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Synthesis, selective cytotoxicities and probable mechanism of action of 7-methoxy-3-arylflavone-8-acetic acids

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ABSTRACT

Thirteen new analogues of flavone-8-acetic acid, that is, compounds **10a**–**m** bearing a methoxy group at the 7-position and diverse subsitiuents on the benzene ring at the 2- and 3-positions of flavone nucleus, were synthesized and evaluated for their direct antiproliferative effects on two human tumor cell lines and for their indirect antiproliferative activities in the transwell co-culture system. The results indicated that most of compounds **10a**–**m** showed moderate direct cytotoxicities. Among them, compound **10i** exhibited higher direct cytotoxicity and selectivity for both cell lines over BJ human foreskin fibroblast cells than 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Interestingly, compared with DMXAA, compound **10e** showed comparable indirect cytotoxicity and higher selectivity. In addition, compound **10e** was found to be able to induce tumor necrosis factor α (TNF-α) production in human peripheral blood mononuclear cells.

1. Introduction

Cancer is one of modern diseases that are increasingly threatening the public health. Therefore, finding efficient therapy for it has been attracting considerable interest from medicinal chemists. In these aspects, vascular disruption of existing tumor blood vessels represents a novel antineoplastic strategy. In preclinical models, tumor vascular disrupting agents (VDAs) have been shown to selectively affect endothelial cells of established tumor blood vessels, resulting in ischemia in the central component of tumor masses with the persistence of a viable layer of cancer cells in the periphery.[1–3] Because VDAs predominantly target the tumor core, a hypoxic region in which cells are known to harbor resistance to traditional DNA-damaging chemotherapy, drug discovery strategy has been evolved to combine VDAs with viable rim-targeting cytotoxic agents to achieve synergistic anti-tumor activity.[4]

Notable example among the VDAs developed to date is 5,6-dimethylxanthenone-4-acetic acid (DMXAA) **1**, an analog of flavone acetic acid (FAA) **2** (Fig. 1). DMXAA **1** has been shown to promote apoptosis of endothelial cells of tumor blood vessels and cause the release of von Willebrand's factor, which then leads to blood clotting and vessel occlusion. Further investigations have revealed that DMXAA **1** is a strongly immunogenic molecule. Its anti-neoplastic properties are largely attributed to the induction of tumor necrosis factor α (TNF-α), which can be detected in the serum and tumor microenvironment within hours of administration.[5] Although phase III trials against lung cancers discontinued because of no survival benefit of DMXAA (i.e., Vadimezan) combination therapy for patients in the late stage of lung cancer,[6,7] the outcomes have clearly demonstrated that DMXAA **1** is safe and well-tolerated in humans.[8]

Extensive structure–activity relationship study has suggested that the (4-oxo-4*H*-chromen-8-yl)acetic acid skeleton in DMXAA **1** and FAA **2** is crucial to maintain the activity of the parent compound.[9–13] For example, compound **3** (Fig. 1) showed 7.3-fold higher indirect cytotoxicty than DMXAA **1**,[11] whereas compound **4** was found to have extensive direct cytotoxicity against leukemia cells (IC_{50} = 9 μM).[13] Isoflavone acetic acid **5** bearing an acetic acid group at the 8-position of biochanin A **6** was found to have high activity against ovarian carcinoma cells.[14] In addition, it is reported that isoflavonoid derivatives bearing aryl groups at the 3-position of the 4*H*-chromen-4-one skeleton, such as Biochanin A **6**,[15] Genistein **7**[16] and Glaziovianin A **8**,[17] exhibit potent anticancer activities. On the other hand, it is known that many flavonoids[18–22] and isoflavonoids showing potent anticancer activities, such as compounds **8** and **9**,[23] have a methoxy group at the 7-position. These

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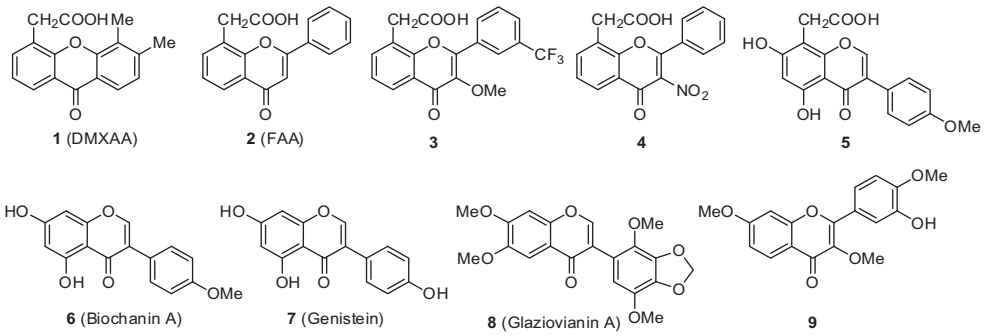


Figure 1. Structures of flavonoid and isoflavonoid analogues **1**–**9** with antitumor activities.

findings make us reasoning that incorporation of the (4-oxo-4*H*-chromen-8-yl)-acetic acid skeleton with aryl groups at the 3-position and a methoxy group at the 7-position of the 4*H*-chromen-4-one skeleton would lead to novel flavonoid analogues having potential anticancer activities. With this rationale in mind, herein we describe the synthesis of novel 7-methoxy-3-arylflavone-8-acetic acids **10a**–**m** (Fig. 2) and their direct and indirect cytotoxicities against HT-29 human colon adenocarcinoma and A549 lung adenocarcinoma cell lines. The probable mechanism of biological action is also briefly discussed.

2. Results and discussion

2.1. Chemistry

The synthetic route for compounds **10a**–**m** is outlined in Scheme 1. Thus, the Wahala–Hase reaction of 2-methylresorcinol **11** with substituted phenylacetic acids **12a**–**d** in the presence of $BF_3{\cdot}OEt_2$ afforded substituted 2,4-dihydroxy-3-methyldeoxy benzoins **13a**–**d** in 53–85% yields.[24] Selective methylation of compounds **13a**–**d** with iodomethane in the presence of K_2CO_3 gave compounds **14a**–**d**. Esterification of compounds **14a**–**d** with aromatic acyl chlorides **15a**–**e** and subsequent heating in freshly distilled anhydrous glycerol at 260 °C gave the corresponding 7-methoxy-8-methyl-3-arylflavonols **16a**–**m**. Compounds **16a**–**m** were then brominated, cyanated and hydrolyzed to give compounds **10a**–**m**. The structures of compounds **10a**–**m** were confirmed on the basis of MS (ESI and HR), NMR (^1H and ^{13}C) and elemental analysis data (see Section 4 and Supporting information).

2.2. Pharmacology

2.2.1. Direct cytotoxicity

The direct antiproliferative activities of compounds **10a**–**m** were evaluated against HT-29 human colon adenocarcinoma, A549 lung adenocarcinoma cell lines and BJ human foreskin fibroblast cell lines by using MTT-based assay.[25] The obtained IC_{50} values, together with those of DMXAA **1** and FAA **2** as positive controls, are listed in Table 1.

It can be seen from Table 1 that most of compounds **10a**–**m** showed moderate cytotoxic activities towards both HT-29 and A549 cells. Among them, compounds **10d**, **10i** and **10l** showed comparable cytotoxicities against A549 cells with DMXAA **1** and FAA **2**, whereas compounds **10d**, **10i** and **10j** decreased the HT-29 cell proliferation with the IC_{50} values being 1.6-, 3.8- and 1.9-fold higher than that of DMXAA **1**, and 14.4-, 34.8- and 17.0-fold higher than that of FAA **2**, respectively. Secondly, most of compounds **10a**–**m** exhibited higher cytotoxicities against HT-29 than against A549. More importantly, compound **10i** exhibited 13.0- and 4.3-fold higher selectivity for tumor cells over BJ human foreskin fibroblast cells. In contrast, DMXAA **1** showed very low selectivity.

