Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: informahealthcare.com/journals/ienz20

Antioxidant and neuroprotective effects of synthesized sintenin derivatives

Lei Xiang Yang, Li Juan Zhang, Ke Xin Huang, Xiao Kun li, Li Hong Hu, Xiao Yu Wang, Joachim StÖckigt & Yu Zhao

To cite this article: Lei Xiang Yang, Li Juan Zhang, Ke Xin Huang, Xiao Kun li, Li Hong Hu, Xiao Yu Wang, Joachim StÖckigt & Yu Zhao (2009) Antioxidant and neuroprotective effects of synthesized sintenin derivatives, Journal of Enzyme Inhibition and Medicinal Chemistry, 24:2, 425-431, DOI: 10.1080/14756360802188214

To link to this article: https://doi.org/10.1080/14756360802188214

Published online: 01 Apr 2009.

Journal of Enzyme Inhibition and Medicinal Chemistry, April 2009; 24(2): 425–431

informa
healthcare

Antioxidant and neuroprotective effects of synthesized sintenin derivatives

LEI XIANG YANG[1], LI JUAN ZHANG[1], KE XIN HUANG[2], XIAO KUN LI[2], LI HONG HU[1], XIAO YU WANG[1], JOACHIM STÖCKIGT[1,3], & YU ZHAO[1,2]

[1]*Department of TCM and Natural Drug Research, College of Pharmaceutical Sciences, Zhejiang University, 388 Yuhangtang Road, Hangzhou 310058, China,* [2]*Pharmacy School of Wenzhou Medical College, Wenzhou 325035, China, and* [3]*Lehrstuhl für Pharmazeutische Biologie, Institut für Pharmazie, Johannes-Gutenberg Universität Mainz, Staudinger Weg 5, D-55099 Mainz, Germany*

(*Received 8 March 2008; accepted 29 April 2008*)

Abstract
Three series of sintenin derivatives (compounds **1**–**14**) were designed and prepared and their antioxidative and neuroprotective effects were evaluated. The *in vitro* models of scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, chelating ferrous ions, inhibiting the rat brain homogenates lipid peroxidation, and protecting neurons damaged by hydrogen peroxide were employed for bioassays. It was found that sintenin derivatives **4** and **13** showed remarkable antioxidative and neuroprotective activities.

Keywords: *Sintenin derivatives, lignanoid, antioxidant, neuroprotective effects*

Introduction

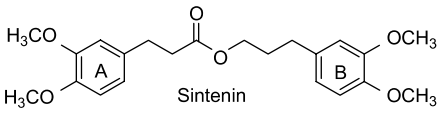
Reactive oxygen species (ROS), generated by the reduction of molecular oxygen, have been established to be involved in various pathological events and the development processes of many diseases [1–3]. Hydrogen peroxide (H_2O_2), one kind of ROS, could be transformed to hydroxyl radical (OH·) at the existence of ferrous ions (Fe^{2+}), and it has been implicated in the pathogenesis of brain injuries and neurodegenerative diseases by impairing cells through direct oxidation of lipids, proteins and DNA, or by acting as a signal molecule to trigger the cellular apoptotic pathways [4]. Lipid peroxidation could cause severe damage to cell membrane structure and consequently, alters its fluidity and ability to function properly. Unbalanced oxidative homeostasis and lipid peroxidation are associated with neurodegenerative diseases, e.g. Alzheimer's disease (AD) and Parkinson's disease (PD) [5].

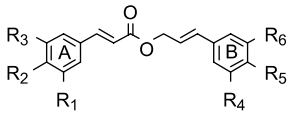
Antioxidants can intervene in the oxidation process by inhibiting the initiation or propagation of oxidizing chain reactions [6]. Therefore, substantially increased attempts are undertaken to discover and develop efficient synthetic or natural antioxidants to prevent or attenuate ROS-induced damages.

