Chapter 5

Phenolics from *Glycyrrhiza glabra* and *G. uralensis* Roots and Their PPAR-γ Ligand-Binding Activity: Possible Application for Amelioration of Type 2 Diabetes

Minpei Kuroda and Yoshihiro Mimaki

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67406

Abstract

The EtOH extract of *Glycyrrhiza glabra* roots and the EtOAc extract of *Glycyrrhiza uralensis* roots exhibited considerable PPAR-γ ligand-binding activity. Bioassay-guided fractionation of these extracts resulted in the isolation of 52 phenolics, including 11 novel ones. The PPAR-γ ligand-binding activity of more than 10 isolated phenolics at 10 μg/mL was approximately three times greater than that of 0.5 μM triglitazone. Glycyrin (**44**), isolated from the EtOAc extract of *G. uralensis* roots as a PPAR-γ ligand, reduced the blood glucose levels of genetically diabetic KK-A^{y} mice through its PPAR-γ ligand-binding activity.

Keywords: phenolics, *Glycyrrhiza glabra*, *Glycyrrhiza uralensis*, PPAR-γ, metabolic syndrome

1. Introduction

Peroxisome proliferator-activated receptor (PPAR)-γ is the primary molecular target for insulin-sensitizing thiazolidinedione drugs. These drugs activate PPAR-γ, increasing the number of small adipocytes that differentiate from preadipocytes and inducing apoptosis in large adipocytes. Because small adipocytes function normally, whereas large adipocytes hyperproduce and hypersecrete adipocytokines, an increased ratio of small adipocytes to large adipocytes improves insulin resistance. Therefore, compounds with PPAR-γ ligand-binding activity may be useful for the prevention and improvement of type 2 diabetes, a representative

insulin resistance syndrome. We found that the EtOH extract of *Glycyrrhiza glabra* roots and the EtOAc extract of *G. uralensis* roots exhibited higher activity than did the other materials tested. Bioassay-guided fractionation of these extracts resulted in the isolation of 52 phenolics, including 11 novel ones [1, 2].

In this chapter, we describe the results of the bioassay-guided fractionation of *G. glabra* and *G. uralensis* roots using a GAL-4-PPAR-γ chimera assay method.

2. PPAR-γ ligand-binding activity

PPAR-γ ligand-binding activity was assessed using a GAL-4-PPAR-γ chimera assay system (**Figure 1**) [3]. CV-1 monkey kidney cells from the American Type Culture Collection (ATCC) were suspended in Dulbecco's Modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 50 IU/mL Penicillin G sodium salt, 50 μg/mL streptomycin sulfate, and 37 mg/L ascorbic acid. The cells were then 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. Cells were washed with OPTI-minimum essential medium (MEM) and pM-hPPAR-γ and p4 × UASg-tk-luc were transfected into cells using LipofectAMINE PLUS (Gibco). pM and p4 × UASg-tk-luc were transfected into CV-1 cells as a mock control. Twenty-four hours after transfection, the medium was changed to DMEM containing 10% charcoal-treated FBS [4] and the cells were further cultured for 24 h. The cells were then washed with phosphate-buffered saline containing Ca^{2+} and Mg^{2+}, and luciferase activity was measured using LucLite (Perkin-Elmer). Luminescence intensity was measured using a TopCount Microplate scintillation/luminescence counter. PPAR-γ ligand-binding activity was expressed as the relative luminescence intensity (test group/control group) determined for each sample.

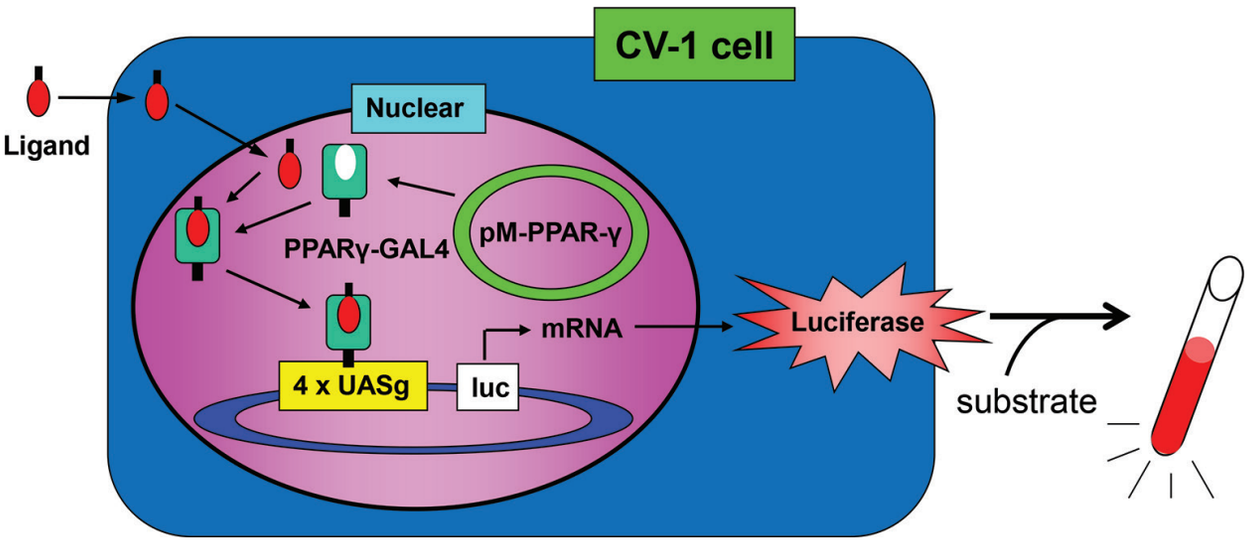

Figure 1. GAL4-PPAR-γ chimera assay system.

