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Novel Selective Estrogen Receptor Downregulators (SERDs) Developed Against Treatment-Resistant Breast Cancer

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Abstract

Resistance to the selective estrogen receptor modulator (SERM) tamoxifen and to aromatase inhibitors that lower circulating estradiol occurs in up to 50% of patients, generally leading to an endocrine-independent ER⁺ phenotype. Selective ER downregulators (SERDs) are able to ablate ER and thus theoretically to prevent survival of both endocrine-dependent and independent ER⁺ tumors. The clinical SERD, fulvestrant, is hampered by intramuscular administration and undesirable pharmacokinetics. Novel SERDs were designed using the 6-OH-benzothiophene (BT) scaffold common to arzoxifene and raloxifene. Treatment-resistant (TR) ER⁺ cell lines (MCF-7:5C and MCF-7:TAM1) were used for optimization, followed by validation in the parent endocrine-dependent cell line (MCF-7:WS8), in 2D and 3D cultures, using ER α in-cell westerns, ERE-luciferase, and cell viability assays, with GDC-0810 (ARN-810) used for comparison. Two BT SERDs with superior in vitro activity to GDC-0810 were studied for bioavailability and shown to cause regression of a TR, endocrine-independent ER⁺ xenograft superior to GDC-0810.

Graphical Abstract

Keywords

Breast cancer; tamoxifen resistance; estrogen receptor; Selective Estrogen Receptor Downregulators (SERDs)

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Rui Xiong and Jiong Zhao contributed equally. The manuscript was written with contributions from all authors. All authors have given approval to the final version of the manuscript.

Supporting information

ER degradation of compound **28a**, **28c**, **24b**, and **24c** (100 nM) verified by western blot; cell survival data (DNA) of compound **16b** in TR MCF-7:5C and MCF-7:WS8 cells.

Introduction

Approximately 70% of breast cancer patients have estrogen receptor positive (ER+) tumors.¹ The selective estrogen receptor modulator (SERM), tamoxifen,^{2–4} and aromatase inhibitors (AIs) represent first-line treatment for ER+ patients;⁵ however, up to 50% of patients either do not respond or acquire resistance within 5 years of treatment.⁶ Multiple mechanisms contribute to the development of an ER+ and treatment resistant (TR) phenotype, in which growth is endocrine independent, including ligand-independent constitutive activation of ER.^{7, 8} Selective ER downregulators (SERDs) have the potential to block endocrine-dependent and endocrine-independent ER signaling by ablation of ER and have been recognized to offer a therapeutic approach to ER+ breast cancer in both early stage and more advanced TR cases.

The first generation SERD, fulvestrant (Figure 1; **1**)⁹, has poor physicochemical and pharmacokinetic (PK) characteristics, requiring intramuscular injection and resulting in a lag of 3–6 month to reach steady-state concentrations.¹⁰ Compound **1**, first reported as the “pure antiestrogen” ICI 182780,⁹ has been widely used to probe ER signaling, and the mechanism of action is now understood to involve rapid, proteasome-dependent degradation of the receptor.^{11, 12} Recent work on **1**, using radiolabelled estradiol, reported a strong correlation between ER degradation and clinical benefit: however, partial engagement of ER, caused by poor physicochemical properties, limited optimum therapeutic efficacy in some patients.¹³

GW 5638 (**3**)¹⁴, a second generation non-steroidal triphenylethylene-based SERD that progressed to phase I clinical trials, has the characteristic acrylate side chain that provides key hydrogen bonds with helix 12 and opens a hydrophobic surface thought to induce degradation by 26S proteasome.^{15–17} The clinical efficacy of **1**, despite its poor physicochemical and pharmacokinetic characteristics, has inspired contemporary interest in orally bioavailable SERDs. The recent intensive interest in discovery and development of novel orally bioavailable SERDs is highlighted by the pursuit of SERDs on multiple scaffolds^{18–21}, and the clinical development of GDC-0810 (ARN-810, **2**)²², RAD-1901²³, and AZD-9496 (**4**)^{24, 25} by Genentech, Radius Health, and Astra Zeneca, respectively.

The SERM raloxifene (**6**)²⁶ has been used in breast cancer chemoprevention and chronic treatment of postmenopausal osteoporosis for almost two decades, representing a clinically proven and safe scaffold that we have exploited in design of novel ER-directed ligands.^{27–29} Herein we report a medicinal chemistry campaign towards development of novel orally bioavailable SERDs based on the benzothiophen-6-ol (BT) core of the SERMs **6** and arzoxifene (**5**)³⁰. Other reports on use of a BT scaffold by Novartis²¹ and Shoda³¹, reported modest potency where data was made available. Our structural optimization of the molecular topology was guided by antiproliferative potency in TR and parent cell lines, potency in ER α degradation assays, and potency against ER α activation in cell-based reporter assays. The use of 2D and 3D cultures and two different TR cell lines, one a model of tamoxifen resistance (extended tamoxifen treatment), and a second a model of AI resistance (extended estrogen deprivation) led to novel orally bioavailable SERDs with subnanomolar potency in

endocrine-sensitive and TR cell lines, and efficacy in a TR ER+ breast cancer mouse xenograft study.

Structure design

We have reported a number of approaches to modify the BT scaffold to diversify biological activity of ER ligands.^{27, 28, 32–37} Replacement of the archetypical SERM 2-phenoxyethylamino side chain of **5** (Figure 1) by an acrylate containing side chain was envisioned simplistically to provide the basis for SERD activity. The acrylate side chain is designed to engage in a hydrogen bonding network with helix 12 *in simile* with GW5638 in complex with ER α (pdb: 1R5K). This side chain is also a key feature of newer generation SERDs. **5** was originally designed to overcome the low oral bioavailability of **6**, although in Phase 3 trials, **5** did not show superiority in efficacy and safety to **6**.^{38, 39} Mindful of the extensive glucuronidation^{40, 41} and low bioavailability of **6**, we attempted to mitigate these effects in design of novel SERDs, using structural knowledge gained from lasofoxifene (**7**)⁴², a phenolic SERM with high reported bioavailability (> 60% in rats), rationalized by the disruption of planar topology. Therefore, to enhance the oral bioavailability of BT-SERDs, we inserted a ketone linker at the BT 2-position and decorated the 2-benzoyl substituent with planarity-breaking groups at the 2', 4', 5' and 6' positions (Figure 2). Molecular docking of a putative SERD (**14a**), designed using these principles, is shown in the ER α ligand binding domain (pdb: 1R5K; Figure 2), demonstrating that the acrylate side chain remains able to make similar contacts to that of GW5638 in complex with ER α . A further analysis of docking data reveals two unoccupied hydrophobic cavities formed by Leu 384 and Leu 428; the latter pocket is fully occupied by the ethyl group of GW5638 in the crystal structure and both pockets can potentially be used to enhance the potency of novel SERDs.

Chemistry

Scheme 1 outlines the general synthetic route for preparing BT-SERDs, **14a–h**. The synthetic strategy utilized a key starting material, 3-chloro-6-methoxybenzo[*b*]thiophene-2-carbonyl chloride (**8**), which allows modification of both 2 and 3 positions of the benzothiophene scaffold. The acylchloride of **8** was converted to a Weinreb amide, followed by Grignard reaction to functionalize this position. The C-O bond formation at the 3-position proved to be troublesome. In the synthesis of **5**, Palkowitz and colleagues reported a reaction sequence to prepare the oxygen-linkage that involved bromination, oxidation of the sulfur to sulfoxide, and subsequent nucleophilic aromatic displacement.³⁰ A subsequent paper examined the possibility of using transition metal-catalyzed reactions, such as the copper iodide-catalyzed or palladium-catalyzed etherification of aryl halides for C-O bond formation.⁴³ Unfortunately, both failed. Herein, we utilized the 2-keto group to activate the 3 position, and directly formed the oxygen-linkage with the corresponding substituted phenols through a nucleophilic aromatic substitution. Selective deprotection of methyl ether over diaryl ether was achieved using BF₃•SMe₂, which also showed better yield compared to the other common demethylating reagent, BBr₃. The acrylate group was installed by standard Heck coupling of aryl bromide with methyl acrylate using a palladium catalyst.

Compounds **16a**, **16b** and **19** were prepared using a similar strategy as depicted in Scheme 2. Methyl 6-hydroxy-2-naphthoate or methyl 7-hydroxyquinoline-3-carboxylate was directly coupled to the BT core through a nucleophilic aromatic substitution reaction. Since **16a** and **16b** do not contain an acrylate group, simultaneous deprotection of phenol methyl ether and ester groups was conducted using $\text{BF}_3 \cdot \text{SMe}_2$ at 35 °C for 48 hours. In the preparation of compound **17**, an unprotected 4-aminophenol was selectively coupled to the BT core with good yield (81%). A similar $\text{S}_{\text{N}}\text{Ar}$ reaction using an unprotected 4-aminophenol that selectively reacts at the phenol group was reported by Wang and colleagues.⁴⁴ Demethylation of compound **17** followed by amide coupling afforded compound **19**.

In the synthesis of **24a–c**, compound **8** was directly used in preference to compound **9** when the corresponding Grignard reagents were insufficiently reactive towards the Weinreb amide (Scheme 3). The remaining steps were analogous to those used in the synthesis of **14a–h** (Scheme 1).

For compounds **28b** and **28c** (Scheme 4) that contain Cl substituents, methyl (*E*)-3-(4-hydroxyphenyl) acrylate was used in the $\text{S}_{\text{N}}\text{Ar}$ coupling to avoid the potential selectivity problem in Heck reactions. Methyl (*E*)-3-(4-hydroxyphenyl) acrylate was less reactive compared to 4-bromophenol in this reaction and the reaction temperature was raised to 90 °C to compensate. Selective deprotection of phenol methyl ether over methyl ester was conducted using $\text{BF}_3 \cdot \text{SMe}_2$ in an ice water bath. A small amount of ester hydrolysis product was also observed by LC-MS, and this amount can be reduced by careful control of reaction temperature. Overall, our concise synthetic route produced a test compound library in 4–6 steps.

