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Natural and synthetic 2′-hydroxy-chalcones and aurones: Synthesis, characterization and evaluation of the antioxidant and soybean lipoxygenase inhibitory activity

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ABSTRACT

A series of 2′-hydroxy-chalcones and their oxidative cyclization products, aurones, have been synthesized and tested for their antioxidant and lipoxygenase inhibitory activity. The natural product aureusidin (**31**) was synthesized in high yield by a new approach. An extensive structure-relationship study was performed and revealed that several chalcones and aurones possess an appealing pharmacological profile combining high antioxidant and lipid peroxidation activity with potent soybean LOX inhibition.

1. Introduction

Chalcones (1,3-diaryl-2-propen-1-ones) (Fig. 1) are flavonoid and isoflavonoid precursors which are abundant in edible plants and display a wide spectrum of biological activities including antioxidant,[1–5] antibacterial,[6,7] antileishmanial,[8–10] anticancer,[11–13] antiangiogenic,[14] anti-infective and anti-inflammatory activities.[15–19] The growing interest in these compounds and their potential use in medicinal applications are proved by the growing number of publications concerning the synthesis and biological evaluation of chalcone analogues.

Representative examples of naturally occurring bioactive chalcones are xanthohumol, the principal prenylated flavonoid of the hop plant, which is characterized as a 'broad spectrum' cancer chemopreventing agent in vitro,[20] cardamonin, a hydroxychalcone isolated from a zingiberous plant species, which possesses antimutagenic, vasorelaxant and anti-inflammatory properties,[21,22] and flavokawains A, B and C, which are found mainly in the kava plant and have been used in traditional medicine practices of the Pacific Islands.[23]

Aurones, (*Z*)-2-benzylidenebenzofuran-3-(2*H*)-ones (Fig. 1), constitute a less studied subclass of flavonoids, which occur rarely in nature: to date approximately 100 aurones have been reported from natural sources, mainly flowering plants, and a few ferns, mosses and marine brown algae.[24] Aurones are responsible for the bright yellow color of some popular ornamental flowers such as snapdragon, cosmos and dahlia and are biosynthesized from chalcones by the key enzyme aureusidin synthase.[25] Representative naturally occurring aurones are aureusidin,[26] sulfuretin[27] and maritimetin,[28] possessing various hydroxylation patterns. A few natural aurones bearing methoxy substituents on either or both rings have been reported.[24,29,30]

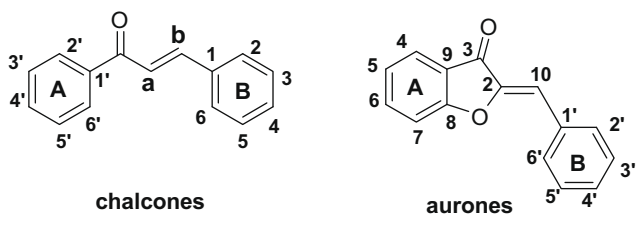

Figure 1. General structure and numbering scheme of the chalcone and aurone molecular scaffolds.

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The spectrum of biological activity of this class of compounds has not been extensively studied. However, the existing data on the bioactivity of natural and synthetic aurones is very promising, thus these heterocyclic compounds can be considered as an attractive scaffold for drug design and development. So far, aurones have been reported to possess insect antifeedant activity,[31] anticancer,[32,33] antileishmanial[30,34] and antibacterial properties,[35] inhibitory activity against a variety of enzymes and proteins[36–42] and have been developed as potential amyloid imaging agents.[43] Surprisingly, only few studies on the antioxidant activity of aurones exist, however these prove their potential as antioxidant agents.[44,45]

The formation of Reactive Oxygen Species (ROS) is characteristic of aerobic organisms, as an unavoidable consequence of cell metabolism. Normally, defense against these highly reactive species can be accomplished by the organism using enzymes, like superoxide dismutase and glutathione peroxidase, and naturally occurring antioxidants such as α-tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene and polyphenolic flavonoids. However, in many pathophysiological conditions the excessive production of ROS overwhelms the natural antioxidant defense mechanisms. This imbalance is termed oxidative stress (OS).

Persistently high levels of ROS can modify essentially biological molecules, such as lipids, proteins and DNA. It is consistent that rates of ROS production are increased in most diseases.[46] Oxidative stress has been associated with several human diseases such as cancer, neurodegenerative syndromes and inflammation. As a result, natural and synthetic small molecules possessing antioxidant activity are becoming increasingly important in disease prevention and therapy.

Lipoxygenases (LOX) are iron-containing enzymes widely distributed in plants and animals. They catalyze the oxidation of polyunsaturated fatty acids such as linoleic acid (in plants) and arachidonic acid (in mammals) at specific positions to hydroperoxides. Lipoxygenase inhibitors are of interest due to the implication of the enzyme to various pathophysiological conditions as well as to a number of food-related processes.

In humans lipoxygenase plays a key role in the biosynthesis of leukotrienes, the proinflammatory mediators mainly released from myeloid cells. Thus, inhibitors of lipoxygenases have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases, but their therapeutic potential has now been expanded to certain types of cancer and cardiovascular diseases.[47–50] The majority of LOX inhibitors are antioxidants or free radical scavengers, since lipoxygenation occurs via a carbon-centered radical, and these compounds can inhibit the formation of the radical or trap it once formed.[51,52]

Moreover, lipoxygenases have applications in food chemistry and technology: they play a positive role in bread making and aroma production; however, their activity may have an impact on the final color or off-flavor formation in various food products.[53,54]

As part of our research program involving the design and synthesis of bioactive small molecules, we decided to study the molecular scaffold of 2′-hydroxy-chalcones and their oxidative cyclization products, aurones, and examine their potential as antioxidant and anti-inflammatory agents. Thus, we present here the synthesis and structural characterization of a series of natural and non-natural 2′-hydroxy-chalcones and aurones as well as their in vitro antioxidant and soybean lipoxygenase inhibitory activity.

2. Results and discussion

2.1. Synthesis and characterization of chalcones and aurones

2′-Hydroxy-chalcones **7**–**18** have been synthesized via the Claisen–Schmidt condensation reaction between appropriately substituted 2′-hydroxy-acetophenones and benzaldehydes in basic conditions (20% aqueous KOH), as depicted in Scheme 1.

In order to contribute to robust structure–activity relationship studies, we decided to prepare a series of chalcones and aurones possessing unsubstituted or methoxy-substituted ring A while bearing methoxy, chloro or methyl substituents on various positions of ring B. Thus, we used as starting material the commercially available 2′-hydroxy-acetophenone (**6**) and we prepared 2′-hydroxy-4′,6′-dimethoxy-acetophenone (**2**), by methylation of 2′,4′,6′-trihydroxyacetophenone (phloroacetophenone) (**1**) using dimethyl sulfate as the alkylating agent (see Section 5). The benzaldehydes used in this study were all commercially available, with the exception of 3,4-dimethoxymethylbenzaldehyde (**5**) which was synthesized from 3,4-dihydroxybenzaldehyde (**4**) using MOM-Cl and K_2CO_3 in acetone (see Section 5).

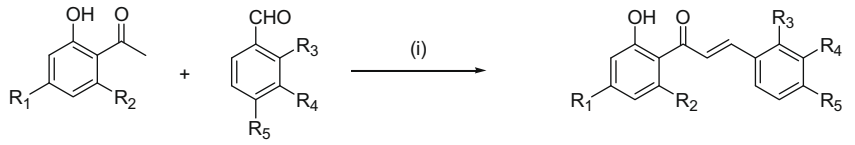
The synthesis of the polyhydroxylated naturally occurring chalcone **19** (Scheme 2) was accomplished via the chalcone **18**, which is fully protected with the methoxymethyl (MOM) protecting group, as proposed by other researchers for the same or analogous compounds.[55–57] The MOM group is stable under the basic conditions of the Claisen–Schmidt condensation and is easily removed by acidic treatment. The required 2′-hydroxy-4′,6′-dimethoxymethylacetophenone (**3**) was synthesized from phloroacetophenone (**1**) as described in Section 5. Removal of the MOM protecting group was achieved by refluxing **18** in 10% aqueous HCl in methanol for 15 min, carefully monitoring the reaction by TLC. After aqueous work-up and purification by silica gel flash column chromatography, we obtained **19** in 53% yield.

The existing methodologies for the synthesis of the aurone scaffold include the oxidative cyclization of 2′-hydroxychalcones,[58,59] the base-catalyzed cyclization of α-bromo-6-alkoxydihydrochalcones (Wheeler synthesis),[60] bromination and cyclization of 2′-acetoxychalcones,[61] condensation of benzofuran-3(2*H*)-ones with benzaldehydes[62] and, recently, the gold-catalyzed cyclization of 2-(1-hydroxyprop-2-ynyl)phenols.[63] Having prepared the series of chalcones **7**–**18**, we decided to proceed to the synthesis of the desired aurones via the oxidative cyclization methodology using mercury(II) acetate in pyridine (Scheme 3). This combination of reagent/solvent was proposed by Subbaraju and co-workers[45] as an alternative to mercury(II) acetate in acetic acid in an attempt to minimize the formation of the flavone cyclization product. We were gratified to find that this was indeed the case with all the chalcones we subjected to this reaction: refluxing equimolar amounts of the chalcone and mercury(II) acetate in pyridine, the major (or exclusive) product was the desired aurone which was subsequently purified either by recrystallization or by flash column chromatography. In all cases the thermodynamically more stable *Z*-geometric isomer was obtained and the yields were moderate to satisfactory (44–70%).

To our knowledge, the synthesis of aurones **21**, **23**–**25**, **27** and **28** has not been previously accomplished using this oxidative cyclization procedure.

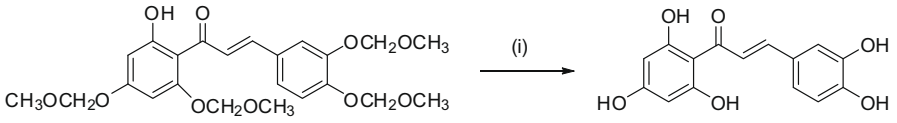
In order to efficiently synthesize aureusidin (4,6,3′,4′-tetrahydroxy-aurone) (**31**) (Scheme 4), one of the most common natural aurones, the corresponding 4,6,3′,4′-tetramethoxymethyl-aurone (**30**) was prepared. Thus, oxidative cyclization of chalcone **18** resulted in the formation of the new aurone **30** which was obtained as yellow crystals in 67% yield, after recrystallization from methanol/dichloromethane. Removal of the MOM protecting groups was effected by refluxing **30** in a solution of 10% aqueous HCl and methanol for 15 min. Extraction with ethyl acetate followed by evaporation of the solvents and purification by flash column chromatography, afforded aureusidin (**31**) as an orange-red solid in 88% yield.

