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Synthesis and Cytotoxic Effects of Sulfonamide-Substituted 5,6,7-Trimethoxyflavones on Human Cancer Cell Lines

Dong Han Kim, Bo Hee Yun, Eun Woo Choi, Sang Min Oh, Md. Maqusood Alam, Kyung-Tae Lee, and Yong Sup Lee*

*Department of Pharmacy, College of Pharmacy & Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul 130-701, Korea. *E-mail: kyslee@khu.ac.kr*
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The root of *Scutellaria baicalensis* Georgi have been traditionally used clinically in China, Korea and Japan to treat various ailments, such as allergy, inflammatory disease, diarrhea, and infections accompanied by fever. Studies have also reported that the extract from *S. baicalensis* exhibits antifungal activity,[1] inhibits skin inflammation,[2] and attenuates oxidative stress in cerebral ischemia.[3] Baicalein (**1**, 5,6,7-trihydroxyflavone) has been suggested as one of active component of *S. baicalensis* since it possesses a diverse range of pharmacological properties including anti-inflammatory,[4] antioxidative,[5] anxiolytic,[6] and anti-HIV[7] activities. In particular, it has been known to inhibit cell growth of human breast cancer,[8] hepatoma,[9] and prostate cancer.[10] Accordingly, baicalein has commonly been served as a lead compound to discover anticancer agents. In this line, 5,6,7-trimethoxyflavone (**2**) was synthesized by alkylating hydroxyl groups on the A ring of baicalein and it showed more potent cell growth inhibitory activity on hepatoma cells than baicalein.[11] The effects of 5,6,7-trimethoxyflavone against breast cancer resistance protein was also evaluated.[12]

Sulfonamide motifs are privileged functional group in drug discovery and widely exist in the structures of many drugs as a key pharmacophore. Further, several sulfonamide derivatives have been reported to show anticancer activity in a variety of mechanisms such as carbonic anhydrase inhibition, cell cycle arrest, disruption of microtubule assembly, and angiogenesis inhibition.[13] In the present study, we first synthesized new 5,6,7-trimethoxyflavone derivatives (**3**), which have a sulfonamide group at B-ring of flavone to further improve anticancer activity of baicalein and 5,6,7-trimethoxyflavone (Figure 1).

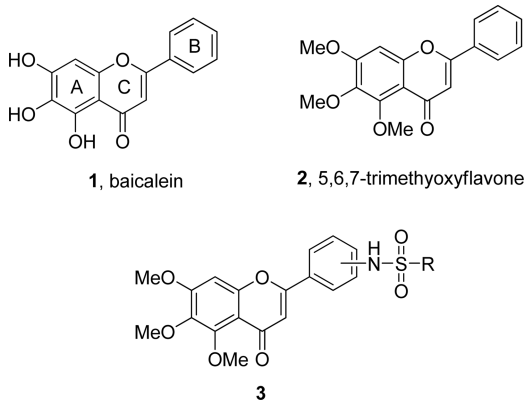
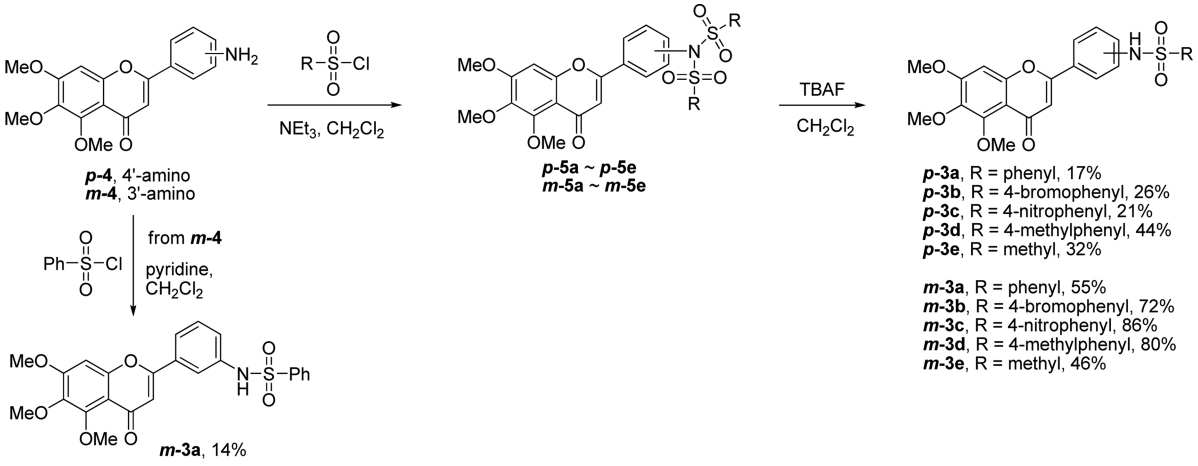


