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Synthesis and biological evaluation of hybrids from farnesylthiosalicylic acid and hydroxylcinnamic acid with dual inhibitory activities of Ras-related signaling and phosphorylated NF-κB

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Abstract:

A series of hybrids (**5a-r**) of farnesylthiosalicylic acid (FTS) and hydroxylcinnamic acid were designed and synthesized. Most of the hybrids displayed potent antiproliferative activity aginst seven cancer cell lines *in vitro*, superior to FTS as well as sorafenib. The most potent compound **5f** selectively inhibited cancer cells but not non-tumor liver cell proliferation *in vitro*, and significantly induced SMMC-7721 cell apoptosis. Interestingly, **5f** could simultaneously inhibit not only Ras-related signaling but also phosphorylated NF-κB, which may synergetically contribute to the cell growth inhibition and apoptosis induction. Moreover, **5f** showed low acute toxicity to mice and significantly inhibited the hepatoma tumor growth.

Key words: Farnesylthiosalicylic acid, Hydroxylcinnamic acids, Cell apoptosis, Ras-related signaling pathway, NF-κB.

Introduction

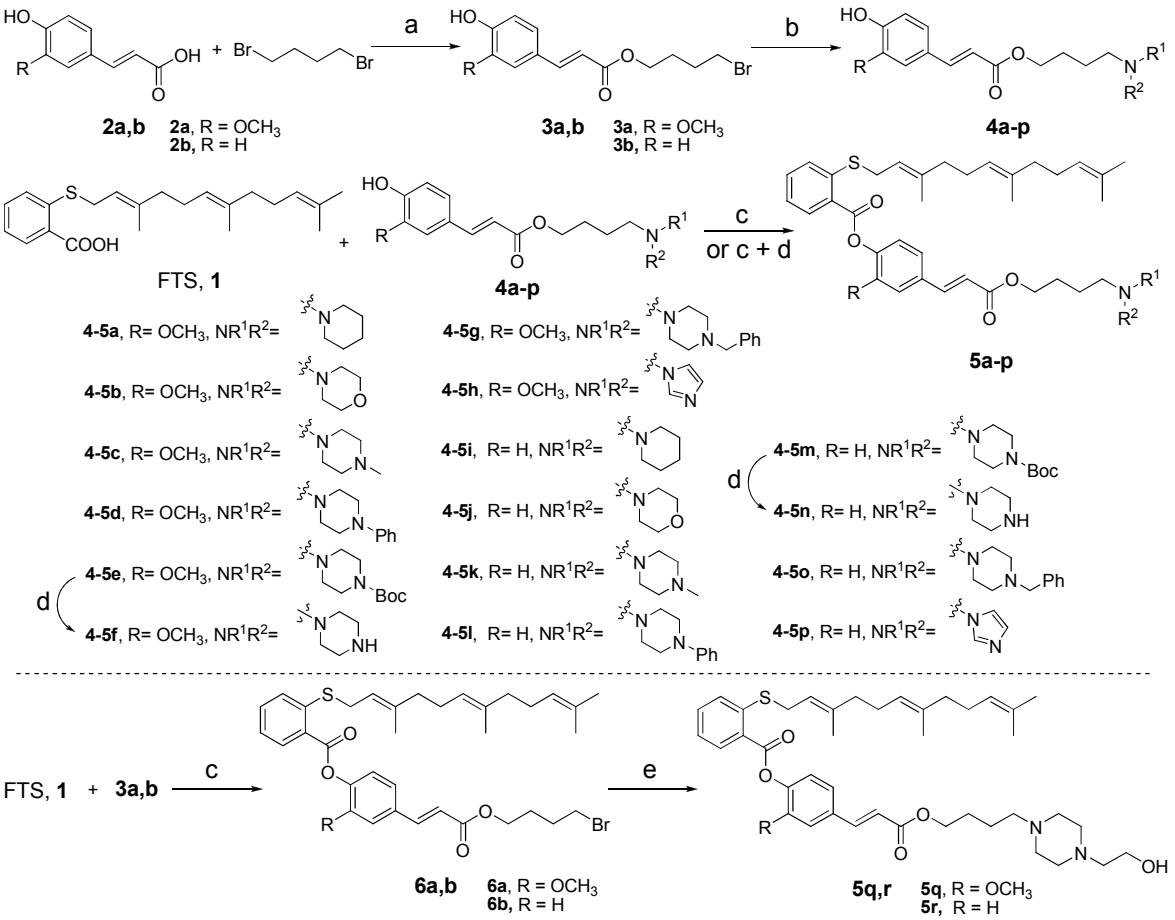
The Ras proteins encoding by *ras* genes are GTP-anchoring proteins and function as binary molecular switches that can mediate intracellular signal transduction between G-protein-coupled receptors and downstream events, such as Raf-MEK1/2-extracellular signal-regulated kinase1/2 (ERK1/2) and phosphosphatidylinositol-3-kinase (PI3K)-Akt.[1-4] This signaling pathway is quite important in the normal physiological setting, regulating cell proliferation, migration, differentiation and apoptosis.[5-6] However, oncogenic mutations occurring approximately 30% in the *ras* genes in human cancers lead to constitutive activation of Ras-GTP proteins, and the overexpressed proteins and consequently aberrant Ras signaling cascades eventually result in the development of disorders, especially tumors in humans. Thus, the Ras proteins and its related signaling pathway are attractive therapeutic targets for several cancers.[7,8]

Farnesylthiosalicylic acid (FTS, **1**) is a well-known Ras inhibitor with structurally mimicking the carboxyl-terminal farnesylcysteine group, and has been preclinically studied for the treatment of malignancies including lung, breast, pancreas and liver cancers.[9-13] By recognizing the anchorage and dislodging the active Ras protein from the cell membrane, FTS can block the initiation of downstream signaling events, thereby inhibiting tumor cell growth and promoting the tumor cell apoptosis.[14] With huge potential but some limitation of therapeutic efficacy in cancers,[15] FTS would be an excellent parent molecule for the development of Ras inhibitors with potent anti-tumor activity.

Hydroxylcinnamic acids, such as ferulic acid and *p*-hydroxycinnamic acid, widely occur in the plant

kingdom, and display antiproliferative activity against some types of cancer cells,[16-18] thus attracting considerable attention from medicinal chemists and pharmacologists. In addition, it was documented that hydroxylcinnamic acids and their derivatives could evidently block transcription factor (NF-κB) pathway and initiate an apoptotic cascade.[19-21] Reportedly, inhibition of both Ras and NF-κB signaling pathways could significantly lead to tumor cell growth inhibition and apoptosis.[22-24] In this regard, we hypothesized that FTS/hydroxylcinnamic acid hybrids might efficaciously prevent the Ras and NF-κB signaling pathways, inhibit tumor cell proliferation, and induce tumor cell apoptosis. Herein, synthesis and biological evaluation of these hybrids are described.

Results and discussion

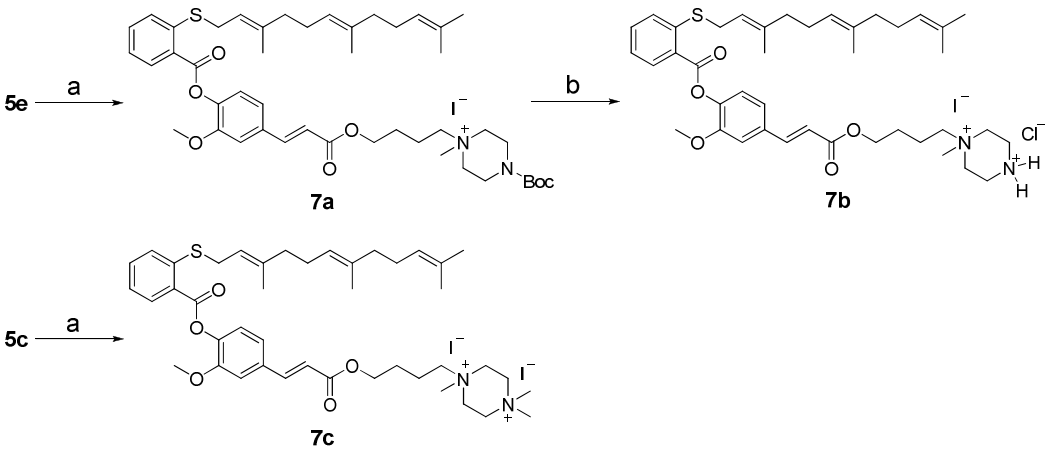


Scheme 1. Reaction conditions and reagents: a) acetone, Et_3N, 50°C, 4 h, 74-77%; b) *N*-containing heterocycles, K_2CO_3, KI, MeCN, 50°C, 5-10 h; c) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 4-dimethylamiopyridine (DMAP), CH_2Cl_2, rt, 15-24 h, 46-64%; d) trifluoroacetic acid, CH_2Cl_2, rt, 2 h, 85-88%; e) *N*-hydroxyethyl piperazine, K_2CO_3, KI, MeCN, 50°C, 6 h, 52-58%.

Chemical Synthesis

The synthetic route of compounds **5a-r** was depicted in Scheme 1. The parent compound FTS was synthesized as previously reported by our group.[25] Hydroxylcinnamic acid **2a** or **2b** was treated with 1,4-dibromobutane in the presence of triethylamine to furnish bromides **3a** or **3b**, which subsequently reacted with different cyclic secondary amines to give amino intermediates **4a-p**. Then treatment of FTS (**1**) with **4a-p**

produced target compounds **5a-p**. Compounds **5f** and 5**n** were obtained from **5e** and **5m**, respectively, via a de-Boc (tert-butyloxygencarbonyl) protection reaction in the presence of trifluoroacetic acid (TFA). Compounds **5q** and **5r** were achieved via an alternative synthetic route, where **3a** and **3b** were treated with **1** to give **6a** and **6b** followed by condensation with *N*-hydroxyethyl piperazine to afford **5q** and **5r**, respectively. The hydrochloride salts **5a'**, **5c'**, **5e'-g'**, and **5n'** were prepared from **5a**, **5c**, **5e-g**, and **5n** in the EtOAc solution containing saturated hydrochloride, respectively. Compound **5e** was further treated with iodomethane followed by deprotection to provide quaternary amine compound **7b**, while **7c** was prepared through methylation of **5c** with iodomethane (Scheme 2).



Scheme 2. Reaction conditions and reagents: a) iodomethane, MeCN, rt, 15-24 h, 84-86%; b) EtOAc, HCl, rt, 3 h, 81%.

In vitro biological evaluations

All the synthesized compounds **5a-r** were preliminarily screened for their cancer cell growth inhibitory activitiy against human hepatocellular carcinoma cells (SMMC-7721), breast cancer cells (MCF-7) and gastric cancer cells (SGC7901) using MTT assay, and with FTS and a well-known Ras-related signal inhibitor sorafenib as reference compounds. It was observed that several compounds strongly inhibited cell growth of these cancer cell lines at 50 μM, superior to FTS (Fig. 1), especially, **5a**, **5c** and **5e-g** even more potent than sorafenib with the inhibition by 90%.

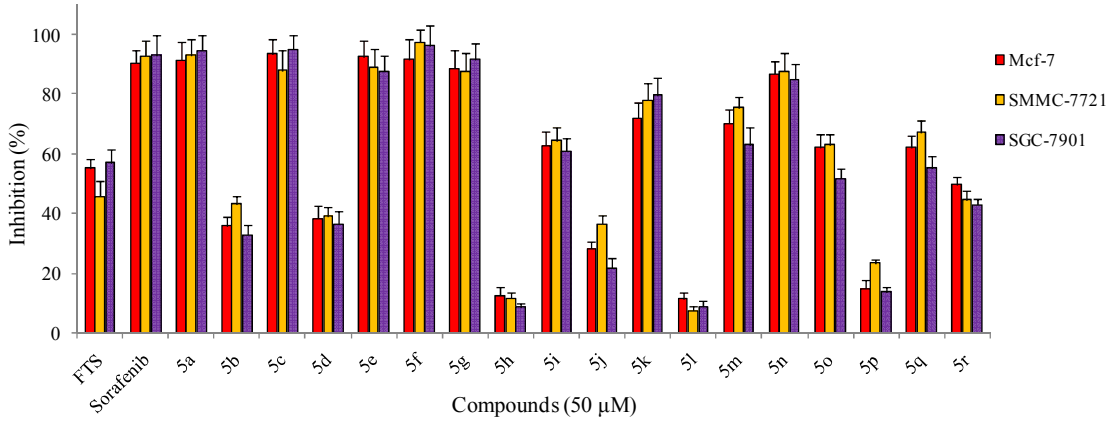


Fig. 1 Inhibition of cell proliferation (%) of target compounds against MCF-7, SMMC-7721, and SGC7901 cell lines after incubation for 48 h at a concentration of 50 μM. Data are expressed as means + SD of each compound from three separate measurements.

