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Short communication

Synthesis and inhibition of PGE_2 production of 6,8-disubstituted chrysin derivatives

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Abstract

A series of 6,8-disubstituted chrysin derivatives have been synthesized and evaluated for their PGE_2 inhibitory activities. 6,8-Disubstituted chrysin derivatives were obtained from naturally occurring chrysin by halogenation, oxidation, thiomethylation and C–C cross coupling reaction. Among the compounds investigated, 6,8-dibromochrysin (**2**), 6,8-diiodochrysin (**4**), 6,8-dimethylthiochrysin (**9**) and 6,8-dimethoxychrysin (**11**) showed as strong inhibitory activities of PGE_2 production from LPS-induced RAW 264.7 cells as wogonin, a well known natural flavone having strong and selective COX-2 inhibitory activity.

Keywords: Chrysin; PGE_2 production; Anti-inflammatory; COX-2; Wogonin; Flavonoids

1. Introduction

Chrysin (5,7-dihydroxyflavone), a naturally wide distributed flavonoid, has been reported to possess diverse biological activities such as anti-oxidant, anti-allergy, anti-inflammatory and anti-cancer [1–4]. It has been proposed that chrysin acts as an agonist of PPAR-γ which results in down-regulation of the key pro-inflammatory enzymes, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) [5]. Furthermore, some natural flavone analogs such as wogonin, baicalein and oroxylin A (Fig. 1) showed much stronger inhibitory activities of PGE_2 production than that of chrysin [6,7]. These flavones have an extra 6- or 8-substituent group at the A ring of the flavone structures compared to the structure of chrysin. Therefore, we assumed that structural modification at both 6- and 8-positions of the A ring of chrysin could be tolerable to the bioactivity.

As an attempt to discover novel synthetic flavones with potent anti-inflammatory activity, recently we have synthesized flavone analogs modified at the A and B ring systems of chrysin and evaluated their inhibitory activities against prostaglandin production [8,9]. The results showed that methylation of 5,7-dihydroxy groups on the A ring as well as substitutions on the B ring of chrysin did not affect to the bioactivity. As part of our continuing research efforts directed at the SARs of natural flavones for the anti-inflammatory activity, we were interested in the effects of hydrophobic groups such as methylthio ($-SCH_3$), halogen (I, Br), alkyl and aryl groups substituted at the 6- and 8-positions of chrysin. Based on the structure–activity relationships (SARs) of these compounds for anti-inflammation, we visualized that the substitutions either at 6- or 8-position on the A ring of chrysin may play important roles in their bioactivities. These substituents are rarely seen in the basic structure of natural flavones, but it would be believed that they have important roles of bioactivities. In this paper, the synthesis of 6,8-disubstituted chrysin derivatives and evaluation of their inhibitory activities on PGE_2 production are reported. These compounds are more structurally similar to wogonin and oroxylin A than chrysin. As observed from the inhibitory activities of PGE_2 production of wogonin, baicalein and oroxylin A, substituention at 6- or 8-position did increase the bioactivity compared to that of chrysin. These results led us to design chrysin derivatives with substituents at 6- and 8-position. All the compounds were easily obtained from chrysin with excellent yields in a few steps.

