Synthesis and Biological Evaluation of Selective Aromatase Expression Regulators in Breast Cancer Cells

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Aromatase converts androgens to estrogens and is a particularly attractive target in the treatment of estrogen receptor positive breast cancer. The enzyme is encoded by the *CYP19* gene, which is expressed in a tissue-specific manner. Prostaglandin E_2 (PGE_2), the major product of cyclooxygenase-2 (COX-2), stimulates aromatase gene expression via protein kinase A and C signaling pathways. Our previous study demonstrated that COX-2 selective inhibitor nimesulide decreased aromatase activity from the transcriptional level in breast cancer cells. In this manuscript, the synthesis and biological evaluation of a series of nimesulide analogues as potential selective aromatase expression regulators are described. Several novel sulfonanilide compounds demonstrate IC_{50} values from 0.33 to 2.68 μM in suppressing aromatase enzyme activity in SK-BR-3 breast cancer cells and are 10- to 80-fold more active than nimesulide. Also, the sulfonanilide compounds selectively decrease aromatase gene expression in breast cancer cells, without exhibiting cytotoxic or apoptotic effects at low micromole concentrations.

1. Introduction

In the United States, breast cancer is the most common cancer diagnosed in women and is the second leading cause of death in women after lung cancer. It is estimated that 212 920 new cases of invasive breast cancer will be detected among women in the United States in the 2006 and 40 970 women will die from this disease in the same year.[1] Approximately 60% of premenopausal and 75% of postmenopausal breast cancer patients have estrogen-dependent carcinoma.[2] Hormone-dependent breast tumors require estrogens for their growth and are characterized by high expression of estrogen receptors (ERs).[3] As a result, efforts to block estrogen action by interfering with the binding to its receptor or by decreasing estrogen production are used as strategies to treat hormone-dependent breast cancer. The first approach involves antiestrogen molecules that can compete with estrogen for binding to the ERs. The second method utilizes aromatase inhibitors (AIs) that inhibit the enzyme catalyzing the final rate-limiting step of the estrogen biosynthesis.[4]

Significant research has focused on developing antiestrogens that can selectively inhibit the estrogen effects in breast tissue without antagonizing the physiological roles of estrogens in other tissues. Agents that exhibit such tissue-specific antiestrogenic/estrogenic activities have been termed selective estrogen receptor modulators (SERMs). Tamoxifen is the most widely used SERM in hormone-dependent breast cancer therapy and has made a substantial contribution to the reduced mortality rate in many developed countries since 1990.[5] Although tamoxifen is still considered the "gold standard" for endocrine therapy in hormone-dependent breast cancer, its use is associated with tumor resistance and increased risk of endometrial cancer.[6] Aromatase inhibitors, such as anastrozole, letrozole, and exemestane, significantly decrease plasma estrogen levels, are emerging as alternatives to tamoxifen due to their clinical efficacy, and have favorable safety profiles for the treatment of hormone-dependent breast cancer in postmenopausal women.[7−9] However, since the AIs inhibit aromatase enzyme in a global fashion, a major long-term side effect of the AIs is the reduction of the bone density which can lead to osteoporosis.[10]

To reduce the risk of the long-term side effects, a new pharmacological approach in the treatment of estrogen-dependent postmenopausal breast cancer is the use of tissue-specific inhibitors of aromatase. This concept is based on the tissue-specific regulation of aromatase expression. Ten different promoter regions have been identified upstream of the coding region of the aromatase gene (*CYP19*).[11] The employment of alternative promoters results in tissue-specific regulation of the *CYP19* expression. Each promoter is regulated by distinct hormones and second messenger pathways. In postmenopausal women, estrogens are produced by adipose tissue, including breast tissue and skin.[12,13] In these tissues aromatase expression is directed by promoter I.4 that is regulated by the synergistic actions of glucocorticoids and class I cytokines such as interleukin (IL)-6, IL-11, and tumor necrosis factor alpha (TNFα).[13] In breast adenocarcinoma, aromatase expression and activity increase significantly compared to normal breast tissue.[14] Breast tumors produce factors that stimulate aromatase expression locally.[15] This stimulation is associated with switching of the aromatase gene promoter from promoter I.4 to c-AMP-dependent promoter I.3 and promoter II in breast cancer and surrounding adipose stromal cells.[16,17] Current evidence suggests that tumor-derived factors, including the cyclooxygenase (COX) product prostaglandin E_2 (PGE_2), can mediate the induction of aromatase expression via promoter II by stimulating adenylate cyclase in adipose stromal cells.[16−18] This biochemical mechanism may explain epidemiological observations of the beneficial effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on breast cancer.

Previously, we demonstrated that COX-2 inhibitors, *N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide (nimesulide),[19] and

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[a] Abbreviations: PGE_2, Prostaglandin E_2; COX-2, cyclooxygenase-2; NSAIDs, nonsteroidal anti-inflammatory drugs; ER, estrogen receptors; AIs, aromatase inhibitors; SERMs, selective estrogen modulators; TNFα, tumor necrosis factor alpha; cAMP, adenosine 3′,5′-cyclic monophosphate.

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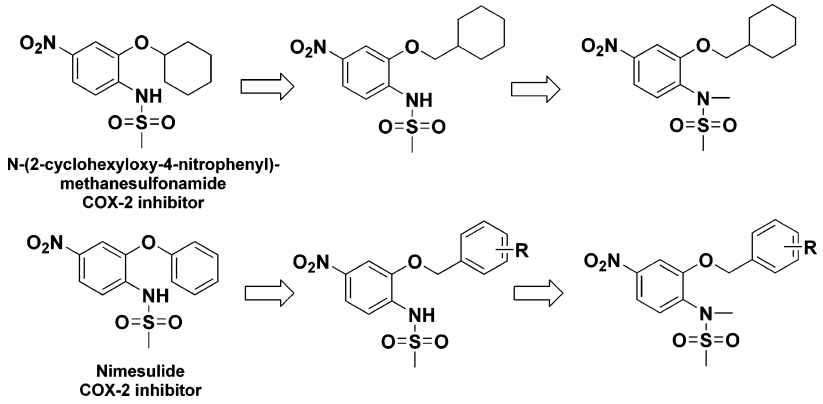
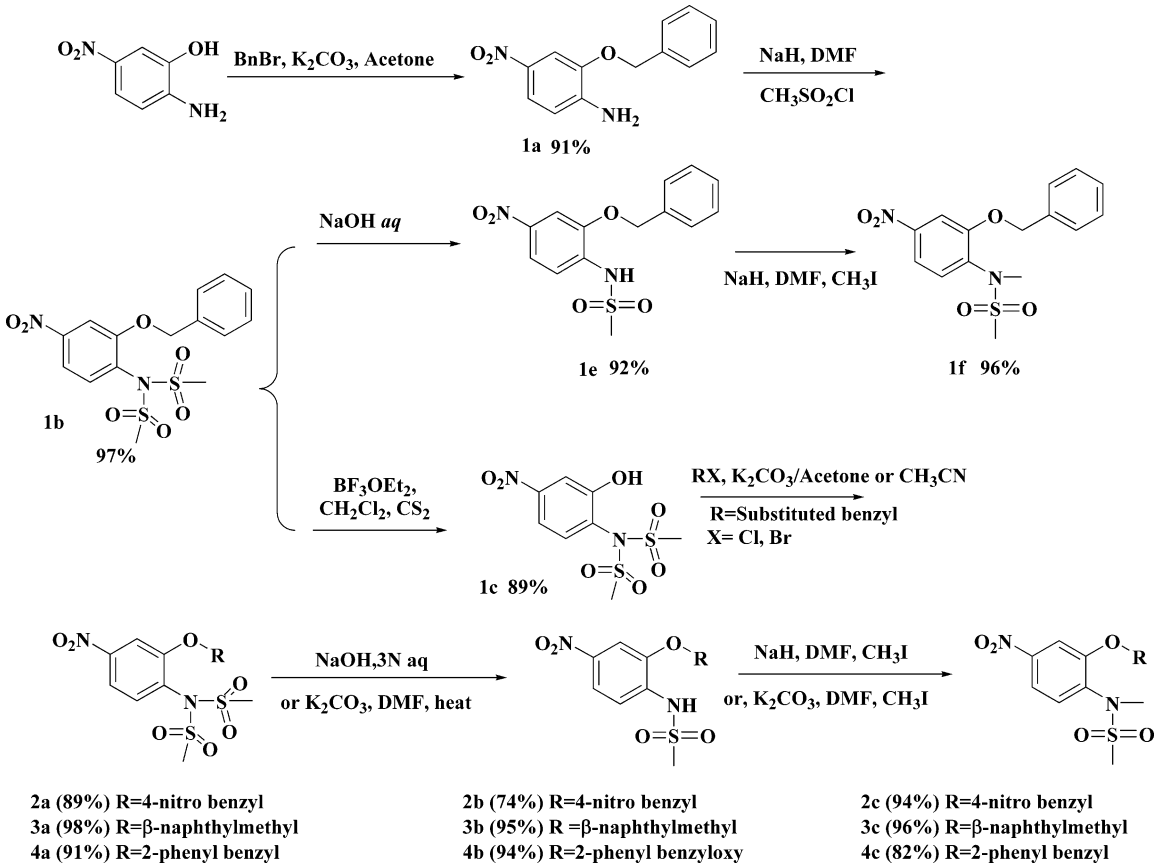


Figure 1. Chemical structures of COX-2 inhibitors nimesulide, *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide, and the target compounds

