Synthesis and Anticancer Activities of 5,6,7-Trimethylbaicalein Derivatives[1)]

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The aim of this study was to develop potential anticancer agents based on a naturally occurring baicalein, a flavonoid from *Scatellariae radix*. Cinnamic acid derivatives were converted to corresponding chlorides and then condensed with 3,4,5-trimethoxyphenol in the presence of $BF_3 \cdot Et_2O$ to give chalcones. Intramolecular cyclization of these intermediates by the actions of DMSO/I_2 afforded the desired trimethylbaicalein derivatives. Cell viability after treatment with the tested compound for 2 d was determined by a colorimetric MTT assay. The results indicated that most of the derivatives showed improved inhibition of proliferation of Hep G2 cells. Compound 9 was the most potent, in which the cell viability was reduced to <2% at the 25 μM level. In the case of Hep 3B cells, 8a, 8b and 8f showed moderate inhibition of their proliferation and 25 μM was required to reduce the viability to *ca.* 30%. On the other hand, prostate DU145 cells were more resistant. Most of the derivatives caused a 60% inhibition of DU145 cells only at a concentration of 100 μM or above.

Key words anticancer agent; trimethylbaicalein derivative; MTT assay

The Chinese herb *Scutellariae baicalensis* has been used since ancient times to treat allergic and inflammatory diseases in China. Recently it has also been used to treat hepatoma and leukemia in the Chinese community. The broad pharmacological properties of the herb have been investigated, including anti-inflammatory, antiallergic, anticarcinogenic, free radical scavenging and antioxidant activities.[2—4)] The major components of *Scutellariae baicalensis* are baicalein (**1**, Fig. 1), and its glycone baicalin, and wogonin. Recent investigations on baicalein indicated that it is an effective antihepatoma agent with minimal influence on noncancer cells.[5)] Ikemoto and co-workers reported that baicalein also exhibited the greatest antiproliferative activity among these components with IC_{50}s of 0.9—4.4 μg/ml against bladder cancer cell lines (KU-1, EJ-1, MBT-2).[6)] Baicalein was also found to suppress cell cycle progression in two prostate cancer cell lines, PC3 and DU-145.[7)] On the other hand, many natural components have been known to be potential leads for the development of anticancer agents, for instance, combretastatin A4 (**2**),[8)] a potent antimitotic agent and hormothamnione (**3**),[9)] a styrylchrome pigment with potent cytotoxicity (IC_{50}=0.53 μg/ml, KB cells). They were potent anticancer leads with trimethoxybenzene or trimethoxyflavone pharmacophores. Chemically, baicalein has a classical trihydroxyflavone skeleton that has been known to possess various novel pharmacological properties. These results suggested that trimethylated baicalein can be a suitable lead to develop promising antiproliferative agents specifically on hepatoma and bladder cancers. In this report, we synthesized certain 5,6,7-trimethylbaicalein derivatives basically modified at B-ring of its structural skeleton and preliminarily evaluated their cytotoxic effects by colorimetric MTT assays.

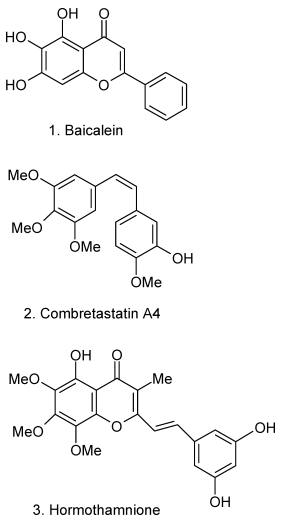
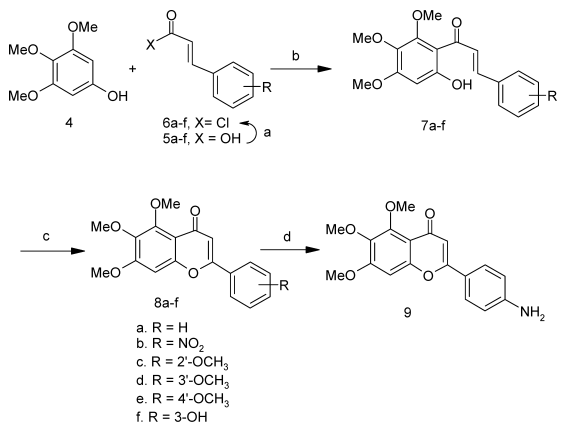


Fig. 1. Chemical Structures of Potent Anticancer Agents

Results and Discussion

Chemistry A concise and efficient synthesis of baicalein derivatives was carried out by the reactions outlined in Chart 1. Cinnamoyl chlorides **6a**—**f** were obtained from corresponding acids **5a**—**f** in good yields by the action of oxalyl



a. $(COCl)_2$, DMF b. $BF_3 Et_2O$, heat c. I_2, DMSO, heat d. $SnCl_2 2H_2O$.