Figure 1. Chemical structures of baicalein (**1**), 5,6,7-trimethoxyflavone (**2**) and target compounds **3**.

Chemistry. The key intermediates 3'-amino- and 4'-amino-5,6,7-trimethoxyflavone, ***m*-4** and ***p*-4** was obtained by the previously reported procedure.[14] Initially the synthesis of sulfonamide-substituted 5,6,7-trimethoxyflavones **3** was tried by direct coupling of amino group in ***p*-4** and ***m*-4** with several sulfonyl chlorides, as was exemplified by the reaction of ***m*-4** with 1 equivalent of benzenesulfonyl chloride in



Scheme 1. Synthesis of sulfonamide-substituted 5,6,7-trimethoxyflavones **3**.

the presence of pyridine to obtain ***m*-3a** (Scheme 1). However, isolation yield of ***m*-3a** was very low (14%) due to concomitant formation of *N,N*-disulfonamido-flavone ***m*-5a** as a major product. Thus, mono-sulfonamide-substituted flavones **3** were prepared by the exclusive formation of *N,N*-disulfonamido-flavones (***p*-5a~e**, ***m*-5a~e**) followed by selective monodesulfonylation. Amino-flavones ***p*-4** and ***m*-4** were reacted with excess sulfonyl chloride in CH_2Cl_2 in the presence of triethylamine as a base to give disulfonamido-flavone (***p*-5a~e**, ***m*-5a~e**) in moderate to good yield. Finally, compounds ***p*-5a~e** and ***m*-5a~e** were treated with TBAF to provide monosulfonamido-flavones (***p*-3a~e**, ***m*-3a~e**).[15]

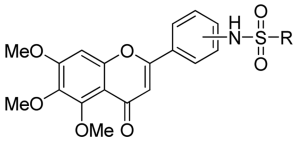
Biological Evaluation. The inhibitory effects of sulfonamide-substitutied 5,6,7-trimethoxyflavones **3** on the growth of human breast cancer (MCF-7) and hepatocellular carcinoma (Hep G2) cell lines were evaluated by measuring their growth inhibition effects using colorimetric MTT assays. To compare these data with those of standards, the growth inhibitory activity of baicalein (**1**) and 5,6,7-trimethoxyflavone (**2**) was inserted in the data set (Table 1). First, we screened the growth inhibitory activity of compounds on the tumor cell lines at 100 μM concentration. For compounds showing more than 50% growth inhibition, we assayed again at five-doses to determine IC_{50} values.

In our assay systems, baicalein (**1**) showed about 40% cell growth inhibition of MCF-7 cells but negligible effect on the growth of Hep G2 cells. As was observed in the preceeding report,[11] 5,6,7-trimethoxyflavone (**2**) showed more potent cytotoxicities than baicalein on MCF-7 and Hep G2 cell lines. Among synthesized, ***p*-3** series compounds of 5,6,7-trimethoxyflavones, possessing sulfonamide substituent at *para* position, showed less cytotoxicities than **2** on these cancer cell lines. However, compound ***p*-3b**, possessing a 4-bromobenzenesulfonamide substituent, exhibited more potent cytotoxicities on MCF-7 and Hep G2 cell lines.

