Supporting Information

Novel synthetic baicalein derivatives caused apoptosis and activated AMP-activated protein kinase in human tumor cells

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Experimental Section

1. Chemistry

1.1 General information

The reagents (chemicals) were purchased from Lancaster (Morecambe, England), Acros (Geel, Belgium) and Shanghai Chemical Reagent Company (Shanghai, China), and were used without further purification. The analytical thin-layer chromatography was HSGF 254 (150–200 μm thickness; Yantai Huiyou Company, Yantai, Shandong, China). The ^{1}H NMR (300 MHz or 400 MHz) spectra were recorded on Varian Mercury-300 or 400 High Performance Digital FT-NMR with TMS as internal standard, and the ^{13}C NMR (300 MHz) spectra were determined using a Varian Mercury-400 High Performance Digital FT-NMR. Chemical shifts were reported in parts per million (ppm, d) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). EI-MS and HRMS were performed with Finnigan MAT 95, EI: 70 eV, R:10000.

1.2 HPLC purity and HRMS results of all final compounds

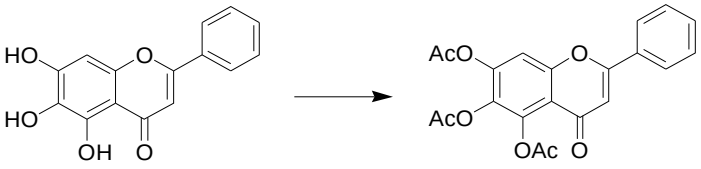
Cpd.	Purity (%)[a]	HRMS result
7	98	HRMS (EI):: *m/z* calcd for $C_{18}H_{14}O_5$, 310.0841, found 310.0852
8	96	HRMS (EI): m/z calcd for $C_{22}H_{16}O_5$, 360.0998, found 360.0998
9	96	HRMS (EI): *m/z* calcd for $C_{22}H_{15}FO_5$, 378.0903, found 378.0920
10	96	HRMS (EI):: *m/z* calcd for $C_{23}H_{18}O_6$, 390.1103, found 390.1114
11	99	HRMS (EI):: *m/z* calcd for $C_{21}H_{15}NO_5$, 361.0950, found 361.0943
17	98	HRMS (EI): *m/z* calcd for $C_{21}H_{21}NO_6$, 383.1369, found 383.1368

18	100	HRMS (EI):: *m/z* calcd for $C_{22}H_{23}NO_5$, 381.1576, found 381.1580
19	100	HRMS (EI):: *m/z* calcd for $C_{21}H_{22}O_5$, 354.1467, found 354.1473
24	99	HRMS (EI):: *m/z* calcd for $C_{22}H_{16}O_5$, 360.0998, found 360.0995
25	99	HRMS (EI): *m/z* calcd for $C_{22}H_{15}FO_5$, 378.0903, found 378.0919
26	99	HRMS (EI): *m/z* calcd for $C_{23}H_{18}FO_6$, 390.1103, found 390.1112
27	100	HRMS (EI): *m/z* calcd for $C_{21}H_{21}NO_6$, 383.1369, found 383.1368

[a] Purity was recorded on Gilson high-performance liquid chromatography (HPLC) (306 pump, uv/vis-156 Detector, 215 liquid handle)

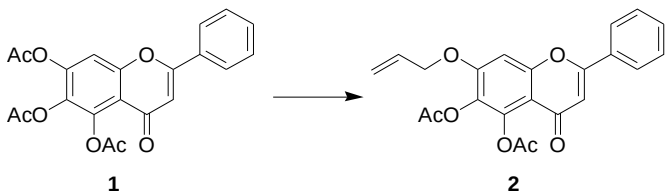
1.3 Experimental procedures

5, 6, 7-Triacetoxy-flavone (**1**)



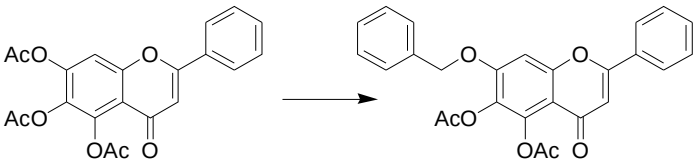
Baicalein (5.4 g, 20 mmol) and NaOAc (2.25 g, 28.0 mmol) was dissolved in acetic anhydride (31.8 mL), and the solution was stirred at 75 ^{o}C until start material disappear. The reaction mixture was poured into ice-water (150 mL), and the precipitate was collected by filtration and washed by EtOH to give compound **1** (7.21 g, 91.1%) as a pale white powder, mp: 191 - 192 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.36 (s, 3H), 2.45 (s, 3H), 6.66 (s, 1H), 7.51 - 7.55 (m, 4H), 7.85 - 7.87 (m, 2H); MS (EI):: *m/z* 396 (M+), 354, 312, 270 (100%).

5, 6-Diacetoxy-7-allyloxy-flavone (**2**)



A mixture of **1** (3.96 g, 10 mmol), allyl bromide (2.54 mL, 30 mmol) and anhydrous K_2CO_3 (5.52 g, 40 mmol) in acetone (250 mL) was refluxed for 24 h with stirring. The reaction mixture was cooled down to room temperature, filtered and the solvent was evaporated under reduced pressure to give the crude compound as a pale white solid. The solid washed with few EtOAc to give compound **2** (3.64 g, 92.4%) as a white powder. mp: 176 - 178 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.45 (s, 3H), 4.68 - 4.70 (m, 2H), 5.34 - 5.46 (m, 2H), 5.97 - 6.07 (m, 1H), 6.60 (s, 1H), 6.95 (s, 1H), 7.50 - 7.53 (m, 3H), 7.84 - 7.86 (m, 2H); MS (EI):: *m/z* 394 (M^+), 310 (100%), 270, 241, 69.

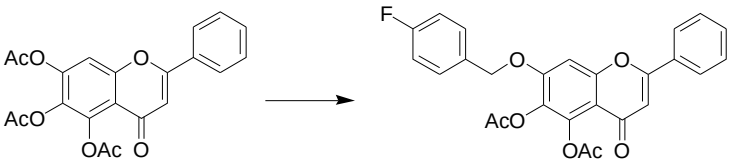
5, 6-Diacetoxy-7-benzyloxy-flavone (**3**)



1 **3**

Compound 3(3.81 g, 85.8%) was prepared from 1(3.96 g) under the same condition as described for compound 2. White solid, mp: 172 - 174 °C; 1HNMR (400 MHz, CDCl3): δ 2.32 (s, 3H), 2.46 (s, 3H), 5.22 (s, 2H), 6.60 (s, 1H), 7.00 (s, 1H), 7.36 - 7.43 (m, 5H), 7.48 - 7.54 (m, 3H), 7.83 - 7.85 (m, 2H); MS (EI):: *m/z* 444 (M+), 402, 360, 269, 91 (100%).

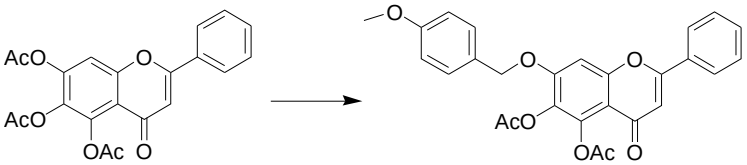
5, 6-Diacetoxy-7-(4'-fluoro-benzyloxy)-flavone (**4**)



1 **4**

Compound 4 (4.09 g, 88.5%) was prepared from 1 (3.96 g) under the same condition as described for compound 2. White solid, mp: 180 - 182 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.45 (s, 3H), 5.15 (s, 2H), 6.60 (s, 1H), 6.99 (s, 1H), 7.08 - 7.12 (m, 2H), 7.37 - 7.40 (m, 2H), 7.49 - 7.54 (m, 3H), 7.82 - 7.85 (m, 2H); IR (KBr): 3433, 1782, 1647, 1612, 1512, 1496, 1456; MS (EI):: *m/z* 462 (M^+), 420, 378, 269 (100%), 241, 109.

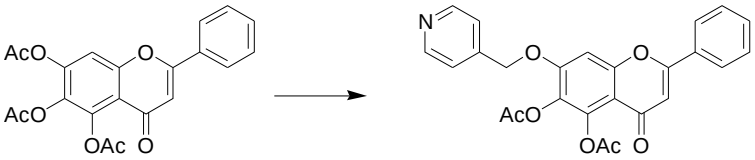
5, 6-Diacetoxy-7-(4'-methoxy-benzyloxy)-flavone (**5**)



1 **5**

Compound 5 (3.84 g, 81.0%) was prepared from 1 (3.96 g) under the same condition as described for compound 2. White solid, mp: 213 - 215 °C; ^{1}HNMR (400 MHz , $CDCl_3$): δ 2.28 (s, 3H), 2.45 (s, 3H), 3.82 (s, 3H), 5.12 (s, 2H), 6.60 (s,1H), 6.92 - 6.94 (m, 2H), 7.01 (s, 1H), 7.32 - 7.34 (d, 2H), 7.49 - 7.54 (m, 3H), 7.83 - 7.85 (m, 2H); IR (KBr): 1768, 1647, 1614, 1514, 1456; MS (EI):: *m/z* 474(M^+), 432, 390, 270, 121 (100%), 84.

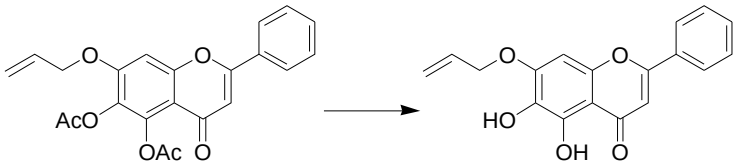
5, 6-Diacetoxy-7-(pyridin-4-ylmethoxy)-flavone (**6**)



1 **6**

Compound 6 (0.230 g, 74.0%) was prepared from 1 (0.277 g) under the same condition as described for compound 2. Pale white solid, mp: 209 - 211 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 2.30 (s, 3H), 2.46 (s, 3H), 5.21 (s, 2H), 6.60 (s, 1H), 7.02 (s, 1H), 7.35 - 7.38 (m, 1H), 7.49 - 7.56 (m, 3H), 7.73 - 7.76 (m, 1H), 7.83 - 7.89 (m, 2H), 8.63 - 8.65 (m, 1H), 8.70 (m, 1H); MS (EI):: *m/z* 445 (M^+), 361, 269 (100%), 241, 93, 69; IR (KBr): 3433, 1768, 1637, 1610, 1493, 1462 cm^{-1}..

