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Research paper

Novel chromone and xanthone derivatives: Synthesis and ROS/RNS scavenging activities

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ABSTRACT

Chromones and xanthones are oxygen-containing heterocyclic compounds acknowledged by their antioxidant properties. In an effort to develop novel agents with improved activity, a series of compounds belonging to these chemical classes were prepared. Their syntheses involve the condensation of appropriate 2-methyl-4*H*-chromen-4-ones, obtained *via* Baker-Venkataraman rearrangement, with (*E*)-3-(3,4-dimethoxyphenyl)acrylaldehyde to provide the corresponding 2-[(1*E*,3*E*)-4-(3,4-dimethoxyphenyl)buta-1,3-dien-1-yl]-4*H*-chromen-4-ones. Subsequent electrocyclization and oxidation of these compounds led to the synthesis of 1-aryl-9*H*-xanthen-9-ones. After cleavage of the protecting groups, hydroxylated chromones and xanthones were assessed as scavenging agents against both reactive oxygen species (ROS) [superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and peroxyl radical ($ROO^{\bullet}$)] and reactive nitrogen species (RNS) [nitric oxide ($^{\bullet}NO$) and peroxynitrite anion ($ONOO^-$)]. Generally, all the tested new hydroxylated chromones and xanthones exhibited scavenger effects dependent on the concentration, with IC_{50} values found in the micromolar range. Some of them were shown to have improved scavenging activity when compared with previously reported analogues, allowing the inference of preliminary conclusions on the structure–activity relationship.

1. Introduction

Chromones (4*H*-chromen-4-ones) constitute an important class of oxygenated heterocyclic compounds that commonly occur in nature [1]. Their large variety of biological and pharmacological effects, dependent on structural features such as the type, number and position of substituents attached to the chromone nucleus, is well-documented and several derivatives are even currently used as therapeutic agents [2–4].

9*H*-Xanthen-9-ones or simply xanthones are another class of naturally occurring oxygen-containing heterocyclic compounds [5]. They are characterized by the presence of a huge diversity of functional groups associated to a range of pharmacological properties that includes anti-inflammatory, antimicrobial, antioxidant, cardiovascular protective, hepatoprotective and cytotoxic activities [6–10].

Despite the successful application of certain natural compounds as drugs, their structurally limited accessibility imposed by biosynthetic pathways, the complex and time consuming process of extraction and purification or even their economically unfeasible synthesis on a gram-scale, often make them not candidate drugs for scientific and pharmaceutical industry in the quest for new therapeutic hits [11]. Being aware of this knowledge, the development of new synthetic procedures applied for the synthesis of more efficient bioactive compounds constitutes an interesting and hot challenge for organic chemists.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) occurred in biological systems through a variety of sources and under normal and controlled conditions mediate and regulate a

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variety of physiological functions. However, ROS and RNS over-accumulation, known as oxidative stress, caused by the imbalance between their generation and elimination, results in severe deleterious effects on various cell constituents as DNA, RNA, lipids, and proteins [12,13] and it is consequently implicated in numerous physiopathologies such metabolic disorders, cellular aging, inflammation, cardiovascular dysfunction, neurodegenerative diseases and carcinogenesis [12–15].

The maintenance of ROS/RNS balance is carried out by endogenous enzymatic antioxidant defenses such as superoxide dismutase, glutathione peroxidase, thioredoxin reductase and catalase and by non-enzymatic compounds such as glutathione, uric acid and coenzyme Q. If the internal production of antioxidants is not enough to neutralize all the ROS/RNS produced, a series of exogenous non-enzymatic antioxidants can be provided from human diet that includes carotenoids, phenolic compounds, and flavonoids, among others [16]. Thus, in the demand for new antioxidant agents, the design of novel and more effective scavengers of ROS and RNS is considered a key topic for several research groups. Continuing our interest in this area herein we report the synthesis of novel chromone and xanthone derivatives, as well as the scavenging activity of their hydroxyl constituents against ROS [superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and peroxyl radical (ROO$^\bullet$)] and RNS [nitric oxide ($^\bullet$NO) and peroxynitrite anion ($ONOO^-$)].

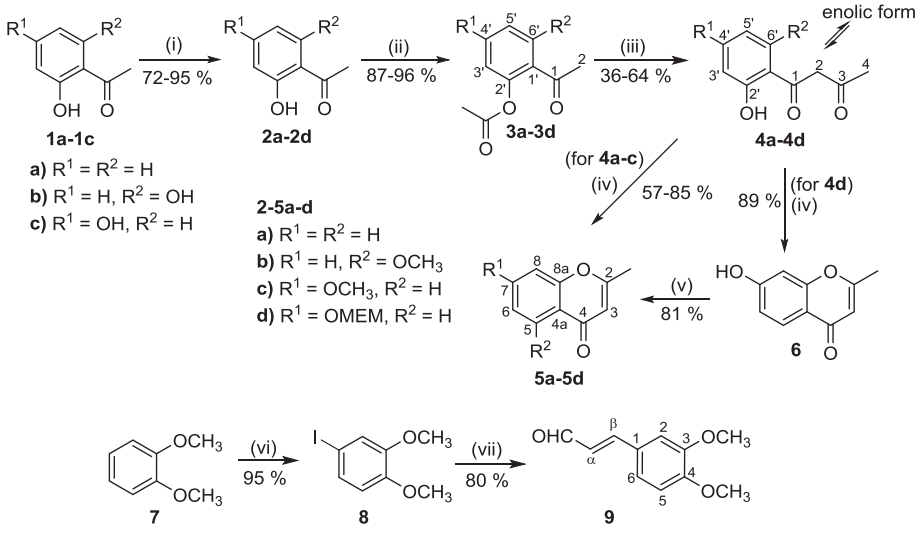
2. Results and discussion

2.1. Chemistry

The required starting 2-methyl-4*H*-chromen-4-ones **5a-5c** were synthesized by the Baker-Venkataraman method in moderate overall yields [17] (Scheme 1). It started by the monoprotection of 2'-hydroxyacetophenones **1b-1c** with dimethyl sulfate in refluxing acetone. The next steps involved the acetylation of the protected 2'-hydroxyacetophenones **2a-2c** followed by the base-catalysed Baker-Venkataraman rearrangement of the formed ester **3a-3c**, leading to the 1,3-diketone derivatives **4a-4c** in good yields. Finally, these 1,3-diketones were treated with a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) in DMSO to afford the desired 2-methyl-4*H*-chromen-4-ones **5a-5c**. The most important structural features in the ^{1}H NMR spectra of chromones **5a-5c** are in each case a singlet at δ 2.30–2.40 ppm corresponding to the resonance of 2-CH_3 protons and a singlet at δ 6.07–6.18 ppm corresponding to **H-3**. Furthermore, the ^{13}C NMR spectra of chromones **5a-5c** showed signals at δ 19.7–20.6, 110.3–111.8 and 166.2–178.0 ppm assigned to resonances of 2-*C*H$_3$, C-3 and C-4, respectively.

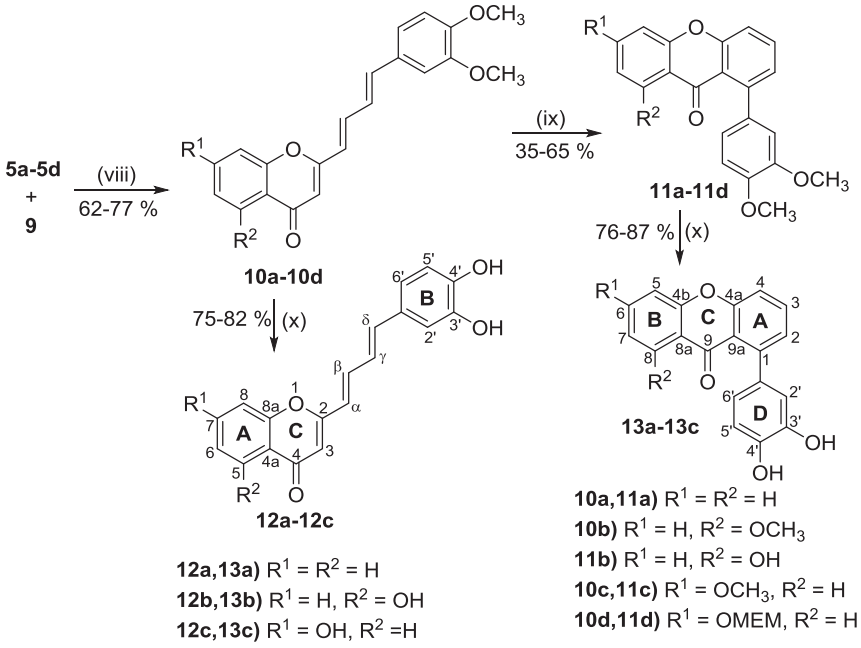
The required (*E*)-3-(3,4-dimethoxyphenyl)acrylaldehyde **9** was synthesized through a two-step method starting with an acid-catalysed iodination of 1,2-dimethoxybenzene 7 with *N*-iodosuccinimide (NIS), followed by a palladium-catalysed cross-coupling reaction with acrolein diethyl acetal (Scheme 1) and its structure was confirmed by NMR spectroscopy. The ^{1}H NMR spectrum of this aldehyde showed: i) two singlets at δ 3.93 and 3.94 ppm due to the resonances of 3- and 4-OC*H*$_3$; two doublets at δ 6.62 and 7.42 ppm attributed to the resonances of the H-α and H-β vinyl protons, respectively, in a *trans* configuration ($J_{H\alpha,H\beta}$ 15.8 Hz); and a doublet at high frequency value (δ 9.66 ppm) assigned to the *C*HO resonance. The structure of this aldehyde was also confirmed by the ^{13}C NMR signals of *C*HO (193.5 ppm), 3,4-(OCH$_3$)$_2$ (55.8 and 55.9 ppm), and of C-α (126.6 ppm) and C-β (152.8 ppm).

The reactivity of 2-methyl-4*H*-chromen-4-ones has been the main topic of several works [18]. Among them, there are a number of condensation reactions with carbonyl compounds to afford new heterocyclic systems. Following our previous work involving condensation, electrocyclization and oxidation reactions [19], we have synthesized chromones **10a-10c** and xanthones **11a-11c**, respectively. Thus, the 2-[(1*E*,3*E*)-4-(3,4-dimethoxyphenyl)buta-1,3-dien-1-yl]-4*H*-chromen-4-ones **10a-10c** were prepared in good yields by the condensation of 2-methyl-4*H*-chromen-4-ones **5a-5c** with (*E*)-3-(3,4-dimethoxyphenyl)acrylaldehyde **9** in the presence of 4 equiv of sodium ethoxide (generated *in situ*), at room temperature (Scheme 2). The most important features in the ^{1}H NMR spectra of 2-[(1*E*,3*E*)-4-(3,4-dimethoxyphenyl)buta-1,3-dien-1-yl]-4*H*-chromen-4-ones **10a-10c** are in each case: i) the absence of the singlet due to the *CH*$_3$ proton resonance of the precursor 2-



(i) Me_2SO_4 or MEMCl (for **1c**), K_2CO_3, acetone, reflux, 20 min or 1 h (for **1c**); (ii) MeCOCl, pyridine, r.t., 2 h; (iii) NaH, THF, reflux, 2 h; (iv) *p*-TSA, DMSO, 100 °C, 2 h; (v) MEMCl, K_2CO_3, acetone, reflux, 1 h; (vi) NIS, TFA, CH_3CN, 80 °C, 2 h; (vii) acrolein diethylacetal, $^nBu_4NOAc$, K_2CO_3, $Pd(OAc)_2$, DMF, 90 °C, 4 h.

Scheme 1.



(viii) Na, EtOH, r.t., 12 h; (ix) I_2, 1,2,4-TCB, reflux, 48 h; (x) BBr_3, dry CH_2Cl_2, -78 ºC to r.t., 3-4 h

Scheme 2.

