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Synthesis of the new *pseudo*-symmetrical tamoxifen derivatives and their anti-tumor activity

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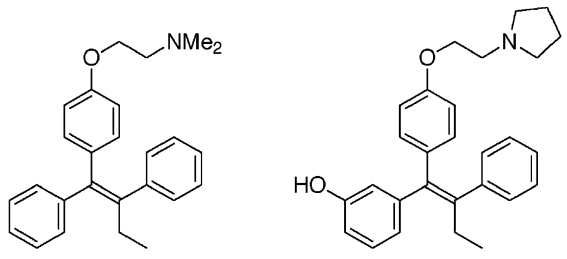
Abstract—Three new *pseudo*-symmetrical tamoxifen derivatives, RID-B (**15**), C (**16**), and D (**17**), were synthesized via the novel three-component coupling reaction, and the structure–activity relationships of the *pseudo*-symmetrical tamoxifen derivatives were examined. It was discovered that **15** strongly inhibits the viability of HL-60 human acute promyelocytic leukemia, whereas **16** possesses medium activity against the cell line and **17** has no effect on the cell viability. The agarose gel electrophoresis for DNA cleavage showed the cell death might be induced by apoptosis.

Tamoxifen (**1**, Fig. 1),[1] the early generation of SERMs (selective estrogen receptor modulators), has been used as the first-line agent for the treatment of estrogen-dependent breast cancer since the 1970s. Accumulative risk-benefit assessment of tamoxifen therapy and comparative studies of **1** and other new types of drugs also established its efficacy and safety. Therefore, the development of an expeditious synthetic route for producing new tamoxifen-type drugs followed by the systematic studies of their biological activities is significantly required. In this communication, we report a novel short-step synthesis of a new class of anti-cancer agents, which are *pseudo*-symmetrical tamoxifen derivatives, via the three-component coupling reaction, and their cytotoxic activity against HL-60 cancer cells.

Recently, we have established a novel three-component coupling reaction among aromatic aldehydes, cinnamyltrimethylsilane (**4**), and aromatic nucleophiles in the presence of a Lewis acid catalyst.[2] Furthermore, we reported that the sequential one-pot allylation and Friedel–Crafts type alkylation reaction can be effectively applied to the preparation of **1** and its halogenated derivatives.[3] The coupling reaction of benzaldehyde, cinnamyltrimethylsilane (**4**), and anisole promoted by 1 equivalent of $HfCl_4$ and 50 mol% of TMSOTf at room temperature afforded 3,4,4-triarylbutene **5**, which is the basic skeleton of the tamoxifen derivatives, in a satisfactory yield (Scheme 1). The coupling reaction also proceeded using 3-pivaloyloxybenzaldehyde and β-chlorophenetole as a second nucleophile, which implemented a new route to droloxifene (**2**), one of 3-hydroxytamoxifen derivatives, through only three steps.[4]



Tamoxifen (**1**) Droloxifene (**2**)

Figure 1. Structures of tamoxifen (**1**) and droloxifene (**2**).

