Bioorganic & Medicinal Chemistry Letters 23 (2013) 3700–3703

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and neuroprotective effect of *E*-3,4-dihydroxy styryl aralkyl ketones derivatives against oxidative stress and inflammation

Xianling Ning [a], Ying Guo [a], Xiaoyan Ma [a], Renzong Zhu [a], Chao Tian [a], Xiaowei Wang [a], Zhizhong Ma [c], Zhili Zhang [a,*], Junyi Liu [a,b,*]

[a] Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China
[b] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China
[c] Department of the Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, Beijing 100191, China

ARTICLE INFO

Article history:
Received 10 April 2013
Revised 3 May 2013
Accepted 7 May 2013
Available online 16 May 2013

Keywords:
E-3,4-Dihydroxy styryl aralkyl ketones
Caffeic acid phenethyl ester
Neuroprotective agents
Antioxidants
Anti-inflammatory

ABSTRACT

E-3,4-Dihydroxy styryl aralkyl ketones as well as their 3,4-diacetylated derivatives as the analogues of neuroprotective agent CAPE were designed and synthesized for improving stability and lipid solubility. The neuroprotective activities of target compounds **10a**–**g** and **11a**–**g** were tested by three models in vitro, including 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity, neuronal protecting effect against damage induced by H_2O_2 in PC12 cells and nitric oxide suppression effect in BV2 microglial cells. The results demonstrated that compounds **10f** and **11f** exhibited the most potent neuroprotective effect against oxidative stress and inflammation, which is higher than that of the lead compound CAPE.

Oxidative stress and inflammation have been implicated in many neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS).[1,2] Oxidative stress induced by overproduction of reactive oxygen species causes damage of basic components in nerve cells, such as lipids, DNA and proteins.[3] Inflammation also plays a vital role in pathogenesis of neurodegenerative diseases. Although this process is vital for normal function in the central nervous system (CNS), it is postulated that this process may spiral out of control with over activation of microglia, over production of cytokines and other proinflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and tumour necrosis factor (TNF)-alpha which at last result in cell injury.[1] Recently, the antioxidative and anti-inflammatory strategies have shown promise in the treatment of neurodegenerative diseases.

Compound **1** (Caffeic acid phenethyl ester, CAPE) (Fig. 1) which is the active component of the propolis produced by the hives of honeybees is found to possess antioxidant,[4] anti-inflammatory,[5] antiviral,[6] antibacterial,[7] antiatherosclerotic,[8] immunostimulatory[9] and antitumor[10] properties. The antioxidant property of **1** is reflected by means of blocking production of reactive oxygen species and the xanthine/xanthine oxidase system.[11] And the anti-inflammatory property is revealed through reducing prostaglandin and leukotriene synthesis by inhibiting cyclooxygenase enzyme activity or the down-regulation of cyclooxygenase gene expression.[12,13] It is deduced that both antioxidative and antiinflammatory properties of **1** can contribute to their neuroprotective effects in kinds of neurodegeneration. Many recent studies have confirmed that **1** has a neuroprotective property. Compound **1** is able to block 6-hydroxydopamine,[14] glutamate,[15] spinal ischemia,[16] hypoxia-ischemic brain injury[17,18] and low potassium[19]-induced neuronal death in vivo models. Thus, **1** can be recognized as a promising neuroprotective agent with multiple targets effects.

Although many preclinical studies have demonstrated biological activities of **1** in vitro and vivo models, pharmacokinetic studies show that **1** as an aryl ester is dramatically degraded by esterases in rats after the rapid oral absorption.[20] Additionally, with two phenolic hydroxyl functions **1** has a poor solubility in lipophilic environment, so most probably only small amount of **1** can pass through blood–brain barrier (BBB). The aim of this study, therefore, is to design and synthesize its analogues with better neuroprotective activity, stability and BBB permeability, and to discuss structure–activity relationships.

