Supporting Information

Synthesis and Biological Evaluation of Novel 7-O-Lipophilic Substituted Baicalein Derivatives as Potential Anticancer Agents

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1. General methods

All chemical reagents in commercial quality were used as received (Sigma-Aldrich, St. Louis, MO, USA) and were used without further purification. Solvents were dried and the synthesized compounds were purified using standard techniques. Reactions-progression was monitored by TLC on aluminum plates coated with silica gel with a fluorescent indicator (Merck 60 F254). Unless otherwise stated. Melting points were determined on in open capillaries using the Fargo MP-2D apparatus and are uncorrected. NMR spectra were recorded using TMS as an internal standard in CDCl3 at 500.13 MHz for 1H and at 125.77 MHz for 13C (Bruker Biospin GmbH AVANCE III 500 MHz, Rheinstetten, Germany). Chemical shift (δ) were reported in parts per million (ppm) measured relative to the internal standards (TMS), and the coupling constant (J) were expressed in Hertz (Hz). Column chromatography was performed with silica gel SiliaFlash® G60 (60–200 μm) purchased from SiliCycle Inc. (Quebec City, Canada). In general, the reactions were carried out under anhydrous conditions in dry solvent and nitrogen atmosphere. The purity of these compounds was based on the analysis of HPLC (Hitachi High-Technologies, Tokyo, Japan) equipped with a 280 nm detector and LiChroCART RP-C18 column (4.6 mm i.d. × 250 mm, 5 μm, Merck, Darmstadt, Germany). The mobile phase was composed of MeOH-H2O (0.05% TFA) (90:10) and the flow rate was 1.0 mL/min. The purity of all compounds was more than 98%. The mass spectra were acquired using a Thermo Finnigan model LXQ (Thermo Electron Co., Waltham, MA, USA) ion trap mass spectrometer equipped with ESI source interference and controlled by Xcalibur 2.06. The mass spectra were acquired in a positive ion mode or a negative ion mode.

2. Extraction and isolation
Baicalein and wogonin were isolated from the acetone extract of the root of *S. baicalensis* Georgi according to previously described procedure. Briefly, dried roots of *S. baicalensis* were cut into small pieces, immersed, and extracted with 10-times volume of acetone twice at room temperature for two weeks. Acetone extracts were concentrated and subjected to column chromatography on silica gel (7.5 cm i.d. × 30 cm) eluted with CHCl$_3$ and CHCl$_3$-MeOH (10:1) to yield the two fractions. The CHCl$_3$ eluent was coated with Celite 540 (Merck) and rechromatographed on silica gel (3 cm i.d. × 20 cm) and eluted with n-hexane-acetone (10:1 to 3:1 gradient) to yield wogonin (3, 653 mg). CHCl$_3$-MeOH (10:1) eluent was subjected to chromatography on silica gel (3 cm i.d. × 20 cm) and eluted with CH$_2$Cl$_2$-acetone (9:1) to yield baicalein (1, 2.30 g).

5,6,7-Trihydroxyflavone (Baicalein, 1)

Yellow powder; mp: 270‒272°C (lit. 1 272–273°C); $^1$H NMR (500 MHz, DMSO-$d_6$): δ$_H$ 12.66 (s, 1H, 5-OH), 10.56 (s, 1H, 7-OH), 8.81 (s, 1H, 6-OH), 8.06‒8.05 (m, 2H, H-2',6'), 7.59‒7.56 (m, 3H, H-3',4',5'), 6.93 (s, 1H, H-3), 6.63 (s, 1H, H-8); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ$_C$ 182.18, 162.90, 153.64, 149.83, 146.97, 131.82, 130.96, 129.31, 129.11, 126.30, 104.48, 104.28, 94.01; LC-MS (ESI, m/z) calculated for C$_{15}$H$_{10}$O$_5$: 270.05, found for 269.04 [M–H]$^-$. 

5,7-Dihydroxy-8-methoxyflavone (Wogonin, 3)

Yellow brown needle crystal; mp: 204–207°C (lit. 2 203–205°C); $^1$H-NMR (500 MHz, DMSO-$d_6$): δ$_H$ 12.50 (s, 1H, 5-OH), 10.84 (s, 1H, 7-OH), 8.07 (d, $J = 7.0$ Hz, 2H), 7.62–7.61 (m, 3H, H-3',4',5'), 7.00 (s, 1H, H-3), 6.31 (s, 1H, H-6), 3.85 (s, 3H, OCH$_3$); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ$_C$ 182.08, 163.06, 157.40, 156.25, 149.65, 132.13, 130.86, 129.31, 127.81, 126.31, 105.08, 103.78, 99.18, 61.10; LC-MS (ESI, m/z) calculated for C$_{16}$H$_{12}$O$_5$: 284.07, found for 282.98 [M–H]$^-$, 267.93 [M–H–CH$_3$]$^-$. 

