Bioorganic & Medicinal Chemistry Letters 19 (2009) 6009–6013

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Glucose-containing flavones—their synthesis and antioxidant and neuroprotective activities

Seung Hwan Kim [a], Ch. Naveen Kumar [b], Hyoung Ja Kim [a], Dong Han Kim [b], Jungsook Cho [c], Changbae Jin [a], Yong Sup Lee [b,*]

[a] Life Sciences Research Division, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea
[b] Department of Life and Nanopharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 130-701, Republic of Korea
[c] Department of Pharmacology, College of Medicine, Dongguk University, Gyeongbuk 780-714, Republic of Korea

ARTICLE INFO

Article history:
Received 2 July 2009
Revised 14 September 2009
Accepted 16 September 2009
Available online 19 September 2009

Keywords:
Reactive oxygen species
Antioxidant
Ischemia
Quercetin 3-*O*-methyl ether
Glucose
Neuroprotection

ABSTRACT

Due to high reactivity, reactive oxygen species can attack biological molecules leading to cell or tissue injury. In this study, glucose moiety was attached at the C-7 position of quercetin 3-*O*-methyl ether (**1**) and luteolin (**2**) through glycosidic bond or ether linkage. The glucose-containing compounds showed potent DPPH and superoxide anion radical scavenging and lipid peroxidation inhibition activities and nearly equivalent protective actions to the parent aglycons against the H_2O_2-induced oxidative neuronal damage in primary cultured rat cortical cells. Among the compounds tested, **3b** and **3c** were the most potent (IC_{50} values = 7.33 and 5.34 μM, respectively), exhibiting nearly equivalent actions to the parent compounds **1** and **2** (IC_{50} = 3.50 and 3.75 μM, respectively).

In aerobic organisms, mitochondria convert glucose into energy for cells in the form of ATP via the electron transport chain. During mitochondrial respiration, oxygen is normally reduced to water, but this process is incomplete and up to 2% of oxygen is converted to the superoxide anion radical O_2^-,[1] which can be transformed to other reactive oxygen species (ROS), such as, hydrogen peroxide, peroxynitrite, or hydroxy radicals.[2,3] ROS are highly reactive due to the presence of an unpaired oxygen valence shell, which allows them to attack biological molecules, such as, lipids, proteins, enzymes, DNA, and RNA, and thus cause cell and tissue injuries.[4] In particular, brain tissue is highly sensitive to oxidative stress due to its high oxygen utilization rate, low levels of antioxidant enzymes,[5] the susceptibility of brain membranes to peroxidation,[6] and its high iron content.[7] Therefore, antioxidants that can protect neuronal cells from oxidative injuries may provide a means for preventing or treating oxidative stress-related neurodegenerative disorders, such as, ischemia,[8] Alzheimer's disease,[9] and Parkinson's disease.[10] During the course of our continued search for antioxidative neuroprotectants from plant sources, we recently identified quercetin 3-*O*-methyl ether (**1**) a potent antioxidant from *Opuntia ficus-indica* var. *saboten*.[11] We found that **1** exhibited a protective effect against oxidative neuronal damage induced by H_2O_2 or superoxide anion radicals in cultured neurons.[12]

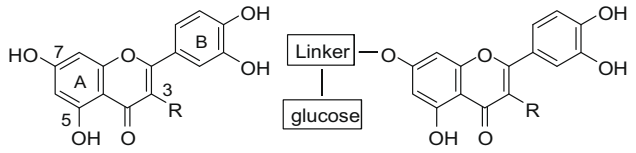
The in vivo biological activities of flavonoids depend on many parameters, including bioavailability. Thus, the lower solubilities of flavonoids in water can limit their bioavailabilities in vivo.[13] On the other hand, most flavonoids present in vegetables or plants are bound to sugars as glycosides. In this context, it has been reported that the absorption of quercetin glycoside is more efficient than that of quercetin aglycon,[14] indicating that the hydrophilic character of glucose probably increased the water solubility of the aglycon, and enhanced its bioavailability. Accordingly, to develop a neuroprotective agent from quercetin 3-*O*-methyl ether (**1**), we modified its structure in an attempt to improve its physicochemical properties. In this report, we describe synthesis of glucose-containing flavones **3a–f** and their antioxidant and neuroprotective activities (Fig. 1).