Chart 1

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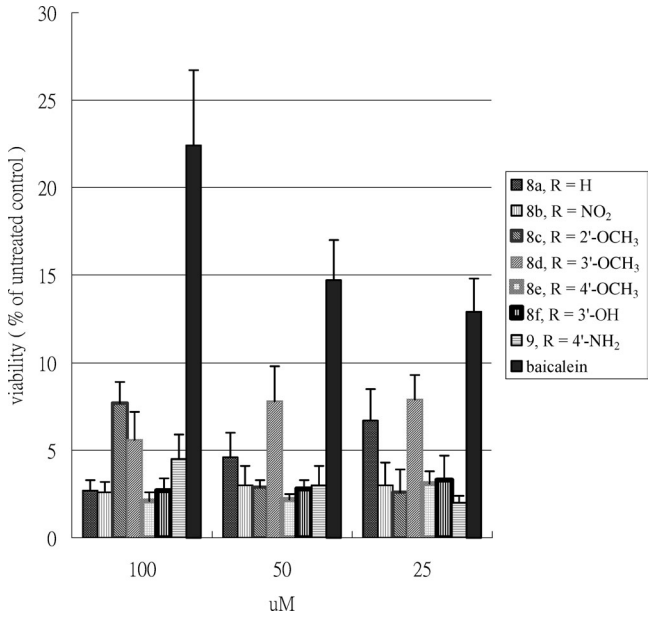

Fig. 2. Effects of **8a**—**f**, **9** Compared with Baicalein on Cell Viability of Hepatoma G2 Cell Lines

Values are the mean±S.D., $n=3$.

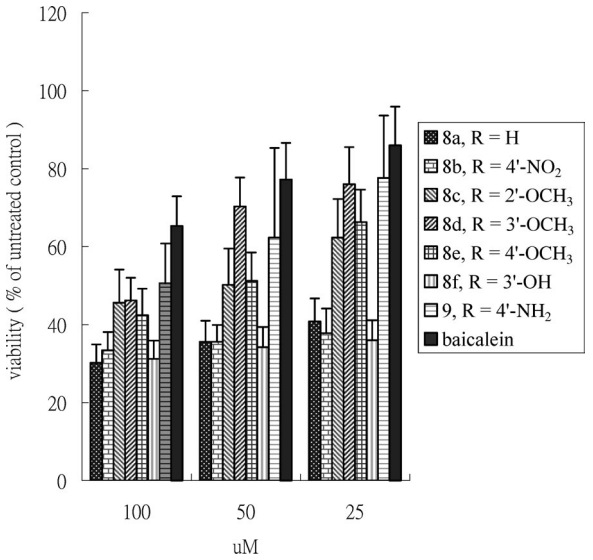

Fig. 3. Effects of **8a**—**f**, **9**, Compared with Baicalein on Cell Viability of Hepatoma 3B Cell Lines

Values are the mean±S.D., $n=3$.

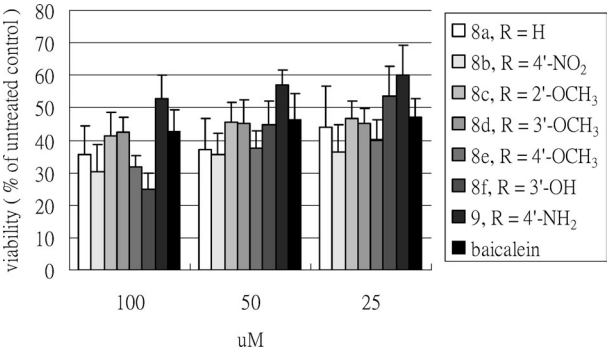

Fig. 4. Effects of **8a**—**f**, **9**, Compared with Baicalein on Cell Viability of Prostate DU-145 Cell Lines

