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Synthesis and Biological Evaluation of Novel Sulfonanilide Compounds as Antiproliferative Agents for Breast Cancer

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Abstract

Combinatorial chemistry approaches facilitate drug discovery processes and result in structural modifications of lead compounds that enhance pharmacological activity, improve pharmacokinetic properties, and/or reduce unwanted side effects. Epidemiological and animal model studies have suggested that nonsteroidal anti-inflammatory drugs (NSAIDs) can act as chemopreventive agents. The cyclooxygenase-2 (COX-2) inhibitor nimesulide shows anticancer effect in several cancer cell lines via COX-2 dependent and independent mechanisms. The molecular structure of nimesulide was used as a starting scaffold to design novel sulfonanilide analogs and examine the structural features that contribute to this anticancer effect. A systematic combinatorial chemical approach was used to generate diversely substituted sulfonanilide derivatives that were tested for their effects on the proliferation of human breast cancer cells. Structure–function analysis indicated that the inhibition of cell growth by compounds derived from the novel sulfonanilides required a bulky terminal phenyl ring, a methanesulfonamide, and a hydrophobic carboxamide moiety.

Keywords

breast cancer; cyclooxygenase-2; NSAIDs; nimesulide; sulfonanilide

1. Introduction

The area of drug discovery and drug development has experienced significant advances with the introduction of combinatorial chemistry approaches. This innovative technology of producing libraries of structurally related compounds is particularly beneficial in the step of lead optimization. Lead optimization involves structural modifications of a "lead" compound that has demonstrated desired biological or pharmacological activities, often in an *in vitro* assay system. Combinatorial chemistry approaches facilitate structural modifications of a lead scaffold to enhance pharmacological activity, improve pharmacokinetic properties, and/or reduce unwanted side effects.

Recent epidemiological and animal model studies have suggested that nonsteroidal anti-inflammatory drugs (NSAIDs) act as chemopreventive agents.[1–8] The premise that COX-2 inhibition is integral to this anticarcinogenic effect is based on the assumption that COX-2 generated prostaglandins promote tumor growth in an autocrine and/or paracrine manner.[9,10]

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6. Supporting Information Available
HPLC Analysis for compounds 45–76. This information is available free of charge via the Internet at http://pubs.acs.org.

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It is well documented that COX-2 is constitutively overexpressed in many types of human cancers.[9] Animal studies have demonstrated that efficient tumor growth requires the presence of COX-2 in the host and that enhanced COX-2 expression in the host was sufficient to induce mammary gland tumorigenesis.[11] Furthermore, increased COX-2 expression appears to be involved in the development of cancer by promoting cell division, inhibiting apoptosis, altering cell adhesion and enhancing metastasis, and stimulating neovascularization.[12–15] The inhibition of COX-2 activity by traditional NSAIDs blocks these activities and thus may account for the anticarcinogenic activity of these drugs. However, an expanding body of evidence suggests that COX-2-independent mechanism may also be involved in the antitumor effect of COX-2 inhibitors.[4,7,8] Each NSAID type appears to have its own non-specific COX-2 independent target. For example, the COX-2 inhibitor celecoxib induces cell apoptosis in prostate cancer cells by inhibiting 3-phosphoinositide-dependent protein kinase via a COX-2 independent mechanism.[16]

Adenocarcinoma of the breast is the most common cancer in women in the United States and ranks second only to lung cancer as a cause of cancer-related mortality. About 178,500 women in the United States will be found to have invasive breast cancer in 2007. About 40,500 women will die from the disease this year. Currently over 2 million women living in the United States have been treated for breast cancer.[17] A growing body of experimental and epidemiological evidence suggests that the use of NSAIDs may decrease the incidence of mammary cancer, tumor burden, and tumor volume.[18–20] Although this effect has been studied within the past few decades, the mechanism by which these benefits occur is unclear. Nimesulide [N-(4-nitro-2-phenoxyphenyl) methane sulfonamide] is a nonsteroidal anti-inflammatory drug with a preferential cyclooxygenase-2 inhibitory activity and has been available in some Asia and European countries since 1985. In fact, the anti-inflammatory activity of nimesulide is almost the same as that of indomethacin, but its ulcerogenic potential is much weaker. Nimesulide can induce apoptosis in liver and lung cancer cells; it also suppressed the development of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced mammary gland carcinogenesis in rats.[21,22] Aromatase, the key enzyme for estrogen synthesis, is elevated in hormone-dependent breast cancer.[23] Research in our laboratory demonstrated that nimesulide also suppressed aromatase activity and expression in several breast cancer cell lines.[24]

Although nimesulide has been reported as effective in suppressing several types of cancer cell growth, the concentrations used in those studies are much higher than the effective dose for COX-2 inhibition.[21,22] This suggests that COX-2 independent mechanisms may be involved. The functionality at the <u>B</u> position is very critical for the COX-2 inhibitory activity (Figure 1).[27] Only when the N-H is available in the ionized form, do the compounds inhibit COX-2. Introduction of any group in <u>B</u> position eliminates this ionization process and produce compounds with no COX-2 inhibitory activity.[25,27] Previously we tested nimesulide derivatives in SK-BR-3 breast cancer cells at their IC_{50} for aromatase suppression and also COX-2 inhibition in breast cancer cells. The results demonstrate that the N-methyl substituted compounds at position **B** did not exhibit COX-2 inhibitory activity.[25]

Two targeted libraries of nimesulide analogs have been synthesized in our laboratory, and their pharmacological effect on aromatase has been extensively studied.[25,26] In this publication, their biological effect on breast cancer cell growth was investigated in a panel of breast cancer cell lines. Two analogs significantly inhibited SK-BR-3 breast cancer cell growth, which is a HER2/neu overexpressed breast cancer cell line. Based on the two compounds, a combinatorial approach was used to generate diversely substituted nimesulide derivatives by parallel synthesis. Their preliminary evaluation as antiproliferation agents in breast cancer agents was also further explored.

2. Results and discussion

2.1. Compounds design

A total of 76 compounds were prepared, which were based on nimesulide structure as a platform (Figure 1). We systematically altered the structure of nimesulide using the combinatorial strategies to modify the four moieties depicted in Figure 1. The **A** position aromatic ring of nimesulide was modified to either alkyl or substituted aryl groups to generate compounds **1–44** published previously.[25,26] Alkyl or substituted benzyl groups were introduced at the **B** position to produce compounds **45–56**. Next, the **C** position was modified with various substituted benzamides to produce compounds **57–72**. Last, the **D** position nitro group was reduced to the amine moiety to generate compound **73**, and then **73** was treated with substituted acyl chloride and K_2CO_3 to generate the carboxamides **74–76,** respectively. The parallel synthesis can generate structure of novel sulfonanilides at the **A**, **B**, **C** and **D** positions diversity. Further biological investigation of these compounds can reveal the structure requirement for the suppression of breast cancer cell growth. In addition, this combinatory strategy can easily be extended to produce hundreds of new analogs for lead optimization and drug development.

2.2. Parallel synthesis of diverse sulfonanilide derivatives

In the current study, the **A** moiety of these two compounds was retained and the **B**, **C**, or **D** position was modified to generate several small libraries. The **A** position modification has been extensively explored previously.[25,26] 2, 5-Dimethylbenzyl and 4-isopropyl benzyl substituted **A** position lead to significantly more active compounds.

Modifications at the **B** position are described in Scheme 1. The starting material 2-amino-5-nitrophenol was refluxed with K_2CO_3 and 2, 5-dimethylbenzyl chloride or 4-isopropyl benzyl chloride to obtain compounds **1a**, **2a,** respectively. Sodium hydride and methanesulfonyl chloride were added to compound **1a** or **2a** in dry dimethylformamide (DMF) at room temperature and the reaction mixture was stirred at room temperature overnight to obtain the N,N-bimethanesulfonamido compound (**1b** or **2b**). Compounds **1b** and **2b** are hydrolyzed with 10% NaOH solution to generate compounds **29** and **43** as monomethanesulfonamido compounds. Compound **29** or **43** were treated with K_2CO_3 and substituted benzyl chloride/bromide, alkyl bromide or iodide in DMF at room temperature or at reflux to obtain compounds **45–56**, respectively.

Modifications at the **C** position are described in Scheme 2. Compounds **1a** or **2a** was treated with different substituted acyl chloride and K_2CO_3 to generate the carboxamides **57–72**.

Modifications at the **D** position are described in Scheme 3. First, the **D** position nitro was reduced to the amine group to obtain compound **73** (Figure 1), then compound **73** was treated with different acyl chlorides and K_2CO_3 to generate the carboxamides **74–76**, respectively.

