Supporting Information

Exploration of Pharmacophore in Chrysosplenol C as Activator in Ventricular Myocyte Contraction

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Contents

1. General experimental information

Melting points were determined on Electro thermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures prior to use. Thin layer chromatography was performed on E Merck silica gel GF-254 pre-coated plates and the identification was done with UV light and colorization with spray 10% phosphomolybdic acid followed by heating. Flash column chromatography was performed with E Merck silica gel (230-400 mesh). Infrared spectrum was recorded by using sample as such on FT-IR spectrum with Nicolet – 380 models. NMR spectra were measured against the peak of tetramethylsilane by JEOL, JNM-AL-400 (Alice) 400 FT-NMR spectrometer. High resolution mass spectra (HRMS) were measured by using Shimadzu LCMS-IT-TOF spectrometer.

2. General synthetic procedure for synthesis of chrysosplenol C analogs

2.1. General procedure for the synthesis of key intermediate chalcones[1] (3)

Appropriately substituted-2-hydroxyacetophenone **1** (1.0 equiv.) was dissolved in 90% aq. ethanol and added potassium hydroxide (2.0 equiv.) and substituted benzaldehydes **2** (1.02 equiv.). The resulting mixture was further stirred for 2 - 3 h at 78 ^{o}C. After completion of reaction, the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane and washed with water three times. The organic layer was dried with anhydrous sodium sulfate and evaporated under reduced pressure to get the crude product, which was further purified by flash column chromatography to give chalcone analogs **3**.[1]

2.2. General Procedure for the synthesis of Chromenone analogs (5)

To a mixture of 10% aqueous sodium hydroxide solution (10 mL) and 2'-hydroxychalcone **3** (0.5 g, 0.32 mmol) in a (1:1) mixture of ethanol and dioxane was added 10 mL of 35% H_2O_2 dropwise at 5-10 °C. Stirring of the mixture at this temperature for 2 h and subsequently at room temperature overnight resulted in a yellow suspension. After acidification with 2 M

HCl, the precipitate was filtered and washed with H_2O. The crude product was recrystallized from ethanol to give **4** as a light yellow solid.

For the preparation of **5a-e**,**5l-p** and **s** (Scheme 1) methylation of 3-hydroxy group and deprotection of benzyl group of **4** were conducted. To a solution of compound **4** in DMF was added NaH (1.0 equiv.) followed by methyl iodide (1.0 equiv.) at ambient temperature. The reaction mixture was stirred up to 4 h followed by quenching with ethylacetate and water workup gave methylated intermediate which was further subjected to 3 volumes of trifluro acetic acid in prescence of catalytic thio-anisole at room temperature for 8 h. After completion of the reaction solvent evaporated and residue was purified by flash column chromatography furnished **5** with moderate yields. For the preparation of **5f –j**, **5q**,**r** and **t** only deprotection of 4 preocedure was followed (Scheme 2).

3. Characterization Data for final Products

3.1. **5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4H-chromen-4-one** (**5a**)

Yield 34%; Light Yellow Solid; mp. 208 - 211 ^{o}C; IR (cm^{-1}) 3115,1579,1433,1251,820; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.21 (br. s., 1H), 7.32 (t, *J* = 8.16, Hz, 1H), 7.19 - 7.15 (m, 2H), 7.07 (d, *J* = 9.27 Hz, 1H), 6.72 (s, 1H), 6.56 (m, 1H), 3.97 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.0, 153.9, 148.7, 148.5, 147.5, 145.8, 137.5, 123.1, 122.6, 122.2, 121.0, 119.8, 115.6, 111.8, 106.8, 55.6; HRMS: calculated for $C_{17}H_{14}O_6$: m/z 314.0790, Found: 314.0784.

3.2. **6-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4H-chromen-4-one** (**5b**)

Yield 52%; Light Yellow Solid; mp. 263 - 269 ^{o}C; IR (cm^{-1}) 3126,1587,1442,1255,823; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, *J* = 2.56, 5.49 Hz, 2H), 7.69 - 7.73 (m, 1H), 7.47 (d, *J* = 9.27 Hz, 1H), 7.22 - 7.25 (m, 1H), 7.06 (d, *J* = 8.29 Hz, 1H), 6.20 (br. s., 1H), 5.97 (s, 1H), 3.99 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 154.2, 148.7, 148.5, 147.5, 145.8, 137.5, 123.0, 122.6, 122.2, 121.8, 119.8, 115.6, 111.8, 106.8, 55.8. HRMS:

calculated for $C_{17}H_{14}O_6$: m/z 314.0790, Found: 314.0789.

