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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and anti-inflammatory activity of dihydroflavonol derivatives

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Abstract Thirty dihydroflavonol derivatives (**D1–D30**) were designed and synthesized, meanwhile the synthesized compounds were characterized on the basis of spectroscopic analyzes. Their inhibitory activity against the pro-inflammatory inducible interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in lipopolysaccharide (LPS)-stimulated murine RAW 264.7 macrophages were evaluated and showed various efficiency. Compounds **D1–D30** showed no toxic effects on RAW 264.7 cells at the concentration 20 μM; among them, compounds **D9**, **D13**, and **D19** exhibited best anti-inflammatory activity through decreasing IL-1β, IL-6, and TNF-α. Furthermore, their structure–activity relationships were discussed preliminarily.

Keywords Dihydroflavonol derivatives · Synthesis · Anti-inflammatory activity · Structure–activity relationship

Introduction

Flavonoids are phenolic substances isolated from a wide range of plants, including over 8000 individual compounds known (Vezza et al. 2016). Among the flavonoids, dihydroflavonols have received more and more attention for novel structure and excellent pharmacological activities, in which the inflammatory response plays an important role in the pathological processes of many diseases, including cancer, cardiovascular disorder and inflammation (Mantovani et al. 2008; Ragab and Raafat 2016; Swaminathan et al. 2014). During the inflammatory process, the mononuclear cells usually differentiate into macrophages, these cells release a plethora of inflammatory mediators, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), that play an important role in promoting the inflammatory response and pathological processes (Raison et al. 2006; Siemieniuch et al. 2016). Inhibition of the expression of inflammatory cytokines by small molecules or antagonism of their actions by antibodies has been considered to be effective strategies for the treatment of inflammation-related diseases (Bengmark 2006; Zhang et al. 2014). In nature, many dihydroflavonols such as dihydromyricetin, astilbin and silybin are found and possess outstanding bioactivity, which constitute the core of various natural products and play a unique role in drug discovery history (Terrier et al. 2009).

Taxifolin (Fig. 1) known as dihydroquercetin, which belongs to dihydroflavonols, is widely found in medicinal plants and has a wide range of biological activities (Manigandan et al. 2015; Marín et al. 2011). Many research showed that taxifolin had a significant anti-inflammatory effect and could inhibit LPS-induced IL-1β, IL-6, and TNF-α production by cytokine assay (Kim et al. 2008). Recently, our group isolated taxifolin from *Smilax China L.* and proved to be the best anti-inflammatory activity in *Smilax China L* (Xie et al. 2012). However, the natural source of taxifolin is very limited and extractive procedures are very costly, which restricts the application of taxifolin widely. To obtain better anti-inflammatory

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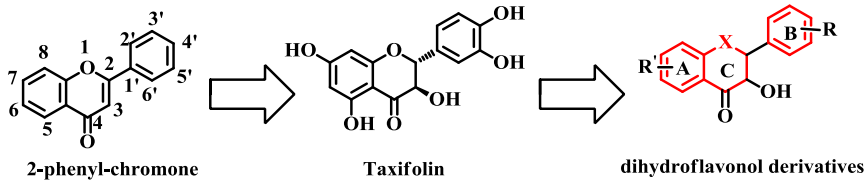
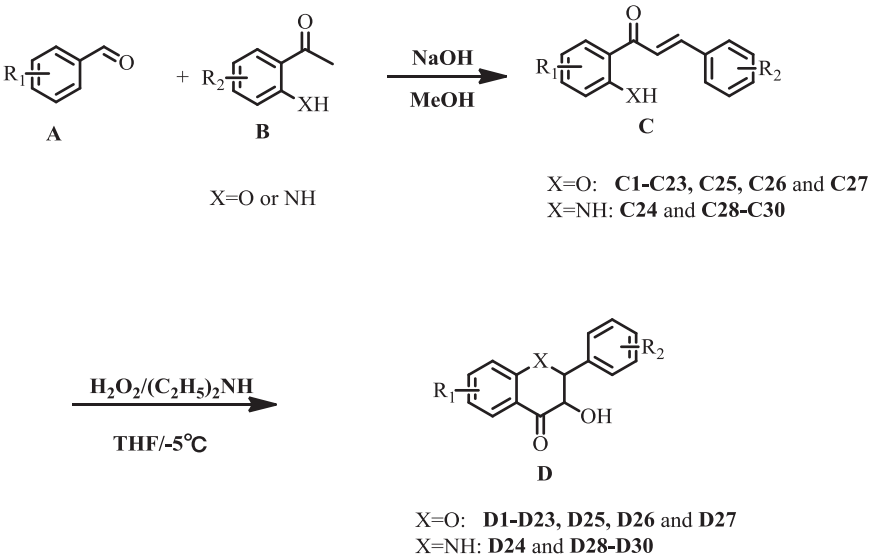


Fig. 1 Structures of 2-phenyl chromone, taxifolin, and dihydroflavonol derivatives



Scheme 1 Synthetic route of compounds (**D1–D30** and **TA-SY**)

activity compounds, the modification and synthesis of taxifolin are very urgent.

Structural analysis indicates that the basic mother-nucleus of flavonoids is 2-phenyl chromone (Fig. 1), which usually has three aromatic rings (A, B, and C). Many studies stated that anti-inflammatory activity of flavonoids was closely related with the electron density distribution in its conjugated system and substitution patterns of the three aromatic rings (Cho et al. 2013; Lu et al. 2015). Dihydroflavonols as typical flavonoids are a kind of flavones, in which 2, 3-position is a single bond and 3-position is substituted by hydroxyl group, the biological activity of dihydroflavonols may also be affected by these factors.

In previous studies, we found a simple and effective synthetic route of dihydroflavonols by optimizing the reaction conditions (Zhou et al. 2016), which is illustrated in the Scheme 1. Herein, we synthesized a series of dihydroflavonol derivatives by introducing different groups as described in Fig. 2 and evaluated their anti-inflammatory activities. Although dihydroflavonols had been reported to suppress the expression of various inflammatory cytokines, their structure–activity relationship (SAR) was not reported (Hernández et al. 2007; Dok-Go et al. 2003). To carry out effective modification and improve anti-inflammatory effect, the preliminary structure–activity relationship of dihydroflavonol derivatives was further discussed.

Materials and methods

Experimental

All the materials and solvents were procured from Sinopharm Chemical Reagent Co., Ltd (China). All the synthesized compounds were checked by thin layer chromatography (TLC) performed on Silica gel 60 GF_{254} coated plates. Infrared spectra were recorded in the range of 4000–600 cm^{-1} on a (Spectrum BX) Perkin Elmer Fourier transform infrared spectrophotometer in KBr phase. Melting points of the compounds were checked on a Shinuo melting point apparatus (WR-2, Shanghai), and the thermometer was uncorrected. Nuclear magnetic resonance (NMR) spectra were processsed in dimethyl sulfoxide (DMSO)-d_6 on a Bruker nuclear magnetic resonance NMR spectrophotometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C at the Analysis and Testing Center, Huazhong University of Science and Technology, China. Mass spectrometer was used for the measurement of molecular masses of compounds.

