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Original article

Pyranoxanthones: Synthesis, growth inhibitory activity on human tumor cell lines and determination of their lipophilicity in two membrane models

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ABSTRACT

The benzopyran and dihydrobenzopyran moieties can be considered as “privileged motifs” in drug discovery being good platforms for the search of new bioactive compounds. These moieties are commonly found fused to the xanthonic scaffold belonging to the biologically important family of the generally designated prenylated xanthones. Several pyranoxanthones have shown promising antitumor activity and since most of them are from natural origin, the biosynthetic pathway only allows a particular pattern of substitution which limits their structural diversity and renders any broad structure–activity study hard to be established. Accordingly, with the aim of rationalizing the importance of the fused ring orientation and oxygenation pattern in pyranoxanthones, this study describes the synthesis of 14 new pyranoxanthones and evaluation of their cell growth inhibitory activity in four human tumor cell lines as well as their lipophilicity in two membrane models. This systematic approach allowed establishing structure–activity and structure–lipophilicity relationships for the obtained compounds in combination with 6 previously described compounds. From this work an angular pyranoxanthone scaffold emerged as particularly promising, presenting a potent cell growth inhibitory activity and suitable drug-like lipophilicity.

1. Introduction

2,2-Dimethylbenzopyran (or 2,2-dimethylchromene) and 2,2-dimethyl-3,4-dihydrobenzopyran (or 2,2-dimethylchroman) are motifs frequently found in secondary metabolites isolated from nature [1,2]. In fact, several well-known bioactive molecules have these motifs in their structure [1–4] and some representative examples are (Fig. 1): β-lapachone (ARQ-501) (**1**), a compound isolated from the heartwood of the Lapacho tree which exhibits a potent antitumor activity [5,6]; nabilone (**2**), a synthetic cannabinoid used as an anti-emetic agent [7]; precocenes (**3a** and **3b**) which are used as insecticides [8]; calanolide A (**4**), an HIV-1 reverse transcriptase inhibitor isolated from *Calophyllum*

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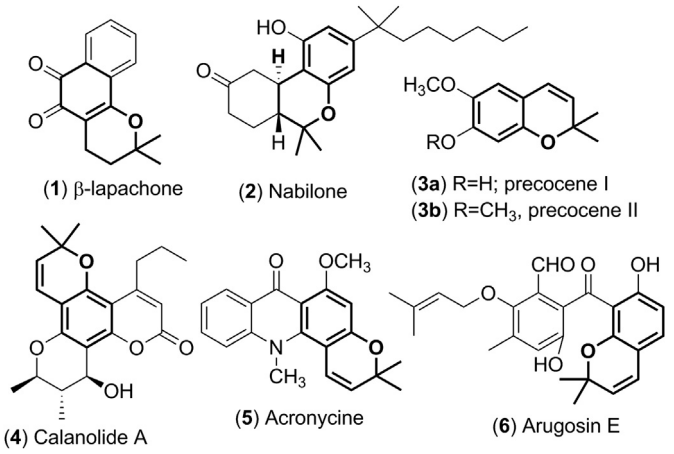


Fig. 1. Representative examples of bioactive compounds bearing 2,2-dimethylbenzopyran or 2,2-dimethyl-3,4-dihydrobenzopyran motifs.

lanigerum [9,10]; acronycine (**5**), a pyranoacridone alkaloid isolated from *Acronychia baueri* with potent antitumor activity [11]; and arugosin E (**6**), a compound isolated from the mycelial extract of *Aspergillus silvaticus* with potent antibacterial activity [12].

These "privileged motifs" [1,2] are frequently found fused to the xanthonic core both in secondary metabolites isolated from higher plants and xanthones obtained by synthesis, with many of them exhibiting promising biological activities [13–15], particularly antitumor [16,17]. In fact, most of the described antitumor pyranoxanthones are from natural origin [13,14,16] and representative compounds [18–26] are depicted in Fig. 2 (only compound **14** is from synthetic origin). Although these pyranoxanthones bear in common the fused pyran or dihydropyran ring, the place of the ring along the xanthone scaffold may vary as well as the position and type of functional groups present. In addition, there has been a big discrepancy in the methodologies and cell lines used when studying the tumor cell growth inhibitory potential of pyranoxanthones, which makes structure–activity relationships hard to establish. Consequently, there is a lack of a systematic study focused on the orientation of the fused pyran and dihydropyran ring as well as the pattern of substitution, which would be able to provide a deeper comprehension of the structural features of pyranoxanthones that are related to their biological activity.

Accordingly, in this work a systematic study of the following structural aspects was envisaged: i) presence or absence of either hydroxyl or methoxyl group on the xanthone core, ii) presence or absence of a double bond in the ring fused to the xanthone core; and iii) relative orientation of the fused ring along the xanthone core (Fig. 3). In order to fulfill these requirements, a chemical library of 21 pyranoxanthones was obtained. The compounds were synthesized either by chemical modification of simple oxygenated xanthones or by total synthesis through benzophenone and diaryl ether routes [27,28]. The cell growth inhibitory activity of the synthesized pyranoxanthones was evaluated *in vitro* in four human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), A375-C5 (melanoma) and HL-60 (acute myeloid leukemia).

In addition, a preliminary assessment of the drug-likeness of the pyranoxanthones synthesized was also made by the evaluation of their lipophilicity. This physicochemical property has a great impact in the pharmacokinetic and pharmacodynamic behavior being determined in an early stage of the drug discovery pipeline [29–32]. In fact, lipophilicity has been correlated with several pharmacokinetic parameters [33] and compounds with high lipophilicity have an increased risk of attrition during clinical trials [32]. Moreover, it is also one of the descriptors of the Lipinski "rule of five" which is commonly used to roughly evaluate the oral bioavailability [34]. In this work, the compounds lipophilicity was determined using two membrane models, namely liposomes and micelles which are able to mimic the anisotropic media found in biomembranes and encode important interactions that take place between the solute and the biomembranes [30,35–41]. This analysis provided an insight into the effects that oxygenation pattern and fused ring orientation had on the lipophilicity, as well as to select the most promising scaffolds, therefore serving as guidance for further chemical modifications.

2. Results and discussion

2.1. Chemistry

Pyranoxanthones are commonly obtained by two general approaches: i) synthesis of simple oxygenated xanthones and then formation of the pyran ring; and ii) synthesis of benzopyran moiety followed by the assembling of the xanthone core [14,42]. Most of

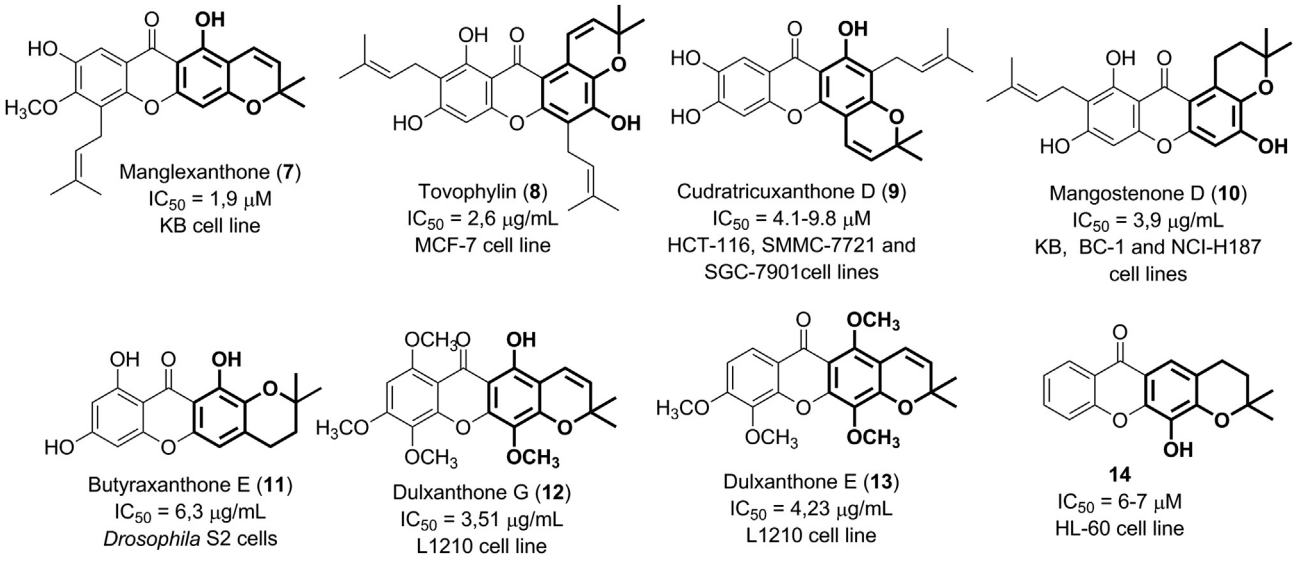


Fig. 2. Representative examples of pyranoxanthones with tumor cell growth inhibitory potential.

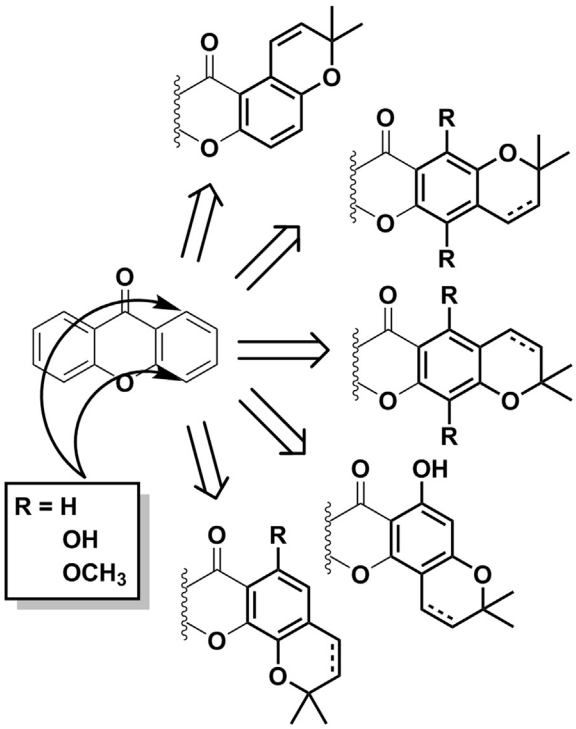


Fig. 3. Targeted compounds by variation of the pattern of oxygenation and the orientation of the fused ring.

the synthetic pyranoxanthones referred in the literature used the first approach, being the later only applied for the total synthesis of highly substituted pyranoxanthones, usually natural products [14]. In order to synthesize the envisaged compounds, both strategies were applied and the synthesis of 21 pyranoxanthones is described in the next two sections.

2.1.1. Synthesis of pyranoxanthones by chemical modification of simple oxygenated xanthones

In Scheme 1 – **I** is represented the synthesis of pyranoxanthones **19**, **20** and **23** using 3,4-dimethoxyxanthone (**15**) as building block. In the case of the pyranoxanthones **19** and **20**, the first step was the mono *O*-demethylation of 3,4-dimethoxyxanthone (**15**) using an odorless thiol reagent [43] to give 3-hydroxy-4-methoxyxanthone (**17**). However, this reaction was slightly regioselective since 4-hydroxy-3-methoxyxanthone (**16**) was also obtained but in lower yield (ratio 17/16 of 2.7). Compound **17** was *O*-dimethylpropargylated using a KI/CuI/K_2CO_3/3-chloro-3-methyl-1-butyne methodology [44,45] to give compound **18** which was then cyclized either by thermal rearrangement, gold (Gagosz's catalyst) [46,47] or platinum ($PtCl_4$) [48,49] to give pyranoxanthone **19**. Pyranoxanthone **20** was obtained from compound **17** by reacting with prenyl bromide under microwave heating and montmorillonite K-10 catalysis, a methodology described in the literature for the synthesis of compound **14** [25,50]. The approach followed for the synthesis of **19** was applied for the synthesis of pyranoxanthone **23** using 3,4-dihydroxyxanthone (**21**) as building block which was obtained from the *O*-demethylation of compound **15** with $AlCl_3$. The *O*-dimethylpropargylation of compound **21** led to the desired aryl dimethylpropargyl ether **22** and also to compound **24** which was formed by the intramolecular nucleophilic attack of the hydroxyl of compound **22** to the alkyne (30% *versus* 47% respectively). The cyclization of compound **22** led to pyranoxanthone **23** and also to compound **24** (33% *versus* 53% respectively).

The pyranoxanthones **26**–**29** were synthesized using 1,3-dihydroxyxanthone (**25**) as building block (Scheme 1 – **II**). The pyranoxanthones **26** and **27** were synthesized using a previously described procedure [50] and pyranoxanthones **28** and **29** were obtained in one step by the condensation of 1,3-dihydroxyxanthone (**25**) with prenal [51].

The pyranoxanthones **32**, **35** and **38** were successfully obtained by the same approach followed for the synthesis of compounds **19** and **23** but using respectively 4-hydroxyxanthone (**30**), 2-hydroxyxanthone (**33**) and 1,2-dihydroxyxanthone (**36**) as building blocks (Scheme 1 – **III**, **IV** and **V**). In the synthesis of the pyranoxanthone **35**, only one isomer was obtained from the cyclization of the propargyl ether catalyzed by platinum and in the *O*-dimethylpropargylation of 1,2-dihydroxyxanthone (**36**), two by-products were obtained besides compound **37** which were formed by the intramolecular nucleophilic attack of the hydroxyl to the alkyne of two propargyl aryl ethers intermediates (**40** and **41**). The pyranoxanthone **39** was synthesized through the hydrogenation of compound **38** by a transfer hydrogenation methodology [52].

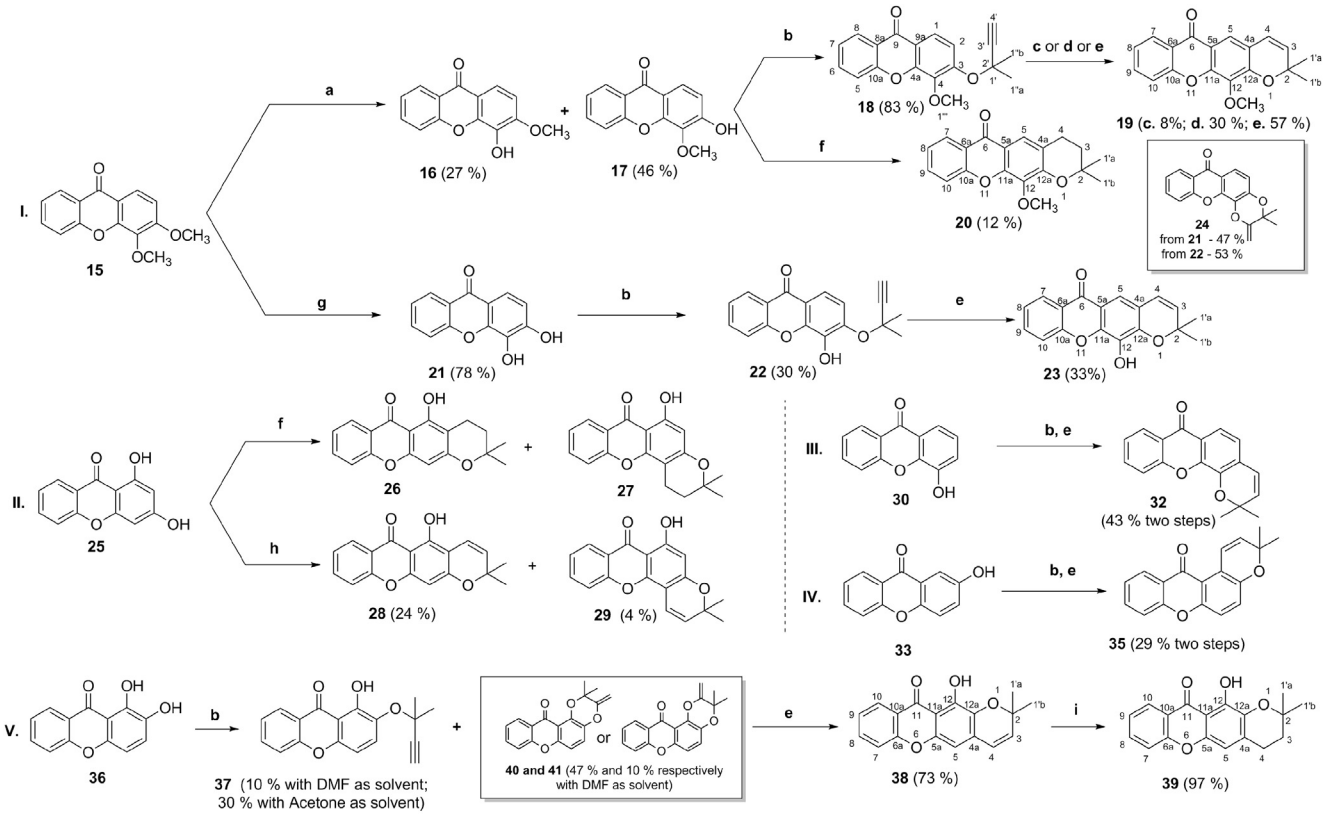
2.1.2. Synthesis of pyranoxanthones by total synthesis

The pyranoxanthone **51** was obtained *via* a diaryl ether intermediate using benzopyran **45** and methyl 2-iodobenzoate (**47**) as building blocks (Scheme 2). The benzopyran **45** was obtained in three steps starting from the reaction of 2,4-dihydroxybenzaldehyde (**42**) with prenal catalyzed by calcium hydroxide [53,54] followed by an *O*-methylation with dimethylsulfate and oxidation by a Baeyer–Villiger type-oxidation [55]. The other building block was synthesized from the esterification of 2-iodobenzoic acid (**46**). The two compounds were coupled by an Ullmann-ether synthesis [56] to give the diaryl ether **48** which was then hydrolyzed with LiOH and transformed into the respective *N*,*N*-diethylamide using TBTU as coupling reagent. Lastly, diaryl ether **50** was cyclized to the pyranoxanthone **51** using a directed remote metalation [57]. The pyranoxanthone **51** was reduced to give pyranoxanthone **52** and *O*-demethylated with BBr_3 to give pyranoxanthone **53**.

