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Synthesis of radioiodine labeled dibenzyl disulfide for evaluation of tumor cell uptake

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Abstract—Benzyl 4-halobenzyl and ally benzyl disulfide were synthesized as diallyl disulfide analogues and their tumor growth inhibitory effects on the cancer cells (SNU C5 and MCF-7) were comparable to that of diallyl disulfide, indicating that the disulfide functional group was responsible for the tumor growth inhibitory effects. Cu(I)-assisted radioiodination of benzyl 4-bromobenzyl disulfide gave benzyl 4-[^{123}I/^{125}I]iodobenzyl disulfide in 30–40% radiochemical yield. The radiolabeled disulfide was taken up by the cancer cells in a time-dependent manner, and the uptake was inhibited by the pretreatment of *S*-methyl methanethiosulfonate (MMTS), phorone and diallyl disulfide. This study suggested that the radiolabeled dibenzyl disulfide was taken up by the cancer cells via thiol-disulfide exchange and retained inside the cells.

1. Introduction

Garlic has been a focus of attention as the dietary anticarcinogen, since the recent studies implicating the correlation between consumption of garlic and low incidence of cancer.[1] In addition to this anticarcinogenic property, garlic has been known to possess antibiotic, fungicidic, antihelmentic, antithrombotic, and antioxidant properties.[1–3] The anticarcinogenicity of garlic has been therefore evaluated in the studies using chemically induced tumors in animals.[4–6] Garlic was shown to inhibit the promotion phase of carcinogenesis in skin tumors induced by 7,12-dimethylbenz[α]anthracene.[7] Reduction of breast cancer risk was also shown related to the consumption of garlic.[8,9] It was suggested that the anticarcinogenic property of the garlic derives from a complex mixture of organosulfur compounds, such as oil-soluble diallyl sulfide, diallyl disulfide (**6**), diallyl trisulfide, and water-soluble *S*-allyl cysteine.[10,11] Dietary allyl sulfur compounds of garlic reduced tumors in rats treated with the typical colon carcinogen, 1,2-dimethylhydrazine.[12,13] The oil-soluble organosulfur compound **6** was shown to inhibit the growth of canine mammary tumor cells (CMT-13) in vitro and *N*-methyl-*N*-nitrosourea-induced rat mammary tumors, whereas water-soluble organosulfur compounds were not effective.[14–16] Disulfide **6** also markedly inhibits the growth of human colon tumor cells (HCT-15).[17] Therefore, diallyl disulfide **6**, a major organosulfur compound in garlic, appears to be the most effective in growth inhibition of human cancer cells such as skin, breast, and colon cancer cells.[17]

The anticarcinogenicity of the organosulfur compounds seems to derive from more than one mechanism, because the organosulfur compounds can inhibit activation and carcinogenicity of chemicals such as diverse carcinogens and also influence in various tissues such as skin, breast, colon, etc.[18] Suppression of cell division rate and induction of apoptosis were reported as another mechanisms for the anticarcinogenicity of **6**.[19] It is likely that the disulfide functional group of **6** is responsible for its anticarcinogenicity. In this study, therefore, benzyl halobenzyl and allyl benzyl disulfide (Fig. 1) were synthesized as diallyl disulfide analogues and evaluated for their inhibitory effects on growth of human breast cancer (MCF-7) cells and colon cancer (SNU C5) cells. Their tumor cell uptake and retention mechanisms were investigated using the radiolabeled

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disulfide, because little is known about the mechanisms of the disulfide. The disulfides containing benzene rings were desirable for radiolabeling, due to facile introduction of radiohalogen to a benzene ring and a long synthetic route required for the synthesis of radioiodine labeled diallyl disulfide. The slight modification in benzyl halobenzyl and allyl benzyl disulfide does not seem to induce a significant change of the physicochemical properties of **6**. Among these disulfides, dibenzyl disulfide was radiolabeled with $^{123}I/^{125}I$ and its tumor cell uptake and retention mechanisms were evaluated.