2.2.2. Indirect cytotoxicity

It is reported that the antitumor effects of both DMXAA **1** and FAA **2** are due to their indirect rather than direct cytotoxicities. In other words, they are deeply involved in the induction of apoptosis in tumor vascular endothelial cells[27] and an indirect vascular effect involving the production of a spectrum of cytokines and chemokines, including TNF-α,[28] interferon-β,[29] interferon-inducible protein[30] and inducible nitric oxide synthase (iNOS).[31] The primary effect of DMXAA **1** on the endothelial hyper-permeability is thought to be indirect by increasing the intratumoral concentrations of TNF-α among other cytokines.[32] On the other hand, it is reported that activated macrophages are able to recognize and kill tumor cells,[33] which is unavoidable especially when tumor cells are co-cultured with human monocytes (i.e., side-by-side mode with contact).[34] However, this may be eliminated by using transwell co-culture system that works through an above-and-below mode without contact (Fig. 3a).[35,36]

Therefore, we evaluated the indirect cytotoxicities induced in Human PBMCs (in the top well) by compounds **10a**–**m** (50 μM) toward A549 lung adenocarcinoma cells (in the bottom wells) after co-cultured for 24 h in HTS Transwell®-96Well Permeable Support

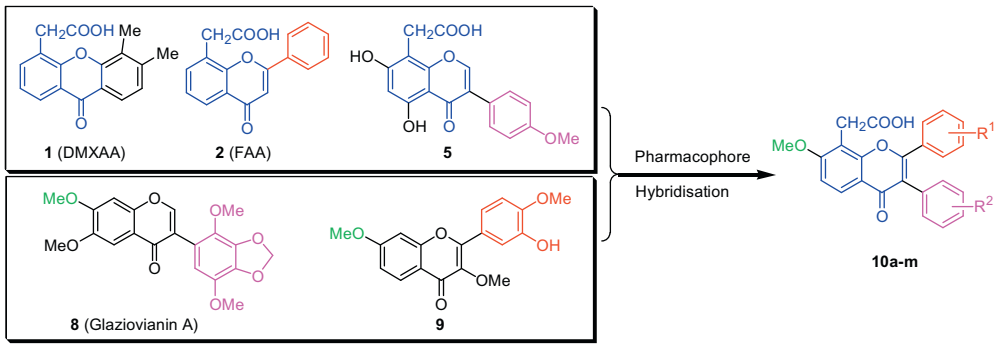
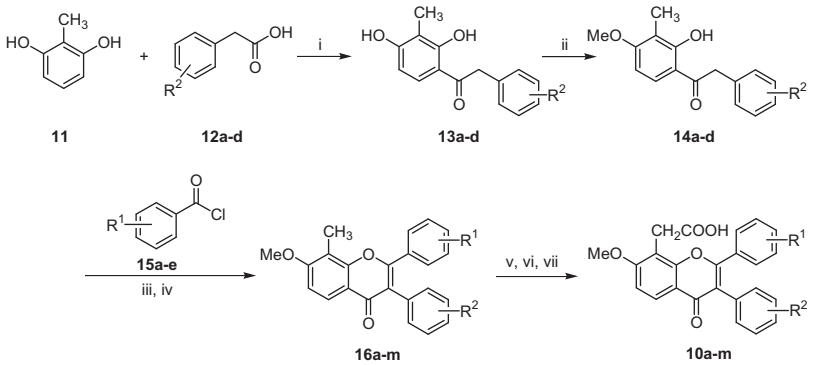
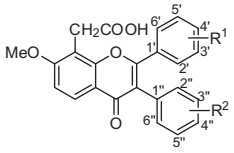


Figure 2. Design of flavone-8-acetic acid derivatives **10a**–**m**.



Scheme 1. Synthetic route for compounds **10a–m**. Reagents and conditions: (i) $BF_3{\cdot}Et_2O$; (ii) MeI, K_2CO_3, acetone, reflux; (iii) pyridine, room temperature; (iv) glycerol, 260 °C, 2 h; (v) NBS, AIBN, CCl_4, reflux; (vi) Et_4NCN, CH_2Cl_2; (vii) H_2SO_4, AcOH, H_2O. Please refer to Table 1 for specific R^1 and R^2.

Table 1
Direct cytotoxicities (IC_{50}, μM) and selectivities of compounds **10a–m** [a]



Compound	R^1	R^2	HT-29			A549			BJ		
			IC_{50}	PR1[b]	PR2[b]	IC_{50}	PR1[b]	PR2[b]	CC_{50}	SR1[c]	SR2[c]
1	\	\	269.5 ± 25.4	1.0	\	345.0 ± 50.7	1.0	\	48.9 ± 8.9	0.18	0.14
2	\	\	2449.5 ± 27.4	\	1.0	341.5 ± 22.8	\	1.0	ND[d]	\	\
10a	H	H	371.2 ± 68.5	0.7	6.6	686.3 ± 66.2	0.5	0.5	ND	\	\
10b	4′-MeO	H	236.6 ± 46.5	1.1	10.3	757.2 ± 70.0	0.5	0.5	ND	\	\
10c	3′, 4′-diMeO	H	794.9 ± 19.6	0.3	3.1	>1000			ND	\	\
10d[e]	4′-Cl	H	170.0 ± 10.3	1.6	14.4	281.7 ± 55.8	1.2	1.2	ND	\	\
10e	H	4″-MeO	307.5 ± 71.3	0.9	8.0	447.4 ± 54.7	0.8	0.8	469.3 ± 24.7	1.5	1.0
10f	4′-MeO	4″-MeO	809.8 ± 57.1	0.3	3.0	>1000	\	\	ND	\	\
10g	3′, 4′-MeO	4″-MeO	574.8 ± 24.7	0.5	4.3	594.8 ± 23.7	0.6	0.6	ND	\	\
10h	H	3″,4″-diMeO	341.3 ± 29.6	0.8	7.2	>1000	\	\	ND	\	\
10i[e]	H	4″-F	70.3 ± 9.6	3.8	34.8	212.0 ± 48.0	1.6	1.6	912.7 ± 28.1	13.0	4.3
10j	4′-MeO	4″-F	143.7 ± 28.6	1.9	17.0	608.4 ± 50.0	0.6	0.6	188.4 ± 14.2	1.3	0.3
10k	3′, 4′-MeO	4″-F	860.4 ± 49.3	0.3	2.8	>1000	\	\	ND	\	\
10l[e]	4-Cl	4″-F	248.5 ± 51.4	1.1	9.9	292.6 ± 21.9	1.2	1.2	ND	\	\
10m	4′-CH_3SO_2	4″-F	683.4 ± 23.2	0.4	3.6	>1000	\	\	ND	\	\

[a] The IC_{50} value represents the concentration of each compound resulting in 50% inhibition in cell growth after 24 h incubation, and was the mean values of three repeated experiments.
[b] PR1 and PR2 denote the potency ratios (PR) relative to DMXAA **1** and FAA **2**, respectively.
[c] SR1 and SR2 denote the selectivity ratios of BJ over HT-29 and A549, respectively.
[d] ND = not detected.
[e] The data for compounds **10d**, **10i** and **10l** were taken from Ref. 26.

