European Journal of Medicinal Chemistry 44 (2009) 3830–3835

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Preliminary communication

Bromoalkoxyxanthones as promising antitumor agents: Synthesis, crystal structure and effect on human tumor cell lines

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ARTICLE INFO

Article history:
Received 17 February 2009
Received in revised form
23 March 2009
Accepted 3 April 2009
Available online 14 April 2009

Keywords:
Antitumor
Bromoalkoxyxanthone
Oxygen heterocycle
X-ray diffraction
Xanthones

ABSTRACT

In a study involving the synthesis of bis-intercalators, a bisxanthone and a minor product, 1-(6-bromohexyloxy)-xanthone were obtained. Although no capacity to inhibit the growth of human tumor cell lines was observed for the bisxanthone, the bromoalkoxyxanthone revealed this biological activity. In light of these results bromoalkylation of 3,4-dihydroxyxanthone furnished two bromohexyloxy-xanthones that were investigated for their effect on the in vitro growth of human tumor cell lines MCF-7 (ER+, breast), MDA-MB-231 (ER−, breast), NCI-H460 (non-small lung), and SF-268 (central nervous system). The X-ray structure of 1-(6-bromohexyloxy)-xanthone revealed that the xanthone skeleton remains essentially planar forming a dihedral angle of 61.3(2)° with the 6-bromohexyl side chain. These results revealed bromoalkoxyxanthones as interesting scaffolds to look for potential anticancer drugs.

1. Introduction

Heterocycles play an important role in the design and discovery of new pharmacologically active compounds. The xanthone framework is an important *O*-heterocycle of documented relevance for development of anticancer agents [1]. Two derivatives, dimethylxanthenone-4-acetic acid (DMXAA), that causes tumor necrosis, and psorospermin, that acts by intercalation into the DNA molecule and by the alkylation of guanine at the topoisomerase II cleavage site, have entered clinical trials. Recently, there has been an increase in the synthesis of new xanthonic structures [1,2], partly due to the ability for their derivatives to bind to different classes of receptors [1].

Due to this interest, natural and synthetic xanthones have been reported by our group to demonstrate an antiproliferative effect on human tumor cell lines [3–5] and on human lymphocytes [3,4] and an effect compatible with protein kinase C (PKC) activation [6,7] and PKC inhibition [8].

DNA is also an important target for prenylated and/or oxygenated xanthones that due to their specific binding are capable of a wide range of antitumor responses such as DNA breaks, DNA–protein cross-links and DNA synthesis suppression [9–12]. The overall results for xanthone binding studies with DNA indicate that the planar tricycle moiety serves as an important feature for designing new DNA intercalators. There are a number of DNA intercalators that recognize one of the DNA groves, although the three-dimensional structures of some complexes reveal differences in the position of the drugs within each grove. Efforts to identify molecules with a greater affinity for DNA have resulted in the development of bis-intercalators in which two intercalating ligands (hydrophilic [13–15] or lipophilic [16]) are linked by a chain.

The primary aim of this work was to obtain potential DNA bis-intercalators with a xanthonic framework for CNS tumors, i.e., with suitable lipophilicity for blood–brain barrier penetration. With that purpose, the synthesis of dimeric compounds with an alkyl bridge was initially planned.

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doi:10.1016/j.ejmech.2009.04.011

2. Chemistry

The preparation of a hexamethylenebis(oxy)-linked assembly is illustrated in Scheme 1. The Nencki reaction [step (I) in Scheme 1] offered a convenient method for preparing 1-hydroxyxanthone (**1**) in 48% yield [17]. The intermediate benzophenone **1a** was also isolated in 5% yield and its structure was determined by X-ray crystallography. 1,1′-[Hexane-1,6-diylbis(oxy)]bis[9*H*-xanthen-9-one] (**2**, 50%) and a minor product 1-(6-bromohexyloxy)-9*H*-xanthen-9-one (**3**, 30%) were obtained from the reaction of 1-hydroxyxanthone (**1**) with dibromohexane in basic conditions [step (II) in Scheme 1].

