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SYNTHESIS AND EVALUATION OF SELENOFLAVONES THAT HAVE POTENTIAL NEUROPROTECTIVE EFFECTS

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Abstract – We synthesized selenoflavones and evaluated their physicochemical properties and antioxidant effects. When oxygen was substituted with selenium, the compounds exhibited improved polarity and lipophilicity, implying that this change could lead to better BBB penetration. Selenoflavones revealed more potent antioxidant activity in our *in-vitro* assay. This suggests that selenoflavones would be more druggable than flavones and have a better potential as a neuroprotective agent.

Flavonoids are phenolic compounds, which are mainly found in fruits, herbs, and grains.^{1,2} In recent years, flavonoids have increasingly attracted scientific and public attention, due to their beneficial effects against oxidative stress, inflammation, and certain cancers.³⁻⁵ Up to date, several synthetic and biological studies have been done to evaluate flavonoids as a potential neuroprotective drug.⁶⁻⁸ However, many hydroxyl- and alkoxy- groups of flavonoids make them strongly polar. The high polarity makes it difficult to pass them through membranes. We hypothesized that polarity and lipophilicity can be improved by substituting selenium for oxygen atom in flavones(1).

It has been known that selenium, as a trace element in the human body, exists in a form of selenoproteins. Glutathione peroxidase (GPx) is one of the selenoproteins that exhibit antioxidant function.^{9,10} Furthermore, synthetic GPx mimetic compounds have been developed. Those synthetic organoselenium compounds show more potent antioxidant effects.¹¹⁻¹³

Based on these previous studies, we hypothesized that selenoflavonoids can pass through blood-brain barrier (BBB) more easily and have a synergetic antioxidant effect. To test this hypothesis, our group focused on synthesizing selenoflavones (2) as a potential neuroprotective agent.



Figure 1. Structure of flavones and selenoflavones

Synthetic pathway to generate selenoflavones is illustrated in Scheme 1. 3-Phenylpropioloyl chloride was prepared from 3-phenylpropiolic acid and used without purifications.¹⁴ Bromobenzenes and 3-phenylpropioloyl chloride were conducted at Friedel-Crafts condition which formed **3**. Then compound **3** was treated with sodium hydrogen selenide (NaHSe), *in-situ* generated from selenium powder and sodium borohydride (NaBH₄) to afford the selenoflavone (**2a**, **2b**).¹⁵



Scheme 1. Reagents and conditions : (a) $COCl_2$, DMF(cat.) CH_2Cl_2 , rt, 1 h; (b) $AlCl_3$, CH_2Cl_2 , 0 °C, 2 h; (c) Se, NaBH₄, DMF, 100 °C, 5 h Treatment of 5,6,7-trihydoxyflavone (baicalein, **1g**) and 5,7-dihydoxyflavone (chrysin, **1f**) with dimethyl sulfate generated corresponding methylated flavones (**1a**, **1b**) (Scheme 2).



Scheme 2. Reagents and conditions : (a) Me₂SO₄, K₂CO₃, acetone, reflux, overnight

Further modification of compounds (**1a-1c**, **2a-2e**) by boron tribromide (BBr₃) at low temperature resulted in selective demethylation to get compounds (**1c-1e**, **2c-2g**) (Scheme 3). All the prepared flavones (**1a-1f**) and selenoflavones (**2a-2f**) are listed in Table 1.



Scheme 3. Reagents and conditions : (a) BBr₃, CH₂Cl₂, -40 °C to rt, 2 h

To confirm the difference between flavones and selenoflavones, topological polar surface area (tPSA) and calculated partition coefficient (ClogP) were generated by Cambridgesoft ChemBioDraw software (Table 2) and retention time of compounds in reversed phase HPLC was measured (Table 3).

Polar surface area (PSA) of a molecule is the total surface of polar atoms. It is one of the druglikeness factors showing the polarity of molecules. Molecules with a PSA of less than 140 $Å^2$ were predicted to have good cell membrane permeability.¹⁶ And a PSA less than 90 $Å^2$ is usually needed to penetrate the BBB.¹⁷ Calculated tPSA was decreased by changing oxygen to selenium. Therefore, selenoflavones were expected to move through BBB more easily than flavones.