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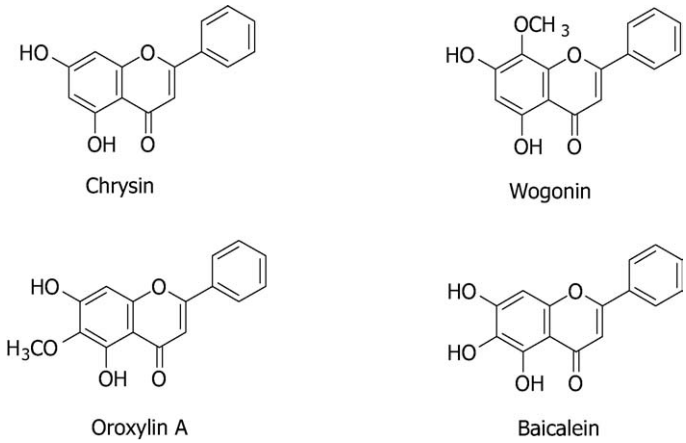
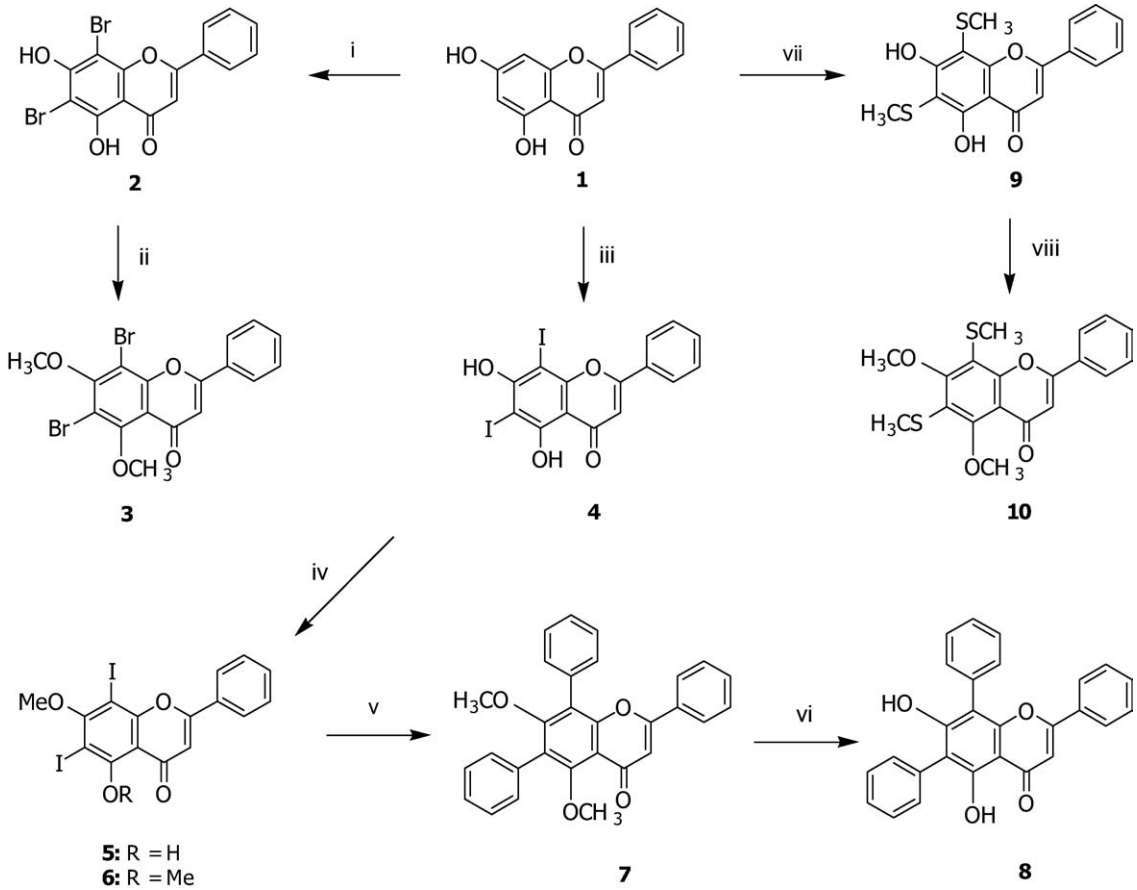


