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Phenolics from *Glycyrrhiza glabra* roots and their PPAR-γ ligand-binding activity

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ABSTRACT

Bioassay-guided fractionation of the EtOH extract of licorice (*Glycyrrhiza glabra* roots), using a GAL-4-PPAR-γ chimera assay method, resulted in the isolation of 39 phenolics, including 10 new compounds (**1**–**10**). The structures of the new compounds were determined by analysis of their spectroscopic data. Among the isolated compounds, 5′-formylglabridin (**5**), (2*R*,3*R*)-3,4′,7-trihydroxy-3′-prenylflavane (7), echinatin, (3*R*)-2′,3′,7-trihydroxy-4′-methoxyisoflavan, kanzonol X, kanzonol W, shinpterocarpin, licoflavanone A, glabrol, shinflavanone, gancaonin L, and glabrone all exhibited significant PPAR-γ ligand-binding activity. The activity of these compounds at a sample concentration of 10 μg/mL was three times more potent than that of 0.5 μM troglitazone.

1. Introduction

Type 2 diabetes, obesity/abdominal obesity, hypertension, and dyslipidemia are closely linked to insulin resistance. The clustering of these risk factors in the same individual has been called metabolic syndrome, which has been recognized as a major public health problem around the world.[1,2] A crucial role in development of the metabolic syndrome is played by adipocytes, which are highly specialized cells involved in energy regulation and homeostasis. Adipocyte differentiation is a tightly controlled process in which determinant genes such as those of peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT/enhancer binding protein-α lead to programmed adipocyte differentiation.[3,4] PPARs are ligand-dependent transcriptional regulatory factors belonging to the nuclear receptor superfamily and regulate the expression of a group of genes that maintain glucose and lipid metabolism. Among PPARs, PPAR-γ is the predominant molecular target for insulin-sensitizing thiazolidinedione drugs such as troglitazone, pioglitazone, and rosiglitazone, which have been approved for use in the treatment of type 2 diabetes patients.[5,6] We previously found that an EtOH extract of *Glycyrrhiza uralensis* F. roots, which have long been used as a traditional Chinese medicine, was effective in preventing and/or ameliorating diabetes, abdominal obesity, and hypertension in KK-A^y mice, high-fat diet-induced obese C57BL/6J mice, and spontaneously hypertensive rats, respectively.[7] The activities were strongly suggested to be associated with PPAR-γ ligand-binding activity, and some prenylflavonoids including glycycoumarin, glycyrin, dehydroglyasperin C, and dehydroglyasperin D were identified as PPAR-γ ligands.[7,8] Among them, glycyrin, one of the main PPAR-γ ligands of *G. uralensis*, significantly decreased blood glucose levels in genetically diabetic KK-A^y mice.[8] On the other hand, we found that a hydrophobic flavonoids-enriched fraction prepared from the EtOH extract of *Glycyrrhiza glabra* L. roots, which exhibited PPAR-γ ligand-binding activity, was also effective in preventing and/or ameliorating diabetes, abdominal obesity, and body weight gain in KK-A^y mice and/or high-fat diet-induced obese C57BL/6J mice.[9,10] These research findings prompted us to identify secondary metabolites with PPAR-γ ligand-binding in the EtOH extract of *G. glabra* roots, which may contribute to the anti-diabetes, anti-abdominal obesity, and anti-obesity effects. Bioassay-guided fractionation of the EtOH extract using a GAL4-PPAR-γ chimera assay method resulted in the isolation of 10 new phenolic compounds (**1–10**), along with 29 known ones, which were identified by comparison of their physical and spectral data with those of reported compounds such as echinatin (**11**),[11] lichocalcone B (**12**),[12] morachalcone A (**13**),[13] 2′,3,4′-trihydroxy-3′-γ,γ-dimethylallyl-6″,6″-dimethylpyrano[2″,3″:4,5]chalcone (**14**),[14] 1-(2′,4′-dihydroxyphenyl)-2-hydroxy-3-(4″-hydroxyphenyl)-1-propanone (**15**),[15] kanzonol Y (**16**),[16] (3*R*)-vestitol (**17**),[17] (3*R*)-2′,3′,7-trihydroxy-4′-methoxyisoflavan (**18**),[18] kanzonol X (**19**),[16] glabridin (**20**),[19] 4′-*O*-methylglabridin (**21**),[20] 3′-hydroxy-4′-*O*-methylglabridin (**22**),[21] hispaglabridin A (**23**),[20] hispaglabridin B

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(**24**),[20] glabrene (**25**),[22] kanzonol W (**26**),[16] glabrocoumarin (**27**),[23] shinpterocarpin (**28**),[24] *O*-methylshinpterocarpin (**29**),[24] licoagrocarpin (**30**),[25] licoflavanone A (**31**),[26] glabrol (**32**),[19] shinflavanone (**33**),[24] euchrenone a5 (**34**),[27] xambioona (**35**),[28] gancaonin L (**36**),[29] glabrone (**37**),[30] kanzonol U (**38**),[17] and 8,8-dimethyl-3,4-dihydro-2*H*,8*H*-pyrano[2,3-*f*]-chromon-3-ol (**39**).[31] This paper reports on the structural determination of the 10 new compounds and the PPAR-γ ligand-binding activity of **1–39**.

2. Results and discussion

The dried roots of *G. glabra* (4.0 kg) were extracted with 95% EtOH (20 L, 2×, 2 h) at 45 °C. Each extract was combined and concentrated under reduced pressure to give a 95% EtOH extract (120 g). The extract exhibited strong PPAR-γ ligand-binding activity and its relative luminescence intensity was 2.2 at a sample concentration of 5 μg/mL, which was almost as potent as that of 0.5 μM troglitazone, a potent synthetic PPAR-γ agonist. The extract was chromatographed on silica gel eluted with $CHCl_3$–MeOH gradients (19:1; 9:1; 2:1) and finally with MeOH. The $CHCl_3$–MeOH (19:1) eluted portion (85 g) showed significant PPAR-γ ligand-binding activity (Fig. 1) and was subjected to a series of chromatographic separations to obtain **1** (8.0 mg), **2** (13.8 mg), **3** (1.6 mg), **4** (6.9 mg), **5** (18.5 mg), **6** (8.6 mg), **7** (18.5 mg), **8** (4.9 mg), **9** (7.3 mg), **10** (30.2 mg), **11** (5.4 mg), **12** (17.3 mg), **13** (14.1 mg), **14** (17.4 mg), **15** (5.8 mg), **16** (2.5 mg), **17** (10.5 mg), **18** (8.1 mg), **19** (37.1 mg), **20** (193 mg), **21** (11.4 mg), **22** (54.7 mg), **23** (13.7 mg), **24** (10.7 mg), **25** (28.8 mg), **26** (3.0 mg), **27** (16.4 mg), **28** (41.0 mg), **29** (32.4 mg), **30** (6.1 mg), **31** (4.8 mg), **32** (13.5 mg), **33** (7.8 mg), **34** (1.5 mg), **35** (8.0 mg), **36** (8.8 mg), **37** (15.5 mg), **38** (21.6 mg), and **39** (17.4 mg) (Figs. 2 and 3).