http://dx.doi.org/10.5772/67406

3. Isolation and structural determination of phenolic compounds from *G. glabra*

The roots of *G. glabra* (4.0 kg) were extracted twice with 20 L of 95% EtOH for 2 h at 45°C. The extracts were combined and concentrated under reduced pressure to give a 95% EtOH extract (120 g). The extract exhibited a relative luminescence intensity of 2.2 at a sample concentration of 5 μg/mL, indicating a PPAR-γ ligand-binding activity that was almost as strong as that of 0.5 μM troglitazone (TRG), a potent synthetic PPAR-γ agonist. The extract was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH gradients (19:1; 9:1; 2:1) and finally with MeOH. After removal of solvent, the fraction eluted with $CHCl_3$-MeOH (19:1) yielded 85 g of material that showed notable PPAR-γ ligand-binding activity (**Figure 2**). A series of chromatographic separations were then performed, resulting in 10 new phenolic compounds [**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)] (**Figure 3**). In addition, 29 known phenolic compounds were obtained and identified by comparison of physical and spectral data with those reported in the literature. The following known phenolic compounds were identified in the $CHCl_3$-MeOH (19:1) fraction: echinatin (**11**, 5.4 mg) [5], lichocalcone B (**12**, 17.3 mg) [6], morachalcone A (**13**, 14.1 mg) [7], 2′,3,4′-trihydroxy-3′-γ,γ-dimethylallyl-6″,6″-dimethylpyrano[2″,3″:4,5]chalcone (**14**, 17.4 mg) [8], 1-(2′,4′-dihydroxyphenyl)-2-hydroxy-3-(4″-hydroxyphenyl)-1-propanone (**15**, 5.8 mg) [9], kanzonol Y (**16**, 2.5 mg) [10], (3*R*)-vestitol (**17**, 10.5 mg) [11], (3*R*)-2′,3′,7-trihydroxy-4′-methoxyisoflavan (**18**, 8.1 mg) [12], kanzonol X (**19**, 37.1 mg) [10], glabridin (**20**, 193 mg) [13], 4′-*O*-methylglabridin (**21**, 11.4 mg) [14], 3′-hydroxy-4′-*O*-methylglabridin (**22**, 54.7 mg) [15],

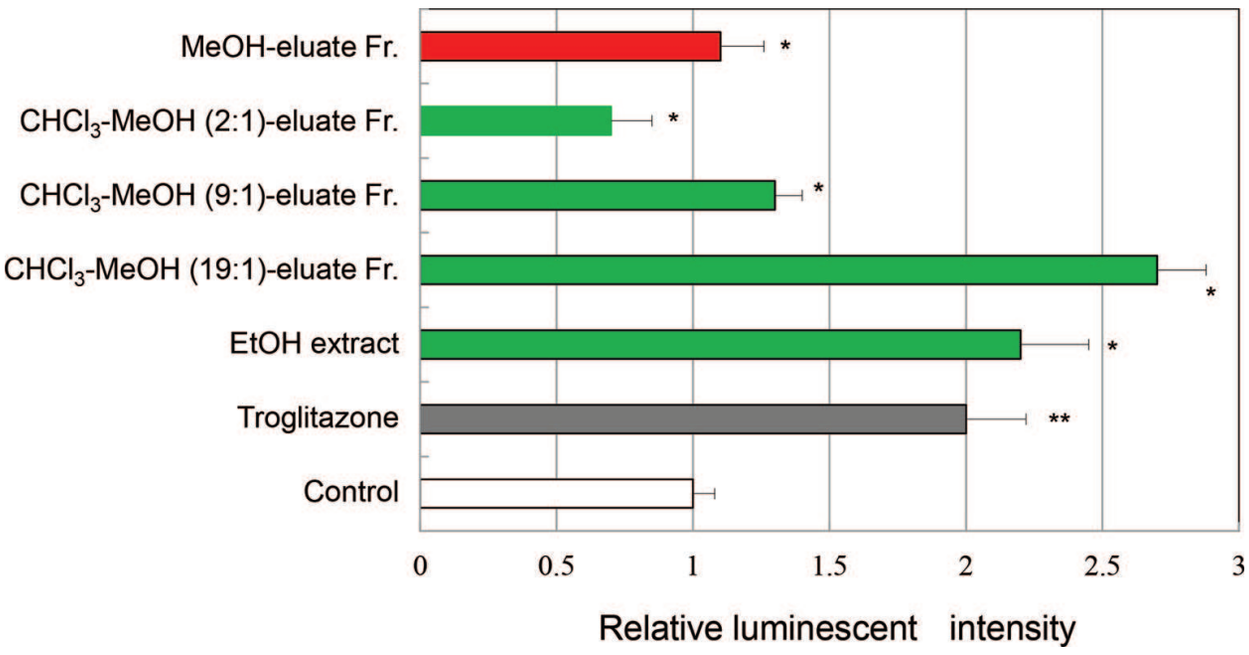

Figure 2. PPAR-γ ligand-binding activity of *G. glabra* extract and fractions [1]. PPAR-γ ligand-binding activity of the extract and fractions (5 μg/mL), as well as that of troglitazone (0.5 μM) used as a positive control, was measured using a GAL-4-PPAR-γ chimera assay. Statistical significance is indicated as * ($p < 0.05$) or ** ($p < 0.01$) as determined by Dunnett's multiple comparison test.

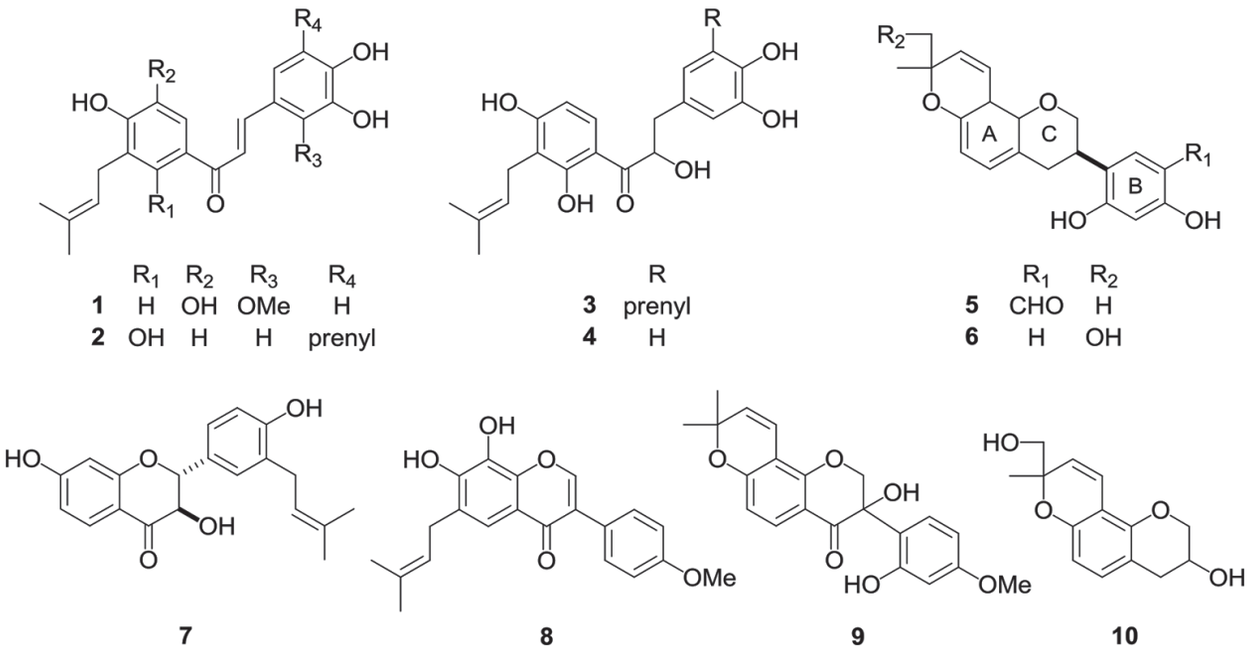

Figure 3. Structures of **1–10** isolated from *G. glabra* roots [1].