The work proceeded with the bromoalkylation of a more potent xanthone, 3,4-dihydroxyxanthone (**4**), previously found as an inhibitor of the growth of human tumor cell lines [3]. Two bromohexyloxyxanthones, 3-(6′-bromohexyloxy)-4-hydroxyxanthone (**5**, 40%) and 3,4-bis(6′-bromohexyloxy)-xanthone (**6**, 10%), were obtained in higher yields with the dropwise addition of dibromohexane (Scheme 2).

3. Pharmacology: in vitro

Being the investigation of secondary products a strategy in drug discovery and having a bromoimidazolxanthone revealed as having high potency in inhibiting P450 17α-hydroxylase-17,20-lyase, a target for the development of drugs for cancer treatment [18], both secondary products (**1a**) and (**3**) were investigated along with compound **2** for their effect on the in vitro growth on four human tumor cell lines: SF-268 (central nervous system cancer), NCI-H460 (non-small lung cancer), MCF-7 (breast cancer, ER+), and MDA-MB-231 (breast cancer, ER−). 3-(6′-Bromohexyloxy)-4-hydroxyxanthone (**5**) and 3,4-bis(6′-bromohexyloxy)-xanthone (**6**) were further investigated for their growth inhibitory effect. The results of this study are summarized in Table 1.

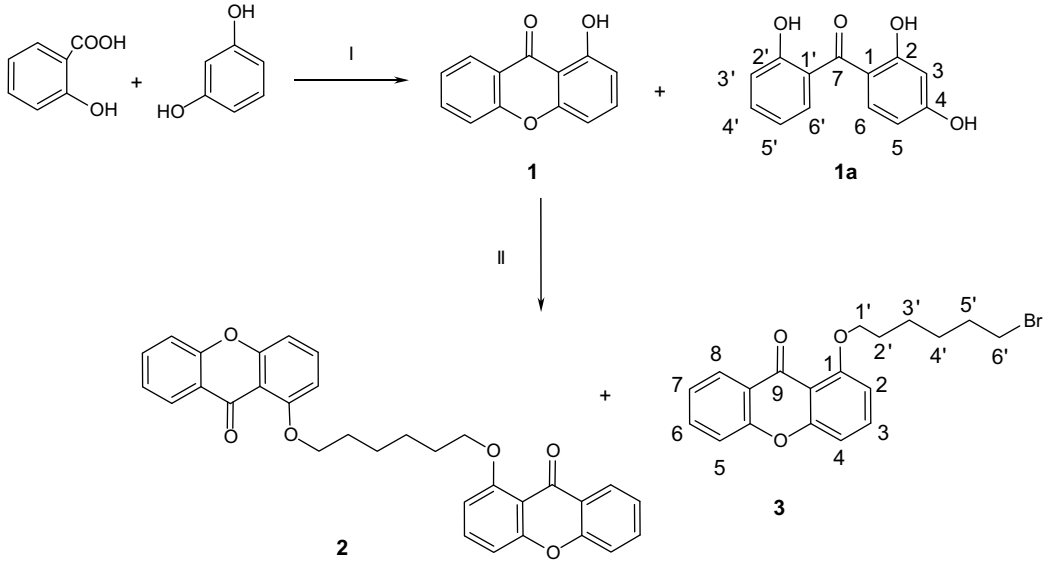
4. Results and discussion

Although for bis-alkoxyxanthone (**2**) and 2,2′,4-trihydroxybenzophenone (**1a**) no relevant growth inhibition was observed on these tumor cell lines ($GI_{50} \geq 100$ μM), the secondary product bromoalkoxyxanthone (**3**) showed an interesting growth inhibitory effect, higher than the parent compound (**1**), against SF-268 ($GI_{50} = 30.2 \pm 3.6$ μM) and NCI-H460 ($GI_{50} = 30.2 \pm 3.6$ μM). Curiously, this molecular modification led to the appearance of a growth inhibitory activity against the estrogen receptor (ER+) MCF-7 ($GI_{50} = 22.7 \pm 1.3$ μM) but not against the ER− MDA-MB-231 ($GI_{50} > 100$ μM). The growth inhibition effects presented by these xanthones and benzophenone derivatives cannot be attributed to a toxic effect, as inferred from the sulforhodamine B assay (data not shown).