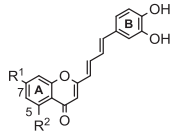
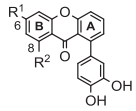
methylchromones **5a-5c**; ii) the change in the multiplicity of protons H-α and H-β from doublets to multiplets, indicating that these protons are now included in a 1,3-diene chain. The resonances of C-α, C-β, C-γ and C-δ carbons appear in ^{13}C NMR spectra at δ 122.1–122.5, 136.9–137.4, 124.8–125.2 and 138.5–138.8 ppm, respectively, while that of the carbonyl carbon appear at 177.7–178.3 ppm. The electrocyclization and oxidation reactions of 2-[(1*E*,3*E*)-4-(3,4-dimethoxyphenyl)buta-1,3-dien-1-yl]-4*H*-chromen-4-ones **10a-10c** were performed under refluxing 1,2,4-trichlorobenzene (TCB) with a catalytic amount of iodine to afford the desired 1-(3,4-dimethoxyphenyl)-9*H*-xanthen-9-ones **11a-11c** in moderate to good yields (Scheme 2). During this step the 5-OMe-protecting group of chromone **10b** was cleaved giving the corresponding 8-hydroxyxanthone **11b**. This was confirmed from the ^1H NMR spectrum by the presence of a singlet at δ 12.60 ppm assigned to the resonance of the 8-O*H* proton. The most relevant structural features present in the ^1H NMR spectra of compounds **11a-11c** are three double doublets at δ 7.17–7.19, 7.65–7.73 and 7.47–7.51 ppm, due to the resonances of H-2, H-3 and H-4, respectively, which means that the cyclization to xanthones occurred. The HMBC and HSQC spectra of xanthones **11a-11c** allowed to assign the carbon resonances of C-2 (δ 127.3–127.8 ppm), C-3 (δ 132.7–134.2 ppm), C-4 (δ 117.2–117.5 ppm) and the newly formed quaternary carbons C-9a (δ 117.9–119.4 ppm) and C-1 (δ 143.7–144.0 ppm. Finally and in order to obtain the desired hydroxylated derivatives **12a-12c** and **13a-13c**, the deprotection reaction of respectively **10a-10c** and **11a-11c**, occurred in the presence of boron tribromide (BBr_3) in dry CH_2Cl_2 (Scheme 2). Unfortunately, the deprotection step was not straightforward for 7-OMe-chromone **10c** and 6-OMe-xanthone **11c**, which was not achieved even after several days of reaction with BBr_3. This drawback was overcome by changing the protecting 4'-hydroxy group of 2′,4'-dihydroxyacetophenone **1c** with methoxyethoxymethyl chloride (MEMCl). The Baker-Venkataraman procedure for the synthesis of chromone **5d** was similar to the described for the methoxylated chromones **5a-5c**, however, it required one more OH–protection step due to the release of the MEM group during the cyclodehydration reaction (Scheme 1, *vide experimental*). The ^1H NMR spectrum of chromone **5d** showed particular signals assigned as two singlets at δ 3.38 and 5.36 ppm and two multiplets at δ 3.55–3.58 and 3.83–3.86 ppm corresponding to the resonances of the protons of MEM moiety. On the other hand, its carbon resonances can be observed at δ 59.0, 68.0, 71.4 and 93.2 ppm in the ^{13}C NMR spectrum of chromone **5d**. The condensation reaction of chromone **5d** with (*E*)-3-(3,4-dimethoxyphenyl)acrylaldehyde **9** and subsequent electrocyclization and oxidation reactions occurred as already mentioned for the methoxylated chromones **5a-5c**. Finally, the MEM group was easily removed in a couple hours by reaction with BBr_3. Thus, the most important characteristics in the ^1H NMR spectra of compounds **12a-12c** and **13a-13c** are: i) the singlets corresponding to the resonances of O*H* protons of the catechol moiety at δ 9.08–9.42 ppm for derivatives **12a-12c** and at δ 8.86–8.94 ppm for derivatives **13a-13c**; ii) the singlets assigned to the resonances of O*H* protons at position 5 of chromone **12b** and of position 8 of xanthone **13b**, at δ 12.59–12.77 ppm, respectively; and iii) the singlets attributed to O*H* protons at position 7 of chromone **12c** and of position 6 of xanthone **13c** at δ 10.81–10.89 ppm, respectively.

2.2. ROS and RNS scavenging activity studies

In this study, novel chromone **12a-12c** and xanthone **13a-13c** derivatives were subjected to preliminary screening of their antioxidant potential in non-cellular systems, through their ability to scavenge ROS and RNS involved in oxidative stress processes. All compounds possess a 3,4-dihydroxyphenyl moiety, known as catechol unit, in the B-ring of chromone and in the D-ring of xanthone, an important feature for the improved scavenging activity of ROS/RNS presented by several phenolic compounds [20–22].

Xanthones **13a-13c** were able to prevent the $O_2^{\bullet-}$-dependent reduction of nitroblue tetrazolium chloride (NBT) in a concentration-dependent manner (Table 1). Derivatives **13a** and **13c** were the most active tested compounds presenting IC_{50} values of 55 ± 4 and 61 ± 2 μM, respectively, and similar to the positive

Table 1
Scavenging activity of the tested compounds against superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$) (IC_{50}, mean ± SEM).

Compound		R^1	R^2	IC_{50} (μM)			
				$O_2^{\bullet-}$	H_2O_2	HOCl	$^1O_2$
Chromones							
12a		H	H	–	143 ± 8	32 ± 3	1.3 ± 0.1
12b		H	OH	–	125 ± 13	94 ± 15	1.1 ± 0.2
12c		OH	H	36%[200μM a]	121 ± 9	17 ± 3	1.6 ± 0.4
Xanthones							
13a		H	H	55 ± 4	12%[1000μM a]	158 ± 18	4.4 ± 0.7
13b		H	OH	80 ± 11	658 ± 23	96 ± 8	10 ± 2
13c		OH	H	61 ± 2	518 ± 26	31 ± 3	7.3 ± 0.6
Positive control							
Quercetin				60 ± 7	1338 ± 42	2.4 ± 0.3	1.5 ± 0.2

[a] Scavenging effect (mean %) at the highest tested concentration (in superscript).

control quercetin (IC_{50} 60 ± 7 μM). Derivative **13b** were less active with an IC_{50} of 80 ± 11 μM. These results seem to indicate that the presence of the OH group in position 8 of the xanthone core is disadvantageous for the $O_2^{\bullet-}$ scavenging activity since this derivative is less effective than their parent compound **13a** and their 6-OH derivative, **13c**. This comes in agreement with previous studies on flavonoids that the strong intramolecular hydrogen bond between the OH group *peri* to the carbonyl group is generally disadvantageous for the scavenging activity of these compounds [23]. In addition, the new 1-arylxanthones **13a-13c** were less potent scavengers than other xanthones possessing a catechol group at position 2 or 3 of their skeleton [24]. It was not possible to determine the scavenging effect of chromones **12a** and **12b** due to the precipitation that occurred in the tested system. Chromone **12c** caused 36% inhibition of absorbance at its maximum concentration (200 μM). The lack of reactivity of these chromones towards $O_2^{\bullet-}$ is not in accordance to the knowledge about structure–activity relationship of several flavonoids and phenolic compounds, bearing a catechol or styryl moiety, to scavenge this ROS [20,21,25].

All the tested compounds proved to be effective scavengers of H_2O_2 in a concentration-dependent manner, except for compound **13a**, which only reached 12% effect at the highest tested concentration (1000 μM) (Table 1). Chromones **12a-12c** (IC_{50} values of 143 ± 8 μM, 125 ± 13 μM and 121 ± 9 μM, respectively) were noticeably more active than xanthones **13a-13c**. All the tested chromones were even more active than the positive control quercetin (IC_{50} 1338 ± 42 μM). These results indicate that the presence of 7-OH and 5-OH groups is favourable to the reaction of chromones with H_2O_2 but no influence was observed for the position of the OH groups in the chromone core since no meaningful differences were found for derivatives **12b** and **12c**. Similar to chromones, the presence of OH groups in B-ring of the xanthone moiety is an important structural feature. However, their presence at C-6 (compound **13c**, IC_{50} 518 ± 26 μM) seems to have a positive effect for the H_2O_2 scavenging effect in comparison to its presence at C-8 (compound **13b**, IC_{50} 658 ± 23 μM). The study developed by Santos et al. [24] showed that xanthones possessing catechol groups at positions 2 or 3 of the xanthone backbone have no effect in preventing the oxidation of lucigenin promoted by H_2O_2. Taking into consideration that these xanthones have no OH groups in B-ring, we can infer that their inclusion appears to be important to enhance the scavenging properties against this ROS. This finding is also corroborated with the high inhibitory potential on H_2O_2 presented by trihydroxyxanthones when compared with dihydroxyxanthones [26,27].

The HOCl-induced oxidation of dihydrorhodamine 123 (DHR) was efficiently prevented by all the tested compounds, in a concentration-dependent manner. Compounds **12a**, **12c** and **13c** proved to be the most active ones, providing IC_{50} values of 32 ± 3 μM, 17 ± 3 μM and 31 ± 3 μM, respectively (Table 1). The results showed that the presence of a 7-OH group in chromone **12c** led to a higher scavenging effect while the introduction of a 5-OH group, chromone **12b**, led to a weaker scavenger then their parent compound, chromone **12a**. The importance of these OH groups on the A-ring were previously reported for the HOCl scavenging activity of flavones (analogues of chromones **12a-12c** without the 1,3-diene system) and 2-styrylchromones (analogues of chromones **12a-12c** lacking one double bond), related to the ability to promote the chlorination at C-6 and C-8 positions, *via* an electrophilic aromatic substitution [28]. On the other hand, the additional double bond found in the new chromones seems to be unfavourable for the HOCl scavenging activity since these compounds were less potent scavengers than the previously tested 2-styrylchromones [29]. Similarly to the above described for the scavenging effect against H_2O_2, the 6-OH substitution (compound **13c**, IC_{50} 31 ± 3 μM) in the tested xanthones favoured the scavenging of HOCl in comparison to the 8-OH derivative (compound **13b**, IC_{50} 96 ± 8 μM) or even their absence in the main core (compound **13a**, IC_{50} 158 ± 18 μM). The 2,3-diarylxanthones already reported were found to be more active than the tested 1-arylxanthones, suggesting the importance of the catechol group at C-2 or C-3 rather than in C-1 to their reaction with this ROS [24].

All of the tested compounds were able to scavenge $^1O_2$ in a concentration-dependent manner. Chromones **12a-12c** (IC_{50} values of 1.3 ± 0.1 μM, 1.1 ± 0.2 μM and 1.6 ± 0.4 μM, respectively) were considerably more effective than xanthones **13a-13c** (IC_{50} values of 4.4 ± 0.7 μM, 10 ± 2 μM and 7.3 ± 0.6 μM, respectively) (Table 1). Moreover, chromones **12a** and **12b** showed a slightly higher effect than the positive control, quercetin (IC_{50} 1.5 ± 0.2 μM). Analysing the results we can conclude that chromones **12a-12c** were shown to be more effective scavengers of $^1O_2$ than the 2-styrylchromones and flavones with comparable structures [29], indicating that the additional double bonds at C-2 of the chromone moiety clearly influence the scavenger effect, increasing its activity. Another important feature is the presence of the 5-OH group to improve the $^1O_2$ scavenging potential in contrast to their substitution at C-7 that

brings no advantage [29]. In what concerns to xanthones, the absence of OH groups in B-ring increases $^1O_2$ scavenging activity when compared to its presence at C-6 or C-8 of the xanthone backbone, the 6-OH derivative (**13c**) being more active than the 8-OH derivative (**13b**). Curiously, a different behaviour is observed to α-mangostin (a trihydroxyxanthone) that presented a stronger scavenger activity than γ-mangostin (a dihydroxyxanthone) [26].