These active compounds were further assayed for their antiproliferative activity against seven cancer cell lines including hepatocellular carcinoma cells (SMMC-7721, HepG2, and H22), human breast cancer cells (Mcf-7), human bladder carcinoma cells (EJ), human ovarian cancer cells (Skov3) and human gastric cancer cells (SGC-7901) by MTT assay (Table 1). All tested compounds exhibited more potent antiproliferative activity (IC_{50s} 3.08-21.02 μM) than FTS (IC_{50s} 41.35-103.28 μM) against all cancer cells, and **5c** and **5f** (IC_{50s} 6.32~9.15 μM, and IC_{50s} 3.08~8.17 μM, respectively) even more strongly inhibited the cancer cell growth relative to sorafenib (IC_{50s} 9.12-22.93 μM). Notably, **5f** exhibited the most potent antiproliferative activity against SMMC-7721 cells (IC_{50} = 3.08 μM).

Table 1 The IC_{50} values of active compounds **5a**, **5c**, **5e-g**, and **5n** against seven cancer cell lines[a]

Compd.	*In vitro* antiproliferative activity (IC_{50}[a], μM)						
	SMMC-7721	Hep G2	H22	Mcf-7	Skov3	EJ	SGC-7901
FTS	69.7 ± 3.85	103.2 ± 7.65	56.3 ± 4.28	49.1 ± 4.66	51.2 ± 5.06	47.6 ± 3.28	41.3 ± 5.56
Sorafenib	18.7 ± 2.65	16.2 ± 2.17	13.8 ± 1.43	9.12 ± 1.80	9.25 ± 2.15	22.9 ± 3.53	11.5 ± 2.71
5a	7.73 ± 0.86	9.37 ± 0.77	7.62 ± 0.61	7.91 ± 1.06	6.89 ± 0.69	6.06 ± 0.48	9.23 ± 1.25
5c	6.93 ± 0.65	8.64 ± 0.69	6.38 ± 0.55	7.23 ± 0.81	6.32 ± 0.58	9.15 ± 1.26	7.08 ± 0.82
5e	13.4 ± 0.93	12.3 ± 1.84	10.5 ± 1.03	16.9 ± 1.85	15.5 ± 1.60	11.9 ± 0.92	18.3 ± 2.15
5f	3.08 ± 0.42	6.21 ± 0.46	3.73 ± 0.72	8.17 ± 0.96	7.07 ± 0.92	4.98 ± 0.55	6.35 ± 0.71
5g	9.38 ± 1.11	13.0 ± 2.02	9.63 ± 0.74	11.5 ± 0.94	13.9 ±1.08	11.2 ± 1.10	9.98 ± 1.14
5n	14.5 ± 1.30	15.9 ± 1.56	13.0 ± 1.21	18.3 ± 2.16	12.4 ± 1.13	16.7 ± 2.37	21.0 ± 3.63

[a] Data are expressed as mean ± SD of three independent experiments.

It was observed that **5f** had better activity than its Boc-protected comparator **5e**, suggesting that the polar piperazine group was beneficial for the activity. So it was interesting to know whether their hydrochloride salts and methylated compounds could further enhance antiproliferative activity. Accordingly, six hydrochloride salts (**5a'**, **5c'**, **5e'-g'**, and **5n')** and two quaternary amine compounds (**7b** and **7c**) were prepared and tested for their antiproliferative activity against SMMC-7721 and Mcf-7 cells (Table 2). The results indicated that the hydrochloride salts displayed significant antiproliferative activity comparable to their free bases. However, the quaternary amine compounds displayed slightly weaker antiproliferative activity than the corresponding amines and hydrochloride salts.

Table 2 The IC_{50} values of hydrochloride salts (**5a'**, **5c'**, **5e'-g'**, and **5n'**) and quaternary amine compounds (**7b** and **7c**) against SMMC-7721 and Mcf-7 cells

Compd.	*In vitro* antiproliferative activity (IC_{50}, μM)		Compd.	*In vitro* antiproliferative activity (IC_{50}, μM)	
	SMMC-7721	Mcf-7		SMMC-7721	Mcf-7
FTS	65.8 ± 3.63	48.2 ± 3.78	**5f'**	3.44 ± 0.66	5.62 ± 0.90
Sorafenib	16.9 ± 3.55	10.3 ± 2.67	**5g'**	13.6 ± 1.89	15.1 ± 2.35
5a'	5.40 ± 0.95	6.71 ± 1.12	**5n'**	10.5 ±1.71	12.8 ± 2.03
5c'	3.61 ± 0.73	4.86 ± 0.49	**7b**	6.25 ± 0.87	7.85 ± 0.59
5e'	15.3 ± 2.17	16.7 ± 3.16	**7c**	7.13 ± 0.92	7.96 ± 0.76

Given the strong growth inhibitory activity of **5f** *in vitro*, the selectivity profile was investigated by examining its inhibitory effects on the growth of both cancer cells SMMC-7721, H22 and normal liver cells LO2 cells. It was found that treatment with increased dose of **5f** had no significant effect on the survival of non-tumor LO2 cells while the same treatment induced majority of SMMC-7721 cell death (Fig. 2A), suggesting **5f** might possess selective antiproliferation activity against tumor cells. Given that **5f** contained both ferulic acid (**4f**) and FTS moieties, we further determined the antiproliferation activity of **4f** and FTS against SMMC-7721 cells. As shown in Fig. 2B, **4f** and FTS were apparently less potent than **5f**, suggesting that the antitumor activity of **5f** may be attributed to the synergetic effects of the ferulic acid and FTS moieties.

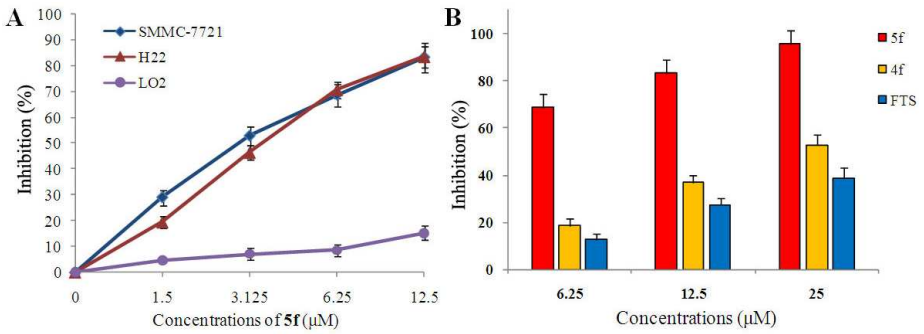


Fig. 2 (A) Inhibitory effects of **5f** on the proliferation of SMMC-7721, H22, and LO2 cells. Cells were incubated with the indicated concentrations of **5f** for 48 h. Cell proliferation was assessed using the MTT assay. Data are means ± SD of the inhibition (%) from three independent experiments. (B) Inhibitory effects of **4f**, **5f** and FTS against SMMC-7721 cells. SMMC-7721 cells were incubated with the indicated compounds at 6.25, 12.5, and 25 μM for 48 h, and cell proliferation was assessed by the MTT assay. Data are means + SD of the inhibition (%) from three independent experiments.

In vivo anti-cancer activity of 5f

To evaluate the safety of **5f** *in vivo*, groups of ICR mice were injected intravenously with a single dose of **5f** or vehicle control, respectively. The survival of mice was monitored up to 14 days after injection. Only three mice that had been treated with **5f** at the highest dose (610.4 μmol/kg) survived (shown in Table 2). In contrast, injection with **5f** at the lowest dose (250 μmol/kg) did not cause any death and abnormality in eating, drinking, body weight, and activity throughout the observation period. As a result, the LD_{50} value of **5f** was calculated to be 495.2 μmol/kg for this train of mice.

Table 2 The acute toxicity of **5f** in mice

Dose (μmol/kg)	Number of mice	Number of dead mice							Total death	Survival (%) on day 14
		1h	4h	1d	2d	3d	4d	5-14d		
610.4	10	0	0	1	1	1	2	1	7	30
488.3	10	0	0	0	1	2	1	0	4	60
390.6	10	0	0	0	1	2	0	0	3	70
312.5	10	0	0	0	1	1	0	0	2	80
250.0	10	0	0	0	0	0	0	0	0	100

Next, we established a mice model which was inoculated subcutaneously with H22 cells to evaluate the *in vivo* antitumor activity of **5f**. The mice were randomly administered with **5f**, FTS, and vehicle, respectively. The changes in tumor volumes and weights were measured over 14 days. Compared with the vehicle control,

treatment with **5f** significantly reduced the tumor volume growth rate in a dose-dependent manner (Fig. 3). It was observed that treatment with **5f** (59.3 μmol/kg) more strongly inhibited the growth of the tumor cells *in vivo* than FTS at the same dose. Importantly, the tumor weights (1.05 ± 0.15, 0.63 ± 0.29 g) in the mice treated with **5f** at 29.7 and 59.3 μmol/kg, respectively, were significantly less than those from the vehicle-treated controls (1.66 ± 0.32 g, $p < 0.01$). Besides, there was no statistical difference in body weight in postinoculation among the four groups of mice. Together, our data clearly demonstrated that **5f** could evidently inhibit the growth of tumor *in vivo*.

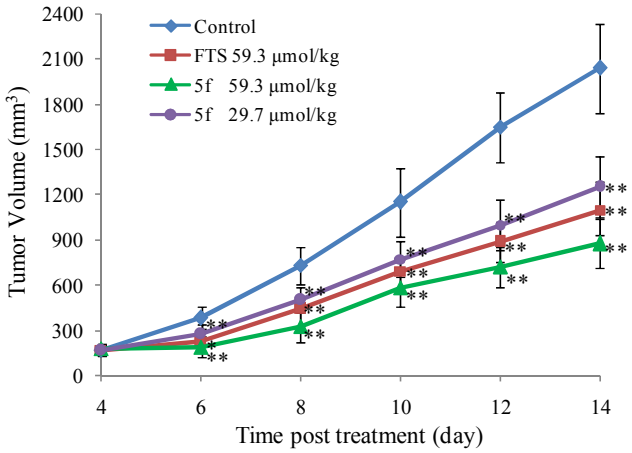


Fig. 3 Inhibitory effects of **5f** on the growth of H22 tumors *in vivo*. Mice inoculated with H22 tumors were randomly treated daily with **5f**, FTS, or vehicle, and the volumes of tumors were measured at the indicated time points. Data are shown as means ± SD from each group of mice ($n = 6$). *P < 0.05, **P < 0.01 vs control.

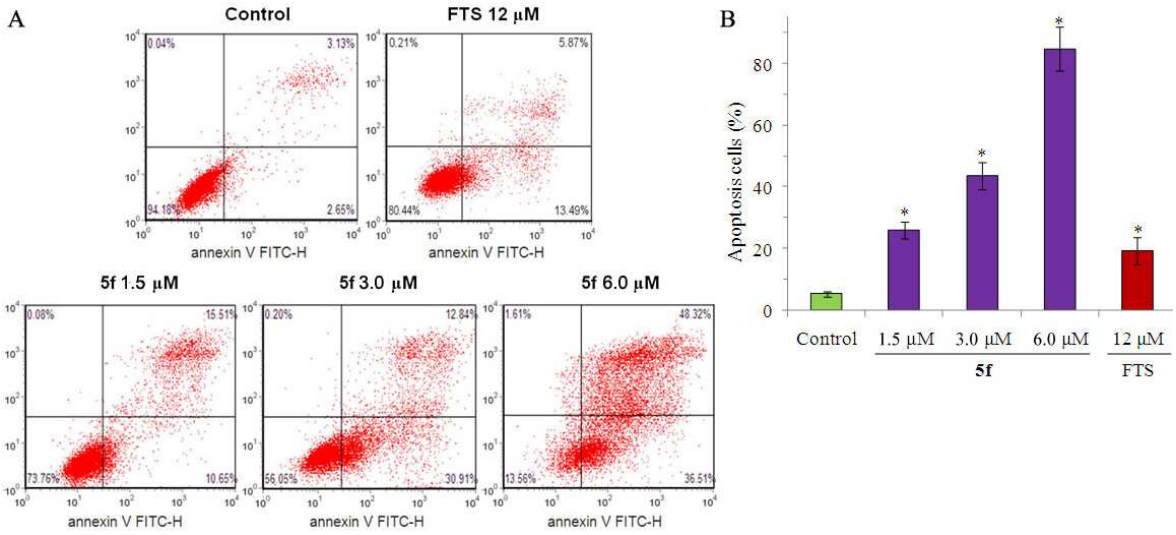


Fig. 4 Compound **5f** induced SMMC-7721 cell apoptosis *in vitro*. SMMC-7721 cells were incubated with the indicated concentrations of **5f**, or FTS (12 μM) for 48 h, and the cells were stained with FITC-Annexin V/PI, followed by flow cytometry analysis. (A) Flow cytometry analysis. (B) Quantitative analysis of apoptotic cells. Data are expressed as means ± SD of the percentages of apoptotic cells from three independent experiments. *$P < 0.01$ vs control.