Because of the obvious structural similarities, luteolin (**2**) was also included in this study. Glucose was attached at the C-7 position of **1** and **2**, because we previous found that modification at this position does not substantially influence antioxidant activity.[15] Flavones were derivatized as glucosides or as ethers using ester or ethylene glycol linkages.

The regioselective 7-O-glucosylations of quercetin 3-*O*-methyl ether (**1**) and luteolin (**2**) were achieved using the Mitsunobu method,[16] which involves the coupling of protected flavones with

* Corresponding author. Tel.: +82 2 961 0370; fax: +82 2 966 3885.
E-mail address: kyslee@khu.ac.kr (Y.S. Lee).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.062

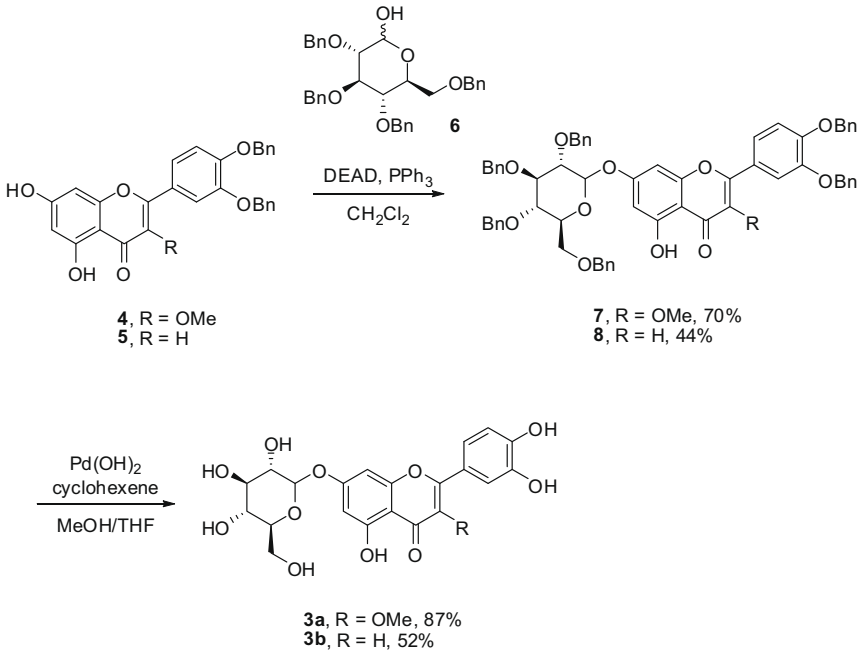


1, R = OCH_3
2, R = H

3a~f, R = OCH_3 or H

Figure 1. Design of glucose-containing flavones.

protected glucose (Scheme 1). 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (**6**) was reacted with benzyl-protected flavones **4**[15] and **5**[17] in the presence of DEAD and triphenylphosphine (PPh_3) in CH_2Cl_2 at room temperature to afford the benzyl-protected flavone glucosides **7** and **8** in 70% and 44% yields, respectively, with predominantly the β anomeric configuration. The benzyl-protecting groups in **7** and **8** were removed by hydrogenolysis in the presence of $Pd(OH)_2$ and cyclohexene in MeOH/THF at 80 °C to afford **3a** and **3b** in 87% and 52% yields, respectively.

