity, suggesting that the mechanism of cytotoxicity is not a result of membrane damage. Getting all the results together, the cytotoxicity of resveratrol and its analogs may be caused by more specific pathway probably involving DNA and protein synthesis instead of membrane damage.

3. Conclusion

The Perkin reaction showed to be a feasible method to prepare stilbene analogs. By using the procedure along with other simple methods it was possible to access the syntheses of seven resveratrol analogs. The compounds revealed fairly in vitro activity against cancer cell lines, particularly compounds **9** and **11**. In addition, compounds **8** and **9** strongly inhibited sea urchin egg development. The cytotoxic effect of the stilbene-based resveratrol analogs may be connected with different cellular pathways relating to proliferation reduction such as cell cycle inhibition, differentiation or apoptosis induction, as shown for resveratrol, however, generally, with a reduced antiproliferative action. Resveratrol showed to be largely more active to the performed biological tests than all the prepared compounds. However, the presented results may be used to draw relevant information, such as SARs that might drive future research.

4. Materials and methods

4.1. Chemistry

All melting points were determined using Uniscience of Brazil Mod. 498 equipment. Absorption FT-IR spectra were obtained using the KBr pellet method or in chloroform solution performed with a Perkin Elmer Mod. 783-FT spectrometer. NMR spectra were recorded on Bruker DPX-300 spectrometer, the chemical shifts were presented in ppm (δ) relative to TMS ($\delta = 0.0$) and $CDCl_3$ was employed as a solvent. High resolution electrospray ionization-mass spectrometry (ESI-MS) analyses were performed using a Q-TOF Micromass spectrometer in both positive and negative ion modes with capillary set at ±3000 V and cone voltage set at ±40 V, and de-solvation temperature at 100 °C.

The solvents employed in the reactions and silica gel column chromatography were previously purified and dried according to the procedures found in literature [34]. Thin-layer chromatography (TLC) was carried out on silica gel plates with a fluorescence indicator F_{254} (0.2 mm, E. Merck); the spots were visualized in UV light, and by spraying with 1% ethanol solution of vanillin or by charring reagent. Purification of compounds was performed using column chromatography; the stationary phase was silica gel 60 (80–230 mesh) from ACROS (Brazil), silica gel 60 (230–400 mesh) from Merck and Celite. All reagents used in the present study were of analytical grade.

4.2. General procedure for Perkin's reaction

Under argon, the corresponding aldehyde (1 equiv.), phenylacetic acid (1 equiv.), acetic anhydride (2 equiv.) and triethylamine (0.7 equiv.) were heated under reflux for 12 h, cooled at room temperature, diluted with 50 mL of water and 50 mL of ethyl acetate. The aqueous layer was extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$ and concentrated under vacuum. A yellowish solid was obtained most of the times and was purified by recrystallization or chromatography on silica gel (hexane/ethyl acetate).

4.2.1. (*E*)-3-(3,4-Dimethoxyphenyl)-2-phenylacrylic acid (**5**)

White solid (0.69 g, 49% yield). M.p. = 223–225 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3444, 1666, 1595, 1424, 1266, 1147. HRMS [ESI(−)-MS]: $C_{17}H_{15}O_4$ $[M-H]^-$ *m/z*, calcd 283.0970, found 283.0624.

4.2.2. (*E*)-3-(4-Acetoxy-3-methoxyphenyl)-2-phenylacrylic acid (**6**)

White solid (0.75 g, 48% yield). M.p. = 191–194 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3444, 1773, 1673. HRMS [ESI(−)-MS]: $C_{18}H_{15}O_5$ $[M-H]^-$ *m/z*, calcd 311.0919, found 311.0972.

4.2.3. (*E*)-3-(4-Methoxyphenyl)-2-phenylacrylic acid (**7**)

White solid (0.62 g, 49% yield). M.p. = 183–185 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3000, 1671, 1603. HRMS [ESI(−)-MS]: $C_{16}H_{13}O_3$ $[M-H]^-$ *m/z*, calcd 253.0865, found 253.0693.

4.2.4. (*E*)-3-(2-Hydroxyphenyl)-2-phenylacrylic acid (**8**)

Gray solid (0.84 g, 48% yield). M.p. = 137–139 °C. HRMS [ESI(−)-MS]: $C_{15}H_{11}O_3$ $[M-H]^-$ *m/z*, calcd 239.0708, found 239.0721.

