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Original article

Synthesis and biological evaluation of sulforaphane derivatives as potential antitumor agents

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ABSTRACT

A series of sulforaphane derivatives were synthesized and evaluated in vitro for their cytotoxicity against five cancer cell lines (HepG2, A549, MCF-7, HCT-116 and SH-SY5Y). The pharmacological results showed that many of the derivatives displayed more potent cytotoxicity than sulforaphane (SFN). Furthermore, SFN and derivative **85** could induce cell cycle arrest at S or G2/M phase and cell apoptosis. SFN and **85** exhibited time- and dose-dependent activation on Nrf2 transcription factor, and **85** acted as a more potent Nrf2 inducer than SFN.

1. Introduction

Isothiocyanates are a class of compounds which are abundant in many cruciferous vegetables, such as broccoli, cauliflower, Brussels sprouts, and cabbage [1,2]. There is increasing epidemiological and clinical evidence that people that intake large amounts of vegetables of the Brassica family, particularly broccoli and other cruciferous vegetables are less likely to develop certain types of cancers [3–5], and thus, many of such analogues containing the isothiocyanates motif have been synthesized for potential medical applications [6]. The isothiocyanates, are hydrolysis products of glucosinolates that are not the putative bioactive compounds in cruciferous vegetables, and the hydrolytic reaction is catalyzed by the endogenous myrosinase which is released by disruption of the plant cell during harvesting, processing, or chewing [7,8].

Sulforaphane (SFN), one of the naturally occurring isothiocyanates, which was first discovered by scientists at Johns Hopkins University in 1992 [9], has attracted considerable research interests due to its important biological and pharmacological properties. SFN is an effective anticancer agent with the ability for both preventing and fighting many types of cancers [10,11]. Early research mainly focused on the “blocking activity” of SFN via Phase 2 enzyme induction, as well as inhibition of enzymes involved in carcinogen activation. In recent years, many studies have showed that SNF could provide protection against tumor development during the post-initiation phase. The mechanisms of SFN suppression effects, including cell cycle arrest and apoptosis, have also been investigated [3,8]. Additionally, pharmacological administration of SFN might be a promising therapeutic approach for cancers treatment. For example, according to a report from the US National Cancer Institute, SFN is considered to be one of the 40 most promising anticancer agents [12–14].

During the past years, a few methods for preparing SFN and its derivatives have been reported in the literature [15–17], and structural modifications have been envisioned to increase their cytotoxicity, especially for their specificity toward tumor cells [18–20]. To the best of our knowledge, there were few reports regarding SFN derivatives that modified the side chain (the methyl group). In our present study, we have designed and synthesized a series of SFN derivatives with different side chains and various lengths of spacers between the isothiocyanato and sulfoxide groups. We evaluated their anticancer activity against several cancer cell lines in vitro, and elucidated the possible mechanism of cell growth inhibition by these SFN derivatives.

2. Chemistry

In order to investigate the structure–activity relationship of the SFN derivatives, we replaced the methyl group in SFN (**77**) with several different alkyl and aryl groups. At the same time, we

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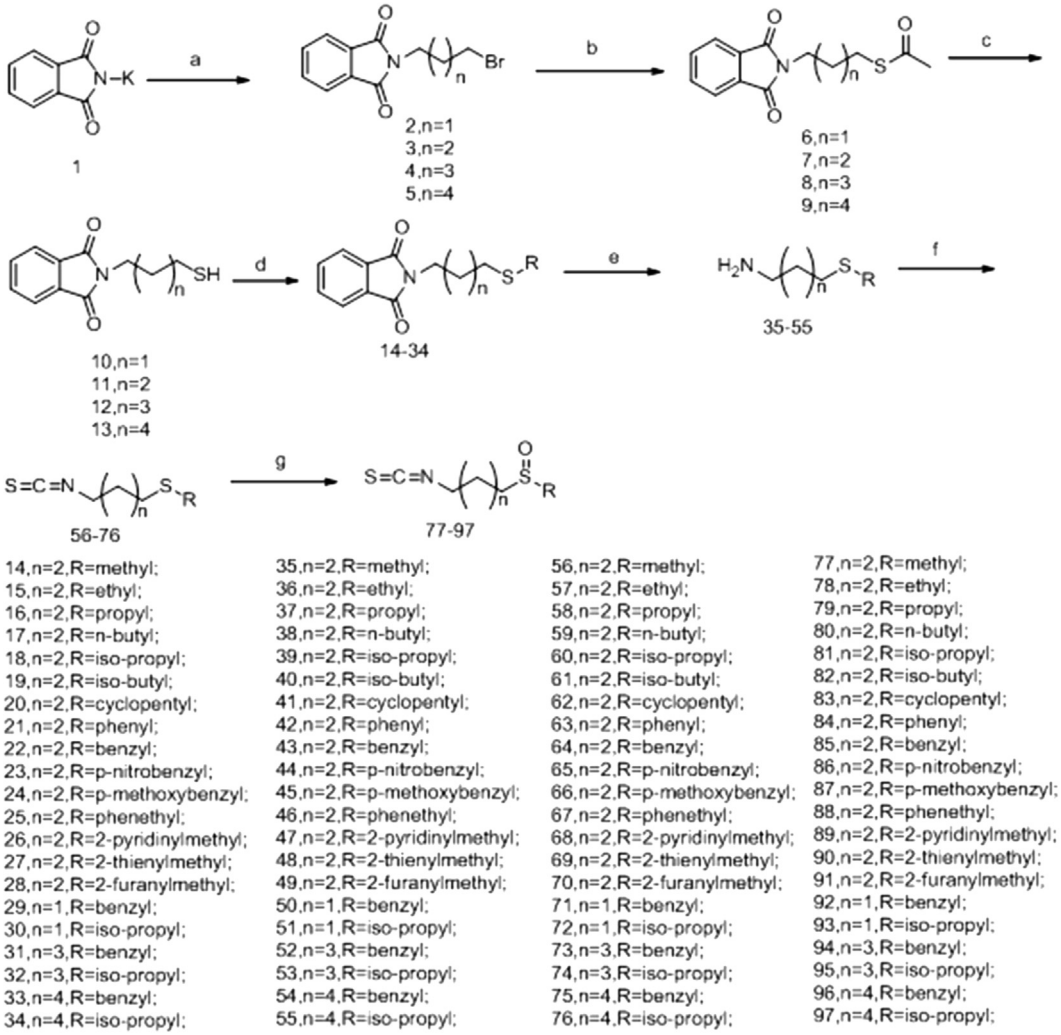
changed the length of spacer between the isothiocyanato and sulfoxide groups. The SFN derivatives (**78**–**97**) were obtained through the synthetic route shown in Scheme 1. Firstly, potassium phthalimide (**1**) was refluxed with different alkyl dibromides in acetone, affording mono-bromides **2**–**5**. Secondly, **2**–**5** were refluxed with potassium thioacetate (KSAc) in THF to give thioacetates **6**–**9** [21,22]. Thiols **10**–**13** were obtained by acidic hydrolysis of **6**–**9** in MeOH. In the following step, **10**–**13** were alkylated with various alkyl bromides in the presence of NaH (method A), affording thio-ethers **14**–**34**. While for phenyl group as the substituent (for synthesizing compound **21**), $Pd_2(dba)_3$ was employed as a catalyst for the alkylation of phenyl bromide (method B) [23]. However, when the same protocol was used for introducing other cyclic and heterocyclic groups, no desired products were obtained. After **14**–**34** were refluxed with 80% hydrazine hydrate in MeOH for 4 h, amines **35**–**55** were produced. By following the known procedure (treated with carbon disulfide, triethylamine and MsCl) [4], isothiocyanates **56**–**76** were obtained in satisfactory yields. Finally, the target compounds **77**–**97** were obtained by oxidizing **56**–**76** with 1 M equiv of MCPBA. When **64** was oxidized by 4 M equiv of MCPBA, sulfone **98** was obtained in 52% yield. The structures of all the final products **77**–**98** were confirmed by their MS, ^{1}H NMR and ^{13}C NMR spectral properties.

3. Results and discussion

3.1. In vitro cytotoxicity

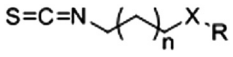
The synthesized SFN derivatives **64**, **78**–**98** were screened for in vitro cytotoxicity against five cancer cell lines, including HepG2 (Liver hepatocellular carcinoma), A549 (human lung adenocarcinoma), MCF-7 (human breast adenocarcinoma), HCT-116 (human colon cancer cell line) and SH-SY5Y (human neuroblastoma cell line), by the standard MTT assay, and using 5-Fu as a positive control. Antitumor potency of the compounds was indicated by IC_{50} values that were calculated by linear regression analysis of the concentration–response curves obtained for each compound, and the results were summarized in Table 1.

As shown in Table 1, all the derivatives exhibited more potent inhibitory activity against HepG2, A549, MCF-7, HCT-116 and SH-SY5Y than SFN, but for A549, HCT-116 and SH-SY5Y cell lines, most of the compounds showed weaker inhibitory activity than 5-Fu. Among the derivatives, **81** and **82** possess significant cytotoxicity against HepG2 with the IC_{50} values at 2.05 μM and 2.16 μM; **82** and **85** had stronger inhibitory activity against MCF-7 with the IC_{50} values at 3.66 μM and 3.30 μM respectively; derivative **91** with the IC_{50} value at 5.64 μM against A549, and derivative **87** with the IC_{50}



Scheme 1. Synthesis of SFN derivatives (**77**–**97**). Reagents and conditions: (a) alkyl dibromide, acetone, reflux, 10 h; (b) KSAc, THF, reflux, 3–5 h; (c) conc. HCl, MeOH, reflux, 3–4 h; (d) method A: NaH, R–Br, THF, rt, 1–2 h; method B: $Pd_2(dba)_3$, DPPF, toluene, bromobenzene, DIPEA, reflux, 2 h; (e) 80% hydrazine hydrate, MeOH, reflux, 3–4 h; (f) (1) Et_3N, CS_2, THF, 0 °C to rt, 90 min; (2) MsCl, 0 °C to rt, 30 min; (g) 85% MCPBA, DCM, 0 °C, 1 h.

