

Investigation on the Anticancer Activity of Symmetric and Unsymmetric Cyclic Sulfamides

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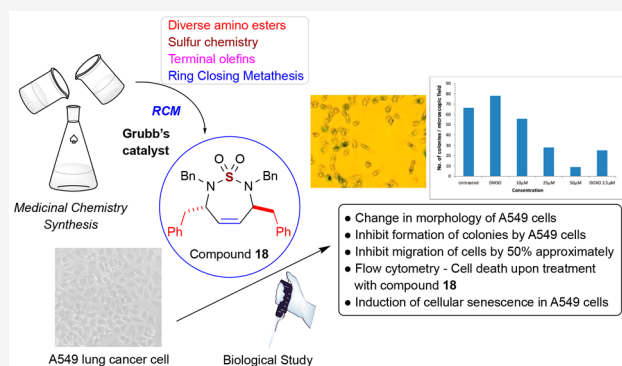
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ABSTRACT: The sulfamide functional group has been extensively employed in organic synthesis to discover probes and drugs in various applications such as cancer, human immunodeficiency virus (HIV), virus, and diabetes. Herein, we describe the synthesis of 7-membered symmetric and unsymmetric sulfamide compounds and their biological evaluation through the National Cancer Institute (NCI) panel of 60 human tumor cell lines (NCI-60) and the mechanism of action study. The results of a study from the NCI-60 cell line exhibited that many synthesized cyclic sulfamide compounds inhibited breast cancer (MDA-MB-468). The mechanism of action study of a representative compound **18** showed the inhibition of proliferation and apoptosis in A549 lung cancer cells.

KEYWORDS: Anticancer activity, Cyclic sulfamide, NCI-60 cell line, A549 lung cancer



The sulfamide is a broadly accepted functional moiety in medicinal chemistry for the design and identification of various biologically active compounds. Sulfamide-based inhibitors have been designed for various enzymes such as carbonic anhydrides (CAs),^{1–6} protease,^{7–10} γ -secretase,¹⁰ serine-protease,¹¹ thrombin,¹² metalloproteinase carboxypeptidase A (CPA),¹³ matrix metalloproteinase (MMP),^{13–15} steroid sulfatase (STS),^{16,17} protein tyrosine phosphatase inhibitors,^{17,18} etc. Also, there has been a report on the anticancer activity of sulfonamide-based compounds.¹⁹ In many cases, a free or substituted sulfamide moiety contributes to binding to the active site of a protein showing better affinity and exhibiting higher aqueous solubility and bioavailability. For example, cyclic sulfamide inhibitors interact with the catalytic aspartic acid residue of HIV protease.^{7,8,19,20} Cyclic sulfamides have been applied in medicinal chemistry to identify several valuable compounds as shown in Figure 1. Prominently, cyclic sulfamides have been used as a structural template applicable for the inhibitor development such as HIV, metalloprotease, serine proteases, and γ -secretase. Compound **I** was discovered by Merck as a γ -secretase targeting therapy in Alzheimer's disease (AD) in 2005.²¹ The series of this cyclic sulfamide was one of the structural substitute analogues to the acyclic sulfonamide derivatives which were reported in the same year.²² Next, compound **II**, an orally bioavailable Factor Xa (FXa) inhibitor, was developed by the Yamanouchi Pharmaceutical Co. Ltd. (currently known as Astellas Pharma Inc.).^{23,24} FXa, a serine protease, is involved in blood clotting by cleaving prothrombin into active thrombin. Novel 5-membered cyclosulfamide compound **III** as a Norwalk virus

inhibitor was developed via structure–activity relationship (SAR) studies.²⁵ *N*-Cyclopropyl sulfamide compound **IV** having a fused 6-membered ring system is another example of γ -secretase inhibitors studied by Merck for the treatment of AD.²⁶ The Korea Institute of Science and Technology (KIST) has developed carbapenem compounds **V** containing a pendant cyclic sulfamide, which was found to display potent antibacterial activity.²⁷

As discussed above, sulfamides have been shown to provide biological activities against different enzymes and have been developed for various diseases including cancer. However, cyclic sulfamides have not been actively investigated in cancer drug development. With this need, we set our objective in this study to explore the anticancer activity of seven-membered sulfamide compounds. Due to the intrinsic properties of this highly polarized moiety when attached to an organic scaffold, it is an attractive consideration to incorporate the sulfamide moiety for inducing desired physicochemical properties such as enhanced water solubility, better bioavailability, etc., to the druglike compounds. Encouraged by the possibilities offered by this moiety, we synthesized a set of new seven-member cyclic sulfamide compounds and investigated their potential as