On the other hand, almost 5,6,7-trimethoxyflavones ***m*-3**, possessing sulfonamide substituent at *meta* position, showed more potent cytotoxicities than **1** and **2** on MCF-7 and Hep G2 cell lines indicating that the position of sulfonamide substituents is important on activity. When the substituents were changed from arylsulfonyl to methanesulfonyl group (***p*-3d** and ***m*-3d**), cytotoxic effects were decreased. Compounds ***p*-3b** and ***m*-3b**, possessing 4-brombenzenesulfonamide group at *meta*- and *para*-position of the B-ring, respectively, exhibited the most potent cytotoxicities against MCF-7 (IC_{50} = 61.31 μM for ***p*-3b** and 37.53 μM for ***m*-3b**) and Hep G2 (IC_{50} = 66.09 μM for ***p*-3b** and 70.78 μM for ***m*-3b**) cell lines while 5,6,7-trimethoxyflavone (**2**) showed 58.42% and 39.78% inhibitions of cell growth on these cancer cell lines at 100 μM concentration.

In summary, we synthesized sulfonamide-substituted 5,6,7-trimethoxyflavones to improve the cytotoxic effect of baicalein on human cancer cell lines. Among synthesized, 4-bromobenzensulfonamide-substituted 5,6,7-trimethoxyflavones ***p*-3b** and ***m*-3b** showed the most potent and enhanced cytotoxicity on MCF-7 and Hep G2 cell lines than baicalein and 5,6,7-trimethoxyflavone (**2**). Accordingly, our finding indicates that the introduction of sulfonamide moiety on flavone structures can enhance their biological activity.

Table 1. The growth inhibition activity of ***p*-3a~e** and ***m*-3a~e** on cancer cell lines *in vitro*



Compds	Position of substituents	R	% Inhibition of cell growth[a]	
			MCF-7	Hep G2
***p*-3a**	4'-	phenyl	32.91	21.38
***p*-3b**	4'-	4-bromophenyl	86.45 (61.31 ± 2.71)[b]	71.98 (66.09 ± 3.93)[b]
***p*-3c**	4'-	4-nitrophenyl	48.41	26.19
***p*-3d**	4'-	4-methylphenyl	46.68	50.49
***p*-3e**	4'-	methyl	46.17	13.67
***m*-3a**	3'-	phenyl	59.79 (70.86 ± 3.36)[b]	51.65
***m*-3b**	3'-	4-bromophenyl	86.38 (37.53 ± 1.27)[b]	64.57 (70.78 ± 6.14)[b]
***m*-3c**	3'-	4-nitrophenyl	86.39 (55.02 ± 3.80)[b]	49.50
***m*-3d**	3'-	4-methylphenyl	68.05 (48.57 ± 1.12)[b]	35.00
***m*-3e**	3'-	methyl	39.76	24.75
1, baicalein			40.28	-[c]
2, 5,6,7-trimethoxyflavone			58.42	39.78

[a]% Cancer cell growth inhibition at 100 μM of compounds. [b]Data in the parenthesis are IC_{50} values in μM. IC_{50} was defined as the concentration resulting in 50% growth inhibition. [c]No inhibition. Data are presented as the means ± SDs of the three independent experiments.

Experimental Section

Materials and Chemicals. Most of the reagents and solvents were purchased from Sigma-Aldrich, Tokyo Chemical Industry, Acros Organics, or Fluka and used without further purification. Reactions were monitored with thin layer chromatography (TLC) conducted on Merck Kieselgel 60F254. ^1H and ^{13}C NMR spectra were recorded on Brucker Avance 400 spectrometer (400 and 100 MHz, respectively).

