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Original article

Synthesis and SAR studies of mono *O*-prenylated coumarins as potent 15-lipoxygenase inhibitors

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ABSTRACT

All of the mono isopentenyloxy, -geranyloxy and -farnesyloxy derivatives of coumarin were synthesized and their inhibitory potency against soybean 15-lipoxygenase (SLO) and human 15-lipoxygenase-1 (HLO-1) were determined. Amongst the synthetic analogs, 5-farnesyloxycoumarin showed the most potent inhibitory activity against SLO (IC_{50} = 0.8 μM) while 6-farnesyloxycoumarin was the strongest HLO-1 inhibitor (IC_{50} = 1.3 μM). The IC_{50} variations of the farnesyl derivatives for HLO-1 (1.3 to ~75 μM) were much higher than that observed for SLO (0.8–5.8 μM). SAR studies showed that hydrogen bonding, CH/π, anion-π and S–O═C interactions with Fe^{III}–OH, Leu408, Glu357 and Met419 were the distinct intermolecular interactions which can lead to important role of the coumarin substitution site in HLO-1 inhibitory potency, respectively.

1. Introduction

Lipoxygenases (LOs) are a main group of the non heme iron-containing proteins which can catalyze the hydroperoxidation of poly unsaturated fatty molecules containing a *cis*,*cis*-1,4-pentadiene structure such as arachidonic and linoleic acid [1]. In mammalians, three classes of signaling compounds leukotrienes, lipoxines and eoxines, are formed *via* hydroperoxidation at 5 or 15 carbon position of the arachidonic acid [2,3]. Amongst the mammalian lipoxygenases involved in the human disease, 15-lipoxygenase-1 (15-LO-1) is recently well-documented as a target for reducing the biosynthesis of eoxines, one of the known pro-inflammatory mediators [3]. It has been showed that the 15-LO-1 pathway can generate the pro-inflammatory eoxines in eosinophils, mast cells, and nasal polyps from allergic subjects, indicating that inhibition of 15-LO-1 might be an attractive target for treatment of inflammatory respiratory disorders such as asthma, rhinitis, and chronic obstructive pulmonary disease (COPD) in humans [3]. 15-LO metabolites such as 15-HETE were also found as important mediators in the development of Th1-allergic inflammation induced by sensitization with an allergen plus dsRNA, and the 15-LO pathway was introduced as a novel therapeutic target for the treatment of virus-associated asthma characterized by a Th1 immune response to inhaled allergens [4]. The critical role of the 15-LO-1 metabolite, 13-(S)-hydroxyoctadecadienoic acid (13-HODE), in the progression of prostate cancers and inhibition of 15-LO-1 activity for apoptosis induction in PC3 cells has been demonstrated [5,6]. It was also found that 15-LO involved in oxidative modification of low-density lipoproteins (LDL) leading to the development of atherosclerosis [7–9]. A recent study on human airway epithelial cells, in cell culture and in human asthmatic epithelial cells, showed that high levels of 15-LO-1 interact with phosphatidylethanolamine-binding protein-1 to displace Raf-1 and sustain MAPK/ERK activation [10].

There is a considerable interest in the development of LO inhibitors for therapeutic applications. Although a numerous LO inhibitors has been prepared and biologically studied, due to their

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side effects, consuming of them is usually forbidden or limited [11]. Explore the alternative strategies to lower the formation of inflammatory mediators and oncogenic agents with the help of natural dietary products are necessary. A group of these natural products are coumarins. Coumarins, consist of fused benzene and a pyrone rings, comprise a very large class of phenolic derivatives occurred in plants. More than 1300 derivative of coumarins, possessing anti-inflammatory and anticancer properties [12], have been identified as primary or secondary metabolites in green plants, fungi and bacteria [13]. Several natural and synthetic derivatives of hydroxycoumarins have been introduced as inhibitors of the lipoxygenase and cyclooxygenase pathways of arachidonate metabolism [14,15].

In previous work, umbelliprenin (7-*trans*,*trans*-farnesyloxy coumarin), was examined for *in vitro* antioxidant activity, *in vitro* inhibitory activity against soybean lipoxygenase, and *in vivo* anti-inflammatory activity [16]. Lipoxygenase inhibitory activity of umbelliprenin was determined by using the 1,3-dien formation method (measuring the decrease in absorbance at 234 nm) where the linoleic acid was used as the substrate [16].

Umbelliprenin did not show any significant antioxidant activity in the presence of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) but exhibited a remarkable and potent inhibition against soybean 15-lipoxygenase (SLO) [16]. This compound, in the *in vivo* anti-inflammatory test, could also inhibit the carrageenin induced paw edema significantly (39%) [16].

In the present study, *O*-prenylated derivatives involving farnesyloxy, geranyloxy and isopentenyloxy substituents at positions 3, 4, 5, 6, 7 and 8 of coumarin ring were synthesized and the lipoxygenase inhibitory activities of them together with the SAR studies were evaluated.

2. Result and discussion

All of the *O*-prenylated coumarins were synthesized according to the previous literature *via* reaction of each hydroxycoumarin with the desired prenyl bromide in the presence of potassium carbonate and acetone (Scheme 1) [17]. 5- and 8-Hydroxycoumarins were synthesized by starting from related dimethoxy benzaldehydes and malonic acid. In the synthetic pathway, 2-hydroxy-6-methoxy benzaldehyde (**1c**) and 2-hydroxy-3-methoxy benzaldehyde (**1f**) reacted with malonic acid in the mixture of acetic acid and benzene at reflux condition to produce corresponding methoxy-3-carboxycoumarins (**2c** and **2f**) [18]. After acidic decarboxylation of the carboxycoumarines [19] and then demethylation of the resulted methoxycoumarins (**3c** and **3f**) by melting with anhydrous aluminum chloride [20], the desired hydroxycoumarins (**4c** and **4f**) were prepared (Scheme 1).

The inhibitory activity of the synthetic compounds against SLO was determined using modified oxidative coupling of 3-methyl-2-benzothiazolinone (MBTH) with 3-(dimethylamino) benzoic acid (DMAB) reported by Anthon et al. [21]. In this method, the basis of the lipoxygenase activity determination is the peroxide concentration measurement.

The enzyme assay was performed according to the end point protocol while in the previous work it had been down by using the kinetic procedure (measurement of the enzyme activity within 60–120 s after addition of the substrate to the enzyme-inhibitor mixture) [16]. By using the last protocol the lower IC_{50} values versus the former is obtained.

Among the three groups of synthetic *O*-prenylated coumarins, farnesyloxy derivatives, almost showed the best inhibitory activity against soybean 15-lipoxygenase (Table 1). The results showed that the inhibitory potential depends on the prenyl length and it decreases by 8–50 fold from farnesyl to isopentenyl substituents. In the assessment, 5-farnesyloxy derivative (**7c**) was the best SLO inhibitor by an IC_{50} value of 0.8 ± 0.1 μM. Amongst the farnesyloxy analogs, **7f** was the weakest inhibitor by an IC_{50} value of 5.8 μM. Complementary studies on inhibitory mechanism showed that the mentioned compounds inhibit lipoxygenase activity by competitive mechanism (Fig. 1).