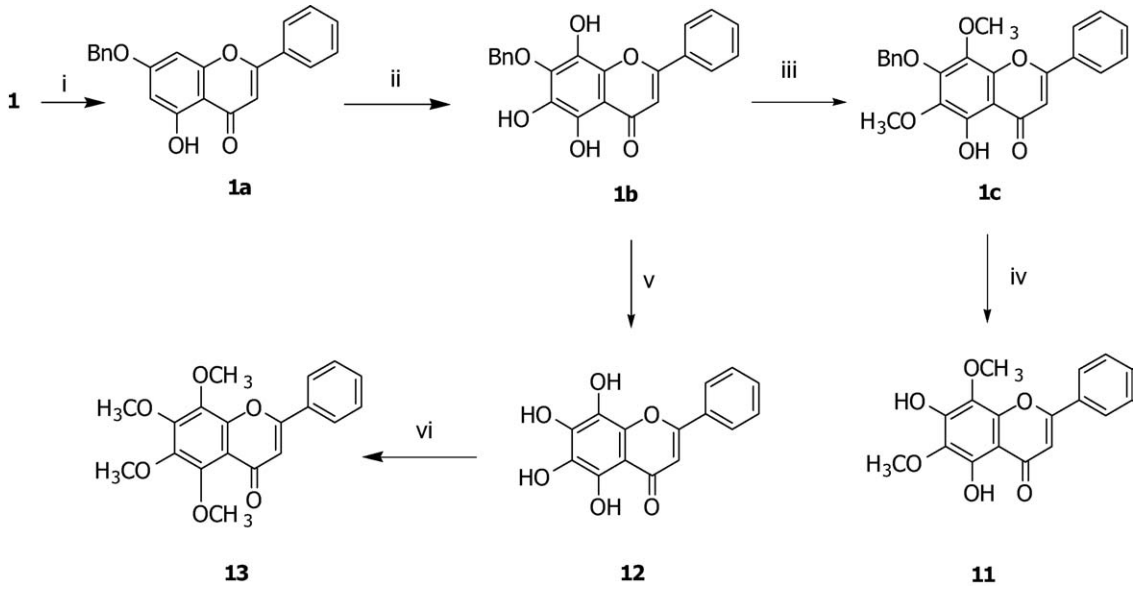
Fig. 1. Structures of some natural flavone analogs.

2. Chemistry

The synthetic procedures and reaction conditions for all investigated compounds are shown in Schemes 1 and 2. Chrysin (**1**) was directly halogenated by process of bromination [10] to form 6,8-dibromochrysin (**2**), or iodination [11] to form 6,8-diiodochrysin (**4**). Methylation reaction of the 6,8-dihalogenated chrysin derivatives with either 1 or 2 M equivalents of dimethylsulfate in anhydrous acetone and potassium carbonate gave 5,7-dimethoxy-6,8-dibromoflavone (**3**), 5-hydroxy-7-methoxy-6,8-diiodoflavone (**5**), and 6,8-diiodo-5,7-dimethoxyflavone (**6**). Suzuki coupling reaction [12] of **6**, benzeneboronic acid and catalytic amount of $Pd(PPh_3)_4$ afforded 5,7-dimethoxy-6,8-diphenylflavone (**7**). Deprotection reaction of **7** with BBr_3 in anhydrous methylene chloride gave 5,7-dihydroxy-6,8-diphenylflavone (**8**). Thiomethylation reaction [13] of chrysin (**1**) gave 6,8-dimethylthiochrysin (**9**) and followed by methylation with dimethylsulfate yielded



Scheme 1. i: Bromine, $CHCl_2$, Me_2S, 0 °C; ii: Me_2SO_4 (2 equiv.), K_2CO_3, acetone, reflux; iii: iodine, acetic acid, 0 °C; iv: Me_2SO_4 (1 equiv. For **5**; 2 equiv. for **6**), K_2CO_3, acetone, reflux; v: benzeneboronic acid, DMF, Pd $(PPh_3)_4$, 90 °C; vi: BBr_3, chloroform, reflux; vii: DMDS, $FeCl_3$, toluene, reflux; viii: Me_2SO_4 (2.5 equiv.), K_2CO_3, acetone, reflux.



Scheme 2. i: Benzyl bromide, K_2CO_3, acetone, 60 °C; ii: $K_2S_2O_8$, pyridine, KOH; iii: Me_2SO_4, K_2CO_3, acetone 60 °C; iv: c-HCl, acetic acid, 80 °C; v: c-HCl, acetic acid, 80 °C; vi: Me_2SO_4, K_2CO_3, acetone 60 °C.

6,8-dithiomethyl-5,7-dimethoxyflavone (**10**). Reactions of chrysin via benzylation, *Elbs persulfate oxidation* [14], methylation and debenzylation yielded 5,6,7,8-tetrahydroxyflavone (**11**), 5,6,7,8-tetra-methoxyflavone (**12**) and 5,7-dihydroxy-6,8-dimethoxyflavone (**13**) as described in Scheme 2. The synthesized compounds were purified by flash column chromatography and analyzed the structures based on ^{1}H-NMR and ^{13}C-NMR spectra.

