Novel Sulfonanilide Analogs Decrease Aromatase Activity in Breast Cancer Cells: Synthesis, Biological Evaluation, and Ligand-Based Pharmacophore Identification

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Aromatase converts androgens to estrogens and is a particularly attractive target in the treatment of estrogen receptor positive breast cancer. Previously, the COX-2 selective inhibitor nimesulide and analogs decreased aromatase expression and enzyme activity independent of COX-2 inhibition. In this manuscript, a combinatorial approach was used to generate diversely substituted novel sulfonanilides by parallel synthesis. Their pharmacological evaluation as agents for suppression of aromatase activity in SK-BR-3 breast cancer cells was extensively explored. A ligand-based pharmacophore model was elaborated for selective aromatase modulation (SAM) using the Catalyst HipHop algorithms. The best qualitative model consisted of four features: one aromatic ring, two hydrogen bond acceptors, and one hydrophobic function. Several lead compounds have also been tested in aromatase transfected MCF-7 cells, and they significantly suppressed cellular aromatase activity. The results suggest that both genomic and nongenomic mechanisms of these compounds are involved within the aromatase suppression effect.

1. Introduction

Breast cancer is the second leading cause of cancer death in women in the United States. About 178480 women in the United States will be found to have invasive breast cancer in 2007. About 40460 women will die from the disease this year. Approximately over 2 million women living in the United States have been treated for breast cancer.[1] Two-thirds of breast cancers are hormone-dependent, contain estrogen receptors (ERs[a]), and require estrogen for tumor growth. These patients are, therefore, suitable candidates for hormonal therapy, which targets blocking estrogen stimulation of breast cancer cells. This can be achieved by different approaches.[2,3] In postmenopausal women, who are characterized by the absence of ovarian production of estrogen, ovarian suppression is not required. Two strategies that ameliorate the effects of estrogen in promoting breast cancer are currently being compared in clinical trials of postmenopausal patients with ER-positive breast cancer. Antiestrogens (e.g., tamoxifen and fulvestrant) block binding of estrogen to its receptor, whereas aromatase inhibitors (e.g., letrozole, anastrozole, and exemestane) block estrogen biosynthesis. AIs are now proving to be more effective and increase survival.[4–6]

Tamoxifen has been the mainstay of hormonal therapy in both early and advanced breast cancer patients for approximately three decades. Almost all patients with ER-positive tumors in Western countries have been treated with this drug either as adjuvant treatment following surgery or as first-line treatment for advanced disease. However, approximately 50% of patients with advanced disease do not respond to first-line treatment with tamoxifen. Furthermore, almost all patients with metastatic disease and approximately 40% of the patients that receive tamoxifen as adjuvant therapy experience tumor relapse and die from their disease. These findings strongly suggest primary or acquired resistance to tamoxifen occurs in breast cancer patients and affect the efficacy of this treatment.[7–10]

Significant improvements in the efficacy of endocrine therapy for ER-positive breast cancer have resulted from the introduction of AIs, which induce estrogen deprivation in postmenopausal women.[7] The "third generation AIs" are potent and highly selective in their inhibition of the cytochrome P450 enzyme, aromatase. As a consequence, they efficiently block conversion of androgens (testosterone and androstenedione) to estrogens (estradiol and estrone, respectively) in peripheral tissues and breast tumors.[11] Of the three aromatase inhibitors (AIs) currently approved for breast cancer treatment, letrozole and anastrozole are nonsteroidal triazole compounds, whereas exemestane is a steroidal analog of androstenedione. In recent years, AIs have become first-line hormonal therapy for ER-positive patients with advanced breast cancer. However, these compounds inhibit aromatase activity throughout the body and could adversely impact sites where estrogen is required for normal function, such as bone and brain. As a consequence, other approaches are being investigated to develop agents that can decrease aromatase activity selectively.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are beneficial in breast cancer treatment.[12] Recently, it has been shown that the COX-2 selective inhibitor, celecoxib, has strong chemopreventive activity against mammary carcinoma in rats.[13] In addition to COX inhibition, these small molecules can also target other molecular pathways. For example, celecoxib can block phosphoinositide 3-kinase (PI3K)/phosphoinositide-dependent kinase (PDK)/Akt pathway to induce apoptosis in prostate cancer cells.[14,15]

The COX-2 inhibitor nimesulide suppressed the development of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary gland carcinogenesis in rats.[16,17] Research in our laboratory demonstrated that nimesulide also suppressed aromatase activity and expression in several breast cancer cell lines.[18] Two small libraries of nimesulide analogs have been

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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.

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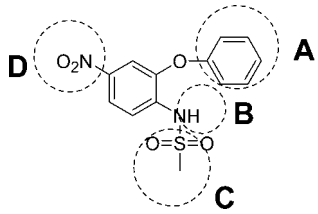


Figure 1. Structural modifications of nimesulide.

synthesized in our laboratory. Their pharmacological effect on aromatase has been extensively studied.[19,20] In this manuscript, a combinatorial chemistry approach was used to generate diversely substituted nimesulide derivatives by parallel synthesis. Their pharmacological evaluation as agents for suppression aromatase activity in breast cancer cells was also further explored. A ligand-based pharmacophore model was identified by a qualitative approach, that is, Catalyst HipHop algorithms, based on the nimesulide derivatives library. The best qualitative model consisted of four features: one aromatic ring, two hydrogen bond acceptors, and one hydrophobic function. Several lead compounds also significantly decreased aromatase activity in aromatase transfected MCF-7 cells. The results suggest that both genomic and nongenomic mechanisms of these compounds are involved within the aromatase suppression effect.

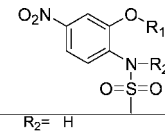
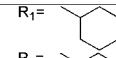
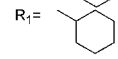
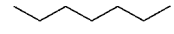
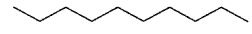
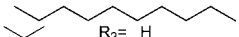
2. Results and Discussion

2.1. Compound Design. In this study, 81 novel sulfonanilides are described and are 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**–**46**, as reported previously.[19,20] Alkyl or substituted benzyl groups at the B position were introduced to produce compounds **47**−**60**. Next, the C position was modified with benzamide, substituted benzamide, or substituted benzenesulfonamide to produce compounds **61**−**72**. Last, the D position nitro group was reduced to the amine moiety and substituted benzamide groups were then introduced to generate compounds **73**−**81**. This parallel combinatory strategy can easily be extended to produce hundreds of new analogs, which is well suited for future lead modification and drug development.

