JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Evaluation of the Potential Hypoglycemic and Beta-Cell Protective Constituents Isolated from Corni Fructus To Tackle Insulin-Dependent Diabetes Mellitus

Mei-Hsiang Lin,[†,‡] Hui-Kang Liu,[‡,§] Wei-Jan Huang,[∥] Chu-Chun Huang,[∥] Tzu-Hua Wu,[†] and Fen-Lin Hsu*[,∥,⊥]

[†]College of Pharmacy, School of Pharmacy, Taipei Medical University, 250 Wuxing Street, Taipei 11031, Taiwan, ROC

[§]Division of Herbal Drugs and Natural Products, National Research Institute of Chinese Medicine, 155-1 Li-Nong Street, Section 2, Taipei 112, Taiwan, ROC

[∥]Graduate Institute of Pharmacognosy, School of Pharmacy, Taipei Medical University, 250 Wuxing St., Taipei 11031, Taiwan, ROC

[⊥]Department of Pharmacy, Taipei Medical University Hospital, 250 Wuxing Street, Taipei, Taiwan, ROC

ABSTRACT: Corni fructus is the fruit of *Cornus officinalis* Sieb. et Zucc. and has attracted much interest due to its traditional applications and active fraction that reportedly possesses antidiabetic effects. In this study, we isolated 12 compounds from Corni fructus including three flavonoids, two iridoid glycosides, three phenolic compounds, and two triterpenoids, together with cornuside (**11**) and 2-butoxybutanedioic acid (**12**). Chemical structures were identified by ^{1}H, ^{13}C NMR, DEPT, COSY, HSQC, and HMBC spectral analyses. Furthermore, the glucose uptake efficiency, messenger (m)RNA expression of phosphoenolpyruvate carboxykinase (PEPCK), and prevention of cytokine-mediated cytotoxicity in the presence of test agents were evaluated. While CH and CB significantly increased glucose uptake from muscle, compounds **3** and **8**, each at 50 μM, significantly suppressed PEPCK mRNA expression. Finally, compound **5**, at 50 and 100 μM, effectively attenuated β-cell death. In conclusion, those compounds could contribute to the antihyperglycemic and β-cell-protective actions of Corni fructus against diabetes mellitus.

KEYWORDS: diabetes mellitus, Liuwei Dihuang Wan, Corni fructus, phosphoenolpyruvate carboxykinase (PEPCK), beta cells, cytokine, glucose uptake

INTRODUCTION

Diabetes mellitus (DM), a complicated metabolic disease caused by hyperglycemia, results in a number of deleterious effects particularly on the macrovascular and microvascular systems.[1] Diabetic patients show a high level of blood glucose caused by an inability to produce insulin or its poor utilization. Diabetes is classified into type-1 diabetes, associated with an insulin deficiency from autoimmunity-mediated depletion of pancreatic β-cells, and type 2, which is associated with impaired secretion of insulin and its resistance.[2] Diabetes ranks high among the top 10 leading causes of death in the world. Long-term and serious complications often result in a high incidence of death. Diabetes treatments exhaust enormous amounts of resources including medicines, diets, physical training, and so on in all countries of the world. It is crucial to develop novel therapies with few side effects for long-term control of this disease.

Insulin plays a central role regulating glucose homeostasis via its actions in the liver and skeletal muscle. In the liver, hepatic glucose output is elevated during fasting and starvation periods mainly through gluconeogenesis, which is controlled by two rate-limiting enzymes of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6 Pase). Several hormones including glucocorticoids, retinoic acid, and glucagon (via its second messenger, cyclic (c)AMP)[3−6] enhance the hepatic transcription of both the PEPCK and G6 Pase genes. In contrast, insulin represses both genes[7−9] to prevent hepatic glucose overproduction.[10,11] Meanwhile, in a fed condition, secreted insulin also activates peripheral glucose uptake to utilize excessive blood glucose. Importantly, insulin activates similar signal transductions to facilitate its antihyperglycemic actions in the liver and muscle. With type-1 and late type-2 diabetes, chronic cytokine attack, glucolipotoxicity, and other extracellular stresses lead to a massive loss of β-cells.[12,13] Nitric oxide (NO), produced by inducible nitric oxide synthase (iNOS), could be considered as one of the key proinflammatory mediators for beta-cell dysfunction and destruction via affecting mitochondrial functions and later endoplasmic reticulum stress.[14,15] As a result, these patients can be diagnosed as having insulin-dependent DM (IDDM). Application of insulin mimetics or β-cell-protective agents can either directly control or delay the onset of dysglycemia in IDDM subjects.

In traditional Chinese medicine, Liuwei Dihuang Wan is a prescription for treating diabetic disorders.[16,17] It consists of six principals: the herbs dioscorea (Dioscoreae rhizoma), cornus (Corni fructus), alisma (Rhizoma alismatis), holelen (Poria), rehmannia (Rehmanniae radix), and tree peony bark (Moutan radicis cortex). This prescription is used alone or modified by adding additional components for diabetic therapy in China and Japan.[18−23] Studies revealed that Corni fructus extract has

Received: April 7, 2011
Accepted: June 19, 2011
Revised: June 13, 2011
Published: June 19, 2011

antidiabetic activity in rats with streptozotocin-induced IDDM[24,25] and is also regarded as the major active principal in the plasma glucose-lowering action of Die-Huang-Wan in normal rats.[26] Moreover, it was reported that a fraction of Corni fructus, the fruit of *Cornus officinalis* Sieb. et Zucc., has an antidiabetic effect.[27,28]

More than 150 compounds were isolated from Corni fructus, including iridoid glycosides,[29] saccharides, tannins,[30−32] flavonoids,[33,34] volatile oils,[35] amino acids, organic acids, vitamins, and other trace elements. Some components (i.e., morroniside, loganin, ursolic acid, oleanic acid, gallic acid, and epigallocatechin gallate (EGCG)) showed antidiabetic effects.[36−44]

Previously, we revealed that the methanol fraction of Corni fructus suppressed transcription of hepatic gluconeogenesis-related genes, and prevented toxin-mediated β-cell destruction.[45] However, the responsible active compounds remain to be elucidated. To address this issue, we further isolated pure comounds from Corni fructus to investigate whether those compounds possess antidiabetic activities related to regulating PEPCK messenger (m)RNA transcription and attenuate β-cell destruction caused by cytotoxic cytokines.

MATERIALS AND METHODS

Chemicals and Reagents. Antibiotics (100 U/mL penicillin and 0.1 g/L streptomycin) and primers were purchased from Invitrogen (Taipei, Taiwan). Phosphate-buffered saline (PBS) was from Sigma (St. Louis, MO, USA). RPMI-1640 tissue culture medium (supplemented with 0.3 g/L glutamine), fetal bovine serum (FBS), and trypsin/EDTA (10× 5.0 g trypsin (1:250) and EDTA in 2.0 g/L normal saline) were purchased from Biological Industries (Beit Haemek, Israel). Recombinant rat interleukin (IL)-1β and interferon (IFN)-γ were purchased from Peprotech (London, U.K.). A RevertAid First Strand cDNA Synthesis kit was purchased from Fermentase (Vilnius, Lithuania). TBE buffer (10× stock) and DNA ladders were bought from Bio-East (Taipei, Taiwan). All other reagents were purchased from either Sigma or BDH Chemicals (Grove, USA). All antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). BRIN-BD 11 cells were kindly provided by Prof. Peter R. Flatt (University of Ulster Coleraine, U.K.). Methanol, CH_2Cl_2, *n*-BuOH, *n*-hexane, EA, and acetone were purchased from Merck (Darmstadt, Germany).

General Chemical Experiment. The melting point was recorded on a Büchi B-545 melting point apparatus and was uncorrected. The optical rotation was measured on a Jasco DIP-1020 digital polarimeter. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AM-500 (500 MHz) FT-NMR spectrometer in D-solvent, using the solvent as internal standard. The EIMS was determined on a Finnigan TSQ-700 mass spectrometer. The ESIMS was determined on a VG Platform electrospray mass spectrometer. The FABMS was determined on a JEOL JMS-700 mass spectrometer. Column chromatography was carried out with Sephadex LH-20 (20−100 μm, Pharmacia Fine Chemicals, China), MCI-gel CHP 20P (75−150 μm, Mitsubishi Chemical Industries, Japan), Cosmosil C_{18}-OPN (75 μm, Nacalai Tesque, USA), Fractogel (TSK, HW-40(s)), and silica gel 60 (70−230 mesh, Merck). TLC was conducted on silica gel plates (60 F-254, Merck), and a 10% sulfuric acid solution was used as the visualizing agent on heating.

Plant Material. Corni fructus (the fruit of *C. officinalis*) was purchased from a market in Taipei, Taiwan. The plant was authenticated, and a voucher specimen was deposited by Dr. I-Jung Lee at the Herbarium of the National Research Institute of Chinese Medicine, Taiwan, with issue number NHP00947.

