Supplementary Information

SYNTHESIS AND EVALUATION OF COUMARIN-RESVERATOL HYBRIDS AS SOYBEAN 15-LIPOXYGENASE INHIBITORS

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Synthesis of 3-arylcoumarins; General procedure

2-Hydroxy-3,4,5-trimethoxybenzaldehyde (**1c**) was synthesized from 2,3,4-trimethoxybenzaldehyde according to the literature [29].

A mixture of salicylaldehyde derivative **1** (0.5 mmol), phenylacetic acid derivative **2** (1 mmol), and DABCO (3 mmol) was heated at 180 °C for the appropriate time (Table 1). After the completion of the reaction (monitored by TLC), it was diluted with ice water and extracted with dichloromethane (3× 15 mL). The organic layer was dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was recrystallized from ethanol to give pure compound. All the products were characterized using ¹H NMR, ¹³C NMR, and CHN analysis.

3-Phenyl-chromen-2-one (3a). Yield: 90, white crystals, mp 138-140 °C. IR (KBr): 1714 (C=O) cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ = 7.32 (t, 1H, *J* = 8.0 Hz, H₆), 7.39 (d, 1H, *J* = 8.0 Hz, H₈), 7.43-7.49 (m, 3H, Ph), 7.54-7.57 (m, 2H, Ph), 7.72 (m, 2H, H₅, H₇), 7.84 (s, 1H, H₄). ¹³C NMR

(125 MHz, CDCl₃): δ = 111.1, 112.0, 116.5, 119.8, 121.2, 124.4, 127.9, 128.9, 131.4, 139.2, 148.5, 153.4, 160.0. Anal. calcd. for C₁₅H₁₀O₂: C, 81.07; H, 4.54. Found: C, 81.21; H, 4.32.

3-(3,4-Dimethoxyphenyl)-2H-chromen-2-one (3b). Yield: 81%, white crystals, mp 133-135 °C. IR (KBr): 1710 (C=O) cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ = 3.94 (s, 3H, OCH₃), 3.96 (s, 3H, OCH₃), 6.95 (d, 1H, J = 8.2 Hz, H_{5'}), 7.29-7.32 (m, 3H, H₆, H₈, H_{2'}), 7.37 (d, 1H, J = 8.2 Hz, H_{6'}), 7.51-7.56 (m, 2H, H₅, H₇), 7.79 (s, 1H, H₄). ¹³C NMR (125 MHz, CDCl₃): δ = 55.8, 55.9, 110.9, 111.7, 116.3, 119.7, 121.2, 124.4, 127.3, 127.7, 127.8, 131.0, 138.7, 148.6, 149.6, 154.12, 159.3. Anal. calcd. for C₁₇H₁₄O₄: C, 72.33; H, 5.00. Found: C, 72.51; H, 4.82.

3-(4-Chlorophenyl)-2H-chromen-2-one (3c). Yield: 88%, white crystals, mp 170-172 °C. IR (KBr): 1712 (C=O) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ = 7.33 (td, J = 8.0, 0.8 Hz, 1H, H₆), 7.39 (d, 1H, J = 8.0 Hz, H₈), 7.44 (d, J = 8.4 Hz, 2H, H_{2'}, H_{6'}), 7.55-7.59 (m, 2H, H₅, H₇), 7.68 (d, J = 8.4 Hz, 2H, H_{3'}, H_{5'}), 7.84 (s, 1H, H₄). ¹³C NMR (100 MHz, CDCl₃): δ = 116.5, 119.5, 124.6, 127.2, 128.0, 128.7, 129.8, 131.7, 133.1, 134.9, 139.9, 153.6, 160.4. Anal. calcd. for C₁₅H₉ClO₂: C, 70.19; H, 3.53. Found: C, 70.28; H, 3.31.