2.1. Pharmacological Evaluation of Known Nimesulide Analogs. We tested some of the nimesulide derivatives in SK-BR-3 breast cancer cells of their IC_{50} for aromatase suppression (compounds in Table 1 and part of the compounds in Table 2) and also their COX-2 inhibition in breast cancer cells.[19] The results suggest that selective aromatase suppression is independent of the COX-2 inhibition. The functionality at the B-position is very critical for the COX-2 inhibitory activity. The compounds inhibit COX-2 only when the N−H is available in the ionized form. Introduction of any group in the B-position eliminates this ionization process and produces compounds with no COX-2 inhibitory activity.[21] However, this ionization is not important for aromatase suppression. On the contrary, blocking this position results in better agents for suppression of aromatase expression and activity.[19]

For the purpose of building detailed structure–activity relationships to facilitate the future drug development, all the compounds synthesized previously were investigated here for their IC_{50} in suppression of aromatase in SK-BR-3 breast cancer cells. The results suggest that introducing short bulky alkyl or bulky substituted benzyl group to the A-position is better for the aromatase suppression (Tables 1 and 2). Compounds **1**, **2**, **5**, **6**, **9**, **10**, **25**, and **26** decreased aromatase activity in SK-BR-3 cells, with IC_{50} around 0.5 μM. At the B-position, methyl is slightly better than hydrogen in most of the compounds. However, methyl and hydrogen are insufficient to fully characterize this position, and additional substituted compounds at the B-position were synthesized and discussed next.

Table 1. Suppression of Aromatase Activity in SK-BR-3 Breast Cancer Cells by Compounds with Modifications at the A-Position with Alkyl Groups

Compd		IC_{50} (μM)[a]
1	R_1=  R_2= H	0.68 ± 0.37
2	R_1=  R_2= CH_3	0.47 ± 0.22
3	R_1= CH_3 R_2= H	>25
4	R_1= CH_3 R_2= CH_3	>25
5	R_1=  R_2= H	0.87 ± 0.64
6	R_1=  R_2= CH_3	0.44 ± 0.15
7	R_1=  R_2= H	1.21 ± 0.34
8	R_1=  R_2= CH_3	0.96 ± 0.27
9	R_1=  R_2= H	0.30 ± 0.20
10	R_1=  R_2= CH_3	0.23 ± 0.12
11	R_1=  R_2= H	2.51 ± 1.04
12	R_1=  R_2= CH_3	0.76 ± 0.22
13	R_1=  R_2= H	2.67 ± 1.13
14	R_1=  R_2= CH_3	4.49 ± 1.97
15	R_1=  R_2= H	0.91 ± 0.50
16	R_1=  R_2= CH_3	3.96 ± 2.35
17	R_1=  R_2= H	5.87 ± 1.72
18	R_1=  R_2= CH_3	2.81 ± 1.36

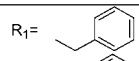
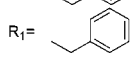
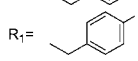
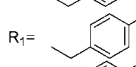
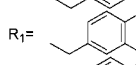
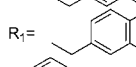
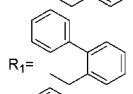
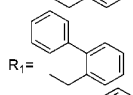
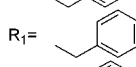
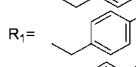
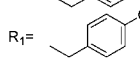
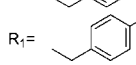
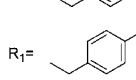
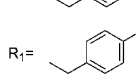
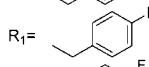
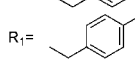
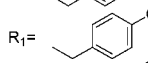
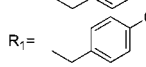
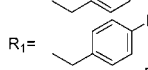
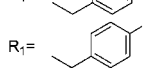
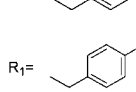
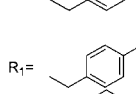
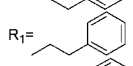
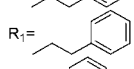
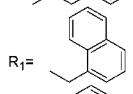
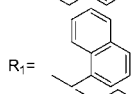
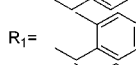
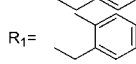
[a] From ref 19.

2.2. Parallel Synthesis of Diversely Substituted Sulfonanilides. Modifications at the A-position have been extensively explored previously.[19,20] In the current study, we mainly focus on modifications of the B-, C-, and D-position of nimesulide.

The B-position modifications are described in Scheme 1. The starting material 2-amino-5-nitrophenol was refluxed with K_2CO_3 and benzyl bromide to obtain compound **1a**. 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**). Compound **1b** was hydrolyzed with 10% NaOH solution to generate compound **19** as a monomethanesulfonamido compound. Compound **19** was treated with K_2CO_3 and substituted benzyl chloride/bromide, alkyl bromide, or iodine in DMF at room temperature or refluxed to obtain compounds **47**–**60**, respectively.

Modifications at the C-position are described in Scheme 2. Compound **1a** was treated with different acyl chlorides and K_2CO_3 to generate the carboxamides **61**–**70**. For aryl sulfonamide compounds, sodium hydride and aryl sulfonyl chloride were added to compound **1a** in dry dimethylformamide (DMF) at room temperature, and the reaction mixture was stirred overnight to obtain compounds **71** and **72**.

Table 2. Suppression of Aromatase Activity in SK-BR-3 Breast Cancer Cells by Compounds with Modifications at the A-Position with Aryl Groups

Compd	R_1	R_2	IC_{50} (μM)
19		H	2.14 ± 0.55
20[b]		CH_3	0.81 ± 0.29
21		H	0.40 ±0.08
22[b]		CH_3	0.49 ± 0.14
23		H	2.17 ± 1.37
24[b]		CH_3	2.68 ± 0.91
25		H	0.26 ± 0.20
26[b]		CH_3	0.33 ± 0.15
27		H	1.75 ± 1.49
28[b]		CH_3	2.33 ± 0.66
29		H	7.82 ± 3.42
30		CH_3	0.47 ± 0.44
31		H	7.45 ± 5.58
32		CH_3	Cytotoxicity
33		H	0.58 ± 0.47
34[b]		CH_3	1.78 ± 0.63
35		H	0.29 ± 0.27
36		CH_3	2.40 ± 1.52
37		H	5.85 ± 2.10
38		CH_3	0.33 ± 0.26
39		H	0.85 ± 0.37
40		CH_3	0.65 ± 0.26
41		H	0.23 ± 0.12
42		CH_3	0.52 ± 0.33
43		H	5.74 ± 2.92
44		CH_3	1.17 ± 0.70
45		H	0.37 ± 0.30
46		CH_3	cytotoxicity

[b] From ref 20.

Modifications at the D-position are described in Scheme 3. First, the D-position nitro was reduced to amine to obtain compound **73** (Scheme 1), then **73** was treated with different acyl chlorides and K_2CO_3 to generate the carboxamides **74**–**81**, respectively.

This parallel combinatory strategy can produce numerous new analogs to facilitate lead optimization and future drug development. The structure of all the synthesized compounds in the following biological study were determined by ^{1}H NMR and HRMS and the purity is confirmed by HPLC.

2.3. Biological Evaluation of the Sulfonanilides Substituted at the B-, C-, and D-Position. The diversely substituted sulfonanilides were evaluated for their ability to suppress aromatase activity in SK-BR-3 breast cancer cells. The results demonstrate that the B-position alkyl- or aryl-substituted compounds lost their biological activity in suppression of aromatase. Compounds **47**–**60** only slightly decreased SK-BR-3 cellular aromatase activity at 50 μM (data not shown).