Values are the mean±S.D., $n=3$.

chloride in the presence of a trace amount of dimethylformamide. Trimethoxyphenol (**4**) was then reacted with the cinnamoyl chloride intermediates in the presence of BF_3–Et_2O complex to proceed with electrophilic aromatic substitution reaction and give the corresponding chalcones **7a**—**f** in moderate yields (46—96%). The following cyclodehydrogenation of chalcones was accomplished with the catalytic amounts of iodine in DMSO[10] in a short reflux time to give the desired trimethylbaicalein derivatives **8a**—**f**. Further reduction of nitro derivative **8b** was problematic. It was unsuccessful under iron/hydrochloric acid and heterogeneous platinum oxide conditions. Finally, the mild reducing agent tin(II) chloride ran the reduction smoothly to give the desired anilide derivative **9**.

Anti-cancer Activities The inhibitory effects of baicalein and its derivatives on the growth of human Hep and prostate cancer cells were assessed by measuring the number of viable cells surviving after treatment with 25, 50, and 100 μM of tested compounds for 2 d by the colorimetric MTT assays.[11] First, the anticancer effects of these trimethylbaicalein derivatives on human Hep G2 cell line are shown in Fig. 2. All the tested derivatives were more potent than baicalein. The viability of Hep G2 cells was impressively reduced to $<10\%$ by 25 μM of all of these trimethylbaicalein derivatives ($p<0.05$), while it was reduced to $<15\%$ by 25 μM of baicalein. At the 100 μM level, the viability of the cells was effectively reduced to $<5\%$ by these derivatives except **8c** ($p<0.01$). Among these compounds, **9** was the most potent, in which the cell viability was reduced to $<2\%$ at the 25 μM level. As shown in Fig. 3 in the case of Hep 3B, **8a**, **8b** and **8f** showed moderate inhibitory effects requiring 25 μM to reduce the viability to 30% ($p<0.05$). These synthetic derivatives showed higher potency as compared with baicalein. **8e** was the most potent, in which the percentage of viable cells was reduced to about 30% in Hep 3B cells after treatment with 25 μM for 2 d.

On the other hand, baicalein and these derivatives demonstrated a weak inhibition on the growth of prostate cancer cell, DU145 (Fig. 4). These trimethylbaicalein derivatives except **9** showed a 60% inhibitory effect only at a concentration higher than 100 μM. Only **8f** was able to inhibit the cell growth of DU145 by over 75% at this concentration. But most of these derivatives were stronger than baicalein against the prostate cancer cells.

The naturally occurring baicalein has been known to possess a broad spectrum of pharmacological activities. Its antiproliferative activities against certain refractory bladder and prostate cancer cells were quite promising, as previously reported.[6,7] The present *in vitro* study has demonstrated that baicalein derivatives with the trimethoxyflavone pharmacophore exhibited an improved inhibitory effect on certain human Hep and prostate cancer cells. In fact, recent investigations indicated that the trimethoxy unit of naturally occurring combretastatins is essential for their anticancer activities by interaction with tubulin.[15] Moreover, the well known colchicine and the marine product hormothamnione also possess the trimethoxybenzene functionality that is required for their antimitotic properties.[9] All of the contributions indicated the trimethoxybenzene unit is critical for promising

anticancer properties. In this study, compound modification at B-ring of the trimethylbaicalein skeleton, *e.g.* **8b** (4′-NO_2), **8e** (4′-OCH_3), **8f** (3′-OH), and **9** (4′-NH_2) showed impressive potency against Hep G2 cells, whereas **8a**, **8b** and **8f** also exhibited potent inhibition of proliferation of Hep 3B cells. These results suggested that triple methoxy groups at A-ring of baicalein skeleton could be a suitable lead toward potent inhibitors against certain Hep cancers. Modification of B-ring with certain common electrowithdrawal or electrodonating groups at the 4′-position would provide improved antiproliferative activities. Recently, Chan and co-workers reported that baicalein caused a 50% inhibition of DU145 cells only at a concentration of 150 μM or above.[12] In our study, derivatives **8b** and **8f** showed more potency against DU145 cells than baicalein.