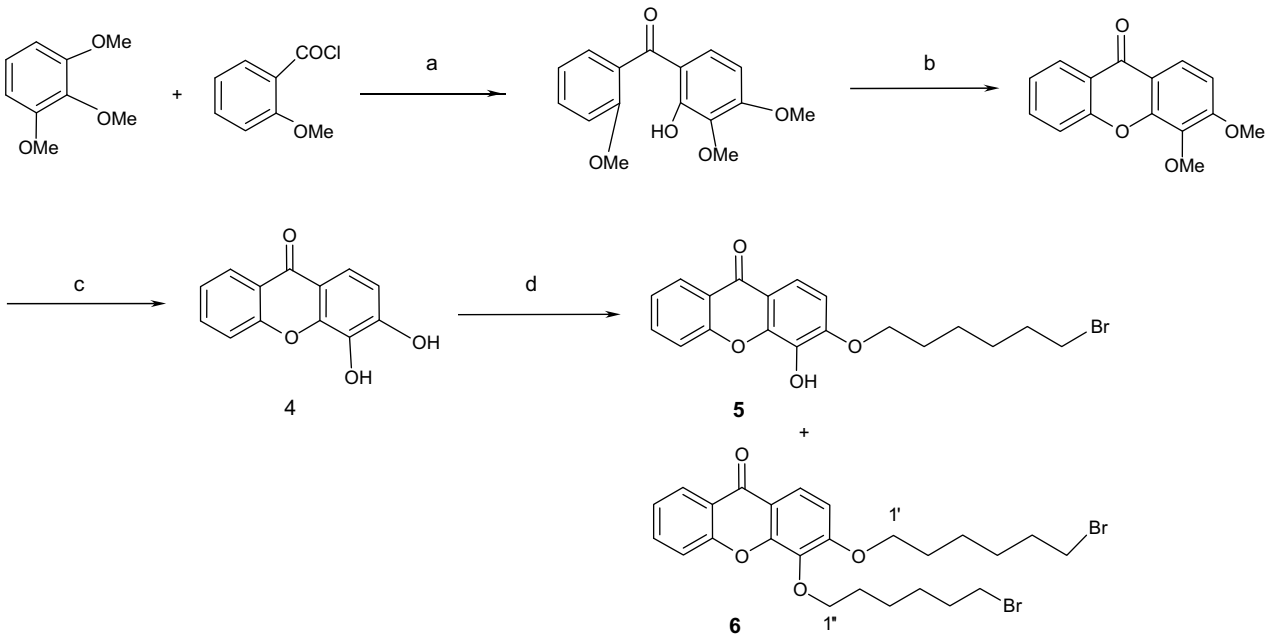
Similarly to bis-alkoxyxanthone **2**, no relevant growth inhibition was observed for bromoalkoxyxanthone **5** on all the tumor cell lines tested ($GI_{50} > 100$ mM). Compound **6** was found to inhibit only the growth of the breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231). Similarly to compound **3**, bromoalkoxyxanthone **6** was also more active than its precursor **4** against ER+ MCF-7 ($P < 0.05$). On the contrary, compound **4** was much more active with glioma SF-268, lung NCI-H460, and MDA-MB-231 cell lines than with MCF-7. Although more studies are needed to establish a structure–activity relationship, results showed that one free hydroxyl group in the bromoalkoxyxanthone scaffold (compound **5**) is unfavorable to the growth inhibitory activity against ER+ MCF-7.

The structure of compounds **1**–**6** was established on the basis of IR, MS, and NMR techniques. For compounds **1** and **4** all the data were in accordance with the literature [8,19]. The ^{1}H NMR and ^{13}C NMR data of compounds **2**, **3**, **5**, and **6** are described for the first time (see Experimental section). The analysis of the ^{1}H and ^{13}C NMR spectra of compounds **2**, **3**, **5**, and **6** allowed the assignment of the AKPX spin system corresponding to the resonance of H-5, H-6, H-7 and H-8. The assignment of the corresponding carbon resonances was made from the correlations found in the HSQC spectra, whereas those of the quaternary carbons of the unsubstituted ring were based on the connectivities found in the HMBC.