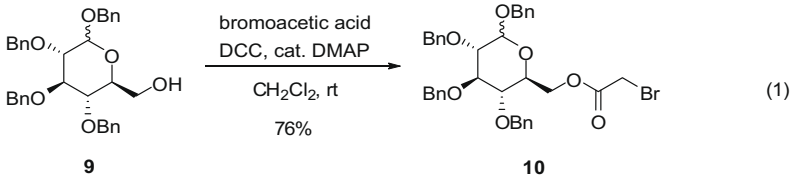


4, R = OMe
5, R = H

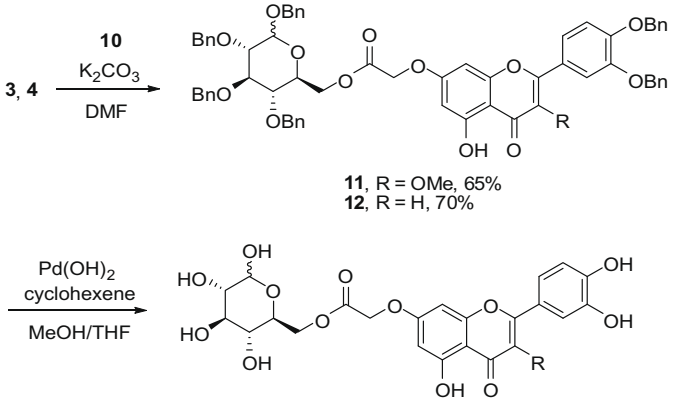
7, R = OMe, 70%
8, R = H, 44%

3a, R = OMe, 87%
3b, R = H, 52%

Scheme 1.



9 **10** (1)



11, R = OMe, 65%
12, R = H, 70%

(2)

3c, R = OMe, 66%
3d, R = H, 39%

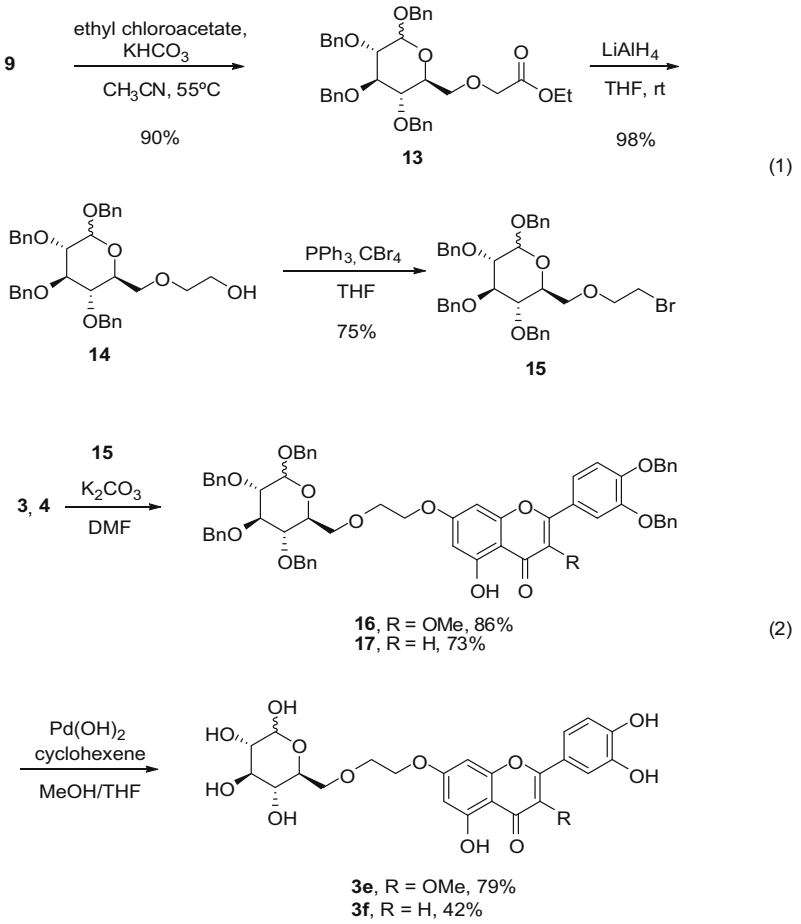
Scheme 2.

