Bioorganic & Medicinal Chemistry Letters 25 (2015) 62–66

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The synthesis of sulforaphane analogues and their protection effect against cisplatin induced cytotoxicity in kidney cells

Taejung Kim [a], Young-Joo Kim [a], Im-Ho Han [a], Dahae Lee [a,b], Jungyeob Ham [a], Ki Sung Kang [b,*], Jae Wook Lee [a,c,*]

[a] Natural Product Research Center, Korea Institute of Science and Technology, Gangneung 210-340, Republic of Korea
[b] College of Korean Medicine, Gachon University, Seongnam 461-701, Republic of Korea
[c] Department of Biological Chemistry, University of Science and Technology, Daejeon 305-350, Republic of Korea

ARTICLE INFO

Article history:
Received 14 July 2014
Revised 17 October 2014
Accepted 5 November 2014
Available online 11 November 2014

Keywords:
Sulforaphane
Cisplatin induced kidney disease
Kidney protection
Reactive oxygen species
Isothiocyanate

ABSTRACT

A series of sulforaphane analogues were synthesized with various amines by treatment of carbon disulfide followed by Boc_2O and DMAP. These synthesized sulforaphane analogues were tested on cisplatin treated cultured LLC-PK1 kidney cell line. Among these analogues, several compounds including SF5 show a potent effect on kidney cell protection assay at the concentration of 2.5 μM. Further studies with compound SF5 revealed that the kidney cell protection effect was related by inhibiting the apoptosis pathway through JNK-p53-caspase apoptotic cascade. Compound SF5 may be considered as a promising candidate for the development of new kidney protection agent against drug induced acute kidney disease.

The increasing incidence of drug-induce acute kidney disease has led to a societal interest in development of new therapeutic strategies to overcome this condition. Kidney disease related to side effect of drug is considered as a limitation to the effective treatment for many patients. In particular, cisplatin is a powerful anticancer chemotherapeutic agent which can dramatically improve the survival rate of cancer patients. Although cisplatin has been used as mainstay of cancer therapy for many years, severe side effects of cisplatin, particularly nephrotoxicity, limits its wide spread use for cancer patients.[1] For many years, various approaches have been taken to curtail the side effect of cisplatin. However, nephrotoxicity still remains a major concern associated with cisplatin based cancer therapy. The mechanism of cisplatin nephrotoxicity is related to various cell signal pathways including cell death promoting apoptosis,[2] MAPK,[3] p53,[4] and reactive oxygen species (ROS)[5] or cytoprotective p21.[6] Cisplatin also induces TNF-α production in tubular cells that triggers an inflammatory response[7] and cell injury and death. There are several pharmacological approaches to address the problem such as using CDK, p53, MAPK inhibitors, antioxidant, and anti-inflammation to protect kidney cell during cisplatin treatment. Yet, most studies of the effects and treatments have been conducted only in cultured cells, mice, or rats.[8] Therefore, there is no verifiable treatment for patients to prevent cisplatin induced nephrotoxicity except for kidney dialysis.

Recently, an appropriate treatment to reduce the risk of these kidney failures is offered by the identification of chemoprotective agents from natural sources. Chemoprotection by natural products increases the cellular major defense mechanism by cells against endogenous and environmental attack resulting from ROS and electrophilic species.[9] The natural chemoprotective agent sulforaphane, a naturally occurring isothiocyanate derived from glucosinolate, is present in cruciferous vegetable such as broccoli, brussels sprouts or cabbages.[5] Sulforaphane has gained attention as a chemoprotective agent which can enhance the expression of classical phase 2 antioxidant enzymes and cytoprotective proteins including NAD(P)H:quinone oxidoreductase (NQO1),[10] thioredoxin, superoxide dismutase (SOD), heme oxidase-1 (HO-1), glutathione peroxidase and glutathione *S*-transferase (GST).[11] Extensive research in vitro and in vivo has already demonstrated the potential of sulforaphane to protect or reduce against cancer,[12] skin damage,[13] and renal damage[14] resulting from reduce oxidative stress,[11a,12a] and UV irradiation.[15]

In our prior study, sulforaphane is identified as a potent Renoprotective agent for cultured LLC-PK1 cells under cisplatin induced oxidative stress. To discover more potent sulforaphane analogues, we conducted structure activity relationship (SAR) studies by considering on the characteristics of side chain such