Table 1
The cytotoxicity of sulforaphane derivatives.



Compd.	IC_{50} (μM)						R	*n*	X
	HepG2	A549	MCF-7	HCT-116	SH-SY5Y	Vero			
64	12.56	51.34	38.28	41.45	20. 71	24. 95	Benzyl	2	S
78	5.35	10.16	8.26	3.85	9.81	7.9	Ethyl	2	S=O
79	3.91	10.74	5.96	3.95	7.66	5.64	Propyl	2	S=O
80	5.93	9.56	5.84	2.81	6.47	5.15	*n*-Butyl	2	S=O
81	2.05	10.41	8.82	3.74	9.42	10.4	Iso-propyl	2	S=O
82	2.16	9.09	3.66	2.06	5.7	4.98	Iso-butyl	2	S=O
83	3.64	10.11	4.45	3.14	7.14	4.6	Cyclopentyl	2	S=O
84	6.34	23.8	7.05	4.53	8.75	4.33	Phenyl	2	S=O
85	2.85	7.41	3.3	3.66	5.21	4.85	Benzyl	2	S=O
86	3.18	14.35	7.77	2.83	7.23	3.36	p-Nitrobenzyl	2	S=O
87	4.62	32.55	8.89	5.46	2.79	3.71	p-Methoxybenzyl	2	S=O
88	6.97	10.21	9.33	8.02	5.6	6.82	Phenethyl	2	S=O
89	5.35	8.33	6.89	3.72	7.83	7.65	2-Pyridinylmethyl	2	S=O
90	3.82	8.41	4.14	3.21	5.31	6.22	2-Thienylmethyl	2	S=O
91	3.86	5.64	5.68	3.47	7.79	5.25	2-Furanylmethyl	2	S=O
92	9.84	11.82	7.92	15.64	8.89	10.14	Benzyl	1	S=O
93	7.14	16.7	7.93	15.92	18.05	8.25	Iso-propyl	1	S=O
94	3.91	6.21	3.83	5.19	7.01	5.85	Benzyl	3	S=O
95	3.69	7.75	6.78	3.42	8.62	10.28	Iso-propyl	3	S=O
96	6.17	14.69	5.49	11.57	7.63	4.33	Benzyl	4	S=O
97	7.82	11.99	9.76	9.46	7.82	7.31	Iso-propyl	4	S=O
98	8.49	8.89	7.55	6. 28	4. 78	7.17	Benzyl	2	O=S=O
SFN (77)	14.05	21.99	17.66	11.59	13.27	13.72	Methyl	2	S=O
5-Fu	24.96	8.71	10.14	3.24	0.97	9.71			

value at 2.79 μM against SH-SY5Y. Compared to 5-Fu, **80**, **82** and **86** possess more potent inhibitory effects against HCT-116 with the IC_{50} values at 2.81 μM, 2.06 μM and 2.83 μM respectively, reflecting the selectivity for a particular human HCT-116 cancer cell type.

From Table 1, the derivatives with branched alkyl groups (**81**, **82**) at side chains were more cytotoxic toward HepG2 cell growth in vitro than the derivatives with straight-chain alkyl groups (**78**, **79**, and **80**), cycloalkyl groups (**83**), phenyl groups (**84**, **86**–**88**) and heterocyclic groups (**89**–**91**). In contrast, the derivatives with heterocyclic side chains (**89**–**91**) were more potent for inhibiting A549 cell growth than those with alkyl groups (**78**–**83**) and aryl groups (**84**, **86**–**88**). For MCF-7, benzyl group substituted compounds (**85**, **94**) possess stronger inhibitory activity than other derivatives. The derivatives with branched alkyl groups (**78**–**82**) and heterocyclic groups (**89**–**91**) were more potent in inhibiting HCT-116 cell growth than those with other substituted groups. For SH-SY5Y, benzyl group substituted derivative (**87**) has the best activity with the IC_{50} value at 2.79 μM. A comparison of activity of benzyl derivatives **85**–**88** against SH-SY5Y indicates that an electron-donating group at para position of benzyl could significantly improve the activity, while an electron with drawing group has a detrimental effect. Compound (**64**) without sulfoxide group showed weaker inhibitory activity than most of the derivatives, and compound (**98**) with sulfone group showed better inhibitory activity against HepG2, A549, MCF-7, HCT-116 and SH-SY5Y than SFN. The results indicate that replacing the sulfoxide group with a sulfone group might be a general approach to boost the biological activity of this type of agents.

It is interesting to note that the cytotoxicity of the SFN derivatives is related to the length of the spacers between the isothiocyanato and sulfoxide groups: the derivatives with a shorter spacer (3-carbon spacer; such as **92** and **93**) and with a longer spacer (6-carbon spacer; such as **96** and **97**) have weaker cytotoxicity against most of the tested cancer cell lines than those with a 4-carbon spacer (**85** and **81**). The cytotoxicity of the SFN derivatives with different spacers has the following tendency: 3-carbon spacer < 6-carbon spacer < 5-carbon spacer < 4-carbon spacer. These results indicated that the introduction of branched alkyl and benzyl groups at the side chain might facilitate increasing the cytotoxic activities of the SFN derivatives, especially for those with a 4-carbon spacer.

3.2. Effect of SFN and derivative **85** on cell cycle arrest in HepG2

To assess whether cell growth inhibition induced by SFN and the most active derivative **85** is mediated via alternations in cell cycle progression, DNA cell cycle analysis was performed. As shown in Fig. 1, compared with the control, SFN and **85** treatment resulted in a dose-dependent accumulation of cells in the S phase accompanied by a decrease in G0/G1 phase (Fig. 1A). At the same time, the G2/M phase arrest could also be observed in the cells treated with **85**. The percentage of cells in the S fraction was increased by 5.9% and 9.11% when treated with 20 μM of SFN and **85**, respectively, compared to a vehicle control. The results indicated that SFN and **85** could induce S or G2/M phase cell cycle arrest, and **85** has more potent inhibitory activity than SFN.

3.3. Effect of SFN and **85** on cell apoptosis in HepG2

In order to evaluate whether the SFN derivatives-mediated inhibition in cell growth is due to the induction of apoptosis, Annexin-V/PI staining was used to detect the apoptotic ratio of SFN and **85** treated cells. As shown in Fig. 2, after treatment with 20 μM of SFN and **85** in HepG2 cells for 48 h, the apoptotic cells represented 5.09% and 25.57% of the total cells, respectively, whereas the control had only 0.43% apoptotic cells. The results indicated a greater induction of apoptosis upon treatment with **85** than SFN.

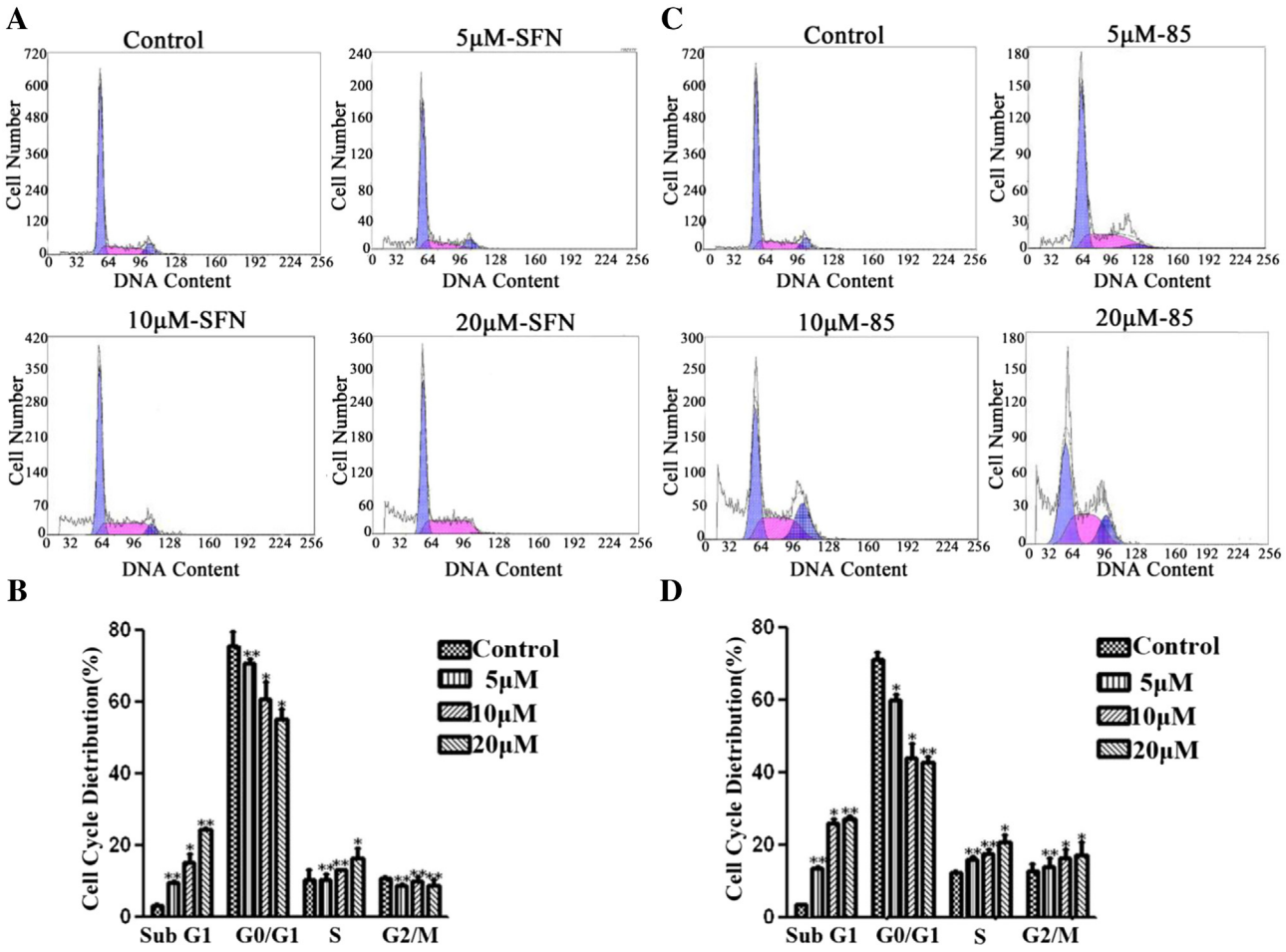


Fig. 1. Effects of SFN and **85** on cell cycle of HepG2 cells. Cells were treated with SFN (5, 10, 20 μM) and **85** (5, 10, 20 μM) for 48 h, and the DNA content of 10,000 events was analyzed by flow cytometry. The profiles showed the cell cycle (A and C) and the proportions (%) in each phase (B and D) of HepG2 cells treated with SFN and **85**, respectively. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to control.