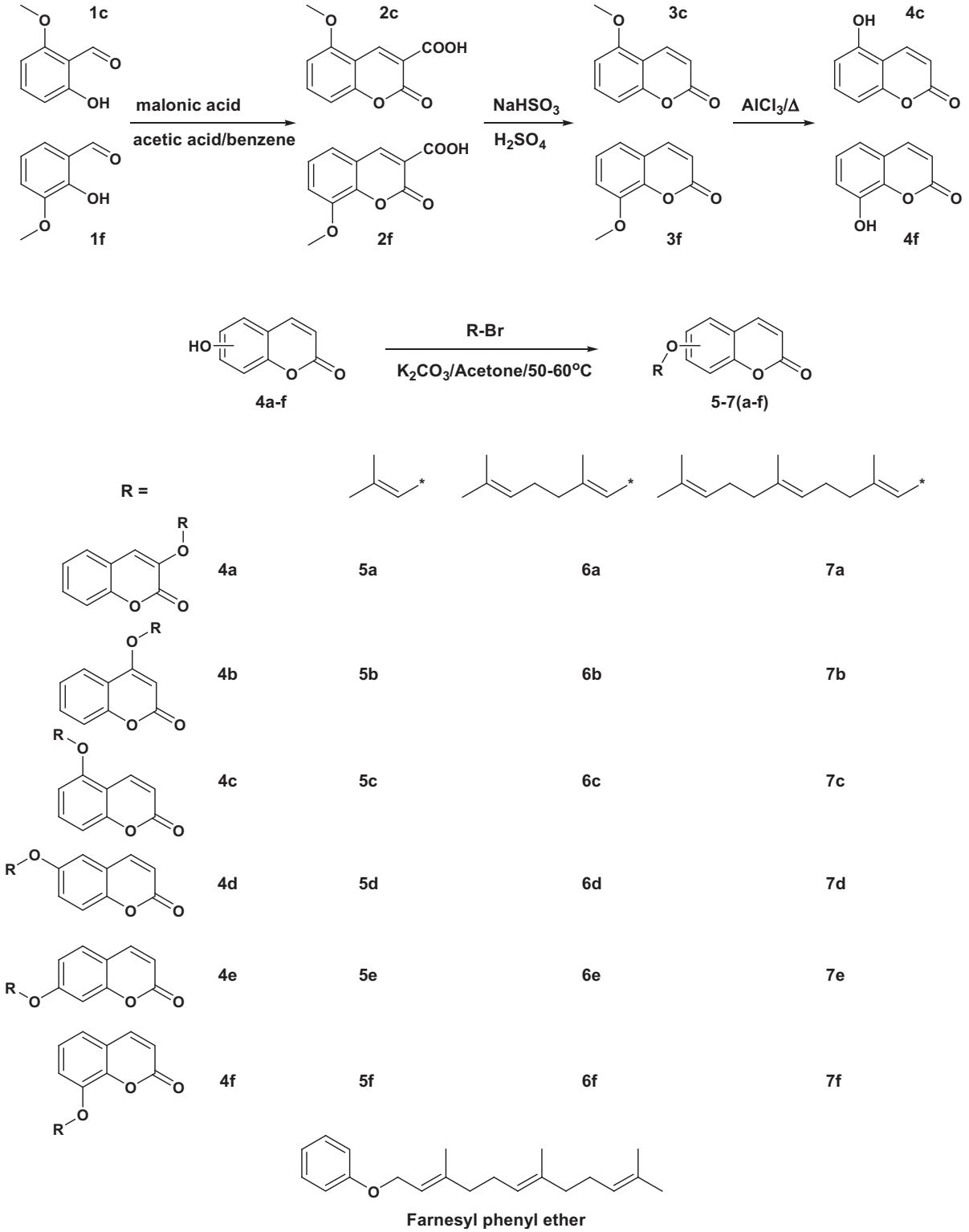
In the next step, the inhibitory potential of the farnesyloxy and geranyloxy coumarins against recombinant human 15-lipoxygenase 1 (HLO-1) were determined (Table 2). In the experiment, compounds **7d** showed the best inhibitory activity (IC_{50} = 1.3 μM) while the lowest activity was observed for **7a** (IC_{50} > 50 μM). Considering the data of Table 2, it is found that like SLO, by decreasing the prenyl length the IC_{50} value increases. It was interesting that the inhibitory results were different when compared with SLO. For example, **7a** with the second rank of SLO inhibition was the weakest HLO-1 inhibitor while the ternate SLO inhibitor, **7d**, showed the highest potency for suppressing HLO-1 activity.

In order to give further proof to the action mechanism of designed inhibitors, molecular models of the complex enzyme-inhibitor were generated for the synthetic coumarins using X-ray 3D structures of soybean 15-LO (SLO) and rabbit 15-LO-1 (RLO-1). There is reasonable homology between the HLO-1 and RLO-1 (identities: 81%, positives: 91%, extracted from NCBI-BLAST [22]). This homology increases (~99%) within 20 Å in the active site pocket. So we can use the 3D structure of RLO-1 for HLO-1 in docking analysis.

There are significant differences in size, sequence, and substrate preference between the plant and animal LOs, but the overall fold and geometry of the non heme iron-binding site are conserved [23]. The structures of SLO and RLO-1 demonstrate a high level of conservation within 10 Å (around the linoleic acid into SLO X-ray view) in the active site pocket. The structure of RLO-1 was superimposed on the SLO with RMS (C-α atoms) = 1.24 Å in the mentioned region (Fig. 2). The largest differences between the SLO and RLO-1 active site pocket were found in residues of initial part of α2 helix. In SLO Leu^{273}–Ala^{280} form α helix (α2 helix) while the similar residues in RLO-1 (Asp^{170}–Leu^{177}) did not shape any secondary structure (Fig. 2).

Bonding affinity of the designed molecular structures (compounds **6a**–**f** and **7a**–**f**) toward SLO and RLO-1 was studied. Docked conformers of **6a**–**f** and **7a**–**f** were generated in AutoDockTools (ADT) software. In docking process, flexible side chain of the active site pocket residues was allowed to be rotatable (Phe353, Leu408, Phe415, Ile418, Met419, Ile593, Leu597 in RLO-1 and Leu277, Leu515, Ile557, Leu560, Leu565, Val566, Ile572, Phe576-Leu773, Ile857 in SLO). A detailed assessment of the output clusters (cluster tolerance/Å = 2.5) of both enzymes revealed that the most bonding conformers (17–25%) belong to cluster in which prenyl portion surrounded by Leu277, Trp519, Ile557, Leu560, Leu565, Val566, Ile572, Leu773 and Ile857 in SLO and by Leu408, Ala404, Arg403, Ile400, Asp174 and Ile173 in RLO-1 (Fig. 3). In these clusters, coumarin ring covered by lipophilic side chains of Ser510, His513, Gln514, Phe576, Gln716, Asp766 and Ile770 in SLO and by Ile593, Glu548, Leu408, Phe415, Met419, His361, Leu362 and Glu357 side chains in RLO-1 (Fig. 3). Amongst the mentioned amino acids Ile557, Leu560, Leu565, Val566, Ile572, Leu773, Ile857, His513, Gln514, Gln716, Asp766 from SLO and Leu408, Ala404, Ile400, Glu548, Leu408, His361, Leu362, Glu357 from RLO-1 (as same as HLO-1) are highly conserved [23]. It is notable that Asp174 and Ile173 (highly conserved residues in mammalian 15-lipoxygenase-1) belong to the α2 helix have been reported as major residues which form a distinguished space when compared with SLO [23].