* Corresponding authors. Tel.: +82 31 750 5402; fax: +82 31 750 5416 (K.S.K.); tel.: +82 33 650 3514; fax: +82 33 650 3529 (J.W.L.).
E-mail addresses: kkang@gachon.ac.kr (K.S. Kang), jwlee5@kist.re.kr (J.W. Lee).

http://dx.doi.org/10.1016/j.bmcl.2014.11.014
0960-894X/

as lipophilicity, chain length, and steric factor. In the previous studies, the characteristic of side chain of isothiocyanate (ITC) influence not only lipophilicity, but also the reactivity of the isothiocyanate moiety.[16] Therefore, we designed 16 aliphatic ITCs or aromatic ITCs. To generate 16 sulforaphane analogues, we followed the previously reported procedure describe below (Fig. 1).[16a]

At first, various sulforaphane analogues were prepared from various aliphatic and aromatic amines (**1**) reacted with carbon disulfide under basic condition followed by the addition of di-*tert*-butyl dicarbonate (Boc_2O) and 4-dimethylaminopyridine (DMAP). These synthesized aliphatic and aromatic sulforaphane analogues were purified by silica gel column chromatography. The chemical structure of synthesized aliphatic and aromatic sulforaphane analogues were characterized by ^1H NMR, ^{13}C NMR, and LC–MS.

To evaluate molecules that enhance cell survival, we utilized a previously developed cell-based assay with UV–vis readout. This cell-based assay format is commonly used for the identification of molecules that enhance cell-survival. In LLC-PK1 cell-based assay condition, cisplatin (25 μM) is treated to generate oxidative stress on LLC-PK1 cells. The oxidative stress activates cell death signal pathway, which results in 60% of cell survival. Using this condition, 16 sulforaphane analogues were tested in a dose response format in the LLC-PK1 cell-based assay. We then evaluated cell survival rate by WST-1 assay using cell counting kit (CCK-8 kit).

We defined the potency of the compounds by calculating the concentration which resulted in more than 60% cell survival. Sulforaphane (10 μM) increase more than 77% of cell survival with minimal effect on cell toxicity in LLC-PK1 cells. Most of sulforaphane analogues showed similar or better potency compared with sulforaphane in cell survival assay. Effects of sulforaphane analogues were tested up to 10 μM, whereas toxic analogues at high doses were tested up to 2.5 μM. SF6, SF8 and SF13 show more than 80% of cell survival at 2.5 μM concentration, which is more potent than sulforaphane. SF5 show strong cell survival effect of kidney epithelial cells (93% at a concentration of 2.5 μM). Analogues SF12 also show 90% cell survival at a concentration of 10 μM, which is similar or slightly better than sulforaphane. 1-Phenylethane group (SF7) and methoxyphenyl group (SF9) led to a decrease in activity due to their steric effect. In contrast, chlorobenzyl (SF6), hydroxyphenethyl (SF8), and diphenethyl group (SF5) appear to be tolerated and increase activity. Based on the SAR, we selected SF5[17] for further study considering a cellular potency (Fig. 2).

To confirm the potency of SF5 to cell survival effect, we observed the image of 25 μM of cisplatin treated LLC-PK1 cell following treatment with different concentration of SF5. In the cell image data, cisplatin treated LLC-PK1 cell showed growth arrest or cell death. However, LLC-PK1 cells treated with different concentration of SF5 showed improved cell survival above 1 μM concentration of SF5. We confirmed that SF5 has a cytoprotective effect to cisplatin treated LLC-PK1 cells (Fig. 3).

To further evaluate the cytoprotective effects of SF5, we performed the image based cytometric assay.[18] Figure 4 shows the effects of SF5 on apoptosis in LLC-PK1 cells. As shown in Figure 4A, the number of dead and apoptotic cells which were stained with red and green color were increased after cisplatin treatment, whereas it was decreased by the co-treatment of SF5. The elevated percentage of apoptotic cells by cisplatin treatment in LLC-PK1 cells was markedly decreased by the co-treatment of SF5 (Fig. 4B).

To investigate the mechanism of protection effect of SF5 in LLC-PK1 cells, we analyzed expression of JNK, p53, and caspase-3 by western blot analysis. In western blot analysis, cisplatin induced high levels of phosphorylated JNK (phospho-JNK) and cleavage of caspase-3, which activated apoptosis of LLC-PK1 cells. While different concentration of SF5 shows reduction of phospho-JNK, p53, and cleaved caspase-3 protein levels.

