European Journal of Medicinal Chemistry 221 (2021) 113543

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Design and synthesis of novel benzothiophene analogs as selective estrogen receptor covalent antagonists against breast cancer

Chengfeng Bai [a, b], Shengnan Ren [a, b], Shuangjie Wu [a, b], Meiqi Zhu [a, b], Guoshun Luo [a], Hua Xiang [a, *]

[a] State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing, 210009, China
[b] Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, Nanjing, 210009, China

ARTICLE INFO

Article history:
Received 9 February 2021
Received in revised form
4 May 2021
Accepted 10 May 2021
Available online 14 May 2021

Keywords:
Breast cancer
Estrogen receptor alpha (ERα)
Endocrine-resistance
Covalent antagonist
Benzothiophene

ABSTRACT

Endocrine therapy (ET) has benefited patients with estrogen receptor alpha (ERα) positive breast cancer for decades. Selective estrogen receptor modulator (SERM) such as Tamoxifen represents the clinical standard of care (SoC). Despite the therapeutic importance of current SoC agents, 30–50% of prolonged treatment patients inevitably generated resistant tumor cells, usually eventually suffered tumor relapse and developed into metastatic breast cancer (MBC), which was the leading cause of female cancer-related mortality. Among these, most resistant tumors remained dependent on ERα signaling, which reignited the need for the next generation of ERα related agents. We hypothesized that selective estrogen receptor covalent antagonists targeting ERα would provide a therapeutic alternative. In the current work, series of novel benzothiophene hybrids bearing electrophile moieties were synthesized and biologically evaluated. The representative analogue **15c** exhibited potent anti-proliferative effect in MCF-7 cell lines *in vitro*, and further mechanism studies confirmed the necessity of covalent bonding. More importantly, **15c** could attenuate the expression of *TFF-1*, *GREB-1* and downregulate the levels of cellular ERα protein.

1. Introduction

Breast cancer is the most common type of invasion cancers among women all over the world. Nearly 70% of these patients express ERα (encoded by *ESR1*) which is the critical driver in oncogenic proliferation and metastasis [1,2]. Over the past 40 years, the treatment has long been relied on endocrine therapies, targeting ERα either by blocking estrogen binding to the receptor, such as tamoxifen (TAM) [3], or preventing the synthesis of estrogens under aromatase catalysis. Despite TAM's clinical success, its ERα agonist activity observed in endometrium and associated with an increased risk of developing endometrial cancer remain great concerns. The efforts on creating novel ERα antagonists without this risk finally led to the discovery of selective estrogen receptor degrader (SERD), such as Fulvestrant (Fig. 1). However, considering its poor physicochemical properties and oral bioavailability, Fulvestrant can only be clinically administered via monthly intramuscular injection [4]. Recently, considerable non-steroidal SERDs with oral bioavailability are in clinical trials, addressing both local breast cancer and peripheral metastases, such as LSZ102 (Novartis) [5], RAD1901 (Radius) [6], AZD9496 (AstraZeneca) [7], GDC-0810 (Genentech) [8], *etc.* (Fig. 1). Notwithstanding these successes in the clinic, intrinsic and acquired drug resistance remains the persistent challenge. Generally, patients who generated resistant tumors would inevitably progress to an antiestrogen-resistant state [9], making TAM ineffective. Patients those burdened with advanced endocrine metastatic diseases are currently incurable and only have a less than 5-year survival [10].

Mechanisms of resistance have been included but not limited to the following aspects: 1) aberrant expression of ERα coactivators/corepressors [11]; 2) "cross-talk" between ERα and other kinases [12]; 3) hotspot mutations in ESR1 which occupied about 30% of endocrine-resistance [13–15]. Of note is the resistance after TAM treatment, it is associated with hotspot mutations activating of Y537 S/C/N and D538G. Mutations that occurred in ligand binding domain (LBD) could prompt activation of ERα in a ligand-independent manner, stabilize an active conformation of Helix 12 (H12), and favor the recruitment of coactivator even in the absence

* Corresponding author.
E-mail address: xianghua@cpu.edu.cn (H. Xiang).

https://doi.org/10.1016/j.ejmech.2021.113543
0223-5234/

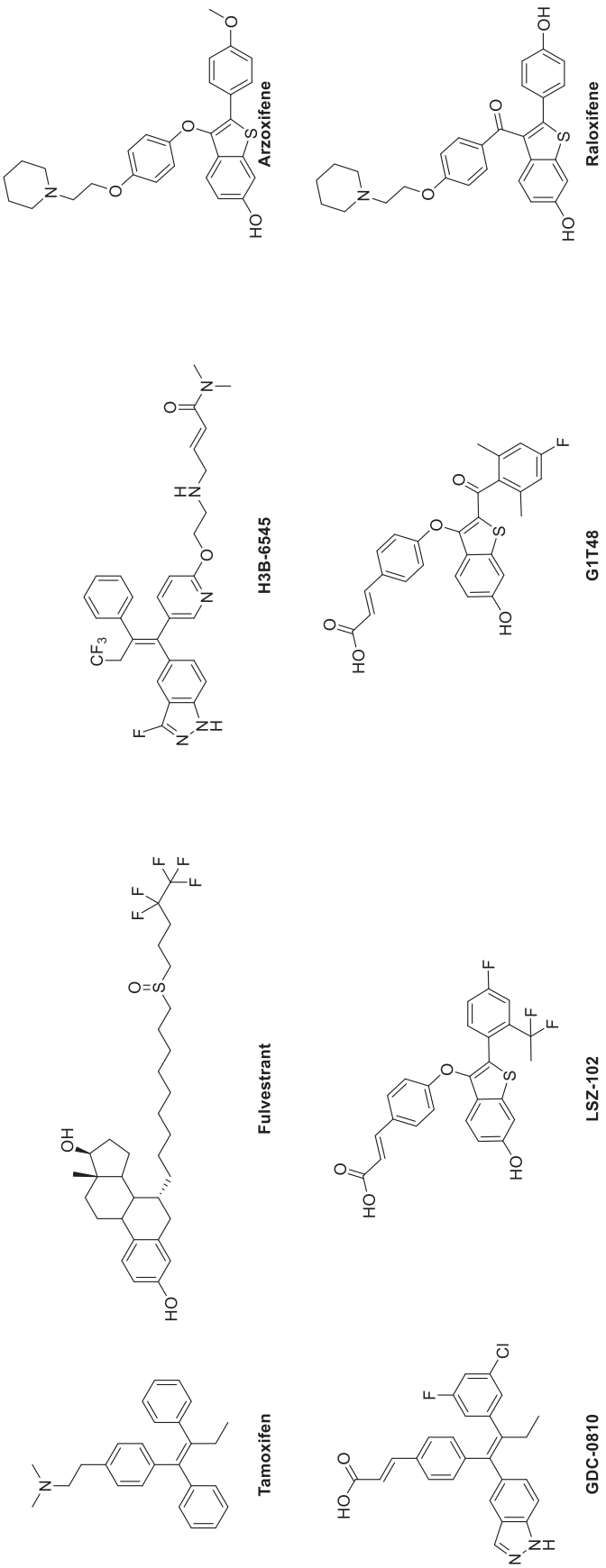


Fig. 1. Structures of representative estrogen receptor ligands.

of estradiol (E2). Consequently, these mutations could result in tumor resistance and reduced potency of antagonists [16]. For the resistance settings, up to 90% of endocrine therapy-resistance tumors remain dependent on ERα signaling for survival and metastasis. Hence, there still exists an urgent need to develop novel binding modes of ERα antagonism and overcome resistance of current SoC endocrine therapies.