From previous studies, **1** seems to exert some of its effects through its catechol ring functionality which provides free radical scavenging and antioxidant activity, and the unsaturated double bond of the side chain which maximizes the stabilization of the phenolic radical.[21,22] Therefore, *E*-3,4-dihydroxy styryl aralkyl

* Corresponding authors. Tel./fax: +86 10 82805203 (Z.Z.).
E-mail addresses: lilybmu@bjmu.edu.cn (Z. Zhang), jyliu@bjmu.edu.cn (J. Liu).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.05.016

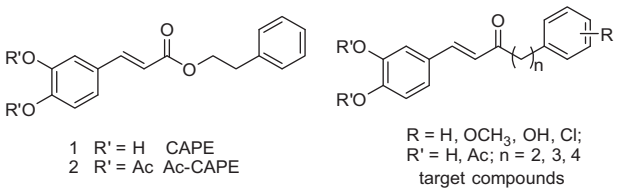


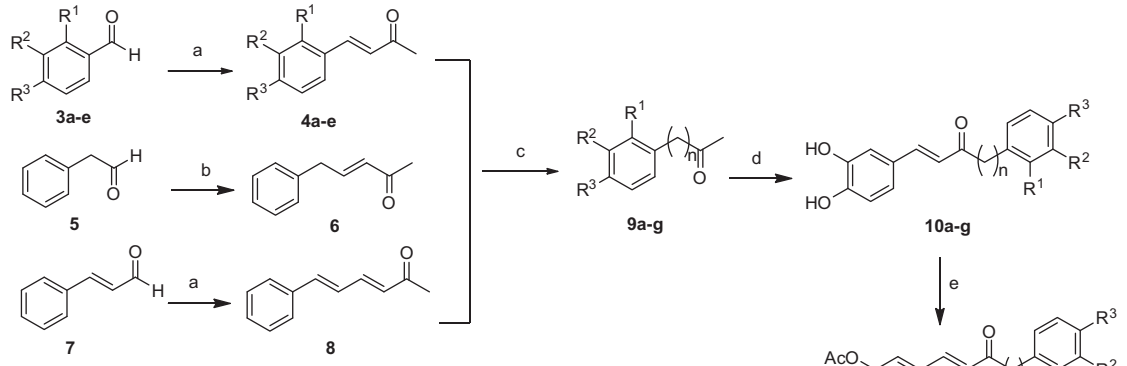
Figure 1. The structures of **1**, **2** and target compounds.

ketones (Fig. 1) are designed which reserve *E*-3,4-dihydroxy styryl group and introduce the ketone group instead of the unstable ester group. In order to improve BBB permeability, two phenolic hydroxyl functions of target compounds are acetylated rather than methylated to get corresponding high liposoluble compounds, because the methylation of phenolic hydroxyl functions may result in the moderate loss of biological activity.[23] It is also reported that acetylated phenolic compounds exhibit the same or higher neuroprotective activities compared with the initial phenolic compounds.[24] To explore the structure–activity relationships, the compounds with various lengths of alky chains and with various substituted groups on the aromatic ring are also designed.

E-3,4-dihydroxy styrene aralkyl ketones were synthesized as showed in Scheme 1. The intermediates, substituted 4-phenyl-but-3-en-2-one (**4a**–**e**), 5-phenyl-pent-3-en-2-one (**6**) and 6-phenyl-hexa-3,5-dien-2-one (**8**) were prepared by different pathways. The **4a–e** and **8** were prepared by the Claisen–Schimidt condensation reaction of substituted benzaldehydes (**3a**–**e**) or cinnamyl aldehyde (**7**) with acetone using a well-known procedure.[25,26] **6** was obtained by Wittig reaction. The 1-chloro-propan-2-one was converted to the corresponding phosphonium salt by heating with triphenylphosphine in $CHCl_3$. The salt reacted with phenylacetaldehyde (**5**), Na_2CO_3 as the catalyst, to produce **6.**[27] In the next step, **4a**–**e**, **6** and **8** were hydrogenated using 10% Pd/C as the catalyst in CH_2Cl_2 to afford saturated compounds **9a**–**g** in high yields.[28,29] Then, the **9a**–**g** reacted with 3,4-dihydroxy benzaldehyde by condensation using the pyrrolidine and acetic acid as the catalysts to obtain *E*-3,4-dihydroxy styryl aralkyl ketones (**10a**–**g**) in desired yields. Further, to improve BBB permeability, *E*-3,4-dihydroxy styryl aralkyl ketones (**10a**–**g**) were acetylated by acetic anhydride with pyridine as the catalyst to afford the corresponding *E*-3,4-diacetyl styryl aralkyl ketones (**11a**–**g**) in high yields. The ^1H NMR, ^{13}C NMR and HRMS data of all compounds synthesized were in full agreement with the proposed structures. The *E* geometry of the target compounds was confirmed by the coupling constants ($J \approx 16$ Hz).

