# New synthetic aliphatic sulfonamido-quaternary ammonium salts as anticancer chemotherapeutic agents

**ARTICLE** *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 2013

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2013.09.022 · Source: PubMed



**9 AUTHORS**, INCLUDING:

Jee Sun Yang
Yonsei University
**16** PUBLICATIONS **136** CITATIONS
SEE PROFILE

Gyoonhee Han
Yonsei University
**73** PUBLICATIONS **891** CITATIONS
SEE PROFILE

European Journal of Medicinal Chemistry 69 (2013) 670–677

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# New synthetic aliphatic sulfonamido-quaternary ammonium salts as anticancer chemotherapeutic agents

Doona Song [a], Jee Sun Yang [a], Changmok Oh [a], Shuolin Cui [a, e], Bo-Kyung Kim [b], Misun Won [b], Jang-ik Lee [c], Hwan Mook Kim [d], Gyoonhee Han [a, e, *]

[a] Translational Research Center for Protein Function Control, Department of Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea
[b] Medical Genomics Research Center, KRIBB, Daejeon 305-806, Republic of Korea
[c] College of Pharmacy, Yonsei University, Incheon 406-840, Republic of Korea
[d] College of Pharmacy, Gachon University, Incheon 406-799, Republic of Korea
[e] Department of Integrated OMICS for Biomedical Sciences (WCU Program), Yonsei University, Seoul 120-749, Republic of Korea

ARTICLE INFO

*Article history:*
Received 16 July 2013
Received in revised form
9 August 2013
Accepted 9 September 2013
Available online 21 September 2013

*Keywords:*
Aliphatic sulfonamido-quaternary ammonium salts
Anticancer chemotherapeutic agents
Open-ring moiety of quaternary ammonium salts
RhoB
Perifosine
NSC126188

ABSTRACT

RhoB is expressed during tumor cell proliferation, survival, invasion, and metastasis. In malignant progression, the expression levels of RhoB are commonly attenuated. RhoB is known to be linked to the regulation of the PI3K/Akt survival pathways. Based on aliphatic amido-quaternary ammonium salts that induce apoptosis via up-regulation of RhoB, we synthesized novel aliphatic sulfonamido-quaternary ammonium salts. These new synthetic compounds were evaluated for their biological activities using an *in vitro* RhoB promoter assay in HeLa cells, and in a growth inhibition assay using human cancer cell lines including PC-3, NUGC-3, MDA-MB-231, ACHN, HCT-15, and NCI-H23. Compound **5b** (ethyl-dimethyl-{3-[methyl-(tetradecane-1-sulfonyl)-amino]-propyl}-ammonium; iodide) was the most promising anticancer agent in the series, based upon the potency of growth inhibition and RhoB promotion. These new aliphatic sulfonamido-quaternary ammonium salts could be a valuable series for development of new anticancer chemotherapeutic agents.



## 1. Introduction

Cancer cells acquire a variety of characteristic changes during carcinogenesis [1]. There is a change in expression of proteins and signaling pathways which regulate cell growth, differentiation, and development. Many of these proteins and signaling pathways currently are under investigation as possible targets for cancer therapy. Among different cancers, gastric cancer is a common malignancy in many countries, and increasing evidence has reported that gastric carcinogenesis displays multiple genetic alterations including activation of oncogenes, inactivation of tumor suppressor genes, loss of heterozygosity in chromosomes, and errors in DNA replication [2,3].

The role of RhoB in human cancer is equivocal. Some studies suggest that RhoB acts as a suppressor or negative modifier in cancer, as evidenced by the down-regulation of RhoB protein expression in head and neck carcinoma and lung cancer [4–6]. Loss of RhoB was found to be associated with an increased susceptibility to chemical carcinogenesis in mice [7]. In addition, targeted deletion of RhoB in mice increased tumor formation initiated by a Ras mutation [1]. In gastric cancer cell lines, RhoB expression suppressed the growth and migration of these cells, and enhanced the chemosensitivity [8]. Conversely, RhoB was found to be overexpressed in breast tumors [9]. Overall, it is reported that RhoB is associated with cell proliferation, survival, invasion, and metastasis curing carcinogenesis, and its levels are commonly attenuated during malignant progression.

The RhoB gene is a short-lived, early response inducible protein involved in receptor endocytosis, apoptosis, and gene expression [10]. In addition, it is rapidly up-regulated by a number of stimuli such as UV irradiation, cytokines, growth factors, and steroid and toxin treatments [11–13]. Expression of this gene is reduced by Ras

* Corresponding author. Department of Integrated OMICS for Biomedical Sciences (WCU Program), Yonsei University, Seoul 120-749, Republic of Korea. Tel.: +82 2 21232882; fax: +82 2 3627265.
*E-mail addresses:* gyoonhee@yonsei.ac.kr, gyoonhee@gmail.com (G. Han).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.09.022

via Akt/PKB, EGFR, and ErbB2 [14]. The function of RhoB also has been linked to the regulation of PI3K/Akt survival pathways [15,16]. PI3K/Akt signaling is essential for tumor progression, because numerous genetic lesions have been discovered in Akt signaling components in human breast cancers and other solid tumors [17]. Furthermore, some studies reported that loss of RhoB decreases Akt phosphorylation and blocks its nuclear translocation in stromal endothelial cells [15]. In tumor cells, regulation of the Akt signaling axis by RhoB controls invasion and migration [18,19].

In a previous study, we found that up-regulation of RhoB by NSC126188 (Fig. 1) is mediated via c-Jun *N*-terminal kinase (JNK) signaling [20], which constitutes the principle member of the mitogen-activated protein kinase (MAPK) family [21,22]. JNK regulates many cellular processes, including cell proliferation, differentiation, survival, and migration. There is also cross-talk and integration with other signaling pathways in a cell specific manner [22]. Recently, it was reported that an alkyl piperazine derivative (NSC126188, Fig. 1) caused apoptosis via up-regulation of RhoB in HeLa cells [23]. In addition, we previously reported synthesis and biological activity of the aliphatic amido-quaternary ammonium salt series as represented by allyl-diethyl-[2-(methyl-tetradecanoyl-amino)-ethyl]-ammonium bromide (Fig. 1, **A913**) which was synthesized as an anticancer agent, based on NSC126188 [24]. In the present study, novel aliphatic sulfonamido-quaternary ammonium salts were designed and synthesized by isosteric replacement of the functional group of NSC126188 and **A913**. A structural activity relationship (SAR) study of these synthesized compounds was also conducted, based on their RhoB promoter assays in HeLa cells and their growth inhibitory activities in six human cancer cell lines including PC-3, NUGC-3, MDA-MB-231, ACHN, HCT-15, and NCI-H23. The results demonstrated that these new aliphatic sulfonamido-quaternary ammonium salts could be new compounds for development of effective anticancer chemotherapeutic agents.

## 2. Chemistry

Based on structural characteristics of NSC126188 and **A913**, we designed and synthesized 24 analogs with three major modifications. First, the carbonyl group was substituted with a sulfonyl group. Second, diverse *N*-substituents were introduced such as methyl, ethyl, and ally groups. Third, various aliphatic moieties were introduced into quaternary ammonium salts. As illustrated in Schemes 1–3, various sulfonate sodium salts **1** were reacted with thionyl chloride to produce sulfonyl chlorides *in situ*, which were coupled with *N*,*N*-diethyl-*N*′-methylethylenediamine, *N*,*N*,*N*′-trimethylethylenediamine, *N*,*N*-dimethyl-1,3-propanediamine, *N*,*N*,*N*′-trimethyl-1,3-diaminopropane, or *N*,*N*,2,2-tetramethyl-1,3-propanediamine to yield sulfonamides **2a**–**2d**, **4a**–**4d**, and **6a**–**6b** (Schemes 1-a, 2-a, 3-a), which were confirmed by $^1$H nuclear magnetic resonance (NMR) spectra. Sulfonamides were converted to sulfonamido-quaternary ammonium salts, **3a**–**3l**, **5a**–**5h**, and **7a**–**7d,** by *N*-alkylation using diverse alkyl halides (Schemes 1-b, 2-b, 3-b). The structures of sulfonamido-quaternary ammonium salts

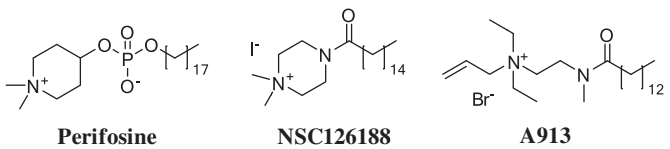



**Fig. 1.** Chemical structures of perifosine, NSC126188 (4-hexadecanoyl-1,1-dimethyl-piperazin-1-ium iodide), and **A913** (a representative analogue of the aliphatic amido-quaternary ammonium salt series).