		R ¹ O			
Compound	М	R ¹	R ²	R ³	
1a	0	OMe	OMe	OMe	
1b	0	OMe	Н	OMe	
1c	0	OH	OMe	OMe	
1d	0	OH	Н	OMe	
1e	0	OH	ОН	OMe	
1f	0	OH	Н	OH	
1g	0	OH	OH	OH	
2a	Se	OMe	OMe	OMe	
2b	Se	OMe	Н	OMe	
2c	Se	OH	OMe	OMe	
2d	Se	OH	Н	OMe	
2e	Se	OH	ОН	OMe	
2 f	Se	OH	Н	OH	
2g	Se	OH	OH	OH	

Table 1. Structure-analytical data of flavone and selenoflavone derivatives

R³

 R^2

 R^1

Table 2. Calculated physicochemical properties of flavones and selenoflavones

Flavone	Physicochemical properties		Salar aflavora	Physicochemical properties	
	tPSA ^a (Å ²)	ClogP ^a	Selenomavone	tPSA ^a (Å ²)	ClogP ^a
1 a	54.0	2.88	2a	44.8	3.37
1b	44.8	3.62	2b	35.5	4.11
1c	65.0	3.52	2c	55.8	4.01
1d	55.8	4.15	2d	46.5	4.64
1e	76.0	3.33	2e	66.8	3.82
1f	66.8	3.56	2f	57.5	4.05
1g	87.0	3.00	2g	77.8	3.49

(a) Generated by Cambridgesoft ChemBioDraw software

Table 3. Retention time of flavones and selenoflavones in reversed phase HPLC

Flavone	Retention time in HPLC (min)		Salanaflavana	Retention time in HPLC (min)	
	Method A ^a	Method B ^b	Selenonavone -	Method A ^a	Method B ^b
1a	1.421	1.862	2a	1.562	2.230
1b	1.515	2.069	2b	1.912	3.285
1c	1.556	2.201	2c	1.776	2.801
1d	1.943	3.248	2d	2.375	4.549
1e	1.316	1.641	2e	1.451	1.986
1f	1.458	1.964	2 f	1.626	2.457
1g	1.265	1.527	2g	1.399	1.860

(a) $MeOH:H_2O = 90:10$, flow 1 mL/min; (b) $MeOH:H_2O = 80:20$, flow 1 mL/min

Log*P* is the concentration ratio of a compound between 1-octanol and water. The $\log P$ is known as a measure of lipophilicity. Compounds with high $\log P$ value are lipophilic which are well distributed to hydrophobic compartment like lipid bilayer of the membrane. Selenoflavones exhibited larger $\operatorname{Clog} P$ value which represents to be more lipophilic than flavones.

For confirming the accuracy of the calculated ClogP value of compounds, it was plotted against the retention time. Because retention time of compounds is also affected by its partition coefficient, LogP can be determined by correlating retention time of compounds with known $\log P$ value of similar compounds.^{18,19} Thus, we inferred that correlation between ClogP and retention time might signify the accuracy of the calculated value. As shown in Figure 2, the slope of Method A was approximately linear with correlation coefficient r = 0.8763. Method B also represented a high correlation between these two factors (r = 0.8769). It means that calculated ClogP is trustworthy and increased ClogP value of compounds reveals actual increment of lipophilicity.



Figure 2. Relationship of the retention time and the calculated ClogP value

To compare the antioxidant activity of flavones and selenoflavones, we treated neuro2a cells with these compounds and quantified reactive oxygen species (ROS) in cells by DCFDA, a ROS-detecting fluorescence dye.²⁰ Decrease in cellular ROS by a chemical represents antioxidant activity. As shown in Figure 3, compounds **1e**, **2e**, **1g**, and **2g** showed higher antioxidant activity compared to that of other compounds. However, these compounds have larger tPSA and smaller ClogP values than others. The results of antioxidant assay were not be correlated by only one factor. The factors could include the oxidation potential, polarity, and lipophilicity of compounds. There is no consistent difference between antioxidant activity of flavones and selenoflavones. However, we did not deduce from this observation

that selenium has no influence to antioxidant effect. It should be necessary to do additional research to ensure the results. Instead, we suspected that antioxidant effect could be in discord with prooxidant effect. The next experiment which showed in Figure 4 could be explained the possible difference between antioxidant effect and prooxidant effect.