hispaglabridin A (**23**, 13.7 mg) [14], hispaglabridin B (**24**, 10.7 mg) [14], glabrene (**25**, 28.8 mg) [16], kanzonol W (**26**, 3.0 mg) [10], glabrocoumarin (**27**, 16.4 mg) [17], shinpterocarpin (**28**, 41.0 mg) [18], *O*-methylshinpterocarpin (**29**, 32.4 mg) [18], licoagrocarpin (**30**, 6.1 mg) [19], licoflavanone A (**31**, 4.8 mg) [20], glabrol (**32**, 13.5 mg) [13], shinflavanone (**33**, 7.8 mg) [18], euchrenone a5 (**34**, 1.5 mg) [21], xambioona (**35**, 8.0 mg) [22], gancaonin L (**36**, 8.8 mg) [23], glabrone (**37**, 15.5 mg) [24], kanzonol U (**38**, 21.6 mg) [11], and 8,8-dimethyl-3,4-dihydro-2*H*,8*H*-pyrano[2,3-*f*]-chromon-3-ol (**39**, 17.4 mg) [25] (**Figure 4**). The structures of isolated compounds were classified into 12 groups: chalcones (**1–4**, **11–16**), isoflavans (**5**, **6**, **17–24**), an isoflavone (**25**), 3-arylcoumarins (**26**, **27**), pterocarpans (**28–30**), a flavone (**31**), a flavanol (**7**), flavanones (**32–35**), isoflavones (**8**, **36**, **37**), an isoflavane (**9**), an 2-aryl benzofuran (**38**), and chromones (**10**, **39**). Compounds **13**, **15**, **17**, **18**, and **36** were isolated from *G. glabra* for the first time.

Compound **1** was isolated as a yellow amorphous powder with a formula of $C_{21}H_{22}O_6$ determined by high-resolution electrospray ionization mass spectrometry (HRESIMS), which showed an accurate $[M+H]^+$ ion at *m/z* 371.1487. Compound **1** had an absorption maxima at 366 and 248 nm in its UV spectrum and absorbance bands at 3427 cm^{-1} (hydroxy groups), 1625 cm^{-1} (conjugated carbonyl group), and 1595, 1507, and 1469 cm^{-1} (aromatic rings) in its IR spectrum. The ^1H NMR spectrum of **1** (acetone-d_6) contained 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 indicated 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 suggested that **1** was a chalcone derivative with four hydroxy groups, a methoxy group, and a prenyl group. Long-range correlations were observed in the heteronuclear multiple bond coherence (HMBC) spectrum of **1** (**Figure 5**) 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)

http://dx.doi.org/10.5772/67406

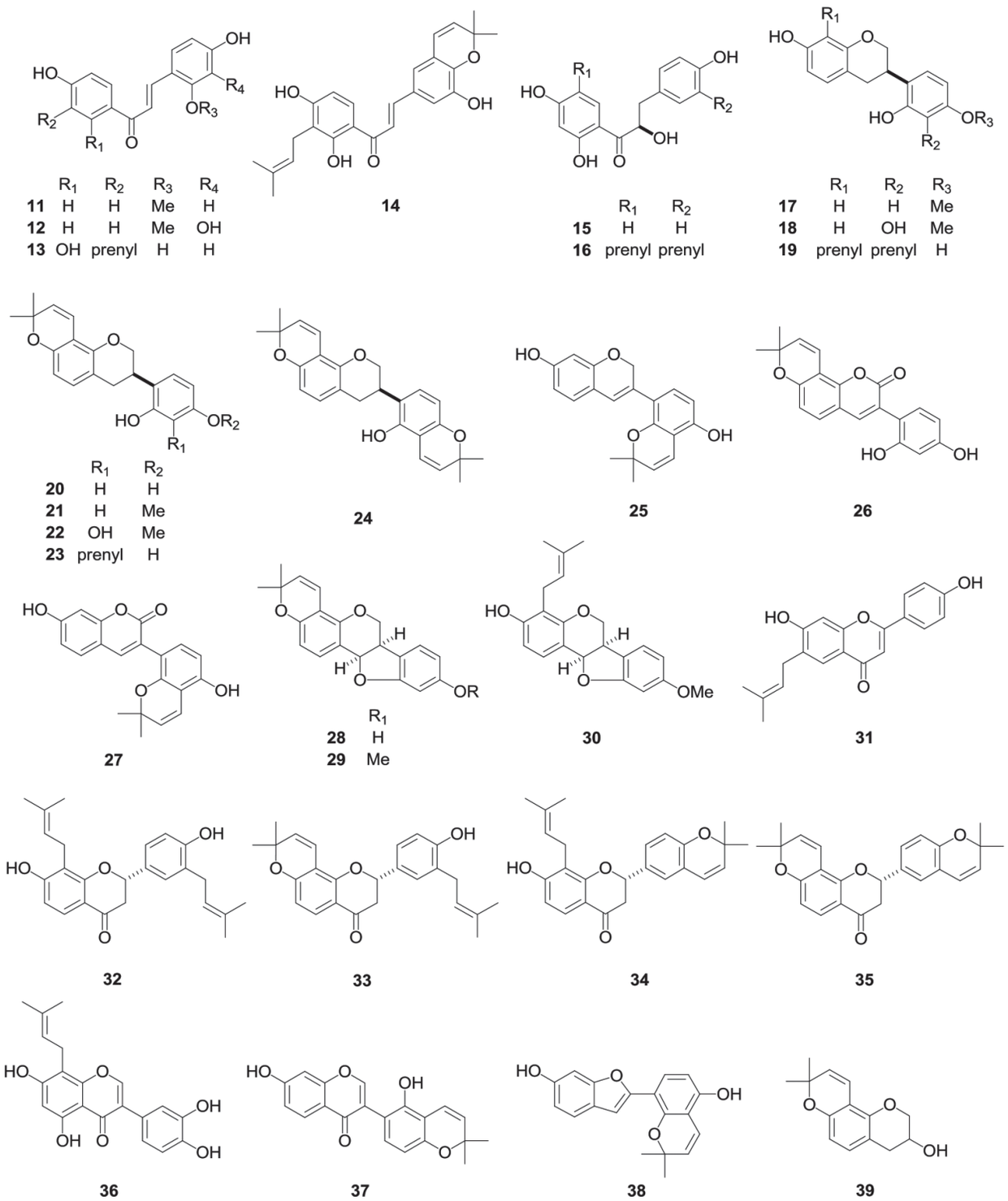

Figure 4. Structures of **11–39** isolated from *G. glabra* roots [1].