Herein, the crystal structures of the inhibitor of human tumor cell lines 1-(6-bromohexyloxy)-9*H*-xanthen-9-one (**3**) and of 2,2′,4-trihydroxybenzophenone (**1a**) are also described. A perspective view of the crystal structure of compound **3** showing the atom-labelling scheme is presented in Fig. 1 and was obtained using ORTEP [20]. The atoms that form the xanthone skeleton (C1–C2–C3–C4–C4A–O4A–C10A–C5–C6–C7–C8–C8A–C9–C9A) define a plane with an rms deviation of 0.03 Å. The C9 and the O4A atoms are the ones that deviate more from this plane: 0.05 Å and 0.06 Å, respectively; while O1 lies on the plane, O9 is 0.104 Å away and this is



Scheme 1. Reagents and conditions: (I) zinc chloride, 200 °C/5 min–180 °C/4 h; (II) potassium carbonate, dry DMF, 1,6-dibromobutane, room temperature, 24 h.



Scheme 2. Reagents and conditions: (a) aluminum chloride, dry Et_2O, room temperature (85%); (b) NaOH, MeOH, H_2O, reflux (98%); (c) aluminum chloride, dry toluene, reflux (75%); (d) potassium carbonate, dry DMF, 1,6-dibromobutane dropwise addition, room temperature, 24 h.

probably due to a repulsion between these two oxygen atoms. The C1′–C2′–C3′–C4′–C5′–C6′–Br atoms also lie in a plane defined with an rms deviation of 0.04 Å and the dihedral angle between both planes is 61.3(2)°. In the crystal structure of compound **3** the packing of the molecules is governed essentially by van der Waals forces. While most of xanthone crystal structures show an efficient packing into columns with the xanthone skeleton planes parallel to one another [21], this compound reveals two stacking planes, each of them with an intermolecular separation of 4.0 Å.

The crystal structure of compound **1a** is shown in Fig. 2. The two aromatic ring planes form an angle of 44.69(6)° and the rotation of the three OH groups around the C–OH bound is less than 10° in relation to the plane of the respective aromatic ring. Moreover, the hydroxyl groups bound to C2 and C2′ are hydrogen bond to O7. Similar interactions are found in crystal structures of xanthone derivatives when –OH are bound to C1 or C8 [21,22].

Several promising compounds with antitumor and antiviral activities share a common scaffold that is pertinent to possible intercalative binding to DNA. They comprise a polycyclic, rigid and planar system coupled with a flanking long chain [23]. The dihedral angle between the polycyclic structure and the long chain is approximately 94° in the case of the widely prescribed anthracycline antitumor antibiotic daunorubicin [24]. In most cases, the polycyclic ring binds to the minor groove of DNA via hydrophobic and stacking effects. Interestingly, a series of substituted imidazothioxanthones were studied in order to find out about their possible role as DNA triple helix-binding ligands [25] and the results suggested that a protonated or positively charged side chain

Table 1
Effect of compounds on the growth of human tumor cell lines.[a]

Compounds	GI_{50} (μM)			
	SF-268 (glioma)	NCI-H460 (lung)	MCF-7 (breast, ER+)	MDA-MB-231 (breast, ER−)
1	70.8 ± 10.9	84.9 ± 13.6	>100	66.0 ± 5.4
1a	>100	91.2 ± 8.7	100	ND
2	>100	>100	>100	>100
3	30.2 ± 3.6	30.7 ± 3.2	22.7 ± 1.3	>100
4	22.6[b]	11.4[b]	52.4 ± 6.3	22.8 ± 4.8
5	>100	>100	>100	>100
6	>100	>100	20.5 ± 1.9	56.8 ± 11.2

[a] Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and represent means ± SEM of 3–5 independent experiments performed in duplicate and carried out independently.

[b] Results of one or two independent experiments performed in duplicate. Doxorubicin was used as positive control, GI_{50}: MCF-7 = 42.8 ± 8.2 nM; MDA-MB-231 = 10.86 ± 1.28 nM; SF-268 = 94.0 ± 7.0 nM; NCI-H460 = 94.0 ± 8.7 nM. ND = not determinate.

