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Development of 3-Alkyl-6-Methoxy-7-Hydroxy-Chromone (AMHC) from Natural Isoflavones, a New Fluorescent Scaffold for Biological Imaging

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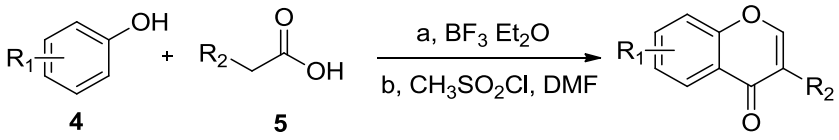
1. Chemistry

1.1 General information

General: All the solvents and chemicals were purchased from commercial sources: Sigma-Aldrich Chemical Co., Beijing Ou-he Reagents Co.. Some of flavones and isoflavones were purchased from Beijing Shiji-Aoke Biotechnology Co. and Shanghai Jingke Chemistry Technology Co. with the purity of more than 95%. Flash column chromatography was performed on Biotage Isolera one. ^{1}H NMR and ^{13}C NMR were recorded on *Mercury300*, *Mercury400*, *Bruker AVANCEIII 400* spectrometer. Chemical shifts are referenced to the residual solvent peak and reported in ppm (δ scale) and all coupling constant (J) values are given in Hertz (Hz). The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. ESI-HRMS data were measured on Thermo Exactive Orbitrap plus spectrometer. Purity was determined using both LCMS and NMR spectroscopy. All of the synthesized compounds have a purity of > 95%.

General Procedure of 3-substituted chromone analogues [1]**:** Acetic acid derivatives (1 eq, 5 mmol) and phenol derivatives (1 eq, 5 mmol) were combined in a round bottom flask and flushed with Argon. Boron trifluoride diethyl etherate (2 ml) was added to the solid in the flask and the mixture was stirred under Argon with heating to 110 ℃ for 2 hours. After cooling to room temperature, all reactions were analyzed by TLC for completion. Methane sulfonyl chloride ($MeSO_2Cl$) (3.1 eq, 1.2 ml.) was added to dried DMF (2.5 ml) in the dropping funnel. This mixture was then added drop wise to the round bottom flask over a period of 10 minutes. Once the addition was complete, the temperature was increased to reflux (110 ℃). The reaction was monitored by TLC and was completed after 3-5 hours. The reaction mixture was cooled to room temperature and poured into ice-water (15 ml) and extracted with EA (15 ml×4). The organic phase was washed with brine, dried and concentrated in vacuo, which was purified by silica gel column (DCM:CH_3OH=100:1).



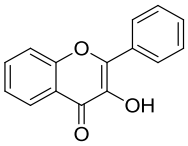
4 5

Scheme 1. Reaction conditions: a), **4** 5 mmol, **5** 5 mmol, solvent BF_3•Et_2O (2 ml), 110 ℃ for 2 hours. b), CH_3SO_2Cl (1.2 ml), DMF (2.5 ml), 110 ℃ for 3 hours.

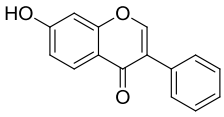
1.2 Compound characterization (^{1}H, ^{13}C and HRMS)

Compounds (**1**, **2**, **6**-**25**) were purchased from Beijing Shiji-Aoke Biotechnology Co. and Shanghai Jingke Chemistry Technology Co. with the purity of more than 95%.

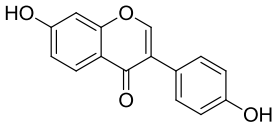
3-hydroxy-2-phenyl-*4H*-chromen-4-one (**1**)



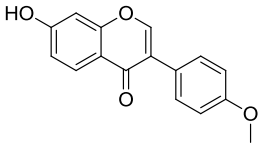
7-hydroxy-3-phenyl-*4H*-chromen-4-one (**2**)



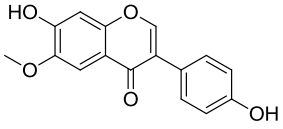
7-hydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**6**)



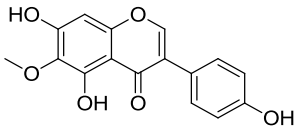
7-hydroxy-3-(4-methoxyphenyl)-*4H*-chromen-4-one (**7**)



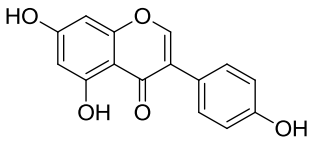
7-hydroxy-3-(4-hydroxyphenyl)-6-methoxy-*4H*-chromen-4-one (**8**)



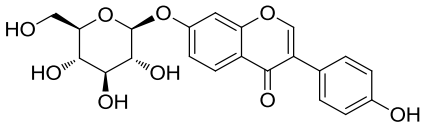
5, 7-dihydroxy-3-(4-hydroxyphenyl)-6-methoxy-*4H*-chromen-4-one (**9**)



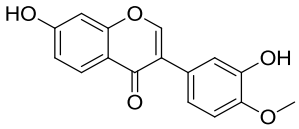
5, 7-dihydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**10**)



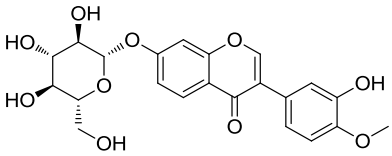
Luteolin (**11**)



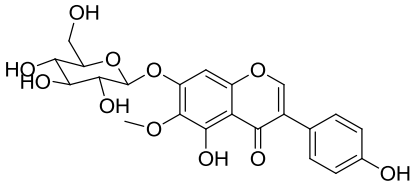
7-hydroxy-3-(3-hydroxy-4-methoxyphenyl)-*4H*-chromen-4-one (**12**)



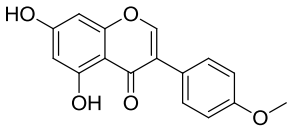
Calycosin-7-glucoside (**13**)



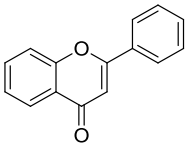
Tectoridin (**14**)



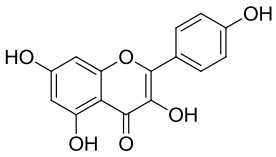
5, 7-dihydroxy-3-(4-methoxyphenyl)-*4H*-chromen-4-one (**15**)



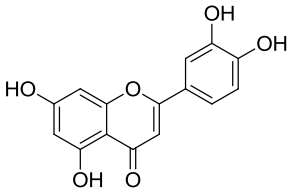
2-phenyl-*4H*-chromen-4-one (**16**)



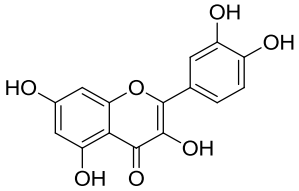
3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-*4H*-chromen-4-one (**17**)



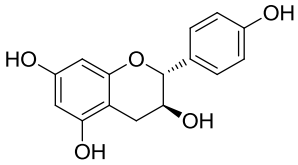
2-(3, 4-dihydroxyphenyl)-5, 7-dihydroxy-*4H*-chromen-4-one (**18**)



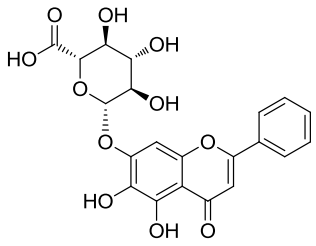
2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-*4H*-chromen-4-one (**19**)



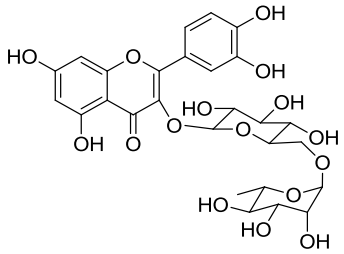
Catechin (**20**)



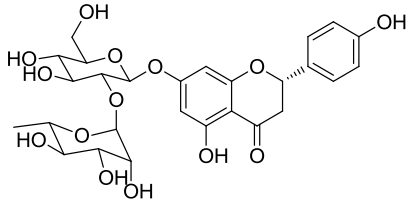
Baicalin (**21**)



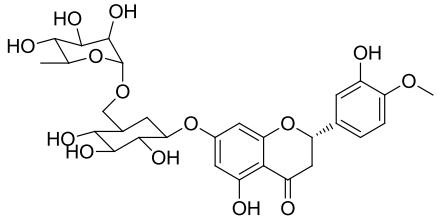
Rutin (**22**)



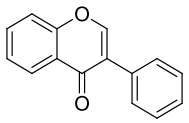
Naringin (**23**)



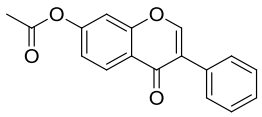
Hesperidin (**24**)



3-phenyl-*4H*-chromen-4-one (**25**)

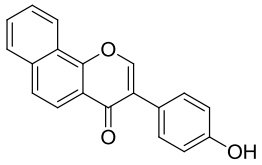


4-oxo-3-phenyl-*4H*-chromen-7-yl acetate (**26**)



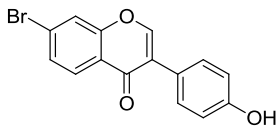
To a solution of 7-hydroxy-3-phenyl-*4H*-chromen-4-one (**2**) (254 mg, 1 mmol) in 2 ml of anhydrous pyridine, 1.0 ml of acetic anhydride was added. The mixture was gently stirred for 4 hours at room temperature. Reaction product was precipitated in 15 ml of ice water, collected by filtration, washed with small portions of cold water, and dried under vacuum. The product was obtained as white solid in 88% yield; ^{1}H NMR (300 MHz, DMSO) δ = 8.56 (s, 1H), 8.19 (d, *J*=8.7 Hz, 1H), 7.66 - 7.56 (m, 3H), 7.50 - 7.37 (m, 3H), 7.33 (dd, *J*=8.7, 2.1 Hz, 1H), 2.35 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.03, 169.14, 156.59, 155.30, 154.95, 132.13, 129.42, 128.66, 128.44, 127.48, 124.50, 122.16, 120.72, 111.98, 21.38; HRMS (ESI) calcd. for $C_{17}H_{13}O_4$ $[M+H]^+$ 281.08084, found: 281.08093.

3-(4-hydroxyphenyl)-*4H*-benzo[h]chromen-4-one (**27**)



Compound 3-(4-hydroxyphenyl)-*4H*-benzo[h]chromen-4-one (**27**) was prepared according to the general procedure and the product was obtained as white solid in 76% yield; ^{1}H NMR (300 MHz, DMSO) δ = 9.62 (s, 1H), 8.65 (s, 1H), 8.53 - 8.43 (m, 1H), 8.15 - 8.05 (m, 2H), 7.94 (d, *J*=8.8 Hz, 1H), 7.87 - 7.72 (m, 2H), 7.51 (d, *J*=8.5 Hz, 2H), 6.87 (d, *J*=8.5 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 175.59, 157.93, 153.33, 153.20, 135.63, 130.59, 130.06, 128.72, 128.16, 125.73, 125.57, 123.83, 122.63, 122.27, 121.07, 120.54, 115.54; HRMS (ESI) calcd. for $C_{19}H_{13}O_3$ $[M+H]^+$ 289.08592, found: 289.08585.

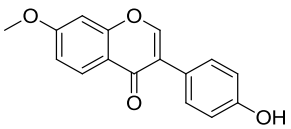
7-bromo-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**28**)



Compound 7-bromo-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**28**) was prepared according to the general procedure and the product was obtained as white solid in 20% yield; ^{1}H NMR (300

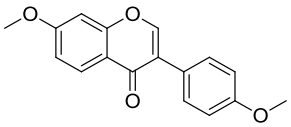
MHz, DMSO) δ = 9.56 (s, 1H), 8.45 (s, 1H), 8.04 (d, *J*=2.1 Hz, 1H), 7.71 (dd, *J*=8.7, 2.1 Hz, 1H), 7.64 (d, *J*=8.7 Hz, 1H), 7.42 (d, *J*=8.5 Hz, 2H), 6.83 (d, *J*=8.5 Hz, 2H); ^{13}C NMR (151 MHz, dmso) δ = 175.27, 157.28, 154.58, 153.63, 135.37, 131.89, 130.06, 125.88, 123.81, 123.49, 122.26, 118.32, 115.00; HRMS (ESI) calcd. for $C_{15}H_{10}O_3Br$ $[M+H]^+$ 316.98078, found: 316.98087,

3-(4-hydroxyphenyl)-7-methoxy-*4H*-chromen-4-one (**29**)



Compound 3-(4-hydroxyphenyl)-7-methoxy-*4H*-chromen-4-one (**29**) was prepared according to the general procedure and the product was obtained as white solid in 68% yield; ^{1}H NMR (300 MHz, DMSO) δ = 9.54 (s, 1H), 8.37 (s, 1H), 8.03 (d, *J*=8.9 Hz, 1H), 7.41 (d, *J*=8.6 Hz, 2H), 7.15 (d, *J*=2.3 Hz, 1H), 7.08 (dd, *J*=8.9, 2.4 Hz, 1H), 6.82 (d, *J*=8.6 Hz, 2H), 3.91 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.18, 164.11, 157.89, 157.71, 153.58, 130.53, 127.40, 124.17, 122.84, 118.07, 115.44, 115.16, 100.98, 56.55; HRMS (ESI) calcd. for $C_{16}H_{13}O_4$ $[M+H]^+$ 269.08084, found: 269.08054.

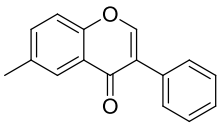
7-methoxy-3-(4-methoxyphenyl)-*4H*-chromen-4-one (**30**)



To a solution of 7-hydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**6**) (1.0 mmol, 254 mg) in 5 ml of DMF was added NaOH (3 mmol, 120 mg) pellets. The mixture was stirred at RT for 5 min and iodomethane (2.2 mmol, 0.136 ml) was added dropwise. The mixture was stirred at RT for another 2 hours and poured into ice water. Product in the water was extracted with Ethyl acetate. The organic phase was washed with brine, dried and concentrated in vacuo, The residue was purified by flash column chromatography on silica gel (DCM:CH_3OH=100:1), the product was obtained as white solid in 71% yield; ^{1}H NMR (400 MHz, DMSO) δ = 8.43 (s, 1H), 8.04 (d, *J*=8.8 Hz, 1H), 7.54 (s, 1H), 7.17 (d, *J*=2.3 Hz, 1H), 7.09 (dd, *J*=8.9, 2.3 Hz, 1H), 7.00 (d, *J*=8.8 Hz, 2H), 3.91 (s, 3H), 3.79 (s, 3H); 13C NMR (101 MHz, DMSO) δ = 174.63, 163.70,

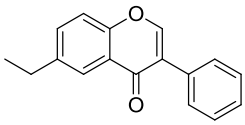
159.00, 157.45, 153.45, 130.06, 126.94, 124.06, 123.37, 117.59, 114.76, 113.61, 100.55, 56.10, 55.13; HRMS (ESI) calcd. for $C_{17}H_{15}O_4$ [M+H]+ 283.09649, found: 283.09659.

6-methyl-3-phenyl-*4H*-chromen-4-one (**31**)



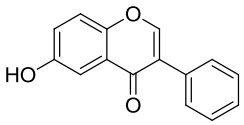
Compound 6-methyl-3-phenyl-*4H*-chromen-4-one (**31**) was prepared according to the general procedure and the product was obtained as white solid in 86% yield; ^{1}H NMR (300 MHz, DMSO) δ = 8.52 (s, 1H), 7.94 (s, 1H), 7.66 (dd, *J*=8.6, 1.8 Hz, 1H), 7.60 (m, 3H), 7.50 - 7.35 (m, 3H), 2.45 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.53, 155.00, 154.40, 135.75, 135.59, 132.47, 129.40, 128.64, 128.30, 125.20, 124.22, 124.03, 118.66, 20.96; HRMS (ESI) calcd. for $C_{16}H_{13}O_2$ $[M+H]^+$ 237.09101, found: 237.09105.

6-ethyl-3-phenyl-*4H*-chromen-4-one (**32**)



Compound 6-ethyl-3-phenyl-*4H*-chromen-4-one (**32**) was prepared according to the general procedure and the product was obtained as white solid in 70% yield; ^{1}H NMR (300 MHz, DMSO) δ = 8.53 (s, 1H), 7.96 (s, 1H), 7.70 (dd, *J*=8.6, 1.9, 1H), 7.65 - 7.57 (m, 3H), 7.42 (m, 3H), 2.76 (q, *J*=7.5, 2H), 1.24 (t, *J*=7.5, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.58, 154.99, 154.55, 141.77, 134.73, 132.48, 129.40, 128.64, 128.30, 124.23, 124.10, 123.95, 118.78, 28.05, 15.99; HRMS (ESI) calcd. for $C_{17}H_{15}O_2$ $[M+H]^+$ 251.10666, found 251.10652.

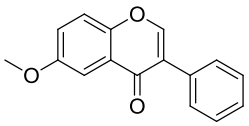
6-hydroxy-3-phenyl-*4H*-chromen-4-one (**33**)



Compound 6-hydroxy-3-phenyl-*4H*-chromen-4-one (**33**) was prepared according to the general procedure and the product was obtained as white solid in 42% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.03 (s, 1H), 8.49 (s, 1H), 7.61 - 7.55 (m, 3H), 7.48 - 7.33 (m, 4H), 7.26 (dd, *J*=9.0, 2.9 Hz,

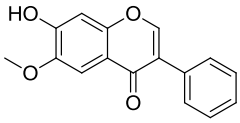
1H); ^{13}C NMR (101 MHz, DMSO) δ = 175.38, 155.40, 154.84, 149.87, 132.65, 129.42, 128.61, 128.20, 125.21, 123.66, 123.29, 120.19, 116.10, 108.45; HRMS (ESI) calcd. for $C_{15}H_{11}O_3$ $[M+H]^+$ 239.07027, found:239.07001.