The results from the Oxygen Radical Absorbance Capacity (ORAC) assay are listed in Table 2. All the tested compounds were able to delay the loss of fluorescence, preventing the ROO•-dependent fluorescein oxidation, in a concentration-dependent manner. In fact, it has been previously referred that the presence of a catechol unit plays an essential role in what concerns the ROO• scavenging activity of phenolic compounds, delaying or preventing lipid peroxidation [21,30]. All of compounds achieved similar ORAC values which were apparently superior to the value presented by the water-soluble vitamin E analogue trolox, used as standard. In fact, if we consider that compounds **12a-12c** and **13a-13c** are expected to trap 4 peroxyl radicals similar to quercetin while trolox only traps 2 [31], the tested compounds are slightly less effective than trolox and clearly less effective than quercetin (Table 2). The results indicate that chromone **12c** (1.63 ± 0.09) has higher scavenging effect, followed by chromones **12a** (1.44 ± 0.01) and **12b** (1.40 ± 0.05), with similar ORAC values. Considering that chromones **12a-12c** are less active than the 2-styrylchromones studied by Gomes et al. we can conclude that the additional double bond at C-2 decreases the scavenging effect against this ROS [29]. Analysing the results of xanthones, compounds **13a** (1.5 ± 0.1) and **13c** (1.58 ± 0.09) are the most active ones, followed by compound **13b** (1.1 ± 0.1). An interesting feature common to all the ROS described is the fact that 6-OH xanthone **13c** was shown to have improved scavenging activity when compared to the 8-OH xanthone **13b**. Similar to the H_2O_2 scavenging assays, the ROO• scavenging effects of the tested 1-arylxanthones showed to be more efficient than the 2,3-diarylxanthones studied by Santos et al. [24]. In fact, they concluded that, unlike to the observed for the other ROS, the introduction of phenolic groups at C-2 or C-3 of the xanthone core is more favourable for the scavenging of ROO• that the addition of catechol groups in these positions.

The •NO-induced oxidation of 4,5-diaminofluorescein (DAF-2) was efficiently inhibited by all of the tested compounds, in a concentration-dependent manner. Compounds **12a**, **12b**, **12c** and **13c** revealed to be the most potent scavengers with IC_{50} values of 0.82 ± 0.07 μM, 1.23 ± 0.08 μM, 1.42 ± 0.09 μM and 1.08 ± 0.05 μM, respectively (Table 3). The absence of OH groups in the A-ring of chromones increases the scavenging activity against •NO when compared to its presence in position 5 or 7, being more effective in the former case. Chromones **12a-12c** were much weaker scavengers than the structurally related 2-styrylchromones tested by Gomes et al., indicating that the introduction of a double bond at C-2 decreases the scavenging capacity [29]. With respect to xanthone derivatives, the more active was compound **13c** followed by compound **13b** (IC_{50} 1.5 ± 0.1 μM) and compound **13a** (IC_{50} 1.99 ± 0.09 μM). These results demonstrate that the presence of an OH group in position 6 of the A-ring increases the scavenging activity of xanthones against •NO when compared to its presence in position 8 and even more in their absence in that ring.

All the tested compounds were shown to efficiently prevent $ONOO^-$-induced oxidation of DHR, in a concentration-dependent manner. These assays were also performed in the presence of 25 mM $NaHCO_3$ in order to mimics the physiological CO_2 concentration *in vivo*. The fast reaction between $ONOO^-$ and CO_2 ($k_2 = 3–5.8 \times 10^4\ M^{-1}\ s^{-1}$) leads to the formation of other highly reactive species that affects the reactivity of $ONOO^-$ scavengers in the reaction medium, either increasing or decreasing their effects [32]. Generally, the compounds were shown to be equally potent scavengers in the absence and presence of $NaHCO_3$, with the exception of xanthones **13a** (more active in the presence of $NaHCO_3$) and **13c** (more active in the absence of $NaHCO_3$). A similar conclusion can be drawn from the scavenging effects presented by several phenolic compounds and flavonoids [29,33,34]. Chromone **12c** proved to be the most active derivative, with IC_{50} of 0.29 ± 0.02 and 0.30 ± 0.04 μM, in the absence and in the presence of $NaHCO_3$ 25 mM, respectively (Table 3).

Concerning the results in the absence of $NaHCO_3$, chromones **12a-12c** (IC_{50} range from 0.29 to 0.34 μM) were visibly more efficient than xanthones **13a-13c** (IC_{50} range from 0.39 to 0.57 μM) (Table 3) and even than the positive control, quercetin (IC_{50} 0.45 ± 0.07 μM) (Table 2). No meaningful differences were observed in the scavenging effect of all chromone derivatives **12a-12c**, which indicates that the catecholic B-ring, a common feature in these compounds, is the main factor responsible for the $ONOO^-$ scavenging capacity [29]. The lower IC_{50} value of xanthone **13c** indicates that the 6-OH substitution improved the $ONOO^-$ scavenging effect when compared to 8-OH substitution (xanthone **13b**, IC_{50} 0.57 ± 0.08 μM) or their parent compound (xanthone **13a**, IC_{50} 0.51 ± 0.08 μM). All 1-arylxanthones tested presented lower efficiency than 2,3-diarylxanthones reported by Santos et al. [24].

In the presence of $NaHCO_3$, the most active scavengers were compounds **12a**, **12c** and **13a** with IC_{50} values of 0.34 ± 0.05 μM,

Table 2
Scavenging activity of the tested compounds against peroxyl radical (ROO•) expressed as ORAC values (mean ± SEM).

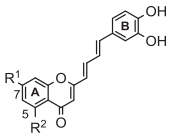
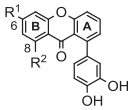
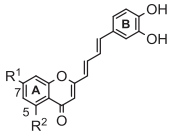
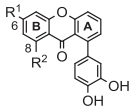
Compound		R^1	R^2	$ORAC_{ROO•}$ ± SEM (μM trolox equiv/μM compound)
Chromones				
12a		H	H	1.44 ± 0.01
12b		H	OH	1.40 ± 0.05
12c		OH	H	1.63 ± 0.09
Xanthones				
13a		H	H	1.5 ± 0.1
13b		H	OH	1.1 ± 0.1
13c		OH	H	1.58 ± 0.09
Positive controls				
Quercetin				3.4 ± 0.3
Trolox				1

Table 3
Scavenging activity of the tested compounds against nitric oxide (•NO) and peroxynitrite anion ($ONOO^-$) (with and without 25 mM $NaHCO_3$) (IC_{50}, mean ± SEM).

Compound		R^1	R^2	IC_{50} (μM)		
				•NO	$ONOO^-$ without $NaHCO_3$	$ONOO^-$ with $NaHCO_3$
Chromones						
12a		H	H	0.82 ± 0.07	0.33 ± 0.02	0.34 ± 0.05
12b		H	OH	1.23 ± 0.08	0.34 ± 0.03	0.4 ± 0.1
12c		OH	H	1.42 ± 0.09	0.29 ± 0.02	0.30 ± 0.04
Xanthones						
13a		H	H	1.99 ± 0.09	0.51 ± 0.08	0.35 ± 0.05
13b		H	OH	1.5 ± 0.1	0.57 ± 0.08	0.56 ± 0.07
13c		OH	H	1.08 ± 0.05	0.39 ± 0.08	0.66 ± 0.07
Positive controls						
Quercetin				0.77 ± 0.07	0.45 ± 0.07	0.68 ± 0.09

0.30 ± 0.04 μM and 0.35 ± 0.05 μM, respectively (Table 3). Analysing the results in the absence and in the presence of $NaHCO_3$, we can state that chromones **12a-12c** were shown to possess a similar behaviour regarding the presence of OH substituents but a higher scavenging activity than the 2-styrylchromones [29]. For xanthone derivatives **13a-13c**, the absence of OH groups in the B-ring enhanced the $ONOO^-$ scavenging activity in comparison to their inclusion at position 8 and even more at position 6 of the xanthone skeleton. The range of the IC_{50} values found for the tested 1-arylxanthones were similar to those obtained from 2,3-diarylxanthones [24].

3. Conclusion

Novel 2-[(1*E*,3*E*)-4-(3,4-dimethoxyphenyl)buta-1,3-dien-1-yl]-4*H*-chromen-4-ones and corresponding 1-aryl-9*H*-xanthen-9-ones were prepared from simple starting materials. Generally, all the tested new hydroxylated chromones and xanthones exhibited scavenger effects dependent on the concentration, with IC_{50} values found in the micromolar range. We can also state that no homogeneous behaviour was observed in the scavenging activity of the tested compounds against the most physiologically relevant ROS and RNS, varying in accordance with the reactive species under study. The 7-hydroxychromone **12c** appeared as the lead compound being the most active for scavenging H_2O_2, HOCl, $ROO^•$ and $ONOO^-$.

Some of the tested compounds were shown to have improved scavenging activity when compared with previously reported analogues, possibly suggesting novel structure–activity relationships that need to be confirmed in future studies: the additional double bond of the new synthesized chromones increased the $^1O_2$ and $ONOO^-$ scavenging ability [29]; the catechol group in position C-1 of the xanthone core has a stronger effect in the H_2O_2 and $ROO^•$ scavenging activity rather than in positions C-2 or C-3 to their reaction with these ROS [24]. In conclusion, the novel synthesized chromone and xanthone derivatives seem to be promising pharmacophores with potential therapeutic applications associated to oxidative stress disorders.

4. Experimental section

4.1. Chemistry, general information

Melting points were measured in a Büchi Melting Point B-540 apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance 300 spectrometer (300.13 for ^1H and 75.47 MHz for ^{13}C), with $CDCl_3$ as solvent, unless otherwise stated. Chemical shifts (d) are reported in ppm, and coupling constants (*J*) in Hz. The internal standard was TMS. Positive-ion ESI mass spectra were acquired with a QTOF 2 instrument. Elemental analyses were obtained with a CHNS 932 LECO analyser (University of Aveiro).

4.2. Reagents and chemicals

All chemicals and solvents used were of analytical grade, obtained from commercial sources and used as received or dried by standard procedures. The endoperoxide disodium 3.3'-(1,4-naphthalene)bispropionate ($NDPO_2$) was synthesized according to procedure previously described by some of the authors [35].

4.3. Synthesis of methoxy-2'-hydroxyacetophenones (2b, 2c)

Dimethyl sulfate (3.1 mL, 32.7 mmol) was added to a solution of the appropriate 2'-hydroxyacetophenone **(1b**, **1c)** (29.7 mmol) in acetone (60 mL) with potassium carbonate (12.3 g, 89.1 mmol). The mixture was stirred at reflux for 20 min. The inorganic salts were removed by filtration and washed with acetone (50 mL). The solvent was evaporated to dryness and the residue was purified by silica gel column chromatography using dichloromethane as eluent.

*6′-Methoxy-2'-hydroxyacetophenone (**2b**)* (4.69 g, 95%) and *4'-methoxy-2'-hydroxyacetophenone (**2c**)* (4.59 g, 93%), which showed spectroscopic and analytical data identical to one previously reported [36].

4.4. Synthesis of 1-{2-hydroxy-4-[(2-methoxyethoxy)methoxy] pheny}ethan-1-one (2d)

Methoxyethoxylmethyl chloride (MEMCl) (4.14 mL, 36.3 mmol) was added to a solution of 2′,4'-dihydroxyacetophenone (**1c**) (33 mmol) in acetone (60 mL) with potassium carbonate (9.12 g, 66 mmol). The mixture was stirred at reflux for 1 h. The inorganic salts were removed by filtration and washed with acetone (50 mL). The solvent was evaporated to dryness and the residue was purified by silica gel column chromatography using dichloromethane as eluent, giving 1-{2-hydroxy-4-[(2-methoxyethoxy)methoxy] phenyl}ethan-1-one (**2d**) in good yield (5.70 g, 72%).

*4.4.1. 1-{2-hydroxy-4-[(2-methoxyethoxy)methoxy]pheny})ethan-1-one (**2d**)*

Colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.57 (s, 3H, H-2), 3.38 (s, 3H, OCH_3), 3.54–3.57 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 3.80–3.83 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 5.30 (s, 1H, CH_3OCH_2-CH_2OCH_2O), 6.57 (dd, 1H, H-5′, *J* 8.8, 2.4 Hz), 6.60 (d, 1H, H-3′, *J* 2.4 Hz),7.65 (d, 1H, H-6′, *J* 8.8 Hz), 12.61 (s, 1H, 2′-O*H*) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.3 (C-2), 59.0 ($CH_3OCH_2CH_2OCH_2O$), 68.1 ($CH_3OCH_2CH_2OCH_2O$), 71.5 ($CH_3OCH_2CH_2OCH_2O$), 93.0 (CH_3OCH_2-CH_2OCH_2O), 103.8 (C-3′), 108.1 (C-5′), 114.7 (C-1′), 132.4 (C-6′), 163.5 (C-2), 164.8 (C-2′), 202.8 (C-1) ppm. MS *m/z* (ESI, %): 241 ([M+H]$^+$, 100), 263 ([M+Na]$^+$, 64). HRMS (ESI) *m/z* calcd. for $C_{12}H_{17}O_5$ [M+H]$^+$ 241.1071; found 241.1073.

