Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 6080–6088
Bioorganic & Medicinal Chemistry

Dihydroxyxanthones prenylated derivatives: Synthesis, structure elucidation, and growth inhibitory activity on human tumor cell lines with improvement of selectivity for MCF-7

Raquel A. P. Castanheiro,[a] Madalena M. M. Pinto,[a,b,*] Artur M. S. Silva,[c] Sara M. M. Cravo,[a,b] Luís Gales,[d,e] Ana M. Damas,[d,e] Naïr Nazareth,[a] Maria S. J. Nascimento[a,f] and Graham Eaton[g]

[a]*Centro de Estudos de Química Orgânica, Fitoquímica e Farmacologia, Universidade do Porto, Portugal*
[b]*Laboratório de Química Orgânica, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, 4050-047 Porto, Portugal*
[c]*Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*
[d]*Unidade de Estrutura Molecular, IBMC—Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal*
[e]*ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, 4099-003 Porto, Portugal*
[f]*Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, 4050-047 Porto, Portugal*
[g]*Department of Chemistry, Leicester University, University Road, Leicester LE 7RH, UK*

Received 17 January 2007; revised 14 June 2007; accepted 19 June 2007
Available online 23 June 2007

Abstract—The synthesis, structure elucidation, and antitumor activity of 11 xanthones are reported, being the compounds **3**, **4**, **6**–**8**, and **9** described for the first time. Xanthones **1** and **2** were used as building blocks to obtain the prenylated derivatives **3**–**8**. Prenylation was carried out using prenyl bromide in alkaline medium. Dihydropyranoxanthones **9**–**11** were obtained from compounds **4** and **5** by an oxidative ring closure. The structure of the compounds was established by IR, UV, MS, and NMR (^{1}H, ^{13}C, COSY, HSQC, and HMBC) techniques and for compounds **4**, **6**, and **11** the structure was confirmed by X-ray crystallographic analysis. The effect of the 11 xanthones on the in vitro growth of four human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (central nervous system cancer), and UACC-62 (melanoma) is also described.

1. Introduction

Xanthonic compounds show interesting biological activities associated with their tricyclic scaffold depending on the nature and/or position of the different substituents.[1]

A relationship between activity and the presence of prenyl groups in key-positions on the xanthone nucleus was associated with some biological activities, such as inhibition of human lymphocyte proliferation,[2] PKC modulation,[3] antitumor,[4] and anti-inflammatory.[5]

The influence of these groups is dual, considering the influence on physicochemical properties, namely lipophilicity, and on three-dimensional properties affecting steric effects on interaction with the biological targets.

Recently, we have investigated the effect of several hydroxy and methoxyxanthones on the in vitro growth of human tumor cell lines.[6] Among 27 xanthones tested 1,3-dihydroxy-2-methylxanthone (**1**) was found to have the best growth inhibitory activity against the three cell lines tested, namely MCF-7 (breast cancer), TK-10 (renal cancer), and UACC-62 (melanoma). Simple dihydroxyxanthone derivatives also showed some interesting tumor growth inhibitory activity.[6]

In order to modulate and improve the antitumor activity of these compounds, we have synthesized new prenylated derivatives, using as building blocks 1,3-dihydroxy-2-methylxanthone (**1**) and the *nor*-derivative 1,3-dihydroxyxanthone (**2**) (Fig. 1) and their activity was evaluated (Table 1).

Keywords: Xanthones; Prenylation; Antitumor activity; X-ray crystallography; NMR spectroscopy.
* Corresponding author. Tel.: +351 222078916; fax: +351 222003977; e-mail: madalena@ff.up.pt

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.06.037

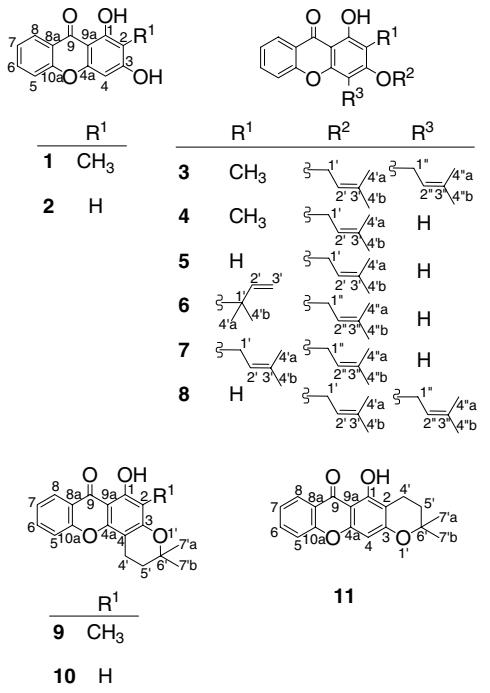


Figure 1. Structures of building blocks **1** and **2** and prenylated derivatives **3**–**11** (the numbering used concerns the NMR discussion).

Table 1. Effects of xanthones **1** and **2** and xanthone derivatives **3**–**11** on the growth of human tumor cell lines

Compound	GI_{50} (μM)			
	MCF-7	NCI-H460	SF-268	UACC-62
1	21.9 ± 0.4	20.6 ± 0.9	33.4 ± 0.2	20.0 ± 0.5
2	50.8 ± 2.2	37.9 ± 2.9	61.4 ± 5.2	38.0 ± 1.6
3	>130	>130	>130	>130
4	>160	>160	>160	>160
5	>160	>160	>160	>160
6	6.0 ± 0.7	>130	>130	>130
7	9.1 ± 1.5	>130	>130	>130
8	112.5 ± 10.1	>130	>130	>130
9	18.4 ± 1.9	>160	>160	ND
10	>160	>160	>160	ND
11	88.6 ± 12.9	>160	>160	ND

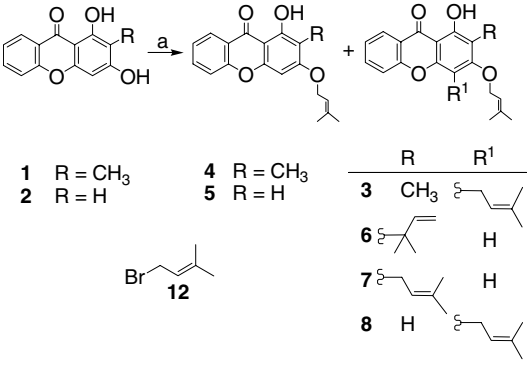
Results expressed as GI_{50}, concentrations of compound that cause 50% inhibition of tumor cell lines growth, are means ± SEM of 3–8 independent experiments performed in duplicate. Doxorubicin was used as positive control, GI_{50}: MCF-7 = 42.8 ± 8.2 nM; NCI-H460 = 94.0 ± 8.7 nM; SF-268 = 93.0 ± 7.0 nM; UACC-62 = 94.0 ± 9.4 nM. ND, not done.

