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•Research article•

Synthesis, and anti-inflammatory activities of gentiopicroside derivatives

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[ABSTRACT] A series of 26 novel derivatives have been synthesized through structural modification of gentiopicroside, a lead COX-2 inhibitor. And their *in vivo* and *in vitro* anti-inflammatory activities have been investigated. The *in vitro* anti-inflammatory activities were evaluated against NO, PGE_2, and IL-6 production in the mouse macrophage cell line RAW264.7 stimulated by LPS. Results showed that most compounds had good inhibitory activity. The *in vivo* inhibitory activities were further tested against xylene-induced mouse ear swelling. Results demonstrated that several compounds were more active than the parent compound gentiopicroside. The inhibition rate of the most active compound P23 (57.26%) was higher than positive control drug celecoxib (46.05%) at dose 0.28 $mmol \cdot kg^{-1}$. Molecular docking suggested that these compounds might bind to COX-2 and iNOS. Some of them, *e.g* P7, P14, P16, P21, P23, and P24, had high docking scores in accordance with their potency of the anti-inflammatory activitiy, that downregulation of the inflammatory factors, NO, PGE_2, and IL-6, was possibly associated with the suppression of iNOS and COX-2. Therefore, these gentiopicroside derivatives may represent a novel class of COX-2 and iNOS inhibitors.

[KEY WORDS] Gentiopicroside derivatives; Structural modification; Anti-inflammatory activity; Selective inhibitors
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Introduction

Inflammatory diseases, including osteoarthritis (OA) and rheumatoid arthritis (RA), are common clinical diseases in the world, which seriously threaten the health of human beings and have an increasing incidence [1-3]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are first-line drugs to suppress inflammation, which mainly include cyclooxygenase-2 (COX-2) selective inhibitors, for example, celecoxib [3]. However, NSAIDs were found to increase the risk of gastrointestinal and cardiovascular diseases [2-4]. Therefore, it is of great significance to develope safer and more efficacious anti-inflammatory drugs. One of the main causes of inflammation is excessive secretion of inflammatory cytokines, such as prostaglandin E2 (PGE_2), nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) [3-5]. Among all of proinflammatory mediators, NO and PGE_2 are two of the most critical chemokines for inflammation. Studies found that NO inhibitors may be considered as potential anti-inflammatory agents for inflammatory diseases. The overproduction of PGE_2 and NO is closely related to the overexpression of COX-2 and iNOS [3-6]. Inducible nitric oxide synthase (iNOS) and COX-2 are two main areas of interest in current inflammation research and treatment. The production of inflammatory cytokines NO and PGE_2 is controlled by inhibiting their activity [3, 7-9]. Thus, the study of selective iNOS and COX-2 inhibitors should be paid enough attention for the treatment of inflammation [3, 4].

Natural product gentiopicroside is an iridoid glycoside with significant anti-inflammatory and analgesic effect. It can protect liver, clear heat, lower blood pressure, and exhibit antioxidative and antitumor activities [3, 10, 11]. Moreover, gentiopicroside is safe and nontoxic [12]. Our research group found that gentiopicroside is also a novel selective inhibitor of COX-2 and iNOS [3, 13]. But it is highly hydrophilic due to the presence of the sugar fragment in its structure (Fig. 1)

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These authors have no conflict of interest to declare.

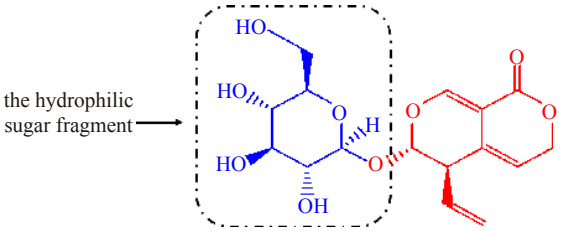


Fig. 1 The structure of the gentiopicroside

offered [14, 15], thereby with a reduced oral bioavailability, a fast metabolism, a short bilogical half-life, and a limited efficacy [10, 11]. Therefore, we attempted to reduce polarity and keep its biological activity at the same time. In this study, we introduced hydrophobic cyclic acetals into the structure of gentiopicroside to reduce its hydrophilicity and enhance its lipophilicity, aiming to obtain some new compounds with excellent anti-inflammatory activity.

Material and Methods

Materials and reagents

Gentiana officinalis H. Smith was collected from Dingxi, Gansu province of China, and identified by Dr. JIN Ling from Gansu University of Chinese Medicine. Gentiopicroside was isolated from *Gentiana officinalis* H. Smith by our group [3]. Benzaldehyde, 4-Chlorobenzaldehyde, 2,4-Dichlorobenzaldehyde, Anisic aldehyde, and other raw materials were purchased from Energy Chemical. Chloroform-*d* and Methanol-*d*4 were purchased from Armar Marchemicals. The mouse macrophage RAW 264.7 was purchased from Procell Life Science and Technology. MTT, LPS, and other biological reagents were purchased from Sigma-Aldrich. Griess assays (using determined NO), PGE_2, and IL-6 ELISA Kits were purchased from Neobioscience.

Animals

KM mice [SPF, weighing (20 ± 2) g] were provided by the Laboratory Animal Center of Gansu University of Chinese Medicine (SCXK2011-0001, Lanzhou, China). The mice were housed under controlled conditions (relative humidity of 60% ± 5% at temperature 25 ± 2 °C) on a 12/12 h day/night cycle. Standard laboratory food and water were provided. All animal experiments were approved by the Institutional Animal Care and Use Committee of Gansu University of Chinese Medicine. The mice were subjected to fasting for at least 12 h before the experiments and had free access to water [3].

Synthesis of gentiopicroside derivatives

26 derivatives of gentiopicroside (Fig. 3) were synthesized according to the procedure shown in Fig. 2. Gentiopicroside 1 mmol, aldehyde (2.0 equiv.), appropriate p-toluenesulfonic acid, were added to a 50 mL two-mouth bottle and sealed, replaced the air with N_2 for three times, then added CH_3CN 25 mL. The mixture was heated, stirred reflux. The reaction process detected by the thin layer chromatography (TLC) until it was completed. Finally, filtrated, evaporated the solvent and the compounds was separated and purified by column chromatography ($CHCl_3$: Me_2CO = 3 : 1). The structures of the newly sythesized compounds were characterized by NMR, MS and IR. Their hydrophilicities were preliminarily judged by the TLC relative shift values. All derivatives were found to exhibit lower polarities than the parent compound gentiopicroside.

In vitro biological evaluation

All newly synthesized compounds showed no cytotoxicity within 100 $\mu g \cdot mL^{-1}$ in the MTT cell viability assay. Their *in vitro* inflammatory activity at 100, 50, and 25 $\mu g \cdot mL^{-1}$ were evaluated against the release of inflammatory factors NO, PGE_2, and IL-6 by the lipopolysaccharide (LPS)-stimulated mouse macrophage cell line RAW264.7. The cells were pretreated with compounds (100, 50, and 25 $\mu g \cdot mL^{-1}$) for 2 h except the normal and model groups, respectively, and then stimulated with LPS (1 $\mu g \cdot mL^{-1}$) for 24 h except the normal group, then the culture medium was collected. The levels of NO were determined by the Griess assay, and the levels of PGE_2 and IL-6 were by ELISA kits. Their values were expressed as mean ± SD.

In vivo biological evaluation

The KM mice were divided randomly into 29 groups: control group, celecoxib group, gentiopicroside group, and 26 dose groups, with ten mice each group. Mice in each group were administered with the doses 0.28 $mmol \cdot kg^{-1}$ for 7 days. On the last day, each animal received 0.1 mL of xylene on the anterior and posterior surfaces of the right ear. The left ear was considered as control. After 15 min, the animals were killed by cervical dislocation and both ears were sampled. Circular sections were taken using a cork borer with a diameter of 9 mm and weighed. The degree of ear swelling was calculated based on the weight of the left ear without receiving xylene.

Molecular docking

Crystal structures of COX-2 (PDB ID: 5ikr) and iNOS

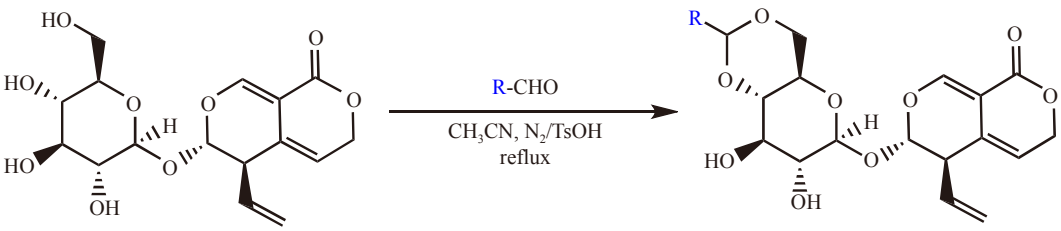
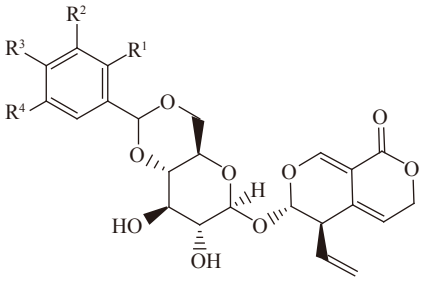


