Biochemical and Biophysical Research Communications 415 (2011) 6–10

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Strongylophorine-8, a pro-electrophilic compound from the marine sponge *Petrosia* (*Strongylophora*) *corticata*, provides neuroprotection through Nrf2/ARE pathway

Shunsuke Sasaki [a], Terumasa Tozawa [a], Ryan M. Van Wagoner [b], Chris M. Ireland [b], Mary Kay Harper [b], Takumi Satoh [a,*]

[a] Department of Welfare Engineering, Faculty of Engineering, Iwate University, Morioka, Iwate 020-8551, Japan
[b] Department of Medicinal Chemistry, University of Utah, 30 South 2000 East, Salt Lake City, UT 84112, United States

ARTICLE INFO

Article history:
Received 18 September 2011
Available online 1 October 2011

Keywords:
Strongylophorine-8
ARE
Nrf2
Pro-electrophilic drugs
Para-hydroquinone

ABSTRACT

Green plant-origin electrophilic compounds are a newly-recognized class of neuroprotective compounds that provide neuroprotection through activation of the Nrf2/ARE pathway. Electrophilic hydroquinones are of particular interest due to their ability to become electrophilic quinones upon auto-oxidation. Although marine organisms frequently produce a variety of electrophilic compounds, the detailed mechanisms of action of these compounds remain unknown. Here, we focused on the neuroprotective effects of strongylophorine-8 (STR8), a *para*-hydroquinone-type pro-electrophilic compound from the sponge *Petrosia* (*Strongylophora*) *corticata*. STR8 activated the Nrf2/ARE pathway, induced phase 2 enzymes, and increased glutathione, thus protecting neuronal cells from oxidative stress. Microarray analysis indicated that STR8 induced a large number of phase 2 genes, the regulation of which is controlled by the Nrf2/ARE pathway. STR8 is the first example of a neuroprotective pro-electrophilic compound from marine organisms.

1. Introduction

Electrophilic compounds have a significant advantage over antioxidant molecules in mediating cytoprotection against oxidative damage because their actions are more sustained and are amplified by transcription-mediated signaling pathways [1–5]. Electrophiles can induce the expression of a set of antioxidant enzymes, called "phase 2 enzymes", including NADPH quinone oxidoreductase 1 (NQO1), glutathione S-transferase (GST), heme oxygenase-1 (HO-1), and glutamyl cysteine ligase catalytic subunit (GCLC), all of which provide efficient cytoprotection by regulating the intracellular redox state [6–8]. Representing a specific transcriptional element located in the 5′ upstream promoter region of genes that encode phase 2 enzymes, the antioxidant-responsive element (ARE) plays a central role in the induction of such enzymes [6–8]. The key cascade involved is the Keap1/Nrf2 pathway, which comprises Keap1, a regulator protein, and Nrf2, a transcriptional factor that binds to the ARE [6–8]. Recently, some electrophilic compounds, including carnosic acid [9–12], curcumin [13,14], sulforaphane [15], (−)-epicatechin [16], cystamine [17], and plumbagin [18], all from green plants, have been reported to protect neurons against various types of stress in addition to the protection afforded by synthetic electrophiles such as D1 [19], *tert*-butyl hydroquinone [20], Neurite outgrowth-Promoting Prostaglandin 11 [21–23], and 3H-1, 2-dimethiole-3-thione [24]. All of these compounds utilize the same intracellular signaling pathway (Nrf2/ARE pathway) for their protective effects [1–24]. Other hopeful candidates of efficient electrophiles are those from marine organisms, but there are few reports of such compounds affording cytoprotection against oxidants to date [1–3]. Given our experience with plant metabolites and the occurrence of similar structural types in marine natural products, we have been searching for electrophiles from marine organisms as candidates for neuroprotective compounds. In this present study, among the vast number of substances produced by marine organisms we have focused on strongylophorine-8 (STR8) because it is a *para*-hydroquinone-type pro-electrophilic compound [25–33]. The STRs are meroditerpenoids that were first isolated from the sponge *Strongylophora durissima*, collected in Papua New Guinea and the Philippines [25–27]. These electrophiles were

Abbreviations: ARE, antioxidant-responsive element; CA, carnosic acid; DMSO, dimethyl sulfoxide; GSH, reduced glutathione; GSSG, oxidized glutathione; HO-1, heme oxygenase-1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NQO-1, NADPH quinone oxidoreductase 1; GCLC, glutamyl cysteine ligase catalytic subunit; GST, glutathione S-transferase; PAT, pathologically-activated therapeutic; PED, pro-electrophilic drug; RT-PCR, reverse transcription-polymerase chain reaction; SD, standard deviation; STR, strongylophorine.

