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Identification of 10-dehydrooxyglycyuralin E as a selective human estrogen receptor alpha partial agonist

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ABSTRACT

Selective estrogen receptor modulators (SERMs) act as either agonist or antagonist of estrogen receptor (ER) in a tissue selective manner and have been used in several diseases such as breast cancer, postmenopausal syndrome, osteoporosis, and cardiovascular diseases. However, current SERMs may also increase the risk of serious side effects and trigger drug resistance. Herein, a screening program, that was designed to search for novel SERMs, resulted in the identification of a series of 2-arylbenzofuran-containing compounds that are ligands for ERα, when applying the *Gaussia*-luciferase reporter assay. One of these compounds, 10-dehydrooxyglycyuralin E (**T9**) was chemically synthesized. **T9** showed anti-estrogenic/proliferative activity in ERα-positive breast cancer cells. Pretreatment of **T9** prevented the mRNA expression of *GREB1*, which is an estrogen response gene. Furthermore, by an *in silico* docking simulation study we demonstrated that **T9** showed interactions directly to ERα. Taken together, these results demonstrated that **T9** is a candidate of SERMs and a useful seed compound for the foundation of the selective activity of SERMs.

1. Introduction

The estrogen receptor (ER) belongs to the nuclear receptor superfamily of ligand-dependent transcription factors [1,2]. ER is classified into two subtypes, the ERα (NR3A1) and the ERβ (NR3A2) [3–5]. Although both subtypes are activated by the hormone estrogens (17β-estradiol), there exists a large differences between ERα and ERβ in their sequence homology, organs/tissues expression, and targeted gene function [5]. ER plays crucial roles in diverse biological effects depending on target organs such as the development of female reproductive system, protection of cardiovascular tissue and maintenance of the bone mass.

Hormone replacement therapy (HRT) is used for the regulation of estrogen levels to alleviate menopausal symptoms (e.g. hot flashes) and treat diseases (e.g. osteoporosis) [6]. In contrast, anti-estrogenic agents are used to fight estrogen related diseases [7], including postmenopausal endometriosis [8], uterine leiomyomas [9], and estrogen-dependent breast cancer [10]. Especially, ERα has been extensively studied in breast cancer [11,12].

Selective estrogen receptor modulators (SERMs), which are competitive partial agonists of the ER, take much advantage of selective effects of ER in estrogen target tissues than ER full agonists [13,14]. Currently available SERMs, such as tamoxifen [15,16], raloxifene [17,18] and bazedoxifene [19,20], have been developed into useful pharmacological agents for the treatment and prevention of breast cancer and menopausal osteoporosis. However, it has also been reported that the unremitting use of SERMs may increase the risk of several serious side effects, such as strokes, deep vein thromboembolic disorders [21], and drug resistance [22]. Therefore, in clinical research studies, the tissue-selective estrogen complex (TSEC), conjugation of

Abbreviations: SERMs, selective estrogen receptor modulators; ER, estrogen receptor; ERE, estrogen response elements; **T9**, 10-dehydrooxyglycyuralin E; GREB1, growth regulation by estrogen in breast cancer 1; E2, 17β-estradiol; HRT, hormone replacement therapy; TSEC, tissue-selective estrogen complex; SERD, selective estrogen receptor degradator

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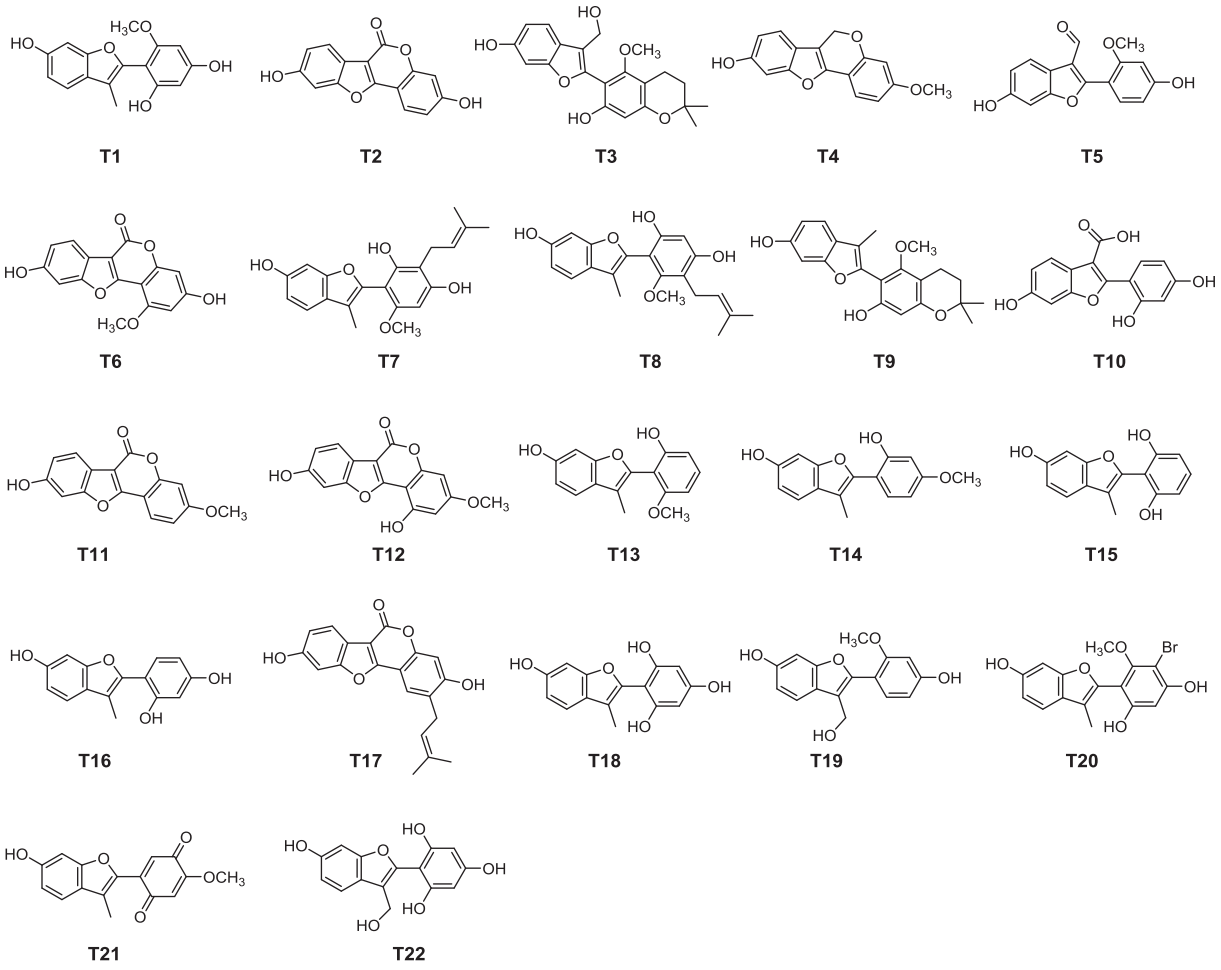

Fig. 1. Chemical structures.

estrogens with a selective SERM, has been expected to be the new menopausal therapy that will improve vasomotor symptoms and protect against osteoporosis without concurrent endometrium and breast stimulation [23,24].

Briefly, discovery of novel SERMs are essential and critical for drug development of estrogen-dependent diseases. In the present study, we conducted a screening program searching for novel SERMs, and resulted in the identification of a scaffold of 2-arylbenzofuran-containing compounds showing ERα agonistic activity (Fig. 1). Among them, a compound named 10-dehydrooxyglycyuralin E (**T9**) exhibited partial agonist activity for ERα and anti-proliferative effect on breast cancer cells.