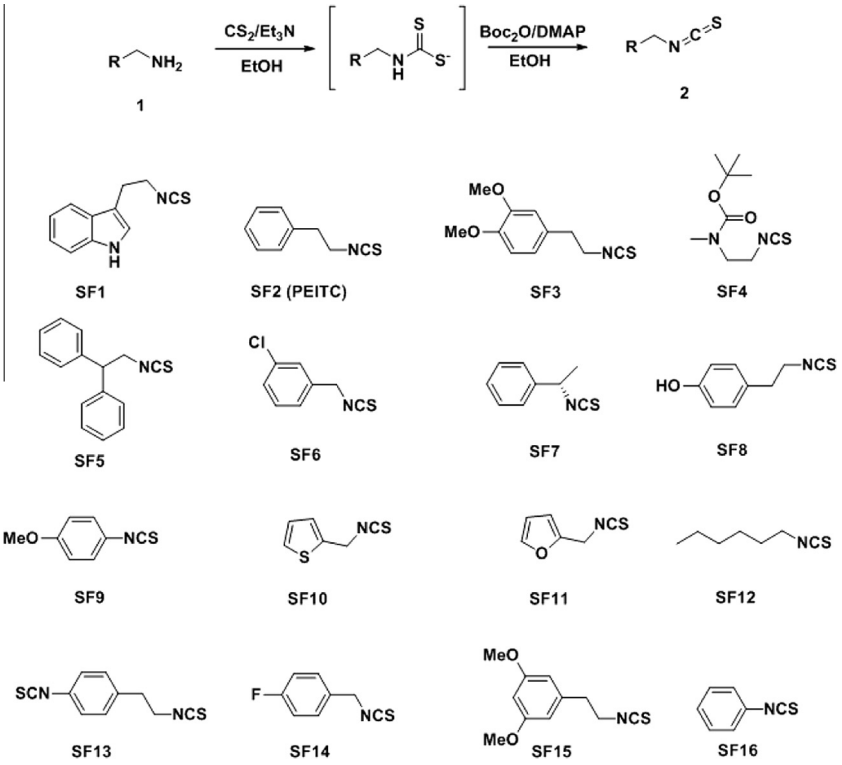

Figure 1. The synthetic scheme of sulforaphane and chemical structure of sulforaphane analogues.