4.2.5. (*Z*)-1-(3,4-Dimethoxyphenyl)-2-phenyl-ethene (**9**)

Compound **5** (0.5 g, 1.7 mmol) and copper chromite (30 mg, 0.2 mmol) were added to quinoline (2.45 g, 19 mmol). The reaction mixture was stirred and heated to reflux for 2 h. The mixture was filtered over Celite and washed with ethyl acetate (10 mL). The organic layer was washed with 2 M hydrochloric acid (3 × 10 mL), dried over anhydrous magnesium sulfate and concentrated to dryness in a rotaevaporator under vacuum at 45 °C. A brown solid was obtained, which was purified by chromatography on silica gel employing hexane/ethyl acetate (3/1) as the eluent. Compound **9** was obtained as yellow oil (0.32 g, 78% yield). FT-IR ($CHCl_3$, cm^{-1}) ν_{max}: 1600, 1514, 1270. HRMS [ESI(+)-MS]: $C_{16}H_{17}O_2$ $[M+H]^+$ *m/z*, calcd 241.1229, found 241.2111.

4.2.6. (*E*)-1-(3,4-Dimethoxyphenyl)-2-phenyl-ethene (**10**)

Compound **9** (0.020 g, 0.083 mmol) was dissolved in methyl alcohol (5 mL) and concentrated hydrochloric acid (1 mL) was added to the solution. The reaction mixture was allowed to stir at room temperature for 48 h. Water was added (10 mL) and the mixture was extracted with ethyl acetate (3 × 5 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated to dryness in a rotaevaporator under vacuum at 45 °C, resulting in a white solid (0.019 g, 98% yield). M.p. = 94–97 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 1591, 1514, 1267. HRMS [ESI(+)-MS]: $C_{16}H_{17}O_2$ $[M+H]^+$ *m/z*, calcd 241.1229, found 241.2111.

4.2.7. (*E*)-3-(4-Hydroxy-3-methoxyphenyl)-2-phenylacrylic acid (**11**)

Compound **6** (0.010 g, 0.035 mmol) was added to a 10% solution of sodium hydroxide (15 mL). The mixture was stirred for 24 h and then diluted with ethyl acetate (3 × 10 mL). The water layer was acidified in an ice bath. Vacuum filtration followed by washing with cooled water (3 × 15 mL) resulted in a white solid (0.0095 g, 95% yield). M.p. = 182–185 °C. HRMS [ESI(−)-MS]: $C_{16}H_{13}O_4$ $[M-H]^-$ *m/z*, calcd 269.0814, found 269.1530.

4.3. MTT assay

The cytotoxicity of the compounds was tested against CEM, HL-60 and K-562 (human leukemias), B-16 (murine melanoma), HCT-8 (human colon), MBA-MD-435 (human melanoma) and PC3 (human prostate) cell lines obtained from the National Cancer Institute, Bethesda, MD, USA. Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 μg/mL streptomycin and 100 U/mL penicillin and incubated at 37 °C with a 5% CO_2 atmosphere. Cells were plated in 96-well plates (10^5 cells/well for adherent cells or 0.5×10^5 cells/well for suspended cells in 100 μL of medium).

After 24 h, the compounds (0.39–25.0 μg/mL) dissolved in 1% DMSO were added to each well and incubated for 72 h. Control group received the same amount of DMSO. Doxorubicin (Doxolem®, Zodiac Produtos Farmacêuticos S/A, Brazil) was used as a positive control. Tumor cell growth was quantified by the ability of the living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a purple formazan product [35]. At the end of the incubation, the plates were centrifuged and the medium was replaced by fresh medium (200 μL) containing 0.5 mg/mL MTT. Three hours later, the MTT formazan product was dissolved in 150 μL DMSO, and the absorbance was measured using a multiplate reader (Spectra Count, Packard, Ontario, Canada). The drug effect was quantified as the percentage of the absorbance of reduced dye at 550 nm in relation to control wells.