* Corresponding author. Fax: +81 19 621 6314.
E-mail address: tsatoh@iwate-u.ac.jp (T. Satoh).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.09.114

previously reported to possess the following biological activities: antibacterial, antifungal, insecticidal, lethal toxicity toward brine shrimp, inhibition of the maturation of starfish oocytes, and HIF-1 activation [28–33]. To date no studies of the effects of these compounds on neurons has been reported. As reported herein, we found that STR8 activated the Nrf2/ARE pathway and protected neuronal cells against oxidative stress. This paper represents the first report of a pro-electrophilic compound from a marine organism that provides neuroprotection through activation of the Nrf2/ARE pathway.

2. Materials and methods

2.1. Chemicals

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), reduced glutathione (GSH), and oxidized glutathione (GSSG) were purchased from Sigma. STRs were prepared as 10 mM stock solutions in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the culture medium was less than 0.1%. STRs were extracted from the sponge *Petrosia* (*Strongylophora*) *corticata* collected in Papua New Guinea as described elsewhere [33].

2.2. HT22 culture and MTT assay

HT22 cells were cultured as described previously [34,35]. The cells were maintained in 75-cm^2 flasks (Invitrogen, Carlsbad, CA) containing 10 mL of Dulbecco's Modified Eagle medium supplemented with 10% (v/v) heat-inactivated (56 °C, 30 min) fetal calf serum (Invitrogen, Carlsbad, CA). HT22 cells were seeded into 48-well plates at a density of 4×10^4 cells/cm^2. After a 5-h incubation, the desired compounds were added to the cultures. Sixty minutes later, 5 mM glutamate was added; and the cells were then incubated further for 24 h. To evaluate cell survival of HT22 cells, we performed an MTT assay [34,35].

2.3. ARE and GSH + GSSG assays

GSTYa ARE-luciferase vector was constructed as described previously [9,36,37]. In order to measure ARE activation by STR8, we conducted luciferase assays as described earlier [9–10] For measurement of reduced and oxidized glutathione (GSH + GSSG) levels, the cells were lysed with 1% sulfosalicylic acid [9–10]. Lysates were incubated on ice for 10 min, and total glutathione (reduced and oxidized) was determined as described previously [9–10]. Experiments presented here were repeated at least three times, with four samples for each determination. The data are presented as the mean ± SD.

2.4. Oligonucleotide microarray analysis

Total RNA was isolated from vehicle (DMSO)- or STR8-treated HT22 cells by using TRIzol Reagent (Invitrogen, Carlsbad, CA) [33,34]. cDNA was synthesized by using the Superscript II system (Invitrogen) with a T7-Oligo(dT) primer. Biotin-labeled cRNA was prepared by *in vitro* transcription and fragmented by incubation at 94 °C for 35 min in 40 mmol/L Tris acetate buffer (pH 8.1) containing 100 mM/L potassium acetate and 30 mM/L magnesium acetate. Fragmented cRNA was hybridized at 45 °C for 16 h to a GeneChip® Mouse 430 2.0 Array (Affymetrix, Santa Clara, CA), which contains over 39,000 transcripts. Probe arrays were washed and stained using a Fluidics Station 450 and scanned with a GeneChip® Scanner 300. Affymetrix GeneChip Operating Software (GCOS v1.4) was used for analysis.

2.5. RT-PCR

Reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed as described previously [9–10,33,34] using the following primers:

β-Actin (β-actin: 287 bp)
Forward 5′-ATC CGT AAA GAC CTC TAT GC-3′
Reverse 5′-AAC GCA GCT CAG TAA CAG TC-3′
NADP(H):quinone oxidoreductase1 (nqo1: 923 bp)
Forward 5′-ATC CTT CCG AGT CAT CTC TA-3′
Reverse 5′-CAA CGA ATC TTG AAT GGA GG-3′
Glutathione S-transferase, alpha 4 (gsta4: 440 bp)
Forward 5′-CCA GGG CCA TCC TCA GCT AC-3′
Reverse 5′-CTC AAC ATA GGG GCC ATC TGG-3′
Heme oxygenase-1 (ho-1: 617 bp)
Forward 5′-AGG TGT CCA GAG AAG GCT T-3′
Reverse 5′-ATC TTG CAC CAG GCT AGC A-3′
Gsta1 (gsta1: 328 bp)
Forward 5′-GAG CTG AGT GGA GAA GAA GC-3′
Reverse 5′-GGG CTC TCT CCT TCA TGT CC-3′
Glutamate-cysteine ligase, catalytic subunit (gclc: 561 bp)
Forward 5′-GTG GAG TAC ATG TTG GTG TC-3′
Reverse 5′-GTA GAT ATG GTC TGG CTG AG-3′

3. Results

3.1. Neuroprotective effects

In order to examine the importance for the *para*-hydroquinone moiety of the strongylophorines for neuroprotection, we compared the protective effects afforded by STR8 and STR2 (Fig. 1). STR8 contains a *para*-hydroquinone moiety that can be converted to a quinone by autooxidation capable of activating the Keap1/Nrf2 pathway. By contrast, the corresponding moiety in STR2 contains an ether and therefore cannot be converted to the quinone. Thus, if the effector for neuroprotective effects is the quinone, as was shown in the case of CA in previous papers [9,10], STR8 should be protective and STR2 should not. We used HT22 cells, a neuronal cell line from mouse hippocampus, as a model for oxidative cell damage. In HT22 cells, high concentrations (mM levels) of glutamate can induce cell death by depleting intracellular GSH through inhibiting the influx of cystine [34,35]. Cell survival was quantified using an MTT assay (Fig. 2). Treatment of cells with 5 mM glutamate induced global cell death by oxidative stress within 24 h. Cotreatment with STR2 afforded no protection from glutamate-mediated toxicity (Fig. 2). By contrast, cotreatment of cells with STR8 led to protection from the effects of glutamate in a dose-dependent fashion (Fig. 2). ED50 (protective effects) and LD50 (killing effects) of STR8 for the cells were 4.1 and 14.24 μM, respectively; and thus this compound did not have a broad safety zone, suggesting that it would not be suitable for *in vivo* use. These results suggest that the presence of a *para*-hydroquinone moiety competent of conversion to a quinone by autooxidation is critical for neuroprotective activity.

3.2. Microarray analysis and RT-PCR

We hypothesized that the protective effects by STR8 should be closely related to its transcriptional activation of the Nrf2/ARE pathway [9,19]. In order to examine this possibility, we initially performed a microarray analysis using HT22 cells exposed to these compounds vs. the control (vehicle, DMSO; Table 1). The top 10 genes induced by 5 μM STR8 are listed in Table 1. STR8 induced several phase 2 genes, such as nqo1, gsta4, and ho-1 suggesting

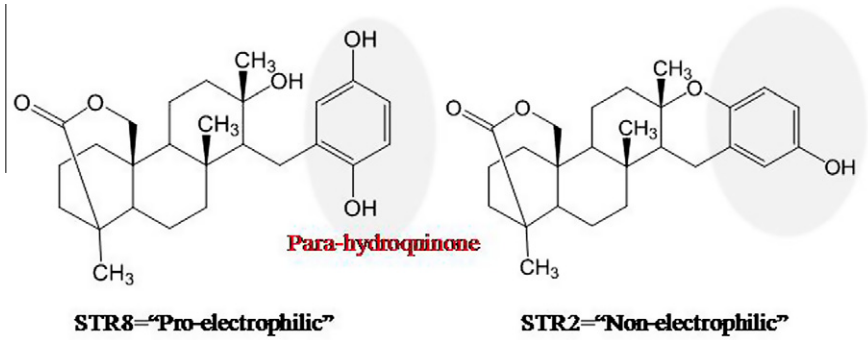

Fig. 1. Chemical structures of STRs. Note that STR8, but not STR2, has a *para*-hydroquinone moiety, although they share the same basic chemical structure of meroditerpenes.