Only two other synthetic approaches to aureusidin have been reported until now: one involving condensation of 4,6-dihydroxy-



	R_1	R_2	R_3	R_4	R_5
7	H	H	H	H	OCH_3
8	H	H	H	H	CH_3
9	H	H	H	H	Cl
10	H	H	H	OCH_3	H
11	H	H	OCH_3	H	H
12	H	H	H	OCH_3	OCH_3
13	OCH_3	OCH_3	H	OCH_3	OCH_3
14	OCH_3	OCH_3	H	H	OCH_3
15	OCH_3	OCH_3	H	H	Cl
16	OCH_3	OCH_3	H	H	CH_3
17	OCH_3	OCH_3	OCH_3	H	H
18	OMOM	OMOM	H	OMOM	OMOM

Scheme 1. Reagents and conditions: (i) 20% aqueous KOH, EtOH, rt, 24–36 h.



18 **19**

Scheme 2. Reagents and conditions: (i) 10% aqueous HCl, EtOH, reflux, 15 min.

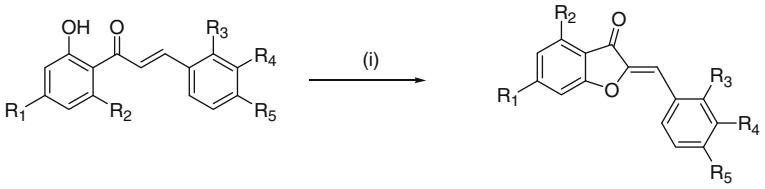
benzofuranone with 3,4-dihydroxybenzaldehyde in acetic acid/HCl,[2,26] giving aureusidin in low yield (7%)[2] and the one by D. Bolek and M. Gütschow,[64] who synthesized aureusidin in 36% yield by demethylation of the corresponding 4,6,3′,4′-tetramethoxy-aurone using BBr_3.

The synthesis proposed in this work via the 4,6,3′,4′-tetramethoxymethyl-aurone (**30**) is advantageous as it involves a mild and quick deprotection step and affords aureusidin (**31**) in high yield.

The ^{1}H NMR spectra of the synthesized chalcones confirmed the *E*-geometry of the double bond, showing a pair of AB doublets with characteristic *J* values of 15.3–15.8 Hz. The chemical shifts of the most characteristic protons are strongly affected by the substitution pattern of rings A and B. Therefore, for compounds **7**–**10** and **12**, bearing no substituents on ring A, the signals of the vinylic protons appear at δ 7.53–7.64 (H_α) and at δ 7.89–7.92 (H_β). A significant downfield shift of these signals is observed in the ^{1}H NMR spectrum of compound **11**, which bears a methoxy group on the 2-position of ring B. The signal of H_α appears at δ 7.79 ($\Delta\delta$ = 0.15–0.26 ppm) and the signal of H_β at 8.23 ($\Delta\delta$ = 0.31–0.47 ppm). This difference can be attributed to the 'ortho' effect of the methoxy group. The ^{1}H NMR spectra of the chalcones **7**–**12** are also characterized by a downfield signal at δ 12.75–12.93 which is attributed to the hydrogen-bonded proton of the 2′-hydroxyl group.

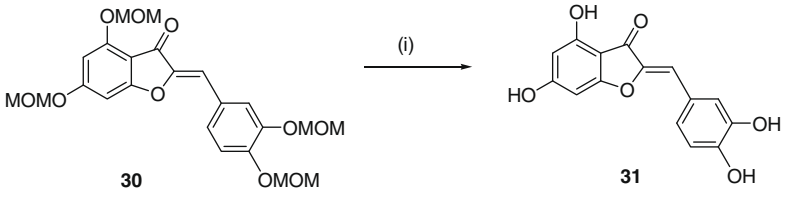
The presence of the methoxy or methoxymethyl groups at positions 4′ and 6′ of ring A (compounds **13**–**18**) does not strongly affect the chemical shifts of the vinylic protons, which appear at δ 7.71–7.77 (H_α) and at δ 7.79–7.87 (H_β), but causes a significant downfield shift of the hydroxyl proton signal (δ 13.91–14.93), indicating a stronger hydrogen bond as expected by the electron-donating nature of these substituents.[65] The 'ortho' effect of the methoxy group present on the 2-position of chalcone **17** results to a downfield shift of the vinylic protons signals (δ 7.96 for H_α and 8.15 for H_β).

In the case of chalcones **13** and **14**, the ^{1}H NMR spectra obtained at a 300 MHz instrument failed to show the expected splitting pattern of the AB doublet system: the signals of H_α and H_β appeared as a doublet at 7.78 ppm (*J* = 2.1 Hz) in the spectrum of **13** and as a singlet at 7.79 ppm in the spectrum of **14**. Obtaining the spectra at a 600 MHz instrument showed the expected splitting pattern



	R_1	R_2	R_3	R_4	R_5
20	H	H	H	H	OCH_3
21	H	H	H	H	CH_3
22	H	H	H	H	Cl
23	H	H	H	OCH_3	H
24	H	H	OCH_3	H	H
25	H	H	H	OCH_3	OCH_3
26	OCH_3	OCH_3	H	H	OCH_3
27	OCH_3	OCH_3	H	H	Cl
28	OCH_3	OCH_3	H	H	CH_3
29	OCH_3	OCH_3	OCH_3	H	H
30	OMOM	OMOM	H	OMOM	OMOM

Scheme 3. Reagents and conditions: (i) $Hg(OAc)_2$, pyridine, 110 °C.



Scheme 4. Reagents and conditions: (i) 10% aqueous HCl, MeOH, reflux, 15 min.

with the appropriate *J* coupling values (see Section 5), in accordance to the spectra obtained for all the synthesized chalcones in this work.

The NMR spectroscopic data of the synthesized aurones prove that this oxidative cyclization procedure yields exclusively the *Z*-geometrical isomer, as has already been reported. As expected, the substitution pattern of both rings of the aurones affects the chemical shifts of the characteristic vinylic proton. More specifically, this proton appears as a singlet at δ 6.84–6.89 for the non-substituted on ring A aurones **20**–**23** and **25** whereas it shifts upfield, at δ 6.57–6.71, for aurones **26**–**28** and **30**, bearing two electron-donating alkoxy groups on ring A. The electron releasing nature and the position of the substituents on ring B cause further differentiation in the chemical shifts of the vinylic proton: the presence of a chloro-substituent on position 4′ results to an upfield shift of the signal (δ 6.84 for **22** and δ 6.57 for **27**) whereas the presence of a methoxy group on position 2′ causes a downfield shift of the signal at δ 7.49 for **24** and 7.32 for **29**. This effect of the 2′-substituent, the same as in the case of the corresponding chalcones, has been reported previously for 2′-substituted aurones and was a reason to doubt the *Z*-configuration of these compounds. However, Sim et al. published recently the X-ray structural analysis of aurone **29** and unambiguously proved the *Z*-configuration of the exocyclic double bond.[41] In general our spectral data were in agreement with the reported data for the same or analogous compounds.

2.2. Antioxidant activity

Taking the multifactorial character of oxidative stress into account, we decided to evaluate the in vitro antioxidant activity of the synthesized molecules using five different antioxidant assays. Therefore, the radical scavenging ability of the compounds was tested against the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical and the superoxide anion radical $O_2^{\cdot -}$ (metal-free systems) as well as against the hydroxyl radical ($HO^{\cdot}$) generated by the Fe^{3+}/ascorbic acid system. The ability of the compounds to scavenge another important ROS, hydrogen peroxide, was tested using the peroxyoxalate chemiluminescence method. Finally, the ability of the synthesized chalcones and aurones to inhibit lipid peroxidation induced by the thermal free radical producer 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was evaluated.

2.2.1. Interaction with the DPPH stable free radical

The scavenging effect of the synthesized compounds on the DPPH radical was evaluated according to the methods of Hadjipavlou et al.[66] and the results are presented in Tables 1 and 2.

The interaction of the synthesized compounds with the stable free radical DPPH indicates their radical scavenging ability in an iron-free system. The interaction of the tested chalcones and aurones with DPPH was found to be time and concentration dependent. Among the tested chalcones, the best DPPH radical scavenging activity was presented by the naturally occurring 2′,3,4,4′,6′-pentahydroxy-chalcone (**19**), which exhibits higher activity than the reference compound nordihydroguaiaretic acid (NDGA). 2′-Hydroxy-3,4,4′,6′-tetra(methoxymethyl)-chalcone, (**18**), and 2′-hydroxy-2-methoxy-chalcone (**11**), bearing a methoxy group on position 2 of the B ring and no substituents on the A ring display satisfactory activity, but much lower than **19**. For chalcones **7**–**12**, not bearing methoxy groups on ring A, the DPPH scavenging ability seems to depend only on the position of the methoxy group on ring B (compare **7**, **10** and **11**), whereas the electronic character of the B ring substituents does not affect activity since compounds **7**, **8** and **9** display the same activity. The same is true for chalcones **13**–**17**, which bear methoxy groups on positions 4′ and 6′ of the A ring: the most active chalcone of this series is **17**, bearing the OCH_3 group on position 2 of the B ring.

The tested aurones are moderate to good DPPH radical scavengers, with the exceptions of aureusidin (**31**) and aurone **23**, substituted with a methoxy group on position 3′, which exhibit scavenging ability comparable to the reference compound NDGA. It is also interesting that compound **23** presents high scavenging activity as good as compound **31**, although they present significantly different lipophilicity, 3.97 and 2.42, respectively. Aurone **22**, bearing a Cl group on position 4′ showed very good activity (76.2%). In the case of these compounds the substitution pattern of ring A is crucial for activity: with the exception of **20**, aurones which do not bear methoxy groups on ring A are more active. As in the case of chalcones, the electron-withdrawing or -donating character of the substituents is not important for this type of activity.

2.2.2. Superoxide anion radical scavenging ability

Superoxide anion radicals $O_2^{-\cdot}$ and H_2O_2 are reactive oxygen species produced in vivo, through a one-electron reduction process of molecular oxygen. The evaluation of superoxide anion radical scavenging activity can be determined using assays involving non-enzymatic or enzymatic production of superoxide anions. In this case, the non-enzymatic assay was used. The superoxide producing system was set up by mixing phenazine methosulfate (PMS), NADH and air–oxygen. The production of superoxide radical was estimated by the nitroblue tetrazolium method.[51]

The results of this assay are presented in Tables 1 and 2. The tested chalcones exhibited high activity at 10 μM. Compounds **12**, **15** and **16** presented the highest activity (100%), followed by compounds **10** and **11**. For the chalcones that do not have any substituents on the A ring (compounds **7**–**12**) the position of the substituents seems to be important for activity: substituents on position 4 do not favor activity regardless if they are electron-donating (–OCH_3 and –CH_3 for compounds **7** and **8,** respectively) or electron-withdrawing (–Cl for compound **9**). On the other hand, the presence of –OCH_3 group on positions 2, 3 or the presence of two methoxy groups on positions 3 and 4 increases the radical scavenging activity of the chalcone. As far as substitution with two methoxy groups on positions 4′ and 6′ of the A ring is concerned, the results show that activity is enhanced when a –CH_3 or a –Cl group is present on position 4 of the B ring (compounds **16** and **15,** respectively).