We report the synthesis of the xanthonic building blocks **1** and **2** (Scheme 1) as well as of nine prenylated derivatives **3**–**11** (Fig. 1). The synthetic approach used to synthesize the prenylated xanthones **3**–**8** was by nucleophilic substitution on the xanthonic building blocks **1** and **2**, with prenyl bromide (**12**) in alkaline medium[7] (Scheme 2). The cyclic derivatives **9** and **10**–**11** were obtained from the prenylated xanthones **4** and **5**, respectively, by refluxing with a catalytic amount of zinc chloride in dry xylene[7] (Scheme 3).

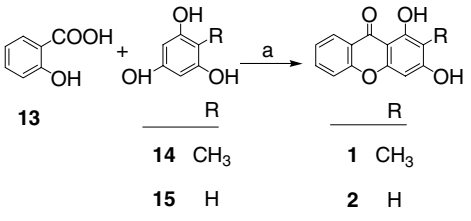
The xanthonic building blocks **1** and **2** were evaluated together with the prenylated derivatives **3**–**11** (Table 1), for their capacity to inhibit the in vitro growth of four human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (central nervous system cancer), and UACC-62 (melanoma).

Considering the *O*-prenylderivatives **3** and **4** from the xanthone **1** (Fig. 1) the antitumor activity for cellular lines tested was not improved (Table 1). However the corresponding cyclic derivative **9**, showed specificity for MCF-7 cell line.

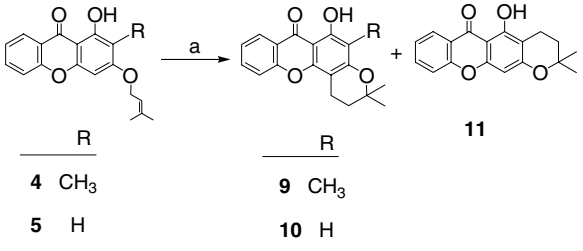
Starting with xanthone **2** the compounds **5**–**8** were obtained and it could be found that compounds **6** and **7** showed a selective and highly potent growth inhibitory activity against the MCF-7 cell line. Considering the cyclic derivatives **10** and **11**, an enhancement on compound **10** activity was not observed, when compared to their precursor **5** (Fig. 1 and Table 1).



Scheme 2. Reagents and condition: (a) Prenyl bromide (**12**), K_2CO_3, Acetone, Reflux, 8 h.



Scheme 1. Reagents and condition: (a) $ZnCl_2$, $POCl_3$, 70 °C, 3 h.



Scheme 3. Reagents and condition: (a) $ZnCl_2$, *o*-xylene, 200 °C, 21 h.

2. Results and discussion

2.1. Synthesis of xanthone prenylated derivatives

Xanthones **1** and **2** were obtained by the Grover, Shah, and Shah method.[7–9] 1,3-dihydroxyxanthones (**1** and **2**) have been obtained by condensation of salicylic acid (**13**) and phloroglucinol derivatives (**14** and **15**)[9] (Scheme 1).

The prenylated derivatives **3**–**8** were obtained by the reaction of xanthones **1** or **2** and prenyl bromide in alkaline medium.[7] Xanthone **1** gave two prenylated derivatives **3** and **4**, and xanthone **2** afforded the prenylated derivatives **5**–**8**, being the compounds **3**, **4**, and **6**–**8** described for the first time (Scheme 2). The cyclic derivatives **9**–**11** were obtained by refluxing the prenylated xanthones **4** or **5** with a catalytic amount of zinc chloride, in dry xylene.[7] The prenylated xanthone **4** gave the novel cyclic derivative **9** (described for the first time) while the prenylated xanthone **5** afforded the derivatives **10** and **11** (Scheme 3).

2.2. Structural elucidation of prenylated xanthones

For compounds **1** and **2** all the data are according to the literature.[7,8,10] The structure elucidation of compounds **3**–**11** was established on the basis of UV, IR, MS, and NMR techniques. The ^{1}H NMR and ^{13}C NMR data of prenylated xanthones are reported in Tables 2 and 3, respectively.

The ^{1}H NMR spectra of all prenylated xanthones had in common the signals of hydrogen-bonded hydroxyl group δ_H 12.64–13.67 ppm (OH-1) and four aromatic protons corresponding to the phenyl ring. The ^{1}H NMR spectra of prenylated xanthones **3**, **4**, and **9** contained a signal due to a methyl group at δ_H 2.09–2.24 ppm. The ^{1}H NMR spectra of xanthones **4** and **5** showed signals that evidence one 3,3-dimethylallyl (prenyl) group, while the spectra of xanthones **3**, **6**–**8** showed signals concerning the presence of two prenyl groups. The spectra of xanthones **9**, **10**, and **11** showed the presence of a fused dihydropyran ring considering two triplets due to the two methylene protons and the signal for the protons of two methyl groups appearing as a singlet. The ^{13}C NMR spectra revealed signals for one carbonyl group (compounds **3**–**11**), two aromatic rings (**3**–**11**), one of them containing a methyl carbon in the case of compounds **3**, **4**, and **9**, two oxygenated carbons (**3**–**11**), one (**4**, **5**) or two (**3**, **6**–**8**) prenyl groups, and a 2,3-dihydropyran ring (**9**, **10**) or a 3,4-dihydropyran ring (**11**). The position of the substituents on the xanthone skeleton was determined on the basis of HSQC and HMBC spectral analysis (Fig. 2).

In HMBC spectra of all prenylated xanthones, the hydrogen-bonded hydroxyl group (OH-1) correlated with C-1 and C-9a, allowing the assignment of these two carbon resonances. In the same way H-1′ and H-1″ of prenyl group, in compounds **3**–**5**, **8**, and **6**, **7**, respectively, correlated with C-3 of xanthone ring indicating that all the prenylated xanthones had a 3,3-dimethylallyloxy group at C-3.

For diprenylated xanthones, it was observed that the H-1″ of the other prenyl group correlated with C-4 and C-4a in compounds **3** and **8**, and the H-1′ correlated with C-2 in compound **7**. The presence of the 1,1-dimethylallyl group in xanthone **6** was assigned by their C-1′ quaternary carbon and by the two double doublets corresponding to 2× H-3′ (J = 17.5, 10.6 and 17.5, 1.3 Hz).

