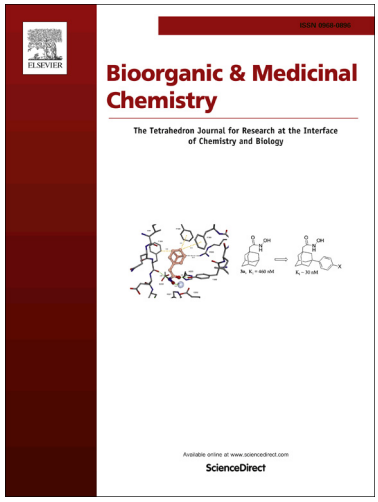

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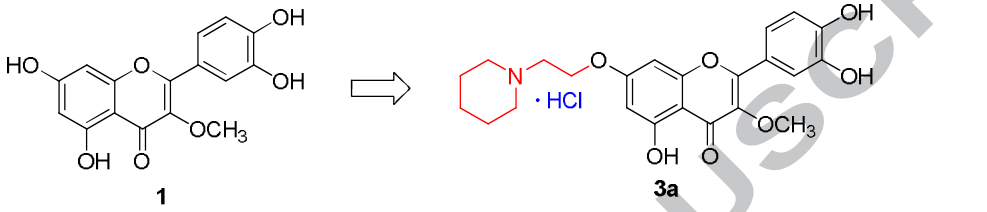

IC_{50}: DPPH radicals, 20.74 μM; O_2^- radicals, 8.15 μM
Lipid peroxidation, 19.18 μM

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Synthesis of (2-amino)ethyl derivatives of quercetin 3-*O*-methyl ether and their antioxidant and neuroprotective effects

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ABSTRACT

Reactive oxygen species have been implicated in several diseases, particularly in ischemia-reperfusion injury. Quercetin 3-*O*-methyl ether has been reported to show potent antioxidant and neuroprotective activity against neuronal damage induced by reactive oxygen species. Several aminoethyl-substituted derivatives of quercetin 3-*O*-methyl ether have been synthesized to increase water solubility while retaining antioxidant and neuroprotective activity. Among such derivatives, compound **3a** shows potent and well-balanced antioxidant activity in three types of cell-free assay systems and has in vivo neuroprotective effects on transient focal ischemic injury induced by the occlusion of the middle cerebral artery in rats.

1. Introduction

In aerobic organisms, most cells have mitochondria, which produce energy for cells in the form of ATP through an electron transport chain. During respiration, oxygen is normally reduced to water, but when this process is incomplete, some reactive oxygen species (ROS) are produced, including superoxide anion radical, hydrogen peroxide, peroxynitrite, and hydroxyl radical.[1] Because ROS are highly reactive, they can damage cells and cause diseases such as cardiovascular disease,[2] ischemic stroke,[3] and Alzheimer's disease.[4] In particular, close attention has been paid to the involvement of oxidative stress in brain dysfunction and neurodegenerative disorders.[5] The brain may be particularly vulnerable to oxidative stress caused by ROS because of its high polyunsaturated lipid content, regions of high iron concentrations, and moderate levels of antioxidant molecules.[6] In ischemic stroke, which is caused by some blockage in the artery to the brain, ROS can exacerbate cell injury and death because the re-supply of oxygen to the brain through reperfusion is relevant during the ischemic phase.[7,8] Therefore, antioxidants that can scavenge oxygen free radicals have the therapeutic potential to treat neuronal injury following ischemia and reperfusion.[9,10] Since no medical treatment is currently approved for the treatment of stroke to reduce brain infarction or neurological disability beyond tissue plasminogen activator, a thrombolytic agent restricted to administration within 3 hours after stroke, it is most important to develop neuroprotective drugs, which can be given to minimize the neuronal damage which follows a stroke.

Quercetin 3-*O*-methyl ether (**1**, Q-3ME) has been identified as a potent antioxidant from *Opuntia ficus-indica var. saboten* (Fig 1).[11] Compound **1** has been found to inhibit neuronal damage induced by H_2O_2 or superoxide anion radicals in primary cultured rat cortical cells[12] and to have in vivo neuroprotective effects on transient focal ischemic brain injury in a middle cerebral artery occlusion (MCAO) rat model.[13] In addition, the synthesis of 7-*O*-glucosyl flavones (for example, **2**) has been reported to enhance the water solubility of **1**. However, an excessive increase in hydrophilicity by introducing the polar glucose group reduces inhibitory activity against lipid peroxidation.[14]

The introduction of the alkylamino group to the structure of compounds is a general strategy for improving water solubility.[15-17] The alkylamino group can increase the water solubility of compounds through the formation of hydrochloride salts. On the other hand, the lipophilic characteristic can be retained by

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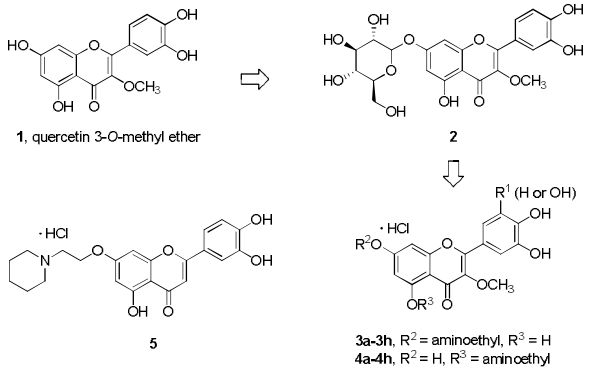


Figure 1. Structures of quercetin 3-*O*-methyl ether (**1**), its 7-*O*-glucoside (**2**), luteolin derivative **5**, and target compounds **3a**–**3h** and **4a**–**4h**.

introducing diverse alkyl groups. To prove whether this strategy works on flavone compounds, we introduced aminoethyl groups at the C-7 position of luteolin derivatives as a preliminary work and found that the synthesized 7-*O*-aminoethyl luteolin derivatives (e.g. **5**) exhibited a similar level of lipid peroxidation inhibitory activities to luteolin.[18] Based on these findings, we expanded this strategy to Q-3ME (**1**), which has a methoxy substituent at the C-3 position in the structure of luteolin, since this compound had been already proven to show in vivo neuroprotective effects on transient focal ischemic brain injury in a MCAO rat model.[13] Accordingly, aminoethyl-substituted Q-3ME derivatives **3a**–**3h** and **4a**–**4h** were derived in hydrochloride salt forms to improve water solubility without affecting antioxidant activities and neuroprotective effects of **1** (Fig 1). Additionally, an aminoethyl group was attached at the C-5 or C-7 position of the Q-3ME structure to examine effects of the position of the substituents on antioxidant activity and how antioxidant activity profiles influence in vivo neuroprotective effects. The B-ring of Q-3ME was also varied through the introduction of an additional hydroxyl group to provide myricetin 3-*O*-methyl ether derivatives (R^1 = OH). Finally, in vivo neuroprotective effects of selected compounds on transient focal ischemic brain injury were investigated using an MCAO rat model to examine whether the neuroprotective effects correlate to their antioxidant activities and are influenced by the introduction of aminoethyl substituents.