Targeted covalent inhibitors (TCIs) have emerged as efficacious pharmacological modalities and been demonstrated multiple advantages, including improved potency, selectivity, and efficacy over conventional reversible inhibitors. As documented, the pocket volume of ER ligand-binding is ~450 $Å^3$ and suitable for ligands 250−380 $Å^3$. Additionally, the plasticity of ER-LBD is reflected by the diversity of ER ligand scaffolds and suits itself to discover novel pharmacological modulators [17]. Consistent with previous reports, four cysteines (amino acids 381, 417, 447, and 530) in LBD were demonstrated as covalent targets of ERα [18]. Further analysis of the X-ray crystal structure of ERα confirmed that both C381 and C530 could be engaged by an electrophile in the ligand-binding pocket, while only C530 is directly related to ligand-dependent activation and suitable for the discovery of effective antagonists [19,20]. Mechanistically, the covalent interaction targeting a nonconserved cysteine at 530 position of Helix 11 might overcome the stabilizing effects of mutations [21]. Consistent with the hypothesis, Puyang et al. (2018) identified a representative selective estrogen receptor covalent antagonist (SERCA) named H3B-6545 (Fig. 1). The novel antagonist emerged with the ability to inactivate both $ERα^{WT}$ and $ERα^{MUT}$ (*e.g.,* Y537S, D538G) *in vitro* and in *vivo*, through enforcing a unique antagonist conformation in ERα. Crystal structure of H3B-6545 bound to the ER LBD showed that the continuous electron density between C530 and the Michael acceptor in the cocrystal structure was observed, which directly confirmed the covalent engagement. Currently, H3B-6545 has been evaluated in an ongoing phase 1/2 study in women with locally advanced or metastatic ER+, HER2-breast cancer (NCT03250676). The compelling activity of H3B-6545 supports the notion of a covalent antagonist, and it also shares some pharmacologic features with SERMs [22−24].

Over the past decades, our group engaged in discovering novel small molecule agents targeting ER-related diseases. For the present study, we identified a series of novel SERCAs that bearing novel skeletons and more satisfactory biological activity.

2. Results and discussion

2.1. Structure design

To date, Raloxifene (Fig. 1) has been applied in the therapy of breast cancer and postmenopausal osteoporosis for decades. Its benzothiophene scaffold was deemed safe and valid in the design of novel ER-targeted ligands [25]. With the purpose of improving potency and pharmacokinetic (PK) properties, various chemical modifications have been accomplished and yielded different ER ligands with satisfactory activity. For instance, arzoxifene [26,27], G1T48 (G1) [28] and LSZ-102 (Novartis) [5] have been demonstrated as potent antineoplastic agents across breast cancer model (Fig. 1).

Based on structural knowledge gained from Raloxifene, its benzothiophene scaffold was chosen as the core structure, as depicted in Fig. 2. Additionally, fluorine substituted at the 4′-position of the skeleton was expected to attenuate the toxicity of ligand while maintaining intrinsic ERα binding affinity and modulating activity [29]. To further improve the anti-ERα potency of designed antagonists, our chemistry efforts on structural optimization focused on modifying the electrophile warheads and length of linkers. On the other hand, influenced in part by the crystal structure of H3B-6545 bound to the ER LBD, various “Michael acceptors” (amine electrophile moieties) that similar to H3B-6545 were introduced and expected to interact with cysteine at 530 position of Helix 11. To explore an optimal binding model and a meaningful structure-activity relationship (SAR), both ethyl and azetidine linkers were respectively applied in compounds **15(a−f)** and **21(a−e)**, as depicted in Fig. 2. Moreover, through replacement of the amide warheads, different acrylamide analogs (**23a-c** and **25a-c)** were also investigated. For the biological assessment, in order to verify the pharmacophore role of “Michael acceptor,” compound **16**, the saturated analogue of **15c**, was designed as the negative control and well biologically evaluated. Structure details of all the newly prepared compounds were depicted in Table 1.

2.2. Chemistry

Synthesis of synthons **2, 4,** and **6(a−f)** followed the procedures depicted in Scheme 1. For the construction of compound **2**, 4-benzyloxyphenol was reacted with *tert*-butyl (2-bromoethyl) carbamate in the presence of $C_{S2}CO_3$, followed by deprotection of benzyl group under Pd/C and H_2, intermediate **4** was obtained with the similar method.

Bromination of (E)-but-2-enoic acid (compound **5**) occurred in the presence of NBS and benzoyl peroxide. Subsequently, intermediates **6(a−f)** were obtained by coupling different types of amines to carboxyl of compound **5** (Scheme 1).

Reagents and conditions. (a) tert-butyl (2-bromoethyl)carbamate, Cs_2CO_3, DMF, 50 °C. (b) H_2, Pd/C, MeOH, rt. (c) 1-Boc-3-iodoazetidine, Cs_2CO_3, DMF, 130 °C. (d) H_2, Pd/C, MeOH, rt. (e). NBS, benzoyl peroxide, CCl_4. (f) Oxalyl chloride, Na_2CO_3, amine, CH_2Cl_2.

Reagents and conditions. (a) KOH, EtOH. (b) PPA, 110−120 °C. (c) N-bromoacetamide, CH_2Cl_2. (d) H_2O_2, CF_3COOH, CH_2Cl_2. (e) Compound **2**, Cs_2CO_3, DMF, 50 °C. (f) $LiAlH_4$, THF, 0 °C (g) HCl/MeOH, rt. (h) **6(a−f)**, DIPEA, DMF. (i) BBr_3, CH_2Cl_2, 0 °C. (j) H_2, Pd/C, MeOH.

Reagents and conditions. (a) Compound **4**, Cs_2CO_3, DMF, 50 °C. (b) $LiAlH_4$, THF, 0 °C (c) HCl/MeOH, rt. (d) **6(a−e)**, DIPEA, DMF. (e) BBr_3, CH_2Cl_2, 0 °C.

Reagents and conditions. (a) various acid chlorides, CH_2Cl_2, DIPEA. (b) BBr_3, CH_2Cl_2, 0 °C.

Scheme 2 outlined the synthesis of target compounds **15(a−f)** and the saturated analogue **16**, which was utilized as the negative control in the bioassay assays. As documented, through nucleophilic substitution and cyclization reaction, compound **8** was obtained in good yield [30]. As initially expected, bromination of **8** should be accomplished with N-bromosuccinimide, followed by introducing fragment **2** or **4** to the core. Unfortunately, both compounds **2** and **4** failed to couple with the brominated intermediate **9** through nucleophilic aromatic substitution reaction. In order to activate the reaction site, the thiophene fragment of compound **9** was oxidized to the corresponding sulfoxide **10**. Desired nucleophilic substitution was accomplished without formation of obvious by-products. Having served its functionality, the sulfoxide group of **11** was removed via reduction in the presence of $LiAlH_4$. Subsequently, deprotection of Boc protecting group with HCl/MeOH solution afforded compound **13**, which was then undergoing SNAr reaction with various synthons **6(a-f**) to obtain compounds **14(a−f)**. Finally, desired compounds **15(a−f)** were obtained through demethylation in the presence of BBr_3/CH_2Cl_2 solution. Additionally, the negative control **16** was obtained through the hydrogenation of **15c**. Synthesis details for **21(a−e)** were outlined in Scheme 3, which were quite similar to the procedure of **15(a−f)**. Meanwhile, amide analogs **23(a−c)** and **25(a−c)** were prepared from compounds **13** and **19** through coupling with the