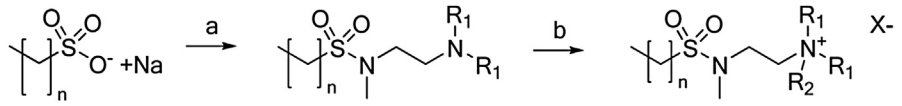
**3**, **5**, and **7** were confirmed by $^1$H and $^{13}$C NMR spectra and high resolution mass spectrometry (HRMS). The newly prepared quaternary ammonium salt analogs were characterized and evaluated for *in vitro* biological activities and RhoB promoter activities.

## 3. Results and discussion

Twenty-four compounds synthesized using Schemes 1–3 were assayed for their growth inhibitory activity against the human cancer cell lines PC-3 (prostate cancer), NUGC-3 (gastric cancer), MDA-MB-231 (breast cancer), ACHN (renal cancer), HCT-15 (colon cancer), and NCI-H23 (non-small cell lung cancer). The $GI_{50}$ values from the sulforhodamine B (SRB) assay for the compounds are listed in Table 1, showing that the synthesized compounds significantly inhibited the gastric cancer cell line. Perifosine (Fig. 1), a known PI3K/Akt inhibitor, and NSC126188 were used as positive references to compare *in vitro* growth inhibitory activities of the synthesized compounds. Structurally, perifosine and NSC126188 share a long aliphatic chain and the same quaternary ammonium salt moiety with *N*,*N*-dimethyl substituent [24]. According to our previous study, aliphatic amido-quaternary ammonium salt compounds with 14 and 16 carbon chain lengths usually showed good inhibitory activity [25]. To identify compounds with better activity than known compounds (perifosine, NSC126188, and **A913**), a structure–activity relationship study was conducted with emphasis on the sulfonyl group, diamine, and/or *N*-substituent of novel aliphatic sulfonamido-quaternary ammonium salts.

First, based on **A913** (14 carbon chain length, *N*,*N*-diethyl-*N*′-methylethane-1,2-diamine, allyl group, $GI_{50}$ = 0.14 μM for the NUGC-3 cancer cell line, $GI_{50}$ = 0.21 μM for the prostate cancer cell line) which showed the most potent inhibitory activity in the previous study, analogs **3a**–**3f** were designed and synthesized. We fixed the carbon chain length at 14 and 16, which resulted in good anti-proliferation activity. The carbonyl group of **A913** was changed to the sulfonyl group because sulfonyl group is more stable than carbonyl group with regard to the chemistry although sulfonyl and carbonyl group are isosteric. In addition, its $R_2$ group (described on Scheme 1) was substituted with the methyl, ethyl, or allyl group. Six analogs showed lower growth inhibitory activity than **A913** for the NUGC-3 and PC-3 cell lines. However, they showed better anti-proliferative activity than perifosine, especially for the NUGC-3 cell line. While **A913** showed good growth inhibitory activities in most cancer cell lines, six analogs exhibited selective anti-proliferative activities for the gastric cancer cell line. In addition, compounds **3c** and **3e** exhibited better growth inhibition activities than NSC126188, with $GI_{50}$ values of 0.21 and 0.22 μM, respectively, in the NUCG-3 cell line. The results demonstrated that the substitution of a sulfonyl group could increase the specificity of inhibitory activities for a gastric cancer cell line.

Second, we assessed the effect of *N*-substituents by diverse alkyl groups such as methyl, ethyl, and allyl. The effect of *N*-substituents were compared among compounds with fixed carbon chain length and *N*,*N*-dialkylethane diamine. The effect of *N*-substituents was barely noticeable for compounds with *N*,*N*-diethylethane diamine (**3a**–**3f**). In contrast, analogs with *N*,*N*-dimethylethane diamine (**3g**–**3l**) displayed the effect of the *N*-substituent on the gastric cancer cell line. Compounds with an ally group (**3i**, $GI_{50}$ = 0.83 μM; **3l,** $GI_{50}$ = 0.76 μM) exhibited poorer inhibitory activity than compounds with a methyl group (**3g**, $GI_{50}$ = 0.39 μM; **3j**, $GI_{50}$ = 0.33 μM) or with an ethyl group (**3h**, $GI_{50}$ = 0.30 μM; **3k**, $GI_{50}$ = 0.36 μM). When comparing two compounds with either a methyl group or ethyl group (**3a** and **3b**, **3d** and **3e**, **3g** and **3h**, **3j** and **3k**), most compounds with an ethyl group showed higher anti-proliferative activity for the NUGC-3 cell line, except **3j** and **3k**. These results demonstrated that a simple alkyl group, especially the




1

2a n=13, $R_1$= Et
2b n=15, $R_1$= Et
2c n=13, $R_1$= Me
2d n=15, $R_1$= Me

3a n=13, $R_1$=Et, $R_2$=Me, X=I
3b n=13, $R_1$=Et, $R_2$=Et, X=I
3c n=13, $R_1$=Et, $R_2$=Ally, X=Br
3d n=15, $R_1$=Et, $R_2$=Me, X=I
3e n=15, $R_1$=Et, $R_2$=Et, X=I
3f n=15, $R_1$=Et, $R_2$=Ally, X=Br
3g n=13, $R_1$=Me, $R_2$=Me, X=I
3h n=13, $R_1$=Me, $R_2$=Et, X=I
3i n=13, $R_1$=Me, $R_2$=Ally, X=Br
3j n=15, $R_1$=Me, $R_2$=Me, X=I
3k n=15, $R_1$=Me, $R_2$=Et, X=I
3l n=15, $R_1$=Me, $R_2$=Ally, X=Br

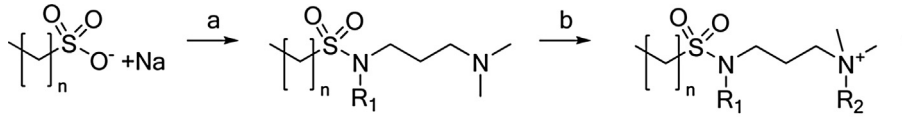
**Scheme 1.** Reaction protocol for the synthesis of aliphatic sulfonamido-quaternary ammonium salts (**3a**–**3l**). Reagents and conditions: (a) i) $SOCl_2$, $CH_2Cl_2$ at 60 °C ii) *N*,*N*-dialkyl-*N*′-methylethylenediamine, $CH_2Cl_2$ at 0 °C (b) alkyl halide, $CH_3CN$ at 95–100 °C or methyl iodide, toluene, 110–115 °C.

ethyl group, on the *N*-substituent position induced inhibitory activities more effectively.

Third, to evaluate the effects of the open-ring moiety on sulfonamido-quaternary ammonium salts, we introduced four aliphatic groups such as *N*,*N*-dialkyl-*N*′-methylethylenediamine (*N*,*N*-diethyl-*N*′-methylethylenediamine, *N*,*N*,*N*′-trimethylethane-1,2-diamine), *N*,*N*-dimethylpropane-1,3-diamine, *N*,*N*,*N*′-trimethylpropane-1,3-diamine, and *N*,*N*,2,2-tetramethylpropane-1,3-diamine (**3a**–**3l**, **5a**–**5h**, **7a**–**7d**). The first group of compounds (*N*,*N*-dialkyl-*N*′-methylethylenediamine derivatives, **3a**–**3l**), which were synthesized with the same diamine moiety as **A913**, exhibited similar or better growth inhibitory activity than **A913**. Compound **3i** showed specific anti-proliferative activity for the PC-3 cancer cell line and compound **3j** displayed specificity for the NUGC-3 cancer cell line. **5a**–**5d** (second group, *N*,*N*,*N*′-trimethylpropane-1,3-diamine derivatives) had longer carbon length of the open-ring moiety than the first group of compounds. These compounds displayed remarkably more potent inhibitory activity than the reference compounds, NSC126188 and perifosine. Among them, compound **5b** ($R_2$ = ethyl group, described in Scheme 2) displayed the most potent growth inhibition of all synthesized analogs for both gastric and prostate cancer cell lines. **5e**–**5g** (third group, *N*,*N*-dimethylpropane-1,3-diamine derivatives), which had hydrogen at the $R_3$ position instead of a methyl group in the second group, displayed similar or lower anti-proliferation activities than **5a**–**5d** and NSC126188. Branched open-ring moieties were introduced to **7a**–**7d** (fourth group, *N*,*N*,2,2-tetramethylpropane-1,3-diamine derivatives), which decreased the inhibitory activity of the analogs. Among four open-ring moiety groups, a group of *N*,*N*,*N*'-trimethylpropane-1,3-diamine compounds induced the most positive effects on activity than any other groups, for both the gastric and prostate cancer cell lines.