2. Result and discussion

2.1. Screening of 2-arylbenzofuran-containing compounds as ERα partial agonists

Primary screening of ERα agonistic activity was carried out by a *Gaussia*-luciferase reporter assay and a few of 2-arylbenzofuran-containing compounds were identified as potential hits (Figs. 1 and 2). Compounds **T1**–**T22** were a series of 2-arylbenzofuran-contaning natural products and their derivates, which were chemically synthetized by a cascade [3,3]-sigmatropic rearrangement/aromatization strategy in a previous investigation [25]. A test concentration of 10 μM was set in primary screening (GAL-RE *Gaussia*-luciferase reporter assay), so that the potential hits possess acceptable effectiveness to endure further evaluation assay for drug development. At a final concentration of 10 μM, **T1**, **T5,** and **T6** showed the most potent ERα agonistic activity comparable to that of estradiol (E2), but no cytotoxic effect was observed. However, these compounds were excluded from further screening as we were searching for partial agonists for ER, as SERM, but not full (perfect) antagonists or agonists. The compounds **T2**, **T7**, **T9**, **T10**, **T12**, **T13**, **T15**, **T17,** and **T19**, which showed partial agonistic activity, were then selected for secondary screening for their ERα agonistic activity by the estrogen response elements (ERE)-dual luciferase assay. As shown in Fig. 3, five compounds (**T2**, **T7**, **T9**, **T10,** and **T12**) showed partial ERα agonistic effects compared to E2.

To test the anti-estrogenic effect of these compounds in ER-positive breast cancer [10], their proliferative effect was evaluated by the MTS assay in the MCF-7 cells (Fig. 4). Whereas most compounds showed estrogenic/proliferative effect, **T9** did not enhance cell proliferation (Fig. 4A), and also prevented estrogen induced cell proliferation (Fig. 4B). These results suggested that **T9** was a candidate compound for SERM, and was selected for further detailed investigation of its ERα agonistic activity.

2.2. Chemical synthesis of T9

The chroman derivative **T9** was synthesized following our previous reported procedure using a cascade [3,3]-sigmatropic rearrangement/aromatization strategy as the key step [25]. The ether precursor **5** was prepared by Mitsunobu reaction from benzofuran-3-ylmethanol **3** and phenol **4**, which were synthesized by known methods starting from compound **1**. The synthesized substrate **5** was mixed with silica gel and heated to 140 °C under neat conditions for 4 h leading to the desired

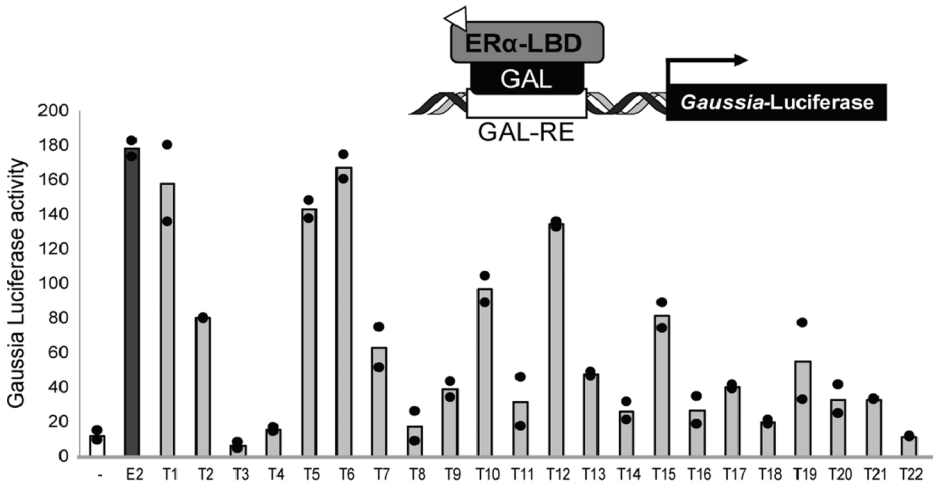

Fig. 2. Effect of compounds **T1–T22** on transactivation of Estrogen receptor alpha using GAL-RE *Gaussia*-luciferase reporter assay.
HEK293 cells were transfected with the expression vector for GAL4/DBD-ERα/LBD and GAL-RE driven *Gaussia*-luciferase reporter vector. The cells were treated with compounds (10 μM) and E2 (10 nM) for 24 h, and *Gaussia*-luciferase activity was measured. The results are the average activity of two different wells (n = 2).

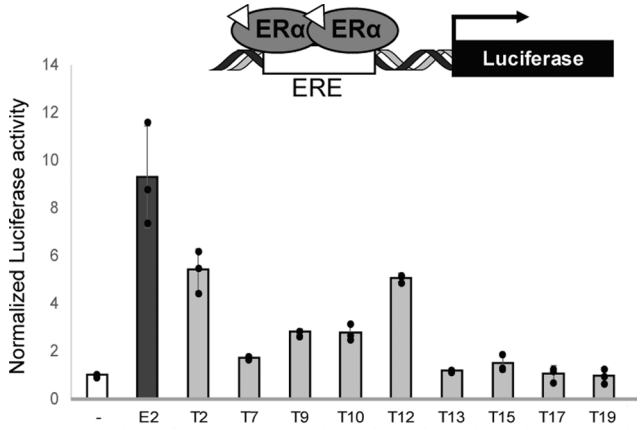

Fig. 3. Effect of compounds **T2**, **T7**, **T9**, **T10**, **T12**, **T13**, **T15**, **T17**, and **T19** on transactivation of ERα using ERE luciferase reporter assay.
HEK293 cells were transfected with the expression vector for ERα, ERE driven luciferase reporter vector, and pGL4.74, *Renilla* reporter vector. The cells were treated with compounds (10 μM) or E2 (10 nM) for 24 h, and luciferase activity was measured. The results are shown as mean ± SD (n = 3).

product **2** with a yield of 73%. After several functional transformations including *O*-methylation, hydrogenation, MOM protection of hydroxyl groups and bromination, the intermediate **10** was obtained in a moderate yield. Finally, the chroman derivative **T9** was generated by a palladium-catalyzed Suzuki coupling between bromide precursor **10** and prenyl boronic ester **11** and a CSA promoted cascade MOM-deprotection and cyclization reaction (Fig. 5).

2.3. ERα agonist activity

To examine the subtype selectivity of **T9** between ERs, HEK293 cells were transfected with ERE luciferase reporter and co-transfected with ERα or ERβ. **T9** repressed the transcriptional activity of ERα at a concentration of 10 μM (Fig. 6A). In contrast, **T9** could not repress transcriptional activity of ERβ at the same concentration (Fig. 6B). These results indicated that **T9** is subtype selective ligand for ERα.

Next, we examined the antagonistic effects of **T9** on ERα-target gene regulation in MCF-7 cells. Cells were pretreated with **T9** for 30 min and co-treated with E2 for 6 h. mRNA expression level of Growth Regulation by Estrogen in Breast cancer 1 (GREB1) [26,27], one of the estrogen response genes, was increased by E2 treatment (Fig. 7A). Pretreatment of **T9** prevents E2-induced *GREB1* mRNA expression. Further, we examined whether **T9** affects the protein levels of ERα, treating the MCF-7 cells with **T9** in the absence or presence of E2; ERα protein levels were not changed (Fig. 7B). Thus, **T9** acts as antagonist of ERα in MCF-7 cells.

To investigate the effect of **T9** on cell viability, the cells were treated with increasing concentration of **T9** (0.1–30 μM) in the absence or presence of E2 and ICI (Fig. 8). E2-dependent cell proliferation was suppressed by **T9**. However, **T9** caused ER-independent cell growth in low concentrations, as this observation was not prevented by the co-treatment with the selective estrogen receptor degradator (SERD), ICI (1 μM) [28].

2.4. Docking simulation

Considering the above experimental results, molecular docking calculation was conducted to explore the binding pattern of **T9** with ERα. The crystal structure of ERα complexed with ligand was selected as a receptor model for the docking study. Meanwhile, the binding mode of ERα with this native ligand provided us with a valuable reference to achieve accuracy in the predicted interactions between **T9** and ERα. The docked pose displaying the best binding affinity was analyzed and compared to that of the co-crystal ligand. The binding energy of **T9** with ERα was −7.13 kcal/mol, while for the native ligand was −8.88 kcal/mol. Navigating the binding pocket, the hydroxy moiety in benzofuran of **T9** is involved in a hydrogen bound interaction with Glu353 of ERα, as that was also observed in the co-crystal structure [29–31]. The hydrophobic interactions between **T9** and several residues such as Leu349, Leu346, Leu391, Leu428, Met343, were also consistent with the binding modes of ERα and its co-crystal ligand (Fig. 9). Furthermore, a surface, generated with **T9** in the docking conformation with the lowest binding affinity, fit the acceptor well, which certified the probability of **T9** to exert its bioactivity with a direct interaction with ERα (Fig. 10).