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{21}H_{22}O_6$ on the basis of its HRESIMS data, showing an accurate $[M+H]^+$ ion at *m/z* 371.1487. The UV spectrum of **1** had absorption maxima at 366 and 248 nm, whereas the IR spectrum showed absorbance bands at 3427 cm^{-1} (hydroxy groups), 1625 cm^{-1} (conjugated carbonyl group), and 1595, 1507 and 1469 cm^{-1} (aromatic rings). The ^1H NMR spectrum of **1** (acetone-d_6) exhibited signals for two *trans*-coupled protons at δ_H 7.91 and 7.67 (each d, J = 15.7 Hz), *ortho*-coupled aromatic protons at δ_H 7.25 and 6.72 (each d, J = 8.5 Hz), *meta*-coupled aromatic protons at δ_H 7.55 and 7.51 (each d, J = 1.9 Hz), and methoxy protons at δ_H 3.88 (s). In addition, the ^1H NMR spectrum showed the presence of a prenyl (3-methyl-2-butenyl) group [δ_H 5.40 (1H, m), 3.43 (2H, br d, J = 7.3 Hz), 1.77 and 1.75 (each 3H, br s)]. These data suggest **1** to be a chalcone derivative with four hydroxy groups, a methoxy group, and a prenyl group. In the HMBC spectrum of **1** (Fig. 4), long-range correlations were observed between H-2 (δ_H 7.51) and C=O (carbonyl, δ_C 188.0)/C-3 (δ_C 144.6)/C-4 (δ_C 148.3), and between H-6 (δ_H 7.55) and C=O/C-1 (δ_C 130.7), indicating that two hydroxy groups are attached to C-3 and C-4. HMBC correlations between H-1″ (δ_H 3.43) and C-4/C-6 (δ_C 122.8), between H-2″ (δ_H 5.40) and C-5 (δ_C 128.2) are indicative of the prenyl group at C-5. The structure of the ring-B moiety assigned to 3′,4′-dihydroxy-2′-methoxyphenyl and its linkage to C-β of the trans-olefinic group were ascertained by long-range correlations between H-5′ (δ_H 6.72) and C-1′ (δ_C 121.0)/C-3′ (δ_C 138.7)/C-4′ (δ_C 149.0), between H-6′ (δ_H 7.25) and C-β (δ_C 138.4)/C-2′ (δ_C 148.7), and between methoxy protons and C-2′. Thus, the structure of **1** was formulated as 3,3′,4,4′-tetrahydroxy-2′-methoxy-5-prenylchalcone.

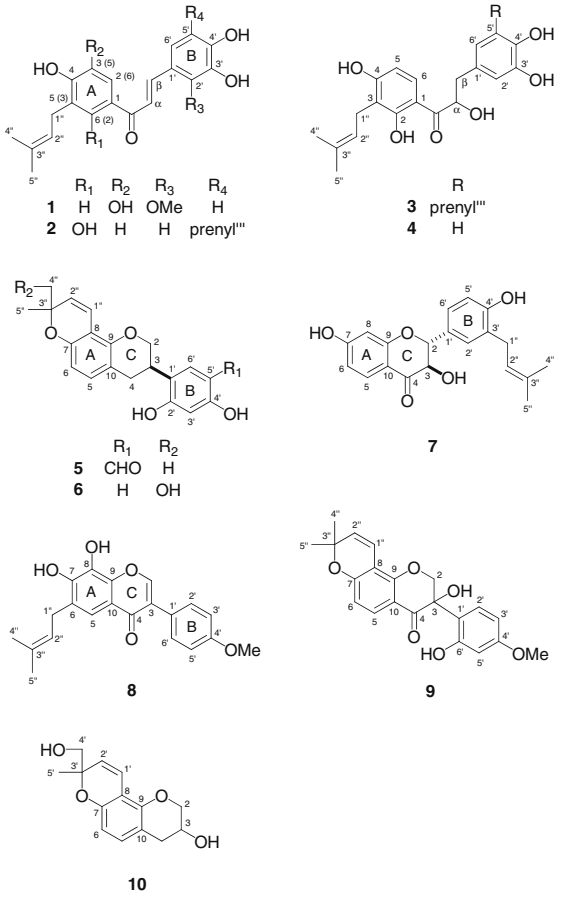

Figure 2. Structures of **1**–**10**.

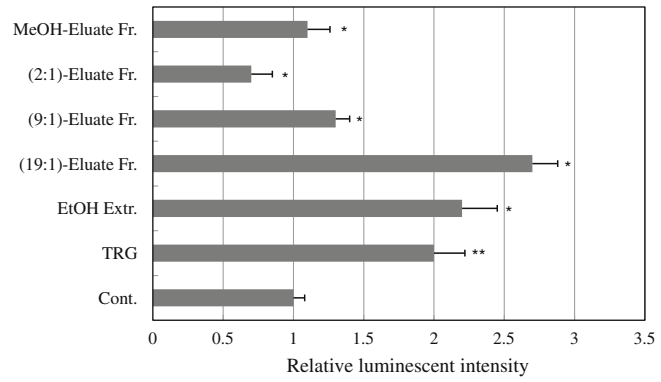

Figure 1. PPAR-γ ligand-binding activity of the licorice extract and fractions. PPAR-γ ligand-binding activity of the licolice extract and fractions (5 μg/mL), as well as that of troglitazone (TRG, 0.5 μM) used as a positive control, were measured using a GAL-4-PPAR-γ chimera assay. All samples were dissolved in dimethyl surfoxide, and added to medium to obtain the indicated concentrations. The luminescence intensity ratio (test group/control group) was determined for each sample, and PPAR-γ ligand-binding activity was expressed as the relative luminescence intensity of the test sample to that of the control sample. Values are means ± SD, n = 4 experiments. Statistical significance is indicated as * (p <0.05) or ** (p <0.01) as determined by Dunnett's multiple comparison test.

Compound **2**, a yellow powder, was determined to be $C_{25}H_{28}O_5$ on the basis of its HRESIMS data (*m/z* 409.2032 $[M+H]^+$). The ^1H NMR spectrum of **2** showed two doublet signals at δ_H 7.75 and 7.64 (each d, J = 15.3 Hz), which are characteristic of the *trans*-olefinic group of chalcone derivatives. Furthermore, the ^1H NMR spectrum exhibited signals for two prenyl groups at δ_H 5.38 and 5.29 (each 1H, m), 3.40 and 3.39 (each 2H, br d, J = 7.1 Hz), 1.79, 1.76, 1.74 and 1.66 (each 3H, br s), and a chelated hydroxy group at δ_H 12.31 (s), as well as signals for *ortho*-coupled aromatic protons at