The synthesized compounds significantly suppressed cell proliferation, especially for the gastric cancer cell line (NUGC-3) and the prostate cancer cell line (PC-3) among the six cancer cell lines tested. Introduction of a sulfonyl group instead of a carbonyl group increased the specificity for the cancer cell lines. Analogs with an introduced sulfonyl group showed more effective growth inhibition activities for the gastric cancer cell lines than for any other cancer cell line. In addition, the ethyl group on the *N*-substituent position and *N*,*N*,*N*′-trimethylpropane-1,3-diamine, among open-ring moieties, increased inhibitory activities for both the gastric and prostate cancer cell lines. Among 24 compounds, compound **5b** (*N*,*N*,*N*′-trimethylpropane-1,3-diamine, $R_2$ = ethyl group) showed the best growth inhibitory activity for both the NUGC-3 and PC-3 cancer cell lines.

For this series of compounds, the activity of RhoB was determined using the luciferase reporter gene assay. The results are

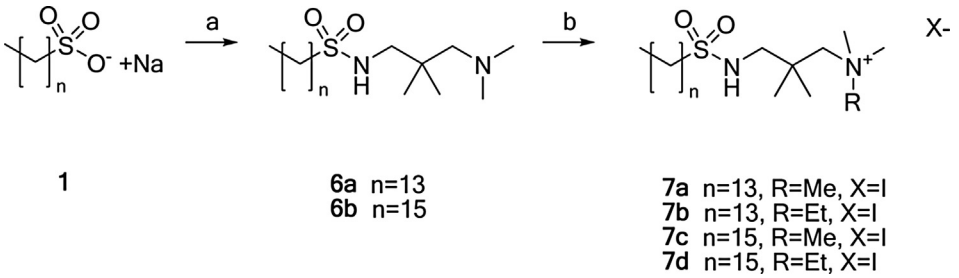



1

4a n=13, $R_1$=Me
4b n=15, $R_1$=Me
4c n=13, $R_1$=H
4d n=15, $R_1$=H

5a n=13, $R_1$=Me, $R_2$=Me, X=I
5b n=13, $R_1$=Me, $R_2$=Et, X=I
5c n=15, $R_1$=Me, $R_2$=Me, X=I
5d n=15, $R_1$=Me, $R_2$=Et, X=I
5e n=13, $R_1$=H, $R_2$=Me, X=I
5f n=13, $R_1$=H, $R_2$=Et, X=I
5g n=15, $R_1$=H, $R_2$=Me, X=I
5h n=15, $R_1$=H, $R_2$=Et, X=I

**Scheme 2.** Reaction protocol for the synthesis of aliphatic sulfonamido-quaternary ammonium salts (**5a**–**5h**). Reagents and conditions: (a) i) $SOCl_2$, $CH_2Cl_2$ at 60 °C ii) *N*,*N*-dimethyl-*N*′-alkylpropanediamine, $CH_2Cl_2$ at 0 °C (b) alkyl halide, $CH_3CN$ at 95–100 °C or methyl iodide, toluene, 110–115 °C.




**Scheme 3.** Reaction protocol for the synthesis of aliphatic sulfonamido-quaternary ammonium salts (**7a**–**7d**). Reagents and conditions: (a) i) $SOCl_2$, $CH_2Cl_2$ at 60 °C ii) *N*,*N*,2,2-tetramethylpropane-1,3-diamine, $CH_2Cl_2$ at 0 °C (b) alkyl halide, $CH_3CN$ at 95–100 °C or methyl iodide, toluene, 110–115 °C.

shown in Fig. 2. Most of the synthesized compounds displayed better RhoB promoter activity than NSC126188. To assess the effects on RhoB expression based on *N*-substituents, the analogs with an *N*-ethyl group on the quaternary ammonium salts were compared with analogs with an *N*-methyl group. In a manner similar to the growth inhibition results, most analogs with an ethyl group showed better expression than analogs with a methyl group. In addition, RhoB promoter activity based on open-ring moieties also displayed a similar tendency as the growth inhibitory activity. Compounds with *N*,*N*-diethyl-*N*′-methylethylenediamine and *N*,*N*,*N*′-trimethylpropane-1,3-diamine generally induced high RhoB promoter activity. These results demonstrated that diamine and *N*-substituents of new aliphatic sulfonamido-quaternary ammonium salts showed the same qualitative effects for both RhoB expression and growth inhibition.

Based on NSC126188 and **A913** which share structural features, we designed and synthesized a new series of aliphatic sulfonamido-quaternary ammonium salts and evaluated their activities using the anti-proliferative assay and the RhoB promoter assay. Using the growth inhibition assay, the sulfonyl group of analogs was generally more effective for the gastric cancer cell line than other tested cancer cell lines. Among 24 synthesized compounds, the analogs with *N*-ethyl substituent and *N*,*N*,*N*′-trimethylpropane-1,3-diamine showed effective results on both the anti-proliferative assay and the RhoB promoter assay. The compound **5b** was the most promising anticancer agent among this series, based upon growth inhibition as well as activation of RhoB expression. Together, the results show that synthetic aliphatic sulfonamido-quaternary ammonium salts are remarkably potent reagents for further development of anti-cancer chemotherapy.

**Table 1**
Growth inhibition of synthesized compounds.

| Compound | Growth inhibition (μM)[a] | | | | | |
|---|---|---|---|---|---|---|
| | NUGC-3 | PC-3 | MDA-MB-231 | ACHN | HCT-15 | NCI-H23 |
| **3a** | 0.52 | 0.82 | 1.22 | 1.74 | 2.35 | 0.71 |
| **3b** | 0.32 | 0.52 | 0.87 | 1.46 | 3.46 | 0.47 |
| **3c** | 0.21 | 0.53 | 0.71 | 1.35 | 2.03 | 0.30 |
| **3d** | 0.39 | 0.56 | 1.12 | 1.29 | 1.49 | 0.62 |
| **3e** | 0.22 | 0.52 | 1.17 | 1.31 | 1.61 | 0.55 |
| **3f** | 0.39 | 0.76 | 1.34 | 1.45 | 1.81 | 0.45 |
| **3g** | 0.39 | 0.47 | 0.92 | 1.40 | 0.75 | 2.04 |
| **3h** | 0.30 | 0.34 | 0.99 | 1.30 | 1.61 | 0.59 |
| **3i** | 0.83 | 0.46 | 1.80 | 1.39 | 1.71 | 0.73 |
| **3j** | 0.33 | 0.62 | 0.93 | 1.60 | 0.70 | 1.29 |
| **3k** | 0.36 | 0.57 | 0.54 | 1.75 | 1.26 | 1.81 |
| **3l** | 0.76 | 0.62 | 1.35 | 3.48 | 4.73 | 0.89 |
| **5a** | 0.15 | 0.30 | 1.26 | 1.01 | 0.68 | 0.21 |
| **5b** | 0.13 | 0.22 | 0.75 | 0.54 | 1.27 | 0.38 |
| **5c** | 0.21 | 0.44 | 1.04 | 1.00 | 0.78 | 0.91 |
| **5d** | 0.20 | 0.36 | 0.69 | 0.95 | 0.78 | 0.43 |
| **5e** | 0.38 | 0.45 | 1.40 | 2.04 | 0.94 | 0.46 |
| **5f** | 0.29 | 0.30 | 1.17 | 1.64 | 1.14 | 0.33 |
| **5g** | 0.56 | 0.58 | 1.39 | 1.89 | 0.86 | 1.56 |
| **5h** | 0.32 | 0.56 | 0.91 | 1.29 | 1.20 | 0.91 |
| **7a** | 0.32 | 0.35 | 2.11 | 2.60 | 1.70 | 0.31 |
| **7b** | 0.51 | 0.46 | 0.72 | 2.52 | NA[b] | 0.73 |
| **7c** | 0.43 | 0.56 | 1.52 | 2.21 | 0.56 | 0.74 |
| **7d** | 0.57 | 0.79 | 2.79 | 2.27 | 1.35 | 0.69 |
| Perifosine | 0.54 | 0.44 | 2.86 | 4.56 | 1.25 | 4.21 |
| NSC126188 | 0.29 | 0.48 | 1.44 | 1.04 | 0.58 | 2.34 |
| **A913** | 0.14 | 0.21 | 0.30 | 0.10 | NA[b] | 0.24 |

[a] Growth inhibition was measured by the SRB (sulforhodamine B) assay. The values are means of three experiments.
[b] NA, not active.