3.3. **2-(4-hydroxy-3-methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one** (**5c**)

Yield 77%; Light Yellow Solid; mp. : 174 - 177 ^{o}C; IR (cm^{-1}) 3118,1582,1433,1248,820; ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.16 (d, *J* = 9.02 Hz, 1H), 7.75 (d, *J* = 1.95 Hz, 1H), 7.69 (dd, *J* = 1.83, 8.41 Hz, 1H), 7.06 (d, *J* = 8.29 Hz, 1H), 6.95 - 7.00 (m, 1H), 6.92 (d, *J* = 2.20 Hz, 1H), 6.05 (s, 1H), 3.99 (s, 3H), 3.93 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 164.1, 157.0, 155.2, 148.0, 146.4, 140.7, 127.2, 123.1, 122.5, 118.1, 114.5, 114.3, 111.0, 99.9, 59.9, 56.1, 55.8. HRMS: calculated for $C_{18}H_{16}O_6$: m/z 328.0947 Found: 328.0942.

3.4. **7-chloro-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4H-chromen-4-one** (**5d**)

Yield 68%; Light Yellow Solid; mp. : 183 - 187 ^{o}C; IR (cm^{-1}) 1621,1477,1288,1257, 823; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.54 Hz, 1H), 7.67 - 7.74 (m, 2H), 7.57 (d, *J* = 1.71 Hz, 1H), 7.33 - 7.39 (m, 1H), 7.06 (d, *J* = 8.54 Hz, 1H), 6.04 (s, 1H), 3.99 (s, 3H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 155.8, 155.3, 148.4, 146.4, 141.0, 139.5, 127.2, 125.6, 122.8, 122.8, 122.5, 118.0, 114.6, 111.0, 59.9, 56.1. HRMS: calculated for $C_{17}H_{13}ClO_5$: m/z 332.0452, Found: 332.0448.

3.5. **2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4H-chromen-4-one** (**5e**)

Yield 44%; Light Yellow Solid; mp. : 170 - 174 ^{o}C; IR (cm^{-1}) 3234,1621, 1511,1256,1131, 820; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.28 (dd, *J*=8.05, 1.71 Hz, 1 H) 7.77 (d, *J*=1.95 Hz, 1 H) 7.65 - 7.75 (m, 2 H) 7.51 - 7.58 (m, 1 H) 7.42 (d, *J*=7.07 Hz, 1 H) 7.07 (d, *J*=8.29 Hz, 1 H) 6.09 (s, 1 H) 3.99 (s, 3 H) 3.89 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 155.7, 155.2, 148.2, 146.3, 140.9, 133.4, 125.8, 124.7, 122.7, 117.9, 114.5, 111.1, 59.9, 56.0. HRMS: calculated for $C_{17}H_{14}O_5$: m/z 298.0841, Found: 298.0837.

3.5. **3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one** (**5f**)

Yield 28 %; Yellow Solid; mp. : 278 - 281 ^{o}C; IR (cm^{-1}) 3237,2935, 1587,1433,1255, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (br. s., 1H), 9.26 (br. s., 1H), 7.22 (t, *J* = 8.10, Hz, 1H), 7.19 - 7.15 (m, 2H), 7.07 (d, *J* = 9.27 Hz, 1H), 6.72 (s, 1H), 6.54 (m, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.0, 153.9, 148.7, 148.5, 147.5, 145.8, 137.5, 123.1, 122.6, 122.2, 121.0, 119.8, 115.6, 111.8, 106.8, 55.6. HRMS: calculated for $C_{16}H_{12}O_6$: m/z 300.0634, Found: 300.0629.

3.7. **3,6-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one** (**5g**)

Yield 35 %; Yellow Solid; mp. : 263 - 270 ^{o}C; IR (cm^{-1}) 3232,2933, 1578,1358,1258, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.91 (br. s., 1H), 9.68 (br. s., 1H), 9.16 (br. s., 1H), 7.77 - 7.82 (m, 1H), 7.73 (dd, *J* = 1.95, 8.54 Hz, 1H), 7.63 (d, *J* = 9.02 Hz, 1H), 7.34 (d, *J* = 2.93 Hz, 1H), 7.22 (dd, *J* = 3.05, 9.15 Hz, 1H), 6.94 (d, *J* = 8.29 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 154.2, 148.7, 148.5, 147.5, 145.8, 137.5, 123.0, 122.6, 122.2, 121.8, 119.8, 115.6, 111.8, 106.8, 55.8. HRMS: calculated for $C_{16}H_{12}O_6$: m/z 300.0633, Found: 314.0628.