The pyranoxanthones **62** and **63** were obtained by total synthesis through the cyclization of the same benzophenone intermediate (**61**), using as building blocks the benzopyran **57** and a MOM-protected methyl salicylate (**59**) (Scheme 3). The synthesis of benzopyran **57** started by the oxidation of 2,4-dimethoxybenzaldehyde (**54**) to give 2,4-dimethoxyphenol (**55**) which was then *O*-dimethylpropargylated through a DBU/$CuCl_2$/MeCN/3-chloro-3-methyl-1-butyne methodology [58] and cyclized thermally to give compound **57**. After the synthesis of the two building blocks, the next step was the synthesis of the benzophenone **60** which was accomplished through the 1,2-nucleophilic addition of a lithiated intermediate of benzopyran **57** formed by an *ortho* lithiation to the ester of the protected methyl salicylate (**59**). The MOM group of benzophenone **60** was removed using $NbCl_5$ [59] and the respective benzophenone **61** cyclized to give pyranoxanthones **62** and **63** by an intramolecular nucleophilic aromatic substitution with cesium carbonate in DMF [60]. However, pyranoxanthone **63** was obtained in a higher yield than **62** (81% *versus* 8%) due to the inductive effect of the oxygen in position 1 which makes carbon 8 more prone to suffer a nucleophilic attack. The pyranoxanthones **62** and **63** were reduced to give pyranoxanthones **64** and **65** respectively. The pyranoxanthones **63** and **65** were also *O*-demethylated to give respectively **66** and **67**.

2.1.3. Structure elucidation

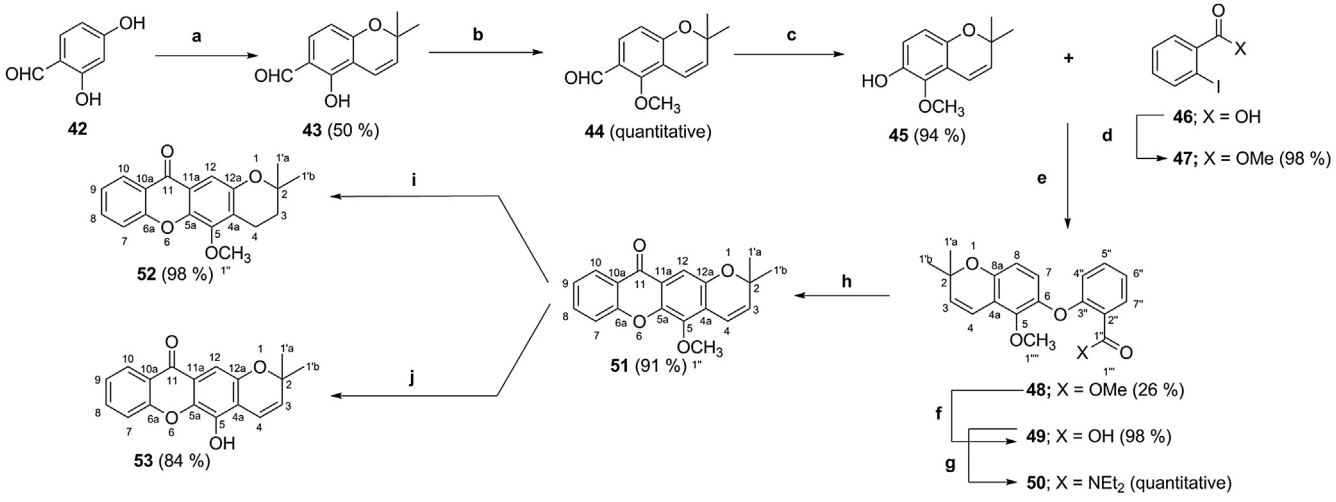
The structure elucidation of the compounds synthesized was established on the basis of IR, HRMS and NMR techniques. The ^1H



Reagents and conditions: **(a)** $(C_2H_5)_2N(CH_2)_2SH.HCl$, NaO*t*Bu, DMF; **(b)** 3-chloro-3-methyl-1-butyne, CuI, KI, K_2CO_3; **(c)** DMF, heating; **(d)** $Ph_3PAuNTf_2$, toluene; **(e)** $PtCl_4$, dioxane; **(f)** prenyl bromide, Montmorillonite K-10, MW, $CHCl_3$; **(g)** $AlCl_3$, toluene; **(h)** prenal, $Ca(OH)_2$, MeOH; **i)** triethylsilane, Pd/C, MeOH

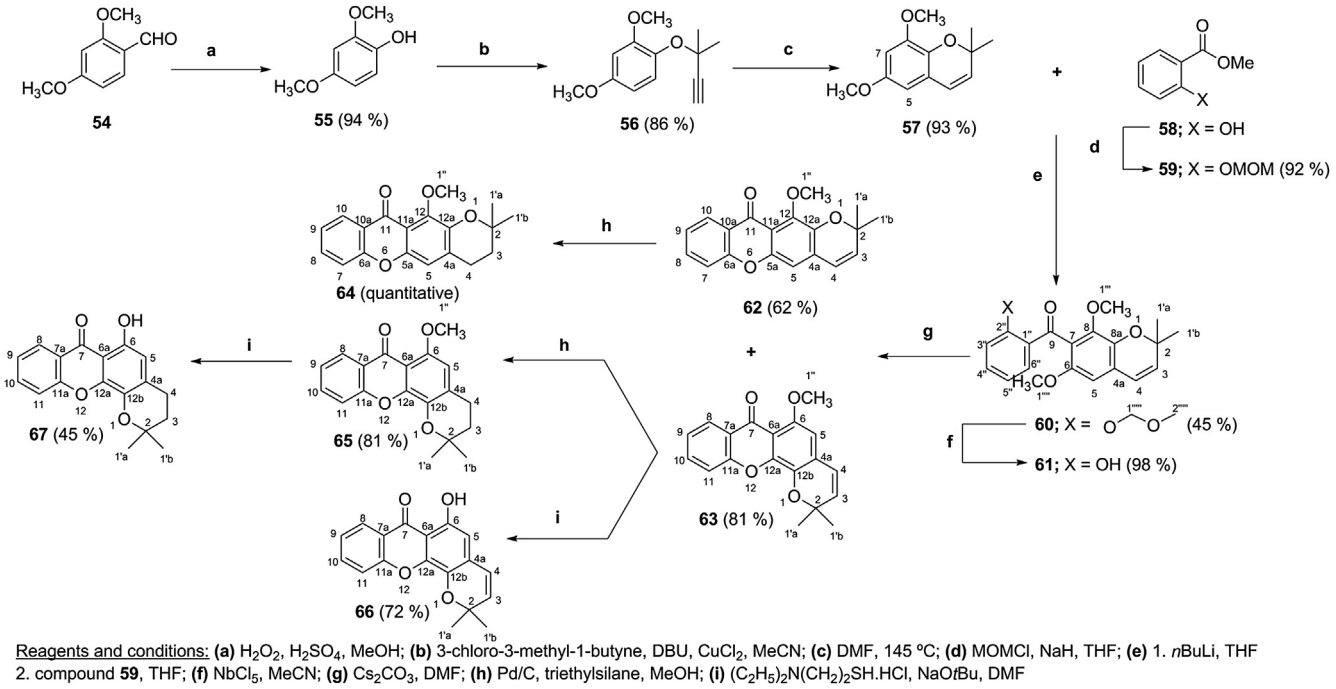
Scheme 1. Synthesis of pyranoxanthones **19**, **20**, **23**, **32**, **35**, **38** and **39**.

and ^{13}C NMR data of 14 pyranoxanthones are reported in Tables 1 and 2. The ^{13}C NMR assignments of pyranoxanthones **19**, **20**, **51**, **52**, **53**, **62**, **63**, **64**, **65**, **66** and **67** were determined by 2D heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments. The ^{13}C NMR chemical shifts assignments of compound **23** were established by comparison with the assignments of compounds **14** [25], **19** and **20**. The ^{13}C NMR chemical shifts assignments of compounds **38** and **39** were established by comparison with the ^{13}C NMR assignments of compounds **62** and **64**. The compound **32** has been previously isolated from *Calophyllum caledonicum* and has been given the name caledinoxanthone B [61]. However, it has never been



Reagents and conditions: **(a)** Prenal, $Ca(OH)_2$, MeOH **(b)** dimethyl sulfate, K_2CO_3, acetone; **(c)** H_2O_2. $KHSO_4$, MeOH; **(d)** MeOH, H_2SO_4; **(e)** CuI, *N*,*N*-dimethylglicine, Cs_2CO_3, dioxane; **(f)** LiOH, THF/MeOH; **(g)** 1. TBTU, triethylamine, THF 2. diethylamine; **(h)** LDA, THF; **(i)** Pd/C, triethylsilane, MeOH; **(j)** BBr_3 CH_2Cl_2

Scheme 2. Synthesis of pyranoxanthones **51**, **52** and **53**.



Scheme 3. Synthesis of pyranoxanthones **62**–**67**.

obtained by synthesis and the spectroscopic data of compound **32** were in accordance with the reported for caledinoxanthone B [61].

The intramolecular cyclization of benzophenone **61** led to xanthones **62** and **63**. The structure of the two isomers was assigned by a Nuclear Overhauser effect spectroscopy (NOESY) experiment where a Nuclear Overhauser effect (NOE) was observed between proton 5 and the methoxyl group (Fig. 4). The assignment of the structure of compound **63** was also confirmed by X-ray crystallography (Fig. 4). The crystal structure of compound **63** reveals the usual structural features [62] with the xanthone skeleton remaining essentially planar and the γ-pyrone in a half chair conformation.

2.2. Biological activity

The effect of 14 synthesized pyranoxanthones (**19**, **32**, **35**, **38**, **39**, **51**, **52**, **53**, **62**, **63**, **64**, **65**, **66** and **67**) was evaluated on the growth of the following four human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), A375-C5 (melanoma) and HL-60 (acute myeloid leukemia). The growth inhibitory activity was determined using the Sulforhodamine B (SRB) assay adopted from the National Cancer Institute (NCI, USA). This assay uses the protein-binding dye SRB to indirectly assess cell growth [63–65]. For each cell line, a dose–response curve was established allowing the determination of the concentration that caused a cell growth inhibition of 50% (GI_{50}) [66] and the results obtained are shown on Table 3. In addition, Table 3 also shows the GI_{50} concentration of compounds **14**, **20**, **26**–**29** which were published elsewhere [67–69].

Regarding the results obtained in the adherent MCF-7, NCI-H460 and A375-C5 cell lines compound **32** was the most potent for MCF-7 cells (GI_{50} = 13.3 ± 1.3 μM) and A375-C5 cells (GI_{50} = 6.2 ± 0.6 μM). Compounds **32** and **38** were the most potent in the NCI-H460 cells, presenting GI_{50} of 32.9 ± 7.1 μM and 31.7 ± 2.6 μM respectively. When analyzing the results obtained in the leukemia cell line (HL-60), compounds **20**, **32**, **51**, **62**, **63** and **66** presented GI_{50} below 12 μM, with compound **32** being the most potent (GI_{50} of 3 μM). Most of the synthesized compounds were shown to be more potent towards the HL-60 tumor cell line than towards the other three cell lines tested.

The data obtained from the evaluation of the cell growth inhibitory activity of the synthesized compounds in the four tumor cell lines allowed to make structure–activity relationships regarding the orientation and type of fused ring (pyran or dihydropyran) and presence or absence of hydroxyl or methoxyl on the xanthone scaffold (Fig. 5). Considering the linear pyranoxanthones A (Fig. 5), the presence of a hydroxyl in position 12 was associated with the moderate activity observed in the four tumor cell lines tested while the methoxyl group associated with growth inhibitory effect only in the HL-60 cells. If the hydroxyl was instead in position 5, none or poor growth inhibitory activity was observed in the MCF-7 and NCI-H460 cell lines. Regarding linear pyranoxanthones B, the presence of a methoxyl group in position 5 was associated with a moderate activity of these compounds in the four tumor cell lines while the presence of a hydroxyl in this position was associated with a loss of activity. Moreover, when comparing compounds **51** and **52**, the presence of a double bond on ring D was associated with a higher potency in the HL-60 cell line. Considering the pyranoxanthone B with a hydroxyl or methoxyl in position 12, the presence of a double bond in the fused ring D was associated with a higher growth inhibitory effect in the four tumor cell lines and the methoxyl with a higher potency observed in the HL-60 tumor cell line. To sum up, in the case of the linear pyranoxanthones, the orientation of the fused ring and the type and position of the hydroxyl or methoxyl may have an important role in the growth inhibitory activity observed in the four human tumor cell lines tested.

Considering the angular pyranoxanthone C, no growth inhibitory activity was observed for MCF-7 and NCI-H460 cell lines. Considering the angular pyranoxanthones D and E without substituents, the former was associated with a higher growth inhibitory effect in the four tumor cell lines tested, which decreased with the introduction of a methoxyl or hydroxyl in position 6 (although less critical to the effect in the HL-60 cells). Moreover, the double

Table 1
^{1}H NMR chemical shifts of compounds **19**, **20**, **23**, **38**, **39**, **51**, **52**, **53**, **62**, **63**, **64**, **65**, **66**, and **67**. (The compounds numbering is referred in Schemes 1–3).