Possible mechanisms underlying the anti-cancer activity of 5f

To test the effects of **5f** on tumour cell apoptosis, SMMC-7721 cells were incubated with different

concentrtions of **5f**, or FTS for 48 h, and the percentages of apoptotic cells were determined by FITC-Annexin V/PI staining and flow cytometry. As shown in Fig. 4, treatment with **5f** dose-dependently induced cancer cells apoptosis. Incubation with 6.0 μM of **5f** induced over 80% of SMMC-7721 cell apoptosis. In sharp contrast, FTS (12 μM) only induced about 20% apoptotic cells.

Next, western blot analysis was conducted. It was revealed that treatment with **5f** dramatically increased the relative levels of pro-apoptotic Bax and caspase 3 expression, but reduced the levels of anti-apoptotic Bcl-2 expression (Fig. 5A and B) in a dose-dependent manner.

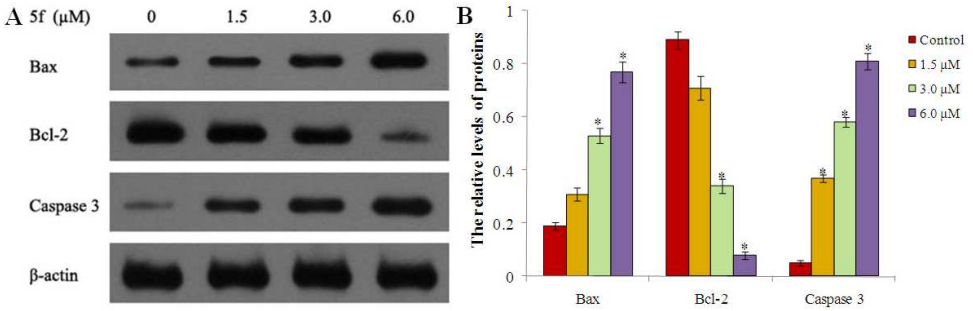


Fig. 5 Effect of **5f** on the expression of apoptosis-related proteins in SMMC-7721 cells. (A) The expression of Bax, Bcl2, caspase 3 and β-actin was examined by Western blot analysis. SMMC-7721 cells were incubated with, or without, **5f** at the indicated concentrations for 48 h and the levels of protein expression were detected using specific antibodies. Data shown are representative images of each protein for three separate experiments. (B) Quantitative analysis: the relative levels of each protein compared to control β-actin were determined by densimetric scanning. Data are expressed as means + SD from three separate experiments. *$P < 0.01$ vs control.

To get insight into the mechanisms underlying the anti-cancer activity of these FTS/hydroxylcinnamic acid hybrids, we examined the inhibitory effects of the active compound **5f** on the expression of Ras-related signaling and NF-κB proteins in SMMC-7721 cells. The cells were incubated with vehicle alone, FTS (12 μM), or **5f** (1.5, 3.0, or 6.0 μM). The expression and phosphorylation levels of the Ras-related signal events, Raf, Akt, ERK, and NF-κB were determined by immunoblotting assays using β-actin as the control (Fig. 6). It was observed that treatment with **5f** dose-dependently inhibited the levels of phospho-Raf, ERK and Akt in SMMC-7721 cells. Importantly, **5f** dramatically inhibited the expression of phospho-NF-κB in SMMC-7721 cells at increased concentrations, while FTS had no significant change relative to the control group. The results suggest that treatment with **5f** could not only inhibit Ras-related signaling but also inhibit phosphorylated NF-κB, generating synergetic anti-cancer effects.

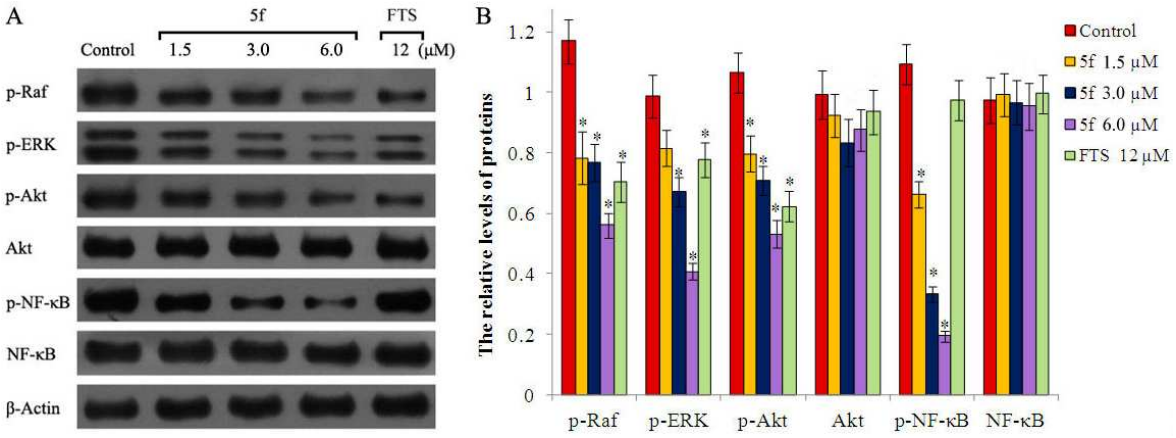


Fig. 6 Immunoblot analysis of the expression and phosphorylation of the Ras-related signal events *in vitro*. (A) SMMC-7721 cells were treated with vehicle (control), different doses of **5f**, or FTS were homogenized, and their lysates were subjected to immunoblot analysis using antiphospho-Raf (Ser259), antiphospho-ERK1/2 (Thr202/Tyr204), anti-Akt, antiphospho-Akt (Ser473), anti-phoshpo-NF-κB p65, anti-NF-κB and anti-β-actin antibodies, respectively. β-Actin was used as the control. (B) Quantitative analysis. The relative levels of each signaling event to control β-actin were determined by densimetric scanning. The data are expressed as means ± SD of three duplicate experiments. $*P < 0.01$ vs control.

Conclusions

In conclusion, we designed and synthesized a series of FTS/hydroxylcinnamic acid hybrids and investigated their *in vitro* and *in vivo* antitumor effects. The structure-activity relationship (SAR) analysis revealed that FTS/ferulic acid hybrids **5a-h** showed more potent antiproliferation activity than the corresponding FTS/*p*-hydroxycinnamic acid hybrids **5i-p**. Several compounds (**5a**, **5c**, **5e-g**, and **5n**) displayed potent cell growth inhibitory activity against six human cancer cells *in vitro*. The most potent compound **5f** possessed stronger antiproliferative activity of cancer cells than FTS, ferulic acid as well as sorafenib, and did not affect proliferation of non-tumor LO-2 cells in the effective dose against cancer cells. In addition, **5f** could significantly induce cell apoptosis by reducing the anti-apoptotic protein levels of Bcl-2 and up-regulating the pro-apoptotic proteins of Bax and caspase-3. Furthermore, **5f** inhibited both Ras-related signaling and phosphorylated NF-κB simultaneously, which may synergistically contribute to the strong activity in the cell growth inhibition and apoptosis induction effects. Moreover, the low acute toxicity and the significant growth inhibition of cancer cells *in vivo* suggested that **5f** may be a promising candidate for the intervention of human cancers.

Experimental section

General procedures

Infrared (IR) spectra (KBr) were recorded on a Nicolet Impact 410 instrument (KBr pellet). ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. MS spectra were recorded on a Mariner Mass Spectrum (ESI). High resolution mass spectra were recorded with Agilent technologies LC/MSD TOF. Element analysis was performed on an Eager 300 instrument. All compounds were routinely checked by TLC and ^{1}H NMR. TLCs and preparative thin-layer chromatography were performed on silica gel GF/UV 254, and the chromatograms were conducted on silica gel (200–300 mesh, Merck) and visualized under UV light at 254 and 365 nm. All solvents were reagent grade and, when necessary, were purified and dried by standards methods. Solutions after reactions and extractions were concentrated using a

rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. The target products **5a**-**r** were purified by column chromatography, and their structures were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, HRMS, and elemental analyses. Each compounds with purity of >95% was determined by high-performance liquid chromatography, and could be used for subsequent experiments.

(*E*)-4-Bromobutyl-3-(4-hydroxy-3-methoxyphenyl)acrylate (3a). To a solution of fumalic acid (**2a**, 5.00 g, 25.80 mmol) in acetone (50 mL) was added 1,4-dibromobutane (21.60 g, 100 mmol) and 10 mL Et_3N, and the mixture was reacted at 50°C for 4 h. Then the solution was cooled to room temperature. After filtration, the filtrate was collected and concentrated in vacuo. The resulting residue was purified by column chromatography (EtOAc-PE = 1:4, v/v as the eluate), affording the title compound as a pale yellow needle-like solid 6.54 g, yield: 77%, mp 85-88°C.

(*E*)-4-Bromobutyl-3-(4-hydroxyphenyl)acrylate (3b). The title compound was synthesized, using a method similar to that used for the preparation of **3a**, starting from 4-hydroxycinnamic acid (**2b**, 5.00 g, 30.5 mmol) in 74% yield as a pale yellow solid 6.75 g, mp 96-98°C.

2-Methoxy-4-((*E*)-3-oxo-3-(4-(piperidin-1-yl)butoxy)prop-1-en-1-yl)phenyl-2-(((2*E*,6*E*)-3,7,11-trimeth yldodeca-2,6,10-trien-1-yl)thio)benzoate (5a). To a solution of compound **3a** (0.16 g, 0.50 mmol) in acetonitrile (10 mL), KI (8.3 mg, 0.05 mmol), K_2CO_3 (0.14 g, 1.00 mmol) and piperidine (44 mg, 0.52 mmol) were added. The mixture was stirred at 50 °C until the starting material was totally consumed. After filtration, the filtrate was collected and concentrated in vacuo to afford product **4a**, which was subsequently added into the mixture of FTS (0.16 g, 0.45 mmol), EDCI (96 mg, 0.50 mmol), and DMAP (6.1 mg, 0.05 mmol) in anhydrous CH_2Cl_2 (15 mL). The reactive solution was stirred at room temperature for 16h. After filtration, the filtrate was evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2-MeOH = 15:1-8:1, v/v as the eluate) to afford **5a** (0.17 g, 0.05 mmol, 52%) as a colorless thick liquid. Analytical data for **5a**: IR (KBr, cm^{-1}): 3426, 2937, 1749, 1616, 1426, 1247, 1138, 1019; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.67 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.60-7.64 (m, 2H, Ar-H), 7.47-7.53 (m, 1H, Ar-H), 7.27-7.35 (m, 3H, Ar-H), 6.75 (d, 1H, J = 12.0 Hz, COCH), 5.26-5.30 (m, 1H, $SCH_2C\underline{H}$), 5.03-5.07 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.15-4.19 (m, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.63-3.65 (d, 2H, J = 6.0 Hz, SCH_2), 2.24-2.30 (m, 6H, NCH_2), 1.97-2.03 (m, 8H, 2 × $CC\underline{H}_2C\underline{H}_2CH$), 1.36-1.70 (m, 22H, 4 × CH=$CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$, $CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.21 (CO), 163.22 (CO), 151.23 (Ar-C), 143.70 (Ar-C=), 140.89 (Ar-C), 140.21 (Ar-C), 134.58 (C=), 133.25 (Ar-C), 133.21 (Ar-C), 131.50 (C=), 126.60 (Ar-C), 126.52 (Ar-C), 124.39 (Ar-C), 124.14 (Ar-C), 124.05 (Ar-C), 124.01 (Ar-C), 123.47 (C=), 123.29 (C=), 121.61 (C=), 118.51 (C=), 118.15 (C=), 112.03 (Ar-C), 63.93 (OCH_2), 57.83 (NCH_2), 56.08 (OCH_3), 53.84 ($N(CH_2)_2$), 32.05 (CH_2), 31.41 (CH_2), 26.23 (SCH_2), 26.11 (CH_2), 25.94 (CH_2), 25.75 (CH_2), 25.69 (CH_3), 25.38 (CH_3), 25.32 (2×CH_2), 23.91 (CH_2), 23.03 (CH_2), 22.59 (CH_3), 15.92 (CH_3); MS (ESI) *m/z* = 674 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{41}H_{56}NO_5S$: 674.3879; found: 674.3888 $[M+H]^+$; Anal. Calcd. for $C_{41}H_{55}NO_5S$: C, 73.07; H, 8.23; N, 2.08; Found: C, 72.81; H, 8.33; N, 2.22.