3.8. **3-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-4H-chromen-4-one** (**5h**)

Yield 56 %; Yellow Solid; mp. : 220 - 225 ^{o}C; IR (cm^{-1}) 3234,2935, 1620,1358,1258, 820; ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.16 (d, *J* = 9.02 Hz, 1H), 7.64 - 7.79 (m, 2H), 7.06 (d, *J* = 8.29 Hz, 1H), 6.95 - 7.00 (m, 1H), 6.92 (d, *J* = 2.20 Hz, 1H), 6.05 (s, 1H), 3.86 - 4.01 (m, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 163.6, 156.4, 148.6, 147.5, 145.3, 137.7, 128.5, 128.3, 126.1, 125.8, 122.5, 121.6, 115.6, 115.2, 114.6, 111.7, 100.4, 56.1, 55.9. HRMS: calculated for $C_{17}H_{14}O_6$: m/z 314.0790, Found: 314.0787.

3.9. **7-chloro-3-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one** (**5i**)

Yield 60 %; Yellow Solid; mp. : 200 - 205 ^{o}C; IR (cm^{-1}) 3120, 1437, 1620,1358,1258, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 9.36 (s, 1H), 8.07 - 8.13 (m, 1H), 7.83 (d, *J* =

1.95 Hz, 1H), 7.75 - 7.80 (m, 3H), 7.46 (ddd, *J* = 2.44, 5.61, 8.05 Hz, 1H), 6.96 (d, *J* = 8.54 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 154.6, 149.0, 147.6, 146.3, 138.3, 137.8, 126.7, 125.1, 122.1, 120.3, 118.4, 115.6, 111.7, 55.8. HRMS: calculated for $C_{16}H_{11}ClO_5$: m/z 318.0295, Found: 318.0290.

3.10. **3-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one** (**5j**)

Yield 58 %; Yellow Solid; mp. : 209 - 211 ^{o}C; IR (cm^{-1}) 3120, 1437, 1620,1358,1258, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H), 9.38 (s, 1H), 8.09 - 8.13 (m, 1H), 7.83 (d, *J* = 1.95 Hz, 1H), 7.77 - 7.80 (m, 2H), 7.42 - 7.49 (m, 1H), 7.08 - 7.17 (m, 1H), 6.95 - 7.04 (m, 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 154.5, 148.9, 147.5, 146.0, 138.1, 133.5, 122.4, 122.0, 121.4, 118.5, 115.6, 55.8. HRMS: calculated for $C_{16}H_{12}O_5$: m/z 284.0685, Found: 284.0679.

3.11. **7-chloro-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one (5k)**

To a solution of the corresponding 3-chalcone (1 equiv) in DMSO (20.0 mL), I_2 (0.05 equiv) was added. The mixture was heated at reflux for 1 h. Then the reaction mixture was poured into water and extracted with ethyl acetate (3x25.0 mL). The organic layer was washed with water and brine, dried over anhydrous sodium sulfate. The solvent was evaporated in vacuum and the residue was purified by flash column chromatography to get compound **5k.** Yield 49 %; White Solid; mp. : 210 - 212 ^{o}C; IR (cm^{-1}) 3130, 1434, 1622,1358,1257, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.96 - 8.05 (m, 2H), 7.60 - 7.64 (m, 2H), 7.53 (dd, *J* = 1.95, 8.54 Hz, 1H), 7.02 (s, 1H), 6.95 (d, *J* = 8.78 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.4, 163.3, 156.0, 150.8, 148.2, 138.2, 126.7, 126.7, 125.9, 122.3, 121.7, 120.5, 118.6, 118.6, 118.5, 115.9, 110.3, 105.3, 56.0. HRMS: calculated for $C_{16}H_{11}ClO_4$: m/z 302.0346, Found: 302.0340.

3.12. **2-(4-hydroxyphenyl)-3,7-dimethoxy-4H-chromen-4-one** (**5l**)

Yield 53 %; Light Yellow Solid; mp. : 223 - 226 ^{o}C; IR (cm^{-1}) 3130, 1437, 1620,1355,1251, 820; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.21 (s, 1H), 7.97 (dd, *J* = 7.32, 8.54 Hz, 3H), 7.26 (d, *J* = 2.20 Hz, 1H), 7.05 (d, *J* = 8.78 Hz, 1H), 6.96 (d, *J* = 8.54 Hz, 2H), 3.91 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 173.2, 163.8, 160.0, 156.6, 154.9, 139.7, 130.1, 126.3, 121.1, 117.4, 115.6, 114.6, 100.6, 59.4, 56.1. HRMS: calculated for $C_{17}H_{14}O_5$: m/z 298.0841, Found: 398.0838.

