Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 7408–7425
Bioorganic & Medicinal Chemistry

Structure–activity relationship studies of flavonoids as potent inhibitors of human platelet 12-hLO, reticulocyte 15-hLO-1, and prostate epithelial 15-hLO-2

Yesseny Vasquez-Martinez,[a,†] Rachana V. Ohri,[b,†] Victor Kenyon,[b] Theodore R. Holman[b,*] and Silvia Sepúlveda-Boza[a,*]

[a]*Laboratorio de Investigación Científica Emory Black, Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Casilla 442, Correo 2 Santiago, Chile*
[b]*Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064, USA*

Received 28 May 2007; revised 11 July 2007; accepted 13 July 2007
Available online 22 August 2007

Abstract—Human lipoxygenase (hLO) isozymes have been implicated in a number of disease states and have attracted much attention with respect to their inhibition. One class of inhibitors, the flavonoids, have been shown to be potent lipoxygenase inhibitors but their study has been restricted to those compounds found in nature, which have limited structural variability. We have therefore carried out a comprehensive study to determine the structural requirements for flavonoid potency and selectivity against platelet 12-hLO, reticulocyte 15-hLO-1, and prostate epithelial 15-hLO-2. We conclude from this study that catechols are essential for high potency, that isoflavones and isoflavanones tend to select against 12-hLO, that isoflavans tend to select against 15-hLO-1, but few flavonoids target 15-hLO-2.

1. Introduction

Lipoxygenases (LO) catalyze the first step in the conversion of polyunsaturated fatty acids to hydroxylated fatty acids.[1–3] They contain a non-heme iron (Fe^{+3}) that promotes hydrogen atom abstraction, dioxygenation of the 1,4-diene moiety, and reduction of the peroxyl radical to form mono-peroxyl fatty acids.[4] A wide variety of human lipoxygenase (hLO) isozymes are found in nature, which are named after the oxygenated carbon atom of the fatty acid product.[5] The three human lipoxygenase isozymes of this investigation, platelet 12-human lipoxygenase (12-hLO), reticulocyte 15-human lipoxygenase-1 (15-hLO-1) and prostate epithelial 15-human lipoxygenase-2 (15-hLO-2), are involved in a number of diseases. Platelet 12-hLO[6,7] is implicated as a critical signaling molecule in tumor metastasis,[8] skin disease,[9] and neuronal degeneration.[10] Reticulocyte 15-hLO-1[11] has been implicated in atherogenic processes,[12,13] and several pro-neoplastic effects, which contribute to prostatic adenocarcinoma by promoting cell proliferation and angiogenesis.[14–16] Prostate epithelial 15-hLO-2,[17] however, is thought to inhibit the growth of prostatic adenocarcinoma[16] because its loss contributes to increased proliferation and reduced differentiation of cancerous cells.[18,19] For these reasons, there is considerable interest in the discovery and development of selective inhibitors of 12-hLO and 15-hLO-1, which do not inhibit 15-hLO-2.

The search for selective lipoxygenase inhibitors has uncovered several classes of natural products with inhibitory activity. Known inhibitors against 12-hLO include, the alkaloids, such as chelerythrine and sanguinarine,[20] the tropolones, such as hinokitiol,[21] the arachidonic acid analogues, such as falcarindiol and panaxynol,[22] and the phenolics, such as rosmarinic acid, caffeic acid,[23] and anthracenones.[24] Known 15-hLO-1 inhibitors include the marine-derived brominated phenol esters,[25] the marine sponge-derived terpenes,[26,27] the nordihydroguaiaretic acid (NDGA) derivatives,[28] and the anthraquinones.[29] Nevertheless, the most effective inhibitors against both 12-hLO and 15-hLO-1 are found in the broad class of phenolic compounds,[30] especially among flavonoids such as baicalein,[31] quercetin,[32] luteolin,[33]

Keywords: Lipoxygenase; Flavonoids; Reductive inhibition; IC_{50}.
* Corresponding authors. Tel.: +1 831 459 5884; fax: +1 831 459 2935; e-mail: tholman@chemistry.ucsc.edu
† These authors contributed equally to this research.

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.07.036

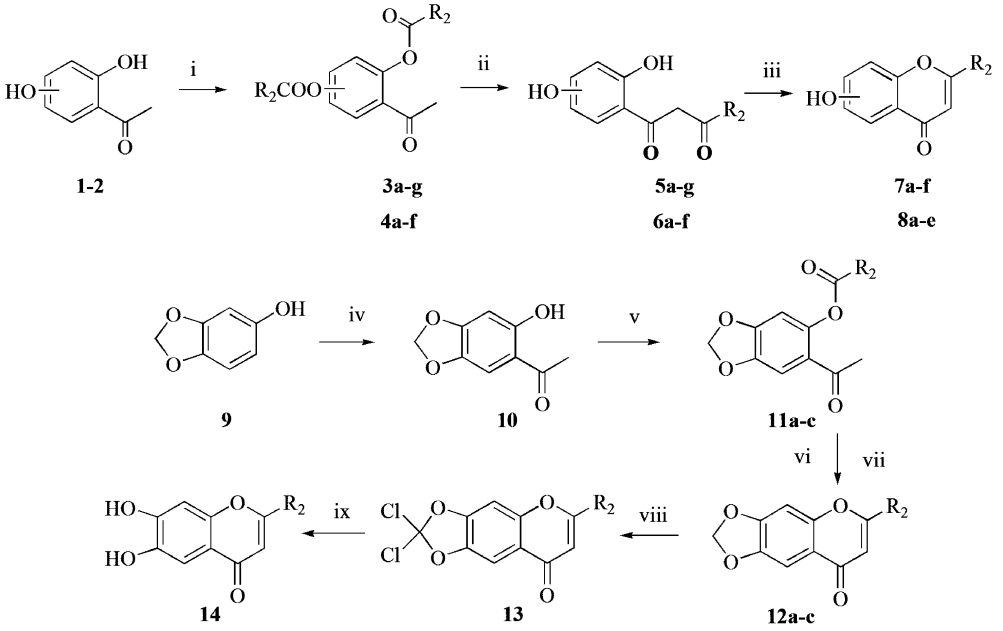
anadanthoflavone,[34] esculetin,[35] and epicatechin.[36] Interestingly, members of this class of potent inhibitors have rarely been simultaneously investigated to determine their relative potency and selectivity against 12-hLO, 15-hLO-1 and 15-hLO-2. This fact, together with the limited structural variability of natural flavonoids, highlighted the need to investigate the structural requirements of this broad class of flavonoid inhibitors against each of these three hLO isozymes.

In the current investigation, we have used the basic structure of flavonoids, the benzopyran skeleton, as a structural template for the design of novel, potent, and selective inhibitors of hLO. We present the synthesis of new benzopyran-4-one analogues with different alkyl and/or aryl substitution at positions C-2 and C-3, and with the scaffold decorated with hydroxyl groups at different positions. This diverse library of flavonoid derivatives was subsequently screened in vitro against 12-hLO, 15-hLO-1, and 15-hLO-2 and compared with known inhibitors in order to determine the relative structural determinants for each isozyme's inhibition.

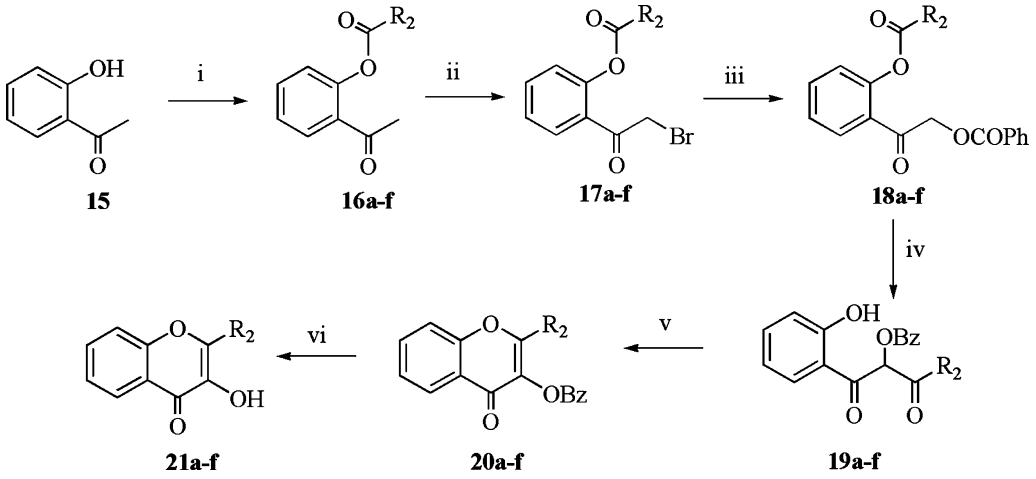
2. Results and discussion

2.1. Synthesis

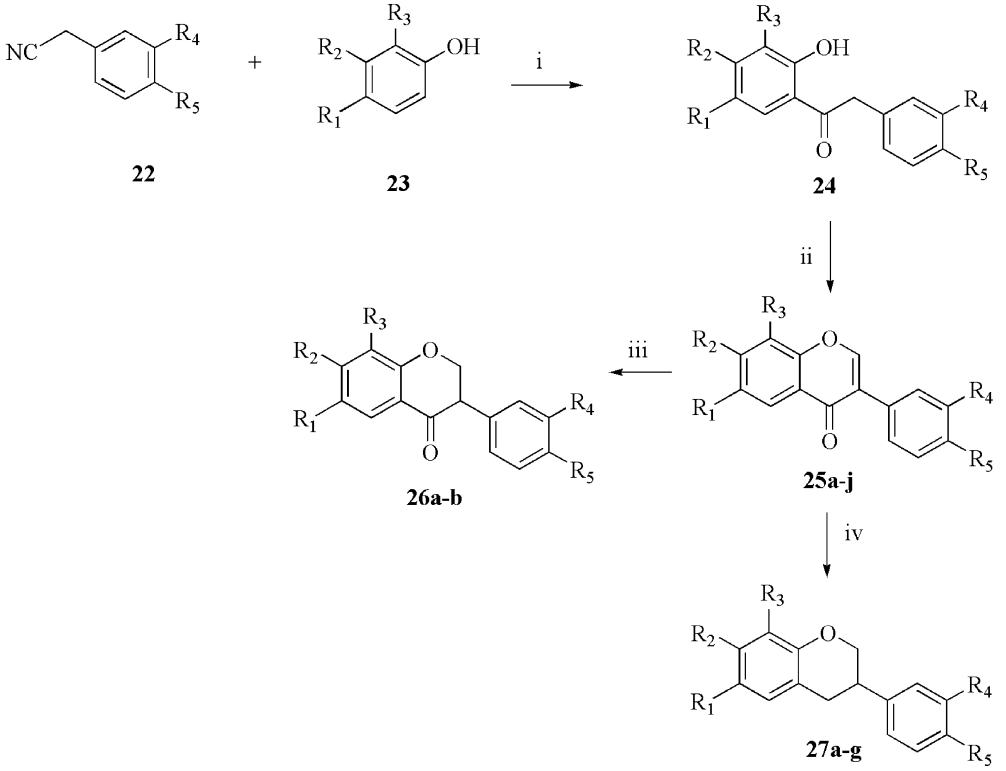
The 2-substituted 6-hydroxychromen-4-ones (**7a**–**f**) were prepared according to Nussbaumer et al.[37] by acylation of 2,5-dihydroxyacetophenone (**1**) to introduce the desired substituent. The doubly acylated intermediate (**3a**–**g**) underwent migration of acyl residues when treated with sodium hydride. The resulting crude products (**5a**–**g**) were then cyclized to the corresponding 6-hydroxychromen-4-ones (**7a**–**f**) with sulfuric and acetic acids. The 2-substituted 7-hydroxychromen-4-ones (**8a**–**e**) were synthesized analogously (Scheme 1), starting from 2,4-dihydroxyacetophenone (**2**). The 2-alkyl-substituted 6,7-methylenedioxybenzopyran-4-ones (**12a**–**c**) were obtained from the corresponding 2-hydroxy-4,5-methylene-dioxyacetophenone (**10**) using the method previously described,[37] and the 6,7-dihydroxy-2-t-butyl-benzopyran-4-one (**14a**) was prepared from **12a** as shown in Scheme 1. The deprotection of the methylenedioxy group could not be achieved with **12b** and **12c**, because a complex mixture was obtained that we were unable to separate and identify. The 2-substituted 3-hydroxychromen-4-ones (**21a**–**f**) were prepared according to Fourgerousse et al.[38] by acylation of the 2-hydroxyacetophenone (**15**) in pyridine solution at room temperature for 2 h. Selective bromination at the alpha position with regard to the carbonyl group was achieved using phenyltrimethylammonium tribromide (PTT). In the next step, the functional chromanol oxygen atom was introduced with potassium benzoate, under vigorous stirring (48 h) at room temperature. The Baker–Venkataraman rearrangement was carried out using a non-nucleophilic base, sodium hydride, in DMF at 0–5 °C. The product was washed with water and used immediately in the cyclization step, which took place in a 0.5% solution of sulfuric acid in glacial acetic acid. Finally, the hydroxyl groups were deprotected by saponification in a 5% alcoholic solution of sodium hydroxide, for 2 h at 60 °C (Scheme 2). The isoflavonoids (**25a**–**j**) were obtained by electrophilic substitution of appropriate phenols with benzyl cyanides (Houben Hoesch reaction). The resulting hydroxylketones were cyclized to the isoflavones using DMF/$MeSO_2Cl$ as a carbon atom donor in the presence of BF_3Et_2O (Scheme 3).[39] The isoflavanone (**26a**–**b**) and isoflavane (**7a**–**g**) were obtained through catalytic hydrogenation with Pd/C (10%) from the corresponding isoflavones. The reduction of isoflavones to the corresponding isoflava-



Scheme 1. Synthesis of 6-and 7-hydroxybenzopyran-4-ones. (i) 2.5 eq. R_2COCl/pyridine/rt; (ii) NaH/DMF/0 °C; (iii) $AcOH/H_2SO_4$/60 °C; (iv) Ac_2O/BF_3OEt/90 °C; (v) 2.5 eq. R_2COCl/pyridine/rt; (vi) NaH/DMF/0 °C; (vii) $AcOH/H_2SO_4$/60 °C; (viii) PCl_5/CH_2Cl_2; (ix) H_2O.



Scheme 2. Synthesis of 3-hydroxy-benzopyran-4-ones. (i) 2.5 eq R_2COCl/pyridine/rt; (ii) $PhMe_3N^+Br_3^-$/THF/rt; (iii) PhCOOK/CH_3CN/rt; (iv) NaH/DMF/0 °C; (v) AcOH/H_2SO_4/60 °C; (vi) NaOH 5%/EtOH-H_2O/60 °C.



Scheme 3. Synthesis of isoflavonoids. (i) $ZnCl_2/H_2O/H^+$; (ii) DMF/$MeSO_2Cl$ in BF_3OEt; (iii) H_2/Pd/C/EtOH-Dioxane/6 h; (iv) H_2/Pd/C/HAc—0.1% conc. H_2SO_4/14 h.

nones (**26a**–**b**) was done by catalytic hidrogenation over Pd/C (10%) in dioxane/ethanol for 6 h. To obtain the isoflavanes (**27a**–**g**), the corresponding isoflavones were hydrogenated over Pd/C (10%) in acetic acid containing 0.1% concentrated sulfuric acid, for 14 h.

2.2. Biological evaluation

Many flavonoids have been investigated over the years as lipoxygenase inhibitors, however, they are rarely screened simultaneously with a variety of hLO isozymes to determine specificity. We present the inhibitory potency of known flavonoid inhibitors, quercetin, baicalein, and fisetin, in conjunction with a library of synthetic flavonoid compounds against the activity of three human lipoxygenases, 12-hLO, 15-hLO-1, and 15-hLO-2.

Initial HTP screening of all forty-seven flavonoid compounds was performed against 12-hLO, 15-hLO-1, and 15-hLO-2, using the Fe^{3+}/xylenol orange screening method[40] in a 384-well format and compared these results with our well-established manual screen.[31,41] The HTP screen uncovered twelve flavonoids with IC_{50} val-

ues lower than 50 μM for 12-hLO, thirteen for 15-hLO-1, and three for 15-hLO-2. Our manual screen of the entire library confirmed these results, with one additional inhibitor found for 12-hLO and 3 for 15-hLO-1. These data indicate that our one-point HTP screen was able to detect hLO inhibitors exceptionally well, with over 95% predictive power for all three of the hLO isozymes, 12-hLO, 15-hLO-1, and 15-hLO-2.