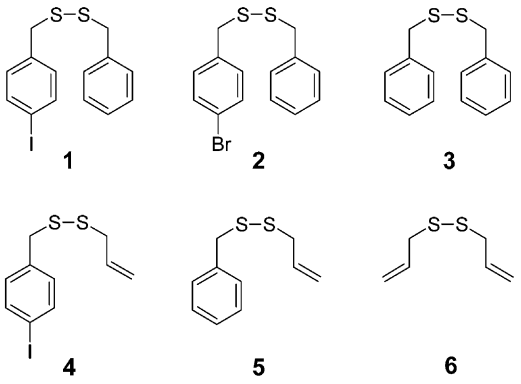

Figure 1. Structures of disulfides: benzyl 4-iodobenzyl disulfide (**1**), benzyl 4-bromobenzyl disulfide (**2**), dibenzyl disulfide (**3**), allyl 4-iodobenzyl disulfide (**4**), allyl benzyl disulfide (**5**) and diallyl disulfide (**6**).

2. Results and discussion

2.1. Synthesis of disulfides

4-Halobenzyl thiol (**9**) was readily prepared from 4-halobenzyl alcohol in three steps (Fig. 2). Although the Mitsunobu reaction does not require activation of a hydroxyl group for displacement with a sulfur nucleophile,[20] thioacetylation of the alcohol followed by cleavage of the thioacetate to thiol under basic condition provided thiol and disulfide in low yields which were also limited in separation due to their similar polarities. Therefore, the hydroxyl group was activated by conversion to the methanesulfonate **7**, followed by displacement with a suitable sulfur nucleophile. Reduction of the resulting thioester **8** with $LiAlH_4$ gave the sole product **9**.[21]

Synthesis of the unsymmetric disulfide was carried out in high yield using 2,2′-dithiobis(benzothiazole) (**10**) as the intermediate disulfide.[22] This symmetric disulfide was reacted with one equivalent of benzyl thiol under the mild conditions to provide unsymmetric disulfides, **11** and **12**. Further thiol-disulfide exchange reaction of this disulfide with 4-halobenzyl thiol or allyl thiol yielded the desired unsymmetric disulfides, **1**, **2**, **4** and **5** (Figs 2 and 3).[22,23] When 4-halobenzyl thiol was reacted with disulfide **10**, the resulting unsymmetric disulfide was not readily separated from **10**. Therefore, benzyl thiol was converted to 2-benzothiazolyl benzyl disulfide (**11**) that was then reacted with 4-halobenzyl thiol to give the desired unsymmetric disulfide in high yield (>89%) and with high purity (>99%).

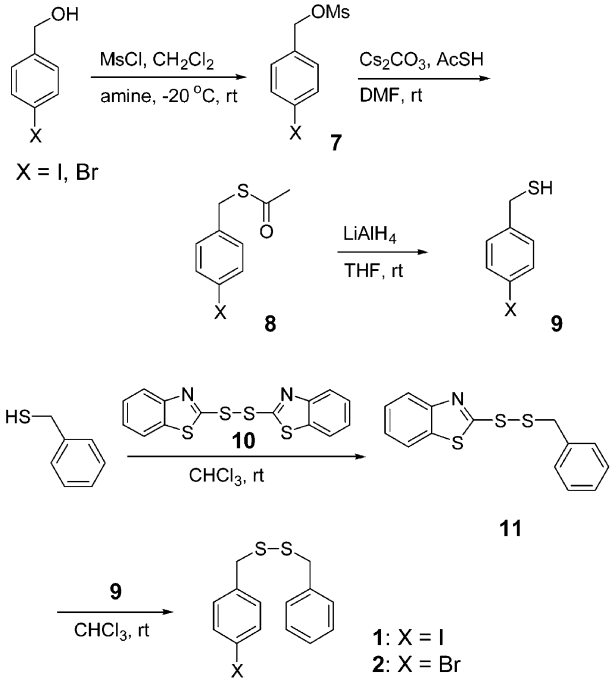

Figure 2. Synthetic pathway of benzyl 4-halobenzyl disulfide (**1** and **2**).