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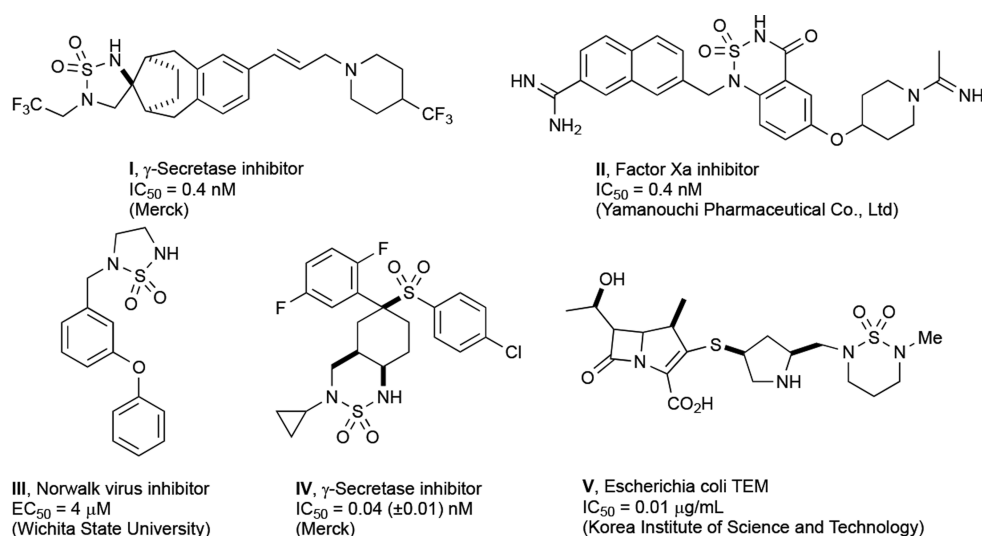
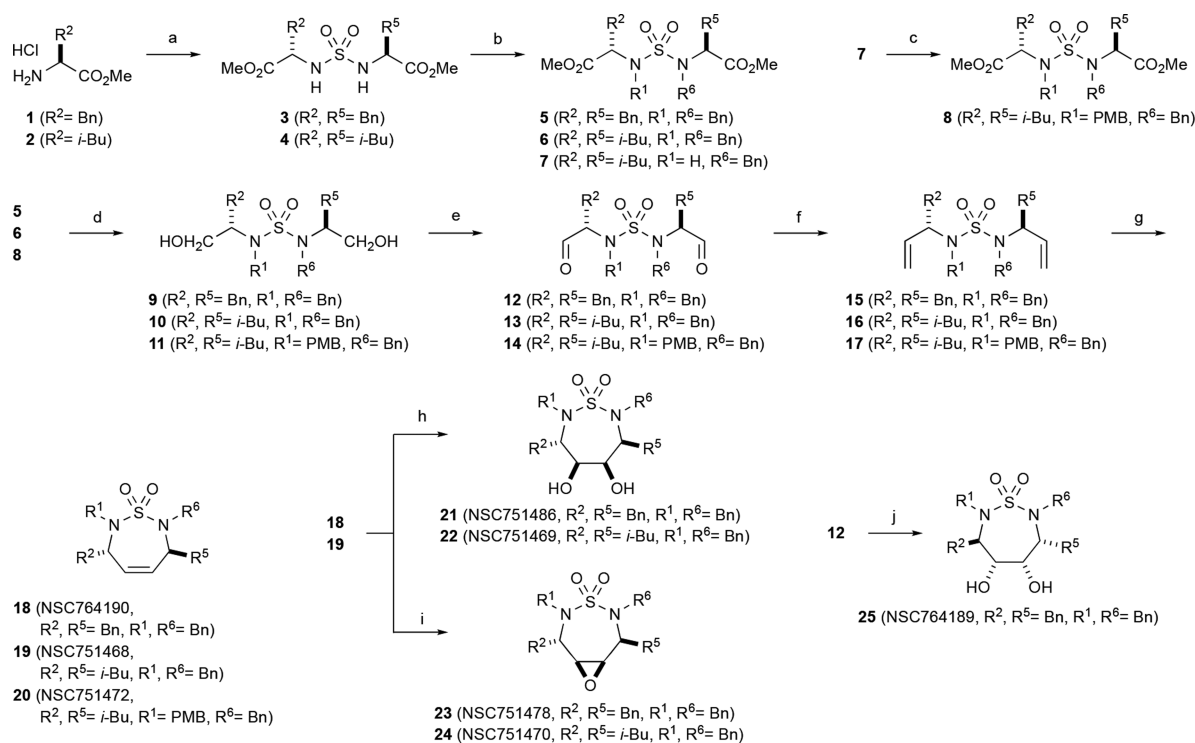


Figure 1. Representative examples of biologically active cyclic sulfamides.