	19[a,b]	**20**[a,b]	**23**[a,c]	**38**[a,e]	**39**[a,e]	**51**[a,b]	**52**[a,b]	**53**[b,d]	**62**[a,b]	**63**[a,b]	**64**[a,b]	**65**[a,b]	**66**[a,b]	**67**[a,b]
H-3	5.75 (*d*, *J* = 10.0)	1.90 (*t*, *J* = 6.8)	5.73 (*d*, *J* = 10.0)	5.94 (*d*, *J* = 9.9)	1.94 (*t*, *J* = 6.8)	5.86 (*d*, *J* = 10.1)	1.86 (*t*, *J* = 6.8)	5.77 (*d*, *J* = 10.0)	5.95 (*d*, *J* = 9.8)	5.89 (*d*, *J* = 9.8)	1.89 (*t*, *J* = 6.8)	1.91 (*t*, *J* = 6.9)	5.86 (*d*, *J* = 9.8)	1.92 (*t*, *J* = 6.8)
H-4	6.46 (*d*, *J* = 10.0)	2.64 (*td*, *J* = 6.8, 0.9)	6.47 (*d*, *J* = 10.0)	6.40 (*d*, *J* = 9.9)	2.90 (*td*, *J* = 6.8; 0.5)	6.71 (*d*, *J* = 10.1)	2.93 (*t*, *J* = 6.8)	6.80 (*d*, *J* = 10.0)	6.41 (*d*, *J* = 9.8)	6.38 (*d*, *J* = 9.8)	2.91 (*t*, *J* = 6.8)	2.88 (*t*, *J* = 6.9)	6.31 (*d*, *J* = 9.8)	2.90 (*t*, *J* = 6.8)
H-5	7.74 (*s*)	7.86 (*t*, *J* = 0.9)	7.59 (*s*)	6.65 (*s*)	6.70 (*broad s*)	–	–	Not observed	6.91 (*s*)	6.47 (*s*)	7.00 (*s*)	6.50 (*s*)	6.42 (*s*)	6.56 (*s*)
H-6	–	–	–	–	–	–	–	–	–	–	–	–	12.14 [OH, s]	12.00 [OH, s]
H-7	8.32 (*dd*, *J* = 8.0, 1.7)	8.32 (*dd*, *J* = 7.9, 1.6)	8.32 (*dd*, *J* = 8.0, 1.7)	7.43 (*dd*, *J* = 8.6, 0.9)	7.44 (broad *d*, *J* = 8.4)	7.46 (*d*, *J* = 8.6)	7.53 (*dd*, *J* = 8.4, 1.0)	7.51 (*dd*, *J* = 8.4, 0.9)	7.38 (*dd*, *J* = 8.5, 1.0)	–	7.37 (*dd*, *J* = 8.5, 0.8)	–	–	–
H-8	7.37 (*ddd*, *J* = 8.0, 7.0, 0.9)	7.36 (*ddd*, *J* = 7.9, 7.0, 1.1)	7.37 (*ddd*, *J* = 8.0, 7.1, 1.1)	7.72 (*ddd*, *J* = 8.6, 7.2, 1.7)	7.71 (*ddd*, *J* = 8.4, 7.1, 1.7)	7.64 (*ddd*, *J* = 8.6, 7.1, 1.7)	7.71 (*ddd*, *J* = 8.4, 7.1, 1.7)	7.63 (*ddd*, *J* = 8.4, 7.1, 1.7)	7.65 (*ddd*, *J* = 8.5, 7.1, 1.6)	8.29 (*dd*, *J* = 8.0, 1.7)	7.64 (*ddd*, *J* = 8.5, 7.0, 1.7)	8.30 (*dd*, *J* = 8.0, 1.7)	8.19 (*dd*, *J* = 8.0, 1.7)	8.30 (*dd*, *J* = 8.0, 1.7)
H-9	7.71 (*ddd*, *J* = 8.5, 7.0, 1.7)	7.70 (*ddd*, *J* = 8.4, 7.0, 1.6)	7.70 (*ddd*, *J* = 8.5, 7.1, 1.7)	7.36 (*ddd*, *J* = 8.0, 7.1, 0.9)	7.35 (*ddd*, *J* = 8.0, 7.0, 1.0)	7.29 (*ddd*, *J* = 8.0, 7.1)	7.36 (*ddd*, *J* = 8.0, 7.1, 1.0)	7.27 (*ddd*, *J* = 8.0, 7.1, 0.9)	7.32 (*ddd*, *J* = 8.0, 7.1, 1.0)	7.32 (*ddd*, *J* = 8.0, 7.0, 1.0)	7.31 (*ddd*, *J* = 8.0, 7.0, 0.8)	7.33 (*ddd*, *J* = 8.0, 6.9, 1.1)	7.31 (*ddd*, *J* = 8.0, 7.0, 1.0)	7.40 (*ddd*, *J* = 8.0, 7.1, 1.1)
H-10	7.57 (*dd*, *J* = 8.5, 0.9)	7.57 (*dd*, *J* = 8.4, 1.1)	7.56 (*dd*, *J* = 8.5, 1.1)	8.28 (*dd*, *J* = 8.0, 1.7)	8.24 (*dd*, *J* = 8.0, 1.7)	8.24 (*dd*, *J* = 8.0, 1.7)	8.32 (*dd*, *J* = 8.0, 1.7)	8.17 (*dd*, *J* = 8.0, 1.7)	8.28 (*dd*, *J* = 8.0, 1.6)	7.66 (*ddd*, *J* = 8.5, 7.0, 1.7)	8.29 (*dd*, *J* = 8.0, 1.6)	7.65 (*ddd*, *J* = 8.4, 6.9, 1.7)	7.67 (*ddd*, *J* = 8.5, 7.0, 1.7)	7.76 (*ddd*, *J* = 8.4, 7.1, 1.7)
H-11	–	–	–	–	–	–	–	–	–	7.53 (*ddd*, *J* = 8.5, 1.0)	–	7.55 (*ddd*, *J* = 8.4, 1.1)	7.52 (*ddd*, *J* = 8.5, 1.0)	7.64 (*ddd*, *J* = 8.4, 1.1)
H-12	–	–	5.67 [broad, OH]	12.66 [OH, *s*]	12.77 [OH, *s*]	7.39 (*s*)	7.49 (*s*)	7.03 (*s*)	–	–	–	–	–	–
H-1′a and **H-1′b**	1.55 (*s*)	1.46 (*s*)	1.55 (*s*)	1.54 (*s*)	1.49 (*s*)	1.40 (*s*)	1.36 (*s*)	1.36 (*s*)	1.52 (*s*)	1.46 (*s*)	1.42 (*s*)	1.46 (*s*)	1.46 (*s*)	1.46 (*s*)
H-1″	4.03 (*s*)	4.00 (*s*)	–	–	–	3.99 (*s*)	4.07 (*s*)	–	4.02 (*s*)	3.97 (*s*)	3.99 (*s*)	3.97 (*s*)	–	–

Abbreviations: singlet (*s*), doublet (*d*), triplet (*t*), quartet (*q*), quintet (*qt*), multiplet (*m*), doublet of doublets (*dd*), double doublet of doublets (*ddd*), doublet of triplets (*dt*) and triplet of doublets (*td*).
[a] Values in ppm (δ_H) measured at 300.13 MHz or 500.13 in $CDCl_3$, *J* values (Hz) are shown in parentheses.
[b] Assignments were confirmed by HSQC and HMBC experiments.
[c] Assignments were established by comparison with the assignments of compounds **14**, **19** and **20**.
[d] Values in ppm (δ_H) measured at 300.13 MHz in DMSO-d_6, *J* values (Hz) are shown in parentheses.
[e] Assignments were established by comparison with the assignments of compounds **62** and **64**.

Table 2
^{13}C NMR chemical shifts of compounds **19**, **20**, **23**, **38**, **39**, **51**, **52**, **53**, **62**, **63**, **64**, **65**, **66**, and **67**. (The compounds numbering is referred in Schemes 1–3).

	19[a,b]	**20**[a,b]	**23**[a,c]	**38**[a,e]	**39**[a,e]	**51**[a,b]	**52**[a,b]	**53**[b,d]	**62**[a,b]	**63**[a,b]	**64**[a,b]	**65**[a,b]	**66**[a,b]	**67**[a,b]
C-2	78.1	76.5	78.7	76.4	74.7	76.2	74.3	75.4	76.5	76.7	74.7	74.9	76.6	74.8
C-3	131.2	32.3	131.0	136.9	32.3	135.6	31.7	132.9	136.5	135.2	32.2	32.5	136.2	32.5
C-4	121.6	22.1	121.5	121.8	23.7	116.4	18.2	116.3	121.5	121.9	23.4	23.5	122.2	23.6
C-4a	119.0	119.3	117.8	129.1	131.3	121.6	123.6	115.3	128.6	126.1	130.6	127.2	129.0	130.9
C-5	118.2	121.2	114.4	103.2	105.4	143.6	146.0	141.1	109.7	102.6	112.3	105.3	106.6	109.0
C-5a	116.1	115.2	116.1	149.9	149.6	144.7	143.4	140.4	151.3	–	149.7	–	–	–
C-6	176.3	176.6	176.4	–	–	–	–	–	–	153.7	–	152.3	154.7	152.8
C-6a	121.6	121.5	121.6	156.3	156.3	155.7	155.8	155.2	155.3	112.7	155.3	112.2	108.9	108.3
C-7	126.6	126.5	126.7	117.7	117.7	118.0	118.0	117.7	117.2	176.4	117.2	176.7	181.9	182.1
C-7a	–	–	–	–	–	–	–	–	–	122.9	–	122.9	120.6	120.6
C-8	124.0	123.6	124.0	135.2	135.2	134.4	134.4	134.0	134.0	126.7	134.0	126.6	125.9	125.8
C-9	134.3	134.2	134.4	123.7	123.5	123.8	123.6	123.2	123.5	123.8	123.3	123.1	124.0	124.0
C-10	118.1	118.0	117.9	125.8	125.9	126.7	126.7	125.8	126.7	133.9	126.7	133.1	135.3	135.1
C-10a	156.0	156.2	155.9	120.1	119.9	121.3	121.2	120.6	128.3	–	122.3	–	–	–
C-11	–	–	–	182.0	182.5	176.4	176.8	175.8	176.1	117.7	176.4	117.8	118.3	118.5
C-11a	135.7	149.1	144.5	109.1	107.7	122.3	121.7	120.8	116.0	155.0	116.0	155.0	156.2	156.5
C-12	134.8	135.7	132.2	148.7	148.4	106.7	107.2	100.7	147.1	–	147.6	–	–	–
C-12a	151.5	153.2	145.5	135.0	137.4	148.9	150.6	148.6	141.9	146.8	130.6	147.5	144.4	146.5
C-12b	–	–	–	–	–	–	–	–	–	134.6	–	136.7	132.3	134.6
C-1′a and **C-1′b**	28.4	27.0	28.5	27.7	26.7	27.7	26.7	27.2	27.7	27.3	26.8	26.4	27.4	26.6
C1″	61.5	61.2	–	–	–	62.3	61.1	–	61.4	56.5	61.1	56.5	–	–

[a] Values in ppm (δ_C) measured at 75.45 MHz or 125.77 in $CDCl_3$.
[b] Assignments were confirmed by HSQC and HMBC experiments.
[c] Assignments were established by comparison with the assignments of compounds **14**, **19** and **20**.
[d] Values in ppm (δ_C) measured at 75.45 MHz in DMSO-d6.
[e] Assignments were established by comparison with the assignments of compounds **62** and **64**.

bond in the fused ring D of analogs D was associated with a higher growth inhibitory effect observed in the four tumor cell lines studied. Pyranoxanthones with this particular fused ring orientation are not commonly found in nature [13] and, as far as we know, no growth inhibitory activity has been previously reported for pyranoxanthones with this geometry.

2.3. Determination of the lipophilicity

The lipophilicity is commonly evaluated by the partition coefficient (K_p) of a solute in a biphasic octanol–water system and designated as Log *P* [30,70]. In spite of being widely used in drug discovery, it fails to mimic the anisotropic media found in membranes and encode all the interactions that take place between a solute and biomembranes [30,35]. As a result, membrane models such as liposomes and micelles have been developed and showed to be able to mimic to a better extent the biomembranes [36–41]. As a result, the lipophilicity of the synthesized compounds was evaluated as the partition coefficient (K_p) of the solute between buffer (HEPES, pH = 7.4) and liposomes or micelles.

The method used for the determination of the K_p considers the variations in the UV–Vis spectra of a solute when it permeates from the aqueous to a non-polar media. This technique evaluate changes in absorption parameters such as molar absorptivity (ε) or maximum wavelength (λ_{max}) and was calculated with the aid of derivative spectroscopy to increase the noise-to-signal ratio and eliminate the light scattering [71–75].

The K_p can be calculated using the following equation [72,74,75]:

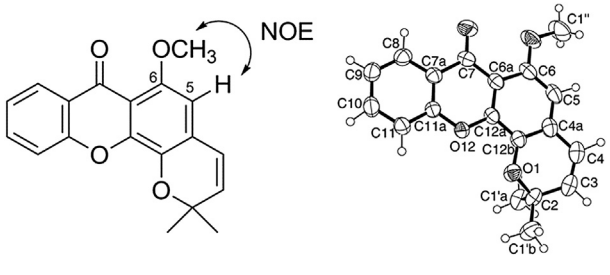


Fig. 4. NOE correlation between proton in position 5 and the protons in the methoxyl group bond to carbon 6 of compound **63**. ORTEP view of the crystal structure of compound **63** showing the atom-labeling scheme. Displacement ellipsoids are drawn at the 50% probability level. Hydrogen atoms are represented by circles of arbitrary size.

Table 3
GI_{50} concentrations (μM) of the synthesized compounds in MCF-7, NCI-H460, A375-C5 and HL-60 cell lines.

Compound	GI_{50} (μM)			
	MCF-7	NCI-H460	A375-C5	HL-60
19	>150[a]	>150[a]	>150[a]	N.R.
32	13.3 ± 1.3	32.9 ± 7.1	6.2 ± 0.6	3.2 ± 0.7
35	50.9 ± 3.5	44.5 ± 1.4	37.9 ± 6.5	36.7 ± 3.3
38	39.6 ± 0.6	31.7 ± 2.6	29.6 ± 4.7	38.9 ± 9.8
39	>150[a]	>150[a]	>150[a]	26.1 ± 9.5
51	N.R.	N.R.	N.R.	11.8 ± 3.8
52	45.1 ± 3.3	47.3 ± 6.0	42.5 ± 5.5	31.8 ± 6.1
53	107.9 ± 13.9	>150[a]	>150[a]	>70[a]
62	N.R.	42.9 ± 16.1	47.4 ± 4.1	9.6 ± 3.2
63	N.R.	N.R.	69.5 ± 5.9	9.6 ± 1.7
64	N.R.	>150[a]	>150[a]	37.6 ± 17.1
65	>100[a]	N.R.	>100[a]	30.9 ± 10.8
66	N.R.	N.R.	>100[a]	6.4 ± 0.3
67	>50[a]	>50[a]	>50[a]	>50[a]
14[b]	39.7 ± 3.2	40.3 ± 3.3	28.9 ± 8.1	23.4 ± 1.1
20[b]	>150	>150	>150	8.8 ± 5.9
26[b]	88.6 ± 12.9	>160	N.D.	N.D.
27[b]	>160	>160	N.D.	N.D.
28[b]	>150	>150	N.D.	N.D.
29[b]	>150	>150	N.D.	N.D.

The values presented refer to mean ± SE of at least three independent experiments. N.R. – not-reproducible. N.D. – not determined. The maximum DMSO concentration used was 0.25% for all compounds tested and was found to not interfere with cell growth (data not shown). Doxorubicin was used as a positive control (MCF-7: 65.4 ± 8.5 nM; NCI-H460: 64.1 ± 6.8 nM, A375-C5: 144.8 ± 9.8 nM and HL60: 28.0 ± 0.6 nM).
[a] These values correspond to two experiments.
[b] Previously published results in Refs. [67–69].

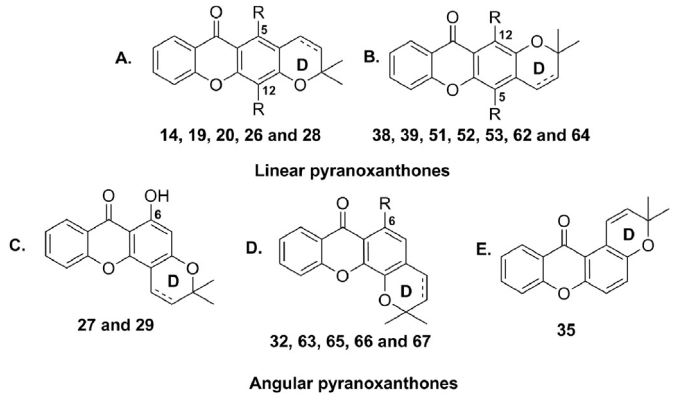


Fig. 5. Synthesized pyranoxanthones with different orientation of the fused ring D.

$$D = D_w + \frac{(D_l - D_w)K_p[L]V\phi}{1 + K_p[L]V\phi} \quad (1)$$

where, $[L]$ is the lipid concentration (mol L^{-1}), D_l the derivative of lipid absorbance, D_w the derivative of water, $V\phi$ the lipid molar volume (L mol^{-1}), K_p the partition coefficient (dimensionless) and:

$$D = \frac{\partial^n Abs}{\partial \lambda^n} \quad (2)$$

Accordingly, the partition coefficient could be calculated by fitting the derivative spectrometric data (D vs $[L]$) to the Equation (1) by a nonlinear regression method, being D_l and K_p the adjustable parameters [72,74,75].

The K_p in liposomes/buffer was determined for **19**, **32**, **35**, **38**, **39**, **51**–**56** and **62**–**65** and in micelles/buffer for all the compounds with the exception of compound **23** which was obtained in low yields (Table 4). The K_p was determined using an adapted procedure developed by Magalhães et al. using a 96-well plate [38] in which DMSO was added to each plate to a maximum of 1% [76]. In the case of the compounds having very low solubility and/or molar absorptivity (ε), the Log K_p was determined in a conventional double-beam UV–Vis spectrophotometer. Moreover, for compounds **26**–**28**, **66** and **67** with both low solubility and molar absorptivity, the light scattering for wavelengths below 300 nm in liposomes did not allow to calculate the Log K_p in this model.

Table 4
Partition coefficients in liposomes-buffer and micelle-buffer for a chemical library of pyranoxanthones.

	Log $K_{p\ liposomes}$	Log $K_{p\ micelles}$
19	3.88 ± 0.09	3.83 ± 0.05
26	–	4.32 ± 0.04
27	–	4.27 ± 0.06
28	–	4.06 ± 0.01
29	–	4.02 ± 0.03
32	3.42 ± 0.02	3.50 ± 0.12
35	4.14 ± 0.08	4.10 ± 0.04
38	4.17 ± 0.06	4.15 ± 0.08
39	3.96 ± 0.03	3.88 ± 0.08
51	3.94 ± 0.09	4.07 ± 0.10
52	3.92 ± 0.04	3.88 ± 0.02
53	3.06 ± 0.16	3.29 ± 0.06
62	3.54 ± 0.01	3.76 ± 0.03
63	3.32 ± 0.12	3.33 ± 0.02
64	3.09 ± 0.18	3.58 ± 0.08
65	3.08 ± 0.06	3.46 ± 0.01
66	–	4.08 ± 0.04
67	–	4.28 ± 0.03
14[a]	3.35 ± 0.02	3.28 ± 0.02
20[a]	3.60 ± 0.08	3.59 ± 0.06

Mean of three independent measurements.
[a] These results have been published elsewhere [69].