General Route to Sulfonamine-Substituted 5,6,7-Trimethoxyflavones *p*-3 and *m*-3. To a solution of amino-flavone ***p*-4** or ***m*-4** (0.61 mmol) and triethylamine (0.92 mmol) in CH_2Cl_2 (10 mL) was added slowly aryl- or methanesulfonyl chloride (3.13 mmol). The resulting mixture was stirred at rt under N_2 atmosphere. The mixture was quenched with water and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography to give the *N,N*-disulfonamide-substituted 5,6,7-trimethoxyflavone ***p*-5** or ***m*-5**. To a solution of ***p*-5** or ***m*-5** (0.25 mmol) in CH_2Cl_2 (10 mL) was added tetra-*n*-butylammonium fluoride

(1 M solution in THF, 0.30 mmol). The mixture was stirred at rt under N_2 atmosphere. The mixture was quenched with water and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography to give ***p*-3** or ***m*-3**.

***N*-(4-(5,6,7-Trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)-benzenesulfonamide (*p*-3a).** Compound ***p*-3a** was obtained according to the above procedure from the compound ***p*-4** and benzenesulfonyl chloride. ***p*-5a**: Yield 25%; ^{1}H NMR ($CDCl_3$) δ 7.97 (4H, d, J = 7.7 Hz, H-2",6"), 7.87 (2H, d, J = 8.5 Hz, H-2',6'), 7.71 (2H, t, J = 7.7 Hz, H-4"), 7.58 (4H, t, J = 7.7 Hz, H-3",5"), 7.17 (2H, d, J = 8.5 Hz, H-3',5'), 6.79 (1H, s, H-3), 6.67 (1H, s, H-8), 4.00 (3H, s, -OCH_3), 3.98 (3H, s, -OCH_3), 3.93 (3H, s, -OCH_3); ***p*-3a**: Yield 66%; ^{1}H NMR (DMSO-d_6) δ 10.86 (1H, s, -NH), 7.95 (2H, d, J = 8.4 Hz, H-3',5'), 7.85 (2H, d, J = 8.0 Hz, H-2",6"), 7.65 (1H, t, J = 7.0 Hz, H-4"), 7.59 (2H, t, J = 7.5 Hz, H-3",5"), 7.26 (2H, d, J = 8.5 Hz, H-2',6'), 7.17 (1H, s, H-3), 6.69 (1H, s, H-8), 3.94 (3H, s, -OCH_3), 3.79 (3H, s, -OCH_3), 3.76 (3H, s, -OCH_3); ^{13}C NMR (DMSO-d_6) δ 175.5, 159.7, 157.5, 153.9, 151.5, 140.7, 139.8, 139.3, 133.2, 129.4(2C), 127.2(2C), 126.6(2C), 125.9, 118.9(2C), 112.0, 106.7, 97.2, 61.8, 61.0, 56.4.

4-Bromo-*N*-(4-(5,6,7-trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)benzenesulfonamide (*p*-3b): Compound ***p*-3b** was obtained according to the above procedure from the compound ***p*-4** and 4-bromobenzenesulfonyl chloride. ***p*-5b**: Yield 37%; ^{1}H NMR ($CDCl_3$) δ 7.90 (2H, d, J = 8.6 Hz, H-2',6'), 7.82 (4H, d, J = 8.7 Hz, H-2",6"), 7.73 (4H, d, J = 8.7 Hz, H-3",5"), 7.17 (2H, d, J = 8.6 Hz, H-3',5'), 6.81 (1H, s, H-3), 6.70 (1H, s, H-8), 4.00 (3H, s, -OCH_3), 3.99 (3H, s, -OCH_3), 3.93 (3H, s, -OCH_3); ***p*-3b**: Yield 70%; ^{1}H NMR (DMSO-d_6) δ 10.91 (1H, s, -NH), 7.96 (2H, d, J = 8.7 Hz, H-2',6'), 7.82 (2H, d, J = 8.6 Hz, H-2",6"), 7.75 (2H, d, J = 8.6 Hz, H-3",5"), 7.26 (2H, d, J = 8.7 Hz, H-3',5'), 7.17 (1H, s, H-3), 6.71 (1H, s, H-8), 3.94 (3H, s, -OCH_3), 3.79 (3H, s, -OCH_3), 3.76 (3H, s, -OCH_3); ^{13}C NMR (DMSO-d_6) δ 176.1, 160.1, 158.0, 154.4, 152.0, 140.8, 140.0, 138.9, 133.1(4C), 129.1(4C), 127.8(4C), 127.7, 126.7, 119.6(4C), 112.5, 107.3, 97.7, 62.3, 61.5, 56.9.