Conclusion

In summary, new trimethylbaicalein derivatives **8b** and **8f** were found to be much more potent than baicalein against certain Hep cancer cells and showed moderate potency to inhibit proliferation of DU145 cells. Although compound **9** had a much weaker inhibitory effect on prostate DU145, it showed impressive potency against Hep G2 cells among these derivatives. Recent investigations on the structure–activity relationships of combretastatin A4 have come up with some potent analogues with antimitotic activities.[16] These results suggested that baicalein would be an attractive and promising leader in the treatment of certain Hep cancers. Further, manipulation of baicalein derivatives with trimethoxyflavone pharmacophore would be worth developing potential candidates for human malignant cancers. The study is in progress and will be reported in due course.

Experimental

Chemistry All reagents were commercial materials and were used directly unless otherwise noted. DMF was dehydrated over 4 Å molecular sieves. NMR spectra were recorded on a Varian Gemini at 300 MHz for ^{1}H and at 75 MHz for ^{13}C. Elemental analyses were determined using a Perkin-Elmer 240 EA analyzer. Chromatography refers to flash chromatography on silica gel (silica gel 60, 230—400 mesh ASTM, E. Merck). Melting points were recorded on a Thomas Hoover capillary melting point apparatus in open capillary tubes and are uncorrected.

General Procedure A A solution of a cinnamic acid derivative (**5**) in dichloromethane (10 ml) was cooled to 0—5 °C under N_2 with an ice-water bath. Oxalyl chloride (12 mmol) was added *via* syringe followed by a trace amount of anhydrous DMF. After maintaining at 0—5 °C for 2 h, the reaction mixture was concentrated in vacuum to give the corresponding chloride **6**, which was used directly without further purification. To a mixture of the acyl chloride **6** and 3,4,5-trimethoxyphenol (**4**, 10 mmol) was slowly added BF_3–Et_2O (10 ml) *via* syringe. The reaction mixture was then heated under reflux for 30 min and cooled to room temperature to give a precipitate, which was washed with ether and collected by filtration to give the desired product **7**.

6′-Hydroxy-2′,3′,4′-trimethoxychalcone (7a) According to general procedure A, the title compound was obtained as an orange solid: mp 194—196 °C; UV (MeOH) λ_{max} nm (log ε)=321 (4.46), 218 (4.48); IR (KBr) ν cm^{-1}=3421 (br, OH), 1618 (C=O), 1595 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.85 (3H, s, 3′-OCH_3), 3.99 (3H, s, 4′-OCH_3), 4.06 (3H, s, 2′-OCH_3), 6.37 (1H, s, H-5′), 7.46—7.51 (3H, m, H-3,4,5), 7.71 (2H, d, J=6.2 Hz, H-2,6), 8.06 (2H, d, J=15.4 Hz, H-α), 8.37 (2H, d, J=15.4 Hz, H-β); EI-MS: m/z [M]$^+$ 314. *Anal.* ($C_{18}H_{18}O_5$) Calcd C, 68.76; H, 5.76. Found C, 68.55; H, 5.97.

6′-Hydroxy-2′,3′,4′-trimethoxy-4-nitrochalcone (7b) According to general procedure A, the title compound was obtained as an orange solid: mp 180—182 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.84 (3H, s, 3′-OCH_3), 3.93 (3H, s, 4′-OCH_3), 3.95 (3H, s, 2′-OCH_3), 6.32 (1H, s, H-5′), 7.78 (2H, d, J=6.2 Hz, H-2, 6), 8.03 (2H, d, J=6.2 Hz, H-3, 5), 8.28 (2H, d, H-α, β); FAB-MS: m/z [M+H]$^+$ 360. *Anal.* ($C_{18}H_{17}NO_7$) Calcd C, 60.17; H, 4.74. Found C, 60.46; H, 4.59.