*4.5. Synthesis of 2-acetylphenyl acetates (**3a-3d**)*

Acetyl chloride (5.2 mL, 73.4 mmol) was added to a solution of the appropriate acetophenone (**2a-d**) (36.7 mmol) in dry pyridine (50 mL). The solution was stirred under nitrogen atmosphere at room temperature for 2 h. After that period, the solution was poured into ice (100 g) and water (100 mL), and the pH was adjusted to 4 with diluted hydrochloric acid. The mixture was vigorously stirred for 15 min and the aqueous layer extracted with dichloromethane (3 × 100 mL). The organic residue was dried over anhydrous sodium sulfate and the solvent was evaporated to dryness. The residue was purified by silica gel column chromatography using dichloromethane as eluent giving the 2-acetylphenyl acetates (**3a-3d**) in excellent yields.

*4.5.1. 2-Acetylphenyl acetate (**3a**)*

(6.15 g, 94%), m.p. 83–85 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, 2′-OCOCH_3), 2.56 (s, 3H, H-2), 7.12 (dd, 1H, H-3′, *J* 7.9, 1.1 Hz), 7.33 (ddd, 1H, H-5′, *J* 7.7, 7.6, 1.1 Hz), 7.54 (ddd, 1H, H-4′, *J* 7.9, 7.6, 1.7 Hz), 7.82 (dd, 1H, H-6′, *J* 7.7, 1.7 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.2 (2′-OCO*C*H_3), 29.3 (C-2), 123.8 (C-3′), 126.0 (C-5′), 130.3 (C-6′), 130.6 (C-1′), 133.4 (C-4′), 149.0 (C-2′), 169.5 (2′-O*C*OCH$_3$), 197.6 (C-1) ppm. MS *m/z* (EI, %): 178 ([M]$^{+\cdot}$, 4), 136 (65), 121 (100), 107 (4), 93 (8), 92 (9), 77 (7), 65 (11), 63 (8), 51 (4). Anal. calcd for $C_{10}H_{10}O_3$: C 67.41, H 5.66; found: C 67.36, H 5.90%

*4.5.2. 2-Acetyl-3-methoxyphenyl acetate (**3b**)*

(6.65 g, 87%), m.p. 76–77 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.23 (s, 3H, 2′-OCOCH_3), 2.49 (s, 3H, H-2), 3.85 (s, 3H, OCH_3), 6.71 (dd, 1H, H-3′, *J* 8.2, 0.6 Hz), 6.82 (dd, 1H, H-5′, *J* 8.2, 0.6 Hz), 7.34 (t, 1H, H-4′, *J* 8.2 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7 (2′-OCO*C*H$_3$), 31.5 (C-2), 55.8 (O*C*H$_3$), 108.6 (C-5′), 115.0 (C-3′), 123.9 (C-1′), 131.0 (C-4′), 147.6 (C-2′), 157.4 (C-6′), 169.2 (2′-O*C*OCH$_3$), 200.4 (C-1) ppm. MS *m/z* (ESI, %): 231 ([M+Na]$^+$, 100); Anal. calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; found: C, 63.50; H, 5.94%

*4.5.3. 2-Acetyl-5-methoxyphenyl acetate (**3c**)*

Colourless oil. (7.33 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.33 (s, 3H, 2′-OCOCH_3), 2.48 (s, 3H, H-2), 3.81 (s, 3H, OCH_3), 6.60 (d, 1H, H-3′, *J* 2.5 Hz), 6.79 (dd, 1H, H-5′, *J* 8.4, 2.5 Hz), 7.81 (d, 1H, H-6′, *J* 8.4 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7 (2′-OCO*C*H$_3$), 28.5 (C-2), 55.2 (O*C*H$_3$), 109.0 (C-3′), 111.1 (C-5′), 122.4 (C-1′), 132.2 (C-6′), 151.0 (C-2′), 163.3 (C-4′), 169.0 (2′-O*C*OCH$_3$), 195.2 (C-1) ppm. MS *m/z* (ESI, %): 231 ([M+Na]$^+$, 100); Anal. calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; found: C, 63.23; H, 5.79%.

*4.5.4. 2-Acetyl-5-[(2-methoxyethoxy)methoxy]phenyl acetate (**3d**)*

Colourless oil. (9.22 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, 2′-OCOCH_3), 2.52 (s, 3H, H-2), 3.37 (s, 3H, $CH_3OCH_2CH_2OCH_2O$), 3.53–3.56 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 3.80–3.83 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 5.31 (s, 2H, $CH_3OCH_2CH_2OCH_2O$), 6.79 (d, 1H, H-3′, *J* 2.5 Hz), 6.98 (dd, 1H, H-5′, *J* 8.8, 2.5 Hz), 7.82 (d, 1H, H-6′, *J* 8.8 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.2 (2′-OCO*C*H$_3$), 29.0 (C-2), 59.0 ($CH_3OCH_2CH_2OCH_2O$), 68.0 ($CH_3OCH_2CH_2OCH_2O$), 71.4 ($CH_3OCH_2CH_2OCH_2O$), 93.2 ($CH_3OCH_2CH_2OCH_2O$), 111.4 (C-3′), 113.4 (C-5′), 123.9 (C-1′), 132.3 (C-6′), 151.1 (C-2′) 161.2 (C-4′), 169.4 (2′-O*C*OCH$_3$), 195.8 (C-1) ppm. MS *m/z* (ESI, %): 305 ([M+Na]$^+$, 100). HRMS (ESI) *m/z* calcd. for $C_{14}H_{19}O_6$ [M+H]$^+$ 283.1176; found 283.1175.

*4.6. Synthesis of 1-(2-hydroxyphenyl)butane-1,3-diones/(Z)-3-hydroxy-1-(2-hydroxyphenyl)but-2-en-1-one (**4a-4d**)*

Sodium hydride (536 mg, 22.3 mmol) was added to a solution of the appropriate 2-acetylphenyl acetate (**3a-3d**) (14.9 mmol) in dry THF (30 mL). The mixture was stirred under reflux for 2 h, under nitrogen atmosphere. After that period, the solution was poured into ice (50 g) and water (50 mL), and the pH was adjusted to 5–6 with diluted hydrochloric acid. The mixture was vigorously stirred for 15 min. The aqueous layer was extracted with dichloromethane (3 × 50 mL) and the solvent was evaporated to dryness. The residue was purified by silica gel column chromatography using dichloromethane as eluent giving 1-(2-hydroxyphenyl)butane-1,3-diones/(*Z*)-3-hydroxy-1-(2-hydroxyphenyl)but-2-en-1-ones (**4a-4d**) in good yields.

*4.6.1. 1-(2-Hydroxyphenyl)buta-1,3-dione (25%)/(Z)-3-Hydroxy-1-(2-hydroxyphenyl)but-2-en-1-one (75%) (**4a**)*

(1.43 g, 54%), m. p. 94–96 °C. ^{1}H NMR (300 MHz, $CDCl_3$): Diketone form δ 2.32 (s, 3H, CH_3), 4.11 (s, 2H, H-2), 7.90 (dd, 1H, H-6′, *J* 7.8, 1.5 Hz), 11.94 (s, 1H, 2′-O*H*); Enolic form δ 2.15 (s, 3H, CH_3), 6.18 (s, 1H, H-2), 6.88 (ddd, 1H, H-5′, *J* 7.8, 7.7, 1.1 Hz), 6.97 (dd, 1H, H-3′, *J* 8.1, 1.1 Hz); 7.44 (ddd, 1H, H-4′, *J* 8.1, 7.7, 1.6 Hz); 7.64 (dd, 1H, H-6′, *J* 7.8, 1.6 Hz); 12.08 (s, 1H, 2′-O*H*); 14.98 (s, 1H, 3-O*H*) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): Diketone form δ 30.6 (*C*H$_3$), 54.5 (C-2), 101.2 (C-5′), 119.3 (C-1′), 130.7 (C-6′), 137.3 (C-4′), 162.8 (C-2′), 199.7 (C-3), 201.4 (C-1); Enolic form δ 22.8 (*C*H$_3$), 95.4 (C-2), 118.7 (C-3′), 119.0 (C-5′), 119.4 (C-1′), 128.5 (C-6′), 135.7 (C-4′), 162.5 (C-2′), 183.0 (C-3), 195.5 (C-1) ppm; MS *m/z* (EI, %): 178 ([M]$^{+\cdot}$, 8), 163 (20), 160 (15), 121 (100), 120 (9), 92 (8); Anal. calcd for $C_{10}H_{10}O_3$: C 67.41, H 5.66; found: C 67.38, H 5.73%.

*4.6.2. 1-(2-Hydroxy-6-methoxyphenyl)buta-1,3-dione (50%)/(Z)-3-Hydroxy-1-(2-hydroxy-6-methoxyphenyl)but-2-en-1-one (50%) (**4b**)*

(1.74 g, 56%), m. p. 91–92 °C. ^{1}H NMR (300 MHz, $CDCl_3$): Diketone form δ 2.25 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.07 (s, 2H, H-2), 6.37 (dd, 1H, H-5′, *J* 8.4, 0.8 Hz), 6.60 (dd, 1H, H-3′, *J* 8.1, 0.9 Hz), 7.37 (t, 1H, H-4′, *J* 8.4 Hz), 12.95 (s, 1H, 2′-O*H*); Enolic form δ 2.12 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.40 (dd, 1H, H-5′, *J* 8.1, 0.9 Hz), 6.57 (dd, 1H, H-3′, *J* 8.4, 0.8 Hz), 6.62 (s, 1H, H-2), 7.30 (t, 1H, H-4′, *J* 8.1 Hz), 12.49 (s, 1H, 2′-O*H*), 15.30 (s, 1H, 3-O*H*) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): Diketone form δ 30.1 (*C*H$_3$), 55.6 (OCH$_3$), 59.9 (C-2), 101.2 (C-5′), 110.9 (C-1′), 111.1 (C-3′), 136.9 (C-4′), 160.7 (C-6′), 165.0 (C-2′), 200.3 (C-3), 202.0 (C-1); Enolic form δ 22.9 (*C*H$_3$), 55.7 (OCH$_3$), 101.6 (C-5′), 101.7 (C-2), 109.8 (C-1′), 111.0 (C-3′), 134.9 (C-4′), 160.2 (C-6′), 163.8 (C-2′), 183.3 (C-3), 194.2 (C-1) ppm; MS *m/z* (ESI, %): 209 ([M+H]$^+$, 10), 231 ([M+Na]$^+$, 100); Anal. calcd for $C_{11}H_{12}O_4$: C 63.45, H 5.81; found: C 63.37, H 6.14%.