Fig. 2 Synthesis of gentiopicroside derivatives



P1: $R^1 = R^2 = R^3 = R^4 = H$;	**P2**: $R^1 = R^2 = R^4 = H$, $R^3 = OCH_3$;
P3: $R^1 = R^2 = R^4 = H$, $R^3 = Cl$;	**P4**: $R^1 = R^2 = R^4 = H$, $R^3 = NO_2$;
P7: $R^1 = R^4 = H$, $R^2 = F$, $R^3 = OCH_3$;	**P8**: $R^1 = R^4 = H$, $R^2 = R^3 = CH_3$;
P9: $R^1 = R^3 = Cl$, $R^2 = R^4 = H$;	**P10**: $R^1 = R^3 = NO_2$, $R^2 = R^4 = H$;
P11: $R^1 = H$, $R^2 = R^3 = R^4 = OCH_3$;	**P13**: $R^1 = R^2 = R^4 = H$, $R^3 = F$;
P14: $R^1 = R^2 = R^4 = H$, $R^3 = Br$;	**P15**: $R^1 = R^2 = R^4 = H$, $R^3 = CF_3$;
P16: $R^1 = R^2 = R^4 = H$, $R^3 = OCF_3$;	**P17**: $R^1 = R^2 = R^4 = H$, $R^3 = CH_3$;
P20: $R^1 = R^3 = R^4 = H$, $R^2 = F$;	**P21**: $R^1 = R^4 = H$, $R^2 = CF_3$, $R^3 = F$;
P22: $R^1 = R^4 = H$, $R^2 = Br$, $R^3 = F$;	**P23**: $R^1 = R^2 = R^4 = H$, $R^3 = OCHF_2$;
P24: $R^1 = F$, $R^2 = R^3 = H$, $R^4 = Br$;	**P25**: $R^1 = R^3 = H$, $R^2 = R^4 = F$;

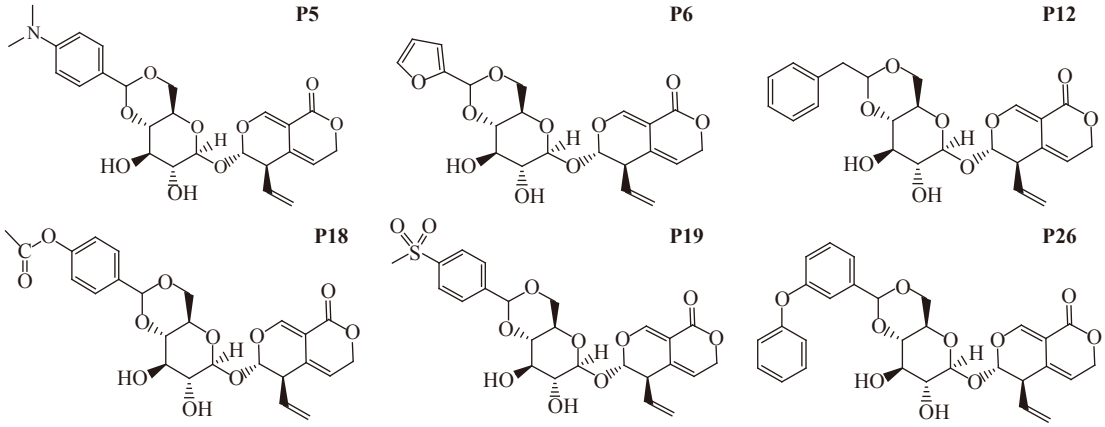


Fig. 3 Structures of gentiopicroside derivatives

(PDB ID: 3e7g) were retrieved from the PDB database. The docking operation was performed in the schrodinger software. The 3D-structures of 26 compounds were generated using the Quantum Mechanics module and docked into the structures of COX-2 and iNOS with the Induced Fit Docking method.

Results and discussion

Chemistry

Compound **P14** was obtained as a white needle crystal in 38.1% yield, mp 134−136 °C. HR-ESI-MS *m/z*: 545.0419 $[M + Na]^+$, Calcd. 545.0418 for $C_{23}H_{23}O_9BrNa$. IR (KBr, *v* in cm^{-1}): 3434.12, 2881.29, 1716.99, 1610.23, 1490.17, 1379.63, and 1272.36. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 7.50 (1H, overlaps, H-3), 7.49 (2H, d, *J* = 8.4 Hz, H-4" and H-6"), 7.37 (2H, d, *J* = 8.4 Hz, H-3" and H-7"), 5.65 (1H, m, H-8), 5.50 (1H, s, H-6), 5.40 (1H, d, *J* = 3.3 Hz, H-1), 5.26 (1H, s, H-10b), 5.23 (1H, d, *J* = 6.8 Hz, H-10a), 5.07 (1H, d, *J* = 17.4 Hz, H-7a), 4.95 (1H, d, *J* = 17.4 Hz, H-7b), 4.74 (1H, d, *J* = 7.8 Hz, H-1'), 4.31 (1H, dd, *J* = 10.4, 4.8 Hz, H-6'a), 3.76 (1H, overlaps, H-3'), 3.70 (1H, d, *J* = 10.4 Hz, H-6'b), 3.48 (1H, t, *J* = 9.3 Hz, H-4'), 3.39 (1H, overlaps, H-5'), 3.42 (1H, overlaps, H-2'), and 3.27 (1H, d, *J* = 6.8 Hz, H-9). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 163.9 (C-11), 149.4 (C-3), 132.8 (C-8), 125.6 (C-5), 119.0 (C-10), 115.9 (C-6), 103.8 (C-4), 97.4 (C-1), 69.5 (C-7), 45.3 (C-9), 136.0 (C-2"), 131.4 (C-4"), 131.4 (C-6"), 128.2 (C-3"), 128.2 (C-7"), 123.3 (C-5"), 101.0 (C-1"), 99.3 (C-1'), 80.0 (C-4'), 73.9 (C-2'), 73.0 (C-3'), 68.4 (C-6') and 66.5 (C-5'). The ^1H-^1H COSY and HMBC spectra (Fig. 4) revealed the relationships as shown in Fig. 5.

Compound **P1** was obtained as a colorless powder in 40.5% yield, mp 132−134 °C. HR-ESI-MS *m/z*: 467.1302 $[M + Na]^+$, Calcd. 467.1313 for $C_{23}H_{24}O_9Na$. IR (KBr, *v* in cm^{-1}): 3435.02, 2917.64, 1719.90, 1610.48, 1071.38. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.34 (1H, d, *J* = 3.3 Hz, H-1), 7.45 (1H, overlaps, H-3), 5.42 (1H, d, *J* = 9.0 Hz, H-6), 4.98 (1H, d, *J* = 17.0 Hz, H-7a), 4.86 (1H, d, *J* = 15.8 Hz, H-7b), 5.57 (1H, dd, *J* = 7.6, 9.7 Hz, H-8), 3.21 (1H, d, *J* = 4.8 Hz, H-9), 5.18 (1H, overlaps, H-10), 4.67 (1H,

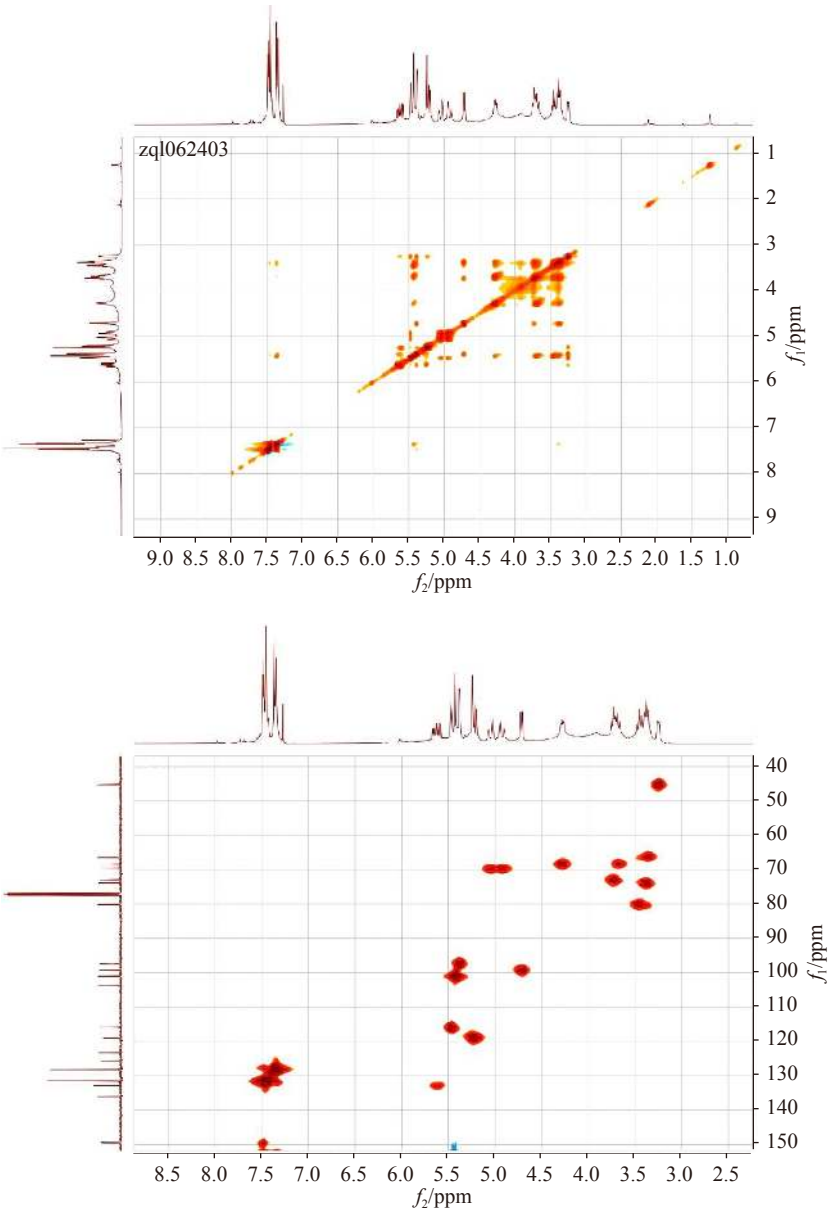


Fig. 4 The ^{1}H-^{1}H COSY and HMBC spectra of compound P14

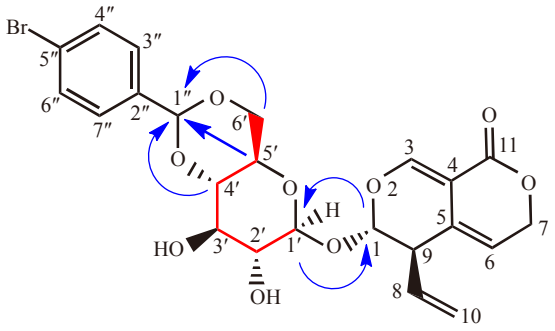


Fig. 5 The structure of compound P14, ^{1}H-^{1}H COSY(–), HMBC(H→C)

d, *J* = 7.8 Hz, H-1′), 3.42 (1H, overlaps, H-2′), 3.65 (1H, overlaps, H-3′), 3.40 (1H, overlaps, H-4′), 3.37 (1H, overlaps, H-5′), 4.23 (1H, dd, *J* = 4.5, 10.2 Hz, H-6′a), 3.66 (1H, dd, *J* = 9.0, 17.0 Hz, H-6′b), 7.30 (2H, d, *J* = 3.5 Hz, H-3″and H-7″), 7.30 (2H, d, *J* = 3.5 Hz, H-4″and H-6″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.5 (C-3), 103.7 (C-4), 126.4 (C-5), 115.8 (C-6), 69.5 (C-7), 132.9 (C-8), 45.2 (C-9), 118.9 (C-10), 163.9 (C-11), 99.3 (C-1′), 73.8 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 101.7 (C-1″), 137.1 (C-2″), 128.3 (C-3″), 129.2 (C-4″), 125.6 (C-5″), 129.2 (C-6″), 128.3 (C-7″).