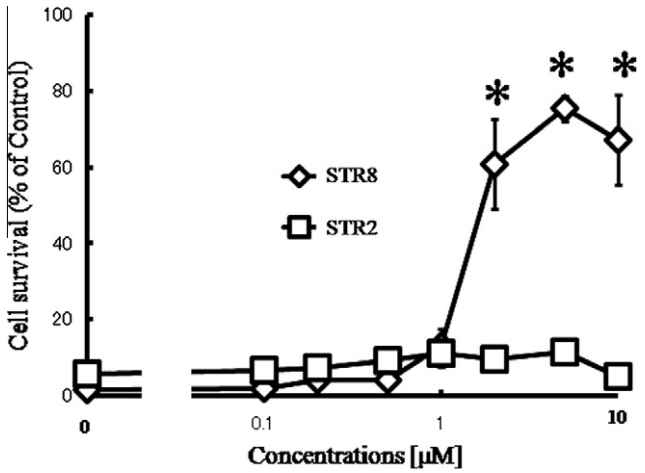

Fig. 2. Protection of HT22 cells by STR8. HT22 cells were seeded into 24-well plates at a density of 4×10^4 cells/cm^2. After a 5-h incubation, test compounds were added. One hour later the cells were treated with glutamate for 24 h and then were subjected to an MTT assay. The values, which represent the percentage of the control MTT value (no glutamate), are means ± S.D ($n = 4$). *Significantly differently ($p < 0.01$) from control (no glutamate) cells by ANOVA.

that STR8 is a potent activator of the Nrf2/ARE pathway. In order to confirm that STR8 indeed induced the genes encoding these phase 2 enzymes, we performed RT-PCR analysis using primers for nqo1, gesta4, and ho-1. All of these in addition to gclc and gsta1 were significantly induced by STR8 (Fig. 3A).

3.3. Activation of ARE and increase in GSH level

Neuroprotective effects by electrophilic compounds may be due to activation of the Keap1/Nrf2 pathway and ARE [1–21]. Here we examined whether STR8 could activate ARE in the neuronal cell line HT22 (Fig. 3B). We examined the activation of GSTYaARE, a transcriptional element that responds to Nrf2 of the Keap1/Nrf2 pathway, by conducting a luciferase assay using HT22 cells transfected with ARE(GSTYa)-luciferase expression vectors (Fig. 3B). Based on the luciferase activity, STR8 (5 μM) stimulated the expression of ARE-regulated genes by >fourfold. The activation of ARE led to an increase in total glutathione level (GSH + GSSG; Fig. 3C), which is not surprising since enzymes of glutathione biosynthesis such as GCLC are regulated under the influence of ARE. Actually, various electrophilic compounds were earlier reported to increase GSH and GSH + GSSG levels through ARE activation [1–5]. STR8 increased the GSH + GSSG level by over threefold relative to control.

4. Discussion

Although electrophiles are capable of inducing neuroprotective pathways in neuronal cells, one of the disadvantages that must be overcome is non-specific reaction of the electrophile with cellular thiols [1–3]. We have proposed that one solution is the use of pro-electrophilic compounds [1,9,19]. Such compounds are not directly electrophilic but can be converted to electrophiles [9,19]. We designated these types of compounds as "pro-electrophilic drugs (PEDs)" [9,19]. One of the best examples of a PED is a hydroquinone that is oxidized to a quinone [9,19]. STR8 possesses a *para*-hydroquinone ring and thus represents a pro-electrophilic compound that can be converted to a quinone-type electrophile by oxidation, as proposed elsewhere for CA and D1 [9,12,19]. We have focused on hydroquinone-type pro-electrophilic compounds because of their distinctively "prodrug"-like properties [9,11,19]. We believe that their activation can lead to effective neuroprotection against oxidative stress [9,11] and ER stress [19]. There are two factors within cells that affect this oxidation from hydroquinone to quinone, i.e., the presence of copper ions and the presence of an

Table 1
Microarray analysis of the genes induced by STR8. The top 10 genes induced by STR8 in HT22 cells are listed. These genes were selected for their significant up-regulation ($p < 0.0003$) in response to STR8. HT22 cells were incubated in the absence or presence of 5 μM STR8 for 24 h in normal medium. Total RNA was isolated and subjected to microarray analysis. Fold change (STR8/control vehicle), GeneBank accession ID, and gene name are indicated. Red characters indicate the phase 2 genes.