Scheme 1. Synthesis of Cyclic Sulfamide Compounds



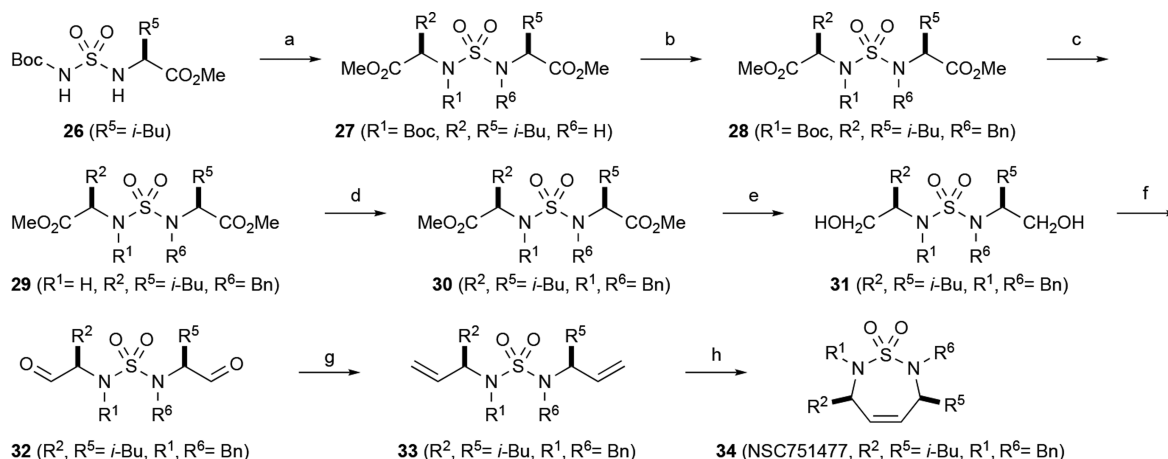
^aSO₂Cl₂, Et₃N, CH₂Cl₂. ^bBenzyl bromide (BnBr) or benzyl chloride (BnCl), K₂CO₃, CH₃CN. ^cPMBCl, K₂CO₃, CH₃CN. ^dLiAlH₄, THF. ^eSwern oxidation or Dess–Martin periodinane. ^fPh₃P⁺CH₃Br[−], *n*-BuLi, THF. ^gGrubbs Cat-II, benzene. ^hOsO₄, NMO, H₂O/acetone. ⁱ*m*-CPBA, CH₂Cl₂. ^jVCl₃(THF)₃, CH₂Cl₂, Zn; PMBCl = *p*-methoxybenzyl chloride, THF = tetrahydrofuran, *m*-CPBA = *m*-chloroperoxybenzoic acid, DIAD = diisopropyl azodicarboxylate, TFA = trifluoroacetic acid, NMO = *N*-methylmorpholine.

anticancer agents. The structural modifications were based on the assumption that the variation of physicochemical properties and thereby different biological activity could be achieved with a change in substituents. Herein we are describing the results of our detailed studies.

We have devoted our efforts to the development of synthetic methods for the sulfur-containing small molecule compounds in various ways.^{28–30} The synthesis of amino ester-derived C₂-symmetric and unsymmetric sulfamides and further synthesis are described in Scheme 1. Amino esters are useful chiral

auxiliary groups that are employed in medicinal chemistry synthesis and have been utilized in a wide range of areas such as peptide synthesis, asymmetric synthesis, and medicinal chemistry. The construction of chiral carbons in different positions of cyclic sulfamide was effectively possible by the employment of amino ester. Condensation of a slight excess of amino ester HCl salt with SO₂Cl₂ in CH₂Cl₂ at 0 °C provided sulfamides **3** and **4** in high yield. For the monobenylation, **1** equiv of benzyl chloride was consumed to afford **7** which was used for next benzylation with *p*-methoxybenzyl chloride to

Scheme 2. Synthesis of C2-Symmetric Cyclic Sulfamide 34



^aMethyl (S)-2-hydroxy-4-methylpentanoate, PPh₃, DIAD, THF. ^bBnCl or BnBr, K₂CO₃, CH₃CN. ^cTFA, CH₂Cl₂. ^dBnCl or BnBr, K₂CO₃, CH₃CN. ^eLiAlH₄, THF. ^fOxalyl chloride, DMSO, Et₃N, CH₂Cl₂, -78 °C. ^gPh₃P⁺CH₃Br⁻, *n*-BuLi, THF. ^hGrubbs Catalyst-II, benzene, reflux.