*4.6.3. 1-(2-Hydroxy-4-methoxyphenyl)buta-1,3-dione (31%)/(Z)-3-Hydroxy-1-(2-hydroxy-4-methoxyphenyl)but-2-en-1-one (69%) (**4c**)*

(1.98 g, 64%), m. p. 62–63 °C. ^{1}H NMR (300 MHz, $CDCl_3$): Diketone form δ 2.24 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 3.96 (s, 2H, H-2), 6.36 (dd, 1H, H-5′, *J* 8.4, 0.8 Hz), 6.60 (d, 1H, H-3′, *J* 0.9 Hz), 7.44

(d, 1H, H-6′, *J* 8.4 Hz), 12.40 (s, 1H, 2′-O*H*); Enolic form *δ* 2.05 (s, 3H, C*H*$_3$), 3.75 (s, 3H, OC*H*$_3$), 5.97 (s, 1H, H-2), 6.40 (dd, 1H, H-5′, *J* 8.1, 0.9 Hz), 6.57 (d, 1H, H-3′, *J* 0.9 Hz),7.75 (d, 1H, H-6′, *J* 8.4 Hz), 12.50 (s, 1H, 2′-O*H*), 14.75 (s, 1H, 3-O*H*) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): Diketone form *δ* 30.0 (*C*H$_3$), 55.6 (OC*H*$_3$), 59.9 (C-2), 101.2 (C-5′), 101.3 (C-3′), 105.4 (C-1′), 136.9 (C-6′), 160.7 (C-2′), 164.9 (C-4′), 197.4 (C-1), 201.8 (C-3); Enolic form *δ* 22.1 (*C*H$_3$), 55.1 (OC*H*$_3$), 94.6 (C-2), 101.0 (C-3′), 107.3 (C-5′), 111.6 (C-1′), 129.9 (C-6′), 164.8 (C-2′), 165.4 (C-4′), 181.0 (C-3), 193.9 (C-1) ppm; MS *m/z* (ESI, %): 209 ([M+H]$^+$, 39), 231 ([M+Na]$^+$, 100). HRMS-EI *m/z* for $C_{11}H_{12}O_4$ $M^{+\cdot}$ calcd 208.0736, found 208.0744.

4.6.4. 1-(2-Hydroxy-4-methoxyethoxymethoxyphenyl)buta-1,3-dione (46%)/(Z)-3-hydroxy-1-(2-hydroxy-4-methoxyethoxymethoxyphenyl)but-2-en-1-one (54%) (**4d**)

Colourless oil. (1.51 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): Diketone form *δ* 2.30 (s, 3H, C*H*$_3$), 3.38 (s, 3H, OC*H*$_3$), 3.54–3.57 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 3.79–3.83 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 4.02 (s, 2H, H-2), 5.30 (s, 2H, $CH_3CH_2CH_2OCH_2O$), 6.53–6.62 (m, 2H, H-3′, H-5′), 7.54–7.58 (m, 1H, H-6′), 12.30 (s, 1H, 2′-O*H*); Enolic form *δ* 2.12 (s, 3H, C*H*$_3$), 3.38 (s, 3H, OC*H*$_3$), 3.54–3.57 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 3.79–3.83 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 5.30 (s, 2H, $CH_3CH_2CH_2OCH_2O$), 6.05 (s, 1H, H-2), 6.53–6.62 (m, 2H, H-3′, H-5′), 7.54–7.58 (m, 1H, H-6′), 12.42 (s, 1H, 2′-O*H*), 14.83 (s, 1H, 3-O*H*) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): Diketone form *δ* 30.5 (*C*H$_3$), 54.5 (C-2), 59.1 (OCH$_3$), 68.2 ($CH_3OCH_2CH_2OCH_2O$), 71.5 ($CH_3OCH_2CH_2OCH_2O$), 93.0 ($CH_3OCH_2CH_2OCH_2O$), 103.9 (C-5′), 108.7 (C-3′), 112.9 (C-1′), 132.5 (C-6′), 164.2 (C-2′), 165.4 (C-4′), 200.0 (C-1); Enolic form *δ* 22.6 (*C*H$_3$), 59.1 (OCH$_3$), 68.2 ($CH_3CH_2CH_2OCH_2O$), 71.5 ($CH_3CH_2CH_2OCH_2O$), 93.0 ($CH_3CH_2CH_2OCH_2O$). 95.0 (C-2), 104.2 (C-5′), 108.2 (C-3′), 112.9 (C-1′), 130.2 (C-6′), 163.1 (C-2′), 164.9 (C-4′), 181.5 (C-3), 194.4 (C-1) ppm. MS *m/z* (ESI, %): 283 ([M+H]$^+$, 17), 305 ([M+Na]$^+$, 53), 587 ([2M + Na]$^+$, 16). HRMS (ESI) *m/z* calcd. for $C_{14}H_{19}O_6$ [M+H]$^+$ 283.1176; found 283.1176.

4.7. Synthesis of 2-methyl-4H-chromen-4-ones (**5a-5c, 6**)

A catalytic amount of *p*-toluenesulfonic acid monohydrate (*p*-TSA, 1.21 g, 6.35 mmol) was added to a solution of the appropriate 1-(2-hydroxyphenyl)buta-1,3-dione/(*Z*)-3-hydroxy-1-(2-hydroxyphenyl)but-2-en-1-one (**4a-4d**) (12.7 mmol) in DMSO (20 mL). The mixture was stirred at 100 °C under nitrogen atmosphere for 2 h. After that period the mixture was poured into ice (100 g) and water (100 mL) and the resulting mixture extracted with ethyl ether (3 × 100 mL), being organic layer dried over anhydrous sodium sulfate. The solvent was evaporated to dryness and the residue purified by silica gel column chromatography using dichloromethane as eluent giving 2-methyl-4*H*-chromen-4-ones (**5a-5c**, **6**) in very good yields.

4.7.1. 2-Methyl-4H-chromen-4-one (**5a**)

(1.73 g, 85%), m.p. 69–71 °C (Lit. 55–56 °C [36]). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 2.40 (s, 3H, C*H*$_3$), 6.18 (s, 1H, H-3), 7.36–7.44 (m, 2H, H-6, H-8), 7.65 (ddd, 1H, H-7, *J* 8.6, 7.1, 1.6 Hz), 8. 19 (dd, 1H, H-5, *J* 8.0, 1.6 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 20.6 (*C*H$_3$), 110.5 (C-3), 117.8 (C-8), 123.5 (C-4a), 124.9 (C-6), 125.6 (C-5), 133.5 (C-7), 150.1 (C-8a), 166.2 (C-2), 207.0 (C-4) ppm. MS *m/z* (ESI, %): 161 ([M+H]$^+$, 100), 183 ([M+Na]$^+$, 23). HRMS (ESI) *m/z* calcd. for $C_{10}H_9O_2$ [M+H]$^+$ 161.0597; found 161.0599

4.7.2. 5-Methoxy-2-methyl-4H-chromen-4-one (**5b**)

(1.69 g, 70%), m.p. 97–98 °C (Lit. 97–98 °C [36]). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 2.30 (s, 3H, C*H*$_3$), 3.96 (s, 3H, OC*H*$_3$), 6.07 (s, 1H, H-3), 6.78 (dd, 1H, H-6, *J* 8.3, 0.6 Hz), 6.97 (dd, 1H, H-8, *J* 8.3, 0.6 Hz), 7.51 (t, 1H, H-7, *J* 8.3 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 19.7 (*C*H$_3$), 56.2 (OCH$_3$), 106.0 (C-6), 109.7 (C-8), 111.8 (C-3), 113.9 (C-4a), 133.3 (C-7), 158.3 (C-8a), 159.5 (C-5), 163.5 (C-2), 178.0 (C-4) ppm. MS *m/z* (ESI, %): 191 ([M+H]$^+$, 100), 213 ([M+Na]$^+$, 90). HRMS-EI *m/z* for $C_{11}H_{10}O_3$ $M^{+\cdot}$ calcd 190.0630, found 190.0641

4.7.3. 7-Methoxy-2-methyl-4H-chromen-4-one (**5c**)

(1.38 g, 57%), m.p. 113–114 °C (Lit. 106–108 °C [34]). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 2.35 (s, 3H, C*H*$_3$), 3.89 (s, 3H, OC*H*$_3$), 6.10 (s, 1H, H-3), 6.81 (d, 1H, H-8, *J* 2.4 Hz), 6.94 (dd, 1H, H-6, *J* 8.8, 2.4 Hz), 8.08 (d, 1H, H-5, *J* 8.8 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 20.4 (*C*H$_3$), 55.7 (OCH$_3$), 100.1 (C-8), 110.3 (C-3), 113.9 (C-6), 117.3 (C-4a), 126.9 (C-5), 158.1 (C-8a), 163.8 (C-7), 165.5 (C-2), 177.7 (C-4) ppm. MS *m/z* (ESI, %): 191 ([M+H]$^+$, 100). Anal. calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; found: C, 69.43; H, 5.63%

4.7.4. 7-Methoxyethoxymethoxy-2-methyl-4H-chromen-4-one (**5d**)

Colourless oil. (1.21 g, 81%). ^{1}H NMR (300 MHz, ($CDCl_3$): *δ* 2.36 (s, 3H, C*H*$_3$), 3.38 (s, 3H, C*H*$_3$OCH$_2$CH$_2$OCH$_2$O), 3.55–3.58 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 3.83–3.86 (m, 2H, $CH_3OCH_2CH_2OCH_2O$), 5.36 (s, 2H, $CH_3OCH_2CH_2OCH_2O$), 6.11 (s, 1H, H-3), 7.04 (dd, 1H, H-6, *J* 8.7, 2.4 Hz), 7.07 (d, 1H, H-8, *J* 2.4 Hz), 8.08 (d, 1H, H-5, *J* 8.7 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 20.4 (*C*H$_3$), 59.0 (*C*H$_3$OCH$_2$CH$_2$OCH$_2$O), 68.0 ($CH_3OCH_2CH_2OCH_2O$), 71.4 ($CH_3OCH_2CH_2OCH_2O$), 93.2 ($CH_3OCH_2CH_2OCH_2O$), 103.0 (C-8), 110.2 (C-3), 115.0 (C-6), 118.1 (C-4a), 126.9 (C-5), 157.8 (C-8a), 161.2 (C-7), 165.8 (C-2), 177.7 (C-4) ppm. MS *m/z* (ESI, %): 265 ([M+H]$^+$, 82), 287 ([M+Na]$^+$, 83), 551 ([2M + Na]$^+$, 100). HRMS (ESI) *m/z* calcd. for $C_{14}H_{17}O_5$ [M+H]$^+$ 265.1076; found 265.1068.

4.7.5. 7-Hydroxy-2-methyl-4H-chromen-4-one (**6**)

(1.99 g, 89%), m.p. 246–247 °C. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 2.33 (s, 3H, C*H*$_3$), 6.09 (s, 1H, H-3), 6.80 (d, 1H, H-8, *J* 2.2 Hz), 6.88 (dd, 1H, H-6, *J* 8.7, 2.2 Hz), 7.83 (d, 1H, H-5, *J* 8.7 Hz), 10.72 (s, 1H, 7-O*H*) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 19.9 (*C*H$_3$), 102.1 (C-8), 109.5 (C-3), 114.7 (C-6), 115.8 (C-4a), 126.5 (C-5), 157.7 (C-8a), 162.4 (C-7), 165.9 (C-2), 176.2 (C-4) ppm. MS *m/z* (ESI, %): 177 ([M+H]$^+$, 100), 199 ([M+Na]$^+$, 15). HRMS (ESI) *m/z* calcd. for $C_{10}H_9O_3$ [M+H]$^+$ 177.0546; found 177.0548

4.8. Synthesis of 4-iodo-1,2-dimethoxybenzene (**8**)

To a stirred solution of 1,2-dimethoxybenzene **7** (1 mL, 7.85 mmol) in acetonitrile (5 mL) was added *N*-iodosuccinimide (NIS) (1.94 g, 8.63 mmol) and trifluoroacetic acid (TFA) (0.18 mL, 2.35 mmol). The mixture was stirred for 2 h at 80 °C. After that period the resulting mixture was poured into ice (20 g) and water (100 mL) and a saturated solution of sodium thiosulfate was added. The aqueous layer was extracted with dichloromethane (3 × 100 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane as eluent to give the desired 4-iodo-1,2-dimethoxybenzene **8** in excellent yield (1.97 g, 95%). This compound showed spectroscopic and analytical data identical to one previously reported [37].

