Glycoside Compounds From *Glycyrrhiza uralensis* and Their Neuroprotective Activities

Guanhua Wei¹, Honghong Da², Kaixue Zhang¹, Junmin Zhang¹,², Jianguo Fang², and Zhigang Yang¹

**Abstract**

This paper concerns the study of the roots and rhizomes of *Glycyrrhiza uralensis* where one new alkaloid glycoside, 3,4-dihydroxyquinoline 4-O-β-D-glucopyranoside, along with 13 known compounds (12 phenolic glycosides and one triterpene glycoside) were isolated and identified. The structure of the new compound and the known ones were identified on the basis of nuclear magnetic resonance (NMR) and mass spectrometric (MS) analysis. All the glycosides were tested for their anti-neuroinflammatory activities by inhibiting nitric oxide (NO) release in lipopolysaccharide (LPS)-induced murine microglial BV-2 cells. Several compounds were tested for their antioxidant activities in rat adrenal pheochromocytoma PC12 cells. A structure–activity relationship (SAR) analysis was carried out and revealed that the position and amount of sugar moieties have significant impact on antioxidant activities.

**Keywords**

*Glycyrrhiza uralensis*, glycosides, 3, 4-dihydroxyquinoline 4-O-β-D-glucopyranoside, anti-neuroinflammatory activity, antioxidant activity

Received: September 18th, 2020; Accepted: January 13th, 2021.

Licorice, one of the most popular Chinese herbal medicines, is, according to the Pharmacopoeia of the People's Republic of China, derived from the dried roots and rhizomes of *Glycyrrhiza uralensis*, *G. inflata*, and *G. glabra* (family Leguminosae). To date, different kinds of products have been isolated from licorice, including flavonoids, triterpenoid saponins, coumarins, polysaccharides and alkaloids.1-4 Pharmacological studies have revealed that licorice extracts and isolated constituents show significant anti-inflammatory,5 antioxidant6 and neuroprotective activities.7

The incidence of neurological disorders, such as Alzheimer's and Parkinson's diseases, is increasing worldwide due to the increase in the number of the aged population; mortality from these diseases accounts for 17% of global deaths.8,9 Considerable evidence has demonstrated that overproduction of reactive oxygen species (ROS) and neuroinflammation are closely related to the pathogenesis of several neurological diseases.10-12 Previous studies have evidenced the efficacy of licorice extract and its purified ingredients as antioxidation and anti-inflammation agents.13-15

Therefore, based on phenolic compounds which have been isolated from licorice in our previous studies,16 our present study focused on investigations targeting neuroprotective effects of isolated compounds against inflammatory and oxidant damage *in vitro*. As a result, one new alkaloid glycoside (1), along with thirteen known compounds (2, 14, Figure 1) were isolated from the n-butyl alcohol (n-BuOH) soluble portion. The structures were determined by analysis of their spectroscopic data and comparison with references. All of the compounds were tested for their anti-neuroinflammatory activities, among which three compounds (4, 5, and 12) were further tested for their antioxidant activities since their structures are similar to those of liquiritin (2) and isoliquiritin (3). Herein, the isolation and structural elucidation of the new compound, and the bioactivity evaluation of compounds 1-14 are described.

¹School of Pharmacy, Lanzhou University, China
²State Key Laboratory of Applied Organic Chemistry and College of Chemistry and Chemical Engineering, Lanzhou University, China

**Corresponding Author:**

Zhigang Yang, School of Pharmacy, Lanzhou University, Lanzhou, China.
Email: yangzg@lzu.edu.cn
Results and Discussion