3.4. Effect of SFN and **85** on Nrf2 transcription factor in HepG2

Nrf2/ARE pathway plays an important role in chemical prevention. It is of great significance to develop new Nrf2 targeted inducers for preventive treatment of tumor and multi-organs diseases. SFN has been found to be the strongest natural Nrf2 inducer. To investigate whether the new SFN derivatives possessed more potent Nrf2 activation effect, we carried out the Nrf2 activation assay by Trans^{AM} Nrf2 Kit. As shown in Fig. 3, SFN and **85** exhibited a time- and dose-dependent activation on Nrf2 transcription factor. Furthermore, **85** acted as a more potent Nrf2 inducer than SFN.

4. Conclusions

We have synthesized 22 SNF derivatives, and evaluated their antitumor activities against five cell lines (HepG2, A549, MCF-7, HCT-116 and SH-SY5Y). All the derivatives exhibited more potent inhibitory activity against HepG2, A549, MCF-7, HCT-116 and SH-SY5Y than SNF, and most of them displayed selective cytotoxicity toward HepG2 cell line. Our results indicate that the introduction of different groups other than methyl group, especially a branched alkyl or benzyl group, at side chain of SNF might contribute to higher cytotoxic activity of the SNF derivatives. The mechanism studies showed that the cytotoxic activity of these derivatives could be attributed to the induction of cell cycle arrest and apoptosis in cancer cells. SFN and derivative **85** exhibited time- and dose-dependent activation on Nrf2 transcription factor. Furthermore, **85** is a more potent Nrf2 inducer than SFN. The present study might provide valuable clues in the search for more potent and selective antitumor agents based on the scaffold of natural occurring isothiocyanates, such as SNF.

5. Experimental protocols

5.1. Synthesis

Reagents and solvents were purchased from commercial sources. Solvents were dried and purified using standard techniques. All reactions involving air or moisture sensitive reagents were performed under nitrogen atmosphere. NMR (^{1}H and ^{13}C) spectra were recorded on a Bruker AVANCE III; 400 and 500 Hz spectrometer. Mass spectra were recorded on a Shimadzu VG-Autospec-3000 mass spectrometer.

5.1.1. General procedures for preparation of **2**–**5**

Acetone (150 mL) and dibromoalkyl (30 mol) were added to a 250 mL three-necked round-bottom flask fitted with a mechanical stirrer and reflux condenser. Potassium phthalimide (11.85 g, 10 mol) was added slowly over a 15-min period, and then the reaction mixture was heated under reflux for 10 h. The reaction mixture was filtered via suction, and the acetone was removed via rotary evaporation. The crude product was purified by flash

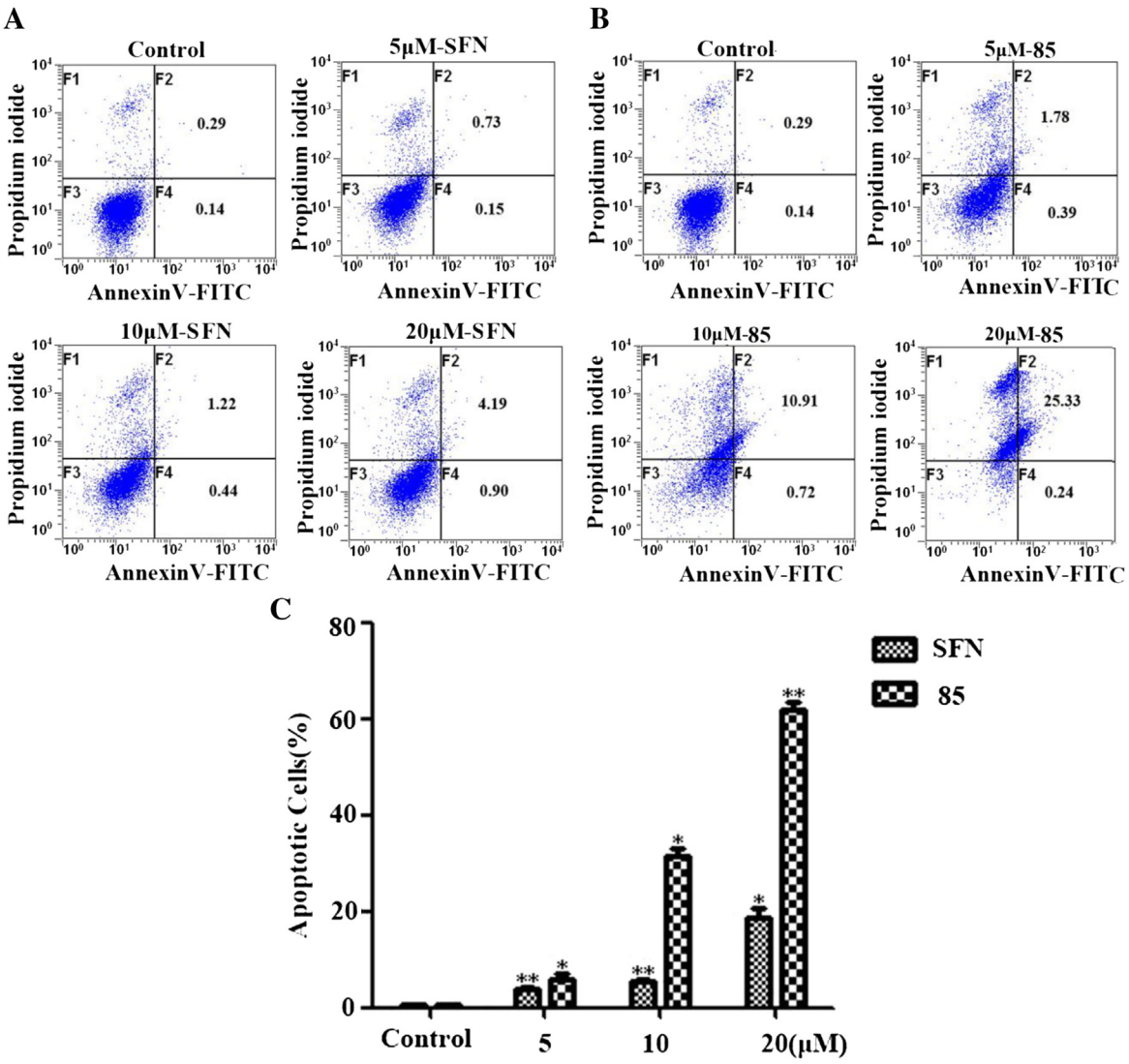


Fig. 2. Effects of SFN and **85** on cell apoptosis of HepG2 cells. (A and B) Induction of apoptosis were measured by Annexin-V/PI double-staining assay after treatment with SFN (5, 10 and 20 μM) and **85** (5, 10 and 20 μM) for 48 h. (C) The profiles showed the apoptotic proportion of HepG2 cells treated with SFN and **85**, respectively. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to control.

chromatography on silica gel (EtOAc:petroleum ether = 1:14) to afford **2**–**5** as white solid.

*5.1.1.1. 2-(4-Bromobutyl)isoindoline-1,3-dione (**3**).* Yield 75%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.84–1.94 (m, 4H), 3.45 (t, 2H, J = 6.0 Hz), 3.73 (t, 2H, J = 6.5 Hz), 7.73 (dd, 2H, J = 3.0, 5.5 Hz), 7.85 (dd, 2H, J = 3.0, 5.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 27.27, 29.89, 32.73, 36.99, 123.27, 132.11, 133.99, 168.36.

*5.1.1.2. 2-(3-Bromobutyl)isoindoline-1,3-dione (**2**).* Yield 68%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.23–2.30 (m, 2H), 3.42 (t, 2H, J = 6.8 Hz), 3.84 (t, 2H, J = 6.8 Hz), 7.73 (dd, 2H, J = 3.2, 5.2 Hz), 7.85 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.79, 31.67, 36.76, 123.33, 132.05, 134.06, 168.23.

*5.1.1.3. 2-(5-Bromobutyl)isoindoline-1,3-dione (**4**).* Yield 72%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.48–1.54 (m, 2H), 1.68–1.73 (m, 2H), 1.87–1.93 (m, 2H), 3.39 (t, 2H, J = 6.8 Hz), 3.70 (t, 2H, J = 7.2 Hz), 7.72 (dd, 2H, J = 3.2, 5.2 Hz), 7.85 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.42, 27.74, 32.22, 33.31, 37.68, 123.22, 132.17, 133.92, 168.39.

*5.1.1.4. 2-(6-Bromobutyl)isoindoline-1,3-dione (**5**).* Yield 71%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.33–1.41 (m, 2H), 1.45–1.53 (m, 2H), 1.66–1.73 (m, 2H), 1.82–1.89 (m, 2H), 3.39 (t, 2H, J = 6.4 Hz), 3.68 (t, 2H, J = 7.2 Hz), 7.72 (dd, 2H, J = 2.8, 5.2 Hz), 7.83 (dd, 2H, J = 2.8, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.02, 26.71, 27.40, 31.61, 32.60, 36.84, 122.17, 131.18, 132.86, 167.40.

*5.1.2. General procedures for preparation of **6**–**9***

To a stirred solution of **2**–**5** (5 mmol) in THF (60 mL) was added potassium thioacetate (15 mmol), and the mixture was refluxed for 3–4 h. And the solvent was evaporated to dryness in vacuo, and then added water (30 mL), and the organic materials were extracted with EtOAc (3 × 30 mL). The combined extracts were washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (EtOAc:petroleum ether = 1:8) to give compounds **6**–**9** as white powder.

