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Lead Optimization of COX-2 Inhibitor Nimesulide Analogs to Overcome Aromatase Inhibitor Resistance in Breast Cancer Cells

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About two thirds of breast cancers are hormone dependent, which contain estrogen receptors (ER) and require estrogen for tu mor growth. These patients are therefore suitable candidates for hormonal therapy, which aims to block estrogen stimulation of breast cancer cell growth.[1] Tamoxifen has been the mainstay of hormonal therapy in both early and advanced breast cancer pa tients for approximately three decades. However, aromatase inhib itors (AIs) are now proving to be more effective and to increase survival more than antiestrogens.[2–5] Over recent years AIs have be come the first line endocrine therapy for ER positive patients with advanced breast cancer.[6] However, after prolonged endocrine ther apy, acquired resistance to AIs is expected to occur in a majority of breast cancer patients.[7–10] The possible resistance mechanism has been investigated in preclinical models in our laboratory and oth ers.[8–11]

The long term estrogen deprivation (LTED) system has been used as a model for AI resistance in several laboratories, mainly due to its lack of a hormone environment that mimics the aroma tase inhibition effect.[8,12–15] It has been reported that the activation of the growth factor signaling pathways in LTED cell lines such as HER2 and insulin like growth factor I receptor, which crosstalk with the ER signaling pathway resulting in an activation of various MAPKs and PI3K/AKT, is responsible for the cell survival and prolif eration.[8–10] Although ER is still functional in LTED cells, the trans activation potential of ER is altered which suggests that ER transcriptional regulation function was partially lost. LTEDaro cell line was generated using an aromatase overexpressing MCF 7 cell line and was suggested to be a late stage endocrine resistance model. Nevertheless, from drug discovery point of view, LTEDaro is a good model for the evaluation of potential compounds to over come AI resistance.[15]

Nonsteroidal anti inflammatory drugs (NSAIDs) are beneficial in breast cancer treatment.[16] It has been reported that COX 2 inhibitor nimesulide suppressed the development of 2 amino 1 methyl 6 phenylimidazo [4,5 *b*] pyridine (PhIP) induced mam mary gland carcinogenesis in rats.[17] Other research demonstrated that nimesulide also suppressed aromatase activity and expression in several breast cancer cell lines.[18] Nimesulide derivatives which do not have COX 2 inhibitory activity were more active than nimesulide to target aromatase.[19,20] Further study reveals that sev eral nimesulide analogs were able to selectively inhibit Her2 over expressing breast cancer cell proliferation, which suggests that they are potentially able to overcome AI resistant breast cancer cell growth.[21] Consequent investigations demonstrated that the com pounds induce LTEDaro cell apoptosis, which exhibited that they can overcome AI resistance for hormone dependent breast cancer. Because of the unique character of nimesulide derivatives, we pro pose that the modification of the structure might change the drug from a COX 2 inhibitor to an anti cancer agent.[20] Furthermore, these new analogs selectively target Her2 overexpressing breast cancer cells which makes them good candidates to overcome AI resistance.

We try to further optimize the structure of nimesulide using the combinatorial strategies to modify the four positions depicted in Figure 1. Previous study demonstrated that B position as proton, or methyl group, is the best fit for the analogs to inhibit cancer cell growth. For C position, small methyl sulfonamide or acetyl groups