This parallel combinatory strategy can produce new analogs for lead optimization and drug development. Structures of all the synthesized compounds were determined by ^{1}H-NMR, their purity confirmed by HPLC with two mobile phases, and the structures of significant biologically active compounds also confirmed by HRMS.

2.3. Pharmacological evaluation of sulfonanilide analogs

In general, various substitutions at the **A** position exhibit suppression of the growth of the SK-BR-3 breast cancer cells. The results suggest that the **A** position bulky group is beneficial for the inhibition of cell growth (Table 1, 2). Compounds **6**, **11**, **12**, **13**, **21**, **23**, **24**,

30, **41**, **42**, **43** and **44** exhibit more than 50% inhibition of SK-BR-3 cell proliferation at 25μM. Particularly, compounds **44** and **30** are more effective than others; showing more than 80% inhibition, with an IC_{50} of 6.5μM and 20.1 μM respectively. Since these two compounds both are methyl substituted **B** position nimesulide derivatives, they are not potential COX-2 inhibitors. This suggests that the cell growth suppression effect of these two analogs should be independent of COX-2 inhibition. Further exploration exhibits that the suppression is time and dose dependent (Figure 2, Table 3). (MCF-7, MDA-MB-231, BT-474). Compounds **44** and **30** also significantly suppressed BT474 (estrogen receptor positive and HER2/neu overexpress) breast cancer cell growth with an IC_{50} of 13.5 μM and 44.7μM, respectively (Table 3). They did not significantly suppress other breast cancer cell growth. These results suggest that compounds **44** and **30** might selectively inhibit cell growth of HER2/neu overexpressing breast cancer cells. Overall, introduction of 2, 5-dimethyl benzyl and 4-isopropyl benzyl groups into the **A** position of nimesulide generated two compounds that significantly suppressed SK-BR-3 breast cancer cell growth.

The **B** position alkyl or aryl substituted analogs overall showed weaker cell growth inhibition comparing with proton or methyl group at **B** position (Table 4). It appears that a smaller group at **B** position, such as proton or methyl, is better for biological activity. Introduction of carboxamides group to the **C** position decreases the cell growth suppression activity (Table 5). However, compound **63**, which has 4-chloro-3-nitro benzamide group at the **C** position, showed significant cell growth inhibition with more than 80% of cell growth at 25 μM. Reduction of the **D** position nitro group to amine decreases the biological activity, with only 63% inhibition compared with compound **44** with 90% inhibition at 25 μM (Table 6). Introduction of acetyl to the **D** position amine leads to much less active compound **74,** with 14.6% cell growth inhibition at 25 μM. However, introduction of benzoyl or cyclohexanacarbonyl group to **D** amine moiety generates more active compounds **75** and **76**, with 73.2% and 75.4% cell growth inhibition activity at 25 μM, respectively. This suggests that a hydrophobic moiety at the **D** position is better for cell growth inhibition.

3. Conclusion

An efficient method has been developed for the parallel synthesis of diversified novel sulfonanilides via a combinatorial chemistry approach. This parallel is highly efficient and suitable for the synthesis of large libraries of analogs. Through biological activity evaluation of the compound library, we have identified novel compounds **30**, **44**, **63**, **75** and **76** which exhibited potent inhibition against SK-BR-3 breast cancer cell growth at sub-micromolar level. Structure–function analysis indicated that the inhibition of breast cancer cell growth by the synthetic compounds require a hydrophobic group substituted bulky phenyl ring, a methanesulfonamide and a hydrophobic carboxamide moiety (Figure 3). Further research on the mechanisms of these compounds in suppression of SK-BR-3 breast cancer cell growth is ongoing in our laboratory.

4. Experimental section

4.1. Chemistry

Chemicals were commercially available and used as received without further purification unless otherwise noted. Moisture sensitive reactions were carried out under a dry argon atmosphere in flame-dried glassware. Solvents were distilled before use under argon. Thin-layer chromatography was performed on precoated silica gel F254 plates (Whatman). Silica gel column chromatography was performed using silica gel 60A (Merck, 230–400 Mesh). High-resolution electrospray ionization mass spectra were obtained on the Micromass QTOF Electrospray mass spectrometer at The Ohio State Chemical Instrumentation Center. All the NMR spectra were recorded on a Bruker DPX 250 and DRX 400 MHz in either

DMSO-d_6 or $CDCl_3$. Chemical shifts (δ) for ^{1}H NMR spectra are reported in parts per million calibrated to residual solvent protons.

For the HPLC analysis, a 1.00 mg/mL stock solution of each standard was prepared in either methanol or acetonitrile. HPLC analysis was performed on a HP1100 system (Hewlett-Packard, Palo Alto, CA), which consists of a binary pump, autosampler, column compartment, and a UV/VIS detector. Reversed phase HPLC was carried out on a C18 column (4.6 × 250 mm, 5μm) from Beckman (Beckman Instruments, Fullenton, CA) at room temperature with a flow rate of 1.0 mL/min. Two mobile phases (mobile phase A: 35%water, 65% acetonitrile; mobile phase B: 25%water, 75% methanol) were employed to run 35 min. An injection volume of 5–15μL was used. The UV detector was set up at 254 and 330nm.

Compounds **1a**, **2a**, **1b**, **2b**, **29**, **30**, **43**, **44** were prepared as described by Su *et al.*[26]

General procedure for the preparation of B position substituted nimesulide analogs compounds 45–56 from compounds 29 and 43—K_2CO_3 (5 mmol, 5*eq*) and aryl or alkyl halide (1 mmol, 1.2 *eq*) were successively added to a solution of compound **29** or **43** (1.0 mmol, 1.0 *eq*) in dry DMF and the mixture was stirred at room temperature or 80 °C from 3 h to overnight. After being cooled, 5 mL H_2O and 1 mL saturated aqueous Na_2CO_3 was added to the mixture and it was stirred at room temperature overnight. The precipitated solid was collected by filtration and washed with H_2O and cold hexane to afford desired compounds.

(45) N-(2,5-Dimethyl benzyl)-N-[2-(2,5-dimethyl benzyloxy)-4-nitro phenyl]-methanesulfonamide: Compound **43** and 2, 5-dimethyl benzyl chloride were stirred in DMF at 80 °C overnight. Pale yellow solid, yield 81%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.00 (1H, d, *J* = 2.4 Hz), 7.77 (1H, dd, *J* = 8.7, 2.4 Hz), 7.49 (1H, d, *J* = 8.6 Hz), 7.34 (1H, s), 7.19 (1H, d, *J* = 7.7 Hz), 7.13 (1H, d, *J* = 7.6 Hz), 6.96 (3H, m), 5.27 (2H, s), 4.75 (2H, s), 2.97 (3H, s), 2.37 (3H, s), 2.30 (3H, s), 2.13 (6H, s).

(46) N-[2-(2,5-Dimethyl benzyloxy)-4-nitro phenyl]-N-naphthalen-2-ylmethyl-methanesulfonamide: Compound **43** and 2-(bromomethyl) naphthalene were stirred at room temperature overnight. Yellow solid, yield 84%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.99 (1H, d, *J* = 2.5 Hz), 7.87 (2H, m), 7.77 (2H, m), 7.66 (1H, s), 7.49 (4H, m), 7.35 (1H, s), 7.21 (1H, d, *J* = 7.7 Hz), 7.15 (1H, d, *J* = 7.9 Hz), 5.29 (2H, s), 4.95 (2H, s), 3.06 (3H, s), 2.38 (3H, s), 2.31 (3H, s).

(47) N-Benzyl-N-[2-(2, 5-dimethyl benzyloxy)-4-nitro phenyl]-methanesulfonamide: Compound **43** and benzyl bromide were stirred at room temperature overnight. Yellow solid, yield 75%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.00 (1H, d, *J* = 2.4 Hz), 7.76 (1H, dd, *J* = 2.4, 8.6 Hz), 7.43 (1H, d, *J* = 8.6 Hz), 7.32 (1H, s), 7.25 (7H, m), 5.28 (2H, s), 4.77 (2H, s), 3.01 (3H, s), 2.36 (3H, s), 2.31 (3H, s).

(48) N-(4-Bromo benzyl)-N-[2-(2,5-dimethyl benzyloxy)-4-nitro phenyl]-methanesulfonamide: Compound **43** and 4- bromobenzyl bromide were stirred at room temperature overnight. Pale yellow solid, yield 73%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.00 (1H, d, *J* = 2.4 Hz), 7.79 (1H, dd, *J* = 2.4, 8.6 Hz), 7.45 (3H, m), 7.30 (1H, s), 7.18 (4H, m), 5.27 (2H, s), 4.74 (2H, s), 3.02 (3H, s), 2.35 (3H, s), 2.30 (3H, s).