General synthesis of dihydroflavonol derivatives (D1–D30)

2′-Hydroxyl chalcone derivatives (1.0 mmol) and DEA (3.0 mmol) were added to stirred in anhydrous THF

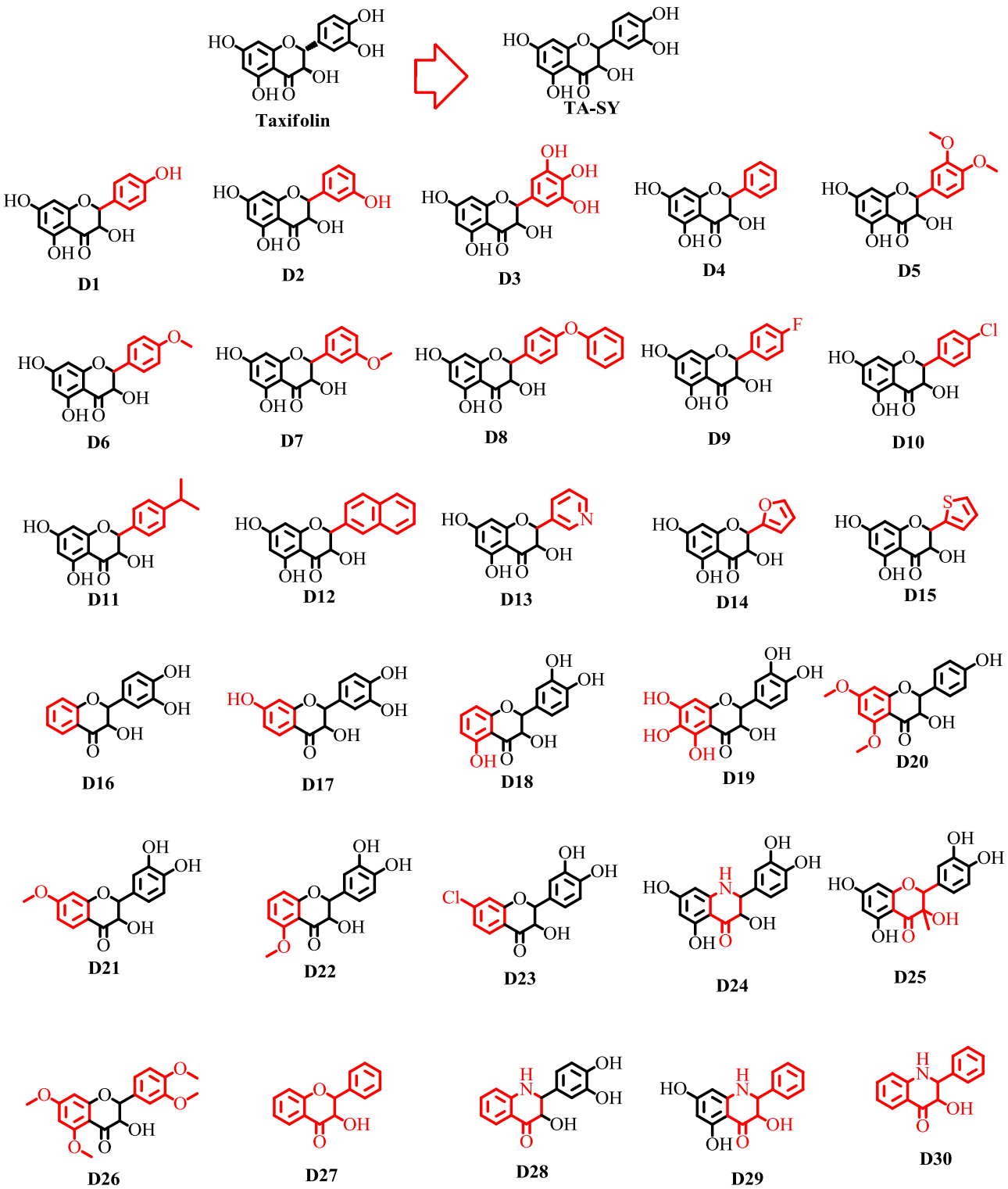


Fig. 2 Structures of **TA-SY** and **D1–D30**

(15.0 ml). To the above reaction mixture 30% H_2O_2 (0.3 ml, 3.0 mmol) was added and stirred at −5 °C. After completion of the reaction (as indicated by TLC), it was poured into ice cold water (20.0 ml) and stirred well for 30 min. Then filtering and washing in cold water, the crude material was recrystallized from petroleum ether and ethyl acetate to give corresponding dihydroflavonol derivatives.

*3,5,7-trihydroxy-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**TA-SY**)*

White solid; yield: 61%; M.p. 229–230 °C, IR (KBr): ν_{max}/cm^{-1}: 1649 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.90 (1H, s, OH-5), 10.80 (1H, s, OH-7), 9.01 (1H, s, OH-4′), 8.96 (1H, s, OH-3′), 6.98–7.14 (3H, m, H-2′, 5′, 6′), 5.90 (1H, d, $J = 2.4$ Hz, H-8), 5.85 (1H, d, $J = 2.4$ Hz, H-6), 5.74 (1H, s, OH-3), 4.97 (1H, d, $J = 12.0$ Hz, H-2), 4.49 (1H, d, $J = 12.0$, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.16 (C-4), 167.22 (C-7), 164.24 (C-5), 163.04 (C-9), 145.99 (C-3′), 145.45 (C-4′), 128.04 (C-1′), 120.75 (C-6′), 115.38 (C-5′), 115.21 (C-2′), 101.44 (C-10), 96.17 (C-6), 96.97 (C-8), 83.05 (C-2), 72.94 (C-3). Mass spectrometry (MS) electrospray ionization (ESI) *m/z*: 304.0 $[M]^+$.

*3,5,7-Trihydroxy-2-(4′-hydroxyphenyl) chroman-4-one (**D1**)*

White solid; yield: 67%; M.p. 219–220 °C, IR (KBr): ν_{max}/cm^{-1}: 1649 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.89 (1H, s, OH-5), 10.78 (1H, s, OH-7), 9.14 (1H, s, OH-4′), 7.12–7.18 (2H, m, H-2′, 3′), 6.98–7.08 (2H, m, H-5′, 6′), 5.95 (1H, d, $J = 2.4$ Hz, H-8), 5.89 (1H, d, $J = 2.4$ Hz, H-6), 5.70 (1H, s, OH-3), 4.99 (1H, d, $J = 12.0$ Hz, H-2), 4.50 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.95 (C-4), 167.18 (C-7), 164.04 (C-5), 163.55 (C-9), 157.77 (C-4′), 129.60 (C-1′), 128.70 (C-3′), 119.98 (C-6′), 115.61 (C-5′), 115.50 (C-2′), 101.24 (C-10), 96.72 (C-6), 96.07 (C-8), 84.18 (C-2), 73.18 (C-3). MS (ESI) *m/z*: 288.2 $[M]^+$.

*3,5,7-Trihydroxy-2-(3′-hydroxyphenyl) chroman-4-one (**D2**)*

White solid; yield: 58%; M.p. 201–203 °C, IR (KBr): ν_{max}/cm^{-1}: 1650 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.02 (1H, s, OH-5), 10.91 (1H, s, OH-7), 8.89 (1H, s, OH-3′), 7.14 (1H, s, H-2′), 6.88–7.09 (3H, m, H-4′, 5′, 6′), 5.98 (1H, d, $J = 2.4$ Hz, H-8), 5.90 (1H, d, $J = 2.4$ Hz, H-6), 5.72 (1H, s, OH-3), 5.01 (1H, d, $J = 11.6$ Hz, H-2), 4.48 (1H, d, $J = 11.6$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 198.14 (C-4), 168.01 (C-7), 164.47 (C-5), 163.68 (C-9), 158.70 (C-3′), 134.98 (C-4′), 130.60 (C-1′), 119.89 (C-6′), 116.03 (C-5′), 115.50 (C-2′), 101.24 (C-10), 96.72 (C-6), 96.07 (C-8), 83.26 (C-2), 72.61 (C-3). MS (ESI) *m/z*: 288.0 $[M]^+$.

*3,5,7-Trihydroxy-2-(3′,4′,5′-trihydroxyphenyl) chroman-4-one (**D3**)*

White solid; yield: 41%; M.p. 234–236 °C, IR (KBr): ν_{max}/cm^{-1}: 1658 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.01 (1H, s, OH-5), 10.89 (1H, s, OH-7), 9.45 (1H, s, OH-4′), 8.89 (1H, s, OH-3′), 8.86 (1H, s, OH-5′), 7.10 (1H, d, $J = 3.2$ Hz, H-2′), 7.12 (1H, d, $J = 3.2$ Hz, H-6′), 5.90 (1H, d, $J = 2.4$ Hz, H-8), 5.87 (1H, d, $J = 2.4$ Hz, H-6), 5.73 (1H, s, OH-3), 5.01 (1H, d, $J = 12.4$ Hz, H-2), 4.48 (1H, d, $J = 12.4$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.90 (C-4), 167.28 (C-7), 164.80 (C-5), 164.27 (C-9), 158.01 (C-4′), 145.90 (C-3′), 133.89 (C-5′), 131.45 (C-1′), 119.89 (C-6′), 115.50 (C-2′), 101.55 (C-10), 96.75 (C-6), 96.10 (C-8), 81.97 (C-2), 72.46 (C-3). MS (ESI) *m/z*: 320.2 $[M]^+$.