Extraction and Isolation. Dried *C. officinalis* fruit (5.9 kg) were extracted with 70% aqueous acetone (with a ratio of solvent volume/dry weight of about 2 mL/g) three times, and then concentrated into a residue (1.5 kg) under a vacuum at 45 °C. The residue was dissolved in H_2O, and partitioned with *n*-hexane, CH_2Cl_2, and *n*-BuOH to obtain four layers: CH (*n*-hexane layer), CM (CH_2Cl_2 layer), CB (*n*-BuOH layer), and CW (H_2O layer). The concentrated CH extract (2.4 g) was subjected to column chromatography over a silica gel column (0.5 × 30 cm) and eluted with CH_2Cl_2−MeOH (from 20/1 to 18/1) to afford oleanic acid (**9**, 0.14 g)[46] and a CM-M mixture. β-Sitosterol (**10**, 20 mg)[47] was obtained from the CM-M mixture (0.15 g) by silica gel column (0.5 × 30 cm) chromatography, using *n*-hexane−EA (from 6/1 to 8/1) as the solvent system. The concentrated CB extract (630 g) was subjected to column chromatography over an MCI-gel CHP 20P column (10.5 × 110 cm) and eluted with a step gradient system (H_2O−MeOH, 0−100%) to afford four fractions of CB-M1−4. Fraction CB-M1 (10.0 g) was divided into subfractions A1−9 by passage through a Sephadex LH-20 column (3.5 × 73 cm) and eluted with acetone−H_2O (from 100% to 92%). Fraction A7 (0.3 g) was further separated on an MCI-gel CHP 20P column (0.5 × 30 cm) and eluted with a step gradient system (H_2O−MeOH, 0−100%) to give five subfractions of B1−5. Quercetin-3-*O*-β-D-(6″-*n*-butyl glucuronide) (**2**, 90 mg)[48] was obtained from subfraction B3 (0.11 g) by Sephadex LH-20 column (2.0 × 39 cm) chromatography, using 40% MeOH as the solvent system. Fraction CB-M2 (4.2 g) was chromatographed on a Sephadex LH-20 column (3.0 × 73 cm) and eluted with 50% MeOH to give four fractions of C1−4. Fraction C2 (3.0 g) was divided into subfractions D1−12 by passage through an MCI-gel CHP 20P column (2.0 × 39 cm) and elution with a step gradient system (H_2O−MeOH, 0−100%). (7R)-*n*-Butyl morroniside (**5**, 0.12 g)[49] was produced from subfraction D12 (0.21 g) by silica gel column (2.0 × 39 cm) chromatography, using CH_2Cl_2−MeOH (from 15/1 to 12/1) as the solvent system. Fraction CB-M3 (12.13 g) was chromatographed on a Sephadex LH-20 column (4.5 × 75 cm) and eluted with a step gradient system (H_2O−MeOH, 0−100%) to obtain two fractions of CB-S1 and CB-S2. Fraction CB-S1 (0.75 g) was divided into subfractions E1−10 by passage through an MCI-gel CHP 20P column (1.8 × 48 cm) and elution with a step gradient system (H_2O−MeOH, 30−100%). The E10 fraction (0.23 g) was further separated through a Sephadex LH-20 column (3.0 × 73 cm) and a step gradient system (H_2O−MeOH, 0−100%) to afford quercetin 3-*O*-β-glucuronide (**1**, 90 mg).[48] Fraction CB-S2 (4.9 g) was divided into subfractions F1−10 by passage through a Sephadex LH-20 column (3.0 × 73 cm) and elution with aqueous acetone (from 2% to 4%). Compound **11** (cornuside, 1.90 g)[32] was yielded from subfraction F8 (3.3 g) by Fractogel column (3.0 × 73 cm) chromatography and elution with a step gradient system (H_2O−MeOH, 0−100%). Fraction CB-M4 (110 g) was subjected to column chromatography over a Sephadex LH-20 column (10 × 110 cm) and eluted with a step gradient system (H_2O−MeOH, 0−100%) to give fractions CB-X1−3. Fraction CB-X1 (3.5 g) was divided into subfractions G1−7 by passage over an MCI-gel CHP 20P column (3.5 × 73 cm), eluted with H_2O−MeOH (from 20% to 100%). Caffeic acid (**7**, 40 mg)[50] was afforded from subfraction G7 (96 mg) by Sephadex LH-20 column (0.5 × 30 cm) chromatography, using 2% aqueous acetone as the solvent system. Fraction CB-X2 (3.7 g) was further separated on an MCI-gel CHP 20P column (3.5 × 73 cm) and eluted with a step gradient system (H_2O−MeOH, 0−100%) to obtain subfractions CB-Y1 and CB-Y2. Subfraction CB-Y1 (0.2 g) was further separated on an MCI-gel CHP 20P column (0.5 × 30 cm; H_2O−MeOH, 10−100%) and a Cosmosil C_{18}−OPN column (0.5 × 30 cm; H_2O−MeOH, 10−100%) to obtain caftaric acid monomethyl ester (**8**, 90 mg).[51,52] Gallic acid (**6**, 100 mg)[53] was yielded from subfraction CB-Y2 (0.18 g) by Sephadex LH-20 column (0.5 × 30 cm) chromatography, using acetone as the solvent system. Fraction CB-X3 (90 g) was further separated on an MCI column (10 × 110 cm) and eluted with a step gradient system (H_2O−MeOH, 0−100%) to obtain subfractions CB-Z1 and CB-Z2. Subfraction CB-Z1 (11.0 g) was crystallized by

acetone-H_2O to give loganin (**4**, 10.0 g).[54] Both (−)-epicatechin 3-*O*-gallate (**3**, 30 mg)[55] and butoxysuccinic acid (**12**, 3.40 g)[56] were obtained from subfraction CB-Z2 (5.9 g) by Sephadex LH-20 column (3.5 × 75 cm) chromatography, using acetone as the solvent system.

Quercetin 3-O-β-D-Glucuronide (***1***). FAB-MS (negative-ion mode): *m/z* 477 ([M − H]$^-$). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.64 (1H, d, *J* = 2.2 Hz, H-2′), 7.62 (1H, dd, *J* = 8.6, 2.2 Hz, H-6′), 6.84 (1H, d, *J* = 8.6 Hz, H-5′), 6.40 (1H, d, *J* = 2.1 Hz, H-8), 6.21 (1H, d, *J* = 2.1 Hz, H-6), 5.33 (1H, d, *J* = 7.7 Hz, H-1″), 3.73 (1H, d, *J* = 9.7 Hz, H-5″), 3.44−3.60 (3H, m, H-2″, 3″, 4″) ^{13}C NMR (CD_3OD, 125 MHz): δ 179.3 (C-4), 172.2 (C-6″), 166.0 (C-7), 163.1 (C-5), 159.1 (C-2), 158.5 (C-9), 149.9 (C-4′), 146.0 (C-3′), 123.5 (C-6′), 122.9 (C-1′), 117.2 (C-2′), 105.7 (C-10), 104.2 (C-1″), 99.9 (C-6), 94.8 (C-8), 77.6 (C-3″), 77.1 (C-5″), 75.4 (C-2″), 72.9 (C-4″).

Quercetin 3-O-β-D-(6″-n-Butyl glucuronide) (***2***). FAB-MS (negative-ion mode): *m/z* 533 ([M − H]$^-$). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.61 (1H, dd, *J* = 8.6, 1.9 Hz, H-6′), 7.59 (1H, d, *J* = 1.9 Hz, H-2′), 6.83 (1H, d, *J* = 8.6 Hz, H-5′), 6.38 (1H, d, *J* = 1.7 Hz, H-8), 6.19 (1H, d, *J* = 1.7 Hz, H-6), 5.27 (1H, d, *J* = 7.7 Hz, H-1″), 4.05 (2H, *t*, *J* = 6.4, *n*-butyl H-1), 3.73 (1H, d, *J* = 9.5 Hz, H-5″), 3.58 (1H, dd, *J* = 9.1, 9.5 Hz, H-4″), 3.52 (1H, dd, *J* = 9.0, 7.7 Hz, H-2″), 3.45 (1H, dd, *J* = 9.0, 9.1 Hz, H-3″), 1.49 (2H, m, *n*-butyl H-3), 1.25 (2H, m, *n*-butyl H-2), 0.82 (3H, *t*, *J* = 7.4, *n*-butyl H-3). ^{13}C NMR (CD_3OD, 125 MHz): δ 179.2 (C-4), 170.2 (C-6″), 166.2 (C-7), 163.0 (C-5), 159.1 (C-2), 158.4 (C-9), 149.9 (C-4′), 146.0 (C-3′), 123.5 (C-6′), 122.9 (C-1′), 117.2 (C-2′), 105.6 (C-10), 104.4 (C-1″), 100.0 (C-6), 94.8 (C-8), 77.4 (C-3″), 77.2 (C-5″), 75.4 (C-2″), 72.7 (C-4″), 66.2 (*n*-butyl C-1), 31.5 (*n*-butyl C-2), 19.9 (*n*-butyl C-3), 13.9 (*n*-butyl C-4).

(−)-Epicatechin-3-O-gallate (***3***). ESI-MS: *m/z* 443 ([M + H]$^+$). ^{1}H NMR (CD_3OD, 500 MHz): δ 6.94 (2H, *s*, H-2″, 6″), 6.93 (1H, d, *J* = 2.0 Hz, H-2′), 6.80 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 6.69 (1H, d, *J* = 8.0 Hz, H-5′), 5.96 (2H, d, *J* = 2.6 Hz, H-6, 8), 5.51 (1H, *br*, H-3), 5.02 (1H, *s*, H-2), 2.92 (2H, dd, *J* = 17.3, 2.2 Hz, H-4). ^{13}C NMR (CD_3OD, 125 MHz): δ 167.6 (−COO-), 157.8 (C-7, 5), 157.3 (C-9), 146.3 (C-3″, 5″), 145.9 (C-4′, 3′), 139.8 (C-4″), 131.4 (C-1′), 121.5 (C-1″), 119.4 (C-6′), 116.0 (C-5′), 115.1 (C-2′), 110.2 (C-2″, 6″), 99.4 (C-10), 96.6 (C-6), 95.9 (C-8), 78.6 (C-2), 70.0 (C-3), 26.8 (C-4).