Figure 3. Antioxidant activity of flavones and selenoflavones at 10 μ M



Figure 4. Dose-response experiments with 1e and 2e

We performed the dose-response experiments with 2e which presented best antioxidant activity among selenoflavones at 10 μ M and corresponding flavone (1e). Compound 2e exhibited antioxidant effect up to

90% at 100 μ M. In contrast, **1e** showed a sharp decline in its antioxidant effect at 100 μ M. Depending on the condition, some substances can behave as either antioxidants or prooxidants.^{21,22} Concentration is one of the important conditions.²³ At high concentration, **1e** might act not as an antioxidant but as a prooxidant that induces oxidative stress. From these results, **2e** is expected to be more potent and safer antioxidant than **1e**.

In summary, we have synthesized selenoflavones and evaluated their physicochemical properties and *in-vitro* antioxidant effects. As we hypothesized, substitution of selenium for oxygen atom in flavone led to improved physicochemical and biological properties. Therefore, selenoflavoids are anticipated to penetrate BBB better and be more druggable as a neuroprotective drug.

EXPERIMENTAL

General Information

CH₂Cl₂ used for the reaction was LC grade reagent and redistilled from calcium hydride. All other reagents were used as received without purification. Reactions were monitored by thin-layer chromatography on 0.25 mm silica plate (F-254) visualizing with UV light (254 nm) and KMnO₄ solution. Flash chromatography was performed using silica gel (230-400 mesh) with hexanes and EtOAc as eluent. ¹H and ¹³C NMR spectra were recorded on Agilent 400 MHz NMR spectrometer. Chemical shifts are reported in parts per million (δ), and coupling constants (*J*) are expressed in Hertz. HR-MS were measured with electrospray ionization (ESI) and Q-TOF mass analyzer. Physicochemical properties were generated by CS ChemProp. Retention time of compounds was measured with Agilent 1200 series HPLC and Agilent ZORBAX Eclipse Plus C18 (4.6 x 100 mm, 3.5 µm) column. Cellular fluorescence was measured with multi-plate reader (Tecan, Männedorf, Switzerland) at 490nm/520nm.

Procedure for the Formation of 3-Phenylpropioloyl Chloride

Oxalyl chloride (1.2 mmol) and DMF (cat.) were added to a solution of 3-phenylpropiolic acid (1.0 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred for 1 h at rt. After the excess reagent and solvent were distilled off, the residue was dried in vacuo. 3-Phenylpropioloyl chloride was used without further purification.

General Procedure for the Friedel-Crafts Acylation

Aluminum chloride powder (2.0 mmol) was suspended in CH_2Cl_2 (10 mL) at 0 °C. 3-Phenylpropioloyl chloride (1.2 mmol) in CH_2Cl_2 (5 mL) was added and the mixture was stirred for 30 min. Then a solution of bromobenzene (1.0 mmol) in CH_2Cl_2 (5 mL) was added dropwise and stirred for another 2 h. After the addition of water (20 mL), the organic layer was separated and the aqueous layer was extracted with

 CH_2Cl_2 (3 x 30 mL). The combined organic extracts were filtered through celite pad, washed with water (3 x 100 mL) and brine (100 mL), dried (MgSO₄), and concentrated. The resulting residue was purified by flash chromatography to give (**3a**, **3b**) in 85-92% yields.