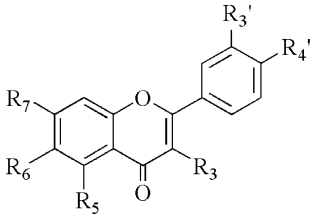
Full IC_{50} values were determined for all the potent flavonoid inhibitors found. As seen in Table 1, the known LO inhibitors, nordihydroguaiaretic acid (NDGA), quercetin (**28**), fisetin (**29**), and baicalein (**30**) are potent inhibitors of 12-hLO and 15-hLO-1, which is consistent with previous data.[42,31,32] Nevertheless, despite the potency of these inhibitors against 12-hLO and 15-hLO-1, only NDGA had some effect on 15-hLO-2, albeit stronger than the published value of 76 μM.[43] Interestingly, quercetin, fisetin, and baicalein are reductive inhibitors against 15-hLO-1, even though the positions of the alcohols are dramatically different among the three. This implies that multiple binding orientations are possible for all three compounds, affording each inhibitor's catechol moiety access to the iron to perform an inner sphere reduction.[44] The conclusion that there are multiple inhibitor binding modes is supported by the fact that both **29** and **30** are potent, reductive inhibitors but **36** is not. The catechol is therefore required for activity but it can be on either ring B or ring C, suggesting at least two spatially distinct orientations of flavonoids to achieve ferric ion binding and reduction.

We also investigated commercial flavonoids, **31**–**36**, to examine the influence of hydroxyl groups on the inhibitory activity of the flavone skeleton (Table 1). We found that none of the monohydroxylated and dihydroxylated flavonoids inhibited 12-hLO or 15-hLO-2. Compound **31**, however, with a single hydroxyl group at the C-3 position, was a weak inhibitor of 15-hLO-1, and the introduction of a second hydroxyl group at C-5 (**34**) led to a 20-fold improvement of the IC_{50}, with an excellent selectivity for 15-hLO-1 (IC_{50} 15-hLO-1/12-hLO < 0.03).

The next class of compounds investigated were the 2-alkylbenzopyran-4-ones, **7a**–**f**, **8a**–**e**, **12a**–**c**, and **14** (Table 2). The first conclusion derived from these data is that the catechol moiety is important for activity. This is clearly seen when comparing **7e**, **8d**, **12a**, and **14**, because all have the same substituent attached to the pyranone ring, but only **14** has a catechol moiety and only **14** is a lipoxygenase inhibitor. This is consistent with our previous results regarding NDGA derivatives, in which the catechol moiety was critical for inhibition due to its ability to reduce the active site ferric species of soybean lipoxygenase to its inactive ferrous form, thus interrupting the catalytic cycle.[45,46] The second conclusion is that this class of compounds, along with the 3-hydroxybenzopyran-4-ones (Table 3), are in general poor inhibitors. The only exception, aside from the catechol containing **14**, is **7f**, which selectively inhibits 15-hLO-1 ($IC_{50} = 38 \pm 10$ μM). Interestingly, the two features that are critical for the inhibitory potency of **7f** are the position of the alcohol (**8e** is inactive) and the length and bulk of the alkane side group (**7b** and **7d** are inactive), indicating a specific binding mode for **7f**. Compounds **21a**–**f** (Table 3) are all poor inhibitors, consistent with the conclusion that a catechol moiety is important for inhibitor potency.

Another critical finding, beyond the presence of a catechols being present, is that the aromaticity and oxidation state of ring C are important for the potency and selectivity of these compounds against LO (Tables 4a, 4b and 4c). Among the 6,7-catechol compounds, the parent structure (**25a**) is not potent against either 12-hLO or 15-hLO-1 (Table 4a). However, if the double bond is removed from ring C, **26a**, the potency and selectivity against 12-hLO increase dramatically (IC_{50}

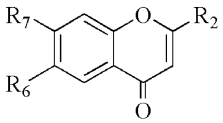
Table 1. Inhibitory activity ($IC_{50} \pm SD$) of commercial flavonoids[a]



	R_3	R_5	R_6	R_7	$R_{3'}$	$R_{4'}$	12-hLO IC_{50} (μM)	15-hLO-1 IC_{50}(μM)	15-hLO-1/12-hLO	15-hLO-2 IC_{50} (μM)	Reduction 15-hLO-1
NDGA	—	—	—	—	—	—	2.6 ± 0.3	0.25 ± 0.02	0.1	11 ± 0.7	Yes
28	OH	OH	H	OH	OH	OH	0.44 ± 0.08	2.2 ± 0.3	5	>100	Yes
29	OH	H	H	OH	OH	OH	0.95 ± 0.2	1.4 ± 0.2	1.5	>100	Yes
30	H	OH	OH	OH	H	H	0.86 ± 0.3	9.1 ± 0.8	12	>100	Yes
31	OH	H	H	H	H	H	>100	73 ± 18	< 0.73	>100	No
32	H	H	OH	H	H	H	>100	>100	—	>100	No
33	H	H	H	OH	H	H	>100	>100	—	>100	—
34	OH	OH	H	H	H	H	>100	3.3 ± 1.6	< 0.03	>100	No
35	OH	H	OH	H	H	H	>100	>100	—	>100	—
36	OH	H	H	OH	H	H	>100	>100	—	>100	—

[a] All IC_{50} values are expressed in micromolar units.

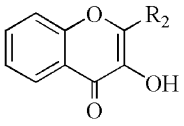
Table 2. Inhibitory activity (IC_{50} ± SD) of 2-alkylbenzopyran-4-ones[a]



	R_2	R_6	R_7	12-hLO IC_{50} (μM)	15-hLO-1 IC_{50} (μM)	15-hLO-1/12hLO	15-hLO-2 IC_{50} (μM)	Reduction 15-hLO-1
7a	CH_2CH_3	OH	H	>100	>100	—	>100	—
7b	$CH_2CH_2CH_3$	OH	H	>100	>100	—	>100	—
7c	$CH(CH_3)_2$	OH	H	>100	>100	—	>100	—
7d	$CH_2CH(CH_3)_2$	OH	H	>100	>100	—	>100	—
7e	$C(CH_3)_3$	OH	H	>100	>100	—	>100	—
7f	$CH_2(CH_2)_3CH_3$	OH	H	>100	38 ± 10	< 0.38	>100	No
8a	$CH_2CH_2CH_3$	H	OH	>100	>100	—	>100	—
8b	$CH(CH_3)_2$	H	OH	>100	>100	—	>100	—
8c	$CH_2CH(CH_3)_2$	H	OH	>100	>100	—	>100	—
8d	$C(CH_3)_3$	H	OH	>100	>100	—	>100	—
8e	$CH_2(CH_2)_3CH_3$	H	OH	>100	>100	—	>100	—
12a	$C(CH_3)_3$	O–CH_2–O		>100	>100	—	>100	—
12b	$CH_2CH(CH_3)_2$	O–CH_2–O		>100	>100	—	>100	—
12c	Ph	O–CH_2–O		>100	> 100	—	>100	—
14	$C(CH_3)_3$	OH	OH	>100	18 ± 3.5	<0.18	>100	No

[a] All IC_{50} values are expressed in micromolar units.

Table 3. Inhibitory activity (IC_{50} ± SD) of 3-hydroxybenzopyran-4-ones[a]



	R_2	12-hLO IC_{50} (μM)	15-hLO-1 IC_{50} (μM)	15-hLO-2 IC_{50} (μM)
21a	CH_2CH_3	>100	>100	>100
21b	$CH_2CH_2CH_3$	>100	>100	>100
21c	$CH(CH_3)_2$	>100	>100	>100
21d	CH2CH$(CH_3)_2$	>100	>100	>100
21e	$C(CH_3)_3$	>100	>100	>100
21f	$CH_2(CH_2)_3CH_3$	>100	>100	>100

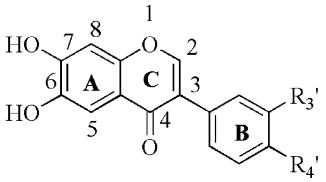
[a] All IC_{50} values are expressed in micromolar units.

15-hLO-1/12-hLO = 12) (Table 4b). If the ketone is also removed, that is, **27a**, the compound remains as a potent inhibitor, but its selectivity is reversed (IC_{50} 15-hLO/12-hLO-1 = 0.019) (Table 4c).

Compound **25b** is a potent inhibitor for 12-hLO, but not as potent against 15-hLO-1. Removal of the double bond from ring C, that is, **26b**, increases the potency toward 12-hLO, while inhibition of 15-hLO-1 is lost (IC_{50} 15-hLO-1/12-hLO > 26) (Table 4b). If the ketone is also removed, that is, **27b**, then the potency against 15-hLO-1 increases dramatically, while the potency against 12-hLO decreases, shifting the IC_{50} 15-hLO-1/12-hLO ratio from 26 for **26b** to 0.03 for **27b**. Compound **25d** is not potent against either 12-hLO or 15-hLO-1, however, removal of the double bond and ketone from ring C, that is, **27c**, improves inhibition of both 12-hLO and 15-hLO-1 (IC_{50} 15-hLO/12-hLO-1 = 0.032) (Table 4c).

These observations indicate that the selectivity is affected by the planarity of the flavonoid, with **27a**–**c** and **27d** being selective against 15-hLO-1, while **25b**, **25c**, **26a**, and **26b** are selective against 12-hLO. These trends suggest that 6,7-dihydroxyisoflavones and 6,7-dihydroxyisoflavanones tend to be selective against 12-hLO, while 6,7-dihydroxyisoflavans tend to be selective

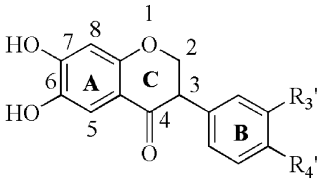
Table 4a. Inhibitory activity (IC_{50} ± SD) of 6,7-dihydroxyisoflavones[a]



	$R_{3'}$	$R_{4'}$	12-hLO IC_{50} (μM)	15-hLO-1 IC_{50} (μM)	15-hLO-1/12-hLO	15-hLO-2 IC_{50} (μM)	Reduction 15-hLO-1
25a	H	OCH_3	>100	>100	—	>100	NA
25b	H	OH	8.7 ± 0.95	49 ± 7.8	6	>100	No
25c	OH	NO_2	5.8 ± 0.54	>100	>17	>100	NA
25d	O–CH_2–O		>100	>100	—	>100	NA

[a] All IC_{50} values are expressed in micromolar units.

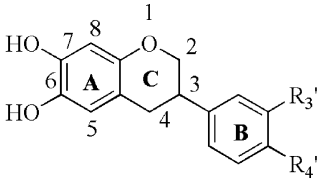
Table 4b. Inhibitory activity (IC_{50} ± SD) of 6,7-dihydroxyisoflavanones[a]



	$R_{3'}$	$R_{4'}$	12-hLO IC_{50} (μM)	15-hLO-1 IC_{50} (μM)	15-hLO-1/12-hLO	15-hLO-2 IC_{50} (μM)	Reduction 15-hLO-1
26a	H	OCH_3	1.6 ± 0.1	19 ± 1.9	12	>100	No
26b	H	OH	3.8 ± 0.29	>100	>26	>100	NA
26c	CH_3	H	14 ± 1.4	0.21 ± 0.02	0.014	>100	Yes

[a] All IC_{50} values are expressed in micromolar units.

Table 4c. Inhibitory activity (IC_{50} ± SD) of 6,7-dihydroxyisoflavans[a]



	$R_{3'}$	$R_{4'}$	12-hLO IC_{50} (μM)	15-hLO-1 IC_{50} (μM)	15-hLO-1/12-hLO	15-hLO-2 IC_{50} (μM)	Reduction 15-hLO-1
27a	H	OCH_3	7.6 ± 0.6	0.15 ± 0.01	0.019	>100	Yes
27b	H	OH	17 ± 1.7	0.51 ± 0.12	0.031	71 ± 28	Yes
27c	O–CH_2–O		11 ± 1.2	0.35 ± 0.06	0.032	16 ± 2.2	Yes
27d	CH_3	H	15 ± 1.4	0.21 ± 0.02	0.014	8.3 ± 0.92	Yes

[a] All IC_{50} values are expressed in micromolar units.

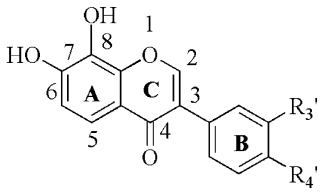
against 15-hLO-1. These inhibitor results for 12-hLO and 15-hLO-1 are consistent with previous inhibition data against porcine 5-lipoxygenase, which indicates that some isoflavans are better inhibitors of the three LO isozymes, 5, 12, and 15-hLO-1, than the corresponding isoflavones.[47] An exception to this trend is **26c**, which is selective against 15-hLO, even though it is an isoflavanone, indicating that certain substituents on ring B can counter the effect of the ring C structure. Finally, the role of ring C also affects the mechanism of inhibition, with **27a** being a reductive inhibitor, while **26a** is not. These data suggest that the non-planarity of ring C in **27a** allows better access to the iron for an inner sphere reduction.

Modifications on ring B also have effects on the inhibition potency of both isoflavones and isoflavanones (Tables 4a and 4b). This is seen by inspecting the structures of compounds **25a** and **25d**, which do not inhibit either 12-hLO or 15-hLO-1, while **25b** and **25c** are both potent and selective inhibitors of 12-hLO (IC_{50} 15-hLO-1/12-hLO = 6 and 17, respectively). Among the isoflavanones, **26a** and **26b** are selective against 12-hLO, while **26c** is selective against 15-hLO-1 (*vide supra*). However, inspection of the four isoflavan compounds (**27a**–**d**), whose ring C lacks both the double bond and ketone (Table 4c), demonstrates that their potency and selectivity are relatively unaffected by R_3' and R_4' modifications. This structure–activity relationship difference between the three classes of inhibitors, isoflavones, isoflavanones, and isoflavans, suggests different binding modes, where the ring B substituent's effects are nullified by the structural flexibility of the isoflavan but not by the more rigid isoflavones and isoflavanones.

The data in Tables 5a and 5b indicate that the 7,8-catechol compounds are potent inhibitors of both 12-hLO and 15-hLO-1 and that in general, shifting the catechol does not greatly affect the inhibitor's ability to reduce the iron. One exception to this trend is the 10-fold decrease in potency of **27e** compared to **27a**. Another observation is that the 7,8-isoflavones are more selective against 12-hLO, while the 7,8- dihydroxyisoflavans are more selective against 15-hLO-1. This is seen by comparing **25e** with **27g**, whose potencies are comparable but with reversed selectivity. These trends are similar to those observed for 6,7-dihydroxyflavonoids (Tables 4a, 4b and 4c) and support our hypothesis that the structure of ring C is important for the selectivity for both 6,7-and 7,8-dihydroxyflavonoids.

In the preceding discussion, we have not mentioned reduction potential as a variable because it is exceedingly hard to establish a trend for two reasons. First, it is difficult to estimate the effect on the reduction potential for substituents that are on ring B because any effect would be weak due to their relative distance from the catechols. This assertion is supported by the fact that **25g** and **25h** have comparable inhibitory potencies, even though the electron-withdrawing chlorine is on the electronically distinct *para*- and *meta*-positions, respectively. Second, it was previously shown that the potency of very simple catechols does not track with their reduction potential,[44] indicating that it would be even harder to dif-

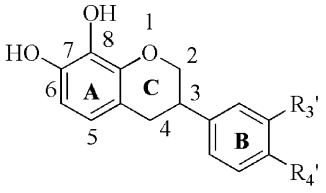
Table 5a. Inhibitory activity (IC_{50} ± SD) of 7,8-dihydroxyisoflavones[a]



	$R_{3'}$	$R_{4'}$	12-hLO IC_{50} (μM)	15-hLO-1 IC_{50} (μM)	15-hLO-1/12-hLO	15-hLO-2 IC_{50} (μM)	Reduction 15-hLO-1
25e	H	CH_3	1.6 ± 0.3	7.8 ± 0.8	5	>100	Yes
25f	H	H	6.4 ± 0.6	13 ± 1.6	2	>100	Yes
25g	H	Cl	0.48 ± 0.09	9.0 ± 1.0	10	>100	Yes
25h	Cl	H	0.78 ± 0.08	6.2 ± 0.7	8	>100	Yes
25i	CH_3	H	3.6 ± 0.3	11 ± 0.7	3	>100	Yes
25j	CF_3	H	0.62 ± 0.06	8.3 ± 0.8	13	>100	Yes

[a] All IC_{50} values are expressed in micromolar units.