Structure Determination of Isolated Compounds

Compound 1 was obtained as a yellow amorphous powder. The molecular formula, C_{15}H_{17}NO_{7}, was determined from the HR-ESI-MS ion at m/z 322.0942 [M–H] – (calcld for C_{15}H_{16}NO_{7}, 322.0927). (Supplemental Figure S1). The UV spectrum of 1 (Supplemental Figure S2) had maximum absorption wavelengths at 213, 250, and 289 nm, which indicated that 1 had a quinoline skeleton.\(^{17}\) The 1H-NMR spectrum (Supplemental Figure S3) showed an \(\alpha\)-substituted benzene ring, consisting of four aromatic protons \(\delta_H 8.02 (1H, dd, J = 4.4, 1.2 Hz); 7.50 (1H, dd, J = 4.4, 1.2 Hz); 7.22 (1H, ddd, J = 0.8, 4.0, 4.8 Hz); 7.20 (1H, ddd, J = 0.8, 4.0, 4.8 Hz),\) a bond group \(\delta_H 8.14 (1H, s),\) and a hydroxyl group at \(\delta_H 12.04 (1H, s).\) In accordance with this molecular formula, 15 carbon signals were observed in the 13C-NMR spectrum (Supplemental Figure S4), with the assistance of DEPT and HSQC experiments, including one methylene, 10 methines, and four quaternary carbons. From the 13C- and 1H-NMR spectra, a \(\beta\)-glucopyranosyl moiety \(\delta_H 5.59 (1H, d, J = 7.2 Hz, H-1'), 3.69 (1H, dd, J = 12.0, 3.6 Hz, H-6'), 3.48 (1H, dd, J = 12.0, 3.6 Hz, H-6'), 3.25-3.40 (m); \(\delta_C 94.2 (C-1'), 78.2 (C-3'), 77.1 (C-5'), 73.1 (C-2'), 70.1 (C-4'), 61.1 (C-6')\) was clearly inferred. Apart from these signals for the substituent group, the remaining proton and carbon signals suggested compound 1 to be a hydroxyquinoline, which was supported by the following HMBC and 1H-1H COSY experiments.

The structure of 1 was further analyzed from its 2D NMR spectroscopic data, including 1H-1H COSY, HSQC, HMBC and NOESY spectra (Supplemental Figures S6-S9). In the 1H-1H COSY spectrum, combined with the HSQC spectrum, the aromatic proton at \(\delta 7.22 (\delta_C 123.0, C-7)\) correlated with two protons at \(\delta 7.20 (\delta_C 121.9, C-6)\) and 8.02 (\(\delta_C 121.0, C-8)\). Additionally, the proton at \(\delta 7.20\) correlated with protons at \(\delta 7.22\) and 7.50 (\(\delta_C 112.9, C-5)\). The proton and carbon signals attribution at different positions of the \(\alpha\)-substituted benzene ring were identified. In the HMBC spectrum, the glycosidic site was established unambiguously by a long-range correlation between H-1' (\(\delta_H 5.59)\) and C-4 (\(\delta_C 163.4)\). In addition, the hydroxyl proton at \(\delta 12.04\) correlated with carbons at C-3 (\(\delta 106.3)\) and C-10 (\(\delta 126.2)\), while the proton at position 2 of the quinolone ring at \(\delta 8.14\) correlated with carbons at C-3 (\(\delta 106.3)\), C-9 (\(\delta 136.9)\), and C-10 (\(\delta 126.2)\) (Figure 2). On the basis of detailed analysis, all the signals in the 1D- and 2D-NMR spectra were assigned. Following acid hydrolysis of 1 with 2N TFA, the sugar moiety was determined to be D-glucose based on HPLC analysis (\(t_R = 18.92\) minutes) after chiral derivatization.\(^{18}\) Thus, the structure of 1 was identified as 3,4-dihydroxyquinoline 4-\(\beta\)-D-glucopyranoside.

The known compounds (2-14) were identified as liquiritin (2),\(^{19}\) isoliquiritin (3),\(^{20}\) neisoliquiritin (4),\(^{21}\) liquiritin apioside (5),\(^{22}\) neoliquiritin (6),\(^{23}\) isoliquiritin apioside (7),\(^{24}\) ononin (8),\(^{25}\) licuriside (9),\(^{26}\) (2S)-liquiritigenin-7-\(\beta\)-D-glucopyranosyl-(2→1)-\(\alpha\)-D-apiofuranoside (10),\(^{27}\) (2R)-liquiritigenin-7-\(\beta\)-D-glucopyranosyl-(2→1)-\(\beta\)-D-apiofuranoside (11),\(^{27}\) liquiritigenin-
Wei et al.