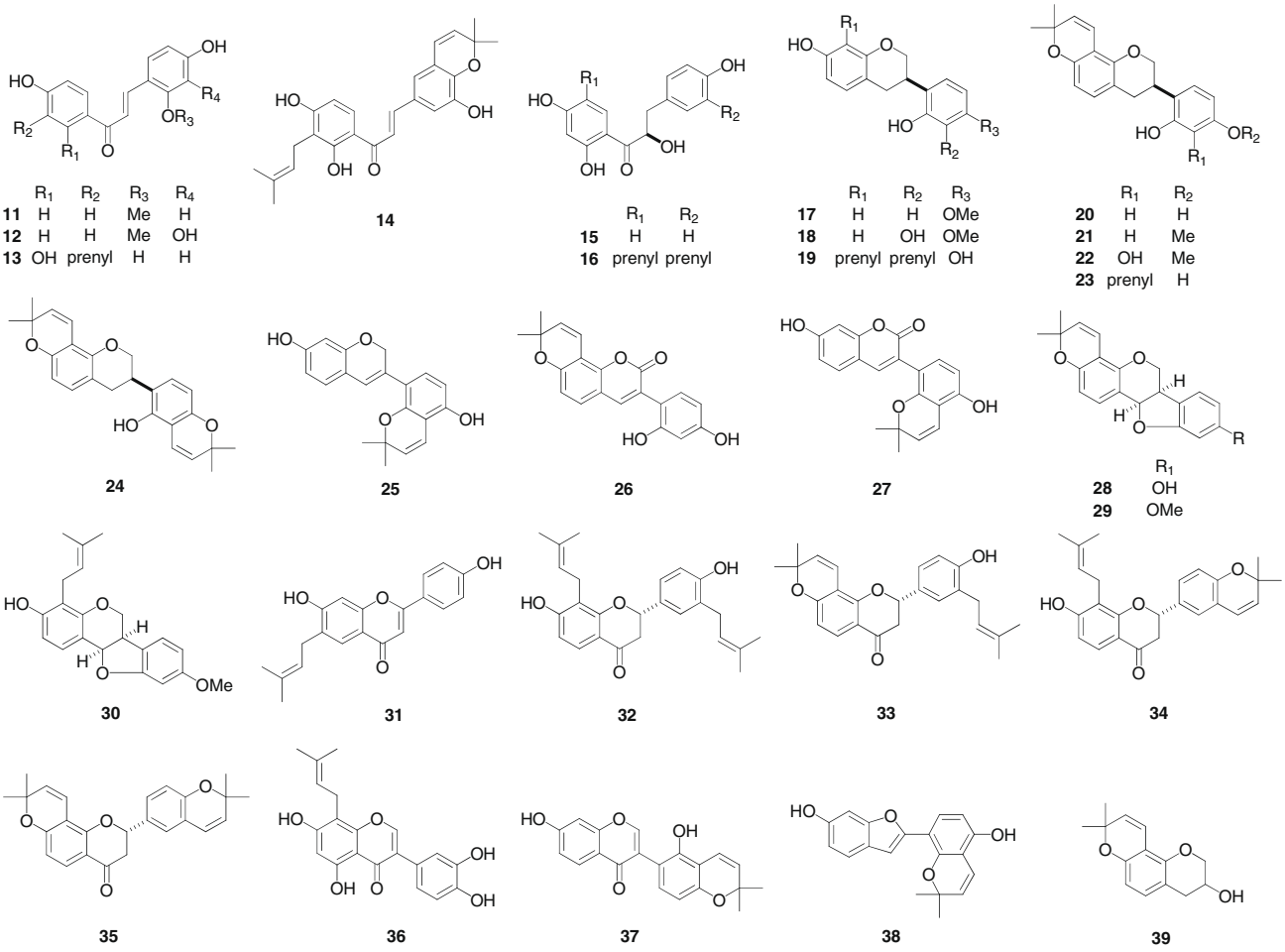

Figure 3. Structures of **11**–**39**.

δ_H 7.90 and 6.56 (each d, J = 8.9 Hz) and *meta*-coupled aromatic protons at δ_H 7.20 and 7.19 (br s). The ^{13}C NMR spectrum showed a total of 25 carbon signals including a carbonyl carbon signal at δ_C 192.5. These data suggest that **2** is a diprenylated tetrahydroxychalcone derivative. In the HMBC spectrum of **2** (Fig. 4), long-range correlations were observed between H-1″ (δ_H 3.39) and C-4 (δ_C 161.2), H-2″ (δ_H 5.29) and C-3 (δ_C 113.9), H-6 (δ_H 7.90) and C-4/C=O (δ_C 192.5), H-2′ (δ_H 7.20) and C-3′ (δ_C 145.1)/C-4′ (δ_C 146.8)/C-α (δ_C 117.8), H-1‴ (δ_H 3.40) and C-4′ (δ_C 146.8)/C-6′ (δ_C 123.4), and between H-2‴ (δ_H 5.38) and C-5′ (δ_C 129.1), indicating that two prenyl units are attached to C-3 and C-5′, and four hydroxy groups to C-2, C-4, C-3′ and C-4′, respectively. The structure of **2** was characterized as 2,3′,4,4′-tetrahydroxy-3,5′-diprenylchalcone.

Compound **3** was obtained as a pale yellow powder and shown to have a molecular formula of $C_{25}H_{30}O_6$ on the basis of its HRESIMS data (*m/z* 427.2133 $[M+H]^+$). The ^1H NMR spectrum of **3** displayed signals for *ortho*-coupled aromatic protons at δ_H 7.71 and 6.53 (each d, J = 8.9 Hz), *meta*-coupled aromatic protons at δ_H 6.64 and 6.49 (each d, J = 2.0 Hz), two prenyl groups at δ_H 5.27 (1H × 2, m), 3.37 and 3.26 (each 2H, br d, J = 7.2 Hz), and 1.78, 1.70, 1.69 and 1.65 (each 3H, br s), and a chelated hydroxy group at δ_H 12.33 (s). Although these spectral features were similar to those of **2**, the trans-olefinic proton signals assignable to H-α and H-β in **2** were displaced by three proton signals with an ABX-type spin system at δ_H 5.16 (m), 2.99 (dd, J = 14.0, 4.8 Hz) and 2.82 (dd, J = 14.0, 7.1 Hz) in **3**. In the HMQC spectrum of **3**, the proton signals at δ_H 2.99 and 2.82 were associated with a methylene carbon signal at δ 42.1, with which the aromatic protons at δ 6.64 and 6.49 (1H × 2, d, J = 2.0 Hz) showed long-range correlations (Fig. 4). Thus, the structure of **3** was characterized as 2,3′,4,4′,α-pentahydroxy-3,5′-diprenyl-dihydrochalcone.

Compound **4** exhibited a molecular formula of $C_{20}H_{22}O_6$ on the basis of its HRESIMS, which was less than that of **3** by C_5H_8, corresponding to one prenyl group. The ^1H NMR spectrum of **4** indicates that it is a dehydrochalcone derivative closely related to **3**. However, signals assignable to the prenyl group at C-5′ of the ring B part could not be detected in the ^1H NMR spectrum of **4**. Instead, signals due to a 1,3,4-trisubstituted aromatic group were observed at δ_H 6.78 (1H, d, J = 2.0 Hz), 6.71 (1H, d, J = 8.1 Hz), and 6.57 (1H, d, J = 8.1, 2.0 Hz), corresponding to H-2′, H-5′, and H-6′, respectively. The HMBC spectrum of **4** showed correlation peaks between H-2′ and C-β (δ_C 41.9), H-6′ and C-β, and between H-α (δ_H 5.17) and C-1′ (δ_C 129.6) (Fig. 4). Thus, the structure of **4** was determined to be 2,3′,4,4′,α-pentahydroxy-3-prenyl-dihydrochalcone.

Compounds **3** and **4** showed no specific rotations, thus being obtained as racemic compounds.

Compound **5** was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{21}H_{20}O_5$ on the basis of its HRESIMS data. The ^1H NMR spectrum of **5** exhibited characteristic signals for an isoflavan skeleton at δ_H 4.43 (ddd, J = 10.2, 3.4, 2.2 Hz, H-2a), 4.07 (dd, J = 10.2, 10.2 Hz, H-2b), 3.54 (m, H-3), 3.07 (dd, J = 15.5, 11.1 Hz, H-4a) and 2.90 (ddd, J = 15.5, 5.0, 2.2 Hz, H-4b) as well as signals assignable to two aromatic protons at δ_H 7.58 and 6.49 (each s), *ortho*-coupled aromatic protons at δ_H 6.87 and 6.32 (each d, J = 8.2 Hz) and a 2,2-dimethylpyran ring at δ_H 6.63 and 5.65 (each 1H, d, J = 9.8 Hz), and δ_H 1.40 and 1.38 (each