*5.1.2.1. S-(4-(1,3-Dioxoisoindolin-2-yl)butyl)ethanethioate (**7**).* Yield 96%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.61–1.66 (m, 2H), 1.72–1.77 (m, 2H), 2.31 (s, 3H), 2.91 (t, 2H, J = 7.0 Hz), 3.69 (t, 2H,

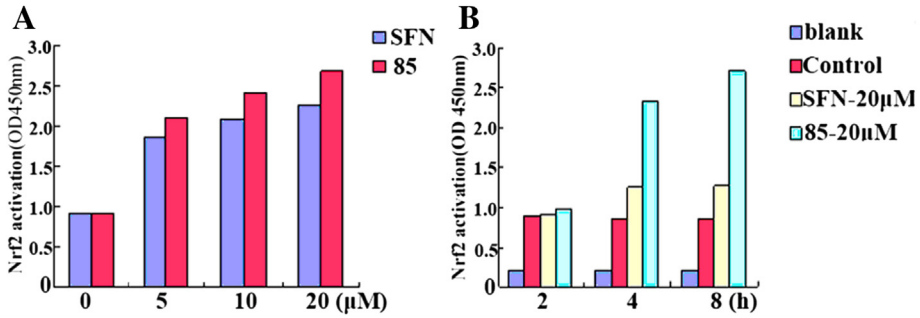


Fig. 3. Effects of SFN and **85** on Trans^{AM} Nrf2 activation of HepG2 cells. (A) Nrf2 activation was measured by Trans^{AM} Nrf2 Kit after treatment with SFN and **85** (20 μM) for different times (2, 4 and 8 h). (B) Nrf2 activation was measured after treatment with different concentrations of SFN and **85** (5, 10 and 20 μM) for 6 h.

J = 7.0 Hz), 7.72 (dd, 2H, J = 3.0, 5.5 Hz), 7.85 (dd, 2H, J = 3.0, 5.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 26.93, 27.70, 28.52, 30.60, 37.42, 123.24, 132.17, 133.93, 168.35, 195.60.

*5.1.2.2. S-(3-(1,3-Dioxoisoindolin-2-yl)propyl)ethanethioate (**6**).* Yield 75%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.94–2.01 (m, 2H), 2.32 (s, 3H), 2.90 (t, 2H, J = 7.2 Hz), 3.76 (t, 2H, J = 6.8 Hz), 7.72 (dd, 2H, J = 3.2, 5.2 Hz), 7.85 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.42, 28.67, 30.58, 36.91, 123.29, 132.11, 133.99, 168.30, 195.37.

*5.1.2.3. S-(5-(1,3-Dioxoisoindolin-2-yl)pentyl)ethanethioate (**8**).* Yield 87%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.37–1.45 (m, 2H), 1.58–1.73 (m, 4H), 2.85 (t, 2H, J = 7.2 Hz), 3.68 (t, 2H, J = 7.2 Hz), 7.70–7.72 (dd, 2H, J = 3.6, 5.2 Hz), 7.83–7.85 (dd, 2H, J = 3.2, 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.00, 28.11, 28.88, 29.11, 30.59, 37.78, 123.20, 132.21, 133.88, 168.39, 195.75.

*5.1.2.4. S-(6-(1,3-Dioxoisoindolin-2-yl)hexyl) ethanethioate (**9**).* Yield 97%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.35–1.40 (m, 4H), 1.53–1.58 (m, 2H), 1.65–1.69 (m, 2H), 2.30 (s, 3H), 2.85 (t, 2H, J = 7.2 Hz), 3.67 (t, 2H, J = 7.6 Hz), 7.70 (dd, 2H, J = 2.8, 5.2 Hz), 7.84 (dd, 2H, J = 2.8, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.35, 27.29, 27.41, 28.00, 28.33, 29.58, 36.89, 122.15, 131.21, 132.83, 167.39, 194.80.

*5.1.3. General procedures for preparation of **10**–**13***

A solution of **6**–**9** (10 mmol) in anhydrous methanol (30 mL) was first degassed and refilled with N_2. Concentrated HCl (4 mL) was added to the solution, and the resulting mixture was refluxed for 5 h under N_2 atmosphere until no more starting material could be detected by TLC. The reaction mixture was quenched with water (20 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give crude product, which was purified by flash chromatography on silica gel (EtOAc:petroleum ether = 1:12) to give compounds **10**–**13** as white powder.

*5.1.3.1. 2-(4-Mercaptobutyl)isoindoline-1,3-dione (**11**).* Yield 94%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.26 (t, 1H, J = 7.0 Hz), 1.63–1.69 (m, 2H), 1.77–1.83 (m, 2H), 2.55–2.59 (dd, 2H, J = 7.0 Hz), 3.70 (t, 2H, J = 7.0 Hz), 7.72 (dd, 2H, J = 3.0, 5.5 Hz), 7.84 (dd, 2H, J = 3.0, 5.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.09, 27.31, 31.10, 37.33, 123.24, 132.11, 133.95, 168.39.

*5.1.3.2. 2-(3-Mercaptopropyl)isoindoline-1,3-dione (**10**).* Yield 60%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.59 (t, 1H, J = 8.4 Hz), 1.97–2.04 (m, 2H), 2.56 (dd, 2H, J = 7.2, 14.8 Hz), 3.82 (t, 2H, J = 6.8 Hz), 7.72 (dd, 2H, J = 3.2, 5.2 Hz), 7.85 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.90, 32.74, 36.46, 123.28, 132.09, 134.01, 168.38.

*5.1.3.3. 2-(5-Mercaptopentyl)isoindoline-1,3-dione (**12**).* Yield 92%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.32 (t, 1H, J = 7.6 Hz), 1.41–1.48 (m, 2H), 1.62–1.73 (m, 4H), 2.52 (dd, 2H, J = 7.6, 14.8 Hz), 3.69 (t, 2H, J = 7.2 Hz), 7.71 (dd, 2H, J = 3.2, 5.2 Hz), 7.84 (dd, 2H, J = 3.2, 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.40, 25.58, 28.07, 33.50, 37.81, 123.20, 132.18, 133.90, 168.41.

*5.1.3.4. 2-(6-Mercaptohexyl)isoindoline-1,3-dione (**13**).* Yield 95%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.25–1.47 (m, 5H), 1.57–1.72 (m, 4H), 2.51 (dd, 2H, J = 7.6, 14.4 Hz), 3.68 (t, 2H, J = 7.2 Hz), 7.72 (dd, 2H, J = 4.0, 8.0 Hz), 7.85 (dd, 2H, J = 4.0, 8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.48, 25.29, 26.85, 27.45, 32.82, 36.89, 122.16, 131.19, 132.85, 167.41.

*5.1.4. General procedures for preparation of **14**–**34***

Method A: R–Br (18 mmol) was added to a mixture containing sodium hydride (30 mmol) and **10**–**13** (15 mmol) in dry THF (50 mL). The mixture was stirred at room temperature for 1–2 h. The reaction mixture was cooled to 0 °C and water (20 mL) was added. The solution was extracted with EtOAc (3 × 30 mL). The organic layer was separated, dried with anhydrous $MgSO_4$, and evaporated under vacuum. The crude product was purified using flash chromatography on silica gel (EtOAc:petroleum ether = 1:10) to give compounds **14**–**34**.

Method B: To a solution of [$Pd_2(dba)_3$] (0.10 mmol) and DPPF (1,1′-bis(diphenylphosphino)ferrocene) (0.20 mmol) in toluene (20 mL) were added bromobenzene (10 mmol), DIPEA (11 mmol) and **11** (10 mmol) at rt. The solution was stirred under reflux for 2 h then cooled to rt. The reaction was quenched by addition of H_2O and extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography on silica gel to afford **21**.

*5.1.4.1. 2-(4-(Methylthio)butyl)isoindoline-1,3-dione (**14**).* Yield 64%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.59–1.68 (m, 2H), 1.76–1.83 (m, 2H), 2.08 (s, 2H), 2.53 (t, 2H, J = 6.8 Hz), 3.71 (t, 2H, J = 7.2 Hz), 7.72 (dd, 2H, J = 3.2, 5.6 Hz), 7.84 (dd, 2H, J = 3.2, 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.51, 26.37, 27.71, 33.64, 37.50, 123.21, 132.12, 133.92, 168.38.

*5.1.4.2. 2-(4-(Ethylthio)butyl)isoindoline-1,3-dione (**15**).* Yield 65%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.24 (t, 3H, J = 7.2 Hz), 1.62–1.67 (m, 2H), 1.76–1.83 (m, 2H), 2.49–2.58 (m, 4H), 3.71 (t, 2H, J = 6.8 Hz), 7.71 (dd, 2H, J = 3.2, 5.2 Hz), 7.84 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.78, 25.91, 26.85, 27.82, 31.05, 37.51, 123.21, 132.12, 133.92, 168.40.

*5.1.4.3. 2-(4-(Propylthio)butyl)isoindoline-1,3-dione (**16**).* Yield 53%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.97 (t, 3H, J = 7.5 Hz), 1.55–1.66 (m, 4H), 1.76–1.82 (m, 2H), 2.47 (t, 2H, J = 7.0 Hz), 2.54 (t, 2H, J = 7.5 Hz), 3.71 (t, 2H, J = 7.0 Hz), 7.71 (dd, 2H, J = 3.0, 5.5 Hz), 7.84 (dd, 2H,

J = 3.0, 5.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.50, 22.99, 26.94, 27.81, 31.51, 34.21, 37.53, 123.22, 132.13, 133.92, 168.42.

*5.1.4.4. 2-(4-(Butylthio)butyl)isoindoline-1,3-dione (**17**).* Yield 55%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, 3H, J = 7.6 Hz), 1.36–1.43 (m, 2H), 1.51–1.67 (m, 4H), 1.76–1.83 (m, 2H), 2.47–2.56 (m, 4H), 3.70 (t, 2H, J = 7.2 Hz), 7.71 (dd, 2H, J = 2.8, 5.2 Hz), 7.84 (dd, 2H, J = 2.8, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.67, 22.01, 26.96, 27.82, 31.59, 31.81, 31.87, 37.55, 123.21, 132.18, 133.90, 168.39.