Scheme 1. Synthesis of Compounds **1b**−**4c**

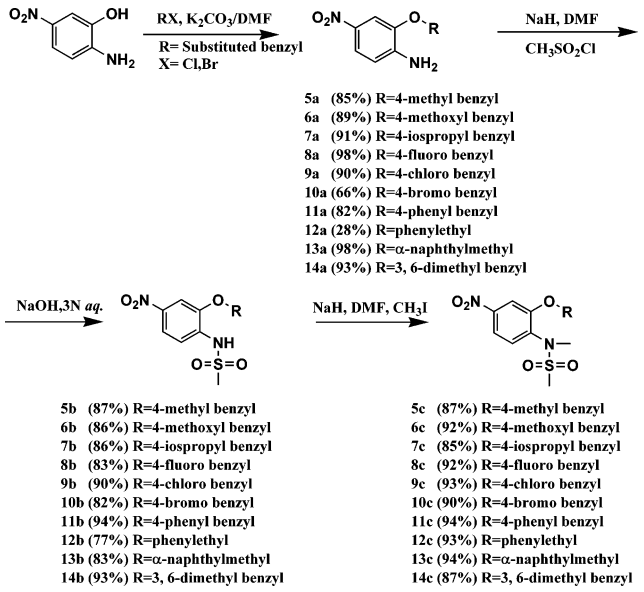


N-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS-398),[20] suppress aromatase activity in breast cancer cells by suppressing aromatase transcription,[21] and these two agents share very similar chemical structures (Figure 1). Interestingly, derivatives of *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide suppresses aromatase activity at similar levels with the compound itself, whereas the *N*-methyl and the 2-methyl derivatives of *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide exhibit no COX-2 inhibition.[22] In the present manuscript, we described the synthesis of novel sulfonanilide compounds based on nimesulide (Figure 1) and the biological effects of these new agents as selective aromatase expression regulators.

2. Chemistry

The synthesis of the target compounds was carried out in Scheme 1 and Scheme 2, in which R represents substituted benzyl moieties and X represents chloride or bromide. The starting material 2-amino-5-nitrophenol was commercially available. In Scheme 1, 2-amino-5-nitrophenol was refluxed with K_2CO_3 and benzyl bromide to obtain compounds **1a**.[23] Sodium hydride and methanesulfonyl chloride were added to compound **1a** in dry dimethylformamide (DMF) at room temperature, and the reaction mixture was stirred at room temperature overnight to obtain the *N*,*N*-bimethanesulfonamido (**1b**). Dealkylation reaction of compound **1b** was performed with $BF_3{\cdot}OEt_2-Me_2S$ in dichloromethane yielding hydroxy compound **1c**. In addition, **1b** can be hydrolyzed with 10% NaOH solution to generate **1e** as a monomethanesulfonamido compound. Compound **1e** was treated with K_2CO_3 and substituted benzyl chloride/bromide in acetone or CH_3CN at room temperature or refluxed to obtain **2a**−**4a**. Hydrolysis of **2a**−**4a** generated **2b**−**4b**. Methylation of **1e** and **2b**−**4b** gave compounds **1f**, **2c**−**4c**, respectively. In Scheme 2, 2-amino-5-nitrophenol was treated with K_2CO_3 and substituted benzyl chloride/bromide in DMF to obtain com-

Scheme 2. Synthesis of Compounds **5a**−**14c**



pounds **5a**−**14a**. Sodium hydride and methanesulfonyl chloride were added and resulted in a mixture of *N*,*N*-bimethanesulfonamido and *N*-methanesulfonamido compounds. The mixture was hydrolyzed with 10% NaOH solution to generate monomethanesulfonamido compounds **5b**−**14b**, respectively. Methylation of **5b**−**14b** gave compounds **5c**−**14c** respectively.[22] The structures of all the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS, and the composition of key compounds in the biological studies was also confirmed by elemental analysis.

3. Biological Evaluation

Aromatase in the endoplasmic reticulum of cells catalyzes the biosynthesis of estrogen. The regulation of aromatase is complex and different in various tissues, and several tissue-specific promoter regions have been identified upstream from the *CYP19* gene. These tissue-specific promoters include promoter PI.1, PI.3, PI.4, PI.6, PI.7, and PII. Promoter PI.1 is the major promoter used in placental tissues, and the PII and I.3 promoters are used in the ovary and in breast cancer tissues. Due to the unique organization of tissue-specific promoters, various promoters employ different signaling pathways and different transcription factors. This use of tissue-specific promoters allows for the development of possible selective aromatase expression regulators.[4] Promoter I.1 (mainly used in placenta) is regulated through a protein kinase C-mediated mechanism.[14,24] Studies from several research groups indicated that promoters II and I.3, which are adenosine 3′,5′-cyclic monophosphate (cAMP)-regulated promoters, are the major promoters driving aromatase expression in breast cancer and surrounding adipose stromal cells. In addition, alternate exon PII and 1.3 were also found to be the major exons in aromatase transcripts in four breast cancer cell lines (MCF-7, T-47D, SK-BR-3, and MDA-MB-231 cells).[15] Therefore, the major promoter used in breast tumors and the four breast cancer cells (i.e., cAMP-stimulated promoters I.3 and II) is different from that placental tissue (i.e., protein kinase C-mediated promoter I.1).

In the current research, evaluations of the synthetic compounds were performed in both SK-BR-3 breast cancer cells (which produce aromatase expression from promoter II and I.3 mediated by cAMP) and JAR choriocarcinoma placental cells (which produce aromatase expression from promoter I.1 mediated by protein kinase C). Comparisons of bioactivity between these two cell lines enable us to differentiate compounds that can only suppress aromatase activity in breast cancer cells and not suppress aromatase activity in placental cells. Those compounds with differential effects on aromatase activity were further investigated for their potential to suppress *CYP19* transcription in breast cancer cells.

The cellular aromatase assays for these compounds were performed in the choriocarcinoma placental JAR cell line and the breast cancer SK-BR-3 cell line according to the modified method of the procedure previously reported by our laboratory.[21,22] These two cell lines, the SK-BR-3 and JAR cells, were used because of their high cellular aromatase enzyme activity and their different regulation of *CYP19* expression.[21] Other human breast cancer cell lines express lower levels of aromatase activity. If the compounds decrease aromatase activity effectively in JAR cells, they may decrease aromatase transcription from promoter I.1 by interfering with protein kinase C pathway. If the compounds decrease aromatase activity effectively in SK-BR-3 breast cancer cells, these compounds may decrease aromatase expression from promoter II and I.3 mediated by cAMP. If the compounds decrease aromatase activity in both cells lines, then several alternative mechanisms for these compounds may be involved: (1) they may directly inhibit aromatase enzyme, (2) they may increase aromatase degradation, or (3) they may decrease aromatase mRNA stability. In addition, the compounds were tested for their general cell cytotoxicity and the ability to induce apoptosis. Compounds only targeting aromatase expression in breast cancer cells and not affecting other cell functions will be the ideal final target compounds.

4. Results and Discussion

The ability of the synthesized compounds to suppress aromatase activity was performed in JAR cells (Figure 2). Unexpectedly, most of the compounds tested exhibited aromatase suppression activity at 15 μM concentration. The results indicate that the compounds may be involved in multiple pathways to decrease aromatase activity. Most of the compounds significantly decrease aromatase activity in SK-BR-3 cells as well at 2.5 μM (Figure 3). Among all the compounds tested, only the ones that do not affect aromatase activity in JAR cells but decrease aromatase activity in SK-BR-3 breast cancer cells might be selective aromatase expression regulators. In Figure 2, compounds **1f**, **2c**, **3c**, **4c**, **5b**, **5c**, **7b**, **8c**, **11b**, **11c**, **13b**, **13c**, **14b**, and **14c** at 15μM decrease aromatase activity less than 50% in JAR cells. In Figure 3, compounds **1e**, **1f**, **2b**, **2c**, **3b**, **3c**, **4b**, **4c**, **5c**, **6c**, **7c**, **8c**, **9b**, **9c**, **10c**, **12b**, **12c**, and **13b** decrease aromatase activity greater than 50% at 2.5μM in SK-BR-3 cells. Compounds **1f**, **2c**, **3c**, **4c**, **5c**, and **8c** may be potential selective aromatase expression regulators because they do not decrease aromatase activity very effectively in JAR cells at 15 μM but significantly suppress aromatase activity in SK-BR-3 breast cancer cells at 2.5 μM.

To further investigate the six compounds, dose−response studies on aromatase activity were performed in SK-BR-3 cells. All six compounds exhibited dose−response of suppression aromatase activity (Figure 4), and the corresponding IC_{50} values are listed in Table 1. The IC_{50} of nimesulide is listed as well. The results of the cellular aromatase assay exhibited that extending one carbon at the 2-position of nimesulide results in a significant increase in suppression of aromatase in breast cancer cells compared with nimesulide. Compound **4c**, which is the most bulky compound, showed the best IC_{50} value with

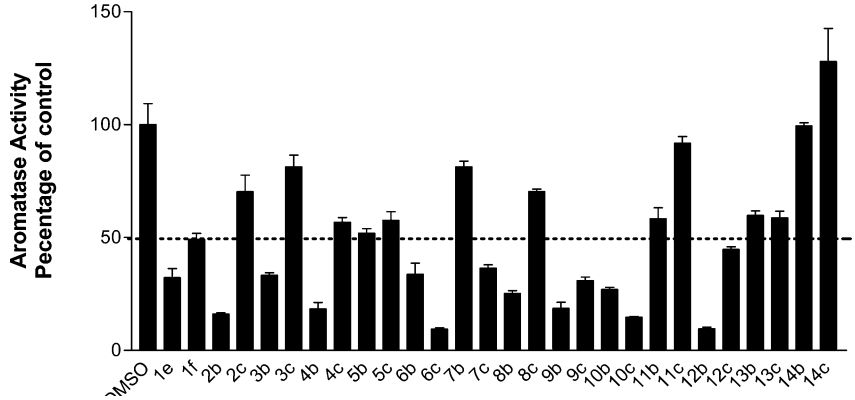


Figure 2. Aromatase activity in JAR cells treated with novel sulfonanilide. JAR cells were treated with indicated compounds at 15 μM, and aromatase activity was measured as described in the Experimental Section. The results were normalized against a control treatment with vehicle, and the value of 100% is equal to 4.0 pmol/h/10^6 cells. Each data bar represents the mean results of three independent determinations.