4-Nitro-*N*-(4-(5,6,7-trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)benzenesulfonamide (*p*-3c): Compound ***p*-3c** was obtained according to the above procedure from the compound ***p*-4** and 4-nitrobenzenesulfonyl chloride. ***p*-5c**: Yield 27%; ^{1}H NMR ($CDCl_3$) δ 8.46 (4H, d, J = 8.7 Hz, H-3",5"), 8.18 (4H, d, J = 8.7 Hz, H-2",6"), 7.94 (2H, d, J = 8.3 Hz, H-2',6'), 7.18 (2H, d, J = 8.3 Hz, H-3',5'), 6.80 (1H, s, H-3), 6.71 (1H, s, H-8), 4.00 (3H, s, -OCH_3), 3.99 (3H, s, -OCH_3), 3.93 (3H, s, -OCH_3); ***p*-3c**: Yield 77%; ^{1}H NMR (DMSO-d_6) δ 11.11 (1H, s, -NH), 8.39 (2H, d, J = 7.9 Hz, H-3",5"), 8.07 (2H, d, J = 7.9 Hz, H-2",6"), 7.96 (2H, d, J = 7.9 Hz, H-2',6'), 7.27 (2H, d, J = 7.9 Hz, H-3',5'), 7.15 (1H, s, H-3), 6.69 (1H, s, H-8), 3.93 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3), 3.75 (3H, s, -OCH_3); ^{13}C NMR (DMSO-d_6) δ 176.1, 160.0, 158.0, 154.4, 152.0, 150.5, 145.0, 140.5, 140.3, 128.8(2C), 127.9(2C), 127.0, 125.3(2C), 119.9(2C), 112.5, 107.3, 97.7, 62.3, 61.5, 56.9.

4-Methyl-*N*-(4-(5,6,7-trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)benzenesulfonamide (*p*-3d): Compound ***p*-3d** was obtained according to the above procedure from the compound ***p*-4** and *p*-toluenesulfonyl chloride. ***p*-5d**: Yield 62%; ^{1}H NMR ($CDCl_3$) δ 7.86 (2H, d, J = 8.4 Hz, H-2',6'), 7.83 (4H, d, J = 8.3 Hz, H-2",6"), 7.36 (4H, d, J = 8.3 Hz, H-3",5"), 7.17 (2H, d, J = 8.4 Hz, H-3',5'), 6.79 (1H, s, H-3), 6.67 (1H, s, H-8), 4.00 (3H, s, -OCH_3), 3.98 (3H, s, -OCH_3), 3.93 (3H, s, -OCH_3), 2.49 (6H, s, -CH_3); ***p*-3d**: Yield 71%; ^{1}H NMR (DMSO-d_6) δ 10.75 (1H, s, -NH), 7.92 (2H, d, J = 8.8 Hz, H-2',6'), 7.72 (2H, d, J = 8.2 Hz, H-2",6"), 7.37 (2H, d, J = 8.2 Hz, H-3",5"), 7.25 (2H, d, J = 8.8 Hz, H-3',5'), 7.16 (1H, s, H-3), 6.66 (1H, s, H-8), 3.94 (3H, s, -OCH_3), 3.79 (3H, s, -OCH_3), 3.76 (3H, s, -OCH_3), 2.33 (3H, s, -CH_3); ^{13}C NMR (DMSO-d_6) δ 175.5, 159.8, 157.4, 153.9, 151.5, 143.5, 141.3, 139.8, 136.6, 129.8(2C), 127.2(2C), 126.7(2C), 125.4, 118.8(2C), 112.0, 106.5, 97.2, 61.8, 61.0, 56.4, 20.9.