*5.1.4.5. 2-(4-(Isopropylthio)butyl)isoindoline-1,3-dione (**18**).* Yield 51%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.24 (d, 6H, J = 6.8 Hz), 1.59–1.66 (m, 2H), 1.76–1.83 (m, 2H), 2.56 (t, 2H, J = 7.2 Hz), 2.85–2.95 (m, 1H), 3.70 (t, 2H, J = 7.2 Hz), 7.71 (dd, 2H, J = 3.2, 5.2 Hz), 7.84 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.41, 23.45, 27.04, 27.92, 29.94, 34.78, 37.53, 123.20, 132.13, 133.90, 168.39.

*5.1.4.6. 2-(4-(Isobutylthio)butyl)isoindoline-1,3-dione (**19**).* Yield 57%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.97 (d, 6H, J = 6.8 Hz), 1.60–1.66 (m, 2H), 1.73–1.81 (m, 3H), 2.38 (d, 2H, J = 6.8 Hz), 2.53 (t, 2H, J = 7.2 Hz), 3.70 (t, 2H, J = 7.2 Hz), 7.71 (dd, 2H, J = 3.2, 5.6 Hz), 7.84 (dd, 2H, J = 3.2, 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.04, 26.99, 27.80, 28.63, 32.20, 37.53, 41.51, 123.20, 132.14, 133.91, 168.39.

*5.1.4.7. 2-(4-(Cyclopentylthio)butyl)isoindoline-1,3-dione (**20**).* Yield 50%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.44–1.83 (m, 10H), 1.93–1.99 (m, 2H), 2.57 (t, 2H, J = 7.2 Hz), 3.03–3.10 (m, 1H), 3.71 (t, 2H, J = 6.8 Hz), 7.71 (dd, 2H, J = 3.2, 5.6 Hz), 7.84 (dd, 2H, J = 3.2, 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.81, 27.07, 27.92, 31.31, 33.87, 37.55, 43.83, 123.20, 132.16, 133.90, 168.40.

*5.1.4.8. 2-(4-(Phenylthio)butyl)isoindoline-1,3-dione (**21**).* Yield 58%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.64–1.71 (m, 2H), 1.80–1.87 (m, 2H), 2.95 (t, 2H, J = 7.2 Hz), 3.69 (t, 2H, J = 7.2 Hz), 7.12–4 (t, 1H, J = 7.6 Hz), 7.23 (d, 2H, J = 7.6 Hz), 7.31 (d, 2H, J = 7.6 Hz), 7.71 (dd, 2H, J = 3.2, 5.2 Hz), 7.83 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.30, 27.60, 33.23, 37.40, 123.21, 125.96, 128.86, 129.40, 132.11, 133.91, 136.29, 168.35.

*5.1.4.9. 2-(4-(Benzylthio)butyl)isoindoline-1,3-dione (**22**).* Yield 69%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.54–1.62 (m, 2H), 1.71–1.78 (m, 2H), 2.45 (t, 2H, J = 7.2 Hz), 3.66 (t, 2H, J = 6.8 Hz), 3.68 (s, 2H), 7.17–7.28 (m, 5H), 7.70 (dd, 2H, J = 3.2, 4.8 Hz), 7.83 (dd, 2H, J = 3.2, 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.47, 27.75, 30.82, 36.29, 37.49, 123.20, 126.90, 128.45, 128.80, 132.11, 133.91, 138.47, 168.36.

*5.1.4.10. 2-(4-((4-Nitrobenzyl)thio)butyl)isoindoline-1,3-dione (**23**).* Yield 65%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.57–1.63 (m, 2H), 1.72–1.79 (m, 2H), 2.46 (t, 2H, J = 7.2 Hz), 3.69 (t, 2H, J = 6.8 Hz), 3.76 (s, 2H), 7.47 (d, 2H, J = 8.0 Hz), 7.70–7.73 (dd, 2H, J = 3.2, 8.0 Hz), 7.83–7.85 (dd, 2H, J = 3.2, 7.6 Hz), 8.16 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.35, 26.64, 30.09, 34.81, 36.32, 122.21, 122.74, 128.57, 131.03, 132.90, 132.98, 145.38, 145.96.

*5.1.4.11. 2-(4-((4-Methoxybenzyl)thio)butyl)isoindoline-1,3-dione (**24**).* Yield 68%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.57–1.63 (m, 2H), 1.71–1.78 (m, 2H), 2.44 (t, 2H, J = 6.8 Hz), 3.67 (t, 4H, J = 7.2 Hz), 3.78 (s, 3H), 6.82 (d, 2H, J = 8.4 Hz), 7.20 (d, 2H, J = 8.4 Hz), 7.71 (dd, 2H, J = 3.2, 4.8 Hz), 7.84 (dd, 2H, J = 3.2, 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.51, 27.79, 30.72, 35.65, 37.51, 55.26, 113.88, 123.19, 129.85, 130.41, 132.13, 133.90, 158.58, 168.36.

*5.1.4.12. 2-(4-(Phenethylsulfinyl)butyl)isoindoline-1,3-dione (**25**).* Yield 55%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.59–1.68 (m, 2H), 1.75–1.82 (m, 2H), 2.54–2.60 (m, 2H), 2.75 (t, 2H, J = 8.0 Hz), 2.86 (t, 2H, J = 7.2 Hz), 3.70 (t, 2H, J = 6.8 Hz), 7.19 (t, 2H, J = 7.2 Hz), 7.29 (d, 2H, J = 7.2 Hz), 7.70 (dd, 2H, J = 2.8, 5.2 Hz), 7.83 (dd, 2H, J = 2.4, 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.87, 26.77, 30.73, 32.64, 35.39, 36.47, 122.20, 125.29, 127.43, 127.47, 131.15, 132.89, 139.60, 167.36.

*5.1.4.13. 2-(4-((Pyridin-2-ylmethyl)thio)butyl)isoindoline-1,3-dione (**26**).* Yield 60%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.58–1.65 (m, 2H), 1.71–1.77 (m, 2H), 2.53 (t, 2H, J = 7.2 Hz), 3.66 (t, 2H, J = 7.2 Hz), 3.82 (s, 2H), 7.11–7.14 (m, 1H), 7.36 (d, 1H, J = 7.6 Hz), 7.61–7.65 (m, 1H), 7.70 (dd, 2H, J = 3.2, 5.6 Hz), 7.84 (dd, 2H, J = 3.2, 5.6 Hz), 8.49 (t, 1H, J = 4.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.55, 27.74, 31.17, 37.49, 38.23, 121.84, 123.00, 123.21, 132.15, 133.90, 136.67, 149.23, 158.96, 168.36.

*5.1.4.14. 2-(4-((Thiophen-2-ylmethyl)thio)butyl)isoindoline-1,3-dione (**27**).* Yield 70%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.56–1.64 (m, 2H), 1.73–1.80 (m, 2H), 2.53 (t, 2H, J = 7.2 Hz), 3.68 (t, 2H, J = 7.2 Hz), 3.90 (s, 2H), 6.86–6.91 (m, 2H), 7.15–7.17 (m, 1H), 7.71 (dd, 2H, J = 3.2, 5.6 Hz), 7.83 (dd, 2H, J = 3.2, 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.46, 27.78, 30.63, 31.10, 37.51, 123.23, 124.80, 125.97, 126.65, 126.88, 132.18, 133.93, 142.18, 168.37.

*5.1.4.15. 2-(4-((Furan-2-ylmethyl)thio)butyl)isoindoline-1,3-dione (**28**).* Yield 56%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.57–1.63 (m, 2H), 1.73–1.80 (m, 2H), 2.54 (t, 2H, J = 7.2 Hz), 3.67 (t, 2H, J = 6.8 Hz), 3.69 (s, 2H), 6.15 (d, 1H, J = 2.8 Hz), 6.26 (s, 1H), 7.32 (s, 1H), 7.71 (dd, 2H, J = 3.2, 5.2 Hz), 7.84 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.52, 27.75, 28.33, 31.24, 37.49, 107.33, 110.37, 123.21, 132.16, 133.92, 142.04, 151.82, 168.37.

*5.1.4.16. 2-(3-(Benzylthio)propyl)isoindoline-1,3-dione (**29**).* Yield 53%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.89–1.97 (m, 2H), 2.43 (t, 2H, J = 7.2 Hz), 3.73 (t, 2H, J = 7.2 Hz), 3.76 (s, 2H), 7.17–7.28 (m, 5H), 7.71 (dd, 2H, J = 3.2, 8.4 Hz), 7.84 (dd, 2H, J = 3.2, 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.05, 28.40, 36.01, 37.13, 123.23, 126.93, 128.46, 128.86, 132.17, 133.92, 138.13, 168.28.

*5.1.4.17. 2-(3-(Isopropylthio)propyl)isoindoline-1,3-dione (**30**).* Yield 50%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.25 (d, 6H, J = 6.8 Hz), 1.93–2.00 (m, 2H), 2.57 (t, 2H, J = 7.6 Hz), 2.89–2.94 (m, 1H), 3.79 (t, 2H, J = 7.2 Hz), 7.71 (dd, 2H, J = 3.2, 5.2 Hz), 7.83 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.38, 27.88, 28.66, 34.83, 37.31, 123.23, 132.14, 133.94, 168.34.

*5.1.4.18. 2-(5-(Benzylthio)pentyl)isoindoline-1,3-dione (**31**).* Yield 85%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.38–1.43 (m, 2H), 1.56–1.69 (m, 4H), 2.40 (t, 2H, J = 7.2 Hz), 3.66 (t, 2H, J = 7.2 Hz), 3.69 (s, 2H), 7.20–7.30 (m, 5H), 7.71 (dd, 2H, J = 3.2, 5.2 Hz), 7.84 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.05, 28.18, 28.76, 31.10, 36.26, 37.81, 123.18, 126.88, 128.45, 128.82, 132.15, 133.88, 138.56, 168.41.