Loganin (***4***). ESI-MS: *m/z* 391 ([M + H]$^+$). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.37 (1H, *s*, H-3), 5.26 (1H, d, *J* = 4.4 Hz, H-1), 4.64 (1H, d, *J* = 7.9 Hz, H-1′), 4.03 (1H, *t*, *J* = 4.05 Hz, H-7), 3.88 (1H, dd, *J* = 1.4, 11.9 Hz, H-6′), 3.68 (3H, *s*, H-12), 3.65 (1H, dd, *J* = 5.6, 11.9 Hz, H-6), 3.34 (1H, m, H-3′), 3.29 (1H, m, H-5′), 3.27 (1H, m, H-4′), 3.18 (1H, m, H-2′), 3.10 (1H, *q*, *J* = 8.0 Hz, H-5), 2.22 (1H, m, H-6), 2.02 (1H, m, H-9), 1.86 (1H, m, H-8), 1.61 (1H, m, H-6), 1.09 (3H, *s*, H-10). ^{13}C NMR (CD_3OD, 125 MHz): δ 169.5 (C-11), 152.1 (C-3), 114.0 (C-4), 100.0 (C-1′), 97.7 (C-1), 78.4 (C-5′), 78.0 (C-3′), 75.1 (C-7), 74.7 (C-2′), 71.6 (C-4′), 62.8 (C-6′), 51.6 (C-12), 46.5 (C-9), 42.7 (C-6), 42.2 (C-8), 32.2 (C-5), 13.4 (C-10).

(7R)-n-Butyl Morroniside (***5***). White amorphorus solid. FAB-MS: *m/z* 485.3 ([M + Na]$^+$), 463.3 ([M + H]$^+$). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.50 (1H, *s*, H-3), 5.88 (1H, d, *J* = 9.2 Hz, H-1), 4.77 (1H, d, *J* = 7.8 Hz, H-1′), 4.79 (1H, overlapped by solvent, H-7), 4.38 (1H, m, H-8), 3.88 (1H, dd, *J* = 1.6, 12.1 Hz, H-6′), 3.68 (3H, *s*, H-12), 3.65 (1H, m, H-6′), 3.63 (2H, m, *n*-butyl H-1), 3.43−3.20 (4H, m, H-2′,3′,4′,5′), 3.07 (1H, *dt*, *J* = 4.6, 12.8 Hz, H-5), 1.91 (1H, dd, *J* = 4.6, 13.0 Hz, H-6), 1.82 (1H, m, H-9), 1.62−1.41 (5H, m, H-6, *n*-butyl H-2′, 3′), 1.33 (3H, d, *J* = 7.0 Hz, H-10), 0.95 (3H, *t*, *J* = 7.4 Hz, *n*-butyl H-4). ^{13}C NMR (CD_3OD, 125 MHz): δ 168.8 (C-11), 154.5 (C-3), 111.7 (C-4), 100.1 (C-1′), 98.4 (C-7), 95.7 (C-1), 78.5 (C-3′), 78.0 (C-5′), 75.0 (C-4′), 71.7 (C-2′), 68.2 (*n*-butyl C-1), 66.4 (C-8), 62.8 (C-6′), 51.8 (C-12), 40.4 (C-9), 34.0 (C-6), 32.8 (*n*-butyl C-2), 28.1 (C-5), 20.5 (*n*-butyl C-3), 19.6 (C-10), 14.2 (*n*-butyl C-4).

Gallic Acid (***6***). EI-MS: *m/z* 170 (M$^+$). ^{1}H NMR (acetone-d_6, 500 MHz): δ 7.15 (2H, *s*, H-2, 6). ^{13}C NMR (acetone-d_6, 125 MHz): δ 167.9 (C-7), 145.8 (C-3, 5), 138.5 (C-4), 121.9 (C-1), 110.0 (C-2, 6).

Caffeic Acid (***7***). EI-MS: *m/z* 180 (M$^+$). ^{1}H NMR (acetone-d_6, 500 MHz): δ 7.53 (1H, d, *J* = 15.9, H-7), 7.15 (1H, d, *J* = 1.9, H-2), 7.03 (1H, dd, *J* = 1.9, 8.2, H-6), 6.88 (1H, d, *J* = 8.2, H-5), 6.26 (1H, d, *J* = 15.9, H-8). ^{13}C NMR (acetone-d_6, 125 MHz): δ 168.2 (C-9), 148.6 (C-4), 146.2 (C-3), 145.9 (C-7), 127.7 (C-1), 122.4 (C-6), 116.3 (C-5), 115.8 (C-8), 115.1 (C-2).

Caftaric Acid Monomethyl Ester (***8***). EI-MS: *m/z* 326 (M$^+$). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.63 (1H, d, *J* = 16.0, H-7), 7.16 (1H, d, *J* = 1.9, H-2), 7.05 (1H, dd, *J* = 1.9, 8.1, H-6), 6.87 (1H, d, *J* = 8.1, H-5), 6.29 (1H, d, *J* = 16.0, H-8), 5.53 (1H, d, *J* = 2.5, H-2′), 4.85 (1H, d, *J* = 2.5, H-3′). ^{13}C NMR (CD_3OD, 125 MHz): δ 171.8 (C-4′), 168.5 (C-1′), 166.3 (C-9), 148.9 (C-4), 147.2 (C-7), 146.2 (C-3), 127.4 (C-1), 122.8 (C-6), 116.3 (C-5), 115.2 (C-2), 114.3 (C-8), 73.8 (C-2′), 71.7 (C-3′).

Oleanolic Acid (***9***). EI-MS: *m/z* 456 (M$^+$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.28 (1H, *t*, *J* = 3.4 Hz, H-12), 3.20 (1H, dd, *J* = 11.4, 4.2 Hz, H-3), 2.80 (1H, dd, *J* = 14.0,4.0 Hz, H-18), 1.11 (3H, *s*, H-27), 0.92 (3H, *s*, H-25), 0.90 (3H, *s*, H-30), 0.88 (3H, *s*, H-29), 0.84 (3H, *s*, H-23), 0.82 (3H, *s*, H-24), 0.72 (3H, *s*, H-26). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.1 (C-28), 143.6 (C-13), 122.6 (C-12), 79.0 (C-3), 55.3 (C-5), 47.5 (C-9), 46.5 (C-17), 45.8 (C-19), 41.6 (C-14), 40.9 (C-18), 39.3 (C-8), 38.1 (C-1), 37.7 (C-4), 37.0 (C-10), 33.8 (C-21), 33.0 (C-29), 32.5 (C-22), 32.5 (C-7), 30.7 (C-20), 28.1 (C-23), 27.7 (C-15), 25.0 (C-27), 23.6 (C-30), 23.5 (C-2), 23.4 (C-11), 22.9 (C-16), 18.2 (C-6), 17.1 (C-26), 15.5 (C-24), 15.3 (C-25).

β-Sitosterol (***10***). EI-MS: *m/z* 414 (M$^+$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.35 (1H, m, H-6), 3.52 (1H, m, H-3), 1.02 (3H, *s*, H-19), 0.92 (3H, d, *J* = 6.6 Hz, H-21), 0.85 (3H, *t*, H-29), 0.82 (3H, d, H-26), 0.80 (3H, d, H-27), 0.68 (3H, *s*, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.1 (C-9), 45.9 (C-24), 42.3 (C-13), 42.3 (C-4), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.1 (C-20), 34.0 (C-22), 31.9 (C-7), 31.9 (C-8), 31.6 (C-2), 29.2 (C-25), 28.2 (C-16), 26.1 (C-23), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.8 (C-21), 12.0 (C-29), 11.9 (C-18).

Cornuside (***11***). ESI-MS: *m/z* 541 ([M + H]$^+$). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.47 (1H, s, H-3), 7.04 (2H, s, H-2′, 6′), 5.79 (1H, ddd, *J* = 8.4, 10.1, 17.3 Hz, H-8), 5.55 (1H, d, *J* = 6.6 Hz, H-1), 5.30 (1H, d, *J* = 17.3 Hz, H-10), 5.25 (1H, d, *J* = 10.1 Hz, H-10), 4.71 (1H, d, *J* = 7.9 Hz, H-1″), 4.24 (2H, m, H-7), 3.90 (1H, dd, *J* = 11.9, 1.5 Hz, H-6″), 3.66 (1H, dd, *J* = 11.9, 6.0 Hz, H-6″), 3.61 (3H, s, H-12), 3.38 (1H, dd, *J* = 8.8, 8.9 Hz, H-3″), 3.31 (1H, overlapped by solvent, H-5″), 3.26 (1H, dd, *J* = 8.8, 9.4 Hz, H-4″), 3.21 (1H, dd, *J* = 7.9, 8.9 Hz, H-2″), 2.94 (1H, q, *J* = 6.5, H-5), 2.66 (1H, q, *J* = 6.6, 8.4, H-9), 2.05 (1H, *dq*, *J* = 7.0, 13.9 Hz, H-6), 1.90 (1H, *dq*, *J* = 6.5, 13.9 Hz, H-6). ^{13}C NMR (CD_3OD, 125 MHz): δ 169.2 (C-11), 168.5 (C-7′), 153.7 (C-3), 146.4 (C-3′, 5′), 139.7 (C-4′), 135.6 (C-8), 121.6 (C-1′), 119.6 (C-10), 111.4 (C-4), 110.1 (C-2′, 6′), 100.1 (C-1″), 97.6 (C-1), 78.2 (C-5″), 77.9 (C-3″), 74.6 (C-2″), 71.5 (C-4″), 64.3 (C-7), 62.7 (C-6″), 51.8 (C-12), 45.3 (C-9), 31.6 (C-5), 30.2 (C-6).