Compound **P2** was obtained as a colorless powder in 36.0% yield, mp 139−141 °C. HR-ESI-MS *m/z*: 475.1601 $[M + H]^+$, Calcd. 475.1599 for $C_{24}H_{26}O_{10}H$. IR (KBr, *v* in cm^{-1}): 3432.70, 2883.52, 1716.20, 1612.20, 1518.62, 1383.79, 1251.55. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.39 (1H, d, *J* = 3.4 Hz, H-1), 7.48 (1H, overlaps, H-3),

5.48 (1H, s, H-6), 4.97 (1H, m, H-7), 5.63 (1H, m, H-8), 3.25 (1H, d, *J* = 4.5 Hz, H-9), 5.22 (1H, m, H-10), 4.72 (1H, d, *J* = 7.8 Hz, H-1′), 3.43 (1H, m, H-2′), 3.87 (1H, s, H-3′), 3.72 (1H, m, H-4′), 3.39 (1H, overlaps, H-5′), 4.27 (1H, dd, *J* = 10.4, 4.8 Hz, H-6′a), 3.72 (1H, m, H-6′b), 7.39 (2H, d, *J* = 8.7 Hz, H-3″and H-7″), 6.86 (2H, *J* = 8.7 Hz, H-4″and H-6″), 3.75 (3H, s, OCH_3).[13]C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.3 (C-3), 103.8 (C-4), 125.7 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 119.0 (C-10), 163.8 (C-11), 99.2 (C-1′), 73.9 (C-2′), 73.1 (C-3′), 80.1 (C-4′), 66.6 (C-5′), 68.4 (C-6′), 101.8 (C-1″), 160.2 (C-2″), 113.7 (C-3″), 127.7 (C-4″), 129.4 (C-5″), 127.7 (C-6″), 113.7 (C-7″), 55.3 (C-8″).

Compound **P3** was obtained as a light yellow powder in 44.9% yield, mp 140−142 °C. HR-ESI-MS *m/z*: 501.0926 $[M + Na]^+$, Calcd. 501.0923 for $C_{23}H_{23}ClO_9Na$. IR (KBr, ν in cm^{-1}): 3434.76, 2881.32, 1716.36, 1609.68, 1494.42, 1380.67, 1272.82. [1]H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.47 (1H, m, H-1), 7.49 (1H, m, H-3), 5.50 (1H, s, H-6), 5.02 (1H, m, H-7), 5.66 (1H, m, H-8), 3.28 (1H, d, *J* = 5.8 Hz, H-9), 5.26 (1H, m, H-10), 4.78 (1H, d, *J* = 7.8 Hz, H-1′), 3.42 (1H, overlaps, H-2′), 3.76 (1H, overlaps, H-3′), 3.48 (1H, m, H-4′), 3.39 (1H, overlaps, H-5′), 4.33 (1H, dd, *J* = 10.4, 4.8 Hz, H-6′a), 3.76 (1H, m, H-6′b), 7.05 (2H, d, m, H-3″and H-7″), 7.28 (2H, d, m, H-4″and H-6″).[13]C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.3 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 119.0 (C-10), 163.7 (C-11), 99.2 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 101.0 (C-1″), 161.9 (C-2″), 126.4 (C-3″), 128.3 (C-4″), 22.7 (C-5″), 129.2 (C-6″), 126.5 (C-7″).

Compound **P4** was obtained as a colorless powder in 59.1% yield, mp 119−121 °C. HR-ESI-MS *m/z*: 512.1174 $[M + Na]^+$, Calcd. 512.1163 for $C_{23}H_{23}NO_{11}Na$. IR (KBr, ν in cm^{-1}): 3442.56, 2882.24, 1705.27, 1611.29, 1523.01, 1349.54, 1272.94, 1207.92, 1073.63. [1]H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.41 (1H, d, *J* = 3.3 Hz, H-1), 8.19 (1H, overlaps, H-3), 5.53 (1H, s, H-6), 5.04 (1H, m, H-7), 5.64 (1H, m, H-8), 3.27 (1H, d, *J* = 6.8 Hz, H-9), 5.24 (1H, m, H-10), 4.78 (1H, d, *J* = 7.8 Hz, H-1′), 3.42 (1H, overlaps, H-2′), 3.80 (1H, dt, *J* = 9.7, 15.3 Hz, H-3′), 3.48 (1H, dt, *J* = 7.2,14.6 Hz, H-4′), 3.30 (1H, dd, *J* = 10.4, 31.3 Hz, H-5′), 4.36 (1H, dd, *J* = 10.5, 4.9 Hz, H-6′a), 3.57 (1H, d, *J* = 9.34 Hz, H-6′b), 7.47 (2H, d, *J* = 1.0 Hz, H-3″and H-7″), 7.67 (1H, d, *J* = 8.7 Hz, H-4″and H-6″). [13]C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.2 (C-3), 103.9 (C-4), 125.6 (C-5), 116.0 (C-6), 69.5 (C-7), 132.7 (C-8), 45.4 (C-9), 119.1 (C-10), 163.7 (C-11), 99.2 (C-1′), 74.0 (C-2′), 73.0 (C-3′), 80.2 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 100.1 (C-1″), 148.4 (C-2″), 123.5 (C-3″), 127.5 (C-4″), 143.2 (C-5″), 127.5 (C-6″), 123.5 (C-7″).

Compound **P5** was obtained as a yellow powder in 15.4% yield, mp 114−116 °C. HR-ESI-MS *m/z*: 488.1917 $[M + H]^+$, Calcd. 488.1915 for $C_{25}H_{29}NO_9H$. IR (KBr, ν in cm^{-1}): 3429.64, 2882.71, 1703.76, 1611.30, 1530.99, 1375.07, 1073.15. [1]H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.41 (1H, dd, *J* = 5.0, 11.3 Hz, H-1), 7.72 (1H, d, *J* = 8.9 Hz, H-3), 5.49 (1H, s, H-6), 4.97 (1H, dt, *J* = 10.3, 17.6 Hz, H-7), 5.63 (1H, ddd, *J* = 7.4, 9.9, 17.4 Hz, H-8), 3.25 (1H, m, H-9), 5.21 (1H, dd, *J* = 11.9, 16.8 Hz, H-10), 4.71 (1H, d, *J* = 7.8 Hz, H-1′), 3.40 (1H, overlaps, H-2′), 3.76 (1H, overlaps, H-3′), 3.42 (1H, tt, *J* = 6.9, 11.1 Hz, H-4′), 3.39 (1H, overlaps, H-5′), 4.26 (1H, dd, *J* = 10.4, 4.7 Hz, H-6′a), 3.70 (1H, m, H-6′b), 7.32 (2H, d, *J* = 8.8 Hz, H-3″and H-7″), 7.48 (2H, d, *J* = 8.6 Hz, H-4″and H-6″).[13]C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.3 (C-3), 103.8 (C-4), 125.7 (C-5), 115.9 (C-6), 69.5 (C-7), 132.9 (C-8), 45.3 (C-9), 118.9 (C-10), 163.8 (C-11), 99.2 (C-1′), 73.8 (C-2′), 73.1 (C-3′), 80.2 (C-4′), 66.7 (C-5′), 68.4 (C-6′), 102.4 (C-1″), 151.2 (C-2″), 128.2 (C-3″), 127.2 (C-4″), 190.4 (C-5″), 127.2 (C-6″), 128.2 (C-7″), 40.1 (C-8″), 40.5 (C-9″)

Compound **P6** was obtained as a colorless powder in 43.1% yield, mp 131−133 °C. HR-ESI-MS *m/z*: 457.1107 $[M + Na]^+$, Calcd. 457.1105 for $C_{21}H_{22}O_{10}Na$. IR (KBr, ν in cm^{-1}): 2886.30, 1710.52, 1507.25, 1079.01. [1]H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.37 (1H, d, *J* = 3.4 Hz, H-1), 7.50 (1H, overlaps, H-3), 5.47 (1H, s, H-6), 4.95 (1H, dt, *J* = 10.3, 17.6 Hz, H-7), 5.60 (1H, m, H-8), 3.23 (1H, d, *J* = 5.7 Hz, H-9), 5.20 (1H, dd, *J* = 3.0, 14.2 Hz, H-10), 4.72 (1H, d, *J* = 7.8 Hz, H-1′), 3.43 (1H, dq, *J* = 8.8, 18.6 Hz, H-2′), 3.76 (1H, overlaps, H-3′), 3.74 (1H, ddd, *J* = 12.7, 27.0, 27.5 Hz, H-4′), 3.39 (1H, overlaps, H-5′), 4.27 (1H, dd, *J* = 10.3, 3.5 Hz, H-6′a), 3.70 (1H, d, *J* = 10.4 Hz, H-6′b), 7.46 (1H, s, H-3″), 7.26 (1H, s, H-4″), 7.37 (1H, d, *J* = 0.9 Hz, H-5″). [13]C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.9 (C-8), 45.3 (C-9), 118.9 (C-10), 163.9 (C-11), 99.3 (C-1′), 73.8 (C-2′), 72.8 (C-3′), 80.2 (C-4′), 66.3 (C-5′), 68.4 (C-6′), 108.5 (C-1″), 30.9 (C-2″), 149.3 (C-3″), 99.7 (C-4″), 96.1 (C-5″).