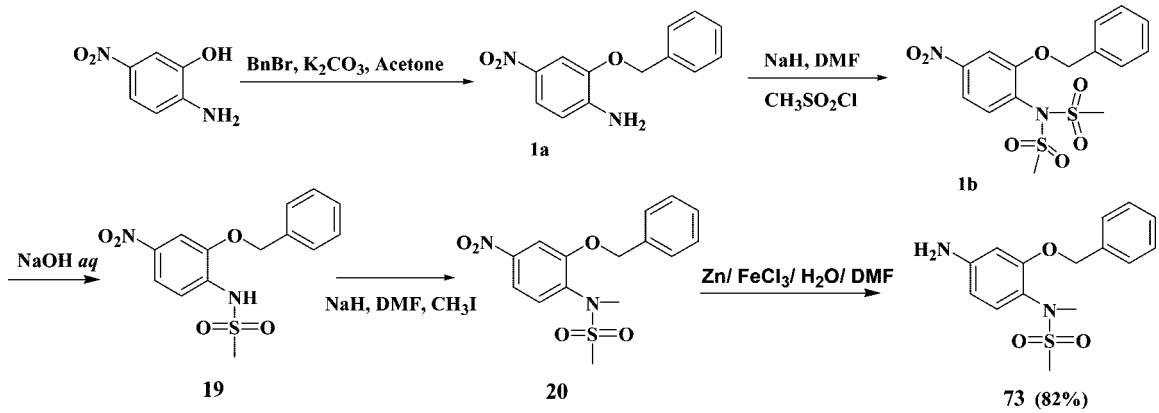
The majority of the C-position benzamide-substituted derivatives did not decrease aromatase activity (Table 3). However, compound **63**, which has an acetyl group at the C-position, showed an IC_{50} of 1.18 μM in suppression of aromatase. Compounds **71** and **72**, which have different phenol sulfonamide groups at the C-position, showed an IC_{50} of 2.17 μM and 17.37 μM, respectively. This suggests that an electron-withdrawing group is better for the aromatase suppression with phenol sulfonamide analogs. Overall, the main factor that controls the suppression of aromatase activity at the C-position is steric effect. Smaller methyl sulfonamide or acetyl groups are the best fit and bulky groups will decrease the pharmacological activity.

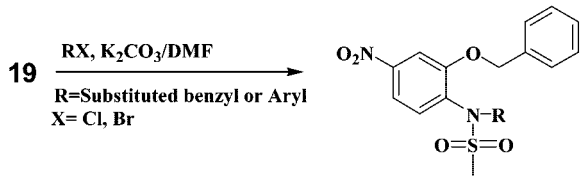
Unexpectedly, introduction of different benzamide groups to the D-position significantly increased the suppression of aromatase activity (Table 4). Compound **73**, which has an amine group at the D-position, showed similar activity as compound **20**, which has a nitro group at the D-position. Introducing an acetyl group to the D-amine (compound **74**, IC_{50} 16.67 μM) significantly decreased the biological activity. On the other hand, other various benzamide-substituted derivatives significantly suppressed cellular aromatase activity, with IC_{50} from 0.12 μM to 1.77 μM (Table 4). Compound **81**, which has a 4-phenol benzamide group at the D-position, is the most active analog in this library, with an IC_{50} of 0.12 μM. Compound **76**, which has a cyclohexaneacetamide group at the D-position, exhibited an IC_{50} of 0.25 μM. These results suggest that bulky hydrophobic groups at the D-position are better for the suppression of aromatase in SK-BR-3 breast cancer cells.

2.4. Ligand-Based Pharmacophore Identification. The range of inhibitory activity (~2 log units) was insufficient to allow us to generate a meaningful activity-based (predictive) pharmacophore model using Catalyst/Hypogen technology. We employed the Catalyst/HipHop approach to evaluate the common feature required for suppression of cellular aromatase activity. Thus, a training set consisting of 10 sulfonanilide derivatives (compounds **2**, **8**, **20**, **22**, **40**, **42**, **63**, **75**, **76**, and **81**), having structural diversity and exhibiting the greatest suppression of aromatase activity, was submitted for pharmacophore model generation based on common chemical features.

The resulting pharmacophore model contained four chemical features: one hydrophobe (blue), two hydrogen bond acceptors (green), and one ring aromatic (yellow) (Figure 2). Compound **41** was mapped onto the model. The hydrophobe maps to the A-position of phenol ring with a two-carbon chain and a hydrogen bond acceptors map to the C-position sulfonamide

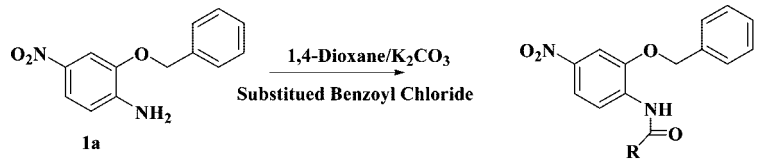
Scheme 1. Modifications of the B-Position



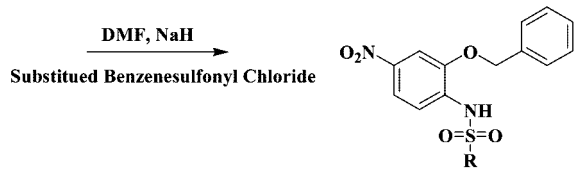


47 R= isopropyl (28%)
48 R= propyl (53%)
49 R= hexyl (66%)
50 R= Benzyl (88%)
51 R= 4-methoxy-benzyl (97%)
52 R= 4-methyl-benzyl (75%)
53 R= 4-fluoro-benzyl (72%)
54 R= 4-chloro-benzyl (75%)
55 R= 4-bromo-benzyl (92%)
56 R= 4-isopropyl-benzyl (91%)
57 R= 2,5-dimethyl-benzyl (75%)
58 R= cyclohexylmethyl (52%)
59 R= biphenyl-4-ylmethyl (71%)
60 R= naphthalen-2-ylmethyl (72%)

Scheme 2. Modifications of the C-Position



61 R= Phenyl (96%)
62 R= Cyclohexanyl (99%)
63 R= Methyl (89%)
64 R= 4-nitro-phenyl (94%)
65 R= 3-nitro-phenyl (91%)
66 R= 4-chloro-3-nitro-phenyl (83%)
67 R= 4-chloro-3-chloro-phenyl (92%)
68 R= 4-cyano-phenyl (84%)
69 R= 2-Naphthalene (91%)
70 R= 4-Biphenyl (57%)



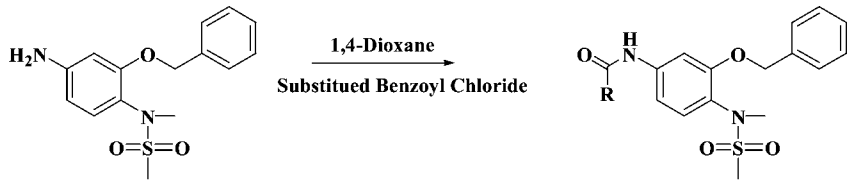
71 R= 4-nitro-phenyl (84%)
72 R= 4-methyl-phenyl (60%)

and the D-position nitro group. The aromatic ring maps with the platform center phenol ring.

From the biological activity and the structure of the respective compounds, the D-position as a hydrophobic area enhances the pharmacological activity. The synthesis of more diverse compounds at the D-position will be necessary to further optimize the model.