2.2. Radiochemical Synthesis of [$^{123}I/^{125}I$]1

Radioiodine labeled disulfide was prepared by halogen exchange reaction of benzyl 4-bromobenzyl disulfide in the presence of $Na^{123}I/^{125}I$ and CuCl at 150 °C for 1 h (Fig. 4). It was suggested that Cu(I) ion forms a tricyclic intermediate between Br and the aromatic carbon which allows substitution of Br with I.[24] Progress of the reaction was monitored by radio-TLC (Fig. 5). At the end of the reaction, the product was purified by reverse phase HPLC (t_R = 10–11 min) (Fig. 6). When a normal

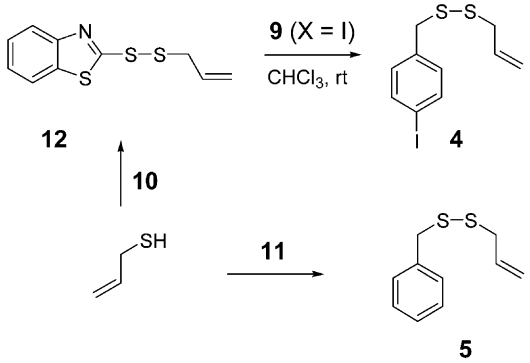

Figure 3. Synthetic pathways of allyl 4-iodobenzyl disulfide (**4**) and allyl benzyl disulfide (**5**).

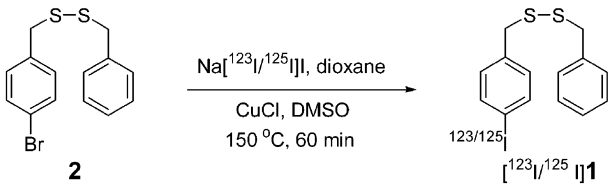

Figure 4. Synthetic pathway of [$^{123}I/^{125}I$]**1**.

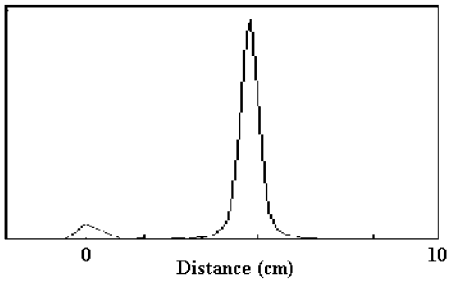

Figure 5. Radio-TLC of the radioiodination mixture. Developing solvents: 49:1 hexane–ethyl acetate.

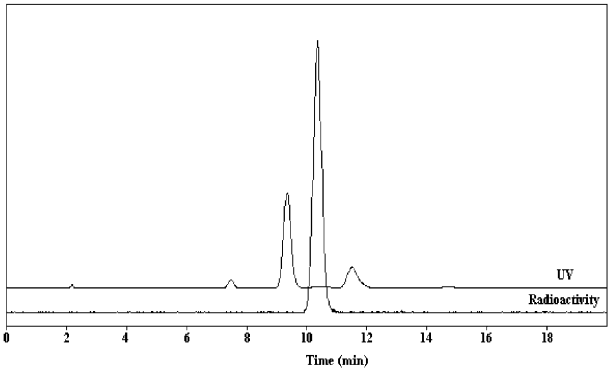

Figure 6. Semipreparative HPLC traces of [^{123}I/^{125}I]**1**. HPLC solvents: 85:15 CH_3OH–H_2O; HPLC column: C18 silica gel; $t_R = 10.4$ min; flow rate: 4 mL/min; UV detector (UV) and a NaI(Tl) radioactivity detector.

phase HPLC system was used for the purification, the product was coeluted with the precursor **2**. Therefore, a reverse phase HPLC system was applied to the purification of the final product using a 85:15 mixture of methanol and water. Removal of the solvents (70 °C, N_2) was troublesome, because this procedure not only required a long period of time for removal of the solvents but also accompanied simultaneous evaporation of the product. The product from the desired HPLC fraction was therefore extracted with CH_2Cl_2 and the solvents were carefully removed at low temperature (40 °C) under a gentle stream of N_2. [^{123}I/^{125}I]**1** was obtained in 30–40% radiochemical yield (decay-corrected) and with high specific activity (420–660 GBq/μmol). The identity of [^{123}I/^{125}I]**1** was confirmed by coelution with the nonradioactive authentic standard (**1**) as determined by HPLC.