3. Conclusions

SERMs exhibiting tissue-selective and partial agonistic activities are useful therapeutic drug candidates for the treatment of various estrogen-dependent diseases, such as breast cancer, postmenopausal syndrome, osteoporosis, and cardiovascular diseases. During a screening program, that was designed to search for novel SERMs targeting ERα, a series of 2-arylbenzofuran-containing compounds were identified as partial ERα agonists. Among them, 10-dehydrooxyglycyuralin E (**T9**) showed the most promising anti-estrogenic/proliferative activity in ERα-positive breast cancer cells. Thus, **T9** was chemically synthesized and further investigated its ERα agonistic activity. **T9** was demonstrated to be ER-subtype selective partial agonist of ERα but not ERβ. Furthermore, pretreatment of **T9** prevented E2-induced *GREB1* mRNA expression, but not affected the protein levels of

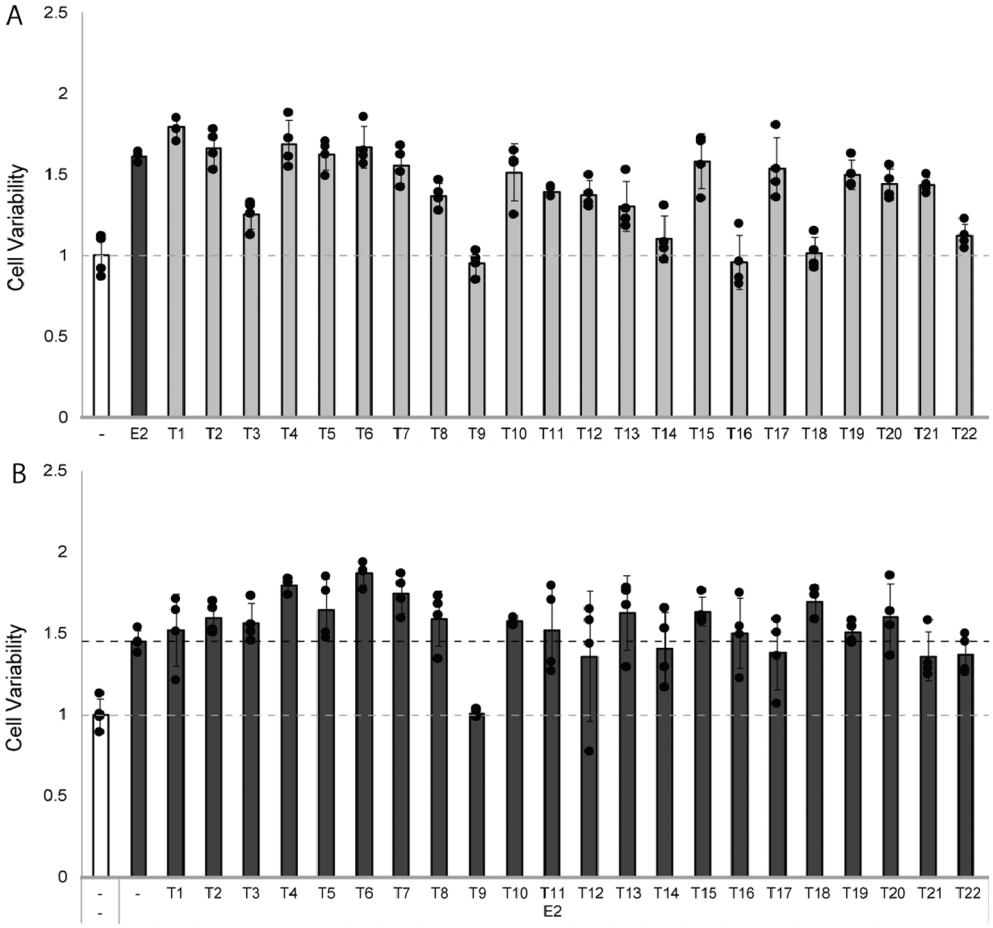

Fig. 4. Effect of compounds **T1–T22** on estrogen-mediated breast cancer cell proliferation.
MCF-7 cells were seeded on 96-well plates (1×10^4 cells/well) with phenol red free DMEM supplemented with 5% charcoal-stripped FBS. The cells were treated with compounds (10 μM) in the absence (A) or presence (B) of E2 (10 nM). After 72 h, cell viability was evaluated by the MTS assay. The results are shown as mean ± SD (n = 4).

ERα, indicating that **T9** is also an antagonist of ERα. The *in silico* molecular docking simulation suggested that **T9** directly interacts with ERα. Taken together, these results demonstrated that **T9** is a potential SERM candidate for further drug development targeting ERα-related diseases, but need further pharmacological investigations.

4. Experimental section

4.1. Experimental procedures and spectroscopic data of the synthesized compounds

2-(2-Acetyl-5-(benzyloxy)phenoxy)acetic acid ethyl ester (**2**). To a solution of 2,4-dihydroxyacetophenone **1** (100.0 g, 0.657 mol) in dry acetonitrile (500 mL) was added anhydrous potassium carbonate (100.0 g, 0.723 mol), and the mixture was refluxed for 1 h. Benzyl bromide (76.6 mL, 0.644 mol) dissolved in dry acetonitrile (50 mL) was added dropwise to the mixture. The reaction was refluxed for 3 h. The reaction mixture was subjected to a silica gel column, eluted with ethyl acetate and evaporated to dryness. The crude product, anhydrous potassium carbonate (90 g, 0.657 mol), and ethyl chloroacetate (96.5 g, 0.788 mol) were dissolved in dry acetone (400 mL) and refluxed for 48 h. The precipitate was filtered off through silica gel, washed with acetone and the filtrate was evaporated to dryness, purified by flash column chromatography (ethyl acetate/petroleum ether = 1:20) to give product **2** (129.3 g) as a white solid with a yield of 60%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.66 (d, J = 8.9 Hz, 1H), 7.45 (m, 2H), 7.41 (m, 2H), 7.4.0 (m, 1H), 6.73 (d, J = 2.4 Hz, 1H), 6.72 (dd, J = 8.9, 2.4 Hz, 1H), 5.18 (s, 2H), 4.95 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 2.57 (s, 3H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.8, 168.1, 163.5, 159.1, 136.2, 133.1, 128.9, 128.5, 127.7, 122.0, 107.0, 100.1, 70.5, 65.7, 61.7, 32.2, 14.3; IR (thin film, cm^{-1}) 3410, 3036, 2933, 2790, 1614, 1501, 1486, 1110; HRMS (ESI-TOF) m/z [M + Na]$^+$ Calcd. for $C_{19}H_{20}O_5Na$ 352.1236; Found 352.1230.

(6-(Benzyloxy)benzofuran-3-yl)methanol (**3**). To a vigorously stirred solution of potassium carbonate (50 g, 0.36 mol) in water (400 mL) 2-(2-acetyl-5-(benzyloxy) phenoxy)acetic acid ethyl ester **2** (100 g, 0.3 mol) was added and the mixture was gently refluxed for 3 h. The solution was cooled down to 5 °C and acidified carefully with a solution of concentrated HCl. The deposited precipitate was filtered off and washed with cold water. The resulting crude product together with anhydrous sodium acetate (96 g, 1.2 mol) was dissolved in acetic anhydride (390 g, 2.8 mol) and the mixture was heated at 160 °C for 3 h. After cooling, the mixture was diluted with water (900 mL) and then extracted with diethyl ether. The combined organic layers were washed with a saturated aqueous solution of Na_2CO_3 and brine, dried over anhydrous Na_2SO_4, and then evaporated to dryness. The mixture of the dry crude product and selenium dioxide (33 g, 0.3 mol) in dry 1,4-dioxane (200 mL) was refluxed for 48 h. The resulting black precipitate was filtered off and the crude product was evaporated to dryness. To the solution of the crude mixture in methanol (100 mL) under stirring were added small quantities of $NaBH_4$ at room temperature until the entire amount of the aldehyde was converted. Then, the reaction mixture was treated with 5 M HCl (10 mL). The crude product was treated with a mixture of ethyl acetate and water (1:1, v/v). The organic layers were separated, washed with water, brined and then dried over anhydrous Na_2SO_4, evaporated to dryness, and purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:5, R_f = 0.1) to give product **3** (48.0 g) as a yellow powder with a yield of 63%. Mp 126.6–127.1 °C;^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 – 7.51 (m, 2H), 7.46 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.34 (d, J = 7.4 Hz, 1H), 7.09 (d, J = 2.2 Hz, 1H), 6.99 (dd, J = 8.5, 2.2 Hz, 1H), 5.11 (s, 2H), 4.80 (s, 2H) ; ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.5,