The introduction of a glucose via an ether bond using acetate as a linker was achieved by the regioselective 7-O-alkylation of the flavones with a bromoacetate **10**, which containing an ester bound glucose group (Scheme 2). The required bromoacetate **10** was prepared in 78% yield by the DCC coupling reaction between benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranoside (**9**)[18] and bromoacetic acid in the presence of a catalytic amount of DMAP in CH_2Cl_2. The reaction between **10** and the benzyl-protected flavones **3** and **4** in the presence of K_2CO_3 in DMF at 80 °C afforded the benzyl-protected flavones **11** and **12** in 65% and 70% yields, respectively. Finally, the benzyl-protecting groups in **11** and **12** were removed using $Pd(OH)_2$ and cyclohexene to afford **3c** and **3d** in 66% and 39% yields, respectively.

The introduction of a glucose via an ether bond using ethylene glycol as a linker was also achieved using a procedure similar to that described above using the bromoethyl-glucose **15** (Scheme 3). Compound **15** was prepared from **9** through a three-step reaction sequence, involving; alkylation of the C-6 hydroxyl group of **9** with ethyl chloroacetate in the presence of $KHCO_3$ in CH_3CN (90%),[19] reduction of the ethyl ester group with $LiAlH_4$ in THF (98%),[20] and bromination of the resulting hydroxyl group using CBr_4 and PPh_3 in THF (75%).[21] Finally, the coupling reactions between **15** and benzyl-protected flavones **3** and **4** in the presence of K_2CO_3 in DMF followed by the removal of the benzyl-protecting groups of the resulting products on $Pd(OH)_2$ and cyclohexene system afforded **3e** and **3f**.

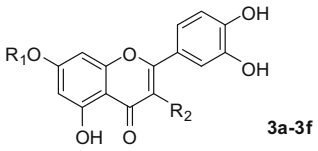
It has been reported that quercetin glucoside is predominantly absorbed by a glucose-transport system[22,23] or by passive transport after deglycosylation in the intestine prior to absorption.[24] In the present study, the glucose moiety was introduced to flavones in two ways; via an ether bond, using ester or ethylene glycol linkages, or via a glucosidic bond. Since the glucose connected to flavones via an ether linkage is unlikely to be cleaved by intestinal microflora, we assessed the antioxidant and neuroprotective activities of **3a–3f** to investigate whether the biological activities of parent flavones **1** and **2** were retained in these glucose-containing flavones. The antioxidant activities of the synthesized flavones **3a–3f** were evaluated by examining DPPH[25] and superoxide anion[26] radical scavenging and the inhibition of lipid peroxidation[27] assays in several cell-free bioassay systems (Table 1). For comparison purposes, the antioxidant activities of ascorbic acid and the parent flavones **1** and **2** were included as positive controls. As shown in Table 1, the synthesized compounds showed DPPH radical scavenging activities (IC_{50} = 15.64–19.74 μM) that were similar to those of the parent compounds **1** and **2** (IC_{50} = 14.17 and 16.18 μM, respectively), which confirmed that the introduction of the glucose moiety had little effect on DPPH radical scavenging activity. Furthermore, DPPH radical scavenging activities did not appear to be altered significantly by the type of glucose linkage.

Xanthine oxidase generates hydrogen peroxide and superoxide anion radicals, which are readily converted to highly reactive hydroxyl radical in the presence of iron,[28] and thus, we examined the superoxide anion radical scavenging activities of **3a**–**3f** using an xanthine/xanthine oxidase system (Table 1).[26] The quercetin 3-*O*-methyl ether series (**3a**, **3c**, **3e**) exhibited variable superoxide radical scavenging activities (IC_{50} = 10.24–32.92 μM) as compared to the parent compound **1** (IC_{50} = 17.39 μM). In contrast, the superoxide radical scavenging activity of the luteolin series (**3b**, **3d**, **3f**) was increased by ca. twofold by the introduction of a glucose group

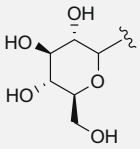
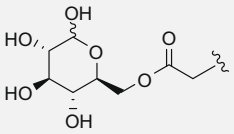
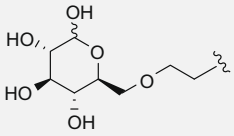


Scheme 3.