(49) N-[2-(2,5-Dimethyl benzyloxy)-4-nitro phenyl]-N-hexyl-methanesulfonamide: Compound **43** and 1-iodohexane were stirred at 80 °C overnight. Yellow solid, yield 60%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.06 (1H, d, *J* = 2.5 Hz), 7.90 (1H, dd, *J* = 2.5, 8.6

Hz), 7.60 (1H, d, *J* = 8.6 Hz), 7.27 (1H, s), 7.16 (1H, d, *J* = 7.7 Hz), 7.10 (1H, d, *J* = 801 Hz), 5.27 (2H, s), 3.54 (2H, dd, *J* = 6.9, 6.9 Hz), 2.90 (3H, s), 2.32 (3H, s), 2.27 (3H, s), 1.32 (9H, m), 0.82 (3H, dd, *J* = 6.7, 6.7 Hz).

(50) N-[2-(2,5-Dimethyl benzyloxy)-4-nitro phenyl]-N-(4-methoxy benzyl)-methanesulfonamide: Compound **43** and 4-methoxy benzyl chloride were stirred at 80 °C overnight. Yellow solid, yield 96%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.99 (1H, d, *J* = 2.4 Hz), 7.76 (1H, dd, *J* = 2.4, 8.6 Hz), 7.38 (1H, d, *J* = 8.8 Hz), 7.31 (1H, s), 7.18 (4H, m), 7.80 (2H, d, *J* = 8.6 Hz), 5.27 (2H, s), 4.69 (2H, s), 3.69 (3H, s), 2.99 (3H, s), 2.35 (3H, s), 2.30 (3H, s).

(51) N-[2-(2,5-Dimethyl benzyloxy)-4-nitro phenyl]-N-(4-methyl benzyl)-methanesulfonamide: Compound **43** and 4-methyl benzyl chloride were stirred at 80 °C overnight. Pale yellow solid, yield 87%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.99 (1H, d, *J* = 2.5 Hz), 7.76 (1H, dd, *J* = 2.5, 8.6 Hz), 7.40 (1H, d, *J* = 8.6 Hz), 7.31 (1H, s), 7.19 (6H, m), 5.27 (2H, s), 4.72 (2H, s), 2.99 (3H, s), 2.35 (3H, s), 2.30 (3H, s), 2.22 (3H, s).

(52) N-[2-(2, 5-Dimethyl benzyloxy)-4-nitro phenyl]-N-(4-fluoro benzyl)-methanesulfonamide: Compound **43** and 4-fluoro benzyl chloride were stirred at 80 °C overnight. Pale yellow solid, yield 89%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.00 (1H, d, *J* = 2.4 Hz), 7.78 (1H, dd, *J* = 2.5, 8.6 Hz), 7.42 (1H, d, *J* = 8.6 Hz), 7.30 (1H, s), 7.26(2H, dd, *J* = 5.6, 8.3 Hz), 7.18(1H, d, *J* = 7.7 Hz), 7.13(1H, d, *J* = 7.6 Hz), 7.08 (2H, dd, *J* = 8.8, 8.8 Hz), 5.27 (2H, s), 4.75 (2H, s), 3.01 (3H, s), 2.35 (3H, s), 2.30 (3H, s).

(53) N-(2,5-Dimethyl benzyl)-N-[2-(4-isopropyl benzyloxy)-4-nitro phenyl]-methanesulfonamide: Compound **29** and 2,5-dimethyl benzyl chloride were stirred at 80 °C overnight. Pale yellow solid, yield 88%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.95 (1H, d, *J* = 1.7 Hz), 7.73 (1H, dd, *J* = 1.8, 8.6 Hz), 7.50 (2H, d, *J* = 8.0 Hz), 7.44 (1H, d, *J* = 8.7 Hz), 7.34 (2H, d, *J* = 7.9 Hz), 6.94 (3H, m), 5.32 (2H, s), 4.78 (2H, s), 3.04 (3H, s), 2.94 (1H, m), 2.15 (3H, s), 2.11 (3H, s), 1.23 (3H, s), 1.21 (3H, s).

(54) N-[2-(4-Isopropyl benzyloxy)-4-nitro phenyl]-N-naphthalen-2-ylmethyl-methanesulfonamide: Compound **29** and 2-(bromomethyl) naphthalene were stirred at 80 °C overnight. Pale yellow solid, yield 94%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.95 (1H, d, *J* = 1.5 Hz), 7.87 (2H, m), 7.77 (1H, m), 7.71 (2H, m), 7.48 (6H, m), 7.32 (2H, d, *J* = 7.7 Hz), 5.34 (2H, s), 4.97 (2H, s), 3.12 (3H, s), 2.95 (1H, m), 1.24 (3H, s), 1.22 (3H, s).

(55) N-Benzyl-N-[2-(4-isopropyl benzyloxy)-4-nitro phenyl]-methanesulfonamide: Compound **29** and benzyl bromide were stirred at room temperature overnight. White solid, yield 85%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.97 (1H, d, *J* = 2.5 Hz), 7.74 (1H, dd, *J* = 2.5, 8.6 Hz), 7.49 (2H, d, *J* = 8.1 Hz), 7.39 (1H, d, *J* = 8.6 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 7.27 (5H, m), 5.32 (2H, s), 4.79 (2H, s), 3.06 (3H, s), 2.95 (1H, m), 1.24 (3H, s), 1.22 (3H, s).

(56) N-(4-Bromo benzyl)-N-[2-(4-isopropyl benzyloxy)-4-nitro phenyl]-methanesulfonamide: Compound **29** and 4-bromobenzyl bromide were stirred at room temperature overnight. White solid, yield 97%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.97 (1H, d, *J* = 2.5 Hz), 7.77 (1H, dd, *J* = 2.5, 8.6 Hz), 7.46 (5H, m), 7.34 (2H, d, *J* = 8.1 Hz), 7.20 (2H, d, *J* = 8.4 Hz), 5.31 (2H, s), 4.77 (2H, s), 3.07 (3H, s), 2.94 (1H, m), 1.24 (3H, s), 1.22 (3H, s).

General procedure for the preparation of C position substituted nimesulide analogs compounds 57–72 from compounds 1a and 2a—K_2CO_3 (5 mmol, 5*eq*) and substituted acyl chloride (1.2 mmol, 1.2 *eq*) were successively added to a solution of compound **1a** or **2a** (1.0 mmol, 1.0 *eq*) in dry 1,4 dioxane and the mixture was stirred at room temperature or 80 °C from 3 h to overnight. After being cooled, 10 mL H_2O and 3 mL saturated aqueous Na_2CO_3 was added to the mixture and it was stirred at room temperature overnight. The precipitated solid was collected by filtration and washed with H_2O and cold ethyl ether/hexane to afford desired compounds.

(57) N-[2-(2, 5-Dimethyl benzyloxy)-4-nitro phenyl]-acetamide: Compound **2a** and acetyl chloride were stirred at room temperature overnight. White solid, yield 94%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.54(1H, s), 8.31 (1H, dd, *J* = 1.7, 8.8 Hz), 7.92 (2H, m), 7.30 (1H, s), 7.15 (1H, d, *J* = 7.3 Hz), 7.09 (1H, d, *J* = 7.6 Hz), 5.30 (2H, s), 2.32 (3H, s), 2.27 (3H, s), 2.16 (3H, s).

(58) N-[2-(2,5-Dimethyl benzyloxy)-4-nitro phenyl]-benzamide: Compound **2a** and benzoyl chloride were stirred at room temperature overnight. White solid, yield 93%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.79(1H, s), 8.23 (1H, d, *J* = 8.8 Hz), 8.07 (1H, d, *J* = 2.4 Hz), 7.99 (1H, dd, *J* = 2.4, 8.8 Hz), 7.95 (2H, d, *J* = 7.3 Hz), 7.64(1H, m), 7.56 (2H, m), 7.38 (1H, s), 7.12 (1H, d, *J* = 7.6 Hz), 7.06 (1H, d, *J* = 7.6 Hz), 5.33 (2H, s), 2.30 (3H, s), 2.22 (3H, s).

(59) Cyclohexanecarboxylic acid [2-(2,5-dimethyl-benzyloxy)-4-nitro phenyl]-amide: Compound **2a** and cyclohexanecarbonyl chloride were stirred at room temperature overnight. Pale yellow solid, yield 93%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.36(1H, s), 8.25 (1H, d, *J* = 9.0 Hz), 7.95 (1H, d, *J* = 2.3 Hz), 7.91 (1H, dd, *J* = 2.4, 8.9 Hz), 7.34 (1H, s), 7.14 (1H, d, *J* = 7.7 Hz), 7.08 (1H, d, *J* = 7.5 Hz), 5.31 (2H, s), 2.60 (1H, m), 2.31 (3H, s), 2.28 (3H, s), 1.83(5H, m), 1.41 (5H, m).