*5.1.4.19. 2-(5-(Isopropylthio)pentyl)isoindoline-1,3-dione (**32**).* Yield 51%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.24 (d, 6H, J = 6.4 Hz), 1.41–1.48 (m, 2H), 1.59–1.73 (m, 4H), 2.51 (t, 2H, J = 7.6 Hz), 2.86–2.93 (m, 1H), 3.68 (t, 2H, J = 7.2 Hz), 7.71 (dd, 2H, J = 3.2, 5.2 Hz), 7.84 (dd, 2H, J = 3.2, 5.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.44, 26.26, 28.23, 29.42, 30.31, 34.80, 37.88, 123.18, 132.22, 133.87, 168.41.

*5.1.4.20. 2-(6-(Benzylthio)hexyl)isoindoline-1,3-dione (**33**).* Yield 77%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.25–1.42 (m, 4H), 1.51–1.58 (m, 2H), 1.61–1.69 (m, 2H), 2.39 (t, 2H, J = 7.2 Hz), 3.66 (t, 4H, J = 7.2 Hz), 7.20–7.30 (m, 5H), 7.70 (dd, 2H, J = 2.8, 5.6 Hz), 7.83 (dd, 2H, J = 2.8, 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.43, 27.33, 27.43, 28.03, 30.25, 35.29, 36.91, 122.14, 125.84, 127.42, 127.80, 131.19, 132.82, 137.62, 167.39.

*5.1.4.21. 2-(6-(Isopropylthio)hexyl)isoindoline-1,3-dione (**34**).* Yield 50%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.24 (d, 6H, J = 6.8 Hz), 1.32–1.47 (m, 4H), 1.54–1.72 (m, 4H), 2.51 (t, 2H, J = 7.6 Hz), 2.86–2.92 (m, 1H), 3.68 (t, 2H, J = 7.2 Hz), 7.70 (dd, 2H, J = 2.8, 5.6 Hz), 7.83 (dd, 2H, J = 2.8, 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.50, 27.48, 27.55, 28.67, 29.43, 33.77, 36.95, 122.14, 131.20, 132.82, 167.40.

*5.1.5. General procedures for preparation of **56**–**76***

N-Cinnamylphthalimide **14**–**34** (10 mmol) was mixed with 80% hydrazine hydrate (25 mmol) and MeOH (60 mL), and the mixture was stirred at 70 °C for 3–4 h. After the solvent was removed by a rotary evaporator, the solid residue was treated with 1 N NaOH (30 mL), and the resulting mixture was extracted with EtOAc (4 × 30 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent under reduced pressure afforded the crude product **35**–**55**, which were used for next step without purification.

Et_3N (16 mmol) was added to a solution of amine **35**–**55** (5 mmol) in anhydrous THF (30 mL), and the mixture was cooled to 0 °C in an ice bath. Carbon disulfide (5.5 mmol) was then added to the reaction mixture slowly. When the addition was completed, the ice bath was removed and the mixture was stirred at room temperature for 90 min. The reaction mixture was cooled to 0 °C and MsCl (5.5 mmol) was added in one portion. The ice bath was removed and the mixture was stirred at room temperature for 30 min. The resulting yellow suspension was diluted with DCM (40 mL), washed successively with 1 M HCl (2 × 15 mL), water (15 mL), and brine (20 mL), and then dried with anhydrous $MgSO_4$. The solvent was concentrated in vacuo and the crude product was purified by flash chromatography (EtOAc:petroleum ether = 1:10) to give a light-yellow oil **56**–**76**.

*5.1.5.1. (4-Isothiocyanatobutyl)(methyl)sulfane (**56**).* Yield 48%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.70–1.86 (m, 4H), 2.11 (s, 3H), 2.54 (t, 2H, J = 6.8 Hz), 3.56 (t, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.45, 25.86, 28.88, 33.32, 44.74, 130.39.

*5.1.5.2. Ethyl(4-isothiocyanatobutyl)sulfane (**57**).* Yield 54%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (t, 3H, J = 7.6 Hz), 1.69–1.86 (m, 4H), 2.51–2.58 (m, 4H), 3.55 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.75, 25.91, 26.35, 28.96, 30.72, 44.73, 130.19.

*5.1.5.3. (4-Isothiocyanatobutyl)(propyl)sulfane (**58**).* Yield 47%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.99 (t, 3H, J = 5.6 Hz), 1.55–1.65 (m, 2H), 1.69–1.75 (m, 2H), 1.79–1.85 (m, 2H), 2.48–2.57 (m, 4H), 3.5 (t, 2H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.50, 22.95, 26.44, 28.97, 31.16, 34.19, 44.74, 130.24.

*5.1.5.4. Butyl(4-isothiocyanatobutyl)sulfane (**59**).* Yield 50%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.92 (t, 3H, J = 7.2 Hz), 1.38–1.46 (m, 2H), 1.53–1.60 (m, 2H), 1.68–1.85 (m, 4H), 2.49–2.57 (m, 4H), 3.56 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.69, 22.02, 26.46, 29.00, 31.25, 31.77, 31.87, 44.76, 130.44.

*5.1.5.5. Isopropyl(4-isothiocyanatobutyl)sulfane (**60**).* Yield 43%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (d, 6H, J = 6.8 Hz), 1.68–1.86 (m, 4H), 2.57 (t, 2H, J = 6.8 Hz), 2.88–2.95 (m, 1H), 3.55 (t, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.36, 23.39, 26.57, 29.05, 29.58, 34.85, 44.71, 130.05.

*5.1.5.6. Isobutyl(4-isothiocyanatobutyl)sulfane (**61**).* Yield 45%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.99 (d, 6H, J = 6.8 Hz), 1.68–1.85 (m, 5H), 2.40 (d, 2H, J = 6.8 Hz), 2.54 (t, 2H, J = 7.2 Hz), 3.55 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.05, 26.52, 28.65, 29.00, 31.89, 41.54, 44.77, 130.50.

*5.1.5.7. Cyclopentyl(4-isothiocyanatobutyl)sulfane (**62**).* Yield 49%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.48–1.86 (m, 10H), 1.96–2.02 (m, 2H), 2.58 (t, 2H, J = 7.2 Hz), 3.05–3.12 (m, 1H), 3.55 (t, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.80, 26.59, 29.07, 30.96, 33.86, 43.85, 44.74, 130.26.

*5.1.5.8. (4-Isothiocyanatobutyl)(phenyl)sulfane (**63**).* Yield 65%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.74–1.86 (m, 4H), 2.95 (t, 2H, J = 7.2 Hz), 3.53 (t, 2H, J = 6.0 Hz), 7.09–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.08, 28.86, 33.09, 44.67, 126.28, 129.03, 129.60, 130.50, 135.95.

*5.1.5.9. Benzyl(4-isothiocyanatobutyl)sulfane (**64**).* Yield 65%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.62–1.79 (m, 4H), 2.43 (t, 2H, J = 6.8 Hz), 3.47 (t, 2H, J = 6.0 Hz), 3.71 (s, 2H), 7.22–7.34 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.95, 28.88, 30.28, 36.20, 44.63, 127.06, 128.54, 128.84, 130.25, 138.25.

*5.1.5.10. (4-Isothiocyanatobutyl)(4-nitrobenzyl)sulfane (**65**).* Yield 62%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.66–1.79 (m, 4H), 2.45 (t, 2H, J = 7.2 Hz), 3.52 (t, 2H, J = 6.0 Hz), 3.78 (s, 2H), 7.50 (d, 2H, J = 8.4 Hz), 8.19 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.94, 28.91, 30.63, 35.67, 44.65, 123.85, 128.33, 129.66, 146.11, 147.07.

*5.1.5.11. (4-Isothiocyanatobutyl)(4-methoxybenzyl)sulfane (**66**).* Yield 61%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.64–1.79 (m, 4H), 2.42 (t, 2H, J = 7.2 Hz), 3.49 (t, 2H, J = 6.0 Hz), 3.67 (s, 2H), 3.80 (s, 3H), 6.85 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.04, 28.98, 30.24, 35.61, 44.70, 55.35, 114.00, 128.38, 129.94, 130.22, 158.72.

*5.1.5.12. (4-Isothiocyanatobutyl)(phenethyl)sulfane (**67**).* Yield 63%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.67–1.81 (m, 4H), 2.54 (t, 2H, J = 6.8 Hz), 2.78 (t, 2H, J = 7.6 Hz), 2.89 (t, 2H, J = 8.0 Hz), 3.52 (t, 2H, J = 5.6 Hz), 7.20–7.32 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.92, 27.94, 30.42, 32.64, 35.35, 43.70, 125.38, 127.48, 127.50, 129.46, 139.44.

*5.1.5.13. 2-(((4-Isothiocyanatobutyl)thio)methyl)pyridine (**68**).* Yield 64%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.65–1.80 (m, 4H), 2.53 (t, 2H, J = 7.2 Hz), 3.49 (t, 2H, J = 6.4 Hz), 3.83 (s, 2H), 7.16–7.19 (m, 1H), 7.37 (d, 1H, J = 7.6 Hz), 7.64–7.69 (m, 1H), 8.52–8.54 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.08, 28.87, 30.67, 38.10, 44.64, 121.97, 123.07, 130.19, 136.77, 149.26, 158.77.

*5.1.5.14. 2-(((4-Isothiocyanatobutyl)thio)methyl)thiophene (**69**).* Yield 51%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.42–1.81 (m, 4H), 2.52 (t, 2H, J = 6.8 Hz), 3.50 (t, 2H, J = 6.4 Hz), 3.93 (s, 2H), 6.93 (d, 2H, J = 5.2 Hz), 7.21 (d, 1H, J = 4.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.91, 28.91, 30.53, 30.56, 44.65, 124.96, 126.11, 126.70, 130.47, 141.90.

*5.1.5.15. 2-(((4-Isothiocyanatobutyl)thio)methyl)furan (**70**).* Yield 50%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.64–1.81 (m, 4H), 2.53 (t, 2H, J = 6.8 Hz), 3.51 (t, 2H, J = 6.4 Hz), 3.72 (s, 2H), 6.18–6.19 (m, 1H), 6.31–6.32 (m, 1H), 7.36–7.37 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.07, 28.31, 28.93, 30.85, 44.69, 107.51, 110.47, 130.54, 142.16, 151.63.