Compound **P7** was obtained as a colorless powder in 40.3% yield, mp 137−139 °C. HR-ESI-MS *m/z*: 493.1511 $[M + H]^+$, Calcd. 493.1505 for $C_{24}H_{25}FO_{10}H$. IR (KBr, ν in cm^{-1}): 3443.49, 2883.60, 1717.14, 1610.81, 1522.70, 1277.59, 1073.57. [1]H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.42 (1H, m, H-1), 7.49 (1H, s, H-3), 5.51 (1H, s, H-6), 5.00 (1H, dt, *J* = 17.7, 10.3 Hz, H-7), 5.65 (1H, ddd, *J* = 7.4, 9.9, 17.4 Hz, H-8), 3.27 (1H, d, *J* = 5.3 Hz, H-9), 5.24 (1H, m, H-10), 4.76 (1H, d, *J* = 7.8 Hz, H-1′), 3.44 (1H, ddd, *J* = 7.3, 13.8, 17.0 Hz, H-2′), 3.72 (1H, ddd, *J* = 18.7, 26.8, 45.7 Hz, H-3′), 3.44 (1H, ddd, *J* = 7.3, 13.8, 17.0 Hz, H-4′), 3.72 (1H, ddd, *J* = 18.7, 26.8, 45.7 Hz, H-5′), 4.30 (1H, dd, *J* = 10.4, 4.8 Hz, H-6), 7.24 (1H, m, H-3″), 6.93 (1H, t, *J* = 8.5 Hz, H-6″), 7.24 (1H, m, H-7″), 3.87 (3H, s, OCH_3). [13]C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.3 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 119.0 (C-10), 163.8 (C-11), 99.2 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 100.8 (C-1″), 136.0 (C-2″), 128.2 (C-3″), 131.4 (C-4″), 123.3 (C-5″), 131.4 (C-6″), 128.2 (C-7″), 56.3 (C-8″).

Compound **P8** was obtained as a colorless powder in

43.6% yield, mp 166−168 °C. HR-ESI-MS *m/z*: 473.1807 $[M + H]^+$, Calcd. 473.1806 for $C_{25}H_{28}O_9H$. IR (KBr, ν in cm^{-1}): 3437.06, 2882.21, 1718.39, 1610.73. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.41 (1H, d, *J* = 3.1 Hz, H-1), 7.53 (1H, overlaps, H-3), 5.50 (1H, d, *J* = 4.4 Hz, H-6), 5.00 (1H, dt, *J* = 29.4, 6.1 Hz, H-7), 5.64 (1H, m, H-8), 3.27 (1H, d, *J* = 6.8 Hz, H-9), 5.28 (1H, td, *J* = 5.2, 16.6 Hz, H-10), 4.76 (1H, d, *J* = 7.8 Hz, H-1′), 3.42 (1H, ddd, *J* = 8.0, 40.1, 70.1 Hz, H-2′), 3.78 (1H, ddd, *J* = 11.7, 22.6, 24.6 Hz, H-3′), 3.42 (1H, ddd, *J* = 8.0, 40.1, 70.1 Hz, H-4′), 3.78 (1H, ddd, *J* = 11.7, 22.6, 24.6 Hz, H-5′), 4.27 (1H, m, H-6′), 7.28 (1H, s, H-3″), 7.20 (1H, d, *J* = 8.3 Hz, H-6″), 7.28 (1H, s, H-7″), 1.27 (6H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.9 (C-8), 45.3 (C-9), 118.9 (C-10), 163.9 (C-11), 99.2 (C-1′), 73.8 (C-2′), 73.1 (C-3′), 80.2 (C-4′), 66.6 (C-5′), 68.4 (C-6′), 102.0 (C-1″), 136.6 (C-2″), 129.5 (C-3″), 137.7 (C-4″), 134.5 (C-5″), 127.4 (C-6″), 123.8 (C-7″), 19.6 (C-8″), 19.7 (C-9″).

Compound **P9** was obtained as a colorless powder in 54.4% yield, mp 104−106 °C. HR-ESI-MS *m/z*: 535.0521 $[M + Na]^+$, Calcd. 535.0533 for $C_{23}Cl_2H_{22}O_9Na$. IR (KBr, ν in cm^{-1}): 3435.76, 2885.06, 1721.39, 1610.46, 1382.31, 1272.86, 1078.10. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.39 (1H, d, *J* = 3.4 Hz, H-1), 7.60 (1H, overlaps, H-3), 5.50 (1H, s, H-6), 5.00 (1H, m, H-7), 5.63 (1H, m, H-8), 3.25 (1H, d, *J* = 5.4 Hz, H-9), 5.22 (1H, m, H-10), 4.75 (1H, d, *J* = 7.8 Hz, H-1′), 3.44 (1H, overlaps, H-2′), 3.74 (1H, overlaps, H-3′), 3.44 (1H, ddt, *J* = 12.9, 22.1, 26.1 Hz, H-4′), 3.39 (1H, overlaps, H-5′), 4.30 (1H, dd, *J* = 4.8, 10.5 Hz, H-6′), 7.48 (1H, s, H-4″), 7.24 (1H, m, H-6″), 7.35 (2H, dd, *J* = 2.0, 4.4 Hz, H-7″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.7 (C-8), 45.3 (C-9), 119.0 (C-10), 163.8 (C-11), 99.2 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.3 (C-4′), 66.5 (C-5′), 68.6 (C-6′), 98.4 (C-1″), 135.7 (C-2″), 133.6 (C-3″), 129.3 (C-4″), 133.0 (C-5″), 129.0 (C-6″), 127.3 (C-7″).

Compound **P10** was obtained as a colorless powder in 16.6% yield, mp 135−137 °C. HR-ESI-MS *m/z*: 557.1017 $[M + Na]^+$, Calcd. 557.1014 for $C_{23}H_{22}N_2O_{13}Na$. IR (KBr, ν in cm^{-1}): 3433.60, 2922.63, 1715.38, 1610.45, 1537.81, 1360.07, 1072.77. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.39 (1H, d, *J* = 3.4 Hz, H-1), 7.47 (1H, overlaps, H-3), 5.50 (1H, d, *J* = 14.1 Hz, H-6), 5.02 (1H, dd, *J* = 17.2, 44.6 Hz, H-7), 5.63 (1H, m, H-8), 3.23 (1H, m, H-9), 5.25 (1H, m, H-10), 4.75 (1H, dd, *J* = 7.8, 20.7 Hz, H-1′), 3.44 (1H, dt, *J* = 8.2, 16.5 Hz, H-2′), 3.77 (1H, overlaps, H-3′), 3.61 (1H, ddd, *J* = 7.5, 16.6, 26.4 Hz, H-4′), 2.13 (1H, dd, *J* = 16.0, 18.3 Hz, H-5′), 3.90 (1H, dt, *J* = 9.8, 19.5 Hz, H-6′), 7.26 (1H, s, H-4″), 6.16 (1H, s, H-6″), 6.48 (2H, s, H-7″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.3 (C-3), 103.8 (C-4), 125.7 (C-5), 115.8 (C-6), 69.5 (C-7), 132.8 (C-8), 45.4 (C-9), 119.0 (C-10), 163.6 (C-11), 99.3 (C-1′), 74.0 (C-2′), 72.8 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 101.0 (C-1″), 136.0 (C-2″), 128.2 (C-3″), 131.4 (C-4″), 123.3 (C-5″), 131.4 (C-6″), 128.2 (C-7″).

Compound **P11** was obtained as a colorless powder in 45.3% yield, mp 117−119 °C. HR-ESI-MS *m/z*: 557.1635 $[M + Na]^+$, Calcd. 557.1629 for $C_{26}H_{30}O_{12}Na$. IR (KBr, ν in cm^{-1}): 3458.68, 2883.16, 1717.92, 1610.36, 1509.09, 1463.20, 1420.25, 1236.86, 1127.70. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.41 (1H, m, H-1), 7.47 (1H, overlaps, H-3), 5.50 (1H, d, *J* = 14.1 Hz, H-6), 5.02 (1H, dt, *J* = 10.3, 17.7 Hz, H-7), 5.63 (1H, ddd, *J* = 7.4, 9.7, 23.6 Hz, H-8), 3.25 (1H, d, *J* = 5.5 Hz, H-9), 5.22 (1H, m, H-10), 4.74 (1H, d, *J* = 7.8 Hz, H-1′), 3.49 (1H, m, H-2′), 3.70 (1H, overlaps, H-3′), 3.88 (1H, m, H-4′), 2.60 (1H, s, H-5′), 4.31 (1H, dd, *J* = 4.6, 10.4 Hz, H-6′), 7.28 (2H, s, H-3″and H-7″), 3.88 (9H, m, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.5 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 119.0 (C-10), 163.8 (C-11), 99.3 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.2 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 101.8 (C-1″), 153.1 (C-2″), 103.5 (C-3″), 138.6 (C-4″), 132.5 (C-5″), 132.4 (C-6″), 103.4 (C-7″), 60.8 (C-8″), 56.1 (C-9″), 56.1 (C-10″).