Table 1
The antioxidant and neuroprotective activities of the glucose-containing flavones **3a–3f** and their parent compounds **1** and **2**



3a-3f

Compds	R_1	R_2	IC_{50}[a] (μM)			
			DPPH scavenging[b]	O_2^- scavenging[c]	LPO inhibition[d]	Inhibition of H_2O_2-induced injury[e]
3a		OMe	16.89 ± 0.52	10.24 ± 0.79	43.21 ± 9.80	30.65 ± 1.14
3b		H	17.28 ± 0.27	3.28 ± 0.20	40.41 ± 5.75	7.33 ± 1.10
3c		OMe	19.74 ± 2.11	32.92 ± 0.17	66.66 ± 4.81	5.34 ± 1.08
3d		H	16.67 ± 1.48	6.22 ± 0.08	47.87 ± 6.67	12.70 ± 1.02
3e		OMe	15.64 ± 1.80	12.77 ± 0.63	77.96 ± 11.58	58.36 ± 1.04
3f		H	18.50 ± 1.73	3.53 ± 0.51	47.80 ± 2.35	15.71 ± 1.27
1	H	OMe	14.17 ± 0.69	17.39 ± 0.50	19.00 ± 0.51	3.50 ± 1.26
2	H	H	16.18 ± 1.85	8.66 ± 0.10	24.66 ± 4.75	3.75 ± 1.16
Ascorbic acid			40.99 ± 6.19	>300	>300	—

[a] IC_{50} values (defined as concentrations that inhibited activity by 50%) were calculated using GraphPad Prism using data obtained from at least three independent experiments.
[b] DPPH radical scavenging activity.[25]
[c] Radical scavenging activity of superoxide anions generated in the xanthine/xanthine oxidase system.[26]
[d] Inhibition of lipid peroxidation induced by iron-ascorbic acid in rat liver homogenate.[27]
[e] Inhibition of the H_2O_2-induced oxidative neuronal damage in primary cultured rat cortical cells.[29] The IC_{50} values are expressed as the means ± SD.

(IC_{50} = 3.28–6.22 μM) versus parent compound **2** (IC_{50} = 8.66 μM). Furthermore, luteolin series compounds (**3b**, **3d**, **3f**) were about 3–4-fold more potent than the quercetin 3-*O*-methyl ether series (**3a**, **3c**, **3e**), which implied that the substituent (R = H or OCH_3) at the C-3 position of the flavone ring played a role in the scavenging of superoxide radicals.

Due to high levels of unsaturated lipids, brain tissue is particularly susceptible to lipid peroxidation by ROS.[6] Thus, we evaluated the effects of the synthesized compounds on lipid peroxidation using rat liver homogenate.[27] Table 1 shows that all the glucose-containing compounds synthesized during this study inhibited lipid peroxidation (Table 1). However, they were 2–3-fold less potent than the parent compounds **1** and **2**. We attribute this reduced inhibition of lipid peroxidation to the hydrophilic character conferred by the attachment of glucose. All synthesized and parent compounds were significantly more potent than ascorbic acid at scavenging radicals and inhibiting lipid peroxidation (Table 1).