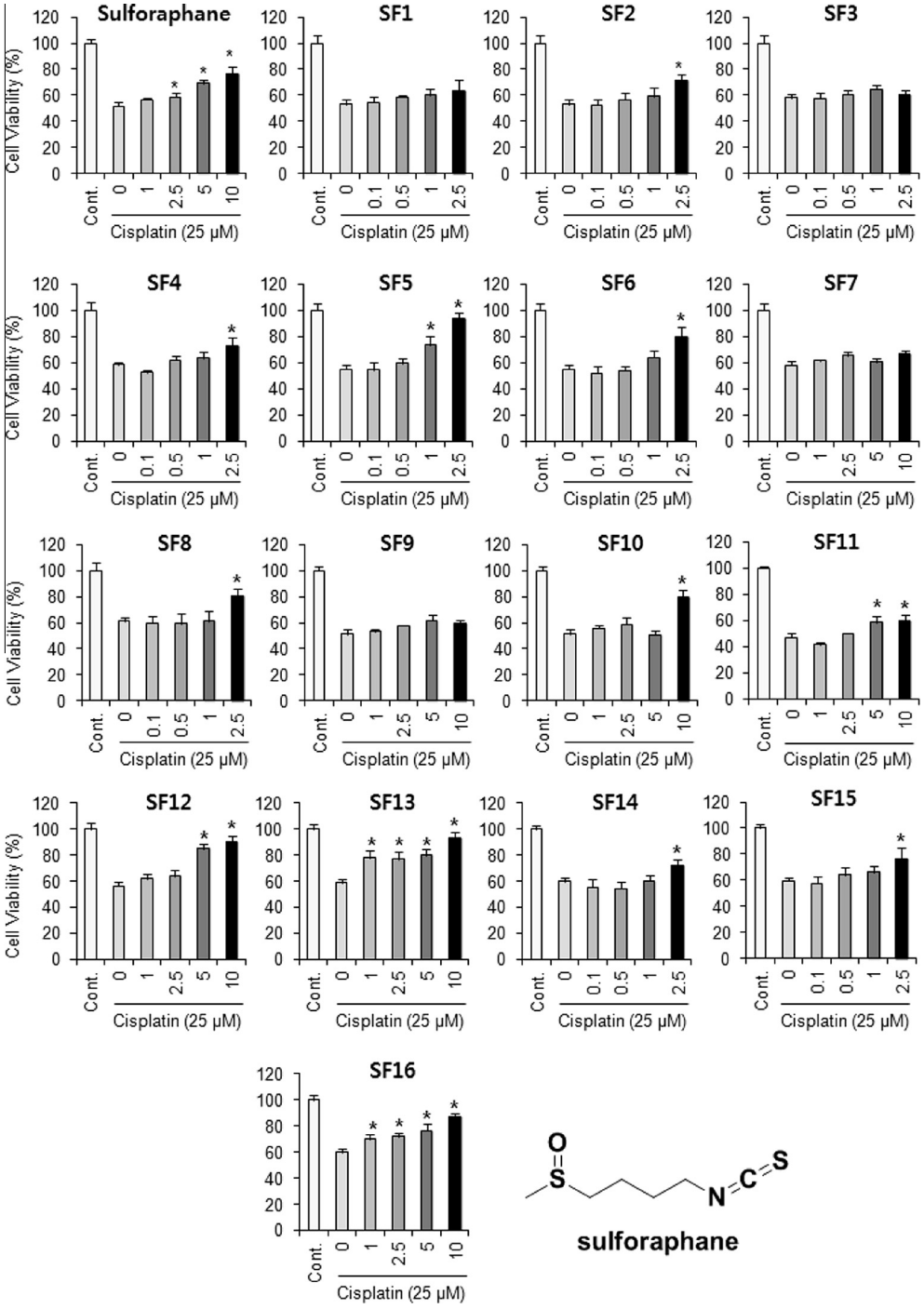

Figure 2. LLC-PK1 protection assay of sulforaphane and sulforaphane analogues under the treatment of 25 μM of cisplatin. $^{*}p < 0.05$ compared to the cisplatin-treated value.

Earlier studies showed that the cisplatin-induced damages in LLC-PK1 cells was largely attenuated by the antioxidant *N*-acetylcysteine, while apoptosis was prevented by the specific inhibitors for caspases-2, -8, and -3 and a p53 inhibitor pifithrin-alpha but not by the p38 MAPK inhibitor SB203580.[19] JNK is another important MAPK which is involved in the cytoprotective effect of selenium on cadmium-induced oxidative renal cell damage.[20]

In the present study, we sought to determine the role of the JNK-p53-caspase-3 apoptotic cascade in mediating the protective effect of SF5 against oxidative cytotoxicity in renal cells. As shown in Figure 5, phosphorylation of JNK was observed at 24 h after cisplatin treatment, and it was decreased after treatment with SF5 in a dose dependent manner. In Figure 5, protein level of p53 was also markedly increased after treatment of cisplatin, whereas the elevated level of p53 was reduced significantly by a high concentration (2.5 μM) of SF5. Similarly, the elevated protein level of cleaved caspase-3 also decreased after treatment with SF5 in a dose dependent manner.

In summary, the results of our present study show that the JNK-p53-caspase-3 signaling cascade plays a critical role in mediating the protective effect of SF5 against oxidative cytotoxicity in cultured LLC-PK1 cells.