3-(4-Methoxyphenyl)-2H-chromen-2-one (3d). Yield: 85%, white crystals, mp 142-144 °C. IR (KBr): 1718 (C=O) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ = 3.90 (s, 3H, OCH₃), 7.34 (td, J = 8.0, 0.8 Hz, 1H, H₆), 7.40 (d, J = 8.0 Hz, 1H, H₈), 7.46 (d, J = 9.0 Hz, 2H H_{3'}, H_{5'}), 7.54-7.60 (m, 2H, H₅, H₇), 7.65-7.69 (d, J = 9.0 Hz, 2H H_{2'}, H_{6'}), 7.83 (s, 1H, H₄). Anal. calcd. for C₁₆H₁₂O₃: C, 76.18; H, 4.79. Found: C, 76.32; H, 4.61.

3-(4-Nitrophenyl)-2H-chromen-2-one (3e). Yield: 93%, white crystals, mp 252-254 °C. IR (KBr): 1719 (C=O) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ = 7.17 (d, J = 8.4 Hz, 2H, H_{2'}, H_{6'}), 7.32 (td, J = 8.0, 2.0 Hz, 1H, H₆), 7.40 (d, J = 8.0, 1H, H₈), 7.56 (m, 2H, H₅, H₇), 7.81 (s, 1H, H₄),

8.24 (d, $J = 8.4$ Hz, 2H, H_{3'}, H_{5'}). Anal. calcd. for C₁₅H₉NO₄: C, 67.42; H, 3.39; N, 5.24. Found: C, 67.28; H, 3.52; N, 5.41.

3-(2,4-Dichlorophenyl)-2H-chromen-2-one (3f). Yield: 86%, white crystals, mp 187-189 °C. IR (KBr): 1717 (C=O) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.33$ -7.39 (m, 3H, H₆, H₈, H_{6'}), 7.41 (d, $J = 8.4$ Hz, 1H, H_{5'}), 7.53 (d, $J = 2.0$ Hz, 1H, H_{3'}), 7.55-7.58 (dd, $J = 8.0$, 1.6 Hz, 1H, H₅), 7.60-7.62 (td, $J = 8.0$, 1.6 Hz, 1H, H₇), 7.78 (s, 1H, H₄). ¹³C NMR (100 MHz, CDCl₃): $\delta = 116.8$, 118.7, 124.7, 126.0, 127.2, 128.2, 129.8, 132.2, 132.2, 132.2, 134.5, 135.4, 143.0, 154.0, 159.7. Anal. calcd. for C₁₅H₈Cl₂O₂: C, 61.89; H, 2.77. Found: C, 61.68; H, 2.52.

3-(4-Fluorophenyl)-2H-chromen-2-one (3g). Yield: 91%, white crystals, mp 191-193 °C. IR (KBr): 1712 (C=O) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 7.14$ -7.18 (t, $J = 9.0$ Hz, 2H, H_{3'}, H_{5'}), 7.33 (td, $J = 7.7$, 1.2 Hz, 1H, H₆), 7.40 (dd, $J = 7.7$, 1.2 Hz, 1H, H₈), 7.54-7.58 (m, 2H, H₅, H₇), 7.72 (dd, $J = 9.0$, 5.5 Hz, 2H, H_{2'}, H_{6'}), 7.81 (s, 1H, H₄). ¹³C NMR (100 MHz, CDCl₃): $\delta = 115.5$ (d, $J_{C-F} = 21.4$ Hz), 116.5, 119.6, 124.6, 127.6 (d, $J_{C-F} = 54.7$ Hz), 130.4 (d, $J_{C-F} = 22.8$ Hz), 130.5, 130.7, 131.5, 139.79, 153.5, 160.6, 161.1 (d, $J_{C-F} = 247.7$ Hz). Anal. calcd. for C₁₅H₉FO₂: C, 75.00; H, 3.78. Found: C, 75.18; H, 3.89.