***N*-(4-(5,6,7-Trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)-methanesulfonamide (*p*-3e):** Compound ***p*-3e** was obtained according to the above procedure from the compound ***p*-4** and methanesulfonyl chloride. ***p*-5e**: Yield 43%; ^{1}H NMR (DMSO-d_6) δ 8.17 (2H, d, J = 8.6 Hz, H-2',6'), 7.73 (2H, d, J = 8.6 Hz, H-3',5'), 7.26 (1H, s, H-3), 6.93 (1H, s, H-8), 3.96 (3H, s, -OCH_3), 3.82 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3), 3.59 (6H, s, -CH_3); ***p*-3e**: Yield 74%; ^{1}H NMR (DMSO-d_6) δ 10.28 (1H, s, -NH), 8.05 (2H, d, J = 8.5 Hz, H-2',6'), 7.35 (2H, d, J = 8.5 Hz, H-3',5'), 7.20 (1H, s, H-3), 6.75 (1H, s, H-8), 3.96 (3H, s, -OCH_3), 3.81 (3H, s, -OCH_3), 3.77 (3H, s, -OCH_3), 3.12 (3H, s, -CH_3); ^{13}C NMR (DMSO-d_6) δ 176.3, 160.6, 158.2, 154.6, 152.2, 142.3, 140.5, 128.1(2C), 126.0, 119.1(2C), 112.7, 107.2, 98.0, 62.5, 61.7, 57.1, 40.4.

***N*-(3-(5,6,7-Trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)-benzenesulfonamide (*m*-3a):** Compound ***m*-3a** was obtained according to the above procedure from the compound ***m*-4** and benzenesulfonyl chloride. ***m*-5a**: Yield. 93%; ^{1}H NMR ($CDCl_3$) δ 7.97-7.95 (5H, m, H-6', H-2",6"), 7.73 (2H, d, J = 7.7 Hz, H-4"), 7.60 (4H, t, J = 7.7 Hz, H-3",5"), 7.53 (1H, t, J = 7.9 Hz, H-5'), 7.52 (1H, t, J = 1.6 Hz, H-2'), 7.18 (1H, d, J = 7.9 Hz, H-4'), 6.75 (1H, s, H-3), 6.59 (1H, s, H-8), 4.02 (3H, s, -OCH_3), 4.00 (3H, s, -OCH_3), 3.93 (3H, s, -OCH_3); ***m*-3a**: Yield 59%; ^{1}H NMR (DMSO-d_6) δ 10.57 (1H, s, -NH), 7.83 (2H, d, J = 7.2 Hz, H-2",6"), 7.71 (1H, dd, J = 8.0, 1.3 Hz, H-6'), 7.66 (1H, t, J = 1.7 Hz, H-2'), 7.63 (1H, m, H-4"), 7.58 (2H, t, J = 7.2 Hz, H-3",5"), 7.42 (1H, t, J = 8.0 Hz, H-5'), 7.27 (1H, dd, J = 8.0, 1.3 Hz, H-4'), 7.05 (1H, s, H-3), 6.60 (1H, s, H-8), 3.98 (3H, s, -OCH_3), 3.80 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3); ^{13}C NMR (DMSO-d_6) δ 175.4, 159.5, 157.6, 153.8, 151.5, 139.8, 139.2, 138.4, 133.1, 131.8, 130.0, 129.3(2C), 126.7(2C), 122.5, 121.7, 117.0, 112.0, 107.8, 96.9, 61.8, 60.9, 56.4.