2-Methoxy-4-((*E*)-3-(4-morpholinobutoxy)-3-oxoprop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodec a-2,6,10-trienylthio)benzoate (5b). According to the procedure of the preparation of **5a**, morpholine (45 mg, 0.52 mmol), **3a** (0.16 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5b** (0.20 g, 0.29 mmol, 58 %) as a colorless thick liquid. Analytical data for **5b**: IR (KBr, cm^{-1}): 3423, 2923, 1712, 1637, 1218, 1033; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.13 (d, 1H, J = 6.0 Hz, Ar-H), 7.67 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.59-7.63 (m, 2H, Ar-H), 7.50 (m, 1H, Ar-H), 7.25-7.36 (m, 3H, Ar-H), 6.75 (d, 1H, J = 12.0 Hz, COCH), 5.26-5.29 (m, 1H, $SCH_2C\underline{H}$), 5.04-5.07 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.16-4.19 (m, 2H, OCH_2), 3.83 (s, 3H,

OCH_3), 3.63-3.65 (d, 2H, J = 6.0 Hz, SCH_2), 3.56-3.57 (m, 4H, OCH_2), 2.29-2.34 (m, 6H, NCH_2), 1.83-2.01 (m, 8H, 2 × CCH_2CH_2CH), 1.47-1.70 (m, 16H, 4 × $CH=CCH_3$, $OCH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.30 (CO), 163.25 (CO), 151.26 (Ar-C), 143.82 (Ar-C=), 140.92 (Ar-C), 140.31 (Ar-C), 133.39 (C=), 133.26 (Ar-C), 131.65 (C=), 131.63 (C=), 126.56 (Ar-C), 126.54 (Ar-C), 124.46 (Ar-C), 124.19 (Ar-C), 124.05 (Ar-C), 123.99 (Ar-C), 123.38 (C=), 122.31 (C=), 121.74 (C=), 118.53 (Ar-C), 118.16 (C=), 112.01 (Ar-C), 66.20 (OCH_2), 63.98 (OCH_2), 57.71 (NCH_2), 56.12 (OCH_3), 53.31 (NCH_2), 32.11 (CH_2), 31.48 (CH_2), 26.19 (SCH_2), 26.12 (CH_2), 26.00 (CH_2), 25.75 (CH_2), 25.50 (CH_2), 25.42 (CH_2), 23.13 (CH_2), 22.39 (CH_3), 15.92 (CH_3); MS (ESI) *m/z* = 676 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{40}H_{54}NO_6S$: 676.3672; found: 676.3681 $[M+H]^+$; Anal. Calcd. for $C_{40}H_{53}NO_6S$: C, 71.08; H, 7.90; N, 2.07; Found: C,70.88; H, 8.05; N, 1.96.

2-Methoxy-4-((*E*)-3-(4-(4-methylpiperazin-1-yl)butoxy)-3-oxoprop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5c). According to the procedure of the preparation of **5a**, 4-methylpiperazine (52 mg, 0.52 mmol), **3a** (0.16 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5c** (0.16 g, 0.23 mmol, 47 %) as a colorless thick liquid. Analytical data for **5c**: IR (KBr, cm^{-1}): 3431, 2924, 1706, 1636, 1461, 1237, 1156, 1032; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.67 (d, 1H, J = 12.0 Hz, $COCH=CH$), 7.60-7.65 (m, 2H, Ar-H), 7.48-7.51 (m, 1H, Ar-H), 7.26-7.35 (m, 3H, Ar-H), 6.75 (d, 1H, J = 12.0 Hz, COCH) , 5.26-5.30 (m, 1H, SCH_2CH), 5.04-5.07 (m, 2H, 2×$CH_2CH=CCH_3$), 4.16-4.19 (m, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.63-3.65 (d, 2H, J = 6.0 Hz, SCH_2), 2.27-2.30 (m, 10H, NCH_2), 2.14 (s, 3H, NCH_3), 1.98-2.02 (m, 8H, 2×CCH_2CH_2CH), 1.50-1.70 (m, 18H, 4×CH =CCH_3, $OCH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.21 (CO), 163.22 (CO), 151.24 (Ar-C), 143.71 (Ar-C=), 140.91 (Ar-C), 140.22 (Ar-C), 134.59 (C=), 133.25 (C=), 133.22 (Ar-C), 131.51 (C=), 126.60 (Ar-C), 126.54 (Ar-C), 124.40 (Ar-C), 124.13 (Ar-C), 124.06 (Ar-C), 124.02 (Ar-C), 123.48 (C=), 123.30 (C=), 121.61 (C=), 118.52 (Ar-C), 118.16 (C=), 112.04 (Ar-C), 63.94 (OCH_2), 57.19 (NCH_2), 56.09 (OCH_3), 54.62 (2×CH_2), 52.47 (2×CH_2), 45.55 (NCH_3), 32.06 (CH_2), 31.42 (CH_2), 26.21 (SCH_2), 26.12 (CH_2), 25.95 (CH_2), 25.76 (CH_2), 25.70 (CH_3), 25.42 (CH_3), 23.05 (CH_2), 22.69 (CH_3), 15.93 (CH_3); MS (ESI) m/z = 689 $[M+H]^+$. HRMS (ESI): *m/z* calcd for $C_{41}H_{57}N_2O_5S$: 689.3988; found: 689.3979 $[M+H]^+$; Anal. Calcd. for $C_{41}H_{56}N_2O_5S$: C, 71.48; H, 8.19; N, 4.07; Found: C, 71.29; H, 8.25; N, 3.89.

2-Methoxy-4-((*E*)-3-oxo-3-(4-(4-phenylpiperazin-1-yl)butoxy)prop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5d). According to the procedure of the preparation of **5a**, 4-phenyl piperazine (84 mg, 0.52 mmol), **3a** (0.16 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5d** (0.21 g, 0.28 mmol, 56 %) as a colorless thick liquid. Analytical data for **5d**: IR (KBr, cm^{-1}): 3449, 2926, 1638, 1460, 1236, 1032; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.68 (d, 1H, J = 12.0 Hz, $COCH=CH$), 7.60-7.64 (m, 2H, Ar-H), 7.48-7.50 (m, 1H, Ar-H), 7.36(d, 1H, J = 6.0 Hz, Ar-H), 7.32 (m, 1H, Ar-H), 7.26 (d, 1H, J = 6.0 Hz, Ar-H), 7.20 (m, 2H, Ar-H), 6.92 (m, 2H, Ar-H), 6.75-6.78 (m, 2H, COCH,Ar-H) , 5.26-5.29 (m, 1H, SCH_2CH), 5.04 (m, 2H, 2×$CH_2CH=CCH_3$), 4.18-4.21 (m, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.63-3.65 (d, 2H, J = 6.0 Hz, SCH_2), 3.10-3.11 (m, 4H, NCH_2), 2.21-2.30 (m, 6H, NCH_2), 1.98-2.02 (m, 8H, 2 × CCH_2CH_2CH), 1.45-1.70 (m, 16H, 4 × $CH=CCH_3$, $OCH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.31 (CO), 163.21 (CO), 151.26 (Ar-C), 151.05 (Ar-C), 143.82 (C=), 140.91 (Ar-C), 140.30 (Ar-C), 134.64 (C=), 133.37 (C=), 133.27 (Ar-C), 131.62 (C=), 128.88 (Ar-C), 126.67 (Ar-C), 126.53 (Ar-C), 124.45 (Ar-C), 124.16 (Ar-C), 124.10 (Ar-C), 124.06 (Ar-C), 123.51 (C=), 123.37 (C=), 121.61 (C=), 118.71 (Ar-C), 118.52 (Ar-C), 118.16 (C=), 112.04 (Ar-C), 64.01 (OCH_2), 57.32 (NCH_2), 56.11 (OCH_3), 52.73 (2×CH_2), 48.20 (2×CH_2), 32.11 (CH_2), 29.54 (CH_2), 26.28 (SCH_2), 25.99 (CH_2), 25.74 (CH_2), 25.50 (CH_3), 23.12 (CH_2), 22.75 (CH_3), 17.52 (CH_3), 17.44 (CH_3), 15.93 (CH_3); MS (ESI) *m/z* = 751 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{46}H_{59}N_2O_5S$: 751.4145; found: 751.4138 $[M+H]^+$; Anal. Calcd. for $C_{46}H_{58}N_2O_5S$: C, 73.56; H, 7.78; N, 3.73; Found: C, 73.39; H, 7.89; N, 3.62.

Tert-butyl-4-(4-((*E*)-3-(3-Methoxy-4-(2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoyloxy)phenyl)acryloyloxy)butyl)piperazine-1-carboxylate (5e). According to the procedure of the preparation of **5a**, 4-tertbutoxycarbonyl piperazine (97 mg, 0.52 mmol), **3a** (0.16 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5e** (0.18 g, 0.23 mmol, 46 %) as a colorless thick liquid. The title compound was synthesized from **3a** (0.16 g, 0.50 mmol), 4-tertbutoxycarbonyl piperazine (97 mg, 0.52mmol), and FTS (0.16 g, 0.45 mmol) according to the preparation of **5a** in 46% yield as a colorless thick liquid 0.16 g. Analytical data for **5e**: IR (KBr, cm^{-1}): 3427, 2927, 1712, 1640, 1463, 1239, 1035; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.67 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.57-7.62 (m, 2H, Ar-H), 7.49 (m, 1H, Ar-H), 7.22-7.29 (m, 3H, Ar-H), 6.73 (d, 1H, J = 12.0 Hz, COCH), 5.23-5.27 (m, 1H, $SCH_2C\underline{H}$), 5.02-5.05 (m, 2H, 2 ×C<u>H</u>=CCH_3), 4.13-4.17 (m, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 3.63-3.65 (d, 2H, J = 6.0 Hz, SCH_2), 3.27 (m, 2H, NCH_2), 2.27-2.33 (m, 8H, NCH_2), 1.96-2.02 (m, 8H, 2 × $CC\underline{H}_2C\underline{H}_2CH$), 1.48-1.68 (m, 16H, 4 × CH=$CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$), 1.36 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.19 (CO), 163.20 (CO), 153.75 (CO), 151.22 (Ar-C), 143.71 (Ar-C=), 140.89 (Ar-C), 140.20 (Ar-C), 134.55 (C=), 133.23 (C=), 133.19 (Ar-C), 131.49 (C=), 126.58 (Ar-C), 126.50 (Ar-C), 124.40 (Ar-C), 124.11 (Ar-C), 124.06 (Ar-C), 124.00 (Ar-C), 123.44 (Ar-C), 123.27 (C=), 121.59 (C=CH_3), 118.49 (C=CH_3), 118.13 (Ar-C=), 112.01 (Ar-C), 78.60 (C), 63.89 (OCH_2), 57.17 (NCH_2), 56.06 (OCH_3), 52.43 (2×CH_2), 44.64 (2×CH_2), 32.03 (CH_2), 31.40 (CH_2), 27.98 (CH_3), 26.15 (SCH_2), 26.10 (CH_2), 25.93 (CH_2), 25.74 (CH_2), 25.68 (CH_3), 25.38 (CH_3), 23.03 (CH_2), 22.58 (CH_3), 15.90 (CH_3); MS (ESI) m/z = 775 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{45}H_{62}N_2NaO_7S$: 797.4175; found: 797.4191 $[M+Na]^+$; Anal. Calcd. for $C_{45}H_{62}N_2O_7S$: C, 69.74; H, 8.06; N, 3.61; Found: C, 69.53; H, 8.19; N, 3.72.