3. General synthesis of baicalein, wogonin and chrysin derivatives

A series of 7-O-substituted baicalein, wogonin and chrysin derivatives were synthesized and evaluated by alkylation using methyl iodide or alkyl bromide with anhydrous potassium carbonate in anhydrous acetone reflux 8–24 h. The synthetic procedure and reaction conditions for all investigated compounds are shown in Scheme 1 and Scheme 2.

5,6-Dihydroxy-7-methoxyflavone (5a)
5,6-Dihydroxy-7-isoprenoxyflavone (5b)

Yellow needle crystal; mp: 221–222°C (lit.3 221–223°C); ¹H NMR (500 MHz, DMSO-d₆): δH 12.48 (s, 1H, 5-OH), 8.79 (s, 1H, 6-OH), 8.08–8.06 (m, 2H, H-2′,6′), 7.60–7.55 (m, 3H, H-3′,4′,5′), 6.96 (s, 1H, H-3), 6.93 (s, 1H, H-8), 3.92 (s, 3H, OCH₃); ¹³C NMR (125 MHz, DMSO-d₆): δC 182.37, 163.21, 154.66, 149.85, 146.13, 132.00, 130.88, 130.14, 129.17, 126.36, 105.34, 104.72, 91.32, 56.38; LC-MS (ESI⁺, m/z) calculated for C₁₆H₁₂O₅: 284.07, found for 307.28 [M+Na]⁺, 590.90 [2M+Na]⁺.

5,6-Dihydroxy-7-geranoxyflavone (5c)

Yellow powder; mp: 156–157°C (lit.¹ 164–165°C); ¹H NMR (500 MHz, DMSO-d₆): δH 12.48 (s, 1H, 5-OH), 8.71 (s, 1H, 6-OH), 8.10–8.08 (m, 2H, H-2′,6′), 7.63–7.56 (m, 3H, H-3′,4′,5′), 6.98 (s, H, H-3), 6.95 (s, 1H, H-8), 5.52 (dt, J = 1.3, 6.6 Hz, 1H, H-2″), 4.69 (d, J = 6.6 Hz, 2H, H-1″), 1.77 (s, 3H, CH₃), 1.74 (s, 3H, CH₃); ¹³C NMR (125 MHz, DMSO-d₆): δC 182.37, 163.24, 153.88, 149.81, 146.20, 137.91, 132.06, 130.93, 130.31, 129.23, 126.40, 119.32, 105.26, 104.74, 92.27, 65.84, 25.54, 18.17; LC-MS (ESI⁺, m/z) calculated for C₂₀H₁₈O₅: 338.12, found for 698.99 [2M+Na]⁺.

5,6-Dihydroxy-7-farnesoxyflavone (5d)

Yellow powder; mp: 106–107°C (lit.¹ 103–104°C); ¹H NMR (500 MHz, DMSO-d₆): δH 12.48 (s, 1H, 5-OH), 8.71 (s, 1H, 6-OH), 8.08 (d, J = 7.7 Hz, 2H, H-2′,6′), 7.63–7.57 (m, 3H, H-3′,4′,5′), 6.98 (s, 1H, H-3), 6.94 (s, 1H, H-8), 5.50 (t, J = 6.3 Hz, 1H, H-2″), 5.06 (t, J = 6.3 Hz, 1H, H-6″), 4.72 (d, J = 6.3 Hz, 2H, H-1″), 2.10–2.06 (m, 4H, H-4″,5″), 1.74 (s, 3H, CH₃), 1.58 (s, 3H, CH₃), 1.55 (s, 3H, CH₃); ¹³C NMR (125 MHz, DMSO-d₆): δC 182.34, 163.19, 153.74, 149.73, 146.17, 140.80, 134.76, 132.03, 130.91, 130.69, 130.36, 129.20, 126.35, 124.07, 123.53, 119.24.
5,6-Dihydroxy-7-butyloxyflavone (5e)