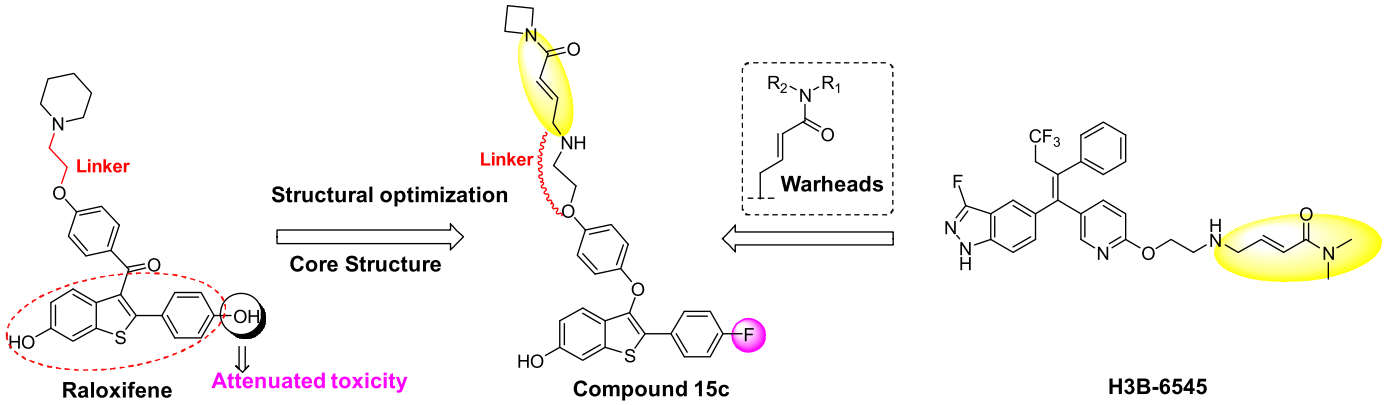
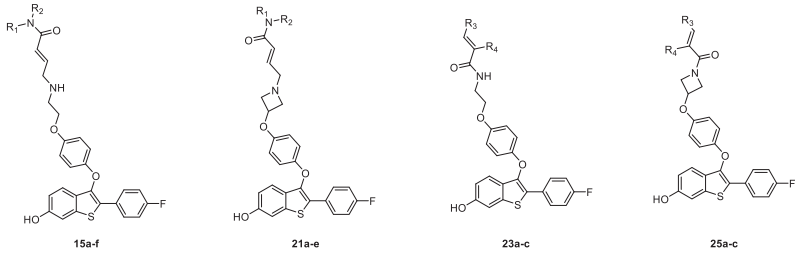


Fig. 2. Design of novel benzothiophene-based SERCAs, compound **15c** was constituted with the core structure that similar to Raloxifene and side chains common to H3B-6545.

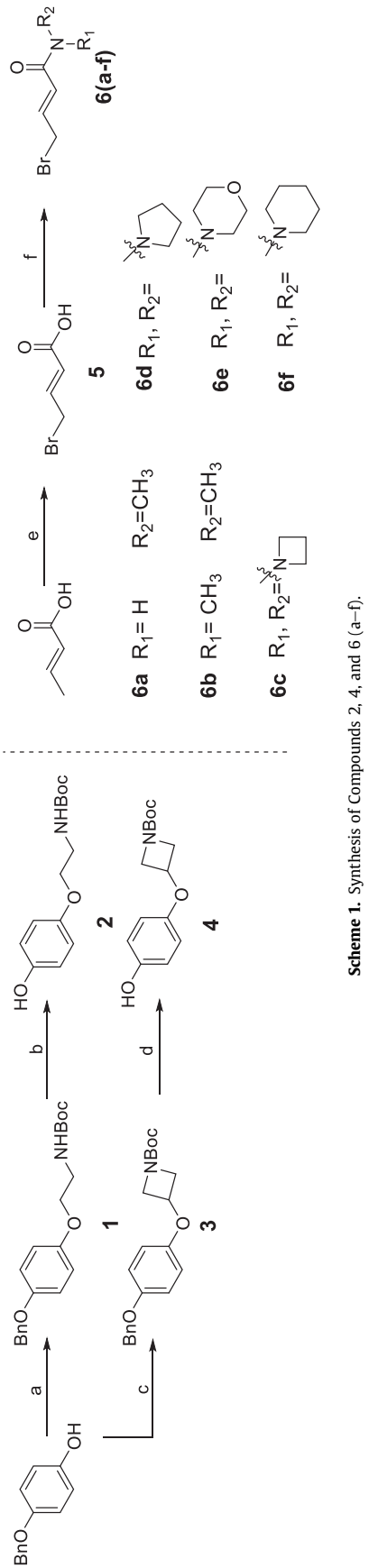
Table 1
Antiproliferation activity of analogs in breast cancers.



Comp.	R_1	R_2	R_3	R_4	Antiproliferation (IC_{50}, μM)		
					MCF-7	Ishikawa	MDA-MB-231
15a	H	$-CH_3$	—	—	16.1	15.53	>50
15b	$-CH_3$	$-CH_3$	-	-	13.83	11.91	>50
15c			-	-	7.34	6.56	>50
15d			-	-	7.61	11.25	>50
15e			-	-	8.24	18.55	>50
15f			-	-	16.38	8.43	>50
21a	H	$-CH_3$	-	-	>25	>25	>50
21b	$-CH_3$	$-CH_3$	-	-	22.33	11.21	>50
21c			-	-	>25	5.45	>50
21d			-	-	8.45	9.15	>50
21e			-	-	8.90	9.84	>50
23a	-	-	H	H	22.96	>25	>50
23b	-	-	H	$-CH_3$	>25	>25	>50
23c	-	-	$-CH_3$	H	21.65	12.44	>50
25a	-	-	H	H	23.53	12.26	>50
25b	-	-	H	$-CH_3$	>25	14.20	>50
25c	-	-	$-CH_3$	H	27.96	9.40	>50
16	-	-	-	-	>25	>25	>50
Raloxifene	-	-	-	-	19.94	12.39	>50
Tamoxifen	-	-	-	-	-	16.78	>50

a. IC_{50}: Concentration that inhibits 50% of cell growth.

corresponding amine and demethylation reactions, details outlined in Scheme 4.



Scheme 1. Synthesis of Compounds 2, 4, and 6 (a–f).

2.3. Antiproliferative activity in vitro

The *in vitro* antiproliferative efficacy of all the newly prepared compounds was evaluated by Cell Counting Kit-8 (CCK-8) assay in MCF-7, Ishikawa, and MDA-MB-231 (ERα-) cells. Results expressed as IC_{50} were summarized in Table 1, and both Raloxifene and Tamoxifen were used as the baseline control.

The result showed that most of the synthesized compounds exerted moderate to potent antiproliferative activity. Of the ethane series, compound **15c** showed significantly more potent activity than Raloxifene against both MCF-7 and Ishikawa cells (IC_{50} = 7.34 μM and 6.56 μM, respectively). On the other hand, compounds **15d** and **15e** bearing different terminal amides exhibited very similar potency and efficacy to **15c**. To further explore the necessity of electrophile moiety in **15c**, compound **16** designed from **15c** was synthesized and evaluated *in vitro*. The saturated analogue **16** notably abolished the anti-proliferative activity previously observed in **15c**, revealing the indispensable pharmacophore role of electrophile moiety in **15c**.

Additionally, compounds **21(a–e)**, through substituting the ethane linker of **15(a–f)** for azetidines, were also investigated. As shown in Table 1, **21d** and **21e** showed almost 2–3 folds more potent activity than Raloxifene against both MCF-7 and Ishikawa cells, while marginally inferior to **15d** and **15e**. As also observed for **21(a–c)**, all these alterations had a slightly negative effect on anti-proliferation potency relative to their corresponding ethane derivatives, suggesting that ethane linker was conducive to antitumor activity. On account of the non-significant activity difference between **15e** and **15f**, only **21e** that corresponding to analogue **15e** and with morpholine "tail", was synthesized and biologically evaluated. A closer analysis of the binding model between **15c** and **21c** through molecular docking indicated that ethane linker facilitated the electrophile moiety closer to target residue Cys 530 (Fig. 6c).

Besides, reversing the carboxyl from terminal acrylamides to directly coupling to the linker led to entirely different "warheads", compounds **23(a–c)** and **25(a–c)**. However, a notable activity reduction was observed. Having evaluated the antiproliferative activity on ERα positive cells, a further investigation focused on ERα negative cells (MDA-MB-231) was accomplished. As expected, all the synthesized compounds resulted with IC_{50} > 50 μM.