Table 5b. Inhibitory activity (IC_{50}± SD) of 7,8-dihydroxyisoflavans[a]



	$R_{3'}$	$R_{4'}$	12-hLO IC_{50} (μM)	15-hLO-1 IC_{50} (μM)	15-hLO-1/12-hLO	15-hLO-2 IC_{50} (μM)	Reduction 15-hLO-1
27e	H	OCH_3	70 ± 8.7	5.7 ± 1.0	0.08	>100	Yes
27f	OCH_3	OCH_3	>100	>100	—	>100	Yes
27g	H	CH_3	26 ± 4.5	3.7 ± 0.66	0.14	>100	Yes

[a] All IC_{50} values are expressed in micromolar units.

ferentiate between steric and reduction potential effects with the complex compounds presented in this investigation.

One of the most intriguing aspects of this study is the weak potency of most of these compounds against 15-hLO-2, even though many are potent against either 12-hLO or 15-hLO-1. The only inhibitors that are potent against 15-hLO-2 are those that have a 6,7-catechol but lack the double bond and ketone group in ring C (**27b–d**). Interestingly, modifications of ring B affect the potency of the isoflavans against 15-hLO-2, with **27d** being more than 10-fold more potent than **27a**. This trend is opposite to that of the 7,8-isoflavans, whose changes in ring B do not affect potency, illustrating a difference in their structure–activity relationship. In addition, the 15-hLO-2 inhibitors are more potent against 15-hLO-1 than 12-hLO, which may suggest a greater similarity in the active sites between 15-hLO-2 and 15-hLO-1, however, differences in the access or exit channels cannot be completely ruled out. This is reasonable given the similar regio-specificity of product generation between 15-hLO-2 and 15-hLO-1. This explanation, however, does not account for the fact that the majority of the 15-hLO-1 and 12-hLO inhibitors do not affect 15-hLO-2, possibly due to the fact that the protein sequence of 15-hLO-2 has limited sequence identity to both 12-hLO (37%) or 15-hLO-1 (37%).

2.3. Molecular modeling analysis

As discussed above, our data suggest that there are multiple modes in which flavonoids can bind to the active site of the hLO isozymes, depending on the position of the catechol moiety with respect to the flavonoid scaffold. To probe this possibility further, we flexibly docked four distinct catechol inhibitors into the active site of the 15-hLO-1 homology model to visualize their mode of binding.[41] Given that these particular catechols are known to directly bind and reduce the ferric center of LO,[44] we analyzed the docking behavior of compounds **25b**, **25e**, **29**, and **30**, each representing one of the four classes of catechols studied. The docking poses of these four inhibitors (Fig. 1) demonstrate that even if we constrain the close proximity of the catechol to the iron (i.e., to act as a reductive inhibitor), the active site of 15-hLO-1 is large enough to accommodate four distinct binding modes of the flavonoid scaffold. This modeling result supports the experimental conclusion that for small catechols, no single binding mode predominates. It should be noted that we were unable to relate the GLIDE score of these inhibitors to their inhibitory potency, most likely due to their small size relative to the 15-hLO-1 active site and the poor estimation of the metal–catechol bond by the docking program.

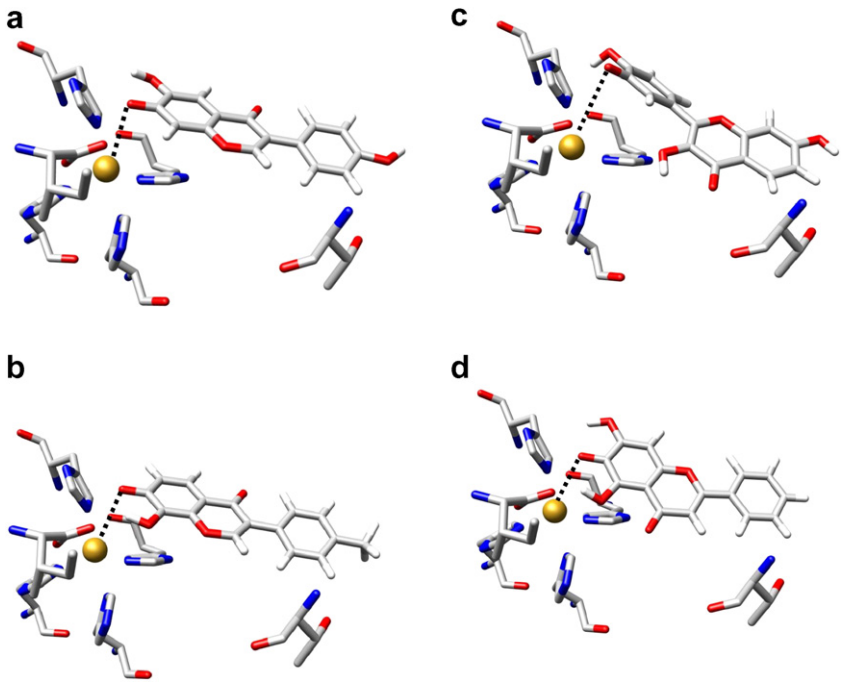


Figure 1. Representative poses of compound docking simulations for 15-hLO-1 with the closest phenolic distance to the iron, for inner sphere reduction, indicated with a dashed line. **25b**, 3.2 Å (a), **25e**, 3.2 Å (b), **29**, 4.2 Å (c), and **30**, 3.1 Å (d). The first coordination sphere ligands that surround the active site iron are also shown, with Thr593 shown in the lower right quadrant.

In summary, the data presented here outline a number of important discoveries. First, the aromaticity and oxidation state of ring C in our flavonoid compounds appear to be important for both the inhibitory potency and selectivity against hLO, with isoflavones and isoflavanones preferentially inhibiting 12-hLO and isoflavanes preferentially inhibiting 15-hLO-1. Second, modification of the basic flavonoid structure has produced a number of selective inhibitors of both 12-hLO (**25c**, **26b**, and **25j**) and 15-hLO-1 (**26c**, **27a**, and **27d**), indicating that the flavonoid skeleton is a viable scaffold for selective inhibitor development. Third, a variety of binding modes are possible for flavonoids in lipoxygenases as seen by the fact that compounds **25b**, **25e**, **29**, and **30** are all potent, reductive inhibitors but their relative positioning of ring B to their catechol moiety is different. Finally, it appears that the structural requirements for 15-hLO-2 inhibition are dramatically different from those of 15-hLO-1 and 12-hLO, which indicates that if 15-hLO-2 has a beneficial role in cancer prevention, inhibitors can be developed that do not target its activity.

3. Experimental

3.1. Chemical synthesis

Melting points were determined on an Electrothermal apparatus and are not corrected. UV spectra were recorded on a Spectronic Genesys 5 instrument. ^{1}H and ^{13}C NMR spectra were recorded at 300 or 400 MHz (^{1}H) and 75 or 100 MHz (^{13}C), respectively (Bruker AMX 300 and Bruker AMX 400 spectrometers), with $(CH_3)_4Si$ as internal standard. Chemical shifts are reported in parts per million, using tetramethylsilane as an internal reference. High-resolution mass spectra were recorded with an EI MS-50 AEI instrument at Bonn University, Germany, and with a MAT 95XP, Thermo-Finnigan spectrometer at the University of Chile, Santiago. All starting materials were commercially available, >98% purity, and used without further purification. Quercetin, baicalein, fisetin, 3-hydroxyflavone, 6-hydroxyflavone, 7-hydroxyflavone, 3,5-dihydroxyflavone, 3,6-dihydroxyflavone, and 3,7-dihydroxyflavone were purchased from Aldrich.

The HPLC analyses of the compounds were performed using a Merck-Hitachi Intelligent L-6200A Pump, an L-4250 UV-Vis Detector, and a D-7000 HSM System Manager Report, a C_{18} reverse phase column (Hypersil ODS-5, 250×4 mm), and a flow rate of 1 mL/min. Compounds **7a**–**14** were detected at 260 nm, **21a**–**f** at 319 nm, **25a**–**j**, **26a**, **26b**, **27b**, **27f**, and **27g** at 255 nm, and **27a** and **27c**–**e** at 295 nm. Two different solvent systems were used: system 1: (**A**) acetonitrile and (**B**) 1% acetic acid and system 2: (**A**) acetonitrile and (**B**) a 1:1 mixture of 1% acetic acid/methanol. A gradient of 30 min of duration was used in both cases, beginning with 30% of (**A**), reaching 99% at 30 min, and (**B**) starting with 70% and ending with 1% in the same period. In both systems all the compounds used were found to be more than 95% pure.

3.2. General procedure for the synthesis of 2-alkyl-6-hydroxy-4-H-benzopyran-4-one (7a–f)

3.2.1. Synthesis of intermediate compounds 3a–g, 4a–f. A solution of **1** (5 g, 32.8 mmol) in dry pyridine (35 mL) was treated with propionyl chloride (10 g, 83 mmol) un-

der cooling in an ice-bath to keep the reaction temperature at about 20 °C. The mixture was stirred for 18 h at room temperature; and then the solvent was partially removed under reduced pressure. The residue was poured onto ice and hydrochloric acid (32%, 30 mL) and extracted with diethyl ether three times. The combined organic layers were washed with aqueous sodium carbonate solution and water, dried ($MgSO_4$), and concentrated to obtain the crude title compound **3a**, which was purified by chromatography on silica gel eluting with dichloromethane to give **3a.**

Compounds **3b**–**g** and **4a**–**f** were prepared in a similar fashion using **1** or **2** and 2.5 eq of the requisite acyl chloride.

3.2.1.1. 2′,5′-Dicyloxypropanoyloxyacetophenone (3a) (77%). ^{1}H NMR ($CDCl_3$) δ_H 7.50 (1H, d, J = 2.8 Hz), 7.24 (1H, dd, J = 8.6 Hz, J = 2.8 Hz), 7.09 (1H, d, J = 8.6 Hz), 2.60 (4H, m), 2.51 (3H, s), 1.25 (3H, t, J = 7.4 Hz), 1.25 (3H, t, J = 7.5 Hz).

3.2.1.2. 2′,5′-Dibutanoyloxyacetophenone (3b) (91%). ^{1}H NMR ($CDCl_3$) δ_H 7.49 (1H, d, J = 2.8 Hz), 7.23 (1H, dd, J = 8.6 Hz; J = 2.8 Hz), 7.09 (1H, d, J = 8.6 Hz), 2.55 (4H, m), 2.50 (3H, s), 1.77 (4H, m), 1.02 (6H, t, J = 7.4 Hz).

3.2.1.3. 2′,5′-Di-(2-methylpropanoyloxy)-acetophenone (3c) (85%). ^{1}H NMR ($CDCl_3$) δ_H 7.46 (1H, d, J = 2.6 Hz), 7.22 (1H, dd, J = 8.9 Hz, J = 2.8 Hz), 7.06 (1H, d, J = 8.7 Hz), 2.80 (2H, m), 2.50 (3H, s), 1.31 (6H, d, J = 6.9 Hz), 1.29 (6H, d, J = 6.9 Hz).

3.2.1.4. 2′5′-Di-(3-methylbutanoyloxy)-acetophenone (3d) (91%). ^{1}H NMR ($CDCl_3$) δ_H 7.47 (1H, d, J = 2.8 Hz), 7.23 (1H, dd, J = 8.6 Hz, J = 2.8 Hz), 7.09 (1H, d, J = 8.8 Hz), 2.51 (3H, s), 2.47 (2H, d, J = 7.1 Hz), 2.42 (2H, d,J = 7.1 Hz), 2.22 (2H, m), 1.04 (12H, d, J = 6.6 Hz).

3.2.1.5. 2′,5′-Di-(2,2-dimethylpropanoyloxy)-acetophenone (3e) (95%). ^{1}H NMR ($CDCl_3$) δ_H7.42 (1H, d, J = 2.6 Hz), 7.19 (1H, dd, J = 8.7 Hz, J = 2.6 Hz), 7.01 (1H, d, J = 8.7 Hz), 2.50 (3H, s), 1.35 (9H, s), 1.33 (9H, s).

3.2.1.6. 2′,5′-Dihexanoyloxyacetophenone (3f) (82%). ^{1}H NMR ($CDCl_3$) δ_H 7.49 (1H, d, J = 2.8 Hz), 7.24 (1H, dd, J = 8.6 Hz, J = 2.8 Hz), 7.09 (1H, d, J = 8.6 Hz) 2.58 (4H, m), 2.54 (3H, s), 1.74 (4H, m), 1.37 (8H, m), 0.90 (6H, m).

3.2.1.7. 2′,5′-Dicinnamoyloxyacetophenone (3g) (87%). ^{1}H NMR ($CDCl_3$) δ_H 7.83 (1H, d, J = 15.9 Hz), 7.82 (1H, d, J = 15.9 Hz), 7.57 (1H, d, J = 2.8 Hz), 7.51 (4H, m), 7.40 (6H, m), 7.30 (1H, dd, J = 8.8 Hz, J = 3.0 Hz), 7.15 (1H, d, J = 8.8 Hz), 6.59 (1H, d, J = 16.1 Hz), 6.56 (1H, d, J = 16.1 Hz), 2.49 (1H, s).

3.2.1.8. 2′,4′-Dipropanoyloxyacetophenone (4a) (88%). ^{1}H NMR ($CDCl_3$) δ_H 7.82 (1H, d, J = 8.6 Hz), 7.08 (1H, dd, J = 8.6 Hz, J = 2.3 Hz), 6.94 (1H, d, J = 2.3 Hz), 2.52 (3H, s), 2.32 (3H, s), 2.28 (3H, s).

3.2.1.9. 2′,4′-Dibutanoyloxyacetophenone (4b) (72%). ^{1}H NMR ($CDCl_3$) δ_H 7.81 (1H, d, J = 8.6 Hz), 7.06 (1H, dd, J = 8.6 Hz, J = 2.3 Hz), 6.92 (1H, d, J = 2.3 Hz), 2.57 (2H, t, J = 7.4 Hz), 2.52 (2H, t, J = 7.3 Hz), 2.51 (3H, s), 1.77 (4H, m), 1.03 (3H, t, J = 7.4 Hz), 1.02 (3H, t, J = 7.4 Hz).

3.2.1.10. 2′,4′-Di-(2-methylpropanoyloxy)-acetophenone (4c) (97%). ^{1}H NMR ($CDCl_3$) δ_H 7.80 (1H, d, J = 8.6 Hz), 7.06 (1H, d, J = 8.6 Hz, J = 2.3 Hz), 6.89 (1H, d, J = 2.3 Hz), 2.80 (3H, s), 2.51 (2H, m), 1.32 (6H, d, J = 7.1 Hz), 1.21 (6H, d, J = 7.1 Hz).

3.2.1.11. 2′4′-Di-(3-methylbutanoyloxy)-acetophenone (4d) (93%). δ^1_H H NMR ($CDCl_3$) 7.78 (1H, d, J = 8.6 Hz), 7.04 (1H, dd, J = 8.6 Hz, J = 2.3 Hz), 6.89 (1H, d, J = 2.3 Hz), 2.49 (3H, s), 2.46 (2H, d), 2.41 (2H, d), 2.198 (2H, m), 1.03 (6H, s), 1.02 (6H, s).

3.2.1.12. 2′,4′-Di-(2,2-dimethylpropanoyloxy)-acetophenone (4e) (89%). ^{1}H NMR ($CDCl_3$) δ_H 7.77 (1H, d, J = 8.6 Hz), 7.02 (1H, dd, J = 8.6 Hz, J = 2.3 Hz), 6.81 (1H, d, J = 2.3 Hz), 2.50 (3H, s), 1.35 (9H, s), 1.32 (9H, s).

3.2.1.13. 2′,4′-Dihexanoyloxyacetophenone (4f) (76%). ^{1}H NMR ($CDCl_3$) δ_H 7.81 (1H, d, J = 8.6 Hz), 7.06 (1H, dd, J = 8.6 Hz, J = 2.3 Hz), 6.92 (1H, d, J = 2.3 Hz), 2.58 (4H, m), 2.51 (3H, s), 1.72 (4H, m), 1.36 (8H, m), 0.91 (6H, t, J = 7.1 Hz).

3.2.2. Synthesis of the intermediate 3-(1,3-dioxopentyl)-4-hydroxyphenyl propanoate (5a–g, 6a–f). Crude **3a** (5.2 g, 20 mmol) was dissolved in dry DMF (30 mL) and added slowly at 0 °C under argon to a suspension of sodium hydride (95% pure, 870 mg, 34.4 mmol) in dry DMF (30 mL). Acetic acid was added to the mixture with caution and then, it was poured into water (300 mL) and extracted with ethyl acetate (3 × 70 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution, dried ($MgSO_4$), and concentrated to yield the crude title compound, which was used in the next step without further purification.