Biological testing

Our therapeutic objective was to discover a novel, potent, orally bioavailable SERD with preclinical efficacy in TR, endocrine-resistant, ER+ breast cancer; capable of inhibiting growth of multiple cell lines in culture, and effecting regression of tumors in a mouse xenograft study. The ER+ cell lines tested were endocrine and tamoxifen-sensitive (MCF-7:WS8), or treatment resistant (TR: MCF-7:5C, MCF-7:TAM1, MCF-7:PKC α). MCF-7:TAM1 cells are a model for tamoxifen resistance, whereas MCF-7:5C cells are a model for AI resistance; however all three TR cell lines are endocrine-independent and tamoxifen-insensitive. Rather than use ER degradation as the primary assay, as described by Callis⁴⁵ and Lai²⁰, we measured as our primary screen the ability of novel SERDs to inhibit growth of TR MCF-7:5C cells, assessed by DNA assay on the 6th day after treatment. The MCF-7:5C cell line was obtained by long-term deprivation of E_2 , and can be seen as a model of AI-resistant ER+ breast cancer.^{27, 46} The clinical SERD, **1**, was used for comparison in early testing of our novel oral SERDs (Supp. Figs S1, S2), but **2** was selected as a more appropriate control for further studies, since it is an orally bioavailable SERD currently in Phase II clinical trials. Results in the primary assay are reported as inhibition of cell growth relative to vehicle (0%) and **2** (1 μM) treated cells (100%) (Table 1 & 2). Cell viability of the MCF-7:WS8 ER+ endocrine-dependent, parental cell line was used as a counterscreen to exclude potential ER agonists or general cytotoxins. The secondary screen was the measurement of ER degradation, obtaining potency and efficacy using in-cell westerns in

MCF-7:WS8 cells (Figure 3, Figure 4, Figure S2 & Table 3). Loss of ER α was verified by standard western blots in both MCF-7:WS8 and TR MCF-7:5C cells (data in Supp. Figure S1). The relative binding affinity of selected SERDs to full length ER α was evaluated in a radioligand displacement assay (Table 3). Tertiary assays relied upon measurement of ER α antagonism in endocrine-dependent MCF-7:WS8 cells co-treated with 0.1 nM E₂, the response being normalized to control treated cells (0%) and E₂ (0.1nM) treated cells (100 %). The representative data from this series of primary to tertiary screens, comparing **2** to BT-SERD **28c**, are depicted in Figure 4 (Panel A shows viability of TR MCF-7:5C cells; Panel B shows viability of MCF-7:WS8 cells; Panel C shows dose-dependent ER downregulation; Panel D shows antagonism of E₂ action at ER α).

SERDs obtained from the primary and secondary screens were characterized in a further TR ER+ cell line (MCF-7:TAM1) obtained from long-term exposure to tamoxifen,⁴⁷ and therefore a model of tamoxifen resistance. To provide an increased level of cellular complexity, we studied drug effects in 3D spheroidal cell cultures that better mimic the *in vivo* tumor microenvironment, including cell-cell interactions, and a hypoxic core (Figure 5; Table 3). Cell viability in 3D cell cultures was quantified by ATP assay on day 14 after compound treatment (100 nM).

Structure optimization

In the crystal structures of SERDs **3** and **4** complexed with ER α (PDB ID: 1R5K and 5ACC), the planar phenyl acrylate side chain simultaneously forms a water-mediated hydrogen bond with Leu-536 and Tyr-537 of helix 12 and a hydrogen bond with Asp-351. The consequent conformational change to the AF2 domain is hypothesized to promote proteosomal ER degradation.¹⁶ Acrylate SERDs are currently in clinical trials; however, the potential susceptibility of acrylate to Michael addition and Phase 1 and 2 metabolism, caused us to explore phenyl acrylate bioisosteres. The BT-SERD **14b** with a cinnamate side chain showed good potency in inhibiting the growth of both MCF-7:5C and MCF-7:WS8 cells (IC₅₀ = 1.3 nM and 0.9 nM, respectively), and in comparison, the fused ring analogues containing naphthalene (**16a**) and quinoline (**16b**) bioisosteres, showed only modestly compromised potency in MCF-7:5C cells (IC₅₀ = 4.8 nM and 32.3 nM, respectively) (Table 1). However, the maximum efficacy of **16b** in inhibiting cell growth was only 52% compared to the positive control compound **2** at 1 μ M (supp. Figure S3). The α -carboxy amide bioisostere (**19**) conserves the sp² centers of the acrylate, but showed reduced efficacy: unable to inhibit growth of MCF-7:5C cells to the same degree as **2**, although potency was high (IC₅₀ = 1.7 nM, E_{max} = 64%). Neither **16b** nor **19** was observed to inhibit growth of the parent MCF-7:WS8 cells. Reduced potency, coupled with reduced efficacy in TR cells or the parent cell line, led us to return to the phenyl acrylate series for further structure optimization.

In our previous work on selective human ER partial agonists (ShERPAs),²⁷ we explored the ability of ER to accommodate a range of substituents exploiting the plasticity of helix 11. Similarly, in the candidate SERDs developed herein, we inserted a ketone linker between the BT core and the phenyl group at the 2-position and subsequently explored a variety of 2-phenyl substituents to fine tune the molecular topology and to gain potency in our primary

and secondary screens. In addition to the normal use of electron-withdrawing substituents to minimize Phase 1 metabolism; as introduced above, we expected that disruption of planarity would attenuate Phase 2 metabolism of the BT-phenol. The activity of these BT-SERDs, derived from measuring responses at >10 concentrations is summarized in Table 2. The prototype of the series, **14a** with an undecorated 2-phenyl ring, displayed high, but comparatively moderate potency against both MCF-7:5C and MCF-7:WS8 cells (IC_{50} = 13 and 2.2 nM, respectively). Adding an *o*-methyl group (**14b**) to exploit the hydrophobic pocket formed by Phe-425 and Leu-428, increased the potency against both TR and parent cell lines (IC_{50} = 1.3 and 0.9 nM, respectively) and an *o*-ethyl group was well tolerated (**14c**) (IC_{50} = 1.2 and 0.8 nM, in TR and parent cell lines respectively). Other monosubstitutions of the 2-phenyl group did not improve activity, although it was interesting that replacement of the 2-phenyl group in the 3-methyl thiophene analog (**14f**) did not lead to complete loss of activity (IC_{50} = 12.5 and 1 nM, in TR and parent cell lines respectively). Exploration of other heterocycles at this position would be a promising approach to modulate the physicochemical and ADMET properties of next generation SERDs. The partial efficacy of some candidate SERDs (**14d**, **14e**, **16b** & **19**) and the differential activity in TR versus parent cell lines is a feature common to ShERPAs²⁷ and other classes of BT-based ER ligands, which will be the focus of future reports.

Towards our objective of high potency in primary screens and disrupted planarity, further 2-phenyl substitutions were explored building on identification of compound **14b** as a potent and efficacious SERD. Introduction of fluorine at the 4' position (compound **14g**) slightly enhanced potency in MCF-7:5C and MCF-7:WS8 cells (IC_{50} = 1.0 and 0.4 nM, in TR and parent cell lines respectively), whereas fluorine at the 5' position (**14h**) reduced potency (IC_{50} = 4.7 and 0.7 nM, in TR and parent cell lines respectively). Adding a second methyl group at either the 4' or 6' position, (**24a** and **28a**) improved potency against MCF-7:5C cells almost three fold compared to compound **14b** (IC_{50} = 0.4 and 0.5 nM, in TR cell lines respectively). A second small hydrophobic pocket formed by Leu-525 and Leu-384 (discernable in crystal structure PDB ID: 1R5K) can explain the improved potency of **28a**. 2',4',6'-Trisubstituted 2-phenyl compounds were highly potent, whether the 4' position substituent was Me (**24b**), F (**24c**), or Cl (**28c**). Methyl and ethyl groups at the 2' position were preferred to exploit the small hydrophobic pocket formed by Phe-425 and Leu-428, and further modification at both the 4' and 6' positions with methyl and halogens enhanced potency in growth inhibition of both TR and parent MCF-7 cell lines.

In summary, although planar mimics of phenyl acrylate, such as naphthalene and quinolone, were potent and efficacious in inhibiting the growth of TR MCF-7:5C and parent MCF-7:WS8 cells, the phenyl acrylate 'warhead' provided superior potency. Incorporation of a substituent at the *o*-position of the 2-phenyl group, in compound **14b**, significantly increased the potency of cell growth inhibition compared to the unsubstituted 2-phenyl homologue. Docking analysis showed that this methyl group could insert into a hydrophobic pocket formed by Phe-425 and Leu-428 (Figure 6). A second methyl group at the 6' position further enhanced activity, putatively by stabilizing ER conformations in which this group occupies a pocket close to Leu 525. A third methyl, or a halogen group at the 4' position of the 2-phenyl group led to SERDs with an excellent profile of activity in cell

cultures. Docking analysis of these 2',4',6'-trisubstituted SERDs showed that the 4' substituent rested in the vicinity of Met 421 in a region of the ligand binding pocket that also contains the His 524 residue that is important in stabilizing the E₂/ER α complex.

Validation of SERD activity

Six compounds (**14b**, **14g**, **24a**, **24c**, **28a**, and **28c**) with high potency and efficacy in growth inhibition of TR MCF-7:5C and parent MCF-7:WS8 cells were further characterized. Most importantly, to define these compounds as SERDs, the level of ER α expression was measured in a cell-based immunofluorescence assay (ICW) at 24 h in MCF-7:WS8 cells. Exemplar ICW images are shown in Figure 3 for compounds **24b**, **24c** and **28c**. We normalized the response to 100% (DMSO vehicle) and 0% as the response to excess, saturating concentrations of **2** (1 μ M); essentially the maximal response to **2**. As control SERD, **2** was a potent ER α downregulator with an IC₅₀ value of 0.8 nM that was consistent with the literature reported number (0.7 nM).²⁰ All six of our compounds “downregulated” ER α with IC₅₀ ranging from 0.07 nM to 1.1 nM. Compound **28c** was the most potent compound in inhibiting cell growth of MCF-7:5C and MCF-7:WS8 cells and was also the most potent ER downregulator with an IC₅₀ of 0.07 nM. Potency data for ER downregulation obtained from 10 concentrations of **14b**, **14g**, **24a**, **24c**, **28a** and **28c** is summarized in Table 3. A direct dose-dependent ER degradation comparison of **1**, **2** **28c** and **24c** is described in Figure S2.