3. Pharmacology

The bioassays were performed according to the published procedure [7]. RAW 264.7 cells obtained from American Type Culture Collection were cultured with DMEM supplemented with 10% FBS and 1% CO_2 at 37 °C and activated with LPS (Lipopolysaccharide, *Escherichia coli* O127:B8). Briefly, cells were plated in 96-well plates (2×10^5 cells per well). Each synthetic flavone was dissolved in dimethyl sulfoxide (DMSO) and LPS (1 μg/ml) were added and incubated for 24 h. Cell viability was assessed with MTT assay based on the experimental procedures described previously [15]. All tested compounds showed no or less than 10% reduction of MTT assay, indicating that they were not significantly cytotoxic to RAW 264.7 cells in the presence or absence of LPS. PGE_2 concentration in the medium was measured using EIA kit for PGE_2 according to the manufacturer's recommendation. All experiments were carried out at least twice and they gave similar results. The inhibitory activities of synthetic flavones on COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells were estimated and shown in Table 1.

4. Results and discussion

Among the synthesized 6,8-disubstituted chrysin derivatives, the compounds which have free hydroxy groups at the 5- and 7-positions (**2**, **4**, **9** and **11**) generally showed very strong inhibitory activities of COX-2 catalyzed PGE_2 production as shown in Table 1. While all 6,8-disubstituted chrysin derivatives with methoxy group(s) at 5- and/or 7-position(s) (**3**, **5**, **6**, **7**, **10** and **13**) showed significantly decreased bioactivities. The compound having hydroxy groups at 6- and 8-positions (**12**) showed much reduced bioactivity though it has the free hydroxy groups at 5- and 7-positions. The compounds having phenyl substituents at 6- and 8-positions (**7** and **8**) showed moderate activities regardless of the free hydroxy groups at 5- and 7-positions. These results may imply that the character of substituents at the 6- and 8-positions is related with bioactivities of chrysin derivatives. Based on our

Table 1
Inhibition of COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells by synthetic chrysin derivatives

Flavones[a]	Percent inhibition of PGE_2 production[b]	IC_{50} (μM)
1 (Chrysin)	11.12	NT[c]
2	99.93	1.34
3	Inactive	NT
4	98.41	1.82
5	Inactive	NT
6	57.13	NT
7	43.73	NT
8	39.38	NT
9	98.53	0.96
10	Inactive	NT
11	99.20	2.85
12	22.43	NT
13	64.78	NT
Wogonin	99.27	1.08

[a] All compounds were treated at 10 μM. Treatment of LPS to raw cells increased PGE_2 production (10 μM) from the basal level of 0.5 μM.

[b] % Inhibition = 100 × [1 – (PGE_2 of LPS with the flavones treated group – PGE_2 of the basal)/(PGE_2 of LPS treated group – PGE_2 of the basal)].

[c] NT: not tested.

present results, it may be concluded that the 5,7-dihydroxy groups as well as the character of substituents at 6- and 8-positions on the A ring of chrysin derivatives play important roles on the inhibition of PGE_2 production. The active compounds with inhibition values over 90% were tested for MTT assay. The results indicated that all tested compounds did not exhibit any cytotoxicity. Therefore, the inhibition of PGE_2 production by flavone derivatives were not associated with their cytotoxicity.

In summary, we prepared chrysin derivatives modified at the 6- and 8-positions of the A ring and evaluated their inhibitory activities on COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells. We found that 6,8-dihalogeno (**2** and **4**), 6,8-dimethylthio (**10**) and 6,8-dimethoxy (**11**) chrysin derivatives with 5,7-dihydroxy groups possessed strong inhibitory activities compared to that of chrysin. The present results indicated that the free hydroxyl groups at 5- and 7-potitions and the character of substituents at the 6- and 8-positions of the A ring play very important roles to biological activity as we premised. The strong inhibitory activities of PGE_2 production by 6,8-disubstituted chrysin derivatives may be explained by the structural similarity of these compounds, wogonin and oroxylin A. In conclusion, substitution at both 6- and 8-posiotion is tolerable to bioactivity but size and electronic character of the substituents seem to closely related with bioactivity. Our present results are quite different from the previous results by us that methylation of 5,7-dihydroxy groups on the A ring of chrysin did not affect to the bioactivity [8]. Further SARs study on the A ring is currently under investigation.

