



## Design, synthesis and biological evaluation of novel aliphatic amido/sulfonamido-quaternary ammonium salts as antitumor agents

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### ABSTRACT

RhoB, one of the upstream signaling proteins of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, is frequently mutated in human cancer. Based on a piperazine alkyl derivative that induced apoptosis via up-regulation of RhoB, we synthesized novel aliphatic amido/sulfonamido-quaternary ammonium salts and evaluated their biological activities using an in vitro growth inhibition assay and RhoB promoter assay in human cancer cells. Compound **3a** was the most promising anticancer agent in the series, based upon its potent growth inhibition via RhoB-mediated signaling. These novel aliphatic amido/sulfonamido-quaternary ammonium salts may be useful as a platform for development of anticancer chemotherapeutic agents.

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## 1. Introduction

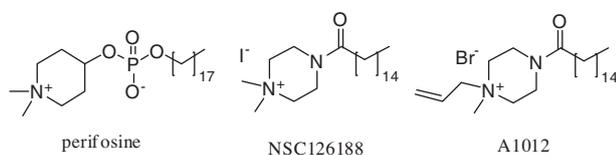
Carcinogenesis can be the result of changes in proteins and signaling pathways which regulate cell growth, differentiation, and development. In these pathways, PI3K/Akt signaling and Ras signaling are often stimulated in human cancers via several different mechanisms.<sup>1–5</sup>

Phosphatidylinositol-3-kinase (PI3K) is a lipid kinase which generates phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P<sub>3</sub>]. PI(3,4,5)P<sub>3</sub> acts as a second messenger and is necessary for the translocation of Akt to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase (PDK)1 and PDK2.<sup>6</sup> Activated Akt plays a crucial role in cell proliferation and survival by phosphorylation of a variety of substrates.<sup>6</sup> With similar structural features as PI(3,4,5)P<sub>3</sub>, phosphatidylinositol (PI) analogues are one class of Akt inhibitors represented by perifosine [octadecyl-(1,1-dimethyl-piperidino-4-yl)-phosphate] (Fig. 1), which is synthetic and orally bioavailable.<sup>7–9</sup>

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The small GTPase Ras, one of the proteins involved in the upstream pathway of PI3K/Akt signaling, is also frequently mutated in human cancer.<sup>10</sup> Mutated Ras results in an altered PI3K, which is a downstream target of Ras signaling.<sup>11</sup> Rho (Ras homologous small GTPase) are members of the Ras-superfamily which is responsible for proliferation, survival, invasion, metastasis, as well as overall angiogenic capacity of cancer cells.<sup>12–15</sup> Rho is composed of subgroups which show 86% sequence homology. Of note is RhoB, a subgroup member with different activities from the RhoA-related subgroups (RhoA and RhoC). RhoB has a tumor suppressive role, whereas most other Rho proteins have promoting roles in cancer.<sup>16,17</sup> Because RhoB expression is frequently suppressed in tumor progression, it is believed that RhoB overexpression prevents oncogenic signaling by modifying oncogene proteins such as EGFR, ErbB2, Ras, and Akt resulting in changes in their localizations.<sup>17</sup> Recently, it was reported that a piperazine alkyl derivative (NSC126188, Fig. 1) induced apoptosis via up-regulation of RhoB in HeLa cells.<sup>18</sup> Structural features of this compound are similar to perifosine. Therefore, it was envisioned that compounds with a cognate structure like perifosine or NSC126188 can trigger apoptosis through the RhoB mediated pathway in cancer.<sup>19,20</sup>

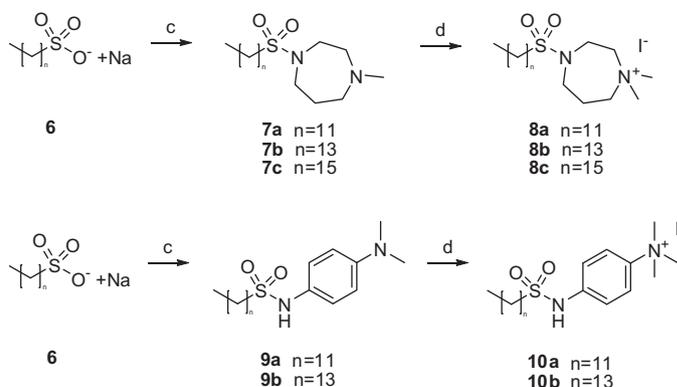


**Figure 1.** Chemical structures of PI analogue (perifosine), NSC126188 (4-hexadecanoyl-1,1-dimethyl-piperazin-1-ium iodide), and A1012<sup>7</sup> (a representative analogue of the aliphatic amido-quaternary ammonium salt series).

We previously reported synthesis and biological activity of the aliphatic amido-quaternary ammonium salt series as represented by A1012 (Fig. 1) as a potential anticancer agent.<sup>7</sup> Herein, design and synthesis of novel aliphatic amido/sulfonamido-quaternary ammonium salts through isosteric replacement of the functional group of NSC126188 and A1012, and biological evaluation of these derivatives are described. We performed a structural–activity relationship (SAR) study of these synthesized compounds, based on their estimation of growth inhibitory activities and RhoB promoter assays in six human cancer cell types. The results suggested that these novel aliphatic amido/sulfonamido-quaternary ammonium salts may be useful lead compounds for development of effective anticancer chemotherapeutic agents.

## 2. Chemistry

Based on structural characteristics of NSC126188, we designed and synthesized 19 analogues, which included three major modifications. First, the piperazine structure was substituted with a phenyl ring or homopiperazine. Second, the carbonyl group was replaced with a sulfonyl group. Third, a variety of N-substituents were introduced. As illustrated in Schemes 1 and 2, compounds were synthesized from various fatty acids by two step procedures. Fatty acids **1** were reacted with thionyl chloride to produce an acid chloride intermediate, which was coupled with 1-methylhomopiperazine or *N,N*-dimethyl benzene-1,4-diamine to yield amides **2a–d** and **4a–d** (Scheme 1). Amides were converted to amido-quaternary ammonium salts **3a–3j** and **5a–5d** by N-alkylation using diverse alkyl halides. As shown in Scheme 2, sulfonamides **7a–c**