In the case of dihydropyranoxanthones, it was observed that H-4′ of the pyran ring of xanthones **9** and **10** correlated with C-3, C-4, C-4a, C-5′, and C-6′ indicating the presence of a 3,4-dihydropyran ring, on the other hand the H-4′ of the pyran ring of xanthone **11** correlated with C-1, C-2, C-3, C-5′, and C-6′ indicating the presence of a 2,3-dihydropyran ring.

The structure of compounds **4**, **6**, and **11** was also determined by X-ray crystallography. A perspective view of the crystal structures, obtained using ORTEP[11] and showing the atomic numbering, is presented in Figure 3.

Like in most of the crystal structures reported until now,[12] the xanthone basic skeletons of **4**, **6**, and **11** are essentially planar and the central pyranoid rings of the three compounds have a partial aromatic character. The C4a–O10–C10a angles are 119.43(11)° (**4**), 119.12(18)° (**6**), and 119.64(13)° (**11**); the C4a–O10 bond lengths are 1.3630(16) Å (**4**), 1.366(3) Å (**6**), and 1.375(2) Å (**11**), and the C10a–O10 bond lengths are 1.3612(16) Å (**4**), 1.363(3) Å (**6**) and 1.373(2) Å (**11**), which are values slightly lower than those observed for diaryl ethers C_{ar}–O–C_{ar}: 1.384(14) Å,[13] suggesting that the p_z electrons of O-10, C-4a, and C-10a are used in conjugation in the central ring.

It was already reported that in hydroxylated or methoxylated xanthones a coplanar conformation with respect to the xanthone skeleton is usually adopted.[14,15] Moreover, when the –OH group is bound to C-1 or C-8, as it happens in **4**, **6**, and **11**, a hydrogen bond to O-11 is always established.[12]

The bond lengths and angles of the prenyl group of **4** and **6** are comparable with those found in the crystal structure of *Emericellin*.[16] Compound **11** comprises four six-membered rings with a dihydropyran ring linearly fused to the xanthone skeleton. Three crystal structures of xanthones with a similar four-ring system arrangement were already elucidated: 1-hydroxy-6,7-dimethoxy-8-(3-methylbut-2-enyl)-6′,6′-dimethylpyrano(2′,3′:3,2)xanthone,[17] *Dulxanthone E*,[18] and *6-Deoxyjacareubin*.[19] In all three crystal structures now available, the xanthone skeleton is almost flat and the dihydropyran ring assumes a half-chair conformation.

2.3. Biological studies

The effects of the prenylated xanthones **3**–**11** and of the two xanthonic building blocks, **1** and **2** on the growth of

Table 2. ^{1}H NMR chemical shifts of prenylated xanthones **3**–**11**[a]

	3	**4**	**5**	**6**	**7**	**8**
H-1	12.98 [OH, s]	12.91 [OH, s]	12.85 [OH, s]	13.67 [OH, s]	12.92 [OH, s]	12.97 [OH, s]
H-2	—	—	6.34 (d, *J* = 2.2)	—	—	6.39 (s)
H-4	—	6.39 (s)	6.42 (d, *J* = 2.2)	6.42 (s)	6.44 (s)	—
H-5	7.47 (d, *J* = 8.4)	7.38 (d, *J* = 8.4)	7.41 (d, *J* = 8.3)	7.40 (d, *J* = 8.6)	7.42 (d, *J* = 8.4)	7.46 (d, *J* = 8.2)
H-6	7.72 (ddd, *J* = 8.4, 7.4, 1.6)	7.67 (ddd, *J* = 8.4, 7.2, 1.6)	7.69 (ddd, *J* = 8.3, 7.2, 1.6)	7.69 (ddd, *J* = 8.6, 7.0, 1.6)	*7.69* (ddd, *J* = 8.4, 7.0, 1.6)	7.71 (ddd, *J* = 8.2, 7.1, 1.6)
H-7	7.37 (t, *J* = 7.8, 7.4)	7.32 (d, *J* = 7.2)	7.36 (t, *J* = 7.9, 7.2)	7.36 (t, *J* = 8.1, 7.0)	7.36 (t, *J* = 8.0, 7.0)	7.36 (t, *J* = 8.0, 7.1)
H-8	8.26 (dd, *J* = 7.8, 1.6)	8.22 (dd, *J* = 7.9, 1.6)	8.23 (dd, *J* = 7.9, 1.6)	8.26 (dd, *J* = 8.1, 1.6)	8.26 (dd, *J* = 8.0, 1.6)	8.24 (dd, *J* = 8.0, 1.6)
H-10	2.24 (s)	2.09 (s)	—	—	—	—
H-1′	4.41 (d, *J* = 7.1)	4.61 (d, *J* = 6.5)	4.59 (d, *J* = 6.7)	—	3.38 (d, *J* = 7.2)	4.63 (d, *J* = 6.6)
H-2′	5.61 (t, *J* = 7.1)	5.51 (t, *J* = 6.5)	5.50 (t, *J* = 6.7)	6.32 (dd, *J* = 17.4, 10.6)	5.25 (t, *J* = 7.2)	5.49 (t, *J* = 6.6)
H-3′	—	—	—	4.88 (dd, *J* = 17.4, 1.2) 4.81 (dd, *J* = 10.6, 1.2)	—	—
H-4′	1.82, 1.72 (2s)	1.82, 1.78 (2s)	1.82, 1.77 (2s)	1.61, 1.59 (2s)	1.79, 1.68 (2s)	1.81, 1.76 (2s)
H-1″	3.57 (d, *J* = 6.9)	—	—	4.58 (d, *J* = 6.5)	4.63 (d, *J* = 6.6)	3.50 (d, *J* = 7.0)
H-2″	5.24 (t, *J* = 6.9)	—	—	5.49 (t, *J* = 6.5)	5.51 (t, *J* = 6.6)	5.23 (t, *J* = 7.0)
H-4″	1.89, 1.69 (2s)	—	—	1.81, 1.76 (2s)	1.82, 1.78 (2s)	1.87, 1.68 (2s)