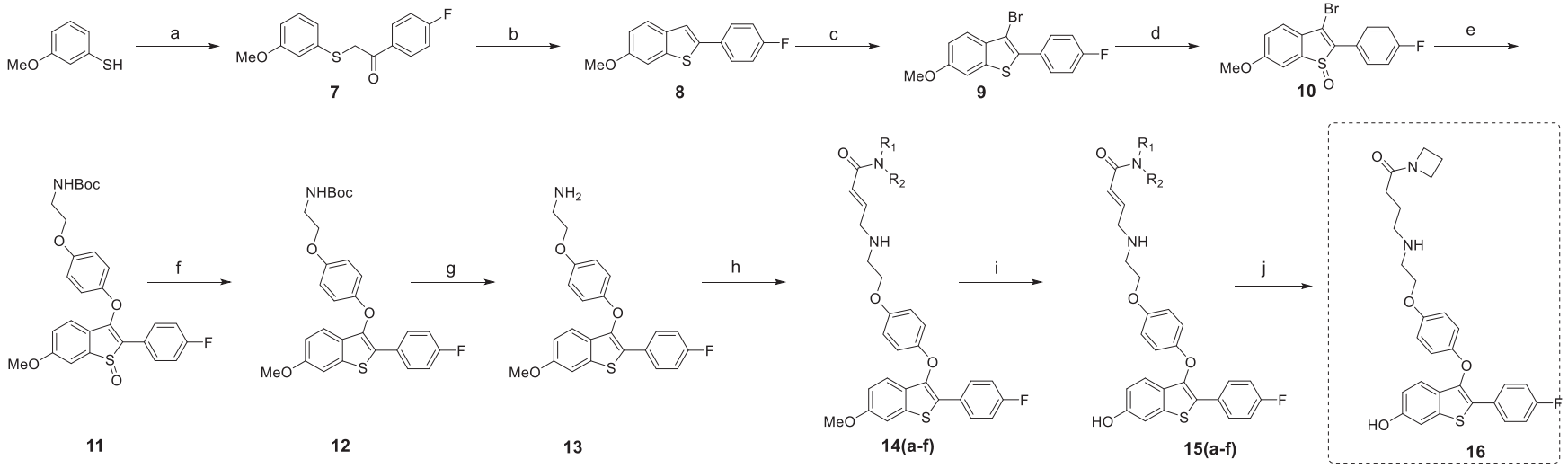
These above-mentioned results collectively revealed that **15c** was a potent inhibitor for ER + tumor cells. More importantly, its antiproliferative activity was closely related to its electrophile moiety.

2.4. ERα binding affinities of the synthetic compounds

To further assess whether the attractive activity of analogs was correlated with the interaction between ERα and the ligands, we evaluated the ERα binding affinities of these agents via a fluorescence polarization protocol. (ER Alpha Competitor Assay Kit, Green).

Several compounds with more potent antiproliferation than Raloxifene were chosen and tested at the concentration of 1 μM, results illustrated in Table 2 (see Table 2).

As shown in Table 2, all the tested compounds showed higher binding affinity for ERα than the positive control Raloxifene, among which compound **15c** exhibited the most potent binding inhibition effect. Additionally, the ethane series **15d**, **15e**, and **15f** were more potent than azetidine derivatives **21d** and **12e**, which was consistent with the *in vitro* anti-proliferative activity. Based on the data collected in antiproliferative activity and ERα binding assay, **15c** was prioritized for further study.



14a, 15a R_1= H R_2=CH_3

14b, 15b R_1= CH_3 R_2=CH_3

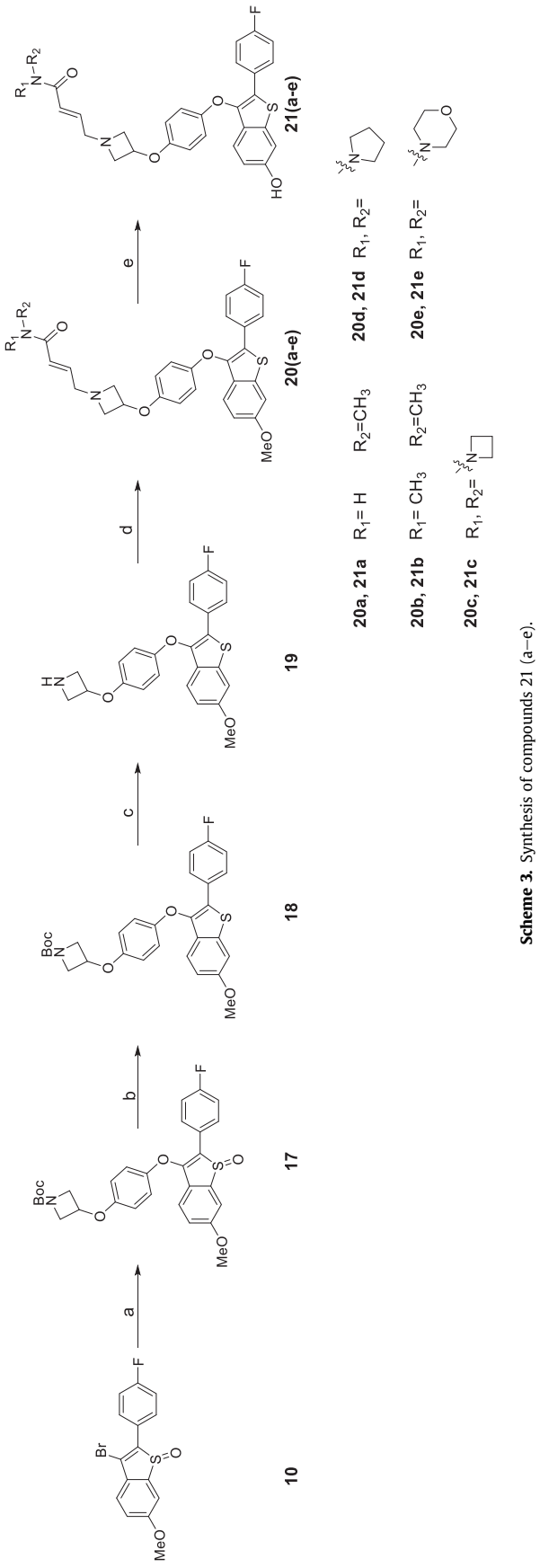
14c, 15c R_1, R_2= 

14d, 15d R_1, R_2= 

14e, 15e R_1, R_2= 

14f, 15f R_1, R_2= 

Scheme 2. Synthesis of compounds 15 (a–f) and 16.



Scheme 3. Synthesis of compounds 21 (a–e).

2.5. Effects of compound 15c on ERα level of MCF-7 cell

To profile the impact of covalent engagement on cellular ERα protein, both western blotting and immunofluorescence microscopy were utilized. Immunofluorescence microscopy analysis was performed at concentration 5 μM, and the result revealed the potential effects of compound **15c** on downregulating the ERα level (Fig. 3A and B). Further investigation at lower concentrations was accomplished by western blotting assay in the $ER\alpha^{WT}$-expressing MCF-7 parental cell line. In contrast to Fulvestrant, following 24 h treatment, compound **15c** showed slightly decreased effects on ERα level at 0.5 μM (Fig. 3c).

2.6. Effects of compound 15c on ERα-dependent transcription

To further profile the impact of covalent interaction, quantitative real-time polymerase chain reaction (RT-PCR) was adopted to interrogate gene expression of two independent ERα target genes, *GREB1* and *TFF1*. Estradiol (E2) was used to stimulate ERα signaling in MCF-7 cells before co-incubated with compounds. As expected, compound **15c** could dramatically decrease *GREB1* and *TFF1* gene expression in a dose-dependent manner. Simultaneously, compound **16** showed distinctly reduced potency relative to **15c**. These data collectively revealed that the cellular potency observed for **15c** was driven by its covalent binding (Fig. 4).