3.2.3. Synthesis of the 2-alkyl-6-hydroxy-4*H*-benzopyran-4-ones (7a–f). A solution of 4.3 mmol of crude **5a** in methanol (30 mL) was treated with 15 mL of concentrated aqueous hydrochloric acid in 20 mL of dioxane. The mixture was heated to 60 °C and then poured into water, and extracted with ethyl acetate. Subsequent drying over magnesium sulfate and evaporation yielded **(7a),** which was crystallized in methanol.

3.2.3.1. 2-Ethyl-6-hydroxy-4*H*-benzopyran-4-one (7a) (60%). ^{1}H NMR (DMSO-d_6) δ_H 9.90 (1H, s, OH-6), 7.46 (1H, d, J = 9.0 Hz, H-5), 7.27 (1H, d, J = 2.8 Hz, H-8), 7.18 (1H, dd, J = 9.0 Hz, J = 3.0 Hz, H-7), 6.11 (1H, s, H-3), 2.63 (2H, c, J = 7.5 Hz, Ar–CH_2–), 1.21 (3H, t, J = 7.5 Hz, Ar–CH_2CH_3). ^{13}C NMR (DMSO-d_6) δ_C177.3, 171.2, 154.9, 150.1, 124.3, 123.1, 119.7, 107.8, 27.0, 11.2. UV: 328 and 226 nm. HRMS $(M+H)^+$ calcd for $C_{11}H_{10}O_3$, 190.0629; found 190.0622, mp 164–165 °C, lit.,[48] mp 165 °C.

3.2.3.2. 6-Hydroxy-2-propyl-4*H*-benzopyran-4-one (7b) (58%). ^{1}H NMR (DMSO-d_6) δ_H 9.89 (1H, s, OH-6), 7.44 (1H, d, J = 8.8 Hz, H-5), 7.27 (1H, d, J = 3.0 Hz, H-8), 7.18 (1H, dd, J = 8.8 Hz; J = 3.0 Hz, H-7), 6.12 (1H, s, H-3), 2.57 (2H, t, J = 7.4 Hz, Ar–CH_2–), 1.66 (2H, m, Ar–CH$_2$CH_2–), 0.93 (3H, t, J = 7.3 Hz, Ar–CH$_2$CH$_2$CH_3). ^{13}C NMR (DMSO-d_6) δ_C 177.2, 169.5, 155.0, 150.1, 124.4, 123.1, 119.8, 108.8, 108.0, 35.6, 20.2, 13.7. UV: 328 nm, 226 nm. HRMS (M+H)$^+$ calcd for $C_{12}H_{12}O_3$, 204.0786; found 204.0792, mp 155–156 °C, lit.,[37] mp 150 °C.

3.2.3.3. 6-Hydroxy-2-isopropyl-4*H*-benzopyran-4-one (7c) (56%). ^{1}H NMR (DMSO-d_6) δ_H 9.89 (1H, s, 6-OH), 7.46 (1H, d, J = 9.0 Hz, H-5), 7.27 (1H, d, J = 2.8 Hz, H-8), 7.18 (1H, dd, J = 9.0 Hz, J = 2.8 Hz, H-7), 6.10 (1H, s, H-3), 2.87 (1H, m, Ar–C*H*–), 1.24 (6H, d, J = 7.0 Hz, Ar–CH(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ_C 177.4, 174.0, 155.0, 150.0, 124.4, 123.2, 119.8, 107.9, 106.4, 32.9, 20.3. UV: 328 and 226 nm. HRMS (M+H)$^+$ calcd for $C_{12}H_{12}O_3$, 204.0786; found 204.0790. Mp 182–183 °C.

3.2.3.4. 6-Hydroxy-2-isobutyl-4*H*-benzopyran-4-one (7d) (50%). ^{1}H NMR (DMSO-d_6) δ_H 9.90 (1H, s, OH-6), 7.44 (1H, d, J = 8.8 Hz, H-5), 7.27 (1H, d, J = 3.0 Hz, H-8), 7.17 (1H, dd, J = 8.8 Hz; J = 3.0 Hz, H-7), 6.12 (1H, s, H-3), 2.48 (2H, d, J = 7.3 Hz, Ar–CH_2), 2.06 (1H, m, Ar–CH$_2$C*H*–), 0.93 (6H, d, J = 6.6 Hz, Ar–CH$_2$CH(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ_C 177.1, 168.7, 155.0, 150.1, 124.4, 123.1, 119.8, 109.7, 108.0, 42.7, 27.0, 22.4. UV: 328 and 229 nm. HRMS (M+H)$^+$ calcd for $C_{13}H_{14}O_3$, 218.0942; found 218.0942. Mp 145–146 °C.

3.2.3.5. 6-Hydroxy-2-*t*-butyl-4*H*-benzopyran-4-one (7e) (50%). ^{1}H NMR (DMSO-d_6) δ_H 9.90 (1H, s, OH-6), 7.48 (1H, d, J = 8.8 Hz, H-5), 7.26 (1H, d,J = 2.8 Hz, H-8), 7.19 (1H, dd, J = 8.8 Hz;J = 2.8 Hz, H-7), 6.13 (1H, s, H-3), 1.30 (9H, s, C(CH_3)$_3$). ^{13}C NMR (DMSO-d_6) δ_C 177.6, 175.8, 162.3, 154.9, 150.1, 123.3, 119.7, 107.8, 105.4, 36.5, 28.1. UV: 328, 226 nm. HRMS (M+H)$^+$ calcd for $C_{13}H_{14}O_3$, 218.0942; found 218.0937, mp 179–180 °C, lit.,[37] mp 170 °C.

3.2.3.6. 6-Hydroxy-2-pentyl-4*H*-benzopyran-4-one (7f) (40%). ^{1}H NMR (DMSO-d_6) δ_H 9.89 (1H, s, OH-6), 7.44 (1H, d, J = 9.1 Hz, H-5), 7.27 (1H, d, J = 3.0 Hz, H-8), 7.16 (1H, dd, J = 9.1 Hz, J = 3.0 Hz, H-7), 6.13 (1H, s, H-3), 3.31 (2H, t, J = 7.6 Hz, Ar–CH_2–), 1.64 (4H, m, Ar–CH$_2$CH_2CH$_2$CH_2–), 1.31 (2H, m, Ar–CH$_2$CH$_2$CH_2–), 0.85 (3H, t, J = 6.8 Hz, Ar–(CH$_2$)$_4$CH_3). ^{13}C NMR (DMSO-d_6) δ_C 177.1, 169.7, 155.0, 150.1, 124.4, 123.1, 119.8, 108.7, 108.0, 33.7, 31.0, 26.4, 22.2, 14.2. UV: 328 and 226 nm. HRMS (M+H)$^+$ calcd for $C_{14}H_{16}O_3$, 232.1099; found 232.1093, mp 118–120 °C.

3.2.3.7. 7-Hydroxy-2-propyl-4*H*-benzopyran-4-one (8a) (42%). ^{1}H NMR (DMSO-d_6) δ_H 10.68 (1H, s, OH-7), 7.82 (1H, d, J = 8.5 Hz, H-5), 6.87 (1H, dd, J = 8.5 Hz; J = 2.5 Hz, H-6), 6.79 (1H, d, J = 2.5 Hz, H-8), 6.06 (1H, s, H-3), 2.56 (2H, t, J = 7.4 Hz, Ar–CH_2–), 1.67 (2H, m, Ar–CH$_2$CH_2-), 0.93 (3H, t, J = 7.4 Hz, Ar–CH$_2$CH$_2$CH_3). ^{13}C NMR (DMSO-d_6) δ_C 176.6, 169.0, 162.9, 158.2, 126.9, 116.3, 115.1, 109.3, 102.6, 35.5, 20.1, 13.7. UV: 292, 247 and 214 nm. HRMS (M+H)$^+$ calcd for $C_{12}H_{12}O_3$, 204.0786; found 204.0778. Mp 150–152 °C, lit.,[37] mp 143 °C.

3.2.3.8. 7-Hydroxy-2-isopropyl-4*H*-benzopyran-4-one (8b) (45%). ^{1}H NMR (DMSO-d_6) δ_H10.68 (1H, s, OH-7), 7.82 (1H, d, J = 8.6 Hz, H-5), 6.87 (1H, dd, J = 8.8 Hz, J = 2.3 Hz, H-6), 6.80 (1H, d, J = 2.0 Hz, H-8), 6.04 (1H, s, H-3), 2.83 (1H, m, Ar–C*H*–) 1.23 (6H, d, J = 7.1 Hz, Ar–CH(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ_C 176.9, 173.6, 163.1, 157.8, 126.9, 116.5, 114.9, 107.0, 102.7, 32.8, 20.5. UV: 295, 247 and 214 nm. HRMS (M+H)$^+$ calcd for $C_{12}H_{12}O_3$, 204.0786; found 204.0791. Mp 156–158 °C.

3.2.3.9. 7-Hydroxy-2-isobutyl-4*H*-benzopyran-4-one (8c) (52%). ^{1}H NMR (DMSO-d_6) δ_H 10.68 (1H, s, OH-7), 7.83 (1H, d, J = 8.6 Hz, H-5), 6.87 (1H, dd, J = 8.8 Hz, J = 2.3 Hz, H-6), 6.80 (1H, d, J = 2.0 Hz, H-8), 6.06 (1H, s, H-3), 2.46 (2H, d, J = 7.3 Hz, Ar–CH_2), 2.05 (1H, m, Ar–CH$_2$C*H*–), 0.93 (6H, d, J = 6.8 Hz, Ar–CH$_2$CH(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ_C 176.6, 168.2, 162.9, 158.3, 126.9, 116.5, 115.2, 110.3, 102.7, 42.5, 26.9, 22.1. UV: 292, 247 and 214 nm. HRMS (M+H)$^+$ calcd for $C_{13}H_{14}O_3$, 218.0942; found 218.0936. Mp 152–153 °C.

3.2.3.10. 7-Hydroxy-2-*t*-butyl-4*H*-benzopyran-4-one (8d) (40%). ^{1}H NMR (DMSO-d_6) δ_H 10.69 (1H, s, OH-7), 7.82 (1H, d, J = 8.9 Hz, H-5), 6.87 (1H, dd, J = 8.7 Hz, J = 2.3 Hz, H-6), 6.82 (1H, d, J = 2.0 Hz, H-8), 6.07 (1H, s, H-3), 1.28 (9H, s, –C(CH_3)$_3$). ^{13}C NMR (DMSO-d_6) δ_C 177.0, 175.2, 163.0, 158.1, 126.8, 116.0, 115.2, 106.0, 102.6, 36.4, 27.9. UV: 295, 247 and 214 nm. HRMS (M+H)$^+$ calcd for $C_{13}H_{14}O_3$, 218.0942; found 218.0943. Mp 222–223 °C.

3.2.3.11. 7-Hydroxy-2-pentyl-4*H*-benzopyran-4-one (8e) (45%). ^{1}H NMR (DMSO-d_6) δ_H 10.65 (1H, s, OH-7), 7.82 (1H, d, J = 8.7 Hz, H-5), 6.87 (1H, dd, J = 8.9 Hz, J = 2.3 Hz, H-6), 6.80 (1H, d, J = 2.3 Hz, H-8), 6.07 (1H, s, H-3), 2.58 (2H, t, J = 7.5 Hz, Ar–CH_2), 1.64 (2H, m, Ar-CH$_2$CH$_2$CH_2–), 1.31 (4H, m, Ar–CH$_2$CH_2CH$_2$CH_2–), 0.86 (3H, m, Ar–(CH$_2$)$_4$CH_3). ^{13}C NMR (DMSO-d_6) δ_C 176.6, 169.3, 162.9, 158.2, 126.9, 116.3, 115.1, 109.2, 102.6, 33.6, 31.0, 26.3, 22.2, 14.2. UV: 247 and 214 nm. HRMS (M+H)$^+$ calcd for $C_{14}H_{16}O_3$, 232.1099; found 232.1094, mp 135–136 °C.

3.3. General procedure for the synthesis of 2-alkyl-6,7-methylenedioxybenzopyran-4-ones (12a–c)

3.3.1. Synthesis of 2-hydroxy-4,5-methylenedioxyacetophenone (10) (77%). To a solution of sesamol (2.76 g, 0.02 mol) in Ac_2O (10 mL) was added 5 mL of the boron trifluoride etherate complex at 0 °C, under argon. The resulting solution was heated to 80–90 °C for 1 h and poured into a saturated NaOAc solution (20 mL). After extracting with Et_2O, the Et_2O solution was washed

with 10% aqueous $NaHCO_3$ solution and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was recrystallized from absolute EtOH to obtain the acetophenone **10**. ^1H NMR ($CDCl_3$) δ_H 12.99 (1H, s), 7.03 (1H, s), 6.43 (1H,s), 5.95 (2H,s), 2.50 (3H,s). ^{13}C NMR ($CDCl_3$) δ_C 201.9. 162.1, 1154.4, 140.5, 112.3, 107.2, 101.9, 98.7, 26.4.

3.3.2. Synthesis of 2-alkyl benzopyran-4-ones (12a–c). A solution of **10** (5 g, 32.8 mmol) in dry pyridine (35 mL) was treated with trimethylacetyl chloride (10 g, 83 mmol) under cooling with an ice-bath to keep the reaction temperature at about 20 °C. The mixture was stirred for 18 h at room temperature; and the solvent was partially distilled off under reduced pressure. The residue was poured onto ice and hydrochloric acid (32%, 30 mL) and extracted with diethyl ether. The combined organic layers were washed with aqueous sodium carbonate solution and water, dried ($MgSO_4$), and concentrated to give the crude title compound **11a**.

Crude **11a** (5.2 g, 19 mmol) was dissolved in dry DMF (30 mL) and added slowly to a suspension of sodium hydride (95% pure, 870 mg, 34.4 mmol) in dry DMF (30 mL), at 0 °C, under argon. Acetic acid was added to the mixture with caution and then, it was poured into water (300 mL) and extracted with ethyl acetate (3×70 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution, dried ($MgSO_4$), and evaporated to yield the crude title compound. A solution of this crude compound in methanol (30 mL) was treated with concentrated aqueous hydrochloric acid in dioxane. The mixture was heated to 60 °C and then poured into water, and washed with ethyl acetate. Subsequent drying over magnesium sulfate and evaporation yielded **(12a).**

3.3.2.1. 6,7-Methylenedioxy-2-*t*-butylbenzopyran-4-one (12a). ^1H NMR ($CDCl_3$) δ_H 7.46 (1H,s, H-5), 6.84 (1H, s, H-8), 6.27 (1H,s, H-3), 6.06 (2H, s, O–C*H*$_2$–O), 1.31 (9H, s, C(C*H*$_3$)$_3$). ^{13}C NMR ($CDCl_3$) δ_C 177.9, 175.5, 153.9, 152.8, 146.1, 118.1, 106.2, 102.4, 102.2, 97.9, 36.4, 28.0. UV: 319, 277, 235 nm. HRMS $(M+H)^+$ calcd for $C_{14}H_{14}O_4$, 246.0892; found 246.0897, mp 214–215 °C.

3.3.2.2. 2-Isobutyl-6,7-methylenedioxybenzopyran-4-one (12b). ^1H NMR ($CDCl_3$) δ_H 7.48 (1H,s, H-5), 6.82 (1H, s, H-8), 6.19 (1H, s, H-3), 6.07 (2H, s, O–C*H*$_2$–O), 2.44 (2H, d, $J = 7.1$ Hz, Ar–C*H*$_2$–), 2.10 (1H, m, Ar–CH$_2$C*H*–), 0.98 (6H, d, $J = 6.6$ Hz, Ar–CH$_2$CH(CH$_3$)$_2$). ^{13}C NMR ($CDCl_3$) δ_C 177.3, 168.5, 154.0, 152.8, 146.1, 118.4, 110.0, 102.3, 97.9, 43.3, 27.2, 22.3. UV: 319, 277 and 235 nm. HRMS $(M+H)^+$ calcd for $C_{14}H_{14}O_4$, 246.0892; found 246.0890, mp 90–92 °C.