For each farnesyl and geranyl derivatives, it was found that there is a satisfactory relationship between IC_{50} values and the lowest calculated inhibitory constants (K_i) of the docked conformer in the mentioned clusters (Fig. 4). The described conformer from each



Scheme 1. General procedure for the synthesis of *O*-prenylated coumarins and the chemical structure of farnesyl phenyl ether (FPE). **5c** = umbelliprenin; **5b** = aurapten.

cluster was named as "consensus structure" and used for further analysis (Fig. 5).

Considering the IC_{50} values of the synthetic compounds, our founding revealed that the prenyl length played an important role in the inhibitory activities and when the prenyl moiety composed of more than 10 carbons (geranyl group), substitution site of coumarin ring would be substantial.

The higher IC_{50} variation of HLO-1 as compared with SLO, implied the important role of the farnesyl substituent situation of coumarin ring in HLO-1 inhibition. This observation originates from the different chemical characteristics of the active site pocket in these enzymes.

By considering the docked models of both enzymes it is found that the coumarin moiety of consensus structures has four

Table 1
SLO inhibitory assessment data of *O*-prenylated coumarins, farnesyl phenyl ether (FPE), caffeic acid and 4-methyl-2-(4-methylpiperazinyl)pyrimido[4,5-b]benzothiazine (4-MMPB: CAS 928853-86-5).

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
5a	54.4 ± 2.2	**6a**	4.7 ± 0.1	**7a**	1.7 ± 0.1
5b	43.9 ± 2.4	**6b**	5.6 ± 0.2	**7b**	3.5 ± 0.2
5c	39.8 ± 2.2	**6c**	2.8 ± 0.1	**7c**	0.8 ± 0.1
5d	69.4 ± 2.6	**6d**	9.6 ± 0.2	**7d**	2.1 ± 0.1
5e	60.4 ± 2.7	**6e**	6.3 ± 0.2	**7e**	3.1 ± 0.1
5f	46.5 ± 2.9	**6f**	8.1 ± 0.2	**7f**	5.8 ± 0.2
4-MMPB	21.2 ± 1.4	Caffeic acid	39.4 ± 1.3	FPE	2.7 ± 0.3

Table 2
HLO-1 inhibitory assessment data of *O*-prenylated coumarins (IC_{50} of FPE > 50 μM). Due to self aggregation the IC_{50} of FPE, **7a** and **6a** was not measurable at higher than 50 and 100 μM respectively.

Compound	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**
IC_{50} (μM)	>50	28.8 ± 2.4	3.6 ± 0.3	1.3 ± 0.1	16.7 ± 1.0	15.6 ± 0.8
Compound	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**
IC_{50} (μM)	>100	42.6 ± 1.7	12.8 ± 0.3	6.8 ± 0.3	23.9 ± 1.2	39.5 ± 2.2

prominent intermolecular interactions with the RLO-1 (HLO-1) active site component which is not seen in SLO (Fig. 6):

1. Hydrogen bond between Fe–OH and phenoxy oxygen of coumarin ring.
2. CH/π interaction between the CH_3 of Leu408 side chain and aromatic portion of coumarin ring.
3. ππ stacking interaction between Glu357 carboxylate anion and coumarin ring.
4. S–O interaction between Met419 sulfur atom and oxygen of coumarin carboxylate.

CH/π and ππ stacking interactions make important contributions to protein structure and function. Experimental evidence of CH/π interactions between methyl groups and aromatic amino acids backbone in proteins has been reported in some literatures [24]. High-level *ab initio* analysis of CH/π interactions between methane and benzene has shown that this interaction is strongest when a CH bond is pointing directly at the center of the aromatic ring [25–27]. In the desired bonding cluster of RLO-1, methyl portion of highly conserved Leu408 [23,28] can axially interact with π electrons of benzene portion of coumarin. The ππ stacking interaction between Glu357 carboxylate and coumarin ring can affect the hydrogen bonding strength between Glu357 and His361 directly bonded to Fe core. The effective role of Glu357–His361 and Glu357–Gln548–His545 hydrogen bond network in oxidation potency of Fe^{III}–OH has been previously studied [29]. The influence of parallel π-stacking of aromatic compounds on the hydrogen bonding ability of purine and pyrimidine bases and also pairwise interaction of aromatic amino acids with anionic amino acids (anion–π interactions) has been recently investigated [30–32]. Similar to the mentioned intermolecular interactions (CH/π and anion–π interactions by Leu408 and Glu357) are seen for aromatic portion of 2-(1-octynyl)-cinnamate (ligand) in 3D view of RLO-1 co-crystal structure (PDB entry: 2P0M) [28].

Non-bonded interactions between a divalent sulfur atom and carbonyl oxygen atom have recently been characterized in proteins. Statistical database analyses of various types of S–O═C interactions implied that the S atom tends to approach the O atom either within the carbonyl plane (in the n_O direction) or from the vertical direction (in the π_O direction) at distance of 3–3.5 Å [33]. In the case of S–O (ester) interactions, the vertical direction is significantly preferred [33]. In the mentioned bonding cluster of RLO-1, carbonyl portion of coumarin ring interacts with the sulfur of Met419. The steric role of the Met419 in peroxidation site of arachidonic acid was proved by site direct mutagenesis [34].

Amongst the consensus structures, ones with the suitable direction of coumarin ring to reach the four mentioned intermolecular interactions would be potent inhibitors. Amongst the synthetic farnesyl derivatives, compound **7d** has the lowest K_i for this kind of bonding conformation (Fig. 6).

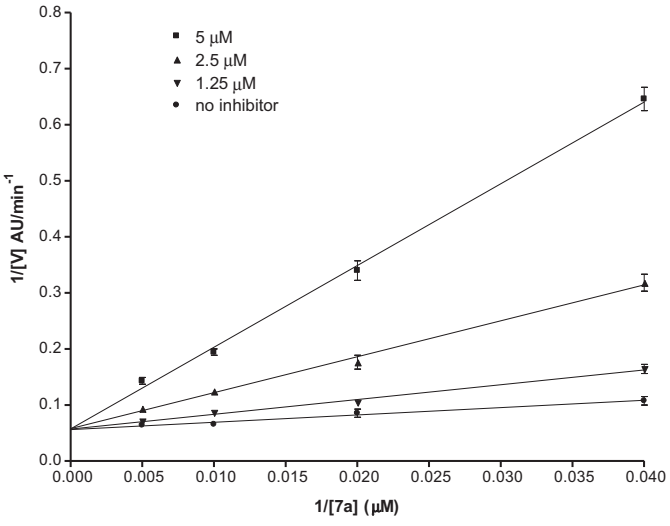


Fig. 1. Lineweaver–Burk plot of SLO inhibition by **7a**. Lipoxygenase activity was measured at 25, 50, 100 and 200 μM concentrations of linoleic acid by MBTH–DMAB method. The *Y*-intercept average ($1/V_{max}$) of the plotted carves is 0.0574 ± 0.0032 (K_m = 23.25 μM).