5. Experimental section

5.1. Chemistry

All chemicals were purchased from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from proper dehydrating agents under nitrogen gas. ^{1}H-NMR spectra were recorded on a Varian Gemini 2000 instrument (200 MHz) spectrometer. ^{13}C-NMR spectra were recorded on a Varian Gemini 2000 instrument (50 MHz) spectrometer. Chemicals shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane as a internal standard on the δ scale. Data are reported as follows: chemical shift, multiplicity (s = single, d = double, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (Hz), integration. Chromatographic purification was carried out by flash chromatography using Kieselgel 60 (230–240 mesh, Merck). Mass spectra were recorded on GCMS (Autospec. M363 series) auto sampler/direct injection (EI). Elemental analyses were recorded on Series II CHN s/o Analyzer PE 2400 AD:6 and the data for C, H, N were with in ±0.4% of the theoretical values.

*5.1.1. 6,8-Dibromo-5,7-dihydroxyflavone (**2**)*

To the flask dried on the flame was placed 1.8 ml of Me_2S and immediately dissolved with 20 ml of anhydrous CH_2Cl_2. After cooling in ice-water bath, a solution of 1.3 ml of bromine in 20 ml of CH_2Cl_2 was added slowly with magnetic stirring over 10 min. Then, a solution of 5.08 g of chrysin in 50 ml of CH_2Cl_2 was added slowly over a few min., and stirring was continued for another 2 h and 30 min. At the end of reaction (monitoring by TLC with the solvent system of chloroform and methanol 20:1), the solvent was removed by evaporation in vacuum, followed by treatment with saturated $NaHCO_3$ solution to remove trace of HBr. The remain solid was washed with water and crystallized from absolute methanol to obtain the title compound. Yield 92%. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 13.75 (s, 1H, OH), 11.77 (s, 1H, OH), 8.12–8.16 (d, 2H, H2', H6'), 7.60–7.64 (t, 3H, H3', H4', H5'), 7.21 (s, 1H, H3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 181.5 (C-4), 163.4 (C-7), 157.9 (C-2), 157.1 (C-10), 152.4 (C-5), 132.5 (C-1'), 130.3 (C-3' and C-5'), 129.3 (C-4'), 126.5 (C-2' and C-6'), 105.1 (C-9), 104.8 (C-3), 94.7 (C-6), 88.6 (C-8). EIMS: *m/z* 413 (M + 1). Anal. $C_{15}H_8Br_2O_4$ (C, H, N).

*5.1.2. 6,8-Dibromo-5,7-dimethoxyflavone (**3**)*

A mixture of 6,8-dibromo-5,7-dihydroxyflavone (1 equiv.) and K_2CO_3 and dimethyl disulfate (2.2 equiv.) in acetone was refluxed for 6 h, monitoring by TLC. The mixture was filtered and filtration was concentrated. Solidified product was filtered, washed with water, and recrystallized from methanol to form the titled flavone. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.18–8.24 (d, 2H, H2', H6'); 7.68–7.75 (m, 3H, H3', H4', H5'); 7.17 (s, 1H, H3); 3.94-4.03 (s, 6H, 2xOCH_3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 175.8 (C-7), 166.5 (C-4), 161.7 (C-5), 160.8 (C-2), 153.5 (C-9), 132.2 (C-1'), 130.4 (C-3' and C-5'), 129.4 (C-4'), 126.3 (C-2' and C-6'), 115.6 (C-10), 108.1 (C-3), 99.0 (C-8), 77.6 (C-6), 61.66 and 61.00 (2 × OCH_3). EIMS: *m/z* 440 (M^+). Anal. $C_{17}H_{12}Br_2O_4$ (C, H, N).