7,4'-di-β-D-glucopyranoside (12), 28 isoliquiritigenin-4,4'-di-β-D-glucopyranoside (13), 29 and 18β-glycyrrhetinic acid-3-O-glucuronide (14), 30 based on the analysis and comparison of their NMR spectroscopic data with those reported in the literature.

Anti-neuroinflammatory Activities In Vitro

It has been reported that neuroinflammation might drive the pathogenic process in several degenerative neurological disorders. 31 To obtain either new anti-neuroinflammatory agents or lead compounds for degenerative neurological diseases, all isolated compounds (1-14) were evaluated for their inhibitory activities on LPS-induced NO production in murine microglial BV-2 cells by the Griess reaction. Curcumin was used as a positive control (IC50 value of 1.9 µg/mL). The NO inhibitory effects shown by all of these compounds are described using IC50 values, which are summarized in Table 1. Based on comparison and analysis of their IC50 values (Table 1), compounds 1, 3, 4, and 6 exerted the more inhibitory activity against LPS-stimulated NO production in BV-2 cells with IC50 values less than 50 µg/mL. MTT assay indicated that none of the assayed compounds exhibited significant cytotoxicity to the BV-2 cells at their effective concentration for the inhibition of NO production (Figure 3).

Antioxidant Activities In Vitro

6-Hydroxydopamine (6-OHDA) is a dopaminergic neurotoxin, the toxic effects of which have been linked to overproduction of ROS, such as hydrogen peroxide (H2O2), superoxide anion and hydroxyl radical. Under physiological conditions 6-OHDA is rapidly and nonenzymatically oxidized by molecular oxygen.
to form $\text{H}_2\text{O}_2$ and the corresponding $\rho$-quinone. High and sustained concentrations of ROS can cause damage to many cellular and extracellular constituents, including DNA, proteins, and lipids. Oxidation of proteins may lead to the formation of insoluble protein aggregates, which are the molecular basis of a number of diseases, particularly neurodegenerative pathologies. Therefore, $\text{H}_2\text{O}_2$ and 6-OHDA are widely used to investigate the pathogenesis of some neurological disorders in vivo and in vitro.

The protective effect of liquiritin and isoliquiritin on corticosterone-induced neurotoxicity in rat adrenal pheochromocytoma PC12 cells has been proved. For the exploration of neuroprotective activities in isolated compounds with similar structures as liquiritin (2) and isoliquiritin (3), compounds 4, 5, and 12 were tested for their protective activities against the $\text{H}_2\text{O}_2$- and 6-OHDA-induced injury model in PC12 cells by MTT assay. None of the assayed compounds exhibited significant cytotoxicity to the PC12 cells at either 20 or 50 $\mu$mol/L (Figure 4). The antioxidant effects were also evaluated for their protective activities against $\text{H}_2\text{O}_2$- or 6-OHDA-induced apoptosis, with the higher concentration (Figure 5). The final results showed that compounds 2 and 3 were capable of relieving the cell injury, notably in the 6-OHDA-treated group ($P < .05$). However, compounds 4, 5, and 12 lacked conspicuous antioxidant activity, and even led to further cellular damage at 50 $\mu$mol/L. The protective effect of 2 and 3 was better than that of 4, 5, and 12, both in the $\text{H}_2\text{O}_2$- and 6-OHDA-treated groups. According to the activity comparison of compounds with the same basic parent structure, the preliminary SAR analysis showed that different positions of the sugar moieties might have a significant impact on their antioxidant activities. Additionally, the more the number of sugar moieties, the less was the antioxidant activity of the compound.