The structural and biological diversity of lignanoid has attracted great attentions of related researchers. Sintenin (3-(3,4-dimethoxyphenyl)propyl-3-(3,4-dimethoxy- phenyl) propanoate), a lignanoid isolated from the leaves and stems of *Piper sintenense* scattered in Taiwan, has been reported to possess selective cytotoxicity against mouse acute lymphocytic leukemia P388 cells [7]. However, to the best of our knowledge, its antioxidative property is not well studied. In continuation of studying the biological activities of sintenin and its derivatives and exploring novel antioxidative agents, three series (A, B and C) of sintenin analogues (**1**–**14**) were designed and

Correspondence: Y. Zhao, Ph.D, Dept. of TCM & Natural Drug Research, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China. Fax: +86 571 88208449. E-mail: dryuzhao@zju.edu.cn; dryuzhao@126.com

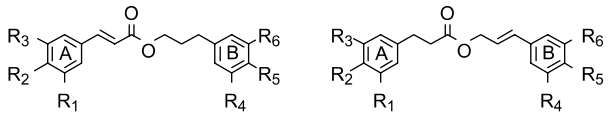
ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/14756360802188214





(Series A)

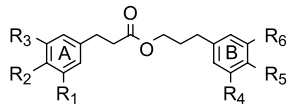
Compd.	R_1	R_2	R_3	R_4	R_5	R_6
1	H	NO_2	H	OCH_3	OCH_3	H
2	OCH_3	OCH_3	OCH_3	H	OCH_3	H
3	H	OCH_2OCH_3	H	OCH_2OCH_3	OCH_2OCH_3	H
4	OH	OH	H	H	H	H



Compound **5** Compounds **6-8**

(Series B)

Compd.	R_1	R_2	R_3	R_4	R_5	R_6
5	H	CH_3	H	H	CH_3	H
6	H	OCH_2OCH_3	H	H	H	H
7	H	OH	H	H	H	H
8	H	H	H	H	H	H



(Series C)

Compd.	R_1	R_2	R_3	R_4	R_5	R_6
9	OCH_3	OCH_3	H	OCH_3	OCH_3	H
10	H	H	H	H	H	H
11	OCH_2OCH_3	OCH_2OCH_3	H	H	OCH_2OCH_3	H
12	H	OH	H	H	OCH_3	H
13	OH	OH	H	H	H	H
14	H	OH	H	H	H	H

Figure 1. The structures of sintenin and three series of synthetic sintenin derivatives.

synthesized for the evaluation of antioxidant properties. Series A (compounds **1**–**4**) and series B (compounds **5**–**8**) were unsaturated esters with either one or two double-bonds between two phenyls, while series C (compounds **9–14**) were saturated esters (Figure 1). Different patterns of substituents on the aromatic A and/or B rings were also systematically introduced in the synthetic molecules, e.g., OCH_2 OCH_3 to replace OCH_3 groups and demethylation to expose the phenolic OH groups.

The antioxidant activities of synthesized compounds were investigated by scavenging 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radicals, chelating of Fe^{2+}, and the inhibition of lipid peroxidation in rat brain homogenates. Moreover, the protective effects of the test compounds on neurons damaged by H_2O_2 were also assayed to preliminarily reveal the diverse bioactivities of synthesized sintenin analogues.

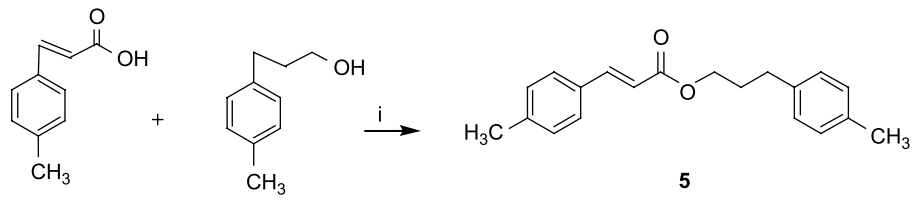
Materials and methods

ESI-MS data were recorded on a Bruker Esquire 3000 + spectrometer. ^1H NMR and ^{13}C NMR spectra were measured on a Varian INOVA 400 spectrometer with TMS as internal standard and $CDCl_3$ as solvent. Preparative TLC was performed on silica gel GF_{254} and RP-18 plates (Merck, Darmstadt, Germany). The silica gel GF_{254} was purchased from Qingdao Marine Chemical Factory, China. DPPH, H_2O_2, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ferrozine were purchased from Sigma Chemical Co. (St. Louis, USA). Tris bases, DMEM medium were obtained from Gibco (Grand Island, NewYork, USA). Quercetin was prepared in our laboratory (HPLC purity of 99%), and rat pheochromocytoma (PC12) cells were provided by Cell Bank of Chinese Academy of Sciences, Shanghai. All other reagents were of the highest purity commercially available. Sprague-Dawley rats were obtained from the Zhejiang Center of Laboratory Animals, China. The use of animals was in accordance with Guideline for the Care and Use of Laboratory Animals of Zhejiang University.