4.9. Synthesis of (E)-3-(3,4-dimethoxyphenyl)acrylaldehyde (**9**)

To a stirred solution of 1-iodo-3,4-dimethoxybenzene **8** (0.132 g, 0.5 mmol) in DMF (2.0 mL) were added acrolein diethyl acetal (0.229 mL, 1.5 mmol), ^{n}Bu$_4$NOAc (0.302 g, 1.0 mmol), K_2CO_3 (0.104 g, 0.75 mmol), and $Pd(OAc)_2$ (0.003 g, 0.015 mmol) and the mixture was stand for 4 h at 90 °C. After that period, the mixture was cooled, hydrochloric acid 2 N was slowly added and the reaction was stirred

at room temperature for 10 min. Then, the mixture was diluted with ethyl ether (50 mL) and washed with water (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with dichloromethane as eluent to give the desired (*E*)-3-(3,4-dimethoxyphenyl)acrylaldehyde **9** in good yield (77 mg, 80%).

*4.9.1. (E)-3-(3,4-dimethoxyphenyl)acrylaldehyde (**9**)*

M.p. 80–82 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (s, 3H, 3-OC*H*$_3$), 3.94 (s, 3H, 4-OC*H*$_3$), 6.62 (dd, 1H, H-α, *J* 15.8, 7.8 Hz), 6.91 (d, 1H, H-5, *J* 8.3 Hz), 7.08 (d, 1H, H-2, *J* 2.0 Hz), 7.17 (dd, 1H, H-6, *J* 8.3, 2.0 Hz), 7.42 (d, 1H, H-β, *J* 15.8 Hz), 9.66 (d, 1H, C*H*O, *J* 7.8 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8 and 55.9 (3,4-OC*H*$_3$), 109.7 (C-2), 111.0 (C-5), 123.3 (C-6), 126.6 (C-α), 126.9 (C-1), 149.3 (C-3), 151.9 (C-4), 152.8 (C-β), 193.5 (*C*HO) ppm. MS *m/z* (ESI$^+$, %): 193 ([M+H]$^+$, 95), 215 ([M+Na]$^+$, 100). HRMS (ESI$^+$) *m/z* calcd. for $C_{11}H_{13}O_3$ [M+H]$^+$ 193.0859; found 193.0855.

*4.10. Synthesis of 2-[(1E,3E)-4-(3,4-dimethoxyphenyl)buta-1,3-dien-1-yl]-4H-chromen-4-ones (**10a-10d**)*

Sodium (0.11 g, 4.8 mmol) was gradually added to dry ethanol (5 mL). The appropriate 2-methyl-4*H*-chromen-4-one **5a-5d** (1.2 mmol) and (*E*)-3-(3,4-dimethoxyphenyl)acrylaldehyde **9** (0.29 g, 1.5 mmol) were added and the reaction mixture allowed to stand at room temperature for 12 h. The solution was then poured into ice (20 g) and water (30 mL) and the pH adjusted to 4 with diluted hydrochloric acid. The obtained solid was removed by filtration, taken in dichloromethane (50 mL) and purified by silica gel column chromatography using dichloromethane as eluent. The solvent was removed to dryness and the residue was recrystallized from ethanol to provide the 2-[(1*E*,3*E*)-4-(3,4-dimethoxyphenyl) buta-1,3-dien-1-yl]-4*H*-chromen-4-ones **10a-10d**, in moderate to good yields.

*4.10.1. 2-[(1E,3E)-4-(3,4-Dimethoxyphenyl)buta-1,3-dien-1-yl]-4H-chromen-4-one (**10a**)*

(249 mg, 62%), m. p. 146–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (s, 3H, 4′-OC*H*$_3$), 3.86 (s, 3H, 3′-OC*H*$_3$), 6.14 (s, 1H, H-3), 6.20 (d, 1H, H-α, *J* 15.2 Hz), 6.68–6.81 (m, 3H, H-γ, H- δ, H-5′), 6.92–6.96 (m, 2H, H-2′, H-6′), 7.24–7.32 (m, 2H, H-β, H-6) 7.38 (dd, 1H, H-8, *J* 8.5, 0.9 Hz), 7.56 (ddd, 1H, H-7, *J* 8.5, 7.0, 1.7), 8.09 (dd, 1H, H-5, *J* 8.0, 1.7) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.5 (3′-O*C*H$_3$), 55.6 (4′-O*C*H$_3$), 108.5 (C-2′), 109.5 (C-3), 110.8 (C-5′), 117.4 (C-8), 120.9 (C-6′), 122.1 (C-α), 123.7 (C-4a), 124.5 (C-6), 124.8 (C-γ), 125.2 (C-5), 129.0 (C-1′), 133.2 (C-7), 137.4 (C-β), 138.8 (C-δ), 148.8 (C-3′), 149.7 (C-4′), 155.6 (C-8a), 161.7 (C-2) 177.9 (C-4) ppm. MS *m/z* (ESI, %): 335 ([M+H]$^+$, 100), 357 ([M+Na]$^+$, 25). Anal. calcd for $C_{21}H_{18}O_4$: C 75.43, H 5.43; found: C 75.54, H 5.71%.

*4.10.2. 5-Methoxy-2-[(1E,3E)-4-(3,4-dimethoxyphenyl)buta-1,3-dien-1-yl]-4H-chromen-4-one (**10b**)*

(332 mg, 76%), m. p. 173–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.91 (s, 3H, 4′-OC*H*$_3$), 3.94 (s, 3H, 3′-OC*H*$_3$), 3.98 (s, 3H, 5-OC*H*$_3$), 6.16 (s, 1H, H-3), 6.27 (d, 1H, H-α, *J* 15.2 Hz), 6.77–6.89 (m, 4H, H-5′, H-6, H-γ, H-δ), 7.03–7.08 (m, 3H, H-2′, H-6′, H-8), 7.33 (ddd, 1H, H-β, *J* 15.2, 7.6, 2.5 Hz), 7.54 (t, 1H, H-7, *J* = 8.4 Hz) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 55.8 (3′-O*C*H$_3$), 55.9 (4′-O*C*H$_3$), 56.4 (5-O*C*H$_3$), 106.2 (C-6), 108.8 (C-2′), 109.9 (C-8), 111.1 (C-5′), 111.5 (C-3), 114.6 (C-4a), 121.0 (C-6′), 122.1 (C-α), 125.2 (C-γ), 129.4 (C-1′), 133.5 (C-7), 136.9 (C-β), 138.5 (C-δ), 149.1 (C-3′), 149.9 (C-4′), 158.0 (C-8a), 159.7 (C-5), 159.7 (C-2), 178.3 (C-4) ppm. MS *m/z* (ESI, %): 365 ([M+H]$^+$, 100), 387 ([M+Na]$^+$, 38), 751 ([2M + Na]$^+$, 89). Anal. calcd for $C_{22}H_{20}O_5$: C 72.51, H 5.53; found: C 72.29, H 5.75%.

*4.10.3. 2-[(1E,3E)-4-(3,4-Dimethoxyphenyl)buta-1,3-dien-1-yl]-7-methoxy-4H-chromen-4-one (**10c**)*

(337 mg, 77%), m. p. 187–189 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.91 (s, 3H, 7-OC*H*$_3$), 3.92 (s, 3H, 4′-OC*H*$_3$), 3.93 (s, 3H, 3′-OC*H*$_3$), 6.16 (s, 1H, H-3), 6.32 (d, 1H, H-α, *J* 15.2 Hz), 6.78–6.90 (m, 2H, H-γ, H-δ), 6.86 (d, 1H, H-5′, *J* 9.0 Hz), 6.88 (d, 1H, H-8, *J* 2.4 Hz), 6.93 (dd, 1H, H-6, *J* 8.8, 2.4 Hz), 7.01 (br s, 1H, H-2′), 7.03 (d, 1H, H-6′, *J* 9.0 Hz), 7.35 (ddd, 1H, H-β, *J* 15.2, 7.7, 2.4 Hz), 8.07 (d, 1H, H-5, *J* 8.8 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.7, 55.8, 55.9 (3 × O*C*H$_3$), 100.2 (C-8), 108.7 (C-2′), 109.8 (C-3), 111.1 (C-5′), 113.8 (C-6), 117.9 (C-4a), 121.0 (C-6′), 122.5 (C-α), 125.2 (C-γ), 126.9 (C-5), 129.3 (C-1′) 137.0 (C-β), 138.6 (C-δ), 149.1 (C-3′), 149.9 (C-4′), 157.6 (C-8a), 161.6 (C-2), 164.0 (C-7), 177.7 (C-4) ppm. MS *m/z* (ESI, %): 365 ([M+H]$^+$, 100), 387 ([M+Na]$^+$, 28). Anal. calcd for $C_{22}H_{20}O_5$: C 72.51, H 5.53; found: C 72.89, H 5.70%.

*4.10.4. 7-[(2-Methoxyethoxy)methoxy]-2-[(1E,3E)-4-(3,4-dimethoxyphenyl)buta-1,3-dien-1-yl]-4H-chromen-4-one (**10d**)*

Orange oil. (395 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.40 (s, 3H, C*H*$_3$OCH$_2$CH$_2$OCH$_2$O), 3.58–361 (m, 2H, CH$_3$OC*H*$_2$CH$_2$OCH$_2$O), 3.86–3.89 (m, 2H, CH$_3$OCH$_2$C*H*$_2$OCH$_2$O), 3.92 (s, 3H, 4′-OC*H*$_3$), 3.95 (s, 3H, 3′-OC*H*$_3$), 5.40 (s, 2H, CH$_3$OCH$_2$CH$_2$OC*H*$_2$O), 6.19 (s, 1H, H-3), 6.32 (d, 1H, H-α, *J* 15.2 Hz), 6.85–9.93 (m, 3H, H-γ, H-δ, H-5′), 7.02–7.08 (m, 3H, H-2′,6′, H-6), 7.18 (d, 1H, H-8, *J* 2.3 Hz), 7.36–7.45 (m, 1H, H-β), 8.09 (d, 1H, H-5, *J* 8.8 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8 (3′-O*C*H$_3$), 55.9 (4′-O*C*H$_3$), 59.0 (*C*H$_3$OCH$_2$CH$_2$O-CH$_2$O), 68.1 (CH$_3$OCH$_2$*C*H$_2$OCH$_2$O), 71.4 (CH$_3$OCH$_2$CH$_2$*C*H$_2$O), 93.3 (CH$_3$OCH$_2$CH$_2$O*C*H$_2$O), 102.9 (C-8), 108.8 (C-2′), 109.8 (C-3), 111.1 (C-5′), 115.1 (C-6), 118.7 (C-4a), 121.1 (C-6′), 122.4 (C-α), 125.2 (C-γ), 126.9 (C-5), 129.4 (C-1′), 137.4 (C-β), 138.9 (C-δ), 149.1 (C-3′), 150.0 (C-4′), 157.4 (C-8a), 161.5 (C-7), 161.8 (C-2), 177.9 (C-4) ppm. MS *m/z* (ESI, %): 439 ([M+H]$^+$, 100), 461 ([M+Na]$^+$, 10). HRMS (ESI) *m/z* calcd. for $C_{25}H_{27}O_7$ [M+H]$^+$ 439.1757; found 439.1736.

*4.11. Synthesis of 1-(3,4-dimethoxyphenyl)-9H-xanthen-9-ones (**11a-11d**)*

Iodine (18 mg, 0.07 mmol) was added to a solution of the appropriate chromone **10a-10d** (0.35 mmol) in 1,2,4-trichlorobenzene (5 mL) and the mixture was refluxed for 48 h. After this period, the solution was directly purified by silica gel column chromatography using light petroleum ether as eluent to remove the 1,2,4-trichlorobenzene and then dichloromethane to recover the desired product. The solvent was evaporated to dryness and the residue was recrystallized from ethanol to give the 1-(3,4-dimethoxyphenyl)-9*H*-xanthen-9-ones **11a-11d**, in moderate yields.