### Experimental

**General Experiments**

The UV spectra were recorded on a Lambda 25 UV-Visible spectrophotometer (Perkin-Elmer, USA), 1D and 2D NMR spectra on a Bruker AVANCE AV III-400 instrument (Bruker, Switzerland, 100 MHz for $^{13}$C and 400 MHz for $^1$H) with tetramethylsilane (TMS) as an internal reference at room temperature, and HR-ESI-MS on an Agilent LC-QTOF-MS (1290, 6560) spectrometer (Palo Alto, CA, USA). Medium pressure liquid chromatography (MPLC) was run using a NP7000 pump with a NU3000 UV detector (Hanbon Sci. & Tech, Jiangsu, People's Republic of China) and a column (26 × 460 mm) filled with octadecylsilyl (75 C$_{18}$-OPN, Nacalai Tesque, Kyoto, Japan). HPLC separations were carried out on an EasyChrom...
system, equipped with the same detector as the MPLC system and a 5C_{18}-PAQ (10 × 250 mm, 10 µm) column (Waters, Milford, USA). Column chromatography was performed with silica gel (100, 200 mesh, Qingdao Haiyang Chemical Co., Ltd., People’s Republic of China) and two kinds of macroporous resin (D101; AB-8, 16-60 mesh, Solarbio, Beijing, People’s Republic of China).

**Plant Material**

The roots and rhizomes of *Glycyrrhiza uralensis* Fisch. were collected from Minqin, Gansu Province, People’s Republic of China, in October 2016. The plant was identified by one of the authors, Dr. Zhigang Yang. A voucher specimen (No. MQG201610) has been deposited at the School of Pharmacy, Lanzhou University.

**Extraction and Isolation**

The dried roots and rhizomes (10 kg) of *G. uralensis* were powdered and extracted with 80% ethanol (EtOH) at room temperature, three times. After evaporation of the solvent under reduced pressure, the residues (810 g) were dispersed in water and successively extracted with chloroform (CHCl₃), ethyl acetate (EtOAc) and n-BuOH, respectively. The n-BuOH-soluble portion (240 g) was subjected to silica gel column chromatography (100, 200 mesh, Qingdao Haiyang Chemical Co., Ltd., People’s Republic of China, in October 2016. The plant was identified by one of the authors, Dr. Zhigang Yang. A voucher specimen (No. MQG201610) has been deposited at the School of Pharmacy, Lanzhou University.

**Acid Hydrolysis of 1 and Determination of Sugar**

Compound 1 (1.0 mg) was heated at 60 °C for 2 hours in 2 ml of 2 N trifluoroacetic acid, and the resulting product was extracted with EtOAc (5 ml × 3). The water-soluble layer was concentrated to dryness in vacuo. The residue was dissolved in pyridine (0.1 ml) and to it was added L-cysteine methyl ester hydrochloride (2.0 mg) at 60 °C for 1 hour. o-Tolylisothiocyanate (10 µL) was added to the mixture,
which was heated at 60 °C for 1 hour. The reaction mixture was analyzed by HPLC using a C<sub>18</sub> column (5 μm, 4.6 × 250 mm, Waters), UV detection at 250 nm, and isocratic conditions of 23% aq. ACN in 0.1% formic acid (1 mL/min, 25 minutes).<sup>36</sup> Under the same conditions, the retention times of authentic samples were 18.95 minutes (D-glucose) and 20.93 minutes (L-glucose). The peak of 1 coincided with that of the derivative of authentic D-glucose (18.92 minutes).