and between H-6 (δ_H 7.55) and C═O/C-1 (δ_C 130.7), indicating that two hydroxy groups were 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) and between H-2″ (δ_H 5.40) and C-5 (δ_C 128.2) indicated the existence of a prenyl group at C-5. The structure of the B-ring moiety attributed to 3′,4′-dihydroxy-2′-methoxyphenyl and its linkage to C-β of the *trans*-olefinic group were determined by HMBC correlations between

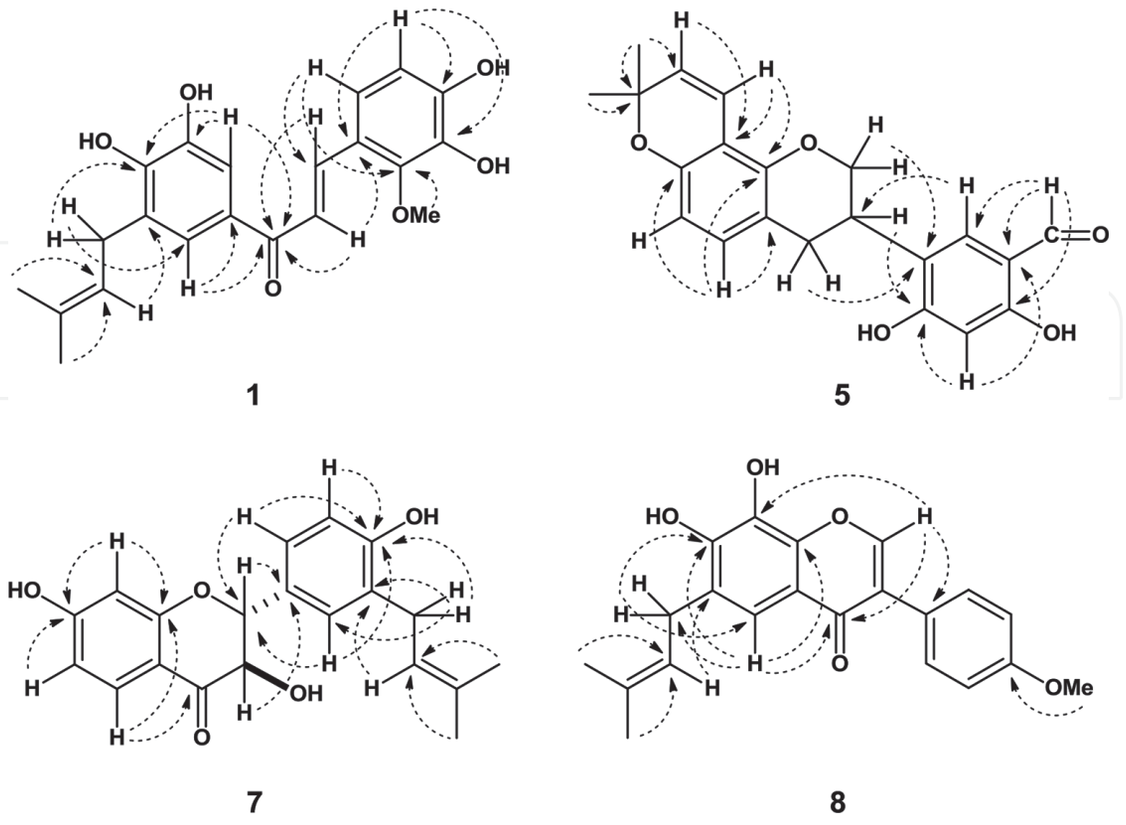

Figure 5. Key HMBC correlations of **1**, **5**, **7**, and **8** [1].

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), H-6′ (δ_H 7.25) and C-β (δ_C 138.4)/C-2′ (δ_C 148.7), and methoxy protons and C-2′. Therefore, the structure of **1** was assigned as 3,3′,4,4′-tetrahydroxy-2′-methoxy-5-prenylchalcone.

Compound **5** was isolated as a yellow amorphous powder with a molecular formula of $C_{21}H_{20}O_5$ determined by HRESIMS. The ^{1}H NMR spectrum of **5** indicated signals characteristic of 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). In addition, the spectrum of **5** indicated signals that we assigned 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 3H, s). Compound **5** and glabridin (**20**) have similar features in their ^{1}H NMR spectra. Moreover, the ^{1}H and ^{13}C NMR signals at δ_H 9.77 and δ_C 195.3 indicated the presence of a formyl group, which was attached at C-5′, as determined by the 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) (**Figure 5**). The circular dichroism (CD) profile of **5** was the same as that of synthetic 5′-formylglabridin prepared by formylation of **20**, indicating that the absolute configuration at C-3 was *R*. Therefore, the structure of **5** was assigned as 5′-formyl glabridin. It was notable that **5** was the first naturally occurring isoflavan with a formyl group in the B-ring portion of the compound.

Compound **7** was isolated as a yellow powder with a molecular formula of $C_{20}H_{20}O_5$ determined by HRESIMS. Compound **7** had an absorption maxima at 313 and 276 nm in its UV

http://dx.doi.org/10.5772/67406

spectrum and absorption bands at 3374 cm^{-1} (hydroxy groups), 1673 cm^{-1} (a carbonyl group), and 1608, 1502, and 1463 cm^{-1} (aromatic rings) in its IR spectrum. The ^1H NMR spectrum of **7** showed signals that we assigned 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, two 1,3,4-trisubstituted aromatic rings were identified from 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) and 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). The above data indicated that **7** was a dihydroxyflavan-3-ol derivative with a prenyl unit. The 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 H-1″ (δ_H 3.38) and C-2′ (δ_C 130.0)/C-3′ (δ_C 128.1)/C-4′ indicated that two hydroxy groups and a prenyl group were attached to C-7, C-4′, and C-3′, respectively (**Figure 5**). In the CD spectrum of **7**, the positive Cotton effects at 210, 240, and 334 nm and the negative Cotton effect at 304 nm indicated absolute configurations of 2*R* and 3*R* [26]. Therefore, the structure of **7** was identified as (2*R*,3*R*)-3,4′,7-trihydroxy-3′-prenylflavanone.