Among the tested aurones, compounds **25** (bearing –OCH_3 groups on positions 3′ and 4′) and **28** (with –OCH_3 groups on positions 4 and 6 and a –CH_3 on position 4′) showed the highest superoxide anion scavenging activity (97.4%), followed by aurones **22**, **27** and **20**. It is interesting to note that there is a correlation between the activity of chalcones and the corresponding aurones in this assay: a highly active chalcone gives a highly active aurone (e.g., **12** is cyclized to **25**, **15** to **27** and **16** to **28** and all these compounds are highly active).

2.2.3. HO· radical scavenging ability

The competition of the synthesized chalcones and aurones with DMSO for HO·, generated by the Fe^{3+}/ascorbic acid system, expressed as percent inhibition of formaldehyde production, was used for the evaluation of their hydroxyl radical scavenging activity.[51] In this experiment, the majority of the tested chalcones and aurones showed remarkable activity at 100 μM (higher than the well known antioxidant trolox) (Tables 1 and 2). Compounds **7**–**9** exhibited 100% inhibition, indicating that the electron-donating

Table 1
Interaction % with DPPH, superoxide radical scavenging ability (PMS %) and competition % with DMSO for the hydroxyl radical (HO· %) of chalcones **7**–**19**

Compound	DPPH (%)				PMS %	HO· (%)	*C* log *P*
	50 μM		100 μM		10 μM	100 μM	
	20 min	60 min	20 min	60 min			
7	0.0	0.0	0.0	40.8	73.7	100.0	3.88
8	0.0	1.5	0.0	43.8	84.2	100.0	4.46
9	0.0	0.0	0.0	40.8	84.2	100.0	4.67
10	0.0	0.0	0.0	34.6	97.4	57.0	3.88
11	0.0	0.0	11.8	57.7	92.1	100.0	3.88
12	0.0	0.0	6.3	28.5	100	84.1	3.62
13	0.0	0.0	12.9	20.8	86.8	42.2	3.66
14	0.0	0.0	6.3	40.8	50.0	78.7	3.92
15	0.0	0.0	15.1	46.9	100	100.0	4.72
16	0.0	0.0	9.6	24.6	100	100.0	4.50
17	9.7	5.9	15.1	47.7	89.5	0.0	3.92
18	5.6	0.0	42.6	65.4	50.0	100.0	2.89
19	n.t. [a]	n.t.	100.0	94.9	n.t.	n.t.	1.57
NDGA[b]	84	83	81	83			
Caffeic acid					5.5		
Trolox						73.4	

Lipophilicity theoretically calculated as *C* log *P* values.
[a] n.t. = not tested.
[b] NDGA—nordihydroguaiaretic acid.

Table 2
Interaction % with DPPH, superoxide radical scavenging ability (PMS %) and competition % with DMSO for the hydroxyl radical (HO· %) of aurones **20**–**29** and **31**

Compound	DPPH (%)				PMS %	HO· (%)	*C* log *P*
	50 μM		100 μM		10 μM	100 μM	
	20 min	60 min	20 min	60 min			
20	13.9	0.0	5.1	23.8	81.6	76.4	3.97
21	5.6	29.4	23.9	49.2	68.4	84.5	4.55
22	11.1	10.3	49.3	76.2	89.5	0.0	4.76
23	11.1	0.0	87.9	83.8	50.0	57.8	3.97
24	4.2	0.0	25.0	43.1	73.7	100.0	3.97
25	n.t.	n.t.	n.t.	n.t.	97.4	76.7	3.71
26	2.8	0.0	0.0	11.5	63.2	100.0	3.95
27	0.0	0.0	6.3	20.8	84.2	91.5	4.75
28	0.0	0.0	8.5	29.2	97.4	100.0	4.53
29	4.2	0.0	1.8	30.8	55.3	77.9	3.95
31	n.t.[a]	n.t.	84.9	97.7	n.t.	n.t.	2.42
NDGA[b]	84	83	81	83			
Caffeic acid					5.5		
Trolox						73.4	

Lipophilicity theoretically calculated as *C* log *P* values.
[a] n.t. = not tested.
[b] NDGA—nordihydroguaiaretic acid.

Table 3
H_2O_2 scavenging ability (IC_{50} (mM) and % inhibition of LP induced by AAPH for chalcones **7**–**19**

Compound	H_2O_2 scavenging ability IC_{50} (mM) ± SD	% Inhibition of LP induced by AAPH 60 s, 10 μM
7	0.500 ± 0.030	61.6
8	1.035 ± 0.089	49.6
9	0.713 ± 0.020	74.4
10	0.940 ± 0.060	75.8
11	0.599 ± 0.035	43.1
12	0.228 ± 0.020	64.6
13	0.088 ± 0.003	73.6
14	0.456 ± 0.025	80.9
15	0.657 ± 0.016	42.7
16	0.665 ± 0.008	100.0
17	0.503 ± 0.034	68.4
18	n.t.[a]	77.1
19	0.076 ± 0.006	0.0
Quercetin	0.162 ± 0.019	
β-Carotene	0.0169 ± 0.0003	
Trolox		63.0

[a] n.t. = not tested.

Table 4
H_2O_2 scavenging ability (IC_{50}) and % inhibition of LP induced by AAPH for aurones **20**–**29** and **31**

Compound	H_2O_2 scavenging ability IC_{50} (mM) ± SD	% Inhibition of LP induced by AAPH 60 s, 10 μM
20	0.291 ± 0.030	64.6
21	2.009 ± 0.040	43.1
22	4.14 ± 0.210	46.4
23	1.992 ± 0.031	62.3
24	0.466 ± 0.030	71.1
25	0.044±0.006	53.3
26	1.089 ± 0.010	42.6
27	n.t.[a]	82.9
28	n.t.	72.0
29	2.401 ± 0.082	87.1
31	n.t.	77.8
		19.0 (10 μM)
Quercetin	0.162 ± 0.019	
β-Carotene	0.0169 ± 0.0003	
Trolox		63

[a] n.t. = not tested.

or -withdrawing character of the substituent on the B ring does not affect activity. The position and number of methoxy groups on ring B seems to be important: compounds **7** and **11** show 100% inhibition whereas chalcones **12** (with two OCH_3 groups on positions 3 and 4) and **10** (bearing a OCH_3 group on position 3 of the B ring) are less active. 100% inhibition was observed also by chalcones **15** and **16**. It is evident that the substitution pattern of the rings is important for the HO· radical scavenging ability of the tested chalcones: the presence of methoxy groups on ring A accompanied by the presence of one or two methoxy groups on ring B do not favor activity (compare **7** with **14**, **11** with **17** and **12** with **13**). On the other hand, the highly oxygenated chalcone **18** is highly active.

The majority of tested aurones showed important radical scavenging ability as well, ranging from 76.4% to 100.0% (Table 1). For aurones **20**–**25**, bearing no substituents on ring A, the electron-donating or -withdrawing character and the position of the substituents on ring B seem to be important for activity: aurone **24**, bearing a methoxy group on 2′-position is highly active (100% inhibition) whereas the other isomers (**20** and **23**) were less active. Moreover, the presence of the electron-withdrawing chlorine atom on the 4′-position results to compound **22** which is devoid of activity. Aurones bearing two methoxy groups on positions 4- and 6- are more active than the corresponding compounds without the methoxy groups: it is interesting to note that even the inactive aurone **22** is converted to a highly active aurone **27** when the two methoxy groups are incorporated in the structure.

2.2.4. H_2O_2 scavenging ability

The ability of the synthesized compounds to scavenge hydrogen peroxide was evaluated using the peroxyoxalate chemiluminescence method.[67] The results are presented in Tables 3 and 4. Two of the tested chalcones, the natural product 2′,3,4,4′,6′-pentahydroxychalcone, (**19**) and the analogue 2′-hydroxy-3,4,4′,6′-tetramethoxychalcone, (**13**) and one aurone [3,4-dimethoxy-aurone, (**25**)] were more potent H_2O_2 scavengers than the reference compound quercetin. The results show that the 3,4,4′,6′-substitution pattern of chalcones **13** and **19** is the most important factor affecting this type of activity: chalcones bearing one less methoxy group on ring B, regardless the position, show lower activity (compare flavokawain A, **14** and **17** with **13**). It seems that the 'catecholic' structure is the most influential factor for this type of activity. Removal of the methoxy group of ring B and substitution with CH_3 or Cl results in further decrease of activity. The chalcones which do not possess methoxy groups on ring A are even less active with the exception of 2′-hydroxy-3,4-dimethoxychalcone (**12**), which is the third more active compound of this series.

Generally, the tested aurones did not show remarkable H_2O_2 scavenging ability with the exception of 3′,4′-dimethoxyaurone (**25**), which is one of the most active compounds tested in this study. Again, it is the 3′,4′-dimethoxy substitution pattern of the B ring that is crucial for activity: removal of the methoxy group from position 3′ results in a compound with lower but still important activity (compound **20**). The position of the remaining methoxy group is significant: moving this group from position 4′ to 2′ lowers activity whereas the 3′-substitution results in a much less active compound. If the methoxy group is replaced by CH_3 or Cl, activity is even lower with compound **22** bearing the electron-withdrawing Cl being the least active compound of this series. Conversely to the chalcones, the two aurones bearing methoxy groups on ring A that were tested were not efficient H_2O_2 scavengers.

2.2.5. Inhibition of lipid peroxidation

AAPH induced linoleic acid oxidation has been developed as a quick and reliable method for measuring the antioxidant activity of raw materials, worts and beers. It is based on the inhibition of lipid oxidation, provides a measure of how efficiently antioxidants protect against lipid oxidation in vitro. Oxidation of exogenous linoleic acid by a thermal free radical producer (AAPH) is followed by UV spectrophotometry in a highly diluted sample.[68]

The majority of the studied compounds effectively inhibit AAPH induced lipid peroxidation, showing higher activity than the reference compound trolox. Chalcone **16** exhibited the highest activity (100% at 10 μM). In general, chalcone derivatives bearing alkoxy substituents (–OCH_3 or –OCH_2OCH_3) on positions 4′ and 6′ of the A ring are more efficient inhibitors of lipid peroxidation than the non-substituted analogues. For these chalcones (compounds **13**, **14**, **16**–**18**), the presence of electron-donating groups on the B ring seems to enhance activity (73–100%) whereas the presence of a Cl group leads to compound **15** with moderate activity (42.7%). The same trend concerning the substitution on the A ring (the fused aromatic ring) is also evident in the aurone analogues: three out of the four tested aurones with methoxy groups on the A ring (compounds **27**–**29**) exhibit the highest inhibitory activity from all the aurone series (72–87%). Aureusidin **31** is also a good inhibitor of lipid peroxidation (77.8%) while it presents the lowest *C* log *P* value among all the aurones. Apparently, for the lipid peroxidation assay, the presence and electron-donating character of the substituents on the A ring plays the most important role in the activity of this series of compounds.