2-Methoxy-4-((*E*)-3-oxo-3-(4-(piperazin-1-yl)butoxy)prop-1-en-1-yl)phenyl-2-(((2*E*,6*E*)-3,7,11-trimeth yldodeca-2,6,10-trien-1-yl)thio)benzoate (5f). A mixture of **5e** (0.16 g, 0.20 mmol) and CH_2Cl_2/TFA (10 mL, v/v = 7/3) was stirred for 3 h at room temperature. Then the solvent was was evaporated in vacuo. The crude product was dissolved in CH_2Cl_2 (30 mL) and washed with saturated $NaHCO_3$ (20 mL). The organic layer was concentrated to afford **5f** (0.12 g, 0.18 mmol, 88 %) as the colorless thick liquid. Analytical data for **5f**: IR (KBr, cm^{-1}): 3401, 2947, 1732, 1618, 1447, 1235, 1159, 1039; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.13 (d, 1H, J = 6.0 Hz, Ar-H), 7.68 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.59-7.64 (m, 2H, Ar-H), 7.45-7.50 (m, 1H, Ar-H), 7.28-7.35 (m, 3H, Ar-H), 6.74 (d, 1H, J = 12.0 Hz, COCH) , 5.26-5.31 (m, 1H, $SCH_2C\underline{H}$), 5.04-5.09 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.18 (m, 2H, OCH_2), 3.80 (s, 3H, OCH_3), 3.64-3.65 (m, 2H, SCH_2), 3.26 (m, 2H, NCH_2), 2.67-2.83 (m, 5H, $\underline{H}N(C\underline{H}_2)_2$), 2.34-2.47 (m, 4H, NCH_2), 1.93-2.01 (m, 8H, 2 × $CC\underline{H}_2C\underline{H}_2CH$), 1.51-1.70 (m, 16H, 4 × CH= $CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 165.95 (CO), 163.05 (CO), 151.06 (Ar-C), 143.75 (Ar-C=), 142.29 (Ar-C), 140.74 (Ar-C), 134.39 (C=), 133.12 (C=), 133.03 (Ar-C), 131.37 (Ar-C), 131.30 (C=), 128.57 (Ar-C), 126.46 (Ar-C), 125.48 (Ar-C), 124.03 (C=), 123.17 (C=), 121.61 (C=), 118.44 (Ar-C), 118.21 (C=), 112.08 (Ar-C), 72.22 (NCH_2), 63.11 (OCH_2), 56.04 (OCH_3), 54.90 (CH_2), 47.41 (CH_2), 31.93 (CH_2), 29.39 (CH_2), 29.26 (SCH_2), 25.72 (CH_2), 25.22 (CH_2), 22.69 (CH_2), 22.13 (CH_2), 21.93 (CH_3), 21.93 (CH_3), 21.93 (CH_3), 15.93 (CH_3); MS (ESI) m/z = 675 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{40}H_{55}N_2O_5S$: 675.3832; found: 675.3846 $[M+H]^+$; Anal. Calcd. for $C_{40}H_{54}N_2O_5S$: C, 71.18; H, 8.06; N, 4.15; Found: C, 71.02; H, 8.21; N, 3.98.

2-Methoxy-4-((*E*)-3-oxo-3-(4-(4-benzylpiperazin-1-yl)butoxy)prop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-tri methyldodeca-2,6,10-trienylthio)benzoate (5g). According to the procedure of the preparation of **5a**, 4-benzylpiperazine (92 mg, 0.52 mmol), **3a** (0.16 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5g** (0.23 g, 0.30 mmol, 59 %) as a colorless thick liquid. Analytical data for **5g**: IR (KBr, cm^{-1}): 3429, 2937, 1619, 1454, 1238, 1039; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.67 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.59-7.63 (m, 2H, Ar-H), 7.49 (m, 1H, Ar-H), 7.25-7.31 (m, 8H, Ar-H), 6.75 (d, 1H, J =

12.0 Hz, COCH), 5.26-5.29 (m, 1H, $SCH_2C\underline{H}$), 5.04-5.07 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.17 (m, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 3.63-3.65 (m, 2H, SCH_2), 3.44 (s, 2H, $ArCH_2$), 2.28-2.36 (m, 10H, NCH_2), 1.93-2.02 (m, 8H, 2 × $CC\underline{H}_2C\underline{H}_2CH$), 1.50-1.70 (m, 16H, 4 × $CH{=}CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.29 (CO), 163.25 (CO), 151.26 (Ar-C), 143.80 (Ar-C=), 140.91 (Ar-C), 140.30 (Ar-C), 138.26 (Ar-C), 134.64 (C=), 133.36 (C=), 133.26 (Ar-C), 131.63 (C=), 128.78 (Ar-C), 128.12 (Ar-C), 126.84 (Ar-C), 126.53 (Ar-C), 124.45 (Ar-C), 124.16 (Ar-C), 124.10 (Ar-C), 124.06 (Ar-C), 123.84 (Ar-C), 123.46 (C=), 123.36 (C=), 121.72 (C=), 118.54 (Ar-C), 118.15 (C=), 111.99 (Ar-C), 64.00 (OCH_2), 62.12 (NCH_2), 57.31 (NCH_2), 56.10 (OCH_3), 52.75 (2×CH_2), 52.68 (2×CH_2), 32.10 (CH_2), 31.47 (CH_2), 26.24 (SCH_2), 25.99 (CH_2), 25.74 (CH_2), 25.71 (CH_2), 25.50 (CH_3), 25.41 (CH_3), 23.12 (CH_2), 22.77 (CH_3), 15.97 (CH_3); MS (ESI) *m/z* = 765 $[M+H]^+$. HRMS (ESI): *m/z* calcd for $C_{47}H_{61}N_2O_5S$: 765.4301; found: 765.4326 $[M+H]^+$; Anal. Calcd. for $C_{47}H_{60}N_2O_5S$: C, 73.79; H, 7.90;N, 3.66; Found: C, 73.59; H, 8.09; N, 3.79.

4-((*E*)-3-(4-(1H-Imidazol-1-yl)butoxy)-3-oxoprop-1-enyl)-2-methoxyphenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5h). According to the procedure of the preparation of **5a**, imidazole (35 mg, 0.52 mmol), **3a** (0.16 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5h** (0.17 g, 0.26 mmol, 52 %) as a colorless thick liquid. Analytical data for **5h**: IR (KBr, cm^{-1}): 3421, 2932, 1655, 1413, 1136, 1014; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.68 (d, 1H, *J* = 12.0 Hz, COCH=C$\underline{H}$), 7.59-7.63 (m, 3H, Ar-H, N-CH=N), 7.48-7.50 (m, 1H, Ar-H), 7.19-7.37 (m, 4H, Ar-H, N-C$\underline{H}$=CH), 6.90-6.91 (m, 1H, N-CH=C$\underline{H}$), 6.75 (d, 1H, *J* = 12.0 Hz, COCH), 5.28-5.29 (m, 1H, $SCH_2C\underline{H}$), 5.04-5.07 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.13-4.18 (m, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.64 (d, 2H, *J* = 6.0 Hz, SCH_2), 3.36 (m, 2H, NCH_2), 1.98-2.02 (m, 8H, 2 × $CC\underline{H}_2C\underline{H}_2CH$), 1.39-1.70 (m, 16H, 4 × $CH{=}CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.26 (CO), 163.26 (CO), 151.26 (Ar-C), 143.93 (Ar-C=), 140.94 (Ar-C), 140.31 (Ar-C), 137.23 (Ar-C), 133.38 (C=), 133.25 (C=), 131.65 (C=), 128.30 (Ar-C), 126.84 (Ar-C), 126.54 (Ar-C), 124.16 (Ar-C), 123.85 (Ar-C), 123.46 (Ar-C), 123.38 (Ar-C), 123.37 (C=), 122.98 (C=), 121.75 (C=), 119.28 (Ar-C), 118.49 (Ar-C), 118.43 (Ar-C), 118.16 (C=), 112.01 (Ar-C), 63.50 (OCH_2), 56.12 (OCH_3), 45.54 (NCH_2), 32.11 (CH_2), 29.54 (CH_2), 27.25 (SCH_2), 26.00 (CH_2), 25.41 (CH_2), 25.38 (CH_2), 25.32 (CH_3), 25.08 (CH_3), 23.13 (CH_2), 22.73 (CH_3), 15.97 (CH_3); MS (ESI) *m/z* = 657 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{39}H_{49}N_2O_5S$: 657.3362; found: 657.3378 $[M+H]^+$; Anal. Calcd. for $C_{39}H_{48}N_2O_5S$: C, 71.31; H, 7.37; N, 4.26; Found: C, 71.09; H, 7.50; N, 4.47.

4-((*E*)-3-Oxo-3-(4-(piperidin-1-yl)butoxy)prop-1-en-1-yl)phenyl-2-(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5i). According to the procedure of the preparation of **5a**, piperidine (44 mg, 0.52 mmol), **3b** (0.15 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5i** (0.20 g, 0.31 mmol, 62 %) as a colorless thick liquid. Analytical data for **5i**: IR (KBr, cm^{-1}): 3420, 2917, 1738, 1632, 1419, 1232, 1119, 1023; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.84-7.86 (d, 2H, Ar-H), 7.69 (d, 1H, *J* = 12.0 Hz, COCH=C$\underline{H}$), 7.61 (m, 1H, Ar-H), 7.64 (m, 1H, Ar-H), 7.32 (m, 3H, Ar-H), 6.67 (d, 1H, *J* = 12.0 Hz, COCH), 5.26-5.29 (m, 1H, $SCH_2C\underline{H}$), 5.03 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.15-4.18 (m, 2H, OCH_2), 3.64 (d, 2H, *J* = 6.0 Hz, SCH_2), 2.24-2.30 (m, 6H, NCH_2), 1.98-2.03 (m, 8H, 2× $CC\underline{H}_2C\underline{H}_2CH$), 1.36-1.71 (m, 22H, 4 × $CH{=}CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$, $CH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.24 (CO), 163.94 (CO), 151.94 (Ar-C), 143.40 (Ar-C=), 140.35 (Ar-C), 133.42 (C=), 133.33 (C=), 131.98 (C=), 129.74 (Ar-C), 126.91 (Ar-C), 126.80 (Ar-C), 124.35 (Ar-C), 124.30 (Ar-C), 124.28 (Ar-C), 123.74 (C=), 122.63 (C=), 122.54 (C=), 122.47 (Ar-C), 118.36 (Ar-C), 118.19 (C=), 64.05 (OCH_2), 58.08 (NCH_2), 54.02 (NCH_2), 32.12 (CH_2), 31.55 (CH_2), 29.72 (CH_2), 29.72 (CH_2), 29.72 (CH_2), 26.31 (SCH_2), 26.17 (CH_2), 26.00 (CH_3), 25.59 (CH_2), 24.18 (CH_2), 23.13 (CH_3), 22.80 (CH_3), 15.97 (CH_3); MS (ESI) *m/z* = 644 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{40}H_{54}NO_4S$: 644.3774; found: 644.3752 $[M+H]^+$; Anal. Calcd. for $C_{40}H_{53}NO_4S$: C, 74.61; H, 8.30;N, 2.18; Found: C, 74.49; H, 8.44; N, 2.02.

4-((*E*)-3-(4-Morpholinobutoxy)-3-oxoprop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5j). According to the procedure of the preparation of **5a**, morpholine (45 mg, 0.52 mmol) **3b** (0.15 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5j** (0.20 g, 0.31 mmol, 62 %) as a colorless thick liquid. Analytical data for **5j**: IR (KBr, cm^{-1}): 3444, 2925, 1712, 1637, 1459, 1208, 1034; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.85 (d, 2H, Ar-H), 7.69 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.62 (m, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 7.32-7.34 (m, 3H, Ar-H), 6.65 (d, 1H, J = 12.0 Hz, COCH), 5.26-5.29 (m, 1H, $SCH_2C\underline{H}$), 5.04 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.16-4.19 (m, 2H, OCH_2), 3.65 (d, 2H, J = 6.0 Hz, SCH_2), 3.54-3.58 (m, 4H, OCH_2), 2.35 (m, 6H, $N(CH_2)$, 1.91-2.01 (m, 8H, 2 × $CC\underline{H_2}C\underline{H_2}CH$), 1.45-1.71 (m, 16H, 4 × CH=$CC\underline{H_3}$, $OCH_2C\underline{H_2}C\underline{H_2}$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.23 (CO), 163.93 (CO), 151.95 (Ar-C), 143.43 (Ar-C=), 142.26 (Ar-C), 134.25 (C=), 133.36 (C=), 133.32 (Ar-C), 131.96 (Ar-C), 131.48 (C=), 129.73 (Ar-C), 126.80 (Ar-C), 126.78 (Ar-C), 124.33 (C=), 124.29 (Ar-C), 124.09 (C=), 123.47 (C=), 122.47 (Ar-C), 118.33 (Ar-C), 118.20 (C=), 66.17 (OCH_2), 63.94 (OCH_2), 57.67 (NCH_2), 53.23 (NCH_2), 34.07 (CH_2), 32.11 (CH_2), 31.15 (CH_2), 30.83 (CH_2), 29.71 (SCH_2), 27.33 (CH_2), 26.14 (CH_3), 25.73 (CH_3), 23.05 (CH_2), 22.69 (CH_3), 15.93 (CH_3); MS (ESI) m/z = 646 $[M+H]^+$. HRMS (ESI): *m/z* calcd for $C_{39}H_{52}NO_5S$: 646.3566; found: 646.3570 $[M+H]^+$; Anal. Calcd. for $C_{39}H_{51}NO_5S$: C, 72.52; H, 7.96; N, 2.17; Found: C, 72.33; H, 8.16; N, 2.08.