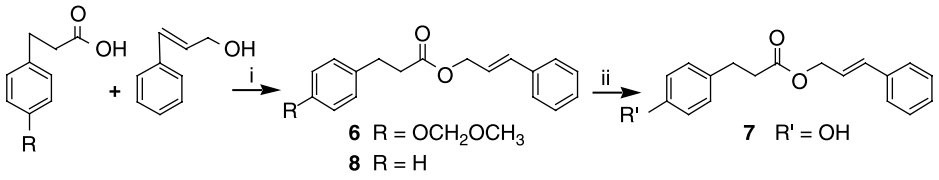
Chemistry

Compounds **1**–**4** and **9**–**14** were synthesized according to the previously reported methods [8]. The structures of the synthetic compounds were identified by MS and NMR measurement, some of which were also directly compared with authentic samples.

The synthetic routes for compounds **5**–**8** are outlined in Schemes 1 and 2. 4-Methylcinnamic acid was treated with 4-methylphenylpropyl alcohol under catalysis of carbonyldiimidazole (CDI) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford **5**



Scheme 1. Synthesis of compound **5**. Reagents and conditions: (i) CDI, THF, reflux, 0.5 h; DBU, THF, reflux, 12 h.



Scheme 2. Synthesis of compounds **6**–**8**. Reagents and conditions: (i) DCC, CH_2Cl_2, rt, 5 min; DMAP, rt, 12 h (ii) 10% HCl, CH_3OH, reflux.

with a yield of 72% [9]. As illustrated in scheme 2, 4-methoxylmethoxylphenylpropionic acid was reacted with phenylallyl alcohol catalyzed by N,N′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to afford **6** with a yield of 70% [10]. Compound **7** was obtained with a yield of 68% by removing the OCH_2OCH_3 group of **6** by treatment of 10% hydrochloric acid. The preparation of **8** was similar to that of **6**, but with a different starting material, and its yield was 69%. The structures of **5** − **8** were elucidated mainly on the basis of ^1H and ^{13}C NMR spectra as well as MS spectral data.

Synthesis of (E)-4-methylcinnamic acid-4′-methyl phenylpropyl ester (5). A mixture of 4-methylcinnamic acid (100 mg, 0.62 mM), CDI (313 mg, 1.23 mM), and dry tetrahydrofuran (THF, 10 mL) was refluxed for 30 min. The solution of 4-methylphenylpropanol (100 mg, 0.68 mM) and DBU (103 mg, 0.68 mM) in dry THF (10 mL) was then added for another 12 h refluxing. The solvent was removed by evaporation and the resulted mixture was purified by column chromatography to afford **5** (131 mg, 72%) as a colorless oil; *Rf* (petroleum ether/EtOAc = 3:1) 0.27; ^1H NMR (400 MHz, $CDCl_3$): δ 7.64 (1H, d, J = 16.0 Hz, H-1), 7.05 − 7.12 (8H, m, ArH), 6.63 (1H, d, J = 16.0 Hz, H-2), 4.09 (2H, t, J = 6.4 Hz, H-5), 2.70 (2H, t, J = 8.0 Hz, H-7), 2.34 (3H, s, CH_3-4′), 2.33 (3H, s, CH_3-4″), 1.92 (2H, m, H-6); ESI-MS *m/z* $[M + NH_4]^+$374.

Synthesis of 4-methoxylmethoxylphenylpropionic acid-(E)-phenylallyl ester (6). The mixture of 4-methoxylmethoxylphenylpropionic acid (50 mg, 0.24 mM) and DCC (54 mg, 0.26 mM) in dry dichloromethane (8 mL) was stirred at room temperature for 5 min, and then phenylallyl alcohol (35 mg, 0.26 mM) and 4-DMAP (5.8 mg, 0.048 mM) were added. The mixture was stirred for another 12 h at room temperature. The white insoluble substance was then removed by filtering and the resulted mixture was purified by column chromatography to give **6** (55 mg, 70%) as a colorless oil; *Rf* (petroleum ether/ EtOAc = 3:1) 0.38; ^1H NMR (400 MHz, $CDCl_3$): δ 6.95 − 7.40 (9H, m, ArH), 6.72 (1H, d, J = 16.0 Hz, H-7), 6.63 (1H, dt, J = 6.4, 15.6 Hz, H-6), 5.14 (2H, s, CH_3OCH_2O-4′), 4.74 (2H, d, J = 6.4 Hz, H-5), 3.47 (3H, s, CH_3OCH_2O-4′), 2.93 (2H, t, J = 8.0 Hz, H-3), 2.66 (2H, t, J = 8.0 Hz, H-2); ESI-MS *m/z* $[M + NH_4]^+$344.

