DR. HAMID SADEGHIAN (Orcid ID : 0000-0003-1571-5458)

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O-prenylated carbostyrils as a novel class of 15-lipoxygenase inhibitors: synthesis, characterization and inhibitory assessment

Seyed Jamal Alavi [a*], Amir Zebarjadi [a], Mahdi Hosseni Bafghi [a], Hossein Orafai [b], Hamid Sadeghian [a,c*]

[*] Corresponding author Sadeghianh@mums.ac.ir

[*] Postal Address: Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad 91857-63788, Iran

[a] Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran

[b] Department of Pharmaceutics, Faculty of Pharmacy, University of Al-Zahraa for Women, Karbala, Iraq

[c] Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

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Abstract

Catalyzed peroxidation of unsaturated lipid in animals and plants intimately is linked to the activity of 15-Lipoxygenase enzymes. Lipoxygenases (LOXs) are well known to play an important role in many acute and chronic syndromes such as inflammation, asthma, cancer, allergy etc. In this study, a series of mono prenyloxycarbostyrils were synthesized and evaluated as potential inhibitors of soybean 15-Lipoxygenase (SLO) and their inhibitory potencies were compared to mono prenyloxycoumarins which had been reported in the previous works. The synthetic compounds inhibit lipoxygenase enzyme by competitive mechanism like the prenyloxy coumarins. The results showed that position and length of the prenyl moiety play the important role in lipoxygenase inhibitory activity. Amongst all of the synthetic compounds (carbostyril and coumarin derivatives), 6-farnesyloxycoumarin and 8-farnesyloxycarbostyril demonstrated the best inhibitory activity by IC_{50} values of 2.1 μM and 0.53 μM respectively.

***Keywords*:** 15-LOX, Carbostyril, Coumarin, Farnesyl, Geranyl.

Introduction

Lipoxygenases (LOX) are one of the main types of enzyme involved in regio- and stereospecific oxidation pathways of polyunsaturated fatty acids [1]. Lipoxygenase is the common name of iron-containing enzymes that play a key role in catalyzing polyunsaturated fatty acids conversion to hydroyperoxyeicosatetraenoic acids (HPETEs), hydroxyeicosatetraenoic acids (HETEs) and subsequently leukotrienes (LTs), which are the well-known mediators of hypersensitivity and inflammatory reactions [2, 3].

Linoleic acid (LA) is the most common substrate for plant LOXs while arachidonic acid (AA) is considered as the main substrate of mammalian LOXs. Moreover, peroxidation of LA, as a preferred substrate, by some of mammalian LOXs has been identified.

There are five different types of mammalian LOXs based on the insertion site of molecular oxygen into AA: 5-LOX (EC 1.13.11.34), 8-LOX (EC 1.13.11.40), 11-LOX, (EC 1.13.11.45), 12-LOX (EC 1.13.11.31), and 15-LOX (EC 1.13.11.33) [1].

Up to now, two kinds of mammalian 15-lipoxygenases have been identified: 15-LOX-1 and 15-LOX-2. LA is the main substrate of 15-LOX-1 that is metabolized to 13(S)-hydroxy-9Z,11E-octadecadienoic acid (13-S-HODE) while the preferred substrate of 15-LOX-2 is AA oxidized to 15(S)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15-S-HETE) [4].

Lipoxygenases by formation of biologically active compounds such as leukotrienes, lipoxins, and eoxins can interfere in signaling pathways of lipids and finally regulate cell metabolism and apoptosis [5].

It has been found that overproduction of the lipoxygenases metabolites causes many acute and chronic diseases such as asthma, allergy, cancer, stroke, ischemia and/or post-ischemic inflammatory response and arthritis [5, 6]. Due to lipoxygenase importance as therapeutic targets, there is much interest for designing and discovering of novel and potent lipoxygenase inhibitors.

Three well-known strategies have been established to inhibit the lipoxygenase activity: (i) redox inhibitors or antioxidants that interfere with the redox cycle of hydroperoxidation, (ii) iron-chelator agents that directly form a complex with iron core of the enzyme, and (iii) non-redox competitive inhibitors that only compete with the substrate for occupying the active pocket of the enzyme [7]. It has been shown that the radical scavenging property of the redox inhibitors is the main factor for lipoxygenase inhibition. They can react with the free radical form of the

polyunsaturated fatty acid after the hydrogen abstraction step of the hydroperoxidation cycle, and cause enzyme deactivation [8].

Coumarin is a heterocyclic molecule containing lactone fused benzene skeleton and some of its derivatives have been introduced as lipoxygenase inhibitors [9-11].

Several natural and synthetic derivatives of hydroxycoumarins have been reported as inhibitors of the lipoxygenase pathways [12- 14]. Most of them inhibit the lipoxygenase activity using redox mechanism via their hydroxyl groups.

