Synthesis of Xanthones and Benzophenones as Inhibitors of Tumor Cell Growth

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Abstract: The synthesis of two new sulfated xanthones and two *O*-substituted benzophenones was carried out to evaluate for their inhibitory effect on growth of human tumor cell lines. The sulfation of 3,6-(**2**) and 3,4-dihydroxyxanthone (**3**) was accomplished using sulfur trioxide-pyridine to give, respectively, xanthone-3,6-*O*,*O*-bis(sulfate) (**4**) and xanthone-3,4-*O*,*O*-bis(sulfate) (**5**). Treatment of 2,2',4,4'-tetrahydroxybenzophenone (**6**) with acetic acid and prenyl bromide furnished 2,2',4,4'- tetraacetoxybenzophenone (**7**) and (5-hydroxy-2,2-dimethylchroman-6-yl)(2'-hydroxy-4'-(*tert*-pentyloxy)phenyl)methanone (**8**), respectively. Compounds **2-8** were tested for their effect on the *in vitro* growth of four representative human tumor cell lines: MCF-7 ER(+) (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (glioma), and A375-C5 (melanoma). Compounds **2** and **7-8** showed an inhibitory activity in the μM range.

Keywords: Antitumor, Benzophenones, Prenylated, Sulfated, Xanthones.

INTRODUCTION

Many naturally occurring and synthetic xanthones have been found to possess interesting biological and pharmacological activities [1-5]. Their chemotherapeutic potential seems rather attractive, since many xanthone derivatives are endowed with potent cytotoxic properties [4]. Consequently, we have focused our attention on the synthesis of xanthone derivatives and their capacity to inhibit the *in vitro* growth of some tumor cell lines, especially MCF-7 ER(+) (breast adenocarcinoma estrogen receptor positive) [6-9]. It was proposed that the growth inhibitory effect of xanthones was associated not only to their tricyclic scaffold, but also that this effect strongly depends on the nature and/or position of the different substituents. Particularly, in the field of anticancer drugs, isopropylfuran and dimethylpyran units fused onto xanthone cores appear interesting, with the remarkable example of a natural furanoxanthone, psorospermin, that was used as model for further chemical and biological developments [10]. Particularly, we have recently synthesized a series of dihydropyranoxanthones to evaluate their effect on the *in vitro* growth of three human tumor cell lines and we have found that some of these derivatives were not only potent growth inhibitors but also selective against the MCF-7 ER(+) cells [6,11]. Besides these derivatives, we have found that bromoalkoxyxanthones and xanthonolignoids also showed selectivity against the growth inhibition of the MCF-7 ER(+) human tumor cells [7,9].

Benzophenones are biosynthetic blocks as well as intermediates in many synthetic pathways of xanthones and constitute another interesting class of tumor cell growth inhibitors. One of the most interesting antiproliferative benzophenones is benzophenone-4,4′-*O*,*O*-bis-sulfamate (**1**, Benzomate), which was obtained during an attempt to design nonsteroidal steroid sulfatase (STS) inhibitors [12]. Structure-activity relationship studies revealed that the presence of the carbonyl group is essential for the activity whereas the bis-sulfamate moiety is required for the irreversible mechanism [12]. Interestingly, it was found that the conformational flexibility of the molecule is not pivotal for this activity. This fact led us to attempt to investigate a more rigid but similar structure by using the xanthonic scaffold to obtain potential STS inhibitors. It is well established that steroid sulfatase (STS) is one of the key enzymes of estrogen biosynthesis; thus blocking this enzyme will result in inhibition of estrogen production. Consequently, the hormone receptors will receive fewer growth signals and thus the growth of the cancer cells will slow down or stop. Interestingly, Di *et al.* [13] have reported the isolation of sulfonated xanthones from *Hypericum sampsonii*, which exhibited a significant cytotoxicity against cancer cell lines. These findings, together with the structural feature of Benzomate (**1**), led us to hypothetize the sulfation of the xanthone scaffold as a strategy of obtaining new xanthone derivatives as potential inhibitors of the cancer cells growth.