*5.1.3. 5,7-Dihydroxy-6,8-diiodoflavone (**4**)*

A mixture of iodine (20 mmol) in 20 ml of CH_2Cl_2 and 5,7-dihydroxyflavone (20 mmol) in 20 ml of glacial acetic acid was stirred at room temperature for 30 min. during the slow addition of 2 g of 65% HNO_3 acid in 10 ml of acetic acid. The iodinated product was precipitated during the addition. The suspension was stirred for 2 h and filtered. The solid was washed with 10% $Na_2S_2O_4$ solution then cooled methanol and water. The product was crystallized as pale yellow crystals. Yield 75%. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 13.89 (s, 1H, OH), 8.08–8.12 (m, 2H, 6.8 Hz, H2', H6'), 7.48–7.53 (m, 3H, H3', H4'), 7.08 (s, 1H, H3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 181.5 (C-4), 178.5 (C-7), 163.8 (C-2), 162.0 (C-5), 161.1 (C-10), 132.6 (C-1'), 130.4 (C-3' and C5'), 129.4 (C-4'), 126.8 (C-2' and C-6'), 105.1 (C-9), 104.7 (C-3), 63.4 (C-6 and C-8). EIMS: *m/z* 506 (M^+). Anal. $C_{15}H_8I_2O_4$ (C, H, N).

*5.1.4. 5-Hydroxy-6,8-diiodo-7-methoxyflavone (**5**)*

A mixture of 6,8-diiodo-5,7-dihydroxyflavone (1 equiv.) and K_2CO_3 and dimethylsulfate (1.1 equiv.) in acetone was refluxed for 4 h, monitoring by TLC. The mixture was fil-

tered and filtration was concentrated. Solidified product was filtered, washed with water, and recrystallized from methanol to form the titled flavone. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 14.06 (s, 1H, OH-5), 8.31–8.35 (m, 2H, H2' and H6′); 7.31–7.61 (m, 3H, H3', H4' and H5'); 7.41 (s, 1H, H3); 3.97 (s, 3H, OCH_3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 182.1 (C-4 and C-7), 164.4 (C-2), 161.3 (C-5 and 9), 132.8 (C-1'), 130.2 (C-3' and C-5'), 129.4 (C-4'), 126.4 (C-2' and C-6'), 107.7 (C-10), 105.3 (C-3), 97.3 (C-6 and C-8), 60.8 (OCH_3). EIMS: *m/z* 520 (M + 1). Anal. $C_{16}H_{10}I_2O_4$ (C, H, N).

*5.1.5. 6,8-Diiodo-5,7-dimethoxyflavone (**6**)*

Methylation of the 5,7-phenolic groups was carried out following the procedure for the synthesis of the compound **3**. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.04-8.09 (d, 2H, J = 7.4 Hz, 2 Hz, H2', H6'); 7.54–7.58 (m, 3H, J = 7.2 Hz, 2.2 Hz, H3', H4', H5'); 6.79 (s, 1H, H3); 3.96–3.99 (s, 6H, 2 × OCH_3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 175.0 (C-7), 163.4 (C-4), 161.0 (C-5), 160.1 (C-2), 157.4 (C-9), 132.1 (C-1'), 130.5 (C-3' and C-5'), 129.2 (C-4'), 126.5 (C-2' and C-6'), 115.7 (C-10), 107.8 (C-3), 89.0 (C-8), 79.4 (C-6), 61.5 and 60.7 (2 × OCH_3). EIMS: *m/z* 534 (M^+). Anal. $C_{17}H_{12}I_2O_4$ (C, H, N).

*5.1.6. 5,7-Dimethoxy-6,8-diphenylflavone (**7**)*

To a solution of 5,7-dimethoxy-6,8-diiodoflavone in DME was added Pd $(PPh_3)_4$ (0.2% mole) The resulting mixture was degassed and stirred at ambient temperature for 20 min. before the addition Na_2CO_3 solution. The mixture was degassed again and then stirred in an atmosphere of nitrogen for several hours. The benzeneboronic acid (3 equiv.) was added, and the reaction mixture was heated at 80 °C for 2–4 h with monitoring by TLC. After cooling to room temperature, the mixture was diluted with dichloromethane and water, the organic phase was separated, washed with water and dried over magnesium sulfate, filtered and evaporated in vacuum. The remain solid was purified by flash column to obtain 5,7-dimethoxy-6,8-diphenylflavone, yield 76%. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.36–7.55 (m, 15H, J = 8.0 Hz, 2.8, H-phenyl); 6.75 (s, 1H, H3); 3.16–3.60 (s, 6H, 2 × OCH_3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 176.3 (C-4), 160.5 (C-2), 132.2 (C-7), 131.8 (C-5), 131.5(C-9), 130.7 (2C-1", 130.4 (C-1'), 128.9 (2C-3" and 2C-5"), 128.3 (2C-3"), 127.9 (2C-2", 2C-4" and 2C-6"), 125.9 (C-2' and C-4'), 124.3 (C-6), 121.2 (C-10), 108.6 (C-3), 61.8 and 60.7 (2 × OCH_3). EIMS: *m/z* 434 (M + 1). Anal. $C_{29}H_{22}O_4$ (C, H, N).