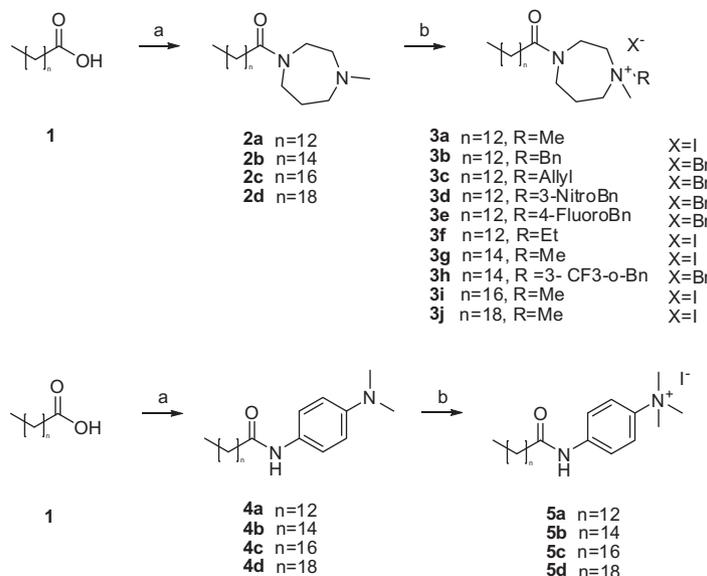


**Scheme 2.** Reaction protocol for the synthesis of aliphatic sulfonamide-quaternary ammonium salts (**8a–8c**, **10a–10b**). Reagents and conditions: (a) (i) SOCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub> at 60 °C (ii) 1-methylhomopiperazine or *N,N*-dimethyl-1,4-phenylenediamine, CH<sub>2</sub>Cl<sub>2</sub> at 0 °C, or DMAP, EDCl, 1-methylhomopiperazine or *N,N*-dimethyl-1,4-phenylenediamine, CH<sub>2</sub>Cl<sub>2</sub>; (b) methyl iodide, CH<sub>3</sub>CN at 95–100 °C or methyl iodide, toluene, 110–115 °C.

and **9a–b** were prepared from various sulfonate sodium salts **6** in a similar fashion. Sulfonamides were further converted to sulfonamide-quaternary ammonium salts **8a–8c** and **10a–10b** by N-alkylation using methyl iodide. The newly prepared quaternary ammonium salt analogues were characterized and evaluated for their biological activities.

## 3. Results and discussion

Nineteen compounds synthesized using Schemes 1 and 2 were assayed for their growth inhibitory activity against six human cancer cell lines: PC-3 (prostate cancer), NUGC-3 (stomach cancer), MDA-MB-231 (breast cancer), ACHN (renal cancer), HCT-15 (colon cancer), and NCI-H23 (non-small cell lung cancer). The GI<sub>50</sub> data for the compounds are listed in Table 1. Perifosine, a known PI3K/Akt inhibitor, was used as a positive reference to compare in vitro growth inhibitory activities of the synthesized compounds. Structurally, perifosine and NSC126188 share a long aliphatic chain



**Scheme 1.** Reaction protocol for the synthesis of aliphatic amido-quaternary ammonium salts (**3a–3j**, **5a–5d**). Reagents and conditions: (a) (i) SOCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub> at 60 °C (ii) 1-methylhomopiperazine or *N,N*-dimethyl-1,4-phenylenediamine, CH<sub>2</sub>Cl<sub>2</sub> at 0 °C, or DMAP, EDCl, 1-methylhomopiperazine or *N,N*-dimethyl-1,4-phenylenediamine, CH<sub>2</sub>Cl<sub>2</sub>; (b) alkyl halide, CH<sub>3</sub>CN at 95–100 °C or alkyl halide, toluene, 110–115 °C.

**Table 1**  
Growth inhibition of synthesized compounds

Compound	Growth inhibition ( $\mu\text{M}$ )					
	PC-3	NUGC-3	MDA-MB-231	ACHN	HCT-15	NCI-H23
<b>3a</b>	0.36	0.08	0.98	0.49	1.70	1.36
<b>3b</b>	0.45	0.31	1.16	1.00	NA	1.07
<b>3c</b>	0.18	0.26	0.45	0.44	NA	0.28
<b>3d</b>	0.18	0.46	0.71	0.82	NA	0.89
<b>3e</b>	0.45	0.94	1.48	1.10	NA	1.48
<b>3f</b>	0.27	0.22	0.68	1.61	1.86	1.18
<b>3g</b>	0.40	0.21	0.94	0.40	0.93	2.38
<b>3h</b>	0.63	0.87	1.34	1.93	NA	1.45
<b>3i</b>	0.24	0.26	0.67	0.57	0.40	1.16
<b>3j</b>	NA	0.36	2.22	2.78	0.99	3.84
<b>5a</b>	0.54	0.74	3.56	4.54	1.36	4.14
<b>5b</b>	0.79	0.70	2.83	3.15	2.37	1.80
<b>5c</b>	1.20	0.41	4.34	1.87	1.22	3.29
<b>5d</b>	1.63	1.28	3.68	1.76	3.02	1.93
<b>8a</b>	0.38	0.24	0.84	1.16	0.96	0.96
<b>8b</b>	0.62	0.25	0.83	1.33	0.38	0.80
<b>8c</b>	0.40	0.38	0.59	2.01	0.77	3.40
<b>10a</b>	1.02	1.21	NA	4.50	NA	3.94
<b>10b</b>	0.90	1.74	4.54	NA	3.38	0.73
Perifosine	0.44	0.54	2.86	4.56	1.25	4.21
NSC126188	0.48	0.29	1.44	1.04	0.58	2.34

and the same quaternary ammonium salt moiety with *N,N*-dimethyl substituents. According to our previous study,<sup>7</sup> aliphatic amido-quaternary ammonium salt compounds were better with carbon chain lengths from 14 to 20 than with carbon lengths under 14 and over 20. Accordingly, we synthesized the analogues which had carbon chain lengths from 14 to 20. In the case of the sulfonamides, chain length, including sulfur, were set to 13–17. To find compounds with better activity and properties than known compounds, a structure–activity relationship study was conducted with emphasis on the carbonyl group, piperazine, and/or *N*-substituent of NSC126188.