Compound **P12** was obtained as a colorless powder in 59.3% yield, mp 129−131 °C. HR-ESI-MS *m/z*: 481.1470 $[M + Na]^+$, Calcd. 481.1469 for $C_{24}H_{26}O_9Na$. IR (KBr, ν in cm^{-1}): 3436.72, 2884.21, 1715.51, 1604.43, 1382.14, 1077.52. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.42 (1H, d, *J* = 3.3 Hz, H-1), 7.51 (1H, s, H-3), 5.52 (1H, s, H-6), 5.01 (1H, dt, *J* = 10.4, 17.7 Hz, H-7), 5.65 (1H, ddd, *J* = 7.4, 9.9, 17.4 Hz, H-8), 3.27 (1H, d, *J* = 6.8 Hz, H-9), 5.24 (1H, m, H-10), 4.76(1H, m, H-1′), 3.65 (1H, overlaps, H-2′), 3.40 (1H, overlaps, H-3′), 3.01 (1H, ddd, *J* = 5.1, 14.2, 20.1 Hz, H-4′), 3.39 (1H, overlaps, H-5′), 4.18 (1H, dd, *J* = 4.2, 10.3 Hz, H-6′), 7.28 (1H, m, H-2″), 2.19(1H, s, H-4″), 1.57 (1H, m, H-5″), 1.39 (1H, m, H-6″), 1.28 (1H, s, H-7″), 0.95 (1H, t, *J* = 7.4 Hz, H-8″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.3 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 119.0 (C-10), 163.8 (C-11), 99.2 (C-1′), 73.9 (C-2′), 73.1 (C-3′), 79.7 (C-4′), 66.7 (C-5′), 68.1 (C-6′), 102.9 (C-1″), 40.8 (C-2″), 136.0 (C-3″), 126.7 (C-4″), 128.3 (C-5″), 129.6 (C-6″), 128.2 (C-7″), 126.8 (C-7″).

Compound **P13** was obtained as a colorless powder in 30.5% yield, mp 133−135 °C. HR-ESI-MS *m/z*: 485.1219 $[M + Na]^+$, Calcd. 485.1218 for $C_{23}H_{23}O_9FNa$. IR (KBr, ν in cm^{-1}): 3443.12, 2924.41, 1720.90, 1609.60, 1515.08, 1379.75, 1226.68, 1073.24. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.45 (1H, m, H-1), 7.49 (1H, m, H-3), 5.49 (1H, s, H-6), 5.02 (1H, m, H-7a), 5.66 (1H, m, H-8), 3.27 (1H, d, *J* = 6.8 Hz, H-9), 5.26 (1H, m, H-10), 4.78 (1H, d, *J* = 7.8 Hz, H-1′), 3.42 (1H, overlaps, H-2′), 3.76 (1H, overlaps, H-3′), 3.78 (1H, dt, *J* = 9.5, 14.9 Hz, H-4′), 3.48 (1H, overlaps, H-5′), 4.33 (1H, dd, *J* = 10.4, 4.8 Hz, H-6′a), 3.65 (1H, d, *J* = 7.6 Hz, H-6′b), 7.28 (2H, m), 7.49 (2H, m, H-4″and H-6″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.3 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 119.0 (C-10), 163.8 (C-11), 99.2

(C-1′), 74.0 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.6 (C-5′), 68.4 (C-6′), 101.2 (C-1″), 161.9 (C-2″), 128.2 (C-3″), 115.3 (C-4″), 22.7 (C-5″), 115.1 (C-6″), 128.3 (C-7″).

Compound **P15** was obtained as a colorless powder in 59.2% yield, mp 138−140 °C. HR-ESI-MS *m/z*: 535.1186 $[M + Na]^+$, Calcd. 535.1186 for $C_{24}H_{23}O_9F_3Na$. IR (KBr, ν in cm^{-1}): 3446.12, 2914.44, 1720.00, 1611.40, 1326.13, 1067.73. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.42 (1H, d, *J* = 3.1 Hz, H-1), 7.64 (1H, overlaps, H-3), 5.53 (1H, d, *J* = 17.8 Hz, H-6), 5.02 (1H, dd, *J* = 17.5, 48.2 Hz, H-7), 5.65 (1H, m, H-8), 3.27 (1H, d, *J* = 6.7 Hz, H-9), 5.24 (1H, m, H-10), 4.74 (1H, d, *J* = 7.8 Hz, H-1′), 3.50 (1H, overlaps, H-2′), 3.78 (1H, overlaps, H-3′), 3.50 (1H, overlaps, H-4′), 3.78 (1H, overlaps, H-5′), 4.34 (1H, dd, *J* = 10.3, 4.6Hz, H-6′), 7.52 (2H, d, *J* = 15.6 Hz, H-3″and H-7″), 7.28 (1H, s, H-4″and H-6″), 2.15 (1H, dd, *J* = 6.9, 14.1 Hz, H-8″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.5 (C-3), 103.8 (C-4), 125.5 (C-5), 115.9 (C-6), 69.6 (C-7), 132.8 (C-8), 45.3 (C-9), 118.9 (C-10), 164.1 (C-11), 99.3 (C-1′), 74.0 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 100.8 (C-1″), 135.6 (C-2″), 120.7 (C-3″), 125.5 (C-4″), 121.7 (C-5″), 125.5 (C-6″), 120.7 (C-7″), 29.7 (C-8″).

Compound **P16** was obtained as a colorless powder in 52.1% yield, mp 138−140 °C. HR-ESI-MS *m/z*: 551.1154 $[M + Na]^+$, Calcd. 551.1136 for $C_{24}H_{23}O_{10}F_3Na$. IR (KBr, ν in cm^{-1}): 3445.08, 2884.28, 1718.80, 1611.50, 1512.66, 1270.54. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.41 (1H, d, *J* = 3.1 Hz, H-1), 7.53 (1H, overlaps, H-3), 5.50 (1H, d, *J* = 4.4 Hz, H-6), 5.00 (1H, dt, *J* = 16.1, 29.4 Hz, H-7), 5.64 (1H, m, H-8), 3.27 (1H, d, *J* = 6.7 Hz, H-9), 5.28 (1H, td, *J* = 5.2, 16.6 Hz, H-10), 4.76 (1H, d, *J* = 7.8 Hz, H-1′), 3.42 (1H, ddd, *J* = 8.0, 40.1, 70.1 Hz, H-2′), 3.78 (1H, ddd, *J* = 11.7, 22.6, 24.6 Hz, H-3′), 3.42 (1H, ddd, *J* = 8.0, 40.1, 70.1 Hz, H-4′), 3.78 (1H, ddd, *J* = 11.7, 22.6, 24.6 Hz, H-5′), 7.20 (2H, d, *J* = 8.3 Hz, H-3″and H-7″), 7.28 (1H, s, H-4″and H-6″), 1.27 (1H, s, H-8″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.5 (C-3), 103.8 (C-4), 125.5 (C-5), 115.9 (C-6), 69.6 (C-7), 132.8 (C-8), 45.3 (C-9), 119.1 (C-10), 164.1 (C-11), 99.3 (C-1′), 74.0 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 100.8 (C-1″), 135.6 (C-2″), 120.7 (C-3″), 128.0 (C-4″), 121.7 (C-5″), 128.0 (C-6″), 120.7 (C-7″), 29.7 (C-8″).

Compound **P17** was obtained as a colorless powder in 39.3% yield, mp 139−141 °C. HR-ESI-MS *m/z*: 481.1470 $[M + Na]^+$, Calcd. 481.1469 for $C_{24}H_{26}O_9Na$. IR (KBr, ν in cm^{-1}): 3440.76, 2923.85, 1719.75, 1610.92, 1073.43. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.44 (1H, m, H-1), 7.51 (1H, d, *J* = 0.9 Hz, H-3), 5.44 (1H, m, H-6), 5.00 (1H, m, H-7), 5.65 (1H, m, H-8), 3.27 (1H, d, *J* = 6.8 Hz, H-9), 5.24 (1H, m, H-10a), 4.74 (1H, d, *J* = 7.8 Hz, H-1′), 3.43 (1H, tt, *J* = 7.2, 9.6 Hz, H-2′), 3.71 (1H, overlaps, H-3′), 3.43 (1H, tt, *J* = 7.2, 9.6 Hz, H-4′), 3.71 (1H, overlaps, H-5′), 4.29 (1H, dd, *J* = 10.4, 4.8 Hz, H-6′), 7.38 (2H, d, *J* = 8.1 Hz, H-3″and H-7″), 7.17 (1H, d, *J* = 8.0 Hz, H-4″and H-6″), 2.34 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.9 (C-8), 45.3 (C-9), 118.9 (C-10), 163.9 (C-11), 99.2 (C-1′), 73.8 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.6 (C-5′), 68.4 (C-6′), 101.9 (C-1″), 139.0 (C-2″), 126.3 (C-3″), 129.0 (C-4″), 134.2 (C-5″), 129.0 (C-6″), 126.3 (C-7″), 21.3 (C-8″).

Compound **P18** was obtained as a colorless powder in 31.2% yield, mp 103−105 °C. HR-ESI-MS *m/z*: 525.1353 $[M + Na]^+$, Calcd. 525.1367 for $C_{25}H_{26}O_{11}Na$. IR (KBr, ν in cm^{-1}): 3457.12, 2882.15, 1719.05, 1611.02, 1510.41, 1371.55, 1073.36. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.41 (1H, d, *J* = 2.9 Hz, H-1), 7.52 (1H, overlaps, H-3), 5.50 (1H, s, H-6), 4.99 (1H, dd, *J* = 17.3, 45.6 Hz, H-7), 5.65 (1H, ddd, *J* = 7.7, 9.5, 17.4 Hz, H-8), 3.27 (1H, d, *J* = 6.8 Hz, H-9), 5.25 (1H, dd, *J* = 10.6, 22.1 Hz, H-10), 4.73 (1H, d, *J* = 7.7 Hz, H-1′), 3.46 (1H, overlaps, H-2′), 3.70 (1H, overlaps, H-3′), 3.46 (1H, overlaps, H-4′), 3.70 (1H, overlaps, H-5′), 4.30 (1H, m, H-6′), 7.28 (2H, d, *J* = 5.8 Hz, H-3″and H-7″), 7.08 (2H, d, *J* = 8.3 Hz, H-4″and H-6″), 1.33 (1H, m, H-8″), 2.30 (3H, m, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.9 (C-8), 45.3 (C-9), 118.9 (C-10), 163.8 (C-11), 99.3 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 101.1 (C-1″), 136.0 (C-2″), 128.2 (C-3″), 131.4 (C-4″), 123.3 (C-5″), 131.4 (C-6″), 128.2 (C-7″), 31.9 (C-8″), 21.1 (C-9″).

