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Synthesis and evaluation of raloxifene derivatives as a selective estrogen receptor down-regulator

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ABSTRACT

Estrogen receptors (ERs) play a major role in the growth of human breast cancer cells. A selective estrogen receptor down-regulator (SERD) that acts as not only an inhibitor of ligand binding, but also induces the down-regulation of ER, would be useful for the treatment for ER-positive breast cancer. We previously reported that tamoxifen derivatives, which have a long alkyl chain, had the ability to down-regulate ERα. With the aim of expanding range of the currently available SERDs, we designed and synthesized raloxifene derivatives, which had various lengths of the long alkyl chains, and evaluated their SERD activities. All compounds were able to bind ERα, and RC10, which has a decyl group on the amine moiety of raloxifene, was shown to be the most potent compound. Our findings suggest that the ligand core was replaceable, and that the alkyl length was important for controlling SERD activity. Moreover, RC10 showed antagonistic activity and its potency was superior to that of 4,4′-(heptane-4,4-diyl)bis(2-methylphenol) (**18**), a competitive antagonist of ER without SERD activity. These results provide information that will be useful for the development of promising SERDs candidates.

1. Introduction

Selective estrogen receptor down-regulators (SERDs) are a class of pure antagonists that not only interfere with the binding of estradiol to estrogen receptors (ERs), but also induce the rapid down-regulation of ER.[1–5] Compared with selective estrogen receptor modulators (SERMs), which act as either agonists or antagonists, depending on the tissue, such as tamoxifen and its active metabolite, 4-OHT (Fig. 1), SERDs showed no agonistic activity in any tissue and are often used as second-line agent for tamoxifen-resistant breast cancer. Fulvestrant[6,7] and GW7604[8] are two well-known SERDs (Fig. 1). Fulvestrant is an estradiol derivative with an 4,4,5,5,5-pentafluoropenthylsulfinyl alkyl group at the 7α position of estradiol. Fulvestrant is an effective therapeutic option for postmenopausal women with ER-positive breast cancers.[9] GW7604 has a triphenylethylene ligand core and an acrylic acid unit. X-ray crystallography of the ERα-GW7604 complex indicates that the carboxylic acid group plays a key role. The direct interaction between the carboxylic acid group and the N terminus of helix 12 of the receptor induced a conformational change and destabilization of the receptor.[10] This result promoted extensive studies into the synthesis and evaluation of compounds with ligand cores and acrylic acid.[11–15] These reports indicate that introducing an acrylic acid to a ligand core is an effective way to develop SERDs. Our group has previously reported the design and synthesis of tamoxifen derivative SERDs, which have a long alkyl chain on the amine moiety.[16,17] The compound with a decyl group, termed C10 (Fig. 1), had the highest ability to induce the down-regulation of ERα among the simple alkyl chain derivatives. Next, we investigated the relation between the ligand core and the SERD activity of the compound. We chose raloxifene (Fig. 1) as the ligand core, which is also known as a non-steroidal SERM that binds effectively to ERα and ERβ subtypes.[18,19] In this manuscript, we describe the synthesis of raloxifene derivatives having a long alkyl chain and evaluate their activities. Our findings suggest that the ligand core is replaceable with other structures, and that an appropriate length of the long alkyl chain of the ligand is necessary for the ability to down-regulate ER.

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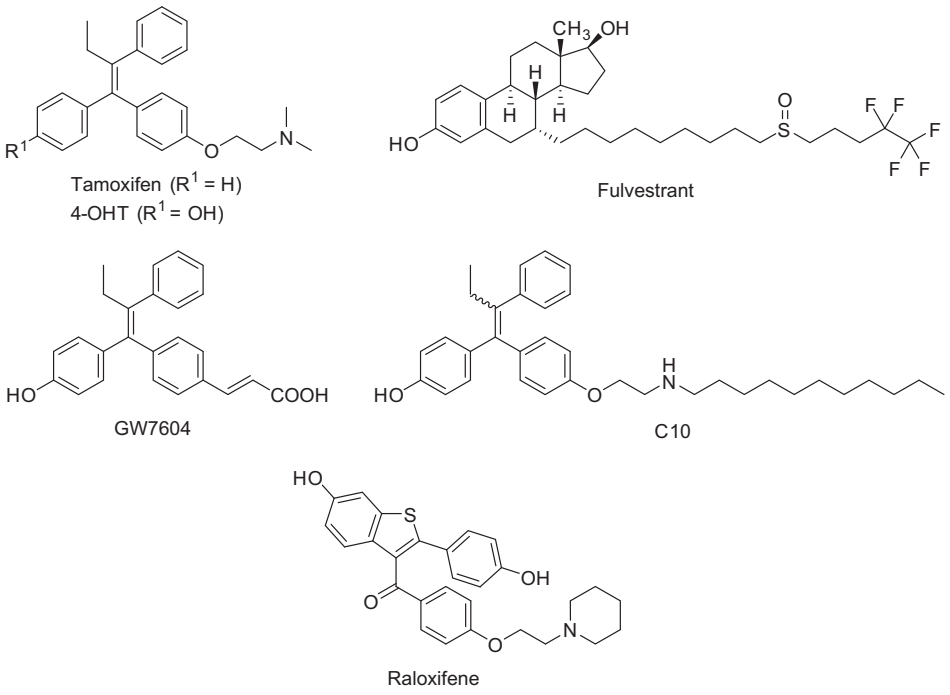