Yellow powder; mp: 166–167°C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 12.47 (s, 1H, 5-OH), 7.88–7.86 (m, 2H, H-2′, H-6′), 7.54–7.48 (m, 3H, H-3′,4′,5′), 6.67 (s, 1H, H-3), 6.59 (s, 1H, H-8), 5.32 (s, 1H, 6-OH), 4.14 (t, $J = 6.6$ Hz, 2H, H-1″), 1.88 (p, $J = 7.1$ Hz, 2H, H-2″), 1.25–1.23 (m, 2H, H-3″), 1.00 (t, $J = 7.4$ Hz, 3H, H-4″); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 182.93, 164.26, 152.54, 150.94, 145.92, 131.97, 131.76, 129.95, 129.30, 126.48, 106.25, 105.69, 91.33, 69.56, 31.17, 19.38, 14.01; LC-MS (ESI$^+$, m/z) calculated for C$_{19}$H$_{18}$O$_5$: 326.12, found for 268.28 [M–C$_4$H$_9$]+, 325.42 [M–H]$^–$.

5,6-Dihydroxy-7-hexyloxyflavone (5f)

Yellow powder; mp: 138–140°C (lit. 140–142°C); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 12.47 (s, 1H, 5-OH), 7.88–7.86 (m, 2H, H-2′,6′), 7.55–7.48 (m, 3H, H-3′,4′,5′), 6.66 (s, 1H, H-3), 6.59 (s, 1H, H-8), 5.34 (s, 1H, 6-OH), 4.13 (t, $J = 6.8$ Hz, 2H, H-1″), 1.89 (p, $J = 6.8$ Hz, 2H, H-2″), 1.52–1.45 (m, 2H, H-3″), 1.37–1.34 (m, 4H, H-4″–7′′), 0.90 (t, $J = 6.9$ Hz, 3H, H-8″); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 182.91, 164.26, 152.54, 150.93, 145.90, 132.96, 131.74, 129.94, 129.29, 126.47, 106.24, 105.67, 91.32, 69.87, 31.71, 29.11, 25.80, 22.78, 14.23; LC-MS (ESI$^+$, m/z) calculated for C$_{21}$H$_{22}$O$_5$: 354.15, found for 268.01 [M–C$_6$H$_{13}$]+, 353.09 [M–H]$^–$.

5,6-Dihydroxy-7-octyloxyflavone (5g)

Yellow powder; mp: 123–124°C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 12.47 (s, 1H, 5-OH), 7.88–7.86 (m, 2H, H-2′,6′), 7.55–7.48 (m, 3H, H-3′,4′,5′), 6.66 (s, 1H, H-3), 6.59 (s, 1H, H-8), 5.33 (s, 1H, 6-OH), 4.13 (t, $J = 6.8$ Hz, 2H, H-1″), 1.89 (p, $J = 6.8$ Hz, 2H, H-2″), 1.51–1.45 (m, 2H, H-3″), 1.37–1.33 (m, 8H, H-4″–7′′), 0.87 (t, $J = 6.9$ Hz, 3H, H-8″); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 182.92, 164.25, 152.54, 150.94, 145.92, 131.96, 131.76, 129.94, 129.30, 126.48, 106.25, 105.69, 91.32, 69.87, 32.01, 29.51, 29.41, 29.14, 26.12, 22.86, 14.31; LC-MS (ESI$^+$, m/z) calculated for C$_{23}$H$_{26}$O$_5$: 382.18, found for 381.08 [M–H]$^–$.

5,6-Dihydroxy-7-decyloxyflavone (5h)

Yellow powder; mp: 117–118°C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 12.46 (s, 1H, 5-OH), 7.87–7.85 (m, 2H, H-2′,6′), 7.54–7.47 (m, 3H, H-3′,4′,5′), 6.65 (s, 1H, H-3),...
6.58 (s, 1H, H-8), 5.33 (s, 1H, 6-OH), 4.12 (t, J = 6.8 Hz, 2H, H-1′′), 1.88 (p, J = 6.8 Hz, 2H, H-2′′), 1.49–1.43 (m, 2H, H-3′′), 1.37–1.33 (m, 12H, H-4′′–9′′), 0.85 (t, J = 6.7 Hz, 3H, H-10′′); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 182.92, 164.25, 152.54, 150.94, 145.91, 131.96, 131.754, 29.94, 129.30, 126.48, 106.24, 105.68, 91.32, 69.87, 32.10, 29.92, 29.75, 29.54, 29.52, 29.15, 26.12, 22.89, 14.33; LC-MS (ESI $^-$, m/z) calculated for C$_{25}$H$_{30}$O$_5$: 410.21, found for 409.15 [M–H]$^-$.