*5.1.7. 5,7-Dihydroxy-6,8-diphenylflavone (**8**)*

Solution 1 M BBr_3 in CH_2Cl_2 (4 equiv.) was added slowly to the solution of 57-Dimethoxy-6,8-diphenylflavone during 10 min. Nitrogen gas was inserted to the reaction flask to remove the oxygen gas, then it was heating at 40 °C for 6–10 h, monitoring by TLC. The reaction mixture was cooled to room temperature, methanol was added to destroy the excess of BBr_3. Methanol and solvent was removed in vacuum to obtain solid. The solid was washed with water and recrystallized twice from acetone-water or dichloromethane - methanol to obtain a pure product. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 13.42 (s, 1H, OH_5), 9.45 (s, 1H, OH_7); 7.75–7.79 (m, 3H, phenyl); 7.48–7.58 (m, 12H, phenyl); 7.10 (s, 1H, H3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 182.7 (C-4), 163.2 (C-2), 158.3 (C-7), 157.6 (C-5), 153.2 (C-9), 132.3 (2C-1"), 131.7 (C-1'), 129.1 (2C-3" and 2C-5"), 128.3 (C-3' and C-5'), 127.8 (C-4'), 127.3 (2C-2", 2C-4" and 2C-6"), 126.2 (C-2' and C-6'), 113.6 (C-6), 109.2 (C-10), 104.8 (C-8), 97.3 (C-3). EIMS: *m/z* 406 (M^+). Anal. $C_{27}H_{18}O_4$ (C, H, N).

*5.1.8. 5,7-Dihydroxy-6,8-dimethylsulfinylflavone (**9**)*

Ferric chloride and dimethyl disulfide were added to a solution of chrysin in dried toluene with vigorously stirring. The mixture was then heated to 105 °C and kept at this temperature for 14 h. After cooling to the room temperature, hydrolysis was carried out using solution of 10% hydrochloric acid. The mixture was extracted with dichloromethane (5 × 15 ml), and the solvent was evaporated. The solid was crystallized from methanol to obtain 6,8-dimethylthiochrysin), 87% yield. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 14.03 (s, 1H, OH), 10.37 (s, 1H, OH), 8.01–8.06 (q, 2H, H2', H6'), 7.57–7.59 (t, 3H, H3', H4', H5'), 6.78 (s, 1H, H3), 2.39–2.41 (s, 6H, 2 × SCH_3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 182.3 (C-4), 165.1 (C-2), 163.5 (C-7), 162.7 (C-10), 157.2 (C-9), 132.4 (C-1'), 130.7 (C-3' and C5'), 129.1 (C-4'), 126.5 (C-2' and C-6'), 105.1 (C-9), 104.8 (C-6 and C-8), 99.6 (C-3), 18.0 and 16.9 (2 × SCH_3). EIMS: *m/z* 346 (M + 1). Anal. $C_{17}H_{14}O_4S_2$ (C, H, N).

*5.1.9. 5,7-Dimethoxy-6,8-dimethylsulfinylflavone (**10**)*

Methylation of the compound **9** was carried out following the procedure for the synthesis of the compound **3**. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 8.02–8.04 (q, 2H, H2', H6'); 7.54–7.59 (t, 3H, H3', H4', H5'); 6.80 (s, 1H, H3); 4.03 and 4.07 (s, 6H, 2-OCH_3); 2.51 and 2.47 (s, 6H, 2 × SCH_3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 176.5 (C-4), 165.7 (C-2), 162.3 (C-7), 160.9 (C-5), 157.3 (C-9), 131.1 (C-1'), 128.7 (C-3' and C5'), 125.9 (C-4'), 125.6 (C-2' and C-6'), 108.4 (C-10), 107.8 (C-8), 104.9 (C-6), 95.4 (C-3), 61.3 (OCH_3), 61.0 (OCH_3), 17.8 (2 × SCH_3). EIMS: *m/z* 374 (M^+). Anal. $C_{19}H_{18}O_4S_2$ (C, H, N).