The neuroprotective properties of *E*-3,4-dihydroxy styryl aralkyl ketones (**10a**–**g**) and their 3,4-diacetylated derivatives (**11a**–**g**) were assessed by way of several experimental pharmacological models in vitro, in which the antioxidant properties were evaluated by two models of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity and neuronal protecting effect against damage induced by hydrogen peroxide (H_2O_2) in PC12 cells, and the anti-inflammatory property was tested by the model of nitric oxide suppression effect in BV2 microglial cells.

The free radicals contribute to the pathogenesis of neurodegenerative disorders, therefore, antioxidant therapy is considered as one of options in treatment of neurodegenerative disorders.[30] DPPH free radicals can be used in preliminary screening of compounds with capability of scavenging reactive free radicals.[31] The free radical scavenging capacities of target compounds **10a**–**g** and **11a**–**g** were evaluated by the published test method[32] over the concentration range of 1–50 μM. The ethanol solution of test compounds and DPPH were mixed. After 60 min of incubation, the capacities of scavenging free radicals were monitored by measuring the change in light absorption at 517 nm. The results of **10a**–**g** are shown in Table 1. From the results, compounds **10a**–**g** show the similar or stronger free radical scavenging capacities than **1**. Especially compound **10f** ($IC_{50} = 9.2 \pm 0.4$ μM) exhibits prominent activity, which is 1.3-fold higher than that of **1** ($IC_{50} = 12.1 \pm 0.3$ μM). The compounds **10f**–**g** ($IC_{50} = 9.2 \pm 0.4$ and 10.6 ± 0.5 μM) with 3C and 4C alkyl chains show more potent free radicals quenching abilities compared with the compound **10a** ($IC_{50} = 12.7 \pm 0.5$ μM) with 2C alkyl chain. It is also noteworthy that the electron-withdrawing chloro substituted compound (**10b**) exhibits more effective activities than electron-donating methoxyl substituted compounds (**10c–e**). In addition, acetylated compounds **2** and **11a**–**g** do not show detectable scavenging capacities under the concentration of 50 μM, which implies that two phenolic



a: n = 2, R^1 = H, R^2 = H, R^3 = H;
b: n = 2, R^1 = H, R^2 = Cl, R^3 = H;
c: n = 2, R^1 = H, R^2 = OCH_3, R^3 = OCH_3;
d: n = 2, R^1 = OCH_3, R^2 = H, R^3 = OCH_3;
e: n = 2, R^1 = H, R^2 = OCH_3, R^3 = OH (**3a-e**, **4a-e**, **9a-g** and **10a-g**); R^3 = Ac (**11a-g**);
f: n = 3, R^1 = H, R^2 = H, R^3 = H;
g: n = 4, R^1 = H, R^2 = H, R^3 = H.

Scheme 1. Synthesis of *E*-3,4-dihydroxy styryl aralkyl ketones and their 3,4-diacetylated derivatives. Reagents and conditions: (a) acetone, NaOH or K_2CO_3, H_2O, rt, 80–95%; (b) 1-chloro-propan-2-one, PPh_3, $CHCl_3$, reflux; 10% Na_2CO_3, rt; phenylacetaldehyde, THF, rt; 62%; (c) 10% Pb/C, H_2, CH_2Cl_2, rt, 90–95%; (d) 3,4-dihydroxy-benzaldehyde, pyrrolidine, acetic acid, THF, reflux, 60–85%; (e) pyridine, acetic anhydride, rt, 92–96%.