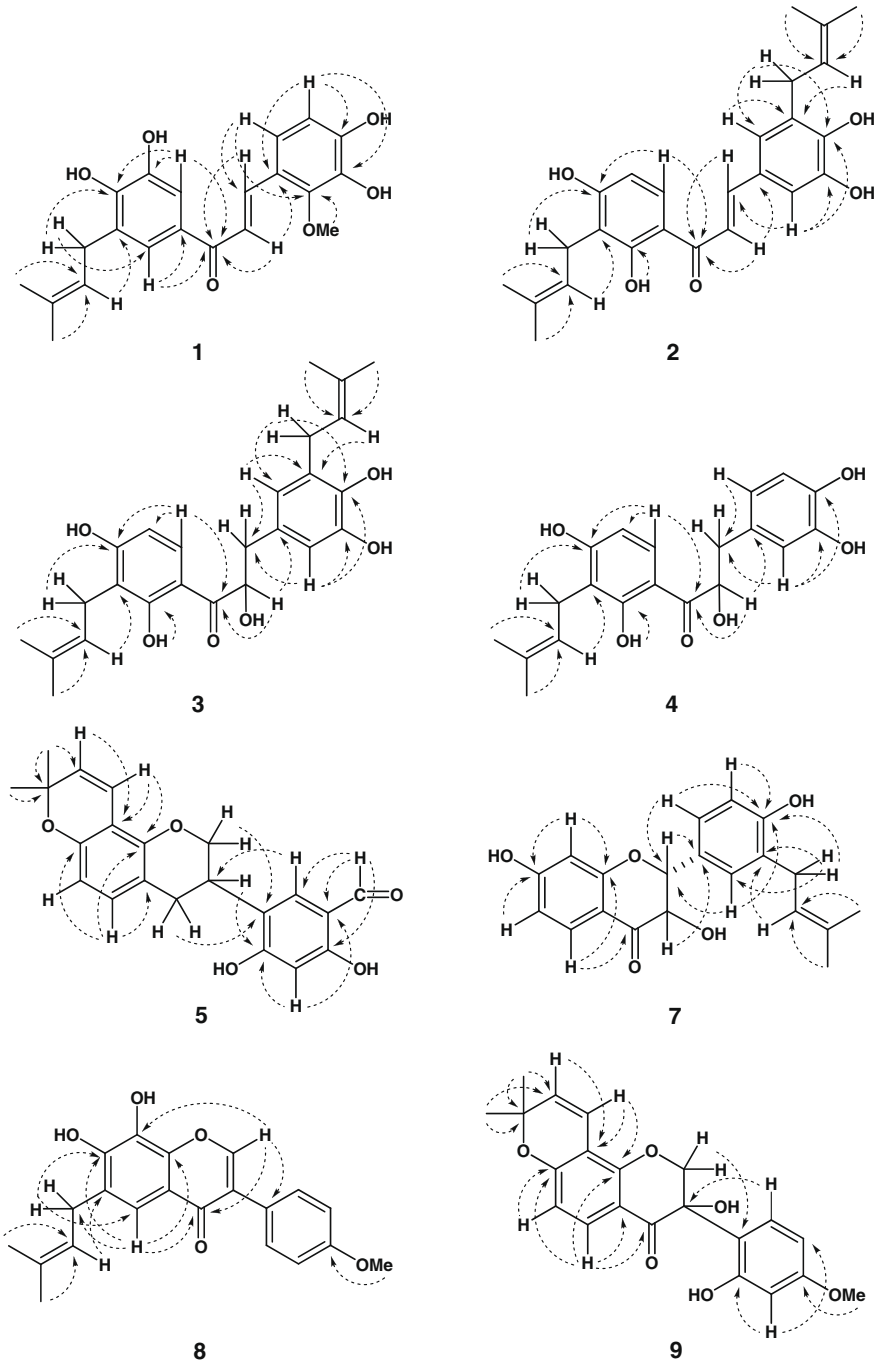

Figure 4. Key HMBC correlations of **1**–**5** and **7**–**9**.

3H, s). These ^{1}H NMR spectral features of **5** are very similar to those of glabridin (**20**). In addition, the presence of a formyl group in the molecule of **5** was shown by the ^{1}H and ^{13}C NMR signals at δ_H 9.77 and δ_C 195.3, and it was attached to the C-5′ position as determined by HMBC correlations between the formyl proton signal and C-4′ (δ_C 163.6)/C-5′ (δ_C 115.4)/C-6′ (δ_C 133.9) (Fig. 4). Since the CD profile of **5** was the same as that of synthetic 5′-formylglabridin prepared from **20** by formylation, the absolute configuration at C-3 was determined to be *R*. Thus, the structure of **5** was assigned as 5′-formyl glabridin.

Compound **6** had a molecular formula of $C_{20}H_{20}O_5$ on the basis of its HRESIMS, which differed from **20** by containing one more oxygen atom. When the ^{1}H NMR spectrum of **6** was compared with that of **20**, one of the germinal dimethyl groups linked to C-3″ at δ_H 1.39 and 1.38 disappeared in **6**, and resonances for a hydroxymethyl group were detected at δ_H 3.63 and 3.54 (each d, J = 11.3 Hz). These ^{1}H NMR spectroscopic data, along with a hydroxymethyl carbon signal at δ_C 67.7 and HMBC correlations between H_2-4″ (δ_H 3.63 and 3.54) and C-2″ (δ_C 126.1)/C-3″ (δ_C 78.8)/C-5″ (δ_C 22.5), allowed the structure of **6** to be assigned as 4″-hydroxyglabridin. The CD spectrum of **6** was consistent with that of **20**, giving evidence of the 3*R* configuration.

Compound **7** was isolated as a yellow powder and its molecular formula was determined to be $C_{20}H_{20}O_5$ by its HRESIMS. The UV spectrum of **7** had absorption maxima at 313 and 276 nm, whereas the IR spectrum showed absorption bands at 3374 cm^{-1} (hydroxy

groups), 1673 cm^{-1} (a carbonyl group), and 1608, 1502 and 1463 cm^{-1} (aromatic rings). The ^1H NMR spectrum of **7** showed signals assignable to a prenyl group at δ_H 5.39 (1H, m), 3.38 (2H, d, J = 7.3 Hz), and 1.74 and 1.72 (each 3H, br s), and two methines bearing an oxygen function at δ_H 5.03 and 4.59 (each d, J = 11.9 Hz)]. Furthermore, six aromatic protons comprising two ABX-type spin-coupling systems at δ_H 7.74 (d, J = 8.6 Hz), 6.64 (dd, J = 8.6, 2.2 Hz), and 6.41 (d, J = 2.2 Hz); δ_H 7.35 (d, J = 2.0 Hz), 7.27 (dd, J = 8.2, 2.0 Hz), and 6.90 (d, J = 8.2 Hz), were assigned to two 1,3,4-trisubstituted aromatic rings. From the above data, **7** was deduced to be dihydroxyflavan-3-ol derivative with a prenyl unit. HMBC correlations between H-5 (δ_H 7.74) and C-4 (δ_C 192.7)/C-9 (δ_C 164.1), H-8 (δ_H 6.41) and C-7 (δ_C 165.4)/C-9, H-6 (δ_H 6.64) and C-7, H-2′ (δ_H 7.35) and C-2 (δ_C 84.6)/C-4′ (δ_C 155.8), H-6′ (δ_H 7.27) and C-2/C-4′, and between H-1″ (δ_H 3.38) and C-2′ (δ_C 130.0)/C-3′ δ_C 128.1)/C-4′ indicate that two hydroxy groups and a prenyl group are attached to C-7, C-4′, and C-3′, respectively (Fig. 4). The positive Cotton effects at 210, 240, and 334 nm and negative Cotton effect at 304 nm in the CD spectrum of **7** allowed the absolute configurations to be assigned as 2*R* and 3*R*.[32] Thus, the structure of **7** was determined to be (2*R*,3*R*)-3,4′,7-trihydroxy-3′-prenylflavanone.