	9	**10**	**11**
H-1	12.88 [OH, s]	12.64 [OH, s]	13.22 [OH, s]
H-2	—	6.26 (s)	—
H-4	—	—	6.35 (s)
H-5	7.44 (dd, *J* = 8.4, 0.9)	7.47 (d, *J* = 8.4)	7.42 (d, *J* = 8.4)
H-6	7.68 (ddd, *J* = 8.4, 7.1, 1.6)	7.71 (ddd, *J* = 8.4, 7.2, 1.6)	7.69 (ddd, *J* = 8.4, 7.1, 1.6)
H-7	7.35 (t, *J* = 7.9, 7.1, 0.9)	7.38 (t, *J* = 8.0, 7.2)	7.35 (t, *J* = 8.0, 7.1)
H-8	8.25 (dd, *J* = 7.9, 1.6)	8.26 (dd, *J* = 8.0, 1.6)	8.24 (dd, *J* = 8.0, 1.6)
H-10	2.09 (s)	—	
H-4′	2.89 (t, *J* = 6.8)	2.88 (t, *J* = 6.8)	2.74 (t, *J* = 6.8)
H-5′	1.88 (t, *J* = 6.8)	1.89 (t, *J* = 6.8)	1.86 (t, *J* = 6.8)
H-7′	1.40 (br s)	1.40 (br s)	1.39 (br s)

[a] Values in ppm (δ_H). Measured in $CDCl_3$, at 300.13 MHz. *J* values (Hz) are shown in parentheses.

Table 3. ^{13}C NMR chemical shifts of prenylated xanthones **3**–**11**[a]

	3	4	5	6	7	8	9	10	11
C-1	158.9	159.6	163.4	161.9	159.6	161.8	157.9	160.9	160.6
C-2	113.4	108.1	97.6	117.0	112.1	95.1	107.5	99.4	104.1
C-3	162.9	164.0	166.0	165.3	163.8	163.6	159.7	161.7	161.8
C-4	113.2	90.3	93.4	91.3	90.7	108.1	99.0	99.8	95.0
C-5	117.7	117.4	117.5	118.8	117.4	117.6	117.4	117.5	117.6
C-6	135.0	134.6	134.9	134.6	134.6	134.9	134.4	134.7	134.8
C-7	123.7	123.7	123.9	123.8	123.8	123.7	123.7	124.0	123.6
C-8	125.9	125.8	125.8	126.0	125.9	125.8	125.8	125.9	125.8
C-9	181.8	180.6	180.7	181.0	180.7	181.2	180.7	180.8	180.8
C-10	8.8	7.3	—	—	—	—	7.2	—	—
C-4a	152.8	155.8	157.6	156.1	156.1	154.1	152.5	154.7	155.6
C-10a	156.1	155.8	156.0	155.6	155.9	156.2	155.8	155.9	156.1
C-8a	120.4	120.7	120.6	120.7	120.8	120.4	120.7	120.7	120.6
C-9a	105.8	103.4	103.8	103.9	103.8	103.4	103.0	103.7	[b]
C-1′	70.7	65.6	65.4	41.1	21.4	65.7	—	—	—
C-2′	119.8	118.9	118.5	150.6	122.1	119.0	—	—	—
C-3′	138.5	138.4	139.4	106.8	131.7	138.6	—	—	—
C-4′	25.9; 18.1	25.8; 18.3	25.8; 18.3	29.7; 29.0	25.8; 17.8	25.8; 18.3	16.4	16.2	16.0
C-5′	—	—	—	—	—	—	31.7	31.8	31.8
C-6′	—	—	—	—	—	—	76.0	76.2	76.3
C-7′	—	—	—	—	—	—	26.8	26.7	26.8
C-1″	22.8	—	—	65.7	65.6	21.7	—	—	—
C-2″	122.8	—	—	118.8	119.0	122.2	—	—	—
C-3″	131.7	—	—	138.1	138.5	131.5	—	—	—
C-4″	25.7; 18.0	—	—	25.7; 18.3	25.8; 18.3	25.8; 17.9	—	—	—

[a] Values in ppm (δ_C). Measured in $CDCl_3$, at 75.47 MHz.
[b] Not observed.

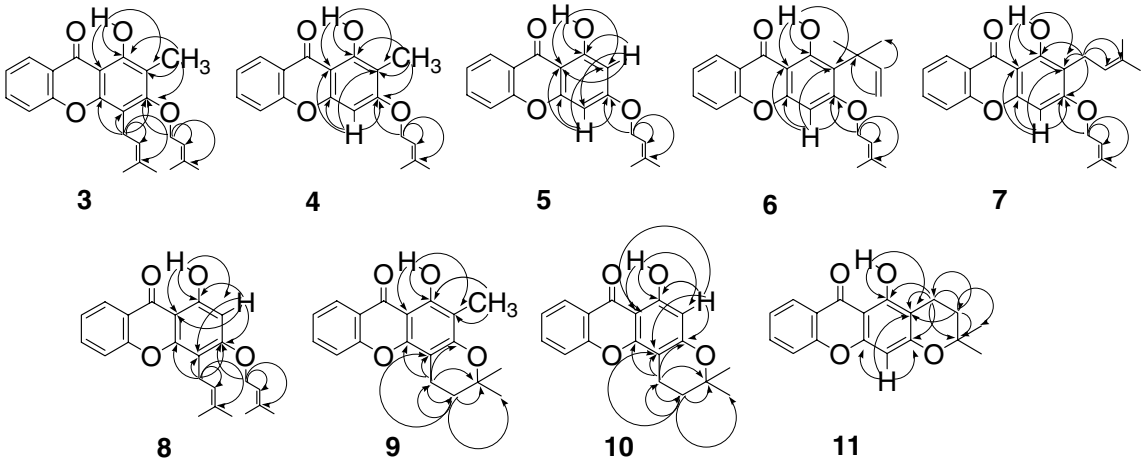


Figure 2. Main connectivities found in the HMBC of prenylated xanthones **3**–**11**.

four human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (central nervous system cancer), and UACC-62 (melanoma), given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}), are summarized in Table 1.

Though the number of compounds is small, some trends in structure-activity relationship are apparent. Considering compounds **1**, **6**, and **7** it seems to be important the existence of an alkyl group at C-2 for the antitumor activity. The size of this alkyl group is also important, since the prenyl groups are associated with more selective compounds. This kind of selectivity for MCF-7 cell line was not previously described for prenylated xanthones and probably can be related with a molecular mechanism concerning interaction with estrogen receptors present in this estrogen receptor positive (ER+) breast cell line.

Comparing the growth inhibitory effects on MCF-7 cell line of compounds **9**–**11**, and their precursors **4** and **5**, it can be concluded that the extra pyran ring led to the appearance of an effect in compounds **9** and **11**, but not in compound **10**.