Bioassay for Anti-neuroinflammatory Effects In Vitro

The anti-neuroinflammatory effects in vitro were examined by inhibiting NO release in LPS-induced murine microglial BV-2 cells. The cells were cultured at 37 °C in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% (v/v) activated fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin under a water-saturated atmosphere of 95% air and in a humidified atmosphere of 5% CO<sub>2</sub>. BV-2 cells were plated in 96-well culture plates (2 × 10<sup>4</sup> cells/well) and preincubated for 24 hours. The cells were incubated for 24 hours either with or without 1 μg/mL of LPS (Beijing Solarbio Science & Technology Co., Ltd., People’s Republic of China) in either the absence or presence of the isolated compounds. Curcumin was used as a positive control. After treatment with various concentrations (10, 25, 50 μg/mL) of compounds and curcumin, the culture supernatant of cells (100 μL) was mixed with an equal volume of Griess reagent (1:1 mixture of 0.1% N-(1-naphthyl)ethylenediamine in H<sub>2</sub>O and 1% sulfanilamide in 5% H<sub>3</sub>PO<sub>4</sub>) at room temperature for 20 minutes. NO concentration was quantified by the absorbance of the mixture read on a Tecan Spark 20M microplate reader (Tecan Inc., Swiss) at 550 nm, using a standard curve of known nitrite concentration versus absorbance at the same wavelength. The IC<sub>50</sub> values were determined with SPSS22.0 software from the corresponding experiments performed in triplicate.

Bioassay for Antioxidant Effects In Vitro

The antioxidant effects in vitro were tested for the protection against the H<sub>2</sub>O<sub>2</sub> or 6-OHDA-induced injury model in rat adrenal pheochromocytoma PC12 cells. The cells were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, and 100 units/mL penicillin/streptomycin, maintained in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C. PC12 cells (1 × 10<sup>4</sup> cells/well) were plated in 96-well plates and allowed to adhere for 24 hours and then treated with different concentrations (10, 20, 50 μmol/L) of compounds without distinct cytotoxicity for 24 hours. After replacing with fresh medium containing 500 μmol/L H<sub>2</sub>O<sub>2</sub> or 200 μmol/L 6-OHDA for 12 hours, the cell viability was determined by the MTT assay.

Acknowledgments

Authors are thankful to the Scientific Research and Experiment Center of the School of Pharmacy, Lanzhou University, and Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences for the measurement of NMR spectra.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant from the Major International S & T Cooperation Project, Ministry of Science and Technology of the People’s Republic of China (2016YFE0129000); Research project of Gansu Provincial Administration of Traditional Chinese Medicine (GZK-2015-21); The Natural Science Foundation of Gansu Province (20J5RA311) and the Fundamental Research Funds for the Central Universities (lzujbky-2017-k26).

ORCID ID

Guanhua Wei  https://orcid.org/0000-0001-5632-3608

Supplemental Material

Supplemental material for this article is available online.