3.3.2.3. 6,7-Methylenedioxy-2-phenylbenzopyran-4-one (12c). ^1H NMR ($CDCl_3$) δ_H8.03, (2H, dd, $J = 8.0$ Hz, $J = 2.1$ Hz, H-2′, H-6′), 7.56 (3H, m, H-3′, H-4′, H-5′), 7.37 (1H, s, H-5), 7.31 (1H, s, H-8), 6.94 (1H, s, H-3), 6.21 (2H, s, O–C*H*$_2$–O). ^{13}C NMR ($CDCl_3$) δ_C 176.6, 162.5, 153.6, 153.3, 146.8, 132.2, 131.8, 129.8, 118.9, 106.9, 103.5, 101.5, 99.2. HRMS $(M+H)^+$ calcd for $C_{16}H_{10}O_4$, 266.5792; found 265.99224 (The molecular ion does not appear with sufficient intensity) mp 210–212 °C.

3.3.3. Synthesis of 6,7-dihydroxy-2-t-butylbenzopyran-4-one (14). A solution of **12c** (0.7 g, 0.65 mmol) in chloroform (8 mL) was treated with PCl_5 (0.3 g, 1.3 mmol). The mixture was refluxed for 7 h and after cooling the solvent was removed under reduced pressure. The residue was hydrolyzed in water refluxing for 2 h, and after cooling was extracted twice with ethyl acetate. The solvent was removed and the product was purified through a silica gel column, eluting with a 2:1 CH_2Cl_2/EtOAc in a 2:1 proportion as eluent to give (**14**) (53%). ^1H NMR ($CDCl_3$) δ_H 8.06 (1H, s, OH-7), 7.98 (1H, s, OH-6), 7.55 (1H, s, H-5), 7.26 (1H, s, H-8), 6.29 (1H, s, H-3), 1.35 (9H, s, –C(CH$_3$)$_3$). ^{13}C NMR ($CDCl_3$) δ_C 179.1, 177.0, 150.5, 150.3, 128.2, 123.1, 119.1, 110.6, 106.1, 37.8, 28.1. HRMS $(M+H)^+$ calcd for $C_{13}H_{14}O_4$, 234.0892; found 234.0894, mp 148–149 °C.

3.3.4. General procedure for the synthesis of the intermediate compounds (16a–f). Synthesis of 2′-propanoyloxy acetophenone (16a). A mixture of (**15**) (1.5 g, 11 mmol) and propionyl chloride (2.62 mL, 22 mmol) was stirred in dry pyridine (5 mL) at room temperature for 2 h. The mixture was then poured into a mixture of crushed ice (25 mL) and concentrated HCl (1.5 mL), extracted twice with dichloromethane, and the combined organics were washed three times with water. The solvent was removed under reduced pressure. The residue was purified through a silica gel column using CH_2Cl_2 as eluent to give (**16a**) (2.3 g, 97%). ^1H NMR ($CDCl_3$) δ_H7.78 (1H, dd, $J = 8.1$ Hz, $J = 2.1$ Hz), 7.50 (1H, ddd, $J = 7.7$ Hz, $J = 8.1$ Hz, $J = 1.7$ Hz), 7.29 (1H, ddd, $J = 7.7$ Hz, $J = 8.1$ Hz, $J = 1.7$ Hz), 7.08 (1H, dd, $J = 8.5$ Hz, $J = 1.1$ Hz), 2.63 (2H, q), 2.52 (3H, s), 1.26 (3H, t, $J = 7.5$ Hz).

3.3.4.1. 2′-Butanoyloxyacetophenone (16b) (90%). ^1H NMR ($CDCl_3$) δ_H7.76 (1H, dd, $J = 7.8$ Hz, $J = 1.5$ Hz), 7.50 (1H, ddd, $J = 7.8$ Hz,$J = 7.6$ Hz, $J = 8.0$ Hz, $J = 1.8$ Hz), 7.29 (1H, ddd, $J = 7.8$ Hz, $J = 7.6$ Hz,$J = 7.6$ Hz, $J = 1.0$ Hz), 7.06 (1H, dd, $J = 8,1$ Hz, $J = 1.3$ Hz), 2.84 (1H, m), 2.53 (3H, s), 1.33 (6H, d, $J = 6.8$ Hz).

3.3.4.2. 2′-(2-Methylpropanoyloxy)-acetophenone (16c) (98%). ^1H NMR ($CDCl_3$) δ_H7.77 (1H, dd, $J = 7.8$ Hz, $J = 1.8$ Hz), 7.50 (1H, ddd, $J = 7.5$ Hz, $J = 8.1$ Hz, $J = 1.8$ Hz, $J = 1.5$ Hz), 7.29 (1H, ddd, $J = 7.6$ Hz, $J = 7.5$ Hz, $J = 7.7$ Hz, $J = 1.3$ Hz), 7.08 (1H, dd, $J = 8.1$ Hz,$J = 1.0$ Hz), 2.58 (2H, t, $J = 7.4$ Hz), 2.53 (3H, s), 1.79 (2H, m), 1.04 (3H, t, $J = 7.3$ Hz).

3.3.4.3. 2′-(3-Methylbutanoyloxy)-acetophenone (16d) (80%). ^1H NMR ($CDCl_3$) δ_H 7.76 (1H, dd, $J = 7.8$ Hz,$J = 1.8$ Hz), 7.49 (1H, ddd, $J = 7.5$ Hz, $J = 8.0$ Hz, $J = 8.2$ Hz, $J = 1.8$ Hz), 7.28 (1H, ddd, $J = 7.6$ Hz,$J = 7.5$ Hz, $J = 7.8$ Hz, $J = 1.3$ Hz), 7.08 (1H, dd, $J = 8.1$ Hz, $J = 1.5$ Hz), 2.52 (3H, s), 2.48 (2H, d, $J = 7.1$ Hz), 2.24 (1H, m), 1.05 (6H, d, $J = 6.6$ Hz).

3.3.4.4. 2′-(2,2-Dimethylpropanoyloxy)-acetophenone (16e) (98%). ^{1}H NMR ($CDCl_3$) δ_H 7.74 (1H, dd, J = 7.8 Hz, J = 1.8 Hz), 7.49 (1H, ddd, J = 7.3 Hz, J = 8.1 Hz,J = 8.1 Hz, J = 1.8 Hz), 7.28 (1H, ddd, J = 7.6 Hz,J = 7.6 Hz, J = 7.6 Hz, J = 1.3 Hz), 7.02 (1H, dd, J = 8.1 Hz, J = 1.0 Hz), 2.5 (3H, s), 1.4 (9H, s).

3.3.4.5. 2′-Hexanoyloxyacetophenone (16f) (49%). ^{1}H NMR ($CDCl_3$) δ_H 7.77 (1H, dd, J = 7.8 Hz, J = 1.8 Hz), 7.50 (1H, ddd, J = 7.4 Hz, J = 8.0 Hz, J = 8.0 Hz, J = 1.8 Hz), 7.28 (1H, ddd, J = 7.6 Hz, J = 7.6 Hz, J = 7.6 Hz, J = 1.3 Hz), 7.08 (1H, dd, J = 8.1 Hz, J = 1.3 Hz), 2.59 (2H, t, J = 7.6 Hz), 2.52 (3H, s), 1.75 (2H, m), 1.38 (4H, m), 0.91 (3H, t, J = 7.1 Hz).

3.3.5. General procedure for the synthesis of 17a–f. Synthesis of 2′-propanoyloxy-2-bromoacetophenone (17a). To a solution of (**16a**) (2.2 g, 11.5 mmol) in anhydrous tetrahydrofuran (10 mL), PTT (4.3 g, 11.5 mmol) was added in portions over a period of 10 min. The reaction mixture was stirred until a colorless precipitate was formed. The residue was recrystallized from ethanol to give (**17a**) (3.0 g, 97%), ^{1}H NMR ($CDCl_3$) δ_H 7.80 (1H, dd, J = 7.7 Hz, J = 1.5 Hz), 7.56 (1H, ddd, J = 7.8 Hz, J = 7.9 Hz, J = 7.7 Hz J = 1.5 Hz), 7.32 (1H, ddd, J = 7.6 Hz, J = 7.7 Hz, J = 7.5 Hz, J = 1.1 Hz), 7.18 (1H, dd, J = 8.1 Hz, J = 0.8 Hz), 4.39 (2H, s), 2.65 (2H, c, J = 7.5 Hz), 1.27 (3H, t, J = 7.5 Hz).

3.3.5.1. 2′-Butanoyloxy-2-bromoacetophenone (17b) (98%). ^{1}H NMR ($CDCl_3$) δ_H 7.77 (1H, dd, J = 7.9 Hz, J = 1.7 Hz), 7.55 (1H, ddd, J = 7.5 Hz, J = 8.2 Hz, J = 8.1, HzJ = 1.7 Hz), 7.31 (1H, ddd, J = 7.6 Hz, J = 7.5 Hz, J = 7.7 Hz, J = 1.1 Hz), 7.13 (1H, dd, J = 8.1 Hz, J = 1.1 Hz), 4.38 (2H, s), 2.84 (1H, m), 1.33 (6H, d, J = 7.2 Hz).

3.3.5.2. 2′-(2-Methylpropanoyloxy)-2-bromoacetophenone (17c) (98%). ^{1}H NMR ($CDCl_3$) 7.79 (1H, dd, J = 7.7 Hz, J = 1.7 Hz), 7.55 (1H, ddd, J = 7.5 Hz, J = 8.2 Hz, J = 7.9 Hz, J =1.5 Hz), 7.31(1H, ddd, J = 7.7 Hz, J = 7.6 Hz, J = 8.1 Hz, J = 1.1Hz), 7.16 (1H, dd, J = 8.3 Hz, J = 1.3 Hz), 4.39 (2H, s), 2.59 (2H, t, J = 7.4 Hz), 1.78 (2H, m), 1.03 (3H, t, J = 7.3 Hz).

3.3.5.3. 2′-(3-Methylbutanoyloxy)-2-bromoacetophenone (17d) (98%). ^{1}H NMR ($CDCl_3$) δ_H 7.78 (1H, dd, J = 7.9 Hz, J = 1.7 Hz), 7.55 (1H, ddd, J = 7.5 Hz, J = 8.1 Hz, J = 7.7 Hz, J = 1.7 Hz), 7.31(1H, ddd, J = 7.7 Hz, J = 7.8 Hz, J = 7.5 Hz, J = 1.1 Hz), 7.15 (1H, dd, J = 8.1 Hz, J = 1.1 Hz), 4.39 (2H, s), 2.50 (2H, d, J = 7.0 Hz), 2.25 (1H, m), 1.05 (6H, s).

3.3.5.4. 2′-(2,2-Dimethylpropanoyloxy)-2-bromoacetophenone (17e) (96%). ^{1}H NMR ($CDCl_3$) δ_H 7.75 (1H, dd, J = 7.7 Hz, J = 1.7 Hz), 7.54 (1H, ddd, J = 7.5 Hz, J = 8.1 Hz, J = 8.1 Hz, J = 1.7 Hz),7.33 (1H, ddd, J = 7.0 Hz, J = 7.4 Hz, J = 7.6 Hz, J = 1.1 Hz), 7.07 (1H, dd, J = 8.1 Hz, J = 1.1 Hz), 4.37 (2H, s), 1.37 (9H, s).

3.3.5.5. 2′-Hexanoyloxy-2-bromoacetophenone (17f) (97%). ^{1}H NMR ($CDCl_3$) δ_H 7.79 (1H, dd, J = 7.9 Hz, J = 1.7 Hz), 7.56 (1H, ddd, J = 7.4Hz, J = 8.3 Hz, J = 8.1 Hz, J = 1.7 Hz), 7.32 (1H, ddd, J = 7.6 Hz, J = 7.9 Hz, J = 7.4 Hz, J = 1.1 Hz), 7.16 (1H, dd, J = 8.1 Hz, J = 1.1 Hz), 4.39 (2H, s), 2.61 (2H, m), 1.75 (2H, m), 1.37 (4H, m), 0.91 (3H,t, J = 7.0 Hz).

3.3.6. General procedure for the synthesis of 18a–f. Synthesis of 2-(2-phenoxyacetyl) phenyl propionate (18a). A mixture of (**18a**) (3.0 g, 11 mmol) and potassium benzoate (2.7 g, 17 mmol) was stirred in acetonitrile (48 mL) at room temperature for 48 h. The salts were filtered off, and the solvent was removed under reduced pressure. The residue was taken up in dichloromethane, washed with aqueous sodium carbonate, and then washed with water. The solvent was removed under reduced pressure, and the residue was recrystallized from ethanol to give **18a** (53%).

3.3.7. General procedure for the synthesis of (20a–f). Synthesis of 2-ethyl-4-oxo-4 H-chromen-3-yl benzoate (20a). To a suspension of sodium hydride (148 mg, 6.2 mmol) in dry DMF (2.5 mL) was added **18a** (1.8 g, 5.6 mmol) in DMF (2.5 mL). The reaction mixture was refluxed for 90 min with stirring, and then the cooled mixture was poured into a mixture of ice (120 g) and concentrated HCl (2 mL). The crude β-diketone **(19a)** precipitated subsequently was washed with water and was used in the following ring closure step without further purification.

To a solution of crude β-diketone **(19a)** in acetic acid (10 mL), concentrated sulfuric acid (0.25 mL) was added dropwise. The reaction mixture was heated at 60 °C for 90 min with stirring, and the solution was poured over ice (60 g). The precipitate was filtered, washed with water, and recrystallized from ethanol to yield the pure (**20a**) (556 mg, 60%) as colorless needles. ^{1}H NMR ($CDCl_3$) δ_H 8.17 (1H, dd, J = 7.6 Hz, J = 1.8 Hz), 8.16 (1H, dd, J = 8.3 Hz, J = 1.3 Hz), 7.61 (1H, ddd, J = 7.8 Hz, J = 7.8 Hz, J = 7.8 Hz, J = 1.5 Hz), 7.56 (1H, dd), 7.43 (3H, m), 7.33 (1H, ddd,J = 7.6 Hz, J = 7.6 Hz, J = 7.6 Hz, J = 1.0 Hz), 2.70 (2H, c, J = 7,6 Hz), 1.27 (3H, t, J = 7,6 Hz).

3.3.7.1. 2-Isopropyl-4-oxo-4*H*-chromen-3-yl benzoate (20b) (80%). ^{1}H NMR ($CDCl_3$) δ_H8.15 (3H, m), 7.59 (2H, m), 7.45 (3H, m), 7.32 (1H, ddd, J = 7.5 Hz, J = 7.5 Hz, J = 7.6 Hz, J = 1.0 Hz), 3.21 (1H, q, J = 6.8 Hz), 1.3 (6H, d, J = 6.8 Hz).

3.3.7.2. 4-Oxo-2-propyl-4*H*-chromen-3-yl benzoate (20c) (55%). ^{1}H NMR ($CDCl_3$) δ_H 8.22 (3H, m), 7.65 (2 H, m), 7.50 (3H, m), 7.40 (1H, ddd, J = 7.6 Hz, J = 7.5 Hz, J = 7.7 Hz, J = 1.0 Hz), 2.70 (2H, t, J = 7.3 Hz), 1.81 (2H, m), 1.01 (3H, t, J = 7.3 Hz).

3.3.7.3. 2-Isobutyl-4-oxo-4*H*-chromen-3-yl benzoate (20d) (92%). ^{1}H NMR ($CDCl_3$) δ_H 8.17 (3H, m), 7.59 (2H, m), 7.43 (3H, m), 7.34 (1H, ddd, J = 7.6 Hz, J = 7.5 Hz, J = 7.7 Hz, J = 1.0 Hz), 2.55 (2H, d, J = 7.3 Hz), 2.16 (1H, m), 0.96 (6H, d, J = 6.8 Hz).

3.3.7.4. 2-*t*-Butyl-4-oxo-4*H*-chromen-3-yl benzoate (20e) (61%). ^{1}H NMR ($CDCl_3$) δ_H 8.12 (3H, m), 7.60 (2H, m), 7.44 (3H, m), 7.31 (1H, ddd, J = 7.6 Hz, J = 7.6 Hz, J = 7.6 Hz, J = 1.01 Hz), 1.36 (1H, s).

3.3.7.5. 4-Oxo-2-pentyl-4*H*-chromen-3-yl benzoate (20f) (85%). ^{1}H NMR ($CDCl_3$) δ_H 8.13 (3H, m), 7.59 (2H, m), 7.43 (3H, m), 7.33 (1H, ddd, J = 7.6 Hz, J = 7.6 Hz, J = 7.6 Hz, J = 0.8 Hz), 2.55 (2H, t, J = 7.6 Hz), 1.68 (2H, t, J = 7,0 Hz), 1.29 (4H, m), (0.82, 3H, t, J = 7.1 Hz).