It is also interesting to point out that the higher activity of compound **9** for MCF-7 corresponds to

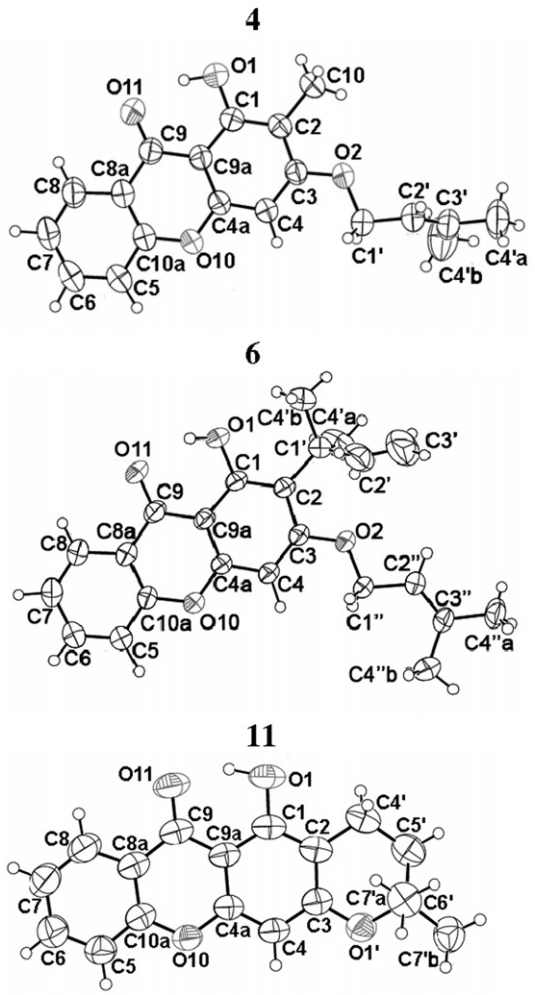


Figure 3. View of the molecular structure of compounds **4**, **6**, and **11** showing the atom-labeling scheme. Displacement ellipsoids are drawn at the 50% probability level. Hydrogen atoms are represented by circles of arbitrary radii.

a global angular structure with a methyl group at C-2, while the compound **10**, although with an angular structure but without substituent at C-2, shows no activity.

3. Conclusions

By a classic method of prenylation six new prenylated xanthones were obtained, **3**–**4** and **6**–**9**. Long-range C, H correlations led to an unambiguous establishment of the structures of different compounds and a detailed structural analysis for three of them (**4**, **6**, and **11**) was obtained by X-ray crystallography.

With the molecular modification concerning prenylation of xanthones the improvement of bioactivity was achieved leading to compounds **6**, **7**, and **9** with a selective and potent growth inhibitory activity against the breast cancer MCF-7 cell line, if compared with their parent compounds **1** and **2**, while the growth inhibitory activity against the other cell lines was lost.

4. Experimental

4.1. General methods

Purifications of compounds were performed by flash chromatography using Merck silica gel 60 (0.040–0.063 mm) and preparative thin layer chromatography (TLC) using Merck silica gel 60 (GF_{254}) plates. Melting points were obtained in a Köfler microscope and are uncorrected. IR spectra were measured on an ATI Mattson Genesis series FTIR (software: WinFirst v.2.10) spectrophotometer in KBr microplates (cm^{-1}). UV spectra were taken in ethanol[20] and were recorded on a Varian CARY 100 spectrophotometer: λ_{max} in nm (software: Cary Win UV, v. 3.0). ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ or DMSO-d_6 at room temperature, on Bruker Avance 300 instrument (300.13 MHz for ^1H and 75.47 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as an internal reference; ^{13}C NMR assignments were made by 2D HSQC and HMBC experiments (long-range C, H coupling constants were optimized to 7 and 1 Hz). EIMS spectra were recorded as EI (electronic impact) mode on a VG Autospec Q spectrometer (*m*/*z*) and HRMS mass spectra were measured on a Kratos Concept III 2 Sector Mass Spectrometer, recorded as FAB (Fast Atom Bombardment) or EI (electronic impact) mode. Prenyl bromide was purchased from Sigma Aldrich. The following materials were synthesized and purified by the described procedures.

4.2. Synthesis of the building blocks 1,3-dihydroxy-2-methylxanthone (1) and 1,3-dihydroxyxanthone (2)

The compounds were obtained, 32% and 53% respectively, and characterized according to the described procedures.[7–10]

4.3. Prenylation of 1,3-dihydroxy-2-methylxanthone (1)

A mixture of 1,3-dihydroxy-2-methylxanthone (**1**) (0.50 g; 2.06 mmol), prenyl bromide (0.66 g; 4.43 mmol), and anhydrous K_2CO_3 (0.69 g; 4.99 mmol) in anhydrous acetone (90 mL) was refluxed at 65 °C for 8 h. After cooling, the solid was filtered and the solvent removed under reduced pressure and afforded the crude product. This crude product was purified by flash chromatography (SiO_2; hexane/AcOEt, 99:1) yielding successively **3**, a mixture of **3** + **4**, and **4**. The isolation of the components of the mixture was then carried out by preparative TLC (SiO_2; hexane/AcOEt, 95:5). Prenylated xanthones **3** and **4** were crystallized from EtOH.

4.3.1. 1-Hydroxy-2-methyl-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3). Three percent as yellow crystals, mp 173–175 °C (EtOH); UV (EtOH) λ_{max} (ε): 374, 304, 259, 234, 214 (14,735; 46,212; 78,674; 84,659; 82,462); (EtOH + NaOH): 417, 308, 274, 233 (18,939; 50,947; 57,045; 88,295); (EtOH + $AlCl_3$): 368, 306, 261, 234, 215 (16,894; 47,841; 72,727; 87,614; 84,053); (EtOH + $AlCl_3$ + HCl): 368, 309, 263, 234, 213 (11,553; 39,583; 55,303; 75,038; 75,152); (EtOH + NaOAc): unchanged; IR (KBr) ν_{max}: 3446,

3230, 2962, 2926, 2856, 1645, 1610, 1570, 1522, 1473, 1433, 1132, 1101, 816, 758; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 378(49, $M^{+\cdot}$), 310(100), 295(85), 267(34), 255(63), 242(63), 225(15), 121(15), 77(13), 69(72); Anal. Calcd for $C_{24}H_{27}O_4$:379.4776; found: 379.1909 or FABHRMS: 379.1909 ([M+H]$^{+\cdot}$, $C_{24}H_{27}O_4^{+\cdot}$; calcd 379.4776).