3. In vitro inhibition of soybean lipoxygenase (LOX)

Flavonoids, such as quercetin, isoquercitrin, apigenin and luteolin are the most potent 5-lipoxygenase inhibitors among phenolic natural products. Studies on the 5-LOX inhibitory activity of naturally occurring flavonoids suggest that the presence of a catechol structure or a lipophilic substituent in polyhydroxylated compounds appears to be essential for 5-lipoxygenase inhibitory activity.[69–71]

The synthesized chalcones and aurones were evaluated for inhibition of soybean lipoxygenase by the UV-absorbance-based enzyme assay[66] and the results are presented in Tables 5 and 6.

The majority of LOX inhibitors are antioxidants or free radical scavengers,[72] since lipoxygenation occurs via a carbon centred radical. Some studies suggest a relationship between LOX inhibition and the ability of the inhibitors to reduce the Fe^{3+} at the active site to the catalytically inactive Fe^{2+}.[73,74] LOXs contain a 'non-heme' iron per molecule in the enzyme active site as high-spin Fe^{2+} in the native state and the high-spin in the activated state Fe^{3+}. Several LOX inhibitors are excellent ligands for Fe^{3+}. It has been demonstrated that their mechanism of action is presumably related to its coordination with a catalytically crucial Fe^{3+}.

Among the tested compounds, chalcones were generally found to exhibit superior LOX inhibitory activity than aurones. Chalcones **8** and **9**, which have no substituents on the A ring, are very good LOX inhibitors, showing IC_{50} values of 52.2 and 56 μM, respectively. Compound **8** bears an electron-donating methyl group on position 4 of the B ring, whereas compound **9** bears an electron-withdrawing Cl group at the same position. It seems that the different electronic character of these substituents does not affect the LOX inhibitory activity in this case. The presence of methoxy groups either on ring A or on ring B was generally accompanied by low inhibitory activity, with the exception of chalcone **16** (with two methoxy groups on positions 4′ and 6′ of ring A and a methyl group on position 4 of ring B), which is a potent LOX inhibitor (IC_{50} 53 μM).

In an analogous work, Hasan et al.[75] have reported that 2′-hydroxychalcones bearing a chloro-substituent on ring A are efficient LOX inhibitors whereas the presence of a chloro or methyl group on ring B dramatically decreases activity. Moreover, the same group reports that chalcones **7**, **10** and **12** do not present any LOX inhibitory activity and these findings are in accordance with our work with the exception of compound **12** which, in our hands, exhibits 67.5% inhibition. The anti-inflammatory potential of 2′-hydroxy-chalcones has been recently reported by Tran et al. who have shown that compounds **7**–**9** possess moderate inhibitory activity against prostaglandin E2 production and that the presence of alkoxy groups on ring B is required for activity.[76]

As far as the aurone series is concerned, the majority of these compounds did not show significant LOX inhibitory activity at 100 μM, with the exception of aurone **26**, bearing two methoxy groups on positions 4 and 6 of the fused aromatic ring and a methyl group on position 4′ of the other aromatic ring, which exhibited the best LOX inhibitory activity among all the tested compounds (IC_{50} 50 μM) in this work. It is noteworthy that the two chalcones **8** and **16** as well as aurone **26**, which share the common structural feature of a methyl substituent on ring B, are equipotent LOX inhibitors, regardless of the substitution of ring A.

The presence of more than one free hydroxyl groups on the chalcone or aurone skeleton proved to be detrimental for activity: neither chalcone **19** nor aureusidin **31** showed any LOX inhibitory activity.

Table 5
In vitro inhibition of soybean lipoxygenase (LOX) (IC_{50})/% at 100 μM for chalcones **7**–**19**

Compound	LOX IC_{50} (μM) (% 100 μM)
7	(5.0%)
8	52.5
9	56
10	(36.4%)
11	100
12	67.5
13	(1.6%)
14	(2.3%)
15	82
16	53
17	(20.0%)
18	(14.1%)
19	(0.0%)
NDGA[a]	40
Caffeic acid	600

[a] NDGA—nordihydroguaiaretic acid.

Table 6
In vitro inhibition of soybean lipoxygenase (LOX) (IC_{50})/% at 100 μM for aurones **20**–**31**

Compound	LOX IC_{50} (μM) (% 100 μM)
20	70.0
21	(32.4%)
22	(26.4%)
23	(34.9%)
24	(39.9%)
26	50
27	(7.8%)
28	(0.0%)
29	100
30	(0.0%)
31	(0.0%)
NDGA[a]	40
Caffeic acid	600

[a] NDGA—nordihydroguaiaretic acid.

4. Conclusions

In this study, a series of chalcones and aurones have been synthesized and characterized. A new efficient approach to the synthesis of the natural product aureusidin has been developed. The antioxidant activity of the synthesized compounds has been studied in vitro using five different assays. Moreover, in an attempt to identify the potential of the compounds as anti-inflammatory agents, their ability to inhibit in vitro soybean lipoxygenase was evaluated.

Our study indicates that high DPPH radical scavenging activity is not always accompanied by high LOX inhibitory activity. Polyphenolic compounds such as chalcone **19** and aureusidin (**31**) are very efficient DPPH radical scavengers however they do not inhibit LOX. On the other hand, chalcones **8** and **9** and aurone **26** are potent LOX inhibitors possessing low to moderate DPPH radical scavenging activity. This is in accordance with the finding of Curini et al.[77] who have studied the antioxidant and LOX inhibitory activity of five natural prenyloxy-carboxylic acids and showed that the most efficient LOX inhibitor (boropinic acid) is not the most active DPPH radical scavenger.

However, a better correlation exists between LOX inhibitory activity and superoxide anion radical or hydroxyl radical scavenging ability: 2′-hydroxy-chalcones **8**, **9** and **16** are highly active in these assays with chalcone **16** presenting a very good H_2O_2 scavenging and lipid peroxidation inhibition profile.

On the basis of the structure–activity relationship studies performed in this work, it is evident that among the tested compounds chalcone **16** and aurone **26**, both bearing methoxy groups on positions 2′ and 4′ of the A ring, possess the most promising combined antioxidant-LOX inhibitory profile. Apparently, the design of this type of dual-acting molecules should be further explored based on the structural features of these compounds.

5. Experimental

5.1. Instruments and materials

^{1}H NMR spectra (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded on a Varian Gemini 2000 300 MHz spectrometer. The ^{1}H NMR spectra (600 MHz) of compounds **13** and **14** were recorded on a Varian 600 MHz spectrometer. Melting points were determined on a Gallenkamp MFB-595 melting point apparatus and are uncorrected. For the in vitro tests a Lambda 20 (Perkin–Elmer) UV–vis double beam spectrophotometer was used.

The LC/MS analysis was performed using an LC/DAD/MS system comprising a Finnigan MAT Spectra System P4000 pump coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer. The separation was performed on a 125 × 2 mm Superspher 100–4 RP-18 column (Macherey-Nagel, 4 lm particle size) at a flow rate of 0.33 mL/min, the column being kept at 40 °C. The detection was monitored at 370, 400 and 278 nm. The MS-ESI(+) spectroscopy at a probe temperature of 350 °C, probe voltage of 4 kV and at 12 and 40 eV in the mass analyzer. The mass range was set at 121–787 amu and the scan rate was at 0.8 scans/s. The following gradient program was used: (A) AcOH (2.5%) and (B) MeOH, 100% A for 5 min, 0% A in 15 min and kept at 0% A for another 5 min. The data were processed using the Xcalibur 1.2 software.

All the chemicals used were of analytical grade and commercially available from FLUKA, Sigma–Aldrich, ACROS Organics and Merck. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and nordihydroguaiaretic acid (NDGA) were purchased from the Aldrich Chemical Co. Milwaukee, WI, (USA). Soybean lipoxygenase, linoleic acid sodium salt and indomethacin were obtained from Sigma Chemical, Co. (St. Louis, MO, USA).

5.1.1. 2-Hydroxy-4,6-dimethoxyacetophenone, (2)

A mixture of 2′,4′,6′-trihydroxyacetophenone (**1**; 2.2 g, 10 mmol), $(CH_3)_2SO_4$ (1.91 mL, 20 mmol) and anhydrous K_2CO_3 (3.04 g, 22 mmol) in dry acetone (45 mL) was refluxed for 5 h. The reaction mixture was then poured into ice-water (50 mL) and the resulting white precipitate was filtered off and washed with cold water. Yield 1.33 g (62%). The compound was used without further purification, as the ^{1}H and ^{13}C NMR spectral data indicated satisfactory purity.

^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 14.02 (1H, s, OH), 6.06 (1H, d, J = 2.4 Hz, H-3), 5.92 (1H, d, J = 2.4 Hz, H-5), 3.85, 3.82 (each 3H, s, 2 × OCH_3), 2.61 (3H, s, $COCH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 203.12, 167.59, 166.08, 162.90, 106.01, 93.48, 90.73, 55.52, 32.88.

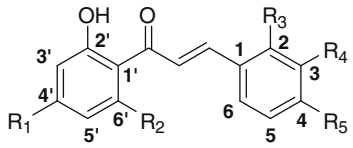
5.1.2. 2-Hydroxy-4,6-bis(methoxymethyl)acetophenone, (3)[2,57]

To a mixture of 2′,4′,6′-trihydroxyacetophenone (**1**; 1.0 g, 5.4 mmol) and anhydrous K_2CO_3 (5.2 g, 40 mmol) in dry acetone (100 mL) was added dropwise chloromethyl methyl ether (1 mL, 13.4 mmol), The mixture was refluxed for 4 h, cooled to room temperature, filtered, washed with acetone and the solvent was evaporated. The resulting yellowish oil was purified using flash column chromatography (petroleum ether/ethyl acetate 9:1) to give **3** as a low melting white solid. Yield 930 mg (63%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 13.70 (1H, s, OH), 6.26 (1H, d, J = 2.7 Hz, H-3), 6.24 (1H, d, J = 2.7 Hz, H-5), 5.25, 5.17 (each 2H, s, 2 × OCH_2O), 3.52, 3.47 (each 3H, s, 3 × OCH_3), 2.65 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 203.30, 166.83, 163.45, 160.35, 106.94, 97.16, 94.48, 94.01, 56.69, 56.43, 32.99.

5.1.3. 3,4-Bis(methoxymethyl)benzaldehyde, (5)[2,57]

Following the procedure described for the synthesis of compound **3**, aldehyde **5** was prepared from 3,4-dihydroxybenzaldehyde (**4**; 500 mg, 3.6 mmol). After evaporation of the solvent, the oily residue was purified using flash column chromatography ((petroleum ether/ethyl acetate 7.5:2.5) to give **5** as a low melting white solid. Yield 702 mg (83%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 9.85 (s, 1H, CHO), 7.67 (d, 1H, J = 1.8 Hz, H-2), 7.50 (dd, 1H, J = 8.4, 1.8 Hz, H-5), 7.27 (d, 1H, J = 8.4 Hz, H-6), 5.32, 5.29 (each 2H, s, 2 × OCH_2O), 3.52, 3.51 (each 3H, s, 3 × OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 190.76, 152.57, 147.42, 131.10, 126.28, 115.88, 115.33, 95.33, 94.92, 56.45, 56.33.