The following suggested that compound **8** ($C_{21}H_{20}O_5$) was an isoflavone derivative: a UV absorption maximum at λ_{max} 263 nm [27], a proton resonance at δ_H 8.16 (1H, s), and a corresponding oxygen-bearing olefinic carbon signal at δ_C 152.2. The ^1H NMR spectrum of **8** 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 × 2, br s)]. In the HMBC spectrum of **8** (**Figure 5**), 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), methoxy protons (δ_H 3.84) and C-4′ (δ_C 159.9), and H-2 (δ_H 8.16) and C-8 (δ_C 145.7), indicating that two hydroxy groups were attached to C-7 and C-8, a methoxy group to C-4′, and a prenyl group to C-6. Therefore, the structure of **8** was found to be 7,8-dihydroxy-4′-methoxy-6-prenylisoflavanone.

In the same way, the structures of **2–4**, **6**, **9**, and **10** were established as shown in **Figure 3**. Compounds **3**, **4**, and **9** showed neither specific rotation nor Cotton effects in their CD spectra, indicating that these compounds were racemates.

4. PPAR-γ ligand-binding activity of compounds 1–39 isolated from *G. glabra*

Compounds **5**, **7**, **11**, **18**, **19**, **26**, **28**, **31–33**, **36**, and **37** showed significant PPAR-γ ligand-binding activity. Among these compounds, the prenylflavone derivative licoflavanone A (**31**) was the most potent (**Figure 6**). These active compounds likely contributed the most to the PPAR-γ ligand-binding activity of the EtOH extract. The isoflavone derivative, kanzonol X (**19**), and flavanone derivative, glabrol (**32**), both had two prenyl units and exhibited potent ligand-binding activity. Hispaglabridin B (**24**) and xambioona (**35**), in which two prenyl units were cyclized to form two six-membered rings, exhibited weaker ligand-binding activities than **19** and **32** did, suggesting

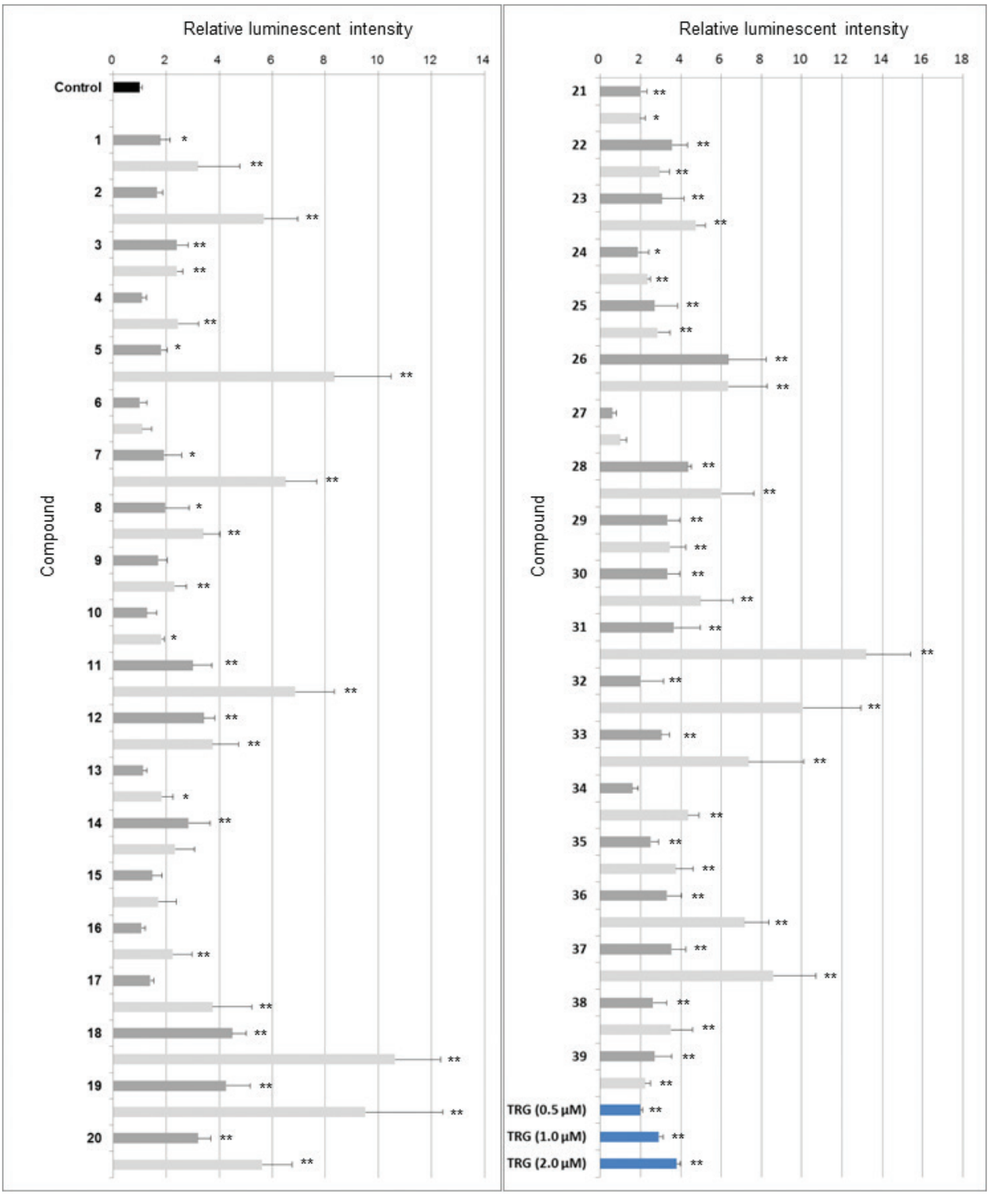

Figure 6. PPAR-γ ligand-binding activity of compounds **1–39** at 2 (■) and 10 (□) μg/mL with a GAL-4-PPAR-γ chimera assay [1]. Troglitazone (TRG) at 0.5, 1.0, and 2.0 μM was used as a positive control, and dimethyl sulfoxide at 1 mL/L was used as a solvent control. Values are means ± SD, $n = 4$ experiments. Statistical significance is indicated by * ($p < 0.05$) or ** ($p < 0.01$) as determined by Dunnett's multiple comparison test.

that the two non-cyclic prenyl moieties were necessary for the potent activity of these compounds (**Figure 7**). Taking together all the above data, the PPAR-γ ligand-binding activity of the phenolic compounds was affected by slight differences in the substitution groups on the aromatic rings.