3.13. **7-chloro-2-(4-hydroxyphenyl)-3-methoxy-4H-chromen-4-one (5m)**

Yield 78 %; Light Yellow Solid; mp. : 138 - 141 ^{o}C; IR (cm^{-1}) 3132, 1477, 1620,1355,1251, 823; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.29 (br. s., 1H), 8.07 (d, *J* = 8.54 Hz, 1H), 7.98 - 8.02 (m, 2H), 7.96 (d, *J* = 1.71 Hz, 1H), 7.52 (dd, *J* = 1.95, 8.54 Hz, 1H), 6.94 - 6.99 (m, 2H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 173.1, 160.3, 155.6, 154.9, 140.0, 138.2, 130.4, 130.4, 130.3, 126.9, 125.5, 122.5, 120.7, 118.3, 115.7, 59.4. HRMS: calculated for $C_{16}H_{11}ClO_4$: m/z 302.0346, Found: 302.0339.

3.14. **2-(4-chlorophenyl)-3,7-dimethoxy-4H-chromen-4-one (5n)**

Yield 61 %; Light Yellow Solid; mp. : 140 - 143 ^{o}C; IR (cm^{-1}) 3130, 1437, 1620,1355,1251, 820; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.06 - 8.12 (m, *J* = 8.54 Hz, 2H), 7.99 (d, *J* = 9.02 Hz, 1H), 7.64 - 7.70 (m, *J* = 8.54 Hz, 2H), 7.28 (d, *J* = 2.20 Hz, 1H), 7.08 (dd, *J* = 2.32, 8.90 Hz, 1H), 3.91 (s, 3H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 173.4, 164.1, 156.7, 153.3, 140.9, 135.6, 130.1, 129.5, 128.9, 126.4, 117.4, 115.0, 100.6, 59.8, 56.2. HRMS: calculated for $C_{17}H_{13}ClO_4$: m/z 316.0502, Found: 316.0498.

3.15. **7-chloro-2-(4-chlorophenyl)-3-methoxy-4H-chromen-4-one (5o)**

Yield 66 %; White Solid; mp. : 168 - 171 ^{o}C; IR (cm^{-1}) 3129, 1433, 1620,1353,1251, 820; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.11 (d, *J* = 8.54 Hz, 1H), 7.94 - 8.01 (m, 2H), 7.96 (d, *J* = 1.71 Hz, 1H), 7.53 (dd, *J* = 1.95, 8.54 Hz, 1H), 6.94 - 6.99 (m, 2H), 3.83 (s, 3H); ^{13}C NMR

(100 MHz, DMSO-d_6) δ 173.1, 160.3, 155.6, 154.9, 140.0, 138.2, 130.4, 130.4, 130.3, 126.9, 125.5, 122.5, 120.7, 118.3, 115.7, 59.4. HRMS: calculated for $C_{16}H_{10}Cl_2O_3$: m/z 320.0007, Found: 320.0001.

3.16. **7-chloro-2-(3-hydroxyphenyl)-3-methoxy-4H-chromen-4-one** (**5p**)

Yield 24 %; White Solid; mp. : 221 - 226 ^oC; IR (cm^{-1}) 3130, 1439, 1620,1345,1258, 820; ^1H NMR (400 MHz, DMSO-d_6) δ 8.10 (d, *J* = 8.53 Hz, 1H), 8.02 (d, *J* = 1.71 Hz, 1H), 7.64 - 7.67 (m, 1H), 7.60 - 7.63 (m, 1H), 7.49 - 7.57 (m, 2H), 7.15 - 7.20 (m, 1H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.5, 159.3, 155.0, 155.0, 141.2, 138.5, 131.5, 130.0, 126.9, 125.8, 122.5, 120.7, 118.6, 116.7, 113.8, 59.7, 55.3. HRMS: calculated for $C_{16}H_{11}ClO_4$: m/z 302.0346, Found: 302.0340.

3.17. **3-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one** (**5q**)

Yield 39 %; White Solid; mp. : 254 - 257 ^oC; IR (cm^{-1}) 3130, 1437, 1620,1355,1251, 820; ^1H NMR (400 MHz, DMSO-d_6) δ 10.06 (br. s., 1H), 9.21 (br. s., 1H), 8.10 (d, *J* = 8.78 Hz, 2H), 7.99 (d, *J* = 9.02 Hz, 1H), 7.26 (d, *J* = 2.20 Hz, 1H), 7.04 (dd, *J* = 2.32, 8.90 Hz, 1H), 6.94 (d, *J* = 8.78 Hz, 2H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.2, 163.6, 159.0, 156.4, 145.5, 137.5, 129.4, 129.4, 129.3, 126.2, 122.2, 115.5, 115.3, 114.5, 100.3, 56.0. HRMS: calculated for $C_{16}H_{12}O_5$: m/z 284.0685, Found: 284.0679.