2.5. Aromatase Assays in Aromatase Transfected MCF-7 Cells. Our previous study demonstrated that sulfonanilide analogs decrease cellular aromatase activity and transcription. However, the concentrations for the suppression of aromatase activity are much lower than the concentrations for the suppression of CYP19 gene expression.[19] This suggests that these compounds might also suppress cellular aromatase activity with

Scheme 3. Modifications of the D-Position



73

74 R= Methyl (80%)
75 R= Phenyl (93%)
76 R= Cyclohexanyl (90%)
77 R= 4-chloro-3-chloro-phenyl (87%)
78 R= 4-chloro-3-nitro-phenyl (98%)
79 R= 4-cyano-phenyl (97%)
80 R= 2-Naphthalene (96%)
81 R= 4-Biphenyl (81%)

Table 3. Suppression of Aromatase Activity in SK-BR-3 Breast Cancer Cells by Compounds with Modifications at the C-Position

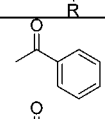
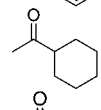
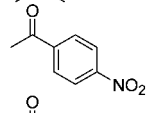
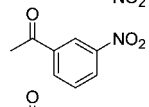
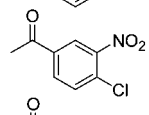
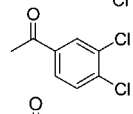
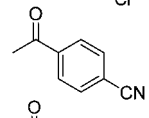
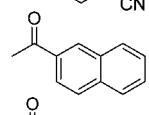
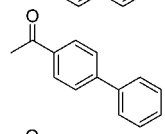
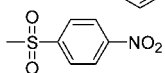
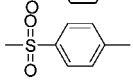
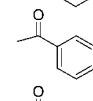
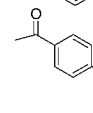
Compd		IC_{50} (μM)
61	R= 	> 25
62	R= 	> 25
63	R= 	1.18 ± 0.34
64	R= 	> 25
65	R= 	> 25
66	R= 	> 25
67	R= 	> 25
68	R= 	> 25
69	R= 	> 25
70	R= 	> 25
71	R= 	2.17 ± 1.22
72	R= 	17.37 ± 11.70

Table 4. Suppression of Aromatase Activity in SK-BR-3 Breast Cancer Cells by Compounds with Modifications at the D-Position

Compd		IC_{50} (μM)
73	R_1 = H R_2 = H	1.10 ± 0.32
74	R_1 =  R_2 = H	16.67 ± 9.75
75	R_1 =  R_2 = H	1.77 ± 0.94
76	R_1 =  R_2 = H	0.25 ± 0.16
77	R_1 =  R_2 = H	0.69 ± 0.49
78	R_1 =  R_2 = H	0.69 ± 0.40
79	R_1 =  R_2 = H	0.44 ± 0.36
80	R_1 =  R_2 = H	1.07 ± 0.59
81	R_1 =  R_2 = H	0.12 ± 0.06

a nongenomic mechanism. Because the compounds do not inhibit aromatase enzyme directly, post-transcriptional modification may be a primary mechanism. Aromatase transfected MCF-7 cells were used to investigate the possible post-transcriptional mechanisms. In these cells, aromatase transcription is controlled by tetracycline, which eliminates the possibility that the compounds decrease CYP19 gene expression.[22] Thus, any suppression of aromatase activity by these compounds in the transfected cells would be the result of nongenomic action.

Compounds **9**, **10**, **25**, **26**, **35**, **38**, **41**, **45**, **76**, **79**, and **81**, which have IC_{50} below 0.5 μM for suppression of aromatase activity in SK-BR-3 cell, were tested in aromatase transfected MCF-7 cells at 1 μM. The results demonstrate that some of the sulfonanilide analogs significantly suppressed cellular aromatase activity (Figure 3). This suggests that these compounds suppress cellular aromatase activity via a nongenomic mechanism in this transfected cell line.

3. Conclusion

In summary, the biological results indicated that novel sulfonanilides significantly decrease aromatase activity at low micromolar to submicromolar concentrations in SK-BR-3 breast cancer cells. These compounds were more active than the lead

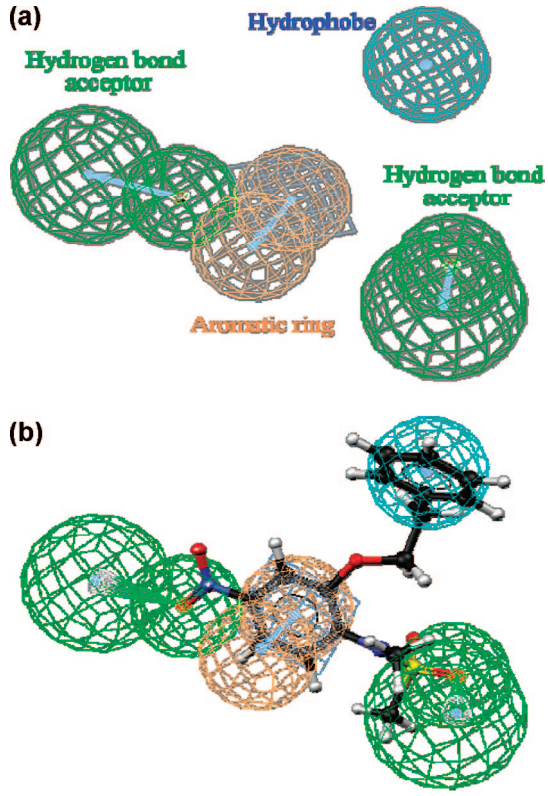


Figure 2. (a) Pharmacophore generated using Catalyst methodology that describes the suppression of aromatase activity of the sulfonanilide derivatives. Pharmacophore features are color-coded (yellow, aromatic ring; blue, hydrophobic group; green, hydrogen bond acceptor). (b) Compound **41** mapped onto the pharmacophore.

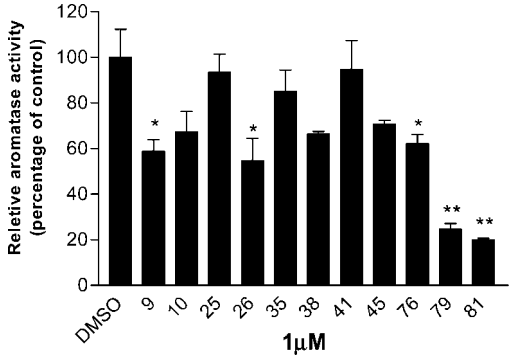


Figure 3. Aromatase activity in MCF-7 transfected aromatase cell treated with sulfonanilide derivatives. Cells were treated with indicated compounds at 1 μM and aromatase activity was measured as described in the Experimental Section. The results were normalized against a control treatment with vehicle. Each data bar represents the mean results of three independent determinations. $*P < 0.05$, $**P < 0.005$.

nimesulide. Structure–activity analysis demonstrated that improved pharmacological activity of the novel sulfonanilides is achieved with substitutions at the A-position as short bulky hydrophobic groups, at the B-position as a hydrogen or methyl group, at the C-position as methyl sulfonamide, and at the D-position as substituted benzamide groups. Ligand-based pharmacophore model identification based on the sulfonanilide library reveals that the best qualitative model consisted of four features: one aromatic ring, two hydrogen bond acceptors, and one hydrophobic function.