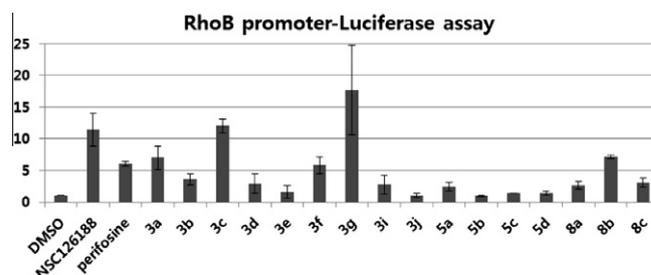
First, we synthesized compounds which were modified in the piperazine ring of NSC126188, while the *N*-methyl-*N*-methyl group was fixed. To evaluate the effects of the piperazine ring group, piperazine was substituted with a phenyl group (**5a–5d**). Compounds **5a–5d** had lower activity than NSC126188 in all cell lines. Next, compounds **10a** and **10b** were substituted with sulfonyl (as isostere of carbonyl) and phenyl (analogue of piperazine) groups. Two compounds showed over 0.5  $\mu\text{M}$  in growth inhibition of all cancer cell lines. In order to assess the effect of the ring moiety, the phenyl ring was replaced with homopiperazine. Generally, compounds **8a–8c** with a sulfonyl group and homopiperazine were more active than those with the phenyl group. In most of the cell lines, **8a–8c** exhibited better activity than **10a–10b**. In addition, this series of compounds showed better activities slightly than NSC126188 in some cell lines. It was determined that homopiperazine was the more active of the two functional groups, therefore the ring group played an important role in cell growth inhibitory activity. Based on the results of **8a–8c** and **5a–5d**, we synthesized analogues with a carbonyl group and homopiperazine (**3a, 3g, 3i, and 3j**), among which, **3a, 3g, 3i, and 3j** showed better or similar growth inhibition than NSC126188 in most cell line. Most notably, the compound **3a** showed very potent growth inhibition in NUGC-3 cells. Interestingly, there was a correlation between the activity and carbon chain length as shown in **5a–d** and **3a, 3g, 3i, and 3j**, indicating lipophilicity was one of the key factors for growth inhibitory activity.

Second, we introduced diverse *N*-substituents, including the ethyl, benzyl, allyl, 3-nitrobenzyl, and 4-fluorobenzyl groups on an *N*-methyl group to evaluate the effects of *N*-substituents. The compounds **3a–3f** had the same 14 carbon chain length, carbonyl

group, and homopiperazine, but different *N*-substituents. Six compounds exhibited good anti-proliferative activity on PC-3 and NUGC cells. The compound **3e** (4-fluorobenzyl) showed selective activity on PC-3 cells. Among the six compounds, the compound **3a**, which had an *N*-methyl group, had the highest growth inhibition of NUGC cells.

For this series, the RhoB promoter activity was also determined using the luciferase reporter gene assay as described in Figure 2. Among 19 analogues, five compounds containing the homopiperazine moiety (**3a, 3c, 3f, 3g, and 8b**) exhibited good reporter expression. Of note, the compounds **3c** and **3g** showed better reporter expression than NSC126188 in the RhoB promoter-based assay. However, **3b, 3d, 3j, 8a, and 8c** showed weak reporter expression activity, while exhibiting better growth inhibition than perifosine in cancer cell lines. Overall, the RhoB promoter assay results did not parallel those of the anti-proliferative activities of the analogues. To understand the reason of this difference, we analyzed the molecular and physical properties of the analogues, particularly *AlogP* (atomic calculation based the octanol–water partition coefficient) and FPSA (Fractional Polar Surface Area, total partially charged and solvent accessible molecular surface area divided by the total molecular surface area) parameters which could be related to the solubility of compounds and/or cell permeability (Table 2). With good promoter and anti-proliferative activities, **3a, 3c, 3f, and 3g** had similar molecular properties; *AlogP* value <5 and FPSA <0.051, respectively. With low growth inhibition and promoter assay value, most compounds showed higher *AlogP* values and/or high FPSA than those of compounds which both growth inhibition and promoter assay value are good. Especially, compound **3d** which had very low promoter activity in spite of the best  $\text{GI}_{50}$  showed higher *AlogP* and FPSA than the compounds with good values both in growth inhibition and promoter assay. It was suggested that the different tendency of  $\text{GI}_{50}$  value and promoter assay activity of some compounds could result from their *AlogP* and FPSA parameters which are involved in solubility and/or cell permeability. Overall, **3a** (14 carbon chain, carbonyl homopiperazine, *N,N*-dimethyl substituents) was considered an excellent anti-cancer agent in the series, with the best inhibitory activity on NUGC cells and a positive value in the RhoB promoter assay. Regarding molecular properties, it showed better drug-like values in terms of *AlogP* and FPSA than did perifosine.

Perifosine inhibited PI3K/Akt, and NSC126188 mediated the RhoB protein. These two compounds which have structural similarities may be responsible for modulating apoptosis through the PI3K/Akt pathway and/or RhoB-mediated pathways. Accordingly, we designed and synthesized a novel series of aliphatic quaternary ammonium salts and evaluated their activities using the growth inhibition assay and the RhoB promoter assay. The effects of ring moiety, carbonyl or sulfonyl group, and *N*-substituent patterns on the aliphatic amido/sulfonamide-quaternary ammonium salt series were further compared with their physical properties. The



**Figure 2.** Activation of RhoB promoter was determined by luciferase activity in cells transfected with the pGL2-RhoB-Luc in the presence of various compounds (5  $\mu\text{M}$ ). NSC126188 (5  $\mu\text{M}$ ) and perifosine (5  $\mu\text{M}$ ) were used as positive controls.

**Table 2**  
Molecular and physical properties

Entry	Compound	AlogP	FPSA
1	<b>3a</b>	3.929	0.051
2	<b>3b</b>	5.513	0.043
3	<b>3c</b>	4.546	0.048
4	<b>3d</b>	5.969	0.131
5	<b>3e</b>	6.281	0.043
6	<b>3f</b>	4.278	0.049
7	<b>3g</b>	4.842	0.047
8	<b>3h</b>	8.545	0.054
9	<b>3i</b>	5.754	0.044
10	<b>3j</b>	6.666	0.041
11	<b>5a</b>	5.347	0.070
12	<b>5b</b>	6.259	0.065
13	<b>5c</b>	7.172	0.060
14	<b>5d</b>	8.084	0.057
15	<b>8a</b>	3.013	0.115
16	<b>8b</b>	3.925	0.106
17	<b>8c</b>	4.838	0.099
18	<b>10b</b>	4.431	0.131
19	Perifosine	4.607	0.135

compound **3a** was the most promising anticancer agent in the series, based upon its potent growth inhibition via RhoB mediated signaling. Together, our results showed that synthetic aliphatic quaternary ammonium salts are valuable lead compounds for development of anticancer chemotherapy.