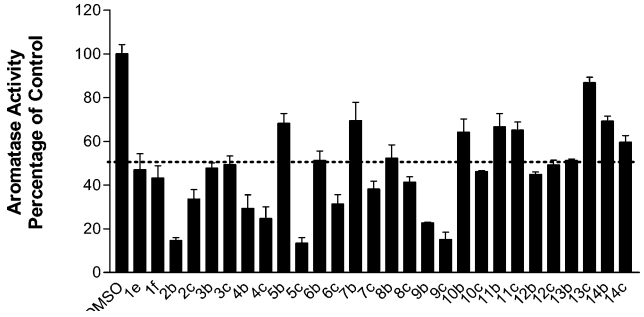


Figure 3. Aromatase activity in SK-BR-3 cells treated with novel sulfonanilide. SK-BR-3 cells were treated with indicated compounds at 2.5 μM, and aromatase activity was measured as described in the Experimental Section. The results were normalized against a control treatment with vehicle, and the value of 100% is equal to 0.03 pmol/h/10^6 cells. Each data bar represents the mean results of three independent determinations.

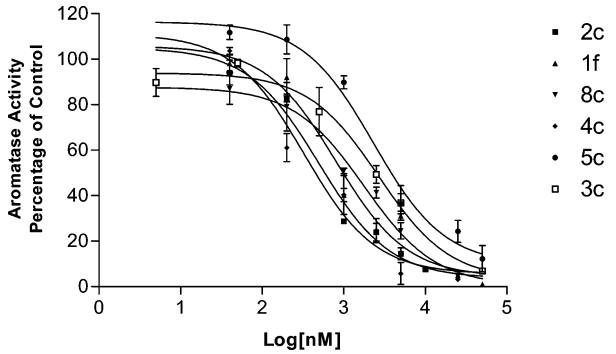


Figure 4. Dose−response suppression of aromatase activity in SK-BR-3 cells by novel sulfonanilide. SK-BR-3 cells were treated with **2c** (■), **1f** (▲), **8c** (▼), **4c** (♦), **5c** (●), and **3c** (□) and aromatase activity was measured as described in the Experimental Section. The results were normalized against a control treatment with vehicle, with the value of 100% equal to 0.03 pmol/h/10^6 cells. Each data point represents the mean results of three independent determinations, and the data were statistically analyzed by a nonlinear regression analysis method.

an 80-fold increase. Furthermore, real time PCR demonstrated that compounds **1f**, **2c**, **3c**, **4c**, and **8c** at 5 μM significantly decreased *CYP19* gene expression in SK-BR-3 cells (Figure 5). This suggests that the suppression of aromatase enzyme activity occurs at the transcriptional level. Compound **12b** was chosen as a negative control because it inhibited aromatase activity in both JAR cells and SK-BR-3 cells. Real time RT-PCR assay of *CYP19* demonstrated that compound **12b** might only decrease aromatase enzyme activity but not affect the *CYP19* gene

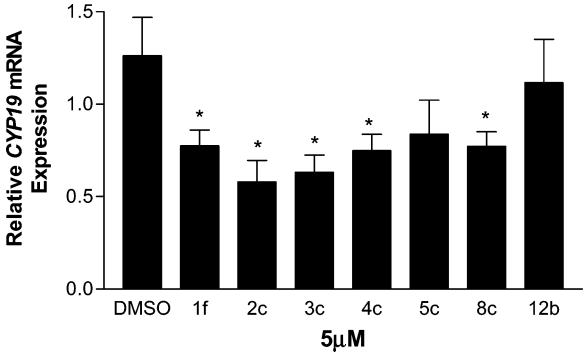


Figure 5. Real-time RT-PCR analysis of *CYP19* mRNA expression in SK-BR-3 cells treated by novel sulfonanilide. Cells were treated for 24 h with the indicated agents at 5μM, and total RNA was isolated. Results are expressed as means of *CYP19* (normalized to 18S rRNA) ± SEM., *P < 0.05 vs control by unpaired t test (n = 9).

expression. This result suggests that **12b** decreases aromatase activity through a posttranscriptional mechanism. In addition, some researchers found that aromatase was also regulated via phosphorylation processes.[25,26] Possible mechanisms by which an agent only decreases aromatase enzyme activity without suppression of its gene expression are direct enzyme inhibition, increased enzyme degradation, or alterations in posttranslational modifications.

Fortunately, all six compounds, **1f**, **2c**, **3c**, **4c**, **5c**, and **8c,** did not cause significant cell cytotoxicity in SK-BR-3 breast cancer cells at higher concentrations (10 μM) than the effective dose for suppression of aromatase expression (Figure 6). However, some of them induce some cytotoxicity at higher concentrations (30 μM). The compounds do not produce apoptotic effects in SK-BR-3 cells at 5 μM, which is effective dose for suppressing aromatase expression (Figure 7). These two results show that, at low micromolar concentrations, the six compounds did not cause any cytotoxicity and apoptotic effects.

In summary, the biological results indicated that several agents selectively decreased aromatase activity and enzyme gene expression at low micromolar concentrations in SK-BR-3 breast cancer cells. These compounds were 10- to 80-fold more active than nimesulide and did not affect the aromatase activity in choriocarcinoma placental JAR cells. Furthermore, this suppression of aromatase activity occurs at the transcriptional level. Several potential lead compounds were developed in this study as selective aromatase expression regulators by structural modifications of the COX-2 inhibitor nimesulide. Because of the limited amount of effective compounds, it is difficult to draw

Table 1. Suppression of Aromatase Activity in SK-BR-3 Breast Cancer Cells

compd	chemical description	IC_{50} (μM)[a]
nimesulide	*N*-(2-phenoxy-4-nitrophenyl)methanesulfonamide	27.0 ± 4.70[b]
1f	*N*-methyl-*N*-(2-benzyloxy-4-nitrophenyl) methanesulfonamide	0.81 ± 0.29
2c	*N*-methyl-*N*-[2-(4′-nitrobenzyloxy)-4-nitrophenyl]methanesulfonamide	0.49 ± 0.14
3c	*N*-methyl-*N*-[2-(β-naphthylmethoxy) 4-nitrophenyl]methanesulfonamide	2.68 ± 0.91
4c	*N*-methyl-*N*-[2-(2′-phenylbenzyloxy)-4-nitrophenyl]methanesulfonamide	0.33 ± 0.15
5c	*N*-methyl-*N*-[2-(4′-methylbenzyloxy)-4-nitrophenyl]methanesulfonamide	2.33 ± 0.66
8c	*N*-methyl-*N*-[2-(4′-fluorobenzyloxy)-4-nitrophenyl]methanesulfonamide	1.78 ± 0.63

[a] IC_{50} values were calculated by a nonlinear regression analysis (GraphPad Prism). Each dose−response curve contained six concentrations, each in triplicate. [b] See ref 21.

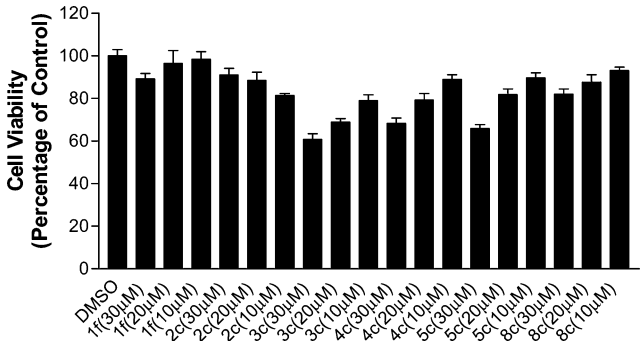


Figure 6. Cell cytotoxicity in SK-BR-3 cells treated with novel sulfonanilide. SK-BR-3 cells were treated with indicated compounds at different concentrations, and cell viability was measured by MTT assay as described in the Experimental Section.

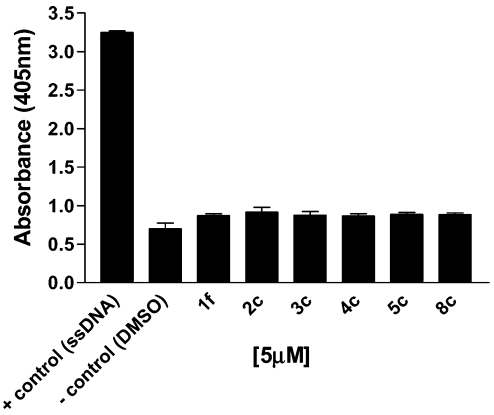


Figure 7. Cell apoptosis in SK-BR-3 cells treated with novel sulfonanilide. SK-BR-3 cells were treated with indicated compounds at different concentrations, and apoptotic cells were measured as described in the Experimental Section.

extensive structure−activity relationships for the compounds. However, these initial findings on the sulfonanilide analogues will enable further lead optimization for novel drug discovery and examination of structural requirements for the selective regulation of aromatase expression. Our current research also focuses on discerning the specific molecular target of the compounds.