On the other hand, the growth inhibitory effect described for prenylated benzophenones [14-18], and particularly, for natural benzophenones with a biciclo 3.3.1-nonane-2,4,9 trione system [19-22], are well recognized. Interestingly, for *O*-substituted benzophenones, only one geranyloxybenzophenone has been investigated for its growth inhibitory activity showing the IC_{50}<10μg/mL against breast tumor cell

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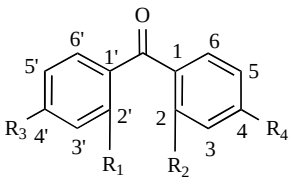
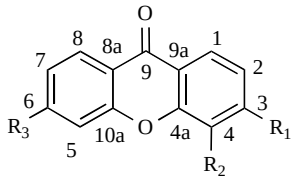


	R_1 and R_2	R_3 and R_4
1	H	OSO_2NH_2
6	OH	OH
7	$OCOCH_3$	$OCOCH_3$



	R_1	R_2	R_3
2	OH	H	OH
3	OH	OH	H
4	OSO_3^-	H	OSO_3^-
5	OSO_3^-	OSO_3^-	H

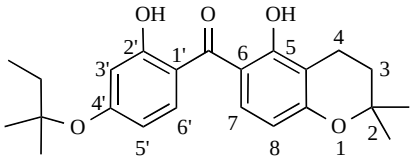


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Fig. (1). Structures of Benzomate (**1**) and benzophenones (**6-8**)/xanthones (**2-5**) investigated.

line MCF-7 [14,15,23]. However, a problem associated to benzophenones activity is that many of them also exhibit estrogenic activity which can interfere with the evaluation of their antiproliferative effect, especially on the MCF-7 ER(+) cells. Thus, is important to dissociate the estrogenic activity from the antiproliferative effect of these compounds. It is well known that a phenolic moiety is important for estrogenicity [24,25]. Additionally, it has been previously shown for a large, structurally diverse group of chemicals that the substitution of the hydroxyl by an alkoxyl group such as a methoxyl group significantly decreases the affinity for the ER [26]. Based on these facts, we have carried out a molecular modification of benzophenones by introducing a hydrophobic prenyl group to the benzophenone scaffold to block phenolic hydroxyl groups, eventually associated to a estrogenic activity.

Consequently, the synthesis of analogues of benzophenone-4,4′-*O*,*O*-*bis*-sulfamate (**1**, Benzomate) with a xanthone/benzophenone sacaffold was planned in order to obtain a series of molecules and to evaluate their effect on the *in vitro* growth of tumor cell lines. The molecular modifications were carried out by the introduction of sulfate groups into the xanthone scaffold for potential reversible STS inhibitors while acetyl/prenyl groups were introduced into the benzophenone scaffold to dissociate the potential estrogenic activity from the antiproliferative activity.

Herein, we report the synthesis of the rigid analogues of a potent STS inhibitor Benzomate (**1**): xanthone-3,6-*O*,*O*-bis(sulfate) (**4**) and xanthone-3,4-*O*,*O*-bis(sulfate) (**5**). Other two analogues of Benzomate (**1**), namely 2,2',4,4'-tetraacetoxybenzophenone (**7**) and (5-hydroxy-2,2-dimethylchroman-6-yl)(2'-hydroxy-4'-(*tert*-pentyloxy)phenyl)methanone (**8**) were also obtained (Fig. **1**). Compounds **4-5** and **7-8**, along with their respective precursors (**2-3** and **6**), were evaluated for their *in vitro* growth inhibitory effect on human tumor cell lines: MCF-7 ER(+), NCI-H460, SF-268, and A375-C5 cell lines.

RESULTS AND DISCUSSION

3,4-Dihydroxyxanthone (**3**) was obtained according to the previously described procedure (65%) [8]. Attempts to synthesize 3,6-dihydroxyxanthone (**2**) were based on base-catalyzed cyclization reactions, by oxidative or dehydrative processes [5]. The dehydrative process was performed by heating 2,2',4,4'-tetrahydroxybenzophenone (**6**) in furnace (180ºC) to afford 3,6-dihydroxyxanthone (**2**) in excellent yield (85%) (Fig. **2A**).

Sulfated xanthonic derivatives **4-5** were successfully obtained, in the presence of sulfur trioxide(SO_3)-pyridine adduct [27], from dihydroxyxanthones **2-3**, respectively, in moderate yields (28-49%) (Fig. **2B**). Sulfur trioxide–pyridine has been used extensively for sulfating alcohols, sterols, phenols and carbohydrates and normally the reactions run under moderate temperatures, usually below 120º C in the presence of pyridine excess [28]. However, in the case of the sulfated xanthones **4-5**, better yields were obtained when dimethylacetamide (DMA) was used as solvent.