Recently, soybean 15-lipoxygenase (Soybean 13-S-Lipoxygenase-1) and human 15-lipoxygenase-1 (15-hLOX-1) inhibitory activities of mono *O*-prenyloxycoumarins (isopentenyloxy, -geranyloxy and -farnesyloxy derivatives) was reported. The structure activity relationship (SAR) studies showed that both intermolecular interactions of coumarin ring (hydrogen bond with Fe-OH core and π-π*/π-δ* between phenyl moiety and active site amino acids) and prenyl substitution site play important roles in lipoxygenase inhibitory potency [9]. It was also found that prenyl moiety with more than 10 carbons (farnesyl and geranyl) had significantly higher lipoxygenase inhibitory potency.

Herein, we tried to study lipoxygenase inhibitory activity of mono *O*-prenyloxycarbostyril. Carbostyrils (2-quinolone), which consist of fused benzene and 2-pyridone rings, are another important group of heterocyclic compounds that are isoelectronic and homolog with coumarins. Several natural and synthetic derivatives of carbostyril have been introduced as antimicrobial, anti-inflammatory, anticancer, antiviral, antithrombotic and antiplatelet agents [15-17].

In the following of our previous studies on lipoxygenase inhibitors, a series of *O*-prenyloxycarbostyril were synthesized and their lipoxygenase inhibitory activity were determined and compared to *O*-prenyloxycoumarins.

Results

The desired prenyloxycarbostyril derivatives were synthesized by condensation of hydroxycarbostyril with the desired pernyl bromide. Starting reagents 6, 7 and 8-hydroxycarbostyril (**10-12**) were synthesized from the related hydroxyquinolines according to previous literatures (Figure 1) [18]. They were produced via the following synthetic steps: (1) *N*-oxidation of hydroxyl quinoline derivatives in present of hydrogen peroxide and acetic acid by under reflux condition; (2) Thermal rearrangement (75 ^{o}C) of prepared N-oxides **4-6** to the related

acetoxycarbostyrils **7-9**; (3) Hydrolysing of *O*-acetyl moiety of compound **7-9** and formation of products **10-12** (Figure 1) [18].

Figure 1

5-hydroxycarbostyril (**14**) was prepared by alkali fusion of 5-hydroxyqunoline at 300 °C (Figure 1) [19]. *O*-prenylation of hydroxycarbostyril with the desired pernyl bromide in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) afforded the corresponded products: **5-8g** and **5-8f** (Figure 2) [20].

Figure 2

The *O*-prenyloxycoumarins were synthesized by reaction of the desired hydroxycoumarins with geranyl and farnesyl bromide in the mixture of potassium carbonate and acetone (Figure 3) [9].

Figure 3

The lipoxygenase inhibitory activities of the *O*-ptrenylated carbostyrils and coumarins was assessed against Soybean 13-S-Lipoxygenase-1 (SLO; type I-B; EC 1,13,11,12) using the modified oxidative reaction of 3-methyl-2-benzothiazolinone (MBTH) with 3-(dimethylamino) benzoic acid (DMAB), which had been reported by Iranshahi et al. [9]. All the evaluations were compared to 4-MMPB (4-methyl-2-(4-methylpiperazinyl)pyrimido[4,5-b]benzothiazine), as a well-known 15-lipoxygenase inhibitor.

Like prenyloxycoumarins, farnesyloxy analogs of carbostyril were more potent than geranyloxy analogs in the lipoxygenase inhibition. Among the carbostyril derivatives, **8f** was the most potent inhibitor by IC_{50} value of 0.53 μM. By changing the position of farnesyloxy group on carbostyril scaffold, decreasing in lipoxygenase inhibition potency was observed for the other analoges as follow: **6f** > **7f** > **5f** by IC_{50} values of 2.13, 4.20 and 7.15 μM respectively (Table 1). Such a variation in lipoxygenase inhibition was also seen for geranyloxycarbostyrils: **8g** > **6g** > **7g** > **5g** by IC_{50} values of 7.31, 11.92, 15.34 and 17.73 μM respectively (Table 1).

Table 1

Complementary studies on inhibitory mechanism showed that the synthetic inhibitors reduced lipoxygenase activity by competitive mechanism (Figure 4). None of the compounds showed DPPH (2,2-diphenyl-1-picrylhydrazyl) bleaching activity at 100 μM.

Figure 4

Discussion

As seen in Table 1, for both carbostyril and coumarin scaffolds', increasing of the prenyl length in a same substitution site has led to increase of the enzyme inhibition ability.

Replacement of carbostyril to coumarin led to significant effect on lipoxygenase inhibition for 5- and 8- substitutions. Compared to the prenyloxycoumarines, insertion of geranyloxy at position 5 of carbostyril, decreased the lipoxygenase inhibitory potency by 4 folds and it extended to 7 folds for farnesyloxy at the same position. About position 8, no considerable differences in the inhibition potency was seen for geranyloxy substituent while it raised around 16 folds for 8-farnesyloxy carbostyril (**8f**) in comparison with its homolog **8f'** (IC_{50} **8f** = 0.53; IC_{50} **8f'** = 8.13 μM).

