Alkenyl group is responsible for the disruption of microtubule network formation in human colon cancer cell line HT-29 cells

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Alk(en)yl trisulfides (R-SSS-R′) are organosulfur compounds produced by crushed garlic and other Allium vegetables. We found that these compounds exhibit potent anticancer effects through the reaction with microtubules, causing cell cycle arrest. Nine alk(en)yl trisulfides including dimethyl trisulfide, diethyl trisulfide, dipropyl trisulfide (DPTS), dibutyl trisulfide, dipentenyl trisulfide, dipentenyldiallyl trisulfide and allyl methyl trisulfide were synthesized and added to cultures of HT-29 human colon cancer cells at a concentration of 10 μM. The trisulfides with alkylengroups such as DATS, but not those with alkyl groups, induced rapid microtubule disassembly at 30–60 min as well as cell cycle arrest during the mitotic phase approximately at 4 h after the treatment. Both DATS-induced microtubule disassembly and the cell cycle arrest were cancelled by the simultaneous treatment of the cancer cells with 2 mM L-cysteine, glutathione (GSH) or N-acetyl-L-cysteine. Reciprocally, L-buthionine-(S,R)-sulfoximine (500 μM), an inhibitor of GSH synthesis, enhanced the power of DATS in inducing the cell cycle arrest. These results indicate that alk(en)yl trisulfides react with sulfhydryl groups in cysteine residues of cellular proteins such as microtubule proteins. Thus, the present study provides evidence that trisulfides with alkylengroups have potent anticancer activities, at least in part, directed toward microtubules. These findings suggest that alkylengroup trisulfides and their structurally related compounds may provide novel and effective anticancer agents.

Materials and methods

Chemicals

Nine compounds of alk(en)yl trisulfides (dimethyl trisulfide, diethyl trisulfide, DPTS, dibutyl trisulfide, dipentenyl trisulfide, DATS, dibutenyldiallyl trisulfide, dipentenyldiallyl trisulfide and allyl methyl trisulfide) were synthesized by the method of Milligan et al. (15). Alk(en)yl trisulfides were purified by high-performance liquid chromatography (Alliance 2695 system, Waters Co., Milford, MA) on an Inertsil ODS-3 column (6 × 250 mm, GL Science, Tokyo, Japan). The molecular mass of the alk(en)yl trisulfides was determined by using an Agilent 6890/5973N GC/MSD System (Agilent Technologies, Santa Clara, CA). L-cysteine, GSH, N-acetyl-L-cysteine and BSO were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan).

Cell culture and treatment with alk(en)yl trisulfides

Human colon adenocarcinoma cell line HT-29, obtained from the American Type Culture Collection, Manassas, VA, was grown and maintained in McCoy’s 5A medium (Sigma–Aldrich Co., St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Biowest, Nuaille, France) at 37°C in 5% air and 5% CO2. The cells were precultured in 10% FBS containing McCoy’s 5A medium for 48 h. Alk(en)yl trisulfides were prepared in dimethyl sulfoxide solution at a concentration of 5 mM and then added to fresh medium to give

Introduction

Alk(en)yl trisulfides (R-SSS-R′) are organosulfur compounds produced by crushed garlic and other Allium vegetables. These compounds have been shown to exhibit a significant protective effect against cancer in experimental carcinogenesis models (1). Recent studies revealed that these compounds can suppress the proliferation of a variety of cancer cell lines and lead to apoptotic cell death (2–14). Alk(en)yl trisulfides (R-SSS-R′/C3/C2/C1) are organosulfur compounds produced by garlic, and contains three sulfur atoms with two allyl groups. We found that these compounds exhibit potent anticancer effects through the reaction with microtubules, causing cell cycle arrest. Nine alk(en)yl trisulfides including dimethyl trisulfide, diethyl trisulfide, dipropyl trisulfide (DPTS), dibutyl trisulfide, dipentenyl trisulfide, dipentenyldiallyl trisulfide and allyl methyl trisulfide were synthesized and added to cultures of HT-29 human colon cancer cells at a concentration of 10 μM. The trisulfides with alkylengroups such as DATS, but not those with alkyl groups, induced rapid microtubule disassembly at 30–60 min as well as cell cycle arrest during the mitotic phase approximately at 4 h after the treatment. Both DATS-induced microtubule disassembly and the cell cycle arrest were cancelled by the simultaneous treatment of the cancer cells with 2 mM L-cysteine, glutathione (GSH) or N-acetyl-L-cysteine. Reciprocally, L-buthionine-(S,R)-sulfoximine (500 μM), an inhibitor of GSH synthesis, enhanced the power of DATS in inducing the cell cycle arrest. These results indicate that alk(en)yl trisulfides react with sulfhydryl groups in cysteine residues of cellular proteins such as microtubule proteins. Thus, the present study provides evidence that trisulfides with alkylengroups have potent anticancer activities, at least in part, directed toward microtubules. These findings suggest that alkylengroup trisulfides and their structurally related compounds may provide novel and effective anticancer agents.