(60) 3,4-Dichloro-N-[2-(2,5-dimethyl benzyloxy)-4-nitro phenyl]-benzamide: Compound **2a** and 3, 4 dichlorobenzoyl chloride were stirred at room temperature overnight. White solid, yield 94%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.12(1H, s), 8.15 (1H, d, *J* = 1.9 Hz), 8.07 (3H, m), 7.97 (1H, dd, *J* = 8.8, 2.4 Hz), 7.92 (1H, dd, *J* = 2.0, 8.4 Hz), 7.84 (1H, d, *J* = 8.4 Hz), 7.32 (1H, s), 7.11 (1H, d, *J* = 7.7 Hz), 7.04 (1H, d, *J* = 7.7 Hz), 5.30 (2H, s), 2.29 (3H, s), 2.20 (3H, s).

(61) Naphthalene-2-carboxylic acid [2-(2,5-dimethyl benzyloxy)-4-nitro phenyl] amide: Compound **2a** and 2-naphthoyl chloride were stirred at 80 °C for three days. Pale yellow solid, yield 39%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.98(1H, s), 8.55 (1H, s), 8.27 (1H, d, *J* = 8.8 Hz), 8.11 (6H, m), 7.68 (2H, m), 7.40 (1H, s), 7.13 (1H, d, *J* = 7.6 Hz), 7.06 (1H, d, *J* = 7.7 Hz), 5.36 (2H, s), 2.33 (3H, s), 2.18 (3H, s).

(62) Biphenyl-4-carboxylic acid [2-(2,5-dimethyl benzyloxy)-4-nitro phenyl] amide: Compound **2a** and biphenyl-4-carbonyl chloride were stirred at 80 °C for three days. Pale yellow solid, yield 39%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.86(1H, s), 8.24 (1H, d, *J* = 8.8 Hz), 8.07 (3H, m), 8.00 (1H, dd, *J* = 2.4, 8.8 Hz), 7.85 (2H, d, *J* = 8.3 Hz), 7.77 (2H, d, *J* = 7.9 Hz), 7.54 (4H, m), 7.13 (1H, d, *J* = 7.7 Hz), 7.06 (1H, d, *J* = 7.8 Hz), 5.34 (2H, s), 2.32 (3H, s), 2.23 (3H, s).

(63) 4-Chloro-N-[2-(2,5-dimethyl benzyloxy)-4-nitro phenyl]-3-nitro benzamide: Compound **2a** and 4-chloro-3-nitrobenzoyl chloride were stirred at room temperature overnight. White solid, yield 89%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.33(1H, s), 8.58 (1H, d, *J* = 2.0 Hz), 8.23 (1H, dd, *J* = 2.1, 8.4 Hz), 8.07 (4H, m), 7.30 (1H, s), 7.08 (1H, d, *J*

= 7.7 Hz), 7.04 (1H, d, *J* = 7.6 Hz), 5.31 (2H, s), 2.29 (3H, s), 2.18 (3H, s); HRMS calculated for $C_{22}H_{18}ClN_3NaO_6$ $(M + Na)^+$ 478.0776, found 478.0774.

(64) 4-Cyano-N-[2-(2,5-dimethyl benzyloxy)-4-nitro phenyl] benzamide: Compound **2a** and 4-cyano benzoyl chloride were stirred at room temperature overnight. White solid, yield 91%: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ 10.17(1H, s), 8.11 (7H, m), 7.32 (1H, s), 7.11 (1H, d, *J* = 7.6 Hz), 7.05 (1H, d, *J* = 7.7 Hz), 5.31 (2H, s), 2.29 (3H, s), 2.20 (3H, s).

(65) N-[2-(4-Isopropyl benzyloxy)-4-nitro phenyl] acetamide: Compound **1a** and acetyl chloride were stirred at room temperature overnight. Pale yellow solid, yield 77%: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ 9.57(1H, s), 8.34 (1H, d, *J* = 8.5 Hz), 7.88 (2H, m), 7.47 (2H, d, *J* = 8.0 Hz), 7.30 (2H, d, *J* = 8.1 Hz), 5.35 (2H, s), 2.91 (1H, m), 1.20 (3H, d, *J* = 1.3 Hz), 1.19 (3H, d, *J* = 1.3 Hz).

(66) N-[2-(4-Isopropyl benzyloxy)-4-nitro phenyl] benzamide: Compound **1a** and benzoyl chloride were stirred at room temperature overnight. White solid, yield 95%: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ 9.75(1H, s), 8.28 (1H, d, *J* = 8.7 Hz), 7.98 (4H, m), 7.64 (5H, m), 7.29 (2H, d, *J* = 7.9 Hz), 5.35 (2H, s), 2.91 (1H, m), 1.20 (3H, s), 1.19 (3H, s).

(67) Cyclohexanecarboxylic acid [2-(4-isopropyl benzyloxy)-4-nitro phenyl] amide: Compound **1a** and cyclohexanecarbonyl chloride were stirred at room temperature overnight. White solid, yield 99%: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ 9.38(1H, s), 8.31 (1H, d, *J* = 9.1 Hz), 7.88 (2H, m), 7.46 (2H, d, *J* = 8.0 Hz), 7.29 (2H, d, *J* = 9.4 Hz), 5.34 (2H, s), 2.91 (1H, m), 2.63 (1H, m), 1.83(5H, m), 1.41 (5H, m), 1.20 (3H, s), 1.19 (3H, s).

(68) 3, 4-Dichloro-N-[2-(4-isopropyl benzyloxy)-4-nitro phenyl] benzamide: Compound **1a** and 3, 4-dichlorobenzoyl chloride were stirred at room temperature overnight. White solid, yield 91%: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ 10.05(1H, s), 8.15 (2H, m), 7.99 (4H, m), 7.49 (2H, d, *J* = 7.4 Hz), 7.28 (2H, d, *J* = 7.2 Hz), 5.33 (2H, s), 2.89 (1H, m), 1.20 (3H, s), 1.18 (3H, s).

(69) Naphthalene-2-carboxylic acid [2-(4-isopropyl benzyloxy)-4-nitro phenyl] amide: Compound **1a** and 2-naphthoyl chloride were stirred at 80 °C for three days. White solid, yield 85%: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ 9.92(1H, s), 8.52 (1H, s), 8.33 (1H, d, *J* = 8.7 Hz), 8.09 (6H, m), 7.68 (2H, m), 7.55 (2H, d, *J* = 7.8 Hz), 7.31 (2H, d, *J* = 7.8 Hz), 5.37 (2H, s), 2.92 (1H, m), 1.21 (3H, d, *J* = 0.5 Hz), 1.18 (3H, d, *J* = 0.5 Hz).

(70) Biphenyl-4-carboxylic acid [2-(4-isopropyl benzyloxy)-4-nitro phenyl] amide: Compound **1a** and biphenyl-4-carbonyl chloride were stirred at 80 °C for three days. Pale yellow solid, yield 56%: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ 9.81(1H, s), 8.30 (1H, d, *J* = 8.7 Hz), 8.05 (4H, m), 7.87 (2H, d, *J* = 8.2 Hz), 7.77 (2H, d, *J* = 7.5 Hz), 7.55 (5H, m), 7.30 (2H, d, *J* = 7.9 Hz), 5.37 (2H, s), 2.89 (1H, m), 1.20 (3H, d, *J* = 1.0 Hz), 1.18 (3H, d, *J* = 1.0 Hz).

(71) 4-Chloro-N-[2-(4-isopropyl benzyloxy)-4-nitro phenyl]-3-nitro benzamide: Compound **1a** and 4-chloro-3-nitrobenzoyl chloride were stirred at room temperature overnight. Pale yellow solid, yield 91%: ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ 10.26(1H, s), 8.58 (1H, d, *J* = 2.0 Hz), 8.23 (1H, dd, *J* = 2.0, 8.4 Hz), 8.15 (1H, d, *J* = 8.8 Hz), 8.00 (3H, m), 7.48 (2H, d, *J* = 8.0 Hz), 7.27 (2H, d, *J* = 8.0 Hz), 5.34 (2H, s), 2.90 (1H, m), 1.20 (3H, s), 1.18 (3H, s).

(72) 4-Cyano-N-[2-(4-isopropyl benzyloxy)-4-nitro phenyl] benzamide: Compound **1a** and 4-cyanobenzoyl chloride were stirred at room temperature overnight. Pale yellow solid, yield 95%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.11(1H, s), 8.18 (1H, d, J = 8.8 Hz), 8.10 (4H, m), 7.99 (2H, m), 7.48 (2H, d, J = 7.8 Hz), 7.28 (2H, d, J = 7.8 Hz), 5.35 (2H, s), 2.88 (1H, m), 1.20 (3H, d, J = 0.7 Hz), 1.18 (3H, d, J = 0.7 Hz).