It is interesting that, replacing of lactone portion by lactam, has led to changing of the inhibitory potencies variation order. For example for farnesyloxy derivatives the inhibitory potencies order is: **8f** > **6f** > **7f** > **5f** compare to **5'f > 6'f > 7'f > 8'f**. As seen in Table 1, 8-prenyloxy derivatives of carbostyril are the best inhibitors while 5-prenyloxy coumarins are the best inhibitors among their isomers.

None of the compounds bleached the DPPH solution. It implies that they don't have radical scavenging activity. By considering the competitive inhibition mechanism of the prenyloxycarbostyrils, it concluded that these compounds belong to the third group of the lipoxygenase inhibitors (non-redox competitive inhibitors).

Conclusion

In summary, mono prenyloxy derivatives of both carbostyril and coumarin were synthesized and their inhibitory potency against soybean 15-LOX was evaluated. The results showed that prenyl moiety length and prenylation position play the important role in lipoxygenase inhibitory activity. It is notable that the synthetic pernyloxycarbostyril like pernyloxycoumarin inhibits lipoxygenase enzyme by non-redox competitive mechanism. Amongst all of the synthetic compounds (coumarin and carbostyril derivatives), 8-farnesyloxycarbostyril and 5-farnesyloxycumarin showed the best lipoxygenase inhibition activity, respectively.

Materials and methods

Instruments

^{1}H NMR (300 MHz) spectra were obtained by using a Bruker Avance DRX-300 Fourier transformer spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS). The mass spectra were scanned on a Varian Mat CH-7 instrument at 70 eV. Elemental analysis was obtained on a Thermo Finnigan Flash EA microanalyzer. The melting points were recorded on an Electrothermal type 9100 melting point apparatus. All measurements of DPPH bleaching and lipoxygenase activities were carried out using Spekol 1500 spectrophotometer and BioTek ELX800 plate reader. All chemicals were purchased from Sigma, Aldrich and Merck Co.

Lipoxygenase Inhibitory Assessment [11]

Two assay solutions (A and B), enzyme and linoleic acid were firstly provided as follow:

Solution A: DMAB (3-dimethylaminobenzoic acid) 50 mM in phosphate buffer (100 mM, PH 7.0).

Solution B: A mixture of MBTH (3-methyl-2-benzothiazolonhydrazone) (10 mM, 3mL) and hemoglobin (5 mg/mL, 3 mL) in 25 mL phosphate buffer (50 mM, PH 5.0).

The enzyme solution was prepared by dissolving 1 mg lyophilized SLO (L1; Type I-B; EC 1, 13, 11, 12; Sigma Co.) in 5 ml phosphate buffer (50 mM, PH 7.5) and then its activity was checked by diene formation method at 234 nm. Finally, its activity was decreased to desired quantity by dilution with the phosphate buffer.

Linoleic acid (LA) solution: A mixture of LA (5.6 mg), ethanol (0.5 mL) and KOH (100 mM) to a final volume of 5 ml.

Assay procedure: the sample solution in ethanol (25 μL), enzyme solution (4000 units/ml: based on Abs increase at 234 nm per min; 25 μL) and phosphate buffer (PH 7.5, 50 mM; 900 μL), were mixed in test-plate and pre-incubated for 5 min at room temperature. Control test was done by 25 μL of pure ethanol. After pre-incubation, LA solution (50 μL) was added (for starting of the enzymetic reaction) and after 10 min, solution A (270 μL) and subsequently solution B (130 μL) were added for color formation. 3 min later, 200 μL of sodium dodecyl sulfate solution (2% W/V) was added to terminate the reaction. The absorbance was recorded at 598 nm. Each experiment

was performed in triplicate. Calculation of IC_{50} values and the significant analysis between them (one-way ANOVA multiple comparisons) was done in GraphPad Prism 6.0.

Inhibitory mechanism

25 µL of the enzyme solution (4000 unit/ml) was added to mixture of phosphate buffer (pH 7.5; 900 µL) and inhibitor (25 µL in ethanol) and incubated for 10 min at room temperature. Subsequently, 50 µL of LA stock solution was added to the mixture (final concentration: 10, 20, 40, 80 and 160 µM) to start the reaction. After 10 min, solution A (270 µL) and then solution B (130 µL) were added to initiate chromogenic reaction (preparation of solutions A and B described in the previous section). 3 min later, 200 µL of a 2% SDS solution was added to terminate the reaction. The absorbance was recorded at 598 nm compared to the control. These experiments were performed in triplicate. Using the reaction rates ($Abs.min^{-1}$), both inhibitor and linoleic acid concentrations (µM), Michaelis-Menten and Lineweaver-Burk plots were graphed in GraphPad Prism 6.0.

Determination of DPPH bleaching activity

An Ethyl alcohol solution of DPPH (50 µM) was prepared. The prepared solution was equally added to 1 mL of the test compounds solutions (solvent: ethyl alcohol). Equal mixture of pure ethyl alcohol and DPPH solution was used as control solution. After 30 min (25 ^{o}C), the absorbance was recorded at 517 nm.