furnish unsymmetric sulfamide **8**. Benzoylation using benzyl bromide (BnBr) or benzyl chloride (BnCl) and reduction by the addition of lithium aluminum hydride (LAH) carefully into a reaction mixture at 0 °C were the straightforward method to generate primary alcohol intermediates **9–11**. When Dess–Martin periodinane or Swern oxidation reagent is added into analogues **9–11**, two hydroxyl groups were transformed to the aldehydes to give intermediates **12–14**. Under the Wittig reaction condition,^{31,32} the reaction of **5–7** using *n*-butyl lithium and Ph₃P⁺CH₃Br⁻ afforded analogues **18–20**, respectively. For the construction of seven-membered ring **18–20** from dienes **15–17**, we explored ring closing metathesis (RCM) utilizing Grubbs catalyst II (3–6 mol %) in heating benzene. The highlight of our synthetic approach represent the innovative way to construct cyclic sulfamide compounds.

Next, we performed dihydroxylation and epoxidation from **18** and **19** which gave dihydroxyl compound **21** and **22** and epoxy sulfamide **23** and **24**. Reaction of the aldehyde **12** employing Pinacol coupling with a vanadium(II) reagent, [V₂Cl₃(THF)₆]¹⁹₂[Zn₂Cl₆]₃,³³ produced a single diastereomer **25**. The nuclear Overhauser effect (NOE) study allowed us to determine the stereochemistry of each chiral center around a seven-membered ring as shown in Scheme 1.

Synthesis of seven-membered cyclic sulfamide compound **34** was conducted from the reaction of chlorosulfonyl isocyanate (CSI), L-valine methyl ester, and *t*-butanol to generate Boc-group protected sulfamide intermediate **26** in good yields. Sulfamide **27** was then successfully obtained by the reaction of methyl (S)-2-hydroxy-4-methylpentanoate and sulfamide **26** under the Mitsunobu reaction condition.^{34,35} The Mitsunobu reaction is a well-known method to invert the configuration of alcohols. Benzoylation on the sulfamide nitrogen gave sulfamide **28** in 77% yield, followed by the deprotection of the Boc group using TFA at room temperature to furnish intermediate **29** in high yield. It was washed with saturated aqueous NaHCO₃ solution to remove TFA salt and further benzoylation constructed *meso* sulfamide **30**. Two methyl ester groups were transformed into primary hydroxyl groups by the LAH reduction to furnish compound **31**. This diol compound was converted to the unstable dialdehyde compound **32** through the Swern oxidation at low temperature (−78 °C). Wittig

reaction of **32** generated terminal olefin compound **33** which was converted to cyclized *meso* sulfamide **34** by RCM using the second-generation Grubbs catalyst in refluxing benzene (see Scheme 2). Other compounds shown in this paper were synthesized as in the cited methods in the Supporting Information. Compounds **18–46** including the Cancer Chemotherapy National Service Center (NSC) numbers are illustrated in Table 1.

The standard cell culture protocol of the National Cancer Institute panel of 60 human tumor cell lines (NCI-60) and for drug sensitivity analysis in these cell lines has been described previously in the hereby cited literature.^{36–38} All cultured cells were incubated in a humidified incubator with 5% CO₂ in the air at 37 °C. To confirm the absence of contamination, the cell lines were tested for *Mycoplasma* periodically. The NCI-60 cancer cell lines were maintained in RPMI 1640 medium with 5% fetal bovine serum (FBS) and 2 mM L-glutamine (Lonza, Walkersville, MD). Cells were distributed into 96-well microtiter plates at a proper density and maintained for 1 day with no drug. Some of the plates were then administered to decide the cell density at time zero. Concisely, the panel is systematized into nine subpanels on behalf of diverse histologies: leukemia, melanoma, and cancers of lung, colon, kidney, ovary, breast, prostate, and central nervous system. All test compounds prepared in DMSO were added to cultivated cells at five concentrations with 10-fold dilutions, the highest being 10^{−4} M and the others being 10^{−5}, 10^{−6}, 10^{−7}, and 10^{−8} M. Control cells were treated with DMSO alone and incubated under standard culture conditions for 48 h. The percentage growth inhibition was determined relative to cells without drug treatment and the time zero control. After the termination of the assay by addition of cold trichloroacetic acid, and the cells were fixed and stained with the treatment of sulforhodamine B. The bound stain is solubilized, and the absorbance detection of samples can be read in an automated microplate reader. 50% growth inhibition (GI₅₀, cytostatic parameter) is the concentration for 50% max inhibition of cell proliferation, and it was calculated from time zero, control growth, and the five-concentration level absorbance. Inhibitory concentrations (LC₅₀, cytotoxic parameter) are the concentration of drug or chemical that kills 50% of cells with a one-time exposure and represent the average of two independent experiments. Cell