*3,5,7-Trihydroxy-2-phenylchroman-4-one (**D4**)*

White solid; yield: 72%; M.p. 177–180 °C, IR (KBr): ν_{max}/cm^{-1}: 1648 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.91 (1H, s, OH-5), 10.88 (1H, s, OH-7), 7.38–7.56 (5H, m, H-2′, 3′, 4′, 5′, 6′), 5.94 (1H, s, H-8), 5.91 (1H, s, H-6), 5.70 (1H, s, OH-3), 5.19 (1H, d, $J = 12.0$ Hz, H-2), 4.64 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 198.05 (C-4), 167.28 (C-7), 164.27 (C-5), 163.53 (C-9), 133.89 (C-1′), 128.66 (C-3′), 128.32 (C-5′), 127.99 (C-4′), 126.13 (C-2′), 125.98 (C-6′), 101.24 (C-10), 96.72 (C-6), 96.07 (C-8), 83.10 (C-2), 71.70 (C-3). MS (ESI) *m/z*: 272.0 $[M]^+$.

*3,5,7-Trihydroxy-2-(3′,4′-dimethoxyphenyl) chroman-4-one (**D5**)*

White solid; yield: 64%; M.p. 169–170 °C, IR (KBr): ν_{max}/cm^{-1}: 1650 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.90 (1H, s, OH-5), 11.01 (1H, s, OH-7), 6.88–7.08 (3H, m, H-2′, 5′, 6′), 5.90 (1H, s, H-8), 5.79 (1H, s, H-6), 5.65 (1H, s, OH-3), 4.97 (1H, d, $J = 12.0$ Hz, H-2), 4.49 (1H, d, $J = 12.0$ Hz, H-3), 3.89 (3H, s, OCH_3), 3.86 (3H, s, OCH_3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.64 (C-4), 167.28 (C-7), 164.27 (C-5), 163.48 (C-9), 148.66 (C-3′), 148.21 (C-4′), 129.50 (C-1′), 120.57 (C-6′), 111.15 (C-5′), 111.05 (C-2′), 101.21 (C-10), 96.77 (C-6), 96.07 (C-8), 83.60 (C-2), 72.41 (C-3), 56.03 (O–CH_3), 55.95 (O–CH_3). MS (ESI) *m/z*: 332.3 $[M]^+$.

*3,5,7-Trihydroxy-2-(4′-methoxyphenyl) chroman-4-one (**D6**)*

White solid; yield: 58%; M.p. 140–142 °C, IR (KBr): ν_{max}/cm^{-1}: 1653 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.90 (1H, s, OH-5), 11.01 (1H, s, OH-7), 7.40–7.44 (2H, m, H-2′, 6′), 6.92–7.14 (2H, m, H-3′, 5′), 6.04 (1H, s, H-8), 5.95 (1H, s, H-6), 5.73 (1H, s, OH-3), 4.93 (1H, d, $J = 12.0$ Hz, H-2), 4.42 (1H, d, $J = 12.0$ Hz, H-3), 3.80 (3H, s, OCH_3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.98 (C-4), 167.21 (C-7), 164.09 (C-5), 163.43 (C-9), 159.50

(C-4′), 129.65 (C-1′), 128.10 (C-2′, 6′), 113.91 (C-3′, 5′), 101.24 (C-10), 96.72 (C-6), 96.07 (C-8), 84.18 (C-2), 73.18 (C-3), 55.35 (O–CH_3), 55.95 (O–CH_3). MS (ESI) *m/z*: 302.2 $[M]^+$.

*3,5,7-Trihydroxy-2-(3′-hydroxyphenyl) chroman-4-one (**D7**)*

White solid; yield: 71%; M.p. 134–136 °C, IR (KBr): ν_{max}/cm^{-1}: 1651 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.02 (1H, s, OH-5), 10.98 (1H, s, OH-7), 7.22 (1H, s, H-2′), 6.92–7.16 (3H, m, H-4′, 5′, 6′), 6.01 (1H, s, H-8), 5.98 (1H, s, H-6), 5.68 (1H, s, OH-3), 5.02 (1H, d, $J = 12.0$ Hz, H-2), 4.49 (1H, d, $J = 12.0$ Hz, H-3), 3.82 (3H, s, OCH_3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.98 (C-4), 167.21 (C-7), 164.09 (C-5), 163.43 (C-9), 159.50 (C-4′), 129.65 (C-1′), 128.10 (C-2′, 6′), 113.89 (C-3′, 5′), 100.94 (C-10), 96.78 (C-6), 96.04 (C-8), 84.18 (C-2), 73.21 (C-3), 55.35 (O–CH_3). MS (ESI) *m/z*: 302.0 $[M]^+$.

*3,5,7-Trihydroxy-2-(4′-phenoxyphenyl) chroman-4-one (**D8**)*

White solid; yield: 45%; M.p. 99–101 °C, IR (KBr): ν_{max}/cm^{-1}: 1651 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.88 (1H, s, OH-5), 10.84 (1H, s, OH-7), 7.10–7.44 (5H, m, Ar–H), 6.88–7.04 (4H, m, H-2′, 3′, 5′, 6′), 6.05 (1H, s, H-8), 5.93 (1H, s, H-6), 5.75 (1H, s, OH-3), 4.95 (1H, d, $J = 12.0$ Hz, H-2), 4.43 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.98 (C-4), 167.21 (C-7), 164.29 (C-5), 163.53 (C-9), 159.50 (C-4′), 129.87 (C-1′), 124.49 (C-2′, 6′), 118.69 (C-3′, 5′), 101.20 (C-10), 96.77 (C-6), 96.05 (C-8), 84.50 (C-2), 72.98 (C-3). MS (ESI) *m/z*: 364.4 $[M]^+$.

*3,5,7-Trihydroxy-2-(4′-fluorophenyl) chroman-4-one (**D9**)*

White solid; yield: 53%; M.p. 213–215 °C, IR (KBr): ν_{max}/cm^{-1}: 1652 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.89 (1H, s, OH-5), 10.78 (1H, s, OH-7), 7.02–7.10 (2H, m, H-2′, 6′), 6.88–7.92 (2H, m, H-3′, 5′), 5.94 (1H, s, H-8), 5.83 (1H, s, H-6), 5.79 (1H, s, OH-3), 4.97 (1H, d, $J = 12.0$ Hz, H-2), 4.45 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 198.05 (C-4), 167.28 (C-7), 164.27 (C-5), 163.57 (C-9), 161.56 (C-4′), 131.12 (C-1′), 126.95 (C-2′, 6′), 115.09 (C-3′, 5′), 101.24 (C-10), 96.72 (C-6), 96.07 (C-8), 83.56 (C-2), 73.18 (C-3). MS (ESI) *m/z*: 290.2 $[M]^+$.

*3,5,7-Trihydroxy-2-(4′-chlorophenyl) chroman-4-one (**D10**)*

White solid; yield: 58%; M.p. 189–192 °C, IR (KBr): ν_{max}/cm^{-1}: 1651 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.01 (1H, s, OH-5), 10.98 (1H, s, OH-7), 7.09–7.14 (2H, m, H-2′, 6′), 6.88–7.92 (2H, m, H-3′, 5′), 5.91 (1H, s, H-8), 5.85 (1H, s, H-6), 5.77 (1H, s, OH-3), 4.87 (1H, d, $J = 12.0$ Hz, H-2), 4.29 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 198.55 (C-4), 167.47 (C-7), 164.51 (C-5), 163.53 (C-9), 134.11 (C-4′), 132.29 (C-1′), 128.75 (C-2′, 6′), 128.67 (C-3′, 5′), 100.99 (C-10), 96.25 (C-6), 95.91 (C-8), 84.86 (C-2), 73.10 (C-3). MS (ESI) *m/z*: 306.7 $[M]^+$.