2.7. Potential SERMs-like activity of 15c on Ishikawa cells

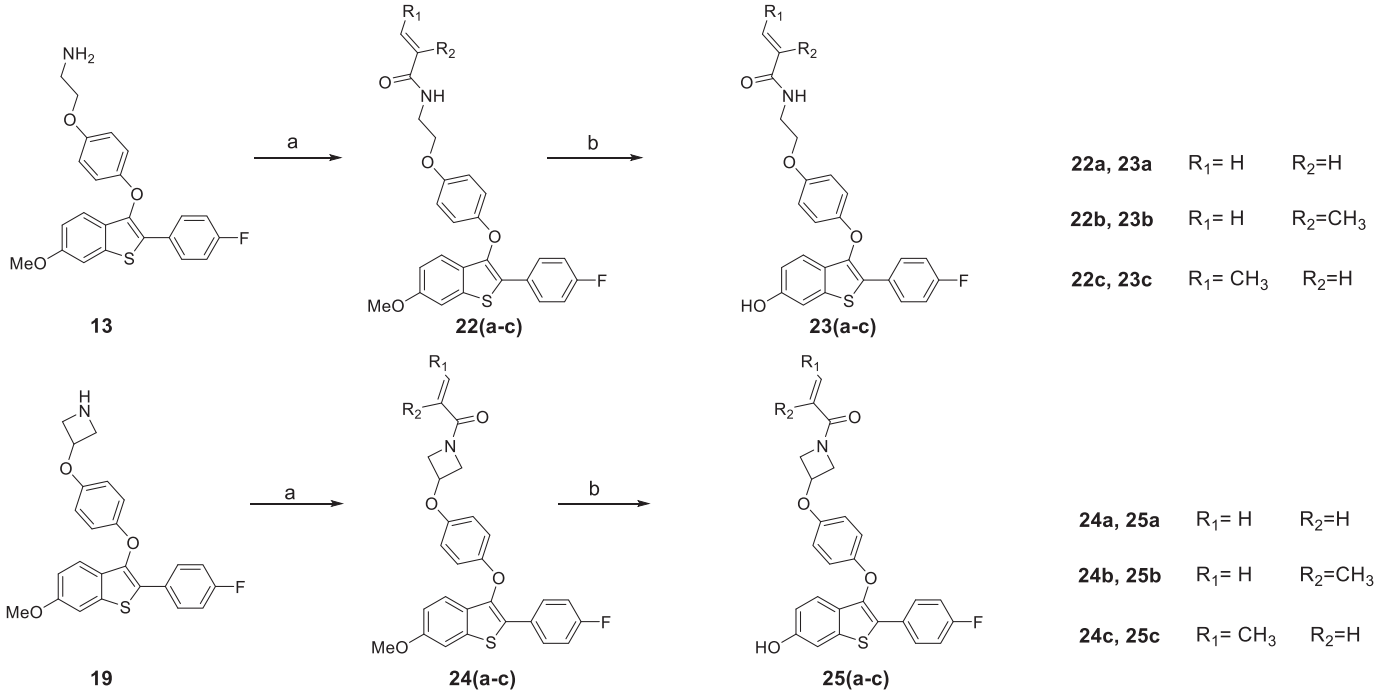
It is pretty clear that 4-hydroxytamoxifen (4-OHT) could induce ERα activity and stimulate signaling in Ishikawa endometrial carcinoma lines, result noted by the increased expression of *PGR* and proliferation of tumor cells [31].

After three days' exposure to **15c** in Ishikawa cells, both **15c** and **16** significantly inhibited the expression of *PGR* in E2-stimulated models (Fig. 5B). Additionally, following 3 days' incubation with **15c** at tested concentrations varying from 156 nM to 5000 nM, proliferation data showed no inducing impact on Ishikawa proliferation, which was in sharp contrast to 4-OHT (Fig. 5A). These data collectively indicated that **15c** didn't share the potential liability of SERMs in endometrial carcinoma cells.

2.8. Compound 15c was dependent on covalent engagement for enhanced ERα antagonism

To investigate the binding model of **15c**, molecular docking was utilized to reveal the details of the interaction between ERα and **15c** (PDB code: 6chw). As shown in Fig. 6A, the hydrogen-bonding network occurred between hydroxyl moiety of A-ring and residues Arg394, Glu353, as well as an irreversible covalent engagement of electrophile targeting Cys530 was observed (Fig. 6A). Comparisons of docking details between saturated analogue **16** and **15c** confirmed similar binding modes for core structure, while linker without electrophile appeared higher degree of flexibility, as shown in Fig. 6B.

Furthermore, to elucidate the binding site of **15c**, a model reaction between **15c** and cysteine was carried out under basic conditions, simulating the interaction between C530 residue, the product was analyzed using ^{1}H NMR. The disappearance of hydrogen in double bond of Michael acceptor demonstrated that cysteine reacted with the β carbon atom of α, β-unsaturated carbonyl in **15c** (Fig. S1).



Scheme 4. Synthesis of compounds 23 (a–c) and 25 (a–c).

Table 2
ERα binding affinity of compounds **15c**, **15d**, **15e**, **15f**, **21d,** and **21e**.

Comp.	Inh% (1 μM)[a]	Comp.	Inh% (1 μM)[a]
15c	98.48	**21d**	88.80
15d	97.87	**21e**	86.81
15e	90.57	**Raloxifene**	74.81
15f	85.81		

[a] Percent inhibition of each compound was calculated from the polarization values.

2.9. Compound 15c induced cell cycle G0/G1 phase arrest and apoptosis in MCF-7 cells

Having demonstrated that the covalent engagement drove the antiproliferative effects of **15c**. Next, we further investigated the impact of **15c** on the cell progression of MCF-7. As illustrated in Fig. 7B, the cell cycle was blocked at G0/G1 phase in a dose-dependent manner in MCF-7 cells, and percentage of cells in G0/G1 phase varied from 22.44 to 42.95%. To profile whether growth inhibition of breast cancer cells was related to induction of apoptosis, **15c**-induced MCF-7 cells were stained with Annexin V-FITC and propidium iodide (PI). The result indicated that **15c** could

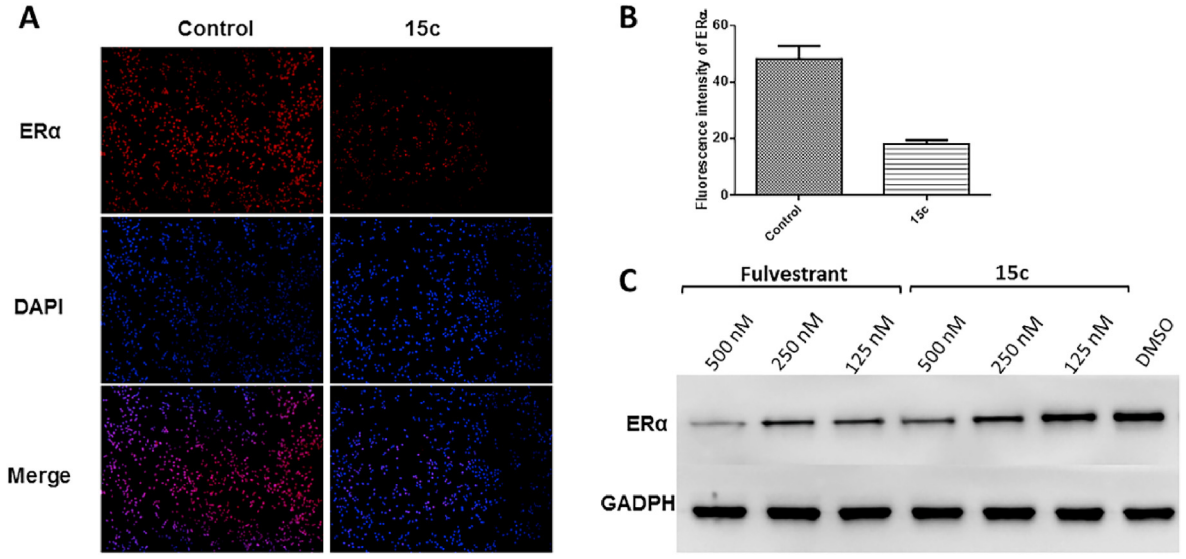


Fig. 3. Effects of **15c** on ERα level. (A) Immunofluorescence microscopy. MCF-7 cells were treated for 24 h with **15c** at 5 μM. (B) fluorescence intensity of ERα. (C) Western blot analysis. MCF-7 cells were incubated with **15c** and Fulvestrant respectively at 125, 250 and 500 nM for 24 h.