5.2. General procedure for the synthesis of chalcones 7–19



To a stirred solution of the appropriate acetophenone (1 equiv) and a substituted benzaldehyde (1 equiv) in ethanol was added KOH (20% w/v aqueous solution) and the mixture was stirred at room temperature for 24–72 h. The reaction mixture was cooled to 0 °C (ice-water bath) and acidified with HCl (10% v/v aqueous solution). In most cases a yellow precipitate was formed, which was filtered and washed with 10% aqueous HCl solution. In the cases where an orange oil was formed, the mixture was extracted with CH_2Cl_2, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give the chalcone as a solid.

5.2.1. 2′-Hydroxy-4-methoxy-chalcone (7)

Prepared following the general procedure starting from 2-hydroxy-acetophenone (**6**, 500 mg, 3.67 mmol) and *p*-methoxybenzaldehyde (500 mg, 3.67 mmol), dissolved in 9 mL ethanol and KOH (20% aqueous solution, 3 mL). The yellow solid was recrystallized from hexane/ethyl acetate. Yield: 624 mg (67%). Mp 84–86 °C (Ref. 78 93–94 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 12.93 (s, 1H, OH), 7.91 (dd, 1H, $J_{6',5'}$ = 8,06 Hz, $J_{6',4'}$ = 1,46 Hz H-6′), 7.906 (d, 1H, $J_{B,A}$ = 15,38 Hz, H_B), 7.63 (d, 2H, $J_{2,3}$ = $J_{6,5}$ = 8,79 Hz, H-2, H-6), 7.54 (d, 1H, $J_{A,B}$ = 15,38 Hz, H_A), 7.51–7.46 (m, 1H, H-5′), 7.02 (d, 1H, $J_{3',4'}$ = 8.79 Hz, H-3′), 6.95 (d, 2H, $J_{3,2}$ = $J_{5,6}$ = 8,79 Hz, H-3, H-5), 6.96–6.91 (m, 1H, H-4′), 3.87 (s, 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 193.66, 163.55, 162.03, 145.34, 136.12, 130.53, 129.51, 127.35, 120.12, 118.73, 118.57, 117.61, 114.51, 55.43; MS (ESI) m/z = 255 $[M+H]^+$.

5.2.2. 2′-Hydroxy-4-methyl-chalcone (8)

Prepared following the general procedure starting from 2-hydroxy-acetophenone (**6**, 500 mg, 3.67 mmol) and *p*-methylbenzaldehyde (441 mg, 3.67 mmol), dissolved in 9 mL ethanol and KOH (20% aqueous solution, 3 mL). The yellow solid was recrystallized from hexane/ethyl acetate. Yield: 610 mg (70%). Mp 116–117 °C (Ref. 78 115–117 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 12.87 (s, 1H OH), 7.93 (dd, 1H, $J_{6',5'}$ = 7.69 Hz, $J_{6',4'}$ = 1.47 Hz H-6′), 7.92 (d, 1H, $J_{B,A}$ = 15.75 Hz, H_B), 7.62 (d,1H, $J_{A,B}$ = 15.75 Hz, H_A), 7.57 (d, 2H, $J_{2,3}$ = $J_{6,5}$ = 7.69 Hz, H-2, H-6), 7.50 (ddd, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 7.87 Hz, $J_{4',6'}$ = 1.47 Hz H-4′), 7.25 (d, 2H, $J_{3,2}$ = $J_{5,6}$ = 7,69 Hz H-3, H-5), 7.03 (dd, 2H, $J_{3',4'}$ = 7.87 Hz, $J_{3',5'}$ = 0.73 Hz H-3′), 6.94 (ddd, 1H, $J_{5',4'}$ = $J_{5',6'}$ = 7.87 Hz, $J_{5',3'}$ = 0,73 Hz H-5′), 2.41 (s, 3H, –CH_3); ^{13}C

NMR ($CDCl_3$, 75 MHz) δ ppm 193.78, 163.58, 145.56, 141.59, 136.25, 131.88, 129.79, 129.61, 128.71, 120.07, 119.04, 118.78, 118.60, 21.56; MS (ESI) m/z = 239 $[M+H]^+$.

5.2.3. 2′-Hydroxy-4-chloro-chalcone (9)

Prepared following the general procedure starting from 2-hydroxy-acetophenone (**6**, 500 mg, 3.67 mmol) and *p*-chlorobenzaldehyde (516 mg, 3.67 mmol), dissolved in 9 mL ethanol and KOH (20% aqueous solution, 3 mL). The yellow solid was recrystallized from methanol to afford yellow crystals. Yield: 617 mg (81%). Mp 153–156 °C (Ref. 78 149–150 °C); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm 12.76 (s, 1H, OH), 7.92 (d, 1H, $J_{6',5'}$ = 8,06 Hz, H-6′), 7.87 (d, 1H, $J_{B,A}$ = 15.60 Hz, H_B), 7.63 (d, 1H, $J_{A,B}$ = 15.60 Hz, H_A), 7.60 (d, 2H, $J_{2,3} = J_{6,5}$ = 8.40 Hz, H-2, H-6), 7.51 (ddd, 1H, $J_{4',3'} = J_{4',5'}$ = 8.42 Hz; $J_{4',6'}$ = 1.47 Hz, H-4′), 7.41 (d, 2H, $J_{3,2} = J_{5,6}$ = 8.42 Hz, H-3, H-5), 7,03 (d, 1H, $J_{3',4'}$ = 8.42 Hz; H-3′), 6.95 (t, 1H, $J_{5',4'} = J_{5',6'}$ = 8.42 Hz; H-5′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 193.38, 163.55, 143.85, 136.79, 136.48, 132.99, 129.71, 129.53, 129.27, 120.48, 119.84, 118.83, 118.62; MS (ESI) m/z = 259/261 $[M+H]^+/[M+2+H]^+$.

5.2.4. 2′-Hydroxy-3-methoxy-chalcone (10)

Prepared following the general procedure starting from 2-hydroxy-acetophenone (**6**, 500 mg, 3.67 mmol) and *m*-methoxybenzaldehyde (500 mg, 3.67 mmol), dissolved in 9 mL ethanol and KOH (20% aqueous solution, 3 mL). The yellow solid was recrystallized from hexane/ethyl acetate. Yield: 760 mg (81%). Mp 90–92 °C (Ref. 78 92–94 °C); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm 12.79 (s, 1H, OH), 7.93 (dd, 1H, $J_{6',5'}$ = 8.05 Hz, $J_{6',4'}$ = 1.47 Hz, H-6′), 7.90 (d, 1H, $J_{B,A}$ = 15.5 Hz, H_B), 7.64 (d, 1H, $J_{A,B}$ = 15.5 Hz, H_A), 7.51 (ddd, 1H, $J_{4',3'} = J_{4',5'}$ = 7.14 Hz, $J_{4',6'}$ = 1.47 Hz, H-4′), 7.35 (d, 1H, J = 8.05 Hz, H-5), 7.27 (d, 1H, J = 7.33 Hz, H-4), 7.18 (m, 1H, H-2), 7.04 (d, 1H, $J_{3',4'}$ = 8.8 Hz, H-3′), 7.01–6.93 (m, 2H, H-5′, H-6), 3.88 (s, 3H, $-OCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 193.72, 163.62, 160.01, 145.38, 136.43, 135.99, 130.04, 129.67, 121.28, 120.46, 120.02, 118.85, 118.65, 116.62, 113.74, 55.40; MS (ESI) m/z = 255 $[M+H]^+$; HRMS calcd for $C_{16}H_{15}O_3$: m/z: 255.1016, found: 255.1009.

5.2.5. 2′-Hydroxy-2-methoxy-chalcone (11)

Prepared following the general procedure starting from 2-hydroxy-acetophenone (**6**, 500 mg, 3.67 mmol) and *o*-methoxybenzaldehyde (500 mg, 3.67 mmol), dissolved in 9 mL ethanol and KOH (20% aqueous solution, 3 mL). The yellow solid was recrystallized from hexane/ethyl acetate. Yield: 793 mg (85%). Mp 111–112 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ ppm 12.93 (s, 1H, OH), 8,23 (d, 1H, $J_{B,A}$ = 15.56 Hz, H_B), 7.93 (dd, 1H, $J_{6',5'}$ = 8.06 Hz, $J_{6',4'}$ = 1.46 Hz, H-6′), 7.79 (d, 1H, $J_{A,B}$ = 15.56 Hz, H_A), 7.65 (dd, 1H, $J_{3',4'}$ = 7.69 Hz; $J_{3',5'}$ = 1.47 Hz, H-3′), 7.49 (ddd, 1H, $J_{4',5'} = J_{4',3'}$ = 7.87 Hz; $J_{4',6'}$ = 1.46 Hz, H-4′), 7.41 (ddd, 1H, $J_{5',4'} = J_{5',6'}$ = 7.87 Hz; $J_{5',3'}$ = 1.46 Hz, H-5′), 7.04–6.92 (m, 4H, H-3, H-4, H-5, H-6), 3.95 (s, 3H, $-OCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 194.31, 163.58, 159.04, 141.12, 136.12, 132.18, 129.70, 129.64, 123.65, 120.80, 120.21, 118.73, 118.55, 111.32, 55.59; MS (ESI) m/z = 255 $[M+H]^+$; HRMS calcd for C_{16} $H_{15}O_3$: m/z: 255.1016, found: 255.1011.

5.2.6. 2′-Hydroxy-3,4-dimethoxy-chalcone (12)

Prepared following the general procedure starting from 2-hydroxy-acetophenone (**6**, 1 g, 7.3 mmol) and 3,4-dimethoxybenzaldehyde (880 mg, 7.3 mmol), dissolved in 18 mL ethanol and KOH (20% aqueous solution, 6 mL). The orange oil produced upon acidification solidified in the refrigerator. Recrystallization from methanol gives purple-brown crystals. Yield: 840 mg (41%). Mp 112–113 °C (Ref. 79 115–117 °C); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm 12.93 (s, 1H, OH), 7.94 (dd, 1H, $J_{6',5'}$ = 7.8 Hz; $J_{6',4'}$ = 0.7 Hz H-6′), 7.89 (d, 1H, $J_{B,A}$ = 15,4 Hz, H_B), 7.53 (d, 1H, $J_{A,B}$ = 15.4 Hz, H_A), 7.50 (ddd, 1H, $J_{5',6'} = J_{5',4'}$ = 7.8 Hz, $J_{5',3'}$ = 1.65 Hz, H-5′), 7.28 (dd, 1H, $J_{6,5}$ = 8,24 Hz, $J_{6,2}$ = 2,02 Hz, H-6), 7.18 (d, 1H, $J_{2,6}$ = 2,0 Hz, H-2), 7.03 (dd, 1H, $J_{5,6}$ = 8.2 Hz, H-5), 6.95 (ddd, 1H, $J_{4',3'} = J_{4',5'}$ = 7,8 Hz, $J_{4',6'}$ = 0.7 Hz, H-4′), 6.92 (d, 1H, $J_{3',4'}$ = 7.8 Hz, H-3′), 3.98 (s, 3H, $-OCH_3$ methoxy group on C-4), 3.95 (s, 3H, $-OCH_3$ methoxy group on C-3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 193.54, 163.53, 151.80, 149.30, 145.62, 136.12, 129.50, 127.57, 123.56, 120.07, 118.69, 118.56, 117.76, 111.15, 110.29, 55.99; MS (ESI) m/z = 285 $[M+H]^+$; HRMS calcd for $C_{17}H_{17}O_4$: m/z: 285.1121, found: 285.1125.