*3,5,7-Trihydroxy-2-(4-isopropylphenyl) chroman-4-one (**D11**)*

White solid; yield: 67%; M.p. 156–157 °C, IR (KBr): ν_{max}/cm^{-1}: 1648 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.94 (1H, s, OH-5), 10.89 (1H, s, OH-7), 7.10–7.25 (2H, m, H-2′, 6′), 6.91–6.99 (2H, m, H-3′, 5′), 6.12 (1H, s, H-8), 5.93 (1H, s, H-6), 5.70 (1H, s, OH-3), 5.01 (1H, d, $J = 12.0$ Hz, H-2), 4.35 (1H, d, $J = 12.0$ Hz, H-3), 2.68 (1H, s, CH_3–C<u>H</u>–CH_3), 1.20 (6H, s, C<u>H</u>$_3$–CH–C<u>H</u>$_3$). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 198.07 (C-4), 167.31 (C-7), 164.30 (C-5), 163.55 (C-9), 148.94 (C-4′), 132.67 (C-1′), 126.06 (C-2′, 6′), 125.56 (C-3′, 5′), 100.06 (C-10), 96.55 (C-6), 96.01 (C-8), 83.04 (C-2), 73.11 (C-3), 33.61 (CH_3–<u>C</u>H–CH_3), 24.00 (<u>C</u>H_3–CH–<u>C</u>H_3). MS (ESI) *m/z*: 314.1 $[M]^+$.

*3,5,7-Trihydroxy-2-(naphthalen-2-yl) chroman-4-one (**D12**)*

White solid; yield: 65%; M.p. 132–133 °C, IR (KBr): ν_{max}/cm^{-1}: 1653 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.00 (1H, s, OH-5), 10.88 (1H, s, OH-7), 7.88–8.01 (3H, m, Ar–H), 714–7.68 (4H, m, Ar–H), 5.99 (1H, s, H-8), 5.88 (1H, s, H-6), 5.72 (1H, s, OH-3), 4.99 (1H, d, $J = 12.0$ Hz, H-2), 4.41 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.48 (C-4), 167.35 (C-7), 163.99 (C-5), 163.44 (C-9), 134.21, 133.02, 132.44, 127.73, 127.42, 126.88, 126.45, 126.11, 125.55, 101.16 (C-10), 96.25 (C-6), 96.12 (C-8), 83.13 (C-2), 73.61 (C-3). MS (ESI) *m/z*: 322.0 $[M]^+$.

*3,5,7-Trihydroxy-2-(pyridin-3-yl) chroman-4-one (**D13**)*

White solid; yield: 52%; M.p. 127–130 °C, IR (KBr): ν_{max}/cm^{-1}: 1652 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.92 (1H, s, OH-5), 10.82 (1H, s, OH-7), 8.80 (1H, s, C-2′), 8.55 (1H, d, $J = 5.2$ Hz, C-4′), 7.95 (1H, d, $J = 8.0$ Hz, C-6′), 7.43 (1H, dd, $J = 5.2$, 8.0 Hz, C-5′), 5.97 (1H, s, H-8), 5.91 (1H, s, H-6), 5.74 (1H, s, OH-3), 5.02 (1H, d, $J = 12.0$ Hz, H-2), 4.52 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.55 (C-4), 167.30 (C-7), 164.19 (C-5), 163.29 (C-9), 148.21 (C-2′), 146.79 (C-4′), 132.84 (C-1′), 131.42 (C-6′), 122.92 (C-5′), 101.21 (C-10),

96.77 (C-6), 96.05 (C-8), 81.21 (C-2), 72.69 (C-3). MS (ESI) *m/z*: 273.0 $[M]^+$.

*3,5,7-Trihydroxy-2-(furan-2-yl) chroman-4-one (**D14**)*

White solid; yield: 58%; M.p. 137–138 °C, IR (KBr): ν_{max}/cm^{-1}: 1651 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.94 (1H, s, OH-5), 10.85 (1H, s, OH-7), 7.46 (1H, s, C-3′), 6.12–6.42 (2H, m, C-4′, 5′), 6.00 (1H, s, H-8), 5.90 (1H, s, H-6), 5.72 (1H, s, OH-3), 4.92 (1H, d, $J = 12.0$ Hz, H-2), 4.52 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 199.51 (C-4), 167.89 (C-7), 164.25 (C-5), 162.29 (C-9), 149.83 (C-1′), 142.12 (C-3′), 113.13 (C-5′), 110.42 (C-4′), 101.22 (C-10), 96.76 (C-6), 95.65 (C-8), 81.17 (C-2), 72.41 (C-3). MS (ESI) *m/z*: 262.2 $[M]^+$.

*3,5,7-Trihydroxy-2-(thiophen-2-yl) chroman-4-one (**D15**)*

White solid; yield: 66%; M.p. 147–138 °C, IR (KBr): ν_{max}/cm^{-1}: 1650 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.90 (1H, s, OH-5), 10.81 (1H, s, OH-7), 7.26 (1H, s, H-3′), 6.13–6.45 (2H, m, H-4′, 5′), 6.04 (1H, s, H-8), 5.95 (1H, s, H-6), 5.69 (1H, s, OH-3), 5.02 (1H, d, $J = 12.0$ Hz, H-2), 4.52 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 199.60 (C-4), 167.31 (C-7), 164.29 (C-5), 162.35 (C-9), 137.53 (C-1′), 126.31 (C-3′), 125.93 (C-5′), 125.34 (C-4′), 101.31 (C-10), 96.72 (C-6), 95.68 (C-8), 79.91 (C-2), 72.59 (C-3). MS (ESI) *m/z*: 278.0 $[M]^+$.

*3-Hydroxy-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D16**)*

White solid; yield: 42%; M.p. 101–104 °C, IR (KBr): ν_{max}/cm^{-1}: 1643 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.05 (1H, s, OH-4′), 9.00 (1H, s, OH-3′), 7.59–7.80 (2H, m, H-5, 7), 7.09–7.11 (2H, m, H-6, 8), 6.80–6.95 (3H, m, H-2′, 3′, 6′), 5.67 (1H, s, OH-3), 5.10 (1H, d, $J = 12.0$ Hz, H-2), 4.60 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 194.24 (C-4), 156.38 (C-9), 145.86 (C-4′), 145.69 (C-3′), 136.49 (C-7), 129.42 (C-1′), 128.38 (C-5), 121.78 (C-10), 120.05 (C-6), 118.27 (C-6′), 118.25 (C-8), 116.58 (C-5′), 115.41 (C-2′), 83.26 (C-2), 73.06 (C-3). MS (ESI) *m/z*: 272.2 $[M]^+$.

*3, 7-Hydroxy-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D17**)*

White solid; yield: 32%; M.p. 189–190 °C, IR (KBr): ν_{max}/cm^{-1}: 1643 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.87 (1H, s, OH-7), 9.12 (1H, s, OH-4′), 9.00 (1H, s, OH-3′), 7.88 (1H, d, $J = 7.6$ Hz, H-5), 6.78–7.01 (4H, m, H-8, 2′, 5′,6′), 6.21 (1H, d, $J = 7.6$ Hz, H-6), 5.77 (1H, s, OH-3), 5.07 (1H, d, $J = 12.0$ Hz, H-2), 4.67 (1H, d, $J =$ 12.0, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 193.02 (C-4), 165.00 (C-9), 161.85 (C-7), 145.86 (C-3′), 145.69 (C-4′), 131.50 (C-5), 129.42 (C-1′), 120.05 (C-6′), 115.58 (C-5′), 115.41 (C-2′), 112.23 (C-10), 112.19 (C-6), 103.70 (C-8), 83.26 (C-2), 73.06 (C-3). MS (ESI) *m/z*: 288.2 $[M]^+$.

*3,5-Trihydroxy-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D18**)*

White solid; yield: 51%; M.p. 201–204 °C, IR (KBr): ν_{max}/cm^{-1}: 1644 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.01 (1H, s, OH-5), 9.11 (1H, s, OH-4′), 8.98 (1H, s, OH-3′), 7.35 (1H, t, H-7), 6.58–7.02 (5H, m, H-6, 8, 2′, 5′,6′), 5.77 (1H, s, OH-3), 5.17 (1H, d, $J = 12.0$ Hz, H-2), 4.54 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 196.76 (C-4), 162.19 (C-9), 157.20 (C-5), 145.86 (C-3′), 145.69 (C-4′), 135.43 (C-7), 129.42 (C-1′), 120.05 (C-6′), 115.58 (C-5′), 115.41 (C-2′), 112.26 (C-8), 111.04 (C-6), 105.45 (C-10), 83.26 (C-2), 72.61 (C-3). MS (ESI) *m/z*: 288.1 $[M]^+$.