No.	Induction	GeneBank	Gene description	Gene symbol
1	4.5	BC004579	NAD(P)H dehydrogenase, quinone 1	Nqo1
2	3.9		Heat shock protein 1B	Hspa1b
3	3.1	BC012639	Glutathione S-transferase, alpha 4	Gsta4
4	3.0	M33203	Heme oxygenase (decycling) 1	Hmox1
5	2.4	BC022913	RIKEN cDNA 4930570C03 gene	4930570C03Rik
6	2.2	BC028755	Protein C receptor, endothelial	Procr
7	2.1	BC016234	Proline rich 13	Prr13
8	2.1	BC017161	DnaJ (Hsp40) homolog, subfamily B, member 4	Dnajb4
9	2.0	BC049957	Sulfiredoxin 1	Srxn1
10	2.0	BC010309	Plasminogen activator, urokinase receptor	Plaur

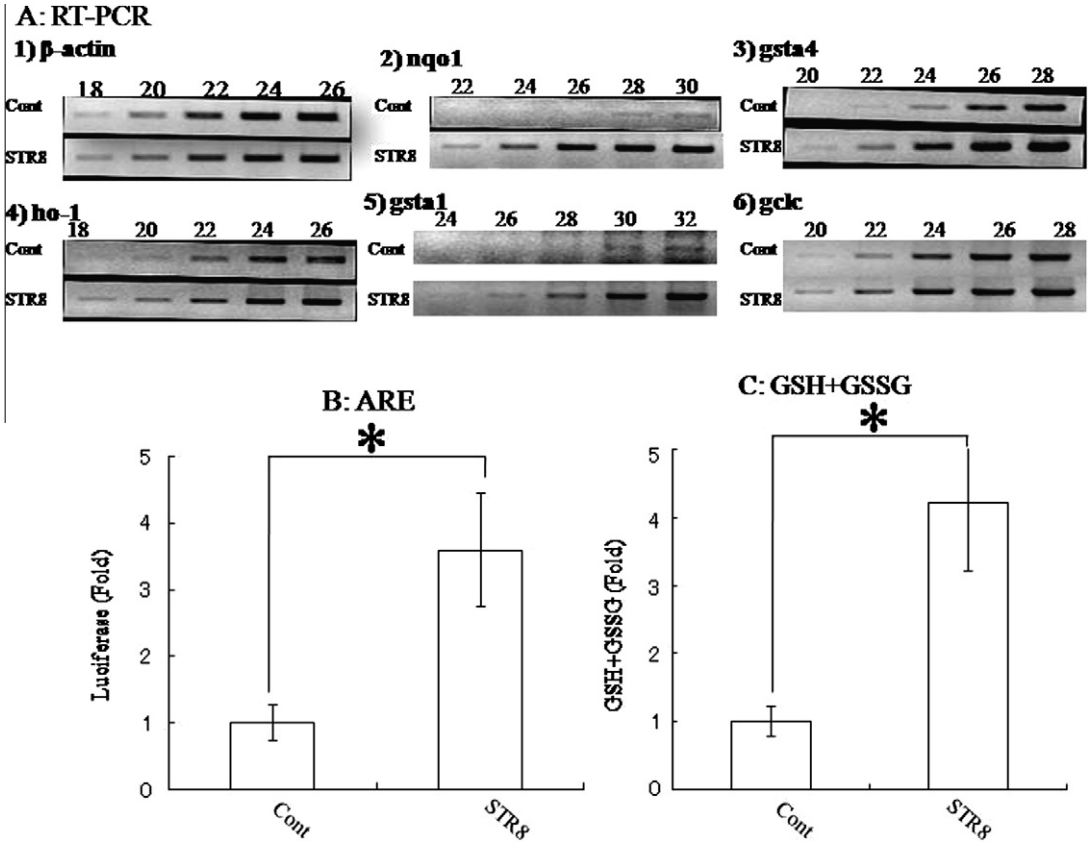

Fig. 3. (A). Induction of phase 2 genes by STR8. Total RNAs were extracted from HT22 cells treated with STR8 (5 μM) for 24 h. RT-PCR was performed for the detection of β-actin, nqo1, gsta4, ho-1, gclc, and gsta1 mRNAs. Aliquots of the reaction products were subjected to electrophoresis after PCR amplification for the indicated number of cycles. (B) ARE activation by STR8. HT22 cells transfected with ARE(GST-Ya)-luciferase assay vector were treated with vehicle or STR8 5 μM for 24 h and then subjected to the luciferase assay. (C) Increase in GSH + GSSH caused by STR8. The HT22 cells treated with vehicle or 5 μM STR8 for 20 h were lysed, and GSH + GSSG levels were then measured. Value are means ± S.D ($n = 4$). *Significantly differently ($p < 0.01$) from control cells by ANOVA.