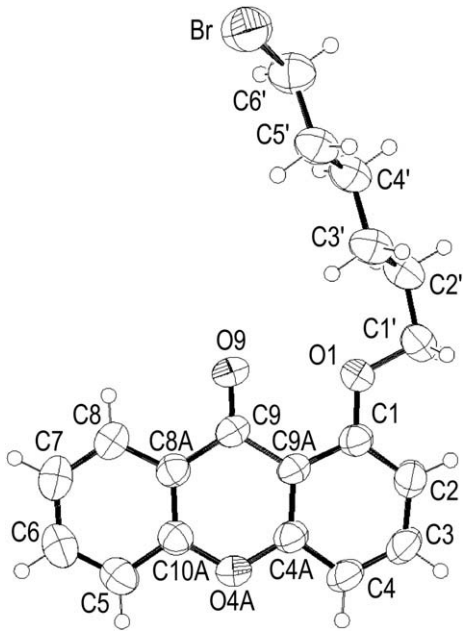


Fig. 1. Molecular structure of compound **3** showing the atom-labelling scheme. H atoms are depicted as spheres of arbitrary radii. Displacement ellipsoids are shown at the 50% probability level.

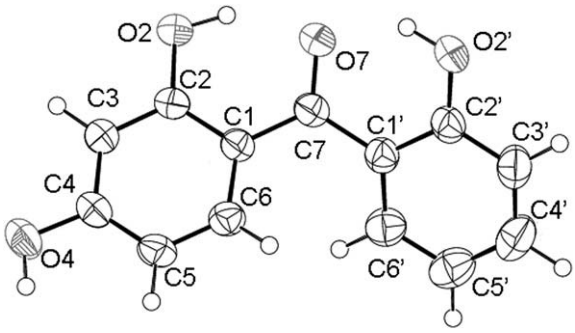


Fig. 2. Molecular structure of compound **1a** showing the atom-labelling scheme.

is necessary for the interaction with DNA. Although the side chain in bromoalkoxyxanthones is not positively charged, the bromine atom could serve as an anchor for the interaction with DNA.

5. Conclusion

In conclusion, the bromoalkoxyxanthone scaffold was revealed as promising for development of new potential antitumor drugs and led to the increase of a growth inhibitory activity against the estrogen receptor (ER+) MCF-7 cell line. With the structure of 1-(6-bromohexyloxy)-xanthone (**3**) resolved by X-ray crystallography, DNA unwinding experiments combined with molecular modeling studies concerning the binding of molecule **3** to DNA or to estrogen receptors might disclose some mechanistic aspects in the future.

6. Experimental

6.1. Chemistry

Purifications of compounds were performed by column chromatography (CC) using Merck silica gel 60 (0.50–0.20 mm) and for monitoring reactions TLC with Merck silica gel 60 (GF_{254}) was used. Melting points were obtained in a Kofler microscope and are uncorrected. IR spectra were recorded on a Perkin Elmer 257 in KBr. ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ or DMSO-d_6 at room temperature, on a Bruker DRX 300 instrument. Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as an internal reference. MS spectra were recorded as EI (electronic impact) mode on a Hitachi Perkin–Elmer. HRMS results were obtained in the services of C.A.C.T.I., Vigo, Spain.

6.1.1. 1-Hydroxyxanthone (*1*), 1,1,3-trihydroxybenzophenone (*1a*) and 3,4-dihydroxyxanthone (*4*)

The synthesis of 1-hydroxyxanthone (**1**) [step (I) in the Scheme 1] was carried out according to the method of Pankajamani and Seshadri [17] and characterized according to the described procedure (48%) [19]. Crystallization of the mother-liquor from acetone rendered compound **1a** in 10% yield.

The synthesis of 3,4-dihydroxyxanthone (**4**) (Scheme 2) was carried out according to the method of Gottlieb et al. [26] and characterized according to the described procedure (70%) [4].

6.1.2. 1,1′-[Hexane-1,6-diylbis(oxy)]bis[9H-xanthen-9-one] (*2*) and 1-(6-oxy)-9H-xanthen-9-one (*3*)

To a solution of 1-hydroxyxanthone (**1**) (215 mg, 1 mmol) in dry DMF (5 mL), was added K_2CO_3 (138 mg, 1 mmol) and the mixture was stirred at room temperature for 30 min. To the suspension formed was added one portion of 1,6-dibromobutane (244 mg, 1 mmol) in dry DMF (1 mL). The reaction mixture was stirred for 24 h at room temperature and then poured into ice-water (40 mL). The yellow pale solid thus obtained was filtered and purified by CC [petroleum ether (313–333 K boiling fraction)/diethyl ether, several proportions]. The collected fractions from petroleum ether/diethyl ether (8:2) furnish a yellow solid that was crystallized from acetone to give compound **3** (30%) and from the collected fractions eluted with petroleum ether/diethyl ether (6:4), a white solid was crystallized from ethyl acetate/petroleum ether (4:1) to give compound **2** (50%).