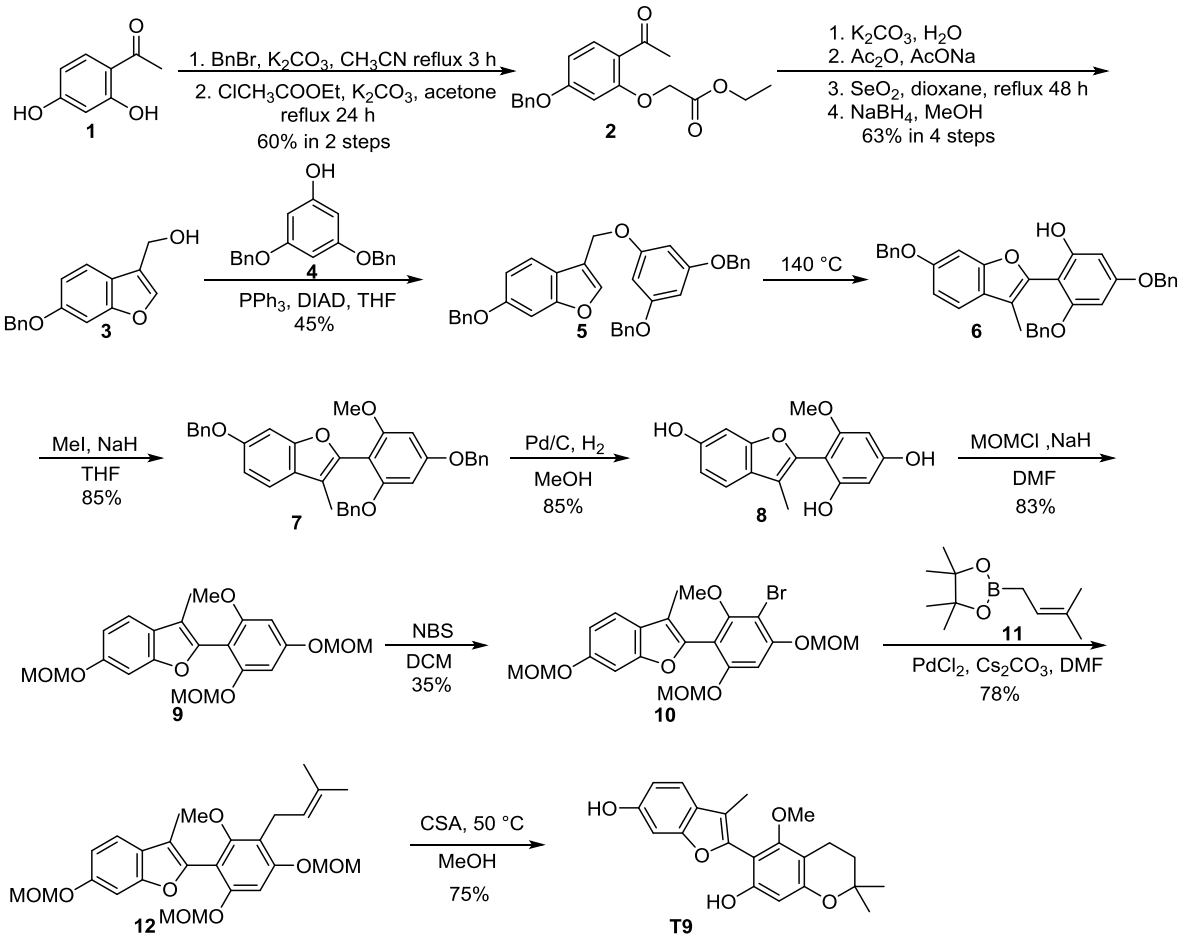

Fig. 5. Synthetic route to chroman derivative **T9**.

156.7, 141.6, 137.0, 128.8, 128.2, 127.6, 120.5, 120.4, 120.2, 112.7, 97.6, 70.7, 56.2; IR (thin film, cm^{-1}) 3402, 3052, 2928, 2843, 1597, 1483, 1120; HRMS (ESI-TOF) *m/z* $[M+H]^+$ Calcd. for $C_{16}H_{15}O_3$ 255.1021; Found 255.1016.

6-(Benzyloxy)-3-((3,5-bis(benzyloxy)phenoxy)methyl)benzofuran (**5**). To the solution of (6-(benzyloxy)benzofuran-3-yl)methanol **3** (2.0 g, 8 mmol), 3,5-dibenzyloxy phenol **4** (2.9 g, 9.6 mmol) and triphenylphosphine (3.2 g, 12 mmol) in dry THF was added diisopropyl azodiformate (2.4 g, 12 mmol) dropwise at 0 °C for 15 min under nitrogen atmosphere. The reaction was stirred at room temperature for 4 h. The crude mixture was evaporated to dryness and purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:50, $R_f = 0.3$) to give product **5** (1.9 g) as a yellow oil with a yield of 45%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 7.4 Hz, 2H), 7.37–7.43 (m, 4H), 7.39 (t, J = 7.5 Hz, 6H), 7.32–7.35 (m, 3H), 7.09 (d, J = 2.2 Hz, 1H), 6.99 (dd, J = 8.5, 2.2 Hz, 1H), 6.29 (s, 3H), 5.12 (s, 2H), 5.10 (s, 2H), 5.01 (s, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.8, 160.6, 157.6, 156.6, 142.5, 137.0, 136.9, 128.8, 128.7, 128.6, 128.2, 128.1, 127.7, 127.6, 127.4, 120.5, 120.3, 116.7, 112.9, 97.6, 95.2, 95.0, 70.7, 70.3, 61.5; IR (thin film, cm^{-1}) 3430, 3031, 2873, 1624, 1599, 1454, 1378, 1164, 1134, 1058; HRMS (ESI) *m/z* $[M+H]^+$ Calcd. for $C_{36}H_{31}O_5$ 543.2166, Found 543.2174.

3,5-Bis(benzyloxy)-2-(6-(benzyloxy)-3-methylbenzofuran-2-yl) phenol (**6**). In a solution of 6-(benzyloxy)-3-((3,5-bis(benzyloxy)

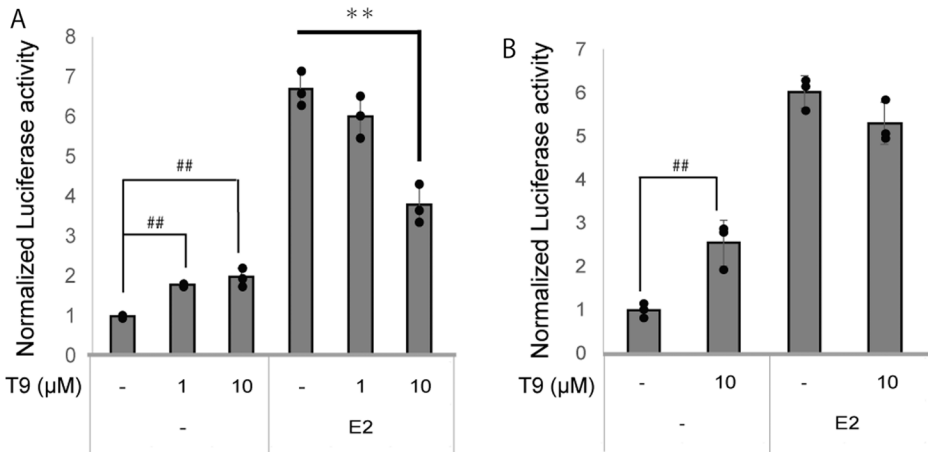

Fig. 6. Effect of **T9** on transactivation of ERα and ERβ using ERE luciferase reporter assay.
HEK293 cells were transfected with the expression vector for ERα (A), ERβ (B), ERE driven luciferase reporter vector, and pGL4.74, *Renilla* reporter vector. The cells were treated with **T9** or E2 (10 nM) for 24 h, and luciferase activity was measured. The results are shown as mean ± SD (n = 3), ** $p < 0.01$, ## $p < 0.01$.