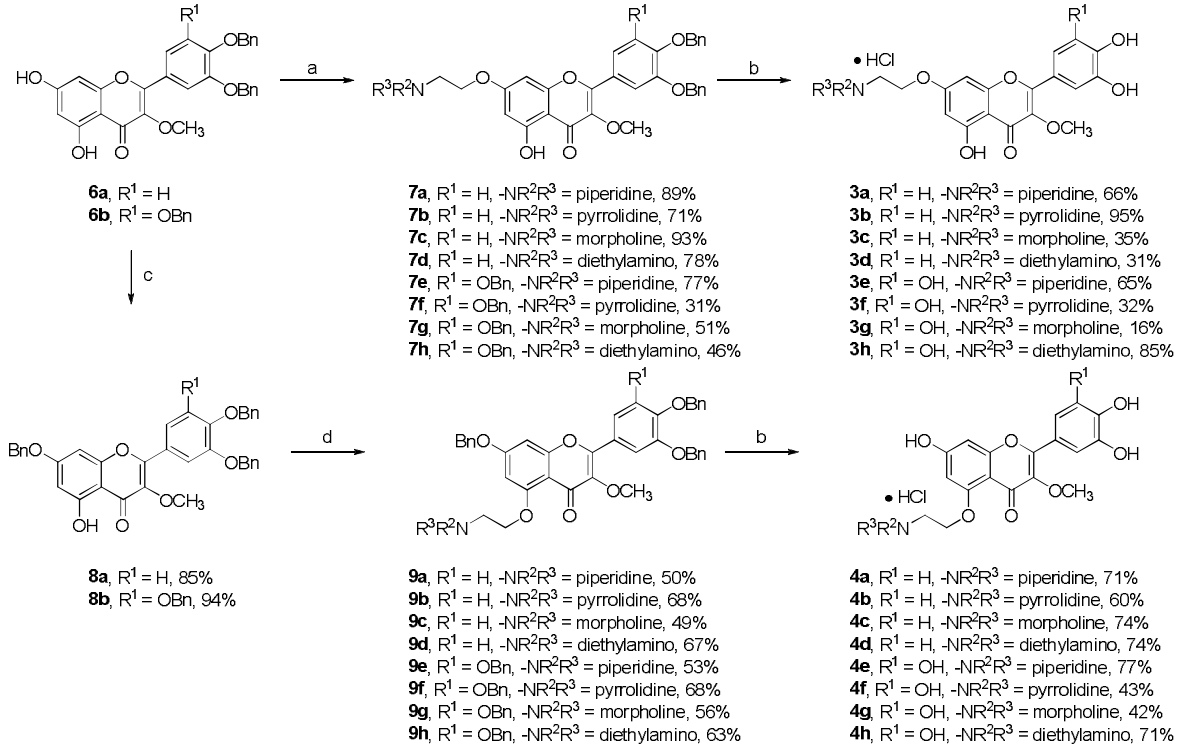
2. Results and discussion

2.1. Chemistry

The synthesis of **3a**–**3h** and **4a**–**4h** started from *O*-benzyl-protected 3-methoxyflavones **6a** and **6b** (Scheme 1). The regioselective 7-*O*-alkylation of **6a** and **6b** with several alkylaminoethyl chlorides in the presence of K_2CO_3 produced **7a**–**7h**.[19] Benzyl-protecting groups in **7a**–**7h** were removed by hydrogenolysis with $Pd(OH)_2$ and cyclohexene in EtOH and then transformed into C-7 aminoethyl-substituted Q-3ME derivatives **3a**–**3h** as hydrochloride salts by treating with HCl gas in ethanol. C-5 aminoethyl-substituted compounds **4a**–**4h** were prepared using a procedure similar to that for the synthesis of **3a**–**3h**. The selective 7-*O*-benzylation of **6a** and **6b** with benzyl bromide in the presence of K_2CO_3, followed by 5-*O*-alkylation with alkylaminoethyl chlorides, produced **9a**–**9h**. Finally, the removal of the benzyl-protecting group in **9a**–**9h** with $Pd(OH)_2$ and cyclohexene and then treatment with HCl provided C-5 aminoethyl derivatives **4a**–**4h** as hydrochloride salts.

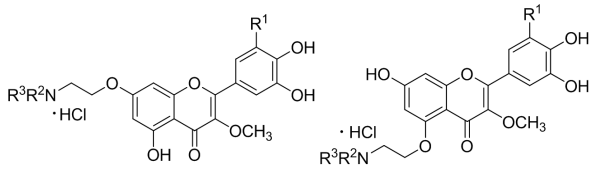
2.2. Antioxidant activity of aminoethyl-substituted Q-3ME derivatives

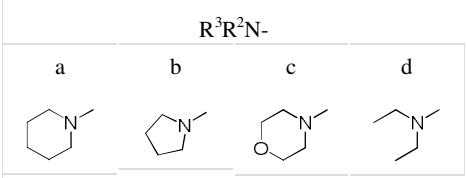
Synthesized compounds were assessed for antioxidant activity



Scheme 1. Reagents and conditions: (a) $R^3R^2NCH_2CH_2Cl$, K_2CO_3, acetone, 60 °C; (b) i) $Pd(OH)_2$/C, EtOH/cyclohexene (1:1), 60–70 °C, ii) HCl, MeOH; (c) benzyl bromide, K_2CO_3, acetone, 55 °C; (d) $R^3R^2NCH_2CH_2Cl$, K_2CO_3, DMF, 70 °C.

Table 1. The antioxidant activity of aminoethyl derivatives **3a**–**3h, 4a**–**4h,** and their parent compound **1**.





Entry	R^1	R^3R^2N-	DPPH radicals[a]	$O_2^{\cdot-}$ radicals[b]	Lipid peroxidation[c]	*c*LogP[e]	Aqueous solubility (mM)[f]
			IC_{50} (μM)[d]				
3a	H	a	20.74 ± 4.55	8.15 ± 0.15	19.18 ± 0.19	3.03	> 50
3b	H	b	18.87 ± 3.05	11.36 ± 1.84	21.80 ± 1.15	2.68	-
3c	H	c	47.16 ± 11.66	7.41 ± 0.28	16.23 ± 0.79	1.86	-
3d	H	d	27.35 ± 3.10	8.14 ± 1.64	15.78 ± 1.90	2.71	-
3e	OH	a	26.32 ± 5.42	> 100	10.54 ± 0.92	2.68	-
3f	OH	b	16.79 ± 1.52	> 100	10.11 ± 0.66	2.34	-
3g	OH	c	25.98 ± 6.74	> 100	10.15 ± 1.24	1.52	-
3h	OH	d	22.93 ± 2.63	> 100	11.54 ± 0.54	2.36	> 50
4a	H	a	> 100	5.80 ± 0.54	25.09 ± 1.34	3.03	-
4b	H	b	84.00 ± 15.93	4.58 ± 0.32	23.29 ± 0.55	2.68	-
4c	H	c	> 100	11.25 ± 3.07	37.37 ± 2.85	1.86	-
4d	H	d	> 100	6.53 ± 2.52	23.32 ± 1.06	2.71	-
4e	OH	a	32.42 ± 8.42	> 100	11.88 ± 0.47	2.68	-
4f	OH	b	27.04 ± 9.82	> 100	10.92 ± 0.96	2.34	-
4g	OH	c	23.18 ± 7.28	> 100	11.96 ± 0.93	1.52	-
4h	OH	d	22.06 ± 1.07	> 100	11.24 ± 0.90	2.36	-
1			14.17 ± 0.69	17.39 ± 0.50	19.00 ± 0.51	1.92	0.011
Ascorbic acid			40.99 ± 6.19	>50	-		
Trolox			-	-	71.44 ± 5.54		

[a] DPPH radical scavenging activity.
[b] Superoxide anion radical scavenging activity generated in the xanthine/xanthine oxidase system.
[c] Iron-dependent lipid peroxidation inhibition activity using a rat liver homogenate.
[d] IC_{50} values with standard deviations are from three independent experiments and expressed as the mean ± S.D.
[e] Values of the free base form, calculated by OSIRIS property explorer.
[f] Solubility in water at room temperature, determined by HPLC.

in three assay systems for DPPH scavenging, superoxide anion radical scavenging, and lipid peroxidation inhibition.[14] The putative antioxidants, ascorbic acid and trolox were used as positive controls. The parent compound Q-3ME (**1**) was also used to assess the variation of their antioxidant activity by the aminoalkyl substituent and the number of hydroxyl groups in the B-ring. First, the effects of the C-7 aminoethyl substituent on antioxidant activity were investigated (Table 1). The DPPH radical scavenging activity (IC_{50} = 18.87–47.16 μM) of C-7 aminoethyl derivatives **3a**–**3d**, which have two hydroxyl groups at the B-ring, was similar to or slightly lower than that of Q-3ME (**1**, IC_{50} = 14.17 μM). The lipid peroxidation inhibitory activity (IC_{50} = 15.78–21.80 μM) of these compounds was comparable to that of the parent compound (IC_{50} = 19.00 μM), indicating that the substitution of the aminoethyl group at C-7 had little effect on antioxidant activity. In addition, the superoxide anion radical scavenging activity (IC_{50} = 8.14–11.36 μM) of these compounds was two times more potent than that of Q-3ME (IC_{50} = 17.39 μM) in the xanthine/xanthine oxidase assay system. By contrast, the lipid peroxidation inhibitory activity (IC_{50} = 10.11–11.54 μM) of C-7 aminoethyl derivatives **3e**–**3h**, which have three hydroxyl groups at the B-ring (myricetin 3-*O*-methyl ether derivatives), was two times more potent than that of Q-3ME. However, to our surprise, these four derivatives showed no noticeable superoxide anion radical scavenging activity under a concentration of 100 μM. From our previous findings, the C-7 aminoethyl luteolin derivatives possessing three hydroxyl groups at the B-ring showed superoxide anion radical scavenging activities to some extent although their potency was 2- to 4-fold less (IC_{50} = 17.56–30.12 μM) than that of luteolin (IC_{50} = 8.66 μM).[18] It is noticeable in that slight difference of a substituent such as the presence or absence of 3-methoxy substituent at the flavones structure can influence the antioxidant activity profile.