To evaluate the neuroprotective effects of the synthesized glucose-containing flavones **3a–3f**, we employed primary cultured rat cortical cells that had been maintained for 10–12 days in vitro.[29] When cultures were exposed to 100 μM H_2O_2 for 5 min prominent neuronal cell death was observed at 20–24 h after exposure, as compared with sham-treated controls. MTT reduction assays showed that 80–90% of cells were damaged under this experimental condition. However, simultaneous cotreatments of cells with **3a**–**3f** and H_2O_2 inhibited oxidative injury, and resulted in concentration-dependent increases in cell survival (Fig. 2). Furthermore, although no clear structure–activity relationship was not found, luteolin series members (**3b**, **3d**, **3f**) were more effective at protecting these neuronal cells against H_2O_2-induced oxidative injury than the quercetin 3-*O*-methyl ether series (**3a**, **3c**, **3e**). Of the compounds tested, **3b** and **3c** most potently inhibited oxidative injury, with IC_{50} values of 7.33 and 5.34 μM, respectively (Table 1); the IC_{50} values of the parent compounds **1** and **2** were 3.50 and 3.75 μM, respectively. Based on these findings, we conclude that glucose addition to the parent structures had only a marginal effect on the protective actions of the parent compounds against H_2O_2-induced oxidative neuronal damage.

In conclusion, we synthesized glucose-containing derivatives of quercetin 3-*O*-methyl ether and luteolin and evaluated their antioxidant and neuroprotective activities. Irrespective of the linkage type examined, glucose-containing compounds exhibited potent DPPH and superoxide radical scavenging activities. Lipid peroxidation in rat liver homogenate was also inhibited by the synthesized compounds, but their potencies were slightly lower than those of the parent compounds. However, the improved physicochemical properties achieved by introducing glucose may compensate for a reduced ability to protect against lipid peroxidation. In addition, the synthesized glucose derivatives retained the neuroprotective effects of their parent compounds against H_2O_2-induced oxidative injuries. Of the synthesized compounds, **3b** and **3c** most protected neuronal cells, and these levels were comparable to those of the parent compounds. Taken together, these findings suggest that the introduction of the glucose moiety to flavones may improve water solubility and only marginally reduced antioxidant and neuroprotective activities.

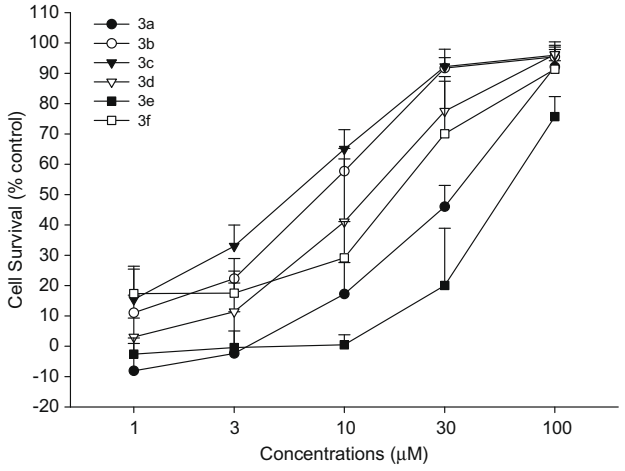


Figure 2. Effects of the glucose-containing flavones **3a–3f** on H_2O_2-induced oxidative damage in cortical cultures. Primary cultured rat cortical cells (10–12 days in vitro) were exposed to 100 μM H_2O_2 for 5 min in the absence or presence of the indicated concentrations of the test compounds. Cell viabilities were assessed using MTT reduction assays after further incubation for 20–24 h at 37 °C.[29] Points represent means ± SEM of three separate experiments performed in duplicate. Cell viabilities were calculated using the following formula: Cell viability (%) = 100 × ($Abs_{insult + sample} - Abs_{insult}$)/($Abs_{control} - Abs_{insult}$).

Acknowledgment

This research was supported by the Plant Diversity Research Center of the 21st Century Frontier Research Program (#PF06216-00).