Preparation of 5-Hydroxycarbostyril (14)

5-hydroxycarbostyril was prepared by alkali fusion technique as previously reported [17]. Briefly, 5-hydroxyquinoline (3.0 g) and potassium hydroxide (20 g) were placed in a stainless-steel beaker with a capacity of 50 ml, and then heated to 300 °C. The resulting mixture (frothing black) was heated for 3 h, when a clear black solution was obtained. After the mixture was cooled to room temperature, water (150 ml) and excess concentrated hydrochloric acid was added to mixture, respectively.

The solid formed (tan-color) was filtered and washed with cold water. The crud product was recrystallized from methanol-water to give a pure product as straw-colored needles melting at 338-340 ^{o}C.

General procedure for preparation of carbostyril N-oxide (4-6)

To a solution of desired hydroxyquinoline (**1-3**) (70 mmol) in acetic acid (100 mL) was added. Hydrogen peroxide (30%, 15 mL) and the resulting mixture were stirred at 75°C for 36 h. After evaporated the solvent under vacuum, the mixture of water and crushed-ice was added and the residue was basified with aqueous ammonia. The crud solid was collected and recrystallized with proper solvent to obtain the pure crystal.

General procedure for preparation of acetoxycarbostyril (7-9)

A mixture of quinolone N-oxide (30 mmol) in Ac_2O (30 mL) was refluxed for 18 h (monitored by TLC). After cooling, the mixture was poured into ice water (100 mL) and neutralized with aqueous ammonia. Then, reaction mixture was extracted with dichloromethane (3×60 mL). The organic extracts were combined and washed with water, dried, and then evaporated to give a brown solid which was crystallized from ethyl acetate [19].

6-Acetoxy-2-hydroxyquinoline (7)

Yellow powder, mp 200-203 °C (Lit. mp 199-200 °C) [22]

7-Acetoxy-2-hydroxyquinoline (8)

Brown powder, mp 247-250 °C (Lit. mp 247-2248 °C) [21]

8-Acetoxy-2-hydroxyquinoline (9)

mp 248-250 °C (Lit. 252-254 °C) [18]

General procedure for preparation of hydroxycarbostyril (10-12)

A stirred suspension of acetoxycarbostyril (25 mmol) in hydrochloric acid (37%, 50 mL) was refluxed for 4 h. After completion the reaction (monitored by TLC), the mixture was poured into ice water (50 mL). The resulting precipitate was filtered off to hydroxyl carbostyril as a brown solid. The crud precipitate crystallized from methanol to give the pure solid [21].

6-Hydroxycarbostyril (10)

White powder, mp 300-305 °C (Lit. White powder, mp 299-300 °C) [22]

7-Hydroxycarbostyril (11)

Brown powder, mp 250-252 °C (Lit. Brown powder, mp 251-252 °C) [21]

8-Hydroxycarbostyril (12)

Brown powder, mp 292-295 °C (Lit. Brown powder, mp 297-299 °C) [18]

General procedure for preparation of *O*-prenyloxycarbostyril (5-8 (g, f))

To a mixture of hydroxycarbostyrils **10-12** (1.5 mmol), DBU (2.3 mmol) in *iso*-propanol (15 mL), prenyl bromide (155 mmol) was added and the mixture refluxed for 6. After cooling, the mixture was diluted with water (10 mL) and then extracted with chloroform (2×15 mL).

The combined extracts were washed with HCl 1 N (2×10 mL), sodium bicarbonate solution (5%, 2×10 mL) and water (2×10 mL), respectively. The solvent was removed under reduced pressure and the crud products were purified by crystallization from hexane or by column chromatography (*n*-hexan: EtOAc).

5-((E)-3,7-dimethylocta-2,6-dienyloxy)quinolin-2(1H)-one (5g)

White solid, , mp 95-97 °C; ^{1}H NMR (301 MHz, Chloroform-*d*) δ 12.25 (s, 1H), 7.64 (d, *J* = 9.5 Hz, 1H), 7.35 (d, *J* = 8.8 Hz, 1H), 6.92 (d, *J* = 2.6 Hz, 1H), 6.72 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.55 (d, *J* = 9.4 Hz, 1H), 5.40 (t, *J* = 6.6 Hz, 1H), 5.05- 4.99 (m, 1H), 4.52 (d, *J* = 6.6 Hz, 2H), 2.04 (m, 4H), 1.70 (s, 3H), 1.61 (s, 3H), 1.52 (s, 3H). ^{13}C NMR (76 MHz, Chloroform-d) δ 164.32, 154.52, 141.68, 140.62, 134.02, 131.90, 123.70, 121.60, 121.05, 120.68, 119.10, 117.52, 110.01, 65.21, 39.75, 26.30, 25.80, 17.03, 16.41. MS (EI, 70 eV): m/z= 297 [M^+]; Anal. calcd for $C_{19}H_{23}NO_2$: C, 78.73; H, 7.80; N, 4.71. Found: C, 78.70; H, 7.79; N, 4.70.