Abbreviations: BSO, L-buthionine-(S,R)-sulfoximine; DATS, diallyl trisulfide; DPTS, dipropyl trisulfide; FBS, fetal bovine serum; GSH, glutathione; PBS, phosphate-buffered saline.

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A concentration of 10 μM Dimethyl sulfoxide was also added to control culture medium to give a final concentration, 0.2%, which was equivalent to that of alk(eny)l trisulfides in the medium.

Indirect immunofluorescence microscopy of β-tubulin
Cells were inoculated on glass slides coated with type I collagen (Cellmatrix type I-C, Nitta Gelatin Inc., Osaka, Japan), precultured for 48 h and then incubated with a given alk(eny)l trisulfide (10 μM) for 30–120 min or 12 h. The glass slides were washed twice with phosphate-buffered saline (PBS), and the cells were fixed with acetone/methanol (1:1) for 2 min at room temperature. After having been washed with PBS, the fixed cells were incubated with mouse anti-β-tubulin monoclonal antibody (TUB2.1, Sigma–Aldrich Co.) for 30 min at room temperature, after which they were incubated with Alexa Fluor 488-labeled goat anti-mouse IgG antibody (Invitrogen Co., Carlsbad, CA) for 30 min. The nuclei were stained with propidium iodide (Sigma–Aldrich Co.). The specific fluorescence was observed with a confocal microscope (FLUOVIEW FV300; Olympus Co., Tokyo, Japan).

Cell cycle analysis
The cell cycle distribution of HT-29 cells was measured by flow cytometry. The cells were incubated with 10 μM alk(eny)l trisulfides as described above. After two washes with PBS, the cells were harvested by trypsinization (5 × 105 cells) and fixed overnight with ice-cold 70% ethanol at −20°C. They were then washed twice with PBS prior to being incubated with 500 μg/ml RNase A (Sigma–Aldrich Co.) and 25 μg/ml propidium iodide (Sigma–Aldrich Co.) at room temperature for 20 min. Then, the cell cycle was analyzed by using a flow cytometer, FACSCanto (BD Biosciences, San Jose, CA) and FlowJo software (Tree Star, Ashland, OR).

Tubulin polymerization assay
Tubulin was purified from pig brain by use of a phosphocellulose column and dissolved in 1,4-piperazinediethane-sulfonic acid buffer (1.5 mg tubulin/ml of 80 mM 1,4-piperazinediethane-sulfonic acid, pH 6.8, 1 mM MgCl2, 1 mM ethyleneglycol-bis(aminooxyether)tetraacetic acid, 10% glycerol and 1 mM guanosine-5′-triphosphate). The tubulin solution was then placed in a temperature-controlled water bath at 37°C for 10 min in the presence or absence of 1 μM DATS or DPTS. In order to initiate tubulin polymerization, the reaction mixture was warmed to 37°C. The tubulin polymerization was monitored by measuring the increase in the absorbance at 340 nm.

Cell growth inhibition assay
HT-29 cells were precultured for 48 h and then exposed to either DATS or DPTS for 16 h. The cell numbers were thereafter counted with a hemocytometer, and the growth inhibition rate was plotted to determine the IC50.