*5.1.5.16. Benzyl(3-isothiocyanatopropyl)sulfane (**71**).* Yield 44%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.84–1.91 (m, 2H), 2.52 (t, 2H, J = 6.8 Hz), 3.59 (t, 2H, J = 6.4 Hz), 3.71 (s, 2H), 7.25–7.34 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.02, 29.35, 36.38, 43.70, 127.19, 128.63, 128.81, 137.98.

*5.1.5.17. Isopropyl(3-isothiocyanatopropyl)sulfane (**72**).* Yield 56%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.28 (d, 6H, J = 6.8 Hz), 1.92–1.99 (m, 2H), 2.65 (t, 2H, J = 6.8 Hz), 2.89–2.95 (m, 1H), 3.66 (t, 2H,

J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.40, 27.19, 30.00, 35.15, 43.88, 130.92.

*5.1.5.18. Benzyl(5-isothiocyanatopentyl)sulfane (**73**).* Yield 47%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.42–1.69 (m, 6H), 2.42 (t, 2H, J = 6.8 Hz), 3.47 (t, 2H, J = 6.8 Hz), 3.70 (s, 2H), 7.22–7.34 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.76, 26.97, 28.44, 29.58, 36.47, 44.95, 127.01, 128.53, 128.86, 130.18, 138.54.

*5.1.5.19. Isopropyl(5-isothiocyanatopentyl)sulfane (**74**).* Yield 55%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (d, 6H, J = 6.8 Hz), 1.42–1.75 (m, 6H), 2.54 (t, 2H, J = 7.2 Hz), 2.88–2.94 (m, 1H), 3.52 (t, 2H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.48, 26.00, 29.05, 29.69, 30.25, 34.95, 45.00, 130.00.

*5.1.5.20. Benzyl(6-isothiocyanatohexyl)sulfane (**75**).* Yield 45%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.36–1.41 (m, 2H), 1.54–1.70 (m, 4H), 2.42 (t, 2H, J = 7.2 Hz), 3.48 (t, 2H, J = 6.4 Hz), 3.70 (s, 2H), 7.21–7.33 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.11, 26.89, 27.89, 28.79, 30.20, 35.39, 43.95, 125.90, 127.44, 127.80, 128.99, 137.57.

*5.1.5.21. Isopropyl(6-isothiocyanatohexyl)sulfane (**76**).* Yield 58%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (d, 6H, J = 6.8 Hz), 1.42–1.47 (m, 4H), 1.57–1.74 (m, 4H), 2.53 (t, 2H, J = 7.2 Hz), 2.86–2.96 (m, 1H), 3.51 (t, 2H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.47, 26.23, 28.15, 29.54, 29.88, 30.37, 34.90, 45.01, 130.01.

*5.1.6. General procedures for preparation of **77–97***

A solution of isothiocyanate **56–76** (3 mmol) in CH_2Cl_2 (20 mL) was chilled to −10 °C in an ice–salt bath. A solution of 85% MCPBA (3 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 5 min, while the internal temperature was kept below 0 °C. When the addition was completed, the mixture was stirred at 0 °C for 1 h, and then the resulting light-yellow suspension was treated with saturated $NaHCO_3$ (15 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined CH_2Cl_2 layers were washed with saturated $NaHCO_3$ (30 mL) and brine (30 mL), then dried with anhydrous $MgSO_4$ and concentrated with a rotary evaporator. The crude product was purified by flash chromatography (MeOH:CH_2Cl_2 = 1:20) to give a light-yellow oil **77–97**.

*5.1.6.1. 1-Isothiocyanato-4-(methylsulfinyl)butane (**77**).* Yield 85%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.83–1.99 (m, 4H), 2.61 (s, 3H), 2.67–2.80 (m, 2H), 3.61 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.11, 29.03, 38.74, 44.68, 53.51, 131.10; MS (ESI, m/z): $[M + 1]^+$ 177.8.

*5.1.6.2. 1-(Ethylsulfinyl)-4-isothiocyanatobutane (**78**).* Yield 90%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.35 (t, 3H, J = 7.2 Hz), 1.83–1.99 (m, 4H), 2.62–2.78 (m, 4H), 3.60 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 6.81, 20.18, 29.10, 44.68, 45.90, 50.55, 130.95; MS (ESI, m/z): $[M + 1]^+$ 191.9.

*5.1.6.3. 1-Isothiocyanato-4-(propylsulfinyl)butane (**79**).* Yield 93%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.10 (t, 3H, J = 7.2 Hz), 1.78–1.99 (m, 6H), 2.58–2.77 (m, 4H), 3.60 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.41, 16.32, 20.20, 29.13, 44.71, 51.31, 54.60, 131.19; MS (ESI, m/z): $[M + 1]^+$ 205.9.

*5.1.6.4. 1-(Butylsulfinyl)-4-isothiocyanatobutane (**80**).* Yield 90%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.97 (t, 3H, J = 7.6 Hz), 1.44–1.55 (m, 2H), 1.70–1.99 (m, 6H), 2.62–2.75 (m, 4H), 3.60 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.68, 20.21, 22.08, 24.62, 29.14, 44.71, 51.29, 52.43, 131.19; MS (ESI, m/z): $[M + 1]^+$ 220.0.

*5.1.6.5. 1-(Isopropylsulfinyl)-4-isothiocyanatobutane (**81**).* Yield 82%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.28 (d, 3H, J = 6.8 Hz), 1.32 (d, 3H, J = 6.8 Hz), 1.83–2.00 (m, 4H), 2.58–2.67 (m, 2H), 2.76–2.82 (m, 1H), 3.60 (t, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.72, 15.97, 20.46, 20.50, 29.14, 44.70, 47.60, 50.56, 130.73; MS (ESI, m/z): $[M + 1]^+$205.9.

*5.1.6.6. 1-(Isobutylsulfinyl)-4-isothiocyanatobutane (**82**).* Yield 83%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.09 (d, 3H, J = 5.2 Hz), 1.11 (d, 3H, J = 5.2 Hz), 1.83–2.00 (m, 4H), 2.18–2.29 (m, 1H), 2.36–2.42 (m, 1H), 2.63–2.75 (m, 3H), 3.60 (t, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.16, 21.71, 22.88, 23.96, 29.11, 44.69, 51.89, 62.16, 131.04; MS (ESI, m/z): $[M + 1]^+$ 220.0.

*5.1.6.7. ((4-Isothiocyanatobutyl)sulfinyl)cyclopentane (**83**).* Yield 81%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.55–2.00 (m, 11H), 2.12–2.21 (m, 1H), 2.64–2.67 (m, 2H), 3.01–3.0 (m, 1H), 3.60 (t, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.69, 26.19, 27.41, 29.15, 44.71, 49.87, 60.00, 130.83; MS (ESI, m/z): $[M + 1]^+$ 232.0.

*5.1.6.8. ((4-Isothiocyanatobutyl)sulfinyl)benzene (**84**).* Yield 90%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.74–1.93 (m, 4H), 2.79–2.85 (m, 2H), 3.53–3.56 (m, 2H), 7.51–7.64 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.56, 28.99, 44.65, 55.97, 123.96, 129.37, 131.17 (2×C), 143.52; MS (ESI, m/z): $[M + 23]^+$ 261.9.

*5.1.6.9. (((4-Isothiocyanatobutyl)sulfinyl)methyl)benzene (**85**).* Yield 90%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.75–1.94 (m, 4H), 2.59 (t, 2H, J = 6.8 Hz), 3.54 (t, 2H, J = 6.4 Hz), 4.02 (dd, 2H, J = 12.8, 43.2 Hz), 7.28–7.42 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.01, 29.08, 44.64, 49.73, 58.48, 128.51, 129.08, 129.22, 129.99, 131.08; MS (ESI, m/z): $[M + 1]^+$ 254.0.

*5.1.6.10. 1-(((4-Isothiocyanatobutyl)sulfinyl)methyl)-4-nitrobenzene (**86**).* Yield 72%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.81–1.99 (m, 4H), 2.66 (t, 2H, J = 7.2 Hz), 3.55 (t, 2H, J = 6.4 Hz), 4.06 (dd, 2H, J = 13.2, 37.2 Hz), 7.50 (d, 2H, J = 8.8 Hz), 8.26 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.19, 29.00, 44.66, 50.52, 57.28, 124.06, 131.09 (2 × C), 137.21, 147.98; MS (ESI, m/z): $[M + 1]^+$ 299.0.

*5.1.6.11. 1-(((4-Isothiocyanatobutyl)sulfinyl)methyl)-4-methoxybenzene (**87**).* Yield 72%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.77–1.94 (m, 4H), 2.57 (t, 2H, J = 6.8 Hz), 3.55 (t, 2H, J = 6.4 Hz), 3.81 (s, 3H), 3.97 (dd, 2H, J = 12.8, 38 Hz), 6.91 (d, 2H, J = 8.4 Hz), 7.21 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.00, 29.09, 44.64, 49.49, 55.32, 57.69, 114.52, 121.35, 130.87, 131.17, 159.80; MS (ESI, m/z): $[M + 23]^+$ 306.0.

*5.1.6.12. (2-((4-Isothiocyanatobutyl)sulfinyl)ethyl)benzene (**88**).* Yield 90%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.82–1.98 (m, 4H), 2.61–2.77 (m, 2H), 2.82–3.18 (m, 4H), 3.57 (t, 2H, J = 6.4 Hz), 7.24–7.34 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.23, 27.81, 28.07, 43.65, 50.42, 53.02, 125.86, 127.56, 127.84, 130.23, 137.73; MS (ESI, m/z): $[M + 1]^+$ 268.0.

*5.1.6.13. 2-(((4-Isothiocyanatobutyl)sulfinyl)methyl)pyridine(**89**).* Yield 85%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.81–1.95 (m, 4H), 2.67–2.82 (m, 2H), 3.56 (t, 2H, J = 6.4 Hz), 4.17 (dd, 2H, J = 12.8, 35.6 Hz), 7.29 (t, 1H, J = 4.8 Hz), 7.37 (d, 1H, J = 7.6 Hz), 7.70–7.72 (m, 1H), 8.62 (d, 1H, J = 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.03, 29.06, 44.61, 50.33, 59.63, 123.71, 125.36, 130.95, 136.91, 150.03, 150.54; MS (ESI, m/z): $[M + 1]^+$ 254.0.