electron acceptor, such as oxygen or oxygen radicals [38]. Thus, it is likely that this process is enhanced under oxidative stress [38]. Moreover, it would be expected that the more severe the oxidative stress, the more effective the conversion of a PED to its active electrophile [38,39]. Further, this chemical reaction appears to be a very slow process, in some cases requiring over 10 h for completion [9]. This slow process makes PEDs distinctive from other electrophilic compounds. Slow conversion of PEDs into their pharmacologically active species may reduce the likelihood of overwhelming cellular pathways, possibly contributing to their ultimate tolerability [9]. We and others compared equimolar concentrations of three hydroquinone isomers (*ortho*-, *meta*-, and *para*-) in terms of their protective effects, ARE activation, S-alkylation, and ability to react with GSH [11,38]. These studies showed the *para*-isomer to offer the highest degree of neuroprotection [11,38]. In several neurodegenerative diseases, oxidative stress plays a critical role in disease progression. By using PEDs, such stress could be harnessed to activate pro-electrophilic compounds via their oxidation at the target site to provide neuroprotection where it is needed [12,38]. This approach using CA [9], D1 [19] or STR8 represents a novel strategy against neurodegenerative disorders, one by which pro-electrophilic drugs may be activated via pathological activity. For drug development, this feature would be highly critical [39]. Lipton [39] designated such compounds as "pathologically-activated therapeutics (PATs)", and thus CA, D1, and STR8 may satisfy these criteria of PATs [9,19,39].

In conclusion, this study has generated an effective molecular probe, i.e., another *para*-hydroquinone type electrophile, that can be used to evaluate the features of PATs [1,39].

Acknowledgments

The authors thank Dr. Larry D. Frye for editorial help with the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 22500282; 2011–2013) from JSPS. This work was further supported by NIH CA36622 (C.M.I.).

References

[1] T. Satoh, S.A. Lipton, Redox regulation of neuronal survival by electrophilic compounds, Trends Neurosci. 30 (2007) 37–45.
[2] H. Wooyoung, S.G. Nathnael, Small molecule modulators of antioxidant response pathway, Curr. Opin. Chem. Biol. 15 (2011) 162–173.
[3] M.P. Mattson, A. Cheng, Neurohormetic phytochemicals: low-dose toxins that induce adaptive neuronal stress response, Trends Neurosci. 29 (2006) 632–639.
[4] T. Nakamura, S.A. Lipton, Cell death: protein misfolding and neurodegenerative diseases, Apoptosis 14 (2009) 455–468.
[5] A.L. Groeger, B.A. Freeman, Signaling actions of electrophiles: anti-inflammatory therapeutic candidates, Mol. Interv. 10 (2010) 39–50.
[6] T. Satoh, S. Okamoto, J. Cui, Y. Watanabe, K. Furuta, M. Suzuki, K. Tohyama, S.A. Lipton, Activation of the Keap1/Nrf2 pathway for neuroprotection by electrophilic phase II inducers, Proc. Natl. Acad. Sci. USA 103 (2006) 768–773.
[7] P. Talalay, Chemoprotection against cancer by induction of phase 2 enzymes, BioFactors 12 (2000) 5–11.
[8] K. Itoh, K.I. Tong, M. Yamamoto, Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles, Free Radic. Biol. Med. 36 (2004) 1208–1213.
[9] T. Satoh, K. Kosaka, K. Itoh, A. Kobayashi, M. Yamamoto, Y. Shimojo, C. Kitajima, J. Cui, J. Kamins, S. Okamoto, T. Shirasawa, S.A. Lipton, Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of specific cysteines, J. Neurochem. 104 (2008) 1116–1131.
[10] T. Satoh, M. Izumi, Y. Inukai, Y. Tsutumi, N. Nakayama, K. Kosaka, C. Kitajima, K. Itoh, T. Yokoi, T. Shirasawa, Carnosic acid protects neuronal HT22 cells through activation of the antioxidant-responsive element in free carboxylic