5,6-Dihydroxy-7-dodecyloxyflavone (5i)

Yellow powder; mp: 120–121°C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 12.47 (s, 1H, 5-OH), 7.88–7.86 (m, 2H, H-2′,6′), 7.55–7.48 (m, 3H, H-3′,4′,5′), 6.67 (s, 1H, H-3), 6.59 (s, 1H, H-8), 5.32 (s, 1H, 6-OH), 4.13 (t, J = 6.8 Hz, 2H, H-1′′), 1.89 (p, J = 6.8 Hz, 2H, H-2′′), 1.50–1.44 (m, 2H, H-3′′), 1.28–1.23 (m, 16H, H-4′′–11′′), 0.86 (t, J = 6.8 Hz, 3H, H-12′′); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 182.93, 164.26, 152.54, 150.94, 145.92, 131.97, 131.76, 129.95, 129.30, 126.48, 106.25, 105.69, 91.33, 69.88, 32.13, 29.87, 29.85, 29.80, 29.75, 29.56, 29.55, 29.15, 26.12, 22.91, 14.34; LC-MS (ESI $^-$, m/z) calculated for C$_{27}$H$_{34}$O$_5$: 438.24, found for 438.26 [M–H]$^-$.

7,8-Dimethoxy-5-hydroxyflavone (6a)

Yellow powder; mp: 158–159°C (lit. 5 181–183°C); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 12.56 (s, 1H, 5-OH), 7.96–7.94 (m, 2H, H-2′,6′), 7.57–7.53 (m, 3H, H-3′,4′,5′), 6.68 (s, 1H, H-3), 6.44 (s, 1H, H-6), 3.96 (s, 1H, OCH$_3$), 3.95 (s, 3H, OCH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 182.74, 163.94, 158.74, 157.59, 149.50, 131.94, 131.35, 129.15, 129.05, 126.33, 105.36, 104.94, 95.84, 61.69, 56.35; LC-MS (ESI $^+$, m/z) calculated for C$_{17}$H$_{14}$O$_5$: 298.08, found for 618.92 [2M+Na]$^+$.

7-Isoprenoxy-5-hydroxy-8-methoxyflavone (6b)

Yellow powder; mp: 173–175°C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H 12.53 (s, 1H, 5-OH), 7.96–7.94 (m, 2H, H-2′,6′), 7.56–7.52 (m, 3H, H-3′,4′,5′), 6.67 (s, 1H, H-3), 6.43 (s, 1H, H-6), 5.51 (dt, J = 1.3, 6.6 Hz, 1H, H-2′′), 4.67 (d, J = 6.6 Hz, 2H, H-1′′), 3.94 (s, 3H, OCH$_3$), 1.81 (s, 1H, CH$_2$), 1.78 (s, 1H, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 182.70, 163.85, 158.13, 157.33, 149.55, 138.91, 131.88, 131.40, 129.34, 129.12, 126.32, 118.80, 105.32, 104.87, 96.98, 66.10, 61.57, 25.79, 18.34; LC-MS (ESI $^+$, m/z) calculated for C$_{21}$H$_{20}$O$_5$: 352.13, found for 726.94 [2M+Na]$^+$.

7-Geranoxy-5-hydroxy-8-methoxyflavone (6c)
Yellow powder; mp: 88–90°C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\)H 12.53 (s, 1H, 5-OH), 7.96–7.94 (m, 2H, H-2′,6′), 7.56–7.54 (m, 3H, H-3′,4′,5′), 6.67 (s, 1H, H-3), 6.43 (s, 1H, H-6), 5.51 (dt, \(J = 0.9, 6.5\) Hz, 1H, H-2″), 5.10–5.08 (m, 1H, H-6″), 4.70 (d, \(J = 6.5\) Hz, 2H, H-1″), 3.94 (s, 3H, OCH\(_3\)), 2.15–2.10 (m, 4H, H-4″,5″), 1.77 (s, 3H, CH\(_3\)), 1.68 (s, 3H, CH\(_3\)) \cdot 1.61 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\)C 182.70, 163.84, 158.13, 157.33, 149.53, 142.04, 131.92, 131.87, 131.40, 129.33, 129.12, 126.31, 123.61, 118.61, 105.31, 104.86, 97.00, 66.14, 61.55, 39.47, 26.20, 25.63, 17.70, 16.77; LC-MS (ESI\(^+\), m/z) calculated for C\(_{26}\)H\(_{28}\)O\(_5\): 420.19, found for 862.96 [2M+Na]\(^+\).