5, 6-Dihydroxy-7-allyloxy-flavone (**7**)

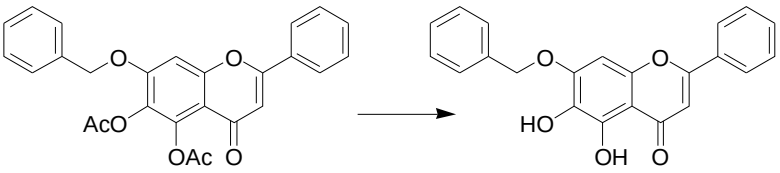


2 **7**

To a 500 mL two-necked round bottom, a mixture of **2** (5.52 g, 14.01 mmoL) and HCl (conc, 6 mL) was dissolved in EtOH (200 mL). The solution was refluxed with stirring until start material was disappeared. Then 50 mL water was added and the most of solvent was removed under reduced pressure. Some yellow solid was precipitated. The precipitate was collected by filtration and washed by EtOH to give compound **7** (3.94 g, 90.7 %) as a yellow powder, mp:161 - 163 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.75 (d, J = 5.0 Hz, 2 H) 5.33 (d, J = 10.6 Hz, 1 H) 5.50 (d, J = 17.3 Hz, 1 H) 6.01 - 6.22 (m, 1 H) 6.98 (d, J = 10.3 Hz, 1 H) 7.49 - 7.72 (m, 3 H) 8.09 (d, J = 7.9 Hz, 2 H) 8.77 (br. s., 1 H) 12.54 (s, 1 H); ^{13}CNMR (400 MHz, DMSO-d_6):

δ 69.4, 92.3, 104.7, 105.3, 118.0, 126.3 (2C), 129.1 (2C), 130.2, 130.8, 131.9, 133.0, 146.3, 149.6, 153.4, 163.2, 182.3; MS (EI): m/z 310(M+), 296 (100%), 241, 139, 69; HRMS (EI):: *m/z* calcd for $C_{18}H_{14}O_5$, 310.0841, found 310.0852; IR (KBr): 3628, 3535, 3066, 1664, 1612, 1585, 1491, 1450, 1425 cm^{-1}.

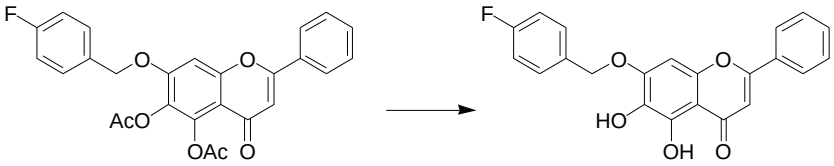
5, 6-Dihydroxy-7-allyloxy-flavone (**8**)



3 **8**

To a 250 mL round bottom, a mixture of **3** (5.33 g, 12.0 mmol) and NaOH (5% in water, 37.4 mL) was dissolved in acetone (150 mL). The solution was protected with argon and stirring at 40~50°C until start material was disappeared. Adjust pH to 4~5 with HCl and 50 mL water was added to the solution. The most of solvent was removed under reduced pressure and the precipitate was collected by filtration. The precipitate washed with EtOH to give compound **8** (4.32 g, 96.8%) as a yellow powder, mp: 198 - 200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.30 (s, 2 H), 7.00 (s, 1 H), 7.07 (s, 1 H), 7.32 - 7.48 (m, 4 H), 7.49 - 7.63 (m, 4 H), 8.00 - 8.19 (m, 2 H), 8.82 (br. s., 1 H), 12.55 (s, 1 H); ^{13}CNMR (400 MHz, DMSO-d6): δ 70.2, 92.6, 104.8, 105.4, 126.3 (2C), 127.8 (2C), 128.0, 128.5 (2C), 129.1 (2C), 130.2, 130.8, 132.0, 136.3, 146.2, 149.7, 153.5, 163.2, 182.2; MS (EI):: *m/z* 360 (M+), 269 (100%), 241, 139, 91, 69; HRMS (EI): m/z calcd for $C_{22}H_{16}O_5$, 360.0998, found 360.0998; IR (KBr): 3419, 1657, 1606, 1578, 1458 cm^{-1}.

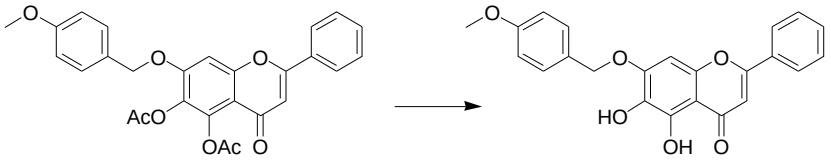
5, 6-Dihydroxy-7-(4'-fluoro-benzyloxy)-flavone (**9**)



4 **9**

Compound 9 (3.20 g, 84.6%) was prepared from 4 (4.62 g) under the same condition as described for compound 8. Yellow powder, mp: 181 - 184 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.28 (s, 2 H), 7.00 (s, 1 H), 7.07 (s, 1 H), 7.26 (t, J = 8.8 Hz, 2 H), 7.54 - 7.65 (m, 5 H), 8.04 - 8.12 (m, 2 H), 8.81 (s, 1 H), 12.54 (s, 1 H); ^{13}CNMR (400MHz, DMSO-d_6): δ 69.6, 92.6, 104.8, 105.4, 115.2, 115.4, 126.3 (2C), 129.2 (2C), 130.0, 130.1, 130.9, 132.0, 132.5, 146.2, 149.7, 153.4, 160.7, 163.1, 163.2, 182.3; MS (EI):: *m/z* 378 (M^+), 270 (100%), 168, 109, 69; HRMS (EI): *m/z* calcd for $C_{22}H_{15}FO_5$, 378.0903, found 378.0920; IR (KBr): 3197, 1660, 1610, 1512, 1578, 1450 cm^{-1}.

5, 6-Dihydroxy-7-(4'-methoxy-benzyloxy)-flavone (**10**)

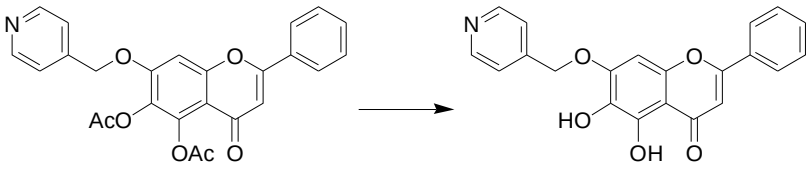


5 **10**

Compound 10 (3.37 g, 86.4%) was prepared from 5 (4.74 g) under the same condition as described for compound 8. Yellow powder, mp: 201 - 204 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.76 (s, 3 H), 5.21 (s, 2 H), 6.95 - 7.01 (m, 3 H), 7.06 (s, 1 H), 7.46 (d, J=8.8 Hz, 2 H), 7.55 - 7.67 (m, 4 H), 8.09 (d, 2 H), 8.77 (s, 1 H), 12.53 (s,

1 H); ^{13}CNMR (400 MHz, DMSO-d_6): δ 55.2, 70.1, 92.6, 104.8, 105.3, 113.9 (2C), 126.4 (2C), 128.1, 129.2 (2C), 129.7 (2C), 103.3, 130.9, 132.0, 146.1, 149.7, 153.6, 159.2, 163.2, 182.3; MS (EI):: *m/z* 390 (M^+), 375, 270, 121 (100%); HRMS (EI):: *m/z* calcd for $C_{23}H_{18}O_6$, 390.1103, found 390.1114; IR (KBr): 3462, 2924, 1666, 1610, 1514, 1462 cm^{-1}.

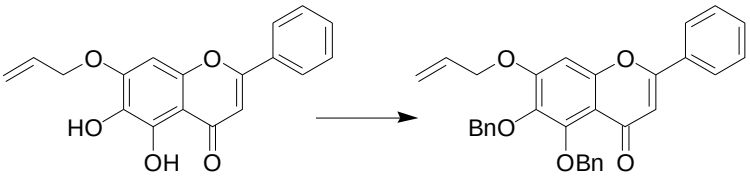
5, 6-Dihydroxy-7-(pyridin-4-ylmethoxy)-flavone (**11**)



6 **11**

Compound 11 (3.04 g, 84.2%) was prepared from 6 (4.45 g) under the same condition as described for compound 8. Yellow powder, mp: 229 - 232 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.45 (s, 2 H), 7.03 (s, 1 H), 7.14 (s, 1 H), 7.53 - 7.68 (m, 3 H), 7.81 - 7.92 (m, 1 H), 8.10 (d, J = 7.6 Hz, 2 H), 8.39 (d, J = 7.3 Hz, 1 H), 8.79 (s, 1 H), 8.99 (s, 1 H), 12.55 (br. s., 1 H); ^{13}CNMR (400 MHz, DMSO-d_6): δ 67.1, 92.6, 104.8, 105.8, 126.3, 126.4 (2C), 129.2 (2C), 130.3, 130.8, 132.1, 135.3, 142.4, 142.8, 143.4, 149.6, 152.7, 163.3, 182.4; MS (EI):: *m/z* 361 (M^+), 270,269 (100%), 241, 139, 93, 69; HRMS (EI):: *m/z* calcd for $C_{21}H_{15}NO_5$, 361.0950, found 361.0943; IR (KBr): 3404, 3059, 1670, 1614, 1581, 1481, 1448 cm^{-1}.

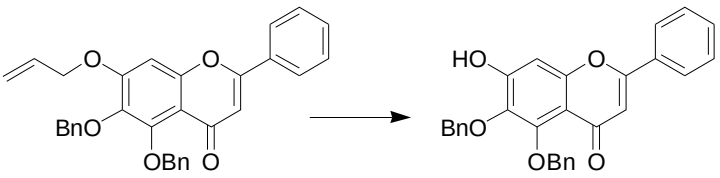
5, 6-Dibenzyloxy-7-allyloxy-flavone (**12**)



7 **12**

To a 500 mL oven-dried three-necked round bottom, a mixture of **7** (4.65 g, 15 mmol), benzyl bromide (7.14 mL, 60 mmol) and anhydrous K_2CO_3 (16.56 g, 120 mmol) was dissolved in anhydrous acetone (200 mL). The solution was refluxed for 24 h with stirring. The reaction mixture filtered and the solvent was evaporated under reduced pressure to give the crude compound as a pale white solid. The solid washed with EtOH to give compound **12** (6.06 g, 82.4%) as a white powder, mp: 149 - 151 °C; ^{1}HNMR (400MHz, $CDCl_3$): δ 4.65 - 4.67 (m, 2H), 5.06 (s, 2H), 5.14 (s, 2H), 5.36 - 5.40 (m, 1H), 5.45 - 5.51 (m, 1H), 6.04 - 6.13 (m, 1H), 6.70 (s, 2H), 6.82 (s, 2H), 7.32 - 7.40 (m, 6H), 7.44 - 7.46 (m, 2H), 7.50 - 7.54 (m, 3H), 7.66 - 7.68 (m, 2H), 7.88-7.90 (m, 2H); MS (EI):: *m/z* 490（M^+）, 399，309，91 (100%); IR (KBr)：3060, 1632, 1601, 1448 cm^{-1}.