1-(6-Bromo-2,3,4-trimethoxyphenyl)-3-phenylprop-2-yn-1-one (3a) ¹H NMR (400 MHz, CDCl₃) δ 7.60 (d, J = 7.1 Hz, 2H), 7.46 (t, J = 7.4 Hz, 1H), 7.38 (t, J = 7.4 Hz, 2H), 6.91 (s, 1H), 3.98 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 177.5, 155.5, 152.5, 141.7, 133.1, 130.8, 128.6, 120.2, 113.1, 112.3, 110.0, 92.9, 89.2, 62.1, 60.9, 56.4; HR-ESI-MS: *m/z* 375.0212 [M+H]⁺ (calcd for C₁₈H₁₆BrO₄⁺, 375.0226)

1-(2-Bromo-4,6-dimethoxyphenyl)-3-phenylprop-2-yn-1-one (3b) ¹H NMR (400 MHz, CDCl₃) δ 7.58 (d, *J* = 7.2 Hz, 2H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.37 (t, *J* = 7.3 Hz, 2H), 6.74 (s, 1H), 6.45 (s, 1H), 3.84 (d, *J* = 0.7 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 177.9, 162.1, 159.2, 133.2, 130.7, 128.5, 124.0, 120.7, 120.4, 109.8, 98.4, 92.3, 89.3, 56.2, 55.8; HR-ESI-MS: *m*/*z* 345.0119 [M+H]⁺ (calcd for C₁₇H₁₄BrO₃⁺, 345.0121)

General Procedure for the Formation of Selenoflavones

To the stirred solution of NaHSe (1.2 mmol) which was prepared from selenium powder (1.2 mmol) and NaBH₄ (1.2 mmol) in DMF (10 mL) at 100 °C for 1 h, a solution of (**3a**, **3b**) (1.0 mmol) in DMF (10 mL) was slowly added and the mixture was stirred for 5 h under these conditions. After the addition of water (10 mL), the mixture was filtered to remove remained selenium. The filtrate was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were washed with water (5 x 20 mL) and brine (20 mL), dried (MgSO₄), and concentrated. The resulting residue was purified by flash chromatography to give (**2a**, **2b**) in 56-62% yields.

5,6,7-Trimethoxyselenoflavone (2a) ¹H NMR (400 MHz, CDCl₃) δ 7.64 – 7.56 (m, 2H), 7.50 – 7.43 (m, 3H), 7.23 (s, 1H), 6.94 (s, 1H), 4.01 – 3.89 (m, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 182.8, 156.6, 156.2, 149.1, 143.0, 137.6, 134.1, 130.5, 129.2, 126.8, 126.8, 120.8, 105.7, 62.0, 61.4, 56.2; HR-ESI-MS: *m/z* 377.0275 [M+H]⁺ (calcd for C₁₈H₁₇O₄Se⁺, 377.0287)

5,7-Dimethoxyselenoflavone (2b) ¹H NMR (400 MHz, CDCl₃) δ 7.65 – 7.55 (m, 2H), 7.50 – 7.41 (m, 3H), 7.21 (s, 1H), 6.73 (d, *J* = 2.3 Hz, 1H), 6.51 (d, *J* = 2.2 Hz, 1H), 3.95 (s, 3H), 3.90 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 183.1, 163.7, 162.1, 147.9, 141.4, 137.5, 130.4, 129.2, 127.8, 126.8, 116.4, 102.9, 99.3, 56.3, 55.7; HR-ESI-MS: *m/z* 347.0207 [M+H]⁺ (calcd for C₁₇H₁₅O₃Se⁺, 347.0181)

General Procedure for the Methylation of Flavones

Dimethyl sulfate (3.5mmol) and K_2CO_3 (4.5 mmol) were added to a solution of (**1g**, **1f**) (1.0 mmol) in acetone (10 mL) and the mixture was stirred for overnight at reflux condition. After the excess solvent was distilled off, the residue was diluted with EtOAc (10 mL) and water (10 mL). Then the organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with water (2 x 20 mL) and brine (20 mL), dried (MgSO₄), and concentrated. The resulting residue was purified by flash chromatography to give (**1a**, **1b**) in 92-95% yields.