5. Experimental Section

5.1. 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 to residual solvent protons. Chemical shifts (δ) for ^{13}C NMR spectra are reported in parts per million relative to residual solvent carbons.

2-Benzyloxy-4-nitroaniline (1a). K_2CO_3 (9.0 g, 65.1 mmol) and benzyl bromide (5.7 g, 33.1 mmol) were successively added to a solution of 2-amino-5-nitrophenol (5.0 g, 32.5 mmol) in acetone (250 mL), and the mixture was refluxed for 8 h. After being cooled, the inorganic precipitate was filtered through a Celite pad, and the filtrate was concentrated *in vacuo*. The resulting solid was recrystallized from AcOEt to afford **1a** (7.2 g, 91%) as yellow needles: mp 148−149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (1H, dd, J = 8.7, 2.4 Hz), 7.80 (1H, d, J = 2.4 Hz), 7.38 (5H, m), 6.69 (1H, d, J = 8.7 Hz), 4.73 (2H, br); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.20, 136.07, 129.19, 128.98, 128.33, 119.72, 112.86, 107.67, 71.32.

***N,N*-(2-Benzyloxy-4-nitrophenyl)dimethanesulfonamide (1b).** NaH (95%, 3.03 g, 120 mmol) was added to a solution of **1a** (7.33 g, 30 mmol) in anhydrous DMF (80 mL) at room temperature. After the mixture was stirred at the same temperature for 30 min, MsCl (10.31 g, 90 mmol) was added slowly and the stirring was continued overnight at room temperature. H_2O (150 mL) and saturated Na_2CO_3 (20 mL) were added to the mixture, and the precipitated solid was collected by filtration and washed with H_2O and a cold ether/hexane mixture to afford **1b** as yellow powder (11.62 g, 97%): mp 188−189 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ8.04 (1H, d, J = 1.7 Hz), 7.89 (2H, m), 7.53 (2H, d, J = 7.7 Hz), 7.38 (3H, m), 5.41 (2H, s), 3.46 (6H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.42, 150.16, 136.25, 134.69, 129.40, 129.38, 129.17, 128.57, 116.70, 109.42, 71.80, 44.59.

***N,N*-(2-Hydroxy-4-nitrophenyl)dimethanesulfonamide (1c).** To a stirred suspension of **1b** (800.7 mg, 2 mmol) in Me_2S (15 mL) and CH_2Cl_2 (15 mL) was slowly added BF_3·OEt_2 (5.07 mL, 40 mmol) at room temperature. The resulting yellow solution was vigorously stirred at room-temperature overnight. After being cooled to 0 °C, the reaction mixture was quenched with water and concentrated under reduced pressure. The insoluble product was collected by filtration and washed with H_2O and $CHCl_3$ to give a pale yellow solid (0.554 g, 89%): mp 249−253 °C (decomp); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (1H, s), 7.69 (3H, m), 3.56 (6H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.29, 149.75, 134.54, 128.32, 114.83, 111.82, 44.74.

***N*-(2-Benzyloxy-4-nitrophenyl)methanesulfonamide (1e).** Compound **1c** (0.4 g, 1 mmol) was added to a 3 N NaOH aq solution and was stirred at 80−90 °C overnight. After cooling, it was neutralized with 5 N HCl until pH 1−2. The precipitated solid was collected and washed with H_2O and cold ether to provide the desired product, and then it was recrystallized from ethyl acetate/hexane to afford a pale yellow solid (0.30 g, 92%): mp 150−152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (1H, dd, J = 8.9, 2.3 Hz), 7.91 (1H, d, J = 2.3 Hz), 7.68 (1H, d, J = 8.9 Hz), 7.43 (5H, m), 7.26 (1H, br), 5.23 (2H, s), 3.09 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.12, 144.14, 134.74, 133.40, 129.63, 129.53, 128.65, 118.30, 116.96, 107.90, 72.30, 40.65; HRMS calculated for $C_{14}H_{14}N_2NaO_5S$ $(M + Na)^+$ 345.0521, found 345.0531.

***N*-Methyl-*N*-(2-benzyloxy-4-nitrophenyl)methanesulfonamide (1f).** Compound **1e** (0.16 g, 0.5 mmol) was dissolved in 3 mL dry DMF, and NaH powder (15.2 mg 95%, 0.6 mmol, 1.2

equiv) was added. The mixture was stirred at room temperature for 10 min, iodomethane (0.6 mmol, 1.2 equiv) was added, and the stirring was maintained for 2 h at room temperature. Then the mixture was taken up with 7 mL of water and 2 mL of Na_2CO_3 aq solution. The precipitated solid was collected by filtration and washed with water and cold ether to afford the desired product, and then it was recrystallized from ethyl acetate/hexane to afford a pale yellow solid (0.16 g, 96%): mp 138−140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (1H, d, J = 2.4 Hz), 7.89 (1H, dd, J = 8.6, 2.5 Hz), 7.56 (1H, d, J = 8.6 Hz), 7.43 (5H, m), 5.23 (2H, s), 3.28 (3H, s), 2.83 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.68, 148.41, 135.77, 134.97, 133.10, 129.52, 129.46, 128.60, 116.98, 108.48, 71.98, 38.91, 37.92; HRMS calculated for $C_{15}H_{16}N_2NaO_5S$ $(M + Na)^+$ 359.0678, found 359.0663. Anal. ($C_{15}H_{16}N_2O_5S$) C, H, N.

General Procedure for the Preparation of 2a−4a. K_2CO_3 (0.69 g, 5 mmol) and aryl halide (5 mmol, 1.0 equiv) were successively added to a solution of compound **1c** (0.77 g, 5 mmol) in CH_3CN or acetone, and the mixture was stirred at room temperature or refluxed from 3 h to overnight. After the mixture was cooled, 20 mL of H_2O and 5 mL of saturated aqueous Na_2CO_3 were added and the precipitated solid was collected by filtration and washed with H_2O and cold ethyl ether to afford the desired compounds.

***N,N*-[2-(4′-Nitrobenzyloxy)-4-nitrophenyl]dimethanesulfonamide (2a).** It is stirred and refluxed in acetone for 2 h. White powder, 89%: mp 265−269 °C (decomp); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (2H, d, J = 8.8 Hz), 8.05 (1H, d, J = 2.3 Hz), 7.93 (1H, dd, J = 8.6, 2.3 Hz), 7.89 (2H, d, J = 8.6 Hz), 7.80 (2H, d, J = 8.8 Hz), 5.59 (2H, s), 3.50 (6H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.10, 150.20, 148.13, 144.10, 135.00, 129.29, 129.21, 124.55, 117.06, 109.48, 70.58, 44.61.

***N,N*-[2-(β-Naphthylmethoxy)-4-nitrophenyl]dimethanesulfonamide (3a).** It is stirred in CH_3CN at room temperature overnight. White powder, 98%: mp 184−185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (1H, d, J = 2.4 Hz), 8.04 (1H, s), 7.95 (2H, dd, J = 8.5, 2.3 Hz), 7.88 (2H, m), 7.64 (1H, dd, J = 8.5, 1.7 Hz), 7.52 (3H, m), 5.44 (2H, s), 3.37 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.35, 150.21, 133.77, 133.57, 133.47, 132.00, 129.24, 128.55, 128.22, 127.69, 127.11, 127.02, 125.74, 116.88, 109.14, 72.52, 44.29.

***N,N*-[2-(2′-Phenylbenzyloxy)-4-nitrophenyl]dimethanesulfonamide (4a).** It is stirred in CH_3CN at room temperature overnight. White powder, 91%: mp 182−184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (1H, dd, J = 8.6, 2.4 Hz), 7.68 (2H, m), 7.40 (9H, m), 5.19 (2H, s), 3.38 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.12, 150.10, 142.79, 140.10, 133.15, 131.84, 130.81, 130.20, 129.59, 129.49, 129.08, 129.03, 128.33, 128.22, 116.68, 109.15, 70.31, 44.24.

General Procedure for the Preparation of 2b−4b. Compound **2a−4a** was added to a 3 N NaOH aq solution or CH_3CN/K_2CO_3 suspension and was stirred at 80−90 °C from 24 to 48 h. After cooling, H_2O and saturated Na_2CO_3 were added, the mixture was stirred for 30 min, and then the insoluble solid was filtered off. The mother liquid was neutralized with 5N HCl until pH 1−2. The precipitated solid was collected and washed with H_2O and cold ether to provide the desired product.

***N*-[2-(4′-Nitrobenzyloxy)-4-nitrophenyl]methanesulfonamide (2b).** It is stirred and refluxed in CH_3CN for 48 h. Yellow powder, 74%: mp 168−169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (2H, d, J = 8.7 Hz), 7.98 (1H, dd, J = 8.9, 2.3 Hz), 7.86 (1H, d, J = 2.3 Hz), 7.71 (1H, d, J = 8.9 Hz), 7.63 (1H, d, J = 8.8 Hz), 5.35 (2H, s), 3.16 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 148.73, 146.40, 143.96, 141.70, 133.38, 128.97, 124.77, 118.88, 116.90, 107.70, 70.74, 40.95; HRMS calculated for $C_{14}H_{13}N_3NaO_7S$ $(M + Na)^+$ 390.0372, found 390.0364.