Compound **8** ($C_{21}H_{20}O_5$) was suggested to be an isoflavone derivative by the UV absorption maximum at λ_{max} 263 nm,[33] and a proton resonance at δ_H 8.16 (1H, s) and its corresponding oxygen-bearing olefinic carbon signal at δ_C 152.2. The ^1H NMR spectrum contained signals for an aromatic proton at δ_H 7.54 (s), *p*-disubstituted aromatic protons at δ_H 7.55 and 6.98 (each d, J = 8.8 Hz) and methoxy protons at δ 3.84 (3H, s). In addition, the ^1H NMR spectrum implied the presence of a prenyl unit [δ_H 5.41 (1H, m), 3.56 2H, d, J = 7.3 Hz), and 1.76 (3H x 2, br s)]. In the HMBC spectrum of **8** (Fig. 4), correlation peaks were observed between H-5 (δ_H 7.54) and C-4 (δ_C 175.4)/C-7 (δ_C 148.7)/C-9 (δ_C 145.7)/C-1″ (δ_C 28.4), between methoxy protons (δ_H 3.84) and C-4′ (δ_C 159.9), and between H-2 (δ_H 8.16) and C-8 (δ_C 145.7), indicating two hydroxy groups to be located at C-7 and C-8, a methoxy group at C-4′, and a prenyl group at C-6, respectively. Thus, the structure of **8** was determined to be 7,8-dihydroxy-4′-methoxy-6-prenylisoflavanone.

Compound **9** had a molecular formula of $C_{21}H_{20}O_6$, as determined by its HRESIMS. The ^1H NMR displayed signals for a 1,2,4-trisubstituted aromatic ring at δH 7.39 (1H, d, J = 8.6 Hz, H-6′), 6.44 (1H, dd, J = 8.6, 2.4 Hz, H-5′) and 6.41 (1H, d, J = 2.4 Hz, H-3′), *ortho*-coupled protons at δ_H 7.71 and 6.52 (each d, J = 8.7 Hz), a 2,2-dimethylpyran ring at δ_H 6.65 and 5.76 (each 1H, d, J = 10.1 Hz), and δ_H 1.46 and 1.45 (each 3H, s), and a methoxy group at δ_H 3.74 (3H, s). The ^1H and ^{13}C NMR spectral data of **9** were essentially analogous to those of glabroisoflavanone B;[21] however, slight differences were recognized in the signals due to the ring C part between the two compounds. Furthermore, the molecular formula of **9** was higher than that of glabroisoflavanone B by one oxygen atom and an oxygenated quaternary carbon signal was observed at δ_C 74.5. A pair of oxymethylene proton signals at δ_H 4.99 and 4.41 (each d, J = 11.8 Hz) were associated with the corresponding oxymethylene carbon signal at δ_C 74.4 (C-2) by the HMQC spectrum, and a long-range correlation was observed from H-6′ (δ_H 7.39) to C-3 (δ_C 74.5) in the HMBC spectrum. Thus, the presence of a hydroxy group at C-3 was verified, and the structure of **9** was established as 2′,3-dihydroxy-4′-methoxy-3″,3″-dimethylpyrano[2″,3″:7,8]isoflavanone. Compound **9** showed no specific rotation nor Cotton effects in the CD spectrum, which is indicative of a racemate.

Compound **10** ($C_{14}H_{16}O_4$) was suggested to be a chromone derivative whose structure was closely related to known compound **39** on the basis of its spectroscopic data. However, the molecular formula of **10** was higher than that of **39** by one oxygen atom. On comparison of the ^1H NMR spectrum of **10** with that of **39**, one of the germinal dimethyl group attached to C-3′ at δ 1.23 and 1.22 (each s) could not be observed in **10**, and signals for a hydroxymethyl group appeared at δ 3.62 and 3.54 (each d, J = 11.3 Hz). HMBC correlations between H_2-4′ (δ_H 3.62 and 3.54) and C-2′ (δ_C 126.2)/C-3′ (δ_C 78.8)/C-5′ (δ_C 22.5) allowed the structure of **10** to be assigned as 8-hydroxymethyl-8-methyl-3,4-dihydro-2*H*,8*H*-pyrano[2,3-*f*]-chromon-3-ol. Compound **10** showed no specific rotation and was obtained as a racemate.

3. PPAR-γ ligand-binding activity

Compounds **5**, **7**, **11**, **18**, **19**, **26**, **28**, **31–33**, **36**, and **37** exhibited significant PPAR-γ ligand-binding activity, among which the prenylflavone derivative, licoflavanone A (**31**), showed the most potent ligand-binding activity (Fig. 5). The isoflavone derivative, kanzonol X (**19**), and flavanone derivative, glabrol (**32**), both having two prenyl units, also showed potent ligand-binding activity. The activity of hispaglabridin B (**24**) and xambioona (**35**), in which the two prenyl units are cyclized to form two six-membered rings,

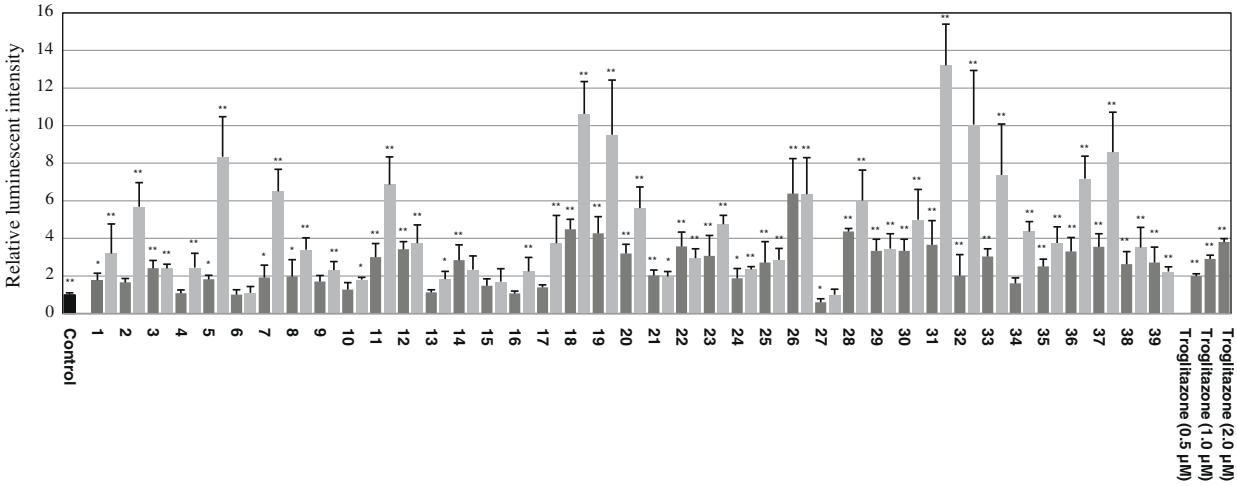

Figure 5. PPAR-γ ligand-binding activity of compounds **1**–**39** at 2 (■) and 10 (□) μg/mL with the GAL-4-PPAR-γ chimera assay. Troglitazone at 0.5, 1.0, and 2.0 μM was used as a positive control, and dimethyl sulfoxide at 1 mL/L as a solvent control. Values are means ± SD, n = 4 experiments. Statistical significance is indicated as * (p <0.05) or ** (p <0.01) as determined by Dunnett's multiple comparison test.

were less than that of **19** and **32**, implying that the two prenyl units are necessary for the appearance of the potent activity in these compounds. As we have previously mentioned, the isoprenyl group at C-6 and the hydroxy group at C-2′ in the aromatic ring-C part in the isoflavan, isoflavene, or arylcoumarin skeleton have been found to be the structural requirements for PPAR-γ ligand-binding activity in the phenolics isolated from *G. uralensis* roots.[8] Considering all of the above data together, PPAR-γ ligand-binding activity in the phenolic compounds are affected by the slight differences of substitution groups on the aromatic rings.