http://dx.doi.org/10.5772/67406

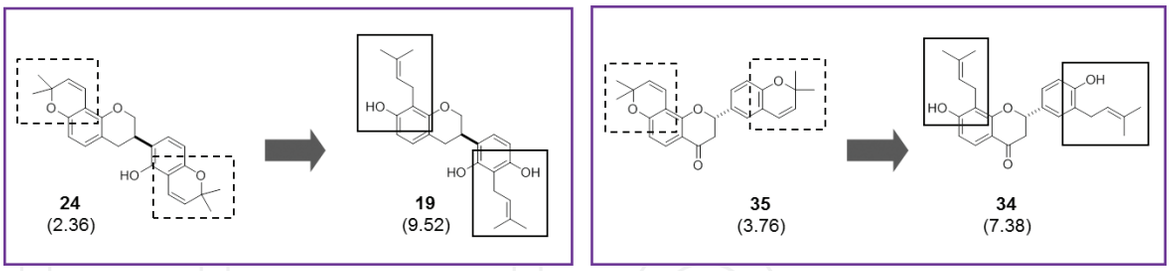

Figure 7. PPAR-γ ligand-binding activity of **19**, **24**, **34**, and **35** isolated from *G. glabra* roots [1]. Values in parentheses are the relative luminescence intensities at 10 μg/mL.

5. Isolation and structural determination of phenolic compounds from *G. uralensis*

The roots of *G. uralensis* (1.2 kg) were extracted with EtOAc (5.5 L) at room temperature for 7 days. The extract was then filtered and concentrated (74.0 g). The EtOAc extract of *G. uralensis* roots exhibited potent PPAR-γ ligand-binding activity and its relative luminescence intensity was 2.8 at a sample concentration of 30 μg/mL. This was almost equivalent to that of 1.0 μM TRG. The EtOAc extract was chromatographed on a silica gel column eluted with $CHCl_3$–MeOH gradients (19:1; 9:1; 4:1) and finally with MeOH to provide four fractions. PPAR-γ ligand-binding activity was concentrated in the $CHCl_3$-MeOH (19:1) eluate (relative luminescence intensity of 2.8 at 30 μg/mL). The $CHCl_3$-MeOH (19:1) eluate (55.4 g) was subjected to multiple chromatographic steps on a silica gel column eluted with $CHCl_3$-MeOH, an ODS silica gel column eluted with MeOH-H_2O and MeCN-H_2O, and to reversed phase HPLC using MeOH-H_2O and MeCN-H_2O mobile phases to obtain compounds **40–52** (22.2, 28.3, 58.7, 225, 80.7, 12.1, 43.5, 17.5, 11.8, 74.5, 51.0, 22.1, and 40.1 mg, respectively). Compounds **41–52** were identified by comparison of their physical and spectral data with those of reported compounds as dehydroglyasperin C (**41**) [28], glyasperin D (**42**) [29], glycycoumarin (**43**) [30], glycyrin (**44**) [31], glyasperin B (**45**) [29], glycyrol (**46**) [32], isoglycyrol (**47**) [32], glicoricone (**48**) [33], licoricone (**49**) [34], licocoumarone (**50**) [30], gancaonin I (**51**) [35], and liquiritigenin (**52**) [36] (**Figure 8**). This was the first isolation of glyasperin B (**45**) from *G. uralensis*. The structure of the new compound, **41**, was determined by 1D and 2D NMR spectroscopic analysis and

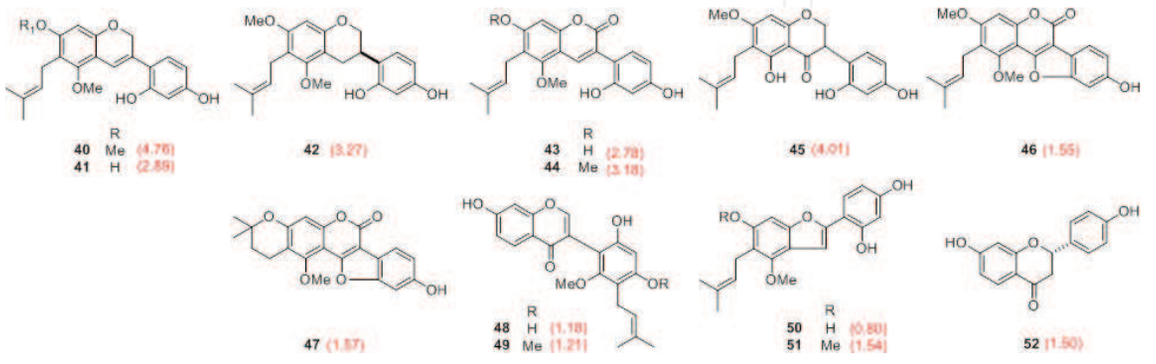

Figure 8. Structures of **40–52** isolated from *G. uralensis* roots [2]. Values in parentheses are the relative luminescence intensities at 5 μg/mL.

HRESIMS as 3-(2,4-dihydroxyphenyl)-5,7-dimethoxy-6-(3-methyl-2-butenyl)-2H-chromene and was named dehydroglyasperin D.

6. PPAR-γ ligand-binding activity of compounds 40–52 isolated from *G. uralensis*

Of the isolated compounds, the new compound **40** and known compounds **41**–**45** exhibited significant PPAR-γ ligand-binding activity (**Figure 6**). The activity of **40** at 5.0 μg/mL (=13.6 μM) was stronger than that of 2.0 μM TRG (relative luminescence intensity of 3.7). The coumestan derivative **46**, which was less active than **40**, was structurally similar to the active compound **43**, and the only detected difference between **43** and **46** was the formation of a five-membered ether ring between C-4 and C-2′ in **46**. This suggested that the presence of a hydroxy group at C-2′ in the isoflavan, isoflavene, or arylcoumarin skeleton is necessary for PPAR-γ ligand-binding activity. Furthermore, the isoflavones, **48** and **49**, which have a hydroxy group at C-2′ and no isoprenyl group at C-6, did not exhibit activity, suggesting that the isoprenyl group at C-6 was also involved in PPAR-γ ligand-binding activity. In conclusion, the isoprenyl group at C-6 and the C-2′ hydroxy group in the aromatic C ring of the isoflavan, isoflavene, or arylcoumarin skeleton were structural requirements for PPAR-γ ligand-binding activity (**Figure 9**).