3.18. **7-chloro-3-hydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one** (**5r**)

Yield 54 %; Yellow Solid; mp. : 248 - 251 ^oC; IR (cm^{-1}) 3129, 1433, 1620,1358,1250, 820; ^1H NMR (400 MHz, DMSO-d_6) δ 10.11 (br. s., 1H), 9.52 (br. s., 1H), 8.12 (d, *J* = 9.02 Hz, 2H), 8.09 (d, *J* = 8.54 Hz, 1H), 7.96 (d, *J* = 1.95 Hz, 1H), 7.50 (dd, *J* = 1.95, 8.54 Hz, 1H), 6.95 (d, *J* = 9.02 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 159.4, 154.7, 146.5, 138.1, 137.8, 129.7, 126.7, 125.1, 121.8, 120.4, 118.3, 115.5. HRMS: calculated for $C_{15}H_9ClO_4$: m/z 288.0189, Found: 288.0183.

3.19. **7-chloro-3-methoxy-2-(3-methoxyphenyl)-4H-chromen-4-one (5s)**

Yield 79 %; White Solid; mp. : 115 - 119 ^{o}C; IR (cm^{-1}) 1437, 1620,1355,1251, 823; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, *J* = 8.54 Hz, 1H), 8.02 (d, *J* = 1.71 Hz, 1H), 7.64 - 7.68 (m, 1H), 7.60 - 7.63 (m, 1H), 7.49 - 7.57 (m, 2H), 7.15 - 7.20 (m, 1H), 3.86 (s, 3H), 3.84 (s, 3H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.5, 159.3, 155.0, 155.0, 141.2, 138.5, 131.5, 130.0, 126.9, 125.8, 122.5, 120.7, 118.6, 116.7, 113.8, 59.7, 55.3. HRMS: calculated for $C_{17}H_{13}ClO_4$: m/z 316.0502, Found: 316.0498.

3.20. **7-chloro-3-hydroxy-2-(3-methoxyphenyl)-4H-chromen-4-one (5t)**

Yield 56 %; Yellow Solid; mp. : 204 - 206 ^{o}C; IR (cm^{-1}) 3135, 1433, 1620,1355,1258, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.11 (d, *J* = 8.54 Hz, 1H), 8.04 (d, *J* = 1.95 Hz, 1H), 7.84 (td, *J* = 0.67, 7.93 Hz, 1H), 7.79 (t, *J* = 2.07 Hz, 1H), 7.44 - 7.55 (m, 2H), 7.11 (dd, *J* = 2.68, 8.29 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 159.3, 154.8, 145.2, 139.6, 138.2, 132.4, 129.8, 126.8, 125.3, 120.3, 118.5, 115.6, 113.3, 55.3. HRMS: calculated for $C_{16}H_{11}ClO_4$: m/z 302.0346, Found: 302.0340.

3.21. **(E)-1-(4-chloro-2-hydroxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (6)**

Yield 81 %; Yellow Solid; mp. : 185 - 189 ^{o}C; IR (cm^{-1}) 3135, 1437, 1648,1251, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.04 (s, 1H), 9.87 (s, 1H), 8.30 (d, *J* = 8.54 Hz, 1H), 7.82 (s, 2H), 7.55 (d, *J* = 1.95 Hz, 1H), 7.34 (dd, *J* = 1.83, 8.17 Hz, 1H), 7.03 - 7.14 (m, 2H), 6.86 (d, *J* = 8.29 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.0, 162.8, 150.6, 148.2, 146.7, 140.1, 132.4, 126.1, 125.1, 119.9, 119.4, 117.9, 117.5, 115.7, 112.1, 55.9. HRMS: calculated for $C_{16}H_{13}ClO_4$: m/z 304.0502, Found: 304.0498.

3.22. **2-(3,4-dimethoxyphenyl)-5-hydroxy-3,6,7-trimethoxy-4H-chromen-4-one** (**7**)

To a solution of Chrysosplenol C in DMF was added NaH (2.2 equiv.) followed by CH_3I (2.2

equiv.) at room temperature. After completion of the reaction, quenched with ethylacetate and washed with water. The organic layer was dried with sodiumsulphate and evaporated, the residue was purified by column chromatography to get compound 7. Yield 32 %; White Solid; mp. : 147 - 152 ^{o}C; IR (cm^{-1}) 3131, 1437, 1620,1579,1355,1259, 820; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 7.74 (dd, *J* = 2.07, 8.66 Hz, 1H), 7.67 (d, *J* = 1.95 Hz, 1H), 7.17 (d, *J* = 8.78 Hz, 1H), 6.95 (s, 1H), 3.94 (s, 3H), 3.87 (s, 6H), 3.83 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.5, 158.9, 155.6, 152.0, 151.8, 151.5, 148.6, 138.2, 131.8, 122.2, 111.7, 111.3, 105.7, 91.6, 60.1, 59.8, 56.6, 55.7. HRMS: calculated for $C_{18}H_{16}O_8$: m/z 360.0845, Found: 360.0839.