The partition coefficients determined in one or two membrane models of the pyranoxanthones are summarized in Table 4. It can be observed that all the compounds showed a Log K_p in liposomes and micelles superior to 3 but below the critical 5 (upper limit referred in the Lipinski "rule of five" [34]). Moreover, it can be observed that the presence of a hydroxyl *ortho* to the carbonyl leads to a dramatic increase in the lipophilicity (compare **14** with **26**, **27**, **38**, **65**; and **53** with **28**, **29**, **39**, **66**). This can be explained by the intramolecular hydrogen bonding that is formed between the carbonyl and the hydroxyl group. Considering the relation between the hydroxyl or methoxyl group with the oxygen of the fused pyran ring, the compounds with an *ortho* relationship seem in most of the cases to have a lower partition coefficient than the compounds with a *meta* relationship (compare **19**, **22** with **51**, **52**; and **39** with **26**). Comparing the linear with the angular arrangement of the pyranoxanthones, it can be observed that in general, the latter have a less partition coefficient than the former (compare **26** with **27**, **38** with **66**; and **62**, **64** with **63** and **65**). Regarding the presence or absence of the double bond in the fused ring, there is not a clear tendency among the different set of compounds.

In order to compare the liposome and micelle models for this class of compounds, a linear regression analysis was made and the following Equation (3) was established:

$$\text{Log } K_{p\ liposomes} = -0.760(\pm 0.604) + 1.182(\pm 0.164)\ \text{Log } K_{p\ micelles} \quad (3)$$

$$n = 14;\ r^2 = 0.814;\ s = 0.178;\ F = 52$$

wherein 95% confidence limits are in parentheses, n is the number of compounds, r^2 the squared correlation coefficient, s the standard deviation, and F Fisher's test. Considering the slope (1.182) and y-intercept (−0.760), we can observe that there is not clear tendency for this class of compounds to increase the affinity for either of the membrane models with an increase of hydrophobicity and that the sum of interactions taking place between these compounds and micelles are in general slightly higher than with liposomes. Moreover, a better correlation was obtained by removing two outliers, namely compounds **64** and **65**, which bare in common the presence of a methoxyl *ortho* to the carbonyl and a fused 2,2-dimethyl-3,4-dihydropyran ring.

$$\text{Log } K_{p\ liposomes} = -0.308(\pm 0.396) + 1.075(\pm 0.106)$$
$$\text{Log } K_{p\ micelles}\ n = 12;\ r^2 = 0.902;\ s = 0.118; F = 102 \quad (4)$$

3. Conclusions

In the present study, 21 pyranoxanthones were synthesized either by molecular modifications of simple oxygenated xanthones or by total synthesis. Regarding molecular modifications of simple oxygenated xanthones, the pyran rings were formed either by the cyclization with platinum of the dimethylpropargyl aryl ethers, or by the condensation with prenal or by the reaction with prenyl bromide catalyzed by Montmorillonite K-10 and microwave heating. The total synthesis of pyranoxanthones was accomplished *via* diaryl ether and benzophenone using the appropriate benzopyrans and carboxylic acid derivatives as building blocks.

The cell growth inhibitory activity of 14 of the synthesized pyranoxanthones was evaluated in four human tumor cell lines, namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell

lung cancer), A375-C5 (melanoma) and HL-60 (acute myeloid leukemia). Among the cell lines tested, higher cell growth inhibitory activity was generally observed in the HL-60 tumor cell line which may indicate that a cellular target may be present or be more relevant in this cell line than in the others. It was observed that the orientation of the fused ring and the oxygenation pattern had an effect in the cell growth inhibitory activity observed in the different cell lines.

The lipophilicity of the pyranoxanthones was evaluated for 20 compounds in two membrane models, namely liposomes and micelles, and all the compounds showed a Log K_p higher than 3 and below 5. Moreover, the micelle model showed a good correlation with liposome model and as a result might be used as a surrogate of liposomes for the determination of the partition coefficient of pyranoxanthones.

The present study allowed to describe, for the first time, three angular pyranoxanthones with a fused ring orientation which have never been related to tumor cell growth inhibitory activity being particularly active in the leukemia (HL-60) tumor cell line. Besides the promising biological activity, the compounds with this particular fused ring orientation showed to have lower lipophilicity than similar structural isomers. These facts make them interesting scaffolds for further studies.

4. Experimental

4.1. General methods

Microwave reactions were performed using glassware setup for atmospheric-pressure reactions and also 12 mL, 50 mL, 100 mL or 270 mL closed glass reactors (internal reaction temperature measurement with a fiber-optic probe sensor) and were carried out using an Ethos MicroSYNTH 1600 Microwave Labstation from Milestone. Reactions were monitored by TLC and/or GC–MS. 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}). ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ or DMSO-d_6 at room temperature, on Bruker Avance 300, 400 and 500 instruments. Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as an internal reference. ^1H NMR spectra were measured at 300.13 MHz or 500.13 MHz and assignment abbreviations are the following: singlet (*s*), doublet (*d*), triplet (*t*), quartet (*q*), quintet (*qt*), multiplet (*m*), doublet of doublets (*dd*), double doublet of doublets (*ddd*), doublet of triplets (*dt*) and triplet of doublets (*td*). ^{13}C NMR spectra were measured at 75.47 MHz, 100.63 MHz or 125.77 MHz. ^{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) or by comparison with the assignments of similar molecules. The EI-MS were recorded on a ThermoQuest Finnigan GC 2000 series/GCQ plus. HRMS spectra were recorded as ESI (electrospray ionization) mode either on an APEXQe FT-ICRMS (Bruker Daltonics), equipped with a 7T actively shielded magnet or VG Autoespec MicroTOF FOCUS (Bruker Daltonics) spectrometer at C.A.C.T.I. – University of Vigo, Spain; or Bruker micrOTOF-Q II (ESI) at the University of Southern Denmark. The X-Ray was determined in a Gemine PX ultra equipped with a CuK_α radiation (λ = 1.54184 Å) and the structure was solved using SHELXS-97 and refined with SHELXL-97. All the reagents were purchased from Sigma Aldrich or Acros and all the solvents were PA used without further purification. The anhydrous solvents were either purchased from Sigma–Aldrich or dried according to the published procedures [77]. Purifications of compounds were performed by column chromatography either by using Merck silica gel 60 (0.040–0.063 mm) (when it is referred to as flash chromatography) or using Merck silica gel 60 (0.2–0.5 mm) (when nothing is referred) and preparative thin layer chromatography (TLC) using Merck silica gel 60 (GF_{254}) plates or GraceResolv® silica gel cartridges (5 g/25 mL).

4.2. Synthesis of pyranoxanthones **14**, **19**, **20** and **23**

4.2.1. Synthesis of compound **15**

3,4-Dimethoxyxanthone (**15**) was synthesized according to the published procedure [78] and the spectroscopic data were in accordance with the literature [79].

4.2.2. Synthesis of compounds **16** and **17**

2-(Diethylamino)ethanethiol HCl (397 mg/2.34 mmol) and 8 mL of DMF anhydrous was placed in a two-necked round-bottom flask and under nitrogen atmosphere. The flask was cooled in an ice bath and when the internal temperature was below 5 °C, solid NaO^{t}Bu (450 mg/4.68 mmol) was added in one portion. After 5 min the ice bath was removed and it was allowed to warm to room temperature. After 15 min, a solution of 3,4-dimethoxyxanthone (**15**) (500 mg/1.95 mmol) in 4 mL of DMF anhydrous was added. The mixture was heated at reflux for 2 h under nitrogen atmosphere. The mixture was allowed to cool to room temperature and then poured over crushed ice. A solution of HCl 1 M was added to the mixture until pH 1. The mixture was extracted with 3 × 50 mL of ethyl acetate. The organic phase was washed with 3 × 50 mL of water, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (petroleum ether (60–80°)/ethyl acetate 7:3). 3-Hydroxy-4-methoxyxanthone (**17**) was crystallized from chloroform/petroleum ether (60–80°) as white solid (221.4 mg/46%) and 4-hydroxy-3-methoxyxanthone (**16**) from methanol as a white solid (83 mg/17%).

The spectroscopic data of 4-hydroxy-3-methoxyxanthone (**16**) and 3-hydroxy-4-methoxyxanthone (**17**) was in accordance with the literature [79].

4.2.3. Synthesis of compound **18**

3-Hydroxy-4-methoxyxanthone (**17**) (120 mg/0.5 mmol), KI (168.5 mg/1 mmol), CuI (11.8 mg/0.062 mmol), K_2CO_3 (135.8 mg/1 mmol) and 5 mL of anhydrous DMF were added to a round-bottom flask. 3-Chloro-3-methyl-1-butyne (430 μL/13.2 mmol) was then added and the mixture was heated under nitrogen atmosphere at 75 °C for 8 h. The solution was allowed to cool to room temperature and poured over crushed ice and 5 mL of HCl 1 M. The aqueous phase was extracted with 3 × 50 mL of ethyl acetate. The organic layer was washed with 3 × 50 mL of distilled water, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was then purified by silica gel flash chromatography (chloroform). Compound **18** was isolated as a white solid (126 mg/83%).

4-Methoxy-3-((2-methylbut-3-yn-2-yl)oxy)xanthone (***18***). Mp: 121–123 °C. IR ν_{max} (cm^{-1}) (KBr): 3432, 3249, 2984, 2934, 2836, 1655, 1602, 1498, 1464, 1448, 1424, 1332, 1278, 1140, 1068, 1034, 743. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.33 (*dd*, J = 8.0, 1.7, H–C(8)), 8.04 (*d*, J = 9.1, H–C(1)), 7.73 (*ddd*, J = 8.5, 7.0, 1.7, H–C(6)), 7.62 (*d*, J = 9.1, H–C(2)), 7.58 (*d*, J = 7.8, H–C(5)), 7.39 (*ddd*, J = 8.0, 7.1, 1.0, H–C(7)), 4.03 (*s*, CH_3O–C(4)), 2.67 (*s*, H–C(3′)), 1.78 (*s*, 6H, H–C(1″a/1″b)). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ (ppm): 176.7 (C9), 156.2 (C10a), 154.2 (C3), 150.8 (C4a), 139.9 (C4), 134.5 (C6), 126.6 (C8), 124.0 (C7), 121.6 (C8a), 121.0 (C1), 118.1 (C5), 118.0 (C9a), 116.8 (C2), 85.2 (C2′), 74.9 (C3′), 74.2 (C1′), 61.5 (C1‴), 29.7 (C1″a/1″b). EIMS m/z (%): 309 (4, $[M+1]^{+\cdot}$), 308 (15, $[M]^{+\cdot}$), 294 (30), 293 (100), 278 (65), 194 (32), 165 (40), 89 (14), 76 (18), 63 (19). HRMS (ESI) m/z calcd for $C_{19}H_{16}NaO_4$ $[M+Na]^+$: 331.0941; found: 317.0925.

*4.2.4. Synthesis of compound **19***

4.2.4.1. Thermal cyclization. A solution of 81 mg of compound **18** in 10 mL of anhydrous DMF was heated at 130 °C under nitrogen atmosphere for 7 h. The solution was allowed to cool to room temperature and the mixture was poured into 50 g of crushed ice and 1 mL of concentrated HCl. The aqueous phase was extracted with 3 × 50 mL of ethyl acetate. The organic phase was washed 3 × 50 mL of distilled water, dried over Na_2SO_4, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (petroleum ether (60–80°)/ethyl ether 7:3). The product was then further purified by preparative TLC to give compound **19** as a white solid (6.3 mg/8%).

4.2.4.2. Hydroarylation of alkynes by gold catalyst. Compound **18** (166.1 mg, 0.539 mmol) and $(Ph_3P)AuNTf_2$ (4.2 mg, 2.695 μmol) (Gagoz's catalyst [47]) were placed in a two-necked round-bottom flask. The two solids were placed under nitrogen atmosphere and 5 mL of anhydrous toluene was added. The mixture was stirred for 7 h at 85 °C. The reaction mixture was allowed to cool to room temperature, filtered, and washed with acetone. The mixture was then concentrated under reduced pressure and the crude product purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1 to 5:5). Compound **19** was obtained as a white crystalline solid (50.1 mg, 30%). Compound **17** was also isolated (83 mg/50%).

4.2.4.3. Hydroarylation of alkynes by $PtCl_4$. Compound **18** (110 mg, 0.35 mmol), $PtCl_4$ (17.7 mg, 52.5 μmol, 15 mol %) and 15 mL dioxane were placed in a round-bottom flask. The mixture was stirred at r.t. for 11 h and then filtered. The solid was washed with diethyl ether and dichloromethane. The organic phases were reunited and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (*n*-hexane/diethyl ether 8:2). Compound **19** was obtained as a white crystalline solid (62.3 mg, 57%).

*12-Methoxy-2,2-dimethylpyrano[3,2-b]xanthen-6(2H)-one (**19**).* Mp: 129–130 °C. IR ν_{max} (cm^{-1}) (KBr): 3254, 2973, 2932, 1656, 1607, 1482, 1438, 1326, 1274, 1128, 1074, 748. ^1H NMR data, see Table 1. ^{13}C NMR data, see Table 2. HRMS (ESI) *m*/*z* calcd for $C_{19}H_{17}O_4$ $[M + H]^+$: 309.11214; found: 309.11204.

*4.2.5. Synthesis of compound **20***

To a Teflon vessel for microwave heating with 3-hydroxy-4-methoxyxanthone (**17**) (110 mg/0.46 mmol), 2 g of clay montmorillonite K-10 and approximately 10 mL of chloroform (just enough to wet the clay), was added prenyl bromide (0.92 mmol). The mixture was heated for 1 h and 15 min at 110 °C under microwave heating. The mixture was allowed to cool to room temperature and the product was filtered and washed with chloroform, acetone and methanol. The organic solvents were reunited and evaporated. The crude product was then purified by silica gel flash chromatography (petroleum ether (60–80°)/ethyl acetate 95:5 to 5:5). Compound **20** was recrystallized from chloroform and petroleum ether (60–80°) as a white solid (17 mg/12%).

*12-Methoxy-2,2-dimethyl-3,4-dihydropyrano[3,2-b]xanthen-6(2H)-one (**20**; 12%).* Mp: 173–174 °C. IR ν_{max} (cm^{-1}) (KBr): 2967, 2936, 2829, 1655, 1609, 1443, 1326, 1114, 1077, 748. ^1H NMR data, see Table 1. ^{13}C NMR data, see Table 2. HRMS (ESI) *m*/*z* calcd for $C_{19}H_{19}O_4$ $[M + H]^+$: 311.12779; found: 311,12772.

*4.2.6. Synthesis of compound **21***

3,4-Dimethoxyxanthone (**15**) (769 mg/3 mmol) and 50 mL of anhydrous toluene were added in a round-bottom flask. The mixture was placed at 0 °C and aluminum chloride (1.2 g/9 mmol) was added slowly. The mixture was heated for 2 h under reflux. The mixture was allowed to cool to room temperature and poured over 150 g of crushed ice and 1 mL of concentrated HCl. The aqueous phase was extracted with 3 × 100 mL of ethyl acetate. The organic phase was washed 3 × 150 mL of distilled water, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel chromatography (chloroform/acetone 9:1). 3,4-Dihydroxyxanthone (**21**) was crystallized from methanol as a pale yellow solid (534 mg/78%).

The spectroscopic data of 3,4-dihydroxyxanthone (**21**) was in accordance with the literature [79].

*4.2.7. Synthesis of compound **14***

12-Hydroxy-2,2-dimethyl-3,4-dihydropyrano[3,2-*b*]xanthen-6(2*H*)-one (**14**) was synthesized according to the published procedure (yield obtained of 21%) and the spectroscopic data was in accordance with the literature [25].

*4.2.8. Synthesis of compound **22***

3,4-Dihydroxyxanthone (**21**) (100 mg/0.44 mmol), potassium carbonate (91 mg/0.66 mmol), potassium iodide (109 mg/0.66 mmol) and copper iodide (13 mg/0.066 mmol) were placed in a round-bottom flask. The flask was placed under nitrogen atmosphere and 20 mL of anhydrous acetone was added. The mixture was stirred for 15 min and 3-chloro-3-methyl-1-butyne (45 mg/0.438 mmol) was added dropwise. The mixture was heated at 40 °C for 2 h and then allowed to cool to room temperature. The crude product was filtered and the organic solvent evaporated. The extract was then purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **22** was isolated as a white solid (39 mg/30%) and compound **24** was isolated as a white solid (60 mg/47%).