Compound **2** mp 76 °C; ^1H NMR (300.13 MHz, DMSO-d_6): $\delta = 8.26$ (H-8, dd, $J = 8.0$ and 1.7 Hz, 2H), 7.65 (H-6, ddd, $J = 9.3$, 6.2 and 1.7 Hz, 2H), 7.40 (H-3 and H-5, m, 4H), 7.32 (H-7, ddd, $J = 9.5$, 6.3 and 1.0 Hz,) 2H, 6.91 (H-4, dd, $J = 7.5$ and 0.8 Hz, 2H), 6.74 (H-2, dd, $J = 7.5$ and 0.5 Hz, 2H), 4.17 (H-1′, t, 4H), 2.04 (H-2′, m, 4H), 1.77 ppm (H-3′, m, 4H); ^{13}C NMR (75.47 MHz, DMSO-d_6): $\delta = 176.2$ (C-9), 160.3 (C-1), 158.0 (C-4a), 155.0 (C-10a), 134.6 (C-3), 133.9 (C-6), 126.7 (C-8), 123.6 (C-7), 123.1 (C-8a), 117.2 (C-5), 113.2 (C-9a), 109.5 (C-4), 106.3 (C-2), 68.8 (C-1′), 28.7 (C-2′), 24.9 ppm (C-3′); IR (KBr): $\nu = 1658$, 1598, 1458, 1354, 1274, 1081, 760 cm^{-1}; MS (EI) m/z (%): 506 (37) $[M]^+$, 225 (32), 212 (100), 196 (24). HRMS-ESI m/z calcd. for $C_{32}H_{26}O_6Na$: 529.1621597, found: 529.1607320.

Compound **3** mp 83–84 °C; ^1H NMR (300.13 MHz, DMSO-d_6): $\delta = 8.30$ (H-8, dd, $J = 8.0$ and 1.7 Hz, 1H), 7.65 (H-6, ddd, $J = 11.7$, 7.7 and 1.7 Hz, 1H), 7.58 (H-3, dd, $J = 8.4$ and 8.5 Hz, 1H), 7.41 (H-5, dd, $J = 8.0$ and 0.6 Hz, 1H), 7.34 (H-7, ddd, $J = 7.7$, 6.7 and 1.0 Hz, 1H), 7.04 (H-4, dd, $J = 8.4$ and 0.7 Hz, 1H), 6.78 (H-2, d, $J = 8.3$ Hz, 1H), 4.14 (H-1′, t, 2H), 3.45 (H-6′, t, 2H), 1.97 (H-2′ and H-5′, m, 4H), 1.61 ppm (H-3′ and H-4′, m, 4H); ^{13}C NMR (75.47 MHz, DMSO-d_6): $\delta = 176.4$ (C-9), 160.2 (C-1), 158.1 (C-4a), 155.0 (C-10a), 134.7 (C-3), 134.1 (C-6), 126.7 (C-8), 123.7 (C-7), 123.0 (C-8a), 117.2 (C-5), 112.7 (C-9a), 109.8 (C-4), 106.3 (C-2), 69.1 (C-1′), 34.0 (C-6′), 32.6 (C-2′), 28.9 (C-5′), 27.9 (C-3′), 25.2 ppm (C-4′); IR (KBr): $\nu = 1656$, 1598, 1479, 1356, 1236, 917, 777 cm^{-1}; MS (EI) m/z (%): 378 (1) $[M+4]^+$, 377 (5) $[M+3]^+$, 376 (15) $[M+2]^+$, 375 (6) $[M+H]^+$, 374 (15) $[M]^+$, 296 (6), 295 (21), 277 (13), 239 (32), 226 (22), 225 (100), 212 (96), 196 (26). HRMS-ESI m/z calcd for $C_{19}H_{19}O_3BrNa$: 397.0409782, found: 397.0404690.