5-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)quinolin-2(1H)-one (5f)

Yellowish solid, ^{1}H NMR (301 MHz, Chloroform-*d*) δ 12.35 (s, 1H), 7.66 (d, *J* = 9.4 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 6.81 (s, 1H), 6.70 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.48 (d, *J* = 9.4 Hz, 1H), 5.40 (t, *J* = 6.3 Hz, 1H), 5.12 – 5.01 (m, 2H), 4.55 (d, *J* = 6.6 Hz, 2H), 2.12 – 1.94 (m, 8H), 1.70 (s, 3H), 1.64 (s, 3H), 1.55 (s, 6H). ^{13}C NMR (76 MHz, Chloroform-*d*) δ 165.11, 160.95, 141.98, 141.01, 140.45, 135.65, 131.41, 129.05, 124.34, 123.63, 118.52, 118.129, 114.46, 113.06, 98.83, 65.24, 39.81, 27.04, 26.43, 25.72, 17.81, 16.82, 16.16. MS (EI, 70 eV): m/z= 365 [M^+]; Anal. calcd for $C_{24}H_{31}NO_2$: C, 78.86; H, 8.55; N, 3.83. Found: C, 78.78; H, 8.52; N, 3.80.

6-((E)-3,7-dimethylocta-2,6-dienyloxy)quinolin-2(1H)-one (6g)

Yellowish solid, mp 50-52 °C; ^{1}H NMR (301 MHz, Chloroform-*d*) δ 12.45 (s, 1H), 7.68 (d, *J* = 9.5 Hz, 1H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.10 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.93 (d, *J* = 2.6 Hz, 1H), 6.65 (d, *J* = 9.5 Hz, 1H), 5.43 (t, *J* = 6.1 Hz, 1H), 5.09 – 4.97 (m, 1H), 4.50 (d, *J* = 6.5 Hz, 2H), 2.04 (m, 4H), 1.69 (s, 3H), 1.60 (s, 3H), 1.53 (s, 3H). ^{13}C NMR (76 MHz, Chloroform-d) δ 164.12, 154.48, 141.64, 140.59, 133.06, 131.89, 123.73, 121.63, 120.90, 120.48, 119.19, 117.43, 109.98, 65.44, 39.56, 26.31, 25.69, 17.73, 16.74. MS (EI, 70 eV): m/z= 297 [M^+]; Anal. calcd for $C_{19}H_{23}NO_2$: C, 78.73; H, 7.80; N, 4.71. Found: C, 78.78; H, 7.80; N, 4.72.

6-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)quinolin-2(1H)-one (6f)

Yellowish solid, mp 75-77 °C; ^{1}H NMR (301 MHz, DMSO-d_6) δ 11.65 (s, 1H), 7.83 (d, *J* = 9.6 Hz, 1H), 7.26 (d, *J* = 2.5 Hz, 1H), 7.21 (d, *J* = 2.6 Hz, 1H), 7.14 (dd, *J* = 8.9, 2.7 Hz, 1H), 6.50 (d, *J* = 9.5 Hz, 1H), 5.45 (t, *J* = 6.0 Hz, 1H), 5.13 – 5.00 (m, 2H), 4.57 (d, *J* = 6.4 Hz, 2H), 2.12 – 1.89 (m, 8H), 1.72 (s, 3H), 1.63 (s, 3H), 1.56 (s, 3H), 1.55 (s, 3H). ^{13}C NMR (76 MHz, DMSO-d_6) δ 161.97, 153.66, 140.62, 140.21, 135.12, 133.77, 131.07, 124.54, 124.01, 122.71, 120.46, 120.20, 120.10, 116.75, 110.81, 65.21, 26.64, 26.13, 25.93, 17.98, 16.83, 16.26. MS (EI, 70 eV): m/z= 365 [M^+]; Anal. calcd for $C_{24}H_{31}NO_2$: C, 78.86; H, 8.55; N, 3.83. Found: C, 78.85; H, 8.54; N, 3.82.

7-((E)-3,7-dimethylocta-2,6-dienyloxy)quinolin-2(1H)-one (7g)

White solid, mp 113-116 °C; ^{1}H NMR (301 MHz, Chloroform-*d*) δ 12.51 (s, 1H), 7.65 (d, *J* = 9.4 Hz, 1H), 7.36 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 2.2 Hz, 1H), 6.75 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.47 (d, *J* = 9.4 Hz, 1H), 5.42 (t, *J* = 6.8 Hz, 1H), 5.03 (q, *J* = 7.7, 6.5 Hz, 1H), 4.56 (d, *J* = 6.7 Hz, 2H), 2.05 (d, *J* = 5.3 Hz, 4H), 1.71 (s, 3H), 1.59 (s, 3H), 1.53 (s, 3H). ^{13}C NMR (76 MHz, Chloroform-d) δ 165.11, 161.24, 142.20, 140.82, 140.43, 131.86, 128.93, 123.75, 118.74, 117.86, 114.17, 113.01, 99.18, 65.29, 39.60, 26.30, 25.68, 17.73, 16.80. MS (EI, 70 eV): m/z= 297 [M^+]; Anal. calcd for $C_{19}H_{23}NO_2$: C, 78.73; H, 7.80; N, 4.71. Found: C, 78.73; H, 7.85; N, 4.72.