Butoxysuccinic Acid (***12***). EI-MS: *m/z* 190 (M$^+$). ^{1}H NMR (acetone-d_6, 500 MHz): δ 4.48 (1H, m, H-2), 4.09 (2H, m, H-1′), 2.73 (2H, m, H-3), 1.58 (2H, m, H-2′), 1.35 (2H, m, H-3′), 0.87 (3H, m, H-4′). ^{13}C NMR (acetone-d_6, 125 MHz): δ 173.7 (C-4), 172.4 (C-1), 68.0 (C-2), 65.4 (C-1′), 52.3 (C-3), 31.2 (C-2′), 19.5 (C-3′), 13.8 (C-4′).

Measurement of Glucose Uptake into the Rat Soleus Muscle. Glucose uptake was determined using the uptake of the radioactive glucose analogue, 2-[1-^{14}C]-deoxy-D-glucose (2-DG) (New England Nuclear, Boston, MA, USA), as previously described.[57] Animals were sacrificed by cervical dislocation, and the soleus muscle was quickly excised using a pair of scissors, dissected free of any adjoining connective tissue, blotted, and divided into long longitudinal strips (35−25 mg per strip). Muscles were placed in 3 mL of Krebs−Ringer bicarbonate buffer

(KRBB) (37 °C, pH 7.4), containing 1 mmol/L glucose and 1% fatty acid-free bovine serum albumin (BSA) under aeration with 5% CO_2 in O_2. After preincubation for 30 min, the muscle tissue was incubated with four concentrated partitioned layers at the desired concentrations for 30 min and then with 50 μL of KRBB containing 2-DG (1 μCi/mL) for 5 min at 37 °C in a shaking water bath under aeration. The reaction was terminated by quickly blotting the muscles and dissolving them in 0.5 mL of 0.5 N NaOH for 45 min before neutralization with 0.5 mL of 0.5 N HCl. After centrifugation, 800 μL of each supernatant was mixed with 1 mL of aqueous counting scintillant, and the radioactivity was determined using a β-counter (Beckman LS6000, Chaska, MN, USA). Nonspecific uptake of 2-DG, assessed after incubation with 20 μmol/L cytochalasin B (Sigma Chemical) for blocking transportation,[57] was subtracted from the total muscle-associated radioactivity.

Measurement of Gene Expression. Total RNA was extracted using the TRI reagent according to the manufacturer's instructions. Pelleted RNA was dissolved in distilled water, quantified with the GeneQuant II RNA/DNA calculator (Pharmacia Biotech, Uppsala, Sweden) and stored at −70 °C. Total RNA (1 μg) was reverse-transcribed to generate templates. Fifty nanograms of complementary (c)DNA was employed for a further polymerase chain reaction (PCR). The sequences of primers for β-actin were 5′-CGTAAAGACCTC-TATGCCAA-3′ and 5′-AGCCATGCCAAATGTGTCAT-3′ and for PEPCK were 5′-AAGGCCGCACCATGTATGTC-3′ and 5′-AG-CAGTGAGTTCCCACCGTAT-3′. In addition, annealing temperatures for amplification of β-actin (57 °C) and PEPCK (57 °C) were employed to generate polymerase chain reaction (PCR) products of 349 and 319 bp, respectively. Once the reaction was completed, PCR products were separated by gel electrophoresis, visualized, photographed with a digital camera, and quantified with Genetools 3.06 (Syngene, Frederick, MD, USA).

Cultivation of BRIN-BD11 and H4IIE Cells. The rat insulin-secreting cell line, BRIN-BD11, was routinely cultured with RPMI-1640 containing 2 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, supplemented with 10% (v/v) FBS (GIBCO, Carlsbad, CA) and 1% (v/v) antibiotics. The rat liver cell line H4IIE was cultured with Dulbecco's modified Eagle's medium (DMEM) containing 1000 mg/L glucose, 5% (v/v) FBS and 1% (v/v) antibiotics. Both cell lines were maintained at 37 °C in an atmosphere of 5% CO_2 and 95% air.

Toxicity Test. The toxicity test was previously described.[58] In brief, BRIN-BD11 cells were trypsinized and seeded into 24-well plates at a density of 1.5×10^5 cells/well. After overnight attachment, cells were incubated with a cytokine mix (rat IL-1β and IFN-γ) at 5 and 100 ng/mL, respectively, in the presence and absence of the isolated compounds. The viability after treatment was determined by a neutral-red assay. It should be noted that the cytokine mix was administrated for 48 h before viability was measured. Acidic ethanol was used to dissolve the imported neutral-red dye, and the absorbance at 540 nm was measured by a Power Wave XS microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA). The absorbance of the control condition was considered to be 100% viable.

Statistical Analysis. The significance of various treatments was determined by Student's *t*-test. The results are expressed as the mean ± standard error of the mean (SEM). Differences were considered significant at $p < 0.05$.

RESULTS

Purification of Constituents from Corni Fructus. The 70% aqueous acetone extract of Corni fructus was successively partitioned with water, *n*-hexane, CH_2Cl_2, and *n*-BuOH to give four layers: a water layer (CW), an *n*-hexane layer (CH), a CH_2Cl_2 layer (CM), and an *n*-BuOH layer (CB). Further compound isolation produced two compounds of **9** and **10** from the *n*-hexane extract. In addition, ten compounds, **1**−**8**, **11**, and **12**, from the *n*-BuOH extract were obtained with yields ranging from 0.02 to 10.0 g (Table 2). The structures of all compounds were elucidated by spectral analyses and identified as known compounds by comparison to data reported in the literature.[44−54] They included three flavonoids of quercetin 3-*O*-β-glucuronide (**1**), quercetin-3-*O*-β-D-(6″-*n*-butyl glucuronide) (**2**), and (−)-epicatechin 3-*O*-gallate (**3**); two iridoid glycosides of loganin (**4**) and (7*R*)-*n*-butyl morroniside (**5**); three phenolic compounds of gallic acid (**6**), caffeic acid (**7**), and caftaric acid monomethyl ester (**8**); two triterpenoids of oleanic acid (**9**) and β-sitosterol (**10**); together with cornuside (**11**) and 2-butoxybutanedioic acid (**12**) (Figure 1). The major component of Corni fructus was loganin (**4**) and yielded 0.17% (10.0 g).

Table 1. Effect of Partitioned Layers from Corni Fructus on Glucose Uptake in Isolated Skeletal Muscle from Rats with STZ-Induced Diabetes[a]

	mean (%) ± SEM
control	100 ± 5.05
CW	106.35 ± 9.45
CH	128.55 ± 9.26**
CM	108.06 ± 7.31
CB	126.66 ± 6.36**
insulin	152.86 ± 6.49***

[a] Values (mean ± SEM) were obtained from each group of eight experiments (**$p < 0.01$, ***$p < 0.001$ vs the control). CW, water layer; CH, *n*-hexane layer; CM, CH_2Cl_2 layer; CB, *n*-BuOH layer.

Table 2. Yield of Compounds 1−12 from Corni Fructus

compd name	no.	wt[a] (g)	yield[a] (%)
Flavonoids			
quercetin 3-*O*-β-D-glucuronide	**1**	0.09	1.5×10^{-3}
quercetin 3-*O*-β-D-(6″-*n*-butyl glucuronide)	**2**	0.09	1.5×10^{-3}
(−)-epicatechin-3-*O*-gallate	**3**	0.03	5.1×10^{-4}
Iridoid Glycosides			
loganin	**4**	10.0	1.7×10^{-1}
(7*R*)-*n*-butyl morroniside	**5**	0.12	2.0×10^{-3}
Phenolic Compounds			
gallic acid	**6**	0.10	1.7×10^{-3}
caffeic acid	**7**	0.04	6.7×10^{-4}
caftaric acid monomethyl ester	**8**[b]	0.09	1.5×10^{-3}
Triterpenoids			
oleanolic acid	**9**	0.14	2.4×10^{-3}
β-sitosterol	**10**	0.02	3.4×10^{-4}
Others			
cornuside	**11**	1.90	3.2×10^{-2}
butoxysuccinic acid	**12**[b]	3.40	5.7×10^{-2}

[a] From 5.9 kg of Corni fructus. [b] First isolated from a natural plant.