6′-Hydroxy-2,2′,3′,4′-tetramethoxychalcone (7c) According to general procedure A, the title compound was obtained as an orange solid: mp 128—131 °C; UV (EtOH): λ_{max} nm (log ε)=363 (4.05), 282 (4.04); IR (KBr): ν cm^{-1}=3419 (OH), 1608 (CO), 1541 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.77 (3H, s, OCH_3) , 4.00 (3H, s, OCH_3) , 4.01 (3H, s, OCH_3), 4.09 (3H, s, OCH_3), 6.59 (1H, s, H-5′), 7.12 (1H, t, J=8.4 Hz, H-5), 7.23 (1H, d, J=8.4 Hz, H-3), 7.60 (1H, t, J=8.4 Hz, H-4), 7.86 (1H, d, J=8.1 Hz, H-6) , 8.22 (1H, d, J=15.6 Hz, H-α), 8.46 (1H, d, J=15.6 Hz, H-β); FAB-MS (NBA as matrix): m/z [M+H]$^+$ 345. *Anal.* ($C_{19}H_{20}O_6$) Calcd C, 66.27; H, 5.85. Found C, 66.52; H, 5.58.

6′-Hydroxy-2′,3,3′,4′-tetramethoxychalcone (7d) According to general procedure A, the title compound was obtained as an orange solid: mp 174—176 °C; UV (EtOH): λ_{max} nm (log ε)=322 (3.02), 209 (3.36); IR (KBr): ν cm^{-1}=3061 (OH), 1635 (CO), 1598 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.72 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 6.41 (1H, s, H-5′), 7.14 (1H, d, H-4), 7.23—7.30 (3H, H-2, 5, 6), 7.57 (2H, s, H-α, β); FAB-MS (NBA as matrix): m/z [M+H]$^+$ 345.1. *Anal.* ($C_{19}H_{20}O_6$) Calcd C, 66.27; H, 5.85. Found C, 66.49; H, 5.67.

6′-Hydroxy-2′,3′,4′,4-tetramethoxychalcone (7e) According to general procedure A, the title compound was obtained as a golden yellow solid: mp 201—204 °C; UV (EtOH): λ_{max} nm (log ε)=364.0 (4.41); ^{1}H-NMR (300 MHz , DMSO-d_6) δ 3.90 (3H, s, –OCH_3), 3.96 (3H, s, –OCH_3), 4.03 (3H, s, –OCH_3), 4.10 (3H, s, –OCH_3), 6.43 (1H, s, H-5′), 7.06 (2H, d, J=9.0 Hz, H-3, 5), 7.75 (2H, d, J=8.7 Hz, H-2, 6), 8.03 (2H, d, J=15.3 Hz, H-α), 8.44 (2H, J=15.0 Hz, H-β); FAB-MS (NBA as matrix): m/z [M+H]$^+$ 345. *Anal.* ($C_{19}H_{20}O_6$) Calcd C, 66.27; H, 5.85. Found C, 66.48; H, 5.76.

3,6′-Dihydroxy-2′,3′,4′-trimethoxychalcone (7f) According to general procedure A, the title compound was obtained as a golden yellow solid: mp 87—89 °C; UV (EtOH): λ_{max} nm (log ε) 322 (4.09), 208 (4.45); IR (KBr): ν cm^{-1}=3323 (OH), 1635 (C=O), 1558 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.84 (3H, s, 3′-OCH_3), 3.89 (3H, s, 4′-OCH_3), 3.91 (3H, s, 2′-OCH_3), 6.30 (1H, s, H-5′), 6.90 (1H, d, J=7.26 Hz, H-4), 7.13 (1H, s, H-2), 7.17 (1H, d, J=7.8 Hz, H-6), 7.26 (1H, t, J=7.8 Hz, H-5), 7.74 (1H, d, J=15.7 Hz, H-α), 7.90 (1H, d, J=15.6 Hz, H-β), EI-MS: m/z [M]$^+$ 330. *Anal.* ($C_{18}H_{18}O_6$) Calcd C, 65.43; H, 5.50. Found C, 65.17; H, 5.79.

General Procedure B To a solution of a chalcone derivative (**7**) in DMSO (30 ml) was added iodine (0.1 g, 0.4 mmol) and the mixture was heated under reflux for 2 h and then poured into ice water to precipitate the product. The crude product was filtered and dissolved in ethyl acetate, and washed with 10% aqueous sodium thiosulfate (2×150 ml). The organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated in vacuum to dryness. The resulting residue, in each case, was crystallized from ethanol to give the desired product **8**.