## 4. Experimental section

All chemicals were obtained from commercial suppliers and used without further purification. All reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 $F_{254}$ (mesh) (E. Merck, Mumbai, India). Spots were visualized under UV light (254 nm). Flash column chromatography was performed with silica (E. Merck, EM9385, 230–400 mesh). $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR, Varian) spectra were recorded at 300 and 75 MHz, at 400 and 100 MHz, or at 500 and 125 MHz, respectively. Proton and carbon chemical shifts were expressed in ppm relative to the internal standard tetramethylsilane, and coupling constants (*J*) were expressed in Hertz. Liquid chromatography–mass spectrometry (LC/MS) spectra were recorded by electrospray ionization (ESI) on a Shimazu LC/MS (Dong-il SHIMADZU Corp., Seoul, Korea) instruments (10% 0.1% TFA in $H_2O$/90% 0.1% TFA in acetonitrile) in scan mode (from 0 to 600 amu/z). The detected ion peaks are $(M^+z)/z$ 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) spectra were obtained from electrospray ionization (ESI) in positive mode using the Micromass Q-TOF (Waters Corp., Seoul, Korea). The capillary and sample cone voltages were 4000 V and 30 V, respectively, the desolvation gas flow was 600 L/h at 200 °C, and the source temperature was 100 °C, using the high resolution tandem mass spectrometer at Yonsei University, Seoul, Korea.

### 4.1. General procedures for the synthesis of *2a–d*, *4a–d*, and *6a–b*

Sulfonate sodium salts **1** (2.9 mmol) were dissolved in anhydrous dichloromethane (0.1 M solution), and thionyl chloride (3.0 equivalents) was added under an argon 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 *N*,*N*,*N*′-alkyl-diamine (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

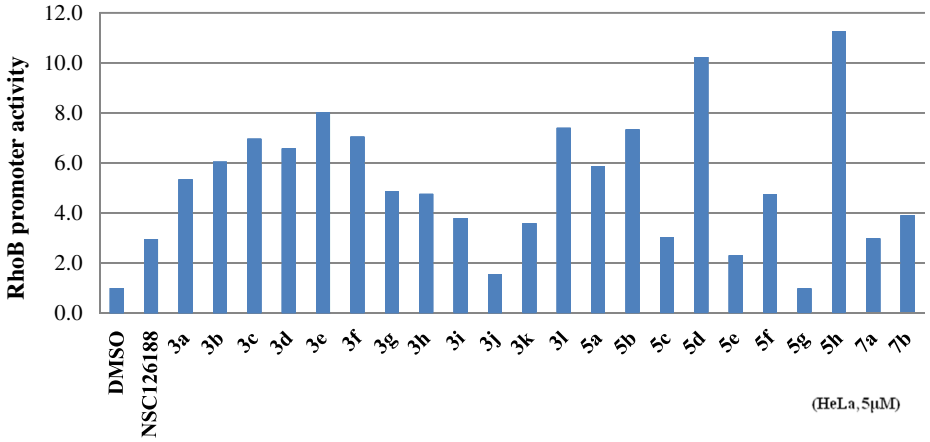



**Fig. 2.** Activation of RhoB promoter was determined by luciferase activity in cells transfected with the pGL2-RhoB-Luc in the presence of various compounds (5 μM). NSC126188 (5 μM) was used as a positive control.

mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with 5% MeOH/$CHCl_3$ to resolve the corresponding amides.

*4.1.1. Tetradecane-1-sulfonic acid (2-diethylamino-ethyl)-methyl-amide (**2a**)*

Sodium 1-tetradecanesulfonate was used and **2a** (53.8%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.14 (t, $J$ = 7.0 Hz, 2H), 3.06 (t, $J$ = 7.5 Hz, 2H), 2.84 (s, 3H), 2.52–2.44 (m, 6H), 1.63–1.60 (m, 2H), 1.36–1.24 (m, 22H), 0.94 (t, $J$ = 7.5 Hz, 6H), 0.85 (t, $J$ = 6.8 Hz, 3H); ESI-MS: $m/z$ = 391 [$M^+$H].

*4.1.2. Hexadecane-1-sulfonic acid (2-diethylamino-ethyl)-methyl-amide (**2b**)*

Sodium 1-hexadecanesulfonate was used and **2b** (50.1%) was obtained. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 3.58 (br m, 2H), 3.00–2.94 (m, 9H), 2.22–2.10 (m, 2H), 1.82–1.76 (m, 2H), 1.42–1.38 (m, 4H), 1.26 (m, 28H), 0.88 (t, $J$ = 6.5 Hz, 3H); ESI-MS: $m/z$ = 419 [$M^+$H].

*4.1.3. Tetradecane-1-sulfonic acid (2-dimethylamino-ethyl)-methyl-amide (**2c**)*

Sodium 1-tetradecanesulfonate was used and **2c** (88.3%) was obtained. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.16 (t, $J$ = 6.4 Hz, 2H), 3.03 (t, $J$ = 7.6 Hz, 2H), 2.78 (s, 3H), 2.36 (t, $J$ = 6.2 Hz, 2H), 2.14 (s, 6H), 1.65–1.55 (m, 2H), 1.38–1.30 (m, 2H), 1.29–1.18 (m, 20H), 0.84 (t, $J$ = 6.6 Hz, 3H); ESI-MS: $m/z$ = 363 [$M^+$H].

*4.1.4. Hexadecane-1-sulfonic acid (2-dimethylamino-ethyl)-methyl-amide (**2d**)*

Sodium 1-hexadecanesulfonate was used and **2d** (52.5%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.17 (t, $J$ = 6.5 Hz, 2H), 3.04 (t, $J$ = 8.0 Hz, 2H), 2.79 (s, 3H), 2.37 (t, $J$ = 6.5 Hz, 2H), 2.15 (s, 6H), 1.66–1.60 (m, 2H), 1.36–1.32 (m, 2H), 1.27–1.25 (m, 24H), 0.85 (t, $J$ = 6.8 Hz, 3H); ESI-MS: $m/z$ = 319 [$M^+$H].

*4.1.5. Tetradecane-1-sulfonic acid (3-dimethylamino-propyl)-methyl-amide (**4a**)*

Sodium 1-tetradecanesulfonate was used and **4a** (37.7%) was obtained. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.08 (t, $J$ = 6.8 Hz, 2H), 2.98 (t, $J$ = 7.0 Hz, 2H), 2.74 (s, 3H), 2.24 (t, $J$ = 6.4 Hz, 2H), 2.15 (s, 6H), 1.67–1.55 (m, 4H), 1.40–1.30 (m, 2H), 1.30–1.15 (m, 20H), 0.84 (t, $J$ = 7.0 Hz, 3H); ESI-MS: $m/z$ = 377 [$M^+$H].

*4.1.6. Hexadecane-1-sulfonic acid (3-dimethylamino-propyl)-methyl-amide (**4b**)*

Sodium 1-hexadecanesulfonate was used and **4b** (54.2%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.09 (t, $J$ = 7.3 Hz, 2H), 2.75 (s, 3H), 2.22 (t, $J$ = 6.7 Hz, 2H), 2.13 (s, 6H), 1.64–1.58 (m, 4H), 1.35 (br s, 2H), 1.24 (s, 26H), 0.85 (t, $J$ = 7.8 Hz, 3H); ESI-MS: $m/z$ = 405 [$M^+$H].

*4.1.7. Tetradecane-1-sulfonic acid (3-dimethylamino-propyl)-amide (**4c**)*

Sodium 1-tetradecanesulfonate was used and **4c** (83.0%) was obtained. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.03–7.01 (m, 1H), 2.97–2.91 (m, 4H), 2.42–2.40 (m, 2H), 2.25 (s, 6H), 1.64–1.58 (m, 2H), 1.24 (br s, 22H), 0.85 (t, $J$ = 7.0 Hz, 3H); ESI-MS: $m/z$ = 363 [$M^+$H].

*4.1.8. Hexadecane-1-sulfonic acid (3-dimethylamino-propyl)-amide (**4d**)*

Sodium 1-hexadecanesulfonate was used and **4d** (72.4%) was obtained. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 6.96 (t, $J$ = 6.8 Hz, 1H), 2.93–2.86 (m, 4H), 2.21 (t, $J$ = 7.0 Hz, 2H), 2.08 (s, 6H), 1.61 (br m, 4H), 1.31 (br s, 2H), 1.20 (br s, 24H), 0.82 (t, $J$ = 6.6 Hz, 3H); ESI-MS: $m/z$ = 391 [$M^+$H].