4.0. Biology

Contractility assay procedure

Single-cell Isolation

Rat ventricular myocytes were enzymatically isolated from male Sprague-Dawley rats (200–300 g) as described[2] previously. Briefly, rats were deeply anesthetized with sodium pentobarbital (150 mg/kg, intraperitoneally), chest cavities were opened, and hearts were excised. This surgical procedure was carried out in accordance with university ethical guidelines. The excised hearts were retrogradely perfused at 7 mL/min through the aorta (at 36.5 ^{o}C), first for 3 minutes with Ca^{2+}-free Tyrode solution composed of (in millimolar) 137 NaCl, 5.4 KCl, 10 HEPES, 1 $MgCl_2$, and 10 glucose (pH 7.3), and then with Ca^{2+} free Tyrode solution containing collagenase (1.4 mg/mL, Type I; Roche, Indianapolis, IN) and protease (0.14 mg/mL, Type XIV; Sigma-Aldrich, St. Louis, MO) for 12 minutes, and finally with Tyrode solution containing 0.2 mM $CaCl_2$ for 8 minutes. The ventricles of the digested heart were then cut into several sections and subjected to gentle agitation to dissociate the cells.

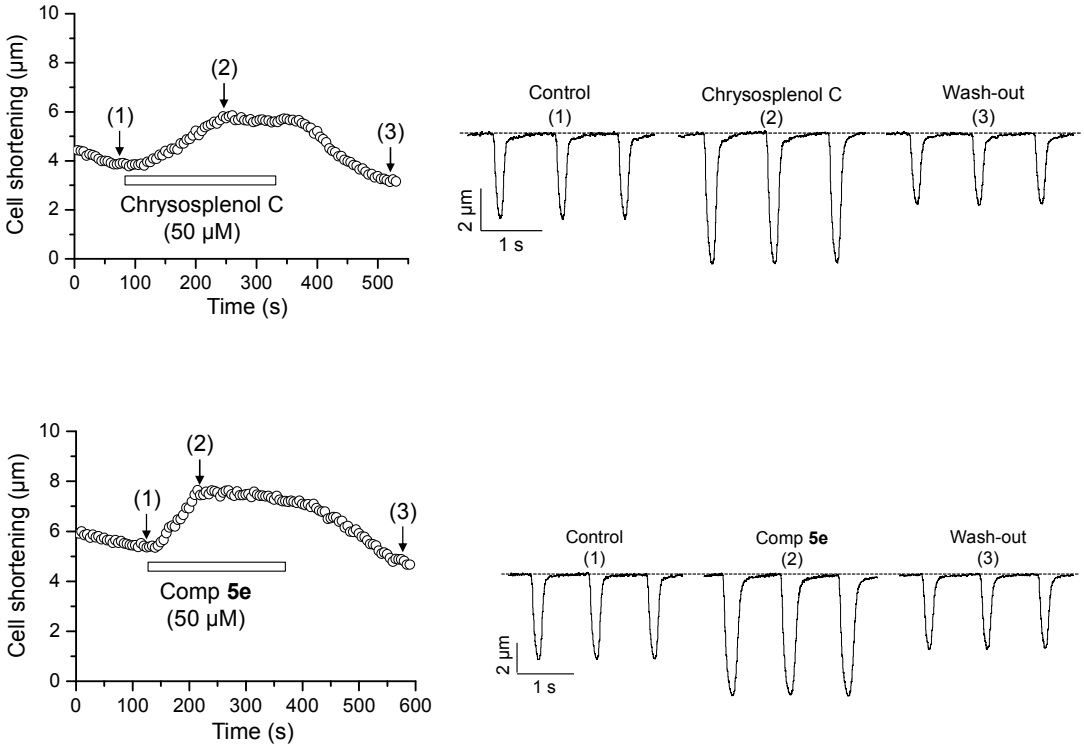
Measurement of Cell Shortenings

Isolated myocytes were continuously superfused with normal Tyrode solution (see above) containing 2 mM Ca^{2+}. Cells were field stimulated with 2 paralleled platinum wires

connected with an electrical stimulator (Stimulator I; Hugo Sach Elektronik, March-Hugstetten, Germany) at 1 Hz. Single-cell shortenings were detected with a video edge detector (Model VED-105; Crescent Electronics, Sandy, UT) connected with a CCD camera (LCL902C; Till Photonics, Graefelting, Germany) and video monitor (ViewFinder III, Polychrome V system; Till Photonics). Analog signals from the edge detector were converted into digital signals by an A/D converter (Digidata 1322A; Molecular Devices). The digitized cell shortening signals were recorded with a PC program, pClamp 9 (Molecular Devices, Sunnyvale, CA).