Several lead compounds also suppressed aromatase activity in aromatase transfected MCF-7 cells, in which aromatase transcription is controlled by tetracycline. This suggests that nongenomic mechanisms are also involved for this suppression. Because these compounds also suppress CYP19 gene expression in SK-BR-3 breast cancer cells,[19,20] both genomic and nongenomic mechanisms are involved in the suppression of cellular aromatase activity. Our current research focuses on discerning the specific molecular target(s) of these compounds. In addition, the pharmacophore identification of the sulfonanilide analogs will enable further lead optimization for novel drug discovery and examination of the structural requirements for the suppression of cellular aromatase.

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 either a Bruker DPX 250 or a DRX 400 MHz spectrometer 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.

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 vacuum degasser, binary pump, autosampler, column compartment, and a UV detector. Reverse-phase HPLC was carried out on a C18 column (3.0 × 150 mm, 5μm) from Beckman (Beckman Instruments, Fullenton, CA) at room temperature, with a flow rate of 0.5 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 5–15 μL was used. The UV detector was set up at 254 and 330 nm.

Compounds **1a**, **1b**, **19**, and **20** were prepared as described by Su et al.[20]

General Procedure for the Preparation of B-Position-Substituted Nimesulide Analogs 47–60 from Compound 19. K_2CO_3 (0.69 g, 5 mmol) and aryl or alkyl halide (1 mmol, 1.0 equiv) were successively added to a solution of compound **19** (1.2 mmol, 1.2 equiv) in dry DMF and the mixture was stirred at room temperature or 80 °C from several hours to overnight. After being cooled, 5 mL of H_2O and 1 mL of satd 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.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-isopropyl-methanesulfonamide (47).** Isopropyl iodine was used and it was stirred in DMF at 80 °C overnight. Pale yellow solid, yield 28%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.02 (1H, d, J = 2.5 Hz), 7.90 (1H, dd, J = 8.6, 2.6 Hz), 7.56 (3H, m), 7.44 (3H, m), 5.32 (2H, s), 4.24 (1H, m), 2.99 (3H, s), 1.21 (3H, d, J = 5.7 Hz), 1.10 (3H, d, J = 5.6 Hz); HRMS calcd for $C_{17}H_{20}N_2NaO_5S$ $(M + Na)^+$, 387.0991; found, 387.0990.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-propyl-methanesulfonamide (48).** Propyl iodine was used and it was stirred in DMF at 80 °C overnight. Pale yellow solid, yield 53%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.03 (1H, d, J = 2.5 Hz), 7.90 (1H, dd, J = 8.6, 2.5 Hz), 7.61 (6H, m), 5.36 (2H, s), 3.58 (2H, dd, J =

6.9, 6.9 Hz), 2.95 (3H, s), 1.39 (2H, m), 0.86 (3H, dd, $J = 7.3$, 7.3 Hz); HRMS calcd for $C_{17}H_{20}N_2NaO_5S$ $(M + Na)^+$, 387.0991; found, 387.0991.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-hexyl-methanesulfonamide (49).** Hexyl iodine was used and it was stirred in DMF at 80 °C overnight. Yellow solid, yield 66%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.03 (1H, d, $J = 2.5$ Hz), 7.90 (1H, dd, $J = 8.6$, 2.5 Hz), 7.60 (6H, m), 5.36 (2H, s), 3.60 (2H, dd, $J = 6.5$, 6.5 Hz), 2.95 (3H, s), 1.32 (8H, m), 0.84 (3H, dd, $J = 7.0$, 7.0 Hz); HRMS calcd for $C_{20}H_{26}N_2NaO_5S$ $(M + Na)^+$, 429.1460; found, 429.1457.

***N*-Benzyl-*N*-(2-benzyloxy-4-nitro-phenyl)-methanesulfonamide (50).** Benzyl bromide was used and it was stirred in DMF at room temperature overnight. Pale yellow solid, yield 88%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.97 (1H, d, $J = 2.4$ Hz), 7.76 (1H, dd, $J = 8.6$, 2.3 Hz), 7.58 (6H, m), 7.25 (5H, s), 5.36 (2H, s), 4.81 (2H, s), 3.06 (3H, s); HRMS calcd for $C_{21}H_{20}N_2NaO_5S$ $(M + Na)^+$, 435.0991; found, 435.0989.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-(4-methoxy-benzyl)-methanesulfonamide (51).** 4-Methoxy-benzyl chloride was used and it was stirred in DMF at 80 °C overnight. Pale yellow solid, yield 97%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.96 (1H, d, $J = 2.1$ Hz), 7.77 (1H, dd, $J = 8.6$, 2.2 Hz), 7.57 (6H, m), 7.15 (2H, d, $J = 8.5$ Hz), 6.82 (2H, d, $J = 8.5$ Hz), 5.35 (2H, s), 4.73 (2H, s), 3.69 (3H, s), 3.04 (3H, s); HRMS calcd for $C_{22}H_{22}N_2NaO_6S$ $(M + Na)^+$, 465.1096; found, 465.1099.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-(4-methyl-benzyl)-methanesulfonamide (52).** 4-Methyl-benzyl chloride was used and it was stirred in DMF at 80 °C overnight. Pale yellow solid, yield 75%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.95 (1H, dd, $J = 1.8$, 1.8 Hz), 7.75 (1H, m), 7.56 (6H, m), 7.12 (2H, d, $J = 7.8$), 7.07 (2H, d, $J = 7.5$ Hz), 5.35 (2H, s), 4.74 (2H, s), 3.04 (3H, s), 2.22 (3H, s); HRMS calcd for $C_{22}H_{22}N_2NaO_5S$ $(M + Na)^+$, 449.1147; found, 449.1147.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-(4-fluoro-benzyl)-methanesulfonamide (53).** 4-Fluoro-benzyl chloride was used and it was stirred in DMF at 80 °C overnight. White solid, yield 72%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.97 (1H, d, $J = 2.5$ Hz), 7.78 (1H, dd, $J = 2.5$, 8.6 Hz), 7.58 (6H, m), 7.30 (2H, m), 7.12 (2H, m), 5.36 (2H, s), 4.79 (2H, s), 3.06 (3H, s); HRMS calcd for $C_{21}H_{19}FN_2NaO_5S$ $(M + Na)^+$, 453.0896; found, 453.0898.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-(4-chloro-benzyl)-methanesulfonamide (54).** 4-Chloro-benzyl chloride was used and it was stirred in DMF at 80 °C overnight. Pale yellow solid, yield 75%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.97 (1H, d, $J = 2.5$ Hz), 7.79 (1H, dd, $J = 2.5$, 8.6 Hz), 7.57 (10H, m), 5.36 (2H, s), 4.79 (2H, s), 3.07 (3H, s); HRMS calcd for $C_{21}H_{19}ClN_2NaO_5S$ $(M + Na)^+$, 469.0601; found, 469.0599.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-(4-bromo-benzyl)-methanesulfonamide (55).** 4-Bromo-benzyl bromide was used and it was stirred in DMF at room temperature overnight. White solid, yield 92%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.97 (1H, d, $J = 2.5$ Hz), 7.75 (1H, dd, $J = 2.6$, 8.6 Hz), 7.57 (8H, m), 7.22 (2H, d, $J = 8.3$ Hz), 5.36 (2H, s), 4.78 (2H, s), 3.06 (3H, s); HRMS calcd for $C_{21}H_{19}BrN_2NaO_5S$ $(M + Na)^+$, 513.0096; found, 513.0095.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-(4-isopropyl-benzyl)-methanesulfonamide (56).** 4-Isopropyl-benzyl chloride was used and it was stirred in DMF at 80 °C overnight. Pale yellow solid, yield 91%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.98 (1H, d, $J = 2.5$ Hz), 7.79 (1H, dd, $J = 2.5$, 8.6 Hz), 7.57 (6H, m), 7.14 (4H, s), 5.35 (2H, s), 4.77 (2H, s), 3.04 (3H, s), 2.85 (1H, m), 1.16 (6H, d, $J = 6.9$ Hz); HRMS calcd for $C_{24}H_{26}N_2NaO_5S$ $(M + Na)^+$, 477.1460; found, 477.1461.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-(2,5-dimethyl-benzyl)-methanesulfonamide (57).** 2,5-Dimethyl-benzyl chloride was used and it was stirred in DMF at 80 °C overnight. Pale yellow solid, yield 75%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.95 (1H, d, $J = 2.5$ Hz), 7.76 (1H, dd, $J = 2.6$, 8.6 Hz), 7.59 (2H, d, $J = 6.7$ Hz), 7.50 (4H, m), 6.96 (3H, m), 5.36 (2H, s), 4.79 (2H, s), 3.04 (3H, s), 2.16 (3H, s), 2.13 (3H, s); HRMS calcd for $C_{23}H_{24}N_2NaO_5S$ $(M + Na)^+$, 463.1304; found, 463.1307.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-cyclohexylmethyl-methanesulfonamide (58).** Cyclohexylmethyl bromide was used and it was stirred in DMF at 80 °C for four days. Pale yellow solid, yield 52%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.03 (1H, d, $J = 2.5$ Hz), 7.90 (1H, dd, $J = 2.5$, 8.6 Hz), 7.62 (2H, d, $J = 8.6$ Hz), 7.54 (5H, m), 5.36 (2H, s), 3.44 (2H, d, $J = 7.1$ Hz), 2.93 (3H, s), 1.71 (5H, m), 1.09 (6H, m); HRMS calcd for $C_{21}H_{26}N_2NaO_5S$ $(M + Na)^+$, 441.1460; found, 441.1461.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-biphenyl-4-ylmethyl-methanesulfonamide (59).** Biphenyl-4-ylmethyl chloride was used and it was stirred in DMF at 80 °C overnight. Yellow solid, yield 71%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.99 (1H, d, $J = 2.5$ Hz), 7.64 (17H, m), 5.37 (2H, s), 4.86 (2H, s), 3.08 (3H, s); HRMS calcd for $C_{27}H_{24}N_2NaO_5S$ $(M + Na)^+$, 511.1304; found, 511.1308.