6-methoxy-3-phenyl-*4H*-chromen-4-one (**34**)



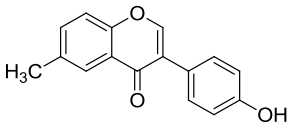
Compound 6-methoxy-3-phenyl-*4H*-chromen-4-one (**34**) was prepared according to the general procedure and the product was obtained as white solid in 85% yield; ^{1}H NMR (300 MHz, DMSO) δ = 8.53 (s, 1H), 7.67 (d, *J*=9.1 Hz, 1H), 7.64 - 7.57 (m, 2H), 7.52 (d, *J*=3.0 Hz, 1H), 7.49 - 7.36 (m, 4H), 3.88 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.26, 157.13, 154.95, 150.86, 132.51, 129.41, 128.65, 128.30, 125.01, 123.84, 123.57, 120.48, 105.67, 56.22; HRMS (ESI) calcd. for $C_{16}H_{13}O_3$ $[M+H]^+$ 253.08592, found: 253.08607.

7-hydroxy-6-methoxy-3-phenyl-*4H*-chromen-4-one (**35**)



Compound 7-hydroxy-6-methoxy-3-phenyl-*4H*-chromen-4-one (**35**) was prepared according to the general procedure and the product was obtained as white solid in 35% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.63 (s, 1H), 8.39 (s, 1H), 7.61 - 7.54 (m, 3H), 7.46 - 7.34 (m, 4H), 3.89 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 174.50, 153.97, 153.47, 152.28, 147.45, 132.80, 129.41, 128.59, 128.12, 123.46, 116.74, 105.28, 103.36, 56.32; HRMS (ESI) calcd. for $C_{16}H_{13}O_4$ $[M+H]^+$ 269.08084, found: 269.08063.

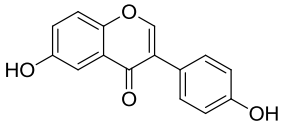
3-(4-hydroxyphenyl)-6-methyl-*4H*-chromen-4-one (**36**)



Compound 3-(4-hydroxyphenyl)-6-methyl-*4H*-chromen-4-one (**36**) was prepared according to the general procedure and the product was obtained as white solid in 80% yield; ^{1}H NMR (300

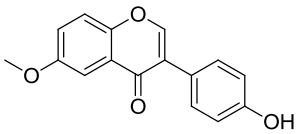
MHz, DMSO) δ = 9.55 (s, 1H), 8.43 (s, 1H), 7.69 - 7.53 (m, 2H), 7.41 (d, *J*=8.6 Hz, 2H), 6.82 (d, *J*=8.6 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.77, 157.71, 154.35, 153.99, 135.56, 135.36, 130.53, 125.16, 124.16, 123.96, 122.86, 118.60, 115.46, 20.96; HRMS (ESI) calcd. for $C_{16}H_{13}O_3$ $[M+H]^+$ 253.08592, found: 253.08565.

6-hydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**37**)



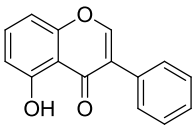
Compound 6-hydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**37**) was prepared according to the general procedure and the product was obtained as white solid in 79% yield; ^{1}H NMR (300 MHz, DMSO) δ = 9.99 (s, 1H), 9.53 (s, 1H), 8.38 (s, 1H), 7.54 (d, *J*=9.0 Hz, 1H), 7.46 - 7.32 (m, 3H), 7.24 (dd, *J*=9.0, 2.9 Hz, 1H), 6.82 (d, *J*=8.4, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 175.62, 157.63, 155.24, 153.85, 149.84, 130.54, 125.14, 123.49, 123.23, 123.05, 120.10, 115.43, 108.41; HRMS (ESI) calcd. for $C_{15}H_{11}O_4$ $[M+H]^+$ 255.06512, found: 255.06515.

3-(4-hydroxyphenyl)-6-methoxy-*4H*-chromen-4-one (**38**)



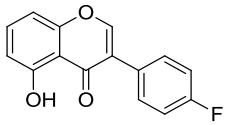
Compound 3-(4-hydroxyphenyl)-6-methoxy-*4H*-chromen-4-one (**38**) was prepared according to the general procedure and the product was obtained as white solid in 82% yield; ^{1}H NMR (300 MHz, DMSO) δ = 9.55 (s, 1H), 8.44 (s, 1H), 7.65 (d, *J*=9.1 Hz, 1H), 7.51 (d, *J*=3.0 Hz, 1H), 7.45 - 7.37 (m, 3H), 6.82 (d, *J*=8.7 Hz, 2H), 3.87 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.02, 157.22, 156.52, 153.49, 150.35, 130.05, 124.45, 123.20, 123.03, 122.41, 119.94, 114.99, 105.14, 55.71; HRMS (ESI) calcd. for $C_{16}H_{13}O_4$ $[M+H]^+$ 269.08084, found: 269.08078.

5-hydroxy-3-phenyl-*4H*-chromen-4-one (**39**)



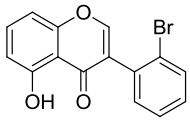
Compound 5-hydroxy-3-phenyl-*4H*-chromen-4-one (**39**) was prepared according to the general procedure, and the product was obtained as white solid in 12% yield; ^{1}H NMR (300 MHz, DMSO) δ = 12.71 (s, 1H), 8.61 (s, 1H), 7.71 (t, *J*=8.4 Hz, 1H), 7.60 (dd, *J*=8.0 Hz, 1.5, 2H), 7.52 - 7.39 (m, 3H), 7.13 (dd, *J*=8.4, 0.7 Hz, 1H), 6.86 (dd, *J*=8.2, 0.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 181.75, 160.86, 156.63, 156.55, 136.60, 131.11, 129.49, 128.76, 128.67, 123.35, 111.49, 111.20, 107.89; HRMS (ESI) calcd. for $C_{15}H_{12}O_3$ $[M+H]^+$ 339.08631, found: 339.08658.

3-(4-fluorophenyl)-5-hydroxy-*4H*-chromen-4-one (**40**)



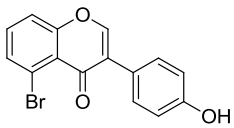
Compound 3-(4-fluorophenyl)-5-hydroxy-*4H*-chromen-4-one (**40**) was prepared according to the general procedure and the product was obtained as white solid in 14% yield; ^{1}H NMR (300 MHz, DMSO) δ = 12.66 (s, 1H), 8.62 (s, 1H), 7.81 - 7.58 (m, 3H), 7.36 - 7.26 (m, 2H), 7.15 - 7.10 (m, 1H), 6.86 (dd, *J*=8.2, 0.5 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 181.63, 160.80, 156.61, 156.53, 136.62, 131.64, 131.55, 127.45, 122.39, 115.76, 115.55, 111.51, 111.14, 107.89; HRMS (ESI) calcd. for $C_{15}H_{10}O_3F$ $[M+H]^+$ 257.06085, found: 257.06082.

3-(2-bromophenyl)-5-hydroxy-*4H*-chromen-4-one (**41**)



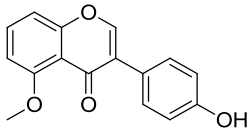
Compound 3-(2-bromophenyl)-5-hydroxy-*4H*-chromen-4-one (**41**) was prepared according to the general procedure and the product was obtained as white solid in 10% yield; ^{1}H NMR (300 MHz, DMSO) δ = 12.10 (s, 1H), 10.94 (s, 1H), 8.01 - 7.66 (m, 1H), 7.62 - 7.44 (m, 3H), 7.19 - 6.94 (m, 1H), 6.44 - 6.29 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 198.48, 166.45, 165.49, 140.02, 135.98, 133.19, 131.92, 128.99, 128.22, 118.75, 112.72, 109.44, 103.11; HRMS (ESI) calcd. for $C_{15}H_{10}O_3Br$ $[M+H]^+$ 316.98078, found: 316.98090.

5-bromo-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**42**)



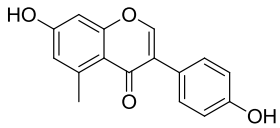
Compound 5-bromo-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**42**) was prepared according to the general procedure and the product was obtained as white solid in 14% yield; ^{1}H NMR (300 MHz, DMSO) δ = 9.58 (s, 1H), 8.47 (s, 1H), 8.05 (m, 2H), 7.69 (dd, *J*=8.6, 1.8 Hz, 1H), 7.42 (d, *J*=8.4 Hz, 2H), 6.83 (d, *J*=8.4 Hz, 2H); δ = 174.93, 157.43, 155.83, 153.74, 130.09, 128.75, 127.47, 127.09, 124.20, 122.93, 121.89, 121.23, 115.06; HRMS (ESI) calcd. for $C_{15}H_{10}O_3Br$ $[M+H]^+$ 316.98078, found: 316.98070.

3-(4-hydroxyphenyl)-5-methoxy-*4H*-chromen-4-one (**43**)



Compound 3-(4-hydroxyphenyl)-5-methoxy-*4H*-chromen-4-one (**43**) was prepared according to the general procedure and the product was obtained as white solid in 9% yield; ^{1}H NMR (300 MHz, DMSO) δ = 9.51 (s, 1H), 8.23 (s, 1H), 7.67 (t, *J*=8.3 Hz, 1H), 7.33 (d, *J*=8.5 Hz, 2H), 7.12 (d, *J*=8.3 Hz, 1H), 6.98 (d, *J*=8.3 Hz, 1H), 6.80 (d, *J*=8.5 Hz, 2H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.08, 160.07, 158.04, 157.59, 151.63, 134.56, 130.66, 125.57, 122.98, 115.32, 114.79, 110.21, 107.45, 56.60; HRMS (ESI) calcd. for $C_{16}H_{13}O_4$ $[M+H]^+$ 269.08084, found: 269.08057.

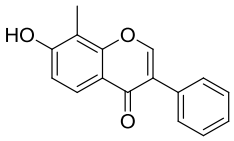
7-hydroxy-3-(4-hydroxyphenyl)-5-methyl-*4H*-chromen-4-one (**44**)



Compound 7-hydroxy-3-(4-hydroxyphenyl)-5-methyl-*4H*-chromen-4-one (**44**) was prepared according to the general procedure and the product was obtained as white solid in 87% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.60 (s, 1H), 9.48 (s, 1H), 8.14 (s, 1H), 7.32 (d, *J*=8.6 Hz, 2H), 6.79 (d, *J*=8.6 Hz, 2H), 6.70 - 6.65 (m, 2H), 2.70 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ =

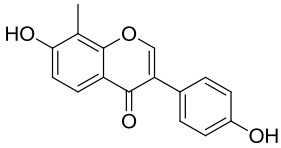
177.05, 161.45, 159.35, 157.50, 151.65, 142.61, 130.71, 124.94, 123.26, 117.38, 115.70, 115.28, 100.96, 23.42; HRMS (ESI) calcd. for $C_{16}H_{13}O_4$ $[M+H]^+$ 269.08084, found: 269.08078.

7-hydroxy-8-methyl-3-phenyl-*4H*-chromen-4-one (**45**)



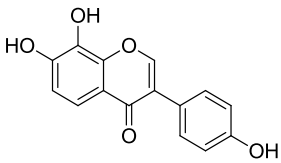
Compound 7-hydroxy-8-methyl-3-phenyl-*4H*-chromen-4-one (**45**) was prepared according to the general procedure and the product was obtained as white solid in 73% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.68 (s, 1H), 8.46 (s, 1H), 7.86 (d, *J*=8.7 Hz, 1H), 7.58 (d, *J*=7.7 Hz, 2H), 7.46 - 7.35 (m, 3H), 7.02 (d, *J*=8.7 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.27, 160.53, 155.99, 154.31, 132.68, 129.39, 128.55, 128.12, 124.28, 123.55, 117.15, 114.44, 111.39, 8.44; HRMS (ESI) calcd. for $C_{16}H_{13}O_3$ $[M+H]^+$ 253.08592, found: 253.08600.

7-hydroxy-3-(4-hydroxyphenyl)-8-methyl-*4H*-chromen-4-one (**46**)



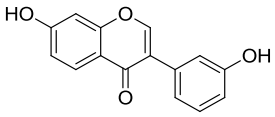
Compound 7-hydroxy-3-(4-hydroxyphenyl)-8-methyl-*4H*-chromen-4-one (**46**) was prepared according to the general procedure and the product was obtained as white solid in 85% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.63 (s, 1H), 9.51 (s, 1H), 8.36 (s, 1H), 7.83 (d, *J*=8.7 Hz, 1H), 7.39 (d, *J*=8.6 Hz, 2H), 6.99 (d, *J*=8.7 Hz, 1H), 6.80 (d, *J*=8.6 Hz, 2H), 2.23 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 175.58, 160.36, 157.60, 155.97, 153.31, 130.53, 124.24, 123.50, 123.11, 117.15, 115.41, 114.32, 111.29, 8.43; HRMS (ESI) calcd. $C_{16}H_{13}O_4$ $[M+H]^+$ 269.08084, found: 269.08090.

7, 8-dihydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**47**)



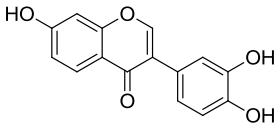
Compound 7,8-dihydroxy-3-(4-hydroxyphenyl)-*4H*-chromen-4-one (**47**) was prepared according to the general procedure and the product was obtained as white solid in 88% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.29 (s, 1H), 9.51 (s, 2H), 8.34 (s, 1H), 7.47 (d, *J*=8.7 Hz, 1H), 7.40 (d, *J*=8.5 Hz, 2H), 6.96 (d, *J*=8.7 Hz, 1H), 6.81 (d, *J*=8.5 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 175.61, 157.58, 153.11, 150.39, 147.18, 133.33, 130.59, 123.39, 123.15, 117.92, 116.12, 115.39, 114.60; HRMS (ESI) calcd. for $C_{15}H_{10}O_5$ [M+H]$^+$ 271.06010, found: 271.06015.

7-hydroxy-3-(3-hydroxyphenyl)-*4H*-chromen-4-one (**48**)



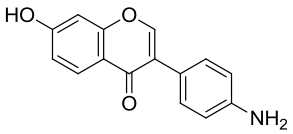
Compound 7-hydroxy-3-(3-hydroxyphenyl)-*4H*-chromen-4-one (**48**) was prepared according to the general procedure and the product was obtained as white solid in 50% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.81 (s, 1H), 9.45 (s, 1H), 8.34 (s, 1H), 7.98 (d, *J*=8.7 Hz, 1H), 7.21 (t, *J*=7.9 Hz, 1H), 7.00 (d, *J*=1.6 Hz, 1H), 6.98 - 6.92 (m, 2H), 6.88 (d, *J*=2.1 Hz, 1H), 6.77 (dd, *J*=8.0, 1.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.81, 163.06, 157.84, 157.47, 154.12, 133.72, 129.53, 127.78, 124.05, 119.93, 117.11, 116.51, 115.68, 115.17, 102.60; HRMS (ESI) calcd. for $C_{15}H_{11}O_4$ [M+H]$^+$ 255.06519, found: 255.06543.

3-(3, 4-dihydroxyphenyl)-7-hydroxy-*4H*-chromen-4-one (**49**)



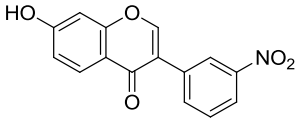
Compound 3-(3,4-dihydroxyphenyl)-7-hydroxy-*4H*-chromen-4-one (**49**) was prepared according to the general procedure and the product was obtained as white solid in 70% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.76 (s, 1H), 9.00 (s, 1H), 8.95 (s, 1H), 8.25 (s, 1H), 7.97 (d, *J*=8.8 Hz, 1H), 7.02 (d, *J*=1.3 Hz, 1H), 6.93 (dd, *J*=8.8, 1.8 Hz, 1H), 6.86 (d, *J*=1.8 Hz, 1H), 6.83 - 6.73 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 175.11, 162.90, 157.80, 153.21, 145.71, 145.23, 127.75, 124.08, 123.45, 120.29, 117.12, 117.05, 115.73, 115.54, 102.52; HRMS (ESI) calcd. for $C_{15}H_{10}O_5$ [M+H]$^+$ 271.06010, found: 271.06015.

3-(4-aminophenyl)-7-hydroxy-*4H*-chromen-4-one (**50**)



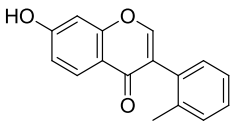
7-hydroxy-3-(4-nitrophenyl)-*4H*-chromen-4-one (100 mg) was dissolved in methanol (10 ml) and 10% palladium on carbon (15 mg) was added. The reaction mixture was hydrogenated for 10 hours at normal pressure and room temperature. It was then filtered through Celite and washed with methanol. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM:CH_3OH=100:1), the product was obtained as white solid in 45% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.74 (s, 1H), 8.23 (s, 1H), 7.96 (d, *J*=8.8 Hz, 1H), 7.24 (d, *J*=8.5 Hz, 2H), 6.92 (dd, *J*=8.8, 2.1 Hz, 1H), 6.84 (d, *J*=2.1 Hz, 1H), 6.59 (d, *J*=8.5 Hz, 2H), 5.19 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 175.33, 162.81, 157.81, 152.55, 148.94, 129.94, 127.73, 124.37, 119.44, 117.12, 115.45, 113.84, 102.48; HRMS (ESI) calcd. for $C_{15}H_{12}O_3N$ $[M+H]^+$ 254.08117, found: 254.08128.

7-hydroxy-3-(3-nitrophenyl)-*4H*-chromen-4-one (**51**)



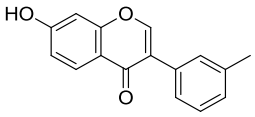
Compound 7-hydroxy-3-(3-nitrophenyl)-*4H*-chromen-4-one (**51**) was prepared according to the general procedure and the product was obtained as white solid in 38% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.91 (s, 1H), 8.63 (s, 1H), 8.52 (s, 1H), 8.34 - 8.13 (m, 1H), 8.05 (d, *J*=7.7 Hz, 1H), 8.01 (d, *J*=8.8 Hz, 1H), 7.74 (t, *J*=8.0 Hz, 1H), 6.99 (dd, *J*=8.8, 2.1 Hz, 1H), 6.92 (d, *J*=2.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.47, 163.40, 157.96, 155.56, 148.08, 135.75, 134.31, 130.12, 127.81, 123.94, 122.98, 121.83, 116.89, 116.00, 102.77; HRMS (ESI) calcd. for $C_{15}H_{10}O_5N$ $[M+H]^+$ 284.05535, found: 284.05557.