*4-Hydroxy-3-((2-methylbut-3-yn-2-yl)oxy)xanthone (**22**).* Mp: 168–169 °C. IR ν_{max} (cm^{-1}) (KBr): 3254, 3152, 3114, 3071, 2992, 2934, 2361, 1639, 1604, 1577, 1455, 1344, 1277, 1247, 1217, 1135, 1065, 1004, 760, 707, 677. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.34 (*dd*, *J* = 8.0, 1.7), 7.86 (*d*, *J* = 8.9), 7.73 (*ddd*, *J* = 8.5, 7.0, 1.7), 7.60 (*dd*, *J* = 8.5, 1.1), 7.55 (*d*, *J* = 8.9), 7.39 (*ddd*, *J* = 8.0, 7.0, 1.1), 5.89 (OH), 2.71 (*s*, 1H), 1.79 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 176.7, 156.2, 147.0, 145.3, 136.6, 134.6, 130.9, 126.7, 124.0, 121.3, 118.1, 116.8, 115.7, 84.7, 75.2, 74.9, 29.7. EIMS *m*/*z* (%): 295 (2, $[M + 1]^{+\cdot}$), 294 (8, $[M]^{+\cdot}$), 279 (56), 205 (16), 165 (16), 146 (21), 121 (36), 115 (39), 102 (23), 92 (26), 91 (26), 77 (100), 76 (30), 75 (40), 74 (30), 65 (39), 63 (33), 51 (84). HRMS (ESI) *m*/*z* calcd for $C_{18}H_{14}NaO_4$ $[M + Na]^+$: 317.0784; found: 317.0780.

*3,3-Dimethyl-2-methylene-2H-[1,4]dioxino[2,3-c]xanthen-7(3H)-one (**24**).* Mp: 173–175 °C. IR ν_{max} (cm^{-1}) (KBr): 3008, 2972, 2920, 1651, 1605, 1501, 1449, 1332, 1303, 1220, 1203, 1165, 1060, 1032, 899, 861, 748, 677. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.32 (*dd*, *J* = 8.0, 1.7), 7.89 (*d*, *J* = 8.9), 7.70 (*ddd*, *J* = 8.5, 7.1, 1.7), 7.59 (*dd*, *J* = 8.5, 1.0), 7.37 (*ddd*, *J* = 8.0, 7.1, 1.0), 6.89 (*d*, *J* = 8.9), 4.97 (*d*, *J* = 2.5, 1H), 4.65 (*d*, *J* = 2.5, 1H), 1.58 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 176.2, 156.0, 155.1, 146.7, 145.3, 134.4, 130.0, 126.7, 124.1, 121.8, 119.6, 118.1, 116.8, 114.0, 91.3, 74.0, 25.1. EIMS *m*/*z* (%): 295 (1, $[M + 1]^{+\cdot}$), 294 (12, $[M]^{+\cdot}$), 251 (25), 228 (69), 200 (19), 172 (21), 171 (31), 115 (53), 114 (44), 88 (23), 77 (26), 76 (30), 75 (21), 67 (74), 65 (100), 63 (39), 62 (24). 53 (35), 51 (53). HRMS (ESI) *m*/*z* calcd for $C_{18}H_{15}O_4$ $[M + H]^+$: 295.09649; found: 295.09787; *m*/*z* calcd for $C_{18}H_{14}NaO_4$ $[M + Na]^+$: 317.07843, found: 317.07867.

*4.2.9. Synthesis of compound **23***

To a solution of compound **22** (30 mg/0.102 mmol) in 2.5 mL of dioxane was added $PtCl_4$ (0.015 mmol/5 mg). The mixture was stirred for 6 h at room temperature. The mixture was filtered and washed with the ethyl ether. The organic solvents were reunited and evaporated. The crude mixture was then purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1 to 7:3).

Compound **23** was isolated as a light yellow solid (10 mg/33%). Compound **24** (16 mg/53%) was also isolated.

12-Hydroxy-2,2-dimethylpyrano[3,2-b]xanthen-6(2H)-one (***23***). Mp: 178–179 °C. IR ν_{max} (cm^{-1}) (KBr): 3247, 2955, 2915, 1843, 1638, 1600, 1449, 1346, 1272, 1208, 1135, 753. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. EIMS *m/z* (%): 294 (3, $[M]^{+\cdot}$), 279 (69), 205 (34), 165 (36), 152 (23), 139 (22), 121 (19), 115 (30), 102 (19), 91 (29), 89 (31), 88 (27), 87 (20), 77 (84), 75 (60), 63 (89), 53 (22), 51 (100). HRMS (ESI) *m/z* calcd for $C_{18}H_{15}O_4$ $[M + H]^+$: 295.09649, found: 295.09584.

4.3. Synthesis of the pyranoxanthones *26*, *27*, *28* and *29*

4.3.1. Synthesis of compound *25*

1,3-Dihydroxyxanthone (**25**) was synthesized according to the published procedure [80] and the spectroscopic data was in accordance with the literature [80,81].

4.3.2. Synthesis of compounds *26* and *27*

5-Hydroxy-2,2-dimethyl-3,4-dihydropyrano[3,2-*b*]xanthen-6(2*H*)-one (**26**) and 6-hydroxy-3,3-dimethyl-2,3-dihydropyrano [2,3-*c*]xanthen-7(1*H*)-one (**27**) were synthesized according to the published procedure [50] and the spectroscopic data are in accordance with the literature [67].

4.3.3. Synthesis of compounds *28* and *29*

To a solution of compound **25** (200 mg/0.88 mmol) and $Ca(OH)_2$ (130.4 mg/1.76 mmol) in 50 mL of methanol was added prenal (370 mg/4.4 mmol). The mixture was stirred for 66 h at room temperature. Methanol was evaporated and the crude product was partitioned between water and ethyl acetate. The aqueous phase was extracted 2 × 50 mL of ethyl acetate. The organic phase was washed 3 × 50 mL HCl 1 M, 3 × 50 mL of distilled water, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel chromatography using Grace ResolvTM 5 g/25 mL cartridge (petroleum ether (60–80°)/ethyl acetate 9:1 to 5:5). Compound **28** (61.2 mg/24%) and **29** (10 mg/4%) were purified as yellow needles.

The spectroscopic data of 5-hydroxy-2,2-dimethylpyrano[3,2-*b*] xanthen-6(2*H*)-one (**28**) and 6-hydroxy-3,3-dimethylpyrano[2,3-*c*] xanthen-7(3*H*)-one (**29**)synthesized was in accordance with the literature [68].

4.4. Synthesis of pyranoxanthone *32*

4.4.1. Synthesis of compound *30*

4-Hydroxyxanthone (**30**) was synthesized according to the published procedure [82] and the spectroscopic data was in accordance with the literature [79].

4.4.2. Synthesis of compound *31*

4-Hydroxyxanthone (**30**) (150 mg/0.707 mmol), potassium carbonate (147 mg/1.06 mmol), potassium iodide (176 mg/1.06 mmol) and copper iodide (20 mg/0.106 mmol) were placed in a round-bottom flask. The flask was placed under nitrogen atmosphere and 15 mL of anhydrous DMF was added. The mixture stirred for 15 min and 3-chloro-3-methyl-1-butyne (145 mg/1.415 mmol) was added dropwise. The mixture was heated to 75 °C and stirred for 14 h. The solution was allowed to cool to room temperature and poured over 100 g of crushed ice. The aqueous phase was extracted 3 × 100 mL of ethyl ether. The organic phase was washed with 2 × 100 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **31** was isolated as a white solid (102 mg/52%).

4-((2-Methylbut-3-yn-2-yl)oxy)xanthone (***31***). Mp: 59–61 °C. IR ν_{max} (cm^{-1}) (KBr): 3266, 2980, 2919, 2866, 2334, 1649, 1590, 1560, 1479, 1457, 1434, 1329, 1216, 1123, 904, 745, 626. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.34 (*dd*, *J* = 8.0, 1.70), 8.08 (*dd*, *J* = 8.0, 1.6), 7.82 (dd, *J* = 8.0, 1.6), 7.73 (ddd, *J* = 8.5, 7.1, 1.7), 7.55 (d, *J* = 8.5), 7.39 (*ddd*, *J* = 8.0, 7.1, 1.1), 7.30 (*t*, *J* = 8.0), 2.56 (*s*, 1H), 1.78 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 177.3, 155.8, 150.1, 144.3, 134.8, 127.8, 126.7, 124.0, 123.0, 121.6, 121.2, 118.2, 85.3, 75.2, 74.5, 29.6. EIMS *m/z* (%): 278 (5, $[M]^{+\cdot}$), 264 (20), 263 (100), 179 (25), 178 (57), 115 (36), 77 (21), 76 (20), 63 (34), 51 (33). HRMS (ESI) *m/z* calcd for $C_{18}H_{14}NaO_3$ $[M + Na]^+$: 301.0835; found: 301.0834.

4.4.3. Synthesis of compound *32*

To a solution of compound **31** (60 mg/0.216 mmol) in 2 mL of dioxane was added $PtCl_4$ (0.032 mmol/11 mg). The mixture stirred overnight at room temperature. The solution was filtered and the solvent evaporated. The crude mixture was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **32** was isolated as yellow solid (45 mg/76%) and also 4-hydroxyxanthone (**30**) as a white solid (7 mg/15%).

2,2-Dimethylpyrano[3,2-c]xanthen-7(2H)-one (***32***). Mp: 182–184 °C. IR ν_{max} (cm^{-1}) (KBr): 2967, 2918, 2880, 1639, 1597, 1449, 1318, 1260, 1209, 1185, 1113, 1057, 905, 761, 737, 680. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.32 (*dd*, *J* = 8.0, 1.6), 7.82 (*d*, *J* = 8.1), 7.71 (*ddd*, *J* = 8.5, 8.0, 1.6), 7.61 (*dd*, *J* = 8.5, 1.1), 7.37 (*ddd*, *J* = 8.0, 6.9, 1.1), 7.01 (*d*, *J* = 8.1), 6.44 (*d*, *J* = 9.8), 5.82 (*d*, *J* = 9.8), 1.57 (*s*, 6H). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ (ppm): 176.9, 156.1, 145.7, 141.3, 134.5, 133.5, 126.6, 126.0, 123.8, 122.4, 122.0, 121.7, 121.3, 118.4, 117.6, 77.5, 27.9. EIMS *m/z* (%): 278 (5, $[M]^{+\cdot}$), 264 (20), 263 (100), 178 (24), 89 (20), 77 (21), 76 (25), 75 (21), 63 (35), 51 (36). HRMS (ESI) *m/z* calcd for $C_{18}H_{15}O_3$ $[M + H]^+$: 279.10157; found: 279.10257; *m/z* calcd for $C_{18}H_{14}NaO_4$ $[M + Na]^+$: 301.08352; found: 308.08303.

4.5. Synthesis of pyranoxanthone *35*

4.5.1. Synthesis of compound *33*

2-Hydroxyxanthone (**33**) was synthesized according to the published procedure [82] and the spectroscopic data was in accordance with the literature [79].

4.5.2. Synthesis of compound *34*

2-Hydroxyxanthone (**33**) (155 mg/0.73 mmol), potassium carbonate (151 mg/1.1 mmol), potassium iodide (183 mg/1.1 mmol) and copper iodide (21 mg/0.11 mmol) were added to a round-bottom flask. The flask was placed under nitrogen atmosphere and 15 mL of anhydrous DMF was added. The mixture was stirred for 15 min and 3-chloro-3-methyl-1-butyne (150 mg/1.46 mmol) was added dropwise. The mixture was heated to 75 °C and stirred for 24 h. The reaction was allowed to cool to room temperature and poured over 100 g of crushed ice. The aqueous phase was extracted with 3 × 100 mL of ethyl ether. The organic phase was washed with 2 × 100 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **34** was isolated as a white solid (80 mg/39%).

2-((2-Methylbut-3-yn-2-yl)oxy)xanthone (***34***). Mp: 103–105 °C. IR ν_{max} (cm^{-1}) (KBr): 3219, 2976, 2917, 2847, 2362, 1645, 1484, 1462, 1314, 1224, 1141, 899, 752. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.34 (*dd*, *J* = 8.0, 1.7), 8.15 (*d*, *J* = 2.9), 7.72 (*ddd*, *J* = 8.6, 7.1, 1.7), 7.57 (*dd*, *J* = 8.9, 2.9), 7.48 (*dd*, *J* = 8.6, 1.0), 7.44 (*d*, *J* = 8.9), 7.37 (*ddd*, *J* = 8.0, 7.1, 1.0), 2.64 (*s*, 1H), 1.70 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 177.1, 156.2, 152.3, 151.6, 134.7, 130.0, 126.7, 123.8, 122.0, 121.3, 118.7, 117.9, 117.3, 85.4, 74.8, 63.4, 29.5. EIMS *m/z* (%):

279 (3, $[M + 1]^{+\cdot}$), 278 (12, $[M]^{+\cdot}$), 264 (18), 263 (100), 235 (16), 220 (16), 205 (30), 180 (40), 152 (26), 131 (18), 115 (16), 103 (15), 89 (16). HRMS (ESI) *m/z* calcd for $C_{18}H_{14}NaO_3$ $[M + Na]^+$: 301.0835; found: 301.0826.

4.5.3. Synthesis of compound **35**

To a solution of compound **34** (60 mg/0.216 mmol) in 2 mL of dioxane was added $PtCl_4$ (0.032 mmol/11 mg). The mixture was stirred overnight at room temperature. The solution was filtered and the solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **35** was isolated as yellow solid (44 mg/75%) and 2-hydroxyxanthone (**33**) as a pale yellow solid (7 mg/15%).

3,3-Dimethylpyrano[3,2-a]xanthen-12(3H)-one (**35**). Mp: 96–98 °C. IR ν_{max} (cm^{-1}) (KBr): 3063, 2968, 2915, 2853, 1642, 1608, 1576, 1466, 1449, 1300, 1234, 1115, 911, 824, 764, 749, 714. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.27 (*dd*, $J = 8.0, 1.7$), 8.09 (*d*, $J = 10.2$), 7.66 (*ddd*, $J = 8.4, 7.1, 1.74$), 7.41 (*dd*, $J = 8.4, 1.1$), 7.32 (*ddd*, $J = 8.0, 7.1, 1.1$), 7.27 (*d*, $J = 9.2$), 7.18 (*d*, $J = 9.2$), 5.82 (*d*, $J = 10.2$), 1.46 (*s*, 6H). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ (ppm): 179.1, 155.3, 151.9, 149.1, 134.3, 132.5, 126.6, 124.1, 123.4, 122.2, 120.9, 120.0, 118.0, 117.4, 116.3, 75.4, 27.3. EIMS *m/z* (%): 278 (5, $[M]^{+\cdot}$), 264 (18), 263 (100), 180 (30), 178 (25), 152 (20), 88 (91), 77 (23), 76 (24), 75 (23), 74 (21), 65 (51), 62 (22), 51 (34). HRMS (ESI) *m/z* calcd for $C_{18}H_{15}O_3$ $[M + H]^+$: 279.10157; found: 279.10227; *m/z* calcd for $C_{18}H_{14}NaO_3$ $[M + Na]^+$: 301.08352; found: 308.09484.

4.6. Synthesis of pyranoxanthones **38** and **39**

4.6.1. Synthesis of compound **36**

1,2-Dihydroxyxanthone (**36**) was synthesized according to the published procedure [83] and the spectroscopic data was in accordance with the literature [84].

4.6.2. Synthesis of compound **37**

4.6.2.1. Method A. 1,2-Dihydroxyxanthone (**36**) (300 mg/1.31 mmol), potassium carbonate (181 mg/1.31 mmol), potassium iodide (328 mg/1.97 mmol) and copper iodide (38 mg/0.197 mmol) were placed in a round-bottom flask. The flask was placed under N_2 atmosphere and 20 mL of anhydrous DMF was added. The mixture was stirred for 15 min and 3-chloro-3-methyl-1-butyne (202 mg/1.97 mmol) was added dropwise. The mixture was heated at 50 °C for 1 h and then allowed to cool to room temperature. The crude product poured over 100 g of crushed ice and extracted 3 × 100 mL of ethyl ether. The organic phase was washed with 2 × 100 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 95:5). Compound **37** was isolated as an orange solid (38 mg/10%), compound **40** (181 mg/47%) as an orange solid and also compound **41** (35 mg/10%) as an orange solid. 1,2-Dihydroxyxanthone (**36**) was also recovered (90 mg/30%).

4.6.2.2. Method B. 1,2-Dihydroxyxanthone (**36**) (250 mg/1.09 mmol), potassium carbonate (181 mg/1.31 mmol), potassium iodide (271 mg/1.635 mmol) and copper iodide (31 mg/0.163 mmol) were placed in a round-bottom flask. The flask was placed under nitrogen atmosphere and 20 mL of anhydrous acetone was added. The mixture was stirred for 15 min and 3-chloro-3-methyl-1-butyne (117 mg/1.314 mmol) was added dropwise. The mixture was heated 40 °C for 12 h and then allowed to cool to room temperature. The crude product was filtered and the organic solvent evaporated. The crude product was then purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 95:5). Compound **37** was isolated as an orange solid (100 mg/31%).