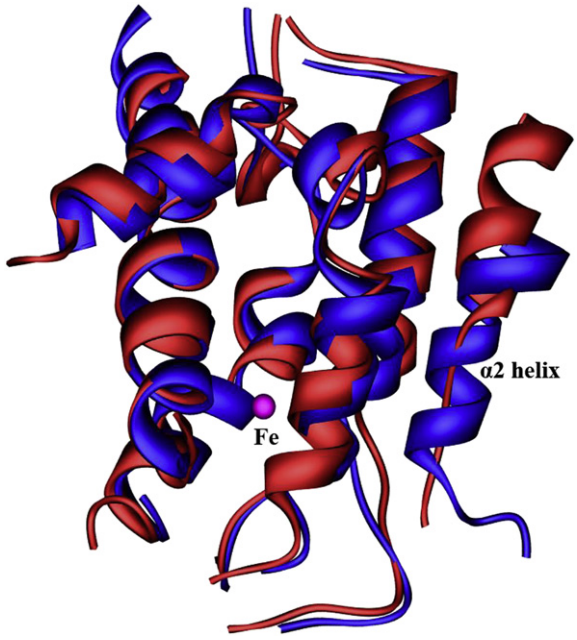


Fig. 2. Comparison of the two 15-lipoxygenase 3D structures (in ribbon view). C-α of SLO and RLO-1 are superposed and colored in blue and red, respectively. The active site pockets were mapped by inserting the Fe atom in pink bull view. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

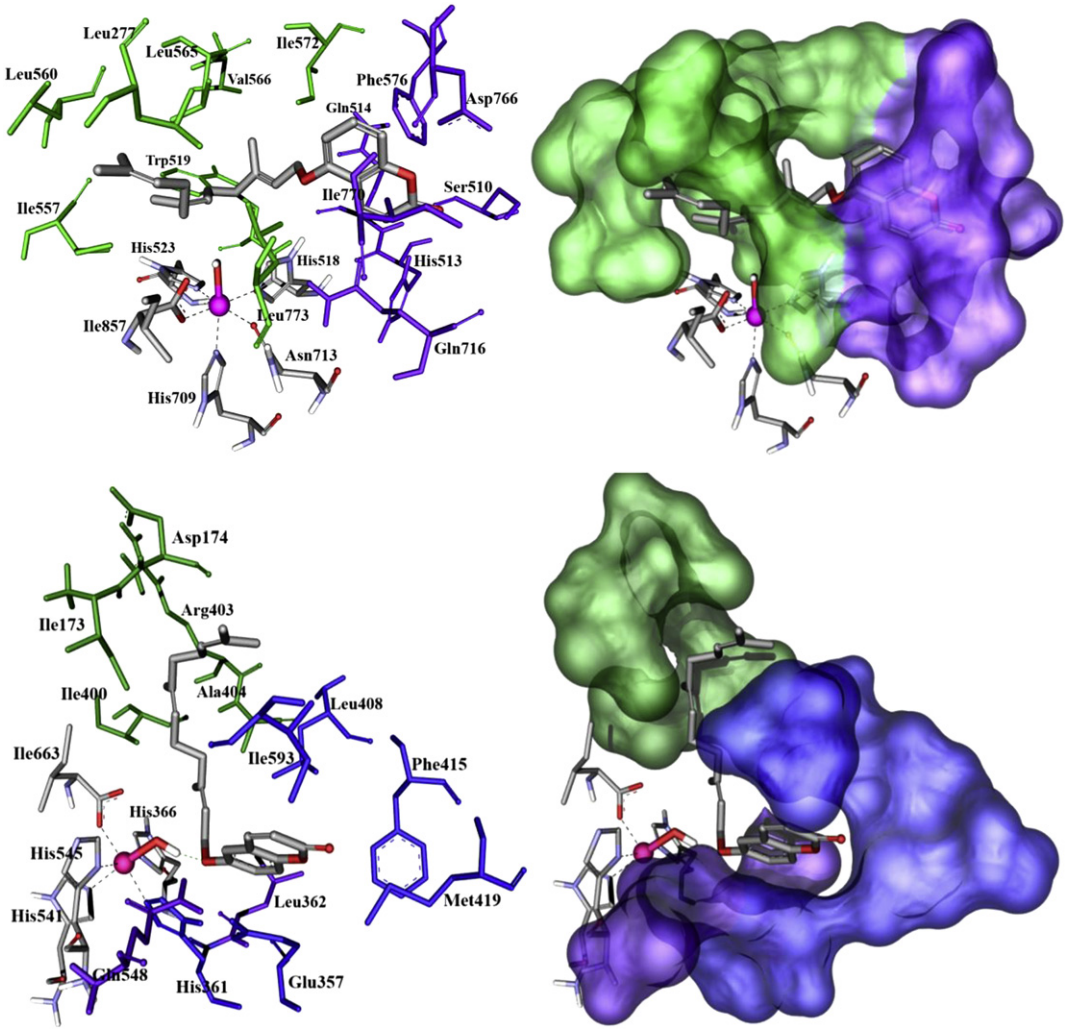


Fig. 3. Stick and solvent surface view of the SLO (above) and RLO-1 (below) active site residues interacted with consensus structure of **7c** and **7d** respectively. The residues which have intermolecular interactions with farnesyl and coumarin moieties are distinguished by green and blue color, respectively. The Fe^{III} bonded to OH is distinguished by pink bull. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In SLO, important factor of the inhibitor bonding tendency is the hydrophobic nature of the active site pocket organized by lipophilic side chain of Leu277, Trp519, Ile557, Leu560, Leu565, Val566, Ile572, Leu773 and Ile857. This claim could be acceptable when we consider the low IC_{50} variation of the prenylated coumarins particularly isopentenyl analogs. For further proof of the assertion, inhibitory activity of farnesyl phenyl ether [35] (Scheme 1) against SLO and HLO-1 was determined. The IC_{50} of SLO was 2.7 μM while it was higher than 50 μM for HLO-1. The results confirm the key role of the prenyl and coumarin moieties in inhibition of SLO and HLO-1 activities respectively.

In summary all of the favorite mono prenyloxycoumarins were synthesized and their inhibitory potency against herbal and mammalian 15-lipoxygenase (SLO and HLO-1) were evaluated. The structure activity relationship (SAR) studies showed the importance of prenyl length in SLO inhibition and influence of coumarin substitution site on inhibitory activity for the longest prenyl chain. It was also found that for farnesyl and geranyl derivatives the role of coumarin substitution site in HLO-1 inhibition is very predominant. The observed inhibition differences between the two mentioned enzymes originated from chemical nature and hydrophobic property of their active side pocket residues.

3. Materials and methods

3.1. Molecular modeling, docking and SAR study

3.1.1. Structure optimization

The structures were designed in chem3D professional; Cambridge software [36]. Output files were minimized under semi-empirical AM1 method (Convergence limit = 0.01; Iteration limit = 50; RMS gradient = 0.05 kcal/mol; Fletcher-Reeves optimizer algorithm) in HyperChem7.5 [37,38].