The relationship between the structure and antioxidant activity of C-5 aminoethyl derivatives **4a**–**4h** was similar to that for C-7 aminoethyl derivatives, except for DPPH radical scavenging activity (Table 1). The lipid peroxidation inhibitory activity of compounds **4a**–**4d**, which have two hydroxyl groups at the B-

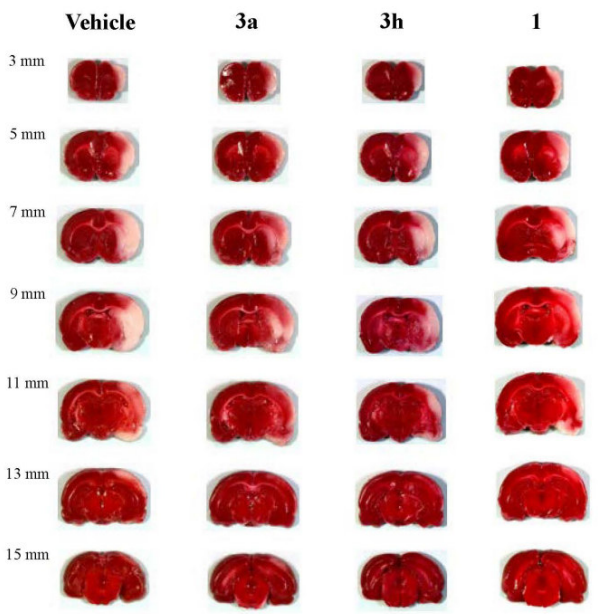


Figure 2. Representative TTC staining showing effects of **3a**, **3h**, and **1** administered at a dose of 10 mg/kg (i.v.) to rats subjected to middle cerebral artery occlusion (MCAO) for 2 h. Each brain was cut into seven serial slices 2 mm thick starting 1 mm from the frontal pole 24 h after MCAO. The posterior surface of each slice was imaged. TTC stained normal brain areas deep red but did not stain infarcted tissue. Values on the left-hand side indicate the distance from the frontal pole.

ring, was similar to or slightly lower than that of Q-3ME, but there was a 2- to 3-fold increase in the superoxide anion radical scavenging activity as were found on C-7 aminoethyl derivatives. However, there was a sharp decrease in their DPPH radical scavenging activity. Noteworthy is that this DPPH radical scavenging activity recovered through the introduction of an additional hydroxyl group at the B-ring (**4e**–**4h**). Compounds **4e**–**4h** showed ca. 2-fold increase in their lipid peroxidation inhibitory activity and no superoxide anion radical scavenging activity under a concentration of 100 μM, as in the case of compounds **3e**–**3h**. This result indicates that the position of aminoethyl substituents and the presence of an additional hydroxyl group at the B-ring had considerable influence on the antioxidant activity profile. The mechanism underlying the effects of these modifications of Q-3ME on antioxidant activity, particularly with respect to DPPH and superoxide anion radical scavenging activity, remains to be investigated. However, the results clearly indicate that C-7 aminoethyl derivatives **3a**–**3d**, which have two hydroxyl groups at the B-ring, showed more potent and well-balanced antioxidant activity in the three assay systems.

Aqueous solubilities of **1** and selected compound **3a** and **3h** are determined. The parent compound Q-3ME showed very poor water solublilty (11.4 μM), however, compound **3a** and **3h** showed much greater aqueous solubilities (> 50 mM) indicating that aminoethyl analogues prepared as HCl salts has more favorable pharmacokinetic properties than the parent compound **1**. (Table 1).

Currently, there are many softwares predicting a lipophilic property of a compound. Calculated LogP values (*c*LogP) of the free base form of **3a**–**3h** and **4a**–**4h** were obtained from the Osiris program[20] and included in the Table 1. Except compounds containing morpholinoethyl substituent (*c*LogP = 1.52–1.86), every compound showed increased predicted lipophilicity (*c*LogP = 2.34–3.03) than Q-3ME (*c*LogP = 1.92). Based on these data, it is expected that the lipophilic characteristic can be increased by introducing aminoethyl groups on the structure of Q-3ME.

In summary, the type of aminoethyl substituents exhibited little effect on antioxidant activity of Q-3ME while the position of aminoethyl substituents exhibit significant effect on DPPH radical scavenging activity. On the other hand, additional hydroxyl group at the B ring increased the lipid peroxidation inhibitory activity and decreased superoxide anion radical scavenging activity regardless of type and position of aminoalkyl substituent. Furthermore, aminoethyl-substituted Q-3ME derivatives showed improved pharmacokinetic properties including water solubility and lipophilicity.

2.3. Neuroprotective effects of compounds 3a and 3h

Compound **3a** was selected to further examine its in vivo neuroprotective activity in a transient focal cerebral ischemic rat model because it showed potent and well-balanced antioxidant activity in the three assay systems. The data of in vivo neuroprotective activity of Q-3ME (**1**)[13] was also included in the Table 2 and 3 for comparisons. Compound **3h**, which showed no superoxide anion radical scavenging activity under a 100 μM concentration, was also included in the experiment to compare and examine whether this activity would be correlated with the neuroprotective effect. The transient MCAO rat model was used to assess neuroprotective effects because most cases of ischemic stroke occur in the MCA region and ROS generated by reperfusion can damage neuronal cells and provoke infarction. Transient focal cerebral ischemia was induced by the occlusion of the right MCA for 2 h with a silicone-coated 4-0 nylon monofilament in male Sprague-Dawley rats.[13] Compound **3a** and **3h** dissolved in 0.9% saline were administered intravenously 30

Table 2. Neuroprotective effects of **3a**, **3h**, and **1** administered intravenously 30 min after the onset of ischemia at a dose of 10 mg/kg in a rat model of transient focal cerebral ischemia for 2 h.

Treatment	*n*	Absolute infarct volume (mm^3)[a]			Corrected total infarct volume (mm^3)[a]	Edema (%)[a]
		Striatum	Cortex	Total		
Vehicle	13	41.9 ± 2.9	195.6 ± 11.5	237.4 ± 13.1	163.2 ± 9.4	13.0 ± 0.7
3a	8	30.1 ± 5.4	93.6 ± 18.8*	128.9 ± 25.1*	92.1 ± 18.3*	6.7 ± 1.1*
3h	14	39.3 ± 5.1	123.4 ± 20.6*	164.0 ± 23.1*	108.9 ± 15.3*	9.4 ± 1.3*
1	12	27.2 ± 4.9	79.9 ± 9.7*	108.8 ± 13.1*	78.8 ± 9.7*	5.5 ± 0.8*

[a] Data is presented as the mean ± S.E.M.

* Significantly different from the vehicle-treated group based on Duncan's multiple-range test ($p < 0.05$)

min after the onset of ischemia at a dose of 10 mg/kg. Infarct size and % edema were measured 24 h after the onset of ischemia by using a 2,3,5-triphenyltetrazolium chloride (TTC) staining method. The area of the absolute infarct in the cortex and striatum and the total area of both hemispheres were measured for each slice by using a computerized image analysis system (Optimas). Absolute infarct volume was calculated by multiplying the area by the slice thickness and summing the volume. The corrected infarct area in a slice was calculated to compensate for the effect of brain edema by subtracting the area of normal tissue in the ipsilateral hemisphere from the total area of the contralateral hemisphere. Corrected total infarct volume was then calculated by multiplying the area by the slice thickness and summing the volume. Hemispheric swelling representing tissue edema was expressed as a percentage increase in the size of the ipsilateral hemisphere in comparison to the contralateral hemisphere (% edema). In addition, neurological scoring was performed 30 min and 24 h after MCAO. The neurobehavioral test consisted of scoring the degree of left-forelimb flexion (0 to 3), the duration of left-forelimb flexion (0 to 4), and the symmetry of movement/forepaw outstretching (0 to 3). Rats were scored on a ranking scale of 0 to 10, which reflected the cumulative score on individual tests with a score of 10 reflecting normal behavior. Fig. 2 shows representative TTC staining to illustrate neuroprotective effects of compound **3a** and **3h**. The area stained red with TTC was considered normal, whereas the rest, infarcted. As shown in Table 2, treatment with 10 mg/kg (i.v.) of compound **3a** significantly reduced the absolute infarct volume of the cortex by 52.2% (93.6 vs. 195.6 mm^3) and absolute total infarct volume by 45.7% (128.9 vs. 237.4 mm^3) in comparison to the vehicle-treated group. In addition, there were significant decreases in corrected total infarct volume and % edema by 43.6% (92.1 vs. 163.2 mm^3) and 48.5% (6.7% vs. 13.0% edema), respectively. This also produced a significant behavioral recovery effect when measured at 24 h after MCAO, increasing the neurological score to 4.87 ± 0.58, whereas it was 2.53 ± 0.21 for the control group (Table 3). This in vivo neuroprotective potency of **3a** is consistent with the in vitro antioxidant activity examined in the cell-free assay system. These neuroprotective effects of **3a** were comparable to those of Q-3ME, which significantly reduced the corrected total infarct volume and % edema by approximately 50% at a dose of 10 mg/kg.[13] However, treatment with **3h** produced weaker neuroprotective effects on ischemic brain injury than that with **3a**. Compound **3h** significantly reduced absolute total infarct volume by 30.9% (164.0 vs. 237.4 mm^3) in comparison to the vehicle-treated group (Table 2). In addition, corrected total infarct volume and % edema were significantly reduced by 33.3% (108.9 vs. 163.2 mm^3) and 27.7% (9.4% vs. 13.0% edema), respectively. However, treatment with **3h** did not produce a significant behavioral recovery effect (Table 3). According to a comparison of neuroprotective effects of treatment between **3a** and **3h** with respect to infarct volume, edema size, and behavioral recovery, the superoxide anion radical scavenging effect was an important parameter. Finally, ability of the compounds to cross the blood-brain barrier (BBB) should be considered in eliciting in vivo neuroprotective activity against ischemic brain injury. It has been known that the penetration rate of flavonoids is dependent on the lipophilicity and interactions with efflux transporters.[21,22] However, the predicted lipophilicity of **3a** (*c*LogP = 3.03) is not so higher than that of **3h** (*c*LogP = 2.36) to explain clearly the differences in their neuroprotective effects by the passage rate of compound through BBB. It is also known that cerebral ischemia induces disruption and hyperpermeability of the BBB, thus enhancing brain penetration of compounds with poor permeability.[23] Accordingly, it remains to measure permeability across the BBB or brain concentration of the compounds.