References and notes

1. Pérez-Campo, R.; Lopez-Torres, M.; Cadenas, S.; Rojas, C.; Barja, G. *J. Comp. Physiol. B* **1998**, *168*, 149.
2. Simonian, N. A.; Coyle, J. T. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 83.
3. Huie, R. E.; Padmaja, S. *Free Radical Res. Commun.* **1993**, *18*, 195.
4. Warner, D. S.; Sheng, H.; Batinic-Haberle, I. *J. Exp. Biol.* **2004**, *207*, 3221.
5. Marklund, S. L.; Westman, M. G.; Lundgreen, E.; Roos, G. *Cancer Res.* **1982**, *42*, 1955.
6. Zaleska, M. M.; Nagy, K.; Floyd, R. A. *Neurochem. Res.* **1989**, *14*, 597.
7. Sorond, F. A.; Ratan, R. R. *Antioxid. Redox Signal.* **2000**, *2*, 421.
8. Nagai, K.; Jiang, M. H.; Hada, J.; Nagata, T.; Yajima, Y.; Yamamoto, S.; Nishizaki, T. *Brain Res.* **2002**, *956*, 319.
9. McIntosh, L. J.; Trush, M. A.; Troncoso, J. C. *Free Radical Biol. Med.* **1997**, *23*, 183.
10. Van Muiswinkel, F. L.; Riemers, F. M.; Peters, G. J.; Lafleur, M. V. M.; Siegel, D.; Jongenelen, C. A. M. *Free Radical Biol. Med.* **2000**, *29*, 442.
11. Lee, E. H.; Kim, H. J.; Song, Y. S.; Jin, C.; Lee, K.-T.; Cho, J.; Lee, Y. S. *Arch. Pharm. Res.* **2003**, *26*, 1018.
12. Dok-Go, H.; Lee, K. H.; Kim, H. J.; Lee, E. H.; Lee, J.; Song, Y. S.; Lee, Y.-H.; Jin, C.; Lee, Y. S.; Cho, J. *Brain Res.* **2003**, *965*, 130.
13. Matsumoto, M.; Chiji, H.; Hara, H. *Free Radical Res.* **2005**, *39*, 1139.
14. Okamoto, T.; Hara, K. *Curr. Top. Pharmacol.* **2005**, *9*, 97.
15. Yoo, H.; Kim, S. H.; Lee, J.; Kim, H. J.; Seo, S. H.; Chung, B. Y.; Jin, C.; Lee, Y. S. *Bull. Korean Chem. Soc.* **2005**, *26*, 2057.
16. Vaccaro, W. D.; Davis, H. R., Jr. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 313.
17. Litkei, G.; Gulasci, K.; Antus, S.; Blasko, G. *Liebigs Ann.* **1995**, 1711.
18. Berkowitz, D. B.; Bose, M.; Pfannenstiel, T. J.; Doukov, T. *J. Org. Chem.* **2000**, *65*, 4498.
19. Ferte, J.; Kuhnel, J. M.; Chapuis, G.; Rolland, Y.; Lewin, G.; Schwaller, M. A. *J. Med. Chem.* **1999**, *42*, 478.
20. Zaveri, N. T. *Org. Lett.* **2001**, *3*, 843.
21. Dubber, M.; Lindhorst, T. K. *Org. Lett.* **2001**, *3*, 4019.
22. Mizuma, T.; Ohta, K.; Hayashi, M.; Awazu, S. *Biochem. Pharmacol.* **1992**, *43*, 2037.
23. Mizuma, T. *Drug Delivery Syst.* **2006**, *21*, 126.
24. Chang, Q.; Zuo, Z.; Chow, M. S. S.; Ho, W. K. K. *Eur. J. Pharm. Biopharm.* **2005**, *59*, 549.
25. *DPPH radical scavenging activity assay.*[30] The antioxidant activities of the synthesized compounds was assessed by examining their abilities to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Reaction mixtures containing test samples (dissolved in EtOH) and 100 μM of DPPH ethanolic solution in 96-well plates were incubated at 37 °C for 30 min. Absorbances were measured at 515 nm. Percent inhibitions were calculated versus ethanol-treated controls. IC_{50} values denote the concentration required to scavenge 50% of DPPH radicals.