In the dehydrative process of **6**, with the presence of acetic anhydride, 2,2',4,4'-tetraacetoxybenzophenone (**7**) was obtained in moderate yield (30%) (Fig. **2C**). The prenylated benzophenone **8** was obtained (6%) by the one-pot reaction

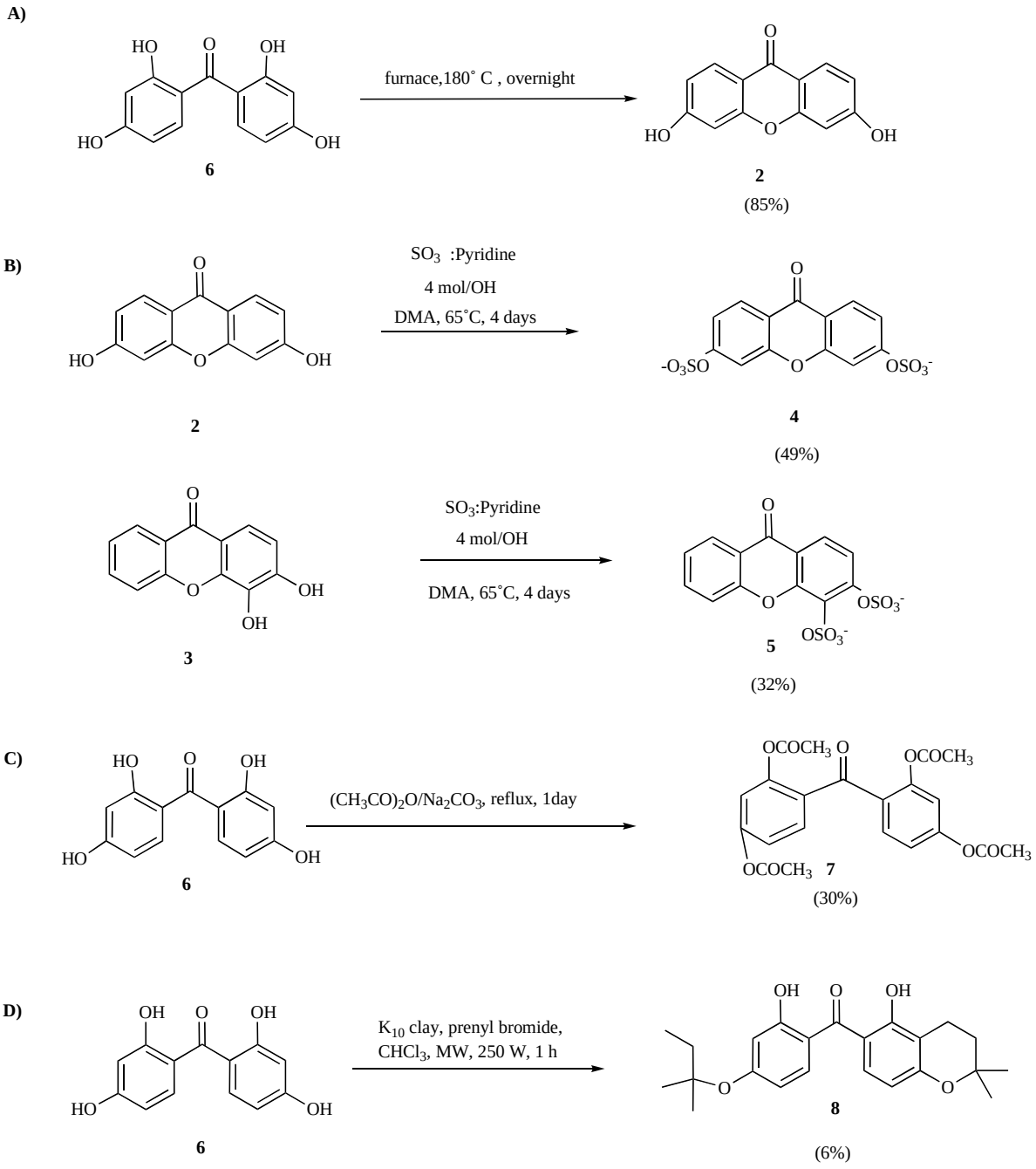

Fig. (2). Reagents and conditions for the synthesis of **A**) 3,6-dihydroxyxanthone (**2**), **B**) xanthone-3,6-*O*,*O*-bis(sulfate) (**4**) and xanthone-3,4-*O*,*O*-bis(sulfate) (**5**), **C**) 2,2',4,4'- tetraacetoxybenzophenone (**7**), and **D**) (5-hydroxy-2,2-dimethylchroman-6-yl)(2'-hydroxy-4'-(*tert*-pentyloxy)phenyl)methanone (**8**).

of benzophenone **6** and prenyl bromide (Fig. **2D**), applying the Montmorillonite K10 clay-catalyzed condensation, as recently described by Castanheiro *et al*. [11]. The formation of this unexpected asymmetrical product (**8**) observed under microwave irradiation, might be favored by the temperature regime that is often described to change selectivities as compared to conventional heat [29,30]. Compound **8** was hypothesized to be formed through a cascade sequence involving clays-induced aldol type reaction of benzophenone **6** with prenyl bromide as previously proposed by Jeso and Nicolaou [31].

Structure Elucidation

The structure of compound **2** was established by comparison of its ^{1}H and ^{13}C NMR data with those reported in the literature [32] while the structures of compounds **4**-**5** and **7**-**8** were elucidated using IR, HRMS, in addition to NMR techniques, and these data are reported in the experimental section.

The structures of the sulfated xanthones **4** and **5** were established not only by comparison of their proton and carbon chemical shift values with those of their respective precursor