*4.1.9. Tetradecane-1-sulfonic acid (3-dimethylamino-2,2-dimethyl-propyl)-amide (**6a**)*

Sodium 1-tetradecanesulfonate was used and **6a** (94.0%) was obtained. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 2.98–2.95 (m, 4H), 2.28–2.27 (m, 8H), 1.82–1.76 (m, 2H), 1.44–1.38 (m, 2H), 1.28–1.25 (m, 20H), 0.87 (t, $J$ = 7.0 Hz, 3H); ESI-MS: $m/z$ = 391 [$M^+$H].

*4.1.10. Hexadecane-1-sulfonic acid (3-dimethylamino-2,2-dimethyl-propyl)-amide (**6b**)*

Sodium 1-hexadecanesulfonate was used and **6b** (91.2%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 6.88–6.86 (m, 1H), 2.94 (t, $J$ = 8.0 Hz, 2H), 2.50–2.49 (m, 2H), 2.21 (s, 6H), 2.08 (s, 2H), 1.65–1.59 (m, 2H), 1.24 (m, 26H), 0.85 (t, $J$ = 7.0 Hz, 3H), 0.82 (s, 6H); ESI-MS: $m/z$ = 419 [$M^+$H].

*4.2. General procedures for the synthesis of **3a–l**, **5a–h**, and **7a–d***

Alkyl halide (1.6 mmol) was added to a solution of **2a–d, 4a–d,** and **6a–b** (0.8 mmol) in anhydrous acetonitrile (0.3 M) at room temperature, and 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*.

*4.2.1. Diethyl-methyl-{2-[methyl-(tetradecane-1-sulfonyl)-amino]-ethyl}-ammonium; iodide (**3a**)*

**2a** was used and **3a** (78.9%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.52 (t, $J$ = 7.3 Hz, 2H), 3.41 (t, $J$ = 7.3 Hz, 2H), 3.40–3.34 (m, 4H), 3.15–3.12 (m, 2H), 2.98 (s, 3H), 2.86 (s, 3H), 1.66–1.63 (m, 2H), 1.37–1.34 (m, 2H), 1.24–1.21 (m, 26H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 56.9, 56.5, 56.5, 48.5, 47.4, 43.6, 35.7, 31.7, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.1, 23.0, 22.5, 14.4, 8.0, 8.0 ppm; ESI-MS: $m/z$ = 405 [M$^+$]; HRMS (ESI) calcd. for $C_{22}H_{49}N_2O_2S$ (M+) 405.3509, found 405.3494.

*4.2.2. Triethyl-{2-[methyl-(tetradecane-1-sulfonyl)-amino]-ethyl}-ammonium; iodide (**3b**)*

**2a** was used and **3b** (82.1%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.48 (t, $J$ = 7.3 Hz, 2H), 3.37–3.34 (m, 2H), 3.30–3.27 (m, 6H), 3.14 (t, $J$ = 7.8 Hz, 2H), 2.87 (s, 3H), 1.66–1.63 (m, 2H), 1.37–1.34 (m, 2H), 1.24 (m, 20H), 1.19 (t, $J$ = 7.0 Hz, 9H), 0.85 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 53.7, 53.0, 53.0, 53.0, 48.5, 43.3, 36.0, 31.7, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4, 7.7, 7.7, 7.7 ppm; ESI-MS: $m/z$ = 419 [M$^+$]; HRMS (ESI) calcd. for $C_{23}H_{51}N_2O_2S$ (M+) 419.3666, found 419.3647.

*4.2.3. Allyl-diethyl-{2-[methyl-(tetradecane-1-sulfonyl)-amino]-ethyl}-ammonium; bromide (**3c**)*

**2a** was used and **3c** (45.8%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 6.05–5.97 (m, 1H), 5.72–5.62 (m, 2H), 3.95 (d, $J$ = 7.0 Hz, 2H), 3.54–3.51 (m, 2H), 3.37–3.34 (m, 2H), 3.31–3.28 (m, 4H), 3.15–3.12 (m, 2H), 2.86 (s, 3H), 1.66–1.63 (m, 2H), 1.37–1.34 (m, 2H), 1.24–1.22 (m, 26H), 0.85 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 127.7, 125.8, 60.0, 54.3, 53.7, 53.7, 48.5, 43.3, 35.9, 31.7, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4, 7.7, 7.7 ppm; ESI-MS: $m/z$ = 431 [M$^+$]; HRMS (ESI) calcd. for $C_{24}H_{51}N_2O_2S$ (M+) 431.3666, found 431.3646.

*4.2.4. Diethyl-{2-[(hexadecane-1-sulfonyl)-methyl-amino]-ethyl}-methyl-ammonium; iodide (**3d**)*

**2b** was used and **3d** (85.3%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.52 (t, $J$ = 7.0 Hz, 2H), 3.42 (t, $J$ = 7.3 Hz, 2H), 3.40–3.35 (m, 4H), 3.13 (t, $J$ = 7.8 Hz, 2H), 2.98 (s, 3H), 2.86 (s, 3H), 1.66–1.63 (m, 2H), 1.37–1.34 (m, 2H), 1.23–1.21 (m, 30H), 0.85 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 56.9, 56.5, 56.5, 48.5, 47.4, 43.6, 35.7, 31.7, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4, 8.0, 8.0 ppm; ESI-MS: $m/z$ = 433 [M$^+$]; HRMS (ESI) calcd. for $C_{24}H_{53}N_2O_2S$ (M+) 433.3822, found 433.3811.

*4.2.5. Triethyl-{2-[(hexadecane-1-sulfonyl)-methyl-amino]-ethyl}-ammonium; iodide (**3e**)*

**2b** was used and **3e** (29.0%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.48 (t, $J$ = 7.3 Hz, 2H), 3.37–3.27 (m, 6H), 3.14 (t, $J$ = 8.0 Hz, 2H), 1.66–1.63 (m, 2H), 1.36–1.34 (m, 2H), 1.24 (br, 24H), 1.19 (t, $J$ = 7.0 Hz, 6H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 53.7, 53.0, 53.0, 53.0, 48.5, 43.3, 36.0 31.7, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4, 7.6, 7.6, 7.6 ppm; ESI-MS: $m/z$ = 445 [M$^+$]; HRMS (ESI) calcd. for $C_{25}H_{55}N_2O_2S$ (M+) 447.3979, found 447.3966.

*4.2.6. Allyl-diethyl-{2-[(hexadecane-1-sulfonyl)-methyl-amino]-ethyl}-ammonium; bromide (**3f**)*

**2b** was used and **3f** (38.1%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 6.05–5.97 (m, 1H), 5.72–5.62 (m, 2H), 3.95 (d, $J$ = 7.0 Hz, 2H), 3.53 (t, $J$ = 7.3 Hz, 2H), 3.44 (t, $J$ = 6.8 Hz, 2H), 3.30–3.10 (m, 6H), 2.84–2.82 (m, 3H), 1.68–1.62 (m, 2H), 1.37–1.34 (m, 2H), 1.24–1.18 (m, 20H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 127.7, 125.8, 60.0, 54.3, 53.7, 48.5, 46.8, 43.3, 35.9, 31.7, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4, 8.8, 7.7 ppm; ESI-MS: $m/z$ = 459 [M$^+$]; HRMS (ESI) calcd. for $C_{26}H_{55}N_2O_2S$ (M+) 459.3979 found 439.3965.

*4.2.7. Trimethyl-{2-[methyl-(tetradecane-1-sulfonyl)-amino]-ethyl}-ammonium; iodide (**3g**)*

**2c** was used and **3g** (68.7%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) 4.14 (br s, 2H), 3.83 (br s, 2H), 3.48 (s, 9H), 3.04 (t, $J$ = 8.0 Hz, 2H), 1.83–1.80 (m, 2H), 1.45–1.42 (m, 2H), 1.31–1.27 (m, 22H), 0.90 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 61.9, 53.0, 48.5, 48.5, 48.5, 44.3, 35.3, 31.7, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4 ppm; ESI-MS: $m/z$ = 377 [M$^+$]; HRMS (ESI) calcd. for $C_{19}H_{43}N_2O_3S$ (M+) 377.3196, found 377.3182.

*4.2.8. Ethyl-dimethyl-{2-[methyl-(tetradecane-1-sulfonyl)-amino]-ethyl}-ammonium; iodide (**3h**)*

**2c** was used and **3h** (83.9%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) 3.55 (t, $J$ = 6.5 Hz, 2H), 3.47 (t, $J$ = 6.5 Hz, 2H), 3.39–3.32 (m, 2H), 3.13 (t, $J$ = 8.0 Hz, 2H), 3.04 (s, 6H), 2.85 (s, 3H), 1.65 (m, 2H), 1.37–1.24 (m, 22H), 0.85 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 59.6, 59.4, 50.3, 48.5, 48.5, 44.0, 35.5, 31.7, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4, 8.3 ppm; ESI-MS: $m/z$ = 391 [M$^+$]; HRMS (ESI) calcd. for $C_{21}H_{47}N_2O_3S$ (M+) 391.3353, found 391.3341.