5, 6-Dibenzyloxy-7-hydroxy-flavone (**13**)

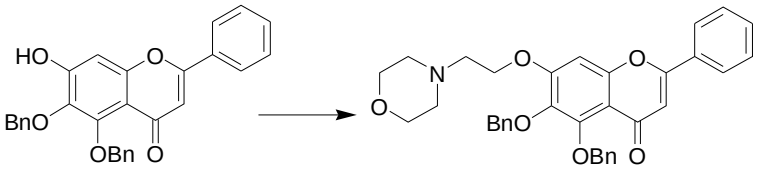


12 **13**

Compound **12** (3.12 g, 6.37 mmol) and $Pd(Ph_3P)_4$ (0.172 g, 0.149 mmol) was dissolved in anhydrous THF (80 mL), and the solution was stirred at room temperature for 5 min. $NaBH_4$ (0.378 g, 9.99 mmol) was added to the solution slowly

and the solution stirring until start material was disappeared. Adjust pH to 4~5 with HCl and the precipitate was collected by filtration. The crude was purified by flash chromatography (PE/EA = 4/1 to 2/1) to afford **13** (2.72 g, 95.0%) as a white solid, mp: 200 - 203 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 5.00 (s, 4H), 6.77 (s, 1H), 6.95 (s, 1H), 7.30 - 7.37 (m, 6H), 7.40 - 7.43 (m, 2H), 7.54 - 7.58 (m, 5H), 8.01 - 8.04 (m, 2H), 11.00 (s, 1H); MS (EI): *m/z* 450 (M^+), 359, 269, 241, 91 (100%); IR (KBr): 3423, 3049, 2551, 1630, 1599, 1583, 1545, 1496, 1448 cm^{-1}.

5, 6-Dibenzyloxy-7-(2-morpholin-4-yl-ethoxy)-flavone (**14**)

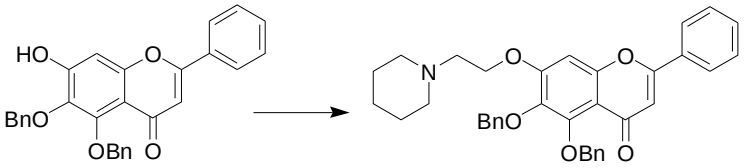


13 **14**

To a 100 mL own-dried three-necked round bottom, a mixture of **13** (0.252 g, 0.56 mmol), 4-(2-Chloroethyl)morpholine hydrochloride (0.130 g, 0.70 mmol), KI (6 mg, 0.035 mmol), anhydrous K_2CO_3 (0.193 g, 1.40 mmol) and anhydrous DMF (5 mL) was dissolved in anhydrous acetone (65 mL). The solution was refluxed for 24 h with stirring. The reaction mixture filtered and the solvent was evaporated under reduced pressure to give the crude compound. The crude was purified by flash chromatography (PE/EA = 4/1, 2/1 to 1/1) to afford **14** (0.260 g, 82.6%) as a white solid, mp: 76 - 79 °C; ^{1}HNMR (400MHz, $CDCl_3$): δ 2.60 - 2.62 (m, 4H), 2.88 (t , *J* = 5.77, 2H), 3.70 - 3.73 (m, 4H), 4.22 (t, *J* = 5.77, 2H), 5.04 (s, 2H), 5.14 (s, 2H), 6.68 (s, 1H), 6.82 (s, 1H), 7.32 - 7.40 (m, 6H), 7.44 - 7.46 (m, 2H), 7.51 - 7.54 (s, 3H),

7.66 - 7.68 (m, 2H), 7.87 - 7.90 (m, 2H); MS (EI):: *m/z* 563 (M^+),472, 382, 114 (100%), 91; IR (KBr): 3427, 2852, 1637, 1602, 1450 cm^{-1}.

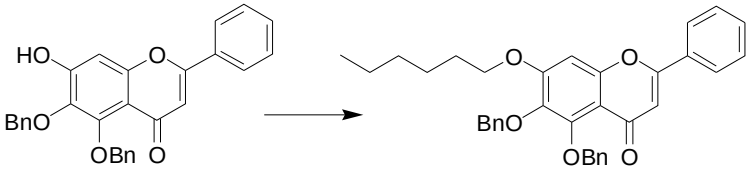
5, 6-Dibenzyloxy-7-(2-piperidin-1-yl-ethoxy)-flavone (**15**)



13 **15**

Compound 15 (0.267 g, 85.1%) was prepared from 13 (0.252 g) under the same condition as described for compound 14. White solid, mp: 89 - 91 °C; ^{1}HNMR (300 MHz, $CDCl_3$): δ 1.46 - 1.50 (m, 2H), 1.59 - 1.67 (m, 4H), 2.56 - 2.61 (m, 4H), 2.87 (t, *J* = 6.00, 2H), 4.23 (t, *J* = 6.00, 2H), 5.04 (s, 2H), 5.12 (s, 2H), 7.00 (s, 1H), 6.86 (s, 1H), 7.28 - 7.41 (m, 6H), 7.42 - 7.55 (m, 5H), 7.65 - 7.68 (m, 2H), 7.87 - 7.92 (m, 2H); MS (EI):: *m/z* 561 (M^+), 470, 270, 98 (100%), 91. IR (KBr): 3440, 2933, 1643, 1603, 1450 cm^{-1}.

5, 6-Dibenzyloxy-7-(2-piperidin-1-yl-ethoxy)-flavone (**16**)

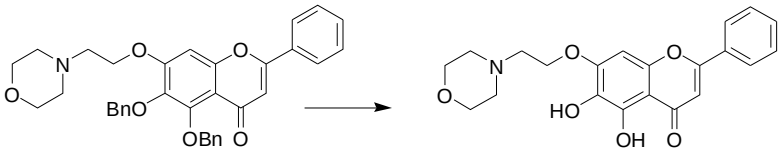


13 **16**

A mixture of **13** (0.252 g, 0.56 mmol), 1-Bromohexane (2.54 mL, 30 mmol) and anhydrous Na_2CO_3 (0.178 g, 1.68 mmol) in anhydrous DMF (15 mL) was refluxed with stirring until start material was disappeared. The reaction mixture was cooled

down to room temperature, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography (PE/EA = 8/1, 4/1 to 2/1) to afford **12** (0.207 g, 69.7%) as a white solid, mp: 120 - 123 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.91 - 0.95 (m, 3H), 1.36 - 1.56 (m, 4H), 1.87 - 1.94 (m, 4H), 4.09 (t, J = 6.46, 2H), 5.04 (s, 2H), 5.14 (s, 2H), 6.81 (s, 1H), 6.84 (s, 1H), 7.33 - 7.40 (m, 6H), 7.45 - 7.47 (m, 2H), 7.51 - 7.54 (s, 3H), 7.66 - 7.68 (m, 2H), 7.89 - 7.92 (m, 2H); ^{13}CNMR (400 MHz, $CDCl_3$): δ 14.0, 22.5, 25.7, 28.9, 31.5, 69.1, 75.8, 76.6, 97.1, 108.3, 113.0, 125.9 (2C), 128.0 (2C), 128.2 (2C), 128.3 (2C), 128.7 (2C), 128.9 (2C), 129.3 (2C), 131.2 , 131.6, 137.1, 137.2, 139.7, 151.5, 154.7, 157.6, 161.0, 177.2; MS (EI):: *m/z* 534 (M^+), 443 (100%), 353, 269, 91 (100%); IR (KBr): 3431, 2929, 2870, 1643, 1603, 1450 cm^{-1}.

5, 6-Dihydroxy-7-(2-morpholin-4-yl-ethoxy)-flavone (**17**)

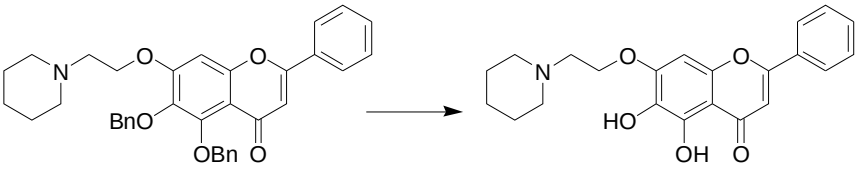


14 **17**

Compound **14** (0.225 g, 0.40 mmol) and 10% Pd/C (0.225g) was dissolved in anhydrous THF (60 mL), and the solution was stirred at room temperature for 5 min. $NaBH_4$ (0.378 g, 9.99 mmol) was added to the solution slowly and the solution stirring until start material was disappeared. Adjust pH to 4~5 with HCl and the precipitate was collected by filtration. The crude was purified by flash chromatography (CH_2Cl_2/CH_3OH = 20/1 to 10/1) to afford **17** (0.096 g, 62.7%) as a

yellow powder, mp: 151 - 153 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.68 (br. s., 4H), 2.77 (t, J = 5.22, 2H), 3.85 (m, 4H), 4.25 (t, J = 5.22, 2H), 6.68 (s, 1H), 6.72 (s, 1H), 7.50 - 7.55 (s, 3H), 7.86 - 7.88 (m, 2H), 12.60 (s, 1H); ^{13}CNMR (400 MHz, DMSO-d_6): δ 53.4 (2C), 56.6, 66.0 (2C), 66.6, 92.7, 104.7, 105.4, 126.3 (2C), 129.2 (2C), 130.4, 130.9, 132.0, 145.9, 149.7, 153.6, 163.2, 182.3; MS (EI):: *m/z* 383 （M^+）,270, 114,100 (100%); HRMS (EI):: *m/z* calcd for $C_{21}H_{21}NO_6$, 383.1368, found 383.1368; IR (KBr): 3384, 2964, 1668, 1612, 1583, 1489 cm^{-1}.