*5.1.10. 5,7-Dihydroxy-6,8-dimethoxyflavone (**11**)*

5,7-Dihydroxyflavone (chrysin) was protected using benzyl bromide (1 equiv.) and K_2CO_3 in the refluxing acetone solvent. Then, the benzyl protected flavone (**1a**) was treated with excess potassium persulfate (3 equiv.) in the present of potassium hydroxide and pyridine to afford 7-benzoyl-5,6,8-trihydroxyflavone (**1b**). This product was methylated with dimethyl sulfate (2 equiv.) to obtain 7-benzoyl-6,8-dimethoxy-5-hydroxyflavone (**1c**), followed by hydrolysis to obtain the titled product. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 12.78 (s, 1H, OH), 10.64 (s, 1H, OH), 8.15–8.19 (q, 2H, H2', H6'), 7.68–7.73 (t, 3H, H3', H4', H5'), 7.12 (s, 1H, H3), 3.88–3.98 (s, 6H, 2 × OCH_3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 182.5 (C-4), 163.1 (C-2), 148.4 (C-5), 147.7 (C-10), 138.7 (C-7), 131.4 (C-1'), 130.6 (C-6), 130.2 (C-8), 128.6 (C-3' and C-5'),

125.7 (C-2', C-4' and C-6'), 104.6 (C-9), 97.2 (C-3), 61.2 and 60.4 (2 × OCH_3). EIMS: *m/z* 314 (M^+). Anal. $C_{17}H_{14}O_6$ (C, H, N).

*5.1.11. 5,6,7,8-Tetrahydroxyflavone (**12**)*

Debenzylation of 7-benzoyl-5,6,8-trihydroxyflavone (**1b**) in aqueous c-HCl solution gave the titled compound ^{1}H-NMR (200 MHz, DMSO-d_6): δ 12.27 (s, 1H, OH), 10.07 (s, 1H, OH); 9.04 (s, 2H, OH); 8.25–8.27 (m, 2H, H2', H6'); 7.67–7.71 (t, 3H, H3', H4', H5'); 7.01 (s, 1H, H3). ^{13}C-NMR (50 MHz, DMSO-d_6): δ 182.6 (C-4), 162.7 (C-2), 142.9 (C-9), 139.8 (C-5), 139.1 (C-7), 131.8 (C-1'), 129.2 (C-3' and C5'), 126.5 (CC4' and C6), 126.4 (C-8), 125.6 (C-2' and C-6'), 104.1 (C-10), 103.0 (C-3). EIMS: *m/z* 286 (M^+). Anal. $C_{15}H_{10}O_6$ (C, H, N).

*5.1.12. 5,6,7,8-Tetramethoxyflavone (**13**)*

A mixture of 5,6,7,8-tetrahydroxyflavone (1 equiv.) and K_2CO_3 and dimethylsulfate (5.2 equiv.) in acetone was refluxed for overnight, monitoring by TLC. The mixture was filtered and filtration was concentrated. Solidified product was filtered, washed with water, and recrystallized from methanol to form the titled flavone. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.90–7.95 (m, 2H, H2' and H6'); 7.43–7.58 (m, 3H, H3', H4' and H5'); 3.95, 3.98, 4.03 and 4.11 (s, 12H, 4 × OCH_3). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 176.8 (C-4), 161.8 (C-2), 146.5 (C-7), 138.8 (C-9), 130.9 (C-1'), 128.5 (C-3' and C5', C-6 and C-8), 125.4 (C-2' and C-6'), 107.4 (C-10), 95.7 (C-3), 61.07, 61.2, 61.4, 61.6 (4 × OCH_3). EIMS: *m/z* 342 (M^+). Anal. $C_{19}H_{18}O_6$ (C, H, N).

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