Table 3. Effects of **3a**, **3h**, and **1** (10 mg/kg, i.v., 30 min after the onset of ischemia) on neurological scores measured 30 min and 24 h after the onset of MCAO.

Treatment	Neurological scores[a]	
	30 min	24 h
Vehicle	2.32 ± 0.11	2.53 ± 0.21
3a	2.25 ± 0.16	4.87 ± 0.58*
3h	2.21 ± 0.11	3.64 ± 0.32
1	2.29 ± 0.12	5.29 ± 0.46*

[a] Data is presented as the mean ± S.E.M.

* Significantly different from the vehicle-treated group based on Duncan's multiple-range test ($p < 0.05$).

3. Conclusion

C-7 or C-5 aminoethyl-substituted Q-3ME derivatives **3a**–**3h** and **4a**–**4h** were synthesized in hydrochloride salt forms to improve water solubility while retaining antioxidant activities. C-7 aminoethyl derivatives **3a**–**3d**, which have two hydroxyl groups at the B-ring, showed well-balanced antioxidant activity. These compounds showed nearly equipotent DPPH radical scavenging and lipid peroxidation inhibitory activity and a 2-fold increase in superoxide anion radical scavenging activity in comparison to Q-3ME, the parent compound. On the other hand, the substitution of the aminoethyl group at C-5 position or the introduction of an additional hydroxyl group at the B-ring increased or reduced the antioxidant activity of Q-3ME depending on the assay system. Compound **3a**, which showed potent and well-balanced antioxidant activity in the three assay systems, had a neuroprotective effect on transient focal ischemic brain injury in an MCAO rat model. More specifically, it significantly reduced corrected total infarct volume and % edema by 43.6% and 48.5%, respectively, with a significant behavioral recovery effect at a dose of 10 mg/kg. The neuroprotective effects of **3a** were generally comparable to those of Q-3ME with minor variations, while the introduction of an aminoethyl group to Q-3ME increased its water solubility. On the other hand, compound **3h**, which showed no superoxide anion radical scavenging activity under a concentration of 100 μM, had a weaker neuroprotective effect than **3a** in the MCAO rat model, suggesting that superoxide anion radical scavenging activity plays an important role in neuroprotective effects.

4. Experimental

4.1. General instrumentation and chemicals

NMR spectra were recorded on a Gemini Varian-300 operating at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR spectra. High resolution mass spectra (HR-MS) were recorded on Jeol accuTOF (JMS-T100TD) equipped with a DART (direct analysis in real time) ion source from Ionsense (Tokyo, Japan) in the positive modes. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel (E. Merck Kiesegel 60F_{254} layer thickness 0.25 mm). Column chromatography was performed with Merck Kiesegel 60 Art 9385 (230-400 mesh). All

solvents, chemicals and reagents were purchased from Sigma-Aldrich, Acros or TCI.

4.2. Synthesis

4.2.1. 2-(3,4-Bis(benzyloxy)phenyl)-5-hydroxy-3-methoxy-7-(2-(piperidin-1-yl)ethoxy)-4*H*-chromen-4-one (7a). To a stirred solution of compound **6a**[18] (1.0 mmol) in acetone (60 mL) was added K_2CO_3 (4.0 mmol) and 1-(2-chloroethyl)piperidine hydrochloride (1.3 mmol). The reaction mixture was heated at reflux for 6 h. The mixture was concentrated and the residue was treated with EtOAc and water. The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 10:1) to afford **7a** (89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (1H, d, *J* = 1.4 Hz), 7.70 (1H, d, *J* = 8.4 Hz), 7.53–7.29 (10H, m), 7.06 (1H, d, *J* = 8.4 Hz), 6.42 (1H, d, *J* = 2.1 Hz), 6.36 (1H, d, *J* = 2.1 Hz), 5.29 (2H, s), 5.28 (2H, s), 4.21 (2H, t, *J* = 6.0 Hz), 3.73 (3H, s), 2.86 (2H, t, *J* = 6.0 Hz), 2.59–2.56 (4H, m), 1.68–1.63 (4H, m), 1.51–1.47 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6, 164.8, 162.2, 156.8, 155.8, 151.5, 148.6, 139.3, 137.2, 136.9, 128.9, 128.8, 128.3, 128.2, 127.5, 127.4, 123.5, 122.8, 115.3, 113.9, 106.3, 98.6, 92.9, 71.6, 71.1, 66.8, 60.3, 57.7, 55.3, 26.0, 25.7, 24.3.

4.2.2. 2-(3,4-Bis(benzyloxy)phenyl)-5-hydroxy-3-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-4*H*-chromen-4-one (7b). The compound **7b** was prepared from **6a** (1.0 mmol) and 1-(2-chloroethyl)pyrrolidine hydrochloride (1.3 mmol) using the procedure described for **7a**. Yield 71%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (1H, d, *J* = 2.0 Hz), 7.69 (1H, d, *J* = 8.7 Hz), 7.49–7.33 (10H, m), 7.31 (1H, d, *J* = 8.7 Hz), 6.39 (1H, d, *J* = 2.1 Hz), 6.34 (1H, d, *J* = 2.1 Hz), 5.26 (2H, s), 5.25 (2H, s), 4.16 (2H, t, *J* = 6.0 Hz), 3.69 (3H, s), 2.93 (2H, t, *J* = 6.0 Hz), 2.66–2.62 (4H, m), 1.83–1.80 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.1, 165.0, 162.3, 157.0, 155.9, 151.7, 148.7, 139.4, 137.3, 137.0, 129.0, 128.5, 128.3, 127.7, 127.6, 123.7, 122.9, 115.4, 114.1, 106.4, 98.7, 93.1, 71.7, 71.3, 68.1, 60.5, 55.1, 23.9, 23.1.

4.2.3. 2-(3,4-Bis(benzyloxy)phenyl)-5-hydroxy-3-methoxy-7-(2-morpholinoethoxy)-4*H*-chromen-4-one (7c). The compound **7c** was prepared from **6a** (1.0 mmol) and 4-(2-chloroethyl)morpholine hydrochloride (1.3 mmol) using the procedure described for **7a**. Yield 93%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (1H, d, *J* = 2.1 Hz), 7.69 (1H, d, *J* = 8.7 Hz), 7.53–7.35 (10H, m), 7.06 (1H, d, *J* = 8.7 Hz), 6.42 (1H, d, *J* = 1.2 Hz), 6.37 (1H, d, *J* = 1.2 Hz), 5.29 (2H, s), 5.28 (2H, s), 4.20 (2H, t, *J* = 6.0 Hz), 3.80–3.76 (4H, m), 3.74 (3H, s), 2.87 (2H, t, *J* = 6.0 Hz), 2.64–2.61 (4H, m).

4.2.4. 2-(3,4-Bis(benzyloxy)phenyl)-7-(2-(diethylamino)ethoxy)-5-hydroxy-3-methoxy-4*H*-chromen-4-one (7d). The compound **7d** was prepared from **6a** (1.0 mmol) and 2-chloro-*N*,*N*-diethylethanamine hydrochloride (1.3 mmol) using the procedure described for **7a**. Yield 78%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (1H, d, *J* = 2.0 Hz), 7.69 (1H, d, *J* = 8.6 Hz), 7.51–7.34 (10H, m), 7.03 (1H, d, *J* = 8.6 Hz), 6.40 (1H, d, *J* = 2.1 Hz), 6.34 (1H, d, *J* = 2.1 Hz), 5.27 (2H, s), 5.26 (2H, s), 4.09 (2H, t, *J* = 6.2 Hz), 3.71 (3H, s), 2.90 (2H, t, *J* = 6.2 Hz), 2.66 (4H, q, *J* = 7.1 H), 1.07 (6H, t, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.1, 165.1, 162.3, 157.0, 155.9, 151.6, 148.7, 139.4, 137.3, 137.0, 129.0, 128.5, 128.3, 127.6, 123.7, 122.9, 115.4, 114.1, 106.4, 98.7, 93.0, 71.7, 71.3, 67.7, 60.5, 51.8, 48.3, 12.3.