References

1. Kuo Y-J, Yang Y-C, Zhang L-J, et al. Flavanone and diphenylpropane glycosides and glycosidic acyl esters from Viscum articulatum. J Nat Prod. 2010;73(2):109-114. doi:10.1021/np9004294
2. Kao T-C, Wu C-H, Yen G-C. Bioactivity and potential health benefits of licorice. J Agric Food Chem. 2014;62(3):542-553. doi:10.1021/jf404939f
3. Han YN, Chung MS, Kim TH, Han BH. Two tetrahydroquinoline alkaloids from Glycyrrhiza uralensis. Arch Pharm Res. 1990;13(1):101-102. doi:10.1007/BF02857842
4. Muttaliflu P, Bobakulov K, Abuduwaili K, et al. Structural characterization and antioxidant activities of a water soluble polysaccharide isolated from Glycyrrhiza glabra. Int J Biol Macromol. 2020;150:677-682. doi:10.1016/j.ijbiomac.2019.11.245
5. Cho HJ, Lim SS, Lee YS, et al. Hexane/ethanol extract of Glycyrrhiza uralensis licorice exerts potent anti-inflammatory effects in murine macrophages and in mouse skin. Food Chem. 2010;121(4):959-966. doi:10.1016/j.foodchem.2010.01.027
6. Li X, Chen W, Chen D. Protective effect against hydroxyl-induced DNA damage and antioxidant activity of Radix Glycyrrhizae
(liquorice root). *Adv Pharm Bull*. 2013;3(1):167-173. doi:10.5681/apb.2013.028
7. Ravanfar P, Namazi G, Borhani Haghighi A, et al. Neurologic effects of licorice: a review. *Pharmagen Res*. 2018;12(23):115-119.
8. Wyss-Coray T. Ageing, neurodegeneration and brain rejuvenation. *Nature*. 2016;539(7628):180-186. doi:10.1038/nature20411
9. Gibbs RM, Lipnick S, Bateman JW, et al. Toward precision medicine for neurological and neuropsychiatric disorders. *Cell Stem Cell*. 2018;23(1):21-24. doi:10.1016/j.stem.2018.05.019
10. Shadfar S, Hwang CJ, Lim M-S, Choi D-Y, Hong JT. Involvement of inflammation in Alzheimer’s disease pathogenesis and therapeutic potential of anti-inflammatory agents. *Arch Pharm Res*. 2015;38(12):2106-2119. doi:10.1007/s12272-015-0648-x
11. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*. 2007;15(6):252-259. doi:10.1007/s10787-007-0013-x
12. Popa-Wagner A, Mitran S, Sivanesan S, Chang E, Buga A-M. Rosmarinus officinalis and brain diseases: the good, the bad, and the ugly. *J Neurochem*. 2013;133:1-4. doi:10.1115/1963520
13. Liu DY, Gao L, Zhang J, Huo XW, Ni H, Cao L. Anti-inflammatory and anti-oxidant effects of licorice flavonoids on ulcerative colitis in mouse model. *Chin Herb Med*. 2017;9(4):358-368. doi:10.1016/S1674-6384(17)60116-3
14. Liu Y-N, Yang Y-N, Feng Z-M, Jiang J-S, Zhang P-C. Eight new triterpenoid saponins with antioxidant activity from the roots of *Glycyrrhiza uralensis* Fisch. *Fitoterapia*. 2019;133:186-192. doi:10.1016/j.fitote.2019.01.014
15. Yang Y-N, Liu Y-Y, Feng Z-M, Jiang J-S, Zhang P-C. Seven new flavonoid glycosides from the roots of *Glycyrrhiza uralensis* and their biological activities. *Carbohydr Res*. 2019;485:107820. doi:10.1016/j.carres.2019.107820
16. Bao F, Bai H-Y, Wu Z-R, Yang Z-G. Phenolic compounds from cultivated *Glycyrrhiza uralensis* and their PD-1/PD-L1 inhibitory activities. *Nat Prod Res*. 2019;1-8. doi:10.1080/14786419.2019.1586698
17. Kamil IA, Smith JN, Williams RT. Studies in detoxication. XLVI. The metabolism of aliphatic alcohols; the glucuronic acid conjugation of acyclic aliphatic alcohols. *Biochem J*. 1953;53(1):129-136. doi:10.1042/bj0530129
18. Kim CS, Oh J, Suh WS, et al. Investigation of chemical constituents from *Spinaeum prunifolia* var. *simpliciflorus* and their biological activities. *Phytomed Lett*. 2017;22:255-260. doi:10.1016/j.phytol.2017.09.014
19. Nakamish T, Inada A, Kambayashi K, Yonedz K, et al. Flavonoid glycosides of the roots of *Glycyrrhiza uralensis*. *Phytochemistry*. 1985;24(2):339-341. doi:10.1016/S0031-9422(00)83548-7