Keywords: Tamoxifen; SERMs; Selective estrogen receptor modulators; *pseudo*-Symmetrical structure; Anti-tumor activity; Apoptosis; Synthesis.
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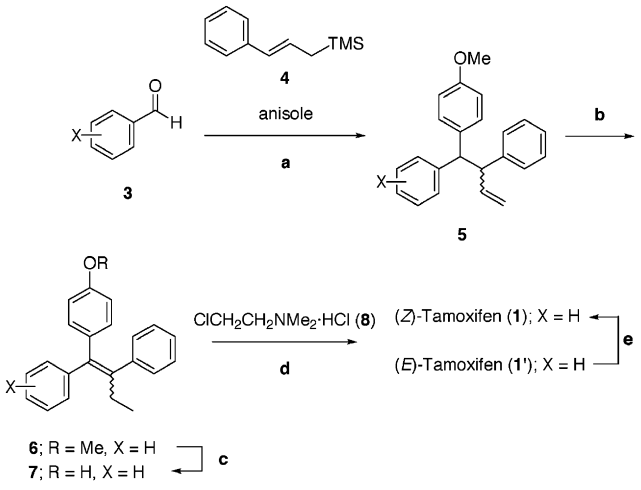
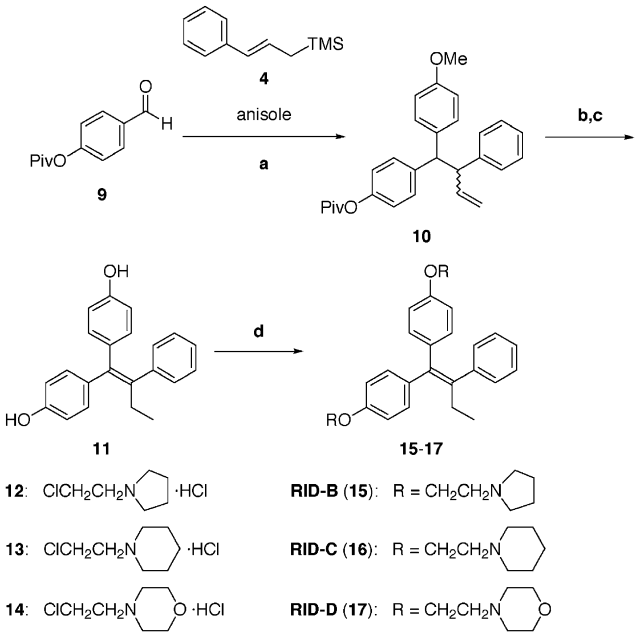


Scheme 1. Reagents and conditions (for X = H): (a) **4**, $HfCl_4$ (1 equiv), TMSOTf (50 mol%), anisole, rt, 2 h, 57%; (b) *t*-BuOK, DMSO, rt, 15 min, 96%; (c) BBr_3, CH_2Cl_2, −78 °C, 2 h, 98%; (d) **8**, NaH, DMF, 50 °C, 30 min, 95% (*Z*/*E* = 54:46); (e) TfOH, CH_2Cl_2, 0 °C, 3 h (51% of **1**, 46% of **1′**).



Scheme 2. Reagents and conditions: (a) **4**, $HfCl_4$ (1 equiv), anisole, rt, 2 h, 78%; (b) *t*-BuOK, DMSO, 90 °C, 1 h, 93%; (c) BBr_3, CH_2Cl_2, 0 °C, 2 h, 86%; (d) NaH, **12**, DMF, 50 °C, 30 min, 84% (for **15**); NaH, **13**, DMF, 50 °C, 30 min, 95% (for **16**); NaH, **14**, DMF, 50 °C, 30 min, 95% (for **17**).

Based on the preliminary results for the synthesis of tamoxifen (**1**) and droloxifene (**2**), the coupling reaction among 4-pivaloyloxybenzaldehyde (**9**), cinnamyltrimethylsilane (**4**), and anisole was carried out for the preparation of *pseudo*-symmetrical tamoxifen analogues (Scheme 2). The three-component coupling reaction smoothly proceeded in the presence of a stoichiometric amount of $HfCl_4$ at room temperature to afford the coupling product **10** in high yield. Bisphenol **11**[5] was prepared from **10** by heating with an excess amount of *t*-BuOK in DMSO via the base-catalyzed double-bond migration reaction and successive deprotection of the *O*-methyl group by BBr_3 in CH_2Cl_2 at 0 °C. Next, **11** was treated with 60% NaH followed by an excess amount of 2-pyrroridinoethylchloride hydrochloric acid salt (**12**), 2-piperidinoethylchloride hydrochloric acid salt (**13**), or 2-morphorinoethylchloride hydrochloric acid salt (**14**) in DMF to afford the novel tamoxifen derivatives, RID-B (**15**),[6] RID-C (**16**),[7] or RID-D (**17**),[8] respectively.

It is well known that *E*-isomer of tamoxifen is not antiestrogenic but functions as an estrogen agonist, although (*Z*)-tamoxifen is effective in treating estrogen-dependent breast cancer. Therefore, the efficiency of the synthesis of tamoxifen derivatives usually depends on the stereoselectivity of the olefination step to generate the desired *Z*-isomer; however, it is not required to use stereogenic reactions to produce the *pseudo*-symmetrical system included in **15**, **16**, and **17**.