*4.2.9. Allyl-dimethyl-{2-[methyl-(tetradecane-1-sulfonyl)-amino]-ethyl}-ammonium; bromide (**3i**)*

**2c** was used and **3i** (51.5%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) 6.04–5.96 (m, 1H), 5.64–5.53 (m, 2H), 4.01–3.99 (d, $J$ = 7.0 Hz, 2H), 3.57–3.42 (m, 2H), 3.27 (t, $J$ = 20.3 Hz, 2H), 3.12–2.96 (m, 6H), 2.83–2.71 (m, 3H), 1.61–1.55 (m, 2H), 1.30–1.27 (m, 2H), 1.21–1.13 (m, 22H), 0.83–0.73 (m, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 128.3, 126.2, 66.1, 60.0, 50.4, 48.5, 48.5, 43.9, 35.5, 31.7, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4 ppm; ESI-MS: $m/z$ = 403 [M$^+$]; HRMS (ESI) calcd. for $C_{22}H_{47}N_2O_2S$ (M+) 403.3353, found 403.3338.

*4.2.10. Trimethyl-{2-[methyl-(tetradecane-1-sulfonyl)-amino]-ethyl}-ammonium; iodide (**3j**)*

**2d** was used and **3j** (27.3%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.56–7.51 (m, 5H), 4.59 (s, 2H), 3.68 (t, $J$ = 7.0 Hz, 2H), 3.52 (t, $J$ = 6.8 Hz, 2H), 3.14 (t, $J$ = 8.0 Hz, 2H), 3.02 (s, 6H), 2.86 (s, 3H), 1.65 (t, $J$ = 7.5 Hz, 2H), 1.36 (t, $J$ = 7.3 Hz, 2H), 1.23 (s, 25H), 0.85 (t, $J$ = 6.5 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 62.0, 53.0, 48.5, 48.5, 48.5, 44.3, 35.3, 31.7, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.1, 29.0, 23.0, 22.5, 14.4 ppm; ESI-MS: $m/z$ = 406 [M$^+$]; HRMS (ESI) calcd. for $C_{23}H_{51}N_2O_2S$ (M+) 405.3509, found 405.3495.

*4.2.11. Ethyl-{2-[(hexadecane-1-sulfonyl)-methyl-amino]-ethyl}-dimethyl-ammonium; iodide (**3k**)*

**2d** was used and **3k** (47.5%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.55 (t, $J$ = 5.5 Hz, 2H), 3.32 (s, 2H), 3.14–3.11 (m, 9H), 2.85 (s, 3H), 1.65 (t, $J$ = 7.5 Hz, 2H), 1.35 (t, $J$ = 7.5 Hz, 3H), 1.23 (s, 23H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 59.1, 58.9, 49.8, 48.0, 43.4, 35.0, 31.3, 29.0, 29.0, 29.0, 29.0, 29.0, 29.0, 29.0, 28.9, 28.8, 28.7, 28.5, 27.7, 22.5, 22.1, 13.9, 7.8 ppm; ESI-MS: $m/z$ = 420 [M$^+$]; HRMS (ESI) calcd. for $C_{22}H_{49}N_2O_2S$ (M+) 419.3666, found 419.3646.

*4.2.12. Allyl-{2-[(hexadecane-1-sulfonyl)-methyl-amino]-ethyl}-dimethyl-ammonium; bromide (**3l**)*

**2d** was used and **3l** (78.1%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 6.06 (m, 1H), 5.65 (t, $J$ = 6.5 Hz, 2H), 4.02–4.01 (d,

$J$ = 7.2 Hz, 2H), 3.59–3.58 (m, 2H), 3.50–3.48 (m, 2H), 3.13 (t, $J$ = 7.8 Hz, 2H), 3.05 (s, 6H), 2.84 (s, 3H), 1.65 (m, 2H), 1.37 (m, 2H), 1.23 (m, 26H), 0.85 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 128.3, 126.2, 66.1, 60.0, 50.4, 48.5, 48.5, 43.9, 35.5, 31.7, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.0, 22.5, 14.4 ppm; ESI-MS: $m/z$ = 431 [M$^+$]; HRMS (ESI) calcd. for $C_{24}H_{51}N_2O_2S$ (M+) 431.3666, found 431.3649.

#### 4.2.13. Trimethyl-{3-[methyl-(tetradecane-1-sulfonyl)-amino]-propyl}-ammonium; iodide (**5a**)

**4a** was used and **5a** (86.9%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.30–3.27 (m, 2H), 3.17 (t, $J$ = 6.5 Hz, 2H), 3.07–3.04 (m, 9H), 2.98 (s, 3H), 1.98–1.93 (m, 2H), 1.65–1.60 (m, 2H), 1.36–1.33 (m, 2H), 1.27–1.24 (m, 22H), 0.85 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 63.5, 52.9, 52.8, 52.8, 48.3, 47.0, 34.8, 31.7, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.1, 22.5, 21.4, 14.4 ppm; ESI-MS: $m/z$ = 391 [M$^+$]; HRMS (ESI) calcd. for $C_{21}H_{47}N_2O_2S$ (M+) 391.3353, found 391.3336.

#### 4.2.14. Ethyl-dimethyl-{3-[methyl-(tetradecane-1-sulfonyl)-amino]-propyl}-ammonium; iodide (**5b**)

**4a** was used and **5b** (56.4%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.25–3.21 (m, 2H), 3.17 (t, $J$ = 6.5 Hz, 2H), 3.05 (t, $J$ = 8.0 Hz, 2H), 3.00 (s, 6H), 2.80 (s, 3H), 1.94–1.90 (m, 2H), 1.64–1.60 (m, 2H), 1.38–1.33 (m, 2H), 1.24–1.21 (m, 25H), 0.83 (t, $J$ = 6.8 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 60.6, 59.2, 50.1, 48.4, 47.1, 35.0 and 34.9, 31.7, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.1, 22.5, 21.1 and 20.8, 14.4, 8.3 and 8.0 ppm; ESI-MS: $m/z$ = 405 [M$^+$]; HRMS (ESI) calcd. for $C_{22}H_{49}N_2O_3S$ (M+) 405.3509, found 405.3497.

#### 4.2.15. {3-[(Hexadecane-1-sulfonyl)-methyl-amino]-propyl}-trimethyl-ammonium; iodide (**5c**)

**4b** was used and **5c** (98.0%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.31–3.26 (m, 2H), 3.26–3.15 (m, 2H), 3.06–3.02 (m, 9H), 2.79 (s, 3H), 1.94 (m, 2H), 1.63 (m, 2H), 1.36–1.23 (m, 26H), 0.86–0.83 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 63.5, 52.9, 52.8, 52.8, 48.3, 47.0, 34.8, 31.7, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.1, 22.5, 21.4, 14.4 ppm; ESI-MS: $m/z$ = 419 [M$^+$]; HRMS (ESI) calcd. for $C_{23}H_{51}N_2O_2S$ (M+) 419.3666, found 419.3657.

#### 4.2.16. Ethyl-{3-[(hexadecane-1-sulfonyl)-methyl-amino]-propyl}-dimethyl-ammonium; iodide (**5d**)

**4b** was used and **5d** (87.8%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.34–3.33 (m, 2H), 3.21–3.17 (t, $J$ = 12.5 Hz, 2H), 3.17–3.15 (t, $J$ = 5.0 Hz, 2H), 3.05–3.02 (t, $J$ = 7.5 Hz, 2H), 2.99 (s, 6H), 2.8 (s, 3H), 1.91 (m, 2H), 1.63 (m, 2H), 1.36–1.21 (m, 29H), 0.86–0.83 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 60.6, 59.2, 50.0, 48.4, 48.4, 47.1, 34.9, 31.7, 29.5, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.3, 29.2, 29.0, 28.2, 23.1, 22.5, 21.1, 14.4, 8.3 ppm; ESI-MS: $m/z$ = 433 [M$^+$]; HRMS (ESI) calcd. for $C_{24}H_{53}N_2O_2S$ (M+) 433.3822, found 433.3805.