20. Liu Q, Liu YL. Studies on chemical constituents of *Glycyrrhiza uralensis* C. F. Li. *Yan Xue Xue Bao*. 1989;24(7):525-531.
21. Lee JE, Lee JY, Kim J, Lee K, Choi SU, Ryu SY. Two minor chalcone acetylglucosides from the roots extract of *Glycyrrhiza uralensis*. *Arch Pharm Res*. 2015;38(7):1299-1303. doi:10.1007/s12272-015-0526-y
22. Montoro P, Maldini M, Russo M, Postorino S, Pacente S, Pizzi C. Metabolic profiling of roots of liquorice (*Glycyrrhiza glabra*) from different geographical areas by ESI/MS/MS and determination of major metabolites by LC-ESI/MS and LC-ESI/MS/MS. *J Pharm Biomed Anal*. 2011;54(3):535-544. doi:10.1016/j.jpba.2010.10.004
23. Li X, Lai GF, Wang YF, et al. Studies on chemical constituents of *Polygonatum kingianum*. *Chin Tradit Herbal Drugs*. 2008;39(6):825-828.
24. Kaur P, Kaur S, Kumar N, et al. Evaluation of antigenotoxic activity of isoliquiritin apioside from *Glycyrrhiza glabra* L. *Toxicol In Vitro ed.*; 2009:23; 680-686.
25. Xiang C, Cheng J, Liang H, Zhao Y-Y, Feng J. Isoflavones from *Milletia nitida* var. *hirsutissima*. *Yan Xue Xue Bao*. 2009;44(2):158-161.
26. Fu B, Li H, Wang X, Lee FSC, Cui S. Identification and identification of flavonoids in licorice and a study of their inhibitory effects on tyrosinase. *J Agric Food Chem*. 2005;53(19):7408-7414. doi:10.1021/jf051258g
27. Wang S, Zhu Y, Shao Q, Wang Y, Fan X, Cheng Y. Identification of chemical constituents in two traditional Chinese medicine formulae by liquid chromatography-mass spectrometry and off-line nuclear magnetic resonance. *J Pharm Biomed Anal*. 2016;117:255-265. doi:10.1016/j.jpba.2015.09.007
28. Gaffield W. Circular dichroism, optical rotatory dispersion and absolute configuration of flavanones, 3-hydroxyflavanones and their glycosides: determination of aglycone chirality in flavanone glycosides. *Tetrahedron*. 1970;26(17):4093-4108.
29. Ji S, Li Z, Song W, et al. Bioactive constituents of *Glycyrrhiza uralensis* (licorice): discovery of the effective components of a traditional herbal medicine. *J Nat Prod*. 2016;79(2):281-292. doi:10.1021/acs.jnatprod.5b00877
30. Kanaoka M, Yano S, Kato H, et al. Synthesis and separation of 18 β-glycyrrhetin monogluconide from serum of a patient with glycyrrhizin-induced pseudo-aldosteronism. *Chem Pharm Bull*. 1986;34(12):4978-4983.
31. Heneka MT, Carson MJ, El Khoury J, et al. Neuroinflammation in Alzheimer’s disease. *Neuron*. 2015;14(4):388-405. doi:10.1016/j.sat.2014.11.016
32. Soto-Otero R, Méndez-Alvarez E, Hermida-Ameijeiras A, Muñoz-Patiño AM, Lahande-Garcia JL. Autooxidation and neurotoxicity of 6-hydroxydopamine in the presence of some antioxidants: potential implication in relation to the pathogenesis of Parkinson’s disease. *J Neurochem*. 2000;74(4):1605-1612. doi:10.1046/j.1471-4159.2000.0741605.x
33. Brigger K, Schiavone S, Miller FJ, Krause K-H. Reactive oxygen species from healthy to disease. *Swiss Med Wkly*. 2012;142:w13659. doi:10.4414/smw.2012.13659
34. Zhou Y-Z, Li X, Gong W-X, et al. Protective effect of isoliquiritin against corticosterone-induced neurotoxicity in PC12 cells. *Food Funct*. 2017;8(3):1235-1244. doi:10.1039/C6FO01503D
35. Wu J, Huang FX, Wang J, Shi CC, Fang GY, et al. Protective effect of liquiritin on corticosterone-induced neurotoxicity in PC12 cells. *Trop J Pharm Res*. 2018;17(10):2013-2017. doi:10.4314/tjpr.v17i10.17
36. Kim J-Y, Son F, Kim D-S. One new veratramine-type alkaloid from *Veratrum maackii var. japonicum* and antioxidative activities of isolated compounds. *Nat Prod Commun*. 2020;15(7):1934578X2093940. doi:10.1177/1934578X20939408