3.4. General procedure for the synthesis of (21a–f)

3.4.1. 2-Ethyl-3-hydroxy-4 H-benzopyran-4-one (21a). Compound (**20a**) (500 mg, 1.7 mmol) was hydrolyzed with 5% aqueous sodium hydroxide solution (5 mL) in ethanol (40 mL) at 60 °C for 2 h. The reaction mixture was poured into a mixture of crushed ice (60 g) and concentrated HCl (1 mL). It was then extracted with ethyl acetate and washed with aqueous sodium hydrogen carbonate and water. The solvent was removed under reduced pressure, and the residue was recrystallized from aqueous ethanol to give **21a** (170 mg, 53%) ^{1}H NMR ($CDCl_3$) δ_H 8.15 (1H, dd, J = 7.3 Hz, J = 1.8 Hz, H-5), 7.57 (1H, ddd, J = 8.7 Hz, J = 7.2 Hz, J = 1.5 Hz, H-7), 7.40 (1H, dd, J = 8.6 Hz, J = 1.0 Hz, H-8), 7.30 (1H, ddd, J = 8.1 Hz, J = 7.1 Hz, J = 1.0 Hz, H-6), 6.08 (1H, s, OH-3), 2.81 (2H, c, Ar–CH_2), 1.28 (3H, t, J = 7.7 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ_C 172.5, 155.7, 153.3, 137.7, 133.0, 125.5, 124.3, 121.5, 118.1, 22.4, 10.9. HRMS $(M+H)^+$ calcd for $C_{11}H_{10}O_3$, 190.0629; found 190.0630. Mp 99–101 °C, UV (MeOH) λ_{max} 319, 283, 232 and 205 nm.

3.4.1.1. 2-Isopropyl-3-hydroxy-4*H*-benzopyran-4-one (21b) (61%). ^{1}H NMR ($CDCl_3$) δ_H 8.15 (1H, dd, J = 8.1 Hz, J = 1.8 Hz, H-5), 7.57 (1H, ddd, J = 8.7 Hz, J = 7.1 Hz, J = 1.8 Hz, H-7), 7.42 (1H, dd, J = 8.8 Hz, J = 1.0 Hz, H-8), 7.30 (1H, ddd, J = 8.0 Hz J = 7.0 Hz, J = 1.2 Hz, H-6), 6.27 (1H, s, OH-3), 3.41 (1H, m, Ar–C*H*–), 1.28 (6H, d, J = 6.8 Hz, Ar–CH(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ_C 172.7, 156.0, 155.7, 136.9, 132.9, 125.5, 124.3, 121.4, 118.1, 28.2, 19.4. HRMS $(M+H)^+$ calcd for $C_{12}H_{12}O_3$, 204.0786; found 204.0788. Mp 152–153 °C, UV (MeOH) λ_{max} 319, 283, 232 and 205 nm.

3.4.1.2. 3-Hydroxy-2-propyl-4*H*-benzopyran-4-one (21c) (63%). ^{1}H NMR ($CDCl_3$) δ_H 8.16 (1H, dd, J = 7.8 Hz, J = 1.5 Hz, H-5), 7.56 (1H, ddd, J = 8.7 Hz, J = 7.0 Hz, J = 1.8 Hz, H-7), 7.39 (1H, dd, J = 8.6 Hz, J = 1.3 Hz, H-8), 7.30 (1H, ddd,J = 8.1 Hz, J = 7.1 Hz, J = 1.1 Hz, H-6), 6.23 (1H, s, OH-3), 2.75 (2H, t, J = 7.4 Hz, Ar–CH_2), 1.74 (2H, m, Ar–CH_2CH_2–), 0.86 (3H, t, J = 7.4 Hz, Ar–$CH_2CH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ_C 172.5, 155.7, 152.3, 138.3, 132.9, 125.5, 124.3, 121.4, 118.1, 30.8, 20.1, 13.7. HRMS $(M+H)^+$ calcd for $C_{12}H_{12}O_3$ 204.0786; found 204.0790. Mp 102–104 °C. UV (MeOH) λ_{max} 319, 283, 232, 205 nm.

3.4.1.3. 2-Isobutyl-3-hydroxy-4*H*-benzopyran-4-one (21d) (45%). ^{1}H NMR ($CDCl_3$) δ_H 8.21 (1H, dd, J = 8.1 Hz, J = 1.7 Hz, H-5), 7.62 (1H, ddd, J = 8.6 Hz, J = 7.0 Hz, J = 1.7 Hz, H-7), 7.44 (1H, dd, J = 8.5 Hz, J = 0.9 Hz, H-8) 7.35 (1H, ddd, J = 8.1 Hz, J = 7.1 Hz, J = 1.0 Hz, H-6), 6.23 (1H, s, OH-3), 2.71 (2H, d, J = 7.2 Hz, Ar–CH_2–), 2.21 (1H, m, Ar–(CH_2CH(CH_3)$_2$)), J = 1.01 (6H, d, J = 6.6 Hz, Ar–(CH_2CH(CH_3)$_2$)). ^{13}C NMR ($CDCl_3$) δ_C 172.4, 155.7, 151.7, 138.8, 133.0, 125.4, 124.2, 121.4, 118.1, 37.9, 27.2, 22.5. HRMS $(M+H)^+$ calcd for $C_{13}H_{14}O_3$, 218.0942; found 218.0947. Mp 130–131 °C, UV (MeOH) λ_{max} 319, 283, 232, 205 nm.

3.4.1.4. 3-Hydroxy-2-*t*-butyl-4*H*-benzopyran-4-one (21e) (40%). ^{1}H NMR ($CDCl_3$) δ_H 8.13 (1H, dd, J = 8.1 Hz, J = 1.5 Hz, H-5), 7.57 (1H, ddd, J = 8.6 Hz, J = 7.0 Hz, J = 1.6 Hz, H-7), 7.41 (1H, dd, J = 8.5 Hz, J = 0.9 Hz, H-8), 7.30 (1H, ddd, J = 8.0 Hz, J = 7.0 Hz, J = 1.1 Hz, H-6), 6.45 (1H, s, OH-3), 1.41 (9H, s, C(CH_3)$_3$). ^{13}C NMR ($CDCl_3$) δ_C 173.3, 162.6, 155.5, 137.1, 133.1, 125.4, 124.4, 121.1, 118.3, 36.5, 27.6. HRMS $(M+H)^+$ calcd for $C_{13}H_{14}O_3$, 218.0942; found 218.0948. Mp 169–171 °C, UV (MeOH) λ_{max} 319, 283, 232 and 205 nm.

3.4.1.5. 3-Hydroxy-2-pentyl-4*H*-benzopyran-4-one (21f) (65%). ^{1}H NMR ($CDCl_3$) δ_H 8.15 (1H,dd, J = 8.3 Hz, J = 1.5 Hz, H-5), 7.57 (1H, ddd, J = 8.6 Hz, J = 7.0 Hz, J = 1.7 Hz, H-7), 7.40 (1H, dd, J = 8.5 Hz, J = 0.6 Hz, H-8), 7.30 (1H, ddd, J = 8.1 Hz, J = 7.0 Hz, J = 1.1 Hz, H-6), 6.18 (1H, s, OH-3), 2.77 (2H, t, J = 7.6 Hz,Ar–CH_2–), 1.71 (2H, m, Ar–CH_2CH_2CH_2–), 1.32 (4H, m, Ar–CH_2CH_2CH_2CH_2–), 0.84 (3H, m, Ar–(CH_2)$_4$CH_3). ^{13}C NMR δ_C 172.5, 155.6, 152.5, 138.2, 133.1, 125.5, 124.3, 121.5, 118.1, 31.4, 28.9, 26.4, 22.4, 14.0. HRMS $(M+H)^+$ calcd for $C_{14}H_{16}O_3$, 232.1099; found 232.1105. Mp 96–99 °C. UV (MeOH) λ_{max} 319, 283, 232 and 205 nm.

3.5. General procedure for the synthesis of compounds 25a–j

Dry HCl (g) was passed into a cooled (0 °C), stirred mixture of the substituted phenylacetonitrile (**22**) (0.34 mol) and anhydrous $ZnCl_2$ (30 g, 0.22 mol) in dry diethyl ether (200 mL). The corresponding polyhydroxybenzene (**23**) (0.28 mol) was added portionwise with constant bubbling of gaseous HCl for a further 4–6 h. The reaction mixture was then stirred at room temperature for 4–20 h, until the complete disappearance of the reagents, as shown by thin-layer chromatography.

The ketiminium chloride intermediate that separated as an oil after 3–4 days was washed with diethyl ether and hydrolyzed by refluxing in 5% HCl (1 L) for 4–5 h. The ketone (**24**), which separated upon cooling, was filtered and recrystallized in the appropriate solvent.

To a solution of benzylketone (**24**) (0.18 mol) in dry DMF (200 mL) BF_3Et_2O (0.88 mol) was added dropwise. This solution was warmed to 50 °C and a solution of methanesulfonyl chloride (0.56 mol) in DMF (100 mL) was slowly added. The resulting mixture was then heated to 100 °C for 2 h. After cooling, it was poured into water (400 mL) and left overnight to give a precipitate, which was then stirred for 2 h in cold methanol (50 mL), filtered, and crystallized in a mixture of water/ethanol.

3.5.1. 6,7-Dihydroxy-4′-methoxyisoflavone (25a) (72%). ^{1}H NMR (DMSO-d_6) δ_H 10.38 (1H, s, 7-OH), 9.75 (1H, s, 6-OH), 8.27 (1H, s, 2-H), 7.49 (2H, d, J = 8.6 Hz, 2′-H, 6′-H), 7.37 (1H, s, 5-H), 6,97 (2H, d, J = 8.6 Hz, 3′-H, 5′-H), 6.89 (1H, s, H-8), 3.76 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6) δ_C 174.9, 159.5, 153.3, 152.9, 151.5,

145.4, 130.7, 125.3, 123.1, 117.2, 114.2, 108.8, 103.4, 55.8. Mp 251 °C.

3.5.2. 4′,6,7-Trihydroxyisoflavone (25b) (53%). ^{1}H NMR (DMSO-d_6) δ_H 9.84 (1H, s, 7-OH), 8.21 (1H, s, 6-OH), 7.36 (1H, s, H-5), 7.35 (2H, d, J = 8.7 Hz, H-2′, H-6′), 6.87 (1H, s, H-8), 6.78 (2H, d, J = 8.7 Hz, H-3′, H-5′), 4.46 (1H, dd, J = 11.0 Hz, J = 13.1 Hz, H-3), 4.43 (1H, dd, J = 5.5 Hz, J = 5.9 Hz, H-2a), 3.96 (1H, dd, J = 5.3 Hz, J = 7.2 Hz, H-2e). ^{13}C NMR (DMSO-d_6) δ_C 187.9, 174.98, 157.8, 153.1, 151.4, 145.3, 130.7, 130.3, 123.4, 117.6, 115.6, 108.6, 103.4. Mp 225 °C.

3.5.3. 6,7-Dihydroxy-4′-nitroisoflavone (25c) (81%). ^{1}H NMR (DMSO-d_6) δ_H 10.11 (1H, s, 7-OH), 8.54 (1H, s, 2-H), 8.26 (2H, d, J = 8.8 Hz, H-3′, H-5′), 7.89 (2H, d, J = 8.8 Hz, H-2′, H-6′), 7.40 (1H, s, H-5), 6.93 (1H, s, H-8), [signal for 8-OH not seen]. ^{13}C NMR (DMSO-d_6) δ_C 174.3, 155.5, 153.3, 151.7, 147.2, 145.8, 140.4, 130.5, 123.9, 121.6, 117.1, 108.9, 103.7. Mp 168 °C.

3.5.4. 6,7-Dihydroxy-3′,4′-methylenedioxyisoflavone (25d) (67%). ^{1}H NMR (DMSO-d_6) δ_H 10.1 (1H, s, 7-OH), 9.63 (1H, s, 6-OH), 8.28 (1H, s, 2-H), 7.37 (1H, s, H-5), 7.12 (1H, d, J = 1.6 Hz, H-2′), 7.03 (1H, dd, J = 1.6 Hz, J = 8.0 Hz, H-6′), 6.94 (1H, d, J = 8.0 Hz, H-5′), 6.88 (1H, s, H-8), 6.02 (2H, s, O–CH_2–O). ^{13}C NMR (DMSO-d_6) δ_C 174.8, 153.6, 153.0, 151.5, 147.4, 145.4, 126.8, 123.1, 123.0, 117.2, 110.1, 108.8, 108.7, 103.4, 101.6. HRMS (M+H)$^+$ calcd for $C_{16}H_{10}O_6$, 298.0478; found 298.0480. Mp 260 °C.

3.5.5. 7,8-Dihydroxy-4′-methylisoflavone (25e) (78%). ^{1}H NMR (DMSO-d_6) δ_H 9.81 (1H, s, 8-OH), 8.38 (1H, s, 2-H), 7.47 (1H, d, J = 8.7 Hz, H-5), 7.45 (2H, d, J = 8.1 Hz, H-2′, H-6′), 7.21 (2H, d, J = 8.1 Hz, H-3′, H-5′), 6.95 (1H, d, J = 8.7 Hz, H-6), 2.32 (3H, s, CH_3), [signal for 7-OH not seen]. ^{13}C NMR (DMSO-d_6) δ_C 175.6, 153.9, 150.8, 147.4, 137.6, 133.6, 129.9, 129.5, 129.3, 123.5, 118.1, 116.3, 114.9, 21.5. Mp 241 °C.

3.5.6. 7,8-Dihydroxyisoflavone (25f) (76%). ^{1}H NMR (DMSO-d_6) δ_H9.83 (1H, s, 8-OH), 8.43 (1H, s, 2-H), 7.57 (2H, d, J = 7.0 Hz, H-3′, H-5′), 7.48 (1H, d, J = 8.6 Hz, H-5), 7.42 (2H, t, J = 7.0 Hz, H-2′, H-6′), 7.37 (1H, d, J = 8.6 Hz, H-6), [signal for 7-OH not seen]. ^{13}C NMR (DMSO-d_6) δ_C 175.5, 154.3, 150.8, 147.4, 133.6, 132.9, 129.7, 128.7, 128.3, 123.7, 118.1, 116.4, 114.9. Mp 211 °C.

3.5.7. 4′-Chloro-7,8-dihydroxyisoflavone (25g) (65%). ^{1}H NMR (DMSO-d_6) δ_H10.35 (1H, s, 7-OH), 9.47 (1H, s, 8-OH), 8.47 (1H, s, H-2), 7.61 (2H, d, J = 8.6 Hz, 2′-H, 6′-H), 7.48 (1H, d, J = 8.7 Hz, H-5), 7.47 (2H, d, J = 8.59 Hz, H-3′, H-5′), 6.96 (1H, d, J = 8.7 Hz, H-8). ^{13}C NMR (DMSO-d_6) δ_C 175.3, 154.6, 150.9, 147.4, 133.6, 133.1, 131.8, 131.4, 128.8, 122.4, 118.0, 116.4, 115.0. HRMS (M+H)$^+$ calcd for $C_{15}H_9O_4Cl$, 288.0189; found 288.0191. Mp 165 °C.

3.5.8. 3′-Chloro-7,8-dihydroxyisoflavone (25h) (69%). ^{1}H NMR (DMSO-d_6) δ_H 9.89 (2H, s, 7-OH, 8-OH), 8.42 (1H, s, 2-H), 7.46 (1H, d, J = 9.0 Hz, 5-H), 7.42-7.68 (4H, m, 2′-H, 4′-H, 5′-H, 6′-H), 7.01 (1H, d, J = 9.0 Hz, 6-H). ^{13}C NMR (DMSO-d_6) δ_C 175.4, 153.3, 151.8, 137.8, 134.3, 134.0, 130.1, 128.1, 126.3, 124.4, 123.6, 118.1, 112.1. HRMS (M+H)$^+$ calcd for $C_{15}H_9O_4Cl$, 288.0189; found 288.0192. Mp 258 °C. UV (MeOH) λ_{max} 261 and 302 nm.

3.5.9. 7,8-Dihydroxy-3′-methylisoflavone (25i) (71%). ^{1}H NMR (DMSO-d_6) δ_H 10.28 (1H, s, 7-OH), 9.69 (1H, s, 8-OH), 8.42 (1H, s, 2-H), 7.51 (1H, d, J = 9.0 Hz, 5-H), 7.24-7.53 (4H, m, 2′-H, 4′-H, 5′-H, 6′-H) 7,0 (1H, d, J = 9.0 Hz, 6-H) 2.37 (3H, s, CH_3). ^{13}C NMR (DMSO-d_6) δ_C 175.7, 153.8, 152.9, 151.6, 138.1, 137.9, 132.4, 128.6, 126.8, 124.7, 123.4, 118.1, 113.2, 23.7. HRMS (M+H)$^+$ calcd for $C_{16}H_{12}O_4$,268.0736; found 269.0740. Mp 268 °C. UV (MeOH) λ_{max} 257 and 309 nm.