Results
Alk(eny)l trisulfides disturb microtubule network formation in HT-29 human colon cancer cells
We have shown previously that DATS treatment caused to disarrange the microtubule structures by acting as a microtubule-depolymerizing agent and to induce cell cycle arrest during the G2/M phase (10). In this study, to determine the effects of alkyl or alk(en)yl trisulfides on microtubule formation, we synthesized and purified nine kinds of alk(en)yl trisulfides (Figure 1). Figure 2 shows the effect of these trisulfides on microtubules in HT-29 human colon cancer cells. The normal microtubule distribution and its network formation were observed in the cytoplasm of untreated control HT-29 cells, as shown in Figure 2A. On the contrary, treatment of HT-29 cells with the series of alk(en)yl trisulfides caused clear disarrangement of the microtubular network, represented as a smeared image due to disassembly of the fibrous network of microtubules (Figure 2C, arrowheads). Such a change could be seen at 30 min (DATS and dibutyl trisulfide) or 120 min (diphenyl trisulfide). There was no obvious change in the microtubule network of the cells treated with the series of alkyl trisulfides (Figure 2B). Among these alkyl compounds, only DPTS gave an abnormal smeared image of the microtubule network at 120 min after the addition of the agent (Figure 2B, DPTS 120 min, arrowhead). Alkyl methyl trisulfide, an asymmetric alk(en)yl trisulfide, also gave similar results to those from DATS (Figure 2D, arrowhead). These disruptions of tubulin structure observed in the DPTS- or alkyl methyl trisulfide-treated HT-29 cells were transient and the normal tubulin structures were observed in the cells at 12 h after the treatment (data not shown).

Statistical analysis
In Figures 3A and 5B, one-way analysis of variance was employed and used to evaluate the difference among multiple groups. If significance was obtained between the groups, Dunnett’s multiple comparison test (Figure 3A) or Tukey–Kramer multiple comparison test (Figure 5B) was used to compare the means between specific groups. The differences were considered statistically significant at P < 0.01. Statistical analysis was performed using statistical package GraphPad Prism software (GraphPad Software, San Diego, CA).
growing naturally or asynchronously showed a typical cell cycle distribution, i.e. 41.7 ± 0.4% in G1 phase, 44.2 ± 0.7% in S phase and 13.2 ± 0.9% in G2/M phase. The presence of alkyl trisulfides did not significantly affect the cell cycle, which maintained the distribution of its G2/M phase with rates between 13 and 16% of all the cells. On the other hand, alkenyl trisulfides markedly increased the G2/M phase arrest as compared with vehicle-treated cells (P < 0.01). The trisulfide having both alkyl and alkenyl groups in the same molecule, i.e. allyl methyl trisulfide, did not change the G2/M phase cells percent. We next examined the time course of the changes in G2/M phase distribution after treatment with 10 μM alk(en)yl trisulfides (Figure 3B and C). The cell cycle arresting effects of alkenyl trisulfides such as DATS, dibutenyl trisulfide and dipentenyl trisulfide were markedly different during the time course for up to 24 h, i.e. the population at the G2/M phase increased as the culture time with the alkenyl trisulfides progressed (Figure 3C). DATS treatment reached maximum at 8–12 h and then gradually returned to the initial pattern (Figure 3C, closed circles). The population of the cells at G2/M phase in dibutenyl or dipentenyl trisulfide-treated cultures reached maximum at 16 h and then slightly decreased (Figure 3C, open circles or
closed triangles). The restoration of the cell cycle suggests that alkenyl trisulfide-induced cell cycle arrest was transient, implying the existence of sulfide clearance mechanisms in the cells. On the contrary, alkyl trisulfides such as dimethyl trisulfide, diethyl trisulfide, DPTS, dibutyl trisulfide and dipentyl trisulfide did not change the cell cycle pattern over the 24 h examined (Figure 3B).