4-((*E*)-3-(4-(4-Methylpiperazin-1-yl)butoxy)-3-oxoprop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca -2,6,10-trienylthio)benzoate (5k). According to the procedure of the preparation of **5a**, 4-methylpiperazine (52 mg, 0.52 mmol), **3b** (0.15 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5k** (0.19 g, 0.29 mmol, 57 %) as a colorless thick liquid. Analytical data for **5k**: IR (KBr, cm^{-1}): 3445, 2965, 1636, 1166, 1034; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.85 (d, 2H, Ar-H), 7.69 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.62 (m, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 7.32-7.34 (m, 3H, Ar-H), 6.67 (d, 1H, J = 12.0 Hz, COCH), 5.26-5.29 (m, 1H, $SCH_2C\underline{H}$), 5.04 (m, 2H, 2×$CH_2C\underline{H}$=CCH_3), 4.15-4.18 (m, 2H, OCH_2), 3.64 (d, 2H, J = 6.0 Hz, SCH_2), 2.27-2.30 (m, 10H, NCH_2), 2.14 (s, 3H, NCH_3), 1.96-2.02 (m, 8H, 2×$CC\underline{H_2}C\underline{H_2}CH$), 1.50-1.71 (m, 18H, 4×CH =$CC\underline{H_3}$, $OCH_2C\underline{H_2}C\underline{H_2}$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.22 (CO), 163.92 (CO), 151.93 (Ar-C), 143.39 (Ar-C=), 142.27 (Ar-C), 134.63 (C=), 133.37 (C=), 133.30 (Ar-C), 131.96 (Ar-C), 131.50 (C=), 129.72 (Ar-C), 126.77 (Ar-C), 126.13 (Ar-C), 124.45 (C=), 124.27 (Ar-C), 124.06 (C=), 123.45 (C=), 122.45 (Ar-C), 118.34 (Ar-C), 118.17 (C=), 64.01 (OCH_2), 57.29 (NCH_2), 54.77 (NCH_2), 52.64 (NCH_2), 45.74 (NCH_3), 32.11 (CH_2), 31.47 (CH_2), 29.73 (CH_2), 26.24 (SCH_2), 25.99 (CH_2), 25.72 (CH_2), 25.50 (CH_3), 22.42 (CH_3), 23.05 (CH_2), 22.69 (CH_3), 15.93 (CH_3); MS (ESI) *m/z* = 659 $[M+H]^+$. HRMS (ESI): *m/z* calcd for $C_{40}H_{55}N_2O_4S$: 659.3883; found: 659.3858 $[M+H]^+$; Anal. Calcd. for $C_{40}H_{54}N_2O_4S$: C, 72.91; H, 8.26; N, 4.25; Found: C, 72.79; H, 8.42; N, 4.09.

4-((*E*)-3-Oxo-3-(4-(4-phenylpiperazin-1-yl)butoxy)prop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5l). According to the procedure of the preparation of **5a**, 4-phenyl piperazine (84 mg, 0.52 mmol), **3b** (0.15 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5l** (0.24 g, 0.33 mmol, 66 %) as a colorless thick liquid. Analytical data for **5l**: IR (KBr, cm^{-1}): 3456, 1639, 1207, 1117, 1035; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.85 (d, 2H, Ar-H), 7.72 (m, 1H, Ar-H), 7.69 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.63 (m, 1H, Ar-H), 7.51(d, 1H, J = 6.0 Hz, Ar-H), 7.32-7.31 (m, 3H, Ar-H), 6.92 (m, 2H, Ar-H), 6.77 (m, 2H, Ar-H) , 6.67 (d, 1H, J = 12.0 Hz, COCH), 5.26-5.31 (m, 1H, $SCH_2C\underline{H}$), 5.05 (m, 2H, 2×$CH_2C\underline{H}$=CCH_3), 4.18-4.21 (m, 2H, OCH_2), 3.64-3.66 (d, 2H, J = 6.0 Hz, SCH_2), 3.12 (m, 4H, NCH_2), 2.36-2.38 (m, 6H, NCH_2),1.98-2.01 (m, 8H, 2×$CC\underline{H_2}C\underline{H_2}CH$), 1.49-1.71 (m, 16H, 4×CH =$CC\underline{H_3}$, $OCH_2C\underline{H_2}C\underline{H_2}$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.24 (CO), 163.94 (CO), 151.94 (Ar-C), 143.43 (Ar-C=), 142.26 (Ar-C), 133.32 (C=), 133.30 (C=), 131.97 (Ar-C), 131.51 (C=), 131.48 (Ar-C), 129.84 (Ar-C), 129.73 (Ar-C), 128.89 (Ar-C), 126.78 (Ar-C), 126.13 (Ar-C), 126.04 (Ar-C), 124.45 (C=), 124.28 (C=),

123.46 (C=), 122.46 (Ar-C), 118.80 (Ar-C), 118.34 (Ar-C), 118.18 (C=), 115.32 (Ar-C), 64.99 (OCH_2), 57.27 (NCH_2), 52.65 (NCH_2), 48.09 (NCH_2), 34.06 (CH_2), 32.10 (CH_2), 30.82 (CH_2), 29.69 (CH_2), 26.22 (SCH_2), 25.99 (CH_2), 25.73 (CH_3), 25.50 (CH_3), 23.12 (CH_2), 22.72 (CH_3), 15.97 (CH_3); MS (ESI) *m/z* = 721 $[M+H]^+$. HRMS (ESI): *m/z* calcd for $C_{45}H_{57}N_2O_4S$: 721.4039; found: 721.4057 $[M+H]^+$; Anal. Calcd. for $C_{45}H_{56}N_2O_4S$: C, 74.96; H, 7.83; N, 3.89; Found: C, 74.68; H, 8.06; N, 3.65.

Tert-butyl-4-(4-((*E*)-3-(4-(2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoyloxy)p-henyl)acryloyloxy)butyl)piperazine-1-carboxylate (5m). According to the procedure of the preparation of **5a**, 4-tertbutoxycarbonyl piperazine (97 mg, 0.52mmol), **3b** (0.15 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5m** (0.18 g, 0.24 mmol, 48 %) as a colorless thick liquid. Analytical data for **5m**: IR (KBr, cm^{-1}): 3427, 2927, 1712, 1640, 1463, 1239, 1035; ^{1}H NMR (DMSO-d_6, 300MHz, δ ppm): 8.11 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.82 (d, 2H, *J* = 6.0 Hz, Ar-H), 7.67 (m, 1H, COCH=C<u>H</u>), 7.59-7.61 (m, 1H, Ar-H), 7.49 (m, 1H, Ar-H), 7.29-7.31 (m, 3H, Ar-H), 6.64 (d, 1H, *J* = 12.0 Hz, COCH), 5.23-5.28 (m, 1H, $SCH_2C\underline{H}$), 5.01-5.05 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.13-4.16 (m, 2H, OCH_2), 3.63 (d, 2H, *J* = 7.2 Hz, SCH_2), 3.27 (m, 2H, NCH_2), 2.28 (m, 8H, 4 × NCH_2), 1.95-2.00 (m, 8H, 2×$CC\underline{H_2}C\underline{H_2}CH$), 1.42-1.67 (m, 16H, 4 × CH=$CC\underline{H_3}$, $OCH_2C\underline{H_2}C\underline{H_2}$), 1.36 (m, 9H, $C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.23 (CO), 163.92 (CO), 153.78 (Ar-C), 151.95 (Ar-C), 148.70 (Ar-C), 148.43 (Ar-C=), 134.64 (C=), 133.31 (C=), 131.96 (Ar-C), 131.51 (C=), 129.73 (Ar-C), 126.14 (Ar-C), 126.06 (Ar-C), 124.45 (C=), 124.28 (Ar-C), 124.10 (Ar-C), 124.07 (C=), 123.51 (C=), 122.46 (Ar-C), 118.32 (Ar-C), 118.18 (C=), 78.72 (C), 75.99 (NCH_2), 63.95 (OCH_2), 57.21 (NCH_2), 52.44 (NCH_2), 34.06 (CH_2), 32.11 (CH_2), 30.83 (CH_2), 28.04 (CH_3), 26.15 (SCH_2), 25.99 (CH_2), 25.73 (CH_3), 25.50 (CH_3), 23.12 (CH_2), 22.56 (CH_3), 17.44 (CH_3), 15.97 (CH_3); MS (ESI) *m/z* = 745 $[M+H]^+$. HRMS (ESI): *m/z* calcd for $C_{44}H_{61}N_2O_6S$: 745.4250; found: 745.4276 $[M+H]^+$; Anal. Calcd. for $C_{44}H_{60}N_2O_6S$: C, 70.93; H, 8.12; N, 3.76; Found: C, 70.78; H, 8.36; N, 3.42.

4-((*E*)-3-Oxo-3-(4-(piperazin-1-yl)butoxy)prop-1-en-1-yl)phenyl-2-(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)thio)benzoate (5n). According to the procedure of the preparation of **5f**, compound **5m** (0.15 g, 0.20 mmol) yielded **5n** (0.11 g, 0.17 mmol, 87 %) as a colorless thick liquid. Analytical data for **5n**: IR (KBr, cm^{-1}): 3409, 2959, 1737, 1626, 1456, 1249, 1166, 1045; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.15 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.85 (d, 2H, *J* = 6.0 Hz, Ar-H), 7.66 (m, 1H, COCH=C<u>H</u>), 7.61-7.64 (m, 1H, Ar-H), 7.56-7.58 (m, 1H, Ar-H), 7.31-7.34 (m, 3H, Ar-H), 6.67 (d, 1H, *J* = 12.0 Hz, COCH), 5.25-5.29 (m, 1H, $SCH_2C\underline{H}$), 5.05-5.09 (m, 2H, 2 × CH_2CH=CCH_3), 4.16-4.17 (m, 2H, OCH_2), 3.62 (d, 2H, *J* = 7.2 Hz, SCH_2), 2.83-2.86 (m, 5H, NH, $NC\underline{H_2}$), 2.33-2.42 (m, 6H, NCH_2), 1.96-2.01 (m, 8H, 2 × $CC\underline{H_2}C\underline{H_2}CH$), 1.52-1.67 (m, 16H, 4 × CH=$CC\underline{H_3}$, $OCH_2C\underline{H_2}C\underline{H_2}$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.02 (CO), 163.85 (CO), 151.88 (Ar-C), 143.47 (C=), 141.98 (Ar-C), 139.89 (C=), 133.32 (C=), 133.19 (Ar-C), 131.85 (Ar-C), 131.36 (C=C), 130.15 (Ar-C), 129.64 (Ar-C), 126.80 (Ar-C), 126.20 (Ar-C), 124.35 (Ar-C), 124.24 (C=), 122.36 (Ar-C), 118.88 (C=), 118.58 (Ar-C), 118.12 (C=), 72.35 (NCH_2), 63.23 (OCH_2), 54.97 (NCH_2), 47.50 (NCH_2), 32.02 (CH_2), 30.87 (CH_2), 29.64 (CH_2), 29.44 (CH_2), 29.36 (CH_2), 25.30 (SCH_2), 22.76 (CH_2), 22.02 (CH_3), 19.98 (CH_3), 19.72 (CH_3), 15.70 (CH_3); MS (ESI) *m/z* = 645 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{39}H_{53}N_2O_4S$: 645.3726; found: 645.3703 $[M+H]^+$; Anal. Calcd. for $C_{39}H_{52}N_2O_4S$: C, 72.63; H, 8.13;N, 4.34; Found: C, 72.42; H, 8.31; N, 4.11.