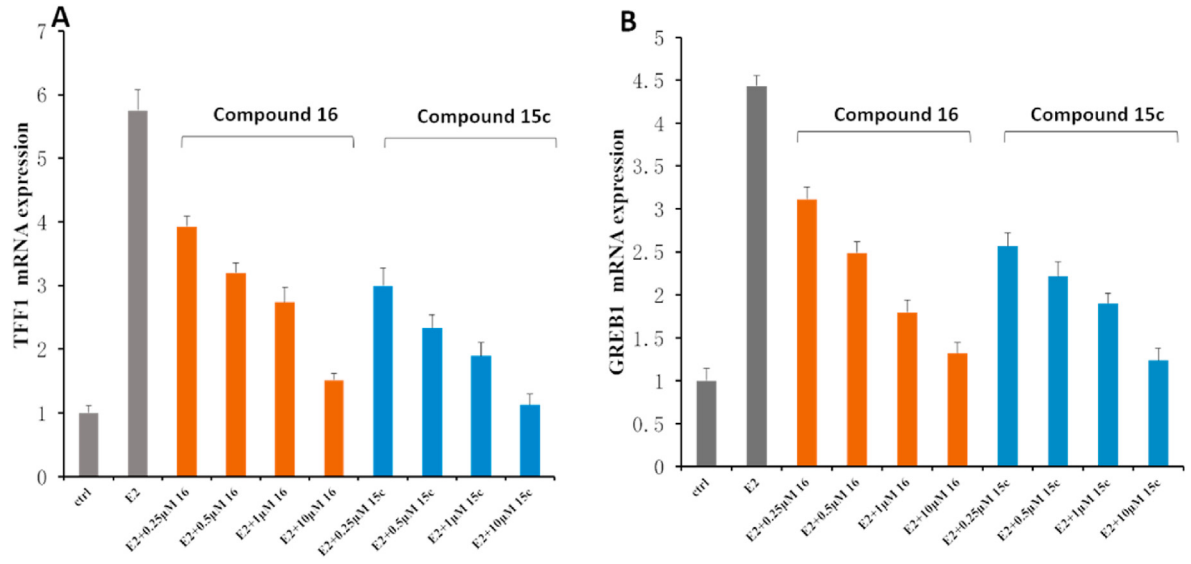


Fig. 4. Dose-response suppression of E2-stimulated model. (A) Expression of *TFF-1* gene when treated with **15c** and **16** respectively at 0.25, 0.5, 1, and 10 μM. (B) Expression of *GREB-1* genes when treated with **15c** and **16** at the indicated concentration. MCF-7 cells were co-incubated with agents for 24 h before determined.

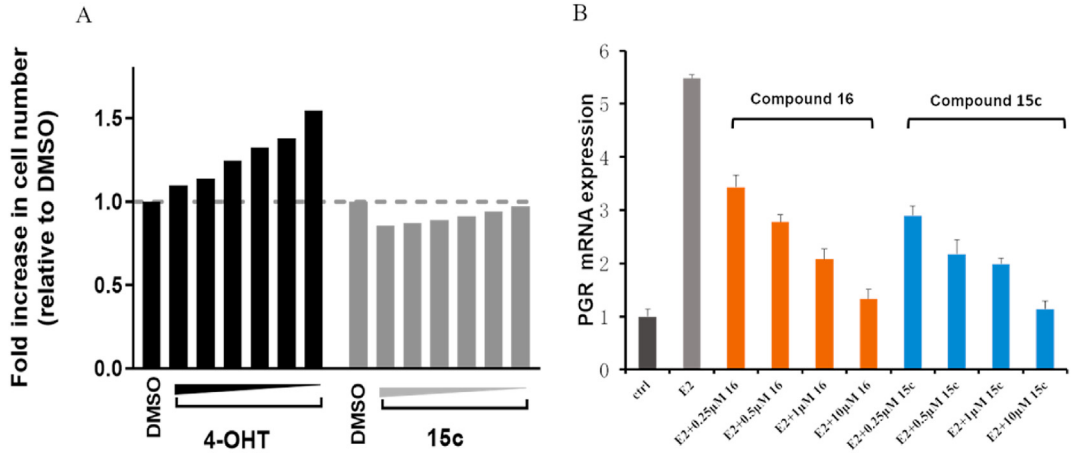


Fig. 5. (A). Proliferation data for Ishikawa cells following two days of treatment respectively with compound **4-OHT** or **15c** at 156, 312, 625, 1250, 2500, and 5000 nM. Data presented as mean fold change relative to DMSO treatments. (B). Dose-response suppression of E2-stimulated expression of *PGR* genes by compound **15c** and **16** at 0.25, 0.5, 1, and 10 μM.

induce a dose-dependent increment in both early- and late-stage apoptosis of MCF-7 cells (2, 11.7, and 25.7%, respectively). (Fig. 7A).

2.10. Early ADME properties assessment of 15c in vitro

We finally set out to assess *in vitro* ADME properties of **15c**, evaluating its pharmacological performance as a candidate drug. As shown in Table 3, the evaluation of **15c** thorough standard microsomal stability and plasma stability assay suggested that the half-life of metabolism was moderate, and it was especially quite stable in rat plasma. Comprehensively considering the two tests, compound **15c** possessed well *in vitro* pharmacodynamic parameters.

3. Conclusion

For the long term of breast cancer endocrine therapy, SERMs and SERDs are standard-of-care representatives. However, both the high recurrence rate and undeniable side effects remained significant concerns. Most recently, evidence demonstrated that SERCAs, through targeting C530 residue of ERα, might inactivate ERα-positive tumors and provide an alternative approach for patients bearing current endocrine resistance.

Our present study identified a series of novel covalent antagonists with preferable binding model to ERα. Most of them acted as suitable ERα ligands and displayed excellent antagonistic activity, among which compound **15c** showed significant potential in ERα antagonistic effect. Moreover, **15c** showed downregulated impacts on the level of cellular ERα.

Mechanically, the loss of potency in compound **16** that relative to **15c**, assisted to reveal the indispensable role of Michael acceptors. Meanwhile, the covalent engagement between C530 residue and **15c** was also clearly observed in docking models. Besides, the reaction model between **15c** and cysteine under basic condition confirmed the binding site of **15c,** simulating the interaction between ER residue.

Our current study extends the understanding and discovery of ERα-targeted covalent antagonists. Further studies focusing on the