5.2.7. 2′-Hydroxy-3,4,4′,6′-tetramethoxy-chalcone (13)

Prepared following the general procedure starting from 2-hydroxy-4,6-dimethoxy-acetophenone (**2**, 430 mg, 2 mmol) and 3,4-dimethoxybenzaldehyde (333 mg, 2 mmol), dissolved in 3.5 mL ethanol and KOH (20% aqueous solution, 2 mL). The solid was recrystallized from hexane/ethyl acetate to afford orange crystals. Yield: 340 mg (50%). Mp 149–151 °C; ^1H NMR ($CDCl_3$, 600 MHz) δ ppm 14.37 (s, 1H, OH), 7.79 (d, 1H, $J_{B,A}$ = 15.5 Hz, H_B), 7.75 (d, 1H, $J_{A,B}$ = 15.5 Hz, H_A), 7.25 (s, 1H, H-2), 7.21 (d, 1H, $J_{6,5}$ = 8.5 Hz, H-6), 6.89 (d, 1H, $J_{5,6}$ = 8.5 Hz, H-5), 6.10 (s, 1H, H-3′), 5.95 (s, 1H, H-5′), 3.93, 3.92, 3.90, 3.83 (each s, each 3H, OCH_3 groups); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 192.45, 168.39, 166.05, 162.40, 153.75, 151.09, 149.16, 142.63, 128.63, 125.46, 122.62, 111.19, 110.52, 106.35, 99.24, 93.85, 91.28, 55.99, 55.86, 55.80, 55.57; MS (ESI) m/z = 345 $[M+H]^+$; HRMS calcd for $C_{19}H_{21}O_6$: m/z: 345.1333, found: 345.1331.

5.2.8. 2′-Hydroxy-4,4′,6′-trimethoxy-chalcone (14)

Prepared following the general procedure starting from 2-hydroxy-4,6-dimethoxy-acetophenone (**2**, 300 mg, 1.4 mmol) and *p*-methoxybenzaldehyde (191 mg, 1.4 mmol), dissolved in 3.5 mL ethanol and KOH (20% aqueous solution, 1.2 mL). The yellow solid was recrystallized from methanol to afford yellow crystals. Yield: 180 mg (41%). Mp 109–112 °C (Ref. 10 109–110 °C); ^1H NMR ($CDCl_3$, 600 MHz) δ ppm 14.40 (s, 1H, OH), 7.80 (d, 1H, $J_{B,A}$ = 15.6 Hz, H_B), 7.77 (d, 1H, $J_{A,B}$ = 15.6 Hz, H_A), 7.55 (d, 2H, $J_{2,3} = J_{6,5}$ = 8.8 Hz, H-2, H-6), 6.92 (d, 2H, $J_{3,2} = J_{5,6}$ = 8.8 Hz, H-3, H-5), 6.10 (d, 1H, $J_{3',5'}$ = 2.3 Hz, H-3′), 5.95 (d, $J_{5',3'}$ = 2.3 Hz, H-5′), 3.91 (s, 3H, $-OCH_3$ methoxy group on C-4), 3.84,3.82 (each s, each 3H, methoxy groups on C-4′ and C-6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 192.58, 168.35, 166.00, 162.44, 161.35, 142.44, 130.08, 128.31, 125.12, 114.34, 106.35, 93.80, 91.21, 55.81, 55.54, 55.37; MS (ESI) m/z = 315 $[M+H]^+$.

5.2.9. 2′-Hydroxy-4-chloro-4′, 6′-dimethoxy-chalcone (15)

Prepared following the general procedure starting from 2-hydroxy-4,6-dimethoxy-acetophenone (**2**, 300 mg, 1.4 mmol) and *p*-chlorobenzaldehyde (197 mg, 1.4 mmol), dissolved in 3.5 mL ethanol and KOH (20% aqueous solution, 1.2 mL). The yellow solid was recrystallized from hexane/ethyl acetate. Yield: 270 mg (61%). Mp 168–170 °C (Ref. 10 173–175 °C); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm 14.21 (s, 1H, OH), 7.86 (d, 1H, $J_{B,A}$ = 15.75 Hz, H_B), 7.71 (d, 1H, $J_{A,B}$ = 15.75 Hz, H_A), 7.53 (d, 2H, $J_{2,3} = J_{6,5}$ = 8.43 Hz, H-2, H-6), 7.37 (d,2H, $J_{3,2} = J_{5,6}$ = 8.43 Hz, H-3; H-5), 6.11 (d, 1H, $J_{3',5'}$ = 2.38 Hz, H-3′), 5.97 (d, 1H, $J_{5',3'}$ = 2,38 Hz, H-5′), 3.92 (s, 3H, $-OCH_3$ methoxy group on C-4′), 3.84 (s, 3H, $-OCH_3$ methoxy group on C-6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 192.34, 168.45, 166.37, 162.47, 140.76, 135.86, 134.10, 129.45, 129.14, 128.06, 106.31, 100.17, 93.83, 91.33, 55.89, 55.61; MS (ESI) m/z = 319/321 $[M+H]^+/[M+2+H]^+$; HRMS calcd for $C_{17}H_{16}ClO_4$: m/z: 319.0732, found: 319.0728.

5.2.10. 2′-Hydroxy-4-methyl-4′,6′-dimethoxy-chalcone (16)

Prepared following the general procedure starting from 2-hydroxy-4,6-dimethoxy-acetophenone (**2**, 500 mg, 2.3 mmol) and *p*-methylbenzaldehyde (278 mg, 2.3 mmol), dissolved in 6 mL

ethanol and KOH (20% aqueous solution, 2.3 mL). The solid was recrystallized from methanol to afford orange crystals. Yield: 580 mg (84%). Mp 132–133 °C (Ref. 10 125–126 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 14.33 (s, 1H, OH), 7.87 (d, 1H, $J_{A,B}$ = 15.3 Hz, H_B), 7.77 (d, 1H, $J_{A,B}$ = 15.3 Hz, H_A), 7.51 (d, 2H, $J_{2,3} = J_{5,6}$ = 8.4 Hz, H-2, H-6), 7.21 (d, 2H, $J_{2,3} = J_{5,6}$ = 8.4 Hz, H-3, H-5), 6.11 (d, 1H, $J_{3',5'}$ = 2.7 Hz, H-3′), 5.96 (d, 1H, $J_{3',5'}$ = 2.7 Hz, H-5′), 3.92 (s, 3H, –OCH_3), 3.84 (s, 3H, –OCH_3), 2.39 ((s, 3H, –CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 192.70, 168.36, 166.11, 162.49, 142.51, 140.50, 132.83, 129.61, 128.38, 126.50, 106.36, 93.79, 91.23, 55.82, 55.55, 21.49; MS (ESI) m/z = 299 $[M+H]^+$; HRMS calcd for $C_{18}H_{19}O_4$: m/z: 299.1278, found: 299.1278.

5.2.11. 2′-Hydroxy-2,4′,6′-trimethoxy-chalcone (17)

Prepared following the general procedure starting from 2-hydroxy-4,6-dimethoxy-acetophenone (**2**, 500 mg, 2.3 mmol) and *o*-methoxybenzaldehyde (313 mg, 2.3 mmol), dissolved in 6 mL ethanol and KOH (20% aqueous solution, 2.3 mL). The yellow oil produced upon acidification was extracted with dichloromethane, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give a yellow solid. Trituration with diethyl ether produces the pure chalcone **17** as a yellow solid. Yield: 380 mg (53%). Mp 170–172 °C (Ref. 80 171–173 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 14.93 (s, 1H, OH), 8.15 (d, 1H, $J_{B,A}$ = 15.8 Hz, H_B), 7.96 (d,1H, $J_{A,B}$ = 15.8 Hz, H_A), 7.61 (dd, 1H, $J_{6,5}$ = 7.3 Hz, $J_{6,4}$ = 1.8 Hz, H-6), 7.36 (ddd, 1H, $J_{4,5} = J_{4,3}$ = 7.8 Hz, $J_{4,6}$ = 1,8 Hz, H-4), 6.98 (dd, $J_{5,6} = J_{5,4}$ = 7.3 Hz, H-5), 6.93 (d, 1H, $J_{3,4}$ = 7.8 Hz, H-3), 6.11 (d, 1H, $J_{3',5'}$ = 2.6 Hz, H-3′), 5.96 (d, 1H, $J_{5',3'}$ = 2.6 Hz, H-5′), 3.91 (s, 6H, two –OCH_3), 3.84 (s, 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 193.05, 168.35, 166.02, 162.50, 158.65, 137.83, 131.30, 128.75, 127.88, 124.59, 111.18, 106.45, 93.77, 91.18, 55.74, 55.53, 55.47; MS (ESI) m/z = 315 $[M+H]^+$.

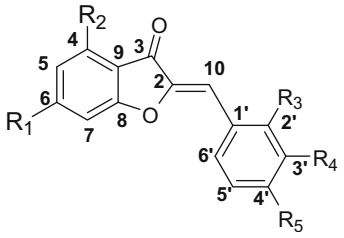
5.2.12. 2′-Hydroxy-3,4,4′,6′-tetra(methoxymethyl)-chalcone (18)

Prepared following the general procedure starting from acetophenone **3** (300 mg, 1.1 mmol) and benzaldehyde **5** (247 mg, 1.1 mmol), dissolved in 3 mL ethanol and KOH (20% aqueous solution, 1 mL). The orange oil produced upon acidification was extracted with dichloromethane, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give a yellow-orange solid, which was recrystallized from hexane/ethyl acetate. Yield: 450 mg (88%). Mp 91–92 °C (Ref. 55 87–88 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 13.91 (s, 1H, OH), 7.85 (d, 1H, $J_{B,A}$ = 15.3 Hz, H_B), 7.73 (d, 1H, $J_{B,A}$ = 15.3 Hz, H_A), 7.50 (d, 1H, $J_{2,6}$ = 1.5 Hz, H-2), 7.20–7.18 (m, 2H, H-5, H-6), 6.31 (d, 1H, $J_{3',5'}$ = 2.4 Hz, H-3′), 6.27 (d, 1H, $J_{3',5'}$ = 2.4 Hz, H-5′), 5.29, 5.28, 5.27, 5.18 (each s, each 2H, –OCH_2O–), 3.54, 3.53, 3.52, 3.48 (each s, each 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 192.71, 167.36, 163.37, 159.90, 149.10, 147.53, 142.34, 129.95, 125.94, 124.10, 116.17, 115.25, 107.47, 97.47, 95.48, 95.14, 94.99, 94.67, 94.03, 56.77, 56.43, 56.31, 56.20; HRMS calcd for $C_{23}H_{28}NaO_{10}$: m/z: 487.1575, found: 487.1579.