7-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)quinolin-2(1H)-one (7f)

White solid, mp 88-90 °C; ^{1}H NMR (301 MHz, Chloroform-*d*) δ 12.34 (s, 1H), 7.65 (d, *J* = 9.4 Hz, 1H), 7.36 (d, *J* = 8.7 Hz, 1H), 6.79 (s, 1H), 6.75 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.47 (d, *J* = 9.4 Hz, 1H), 5.42 (t, *J* = 6.3 Hz, 1H), 5.08 – 4.96 (m, 2H), 4.56 (d, *J* = 6.6 Hz, 2H), 2.10 – 1.88 (m, 8H), 1.72 (s, 3H), 1.60 (s, 3H), 1.53 (s, 6H). ^{13}C NMR (76 MHz, Chloroform-*d*) δ 165.00, 161.25, 142.22,

140.81, 140.39, 135.50, 131.32, 128.95, 124.34, 123.63, 118.74, 117.89, 114.16, 112.96, 99.15, 65.29, 39.69, 39.61, 26.73, 26.23, 25.72, 17.71, 16.82, 16.06. MS (EI, 70 eV): m/z= 365 [M^+]; Anal. calcd for $C_{24}H_{31}NO_2$: C, 78.86; H, 8.55; N, 3.83. Found: C, 78.85; H, 8.54; N, 3.82.

8-((E)-3,7-dimethylocta-2,6-dienyloxy)quinolin-2(1H)-one (8g)

Yellowish solid, mp 72-74 °C; ^{1}H NMR (301 MHz, Chloroform-*d*) δ 9.16 (s, 1H), 7.65 (d, *J* = 9.6 Hz, 1H), 7.05 (m, 2H), 6.91 (dd, *J* = 7.0, 2.1 Hz, 1H), 6.59 (d, *J* = 9.6 Hz, 1H), 5.45 (d, *J* = 6.5 Hz, 1H), 5.07 – 4.97 (m, 1H), 4.59 (d, *J* = 6.7 Hz, 2H), 2.06 (d, *J* = 5.2 Hz, 4H), 1.68 (s, 3H), 1.61 (s, 3H), 1.56 (s, 3H). ^{13}C NMR (76 MHz, Chloroform-d) δ 164.32, 159.12, 141.21, 140.74, 139.41, 130.86, 129.03, 123.55, 119.74, 117.95, 114.25, 113.22, 99.18, 65.29, 39.65, 26.30, 25.65, 17.70, 16.75. MS (EI, 70 eV): m/z= 297 [M^+]; Anal. calcd for $C_{19}H_{23}NO_2$: C, 78.73; H, 7.80; N, 4.71. Found: C, 78.69; H, 7.80; N, 4.70.

8-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)quinolin-2(1H)-one (8f)

colorless liquid, mp <30 °C; ^{1}H NMR (301 MHz, Chloroform-*d*) δ 9.26 (s, 1H), 7.75 (d, *J* = 9.6 Hz, 1H), 7.19 – 7.11 (m, 2H), 7.00 (dd, *J* = 7.0, 2.0 Hz, 1H), 6.68 (d, *J* = 9.5 Hz, 1H), 5.54 (t, *J* = 6.4 Hz, 1H), 5.19 – 5.08 (m, 2H), 4.69 (d, *J* = 6.7 Hz, 2H), 2.19 – 1.98 (m, 8H), 1.78 (s, 3H), 1.70 (s, 3H), 1.65 (s, 3H), 1.62 (s, 3H). ^{13}C NMR (76 MHz, Chloroform-*d*) δ 164.10, 161.20, 142.22, 140.81, 140.21, 135.52, 131.42, 129.05, 124.45, 123.60, 118.80, 117.89, 114.20, 112.90, 99.10, 65.25, 39.71, 39.63, 26.70, 26.20, 25.82, 17.75, 16.82, 16.16. MS (EI, 70 eV): m/z = 365 [M^+]; Anal. calcd for $C_{24}H_{31}NO_2$: C, 78.86; H, 8.55; N, 3.83. Found: C, 78.85; H, 8.50; N, 3.85.

General procedure for preparation of *O*-prenyloxycoumarin (5'-8' (g, f)):

O-prenyloxycoumarin derivatives were prepared according to previously reported method [9]. Briefly, a mixture of hydroxycoumarins (5 mmol), prenyl bromide (6 mmol) and anhydrous potassium carbonate (5 mmol) in dry acetone (3 mL) was refluxed for 12 h. After cooling the mixture, 10 mL water was added and then extracted with ether (2×20 mL). The combined extracts were washed with sodium hydroxide solution (10%, 2×10 mL) and dried with anhydrous sodium carbonate. The solvent was removed under reduced pressure and the crud products were purified.