#### 4. Experimental section

All chemicals were obtained from commercial suppliers and used without further purification. All reactions were monitored by thin layer chromatography on precoated silica gel 60 F254 (mesh; E. Merck, Mumbai, India) and spots were visualized under ultraviolet light (254 nm). Flash column chromatography was performed with silica (Merck EM9385, 230–400 mesh). <sup>1</sup>H nuclear magnetic resonance (NMR; Varian) spectra were recorded at 300 and 75 MHz, at 400 and 100 MHz, or at 500 and 125 MHz. Proton chemical shifts were expressed in ppm relative to internal tetramethylsilane, and coupling constants (*J*) were expressed in Hertz. Liquid chromatography-mass spectrometry spectra were recorded by electrospray ionization (ESI) on liquid chromatography-mass spectrometry instruments (Shimadzu Kyoto, Japan, 10% 0.1% trifluoroacetic acid in H<sub>2</sub>O/90% 0.1% trifluoroacetic acid in acetonitrile), in scanned mode (from 0 to 600 amu/*z*), with detected ion peaks (*M+z*)/*z* and (*M-z*)/*z* in positive and negative ion modes, respectively, where *M* represents the molecular weight of the compound and *z* represents the charge (number of protons). High-resolution mass spectrometry (HRMS) were obtained from ESI-positive mode of the Micromass Q-TOF (Waters Corp, Manchester, UK, The capillary and sample cone voltages were 4000 V and 30 V, the desolvation gas flow was 600 L/h at 200 °C and the source temperature was 100 °C) High resolution tandem mass spectrometry was conducted at the Yonsei University Center for Research Facilities in Seoul, Korea.

##### 4.1. Procedures for 2a–d

Fatty acid **1** (2.9 mmol) was dissolved in anhydrous dichloromethane (0.1 M solution), and thionyl chloride (3.0 equiv) was added under Argon (Ar) atmosphere. The stirred suspension was heated and refluxed for 4 h. The reaction mixture was cooled and poured onto crushed ice for 1 h, and 1-methylhomopiperazine (5.8 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. NaOH solution

(10%) was added (final pH 13), and the mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by chromatography on silica gel with 5% MeOH/CHCl<sub>3</sub> to give the corresponding amides.

##### 4.1.1. 1-(4-Methyl-1,4-diazepan-1-yl)tetradecan-1-one (2a)

Myristic acid was used and **2a** (23.1%) was obtained as a yellowish oil. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.47–3.41 (m, 4H), 2.45–2.24 (m, 4H), 2.28–2.21 (m, 5H), 1.81–1.69 (m, 2H), 1.48 (br, 2H), 1.24 (m, 20H), 0.85 (t, *J* = 6.4 Hz, 3H); ESI-MS: *m/z* = 325 [M<sup>+</sup>H].

##### 4.1.2. 1-(4-Methyl-1,4-diazepan-1-yl)-hexadecan-1-one (2b)

Palmitic acid was used and **2b** (95.3%) was obtained as a yellowish oil. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.48–3.41 (m, 4H), 2.45–2.40 (m, 4H), 2.28–2.22 (m, 5H), 1.81–1.71 (m, 2H), 1.47 (br s, 2H), 1.24 (m, 24H), 0.85 (t, *J* = 6.3 Hz, 3H); ESI-MS: *m/z* = 353 [M<sup>+</sup>H].

##### 4.1.3. 1-(4-Methyl-[1,4]diazepan-1-yl)-octadecan-1-one (2c)

Stearic acid was used and **2c** (98.0%) was obtained as a yellowish oil. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.50–3.41 (m, 4H), 2.47–2.38 (m, 3H), 2.29–2.18 (m, 4H), 1.80–1.70 (m, 2H), 1.50–1.43 (m, 2H), 1.32–1.21 (m, 30H), 0.85 (t, *J* = 6.6 Hz, 3H); ESI-MS: *m/z* = 381 [M<sup>+</sup>H].

#### 4.2. General procedures 1 (amidation step in Scheme 1)

Carboxylic acid **1** (2.9 mmol) was dissolved in anhydrous dichloromethane (0.2 M) at room temperature under an Ar atmosphere, and 1-methylhomopiperazine (4.3 mmol), EDC (1-ethyl-3-[3-dimethylaminopropyl]carboimide hydrochloride, 4.3 mmol) and DMAP (4-dimethylaminopyridine, 0.9 mmol) were added. The mixture was stirred for 8 h at room temperature. Saturated NH<sub>4</sub>Cl solution was added, and the mixture was extracted with dichloromethane (3×). The organic layer was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by chromatography on silica gel with 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub> to resolve the amides.

#### 4.3. Procedures for 3a–j

Alkyl halide (1.6 mmol) was added to a solution of **2a–d** (0.8 mmol) in anhydrous acetonitrile (0.3 M) at room temperature. The reaction mixture was heated to 140–150 °C for 7 h. After the reaction mixture equilibrated to room temperature, the precipitate was granulated for 1 h at 0 °C. Solids were collected by filtration, washed with ethyl acetate, and dried in vacuo.

##### 4.3.1. 1,1-Dimethyl-4-tetradecanoyl-[1,4]diazepan-1-ium; iodide (3a)

Compound **2a** was used and **3a** (71.4%) was obtained as a white solid. Mp: 189–191 °C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.80–3.71 (m, 2H), 3.58–3.40 (m, 6H), 3.11 (s, 6H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.20–2.05 (m, 2H), 1.55–1.46 (m, 2H), 1.34–1.22 (m, 20H), 0.85 (t, *J* = 6.6 Hz, 3H); ESI-MS: *m/z* = 339 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>21</sub>H<sub>43</sub>N<sub>2</sub>OI (MH<sup>+</sup>) 339.3370, found 339.3384.