4-((*E*)-3-Oxo-3-(4-(4-benzylpiperazin-1-yl)butoxy)prop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5o). According to the procedure of the preparation of **5a**, 4-benzylpiperazine (92 mg, 0.52 mmol), **3b** (0.15 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5o** (0.24 g, 0.33 mmol, 66 %) as a colorless thick liquid. Analytical data for **5o**: IR (KBr, cm^{-1}): 3426, 2918, 1602, 1413, 1219, 1033; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.84 (d, 2H, Ar-H), 7.68 (d, 1H, *J* = 12.0 Hz, COCH=C<u>H</u>), 7.63 (m, 1H, Ar-H), 7.51(d, 1H, *J* = 6.0 Hz, Ar-H), 7.23-7.33 (m, 8H, Ar-H), 6.68 (d,

1H, *J* = 12.0 Hz, COCH), 5.26-5.31 (m, 1H, $SCH_2C\underline{H}$), 5.04 (m, 2H, $2\times CH_2C\underline{H}=CCH_3$), 4.15-4.17 (m, 2H, OCH_2), 3.64-3.66 (d, 2H, *J* = 6.0 Hz, SCH_2), 3.42 (s, 2H, Ar-$C\underline{H}_2$), 2.28-2.36 (m, 12H, NCH_2), 1.98-1.99 (m, 8H, 2 × CCH_2CH_2CH), 1.49-1.71 (m, 16H, 4×CH =$CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.22 (CO), 163.93 (CO), 151.93 (Ar-C), 144.63 (Ar-C), 143.39 (Ar-C=), 140.29 (Ar-C), 138.26 (Ar-C), 134.40 (C=), 133.41 (C=), 133.31 (C=), 132.00 (Ar-C), 132.97 (Ar-C), 131.50 (C=), 129.72 (Ar-C), 128.79 (Ar-C), 128.12 (Ar-C), 126.85 (Ar-C), 124.41 (C=), 124.29 (Ar-C), 124.07 (C=), 123.85 (C=), 122.45 (Ar-C), 118.35 (Ar-C), 118.18 (C=), 64.01 (OCH_2), 62.11 (CH_2), 57.30 (NCH_2), 52.74 (NCH_2), 52.67 (NCH_2), 32.11 (CH_2), 31.14 (CH_2), 29.73 (CH_2), 26.23 (SCH_2), 25.73 (CH_3), 25.50 (CH_3), 25.42 (CH_2), 23.12 (CH_2), 22.74 (CH_3), 17.45 (CH_3), 15.97 (CH_3); MS (ESI) *m/z* = 735 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{46}H_{59}N_2O_4S$: 735.4196; found: 735.4227 $[M+H]^+$; Anal. Calcd. for $C_{46}H_{58}N_2O_4S$: C, 75.17; H, 7.95; N, 3.81; Found: C, 74.96; H, 8.11; N, 3.68.

4-((*E*)-3-(4-(1H-Imidazol-1-yl)butoxy)-3-oxoprop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5p). According to the procedure of the preparation of **5a**, imidazole (35 mg, 0.52 mmol), **3b** (0.15 g, 0.50 mmol), and FTS (0.16 g, 0.45 mmol) yielded **5p** (0.21 g, 0.34 mmol, 68 %) as a colorless thick liquid. Analytical data for **5p**: IR (KBr, cm^{-1}) : 3452, 2937, 1702, 1632, 1452, 1170; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.85 (d, 2H, *J* = 6.0 Hz, Ar-H), 7.70 (d, 1H, *J* = 6.0 Hz, COCH=C$\underline{H}$), 7.61-7.66 (m, 2H, Ar-H), 7.51 (m, 1H, Ar-H), 7.32-7.34 (m, 3H, Ar-H), 7.19 (m, 1H, Ar-H), 6.89 (m, 1H, Ar-H), 6.67 (d, 1H, *J* = 12.0 Hz, COCH), 5.26-5.29 (m, 1H, $SCH_2C\underline{H}$), 5.03-5.07 (m, 2H, 2 × $CH_2C\underline{H}=CCH_3$), 4.17 (m, 2H, OCH_2), 4.02 (m, 2H, NCH_2), 3.65-3.66 (m, 2H, SCH_2), 1.95-2.02 (m, 8H, 2 × $CC\underline{H}_2C\underline{H}_2CH$), 1.47-1.71 (m, 16H, 4 × $CH=CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.78 (CO), 163.52 (CO), 152.54 (Ar-C), 144.91 (Ar-C), 144.11 (Ar-C=), 137.82 (Ar-C), 134.98 (C=), 133.93 (C=), 132.53 (Ar-C), 132.10 (C=), 130.92 (Ar-C), 130.76 (Ar-C), 130.33 (Ar-C), 128.91 (Ar-C), 127.73 (Ar-C), 126.70 (Ar-C), 124.41 (C=), 123.05 (Ar-C), 120.35 (C=), 119.85 (C=), 118.82 (Ar-C), 118.77 (C=), 115.41 (Ar-C), 64.10 (OCH_2), 46.11 (NCH_2), 32.09 (CH_2), 30.25 (CH_2), 27.83 (SCH_2), 26.57 (CH_2), 26.31 (CH_2), 25.97 (CH_3), 25.85 (CH_3), 25.56 (CH_2), 23.70 (CH_2), 23.31 (CH_3), 16.55 (CH_3); MS (ESI) *m/z* = 627 $[M+H]^+$. HRMS (ESI): *m/z* calcd for $C_{38}H_{47}N_2O_4S$: 627.3257; found: 627.3281 $[M+H]^+$; Anal. Calcd. for $C_{38}H_{46}N_2O_4S$: C, 72.81; H, 7.40; N, 4.47; Found: C, 72.63; H, 7.57; N, 4.26.

4-((*E*)-3-(4-Bromobutoxy)-3-oxoprop-1-enyl)-2-methoxyphenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (6a). To a solution of FTS (2.40 g, 6.70 mmol) in CH_2Cl_2 (50 mL) was added **3a** (2.31 g, 7.04 mmol), EDCI (1.54 g, 8.04 mmol), and DMAP (0.70 g, 5.70 mmol) and the mixture was reacted at room temperature for 28 h, The solution was concentrated *in vacuo*. The resulting residue was purified by column chromatography (EtOAc-PE = 1:4, v/v as the eluate), affording **6a** (3.40 g, 5.09 mmol, 76%) as a pale yellow oil. Analytical data for **6a**: IR (KBr, cm^{-1}): 3423, 2937, 1665, 1415, 1136, 1014; ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 8.23 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.66 (d, 1H, *J* = 12 Hz, CH=), 7.51 (m, 1H, Ar-H), 7.36 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.24 (m, 1H , Ar-H), 7.20 (m, 1H, Ar-H), 7.17 (m, 1H, Ar-H), 7.13 (m, 1H, Ar-H), 6.40 (d, 1H, *J* = 12 Hz, CH=), 5.34 (m, 1H, $SCH_2C\underline{H}$), 5.08 (m, 2H, 2 × $CH_2C\underline{H}=CCH_3$), 4.73 (m, 2H, OCH_2), 4.25 (s, 3H, OCH_3), 3.85 (m, 2H, SCH_2), 3.60 (m, 2H, CH_2Br), 1.91-1.96 (m, 8H, 2 × $CC\underline{H}_2C\underline{H}_2CH$), 1.50-1.88 (m, 16H, 4 × $CH=CC\underline{H}_3$, $OCH_2C\underline{H}_2C\underline{H}_2$); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 166.81 (CO), 163.98 (CO), 151.71 (Ar-C), 144.36 (C=), 141.68 (Ar-C), 141.07 (Ar-C), 133.24 (C=), 132.96 (Ar-C), 132.85 (Ar-C), 132.08 (C=), 126.16 (Ar-C), 124.93 (Ar-C), 124.52(Ar-C), 124.30 (Ar-C), 124.19 (Ar-C), 124.07 (C=), 123.81 (Ar-C), 123.63 (C=), 121.22 (C=), 118.02 (C=), 117.76 (C=), 111.29 (Ar-C), 63.58 (OCH_2), 55.98 (OCH_3), 33.11 (CH_2), 32.48 (CH_2), 32.44 (CH_2), 31.97 (CH_2), 30.95 (SCH_2), 30.55 (CH_3), 30.34 (CH_3), 29.69 (CH_2), 29.35 (CH_2), 27.43 (CH_3), 25.69 (CH_3), 17.68 (CH_3); MS (ESI) m/z = 669 $[M+H]^+$.

4-((*E*)-3-(4-Bromobutoxy)-3-oxoprop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylt

hio)benzoate (6b). According to the procedure of the preparation of **6a**, compound **3b** (2.10 g, 7.04 mmol), EDCI (1.54 g, 8.04 mmol), DMAP (0.70 g, 5.70 mmol), and FTS (2.40 g, 6.70 mmol) yielded **6b** (3.12 g, 4.89 mmol, 73 %) as a colorless thick liquid. Analytical data for **6b**: IR (KBr, cm^{-1}): 3449, 2910, 1678, 1432, 1149, 1031; ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 8.19 (d, 1H, J = 6.0 Hz, Ar-H), 7.68 (d, 1H, J = 12 Hz, CH=), 7.48-7.53 (m, 3H, Ar-H), 7.37 (d, 1H, J = 6.0 Hz, Ar-H), 7.27 (m, 1H , Ar-H), 7.23 (m, 1H, Ar-H), 7.20 (m, 1H, Ar-H), 6.40 (d, 1H, J = 12 Hz, CH=), 5.34 (m, 1H, SCH_2CH), 5.08 (m, 2H, 2 × CH_2CH=CCH_3), 4.73 (m, 2H, $OCH_2CH_2CH_2CH_2$), 3.61 (m, 2H, SCH_2), 3.47(m, 2H, CH_2Br), 1.86-2.03 (m, 8H, 2 × CCH_2CH_2CH), 1.50-1.88 (m, 16H, 4 × CH=CCH_3, $OCH_2CH_2CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 166.34 (CO), 163.95 (CO), 151.84 (Ar-C), 143.41 (C=), 140.25 (Ar-C), 132.48 (C=), 131.57 (C=), 131.33 (Ar-C), 128.70 (Ar-C), 125.81 (Ar-C), 125.70 (Ar-C), 124.35 (Ar-C), 124.10 (Ar-C), 123.99 (Ar-C), 123.84 (C=), 123.65 (C=), 123.38 (C=), 121.91 (C=), 117.53 (Ar-C), 117.28 (C=), 63.06 (OCH_2), 32.60 (CH_2), 31.94 (CH_2), 31.47 (CH_2), 30.13 (CH_2), 29.93 (SCH_2), 28.84 (CH_3), 26.92 (CH_3), 25.67 (CH_2), 25.20 (CH_2), 23.00 (CH_3), 22.86 (CH_3), 17.19 (CH_3); MS (ESI) m/z = 639 $[M+H]^+$.

4-((*E*)-3-(4-(4-(2-Hydroxyethyl)piperidin-1-yl)butoxy)-3-oxoprop-1-enyl)-2-methoxyphenyl2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate(5q). To a solution of compound **6a** (0.27 g, 0.40 mmol) in acetonitrile (15 mL), KI (8.3 mg, 0.05 mmol), K_2CO_3 (0.14 g, 1.00 mmol), and 2-(piperazin-1-yl)ethanol (68 mg, 0.52 mmol) were added. The mixture was stirred at 50 °C until the starting material was totally consumed. After filtration, the filtrate was collected and concentrated in vacuo. The crude product was purified by column chromatography (EtOAc-MeOH = 10:1-6:1, v/v as the eluate) to afford **5q** (0.15 g, 0.21 mmol, 52%) as a colorless thick liquid. Analytical data for **5q**: IR (KBr, cm^{-1}): 3442, 2927, 1703, 1637, 1453, 1209, 1038; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.68 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.59-7.63 (m, 2H, Ar-H), 7.50 (m, 1H, Ar-H), 7.25-7.35 (m, 3H, Ar-H), 6.75 (d, 1H, J = 12.0 Hz, COCH), 5.26-5.28 (m, 1H, SCH_2CH), 5.03-5.07 (m, 2H, 2 × CH_2CH=CCH_3), 4.17 (m, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.63-3.65 (m, 2H, SCH_2), 3.48 (m, 2H, OCH_2), 2.27-2.40 (m, 12H, NCH_2), 1.97-2.02 (m, 8H, 2 × CCH_2CH_2CH), 1.47-1.77 (m, 16H, 4 × CH=CCH_3, $OCH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.31 (CO), 163.26 (CO), 151.27 (Ar-C), 143.81 (Ar-C=), 140.92 (Ar-C), 140.31 (Ar-C), 134.65 (C=), 133.41 (C=), 133.27 (Ar-C), 132.50 (Ar-C), 131.64 (C=), 125.49 (Ar-C), 124.46 (Ar-C), 124.16 (Ar-C), 124.11 (Ar-C), 124.07 (C=), 123.85 (C=), 123.53 (C=), 123.38 (Ar-C), 121.74 (Ar-C), 118.55 (Ar-C), 118.18 (C=), 112.01 (Ar-C), 63.01 (OCH_2), 60.29 (OCH_2), 57.19 (NCH_2), 57.35 (NCH_2), 56.12 (OCH_3), 53.24 (2×CH_2), 52.75 (2×CH_2), 32.12 (CH_2), 31.48 (CH_2), 29.54 (CH_2), 26.26 (SCH_2), 26.15 (CH_2), 26.00 (CH_2), 25.75 (CH_3), 25.51 (CH_3), 23.13 (CH_2), 22.74 (CH_3), 15.98 (CH_3); MS (ESI) m/z = 719 $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{42}H_{59}N_2O_6S$: 719.4094; found: 719.4075 $[M+H]^+$; Anal. Calcd. for $C_{42}H_{58}N_2O_6S$: C, 70.16; H, 8.13; N, 3.90; Found: C, 69.97; H, 8.40; N, 4.11.