acid- and catechol hydroxyl moieties-dependent manners, Neurosci. Lett. 434 (2008) 260–265.
[11] T. Satoh, S. Saitoh, H. Hosaka, K. Kosaka, Simple ortho- and para-hydroquinones as neuroprotective compounds against oxidative stress associated with a specific transcriptional activation, Biochem. Biophys. Res. Comm. 379 (2009) 537–541.
[12] T. Satoh, N. Harada, T. Hosoya, K. Tohyama, M. Yamamoto, K. Itoh, Keap1/Nrf2 system regulates neuronal survival as revealed through study of keap1 gene knockout mice, Biochem. Biophys. Res. Comm. 380 (2009) 298–302.
[13] M.N. Mandel, J.M. Patlolla, L. Zheng, M.P. Agbaga, J.T. Tran, L. Wicker, A. Kasus-Jacobi, M.H. Elliott, C.V. Rao, R.E. Anderson, Curcumin protects retinal cells from light-and oxidant stress-induced cell death, Free Radic. Biol. Med. 46 (2009) 672–679.
[14] H. Jiang, X. Tian, Y. Guo, W. Duan, H. Bu, C. Li, Activation of nuclear factor erythroid 2-related factor 2 cytoprotective signaling by curcumin protect primary spinal cord astrocytes against oxidative toxicity, Biol. Pharm. Bull. 34 (2011) 1194–1197.
[15] H.M. Park, J.A. Kim, M.K. Kwak, Protection against amyloid beta cytotoxicity by sulforaphane: role of the proteasome, Arch. Pharm. Res. 32 (2009) 109–115.
[16] Z.A. Shah, R.C. Li, A.S. Ahmad, T.W. Kensler, M. Yamamoto, S. Biswal, S. Doré, The flavanol (−)-epicatechin prevents stroke damage through the Nrf2/HO1 pathway, J. Cereb. Blood Flow Metab. 30 (2010) 1951–1961.
[17] M.J. Calkins, J.A. Townsend, D.A. Johnson, J.A. Johnson, Cystamine protects from 3-nitropropionic acid lesioning via induction of nf-e2 related factor 2 mediated transcription, Exp. Neurol. 224 (2010) 307–317.
[18] T.G. Son, S. Camandola, T.V. Arumugam, R.G. Cutler, R.S. Telljohann, M.R. Mughal, T.A. Moore, W. Luo, Q.S. Yu, D.A. Johnson, J.A. Johnson, N.H. Greig, M.P. Mattson, Plumbagin, a novel Nrf2/ARE activator, protects against cerebral ischemia, J. Neurochem. 112 (2010) 1316–1326.
[19] T. Satoh, R. Rezaie, M. Seki, C.R. Sunico, T. Tabuchi, T. Kitagawa, Y. Yanagitai, M. Senzaki, C. Kosegawa, T. Taira, S.R. McKercher, J.K. Hoffman, G.P. Roth, S.A. Lipton, Dual neuroprotective pathways of a pro-electrophilic compound via HSF-1-activated heat shock proteins and Nrf2-activated phase 2 antioxidant response enzymes, J. Neurochem. 119 (2011) 569–578.
[20] A.D. Kraft, D.A. Johnson, J.A. Johnson, Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult [OK], J. Neurosci. 24 (2004) 1101–1112.
[21] T. Satoh, K. Furuta, K. Tomokiyo, D. Nakatsuka, M. Miura, M. Hatanaka, K. Ikuta, M. Suzuki, Y. Watanabe, Facilitatory roles of novel compounds designed from cyclopentenone prostaglandins on the neurite outgrowth-promoting activities of NGF, J. Neurochem. 75 (2000) 1092–1102.
[22] T. Satoh, K. Furuta, K. Tomokiyo, S. Namura, D. Nakatsuka, Y. Sugie, Y. Ishikawa, H. Hatanaka, M. Suzuki, Y. Watanabe, Neurotrophic actions of novel compounds designed from cyclopentenone prostaglandins, J. Neurochem. 77 (2001) 50–62.
[23] T. Satoh, M. Baba, D. Nakatsuka, Y. Ishikawa, H. Aburatani, K. Furuta, T. Ishikawa, H. Hatanaka, M. Suzuki, Y. Watanabe, Role of heme oxygenase-1 protein in the neuroprotective effects by cyclopentenone prostaglandin derivatives as a sustained phase of neuronal survival promoting mechanism under oxidative stress, Eur. J. NeuroSci. 17 (2003) 2249–2255.