xanthones (**2** and **3**), but also by the IR and HRMS spectra. The IR spectra of the sulfated derivatives **4** and **5** revealed two strong bands, characteristic of the S=O (1178 cm^{-1} and 1184 cm^{-1}) and the C-O-S (1067 cm^{-1} and 1004 cm^{-1}) groups, respectively. The HRMS allowed determining the number of sulfate groups for compounds **4** and **5** as two groups.

The multiplicity and coupling constants of the protons observed in the ^{1}H NMR spectrum of 2,2',4,4'- tetraacetoxybenzophenone (**7**) showed, besides the existence of two symmetrical 1,2,4-trisubstituted benzene rings, the signals of the protons of the acetoxyl groups at δ1.84 and 2.31, respectively. The ^{13}CNMR spectrum showed, besides the carbon signals of the aromatic rings and the carbonyl carbon of benzophenone (δ190.3), the carbonyl and methyl groups of the acetates (δ117.3 and 20.0, respectively).

In turn, the ^{1}H NMR spectrum of (5-hydroxy-2,2-dimethylchroman-6-yl)(2'-hydroxy-4'-(*tert*-pentyloxy)phenyl) methanone (**8**) showed the proton signals of two methylene groups (δ1.83t, J=6.8 and 2.73t, J=6.8) and two methyl groups (δ1.37s), characteristic of the fused dihydropyran ring. This was corroborated by the chemical shift values observed in the ^{13}C NMR spectrum (δ16.9CH_2, 26.9CH_3, 32.7CH_2, 67.4C). The fusion of the dihydropyran ring with C3-C4 of the aromatic ring of the benzophenone is evidenced by the lack of H-3 signal and the presence of two *ortho* coupled aromatic protons, corresponding to H-7 and H-8. *O*-Prenylation also occurred on the other aromatic ring since its pattern of substitution was maintained as can be observed by the multiplicities and coupling constants of its protons. The presence of 4'-(*tert*-pentyloxy) substituent on this ring was confirmed by an observation of the proton (δ1.26s, 1.28t, 1.36m) and carbon signals (δ21.7CH_3, 27.1CH_3, 54.8CH_3 and 69.9C) from the ^{1}H and ^{13}C NMR spectra. The proposed structure was in agreement with the result obtained from the high-resolution mass spectrometry (HRMS).