7-hydroxy-3-(*o*-tolyl)-*4H*-chromen-4-one (**52**)



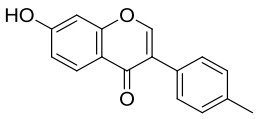
Compound 7-hydroxy-3-(*o*-tolyl)-*4H*-chromen-4-one (**52**) was prepared according to the general procedure and the product was obtained as white solid in 66% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.82 (s, 1H), 8.20 (s, 1H), 7.96 (d, *J*=8.7 Hz, 1H), 7.34 - 7.14 (m, 4H), 6.96 (dd, *J*=8.7, 2.1 Hz, 1H), 6.90 (d, *J*=2.1 Hz, 1H), 2.15 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 174.71, 163.12, 158.16, 154.47, 138.06, 132.68, 131.10, 130.12, 128.58, 127.69, 125.98, 125.36, 116.93, 115.70, 102.69, 20.14; HRMS (ESI) calcd. for $C_{16}H_{13}O_3$ $[M+H]^+$ 253.08592, found: 253.08575.

7-hydroxy-3-(*m*-tolyl)-*4H*-chromen-4-one (**53**)



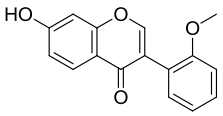
Compound 7-hydroxy-3-(*m*-tolyl)-*4H*-chromen-4-one (**53**) was prepared according to the general procedure and the product was obtained as white solid in 77% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.81 (s, 1H), 8.36 (s, 1H), 7.98 (d, *J*=8.8 Hz, 1H), 7.40 - 7.27 (m, 3H), 7.19 (d, *J*=7.2 Hz, 1H), 6.98 - 6.93 (m, 1H), 6.89 (s, 1H), 2.35 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 174.87, 163.09, 157.89, 154.16, 137.58, 132.48, 129.96, 128.79, 128.46, 127.76, 126.51, 124.10, 117.10, 115.70, 102.62, 21.52; HRMS (ESI) calcd. for $C_{16}H_{13}O_3$ $[M+H]^+$ 253.08592, found: 253.08580.

7-hydroxy-3-(*p*-tolyl)-*4H*-chromen-4-one (**54**)



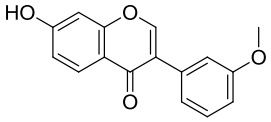
Compound 7-hydroxy-3-(*p*-tolyl)-4H-chromen-4-one (**54**) was prepared according to the general procedure and the product was obtained as white solid in 76% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.81 (s, 1H), 8.34 (s, 1H), 7.98 (d, *J*=8.7 Hz, 1H), 7.46 (d, *J*=8.0 Hz, 2H), 7.23 (d, *J*=8.0 Hz, 2H), 6.95 (dd, *J*=8.7, 1.6 Hz, 1H), 6.88 (d, *J*=1.6 Hz, 1H), 2.34 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 174.94, 163.05, 157.89, 153.90, 137.46, 129.60, 129.30, 129.27, 129.20, 129.13, 127.75, 123.89, 117.10, 115.66, 102.60, 21.26; HRMS (ESI) calcd. for $C_{16}H_{13}O_3$ $[M+H]^+$ 253.08592, found: 253.08592.

7-hydroxy-3-(2-methoxyphenyl)-*4H*-chromen-4-one (**55**)



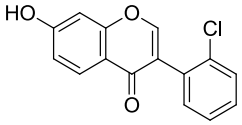
Compound 7-hydroxy-3-(2-methoxyphenyl)-*4H*-chromen-4-one (**55**) was prepared according to the general procedure and the product was obtained as white solid in 74% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.78 (s, 1H), 8.18 (s, 1H), 7.93 (d, *J*=8.7 Hz, 1H), 7.42 - 7.34 (m, 1H), 7.22 (dd, *J*=7.4, 1.5 Hz, 1H), 7.08 (d, *J*=8.3 Hz, 1H), 6.99 (t, *J*=7.4 Hz, 1H), 6.94 (dd, *J*=8.7, 2.1 Hz, 1H), 6.88 (d, *J*=2.1 Hz, 1H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 174.59, 162.97, 158.00, 157.91, 154.40, 132.03, 130.02, 127.65, 122.38, 121.67, 120.51, 117.01, 115.57, 111.71, 102.65, 55.95; HRMS (ESI) calcd. for $C_{16}H_{13}O_4$ $[M+H]^+$ 269.08084, found: 269.08081.

7-hydroxy-3-(3-methoxyphenyl)-*4H*-chromen-4-one (**56**)



Compound 7-hydroxy-3-(3-methoxyphenyl)-*4H*-chromen-4-one (**56**) was prepared according to the general procedure and the product was obtained as white solid in 71% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.82 (s, 1H), 8.41 (s, 1H), 7.99 (d, *J*=8.8 Hz, 1H), 7.34 (t, *J*=7.9 Hz, 1H), 7.17 - 7.12 (m, 2H), 6.98 - 6.93 (m, 2H), 6.89 (d, *J*=1.6 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 174.78, 163.11, 159.44, 157.85, 154.42, 133.89, 129.57, 127.79, 123.78, 121.62, 117.10, 115.72, 115.08, 113.69, 102.62, 55.53; HRMS (ESI) calcd. for $C_{16}H_{13}O_4$ $[M+H]^+$ 269.08084, found: 269.08084.

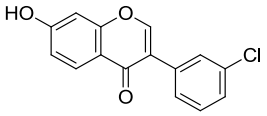
3-(2-chlorophenyl)-7-hydroxy-*4H*-chromen-4-one (**57**)



Compound 3-(2-chlorophenyl)-7-hydroxy-*4H*-chromen-4-one (**57**) was prepared according to the general procedure and the product was obtained as white solid in 69% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.87 (s, 1H), 8.31 (s, 1H), 7.96 (d, *J*=8.7 Hz, 1H), 7.56 (d, *J*=7.8 Hz, 1H), 7.48 - 7.38 (m, 3H), 6.97 (dd, *J*=8.7, 2.0 Hz, 1H), 6.92 (d, *J*=2.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.06, 163.26, 158.13, 154.99, 134.44, 133.03, 131.98, 130.42, 129.62, 127.68,

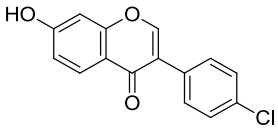
127.46, 123.65, 116.84, 115.83, 102.80; HRMS (ESI) calcd. for $C_{15}H_{10}ClO_3$ $[M+H]^+$ 273.03130, found: 273.03156.

3-(3-chlorophenyl)-7-hydroxy-*4H*-chromen-4-one (**58**)



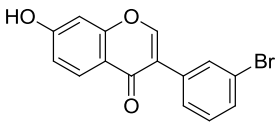
Compound 3-(3-chlorophenyl)-7-hydroxy-*4H*-chromen-4-one (**58**) was prepared according to the general procedure and the product was obtained as white solid in 73% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.86 (s, 1H), 8.49 (s, 1H), 7.99 (d, *J*=8.7 Hz, 1H), 7.68 (s, 1H), 7.57 - 7.53 (m, 1H), 7.50 - 7.41 (m, 2H), 6.97 (dd, *J*=8.7, 2.0 Hz, 1H), 6.90 (d, *J*=2.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.56, 163.26, 157.89, 154.97, 134.73, 133.20, 130.41, 129.04, 128.04, 127.94, 127.79, 122.56, 116.97, 115.87, 102.69; HRMS (ESI) calcd. for $C_{15}H_{10}O_3Cl$ $[M+H]^+$ 273.03130, found: 273.03140.

3-(4-chlorophenyl)-7-hydroxy-*4H*-chromen-4-one (**59**)



Compound 3-(4-chlorophenyl)-7-hydroxy-*4H*-chromen-4-one (**59**) was prepared according to the general procedure and the product was obtained as white solid in 75% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.85 (s, 1H), 8.44 (s, 1H), 7.99 (d, *J*=8.8 Hz, 1H), 7.62 (d, *J*=8.2 Hz, 2H), 7.50 (d, *J*=8.2 Hz, 2H), 6.99 - 6.94 (m, 1H), 6.91 - 6.88 (m, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.65, 163.21, 157.91, 154.57, 132.93, 131.46, 131.10, 128.57, 127.77, 122.78, 116.98, 115.82, 102.67; HRMS (ESI) calcd. for $C_{15}H_{10}O_3Cl$ $[M+H]^+$ 273.03130, found: 273.03134.

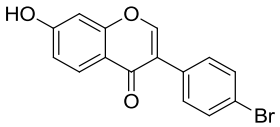
3-(3-bromophenyl)-7-hydroxy-*4H*-chromen-4-one (**60**)



Compound 3-(3-bromophenyl)-7-hydroxy-*4H*-chromen-4-one (**60**) was prepared according to the general procedure and the product was obtained as white solid in 70% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.87 (s, 1H), 8.48 (s, 1H), 7.99 (d, *J*=8.7 Hz, 1H), 7.81 (s, 1H), 7.61 - 7.56

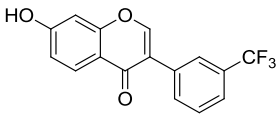
(m, 2H), 7.40 (t, *J*=7.9 Hz, 1H), 6.97 (dd, *J*=8.7, 2.0 Hz, 1H), 6.90 (d, *J*=2.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.56, 163.26, 157.88, 154.95, 134.99, 131.86, 130.93, 130.68, 128.32, 127.77, 122.50, 121.77, 116.96, 115.86, 102.69; HRMS (ESI) calcd. for $C_{15}H_{10}O_3Br$ $[M+H]^+$ 316.98078, found: 316.98141.

3-(4-bromophenyl)-7-hydroxy-*4H*-chromen-4-one (**61**)



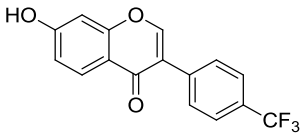
Compound 3-(4-bromophenyl)-7-hydroxy-*4H*-chromen-4-one (**61**) was prepared according to the general procedure and the product was obtained as white solid in 60% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.86 (s, 1H), 8.45 (s, 1H), 7.98 (d, *J*=8.8 Hz, 1H), 7.63 (d, *J*=8.5 Hz, 2H), 7.55 (d, *J*=8.5 Hz, 2H), 6.96 (dd, *J*=8.8, 2.1 Hz, 1H), 6.90 (d, *J*=2.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.60, 163.22, 157.91, 154.56, 131.85, 131.51, 131.43, 127.78, 122.83, 121.53, 116.98, 115.84, 102.68; HRMS (ESI) calcd. for $C_{15}H_{10}O_3Br$ $[M+H]^+$ 316.98078, found: 316.98108.

7-hydroxy-3-(3-trifluoromethylphenyl)-*4H*-chromen-4-one (**62**)



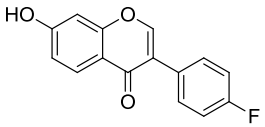
Compound 7-hydroxy-3-(3-trifluoromethylphenyl)-*4H*-chromen-4-one (**62**) was prepared according to the general procedure and the product was obtained as white solid in 43% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.89 (s, 1H), 8.55 (s, 1H), 8.02 - 7.97 (m, 2H), 7.88 (d, *J*=7.6 Hz, 1H), 7.74 (d, *J*=7.6, 1H), 7.68 (t, *J*=7.6 Hz, 1H), 6.98 (dd, *J*=8.7,0.7 Hz, 1H), 6.92 (d, *J*=0.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.15, 162.87, 157.50, 154.75, 133.23, 132.82, 129.39, 129.23, 129.07, 128.76, 128.44, 127.33, 125.60, 125.46, 125.42, 124.37, 122.89, 122.05, 116.49, 115.46, 102.26; HRMS (ESI) calcd. for $C_{16}H_{10}O_3F_3$ $[M+H]^+$ 307.05766, found: 307.05765.

7-hydroxy-3-(4-(trifluoromethyl)phenyl)-*4H*-chromen-4-one (**63**)



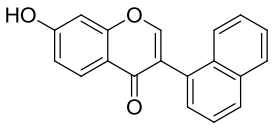
Compound 7-hydroxy-3-(4-(trifluoromethyl)phenyl)-*4H*-chromen-4-one (**63**) was prepared according to the general procedure and the product was obtained as white solid in 47% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.88 (s, 1H), 8.53 (s, 1H), 8.00 (d, *J*=8.7 Hz, 1H), 7.87 - 7.74 (m, 4H), 6.98 (dd, *J*=8.7, 1.0 Hz, 1H), 6.92 (d, *J*=1.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.03, 162.86, 157.45, 154.79, 136.49, 129.60, 129.03, 128.84, 128.24, 127.93, 127.61, 127.33, 125.65, 124.93, 124.89, 124.85, 122.94, 122.23, 116.50, 115.45, 102.26; HRMS (ESI) calcd. for $C_{16}H_{10}O_3F_3$ $[M+H]^+$ 307.05766, found: 307.05756.

3-(4-fluorophenyl)-7-hydroxy-*4H*-chromen-4-one (**64**)



Compound 3-(4-fluorophenyl)-7-hydroxy-*4H*-chromen-4-one (**64**) was prepared according to the general procedure and the product was obtained as white solid in 68% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.84 (s, 1H), 8.41 (s, 1H), 7.99 (d, *J*=8.8 Hz, 1H), 7.66 - 7.58 (m, 2H), 7.32 - 7.22 (m, 2H), 6.96 (dd, *J*=8.8, 2.1 Hz, 1H), 6.89 (d, *J*=2.1 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 174.80, 163.16, 157.93, 154.31, 131.45, 131.37, 128.90, 127.76, 123.02, 117.01, 115.77, 115.53, 115.32, 102.64; HRMS (ESI) calcd. for $C_{15}H_{10}O_3F$ $[M+H]^+$ 257.06085, found: 257.06064.

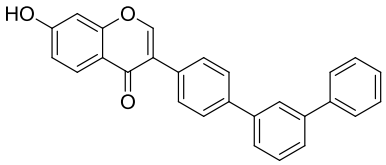
7-hydroxy-3-(naphthalen-1-yl)-*4H*-chromen-4-one (**65**)



Compound 7-hydroxy-3-(naphthalen-1-yl)-*4H*-chromen-4-one (**65**) was prepared according to the general procedure and the product was obtained as white solid in 37% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.87 (s, 1H), 8.36 (s, 1H), 8.01 - 7.95 (m, 3H), 7.64 (d, *J*=8.3 Hz, 1H), 7.60 - 7.50 (m, 2H), 7.49 - 7.42 (m, 2H), 7.02 - 6.93 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ =

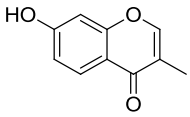
175.27, 163.24, 158.34, 155.02, 133.52, 132.65, 130.94, 128.99, 128.72, 128.53, 127.76, 126.55, 126.41, 126.37, 125.89, 124.39, 116.96, 115.80, 102.81; HRMS (ESI) calcd. for $C_{19}H_{13}O_3$ $[M+H]^+$ 289.08592, found: 289.09566.

3-([1,1':3',1"-terphenyl]-4-yl)-7-hydroxy-*4H*-chromen-4-one (**66**)



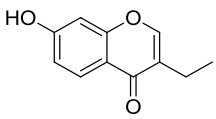
Compound 3-([1,1':3',1"-terphenyl]-4-yl)-7-hydroxy-*4H*-chromen-4-one (**66**) was prepared according to the general procedure and the product was obtained as white solid in yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.85 (s, 1H), 8.49 (s, 1H), 8.02 (d, *J*=8.8 Hz, 1H), 7.95 (s, 1H), 7.85 (d, *J*=6.8 Hz, 2H), 7.79 (d, *J*=6.8 Hz, 2H), 7.75 - 7.65 (m, 4H), 7.62 - 7.57 (m, 1H), 7.55 - 7.46 (m, 2H), 7.41 (t, *J*=9.1 Hz, 1H), 6.98 (d, *J*=8.2 Hz, 1H), 6.91 (s, 1H); ^{13}C NMR (151 MHz, MeOD) δ = 175.73, 163.98, 158.76, 155.21, 142.26, 141.77, 141.43, 140.72, 132.72, 130.90, 130.70, 130.23, 128.89, 128.64, 128.25, 127.93, 127.26, 127.08, 126.38, 124.36, 117.92, 116.60, 103.49; HRMS (ESI) calcd. for $C_{27}H_{19}O_3$ $[M+H]^+$ 391.13287, found: 391.13232.

7-hydroxy-3-methyl-*4H*-chromen-4-one (**67**)



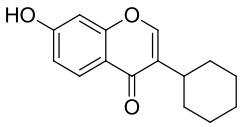
Compound 7-hydroxy-3-methyl-*4H*-chromen-4-one (**67**) was prepared according to the general procedure and the product was obtained as white solid in 32% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.70 (s, 1H), 8.11 (s, 1H), 7.90 (d, *J*=8.7 Hz, 1H), 6.90 (dd, *J*=8.7, 2.1 Hz, 1H), 6.81 (d, *J*=2.1 Hz, 1H), 1.87 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 176.77, 162.77, 158.38, 152.56, 127.15, 119.63, 116.37, 115.34, 102.54, 11.15; HRMS (ESI) calcd. for $C_{10}H_9O_3$ $[M+H]^+$ 177.05462, found: 177.05453.

3-ethyl-7-hydroxy-*4H*-chromen-4-one (**68**)



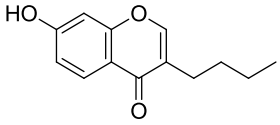
Compound 3-ethyl-7-hydroxy-*4H*-chromen-4-one (**68**) was prepared according to the general procedure and the product was obtained as white solid in 88% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.69 (s, 1H), 8.07 (s, 1H), 7.89 (d, *J*=8.7 Hz, 1H), 6.89 (dd, *J*=8.7, 2.2 Hz, 1H), 6.80 (d, *J*=2.2 Hz, 1H), 2.35 (q, *J*=7.4 Hz, 2H), 1.09 (t, *J*=7.4 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 176.28, 162.79, 158.22, 152.52, 127.22, 125.07, 116.60, 115.33, 102.51, 18.93, 13.63; HRMS (ESI) calcd. for $C_{11}H_{11}O_3$ $[M+H]^+$ 191.07027, found: 191.07027.