Verification that these compounds were ligands for ER α was obtained by measuring relative binding affinity (RBA) using a standard radioligand displacement assay with full-length ER α . RBA values were calculated as 100x (IC₅₀ E₂/IC₅₀ test compound), with RBA for E₂ = 100%. The RBA data are compatible with nanomolar potency in ER-mediated events, showing that all compounds have 10% of the affinity of the potent, endogenous ligand, E₂, for recombinant ER α . Measured RBA for ER α varied from 9.8% to a maximum of 40.3% for compound **28a**, with RBA for **2** measured at 53.4%. A quantitative correlation between RBA and cell-based transcriptional potency is not anticipated,²⁷ because ligand binding affinity to ER as part of a functional, multi-protein complex at DNA is not expected to be identical to affinity for recombinant ER alone.

The final validation of the potent ER ligands discovered in this work was to verify antagonist activity at ER. SERDs have come to be defined as estrogen antagonists with relation to signaling via estrogen response element (ERE) and indeed the archetypical SERD, **1**, was described in early work as a pure antiestrogen.⁹ Antagonist activity was measured using an ERE-luciferase reporter assay in the presence of E₂ (0.1 nM). All compounds were observed to be full pharmacological antagonists at ER α with potency ranging from **2** as the weakest antagonist (IC₅₀ = 11.1 nM; consistent with literature reports^{20, 48}) to **28c** as the most potent antagonist (IC₅₀ = 2.4 nM). Notably, although **14g** was slightly less potent in ER downregulation than **2**, this BT-SERD was twice as potent as **2** as an ER antagonist.

Spheroid models are argued to provide a better prediction of therapeutic efficacy than “in plastic” monolayers that grow on hydrocarbon polymer surfaces, through better mimicking of the tumor microenvironment, including cell-cell interactions, lack of contact with a plastic

surface, secreted extracellular matrix, and a hypoxic core.⁴⁹ Therefore, newly discovered SERDs were tested in 3D spheroid cultures of the parent ER+ breast cancer cell line, MCF-7:WS8, and the TR cell line MCF-7:5C. Spheroids were grown for 1 day before initiating treatment with test compound (100 nM) in media. Media (100 μ l) was replaced every 3 days until day 14. Measurement of spheroid/cell growth at day 15 by ATP assay showed significant inhibition of spheroid growth by SERDs relative to DMSO vehicle control; for example, **2** treated spheroids had cell viability 15% of the vehicle control. Compounds **14g**, **24c**, and **28c** demonstrated strong growth inhibition effects restricting spheroid formation to < 5% of control. A second TR cell line, MCF-7:TAM1, was explored in 3D spheroid cultures to study both the effects of novel SERD **28c** versus **2**, and prospective combination therapy. The ability of both SERDs to disrupt spheroid growth is demonstrated in Figure 5 and quantified in Figure 7. To determine the potential of CDK4/6 inhibitor combination therapy in TR and parent ER+ breast cancer, doses of PD-0332991 (palbociclib, PD) were chosen that produced no significant (10 nM) or modest but significant (100 nM) inhibition of spheroid growth (Figure 7). In TR MCF-7:TAM1 and parent MCF-7:WS8 spheroid cultures, combination of both **28c** and **2** with CDK 4/6 inhibitor showed synergistic inhibition of spheroid growth, with concentration dependence on SERD and kinase inhibitor.

In vivo bioavailability and efficacy

The plasma concentrations of **14b**, **14g**, **24a**, **24c**, **28a**, and **28c** at 0.5 and 4 h (100 mg/kg in a 0.5% CMC suspension p.o.) were measured as a preliminary screen to select a BT-SERD for study in an ectopic xenograft mouse model of endocrine-resistant ER+ breast cancer (Table S1). We anticipated that the halide substituted SERDs would demonstrate superior bioavailability, because of attenuated Phase 2 metabolism. SERDs **24c**, **28a**, and **28c** showed the highest plasma levels in this preliminary study and were subjected to a further more detailed study of plasma bioavailability (Figure 8A). SERDs **28a** and **28c** were ultimately selected for animal study in direct comparison to tamoxifen and **2**. The MCF-7:TAM1 xenograft model was allowed to establish for 5.5 weeks prior to treatment and was randomized to six treatment groups with an average tumor area of 0.325 cm². Tamoxifen (100 mg/kg) was entirely without effect, demonstrating the anticipated resistance of this tumorigenic TR breast cancer cell line to tamoxifen (Figure 8B). SERD **2** at a dose of 100 mg/kg, used previously in the literature,¹⁷ caused regression of tumor size by 21% at day 23 after treatment. Compound **28a** (100 mg/kg) also caused tumor regression similar to **2** (26.7% in tumor area reduction at day 23), whereas **28c** showed the best efficacy in tumor regression (49% reduction) at a dose of 100 mg/kg. Regression was dose-dependent for **28c**: at 30 mg/kg average tumor area was reduced 27%. Injection of tumorigenic cells into mammary fat pads of nude mice produces distinct mammary tumors allowing assessment of individual tumor response (Figure 8C), again demonstrating the efficacy of SERD **28c**. No weight loss was observed during the course of the animal study.

Discussion

The SERM, tamoxifen, and third generation AIs, such as anastrozole, represent standard-of-care, first-line therapy for treatment of ER+ breast cancer. Although, tamoxifen therapy is

associated with higher risk of uterine sarcoma, this is outweighed by the proven benefits of 5–10 years of tamoxifen pharmacotherapy.⁵⁰ However, both tamoxifen and AI therapy are marked by high levels of resistance in which the cancer remains ER+, but does not require estrogen for growth and is insensitive to antagonism by tamoxifen and depletion of estrogen by AIs.^{51–53} A clinically effective oral SERD that is able to block and ablate ER is theoretically a preferred therapeutic approach. Clinical trials revealing the efficacy of **1** (500 mg) as a SERD for first line therapy in breast cancer are recent^{54–59}; for example, the FIRST (Fulvestrant First-Line Study Comparing Endocrine Treatments) Phase II clinical trial showed that **1** (500 mg monthly injection) improved time to progression and overall survival versus anastrozole.^{54–56}

Cyclin D1 is highly expressed in ER+ breast cancer and through inhibitors of CDK4 and CDK6 provides an effective target for breast cancer therapy.⁶⁰ The clinical emergence of CDK4/6 inhibitors, beginning with palbociclib⁶¹, and the potential for rapid, early resistance to these inhibitors⁶², has provided impetus for combination therapy. The PALOMA3 (Palbociclib combined with fulvestrant in hormone receptor-positive HER2-negative metastatic breast cancer after endocrine failure) trial showed that combination therapy of SERD and CDK4/6 inhibitor gave clinical benefit for heavily endocrine pre-treated, advanced breast cancer patients.⁵⁷ Nevertheless, the optimum clinical benefit of **1** was limited by its poor physicochemical and pharmacokinetic properties. GW 5638 and **2** are both triphenylethylene-based next generation SERDs.⁸ In **2**, the phenolic group of GW 7604 was replaced with an indazole bioisostere that greatly boosted bioavailability.²² The discovery of a further next generation SERD, **4**, resulted from a high-throughput screening campaign: **4** contains a 1-aryl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole scaffold, apparently devoid of any hydrogen-bonding interactions with either Glu353 or Arg394, and with a good pharmacokinetic profile.²⁴ Both **2** and **4** were reported to have physicochemical properties superior to **1** and are actively being studied in clinical trials.^{20, 24, 25, 63}

Previously we have reported design and synthesis of novel ER ligands based upon the 6-benzothiophen-ol (BT) core, common to **5** and **6**, which manifest biological activity as SERMs, ER agonists (SEMs), and ShERPAs.²⁷ The BT scaffold has the advantage of broad knowledge on metabolic and pharmacokinetic properties derived from **5** and **6**,^{27, 28, 32–37} and provides highly potent modulation of ER-mediated biological activity. Herein, we have extended the utility of this scaffold to the design and synthesis of novel ER ligands by substituting an acrylate side chain at the BT 3-position and diversifying substituents at the 2-position, yielding potent, orally bioavailable SERDs.

Of the novel SERDs developed in this research, five (**14g**, **24a**, **24c**, **28a**, and **28c**) showed sub-nanomolar potency in cell cultures towards downregulation/degradation of ER α , and inhibition of growth of both TR and parent endocrine-dependent breast cancer cell lines. These SERDs were marginally more potent than **2** in these assays, and all were more efficacious than **2** in inhibiting growth of 3D spheroids; and the 2 BT-SERDs tested in xenograft studies in comparison with **2** were equal or superior to **2**. The potency towards antagonism of E₂ at ER α /ERE was roughly an order of magnitude less than for ER degradation for all SERDs, compatible with growth inhibition in cell cultures being mediated by induction of ER degradation.

Multiple mechanisms of resistance to endocrine treatment (tamoxifen/AI) have been proposed in ER+ breast cancer.^{51, 52, 64–72} In ER+ resistant (TR) cells, growth does not require E₂ and is insensitive to tamoxifen. It is argued that SERD binding to ER α induces a conformational change that opens a hydrophobic surface leading to accelerated ER degradation through the 26S proteosomal pathway.²⁵ Therefore, for a SERD to be effective in TR ER+ cell cultures and xenografts, requires ligand binding to ER α and appropriate conformational change. It was seen as essential to develop SERDs capable of binding to ER α in TR breast cancer cell lines in which resistance was acquired to extended tamoxifen treatment (MCF-7:TAM1) and to extended E₂ deprivation (MCF-7:5C). Both cell lines responded to our novel BT-SERDs and to **2** and the novel SERDs retained activity in the parent MCF-7:WS8 cell line.