4-Bromo-*N*-(3-(5,6,7-trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)benzenesulfonamide (*m*-3b): Compound ***m*-3b** was obtained according to the above procedure from the compound ***m*-4** and 4-bromobenzenesulfonyl chloride. ***m*-5b**: Yield 75%; ^{1}H NMR (DMSO-d_6) δ 10.68 (1H, s, -NH), 7.81 (2H, d, J = 8.3 Hz, H-2",6"), 7.75-7.74 (3H, m, H-6', H-3",5"), 7.69 (1H, br s, H-2'), 7.45 (1H, t, J = 7.3 Hz, H-5'), 7.27 (1H, d, J = 7.3 Hz, H-4'), 7.07 (1H, s, H-3), 6.65 (1H, s, H-8), 3.98

(3H, s, -OCH_3), 3.81 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3); ***m*-3b**: Yield 96%; ^{1}H NMR (DMSO-d_6) δ 8.27 (1H, d, J = 7.7 Hz, H-6'), 7.97 (4H, d, J = 8.2 Hz, H-2",6"), 7.83 (2H, d, J = 8.2 Hz, H-3",5"), 7.70-7.66 (2H, m, H-2',5'), 7.27 (1H, d, J = 7.7 Hz, H-4'), 7.09 (1H, s, H-3), 6.91 (1H, s, H-8), 4.03 (3H, s, -OCH_3), 3.82 (3H, s, -OCH_3), 3.79 (3H, s, -OCH_3); ^{13}C NMR (DMSO-d_6) δ 176.1, 158.7, 158.2, 154.4, 152.1, 140.5, 137.7(2C), 134.5, 134.4, 133.4(4C), 133.1, 131.1, 130.7(4C), 129.8(2C), 128.8, 112.6, 109.0, 97.7, 62.4, 61.5, 57.1.

4-Nitro-*N*-(3-(5,6,7-trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)benzenesulfonamide (*m*-3c): Compound ***m*-3c** was obtained according to the above procedure from the compound ***m*-4** and 4-nitrobenzenesulfonyl chloride. ***m*-5c**: Yield 91%; ^{1}H NMR (DMSO-d_6) δ 8.55 (4H, d, J = 6.9 Hz, H-3",5"), 8.31 (1H, d, J = 7.3 Hz, H-6'), 8.18 (4H, d, J = 6.9 Hz, H-2",6"), 7.83 (1H, br s, H-2'), 7.69 (1H, t, J = 7.3 Hz, H-5'), 7.30 (1H, d, J = 7.3 Hz, H-4'), 7.12 (1H, s, H-3), 6.95 (1H, s, H-8), 3.97 (3H, s, -OCH_3), 3.82 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3); ***m*-3c**: Yield 95%; ^{1}H NMR (DMSO-d_6) δ 10.91 (1H, s, -N*H*), 8.40 (2H, d, J = 8.8 Hz, H-3",5"), 8.07 (2H, d, J = 8.8 Hz, H-2",6"), 7.77 (1H, d, J = 7.8 Hz, H-6'), 7.71 (1H, br s, H-2'), 7.45 (1H, t, J = 7.8 Hz, H-5'), 7.28 (1H, d, J = 7.8 Hz, H-4'), 7.08 (1H, s, H-3), 6.66 (1H, s, H-8), 3.98 (3H, s, -OCH_3), 3.80 (3H, s, -OCH_3), 3.77 (3H, s, -OCH_3); ^{13}C NMR (DMSO-d_6) δ 176.0, 159.9, 158.2, 154.4, 152.1, 150.4, 145.3, 140.4, 138.4, 132.6, 130.7, 128.8(2C), 125.2(2C), 123.7, 123.0, 118.4, 112.6, 108.5, 97.6, 62.3, 61.5, 56.9.