3-cyclohexyl-7-hydroxy-*4H*-chromen-4-one (**69**)



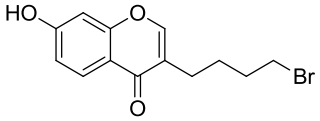
Compound 3-cyclohexyl-7-hydroxy-*4H*-chromen-4-one (**69**) was prepared according to the general procedure and the product was obtained as white solid in 65% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.69 (s, 1H), 7.98 (s, 1H), 7.89 (d, *J*=8.8 Hz, 1H), 6.89 (dd, *J*=8.8, 2.2 Hz, 1H), 6.79 (d, *J*=2.2 Hz, 1H), 2.70 - 2.59 (m, 1H), 1.82 - 1.66 (m, 5H), 1.41 - 1.10 (m, 5H); ^{13}C NMR (101 MHz, DMSO) δ = 175.72, 162.79, 157.85, 152.46, 128.43, 127.41, 116.66, 115.32, 102.41, 34.40, 32.03, 26.80, 26.21; HRMS (ESI) calcd. for $C_{15}H_{17}O_3$ $[M+H]^+$ 245.11722, found: 245.11728.

3-butyl-7-hydroxy-*4H*-chromen-4-one (**70**)



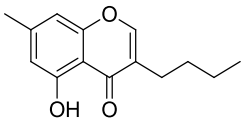
Compound 3-butyl-7-hydroxy-*4H*-chromen-4-one (**70**) was prepared according to the general procedure and the product was obtained as white solid in 60% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.70 (s, 1H), 8.07 (s, 1H), 7.89 (d, *J*=8.8 Hz, 1H), 6.90 (dd, *J*=8.8, 2.1 Hz, 1H), 6.80 (d, *J*=2.1 Hz, 1H), 2.35 - 2.30 (m, 2H), 1.54 - 1.42 (m, 2H), 1.36 - 1.25 (m, 2H), 0.89 (t, *J*=7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 176.31, 162.66, 158.18, 152.87, 127.23, 123.64, 116.60, 115.32, 102.51, 30.71, 25.12, 22.32, 14.20; HRMS (ESI) calcd. for $C_{13}H_{15}O_3$ $[M+H]^+$ 219.10157, found: 219.10144.

3-(4-bromobutyl)-7-hydroxy-*4H*-chromen-4-one (**71**)



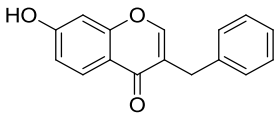
Compound 3-(4-bromobutyl)-7-hydroxy-*4H*-chromen-4-one (**71**) was prepared according to the general procedure and the product was obtained as white solid in 32% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.71 (s, 1H), 8.11 (s, 1H), 7.89 (d, *J*=8.7 Hz, 1H), 6.89 (d, *J*=8.7 Hz, 1H), 6.81 (s, 1H), 3.55 (t, *J*=6.7 Hz, 2H), 2.36 (t, *J*=7.5 Hz, 2H), 1.86 - 1.76 (m, 2H), 1.66 - 1.57 (m, 2H); ^{13}C NMR (151 MHz, MeOD) δ = 169.77, 155.00, 150.57, 145.06, 118.54, 115.09, 108.20, 106.79, 93.71, 24.48, 24.12, 18.94, 16.25.HRMS (ESI) calcd. for $C_{13}H_{14}O_3$ Br $[M+H]^+$ 297.01208, found: 297.10233.

3-butyl-5-hydroxy-7-methyl-*4H*-chromen-4-one (**72**)



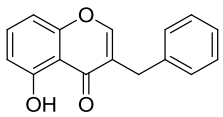
Compound 3-butyl-5-hydroxy-7-methyl-*4H*-chromen-4-one (**72**) was prepared according to the general procedure and the product was obtained as white solid in 32% yield; ^{1}H NMR (400 MHz, DMSO) δ = 12.59 (s, 1H), 8.23 (s, 1H), 6.83 (s, 1H), 6.62 (s, 1H), 2.39 - 2.33 (m, 5H), 1.54 - 1.45 (m, 2H), 1.37 - 1.27 (m, 2H), 0.90 (t, *J*=7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 182.13, 159.63, 156.23, 154.50, 147.05, 122.34, 111.21, 108.18, 107.37, 29.98, 23.92, 21.81, 21.72, 13.65; HRMS (ESI) calcd. for $C_{14}H_{17}O_3$ $[M+H]^+$ 233.11722, found: 233.11720.

3-benzyl-7-hydroxy-*4H*-chromen-4-one (**73**)



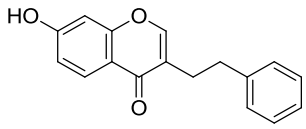
Compound 3-benzyl-7-hydroxy-*4H*-chromen-4-one (**73**) was prepared according to the general procedure and the product was obtained as white solid in 67% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.74 (s, 1H), 8.20 (s, 1H), 7.87 (d, *J*=8.8, 1H), 7.31 - 7.23 (m, 4H), 7.19 - 7.13 (m, 1H), 6.90 (dd, *J*=8.8, 2.0 Hz, 1H), 6.82 (d, *J*=2.0 Hz, 1H), 3.67 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 175.91, 162.94, 158.27, 153.84, 140.20, 128.97, 128.67, 127.27, 126.46, 123.35, 116.71, 115.48, 102.62, 31.16; HRMS (ESI) calcd. for $C_{16}H_{13}O_3$ $[M+H]^+$ 253.08592, found: 253.08607.

3-benzyl-5-hydroxy-*4H*-chromen-4-one (**74**)



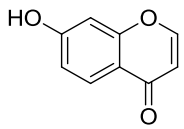
Compound 3-benzyl-5-hydroxy-*4H*-chromen-4-one (**74**) was prepared according to the general procedure and the product was obtained as white solid in 11% yield; ^{1}H NMR (400 MHz, DMSO) δ = 12.52 (s, 1H), 8.45 (s, 1H), 7.65 (t, *J*=8.4 Hz, 1H), 7.37 - 7.25 (m, 4H), 7.22 - 7.16 (m, 1H), 7.06 (d, *J*=8.4 Hz, 1H), 6.80 (d, *J*=8.2 Hz, 1H), 3.73 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 182.65, 160.37, 156.85, 156.38, 139.45, 136.39, 128.95, 128.79, 126.68, 122.62, 111.10, 110.87, 107.92, 30.53; HRMS (ESI) calcd. for $C_{16}H_{13}O_3$ [M+H]$^+$ 253.08592, found: 253.08598.

7-hydroxy-3-phenethyl-*4H*-chromen-4-one (**75**)



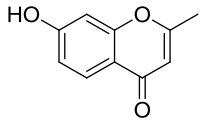
Compound 7-hydroxy-3-phenethyl-*4H*-chromen-4-one (**75**) was prepared according to the general procedure and the product was obtained as white solid in 72% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.73 (s, 1H), 7.99 (s, 1H), 7.93 (d, *J*=8.8 Hz, 1H), 7.33 - 7.26 (m, 2H), 7.24 - 7.16 (m, 2H), 6.92 (dd, *J*=8.8, 0.9 Hz, 1H), 6.81 (d, *J*=0.9 Hz, 1H), 2.82 (t, *J*=7.5 Hz, 2H), 2.63 (t, *J*=7.5 Hz, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 176.28, 162.86, 158.19, 153.19, 141.84, 128.79, 128.76, 127.26, 126.34, 122.77, 116.62, 115.41, 102.56, 34.44, 27.52; HRMS (ESI) calcd. for $C_{17}H_{15}O_3$ [M+H]$^+$ 267.10157, found: 267.10126.

7-hydroxy-*4H*-chromen-4-one (**76**)



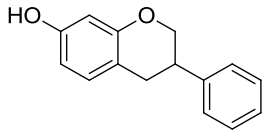
^{1}H NMR (400 MHz, DMSO) δ = 10.79 (s, 1H), 8.15 (d, *J*=6.0 Hz, 1H), 7.89 (d, *J*=8.7 Hz, 1H), 6.92 (d, *J*=8.7 Hz, 1H), 6.85 (s, 1H), 6.22 (d, *J*=6.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 175.64, 162.62, 157.77, 156.11, 126.72, 117.09, 115.06, 111.94, 102.37; HRMS (ESI) calcd. for $C_9H_7O_3$ [M+H]$^+$ 163.03897, found: 163.03870.

7-hydroxy-2-methyl-*4H*-chromen-4-one (**77**)[2]



A solution of 2, 4-dihydroxyacetophenone (2g) in pyridine (5ml) and acetic anhydride (4 ml) was stirred at room temperature for 16 h. The mixture was evaporated to a small volume and the residue dissolved in diethyl ether (10 ml) and the solution washed with water (2×5 ml), dilute hydrochloric acid (2×5 ml), water (5 ml), and aqueous sodium hydrogen carbonate (2×5ml); it was then dried and evaporated to give 2,4-diacetoxyacetophenon. Sodium amide (0.3g) was added to a solution of the 2, 4-diacetoxyacetophenon (1g) in toluene (150 ml) and the mixture was heated at reflux for 6 h. The toluene was decanted and evaporated and dilute hydrochloric acid (5ml) was added to the residue; this was extracted with ether (2×5 ml). The extract was dried and evaporated give the product, which was used for the next step without further purification, was heated at reflux in 10% aqueous sodium carbonate for 15 min. The solution was cooled, acidified with concentrated hydrochloric acid, and set aside at 0 ℃ for 2 h. The product was collected and the residue was purified by flash column chromatography on silica gel (DCM:CH_3OH=100:1), the product was obtained as a solid in 22% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.71 (s, 1H), 7.83 (d, *J*=8.7 Hz, 1H), 6.88 (dd, *J*=8.7 Hz, 2.0, 1H), 6.80 (d, *J*=2.0 Hz, 1H), 6.10 (s, 1H), 2.33 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 176.60, 166.27, 162.88, 158.17, 126.98, 116.23, 115.10, 109.92, 102.60, 20.32; HRMS (ESI) calcd. for $C_{10}H_9O_3$ $[M+H]^+$ 177.05462, found: 177.05437.

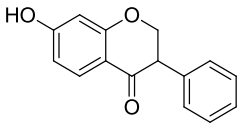
3-phenylchroman-7-ol (**78**)



To a solution of 7-hydroxy-3-phenyl-*4H*-chromen-4-one (**2**) (200 mg) in Ethanol(2 ml), Acetate (2 ml) and 10% palladium on carbon (50 mg) was added. The reaction mixture was hydrogenated for 8 hours at normal pressure and room temperature. It was then filtered through Celite and washed with ethanol. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM:CH_3OH=100:1), the product was

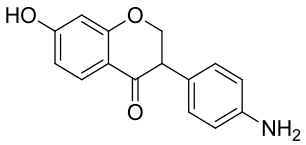
obtained as white solid in 48% yield; ^{1}H NMR (400 MHz, DMSO) δ = 9.17 (s, 1H), 7.39 - 7.30 (m, 4H), 7.28 - 7.22 (m, 1H), 6.88 (d, *J*=8.2 Hz, 1H), 6.30 (dd, *J*=8.2, 1.7 Hz, 1H), 6.21 (d, *J*=1.7 Hz, 1H), 4.24 - 4.19 (m, 1H), 4.00 (t, *J*=10.3 Hz, 1H), 3.19 - 3.08 (m, 1H), 2.95 - 2.79 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ = 157.00, 154.96, 142.13, 130.54, 128.95, 127.92, 127.21, 112.83, 108.53, 102.97, 70.33, 38.42, 31.53; HRMS (ESI) calcd. for $C_{15}H_{14}O_2$ $[M+H]^+$ 227.10666, found: 227.10648.

7-hydroxy-3-phenylchroman-4-one (**79**)



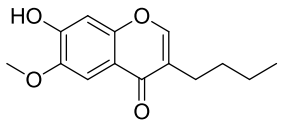
Compound 7-hydroxy-3-phenylchroman-4-one (**79**) was prepared according to the general procedure and the product was obtained as white solid in 70% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.62 (s, 1H), 7.68 (d, *J*=8.7 Hz, 1H), 7.36 - 7.24 (m, 5H), 6.52 (d, *J*=8.7 Hz, 1H), 6.35 (s, 1H), 4.70 - 4.57 (m, 2H), 4.04 (dd, *J*=8.1, 5.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 190.65, 165.06, 163.61, 136.64, 129.52, 129.15, 128.89, 127.64, 114.06, 111.21, 102.79, 71.54, 51.39; HRMS (ESI) calcd. for $C_{15}H_{12}O_3$ $[M+H]^+$ 241.08592, found: 241.08601.

3-(4-aminophenyl)-7-hydroxychroman-4-one (**80**)



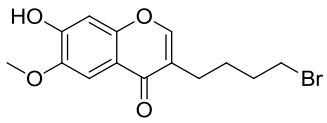
7-hydroxy-3-(4-nitrophenyl)-*4H*-chromen-4-one (100 mg) was dissolved in methanol (10 ml) and 10% palladium on carbon (15 mg) was added. The reaction mixture was hydrogenated for 10 hours at normal pressure and room temperature. It was then filtered through Celite and washed with methanol. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM:CH_3OH=100:1), the product was obtained as white solid in 22% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.55 (s, 1H), 7.65 (d, *J*=8.7 Hz, 1H), 6.88 (d, *J*=8.3 Hz, 2H), 6.56 - 6.45 (m, 3H), 6.33 (d, *J*=2.1 Hz, 1H), 5.00 (s, 2H), 4.56 - 4.48 (m, 2H), 3.76 (t, *J*=6.5 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ = 191.36, 164.85, 163.52, 148.25, 129.45, 123.24, 114.28, 114.11, 111.03, 102.72, 71.85, 50.65; HRMS (ESI) calcd. For $C_{15}H_{14}O_3N$ $[M+H]^+$ 256.09682, found: 256.09668.

3-butyl-7-hydroxy-6-methoxy-*4H*-chromen-4-one (**81**)



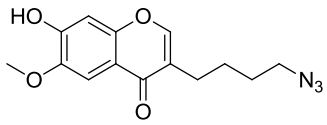
Compound 3-butyl-7-hydroxy-6-methoxy-*4H*-chromen-4-one (**81**) was prepared according to the general procedure and the product was obtained as white solid in 35% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.49 (s, 1H), 8.07 (s, 1H), 7.36 (s, 1H), 6.89 (s, 1H), 3.86 (s, 3H), 2.34 (t, *J*=7.5 Hz, 2H), 1.56 - 1.41 (m, 2H), 1.36 - 1.19 (m, 2H), 0.89 (t, *J*=7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 180.77, 157.90, 157.33, 157.27, 151.93, 127.81, 120.95, 109.52, 108.05, 61.01, 35.52, 29.99, 27.07, 18.98; HRMS (ESI) calcd. for $C_{14}H_{17}O_4$ $[M+H]^+$ 249.11214, found: 249.11201.

3-(4-bromobutyl)-7-hydroxy-6-methoxy-*4H*-chromen-4-one (**82**)



Compound 3-(4-bromobutyl)-7-hydroxy-6-methoxy-*4H*-chromen-4-one (**82**) was prepared according to the general procedure and the product was obtained as white solid in 30% yield; ^{1}H NMR (300 MHz, DMSO) δ = 10.51 (s, 1H), 8.11 (s, 1H), 7.36 (s, 1H), 6.89 (s, 1H), 3.86 (s, 3H), 3.65 (t, *J*=6.4 Hz, 2H), 2.37 (t, *J*=7.1 Hz, 2H), 1.84 - 1.53 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ = 175.96, 153.18, 152.79, 152.52, 147.19, 122.61, 116.16, 104.74, 103.30, 56.24, 45.64, 32.09, 25.90, 24.75; HRMS (ESI) calcd. for $C_{14}H_{16}O_4Br$ $[M+H]^+$ 327.02265, found: 327.02219.

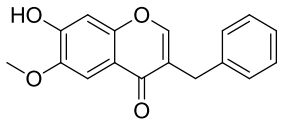
3-(4-azidobutyl)-7-hydroxy-6-methoxy-*4H*-chromen-4-one (**83**)



To a solution of 3-(4-bromobutyl)-7-hydroxy-6-methoxy-*4H*-chromen-4-one (**82**) (33 mg, 0.1 mmol) in DMF(2 ml), Sodium azide (39 mg, 0.6 mmol) was added. The reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was quenched with water, and then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica

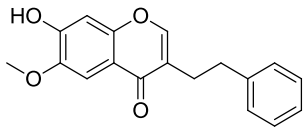
gel (eluting with DCM/CH_3OH (100 then 100/1)). the product was obtained as white solid in 67% yield; ^{1}H NMR (300 MHz, CD_3OD) δ = 8.00 (s, 1H), 7.50 (s, 1H), 6.89 (s, 1H), 3.96 (s, 3H), 3.37 - 3.29 (m, 2H), 2.54 - 2.45 (m, 2H), 1.72 - 1.61 (m, 4H); ^{13}C NMR (101 MHz, MeOD) δ = 177.54, 153.45, 153.28, 152.84, 147.03, 122.70, 115.88, 103.54, 102.47, 55.15, 50.81, 28.12, 25.62, 24.77; HRMS (ESI) calcd. for $C_{14}H_{16}O_4N_3$ $[M+H]^+$ 290.11325, found:290.11353.

3-benzyl-7-hydroxy-6-methoxy-*4H*-chromen-4-one (**84**)



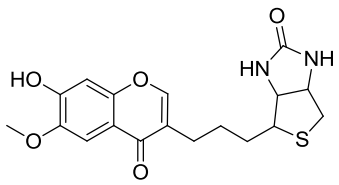
Compound 3-benzyl-7-hydroxy-6-methoxy-*4H*-chromen-4-one (**84**) was prepared according to the general procedure and the product was obtained as white solid in 36% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.53 (s, 1H), 8.20 (s, 1H), 7.34 (s, 1H), 7.31 - 7.23 (m, 4H), 7.16 (t, *J*=6.9 Hz, 1H), 6.90 (s, 1H), 3.84 (s, 3H), 3.68 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 180.41, 158.24, 158.04, 157.34, 152.03, 145.12, 133.74, 133.41, 131.19, 127.62, 120.97, 109.58, 108.11, 61.00, 35.94; HRMS (ESI) calcd. for $C_{17}H_{15}O_4$ $[M+H]^+$ 283.09649, found: 283.09628.