4. Conclusion

The EtOH extract of *G. glabra* roots exhibited considerable PPAR-γ ligand-binding activity and bioassay-guided fractionation of the extract resulted in the isolation of nine chalcones (**1–4**, **11–16**), 10 isoflavans (**5**, **6**, **17–24**), an isoflaven (**25**), two 3-arylcoumarin (**26**, **27**), three pterocarpans (**28–30**), a flavone (**31**), a flavanol (**7**), four flavanones (**32–35**), three isoflavone (**8**, **36**, **37**), an isoflavane (**9**), an 2-arybenzofuran (**38**), and two chromones (**10**, **39**), including 10 new compounds (**1–10**). It is notable that **5** is the first naturally occurring isoflavan with a formyl group at the B ring part. Compounds **13**, **15**, **17**, **18** and **36** were isolated from *G. glabra* for the first time. The isolated compounds were evaluated for their PPAR-γ lignd-binding activity. Compounds **5**, **7**, **11**, **18**, **19**, **26**, **28**, **31–33**, **36**, and **37** showed significant PPAR-γ ligand-binding activity, among which the prenylflavone derivative, licoflavone A (**31**), showed the most potent ligand-binding activity. These active compounds were considered to contribute mainly to the PPAR-γ ligand-binding activity of the EtOH extract, which suggested that the hydrophobic phenolics of *G. glabra* roots showed anti-diabetes, anti-abdominal obesity and anti-obesity effects in disease animal models, possibly mediated via activation of PPAR-γ.

5. Experimental

5.1. General experimental procedures

Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer, UV spectra on a JASCO V-630 spectrophotometer, and CD spectra on a JASCO J-720 instrument. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ-value with reference to tetramethylsilane (TMS) as an internal standard. HRESIMS data were obtained on a Waters-Micromass LCT mass spectrometer (Manchester, UK). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai-Tesque, Kyoto, Japan) were used for column chromatography (CC). TLC was carried out on precoated Silica Gel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254S} (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh), and a Rheodyne injection port. A Capcell Pak C_{18} UG120 column (10 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC.

5.2. Plant material

The roots of *G. glabra* were collected in the northwestern regions of the People's Republic of China in May 2002 and identified by Dr. Yutaka Sashida, emeritus professor of Medicinal Pharmacognosy at Tokyo University of Pharmacy and Life Sciences. A voucher specimen (No. 02-5-GG) is maintained at the School of Pharmacy, Tokyo University of Pharmacy and Life Sciences.

5.3. Extraction and isolation

The dried roots of *G. glabra* (4.0 kg) were extracted with EtOH (20 L, 45 °C, 2 h). The EtOH extract (120 g) was chromatographed on silica gel eluted with $CHCl_3$–MeOH gradients (19:1; 9:1; 2:1), and finally with MeOH alone, to give four fractions (I–IV). Since the activity was found to be concentrated in fraction I (85 g), the fraction was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (99:1), and finally with MeOH alone, to give eight fractions (I-A–H). Fraction I-B (12 g) was separated by silica gel column chromatography using hexane–Me_2CO (3:1) to afford eight fractions (I-B1–8). Fraction I-B2 (1.3 g) was subjected to silica gel column chromatography eluted with hexane–EtOAc (10:1) to give seven fractions (I-B2a–g). Fraction I-B2c (44.7 mg) was subjected to preparative HPLC (flow rate, 0.8 mL/min) using MeOH–H_2O (20:1) to give **29** (32.4 mg, t_R 34 min). Fraction I-B2e (274 mg) was subjected to preparative HPLC using MeOH–H_2O (4:1) to give **35** (8.0 mg, t_R 126 min). Fraction I-B4 (3.5 g) was separated by ODS silica gel column chromatography eluted with MeCN–H_2O (2:1) to give five fractions (I-B4a–e). Fraction I-B4a (1.02 g) was subjected to ODS silica gel column chromatography eluted with MeCN–H_2O (2:1) to give nine fractions (I-B4a1–9). Compound **39** (17.4 mg, t_R 29 min) was obtained from fraction I-B4a1 (92.8 mg) by subjecting it to preparative HPLC using MeCN–H_2O (2:1). Fraction I-B4a4 (83.7 mg) was subjected to preparative HPLC using MeCN–H_2O (2:1) to give **28** (41.0 mg, t_R 45 min) and **30** (6.1 mg, t_R 58 min). Similarly, purification of fraction I-B4a5 (124 mg) yielded **6** (8.6 mg, t_R 53 min), and fraction I-B4c (70 mg) gave **21** (11.4 mg, t_R 67 min), **23** (13.7 mg, t_R 81 min), **24** (10.7 mg, t_R 133 min) and **33** (7.8 mg, t_R 87 min). Fraction I-B6 (1.8 g) was separated by ODS silica gel column chromatography eluted with MeCN–H_2O (3:1) to give eight fractions (I-B6a–h). Fraction I-B6b (215 mg) was subjected to preparative HPLC using MeCN–H_2O (2:1) to give **9** (7.3 mg, t_R 33 min), **19** (37.1 mg, t_R 51 min) and **22** (54.7 mg, t_R 58 min). Similarly, purification of fraction I-B6d (201 mg) yielded **14** (17.4 mg, t_R 83 min) and **34** (1.5 mg, t_R 79 min). Fraction I-C (13.2 g) was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (99:1), and finally with MeOH alone, to give seven fractions (I-C1–7). Fraction I-C5 (1.5 g) was subjected to ODS silica gel column chromatography eluted with MeOH–H_2O (5:1) and MeCN–H_2O (1:1) to give three fractions (I-C5a–c). Fraction I-C5a (458 mg) was chromatographed on ODS silica gel eluted with MeOH–H_2O (5:2) to give a mixture of **8**, **16**, and **37**, which was then separated by preparative HPLC using MeOH–H_2O (5:2) to yield **8** (4.9 mg, t_R 72 min), **16** (2.5 mg, t_R 119 min) and **37** (15.5 mg, t_R 82 min). Compound **32** (13.5 mg) was obtained from fraction I-C5c (350 mg) by subjecting it to ODS silica gel column chromatography eluted with MeOH–H_2O (5:1). Fraction I-D (11.3 g) was subjected to an ODS silica gel column eluted with MeOH–H_2O (4:1; 7:3) and MeCN–H_2O (1:1), a silica gel column with hexane–Me_2CO (2:1), and to preparative HPLC using MeCN–H_2O (1:1) to afford **13** (14.1 mg). Fraction I–E (10.7 g) was subjected to ODS silica gel column chromatography using MeCN–H_2O (3:2) to afford four fractions (I-E1–4). Fraction I-E1 (756 mg) was separated by ODS silica gel column chromatography eluted with MeCN–H_2O (2:3) to give seven fractions (I-E1a–g). Purification of fractions I-E1a (77.7 mg), I-E1c (55.4 mg) and I-E1d (171 mg) by preparative HPLC using MeOH–H_2O (7:3) led to the isolation of compounds **10** (30.2 mg, t_R 22 min), **18** (8.1 mg, t_R 32 min), and **7** (18.5 mg, t_R 48 min) and