[24] M.K. Kwak, K. Itoh, M. Yamamoto, T.R. Sutter, T.W. Kensler, Role of transcription factor Nrf2 in the induction of hepatic phase 2 and antioxidative enzymes in vivo by the cancer chemoprotective agent, 3H-1,2-dimethiole-3-thione, Mol. Med. 7 (2001) 135–145.
[25] J.C. Braekman, D. Daloze, G. Hulot, B. Tursch, J.P. Declercq, G. Germain, M. Van Meerssche, Chemical studies of marine invertebrates. XXXVII. Three novel meroditerpenoids from the sponge *Strongylophora durissima*, Bull. Soc. Chim. Belg. 87 (1978) 917–926.
[26] J. Salvá, D.J. Faulkner, Metabolites of the sponge *Strongylophora durissima* from Maricaban Island, Philippines, J. Org. Chem. 55 (1990) 1941–1943.
[27] M. Balbin-Oliveros, R.A. Edrada, P. Proksch, V. Wray, L. Witte, R.W.M. Van Soest, A new meroditerpenoid dimer from an undescribed Philippine marine sponge of the genus *Strongylophora*, J. Nat. Prod. 61 (1998) 948–952.
[28] K. Warabi, B.O. Patrick, P. Austin, C.D. Roskelley, M. Roberge, R.J. Andersen, Strongylophorine-26, an inhibitor of cancer cell invasion: SAR revealed by synthesis of analogues, J. Nat. Prod. 70 (2007) 736–740.
[29] H. Liu, M. Namikoshi, K. Akano, H. Kobayashi, H. Nagai, X. Yao, Seven new meroditerpenoids, from the marine sponge *Strongylophora strongylata*, that inhibited the maturation of starfish oocytes, J. Asian Nat. Prod. Res. 7 (2005) 661–670.
[30] L.M. McHardy, K. Warabi, R.J. Andersen, C.D. Roskelley, M. Roberge, Strongylophorine-26, a Rho-dependent inhibitor of tumor cell invasion that reduces actin stress fibers and induces nonpolarized lamellipodial extensions, Mol. Cancer Ther. 4 (2005) 772–778.
[31] K. Warabi, L.M. McHardy, L. Matainaho, R. Van Soest, C.D. Roskelley, M. Roberge, R.J. Andersen, Strongylophorine-26, a new meroditerpenoid isolated from the marine sponge *Petrosia* (*Strongylophora*) *corticata* that exhibits anti-invasion activity, J. Nat. Prod. 67 (2004) 1387–1389.
[32] Y.C. Shen, C.V. Prakash, Two new acetylenic derivatives and a new meroditerpenoid from a Taiwanese marine sponge *Strongylophora durissima*, J. Nat. Prod. 63 (2000) 1686–1688.
[33] K.A. Mohammed, R.C. Jadulco, T.S. Bugni, M.K. Harper, M. Sturdy, C.M. Ireland, Strongylophorines: Natural product inhibitors of hypoxia-inducible factor-1 transcriptional pathway, J. Med. Chem. 51 (2008) 1402–1405.
[34] T. Satoh, K. Ishige, Y. Sagara, Protective effects on neuronal cells by ebselen against oxidative stress at multiple steps, Neurosci. Lett. 16 (2004) 1–5.
[35] T. Satoh, M. Izumi, Neuroprotective effects of phenylenediamine derivatives independent of an antioxidant pathway in neuronal HT22 cells, Neurosci. Lett. 418 (2007) 102–105.
[36] T. Takahashi, T. Tabuchi, Y. Tamaki, K. Kosaka, Y. Takikawa, T. Satoh, Carnosic acid and carnosol inhibit adipocyte differentiation in mouse 3T3-L1 cells through induction of phase 2 enzymes and activation of glutathione metabolism, Biochem. Biophys. Res. Commun. 382 (2009) 549–554.
[37] Y. Tamaki, T. Tabuchi, T. Takahashi, K. Kosaka, T. Satoh, Activated glutathione metabolism participates in protective effects of carnosic acid against oxidative stress in neuronal HT22 cells, Planta Med. 76 (2010) 683–688.
[38] X.J. Wang, J.D. Hayes, L.J. Higgins, C.R. Wolf, A.T. Dinkova-Kostova, Activation of the NRF2 signaling pathway by copper-mediated redox cycling of *para*- and *ortho*-hydroquinones, Chem. Biol. 17 (2010) 75–85.
[39] S.A. Lipton, Pathologically-activated therapeutics, Nat. Rev. Neurosci. 8 (2007) 803–808.