Next, the potency of the anti-tumor activities of **15**, **16**, and **17** was assessed in this study. In order to evaluate the anti-tumor activities of these newly prepared compounds against HL-60 human acute promyelocytic leukemia, we tried to determine the efficiency of the *pseudo*-symmetrical compounds decreasing the cell viability by MTT assay, a method of determining cell death by measuring the mitochondrial succinic dehydrogenase activity. Furthermore, it was examined whether this cell death was due to apoptosis or necrosis by agarose gel electrophoresis for oligonucleosomal DNA cleavage.[9]

We first investigated the effects on the viability of the HL-60 cells treated with **15**, **16**, and **17** at various concentrations for 0–6 h (Fig. 2). RID-B (**15**) in the final concentrations of 5, 7.5, and 10 μM decreased the cell viability in a time-dependent manner. A 6-h incubation with 5, 7.5, and 10 μM final concentrations of **15** inhibited the cell viability in more than 90% as measured by the MTT assay. RID-C (**16**) in 7.5 and 10 μM concentrations also inhibited the cell viability in more than 80% after a 6-h treatment. Contrarily, RID-D (**17**) showed no effect on the viability of the HL-60 cells after 6 h. Among them, **15** and **16** clearly induced, dose-dependently, cell death to a greater extent than **17** as shown in Figure 2. Although **15** and **16** have strong cytotoxic activity inducing apoptosis of the HL-60 cells, **17** including morpholine side chain shows no activity on the same cells; therefore, it is indicated that oxygen in morpholine side chain varied cytotoxic characters of **15** and **16** to non-cytotoxic by lowering its basicity.[10] The specific moieties as well as the hydrophobicity derived from the long alkyl side chain might be both important for the strong cytotoxic activity against the HL-60 cells.

The effect of tamoxifen itself on growth of the HL-60 human cell line was measured by the MTT assay at 5 μg/mL after a 4-h incubation. The final growth rate of the cells was inhibited to be ca. 50% content compared with the growth rate at 0 h. On the other hand, the biological activity of 5 μg/mL **15** is very strong to inhibit the cell viability in more than 90% after 4 h under the same conditions.

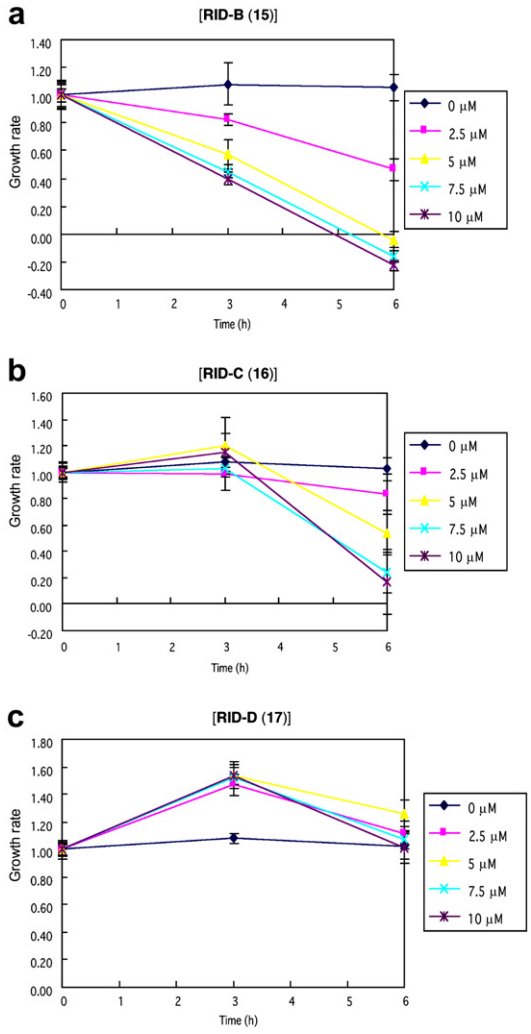


Figure 2. Effects of RID-B (**15**), C (**16**), and D (**17**) on growth of cells. Time-dependent effects of **15**, **16**, and **17** on HL-60 cells were measured by the MTT assay. Data are expressed as growth rate (growth rate has shown that absorbance of formazan at 0 h was 1.0), and error bars show SD ($n = 5$). HL-60 cells were treated at the following four different final concentrations: 2.5, 5, 7.5, and 10 μM, respectively.