4.4. Assay on sea urchins

The assay was performed following the method described by Jimenez et al. [36]. Adult sea urchins (*Lytechinus variegatus*) were collected at Lagoinha beach, on the northeastern coast of Brazil. Gamete elimination was induced by injecting 3 mL of 0.5 M KCl into the urchin's coelomic cavity. For fertilization, 1 mL of a sperm suspension (0.05 mL of concentrated sperm in 2.45 mL of filtered sea water) was added to every 50 mL of egg solution. The assay was carried out in 24-multiwell plates. The compounds were added immediately after fecundation (within 2 min) to get concentrations of 10 and 100 μg/mL in a final volume of 2 mL. Doxorubicin (Doxolem®, Zodiac Produtos Farmacêuticos S/A, Brazil) was used as a positive control. At appropriate intervals, aliquots of 200 μL were fixed in the same volume of 10% formaldehyde to obtain first and third cleavages and blastulae. One hundred eggs were counted for each concentration to obtain the percentage of normal cells.

4.5. Hemolytic assay

The test was performed in 96-well plates using a 2% mouse erythrocyte suspension in 0.85% NaCl containing 10 mM $CaCl_2$, following the method described by Jimenez et al. [36]. The compounds were tested at concentrations ranging from 1.5 to 200 μg/mL. After incubation at room temperature for 30 min and centrifugation, the supernatant was removed and the liberated hemoglobin was measured spectrophotometrically as the absorbance at 540 nm.

4.6. Statistical analysis

Data are presented as mean ± SEM. The IC_{50} or EC_{50} and their confidence intervals were obtained by nonlinear regression using the GRAPHPAD program (Intuitive Software for Science, San Diego, CA).

Acknowledgements

We wish to thank CNPq, Kardol Ind. Química Ltda., Instituto Claude Bernard, FUNCAP, Banco do Nordeste, FINEP and PROPP-UFMS for the financial support in the form of grants and fellowship awards. The authors thank Silvana França dos Santos, Luciana França, and Maria de Fátima Teixeira for technical assistance. We are also indebted to Nanocore Biotecnologia Ltda. for logistic support and for making proposals for future projects in this field.