Crystal structure of soybean 15-lipoxygenase (SLO) complex with 13(S)-hydroproxy-9(Z)-2,11(E)-octadecadienoic acid and rabbit reticulocyte 15-lipoxygenase (RLO-1) complex with 2-(1-octynyl)-cinnamic acid was retrieved from RCSB Protein Data Bank (PDB entry: 1IK3 and 2P0M respectively).

3.1.2. Molecular docking

The ligands of the SLO and RLO-1 3D structure were omitted. Then the Fe was modified to Fe^{III}–OH, geometrically optimized by MM+ method in HyperChem7.5 and outputted in .pdb format for docking process.

Automated docking simulation was implemented to dock the minimized structures into the active site of SLO and RLO-1 with

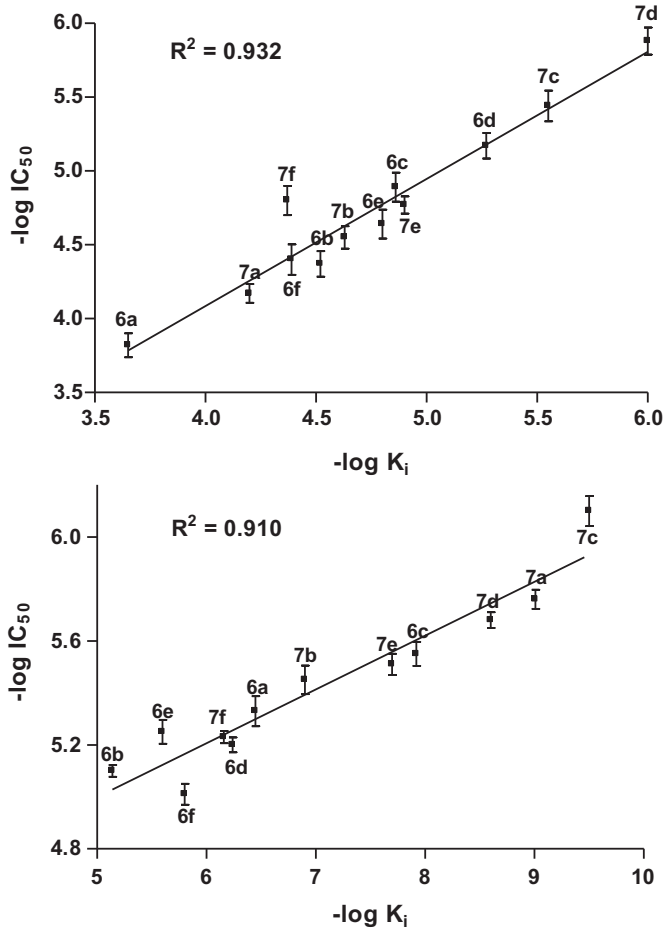


Fig. 4. Log IC_{50} versus $-\log K_i$ for consensus structures of **7a**–**f** and **6a**–**f** for HLO-1 (above) and SLO (below). The IC_{50} of **7a** and **6a** against HLO-1 was predicted as 75 and 150 μM *via* extrapolation respectively.

AutoDock 4.2 [39] using Lamarckian genetic algorithm [40]. This method has been previously shown to produce bonding models similar to the experimentally observed models [41,42]. The torsion angles of the ligands were identified, hydrogens were added to the macromolecule, bond distances were edited and solvent parameters were added to the enzyme 3D structure. Partial atomic charges were then assigned to the macromolecule as well as ligands (Gasteiger for the ligands and Kollman for the protein).

The docking regions of SLO and RLO-1 were defined by considering Cartesian chart 18.3, 4.8,19.2 and −50.7, 165.3, 32.7 as the central of a grid size with 44, 56, 62 and 56, 48, 54 points in *X*, *Y* and *Z* axes respectively. The docking parameter files were generated using Genetic Algorithm and Local Search Parameters (GALS) while number of generations (GA-LS runs) and maximum number of energy evaluations were set to 200 and 2,500,000 respectively (number of individuals in population = 300; number of top individuals to survive to next generation = 1). The 200 docked complexes were clustered with a root-mean-square deviation tolerance (RMSD) of 2.5 Å. Docking results were submitted to Accelrys DS Visualizer 2.0.1 [43] for further evaluations.

3.1.3. 15-LO inhibitory assessment

Linoleic acid and two assay solutions (A and B) were prepared in advance.

Solution A was 50 mM DMAB in a 100 mM phosphate buffer (pH 7.0). Solution B was a mixture of 10 mM MBTH (3 mL), hemoglobin (5 mg/mL, 3 mL) in 50 mM phosphate buffer at pH 5.0 (25 mL). A linoleic acid solution was prepared by mixing 5 mg of linoleic acid with 50 mg Tween 20 in 3 mL water and then diluting with KOH 100 mM to a final volume of 5 mL.

In the standard assay, the sample in ethanol (25 μL), SLO (4000 units/mL in 50 mM phosphate buffer pH 7.0; 25 μL) and phosphate buffer pH 7.0 (50 mM; 900 μL) were mixed in a test tube and preincubation was carried out for 5 min at room temperature. A control test was done with the same volume of ethanol. After the preincubation, linoleic acid solution (50 μL) was added to start the peroxidation reaction, and, 7 min later, solution A (270 μL) and then solution B (130 μL) was added to start the color formation. Further 5 min later, 200 μL of a 2% SDS solution was added to terminate the reaction. The absorbance at 598 nm was compared with control test.

3.2. Experimental section

3.2.1. Instruments

^{1}H NMR (300 & 500 MHz), ^{13}C NMR (75 & 125 MHz) were obtained by using a Bruker Avance DRX-500 Fourier transformer spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS). Elemental analysis was obtained on a Thermo Finnigan Flash EA microanalyzer. All measurements of lipoxygenase activities were carried out using a Spekol 1500 spectrophotometer. HLO-1 was purchased from Novus Biologicals Co. SLO and other chemicals were purchased from Sigma, Aldrich and Merck Co. respectively.

3.2.2. General procedure for preparation of hydroxycoumarins

Desired methoxysalicylaldehyde (0.1 mol; 15.3 g), malonic acid (0.1 mol; 10.4 g), acetic acid (50 mmol; 3.0 g), aniline (4 mmol; 0.37 g) and benzene (30 mL) were heated together. After 8 h the reaction mixture was cooled and filtered. The produced methoxy-3-carboxycoumarin (**2c** and **2f**) was washed with benzene and after drying used for next step without further purification.

*8-Methoxy-3-coumarincarboxylic acid (**2c**)*: Yellow solid, mp: 203–205 °C; ^{1}H NMR ($CDCl_3$): δ 4.00 (s, 3H, $-OCH_3$), 6.9 (s, 1H, H-8 (coumarin)), 7.1 (s, 1H, H-6 (coumarin)), 7.8 (t, 1H, H-7 (coumarin)), 9.00 (s, 1H, H-4 (coumarin)).

*5-Methoxy-3-coumarincarboxylic acid (**2f**)*: Yellow solid, mp: 208–210 °C (Lit. [44]: 210 °C.); ^{1}H NMR ($CDCl_3$): δ 4.00 (s, 3H, $-OCH_3$), 7.32 (s, 3H, H-5, H-6, H-7 (coumarin)), 8.91 (s, 1H, H-4 (coumarin)).