Synthesis of 4-hydroxylphenylpropionic acid-(E)-phenylallyl ester (7). Compound **6** (25 mg, 0.078 mM) was dissolved in 6 mL methanol, and then 10% HCl (4 mL) was added to the solution. The mixture was refluxed for 40 min and was cooled to room temperature. Saturated sodium bicarbonate solution was then added to adjust the pH value to 7.0. After removing methanol by evaporation, the mixture was extracted with ethyl acetate for 3 times. The combined ethyl acetate layer was washed by saturated brine and dried over $MgSO_4$. Compound **7** (15 mg, 68%) was obtained as a colorless oil by column chromatography. *Rf* (petroleum ether/ EtOAc = 3:1) 0.15; ^1H NMR (400 MHz, $CDCl_3$): δ 6.74 − 7.40 (9H, m, ArH), 6.62 (1H, d, J = 16.0 Hz, H-7), 6.38 (1H, dt, J = 6.4, 15.6 Hz, H-6), 4.34 (2H, d, J = 6.4 Hz, H-5), 2.88 (2H, t, J = 8.0 Hz, H-3), 2.61 (2H, t, J = 8.0 Hz, H-2); ESI-MS *m/z* $[M + NH_4]^+$300.

Synthesis of phenylpropionic acid-(E)-phenylallyl ester (8). The preparation of **8** was similar to that of **6**. Colorless oil; *Rf* (petroleum ether/ EtOAc = 3:1) 0.47; ^1H NMR (400 MHz, $CDCl_3$): δ 7.21 − 7.40 (10H, m, ArH), 6.63 (1H, d, J = 16.0 Hz, H-7), 6.26 (1H, dt, J = 6.4, 15.6 Hz, H-6), 4.74 (2H, d, J = 6.4 Hz, H-5), 2.99 (2H, t, J = 8.0 Hz, H-3), 2.70 (2H, t, J = 8.0 Hz, H-2); ESI-MS *m/z* $[M + NH_4]^+$284.

Biology

DPPH radicals scavenging activity. The quenching of free radicals by the compounds was assayed spectrophotometrically at 517 nm against the absorbance of the stable DPPH radicals [11]. The reaction mixture contained in a total volume of 0.25 mL, 25 μL of various concentrations of the test compounds which were dissolved in dimethyl sulfoxide (DMSO) and 40 μL of DPPH (0.4 mg/mL) dissolved in methanol. The methanolic solution of DPPH served as a control; meanwhile quercetin was applied as a

reference. The absorbance was measured at 517 nm after incubating the mixture at 37 °C for 30 min.

Measurement of rat brain homogenates lipid peroxidation. The formation of MDA was used as an indicator of inhibiting lipid peroxidation and was determined by the thiobarbituric acid (TBA) assay on freshly prepared Sprague-Dawley rat brain homogenates using colorimetric analysis [12]. The reaction mixtures which were composed of 200 μL of solution containing an aqueous $FeSO_4$ (4 μM), vitamin C (50 μM), 50 μL of rat brain homogenate and 5 μL of the test compounds (concentrations from 25 to 100 μg/mL), were incubated at 37 °C in capped tubes for 1 h before 100 μL of trichloroacetic acid (20%, v/v) was added. The mixture reacted at room temperature for 30 min. Finally, 200 μL of HCl (0.1 M) and 100 μL of TBA (1%, w/v) were added into each tube and the mixture was incubated at 100 °C for another 1 h. Centrifugation was then carried out at 5000 rpm for 5 min, and the absorbance of the supernatant was measured at 532 nm. Quercetin and curcumin acted as positive standards in this assay; results are shown as the means ± SD, $n = 3$.

Ferrous ions chelating assay. The ferrous ions chelating by the test compounds were estimated as reported previously [13]. Briefly, the samples with final concentration at 110 μg/mL were added to a solution of 2 mM $FeSO_4$ (5 μL) and 80% DMSO (200 μL). The reaction was initiated by adding of 5 mM ferrozine (10 μL), and the mixture was shaken vigorously before left standing at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. Quercetin served as a positive standard.