Conflict of Interest

The authors declare that they have no conflict of interest.

Supporting Information Available

Copies of ^{1}H NMR,^{13}C NMR and Mass spectra of compounds **5g-8g**, **5f-8f** and significant analysis of the IC_{50} values.

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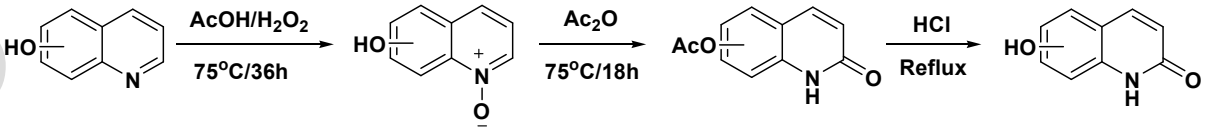
Table 1: Lipoxygenase Inhibitory assessment and DPPH bleaching data of the *O*-prenyloxycarbostyrils and *O*-prenyloxycoumarins in comparison with 4-MMPB (± SD). Significant analysis of the data is accessible in supplementary section. (NE = no effect)

Compound	IC_{50} (µM)	% DPPH bleaching (100 µM)	Compound	IC_{50} (µM)	% DPPH bleaching (100 µM)
5g	17.73 ± 1.81	NE	5'g	3.93 ± 0.92	NE
6g	11.92 ± 1.62	NE	6'g	13.46 ± 1.66	NE
7g	15.34 ± 1.43	NE	7'g	8.86 ± 1.21	NE
8g	7.31 ± 0.73	NE	8'g	11.35 ± 1.41	NE
5f	7.15 ± 0.64	NE	5'f	1.10 ± 0.11	NE
6f	2.13 ± 0.21	NE	6'f	2.93 ± 0.26	NE
7f	4.20 ± 0.17	NE	7'f	4.45 ± 0.71	NE
8f	0.53 ± 0.06	NE	8'f	8.15± 1.13	NE
4-MMPB	18.6 ± 1.44	57.5 ± 3.6			



1-3 4-6 7-9 10-12

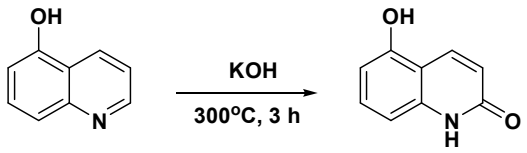


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Figure 1: General procedure for the synthesis of compounds 10-14.