5,6,7-Trimethoxyflavone (1a) ¹H NMR (400 MHz, CDCl₃) δ 7.88 (s, 2H), 7.52 (s, 3H), 6.83 (s, 1H), 6.68 (s, 1H), 4.16 – 3.72 (m, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 177.2, 161.1, 157.7, 154.5, 152.5, 140.3, 131.5, 131.3, 129.0, 125.9, 112.9, 108.4, 96.3, 62.2, 61.6, 56.3; HR-ESI-MS: *m*/*z* 313.1071 [M+H]⁺ (calcd for C₁₈H₁₇O₅⁺, 313.1071)

5,7-Dimethoxyflavone (1b) ¹H NMR (400 MHz, CDCl₃) δ 7.90 – 7.84 (m, 2H), 7.54 – 7.48 (m, 3H), 6.68 (s, 1H), 6.58 (d, J = 2.3 Hz, 1H), 6.38 (d, J = 2.2 Hz, 1H), 3.96 (s, 3H), 3.92 (s, 3H).; ¹³C NMR (101 MHz, CDCl₃) δ 177.6, 164.1, 160.9, 160.6, 159.9, 131.5, 131.2, 128.9, 125.9, 109.3, 109.1, 96.2, 92.8, 56.4, 55.8; HR-ESI-MS: *m/z* 283.0961 [M+H]⁺ (calcd for C₁₇H₁₅O₄⁺, 283.0965)

General Procedure for the Demethylation of Flavones and Selenoflavones

1M BBr₃ in CH₂Cl₂ (1 mL, 1 mmol) was added dropwise to a solution of (**1a-1c**, **2a-2e**) (1.0 mmol) in CH₂Cl₂ (10 mL) at -40 °C. Then temperature was slowly raised to rt during 2 h. After the addition of water (10 mL), the organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (3 x 10 mL). The combined organic extracts were washed with water (3 x 20 mL) and brine (20 mL), dried (MgSO₄), and concentrated. The resulting residue was purified by flash chromatography to give (**1c-1e**, **2c-2g**) in 71-89% yields.

5-Hydroxy-6,7-dimethoxyflavone (1c) ¹H NMR (400 MHz, CDCl₃) δ 12.70 (s, 1H), 7.95 – 7.84 (m, 2H), 7.63 – 7.49 (m, 3H), 6.69 (s, 1H), 6.58 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 182.7, 163.9, 158.9, 153.3, 153.0, 132.6, 131.9, 131.3, 129.1, 126.3, 112.5, 105.6, 90.6, 60.9, 56.3; HR-ESI-MS: *m*/*z* 299.0917 [M+H]⁺ (calcd for C₁₇H₁₅O₅⁺, 299.0914)

5-Hydroxy-7-methoxyflavone (1d) ¹H NMR (400 MHz, CDCl₃) δ 12.72 (s, 1H), 7.88 (dd, *J* = 7.9, 1.6 Hz, 2H), 7.58 – 7.49 (m, 3H), 6.66 (s, 1H), 6.50 (d, *J* = 2.2 Hz, 1H), 6.37 (d, *J* = 2.2 Hz, 1H), 3.88 (s,

3H); ¹³C NMR (101 MHz, CDCl₃) δ 182.5, 165.6, 164.0, 162.2, 157.8, 131.8, 131.3, 129.1, 126.3, 105.8, 105.7, 98.2, 92.7, 55.8; HR-ESI-MS: *m/z* 269.0807 [M+H]⁺ (calcd for C₁₆H₁₃O₄⁺, 269.0808)

5,6-Dihydroxy-7-methoxyflavone (1e) ¹H NMR (400 MHz, CDCl₃) δ 12.51 (s, 1H), 7.90 (d, *J* = 6.8 Hz, 2H), 7.59 – 7.48 (m, 3H), 6.70 (s, 1H), 6.63 (s, 1H), 4.02 (s, 3H); ¹³C NMR (101 MHz, cdcl₃) δ 182.7, 164.1, 152.9, 150.7, 145.6, 138.3, 131.8, 131.4, 129.6, 129.1, 126.3, 105.5, 90.5, 56.5; HR-ESI-MS: *m/z* 285.0753 [M+H]⁺ (calcd for C₁₆H₁₃O₅⁺, 285.0757)

5-Hydroxy-6,7-dimethoxyselenoflavone (2c) ¹H NMR (400 MHz, CDCl₃) δ 7.64 – 7.59 (m, 2H), 7.54 – 7.48 (m, 3H), 7.23 (s, 1H), 6.76 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 187.8, 158.9, 157.2, 155.5, 137.4, 135.3, 134.7, 131.0, 129.4, 126.8, 124.4, 112.5, 100.6, 60.7, 56.3; HR-ESI-MS: *m/z* 363.0120 [M+H]⁺ (calcd for C₁₇H₁₅O₄Se⁺, 363.0130)