Evaluation of Insulin-Mimetic Constituents of Corni Fructus. Insulin-mimetic hypoglycemic activities include both stimulation of glucose uptake and inhibition of hepatic gluconeogenesis. Initial exploration of insulin-mimetic partitions from Corni fructus was carried out using a glucose uptake assay. As shown in Table 1, in the presence of two layers, CH and CB,

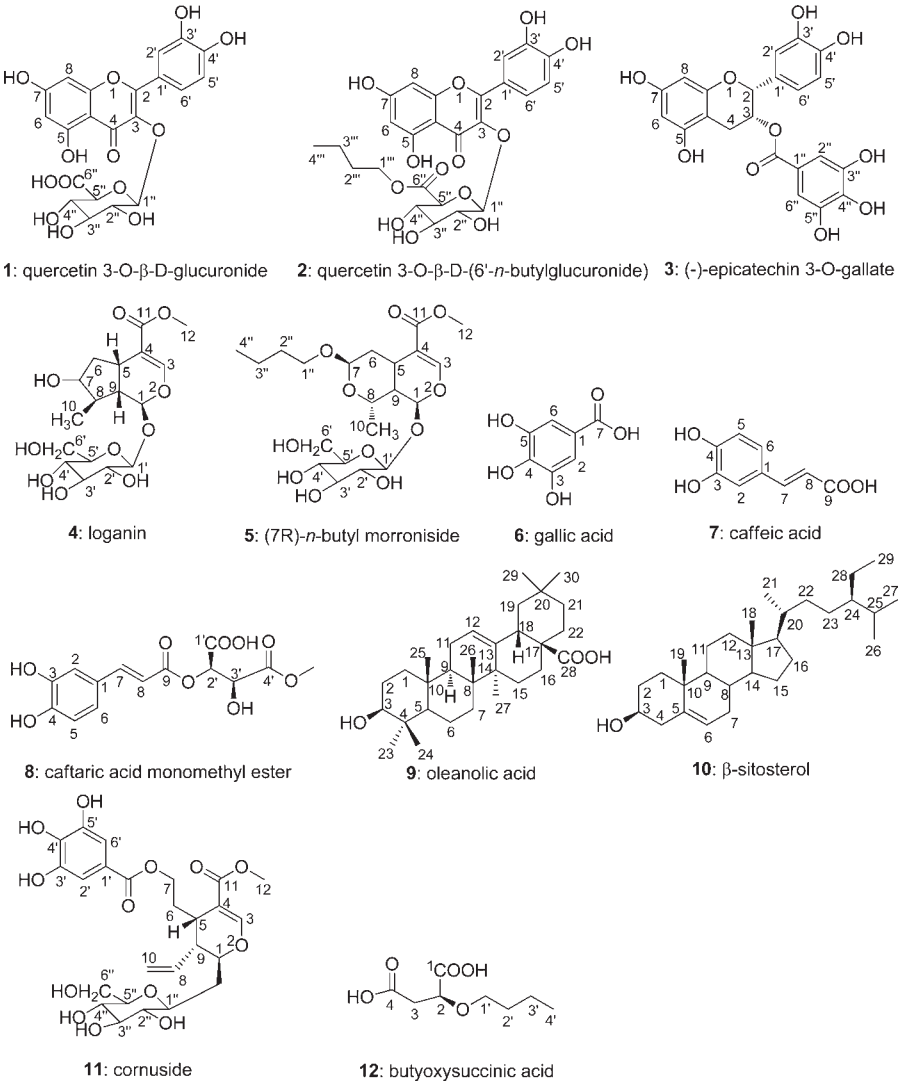

Figure 1. Chemical structures of the isolated compounds **1**−**12** from Corni fructus.

glucose uptake in rat soleus muscle was significantly stimulated, although the stimulatory efficacy was less than that of insulin. Purified constituents from CH and CB were further employed to measure the inhibitory activities of PEPCK mRNA transcription in H4IIE cells under stimulation with dexamethasone and 8-bromo-cAMP. In Figure 2A, similar to insulin's action, most compounds, except **1**, at a concentration of 50 μM effectively inhibited PEPCK mRNA expression induced by dexamethasone and 8-bromo-cAMP. Among those tested compounds, dose-dependent effects of compounds **3** and **8** exhibited comparable activities to that of insulin. As shown in Figure 2B, compounds **3** and **8** at concentrations of 12.5−100 μM showed 10−20% inhibition. Such activity was similar to that of insulin but less potent than that of EGCG (50 μM).

Evaluation of β-Cell Protective Bioactivity of Insulin Mimetics Identified from Corni Fructus. In the presence of the cytokine mix (IL-1β and IFN-γ), significant β-cell apoptosis occurred. Therefore, this cell-based system was used to further evaluate possible β-cell protective properties of preselected insulin-mimetic compounds. As shown in Figure 3A, seven insulin-mimetic compounds, **3**−**6**, **9**, **10**, and **12**, were tested for cytokine-mediated cell death. Compounds **5** and **6** had attenuated cytokine mediated β-cell death by >50% over a period of 48 h at a concentration of 50 μM. A dose-dependent effect of compound **5** at concentrations of 50−100 μM on cytokine-mediated cell death is shown in Figure 3B, and compound **5** demonstrated >50% protection. On the other hand, nitric oxide (NO) production is also an outcome of an inflammatory response after cytokine exposure. Therefore, anti-inflammatory activities of those compounds were also analyzed. As shown in Figure 4A, cytokine-mediated NO production was significantly inhibited by compounds **3**, **5**, and **9**. While compound **5** had similar activity to that of EGCG, compounds **3** and **9** had better activity than EGCG. As shown in Figure 4B, the inhibitory effects of both compounds were dose-dependent but did not reach the level of the unstimulated condition (control).

DISCUSSION

As mentioned previously, insulin normalizes hyperglycemia via stimulating glucose absorption and suppressing glucose production. As a result, dysglycemia in IDDM occurs as a result of insulin deficiency due to the loss of β-cells. Both type-1 diabetes and late type-2 diabetes can be considered IDDM

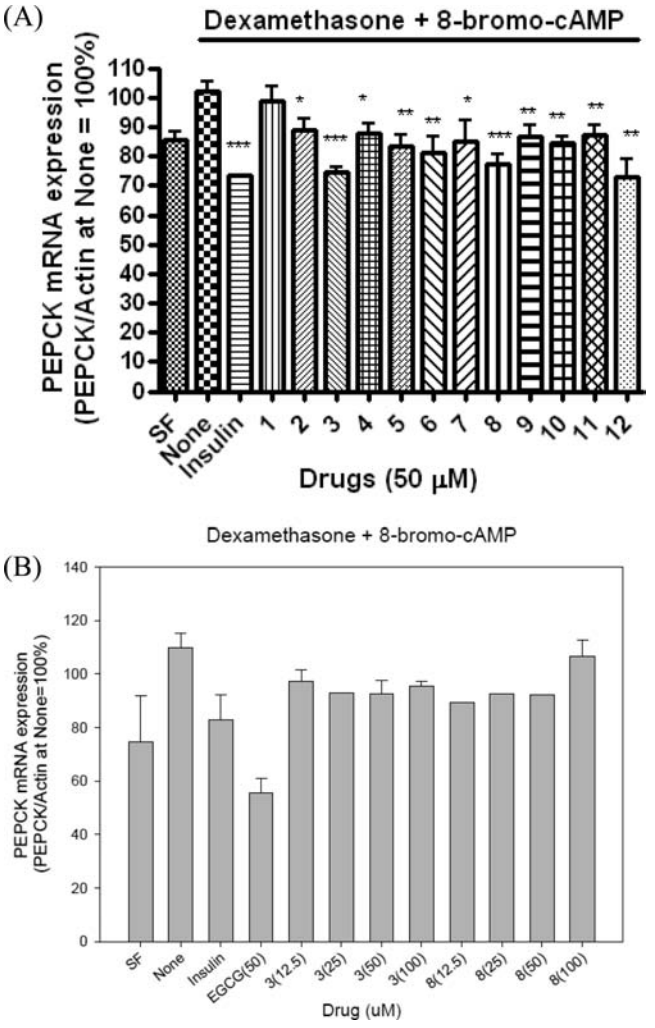

Figure 2. (A) Compounds **1**−**12** mimic insulin's action on PEPCK expression induced by dexamethasone and 8-bromo-cAMP. Quantified results of dexamethasone (500 nM) and 8-bromo-cAMP (100 μM)-induced PEPCK mRNA expression in the presence of compounds **1**−**12** from partitioned Corni fructus at a concentration of 50 μM are shown. (B) Dose-dependent effects of compounds **3** and **8** on PEPCK mRNA and protein expressions in response to dexamethasone and 8-bromo-cAMP. Quantified results of dexamethasone- and 8-bromo-cAMP-induced PEPCK mRNA expressions in the presence of EGCG (50 μM) or various concentrations of compounds **3** and **8** are shown. Insulin (10 nM) served as the experimental control. Quantified data are presented as the mean ± SEM (n = 4). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the control (None). SF, serum-free.

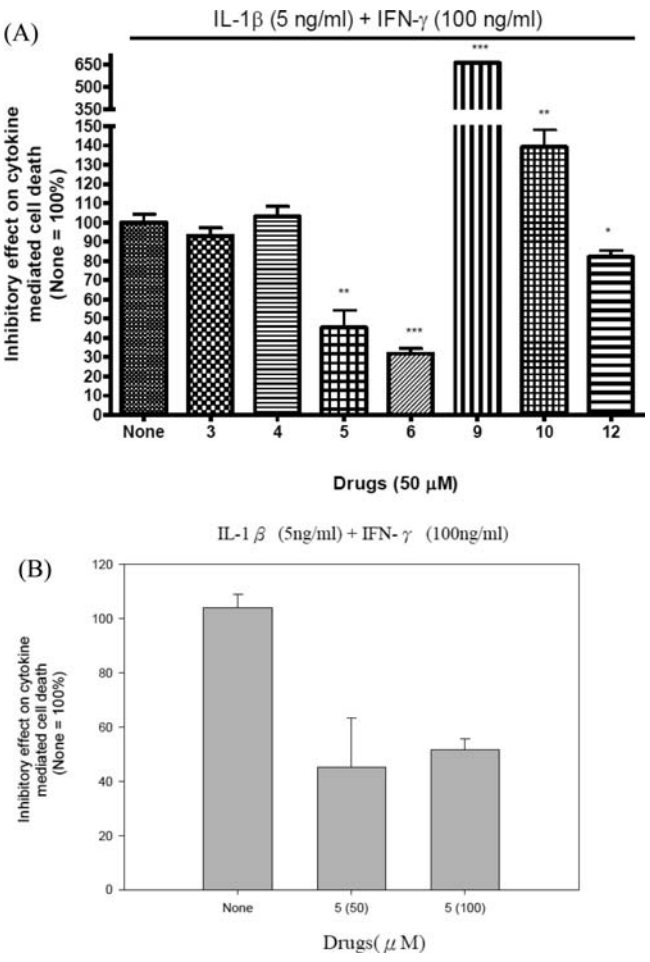

Figure 3. (A) Compounds **3**−**6**, **9**, **10**, and **12** from Corni fructus ameliorated cytokine-mediated cell death. The viability of BRIN-BD11 cells was measured after cells were treated with cytokine mix (IL-1β + IFN-γ) in the presence of the test compounds at a concentration of 50 μM. The maximal reduction of cell viability by cytokine mix alone (None) was regarded as 100%. (B) Dose-dependent effects of compound **5** on cytokine-mediated cell death. The viability of BRIN-BD11 cells treated with cytokine mix in the presence of different concentrations of compound **5** was measured and converted into inhibitory effect on cytokine mediated cytotoxicity. Data are presented as the mean ± SEM (n = 4). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the control (None).