2.3. Tumor growth inhibition by disulfides

Disulfides were evaluated for their abilities to inhibit the growth of MCF-7 and SNU C5 cells in vitro. Treatment of the cancer cells with the disulfide (500 μM) resulted in significant inhibition on the growth of cancer cells, showing growth inhibition (>90%) comparable to that by **6** (Table 1). The result suggested that the presence of disulfide functional group might play a major role in inhibiting the growth of the cancer cells.

2.4. Uptake of [^{125}I]1 by the cancer cells

[^{125}I]**1** was incubated at 37 °C with MCF-7 or SNU C5 cells which were grown at the same temperature for 48 h.

Table 1. Percentage of growth inhibition on MCF-7 and SNU C5 cells by disulfide analogues[a]

Disulfide[a]	**1** (%)	**2** (%)	**3** (%)	**4** (%)	**5** (%)	**6** (%)
MCF-7	96	90	94	97	91	100
SNU C5	100	103	101	—	101	95

[a] Disulfide: benzyl 4-iodobenzyl disulfide (**1**), benzyl 4-bromobenzyl disulfide (**2**), dibenzyl disulfide (**3**), allyl 4-iodobenzyl disulfide (**4**), allyl benzyl disulfide (**5**) and diallyl disulfide (**6**). Data are expressed as mean ($n = 3$).

Uptake of the radiolabeled disulfide by the cancer cells showed a time-dependent increase as shown in Figure 7, demonstrating a two-fold increase at 1 h compared to 15 min by both cells and a four-fold increase at 2 h by SNU C5 cells. This result demonstrated that the radiotracer was more efficiently taken up by SNU C5 cells than by MCF-7 cells.

2.5. Inhibition of the uptake by various compounds

Morphologic change of the cells was observed at the concentrations higher than 500 μM of the disulfide, which was consistent with the results reported in the literature.[16] Thus the concentrations lower than 500 μM were used in this study. Pretreatment of MMTS, phorone, and **6** reduced the uptake of [^{125}I]**1** by the cancer cells in a dose-dependent manner (Fig. 8), showing a similar pattern of the inhibition in both cancer cells. Percentage of the inhibition was well correlated with the concentration of the compounds added (5, 50, and 500 μM). This result can be explained by dose-dependent reduction of the intracellular enzyme-SH and glutathione (GSH) level by MMTS, a temporary blocking agent for enzyme-SH groups, and phorone, a GSH depleting agent, respectively,[25] which leads to lowering the uptake of the radiolabeled disulfide by the cancer cells. It was reported that tumor growth inhibitory effect of **6** was modified by intracellular GSH level.[16] Attempts were therefore made to find the direct effect of intracellular GSH on the uptake of [^{125}I]**1**. The cancer cells were pretreated with GSH for 30 min prior to the incubation with [^{125}I]**1**, however, there was no change in the uptake (data not shown), probably due to impermeability of GSH through the cell membranes. Although the uptake mechanism of the disulfide is not well known, dose-dependent inhibition of the uptake of [^{125}I]**1** by MMTS and phorone suggested that the uptake was mediated by

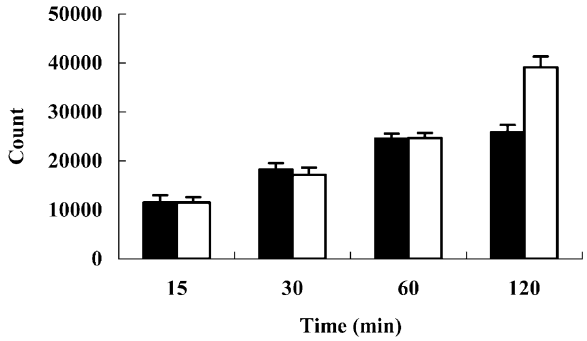

Figure 7. Uptake of [^{125}I]**1** by MCF-7 cells (closed bar) and SNU C5 cells (open bar). Data are expressed as mean ± SD ($n = 3$, $p < 0.05$).