***N*-(2-Benzyloxy-4-nitro-phenyl)-*N*-naphthalen-2-ylmethyl-methanesulfonamide (60).** Naphthalen-2-ylmethyl bromide was used and it was stirred in DMF at room temperature overnight. Pale yellow solid, yield 72%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.95 (1H, d, $J = 2.5$ Hz), 7.86 (5H, m), 7.59 (9H, m), 5.38 (2H, s), 4.98 (2H, s), 3.11 (3H, s); HRMS calcd for $C_{25}H_{22}N_2NaO_5S$ $(M + Na)^+$, 485.1147; found, 485.1148.

General Procedure for the Preparation of C-Position-Substituted Nimesulide Analog Compounds 61–70 from Compounds 1a. K_2CO_3 (0.69 g, 5 mmol) and substituted acyl chloride (1.2 mmol, 1.2 equiv) were successively added to a solution of compound **1a** (1.0 mmol, 1.0 equiv) 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 of H_2O and 3 mL of satd 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 the desired compounds.

***N*-(2-Benzyloxy-4-nitro-phenyl)-benzamide (61).** Benzoyl chloride was used and it was stirred at room temperature overnight. White solid, yield 96%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.77 (1H, s), 8.30 (1H, d, $J = 9.6$ Hz), 7.99 (4H, m), 7.65 (5H, m), 7.39 (3H, m), 5.41 (2H, s); HRMS calcd for $C_{20}H_{16}N_2NaO_4$ $(M + Na)^+$, 371.1008; found, 371.1009.

Cyclohexanecarboxylic Acid (2-Benzyloxy-4-nitro-phenyl)-amide (62). Cyclohexanecarbonyl chloride was used and it was stirred at room temperature overnight. White solid, yield 99%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.39 (1H, s), 8.32 (1H, dd, $J = 5.3$, 9.6 Hz), 7.88 (2H, m), 7.56 (2H, d, $J = 7.8$ Hz), 7.42 (3H, m), 5.41 (2H, s), 2.64 (1H, m), 1.86 (5H, m), 1.43 (5H, m); HRMS calcd for $C_{20}H_{22}N_2NaO_4$ $(M + Na)^+$, 377.1477; found, 377.1477.

***N*-(2-Benzyloxy-4-nitro-phenyl)-acetamide (63).** Acetyl chloride was used and it was stirred at room temperature overnight. White solid, yield 89%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.59 (1H, s), 8.36 (1H, d, $J = 9.6$ Hz), 7.89 (2H, m), 7.57 (2H, dd, $J = 1.6$, 6.6 Hz), 7.45 (3H, m), 5.41 (2H, s), 2.21 (3H, s); HRMS calcd for $C_{15}H_{14}N_2NaO_4$ $(M + Na)^+$, 309.0851; found, 309.0850.

***N*-(2-Benzyloxy-4-nitro-phenyl)-4-nitro-benzamide (64).** 4-Nitro-benzoyl chloride was used and it was stirred at room temperature overnight. Pale yellow solid, yield 94%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.26 (1H, s), 8.42 (1H, d, J = 8.9 Hz), 8.20 (3H, m), 7.99 (2H, d, J = 7.3 Hz), 7.58 (2H, d, J = 7.2 Hz), 7.45 (3H, m), 5.41 (2H, s); HRMS calcd for $C_{20}H_{15}N_3NaO_6$ $(M + Na)^+$, 416.0859; found, 416.0859.

***N*-(2-Benzyloxy-4-nitro-phenyl)-3-nitro-benzamide (65).** 3-Nitro-benzoyl chloride was used and it was stirred at room temperature overnight. Pale yellow solid, yield 91%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.31 (1H, s), 8.77 (1H, d, J = 2.0 Hz), 8.47 (2H, dd, J = 1.0, 8.8 Hz), 8.20 (1H, d, J = 8.6 Hz), 8.00 (3H, m), 7.60 (2H, d, J = 7.7 Hz), 7.42 (3H, m), 5.41 (2H, s); HRMS calcd for $C_{20}H_{15}N_3NaO_6$ $(M + Na)^+$, 416.0859; found, 416.0856.