A Stock solution of chrysosplenol C and their analogs were made in dimethyl sulfoxide (DMSO), which was diluted in the external normal Tyrode solution to make the final testing solutions. The drug solutions were applied to the cells by super fusion using custom-made solution switching apparatus. The experiments were all performed at room temperature (22–25 ^{o}C).

Chrysosplenol and weak active compounds time course shown below:



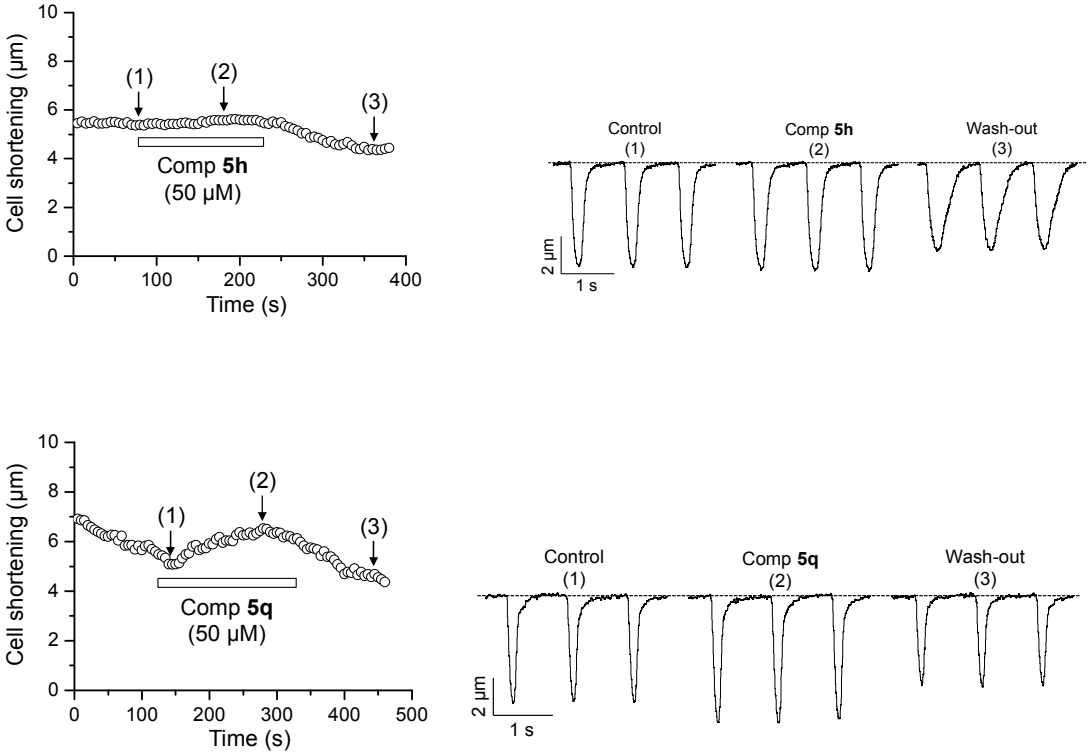
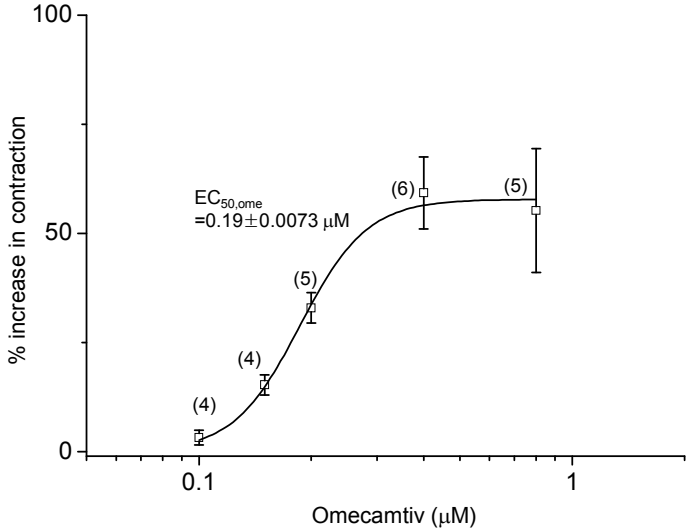


Figure shown below displays the concentration-response data on cell contractility with omecamtiv. Number in the parentheses represents the number of cells tested. This result is based on our assay.