8-Methoxy-3-phenyl-2H-chromen-2-one (3h). Yield: 79%, white crystals, mp 140-142 °C. IR (KBr): 1720 (C=O) cm⁻¹. ¹H NMR (400 MHz, CDCl₃): $\delta = 4.00$ (s, 3H, OCH₃), 7.08 (dd, 1H, $J = 8.0$, 1.0 Hz, H₇), 7.13 (dd, 1H, $J = 8.0$, 1.0, H₅), 7.24 (t, 1H, $J = 8.0$ Hz, H₆), 7.40-7.48 (m, 3H, Ph), 7.72-7.74 (m, 2H, Ph), 7.80 (s, 1H, H₄). ¹³C NMR (100MHz, CDCl₃): $\delta = 56.3$, 113.2, 119.3, 120.3, 124.4, 128.5, 128.5, 128.8, 134.7, 140.0, 143.1, 143.2, 147.0, 160.0. Anal. calcd. for C₁₆H₁₂O₃: C, 76.18; H, 4.79. Found: C, 76.32; H, 4.48.

3-(3,4-Dimethoxyphenyl)-8-methoxy-2H-chromen-2-one (3i). Yield: 74%, white crystals, mp 137-139 °C. IR (KBr): 1714 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.82 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 7.30-7.34 (dd, J = 8.0, 1.5 Hz, 1H, H_7), 7.39-7.50 (m, 3H, H_2 , H_5 , H_6), 7.56 (td, J = 8.0, 1.5 Hz, 1H, H_6), 7.73 (dd, J = 8.0, 1.5 Hz, 1H, H_5), 7.84 (s, 1H, H_4). Anal. calcd. for $\text{C}_{18}\text{H}_{16}\text{O}_5$: C, 69.22; H, 5.16. Found: C, 69.41; H, 5.27.

3-(4-Chlorophenyl)-8-methoxy-2H-chromen-2-one (3j). Yield: 78%, white crystals, mp 179-181 °C. IR (KBr): 1710 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.99 (s, 3H, OCH_3), 6.97-6.99 (dd, J = 8.0, 2.0 Hz, 1H, H_7), 7.07 (dd, J = 8.0, 2.0 Hz, 1H, H_5), 7.12 (d, J = 8.0 Hz, 2H, H_2 , H_6), 7.23 (t, J = 8.0 Hz, 1H, H_6), 7.70 (d, J = 8.0 Hz, 2H, H_3 , H_5), 7.75 (s, 1H, H_4). Anal. calcd. for $\text{C}_{16}\text{H}_{11}\text{ClO}_3$: C, 67.03; H, 3.87. Found: C, 66.89; H, 3.71.

8-Methoxy-3-(4-methoxyphenyl)-2H-chromen-2-one (3k). Yield: 75%, white crystals, mp 144-146 °C. IR (KBr): 1711 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.86 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.98 (d, J = 9.0 Hz, 2H, H_3 , H_5), 7.06 (dd, J = 8.0, 1.2 Hz, 1H, H_7), 7.11 (dd, J = 8.0, 1.2 Hz, 1H, H_5), 7.23 (t, J = 8.0 Hz, 1H, H_6), 7.70 (d, J = 9.0 Hz, 2H, H_2 , H_6), 7.74 (s, 1H, H_4). ^{13}C NMR (100 MHz, CDCl_3): δ = 55.4, 56.2, 112.9, 113.9, 119.2, 120.5, 124.3, 127.1, 128.0, 129.8, 138.5, 142.9, 147.0, 160.1, 160.2. Anal. calcd. for $\text{C}_{17}\text{H}_{14}\text{O}_4$: C, 72.33; H, 5.00. Found: C, 72.51; H, 4.86.

8-Methoxy-3-(4-nitrophenyl)-2H-chromen-2-one (3l). Yield: 80%, white crystals, mp 263-265 °C. IR (KBr): 1700 (C=O) cm^{-1} . ^1H NMR (400MHz, CDCl_3): δ = 4.00 (s, 3H, OCH_3), 7.09-7.18 (m, 3H, H_7 , H_2 , H_6), 7.23-7.27 (m, 2H, H_5 , H_6), 7.72 (s, 1H, H_4), 8.11-8.19 (d, J = 8.5 Hz, 2H, H_3 , H_5); Anal. calcd. for $\text{C}_{16}\text{H}_{11}\text{NO}_5$: C, 64.65; H, 3.73; N, 4.71. Found: C, 64.46; H, 3.51; N, 4.93.