Systems (Fig. 3a). DMXAA **1** was used as a control. The viability of the Human PBMCs was assessed by the MTT assay and always higher than 95% at the concentrations of 50 μM. The results showed that compounds **10**e and **10j** substantially reduced A549 cells proliferation to an extent similar to that of DMXAA **1** (Fig. 3b).

To further determine the pharmacological activities of compounds **10e** and **10j**, their indirect antiproliferative activities were measured on A549 cells co-cultured with murine macrophages (RAW264.7) or human PBMCs. The IC_{50} values of compounds **10e** and **10j** are shown in Table 2. It can be seen that the indirect cytotoxicities of compounds **10e** and **10j** induced in human PBMCs are apparently higher than those induced in RAW264.7, and substantially higher than their direct cytotoxicities (IC_{50} = 447.4 μM and 608.4 μM, respectively, Table 1). These results suggest that the antitumor activities of compounds **10e** and **10j** may be a result of the indirect effects. The finding that the indirect cytotoxicity of compound **10e** was comparable with that of DMXAA **1**, suggests that this compound is exploitable as a potential lead compound for the design of new antitumor agents.

2.2.3. TNF-α production

Because it is reported that TNF-α plays a crucial role in the antitumor effect of DMXAA **1** in mice[37] and the in vitro analysis of the response of human PBMCs to DMXAA **1** could be a useful indicator of the activity of this class of agents,[38] the effects of compounds **10e** and **10j** on the TNF-α production by human PBMCs were investigated (Fig. 4). Their responses were compared with that obtained with lipopolysaccharide (LPS), a known inducer of TNF-α synthesis.[39]

As shown in Figure 4, no significant cytokine production was stimulated by DMXAA **1** alone at any tested concentrations, which is in agreement with Gobbi's and Philpott's results.[11,40] The response of HPBMC to compound **10j** after 24 h incubation was also significantly lower than those to LPS and the culture media.

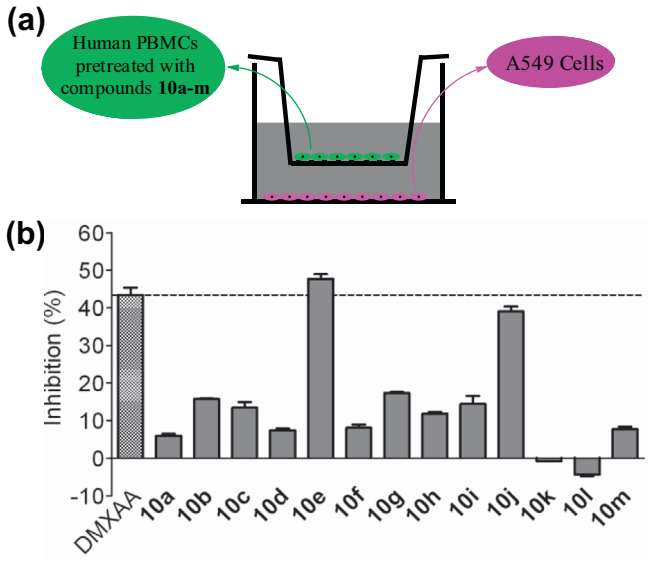


Fig. 3. (a) HTS Transwell®-96Well Permeable Support Systems; (b) Cytotoxicity induced in Human PBMCs by compounds **10a–m** (50 μM) toward A549 cells after co-cultured for 24 h, in HTS Transwell®-96Well Permeable Support Systems with 3 μm pore polycarbonate membranes. Cell viability was then determined by MTT assay.

Table 2
Indirect cytotoxicities (IC_{50}, μM) of compounds **10e** and **10j** induced in murine macrophages (RAW264.7) and human PBMCs in HTS Transwell®-96Well Permeable Support Systems

Compound	RAW264.7		Human PBMCs	
	IC_{50}	PR[a]	IC_{50}	PR[a]
1	207.6 ± 4.5	1.0	91.0 ± 4.3	1.0
10e	370.2 ± 14.0	0.6	81.7 ± 3.5	1.1
10j	550.2 ± 21.0	0.4	121.3 ± 14.3	0.8

[a] PR denotes the potency ratio (PR) relative to DMXAA **1**.

In contrast, the level of TNF-α was significantly increased by compound **10e** at all tested doses and higher than those of DMXAA **1** and the culture media, suggesting that compound **10e** was capable of stimulating the production of TNF-α. In addition, it is reported that DMXAA stimulates TNF synthesis in cultured human leucocytes by amplifying a signal induced by LPS.[40] Thus, it might be interesting to study the potential synergic effect between compound **10e** and LPS.

3. Concluding remarks

In summary, thirteen new flavone-8-acetic acid derivatives bearing different substituents on the phenyl ring at the 2- or 3-position of the flavone nuclei, have been synthesized and fully characterized. These compounds exhibited higher direct cytotoxicities against HT-29 cell than FAA, whereas compounds **10d**, **10i** and **10l** exhibited higher direct cytotoxicities against HT-29 cell and A549 cell than DMXAA. Interestingly, the indirect cytotoxicity of compound **10e** induced in human PBMCs, is substantially higher than its direct cytotoxicity, and comparable with that of DMXAA. These results suggest that compound **10e** is exploitable as a lead compound for further structural optimization. In addition, compared with DMXAA, compounds **10i** and **10e** showed very low cytotoxicities to BJ human foreskin fibroblast cells, and compound **10e** was found to be able to induce TNF-α production in human peripheral blood mononuclear cells. Further efforts aimed at creating novel flavonoid analogues having higher anticancer activities are presently under active investigation in our laboratories.

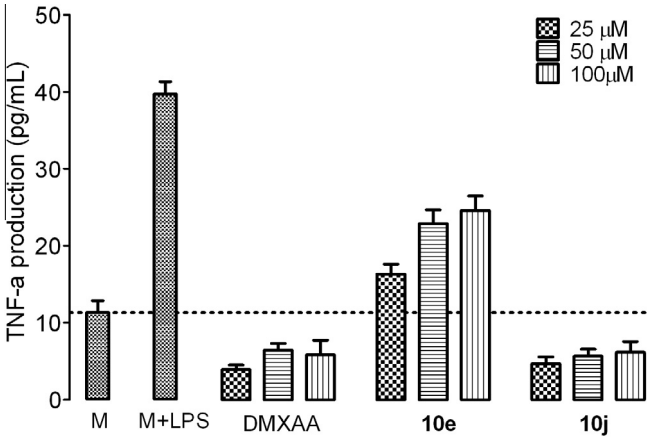


Fig. 4. TNF-α released by HPBMC treated with compounds **10e** and **10j** alone for 24 h. Data were represented as mean ± SED and a paired *t*-test was used. M stands for culture medium.

4. Experimental section

4.1. Chemistry

Melting points were determined in open glass capillaries using an X-5 apparatus and are uncorrected. ESI and HR-ESI mass spectra were measured on a water UPLC/Quattro Premier XE and Thremo High Resolution mass spectrometer (MAT95XP) LCQ DECA XP mass spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using a Varian Mercury 400 spectrometer and TMS as an internal reference. Element analysis was carried out on a Vario ELIII CHNSO elemental analyzer.

DMXAA **1** and FAA **2** were purchased from Sigma (St. Louis, MO, USA). All the other chemicals were of analytical grade and used without further purification.