### 7-Farnesoxy-5-hydroxy-8-methoxyflavone (6d)

Yellow powder; mp: 76–77°C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\)H 12.52 (s, 1H, 5-OH), 7.96–7.94 (m, 2H, H-2′,6′), 7.56–7.52 (m, 3H, H-3′,4′,5′), 6.67 (s, 1H, H-3), 6.43 (s, 1H, H-6), 5.51 (tt, \(J = 0.8, 6.5\) Hz, 1H, H-2″), 5.12–5.06 (m, 2H, H-6″,10″), 4.69 (d, \(J = 6.5\) Hz, 2H, H-1″), 3.94 (s, 3H, OCH\(_3\)), 2.16–2.11 (m, 4H, H-4″,5″), 2.05–2.02 (m, 2H, H-8″), 1.98–1.95 (m, 2H, H-9″), 1.77 (s, 3H, CH\(_3\)), 1.67 (s, 1H, CH\(_3\)), 1.61 (s, 1H, CH\(_3\)), 1.59 (s, 1H, CH\(_3\)); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\)C 182.68, 163.82, 158.11, 157.32, 149.53, 142.09, 135.55, 131.86, 131.39, 131.29, 129.32, 129.11, 126.30, 124.27, 123.47, 118.59, 105.30, 104.85, 96.98, 66.12, 61.54, 39.47, 26.68, 26.14, 25.66, 17.65, 16.79, 16.02; LC-MS (ESI\(^+\), m/z) calculated for C\(_{31}\)H\(_{30}\)O\(_5\): 488.26, found for 998.82 [2M+Na]\(^+\).

### 7-Methoxy-5-hydroxyflavone (7a)

Yellow powder; mp: 168–169°C (lit.\(^6\) 166–168°C); \(^1\)H NMR (500 MHz, DMSO-d\(_6\)): \(\delta\)H 12.75 (s, 1H, 5-OH), 7.92–7.90 (m, 2H, H-2′,6′), 7.58–7.53 (m, 3H, H-3′,4′,5′), 6.69 (s, 1H, H-3), 6.53 (d, \(J = 2.2\) Hz, 1H, H-6), 6.40 (d, \(J = 2.2\) Hz, 1H, H-8), 3.91 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (125 MHz, DMSO-d\(_6\)): \(\delta\)C 182.49, 165.60, 163.98, 162.19, 157.79, 131.82, 131.31, 129.07, 126.28, 105.87, 105.71, 98.18, 92.67, 55.80; LC-MS (ESI\(^+\), m/z) calculated for C\(_{16}\)H\(_{12}\)O\(_2\): 268.07, found for 558.76 [2M+Na]\(^+\).

### 7-Isoprenoxy-5-hydroxyflavone (7b)

Yellow powder; mp: 111–112°C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\)H 12.69 (s, 1H, 5-OH), 7.88–7.86 (m, 2H, H-2′,6′), 7.55–7.49 (m, 3H, H-3′,4′,5′), 6.65 (s, 1H, H-3), 6.49 (d, \(J = 2.1\) Hz, 1H, H-6), 6.37 (d, \(J = 2.1\) Hz, 1H, H-8), 5.48 (t, \(J = 6.6\) Hz, 1H, H-2″), 4.57 (d, \(J = 6.6\) Hz, 2H, H-1″), 1.80 (s, 3H, CH\(_3\)) \cdot 1.75 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\)C 182.47, 164.94, 163.95, 162.12, 157.76, 139.31, 131.79, 131.60, 129.07, 126.29, 118.55, 105.87, 105.62, 98.76, 93.33, 65.45, 25.83, 18.28; LC-MS (ESI\(^+\), m/z) calculated for C\(_{28}\)H\(_{18}\)O\(_4\): 322.12, found for 666.92 [2M+Na]\(^+\).
7-Geranyoxy-5-hydroxyflavone (7c)