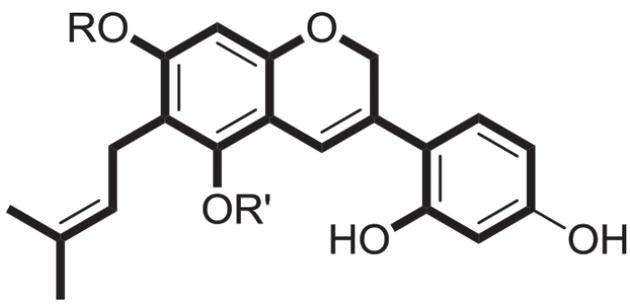

Figure 9. Structural requirements for the isoflavan skeleton for PPAR-γ ligand binding [2].

7. Ameliorative effects on diabetic KK-A^y mice

The ameliorative effects of glycyrin (**44**) in KK-A^y mice, an animal model of genetic type 2 diabetes, were studied using pioglitazone as a positive control. There was no difference in the food intake or body weight of mice between the treated groups and the control group. Test compound intake, calculated from the food intake and body weight of the mice, was approximately 100 mg/(kg day) in the glycyrin and glycyrol (**46**) groups and 23 mg/(kg day) in the pioglitazone group. Blood glucose levels significantly decreased after 4 days of feeding in both the glycyrin- and pioglitazone-treated groups compared to that in the control group, whereas the blood glucose levels of the glycyrol-treated group were comparable to those of the control group (**Table 1**).

http://dx.doi.org/10.5772/67406

	Control	Glycyrin (0.10%)	Glycyrol (0.10%)	Pioglitazone (0.02%)
Body weight (g)				
Day 0	52.6 ± 0.53	54.1 ± 1.78	52.6 ± 1.07	55.1 ± 0.69
Day 4	48.9 ± 0.48	50.4 ± 1.58	49.1 ± 1.02	53.6 ± 1.07
Day 7	50.4 ± 0.56	51.9 ± 1.62	50.3 ± 0.91	55.8 ± 1.50
Day 10	46.9 ± 0.42	48.1 ± 1.57	46.8 ± 0.08	52.5 ± 11.41
Average food intake (g/mouse/day)[b]	5.43	5.34	5.47	6.34
Average test compound intake (mg/kg/day)[c]	0	102	108	23
Blood glucose level (mg/dL)				
Day 0	476 ± 22	474 ± 27	427 ± 24	486 ± 26
Day 4	420 ± 14	278 ± 14**	421 ± 19	191 ± 6**

[a]Body weights and blood glucose levels are expressed as means ± SE of five mice.

[b]Calculated as (total food intake) (number of mice day).

[c]Calculated as (average food intake/average body weight of mice).

Statistical significance is indicated as ** ($P < 0.01$) as determined by Dunnett's multiple comparison test.

Table 1. Effect of feeding glycyrin (**44**) on KK-A^y mice in experiments for the preventing diabetes [2][a].

Pioglitazone, a potent PPAR-γ agonist that activates PPAR-γ, resulted in the improvement of insulin resistance and type 2 diabetes mellitus. Glycyrin exhibited significant PPAR-γ ligand-binding activity and appeared to reduce the blood glucose levels of KK-A^y mice by the same biological mechanism as pioglitazone. This finding was supported by the observation that glycyrol, structurally related to glycyrin but lacking PPAR-γ ligand-binding activity, failed to improve the hyperglycemia of KK-A^y mice.

8. Conclusion

Fractionation of the EtOH extract of *G. glabra* roots and the EtOAc extract of *G. uralensis* roots, guided by a GAL-4-PPAR-γ chimera assay method, resulted in the isolation of 52 phenolics, including 11 new compounds. The structures of the new compounds were determined by spectroscopic analysis. Of the isolated compounds, more than 10 phenolics exhibited significant PPAR-γ ligand-binding activity and the prenylflavone derivative, licoflavanone A (**31**), exhibited the most potent ligand-binding activity. The activity of these compounds at a sample concentration of 10 μg/mL was approximately three times greater than that of 0.5 μM TRG. Six phenolics were isolated from the EtOAc extract of *G. uralensis* roots as PPAR-γ ligands and one, glycyrin (**44**), reduced the blood glucose levels of genetically diabetic KK-A^y mice through its PPAR-γ ligand-binding activity. We have therefore discovered a possible new application of *G. glabra* and *G. uralensis* roots and their constituents for the amelioration of type 2 diabetes, a representative insulin resistance syndrome that is becoming a serious worldwide public health problem.

Author details

Minpei Kuroda* and Yoshihiro Mimaki

*Address all correspondence to: kurodam@toyaku.ac.jp

Department of Medicinal Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan

References

[1] Kuroda M, Mimaki Y, Honda S, Tanaka H, Yokota S, Mae T. Phenolics from *Glycyrrhiza glabra* roots and their PPAR-γ ligand-binding activity. Bioorganic & Medicinal Chemistry 2010; 18: 962–970.

[2] Kuroda M, Mimaki Y, Sashida Y, Mae T, Kishida H, Nishiyama T, Tsukagawa M, Konishi E, Takahashi K, Kawada T, Nakagawa K, Kitahara M. Phenolics with PPAR-γ ligand-binding activity obtained from licorice (*Glycyrrhiza uralensis* Roots) and ameliorative effects of glycyrin on genetically diabetic KK-Ay mice. Bioorganic & Medicinal Chemistry Letters 2003; 13: 4267–4272.

[3] Takahashi N, Kawada T, Goto T, Yamamoto T, Taimatsu A, Matsui N, Kimura K, Saito M, Hosokawa M, Miyashita K, Fushiki T. Dual action of isoprenols on activation of both PPARγ and PPARα in 3 T3-L1 adipocytes and HepG2 hepatocytes. FEBS Letter 2002; 514: 315–322.

[4] 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.

[5] Kajiyama K, Demizu S, Hiraga Y, Kinoshita K, Koyama K, Takahashi K, Tamura Y, Okada K, Kinoshita T. Two prenylated retrochalcones from *Glycyrrhiza inflate.* Pytochemistry 1992; 31: 3229–3232.

[6] Saitoh T, Shibata S. New type chalcone from licorice root. Tetrahedron Letters 1975; 16: 4461–4462.

[7] Delle Monache G, De Rosa M C, Scurria R, Vitali A, Cuteri A, Monacelli B, Pasqua G, Botta B. Comparison between metabolite productions in cell culture and in whole plant of *Maclura pomifera*. Phytochemistry 1995; 39: 575–580.

[8] Kinoshita T, Kajiyama K, Hiraga Y, Takahashi K, Tamura Y, Mizutani K. The isolation of new pyrano-2-arylbenzofuran derivatives from the root of *Glycyrrhiza glabra*. Chemical and Pharmaceutical Bulletin 1996; 44: 1218–1221.

[9] Ferrari F, Botta B, Alves de L R. Flavonoids and isoflavonoids from *Zollernia paraensis*. Phytochemistry 1983; 22: 1663–1664.