***N*-[2-(β-Naphthylmethoxy)-4-nitrophenyl]methanesulfonamide (3b).** It is stirred in 3 N NaOH aq solution at 85−90 °C overnight. Pale yellow solid, 95%: mp 239−243 °C (decomp); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (1H, s), 7.92 (3H, m), 7.83 (1H, s), 7.80 (1H, d, J = 2.5 Hz), 7.68 (1H, dd, J = 8.4, 1.5 Hz), 7.53 (2H, m), 7.45 (1H, J = 8.4 Hz), 5.44 (2H, s), 3.01 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.72, 135.00, 133.60, 133.48, 128.91, 128.67, 128.51, 127.40, 127.23, 127.06, 126.72, 119.89, 118.77, 109.17, 71.30, 41.54; HRMS calculated for $C_{18}H_{16}N_2NaO_5S$ $(M + Na)^+$ 395.0678, found 395.0685.

***N*-[2-(2′-Phenylbenzyloxy)-4-nitrophenyl]methanesulfonamide (4b).** It is stirred in 3 N NaOH aq solution at 85−90 °C overnight. Yellow solid, 94%: mp 116−119 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (2H, dd, J = 9.1, 3.0 Hz), 7.51 (2H, d, J = 7.0 Hz), 7.31 (7H, m), 7.19 (1H, d, J = 9.2 Hz), 4.93 (2H, s), 2.73 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.33, 142.49, 140.87, 135.02, 130.63, 130.52, 130.02, 129.10, 129.00, 128.33, 128.21, 120.32, 116.59, 109.09, 69.14, 41.27; HRMS calculated for $C_{20}H_{18}N_2NaO_5S$ $(M + Na)^+$ 421.0834, found 421.0840.

***N*-Methyl-*N*-[2-(4′-nitrobenzyloxy)-4-nitrophenyl]methanesulfonamide (2c).** Compound **2b** (0.11 g, 0.3 mmol) was dissolved in 4 mL of dry DMF, and K_2CO_3 powder (0.083 g, 0.6 mmol) was added. The mixture was stirred at room temperature for 10 min, iodomethane (0.085 g, 0.6 mmol) was added, and then it was stirred at 45−50 °C overnight. Then the mixture was taken up with 7 mL of water and 2 mL of Na_2CO_3 aq solution. The precipitated solid was collected by filtration and washed with water and cold ether to afford a white solid, 108 mg (94%): mp 209−211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (2H, d, J = 8.7 Hz), 8.00 (1H, d, J = 2.3 Hz), 7.89(1H, dd, J = 8.7, 2.4 Hz), 7.80 (2H, d, J = 8.6 Hz), 7.64 (1H, d, J = 8.7 Hz), 5.53 (2H, s), 3.22 (3H, s), 3.05 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.92, 148.23, 148.12, 144.44, 136.65, 132.64, 129.43, 124.65, 117.12, 109.49, 70.33, 39.17, 38.12. HRMS calculated for $C_{15}H_{15}N_3NaO_7S$ $(M + Na)^+$ 404.0528, found 404.0501. Anal. ($C_{15}H_{15}N_3O_7S$) C, H, N.

General Procedure for the Preparation of 3c−14c. Methanesulfonamide compound (0.5 mmol) was dissolved in 3 mL of dry DMF, and NaH powder (15.2 mg 95%, 0.6 mmol, 1.2 equiv) was added. The mixture was stirred at room temperature for 10 min, iodomethane (0.6 mmol, 1.2 equiv) was added, and stirring was maintained for 2 h at room temperature. Then the mixture was taken up with 7 mL of water and 2 mL of Na_2CO_3 aq solution. The precipitated solid was collected by filtration, washed with water and cold ether to afford the desired product, and then recrystallized from ethyl acetate/hexane.

***N*-Methyl-*N*-[2-(β-naphthylmethoxy)-4-nitrophenyl]methanesulfonamide (3c).** Pale yellow solid, 96%: mp 119−121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (1H, d, J = 2.4 Hz), 7.96 (1H, s), 7.89 (4H, m), 7.55 (4H, m), 5.39 (2H, s), 3.30 (3H, s), 2.84 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.73, 148.43, 135.84, 133.79, 133.58, 133.06, 132.30, 129.44, 128.45, 128.27, 128.08, 127.29, 127.23, 125.80, 117.03, 108.52, 72.16, 38.98, 37.96. HRMS calculated for $C_{19}H_{18}N_2NaO_5S$ $(M + Na)^+$ 409.0834, found 409.0825. Anal. ($C_{19}H_{18}N_2O_5S$) C, H, N.

***N*-Methyl-*N*-[2-(2′-phenylbenzyloxy)-4-nitrophenyl]methanesulfonamide (4c).** Pale yellow solid, 82%: mp 128−129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (1H, dd, J = 8.6, 2.4 Hz), 7.66 (1H, d, J = 2.4 Hz), 7.37 (10H, m), 5.19 (2H, s), 3.24 (3H, s), 2.83 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.51, 148.34, 142.74, 140.20, 132.81, 132.31, 131.05, 129.88, 129.55, 129.41, 129.04, 128.38, 128.24, 116.88, 108.59, 69.76, 38.94, 38.00. HRMS calculated for $C_{21}H_{20}N_2NaO_5S$ $(M + Na)^+$ 435.0991, found 435.0996. Anal. ($C_{21}H_{20}N_2O_5S$) C, H, N.

General Procedure for the Preparation of 5a−14a. K_2CO_3 (0.69 g, 5 mmol) and aryl halide (5 mmol, 1.0 equiv) were successively added to a solution of 2-amino-5-nitrophenol (0.77 g, 5 mmol) in DMF or acetone (10 mL), and the mixture was stirred at room temperature or 75−80 °C from 3 h to overnight. After the mixture was cooled, 20 mL of H_2O and 5 mL of saturated aqueous Na_2CO_3 were added to the mixture and the precipitated solid was collected by filtration and washed with H_2O and cold ethyl ether. If the product precipitated as an oil, the aqueous phase was extracted with CH_2Cl_2. The organic solution was washed with saturated aqueous Na_2CO_3 solution and H_2O, dried over anhydrous $MgSO_4$, and concentrated. The residue was chromatographed on silica gel [AcOEt−hexane (1:5)] to afford desired compounds.

2-(4′-Methylbenzyloxy)-4-nitroaniline (5a). 4-Methylbenzyl chloride was used, and it was stirred in DMF at 75−80 °C for 3 h.

Yellow solid, 85%: mp 149−150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (1H, dd, J = 8.7, 2.3 Hz), 7.79 (1H, d, J = 2.3 Hz), 7.35 (2H, d, J = 8.0 Hz), 7.24 (2H, d, J = 7.8 Hz), 6.66 (1H, d, J = 8.7 Hz), 5.12 (2H, s), 4.61(2H, s), 2.41 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.97, 143.93, 138.97, 138.87, 133.11, 129.86, 128.49, 119.75, 112.31, 107.63, 71.23, 21.67.

2-(4′-Methoxylbenzyloxy)-4-nitroaniline (6a). 4-Methoxylbenzyl chloride was used, and it was stirred in DMF at 75−80 °C for 3 h. Yellow solid, 89%: mp 131−132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (1H, dd, J = 8.7, 2.3 Hz), 7.79 (1H, d, J = 2.3 Hz), 7.38 (2H, d, J = 8.7 Hz), 6.95 (2H, d, J = 8.7 Hz), 6.65 (1H, d, J = 8.7 Hz), 5.09 (2H, s), 4.60(2H, s), 3.85 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.25, 144.96, 143.94, 138.96, 130.14, 128.17, 119.74, 114.54, 112.30, 107.65, 71.10, 55.75.

2-(4′-Isopropylbenzyloxy)-4-nitroaniline (7a). 4-Isopropylbenzyl chloride was used, and it was stirred in DMF at 75−80 °C for 3 h. Yellow solid, 91%: mp 99−101 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (1H, dd, J = 8.7, 2.4 Hz), 7.80 (1H, d, J = 2.4 Hz), 7.39 (2H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.0 Hz), 6.66 (1H, d, J = 8.7 Hz), 5.13 (2H, s), 4.60(2H, s), 2.95 (1H, m), 1.29 (6H, d, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.84, 145.04, 143.88, 133.48, 128.57, 127.25, 119.74, 112.30, 107.54, 71.23, 34.34, 24.38.

2-(4′-Fluorobenzyloxy)-4-nitroaniline (8a). 4-Fluorobenzyl chloride was used, and it was stirred in DMF at room temperature overnight. Yellow solid, 98%: mp 125−127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (1H, dd, J = 8.7, 2.3 Hz), 7.78 (1H, d, J = 2.3 Hz), 7.43 (2H, m), 7.08 (2H, m), 6.73 (1H, d, J = 8.7 Hz), 5.15 (2H, s), 5.05 (2H, br); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.43, 161.97, 145.28, 131.83, 130.32, 130.24, 119.70, 116.26, 116.04, 113.38, 107.69, 70.66.

2-(4′-Chlorobenzyloxy)-4-nitroaniline (9a). 4-Chlorobenzyl chloride was used, and it was stirred in DMF at room temperature overnight. Yellow solid, 90%: mp 128−130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (1H, dd, J = 8.7, 2.3 Hz), 7.75 (1H, d, J = 2.3 Hz), 7.41 (4H, s), 6.68 (1H, d, J = 8.7 Hz), 5.13 (2H, s), 4.61 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.64, 143.84, 138.95, 134.86, 134.60, 129.64, 129.41, 119.97, 112.50, 107.64, 70.49.