*4.11.1. 1-(3,4-Dimethoxyphenyl)-9H-xanthen-9-one (**11a**)*

(76 mg, 65%), m. p. 164.0–164.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 1H, 3′-OC*H*$_3$), 3.96 (s, 1H, 4′-OC*H*$_3$), 6.87–6.97 (m, 3H, H-2′, H-5′, H-6′), 7.19 (dd, 1H, H-2, *J* 7.3, 1.2 Hz), 7.33 (ddd, 1H, H-7, *J* 7.8, 7.1, 1.1 Hz), 7.48 (d, 1H, H-5, *J* 8.7 Hz), 7.51 (dd, 1H, H-4, *J* 9.1, 1.2 Hz), 7.66–7.73 (m, 2H, H-3, H-6), 8.20 (dd, 1H, H-8, *J* 7.8, 1.6 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8 (2 × O*C*H$_3$), 110.4 (C-5′), 112.2 (C-2′), 117.4 (C-5), 117.5 (C-4), 119.4 (C-9a), 120.8 (C-6′), 122.8 (C-8a), 123.8 (C-7), 126.9 (C-8), 127.4 (C-2), 133.4 (C-3), 134.4 (C-1′), 134.5 (C-6), 144.0 (C-1), 148.1 (C-4′), 148.1 (C-3′), 155.3 (C-4b), 157.3 (C-4a), 176.9 (C-9) ppm. MS *m/z* (ESI, %): 333 ([M+H]$^+$, 75), 355 ([M+Na]$^+$, 100). HRMS-EI *m/z* for $C_{21}H_{16}O_4$ M$^{+\cdot}$ calcd 332.1049, found 332.1041.

*4.11.2. 8-Hydroxy-1-(3,4-dimethoxyphenyl)-9H-xanthen-9-one (**11b**)*

(43 mg, 35%), m. p. 167–168 °C. ^{1}H NMR (300 MHz, $CDCl_3$):

δ 3.88 (s, 3H, 4′-OC*H*$_3$), 3.96 (s, 3H, 3′-OC*H*$_3$), 6.74 (dd, 1H, H-5, *J* 8.3, 0.8 Hz), 6.88–6.97 (m, 4H, H-2′, H-5′, H-6′, H-7), 7.18 (dd, 1H, H-2, *J* 7.6, 1.2 Hz), 7.47 (dd, 1H, H-4, *J* 8.2, 1.2 Hz), 7.56 (t, 1H, H-6, *J* 8.3 Hz), 7.69 (dd, 1H, H-3, *J* 8.2, 7.6 Hz), 12.60 (s, 1H, O*H*) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.7 (3′-OCH_3), 55.9 (4′-OCH_3), 106.4 (C-5′), 109.4 (C-8a), 110.3 (C-5), 110.4 (C-7), 112.4 (C-2′), 117.3 (C-4), 117.9 (C-9a), 120.7 (C-6′), 127.8 (C-2), 133.9 (C-1′) 134.2 (C-3), 136.4 (C-6), 143.7 (C-1), 148.0 (C-4′), 148.4 (C-3′), 155.5 (C-4b), 157.3 (C-4a), 161.9 (C-8), 182.5 (C-9) ppm. MS *m/z* (ESI, %): 349 ([M+H]$^+$, 73), 371 ([M+Na]$^+$, 100). Anal. calcd for $C_{21}H_{16}O_5$: C 72.41, H 4.63; found: C 72.23, H 4.76%.

4.11.3. 6-Methoxy-1-(3,4-dimethoxyphenyl)-9H-xanthen-9-one (**11c**)

(53 mg, 42%), m. p. 189–190 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H, 4′-OC*H*$_3$), 3.90 (s, 3H, 6-OC*H*$_3$), 3.94 (s, 3H, 3′-OC*H*$_3$), 6.84–6.95 (m, 5H, H-2′, H-5′, H-6′, H-5, H-7), 7.15 (dd, 1H, H-2, *J* 7.7, 1.2 Hz), 7.44 (dd, 1H, H-4, *J* 8.2, 1.2 Hz), 7.62 (dd, 1H, H-3, *J* 8.2, 7.7 Hz), 8.09 (d, 1H, H-8, *J* 9.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.69, 55.70, 55.74 (6.3′,4′-OCH_3), 99.5 (C-5), 110.2 (C-5′), 112.1 (C-2′), 112.9 (C-7), 116.6 (C-8a), 117.2 (C-4), 119.3 (C-9a), 120.7 (C-6′), 127.3 (C-2), 128.4 (C-8) 132.7 (C-3), 134.5 (C-1′), 143.8 (C-1), 147.9 (C-4′), 148.0 (C-3′), 157.0 (C-4b), 157.2 (C-4a), 164.7 (C-6), 175.9 (C-9) ppm. MS *m/z* (ESI, %): 363 ([M+H]$^+$, 60), 385 ([M+Na]$^+$, 100), 747 ([2M + Na]$^+$, 76). Anal. calcd for $C_{22}H_{18}O_5$: C 72.92, H 5.01; found: C 72.97, H 5.24%.

4.11.4. 6-[(2-Methoxyethoxy)methoxy]-1-(3,4-dimethoxyphenyl)-9H-xanthen-9-one (**11d**)

Yellow oil. (73 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.39 (s, 3H, C*H*$_3$OCH$_2$CH$_2$OCH$_2$O), 3.56–3.59 (m, 2H, CH$_3$OC*H*$_2$CH$_2$OCH$_2$O), 3.85–3.88 (m, 2H, CH$_3$OCH$_2$C*H*$_2$OCH$_2$O), 3.88 (s, 3H, 4′-OC*H*$_3$), 3.94 (s, 3H, 3′-OC*H*$_3$), 5.39 (s, 2H, CH$_3$OCH$_2$CH$_2$OC*H*$_2$O), 6.86 (d, 1H, H-2′, *J* 2.1 Hz), 6.88 (dd, 1H, H-6′, *J* 8.1, 2.1 Hz), 6.94 (d, 1H, H-5′, *J* 8.1 Hz), 6.98 (dd, 1H, H-7, *J* 8.9, 2.3 Hz), 7.11 (d, 1H, H-5, *J* 2.3 Hz), 7.17 (dd, 1H, H-2, *J* 7.7, 1.1 Hz), 7.47 (dd, 1H, H-4, *J* 8.4, 1.1 Hz), 7.65 (dd, 1H, H-3, *J* 8.4, 7.7 Hz), 8.11 (d, 1H, H-8, *J* 8.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8 (3′,4′-OCH_3), 59.1 (*C*H$_3$OCH$_2$CH$_2$OCH$_2$O), 68.2 (CH$_3$OCH$_2$*C*H$_2$OCH$_2$O), 71.5 (CH$_3$O*C*H$_2$CH$_2$OCH$_2$O), 93.3 (CH$_3$OCH$_2$-CH$_2$O*C*H$_2$O), 102.5 (C-5), 110.3 (C-5′), 112.1 (C-2′), 114.0 (C-7), 117.4 (C-4), 117.5 (C-8a), 119.4 (C-9a), 120.8 (C-6′), 127.4 (C-2), 128.5 (C-8), 133.0 (C-3), 134.5 (C-1′), 143.9 (C-1), 148.0 and 148.1 (C-3′,4′), 156.8 (C-4b), 157.4 (C-4a), 162.2 (C-6), 176.1 (C-9) ppm. MS *m/z* (ESI, %): 437 ([M+H]$^+$, 100), 459 ([M+Na]$^+$, 73), 895 ([2M + Na]$^+$, 34). HRMS (ESI): *m/z* calcd. for $C_{25}H_{25}O_7$ [M+H]$^+$ 437.1600; found 437.1611.

4.12. Synthesis of 2-[(1E,3E)-4-(3,4-dihydroxyphenyl)buta-1,3-dien-1-yl]-4H-chromen-4-ones (**12a-12c**) and 1-(3,4-dihydroxyphenyl)-9H-xanthen-9-ones (**13a-13c**)

A solution of 1 M boron tribromide in dichloromethane (2.5 equiv per methyl or MEM group to be removed) was added to a solution of the appropriate 2-[(1*E*,3*E*)-4-(3,4-dimethoxyphenyl) buta-1,3-dien-1-yl]-4*H*-chromen-4-ones **10a-10c** or 1-(3,4-dimethoxyphenyl)-9*H*-xanthen-9-ones **11a-11c** (0.27 mmol) in freshly distilled dichloromethane (10 mL), under nitrogen atmosphere at −78 °C. The mixture was stirred at room temperature for 3–4 h. After that period, the mixture was slowly added of ice (50 g) and water (100 mL) and vigorously stirred for 2 h to allow the formation of a precipitate. The obtained solid was removed by filtration, washed with water (3 × 50 mL) and light petroleum ether (3 × 50 mL), and finally dried under vacuum to afford the expected hydroxylated derivatives **12a-12c** and **13a-13c**, in good yields.

4.12.1. 2-[(1E,3E)-4-(3,4-Dihydroxyphenyl)buta-1,3-dien-1-yl]-4H-chromen-4-one (**12a**)

(65 mg, 79%), m. p. 254–256 °C. ^{1}H NMR [300 MHz, $(CD_3)_2SO$]: δ 6.37 (s, 1H, H-3), 6.55 (d, 1H, H-α, *J* 15.2 Hz), 6.76 (d, 1H, H-5′, *J* 8.1 Hz), 6.81–6.95 (m, 3H, H-γ, H-δ, H-6′), 6.99 (d, 1H, H-2′, *J* 2.0 Hz), 7.43–7.52 (m, 2H, H-6, H-β), 7.66 (d, 1H, H-8, *J* 8.4 Hz), 7.80 (ddd, 1H, H-7, *J* 8.4, 7.0, 1.6 Hz), 8.00 (dd, 1H, H-5, *J* 7.8, 1.6 Hz), 9.11 (s, 1H, 4′-O*H*), 9.42 (s, 1H, 3′-O*H*) ppm; ^{13}C NMR [75 MHz, $(CD_3)_2SO$]: δ 108.9 (C-3), 113.8 (C-2′), 115.9 (C-5′), 118.1 (C-8), 119.9 (C-6′), 121.5 (C-α), 123.5 (C-γ), 124.2 (C-5), 124.8 (C-6), 125.2 (C-10), 127.9 (C-1′), 134.1 (C-7), 138.3 (C-β), 139.9 (C-δ), 145.6 (C-3′), 147.1 (C-4′), 155.5 (C-9), 162.1 (C-2), 176.8 (C-4) ppm. MS *m/z* (ESI, %): 307 ([M+H]$^+$, 100), 353 ([M+2Na]$^+$, 48). HRMS (ESI) *m/z* calcd. for $C_{19}H_{15}O_4$ [M+H]$^+$ 307.0970; found 307.0958.

4.12.2. 5-Hydroxy-2-[(1E,3E)-4-(3,4-dihydroxyphenyl)buta-1,3-dien-1-yl]-4H-chromen-4-one (**12b**)

(65 mg, 75%), m. p. 269–270 °C. ^{1}H NMR [300 MHz, $(CD_3)_2SO$]: δ 6.43 (s, 1H, H-3), 6.54 (d, 1H, H-α, *J* 15.3 Hz), 6.76 (d, 1H, H-5′, *J* 8.0 Hz), 6.79 (d, 1H, H-6, *J* 8.2 Hz), 6.84–6.95 (m, 3H, H-6′, H-γ, H-δ), 7.00 (d, 1H, H-2′, *J* 1.8 Hz), 7.08 (d, 1H, H-8, *J* 8.2 Hz), 7.53 (dd, 1H, H-β, *J* 15.3, 9.9 Hz), 7.65 (t, 1H, H-7, *J* 8.2 Hz), 9.08 (s, 1H, 4′-O*H*), 9.41 (s, 1H, 3′-O*H*), 12.77 (s, 1H, 5-O*H*) ppm; ^{13}C NMR [75 MHz, $(CD_3)_2SO$]: δ 107.1 (C-8), 107.3 (C-3), 110.1 (C-10), 110.8 (C-6), 113.9 (C-2′), 115.9 (C-5′), 120.1 (C-6′), 120.7 (C-α), 124.0 (C-γ), 127.7 (C-1′), 135.8 (C-7), 139.7 (C-β), 140.9 (C-δ), 145.6 (C-3′), 147.3 (C-4′), 155.7 (C-9), 159.9 (C-5), 163.7 (C-2), 182.8 (C-4) ppm. MS *m/z* (ESI, %): 323 ([M+H]$^+$, 80), 345 ([M+Na]$^+$, 23). HRMS (ESI) *m/z* calcd. for $C_{19}H_{15}O_5$ [M+H]$^+$ 323.0919; found 323.0911.