(73) N-[4-Amino-2-(2,5-dimethyl benzyloxy)-phenyl]-N-methyl-methanesulfonamide: A mixture of ferric chloride (4mmol, 4*eq*), and compound **44** (1mmol, 1*eq*) was added to a solvent mixture of dimethylformamide and water (6:1, 7mL). It was stirred for 30min and then zinc dust (10mmol, 10*eq*) was added slowly. After completion of the reaction (10min, monitored by TLC), the reaction mixture was filtered by passing celite pad. The filtrate was diluted with water and basified by adding saturated aqueous Na_2CO_3. The precipitated solid was collected by filtration and dried, and then it was dissolved in acetone. After filtration of the insoluble residues, the desired compound was recovered by distillation of the acetone under reduce pressure. White solid, yield 78%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.25(1H, s), 7.13 (2H, d, J = 7.6 Hz), 7.07 (2H, d, J = 7.1 Hz), 6.36 (1H, s), 6.13 (1H, d, J = 7.2 Hz), 5.32 (2H, s), 4.99 (2H, s), 3.04 (3H, s), 2.76 (3H, s), 2.28 (3H, s), 2.27 (3H, s).

General procedure for the preparation of the D position substituted nimesulide analogs compounds 74–76 from compound 73—K_2CO_3 (5 mmol, 5*eq*) and substituted acyl chloride (1.2 mmol, 1.2 *eq*) were successively added to a solution of compound **73** (1.0 mmol, 1.0 *eq*) in dry 1, 4 dioxane and the mixture was stirred at room temperature overnight. After being cooled, 10 mL H_2O and 3 mL saturated aqueous Na_2CO_3 was added to the mixture and it was stirred at room temperature over night. The precipitated solid was collected by filtration and washed with H_2O and cold ethyl ether/hexane to afford desired compounds.

(74) N-[3-(2,5-Dimethyl benzyloxy)-4-(methanesulfonyl-methyl-amino)-phenyl] acetamide: Compound 73 and acetyl chloride were stirred at room temperature overnight. White solid, yield 97%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.15 (1H, s), 7.54 (1H, d, J = 1.8 Hz), 7.28(1H, s), 7.22(4H, m), 5.06 (2H, s), 3.09 (3H, s), 2.85 (3H, s), 2.30 (3H, s), 2.27 (3H, s), 2.05 (3H, s).

(75) N-[3-(2,5-Dimethyl benzyloxy)-4-(methanesulfonyl-methyl-amino)-phenyl] benzamide: Compound 73 and benzoyl chloride were stirred at room temperature overnight. White solid, yield 97%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.38 (1H, s), 7.98 (2H, m), 7.78 (1H, d, J = 2.2 Hz), 7.62(3H, m), 7.43(1H, dd, J = 8.5, 2.2 Hz),7.32(2H, m), 7.13(2H, m), 5.10 (2H, s), 3.11 (3H, s), 2.87 (3H, s), 2.33 (3H, s), 2.28 (3H, s); HRMS calculated for $C_{24}H_{26}N_2NaO_4S$ $(M + Na)^+$ 461.1511, found 461.1511.

(76) Cyclohexanecarboxylic acid [3-(2,5-dimethyl benzyloxy)-4-(methanesulfonyl-methyl-amino)-phenyl] amide: Compound 73 and cyclohexanecarbonyl chloride were stirred at room temperature overnight. White solid, yield 92%: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.97 (1H, s), 7.64 (1H, d, J = 2.1 Hz), 7.29(1H, s), 7.21(4H, m), 5.05 (2H, s), 3.08 (3H, s), 2.83 (3H, s), 2.33 (1H, m), 2.32 (3H, s), 2.27 (3H, s), 1.81(5H, m), 1.39 (5H, m); HRMS calculated for $C_{24}H_{32}N_2NaO_4S$ $(M + Na)^+$ 467.1980, found 467.1977.

4.2. Biological study

4.2.1. Cell culture—SK-BR-3, MDA-MB-231, MCF-7 and BT474 cells were obtained from ATCC (Rockville, MD). All cells were maintained in DMEM/F12 medium supplemented with 5% fetal bovine serum (FBS) and 20 mg/L gentamycin. Fetal bovine serum was heat inactivated for 30 min in a 56 °C water bath before use. Cell cultures were grown at 37 °C, in a humidified atmosphere of 5% CO_2 in a Hereaus CO_2 incubator.

4.2.2. Cell Viability Analysis—The effect of nimesulides derivatives on breast cancer cell viability was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay in six replicates. Cells were grown in custom medium in 96-well, flat-bottomed plates for 24 h, and were exposed to various concentrations of nimesulide derivatives dissolved in DMSO (final concentration ≤ 0.1%) in media for different time intervals. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. The medium was removed, replaced by 200 μl of 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in fresh media, and cells were incubated in the CO_2 incubator at 37°C for 2 h. Supernatants were removed from the wells, and the reduced 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide dye was solubilized in 200 μl/well DMSO. Absorbance at 570 nm was determined on a plate reader.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATION LIST

PGE_2	Prostaglandin E_2
COX-2	cyclooxygenase-2
NSAIDs	nonsteroidal anti-inflammatory drugs

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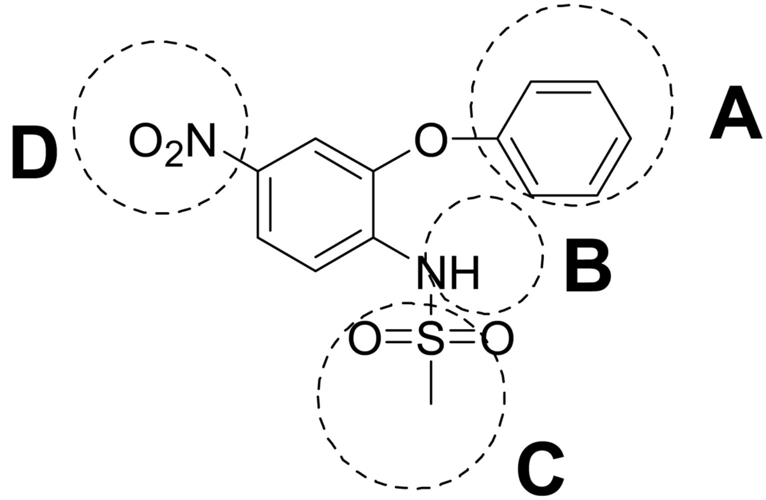