Table 1
Free radical scavenging capacities of **1** and target compounds **10a–g** by the DPPH method

Compound	IC_{50}[a] (μM)	Compound	IC_{50}[a] (μM)
10a	12.7±0.5	**10e**	12.1±0.4
10b	11.1±0.3	**10f**	9.2±0.4
10c	13.2±0.6	**10g**	10.6±0.5
10d	14.0±0.5	**1**	12.1±0.3

[a] IC_{50}: the concentration that produces 50% inhibitory effect; data are expressed as the mean ± SD, $n = 3$.

hydroxyl groups of target compounds really play an important role in free radical scavenging capacity.

In order to determine further the antioxidant properties of target compounds in nerve cells, target compounds **10a–g** and **11a–g** were also assayed by the H_2O_2 model on PC12 cells. H_2O_2 could injure nerve cells for the generation of exogenous free radicals. PC12 cells, commonly as a screening model for studies of neurodegenerative diseases, were used in this study.[33] The protection effect of compounds against H_2O_2 can be conveniently evaluated by the cell viability. PC12 cells were pretreated with test compounds for 3 h, then were exposed to 500 μM H_2O_2 for 5 h. Levels of cell viability were measured by the MTT assay. The cell viabilities attributable to the protective efficiency of target compounds (**10a–g** and **11a–g**) against H_2O_2 at 10 μM are listed in Figure 2. From the result, all target compounds exhibit protection effects against damage induced by H_2O_2, although the activities of target compounds display lower activities compared with **1** and its diacetylated derivative (**2**). Acetylated compounds **11a–g** display similar protection effects against damage induced by H_2O_2 to corresponding un-acetylated compounds **10a–g**. Compounds with 3C–4C alkyl chains (**10f–g** and **11f–g**) show stronger protection effects compared with compounds with 2C alkyl chains (**10a** and **11a**), which is consistent with the result of DPPH model. Additionally, two methoxyl substituted compounds **10c–d** and **11c–d** display weaker protection effects than un-substituted compounds **10a** and **11a**.

The anti-neuroinflammatory properties of target compounds were evaluated by assaying the nitric oxide suppression effect in BV2 microglial cells. The microglial cells are believed to play a key role in the pathway that leads to inflammation-mediated neuronal cell death in a number of neurodegenerative diseases. The uncontrolled activation of microglia cells may cause neuronal damage through the overproduction of proinflammatory substances, such as nitric oxide.[34] Therefore, suppression nitric oxide is a useful strategy for treating neurodegenerative disorders. The inhibition effect of target compounds **10a–g** and **11a–g** on nitric oxide can be measured by the Griess assay.[35] The culture media supernatants were incubated in the dark with equal amounts of Griess reagent. And the absorbance was subsequently read at 540 nm. The nitric oxide suppression activities of tested compounds are summarized in Table 2. All target compounds significantly exhibit nitric oxide suppression activities in LPS-stimulated BV2 microglial cells. Among those compounds, **10e–g** and **11e–g** display similar or higher activities compared with **1** and **2**. Especially, compound **11g** ($IC_{50} = 4.2 \pm 0.3$ μM) shows the strongest nitric oxide suppression activity and has 1.5-fold higher activity than that of **1** ($IC_{50} = 6.4 \pm 0.4$ μM) and **2** ($IC_{50} = 6.2 \pm 0.2$ μM). Compounds with 3C–4C alkyl chain, **10f–g** and **11f–g**, show more potent activities compared with compounds **10a** ($IC_{50} = 14.1 \pm 0.4$ μM) and **11a** ($IC_{50} = 12.3 \pm 0.6$ μM) with shorter 2C chain. All of acetylated target compounds (**11a–g**) reveal the higher nitric oxide suppression effects than corresponding un-acetylated compounds (**10a–g**) which is presumably due to their higher lipophilicity. The acetylated derivatives probably could be hydrolyzed into hydroxyl compounds by the esterase in cells to produce nitric oxide suppression effects. The nitric oxide suppression mechanism, based on the literatures, is presumably that target compounds could inhibit the activity of COX-2 and iNOS which play important roles in the inflammatory mechanism.[36] Moreover, it is observed that compounds **10e** and **11e** with three phenolic hydroxyl groups on the aromatic ring have more effective activities compared with compounds **10a–d** and **11a–d** with two phenolic hydroxyl groups, therefore, introducing of phenolic hydroxyl groups on the aromatic ring is presumably conductive to improvement of nitric oxide suppression activities**.** Additionally, the concentrations of target compounds used in our experiment do not lead to any significant cytotoxicity even at a high concentration (50 μM) up to 24 h of incubation, and in all cases cell viability is above 90% by MTT assay, confirming that inhibition of nitric oxide production in LPS-stimulated BV2 microglial cells is not due to a cytotoxic action of target compounds.