##### 4.3.2. 1-Benzyl-1-methyl-4-tetradecanoyl-[1,4]diazepan-1-ium; bromide (3b)

Compound **2a** was used and **3b** (93.6%) was obtained as a white solid. Mp: 151–153 °C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 7.54(s, 5H), 4.65 (s, 2H), 4.00–3.85 (m, 1H), 3.76–3.60 (m, 1H), 3.59–3.53 (m, 2H), 3.46–3.39 (m, 4H), 2.97(s, 2H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.28–2.10 (m, 2H), 1.58–1.41 (m, 2H), 1.32–1.22 (m, 20H), 0.85 (t, *J* = 6.6 Hz, 3H); ESI-MS: *m/z* = 415 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>27</sub>H<sub>47</sub>N<sub>2</sub>OBr (MH<sup>+</sup>) 415.3680, found 415.3691.

#### 4.3.3. 1-Allyl-1-methyl-4-tetradecanoyl-1,4-diazepan-1-ium bromide (3c)

Compound **2a** was used and **3c** (71.6%) was obtained as a white solid. Mp: 189–191 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 6.11–6.00 (m, 1H), 5.67–5.62 (m, 2H), 4.08–4.03 (m, 2H), 3.85–3.73 (m, 2H), 3.55–3.39 (m, 6H), 3.02 (s, 3H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.17–2.06 (m, 2H), 1.52–1.45 (m, 2H), 1.30–1.18 (m, 20H), 0.85 (t, *J* = 7.0 Hz, 3H); ESI-MS: *m/z* = 365 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>23</sub>H<sub>45</sub>N<sub>2</sub>OBr (MH<sup>+</sup>) 365.3526, found 365.3539.

#### 4.3.4. 1-Methyl-1-(3-nitrobenzyl)-3-tetradecanoyl-1,3-diazepan-1-ium bromide (3d)

Compound **2a** was used and **3d** (91.8%) was obtained as a pale yellow solid. Mp: 156–158 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.47 (d, *J* = 3.7 Hz, 1H), 8.41–8.37 (m, 1H), 8.03 (t, *J* = 7.5 Hz, 1H), 7.85–7.80 (m, 1H), 4.87–4.82 (m, 2H), 4.02–3.84 (m, 1H), 3.74–3.62 (m, 1H), 3.61–3.42 (m, 6H), 3.04 (s, 3H), 2.37–2.31 (m, 2H), 2.25–2.18 (m, 2H), 1.54–1.45 (m, 2H), 1.33–1.19 (m, 20H), 0.85 (t, *J* = 6.5 Hz, 3H); ESI-MS: *m/z* = 460 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>27</sub>H<sub>46</sub>N<sub>3</sub>O<sub>3</sub>Br (MH<sup>+</sup>) 460.3534, found 460.3548.

#### 4.3.5. 1-Methyl-1-(3-nitrobenzyl)-3-tetradecanoyl-1,3-diazepan-1-ium bromide (3e)

Compound **2a** was used and **3e** (31.0%) was obtained as a white solid. Mp: 189–191 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 7.66–7.61 (m, 2H), 7.38–7.34 (m, 2H), 4.71–4.66 (m, 2H), 4.01–3.88 (m, 1H), 3.76–3.68 (m, 1H), 3.61–3.38 (m, 6H), 2.98 (s, 3H), 2.34–2.29 (m, 2H), 2.23–2.13 (m, 2H), 1.52–1.45 (m, 2H), 1.30–1.19 (m, 20H), 0.85 (t, *J* = 6.5 Hz, 3H); ESI-MS: *m/z* = 433 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>27</sub>H<sub>46</sub>N<sub>3</sub>O<sub>2</sub>Br (MH<sup>+</sup>) 433.3584, found 433.3574.

#### 4.3.6. 1-Ethyl-1-methyl-4-tetradecanoyl-[1,4]diazepan-1-ium; iodide (3f)

Compound **2a** was used and **3f** (88.7%) was obtained as a pale yellow solid. Mp: 162–163 °C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.47–3.41 (m, 4H), 2.45–2.24 (m, 4H), 2.28–2.21 (m, 5H), 1.81–1.69 (m, 2H), 1.48 (br, 2H), 1.24 (m, 20H), 0.85 (t, *J* = 6.4 Hz, 3H); ESI-MS: *m/z* = 353 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>26</sub>H<sub>53</sub>N<sub>2</sub>OI (MH<sup>+</sup>) 353.3526, found 353.3533.

#### 4.3.7. 4-Hexadecanoyl-1,1-dimethyl-[1,4]diazepan-1-ium; iodide (3g)

Compound **2b** was used and **3g** (54.9%) was obtained as a white solid. Mp: 180–181 °C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 3.80–3.73 (m, 2H), 3.58–3.48 (m, 4H), 3.48–3.40 (m, 2H), 3.12 (s, 6H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.26–2.03 (m, 2H), 1.57–1.44 (m, 2H), 1.35–1.17 (m, 24H), 0.85 (t, *J* = 6.6 Hz, 3H); ESI-MS: *m/z* = 367 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>23</sub>H<sub>47</sub>N<sub>2</sub>OI (MH<sup>+</sup>) 367.3680, found 367.3785.

#### 4.3.8. 1-Methyl-4-palmitoyl-1-(3-(trifluoromethoxy)benzyl)-1,4-diazepan-1-ium bromide (3h)

Compound **2b** was used and **3h** (71.8%) was obtained as a white solid. Mp: 181–182 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.71–7.66 (m, 2H), 7.49–7.44 (m, 2H), 7.31 (d, *J* = 8.6 Hz, 1H), 5.42 (br m, 2H), 4.26–3.69 (m, 8H), 3.33 (s, 3H), 2.37–2.18 (m, 4H), 1.56 (br s, 2H), 1.23 (br m, 24H), 0.85 (t, *J* = 6.7 Hz, 3H); ESI-MS: *m/z* = 528 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>30</sub>H<sub>50</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub>Br (MH<sup>+</sup>) 527.3819, found 527.3824.

#### 4.3.9. 1,1-Dimethyl-4-stearoyl-1,4-diazepan-1-ium iodide (3i)

Compound **2c** was used and **3i** (79.4%) was obtained as a pale yellow solid. Mp: 188–189 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 3.80–3.72 (m, 2H), 3.55–3.43 (m, 6H), 3.12 (s, 6H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.18–2.05 (m, 2H), 1.52–1.46 (m, 2H), 1.31–1.19 (m, 28H), 0.85 (t, *J* = 7.5 Hz, 3H); ESI-MS: *m/z* = 395 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>25</sub>H<sub>51</sub>N<sub>2</sub>OI (MH<sup>+</sup>) 395.3996, found 395.3995.

