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Design and synthesis of tamoxifen derivatives as a selective estrogen receptor down-regulator

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ABSTRACT

We designed and synthesized an estrogen receptor (ER) down-regulator (**5**), which is a derivative of tamoxifen with a long alkyl side chain. Compound **5** effectively reduced ER protein levels in MCF-7 cells and had an antagonistic effect.

Estrogen receptor (ER) belongs to the nuclear receptor superfamily and is often overexpressed in the tissues of breast cancer patients, which promotes the estrogen-dependent proliferation of cancer cells.[1–4] Tamoxifen (Fig. 1) is an orally available ER antagonist, which competitively blocks the binding of estrogen, such as 17β-estradiol (E2), to the receptor and is effective at treating breast cancer in pre- and post-menopausal women.[5,6] Tamoxifen is one of the selective estrogen receptor modulators (SERMs), which act as either agonists or antagonists depending on the target tissue. Therapy using tamoxifen is often limited because tamoxifen possesses agonistic effects in uterine cancer cells and increases the risk of endometrial cancer.[7–9] Another class of currently available antagonists is selective estrogen receptor down-regulators (SERDs).[10,11] This class of compounds not only interferes with the binding of E2 to ER but also induces the rapid down-regulation of ER.[10–13] The latter property causes no agonistic activity in any tissues. SERDs are structurally divided into two groups. One is steroidal compounds, such as fulvestrant, which is a steroidal analogue with a long alkyl side chain from the 7α position of E2 (ICI182,780, Fig. 1). Although clinical studies have shown some success with fulvestrant,[13–15] its poor pharmacodynamic properties and lack of oral bioavailability have limited its clinical

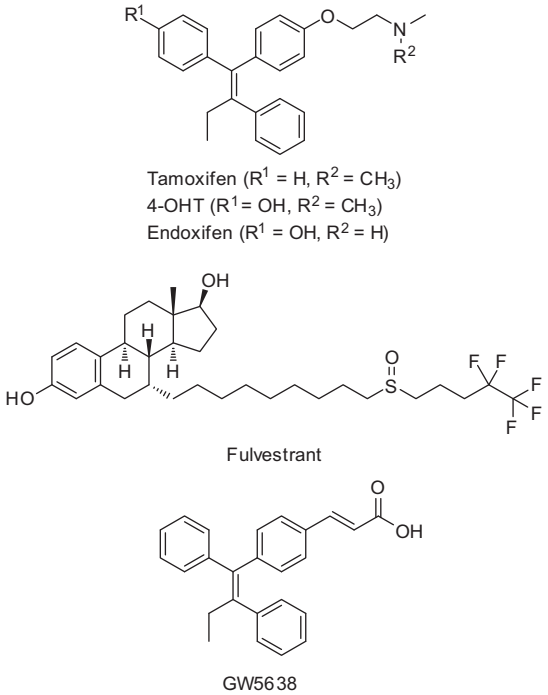

Figure 1. Structures of tamoxifen, tamoxifen active metabolites, fulvestrant, and GW5638.

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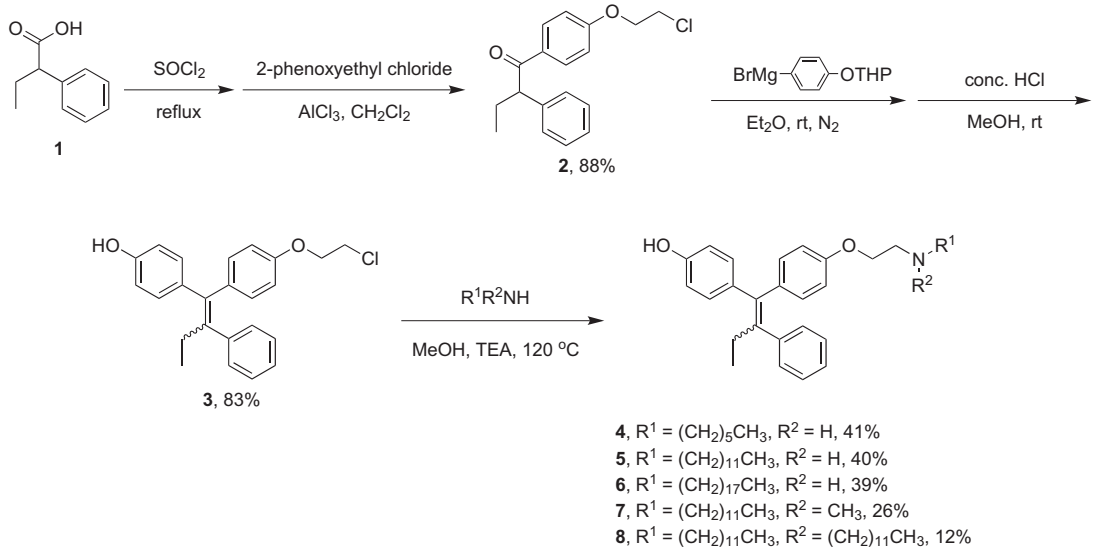