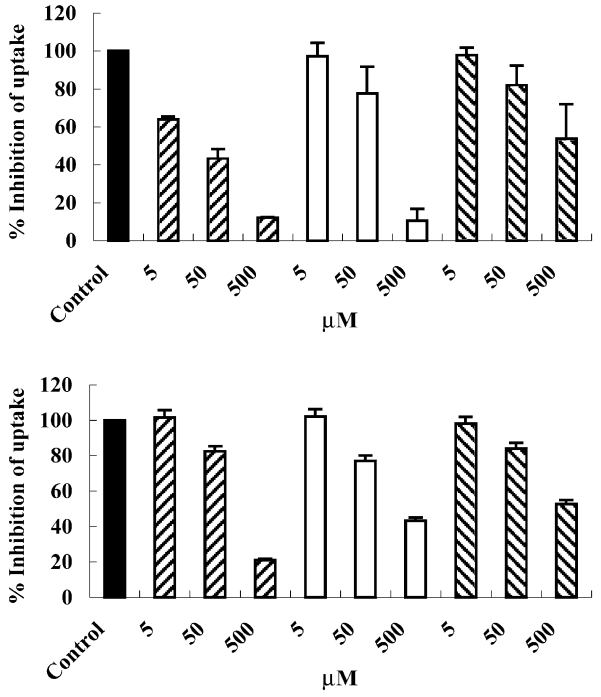

Figure 8. Effect of various compounds (5, 50, and 500 μM) on the uptake of [^{125}I]**1** by MCF-7 cells (top) and SNU C5 cells (bottom).% Inhibition of the uptake by the compounds was expressed relative to the control (100%) at 2 h after administration of the radiotracer ($n=3$); Control (closed bar), **6** (right-handed striped bar), MMTS (open bar), and phorone (left-handed striped bar). Data are expressed as mean ± SD.

thiol-disulfide exchange which has been proposed as a mechanism of enzyme activity control.[25,26] Once the disulfide is taken up by the tumor cells via thiol-disulfide exchange, it is likely that the radioactivity is trapped inside the cells after binding to either enzyme-SH or GSH. Inhibition of the uptake of [^{125}I]**1** by **6** might result from competitive uptake between the two disulfides.

3. Conclusion

Diallyl disulfide found in garlic has been known to inhibit the growth of various human cancer cells. In this study, therefore, benzyl 4-halobenzyl and allyl benzyl disulfide were synthesized as analogues of **6** and they were all effective on the growth inhibition of MCF-7 and SNU C5 cells, indicating that the disulfide functional group might be responsible for the tumor growth inhibitory effects. Unsymmetric radioiodine labeled disulfide, [^{123}I/^{125}I]**1** was synthesized in high radiochemical yield and taken up by the cancer cells in a time-dependent manner. The uptake of [^{125}I]**1** was inhibited by pretreatment of MMTS and phorone, suggesting that the uptake was mediated by thiol-disulfide exchange. It is highly probable that this exchange allows the retention of the radioactivity inside the cells, which would facilitate the tumor imaging by this radiotracer. Further studies are warranted to investigate the biodistribution of [^{123}I]**1** in tumor cell implanted animal models using single photon emission computed tomography (SPECT).

4. Experimental

4.1. General methods

^{1}H NMR spectra were performed on a JNM-LA 300 (JEOL Ltd) and Varian 500NB spectrometer. Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane as an internal reference. Electron impact (EI) and fast atom bombardment (FAB) mass spectra were obtained on a JMS-700 Mstation (JEOL Ltd) instrument in the positive ion mode. For the FAB mass spectrometry, EtOAc or MeOH was used as the solvent and 3-nitrobenzyl alcohol saturated with NaI as matrix. HPLC was carried out on a Thermo Separation Products System using a semipreparative column (Alltech Econosil, C18, 10 μ, 10×250 mm) and an analytical column (YMC, C18, 5 μ, 4.6×250 mm). The eluant was simultaneously monitored by a UV (254 nm) detector and a NaI(Tl) radioactivity detector. Radio-TLC was performed on a radio-TLC scanner (Bioscan). GSH was purchased from Sigma Chemical Company and all other chemicals from Aldrich Chemical Company. Solvents were distilled prior to use.