4.1.1. Synthesis of substituted 2-hydroxy-4-methoxy-3-methyldeoxy benzoins 14a–d

4.1.1.1. General procedures. A mixture of 2-methylresorcinol **11** (15 mmol) and substituted phenylacetic acids **12a–d** (15 mmol) in $BF_3{\cdot}OEt_2$ (15 mL) was heated at 100 °C under the atmosphere of nitrogen for 1.5 h. Then the mixture was poured into ice cold H_2O (500 mL) and allowed to stand at 4 °C for 12 h. The formed precipitate was collected by filtration and re-crystallized from EtOH to give compounds **13a–d**. Then, to a solution of compounds **13a–d** (10 mmol) in anhydrous acetone (60 mL) were added K_2CO_3 (10 mmol) and iodomethane (12 mmol). The resulting mixture was refluxed for 6 h and then filtered. The filtrate was concentrated under reduced pressure and the obtained residue was re-crystallized from EtOH to give compounds **14a–d**.

4.1.1.2. 4-Methoxy-2-hydroxy-3-methyldeoxybenzoin (14a). 53% yield. Mp 108.3–109.3 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 2.08 (s, 3H, CH_3), 3.87 (s, 3H, OMe), 4.22 (s, 2H, CH_2), 6.46 (d, J = 9.2 Hz, 1H), 7.25–7.33 (m, 5H), 7.72 (d, J = 8.8 Hz, 1H), 12.73 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.56, 55.76, 101.99, 113.42, 113.76, 127.95, 128.75, 129.34, 129.72, 134.70, 162.57, 163.57, 202.36; ESI-MS m/z: 257.1 ($[M+H]^+$).

4.1.1.3. 4,4′-Dimethoxy-2-hydroxy-3-methyldeoxybenzoin (14b). 75% yield. Mp 86.5–88.0 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 2.08 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.16 (s,

2H), 6.44 (d, J = 9.0 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 7.17 (d, J = 8.6 Hz, 2H), 7.71 (d, J = 8.9 Hz, 1H), 12.74 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.45, 43.93, 55.19, 55.67, 101.87, 113.32, 113.72, 114.14, 126.57, 129.56, 130.27, 158.58, 162.47, 163.45, 202.6; ESI-MS m/z: 283.1 ([M+H]$^+$).

4.1.1.4. 4,3′,4′-Trimethoxy-2-hydroxy-3-methyldeoxybenzoin (14c). 85% yield. Mp 135.6–136.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 (s, 3H, CH_3), 3.83 (s, 3OMe), 4.13 (s, 2H, CH_2), 6.42 (d, J = 8.9 Hz, 1H), 6.79 (s, 3H), 7.70 (d, J = 8.9 Hz, 1H), 12.77 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.46, 44.42, 55.83, 101.88, 111.34, 112.36, 113.32, 113.73, 121.42, 127.04, 129.55, 148.07, 149.05, 162.48, 163.49, 202.48; ESI-MS m/z: 313.1 ([M+H]$^+$), 335.13 ([M+Na]$^+$).

4.1.1.5. 4′-Fluoro-2-hydroxy-4-methoxy-3-methyldeoxybenzoin (14d). 75% yield. Mp126.7–127.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.08 (s, 3H, CH_3), 3.87 (s, 3H, OMe), 4.18 (s, 2 H, CH_2), 6.45 (d, J = 9.0 Hz, 1H), 6.98–7.03 (m, 2H), 7.14–7.24 (m, 2H), 7.68 (d, J = 9.0 Hz, 1H), 12.66 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.46, 43.81, 55.70, 101.97, 113.24, 113.83, 115.42, 115.64, 129.42, 130.20, 130.23, 130.80, 130.88, 160.67 (C-F), 162.47, 163.11 (C-F), 163.60, 201.92; ESI-MS m/z: 271.0 ([M+H]$^+$), 293.0 ([M+Na]$^+$).

4.1.2. Synthesis of 3-aryl-8-methylflavones 16a–m

4.1.2.1. General procedures. A solution of compounds **14a–d** (1.0 mmol) and benzoyl chlorides **15a–e** (2.7 mmol) in anhydrous pyridine (5 mL) was stirred at room temperature for 5 h. The reaction mixture was concentrated under reduced pressure, poured into water (25 mL) and then extracted with ether (10 mL × 2). The combined organic layer was washed subsequently with 5% aqueous HCl (10 mL) and water (10 mL × 2), dried over anhydrous Na_2SO_4 and concentrated to yield an oily residue. Purification was accomplished by chromatography on a silica gel column, eluting with petroleum ether–acetone (l0/l, v/v), to give crude esters. Then, a solution of the crude esters in freshly distilled anhydrous glycerol (8 mL) was heated at 260 °C for 2 h under the atmosphere of nitrogen. The reaction mixture, after cooled to room temperature, was poured into water (20 mL), and adjusted to pH 10 with 4 N NaOH. The resulting mixture was stirred at room temperature for 15 min and allowed to stand at 0 °C for 24 h. The formed precipitate was collected by filtration and re-crystallized from EtOH to give compounds **16a–m**.

4.1.2.2. 7-Methoxy-8-methyl-2,3-diphenyl-4*H*-chromen-4-one (16a). White solid; 65% yield. Mp 241.4–242.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.97 (s, 3H, OMe), 7.01 (d, J = 7.2 Hz, 1H), 7.22–7.25 (m, 3H), 7.28–7.35 (m, 5H), 7.40 (d, J = 7.6 Hz, 2H), 8.16 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 8.35, 56.17, 108.64, 113.78, 117.41, 122.02, 124.90, 127.51, 128.10, 128.26, 129.60, 129.91, 131.34, 133.16, 133.67, 155.16, 160.98, 161.42, 177.43; ESI-MS m/z: 343.4 ([M+H]$^+$); HR-ESI-MS for $C_{23}H_{19}O_3$ ([M+H]$^+$) Calcd: 343.1329; Found: 343.1314.

4.1.2.3. 7-Methoxy-2-(4-methoxyphenyl)-8-methyl-3-phenyl-4*H*-chromen-4-one (16b). White solid; 75% yield. Mp 194.5–196.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 3.75 (s, 3H, OMe), 3.95 (s, 3H, OMe), 6.87 (d, J = 8.8 Hz, 2H), 7.17–7.19 (m, 2H), 7.20 (d, J = 8.8 Hz, 1H), 7.30–7.35 (m, 5H), 7.94 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 8.12, 55.26, 56.24, 109.22, 112.89, 113.64, 116.54, 120.48, 124.05, 125.09, 127.27, 128.03, 130.97, 131.12, 133.51, 154.29, 160.40, 160.50, 160.96, 175.89; ESI-MS m/z: 373.5 ([M+H]$^+$); HR-ESI-MS for $C_{24}H_{21}O_4$ ([M+H]$^+$) Calcd: 373.1434; Found: 373.1429.

4.1.2.4. 2-(3,4-Dimethoxyphenyl)-7-methoxy-8-methyl-3-phenyl-4*H*-chromen-4-one (16c). Light yellow solid; 65% yield. Mp 211.9–213.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.41 (s, 3H, CH_3), 3.47 (s, 3H, OMe), 3.88 (s, 3H, OMe), 3.97 (s, 3H, OMe), 6.74 (s, 1H), 6.83 (d, J = 8.8 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 7.28–7.37 (m, 6H), 8.13 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 8.04, 54.91, 55.46, 56.23, 109.22, 111.17, 112.91, 113.02, 116.53, 120.50, 122.36, 124.00, 124.87, 127.20, 128.09, 131.01, 133.82, 147.60, 150.50, 154.24, 160.14, 160.97, 175.89; ESI-MS m/z: 403.6 ([M+H]$^+$), 425.5 ([M+Na]$^+$); HR-ESI-MS for $C_{25}H_{23}O_5$ ([M+H]$^+$) Calcd: 403.1540; Found: 403.1530.