Effect of the tested compounds on protecting PC 12 cells against hydrogen peroxide-induced injury. The protective effects of the compounds on PC 12 cells against hydrogen peroxide-induced injury were assessed with MTT assay as reported previously [14]. PC 12 cells were maintained at 37 °C in a humidified atmosphere containing 5% of CO_2. Cells were seeded into 96 well cell culture plates (Shengyou, China) at a density of 8000 cells per well in DMEM medium which was supplemented with 10% heat-inactivated calf serum, 100 units/mL of penicillin and 100 μg/mL of streptomycin. Following protocols were carried out 48 h after cells were seeded. H_2O_2 was prepared in phosphate buffered saline (PBS) on the day of application to cultures at a final concentration of 600 μM. Samples were dissolved in DMSO and diluted with the medium. The PC 12 cells were preincubated with samples 2 h before the H_2O_2 was added. A fresh solution of MTT (5 mg/mL) prepared in NaCl solution (0.9%) was added to the 96-well cell culture plates 3 h after H_2O_2 was added. The plates were incubated in the CO_2 incubator for 3 h, and the formazan were lysed with DMSO. The plates were analyzed in a multi-well-plate reader at 570 nm; data are shown as means ± SD, $n = 3$. The cytotoxicity of the test compounds against PC 12 cells was routinely examined with MTT method.

Results and discussion

Chemistry

Totally, 14 sintenin derivatives were synthesized by the procedure described by Hu et al. [8] and their structures were identified by MS and NMR measurement, some of which were also directly compared with the authentic samples. The yields of compounds **1**–**14** were 74%, 70%, 82%, 33%, 72%, 70%, 68%, 69%, 83%, 90%, 92%, 79%, 69%, and 94%, respectively. The esterification under the catalysis of CDI and DBU is beneficial for the isolation of the target compounds.

DPPH radicals scavenging activity

DPPH is a stable free radical existed *in vitro*, and bleaching of DPPH absorption is representative of the capacity of the test compounds to scavenge free radicals with aryl and stable characters and independent from any enzyme activity. Among the test compounds, compounds **4** and **13** showed noticeable DPPH scavenging activities (Table I). It should be pointed out that the inhibitory activity of compound **13** ($IC_{50} = 20.0 \pm 4.0$ μM) is comparable with quercetin ($IC_{50} = 11.9 \pm 0.43$ μM).

Table I. DPPH radicals scavenging activity by the test compounds.

Compounds	DPPH inhibition (50 μg/mL)
1	11.20%
2	18.60%
3	16.80%
4	35.8 ± 5.4[a]
5	13.40%
6	3.10%
7	14.20%
8	_[b]
9	14.00%
10	_[b]
11	0.70%
12	1.10%
13	20.0 ± 4.0[a]
14	_[b]
Quercetin	11.9 ± 0.43[a]

[a] IC_{50} ± SD (μM): IC_{50} values were further generated for those inhibitions greater than 50% at the concentration of 50 μg/mL. Data are expressed means ± SD, $n = 3$; [b] No detectable activity.

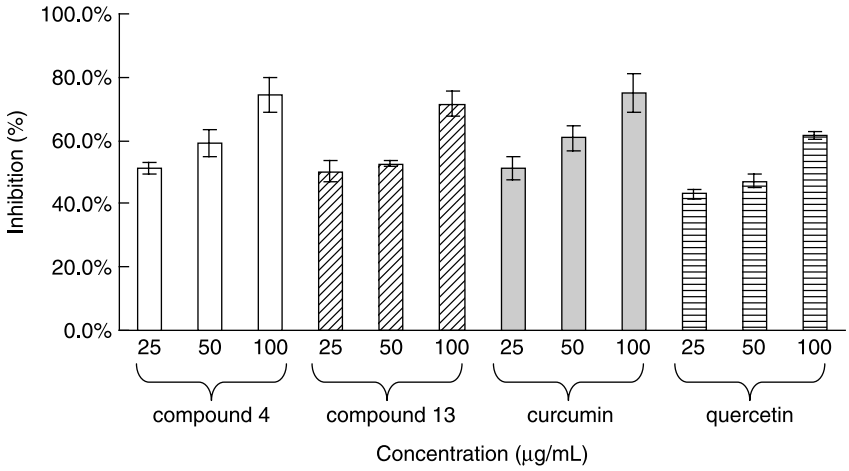


Figure 2. The inhibitory effects of compounds **4**, **13**, curcumin, and quercetin on iron-induced lipid peroxidation in rat brain homogenates.