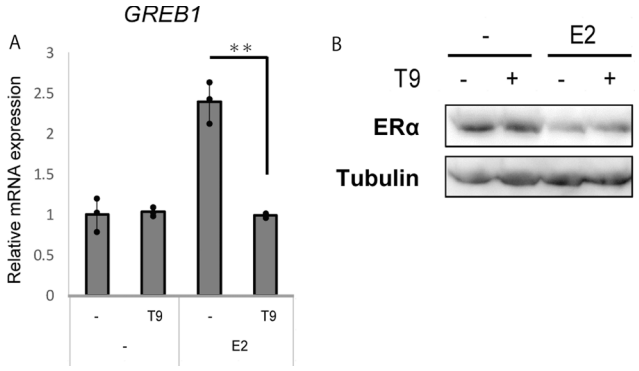

Fig. 7. Effect of **T9** on ER-mediated gene expression and protein levels of ERα in MCF-7 cells.
MCF-7 cells were pretreated with **T9** for 30 min and co-treated with E2 for 6 h. The cells were harvested. (A) The mRNA expression of *GREB1* was measured by RT-qPCR. The results were normalized against *β-actin*, and expressed as the average of the fold induction over the solvent control (mean ± S.D., n = 3), ** $p < 0.01$. Results show one representative result of two independent experiments. (B) Whole-cell lysates of MCF-7 cells were resolved by SDS PAGE electrophoresis and proteins were detected by immunoblotting using antibodies against ERα and Tubulin.

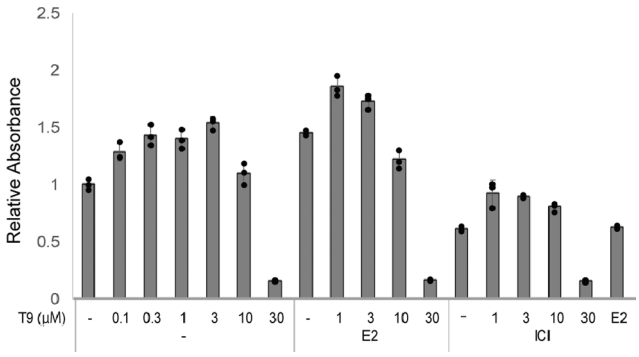

Fig. 8. Effect of **T9** on breast cancer cell proliferation in the absence or presence of E2 and ICI.
MCF-7 cells were seeded on 96-well plate (1×10^4 cells/well) with phenol red free DMEM supplemented with 5% charcoal-stripped FBS. The cells were treated with **T9** (1–30 μM) in the absence or presence of E2 (10 nM) and ICI (1 μM). After 72 h, cell viability was evaluated by the MTS assay. The results are shown as mean ± SD (n = 3).

phenoxy)methyl)benzofuran **5** (1.0 g, 1.8 mmol) in DCM (10 mL) silica gel was added (2.0 g) and DCM was removed under vacuum. The mixture was heated at 140 °C for 4 h. The crude mixture was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:10, R_f = 0.2) to give product **6** (713 mg) as a yellow oil with a yield of 73%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (dd, J = 7.9, 0.9 Hz, 2H), 7.44–7.42 (m, 2H), 7.41–7.38 (m, 5H), 7.36–7.32 (m, 2H), 7.29–7.27 (m, 3H), 7.26–7.23 (m, 2H), 7.09 (d, J = 2.1 Hz, 1H), 6.98 (dd, J = 8.5, 2.2 Hz, 1H), 6.32 (d, J = 2.3 Hz, 1H), 6.29 (d, J = 2.3 Hz, 1H), 5.95 (s, 1H), 5.13 (s, 2H), 5.05 (s, 2H), 5.03 (s, 2H), 2.10 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.7, 158.6, 157.3, 156.7, 155.6, 143.9, 137.1, 136.8, 136.7, 128.8, 128.7, 128.6, 128.3, 128.1, 127.8, 127.7, 127.6, 127.1, 124.1, 119.7, 115.8, 112.2, 100.4, 97.3, 94.7, 94.2, 70.8, 70.6, 70.3, 9.3; IR (thin film, cm^{-1}) 3433, 3032, 2921, 2854, 1629, 1452, 1384, 1151; HRMS (ESI) m/z $[M + H]^+$ Calcd. for $C_{36}H_{31}O_5$ 543.2166, Found 543.2173.

6-(Benzyloxy)-2-(2,4-bis(benzyloxy)-6-methoxyphenyl)-3-methylbenzofuran (**7**). To a suspension of NaH (60% dispersion in paraffin oil) (40 mg, 1.0 mmol) in THF (4 mL) was added a solution of 3,5-bis(benzyloxy)-2-(6-(benzyloxy)-3-methylbenzo furan-2-yl)phenol **6** (488 mg, 0.9 mmol) in dry THF (1 mL) at 0 °C with stirring under a nitrogen atmosphere. The mixture was stirred for 1 h at room temperature and iodomethane (255 mg, 1.8 mmol) was added dropwise over 10 min. The mixture was stirred at room temperature overnight; the reaction mixture was quenched by the addition of a saturated aqueous solution of ammonium chloride (20 mL) and extracted with ethyl acetate (20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, evaporated to dryness, and purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:20, R_f = 0.3) to give product **7** (473 mg) as a yellow oil with a yield of 85%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49–7.29 (m, 11*H*), 7.25–7.18 (m, 5H), 7.09 (d, J = 2.2 Hz, 1H), 6.94 (dd, J = 8.5, 2.2 Hz, 1H), 6.30 (dd, J = 12.4, 2.2 Hz, 2H), 5.12 (s, 2H), 5.06 (s, 2H), 5.02 (s, 2H), 3.75 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.6, 160.4, 159.4, 156.7, 155.6, 145.2, 137.4, 137.1, 136.7, 128.8, 128.7, 128.5, 128.0, 127.8, 127.7, 127.6, 126.9, 124.4, 119.3, 114.4, 111.4, 102.7, 97.4, 93.6, 92.4, 70.8, 70.7, 70.4, 56.1, 9.0; IR (thin film, cm^{-1}) 3428, 2923, 2855, 1630, 1384, 1101; HRMS (ESI) m/z $[M+H]^+$ Calcd. for $C_{37}H_{33}O_5$ 557.2323, Found 557.2332.

4-(6-Hydroxy-3-methylbenzofuran-2-yl)-5-methoxybenzene-1,3-diol (**8**). To the solution of 6-(benzyloxy)-2-(2,4-bis(benzyloxy)-6-methoxyphenyl)-3-methyl-benzofuran **7** (390 mg, 0.7 mmol) in methanol (10 mL) was added 10% Pd/C (40 mg). The reaction vessel was then evacuated, and the atmosphere replaced with hydrogen. After

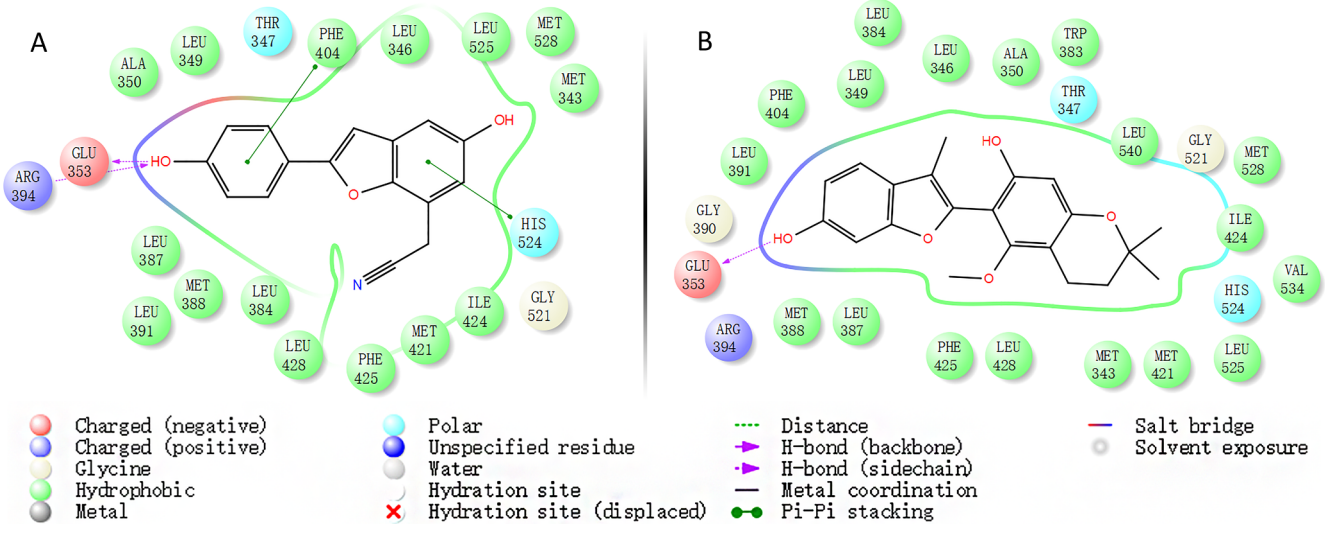

Fig. 9. The key interactions between the co-crystal ligand (A) and **T9** (B) and ERα were displayed in 2D style.