because loss of β-cell mass is associated with both types of diabetes. In type-1 diabetes, β-cells are exposed to local high concentrations of cytokines and reactive oxygen species released from cytotoxic T cells or macrophages.[59] On the other hand, chronic dysglycemia in type-2 diabetes leads to ultimate β-cell failure due to glucotoxicity and/or IL-β-mediated subsequent insulitis.[60] Therefore, to tackle this abnormality, supplying insulin mimetics and preventing loss of β-cell mass are two different approaches leading to similar outcomes. In our previous investigation, we demonstrated that Corni fructus appears to have some yet unidentified constituents that possess dual bioactivities to prevent or control dysglycemia in IDDM. In addition, this unknown fraction appeared to have characteristics of a phenolic compound according to $FeCl_3$ staining results.[45]

In the current study, we re-explored the isolated constituents from Corni fructus to examine the possibility that there is a single constituent which possesses dual action. Therefore, we initially screened for insulin-mimetic constituents. Subsequently, those compounds possessing insulin-mimetic action were tested for β-cell-protective activity. Based on our results, only compound **1** possessed no insulin-stimulatory activity. In addition, our results indicate that both compound **5** and **6** had such dual actions.

Compound **5**, the n-butyl ether linkage of morroniside, attenuated both cytokine-mediated β-cell death and an inflammatory response (NO production). On the other hand, it was shown that morroniside ameliorated the inflammatory response and normalized lipid metabolism in the liver of db/db mice.[61] Thus, it appears that morroniside and ether derivative **5** have

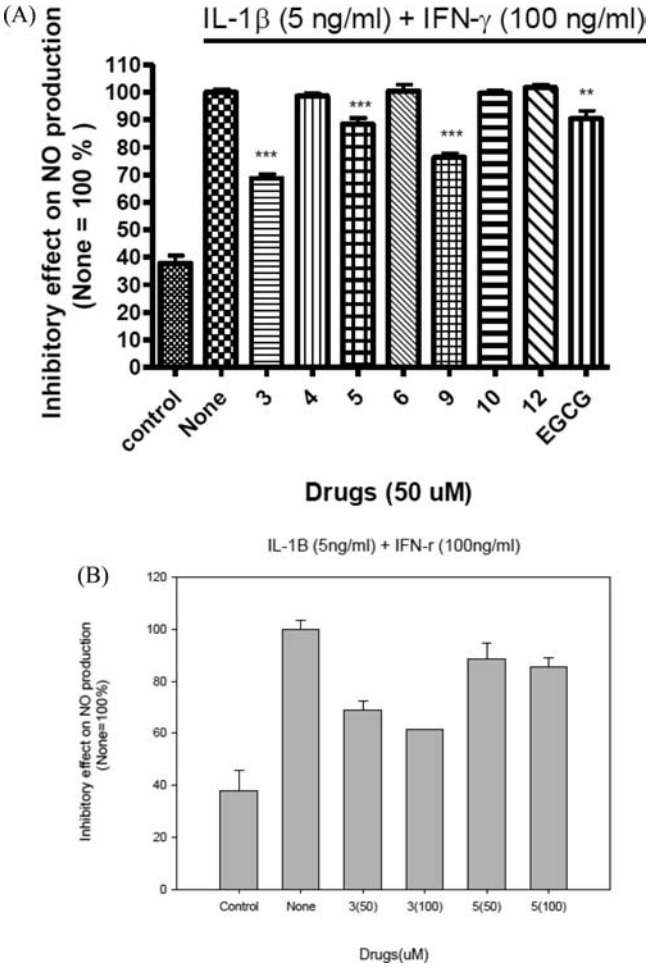

Figure 4. (A) Inhibitory effects of compounds **3**−**6**, **9**, **10**, and **12** from Corni fructus on nitric oxide (NO) production stimulated by cytokine mix (IL-1β + IFN-γ). The NO production from cytokine stimulated BRIN-BD11 cells was measured in the presence of EGCG or compounds **3**−**6** and **9**−**12** at 50 μ*M*. Maximal NO production (none) in the presence of cytokine mix was considered 100%. (B) Dose-dependent effects of compounds **3** and **5** on NO production. The NO production from cytokine stimulated BRIN-BD11 cells was measured in the presence of different concentrations of compounds **3** and **5**. Data are presented as the mean ± SEM (n = 4). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the control (None).

similar β-cell-protective functions. Whether morroniside can suppress PEPCK mRNA transcription remains to be evaluated.

To our knowledge, this seems to be the first report about PEPCK mRNA-suppressive activity by compound **6** (gallic acid), although gallic acid-stimulated GLUT4 translocation in 3T3-L1 adipocytes was recently reported.[62] In addition, gallic acid also protected RINm5F β-cells from glucolipotoxicity by its antiapoptotic and insulin-secretagogue actions according to the literature.[41] Therefore, our results are consistent with those findings.

Loganin (compound **4**) was the major constituent isolated from Corni fructus in the present investigation. In the present study, compound **4** exhibited a slightly potent insulin-mimetic inhibitory effect on PEPCK gene expression, which was similar to that described in a previous report.[45] However, it is also known that loganin possesses a protective effect against advanced glycation end product (AGE)-induced rat mesangial cell proliferation.[36] Therefore, although this compound did not attenuate cytokine-mediated β-cell death of an inflammatory response, it might still play some protective role in the glucotoxicity against β-cells.

Compound **3** ((−)-epicatechin-3-*O*-gallate) and EGCG share similarities in various bioactivities including (i) inhibition of glutamate dehydrogenase which regulates insulin secretion,[63] (ii) insulin-mimetic action on dexamethasone- and 8-bromo-cAMP-stimulated PEPCK transcription in H4IIE cells and mice liver,[41,42] and (iii) β-cell-protective action agianst cytokine-mediated cytotoxicity.[64] However, our results indicate that compound **3** had lower potency than EGCG. Therefore, it appears that 2-pyrogallol had better bioactivity than the 2-catechol substitution on flavan-3-*O*-gallate.

It was reported that oleanolic acid (**9**) has hypoglycemic and hypolipidemic effects in diabetic rats.[40] In the present results, the presence of oleanic acid did inhibit PEPCK mRNA expression. Thus, such antihepatic gluconeogenesis by oleanolic acid might account for the reported hypoglycemic effects in diabetic rats.

Based on these results, it was also possible to make a number of correlations between structure and inhibition of PEPCK mRNA expression by these 12 isolated compounds.

- It was found that inhibition of PEPCK mRNA expression activity of 3-subsititued 3′,4′,5,7-tetrahydroxyflavan-3-ol/flavones decreased in the following order: **3** > **2** > **1** (i.e., *O*-galloyl > *O*-6″-*n*-butyl glucuronyl > *O*-glucuronyl). It was shown that the *n*-butyl ester of 6-glucose was better than the free carboxylic acid, and the galloyl group was the most important for bioactivity.
- Comparing the bioactivities of compounds **5** and **4** (both of which are iridoid glycosides) revealed that the compound with 7-*n*-butyl ether had a higher level of activity, suggesting that the alkoxy of morroniside made a greater contribution to the inhibition of PEPCK mRNA expression activity than did the hydroxyl substituent on position 7 of loganin.
- A 5-hydroxyl substitution appeared to enhance the bioactivity of **6**. Esterification of **7** carboxyl enhanced the inhibition of PEPCK mRNA expression activity of **8**, and the bioactivity decreased in the following sequence: **8** > **6** > **7**. It was shown that the 2,3-dihydroxy-4-methoxy-4-oxobutanoic acid moiety was very important for bioactivity.
- Comparing the bioactivities of compounds **9** and **10** (both of which are triterpenoids) revealed that five cyclic rings produced the same inhibition of PEPCK mRNA expression activity as four cyclic rings, and the carboxylic acid moiety had no effect on the bioactivity.
- It was revealed that the large alkoxy moiety cannot have an effect on bioactivity by comparing the inhibition of PEPCK mRNA expression activity of compounds **6** and **11**.

In the cytokine-mediated NO production assay, it was found that, among the 12 isolated compounds, three compounds (**3**, **5**, and **9**) had anti-inflammatory effects. Their relative bioactivities decreased in the following sequence: **3** > **9** > **5**. This shows that the polyphenol moiety was most important for anti-inflammation.