Inhibitory effects of the test compounds in rat brain homogenates lipid peroxidation

The inhibition of lipid peroxidation is another practical index for the evaluation of antioxidants. Rat brain homogenates, widely adopted biomaterial for the investigation of lipid peroxidation [15], were carried out to examine the antioxidant properties of the compounds **1**–**14**. The extent of *in vitro* lipid peroxidation was measured by lipid-derived malondialdehyde (MDA) production. As shown in Figure 2, compounds **4** and **13** exhibited obvious antioxidant activities in rat brain homogenates in dose-dependent manner. Moreover, the IC_{50} values of these two compounds (86.8 ± 8.1 and $99.0 \pm 13.7\,\mu M$ for compounds **4** and **13**, respectively) were lower than that of quercetin (one of the positive controls, $IC_{50} = 157.5 \pm 19.5\,\mu M$) and were comparable to that of curcumin (the other positive control, $IC_{50} = 69.2 \pm 10.6\,\mu M$).

Chelating ferrous ions by the test compounds

Fe^{2+} chelation may lead to important antioxidative effects by retarding metal-catalysed oxidation. The ferrous ions chelation properties of the test compounds were evaluated and the results were illustrated in Table II. It could be found that only compound **4** showed a notable iron affinity with the inhibitory rate of $75.1 \pm 4.9\%$ at the concentration of 110 μg/mL among all the synthetic compounds.

Protective effects of synthetic compounds on H_2O_2 induced insult in PC 12 cells

PC 12 cells, affording homogeneous populations and being easy of manipulation, provide a useful model system for the investigation of neural injury. The protective effects of the test compounds on PC 12 cells damaged with the direct application of hydrogen proxide were investigated. The results of morphological images of the PC 12 cells cultivated together with compounds **4** and **13** are illustrated in Figure 3. It could be observed that most of the cells became round shapes, while the membrane lesions of cells were also observable after 3 h exposure to 600 μM of H_2O_2 in Figure 3B. Though the round shapes of PC 12 cells could be observed in the presence of compounds **4** and **13**, the cell viabilities and integralities were assuredly improved (Figure 3C and 3D). This indicated that compounds **4** and **13** possess clear protective effects against H_2O_2 insults. As shown in Figure 4, compounds **4** and **13** demonstrated significant neuroprotective activities in a dose-dependent manner. In addition, the experiments of removing the compounds from the pre-incubated medium of PC 12 cells prior to the injury initiated by H_2O_2 were also carried out. The neuroprotective effects of quercetin declined, while the neuroprotective activities of compounds **4** and **13**

Table II. Ferrous ion chelating ability by the test compound.

Compounds	Ferrous ions chelation (110 μg/mL)
1	10.0%
2	2.4%
3	21.2%
4	$75.1 \pm 4.9\%$[a]
5	6.4%
6	10.4%
7	7.2%
8	4.8%
9	12.4%
10	11.2%
11	10.4%
12	14.4%
13	40.4%
14	14.8%
Quercetin	46.8%

[a] Data are shown as means ± SD, $n = 3$.

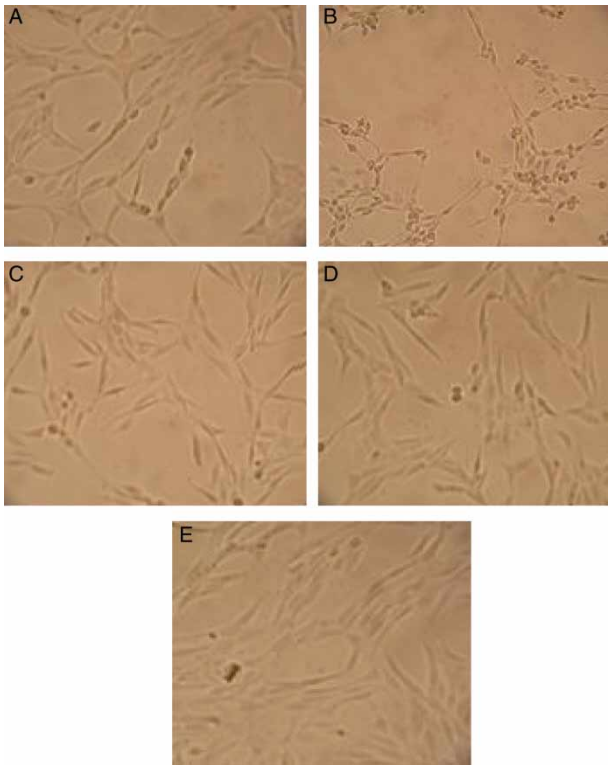


Figure 3. Effects of compounds **4**, **13** and positive control on PC 12 cell injury induced by H_2O_2. (A) PC 12 control cells. (B) PC 12 cells exposed to 600 μM of H_2O_2 for 3 h. Most of the cells become round shapes. The membrane lesion of cells was also observed. (C, D and E) PC 12 cells were pre-incubated with 20 μg/mL of compounds **4**, **13** and quercetin, respectively before exposed to 600 μM H_2O_2 for 3 h.