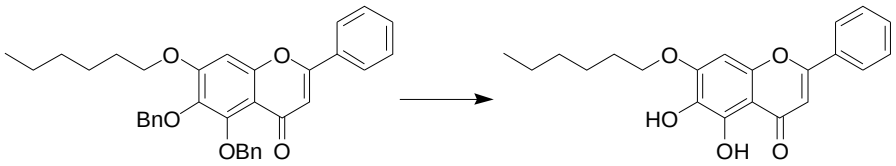
5, 6-Dihydroxy-7-(2-piperidin-1-yl-ethoxy)-flavone (**18**)



15 **18**

Compound 18 (0.121 g, 63.0%) was prepared from 15 (0.280 g) under the same condition as described for compound 17. Yellow powder, mp: 190 - 192 °C; ^{1}HNMR(300 MHz, DMSO-d_6): δ 1.34 - 1.41 (m, 2H), 1.49 - 1.56 (m, 4H), 2.48 - 2.50 (m, 4H), 2.71 (t, J = 5.50, 2H), 4.22 (t, J = 5.50, 2H), 6.99 (s, 1H), 7.02 (s, 1H), 7.55 - 7.62 (m, 3H), 8.08 - 8.11 (m, 2H); ^{13}CNMR (400 MHz, DMSO-d_6): δ 23.8, 25.3 (2C), 54.1 (2C), 56.9, 67.2, 93.4, 104.7, 105.7, 126.4 (2C), 129.2 (2C), 130.9, 131.0, 132.0, 146.1, 149.5, 153.6, 163.2, 182.4; MS (EI):: *m/z* 381 (M^+), 270, 149, 112, 98, 59 (100%); HRMS (EI):: *m/z* calcd for $C_{22}H_{23}NO_5$, 381.1576, found 381.1580; IR (KBr): 3423, 3199, 2945, 2538, 1668, 1612, 1585, 1491, 1448 cm^{-1}.

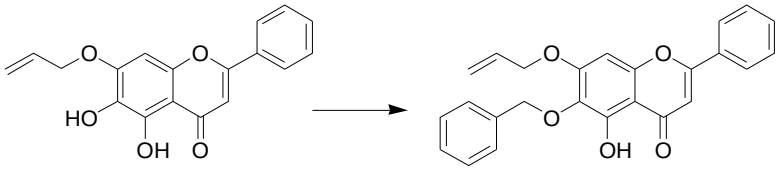
5, 6-Dihydroxy-7-Hexyloxy-flavone (**19**)



16 **19**

Compound 19 (0.111 g, 62.6%) was prepared from 16 (0.267 g) under the same condition as described for compound 17. Yellow powder, mp: 140 - 142 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84 - 0.99 (m, 3 H), 1.27 - 1.37 (m, 4 H), 1.40 - 1.52 (m, 2 H), 1.73 - 1.86 (m, *J*=6.9, 6.9, 6.8, 6.6 Hz, 2 H), 4.12 (t, *J*=6.3 Hz, 2 H), 6.99 (d, *J*=14.1 Hz, 2 H), 7.56 - 7.66 (m, 3 H), 8.11 (d, *J*=7.8 Hz, 1 H), 8.67 (s, 1 H), 12.51 (s, 1 H); ^{13}CNMR (400 MHz, $CDCl_3$): δ 14.0, 22.5, 25.5, 28.8, 31.5, 69.6, 91.0, 105.3, 105.9, 126.2 (2C), 129.0 (2C), 129.7, 131.4, 131.7, 145.6, 150.6, 152.3, 163.9, 182.6; MS (EI):: *m/z* 354 (M^+), 283, 270 (100%), 168; HRMS (EI):: *m/z* calcd for $C_{21}H_{22}O_5$, 354.1467, found 354.1473; IR (KBr): 3550, 3435, 2935, 2852, 1666, 1616, 1587, 1468, 1358 cm^{-1}.

5-Hydroxy-6-benzyloxy-7-allyloxy-flavone (**20**)

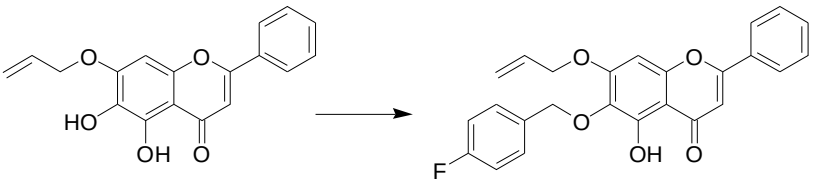


7 **20**

To a 100 mL own-dried three-necked round bottom, a mixture of **7** (0.186 g, 0.6 mmoL), benzyl bromide (99 μL, 0.84 mmoL) , anhydrous K_2CO_3 (0.21 g, 1.52 mmoL) and KI (0.10 g, 0.6 mmol) was dissolved in anhydrous acetone (50 mL). The solution was refluxed with stirring until the reaction completed. The reaction mixture filtered

and the solvent was evaporated under reduced pressure to give the crude compound as a pale white solid. The crude was purified by flash chromatography (PE/EA = 8/1, 4/1 to 2/1) to afford **20** (0.153 g, 63.8 %) as a white powder, mp: 122 - 125 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 4.62 - 4.64 (m, 2H), 5.14 (s, 2H), 5.32 - 5.46 (m, 2H), 6.01 - 6.08 (m, 1H), 6.51 (s, 1H), 6.67 (s, 1H), 7.30 - 7.37 (m, 3H), 7.50 - 7.56 (m, 5H), 7.86 - 7.89 (m, 2H), 12.70 (s, 1H); MS (EI):: *m/z* 400 (M^+), 309 (100%), 269, 253, 91; IR (KBr): 3421, 3064, 3030, 2926, 1660, 1614, 1585, 1492, 1464 cm^{-1}.

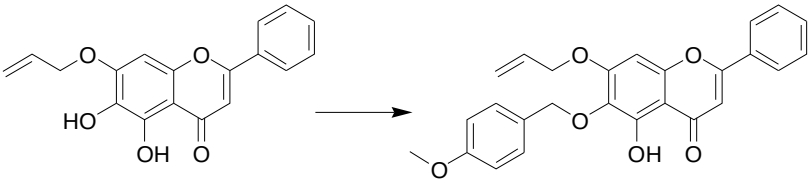
5-Hydroxy-6-(4-fluoro-benzyloxy)-7-allyloxy-flavone (**21**)



7 **21**

Compound 21 (0.183 g, 73.0%) was prepared from 7 (0.186 g) under the same condition as described for compound 20. White powder, mp: 126-128 °C; ^{1}HNMR (300 MHz, $CDCl_3$): δ 4.62 - 4.65 (m, 2H), 5.09 (s, 2H), 5.33 - 5.47 (m, 2H), 5.99 - 6.12 (m, 1H), 6.51 (s, 1H), 6.68 (s, 1H), 6.98 - 7.06 (m, 2H), 7.47 - 7.59 (m, 5H), 7.87 - 7.91 (m, 2H), 12.75 (s, 1H); MS (EI):: *m/z* 418 (M^+), 309 (100%), 253, 109; IR (KBr): 3450, 3076, 2868, 1662, 1612, 1589, 1510, 1495, 1456 cm^{-1}.

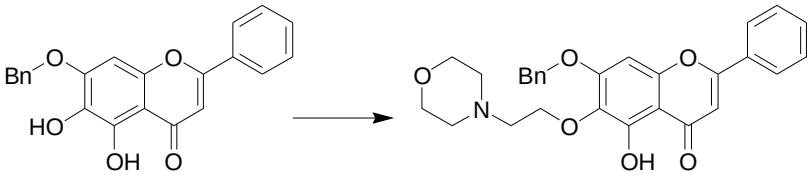
5-Hydroxy-6-(4-methoxy-benzyloxy)-7-allyloxy-flavone (**22**)



7 **22**

Compound 22 (0.185 g, 71.6%) was prepared from 7 (0.186 g) under the same condition as described for compound 20. White powder, mp: 143 - 144 °C; ^{1}HNMR (300 MHz, $CDCl_3$): δ 3.80 (s, 3H), 4.60 - 4.63 (m, 2H), 5.08 (s, 2H), 5.32 - 5.46 (m, 2H), 5.98 - 6.09 (m, 1H), 6.49 (s, 1H), 6.67 (s, 1H), 6.83 - 6.88 (m, 2H), 7.41 - 7.57 (m, 6H), 7.85 - 7.89 (m, 2H), 12.54 (s, 1H); MS (EI):: *m/z* 430 (M^+), 309 , 269, 241, 121(100%); IR (KBr): 3480, 2939, 1662, 1614, 1589, 1491, 1456 cm^{-1};.

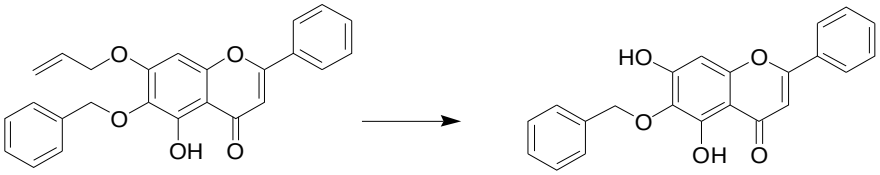
5-Hydroxy-6-(2-morpholin-4-yl-ethoxy)- 7-benzyloxy-flavone (**23**)



8 **23**

Compound 23 (0.161 g, 68.0%) was prepared from 8 (0.180 g) under the same condition as described for compound 14. Pale yellow powder; mp: 141 - 143 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 2.53 (b, 4H), 2.79 (t, J = 5.5, 2H), 3.61 - 3.63 (m, 4H), 4.19 (t, J = 5.5, 2H), 5.18 (s, 2H), 6.63 (s, 1H), 6.67 (s, 1H), 7.35 - 7.57 (m, 8H), 7.86 - 7.89 (m, 2H), 12.68 (s, 1H); MS (EI):: *m/z* 473 (M^+), 360, 269(100%), 241, 114, 100, 91; IR (KBr): 3433, 2956, 1658, 1614, 1587, 1493, 1452 cm^{-1}.