Yellow powder; mp: 80‒82°C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_H$ 12.69 (s, 1H, 5-OH), 7.87‒7.85 (m, 2H, H-2′,6′), 7.55‒7.48 (m, 3H, H-3′,4′,5′), 6.64 (s, 1H, H-3), 6.49 (d, $J$ = 2.1 Hz, 1H, H-6), 6.36 (d, $J$ = 2.1 Hz, 1H, H-8), 5.47 (t, $J$ = 6.5 Hz, 1H, H-2′′), 5.48 (t, $J$ = 6.2 Hz, 1H, H-6′′), 4.68 (d, $J$ = 6.5 Hz, 2H, H-1′′), 2.13‒2.06 (m, 4H, H-4′′,5′′), 1.74 (s, 3H, CH$_3$), 1.66 (s, 3H, CH$_3$), 1.59 (s, 3H, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta_C$ 182.69, 165.18, 164.16, 162.33, 157.98, 142.56, 132.19, 132.01, 131.60, 129.29, 126.51, 123.84, 118.58, 106.09, 105.85, 99.01, 93.57, 65.75, 39.73, 26.46, 25.88, 17.93, 16.97; LC-MS (ESI$^+$, $m/z$) calculated for C$_{25}$H$_{26}$O$_4$: 390.18, found for 802.99 [2M+Na]$^+$. 

7-Farnesoxy-5-hydroxyflavone (7d)

Yellow powder; mp: 56‒57°C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_H$ 12.69 (s, 1H, 5-OH), 7.87‒7.86 (m, 2H, H-2′,6′), 7.55‒7.48 (m, 3H, H-3′,4′,5′), 6.64 (s, 1H, H-3), 6.49 (d, $J$ = 2.1 Hz, 1H, H-6), 5.47 (t, $J$ = 6.5 Hz, 1H, H-2′′), 5.10‒5.04 (m, 2H, H-6′′,10′′), 4.60 (d, $J$ = 6.5 Hz, 2H, H-1′′), 2.15‒2.09 (m, 4H, H-4′′,5′′), 2.05‒2.01 (m, 2H, H-8′′), 1.96‒1.93 (m, 2H, H-9′′), 1.75 (s, 3H, CH$_3$), 1.65 (s, 3H, CH$_3$), 1.59 (s, 3H, CH$_3$), 1.57 (s, 3H, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta_C$ 182.70, 165.19, 164.16, 162.35, 157.98, 142.59, 135.83, 132.02, 131.62, 131.57, 129.30, 126.51, 124.50, 123.72, 118.60, 106.10, 105.85, 99.01, 93.57, 65.74, 39.79, 39.74, 26.92, 26.37, 25.91, 17.90, 16.99, 16.27; LC-MS (ESI$^+$, $m/z$) calculated for C$_{30}$H$_{34}$O$_4$: 458.25, found for 938.92 [2M+Na]$^+$. 

3. DPPH radical scavenging activity

The quenching of free radicals by baicalein and its derivatives and oroxylin A evaluated at 517 nm against the absorbance of the DPPH free radical.$^7$ The sample solution which is dissolved in ethanol 100 μL was added to 100 μL of 200 μM DPPH solutions in ethanol in 96-well plates. The mixture solution was incubated at room temperature for 30 min, and then the absorbance of mixed solution was read at 517 nm using ELISA reader and remaining DPPH was calculated. Samples were dissolved in ethanol and the ethanolic solution of DPPH served as a control. All experiments were carried out in triplicate. The IC$_{50}$ values were defined as the concentration that could scavenge 50% DPPH free radical. Ascorbic acid, trolox and quercetin were used as a reference free radical scavenger.

4. ABTS$^{•+}$ decoloration assay
The antioxidant activity was measured by the ability of hydrogen-donating antioxidants to scavenge the ABTS$^{•+}$ radical cation using previously reported method. The radical cation was prepared by mixing a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 12–16 h until the reaction was complete and absorbance was stable. The ABTS$^{•+}$ solution was diluted in phosphate buffer (PBS) to an absorbance of 0.70 ± 0.02 at 734 nm for measurements. The photometric assay was conducted on in 1.9 mL of the ABTS$^{•+}$ solution and 0.1 mL of baicalein and its derivatives dissolved in an ethanol solution and mixed for 6 min measurements were taken immediately at 734 nm. The percentage inhibition (expressed as percent decrease of absorbance at 734 nm) is calculated as a function of concentration of baicalein derivatives, oroxylin A, and of Trolox for the standard reference data. All experiments were carried out in triplicate. The IC$_{50}$ values were defined as the concentration that cause 50% decrease of ABTS$^{•+}$ radical cation. Ascorbic acid and quercetin were used as a reference free radical scavenger.