Conclusion

In summary, novel, orally bioavailable SERDs were discovered possessing potent dual activity as degraders and antagonists of ER α . These SERDs inhibited growth of endocrine-independent breast cancer cells with IC₅₀ ranging from 0.3 to 12.5 nM. Six compounds were measured for oral drug exposure and two novel SERDs were further validated in a TR xenograft model, demonstrating dose-dependent tumor regression. The potential of combination therapy with SERD **28c** and a CDK4/6 inhibitor was shown in the effects of palbociclib/**28c** co-administration in inhibiting growth of ER+ TR 3D spheroid cell cultures. The comparison with **2** in all in vitro and in vivo assays of potency and efficacy demonstrated the equivalence or superiority of the novel BT-SERDs reported for the first time herein.

EXPERIMENTAL SECTION

Cell Lines and Culture Conditions

MCF-7:WS8, MCF-7:5C and MCF-7:TAM-1 cells were gifts from Dr. Tonetti's lab at UIC. MCF-7:WS8 is hormone-dependent human breast cancer cell clones maintained in phenol red containing RPMI-1640 medium supplemented with 10% FBS at 37 °C, 5% CO₂ that have been previously described.^{46, 73} MCF-7:5C cells were maintained in phenol-red free RPMI 1640 medium supplemented with 10% charcoal-dextran treated fetal bovine serum at 37 °C, 5% CO₂ as previously described.^{74, 75} The MCF-7:5C cells served as AI resistant cells and were generated from MCF-7:WS8 cells by long-term estrogen deprivation.

Cell Growth Assay

Cells were grown in phenol red-free media for 2 days prior to each experiment. On the day of the experiment, cells were seeded in 96-well plate at a density of 5000 cells/well and treated with either 0.1% (v/v) DMSO, 1nM E₂, or compounds prepared in phenol red free media. All compounds were dissolved in DMSO and added to the medium at a final 1:1000 dilution. DNA content was determined on Day 5 (WS8) or Day 6 (5C) by Hoechst 33258 dye.^{76, 77} Fluorescence signals were read by the Synergy H4 (BioTek).

In-cell Western Analysis

MCF-7:WS8 cells were kept in stripped medium 2 days, and 2.0×10^4 /well of the cells were plated in clear bottom 96-well black plates for 48 hrs prior to addition of compounds for 24 hrs. Fixation, detection of ESR1 (sc-8002) and analysis were performed per LI-COR manufacturer's protocol using the In-Cell Western™ Assay Kits and LI-COR ODYSSEY infra-red imaging system. Data was normalized to CellTag 700 stain.

3D-spheroid growth assay

Spheroids were plated at 1000 cells/well in Corning® 96-well black, clear round bottom, ultra-low attachment spheroid microplates and grown in the absence of treatment for 24 hours. Spheroids were then treated with 2X treatment media following the removal of 100 µl media from each well. Treatment was repeated every 2–3 days for 14 days. CellTiter-Glo® 3D Cell Viability Assay protocol is used to determine growth inhibition of the spheroids. On day 15, spheroid plates and reagent (CellTiter-Glo® 3D Reagent) were allowed to come to room temperature for 30 minutes. During this time, the spheroids were washed with PBS by removing 100 µl media and replacing with PBS. 100 µl from each well is then removed and replaced with 100 µl of the reagent, and spheroids were disrupted by pipetting. The plates were placed on a shaker for 5 minutes before equilibrating in dark for 25 minutes. 125 µl from each well is then transferred to a white 96-well plate before recording luminescence using an empty well for the background reading.

Binding affinity studies

Binding affinities were also determined by a competitive radiometric binding assay using 2 nM [³H]estradiol as tracer (PerkinElmer, Waltham, MA) and full-length purified human ERα (Pan Vera/Invitrogen, Carlsbad, CA), as reported previously.^{78, 79} The RBA values were calculated using the following equation: $IC_{50} \text{ estradiol} / IC_{50} \text{ compound} \times 100$.

Estrogen Response Elements (ERE) Luciferase Assay in MCF-7 Cells

MCF-7:WS8 cells were kept in stripped medium 3 days prior to treatment. Cells were plated at a density of 2×10^4 cells/well in 96-well plates and were co-transfected with 5 µg of the pERE-luciferase plasmid per plate, which contains three copies of the Xenopus laevis vitellogenin A2 ERE upstream of firefly luciferase and 0.5 µg of pRL-TK plasmid (Promega, Madison, WI) containing a cDNA encoding Renilla luciferase. Transfection was performed for 6 h using the Lipofectamine 2000 transfection reagent (Invitrogen) in Opti-MEM medium according to the manufacturer's instructions. Cells were treated with test compounds after 6 h, and the luciferase activity was measured after 18 h of treatment using the dual luciferase assay system (Promega) with Synergy H4 (Bio Tek).

Animal Experiments

The Animal Care and Use Committee of the University of Illinois at Chicago approved all of the procedures involving animals. MCF-7:Tam1 tumors were established in 4–6 week old ovariectomized athymic nude mice (Harlan Laboratories) and E2 was administered via silastic capsules (1.0 cm) implanted subcutaneously between the scapulae as previously described.^{47, 80, 81} SERD1 and SERD2 were administered per os at a dose of 100 mg/kg or

30 mg/kg daily for 3.5 weeks in a formulation of 0.5% CMC: PEG-400: Tween-80: PVP (90: 9: 05: 0.5) solution. Tumor cross-sectional area was determined weekly using Vernier calipers and calculated using the formula $(\text{length}/2) \times (\text{width}/2) \times \pi$. Mean tumor area was plotted against time (in weeks) to monitor tumor growth.

General

3-chloro-6-methoxybenzo[b]thiophene-2-carbonyl chloride was purchased from Frontier Scientific Services, Inc. All chemicals and solvents were purchased from Sigma Aldrich, Fisher Scientific, Matrix Scientific or Oakwood Chemical and were used without further purification. **2** was synthesized using the protocols reported by Lai and colleagues.²⁰ Synthetic intermediates were purified using Biotage flash chromatography system on 230–400 mesh silica gel. ¹H and ¹³C NMR spectra were obtained using Bruker DPX-400 or AVANCE-400 spectrometer at 400 and 100 MHz, respectively. NMR chemical shifts were described in δ (ppm) using residual solvent peaks as standard (CDCl₃, 7.26 ppm (¹H), 77.16 ppm (¹³C); CD₃OD, 3.31 ppm (¹H), 49.00 ppm (¹³C); DMSO-*d*₆, 2.50 ppm (¹H), 39.52 ppm (¹³C); Acetone-*d*₆, 2.05 ppm (¹H), 29.84 ppm (¹³C)). Data were reported in a format as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, br = broad, m = multiplet, abq = ab quartet), number of protons, and coupling constants. High resolution mass spectral data were measured in-house using a Shimadzu IT-TOF LC/MS for all final compounds. All compounds submitted for biological testing were confirmed to be 95% pure by analytical HPLC. Synthetic methods, spectral data, and HRMS for novel compounds are described in detail below.

General method for preparing Grignard reagent—To an oven-dried round bottom flask, aryl bromide (1 equiv) in anhydrous tetrahydrofuran and magnesium turnings (1.1 equiv) were added under argon atmosphere. One granule of iodine was added to initiate the reaction. The solution turned pale white and then brownish color along with strong heat release. The Grignard reagent was ready for use without further purification when the magnesium was consumed.

3-chloro-*N*,6-dimethoxy-*N*-methylbenzo[b]thiophene-2-carboxamide (9): To an oven dried round bottom flask, 3-chloro-6-methoxybenzo[b]thiophene-2-carbonyl chloride (8.9 g, 34.9 mmol) was dissolved in 50 mL of anhydrous dichloromethane under argon atmosphere. *N*,*O*-Dimethylhydroxylamine hydrochloride (3.75g, 38.4 mmol) was added in one portion. After stirring for 10 mins, Et₃N (17.6g, 174.5 mmol) was added dropwise. The reaction mixture was stirred overnight until TLC showed the consumption of all starting materials, then quenched by ice water. The solution was extracted with ethyl acetate and washed with brine. The organic extracts were combined, dried over anhydrous Na₂SO₄, concentrated in vacuum, and then purified by flash chromatography (5% - 50% ethyl acetate in hexane) to give 7.6 g white solid (yield, 76%). ¹H NMR (400 MHz, CDCl₃) δ 7.82 (d, *J* = 8.9 Hz, 1H), 7.23 (s, 1H), 7.10 (dd, *J* = 8.9, 2.3 Hz, 1H), 3.90 (s, 3H), 3.73 (s, 3H), 3.39 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 162.03, 159.89, 140.36, 130.19, 125.06, 124.16, 123.24, 116.11, 104.29, 62.05, 55.88, 33.75.

(3-chloro-6-methoxybenzo[*b*]thiophen-2-yl)(phenyl)methanone (10a): To a solution of intermediate (**8**) (500 mg, 1.75 mmol) in anhydrous tetrahydrofuran under argon atmosphere was added a 3 M solution of phenylmagnesium bromide (0.65 ml, 1.93, 1.1 equiv) dropwise. The reaction mixture was stirred overnight and quenched by 1 N HCl/ice water. The solution was extracted with ethyl acetate and washed with brine. The organic extracts were combined, dried over anhydrous Na₂SO₄, concentrated in vacuum, and then purified by flash chromatography (1% – 15% ethyl acetate in hexane) to give 481 mg white solid (yield, 90%). ¹H NMR (400 MHz, CDCl₃) δ 7.92 – 7.78 (m, 3H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.26 (d, *J* = 2.3 Hz, 1H), 7.12 (dd, *J* = 9.0, 2.3 Hz, 1H), 3.92 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 188.94, 160.37, 140.96, 138.08, 132.88, 131.76, 131.05, 129.56, 128.34, 124.86, 124.77, 116.59, 104.31, 55.76.