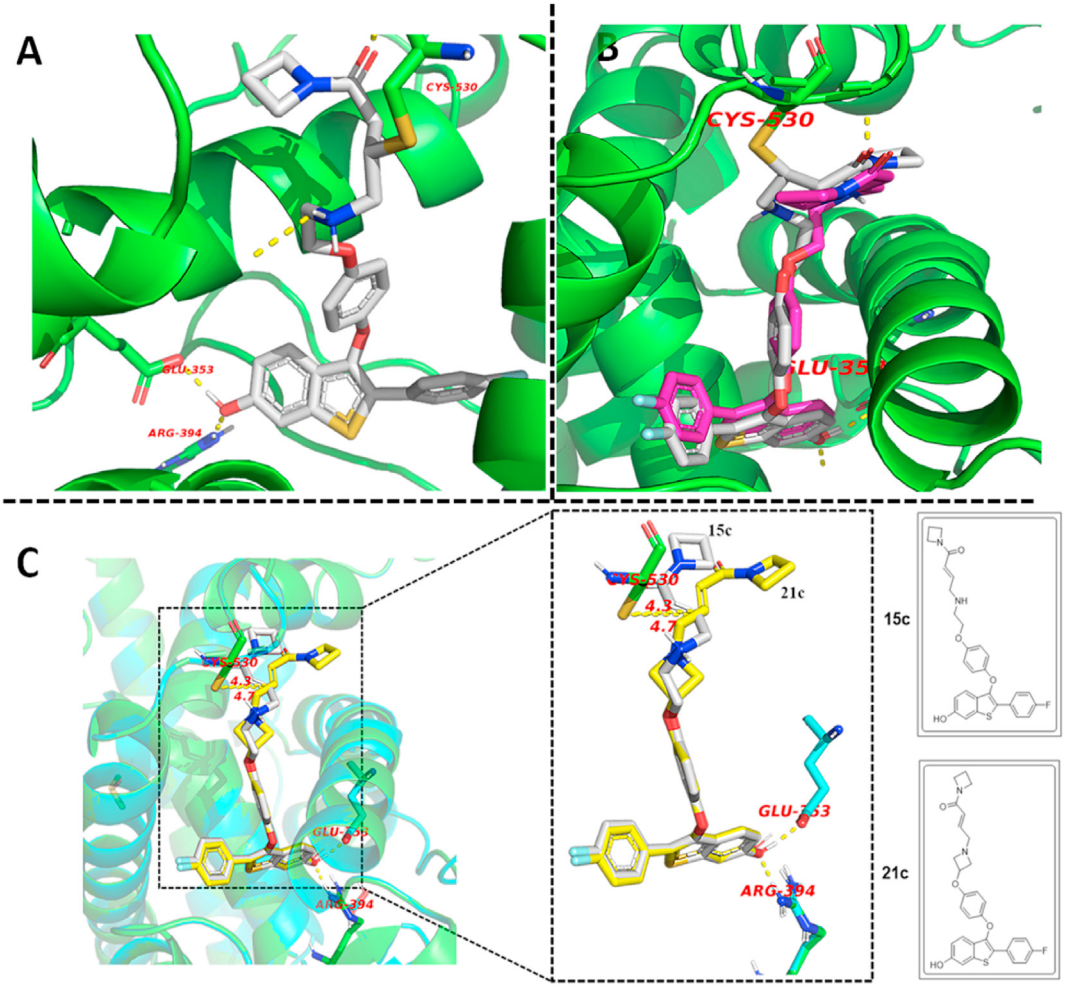


Fig. 6. (A). Compound **15c** was docked to ER LBD (PDB ID: 6chw), demonstrating covalent engagement with C530 of ERα. (B). Comparison of docking details between **15c** and **16**. The red structure stands for compound **16**, and the gray structure stands for **15c**. (C). Docking details between **15c** and **21c**. Distance between Cys530 residue and Michael acceptor was measured, resulting with 4.3 Å and 4.7 Å individually. The yellow structure stands for compound **21c**, and the gray structure stands for compound **15c**.

improvement of *in vivo* activity and direct covalent binding evidence have been accomplished, and additional findings will be reported in due course.

4. Experimental section

4.1. General

All chemicals and solvents were purchased from Energy-chemical, Sigma-Aldrich, and Alladin-chemistry, and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance 300 MHz/400 MHz spectrometer at 300 K, using TMS as an internal standard. NMR chemical shifts are described in δ (ppm) using residual solvent peaks as standard ($CDCl_3$, 7.26 ppm (^{1}H), 77.16 ppm (^{13}C); CD_3OD, 3.31 ppm (^{1}H), 49.00 ppm (^{13}C); DMSO-d_6, 2.50 ppm (^{1}H), 39.52 ppm (^{13}C). MS spectra was recorded on a Shimadzu GC-MS 2050 (ESI) or an Agilent 1946A-MSD (ESI) Mass Spectrum. The TianGuang RY-1 apparatus was used to test melting points (m.p.).

Tert-butyl (2-(4-(benzyloxy)phenoxy)ethyl)carbamate (**1**)

4-(benzyloxy)phenol (500 mg, 2.5 mmol) and *tert*-butyl (2-bromoethyl) carbamate (669 mg, 3 mmol) were dissolved in 10 mL DMF, $C_{S2}CO_3$ (1.63 g, 5 mmol) was added. The solution was stirred at 50 °C for 3 h. After completion, the reaction was quenched with water and resulting mixture was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum to deliver the title compound as white solid (620 mg, 72% yield).

Tert-butyl (2-(4-hydroxyphenoxy)ethyl)carbamate (**2**)

To a solution of compound **1** (1 g, 2.9 mmol) dissolved in 10 mL MeOH, a catalytic amount of Pd/C was added. The solution was stirred at rt under H_2 protected for 2h. After completion, the reaction mixture was filtered, filtrate was concentrated to dryness and purified by column chromatography (SiO_2, CH_2Cl_2: MeOH = 15 : 1–10 : 1) to obtain compound **2** (515 mg, 70% yield) as off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95–6.80 (m, 4H), 4.03 (t, J = 5.1 Hz, 2H), 3.60–3.57 (m, 3H), 1.58 (s, 9H).

Tert-butyl 3-(4-(benzyloxy)phenoxy)azetidine-1-carboxylate (**3**)

4-(benzyloxy)phenol (500 mg, 2.5 mmol) and *tert*-butyl 3-iodoazetidine-1-carboxylate (849 mg, 3 mmol) were dissolved in 10 mL DMF, $C_{S2}CO_3$ (1.63 g, 5 mmol) was added. The solution was stirred at 130 °C for 3 h. After completion, the reaction was quenched with water and resulting mixture was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layer was dried and concentrated under vacuum to deliver the title compound as white solid which was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.29 (m, 5H), 6.94–6.86 (m, 2H),

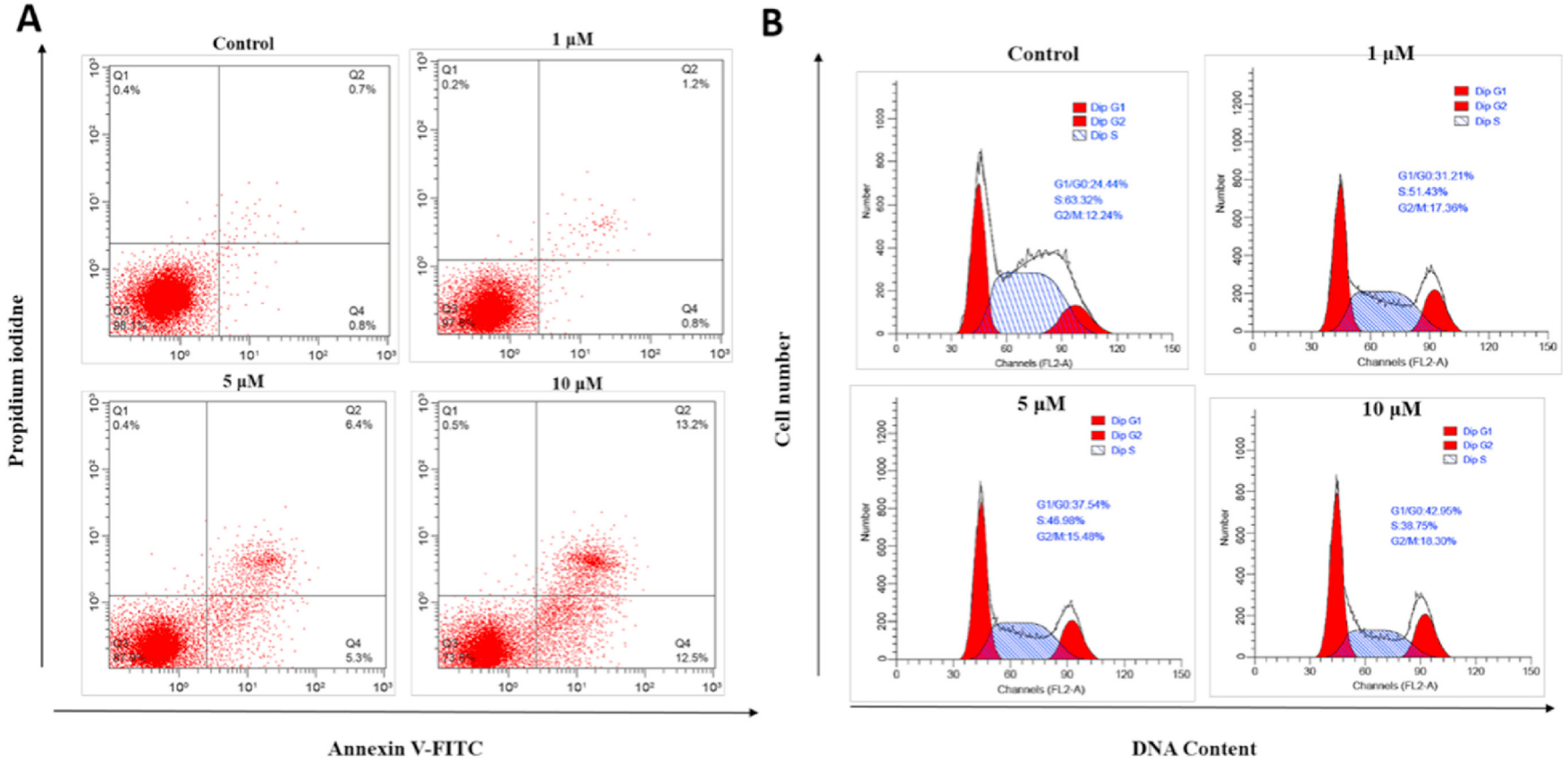


Fig. 7. Compound **15c** induced cell cycle G0/G1 phase arrest and apoptosis in MCF-7 cells. (A) After treated with **15c** for 24 h, MCF-7 cells were harvested and stained with PI, analyzed by flow cytometry. (B) After treated with **15c** at 1, 5, and 10 μM for 48 h, flow cytometry analysis was performed in MCF-7 cells.