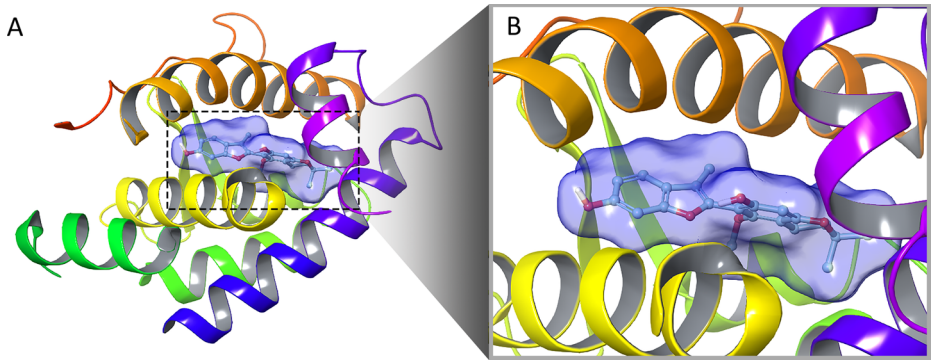

Fig. 10. Docking pose of **T9** binding at the active pocket of ERα.
The protein is depicted in ribbon and the molecule was displayed as a ball and stick model with carbon atoms painted blue. A surface was generated to describe the shape of the binding pocket.

vigorous stirring for 8 h, the reaction mixture was filtered through silica gel and the filtrate was evaporated to dryness and purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:2, R_f = 0.1) to give product **8** (170 mg) as a colorless oil with a yield of 85%. ^{1}H NMR (600 MHz, CD_3OD) δ 7.25 (d, J = 8.3 Hz, 1H), 6.80 (d, J = 2.1 Hz, 1H), 6.70 (dd, J = 8.3, 2.1 Hz, 1H), 6.04 (dd, J = 6.7, 2.1 Hz, 2H), 3.68 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.5, 159.9, 157.8, 155.6, 154.5, 145.1, 123.2, 118.4, 113.4, 110.3, 98.9, 97.0, 95.0, 90.7, 54.6, 7.4; IR (thin film, cm^{-1}) 3450, 2967, 2788, 1695, 1314; HRMS (ESI) m/z $[M+H]^+$ Calcd. for $C_{16}H_{16}O_5$ 287.0914, Found 287.0910.

2-(2-Methoxy-4,6-bis(methoxymethoxy)phenyl)-6-(methoxymethoxy)-3-methylbenzofuran (**9**). To a suspension of NaH (60% dispersion in paraffin oil) (40 mg, 1.0 mmol) in dry dimethylformamide (5 mL) was added a solution of 4-(6-hydroxy-3-methylbenzo furan-2-yl)-5-methoxybenzene-1,3-diol **8** (286 mg, 1.0 mmol) in dry dimethylformamide (2 mL) at 0 °C under stirring in a nitrogen atmosphere. The mixture was stirred at room temperature for 1 h and chloromethyl methyl ether (161 mg, 2.0 mmol) was added dropwise at 0 °C for over 30 min. The reaction was stirred at room temperature for 6 h, quenched by a saturated aqueous solution of NH_4Cl (20 mL) and extracted with ethyl acetate (20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude material was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:10, R_f = 0.1) to give product **9** (347 mg) as a colorless oil with a yield of 83%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.44 (d, J = 8.5 Hz, 1H), 7.17 (d, J = 2.1 Hz, 1H), 6.95 (dd, J = 8.5, 2.1 Hz, 1H), 6.52 (d, J = 2.1 Hz, 1H), 6.47 (d, J = 2.1 Hz, 1H), 5.26 (s, 2H), 5.22 (s, 2H), 5.11 (s, 2H), 3.70 (s, 3H), 3.43 (s, 3H), 3.40 (s, 3H), 3.25 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.8, 159.7, 157.3, 154.5, 154.4, 145.2, 124.4, 119.2, 113.4, 112.3, 102.4, 98.9, 95.6, 94.5, 94.2, 94.0, 93.8, 55.9, 55.8, 55.6, 55.5, 8.4; IR (thin film, cm^{-1}) 3422, 2922, 2853, 1804, 1697, 1492, 1454, 1153, 1025; HRMS (ESI) $[M+H]^+$ m/z Calcd. for $C_{22}H_{27}O_8$ 419.1700, Found 419.1709.

2-(3-Bromo-2-methoxy-4,6-bis(methoxymethoxy)phenyl)-6-(methoxymethoxy)-3-methylbenzofuran (**10**). To a solution of 2-(2-methoxy-4,6-bis(methoxymethoxy)phenyl)-6-(methoxymethoxy)-3-methylbenzofuran **9** (418 mg, 1.0 mmol) in DCM (10 mL) was added a solution of *N*-bromosuccinimide (178 mg, 1.0 mmol) in DCM (5 mL) dropwise under stirring in a nitrogen atmosphere at 0 °C. The mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness. The crude material was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:10, R_f = 0.1) to give product **10** (174 g) as a colorless oil with a yield of 35%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (d, J = 8.4 Hz, 1H), 7.20 (d, J = 2.1 Hz, 1H), 7.00 (dd, J = 8.4, 2.1 Hz, 1H), 6.90 (s, 1H), 5.30 (s, 2H), 5.22 (s, 2H), 5.09 (s, 2H), 3.58 (s, 3H), 3.56 (s, 3H), 3.52 (s, 3H), 3.38 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.2, 156.9, 156.3, 155.4, 155.3, 144.5, 124.9, 119.5, 115.0, 112.6, 110.2, 101.1, 99.6, 99.5, 95.3, 95.2, 61.5, 56.8, 56.4, 56.1, 8.8; IR (thin film, cm^{-1}) 3422, 2922, 2253, 2126, 1652, 1384, 1051, 1026, 1005; HRMS (ESI) m/z $[M+H]^+$ Calcd. for $C_{22}H_{26}O_8Br$ 497.0811, Found 497.0815.

2-(2-Methoxy-4,6-bis(methoxymethoxy)-3-(3-methylbut-2-en-1-yl) phenyl)-6-(methoxymethoxy)-3-methylbenzofuran (**12**). To a solution of 2-(3-bromo-2-methoxy-4,6-bis(methoxymethoxy)phenyl)-6-(methoxymethoxy)-3-methylbenzofuran **10** (99 mg, 0.2 mmol) in dry dimethylformamide (5 mL) was added cesium carbonate (130 mg, 0.4 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium (II) (7 mg, 0.01 mmol), and 4,4,5,5-tetramethyl-2-(3-methylbut-2-en-1-yl)-1,3,2-dioxaborolane **11** (58 mg, 0.3 mmoml) under a nitrogen atmosphere. The mixture was stirred at 90 °C for 9 h. The reaction was monitored by thin layer chromatography (TLC) till the complete consumption of the starting material. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (15 mL). The organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude material was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:10, R_f = 0.1) to obtain product **12** (76 mg) as a colorless oil with a yield of 78%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 6.97 (dd, J = 8.4, 2.1 Hz, 1H), 6.61 (s, 1H), 5.25 (s, 2H), 5.24 – 5.22 (m, 1H), 5.21 (s, 2H), 4.64 (s, 2H), 3.75 (s, 3H), 3.51 (s, 6H), 3.40 (d, J = 6.7 Hz, 2H), 3.16 (s, 3H), 2.09 (s, 3H), 1.78 (s, 3H), 1.68 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.1, 157.9, 156.3, 155.4, 155.1, 146.1, 131.1, 125.2, 123.6, 119.3, 117.0, 114.5, 112.4, 107.3, 99.8, 99.7, 95.4, 94.8, 94.5, 57.3, 56.21, 56.17, 56.1, 25.9, 23.3, 18.0, 8.8; IR (thin film, cm^{-1}) 3425, 2921, 2827, 1589, 1469, 1381, 1152, 1105; HRMS (ESI) $[M+H]^+$ m/z Calcd. for $C_{27}H_{35}O_8$ 487.2326, Found 487.2335.