5.2.13. 2′,3,4,4′,6′-Pentahydroxy-chalcone (19)[2,55]

Chalcone **18** (190 mg, 0.41 mmol) was added to methanol (8 mL) followed by dropwise addition of HCl (10% aqueous solution, 3.3 mL). The mixture was refluxed for 15 min, until a clear orange solution was obtained. The mixture was diluted with water and extracted with ethyl acetate. After drying and evaporation of solvents an orange residue was obtained, which was purified by flash column chromatography (CH_2Cl_2/CH_3OH 95:5) to give an orange solid. Yield: 63.2 mg (53%). Mp 157–158 °C (Ref. 55 157–158 °C); ^{1}H NMR (CD_3COCD_3, 300 MHz) δ ppm 8.07 (d, 1H, $J_{B,A}$ = 15.8 Hz, H_B), 7.70 (d, 1H, $J_{A,B}$ = 15.8 Hz, H_A), 7.20 (d, $J_{2,6}$ = 1.8 Hz, H-2), 7.08 (dd, 1H, $J_{6,5}$ = 8.4 Hz, $J_{6,2}$ = 1.8 Hz, H-6), 6.88 (d, 1H, $J_{5,6}$ = 8,4 Hz, H-5), 5.96 (s, 2H, H-3′, H-5′); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ ppm 193.22, 165.63, 165.54, 148.75, 146.30, 143.69, 128.80, 125.34, 122.96, 116.44, 115.35, 105.67, 96.05; MS (ESI) m/z = 289 $[M+H]^+$.

5.3. General procedure for the preparation of *Z*-aurones 20–32[45]



To a solution of mercuric acetate (1 equiv) in pyridine was added chalcone (1 equiv) at room temperature and the mixture was stirred at 110 °C for 1 h. The cooled reaction mixture was poured into ice cold water and acidified with HCl (10% aqueous solution). The precipitated solid was extracted with dichloromethane or ethyl acetate, the extracts were dried (Na_2SO_4) and the solvent was evaporated to give a solid which was further purified either by flash column chromatography or recrystallization.

5.3.1. 4′-Methoxy-aurone (20)

Prepared following the general procedure starting from chalcone **7** (254 mg, 1 mmol) in 10 mL pyridine. Recrystallization from $CHCl_3$/CH_3OH afforded yellow crystals. Yield: 152 mg (60%). mp137–138.5 °C (Ref. 63 135 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.90 (d, 2H, $J_{2',3'} = J_{5',6'}$ = 8.8 Hz, H-2′, H-6′), 7.80 (dd, 1H, $J_{4,5}$ = 7.69 Hz, $J_{4,6}$ = 1.47, H-4), 7.64 (ddd, 1H, $J_{6,7}$ = 7.69 Hz, $J_{6,5}$ = 7.33 Hz, $J_{4,6}$ = 1.47, H-6), 7.33 (d, 1H, $J_{6,7}$ = 7.69 Hz, H-7), 7.21 (ddd, 1H, $J_{5,4}$ = 7.69 Hz, $J_{5,6}$ = 7.33 Hz, $J_{5,7}$ = 0.74 Hz, H-5), 6.99 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.79 Hz, H-3′, H-5′), 6.89 (s, 1H, H-10), 3.88 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 184.70, 165.85, 161.08, 145.90, 136.51, 133.44, 125.07, 124.56, 123.26, 121.96, 114.51, 113.39, 112.86, 55.39; MS (ESI) m/z = 253 $[M+H]^+$.

5.3.2. 4′-Methyl-aurone (21)

Prepared following the general procedure starting from chalcone **8** (238 mg, 1 mmol) in 10 mL pyridine. The solid residue was purified by flash column chromatography (petroleum ether/ethyl acetate 95:5). Yield: 121 mg (51%, yellow solid). Mp 98–99.5 °C (Ref. 81 98.5–100 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.82–7.78 (m, 2H, H-2′, H-6′, H-4), 7.63 (ddd, 1H, $J_{6,7}$ = 7.69 Hz, $J_{6,5}$ = 7.33 Hz, $J_{6,4}$ = 1.46 Hz, H-6), 7.32–7.17 (m, 4H, H-7, H-5, H-3′, H-5′), 6.87 (s, 1H, H-10), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 184.70, 166.00, 146.51, 140.46, 136.68, 131.56, 129.68, 129.51, 126.17, 124.58, 123.32, 121.77, 113.32, 112.90, 21.60; MS (ESI) m/z = 237 $[M+H]^+$; HRMS calcd for $C_{16}H_{13}O_2$: m/z: 237.0910, found: 237.0908.

5.3.3. 4′-Chloro-aurone (22)

Prepared following the general procedure starting from chalcone **9** (130 mg, 0.5 mmol) in 5 mL pyridine. The solid residue was purified by flash column chromatography (petroleum ether/ethyl acetate 95:5). Yield: 56 mg (44%, light yellow solid). Mp 152–160 °C (Ref. 63 150–152 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.86 (dd, 2H, $J_{2',3'} = J_{6','5}$ = 8.43 Hz, $J_{2',6'}$ = 1.84 Hz, H-2′, H-6′), 7.83–7.80 (m, 1H, H-4), 7.68 (ddd, 1H, $J_{6,7}$ = 8.42 Hz, $J_{6,5}$ = 7.32 Hz, $J_{6,4}$ = 1.46 Hz, H-6), 7.43 (dd, 2H, $J_{2',3'} = J_{5',6'}$ = 8.79 Hz, $J_{3',5'}$ = 1.83 Hz, H-3′, H-5′), 7.34 (d, 1H, $J_{6,7}$ = 8.06 Hz, H-7), 7.27–7.22 (m, 1H, H-5), 6.84 (s, 1H, H-10); MS (ESI) m/z = 257/259 $[M+H]^+$/$[M+2+H]^+$.

5.3.4. 3′-Methoxy-aurone (23)

Prepared following the general procedure starting from chalcone **10** (500 mg, 2 mmol) in 20 mL pyridine. Recrystallization from chloroform/methanol afforded a light yellow solid. Yield: 310 mg (62%). Mp 120–121 °C (Ref. 31 117–119 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.81 (ddd, 1H, $J_{4,5}$ = 7.69 Hz, $J_{4,6}$ = 1.46 Hz, $J_{4,7}$ = 0.73 Hz, H-4), 7.66 (ddd, 1H, $J_{6,7}$ = 7.33 Hz, $J_{6,4}$ = 1.46 Hz, H-6), 7.51–7.50 (m, 2H, H-4′, H-2′), 7.38 (d, 1H, $J_{6',5'}$ = 8.42 Hz H-6′), 7.33 (dd,1H, $J_{7,6}$ = 8.42 Hz; $J_{7,5}$ = 0.73 Hz, H-7), 7.23 (dd, 1H, $J_{5,6}$ = $J_{5,4}$ = 7.33 Hz, H-5), 6.97 (dd, 1H, $J_{5',4'}$ = 8.42 Hz; $J_{5',6'}$ = 2.56 Hz, H-5′), 6.87 (s, 1H, H-10), 3.88 (s, 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 184.93, 166.32, 159.94, 147.14, 137.07, 133.67, 129.98, 124.84, 124.46, 123.66, 121.77, 116.67, 115.90, 113.11, 113.08, 55.49; MS (ESI) m/z = 253 $[M+H]^+$.

5.3.5. 2′-Methoxy-aurone (24)

Prepared following the general procedure starting from chalcone **11** (254 mg, 1 mmol) in 10 mL pyridine. The solid residue was purified by flash column chromatography (petroleum ether/ethyl acetate 92:8 and then 80:20). Yield: 122 mg (48%, yellow solid). Mp 173–174 °C (Ref. 63 169 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 8.31 (dd, 1H, $J_{4,5}$ = 7.69 Hz, $J_{4,6}$ = 1.46 Hz, H-4), 7.82 (dd, 1H, $J_{7,6}$ = 7.69 Hz, $J_{7,5}$ = 1.46 Hz, H-7), 7.64 (ddd, 1H, $J_{5,4}$ = 8.42 Hz, $J_{5,6}$ = 7.33 Hz, $J_{5,7}$ = 1.46 Hz, H-5), 7.49 (s, 1H, H-10), 7.38 (ddd, 1H, $J_{6,7}$ = 8.42 Hz, $J_{6,5}$ = 7.32 Hz, $J_{6,7}$ = 1.46 Hz, H-6), 7.32 (dd, 1H, $J_{6',5'}$ = 8.40 Hz, $J_{6',4'}$ = 0.73 Hz, H-6′), 7.21 (ddd, 1H, $J_{4',3'}$ = 8.06 Hz, $J_{4',5'}$ = 7.69 Hz, $J_{4',6'}$ = 0.73 Hz, H-4′), 7.09–7.04 (m, 1H, H-5′), 6.93 (dd, 1H, $J_{4',3'}$ = 8.42 Hz, $J_{3',5'}$ = 0.73 Hz, H-3′), 3.91 (s, 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 184.68, 165.96 (C-8), 158.87 (C-2′), 146.94, 136.57, 132.00, 131.47, 124.61, 123.27, 121.89, 121.33, 120.82, 112.89, 110.76, 107.26, 55.60 (OCH_3); MS (ESI) m/z = 253 $[M+H]^+$.

5.3.6. 3′,4′-Dimethoxy-aurone (25)

Prepared following the general procedure starting from chalcone **12** (300 mg, 1 mmol) in 13 mL pyridine. Recrystallization from methanol/pentane afforded bright yellow crystals. Yield: 188 mg (64%). Mp 160–162 °C (Ref. 31 175–178 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.82 (dd, 1H, $J_{4,5}$ = 7.69 Hz, $J_{4,6}$ = 1.46 Hz, H-4), 7.65 (ddd, 1H, $J_{6,7}$ = 8.05 Hz, $J_{6,5}$ = 7.69 Hz, $J_{6,4}$ = 1.46 Hz, H-6), 7.55 (d, 1H, $J_{2',6'}$ = 1.83 Hz, H-2′), 7.51 (dd, 1H, $J_{6',5'}$ = 8.43 Hz, $J_{6',2'}$ = 1.84 Hz, H-6′), 7.32 (d, 1H, $J_{7,6}$ = 8.42 Hz, H-7), 7.21 (dd, 1H, $J_{5,4}$ = 8.05 Hz, $J_{5,6}$ = 0.73 Hz, H-5), 6.95 (d, 1H, $J_{5',6'}$ = 8.42 Hz, H-5′), 6.88 (s, 1H, H-10), 3.98 (s, 3H, –OCH_3), 3.95 (s, 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 184.40, 165.73, 150.88, 149.06, 145.91, 136.48, 125.98, 125.26, 124.55, 123.31, 121.88, 113.78, 113.59, 112.80, 112.21, 55.93, 55.90; MS (ESI) m/z = 283 $[M+H]^+$.