Table 1. Compounds Submitted to the NCI-60 Cell Lines

| Compd No. (NSC No.) | Structure | Compd No. (NSC No.) | Structure | Compd No. (NSC No.) | Structure |
|------------------------|-----------|------------------------|-----------|------------------------|-----------|
| 18 764190 | | 19 751468 | | 20 751472 | |
| 21 751486 | | 22 751469 | | 23 751478 | |
| 24 751470 | | 25 764189 | | 34 751477 | |
| 35 751473 | | 36 751479 | | 37 751483 | |
| 38 767525 | | 39 767526 | | 40 767527 | |
| 41 767529 | | 42 767530 | | 43 767528 | |
| 44 767531 | | 45 767532 | | 46 751467 | |

Table 2. Cytostatic (GI_{50} and TGI) and Cytotoxic (LC_{50}) Parameters of Active Compounds

| | | compounds | | | |
|---------------|--------|--------------------|--------------------|--------------------|--------------------|
| | | 18 (NSC 764190) | 21 (NSC 751486) | 23 (NSC 751478) | 25 (NSC 764189) |
| log GI_{50} | median | -4.94 | -5.49 | -5.31 | -5.30 |
| | delta | 1.63 | 0.58 | 1.25 | 0.00 |
| | range | 2.57 | 0.85 | 1.44 | 0.00 |
| log TGI | median | -4.00 | -5.10 | -5.12 | -5.30 |
| | delta | 0.22 | 0.22 | 0.00 | 0.00 |
| | range | 0.22 | 0.62 | 0.00 | 0.00 |
| log LC_{50} | median | -4.00 | -4.80 | -5.12 | -5.30 |
| | delta | 0.00 | 0.18 | 0.00 | 0.00 |
| | range | 0.00 | 0.28 | 0.00 | 0.00 |

lines with a horizontal bar pointing to the left have a GI_{50} that is greater than the mean and are more resistant than the mean, whereas cell lines with a horizontal bar pointing to the right have a GI_{50} that is less than the mean and are more sensitive. The in vitro NCI-60 cell screening is a two-stage process started with the evaluation of the submitted compound at a single high dose (one-dose screen, 10–5 μM) in the full cell panel. Compounds showing compelling growth inhibition in the one-dose screen are evaluated for five-dose screening (five concentration levels). Only the compounds exhibiting more than 60% of growth inhibition in at least 8 tumor cell lines were selected for further testing, and the others were assumed inactive. All 22 compounds were initially evaluated at a single dose of 10 μM . Only the compounds exhibiting more than 60% of growth inhibition in at least 8 tumor cell lines were selected for further testing, and the others were assumed