4.2. Synthesis of disulfides

4.2.1. 4-Halobenzyl methanesulfonate (7). 4-Halobenzyl alcohol (6.42 mmol) was dissolved in dry CH_2Cl_2 (30 mL), and to this was added *N*,*N*′-diisopropylethylamine (1.5 mL, 8.61 mmol). The reaction mixture was allowed to stir at rt for 30 min. At the end of the reaction, the mixture was cooled to −20 °C and then methanesulfonyl chloride (0.6 mL, 7.75 mmol) was slowly added while maintaining the temperature below −10 °C. The resulting solution was stirred for 3 h and then quenched with 1 N HCl. The solution was then poured into water (15 mL) and extracted with CH_2Cl_2 (30 mL×3). The product isolation afforded a pale brown solid **7** in 99% yield.

4.2.2. 4-Iodobenzyl methanesulfonate. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.74 (d, $J=8.5$ Hz, 2H), 7.15 (d, $J=8.5$ Hz, 2H), 5.17 (s, 2H), 2.93 (s, 3H); MS (FAB) *m*/*z* 335 ($M^+ + Na$); HRMS calcd for $C_8H_9IO_3SNa$ 334.9215, found 334.9216.

4.2.3. 4-Bromobenzyl methanesulfonate. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54 (d, $J=8.5$ Hz, 2H), 7.29 (d, $J=7.5$ Hz, 2H), 5.18 (s, 2H), 2.94 (s, 3H); MS (FAB) *m*/*z* 289 ($M^+ + Na$, ^{81}Br), 287 ($M^+ + Na$, ^{79}Br); HRMS calcd for $C_8H_9{}^{79}BrO_3SNa$ 286.9353, found 286.9374.

4.2.4. 4-Halobenzyl thioacetate (8). 4-Halobenzyl methanesulfonate (6.79 mmol) was dissolved in DMF (32 mL) and Cs_2CO_3 (6.64 g, 20.37 mmol) was added. After the reaction mixture was stirred at rt for 30 min, thiolacetic acid (1.46 mL, 20.37 mmol) was added. The mixture was stirred for another 2 h and then extracted with ethyl acetate (30 mL×3), and the combined organic extracts were then dried over anhydrous $MgSO_4$. Flash column chromatography (3:1 hexane–ethyl acetate) afforded a yellow solid **8** in 95% yield.

4.2.5. 4-Iodobenzyl thioacetate. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.64 (s, 2H), 7.06 (s, 2H), 4.06 (d, $J=26$ Hz,

2H), 2.36 (s, 3H); MS (FAB) *m/z* 293 ($M^+ + H$); HRMS calcd for $C_9H_{10}IOS$ 292.9497, found 292.9503.

4.2.6. 4-Bromobenzyl thioacetate. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.39 (s, 2H), 7.14 (s, 2H), 4.01 (d, $J = 15.0$ Hz, 2H), 2.32 (s, 3H); MS (EI) *m/z* 244 (M^+) (Sigma Chemical Company).

4.2.7. 4-Halobenzyl thiol (9). 4-Halobenzyl thioacetate (2.15 mmol) in dry THF (3 mL) was added dropwise to a slurry of $LiAlH_4$ in dry THF (12 mL). The reaction mixture was stirred at rt for 1 h. The mixture was cooled, treated with water and then filtered. The filtrate was washed with water and dried over $MgSO_4$. Flash column chromatography (5:1 hexane–ethyl acetate) afforded a white solid **9** in 83% yield. 4-Iodobenzyl thiol was used without analysis due to its instability.

4.2.8. 4-Bromobenzyl thiol. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.45 (s, 2H), 7.19 (s, 2H), 3.67 (d, $J = 7.5$ Hz, 2H), 1.79 (t, $J = 7.0$ Hz, 1H); MS (EI) *m/z* 201 (M^+) (Sigma Chemical Company).

4.2.9. 2-Benzothiazolyl benzyl disulfide (11). Benzyl thiol (0.12 mL, 1.02 mmol) in $CHCl_3$ (2.5 mL) was added dropwise to a suspension of 2,2′-dithiobis(benzothiazole) (**10**, 339.1 mg, 1.02 mmol) in $CHCl_3$ (14 mL) with stirring at rt.[4,22] After stirred for 1 h, the reaction mixture was washed successively with 5% NaOH (aq, 15 mL×2) and water (15 mL×2) and then dried over $MgSO_4$. The solvent was removed in vacuo and the residue was purified by flash column chromatography (25:1 hexane–ethyl acetate) to give **11** (242 mg, 92.7%) as a white solid. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.87 (d, $J = 7.5$ Hz, 1H), 7.80 (d, $J = 7.0$ Hz, 1H), 7.26–7.44 (m, 7H), 4.18 (s, 2H); MS (FAB) *m/z* 312 ($M^+ + Na$); HRMS calcd for $C_{14}H_{11}NS_3Na$ 311.9951, found 311.9957, identical with the literature values.[22]