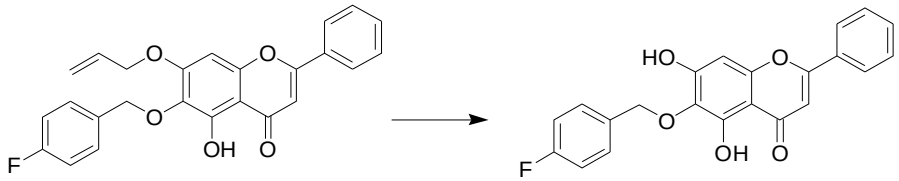
5, 7-Hydroxy-6-benzyloxy-flavone (**24**)



20 **24**

Compound 24 (0.063 g, 87.1%) was prepared from 20 (0.080 g) under the same condition as described for compound 13. Pale yellow powder, mp: 146 - 148 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.03 (s, 2 H), 6.64 (s, 1 H), 6.96 (s, 1 H), 7.36 (m, 3 H), 7.46 - 7.69 (m, 5 H), 8.07 (m, 2 H), 13.00 (s, 1 H); ^{13}CNMR (400 MHz, $CDCl_3$): δ 74.9, 93.4, 105.2, 105.8, 126.2 (2C), 128.8 (6C), 129.1 (2C), 131.2, 131.8, 136.6, 152.2, 153.3, 155.5, 164.1,183.0; MS (EI): *m/z* 360 (M^+), 269 (100%), 241, 91; HRMS (EI):: *m/z* calcd for $C_{22}H_{16}O_5$, 360.0998, found 360.0995; IR (KBr): 3419, 2925, 2630, 1649, 1616, 1578, 1448 cm^{-1}.

5, 7-Hydroxy-6-(4-fluoro-benzyloxy)-flavone (**25**)

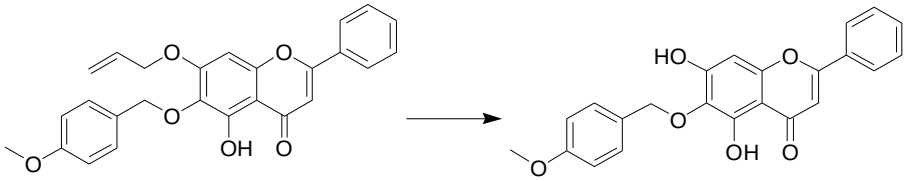


21 **25**

Compound 25 (0.068 g, 89.5%) was prepared from 21 (0.084 g) under the same condition as described for compound 13. Pale yellow powder, mp: 179 - 182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.02 (s, 2 H), 6.64 (s, 1 H), 6.97 (s, 1 H), 7.14 - 7.28 (m, 2 H), 7.47 - 7.68 (m, 5 H), 8.00 - 8.14 (m, 2 H), 10.90 (br. s., 1 H), 13.02 (s, 1 H); ^{13}CNMR (400 MHz, $CDCl_3$): δ 73.9, 93.5, 105.2, 105.7, 115.6, 115.8, 126.2 (2C), 128.5, 129.1 (2C), 130.7, 130.8, 131.2, 131.9,132.5, 152.1, 153.3, 155.4, 164.1, 183.0;

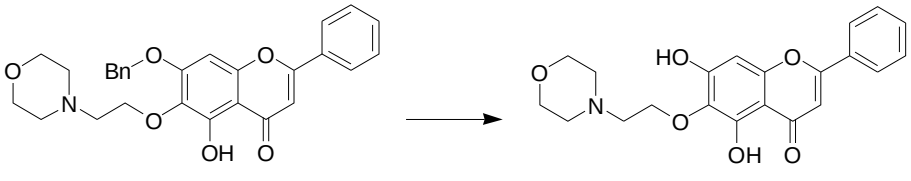
MS(EI): *m/z* 378 (M^+), 269 (100%), 241, 91; HRMS (EI): *m/z* calcd for $C_{22}H_{15}FO_5$, 378.0903, found 378.0919; IR (KBr): 3425, 2935, 2632, 1649, 1618, 1579, 1512 cm^{-1}.

5, 7-Hydroxy-6-(4-methoxy-benzyloxy)-flavone (**26**)



Compound 26 (0.71 g, 91.0%) was prepared from 22 (0.086 g) under the same condition as described for compound 13. Pale yellow powder, mp: 140 - 142 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (s, 3 H), 4.97 (s, 2 H), 6.63 (s, 1 H), 6.92 (d, *J*=8.3 Hz, 2 H), 6.97 (s, 1 H), 7.43 (d, *J*=8.8 Hz, 2 H), 7.54 - 7.66 (m, 3 H), 8.06 (d, *J*=6.8 Hz, 2 H), 10.83 (br. s., 1 H), 13.01 (s, 1 H); ^{13}CNMR (400 MHz, $CDCl_3$): δ 55.2, 74.5, 93.4, 105.2, 105.7, 114.0 (2C), 126.2 (2C), 128.6, 128.7, 129.0 (2C), 130.5 (2C), 131.2, 131.8, 152.2, 153.2, 155.6, 159.9, 164.0, 183.0; MS (EI): *m/z* 390 (M^+), 270, 121 (100%); HRMS (EI): *m/z* calcd for $C_{23}H_{18}FO_6$, 390.1103, found 390.1112; IR(KBr): 3423, 2935, 2634, 1643, 1616, 1578, 1514 cm^{-1}.

5, 7-Hydroxy-6-(2-morpholin-4-yl-ethoxy)-flavone (**27**)



Compound 27 (0.152 g, 99.2%) was prepared from 23 (0.189 g, 0.4mmol) under

the same condition as described for compound 17. Yellow powder, mp: 188 – 191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 - 2.73 (m, 6 H), 3.60 - 3.79 (m, 4 H), 4.15 (t, *J*=5.1 Hz, 2 H), 6.61 (s, 1 H), 6.96 (s, 1 H), 7.54 - 7.66 (m, 3 H), 8.07 (d, *J*=7.8 Hz, 2 H), 12.93 (br. s., 1 H); ^{13}CNMR (400 MHz, DMSO-d_6): δ 52.4 (2C), 56.2, 65.5 (2C), 68.2, 95.1, 104.0, 104.6, 126.4 (2C), 129.2 (2C), 129.8, 130.8, 132.0, 152.8, 153.2, 159.7,163.1, 182.1; MS(ESI) *m/z* 383 (M^+), 269 (100%); IR (KBr): 3433, 3064, 2856, 1649, 1618, 1566, 1496,1450 cm^{-1}.

2. Biology

2.1 Materials

Anti-phospho-AMPKα (Thr 172) and AMPK antibodies, anti-β-actin antibody were purchased from Cell Signaling Technology (MA, UAS). Anti-mouse and anti-rabbit antibodies conjugated to horseradish peroxidase were obtained from Kirkegaard and Perry Laboratories (Gaithersburg, MD). Minimum essential medium (MEM), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution (Pen-Strep), sodium pyruvate, and Hoechst 33342 were purchased from Invitrogen. Baicalin (HPLC content 98%) was purchased from Nanjing Chongyuan Bio-tech Co Ltd (Nanjing, China). 5-aminoimidazole-4-carboxamide riboside (AICAR), and protease inhibitor cocktail set I were purchased from Calbiochem. Sulforhodamine B (SRB) was purchased from Sigma. All other reagents were of analytical grade.

2.2 Cell culture

Four human tumor cell lines, A431, SK-OV-3, DU 145 and HeLa cells (American Type Culture Collection, Rockville, Md., USA), were cultured in MEM (DMEM for HeLa cells) supplemented with 10% FBS and 1% Pen-Strep in a 5% CO2 atmosphere at 37 °C. AICAR, baicalein and its derivatives (dissolved in DMSO) were prepared as stock solutions just before treatment.

2.3 Cytotoxicity Assays

Cytotoxicity was assessed using the SRB assay as described previously.[1] Briefly, 1×10^4 cells $well^{-1}$ were seeded into 96-well flat-bottom plates in 200 μl culture medium. 24 hours later, the indicated concentrations of compouds were added to each well, with each replicated five times. 48 hours hours post-treatment, cells were fixed with 0.1% (w/v) trichloroacetic acid at 4°C for 1 hour, washed with water five times and allowed to dry overnight. Dried cells were stained with 0.4% (w/v) SRB dissolved in 1% acetic acid for 30 min. Wells were washed five times with 1% acetic acid, allowed to dry, and dye was solubilized in 200 μL 10 mM Tris-base(pH 10.5). Absorbance was read at 510 nm (Synergy™ 2, BioTek) and growth inhibition relative to vehicle-treated cells was determined.

2.4 Western blot analysis

Cells were harvested in a lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM

β-glycerolphosphate, 1 mM Na3VO4, 1 μg mL^{-1} leupeptin, 1 mM PMSF). Samples were sonicated three times for 5 s with 15 s breaks between cycles and subsequently centrifuged at 16 000×*g* for 60 min at 4 °C. The protein concentrations of the supernatants were determined with a protein assay kit (Bio-Rad). Equal amounts of total cellular protein were resolved by 10% SDS-PAGE, transferred onto polyvinylidene difluoride membranes (Amersham Biosciences), and then probed with primary antibody for AMPK, phospho-AMPK-α (Thr172), or β-actin followed by secondary antibody conjugated with horseradish peroxidase. The immunocomplexes were visualized via enhanced chemiluminescence (Amersham Biosciences).

2.5 Annexin V-FITC/PI analysis for apoptosis

Apoptosis was quantified by detecting surface exposure of phosphatidylserine in cells using Annexin V-FITC kit (Invitrogen, USA) according to the manufacturer's protocol. A431, SKOV-3, DU-145 and HeLa cells were cultured at 6×10^5 cells mL^{-1} and seeded in 60 mm dish. The cells were treated with free medium containing 5 μM and 25 μM compounds **8** and **9**, or 1 mM AICAR for 48 hour. Cells were collected and washed twice with ice cold PBS. About 1×10^5 cells were resuspended and incubated with AnnexinV–FITC and PI at room temperature for 15 min in the dark. Flow cytometric analysis was performed in a FACSCalibur flow cytometer (Becton Dickinson). At least 10000 events were acquired, obtaining data from FL1 (FITC) and FL2 (IP) detectors. Data were analyzed using Cell Quest Pro software (Becton Dickinson). Early apoptosis and late apoptosis/necrosis were expressed as the

percentages of annexin V+/PI– and annexin V+/PI+ cells, respectively.

2.6 Hoechst 33342 staining

To assess changes in nuclear morphology during apoptosis, staining using fluorescent Hoechst 33342 was performed. Cells were seeded into a Lab-Tek® II Chamber Slide™ System (Nalgene Nunc International, Naperville, IL). Compound was added to the medium and incubated for 48 h. Cells were subsequently exposed to 5 μg mL^{-1} of Hoechst 33342 at room temperature for 30 min in the dark and rinsed twice with PBS. Slides were observed under a fluorescence microscope (Olympus, IX71-F22FL/PH, Japan).