6.1.3. 3-(6′-Bromohexyloxy)-4-hydroxy-9H-xanthen-9-one (*5*) and 3,4-bis(6′-bromohexyloxy)-9H-xanthen-9-one (*6*)

To a solution of compound **4** (1.0 g, 4.4 mmol) in dry DMF (15 mL) was added K_2CO_3 (605 mg, 4.4 mmol) and the mixture was stirred at room temperature for 15 min. To this suspension was added, in 30 min a solution of 1,6-dibromobutane (1.1 g, 4.4 mmol) in dry DMF (5 mL). The reaction mixture was stirred for 24 h at room temperature and then poured into ice-water (50 mL). The brown solid thus obtained was filtered and purified by CC [petroleum ether (313–333 K boiling fraction)/diethyl ether, several proportions]. The collected fractions from petroleum ether/diethyl ether (55:45) furnish a yellow solid that was further isolated by prep. TLC petroleum ether/diethyl ether (35:65) to give compound **6** (10%), from the collected fractions eluted with petroleum ether/diethyl ether (4:6) a white solid was obtained which was crystallized from ethyl acetate/petroleum ether (3:1) to give compound **5** (40%).

Compound **5** mp 173 °C; ^1H NMR (300.13 MHz, DMSO-d_6): $\delta = 8.34$ (H-8, dd, $J = 8.0$ and 1.6 Hz, 1H), 7.91 (H-1, d, $J = 8.9$ Hz, 1H), 7.73 (H-6, ddd, $J = 7.0$, 7.0 and 1.5 Hz, 1H), 7.60 (H-5, d, $J = 7.9$ Hz, 1H), 7.38 (H-7, ddd, $J = 7.0$, 7.0 and 1.5 Hz, 1H), 6.99 (H-2, d, $J = 8.9$ Hz, 1H), 5.75 (*HO*–C(4), s, 1H), 4.23 (H-1′, t, 2H), 3.44 (H-6′, t, 2H), 1.93 (H-2′ and H-5′, m, 4H), 1.59 ppm (H-3′ and H-4′, m, 4H); ^{13}C NMR (75.47 MHz, DMSO-d_6): $\delta = 175.1$ (C-9), 166.0 (C-10a), 150.3 (C-3), 144.9 (C-4a), 134.6 (C-6), 133.5 (C-4), 126.7 (C-8), 123.9 (C-7), 121.6 (C-8a), 118.2 (C-5), 118.2 (C-1), 116.6 (C-9a), 108.3 (C-2), 69.5 (C-1′), 33.7 (C-6′), 32.9 (C-2′), 29.7 (C-5′), 28.0 (C-3′), 25.1 ppm

(C-4′); IR (KBr): $\nu = 1645$, 1606, 1455, 1334, 1250, 1184, 1081, 757 cm^{-1}; HRMS-ESI m/z calcd for $C_{19}H_{20}BrO_4$: 391.05395, found: 391.05375.

Compound **6** mp 63 °C; ^1H NMR (300.13 MHz, DMSO-d_6): $\delta = 8.33$ (H-8, dd, $J = 7.9$ and 1.6 Hz, 1H), 8.06 (H-1, d, $J = 9.0$ Hz, 1H), 7.72 (H-6, ddd, $J = 8.5$, 6.9 and 1.7 Hz, 1H), 7.53 (H-5, dd, $J = 8.0$ and 0.6 Hz, 1H), 7.38 (H-7, ddd, $J = 7.6$, 6.7 and 1.0 Hz, 1H), 6.99 (H-2, d, $J = 9.0$ Hz, 1H), 4.15 (H-1′, t, 4H), 3.45 (H-6′, t, 4H), 1.92 (H-2′ and H-5′, m, 8H), 1.60 ppm (H-3′ and H-4′, m, 8H); ^{13}C NMR (75.47 MHz, DMSO-d_6): $\delta = 176.6$ (C-9), 157.3 (C-3), 156.1 (C-10a), 150.8 (C-4a), 134.4 (C-6), 133.5 (C-4), 126.6 (C-8), 123.9 (C-4), 122.1 (C-7), 121.5 (C-8a), 118.0 (C-5), 117.9 (C-1), 116.6 (C-9a), 109.5 (C-2), 73.9 (C-1′), 69.0 (C-1″), 33.8 (C-6′), 33.6 (C-6″), 32.8 (C-2′), 32.6 (C-2″), 29.6 (C-5′), 29.0 (C-5″), 28.0 (C-3′), 27.8 (C-3″), 25.3 (C-4′), 25.2 ppm (C-4″);; IR (KBr): $\nu = 1655$, 1600, 1461, 1436, 1330, 1288, 1084, 745 cm^{-1}; MS (EI) m/z (%): 555 (1) $[M]^+$, 310 (26), 228 (100), 171 (10). HRMS-ESI m/z calcd for $C_{25}H_{31}Br_2O_4$: 553.05836, found: 553.05765.