still remained visible (Figure 5) after the evacuation of pre-incubated medium containing these compounds. These results suggested that compounds **4** and **13** might work both intracellular and extracellular, whether they accumulate in the cell membrane to attenuate the damage of ROS needs to be identified. Additionally, the cytotoxicities of the active compounds were examined in parallel at the same concentrations. No noticeable cytotoxicities on PC 12 cells were found among these hits. The growth inhibition of PC 12 cells by compounds **4** and **13** was 17.0% and 25.9%, respectively, at 20 μg/mL.

Among all of the test compounds, those with *ortho*-diphenolic hydroxyl groups, i.e., compounds **4** and **13** possessed remarkable antioxidative activities and potential neuroprotective properties. The absence of *ortho*-diphenolic hydroxyl moieties in series A and C diminished their capabilities as antioxidants (Figure 2, Figure 4, and Table II). Results derived from the DPPH radicals scavenging assays demonstrated that compound **13**, which contained free phenolic OH groups on its aromatic A ring of sintenin, is the best DPPH scavenger (Table I). It seems that *ortho*-diphenolic hydroxyl groups play more important roles in scavenging DPPH radicals than the double bonds existing between two phenyls do.

In addition to the potential of free radical scavenging, compound **4** showed stronger antioxidant activities than compound **13** in the implemented bioassays, especially the capacity of Fe^{2+} chelating; this might indicate that the unsaturated ester moiety is benefit for its bioactivity via the enhancement of the conjugated system. Furthermore, the mechanisms of ferrous ion-induced lipid peroxidation of brain homogenates were hypothesized as the decomposition of lipid peroxides, the generation of hydroxyl radicals, or the formation of perferryl or ferryl species [15]. According to the abovementioned results, it could be deduced that the Fe^{2+}-chelating property of compound **4** might be one of the contributors to its anti-peroxidation (Table II).

In conclusion, sintenin derivatives **4** and **13** exhibited remarkable antioxidative and neuroprotective activities. Their DPPH radicals scavenging activity might be due to the diphenolic hydroxyl groups, and compared with the performances of compound **13**, the unsaturated ester moiety of **4** played some positively effects on inhibiting lipid

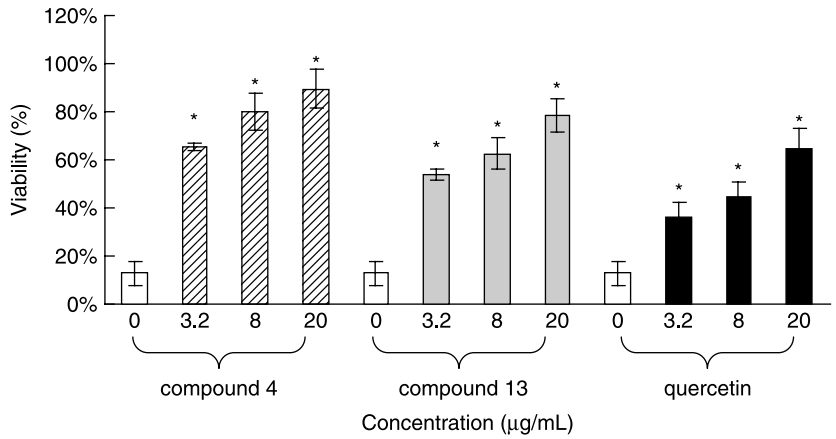


Figure 4. Protective effects of compounds **4**, **13**, and quercetin against $H_2O_2^-$ induced insult in PC 12 cells. The experiments were repeated three times. $\star p < .01$, compared with the negative control.