Sarcomere Assay Procedure:

In the sarcomere, force generation is directly coupled to ATP hydrolysis. Compounds that activate the sarcomere were identified by measuring the increase in myosin ATPase activity in a sarcomere assay.[3]

Actin stimulated ATPase activity was assayed spectrophometrically as reported previously[3,4] with modifications. The standard reaction mixture contained 20 mM Tris HCl (pH 7.5), 15 mM KCl, 6 mM $MgCl_2$, 1 mM ATP, S1 myosin (CS-MYS03) and actin thin filament complex (CS-TFC01) with pCa=6.5. The reaction was stopped by addition of Cytophos reagent (Cytoskeleton BK054 kit) After 10 min incubation at room temperature, samples were analyzed for inorganic phosphate liberated by taking the absorbance at 650 nm on TECAN Infinite .

Selectivity Studies

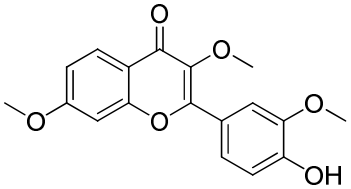
Proteins:

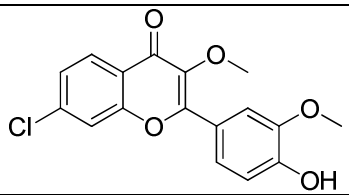
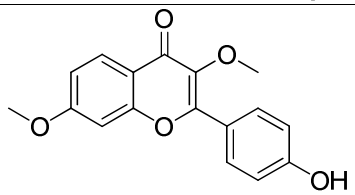
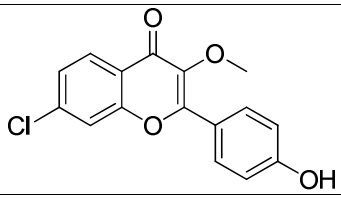
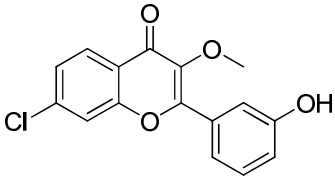
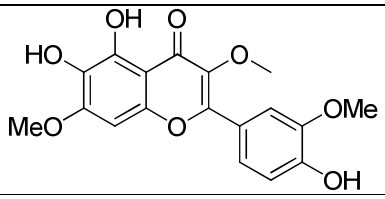
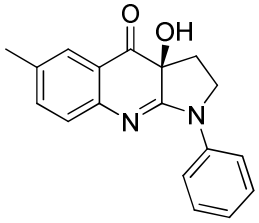
Cardiac myosin S1 (CS-MYS03), Bovine
Skeletal myosin S1 (CS-MYS04), Rabbit Psoas Muscle
Smooth muscle myosin S1(CS-MYS05),Chicken gizzard
Thin filament complex (TFC01), Bovine
Cardiac Actin (AD 99), Bovine
Tropomyosin (T2400, Sigma)

Final Concentrations of Proteins used:

1. S1= 0.006 mg/ml
2. TFC01=0.2 mg/ml
3. pCa=6.5
4. Tropomyosin=0.029 mg/ml (used for smooth muscle)
5. Actin=0.2 mg/ml (used for smooth muscle)
6. Samples used at 10 μM (for specificity test)

Results:

Comp.No	Structure	% ATPase activity		
		Skeletal 10 μM	Smooth 10 μM	Cardiac 10 μM
5c		-2.75 ± 6.15	1.40 ± 4.60	33.74 ± 2.64

5d		-2.90 ± 9.90	3.15 ± 3.35	36.40 ± 1.32
5l		1.40 ± 5.50	3.40 ± 3.70	30.92 ± 1.01
5m		-9.40±2.30	5.65 ± 1.45	28.94 ± 2.29
5p		-5.90 ± 2.00	0.15 ± 4.15	38.71 ± 1.09
Chrysosplenol C		-5.60 ± 7.70	1.75 ± 5.70	28.10 ± 1.20
(-) Blebbistatin @ 100 µm		-52.40±8.50	-17.40±3.40	

% Activity is represented as Average of the two experiments with an average deviation (±)

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4. Hartman J. J.; Malik, F.; Sakowicz, R.; Finer, J. T. High throughput sarcomeric assay. U.S. Patent 6495337 B1, **2002**, 12 pp.