A mixture of methoxy-3-carboxycoumarin (10 g) and sodium bisulfite (12 g) in water (40 mL) was warmed until no more was evolved. Then concentrated sulfuric acid was added and refluxing was continued for 1 h. The mixture was poured on crushed ice and filtered. The produced methoxycoumarin (**3c** and **3f**) was washed with water and after drying used for next step without further purification.

*5-Methoxycoumarin (**3c**)*: White solid, mp: 87 °C (from water); Lit. [20]: 85–87 °C.

*8-Methoxycoumarin (**3f**)*: White solid, mp: 89 °C (from water); Lit. [44]: 89 °C.

A mixture of desired methoxycoumarin (2 g) and aluminum chloride (4 g) was heated at 145–150 °C for 3 h. After cooling a mixture of ice and concentrated hydrochloric acid was added and the precipitated tiny needles of hydroxycoumarin (**4c** and **4f**) were separated.

*5–Hydroxycoumarin (**4c**)*: White solid, mp: 222–224 °C (from water); Lit. [20]: 223–225 °C.

*8-Hydroxycoumarin (**4f**)*: White solid, mp: 152 °C (from water); Lit. [45]: 148–150 °C.

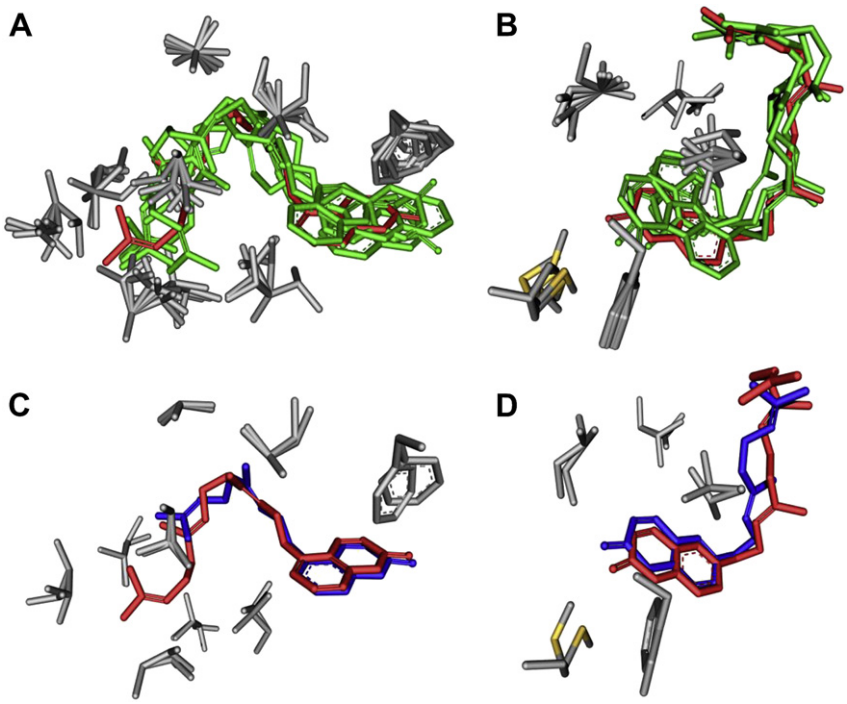


Fig. 5. (Above) Consensus structures of compounds **7a**–**f** in the SLO (A) and RLO-1 (B) active site pockets with presentation of some of the flexible residues side chain. In both figures compounds **7c** and **7d** are distinguished by red color. (Below) Consensus structures of compounds **6c** (bleu) & **7c** (red) in SLO (C) and **6d** (blue) & **7d** (red) in RLO-1 (D) active site pockets. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.3. General procedure for preparation of prenyloxycoumarin

A mixture of hydroxycoumarins **4a**–**f** (5 mmol), prenyl bromide (6 mmol) and anhydrous potassium carbonate (0.70 g; 5 mmol) in dry acetone (3 mL) was refluxed for 12 h and then cooled. The mixture was diluted with water (10 mL) and then extracted with ether (2 × 20 mL). The combined extracts were washed with NaOH 10% (2 × 10 mL) and dried with anhydrous sodium carbonate. After removal of the solvent the products were purified by crystallization from methanol or by column chromatography (silicagel 60; 230–400; heptane).

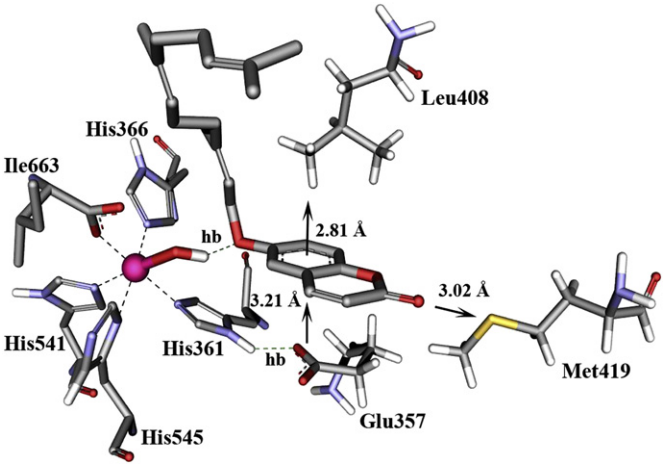


Fig. 6. Suitable situation of **7d** in the RLO-1 active site pocket for formation of hydrogen bond (hb) with Fe–OH, π (coumarin) → σ^* (H-C of Leu408), π (carboxylate anion of Glu357) → π^* (coumarin) and (Met419) S–O=C (coumarin) interaction. The Van der Waals intermolecular interactions are shown by black arrows.

3-(3-Methylbut-2-enyloxy)-2H-chromen-2-one (***5a***): White solid, mp: 108 °C (from methanol); Lit. [46]: 108–109 °C.

4-(3-Methylbut-2-enyloxy)-2H-chromen-2-one (***5b***): White solid, mp: 96–97 °C (from methanol); Lit. [47]: 98 °C.

5-(3-Methylbut-2-enyloxy)-2H-chromen-2-one (***5c***): White solid, mp: 64–65 °C (from methanol); Lit. [48]: 62–64 °C.

6-(3-Methylbut-2-enyloxy)-2H-chromen-2-one (***5d***): White solid, mp: 128–130 °C (from methanol); Lit. [49]: 129–130 °C.

7-(3-Methylbut-2-enyloxy)-2H-chromen-2-one (***5e***): White solid, mp: 76–77 °C (from methanol); Lit. [50]: 74–75 °C.

4-((E)-3,7-dimethylocta-2,6-dienyloxy)-2H-chromen-2-one (***6b***): White solid, mp: 47–48 °C (from methanol); Lit. [51]: 48–50 °C.

7-((E)-3,7-dimethylocta-2,6-dienyloxy)-2H-chromen-2-one (***6e***): White solid, mp: 64–65 °C (from methanol); Lit. [50]: 62–63 °C.

7-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-2H-chromen-2-one (***7e***): white solid, mp: 59–60 °C (from methanol); Lit. [50]: 57–59 °C.