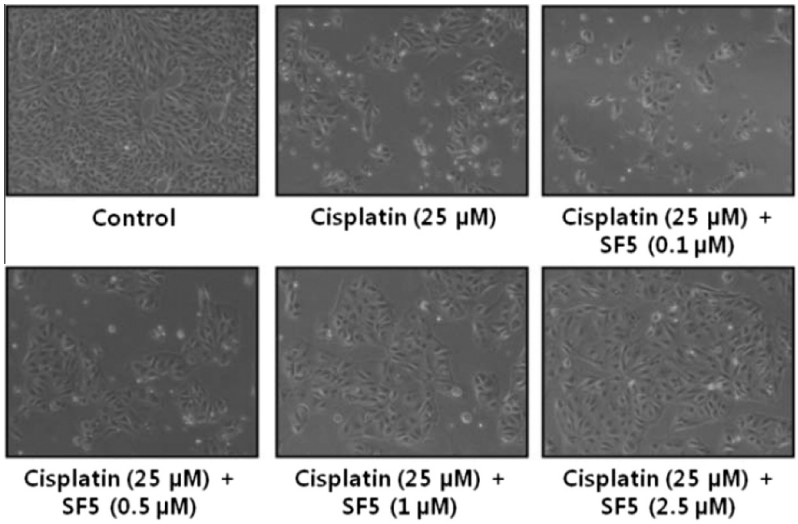

Figure 3. The image of nephroprotection effect with SF5 under the treatment of 25 μM of cisplatin.

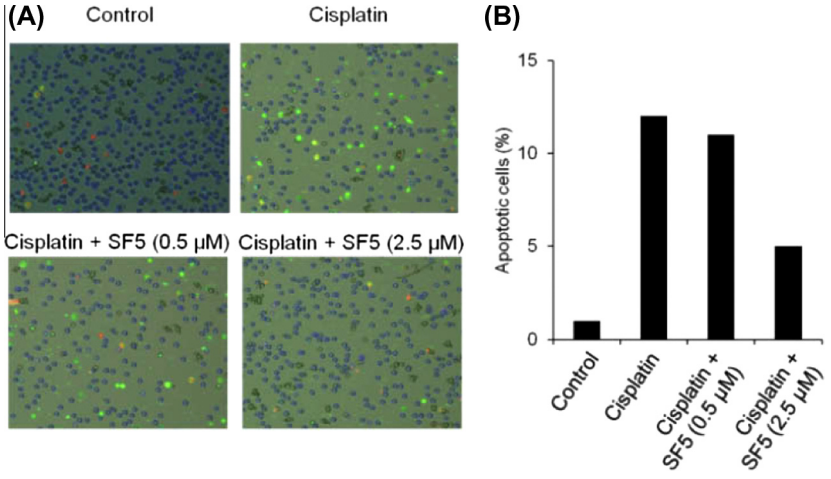

Figure 4. Effects of SF5 on apoptosis in LLC-PK1 cells. (A) Representative images for apoptosis detection. (B) Percentage of Annexin V-positive-stained apoptotic cells. Dead cell and apoptotic cells were stained with red and green color. Apoptosis was determined by a Tali image-based cytometer.

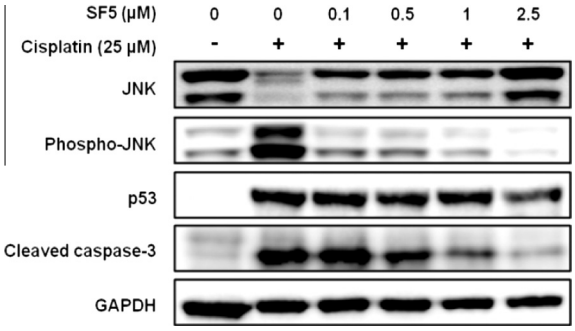

Figure 5. The mechanism of nephroprotection effect of SF5 on cisplatin-induced LLC-PK1 cell damage (Involvement of JNK-p53-caspase-3 signaling pathway in the protective effect of SF5 against oxidative cytotoxicity in cultured LLC-PK1 cells).

Acknowledgment

This work was supported by the Korea Institute of Science and Technology Institutional Program (2Z04211).

Supplementary data

Supplementary data (experimental procedures, characterization of final compounds and biological assay protocols) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.014.