In conclusion, insulin-mimetic effects on muscular glucose uptake and hepatic gluconeogenic enzyme regulation were demonstrated using fractions or isolated compounds. CH and CB partitions from Corni fructus stimulated glucose uptake. Twelve known compounds, **1**−**12**, were isolated from Corni fructus by column chromatography, and the major component was loganin. Compounds **8** and **12** were first isolated from natural plants herein. Among the isolated compounds, **2**−**12**

possessed various extents of insulin-mimetic effects on dexamethasone- and 8-bromo-cAMP-induced PEPCK expression. Compounds **5** and **6** inhibited cytokine-mediated β-cell death, while compounds **3**, **5**, and **9** had anti-inflammatory effects as judged by NO production. Therefore, these components could contribute to the therapeutic actions of Corni fructus against insulin-dependent diabetes mellitus via preserving β-cell mass and correcting insulin deficiency-mediated dysglycemia.

AUTHOR INFORMATION

Corresponding Author

*Graduate Institute of Pharmacognosy, School of Pharmacy, Taipei Medical University, 250 Wuxing Street, Taipei 11031, Taiwan, ROC. Tel: +886-2-27361661 ext 6132. Fax: +886-2-27370903. E-mail: hsu0320@tmu.edu.tw.

Author Contributions

‡These authors contributed equally to this work.

Funding Sources

We gratefully acknowledge the financial support through grants from the National Science Council (NSC96-2323-B-038-002) and National Research Institute of Chinese Medicine (NRICM-100-DHM-5), Taiwan, ROC.

ABBREVIATIONS USED

COSY, correlation spectroscopy; DEPT, distortionless enhancement via polarization transfer; EGCG, epigallocatechin gallate; EIMS, electron impact mass spectroscopy; HMBC, heteronuclear multiple-bond coherence; HSQC, heteronuclear single quantum coherence; IFN-γ, interferon-γ; IL-1β, interleukin-1β; NOESY, nuclear Overhauser effect spectroscopy; PEPCK, phosphoenolpyruvate carboxykinase

REFERENCES

(1) Zimmet, P.; Alberti, K. G.; Shaw, J. Global and societal implications of the diabetes epidemic. *Nature* **2001**, *414*, 782–787.

(2) Pickup, J. C.; Williams, G. *Textbook of Diabetes*; Blackwell Science: Oxford, U.K., 2003.

(3) Granner, D.; Pilkis, S. The gene of hepatic metabolism. *J. Biol. Chem.* **1990**, *265*, 10173–10176.

(4) Lucas, P. C.; Granner, D. K. Hormone response domains in gene transcription. *Annu. Rev. Biochem.* **1992**, *61*, 1131–1173.

(5) Hanson, R. W.; Reshef, L. Regulation of phosphoenolpyruvate carboxylase (GTP) gene expression. *Annu. Rev. Biochem.* **1997**, *66*, 581–611.

(6) Hall, R. K.; Scott, D. K.; Noisin, E. L.; Lucas, P. C.; Granner, D. K. Activation of the phosphoenolpyruvate carboxykinase gene retinoic acid response element is dependent on a retinoic acid receptor/coregulator complex. *Mol. Cell. Biol.* **1992**, *12*, 5527–5535.

(7) Granner, D.; Andreone, T.; Sasaki, K.; Beale, E. Inhibition of transcription of the phosphoenolpyruvate carboxykinase gene by insulin. *Nature* **1983**, *305*, 549–551.

(8) Sasaki, K.; Cripe, T. P.; Koch, S. R.; Andreone, T. L.; Petersen, D. D.; Beale, E. G.; Granner, D. K. Multihormonal regulation of phosphoenolpyruvate carboxykinase gene transcription. The dominant role of insulin. *J. Biol. Chem.* **1984**, *259*, 15242–15251.

(9) O'Brien, R. M.; Granner, D. K. Regulation of gene expression by insulin. *Physiol. Rev.* **1996**, *76*, 1109–1161.

(10) Saltiel, A. R.; Kahn, C. R. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **2001**, *414*, 799–806.

(11) Krones-Herzig, A.; Mesaros, A.; Metzger, D.; Ziegler, A.; Lemke, U.; Bruning, J. C.; Herzig, S. Signal-dependent control of gluconeogenic key enzyme genes through coactivator-associated arginine methyltransferase 1. *J. Biol. Chem.* **2006**, *281*, 3025–3029.

(12) Bergholdt, R.; Heding, P.; Nielsen, K.; Nolsoe, R.; Sparre, T.; Storling, J.; Nerup, J.; Pociot, F.; Mandrup-Poulsen, T. Type 1 database mellitus: an inflammatory disease of the islet. *Adv. Exp. Med. Biol.* **2004**, *552*, 129–153.

(13) Donath, M. Y.; Boni-Schnetzler, M.; Ellingsgaard, H.; Ehses, J. A. Islet inflammation impairs the pancreatic cell in type 2 diabetes. *Physiology* **2009**, *24*, 325–331.

(14) Tripathi, P.; Tripathi, P.; Kashyap, L.; Singh, V. The role of nitric oxide in inflammatory reactions. *FEMS Immunol. Med. Microbiol.* **2007**, *51*, 443–452.

(15) McDaniel, M. L.; Kwon, G.; Hill, J. R.; Marshall, C. A.; Corbett, J. A. Cytokines and nitric oxide in islet inflammation and diabetes. *Proc. Soc. Exp. Biol. Med.* **1996**, *211*, 24–32.

(16) Suzuki, J.; Kimura, M. Hyperoglycemic effects of the blended chinese traditional medicines in geretically and chemically diabetic mice. *Nippon Yakurigaku Zasshi* **1984**, *83*, 1–10.

(17) kamei, A.; hisada, T.; Iwata, S. The evaluation of therapeutic efficacy of hachimi-jio-gan (traditional chinese medicine) to rat galactosemic cataract. *J. Ocul. Pharmacol.* **1987**, *3*, 239–48.

(18) Kitagawa, I.; Nishimura, T.; Furubayashi, A.; Yosioka, I. On the constituents of rhizome of *Rehmannia glutinosa* Libosch. forma hueichingensis Hsiao. *Yakugaku Zasshi* **1971**, *91*, 593–596.

(19) Kiho, T.; Watanabe, T.; Nagai, K.; Ukai, S. Hypoglycemic activity of polysaccharide fraction from rhizome of *Rehmannia glutinosa* Libosch.f.hueichingesnsis Hsiao and the effect on Carbohydrate metabolism in normal mouse liver. *Yakugaku Zasshi* **1992**, *112*, 393–400.

(20) Zhang, R. X.; Gu, G. M.; Zhang, Y. X.; Zhou, J. H. Pharmacology and Clinical of Chinese Traditional medicine (Chinese). *Pharmacol. Clin. Chin. Mater. Med.* **1996**, *1*, 14–16.

(21) Yang, X. B.; Huang, Z. M.; Cao, W. B.; Cheng, H. Y.; Wang, J. H.; Xu, L. Therapeutic and protective effects ofwater ethanolic extract from *Rhizoma alismatis* on streptozotocin induced diabetic mice. *Pharm. J. Chin. People's Liberation Army* **2002**, *18*, 336–338.

(22) Yokozawa, T.; Kim, H. Y.; Yamabe, N. Amelioration of diabetic nephropathy by dried *Rehmanniae radix* (Di Huang) extract. *Am. J. Chin. Med.* **2004**, *32*, 829–839.

(23) He, H.; Yang, X.; Zeng, X.; Shi, M.; Yang, J.; Wu, L.; Li, L. Protective effect of Liuwei Dihuang decoction on early diabetic nephropathy induced by streptozotocin via modulating ET-ROS axis and matrix metalloproteinase activity in rats. *J. Pharm. Pharmacol.* **2007**, *59*, 1297–1305.

(24) Yamahara, J.; Mibu, H.; Sawada, T.; Fujimura, H.; Takino, S.; Yoshikawa, M.; Kitagawa, I. Biologically active principles of crude drugs. Antidiabetic principles of corni fructus in experimental diabetes induced by streptozotocin. *Yakugaku Zasshi* **1981**, *101*, 86–90.

(25) Qian, D. S.; Zhu, Y. F.; Zhu, Q. Effect of alcohol extract of *Cornus officinalis* Sieb. et Zucc. on GLUT4 expression in skeletal muscle in type 2 (non-insulin-dependent) diabetic mellitus rats. *China J. Chin. Mater. Med.* **2001**, *26*, 859–862.

(26) Liou, S. S.; Liu, I. M.; Hsu, S. F.; Cheng, J. T. Corni fructus as the major herb of Die-Huang-Wan for lowering plasma glucose in Wistar rats. *J. Pharm. Pharmacol.* **2004**, *56*, 1443–7.

(27) Yamabe, N.; Kang, K. S.; Goto, E.; Tanaka, T.; Yokozawa, T. Beneficial effect of *Corni fructus*, a constituent of Hachimi-jio-gan, on advanced glycation end product-mediated renal injury in Streptozotocin-treated diabetic rats. *Biol. Pharm. Bull.* **2007**, *30*, 520–526.

(28) Yamabe, N.; Kang, K. S.; Matsuo, Y.; Tanaka, T.; Yokozawa, T. Identification of antidiabetic effect of iridoid glycosides and low molecular weight polyphenol fractions of *Corni fructus*, a constituent of Hachimi-jio-gan, in streptozotocin induced diabetic rats. *Biol. Pharm. Bull.* **2007**, *30*, 1289–1296.

(29) Endo, T.; Taguchi, H. Study on the constituents of *Cornus* officinalis Sieb. et Zucc. *Yakugaku Zasshi* **1973**, *93*, 30–32.

(30) Hatano, T.; Ogawa, N.; Kira, R.; Yasuhara, T.; Okuda, T. Tannins of cornaceous plants, Cornusiins A, B and C, dimeric monomeric and trimeric hydrolysable tannins from *Cornus* officinalis, and

orientation of valoneoyl group in related tannins. *Chem. Pharm. Bull.* **1989**, *37*, 2083–2090.