Effect on the Growth of Tumor Cell Lines

The hydroxy- (**6**) acetoxy- (**7**) and prenyloxy-(**8**) benzophenone derivatives, as well as 3,6-dihydroxyxanthone (**2**) and the sulfated xanthones **4** and **5** were evaluated for their effect on the *in vitro* growth of four human tumor cell lines: MCF-7 ER(+) (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), SF-268 (glioma), and A375-C5 (melanoma) after a continuous exposure of 48 hours and results are shown in Table **1**. Compound **3** has previously shown a moderate growth inhibitory effect on human tumor cell lines [8].

The growth inhibitory effect for compounds **2** and **7-8** was in general moderate but was shown to be dose-dependent (see Supportive/Supplementary Material) and due to growth arrest and not to cell death, as inferred from the sulforhodamine B (SRB) assay. 3,6-Dihydroxyxanthone (**2**) and benzophenones **6-8** were found to be active in the four human tumor cell lines but with benzophenones **7** and **8** showing a significant inhibitory activity against an estrogen dependent MCF-7 ER(+) cell line (GI_{50} < 30 μM, Table **1**). However, no activity was observed for the sulfated xanthones **4** and **5** on the growth of the human cancer cell lines tested (GI_{50}>100 μM, Table **1**).

It can be inferred that the introduction of hydrophobic groups, a hindrance of the hydroxyl groups, led to the enhancement of the inhibitory growth effects on MCF-7 ER(+) when compared the growth inhibitory effects exhibited by *O*-acetylated (**7**) and *O*-prenylated (**8**) benzophenones, with that of their precursor, 2,2',4,4'-tetrahydroxybenzophenone (**6**), (Table **1**). Nonetheless, the selectivity shown by compounds **7** and **8** for MCF-7 cells over the other cells was not evident and therefore modulation of the estrogenic pathway by these compounds is very unlikely. Since compounds **7** and **8** are Benzomate (**1**) analogues, should be interesting to further investigate if their antiproliferative mechanism of action is related to the interaction of compound **7** and **8** with steroid sulfatase (STS).

MATERIALS AND METHODS

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. IR spectra were measured on an ATI Mattson Genesis series FTIR (software: WinFirst

Table 1. GI_{50} Values of Benzophenones 6, 7, and 8, Xanthone 2 and Sulfated Xanthone Derivatives 4 and 5 on the *In Vitro* Growth of Human Tumor Cell Lines

Compounds	GI_{50} (μM)[a]			
	MCF-7 R(+) (breast)	NCI H460 (lung)	A375-C5 (melanoma)	SF-268 (glioma)
2	74.6[b]	31.1[b]	ND	149.1[b]
4	118.5[b]	145.0[b]	140.0[b]	> 150
5	> 150	> 150	> 150	> 150
6	66.1 ± 3.8	105.7[b]	ND	142.3[b]
7	27.4[b]	41.0 ± 1.2	ND	20.1[b]
8	20.3 ± 0.7	23.0 ± 1.0	22.5 ± 0.4	ND
Doxorubicin	43.3 ± 2.6[c]	35.6 ± 1.6[c]	130.2 ± 10.1[c]	94.0 ± 7.0[c]

[a]Results expressed as GI_{50}, concentrations of the compound that cause 50% inhibition of cell growth, are mean ±SEM of 3-5 independent experiments performed in duplicate carried out independently. ND= not determined. [b]Data based on two independently run duplicate experiments. [c]GI_{50} values are expressed in nM for the positive control doxorubicin.

v.2.10) spectrophotometer in KBr microplates (cm^{-1}). ^1H and ^{13}CNMR 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. HRMS results were obtained in the services of C.A.C.T.I., Vigo, Spain. 3,6-Dihydroxyxanthone (**2**) [33] and 3,4-dihydroxyxanthone (**3**) [8] were synthesized according to described methods. The following materials were synthesized and purified by the described procedures.

Xanthone-3,6-O,O-bis(sulfate) (4)