2-(4′-Bromobenzyloxy)-4-nitroaniline (10a). 4-Bromobenzyl bromide was used, and it was stirred in DMF at room temperature overnight. Yellow solid, 66%: mp 131−133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (1H, dd, J = 8.7, 2.3 Hz), 7.75 (1H, d, J = 2.3 Hz), 7.55 (2H, d, J = 8.4 Hz), 7.33 (2H, d, J = 8.5 Hz), 6.67 (1H, d, J = 8.7 Hz), 5.12 (2H, s), 4.62 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.62, 143.84, 138.94, 135.12, 132.36, 129.92, 129.09, 122.99, 119.98, 112.51, 107.63, 70.51.

2-(4′-Phenylbenzyloxy)-4-nitroaniline (11a). 4-Phenylbenzyl chloride was used, and it was stirred in DMF at room temperature for 48 h. Yellow solid, 82%: mp 151−153 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (1H, dd, J = 8.7, 2.3 Hz), 7.82 (1H, d, J = 2.3 Hz), 7.61 (4H, m), 7.54 (2H, d, J = 8.4 Hz), 7.46 (2H, m), 7.40 (1H, m), 6.74 (1H, d, J = 8.6 Hz), 5.21 (2H, s), 5.01 (2H, br); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.33, 141.94, 140.88, 139.56, 134.99, 129.28, 128.84, 128.53, 128.01, 127.90, 119.69, 113.11, 107.70, 71.09.

2-Phenylethoxy-4-nitroaniline (12a). Phenylethyl bromide was used, and it was refluxed in DMF for 48 h. Yellow solid, 28%: mp 83−85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (1H, dd, J = 8.7, 2.4 Hz), 7.68 (1H, d, J = 2.4 Hz), 7.29 (5H, m), 6.62 (1H, d, J = 8.7 Hz), 4.48 (2H, br), 4.31 (2H, dd, J = 6.8, 6.8 Hz), 3.17 (2H, dd, J = 6.7, 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.92, 143.82, 139.04, 138.12, 129.30, 129.08, 127.18, 119.65, 112.30, 107.39, 69.74, 35.96.

2-(α-Naphthylmethoxy)-4-nitroaniline (13a). α-Naphthylmethyl chloride was used, and it was stirred in DMF at room temperature overnight. Yellow solid, 98%: mp 197−199 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (1H, d, J = 8.0 Hz), 7.94 (2H, m), 7.90 (1H, d, J = 2.4 Hz), 7.76 (2H, m), 7.52 (3H, m), 6.70 (1H, d, J = 8.9 Hz), 6.39 (2H, br), 5.70 (2H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.08, 144.24, 136.39, 134.15, 132.85, 131.91, 129.57, 129.32, 127.41, 127.35, 126.88, 126.27, 124.81, 120.78, 112.06, 108.25, 69.17.

2-(3′,6′-Dimethylbenzyloxy)-4-nitroaniline (14a). 3,6-Dimethylbenzyl chloride was used, and it was stirred in DMF at 75−80 °C for 3h. Yellow solid, 93%: mp 160−161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (1H, dd, J = 8.7, 2.2 Hz), 7.73 (1H, d, J = 2.3 Hz), 7.32 (1H, s), 7.11 (1H, d, J = 7.7 Hz), 7.07 (1H, d, J = 7.7 Hz), 6.70 (1H, dd, J = 8.7, 1.0 Hz), 6.39 (2H, br), 5.15(2H, s), 2.30 (3H, s), 2.29 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.05, 144.37, 136.36, 135.57, 135.00, 134.41, 130.94, 130.14, 129.54, 120.68, 112.00, 107.83, 69.45, 21.44, 18.93.

General Procedure for the Preparation of Compounds 5b−14b. NaH (95% powder, 0.265 g, 10.5 mmol, 3.5 equiv) was added to a solution of aryl-substituted 2-amino-5-nitrophenol (3.0 mmol) in anhydrous DMF (8 mL) at room temperature. After the mixture was stirred at the same temperature for 30 min, MsCl (1.031 g, 9.0 mmol, 3 equiv) was added and the stirring was continued overnight at room temperature. H_2O was added to the mixture, and then it was neutralized with 5 N HCl until pH 1−2. The intermediate precipitated as a yellow solid. It was collected by filtration, washed with H_2O, and then used without further purification in the next reaction. The intermediate was added to a 3 N NaOH aq solution and was stirred at 80−90 °C overnight. After cooling, it was neutralized with 5 N HCl until pH 1−2. The precipitated solid was collected and washed with H_2O and cold ether to provide the desired product, and then it was recrystallized from ethyl acetate/hexane.

***N*-[2-(4′-Methylbenzyloxy)-4-nitrophenyl]methanesulfonamide (5b).** Pale yellow solid, 87%: mp 151−152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (1H, dd, J = 8.9, 2.4 Hz), 7.91 (1H, d, J = 2.3 Hz), 7.67 (1H, d, J = 8.8 Hz), 7.32 (2H, d, J = 8.0 Hz), 7.25 (2H, d, J = 7.9 Hz), 5.19 (2H, s), 3.08 (3H, s), 2.41 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.20, 144.15, 139.64, 133.42, 131.73, 130.17, 128.77, 118.19, 116.96, 107.91, 72.25, 40.59, 21.68; HRMS calculated for $C_{15}H_{16}N_2NaO_5S$ $(M + Na)^+$ 359.0678, found 359.0670.

***N*-[2-(4′-Methoxylbenzyloxy)-4-nitrophenyl]methanesulfonamide (6b).** Pale yellow solid, 86%: mp 150−152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (1H, dd, J = 8.8, 2.4 Hz), 7.91 (1H, d, J = 2.2 Hz), 7.67 (1H, d, J = 8.8 Hz), 7.36 (2H, d, J = 8.6 Hz), 6.97 (2H, d, J = 8.6 Hz), 5.16 (2H, s), 3.86 (3H, s), 3.08 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.72, 147.19, 144.13, 133.42, 130.50, 126.73, 118.16, 116.91, 114.87, 107.90, 72.12, 55.80, 40.60; HRMS calculated for $C_{15}H_{16}N_2NaO_6S$ $(M + Na)^+$ 375.0627, found 375.0625.

***N*-[2-(4′-Isopropylbenzyloxy)-4-nitrophenyl]methanesulfonamide (7b).** Pale yellow solid, 86%: mp 171−172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (1H, dd, J = 8.8, 2.5 Hz), 7.92 (1H, d, J = 2.4 Hz), 7.68 (1H, d, J = 8.8 Hz), 7.35 (2H, d, J = 8.2 Hz), 7.31 (2H, d, J = 8.3 Hz), 7.25 (1H, br), 5.18 (2H, s), 3.08 (3H, s), 2.96 (1H, m), 1.30 (6H, d, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.63, 147.25, 144.18, 133.39, 132.02, 128.97, 127.60, 118.20, 116.92, 107.84, 72.30, 40.58, 34.39, 24.33; HRMS calculated for $C_{17}H_{20}N_2NaO_5S$ $(M + Na)^+$ 387.0991, found 387.0981.

***N*-[2-(4′-Fluorobenzyloxy)-4-nitrophenyl]methanesulfonamide (8b).** Yellow solid, 83%: mp 162−164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (1H, dd, J = 8.9, 2.4 Hz), 7.89 (1H, d, J = 2.4 Hz), 7.68 (1H, d, J = 8.9 Hz), 7.41 (2H, m), 7.25 (1H, br), 7.13 (2H, m), 5.19 (2H, s), 3.11 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.79, 162.32, 146.93, 144.06, 133.38, 130.76, 130.68, 130.60, 130.56, 118.38, 116.86, 116.67, 116.46, 107.76, 71.54, 40.71; HRMS calculated for $C_{14}H_{13}FN_2NaO_5S$ $(M + Na)^+$ 363.0427, found 363.0417.

***N*-[2-(4′-Chlorobenzyloxy)-4-nitrophenyl]methanesulfonamide (9b).** Pale yellow solid, 90%: mp 171−173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (1H, dd, J = 8.9, 2.4 Hz), 7.87 (1H, d, J = 2.3 Hz), 7.68 (1H, d, J = 8.9 Hz), 7.43(2H, d, J = 8.5 Hz), 7.37(2H, d, J = 8.5 Hz), 5.20 (2H, s), 3.11 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.85, 144.04, 135.62, 133.38, 133.19, 130.01, 129.76, 118.46, 116.89, 107.77, 71.43, 40.74; HRMS calculated for $C_{14}H_{13}ClN_2NaO_5S$ $(M + Na)^+$ 379.0131, found 379.0136.

***N*-[2-(4′-Bromobenzyloxy)-4-nitrophenyl]methanesulfonamide (10b).** Pale yellow solid, 82%: mp 183−185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (1H, dd, J = 8.9, 2.4 Hz), 7.87 (1H, d, J = 2.3 Hz), 7.68 (1H, d, J = 8.9 Hz), 7.59 (2H, d, J = 8.4 Hz), 7.31(2H, d, J = 8.4 Hz), 5.18 (2H, s), 3.11 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.81, 144.04, 133.69, 133.38, 132.73, 130.25, 123.78, 118.48, 116.89, 107.78, 71.47, 40.75; HRMS calculated for $C_{14}H_{13}BrN_2NaO_5S$ $(M + Na)^+$ 422.9626, found 422.9636.