Scheme 1. Synthesis of tamoxifen derivatives.

utility.[13–16] The other group is non-steroidal compounds, such as GW5638 (Fig. 1). The structure of GW5638 is similar to tamoxifen and contains an acrylic acid side chain extending from the triphenylethylene core.[17] GW5638 showed no agonistic effects in the uterus[17] due to a decrease in stability of the ER.[18] Additionally, it has been shown that GW5638 is useful for inhibiting the growth of tamoxifen-resistant breast tumors.[19]

Thus, the concept of SERDs has provided very important information; however, it is clear that there is an unmet medical need for SERDs with improved pharmaceutical properties. In this Letter, we report the design and synthesis of tamoxifen derivatives that induced down-regulation of the ER. One of the compounds showed the ability to down-regulate the ER in MCF-7 cells and antagonistic activity against ER-mediated gene expression.

Fulvestrant has a long alkyl side chain, which protrudes from the ligand binding pocket, binds along the coactivator recruitment site, and physically prevents H12 interaction.[20] We thought that this long alkyl side chain might play a key role in down-regulation of the ER. Therefore, we designed tamoxifen derivatives into which simple alkyl chains were introduced on the amine moiety of tamoxifen. The alkyl chains is expected to be outside the ER, as determined from the X-ray structure (PDB ID: 3ERT),[21] as shown in our previous report.[22]

Tamoxifen is metabolized by metabolizing enzymes into 4-hydroxytamoxifen (4-OHT) and endoxifen (Fig. 1),[23,24] which have 100 times more affinity for the estrogen receptor than tamoxifen itself.[25,26] The synthetic route to these compounds is shown in Scheme 1. We synthesized (*E*/*Z*)-4-[1-{4-(2-chloroethoxy)phenyl}-2-phenylbut-1-en-1-yl]phenol (**3**) according to a previous report[27] with slight modification. Then, compounds **4**–**8** were synthesized from corresponding amines at moderate yields.[28]

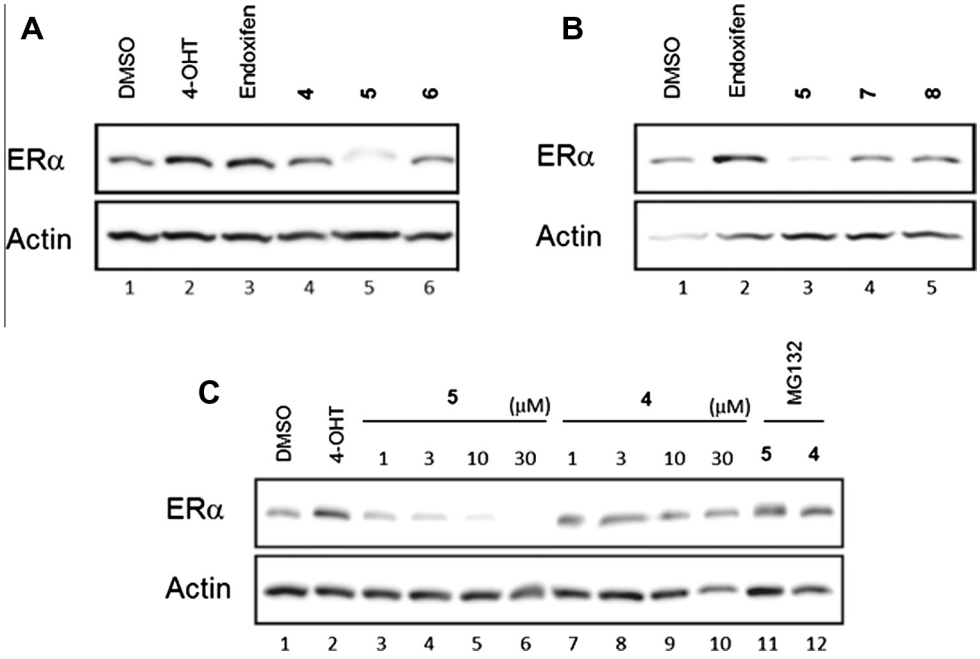

Figure 2. Western blot analyses of ERα protein levels in MCF-7 cells. Cells were incubated with DMSO or compound (10 μM) for 6 h. Whole proteins were extracted and ERα protein levels were analyzed by Western blotting. (A) Effects of the length of the alkyl side chain. (B) Effects of the substitution of amine moiety. (C) Dose dependence of ERα degradation induced by compounds **5** and **4**, and the effects of a protease inhibitor on these responses.