In summary, we report the design, synthesis and biological evaluation of *E*-3,4-dihydroxy styryl aralkyl ketones and their

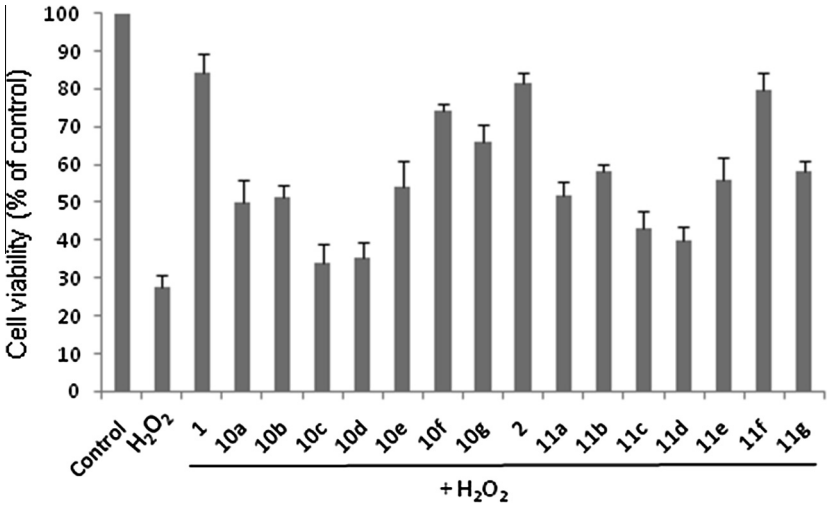


Figure 2. Protective effects of **1**, **2** and target compounds **10a–11g** against H_2O_2 induced injury in PC12 cells at 10 μM, respectively. PC12 cells were pretreated with test compounds for 3 h, then were exposed to 500 μM H_2O_2 for 5 h. Levels of cell viability were measured by the MTT assay. The viability of control cells untreated with tested compounds and H_2O_2 was defined as 100%. The viability of cells treated with drugs was calculated by the following formula: OD (drug-treated)/OD (control) ×100%. Data are expressed as the mean ± SD, $n = 3$.

Table 2
Nitric oxide suppression activities of **1**, **2** and target compounds **10a–11g** in LPS-stimulated BV2 microglial cells

Compound	IC_{50}[a] (μM)	Compound	IC_{50}[a] (μM)
10a	14.1 ± 0.4	**11a**	12.3 ± 0.6
10b	10.3 ± 0.3	**11b**	8.3 ± 0.4
10c	14.5 ± 0.5	**11c**	12.6 ± 0.2
10d	10.6 ± 0.2	**11d**	9.3 ± 0.2
10e	6.3 ± 0.3	**11e**	5.5 ± 0.4
10f	6.4 ± 0.5	**11f**	5.3 ± 0.2
10g	5.9 ± 0.3	**11g**	4.2 ± 0.3
1	6.4 ± 0.4	**2**	6.2 ± 0.2

[a] IC_{50}: the concentration that produces 50% inhibitory effect; data are expressed as the mean ± SD, $n = 3$.