Compound **P19** was obtained as a light yellow powder in 59.4% yield, mp 128−130 °C. HR-ESI-MS *m/z*: 545.1088 $[M + Na]^+$, Calcd. 545.1088 for $C_{24}H_{26}O_{11}SNa$. IR (KBr, ν in cm^{-1}): 3476.85, 2882.87, 1713.92, 1610.64, 1298.36, 1073.61. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.41 (1H, d, *J* = 3.2 Hz, H-1), 7.89 (1H, d, *J* = 8.3 Hz, H-3), 5.50 (1H, s, H-6), 4.99 (1H, dd, *J* = 16.4, 44.0 Hz, H-7), 5.61 (1H, m, H-8), 3.26 (1H, d, *J* = 5.2 Hz, H-9), 5.22 (1H, m, H-10), 4.76 (1H, d, *J* = 7.7 Hz, H-1′), 3.42 (1H, dd, *J* = 9.6, 17.9 Hz, H-2′), 3.75 (1H, dd, *J* = 9.1, 18.0 Hz, H-3′), 3.53 (1H, t, *J* = 9.2 Hz, H-4′), 3.39 (1H, overlaps, H-5′), 4.32 (1H, d, *J* = 5.7 Hz, H-6′), 7.69 (2H, d, *J* = 8.3 Hz, H-3″and H-7″), 7.46 (1H, s, H-4″and H-6″), 3.00 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.4 (C-3), 103.8 (C-4), 125.5 (C-5), 115.9 (C-6), 69.6 (C-7), 132.8 (C-8), 45.2 (C-9), 119.0 (C-10), 163.9 (C-11), 99.3 (C-1′), 74.0 (C-2′), 73.0 (C-3′), 80.2 (C-4′), 66.4 (C-5′), 68.5 (C-6′), 100.2 (C-1″), 142.6 (C-2″), 127.3 (C-3″), 127.6 (C-4″), 140.9 (C-5″), 127.6 (C-6″), 127.3 (C-7″), 44.4 (C-8″).

Compound **P20** was obtained as a colorless powder in 43.5% yield, mp 122−124 °C. HR-ESI-MS *m/z*: 485.1219 $[M + Na]^+$, Calcd. 485.1218 for $C_{23}H_{23}O_9FNa$. IR (KBr, ν in cm^{-1}): 3438.64, 2882.80, 1717.79, 1610.79, 1452.28, 1272.51, 1073.61. ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.40 (1H, d, *J* = 3.4 Hz, H-1), 7.49 (1H, overlaps, H-3), 5.48 (1H, d, *J* = 3.7 Hz, H-6), 4.98 (1H, m, H-7), 5.64 (1H, m, H-8), 3.26 (1H, d, *J* = 5.5 Hz, H-9), 5.23 (1H, m, H-10), 4.74 (1H, d, *J* = 7.8 Hz, H-1′), 3.44 (1H, overlaps, H-2′), 3.73 (1H, overlaps, H-3′), 3.44 (1H, overlaps, H-4′), 3.73 (1H, overlaps, H-5′), 4.29 (1H, dd, *J* = 10.4, 4.8 Hz, H-6′), 7.29

(H, m, H-3″), 7.03 (1H, m, H-5″), 7.29 (H, m, H-6″), 7.03 (1H, m, H-7″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 118.9 (C-10), 163.9 (C-11), 99.3 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.4 (C-5′), 68.4 (C-6′), 100.7 (C-1″), 139.4 (C-2″), 129.9 (C-3″), 161.4 (C-4″), 122.2 (C-5″), 113.7 (C-6″), 113.4 (C-7″).

Compound **P21** was obtained as a colorless powder in 64.5% yield, mp 138−140 °C. HR-ESI-MS *m*/*z*: 553.1094 $[M + Na]^+$, Calcd. 553.1092 for $C_{24}H_{22}O_9F_4Na$. IR (KBr, ν in cm^{-1}): 3441.58, 2885.16, 1718.44, 1610.80, 1509.95, 1076.34. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.42 (1H, d, *J* = 3.3 Hz, H-1), 7.49 (1H, overlaps, H-3), 5.52 (1H, s, H-6), 5.00 (1H, dt, *J* = 17.7, 10.3 Hz, H-7), 5.65 (1H, ddd, *J* = 7.4, 9.8, 17.4 Hz, H-8), 3.27 (1H, d, *J* = 5.8 Hz, H-9), 5.24 (1H, m, H-10), 4.77 (1H, d, *J* = 7.8 Hz, H-1′), 3.48 (1H, overlaps, H-2′), 3.48 (1H, overlaps, H-3′), 3.72 (1H, tt, *J* = 7.5, 46.9 Hz, H-4′), 3.39 (1H, overlaps, H-5′), 4.74 (1H, dd, *J* = 4.8, 10.5 Hz, H-6′), 7.77 (1H, dd, *J* = 1.5, 6.7 Hz, H-3″), 7.70 (1H, m, H-6″), 7.18 (1H, t, *J* = 9.3 Hz, H-7″), 2.17 (1H, s, H-8″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.7 (C-8), 45.3 (C-9), 118.9 (C-10), 164.0 (C-11), 99.3 (C-1′), 74.0 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.4 (C-5′), 68.4 (C-6′), 100.1 (C-1″), 133.4 (C-2″), 132.7 (C-3″), 133.4 (C-4″), 123.8 (C-5″), 132.3 (C-6″), 132.2 (C-7″), 134.1 (C-8″).

Compound **P22** was obtained as a colorless powder in 43.1% yield, mp 122−124 °C. HR-ESI-MS *m*/*z*: 563.0322 $[M + Na]^+$, Calcd. 563.0323 for $C_{23}H_{22}O_9FBrNa$. IR (KBr, ν in cm^{-1}): 3431.23, 2881.85, 1714.78, 1609.84, 1500.36, 1373.52, 1261.47, 1074.25. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.41 (1H, m, H-1), 7.48 (1H, overlaps, H-3), 5.41 (1H, m, H-6), 5.00 (1H, dd, *J* = 16.4, 46.6 Hz, H-7), 5.63 (1H, m, H-8), 3.25 (1H, d, *J* = 5.5 Hz, H-9), 5.22 (1H, m, H-10), 4.74 (1H, d, *J* = 7.8 Hz, H-1′), 3.43 (1H, overlaps, H-2′), 3.70 (1H, overlaps, H-3′), 3.99 (1H, s, H-4′), 3.39 (1H, overlaps, H-5′), 4.31 (1H, dd, *J* = 10.4, 4.8 Hz, H-6′), 7.71 (1H, dd, *J* = 1.6, 8.4 Hz, H-3″), 7.41 (1H, m, H-6″), 7.08 (1H, t, *J* = 8.4 Hz, H-7″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.5 (C-3), 103.7 (C-4), 125.6 (C-5), 115.8 (C-6), 69.6 (C-7), 132.8 (C-8), 45.3 (C-9), 119.0 (C-10), 164.0 (C-11), 99.3 (C-1′), 73.9 (C-2′), 72.9 (C-3′), 80.1 (C-4′), 66.4 (C-5′), 68.4 (C-6′), 100.0 (C-1″), 134.6 (C-2″), 127.4 (C-3″), 160.5 (C-4″), 158.6 (C-5″), 131.8 (C-6″), 127.3 (C-7″).

Compound **P23** was obtained as a colorless powder in 29.8% yield, mp 121−123 °C. HR-ESI-MS *m*/*z*: 533.1235 $[M + Na]^+$, Calcd. 533.1230 for $C_{24}H_{24}O_{10}F_2Na$. IR (KBr, ν in cm^{-1}): 3445.98, 2883.51, 1715.43, 1611.51, 1514.85, 1382.31, 1225.66, 1074.04. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.40 (1H, d, *J* = 3.3 Hz, H-1), 7.50 (1H, overlaps, H-3), 5.47 (1H, m, H-6), 4.99 (1H, m, H-7), 5.64 (1H, m, H-8), 3.26 (1H, d, *J* = 6.4 Hz, H-9), 5.23 (1H, m, H-10), 4.74 (1H, d, *J* = 7.8 Hz, H-1′), 3.44 (1H, ddd, *J* = 8.6, 15.2, 16.7 Hz, H-2′), 3.71 (1H, overlaps, H-3′), 4.51 (1H, d, *J* = 10.3 Hz, H-4′), 3.39 (1H, overlaps, H-5′), 4.30 (1H, dd, *J* = 10.4, 4.8 Hz, H-6′a), 3.71 (1H, m, H-6′b), 7.28 (2H, s, H-3″ and H-7″), 7.09 (2H, d, *J* = 8.5 Hz, H-4″ and H-6″), 2.09 (1H, s H-8″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 119.2 (C-10), 164.0 (C-11), 99.3 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 101.0 (C-1″), 134.2 (C-2″), 119.2 (C-3″), 128.1 (C-4″), 151.1 (C-5″), 128.1 (C-6″), 118.4 (C-7″), 113.2 (C-8″).

Compound **P24** was obtained as a colorless powder in 39.4% yield, mp 122−124 °C. HR-ESI-MS *m*/*z*: 563.0305 $[M + Na]^+$, Calcd. 563.0323 for $C_{23}H_{22}O_9BrFNa$. IR (KBr, ν in cm^{-1}): 3434.90, 2883.37, 1717.75, 1611.07, 1487.80, 1406.66, 1076.30. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.42 (1H, d, *J* = 3.3 Hz, H-1), 7.49 (1H, overlaps, H-3), 5.50 (1H, s, H-6), 5.00 (1H, m, H-7), 5.65 (1H, m, H-8), 3.27 (1H, d, *J* = 6.8 Hz, H-9), 5.24 (1H, m, H-10), 4.76 (1H, d, *J* = 7.8 Hz, H-1′), 3.47 (1H, overlaps, H-2′), 3.74 (1H, overlaps, H-3′), 3.44 (1H, overlaps, H-4′), 3.74 (1H, overlaps, H-5′), 4.31 (1H, dd, *J* = 10.4, 4.7 Hz, H-6′), 5.75 (1H, s, *J* = 6.0 Hz, H-1″), 7.74 (1H, dd, *J* = 2.5, 6.0 Hz, H-3″), 7.44 (1H, ddd, *J* = 2.6, 4.5, 8.7 Hz, H-5″), 6.94 (1H, t, *J* = 9.1 Hz, H-6″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.3 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 118.9 (C-10), 164.0 (C-11), 99.2 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.3 (C-4′), 66.4 (C-5′), 68.6 (C-6′), 99.2 (C-1″), 126.2 (C-2″), 133.8 (C-3″), 160.3 (C-4″), 130.9 (C-5″), 131.4 (C-6″), 117.5 (C-7″).