4.3.2. 1-Hydroxy-2-methyl-3-(3-methylbut-2-enyloxy)xanthone (4). Forty-eight percent as yellow solid, mp 140–142 °C (EtOH); UV (EtOH) λ_{max} (ε): 310, 239, 217 (40,590; 63,323; 62,640); (EtOH + NaOH): 395, 301, 277, 213 (6615; 22,081; 23,882; 103,385); (EtOH + $AlCl_3$): 334, 263, 238, 222 (56,056; 48,106; 62,733; 71,149); (EtOH + $AlCl_3$ + HCl): 329, 262, 237, 221 (50,559; 37,826; 55,373; 67,547); (EtOH + NaOAc): unchanged; IR (KBr) ν_{max}: 3086, 3043, 2960, 2927, 2856, 1645, 1608, 1570, 1514, 1313, 1290, 1228, 1205, 1132, 1099, 818, 775, 754; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 310(30, $M^{+\cdot}$), 242(100), 213(17), 69(30); Anal. Calcd for $C_{19}H_{19}O_4$: 311.3586; found: 311.1284 or FABHRMS: 311.1284 ([M+H]$^{+\cdot}$, $C_{19}H_{19}O_4^{+\cdot}$; calcd 311.3586).

4.4. Prenylation of 1,3-dihydroxyxanthone (2)

A mixture of 1,3-dihydroxyxanthone (**2**) (0.50 g; 2.19 mmol), prenyl bromide (0.67 g; 4.50 mmol), and anhydrous K_2CO_3 (0.60 g; 4.34 mmol) in acetone (90 mL) was refluxed at 65 °C for 8 h. After cooling, the solid was filtered and the solvent removed under reduced pressure affording the crude product. This crude product was purified by flash chromatography (SiO_2; CH_2Cl_2/petroleum ether/Et_2O, 5:90:5) yielding successively **6**, a mixture of **6** + **7**, **7**, a mixture of **8** + **5**, and **5**. The isolation of the components of the mixture **6** + **7** was carried out by preparative TLC (SiO_2; CH_2Cl_2/petroleum ether/Et_2O, 5:90:5) and for mixture **8** + **5** by preparative TLC (SiO_2; hexane/AcOEt, 8:2). Prenylated xanthones **5**–**8** were crystallized from CH_2Cl_2/petroleum ether (60–80).

4.4.1. 1-Hydroxy-3-(3-methylbut-2-enyloxy)xanthone (5). Twenty-five percent as yellow thick needles, mp 137–139 °C (CH_2Cl_2/petroleum ether (60–80)); UV (EtOH) λ_{max} (ε): 347, 305, 254, 236 (12,374; 41,128; 58,932; 66,736); (EtOH + NaOH): 385, 308, 294, 269, 221 (18,487; 31,662; 33,086; 53,175; 95,727); (EtOH + $AlCl_3$): 376, 328, 266, 224 (13,946; 53,116; 60,623; 57,507); (EtOH + $AlCl_3$ + HCl): 383, 323, 264, 224 (11,484; 42,374; 49,763; 47,893); (EtOH + NaOAc): unchanged; IR (KBr) ν_{max}: 3427, 2964, 2924, 2856, 1656, 1606, 1566, 1462, 1429, 1296, 1213, 1155, 1072, 820, 787, 750; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 296(42, $M^{+\cdot}$), 228(100), 199(16), 69(38); Anal. Calcd for $C_{18}H_{17}O_4$: 297.3316; found: 297.1127 or FABHRMS: 297.1127 ([M+H]$^{+\cdot}$, $C_{18}H_{17}O_4^{+\cdot}$; calcd 297.3316).

4.4.2. 1-Hydroxy-3-(3-methylbut-2-enyloxy)-2-(1,1-dimethylprop-2-enyl)xanthone (6). Five percent as yellow crystals, mp 102–104 °C (CH_2Cl_2/petroleum ether (60–80)); UV (EtOH) λ_{max} (ε): 312, 240, 219 (32,299; 55,036; 56,022); (EtOH + NaOH): 408, 303, 283, 218 (7372; 32,774; 29,380; 87,628); (EtOH + $AlCl_3$): 318, 240, 223, 204 (37,737; 65,000; 62,555; 48,759); (EtOH + $AlCl_3$ + HCl): 323, 239, 222, 203 (32,701; 52,956; 52,226; 48,723); (EtOH + NaOAc): unchanged; IR (KBr) ν_{max}: 3433, 2958, 2922, 2850, 1633, 1595, 1558, 1450, 1282, 1144, 1084, 847; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 364(60, $M^{+\cdot}$), 295(80), 281(100), 267(41), 253(52), 241(56), 121(16), 69(52); Anal. Calcd for $C_{23}H_{25}O_4$: 365.4506; found: 365.1753 or FABHRMS: 365.1753 ([M+H]$^{+\cdot}$, $C_{23}H_{25}O_4^{+\cdot}$; calcd 365.4506).

4.4.3. 1-Hydroxy-2-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (7). Two percent as yellow solid, mp 112–114 °C (CH_2Cl_2/petroleum ether (60–80)); UV (EtOH) λ_{max} (ε): 309, 241, 219 (60,584; 91,350; 86,168); (EtOH + NaOH): 400, 300, 279, 226 (16,752; 57,810; 62,153; 90,474); (EtOH + $AlCl_3$): 333, 263, 239, 223 (67,628; 73,686; 83,869; 89,234); (EtOH + $AlCl_3$ + HCl): 328, 263, 238, 222 (59,708; 59,526; 72,591; 85,255); (EtOH + NaOAc): unchanged; IR (KBr) ν_{max}: 3435, 2964, 2922, 2858, 1643, 1608, 1558, 1464, 1306, 1217, 1120, 1082, 1030, 955; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 364(50, $M^{+\cdot}$), 309(19), 295(73), 281(36), 253(65), 241(100), 228(15), 69(33); Anal. Calcd for $C_{23}H_{25}O_4$: 365.4506; found: 365.1754 or FABHRMS: 365.1754 ([M+H]$^{+\cdot}$, $C_{23}H_{25}O_4^{+\cdot}$; calcd 365.4506).