4.2.5. 5-Hydroxy-3-methoxy-7-(2-(piperidin-1-yl)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (7e). The compound **7e** was prepared from **6b** (1.0 mmol) and 1-(2-chloroethyl)piperidine hydrochloride (1.3 mmol) using the procedure described for **7a**. Yield 77%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.09 (17H, m), 6.19 (1H, d, *J* = 1.8 Hz), 6.14 (1H, d, *J* = 1.8 Hz), 4.99 (6H, s), 3.97 (2H, t, *J* = 5.9 Hz), 3.44 (3H, s), 2.60 (2H, t, *J* = 5.9 Hz), 2.33–2.31 (4H, m), 1.45–1.39 (4H, m), 1.35–1.32 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.1, 165.1, 162.3, 157.0, 155.5, 153.0, 141.1, 139.8, 137.9, 137.2, 129.0, 128.7, 128.4, 127.8, 125.9, 108.8, 106.4, 98.8, 93.1, 75.7, 71.7, 67.2, 60.5, 57.9, 55.5, 26.4, 24.5.

4.2.6. 5-Hydroxy-3-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (7f). The compound **7f** was prepared from **6b** (1.0 mmol) and 1-(2-chloroethyl)pyrrolidine hydrochloride (1.3 mmol) using the procedure described for **7a**. Yield 31%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52–7.31 (17H, m), 6.45 (1H, d, *J* = 2.1 Hz), 6.40 (1H, d, *J* = 2.1 Hz), 5.24 (6H, s), 4.23 (2H, t, *J* = 5.7 Hz), 3.70 (3H, s), 2.99 (2H, t, *J* = 5.7 Hz), 2.73–2.69 (4H, m), 1.90–1.86 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6, 164.6, 161.8, 156.5, 155.2, 152.6, 140.7, 139.4, 137.4, 136.7, 128.6, 128.5, 128.2, 127.9, 127.3, 125.4, 108.4, 105.9, 98.3, 92.6, 75.2, 71.3, 67.7, 60.0, 54.7, 23.5.

4.2.7. 5-Hydroxy-3-methoxy-7-(2-morpholinoethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (7g). The compound **7g** was prepared from **6b** (1.0 mmol) and 4-(2-chloroethyl)morpholine hydrochloride (1.3 mmol) using the procedure described for **7a**. Yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.30 (17H, m), 6.43 (1H, d, *J* = 2.1 Hz), 6.38 (1H, d, *J* = 2.1 Hz), 5.23 (6H, s), 4.25 (2H, t, *J* = 5.4 Hz), 3.83–3.79 (4H, m), 3.68 (3H, s), 2.91 (2H, t, *J* = 5.4 Hz), 2.69–2.65 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.7, 164.4, 161.9, 156.6, 155.3, 152.6, 139.4, 137.4, 136.7, 128.6, 128.5, 128.3, 128.0, 127.4, 125.4, 108.4, 106.1, 98.7, 93.0, 75.3, 71.3, 66.7, 60.1, 57.2, 54.0, 25.2, 24.8.

4.2.8. 7-(2-(Diethylamino)ethoxy)-5-hydroxy-3-methoxy-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (7h). The compound **7h** was prepared from **6b** (1.0 mmol) and 2-chloro-*N*,*N*-diethylethanamine hydrochloride (1.3 mmol) using the procedure described for **7a**. Yield 46%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52–7.31 (17H, m), 6.44 (1H, d, *J* = 2.1 Hz), 6.40 (1H, d, *J* = 2.1 Hz), 5.25 (6H, s), 4.17 (2H, t, *J* = 6.0 Hz), 3.71 (3H, s), 2.97 (2H, t, *J* = 6.0 Hz), 2.71 (4H, q, *J* = 7.2 Hz), 1.15 (6H, t, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.7, 164.7, 161.9, 156.5, 155.1, 152.6, 140.7, 139.4, 137.4, 136.7, 128.6, 128.2, 127.9, 127.3, 125.4, 108.4, 105.9, 98.7, 93.4, 75.2, 71.3, 60.1, 51.4, 47.9, 11.8.

4.2.9. 2-(3,4-Dihydroxyphenyl)-5-hydroxy-3-methoxy-7-(2-(piperidin-1-yl)ethoxy)-4*H*-chromen-4-one hydrochloride (3a). To a solution of **7a** in EtOH and cyclohexene (1:1) was added a catalytic amount of $Pd(OH)_2$/C. The reaction mixture was refluxed (60–70 °C) for the period of time (TLC monitoring), filtered through Celite and concentrated. The residue was dissolved in absolute EtOH and then saturated with HCl gas at 0 °C until the mixture become acidic. The resulting precipitate was filtered and dried to afford **3a** (66%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (1H, d, *J* = 1.8 Hz), 7.49 (1H, d, *J* = 8.4 Hz), 6.95 (1H, d, *J* = 8.4 Hz), 6.80 (1H, d, *J* = 2.1 Hz), 6.45 (1H, d, *J* = 2.1 Hz), 4.49 (2H, br s), 3.80 (3H, s), 3.67–3.63 (4H, m), 3.51 (2H, br s), 1.80–1.78 (4H, m), 1.51–1.49 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 178.32, 163.40, 160.94, 156.46, 148.96, 145.37, 138.29, 120.98, 116.00, 115.98, 105.83, 98.56, 93.41, 60.03, 54.74, 53.01, 47.87, 22.68, 21.35; HRMS (ESI) *m/z*: calcd for $C_{23}H_{25}NO_7^+$ ([M-HCl]$^+$) 427.1626, found 427.1653.

4.2.10. 2-(3,4-Dihydroxyphenyl)-5-hydroxy-3-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-4*H*-chromen-4-one hydrochloride (3b). The compound **3b** was prepared from **7b** using the procedure described for **3a**. Yield 95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.57 (1H, s), 7.48 (1H, d, *J* = 6.5 Hz), 6.85 (1H, d, *J* = 6.5 Hz), 6.72 (1H, s), 6.34 (1H, s), 4.16 (2H, t, *J* = 5.6 Hz), 3.79 (3H, s), 3.78–3.76 (4H, m), 2.79 (2H, t, *J* = 5.6 Hz), 1.69–1.67 (4H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 179.3, 164.4, 160.7, 157.2, 156.9, 149.2, 145.4, 139.1, 122.9, 122.3, 117.1,

116.6, 106.4, 99.6, 94.2, 64.8, 61.2, 55.9, 54.6, 24.1; HRMS (ESI) *m/z*: calcd for $C_{22}H_{23}NO_7^+$ ([M-HCl]$^+$) 413.1469, found 413.1522.

4.2.11. 2-(3,4-Dihydroxyphenyl)-5-hydroxy-3-methoxy-7-(2-morpholinoethoxy)-4*H*-chromen-4-one hydrochloride (3c). The compound **3c** was prepared from **7c** using the procedure described for **3a**. Yield 35%. ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD) δ 7.67 (1H, s), 7.55 (1H, d, *J* = 8.5 Hz), 6.95 (1H, d, *J* = 8.5 Hz), 6.47 (1H, s), 6.33 (1H, s), 4.20 (2H, br s), 3.83 (3H, s), 3.77–3.75 (4H, m), 2.87 (2H, br s), 2.63–2.60 (4H, m); ^{13}C NMR (75 MHz, D_2O) δ 177.8, 162.8, 159.3, 155.4, 155.2, 147.7, 143.7, 137.4, 121.5, 120.5, 115.5, 114.9, 104.8, 98.1, 92.8, 63.9, 61.8, 59.6, 55.6, 52.2; HRMS (ESI) *m/z*: calcd for $C_{22}H_{23}NO_8^+$ ([M-HCl]$^+$) 429.1418, found 429.1457.

4.2.12. 7-(2-(Diethylamino)ethoxy)-2-(3,4-dihydroxyphenyl)-5-hydroxy-3-methoxy-4*H*-chromen-4-one hydrochloride (3d). The compound **3d** was prepared from **7d** using the procedure described for **3a**. Yield 31%. ^{1}H NMR (300 MHz, D_2O) δ 7.11 (1H, s), 7.07 (1H, d, *J* = 8.3 Hz), 6.60 (1H, d, *J* = 8.3 Hz), 5.96 (1H, s), 5.86 (1H, s), 3.94 (2H, br s), 3.46 (3H, s), 3.23 (2H, br s), 3.13–3.04 (4H, m), 1.12 (6H, t, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, D_2O) δ 178.3, 163.1, 159.8, 156.3, 155.9, 148.2, 144.3, 137.9, 121.2, 115.9, 115.4, 105.3, 98.2, 93.5, 66.3, 59.9, 57.8, 47.5, 8.4; HRMS (ESI) *m/z*: calcd for $C_{22}H_{25}NO_7^+$ ([M-HCl]$^+$) 415.1626, found 415.1649.

4.2.13. 5-Hydroxy-3-methoxy-7-(2-(piperidin-1-yl)ethoxy)-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one hydrochloride (3e). The compound **3e** was prepared from **7e** using the procedure described for **3a**. Yield 65%. ^{1}H NMR (300 MHz, D_2O) δ 6.44 (2H, s), 5.60 (2H, s), 4.69 (2H, t, *J* = 9.0 Hz), 3.47 (3H, s), 3.33 (2H, s), 3.10 (2H, s), 2.80 (2H, t, *J* = 9.0 Hz), 1.77–1.34 (6H, m); ^{13}C NMR (75 MHz, D_2O) δ 177.7, 162.7, 159.2, 154.9, 144.5, 137.4, 136.1, 119.5, 107.7, 104.3, 97.8, 92.6, 79.6, 59.5, 54.9, 53.6, 22.6, 21.0; HRMS (ESI) *m/z*: calcd for $C_{23}H_{25}NO_8^+$ ([M-HCl]$^+$) 443.1575, found 443.1581.