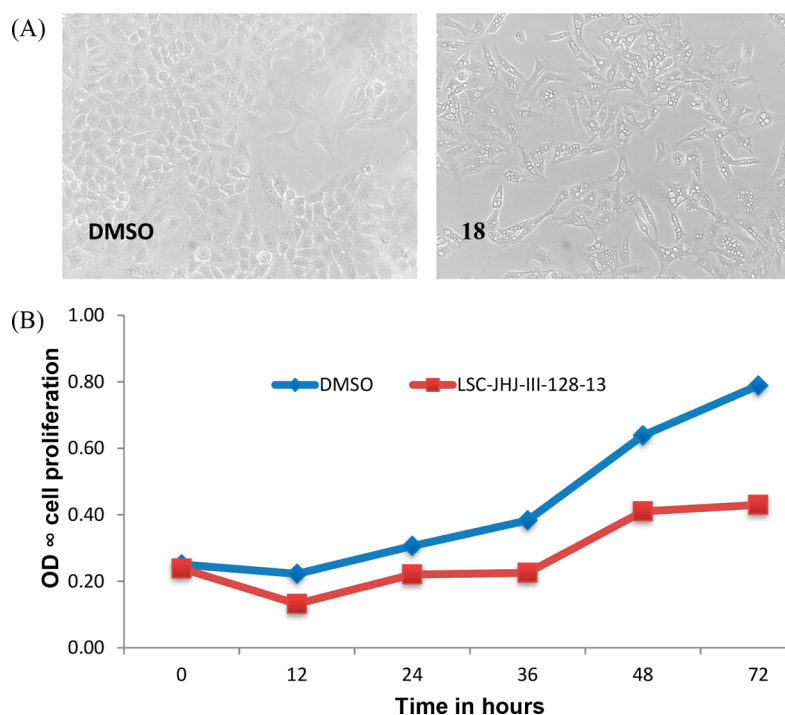


Figure 2. (A) A549 cells were treated with compound **18** (same as LSC-JHJ-III-128-13) at μM or DMSO. On exposure of A549 cells to **18**, the change in morphology of cells was observed. (B) The A549 cell was treated with compound **18** at $10\ \mu\text{M}$ or DMSO. On exposure of A549 cells to compound, 50% growth inhibition is observed after 36 h.

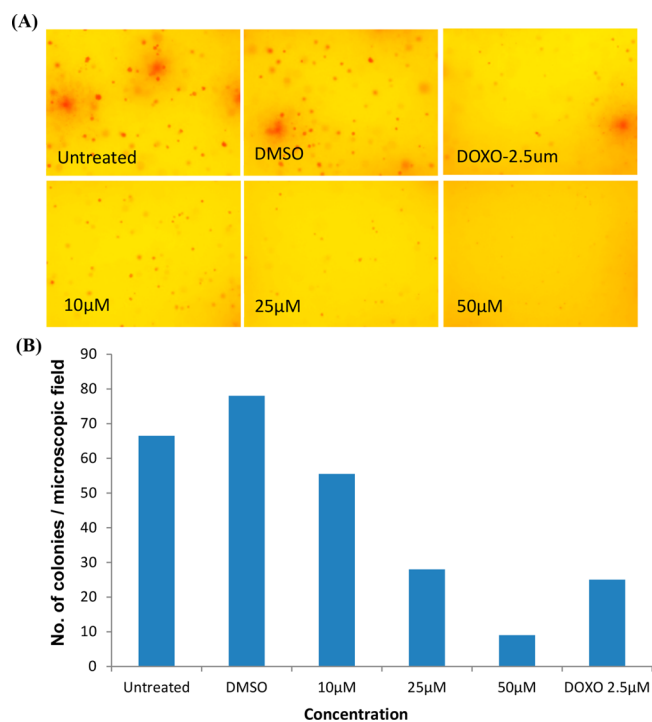


Figure 3. Effect of compound **18** on the anchorage-independent growth of A549 cells. In clonogenic assays, cells were allowed to grow in soft agar containing **18** (0–50 μM) for 9 days to form colonies. (A) Images of the colony-forming assay and (B) number of colonies stained with crystal violet.

inactive. All 22 compounds were initially evaluated at a single-dose screening. Four of our compounds showed significant antiproliferative activity at $10\ \mu\text{M}$. The results of the single-dose screen are given in the Supporting Information.

Subsequently, the antiproliferative activity of the most active four compounds was determined at different concentrations of 10-fold scale such as 100, 10, 1.0, 0.1, and 0.01 μM . The mean GI_{50} and LC_{50} values against a 60 cell line panel are summarized in Table S1 (please refer to the Supporting Information).

These compounds showed varied patterns against all cell lines. Compounds **25** showed nearly the same value for GI_{50} , total growth inhibition (TGI), and LC_{50} suggesting potential toxicity and, therefore, should not be considered for further investigation. Compounds **21** and **23** showed a small differential range between cytostatic markers (GI_{50} , TGI) and the cytotoxicity marker (LC_{50}) suggesting a narrow therapeutic index. However, compound **18** displayed a very interesting selective cytotoxicity pattern against several cell lines. It was particularly potent at a nanomolar concentration against RPMI-8226 (leukemia), HCT-116 (colon cancer), SF-295 (CNS cancer), OVCAR-4 (ovarian cancer), PC-3 (prostate cancer), and MDA-MB-468 (breast cancer) cancer cell lines. Also, comparing the cytostatic data (GI_{50} , TGI) and cytotoxic data (LC_{50}), it is evident that compound **18** showed a remarkable difference between cytostatic indicators (GI_{50} , TGI) and the cytotoxic indicator (LC_{50}) suggesting a broad therapeutic index. In an earlier study,¹⁹ sulfonamide had shown the antiproliferative properties against A549 nonsmall cell lung cancer (NSCLS) cells in vitro. Therefore, to understand the mode of action, we further investigated the mechanism using A549. The five-dose graph and dose–response curves of **18** for the individual disease wise subpanel of NCI-60 cells are presented in Figure S1 (please refer to the Supporting Information).