#### 4.2.17. Trimethyl-[3-(tetradecane-1-sulfonylamino)-propyl]-ammonium; iodide (**5e**)

**4c** was used and **5e** (52.7%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.13 (t, $J$ = 6.1 Hz, 1H), 3.10–2.95 (m, 13H), 2.20–1.96 (m, 3H), 1.91–1.82 (m, 2H), 1.61 (d, $J$ = 7.6 Hz, 2H), 1.36 (s, 3H), 1.24 (s, 21H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 63.77, 63.74, 63.71, 52.83, 52.79, 52.76, 51.13, 36.87, 31.73, 29.50, 29.47, 29.41, 29.26, 29.15, 29.06, 28.04, 23.77, 23.61, 22.53, 14.40 ppm; ESI-MS: $m/z$ = 377 [M$^+$]; HRMS (ESI) calcd. for $C_{20}H_{45}N_2O_2S$ (M+) 377.3196, found 377.3184.

#### 4.2.18. Ethyl-dimethyl-[3-(tetradecane-1-sulfonylamino)-propyl]-ammonium; iodide (**5f**)

**4c** was used and **5f** (58.2%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.15–7.13 (m, 1H), 3.34–3.33 (m, 2H), 3.28–3.24 (br m, 2H), 3.02–2.98 (br m, 10H), 1.86–1.80 (m, 2H), 1.65–1.59 (m, 2H), 1.23 (br s, 25H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 60.63, 59.16, 51.13, 50.13, 50.13, 50.13, 42.98, 39.86, 31.74, 29.50, 29.47, 29.41, 29.27, 29.16, 29.06, 28.04, 23.63, 23.34, 22.54, 14.40, 8.25 ppm; ESI-MS: $m/z$ = 391 [M$^+$]; HRMS (ESI) calcd. for $C_{21}H_{47}N_2O_2S$ (M+) 391.3353, found 391.3339.

#### 4.2.19. [3-(Hexadecane-1-sulfonylamino)-propyl]-trimethyl-ammonium; iodide (**5g**)

**4d** was used and **5g** (84.4%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 3.30 (t, $J$ = 7.5 Hz, 2H), 3.05 (s, 9H), 3.01–2.97 (m, 4H), 1.88–1.84 (m, 2H), 1.65–1.59 (m, 2H), 1.36–1.23 (m, 26H), 0.83 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 63.77, 63.74, 63.71, 52.84, 52.80, 52.76, 51.15, 39.87, 31.74, 29.50, 29.50, 29.50, 29.45, 29.42, 29.27, 29.15, 29.07, 28.05, 23.77, 23.61, 22.54, 14.39 ppm; ESI-MS: $m/z$ = 405 [M$^+$]; HRMS (ESI) calcd. for $C_{22}H_{49}N_2O_2S$ (M+) 405.3509, found 405.3497.

#### 4.2.20. Ethyl-[3-(hexadecane-1-sulfonylamino)-propyl]-dimethyl-ammonium; iodide (**5h**)

**4d** was used and **5h** (82.8%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.14 (m, 1H), 3.33–3.31 (m, 4H), 3.24 (t, $J$ = 7.5 Hz, 2H), 3.01–2.98 (m, 8H), 1.83 (m, 2H), 1.62 (m, 2H), 1.36–1.23 (m, 26H), 0.83 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 60.62, 59.15, 51.11, 50.15, 50.12, 50.09, 39.85, 31.74, 29.50, 29.50, 29.50, 29.50, 29.45, 29.42, 29.27, 29.15, 29.07, 28.05, 23.63, 23.33, 22.54, 14.39, 8.24 ppm; ESI-MS: $m/z$ = 419 [M$^+$]; HRMS (ESI) calcd. for $C_{23}H_{51}N_2O_2S$ (M+) 419.3666, found 419.3654.

#### 4.2.21. [2,2-Dimethyl-3-(tetradecane-1-sulfonylamino)-propyl]-trimethyl-ammonium; iodide (**7a**)

**6a** was used and **7a** (80.7%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.18–7.15 (m, 1H), 3.28 (s, 2H), 3.15 (s, 9H), 3.0 (t, $J$ = 7.8 Hz, 2H), 2.86 (d, $J$ = 7.0 Hz, 2H), 1.66–1.60 (m, 2H), 1.35–1.34 (m, 2H), 1.24 (br s, 20H), 1.11 (s, 6H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 72.68, 55.27, 55.27, 55.27, 52.74, 51.45, 36.92, 31.74, 29.50, 29.50, 29.50, 29.50, 29.50, 29.50, 29.47, 29.24, 29.03, 28.05, 25.04, 23.51, 22.54, 14.40 ppm; ESI-MS: $m/z$ = 405 [M$^+$]; HRMS (ESI) calcd. for $C_{22}H_{49}N_2O_2S$ (M+) 405.3509, found 405.3496.

#### 4.2.22. [2,2-Dimethyl-3-(tetradecane-1-sulfonylamino)-propyl]-ethyl-dimethyl-ammonium; iodide (**7b**)

**6a** was used and **7b** (85.9%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.20 (m, 1H), 3.43–3.41 (m, 2H), 3.21 (s, 2H), 3.02 (s, 6H), 2.98 (m, 2H), 2.86–2.85 (m, 2H) 1.29–1.24 (m, 27H), 1.12 (s, 6H), 0.85 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 69.84, 62.94, 53.16, 51.75, 51.44, 36.82, 31.74, 29.50, 29.50, 29.50, 29.50, 29.50, 29.47, 29.40, 29.25, 29.16, 29.04, 28.05, 25.09, 23.52, 22.54, 14.40, 8.67 ppm; ESI-MS: $m/z$ = 419 [M$^+$]; HRMS (ESI) calcd. for $C_{23}H_{51}N_2O_2S$ (M+) 419.3666, found 419.3652.

#### 4.2.23. [3-(Hexadecane-1-sulfonylamino)-2,2-dimethyl-propyl]-trimethyl-ammonium; iodide (**7c**)

**6b** was used and **7c** (87.3%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.19–7.16 (m, 1H), 3.28 (s, 2H), 3.15 (s, 9H), 3.00 (t, $J$ = 7.7 Hz, 2H), 2.85 (d, $J$ = 7.0 Hz, 2H), 1.62 (br s, 2H), 1.35 (br s, 2H), 1.23 (br s, 24H), 1.11 (s, 6H), 0.84 (t, $J$ = 6.5 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 72.68, 55.27, 52.75, 51.46, 36.92, 31.74, 29.49, 29.49, 29.49, 29.49, 29.49, 29.49, 29.49, 29.45, 29.41, 29.25, 29.15, 29.04, 28.06, 25.05, 26.51, 22.54, 14.39 ppm; ESI-MS: $m/z$ = 433

[$M^+$]; HRMS (ESI) calcd. for $C_{24}H_{53}N_2O_2S$ (M+) 433.3822, found 433.3809.

#### 4.2.24. Ethyl-[3-(hexadecane-1-sulfonylamino)-2,2-dimethyl-propyl]-dimethyl-ammonium; iodide (**7d**)

**6b** was used and **7d** (78.9%) was obtained. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.22–7.20 (m, 1H), 3.42–3.41 (m, 2H), 3.33 (s, 3H), 3.21 (s, 2H), 3.07 (s, 3H), 3.00 (t, $J$ = 8.0 Hz, 2H), 2.85 (d, $J$ = 6.5 Hz, 2H), 1.62 (br s, 2H), 1.29–1.23 (m, 29H), 1.11 (s, 6H), 0.84 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 69.83, 62.94, 55.26, 53.17, 51.74, 51.44, 36.92, 36.82, 31.74, 29.50, 29.50, 29.50, 29.50, 29.46, 29.41, 29.26, 29.15, 29.04, 28.06, 25.09, 25.04, 23.52, 22.54, 14.40, 8.67 ppm; ESI-MS: $m/z$ = 447 [$M^+$]; HRMS (ESI) calcd. for $C_{25}H_{55}N_2O_2S$ (M+) 447.3979, found 447.3961.

### 4.3. SRB assay

Growth inhibition of cancer cell lines in the presence of NSC126188 and perifosine was determined using the SRB assay. After an incubation period, cells were fixed with 10% formalin for 30 min, washed with distilled water, and then stained for 30 min with 0.4% sulforhodamine B. The excess dye was removed by washing with 0.1% acetic acid. The amount of SRB dye that bound to the cell matrix was dissolved in 10 mM Tris (pH 10.5) and was quantified using a microplate reader at 540 nm. The concentration of NSC126188 required to inhibit growth of 50% of cells ($GI_{50}$) was determined after 48 h of drug treatment [26].