7-hydroxy-6-methoxy-3-phenethyl-*4H*-chromen-4-one (**85**)



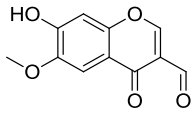
Compound 7-hydroxy-6-methoxy-3-phenethyl-*4H*-chromen-4-one (**85**) was prepared according to the general procedure and the product was obtained as white solid in 33% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.52 (s, 1H), 7.98 (s, 1H), 7.39 (s, 1H), 7.31 - 7.25 (m, 2H), 7.21 - 7.15 (m, 3H), 6.88 (s, 1H), 3.87 (s, 3H), 2.82 (t, *J*=7.6 Hz, 2H), 2.64 (t, *J*=7.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 180.73, 157.98, 157.64, 157.26, 151.99, 146.64, 133.57, 133.53, 131.10, 126.99, 120.96, 109.52, 108.08, 61.02, 39.22, 32.38; HRMS (ESI) calcd. for $C_{18}H_{17}O_4$ $[M+H]^+$ 297.11214, found: 297.11191.

4-(3-(7-hydroxy-6-methoxy-4-oxo-*4H*-chromen-3-yl)propyl)tetrahydro-*1H*-thieno[3,4-d]imidazol-2(3H)-one (**86**)



Compound (**86**) was prepared according to the general procedure and the product was obtained as white solid in 8.5% yield; ^{1}H NMR (400 MHz, DMSO) δ = 10.50 (s, 1H), 8.06 (s, 1H), 7.36 (s, 1H), 6.89 (s, 1H), 6.43 (s, 1H), 6.35 (s, 1H), 4.44 - 4.26 (m, 1H), 4.20 - 4.12 (m, 1H), 3.86 (s, 3H), 3.20 - 3.09 (m, 1H), 2.83 (dd, *J*=12.4, 4.9 Hz, 1H), 2.58 (d, *J*=12.4 Hz, 1H), 2.37 (t, *J*=6.7 Hz, 2H), 1.68 - 1.43 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ = 175.39, 162.58, 152.60, 152.19, 151.92, 146.60, 122.01, 115.59, 104.18, 102.71, 60.97, 59.08, 55.67, 55.16, 40.11, 27.85, 27.47, 24.92; HRMS (ESI) calcd. for $C_{18}H_{21}O_5N_2S$ $[M+H]^+$ 377.11657, found: 377.11627.

7-hydroxy-6-methoxy-4-oxo-*4H*-chromene-3-carbaldehyde (**87**)



A solution of 1-(2,4-dihydroxy-5-methoxyphenyl)ethanone (182 mg, 1 mmol) in DMF (2.5 ml) was prepared in a 25 ml three-neck round-bottom flask under argon and cooled down to -78 ℃. Then phosphorus oxychloride (0.275 ml, 3 mmol) was added dropwise. The mixture was stirred at room temperature for 24 h before the reaction was quenched by addition of H_2O (5 ml) and then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (DCM/CH_3OH =100/1). the product was obtained as brown solid in 81% yield; ^{1}H NMR (400 MHz, DMSO) δ = 11.19 - 11.02 (m, 1H), 10.13 (s, 1H), 8.78 (s, 1H), 7.43 (s, 1H), 7.14 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 189.31, 174.26, 162.60, 154.37, 152.16, 148.16, 119.66, 117.11, 104.95, 104.06, 56.40; HRMS (ESI) calcd. for $C_{11}H_9O_5$ $[M+H]^+$ 221.04445, found: 221.04411.

2. Measurement of photo-physical properties

2.1 Comparison of fluorescence intensity under UV light[3]

All compounds were assigned to various groups according to the structures, they were dissolved in 0.1M Tris-HCl (pH 8.0) at the concentration of 100 μM. 200 μl of each compound solution was added into 96-well plate and was imaged under UV light to compare the fluorescence intensity. Monochrome photo was taken by monochrome digital CDD camera from the system of ChampGel-500^{TM} of SAGECREATION company and color photo was imaged by Canon EOS 600D color digital camera.

(1). Comparison of fluorescence intensity of ***natural products of isoflavones*** (100 μM) in 0.1 M Tris-HCl, pH8.0

Isoflavones (**6**, **7**, **8** and **12**) were observed weak fluorescence intensity under the UV light, but isoflavones (**9**, **10**, **11**, **13**, **14** and **15**) do not show any fluorescence. A trendy can be summarized that 7-hydroxyl group is important to activate the fluorescence of isoflavone core, and 5-hydroxyl will quench the fluorescence of 7-hydroxy-isoflavone.

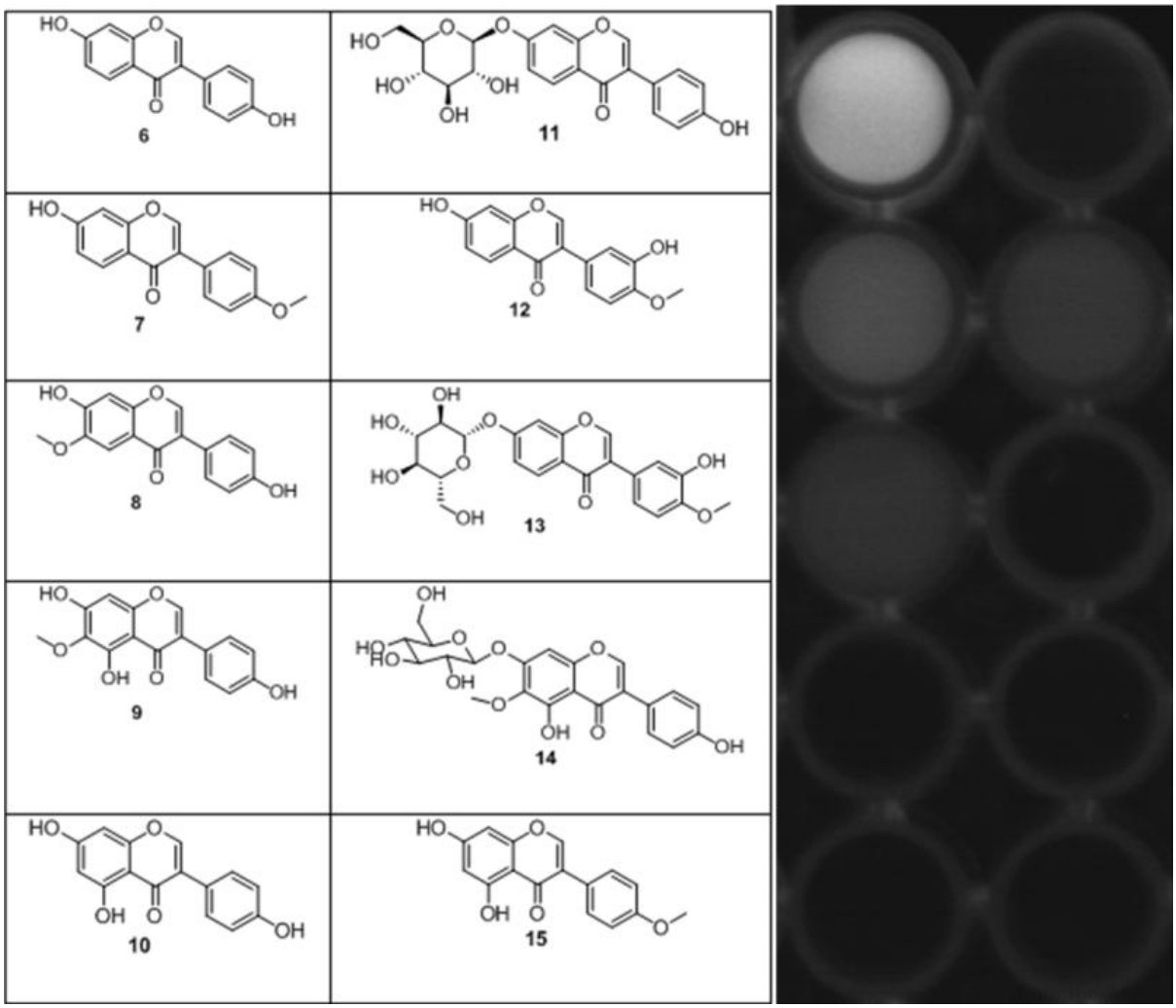


Figure S1: Screening fluorescence intensity of isoflavones (**6-15**) in a microtiter plate under UV light.

(2). Comparison of fluorescence intensity of ***natural products of flavones*** (100 μM) in 0.1M Tris-HCl, pH8.0

All flavones or similar analogues (**16**-**24**) tested in this study do not show the fluorescence intensity under UV light. However, most of flavones in this study also contain a 5-OH group, which is known to cause the flavones and isoflavones hard to be activated.

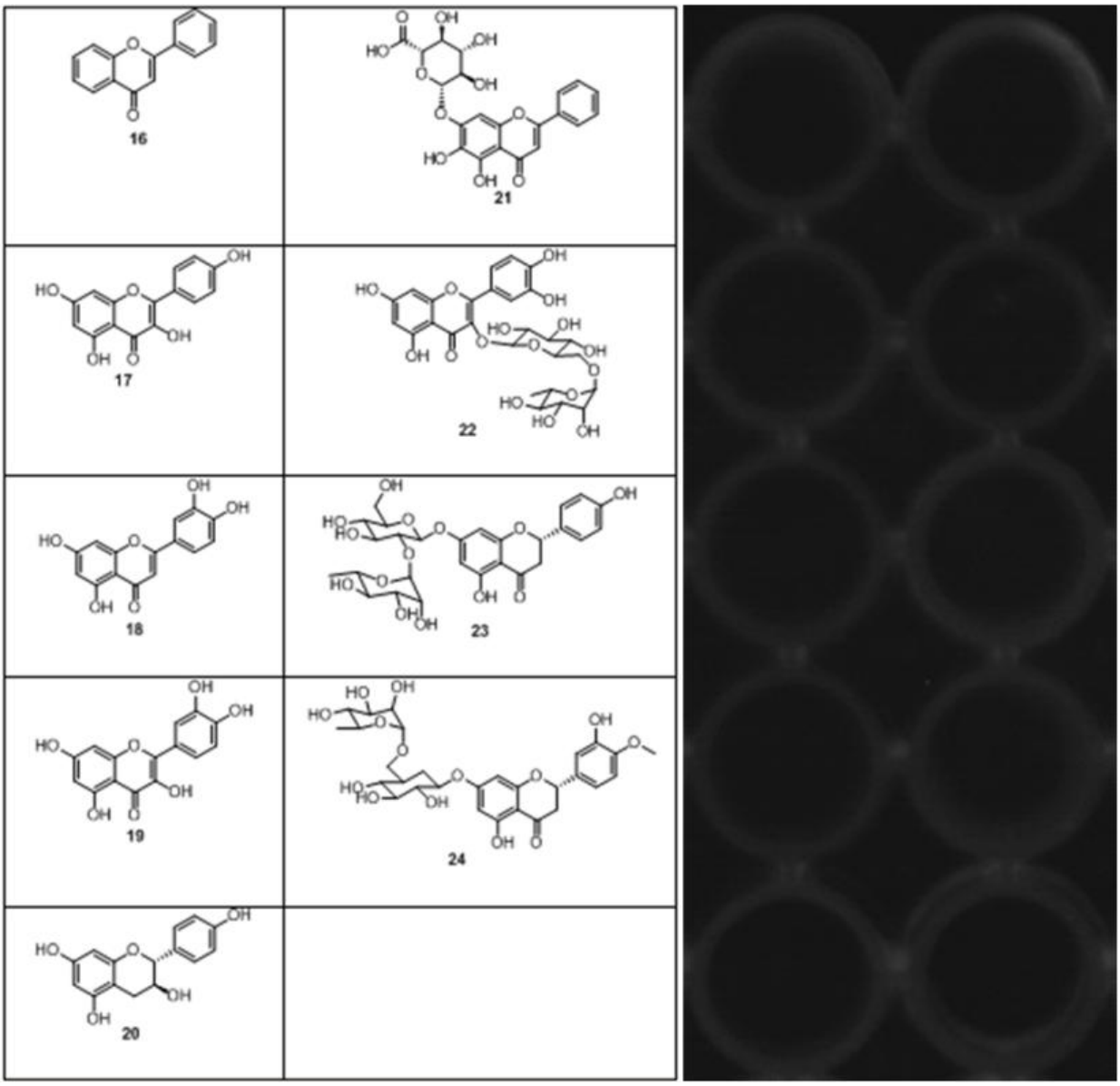


Figure S2: Screening fluorescence intensity of flavones or analogues (**16-24**) in a microtiter plate under UV light.

(3). Comparison of fluorescence intensity of ***3-hydroxy-2-phenylchromone (3-hydroxyflavone, 1)*** and ***7-hydroxy-3-phenylchromone (7-hydroxyisoflavone, 2)*** (100 μM) in 0.1M Tris-HCl, pH8.0

3-Hydroxy-flavone **1** and 7-hydroxy-isoflavone **2** were previously reported as fluorogenic chromone derivatives. However, **1** has a bad aqueous solubility and thus show a rather weak fluorescence. In comparison, **2** can be well dissolved in 0.1M Tris-HCl, pH 8.0, and thus show a relative strong fluorescence (Figure S3a).

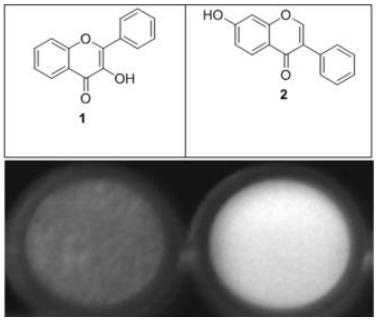


Figure S3a: Screening fluorescence intensity of **1** and **2** in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

In order to better access of fluorescence intensity of **1** and **2**, both of them were dissolved in 0.1M Tris-HCl, pH 8.0 with 1% BSA. 1% BSA was used to assist **1** and **2** to be well dissolved. Interestingly, under this condition, **1** shows stronger fluorescence intensity than **2** (Figure S4b). This indicates that the rather weak fluorescence of **1** in 0.1M Tris-HCl, pH 8.0 is due to the solubility issue.

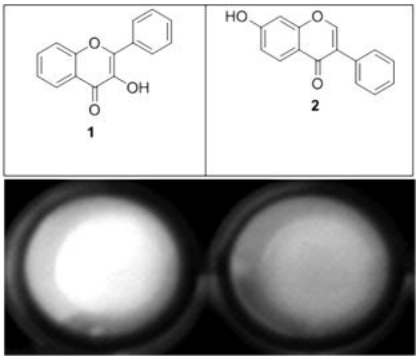


Figure S3b: Screening fluorescence intensity of **1** and **2** in 1% BSA, 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(4). Comparison of fluorescence intensity of ***7-substituted 3-phenylchromone*** (100 μM) in 0.1M Tris-HCl, pH8.0

7-position: 7-hydroxyl is critical for 3-phenylchromone (isoflavone) of being fluorogenic. Once 7-OH was acetylated or methylated, fluorescence will be subsequently decreased or quenched from 3-phenylchromone core.

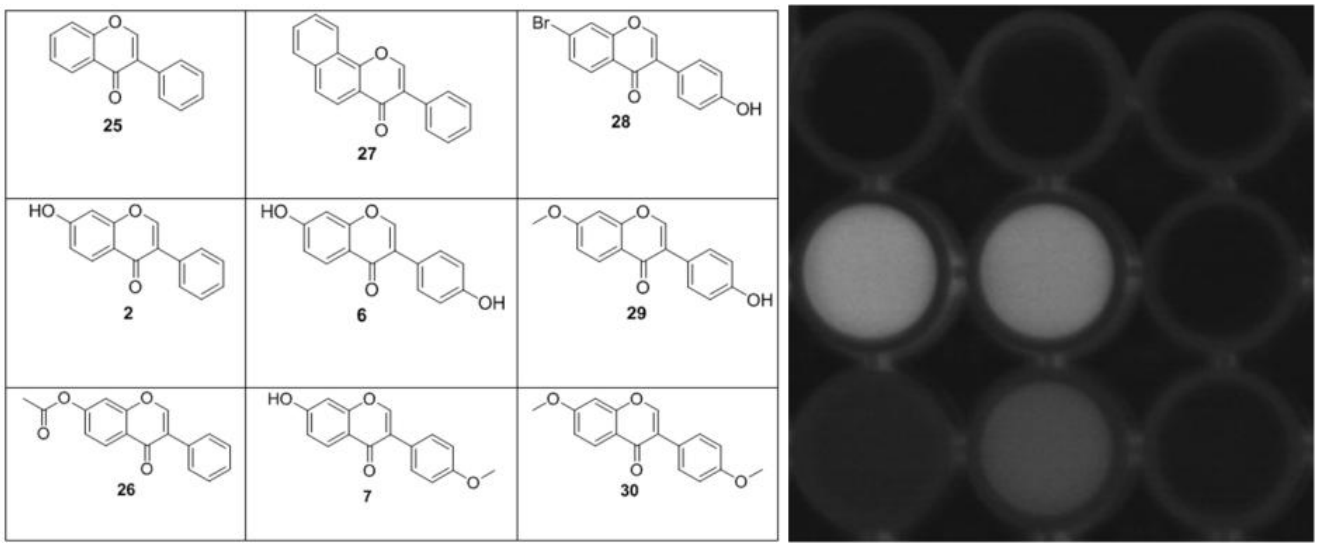


Figure S4: Screening fluorescence intensity of 7-substituted 3-phenylchromone in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(5a). Comparison of fluorescence intensity of ***6-substituted 3-phenylchromone*** (100 μM) in 0.1M Tris-HCl, pH8.0

6-position: 6-OMe alone is able to afford 3-phenylchromone a decent fluorescence quantum yield (Φ=0.21), but this does not work for other substituents (6-OH, 6-CH_3) at this position. Unfortunately, due to solubility issue, 6-methyoxy-3-phenylchromone (100 μM in 0.1M Tris-HCl buffer, pH8.0) displays weak fluorescence intensity under UV light.