#### 4.3.10. 4-Icosanoyl-1,1-dimethyl-[1,4]diazepan-1-ium; iodide (3j)

Compound **2d** was used and **3j** (90.3%) was obtained as a white solid. Mp: 176–177 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 3.73 (d, *J* = 16.0 Hz, 2H), 3.52–3.41 (m, 6H), 3.09 (d, *J* = 8.0 Hz, 6H), 2.28 (t, *J* = 8.0 Hz, 2H), 2.07 (d, *J* = 28.0 Hz, 2H), 1.46 (s, 2H), 1.20 (s, 32H), 0.82 (t, *J* = 6.0 Hz, 3H); ESI-MS: *m/z* = 423 [M<sup>+</sup>]; HRMS (ESI) calcd for C<sub>27</sub>H<sub>55</sub>N<sub>2</sub>OI (MH<sup>+</sup>) 423.4309, found 423.4300.

### 4.4. Procedures for 4a–d

Carboxylic acid **1** (2.9 mmol) was dissolved in anhydrous dichloromethane (0.2 M) at room temperature under an Ar atmosphere. *N,N*-dimethyl-1,4-phenylenediamine (4.3 mmol), EDC (4.3 mmol) and DMAP (0.9 mmol) were added, and the mixture was stirred for 8 h at room temperature. Saturated NH<sub>4</sub>Cl solution was added, and the mixture was extracted with dichloromethane (3×). The organic layer was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The residue was purified by chromatography on silica gel with 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub> to resolve amides.

#### 4.4.1. Tetradecanoic acid (4-dimethylamino-phenyl)-amide (4a)

Myristic acid was used and **4a** (19.5%) was obtained as a yellowish oil. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 9.52 (s, 1H), 7.38 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 9.0 Hz, 2H), 2.82 (s, 6H), 2.21 (t, *J* = 7.5 Hz, 2H), 1.55 (m, 2H), 1.27–1.23 (m, 20H), 0.86–0.84 (m, 3H); ESI-MS: *m/z* = 347 [M<sup>+</sup>H].

#### 4.4.2. Hexadecanoic acid (4-dimethylamino-phenyl)-amide (4b)

Palmitic acid was used and **4b** (47.3%) was obtained as a yellowish oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.52 (s, 1H), 7.38 (d, *J* = 6.9 Hz, 2H), 6.66 (d, *J* = 6.6 Hz, 2H), 2.82 (s, 6H), 2.21 (t, *J* = 7.6 Hz, 2H), 1.56–1.53 (m, 2H), 1.27–1.23 (br m, 24H), 0.85 (t, *J* = 6.8 Hz, 3H); ESI-MS: *m/z* = 375 [M<sup>+</sup>H].

#### 4.4.3. Octadecanoic acid (4-dimethylamino-phenyl)-amide (4c)

Stearic acid was used and **4c** (21.4%) was obtained as a brownish oil. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 10.15 (s, 1H), 7.67 (d, *J* = 9.0 Hz, 2H), 7.30 (d, *J* = 9.0 Hz, 2H), 3.44–3.38 (m, 6H), 2.31 (t, *J* = 6.1 Hz, 2H), 1.55 (t, *J* = 4.5 Hz, 2H), 1.27–1.20 (m, 28H), 0.85 (t, *J* = 6.8 Hz, 3H); ESI-MS: *m/z* = 403 [M<sup>+</sup>H].

#### 4.4.4. Icosanoic acid (4-dimethylamino-phenyl)-amide (4d)

Arachidic acid was used and **4d** (38.4%) was obtained as a brownish oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.38 (d, *J* = 6.8 Hz, 2H), 6.66 (d, *J* = 6.3 Hz, 2H), 5.76 (s, 1H), 2.82 (s, 6H), 2.21 (t, *J* = 7.0 Hz, 2H), 1.97–1.94 (m, 2H), 1.57–1.55 (m, 2H), 1.23 (br m, 30H), 0.85–0.83 (m, 3H); ESI-MS: *m/z* = 431 [M<sup>+</sup>H].

### 4.5. Procedures for 5a–d

Methyl iodide (1.6 mmol) was added to a solution of **5a–d** (0.8 mmol) in anhydrous acetonitrile (0.3 M) at room temperature. The reaction mixture was heated to 140–150 °C for 7 h. After the reaction mixture equilibrated to room temperature, the precipitate was granulated for 1 h at 0 °C. Solids were collected by filtration, washed with ethyl acetate, and dried in vacuo.

#### 4.5.1. Trimethyl-(4-tetradecanoylamino-phenyl)-ammonium; iodide (5a)

Compound **4a** was used and **5a** (78.7%) was obtained as a white solid. Mp: 148–149 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 10.21 (s, 1H), 7.87 (d, *J* = 9.5 Hz, 2H), 7.76 (d, *J* = 9.5 Hz, 2H), 3.56 (s, 9H), 2.32 (t, *J* = 7.5 Hz, 2H), 1.59–1.56 (m, 2H), 1.28–1.24 (br m, 20H),

0.85 (t,  $J = 6.8$  Hz, 3H); ESI-MS:  $m/z = 361$  [ $M^+$ ]; HRMS (ESI) calcd for  $C_{23}H_{41}N_2OI$  (MH<sup>+</sup>) 361.3213, found 361.3210.

#### 4.5.2. (4-Hexadecanoylamino-phenyl)-trimethyl-ammonium; iodide (5b)

Compound **4b** was used and **5b** (20.5%) was obtained as a pale gray solid. Mp: 145–146 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  10.18 (s, 1H), 7.88–7.86 (m, 2H), 7.77–7.75 (m, 2H), 3.56 (s, 9H), 2.32 (t,  $J = 7.4$  Hz, 2H), 1.58 (m, 2H), 1.27–1.23 (m, 24H), 0.85 (t,  $J = 6.8$  Hz, 3H); ESI-MS:  $m/z = 389$  [ $M^+$ ]; HRMS (ESI) calcd for  $C_{25}H_{45}N_2OI$  (MH<sup>+</sup>) 389.3526, found 389.3519.