3.5.10. 7,8-Dihydroxy-3′-trifluoromethylisoflavone (25j) (79%). ^{1}H NMR (DMSO-d_6) δ_H 10.00 (1H, broad, s, 8-OH), 8.64 (1H, s, 2-H), 8.04 (1H, s, 2′-H), 7.71-7.88 (3H, m, 4′-H, 5′-H, 6′-H), 7.55 (1H, d, J = 9.0 Hz, 5-H) 7.04 (1H, d, J = 9.0 Hz, 6-H) [signal for 7-OH not seen]. ^{13}C NMR (DMSO-d_6) δ_C 174.7, 154.5, 150.5, 146.8, 133.5, 133.1, 132.8, 129.2, 129.0, 125.6, 124.4, 124.3, 121.6, 117.4, 115.8, 114.5. HRMS (M+H)$^+$ calcd for $C_{16}H_9O_4F_3$, 322.0453; found 322.0454. Mp 225 °C.

3.6. General procedure for the synthesis of compounds 26a and 26b

3.6.1. Reduction of isoflavone to isoflavanone. To a stirred solution of 1.0 g of the isoflavone in 200 ml of ethanol at room temperature was added 0.5 g of Pd/C (10%) and the mixture was hydrogenated at normal pressure for 6 h. Once the hydrogenation was completed, as verified by thin-layer chromatography, the solution was filtered and concentrated to dryness under reduced pressure. The resulting residue was taken up with 10 mL of water, filtered, washed with water, and dried to afford the corresponding isoflavanone, which was purified by chromatography over a silica gel column with a 2:1 benzene/ether mixture as eluent. The yields were 60–75%.

3.7. General procedure for the synthesis of compounds 27a–g

3.7.1. Reduction of isoflavone to isoflavan. To a stirred solution of 5 mmol of the isoflavone in 100 ml of acetic acid containing 0.1% sulfuric acid was added 0.5 g of Pd/C (10%) and the mixture was hydrogenated for 14 h at normal pressure and room temperature. Once the hydrogenation was completed, verified by thin-layer chromatography, the solution was filtered and concentrated under reduced pressure. The resulting residue was taken up with 50 mL of water, extracted with ether (3 × 50 mL), and the organic layer dried with sodium sulfate to give the corresponding isoflavan, which was purified by chromatography over a silica gel column using ether/methanol mixtures of increasing polarity as eluent. The yields were 40–50%.

3.7.1.1. 6,7-Dihydroxy-4′-methoxyisoflavanone (26a) (52%). ^{1}H NMR (DMSO-d_6) δ_H9.80 (1H, s, 7-OH), 7.56 (1H, s, H-5), 7.14 (2H, d, J = 8.7 Hz, H-2′, H-6′), 7.09

(1H, s, H-8), 6.86 (2H, d, J = 8.6 Hz, H-3′, H-5′), 4.49 (1H, dd, J = 11.2 Hz, J = 13.9 H, H-3z), 4.47 (1H, dd, J = 5.6 Hz, J = 5.7 Hz, H-2a), 3.85 (1H, dd, J = 5.5 Hz, J = 7.6 Hz, H-2e), 3.70 (3H, s, OCH_3), [signal for 6-OH not seen]. ^{13}C NMR (DMSO-d_6) δ_C 191.1, 159.1, 157.1, 154.9, 141.7, 130.3, 129.1, 114.5, 113.0, 111.4, 103.6, 72.0, 55.7, 50.9. Calcd for $C_{16}H_{14}O_5$, C, 67.13%; H, 4.89%, found C, 66.95%; H, 4.91%. Mp 214 °C.

3.7.1.2. 4′,6,7-Trihydroxyisoflavanone (26b) (48%). ^{1}H NMR (DMSO-d_6) δ_H9.84 (1H, s, 7-OH), 8.21 (1H, s, 4′–OH), 7.56 (1H, s, H-5), 6.95 (2H, d, J = 8.7 Hz, H-2′, H-6′), 6.68 (2H, d, J = 8.6 Hz, H-3′, H-5′), 6.15 (1H, s, H-8), 4.79 (1H, dd, J = 11.2 Hz, J = 13.9 H, H-3z), 4.55 (1H, dd, J = 5.6 Hz, J = 5.7 Hz, H-2a), 4.20 (1H, dd, J = 5.5 Hz, J = 7.6 Hz, H-2e), 3.70 (3H, s, OCH_3), [signal for 6-OH not seen]. ^{13}C NMR (DMSO-d_6) δ_C 193.1, 157.4, 152.3, 150.9, 138.8, 131.1, 129.0, 116.6, 116.4, 114.4, 103.4, 70.9, 56.1. Calcd for $C_{15}H_{12}O_5$, C, 66.17%; H, 4.41%, found C, 66.32%; Mp 235 °C.

3.7.1.3. 6,7-Dihydroxy-4′-methoxyisoflavan (27a) (49%). ^{1}H NMR (DMSO-d_6) δ_H8.68 (1H, s, OH-7), 8.25 (1H, s, OH-6), 7.19 (2H, d, J = 8.7 Hz, H-2′, H-6′), 6.87 (2H, d, J = 8.7 Hz, H-3′, H-5′), 6.43 (1H, s, H-5), 6.19 (1H, s, H-8), 4.07 (1H, ddd, J = 3.2 Hz, J = 10.3 Hz, J = 1.6 Hz; H-2e), 3.83 (1H, dd, J = 10.4 Hz, J = 3.2 Hz, H-2a), 3.71 (3H, s, OCH_3), 3.03 (1H, dddd, J = 10.0 Hz, J = 10.1 Hz, J = 5.7 Hz, J = 4.1 Hz, H-3a), 2.80 (1H, dd, J = 10.5 Hz, J = 15.8 Hz, H-4a), 2.71 (1H, ddd, J = 5.5 Hz, J = 16 Hz, J = 1.6 Hz, H-4e). ^{13}C NMR (DMSO-d_6) δ_C 158.7, 147.1, 144.9, 139.7, 134.4, 129.1, 116.3, 114.6, 112.2, 104.0, 70.6, 55.7, 37.9, 31.9. HRMS $(M+H)^+$ calcd for $C_{16}H_{16}O_4$ 272.10486; found 272.10363. Mp 144 °C.

3.7.1.4. 4′6,7-Trihydroxyisoflavan (27b) (55%). ^{1}H NMR (DMSO-d_6) δ_H9.22 (1H, s, OH-4′), 8.66 (1H, s, OH-7), 8,24 (1H, s, OH-6), 7.07 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.69 (2H, d, J = 8.6 Hz, H-3′, H-5′), 6.41 (1H, s, H-5), 6.18 (1H, s, H-8), 4.05 (1H, ddd, J = 3.2 Hz, J = 10.5 Hz, J = 1.6 Hz; H-2e), 3.78 (1H, dd, J = 10.3 Hz, J = 10.3 Hz, H-2a), 3.06 (1H, dddd, J = 10.1 Hz, J = 10.1 Hz, J = 5.8 Hz, J = 4.1 Hz, H-3), 2.77 (1H, dd, J = 10.4 Hz, J = 15.7 Hz, H-4a), 2.68 (1H, ddd, J = 5.6 Hz, J = 16,0 Hz, J = 1.1 Hz, H-4e).^{13}C NMR (DMSO-d_6) δ_C 156.7, 147.1, 144.9, 139.7, 132.1, 128.9, 116.4, 115.9, 112.4, 104.0, 70.8, 38.0, 30.1. HRMS $(M+H)^+$ calcd for $C_{15}H_{14}O_4$ 258.08920; found 258.08590. Mp 176–177 °C.

3.7.1.5. 6,7-Dihydroxy-3′,4′-methylenedioxyisoflavan (27c) (52%). ^{1}H NMR (DMSO-d_6) δ_H8.71 (1H, s, OH-7), 8.26 (1H, s, OH-6), 6.91 (1H, d, J = 2.0 Hz, H-2′), 6.84 (1H, d, J = 8.0 Hz, H-5′), 6.76 (1H, dd, J = 2.0 Hz, J = 8.0 Hz, H-6′), 6.42 (1H, s, H-5), 6.19 (1H, s, H-8), 5.96 (2H, s, O–CH_2–O), 4.06 (1H, ddd, J = 3.5 Hz, J = 10.5 Hz, J = 1.5 Hz, H-2e), 3.84 (1H, dd, J = 10.5 Hz, J = 10,5 Hz, H-2a), 3.02 (1H, ddd, J = 10.5 Hz, J = 10.5 Hz, J = 5.5 Hz, H-3a), 2.79 (1H, dd, J = 10.5 Hz, J = 16,0 Hz, H-4a), 2.71 (1H, ddd, J = 5.5 Hz, J = 16,0 Hz, J = 1.5 Hz, H-4e).^{13}C NMR (DMSO-d_6) δ_C 147.4, 146.5, 145.9, 144.3, 139.4, 135.9, 120.5, 115.7, 111.6, 108.2, 107.9, 103.4, 100.8, 69.9, 37.9, 31.4. HRMS $(M+H)^+$ calcd for $C_{16}H_{14}O_5$, 286.0841; found 286.0842, mp 162 °C.

3.7.1.6. 6,7-Dihydroxy-3′-methylisoflavan (27d) (90%). ^{1}H NMR (DMSO-d_6) δ_H8.75 (1H, s, OH-7), 8.25 (1H, s, OH-6), 7.19 (1H, dd, J = 8.0 Hz, H-5′), 7.00–7.10 (3H, m, H-2′, H-4′, H-6′), 6.46 (1H, s, H-5), 6.24 (1H, s, H-8), 4.12 (1H, ddd, J = 3.0 Hz, J = 10.5, Hz J = 1.6 Hz, H-2e), 3.86 (1H, dd, J = 16 Hz; J = 16 Hz, H-2a), 3.05 (1H, dddd, J = 10.5 Hz, J = 16.0 Hz, J = 5.5 Hz, J = 4.6 Hz H-3a), 2.86 (1H, dd, J = 10.5 Hz, J = 16.0 Hz, H-4a), 2.26 (1H, ddd, J = 16.0 Hz, J = 16,0 Hz, J = 1.5 Hz, H-4e). ^{13}C NMR (DMSO-d_6) δ_C 146.5, 144.3, 141.8, 139.1, 137.5, 128.6, 128.1, 127.3, 124.6, 115.7, 111.5, 103.4, 69.8, 37.3, 31.2, 21.1. HRMS $(M+H)^+$ calcd for $C_{16}H_{16}O_3$, 256.1099; found 256.1101, mp 164 °C.

3.7.1.7. 7,8-Dihydroxy-4′-methylisoflavan (27e) (68%). ^{1}H NMR (DMSO-d_6) δ_H 8.54 (1H, s, OH-7), 8.09 (1H, s, OH-8), 7.19 (2H, d, J = 8.1 Hz, H-2′, H-6′), 7.12 (2H, d, J = 8.1 Hz, H-3′, H-5′), 6.36 (1H, d, J = 8.2 Hz, H-5), 6.28 (1H, d, J = 8.2 Hz, H-6), 4.23 (1H, ddd, J = 3.4 Hz, J = 10.4 Hz, J = 1.7 Hz, H-2e), 3.97 (1H, dd, J = 10.2 Hz; J = 10.2 Hz, H-2a), 3.09 (1H, ddd, J = 4.0 Hz, J = 5.7 Hz, J = 10.0 Hz, J = 8.9 Hz, H-3), 2.89 (1H, dd, J = 10.4 Hz, J = 16.1 Hz, H-4a), 2.80 (1H, ddd, J = 5.4 Hz, J = 15.6 Hz, J = 1.2 Hz, H-4e). ^{13}C NMR (DMSO-d_6) δ_C144.7, 143.6, 139.5, 136.5, 133.7, 129.7, 127.9, 119.1, 113.9, 108.7, 70.5, 38.2, 32.0, 21.3. HRMS $(M+H)^+$ calcd for $C_{16}H_{16}O_3$, 256.1099; found 256.1100, mp 125–126. °C.

3.7.1.8. 7,8-Dihydroxy-3′,4′-dimethoxyisoflavan (27f) (49%). ^{1}H NMR (DMSO-d_6) δ_H 8.53 (1H, s, OH-7), 8.09 (1H, s, OH-6), 6.94 (1H, d, J = 1.8 Hz, H-2′), 6.88 (1H, d, J = 8.4 Hz, H-5′), 6.81 (1H, dd, J = 8.4 Hz, J = 1.8 Hz, H-6′), 6.35 (1H, d, J = 8.2 Hz, H-5), 6.27 (1H, d, J = 8.2 Hz, H-6), 4.24 (1H, ddd, J = 3.2 Hz, J = 10.2 Hz, J = 1.6 Hz, H-2e), 3.96 (1H, dd, J = 10.2 Hz, J = 10.2 Hz, H-2a), 3.73 (3H, s, OCH_3-4′), 3.71 (3H, s, OCH_3-3′), 3.06 (1H, dddd, J = 10.0 Hz, J = 16.0 Hz, J = 5.4 Hz, J = 4.6 Hz, H-3), 2.90 (1H, dd, J = 10.7 Hz, J = 15.7 Hz, H-4a), 2.79 (1H, ddd, J = 5.3 Hz, J = 15.7 Hz, J = 1.1 Hz, H-4e). ^{13}C NMR (DMSO-d_6) δ_C 149.7, 148.4, 144,7, 143.7, 134.9, 133.8, 119.9, 118.9, 114.0, 112.8, 108.7, 70.8, 56.3, 56.2, 38.2, 32.2. HRMS $(M+H)^+$ calcd for $C_{17}H_{18}O_5$, 302.1154; found 302.1156. Mp 159–160 °C.

3.7.1.9. 7,8-Dihydroxy-4′-methoxyisoflavan (27g) (56%). ^{1}H NMR (DMSO-d_6) δ_H 8.54 (1H, s, OH-7), 8.09 (1H, s, OH-8), 7.22 (2H, d, J = 8.8 Hz, H-2′, H-6′), 6.89 (2H, d, J = 8.8 Hz, H-3′, H-5′), 6.35 (1H, d, J = 8.2 Hz, H-5), 6.29 (1H, d, J = 8.2 Hz, H-6), 4.22 (1H, ddd, J = 3.3 Hz, J = 16 Hz, J = 1.5 Hz, H-2e), 3.92 (1H, dd, J = 10.2 Hz, J = 10.2 Hz, H-2a), 3.72 (3H, s, OCH_3), 3.07 (1H, dddd, J = 10.0 Hz, J = 9.9 Hz, J = 5.7 Hz; J = 3.9 Hz, H-3), 2.88 (1H, dd, J = 10.1 Hz, J = 15.6 Hz, H-4a), 2.79 (1H, ddd,

J = 5.5 Hz, J = 15.7 Hz, J = 1.8 Hz, H-4e). ^{13}C NMR (DMSO-d_6) δ_C 158.8, 144.8, 143.7, 134.3, 133.8, 129.1, 119.1, 115.3, 113.9, 108.1, 70.8, 55.7, 37.8, 32.1. HRMS $(M+H)^+$ calcd for $C_{16}H_{16}O_4$, 272.1049; found 272.1049. Mp 128–129 °C.

3.8. Plasmid construction, expression, and purification of human lipoxygenases

The histidine-tagged proteins, 12-hLO and 15-hLO-1, were expressed and purified as described previously.[42,49] The SF9 expression vector for human prostate epithelial 15-lipoxygenase-2 (15-hLO-2) was constructed as follows. The previously published plasmid, pCRII-TOPO-15hLO-2,[19] was cut with EcoRI, to liberate the 15-hLO-2 fragment. This 15-hLO-2 fragment was then ligated into EcoRI cut, pFastBac1 (GibcoBRL), to generate the complete plasmid, pFastBac-15-hLO-2, which was digested to determine the correct orientation. The pFastBac-15-hLO-2 was then transposed into a recombinant FastBac bacmid by DH10Bac cells (GibcoBRL) and then transfected into SF9 cells, as described in the product literature for pFastBac1 (GibcoBRL). The virus was subsequently amplified to $\approx 2 \times 10^{10}$ plaque-forming units (pfu), added to SF9 cells ($\approx 2 \times 10^6$ cells/ml) at a concentration of $\approx 2 \times 10^7$ pfu/ml, and allowed to shake for 72 h. The cells were then harvested and frozen. 15-hLO-2 was purified by douncing the thawed cells and loading the cell extracts onto a 20 mL column of Macro-prep High Q, strong anion exchange column, from Bio-Rad. The protein was eluted with a 50–300 mM sodium chloride gradient (25 mM HEPES, pH 8.0). The collected fractions contained 95% purified protein and 5% glycerol and were frozen at −80 °C. Subsequent thawing produced no decrease in enzymatic activity.