4.2.10. Benzyl 4-halobenzyl disulfide (1 and 2). 4-Halobenzyl thiol (0.84 mmol) in $CHCl_3$ (2.5 mL) was added dropwise to 2-benzothiazolyl benzyl disulfide (**11**) (0.84 mmol) in $CHCl_3$ (12 mL) with vigorous stirring at rt. The reaction mixture was stirred for 4 h. The product isolation and purification (hexane) afforded the unsymmetric disulfides, **1** and **2** (95–97%).

4.2.11. Benzyl 4-iodobenzyl disulfide (1). ^1H NMR ($CDCl_3$, 500 MHz) δ 7.63 (d, $J = 6.5$ Hz, 2H), 7.25–7.35 (m, 5H), 6.94 (d, $J = 6.5$ Hz, 2H), 3.70 (s 2H); 3.50 (s, 2H); MS (FAB) *m/z* 373 ($M^+ + H$); HRMS calcd for $C_{14}H_{14}IS_2$ 372.9581, found 372.9573.

4.2.12. Benzyl 4-bromobenzyl disulfide (2). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (d, $J = 8.3$ Hz, 2H), 7.24–7.36 (m, 5H), 7.06 (d, $J = 8.3$ Hz, 2H), 3.66 (s, 2H), 3.48 (s, 2H); MS (FAB) *m/z* 326 (M^+, ^{81}Br), 324 (M^+, ^{79}Br); HRMS calcd for $C_{14}H_{13}{}^{79}BrS_2$ 323.9642, found 323.9652.

4.2.13. 2-Benzothiazolyl allyl disulfide (12). Allyl thiol (1.3 mL, 1.66 mmol) in $CHCl_3$ (4 mL) was added dropwise to a suspension of **10** (1.66 mmol) in $CHCl_3$ (23 mL) with stirring at rt.[4,22] The reaction mixture was stirred at rt for 1 h. The product isolation and purification (25:1 hexane–ethyl acetate) gave **12** (350 mg, 89%) as a colorless oil. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.86 (dd, $J = 4.8$, 3 Hz, 1H), 7.79 (dd, $J = 7.8$, 0.5, 1H), 7.42 (td, $J = 8.3$, 1.5 Hz, 1H), 7.32 (td, $J = 8$, 1 Hz, 1H), 5.88 (m, 1H), 5.21 (ddd, $J = 34.5$, 17, 10 Hz, 2H), 3.58 (d, $J = 7.5$, 2H); MS (FAB) *m/z* 240 ($M^+ + H$); HRMS calcd for $C_{10}H_{10}NS_3$ 239.9975, found 239.9976.

4.2.14. Allyl 4-iodobenzyl disulfide (4). To **12** (182 mg, 0.76 mmol) in $CHCl_3$ (15 mL) was added dropwise 4-iodobenzyl thiol (190 mg, 0.76 mmol) in $CHCl_3$ (3 mL) with vigorous stirring. After stirred at rt for 4 h, the product isolation and purification (hexane) gave allyl 4-iodobenzyl disulfide (**4**) as a white solid in 95% yield: ^1H NMR ($CDCl_3$, 500 MHz) δ 7.65 (d, $J = 10.5$ Hz, 2H), 7.06 (d, $J = 8.5$ Hz, 2H), 5.73–5.78 (m, 1H), 5.12 (s, 1H), 5.08 (dd, $J = 18.5$, 1.5 Hz, 1H), 3.82 (s, 2H), 3.08 (dd, $J = 28.5$, 7.0 Hz, 2H); MS (FAB) *m/z* 322 (M^+); HRMS calcd for $C_{10}H_{11}IS_2$ 321.9347, found 321.9331.