4.12.3. 7-Hydroxy-2-[(1E,3E)-4-(3,4-dihydroxyphenyl)buta-1,3-dien-1-yl]-4H-chromen-4-one (**12c**)

(71 mg, 82%), m. p. 281–282 °C. ^{1}H NMR [300 MHz, $(CD_3)_2SO$]: δ 6.22 (s, 1H, H-3), 6.49 (d, 1H, H-α, *J* 15.3 Hz), 6.75 (d, 1H, H-5′, *J* 8.2 Hz), 6.80–6.94 (m, 5H, H-6, H-8, H-6′, H-γ, H-δ), 6.97 (d, 1H, H-2′, *J* 1.9 Hz), 7.41 (dd, 1H, H-β, *J* 15.3, 9.4 Hz), 7.83 (d, 1H, H-5, *J* 9.3 Hz), 9.12 (s, 1H, 4′-O*H*), 9.41 (s, 1H, 3′-O*H*), 10.81 (s, 1H, 7-O*H*) ppm; ^{13}C NMR [75 MHz, $(CD_3)_2SO$]: δ 102.3 (C-8), 108.8 (C-3), 113.7 (C-2′), 114.6 (C-6), 115.9 (C-5′), 116.3 (C-4a), 119.8 (C-6′), 121.7 (C-α), 124.2 (C-γ), 126.5 (C-5), 127.9 (C-1′), 137.5 (C-β), 139.4 (C-δ), 145.6 (C-3′), 147.0 (C-4′), 157.3 (C-8a), 161.4 (C-2), 162.7 (C-7), 176.3 (C-4) ppm. MS *m/z* (ESI, %): 323 ([M+H]$^+$, 35), 345 ([M+Na]$^+$, 27). HRMS (ESI) *m/z* calcd. for $C_{19}H_{15}O_5$ [M+H]$^+$ 323.0919; found 323.0908.

4.12.4. 1-(3,4-Dihydroxyphenyl)-9H-xanthen-9-one (**13a**)

(62 mg, 76%), m. p. 259–260 °C. ^{1}H NMR [300 MHz, $(CD_3)_2SO$]: δ 6.56 (dd, 1H, H-6′, *J* 8.0, 2.1 Hz), 6.68 (d, 1H, H-2′, *J* 2.1 Hz), 6.74 (d, 1H, H-5′, *J* 8.0 Hz), 7.15 (dd, 1H, H-2, *J* 7.4, 0.8 Hz), 7.43 (dd, 1H, H-7, *J* 8.5, 7.2 Hz), 7.60 (dd, 1H, H-4, *J* 7.8, 0.8 Hz), 7.64 (d, 1H, H-5, *J* 8.4 Hz), 7.79 (dd, 1H, H-3, *J* 7.8, 7.4 Hz), 7.84 (ddd, 1H, H-6, *J* 8.4, 7.2, 1.5 Hz), 8.02 (dd, 1H, H-8, *J* 8.5, 1.5 Hz), 8.87 (s, 1H, 3′-O*H*), 8.94 (s, 1H, 4′-O*H*) ppm; ^{13}C NMR [75 MHz, $(CD_3)_2SO$]: δ 114.8 (C-5′), 116.5 (C-2′), 117.1 (C-4), 117.6 (C-5), 118.6 (C-9a), 119.7 (C-6′), 122.2 (C-8a), 124.1 (C-7), 126.1 (C-8), 127.4 (C-2), 132.6 (C-1′), 134.0 (C-3), 135.0 (C-6), 143.8 (C-1), 144.2 (C-3′), 144.6 (C-4′), 154.7 (C-4b), 156.8 (C-4a), 175.8 (C-9) ppm. MS *m/z* (ESI, %): 305 ([M+H]$^+$, 100), 327 ([M+Na]$^+$, 81), 343 ([M+K]$^+$, 15), 631 ([2M + Na]$^+$, 41). HRMS (ESI) *m/z* calcd. for $C_{19}H_{13}O_4$ [M+H]$^+$ 305.0814; found 305.0802.

4.12.5. 8-Hydroxy-1-(3,4-dihydroxyphenyl)-9H-xanthen-9-one (**13b**)

(75 mg, 87%), m. p. 266–268 °C. ^{1}H NMR [300 MHz, $(CD_3)_2SO$]: δ 6.60 (dd, 1H, H-6′, *J* 8.0, 2.1 Hz), 6.72 (d, 1H, H-2′, *J* 2.1 Hz), 6.75 (d, 1H, H-5′, *J* 8.0 Hz), 6.77 (dd, 1H, H-7, *J* 8.4, 0.8 Hz), 7.05 (dd, 1H, H-5, *J* 8.4, 0.8 Hz), 7.17 (dd, 1H, H-4, *J* 7.5, 1.1 Hz), 7.60 (dd, 1H, H-2, *J* 8.5,

1.1 Hz), 7.70 (t, 1H, H-6, *J* 8.4 Hz), 7.83 (dd, 1H, H-3, *J* 8.5, 7.5 Hz), 12.59 (s, 1H, 8-O*H*) ppm; ^{13}C NMR [75 MHz, $(CD_3)_2SO$]: δ 106.7 (C-5), 108.9 (C-8a), 110.1 (C-7), 114.9 (C-5′), 116.4 (C-2′), 117.0 (C-2), 117.3 (C-9a), 119.7 (C-6′), 127.8 (C-4), 132.3 (C-1′), 135.0 (C-3), 137.1 (C-6), 143.7 (C-1), 144.3 (C-3′), 144.8 (C-4'), 155.0 (C-4b), 156.9 (C-4a), 161.1 (C-8), 182.2 (C-9) ppm; MS *m/z* (ESI, %): 321 ($[M+H]^+$, 100), 343 ($[M+Na]^+$, 47). HRMS (ESI) *m/z* calcd. for $C_{19}H_{13}O_5$ $[M+H]^+$ 321.0763; found 321.0752.

4.12.6. 6-Hydroxy-1-(3,4-dihydroxyphenyl)-9H-xanthen-9-one (**13c**)

(67 mg, 78%), m. p. 306–307 °C. ^{1}H NMR [300 MHz, $(CD_3)_2SO$]: δ 6.53 (dd, 1H, H-6′, *J* 8.0, 2.0 Hz), 6.64 (d, 1H, H-2′, *J* 2.0 Hz), 6.72 (d, 1H, H-5′, *J* 8.0 Hz), 6.84–6.87 (m, 2H, H-5, H-7), 7.10 (d, 1H, H-2, *J* 7.5 Hz), 7.53 (d, 1H, H-4, *J* 8.1 Hz), 7.72 (dd, 1H, H-3, *J* 8.1, 7.5 Hz), 7.85 (d, 1H, H-8, *J* 9.3 Hz), 8.86 (s, 1H, 4′-O*H*), 8.90 (s, 1H, 3′-O*H*), 10.89 (s, 1H, 6-O*H*) ppm; ^{13}C NMR [75 MHz, $(CD_3)_2SO$]: δ 101.7 (C-5), 114.0 (C-7), 114.8 (C-5′), 115.2 (C-8a), 116.6 (C-2′), 117.0 (C-4), 118.7 (C-9a), 119.8 (C-6′), 127.4 (C-2), 128.2 (C-8), 133.0 (C-1′), 133.5 (C-3), 143.8 (C-1), 144.2 (C-3′), 144.6 (C-4′), 156.7 (C-4a), 156.9 (C-4b), 163.6 (C-6), 174.8 (C-9) ppm. MS *m/z* (ESI, %): 321 ($[M+H]^+$, 25), 343 ($[M+Na]^+$, 31). HRMS (ESI): *m/z* calcd. for $C_{19}H_{13}O_5$ $[M+H]^+$ 321.0763; found 321.0752.

4.13. ROS and RNS scavenging assays

4.13.1. General information

To perform all ROS and RNS-scavenging assays, the compounds under study as well as the positive control, quercetin, were dissolved in DMSO, except for the HOCl scavenging assay (dissolved in ethanol). The assays were undertaken at 37 °C, except for superoxide radical scavenging assay, that was performed at room temperature. Quercetin was used as positive control. In each assay, at least four independent experiments were performed, using 5–7 concentrations in triplicate, to obtain the IC_{50} values, which was calculated from the curves of percentage of inhibition *versus* compound concentration, using the GraphPad Prism 5 software (GraphPad Inc. La Jolla, CA). The results were expressed as the percentage inhibition of the reactive specie-induced oxidation of the probe.

A microplate reader (Synergy HT, BIO-TEK) for fluorescence, absorbance in UV–Vis and luminescence measurements, plus temperature control capacity, was used for all of the ROS and RNS scavenging assays.

4.13.2. Superoxide radical scavenging assay

$O_2^{\bullet-}$ was generated by the NADH/PMS/O_2 system. The scavenging activity was determined by monitoring $O_2^{\bullet-}$-induced reduction of NBT, as previously reported [29]. The effects of the tested compounds (final concentrations of 25–200 μM) were determined spectrophotometrically at 560 nm during 2 min.

4.13.3. Hydrogen peroxide scavenging assay

The H_2O_2 scavenging activity was measured using a previously reported chemiluminescence procedure based on the H_2O_2-induced oxidation of lucigenin [29]. The chemiluminescence signal was recorded immediately after the plate introduction in the microplate reader using the tested compounds at final concentrations of 31.25–1000 μM.

4.13.4. Hypochlorous acid scavenging assay

The HOCl was measured by monitoring the HOCl-induced oxidation of DHR to rhodamine 123, as previously described [29]. HOCl was daily prepared by adjusting the pH of a 1% solution of NaOCl to 6.2 with dropwise addition of 10% H_2SO_4. The fluorimetric assays were performed for the tested compounds (final concentrations of 6.25–400 μM) at the emission wavelength 528 ± 20 nm with excitation at 480 ± 20 nm and the signals were measured immediately after the plate introduction.

4.13.5. Singlet oxygen scavenging assay

The $^1O_2$ was generated by the thermal decomposition at 37 °C of the previously synthesized $NDPO_2$ [35]. The $^1O_2$ scavenging activity was measured by monitoring the oxidation of non-fluorescent DHR to fluorescent rhodamine 123, in the presence of the tested compounds (final concentrations of 0.3125–20 μM for compounds **12a-12c** and 0.625–80 μM for compounds **13a-c**), after a 30 min incubation period, according to a described procedure [29].

4.13.6. Peroxyl radical scavenging assay

The $ROO^{\bullet}$ scavenging activity was measured by monitoring the fluorescence decay resulting from $ROO^{\bullet}$-induced oxidation of fluorescein and expressed as the 'Oxygen Radical Absorbance Capacity' (ORAC), as reported in the literature [38]. $ROO^{\bullet}$ was generated by thermal decomposition of AAPH at 37 °C and the tested compounds used at final concentrations of 0.5–10 μM. Trolox (final concentrations of 0.156–5 μM) was used as the standard control in each study.

4.13.7. Nitric oxide scavenging assay

The $^{\bullet}NO$ scavenging activity was measured by monitoring the $^{\bullet}NO$-induced oxidation of non-fluorescent DAF-2 to the fluorescent triazolofluorescein (DAF-2T), as previously reported [29]. $^{\bullet}NO$ was generated by NOC-5, the tested compounds used at final concentrations of 0.25–8 μM and the fluorimetric signal was detected after a 30 min incubation period, at the emission wavelength 528 ± 20 nm with excitation at 485 ± 20 nm.

4.13.8. Peroxynitrite scavenging assay

The $ONOO^-$ scavenging activity was measured by monitoring the $ONOO^-$-induced oxidation of non-fluorescent DHR to fluorescent rhodamine 123, as previously reported [29]. $ONOO^-$ was synthesized as described before [39]. The fluorimetric signal detected after a 2 min incubation period and the tested compounds with final concentrations of 0.156–5 μM. In a parallel set of experiments, the assays were performed in the presence of 25 mM $NaHCO_3$ in order to simulate the physiological CO_2 concentration *in vivo*. This monitorization is important because, under physiological conditions, the reaction between $ONOO^-$ and CO_2 is predominant, with a very fast rate constant ($k_2 = 3–5.8 \times 10^4\ M^{-1}\ s^{-1}$) [32].

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