3,4-diacetylated derivatives as neuroprotective agents. The results show that target compounds demonstrate multifunctional neuroprotective activities against oxidative and inflammatory injury. Among target compounds **10a–g**, compound **10f** displays the highest neuroprotective activity. It has stronger scavenging reactive free radical effect and higher suppression nitric oxide effect, compared to the lead compound **1**. And its protection effect of nerve cells against damage induced by H_2O_2 is close to **1**. Moreover, its acetylated compound **11f** reveals more potent anti-neuroinflammatory property and similar antioxidant effect, compared to **10f**. In view of the more metabolic stable of ketone group on **10f** and **11f** than ester group on **1**, therefore, we can consider that compounds **10f** and **11f** are the most potent compound which would be a promising structural template for the development of novel and more efficient neuroprotective agents. Further studies are in progress in order to clarify the precise mechanism of their neuroprotective effects.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant nos. 20972011, 21042009 and 21172014) and Ministry of Science and Technology of China (2009ZX09301-010).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.05.016.

References and notes

1. Heneka, M. T. *Nervenarzt* **2007**, *78*, 148.
2. Behl, C.; Davis, J. B.; Lesley, R.; Schubert, D. *Cell* **1994**, *77*, 817.
3. Cini, M.; Moretti, A. *Neurobiol. Aging* **1995**, *16*, 53.
4. Chen, Y. J.; Shiao, M. S.; Wang, S. Y. *Anticancer Drugs* **2001**, *12*, 143.
5. Orban, Z.; Mitsiades, N.; Burke, T. R., Jr.; Tsokos, M.; Chrousos, G. P. *Neuroimmunomodulation* **2000**, *7*, 99.
6. Fesen, M. R.; Kohn, K. W.; Leteurtre, F.; Pommier, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2399.
7. Kujumgiev, A.; Bankova, V.; Ignatova, A.; Popov, S. *Pharmazie* **1993**, *48*, 785.
8. Natarajan, K.; Singh, S.; Burke, T. R., Jr.; Grunberger, D.; Aggarwal, B. B. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9090.
9. Lefkovits, I.; Su, Z.; Fisher, P.; Grunberger, D. *Int. J. Oncol.* **1997**, *11*, 59.
10. Grunberger, D.; Banerjee, R.; Eisinger, K.; Oltz, E. M.; Efros, L.; Caldwell, M.; Estevez, V.; Nakanishi, K. *Experientia* **1988**, *44*, 230.
11. Sud'ina, G. F.; Mirzoeva, O. K.; Pushkareva, M. A.; Korshunova, G. A.; Sumbatyan, N. V.; Varfolomeev, S. D. *FEBS Lett.* **1993**, *329*, 21.
12. Toyoda, T.; Tsukamoto, T.; Takasu, S.; Shi, L.; Hirano, N.; Ban, H.; Kumagai, T.; Tatematsu, M. *Int. J. Cancer* **2009**, *125*, 1786.
13. Michaluart, P.; Masferrer, J. L.; Carothers, A. M.; Subbaramaiah, K.; Zweifel, B. S.; Koboldt, C.; Mestre, J. R.; Grunberger, D.; Sacks, P. G.; Tanabe, T.; Dannenberg, A. J. *Cancer Res.* **1999**, *59*, 2347.
14. Noelker, C.; Bacher, M.; Gocke, P.; Wei, X.; Klockgether, T.; Du, Y. S.; Dodel, R. *Neurosci. Lett.* **2005**, *383*, 39.