5.3.7. 4,4′,6-Trimethoxy-aurone (26)

Prepared following the general procedure starting from chalcone **14** (100 mg, 0.3 mmol) in 3 mL pyridine. The solid residue was purified by flash column chromatography (petroleum ether/ethyl acetate 7:3). Yield: 46 mg (50%). Mp 166–169 °C (Ref. 41 170–171 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.80 (d, 2H, $J_{2',3'}$ = $J_{5',6'}$ = 8.9 Hz, H-2′, H-6′), 6.93 (d, 2H, $J_{2',3'}$ = $J_{5',6'}$ = 8.9 Hz, H-3′, H-5′), 6.72 (s, 1H, H-10), 6.35 (d, 1H, $J_{5,7}$ = 1.83 Hz, H-7), 6.10 (d, 1H, $J_{5,7}$ = 1.83 Hz, H-5), 3.93, 3.88, 3.83 (each s, each 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 180.53, 168.71, 168.65, 160.50, 159.23, 146.70, 132.80, 130.01, 125.23, 114.45, 114.27, 110.91, 105.35, 93.84, 89.08, 56.08, 55.99, 55.74, 55.26; MS (ESI) m/z = 313 $[M+H]^+$.

5.3.8. 4′-Chloro-4, 6-dimethoxy-aurone (27)

Prepared following the general procedure starting from chalcone **15** (150 mg, 0.44 mmol) in 5 mL pyridine. Recrystallization from methanol/hexane afforded cream crystals. Yield: 90 mg (65%). Mp 171–173 °C (Ref. 41 179–180 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.66 (d, 2H, $J_{2',3'}$ = $J_{5',6'}$ = 8.43 Hz, H-2′, H-6′), 7.27 (d, 2H, $J_{2',3'}$ = $J_{5',6'}$ = 8.42 Hz, H-3′, H-5′), 6.57 (s, 1H, H-10), 6.26 (d, 1H, $J_{7,5}$ = 1.84 Hz, H-7), 6.01 (d, 1H, $J_{7,5}$ = 1.83 Hz, H-5), 3.85, 3.81 (each s, each 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 180.27, 169.00, 168.83, 159.32, 147.92, 135.02, 132.06, 131.00, 128.91, 109.07, 104.93, 94.04, 89.20, 56.12, 56.05; MS (ESI) m/z = 317/319 $[M+H]^+$/$[M+2+H]^+$; HRMS calcd for $C_{17}H_{14}ClO_4$: $m_{/z}$: 317.0575, found: 317.0573.

5.3.9. 4,6-Dimethoxy-4′-methyl-aurone (28)

Prepared following the general procedure starting from chalcone **16** (300 mg, 1 mmol) in 10 mL pyridine. Recrystallization from methanol/hexane afforded yellow crystals. Yield: 140 mg (48%). Mp 178–180 °C (Ref. 41 186–188 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.76 (d, 2H, $J_{2',3'}$ = $J_{5',6'}$ = 8.06 Hz, H-2′, H-6′), 7.23 (d, 2H, $J_{2',3'}$ = $J_{5',6'}$ = 8.06 Hz, H-3′, H-5′), 6.76 (s, 1H, H-10), 6.38 (d, 1H, $J_{7,5}$ = 1.83 Hz, H-7), 6.12 (d, 1H, $J_{7,5}$ = 1.83 Hz, H-5), 3.95, 3.91 (each s, each 3H, –OCH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 180.69, 168.97, 168.84, 159.38, 147.45, 139.73, 131.11, 129.77, 129.54, 111.00, 105.35, 93.98, 89.19, 56.18, 56.08, 21.54; MS (ESI) m/z = 297 $[M+H]^+$; HRMS calcd for $C_{18}H_{17}O_4$: m/z: 297.1121, found: 297.1118.

5.3.10. 2′,4,6-Trimethoxy-aurone (29)

Prepared following the general procedure starting from chalcone **17** (250 mg, 0.8 mmol) in 7.5 mL pyridine. Recrystallization from methanol/pentane afforded light yellow crystals. Yield: 172 mg (68%). Mp 216–218 °C (Ref. 41 215–217 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 8.22 (dd, 1H, $J_{6',5'}$ = 7.69 Hz, $J_{6',4'}$ = 1.83 Hz, H-6′), 7.33 (ddd, 1H, $J_{5',4'}$ = $J_{5',6'}$ = 7.69 Hz, $J_{5',3'}$ = 1.46 Hz, H-5′), 7.32 (s, 1H, H-10), 7.03 (dd, $J_{4',3'}$ = $J_{4',5'}$ = 7.69 Hz, H-4′), 6.92 (d, 1H, $J_{3,4}$ = 7.69, H-3′), 6.38 (d, 1H, $J_{7,5}$ = 1.83 Hz, H-7), 6.13 (d, 1H, $J_{7,5}$ = 1.83 Hz, H-5), 3.95, 3.91, 3.90 (each s, each 3H, –OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 180.57, 168.84, 168.65, 159.30, 158.46, 147.84, 131.51, 130.73, 121.5, 120.57, 110.62, 105.37, 104.87, 93.86, 89.10, 56.11, 55.98, 55.45; MS (ESI) m/z = 313 $[M+H]^+$; HRMS calcd for $C_{18}H_{17}O_5$: m/z: 313.1071, found: 313.1052.

5.3.11. 3′,4,4′,6-Tetra(methoxymethyl)-aurone (30)

Prepared following the general procedure starting from chalcone **18** (500 mg, 1.1 mmol) in 11 mL pyridine. Recrystallization from methanol/dichloromethane afforded yellow crystals. Yield: 220 mg (60%). Mp 85–88 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 7.69 (d, 1H, $J_{2',6'}$ = 1.8 Hz, H-2′), 7.53 (dd, 1H, $J_{6',5'}$ = 8.4 Hz, $J_{2',6'}$ = 1.8 Hz, H-6′), 7.21 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H-5′), 6.71 (s, 1H, H-10), 6.59 (d, 1H, $J_{7,5}$ = 1.8 Hz, H-7), 6.52 (d, 1H, $J_{7,5}$ = 1.8 Hz, H-5), 5.37 (s, 2H, CH_2OCH$_3$ on C-4′), 5.29 (s, 4H, CH_2OCH$_3$ on C-4 and C-6), 5.25 (s, 2H, CH_2OCH$_3$ on C-3′), 3.56, 3.54, 3.53, 3.52 (each s, each 3H, –CH_2OCH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 180.62, 168.35, 165.92, 156.82, 148.63, 147.08, 147.04, 126.96, 126.39, 119.65, 116.30, 111.02, 106.59, 98.71, 95.71, 95.12, 95.02, 94.48, 92.91, 56.59, 56.57, 56.31. MS (ESI) m/z = 463 $[M+H]^+$.

5.3.12. Aureusidin (31)

Aurone **31** (220 mg, 0.48 mmol) was added to methanol (9 mL) followed by dropwise addition of HCl (10% aqueous solution, 4 mL). The mixture was refluxed for 15 min, until a clear orange solution was obtained. The mixture was diluted with water and extracted with ethyl acetate. After drying and evaporation of solvents an orange solid was obtained, which was purified by flash column chromatography (CH_2Cl_2/MeOH 8:2, then pure MeOH). Yield: 121 mg (88%). Mp 270–273 °C (dec.) (Ref. 64 265–270 °C); ^{1}H NMR (CD_3OD, 300 MHz) δ ppm 7.47 (d, 1H, $J_{2',6'}$ = 1.83 Hz, H-2′),

7.18 (dd, 1H, $J_{6',5'}$ = 8.43 Hz, $J_{6',2'}$ = 1.83 Hz, H-6′), 7.82 (d, $J_{5',6'}$ = 8.43 Hz, H-5′), 6.57 (s, 1H, H-10), 6.17 (br s, 1H, H-5), 6.00 (br s, 1H, H-7); ^{13}C NMR (CD_3OD, 75 MHz) δ ppm 182.95, 169.36, 148.88, 147.99, 146.63, 132.51, 125.85, 125.80, 118.63, 116.60, 112.74, 104.93, 91.41; MS (ESI) m/z = 287 $[M+H]^+$; HRMS calcd for $C_{15}H_{11}O_6$: m/z: 287.0550, found: 287.0536.

5.4. Biological assays

Each in vitro experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean

5.4.1. Determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH)[66]

To an ethanolic solution of DPPH (0.05 mM) in absolute ethanol the compounds dissolved in DMSO was added (final concentration 0.1 mM). The mixture was shaken vigorously and allowed to stand for 20 min or 60 min; absorbance at 517 nm was determined spectrophotometrically and the percentage of activity was calculated. All tests were undertaken on three replicates and the results presented in Table 1 were averaged.

5.4.2. Competition of the tested compounds with DMSO for hydroxyl radicals[51]

The hydroxyl radicals generated by the Fe^{3+}/ascorbic acid system, were detected according to Nash, by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (0.1 mM), Fe^{3+} (167 μM), DMSO (33 mM) in phosphate buffer (50 mM, pH 7.4), the tested compounds (concentration 0.1 mM) and ascorbic acid (10 mM). After 30 min of incubation (37 °C) the reaction was stopped with CCl_3COOH (17% w/v) (Table 2). Trolox was used as an appropriate standard.

5.4.3. Non-enzymatic assay of superoxide radicals-measurement of superoxide radical scavenging activity[51]

The superoxide producing system was set up by mixing PMS, NADH and air–oxygen. The production of superoxide was estimated by the nitroblue tetrazolium method. The reaction mixture containing compounds, 3 μM PMS, 78 μM NADH, and 25 μM NBT in 19 μM phosphate buffer pH 7.4 was incubated for 2 min at room temperature and the absorption measured at 560 nm against a blank containing PMS. The tested compounds were preincubated for 2 min before adding NADH. Caffeic acid was used as an appropriate standard.

5.4.4. Inhibition of linoleic acid lipid peroxidation[68]

Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion is monitored at 234 nm. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) is used as a free radical initiator. Ten microliters of the 16 mM linoleic acid dispersion was added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4 prethermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of aliquots (10 μL) of KukA and its analogues. In the assay without antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37 °C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides.

5.4.5. Soybean lipoxygenase inhibition study in vitro[66]

The tested compounds dissolved in DMSO were incubated at room temperature with sodium linoleate (0.1 mL) and 0.2 mL of enzyme solution ($1/9 \times 10^{-4}$ w/v in saline) in Tris buffer pH 9. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor.

5.4.6. Physicochemical studies

Since lipophilicity is a significant physicochemical property determining distribution, bioavailability, metabolic activity, and elimination, we tried to determine theoretically calculated $C\log P$ values[82,83] in *n*-octanol-buffer (Tables 1 and 2).

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