1-Hydroxy-2-((2-methylbut-3-yn-2-yl)oxy)xanthone (**37**). Mp: 103–104 °C. IR ν_{max} (cm^{-1}) (KBr): 3465, 3241, 2978, 2917, 2847, 2324, 1647, 1606, 1580, 1460, 1283, 1232, 1133, 1053, 888, 760. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 12.81 (OH), 8.29 (*dd*, $J = 8.0, 1.7$), 7.83 (*d*, $J = 9.0$), 7.76 (*ddd*, $J = 8.3, 7.1, 1.7$), 7.47 (*d*, $J = 8.3$), 7.40 (*ddd*, $J = 8.0, 7.1, 0.9$), 6.90 (*d*, $J = 9.0$), 2.57 (*s*, 1H), 1.72 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 182.7, 156.3, 155.1, 152.4, 137.6, 135.6, 132.6, 126.0, 124.0, 120.1, 117.8, 109.5, 105.7, 86.3, 75.3, 73.9, 29.3. EIMS *m/z* (%): 295 (2, $[M + 1]^{+\cdot}$), 294 (8, $[M]^{+\cdot}$), 279 (56), 121 (36), 115 (39), 77 (100), 76 (30), 75 (40), 74 (30), 65 (39), 63 (33), 51 (84). HRMS (ESI) *m/z* calcd for $C_{18}H_{15}O_4$ $[M + H]^+$: 295.0965, found: 295.0961.

3,3-Dimethyl-2-methylene-2*H*-[1,4]dioxino[2,3-*a*]xanthen-12(3*H*)-one or 2,2-dimethyl-3-methylene-2*H*-[1,4]dioxino[2,3-*a*]xanthen-12(3*H*)-one (**40** or **41**).

Compound **40**. Mp: 90–93 °C. IR ν_{max} (cm^{-1}) (KBr): 2976, 2929, 2847, 1651, 1613, 1460, 1299, 1231, 1198, 1161, 1043, 859, 801, 761. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.28 (*dd*, $J = 8.0, 1.6$), 7.66 (*ddd*, $J = 8.5, 7.0, 1.6$), 7.40 (*d*, $J = 8.5, 1.1$), 7.33 (*ddd*, $J = 8.0, 7.0, 1.1$), 7.31 (*d*, $J = 9.0$), 7.03 (*d*, $J = 9.0$), 4.72 (*d*, $J = 2.3$, 1H), 4.52 (*d*, $J = 2.3$, 1H), 1.64 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 176.4, 155.7, 155.3, 152.2, 141.2, 137.5, 134.2, 126.6, 124.0, 123.7, 122.3, 122.0, 117.4, 109.8, 89.3, 73.7, 25.1. EIMS *m/z* (%): 295 (2, $[M + 1]^{+\cdot}$), 294 (9, $[M]^{+\cdot}$), 253 (27), 251 (45), 228 (70), 200 (27), 199 (47), 115 (35), 114 (24), 92 (12), 89 (10), 88 (16), 78 (19), 77 (100), 65 (38), 63 (40), 51 (62). HRMS (ESI) *m/z* calcd for $C_{18}H_{14}NaO_4$ $[M + Na]^+$: 317.0784, found: 317.0771.

Compound **41**. Mp: 116–119 °C. IR ν_{max} (cm^{-1}) (KBr): 2955, 2923, 2849, 1653, 1615, 1592, 1462, 1301, 1232, 1163, 1044, 862, 799, 761. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.31 (*dd*, $J = 8.0, 1.7$), 7.67 (*ddd*, $J = 8.5, 7.1, 1.7$), 7.42 (*d*, $J = 8.5$), 7.35 (*ddd*, $J = 8.0, 7.1, 1.1$), 7.24 (*d*, $J = 9.0$), 7.03 (*d*, $J = 9.0$), 5.01 (*d*, $J = 2.3$, 1H), 4.61 (*d*, $J = 2.3$, 1H), 1.64 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 176.3, 155.5, 155.3, 151.7, 140.9, 137.0, 134.3, 126.7, 123.9, 123.7, 122.2, 117.4, 111.4, 110.6, 91.0, 72.3, 24.9. EIMS *m/z* (%): 295 (3, $[M + 1]^{+\cdot}$), 294 (17, $[M]^{+\cdot}$), 251 (50), 228 (90), 195 (54), 115 (34), 77 (100), 67 (31), 65 (44), 63 (40), 51 (69). HRMS (ESI) *m/z* calcd for $C_{18}H_{14}NaO_4$ $[M + Na]^+$: 317.0784, found: 317.0784.

4.6.3. Synthesis of compound **38**

To a solution of compound **37** (81 mg/0.275 mmol) in 2.5 mL of dioxane was added $PtCl_4$ (0.041 mmol/14 mg). The mixture was stirred for 24 h at room temperature. The solution was filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 95:5). Compound **38** was isolated as an orange solid (60 mg/73%) and also 1,2-dihydroxyxanthone (**36**) (14 mg/22%).

12-Hydroxy-2,2-dimethylpyrano[2,3-b]xanthen-11(2H)-one (**38**). Mp: 131–133 °C. IR ν_{max} (cm^{-1}) (KBr): 3412, 2961, 2914, 2857, 1651, 1599, 1475, 1439, 1303, 1209, 1135, 1072, 902, 788, 610. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. EIMS *m/z* (%): 294 (24, $[M]^{+\cdot}$), 280 (17), 279 (84), 262 (10), 213 (28), 205 (48), 197 (20), 165 (42), 151 (32), 121 (44), 115 (42), 77 (100), 63 (67), 51 (83). HRMS (ESI) *m/z* calcd for $C_{18}H_{15}O_4$ $[M + H]^+$: 295.09649, found: 295.09704; *m/z* calcd for $C_{18}H_{14}NaO_4$ $[M + Na]^+$: 317.07843; found: 317.07825.

4.6.4. Synthesis of compound **39**

Compound **38** (25 mg/0.08 mmol) and 5 mL of anhydrous methanol in nitrogen atmosphere were placed in a round-bottom flask. To the solution was added Pd/C 10% (15% weight/4 mg) in one portion. Triethylsilane (0.136 mL, 0.8 mmol) was then added dropwise. The mixture was stirred for 15 min at room temperature. The product was filtered over celite and washed 3 times with methanol. The methanol was evaporated and the crude product was purified by silica gel column chromatography (*n*-hexane/ethyl

acetate 95:5). Compound **39** was isolated as light orange solid (23 mg/97%).

*12-Hydroxy-2,2-dimethyl-3,4-dihydropyrano[2,3-b]xanthen-11(2H)-one (**39**).* Mp: 172–173 °C. IR ν_{max} (cm^{-1}) (KBr): 3443, 2972, 2917, 2858, 1650, 1602, 1478, 1444, 129, 1182, 1073, 921, 774. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. EIMS *m/z* (%): 297 (2, $[M + 1]^{+\cdot}$), 296 (100, $[M]^{+\cdot}$), 240 (43), 212 (48), 128 (77), 115 (27), 102 (41), 92 (46), 77 (85), 76 (31), 75 (32), 74 (26), 65 (32), 63 (100), 62 (39). HRMS (ESI) *m/z* calcd for $C_{18}H_{17}O_4$ $[M + H]^+$: 297.11214; found: 297.11353; *m/z* calcd for $C_{18}H_{16}NaO_4$ $[M + Na]^+$: 319.09408; found: 319.09250.

4.7. Synthesis of pyranoxanthones **51**, **52** and **53**

4.7.1. Synthesis of compound **43**

2,4-Dihydroxybenzaldehyde (**42**) (3 g/20.17 mmol), calcium hydroxide (1.543 g/20.8 mmol) and 150 mL of methanol were placed in a round-bottom flask. Then, prenal (9.135 g/108.6 mmol) was added dropwise. The mixture was stirred at room temperature for 48 h. The reaction was quenched with HCl 1 M until pH 1–2. The methanol was evaporated and the aqueous phase was extracted with 3 × 150 mL of ethyl acetate. The organic phase was washed with 2 × 100 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **43** was crystallized from *n*-hexane/ethyl acetate 3:1 as a yellow solid (2.06 g/50%).

*6-Formyl-5-hydroxy-2,2-dimethyl-2H-benzopyran (**43**).* Mp: 68–69 °C. IR ν_{max} (cm^{-1}) (KBr): 3464, 2967, 2922, 2857, 1628, 1484, 1330, 1294, 1247, 1176, 1107, 1081, 748. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 11.65 (OH), 9.66 (*s*, CHO), 7.29 (*d*, $J = 8.6$), 6.88 (*d*, $J = 10.0$), 6.44 (*d*, $J = 8.6$), 5.61 (*d*, $J = 10.0$), 1.46 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 194.5, 160.6, 158.7, 134.7, 128.6, 115.2, 115.1, 109.4, 108.8, 78.2, 28.4. EIMS *m/z* (%): 205 (5, $[M + 1]^{+\cdot}$), 204 (10 $[M]^{+\cdot}$), 190 (15), 189 (100), 187 (60), 159 (12), 131 (12), 103 (10), 77 (12), 51 (6). HRMS (ESI) *m/z* calcd for $C_{12}H_{13}O_3$ $[M + H]^+$: 205.08570, found: 205.08592.

4.7.2. Synthesis of compound **44**

Potassium carbonate (2.43 g/17.6 mmol) was placed in a round-bottom flask and under nitrogen atmosphere. Then, compound **43** (1.7976 g/8.8 mmol) solubilized in 20 mL of anhydrous acetone was added by syringe. Dimethylsulfate (1.667/13.2 mmol) was added dropwise and the mixture was stirred for 20 h at room temperature and under nitrogen atmosphere. The reaction was quenched by the addition of 20 mL of distilled water and the acetone was evaporated. The aqueous phase was acidified with HCl 1 N until pH 2–3 and extracted with 3 × 100 mL of ethyl acetate. The organic phase was washed with 2 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. Compound **44** was obtained in quantitative yield as yellow oil.

*6-Formyl-5-methoxy-2,2-dimethyl-2H-benzopyran (**44**).* IR ν_{max} (cm^{-1}) (KBr): 2974, 2932, 2840, 2747, 1679, 1634, 1463, 1370, 1281, 1249, 1213, 1158, 1110, 1068, 984, 736. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 10.18 (*s*, CHO), 7.66 (*d*, $J = 8.6$), 6.65 (*d*, $J = 8.6$), 6.60 (*dd*, $J = 9.9$, 0.6), 5.70 (*d*, $J = 9.9$), 3.90 (*s*, OCH_3), 1.47 (*s*, 6H). EIMS *m/z* (%): 219 (5, $[M + 1]^{+\cdot}$), 218 (10, $[M]^{+\cdot}$), 204 (18), 205 (100), 174 (10), 160 (60), 133 (16), 105 (5), 91 (8), 78 (10), 51 (6). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 188.3, 160.0, 159.9, 130.5, 129.8, 122.5, 115.9, 114.4, 113.4, 64.4, 28.2. HRMS (ESI) *m/z* calcd for $C_{13}H_{15}O_3$ $[M + H]^+$: 219.10177; found: 219.10157.

4.7.3. Synthesis of compound **45**

Compound **44** (1.0139 g/4.64 mmol) was solubilized in 20 mL of methanol and H_2O_2 (540 μL of a solution of H_2O_2 30% in water). Then, $KHSO_4$ (94.7 mg/0.7 mmol) was added in one portion and the solution was allowed to stir at room temperature for 8 h. The reaction was quenched by the addition 10 mL of distilled water and the methanol evaporated. The aqueous phase was extracted with 3 × 20 mL of dichloromethane. The organic phase was washed with 2 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **45** was isolated as orange oil (898 mg/4.36 mmol/94%).

*6-Hydroxy-5-methoxy-2,2-dimethyl-2H-benzopyran (**45**).* IR ν_{max} (cm^{-1}) (KBr): 3422, 3045, 2973, 2934, 2836, 1728, 1634, 1584, 1470, 1437, 1373, 1295, 1260, 1215, 1152, 1114, 960, 811, 731. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 6.72 (*d*, $J = 8.6$), 6.55 (*d*, $J = 10$), 6.50 (*d*, $J = 8.6$), 5.69 (*d*, $J = 9.9$), 5.15 (OH), 3.82 (*s*, OCH_3), 1.41 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 145.5, 142.5, 142.4, 131.6, 116.8, 114.90, 114.86, 112.4, 75.3, 62.2, 27.4. EIMS *m/z* (%): 208 (5, $[M + 2]^{+\cdot}$), 207 (15, $[M + 1]^{+\cdot}$), 206 (60, $[M]^{+\cdot}$), 191 (100), 177 (50), 176 (30), 148 (18), 91 (10), 77 (5). HRMS (ESI) *m/z* calcd for $C_{12}H_{15}O_3$ $[M + H]^+$: 207.10139; found: 207.10157.

4.7.4. Synthesis of compound **47**

To a solution of 2-iodobenzoic acid (**46**) (15 g/60 mmol) in 200 mL of methanol were added 8.8 mL of sulfuric acid dropwise. The solution was refluxed for 16 h. The mixture was allowed to cool to room temperature and most of the methanol was evaporated by rotary evaporator until approximately 20 mL of solution. The product was then partitioned between water and dichloromethane. The aqueous phase was extracted 2 × 150 mL of dichloromethane. The organic layer was then washed with 3 × 100 mL of saturated solution of bicarbonate, 3 × 100 mL of water, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. Compound **47** was isolated as light yellow oil (15.4 g/98%).

*Methyl 2-iodobenzoate (**47**).* IR ν_{max} (cm^{-1}) (KBr): 3444, 3057, 2994, 2946, 2894, 2836, 1728, 1579, 1459, 1428, 1290, 1249, 1127, 1100, 1013, 738.

4.7.5. Synthesis of compound **48**

Methyl 2-iodobenzoate (**47**) (9.369 g/35.76 mmol), CuI (1.7 g/8.94 mmol), cesium carbonate (19.4 g/59.6 mmol), *N*,*N*-dimethylglycine (495.6 mg/3.576 mmol) were placed in a round-bottom flask and under nitrogen atmosphere. To this flask was added compound **45** (6.1 g/29.8 mmol) solubilized in 20 mL of anhydrous dioxane. The reaction was heated for 18 h at 90 °C and under nitrogen atmosphere. The reaction was allowed to cool to room temperature and filtered. The solid was washed several times with acetone and then the organic phase was concentrated. The mixture was partitioned between ethyl acetate and water. The aqueous phase was then extracted with 2 × 50 mL of ethyl acetate. The organic phase was washed 2 × 50 mL with brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate (9:1)). Compound **48** was recrystallized from ethyl acetate/*n*-hexane 1:1 as a white solid (2.5655 g/26%).

*Methyl 2-((5-methoxy-2,2-dimethyl-2H-benzopyran-6-yl)oxy) benzoate (**48**).* Mp: 88–89 °C. IR ν_{max} (cm^{-1}) (KBr): 3072, 2974, 2943, 2869, 2838, 1729, 1599, 1472, 1369, 1298, 1250, 1218, 1075, 959, 751. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 7.87 (*dd*, $J = 7.9$, 1.8, H–C(7″)), 7.36 (*ddd*, $J = 8.4$, 7.5, 1.8, H–C(5″)), 7.05 (*dd*, $J = 7.5$, 0.8, H–C(6″)), 6.78 (*d*, $J = 8.7$, H–C(7)), 6.75 (*d*, $J = 8.4$, H–C(4″)), 6.63 (*d*, $J = 9.9$, H–C(4)), 6.54 (*d*, $J = 8.7$, H–C(8)), 5.66 (*d*, $J = 9.9$, H–C(3)), 3.90 (*s*, H_3CO–C(1″)), 3.85 (*s*, H_3CO–C(5)), 1.44 (*s*, $(CH_3)_2$–C(2)). ^{13}C NMR (75.47 MHz, $CDCl_3$): 166.5 (C1″), 157.8 (C3″), 150.2 (C6), 147.2 (C5), 141.1 (C8a), 133.4 (C5″), 131.6 (C7″), 130.9 (C3), 121.7 (C6″), 121.7 (C7), 120.5 (C2″), 116.9 (C4), 116.3 (C4″), 116.0 (C4a), 112.0 (C8),

75.9 (C2), 61.6 (C1‴), 52.1 (C1⁗), 27.7 (C1′a and C1′b); HRMS (ESI) *m*/*z* calcd for $C_{19}H_{17}O_4$ $[M - OCH_3]^+$: 309.11214, found: 309.11203.

*4.7.6. Synthesis of compound **49***

Compound **48** (129.6 mg/0.38 mmol) and 10 mL of a solution of THF/methanol 1:1 were placed in a round-bottom flask. Then 250 μL of a solution of LiOH 3 N was added dropwise. The mixture was stirred at room temperature for 46 h. To the flask were added 50 mL of water and the aqueous phase was acidified with HCl 5% until pH 2–3 and extracted with 3 × 50 mL of dichloromethane. The organic phase was washed with 2 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. Compound **49** was obtained as a white solid (121.7 mg/98%).