5-Hydroxy-7-methoxyselenoflavone (2d) ¹H NMR (400 MHz, CDCl₃) δ 7.61 – 7.57 (m, 2H), 7.49 (d, *J* = 7.0 Hz, 3H), 7.19 (s, 1H), 6.71 (d, *J* = 2.3 Hz, 1H), 6.47 (d, *J* = 2.3 Hz, 1H), 3.86 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 187.4, 167.2, 163.7, 155.0, 140.1, 137.4, 131.0, 129.4, 126.7, 124.6, 111.1, 104.3, 100.7, 55.6; HR-ESI-MS: *m/z* 333.0054 [M+H]⁺ (calcd for C₁₆H₁₃O₃Se⁺, 333.0024)

5,6-Dihydroxy-7-methoxyselenoflavone (2e) ¹H NMR (400 MHz, CDCl₃) δ 7.65 – 7.59 (m, 2H), 7.54 – 7.48 (m, 3H), 7.24 (s, 1H), 6.78 (s, 1H), 5.65 (s, 1H), 4.01 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 187.6, 156.5, 151.6, 151.2, 137.6, 132.3, 131.0, 129.9, 129.4, 126.8, 124.2, 111.7, 100.6, 56.4; HR-ESI-MS: *m/z* 348.9979 [M+H]⁺ (calcd for C₁₆H₁₃O₄Se⁺, 348.9974)

5,7-Dihydroxyselenoflavone (2f) ¹H NMR (400 MHz, CD₃OD) δ 7.66 – 7.56 (m, 2H), 7.48 (d, *J* = 3.9 Hz, 3H), 7.04 (s, 1H), 6.57 (d, *J* = 1.6 Hz, 1H), 6.14 (d, *J* = 1.6 Hz, 1H); ¹³C NMR (101 MHz, CD₃OD) δ 186.1, 169.5, 166.6, 154.0, 140.1, 137.6, 130.5, 129.0, 126.3, 123.6, 107.8, 107.5, 103.6; HR-ESI-MS: *m/z* 318.9888 [M+H]⁺ (calcd for C₁₅H₁₁O₃Se⁺, 318.9868)

5,6,7-Trihydroxyselenoflavone (2g) ¹H NMR (400 MHz, CD₃OD) δ 7.58 (d, J = 6.7 Hz, 2H), 7.51 – 7.39 (m, 3H), 7.08 (s, 1H), 6.75 (s, 1H), 3.27 (s, 2H); ¹³C NMR (101 MHz, CD₃OD) δ 188.8, 157.7, 154.1, 152.8, 139.0, 133.3, 132.0, 130.8, 130.5, 127.7, 124.5, 111.4, 106.1; HR-ESI-MS: *m*/*z* 334.9810 [M+H]⁺ (calcd for C₁₅H₁₁O₄Se⁺, 334.9817)

Antioxidant Assay

To measure the antioxidant activities of flavones and selenoflavones, we quantified reactive oxygen species (ROS) in cells treated with the compounds. Dichlorofluorescin diacetate (DCFDA) is cell-permeable, but once it enters cells, it is deacetylated by cellular esterases and cannot exit cells. Since non-fluorescent dichlorofluorescin is oxidized by intracellular ROS to a fluorescent dye, dichlorofluorescein (DCF), if a compound reduces the fluorescence of cells loaded with DCFDA, it has antioxidant activity. Neuro2a cells were plated in 96-well plates and were grown to 80% confluence. Media were changed to new media containing each compound at the indicated concentration 1 h before loading of DCFDA at 10 μ M for 20 min. After further incubation with 10 μ M H₂O₂, cellular fluorescence was measured with multi-plate reader (Tecan, Männedorf, Switzerland) at 490nm/520nm. Antioxidant activity of a compound was measured as decrease in fluorescence compared to DMSO treated group and expressed as % \pm SD.

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