*3,5,6,7-Tetrahydroxy-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D19**)*

White solid; yield: 44%; M.p. 254–256 °C, IR (KBr): ν_{max}/cm^{-1}: 1653 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.99 (1H, s, OH-5), 10.89 (1H, s, OH-7), 10.14 (1H, s, OH-6), 9.15 (1H, s, OH-4′), 8.99 (1H, s, OH-3′), 6.89–7.02 (2H, m, H-5′, 6′), 6.52 (1H, d, $J = 2.4$ Hz, H-8), 5.67 (1H, s, OH-3), 5.03 (1H, d, $J = 12.0$ Hz, H-2), 4.50 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 195.93 (C-4), 159.34 (C-9), 154.48 (C-5), 153.94 (C-7), 145.86 (C-4′), 145.69 (C-3′), 132.72 (C-1′), 128.84 (C-6), 120.05 (C-6′), 115.58 (C-5′), 115.41 (C-2′), 101.11 (C-10), 94.85 (C-8), 83.73 (C-2), 72.35 (C-3). MS (ESI) *m/z*: 320.0 $[M]^+$.

*3-Hydroxy-5,7-dimethoxy-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D20**)*

White solid; yield: 51%; M.p. 178–180 °C, IR (KBr): ν_{max}/cm^{-1}: 1642 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.99 (1H, s, OH-4′), 8.90 (1H, s, OH-3′), 6.68–7.00 (3H, m, H-2′, 5′, 6′), 5.95 (1H, d, $J = 2.4$ Hz, H-8), 5.89 (1H, d, $J = 2.4$ Hz, H-6), 5.72 (1H, s, OH-3), 5.03 (1H, d, $J = 12.0$ Hz, H-2), 4.49 (1H, d, $J = 12.0$ Hz, H-3), 3.91 (3H, s, $OC\underline{H}_3$), 3.84 (3H, s, $OC\underline{H}_3$). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 189.70 (C-4), 165.13 (C-7), 163.70 (C-5), 161.70 (C-9), 145.86 (C-4′), 145.69 (C-3′), 128.84 (C-1′), 120.05 (C-6′), 115.58 (C-5′), 115.41 (C-2′), 103.70 (C-10), 93.80 (C-6), 93.10 (C-8), 83.25 (C-2), 72.50 (C-3), 56.13 ($O\underline{C}H_3$), 55.87 ($O\underline{C}H_3$). MS (ESI) *m/z*: 332.3 $[M]^+$.

*3-Hydroxy-7-methoxy-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D21**)*

White solid; yield: 62%; M.p. 152–155 °C, IR (KBr): ν_{max}/cm^{-1}: 1644 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.02 (1H, s, OH-4′), 8.97 (1H, s, OH-3′), 7.75 (1H, d, $J = 7.2$ Hz, H-5), 6.60–6.99 (5H, m, H-6, 8, 2′, 5′, 6′), 5.71 (1H, s, OH-3), 5.04 (1H, d, $J = 12.0$ Hz, H-2), 4.51 (1H, d, $J = 12.0$ Hz, H-3), 3.78 (3H, s, O$\underline{C}H_3$). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 194.22 (C-4), 167.40 (C-7), 162.72 (C-9), 145.88 (C-4′), 145.79 (C-3′), 129.42 (C-5), 129.10 (C-1′), 120.05 (C-6′), 115.59 (C-5′), 115.11 (C-2′), 112.00 (C-10), 111.25 (C-6), 101.25 (C-8), 83.16 (C-2), 73.16 (C-3), 55.64 (O$\underline{C}H_3$). MS (ESI) *m/z*: 302.2 $[M]^+$.

*3-Hydroxy-5-methoxy-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D22**)*

White solid; yield: 58%; M.p. 172–174 °C, IR (KBr): ν_{max}/cm^{-1}: 1645 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.01 (1H, s, OH-4′), 8.96 (1H, s, OH-3′), 7.41 (1H, s, H-7), 6.63–7.01 (5H, m, H-6, 8, 2′, 5′, 6′), 5.70 (1H, s, OH-3), 5.03 (1H, d, $J = 12.0$ Hz, H-2), 4.48 (1H, d, $J = 12.0$ Hz, H-3), 3.91 (3H, s, O$\underline{C}H_3$). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 195.92 (C-4), 159.87 (C-5), 157.04 (C-9), 145.86 (C-3′), 145.69 (C-4′), 135.09 (C-7), 129.42 (C-1′), 120.05 (C-6′), 115.58 (C-5′), 115.41 (C-2′), 113.16 (C-10), 108.88 (C-6), 107.47 (C-8), 83.26 (C-2), 72.61 (C-3), 56.45 (O$\underline{C}H_3$). MS (ESI) *m/z*: 302.0 $[M]^+$.

*3-Hydroxy-7-chloro-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D23**)*

White solid; yield: 68%; M.p. 166–168 °C, IR (KBr): ν_{max}/cm^{-1}: 1641 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.11 (1H, s, OH-4′), 9.00 (1H, s, OH-3′), 7.80 (1H, d, $J = 7.6$ Hz, H-5), 7.13–7.27 (2H, m, H-6, 8), 6.71–6.93 (3H, m, H-2′, 5′, 6′), 5.70 (1H, s, OH-3), 5.06 (1H, d, $J =$ 12.0 Hz, H-2), 4.51 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 195.39 (C-4), 155.33 (C-9), 145.86 (C-4′), 145.69 (C-3′), 135.79 (C-7), 129.42 (C-5), 127.06 (C-1′), 126.96 (C-6), 120.05 (C-6′), 119.76 (C-10), 119.12 (C-8), 115.59 (C-5′), 115.41 (C-2′), 83.26 (C-2), 73.06 (C-3). MS (ESI) *m/z*: 306.4 $[M]^+$.

*2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydroquinolin-4(1*H*)-one (**D24**)*

White solid; yield: 61%; M.p. >300 °C, IR (KBr): ν_{max}/cm^{-1}: 1644 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.85 (1H, s, OH-5), 10.75 (1H, s, OH-7), 9.11 (1H, s, OH-4′), 8.82 (1H, s, OH-3′), 6.68–6.86 (3H, m, H-2′, 5′, 6′), 5.94 (1H, d, $J = 2.4$ Hz, H-8), 5.85 (1H, d, $J = 2.4$ Hz, H-6), 5.79 (1H, s, OH-3), 5.38 (1H, s, NH-1), 4.57 (1H, d, $J =$ 12.0 Hz, H-2), 4.29 (1H, d, $J = 12.0$, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 199.04 (C-4), 164.32 (C-7), 163.88 (C-5), 153.45 (C-9), 145.37 (C-4′), 145.32 (C-3′), 136.30 (C-1′), 120.53 (C-6′), 116.42 (C-5′), 115.41 (C-2′), 100.54 (C-10), 97.27 (C-6), 95.56 (C-8), 72.00 (C-2), 61.41 (C-3). MS (ESI) *m/z*: 303.0 $[M]^+$.

*3,5,7-Trihydroxy-3-methyl-2-(3′,4′-dihydroxyphenyl) chroman-4-one (**D25**)*

White solid; yield: 25%; M.p. 201–202 °C, IR (KBr): ν_{max}/cm^{-1}: 1642 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.99 (1H, s, OH-5), 10.82 (1H, s, OH-7), 9.08 (1H, s, OH-4′), 8.91 (1H, s, OH-3′), 6.75–6.93 (3H, m, H-2′, 5′, 6′), 5.98 (1H, d, $J = 2.4$ Hz, H-8), 5.89 (1H, d, $J = 2.4$ Hz, H-6), 5.67 (1H, s, OH-3), 5.27 (1H, s, H-2), 1.45 (3H, s, C$\underline{H}_3$). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 198.89 (C-4), 166.84 (C-7), 164.20 (C-5), 161.83 (C-9), 147.80 (C-4′), 146.19 (C-3′), 128.05 (C-1′), 121.08 (C-6′), 116.29 (C-5′), 116.20 (C-2′), 102.29 (C-10), 96.52 (C-6), 95.26 (C-8), 87.20 (C-2), 76.59 (C-3), 19.47 (C$\underline{H}_3$). MS (ESI) *m/z*: 318.2 $[M]^+$.