The IC50 values for growth inhibition were calculated to be 11.0 ± 1.4 μM for DATS and 25.2 ± 3.5 μM for DPTS, showing the growth inhibitory activity of DPTS was weaker than that of DATS. Other alkenyl trisulfides also showed growth inhibition at low concentration (<10 μM) in comparison with alkyl trisulfides (data not shown). These growth-inhibiting potencies correlate with the potencies for microtubule disruption and the inhibition of tubulin polymerization. The trisulfides with alkenyl groups showed both properties (i) antimicrotubule and (ii) cell cycle arrest at G2/M phase. Thus, the

![Fig. 3. Cell cycle progression of HT-29 cells cultured in the presence of alk(en)yl trisulfides having various structures. HT-29 cells were cultured in the presence or absence of alkyl trisulfides (dimethyl trisulfide, diethyl trisulfide, DPTS, dibutyl trisulfide and dipentyl trisulfide), alkenyl trisulfides (DATS, dibutenyl trisulfide and dipentenyl trisulfide) or mixed ank(en)yl trisulfide (allyl methyl trisulfide) at a concentration of 10 μM for 12 h. Then, the cell cycle distribution in G2/M phase was analyzed by using a flow cytometer, as described in Materials and Methods (panel A). Values are the mean ± SE of three independent experiments. ** (P < 0.01) represents the significant differences from value of control group (vehicle) determined by Dunnett’s multiple comparison test. Time-course estimations of the effects of alkyl trisulfides or alkenyl trisulfides (10 μM) on the cell cycle progression are shown in panels B and C, respectively. Panel D shows growth inhibition curves for HT-29 cells cultured with DATS or DPTS. Data represent the average ± SEM of three experiments.](https://academic.oup.com/carcin/article-abstract/29/7/1400/2476739)

![Fig. 4. Induction of mitotic arrest by DATS through the inhibition of mitotic spindle formation. Detection of Ser10-phosphorylated histone H3, a sensitive marker for cells at the M phase, was detected in HT-29 cells cultured with DATS (10 μM) for 12 h by using a flow cytometer (panel A). Spindle formation of HT-29 cells cultured with vehicle or DATS (10 μM) was assessed by the immunofluorescence method using anti-β-tubulin antibody, as described in Materials and Methods (green; panel B). The nucleus was counterstained with propidium iodide (magenta). Scale bar 20 μm.](https://academic.oup.com/carcin/article-abstract/29/7/1400/2476739)
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Disarrangement of microtubules and cell cycle arrest induced by trisulfide is completely blocked by L-cysteine

We reported previously that DATS-induced microtubule disarrangement was due to the microtubule depolymerization attributed to oxidative modification of specific cysteine residues in the β-tubulin molecule (10). So, we examined if the alkenyl trisulfide-induced microtubule disarrangement could be attenuated by coinubcation of the cells with L-cysteine. The cells were preincubated in medium containing 2 mM L-cysteine for 1 h and then 10 μM DATS was added; then they were cultured for 2 h. DATS-induced microtubule network depolymerization [Figure 5A(c), arrowhead] was completely cancelled by the pretreatment of the cells with L-cysteine [Figure 5A(d)]. Incubation of the cells with 10 μM DATS for 12 h caused ~4.5-fold increase in the percentage of cells at the G2/M phase (44.6 ± 1.8% in G2/M phase, Figure 5B). L-Cysteine also completely blocked the DATS-induced cell cycle arrest at the G2/M phase (9.4 ± 1.7% by the coincubation with L-cysteine). These results strongly suggest that exogenous alkenyl trisulfides modify growth-relating proteins at their sulfhydryl groups and hamper their normal functions.