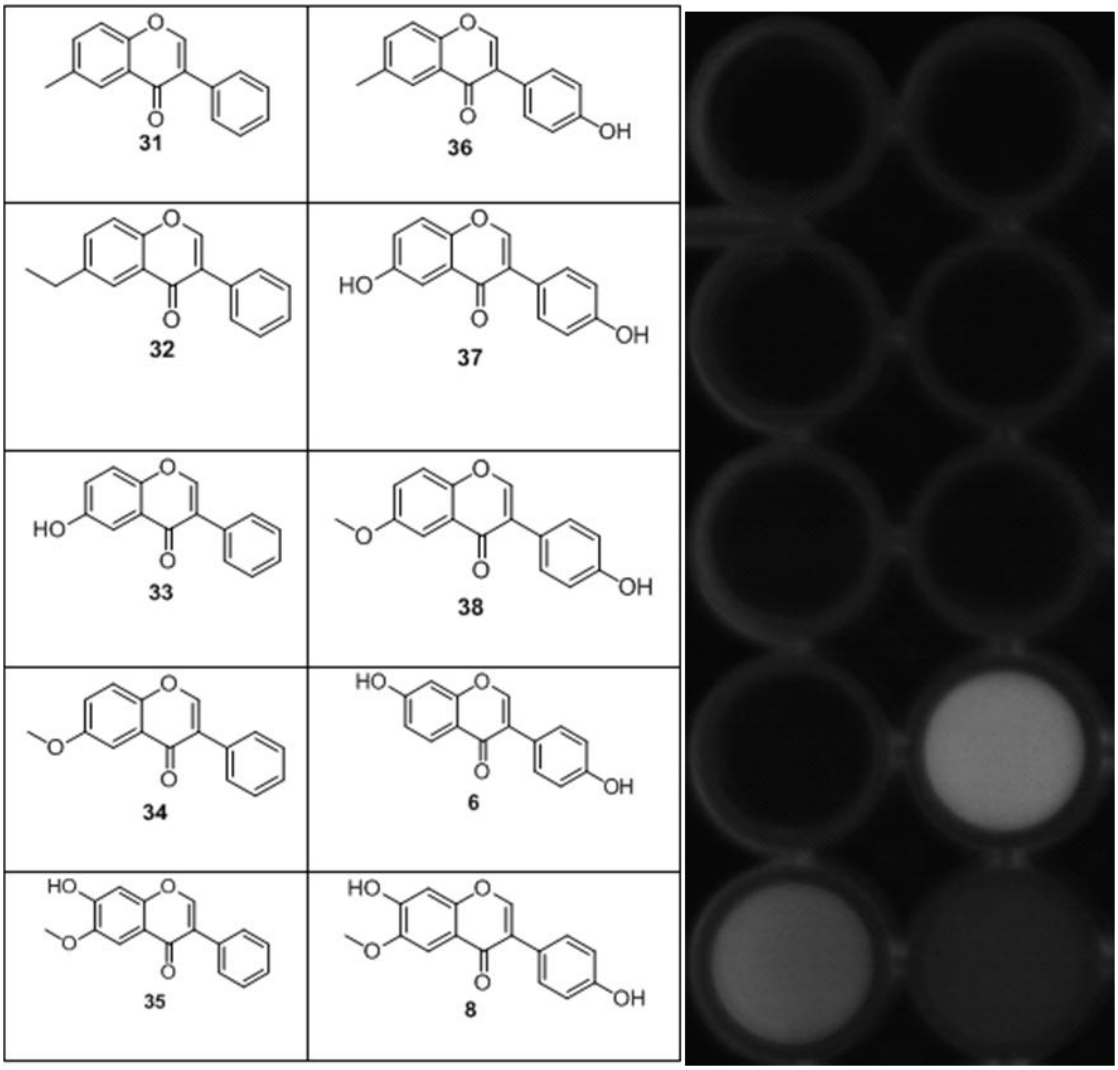


Figure S5a: Screening fluorescence intensity of 6-substituted 3-phenylchromone in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(5b). Comparison of fluorescence intensity of ***6-substituted 3-phenylchromone*** (100 μM) in **1% BSA** 0.1M Tris-HCl buffer, pH8.0

1% BSA was added into the buffer to facilitate the dissolution of 6-methyoxy-3-phenyl-chromone **34**, an improved fluorescence intensity was observed compared to that in figure 5a. In addition, 6-OMe marginally affects fluorescence quantum yield of 7-hydroxy-3-phenylchromone.

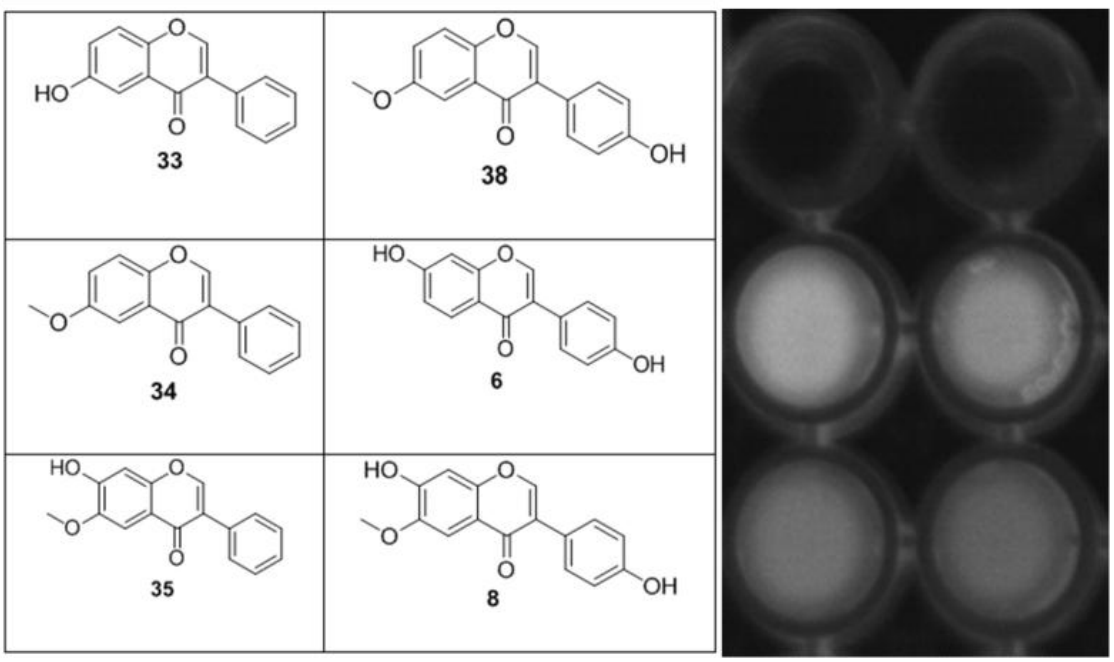


Figure S5b: Screening fluorescence intensity of 6-substituted 3-phenylchromone in 1% BSA, 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(6). Comparison of fluorescence intensity of ***5-substituted 3-phenylchromone*** (100 μM) in 0.1M Tris-HCl, pH8.0

5-position: 5-hydroxyl interacts with 4-carbonyl group and causes the flavones and isoflavones hard to be activated,[4] this results in no fluorescence of 7-hydroxy-3-phenyl-chromone. All of the 5-substituted 3-phenylchromone are not fluorogenic.

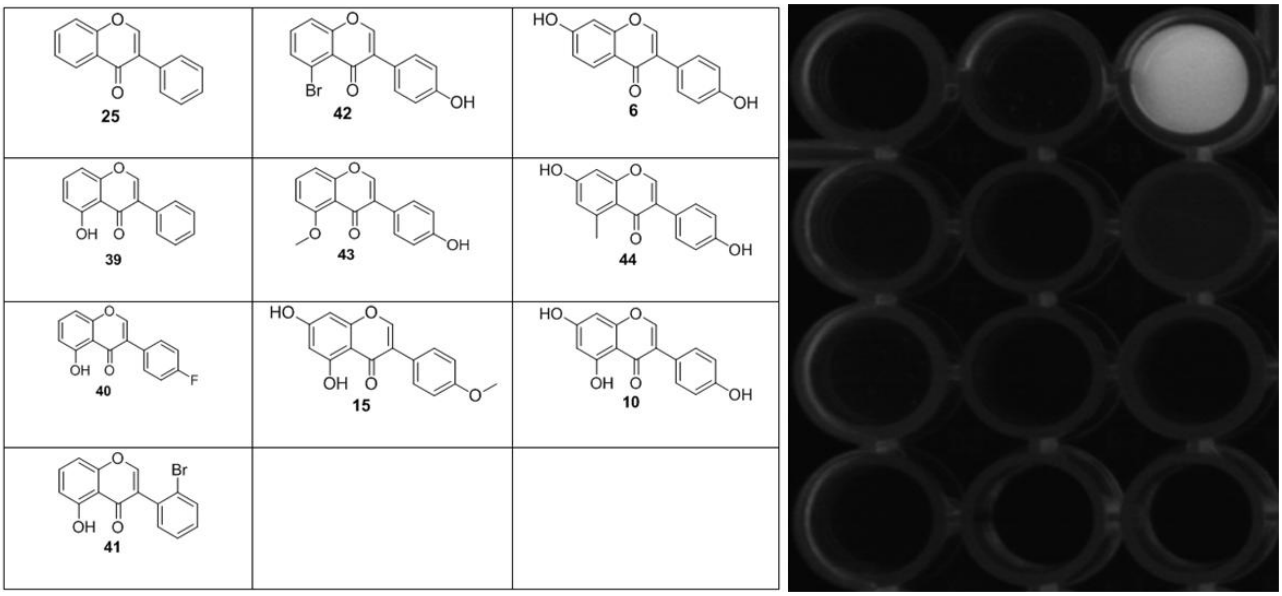


Figure S6: Screening fluorescence intensity of 5-substituted 3-phenylchromone in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(7). Comparison of fluorescence intensity of ***8-substituted 3-phenylchromone*** (100 μM) in 0.1M Tris-HCl, pH8.0

8-position: 8-substituent is not favourable to increase fluorescence quantum yield of 7-hydroxy-3-phenylchromone. 8-OH can also quench fluorescence of 7-hydroxy-3-phenyl-chromone core.

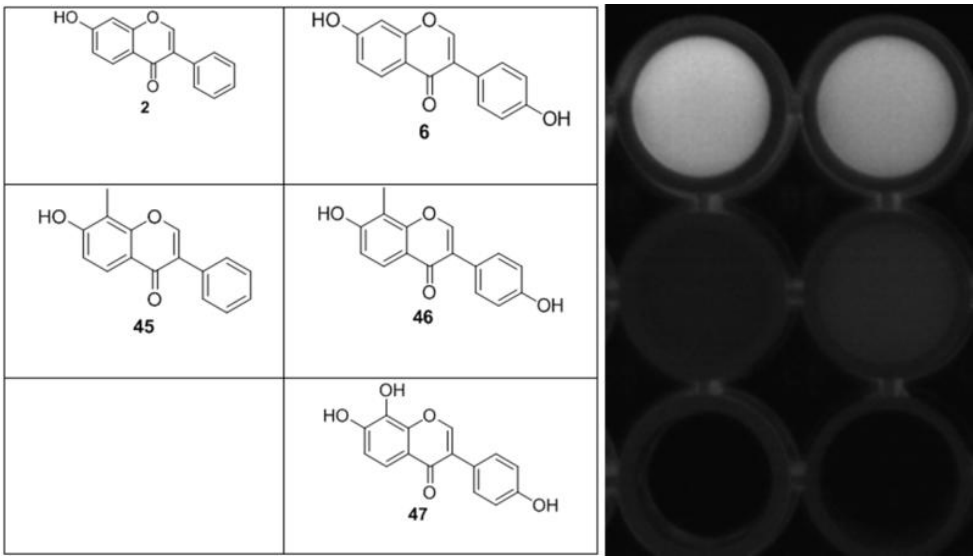


Figure S7: Screening fluorescence intensity of 8-substituted 3-phenylchromone in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(8). Comparison of fluorescence intensity of ***3-substituted-phenyl-7-hydroxychromone*** (100 μM) in 0.1M Tris-HCl, pH8.0

3-position: for 7-hydroxy-isoflavone core, *2', 3', 4'* positions of 3-phenyl group were substituted with various groups to evaluate the effect of them towards fluorescence. The results show that 2' substituents of electro-donating groups (CH_3 > OMe > Cl) can increase fluorescence intensity of 7-hydroxy-isoflavone core.

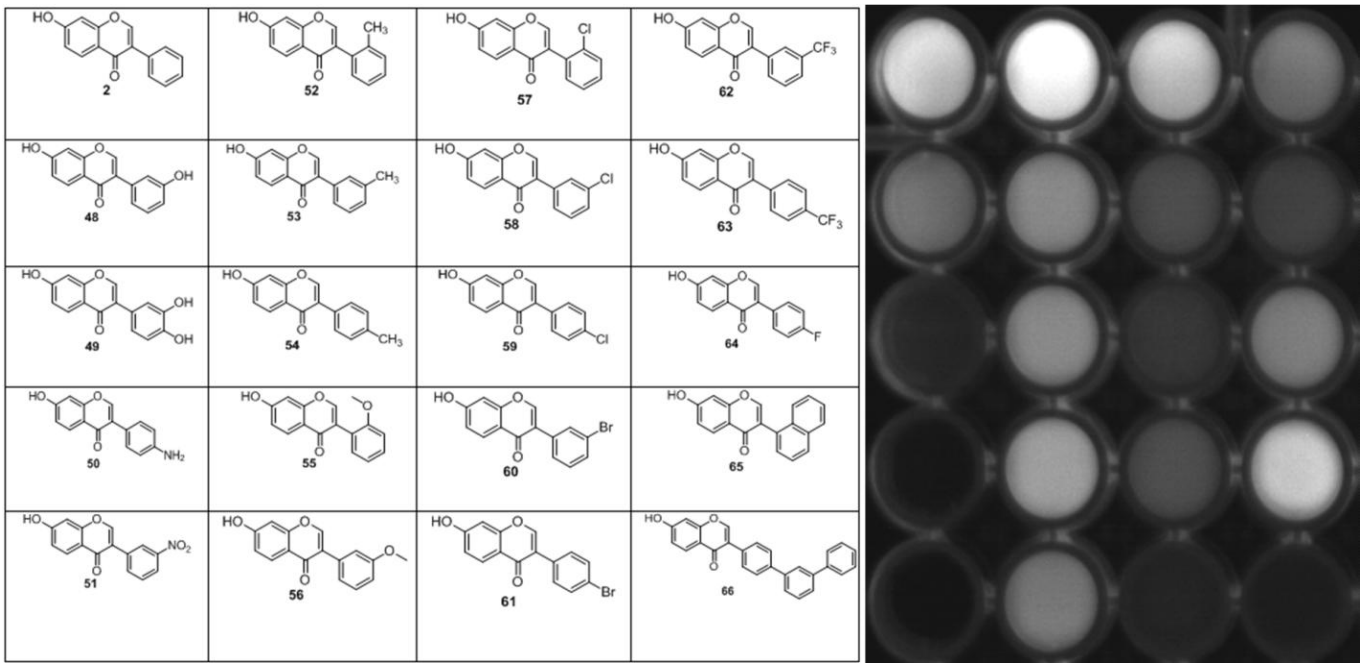


Figure S8: Screening fluorescence intensity of 3-substituted-phenyl-chromone in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(9a). Comparison of fluorescence intensity of ***3-alkyl-7-hydroxychromone*** (100 μM) in 0.1M Tris-HCl, pH8.0

To our surprise, 3-alkylation of chromone made this series of compounds increased fluorescence intensity. But **75** has a rather low fluorescence intensity, which is due to low solubility.

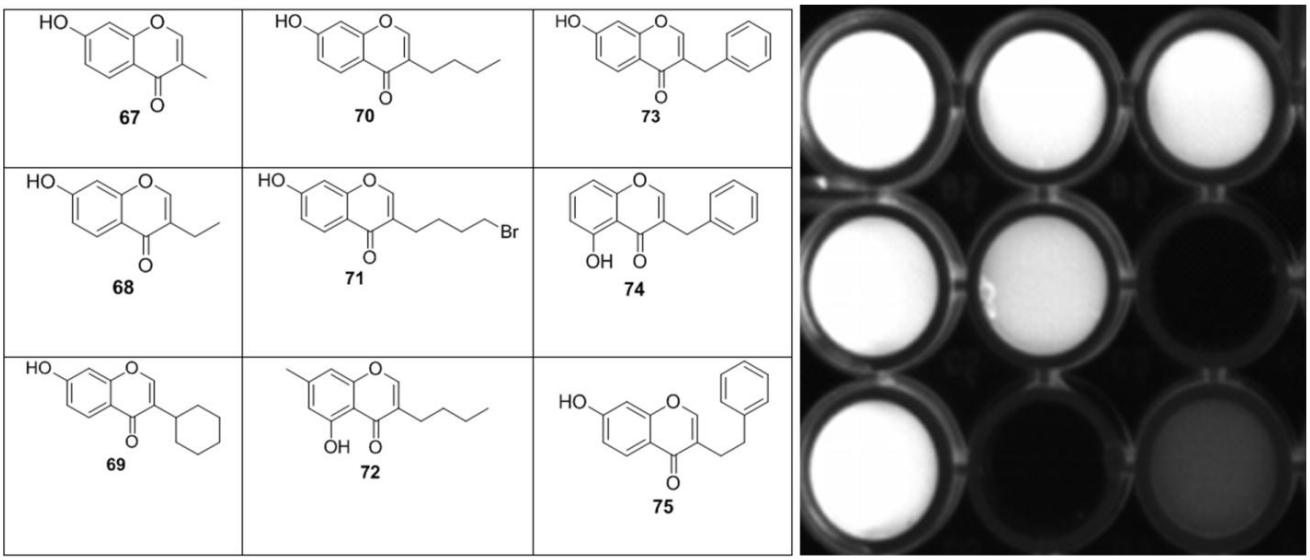


Figure S9a: Screening fluorescence intensity of 3-alkyl-chromone in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(9b). Comparison of fluorescence intensity of ***3-alkyl-7-hydroxychromone*** (100 μM) in 1% BSA 0.1M Tris-HCl, pH8.0

In order to prove the weak fluorescence of **75**, 3-alkyl-7-hydroxychromone analogues were dissolved in 1% BSA 0.1M Tris-HCl, pH8.0 to compare fluorescence intensity. We observed **75** with improved fluorescence intensity.