3-(2,4-Dichlorophenyl)-8-methoxy-2H-chromen-2-one (3m). Yield: 78%, white crystals, mp 189-201 °C. IR (KBr): 1705 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.98 (s, 3H, OCH_3), 7.32-7.39 (m, 2H, H_7 , H_6), 7.43 (d, J = 8.4 Hz, 1H, H_5), 7.54 (s, 1H, H_3), 7.58-7.62 (m, 2H, H_5 , H_6), 7.78 (s, 1H, H_4). Anal. calcd. for $\text{C}_{16}\text{H}_{10}\text{Cl}_2\text{O}_3$: C, 59.84; H, 3.14. Found: C, 59.69; H, 3.30.

3-(4-Fluorophenyl)-8-methoxy-2H-chromen-2-one (3n). Yield: 80%, white crystals, mp 205-207 °C. IR (KBr): 1716 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 4.00 (s, 3H, OCH_3), 7.09-7.16-7.19 (m, 4H, H_5 , H_7 , H_3 , H_5), 7.26 (t, J = 8.0 Hz, 1H, H_6), 7.71-7.75 (dd, J = 9.0, 5.5 Hz, 2H, H_2 , H_6), 7.79 (s, 1H, H_4). ^{13}C NMR (100 MHz, CDCl_3): δ = 56.27, 113.3, 115.5 (d, $J_{\text{C-F}}$ = 21.4 Hz), 119.3, 120.2, 124.4, 127.5, 130.4 (d, $J_{\text{C-F}}$ = 7.2 Hz), 130.7 (d, $J_{\text{C-F}}$ = 3.5 Hz), 139.8, 143.1, 147.0, 160.0, 163.1 (d, $J_{\text{C-F}}$ = 247.4 Hz). Anal. calcd. for $\text{C}_{16}\text{H}_{11}\text{FO}_3$: C, 71.11; H, 4.10. Found: C, 71.28; H, 4.27.

6,7,8-Trimethoxy-3-phenyl-2H-chromen-2-one (3o). Yield: 67%, yellow crystals, mp 147-149 °C. IR (KBr): 1720 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.92 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 6.76 (s, 1H, H_5), 7.58-7.61 (m, 3H, Ph), 7.79-7.83 (m, 2H, Ph), 7.84 (s, 1H, H_4). ^{13}C NMR (100 MHz, CDCl_3): δ = 56.4, 61.5, 61.9, 103.7, 115.1, 123.1, 125.7, 129.3, 131.9, 133.8, 139.9, 141.1, 142.4, 145.8, 150.7, 160.0. Anal. calcd. for $\text{C}_{18}\text{H}_{16}\text{O}_5$: C, 69.22; H, 5.16. Found: C, 69.31; H, 5.29.

3-(3,4-Dimethoxyphenyl)-6,7,8-trimethoxy-2H-chromen-2-one (3p). Yield: 61%, yellow crystals, mp 150-152 °C. IR (KBr): 1757 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.83 (s, 6H, $2\times\text{OCH}_3$), 3.89 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.72 (s, 1H, H_5), 7.04 (d, J = 6.5 Hz, 1H, H_5), 7.41 (s, 1H, H_2), 7.82 (d, J = 6.5 Hz, 1H, H_6), 7.84 (s, 1H, H_4). Anal. calcd. for $\text{C}_{20}\text{H}_{20}\text{O}_7$: C, 64.51; H, 5.41. Found: C, 64.34; H, 5.58.

3-(4-Chlorophenyl)-6,7,8-trimethoxy-2H-chromen-2-one (3q). Yield: 65%, yellow crystals, mp 183-185 °C. IR (KBr): 1728 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.93 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 6.75 (s, 1H, H_5), 7.57-7.63 (m, 4H, H_2 , H_3 , H_5 , H_6), 7.75 (s, 1H, H_4). Anal. calcd. for $\text{C}_{18}\text{H}_{15}\text{ClO}_5$: C, 62.35; H, 4.36. Found: C, 62.14; H, 4.48.