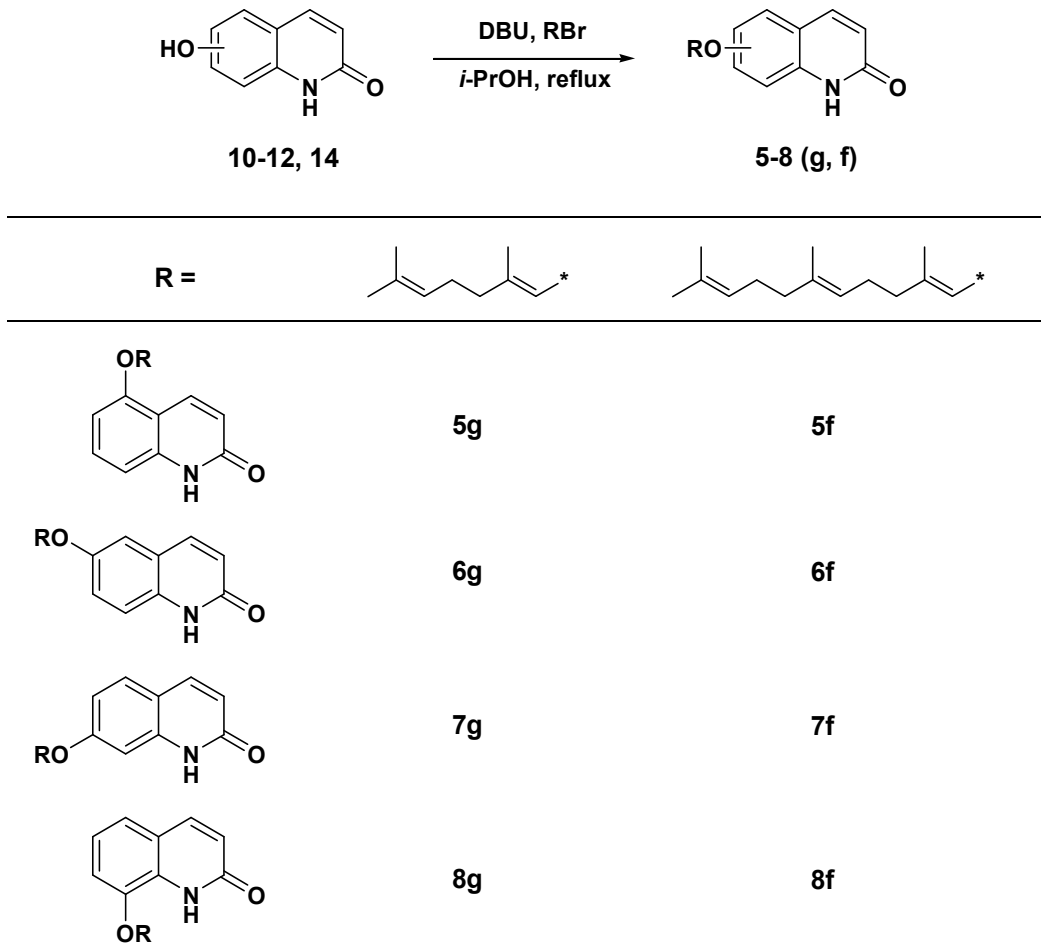

Figure 2: General procedure for the synthesis of compounds 5-8 (g, f). (DBU = 1,8-diazabicyclo[5,4,0]undec-7-ene)

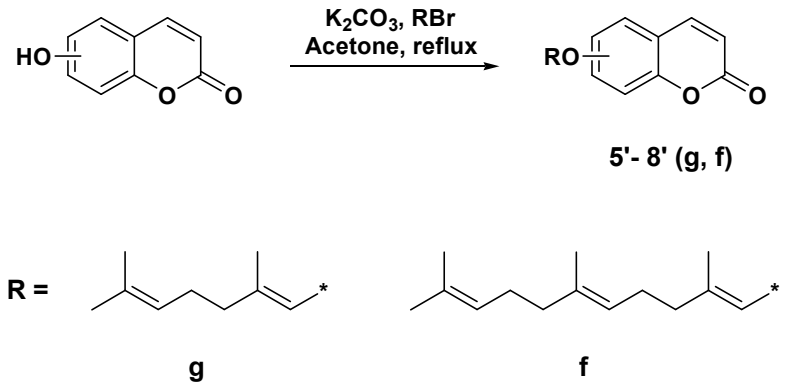

Figure 3: General procedure for the synthesis of *O*-prenyloxycoumarins 5'-8' (g, f).

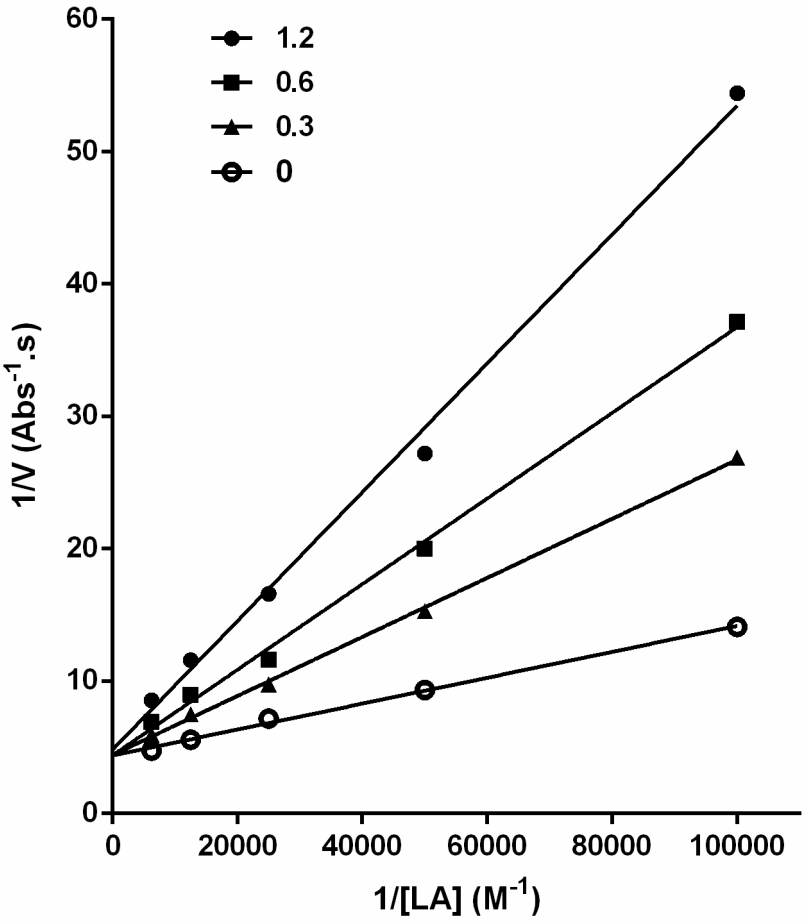

Figure 4: Lineweaver-Burk plot of SLO inhibition by 8f at concentrations 0, 0.3, 0.6 and 1.2 µM (LA: Linoleic acid). K_m = 22.15 ± 1.65 µM and K_i = 0.24 ± 0.018 µM.