15. Wei, X.; Ma, Z.; Fontanilla, C. V.; Zhao, L.; Xu, Z. C.; Taggliabraci, V.; Johnstone, B. H.; Dodel, R. C.; Farlow, M. R.; Du, Y. *Neuroscience* **2008**, *155*, 1098.
16. Irmak, M. K.; Fadillioglu, E.; Sogut, S.; Erdogan, H.; Gulec, M.; Ozer, M.; Yagmurca, M.; Gozukara, A. E. *Cell Biochem. Funct.* **2003**, *21*, 283.
17. Wei, X.; Zhao, L.; Ma, Z.; Holtzman, D. M.; Yan, C.; Dodel, R. C.; Hampel, H.; Oertel, W.; Farlow, M. R.; Du, Y. *Brain* **2004**, *127*, 2629.
18. Altug, M. E.; Serarslan, Y.; Bal, R.; Kontas, T.; Ekici, F.; Melek, I. M.; Aslan, H.; Duman, T. *Brain Res.* **2008**, *1201*, 135.
19. Amodio, R.; De Ruvo, C.; Di Matteo, V.; Poggi, A.; Di Santo, A.; Martelli, N.; Lorenzet, R.; Rotilio, D.; Cacchio, M.; Esposito, E. *Int. J. Dev. Neurosci.* **2003**, *21*, 379.
20. Celli, N.; Mariani, B.; Dragani, L. K.; Murzilli, S.; Rossi, C.; Rotilio, D. *J. Chromatogr., B Anal. Technol. Biomed. Life Sci.* **2004**, *810*, 129.
21. Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radical Biol. Med.* **1996**, *20*, 933.
22. Lien, E. J.; Ren, S.; Bui, H. H.; Wang, R. *Free Radical Biol. Med.* **1999**, *26*, 285.
23. Deng, D.; Zhang, J.; Cooney, J. M.; Skinner, M. A.; Adaim, A.; Jensen, D. J.; Stevenson, D. E. *FEBS Lett.* **2006**, *580*, 5247.
24. Fragopoulou, E.; Nomikos, T.; Karantonis, H. C.; Apostolakis, C.; Pliakis, E.; Samiotaki, M.; Panayotou, G.; Antonopoulou, S. *J. Agric. Food Chem.* **2007**, *55*, 80.
25. Wang, Z.; Yin, G.; Qin, M.; Gao, L.; Wu, A. *Synthesis* **2008**, 3565.
26. Handayani, S.; Arty, I. S. *J. Phys. Sci.* **2008**, *92*, 61.
27. Gravil, S.; Veschambre, H.; Chênevert, R.; Bolte, J. *Tetrahedron Lett.* **2006**, *47*, 6153.
28. Erathodiyil, N.; Ooi, S.; Seayad, A. M.; Han, Y.; Lee, S. S.; Ying, J. Y. *Chemistry* **2008**, *14*, 3118.
29. Dhuru, S.; Bhedi, D.; Gophane, D.; Hirbhagat, K.; Nadar, V.; More, D.; Parikh, S.; Dalal, R.; Fonseca, L. C.; Kharas, F.; Vadnal, P. Y.; Vishwakarma, R. A.; Sivaramakrishnan, H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3784.
30. Nishida, J.; Kawabata, J. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 193.
31. Cotelle, N.; Bernier, J. L.; Catteau, J. P.; Pommery, J.; Wallet, J. C.; Gaydou, E. M. *Free Radical Biol. Med.* **1996**, *20*, 35.
32. Sapkota, K.; Roh, E.; Lee, E.; Ha, E. M.; Yang, J. H.; Lee, E. S.; Kwon, Y.; Kim, Y.; Na, Y. *Bioorg. Med. Chem.* **2011**, *19*, 2168.
33. Halleck, M. M.; Richburg, J. H.; Kauffman, F. C. *Free Radical Biol. Med.* **1992**, *12*, 137.
34. Kim, Y. S.; Joh, T. H. *Exp. Mol. Med.* **2006**, *38*, 333.
35. Senra-Ferreiro, S.; Pena-Pereira, F.; Lavilla, I.; Bendicho, C. *Anal. Chim. Acta* **2010**, *668*, 195.
36. Jung, W. K.; Choi, I.; Lee, D. Y.; Yea, S. S.; Choi, Y. H.; Kim, M. M.; Park, S. G.; Seo, S. K.; Lee, S. W.; Lee, C. M.; Park, Y. M.; Choi, I. W. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 2572.