The $\log\ \text{GI}_{50}$, TGI (cytostatic parameter), and LC_{50} (cytotoxic parameter) for the active compounds **18**, **21**, **23** and **25** against the 60 cell lines are summarized in Table 2,

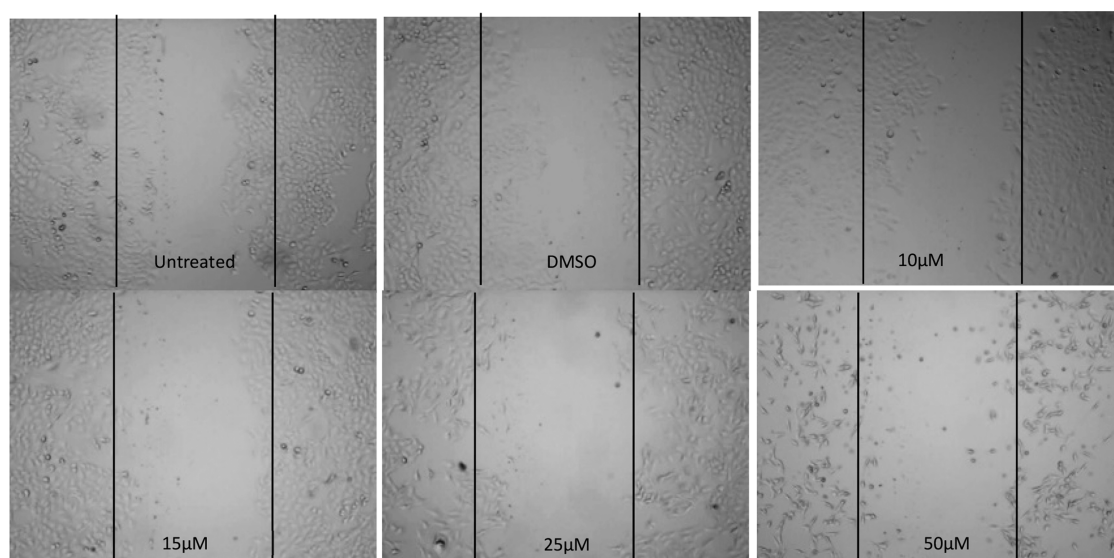


Figure 4. Effect of compound **18** (NSC 764190) on migration of the A549 cell. The representative images of the in vitro scratch assay are shown here. After creating scratches of A549 cell monolayers, cells growing in media containing the test compound (0–50 μM) or DMSO vehicle were analyzed with a phase-contrast microscope, and images are presented. Compound **18** inhibited migration of A549 cells.