The median growth-inhibitory concentrations (IC_{50s}) against the HL-60 cells after a 6-h incubation with **15** and **16** were as follows: 3 and 5 μM final concentrations, respectively. Furthermore, the IC_{50} of **15** after a 3-h treatment was a 4 μM final concentration. We also determined the IC_{50s} by neutral red assay. Neutral red solution is taken through the cell membrane by living cells and accumulated in the lysosomes, whereas MTT is a substrate for mitochondrial succinic dehydrogenase. The IC_{50s} determined by the neutral red assay were lower than those determined by the MTT assay for almost all of the tested compounds, however, the two most effective products were still **15** and **16**. On the basis of these results, we selected these two *pseudo*-symmetrical compounds **15** and **16** for further examinations.

To clarify whether the two products (**15** and **16**) induced cell death by apoptosis or necrosis, we found evidence obtained by agarose gel electrophoresis for the oligonucleosomal DNA cleavage after treatment of the HL-60 cells

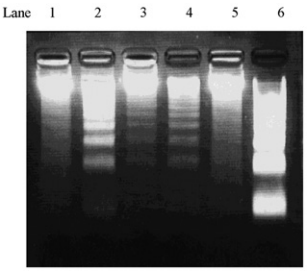


Figure 3. Results of determination of the nature of the cell death assay of *pseudo*-symmetrical tamoxifens for 6 h [for only medium (lane 1), tamoxifen (lane 2), RID-B (**15**) (lane 3), RID-C (**16**) (lane 4), ethanol (lane 5), or actinomycin D (lane 6)].

with a 9 μM final concentration of any of the compounds (Fig. 3). The HL-60 cells were treated with **15** (lane 3), **16** (lane 4), and with only medium, tamoxifen, ethanol, or actinomycin D as the controls (lanes 1, 2, 5, or 6, respectively). The cell death assay of all molecules was carried out for 6 h although the time required for the DNA fragmentation clearly varied for each tested sample.

DNA fragmentation was observed after treatment of the HL-60 cells with the *pseudo*-symmetrical compounds **15** and **16** using the chromatin DNA analysis, and the DNA ladder patterns of **15** (lane 3) and **16** (lane 4) are similar to that of tamoxifen (lane 2). Recently, it has been reported that tamoxifen and some derivatives such as nafoxidine induce apoptosis in HL-60 leukemic cells;[11] therefore, these results support the conclusion that **15** and **16** induced cell death by apoptosis.

In summary, we developed the effective synthesis of the new *pseudo*-symmetrical tamoxifen derivatives RID-B (**15**), C (**16**), and D (**17**) from very simple molecules in four steps. Although **17** (involving morpholine part) does not have any biological activity in the MTT assay, **15** (involving pyrrolidine moiety) and **16** (involving piperidine moiety) showed significant anti-tumor activities. The latter two compounds **15** and **16** might be potent candidates as anti-tumor agents with different modes of action from existing drugs for cancer treatment. It was also discovered that the time-dependent activity of **15** is superior to that of **16** as shown in Figure 2. The synthesis of other derivatives and further studies of the anti-tumor activities of these new entries as *pseudo*-symmetrical tamoxifen agents are now in progress.