References

[1] D. Simoni, M. Roberti, F.P. Invidiata, E. Aiello, S. Aiello, P. Marchetti, R. Baruchello, M. Eleopra, A. Di Cristina, S. Grimaudo, N. Gebbia, L. Crosta, F. Dieli, M. Tolomeo, Bioorg. Med. Chem. Lett. 16 (2006) 3245–3248.
[2] J. Griggs, J.C. Metcalfe, R. Hesketh, Lancet Oncol. 2 (2001) 82–87.
[3] M. Mokni, S. Elkahoui, F. Limam, M. Amri, E. Aouani, Neurochem. Res. 32 (2007) 981–987.
[4] A. Cirla, J. Mann, Nat. Prod. Rep. 20 (2003) 558–564.
[5] G.R. Pettit, M.R. Rhodes, D.L. Herald, E. Hamel, J.M. Schmidt, R.K. Pettit, J. Med. Chem. 48 (2005) 4087–4099.
[6] N. Latruffe, D. Delmas, B. Jannin, M. Cherkaoui Malki, P. Passilly-Degrace, J.P. Berlot, Int. J. Mol. Med. 10 (2002) 755–760.
[7] K.P. Bhat, J.M. Pezzuto, Cancer Res. 61 (2001) 6137–6144.
[8] H.P. Ciolino, G.C. Yeh, Adv. Exp. Med. Biol. 492 (2001) 183–193.
[9] J.H. Kang, Y.H. Park, S.W. Choi, E.K. Yang, W.J. Lee, Exp. Mol. Med. 35 (2003) 467–474.
[10] G. Solladié, Y. Pasturel-Jacopé, J. Maignan, Tetrahedron 59 (2003) 3315–3321.
[11] C.J. Kelley, J.R. Mahajan, L.C. Brooks, L.A. Neubert, W.R. Breneman, M. Carmack, J. Org. Chem. 40 (1975) 1804–1815.
[12] C.J. Kelley, R.C. Harruff, M. Carmack, J. Org. Chem. 41 (1976) 449–455.
[13] M. Bartok, G. Wittmann, G. Gondos, G.V. Smith, J. Org. Chem. 52 (1987) 1139–1141.
[14] T. Sugimura, J. Watanabe, T. Okuyama, Y. Nitta, Tetrahedron: Asymmetry 16 (2005) 1573–1575.
[15] T. Majima, S. Tojo, A. Ishida, S. Takamuku, J. Org. Chem. 61 (1996) 7793–7800.
[16] J.C. Roberts, J.A. Pincock, J. Org. Chem. 69 (2004) 4279–4282.
[17] J.C. Roberts, J.A. Pincock, J. Org. Chem. 71 (2006) 1480–1492.
[18] J.J. Heynekamp, W.M. Weber, L.A. Hunsaker, A.M. Gonzales, R.A. Orlando, L.M. Deck, D.L.V. Jagt, J. Med. Chem. 49 (2006) 7182–7189.
[19] J. Liu, Y. Zhao, Y. Zhou, L. Li, T.Y. Zhang, H. Zhang, Org. Biomol. Chem. 1 (2003) 3227–3231.
[20] V. Kumar, A. Sharma, A. Sharma, A.K. Sinha, Tetrahedron 63 (2007) 7640–7646.
[21] J. Dorrie, H. Gerauer, Y. Wachter, S.J. Zunino, Cancer Res. 61 (2001) 4731–4739.
[22] Z. Ma, O. Molavi, A. Haddadi, R. Lai, R.A. Gossage, A. Lavasanifar, Cancer. Chemother. Pharmacol., in press. doi:10.1007/s00280-008-0704-z, <http://www.springerlink.com/content/8w31220m82583497/>
[23] M. Murias, W. Jager, N. Handler, T. Erker, Z. Horvath, T. Szekeres, H. Nohl, L. Gille, Biochem. Pharmacol. 69 (2005) 903–912.
[24] Y.J. Cai, J.G. Fang, L.P. Ma, L. Yang, Z.L. Liu, Biochim. Biophys. Acta 1637 (2003) 31–38.
[25] K. Gaukroger, J.A. Hadfield, L.A. Hepworth, N.J. Lawrence, A.T. McGown, J. Org. Chem. 66 (2001) 8135–8138.
[26] R.S. Jacobs, L. Wilson, in: A. Aszalor (Ed.), Modern Analysis of Antibiotics, Marcel Dekker Inc., New York, 1986, pp. 481–493.
[27] P.R. Gross, L.I. Malkin, W.A. Moyer, Proc. Natl. Acad. Sci. U S A 51 (1964) 407–414.

[28] B.P. Brandhorst, Development Biology: A Comprehensive Synthesis, in: L.W. Browder (Ed.), Oogenesis, Plenum, New York, 1985, pp. 525–576.
[29] L.V. Costa-Lotufo, P.C. Jimenez, D.V. Wilke, L.K. Leal, G.M. Cunha, E.R. Silveira, K.M. Canuto, G.S. Viana, M.E. Moraes, M.O. de Moraes, C. Pessoa, Z. Naturforsch. C 58 (2003) 675–680.
[30] L.V. Costa-Lotufo, M.T. Khan, A. Ather, D.V. Wilke, P.C. Jimenez, C. Pessoa, M.E. de Moraes, M.O. de Moraes, J. Ethnopharmacol. 99 (2005) 21–30.
[31] N. Fusetani, in: P. Scheuer (Ed.), Bioorganic Marine Chemistry, Springer Verlag, Berlin, 1987, pp. 61–91.
[32] G. Pagano, A. de Biase, I.B. Deeva, P. Degan, Y.K. Doronin, M. Iaccarino, R. Oral, N.M. Trieff, M. Warnau, L.G. Korkina, Life Sci. 68 (2001) 1735–1749.
[33] P. Sharma, J.D. Sharma, J. Ethnopharmacol. 74 (2001) 239–243.
[34] D.D. Perrin, W.L.F. Armarego (Eds.), Purification of Laboratory Chemicals, Pergamon Press, Oxford, 1988.
[35] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[36] P.C. Jimenez, S.C. Fortier, T.M.C. Lotufo, C. Pessoa, M.E.A. Moraes, M.O. de Moraes, L.V. Costa-Lotufo, J. Exp. Mar. Biol. Ecol. 287 (2003) 93–101.