*5.1.6.14. 2-(((4-Isothiocyanatobutyl)sulfinyl)methyl)thiophene (**90**).* Yield 73%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.79–1.94 (m, 4H), 2.61 (t,

2H, J = 5.2 Hz), 3.55 (t, 2H, J = 6.4 Hz), 4.12 (s, 2H), 7.05 (t, 2H, J = 3.2 Hz), 7.32 (t, 1H, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.94, 29.08, 44.63, 49.74, 52.31, 126.83, 127.69, 128.79, 130.03, 131.16; MS (ESI, m/z): $[M + 1]^+$ 259.9.

*5.1.6.15. 2-(((4-Isothiocyanatobutyl)sulfinyl)methyl)furan (**91**).* Yield 77%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.82–1.94 (m, 4H), 2.64–2.68 (m, 2H), 3.57 (t, 2H, J = 6.4 Hz), 4.08 (dd, 2H, J = 14.0, 22 Hz), 6.40–6.42 (m, 2H), 7.44 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.88, 29.11, 44.63, 50.45, 50.76, 111.29, 111, 39, 131.08, 143.57, 143.71; MS (ESI, m/z): $[M + 1]^+$ 243.9.

*5.1.6.16. (((3-Isothiocyanatopropyl)sulfinyl)methyl)benzene (**92**).* Yield 86%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.13–2.20 (m, 2H), 2.60–2.74 (m, 2H), 3.68 (t, 2H, J = 6.4 Hz), 4.04 (dd, 2H, J = 12.8, 38 Hz), 7.29–7.42 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.52, 44.15, 47.23, 58.63, 128.64, 129.17, 129.27, 130.00, 132.12; MS (ESI, m/z): $[M + 1]^+$ 239.9.

*5.1.6.17. 1-(Isopropylsulfinyl)-3-isothiocyanatopropane (**93**).* Yield 82%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.29 (d, 3H, J = 6.8 Hz), 1.34 (d, 3H, J = 6.8 Hz), 2.19–2.26 (m, 2H), 2.65–2.75 (m, 2H), 2.77–2.85 (m, 1H), 3.68–3.82 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.80, 15.90, 24.01, 44.26, 45.19, 50.97, 53.45, 132.29; MS (ESI, m/z): $[M + 1]^+$ 191.9.

*5.1.6.18. (((5-Isothiocyanatopentyl)sulfinyl)methyl)benzene (**94**).* Yield 87%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.47–1.63 (m, 2H), 1.67–1.86 (m, 4H), 2.56–2.60 (m, 2H), 3.51 (t, 2H, J = 6.4 Hz), 4.01 (dd, 2H, J = 12.8, 48 Hz), 7.28–7.41 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.92, 25.87, 29.58, 44.75, 50.46, 53.48, 58.51, 128.46, 129.06, 129.75, 129.99, 130.30; MS (ESI, m/z): $[M + 1]^+$ 267.9.

*5.1.6.19. 1-(Isopropylsulfinyl)-5-isothiocyanatopentane (**95**).* Yield 83%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (d, 3H, J = 6.8 Hz), 1.32 (d, 3H, J = 6.8 Hz), 1.53–1.90 (m, 6H), 2.56–2.66 (m, 2H), 2.75–2.80 (m, 1H), 3.55 (t, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.77, 16.00, 22.36, 26.03, 29.68, 44.82, 48.29, 50.50, 53.48, 130.33; MS (ESI, m/z): $[M + 1]^+$ 220.0.

*5.1.6.20. (((6-Isothiocyanatohexyl)sulfinyl)methyl)benzene (**96**).* Yield 88%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.39–1.50 (m, 4H), 1.65–1.82 (m, 4H), 2.56 (t, 2H, J = 7.6 Hz), 3.49 (t, 2H, J = 6.8 Hz), 3.99 (dd, 2H, J = 12.8, 41.6 Hz), 7.28–7.40 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.27, 25.10, 26.90, 28.57, 43.85, 49.63, 57.46, 127.33, 127.95, 128.87, 128.94 (2 × C); MS (ESI, m/z): $[M + 23]^+$ 303.9.

*5.1.6.21. 1-(Isopropylsulfinyl)-6-isothiocyanatohexane (**97**).* Yield 84%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (d, 3H, J = 6.8 Hz), 1.31 (d, 3H, J = 6.8 Hz), 1.48–1.86 (m, 8H), 2.53–2.65 (m, 2H), 2.71–2.81 (m, 1H), 3.52 (t, 2H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.69, 16.03, 22.73, 26.21, 28.09, 29.67, 44.94, 48.41, 50.35, 130.16; MS (ESI, m/z): $[M + 23]^+$ 256.0.

*5.1.7. Procedures for preparation of **98***

A solution of 85% MCPBA (12 mmol) in CH_2Cl_2 (20 mL) was added to a solution of isothiocyanate **64** (3 mmol) in CH_2Cl_2 (20 mL) over 15 min at room temperature, when the addition was completed, the mixture was stirred for 3 h, and then the resulting white suspension was treated with saturated $NaHCO_3$ (50 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined CH_2Cl_2 layers were washed with saturated $NaHCO_3$ (50 mL) and brine (30 mL), then dried with anhydrous $MgSO_4$ and concentrated with a rotary evaporator. The crude product was purified by flash chromatography (CH_2Cl_2) to give a white solid **98**. Yield 52%; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.78–1.84 (m, 2H), 1.90–1.96 (m, 2H), 2.85 (t, 2H, J = 7.5 Hz), 3.54 (t, 2H, J = 6.5 Hz), 4.25 (s, 2H), 7.42 (s, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.24, 28.61, 44.45, 49.86, 59.83, 127.85, 129.22, 130.50, 131.09; MS (ESI, m/z): $[M + 23]^+$ 291.95.

5.2. Cell line and culture condition

HepG2 (Liver hepatocellular carcinoma), A549 (human lung adenocarcinoma), MCF-7 (human breast adenocarcinoma), HCT-116 (human colon cancer cell line), SH-SY5Y (human neuroblastoma cell line) and Vero (Africa green monkey kidney cell line) were kindly provided by Shanghai Jiao Tong University. The cell was routinely cultured in RPMI-1640 medium or DMEM, supplemented with 10% neonatal bovine serum (NBS) or 10% fetal bovine serum (FBS). The culture was maintained at 37 °C with a gas mixture of 5% CO_2/95% air. All media were supplement with 100 U/mL penicillin and 100 μg/mL streptomycin.

5.3. Cell viability assay

The cytotoxic activity in vitro was measured using the MTT assay. The MTT solution (10.0 μL/well) was added in culture media after cells were treated with various concentrations of compounds for 72 h, and cells were incubated for further 4 h at 37 °C. The purple formazan crystals were dissolved in 100 mL DMSO. After 10 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 570 nm and 630 nm. Assays were performed in triplicate on three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software "Dose–Effect Analysis with Microcomputers". The tumor cell lines panel consisted of HepG2, A549, MCF-7, HCT-116 and SH-SY5Y. In all of these experiments, three replicate wells were used to determine each point.

5.4. Cell cycle analysis

The cell cycle was analyzed by flow cytometry. Briefly, cells were treated with different concentrations of SFN and **85** for 48 h. After incubation, a total of 1×10^8 cells were harvested from the treated and normal samples. The cells were washed twice with PBS, staining was carried out by incubating cells with 500 μL of 4 mg/mL DAPI for 15 min in darkness. The cell cycle distribution was then detected by flow cytometry using a Cell Lab Quanta SC (Beckman Coulter, Fullerton, CA). All experiments were performed three times.

5.5. Annexin V-FITC/PI assay

Cells in different states were observed with an annexin V-FITC/PI double-staining kit by flow cytometry. Cells were treated with different concentrations of SFN and **85** for 48 h. After incubation, cells were collected and washed twice in cold PBS and resuspended in 200 mL of binding buffer at 1×10^5 cells/mL. The samples were incubated with 5 μL of Annexin V-FITC and propidium iodide (PI) in the dark for 15 min at room temperature. Finally, samples were analyzed by flow cytometry and evaluated based on the percentage of cells for Annexin V positive. All experiments were performed three times.

5.6. Preparation of nuclear and cytosolic extracts

HepG2 cells grown on 100-mm Petri dishes were treated for different hours with various concentrations of SFN and **85**. Thereafter, cells were washed twice with cold PBS/phosphatase

inhibitors, and suspended in cold buffer. Centrifuged cell suspension for 5 min at 500 rpm in a centrifuge pre-cooled at 4 °C. Discarded supernatant and gently resuspended cells in 500 μL of 1× hypotonic buffer by pipetting up and down several times. After 15 min on ice, 25 μL of detergent was added, and vortexed for 10 s at highest setting. The supernatant, cytosolic fraction, was removed into new microcentrifuge tubes; and the pellets were suspended again in 50 μL of complete lysis buffer to extract nuclear proteins. The cells were then incubated on ice with vigorous agitation every 5 min for 30 s followed by centrifugation for 10 min at 14,000 *g* at 4 °C, and the supernatant (nuclear extract) was removed into new microcentrifuge tubes. The cytosolic and nuclear extracts were stored at −80 °C until further use.

5.7. Nrf2 transcription factor assay

Trans^{AM} Nrf2 Kit (Active Motif, Carlsbad, North America) as used to detect the activation of Nrf2 transcription factor. Briefly, 10 μL nuclear extract was diluted in complete lysis buffer per well. After incubating for 1 h at room temperature with mild agitation, each well was washed 3 times with 200 μL wash buffer. The nuclear fractions were incubated 1 h at room temperature with Nrf2 (1:1000) diluted in 1 × antibody binding buffer. After washing with 200 μL of wash buffer, each well was incubated with horseradish peroxidase-conjugated antibody (1:1000). Then 100 μL of developing solution was added to all wells, incubated for 2–15 min until blue color developed in the sample. Finally, 100 μL of stop solution was added, and the absorbance was read on a spectrophotomer within 5 min at 450 nm with a reference wavelength of 655 nm.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.03.045.

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