DATS-induced cell cycle arrest was sustained by the depletion of GSH

The effect of cellular GSH depletion on the DATS-induced cell cycle arrest was studied. BSO depletes cellular GSH by inhibiting the activity of γ-glutamylcysteine synthetase, and at the same time, it enhances the cytotoxic effects of DNA topoisomerase inhibitor or alkylating agents (20–24). The cells were incubated with or without 500 μM BSO for 24 h and then treated with 10 μM DATS or DPTS. The treatment of the cells with BSO alone did not show any effect on the cell cycle distribution (data not shown). Pretreatment of the cells with BSO enhanced the effect of DATS and sustained the cell cycle arrest during the G2/M phase for a longer time than without BSO. The population of the cells during the G2/M phase at 12 h was 59% in the cultures with both DATS and BSO and 42% in those with DATS alone (Figure 6A and C). On the other hand, BSO showed little effect on the size of the population of DPTS-treated cells during the G2/M phase (Figure 6B and D). These results indicate that the depletion of cellular GSH enhanced the effect of alkenyl trisulfide on the cell cycle.

Discussion

Recent studies revealed that DATS, a garlic-derived allyl sulfide compound, has a potent antiproliferative effect on several human cancer cell lines (2,5,8,10–13). However, neither diallyl monosulfide nor diallyl disulfide showed such effect on the cells (10). As part of our basic study to determine the nature of more potent anticancer principles in garlic and its related organosulfur compounds, we compared the effect of nine kinds of alk(en)yl trisulfides on microtubule network formation as well as on cell cycle progression by using HT-29 human colon cancer cells. Among the alk(en)yl trisulfides, DATS, dibutyl...
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Fig. 6. BSO enhanced and sustained the DATS-induced cell cycle arrest but had little influence on DPTS-treated cells. HT-29 cells were pretreated with 500 μM BSO for 24 h and then the cells were treated with 10 μM DATS (A and C) or DPTS (B and D) for the times as indicated. The cell cycle distribution of the cells was analyzed by using a flow cytometer.

trisulfide and dipentenyl trisulfide, all unsaturated sulfide compounds, caused marked disruption of microtubules and prominent cell cycle arrest during the mitotic phase; whereas the saturated sulfide compounds showed very little effect (Figures 2 and 3). DPTS and allyl methyl trisulfide showed disruption of microtubule formation at 30–120 min after the treatment; however, they did not influence the cell cycle progression in the time-course study (Figure 3B and C). Both DPTS and allyl methyl trisulfide caused only a transient change in microtubule formation at 120 min and it recovered to the normal structure at 12 h (data not shown). Such transient effect of DPTS might be due to its lower reactivity with sulfhydryl group than that of DATS.

Other organosulfur compounds, Z-ajoene and S-allylmercaptocysteine, which are known to induce microtubule disarrangement, also have alkenyl structures (4,9,14). These results indicate that with respect to the side chain structures of trisulfide compounds, the two alkenyl groups, but not the alkyl group, play a critical role in both microtubule disassembly and cell cycle arrest. However, the mechanisms by which trisulfides cause the microtubule network disarrangement has not yet been clarified. In our previous study, DATS caused the sulfhydryl groups of tubulin molecules to undergo a thiol–disulfide exchange reaction. Taken together, our present data strongly suggest that the reactivity of alkenyl trisulfides toward the sulfhydryl group provided by GSH, cysteine or dithiothreitol (41). As observed for DATS, cytotoxicity of arsenic trioxide is cancelled by cysteine: 9.5 ± 1.3 versus vehicle in Figure 5). These data strongly suggest that some sulfhydryl modification of proteins including tubulin by alkenyl trisulfide is required for their effectiveness.

In summary, the present study provides novel evidence that the cellular sulfhydryl-containing molecules might be a determinant of susceptibility of cancer cells to the alkenyl trisulfides. Reciprocally, the depletion of cellular GSH by BSO potentiated the effects of DATS on the cell cycle arrest (Figure 6). Similar results have been reported for arsenic trioxide (As2O3), i.e. BSO treatment enhances the arsenic trioxide-induced anticancer activities (38–40). As observed for DATS, cytotoxicity of arsenic trioxide is cancelled by the sulfhydryl group provided by GSH, cysteine or dithiothreitol (41). The cellular sulfhydryl-containing molecules might be a determinant of susceptibility of cancer cells to the alkenyl trisulfides.

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References

1. Ross,S.A. et al. (2006) Allyl sulfur compounds from garlic modulate aberrant crypt formation. J. Natr., 136, 8525–8545.