4.1.2.5. 2-(4-Chlorophenyl)-7-methoxy-8-methyl-3-phenyl-4*H*-chromen-4-one (16d). White solid; 71% yield. Mp 234.0–235.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.98 (s, 3 H, OMe), 7.02 (d, J = 8.8 Hz, 1H), 7.21–7.25 (m, 4H), 7.29–7.35 (m, 5H), 8.14 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.32, 56.15, 108.73, 113.73, 117.34, 122.22, 124.94, 127.73, 128.42, 128.45, 130.86, 131.21, 132.10, 132.85, 136.08, 155.07, 159.68, 161.52, 177.24; ESI-MS m/z: 400.0 ([M+Na]$^+$); HR-ESI-MS for $C_{23}H_{18}ClO_3$ ([M+H]$^+$) Calcd: 377.0939; Found: 377.0926.

4.1.2.6. 7-Methoxy-3-(4-methoxyphenyl)-8-methyl-2-phenyl-4*H*-chromen-4-one (16e). White solid; 57% yield. Mp 224.6–226.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.80 (s, 3H, OMe), 3.97 (s, 3H, OMe), 6.85 (d, J = 8.0 Hz, 2H), 7.01 (d, J = 8.8 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 7.28–7.34 (m, 3H), 7.43 (d, J = 7.6 Hz, 2H), 8.16 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.33, 55.24, 56.13, 108.58, 113.74, 113.84, 117.37, 121.53, 124.86, 125.23, 128.12, 129.56, 129.79, 132.44, 133.85, 155.14, 158.76, 160.76, 161.37, 177.71; ESI-MS m/z: 373.9 ([M+H]$^+$), 395.8 ([M+Na]$^+$); HR-ESI-MS for $C_{24}H_{21}O_4$ ([M+H]$^+$) Calcd: 373.1440; Found: 373.1439.

4.1.2.7. 7-Methoxy-2,3-bis(4-methoxyphenyl)-8-methyl-4*H*-chromen-4-one (16f). Light yellow solid; 51% yield. Mp 197.2–198.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.79 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.96 (s, 3H, OMe), 6.79 (d, J = 8.0 Hz, 2H), 6.87 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 8.8 Hz, 1H), 7.16 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 8.13 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.35, 55.25, 56.31, 56.11, 108.44, 113.56, 113.61, 113.92, 117.33, 120.60, 124.77, 125.66, 126.01, 131.16, 131.40, 155.03, 158.88, 160.65, 160.70, 161.26, 177.70; ESI-MS m/z: 403.8 ([M+H]$^+$), 425.7 ([M+Na]$^+$); HR-ESI-MS for $C_{25}H_{23}O_5$ ([M+H]$^+$) Calcd: 403.1545; Found: 403.1556.

4.1.2.8. 2-(3,4-Dimethoxyphenyl)-7-methoxy-3-(4-methoxyphenyl)-8-methyl-4*H*-chromen-4-one (16g). Light yellow solid; 54% yield. Mp 211.3–212.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3H, CH_3), 3.44 (s, 3H, OMe), 3.75 (d, 6H, OMe × 2), 3.95 (s, 3H, OMe), 6.80 (s, 1H), 6.91–6.97 (m, 3H), 7.10 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.8 Hz, 1H), 7.19 (d, J = 8.8 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 8.06, 54.94, 55.06, 55.44, 56.20, 109.12, 111.08, 112.82, 112.88, 113.64, 116.51, 120.05, 122.36, 124.01, 125.09, 125.64, 132.20, 147.59, 150.15, 154.20, 158.48, 159.99, 160.88, 176.13; ESI-MS m/z: 433.7 ([M+H]$^+$), 455.7 ([M+Na]$^+$); HR-ESI-MS for $C_{26}H_{25}O_6$ ([M+H]$^+$) Calcd: 433.1651; Found: 433.1658.

4.1.2.9. 3-(3,4-Dimethoxyphenyl)-7-methoxy-8-methyl-2-phenyl-4*H*-chromen-4-one (16h). Light yellow solid; 62% yield. Mp 197.2–197.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.73 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.96 (s, 3H, OMe), 6.80 (t, J = 8.0 Hz, 3H), 7.00 (d, J = 8.8 Hz, 1H), 7.28–7.34 (m, 3H), 7.44 (d, J = 6.8 Hz, 2H), 7.14 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.29, 55.79, 56.05, 108.56, 111.08, 113.69, 114.61,

117.33, 121.57, 123.92, 124.76, 125.52, 128.11, 129.42, 129.80, 133.81, 148.45, 148.64, 155.05, 160.86, 161.36, 177.54; ESI-MS *m*/*z*: 403.9 ([M+H]$^+$), 425.8 ([M+Na]$^+$); HR-ESI-MS for $C_{25}H_{23}O_5$ ([M+H]$^+$) Calcd: 403.1545; Found: 403.1557.

4.1.2.10. 3-(4-Fluorophenyl)-7-methoxy-8-methyl-2-phenyl-4*H*-chromen-4-one (16i). White solid; 78% yield. Mp 217.4–217.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.95 (s, 3H, OMe), 6.99 (t, *J* = 8.6 Hz, 3H), 7.18–7.22 (m, 2H), 7.25–7.34 (m, 3H), 7.40 (d, *J* = 7.2 Hz, 2H), 8.12 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.31, 56.11, 108.70, 113.81, 115.17, 115.39, 117.29, 121.01, 124.78, 128.22, 129.06, 129.09, 129.56, 130.06, 133.03, 133.11, 133.49, 155.11, 161.01, 161.17, 161.51, 163.47, 177.36; ESI-MS *m*/*z*: 361.8 ([M+H]$^+$), 383.8 ([M+Na]$^+$); HR-ESI-MS for $C_{23}H_{18}FO_3$ ([M+H]$^+$) Calcd: 361.1240; Found: 361.1234.

4.1.2.11. 3-(4-Fluorophenyl)-7-methoxy-2-(4-methoxyphenyl)-8-methyl-4*H*-chromen-4-one (16j). White solid; 41% yield. Mp 179.8–181.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.80 (s, 3H, OMe), 3.96 (s, 3H, OMe), 6.80 (d, *J* = 8.8 Hz, 2H), 6.98–7.04 (m, 3H), 7.19–7.23 (m, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 8.12 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.32, 55. 33, 56.09, 108.55, 113.65, 113.69, 115.25, 115.46, 117.20, 120.03, 124.72, 125.59, 129.45, 129.48, 131.18, 132.99, 133.07, 155.00, 160.91, 160.95, 161.06, 161.40, 163.40, 177.33; ESI-MS *m*/*z*: 391.8 ([M+H]$^+$), 413.8 ([M+Na]$^+$); HR-ESI-MS for $C_{24}H_{20}FO_4$ ([M+H]$^+$) Calcd: 391.1346; Found: 391.1341.

4.1.2.12. 2-(3,4-Dimethoxyphenyl)-3-(4-fluorophenyl)-7-methoxy-8-methyl-4*H*-chromen-4-one (16k). Light yellow solid; 41% yield. Mp 212.5–213.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.34 (s, 3H, CH_3), 3.47 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.97 (s, 3H, OMe), 6.82 (s, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 7.12–7.23 (m, 6H), 7.94 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 8.05, 54.98, 55.47, 56.23, 109.23, 111.15, 112.85, 112.89, 114.57, 114.89, 115.10, 116.43, 119.51, 122.54, 123.97, 124.76, 129.96, 133.08, 133.16, 147.69, 150.31, 154.23, 160.43, 160.99, 162.61, 175.85; ESI-MS *m*/*z*: 421.7 ([M+H]$^+$), 443.7 ([M+Na]$^+$); HR-ESI-MS for $C_{25}H_{22}FO_5$ ([M+H]$^+$) Calcd: 421.1451; Found: 421.1460.