3-((E)-3,7-dimethylocta-2,6-dienyloxy)-2H-chromen-2-one (***6a***): White solid, mp: 70–71 °C; ^{1}H NMR ($CDCl_3$): δ 1.58 (s, 3H, $-CH_3-$, (geranyl)), 1.63 (s, 3H, CH_3-, (geranyl)), 1.75 (s, 3H, CH_3-, (geranyl)), 2.07–2.12 (m, 4H, $-2CH_2-$, (geranyl)), 4.62 (d, 2H, J = 6.5 Hz ($-OCH_2$), (geranyl)), 5.044–5.073 (m, 1H, =CH– (geranyl)), 5.48–5.51 (m, 1H, =CH– (geranyl)), 6.82 (s, 1H, H-4 (coumarin)), 7.21–7.39 (m, 4H, H-5, H-6, H-7, H-8 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 157.73, 149.47, 143.62, 142.46, 131.93, 128.29, 126.39, 124.63, 123.58, 119.78, 117.95, 116.18, 113.69, 110.03, 66.21, 39.47, 26.14, 25.63, 17.69, 16.78. Found: C, 76.48; H, 7.34. $C_{19}H_{22}O_3$ requires: C, 76.51; H, 7.38%.

3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-2H-chromen-2-one (***7a***): White solid, mp: 66–67 °C; ^{1}H NMR ($CDCl_3$): δ 1.58 (s, 6H, $2-CH_3-$, (farnesyl)), 1.66 (s, 3H, CH_3-, (farnesyl)), 1.76 (s, 3H, CH_3-, (farnesyl)), 1.99–2.16 (m, 8H, $-4CH_2-$, (farnesyl)), 4.61 (d, 2H, J = 6.50 Hz, ($-OCH_2$), (farnesyl)), 5.03–5.08 (m, 2H, =CH– (farnesyl)), 5.48–5.50 (t, 1H, J = 5.50 Hz, =CH– (farnesyl)), 6.82 (s,

1H, H-4 (coumarin)), 7.20–7.39 (m, 4H, H-5, H-6, H-7, H-8 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 157.72, 149.46, 143.62, 142.54, 135.56, 131.28, 128.29, 126.40, 124.63, 124.25, 123.47, 119.78, 117.92, 116.18, 113.65, 66.20, 39.65, 39.50, 26.68, 26.10, 25.70, 17.68, 16.82, 16.02. Found: C, 78.62; H, 8.10. $C_{24}H_{30}O_3$ requires: C, 78.69; H, 8.20%.

*4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-2H-chromen-2-one (**7b**)*: White liquid, ^{1}H NMR ($CDCl_3$): δ 1.57 (s, 6H, 2–CH_3–, (farnesyl)), 1.67 (s, 3H, CH_3–, (farnesyl)), 1.74 (s, 3H, CH_3–, (farnesyl)), 1.91–2.13 (m, 8H, –4CH_2–, (farnesyl)), 4.62 (d, 2H, J = 6.50 Hz, (–OCH_2), (farnesyl)), 5.03–5.07 (m, 2H, =CH– (farnesyl)), 5.42–5.45 (t, 1H, J = 5.50 Hz, =CH– (farnesyl)), 5.77 (s, 1H, H-3 (coumarin)), 7.33–7.51 (m, 4H, H-5, H-6, H-7, H-8 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 165.10, 161.22, 155.81, 143.46, 142.25, 135.50, 131.24, 125.71, 124.28, 116.27, 115.35, 113.12, 112.85, 112.38, 101.52, 65.43, 39.64, 39.49, 26.65, 26.10, 25.70, 16.78, 16.75, 16.02. Found: C, 78.59; H, 8.12. $C_{24}H_{30}O_3$ requires: C, 78.69; H, 8.20%.

*5-((E)-3,7-dimethylocta-2,6-dienyloxy)-2H-chromen-2-one (**6c**)*: White liquid; ^{1}H NMR ($CDCl_3$): δ 1.61 (s, 3H, –CH_3–, (geranyl)), 1.69 (s, 3H, CH_3–, (geranyl)), 1.76 (s, 3H, CH_3–, (geranyl)), 2.09–2.17 (m, 4H, –2CH_2–, (geranyl)), 4.62 (d, 2H, J = 6.00 Hz, (–OCH_2), (geranyl)), 5.08–5.11 (m, 1H, =CH– (geranyl)), 5.48–5.51 (m, 1H, =CH– (geranyl)), 6.32 (d, 1H, J = 9.00 Hz, H-3 (coumarin)), 6.72 (d, 1H, J = 7.5 Hz, H-8 (coumarin)), 6.91 (d, 1H, J = 8.00 Hz, H-6 (coumarin)), 7.41–7.44 (t, 1H, J = 8.5 Hz, H-7 (coumarin)), 8.12 (d, 1H, J = 7.00 Hz, H-4 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 161.05, 155.51, 155.18, 142.05, 138.84, 132.24, 131.96, 123.59, 118.66, 114.39, 109.89, 108.98, 106.28, 65.75, 39.49, 26.23, 25.69, 17.72, 16.73. Found: C, 76.45; H, 7.34. $C_{19}H_{22}O_3$ requires: C, 76.51; H, 7.38%.

*5-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-2H-chromen-2-one (**7c**)*: White liquid; ^{1}H NMR ($CDCl_3$): δ 1.61 (s, 3H, –CH_3–, (farnesyl)), 1.61 (s, 3H, CH_3–, (farnesyl)), 1.68 (s, 3H, CH_3–, (farnesyl)), 1.76 (s, 3H, CH_3–, (farnesyl)), 1.96–2.17 (m, 8H, –4CH_2–, (farnesyl)), 4.65 (d, 2H, J = 6.50 Hz, (–OCH_2), (farnesyl)), 5.07–5.13 (m, 2H, =CH– (farnesyl)), 5.49–5.51 (t, 1H, J = 5.50 Hz, =CH– (farnesyl)), 6.32 (s, 1H, J = 9.50 Hz, H-3 (coumarin)), 6.72 (d, 1H, J = 7.5 Hz, H-8 (coumarin)), 6.90 (d, 1H, J = 8.50 Hz, H-6 (coumarin)), 7.40–7.43 (t, 1H, J = 8.00 Hz, H-7 (coumarin)), 8.12 (d, 1H, J = 7.50 Hz, H-4 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 161.03, 155.51, 155.17, 142.03, 138.83, 135.58, 132.25, 131.37, 124.24, 123.47, 118.69, 114.37, 109.86, 108.96, 106.25, 65.74, 39.69, 39.49, 26.70, 26.12, 25.70, 25.51, 17.69, 16.74, 16.06. Found: C, 78.60; H, 8.12. $C_{24}H_{30}O_3$ requires: C, 78.69; H, 8.20%.