***N*-[2-(4′-Phenylbenzyloxy)-4-nitrophenyl]methanesulfonamide (11b).** Yellow solid, 94%: mp 253−255 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (1H, br), 7.87 (1H, s), 7.84 (1H, d, J = 2.5 Hz), 7.64 (6H, m), 7.46 (3H, m), 7.36 (1H, m), 5.35 (2H, s), 3.08 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.70, 140.76, 140.61, 136.25, 129.82, 129.43, 128.41, 127.61, 127.56, 118.32, 108.80, 70.89, 41.53; HRMS calculated for $C_{20}H_{18}N_2NaO_5S$ $(M + Na)^+$ 421.0834, found 421.0815.

***N*-(2-Phenylethoxy 4-nitrophenyl)methanesulfonamide (12b).** Yellow solid, 77%: mp 120−121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (1H, dd, J = 8.9, 2.5 Hz), 7.78 (1H, d, J = 2.4 Hz), 7.63 (1H, d, J = 8.9 Hz), 7.38 (2H, m), 7.29(3H, m), 7.05 (1H, br), 4.38 (2H, dd, J = 6.6, 6.6 Hz), 3.19 (2H, dd, J = 6.6, 6.6 Hz), 2.89 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.40, 144.33, 137.55, 133.23, 129.43, 129.21, 127.52, 118.10, 117.42, 107.43, 70.54, 40.27, 35.76; HRMS calculated for $C_{15}H_{16}N_2NaO_5S$ $(M + Na)^+$ 359.0678, found 359.0668. Anal. ($C_{15}H_{16}N_2O_5S$) C, H, N.

***N*-[2-(α-Naphthylmethoxy) 4-nitrophenyl]methanesulfonamide (13b).** Pale yellow solid, 83%: mp 193−195 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (1H, d, J = 2.4 Hz), 7.96 (4H, m), 7.69 (1H, d, J = 8.9 Hz), 7.58 (3H, m), 7.53(1H, d, J = 8.1 Hz), 7.14 (1H, br), 5.66 (2H, s), 2.90 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.24, 144.24, 134.39, 133.62, 132.03, 130.81, 130.23, 129.58, 128.62, 127.58, 126.83, 125.71, 123.37, 118.44, 117.33, 108.20, 71.07, 40.35; HRMS calculated for $C_{18}H_{16}N_2NaO_5S$ $(M + Na)^+$ 395.0678, found 395.0686.

***N*-[2-(3′,6′-Dimethylbenzyloxy)-4-nitrophenyl]methanesulfonamide (14b).** Pale yellow solid, 93%: mp 178−179 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (1H, d, J = 2.5 Hz), 7.88 (1H, dd, J = 8.9, 2.5 Hz), 7.55 (1H, d, J = 8.9 Hz), 7.32 (1H, s), 7.14 (1H, d, J = 7.7 Hz), 7.08 (1H, d, J = 7.6 Hz), 5.25 (2H, s), 3.10 (3H, s), 2.32 (3H, s), 2.28 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.09, 144.19, 135.59, 134.80, 134.47, 131.01, 130.73, 129.85, 121.28, 117.81, 108.61, 70.30, 41.61, 21.45, 18.97; HRMS calculated for $C_{16}H_{18}N_2NaO_5S$ $(M + Na)^+$ 373.0834, found 373.0829.

Preparation of 5c−14c with General Procedures: *N*-Methyl-*N*-[2-(4′-methylbenzyloxy)-4-nitrophenyl]methanesulfonamide (5c). Yellow solid, 87%: mp 96−98 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (1H, d, J = 2.4 Hz), 7.88 (1H, dd, J = 8.6, 2.4 Hz), 7.55 (1H, d, J = 8.6 Hz), 7.33 (2H, d, J = 8.0 Hz), 7.25 (2H, d, J = 7.8 Hz), 5.18 (2H, s), 3.27 (3H, s), 2.83 (3H, s), 2.41 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.72, 148.42, 139.48, 135.73, 133.14, 131.93, 130.10, 128.73, 116.86, 108.46, 71.89, 38.90, 37.90, 21.69; HRMS calculated for $C_{16}H_{18}N_2NaO_5S$ $(M + Na)^+$ 373.0834, found 373.0825. Anal. ($C_{16}H_{18}N_2O_5S$) C, H, N.

***N*-Methyl-*N*-[2-(4′-methoxylbenzyloxy)-4-nitrophenyl]methanesulfonamide (6c).** Pale yellow solid, 92%: mp 108−109 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (1H, d, J = 2.4 Hz), 7.87 (1H, dd, J = 8.6, 2.5 Hz), 7.54 (1H, d, J = 8.6 Hz), 7.37 (2H, d, J = 8.6 Hz), 6.96 (2H, d, J = 8.7 Hz), 5.15 (2H, s), 3.86 (3H, s), 3.25 (3H, s), 2.81(3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.58, 155.70, 148.39, 135.78, 133.05, 130.42, 126.95, 116.83, 114.76, 108.44, 71.73, 55.75, 38.88, 37.88; HRMS calculated for $C_{16}H_{18}N_2NaO_6S$ $(M + Na)^+$ 389.0783, found 389.0774.

***N*-Methyl-*N*-[2-(4′- isopropylbenzyloxy)-4-nitrophenyl]methanesulfonamide (7c).** Yellow solid, 85%: mp 83−85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (1H, d, J = 2.4 Hz), 7.88 (1H, dd, J = 8.6, 2.4 Hz), 7.56 (1H, d, J = 8.6 Hz), 7.37 (2H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.1 Hz), 5.19 (2H, s), 3.28 (3H, s), 2.95 (1H, m), 2.83 (3H, s), 1.29 (6H, d, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.74, 150.40, 148.43, 135.72, 133.16, 132.28, 128.70, 127.49, 116.87, 108.48, 71.87, 38.89, 37.93, 34.32, 24.32; HRMS calculated for $C_{18}H_{22}N_2NaO_5S$ $(M + Na)^+$ 401.1147, found 401.1143.

***N*-Methyl-*N*-[2-(4′-fluorobenzyloxy)-4-nitrophenyl]methanesulfonamide (8c).** Pale yellow solid, 92%: mp 168−169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (1H, d, J = 2.4 Hz), 7.90 (1H, dd, J = 8.4, 2.4 Hz), 7.55 (1H, d, J = 8.6 Hz), 7.44 (2H, m), 7.13 (2H, m), 5.20 (2H, s), 3.27 (3H, s), 2.84 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.67, 162.20, 155.57, 148.36, 135.86, 132.87, 130.89, 130.86, 130.63, 130.55, 117.10, 116.62, 116.41, 108.46, 71.24, 38.97, 37.93; HRMS calculated for $C_{15}H_{15}FN_2NaO_5S$ $(M + Na)^+$ 377.0583, found 377.0586. Anal. ($C_{15}H_{15}FN_2O_5S$) C,H,N.

***N*-Methyl-*N*-[2-(4′-chlorobenzyloxy)-4-nitrophenyl]methanesulfonamide (9c).** Pale yellow solid, 93%: mp 168−170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (2H, m), 7.55 (1H, m), 7.40 (4H, m), 5.20 (2H, s), 3.27 (3H, s), 2.86 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.54, 148.36, 135.85, 135.49, 133.48, 132.87, 129.91, 129.72, 117.17, 108.48, 71.15, 39.03, 37.95; HRMS calculated for $C_{15}H_{15}ClN_2NaO_5S$ $(M + Na)^+$ 393.0288, found 393.0268.

***N*-Methyl-*N*-[2-(4′-bromobenzyloxy)-4-nitrophenyl]methanesulfonamide (10c).** Pale yellow solid, 90%: mp 151−152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (2H, m), 7.55 (3H, m), 7.34 (2H, d, J = 8.3 Hz), 5.19 (2H, s), 3.28 (3H, s), 2.86 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.52, 148.36, 135.86, 133.99, 132.87, 132.68, 130.15, 123.63, 117.19, 108.48, 71.18, 39.05, 37.96; HRMS calculated for $C_{15}H_{15}BrN_2NaO_5S$ $(M + Na)^+$ 436.9783, found 436.9791.

***N*-Methyl-*N*-[2-(4′-phenylbenzyloxy)-4-nitrophenyl]methanesulfonamide (11c).** Pale yellow solid, 94%: mp 152−153 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (1H, d, J = 2.3 Hz), 7.90 (1H, dd, J = 8.6, 2.3 Hz), 7.68 (2H, d, J = 8.1 Hz), 7.63 (2H, dd, J = 7.8, 1.4 Hz),7.48 (5H, m), 7.41 (1H, d, J = 7.4 Hz), 5.28 (2H, s), 3.31 (3H, s), 2.88 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.70, 148.42, 142.42, 140.56, 135.80, 133.87, 133.08, 129.35, 129.05, 128.20, 128.12, 127.52, 117.02, 108.53, 71.71, 39.00, 37.97; HRMS calculated for $C_{21}H_{20}N_2NaO_5S$ $(M + Na)^+$ 435.0991, found 435.0985.

***N*-Methyl-*N*-(2-phenylethoxy 4-nitrophenyl)methanesulfonamide (12c).** Yellow solid, 93%: mp 126−128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (2H, m), 7.52 (1H, m), 7.35 (2H, m), 7.30 (2H, m), 4.45 (2H, dd, J = 6.5, 6.5 Hz), 3.21 (2H, dd, J = 6.4, 6.4 Hz), 3.13 (3H, s), 2.63 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.73, 148.45, 137.51, 135.20, 133.53, 129.32, 129.08, 127.55, 116.73, 108.00, 69.83, 38.34, 37.60, 35.76; HRMS calculated for $C_{16}H_{18}N_2NaO_5S$ $(M + Na)^+$ 373.0834, found 373.0829.