3. Figure 5

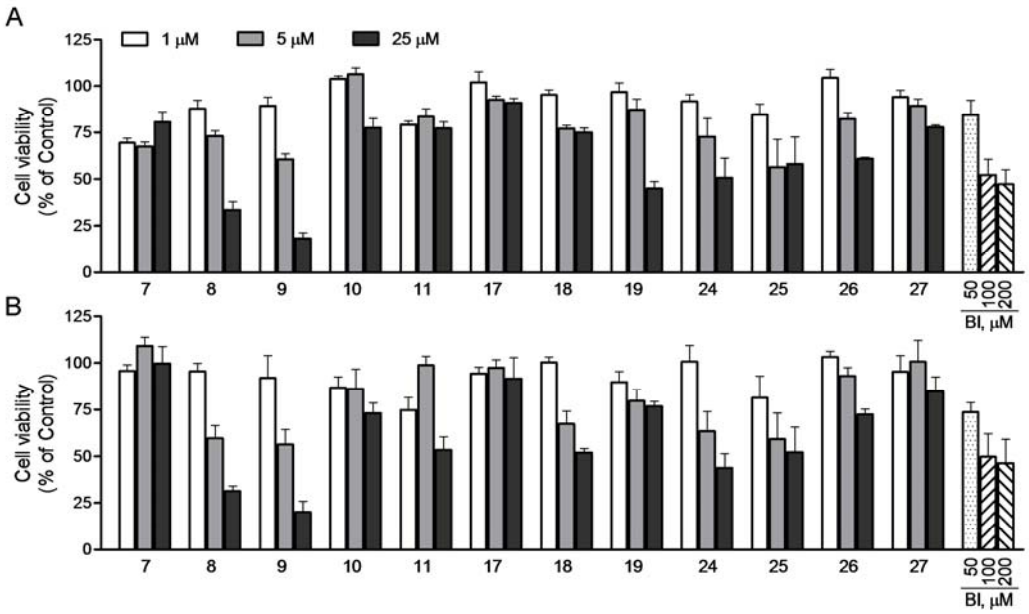


Fig. 5 The cytotoxicity of baicalein derivatives in A431 (A) and SK-OV-3 cells (B):

Supporting Information

Cells were treated with baicalein derivatives or baicalein (BI) as indicated concentrations for 48 hours. Cell viability was measured by SRB. Results represent mean±SE of 3-4 independent experiments done in quintuplicates.

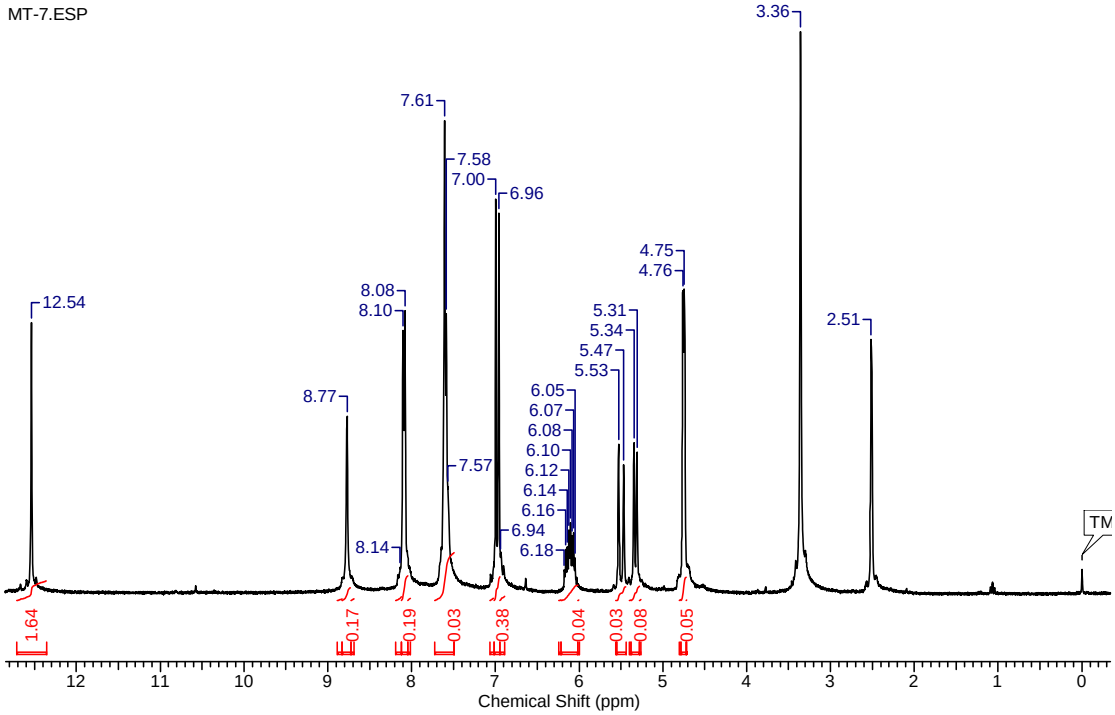
References:

1 P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107-1112.

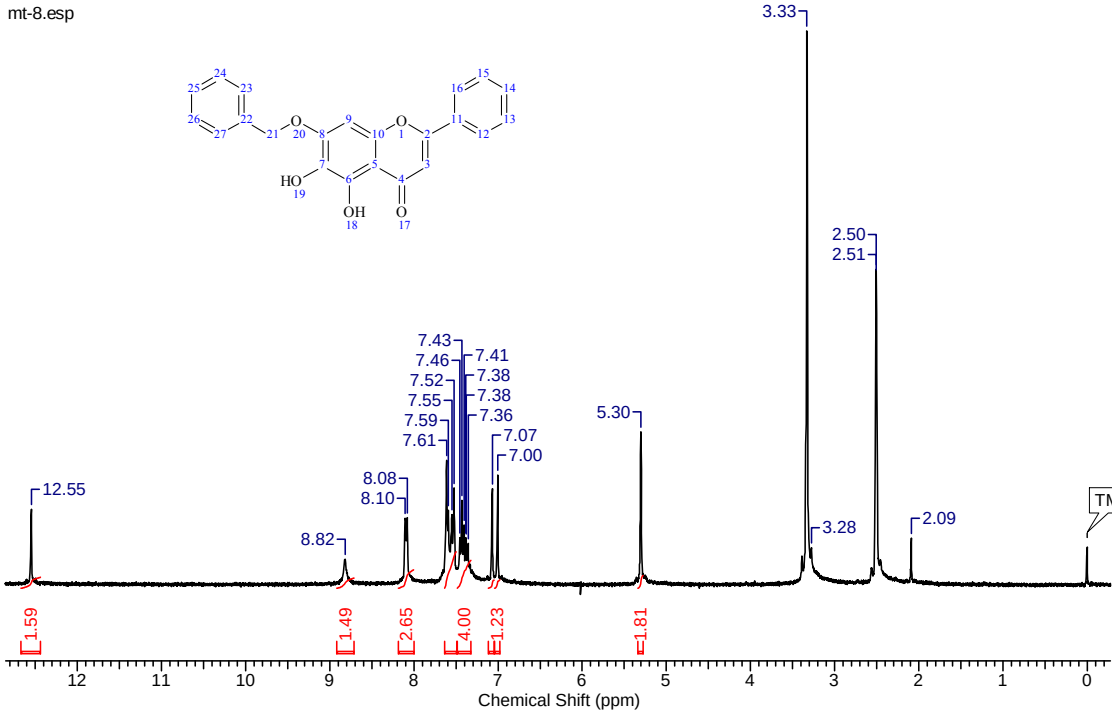
Supporting Information

Copies of selected ^{1}H spectra

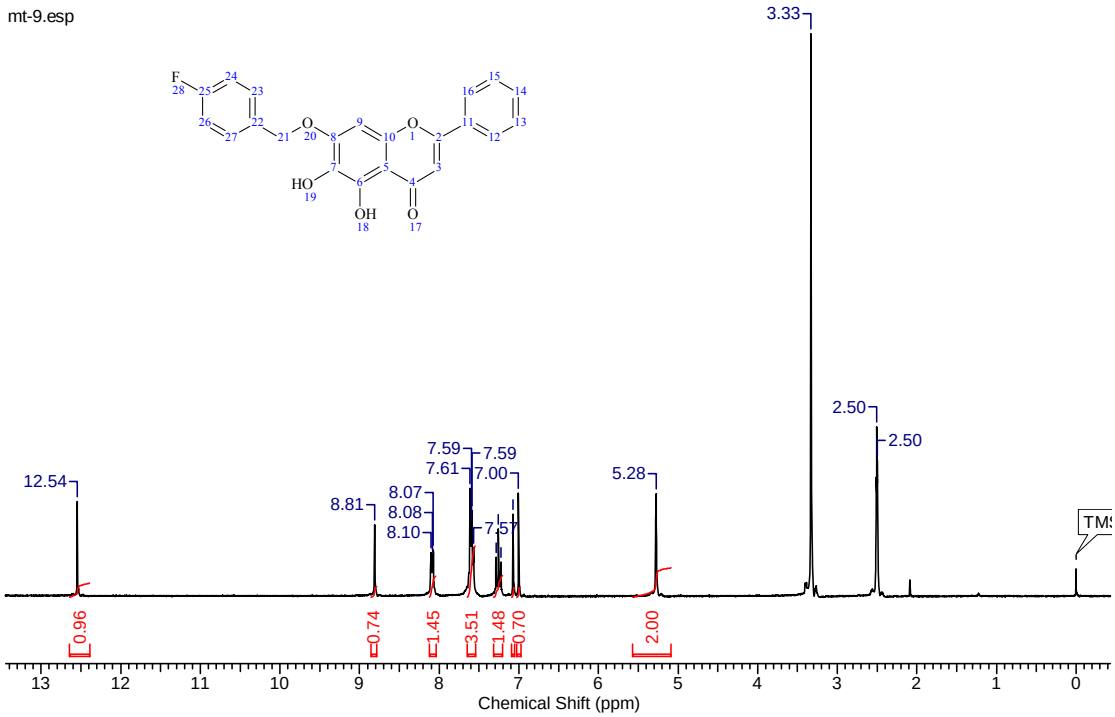
5, 6-Dihydroxy-7-allyloxy-flavone (7)