4.2.14. 5-Hydroxy-3-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one hydrochloride (3f). The compound **3f** was prepared from **7f** using the procedure described for **3a**. Yield 32%. ^{1}H NMR (300 MHz, D_2O) δ 6.59 (2H, s), 5.84 (2H, s), 4.64 (2H, t, *J* = 5.4 Hz), 3.85 (2H, br s), 3.49 (3H, s), 3.30–3.28 (4H, m), 1.95–1.93 (4H, m); HRMS (ESI) *m/z*: calcd for $C_{22}H_{23}NO_8^+$ ([M-HCl]$^+$) 429.1418, found 429.1412.

4.2.15. 5-Hydroxy-3-methoxy-7-(2-morpholinoethoxy)-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one hydrochloride (3g). The compound **3g** was prepared from **7g** using the procedure described for **3a**. Yield 16%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.11 (2H, s), 6.59 (1H, s), 6.50 (1H, s), 4.49 (2H, s), 4.04–3.62 (10H, m), 3.73 (3H, s); HRMS (ESI) *m/z*: calcd for $C_{22}H_{23}NO_9^+$ ([M-HCl]$^+$) 445.1367, found 445.1492.

4.2.16. 7-(2-(Diethylamino)ethoxy)-5-hydroxy-3-methoxy-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one hydrochloride (3h). The compound **3h** was prepared from **7h** using the procedure described for **3a**. Yield 85%. ^{1}H NMR (300 MHz, D_2O) δ 6.51 (2H, s), 5.63 (1H, s), 5.60 (1H, s), 4.70–4.66 (2H, m), 3.50 (3H, s), 3.06–3.02 (6H, m), 1.12 (6H, t, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, D_2O) δ 177.6, 167.87, 162.66, 159.16, 154.86, 144.59, 137.42, 136.26, 119.59, 107.68, 104.36, 97.90, 92.40, 79.7, 59.49, 49.80, 48.04, 8.17; HRMS (ESI) *m/z*: calcd for $C_{22}H_{25}NO_8^+$ ([M-HCl]$^+$) 431.1575, found 431.1604.

4.2.17. 7-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-hydroxy-3-methoxy-4*H*-chromen-4-one (8a). To a stirred solution of compound **6a** (2.0 mmol) in acetone (40 mL) was added K_2CO_3 (3.0 mmol) and benzyl bromide (2.6 mmol). The reaction mixture was heated at reflux for 4 h. The mixture was concentrated and the residue was treated with EtOAc and water. The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was solidified by treating EtOH to afford **8a**. (85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.68 (1H, s, OH), 7.80 (1H, s), 7.70 (1H, d, *J* = 8.7 Hz), 7.54–7.35 (15H, m), 7.06 (1H, d, *J* = 8.7 Hz), 6.50 (1H, d, *J* = 1.5 Hz), 6.46 (1H, d, *J* = 1.5 Hz), 5.30 (4H, s), 5.15 (2H, s), 3.75 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6, 164.4, 161.9, 156.6, 155.5, 151.3, 148.3, 138.9, 136.9, 136.6, 135.7, 128.7, 128.6, 128.5, 128.3, 127.9, 127.8, 127.4, 127.2, 127.1, 123.2, 122.5, 115.1, 113.7, 106.1, 98.5, 92.9, 71.3, 70.8, 70.4, 60.0.

4.2.18. 7-(Benzyloxy)-5-hydroxy-3-methoxy-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (8b). The compound **8b** was prepared from **6b** (2.0 mmol) using the procedure described for **8a**. Yield 94%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.56 (1H, b, OH), 7.58–7.30 (22H, m), 6.90 (1H, d, *J* = 2.4 Hz), 6.52 (1H, d, *J* = 2.4 Hz), 5.26 (6H, s), 5.11 (2H, s), 3.70 (3H, s).

4.2.19. 7-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-3-methoxy-5-(2-(piperidin-1-yl)ethoxy)-4*H*-chromen-4-one (9a). To a stirred solution of compound **8a** (1.0 mmol) in acetone (60 mL) was added K_2CO_3 (4.0 mmol) and 1-(2-chloroethyl)piperidine hydrochloride (1.3 mmol). The reaction mixture was heated at reflux for 6 h. The mixture was concentrated and the residue was treated with EtOAc and water. The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 10:1) to afford **9a** (50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (1H, d, *J* = 1.8 Hz), 7.67 (1H, d, *J* = 8.7 Hz), 7.53–7.34 (15H, m), 7.04 (1H, d, *J* = 8.7 Hz), 6.55 (1H, d, *J* = 1.8 Hz), 6.46 (1H, d, *J* = 1.8 Hz), 5.29 (4H, s), 5.15 (2H, s), 4.27 (2H, t, *J* = 6.0 Hz), 3.73 (3H, s), 3.05 (2H, t, *J* = 6.0 Hz), 2.70–2.68 (4H, m), 1.71–1.67 (4H, m), 1.52–1.50 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 162.7, 159.9, 158.5, 152.3, 150.6, 148.2, 141.1, 136.9, 136.6, 135.6, 128.7, 128.5, 128.4, 128.3, 127.9, 127.8, 127.4, 127.2, 127.1, 114.9, 113.7, 109.6, 97.2, 93.6, 71.2, 70.8, 70.3, 67.2, 59.8, 57.1, 54.9, 25.5, 23.7.

4.2.20. 7-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-3-methoxy-5-(2-(pyrrolidin-1-yl)ethoxy)-4*H*-chromen-4-one (9b). The compound **9b** was prepared from **8a** (1.0 mmol) and 1-(2-chloroethyl)pyrrolidine hydrochloride (1.3 mmol) using the procedure described for **9a**. Yield 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (1H, s), 7.65 (1H, d, *J* = 8.4 Hz), 7.47–7.32 (15H, m), 7.03 (1H, d, *J* = 8.4 Hz), 6.53 (1H, s), 6.44 (1H, s), 5.27 (4H, s), 5.13 (2H, s), 4.27 (2H, t, *J* = 5.7 Hz), 3.70 (3H, s), 3.18 (2H, t, *J* = 5.7 Hz), 2.85–2.83 (4H, m), 1.87–1.85 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1, 163.2, 160.3, 159.0, 151.1, 148.6, 141.6, 137.4, 137.1, 136.1, 129.2, 129.0, 128.8, 128.4, 128.3, 128.0, 127.6, 124.1, 122.4, 115.3, 114.2, 97.7, 94.2, 71.7, 71.3, 70.9, 68.6, 60.3, 55.2, 54.7, 23.9.

4.2.21. 7-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-3-methoxy-5-(2-morpholinoethoxy)-4*H*-chromen-4-one (9c). The compound **9c** was prepared from **8a** (1.0 mmol) and 4-(2-chloroethyl)morpholine hydrochloride (1.3 mmol) using the procedure described for **9a**. Yield 49%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (1H, d, J = 2.1 Hz), 7.67 (1H, d, *J* = 8.7 Hz), 7.53–7.34 (15H, m), 7.05 (1H, d, *J* = 8.7 Hz), 6.56 (1H, d, *J* = 1.8 Hz), 6.45 (1H, d, *J* = 1.8 Hz), 5.29 (4H, s), 5.17 (2H, s), 4.23 (2H, t, *J* = 6.0 Hz), 3.79–3.76 (4H, m), 3.72 (3H, s), 3.01 (2H, t, *J* = 6.0 Hz), 2.74–2.71 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 162.7, 159.9, 158.5, 152.3, 150.6, 148.2, 141.1, 136.9, 136.6, 135.6, 128.7, 128.5, 128.4, 128.3, 127.9, 127.8, 127.5, 127.1, 127.0, 123.6, 121.9, 114.9, 113.7, 109.7, 97.3, 93.6, 71.2, 70.8, 70.4, 67.6, 66.9, 59.8, 56.9, 54.1.

4.2.22. 7-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-5-(2-(diethylamino)ethoxy)-3-methoxy-4*H*-chromen-4-one (9d). The compound **9d** was prepared from **8a** (1.0 mmol) and 2-chloro-*N*,*N*-diethylethanamine hydrochloride (1.3 mmol) using

the procedure described for **9a**. Yield 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (1H, s), 7.46 (1H, d, *J* = 8.7 Hz), 7.30–7.13 (15H, m), 6.83 (1H, d, *J* = 8.7 Hz), 6.34 (1H, s), 6.27 (1H, s), 5.08 (4H, s), 4.95 (2H, s), 3.97 (2H, t, *J* = 6.3 Hz), 3.53 (3H, s), 2.89 (2H, t, *J* = 6.3 Hz), 2.53 (4H, q, *J* = 6.9 Hz), 0.92 (6H, t, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.8, 163.2, 160.6, 159.0, 152.7, 151.1, 148.6, 141.6, 137.4, 137.1, 136.1, 129.2, 129.0, 128.8, 128.4, 128.3, 128.0, 127.6, 124.2, 122.4, 115.3, 114.2, 110.1, 97.6, 94.0, 71.7, 71.3, 70.8, 68.5, 60.3, 51.8, 48.3, 12.2.