5,6,7-Trimethylbaicalein (8a) According to general procedure B, **7a** (3.14 g, 10 mmol) was oxidized to yield the title compound (2.81 g, 90%) as a yellow solid: mp 161—163 °C (lit.[13] 160—162 °C); UV (EtOH): λ_{max} nm (log ε)=306.5 (4.11), 263.5 (4.14), 214.0 (4.36); IR (KBr): ν cm^{-1}=3419 (OH), 1608 (CO), 1546 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.99 (3H, s, 6-OCH_3), 4.05 (3H, s, 7-OCH_3), 4.06 (3H, s, 5-OCH_3), 6.74 (1H, s, H-8), 6.89 (1H, s, H-3), 7.57—7.59 (3H, m, H-3′,4′,5′); 7.95 (2H, dd, J=6.6, 1.3 Hz, H-2′,6′); EI-MS (70 eV): m/z [M]$^+$ 312. *Anal.* ($C_{18}H_{16}O_5$) Calcd C, 69.24; H, 5.12. Found C, 68.95; H, 4.88.

4′-Nitro 5,6,7-Trimethylbaicalein (8b) According to general procedure B, **7b** (3.59 g, 10 mmol) was oxidized to yield the title compound (3.03 g, 85%) as a brown solid: mp 182—185°C; UV (EtOH): λ_{max} nm (log ε)=205.5 (4.24), 316.5 (4.08); IR (KBr): ν cm^{-1}=1649 (CO); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.91 (3H, s, 6-OCH_3), 3.98 (3H, s, 7-OCH_3), 3.99 (3H, s, 5-OCH_3); 6.74 (1H, s, H-8), 6.82 (1H, s, H-3), 8.04 (2H, d, J=9.3 Hz, H-2′,6′); 8.35 (2H, d, J=8.7 Hz, H-3′,5′); EI-MS (70 eV): m/z [M]$^+$ 357. *Anal.* ($C_{18}H_{15}NO_7$) Calcd C, 60.52; H, 4.20. Found C, 60.24, H 3.96.

2′-Methoxy-5,6,7-trimethylbaicalein (8c) According to general procedure B, **7c** (3.44 g, 10 mmol) was oxidized to yield the title compound (2.94 g, 86%) as a yellow solid: mp 76—78 °C; UV (EtOH): λ_{max} nm (log ε)=318.0 (4.64), 263.0 (4.71); IR (KBr): ν cm^{-1}=1636 (CO), 1560 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.91 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.76 (1H, s, H-8), 6.96 (1H, s, H-3), 7.04 (1H, d, J=8.4 Hz, H-3′), 7.08 (1H, t, J=8.4 Hz, H-4′), 7.45 (1H, t, J=8.4 Hz, H-5′), 7.83 (1H, dd, J=7.7, 1.2 Hz, H-6′); FAB-MS (NBA as matrix): m/z [M+H]$^+$ 343. *Anal.* ($C_{19}H_{18}O_6$) Calcd C, 66.66; H, 5.30. Found C, 66.94; H, 4.98.

3′-Methoxy-5,6,7-trimethylbaicalein (8d) According to general proce-

dure B, **7d** (3.44 g, 10 mmol) was oxidized to yield the title compound (2.22 g, 65%) as a brown solid: mp 63—65 °C; UV (EtOH): λ_{max} nm (log ε)=309 (4.46), 265 (4.40), 241 (4.45); IR (KBr): ν cm^{-1}=1637 (CO), 1560 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.96 (6H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 6.64 (1H, s, H-8), 6.79 (1H, s, H-3), 7.03 (1H, d, J=7.5 Hz, H-4′), 7.36—7.43 (3H, m, H-2′,5′,6′); FAB-MS (NBA as matrix): m/z $[M+H]^+$ 343. *Anal.* ($C_{19}H_{18}O_6$) Calcd C, 66.66; H, 5.30. Found C, 66.94; H, 4.90.

4′-Methoxy-5,6,7-trimethylbaicalein (8e) According to general procedure B, **7e** (3.44 g, 10 mmol) was oxidized to yield the title compound (1.16 g, 34%) as a brown solid: mp 164—166°C (lit.[14] 158—159°C); UV (EtOH): λ_{max} nm (log ε)=267.0 (4.45), 212.0 (4.58); IR (KBr): ν cm^{-1}=1638 (CO), 1589 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.97 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.90 (3H, s, OCH_3); 3.85 (3H, s, OCH_3), 6.54 (1H, s, H-8), 6.78 (1H, s, H-3), 6.96 (2H, d, J=8.7 Hz, H-3′,5′), 7.78 (2H, d, J=8.7 Hz, H-2′,6′); FAB-MS (NBA as matrix): m/z $[M+H]^+$ 343. *Anal.* ($C_{19}H_{18}O_6$) Calcd C, 66.66; H, 5.30. Found C, 66.52; H, 5.41.