A mixture of 3,6-dihydroxyxanthone (**2**, 285 mg; 1.25 mmol), DMA (4 mL), and SO_3-pyridine adduct (10 mmol; 1.59 g) was kept under reflux for 4 days. The reaction mixture (pH 4) was basified with triethylamine to pH 7, and 10 mL of acetone was added. After cooling, the brown oil formed was washed with acetone and ether and suspended in aqueous solution of sodium acetate 30% (2mL). Absolute ethanol (50 mL) was added to the suspension and brown oil was formed after cooling. The crude oil (500 mg) was purified by solid phase extraction with a cation exchange cartridge Discovery® DSC-SCX, with a sulfonic acid moiety following the steps: condition with methanol (5 mL); loading [(500 mg of sample; 2 mL (1:1 MeOH: CH_3COOH 1% in water)]; elution with CH_3COOH (15 mL; 1% in water); the acidic fractions were collected and the solvent evaporated under reduced pressure to afford a yellow solid corresponding to xanthone-3,6-*O*,*O*-bis(sulfate) (**4**, 250 mg; 49%); mp > 300 ºC; IR (KBr) υ_{max} : 1632; 1533; 1484, 1178; 1067; ^1H NMR (DMSO, 300.13 MHz) δ: 7.99 (2H, d, *J*=8.6, H-1; H-8), 6.87 (2H, dd, *J*=8.6 and 2.2, H-2; H-7), 6.83 (2H, d, *J*=2.2, H-4, H-5); ^{13}C NMR (DMSO, 75.47 MHz) δ: 174.6 (CO), 163.4 (C-3 and C-6), 157.5 (C-4a and C-10a), 127.9 (C-1 and C8), 114.0 (C-8a and C-9a), 114.0 (C-2 and C-7), 102.2 (C-4 and C-5); HRMS: 479.07387, $C_{13}H_9O_{10}S_2.5H_2O$; calcd 479.01652.

Xanthone-3,4-O,O-bis(sulfate) (5)

A mixture of 3,4-dihydroxyxanthone (**3**, 285 mg; 1.25 mmol), DMA (4 mL) and SO_3-pyridine adduct (10 mmol; 1,59 g) was kept under reflux for 4 days. The same procedure used above for compound **4** was followed in the work-up. The acidic fractions were collected and the solvent evaporated under reduced pressure to afford a yellow solid corresponding to xanthone-3,4-*O*,*O*-bis(sulfate) (**5**, 170 mg, 32%); mp > 300ºC, IR (KBr) υ_{max} : 1630; 1531; 1481; 1184; 1004; ^1H NMR (DMSO, 300.13 MHz) δ: 8.16 (1H, dd , *J*=8.0,1.5, H-8), 7.84 (1H, dd, *J*=8.0, 8.0, 1.5, H-6), 7.64 (1H, dd, *J*=8.0, H-5), 7.57 (1H, d, *J*=8.8, H-1), 7.45 (1H, ddd, *J*=8.0, 8.0, 1.5, H-7), 6.95 (1H, d, *J*=8.8, H-2); HRMS: 479.07403, $C_{13}H_9O_{10}S_2.5H_2O$; calcd 479.01652.

2,2',4,4'- Tetraacetoxybenzophenone (7)

A mixture of 2,2',4,4'-dihydroxybenzophenone (**6**, 1 g; 4.06 mmol) and acetic anhydride (20 mL) was refluxed at 140ºC, for 2 days. After the reaction was finished, aqueous sodium bicarbonate 10% (10 mL) was added to the reaction mixture, which was then poured onto 50 g of crushed ice. The solid thus obtained was filtered, washed with water and dried to furnish a white solid of 2,2',4,4'- tetraacetoxybenzophenone (**7**, 418 mg, 30%); mp 184-185ºC; IR (KBr) υ_{max} : 1762; 1661; 1605; 1372; 1198; 1140; ^1H NMR ($CDCl_3$, 300.13 MHz) δ: 7.60 (1H, d, *J* = 8.5 Hz, H-6; H-6'), 7.24 (2H, dd, *J* = 8.5; 2.2 Hz, H-5; H-5'), 7.17 (2H, d, *J* = 2.0 Hz, H-3;H-3'), 2.31 (6H, s, 2-OCOC$\underline{H}_3$, 2'-OCOC$\underline{H}_3$), 1.84 (6H, s, 4-OCOC$\underline{H}_3$, 4'-OCOC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ: 190.3 (CO), 153.8 (C-2,C-2',C-4,C-4'), 120.0 (C-1; C-1'), 117.3 (C-3,C-3',C-5,C-5', 2-O$\underline{C}$OCH$_3$, 2'-O$\underline{C}$OCH$_3$, 4-O$\underline{C}$OCH$_3$, 4'-O$\underline{C}$OCH$_3$), 131.5 (C-6, C-6'), 20.0 (2-OCO$\underline{C}$H$_3$, 2'-OCO$\underline{C}$H$_3$, 4-OCO$\underline{C}$H$_3$, 4'-OCO$\underline{C}$H$_3$); HRMS: 415.1027 $[M+H]^+$, $C_{21}H_{19}O_9^+$; calcd 415.1029.