Figure 1. Structures of tamoxifen, 4-OHT, fulvestrant, GW7604, C10, and raloxifene.

2. Results

2.1. Chemistry

Raloxifene, a benzothiophene derivative tethered to a basic side chain, is well known as a SERM that binds effectively to ERα and ERβ subtypes. The binding affinity[18,19] and the binding mode[20] of raloxifene is almost identical to that of tamoxifen.

We designed seven novel raloxifene derivatives with long alkyl chains on the amine moiety. Synthetic route of compounds are summarized in Scheme 1. Firstly, we synthesized [4-(2-chloroethoxy)phenyl][6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thiophen-3-yl]methanone (**4**) according to the patent,[21] with slight modification. Commercially available methyl 4-hydroxybenzoate was reacted with 1,2-dichloroethane in the presence of K_2CO_3 as a base to afford **1** (93% yield), which was then hydrolyzed to afford **2** (91% yield). The crude acid chloride **3** was obtained by refluxing in $SOCl_2$ as solvent, and used without further purification. Acid chloride **3** was then reacted with 6-methoxy-2-(4-methoxyphenyl)benzo[*b*]thiophene in the presence of BCl_3 to afford the demethylated compound **4** (94% yield). The chloride group was converted to iodide to afford **5**. Then, compounds **6**–**12** were synthesized from corresponding amines in moderate yields, as indicated in Scheme 1.

2.2. Down-regulation of ERα

We first examined the effects of the length of the long alkyl chain on raloxifene for 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 previously reported.[16,22] As shown in Figure 2, reduction of the ER protein level was observed in the cells treated with 10 μM RC10 and RC12 (lanes 5, 6), compared with raloxifene and other compounds (lane 2–4 and 7–9). RC10 was the most potent of all compounds examined. Next, we examined the dose-dependency of RC10 and RC12. After treatment with RC10 and RC12, ERα protein level decreased in a dose-dependent manner (Fig. 3, lane 3–5 and 7–9, respectively). These reductions were inhibited by addition of a proteasome inhibitor, MG132 (lane 6 and 10), suggesting that RC10 and RC12 have the ability to induce proteasomal degradation of ERα in MCF-7 cells.

2.3. ER binding affinity

To evaluate the ability of compounds to bind to ERα, a fluorescence polarization based competitive binding assay was conducted. The mean IC_{50} values of the compounds tested in the binding assay are summarized in Table 1. The IC_{50} values of all synthesized compounds were between 1.8–61 nM, and the IC_{50} of raloxifene was 0.47 nM. These findings suggest that the binding affinity for ERα was decreased by the introduction of a long alkyl chain to the amine moiety of the ligand core, and that the length of the alkyl chain does not significantly affect the binding affinity. Moreover, no correlation was observed between binding affinity and ER degradation. A similar pattern of results was found with the corresponding tamoxifen derivatives.[17] We also measured binding affinity of compound **18**, 4,4′-(heptane-4,4-diyl)bis(2-methylphenol), a non-steroidal antagonist developed by Maruyama et al.,[23] that has an IC_{50} value of 2.4 nM. The binding affinity of compound **18** was similar to that of our compounds.

2.4. ER antagonistic activity

Finally, we examined the antagonistic effects of RC10 and RC12, both of which had the ability to down-regulate ERα. The ER-antagonistic activities were evaluated using previously established methods.[23–27] Transcriptional activity was activated by 0.3 nM estradiol, and the IC_{50} values were calculated using the luciferase activity value divided by the β-galactosidase activity value. These compounds showed dose-dependent inhibition of transcriptional activity, suggesting that RC10 and RC12 exhibit antagonistic effects. IC_{50} values are summarized in Table 2.