6-(6-Hydroxy-3-methylbenzofuran-2-yl)-5-methoxy-2,2-dimethylchroman-7-ol (**T9**). To a solution of 2-(2-methoxy-4,6-bis (methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-6-(methoxymethoxy)-3-methylbenzofuran **12** (71 mg, 0.2 mmol) in methanol (5 mL) was added CSA (23 mg, 0.1 mmol) and the resulting mixture was stirred at 50 °C for 12 h. The reaction was monitored by TLC till the complete consumption of the starting material. The reaction mixture was quenched by the addition of a saturated aqueous solution of Na_2CO_3 (10 mL) and extracted with ethyl acetate (10 mL). The organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude material was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether = 1:2, R_f = 0.2) to give product **T9** (39 mg) as a colorless oil with a yield of 75%. The spectroscopic data of **T9** was identical with previous reported [25].

4.2. Cell culture

Cells of the human embryonic kidney cell line HEK293 were cultured in Dulbecco's modified Eagle's medium (DMEM; Wako, Osaka, Japan) containing 10% fetal bovine serum (FBS) and penicillin-streptomycin in a humidified atmosphere containing 5% CO_2 at 37 °C. Human breast cancer cell line MCF-7 was cultured in DMEM containing 5% FBS and penicillin-streptomycin in a humidified 5% CO_2 at 37 °C.

4.3. Plasmid construction

Full-length cDNA encoding human ERα and ERβ were inserted into pcDNA5 vector (Invitrogen/Thermo Fisher Scientific, Waltham, MA). The construction of expression vector encoding a GAL4-DBD fused ERα-LBD (GAL4/DBD-ERα/LBD) was cloned into the pcDNA5 vector (Invitrogen). The GAL-responsible elements (GALREs)/TATA-box were inserted into pMCS-*Gaussia Luc* vector (Thermo Fisher Scientific) to insert the GAL-RE driven *Gaussia*-luciferase reporter plasmid. ERE driven luciferase reporter vector was previously constructed [32].

4.4. Gaussia-luciferase reporter assay

HEK293 cells maintained in phenol-red-free DMEM, containing 5% charcoal-stripped FBS (csFBS) were transfected with the expression vector for GAL4/DBD-ERα/LBD, GAL-RE driven *Gaussia*-luciferase reporter vector, using PEI Max Reagent (Polysciences Inc., Warrington, PA). After overnight incubation, the cells were treated with compounds **T1–T22** (10 μM) or Estradiol (10 nM) for 24 h, and the *Gaussia*-luciferase activity was measured using the BioLux Gaussia Luciferase Assay Kit (BioRad, Hercules, CA).

4.5. Dual-luciferase reporter assay

HEK293 cells maintained in phenol-red-free DMEM containing 5% csFBS were transfected with estrogen receptor expression vector, ERE driven luciferase reporter vector, and pGL4.74 (hRluc/TK; Promega, Madison, WI); as an internal standard, we used PEI Max Reagent (Polysciences Inc.). After overnight incubation, the cells were treated with compounds (**T2**, **T7**, **T9**, **T10**, **T12**, **T13**, **T15**, **T17**, and **T19**) or E2 for 24 h and the luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega). The activities of *firefly* luciferase were normalized to those of *Renilla* luciferase.

4.6. Cell proliferation assay

MCF-7 cells were seeded at 1×10^4 cells/ml in phenol-red-free DMEM containing 5% csFBS in 96-well plates. The cells were treated with compounds in the absence or presence of E2 (10 nM) and a selective estrogen receptor degradator (SERD; ICI 182780; Sigma Aldrich, St Louis, MO) (1 μM) [28] as indicated. After 72 h of incubation, the viability of MCF-7 cells was evaluated using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit (Promega) according to the manufacturer's instructions. The number of living cells was directly proportional to the absorbance at 490 nm of a formazan product reduced from living cells by MTS. The absorbance at 490 nm was measured in a multimode detector (BECKMAN COUNTER, CA, USA).

4.7. Western blotting

The cells were seeded in 24-well plates in phenol red-free DMEM containing 5% csFBS. The next day, the cells were treated with **T9** (10 μM) or E2 (10 nM) for 6 h. Total protein was extracted with sodium dodecyl sulfate (SDS) sample buffer [125 mM Tris-HCI pH 6.8, 4% SDS, 20% glycerol, 10% dithiothreitol, and 0.01% bromophenol blue]. The protein samples were separated by SDS-polyacrylamide gel electrophoresis (PAGE). Immunoblotting was performed with rabbit anti-ERα (D8H8) antibody (1: 1000 dilution; Cell Signaling Technology, Danvers, MA) and anti-Tubulin (1: 10,000 dilution; MBL, Nagoya, Japan) as primary antibodies and horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulin G (IgG) (1: 4000 dilution; Cell Signaling Technology) as a secondary antibody. Band intensity was detected using a Luminograph Cheminluminescent Imaging System (ATTO, Tokyo, Japan) and analyzed by ImageJ software (National Institutes of Health).

4.8. Reverse transcription quantitative real-time PCR (RT-qPCR)

Total RNA was isolated using the ISOGEN II (Nippon Gene, Tokyo, Japan) according to the manufacturer's protocol. cDNA was synthesized using a ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan). Quantitative real-time PCR (qPCR) was conducted using SYBR qPCR Mix (Toyobo) according to the manufacturer's protocol and using the 7500 fast system SDS software 2.0.6 (Applied Biosystems, Foster City, CA). The following pairs of primer were used *GREB1*: forward 5′-CAC ATCTATCCTAGACATTTA-3′, reverse 5′-CGCGGACTTTTTTTCTTA GGA-3′ and *β-actin*: forward 5′-TCCTCCTGAGCGCAAGTACTC-3′, reverse 5′-CTGCTTGCTGATCCACATCTG-3′.

4.9. Docking

AutoDock 4 [33] was applied to investigate the possible binding mode of a certain molecule with a certain protein. The crystal structure of human Estrogen Receptor (PDB code: 1X7E [31]) was used in docking calculation. A grid box was generated with a grid spacing of 0.375 Å and its center was defined with the coordination of the co-crystal ligand. Structure of **T9** was built and optimized in SYBYL 6.9.1 software package (Tripos Inc.) with Tripos force field and Gasteiger–Hückel charges. The Lamarckian genetic algorithm was used during the molecular docking, the optimized parameters were set as follows: the maximum number of energy evaluations per run was set to 25 000 000, the iterations of Solis & Wets local search were set to 3000, the number of the individuals was up to 300, and the number of generations was set to 100. The poses generated with a positional root mean square deviation (RMSD) less than 2 Å were divided into one cluster, and the conformation with the lowest binding energy and the highest percentage of frequency was selected as a representative result. All other parameters were kept as default.

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References

[1] S. Green, P. Walter, V. Kumar, A. Krust, J.M. Bornert, P. Argos, P. Chambon, Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A, Nature 320 (1986) 134–139, https://doi.org/10.1038/320134a0.
[2] D.J. Mangelsdorf, C. Thummel, M. Beato, P. Herrlich, G. Schütz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, R.M. Evans, The nuclear receptor superfamily: the second decade, Cell 83 (1995) 835–839 http://www.ncbi.nlm.nih.gov/pubmed/8521507.
[3] G.G. Kuiper, E. Enmark, M. Pelto-Huikko, S. Nilsson, J.A. Gustafsson, Cloning of a novel receptor expressed in rat prostate and ovary, Proc. Natl. Acad. Sci. USA 93 (1996) 5925–5930 http://www.ncbi.nlm.nih.gov/pubmed/8650195.
[4] S. Mosselman, J. Polman, R. Dijkema, ER beta: identification and characterization of a novel human estrogen receptor, FEBS Lett. 392 (1996) 49–53 http://www.ncbi.nlm.nih.gov/pubmed/8769313.
[5] S. Nilsson, J.-å Gustafsson, Estrogen receptors: therapies targeted to receptor subtypes., Clin. Pharmacol. Ther. 89 (2011) 44–55. doi: 10.1038/clpt.2010.226.
[6] A.M. Kaunitz, J.E. Manson, Management of menopausal symptoms, Obstet. Gynecol. 126 (2015) 859–876, https://doi.org/10.1097/AOG.0000000000001058.
[7] K.A. Burns, K.S. Korach, Estrogen receptors and human disease: an update, Arch. Toxicol. 86 (2012) 1491–1504, https://doi.org/10.1007/s00204-012-0868-5.