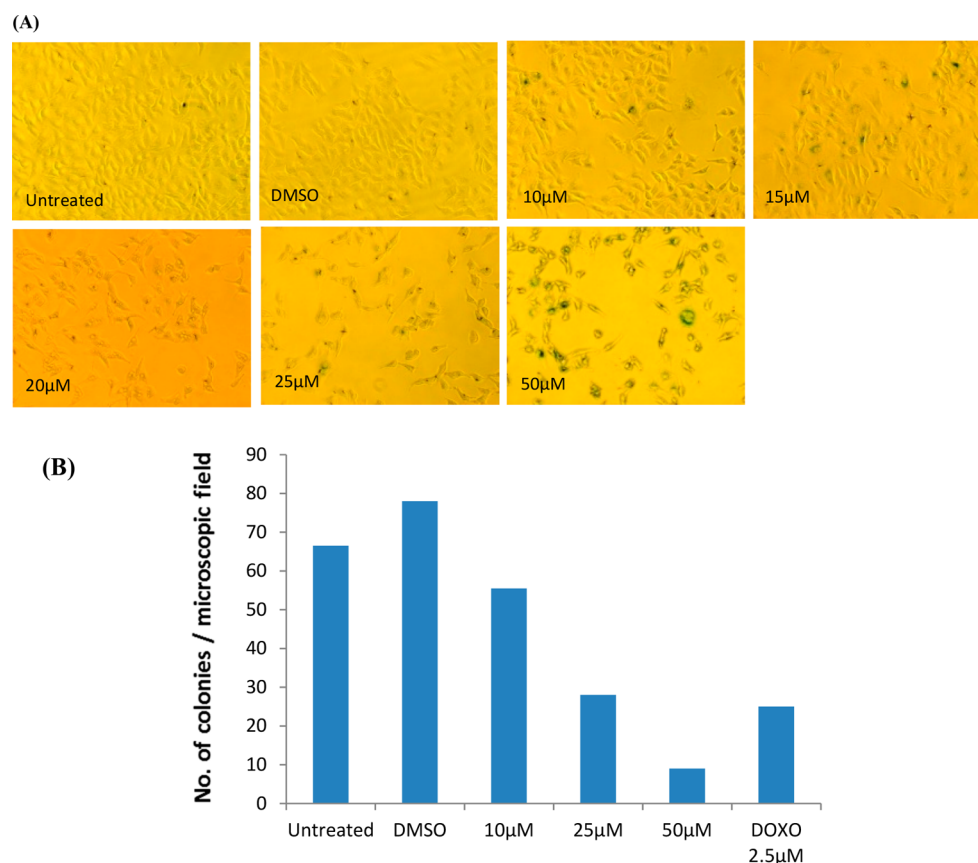


Figure 5. Senescence induced by compound **18** was quantified using SA- β -gal-staining. SA- β -gal positive cells are indicated with the arrow mark. The number of SA- β -gal positive cells increased with increasing concentration of compound indicating that compound **18** induces senescence of A549 cells.

along with the log median values (median value of multiple experiments), log delta values (the maximum sensitivity over the mean), and the log range values (the maximum difference between the least sensitive and the most sensitive cell lines).^{39,40} These parameters offer important information about potency and selectivity of anticancer agents. Large

values of the delta and range imply high selectivity for some histological cancers over others.⁴¹

Inhibitory effects of **18** on the proliferation of A549 lung epithelial cancer cells were evaluated by treating the overnight grown cells with test compound (1 μM). As shown in Figure 2A, upon treatment with test compound, A549 cells lost

morphology and appeared as dead cells with no further proliferation. In a time-dependent experiment with A549 cells, 50% growth inhibition was observed with **18** after 36 h (Figure 2B).

As we observed the antiproliferative activity of **18** against A549 cells and the effect of compound **18** on anchorage-independent growth A549 cells, a soft agar-based colony-forming assay was performed. The colonies stained in soft agar plates have clearly confirmed that the compound **18** inhibits the formation of colonies by cancer cells. In these assays, a dose-dependent inhibition on clonogenic growth by A549 cells by **18** indicates the efficacy of **18** toward inhibition of A549 cell growth (Figure 3A,B).

Toward identifying NCEs with novel anticancer potential, understanding their effects on migration properties of cancer cells is also important. In vitro scratch or wound healing assays are the suitable assay to evaluate the effect of test compounds on cell migration for epithelial or mesenchymal cells. As shown in Figure 4, the results confirm that compound **18** (NSC764190) inhibited migration of cells by 50% approximately even at the lowest tested concentration whereas, in DMSO plates, cells showed migration the same as the control untreated assays (Figure 4, upper left panel).

As we observed the effect of **18** on proliferation and migration of A549 cells, we next determined whether **18** induces apoptosis and/or inhibits cell proliferation. Hence, we determined cell death and cell cycle progression using flow cytometry. After postexposure of A549 cells to **18** for 48 h, propidium iodide (PI) was applied to measure cell cycle progression and cell death.

The flow cytometer results showed an increase in SubG1 population, indicating that compound **18** is inducing cell death (Figure 5).

To gain additional insight into the mechanisms by which **18** treatment induces cell death and inhibits proliferation, we have investigated caspase activation and senescence in A549 cells treated with the compound. A significant increase in the caspase activity could not be observed in compound treated cells compared to the control (data not shown). Therefore, we assessed the endogenous level of β -galactosidase as an indicator for senescence in A549 cells upon treatment with **18**. Interestingly, we could observe more cells with an increased level of SA- β -gal in cells upon treatment with the compound, and the number of cells positive for SA- β -gal also increased with increasing concentration. These results support the hypothesis that the exposure of cells to compound **18** leads to the induction of cellular senescence in A549 cells for cell cycle arrest.

In conclusion, a set of seven-member cyclic sulfamide has been synthesized and tested for potential anticancer activity against nine different cancer panels. Generally, compounds showed selectively sensitivity against a host of cell lines. Almost every compound is strongly inhibited on breast cancer (MDA-MB-468). A mechanism study with a representative compound **18** (NSC764190) using A549 (nonsmall cell lung cancer) cells suggests inhibition caused by apoptosis.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmchemlett.0c00460>.

Characterization data for the synthesized compounds, copies of their NMR spectra, and biochemical assays protocol (PDF)

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Notes

The authors declare no competing financial interest.

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