### 4.4. Luciferase assay

Transactivation of RhoB was determined by a reporter assay using the dual-luciferase reporter assay system (Promega, Madison, WI, USA), as previously described [27]. HeLa cells at 75–90% confluency were transiently cotransfected with a plasmid encoding pGL2-RhoB-firefly luciferase under control of 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.

## Acknowledgments

This research was supported by a grant from the National Research Foundation (grant 2012-0000884), the Translational Research Center for Protein Function Control (NRF-2009-0083522), the Basic Science Research Program (NRF-2013R1A1A2008165), the Korea Health21 R&D Project, Ministry of Health & Welfare (A120478), and the Brain Korea 21 Program, Republic of Korea.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.09.022.

## References

[1] M. Huang, G.C. Prendergast, RhoB in cancer suppression, Histol. Histopathol. 21 (2006) 213–218.
[2] C.T. Stadtlander, J.W. Waterbor, Molecular epidemiology, pathogenesis and prevention of gastric cancer, Carcinogenesis 20 (1999) 2195–2208.
[3] Y.H. Kim, N.G. Kim, J.G. Lim, C. Park, H. Kim, Chromosomal alterations in paired gastric adenomas and carcinomas, Am. J. Pathol. 158 (2001) 655–662.
[4] J. Mazieres, T. Antonia, G. Daste, C. Muro-Cacho, D. Berchery, V. Tillement, A. Pradines, S. Sebti, G. Favre, Loss of RhoB expression in human lung cancer progression, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 10 (2004) 2742–2750.
[5] J. Adnane, C. Muro-Cacho, L. Mathews, S.M. Sebti, T. Munoz-Antonia, Suppression of rho B expression in invasive carcinoma from head and neck cancer patients, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 8 (2002) 2225–2232.
[6] N. Sato, T. Fukui, T. Taniguchi, T. Yokoyama, M. Kondo, T. Nagasaka, Y. Goto, W. Gao, Y. Ueda, K. Yokoi, J.D. Minna, H. Osada, Y. Kondo, Y. Sekido, RhoB is frequently downregulated in non-small-cell lung cancer and resides in the 2p24 homozygous deletion region of a lung cancer cell line, Int. J. Cancer 120 (2007) 543–551.
[7] A.X. Liu, N. Rane, J.P. Liu, G.C. Prendergast, RhoB is dispensable for mouse development, but it modifies susceptibility to tumor formation as well as cell adhesion and growth factor signaling in transformed cells, Mol. Cell. Biol. 21 (2001) 6906–6912.
[8] J. Zhou, Y. Zhu, G. Zhang, N. Liu, L. Sun, M. Liu, M. Qiu, D. Luo, Q. Tang, Z. Liao, Y. Zheng, F. Bi, A distinct role of RhoB in gastric cancer suppression, Int. J. Cancer 128 (2011) 1057–1068.
[9] G. Fritz, C. Brachetti, F. Bahlmann, M. Schmidt, B. Kaina, Rho GTPases in human breast tumours: expression and mutation analyses and correlation with clinical parameters, Br. J. Cancer 87 (2002) 635–644.
[10] K. Stamatakis, E. Cernuda-Morollon, O. Hernandez-Perera, D. Perez-Sala, Isoprenylation of RhoB is necessary for its degradation. A novel determinant in the complex regulation of RhoB expression by the mevalonate pathway, J. Biol. Chem. 277 (2002) 49389–49396.
[11] G. Fritz, B. Kaina, K. Aktories, The ras-related small GTP-binding protein RhoB is immediate-early inducible by DNA damaging treatments, J. Biol. Chem. 270 (1995) 25172–25177.
[12] T. Trapp, L. Olah, I. Holker, M. Besselmann, C. Tiesler, K. Maeda, K.A. Hossmann, GTPase RhoB: an early predictor of neuronal death after transient focal ischemia in mice, Mol. Cell. Neurosci. 17 (2001) 883–894.
[13] R. Gerhard, H. Tatge, H. Genth, T. Thum, J. Borlak, G. Fritz, I. Just, Clostridium difficile toxin A induces expression of the stress-induced early gene product RhoB, J. Biol. Chem. 280 (2005) 1499–1505.
[14] K. Jiang, J. Sun, J. Cheng, J.Y. Djeu, S. Wei, S. Sebti, Akt mediates Ras down-regulation of RhoB, a suppressor of transformation, invasion, and metastasis, Mol. Cell. Biol. 24 (2004) 5565–5576.
[15] I. Adini, I. Rabinovitz, J.F. Sun, G.C. Prendergast, L.E. Benjamin, RhoB controls Akt trafficking and stage-specific survival of endothelial cells during vascular development, Genes Develop. 17 (2003) 2721–2732.
[16] A. Liu, G.C. Prendergast, Geranylgeranylated RhoB is sufficient to mediate tissue-specific suppression of Akt kinase activity by farnesyltransferase inhibitors, FEBS Lett. 481 (2000) 205–208.
[17] K. Stemke-Hale, A.M. Gonzalez-Angulo, A. Lluch, R.M. Neve, W.L. Kuo, M. Davies, M. Carey, Z. Hu, Y. Guan, A. Sahin, W.F. Symmans, L. Pusztai, L.K. Nolden, H. Horlings, K. Berns, M.C. Hung, M.J. van de Vijver, V. Valero, J.W. Gray, R. Bernards, G.B. Mills, B.T. Hennessy, An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer, Cancer Res. 68 (2008) 6084–6091.
[18] Y.C. Lee, T.H. Cheng, J.S. Lee, J.H. Chen, Y.C. Liao, Y. Fong, C.H. Wu, Y.W. Shih, Nobiletin, a citrus flavonoid, suppresses invasion and migration involving FAK/PI3K/Akt and small GTPase signals in human gastric adenocarcinoma AGS cells, Mol. Cell. Biochem. 347 (2011) 103–115.
[19] E. Bousquet, J. Mazieres, M. Privat, V. Rizzati, A. Casanova, A. Ledoux, E. Mery, B. Couderc, G. Favre, A. Pradines, Loss of RhoB expression promotes migration and invasion of human bronchial cells via activation of AKT1, Cancer Res. 69 (2009) 6092–6099.
[20] B.K. Kim, H.M. Kim, K.S. Chung, D.M. Kim, S.K. Park, A. Song, K.J. Won, K. Lee, Y.K. Oh, K.B. Song, J.A. Simon, G. Han, M. Won, Upregulation of RhoB via c-Jun *N*-terminal kinase signaling induces apoptosis of the human gastric carcinoma NUGC-3 cells treated with NSC12618, Carcinogenesis 32 (2011) 254–261.
[21] G.L. Johnson, R. Lapadat, Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases, Science 298 (2002) 1911–1912.
[22] E.F. Wagner, A.R. Nebreda, Signal integration by JNK and p38 MAPK pathways in cancer development, Nat. Rev. Cancer 9 (2009) 537–549.
[23] B.K. Kim, D.M. Kim, K.S. Chung, S.K. Park, S.J. Choi, A. Song, K. Lee, C.W. Lee, K.B. Song, G. Han, J. Simon, H.M. Kim, M. Won, NSC126188, a piperazine alkyl derivative, induces apoptosis via upregulation of RhoB in HeLa cells, Invest. New Drugs 29 (2011) 853–860.
[24] J.S. Yang, D. Song, W.J. Ko, B. Kim, B.K. Kim, S.K. Park, M. Won, K. Lee, H.M. Kim, G. Han, Synthesis and biological evaluation of novel aliphatic amido-quaternary ammonium salts for anticancer chemotherapy: part II, Eur. J. Med. Chem. 63 (2013) 621–628.
[25] J.S. Yang, D. Song, B. Lee, W.J. Ko, S.K. Park, M. Won, K. Lee, H.M. Kim, G. Han, Synthesis and biological evaluation of novel aliphatic amido-quaternary ammonium salts for anticancer chemotherapy: part I, Eur. J. Med. Chem. 46 (2011) 2861–2866.
[26] D.M. Kim, S.Y. Koo, K. Jeon, M.H. Kim, J. Lee, C.Y. Hong, S. Jeong, Rapid induction of apoptosis by combination of flavopiridol and tumor necrosis factor (TNF)-alpha or TNF-related apoptosis-inducing ligand in human cancer cell lines, Cancer Res. 63 (2003) 621–626.
[27] M.S. Won, N. Im, S. Park, S.K. Boovanahalli, Y. Jin, X. Jin, K.S. Chung, M. Kang, K. Lee, S.K. Park, H.M. Kim, B.M. Kwon, J.J. Lee, A novel benzimidazole analogue inhibits the hypoxia-inducible factor (HIF)-1 pathway, Biochem. Biophys. Res. Commun. 385 (2009) 16–21.