(3-(4-bromophenoxy)-6-methoxybenzo[*b*]thiophen-2-yl)(phenyl)methanone (11a): Cesium carbonate (651.6 mg, 2.9 mmol, 2 equiv) was added in one portion to a solution of **10a** (440 mg, 1.45 mmol, 1 equiv) and 4-bromophenol (1.6 mmol, 1.1 equiv) in 5 mL DMF. The reaction mixture was raised to 65 °C and stirred overnight. The reaction mixture was quenched by ice water and extracted with ethyl acetate and washed with brine. The organic extracts were combined, dried over anhydrous Na₂SO₄, concentrated in vacuum, and then purified by flash chromatography (1% - 25% ethyl acetate in hexane) to give 491 mg white solid (yield, 77%). ¹H NMR (400 MHz, CDCl₃) δ 7.72 – 7.65 (m, 2H), 7.52 – 7.44 (m, 2H), 7.34 (t, *J* = 7.7 Hz, 2H), 7.28 (d, *J* = 2.1 Hz, 1H), 7.21 – 7.15 (m, 2H), 6.97 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.49 – 6.43 (m, 2H), 3.92 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 188.95, 160.72, 157.40, 147.59, 141.76, 138.49, 132.47, 132.40, 129.02, 128.09, 126.72, 125.92, 124.44, 117.33, 116.40, 115.07, 105.10, 55.88.

(3-(4-bromophenoxy)-6-hydroxybenzo[*b*]thiophen-2-yl)(phenyl)methanone (12a): **11a** (450 mg, 1.02 mmol) was dissolved in 10 mL of anhydrous dichloromethane at room temperature under argon atmosphere. BF₃•SMe₂ (2.5 ml, 10.4 mmol) was added dropwise to this solution in an ice/water bath. The reaction mixture was stirred until starting material was consumed as monitored by TLC and then quenched by saturated NaHCO₃/ice water. The reaction mixture was extracted with ethyl acetate and washed with brine. The organic extracts were combined, dried over anhydrous Na₂SO₄, concentrated *in vacuo*, and then purified by flash chromatography (5%–60% ethyl acetate in hexane) to give 410 mg white powder (yield, 94%). ¹H NMR (400 MHz, MeOD) δ 7.62 (d, *J* = 7.2 Hz, 2H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.44 – 7.32 (m, 3H), 7.25–7.21 (m, 3H), 6.91 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.49 (d, *J* = 9.0 Hz, 2H). ¹³C NMR (101 MHz, MeOD) δ 190.76, 160.50, 158.77, 149.54, 143.24, 139.95, 133.47, 133.38, 129.69, 129.14, 126.80, 125.81, 125.53, 118.42, 117.39, 115.88, 108.74.

Methyl (E)-3-(4-((2-benzoyl-6-hydroxybenzo[*b*]thiophen-3-yl)oxy)phenyl)acrylate (13a): To a sealed tube, **12a** (400 mg, 0.94 mmol), methyl acrylate (400 mg, 4.65 mmol), and Pd(PPh₃)₂Cl₂ (15% mol) were suspended in DMF (3 ml) and triethylamine (470 mg, 4.64 mmol). The reaction was heated at 110 °C for 6 hours. The reaction mixture was quenched by water and extracted with ethyl acetate. The organic layers was collected and purified by flash chromatography (5%-60% ethyl acetate in hexane) to give 251 mg white

powder (yield, 62%). ^1H NMR (400 MHz, DMSO- d_6) δ 10.39 (s, 1H), 7.65 (d, J = 7.9 Hz, 2H), 7.56-7.52 (m, 4H), 7.39-7.34 (m, 4H), 6.93 (d, J = 8.8 Hz, 1H), 6.66 (d, J = 8.5 Hz, 2H), 6.47 (d, J = 16.1 Hz, 1H), 3.69 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 187.95, 166.69, 159.08, 158.90, 147.19, 143.62, 141.00, 138.11, 132.27, 130.10, 128.67, 128.32, 128.03, 124.63, 124.32, 124.29, 116.55, 116.51, 115.66, 108.03, 51.37.

(E)-3-(4-((2-benzoyl-6-hydroxybenzo[*b*]thiophen-3-yl)oxy)phenyl)acrylic acid (14a): To a solution of intermediate **13a** (105 mg, 0.16 mmol) in methanol (4 ml) was added 10% LiOH solution (4 ml) dropwise. The reaction was monitored by TLC and quenched by 1 N HCl/ice water. After stirring for 10 mins, the mixture was extracted with ethyl acetate. The organic layers was collected and purified by C18 chromatography (5%–60% ethyl methanol in water) to give 77 mg white powder (yield, 77%). ^1H NMR (400 MHz, MeOD) δ 7.66 – 7.58 (m, 2H), 7.55 (d, J = 16.0 Hz, 1H), 7.50 (d, J = 7.4 Hz, 1H), 7.43-7.34 (m, 5H), 7.28 (d, J = 2.0 Hz, 1H), 6.92 (dd, J = 8.8, 2.1 Hz, 1H), 6.61 (d, J = 8.8 Hz, 2H), 6.32 (d, J = 16.0 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 190.76, 170.39, 161.08, 160.51, 149.38, 145.33, 143.23, 139.90, 133.38, 130.77, 130.47, 129.70, 129.13, 126.82, 125.95, 125.55, 118.13, 117.38, 116.96, 108.75. ESI-HRMS (m/z): $[\text{M} + \text{H}]^+$ calcd. for $\text{C}_{24}\text{H}_{17}\text{O}_5\text{S}$: 417.0797; observed, 417.0787.

Methyl 6-((6-methoxy-2-(2-methylbenzoyl)benzo[*b*]thiophen-3-yl)oxy)-2-naphthoate (15a): This compound was prepared by a procedure identical to the preparation of **10a** (159 mg, yield 33%). ^1H NMR (400 MHz, CDCl_3) δ 8.48 (s, 1H), 7.96 (dd, J = 8.6, 1.5 Hz, 1H), 7.64 (d, J = 9.0 Hz, 1H), 7.50 (d, J = 8.7 Hz, 1H), 7.47 (d, J = 8.9 Hz, 1H), 7.32 – 7.27 (m, 2H), 7.16 (t, J = 7.5 Hz, 1H), 6.98 (t, J = 7.5 Hz, 1H), 6.95 – 6.86 (m, 2H), 6.77 (d, J = 2.2 Hz, 1H), 6.73 (dd, J = 8.9, 2.5 Hz, 1H), 3.93 (s, 3H), 3.86 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 190.43, 167.05, 160.88, 158.00, 148.20, 142.08, 139.06, 136.21, 135.72, 131.02, 130.70, 130.36, 130.05, 128.52, 127.67, 127.47, 127.05, 126.85, 126.09, 126.02, 125.04, 124.43, 117.70, 116.38, 109.49, 105.09, 55.69, 52.11, 19.06.

6-((6-hydroxy-2-(2-methylbenzoyl)benzo[*b*]thiophen-3-yl)oxy)-2-naphthoic acid (16a): **14a** (100 mg, 0.21 mmol) was dissolved in 3 mL of anhydrous dichloromethane at room temperature under argon atmosphere. $\text{BF}_3 \cdot \text{SMe}_2$ (1 ml, 4.2 mmol) was added dropwise to this solution in an ice water bath. After stirring for 30 mins, the solution was allowed to 35 °C. The reaction mixture was stirred until starting material was consumed as monitored by TLC and then quenched by saturated NaHCO_3 /ice water. The reaction mixture was extracted with ethyl acetate and washed with brine. The organic extracts were combined, dried over anhydrous Na_2SO_4 , concentrated *in vacuo*, and then purified by flash chromatography (5%–60% ethyl acetate in hexane) to give 37 mg white powder (yield, 38%). ^1H NMR (400 MHz, MeOD) δ 8.47 (s, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 8.9 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.30 (d, J = 1.9 Hz, 1H), 7.28 – 7.08 (m, 2H), 7.01 (t, J = 7.4 Hz, 1H), 6.94 (d, J = 7.6 Hz, 1H), 6.88 (dd, J = 8.8, 2.1 Hz, 1H), 6.79 (s, 1H), 6.74 (dd, J = 8.9, 2.4 Hz, 1H), 1.95 (s, 3H). ^{13}C NMR (100 MHz, MeOD) δ 192.50, 169.84, 160.87, 159.24, 150.50, 143.78, 140.65, 137.54, 136.54, 132.21, 131.70, 131.42, 131.18, 130.09, 128.34, 128.08, 127.69, 127.38, 127.05, 126.27, 125.78, 118.69,

117.51, 110.58, 108.92, 19.19. ESI-HRMS (m/z): [M + H]⁺ calcd. for C₂₇H₁₈O₅S: 455.0953; observed, 455.0939.

(3-(4-aminophenoxy)-6-methoxybenzo[b]thiophen-2-yl)(4-fluoro-2-

methylphenyl)methanone (17): This compound was prepared by a procedure similar to the preparation of **10a** (309 mg, yield 81%). ¹H NMR (400 MHz, CDCl₃) δ 7.42 (d, *J* = 9.0 Hz, 1H), 7.36 – 7.29 (m, 1H), 7.25 (d, *J* = 2.1 Hz, 1H), 6.90 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.82 – 6.74 (m, 2H), 6.45 – 6.38 (m, 2H), 6.34 – 6.27 (m, 2H), 3.89 (s, 3H), 2.19 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 189.86, 163.56 (d, *J*_{C-F} = 249.4 Hz), 160.78, 151.44, 150.01, 142.22, 141.68, 139.52 (d, *J*_{C-F} = 8.5 Hz), 135.61 (d, *J*_{C-F} = 3.0 Hz), 130.22 (d, *J*_{C-F} = 9.2 Hz), 127.25, 126.91, 125.07, 117.30 (d, *J*_{C-F} = 21.4 Hz), 116.09, 116.03, 115.93, 112.03 (d, *J*_{C-F} = 21.6 Hz), 105.05, 55.81, 19.56 (d, *J*_{C-F} = 1.2 Hz).