We examined the effects of the length of the long alkyl side chain on reducing ERα protein levels in MCF-7 breast cancer cells. MCF-7 cells were treated with these compounds, whole protein was extracted, and ERα protein levels were analyzed by Western blotting, as reported previously.[22] As shown in Figure 2A, reduction of the ERα protein level was observed in the cells treated with 10 μM **5** (lane 5), but no significant differences in the activities of **4** and **6** were observed under these conditions (lanes 4 and 6, respectively). The protein level of ERα was slightly increased by treatment with 10 μM 4-OHT and endoxifen (lanes 2 and 3), which was identical to findings previously reported.[29,30]

Next, to investigate the substitution of long alkyl side chains on the amine moiety, we examined the ERα level upon treatment with compounds **5**, **7**, and **8** (Fig. 2B). The result showed that compound **7**, a methyl derivative of **5**, and **8**, a dodecyl derivative of **5**, did not affect the ERα level. This result indicated that the secondary amine is important for the down-regulation of ERα.

We performed dose-response studies in MCF-7 cells. Figure 2C shows that **5** reduced the ERα level in MCF-7 cells in a concentration-dependent manner at the dose range from 1 to 30 μM (Fig. 2C, lanes 3–6) upon 6-h incubation. At the highest concentration, namely, 30 μM, the band of ERα was completely disappeared (Fig. 2C, lane 6). In the case of **4**, there was no dose dependence (Fig. 2C, lanes 7–10) at the range from 1 to 30 μM. To examine the reduction of ERα due to protein degradation, we used MG132 (*N*-benzyloxycarbonylleucylleucylleucinal, a proteasome inhibitor) to inhibit the proteasome in MCF-7 cells (Fig. 2C, lane 11). We found that the inhibition of proteasome activity completely blocked ERα degradation by **5**. Together, these data show that **5** has the ability to induce the proteasomal degradation of ERα protein in MCF-7 cells.

Finally, we examined the antagonistic effects of **5**. Transcriptional analysis of ERα target genes was carried out on MCF-7 cells treated with **5** in the absence or presence of 10 nM E2 for 9-h incubation. Total RNA was extracted, reverse-transcribed, and analyzed by real-time PCR as reported previously.[31] As shown in Figure 3, the mRNA level of pS2, one of the E2 up-regulated genes, decreased in a dose-dependent manner at the dose range from 0.1 to 10 μM **5**. This result clearly showed that **5** has the ability to exibit an antagonistic effect.

In summary, we designed and synthesized new tamoxifen derivatives with a simple long alkyl side chain, which down-regulate the ERα protein level in MCF-7 cells. The reduction of ERα by **5** is due to proteasomal degradation. This molecule may be included in a novel SERD against breast cancer. Further optimization and experiments are currently ongoing in our lab.

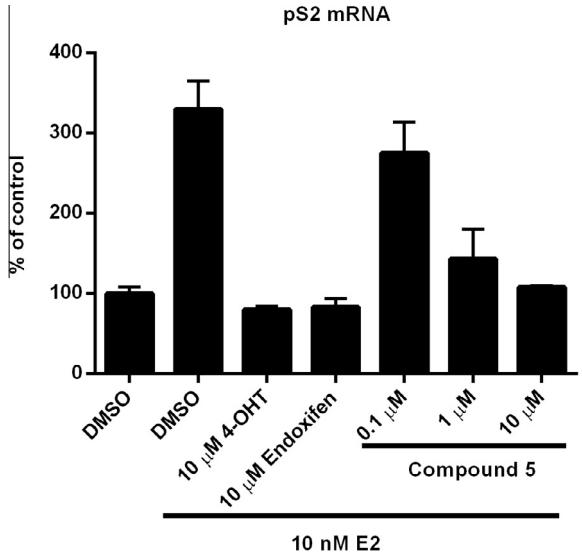

Figure 3. Antagonistic effect of ERα-mediated gene expression in MCF-7 cells.

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28. Experimental method and spectroscopic data for compound **5**: Compound **3** (0.11 mmol), dodecylamine (0.63 mmol), and triethylamine (0.3 mmol) were dissolved in methanol. The mixture was heated in a pressure vessel at 120 °C until the reaction was completed. The mixture was evaporated in vacuo and the products were purified by column chromatography (23.3 mg, 40%). Clear viscous compound; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.65 (br, 1H), 6.37–7.13 (m, 13H), 3.86–4.05 (m, 4H), 3.07–3.18 (m, 2H), 2.87–2.94 (m, 2H), 2.40–2.47 (m, 2H), 1.64–1.68 (m, 2H), 1.25 (s, 18H), 0.86–0.91 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 156.0, 155.6, 155.1, 142.9, 142.8, 141.3, 141.0, 138.0, 137.7, 137.2, 135.2, 134.8, 132.3, 132.2, 130.9, 130.8, 129.9, 128.1, 128.0, 126.1, 115.5, 114.8, 114.2, 113.5, 63.4, 63.2, 50.8, 48.1, 46.6, 46.5, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.3, 27.9, 26.9, 26.4, 26.3, 22.9, 14.4, 13.9; [HR-ESI(+)]: *m*/*z* calcd for $C_{36}H_{49}NO_2$ $[M+H]^+$ 528.3836, found 528.3820.
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