Table 1
^{1}H NMR spectral data of **1**–**10** in acetone-d_6

Position	**1**		**2**		**3**		**4**		Position	**5**		**6**		**7**		**8**		**9**		**10**	
	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)		δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)	δ_H	J (Hz)
1	–		–		–				2	4.43 ddd	10.2, 3.4, 2.2	4.34 ddd	10.2, 3.3, 2.1	5.03 d	11.9	8.16 s	–	4.99 d	11.8	4.16 m	
2	7.51 d	1.9	–		–		–			4.07 dd	10.2, 10.2	4.03 dd	10.2, 10.2			–		4.41 d	11.8	3.90 m	
3	–		–		–		–		3	3.54 m		3.47 m		4.59 d	11.9	–		–		4.13 m	
4	–		–		–		–		4	3.07 dd	15.5, 11.1	2.99 dd	15.1, 11.0	–		–		–		2.95 dd	15.7, 4.6
5	–		6.56 d	8.9	6.53 d	8.9	6.55 d	8.9		2.90 ddd	15.5, 5.0, 2.2	2.80 ddd	15.1, 4.9, 1.9			–				2.63 dd	15.7, 6.7
6	7.55 d	1.9	7.90 d	8.9	7.71 d	8.9	7.76 d	8.9	5	6.87 d	8.2	6.84 d	8.2	7.74 d	8.6	7.54 s	–	7.71 d	8.7	6.78 d	8.2
2-OH	–		12.31 s		12.33 s		12.33 s		6	6.32 d	8.2	6.29 d	8.2	6.64 dd	8.6, 2.2	–		6.52 d	8.7	6.28 d	8.2
1′	–		–		–		–		7	–		–		–		–		–		–	
2′	–		7.20 br s		6.64 d	2.0	6.78 d	2.0	8	–		–		6.41 d	2.2	–		–		–	
3′	–		–		–		–		9	–		–		–		–		–		–	
4′	–		–		–		–		10	–		–		–		–		–		–	
5′	6.72 d	8.5	–		–		6.71 d	8.1	1′	–		–		–		–		–		6.68 d	10.1
6′	7.25 d	8.5	7.19 br s		6.49 d	2.0	6.57 dd	8.1, 2.0	2′	–		–		7.35 d	2.0	7.55 d	8.8	–		5.64 d	10.1
α	7.67 d	15.7	7.64 d	15.3	5.16 m		5.17 m		3′	6.49 s		6.47 d	2.3	–		6.98 d	8.8	6.41 d	2.4	–	
β	7.91 d	15.7	7.75 d	15.3	2.99 dd	14.0, 4.8	3.02 dd	14.0, 4.4	4′	–		–		–		–		–		3.62 d	11.3
					2.82 dd	14.0, 7.1	2.81 dd	14.0, 7.7								–		–		3.54 d	11.3
C=O	–		–		–		–		5′	–		6.36 dd	8.3, 2.3	6.90 d	8.2	6.98 d	8.8	6.44 dd	8.6, 2.4		
1″	3.43 d (2H)	7.3	3.39 d (2H)	7.1	3.37 d (2H)	7.2	3.37 d (2H)	7.2	6′	7.58 s		6.98 d	8.3	7.27 dd	8.2, 2.2	7.55 d	8.8	7.39 d	8.6	1.34 s	
2″	5.40 m		5.29 m		5.27 m		5.27 m		1″	6.63 d	9.8	6.71 d	10.0	3.38 d (2H)	7.3	3.56 d (2H)	7.3	6.65 d	10.1		
3″	–		–		–		–		2″	5.65 d	9.8	5.64 d	10.0	5.39 m		5.41 m		5.76 d	10.1		
4″	1.77 br s		1.66 br s		1.65 br s		1.66 br s		3″	–		–		–		–		–			
5″	1.75 br s		1.79 br s		1.78 br s		1.78 br s		4″	1.40 s		3.63 d	11.3	1.72 br s		1.76 br s		1.46 s			
1‴			3.40 d (2H)	7.1	3.26 d (2H)	7.2						3.54 d	11.3								
2‴			5.38 m		5.27 m				5″	1.38 s		1.36 s	–	1.74 br s		1.76 br s		1.45 s			
3‴			–		–				CHO	9.77 s (1H)											
4‴			1.76 br s		1.70 br s				OMe							3.84 s (3H)		3.74 s (3H)			
5‴			1.74 br s		1.69 br s																
OMe	3.88 s (3H)																				

17 (10.5 mg, t_R 42 min), respectively. Fraction I-E1f (84.7 mg) was subjected to preparative HPLC (flow rate, 0.6 mL/min) with MeOH–H_2O (8:2) to give **31** (4.8 mg, t_R 50 min). Fraction I-E2 (2.9 g) was separated by silica gel column chromatography eluted with $CHCl_3$–EtOAc (10:1) to give nine fractions (I-E2a–i). Purification of fraction I-E2b (160 mg), I-E2f (261 mg) and I-E2 g (305 mg) by preparative HPLC (flow rate, 0.7 mL/min) with MeCN–H_2O (3:2) to give **25** (28.8 mg, t_R 39 min) and **38** (21.6 mg, t_R 44 min), **26** (3.0 mg, t_R 42 min) and **3** (1.6 mg, t_R 67 min), respectively. Fraction I-E3 (6.5 g) was subjected to a Sephadex LH-20 column eluted with MeOH and an ODS silica gel column with MeCN–H_2O (3:2), and to preparative HPLC using MeCN–H_2O (2:1) to give **27** (16.4 mg, t_R 38 min). Fraction I-E4 (1.6 g) was subjected to an ODS silica gel column eluted with MeCN–H_2O (2:1) and a silica gel column with $CHCl_3$–MeOH (49:1) to give **2** (13.8 mg) and **4** (6.9 mg). Fraction I-G (3.6 g) was subjected to ODS silica gel column chromatography using MeOH–H_2O (4:1) to afford four fractions (I-G1–4). Column chromatography of fraction I-G1 (912 mg) on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH (100:1; 50:1; 20:1), and finally with MeOH alone, gave four fractions (I-G1a–d). Fraction I-G1b (178 mg) was subjected to ODS silica gel column chromatography using MeCN–H_2O (1:1) to give a mixture of **11** and **15**. The mixture was separated by preparative HPLC using MeOH–H_2O (7:3) to furnish **11** (5.4 mg, t_R 26 min) and **15** (5.8 mg, t_R 22 min). Fraction I-G1c (143 mg) was subjected to ODS silica gel column chromatography using MeCN–H_2O (2:3) to give three fractions (fraction I-G1c1–3). Compound **12** (17.3 mg, 22 min) was obtained from fraction I-G1c1 (41.0 mg) by subjecting it to preparative HPLC with MeCN–H_2O (7:3). Purification of fractions I-G1c2 (16.6 mg) and I-G1c3 (36.5 mg) by preparative HPLC using MeCN–H_2O (1:1) led to the isolation of **5** (18.5 mg, 33 min) and **1** (8.0 mg, 41 min), respectively. Column chromatography of fraction I-G2 (1.44 g) on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH (30:1; 9:1), and finally with MeOH alone, gave nine fractions (fraction I-G2a–i). Fraction I-G2b (185 mg) was divided by ODS silica gel column chromatography using MeOH–H_2O (4:1) into two fractions (I-G2b1 and I-G2b2). Fraction I-G2b1 (43.7 mg) was submitted to preparative HPLC using MeCN–H_2O (1:1) to give **36** (8.8 mg, 52 min). Fraction I-G2b2 (351 mg) was purified by preparative HPLC using MeOH–H_2O (4:1) to give **20** (193 mg, 39 min).

5.3.1. Compound 1

Yellow powder; UV (MeOH) λ_{max} (log ε) 248 (4.15), 366 (4.40); IR (film) ν_{max} 3427 (OH), 1625 (C=O), 1595, 1507 and 1469 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 371.1487 $[M+H]^+$ (calcd for $C_{21}H_{23}O_6$, 371.1495).

5.3.2. Compound 2

Yellow powder; UV (MeOH) λ_{max} (log ε) 383 (4.39); IR (film) ν_{max} 3375 (OH), 1702, 1614 (C=O), 1566 and 1494 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 409.2032 $[M+H]^+$ (calcd for $C_{25}H_{29}O_5$, 409.2015).

5.3.3. Compound 3

Pale yellow powder; UV (MeOH) λ_{max} (log ε) 289 (4.09); IR (film) ν_{max} 3389 (OH), 1703, 1621 (C=O), 1496 and 1445 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 427.2133 $[M+H]^+$ (calcd for $C_{25}H_{31}O_6$, 427.2121).