(31) Wang, Y.; Li, Z.; Chen, L.; Xu, X. Antiviral compounds and one new iridoid glycoside from *Cornus* officinalis. *Prog. Nat. Sci.* **2006**, *16*, 142–146.

(32) Hatauo, T.; Yasuhara, T.; Abe, R.; Okuda, T. A golloylated monoterpene glucoside and a dimeric hydrolyzable tannin from *Cornus* officinalis. *Phytochemistry* **1990**, *29*, 2975.

(33) Kim, D. K.; Kwak, J. H. A furan derivative from *Cornus* officinalis. *Arch. Pharm. Res.* **1998**, *21*, 787–789.

(34) Delaveau, P.; Paris, R. On the presence of rutoside and gallic acid derivatives in the flowers of *Cornus* mas L. Preliminary research in *C. sanguinea* L. *Bull. Soc. Chim. Biol.* **1961**, *43*, 661–666.

(35) Miyazawa, M.; kamecoka, H. Volatile flavor components of Corni fructus (*cornus officinalis* Sieb. et Zucc.). *Agric. Biol. Chem.* **1989**, *53*, 3337–3340.

(36) Xu, H.; Shen, J.; Liu, H.; Shi, Y.; Li, L.; Wei, M. Morroniside and loganin extracted from *Cornus officinalis* have protective effects on rat mesangial cell proliferation exposed to advanced glycation end products by preventing oxidative stress. *Can. J. Physiol. Pharmacol.* **2006**, *84*, 1267–1273.

(37) Zhang, W.; Hong, D.; Zhou, Y.; Zhang, Y.; Shen, Q.; Li, J. Y.; Hu, L. H.; Li, J. Ursolic acid and its derivative inhibit protein tyrosine phosphatase 1B, enhancing insulin receptor phosphorylation and stimulating glucose uptake. *Biochim. Biophys. Acta* **2006**, *1760*, 1505–1512.

(38) Jang, S. M.; Yee, S. T.; Choi, J.; Choi, M. S.; Do, G. M.; Jeon, S. M.; Yeo, J.; Kim, M. J.; Seo, K. I.; Lee, M. K. Ursolic acid enhances the cellular immune system and pancreatic beta-cell function in streptozotocin-induced diabetic mice fed a high-fat diet. *Int. Immunopharmacol.* **2009**, *9*, 113–119.

(39) Wang, Z. H.; Hsu, C. C.; Huang, C. N.; Yin, M. C. Anti-glycative effects of oleanolic acid and ursolic acid in kidney of diabetic mice. *Eur. J. Pharmacol.* **2010**, *628*, 255–260.

(40) Gao, D.; Li, Q.; Li, Y.; Liu, Z.; Fan, Y.; Han, Z.; Li, J.; Li, K. Antidiabetic potential of oleanolic acid from Liqustrum lucidum Ait. *Can. J. Physiol. Pharmacol.* **2007**, *85*, 1076–1083.

(41) Koyama, Y.; Abe, K.; Sano, Y.; Ishizaki, Y.; Njelekela, M.; Shoji, Y.; Hara, Y.; Isemura, M. Effects of green tea on gene expression of hepatic gluconeogenic enzymes in vivo. *Planta Med.* **2004**, *70*, 1100–1102.

(42) Wolfram, S.; Raederstorff, D.; Preller, M.; Wang, Y.; Teixeira, S. R.; Riegger, C.; Weber, P. Epigallocatechin gallate supplementation alleviates diabetes in rodents. *J. Nutr.* **2006**, *136*, 2512–2518.

(43) Sameermahmood, Z.; Raji, L.; Saravanan, T.; Vaidya, A.; Mohan, V.; Balasubramanyam, M. Gallic acid protects RINm5F beta-cells from glucolipotoxicity by its antiapoptotic and insulin-secretagogue actions. *Phytother. Res.* **2010**, *24*, 83–94.

(44) Waltner-Law, M. E.; Wang, X. L.; Law, B. K.; Hall, R. K.; Nawano, M.; Granner, D. K. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. *J. Biol. Chem.* **2002**, *277*, 34933–34940.

(45) Chen, C. C.; Hsu, C. Y.; Chen, C. Y.; Liu, H. K. Fructus Corni suppresses hepatic gluconeogenesis related gene transcription, enhances glucose responsiveness of pancreatic beta-cells, and prevents toxin induced beta-cell death. *J. Ethnopharmacol.* **2008**, *117*, 483–490.

(46) Seebacher, W.; Simic, N.; Wels, R.; Sat, R.; Kunert, O. Complete assignments of ^{1}H and ^{13}C NMR resonances of oleanolic acid, 18α-oleanolic acid, ursolic acid and their 11-oxo derivatives. *Magn. Reson. Chem.* **2003**, *41*, 636–638.

(47) Kojima, H.; Sato., N.; Hatano, A. Sterol glucosides from *Prunella vulgaris*. *Phytochemistry* **1990**, *29*, 2351–2355.

(48) Nakanishi, T.; Inatomi, Y.; Murata, H.; Ishida, S. S.; Fujino, Y.; Miura, K.; Yasuno, Y.; Inada, A.; Lang, F. A.; Murata, J. Triterpenes and flavonol glucuronides from Oenothera cheiranthifolia. *Chem. Pharm. Bull.* **2007**, *55*, 334–336.

(49) Sunghwa, F.; Sakurai, H.; Saiki, I.; Koketsu, M. Iodine-catalyzed etherification of morroniside. *Chem. Pharm. Bull.* **2009**, *57*, 112–115.

(50) Teng, R. W.; Wang, D. Z.; Wu, Y. S.; Lu, Y.; Zheng, Q. T.; Yang, C. R. NMR assignments and single-crystal X-ray diffraction analysis of deoxyloganic acid. *Magn. Reson. Chem.* **2005**, *43*, 92–96.

(51) Budzianowski, J. Coumarins, caffeoyltartaric acids and their artifactual methyl esters from *Taraxacum officinale* leaves. *Planta Med.* **1997**, *63*, 288.

(52) Saleem, M.; Kim, H. J.; Jin, C.; Lee, Y. S. Antioxidant caffeic acid derivatives from leaves of *Parthenocissus tricuspidata*. *Arch. Pharm. Res.* **2004**, *27*, 300–304.

(53) Wawer, I.; Zielinska, A. ^{13}C-CP-MAS-NMR studies of flavonoids. I. Solid-state conformation of quercetin, quercetin 5′-sulphonic acid and some simple polyphenols. *Solid State Nucl. Magn. Reson.* **1997**, *10*, 33–38.

(54) Garcia, J.; Mpondo, M. E.; Nardin, R. Loganin and a New Iridoid Glucoside from *Gentiana pyrenaica*. *J. Nat. Prod.* **1989**, *52*, 423–425.

(55) Adrienne, L.; Davis, Y. C.; Alan, P.; Davies, J. R. L. ^{1}H and ^{13}C NMR Assignments of Some Green Tea Polyphenols. *Magn. Reson. Chem.* **1996**, *34*, 887–890.

(56) Mnozhoyan, O. L.; Toskunina, L. M. Synthesis of derivatives of dicarboxylic acids. XXXI. Some dimethylaminoethyl esters of α-alkoxysuccinic acids. *Arm. Khim. Zh.* **1968**, *21*, 311–314.

(57) Liu, I. M.; Hsu, F. L.; Chen, C. F.; Cheng, J. T. Antihyperglycemic action of isoferulic acid in streptozotocin-induced diabetic rats. *Br. J. Pharmacol.* **2000**, *129*, 631–636.

(58) Liu, H. K.; Green, B. D.; McClenaghan, N. H.; McCluskey, J. T.; Flatt, P. R. Long-term beneficial effects of vanadate, tungstate, and molybdate on insulin secretion and function of cultured beta cells. *Pancreas* **2004**, *28*, 364–368.

(59) Donath, M. Y.; Storling, J.; Berchtold, L. A.; Billestrup, N.; Mandrup-Poulsen, T. Cytokines and beta-cell biology: from concept to clinical translation. *Endocr. Rev.* **2008**, *29*, 334–350.

(60) Donath, M. Y.; Böni-Schnetzler, M.; Ellingsgaard, H.; Ehses, J. A. Islet inflammation impairs the pancreatic cell in type 2 diabetes. *Physiology* **2009**, *24*, 325–331.

(61) Park, C. H.; Yamabe, N.; Noh, J. S.; Kang, K. S.; Tanaka, T.; Yokozawa, T. The beneficial effects of morroniside on the inflammatory response and lipid metabolism in the liver of db/db mice. *Biol. Pharm. Bull.* **2009**, *32*, 1734–1740.

(62) Vishnu Prasad, C. N.; Anjana, T.; Banerji, A.; Gopalakrishnapillai, A. Gallic acid induces GLUT4 translocation and glucose uptake activity in 3T3-L1 cells. *FEBS lett.* **2010**, *584* (3), 531–536.

(63) Smith, T.; Stanley, C.; Matschinsky, F. Pharmaceutical compositions containing epigallocatechin gallate and epicatechin gallate as glutamate dehydrogenase inhibitors for regulating insulin secretion. *U.S. Pat. Appl. Publ.* **2006**, 1–25.

(64) Han, M. K. Epigallocatechin gallate, a constituent of green tea, suppresses cytokine-induced pancreatic beta-cell damage. *Exp. Mol. Med.* **2003**, *35*, 136–139.