4.2.15. Allyl benzyl disulfide (5). Allyl thiol (0.13 mL, 1.66 mmol) in $CHCl_3$ (3 mL) was added dropwise to **11** (1.66 mmol) in $CHCl_3$ (21 mL) with vigorous stirring. After stirred at rt for 5 h, the product isolation and purification (hexane) gave allyl benzyl disulfide (**5**) as a yellow oil in 95% yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.25–7.33 (m, 5H), 5.73–5.75 (m, 1H), 5.11 (s, 1H), 5.05 (dd, $J = 17.4$, 1.2 Hz, 1H), 3.90 (s, 2H), 3.01 (d, $J = 7.5$ Hz, 2H); MS (EI) *m/z* 196 (M^+); HRMS calcd for $C_{10}H_{12}S_2$ 196.0380, found 196.0385.

4.2.16. Benzyl 4-[^{123}I/^{125}I]iodobenzyl disulfide ([^{123}I/^{125}I]1). Benzyl 4-bromobenzyl disulfide (**2**, 1 mg, 3.1 μmol) was dissolved in dioxane (100 μL), and to this solution were added CuCl (76 μg, 0.08 mmol) in DMSO (20 μL) and an appropriate amount of $Na^{123}I/^{125}I$. The reaction mixture was heated at 150 °C for 1 h. The mixture was extracted with CH_2Cl_2 (1.5×3 mL) and the solvents were removed under a gentle stream of N_2. The residue was redissolved in 1 mL of HPLC solvents and purified by HPLC using a 85:15 mixture of methanol and water at a flow rate of 4 mL/min. The desired product was eluted at the retention time of 10–11 min. [^{123}I/^{125}I]**1** collected from HPLC was extracted with CH_2Cl_2 and then concentrated at 40 °C under a gentle stream of N_2. The residue was redissolved in ethanol and diluted with saline to give a final solution of 18% ethanol in saline. Specific activity of [^{123}I/^{125}I]**1** was determined by using a standard curve obtained from **1** with different concentrations injected on an analytical HPLC column (flow rate: 1 mL/min) versus UV absorbance at 254 nm. Another aliquot of [^{123}I/^{125}I]**1** was coinjected with unlabeled compound **1** on HPLC to confirm its identity.

4.3. Tumor growth inhibition by disulfides

Cells plated from a single pellet into 25 cm^2 tissue culture flasks were grown at 37 °C under a humidified 5% CO_2 atmosphere in RPMI-1640 medium for 24 h.[16] MCF-7 and SNU C5 cells were treated with disulfide (500 μM) in DMSO and incubated at 37 °C under a humidified 5% CO_2 atmosphere in RPMI-1640 medium

for 48 h. Control experiment was simultaneously carried out in the absence of disulfide under the same conditions. Cells were harvested by trypsinization (0.25% trypsin-EDTA), diluted with RPMI-1640, removed by gentle scraping and centrifuged. The cell pellet was suspended in phosphate-buffered saline (pH 7.0) and treated with an equal volume of 0.4% trypan blue. The mixture was allowed to incubate at rt for 3–5 min. Viable cells were counted under hemocytometer. Growth inhibition (%) of the cancer cells by disulfide was determined according to Sundaram and Milner.[16]

4.4. Uptake of [^{125}I]1 by the cancer cells

SNU C5 and MCF-7 cells were prepared as described previously. Radiotracer in 18% ethanol-saline (185 kBq in 5 μL) was added to each well containing the cancer cells (total volume of 2 mL) and, the final concentration of ethanol in each well was 0.045%. The mixture was incubated at 37 °C. At the indicated time points (15, 30, 60, and 120 min, $n=3$), the cells were washed with phosphate-buffered saline (1 mL×3, pH 7.0), and the radioactivity was counted on a gamma counter.

4.5. Inhibition of the uptake by various compounds

Three different concentrations of MMTS, phorone, **6**, or GSH dissolved in DMSO (0.1 mL) were added to each well (total volume of 2 mL) containing MCF-7 or SNU C5 cells which were grown at 37 °C for 48 h. Final concentrations of the compounds in each well were 5, 50, and 500 μM, respectively. Control experiment was also carried out using the same volume of DMSO. After preincubation at 37 °C for 30 min, [^{125}I]**1** was added to the mixture which was further incubated under the same conditions for 2 h. The cells were washed as described above and the radioactivity was counted on a gamma counter.

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