4.1.2.13. 2-(4-Chlorophenyl)-3-(4-fluorophenyl)-7-methoxy-8-methyl-4*H*-chromen-4-one (16l). White solid; 40% yield. Mp 250.7–251.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.36 (s, 3H, CH_3), 3.98 (s, 3H, OMe), 6.70–7.04 (m, 3H), 7.17–7.21 (m, 2H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 8.13 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.31, 56.15, 108.80, 113.76, 115.40, 115.62, 117.19, 121.18, 124.91, 128.59, 128.67, 128.71, 130.83, 131.91, 132.92, 133.00, 136.26, 155.04, 159.87, 161.12, 161.60, 163.58, 177.16. ESI-MS *m*/*z*: 396.0 ([M+H]$^+$); HR-ESI-MS for $C_{23}H_{17}ClFO_3$ ([M+H]$^+$) Calcd: 395.0850; Found: 395.0849.

4.1.2.14. 3-(4-Fluorophenyl)-7-methoxy-8-methyl-2-(4-(methylsulfonyl) phenyl)-4*H*-chromen-4-one (16m). White solid; 51% yield. Mp 172.3–174.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 3.32 (s, 3H, CH_3), 3.98 (3H, OMe), 7.16 (t, *J* = 8.8 Hz, 2H), 7.22–7.26 (m, 2H), 7.27 (d, *J* = 9.2 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 2H), 8.00 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, 5/1 DMSO-d_6-$CDCl_3$, v/v) δ 8.08, 43.09, 56.31, 109.61, 113.05, 114.96, 115.18, 116.51, 121.41, 124.20, 126.82, 128.62, 128.65, 130.36, 133.18, 133.26, 137.81, 141.79, 154.44, 159.00, 160.39, 161.23, 162.82, 175.79; ESI-MS *m*/*z*: 439.4 ([M+H]$^+$); HR-ESI-MS for $C_{24}H_{19}FNaO_5S$ ([M+Na]$^+$) Calcd: 461.0835; Found: 461.0829.

4.1.3. Synthesis of 7-methoxy-3-arylflavone-8-acetic acids 10a–m

4.1.3.1. General procedures. A mixture of compounds **16a–m** (1 mmol), NBS (1.1 mmol) and AIBN (0.12 mmol) in anhydrous CCl_4 (40 mL) was refluxed and monitored by TLC. After refluxing for 5 h, the reaction mixture was filtered. The filtrate was concentrated under reduced pressure and the obtained residue was chromatographed to give 8-bromomethyl-3-arylflavonols. Then, to a solution of 8-bromomethyl-3-arylflavonols (1.05 mmol) in dry CH_2Cl_2 (20 mL) was added tetraethylammonium cyanide (1.05 mmol). The resulting reaction mixture was stirred at room temperature until the starting material disappeared. Then the solvent was evaporated under reduced pressure and the residue was re-crystallized from EtOH to give 3-arylflavone-8- acetonitriles. The obtained compounds were dissolved in a mixture of acetic acid (9 mL), H_2O (9 mL) and concentrated H_2SO_4 (9 mL). After the mixture was refluxed for 2 h, water (375 mL) was added. The formed precipitate was collected by filtration and re-crystallized from EtOH to give compounds **10a–m**.

4.1.3.2. 7-Methoxy-3-phenylflavone-8-acetic acid (10a). White solid; 58% yield. Mp 214.8–215.4 °C; ^{1}H NMR (400 MHz, DMSO-$\boldsymbol{d_6}$) $\boldsymbol{\delta}$ 3.82 (s, 2H, CH_2), 3.97 (s, 3H, OMe), 7.15–7.18 (m, 2H), 7.30–7.33 (m, 6H), 7.36–7.40 (m, 3H), 8.06 (d, ***J*** = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, 5/1 DMSO-$\boldsymbol{d_6}$-$\boldsymbol{CDCl_3}$, v/v) $\boldsymbol{\delta}$ 28.43, 56.55, 109.76, 111.31, 116.56, 121.67, 125.66, 127.42, 127.99, 128.18, 129.27, 130.18, 131.12, 132.85, 132.97, 154.59, 160.46, 161.18, 171.61, 175.78; ESI-MS ***m*/*z***: 388.0 ([M+H]$^+$); HR-ESI-MS for $\boldsymbol{C_{24}H_{19}O_5}$ ([M+H]$^+$) Calcd: 387.1227; Found: 387.1208; Anal. Calcd for $\boldsymbol{C_{24}H_{18}O_5}$: C, 74.60; H, 4.70. Found: C, 74.81; H, 4.87.

4.1.3.3. 7-Methoxy-3-phenyl-4′-methoxyflavone-8-acetic acid (10b). Light yellow solid; 52% yield. Mp 249.3–251.2 °C; ^{1}H NMR (400 MHz, 5/1 DMSO-d_6-$CDCl_3$, v/v) δ 3.75 (s, 3H, OMe), 3.83 (s, 2H, CH_2), 3.96 (s, 3H, OMe), 6.86 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 6.4 Hz, 2H), 7.27–7.34 (m, 6H), 8.03 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.40, 55.22, 56.44, 109.46, 111.18, 113.57, 116.53, 120.66, 124.84, 125.56, 127.26, 128.01, 130.90, 131.04, 133.33, 154.46, 160.30, 160.54, 161.05, 171.59, 175.71; ESI-MS *m*/*z*: 417.5 ([M+H]$^+$); HR-ESI-MS for $C_{25}H_{21}O_6$ ([M+H]$^+$) Calcd: 417.1333; Found: 417.1321] Anal. Calcd for $C_{25}H_{20}O_6$: C, 72.11; H, 4.84. Found: C, 71.87; H, 4.95.

4.1.3.4. 7-Methoxy-3-phenyl-3′,4′-dimethoxyflavone-8-acetic acid (10c). Light yellow solid; 58% yield. Mp 258.2–260.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.44 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.86 (s, 2H, CH2), 3.97 (s, 3 H, OMe), 6.82 (d, *J* = 1.6 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 7.03 (dd, *J* = 8.4 and 1.6 Hz, 1H), 7.20 (d, *J* = 6.8 Hz, 2H), 7.26 (d, *J* = 9.2 Hz, 1H), 7.33–7.38 (m, 3H), 8.03 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, 5/1 DMSO-d_6-$CDCl_3$, v/v) δ 28.40, 54.90, 55.44, 56.43, 109.45, 110.92, 111.09, 112.63, 116.51, 120.65, 122.47, 124.63, 125.56, 127.24, 128.11, 131.02, 133.66, 147.72, 150.28, 154.46, 160.03, 161.02, 171.57, 175.73; ESI-MS *m*/*z*: 447.7 ([M+H]$^+$); HR-ESI-MS for $C_{26}H_{23}O_7$ ([M+H]$^+$) Calcd: 447.1438; Found: 447.1424. Anal. Calcd for $C_{26}H_{22}O_7$: C, 69.95; H, 4.97. Found: C, 69.73; H, 4.78.