*3-Hydroxy-5,7-dimethoxy-2-(3,4-dimethoxyphenyl) chroman-4-one (**D26**)*

White solid; yield: 75%; M.p. 209–210 °C, IR (KBr): ν_{max}/cm^{-1}: 1646 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 6.81–7.04 (3H, m, H-2′, 5′, 6′), 5.97 (1H, d, $J = 2.4$ Hz, H-8), 5.88 (1H, d, $J = 2.4$ Hz, H-6), 5.81 (1H, s, OH-3), 4.99 (1H, d, $J = 12.0$ Hz, H-2), 4.47 (1H, d, $J = 12.0$ Hz, H-3), 3.81–3.90 (12H, m, OC$\underline{H}_3$). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 189.70 (C-4), 165.13 (C-7), 163.70 (C-5), 161.70 (C-9), 148.66 (C-4′), 148.21 (C-3′), 129.50 (C-1′), 120.57 (C-6′), 111.15 (C-5′), 111.05 (C-2′), 103.70 (C-10), 93.80 (C-6), 93.10 (C-8), 83.60 (C-2), 72.50 (C-3), 56.13 (O$\underline{C}H_3$), 56.03 (O$\underline{C}H_3$), 55.95 (O$\underline{C}H_3$), 55.87 (O$\underline{C}H_3$). MS (ESI) *m/z*: 360.3 $[M]^+$.

*3-Hydroxy-2-phenylchroman-4-one (**D27**)*

White solid; yield: 75%; M.p. 186–188 °C, IR (KBr): ν_{max}/cm^{-1}: 1643 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.92–7.94 (1H, m, H-7), 7.50–7.58 (6H, m, H-5, 2′, 3′, 4′, 5′, 6′), 7.08–7.18 (2H, s, H-6, 8), 5.13 (1H, d, $J = 12.0$ Hz, H-2), 4.62 (1H, d, $J = 12.0$ Hz, H-3), 3.69 (1H, s, OH-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 194.23 (C-4), 161.74 (C-9), 136.92 (C-1′), 136.36 (C-7), 129.30 (C-3′, 5′), 128.69 (C-4′), 127.57 (C-5), 127.35 (C-2′, 6′), 122.11 (C-10), 118.14 (C-6), 118.75 (C-8), 83.91 (C-2), 73.67 (C-3). MS (ESI) *m/z*: 340.2 $[M]^+$.

*3,5,7-Trihydroxy-2-(pyridin-3-yl)-2,3-dihydroquinolin-4 (1H)-one (**D28**)*

White solid; yield: 42%; M.p. 188–190 °C, IR (KBr): ν_{max}/cm^{-1}: 1647 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.89 (1H, s, OH-5), 10.91 (1H, s, OH-7), 9.10 (1H, s, OH-4′), 8.95 (1H, s, OH-3′), 7.35–8.51 (3H, m, H-2′, 5′, 6′), 5.92 (1H, d, $J = 2.4$ Hz, H-8), 5.86 (1H, d, $J = 2.4$ Hz, H-6), 5.74 (1H, s, OH-3), 5.28 (1H, s, NH), 4.80 (1H, d, $J = 12.0$ Hz, H-2), 4.32 (1H, d, $J = 12.0$, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 199.04 (C-4), 164.32 (C-7), 163.88 (C-5), 153.45 (C-9), 145.37 (C-2′), 145.32 (C-4′), 136.30 (C-1′), 120.53 (C-6′), 116.42 (C-5′), 100.54 (C-10), 97.27 (C-6), 95.56 (C-8), 72.00 (C-2), 61.41 (C-3). MS (ESI) *m/z*: 272.2 $[M]^+$.

*2-(3,4-Dihydroxyphenyl)-3-hydroxy-2,3-dihydroquinolin-4 (1H)-one (**D29**)*

White solid; yield: 52%; M.p. 225–228 °C, IR (KBr): ν_{max}/cm^{-1}: 1645 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.01 (1H, s, OH-4′), 9.05 (1H, s, OH-3′), 6.68–7.40 (7H, m, H-5, 6, 7, 8, 2′, 5′, 6′), 5.89 (1H, s, OH-3), 4.74 (1H, d, $J = 12.0$ Hz, H-2), 4.32 (1H, d, $J = 12.0$ Hz, H-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 195.89 (C-4), 148.73 (C-9), 145.37 (C-4′), 145.32 (C-3′), 136.30 (C-1′), 133.30 (C-7), 128.79 (C-5), 120.67 (C-6′), 120.53 (C-6), 118.95 (C-8), 116.57 (C-5′), 116.42 (C-10), 115.41 (C-2′), 71.98 (C-2), 61.41 (C-3). MS (ESI) *m/z*: 271.2 $[M]^+$.

*3-Hydroxy-2-phenyl-2,3-dihydroquinolin-4(1H)-one (**D30**)*

White solid; yield: 71%; M.p. 158–160 °C, IR (KBr): ν_{max}/cm^{-1}: 1640 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.40–7.68 (4H, m, H-5, 7, 3′, 5′), 6.85–7.28 (5H, m, H-6, 8, 2′, 4′, 6′), 4.57 (1H, d, $J = 12.0$ Hz, H-2), 4.29 (1H, d, $J = 12.0$ Hz, H-3), 3.61 (1H, s, OH-3). ^{1}C NMR (100 MHz, DMSO-d_6) δ ppm: 195.89 (C-4), 148.73 (C-9), 138.13 (C-1′), 133.30 (C-7), 128.81 (C-3′, 5′), 128.15 (C-4′), 127.51 (C-5), 120.67 (C-2′, 6′), 118.95 (C-10), 116.54 (C-6), 115.75 (C-8), 71.98 (C-2), 60.76 (C-3). MS (ESI) *m/z*: 239.0 $[M]^+$.

Biological assays

Cell cultures

The RAW 264.7 macrophage cells were obtained from Cell Bank of Academy of Sciences in Huazhong University of Science and Technology (China). The macrophage cells were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Bovine, China), 100 U/ml of penicillin (Gibco), and 100 mg/ml of streptomycin (Gibco). Cell cultures were maintained at an atmosphere of 5% CO_2 humidified incubator at 37 °C.

Cell viability assay

The cytotoxicity of the tested compounds was evaluated by MTT (Sigma, USA) colorimetric assay (Hanelt et al. 1994). RAW 264.7 cells at 5×10^3 per well were seeded in 96-well plates (Corning, NY, USA), and the testing compounds at seven concentrations (320, 160, 80, 40, 20, 10, and 5 μM) were incorporated into the cell culture in DMEM medium in humidified incubator within a 5% CO_2 atmosphere at 37 °C for 24 h. Then, 20 μl MTT solutions (5 mg/ml) were added to each well for another 4 h. The resulting crystals were dissolved in DMSO. The optical density was measured at 490 nm. The cytotoxicity was calculated from the plotted results using untreated cells at 100%.

In vitro anti-inflammatory assay

Pro-inflammatory cytokine production by LPS in the RAW 264.7 cells was measured according to the method described by Sudsai et al. (2014) and Wang et al. (2016). The RAW 264.7 cells were stimulated with LPS (1 μg/ml) for 22 h. Then, the tested compounds in 0.1% DMSO were given and the concentration was adjusted to 20 μM with the medium for 24 h. The culture media were collected and centrifuged (1000 rpm) at 4 °C for 10 min, and the supernatant was collected. IL-1β, IL-6, and TNF-a levels in the medium were determined with an ELISA kit (eBioScience, Inc.) according to the manufacturer's instructions.

Result and discussion

Chemistry

The dihydroflavonol derivatives described in this paper were prepared according to the synthetic route in Scheme 1. In general, the 2′-hydroxyl chalcone derivatives were synthesized via Claisen–Schmidt reaction (Sui et al. 2012; Guan et al. 2013; Sun et al. 2012). According to Scheme 1, treatment of 2′-hydroxyl chalcone derivatives with 30% H_2O_2 and diethylamine in tetrahydrofuran under −5 °C, afforded a white crystalline product identified as dihydroflavonol derivatives (**D1–D30** and **TA-SY**). The infrared (IR) spectrum of compounds **D1–D30** and **TA-SY** which showed sharp peak at around 1650 cm^{-1} indicates the presence of ketone (–C=O) functional group (4-position) of C ring. NMR spectroscopy was very important for the identification of flavonoids. NMR datas of the target compounds (**D1–D30** and **TA-SY**) structure confirmed by the presence

of 2, 3-position position of C ring. ^{1}H NMR spectrum displayed one double doublet at δH = 4.50 ppm (1H, d, H-3) and one doublet at δH = 5.00 ppm (1H, d, H-2). The value of coupling constant (J = 12.0 Hz) for H-2 and H-3 protons indicate that the compounds exist in the E-configuration, which is in complete agreement with the results recentl reported (Slade et al. 2005). Moreover, all the aromatic protons were observed at 8.10–5.80 ppm. ^{13}C NMR spectral assigned signals in the range at near δ 80.00 ppm and 70.00 ppm due to presence of C-2 and C-3 of C ring (Pelter et al. 1976). Mass spectroscopy helps to find the molecular weight of the synthesized compounds. The dihydroflavonol derivatives showed the molecular ion peak that equivalent to the molecular weight of proposed compound. The synthesized compounds were supported by spectral data. The IR, ^{1}H NMR, ^{13}C NMR, and MS results are consistent with the proposed structures.