5. Biological activity

5.1 Cell lines and cell culture conditions

All cells were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). Human colon cancer cells (HT-29) and Human colon cancer cells (DLD-1) cells were cultured in RPMI 1640 (HyClone, Logan, USA) medium, supplemented with 10% fetal bovine serum (FBS, Gibco, Life Technologies, New York, USA) and antibiotics (100 mg/mL streptomycin and 100 units/mL penicillin, Gibco, Life Technologies, USA). Human colon cancer cells (SW480), human liver carcinoma cell lines (HepG2), and normal murine embryonic liver cell lines (BNL CL.2) were cultured in Dulbecco’s modified Eagle medium (DMEM, HyClone, Logan, USA) medium, supplemented with 10% fetal bovine serum (FBS) and antibiotics. Human cancer cells were maintained in humidified atmosphere with 5% CO$_2$ and 95% air at 37°C in a carbon dioxide incubator (SANYO, CO$_2$ incubator, Osaka, Japan).

5.2 Cell viability inhibition assay

Cell viability was assessed by staining with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Bionovas Biotechnology Co., Ltd., Toronto, Ontario, Canada). Cancer cells were plated at 5 × 10$^3$ – 1 × 10$^4$ cells into 96-well plates and HL-60 cells were plated at 1 × 10$^5$ cells/mL into 12-well plates. After overnight growth, cells were treated with various concentrations of lawsone
derivatives for 24 h, 48 h and 72 h. At the end of treatment, final concentration of 0.5 mg/mL MTT was added, and cells were incubated for a further 1.5 h. The absorbance was recorded on an ELISA plate reader (SpectraMax 340PC, Molecular Devices, Sunnyvale, CA, USA) at a 540 nm wavelength. Inhibition ratio (%) was calculated using the following equation:

\[
\text{Inhibition ratio (\%)} = \left(\frac{A_{\text{control}} - A_{\text{treated}}}{A_{\text{control}}}\right) \times 100\%
\]

\(A_{\text{treated}}\) and \(A_{\text{control}}\) are the average absorbance of three independent experiments from treated and control groups, respectively.

Cell viability was determined the test compound concentration required to inhibit tumor cell proliferation by 50% (IC\(_{50}\)) from the dose-response curves. All data average values from triplicate samples and the experiments were repeated at least three times.

5.3 Cell morphological assessment

To detect morphological evidence of apoptosis, cells were visualized following DNA staining with the fluorescent dye Hoechst 33258.10,11 The SW480 cells were plated in 6-cm dish at a density of 7 \times 10^5 cells/well and were incubated overnight. The cells were treated with 5–20 μM baicalein (1), compounds 5d and 5i for 48 h. The cells of each dish were stained with 5 mM Hoechst 33258 (Sigma-Aldrich, St. Louis, MO, USA). After Cells were incubated in a dark room for 5 min, the cells were washed with PBS and then were observed by a fluorescent microscope (Zeiss, Axio Observer A1, Japan).

5.4 Cell cycle distribution analysis

Flow cytometry was used to obtain the cell cycle distribution and the apoptotic rate.12 The SW480 cells were plated at 1 \times 10^6 cells per 6-cm dish and cultured overnight. Then various concentration of 5 – 20 μM baicalein (1), compounds 5d and 5i were added for 48 h. The cells of each dish were harvested, washed once with PBS and fixed with methanol at 4°C. After 18 h, the cells of each dish were harvested, washed once with PBS. The cells were resuspended in 473 μL of PBS containing 40 μg/mL propidium iodide (PI) and 40 μg/mL RNase. Cells were incubated in a dark room for 30 min at room temperature then subjected to cell cycle analysis using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and analyzed using the ModFit 3.0 software (Verity Software House, ME, USA).

5.5 Assessment of apoptotic analysis
The SW480 cells were plated at the concentration of $1 \times 10^6$ cells per 6-cm dish. After overnight growth, cells were treated with $5 – 20 \mu M$ baicalein (I), compounds 5d and 5i for 48 h. The cells of each dish were harvested, washed once with PBS. The cells were resuspended in 500 μL of PBS containing 4 μg/mL propidium iodide (PI) and 4 μg/mL annexin-V-FITC. Cells were incubated in a dark room for 30 min at room temperature then subjected to cell cycle analysis using a FACScan flow cytometer. Annexin V-FITC and PI emission were detected in the FL1 and FL2 channels of flow cytometry, respectively. Flow cytometer data show three distinct populations of SW480 cells. Early apoptosis and late apoptosis/necrosis were represented as the percentage of annexin V+/PI– and annexin V+/PI+ cells, respectively. The data were analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and analyzed using the ModFit 3.0 software (Verity Software House, ME, USA).