5.3.4. Compound 4

Pale yellow powder; UV (MeOH) λ_{max} (log ε) 289 (4.09); IR (film) ν_{max} 3389 (OH), 1703, 1621 (C=O), 1496 and 1445 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 359.1499 $[M+H]^+$ (calcd for $C_{20}H_{23}O_6$, 359.1495).

5.3.5. Compound 5

Yellow powder; $[\alpha]_D^{25}$ −10.0 (*c* 0.01, MeOH); CD [MeOH, nm, $\Delta\varepsilon$]: 221 (+2.41), 239 (−2.38), 290 (+1.13); UV (MeOH) λ_{max} (log ε) 318 (3.80), 280 (4.16); IR (film) ν_{max} 3252 (OH), 1702, 1633 (C=O), 1506 and 1478 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 353.1407 $[M+H]^+$ (calcd for $C_{21}H_{21}O_5$, 353.1389).

Table 2
^{13}C NMR spectral data of **1**–**10**

Position	1	2	3	4	Position	5	6	7	8	9	10
1	130.7	115.7	111.2	111.1	2	70.0	70.4	84.6	152.2	74.4	70.4
2	113.1	164.6	163.8	163.9	3	31.8	32.0	73.4	124.1	74.5	62.8
3	144.6	113.9	115.7	115.7	4	30.4	30.8	192.7	175.4	189.9	33.6
4	148.3	162.1	162.6	162.7	5	129.7	129.7	129.3	116.2	129.6	130.1
5	128.2	107.5	107.9	107.9	6	109.0	108.7	111.2	127.4	111.5	109.0
6	122.8	129.6	130.1	130.2	7	152.4	152.2	165.4	148.7	159.6	152.3
1′	121.0	126.8	128.5	129.6	8	110.0	109.9	103.1	145.7	109.5	109.8
2′	148.7	113.3	114.5	117.0	9	150.0	150.2	164.1	145.7	157.4	149.8
3′	138.7	145.1	144.5	145.1	10	114.5	115.1	112.5	117.7	113.9	112.8
4′	149.0	146.8	142.0	144.0	1′	121.5	118.8	128.9	125.3	117.5	118.5
5′	112.0	129.1	128.1	115.3	2′	163.6	156.3	130.0	130.6	156.8	126.2
6′	119.6	123.4	121.9	121.2	3′	102.7	103.1	128.1	113.8	102.7	78.8
α	120.5	117.8	73.6	73.6	4′	163.6	157.5	155.8	159.9	161.6	67.8
β	138.4	145.2	42.1	41.9	5′	115.4	107.3	115.0	113.8	105.3	22.5
C=O	188.0	192.5	204.9	204.9	6′	133.9	128.2	127.1	130.6	128.3	
1″	28.6	21.8	21.8	21.7	1″	117.1	118.7	28.7	28.4	115.6	
2″	122.9	122.8	122.6	122.6	2″	129.2	126.1	123.1	122.4	128.9	
3″	132.3	131.0	131.2	131.2	3″	75.6	78.8	132.0	132.8	77.9	
4″	25.4	25.4	25.4	25.4	4″	27.5	67.7	25.4	25.4	27.9	
5″	17.4	17.4	17.4	17.4	5″	27.3	22.5	17.4	17.4	27.9	
1‴		28.5	28.5		CHO	195.3					
2‴		122.9	123.4		OMe				55.1	55.0	
3‴		132.2	131.5								
4‴		25.4	25.4								
5‴		17.4	17.3								
OMe	61.0										

5.3.6. Formation of 5 from glabridin (20)

A mixture of **20** (60.5 mg), dry aluminum chloride (24.1 mg), ethyl orthoformate (3.1 mL), and dry benzene (23 mL) was stirred at room temperature for 30 min after 5% hydrochloric acid (70 mL) was added to it.[34] The products were extracted with ethyl ether and washed with water, dried and evaporated to dryness. The residue was purified by preparative HPLC using MeCN–H_2O (1:1) to give **5** (1.2 mg, 34 min).

5.3.7. Compound 6

Pale yellow powder; $[\alpha]_D^{25}$ −23.0 (*c* 0.01, MeOH); CD [MeOH, nm, $\Delta\varepsilon$]: 274 (−1.02), 293 (+4.88), 319 (−4.09), 358 (+0.98); UV (MeOH) λ_{max} (log ε) 283 (4.09); IR (film) ν_{max} 3357 (OH), 1520, 1474 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 25 MHz), see Table 2; HRESIMS *m/z* 341.1414 $[M+H]^+$ (calcd for $C_{20}H_{21}O_5$, 341.1389).

5.3.8. Compound 7

Pale yellow powder; $[\alpha]_D^{25}$ −38.0 (*c* 0.01, MeOH); CD [MeOH, nm, $\Delta\varepsilon$]: 210 (+3.81), 240 (+1.45), 304 (−4.01), 334 (+1.21); UV (MeOH) λ_{max} (log ε) 313 (3.84), 276 (4.10); IR (film) ν_{max} 3374 (OH), 1673 (C=O), 1608 and 1502 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 341.1411 $[M+H]^+$ (calcd for $C_{20}H_{21}O_5$, 341.1389).

5.3.9. Compound 8

Yellow powder; UV (MeOH) λ_{max} (log ε) 310 (4.06), 263 (3.57); IR (film) ν_{max} 3283 (OH), 2970, 2920, 1601 (C=O), 1512 and 1471 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 353.1390 $[M+H]^+$ (calcd for $C_{21}H_{21}O_5$, 353.1390).

5.3.10. Compound 9

Yellow powder; UV (MeOH) λ_{max} (log ε) 296 (3.55); IR (film) ν_{max} 3374 (OH), 1673 (C=O), 1608, 1502, and 1463 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 369.1320 $[M+H]^+$ (calcd for $C_{21}H_{21}O_6$, 369.1338).

5.3.11. Compound 10

Pale yellow powder; UV (MeOH) λ_{max} (log ε) 283 (3.88); IR (film) ν_{max} 3376 (OH), 1608, 1478 and 1455 (aromatic ring); ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 249.1142 $[M+H]^+$ (calcd for $C_{14}H_{17}O_4$, 249.1127).

5.4. PPAR-γ ligand-binding activity

PPAR-γ ligand-binding activity was carried out using a GAL-4-PPAR-γ chimera assay system.[35] CV-1 monkey kidney cells from the American Type Culture Collection (ATCC) (Manassas, VA, USA) were inoculated into a 96-well culture plate at 6×10^3 cells/well and incubated in 5% CO_2/air at 37 °C for 24 h. As medium, Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS), 10 mL/L penicillin-streptomycin (5000 IU/mL and 5000 μg/mL, Gibco), and 37 mg/L ascorbic acid (Wako Pure Chemical, Tokyo, Japan) was used. Cells were washed with OPTI-minimum essential medium (OPTI-MEM) (Gibco) and transfected with pM-hPPAR-γ and p4 × UASg-tk-luc using LipofectAMINE PLUS (Gibco). In a mock control, pM and p4 × UASg-tk-luc were transfected into CV-1 cells. After 24 h of transfection, the medium was changed to DMEM containing 10% charcoal-treated FBS and each sample,[36] and the cells were further cultured for 24 h. Then, the cells were washed with Ca^{2+}- and Mg^{2+}-containing phosphate-buffered saline (PBS+), to which Luc-Lite (Perkin–Elmer, Wellesley, MA, USA) was added. The intensity of emitted luminescence was determined using a TopCount microplate scintillation/luminescence counter (Perkin–Elmer). The luminescence intensity ratio (test group/control group) was determined for each sample, and PPAR-γ ligand-binding activity was expressed as the relative luminescence intensity of the test sample to that of the control sample.

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36. The samples were dissolved in DMSO, to which the medium was added to obtain the final concentration of 0.1% (v/v) of DMSO. DMSO was also added to the control wells.