*2-((5-Methoxy-2,2-dimethyl-2H-benzopyran-6-yl)oxy)benzoic acid (**49**).* Mp: 143–144 °C. IR ν_{max} (cm^{-1}) (KBr): 3065, 2974, 2934, 2869, 2809, 2640, 2592, 2809, 1684, 1565, 1464, 1250, 1233, 1060, 953, 772, 730. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 8.20 (*dd*, *J* = 7.9, 1.8), 7.45 (*ddd*, *J* = 8.4, 7.1, 1.8), 7.19 (*ddd*, *J* = 7.9, 7.1, 1.0), 6.79 (*d*, *J* = 8.4), 6.92 (*d*, *J* = 8.8), 6.63–6.57 (*m*, 2H), 5.71 (*d*, *J* = 10.0), 3.74 (*s*, OCH_3), 1.46 (*s*, 6H). ^{13}C NMR (75.47 MHz, $CDCl_3$): 166.7, 158.0, 151.7, 147.7, 138.9, 134.9, 133.5, 131.4, 123.0, 122.4, 117.8, 116.4, 115.5, 112.5, 76.3, 62.1, 27.7. HRMS (ESI) *m*/*z* calcd for $C_{19}H_{17}O_4$ $[M - OH]^+$: 309.11214, found: 309.11203.

*4.7.7. Synthesis of compound **50***

TBTU (2.44 g/7.6 mmol) was placed in a round-bottom flask and then compound **49** (2.48 g/7.6 mmol) solubilized in 250 mL of THF was added. Then triethylamine (1.538 g/15.2 mmol) was added dropwise and the mixture stirred at room temperature for 15 min. Diethylamine (1.112 g/15.2 mmol) was then added and stirred overnight at room temperature. The mixture was concentrated and partitioned between ethyl acetate (250 mL) and water (250 mL). The aqueous phase was extracted with 2 × 250 mL of ethyl acetate. The organic phase was washed 2 × 150 mL of a saturated solution of $NaHCO_3$, 2 × 150 mL with brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 7:3). Compound **50** was obtained as colorless oil in quantitative yield.

*2-((5-Methoxy-2,2-dimethyl-2H-benzopyran-6-yl)oxy)-N,N-diethylbenzamide (**50**).* IR ν_{max} (cm^{-1}) (KBr): 2974, 2931, 2870, 1633, 1470, 1432, 1370, 1293, 1255, 1217, 1113, 1074, 960, 753. ^{1}H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 7.28 (*dd*, *J* = 7.4, 1.7), 7.22 (*ddd*, *J* = 8.4, 7.6, 1.7), 7.03 (*ddd*, *J* = 7.6, 7.4, 0.8), 6.79 (*d*, *J* = 8.8), 6.67 (*d*, *J* = 8.4), 6.63 (*d*, *J* = 10.0), 6.53 (*d*, *J* = 8.8), 5.66 (*d*, *J* = 10.0), 3.80 (*s*, OCH_3), 3.50 (*broad*, 4H), 1.44 (*s*, 6H), 1.22 (*t*, 3H, *J* = 7.1), 1.11 (*t*, 3H, *J* = 7.1). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 168.2, 154.1, 150.3, 147.6, 140.1, 130.8, 129.9, 127.7, 127.3, 122.2, 122.0, 117.0, 116.0, 114.9, 111.9, 75.9, 61.7, 42.9, 39.0, 27.7, 14.2, 12.9. EIMS *m*/*z* (%): 382 (18, $[M]^{+\cdot}$), 366 (28), 295 (35), 263 (100), 235 (45), 189 (25), 176 (34), 160 (32), 72 (38). HRMS (ESI) *m*/*z* calcd for $C_{23}H_{28}NO_4$ $[M + H]^+$: 382.20118; found: 382.20128.

*4.7.8. Synthesis of compound **51***

Compound **50** (2.555 g/6.7 mmol) was placed in a three-necked round-bottom flask and under nitrogen flow. Then 150 mL of anhydrous THF was added and the solution was placed in an ice bath. After 10 min, LDA (16.75 mmol of a solution 2 M in THF) was added dropwise and the reaction was stirred for 15 min at 0 °C, and allowed to warm slowly to room temperature. After 1 h the reaction was quenched by the addition of a saturated solution of NH_4Cl. The mixture was partitioned between ethyl acetate (150 mL) and water (150 mL). The aqueous phase was extracted with 2 × 150 mL of ethyl acetate. The organic phase was washed with 2 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **51** was obtained as yellow solid (1.9 g/91%).

*5-Methoxy-2,2-dimethylpyrano[2,3-b]xanthen-11(2H)-one (**51**).* Mp: 124–125 °C. IR ν_{max} (cm^{-1}) (KBr): 2975, 2935, 2841, 1652, 1605, 1472, 1426, 1315, 1072, 961, 760. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. EIMS *m*/*z* (%): 308 (12, $[M]^{+\cdot}$), 294 (12), 293 (55), 279 (16), 278 (100), 235 (40), 221 (30), 179 (30), 165 (90), 152 (15), 77 (20). HRMS (ESI) *m*/*z* calcd for $C_{19}H_{17}O_4$ $[M + H]^+$: 309.11286; found: 309.11214.

*4.7.9. Synthesis of compound **52***

Pd/C 10% (10–20% weight for mass) was added to a two-necked round-bottom flask and placed under N_2 atmosphere. Then, MeOH 40 mL and compound **51** (270 mg/0.87 mmol) was added dropwise. To this mixture was added triethylsilane (8.7 mmol/1 g) in a pressure-equalizer dropping-funnel. The mixture was allowed to react for 15 min and then the crude product was filtered through a pad of celite and washed with methanol. The organic solvent was evaporated and the crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **52** was obtained as a white solid (267 mg, 98%).

*5-Methoxy-2,2-dimethyl-3,4-dihydropyrano[2,3-b]xanthen-11(2H)-one (**52**).* Mp: 135–136 °C. IR ν_{max} (cm^{-1}) (KBr): 2983, 2936, 2840, 1658, 1473, 1441, 1312, 1074, 758. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. EIMS *m*/*z* (%): 311 (12, $[M + 1]^{+\cdot}$), 310 (30, $[M]^{+\cdot}$), 255 (96), 254 (100), 225 (84), 211 (55), 197 (48), 169 (30), 141 (60), 128 (60), 115 (30), 77 (42), 51 (38). HRMS (ESI) *m*/*z* calcd for $C_{19}H_{19}O_4$ $[M + H]^+$: 311.12779; found: 311.12767.

*4.7.10. Synthesis of compound **53***

Compound **51** (247.3 mg/0.8 mmol) was placed in a round-bottom flask and under nitrogen flow. Then 30 mL of anhydrous dichloromethane was added and the solution was placed in at −78 °C. It was allowed to stay for 15 min and BBr_3 (2 mmol of a solution 1 M in dichloromethane) was added dropwise. The mixture was stirred for 1 h at −78 °C and then allowed to warm slowly to room temperature. After reaching room temperature, the reaction was quenched by the addition of methanol. It was added 20 mL of water to the mixture and the organic solvent evaporated. The aqueous phase was extracted with 2 × 50 mL of dichloromethane. The organic phase was washed with 2 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 8:2). Compound **53** was obtained as yellow solid (198.1 mg/84%).

*5-Hydroxy-2,2-dimethylpyrano[2,3-b]xanthen-11(2H)-one (**53**).* Mp: 245–246 °C. IR ν_{max} (cm^{-1}) (KBr): 2973, 2911, 2840, 1622, 1447, 754. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. HRMS (ESI) *m*/*z* calcd for $C_{18}H_{15}O_4$ $[M + H]^+$: 295.09653; found: 295.09649.

*4.8. Synthesis of pyranoxanthones **62**, **63**, **64**, **65**, **66** and **67***

*4.8.1. Synthesis of compound **55***

To a solution of 2,4-dimethoxybenzaldehyde (**54**) (5.0 g/30 mmol) and 5 mL of H_2O_2 30% in 50 mL of methanol, was added dropwise 0.5 mL of sulfuric acid. The mixture was stirred for 20 h at room temperature. Approximately 45 mL of methanol were evaporated by rotary evaporator and the solution was partitioned between dichloromethane and distilled water. The aqueous phase was extracted 3 × 50 mL of dichloromethane. The organic phase was washed with 3 × 50 mL of distilled water, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by column chromatography

(chloroform). 2,4-Dimethoxyphenol (**55**) was obtained as light yellow oil (4.33 g/94%).

*2,4-Dimethoxyphenol (**55**).* IR ν_{max} (cm^{-1}) (KBr): 3443, 2997, 2939, 2835, 1610, 1512, 1461, 1433, 1374, 1299, 1263, 1228, 1202, 1151, 1116, 1033, 918, 830, 792. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 6.75 (*d*, $J = 5.2$), 6.42 (*d*, $J = 1.7$), 6.31 (*dd*, $J = 5.2, 1.7$), 3.79 (*s*, OCH_3), 3.69 (*s*, OCH_3). EIMS *m*/*z* (%): 156 (4, $[M + 2]^{+\cdot}$), 155 (40, $[M + 1]^{+\cdot}$), 154 (100 $[M]^{+\cdot}$), 140 (4), 139 (58), 112 (4), 111 (90), 96 (22), 79 (10), 69 (10), 51 (20).

4.8.2. Synthesis of compound **56**

2,4-Dimethoxyphenol (**55**) (3 g/19 mmol) and $CuCl_2$ anhydrous (2.5 mg/0.019 mmol) were placed in a round-bottom flask and under N_2 atmosphere. 25 mL of anhydrous CH_3CN were added and the mixture was cooled to 0 °C on an ice bath. Then, DBU (3.76 g/24.7 mmol) was added and 10 min after 3-chloro-3-methyl-1-butyne (2.53 g/24.7 mmol) was added dropwise to the mixture. The mixture was stirred at 0 °C for 10 h. The mixture was allowed to warm to room temperature and then partitioned between 50 mL of distilled water and 50 mL of diethyl ether. The aqueous solution was extracted with 2 × 50 mL of diethyl ether. The organic phase was washed with 2 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 95:5) obtaining compound **56** as yellowish oil (3.5792 g/86%).

*2,4-Dimethoxy-1-(2-methylbut-3-yn-2-yloxy)benzene (**56**).* IR: ν_{max} (cm^{-1}) (KBr): 3273, 2981, 2928, 2826, 2354, 2325, 1598, 1497, 1453, 143, 1200, 1148, 1127, 1030, 872. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 7.30 (*d*, $J = 8.7$), 6.48 (*d*, $J = 2.8$), 6.39 (*dd*, $J = 8.7, 2.8$), 3.79 (*s*, OCH_3), 3.78 (*s*, OCH_3), 2.48 (*s*, 1H), 1.61 (*s*, 6H). EIMS *m*/*z* (%): 222 (10, $[M + 2]^{+\cdot}$), 221 (20, $[M + 1]^{+\cdot}$), 220 (42 $[M]^{+\cdot}$), 205 (25), 189 (10), 155 (15), 154 (100), 139 (40), 125 (30), 111 (30), 93 (15). HRMS (ESI) *m*/*z* calcd for $C_{13}H_{17}O_3$ $[M + H]^+$: 221.11722; found: 221.11705.

4.8.3. Synthesis of compound **57**

Compound **56** (700 mg/2.3 mmol) was placed in a round-bottom flask and under nitrogen atmosphere. 15 mL of anhydrous DMF were added and the solution was stirred for 4 h at 145 °C. The mixture was allowed to cool to room temperature and poured over 50 g of crushed ice. The aqueous phase was extracted with 3 × 50 mL of diethyl ether. The organic phase was washed with 2 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 85:15) and compound **57** was obtained as yellow oil (649 mg/93%).

*6,8-Dimethoxy-2,2-dimethyl-2H-benzopyran (**57**).* IR ν_{max} (cm^{-1}) (KBr): 3033, 2967, 2930, 2834, 1631, 1602, 1578, 1478, 1382, 1251, 1199, 1151, 1085, 1047, 824. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 6.40 (*d*, $J = 2.8$), 6.27 (*d*, $J = 9.8$), 6.18 (*d*, $J = 2.8$), 5.64 (*d*, $J = 9.8$), 3.84 (*s*, OCH_3), 3.76 (*s*, OCH_3), 1.46 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 153.6, 150.0, 136.0, 131.7, 122.5, 121.9, 101.9, 100.4, 75.9, 56.2, 55.6, 27.5. EIMS *m*/*z* (%): 221 (10, $[M + 1]^{+\cdot}$), 220 (24 $[M]^{+\cdot}$), 205 (100), 190 (20), 162 (20), 133 (22), 119 (26), 115 (32), 91 (80). HRMS (ESI) *m*/*z* calcd for $C_{13}H_{17}O_3$ $[M + H]^+$: 221.11722; found: 221.11707.

4.8.4. Synthesis of compound **59**

Sodium hydride (3.63 g/90.53 mmol of 60% sodium hydride in mineral oil) was placed in a round-bottom flask and washed with anhydrous *n*-hexane. The flask was then placed under nitrogen atmosphere and on an ice-bath. Methyl salicylate (**58**) (6.88 g/45.26 mmol) was solubilized in 60 mL of anhydrous THF and added slowly to the flask containing the sodium hydride. The mixture was stirred for 15 min and MOMCl (7.288 g/90.53 mmol) was added dropwise and the flask placed under reflux for 16 h. The mixture was cooled to room temperature and poured over 100 g of crushed ice. The aqueous phase was extracted with 3 × 150 mL of ethyl acetate. The organic phase was washed with 2 × 100 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 95:5). Compound **59** was obtained as colorless oil (8.1522 g/92%).

*Methyl 2-(methoxymethoxy)benzoate (**59**).* IR: ν_{max} (cm^{-1}) (KBr): 2991, 2947, 2903, 2825, 1725, 1596, 1485, 1449, 1432, 1296, 1249, 1150, 1128, 1073, 984, 755. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 7.78 (*dd*, $J = 7.8, 1.8$), 7.44 (*ddd*, $J = 8.4, 7.5, 1.8$), 7.20 (*dd*, $J = 8.4, 0.9$), 7.05 (*ddd*, $J = 7.8, 7.5, 0.9$), 5.26 (*s*, 2H), 3.89 (*s*, OCH_3), 3.52 (*s*, OCH_3). EIMS *m*/*z* (%): 197 (16, $[M]^{+\cdot}$), 165 (100), 135 (26), 92 (45), 63 (34).

4.8.5. Synthesis of compound **60**

A solution of *n*-BuLi in hexanes (1.6 M, 6.25 mL, 11 mmol) was placed in a dropping funnel with pressure equalizing tube. This solution was then added dropwise over a 5 min period, to a solution of compound **57** (2.350 g, 0.01 mol) in 70 mL of anhydrous THF at 0 °C under nitrogen and stirred for 30 min. Then, compound **59** (3.9 g, 0.02 mol) dissolved in 30 mL of anhydrous THF was added dropwise, *via* cannula, at 0 °C under nitrogen. The reaction mixture was stirred for 5 h at 0 °C. The reaction was quenched with a saturated solution of NH_4Cl. The mixture was extracted with 3 × 100 mL of diethyl ether. The organic phase was washed with 2 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 95:5). Compound **60** was obtained as orange oil (1.716 g, 45%).

*(6,8-Dimethoxy-2,2-dimethyl-2H-benzopyran-7-yl)(2-(methoxymethoxy)phenyl) methanone (**60**).* IR ν_{max} (cm^{-1}) (KBr): 2971, 2934, 2833, 1661, 1596, 1568, 1455, 1417, 1372, 1291, 1236, 1204, 1157, 1112, 1080, 980, 913, 756. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 7.72 (*dd*, $J = 7.7, 1.8$; H–C(6″)), 7.43 (*ddd*, $J = 8.5, 7.0, 1.8$; H–C(4″)), 7.12 (*d*, $J = 8.5$; H–C(3″)), 7.04 (*dd*, $J = 7.7, 1.0$; H–C(5″)), 6.35 (*s*; H–C(5)), 6.31 (*d*, $J = 9.8$; H–C(4)), 5.69 (*d*, $J = 9.8$; H–C(3)), 5.05 (*s*, H2–C(1‴″)), 3.71 (*s*, H_3CO–C(6)), 3.66 (*s*, H_3CO–C(8)), 3.35 (*s*, H_3–C(2‴″)), 1.46 (*s*, $(CH_3)_2$–C(2)). ^{13}C NMR (75.47 MHz, $CDCl_3$): 193.4 (C9), 156.6 (C2″), 150.3 (C8), 145.7 (C6), 139.4 (C8a), 133.6 (C4″), 132.1 (C3), 131.5 (C6″), 129.6 (C1″), 125.0 (C7), 123.9 (C4a), 122.3 (C4), 121.6 (C5″), 115.8 (C3″), 104.3 (C5), 94.7 (C1‴″), 76.1 (C2), 61.1 (C1‴), 56.4 (C1″), 56.0 (C2‴″), 27.5 (C2′a and C2′b). HRMS (ESI) *m*/*z* calcd for $C_{22}H_{24}NaO_6$ $[M + Na]^+$: 407.14651; found: 407.14638.