References and notes

1. (a) Rosenberg, B.; Vancamp, L.; Krigas, T. *Nature* **1965**, *205*, 698; (b) Siddik, Z. H. *Oncogene* **2003**, *22*, 7265; (c) Wang, D.; Lippard, S. J. *Nat. Rev. Drug Discov.* **2005**, *4*, 307; (d) Cohen, S. M.; Lippard, S. J. *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *67*, 93; (e) Arany, I.; Safirstein, R. L. *Semin. Nephrol.* **2003**, *23*, 460.
2. (a) Green, D. R.; Reed, J. C. *Science* **1998**, *281*, 1309; (b) Lee, R. H.; Song, J. M.; Park, M. Y., et al. *Biochem. Pharmacol.* **2001**, *62*, 1013; (c) Park, M. S.; De Leon, M.; Devarajan, P. *J. Am. Soc. Nephrol.* **2002**, *13*, 858; (d) Jiang, M.; Wei, Q.; Wang, J., et al. *Oncogene* **2006**, *25*, 4056; (e) Jiang, M.; Pabla, N.; Murphy, R. F., et al. *J. Biol. Chem.* **2007**, *282*, 2636.
3. (a) Nowak, G. *J. Biol. Chem.* **2002**, *277*, 43377; (b) Arany, I.; Megyesi, J. K.; Kaneto, H., et al. *Am. J. Physiol. Ren. Physiol.* **2004**, *287*, F543; (c) Kim, Y. K.; Kim, H. J.; Kwon, C. H., et al. *J. Appl. Toxicol.* **2005**, *25*, 374; (d) Jo, S. K.; Cho, W. Y.; Sung, S. A., et al. *Kidney Int.* **2005**, *67*, 458; (e) Mishima, K.; Baba, A.; Matsuo, M., et al. *Free Radic. Biol. Med.* **2006**, *40*, 1564; (f) Francescato, H. D.; Costa, R. S.; Junior, F. B., et al. *Nephrol. Dial. Transpl.* **2007**, *22*, 2138.
4. (a) Wei, Q.; Dong, G.; Yang, T., et al. *Am. J. Physiol. Ren. Physiol.* **2007**, *293*, F1282; (b) Chipuk, J. E.; Kuwana, T.; Bouchier-Hayes, L., et al. *Science* **2004**, *303*, 1010;