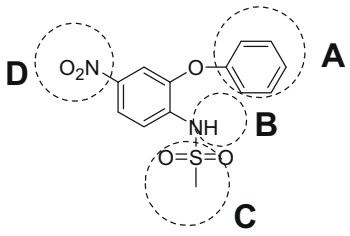
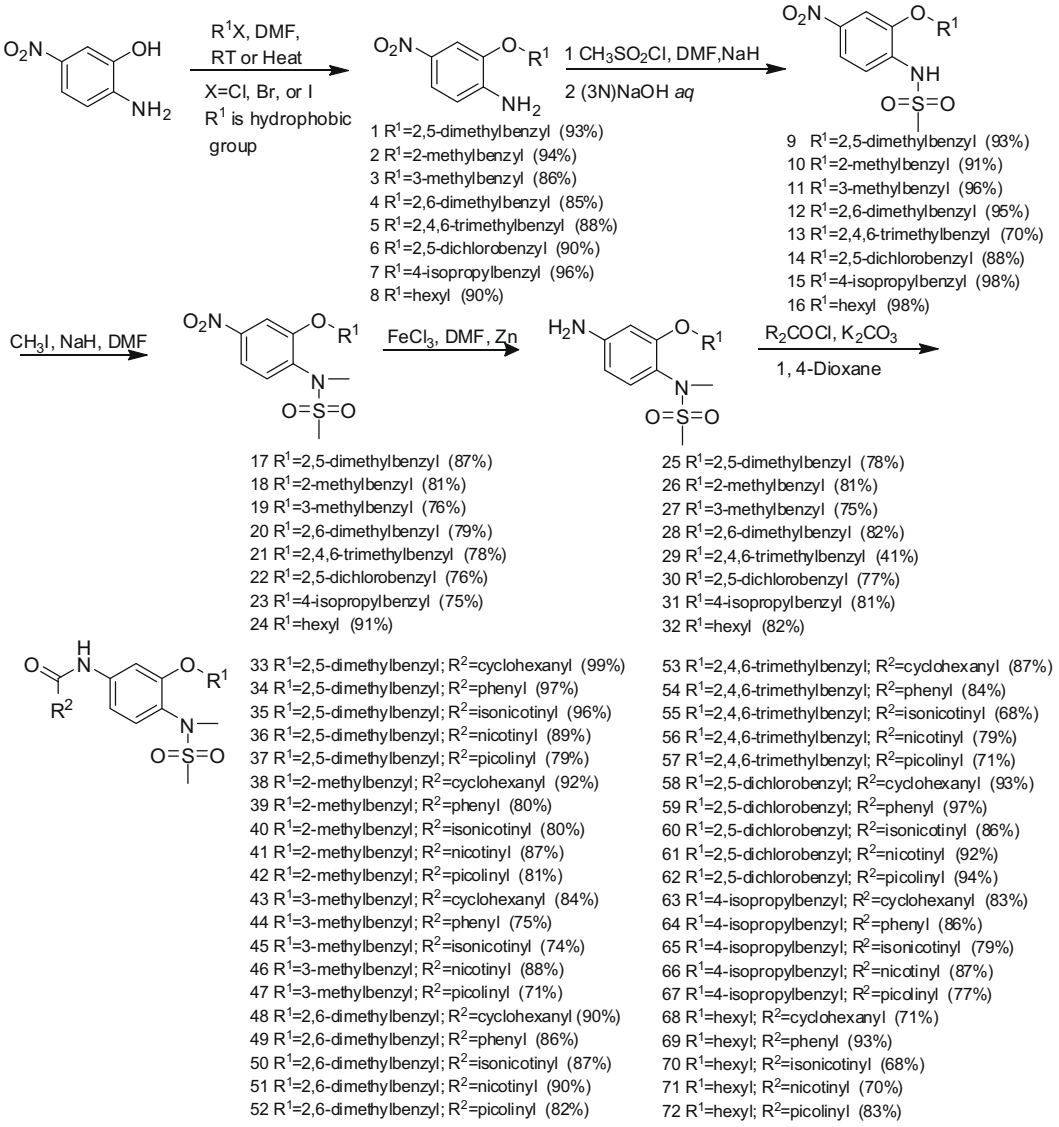


Figure 1. Chemical structure of nimesulide.

is the best fit. Bulky groups will decrease the pharmacological activity.[20] For A position, methyl group substituted benzyl is better for the activity.[20] For D position, we will try pyridine group in this study. Since nitrogen containing heterocyclics can increase aroma tase inhibition activity, according to several other reports.[22,23] In the newly designed derivatives, we will keep B position as methyl group and C position as methyl sulfonamide. A was modified by using different positions and numbers of methyl group substituted benzyls. D will be kept as pyridine or hydrophobic groups (Scheme 1). These compounds and their biological activity will enable us to identify the key pharmacophore of this scaffold on the suppression of LTEDaro breast cancer cell growth.

The results suggest that A position as 2,5 dimethyl or dichloro benzyl is the best fit. Compounds **33** **36** and **58** **61** are relatively more active, except compounds 37 and **62** (Table 1). It seems that D position as a picolinyl group harms the biological activity. Only one methyl group substituted benzyl group at A position definitely decreases the activity. Compounds **38** **47** show much lower activ ity compared with compounds **33** **36**. Compounds **48** **57** are not as active as compounds **33** **36**, which suggests that the methyl group at 2,5 position of benzyl at A position is very critical for the activity. Tri methyl groups clearly do not increase the activity, which has been demonstrated by relatively low activity of com pounds **53** **57**. 4 Isopropyl benzyl group or hexyl group at A posi tion does not help the activity based on the biological results of compounds **63** **72**. However, 2,5 dichloro benzyl group at A posi tion can slightly increase the activity. Compounds **58** and **59** show better activity compared with compounds **33** and **34**.[24] Overall, nitrogen containing aromatic group at D position does not increase



Scheme 1. Synthesis of nimesulide analogs.