Acknowledgments

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6. 1,1-Bis[4-[2-(pyrroridin-1-yl)ethoxy]phenyl]-2-phenyl-1-butene (RID-B, **15**): Colorless crystals: mp 108–109 °C (acetonitrile); IR (KBr): 2929, 2789, 1606, 1511, 1460, 1280, 1242, 1174, 1040, 836, 821 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.19–7.09 (m, 7H, Ar), 6.89 (d, J = 8.4 Hz, 2H, Ar), 6.75 (d, J = 8.7 Hz, 2H, Ar), 6.55 (d, J = 8.7 Hz, 2H, Ar), 4.13 and 3.97 (t, J = 6.0 Hz, 4H, OCH_2), 2.92 and 2.81 (t, J = 6.0 Hz, 4H, NCH_2), 2.66–2.55 (m, 8 H, pyrroridinyl 2-H), 2.48 (q, J = 7.4 Hz, 2H, 3-H), 1.84–1.75 (m, 8H, pyrroridinyl 3-H), 0.93 (t, J = 7.4 Hz, 3H, 4-H); ^{13}C NMR ($CDCl_3$) δ 157.5, 156.7, 142.6, 140.9, 137.8, 136.3, 135.8, 131.8, 130.5, 129.7, 127.8, 125.8, 114.0, 113.3, 66.9, 66.7, 54.7, 54.7, 55.1, 55.1, 29.7, 29.0, 23.5, 23.4, 13.6; HR MS (ESI): calcd for $C_{34}H_{43}N_2O_2$ ($M+H^+$), 511.3319; found, 511.3319.
7. 1,1-Bis[4-[2-(piperidin-1-yl)ethoxy]phenyl]-2-phenyl-1-butene (RID-C, **16**): Colorless crystals: mp 109–110 °C (acetonitrile); IR (KBr): 2928, 1606, 1510, 1460, 1244, 1174, 1033, 836, 820 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.18–7.07 (m, 7H, Ar), 6.88 (d, J = 8.7 Hz, 2H, Ar), 6.75 (d, J = 8.7 Hz, 2H, Ar), 6.54 (d, J = 8.7 Hz, 2H, Ar), 4.12 and 3.96 (t, J = 6.0 Hz, 4H, OCH_2), 2.79 and 2.68 (t, J = 6.0 Hz, 4H, NCH_2), 2.54–2.45 (m, 10H, 3-H and piperidinyl 2-H), 1.66–1.54 (m, 8H, piperidinyl 3-H), 1.49–1.42 (m, 4H, piperidinyl 4-H), 0.93 (t, J = 7.4 Hz, 3H, 4-H); ^{13}C NMR ($CDCl_3$) δ 157.5, 156.7, 142.6, 140.9, 137.8, 136.3, 135.7, 131.8, 130.5, 129.7, 127.8, 125.8, 114.0, 113.3, 65.8, 65.6, 58.0, 57.9, 55.0, 55.0, 29.0, 25.9, 25.9, 24.2, 24.1, 13.6; HR MS (ESI): calcd for $C_{36}H_{47}N_2O_2$ ($M+H^+$), 539.3632; found, 539.3631.
8. 1,1-Bis[4-[2-(morphorin-1-yl)ethoxy]phenyl]-2-phenyl-1-butene (RID-D, **17**): Colorless crystals: mp 111–112 °C (acetonitrile); IR (KBr): 2967, 2853, 2807, 1607, 1510, 1460, 1454, 1281, 1242, 1175, 1115, 1046, 963, 933, 858, 836, 821 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.19–7.08 (m, 7H, Ar), 6.88 (d, J = 8.7 Hz, 2H, Ar), 6.76 (d, J = 8.7 Hz, 2H, Ar), 6.54 (d, J = 8.7 Hz, 2H, Ar), 4.13 and 3.97 (t, J = 5.7 Hz, 2H, OCH_2), 3.75 and 3.70 (t, J = 4.5 Hz, 8H, morphorinyl 3-H), 2.82 and 2.71 (t, J = 5.7 Hz, 4H, NCH_2), 2.60 and 2.52 (t, J = 4.5 Hz, 8H, morphorinyl 2-H), 2.48 (q, J = 7.4 Hz, 2H, 3-H), 0.93 (t, J = 7.4 Hz, 3H, 4-H); ^{13}C NMR ($CDCl_3$) δ 157.3, 156.5, 142.5, 141.0, 137.6, 136.4, 135.9, 131.9, 130.5, 129.6, 127.8, 125.8, 114.0, 113.3, 66.9, 66.8, 65.6, 65.4, 57.7, 57.6, 54.0, 54.0, 29.0, 13.6; HR MS (ESI): calcd for $C_{34}H_{43}N_2O_4$ ($M+H^+$), 543.3217; found, 543.3193.
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