#### 4.5.3. Trimethyl-(4-octadecanoylamino-phenyl)-ammonium; iodide (5c)

Compound **4c** was used and **5c** (36.7%) was obtained as a brown solid. Mp: 127–128 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  10.11 (s, 1H), 7.71 (d,  $J = 9.0$  Hz, 2H), 7.41 (d,  $J = 9.0$  Hz, 2H), 3.93 (s, 9H), 2.30 (t,  $J = 4.8$  Hz, 2H), 1.57 (t,  $J = 4.5$  Hz, 2H), 1.27–1.23 (m, 28H), 0.85 (t,  $J = 6.8$  Hz, 3H); ESI-MS:  $m/z = 417$  [ $M^+$ ]; HRMS (ESI) calcd for  $C_{27}H_{49}N_2OI$  (MH<sup>+</sup>) 417.3839, found 417.3839.

#### 4.5.4. (4-Icosanoylamino-phenyl)-trimethyl-ammonium; iodide (5d)

Compound **4d** was used and **5d** (93.2%) was obtained as a pale purple solid. Mp: 155–156 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.88–7.86 (m, 2H), 7.77–7.75 (m, 2H), 5.75–5.75 (m, 1H), 3.55 (s, 9H), 2.31 (t,  $J = 7.3$  Hz, 2H), 1.26–1.23 (m, 34H), 0.84 (t,  $J = 6.9$  Hz, 3H); ESI-MS:  $m/z = 445$  [ $M^+$ ]; HRMS (ESI) calcd for  $C_{29}H_{53}N_2OI$  (MH<sup>+</sup>) 445.4152, found 445.4152.

#### 4.6. Procedures for 7a–c

Sulfonate sodium salts **6** (3.8 mmol) were dissolved in anhydrous dichloromethane (0.1 M solution), and thionyl chloride (3.0 equiv) was added under an Ar atmosphere. The stirred suspension was heated and refluxed for 4 h. The reaction mixture was cooled and poured onto crushed ice for 1 h. The compound 1-methylhomopiperazine (5.8 mmol) was added dropwise, and the reaction mixture was allowed to warm to room temperature and stirred for 2 h. NaOH solution (10%) was added (final pH 13), and the mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by chromatography on silica gel with 5% MeOH/CHCl<sub>3</sub> to resolve the corresponding amides.

##### 4.6.1. 1-(Dodecane-1-sulfonyl)-4-methyl-[1,4]diazepane (7a)

Sodium 1-dodecanesulfonate was used and **7a** (94.3%) was obtained as a yellowish oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  3.69 (br m, 2H), 3.53 (br m, 2H), 3.10 (br m, 2H), 3.04–3.01 (m, 2H), 2.99–2.93 (m, 2H), 2.67 (s, 3H), 1.80–1.74 (m, 2H), 1.42–1.38 (m, 2H), 1.31–1.27 (m, 18H), 0.89 (t,  $J = 7.0$  Hz, 3H); ESI-MS:  $m/z = 347$  [ $M^+H$ ].

##### 4.6.2. 1-Methyl-4-(tetradecane-1-sulfonyl)-[1,4]diazepane (7b)

Sodium 1-tetradecanesulfonate was used and **7b** (79.9%) was obtained as a yellowish oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.32 (t,  $J = 6.0$  Hz, 4H), 3.01 (t,  $J = 8.0$  Hz, 2H), 2.74 (s, 1H), 2.53 (t,  $J = 5.0$  Hz, 4H), 2.25 (s, 3H), 1.77–1.74 (m, 2H), 1.61–1.57 (m, 2H), 1.34–1.32 (m, 2H), 1.24 (br s, 20H), 0.85 (t,  $J = 6.8$  Hz, 3H); ESI-MS:  $m/z = 375$  [ $M^+H$ ].

##### 4.6.3. 1-(Hexadecane-1-sulfonyl)-4-methyl-[1,4]diazepane (7c)

1-Hexadecanesulfonyl chloride was used and **7c** (49.0%) was obtained as a yellowish oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  3.65 (br m, 2H), 3.52 (br m, 2H), 2.99–2.96 (m, 6H), 2.62 (s, 3H), 1.81–

1.74 (m, 2H), 1.42–1.38 (m, 2H), 1.29–1.27 (m, 26H), 0.89 (t,  $J = 6.8$  Hz, 3H); ESI-MS:  $m/z = 403$  [ $M^+H$ ].

#### 4.7. Procedures for 8a–c

Methyl iodide (1.6 mmol) was added to a solution of **7a–c** (0.8 mmol) in anhydrous acetonitrile (0.3 M) at room temperature. The reaction mixture was heated to 140–150 °C for 7 h. After the reaction mixture equilibrated to room temperature, the precipitate was granulated for 1 h at 0 °C. Solids were collected by filtration, washed with ethyl acetate, and dried in vacuo.

##### 4.7.1. 4-(Dodecane-1-sulfonyl)-1,1-dimethyl-[1,4]diazepan-1-ium; iodide (8a)

Compound **7a** was used and **8a** (47.5%) was obtained as a pale yellow solid. Mp: 171–172 °C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  3.69 (br m, 2H), 3.53 (br m, 2H), 3.10 (br m, 2H), 3.04–3.01 (m, 2H), 2.99–2.93 (m, 2H), 2.67 (s, 3H), 1.80–1.74 (m, 2H), 1.42–1.38 (m, 2H), 1.31–1.27 (m, 18H), 0.89 (t,  $J = 7.0$  Hz, 3H); ESI-MS:  $m/z = 347$  [ $M^+$ ]; HRMS (ESI) calcd for  $C_{19}H_{41}N_2O_2SI$  (MH<sup>+</sup>) 361.2883, found 361.2877.

##### 4.7.2. 1,1-Dimethyl-4-(tetradecane-1-sulfonyl)-[1,4]diazepan-1-ium; iodide (8b)

Compound **7b** was used and **8b** (65.9%) was obtained as a white solid. Mp: 178–179 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.73 (d,  $J = 16.0$  Hz, 2H), 3.52–3.39 (m, 6H), 3.09 (d,  $J = 7.6$  Hz, 6H), 2.28 (t,  $J = 7.4$  Hz, 2H), 2.07 (d,  $J = 29.6$  Hz, 2H), 1.46 (s, 2H), 1.20 (s, 22H), 0.82 (t,  $J = 6.4$  Hz, 3H); ESI-MS:  $m/z = 389$  [ $M^+$ ]; HRMS (ESI) calcd for  $C_{21}H_{45}N_2O_2SI$  (MH<sup>+</sup>) 389.3196, found 389.3215.