4.4.4. 1-Hydroxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (8). Three percent as yellow thin needles, mp 132–134 °C (CH_2Cl_2/petroleum ether (60–80)); UV (EtOH) λ_{max} (ε): 364, 310, 260, 235 (7336; 22,956; 47,080; 47,628); (EtOH + NaOH): 398, 294, 272, 220 (12,664; 27,956; 37,847; 98,358); (EtOH + $AlCl_3$): 364, 326, 273, 233 (13,577; 35,109; 45,949; 51,971); (EtOH + $AlCl_3$ + HCl): 368, 325, 272, 234 (9964; 27,263; 36,971; 41,058); (EtOH + NaOAc): unchanged; IR (KBr) ν_{max}: 3469, 2956, 2922, 2854, 1655, 1604, 1570, 1468, 1423, 1369, 1292, 1232, 1080, 810, 783, 756; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 364(43, $M^{+\cdot}$), 296(65), 281(100), 253(30), 241(47), 228(52), 121(12), 77(11), 69(53); Anal. Calcd for $C_{23}H_{25}O_4$: 365.4506; found: 365.1753 or FABHRMS: 365.1753 ([M+H]$^{+\cdot}$, $C_{23}H_{25}O_4^{+\cdot}$; calcd 365.4506).

4.5. Synthesis of dihydropyranoxanthone 9 from 4

To a solution of the xanthone (**4**) (0.10 g; 0.32 mmol) in dry *o*-xylene (1 mL), anhydrous $ZnCl_2$ (3.00 mg; 0.02 mmol) was added and heated at 200 °C for 21 h. The reaction mixture was cooled and purified by flash chromatography (SiO_2; hexane/AcOEt, 99.5:0.5) and by preparative TLC (SiO_2; hexane/AcOEt, 8:2). Prenylated xanthone **9** was crystallized from EtOH.

4.5.1. 1-Hydroxy-2,6′,6′-trimethyl-4′,5′-dihydropyrano(2′,3′:3,4)xanthone (9). Twenty-two percent as yellow solid, mp 188–190 °C (EtOH); UV (EtOH) λ_{max} (ε): 316, 257, 239, 216 (36,366; 43,385; 64,503; 57,205); (EtOH + NaOH): 405, 301, 282, 220 (9348; 39,876;

37,174; 105,590); (EtOH + $AlCl_3$): 338, 265, 239, 222 (41,584; 43,106; 59,348; 75,280); (EtOH + $AlCl_3$ + HCl): 423, 333, 278, 263, 239, 221 (6553; 41,677; 37,981; 35,342; 53,230; 71,863); (EtOH + NaOAc): unchanged; IR (KBr) ν_{max}: 2970, 2925, 2854, 1653, 1612, 1570, 1475, 1437, 1332, 1267, 1153, 1107, 812, 787, 754; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 310(89, $M^{+\cdot}$), 295(29), 255(100), 242(18), 225(26), 197(16), 121(9), 77(7); Anal. Calcd for $C_{19}H_{18}O_4$: 310.3506; found: 310.1205 or EIHRMS: 310.1205 ($M^{+\cdot}$, $C_{19}H_{18}O_4^{+\cdot}$; calcd 310.3506).

4.6. Synthesis of dihydropyranoxanthones 10 and 11 from 5

To a solution of the xanthone (**5**) (0.09 g; 0.30 mmol) in dry *o*-xylene (1 mL), anhydrous $ZnCl_2$ (0.003 g; 0.02 mmol) was added and heated at 200 °C for 20 h 30 min. The reaction mixture was cooled and purified by a mini-column chromatography (SiO_2; hexane, AcOEt, and Me_2CO) and by preparative TLC (SiO_2; hexane/AcOEt, 95:5). Prenylated xanthones **10** and **11** were crystallized from CH_2Cl_2/petroleum ether (60–80).

4.6.1. 1-Hydroxy-6′,6′-dimethyl-4′,5′-dihydropyrano(2′,3′:3,4)xanthone (10). Four percent as yellow solid, mp 187–190 °C (CH_2Cl_2/petroleum ether (60–80)); UV (EtOH) λ_{max} (ε): 310, 259, 238, 212 (12,018; 18,249; 23,798; 20,059); (EtOH + NaOH): 390, 274, 225 (6558; 22,967; 69,525); (EtOH + $AlCl_3$): 363, 324, 263, 240, 204 (17,122; 24,718; 27,122; 40,593; 28,991); (EtOH + $AlCl_3$ + HCl): 363, 322, 239, 203 (13,264; 20,653; 33,591; 30,504); (EtOH + NaOAc): unchanged; IR (KBr) ν_{max}: 3427, 2968, 2924, 2854, 1662, 1606, 1571, 1471, 1429, 1329, 1290, 1232, 1157, 1116, 1078, 817, 752; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 296(68, $M^{+\cdot}$), 281(31), 241(100), 228(18), 212(20), 149(17), 83(16), 71(21), 57(35); Anal. Calcd for $C_{18}H_{16}O_4$: 296.3236; found: 296.1049 or EIHRMS: 296.1049 ($M^{+\cdot}$, $C_{18}H_{16}O_4^{+\cdot}$; calcd 296.3236).

4.6.2. 1-Hydroxy-6′,6′-dimethyl-4′,5′-dihydropyrano(2′,3′:3,2)xanthone (11). Three percent as yellow solid, mp 147–148 °C (CH_2Cl_2/petroleum ether (60–80)); UV (EtOH) λ_{max} (ε): 313, 256, 237, 218 (24,985; 35,104; 46,706; 37,834); (EtOH + NaOH): 396, 274, 223 (8487; 29,110; 69,050); (EtOH + $AlCl_3$): 329, 263, 239, 222, 205 (36,202; 43,531; 54,421; 49,199; 37,448); (EtOH + $AlCl_3$ + HCl): 325, 262, 239, 222, 204 (29,347; 34,065; 43,591; 38,843; 36,469); (EtOH + NaO Ac): unchanged; IR (KBr) ν_{max}: 3471, 2972, 2924, 2856, 1647, 1606, 1570, 1450, 1300, 1263, 1219, 1134, 1080, 823, 754; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; EIMS: 296(67, $M^{+\cdot}$), 281(20), 253(32), 241(100), 228(11), 212(14), 149(16), 121(13), 71(20), 57(33); Anal. Calcd for $C_{18}H_{16}O_4$: 296.3236; found: 296.1048 or EIHRMS: 296.1048 ($M^{+\cdot}$, $C_{18}H_{16}O_4^{+\cdot}$; calcd 296.3236).