4.1.3.5. 7-Methoxy-3-phenyl-4′-chloroflavone-8-acetic acid (10d). White solid; 72% yield. Mp 220.3–221.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 2H, CH_2), 3.97 (s, 3H, OMe), 7.17–7.19 (m, 2H), 7.30–7.42 (m, 8H), 8.05 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, 5/1 DMSO-d_6-$CDCl_3$, v/v) δ 28.42, 56.55, 109.82, 111.30, 116.52, 121.89, 125.66, 127.57, 128.08, 128.32, 131.07, 131.70, 132.63, 135.00, 154.52, 159.30, 161.23, 171.51, 175.64. ESI-MS *m*/*z*: 421.7 ([M+H]$^+$); HR-ESI-MS for $C_{24}H_{18}ClO_5$ ([M+H]$^+$)

Calcd: 421.0837; Found: 421.0832. Anal. Calcd for $C_{24}H_{17}ClO_5$: C, 68.50; H, 4.07. Found: C, 68.37; H, 4.13.

4.1.3.6. 7-Methoxy-3-(4-methoxyphenyl)-flavone-8-acetic acid (10e). White solid; 65% yield. Mp 243.2–243.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (s, 3H, OMe), 3.81 (s, 2H, CH2), 3.96 (s, 3H, OMe), 6.86 (d, J = 8.4 Hz, 2H), 7.08 (d, J = 8.4 Hz, 2H), 7.28–7.38 (m, 6H), 8.05 (d, J = 8.8 Hz, 1H), 12.49 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.43, 55.01, 56.54, 109.71, 111.26, 113.53, 116.55, 121.23, 124.82, 125.67, 128.23, 129.25, 130.09, 132.29, 133.09, 154.57, 158.53, 160.25, 161.12, 171.60, 176.01; ESI-MS m/z: 417.9 ([M+H]$^+$), 439.9 ([M+Na]$^+$); HR-ESI-MS for $C_{25}H_{21}O_6$ ([M+H]$^+$) Calcd: 417.1338; Found: 417.1365. Anal. Calcd for $C_{25}H_{20}O_6$: C, 72.11; H, 4.84. Found: C, 71.96; H, 4.69.

4.1.3.7. 7-Methoxy-3-(4-methoxyphenyl)-4′-methoxyflavone-8-acetic acid (10f). Light yellow solid; 50% yield. Mp 222.1–223.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.76 (s, 6H, OMe × 2), 3.82 (s, 2H, CH_2), 3.96 (s, 3H, OMe), 6.85–6.95 (m, 4H), 7.07 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.8 Hz, 1H), 7.33 (d, J = 8.0 Hz, 2H), 8.03 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.44, 55.02, 55.31, 56.53, 109.56, 111.18, 113.64, 113.71, 116.52, 120.30, 125.11, 125.20, 125.61, 130.93, 132.24, 154.45, 158.46, 160.16, 160.51, 161.04, 171.64, 175.97; ESI-MS m/z: 447.9 ([M+H]$^+$); HR-ESI-MS for $C_{26}H_{23}O_7$ ([M+H]$^+$) Calcd: 447.1444; Found: 447.1454. Anal. Calcd for $C_{26}H_{22}O_7$: C, 69.95; H, 4.97. Found: C, 69.62; H, 4.79.

4.1.3.8. 7-Methoxy-3-(4-methoxyphenyl)-3′,4′-dimethoxyflavone-8-acetic acid (10g). Light yellow solid; 31% yield. Mp 288.0–290.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.50 (s, 3H, OMe), 3.76 (s, 6H, OMe × 2), 3.84 (s, 2H, CH2), 3.96 (s, 3H, OMe), 6.87–6.93 (m, 4H), 7.02 (dd, J = 8.8 Hz and 2.0 Hz, 1H), 7.10 (dd, J = 8.8 Hz and 2.2 Hz, 2H), 7.26 (dd, J = 9.0 Hz and 4.0 Hz, 1H), 8.02 (dd, J = 8.9 Hz and 3.7 Hz, 1H); ^{13}C NMR (100 MHz, 5/1 DMSO-d_6-$CDCl_3$, v/v) δ 28.49, 54.98, 55.07, 55.47, 56.46, 109.46, 110.96, 111.18, 112.57, 113.70, 116.51, 120.28, 122.54, 124.90, 125.53, 132.18, 147.79, 150.22, 150.27, 154.45, 158.56, 159.90, 160.99, 171.68, 175.98; ESI-MS m/z: 477.4 ([M+H]$^+$), 499.3 ([M+Na]$^+$); HR-ESI-MS for $C_{27}H_{25}O_8$ ([M+H]$^+$) Calcd: 477.1549; Found: 477.1545; Anal. Calcd for $C_{27}H_{24}O_8$: C, 68.06; H, 5.08. Found: C, 67.92; H, 4.93.

4.1.3.9. 7-Methoxy-3-(3,4-dimethoxyphenyl)-flavone-8-acetic acid (10h). Light yellow solid; 48% yield. Mp 217.2–217.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.32 (s, 3H, OMe), 3.57 (s, 3H, OMe), 3.81 (s, H, CH_2), 3.96 (s, 3H, OMe), 6.66 (d, J = 8.4 Hz, 1H), 6.76 (d, J = 1.6 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 7.29–7.42 (m, 6H), 8.05 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.45, 55.39, 56.52, 109.69, 111.27, 111.36, 115.14, 116.59, 121.42, 123.56, 125.07, 125.64, 128.18, 129.15, 130.05, 133.15, 148.18, 154.53, 160.37, 161.10, 171.59, 175.93; ESI-MS m/z: 447.9 ([M+H]$^+$), 469.9 ([M+Na]$^+$); HR-ESI-MS for $C_{26}H_{23}O_7$ ([M+H]$^+$) Calcd: 447.1444; Found: 447.1468; Anal. Calcd for $C_{26}H_{22}O_7$: C, 69.95; H, 4.97. Found: C, 70.06; H, 4.79.

4.1.3.10. 7-Methoxy-3-(4-fluorophenyl)-flavone-8-acetic acid (10i). White solid; 54% yield. Mp 260.2–261.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 2H, CH_2), 3.96 (s, 3H, OMe), 7.14–7.21 (m, 4H), 7.28–7.37 (m, 6H), 8.06 (d, J = 8.4 Hz, 1H), 12.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.41, 56.56, 109.81, 111.30, 114.86, 115.07, 116.51, 120.74, 125.69, 128.31, 129.32, 130.31, 132.77, 133.18, 133.26, 154.60, 160.31, 160.68, 161.24, 162.74, 171.59, 175.74; negative ESI-MS m/z: 404.0 (M$^-$); HR-ESI-MS for $C_{24}H_{18}FO_5$ ([M+H]$^+$) Calcd: 405.1138; Found: 405.1191. Anal. Calcd for $C_{24}H_{17}FO_5$: C, 71.28; H, 4.24. Found: C, 71.03; H, 3.93.

4.1.3.11. 7-Methoxy-3-(4-fluorophenyl)-4′-methoxyflavone-8-acetic acid (10j). White solid; 65% yield. Mp 262.5–264.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.76 (s, 3H, OMe), 3.83 (s, 2H, CH_2), 3.96 (s, 3H, OMe), 6.90 (d, J = 8.4 Hz, 2H), 7.16–7.22 (m, 4H), 7.27–7.33 (m, 3H), 8.02 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.41, 55.32, 56.56, 109.68, 111.22, 113.77, 114.95, 115.16, 116.45, 119.79, 124.76, 125.62, 129.59, 131.01, 133.13, 133.20, 154.49, 160.24, 160.56, 160.67, 161.15, 162.67, 171.57, 175.68; ESI-MS m/z: 435.8 ([M+H]$^+$), 457.3 ([M+Na]$^+$); HR-ESI-MS for $C_{25}H_{20}FO_6$ ([M+H]$^+$) Calcd: 435.1244; Found: 435.1248; Anal. Calcd for $C_{25}H_{19}FO_6$: C, 69.12; H, 4.41. Found: C, 69.26; H, 4.24.