(c) Zhou, H.; Kato, A.; Yasuda, H., et al. *Toxicol. Appl. Pharmacol.* **2004**, *200*, 111; (d) Wang, J.; Pabla, N.; Wang, C. Y., et al. *Am. J. Physiol. Ren. Physiol.* **2006**, *291*, F1300; (e) Pabla, N.; Huang, S.; Mi, Q. S., et al. *J. Biol. Chem.* **2008**, *283*, 6572.
5. Berndtsson, M.; Hägg, M.; Panaretakis, T.; Havelka, A. M.; Shoshan, M. C.; Linder, S. *Int. J. Cancer* **2007**, *120*, 175.
6. (a) Megyesi, J.; Safirstein, R. L.; Price, P. M. *J. Clim. Invest.* **1998**, *101*, 777; (b) Megyesi, J.; Udvarhelyi, N.; Safirstein, R. L., et al. *Am. J. Physiol. Ren. Physiol.* **1996**, *271*, F1211; (c) Price, P. M.; Safirstein, R. L.; Megyesi, J. *Am. J. Physiol. Ren. Physiol.* **2004**, *286*, F378; (d) Yu, F.; Megyesi, J.; Safirstein, R. L., et al. *Am. J. Physiol. Ren. Physiol.* **2005**, *289*, F514; (e) Price, P. M.; Yu, F.; Kaldis, P., et al. *J. Am. Soc. Nephrol.* **2006**, *17*, 2434; (f) Yu, F.; Megyesi, J.; Safirstein, R. L., et al. *Am. J. Physiol. Ren. Physiol.* **2007**, *293*, F52.
7. (a) Bonventre, J. V.; Weinberg, J. M. *J. Am. Soc. Nephrol.* **2003**, *14*, 2199; (b) Devarajan, P. *J. Am. Soc. Nephrol.* **2006**, *17*, 1503; (c) Lu, C. Y.; Hartono, J.; Senitko, M., et al. *Curr. Opin. Nephrol. Hypertens.* **2007**, *16*, 83.
8. Pabla, N.; Dong, Z. *Inter. Soc. Nephrol.* **2008**, *73*, 994.
9. (a) Zhang, Y.; Talalay, P.; Cho, C. G.; Posner, G. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399; (b) Talalay, P.; Fahey, J. W. *J. Nutr.* **2001**, *131*, 3027S; (c) Surh, Y. J. *Nat. Rev. Cancer* **2003**, *3*, 768.
10. Juge, N.; Mithen, R. F.; Traka, M. *Cell. Mol. Life Sci.* **2007**, *64*, 1105.
11. (a) Kensler, T. W.; Wakabayashi, N.; Biswal, S. *Ann. Rev. Pharmacol. Toxicol.* **2007**, *47*, 89; (b) Wang, X.; Tomso, D. J.; Chorley, B. N.; Cho, H. Y.; Cheung, V. G.; Kleeberger, S. R.; Bell, D. A. *Hum. Mol. Genet.* **2007**, *16*, 1188.
12. (a) Zhang, Y. et al *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3147; (b) Cornblatt, B. S. et al *Carcinogenesis* **2007**, *28*, 1485; (c) Conway, C. C. et al *Cancer Res.* **2005**, *65*, 8548.
13. (a) Dinkova-Kostova, A. T. et al *Cancer Lett.* **2006**, *240*, 243; (b) Dinkova-Kostava, A. T. et al *Cancer Epidemiol. Biomarkers Preve.* **2007**, *16*, 847.
14. Kong, L. et al *J. Neurochem.* **2007**, *101*, 1041.
15. (a) Talalay, P. et al *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 17500; (b) Kong, L. et al *J. Neurochem.* **2007**, *101*, 1041.
16. (a) Munch, H.; Hansen, J. S.; Pittelkow, M.; Christensen, J. B.; Boas, U. *Tetrahedron Lett.* **2008**, *49*, 3117; (b) Melchini, A.; Richard, P. W.; Mithen, R. F.; Traka, M. H. *J. Med. Chem.* **2012**, *55*, 9682; (c) Goosen, T. C.; Kent, U. M.; Brand, L.; Hollenberg, P. E. *Chem. Res. Toxicol.* **2000**, *13*, 1349; (d) Moreno, R. L.; Goosen, T.; Kent, U. M.; Chung, F. L.; Hollenberg, P. F. *Arch. Biochem. Biophys.* **2001**, *391*, 99.
17. *The synthesis of (2-isothiocyanatoethane-1,1-diyl)dibenzene (**SF5**)*: Absolute ethanol (1 mL) was added to the 2,2-diphenylethylamine (98.6 mg, 0.5 mmol), carbon disulfide (114.2 mg, 1.5 mmol) and Et_3N (50.6 mg, 0.5 mmol) were added while stirring, resulting in the precipitation of the dithiocarbamate. The reaction mixture was stirred for 30 min at room temperature and then cooled on an ice bath. Boc_2O (109.1 mg, 0.5 mmol), dissolved in absolute ethanol (0.5 mL), was added followed by the immediate addition of a catalytic amount of DMAP. The reaction mixture was kept in the ice bath for 5 min, and was then allowed to reach room temperature. After evolution of gas from the reaction mixture had ceased, the reaction mixture was stirred for a further 5 min at room temperature and evaporated thoroughly in vacuo. The crude product was purified by column chromatography to give pure product (115.8 mg) in 97% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.19 (m, 10H), 4.34 (t, 1H, J = 7.2 Hz), 4.04 (d, 2H, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.3, 131.9, 128.9, 128.0, 127.4, 51.4, 49.4. ESI MS: m/z calcd for $C_{15}H_{13}NS$ $[M+H]^+$ 239.08, found 240.10.
18. *Image-based cytometric assay*. The LLC-PK1 cells were used for an image-based apoptosis assay system. All assays were conducted in accordance with the guideline for the Tali image-based cytometer (Invitrogen, CA, USA). Cells were treated with 25 μM of compounds #6 and #14 for 24 h at 37 °C and 5% CO_2. Cells were harvested by trypsin treatment using TrypLE reagent and stained using the Tali apoptosis kit. The sample was divided and analyzed independently on both the Tali image cytometer and a flow cytometer following the manufacturer's recommended protocol. To determine the portion of the population that had become apoptotic, cells were stained with the annexin V-Alexa Fluor 488 conjugate. Propidium iodide (PI) was used to differentiate the cells that were dead (annexin V positive/PI positive or annexin V negative/PI positive) from those that were apoptotic (annexin V positive/PI negative). The percentages of the population reported as viable, apoptotic, and dead by the Tali cytometer were comparable with data from the same samples independently run on a flow cytometer.

19. Xiao, T.; Choudhary, S.; Zhang, W.; Ansari, N. H.; Salahudeen, A. *J. Toxicol. Environ. Health A* **2003**, *66*, 469.
20. Liu, Y.; Zhang, S. P.; Cai, Y. Q. *Toxicol. In Vitro* **2007**, *21*, 677.