##### 4.7.3. 4-(Hexadecane-1-sulfonyl)-1,1-dimethyl-[1,4]diazepan-1-ium; iodide (8c)

Compound **7c** was used and **8c** (85.4%) was obtained as a white solid. Mp: 159–160 °C; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.67 (br m, 2H), 3.59–3.54 (m, 4H), 3.42–3.39 (m, 2H), 3.13 (s, 6H), 2.12 (br m, 2H), 1.66–1.60 (m, 2H), 1.36–1.35 (m, 2H), 1.24 (br m, 26H), 0.85 (t,  $J = 6.8$  Hz, 3H); ESI-MS:  $m/z = 417$  [ $M^+$ ]; HRMS (ESI) calcd for  $C_{23}H_{49}N_2O_2SI$  (MH<sup>+</sup>) 417.3509, found 417.3496.

#### 4.8. Procedures for 9a–b

Sulfonate sodium salts **6** (3.8 mmol) were dissolved in anhydrous dichloromethane (0.1 M solution), and thionyl chloride (3.0 equiv) was added under an Ar atmosphere. The stirred suspension was heated and refluxed for 4 h. The reaction mixture was cooled and poured onto crushed ice for 1 h. *N,N*-dimethylbenzene-diamine (5.8 mmol) was added dropwise, and the reaction mixture was warmed to room temperature and stirred for 2 h. NaOH solution (10%) was added (final pH 13), and the mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by chromatography on silica gel with 5% MeOH/CHCl<sub>3</sub> to resolve the corresponding amides.

##### 4.8.1. Dodecane-1-sulfonic acid (4-dimethylamino-phenyl)-amide (9a)

Sodium 1-dodecanesulfonate was used and **9a** (83.3%) was obtained as a brownish oil. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.32–7.25 (m, 2H), 7.23–7.15 (m, 2H), 3.07–2.94 (m, 8H), 2.82 (s, 1H), 1.87–1.78 (m, 2H), 1.42–1.28 (m, 18H), 0.90 (t,  $J = 7.0$  Hz, 3H); ESI-MS:  $m/z = 369$  [ $M^+H$ ].

#### 4.8.2. Tetradecane-1-sulfonic acid (4-dimethylamino-phenyl)-amide (9b)

Sodium 1-tetradecanesulfonate was used and **9b** (73.7%) was obtained as a brownish oil.  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ )  $\delta$  9.13 (s, 1H), 7.03 (d,  $J = 7.9$  Hz, 2H), 6.67 (d,  $J = 8.3$  Hz, 2H), 2.85 (t,  $J = 8.1$  Hz, 9H), 1.63 (s, 2H), 1.29 (s, 3H), 1.21 (d,  $J = 15.7$  Hz, 23H), 0.84 (t,  $J = 6.5$  Hz, 3H); ESI-MS:  $m/z = 397$  [ $\text{M}^+$ ].

#### 4.9. Procedures for 10a–b

Methyl iodide (1.6 mmol) was added to a solution of **9a–b** (0.8 mmol) in anhydrous acetonitrile (0.3 M) at room temperature. The reaction mixture was heated to 140–150 °C for 7 h. After the reaction mixture equilibrated to room temperature, the precipitate was granulated for 1 h at 0 °C. Solids were collected by filtration, washed with ethyl acetate, and dried in vacuo.

##### 4.9.1. [4-(Dodecane-1-sulfonylamino)-phenyl]-trimethylammonium; bromide (10a)

Compound **9a** was used and **10a** (33.3%) was obtained as a gray solid. Mp: 119–120 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ )  $\delta$  7.16 (t,  $J = 5.8$  Hz, 1H), 3.33–3.30 (m, 1H), 3.06 (s, 6H), 3.02–2.97 (m, 4H), 1.89–1.83 (m, 2H), 1.65–1.59 (m, 2H), 1.37–1.33 (m, 2H), 1.28–1.20 (m, 20H), 0.85 (t,  $J = 7.0$  Hz, 3H); ESI-MS:  $m/z = 397$  [ $\text{M}^+$ ]; HRMS (ESI) calcd for  $\text{C}_{21}\text{H}_{39}\text{N}_2\text{O}_2\text{SI}$  ( $\text{MH}^+$ ) 383.2727, found 383.2738.

##### 4.9.2. Trimethyl-[4-(tetradecane-1-sulfonylamino)-phenyl]-ammonium; iodide (10b)

Compound **9b** was used and **10b** (86.7%) was obtained as a white solid. Mp: 140–141 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ )  $\delta$  10.25 (s, 1H), 7.91–7.89 (d,  $J = 9.0$  Hz, 2H), 7.34–7.32 (d,  $J = 9.0$  Hz, 2H), 3.56 (s, 9H), 3.16 (t,  $J = 7.5$  Hz, 2H), 1.65–1.63 (m, 2H), 1.32–1.21 (m, 22H), 0.85 (t,  $J = 6.8$  Hz, 3H); ESI-MS:  $m/z = 411$  [ $\text{M}^+$ ]; HRMS (ESI) calcd for  $\text{C}_{23}\text{H}_{43}\text{N}_2\text{O}_2\text{SI}$  ( $\text{MH}^+$ ) 411.3040, found 411.3047.

#### 4.10. Sulforhodamine (SRB) assay

Growth inhibition of cancer cell lines in the presence of NSC126188 was determined using the SRB assay as previously described.<sup>10</sup> SRB dye bound to the cell matrix was quantified using a spectrophotometer at 530 nm.

#### 4.11. Luciferase assay

Transactivation of RhoB was determined by reporter assay using the dual-luciferase reporter assay system (Promega, Madison, WI, USA), as previously described.<sup>11</sup> HeLa cells at 75–90% confluency were transiently cotransfected with the pGL2-RhoB-firefly

luciferase plasmid containing the RhoB promoter, and pRL-SV40-renilla luciferase. Luciferase activity was integrated over a 10 s period and measured using a luminometer (Victor X Light; Perkin Elmer, Waltham, MA, USA). The results were normalized to the levels of renilla luciferase.

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#### Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.bmc.2012.11.027>. These data include MOL files and InChIKeys of the most important compounds described in this article.

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