*6-((E)-3,7-dimethylocta-2,6-dienyloxy)-2H-chromen-2-one (**6d**)*: White solid, mp: 96–97 °C; ^{1}H NMR ($CDCl_3$): δ 1.60 (s, 3H, –CH_3–, (geranyl)), 1.66 (s, 3H, CH_3–, (geranyl)), 1.74 (s, 3H, CH_3–, (geranyl)), 2.07–2.15 (m, 4H, –2CH_2–, (geranyl)), 4.56 (d, 2H, J = 6.50 Hz, (–OCH_2), (geranyl)), 5.06–5.09 (m, 1H, =CH– (geranyl)), 5.46–5.49 (m, 1H, =CH– (geranyl)), 6.41 (d, 1H, J = 9.50 Hz, H-3 (coumarin)), 6.93 (d, 1H, J = 3.00 Hz, H-5 (coumarin)), 7.1–7.12 (dd, 1H, J_1 = 9.00, J_2 = 2.5 Hz, H-7 (coumarin)), 7.24 (d, 1H, J = 9.00 Hz, H-8 (coumarin)), 7.65 (d, 1H, J = 9.50 Hz, H-4 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 161.05, 155.32, 148.36, 143.31, 141.89, 131.89, 123.65, 120.15, 119.18, 118.90, 117.77, 116.94, 111.07, 65.55, 39.50, 32.43, 26.24, 25.68, 17.70, 16.71. Found: C, 76.44; H, 7.32. $C_{19}H_{22}O_3$ requires: C, 76.51; H, 7.38%.

*6-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-2H-chromen-2-one (**7d**)*: White solid, mp: 90–92 °C; ^{1}H NMR ($CDCl_3$): δ 1.59 (s, 3H, –CH_3–, (farnesyl)), 1.60 (s, 3H, CH_3–, (farnesyl)), 1.67 (s, 3H, CH_3–, (farnesyl)), 1.75 (s, 3H, CH_3–, (farnesyl)), 1.95–2.17 (m, 8H, –4CH_2–, (farnesyl)), 4.56 (d, 2H, J = 6.50 Hz, (–OCH_2), (farnesyl)), 5.06–5.11 (m, 2H, =CH– (farnesyl)), 5.47–5.50 (t, 1H, J = 6.00 Hz, =CH– (farnesyl)), 6.41 (s, 1H, J = 9.50 Hz, H-3 (coumarin)), 6.93 (d, 1H, J = 3.00 Hz, H-5 (coumarin)), 7.10–7.13 (dd, 1H, J_1 = 8.50, J_2 = 3.00 Hz, H-7 (coumarin)), 7.25 (d, 1H, J = 9.00 Hz, H-8 (coumarin)), 7.65 (d, 1H, J = 9.50 Hz, H-4 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 161.04, 155.33, 148.37, 143.29, 141.91, 135.53, 131.34, 124.26, 123.52, 120.13, 119.13, 118.92, 117.79, 116.96, 111.04, 65.54, 39.68, 39.52, 26.69, 26.14, 25.70, 17.69, 16.73, 16.03. Found: C, 78.63; H, 8.14. $C_{24}H_{30}O_3$ requires: C, 78.69; H, 8.20%.

*8-(3-Methylbut-2-enyloxy)-2H-chromen-2-one (**5f**)*: White solid, mp: 61–62 °C; ^{1}H NMR ($CDCl_3$): δ 1.73 (s, 3H, –CH_3–, (prenyl)), 1.76 (s, 3H, CH_3–, (prenyl)), 4.64 (d, 2H, J = 7.00 Hz, (–OCH_2), (prenyl)), 5.48–5.51 (m, 1H, =CH– (prenyl)), 6.41 (d, 1H, J = 9.50 Hz, H-3 (coumarin)), 6.93 (d, 1H, J = 3.00 Hz, H-5 (coumarin)), 7.102–7.126 (dd, 1H, J_1 = 9.00, J_2 = 2.5 Hz, H-7 (coumarin)), 7.24 (d, 1H, J = 9.00 Hz, H-8 (coumarin)), 7.65 (d, 1H, J = 9.50 Hz, H-4 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 160.39, 146.43, 144.10, 143.72, 138.43, 124.22, 119.56, 119.31, 119.28, 116.75, 115.51, 66.17, 25.77, 18.26. Found: C, 72.94; H, 6.00. $C_{14}H_{14}O_3$ requires: C, 73.04; H, 6.08%.

*8-((E)-3,7-dimethylocta-2,6-dienyloxy)-2H-chromen-2-one (**6f**)*: white liquid; ^{1}H NMR ($CDCl_3$): δ 1.58 (s, 3H, –CH_3–, (geranyl)), 1.64 (s, 3H, CH_3–, (geranyl)), 1.73 (s, 3H, CH_3–, (geranyl)), 2.04–2.12 (m, 4H, –2CH_2–, (geranyl)), 4.68 (d, 2H, J = 6.50 Hz, (–OCH_2), (geranyl)), 5.04–5.07 (m, 1H, =CH– (geranyl)), 5.49–5.52 (m, 1H, =CH– (geranyl)), 6.40 (d, 1H, J = 9.50 Hz, H-3 (coumarin)), 7.02–7.04 (dd, 1H, J_1 = 3.00, J_2 = 1.5 Hz, =CH– H-7 (coumarin)), 7.05–7.65 (dd, 1H, J_1 = 8.00, J_2 = 1.5 Hz, H-5 (coumarin)), 7.14–7.16 (m, 1H, H-6 (coumarin)), 7.67 (d, 1H, J = 9.50 Hz, H-4 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 160.40, 146.41, 144.13, 143.71, 141.71, 131.81, 124.21, 123.72, 120.04, 119.56, 119.32, 119.10, 116.76, 115.61, 66.27, 39.48, 32.46, 26.22, 25.76, 17.69, 16.73. Found: C, 76.40; H, 7.30. $C_{19}H_{22}O_3$ requires: C, 76.51; H, 7.38%.

*8-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-2H-chromen-2-one (**7f**)*: white liquid; ^{1}H NMR ($CDCl_3$): δ 1.59 (s, 3H, –CH_3–, (farnesyl)), 1.60 (s, 3H, CH_3–, (farnesyl)), 1.67 (s, 3H, CH_3–, (farnesyl)), 1.75 (s, 3H, CH_3–, (farnesyl)), 1.95–2.15 (m, 8H, –4CH_2–, (farnesyl)), 4.71 (d, 2H, J = 6.50 Hz, (–OCH_2), (farnesyl)), 5.06–5.11 (m, 2H, =CH– (farnesyl)), 5.52–5.55 (m, 1H, =CH– (farnesyl)), 6.42 (s, 1H, J = 9.50 Hz, H-3 (coumarin)), 7.04–7.05 (dd, 1H, J_1 = 8.00, J_2 = 1.5 Hz, H-7 (coumarin)), 7.07–7.09 (dd, 1H, J_1 = 8.50, J_2 = 15.00 Hz, H-5 (coumarin)), 7.16–7.19 (m, 1H, H-6 (coumarin)), 7.68 (d, 1H, J = 9.50 Hz, H-4 (coumarin)). ^{13}C NMR ($CDCl_3$) δ 160.38, 146.47, 144.19, 143.65, 141.61, 135.47, 131.29, 124.32, 124.19, 123.61, 119.58, 119.58, 119.29, 119.08, 116.83, 115.62, 66.29, 39.67, 39.51, 26.70, 26.19, 25.70, 17.69, 16.77, 16.03. Found: C, 78.59; H, 8.10. $C_{24}H_{30}O_3$ requires: C, 78.69; H, 8.20%.

Acknowledgments

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.09.006.

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