(3-(4-aminophenoxy)-6-hydroxybenzo[b]thiophen-2-yl)(4-fluoro-2-

methylphenyl)methanone (18): This compound was prepared by a procedure identical to the preparation of **11a** (86 mg, yield 30%). ¹H NMR (400 MHz, MeOD) δ 7.34 – 7.25 (m, 2H), 6.86 – 6.80 (m, 1H), 6.85 – 6.76 (m, 3H), 6.49 (d, *J* = 8.7 Hz, 2H), 6.26 (d, *J* = 8.8 Hz, 2H), 2.13 (s, 3H). ¹³C NMR (100 MHz, MeOD) δ 191.63, 164.75 (d, *J*_{C-F} = 248.2 Hz), 160.50, 152.55, 152.31, 143.72, 140.33 (d, *J*_{C-F} = 8.6 Hz), 137.11 (d, *J*_{C-F} = 3.1 Hz), 131.07 (d, *J*_{C-F} = 9.2 Hz), 127.24, 126.60, 126.23, 118.00 (d, *J*_{C-F} = 21.8 Hz), 117.43, 116.99, 116.82, 112.91 (d, *J*_{C-F} = 21.9 Hz), 108.76, 19.49.

2-((4-((2-(4-fluoro-2-methylbenzoyl)-6-hydroxybenzo[b]thiophen-3-

yl)oxy)phenyl)amino)-2-oxoacetic acid (19): To an oven-dried flask charged with **17** (76 mg, 0.19 mmol), methyl 2-chloro-2-oxoacetate (28 mg, 0.23 mmol) was added with 3 mL of anhydrous tetrahydrofuran at room temperature under argon atmosphere. Triethylamine (58.6 mg, 0.58 mmol) was added dropwise to this solution in an ice/water bath. The reaction mixture was stirred until starting material was consumed as monitored by TLC and then quenched by saturated NaHCO₃/ice water. The reaction mixture was extracted with ethyl acetate and washed with brine. The organic extracts were combined, dried over anhydrous Na₂SO₄, concentrated *in vacuo*. The crude product was dissolved in methanol (2 ml). 10% LiOH solution (2 ml) was added dropwise. The reaction was monitored by TLC and quenched by 1 N HCl/ice water. After stirring for 10 mins, the mixture was extracted with ethyl acetate. The organic layers were collected and purified by C18 chromatography (5%–80% ethyl methanol in water) to give 47 mg white powder (yield, 52%). ¹H NMR (400 MHz, MeOD) δ 7.46 (d, *J* = 8.3 Hz, 2H), 7.38 – 7.27 (m, 2H), 7.24 (s, 1H), 6.90 – 6.77 (m, 3H), 6.46 (d, *J* = 8.2 Hz, 2H), 2.11 (s, 3H). ¹³C NMR (100 MHz, MeOD) δ 191.26, 164.87 (d, *J*_{C-F} = 248.6 Hz), 162.90, 160.76, 157.91, 156.78, 150.82, 143.70, 140.40 (d, *J*_{C-F} = 8.6 Hz), 136.89 (d, *J*_{C-F} = 2.9 Hz), 133.35, 131.10 (d, *J*_{C-F} = 9.1 Hz), 127.30, 127.03, 125.84, 123.09, 118.11 (d, *J*_{C-F} = 21.7 Hz), 117.38, 116.16, 113.05 (d, *J*_{C-F} = 21.9 Hz), 108.85, 19.46. ESI-HRMS (m/z): [M + H]⁺ calcd. for C₂₄H₁₅FNO₆S: 464.0604; observed, 464.0587.

(3-chloro-6-methoxybenzo[b]thiophen-2-yl)(2,6-dimethylphenyl)methanone (20a): To a solution of 3-chloro-6-methoxybenzo[b]thiophene-2-carbonyl chloride (**7**) (522 mg, 2

mmol) in anhydrous tetrahydrofuran under argon atmosphere was added a 0.5 M solution of (2,6-dimethylphenyl)magnesium bromide (2.2 mmol, 1.1 equiv) dropwise. The reaction mixture was stirred overnight and quenched by 1 N HCl/ice water. The solution was extracted with ethyl acetate and washed with brine. The organic extracts were combined, dried over anhydrous Na₂SO₄, concentrated in vacuum, and then purified by flash chromatography (1% – 15% ethyl acetate in hexane) to give 386 mg white solid (yield, 58%). ¹H NMR (400 MHz, CDCl₃) δ 7.79 (d, *J* = 9.0 Hz, 1H), 7.26 – 7.18 (m, 2H), 7.12 – 7.02 (m, 3H), 3.90 (s, 3H), 2.22 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 192.58, 161.14, 142.18, 140.22, 134.18, 131.75, 129.31, 128.69, 127.84, 126.81, 125.55, 116.91, 104.53, 55.91, 19.31.

(3-(4-bromophenoxy)-6-methoxybenzo[*b*]thiophen-2-yl)(2,6-

dimethylphenyl)methanone (21a): This compound was prepared by a procedure identical to the preparation of **11a** (480 mg, yield 94%). ¹H NMR (400 MHz, CDCl₃) δ 7.34 (d, *J* = 8.9 Hz, 1H), 7.28 (d, *J* = 2.1 Hz, 1H), 7.17 (d, *J* = 8.9 Hz, 2H), 7.04 (t, *J* = 7.6 Hz, 1H), 6.92 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.86 (d, *J* = 7.7 Hz, 2H), 6.34 (d, *J* = 8.9 Hz, 2H), 3.88 (s, 3H), 2.11 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 192.08, 160.94, 156.72, 148.28, 142.22, 140.16, 133.71, 131.95, 128.73, 128.49, 127.35, 126.59, 124.67, 116.52, 116.40, 114.74, 105.22, 55.75, 19.18.

(3-(4-bromophenoxy)-6-hydroxybenzo[*b*]thiophen-2-yl)(2,6-dimethylphenyl)methanone

(22a): This compound was prepared by a procedure identical to the preparation of **12a** (429 mg, yield 86%). ¹H NMR (400 MHz, acetone-*d*₆) δ 9.29 (s, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.35 (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 9.0 Hz, 2H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.98 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.90 (d, *J* = 7.5 Hz, 2H), 6.46 (d, *J* = 8.9 Hz, 2H), 2.08 (s, 6H). ¹³C NMR (100 MHz, acetone-*d*₆) δ 191.96, 159.98, 157.87, 149.06, 142.90, 141.45, 134.37, 132.90, 129.46, 128.64, 128.13, 126.65, 125.75, 117.72, 117.26, 115.12, 109.12, 19.29.

Methyl (*E*)-3-(4-((2-(2,6-dimethylbenzoyl)-6-hydroxybenzo[*b*]thiophen-3-yl)oxy)phenyl)acrylate (23a):

This compound was prepared by a procedure identical to the preparation of **13a** (240 mg, yield 86%). ¹H NMR (400 MHz, MeOD) δ 7.57 (d, *J* = 16.0 Hz, 1H), 7.35 (d, *J* = 8.7 Hz, 2H), 7.30 – 7.24 (m, 2H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.88 – 6.80 (m, 3H), 6.45 (d, *J* = 8.7 Hz, 2H), 6.36 (d, *J* = 16.0 Hz, 1H), 3.75 (s, 3H), 2.06 (s, 6H). ¹³C NMR (100 MHz, MeOD) δ 194.05, 169.17, 160.99, 160.62, 150.45, 145.33, 143.89, 141.52, 134.80, 130.57, 130.30, 129.92, 128.47, 126.75, 125.99, 117.57, 117.27, 116.42, 109.04, 52.10, 19.34.

(*E*)-3-(4-((2-(2,6-dimethylbenzoyl)-6-hydroxybenzo[*b*]thiophen-3-yl)oxy)phenyl)acrylic acid (24a):

This compound was prepared by a procedure identical to the preparation of **14a** (81 mg, yield 84%). ¹H NMR (400 MHz, MeOD) δ 7.55 (d, *J* = 16.0 Hz, 1H), 7.32 (d, *J* = 8.7 Hz, 2H), 7.27 – 7.19 (m, 2H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.87 – 6.77 (m, 3H), 6.43 (d, *J* = 8.6 Hz, 2H), 6.31 (d, *J* = 16.0 Hz, 1H), 2.04 (s, 6H). ¹³C NMR (100 MHz, MeOD) δ 194.06, 170.40, 160.93, 160.50, 150.47, 145.38, 143.86, 141.47, 134.76, 130.48, 130.35, 129.90, 128.44, 126.74, 125.99, 117.97, 117.55, 116.38, 109.04, 19.35. ESI-HRMS (*m/z*): [M + H]⁺ calcd. for C₂₆H₂₁O₅S: 445.1110; observed, 445.1098.

(3-chloro-6-methoxybenzo[*b*]thiophen-2-yl)(2,4-dimethylphenyl)methanone (25a): This compound was prepared by a procedure identical to the preparation of **10a** (300 mg, yield 52%). ¹H NMR (400 MHz, CDCl₃) δ 7.80 (d, *J* = 9.0 Hz, 1H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.12 – 7.04 (m, 3H), 3.90 (s, 3H), 2.39 (s, 3H), 2.38 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 190.85, 160.72, 141.57, 141.27, 136.74, 136.38, 134.01, 131.94, 131.52, 128.73, 126.33, 125.82, 125.16, 116.72, 104.45, 55.87, 21.60, 19.81.

(4-chloro-2,6-dimethylphenyl)(3-chloro-6-methoxybenzo[*b*]thiophen-2-yl)methanone (25c): This compound was prepared by a procedure identical to the preparation of **10a** (2.9 g, yield 42%). ¹H NMR (400 MHz, CDCl₃) δ 7.80 (d, *J* = 9.0 Hz, 1H), 7.25 (d, *J* = 2.2 Hz, 1H), 7.12 – 7.06 (m, 3H), 3.92 (s, 3H), 2.20 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 191.57, 161.34, 142.36, 138.73, 136.32, 134.85, 134.11, 131.70, 127.88, 127.13, 125.67, 117.09, 104.56, 55.96, 19.23.