[10] Fukai T, Sheng C B, Horikoshi T, Nomura T. Isoprenylated flavonoids from underground parts of *Glycyrrhiza glabra*. Phytochemistry 1996; 43: 1119–1124.

[11] Gottlieb O.R., Braga de O.A., Goncalves T.M.M., De Oliveira G.G., Pereira S.A. Isoflavonoids from *Cyclolobium species*. Phytochemistry 1975; 14: 2495–2499.

[12] Song C, Zheng Z, Liu D, Hu Z. Antimicrobial isoflavans from *Astragalus membranaceus* (Fisch.) Bunge. Acta Botanica Sinica 1997; 39: 486–488.

[13] Saitoh T, Kinoshita T, Shibata S. New isoflavan and flavanone and licorice root. Chemical and Pharmaceutical Bulletin 1976; 24: 752–755.

[14] Mitscher L A, Park Y H, Omoto S, Clark G W, Clark D. Antimicrobial agents from higher plants, *Glycyrrhiza glabra* L. (var. *Spanish*). I. Some antimicrobial isoflavans, isoflavenes, flavanones and isoflavones. Heterocycles 1978; 9: 1533–1538.

[15] Kinoshita T, Kajiyama K, Hiraga Y, Takahashi K, Tamura Y, Mizutani K. Isoflavan derivatives from *Glycyrrhiza glabra* (Licorice). Heterocycles 1996; 43:581–588.

[16] Mitscher L A, Park Y H, Clark D, Beal J L. Antimicrobial agents from higher plants. Antimicrobial isoflavanoids and related substances from *Glycyrrhiza glabra* L. var. typica. Journal of Natural Products 1980; 43: 259–269.

[17] Kinoshita T, Tamura Y, Mizutani K. The isolation and structure elucidation of minor isoflavonoids from licorice of *Glycyrrhiza glabra* Origin. Chemical and Pharmaceutical Bulletin 2005; 53: 847–849.

[18] Kitagawa I, Chen W Z, Hori K, Harada E, Yasuda N, Yoshikawa M, Ren J. Chemical studies of Chinese licorice-roots. I. Elucidation of five new flavonoid constituents from the roots of *Glycyrrhiza glabra* L. collected in Xinjiang. Chemical and Pharmaceutical Bulletin 1994; 42: 1056-1062.

[19] Asada Y, Li W, Yoshikawa T. Isoprenylated flavonoids from hairy root cultures of *Glycyrrhiza glabra*. Phytochemistry 1998; 47: 389–392.

[20] Fukui H, Goto K, Tabata M. Two antimicrobial flavanones from the leaves of *Glycyrrhiza glabra*. Chemical and Pharmaceutical Bulletin 1988; 36: 4174–4176.

[21] Mizuno M, Tamura K, Tanaka T, Iinuma M. Six flavanones from the roots of *Euchresta formosana*. Phytochemistry 1989; 28: 2811–2812.

[22] Pereira M O da S, Fantine E C, De Sousa J R. Prenylated flavonoids from seeds of *Calopogonium mucunoides*. Phytochemistry 1982; 21; 488–489.

[23] Fukai T, Wang Q H, Takayama M, Nomura T. Structure of five new prenylated flavonoids L, M, N, O, and P from aerial parts of *Glycyrrhiza uralensis.* Heterocycles 1990; 31: 373–382.

[24] Kinoshita T, Saitoh T, Shibata S. The occurrence of an isoflavene and the corresponding isoflavone in licorice root. Chemical and Pharmaceutical Bulletin 1976; 24: 991–994.

[25] Nomura T, Fukai T. "Phenolic constituents of licorice (*Glycyrrhiza* Species)" in "Progress in the chemistry of organic natural products" Herz W, Kirby G W, Moore R E, Steglich W, Tamm C, Eds: Springer Wien: New York, 1998, Vol. 73, p. 27.

[26] Gaffield W. Circular dichroism, optical rotatory dispersion and absolute configuration of flavanones, 3-hydroxyflavanones and their glycosides: Determination of aglycone chirality in flavanone. Tetrahedron 1970; 26: 4093–4108.

[27] Kiuchi F, Chen X, Tsuda Y. Four new phenolic constituents from licorice (root of *Glycyrrhiza* sp.). Heterocycles 1990; 31: 629–636.

[28] Shibano M, Henmi A, Matsumoto Y, Kusano G, Miyase T, Hatakeyama Y. Studies on the index compounds for HPLC analysis of *Glycyrrhiza uralensis*. Heterocycles 1997; 45: 2053–2060.

[29] Zeng L, Fukai T, Nomura T, Zhang R Y, Lou Z C. Four new prenylated flavonoids, glyasperins A, B, C, and D from the roots of *Glycyrrhiza aspera*. Heterocycles 1992; 34: 575–587.

[30] Demizu S, Kajiyama K, Takahashi K, Hiraga Y, Yamamoto S, Tamura Y, Okada K, Kinoshita T. Antioxidant and antimicrobial constituents of licorice: Isolation and structure elucidation of a new benzofuran derivative. Chemical and Pharmaceutical Bulletin 1988; 36: 3474–3479.

[31] Kinoshita T, Saitoh T, Shibata S. A new 3-arylcoumarin from licorice root. Chemical and Pharmaceutical Bulletin 1978; 26: 135–140.

[32] Shiozawa T, Urata S, Kinoshita T, Saitoh T. Revised structures of glycyrol and isoglycyrol, constituents of the root of *Glycyrrhiza uralensis*. Chemical and Pharmaceutical Bulletin 1989; 37: 2239–2240.

[33] Hatano T, Fukuda T, Miyase T, Noro T, Okuda T. Phenolic constituents of licorice. III. Structures of glicoricone and licofuranone, and inhibitory effects of licorice constituents of monoamine oxidase. Chemical and Pharmaceutical Bulletin 1991; 39: 1238–1243.

[34] Saitoh T, Kinoshita T, Shibata S. Flavonols of licorice root. Chemical and Pharmaceutical Bulletin 1976; 24: 1242–1245.

[35] Fukai T, Wang Q H, Kitagawa T, Kusano K, Nomura T, Iitake Y. Structures of six isoprenoid-substituted flavonoids, gancaonins F, G, H, I, glycyrol, and isoglycyrol from Xibei licorice (*Glycyrrhiza* Sp.). Heterocycles 1989; 29: 1761–1772.

[36] Nakanishi T, Inada A, Kambayashi K, Yoneda K. Flavonoid glycosides of the roots of *Glycyrrhiza uralensis*. Phytochemistry 1985; 24: 339–341.