5.6 Measurement of intracellular ROS production

Intracellular oxidant stress was monitored by measuring changes in fluorescence resulting from intracellular probe oxidation. Intracellular production of ROS, namely superoxide anion ($O_2^{•−}$), was measured using dihydroethidium (DHE, Molecular Probes, USA) probe, respectively. The level of intracellular ROS was detected using fluorescent dye DHE sensitivity. Briefly, the SW480 cells were pretreated with 5–20 μM baicalein (1), compounds 5d and 5i for 24 h. The cells were then washed with PBS, and incubated with 10 μM DHE attenuated with serum-free medium for 30 min at 37°C in the dark according to the manufacturer’s instructions. The fluorescence intensity in cells was determined using FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and analyzed using the ModFit 3.0 software (Verity Software House, ME, USA) with settings at excitation and emission equal to 545/605 nm.

5.7 Statistical analysis

The values shown are mean ± SD of three independent experiments. Data are statistically evaluated by Student’s t-test of SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA) and shown significantly different in *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Notes and references:
Figure S1. (A) $^1$H NMR spectra of baicalein (1); (B) $^{13}$C NMR spectra of baicalein (1).
Figure S2. (A) $^1$H NMR spectra of wogonin (3); (B) $^{13}$C NMR spectra of wogonin (3).
Figure S3. (A) $^1$H NMR spectra of 5,6-dihydroxy-7-methoxyflavone (5a); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-methoxyflavone (5a).
Figure S4. (A) $^1$H NMR spectra of 5,6-dihydroxy-7-isoprenoxyflavone (5b); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-isoprenoxyflavone (5b).
**Figure S5.** (A) $^1$H NMR spectra of 5,6-dihydroxy-7-geranoxyflavone (5c); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-geranoxyflavone (5c).
Figure S6. (A) $^1$H NMR spectra of 5,6-dihydroxy-7-farnesoxyflavone (5d); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-farnesoxyflavone (5d).
Figure S7. (A) $^1$H NMR spectra of 5,6-dihydroxy-7-butyloxyflavone (5e); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-butyloxyflavone (5e).
Figure S8. (A) $^1$H NMR spectra of 5,6-dihydroxy-7-hexyloxyflavone (5f); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-hexyloxyflavone (5f).
Figure S9. (A) $^1$H NMR spectra of 5,6-dihydroxy-7-octyloxyflavone (5g); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-octyloxyflavone (5g).
**Figure S10.** (A) $^1$H NMR spectra of 5,6-dihydroxy-7-decyloxyflavone (5h); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-decyloxyflavone (5h).
Figure S11. (A) $^1$H NMR spectra of 5,6-dihydroxy-7-dodecyloxyflavone (5i); (B) $^{13}$C NMR spectra of 5,6-dihydroxy-7-dodecyloxyflavone (5i).
Figure S12. (A) $^1$H NMR spectra of 7,8-dimethoxy-5-hydroxyflavone (6a); (B) $^{13}$C NMR spectra of 7,8-dimethoxy-5-hydroxyflavone (6a).
Figure S13. (A) $^1$H NMR spectra of 7-isoprenoxy-5-hydroxy-8-methoxyflavone (6b); (B) $^{13}$C NMR spectra of 7-isoprenoxy-5-hydroxy-8-methoxyflavone (6b).
Figure S14. (A) $^1$H NMR spectra of 7-geranoxy-5-hydro-8-methoxyflavone (6c); (B) $^{13}$C NMR spectra of 7-geranoxy-5-hydroxy-8-methoxyflavone (6c).
Figure S15. (A) $^1$H NMR spectra of 7-farnesoxy-5-hydroxy-8-methoxyflavone (6d); (B) $^{13}$C NMR spectra of 7-farnesoxy-5-hydroxy-8-methoxyflavone (6d).
Figure S16. (A) $^1$H NMR spectra of 7-methoxy-5-hydroxyflavone (7a); (B) $^{13}$C NMR spectra of 7-methoxy-5-hydroxyflavone (7a).
Figure S17. (A) $^1$H NMR spectra of 7-isoprenoxy-5-hydroxyflavone (7b); (B) $^{13}$C NMR spectra of 7-isoprenoxy-5-hydroxyflavone (7b).
Figure S18. (A) $^1$H NMR spectra of 7-geranyloxy-5-hydroxyflavone (7c); (B) $^{13}$C NMR spectra of 7-geranyloxy-5-hydroxyflavone (7c).
Figure S19. (A) $^1$H NMR spectra of 7-farnesoxy-5-hydroxyflavone (7d); (B) $^{13}$C NMR spectra of 7-farnesoxy-5-hydroxyflavone (7d).