4.8.6. Synthesis of compound **61**

To a solution of compound **60** (152 mg, 0.396 mmol) in 10 mL of CH_3CN at 0 °C was added in one portion $NbCl_5$ (107 mg, 0.396 mmol). The mixture was stirred at 0 °C for 10 min and then allowed to warm to room temperature and let stirring for more 50 min. After this time, the reaction mixture was quenched with saturated solution of $NaHCO_3$. The mixture was extracted with 3 × 50 mL of ethyl acetate. The organic phase was washed with 2 × 50 mL of brine, dried over anhydrous sodium sulfate and organic solvent evaporated. Compound **61** was obtained as orange oil (131.9 mg/98%).

*(6,8-Dimethoxy-2,2-dimethyl-2H-benzopyran-7-yl)(2-hydroxyphenyl)methanone (**61**).* IR ν_{max} (cm^{-1}) (KBr): 3415, 3343, 2973, 2935, 1623, 1568, 1461, 1119, 1071, 1029, 916, 808, 756. ^1H NMR (300.13 MHz, $CDCl_3$) δ (ppm): 12.09 (*s*, OH), 7.45 (*ddd*, $J = 8.5, 7.1, 1.7$), 7.34 (*dd*, $J = 8.0, 1.7$), 7.01 (*dd*, $J = 8.4, 1.0$), 6.79 (*ddd*, $J = 8.2, 7.1, 1.0$), 6.38 (*s*), 6.33 (*d*, $J = 9.8$), 5.73 (*d*, $J = 9.8$), 3.79 (*s*, OCH_3), 3.68 (*s*, OCH_3), 1.48 (*s*, 6H). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ (ppm): 200.6, 162.4, 150.0, 145.6, 139.2, 136.5, 133.3, 132.6, 124.0, 122.2, 121.6, 121.0, 119.0, 117.8, 104.2, 76.4, 61.4, 56.2. EIMS *m*/*z* (%): 342 (1,

$[M + 2]^+$), 325 (4), 309 (10), 294 (4), 279 (12), 253 (4), 207 (5), 206 (13), 205 (100), 190 (7), 173 (8), 147 (11), 121 (16), 91 (14), 77 (17), 65 (17). HRMS (ESI) *m/z* calcd for $C_{20}H_{21}O_5$ $[M + H]^+$: 341.13835; found: 341.13816; *m/z* calcd for $C_{20}H_{20}NaO_5$ $[M + Na]^+$: 363.12029; found: 363.12021.

4.8.7. Synthesis of compound **62** and **63**

To a solution of compound **61** (119 mg, 0.35 mmol) in 15 mL of DMF was added Cs_2CO_3 (171 mg, 0.525 mmol). The mixture was stirred at 50 °C for 25 h. The mixture was allowed to cool to room temperature and poured into crushed ice. The aqueous phase was then extracted with 3 × 50 mL of diethyl ether. The organic phase was washed with 2 × 50 mL of brine, dried over anhydrous sodium sulfate, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 8:2). Compound **63** was obtained as deep green solid (87.6 mg, 81%) and compound **62** as deep green oil (8.6 mg, 8%).

12-Methoxy-2,2-dimethylpyrano[2,3-b]xanthen-11(2H)-one (**62**). IR ν_{max} (cm^{-1}) (KBr): 2958, 2923, 2853, 1653, 1614, 1463, 1429, 1111, 1083. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. EIMS *m/z* (%): 308 (10, $[M]^+$), 293 (18), 276 (22), 265 (85), 349 (64), 221 (25), 191 (35), 173 (65), 165 (100), 152 (24), 121 (30), 115 (50), 95 (25), 89 (40), 77 (65), 63 (50), 51 (28). HRMS (ESI) *m/z* calcd for $C_{19}H_{17}O_4$ $[M + H]^+$: 309.11214, found: 309.11206.

6-Methoxy-2,2-dimethylpyrano[3,2-c]xanthen-7(2H)-one (**63**). Mp: 164–165 °C. IR ν_{max} (cm^{-1}) (KBr): 2974, 2927, 1652, 1615, 1554, 1468, 1420, 1344, 1305, 1265, 1219, 1120, 1077. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. EIMS *m/z* (%): 308 (20, $[M]^+$), 294 (20), 293 (45), 276 (52), 265 (45), 250(55), 249 (60), 221 (40), 205 (35), 178 (45), 165 (100), 152 (20), 121 (55), 115 (35), 89 (20), 77 (45), 63 (40), 51 (45). HRMS (ESI) *m/z* calcd for $C_{19}H_{17}O_4$ $[M + H]^+$: 309.11214, found: 309.11203.

4.8.8. Synthesis of compound **64**

Pd/C 10% (10–20% weight for mass) was placed in a round-bottom flask and under nitrogen atmosphere. Then, compound **62** (28 mg/0.09 mmol) solubilized in 10 mL of methanol was added dropwise. To this mixture was added triethylsilane (0.9 mmol/104 mg) in a pressure-equalizing dropping-funnel. The mixture was allowed to react for 10 min and then the crude product was filtered through celite and washed repeatedly with methanol. The solvent was evaporated and the crude product was purified by Grace cartridge flash chromatography (*n*-hexane/ethyl acetate 8:2). Compound **64** was obtained as a green solid in quantitative yield.

12-Methoxy-2,2-dimethyl-3,4-dihydropyrano[2,3-b]xanthen-11(2H)-one (**64**). Mp: 132–133 °C. IR ν_{max} (cm^{-1}) (KBr): 2972, 2919, 2849, 1655, 1616, 1458, 1421, 1320, 1122, 1078, 746. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. HRMS (ESI) *m/z* calcd for $C_{19}H_{19}O_4$ $[M + H]^+$: 311.12779; found: 311.12769.

4.8.9. Synthesis of compound **65**

Pd/C 10% (10–20% weight for mass) was placed on a round-bottom flask and under nitrogen atmosphere. Then, 20 mL of methanol and compound **63** (166 mg/0.54 mmol) was added dropwise. To this mixture was added triethylsilane (5.4 mmol/627 mg) in a pressure-equalizing dropping-funnel. The mixture was allowed to react for 10 min and then the crude product was filtered by celite and washed repeatedly with methanol. The organic solvent was evaporated and the crude product was purified by Grace cartridge chromatography (*n*-hexane/ethyl acetate 8:2). Compound **65** was obtained as a light yellow solid (138 mg, 81%).

6-Methoxy-2,2-dimethyl-3,4-dihydropyrano[3,2-c]xanthen-7(2H)-one (**65**). Mp: 204–205 °C. IR ν_{max} (cm^{-1}) (KBr): 2971, 2933, 2874, 2844, 1661, 1603, 1481, 1082, 749. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. EIMS *m/z* (%): 308 (10, $[M]^+$), 293 (18), 276 (22), 265 (85), 349 (64), 221 (25), 191 (35), 173 (65), 165 (100), 152 (24), 121 (30), 115 (50), 95 (25), 89 (40), 77 (65), 63 (50), 51 (28). HRMS (ESI) *m/z* calcd for $C_{19}H_{19}O_4$ $[M + H]^+$: 311.12779; found: 311.12769.

4.8.10. Synthesis of compound **66**

N,*N*-2-(Diethylamino)ethanethiol HCl (98.9 mg/0.584 mmol) and 5 mL of DMF anhydrous were placed on a round-bottom flask and under nitrogen atmosphere. The flask was cooled in an ice bath and when the internal temperature was below 5 °C, solid NaO*t*Bu (117.2 mg/1.22 mmol) was added in one portion. After 5 min the ice bath was removed and it was allowed to warm to room temperature. After 15 min, a solution of compound **63** (150 mg/0.487 mmol) in 2 mL of DMF anhydrous was added. The mixture was heated at reflux for 30 min under nitrogen atmosphere. The mixture was allowed to cool to room temperature and then was placed in an ice bath. A solution of HCl 1 M was added to the mixture until pH 1 and 20 mL of water was added to the mixture. The mixture was extracted with 3 × 50 mL of diethyl ether. The organic phase was washed with 3 × 50 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **66** was obtained as orange crystalline solid (102.8 mg/72%).

6-Hydroxy-2,2-dimethylpyrano[3,2-c]xanthen-7(2H)-one (**66**). Mp: 191–192 °C. IR ν_{max} (cm^{-1}) (KBr): 3488, 2970, 2919, 1648, 1606, 1464, 1356, 1272, 1214, 1110, 762. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. HRMS (ESI) *m/z* calcd for $C_{18}H_{15}O_4$ $[M + H]^+$: 295.09649; found: 295.09614.

4.8.11. Synthesis of compound **67**

N,*N*-2-(Diethylamino)ethanethiol HCl (35.6 mg/0.193 mmol) and 3 mL of DMF anhydrous were placed in a round-bottom flask and under nitrogen atmosphere. The flask was cooled in an ice bath and when the internal temperature was below 5 °C, solid NaO^{t}Bu (41.7 mg/0.43 mmol) was added in one portion. After 5 min the ice bath was removed and it was allowed to warm to room temperature. After 15 min, a solution of compound **65** (54 mg/0.16 mmol) in 2 mL of DMF anhydrous was added. The mixture was heated at reflux for 2 h under nitrogen atmosphere. The mixture was allowed to cool to room temperature and then was placed in an ice bath. A solution of HCl 1 M was added to the mixture until pH 1 and 10 mL of water was added to the mixture. The mixture was extracted with 3 × 25 mL of diethyl ether. The organic phase was washed with 3 × 25 mL of brine, dried over sodium sulfate anhydrous, filtered and the organic solvent evaporated. The crude product was purified by silica gel flash chromatography (*n*-hexane/ethyl acetate 9:1). Compound **67** was obtained as orange crystalline solid (21 mg/45%).

6-Hydroxy-2,2-dimethyl-3,4-dihydropyrano[3,2-c]xanthen-7(2H)-one (**67**). Mp: 205–206 °C. IR ν_{max} (cm^{-1}) (KBr): 3500, 2924, 2857, 1654, 1607, 1467, 1375, 1271, 751. ^{1}H NMR data, see Table 1. ^{13}C NMR data, see Table 2. HRMS (ESI) *m/z* calcd for $C_{18}H_{17}O_4$ $[M + H]^+$: 297.11214; found: 297.11204.

4.9. X-ray crystallography

Crystals of compound **63** suitable for X-ray diffraction were obtained by slow evaporation of a solution in acetone. They were orthorhombic, had a $P2_12_12_1$ space group and a cell volume of 1508.90(11) Å^3, and unit cell dimensions were a = 5.6701(2) Å, b = 15.5612(6) Å and c = 17.1013(9) Å (uncertainties in parenthesis). The calculated density was 1.357 g/ml. Diffraction data were collected at 293 K with a Gemini PX Ultra equipped with CuK_α radiation (λ = 1.54184 Å). A total of 4847 reflections were measured,

of which 2484 were independent, and 2393 were observed ($I > 2\sigma(I)$). The structure was solved by direct methods using SHELXS-97 and refined with SHELXL-97. Carbon and oxygen atoms were refined anisotropically. C1″ bound hydrogens were positioned with idealized geometry and refined riding on their parent C atom at a distance of 0.93 Å, with $U_{iso}(H) = 1.2\ U_{eq}$ (C). Other hydrogen atoms were refined freely with isotropic displacement parameters. The refinement converged to R (all data) = 5.15% and wR_2 (all data) = 13.50%. Tables containing the final fractional coordinates, temperature parameters, bond distances, and bond angles were deposited with the Cambridge Crystallographic Data Centre (CCDC reference number 933804).

4.10. Biological activity

4.10.1. Cell culture

The adherent cell lines MCF-7 (breast adenocarcinoma, ECACC, UK), NCI-H460 (non-small cell lung cancer, a kind gift from NCI, Bethesda, USA), A375-C5 (melanoma, ECACC, UK) and the suspension cell line HL-60 (acute myeloid leukemia, DSMZ, Germany) were routinely maintained in RPMI-1640 (with Glutamax, Lonza), supplement with 5% heat inactivated fetal bovine serum (FBS, PAA) at 37 °C in a humidified incubator with 5% CO_2. Cell number and viability were routinely determined with Trypan blue (Sigma). All the experiments were performed with cells in exponential growth and presenting more than 90% viability.

4.10.2. Cell growth inhibition assay

Cells were plated into 96-well tissue culture plates at appropriate densities (MCF-7 and NCI-H460 at 5×10^3 cells/well, A375-C5 at 7.5×10^3 cells/well and HL-60 cells at 1×10^4 cells/well) and incubated for 24 h. Cells were then treated with serial dilutions of the different compounds (ranging from 0 to 150 μM whenever possible). Following 48 h of incubation, the effect of the compounds in the growth of the different cell lines was analyzed with the Sulforhodamine B (SRB) assay, as adopted by the National Cancer Institute (NCI, USA) [63–65]. Briefly, following incubation, cells were fixed *in situ* with ice cold trichloroacetic acid (at 10% for adherent cells and at 16.7% for the cells growing in suspension). Following the SRB staining, plates were washed with 1% acetic acid, the bound dye was solubilized with 10 mM Tris Base and absorbance was measured at 510 nm in a microplate reader (Biotek Instruments Inc, Synergy XS, Winooski, USA). The GI_{50} values for the compounds synthesized (concentration causing a 50% inhibition of cell growth) were calculated from the plotted results. Doxorubicin was used as a positive control. The effect of the solvent (DMSO) on the growth of these cell lines was evaluated in preliminary experiments, by exposing untreated control cells to the maximum concentration of DMSO used in each assay (0.25%).

All experimental data is presented as means of GI_{50} values ± SE from at least three independent experiments (except when indicated otherwise).

4.11. Lipophilicity

4.11.1. Materials

The egg yolk phosphatidylcholine (EPC), HEPES, DMSO and NaCl were acquired from Sigma–Aldrich, the hexadecylphosphocholine (HDPC) from Cayman chemicals and the water used was double-deionized with conductivity less than 0.1 μS cm^{-1}. The 96-well plate reader used was a Synergy HT from Bio-Tek Instruments and the double beam spectrophotometer was a JASCO V660. The extrusion device used was a Lipex® Extruder manufactured from Northern Lipids and the filters were acquired from Whatman. The determination of the UV-spectrum was made either in a flat-bottomed 96-well UV-plates acquired from BD Biosciences or fused quartz cuvettes of 1.4 mL from Hellma.

4.11.2. Liposome preparation

Liposomes were prepared by evaporation to dryness with a nitrogen stream of an EPC solution prepared with chloroform/methanol (9/1). The resulting dried lipid film was dispersed with a buffer (HEPES: 10 mmol L^{-1}, $I = 0.1$ mol L^{-1} with NaCl, pH 7.4) and the mixture was vortex mixed to give multilamellar liposomes (MLVs). The MLVs were extruded 10 times through polycarbonate filters with a pore diameter of 100 nm to form large unilamellar vesicles (LUVs).

4.11.3. Micelle preparation

Micelle solutions were prepared by dissolution of hexadecylphosphocholine (HDPC) in buffer (HEPES: 10 mmol L^{-1}, $I = 0.1$ mol L^{-1} with NaCl, pH 7.4) and mixed by vortex.

4.11.4. General procedure for K_p determination

The procedure used was adapted from the literature [38] and was the following: i) 3 μL of compound solubilized in DMSO (concentration established before in 4.11.5) was added to each well; ii) increasing volumes of liposome or micelles were added to each well and the final concentration for 300 μL were 0 (only buffer), 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 μM. In the end, for the same concentration of xanthone, increasing concentrations of lipid were prepared; iii) HEPES buffer was added to make up 300 μL of final volume; iv) a blank was used for each concentration of lipid containing 1% of DMSO; v) the plate was incubated at 37 ± 0.1 °C for 30 min and then the spectrum was traced from 240 nm to 600 nm. For poorly soluble compounds, the solutions were prepared with the same concentration but in eppendorf (1.5 mL) instead. Each solution was mixed by vortex and incubated for 30 min. The spectrum ranging from 240 nm to 600 nm was traced individually in a double-beam spectrophotometer for each sample at 37 °C. The compound concentration used in the determination of the K_p varied from 2.5 μM for the most poorly soluble compounds to 20 μM for the most soluble.

Partition coefficient (K_p) was calculated by adjusting experimental data through a nonlinear regression of Levenberg–Marquardt in where the adjustable parameter was K_p. The program was available free of charge in the Supplementary information from Ref. [38].

All experimental data are presented as means ± SE from at least three independent experiments. The linear regression analysis was calculated using the IBM SPSS Statistics 20.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.09.

012. These data include MOL files and InChiKeys of the most important compounds described in this article.

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