4.2.23. 7-(Benzyloxy)-3-methoxy-5-(2-(piperidin-1-yl)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (9e). The compound **9e** was prepared from **8b** (1.0 mmol) and 1-(2-chloroethyl)piperidine hydrochloride (1.3 mmol) using the procedure described for **9a**. Yield 53%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48–7.30 (22H, m), 6.54 (1H, d, *J* = 1.5 Hz), 6.48 (1H, d, *J* = 1.5 Hz), 5.23 (6H, s), 5.17 (2H, s), 4.28 (2H, t, *J* = 6.3 Hz), 3.70 (3H, s), 3.05 (2H, t, *J* = 6.3 Hz), 2.70–2.68 (4H, m), 1.70–1.68 (4H, m), 1.52–1.50 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 162.8, 159.9, 158.5, 152.5, 152.0, 141.5, 137.5, 136.8, 135.6, 128.7, 128.5, 128.1, 127.8, 127.5, 127.3, 125.8, 109.6, 108.2, 97.2, 93.6, 75.1, 71.3, 70.4, 67.3, 59.8, 57.2, 54.9, 25.6, 23.8.

4.2.24. 7-(Benzyloxy)-3-methoxy-5-(2-(pyrrolidin-1-yl)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (9f). The compound **9f** was prepared from **8b** (1.0 mmol) and 1-(2-chloroethyl)pyrrolidine hydrochloride (1.3 mmol) using the procedure described for **9a**. Yield 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.32 (22H, m), 6.54 (1H, d, *J* = 1.5 Hz), 6.47 (1H, d, *J* = 1.5 Hz), 5.23 (6H, s), 5.18 (2H, s), 4.29 (2H, t, *J* = 5.4 Hz), 3.69 (3H, s), 3.19 (2H, t, *J* = 5.4 Hz), 2.86–2.84 (4H, m), 1.90–1.88 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 162.8, 159.8, 158.5, 152.5, 152.1, 141.5, 140.2, 137.4, 136.8, 135.5, 128.7, 128.4, 128.1, 127.8, 127.4, 127.2, 125.8, 109.5, 108.2, 97.2, 93.7, 75.1, 71.2, 70.4, 68.1, 59.8, 54.7, 54.2, 50.5, 23.4.

4.2.25. 7-(Benzyloxy)-3-methoxy-5-(2-morpholinoethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (9g). The compound **9g** was prepared from **8b** (1.0 mmol) and 4-(2-chloroethyl)morpholine hydrochloride (1.3 mmol) using the procedure described for **9a**. Yield 56%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48–7.32 (22H, m), 6.54 (1H, d, *J* = 2.1 Hz), 6.45 (1H, d, *J* = 2.1 Hz), 5.22 (6H, s), 5.17 (2H, s), 4.24 (2H, t, *J* = 5.7 Hz), 3.79–3.76 (4H, m), 3.68 (3H, s), 2.99 (2H, t, *J* = 5.7 Hz), 2.74–2.72 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 162.8, 159.9, 158.5, 152.5, 152.1, 141.5, 140.2, 137.5, 136.8, 135.6, 128.7, 128.5, 128.1, 127.8, 127.5, 127.2, 125.8, 109.7, 108.2, 97.4, 93.6, 75.1, 71.3, 70.4, 67.7, 66.9, 59.8, 57.0, 54.2, 36.4, 31.3.

4.2.26. 7-(Benzyloxy)-5-(2-(diethylamino)ethoxy)-3-methoxy-2-(3,4,5-tris(benzyloxy)phenyl)-4*H*-chromen-4-one (9h). The compound **9h** was prepared from **8b** (1.0 mmol) and 2-chloro-*N*,*N*-diethylethanamine hydrochloride (1.3 mmol) using the procedure described for **9a**. Yield 63%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52–7.33 (22H, m), 6.54 (1H, d, *J* = 2.1 Hz), 6.49 (1H, d, *J* = 2.1 Hz), 5.24 (6H, s), 5.19 (2H, s), 4.19 (2H, t, *J* = 6.6 Hz), 3.72 (3H, s), 3.11 (2H, t, *J* = 6.6 Hz), 2.74 (4H, q, *J* = 7.2 Hz), 1.14 (6H, t, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 162.8, 160.2, 158.5, 152.5, 151.9, 141.5, 140.2, 137.5, 136.8, 135.6, 128.7, 128.5, 128.3, 128.1, 127.8, 127.4, 127.3, 125.9, 109.6, 108.2, 97.1, 93.6, 75.3, 71.3, 70.4, 68.2, 59.9, 51.4, 47.9, 11.8.

4.2.27. 2-(3,4-Dihydroxyphenyl)-7-hydroxy-3-methoxy-5-(2-(piperidin-1-yl)ethoxy)-4*H*-chromen-4-one hydrochloride (4a). To a solution of **9a** in EtOH and cyclohexene (1:1) was added a catalytic amount of $Pd(OH)_2/C$. The reaction mixture was refluxed (60–70 °C) for the period of time (TLC monitoring), filtered through Celite and concentrated. The residue was dissolved in absolute EtOH and then saturated with HCl gas at 0 °C until the mixture become acidic. The resulting precipitate was filtered to afford **4a** (71%). ^{1}H NMR (300 MHz, D_2O) δ 6.88 (1H, s), 6.76 (1H, d, *J* = 8.4 Hz), 6.34 (1H, d, *J* = 8.4 Hz), 5.88 (1H, s), 5.86 (1H, s), 4.02 (2H, br s), 3.50–3.40 (4H, m), 3.43 (3H, s), 2.96 (2H, t, *J* = 7.2 Hz), 1.92–1.43 (6H, m); HRMS (ESI) *m/z*: calcd for $C_{23}H_{25}NO_7^+$ ([M-HCl]$^+$) 427.1626, found 427.1795.

4.2.28. 2-(3,4-Dihydroxyphenyl)-7-hydroxy-3-methoxy-5-(2-(pyrrolidin-1-yl)ethoxy)-4*H*-chromen-4-one hydrochloride (4b). The compound **4b** was prepared from **9b** using the procedure described for **4a**. Yield 60%. ^{1}H NMR (300 MHz, D_2O) δ 6.57 (1H, s), 6.45 (1H, d, *J* = 8.4 Hz), 6.06 (1H, d, *J* = 8.4 Hz), 5.66 (1H, s), 5.56 (1H, s), 3.81 (2H, t, *J* = 7.2 Hz), 3.50–3.36 (4H, m), 3.24 (3H, s), 2.98 (2H, br s), 2.01–1.83 (4H, m); ^{13}C NMR (75 MHz, D_2O) δ 174.8, 161.4, 157.7, 156.9, 152.7, 146.7, 143.3, 139.0, 120.3, 114.9, 114.3, 106.7, 97.6, 96.1, 64.1, 59.0, 54.3, 53.4, 22.8; HRMS (ESI) *m/z*: calcd for $C_{22}H_{23}NO_7^+$ ([M-HCl]$^+$) 413.1469, found 413.1605.

4.2.29. 2-(3,4-Dihydroxyphenyl)-7-hydroxy-3-methoxy-5-(2-morpholinoethoxy)-4*H*-chromen-4-one hydrochloride (4c). The compound **4c** was prepared from **9c** using the procedure described for **4a**. Yield 74%. ^{1}H NMR (300 MHz, D_2O) δ 6.75 (1H, s), 6.65 (1H, d, *J* = 8.7 Hz), 6.21 (1H, d, *J* = 8.7 Hz), 5.87 (1H, s), 5.79 (1H, s), 4.13–4.08 (4H, m), 3.84 (2H, t, *J* = 7.2 Hz), 3.53–3.50 (4H, m), 3.42 (3H, s), 3.26 (2H, t, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, D_2O) δ 174.7, 161.4, 157.5, 157.0, 152.7, 146.7, 143.3, 139.0, 120.3, 114.9, 106.7, 97.5, 96.2, 63.9, 62.1, 59.3, 55.8, 52.1; HRMS (ESI) *m/z*: calcd for $C_{22}H_{23}NO_8^+$ ([M-HCl]$^+$) 429.1418, found 429.1479.

4.2.30. 5-(2-(Diethylamino)ethoxy)-2-(3,4-dihydroxyphenyl)-7-hydroxy-3-methoxy-4*H*-chromen-4-one hydrochloride (4d). The compound **4d** was prepared from **9d** using the procedure described for **4a**. Yield 74%. ^{1}H NMR (300 MHz, D_2O) δ 6.61 (1H, s), 6.44 (1H, d, *J* = 8.7 Hz), 6.07 (1H, d, *J* = 8.7 Hz), 5.68 (1H, s), 5.54 (1H, s), 4.67–4.66 (2H, m), 3.27–3.26 (2H, m), 3.15 (3H, s), 3.13–3.03 (4H, m), 1.13 (6H, t, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, D_2O) δ 174.6, 161.4, 157.5, 156.9, 152.7, 146.7, 143.4, 139.1, 127.1, 120.4, 114.4, 106.7, 100.2, 97.7, 96.0, 62.7, 59.2, 50.9, 47.5, 8.4; HRMS (ESI) *m/z*: calcd for $C_{22}H_{25}NO_7^+$ ([M-HCl]$^+$) 415.1626, found 415.1640.