4.7. X-ray crystallography

Suitable crystals of **4**, **6**, and **11** were obtained by slow evaporation of solutions of the compounds in ethanol. The crystals were mounted on glass fibers and diffraction data were collected at 293 K with a Stoe IPDS plate equipped with Mo-Kα radiation (λ = 0.71073 Å). The structures of **4**, **6**, and **11** were solved by direct methods using SHELXS[21] and refined using SHELXL[22] program. All non-H-atoms were refined anisotropically. The H-4′a and H-4′b of **4**, the H-3′, H-4″a, H-4′a, and H-4′b of **6**, and all hydrogen atoms of **11** were positioned with idealized geometry and their coordinates were only altered in accordance with the refinement of their parent C or O atoms. The rest of the hydrogen atoms of **4** and **6** were refined freely with isotropic displacement parameters.

CCDC-649346 (**4**), -649347 (**6**), and -649348 (**11**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

4.8. Tumor cell growth assay

Stock solutions of compounds were prepared in DMSO (Sigma Chemical Co.) and stored at −20 °C. The frozen samples were freshly diluted with culture medium just prior to the assays. Final concentrations of DMSO (⩽25%) did not interfere with the biological activity tested.

The effects of compounds on the growth of the human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the in vitro anticancer drug discovery screen which uses the protein-binding dye sulforhodamine B (Sigma Chemical Co.) to assess cell growth.[23,24] Four human tumor cell lines were used, namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (CNS cancer), and UACC-62 (melanoma). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium (Gibco-BRL) supplemented with 5% heat-inactivated fetal bovine serum (Gibco-BRL), 2 mM glutamine (Sigma Chemical Co.), and 50 μg/mL of gentamicin (Sigma Chemical Co.) at 37 °C in an humidified atmosphere containing 5% CO_2. The optimal plating density of each cell line, that ensures exponential growth throughout all the experimental period, was the same as originally published[23] and was, respectively, 1.5×10^5 cells/mL for MCF-7 and SF-268, 1.0×10^5 cells/mL for UACC-62 and 7.5×10^4 cells/mL to NCI-H460. Cells in 96-well plates were allowed to attach overnight and then exposed for 48 h to five concentrations of compounds. Following this incubation period the adherent cells were fixed in situ, washed, and dyed with SRB. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (EAR 400, STL-Labinstruments). For each compound tested a dose–response curve was generated and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of compound that inhibits 50% of the net cell growth, was determined as described.[23] Doxorubicin (Sigma Chemical Co.), used as a positive control, was tested

in the same manner. Final concentrations of DMSO did not interfere with the growth of cells.

Acknowledgments

The authors thank *Fundação para a Ciência e a Tecnologia* (*FCT*) (I&D 226/94), *FEDER*, *POCTI*, *POCI* and Project No. POCI/SAU-NEU/58735/2004 for financial support and *FCT* for the Ph.D. grant to Raquel Castanheiro (SFRH/BD/13167/2003). The authors are also indebted to the National Cancer Institute, Bethesda, MD, USA, for the generous provision of the human tumor cell lines.
We thank Dr. Bruce F. Milne for English language revision.

References and notes

1. Pinto, M. M. M.; Sousa, M. E.; Nascimento, M. S. J. *Curr. Med. Chem.* **2005**, *12*, 2517.
2. Gonzalez, M. J.; Nascimento, M. S. J.; Cidade, H. M.; Pinto, M. M. M.; Kijjoa, A.; Anantachoke, C.; Silva, A. M. S.; Herz, W. *Planta Med.* **1999**, *65*, 368.
3. (a) Lu, Z. X.; Hasmeda, M.; Mahabusarakam, W.; Ternai, B.; Ternai, P. C.; Polya, G. M. *Chem.-Biol. Interact.* **1998**, *114*, 121; (b) Jinsart, W.; Ternai, B.; Buddhasukh, D.; Polya, G. M. *Phytochemistry* **1992**, *31*, 3711.
4. Ho, C.-K.; Huang, Y.-L.; Chen, C.-C. *Planta Med.* **2002**, *68*, 975.
5. Nakatani, K.; Nakahata, N.; Arakawa, T.; Yasuda, H.; Ohizumi, Y. *Biochem. Pharmacol.* **2002**, *63*, 73.
6. Pedro, M.; Cerqueira, F.; Sousa, M. E.; Nascimento, M. S. J.; Pinto, M. *Bioorg. Med. Chem.* **2002**, *10*, 3725.
7. Subba Rao, G. S. R.; Raghavan, S. *J. Indian Inst. Sci.* **2001**, *81*, 393.
8. Pinto, M. M.; Polónia, J. *Helv. Chim. Acta* **1974**, *57*, 2613.
9. Grover, P. K.; Shah, G. D.; Shah, R. C. *J. Chem. Soc.* **1995**, 3982.
10. Fernandes, E. G. R.; Silva, A. M. S.; Cavaleiro, J. A. S.; Silva, F. M.; Borges, M. F. M.; Pinto, M. M. *Magn. Reson. Chem.* **1998**, *36*, 305.
11. Farrugia, L. J. *J. Appl. Cryst.* **1997**, *30*, 565.
12. Gales, L.; Damas, A. M. *Curr. Med. Chem.* **2005**, *12*, 2499.
13. Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc. Perkin Trans., 2* **1987**, *12*, S1.
14. Gales, L.; Sousa, M. E.; Pinto, M. M. M.; Kijjoa, A.; Damas, A. M. *Acta Cryst.* **2001**, *C57*, 1319.
15. Gales, L.; Sousa, M. E.; Pinto, M. M. M.; Damas, A. M. *Acta Cryst.* **2005**, *E61*, o2213.
16. Fukuyama, K.; Tsukihara, T.; Kishida, S.; Katsube, Y.; Ishida, M.; Hamasaki, T.; Hatsuda, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2947.
17. Ravikumar, K.; Rajan, S. S. *Acta Cryst.* **1987**, *C43*, 553.
18. Kosela, S.; Hu, L.; Yip, S.; Rachmatia, T.; Sukri, T.; Daulay, T. S.; Tan, G.; Vittal, J. J.; Sim, K. *Phytochemistry* **1999**, *52*, 1375.
19. Doriguetto, A. C.; Santos, M. H.; Ellena, J. A.; Nagem, T. J. *Acta Cryst.* **2001**, *C57*, 1095.
20. Mesquita, A.; Corrêa, D.; Gottlieb, O.; Magalhães, M. *Anal. Chim. Acta* **1968**, *42*, 311.
21. Sheldrick, G. M. *Acta Cryst.* **1990**, *A46*, 467.
22. Sheldrick, G. M. In *SHELXL97*, University of Göttingen: Germany, 1997.
23. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigrowolff, A.; Graygoodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
24. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokessch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.