4-((*E*)-3-(4-(4-(2-Hydroxyethyl)piperidin-1-yl)butoxy)-3-oxoprop-1-enyl)phenyl-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)benzoate (5r). According to the procedure of the preparation of **5q**, 2-(piperazin-1-yl)ethanol (68 mg, 0.52 mmol) and **6b** (0.26 g, 0.40 mmol) yielded **5r** (0.16 g, 0.23 mmol, 58 %) as a colorless thick liquid. Analytical data for **5r**: IR (KBr, cm^{-1}): 3447, 2929, 1709, 1632, 1451, 1205, 1030; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.84 (d, 2H, Ar-H), 7.68 (d, 1H, J = 12.0 Hz, COCH=C<u>H</u>), 7.63 (m, 1H, Ar-H), 7.51(d, 1H, J = 6.0 Hz, Ar-H), 7.23-7.33 (m, 3H, Ar-H), 6.66 (d, 1H, J = 12.0 Hz, COCH), 5.26-5.31 (m, 1H, SCH_2CH), 5.03-5.07 (m, 2H, 2×CH_2CH=CCH_3), 4.15-4.18 (m, 2H, OCH_2), 3.65-3.66 (d, 2H, J = 6.0 Hz, SCH_2), 3.48 (m, 2H, OCH_2), 2.28-2.38 (m, 12H, NCH_2), 1.98-2.01 (m, 8H, 2 × CCH_2CH_2CH), 1.50-1.71 (m, 16H, 4×CH =CCH_3, $OCH_2CH_2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.24 (CO), 163.94 (CO), 151.94 (Ar-C), 143.41 (Ar-C=), 142.23 (Ar-C), 133.33 (C=), 131.98 (Ar-C), 131.51 (C=), 130.89 (C=), 129.73 (Ar-C), 127.61 (Ar-C), 126.81 (Ar-C), 124.46 (Ar-C), 124.29 (Ar-C),

124.07 (C=), 123.47 (C=), 122.65 (C=), 122.47 (Ar-C), 118.35 (Ar-C), 118.20 (C=), 64.02 (OCH_2), 60.26 (OCH_2), 58.44 (NCH_2), 57.33 (NCH_2), 53.20 (2×CH_2), 52.70 (2×CH_2), 32.11 (CH_2), 29.73 (CH_2), 29.01 (CH_2), 26.24 (SCH_2), 26.00 (CH_2), 25.73 (CH_3), 23.13 (CH_2), 22.72 (CH_3), 19.71 (CH_3), 15.97 (CH_3); MS (ESI) *m/z* = 689 $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{41}H_{57}N_2O_5S$: 689.3988; found: 689.3976 $[M+H]^+$; Anal. Calcd. for $C_{41}H_{56}N_2O_5S$: C, 71.48; H, 8.19; N, 4.07; Found: C, 71.33; H, 8.25; N, 4.13.

1-(4-(((E)-3-(3-methoxy-4-((2-(((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)thio)benzoyl)oxy)phenyl)acryloyl)oxy)butyl)-1-methylpiperazine-1,4-diium chloride iodide (7b)

A solution of **5e** (60 mg, 0.078 mmol) and iodomethane (1.10 g, 7.8 mmol) in acetonitrile (3 mL) was stirred at room temperature overnight. The solvent was removed under reduced pressure and 4 M HCl/EtOAc solution (3 mL) was added and the mixture was stirred at room temperature for 3 h. After the ethyl ether was added, the precipitate was collected by filtration and was washed with dry diethyl ether to give the product (53 mg, 0.063 mmol, 81%) as white solid. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 3.21 (s, 2H, NH_2), 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.73 (d, 1H, J = 12.0 Hz, Ar-CH=), 7.60-7.68 (m, 2H, Ar-H), 7.50 (m, 1H, Ar-H), 7.37-7.31 (m, 2H , Ar-H), 7.26 (m, 1H, Ar-H), 6.76 (d, 1H, J = 12 Hz, CH=), 5.29 (m, 1H, $SCH_2C\underline{H}$), 5.04 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.22 (m, 2H, OCH_2), 3.73-3.83 (m, 5H, SCH_2, OCH_3), 3.64-3.66 (m, 8H, 2 × $N(CH_2)_2$), 2.36-2.50 (m, 5H, NCH_3, NCH_2), 2.01-2.11 (m, 8H, 2 × $CC\underline{H_2}C\underline{H_2}CH$), 1.36-1.90 (m, 16H, 4 × CH=$CC\underline{H_3}$, $OCH_2C\underline{H_2}C\underline{H_2}$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.22 (CO), 163.22 (CO), 151.26 (Ar-C), 144.00 (C=C), 142.58 (Ar-C), 140.95 (Ar-C), 139.99 (Ar-C), 139.92 (Ar-C), 134.39 (Ar-C), 133.35 (C=), 133.19 (Ar-C), 131.58 (Ar-C), 131.30 (C=), 128.57 (Ar-C), 126.57 (Ar-C), 125.49 (C=), 124.17 (C=), 123.38 (C=), 121.68 (C=), 118.58 (Ar-C), 118.35 (C=), 112.09 (Ar-C), 72.45 (NCH_2), 63.29 (OCH_2), 56.16 (OCH_3), 55.87 (CH_2), 47.22 (CH_2), 45.09 (CH_3), 37.00 (CH_2), 32.09 (CH_2), 29.49 (CH_2), 29.42 (SCH_2), 25.01 (CH_2), 22.69 (CH_2), 22.09 (CH_2), 20.05 (CH_3), 18.05 (CH_3), 15.77 (CH_3), 15.09 (CH_3); HRMS (ESI): *m/z* calcd for $C_{41}H_{58}N_2O_5S$: 690.4055; found: 690.4030 $[M]^{2+}$.

1-(4-(((*E*)-3-(3-methoxy-4-((2-(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)thio)benzoyl)oxy)phenyl)acryloyl)oxy)butyl)-1,4,4-trimethylpiperazine-1,4-diium iodide (7c)

A solution of **5c** (60 mg, 0.087 mmol) in acetonitrile (3 mL) was added iodomethane (1.23 g, 8.7 mmol) and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and dry diethyl ether was added. The precipitate was collected by filtration and was washed with dry diethyl ether to give the product (73 mg, 0.075 mmol, 86%) as white solid. ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 8.14 (d, 1H, J = 6.0 Hz, Ar-H), 7.70 (d, 1H, J = 12.0 Hz, Ar-CH=), 7.58-7.68 (m, 2H, Ar-H), 7.50 (m, 1H, Ar-H),7.38 (d, 1H, Ar-H), 7.32 (m, 1H , Ar-H), 7.27 (m, 1H, Ar-H), 6.75 (d, 1H, J = 12.0 Hz, CH=), 5.27 (m, 1H, $SCH_2C\underline{H}$), 5.04 (m, 2H, 2 × $CH_2C\underline{H}$=CCH_3), 4.23 (m, 2H, $OC\underline{H_2}CH_2CH_2CH_2$), 3.83 (m, 5H, SCH_2, OCH_3), 3.54-3.62 (m, 8H, 2 × $N(CH_2)_2$), 3.39 (s, 6H, $N(CH_3)_2$), 3.17-3.32 (m, 5H, NCH_3, NCH_2), 2.00-2.45 (m, 8H, 2 × $CC\underline{H_2}C\underline{H_2}CH$), 1.46-1.73 (m, 16H, 4 × CH=$CC\underline{H_3}$, $OCH_2C\underline{H_2}C\underline{H_2}$); ^{13}C NMR (DMSO-$d_6$, 75 MHz, δ ppm): 166.22 (CO), 163.23 (CO), 151.28 (Ar-C), 144.04 (C=C), 140.98 (Ar-C), 140.23 (Ar-C), 134.51 (Ar-C), 133.62 (C=), 133.17 (Ar-C), 131.78 (C=), 126.61 (Ar-C), 126.58 (Ar-C), 124.41 (Ar-C), 124.16 (Ar-C), 124.07 (Ar-C), 123.96 (C=), 123.82 (C=), 123.40 (C=), 121.64 (C=), 118.31 (Ar-C), 118.19 (C=), 112.07 (Ar-C), 73.86 (NCH_2), 63.27 (OCH_2), 56.11 (OCH_3), 54.33 (CH_2), 53.27 (CH_2), 52.85 (CH_3), 32.07 (CH_2), 29.58 (CH_2), 26.08 (SCH_2), 25.75 (CH_2), 25.43 (CH_2), 25.21 (CH_3), 22.74 (CH_3), 22.55 (CH_2), 17.99 (CH_3), 15.94 (CH_3), 14.68 (CH_3); HRMS (ESI): *m/z* calcd for $C_{43}H_{62}N_2O_5S$: 718.4368; found: 718.4356 $[M]^{2+}$.

Biological evaluation

MTT assay. human hepatocellular carcinoma cells (SMMC-7721 and HepG2), human breast cancer cells

(MCF-7), human gastric cancer cells (SGC7901), human bladder carcinoma cells (EJ), human ovarian cancer cells (SKOV-3) at 10^4 cells per well were cultured in 10% FBS DMEM in 96-well flat-bottom microplates overnight. The cells were incubated in triplicate with, or without, different concentrations of each test compound for 48 h. During the last 4 h incubation, 30 μL of tetrazolium dye (MTT) solution (5 mg/mL) was added to each well. The resulting MTT-formazan crystals were dissolved in 150 μL DMSO, and absorbance was measured spectrophotometrically at 570 nm using an ELISA plate reader. The inhibition induced by each test compound at the indicated concentrations was expressed as a percentage. The concentration required for 50% inhibition (IC_{50}) was calculated using the software (GraphPadPrism Version 4.03).

Antitumor effects of 5f in mice. H22 cells (3×10^6) were subcutaneously injected into the right armpit of the mice that were randomly divided into five groups of six mice each. Treatments were initiated when tumors reached a mean group size of approximately 100 mm^3. The groups with compound **5f** treatment received two dosages (29.7 or 59.3 μmol/kg) by intravenous injection. The negative control group received 0.9% normal saline, and the positive group was treated with FTS by intraperitoneal injection (59.3 μmol/kg). The corresponding agent for each group was administered every day for 14 days. All mice were then sacrificed, and their tumors were segregated and weighed. The tumor inhibitory ratio was calculated by the following formula: tumor inhibitory ratio (%) = $[(W_{control} - W_{treated})/W_{control}] \times 100\%$. $W_{treated}$ and $W_{control}$ were the average tumor weights of the treated and control mice, respectively. The tumor diameters were measured with calipers every other day, and the tumor volume was calculated by the formula V (mm^3) = $d^2 \times D/2$, where D is the largest diameter and d the smallest diameter.

Flow cytometry assay of cell apoptosis. SMMC-7721 cells were cultured overnight and incubated in triplicate with different concentrtions of **5f** (1.5, 3.0, and 6.0 μM), FTS (12 μM), or vehicle for 48 h. The cells were harvested and stained with FITC-Annexin V and PI (BioVision) at room temperature for 15 min. The percentage of apoptotic cells was determined by flow cytometry (Beckman Coulter) analysis.

Western blot assay. The mechanisms of the cell apoptosis and the inhibitory activity of Ras-related signaling were determined by western blot assay. SMMC-7721 cells at 1.5×10^5/mL were treated with 1.5, 3.0 or 6.0 μM **5f** or vehicle control for 48 h. After harvested and lyzed, the cell lysates (50 μg/lane) were separated by SDS-PAGE (12% gel) and transferred onto nitrocellulose membranes. After blocked with 5% fat-free milk, the target proteins were probed with anti-Bcl-2, anti-Bax, anti-caspase-3, anti-Akt, anti-phospho-Akt (Ser473), anti-phospho-ERK (Thr202/Tyr204), anti-Phospho-Raf (Ser259), anti-NF-κB, anti-phospho-NF-κB and anti-β-actin antibodies (Cell Signaling, Boston), respectively. The bound antibodies were detected by HRP-conjugated second antibodies and visualized using the enhanced chemiluminescent reagent. The relative levels of each signaling event to control β-actin were determined by densimetric scanning.

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