Methyl (E)-3-(4-((2-(2,4-dimethylbenzoyl)-6-methoxybenzo[*b*]thiophen-3-yl)oxy)phenyl)acrylate (26a): To an oven-dried round-bottom flask, cesium carbonate (6.3 g, 19.2 mmol) was added in one portion to a solution of **25a** (3.2 g, 9.6 mmol) and methyl (E)-3-(4-hydroxyphenyl)acrylate (2.6 g, 14.4 mmol) in 12 mL DMF. The reaction mixture was raised to 90 °C and stirred overnight. The reaction mixture was quenched by ice/water and extracted with ethyl acetate and washed with brine. The organic extracts were combined, dried over anhydrous Na₂SO₄, concentrated in vacuum, and then purified by flash chromatography (5% – 35% ethyl acetate in hexane) to give 3.5 g white solid (yield, 78%). ¹H NMR (400 MHz, CDCl₃) δ 7.57 (d, *J* = 16.0 Hz, 1H), 7.46 (d, *J* = 8.9 Hz, 1H), 7.28 (d, *J* = 2.1 Hz, 1H), 7.23 (t, *J* = 8.9 Hz, 3H), 6.96 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.85 (d, *J* = 11.2 Hz, 2H), 6.47 (d, *J* = 8.8 Hz, 2H), 6.27 (d, *J* = 16.0 Hz, 1H), 3.91 (s, 3H), 3.79 (s, 3H), 2.29 (s, 3H), 2.09 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 190.48, 167.65, 160.87, 160.01, 147.81, 144.09, 141.92, 140.64, 136.30, 136.22, 131.40, 129.33, 128.91, 128.29, 127.79, 127.05, 125.79, 124.43, 116.48, 116.43, 115.58, 105.11, 55.87, 51.79, 21.47, 19.38.

Methyl (E)-3-(4-((2-(4-chloro-2,6-dimethylbenzoyl)-6-methoxybenzo[*b*]thiophen-3-yl)oxy)phenyl)acrylate (26c): This compound was prepared by a procedure identical to the preparation of **10a** (11.1 g, yield 84%). ¹H NMR (400 MHz, CDCl₃) δ 7.59 (d, *J* = 16.0 Hz, 1H), 7.38 (d, *J* = 8.9 Hz, 1H), 7.31 – 7.26 (m, *J* = 8.8 Hz, 3H), 6.94 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.79 (s, 2H), 6.45 (d, *J* = 8.7 Hz, 2H), 6.30 (d, *J* = 16.0 Hz, 1H), 3.91 (s, 3H), 3.79 (s, 3H), 2.05 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 191.15, 167.64, 161.29, 159.25, 148.51, 143.98, 142.52, 138.57, 136.01, 134.39, 130.08, 129.27, 129.19, 127.35, 126.80, 124.85, 116.77, 116.76, 115.09, 105.30, 55.94, 51.84, 19.21.

Methyl (E)-3-(4-((2-(2,4-dimethylbenzoyl)-6-hydroxybenzo[*b*]thiophen-3-yl)oxy)phenyl)acrylate (27a): This compound was prepared by a procedure identical to the preparation of **12a** (2.1 g, yield 61%). ¹H NMR (400 MHz, CDCl₃) δ 7.58 (d, *J* = 16.0 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.25 – 7.15 (m, 4H), 7.04 (s, 1H), 6.91 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.86 (d, *J* = 10.0 Hz, 2H), 6.48 (d, *J* = 8.7 Hz, 2H), 6.27 (d, *J* = 16.0 Hz, 1H), 3.80 (s, 3H), 2.28 (s, 3H), 2.09 (s, 3H). ¹³C NMR (100 MHz, DMSO) δ 191.12, 168.01, 160.03,

157.77, 148.39, 144.34, 141.94, 140.80, 136.24, 136.15, 131.45, 129.39, 128.86, 128.26, 127.26, 126.80, 125.86, 124.84, 116.36, 116.28, 115.61, 108.39, 51.94, 21.48, 19.38.

Methyl(E)-3-(4-((2-(4-chloro-2,6-dimethylbenzoyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenyl)acrylate (27c): This compound was prepared by a procedure identical to the preparation of **12a** (6.2 g, yield 65%). ¹H NMR (400 MHz, acetone-*d*6) δ 7.60 (d, *J* = 16.0 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 2.0 Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 6.99 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.89 (s, 2H), 6.57 (d, *J* = 8.7 Hz, 2H), 6.41 (d, *J* = 16.0 Hz, 1H), 3.73 (s, 3H), 2.08 (s, 6H). ¹³C NMR (100 MHz, acetone-*d*6) δ 190.88, 167.53, 160.15, 160.05, 149.24, 144.45, 143.05, 139.95, 136.83, 134.52, 130.28, 130.03, 128.38, 127.86, 126.65, 125.79, 117.45, 117.38, 115.90, 109.12, 51.67, 19.12.

(E)-3-(4-((2-(2,4-dimethylbenzoyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenyl)acrylic acid (28a): This compound was prepared by a procedure identical to the preparation of **14a** (1.6 g, yield 83%). ¹H NMR (400 MHz, acetone-*d*6) δ 7.58 (d, *J* = 16.0 Hz, 1H), 7.50 – 7.38 (m, 4H), 7.25 (d, *J* = 7.7 Hz, 1H), 7.01 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.96 – 6.86 (m, 2H), 6.57 (d, *J* = 8.7 Hz, 2H), 6.38 (d, *J* = 16.0 Hz, 1H), 2.29 (s, 3H), 2.07 (s, 3H). ¹³C NMR (100 MHz, acetone-*d*6) δ 190.44, 167.80, 160.72, 159.80, 148.66, 144.67, 142.52, 141.14, 137.47, 136.47, 131.96, 130.35, 130.01, 128.72, 127.86, 126.99, 126.55, 125.39, 117.81, 117.15, 116.34, 108.90, 21.31, 19.33. ESI-HRMS (*m/z*): [*M* + *H*]⁺ calcd. for C₂₆H₂₁O₅S: 445.1110; observed, 445.1100.

(E)-3-(4-((2-(4-chloro-2,6-dimethylbenzoyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenyl)acrylic acid (28c): This compound was prepared by a procedure identical to the preparation of **14a** (2.1 g, yield 88%). ¹H NMR (400 MHz, acetone-*d*6) δ 7.60 (d, *J* = 16.0 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.45 (d, *J* = 1.7 Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.00 (dd, *J* = 8.8, 1.9 Hz, 1H), 6.91 (s, 2H), 6.58 (d, *J* = 8.6 Hz, 2H), 6.40 (d, *J* = 16.0 Hz, 1H), 2.09 (s, 6H). ¹³C NMR (100 MHz, acetone-*d*6) δ 190.91, 167.77, 160.17, 160.00, 149.30, 144.62, 143.07, 139.99, 136.85, 134.53, 130.26, 130.17, 128.40, 127.88, 126.67, 125.82, 117.94, 117.38, 115.91, 109.13, 19.12. ESI-HRMS (*m/z*): [*M* + *H*]⁺ calcd. for C₂₆H₂₀ClO₅S: 479.0720; observed, 479.0706.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SERDs	selective estrogen receptor downregulators (or degraders)
ER+	estrogen receptor positive

SERM	selective estrogen receptor modulator
ERα	estrogen receptor α
ERβ	estrogen receptor β
E₂	estradiol
BT	benzothiophene
TR	treatment resistant
AIs	aromatase inhibitors
PK	pharmacokinetic
RBA	relative binding affinity
CDK	cyclin-dependent kinases
ERE	estrogen response element
ICW	in-cell western
ShERPAs	selective human ER partial agonists
LBD	ligand-binding domain
ERE	estrogen response element
PD	palbociclib
RT	room temperature
TLC	thin layer chromatography

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Figure 1.
Representative selective estrogen receptor downregulators (SERDs) and selective estrogen receptor modulators (SERMs)

Figure 2. Design of novel benzothiophene-based SERDs

(A) The BT core common to **5** and **6** provided the scaffold for design of BT-SERDs by replacing the basic side chain with an acrylate side chain and inserting a ketone linker at the 2-position, with further diversification of the 2-substituent. (B) Prototype BT-SERD (**14a**) docked to ER α LBD (pDB ID: 1R5K), showing similar global topology compared to the GW5638-ER complex. The acrylate side chain interaction with helix 12 is a key structural feature of SERD-ER complexes. (C) Residues within 5 Å of **14a** are highlighted and two hydrophobic cavities in the vicinity of Leu384 and Phe 425 are circled in red.

Figure 3. In-Cell Western Assay of ERα in MCF-7:WS8 cell cultures

Cells were incubated with increasing concentrations of **2** (ARN-810), **24b**, **24c**, **28c**, or E₂ for 24h before measurement of ERα protein levels were measured using in-cell western as described in the experimental section and imaged using a LI-COR Odyssey NIR system: LEFT ERα (green) is seen to decrease with increasing SERD concentration (green anti-ERα antibody); MIDDLE CellTag 700 (red) is used for normalization; and, RIGHT the color merged plate clearly shows loss of ERα in response to SERD treatment. Data quantitation is shown in Figure 4C, Figure S2 and Table 3.

Figure 4. Profiling SERDs: 2 and 28c

(A) Cell viability of TR MCF-7:5C breast cancer cells 4 days after drug treatment, normalized to vehicle (100%). (B) Cell viability of parent, endocrine-sensitive MCF-7:WS8 breast cancer cells 4 days after drug treatment, normalized to vehicle (100%). (C) Estrogen receptor downregulation using in-cell western assay in MCF-7:WS8 cells. Data were normalized to 1 μ M **2** as 0% and DMSO control as 100%. (D) ER α antagonism using ERE-luciferase reporter assay in MCF-7:WS8 cells. Data was normalized to 1 μ M **2** as 0% and 0.1 nM E₂ as 100%. Data show mean and s.e.m. from at least 3 cell passages.