***N*-(2-Benzyloxy-4-nitro-phenyl)-4-chloro-3-nitro-benzamide (66).** 4-Chloro-3-nitro-benzoyl chloride was used and it was stirred at room temperature overnight. White solid, yield 83%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.31 (1H, s), 8.61 (1H, d, J = 2.1 Hz), 8.26 (1H, dd, J = 2.1, 8.4 Hz), 8.16 (1H, dd, J = 1.1, 9.1 Hz), 8.02 (3H, m), 7.58 (2H, d, J = 7.8 Hz), 7.44 (3H, m), 5.41 (2H, s); HRMS calcd for $C_{20}H_{14}ClN_3NaO_6$ $(M + Na)^+$, 450.0469; found, 450.0468.

***N*-(2-Benzyloxy-4-nitro-phenyl)-4-chloro-3-chloro-benzamide (67).** 3,4-Dichloro-benzoyl chloride was used and it was stirred at room temperature overnight. White solid, yield 92%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.07 (1H, s), 8.17 (2H, dd, J = 1.9, 9.0 Hz), 7.97 (4H, m), 7.59 (2H, d, J = 7.0 Hz), 7.44 (3H, m), 5.39 (2H, s); HRMS calcd for $C_{20}H_{14}Cl_2N_2NaO_4$ $(M + Na)^+$, 439.0228; found, 439.0225.

***N*-(2-Benzyloxy-4-nitro-phenyl)-4-cyano-benzamide (68).** 4-Cyano-benzoyl chloride was used and it was stirred at room temperature overnight. White solid, yield 84%: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (1H, s), 8.18 (2H, d, J = 9.0 Hz), 8.11 (4H, m), 7.97 (2H, m), 7.56 (2H, d, J = 4.6 Hz), 7.43 (3H, m), 5.40 (2H, s); HRMS calcd for $C_{21}H_{15}N_3NaO_4$ $(M + Na)^+$, 396.0960; found, 396.0958.

Naphthalene-2-carboxylic Acid (2-Benzyloxy-4-nitro-phenyl)-amide (69). Naphthalene-2-carbonyl chloride was used and it was stirred at room temperature for two days. White solid, yield 91%: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (1H, s), 8.57 (1H, s), 8.33 (1H, dd, J = 0.9, 5.7 Hz), 8.10 (6H, m), 7.69 (4H, m), 7.45 (3H, m), 5.43 (2H, s); HRMS calcd for $C_{24}H_{18}N_2NaO_4$ $(M + Na)^+$, 421.1164; found, 421.1165.

Biphenyl-4-carboxylic Acid (2-Benzyloxy-4-nitro-phenyl)-amide (70). Biphenyl-4-carbonyl chloride was used and it was stirred at 80 °C for three days. Pale yellow solid, yield 57%: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (1H, s), 8.31 (1H, d, J = 9.3 Hz), 8.06 (2H, d, J = 8.5 Hz), 7.98 (2H, dd, J = 2.0, 7.1 Hz), 7.88 (2H, d, J = 8.4 Hz), 7.79 (2H, dd, J = 1.3, 8.5 Hz), 7.61 (2H, d, J = 7.5 Hz), 7.54 (2H, dd, J = 7.3, 7.3 Hz), 7.46 (3H, m), 7.37 (1H, m), 5.42 (2H, s); HRMS calcd for $C_{26}H_{20}N_2NaO_4$ $(M + Na)^+$, 447.1321; found, 447.1322.

General Procedure for the Preparation of C-Position-Substituted Nimesulide Analogs, Compounds 71 and 72 from Compounds 1a. NaH (95%, 1 mmol) was added to a solution of **1a** (0.4 mmol) in anhydrous DMF (3 mL) at room temperature. After being stirred at the same temperature for 30 min, substituted benzene sulfonyl chloride (0.4 mmol) was added to the mixture slowly and the stirring was continued for 30 min at room temperature. H_2O (5 mL) was added, and the solution was acidified by adding 2 N HCl until pH = 2–3. It was stirred at room temperature overnight. The precipitated solid was collected by filtration and washed with H_2O and a cold ether/hexane mixture to afford the desired compounds.

***N*-(2-Benzyloxy-4-nitro-phenyl)-4-nitro-benzenesulfonamide (71).** 4-Nitro-benzene sulfonyl chloride was used. Pale yellow solid, yield 60%: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (1H, s), 8.24 (2H, d, J = 8.9 Hz), 7.98 (2H, d, J = 8.7 Hz), 7.87 (1H, dd, J = 2.4, 8.7 Hz), 7.81 (1H, d, J = 2.3 Hz), 7.55 (1H, d, J = 8.8 Hz), 7.34 (5H, m), 5.11 (2H, s); HRMS calcd for $C_{19}H_{15}N_3NaO_7S$ $(M + Na)^+$, 452.0528; found, 452.0531.

***N*-(2-Benzyloxy-4-nitro-phenyl)-4-methyl-benzenesulfonamide (72).** 4-Methyl-benzene sulfonyl chloride was used. Pale yellow solid, yield 84%: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (1H, s), 7.81 (4H, m), 7.52 (1H, d, J = 8.8 Hz), 7.39 (7H, m), 5.21 (2H, s), 2.34 (3H, s); HRMS calcd for $C_{20}H_{18}N_2NaO_5S$ $(M + Na)^+$, 421.0834; found, 421.0836.

***N*-(4-Amino-2-benzyloxy-phenyl)-*N*-methyl-methanesulfonamide (73).** A mixture of ferric chloride (4 mmol, 4 equiv) and nitrobenzene compound **20** (1 mmol, 1 equiv) were added to a solvent mixture of dimethyl formamide and water (6:1, 7 mL). It was stirred for 30 min, and then zinc dust (10 mmol, 10 equiv) was added slowly. After completion of the reaction (10 min, monitored by TLC), the reaction mixture was filtered, bypassing the celite pad. The filtrate was diluted with water and basified by adding satd aqueous Na_2CO_3. The precipitated solid was collected by filtration, dried, and then dissolved in acetone. After filtration of the insoluble residues, the desired compound was recovered by distillation of the acetone under reduce pressure. Pale yellow solid, yield 82%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.50 (5H, m), 6.91 (1H, d, J = 8.3 Hz), 6.36 (1H, d, J = 2.2 Hz), 6.14 (1H, dd, J = 2.3, 8.3 Hz), 5.29 (2H, s), 5.07 (2H, s), 3.07 (3H, s), 2.79 (3H, s); HRMS calcd for $C_{15}H_{18}N_2NaO_3S$ $(M + Na)^+$, 329.0936; found, 329.0936.

General Procedure for the Preparation of D-Position-Substituted Nimesulide Analogs 74–81 from Compound 73. K_2CO_3 (0.69 g, 5 mmol) and substituted acyl chloride (1.2 mmol, 1.2 equiv) were successively added to a solution of compound **73** (1.0 mmol, 1.0 equiv) 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 of H_2O and 3 mL of satd 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.

***N*-[3-Benzyloxy-4-(methanesulfonyl-methyl-amino)-phenyl]-acetamide (74).** Acetyl chloride was used and it was stirred at room temperature overnight. White solid, yield 80%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.09 (1H, s), 7.56 (6H, m), 7.23 (2H, m), 5.13 (2H, s), 3.12 (3H, s), 2.87 (3H, s), 2.05 (3H, s); HRMS calcd for $C_{17}H_{20}N_2NaO_4S$ $(M + Na)^+$, 371.1041; found, 371.1044.