4.1.3.12. 7-Methoxy-3-(4-fluorophenyl)-3′,4′-dimethoxyflavone-8-acetic acid (10k). Light yellow solid; 32% yield. Mp 250.7–252.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.53 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.85 (s, 2H, CH_2), 3.97 (s, 3H, OMe), 6.88–6.93 (m, 2H), 6.97 (dd, J = 1.8 Hz and 8.6 Hz, 1H), 7.16–7.25 (m, 4H), 7.27 (d, J = 9.2 Hz, 1H), 8.04 (d, J = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.53, 55.05, 55.50, 55.52, 109.67, 111.10, 111.34, 112.64, 114.94, 115.15, 116.40, 119.75, 122.67, 124.56, 125.49, 129.86, 133.06, 133.13, 147.89, 150.39, 154.46, 160.24, 160.31, 161.09, 162.67, 171.57, 175.68; ESI-MS m/z: 465.4 ([M+H]$^+$); HR-ESI-MS for $C_{26}H_{22}FO_7$ ([M+H]$^+$) Calcd: 465.1350; Found: 465.1354; Anal. Calcd for $C_{26}H_{21}FO_7$: C, 67.24; H, 4.56. Found: C, 66.99; H, 4.30.

4.1.3.13. 7-Methoxy-3-(4-fluorophenyl)-4′-chloroflavone-8-acetic acid (10l). White solid; 71% yield. Mp 258.2–260.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 2H, CH_2), 3.97 (s, 3H, OMe), 7.16 (t, J = 8.8 Hz, 2H), 7.21–7.24 (m, 2 H), 7.30 (d, J = 8.8 Hz, 1H), 7.39 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.8 Hz, 2H), 8.05 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.38, 56.57, 109.90, 111.31, 114.93, 115.14, 116.45, 120.96, 125.66, 128.41, 128.88, 131.11, 131.60, 133.11, 133.19, 135.07, 154.51, 159.50, 161.28, 171.45, 175.59; ESI-MS m/z: 439.3 ([M+H]$^+$); HR-ESI-MS for $C_{24}H_{17}ClFO_5$ ([M+H]$^+$) Calcd: 439.0749; Found: 439.0748; Anal. Calcd for $C_{24}H_{16}ClFO_5$: C, 65.69; H, 3.67. Found: C, 65.83; H, 3.57.

4.1.3.14. 7-Methoxy-3-(4-fluorophenyl)-4′-methylsulfonylflavone-8-acetic acid (10m). White solid; 88% yield. Mp 314.2–316.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.25 (s, 3H, Me), 3.82 (s, 2H, CH_2), 3.97 (s, 3H, OMe), 7.16 (t, J = 8.8 Hz, 2H), 7.23–7.26 (m, 2H), 7.32 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 8.07 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.39, 43.00, 56.60, 110.04, 111.38, 114.97, 115.19, 116.47, 121.75, 125.71, 126.71, 128.49, 130.29, 133.17, 133.25, 137.52, 141.91, 154.57, 158.82, 160.43, 161.40, 162.85, 171.42, 175.59. ESI-MS m/z: 495.4 ([M+H]$^+$), 517.4 ([M+Na]$^+$); HR-ESI-MS for $C_{25}H_{20}FO_7S$ ([M+H]$^+$) Calcd: 483.0914; Found: 483.0921; Anal. Calcd for $C_{25}H_{19}FO_7S$: C, 62.23; H, 3.97. Found: C, 62.37; H, 3.78.

4.2. Pharmacology

4.2.1. Compounds

Compounds **10a**–**m** were dissolved in DMSO and stored as stock solutions (100 mM) at −20 °C. For experimental use, all the compounds were prepared from stock solutions, diluted with growth medium and used immediately.

4.2.2. Cell culture

The murine macrophage cell line RAW264.7 was a gift from Professor Jiake Xu (University of Western Australia). RAW264.7, A549 and HT-29 cells were cultured in RPMI 1640, supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 100 μg/mL streptomycin (all from Invitrogen) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Human PBMCs were isolated from heparinized whole blood by centrifugation over Ficoll-Paque Plus (TBD Science). The BJ human foreskin fibroblast cells were cultured in MEM (Cellgro), supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin (from Invitrogen) and 1% Nonessential Amino Acids (Corning).

4.2.3. Direct cytotoxicity

Cells were seeded for attachment in 96-well microculture plates overnight and then incubated with DMXAA **1**, FAA **2** and compounds **10a**–**m** of varying concentrations for 24 h. Then, MTT (0.5 mg/mL) was added to each well, and the cells were further incubated for 3 h. The stained formazan product was determined spectrophotometrically at 570 nm in GENios Pro microplate reader (TECAN).

4.2.4. Indirect cytotoxicity

Murine Macrophages. RAW264.7 cells were placed at the concentration of 5.0×10^3 cells per well in the top wells of a 0.3 μm 96-transwell plate (CORNING) for attachment overnight, and then activated by DMXAA **1**, FAA **2** and compounds **10a**–**m** of varying concentrations, using triplicate wells per drug dose. After 24 h, the medium was discarded and the A549 cells were seeded at a density of 3×10^3 cells per well in the bottom wells for another 24 h. The cell viability of A549 cells in the bottom wells was assessed by the MTT assay. The inhibition percentage was calculated according to the following formula.

$$\%\text{inhibition} = \left[1 - \frac{\text{OD(A549)of Compounds}}{\text{OD(A549)of Blank Control}}\right] \times 100\%$$

Human Peripheral Blood Mononuclear Cells. Human PBMCs (1×10^6 cells/well) were placed at the top well of a 3 μm 96-transwell plate (CORNING) for attachment overnight, and then activated by DMXAA **1**, FAA **2** and compounds **10a**–**m** of varying concentrations, using triplicate wells per drug dose. After 24 h, the medium was discarded and the A549 cells were seeded at a density of 3×10^3 cells per well in the bottom wells for another 24 h. The cell viability of the A549 cells in the bottom wells was assessed by the MTT assay.

4.2.5. Quantification of TNF-α production

Human PBMCs were isolated as described above and treated with culture medium, DMXAA **1**, compounds **10e** and **10j** at the concentrations of 25, 50, and 100 μM. LPS (from *Escherichia coli* serotype 0127; B8, Sigma) was used as a positive control at the final concentration of 1 μg/mL. After 24 h incubations, the medium was carefully collected and stored at −70 °C for assay. The viability of the cells was assessed by Trypan blue dye exclusion and always higher than 95%.

The concentration of TNF-α was determined according to the manufacturer's instructions of the commercially available enzymes-linked immunosorbent assay (ELISA) kits (Sigma).

4.2.6. Cell viability assay

The BJ human foreskin fibroblast cells were seeded in 96-well microculture plates for attachment overnight and then incubated with DMXAA **1**, compounds **10e**, **10i** and **10j** of varying concentrations for 24 h. Then, MTT (0.5 mg/mL) was added to each well, and the cells were further incubated for 3 h. The stained formazan product was determined spectrophotometrically at 570 nm in GENios Pro microplate reader (TECAN).

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Supplementary data

Supplementary data (structural characterization of compounds **16a**–**m** and **10a**–**m**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.042.

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