Cell viability

MTT assay was used to screen the influences of dihydroflavonol derivatives on the viability of RAW 264.7 cells. Seven concentrations (320, 160, 80, 40, 20, 10, and 5 μM) of Taxifolin and dihydroflavonol derivatives and the stimulator LPS (1 μg/ml) were choosed to determine the cell viability. As shown in Fig. 3, there was no significant difference between the control group (normal) and all the treated groups. It indicated that all the compounds were no significant proliferation effects on RAW 264.7 cells at the concentration 20 μM. Thus, we selected the concentration 20 μM and 1 μg/ml of LPS as the effective concentration in the next experiments.

Anti-inflammatory activity

The precision of the assays for IL-1 beta, IL-6, and TNF alpha is mainly studied by plotting standard curves. According to the manufacturer's instructions, we take the standard density (standard curves use the following concentrations: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/ml) as the abscissa, the OD value for the vertical, draw the standard curve. Finally, the linear correlations R^2 between the sample linear regression and the expected concentration coefficient were more than 0.995. This shows that the kits performance is excellent according to the kits instructions. Therefore, the data determined later is feasible. Having secured a series of structurally diverse dihydroflavonol derivatives, next their anti-inflammatory activity was evaluated. The results of the anti-inflammatory are collected in Figs. 4, 5, and 6 respectively, Meloxicam was used as the positive control. Taxifolin is a natural compound with a single configuration, and **TA-SY** with similar structure of taxifolin is only a synthetic dihydroflavonol compound. So we mainly wanted to investigate whether different configurations of dihydroflavonol have an impact on their anti-inflammatory activity. In Fig. 4, the results revealed that there was no significant difference in the anti-inflammatory activity between the natural product (taxifolin) and synthetic product (**TA-SY**). Among all the compounds screened, Meloxicam, compounds **D9**, **D13**, and **D19** showed better inhibitory effect than Taxifolin on LPS-induce IL-1β, IL-6, and TNF-α in Fig. 5; however, compound **D12** substituted by naphthaline showed the lowest effect on LPS-induce IL-1β, IL-6, and TNF-α, which may be due to the bad solubility.

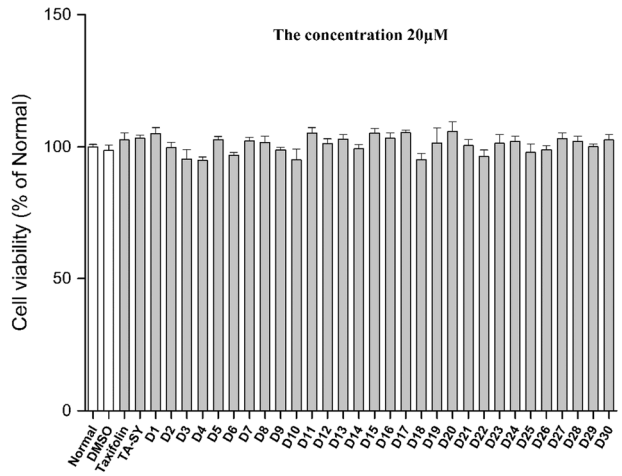


Fig. 3 Cell viability of Taxifolin and dihydroflavonol derivatives on RAW 264.7 cells at 20 μM. Cell viability was determined for Taxifolin and tested compounds (**TA-SY** and **D1–D30**) at 20 μM. The values are mean ± SEM (n = 3)

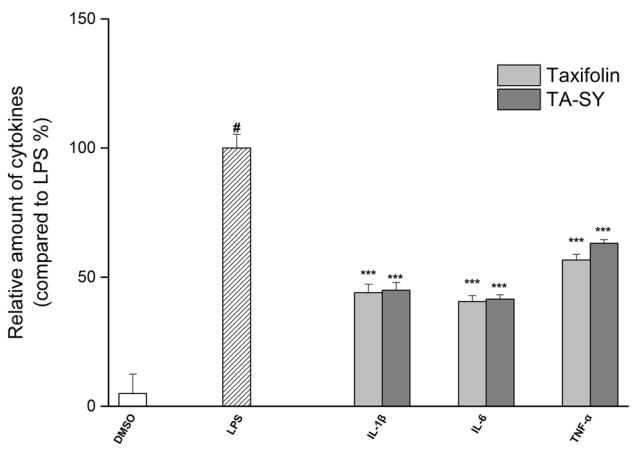


Fig. 4 Effects of Taxifolin and TA-SY on IL-1β, IL-6 and TNF-α production at 20 μM. Values represent the mean ± SD for three independent experiments. Statistical significance relative to the DMSO group was indicated, #$p < 0.05$. Statistical significance relative to the LPS group was indicated, ***$p < 0.001$

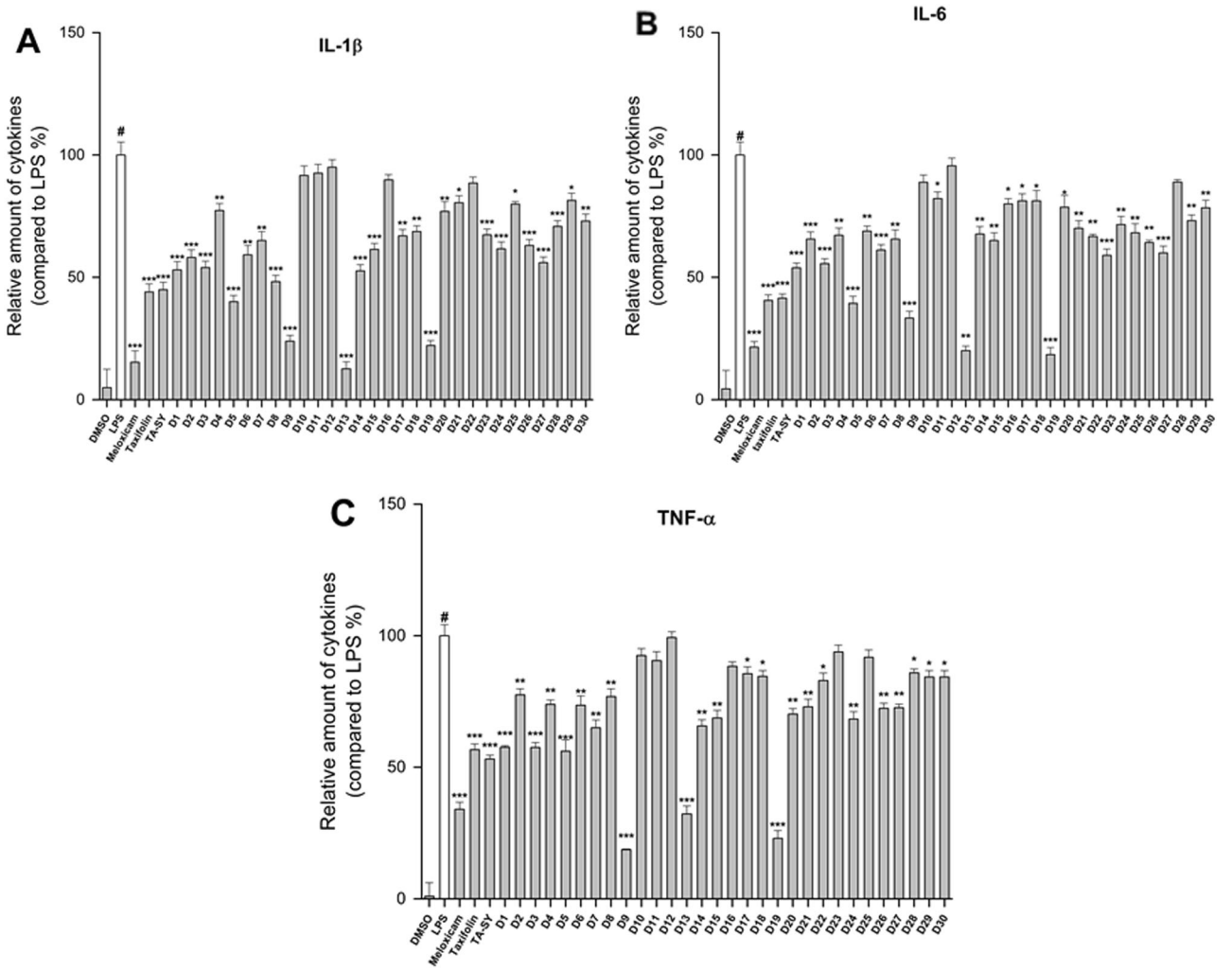


Fig. 5 Effects of dihydroflavonol derivatives on IL-1β (**a**), IL-6 (**b**), and TNF-α (**c**) production at 20 μM. Values represent the mean ± SD for three independent experiments. Statistical significance relative to the DMSO group was indicated, #$p < 0.05$. Statistical significance relative to the LPS group was indicated, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