6,7,8-Trimethoxy-3-(4-methoxyphenyl)-2H-chromen-2-one (3r). Yield: 63%, yellow crystals, mp 149-151 °C. IR (KBr): 1716 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.87 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.09 (s, 3H, OCH_3), 6.73 (s, 1H, H_5), 7.00 (d, J = 8.8 Hz, 2H, H_3 , H_5), 7.68 (d, J = 8.8 Hz, 2H, H_2 , H_6), 7.70 (s, 1H, H_4). ^{13}C NMR (100 MHz, CDCl_3): δ = 55.4, 56.3, 61.6, 61.9, 103.5, 113.9, 115.4, 126.5, 127.2, 129.8, 138.6, 141.5, 142.3, 147.9, 148.2, 150.2, 160.0. Anal. calcd. for $\text{C}_{19}\text{H}_{18}\text{O}_6$: C, 66.66; H, 5.30. Found: C, 66.84; H, 5.13.

6,7,8-Trimethoxy-3-(4-nitrophenyl)-2H-chromen-2-one (3s). Yield: 72%, yellow crystals, mp 269-271 °C. IR (KBr): 1733 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.91 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 6.74 (s, 1H, H_5), 7.48 (d, J = 8.8 Hz, 2H, H_2 , H_6), 7.77 (s, 1H, H_4), 8.43 (d, J = 8.8 Hz, 2H, H_3 , H_5). ^{13}C NMR (100 MHz, CDCl_3): δ = 56.3, 61.6, 61.9, 103.7, 115.0, 123.0, 125.6, 130.0, 131.6, 133.7, 140.0, 141.0, 142.6, 145.9, 150.3, 160.0. Anal. calcd. for $\text{C}_{18}\text{H}_{15}\text{NO}_7$: C, 60.51; H, 4.23; N, 3.92. Found: C, 60.37; H, 4.41; N, 4.21.

3-(2,4-Dichlorophenyl)-6,7,8-trimethoxy-2H-chromen-2-one (3t). Yield: 65%, yellow crystals, mp 215-217 °C. IR (KBr): 1727 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.90 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.72 (s, 1H, H_5), 7.01-7.04 (m, 2H, H_5 , H_6), 7.75-7.77 (s, 1H, H_3), 7.78 (s, 1H, H_4). Anal. calcd. for $\text{C}_{18}\text{H}_{14}\text{Cl}_2\text{O}_5$: C, 56.71; H, 3.70. Found: C, 56.86; H, 3.57.

3-(4-Fluorophenyl)-6,7,8-trimethoxy-2H-chromen-2-one (3u). Yield: 70%, yellow crystals, mp 220-222 °C. IR (KBr): 1742 (C=O) cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ = 3.81 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.71 (s, 1H, H_5), 7.04 (d, J = 9.0 Hz, 2H, H_3 , H_5), 7.26 (dd, J = 9.0, 5.4 Hz, 2H, H_2 , H_6), 7.76 (s, 1H, H_4). Anal. calcd. for $\text{C}_{18}\text{H}_{15}\text{FO}_5$: C, 65.45; H, 4.58. Found: C, 65.62; H, 4.71.

15-LOX inhibition assay. The stock solutions were prepared by dissolving compounds in DMSO (1 ml) and then diluted in DMSO to prepare test solutions. To obtain IC_{50} values, five different concentrations of compounds were prepared as above and then added to test solution containing enzyme (final concentration: 167 U/ml), phosphate buffer (pH = 8) to achieve final concentrations in range of 10^{-3} to 10^{-6} M. Being incubated for 4 minutes, substrate (Linoleic acid, final concentration: 134 μM) was added and the change in the absorbance was measured for 60 seconds at 234 nm. To adjust the effect of DMSO on enzyme activity, 50 μl DMSO also added to enzyme activity assay solution. The enzyme solution was kept in ice throughout the experimental period and quercetin was used as the reference compound.