Figure 1.
Chemical structure of nimesulide

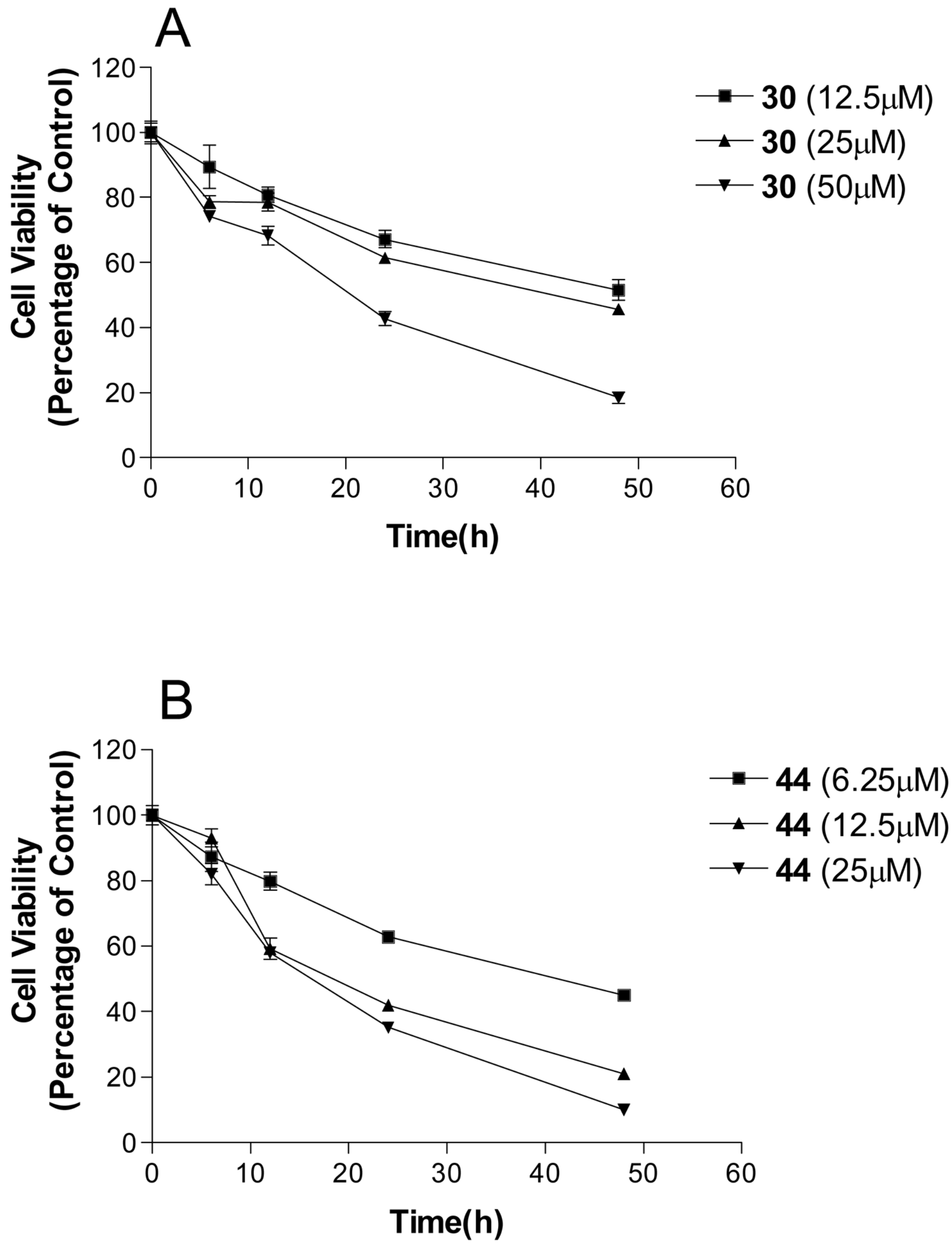

Figure 2. Time-and dose-dependent effects of compound 30 and 44 on the SK-BR-3 cell growth
(A) Compound **30**, (B) Compound **44**. Values obtained from six replicates were plotted for each time point at the indicated concentration of compound **30** or **44.** Control SK-BR-3 cells were treated with a dimethyl sulfoxide (DMSO) vehicle.

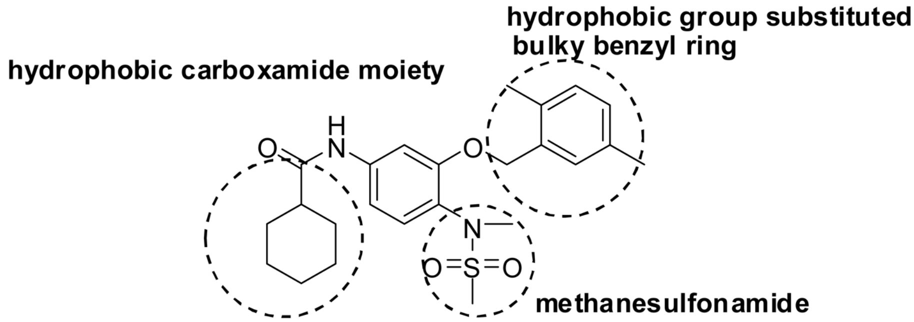

Figure 3.
Chemical structure of compound 76

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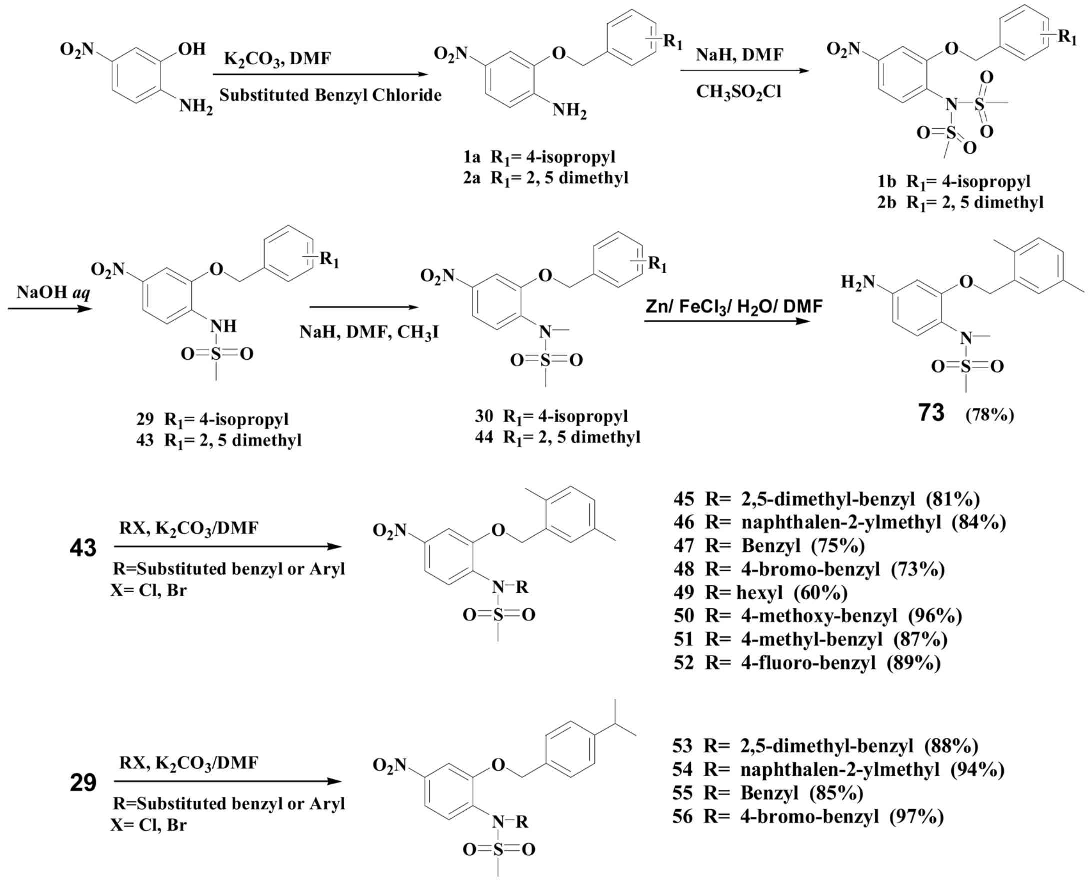
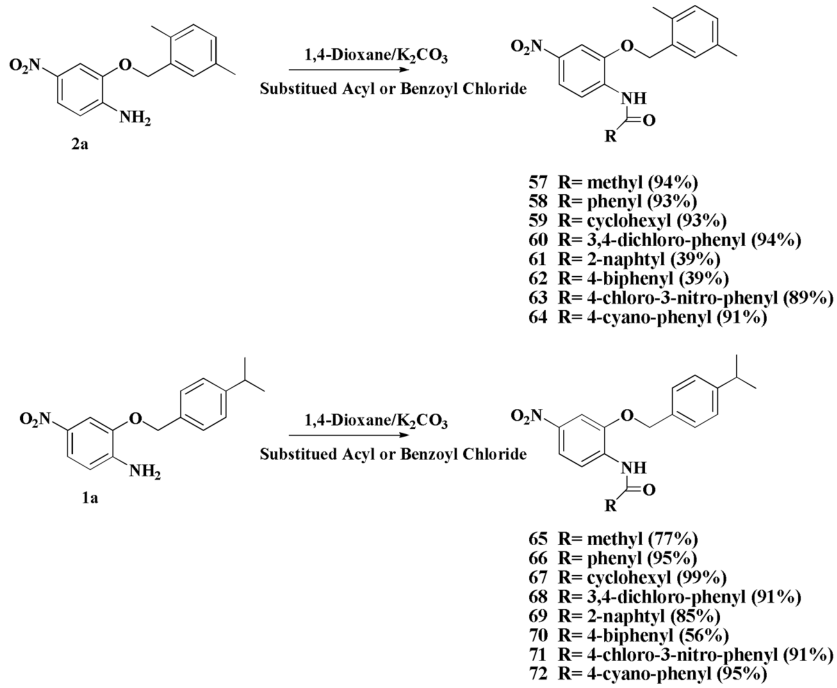
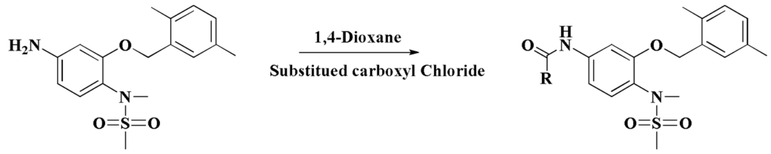