As shown in the Fig. 6, There were significant differences between the low dose groups and the high dose groups ($p < 0.05$). However, there were no significant differences between the high dose groups and the medium dose groups. At the same time, the relationship between the medium dose groups and the low dose groups were very complicated. For example, for compounds **D9**, it had an inhibitory effect on inflammatory factors (IL-1β and TNF-α), where there was a significant difference between the medium dose group and the low dose group ($p < 0.05$). However, there was no significant difference between the medium dose group and the low dose group in the suppression of inflammatory factor (IL-6). From the above results, we analyzed that it was noteworthy that compounds **D9**, **D13**, and **D19** decreased obviously the content of IL-1β, IL-6, and TNF-α in a concentration-dependent manner.

The inhibitory activity of dihydroflavonols against pro-inflammatory cytokines production in inflammatory cells had a close relationship with 3′- and or 4′-substituted B-ring (Jiang et al. 2015). In comparison of functional group effects at 3′- or 4′-position of B-ring for inflammatory cytokines inhibition, the rank of the orders of the inhibitory activities for dihydroflavonol derivatives was fluoro (**D9**) > phenolic hydroxyl (**D1**) > methoxy (**D6**) > isopropyl (**D11**) for 4′-substituent, and phenolic hydroxyl (**D2**) = methoxy (**D7**) for 3/- substituent, respectively. Compared to the inhibitory activities of inflammatory cytokines on B-ring, pyridine ring (**D13**) showed more potent activity than that of several B-ring analogs (**D4**, **D12**, **D14**, and **D15**). SAR study around 5- and/or 7-substituted A-ring gave unique results as well. Compared to the inhibitory activities of inflammatory cytokines for phenolic hydroxyl substituent on A-ring, 5, 6, 7-phenolic hydroxyl substituent on A-ring (**D19**) showed more potent than other analogs. However, SAR study around 1- and 3-substituted C-ring gave bad results (**D24**, **D25**), which suggested that the C-ring is a

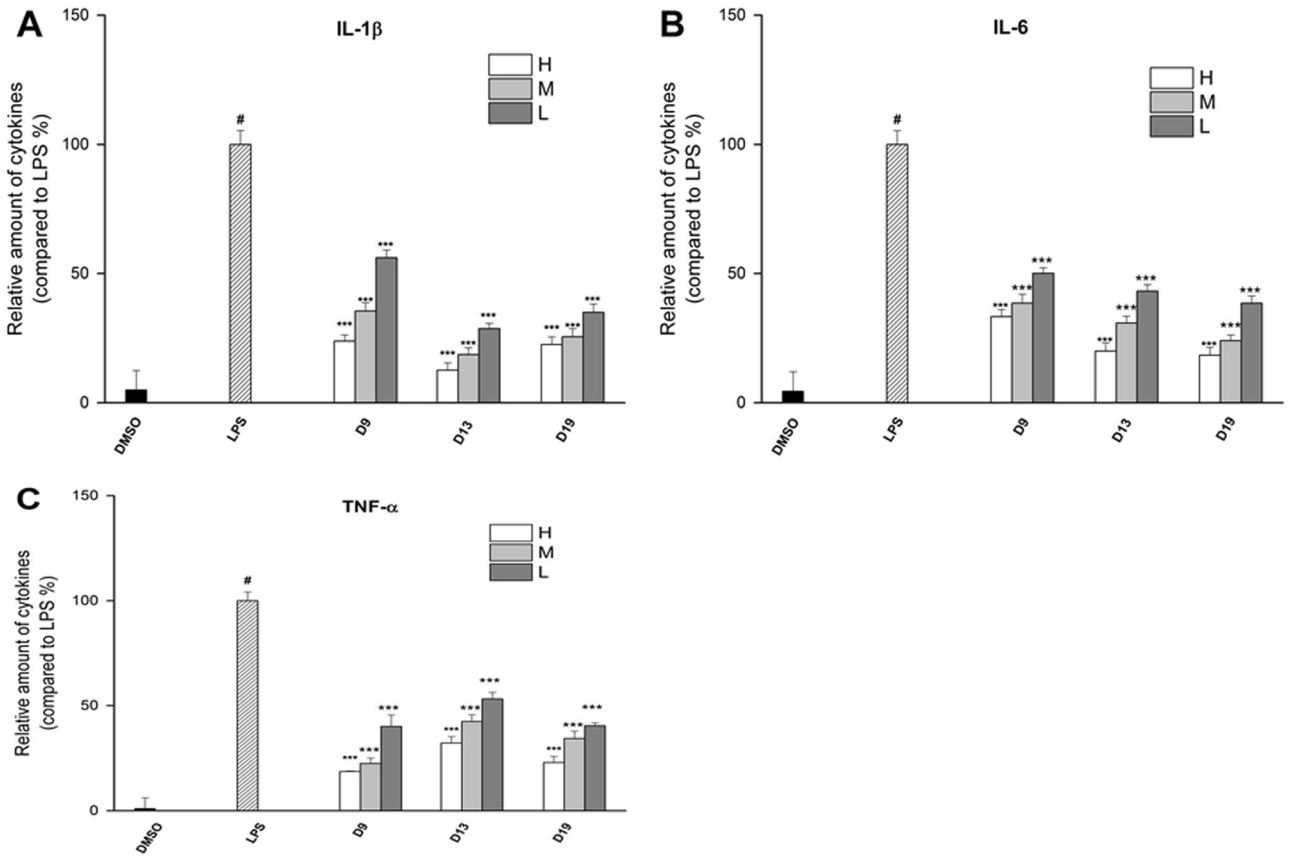


Fig. 6 Compounds **D9**, **D13**, and **D19** inhibited LPS-induced IL-1β (**a**), IL-6 (**b**), and TNF-α (**c**) release in a dose-dependent manner. Values represent the mean ± SD for three independent experiments. Statistical significance relative to the DMSO group was indicated, #$p < 0.05$. Statistical significance relative to the LPS group was indicated, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

necessary structure for its anti-inflammatory activity. With the above analysis, the various substituent-effects toward potent dihydroflavonol derivatives clarified in the present SAR study in vitro might be useful for designing further dihydroflavonol anti-inflammatory drugs.

Conclusions

In conclusion, thirty dihydroflavonol derivatives (**D1–D30**) had been designed and synthesized by the new method, and their anti-inflammatory activities were evaluated. Besides, their structure–activity relationships were discussed preliminarily. The results showed that the A ring, B ring, and C ring substituted by different group exhibited different anti-inflammatory activities. When the 4′-position of B-ring substituted by the fluorine atom or the B-ring was pyridine ring, the anti-inflammatory activity of compounds **D9**, **D13** were increased. With the amounts of hydroxyl group substituted on A-ring increasing, the anti-inflammatory activity of compound **D19** was better. Among these derivatives, compounds **D9**, **D13**, and **D19** showed impressive anti-inflammatory activities at micro molar concentration, which was like positive control and exhibited an obvious dose–effect relationship. In summary, compounds **D9**, **D13**, and **D19** may present a potential therapeutical use as an anti-inflammatory agent, being also useful as a precursor of new flavonoids endowed with such activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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