5-hydroxy-2,2-dimethylchroman-6-yl)(2'-hydroxy-4'-(tert-pentyloxy)phenyl)methanone (8)

A mixture of 2,2',4,4'-tetrahydroxybenzophenone (**6**, 0.11 g; 0.39 mmol), prenyl bromide (0.2 mL; 1.64 mmol), and Montmorillonite K_{10} Clay (2.04 g), in a 12 ml closed microwave reactor under stirring was irradiated at 250 W for 3 × 20 min at a final temperature of 90ºC. After cooling, the solid was filtered and washed and the solvent removed under reduced pressure to furnish a brown oil. This crude product was purified by liquid chromatography (SiO_2; $CHCl_3$/hexane, 70:30) yielding an impure solid. The purification was then carried out by preparative TLC (SiO_2; hexane/AcOEt, 80:20) affording a white compound corresponding to (5-hydroxy-2,2-dimethylchroman-6-yl)(2'-hydroxy-4'-(*tert*-pentyloxy)phenyl)methanone (**8,** 10 mg, 6%); mp 238-240ºC, IR (KBr) υ_{max} :2922; 1609, 1585, 1484, 1461, 1361, 1259, 1227, 1154, 1116, 879, 790, 757, ^1H NMR ($CDCl_3$, 300.13 MHz) δ: 7.50 (1H, d, *J*=8.6, H-6'), 7.38 (1H, d, *J*=9.0, H-7), 6.46 (1H, s, H-3'), 6.43 (1H, d, *J*=8.6, H-5'), 6.35 (1H, d, *J*=9.0, H-8), 2.73 (2H, t, *J* = 6.8, H-4), 1.83 (2H, t, *J*=6.8, H-3), 1.37 (6H, s, CH_3-1''), 1.36 (m, CH_2-3'''), 1.28 (t, CH_3-4'''), 1.26 (6H, s, CH_3-1'''); ^{13}C NMR ($CDCl_3$, 125.77 MHz) δ: 199.1 (CO), 164.4 (C-2'), 162.2 (C-4'), 161.9 (C-4), 160.8 (C-2), 134.3 (C-6'), 129.1 (C-7), 113.9 (C-1'), 113.5 (C-3), 112.6 (C-6), 107.3 (C-5'), 105.2 (C-8), 103.9 (C-3'), 69.9 (C-2'''), 67.4 (C-2), 54.8 (C-3'''), 32.7 (C-3), 27.1 (C-1'''), 26.9 (C-1''), 21.7 (C-4'''), 16.9 (C-4); HRMS: 383.18499, $C_{23}H_{28}O_5$ calcd 383.18530.

Tumor Cell Growth Assay

The effects of **2–8** on the *in vitro* growth of 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' that uses the protein-binding dye sulforhodamine B to assess cell growth.[34,35] Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound (1:3 to 1:2 dilutions), starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Powerwave XS, Wincoski, USA). For each test compound (**2-8**) and cell line, a dose–response curve was obtained (see supportive/supplementary material). The growth inhibition of 50% (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell protein increase in control cells during compounds incubation, was calculated in terms of %T/C [(OD of treated cells/OD of control

cells)×100)] as described elsewhere [34]. Doxorubicin was used as a positive control and tested in the same manner.

CONCLUSION

The synthesis of two new sulfated xanthones was successfully achieved by using sulfur trioxide-pyridine adduct in dimethylacetamide. Though, these new compounds did not exhibit an *in vitro* growth inhibitory effect on the human tumor cell line tested, they represent the new class of xanthonic derivatives waiting for other models of biological evaluation, such as anticoagulant activity in which sulfated small molecules have promising representatives [36].

On the contrary, introduction of the hydrophobic groups to the benzophenone scaffold (**7** and **8**) was found to enhance the *in vitro* growth inhibitory effect, in a micromolar range, on the human tumor cells, especially the MFC-7 ER(+). The activity exhibited by these two derivatives provides an interesting clue for further molecular modifications to be performed in order to improve their potency.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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