6.2. X-ray crystallography

6.2.1. 1,1,3-Trihydroxybenzophenone (*1a*)

Crystals suitable for X-ray diffraction were obtained by slow evaporation of an acetone solution. They were monoclinic, space group $P2_1/c$, cell volume $V = 1054.8(3)$ Å^3 and unit cell dimensions $a = 12.386(2)$ Å, $b = 5.1781(7)$ Å, $c = 17.064(3)$ Å, $\beta = 105.47(2)°$ (uncertainties in parenthesis). The calculated density was 1.450 g/L.

Diffraction data were collected at 293 K with a Gemini PX Ultra equipped with Mo Kα radiation ($\lambda = 0.71073$ Å). A total of 7072 reflections were measured, of which 2161 were independent and 1745 were observed ($I > 2\sigma(I)$). The structure was solved by direct methods using SHELXS-97 [27] and refined with SHELXL-97 [28]. Non-hydrogen atoms were refined anisotropically and the refinement converged to R (all data) = 4.72% and wR^2 (all data) = 10.36%.

6.2.2. 1-(6-Bromohexyloxy)-9H-xanthen-9-one (*3*)

Crystals were obtained by slow evaporation of an acetone solution. They were found to be monoclinic, space group $C2/c$, cell volume $V = 3413.9(14)$ Å^3 and unit cell dimensions $a = 27.744(4)$ Å, $b = 11.833(3)$ Å, $c = 15.849(3)$ Å, $\beta = 139.00(1)°$. The calculated density was 1.460 g/L.

Diffraction data were collected at 292 K with a Stoe IPDS image plate equipped with Mo Kα radiation ($\lambda = 0.71073$ Å). A total of 13 028 reflections were measured, of which 3131 were independent and 2308 were observed ($I > 2\sigma(I)$). The structure was solved by direct methods using SHELXS-97 [27] with atomic positions and displacement parameters refined with SHELXL-97 [28]. Non-hydrogen atoms were refined anisotropically and the refinement converged to R (all data) = 6.25% and wR^2 (all data) = 12.59%.

CCDC 728659 (**1a**) and -728660 (**3**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html (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).

6.3. Biological activity

6.3.1. Samples

Stock solutions of compounds **1**–**6** were prepared in DMSO and kept at −20 °C. Appropriate dilutions of the compounds were freshly prepared just prior to the assays. The final concentrations of DMSO did not interfere with the cell growth.

6.3.2. Cell cultures

Four human tumor cell lines, MCF-7 (breast adenocarcinoma, estrogen-dependent ER (+)), MDA-MD-231 (breast adenocarcinoma, estrogen-independent ER (−)), SF-268 (CNS cancer) and NCI-H460 (non-small cell lung cancer) were used. MCF-7 and MDA-MB-231 were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Bethesda, USA). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat-inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7 and SF-268 and 0.75×10^4 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

6.3.3. Tumor cell growth assay

The effects of **1**–**6** 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 [29,30]. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 200 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 and cell line, a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth, was calculated as described elsewhere [29]. Doxorubicin was used as a positive control and tested in the same manner. Unpaired Student's t-tests were used. Differences with P values below 0.05 were considered statistically significant.

Acknowledgements

Financial support from FCT (I&D no. 4040/07), FEDER, POCI, FCT (PTDC/SAU-NEU/69123/2006), is acknowledged. The authors are also indebted to the National Cancer Institute, Bethesda, MD, USA, for the generous provision of the human tumor cell lines and to Sara Cravo for technical support.

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