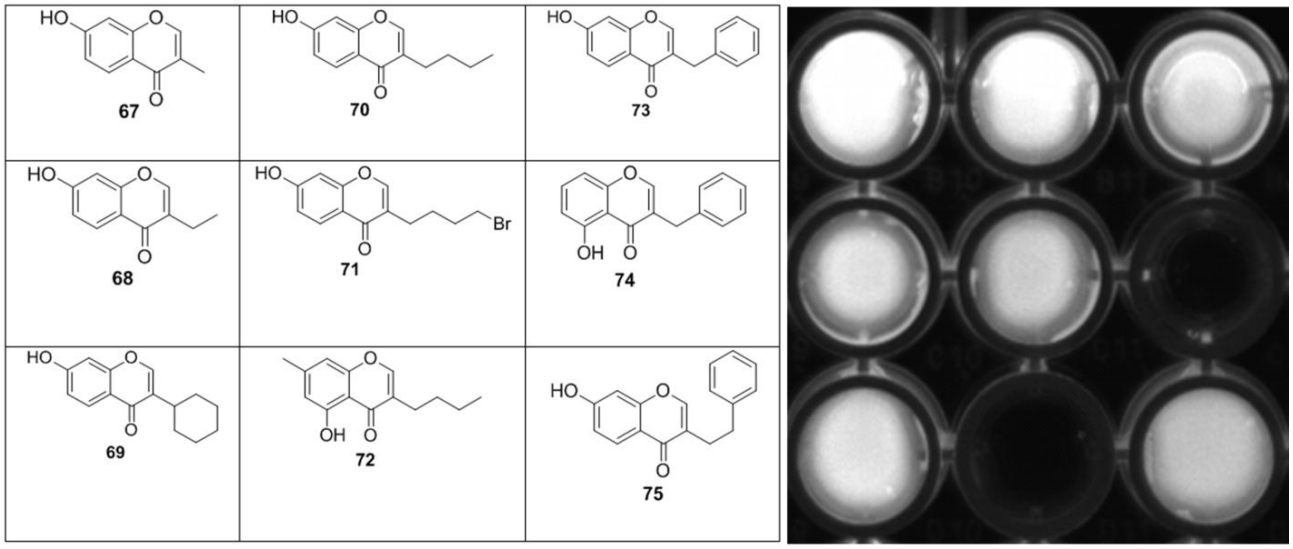


Figure S9b: Screening fluorescence intensity of 3-alkyl-chromone in 1% BSA 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(10). Comparison of fluorescence intensity of ***7-hydroxychromone, 3-methyl-7-hydroxychromone, 2-methyl-7-hydroxychromone*** (100 μM) in 0.1M Tris-HCl, pH8.0

7-hydroxychromone **76**, 3-methyl-7-hydroxychromone **67**, 2-methyl-7-hydroxychromone **77** were compared the fluorescence intensity, and found that **67** and **77** show slightly improved fluorescence intensity than **76**. Thus, the alkylation of 2 and 3 position of chromone can help 7-hydroxychromone core to improve the fluorescence. However, 3-methyl-7-hydroxychromone **67** and 2-methyl-7-hydroxychromone **77** are in a similar range of fluorescence intensity.

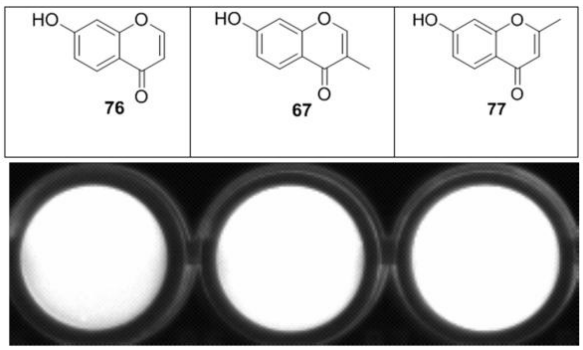


Figure S10: Screening fluorescence intensity of 7-hydroxychromone, 3-methyl-7-hydroxychromone, 2-methyl-7-hydroxychromone in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(11). Comparison of the fluorescence intensity of ***chroman, chroman-4-one*** (100 μM) in 0.1M Tris-HCl, pH8.0

2, 3-double bond were reduced to break the conjugated system, and formed chroman and chroman-4-one. These three new formed compounds do not show any fluorescence.

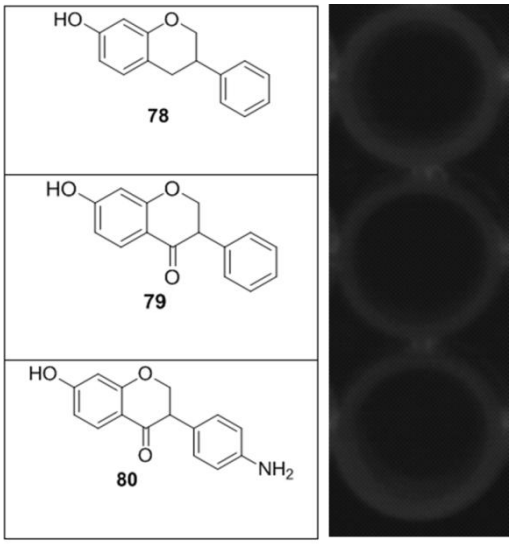


Figure S11: Screening fluorescence intensity of chroman, chroman-4-one in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(12). Comparison of fluorescence intensity of ***3-alkyl-6-methoxy-7-hydroxychromone*** (100 μM) in 0.1M Tris-HCl, pH8.0

6-Methoxyl substitution significantly increases the fluorescence of 3-alkyl-7-hydroxychromone core. A further improved fluorescence intensity was observed for the newly designed 3-alkyl-6-methoxy-7-hydroxychromone derivatives. For compound **87**, the carbonyl group next to 3 position affects the conjugated system of chromone, so compound **87** has low fluorescence intensity.

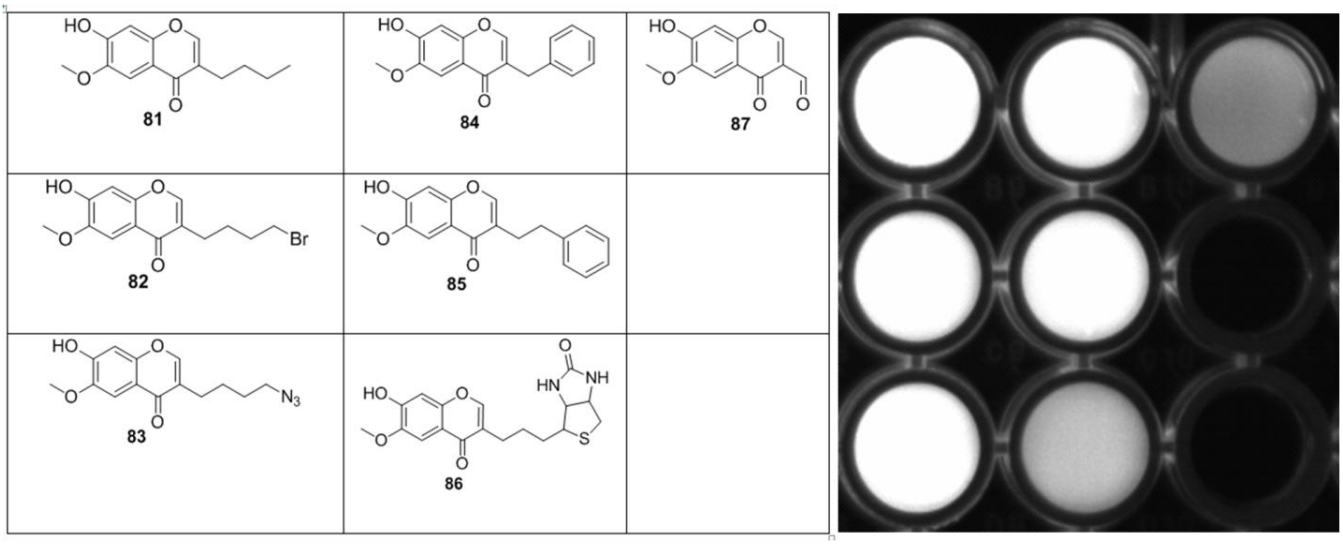


Figure S12: Screening fluorescence intensity of 3-alkyl-6-methoxy-7-hydroxychromone in 0.1M Tris-HCl, pH8.0 in a microtiter plate under UV light.

(13). Compound details that were imaged in Figure 2

Table S1. Compound details for Figure 2.

6	44	49	57	63	67	81	AMC
7	2	52	58	64	68	82	
8	45	53	59	65	69	83	
	35	54	60	66	71	84	
	46	55	61		70	85	
	48	56	62		73		

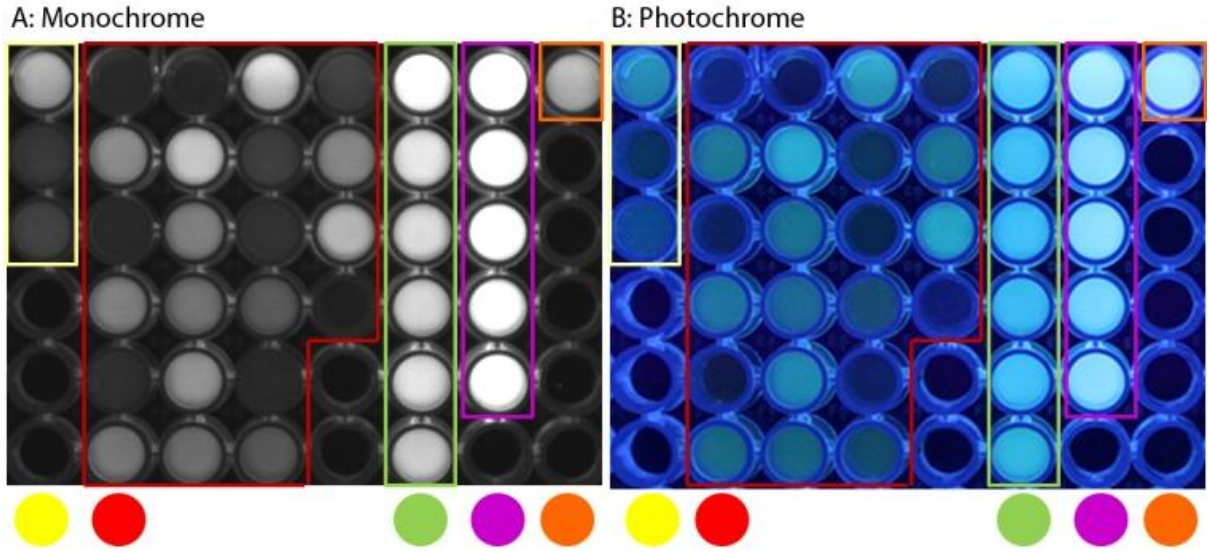


Figure 2. Screening fluorescence intensity of selected compounds (100 μM) in 0.1M Tris-HCl, pH 8.0 in a microtiter plate. A: Imaged by monochrome camera, B: Imaged by color camera. Yellow label, natural products of isoflavones; Red label, 3-phenyl-7-hydroxychromone analogues; Green label, 3-alkyl-7-hydroxychromone derivatives; Purple label, 3-alkyl-6-methoxy-7-hydroxychromone derivatives; Orange label: AMC.

2.2 Measurement of photophysical properties

Compounds that show the fluorescence under UV light were further measured fluorescence quantum yield, molar extinction coefficient, excitation and emission spectra, fluorescence lifetime (Table S2 and S3).[5] UV-visible spectra were acquired with SHIMADU UV-2700, UV-VIS Spectrophotometer. Fluorescence quantum yield, excitation and emission spectra were measured and calculated with HITACHI F-7000 Fluorescence Spectrophotometer. Fluorescence lifetime was measured with Edinburgh Analytical Instruments F900.

To obtain fluorescence excitation and emission spectra, we dissolved compounds **6** and **81** in 0.1M Tris-HCl buffer, pH 8.0 at the concentration of 10 μM. For compound **6**, we set the λ_{em}= 468 nm to get the emission spectra, we then set λ_{ex}= 338 nm to obtain the excitation spectra. For compound **81**, we set the λ_{em}= 447 nm to get the emission spectra, we then set λ_{ex}= 345 nm to obtain the excitation spectra (Figure S13).

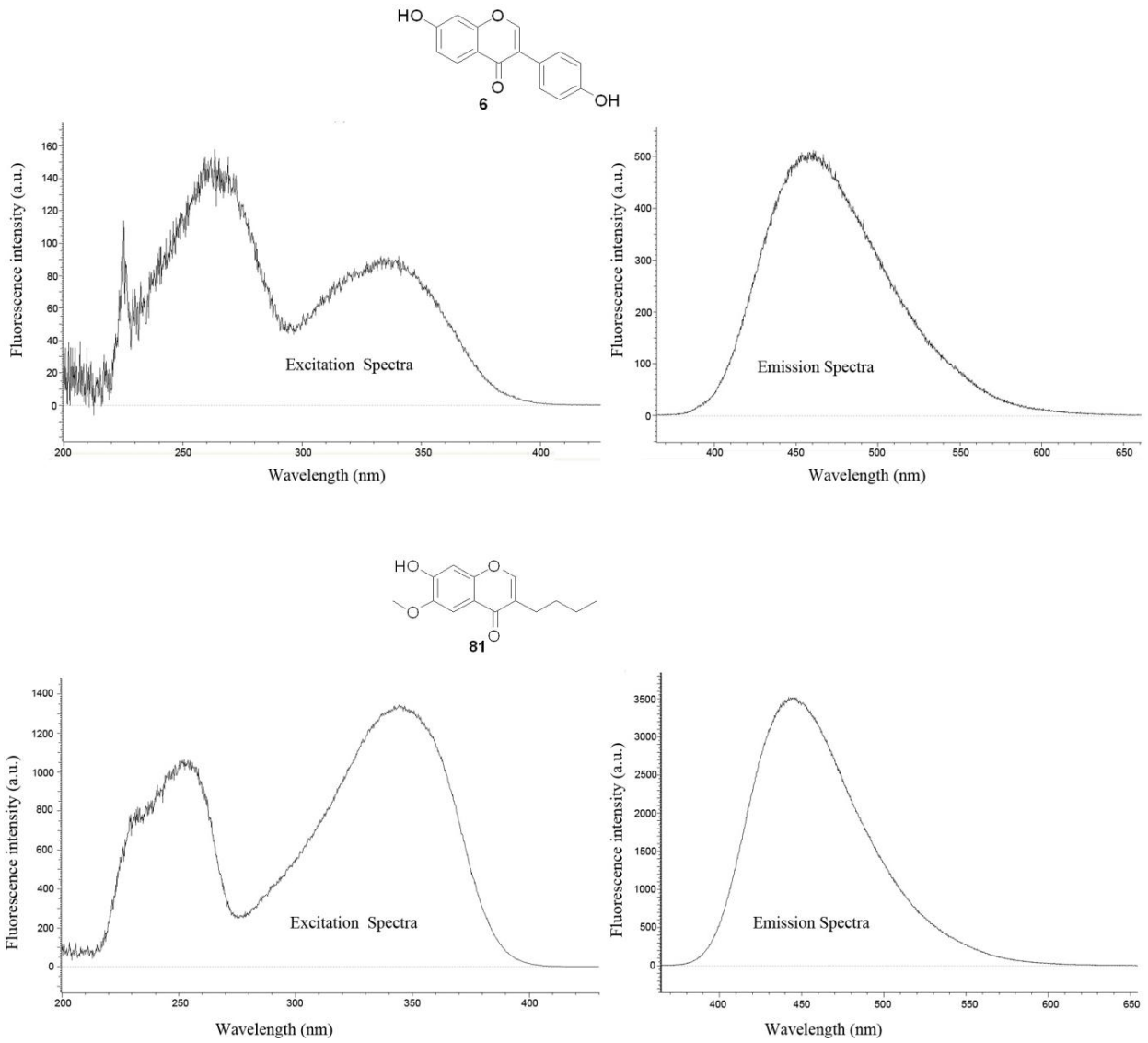


Figure S13: Excitation and Emission spectra of compound **6** and **81** (10 μM) in 0.1M Tris-HCl, pH 8.0.

For the absorption spectra, we dissolved compounds **6** and **81** in 0.1M Tris-HCl buffer, pH 8.0 at the concentration of 10 μM and measured under SHIMADU UV-2700, UV-VIS Spectrophotometer to record the absorption spectra of these two compounds between 190-600 nm. (Figure S14)

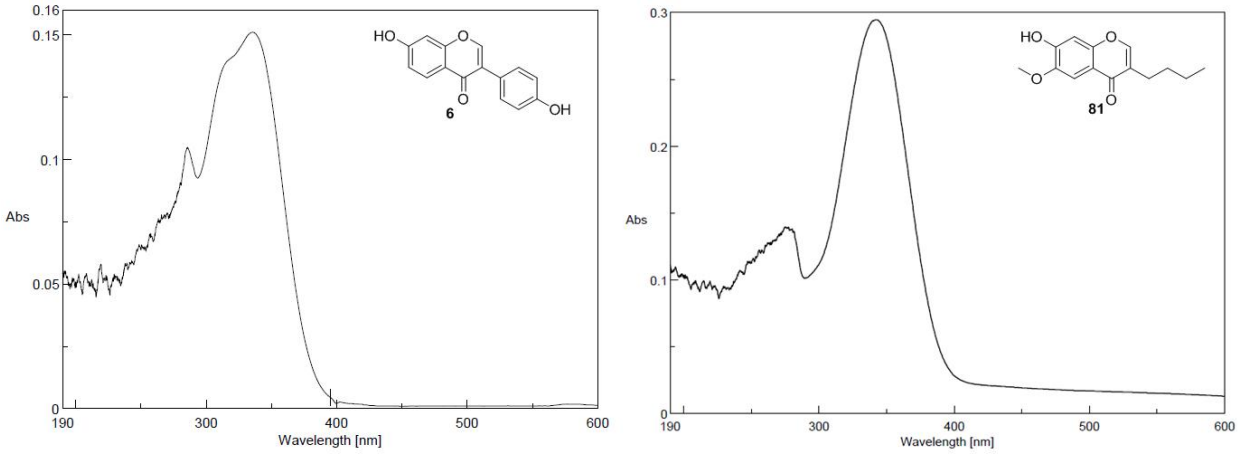


Figure S14: Absorption spectra of compounds **6** and **81** in 0.1M Tris-HCl, pH 8.0.