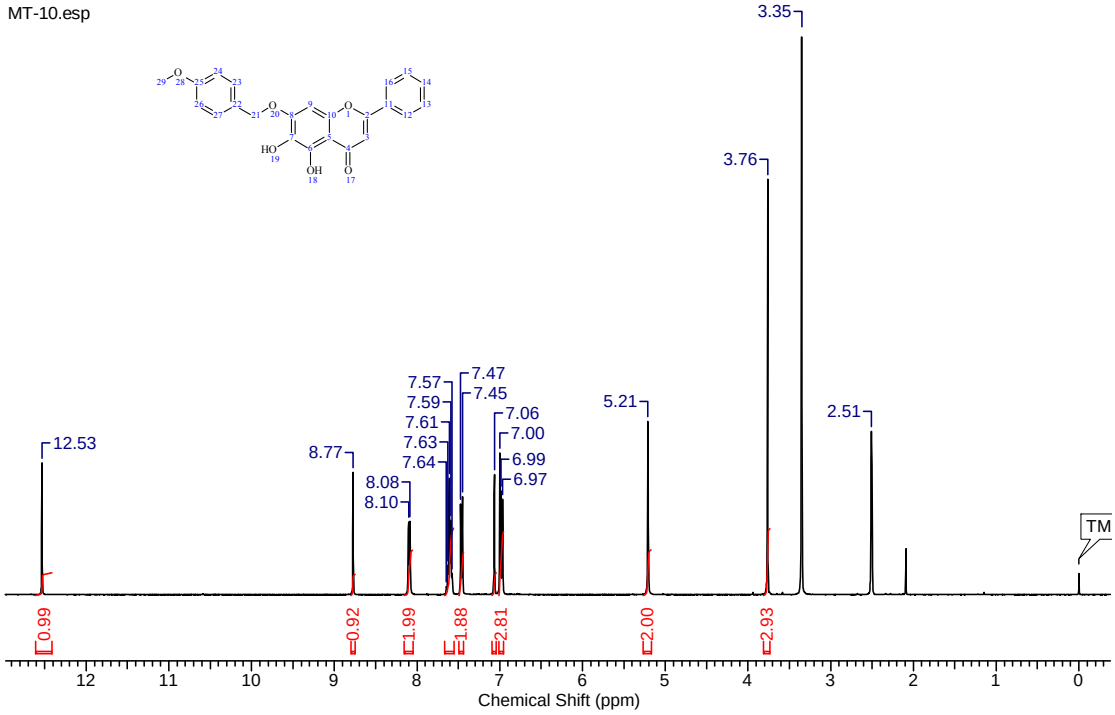
5, 6-Dihydroxy-7-allyloxy-flavone (**8**)



5, 6-Dihydroxy-7-(4'-fluoro-benzyloxy)-flavone (**9**)

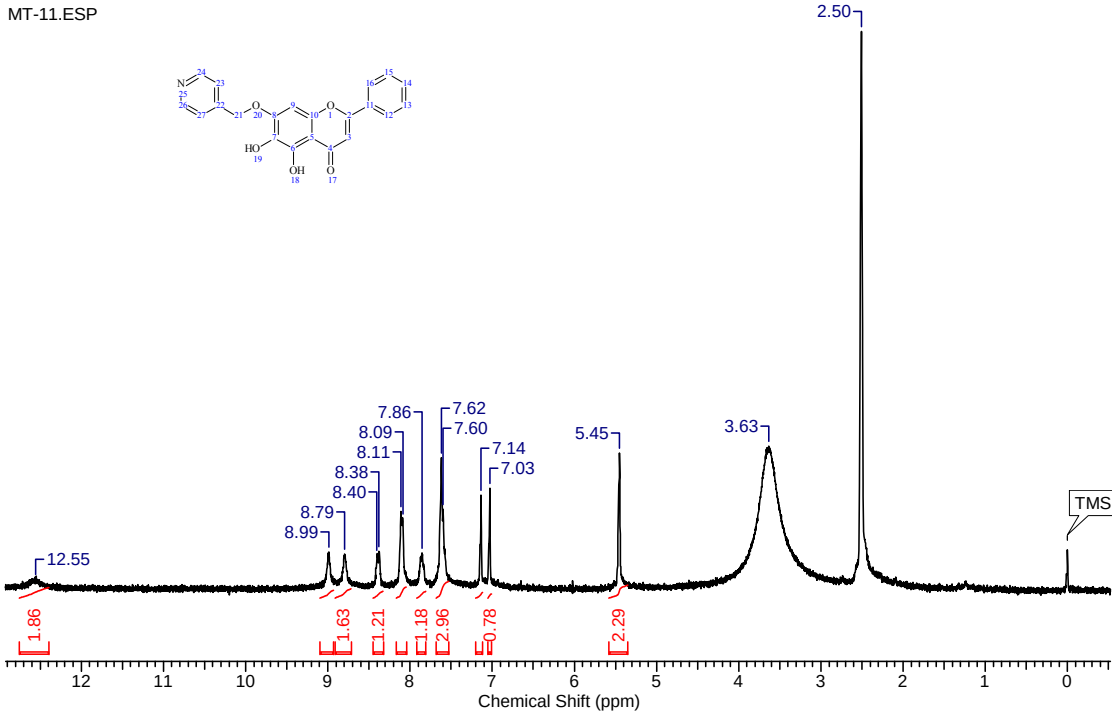


5, 6-Dihydroxy-7-(4'-methoxy-benzyloxy)-flavone (**10**)

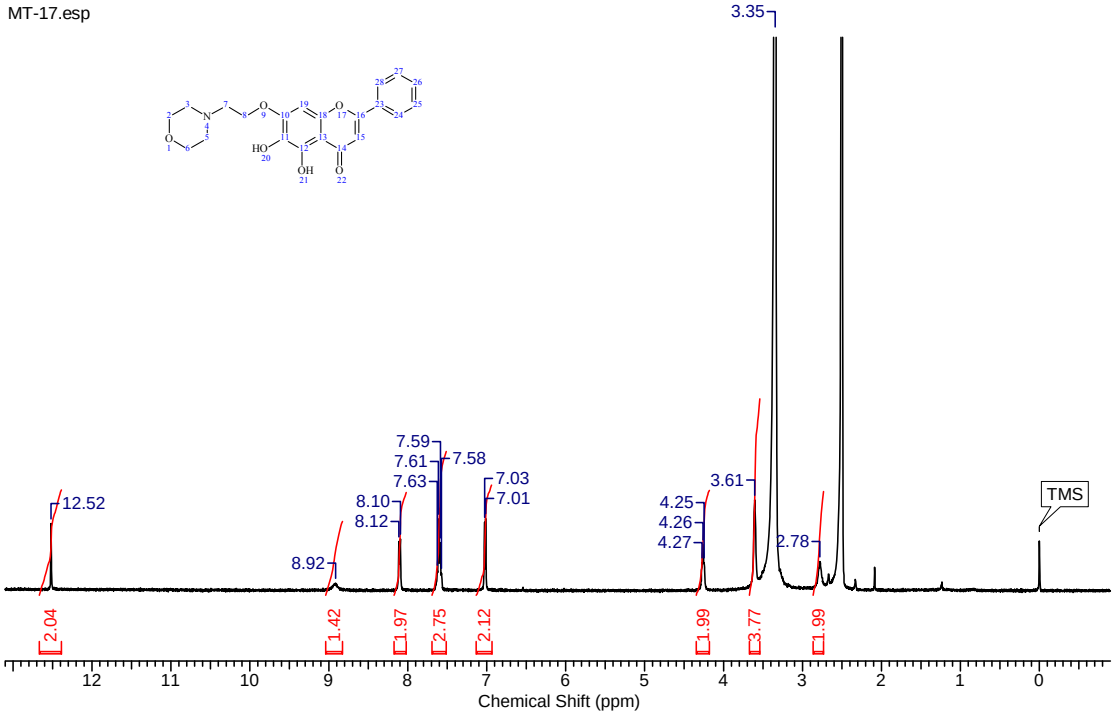


Supporting Information

5, 6-Dihydroxy-7-(pyridin-4-ylmethoxy)-flavone (**11**)

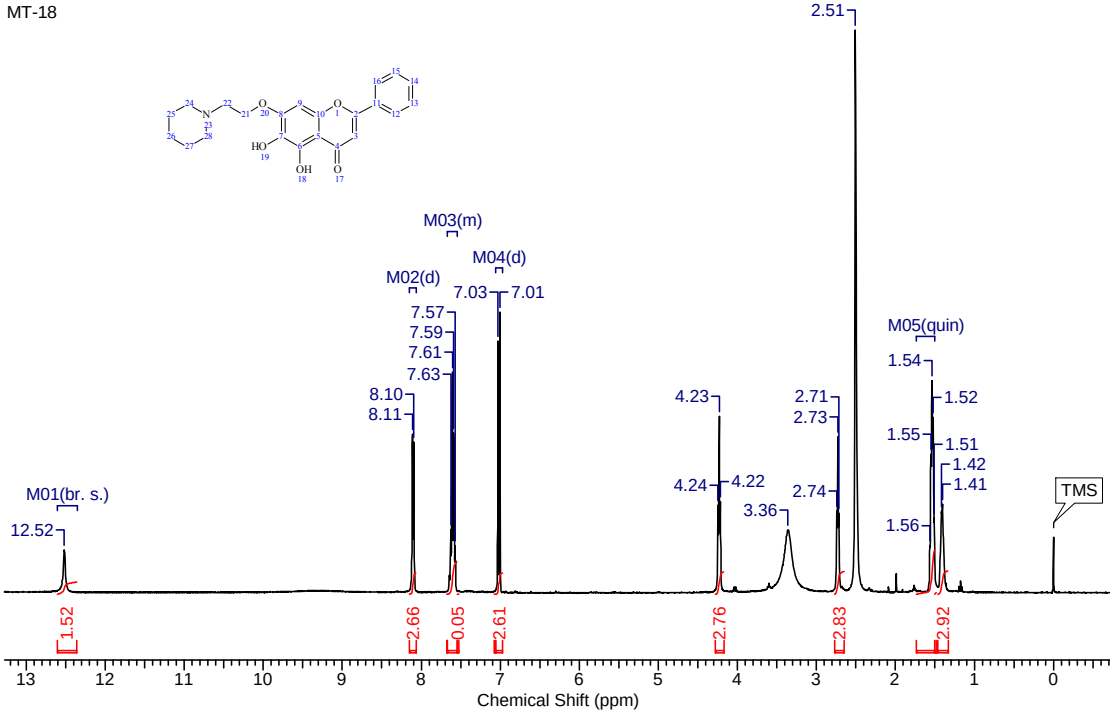


5, 6-Dihydroxy-7-(2-morpholin-4-yl-ethoxy)-flavone (**17**)