3′-Hydroxy-5,6,7-trimethylbaicalein (8f) According to general procedure B, **7f** (3.30 g, 10 mmol) was oxidized to yield the title compound (3.37 g, 98%) as a yellow solid: mp 226—228°C; UV (EtOH): λ_{max} nm (log ε)=309.5 (4.00), 213.5 (4.30); IR (KBr): ν cm^{-1}=3079 (OH), 1631 (CO), 1593 (C=C), ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.76 (3H, s, 6-OCH_3), 3.79 (3H, s, 7-OCH_3), 3.94 (3H, s, 5-OCH_3), 6.68 (1H, s, H-8), 7.19 (1H, s, H-3), 6.96 (1H, d, J=8.1 Hz, H-4′), 7.31—7.48 (3H, m, H-1′,5′,6′); FAB-MS (NBA as matrix): m/z $[M+H]^+$ 329. *Anal.* ($C_{18}H_{16}O_6$) Calcd C, 65.85; H, 4.91. Found C, 65.59; H, 4.68.

4′-Amino-5,6,7-trimethylbaicalein (9) To a solution of **8b** (0.6 g, 1.68 mmol) in ethanol (70 ml) was added tin(II) chloride hydrate (1.17 g, 5.2 mmol). The resulting suspension was heated under reflux for 5 h. After cooling to room temperature, the mixture was poured into water, adjusted to pH 9 with sodium bicarbonate, and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated to give the residue, which was recrystallized with ethanol to yield the title compound (0.34 g, 42%) as a deep yellow solid: mp 73—75°C; UV (EtOH): λ_{max} nm (log ε)=365.0 (4.19), 220.0 (4.31); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.91 (3H, s, 6-OCH_3), 3.96 (3H, s, 7-OCH_3), 3.98 (3H, s, 5-OCH_3), 6.51 (1H, s, H-8), 6.78 (1H, s, H-3), 6.72 (2H, d, J=8.7 Hz, H-3′,5′), 7.64 (2H, d, J=8.4 Hz, H-2′,6′); FAB-MS (NBA as matrix): m/z $[M+H]^+$ 343. *Anal.* ($C_{19}H_{17}NO_5$) Calcd C, 66.66; H, 5.30. Found C, 66.52; H, 5.41.

Cell Lines and Incubation Two hepatoma cell lines (Hep G2 and Hep 3B) and a prostate cancer cell line (DU 145) were used. They were cultured in Dulbecco's modified Eagle's medium overnight and the medium was refreshed after 24 h. The stock solution of baicalein and its derivatives were dissolved in 100% DMSO, and the experimental concentrations were prepared in the aforementioned basal medium with a final DMSO concentration of 0.1%.

Drug Treatment and MTT Cell Viability Assay Cells in 24-well plates (5×10^4/well) were cultured overnight before replacement with medium in the presence or absence of baicalein or its derivatives. Various final concentrations of tested compounds were used in the experiments. The untreated and vehicle control groups contained the basal medium only and 0.1% DMSO in the basal medium, respectively. A general MTT assay was performed. At the end of the experiments, the medium was substituted with MTT solution (0.5 mg/ml) for 4 h. Sodium dodecyl sulfate (10%) was then added for another 12 h to thoroughly dissolve the dark blue crystals. The absorbance was read at 570 nm using a Shimadzu UV-160A reader. The cell viability was calculated as the ratio of the absorbance of the treated cells to the absorbance of the untreated control groups of each cell line at the indicated time. All incubations were carried out at 37°C in a humidified 5% CO_2 atmosphere.

The percentage of cell survival of each well was calculated from the following equation, in which the test consisted of the cells plus the tested compound and the control contained the cells alone.

$$\text{percentage of cell survival}=(T-B)/(C-B)\times100\%.$$

T: absorbance of test; B: absorbance of blank; C: absorbance of control.

Statistical Analysis All values are expressed as the mean±S.D. Statistical analysis was performed using Student's t-test.

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References and Notes

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