Figure 5.
SERDs inhibit growth of MCF-7:TAM1 spheroids at day 10: DMSO (A), **28c** (1 nM) (B), **28c** (1 nM) (C) and **28c** (10 nM) (D).

Figure 6.

Compound **14a** (A), **14b** (B), and **28c** (C) were docked to ER LBD (pdb ID: 5ak2).

Compound **14a** has minimum contacts with hydrophobic pockets (close to Phe 425 and Leu 384), while compound **14b** and **28c** have methyl groups tightly fit into the hydrophobic cavity, corresponding to better potency in cell viability assays.

Figure 7.

Synergistic or additive effects of combination therapy of SERDs and CDK4/6 inhibitor, PD-0332991 (PD), in MCF-7:WS8 and TR MCF-7:TAM1 spheroids were quantified by ATP assay normalized to vehicle (DMSO) as 1.0. Data show mean and SEM with one-way ANOVA comparison with Tukey's post-test: relative to DMSO control group; or comparison of SERD alone (10 nM) with SERD + PD (10 nM): $p < *$ 0.05; $**$ 0.01; $***$ 0.005; $****$ 0.001.

Figure 8.

(A) Plasma concentration of **24c**, **28a**, and **28c** after oral administration in PEG400/PVP/TW80/CMC in water, 9:0.5:0.5:90. The data was the average plasma concentration of three mice at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h and 12 h. (B) MCF7:TAM1 tumors were grown to an average section area of 0.32 cm² (as described in the experimental section). Mice were then randomized into six treatment groups: control, tamoxifen, **2**, **28a**, and two doses of **28c**. Compounds were administrated by oral gavage daily: (B) mean tumor growth over 9 weeks showing mean and SEM; (C) individual tumor area change in percentage at day 23.

Scheme 1^a

^a Reagents and conditions: (a) N,O-Dimethylhydroxylamine hydrochloride, Et₃N, DCM, RT, overnight, 76%; (b) Grignard reagent, THF, 0°C to RT, 2–12 h; (c) 4-bromophenol, Cs₂CO₃, DMF, 70°C; (d) BF₃•SMe₂, DCM, 0°C to RT, 24–48 h; (e) Methyl acrylate, Pd(PPh₃)₂Cl₂, Et₃N, DMF, 110 °C; (f) LiOH, MeOH, H₂O, RT, 4h.

Scheme 2^a

^a Reagents and conditions: (a) methyl 6-hydroxy-2-naphthoate, methyl 7-hydroxyquinoline-3-carboxylate, or 4-aminophenol, Cs₂CO₃, DMF, 70°C; (b) BF₃•SMe₂, DCM, 0°C to RT or 35 °C, 48 h; (c) i) methyl 2-chloro-2-oxoacetate, Et₃N, DCM; ii) LiOH, MeOH, H₂O, RT, 4h.

Scheme 3^a

^a Reagents and conditions: (a) Grignard reagent, THF, 0 °C to RT, 2–12 h; (b) 4-bromophenol, Cs₂CO₃, DMF, 70 °C; (c) BF₃•SMe₂, DCM, 0 °C to RT, 24–48 h; (d) Pd(PPh₃)₂Cl₂, Et₃N, DMF, 110 °C; (e) LiOH, MeOH, H₂O, RT, 4h.

Scheme 4^a

^a Reagents and conditions: (a) Grignard reagent, THF, 0 °C to RT, 2–12 h; (b) methyl (E)-3-(4-hydroxyphenyl)acrylate, Cs₂CO₃, DMF, 90°C; (c) BF₃•SMe₂, DCM, 0°C to RT, 24–48 h; (d) LiOH, MeOH, H₂O, RT, 4h.

Table 1

Optimization of “warhead” using growth inhibition data

Compounds	R ₁	R ₂	MCF-7:5C IC ₅₀ (nM) ^a /E _{max} ^b	MCF-7:WS8 IC ₅₀ (nM)
2			1.2 ± 0.05	0.2 ± 0.09
14b		H	1.3 ± 0.06	0.9 ± 0.07
16a		H	4.8 ± 0.06	2.4 ± 0.12
16b		H	32.3 ± 0.19 (52%)	NI

Compounds	R ₁	R ₂	MCF-7:5C IC ₅₀ (nM) ^a /E _{max} ^b	MCF-7:WS8 IC ₅₀ (nM)
19		F	1.7 ± 0.07 (64%)	NI

^aCell survival was normalized to DMSO control (100 %) and **2** (1 uM) treatment (0 %).

^bMaximum efficacy < 100% is observed when treatment fails to inhibit cell growth to the level observed for **2** (100%) at 100 x IC₅₀.

NI = no inhibition. Data show mean ± s.e.m. from at least three cell passages (triplicates in each passage)

Table 2

Optimization of 2-benzothiophene substituent using growth inhibition data

14a-h, 24a-c, and 28a-c

Compounds	R ₁	MCF-7:5C IC ₅₀ (nM) ^a	MCF-7:WS8 IC ₅₀ (nM) ^b
2		1.2 ± 0.05	0.2 ± 0.09
14a		13 ± 0.08	2.2 ± 0.1
14b		1.3 ± 0.06	0.9 ± 0.07
14c		1.2 ± 0.04	0.9 ± 0.04
14d		2.7 ± 0.11 (61%)	1.2 ± 0.08 (65%)

14a-h, 24a-c, and 28a-c

Compounds	R ₁	MCF-7:5C IC ₅₀ (nM) ^a	MCF-7:WS8 IC ₅₀ (nM) ^b
14e		3.9 ± 0.06 (54%)	NI
14f		12.5 ± 0.01	2.8 ± 0.16
14g		1.0 ± 0.05	0.4 ± 0.07
14h		4.7 ± 0.04	0.70 ± 0.03

14a-h, 24a-c, and 28a-c

Compounds	R ₁	MCF-7:5C IC ₅₀ (nM) ^a	MCF-7:WS8 IC ₅₀ (nM) ^b
28b		2.2 ± 0.12	0.4 ± 0.13
28a		0.4 ± 0.04	0.1 ± 0.08
24a		0.5 ± 0.04	0.1 ± 0.07
24b		0.5 ± 0.03	< 0.1

14a-h, 24a-c, and 28a-c

Compounds	R ₁	MCF-7:5C IC ₅₀ (nM) ^a	MCF-7:WS8 IC ₅₀ (nM) ^b
24c		0.4 ± 0.03	< 0.1
28c		0.3 ± 0.04	< 0.1

^a Cell survival was normalized to DMSO control (100 %) and 1 μ M **2** (0 %) assessed in a 6 day cell viability assay with one time drug treatment.

^b Cell survival was normalized to DMSO control (100 %) and 1 μ M **2** (0 %) assessed in a 4 day cell viability assay with one time drug treatment.

^{ab} Figures in parentheses indicate <100% maximal efficacy (E_{max}).

Data show mean ± s.e.m. from at least three cell passages (triplicates in each passage). NI = no inhibition.

Table 3

ER α degradation, antagonism of E2 signaling, ER α relative binding affinity, and inhibition of growth of ER+ cells cultured in 3D spheroids.

14b, 14g, 28a, 24a, 28c and 24c

Compounds	R ₁	ER α ICW EC ₅₀ (nM) ^a	ERE luciferase IC ₅₀ (nM) ^b	% growth of MCF-7; ws8 3D spheroids ^c	ER α binding K _i (nM) ^d	RBA % (relative to E ₂) ^e
2		0.8 ± 0.07	11.1 ± 0.14	15 ± 3.00	0.37 ± 0.1	53.4 ± 15.0
14b		1.1 ± 0.05	16.7 ± 0.07	12 ± 0.02	1.29 ± 0.4	15.5 ± 4.2
14g		0.71 ± 0.05	8.8 ± 0.11	3.3 ± 0.01	0.65 ± 0.2	30.6 ± 8.7

14b, 14g, 28a, 24a, 28c and 24c

Compounds	R ₁	ERα IC ₅₀ (nM) ^a	ERE luciferase IC ₅₀ (nM) ^b	% growth of MCF-7:ws8 3D spheroids ^c	ERα binding K _i (nM) ^d	RBA % (relative to E ₂) ^e
28a		0.92 ± 0.05	4.5 ± 0.07	12 ± 0.01	0.50 ± 0.1	40.3 ± 4.8
24a		0.65 ± 0.06	4.2 ± 0.05	14 ± 1.00	2.0 ± 0.2	9.8 ± 0.7

14b, 14g, 28a, 28c and 24c

Compounds	R ₁	ERα ICW EC ₅₀ (nM) ^a	ERE luciferase IC ₅₀ (nM) ^b	% growth of MCF-7:ws8 3D spheroids ^c	ERα binding K _i (nM) ^d	RBA % (relative to E ₂) ^e
28c		0.07 ± 0.13	2.4 ± 0.10	1.3 ± 0.01	0.57 ± 0.1	34.8 ± 6.2
24c		0.24 ± 0.16	3.1 ± 0.07	2.1 ± 0.01	0.73 ± 0.2	27.5 ± 7.0

^a Potency for induction of ER degradation measured at 10 concentrations using in-cell westerns (ICW).

^b Potency of antagonism of ERE-luciferase reporter.

^c Spheroid growth inhibition after SERD treatment (100 nM) expressed as % relative to DMSO vehicle control (100%).

Data show mean and s.e.m.

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_p Binding affinities calculated by the formula: $K_i = (K_d[\text{estradiol}]/\text{RBA}) * 100$, where the K_d for estradiol is 0.2 nM.

e Relative binding affinity (RBA) values, determined by radioligand displacement assays expressed as $\text{IC}{50} \text{ estradiol} / \text{IC}_{50} \text{ compound} \times 100$ (RBA, estradiol = 100%).