Table 1
IC_{50} of inhibition of LTEDaro breast cancer cells growth by compounds **33–72**

Compd	Inhibition of LTEDaro cell growth (IC_{50} μM)
Nimesulide	173.30 ± 20.30
33	2.66 ± 0.57
34	4.68 ± 0.54
35	2.37 ± 0.44
36	1.69 ± 0.25
37	174.20 ± 79.33
38	28.46 ± 5.74
39	175.60 ± 94.37
40	11.76 ± 2.34
41	71.49 ± 23.07
42	93.89 ± 30.52
43	44.18 ± 16.04
44	16.07 ± 3.65
45	16.08 ± 3.08
46	93.63 ± 59.03
47	14.89 ± 2.08
48	39.38 ± 13.88
49	16.24 ± 3.32
50	19.91 ± 5.58
51	22.53 ± 6.50
52	41.37 ± 15.70
53	18.49 ± 2.75
54	10.30 ± 3.10
55	13.18 ± 2.23
56	10.36 ± 2.42
57	51.27 ± 14.91
58	1.00 ± 0.39
59	2.15 ± 0.54
60	7.64 ± 1.67
61	14.05 ± 4.16
62	23.58 ± 8.78
63	16.06 ± 4.94
64	7.93 ± 2.85
65	11.46 ± 2.75
66	8.26 ± 3.04
67	11.41 ± 4.11
68	6.98 ± 2.93
69	12.04 ± 2.36
70	9.56 ± 1.90
71	12.07 ± 2.86
72	7.68 ± 2.45

LTEDaro cells were treated with indicated compounds at various concentrations by triplicates for 72 h and cell viability was measured by MTT assay.[25]

the biological activity, even though compound **36** is slightly more potent than compounds **33** and **34**.

In brief, we optimized nimesulide structure and developed sev eral more potent analogs, such as compounds **36**, 58, and **59**, which inhibit LTEDaro cell growth with IC_{50} of 1.69 ± 0.25 μM, 1.00 ± 0.39 μM, and 2.15 ± 0.54 μM, respectively. Compared with nimesulide with IC_{50} of 173.30 ± 20.30 μM, the new derivatives have much more potent pharmacological activity against LTEDaro breast cancer cell growth. Structure activity relationship study suggests that A position needs 2,5 dimethyl or dichloro benzyl group to increase the biological activity. The exact biological mech anism of the compound is still under investigation in our laboratory.

Acknowledgment

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24. *Compound* **36**: White powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.51 (1H, s), 9.08 (1H, s), 8.74 (1H, s), 8.27 (1H, d, J = 8.0 Hz), 7.72 (1H, s), 7.55 (1H, d, J = 8.0 Hz), 7.37 (5H, m), 5.07 (2H, s), 3.08 (3H, s), 2.83 (3H, s), 2.28 (3H, s), 2.24 (3H, s); HRMS calculated for $C_{23}H_{26}N_3O_4S$ $(M+H)^+$ 440.1639, found 440.1638. *Compound* **58**: White powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.91 (1H, s), 7.71 (1H, d, J = 2.5 Hz), 7.56 (2H, m), 7.47 (1H, m), 7.22 (2H, m), 5.13 (2H, s), 3.07 (3H, s), 2.86 (3H, s), 2.27 (1H, m), 1.76 (4H, m) 1.62 (1H, m), 1.37 (2H, m), 1.24 (3H, m); HRMS calculated for $C_{22}H_{27}Cl_2N_2O_4S$ $(M+H)^+$ 485.1063, found 485.1061. *Compound* **59**: White powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.34 (1H, s), 7.93 (2H, d, J = 1.0 Hz), 7.92 (2H, m), 7.59 (6H, m), 7.30 (1H, d, J = 8.5 Hz), 5.18 (2H, s), 3.11 (3H, s), 2.89 (3H, s); HRMS calculated for $C_{22}H_{20}Cl_2N_2NaO_4S$ $(M+Na)^+$ 501.0413, found 501.0410.
25. The effect of nimesulides derivatives on LTEDaro breast cancer cell viability was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide assay (MTT) in triplicates. Cells were grown in custom medium in 96-well, flat-bottomed plates for 24 h, and were exposed to various concentrations of nimesulide derivatives dissolved in DMSO (final concentration $\leqslant$ 0.1%) in media for 72 h. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. The medium was removed, replaced by 200 μl of 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-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.