Compound **P25** was obtained as a colorless powder in 52.5% yield, mp 119−121 °C. HR-ESI-MS *m*/*z*: 503.1129 $[M + Na]^+$, Calcd. 503.1124 for $C_{23}H_{22}O_9F_2Na$. IR (KBr, ν in cm^{-1}): 3435.72, 2884.21, 1715.51, 1604.43, 1382.14, 1077.52. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.44 (1H, dd, *J* = 7.4, 9.9, 17.4 Hz, H-1), 7.48 (1H, overlaps, H-3), 5.44 (1H, dd, *J* = 7.4, 9.9, 17.4 Hz, H-6), 4.99 (1H, dt, *J* = 10.2, 17.7 Hz, H-7), 5.64 (1H, ddd, *J* = 7.4, 9.9, 17.4 Hz, H-8), 3.26 (1H, d, *J* = 5.7 Hz, H-9), 5.22 (1H, m, H-10a), 4.76 (1H, d, *J* = 7.8 Hz, H-1′), 3.93 (1H, d, *J* = 7.7 Hz, H-2′), 3.74 (1H, overlaps, H-3′), 3.44 (1H, m, H-4′), 3.39 (1H, overlaps, H-5′), 4.30 (1H, dd, *J* = 10.4, 4.8 Hz, H-6′), 7.28 (1H, s, H-3″), 7.05 (1H, m, H-5″), 6.77 (1H, tt, *J* = 2.3, 8.8 Hz, H-7″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.8 (C-6), 69.5 (C-7), 132.8 (C-8), 45.3 (C-9), 118.9 (C-10), 163.9 (C-11), 99.3 (C-1′), 73.9 (C-2′), 72.9 (C-3′), 80.1 (C-4′), 66.4 (C-5′), 68.4 (C-6′), 99.8 (C-1″), 140.6 (C-2″), 109.7 (C-3″), 161.6 (C-4″), 109.5 (C-5″), 161.5 (C-6″), 104.3 (C-7″).

Compound **P26** was obtained as a colorless powder in 35.6% yield, mp 118−120 °C. HR-ESI-MS *m*/*z*: 559.1583 $[M + Na]^+$, Calcd. 559.1575 for $C_{29}H_{28}O_{10}Na$. IR (KBr, ν in cm^{-1}): 3437.65, 2880.74, 1719.70, 1610.42, 1489.18, 1250.71, 1073.51. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, *J* in Hz): 5.43 (1H, m, H-1), 7.51 (1H, overlaps, H 3), 5.43 (1H, m, H-6), 5.01 (1H, m, H-7), 5.65 (1H, m, H-8), 3.27 (1H, d, *J* = 6.0 Hz, H-9), 5.23 (1H, m, H-10), 4.75 (1H, d, *J* = 7.8 Hz,

H-1′), 3.46 (1H, m, H-2′), 3.70 (1H, ddd, J = 7.4, 23.8, 39.5 Hz, H-3′), 3.46 (1H, m, H-4′), 3.70 (1H, ddd, J = 7.4, 23.8, 39.5 Hz, H-5′), 4.29 (1H, dd, J = 10.2, 4.5 Hz, H-6′), 7.30 (1H, m, H-3″), 7.00 (1H, m, H-4″), 7.11 (1H, t, J = 7.4 Hz, H-5″), 7.10 (1H, t, J = 7.4 Hz, H-7″), 2.14 (2H, t, J = 14.3 Hz, H-9″and H-13″), 1.28 (2H, s, H-10″and H-12″), 0.02 (1H, s, H-11″). ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm): 97.4 (C-1), 149.4 (C-3), 103.8 (C-4), 125.6 (C-5), 115.9 (C-6), 69.5 (C-7), 132.9 (C-8), 45.3 (C-9), 118.9 (C-10), 163.9 (C-11), 99.2 (C-1′), 73.9 (C-2′), 73.0 (C-3′), 80.1 (C-4′), 66.5 (C-5′), 68.4 (C-6′), 101.0 (C-1″), 138.9 (C-2″), 121.3 (C-3″), 119.5 (C-4″), 123.4 (C-5″), 157.0 (C-6″), 123.4 (C-7″), 157.2 (C-8″), 129.7 (C-9″), 129.8 (C-10″), 117.1 (C-11″), 129.8 (C-12″), 129.7 (C-13″).

Anti-inflammatory activity

The *in vivo* and *in vitro* anti-inflammatory activities of all newly synthesized compounds (**P1**−**P26**) were investigated with the literature methods [5]. The *in vitro* results showed that the NO production was strongly reduced after treatment with **P8**, **P13**, **P15**, **P20**, **P21**, **P24**, and **P26** at 100, 50, and 25 μg·mL^{-1}, compared with the model group (Fig. 6A) ($P < 0.05$). The PGE_2 production was potently decreased after treatment with **P2**, **P5**, **P6**, **P10**, **P11**, and **P23** at 100, 50, and 25 μg·mL^{-1} (Fig. 6B) ($P < 0.05$). The IL-6 pro-

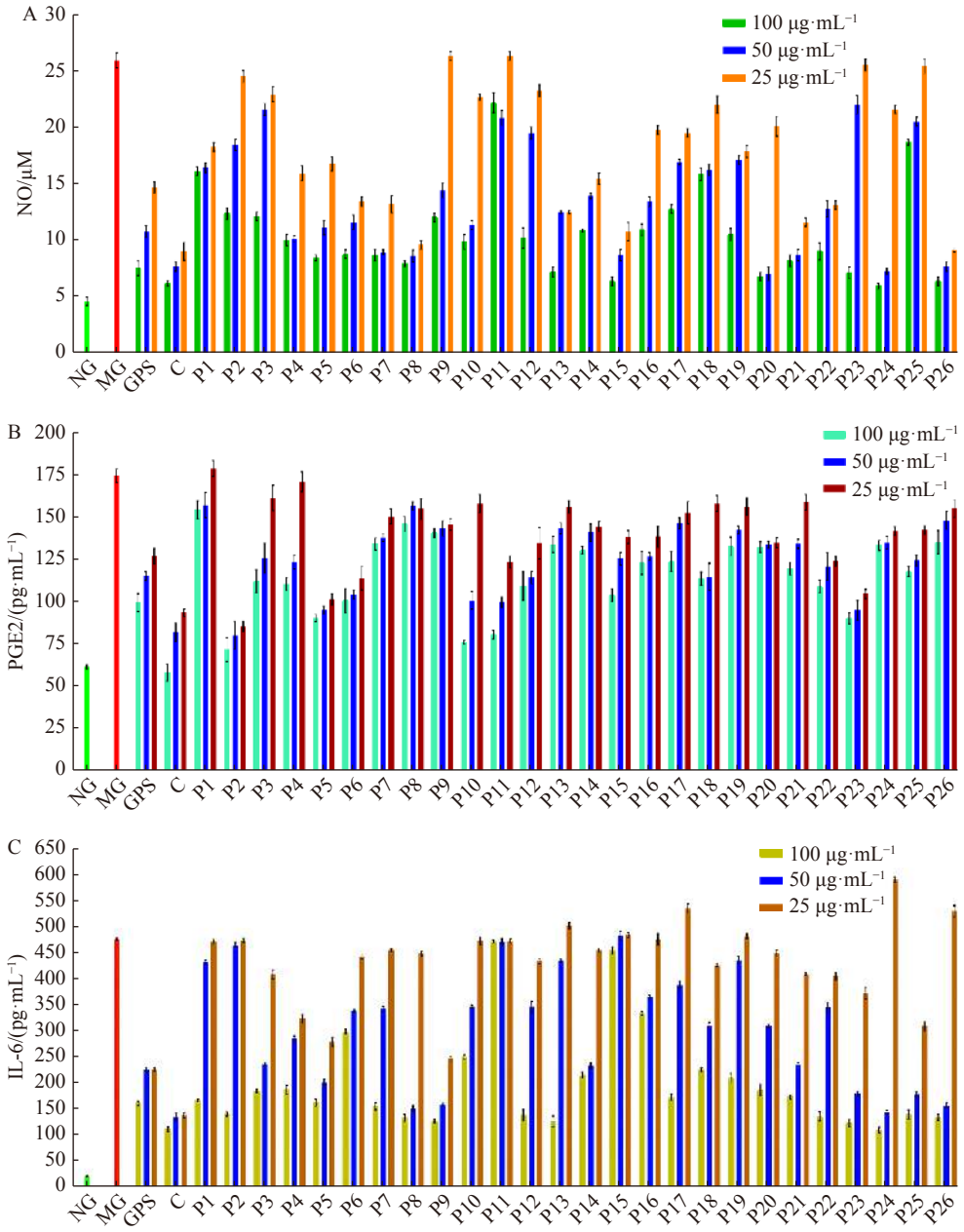


Fig. 6 Effect of gentiopicroside derivatives on LPS-induced NO(A), PGE_2(B), and IL-6(C) production in RAW 264.7 macrophages NG: Normal groups; MG: Model groups; GPS: Gentiopicroside; C: Celecoxib

Table 1 Effects of gentiopicroside derivatives on xylene swelling in mice ($\bar{x}$± s)