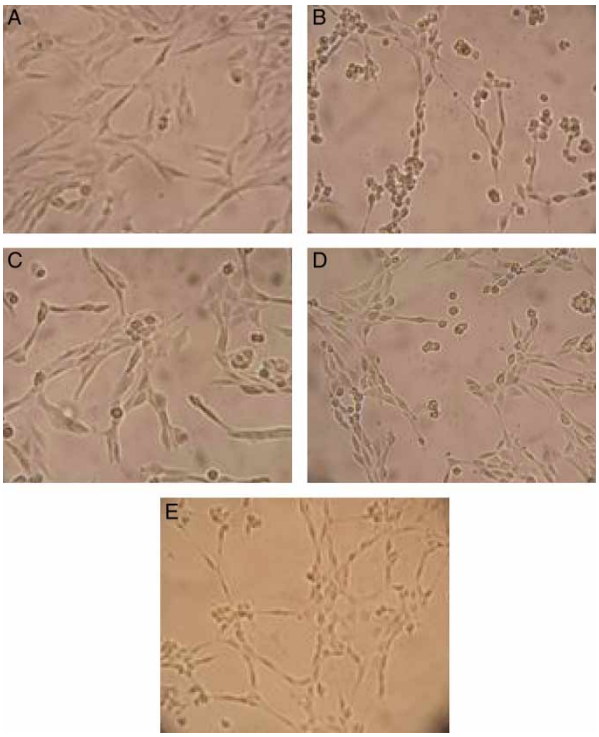


Figure 5. The results of removing test compounds from the pre-incubated medium of PC 12 cells prior to the injury initiated by H_2O_2. (A) PC 12 control cells. (B) PC 12 cells exposed to 600 μM H_2O_2 for 3 h. Most of the cells become round shapes. The membrane lesion of cells was also observed. (C, D and E) PC 12 cells were pre-incubated with 20 μg/mL of compounds **4**, **13** and quercetin, respectively, and then the compounds were removed from the medium and the cells were exposed to 600 μM H_2O_2 for 3 h. The cells cultured with compounds **4** and **13** were remarkably improved in cell viabilities and integralities.

peroxidtion, chelating Fe^{2+}, and neuroprotection. This study might offer some useful information for further development of sintenin analogues as novel antioxidants.

Acknowledgements

This work was partially supported by intramural foundation from Zhejiang University. We thank the Zhejiang Hisun Group for instrumental assistances. One of the authors (Y. Zhao) is grateful to Chinese Ministry of Education and Mr. Ka-Ching Lee for the award of a "Cheung Kong Scholars Professorship". The authors acknowledge helpful discussions with Prof. H.K. Cheng at Chinese University of Hong Kong.

Declaration of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Offen D. Antioxidant therapy in acute central nervous system injury: Current state. Pharmacol Rev 2002;54:271–284.
[2] Lefer DJ, Granger DN. Oxidative stress and cardiac disease. Am J Med 2000;109:315–323.
[3] Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett 1995;358:1–3.
[4] Lin HJ, Wang X, Shaffer KM, Sasaki CY, Ma W. Characterization of H_2O_2-induced acute apoptosis in cultured neural stem/progenitor cells. FEBS Lett 2004;570:102–106.
[5] Gilgun-Serki Y, Melamed E, Offen D. Oxidative stress induced neurodegenerative diseases: The need for antioxidants that penetrate the bood brain barrier. Neuropharmacol 2001;40: 959–975.
[6] Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc 1998;75: 199–212.
[7] Chen JJ, Duh CY, Huang HY, Chen IS. Cytotoxic constituents of *Piper sintenense*. Helv Chim Acta 2003;86:2085–2064.
[8] Hu LH, Zou HB, Gong JX, Li HB, Yang LX, Cheng W, Zhou CX, Bai H, Guérrite F, Zhao Y. Synthesis and biological evaluation of a natural ester sintenin and its synthetic analogues. J Nat Prod 2005;68:342–348.
[9] Rajapakse HA, Zhu H, Young MB, Mott BT. A mild and efficient one pot synthesis of 1,3,4-oxadiazoles from carboxylic acids and acyl hydrazides. Tetrahedron Lett 2006;47:4827–4830.
[10] Plata DJ, Kallmerten J. Total synthesis of 18-deoxynargenicin A1. J Am Chem Soc 1988;110:4041–4042.
[11] Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40: 945–948.
[12] Lee SE, Ju EM, Kim JH. Antioxidant activity of extracts from *Euryale ferox* seed. Exp Mol Med 2002;34:100–106.
[13] Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994;315:161–169.
[14] Xiao QX, Yang JW, Tang XC. Huperzine A protects rat pheochromocytoma cells against hydrogen peroxide-induced injury. Neurosci Lett 1999;275:73–76.
[15] Ko FN, Cheng ZJ, Lin CN, Teng CM. Scavenger and antioxidant properties of prenylflavones isolated from *Artocarpus heterophyllus*. Free Radic Biol Med 1998;25:160–168.