4-Methyl-*N*-(3-(5,6,7-trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)benzenesulfonamide (*m*-3d): Compound ***m*-3d** was obtained according to the above procedure from the compound ***m*-4** and *p*-toluenesulfonyl chloride. ***m*-5d**: Yield 89%; ^{1}H NMR ($CDCl_3$) δ 7.96 (1H, d, J = 8.0 Hz, H-6'), 7.82 (4H, d, J = 8.4 Hz, H-2",6"), 7.52 (1H, t, J = 2.0 Hz, H-2'), 7.51 (1H, t, J = 8.0 Hz, H-5'), 7.37 (4H, d, J = 8.4 Hz, H-3",5"), 7.18 (1H, d, J = 8.0 Hz, H-4'), 6.78 (1H, s, H-3), 6.57 (1H, s, H-8), 4.01 (3H, s, -OCH_3), 4.00 (3H, s, -OCH_3), 3.94 (3H, s, -OCH_3), 2.49 (6H, s, -CH_3); ***m*-3d**: Yield 90%; ^{1}H NMR (DMSO-d_6) δ 10.55 (1H, s, -N*H*), 7.73-7.68 (4H, m, H-2',6', H-2",6"), 7.42 (1H, t, J = 7.8 Hz, H-5'), 7.38 (2H, d, J = 8.0 Hz, H-3",5"), 7.28 (1H, d, J = 7.8 Hz, H-4'), 7.06 (1H, s, H-3), 6.61 (1H, s, H-8), 3.98 (3H, s, -OCH_3), 3.81 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3), 2.33 (3H, s, -CH_3); ^{13}C NMR (DMSO-d_6) δ 175.4, 159.6, 157.6, 153.8, 151.6, 143.4, 139.9, 138.6, 136.4, 131.8, 130.0, 129.7(2C), 126.7(2C), 122.3, 121.6, 116.8, 112.1, 107.8, 96.9, 61.8, 60.9, 56.3, 20.9.

***N*-(3-(5,6,7-Trimethoxy-4-oxo-4*H*-chromen-2-yl)phenyl)methanesulfonamide (*m*-3e):** Compound ***m*-3e** was obtained according to the above procedure from the compound ***m*-4** and methanesulfonyl chloride. ***m*-5e**: Yield 66%; ^{1}H NMR (DMSO-d_6) δ 8.23 (1H, d, J = 7.9 Hz, H-6'), 8.19 (1H, br s, H-2'), 7.76 (1H, d, J = 7.9 Hz, H-4'), 7.70 (1H, t, J = 7.9 Hz, H-5'), 7.28 (1H, s, H-3), 7.05 (1H, s, H-8), 3.97 (3H, s, -OCH_3), 3.82 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3), 3.63 (6H, s, -CH_3); ***m*-3e**: Yield 70%; ^{1}H NMR (DMSO-d_6) δ 10.01 (1H, s, -N*H*), 7.81-7.79 (2H, m, H-2',6'), 7.54 (1H, t, J = 8.0 Hz, H-5'), 7.43 (1H, d, J = 8.0 Hz, H-4'), 7.12 (1H, s, H-3), 6.71 (1H, s, H-8), 3.97 (3H, s, -OCH_3), 3.81 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3), 3.01 (3H, s, -CH_3); ^{13}C NMR (DMSO-d_6) δ 175.5, 159.8, 157.6, 153.9, 151.6, 139.9, 139.2, 132.0, 130.1, 122.6, 121.5, 117.0, 112.1, 107.9, 97.1, 61.9, 61.0, 56.4, 54.9.

Cell Lines and Culture. MCF-7 (human breast cancer cell) and Hep G2 (hepatocellular carcinoma cell) cell lines were purchased from Korean Cell Line Bank (KCLB). These cell lines were grown under humidified 5% CO_2 at 37 °C with DMEM (Gibco BRL)-10% (v/v) heat inactivated Fetal Bovine Serum (FBS).

MTT Assay. Cells were placed into 96-well plates at a density of 5×10^3 cells/mL per well. After 2 day growth under humidified 5% CO_2 at 37 °C, cells were treated with selected compounds. The cells were then harvested, treated with 10 μL of Dye Solution (CellTiter96, Promega, Madison, WI) each well and incubated at 37 °C under 5% CO_2 for 4 h. Then, 100 μL of solubilization solution/stop mix was added and the plates were left to stand for overnight. The absorbances at 570 nm were measured with a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA) and the data were presented as % inhibitions of the cell growth or IC_{50} values.

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