***N*-[3-Benzyloxy-4-(methanesulfonyl-methyl-amino)-phenyl]-benzamide (75).** Benzoyl chloride was used and it was stirred at room temperature overnight. Pale yellow solid, yield 93%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.39 (1H, s), 7.98 (2H, d, J = 6.8 Hz), 7.79 (1H, s), 7.63 (10H, m), 5.18 (2H, s), 3.15 (3H, s), 2.89 (3H, s); HRMS calcd for $C_{22}H_{22}N_2NaO_4S$ $(M + Na)^+$, 433.1198; found, 433.1197.

Cyclohexanecarboxylic Acid [3-Benzyloxy-4-(methanesulfonyl-methyl-amino)-phenyl]-amide (76). Cyclohexanecarbonyl chloride was used and it was stirred at room temperature overnight. Pale yellow solid, yield 90%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.96 (1H, s), 7.65 (1H, s), 7.53 (5H, m), 7.22 (2H, m), 5.13 (2H, s), 3.11 (3H, s), 2.86 (3H, s), 2.37 (1H, m), 1.77

(5H, m), 1.48 (5H, m); HRMS calcd for $C_{22}H_{28}N_2NaO_4S$ (M + Na)$^+$, 439.1667; found, 439.1667.

***N*-[3-Benzyloxy-4-(methanesulfonyl-methyl-amino)-phenyl]-3,4-dichloro-benzamide (77).** 3,4-Dichloro benzoyl chloride was used and it was stirred at room temperature overnight. White solid, yield 87%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.25 (1H, s), 8.23 (1H, s), 7.94 (2H, m), 7.74 (1H, s), 7.55 (7H, m), 5.18 (2H, s), 3.15 (3H, s), 2.90 (3H, s); HRMS calcd for $C_{22}H_{20}Cl_2N_2NaO_4S$ (M + Na)$^+$, 501.0419; found, 501.0420.

***N*-[3-Benzyloxy-4-(methanesulfonyl-methyl-amino)-phenyl]-4-chloro-3-nitro-benzamide (78).** 4-Chloro-3-nitro benzoyl chloride was used and it was stirred at room temperature overnight. White solid, yield 98%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.67 (1H, s), 8.65 (1H, s), 8.29 (1H, d, J = 8.5 Hz), 8.02 (1H, d, J = 8.5 Hz), 7.73 (1H, s), 7.46 (7H, m), 5.19 (2H, s), 3.15 (3H, s), 2.90 (3H, s); HRMS calcd for $C_{22}H_{20}ClN_3NaO_6S$ (M + Na)$^+$, 512.0659; found, 512.0658.

***N*-[3-Benzyloxy-4-(methanesulfonyl-methyl-amino)-phenyl]-4-cyano-benzamide (79).** 4-Cyano benzoyl chloride was used and it was stirred at room temperature overnight. White solid, yield 97%: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.59 (1H, s), 8.12 (2H, d, J = 8.3 Hz), 8.06 (2H, d, J = 8.3 Hz), 7.76 (1H, d, J = 1.8 Hz), 7.54 (2H, d, J = 7.2 Hz), 7.45 (5H, m), 5.18 (2H, s), 3.14 (3H, s), 2.89 (3H, s); HRMS calcd for $C_{23}H_{21}N_3NaO_4S$ (M + Na)$^+$, 458.1150; found, 458.1151.

Naphthalene-2-carboxylic Acid [3-Benzyloxy-4-(methanesulfonyl-methyl-amino)-phenyl]-amide (80). Naphthalene-2-carbonyl chloride was used and it was stirred at room temperature overnight. Pale yellow solid, yield 96%: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.58 (1H, s), 8.60 (1H, s), 8.13 (4H, m), 7.84 (1H, s), 7.67 (9H, m), 5.21 (2H, s), 3.16 (3H, s), 2.91 (3H, s); HRMS calcd for $C_{26}H_{24}N_2NaO_4S$ (M + Na)$^+$, 483.1354; found, 483.1355.

Biphenyl-4-carboxylic Acid [3-Benzyloxy-4-(methanesulfonyl-methyl-amino)-phenyl]-amide (81). Biphenyl-4-carbonyl chloride was used and it was stirred at room temperature overnight. Pale yellow solid, yield 81%: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (1H, s), 8.09 (1H, d, J = 8.2 Hz), 7.88 (5H, m), 7.56 (10H, m), 5.19 (2H, s), 3.15 (3H, s), 2.90 (3H, s); HRMS calcd for $C_{28}H_{26}N_2NaO_4S$ (M + Na)$^+$, 509.1511; found, 509.1513.

4.2. Biological Study. 4.2.1. Cell Culture. SK-BR-3 cells were obtained from ATCC (Rockville, MD). MCF-7 transfected with aromatase was kindly provided by Dr. Yue Wei (University of Virginia). SK-BR-3 cells were maintained in phenol red-free custom media (MEM, Earle's salts, 1.5× amino acids, 2× nonessential amino acids, L-glutamine, 1.5× vitamins, Gibco BRL) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 20 mg/L gentamycin. MCF-7 transfected with aromatase cells were maintained in DMEM/F12 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. 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 transferin, and 5.0 mg/L bovine insulin.

4.2.2. Tritiated Water-Release Assay in Breast Cancer Cells. 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. For the IC_{50} of aromatase suppression in breast cancer cells, five concentrations are used and each in triplicate. After a 24 h treatment, the cells were incubated 3 h 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.

4.2.3. Diphenylamine DNA Assay. To determine the amount of viable cells in each well, the cells were lysed with 0.5 N NaOH aqueous solution and analyzed in triplicate 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 directly to the wells. A uniform cell suspension was prepared from the six-well-plate in 300 μL 0.5 N NaOH aqueous solution, and 60 μL of the unknown samples were added 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 of the 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.

4.2.4. Computational Methods. All molecular modeling studies were performed using Catalyst 4.11 (Accelrys Inc., San Diego, CA) installed on Silicon Graphics O_2 work station equipped with a 300 MHz MIPS R5000 processor (128 MB RAM) running the Irix 6.5 operating system.

All structures were built and minimized within the Catalyst software package, and conformational analysis for each molecule was implemented using the Poling algorithm. The settings in conformer generation were 250 as the maximum number of conformers, best quality generation type, and an energy range of 20 kcal/mol beyond the calculated potential energy minimum. All other parameters used were kept at their default settings.

The range of inhibitory activity was not broad enough (4–5 orders of magnitude are required) for us to generate a meaningful activity-based (predictive) pharmacophore model using Catalyst/Hypogen technology. We employed the Catalyst/HipHop approach to evaluate the common feature required for suppression of cellular aromatase. Therefore, 10 nimesulide derivatives that have the highest aromatase suppression activity (compounds **2**, **8**, **20**, **22**, **40**, **42**, **63**, **75**, **76**, and **81**) and also structure diversity were submitted for pharmacophore model generation based on common chemical features.

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Supporting Information Available: HPLC analysis for compounds **47**–**81**. This material is available free of charge via the Internet at http://pubs.acs.org.

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