4.2.31. 7-Hydroxy-3-methoxy-5-(2-(piperidin-1-yl)ethoxy)-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one hydrochloride (4e). The compound **4e** was prepared from **9e** using the procedure described for **4a**. Yield 77%. ^{1}H NMR (300 MHz, D_2O) δ 6.15 (2H, s), 5.52 (2H, s), 4.68–4.65 (2H, m), 3.50–3.24 (4H, m), 3.49 (3H, s), 2.82 (2H, br s), 1.75–1.33 (6H, m); HRMS (ESI) *m/z*: calcd for $C_{23}H_{25}NO_8^+$ ([M-HCl]$^+$) 443.1575, found 443.1665.

4.2.32. 7-Hydroxy-3-methoxy-5-(2-(pyrrolidin-1-yl)ethoxy)-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one hydrochloride (4f). The compound **4f** was prepared from **9f** using the procedure described for **4a**. Yield 43%. ^{1}H NMR (300 MHz, D_2O) δ 6.14 (2H, s), 5.56 (2H, s), 4.70–4.65 (2H, m), 3.52–3.35 (4H, m), 3.26 (3H, s), 2.99–2.98 (2H, m), 2.01–1.84 (4H, m); ^{13}C NMR (75 MHz, D_2O) δ 174.7, 161.4, 157.5, 156.8, 152.2, 143.9, 139.2, 135.1, 119.3, 107.1, 106.4, 97.1, 95.8, 79.7, 63.7, 59.1, 54.5, 53.4, 22.7; HRMS (ESI) *m/z*: calcd for $C_{22}H_{24}NO_8^+$ ([M-Cl]$^+$) 430.1496, found 430.1485.

4.2.33. 7-Hydroxy-3-methoxy-5-(2-morpholinoethoxy)-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one hydrochloride (4g). The compound **4g** was prepared from **9g** using the procedure described for **4a**. Yield 42%. ^{1}H NMR (300 MHz, D_2O) δ 6.27 (2H, s), 5.74 (2H, s), 4.02–3.93 (4H, m), 3.78 (2H, br s), 3.46–3.40 (4H, m), 3.39 (3H, s), 3.20–3.18 (2H, m); HRMS (ESI) *m/z*: calcd for $C_{22}H_{23}NO_9^+$ ([M-HCl]$^+$) 445.1367, found 445.1391.

4.2.34. 5-(2-(Diethylamino)ethoxy)-7-hydroxy-3-methoxy-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one hydrochloride (4h). The compound **4h** was prepared from **9h** using the

procedure described for **4a**. Yield 71%. ^{1}H NMR (300 MHz, D_2O) δ 6.15 (2H, s), 5.56 (1H, s), 5.50 (1H, s), 4.71–4.70 (2H, m), 3.25–3.23 (2H, m), 3.23 (3H, s), 3.08–3.07 (4H, m), 1.13–1.12 (6H, m); ^{13}C NMR (75 MHz, D_2O) δ 174.6, 161.4, 157.3, 156.7, 152.1, 143.9, 139.2, 135.2, 127.1, 119.3, 107.1, 106.4, 97.2, 95.7, 62.3, 59.0, 50.9, 47.4, 8.3; HRMS (ESI) *m/z*: calcd for $C_{22}H_{25}NO_8^+$ ([M-HCl]$^+$) 431.1575, found 431.1672.

4.3. Antioxidant effects of compounds 3a-3h and 4a-4h

4.3.1. DPPH radical scavenging activity assay

The antioxidant activities of the synthesized compounds were 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 ethanolic DPPH 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.

4.3.2. Superoxide anion radical scavenging activity assay by xanthine oxidase system

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 bovine serum albumin, 25 mM NBT and 1.4×10^{-8} units xanthine oxidase (EC 1.2.3.2) in final volume of 200 μl. After incubation at 25 °C for 20 min, the reaction was terminated by addition of 6.6 μl of 6 mM $CuCl_2$. The absorbance of formazan was determined at 560 nm. IC_{50} values denote the concentration of samples required to scavenge 50% superoxide anion radicals.

4.3.3. Lipid peroxidation inhibitory activity assay

The effects of the synthesized compounds on lipid peroxidation induced by an iron-ascorbic acid mixure were 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 compounds in 50 mM Tris-HCl (pH 7.5) in a total volume of 1 ml at 37 °C for 30 min. 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.

4.4. Neuroprotective effects of compounds 3a and 3h

4.4.1. Preparation of a rat model of transient focal cerebral ischemia

All procedures were conducted in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines of Korea Institute of Science and Technology. All procedures were approved by Korea Institute of Science and Technology IACUC (Approval Number: AP-2010L1001). Under anesthesia of male Sprague-Dawley rats (250 to 300 g) purchased from Daehan Biolink (Chungbuk, Korea) with a gas mixture of 70% N_2O and 2% isoflurane (the balance being O_2), the right MCA was occluded with a silicone rubber cylinder introduced from the bifurcation of the internal carotid artery (ICA) immediately after ligation of the ipsilateral common and external carotid artery. The cylinder was made of 4-0 nylon surgical thread 17 mm long coated with silicone mixed with a hardener to increase the thickness of the distal 5 mm to 0.3–0.4 mm. The proximal tip of the thread was heated, creating a globular stopper for embolization and for easy removal of the cylinder. After introducing the embolus, the ICA was ligated just distal to the point of insertion. The embolus extended from the bifurcation of the ICA to the proximal portion of the anterior cerebral artery. The origins of the right MCA and posterior communicating artery were occluded by the silicone rubber cylinder. Surgery was performed within 15 min and body temperature was kept within normal limits with a heating pad. Following surgery, anesthesia was discontinued and only rats showing neurological deficits characterized by left hemiparesis with upper extremity dominant and/or circling to the left were included in cerebral ischemic groups. After 2-h transient MCAO, reperfusion was achieved by pulling the thread out of the ICA about 10 mm under the same anesthetic conditions as during surgery.

4.4.1. Histological evaluation of brain injury

To examine cerebral infarction caused by transient MCAO, rats were sacrificed by decapitation 24 h after onset of ischemia. After removing brains, seven serial coronal slices of 2-mm thickness were made starting at 1 mm from the frontal pole, incubated in a 2% solution of TTC in normal saline at 37 °C for 60 min for vital staining, and fixed in 10% phosphate-buffered formalin for photography. The areas of the absolute infarct area in the cortex and striatum, and the total areas of both hemispheres were measured for each slice using a computerized image analysis system (Optimas). The absolute infarct volume was calculated by multiplying the area by the slice thickness and summing the volumes. The corrected infarct area in a slice was calculated to compensate for the effect of brain edema by subtracting the area of normal tissue in the ipsilateral hemisphere from the total area of the contralateral hemisphere. Corrected total infarct volume was then calculated by multiplying the area by the slice thickness and summing the volumes. Hemispheric swelling representing tissue edema was expressed as the percent increase in the size of the ipsilateral hemisphere compared with the contralateral hemisphere (% edema).

4.4.2. Evaluation of neurological deficits

Neurological deficits in the rats were measured 30 min and 24 h after onset of ischemia. The neurobehavioral tests consisted of the following. (1) In the forelimb flexion test, the rat was held in the air by the tail, and the degree of left forelimb flexion was scored between 0 and 3 (0, no movement on left side; 1, limited movement on left side; 2, less extended or slower movement on left side; 3, symmetrical movement). (2) In the duration of forelimb flexion test, the rat was held in the air by the tail, and the score (0 to 4) was determined by the duration of left forelimb flexion during a 10-second period (0, 8–10 s; 1, 6–8 s; 2, 4–6 s; 3, 2–4 s; 4, 0–2 s). (3) In the symmetry of movement/forepaw outstretching test, the rat was made to walk along the bench on its forelimbs while being held by the tail to keep the hindlimbs in the air. Symmetry in forepaw outstretching was observed and scored between 0 and 3 (0, left forelimb does not move; 1, left forelimb moves minimally and rat circles; 2, left forelimb outstretches less than right; 3, forelimbs outstretched and rat walks normally). Rats were scored on a ranking scale of 0 to 10, which reflects the cumulative score of the individual tests, with a score of 10 reflecting normal behavior.

4.5. Determination of Aqueous Solubility

Stock solutions of drugs were made up in MeOH and used to calibrate the HPLC (peak area in nanomoles, assuming a linear

response). Accurately weighed amount of compound **1** (1 mg) was saturated at room temperature and the saturated solution was sonicated for 30 min at the same temperature. After standing for an additional 30 min, the samples were centrifuged at 13000 rpm for 3 min, and the concentration of drug in the supernatant was determined by HPLC, using the calibration curve determined previously.[24]

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