2. Sakamoto,K. et al. (1997) Allyl sulfides from garlic suppress the in vitro proliferation of human A549 lung tumor cells. Nutr. Cancer, 29, 152–156.

3. Filomeni,G. et al. (2003) Reactive oxygen species-dependent c-Jun NH2-terminal kinase/c-Jun signaling cascade mediates neuroblastoma cell death induced by diallyl disulfide. Cancer Res., 63, 5940–5949.

4. Xiao,D. et al. (2003) Induction of apoptosis by the garlic-derived compound S-allylmercaptoceysteine (SAMC) is associated with microtubule depolymerization and c-Jun NH2-terminal kinase 1 activation. Cancer Res., 63, 6825–6837.

5. Xiao,D. et al. (2004) Diallyl trisulfide-induced apoptosis in human prostate cancer cells involves c-Jun N-terminal kinase and extracellular-signal regulated kinase-mediated phosphorylation of Bcl-2. Oncogene, 23, 5594–5606.

6. Lund,T. et al. (2005) Garlic arrests MDA-MB-435 cancer cells in mitosis, phosphorylates the proapoptotic BH3-only protein BimEL, and induces apoptosis. Br. J. Cancer, 92, 1773–1781.

7. Wu,X.J. et al. (2005) The role of reactive oxygen species (ROS) production on diallyl disulfide (DADS) induced apoptosis and cell cycle arrest in human A549 lung carcinoma cells. Mutat. Res., 579, 115–124.

8. Xiao,D. et al. (2005) Diallyl trisulfide-induced G2/M phase cell cycle arrest in human prostate cancer cells is caused by reactive oxygen species-dependent destruction and hyperphosphorylation of Cdc 25 C. Oncogene, 24, 6256–6268.

9. Xiao,D. et al. (2005) Effects of a series of organosulfur compounds on mitotic arrest and induction of apoptosis in colon cancer cells. Mol. Cancer Ther., 4, 1388–1398.

10. Hosono,T. et al. (2005) Diallyl trisulfide suppresses the proliferation and induces apoptosis of human colon cancer cells through oxidative modification of beta-tubulin. J. Biol. Chem., 280, 41487–41493.

11. Xiao,D. et al. (2006) Diallyl trisulfide, a constituent of processed garlic, inactivates Akt to trigger mitochondrial translocation of BAD and caspase-mediated apoptosis in human prostate cancer cells. Carcinogenesis, 27, 533–540.

12. Antoniewicz,J. et al. (2006) c-Jun NH2-terminal kinase signaling axis regulates diallyl trisulfide-induced generation of reactive oxygen species and cell cycle arrest in human prostate cancer cells. Cancer Res., 66, 5379–5386.

13. Kim,Y.A. et al. (2007) Mitochondria-mediated apoptosis by diallyl trisulfide in human prostate cancer cells is associated with generation of reactive oxygen species and regulated by Bax/Bak. Mol. Cancer Ther., 6, 1599–1609.

14. Li,M. et al. (2002) Antitumor activity of Z-ajoene, a natural compound purified from garlic: antimotic and microtubule-interaction properties. Carcinogenesis, 23, 573–579.

15. Milligan,B. et al. (1962) New syntheses of trisulfides. J. Chem. Soc., 4850–4853.

16. Wei,Y. et al. (1999) Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. Cell, 97, 99–109.

17. Goto,H. et al. (1999) Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation. J. Biol. Chem., 274, 25543–25549.

18. Hendzel,M.J. et al. (1997) Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. Chromosoma, 106, 348–360.

19. Nigg,E.A. (2001) Mitotic kinases as regulators of cell division and its checkpoints. Nat. Rev. Mol. Cell Biol., 2, 21–32.

20. Mans,D.R. et al. (1992) Modulation by D-L-buthionine-SR-sulphoximine of etoposide cytotoxicity on human non-small cell lung, ovarian and breast carcinoma cell lines. Eur. J. Cancer, 28A, 1447–1452.

21. Sawyer,T.E. et al. (1996) The interaction of buthionine sulphoximide (BSO) and the topoisomerase I inhibitor CPT-11. Br. J. Cancer, 72 (suppl), S109–S113.