***N*-Methyl-*N*-[2-(α-naphthylmethoxy) 4-nitrophenyl]methanesulfonamide (13c).** Pale yellow solid, 94%: mp 163−165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (1H, d, J = 2.4 Hz), 8.01 (1H, dd, J = 6.2, 3.5 Hz), 7.92 (3H, m), 7.51 (5H, m), 5.65 (2H, s), 3.13 (3H, s), 2.55 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.79, 148.46, 135.89, 134.28, 133.22, 131.88, 130.70, 130.57, 129.46, 128.38, 127.32, 126.84, 125.77, 123.74, 117.07, 108.37, 71.37, 38.62, 37.96; HRMS calculated for $C_{19}H_{18}N_2NaO_5S$ $(M + Na)^+$ 409.0834, found 409.0842.

***N*-Methyl-*N*-[2-(3′,6′-dimethylbenzyloxy)-4-nitrophenyl]-methanesulfonamide (14c).** Yellow solid, 87%: mp 137−139 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (1H, d, J = 2.5 Hz), 7.86 (1H, dd, J = 8.6, 2.5 Hz), 7.61 (1H, d, J = 8.6 Hz), 7.30 (1H, s), 7.14 (1H, d, J = 7.7 Hz), 7.11 (1H, d, J = 7.6 Hz), 5.28 (2H, s), 3.15 (3H, s), 2.94 (3H, s), 2.32 (3H, s), 2.28 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.34, 148.19, 136.80, 135.72, 134.52, 134.36, 132.01, 131.06, 130.56, 129.97, 116.75, 109.33, 70.17, 39.04, 38.07, 21.44, 18.84; HRMS calculated for $C_{17}H_{20}N_2NaO_5S$ $(M + Na)^+$ 387.0991, found 387.0983.

5.2. Biological Study. 5.2.1. Cell Culture. JAR and SK-BR-3 cells were obtained from ATCC (Rockville, MD). SK-BR-3 cells were maintained in phenol red-free custom media (MEM, Earle's salts, 1.5x amino acids, 2x nonessential amino acids, L-glutamine, 1.5x vitamins, Gibco BRL) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 20 mg/L gentamycin. JAR cells were maintained in RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L

glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, 90%/FBS, 10%. 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. For all experiments, cells were plated in six-well plates and grown to subconfluency. Before treatment, the media was changed to a defined one containing DMEM/F12 media (Sigma) with 1.0 mg/mL human albumin (OSU Hospital Pharmacy), 5.0 mg/L human transferrin, and 5.0 mg/L bovine insulin.

5.2.2. Tritiated Water-Release Assay in JAR and SK-BR-3 Cell Lines. Measurement of aromatase enzyme activity was based on the tritium water release assay. Cells in six-well plates were treated with 0.1% DMSO (control) and inhibitors at the indicated concentrations. After 24 h, the cells were incubated 3 h (SK-BR-3 cells) or 1 h (JAR cells) with fresh media along with 100 nM [1β-^{3}H]-androst-4-ene-3,17-dione (1 μCi). Subsequently, the reaction mixture was removed, and proteins were precipitated using 10% trichloroacetic acid at 42 °C for 20 min. After a brief centrifugation, the media was extracted three times with an equal amount of chloroform to remove remaining substrate and further treated with dextran-treated charcoal. After centrifugation, a 250-μL aliquot containing the product was counted in 5 mL of liquid scintillation mixture. Results were corrected for blanks and for the cell contents of culture flasks, and results were expressed as picomoles of ^3H_2O formed per hour incubation time per million live cells (pmol/h/10^6 cells). To determine the amount of cells in each flask, the cells were lysed and analyzed using the diphenylamine DNA assay adapted to a 96-well plate. IC_{50} sigmoidal dose−response data were analyzed with Microsoft Excel and the Graphpad Prism (Version 3.0) program.

5.2.3. Diphenylamine DNA Assay. To determine the amount of viable cells in each flask, the cells were lysed with 0.5 N NaOH aqueous solution and analyzed using the diphenylamine DNA assay adapted to a 96-well plate. DNA standards (0−30 μg) were prepared using double-stranded DNA reconstituted in PBS and added in triplicate directly to the wells. A uniform cell suspension was prepared from the six-well plate in 300 μL of 0.5 N NaOH aqueous solution, and 60 μL of the unknown samples was added in triplicate to separate wells. A solution of 0.16% acetaldehyde in water was prepared and mixed at a 1:5 ratio with perchloric acid (20% vol/vol). This solution (60 μL) was added to each well along with 100 μL of a 4% diphenylamine solution in glacial acetic acid. The plates were incubated at 37 °C for 24 h. After centrifugation, 100 μL supernatant of each well was transferred to a new 96-well plate, and the OD_{595} was measured using a microplater reader. The DNA concentration was determined by extrapolation to the standard curve, and the amount of cells/well was calculated using the equation:

$$1 \text{ cell} \approx 7 \text{ pg DNA}.$$

5.2.4. RNA Extraction. Total RNA was isolated using the TRIzol reagent according to the manufacturer's protocol. Total RNA pellets were dissolved in Dnase,RNase-free water and quantitated using a spectrophotometer. The quality of RNA samples was determined by electrophoresis through agarose gels and staining with ethidium bromide; the 18S and 28S rRNA bands were visualized under ultraviolet light.

5.2.5. cDNA Synthesis. Isolated total RNA (2 μg) was treated with DNase I Amplification grade, according to the recommended protocol to eliminate any DNA before reverse transcription. Treated total RNA was denatured at 65 °C for 5 min in the presence of 2.5 ng/μL random hexamers and 0.5 mM dNTP mix. The samples were snap-cooled on ice and centrifuged briefly. Complementary DNA (cDNA) was synthesized using Superscript II reverse transcriptase according to the recommended protocol. Briefly, the reactions were conducted in the presence of 1X First-Strand Buffer and 20 mM DTT at 42 °C for 50 min and consequently inactivated at 70 °C for 15 min. The cDNA generated was used as a template in real-time PCR reactions.

5.2.6. Real-Time PCR. Real-time PCR was performed using the Opticon 2 system from MJ Research (Waltham, MA). For the *CYP19* total gene, the PCR reaction mixture consisted of Taqman Universal PCR Master Mix (Applied Biosystems), 600 nM *CYP19* primer (sense: 5′-TGT CTC TTT GTT CTT CAT GCT ATT TCT C-3′; antisense: 5′-TCA CCA ATA ACA GTC TGG ATT TCC-3′); 250 nM Taqman probe (6FAM 5′-TGC AAA GCA CCC TAA TGT TGA AGA GGC AAT-3′TAMRA)(Invitrogen), and 2.0 μL of each cDNA sample in a final volume of 20 μL. For the 18S house-keeping total gene, the PCR reaction mixture consisted of Taqman Universal PCR Master Mix (Applied Biosystems), 500 nM 18S primer (sense: 5′-CAG TTC ATA CAG CGG AAC ACT G-3′; antisense: 5′-TTT GCT GGA GAA CAG GGC TG-3′), 50 nM Taqman probe (6FAM 5′-TGC TGG CAC CAG ACT TGC CCT C-3′TAMRA) (Invitrogen), and 2.0 μL of each cDNA sample in a final volume of 20 μL. The Taqman probes for aromatase and 18S were designed to anneal to a specific sequence of the aromatase and 18S gene correspondingly between the forward and the reverse primers. Cycling conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 50 cycles at 95 °C for 15 s and 60 °C for 1 min.

5.2.7. Cell Viability Analysis. The effect of nimesulides derivatives on SK-BR-3 cell viability was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide assay in six replicates. Cells were grown in custom media 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 defined media for different time intervals. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. The medium was removed and replaced by 200 μL of 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-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-2*H*-tetrazolium bromide dye was solubilized in 200 μL/well DMSO. Absorbance at 570 nm was determined on a plate reader.

5.2.8. Analysis of Apoptosis. Apoptosis was determined by selective denaturation of DNA in apoptotic cells by formamide and detection of denatured DNA with a monoclonal antibody to single-stranded DNA using an ELISA kit (CHEMICON, Temecula, CA). Cells were plated in a 96-well flat bottom plate from 0.5×10^4 to 1×10^4 cells/well in custom media. Cells were allowed to adhere to wells overnight. Following incubation, compounds were made up in defined media, and a 5 μM screen was performed in each cell line with respective compounds in triplicate for 24 h. Following treatment, the plate was centrifuged at 200*g* for 5 min and media was removed followed by the addition of 200 μL of fixative. The plate was incubated for 30 min at 37 °C, at which point the fixative was removed and the plate dried for 1−2 h at room temperature. Fifty microliters of formamide was added to each well following a brief incubation at room temperature for 10 min. The DNA in apoptotic cells was denatured by heating the plate for 10 min and then briefly cooling the plate for 5 min at 4 °C following removal of formamide. The plate was rinsed three times with 200 μL of PBS following 1 h incubation at 37 °C with 200 μL of 3% blocking agent. After removal of the blocking agent, 100 μL of antibody mixture was added to each well for 30 min at room temperature. The plate was washed three times with 1X wash buffer using 250 μL of wash buffer/well followed by the addition of 200 μL of ABTS solution added to each well for a 15−60 min incubation. The reaction was stopped by the addition of 100 μL of a stop solution added to each well, and absorbance was measured at 405 nm on a SpectroMax 340 UV PlateReader.

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Supporting Information Available: Elemental analysis for compounds **1f**, **2c**, **3c**, **4c**, **5c**, **8c**, and **12b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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