[8] T. Harada, Endometriosis: pathogenesis and treatment, Endometr. Pathog. Treat. 10 (2014) 1–477, https://doi.org/10.1007/978-4-431-54421-0.
[9] G.P. Flake, J. Andersen, D. Dixon, Etiology and pathogenesis of uterine leiomyomas: a review, Environ. Health Perspect. 111 (2003) 1037–1054, https://doi.org/10.1289/ehp.5787.
[10] M. Clemons, P. Goss, Estrogen and the risk of breast cancer, N. Engl. J. Med. 344 (2001) 276–285, https://doi.org/10.1056/NEJM200101253440407.
[11] B. Manavathi, O. Dey, V.N.R. Gajulapalli, R.S. Bhatia, S. Bugide, R. Kumar, Derailed estrogen signaling and breast cancer: an authentic couple, Endocr. Rev. 34 (2013) 1–32, https://doi.org/10.1210/er.2011-1057.
[12] M. Dalvai, K. Bystricky, Cell cycle and anti-estrogen effects synergize to regulate cell proliferation and ER target gene expression, PLoS One 5 (2010) e11011. doi: 10.1371/journal.pone.0011011.
[13] B.L. Riggs, L.C. Hartmann, Selective estrogen-receptor modulators – mechanisms of action and application to clinical practice, N. Engl. J. Med. 348 (2003) 618–629, https://doi.org/10.1056/NEJMra022219.
[14] P.Y. Maximov, T.M. Lee, V.C. Jordan, The discovery and development of selective estrogen receptor modulators (SERMs) for clinical practice, Curr. Clin. Pharmacol. 8 (2013) 135–155, https://doi.org/10.1016/j.mce.2014.11.015.
[15] V.C. Jordan, Tamoxifen: a most unlikely pioneering medicine, Nat. Rev. Drug Discov. 2 (2003) 205–213, https://doi.org/10.1038/nrd1031.
[16] J. Cuzick, J. Forbes, R. Edwards, M. Baum, S. Cawthorn, A. Coates, A. Hamed, A. Howell, T. Powles, IBIS investigators, first results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial, Lancet (London, England) 360 (2002) 817–824, https://doi.org/10.1016/S0140-6736(13)60140-3.
[17] C. Jochems, U. Islander, A. Kallkopf, M. Lagerquist, C. Ohlsson, H. Carlsten, Role of raloxifene as a potent inhibitor of experimental postmenopausal polyarthritis and osteoporosis, Arthritis Rheum. 56 (2007) 3261–3270, https://doi.org/10.1002/art.22873.
[18] P.D. Delmas, N.H. Bjarnason, B.H. Mitlak, A.C. Ravoux, A.S. Shah, W.J. Huster, M. Draper, C. Christiansen, Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women, N. Engl. J. Med. 337 (1997) 1641–1647, https://doi.org/10.1056/NEJM199712043372301.
[19] R.A. Lobo, J.V. Pinkerton, M.L.S. Gass, M.H. Dorin, S. Ronkin, J.H. Pickar, G. Constantine, Evaluation of bazedoxifene/conjugated estrogens for the treatment of menopausal symptoms and effects on metabolic parameters and overall safety profile, Fertil. Steril. 92 (2009) 1025–1038, https://doi.org/10.1016/j.fertnstert.2009.03.113.
[20] B.S. Komm, Y.P. Kharode, P.V.N. Bodine, H.A. Harris, C.P. Miller, C.R. Lyttle, Bazedoxifene acetate: a selective estrogen receptor modulator with improved selectivity, Endocrinology 146 (2005) 3999–4008, https://doi.org/10.1210/en.2005-0030.
[21] H.B. Nichols, L.A. DeRoo, D.R. Scharf, D.P. Sandler, Risk-benefit profiles of women using tamoxifen for chemoprevention, J. Natl. Cancer Inst. 107 (2015) 354, https://doi.org/10.1093/jnci/dju354.
[22] C.K. Osborne, Tamoxifen in the treatment of breast cancer, N. Engl. J. Med. 339 (1998) 1609–1618, https://doi.org/10.1056/NEJM199811263392207.
[23] R. Lindsay, J.C. Gallagher, R. Kagan, J.H. Pickar, G. Constantine, Efficacy of tissue-selective estrogen complex of bazedoxifene/conjugated estrogens for osteoporosis prevention in at-risk postmenopausal women, Fertil. Steril. 92 (2009) 1045–1052, https://doi.org/10.1016/j.fertnstert.2009.02.093.
[24] A. Andersson, A.I. Bernardi, M. Nurkkala-Karlsson, A. Stubelius, L. Grahnemo, C. Ohlsson, H. Carlsten, U. Islander, Suppression of experimental arthritis and associated bone loss by a tissue-selective estrogen complex, Endocrinology 157 (2016) 1013–1020, https://doi.org/10.1210/en.2015-1820.
[25] Y. Tang, C. Jiang, X. Zhang, C. Liu, J. Lin, Y. Wang, C. Du, X. Peng, W. Li, Y. Liu, M. Cheng, Collective syntheses of 2-(3-methylbenzofuran-2-yl)phenol-derived natural products by a cascade [3,3]-sigmatropic rearrangement/aromatization strategy, J. Org. Chem. 82 (2017) 11102–11109, https://doi.org/10.1021/acs.joc.7b02066.
[26] M.G. Ghosh, D.A. Thompson, R.J. Weigel, PDZK1 and GREB1 are estrogen-regulated genes expressed in hormone-responsive breast cancer, Can. Res. 60 (2000) 6367–6375 http://www.ncbi.nlm.nih.gov/pubmed/11103799.
[27] H. Mohammed, C. D'Santos, A.A. Serandour, H.R. Ali, G.D. Brown, A. Atkins, O.M. Rueda, K.A. Holmes, V. Theodorou, J.L.L. Robinson, W. Zwart, A. Saadi, C.S. Ross-Innes, S. Chin, S. Menon, J. Stingl, C. Palmieri, C. Caldas, J.S. Carroll, Endogenous purification reveals GREB1 as a key estrogen receptor regulatory factor, Cell Rep. 3 (2013) 342–349, https://doi.org/10.1016/j.celrep.2013.01.010.
[28] A.E. Wakeling, M. Dukes, J. Bowler, A potent specific pure antiestrogen with clinical potential, Cancer Res. 51 (1991) 3867–3873, https://doi.org/10.1158/0008-5472.CAN-10-3145.
[29] V. Delfosse, M. Grimaldi, J.-L. Pons, A. Boulahtouf, A. le Maire, V. Cavailles, G. Labesse, W. Bourguet, P. Balaguer, Structural and mechanistic insights into bisphenols action provide guidelines for risk assessment and discovery of bisphenol A substitutes, Proc. Natl. Acad. Sci. 109 (2012) 14930–14935, https://doi.org/10.1073/pnas.1203574109.
[30] R.W. Hsieh, S.S. Rajan, S.K. Sharma, G.L. Greene, Molecular characterization of a B-ring unsaturated estrogen: implications for conjugated equine estrogen components of premarin, Steroids 73 (2008) 59–68, https://doi.org/10.1016/j.steroids.2007.08.014.
[31] E.S. Manas, R.J. Unwalla, Z.B. Xu, M.S. Malamas, C.P. Miller, H.A. Harris, C. Hsiao, T. Akopian, W.-T. Hum, K. Malakian, S. Wolfrom, A. Bapat, R.A. Bhat, M.L. Stahl, W.S. Somers, J.C. Alvarez, Structure-based design of estrogen receptor-beta selective ligands, J. Am. Chem. Soc. 126 (2004) 15106–15119, https://doi.org/10.1021/ja047633o.
[32] Y. Kanno, Y. Takane, Y. Takizawa, Y. Inouye, Suppressive effect of aryl hydrocarbon receptor repressor on transcriptional activity of estrogen receptor alpha by protein–protein interaction in stably and transiently expressing cell lines, Mol. Cell. Endocrinol. 291 (2008) 87–94, https://doi.org/10.1016/j.mce.2008.05.004.
[33] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791, https://doi.org/10.1002/jcc.21256.