22. Gantchev,T.G. et al. (1997) Enhancement of etoposide (VP-16) cytotoxicity by enzymatic and photodynamically induced oxidative stress. Anticancer Drugs, 8, 164–173.

23. Troyano,A. et al. (2001) Effect of glutathione depletion on antitumor drug toxicity (apoptosis and necrosis) in U-937 human promonocytic cells. The role of intracellular oxidation. J. Biol. Chem., 276, 47107–47115.

24. Yoshida,F. et al. (2004) Enhancement of sodium borocaptate (BSH) uptake by tumor cells induced by glutathione depletion and its radiobiological effect. Cancer Lett., 215, 61–67.

25. Gilbert,H.F. (1995) Thioldisulfide exchange equilibria and disulfide bond stability. Methods Enzymol., 251, 8–28.

26. Casini,A. et al. (2002) Cysteine-modifying agents: a possible approach for effective anticancer and antiviral drugs. Environ. Health Perspect., 110 (suppl 5), 801–806.

27. Cross,J.V. et al. (2006) Regulation of signal transduction through protein cysteine oxidation. Antioxid. Redox. Signal., 8, 1819–1827.

28. Kim,E.H. et al. (2006) 15-Deoxy-Delta12,14-prostaglandin J2 as a potential endogenous regulator of redox-sensitive transcription factors. Biochem. Pharmacol., 72, 1516–1528.

29. Lipton,S.A. et al. (2002) Cysteine regulation of protein function—as exemplified by NMDA-receptor modulation. Trends Neurosci., 25, 474–480.

30. Gom,J.A. et al. (2004) Biological significance of nitric oxide-mediated protein modifications. Am. J. Physiol. Lung Cell. Mol. Physiol., 287, L262–L268.

31. Gopalakrishna,R. et al. (2000) Protein kinase C signaling and oxidative stress. Free Radic. Biol. Med., 28, 1349–1361.

32. Straus,D.S. et al. (2000) 15-Deoxy-Delta12,14-prostaglandin J2 inhibits multiple steps in the NF-kB signaling pathway. Proc. Natl Acad. Sci. USA, 97, 4848–4849.

33. Cernuda-Morollon,E. et al. (2001) 15-Deoxy-Delta12,14-prostaglandin J2 inhibition of NF-kB-DNA binding through covalent modification of the p50 subunit. J. Biol. Chem., 276, 35530–35536.

34. Perez-Sala,D. et al. (2003) Molecular basis for the direct inhibition of AP-1 DNA binding by 15-deoxy-Delta12,14-prostaglandin J2. J. Biol. Chem., 278, 51251–51260.

35. Shibata,T. et al. (2003) Thioredoxin as a molecular target of cyclopentenone prostaglandins. J. Biol. Chem., 278, 26046–26054.

36. Stamatakis,K. et al. (2006) Identification of novel protein targets for modification by 15-deoxy-Delta12,14-prostaglandin J2 in mesangial cells reveals multiple interactions with the cytoskeleton. J. Am. Soc. Nephrol., 17, 89–98.

37. Cuming,R.C. et al. (2004) Protein disulfide bond formation in the cytoplasm during oxidative stress. J. Biol. Chem., 279, 21749–21758.

38. Zhu,X.H. et al. (1999) Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations. J. Natl Cancer Inst., 91, 772–778.

39. Davison,K. et al. (2004) JNK activation is a mediator of arsenic trioxide-induced apoptosis in acute promyelocytic leukemia cells. Blood, 103, 3496–3502.

40. Chen,D. et al. (2006) Buthionine sulphoximine enhancement of arsenic trioxide-induced apoptosis in leukemia and lymphoma cells is mediated via activation of c-Jun NH2-terminal kinase and up-regulation of death receptors. Cancer Res., 66, 11416–11423.

41. Watson,R.W. et al. (1996) Mechanisms involved in sodium arsenite-induced apoptosis of human neutrophils. J. Leukoc. Biol., 60, 625–632.

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