Scheme 1.
Modification of B position



Scheme 2.
Modification of C position



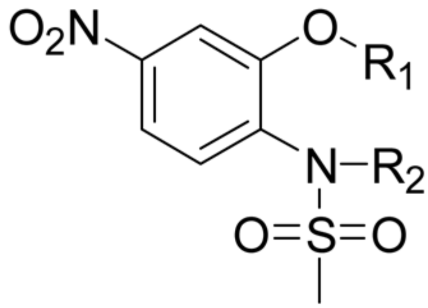
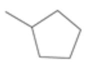
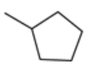
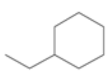
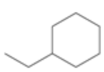
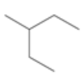
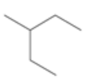
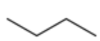
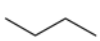
73

74 R= methyl (97%)
75 R= phenyl (97%)
76 R= cyclohexyl (92%)

Scheme 3.
Modification of D position

Table 1

A and B moiety alkyl substituted nimesulide and their inhibition of SK-BR-3 cell growth

Compd		SKBR-3 cell growth inhibition (%) at 25μM
1	R_1= CH_3 R_2= H	13.9 ± 1.6
2	R_1= CH_3 R_2= CH_3	13.7 ± 3.1
3	R_1=  R_2= H	16.6 ± 3.2
4	R_1=  R_2= CH_3	17.1 ± 3.4
5	R_1=  R_2= H	39.3 ± 2.9
6	R_1=  R_2= CH_3	57.5 ± 1.3
7	R_1=  R_2= H	29.1 ± 1.8
8	R_1=  R_2= CH_3	6.1 ± 2.0
9	R_1=  R_2= H	13.8 ± 2.3
10	R_1=  R_2= CH_3	10.6 ± 2.6
11	R_1=  R_2= H	61.6 ± 1.4

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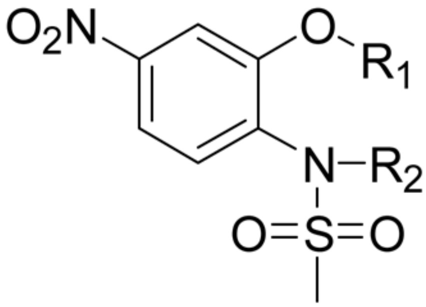
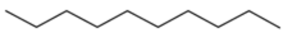
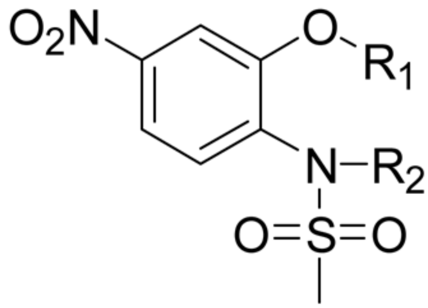
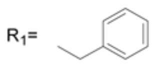
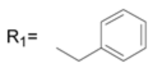
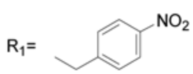
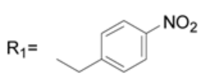
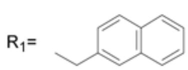
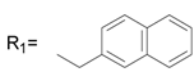
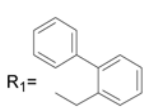
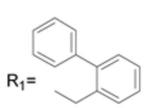
Compd		**SKBR-3 cell growth inhibition (%) at 25μM**
12	R_1=  R_2= CH_3	62.8 ± 0.8
13	R_1=  R_2= H	66.5 ± 0.3
14	R_1=  R_2= CH_3	35.2 ± 2.2
15	R_1=  R_2= H	31.3 ± 2.8
16	R_1=  R_2= CH_3	43.0 ± 3.7

SK-BR-3 cells were treated with indicated compounds at 25μM for 48 h and cell viability was measured by MTT assay as described in the experimental section, n = 6.

Table 2

A and B moiety aryl substituted nimesulide and their inhibition of SK-BR-3 cell growth

Compd		SKBR-3 cell growth inhibition (%) at 25μM
17	 R_2= H	34.1 ± 2.9
18	 R_2= CH_3	38.5 ± 2.2
19	 R_2= H	53.6 ± 1.6
20	 R_2= CH_3	31.5 ± 4.9
21	 R_2= H	55.4 ± 2.4
22	 R_2= CH_3	41.7 ± 3.0
23	 R_2= H	65.7 ± 1.5
24	 R_2= CH_3	53.0 ± 2.9

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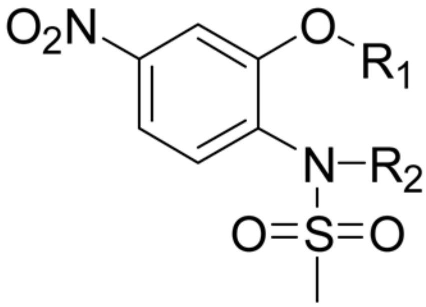
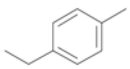
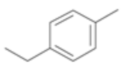
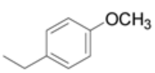
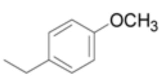
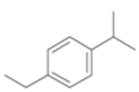
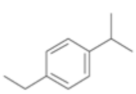
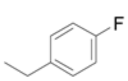
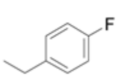
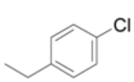
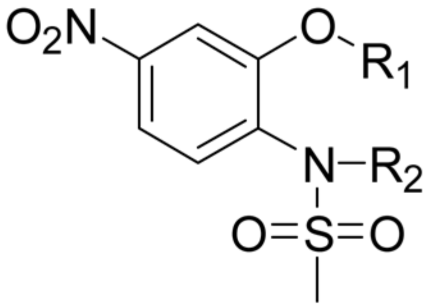
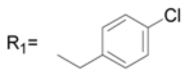
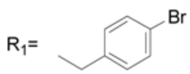
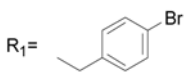
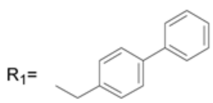
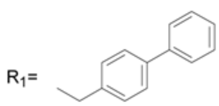
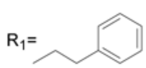
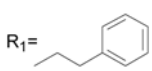
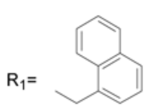
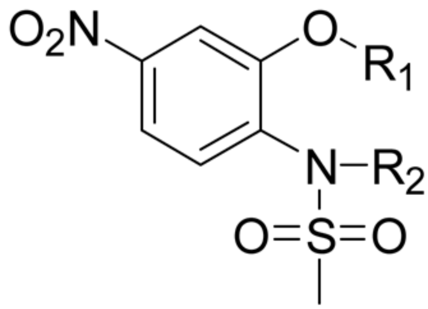
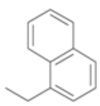
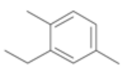
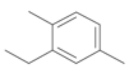
Compd		SKBR-3 cell growth inhibition (%) at 25μM
25	R_1=  R_2= H	27.8 ± 2.6
26	R_1=  R_2= CH_3	37.6 ± 3.3
27	R_1=  R_2= H	26.4 ± 1.9
28	R_1=  R_2= CH_3	26.8 ± 3.7
29	R_1=  R_2= H	24.6 ± 3.2
30 *	R_1=  R_2= CH_3	81.6 ± 1.0
31	R_1=  R_2= H	41.8 ± 1.4
32	R_1=  R_2= CH_3	20.9 ± 5.0
33	R_1=  R_2= H	47.7 ± 3.1

Compd		SKBR-3 cell growth inhibition (%) at 25μM
34	 R_2= CH_3	7.0 ± 1.0
35	 R_2= H	42.7 ± 1.9
36	 R_2= CH_3	13.4 ± 2.2
37	 R_2= H	36.7 ± 1.2
38	 R_2= CH_3	38.6 ± 3.4
39	 R_2= H	28.6 ± 2.9
40	 R_2= CH_3	29.0 ± 4.6
41	 R_2= H	53.8 ± 2.1

Compd		**SKBR-3 cell growth inhibition (%) at 25μM**
42	R_1=  R_2= CH_3	61.4 ± 1.1
43	R_1=  R_2= H	63.7 ± 1.3
44*	R_1=  R_2= CH_3	89.7 ± 0.4

SK-BR-3 cells were treated with indicated compounds at 25μM for 48 h and cell viability was measured by MTT assay as described in the experimental section, n = 6.