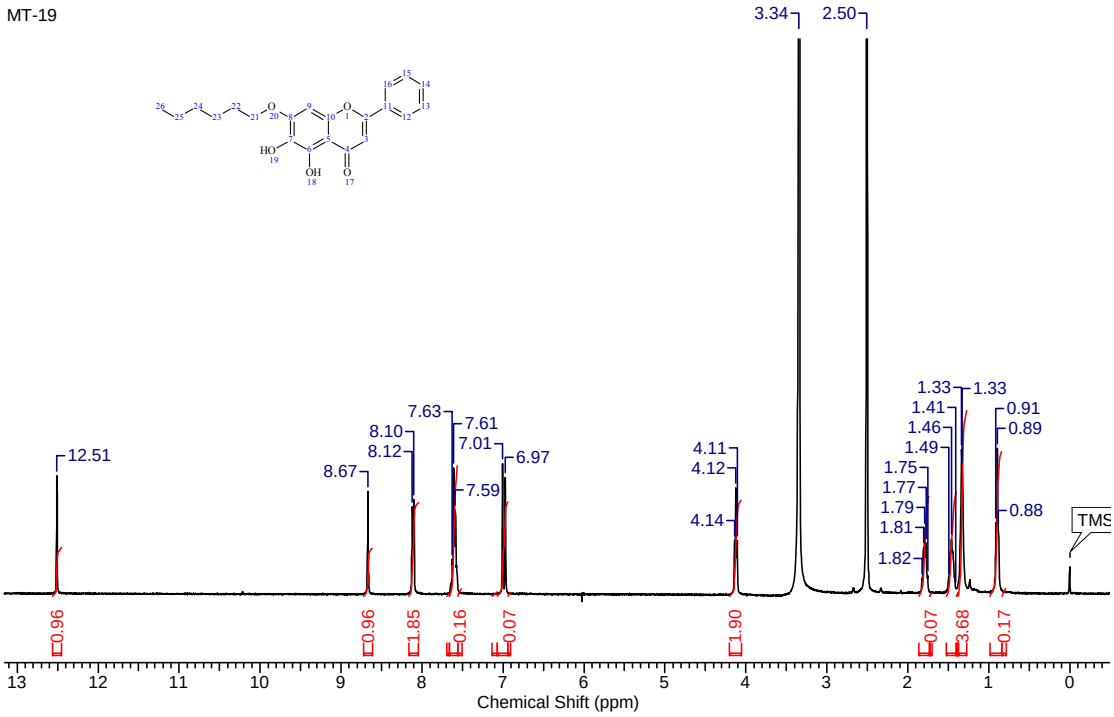


Supporting Information

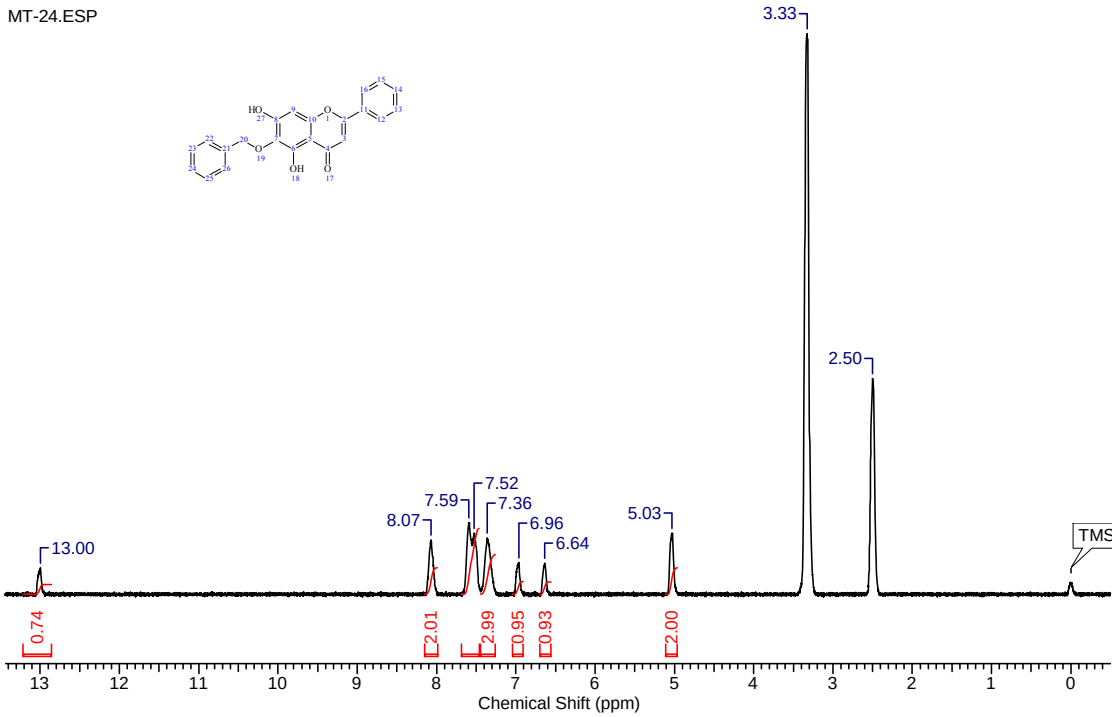
5, 6-Dihydroxy-7-(2-piperidin-1-yl-ethoxy)-flavone (**18**)



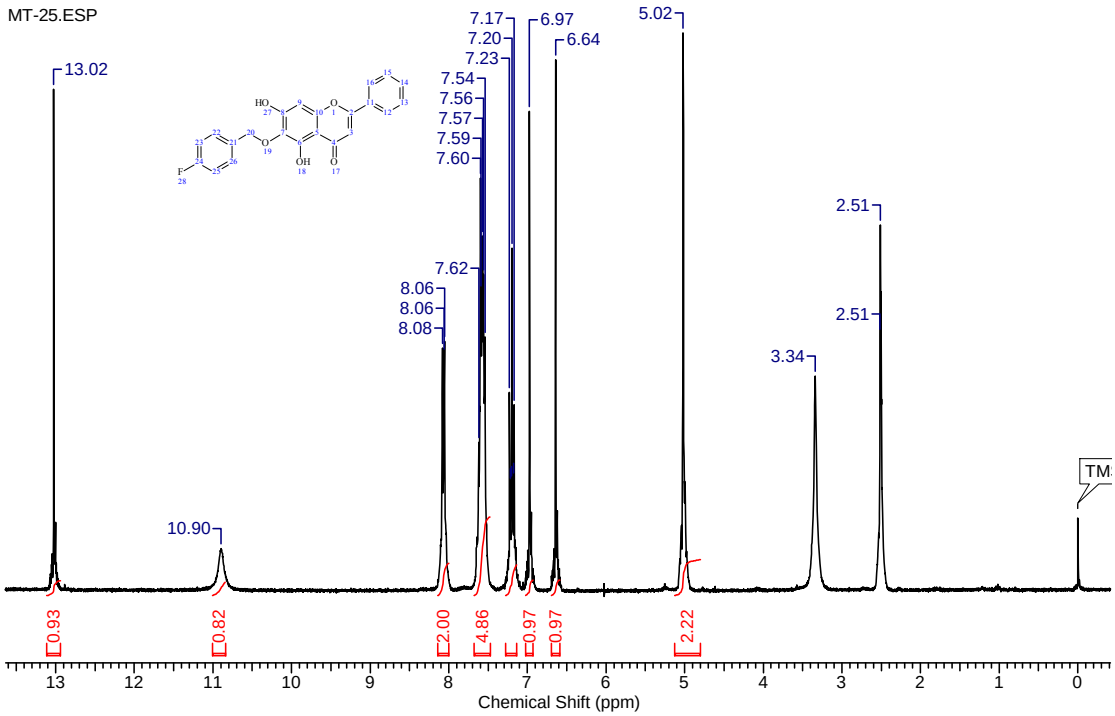
5, 6-Dihydroxy-7-Hexyloxy-flavone (**19**)



5, 7-Hydroxy-6-benzyloxy-flavone (**24**)

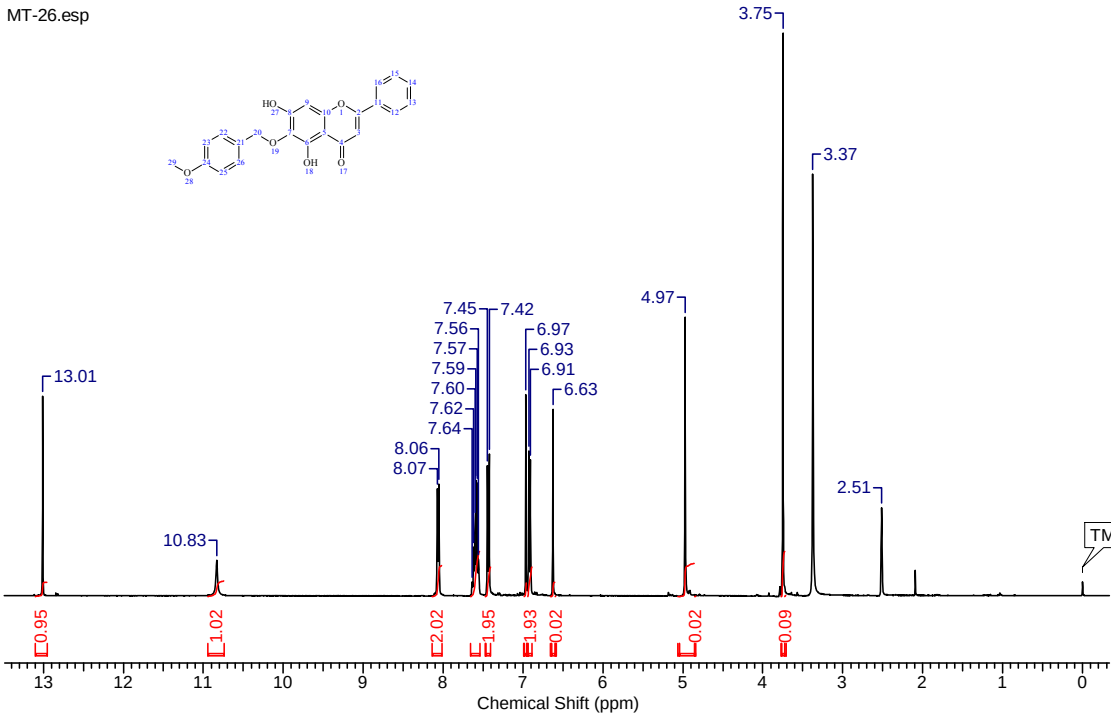


5, 7-Hydroxy-6-(4-fluoro-benzyloxy)-flavone (**25**)



Supporting Information

5, 7-Hydroxy-6-(4-methoxy-benzyloxy)-flavone (**26**)



5, 7-Hydroxy-6-(2-morpholin-4-yl-ethoxy)-flavone (**27**)