Group	Edema degree (mg)	Inhibition (%)	Group	Edema degree (mg)	Inhibition (%)
vehicle control	12.81 ± 3.59	—	**P13**	13.55 ± 4.29	−5.78
celecoxib	6.91 ± 2.67**	46.05	**P14**	11.20 ± 3.68	12.57
gentiopicroside	8.40 ± 3.14*	34.17	**P15**	10.57 ± 2.03	17.49
P1	11.87 ± 3.75	7.30	**P16**	10.66 ± 3.32	16.78
P2	7.83 ± 4.21*	38.88	**P17**	16.75 ± 4.76	−30.76
P3	7.00 ± 1.94**	45.06	**P18**	8.22 ± 1.52*	35.83
P4	7.40 ± 5.43*	42.23	**P19**	7.34 ± 3.84*	43.37
P5	14.36 ± 6.62	−11.92	**P20**	9.88 ± 3.44	22.85
P6	15.12 ± 3.21	−13.03	**P21**	9.49 ± 4.01	25.91
P7	14.21 ± 2.98	−10.93	**P22**	9.35 ± 7.40*	27.57
P8	7.72 ± 5.14*	40.08	**P23**	5.47 ± 3.32**	57.26
P9	13.89 ± 3.68	−8.43	**P24**	10.29 ± 4.81	19.67
P10	14.60 ± 4.68	−13.97	**P25**	11.72 ± 7.44	16.90
P11	13.46 ± 4.83	−5.07	**P26**	7.13 ± 4.88*	44.28
P12	8.37 ± 4.42*	34.64			

Note: Significance level $^{*}P < 0.05$,$^{**}P < 0.01$ compared to the control group, $n = 10$. The statistical significance was calculated between each compound group and control group by using Student's *t*-test of unpaired data. *P* values < 0.05 were considered statistically significant.

duction significantly decreased after treatment with **P8**, **P9**, **P23**, **P24**, **P25**, and **P26** at 100 and 50 μg·mL^{-1} (Fig. 6C) ($P < 0.05$). Together, several compounds displayed stronger inhibitory effects on the secretion of inflammatory cytokines than the parent compound gentiopicroside.

The *in vivo* activity of all compounds was further tested against mouse ear swelling. The results showed that compounds **P3**, **P4**, **P8**, **P19**, **P23**, and **P26** had higher inhibition rates (45.06%, 42.23%, 40.08%, 43.37%, 57.26%, and 44.28%, respectively) than the parent compound gentiopicroside (34.17%) at dose 0.28 mmol·kg^{-1}. The compound **P23** had higher inhibition rate (57.26%) even than the well-known drug celecoxib (46.05%) at dose 0.28 mmol·kg^{-1}.

Molecular docking

The molecular docking results showed that gentiopicroside derivatives might bind to the inflammation targets iNOS and COX-2. For example, **P23** and **P14** could be well docked into the pockets of iNOS and COX-2 [16], making hydrogen bonds with Tyr373, Asp382, Arg381, Val352, and Gln263 of iNOS and Tyr355, Tyr385, Ser530, Met522, and Arg120 of COX-2 (Figs. 7 and 8). Several compounds (**P5**, **P7**, **P9**, **P14**, **P15**, **P16**, **P21**, **P22**, **P23**, **P25**, and **P26**) had high absolute values of docking score and binding energy (Table 2), indicating strong interactions with iNOS and COX-2. This is consistent with their potencies of anti-inflammatory activities.

Structure-activity relationship analysis

26 novel derivatives have been synthesized through structural modification and their hydrophilicities were reduced than the parent compound gentiopicroside, the *in vivo*

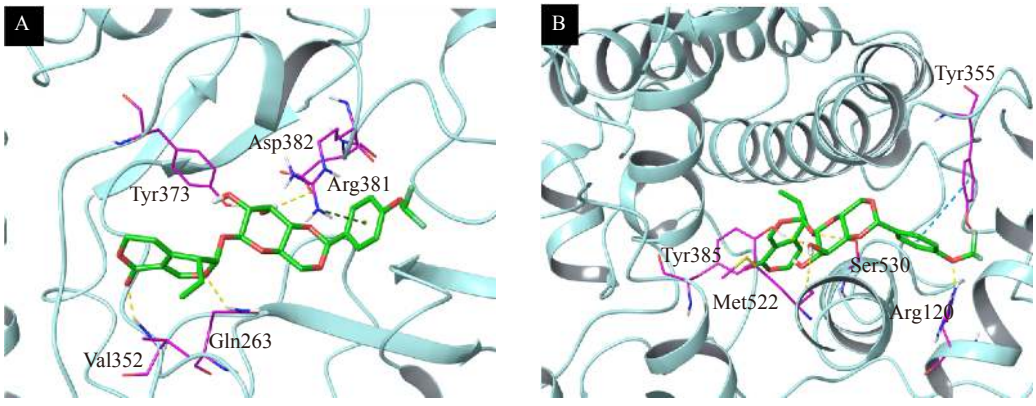


Fig. 7 3D docking model of gentiopicroside derivative P23 with iNOS (A) and COX-2 (B)

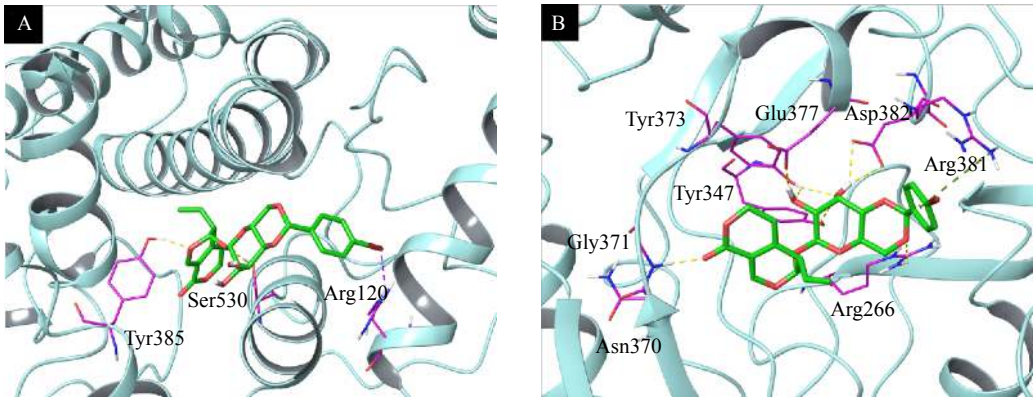


Fig. 8 3D docking model of gentiopicroside derivative P14 with COX-2 (A) and iNOS (B)

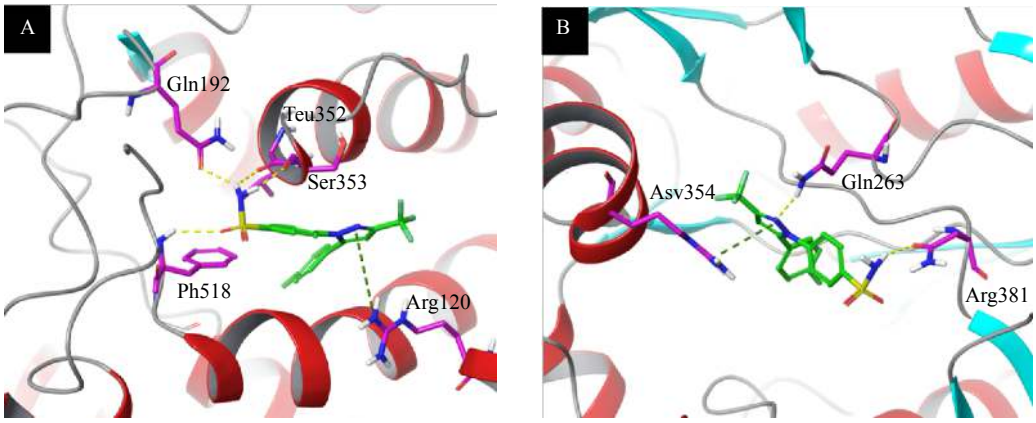


Fig. 9 3D docking model of celecoxib with COX-2 (A) and iNOS (B)

Table 2 Docking scores and binding energy of some gentiopicroside derivatives with iNOS and COX-2

Compounds	iNOS (3e7g)		COX-2 (5ikr)	
	docking score	mmGBSA(kcal·mol^{-1})	docking score	mmGBSA(kcal·mol^{-1})
gentiopicroside	−11.386	−86.877	−13.329	−96.05
celecoxib	−6.090	−42.501	−12.772	−104.082
P5	—	—	−14.689	−134.652
P7	−7.84	−67.277	−14.144	−112.686
P9	−6.096	−75.58	−15.575	−126.766
P14	−7.184	−55.799	−14.503	−111.287
P15	—	—	−15.178	−104.276
P16	−7.401	−75.336	−15.03	−108.928
P21	−7.235	−75.106	−15.507	−101.315
P22	−8.62	−66.663	−14.007	−124.688
P23	−7.788	−74.525	−14.808	−112.288
P25	−7.694	−68.527	−13.727	−111.968
P26	−6.658	−71.078	−13.519	−112.294

and *in vitro* anti-inflammatory activities of some compounds were kept or improved at the same time. Among them, the most potent compound **P23** with the 4-difluoromethoxyphenyl moiety was more active than gentiopicroside and even comparable to celecoxib. In addition, the derivatives with the groups NO_2, F, Cl, CH_3, CF_3, and OCF_2H also showed good activity. It was presumed that para-substitution with the electron-withdrawing groups might benefit the anti-

inflammatory activity.

Conclusions

The natural products play a vital role in discovering anti-inflammatory drug candidates, and their structure modification is the significant research direction in developing safer, more economic, more effective and less toxic anti-inflammatory agents. Gentiopicroside is extracted from the traditional Chinese medicine and its anti-inflammatory effect attracted more attention. In this study, in order to reduce polarity and keep its biological activity, we introduced hydrophobic cyclic acetals into the structure, synthesized a series of novel gentiopicroside derivatives and evaluated their anti-inflammatory activities. Most derivatives displayed significant *in vitro* and *in vivo* anti-inflammatory activities. The anti-inflammatory mechanism of these gentiopicroside derivatives are possibly associated with the downregulation of inflammatory cytokines NO, PGE_2, and IL-6 by the suppression of iNOS and COX-2. These compounds may represent a novel class of selective COX-2 and iNOS inhibitors with potential to be developed as new anti-inflammatory agents.

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