Fluorescence quantum yield was recorded using a comparative method of Williams *et al*.[5] The detection was carried out in 0.1M Tris-HCl buffer (pH 8.0) at the concentration of 0.5 μg/ml using quinine sulfate (0.5 μg/L in 0.1M H_2SO_4, Φ=0.54) as a reference. The quantum yield was calculated using the following equation:

$$\Phi_X = \Phi_{ST}\,(A_{ST}F_x/A_XF_{ST})(n_X/n_{ST})^2$$

Where the subscripts X and ST denote test and standard respectively, Φ is the fluorescence quantum yield, *A* is the absorbance at the excitation wavelength, *F* is the area under the emission curve, and *n* is the refractive index of the solvents used. For the tested compounds and the standard, the excitation wavelength was at 345nm while keeping the absorption below 0.05.

Absorbance maximum wavelength, molar extinction coefficient at absorbance max, and excitation, emission maximum wavelength of each compound (10 μM) were also measured in aqueous buffer, 0.1 M Tris-HCl, pH 8.0.

Measurement of lifetime in 0.1M Tris-HCl buffer, pH8.0: Compounds were dissolved in 0.1M Tris-HCl buffer, pH 8.0 at the concentration of 10 μM. Values of compound lifetimes in 0.1M Tris-HCl buffer, pH 8.0 were obtained by using the Edinburgh Analytical Instruments F900. The excitation wavelength was set at λ_{ex} = 375 nm; τ is the characteristic lifetime of the presumed exponential model. For a single exponential decay model, τ would be the time it takes to decay from the initial amplitude to a value of 37% of this amplitude (1/e = 0.3675). For a

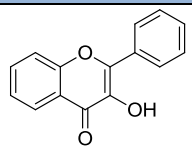
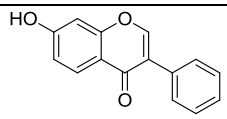
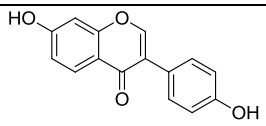
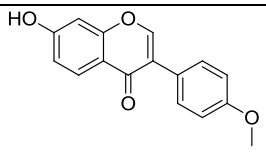
model with multiple exponential terms one can define an "average lifetime" <τ>. The average lifetime is calculated using the displayed parameters:

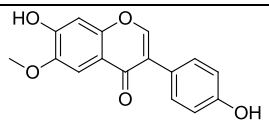
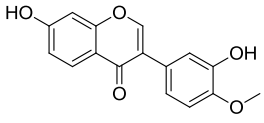
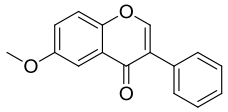
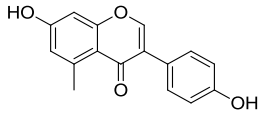
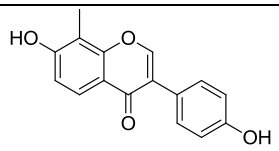
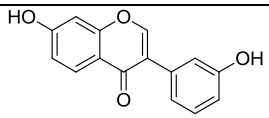
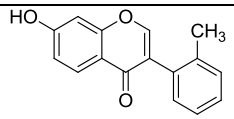
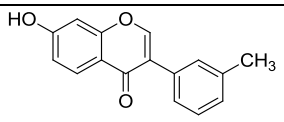
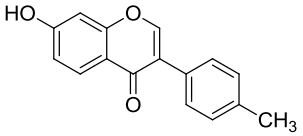
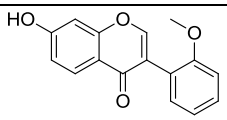
$$\langle \tau \rangle = \frac{B_1 \tau_1^2 + B_2 \tau_2^2}{B_1 \tau_1 + B_2 \tau_2}$$

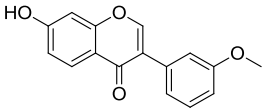
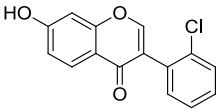
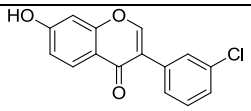
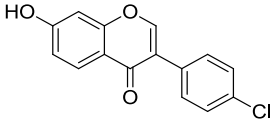
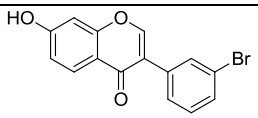
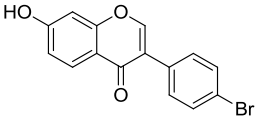
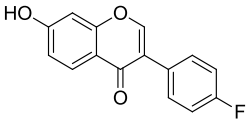
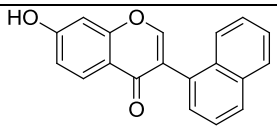
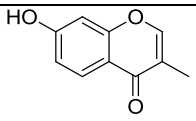
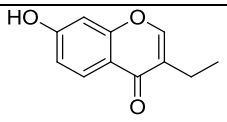
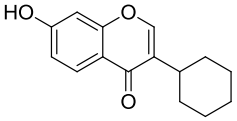
Table S2: Measurement of the fluorescence lifetime of selected compounds

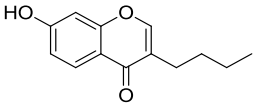
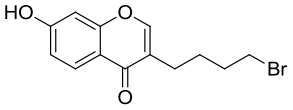
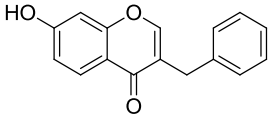
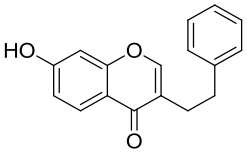
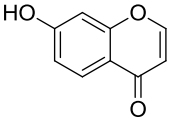
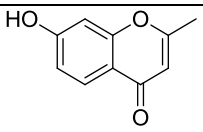
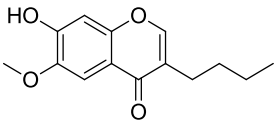
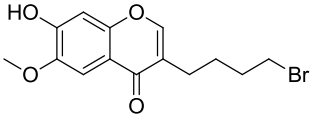
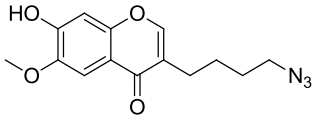
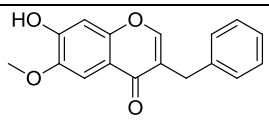
Cmpd	τ_1	τ_2	B_1	B_2	<τ>
6	1.49E-09	5.14E-09	3.60E-02	6.76E-04	1.72 ns
34	1.37E-09	6.31E-09	1.37E-09	1.32E-02	5.24 ns
70	3.41E-09	5.00E-08	2.90E-02	-5.30E-05	2.11 ns
81	4.49E-09		2.71E-02		4.49 ns
83	4.31E-09		2.75E-02		4.31 ns

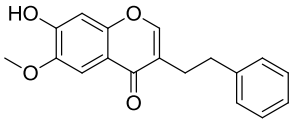
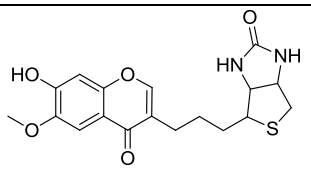
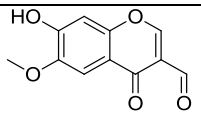
Table S3: Measurement of the photo-physical properties of selected compounds

Cmpd	Structure	λ_{abs} nm	ε $M^{-1}cm^{-1}$	λ_{ex1} nm	λ_{ex2} nm	λ_{em} nm	Φ
1		346	6300	260	363	408	0.10
2		336	12900	265	333	465	0.06
6		311	12600	261	338	468	0.05
7		287	13300	263	338	468	0.05

8		347	12500	259	346	460	0.04
12		334	15700	259	339	472	0.03
34		288	7300	252	335	419	0.21
44		287	17900	269	326	468	0.03
46		287	16500	269	352	496	0.02
48		287	13500	265	336	469	0.05
52		337	17400	261	337	462	0.10
53		336	14200	266	334	469	0.05
54		336	14300	265	339	470	0.05
55		335	14600	294	430	467	0.06

56		337	14000	263	430	469	0.05
57		338	15700	259	339	469	0.05
58		337	16100	264	340	469	0.04
59		286	9300	265	340	459	0.04
60		337	13000	298	340	471	0.05
61		287	11800	266	339	474	0.04
64		287	10000	262	338	472	0.07
65		288	13200	260	340	462	0.10
67		334	13500	259	336	456	0.21
68		334	14200	259	333	456	0.21
69		334	13600	257	334	456	0.27

70		334	12700	258	333	454	0.21
71		336	4600	257	334	456	0.19
73		335	14000	259	334	463	0.19
75		332	6300	258	334	453	0.30
76		330	10200	257	334	461	0.19
77		333	16200	255	333	448	0.21
81		345	16400	252	345	447	0.48
82		346	12200	251	343	447	0.54
83		346	16300	251	342	445	0.41
84		346	16900	255	346	449	0.47

85		346	18000	251	343	445	0.47
86		346	12800	251	346	447	0.56
87		353	17900	254	365	466	0.06

[a], Determined with quinine sulfate (Φ =0.54, 0.1 M H_2SO_4) as reference. [5]

[b], Under the condition of pH of 8.0, two excitation wavelength peaks can be observed for this series of compounds, and both of them were recorded. This is agreed with previous study of photo-physical properties of isoflavones.[4]

3. Biology

3.1 Cell Viability Assay

Human colon cancer cell HCT-116, human liver cancer cell HepG2, human gastric cancer cell BGC-823, human lung cancer cell NCI-H1650, and human ovary cancer cell A2780 were obtained from cell center of Chinese Academy of Medical Sciences & Peking Union Medical College. They were cultured in DMEM medium (Invitrogen) with 10% fetal bovine serum (Gibco) at 37 ℃ with 5% CO_2.

Five compounds (**81**, **82**, **83**, **84** and **85**) from 3-alkyl-6-methoxyl-7-hydroxychromone (AMHC) series were selected and assessed the cyto-toxicity in five human cancer cell lines,[6] namely HCT-116, HepG2, BGC-823, NCI-H1650, and A2780.

All of these five AMHC compounds show no obvious toxicity to human mammalian cell lines with an IC_{50} over than 50 μM.

Table S4: Evaluation of cell cyto-toxicity of five AMHC compounds (**81**-**85**)

Cmpd	IC_{50} (M)				
	HCT-116	HepG2	BGC-823	NCI-H1650	A2780
81	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$
82	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$
83	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$
84	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$
85	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$	$>50\times10^{-6}$

3.2 Detection of EdU incorporation in HepG2 cells using AMHC 83[7]

5-Ethynyl-2-deoxyuridine (EdU) was purchased from commercial source with the purity over than 98%. HepG2 cells were cultured in DMEM medium (Invitrogen) with 10% fetal bovine serum (Gibco) at 37 ℃ with 5% CO_2. HepG2 cells were incubated with 10 μM EdU for 24 h. HepG2 cells were fixed with 4% formaldehyde in PBS buffer for 15 min and then penetrated with 0.5% Triton X-100 in PBS buffer for another 10 min. After washing HepG2 cells with PBS buffer, 5 μM of compound **83** in a click chemistry reaction buffer (Click-iT® cell reaction buffer kit, molecular probes®) was added into fixed HepG2 cells for 30 min, and un-reacted **83** were washed away with 0.1M Tris-HCl, pH8.0 for 3 times. HepG2 cells were imaged under fluorescent microscope (Olympus IX71). Notably, in certain harsh conditions, the fluorescence intensity for imaging can be further improved using the biological buffer with higher pH value (pH 9-10) to substitute 0.1M Tris-HCl pH8.0 for the last step of cell washing.

3.3 Staining rat bone marrow in the absence of EdU using AMHC 83 and Alexa488®

Commercial fluorescein derived Alexa488-azide and Alexa594-azide (Molecular Probes®) can false positively stain rat bone marrow cells with the rate of 13% in the absence of EdU.[7, 8] Here, we inspected if AMHC dye **83** can also stain rat bone marrow cells in the absence of EdU. All animal experiments were conducted in compliance with the Care and Use of Laboratory Animals with the approval of Peking Union Medical College and Chinese Academy of Medical Sciences' Animal Studies Committee.

Male SD rats of 6-8 weeks old were sacrificed and their bone marrows were separated. After gently homogenated, the bone marrow cells were collected though a 30 μm filter, and washed with PBS for 3 times. Bone marrow cells were collected by centrifugation at 1000 rpm at 4 ℃ for 5 min. Then, cells were fixed in 4% paraformaldehyde for 10 min followed by incubation with 0.1% Triton-X100 for 5 min. Alexa488-azide and **83** (final concentration 5 μM) in a click chemistry reaction buffer (Click-*i*T® cell reaction buffer kit, molecular probes®) was mixed with bone marrow cells for 30 min, and then washed away using PBS buffer for three times. For control, only use the click chemistry reaction buffer. Rat bone marrow cells were imaged under fluorescent microscope (Olympus IX71), and we observed that our AMHC dye 83 cannot falsely stain any rat bone marrow cells (Figure S15, A, B, C). But, coincident with the results published by Lin *et al*, [8] we also observed that Alexa488-azide can positively stain certain bone marrow cells, which was not pre-treated with EdU or other azide-contained compounds (Figure S15, D, E, F).

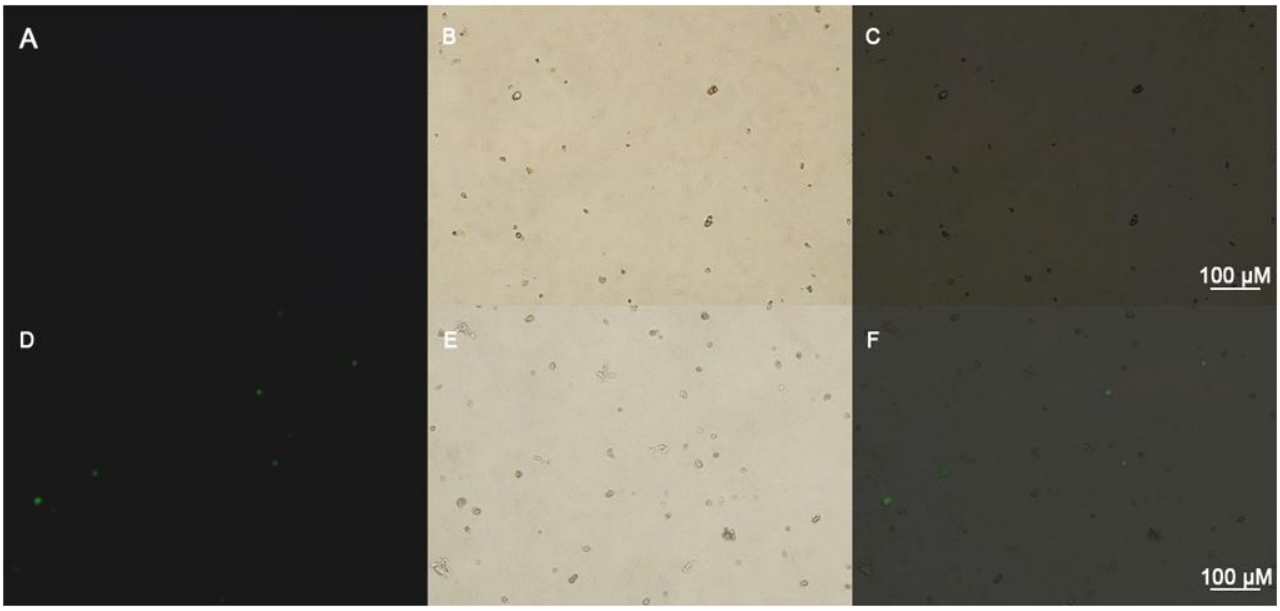


Figure S15. Rat bone marrow in the absence of EdU pre-treatment was stained with the Click-iT kit containing AMHC **83** (azide derivative) A, B, and C; and Alexa488-azide D, E, and F. In figure D and F, green spot can be observed, which show the unspecific staining of rat bone marrow cells by Alexa488-azide.

3.4 Detection of EdU in tumor tissue[7-9]

The application of AMHC **83** staining tissue was also examined. Adult female C57BL mice (8-10 weeks of age) were injected subcutaneously over the right flank with 1×10^6 lewis lung cancer cells. The mice were allowed to run for 10 days. On day 11, they were treated with EdU (50 mg/kg body weight) via intraperitoneal injection. Mice were sacrificed after another 2 days of feeding. The tumor tissues were excised and post-fixed overnight in 4% paraformaldehyde. After embedded with paraffin, the tumors were sectioned into 5-10 micrometers. The sections were de-paraffinizated. Similar to fixed cell imaging, 5 μM of compound **83** in a click chemistry reaction buffer (Click-*i*T® cell reaction buffer kit, molecular probes®) was added to cover the tumor sections for 30 minutes, and then washed out with PBS for three times. Tumor section was imaged under fluorescent microscope NIKON ECLIPSE 80i. Notably, in certain harsh conditions, the fluorescence intensity for imaging can be further improved using the biological buffer with higher pH value (pH 9-10) to substitute 0.1M Tris-HCl, pH8.0 for the last step of tissue washing.

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