13-(S)-HPOD was prepared as before,[50] while LA and AA were purchased from Aldrich Chemical, and BWB70C and BWA4C were purchased from Sigma Chemical. All other reagents were of reagent grade or better and were used without further purification.

3.9. High-throughput inhibitor screen

The high-throughput (HTP) screen was developed in 384-well plates where the hydroperoxide product was detected with iron/xylenol orange.[40,51] Briefly, the lipid peroxide formed by the human lipoxygenase enzymes reacts with Fe^{2+} present in solution and converts it to Fe^{3+} which in turn forms an Fe^{3+}-xylenol orange complex which absorbs at 560 nm. The HTP screen was performed as published earlier with the following changes.[40] The buffer for 12-hLO was 25 mM Hepes (pH 8.0) with 0.01% Triton X-100 and for 15-hLO-1 and 15-hLO-2 was 25 mM Hepes (pH 7.5) with 0.01% Triton X-100. Arachidonic acid was used as a substrate for 12-hLO and 15-hLO-2, while linoleic acid was used for 15-hLO-1. The first step is the addition of 45 μl of a 30 μM substrate buffer solution to each well using the Multidrop 384 by Thermo Labsystems. The inhibitor (1.2 μl at 2.5 mg/ml in DMSO) was then added using a specially designed manual liquid pin transfer device from V&P Scientific. Addition of 5 μL of enzyme, which gives a rate of approximately 0.0020 abs/s using the multidrop, started the reaction (approximately 40 nM for 12-hLO and 15-hLO-1 and 125 nM for 15-hLO-2, final concentration). The reaction was stopped well before completion (after approximately 5 min) by adding appropriate amount of reagents to give a final concentration of 100 μM of xylenol orange dye and 150 μM Fe^{2+} in 25 mM H_2SO_4, with a final volume of 100 μL in each well. The plates were stirred using a gyratory shaker in the dark for 30 min and read at 544 nm using Perkin-Elmer plate reader Victor2. The negative control for the assay was 0.2 μL of DMSO (with no inhibitor) added to reaction buffer, with no enzyme. The positive control for the assay was 0.2 μL of DMSO added to reaction buffer with enzyme, as mentioned above. All the assays were done in triplicate. The percent inhibition was calculated by subtracting the absorbance of the negative control from that of the reaction samples and dividing by the absorbance of the positive control. The value was then subtracted from 1 and multiplied by 100 to get the percent inhibition. In order to confirm the accuracy of the HTP screen, all samples giving greater than 40% inhibition by the HTP assay were screened manually to determine an accurate IC_{50} value (*vida infra*). From this additional screening, it was determined that compounds with less than 45% inhibition from the HTP screen for all three hLOs had IC_{50} values greater than 100 μM.

3.10. Manual absorption IC_{50} inhibition assay

IC_{50} inhibition constants for compounds with greater than 40% inhibition from the HTP screen were determined manually as described previously,[52] with the following changes. The reaction buffer was 25 mM Hepes (pH 8.0) with 0.01% Triton X-100 for 12-hLO and was 25 mM Hepes (pH 7.5) with 0.01% Triton X-100 for 15-hLO-1 and 15-hLO-2. Arachidonic acid was used as a substrate for 12-hLO (3 μM) and 15-hLO-2 (30 μM), while linoleic acid was used for 15-hLO-1 (3 μM). Enzyme rates were determined with approximately 40 nM for 12-hLO and 15-hLO-1 and 125 nM for 15-hLO-2, final concentration, by monitoring product formation at 234 nm ($\varepsilon_M = 2.5 \times 10^4$) with an HP 8435 spectrophotometer at room temperature. Inhibitor (1 mg/mL in DMSO) was added to the substrate buffer and the reaction initiated by addition of enzyme after a stable baseline was observed at 234 nm. Control rates were measured using the same volume of DMSO as the volume of inhibitor. IC_{50}values were determined by measuring the enzymatic rate at a variety of inhibitor concentrations (depending on the inhibitor potency) and plotting their percent inhibition versus inhibitor concentration. The corresponding data were fit to a simple saturation curve using KaleidaGraph software (Synergy) on a Macintosh computer and the inhibitor concentration at 50% activity was calculated.

3.11. Pseudoperoxidase activity assay

The pseudoperoxidase activity was determined using a Perkin-Elmer Lambda 40 spectrophotometer by follow-

ing the decrease in HPOD absorbance at 234 nm due to inhibitor-dependent consumption of HPOD.[53] The reaction mixture contained 15-hLO-1 (185 μg), 25 mM Hepes, pH 7.5, HPOD (5 μM), and inhibitor (10 μM). The reaction was initiated by adding enzyme at room temperature and the final volume of the reaction solution was 2 ml. BWB70C and BWA4C were used as positive controls for this experiment as they are known redox-type inhibitors for human 5-lipoxygenase.[54,55]

3.12. Molecular modeling studies

The 15-hLO-1 homology model was created using the Protein Local Optimization Program (PLOP, commercially distributed as Prime), which uses loop prediction,[56] side chain prediction,[57,58] and energy minimization to align the target and template sequences, as previously reported.[41] Docking of inhibitors to this model has been verified previously by predicting experimental inhibitor potency based solely on docking parameters.[41] The structures of compounds **25b** and **25e**, **29**, and **30** were prepared for docking using the LigPrep (Schrödinger, Inc) ligand preparation software, which generates a minimized conformation of each ligand, and multiple protonation/tautomerization states when appropriate. Flexible ligand docking was performed using the Glide (Schrödinger, Inc.) program,[59,60] which uses a modified version of the Chemscore energy function to score the protein-ligand interactions.[61]

Acknowledgments

T.R.H. gratefully acknowledges the gift of the original 15-hLO-2 gene from Dr. D. Tang. The 2-alkyl-4-H-benzopyran-4-ones described were synthesized by Y.V.M. during a stay in Prof. E. Breitmaier's laboratory at the University of Bonn as the recipient of a DAAD fellowship. S.S.B. thanks the MECESUP UCH 0116 program for the HRMS. The research was supported by NASA # 13171 (S.S.B.), NIH (GM56062-6) (T.R.H.), and the American Cancer Society (RPG-00-219-01-CDD) (TRH) Grants.

References and notes

1. Yamamoto, S. *Biochim. Biophys. Acta* **1992**, *1128*, 117–131.
2. Brash, A. R. *J. Biol. Chem.* **1999**, *274*, 23679–23682.
3. Solomon, E. I.; Zhou, J.; Neese, F.; Pavel, E. G. *Chem. Biol.* **1997**, *4*, 795–808.
4. Prigge, S. T.; Boyington, J. C.; Faig, M.; Doctor, K. S.; Gaffney, B. J.; Amzel, L. M. *Biochimie* **1997**, *79*, 629–636.
5. Kuhn, H. *Prostaglandins Other Lipid Mediat.* **2000**, *62*, 255–270.
6. Yoshimoto, T.; Takahashi, Y. *Prostaglandins Other Lipid Mediat.* **2002**, *68–69*, 245–262.
7. Dailey, L. A.; Imming, P. *Curr. Med. Chem.* **1999**, *6*, 389–398.
8. Honn, K. V.; Tang, D. G.; Gao, X.; Butovich, I. A.; Liu, B.; Timar, J.; Hagmann, W. *Cancer Metastasis Rev.* **1994**, *13*, 365–396.
9. Arenberger, P.; Raap, A.; Armah, B.; Kemény, L.; Ruzicka, T. *Skin Pharmacol.* **1993**, *6*, 148–151.
10. Zhang, Y.; Wang, H.; Li, J.; Jimenez, D. A.; Levitan, E. S.; Aizenman, E.; Rosenberg, P. A. *J. Neurosci.* **2004**, *24*, 10616–10627.
11. Kuhn, H.; Walther, M.; Kuban, R. J. *Prostaglandins Other Lipid Mediat.* **2002**, *68–69*, 263–290.
12. Cathcart, M. K.; Folcik, V. A. *Free Radic. Biol. Med.* **2000**, *28*, 1726–1734.
13. Neuzil, J.; Upston, J. M.; Witting, P. K.; Scott, K. F.; Stocker, R. *Biochemistry* **1998**, *37*, 9203–9210.
14. Schewe, T. *Biol. Chem.* **2002**, *383*, 365–374.
15. Ikawa, H.; Kamitani, H.; Calvo, B. F.; Foley, J. F.; Eling, T. E. *Cancer Res.* **1999**, *59*, 360–366.
16. Kelavkar, U. P.; Glasgow, W.; Olson, S. J.; Foster, B. A.; Shappell, S. B. *Neoplasia* **2004**, *6*, 821–830.
17. Brash, A. R.; Boeglin, W. E.; Chang, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6148–6152.
18. Shappell, S. B.; Boeglin, W. E.; Olson, S. J.; Kasper, S.; Brash, A. R. *Am. J. Pathol.* **1999**, *155*, 235–245.
19. Tang, S.; Bhatia, B.; Maldonado, C. J.; Yang, P.; Newman, R. A.; Liu, J.; Chandra, D.; Traag, J.; Klein, R. D.; Fischer, S. M.; Chopra, D.; Shen, J.; Zhau, H. E.; Chung, L. W.; Tang, D. G. *J. Biol. Chem.* **2002**, *277*, 16189–16201.
20. Vavrecková, C.; Gawlik, I.; Müller, K. *Planta Med.* **1996**, *62*, 397–401.
21. Suzuki, H.; Ueda, T.; Juránek, I.; Yamamoto, S.; Katoh, T.; Node, M.; Suzuki, T. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 885–889.
22. Alanko, J.; Kurahashi, Y.; Yoshimoto, T.; Yamamoto, S.; Baba, K. *Biochem. Pharmacol.* **1994**, *48*, 1979–1981.
23. Cho, H.; Ueda, M.; Tamaoka, M.; Hamaguchi, M.; Aisaka, K.; Kiso, Y.; Inoue, T.; Ogino, R.; Tatsuoka, T.; Ishihara, T., et al. *J. Med. Chem.* **1991**, *34*, 1503–1505.
24. Müller, K.; Altmann, R.; Prinz, H. *Eur. J. Med. Chem.* **2001**, *36*, 569–575.
25. Segraves, E. N.; Shah, R. R.; Segraves, N. L.; Johnson, T. A.; Whitman, S.; Sui, J. K.; Kenyon, V. A.; Cichewicz, R. H.; Crews, P.; Holman, T. R. *J. Med. Chem.* **2004**, *47*, 4060–4065.
26. Cichewicz, R. H.; Kenyon, V. A.; Whitman, S.; Morales, N. M.; Arguello, J. F.; Holman, T. R.; Crews, P. *J. Am. Chem. Soc.* **2004**, *126*, 14910–14920.
27. Amagata, T.; Whitman, S.; Johnson, T.; Stessmann, C. C.; Carroll, J.; Loo, C.; Clardy, J.; Lobkovsky, E.; Crews, P.; Holman, T. R. *J. Nat. Prod.* **2003**, *66*, 230–235.
28. Whitman, S.; Gezginci, M.; Timmermann, B. N.; Holman, T. R. *J. Med. Chem.* **2002**, *45*, 2659–2661.
29. Malterud, K. E.; Farbrot, T. L.; Huse, A. E.; Sund, R. B. *Pharmacology* **1993**, *47 Suppl 1*, 77–85.
30. Schewe, T.; Sadik, C.; Klotz, L. O.; Yoshimoto, T.; Kuhn, H.; Sies, H. *Biol. Chem.* **2001**, *382*, 1687–1696.
31. Deschamps, J. D.; Kenyon, V. A.; Holman, T. R. *Bioorg. Med. Chem.* **2006**, *14*, 4295–4301.
32. Hsieh, R. J.; German, J. B.; Kinsella, J. E. *Lipids* **1988**, *23*, 322–326.
33. Yamamoto, H.; Sakakibara, J.; Nagatsu, A.; Sekiya, K. *J. Agric. Food Chem.* **1998**, *46*, 862–865.
34. Gutierrez-Lugo, M. T.; Deschamps, J. D.; Holman, T. R.; Suarez, E.; Timmermann, B. N. *Planta Med.* **2004**, *70*, 263–265.
35. Sekiya, K.; Okuda, H.; Arichi, S. *Biochim. Biophys. Acta* **1982**, *713*, 68–72.
36. Sadik, C. D.; Sies, H.; Schewe, T. *Biochem. Pharmacol.* **2003**, *65*, 773–781.
37. Nussbaumer, P.; Lehr, P.; Billich, A. *J. Med. Chem.* **2002**, *45*, 4310–4320.
38. Fougerousse, A.; Gonzalez, E.; Brouillard, R. *J. Org. Chem.* **2000**, *65*, 583–586.

39. Sepúlveda-Boza, S.; Walizei, S. H. G.; Rezende, M. C.; Vásquez, Y.; Mascayano, C.; Mejías, L. *Synth. Commun.* **2001**, *31*, 1933–1940.
40. Waslidge, N. B.; Hayes, D. J. *Anal. Biochem.* **1995**, *231*, 354–358.
41. Kenyon, V.; Chorny, I.; Carvajal, W. J.; Holman, T. R.; Jacobson, M. P. *J. Med. Chem.* **2006**, *49*, 1356–1363.
42. Amagata, T.; Whitman, S.; Johnson, T. A.; Stessman, C. C.; Loo, C. P.; Lobkovsky, E.; Clardy, J.; Crews, P.; Holman, T. R. *J. Nat. Prod.* **2003**, *66*, 230–235.
43. Kilty, I.; Logan, A.; Vickers, P. J. *Eur. J. Biochem.* **1999**, *266*, 83–93.
44. Nelson, M. J. *Biochemistry* **1988**, *27*, 4273–4278.
45. Nelson, M. J.; Batt, D. G.; Thompson, J. S.; Wright, S. W. *J. Biol. Chem.* **1991**, *266*, 8225–8229.
46. Kemal, C.; Louis-Flamberg, P.; Krupinski-Olsen, R.; Shorter, A. L. *Biochemistry* **1987**, *26*, 7064–7072.
47. Voss, C.; Sepulveda-Boza, S.; Zilliken, F. W. *Biochem. Pharmacol.* **1992**, *44*, 157–162.
48. Kostanecki, S.; Tambor *J. Chem. Ber.* **1901**, *34*, 1691.
49. Holman, T. R.; Zhou, J.; Solomon, E. *J. Am. Chem. Soc.* **1998**, 120.
50. Lewis, E. R.; Johansen, E.; Holman, T. R. *J. Am. Chem. Soc.* **1999**, *121*, 1395–1396.
51. Gay, C.; Collins, J.; Gebicki, J. M. *Anal. Biochem.* **1999**, *273*, 149–155.
52. Carroll, J.; Jonsson, E. N.; Ebel, R.; Hartman, M. S.; Holman, T. R.; Crews, P. *J. Org. Chem.* **2001**, *66*, 6847–6851.
53. Riendeau, D.; Falgueyret, J. P.; Guay, J.; Ueda, N.; Yamamoto, S. *Biochem. J.* **1991**, *274*, 287–292.
54. Falgueyret, J. P.; Hutchinson, J. H.; Riendeau, D. *Biochem. Pharmacol.* **1993**, *45*, 978–981.
55. Hussey, H. J.; Tisdale, M. J. *Br. J. Cancer* **1996**, *74*, 683–687.
56. Jacobson, M. P.; Pincus, D. L.; Rapp, C. S.; Day, T. J.; Honig, B.; Shaw, D. E.; Friesner, R. A. *Proteins* **2004**, *55*, 351–367.
57. Jacobson, M. P.; Friesner, R. A.; Xiang, Z.; Honig, B. *J. Mol. Biol.* **2002**, *320*, 597–608.
58. Jacobson, M. P.; Kaminski, G. A.; Friesner, R. A.; Rapp, C. S. *J. Phys. Chem. B* **2002**, *106*, 11673–11680.
59. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739–1749.
60. Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. *J. Med. Chem.* **2004**, *47*, 1750–1759.
61. Eldridge, M. D.; Murray, C. W.; Auton, T. R.; Paolini, G. V.; Mee, R. P. *J. Comput. Aided Mol. Des.* **1997**, *11*, 425–445.