*Significantly active compounds

Table 3

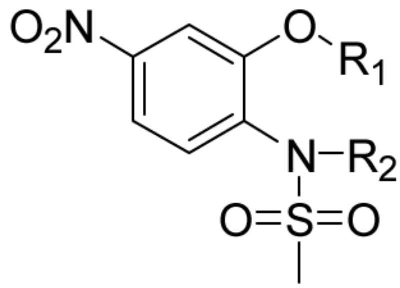
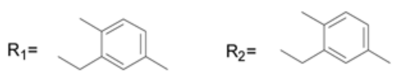
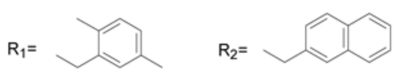
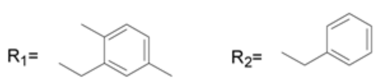
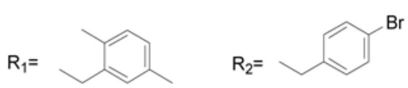
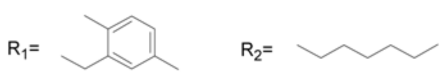
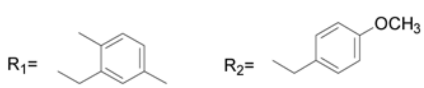
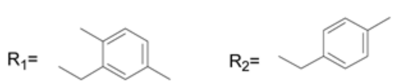
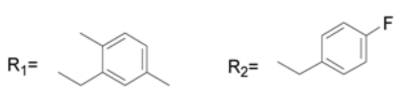
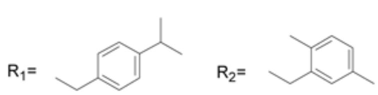
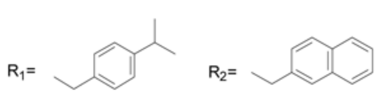
IC_{50} of inhibition of breast cancer cells growth by compounds 44, 30 and nimesulide

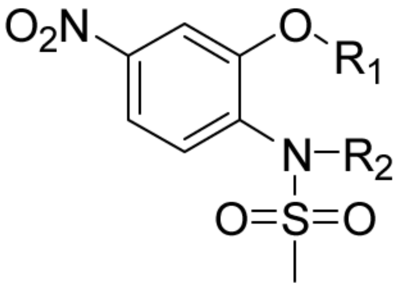
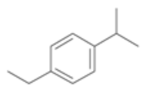
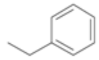
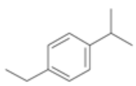
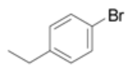
Breast cancer cell lines	Comp 44	Comp 30	Nimesulide
SK-BR-3	6.2 ± 1.8 μM	20.1 ± 5.5 μM	111.8 ± 32.3 μM
MDA-MB-231	>50 μM	>50 μM	123.4 ± 10.8 μM
MCF-7	>50 μM	>50 μM	120.4 ± 8.6 μM
BT-474	13.5 ± 2.6 μM	44.7 ± 16.3 μM	165.9 ± 22.5 μM

Cells were treated with indicated compounds at various concentrations for 48h and cell viability was measured by MTT assay as described in the experimental section, n = 6.

Table 4

Inhibition of SK-BR-3 cells growth by B moiety substituted nimesulide derivatives

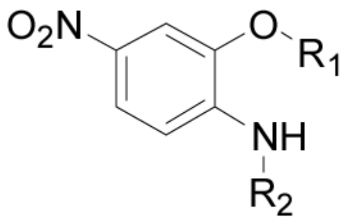
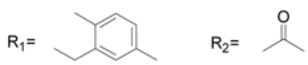
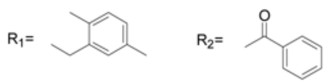
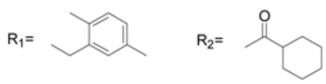
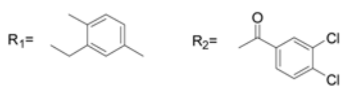
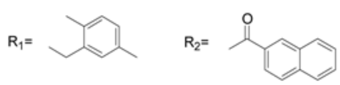
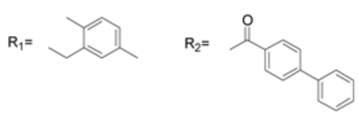
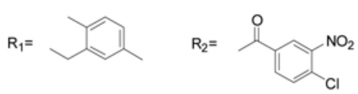
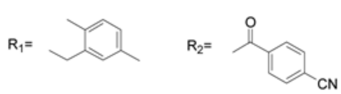
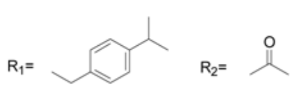
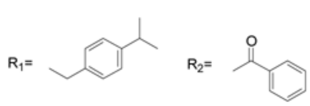
Compd		SKBR-3 cell growth inhibition (%) at 25μM
45		46.3 ± 4.4
46		42.6 ± 2.7
47		41.7 ± 2.4
48		52.3 ± 3.1
49		60.5 ± 2.3
50		26.1 ± 0.7
51		55.1 ± 2.0
52		49.4 ± 0.8
53		54.9 ± 1.2
54		40.1 ± 1.3

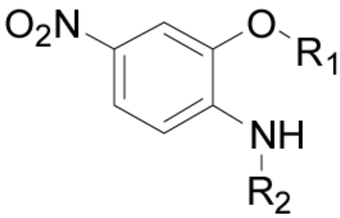
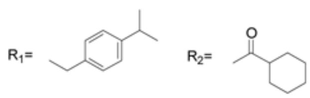
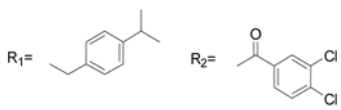
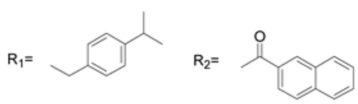
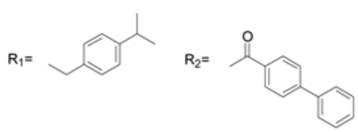
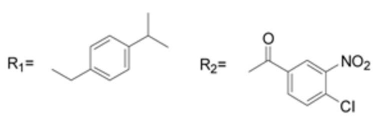
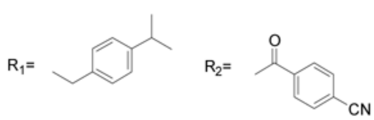
Compd		**SKBR-3 cell growth inhibition (%) at 25μM**
55	R_1=  R_2= 	25.9 ± 0.7
56	R_1=  R_2= 	59.7 ± 0.6

SK-BR-3 cells were treated with indicated compounds at 25μM for 48 h and cell viability was measured by MTT assay as described in the experimental section, n = 6.

Table 5

Inhibition of SK-BR-3 cells growth by C moiety substituted nimesulide derivatives

Compd		SKBR-3 cell growth inhibition (%) at 25μM
57		44.0 ± 1.2
58		36.1 ± 1.1
59		49.5 ± 1.8
60		53.4 ± 1.8
61		25.4 ± 1.6
62		59.7 ± 0.7
63 *		88.3 ± 1.1
64		58.9 ± 1.7
65		38.9 ± 2.4
66		53.4 ± 1.0

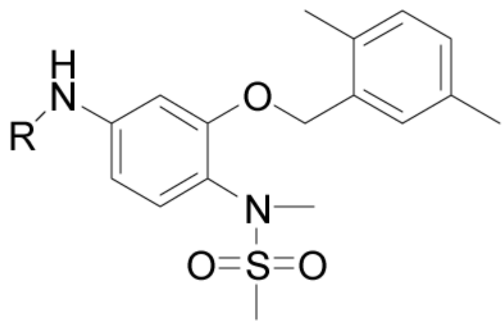
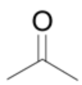
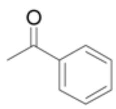
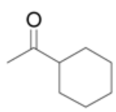
Compd		SKBR-3 cell growth inhibition (%) at 25μM
67		49.7 ± 1.2
68		42.1 ± 2.0
69		35.5 ± 1.1
70		44.1 ± 1.0
71		36.7 ± 0.8
72		65.5 ± 1.2

SK-BR-3 cells were treated with indicated compounds at 25μM for 48 h and cell viability was measured by MTT assay as described in the experimental section, n = 6.

*Significantly active compounds

Table 6

Inhibition of SK-BR-3 cells growth by D moiety substituted nimesulide derivatives

Compd		SKBR-3 cell growth inhibition (%) at 25μM
73	R= H	62.8 ± 1.0
74	R= 	14.6 ± 1.9
75*	R= 	73.2 ± 1.2
76*	R= 	75.4 ± 0.4

SK-BR-3 cells were treated with indicated compounds at 25μM for 48 h and cell viability was measured by MTT assay as described in the experimental section, n = 6.

*Significantly active compounds

Table 7

Inhibition of SK-BR-3 cells growth by compound 63, 75 and 76

Compd	IC_{50} of inhibiting SK-BR-3 cell growth (μM)
63	18.90 ± 4.18
75	3.65 ± 0.19
76	1.38 ± 0.10

SK-BR-3 cells were treated with indicated compounds at various concentrations for 48 h and cell viability was measured by MTT assay as described in the experimental section, n = 6.