26. *Superoxide anion radical scavenging activity assay.*[31] The reaction mixture consisted of 40 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.1 mM EDTA, 50 μg protein/ml of bovine serum albumin, 25 mM nitroblue tetrazolium, 1.4×10^{-8} unit xanthine oxidase (EC 1.2.3.2), and various concentrations of test samples in a final volume of 200 μl. After incubation these mixtures at 25 °C for 20 min, reactions were terminated by adding 6.6 μl of 6 mM $CuCl_2$. The absorbance of the formazan produced was measured at 560 nm. IC_{50} values denote the concentrations required to scavenge 50% of superoxide anion radicals.
27. *Inhibition of lipid peroxidation.*[32] The effects of the synthesized compounds on lipid peroxidation induced by an iron-ascorbic acid mix was determined in rat liver homogenate. In brief, rat liver homogenate (300 μl, 11 mg protein/ml) was incubated with 10 μM Fe_2SO_4, 0.4 mM ascorbic acid and various concentrations of the test samples in 50 mM Tris–HCl (pH 7.5) at 37 °C for 30 min in a total volume of 1 ml. After incubation, lipid peroxidation levels were determined by measuring the formation of thiobarbituric acid-reactive substance (TBARS). Reactions were terminated by adding 2 ml of a solution of 0.375% thiobarbituric acid in 15% trichloroacetic acid containing 0.25 N HCl and 0.01% butylated hydroxytoluene (TBA-TCA reagent). Mixtures were then heated at 95 °C for 30 min, cooled, and centrifuged at 5000*g* for 10 min. The absorbances of supernatants were measured at 535 nm. Protein contents in liver homogenates were determined using the Bradford method using bovine serum albumin as a standard.
28. Atlante, A.; Gagliardi, S.; Minervini, G. M.; Ciotti, M. T.; Marra, E.; Calissano, P. *J. Neurochem.* **1997**, *68*, 2038.
29. *Primary cultures of rat cortical cells, experimental treatments, and the assessments of neuronal injuries.* Cortical cell cultures were prepared from the cerebral cortices of Sprague-Dawley rat embryos at 16–18 days of gestation and maintained at 37 °C in a humidified atmosphere of 95% air/5% CO_2 as previously described.[12] Cultures were used for experiments at 10–12 days after plating. Oxidative neuronal injuries were induced by exposing cultures to H_2O_2 (100 μM) for 5 min in HEPES-buffered salt solution (HBSS). Cultures were then washed and cells were maintained in MEM supplemented with 21 mM glucose for 20–24 h. Neuronal damage was quantified using MTT reduction assays.[33] To evaluate the effects of the glucose-containing flavones on oxidative neuronal injury, cultures were simultaneously exposed to various concentrations of the test compounds and H_2O_2. Cell viabilities were calculated using the following formula: Cell viability (%) = 100 × ($Abs_{insult + sample} - Abs_{insult}$)/($Abs_{control} - Abs_{insult}$). The mean values of three separate experiments performed in duplicate were analyzed by non-linear regression using Prism GraphPad Software and IC_{50} values were calculated.
30. Lee, J. S.; Kim, H. J.; Park, H.; Lee, Y. S. *J. Nat. Prod.* **2002**, *65*, 1367.
31. Toda, S.; Kumura, M.; Ohnishi, M. *Planta Med.* **1991**, *57*, 8.
32. Sanz, M. J.; Ferrandiz, M. L.; Cejudo, M.; Terencio, M. C.; Gil, B.; Bustos, G.; Ubeda, A.; Gunasegaran, R.; Alcaraz, M. J. *Xenobiotica* **1994**, *24*, 689.
33. Cho, J.; Kim, H. M.; Ryu, J.-H.; Jeong, Y. S.; Lee, Y. S.; Jin, C. *Biol. Pharm. Bull.* **2005**, *28*, 455.