Table 3
Rat plasma and liver microsome were used in the two assays, Ketanserin and Benfluorex were used as the control. -: Stands for not performed.

Compd.	LogD	$T_{1/2}$ (min, plasma)	$T_{1/2}$ (min, Liver microsomes)	CL_{int} (mL/min/Kg)
Ketanserin	–	–	18.83	131.91
Benfluorex	–	28.31	–	–
15c	1.67	1017.95	38.15	65.11

6.71–6.63 (m, 2H), 5.01 (s, 2H), 4.81–4.79 (m, 1H), 4.26–4.24 (m, 2H), 3.99–3.96 (m, 2H).

Tert-butyl 3-(4-hydroxyphenoxy)azetidine-1-carboxylate (**4**)

To a solution of compound **3** (1 g, 2.8 mmol) dissolved in 10 mL MeOH, catalytic amount of Pd/C was added. The solution was stirred at rt under H_2 protected for 2h. The reaction mixture was filtered, and filtrate was concentrated to dryness and purified by column chromatography (CH_2Cl_2: MeOH = 15 : 1–10 : 1) to obtain compound **4** (800 mg, 80% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.81–6.73 (m, 2H), 6.62–6.54 (m, 2H), 4.77–4.73 (m, 1H), 4.30–4.16 (m, 2H), 3.97–3.94 (m, 2H).

(E)-4-bromo-N-methylbut-2-enamide (**6a**)

To a solution of (E)-4-bromobut-2-enoic acid (500 mg, 3.0 mmol) dissolved in 10 mL CH_2Cl_2 under N_2 protected at 0 °C, 0.1 mL DMF and oxalyl chloride (571 mg, 4.5 mmol) were added. The solution was stirred at 0 °C for 2 h, followed by addition of Na_2CO_3 (954 mg, 9 mmol) and methylamine hydrochloride (303 mg, 4.5 mmol). Resulting solution was stirred at 0 °C until completion. The mixture was quenched with 10 mL H_2O and then extracted with CH_2Cl_2. Organic layer was washed with 2 × 20 mL brine, then dried and concentrated, purified by column chromatography (SiO_2, CH_2Cl_2: MeOH = 15 : 1–10 : 1) to obtain the title compound as yellow solid (358 mg, 67% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94–6.84 (m, 1H), 6.05–5.99 (m, 1H), 4.02 (d, J = 7.2 Hz, 2H), 2.88 (d, J = 4.9 Hz, 3H).

(E)-4-bromo-N, N-dimethylbut-2-enamide (**6b**)

Compounds **6b** was synthesized with the procedure similar to **6a** (53% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88–6.83 (m, 1H), 6.57–6.53 (m, 1H), 4.19–4.02 (m, 2H), 3.08 (s, 3H), 3.00 (s, 3H).

(E)-1-(azetidin-1-yl)-4-bromobut-2-en-1-one (**6c**)

Compounds **6c** was synthesized with the procedure similar to **6a** (57% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.0–6.82 (m, 1H), 6.22–5.83 (m, 1H), 4.33–4.05 (m, 6H), 2.34–2.29 (m, 3H).

(E)-4-bromo-1-(pyrrolidin-1-yl)but-2-en-1-one (**6d**)

Compounds **6d** was synthesized with the procedure similar to **6a** (73% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01–6.93 (m, 1H), 6.43–6.39 (m, 1H), 4.21–4.19 (m, 2H), 3.58–3.53 (m, 4H), 2.02–1.96 (m, 2H), 1.92–1.88 (m, 2H).

(E)-4-bromo-1-morpholinobut-2-en-1-one (**6e**)

Compounds **6e** was synthesized with the procedure similar to **6a** (59% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.83–6.66 (m, 2H), 4.43–4.22 (m, 2H), 3.60–3.54 (m, 6H), 3.52–3.48 (m, 2H).

(E)-4-bromo-1-(piperidin-1-yl)but-2-en-1-one (**6f**)

Compounds **6f** was synthesized with the procedure similar to **6a** (41% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83–6.79 (m, 1H), 6.58–6.40 (m, 1H), 4.21–4.01 (m, 2H), 3.52–3.48 (m, 4H), 1.59–1.47 (m, 6H).

1-(4-fluorophenyl)-2-((3-methoxyphenyl)thio)ethan-1-one (**7**)

3-Methoxybenzenethiol (1 g, 7.1 mmol) was added in one portion to a freshly prepared solution of 7.5 mL of ethanol, 3 mL of water, and 470 mg of KOH (8.4 mmol). The solution was cooled to 5–10 °C. followed by addition of 2-bromo-1-(4-fluorophenyl) ethanone (1.54 g, 7.1 mmol) solution in 2.5 mL of EtOAc at a rate such that the temperature did not exceed 25 °C. The final mixture was allowed to stir overnight at room temperature. After completion, removed solvent under reduced pressure, and the residue was partitioned between water and ethyl acetate. The aqueous layer was isolated and extracted several times with ethyl acetate, combined extracts were washed with consecutive portions of 10% HCl aqueous solution, water and saturated $NaHCO_3$ aqueous solution, dried over anhydrous Na_2SO_4. The final crude product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99–7.94 (m, 2H), 7.21–7.10 (m, 3H), 6.98–6.90 (m, 2H), 6.76–6.75 (m, 1H), 4.24 (s, 2H), 3.77 (s, 3H).

2-(4-fluorophenyl)-6-methoxybenzo[b]thiophene (**8**)

A 50 mL beaker fitted with a mechanical stirrer was placed in an oil bath with temperature of 85 °C, polyphosphoric acid (50 g) was placed into the beaker. 1-(4-fluorophenyl) 2-(3-methoxyphenylsulfanyl) ethanone (5 g, 18 mmol) was added portion wise at a rate such that the temperature never exceeded 100 °C. After the addition was completed, the reaction mixture was stirred at ~115 °C for 1 h, and followed by slowly poured into the rapidly stirring ice water. The aqueous solution was extracted several times with ethyl acetate, and the combined organic layer was successively washed with water, saturated $NaHCO_3$ aqueous solution, brine, and dried over anhydrous Na_2SO_4. The organic layer was concentrated in vacuo, and the residue was purified by flash chromatography (CH_2Cl_2: MeOH = 30 : 1–25 : 1) to give the desired compound as white solid (3.2 g, 68% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79–7.70 (m, 4H), 7.56 (d, J = 2.3 Hz, 1H), 7.34–7.27 (m, 2H), 7.01–6.97 (m, 1H).

3-bromo-2-(4-fluorophenyl)-6-methoxybenzo[b]thiophene (**9**)

To a solution of 2-(4-fluorophenyl)-6-methoxybenzo [b]thiophene (2.58 g, 10 mmol) dissolved in 30 mL CH_2Cl_2, N-Bromoacetamide (1.46 g, 10.5 mmol) was added dropwise. The mixture was stirred at room temperature for 1 h, the solvent was removed in vacuo. Subsequently, the residue was titrated with ethanol and filtered to give desired compound as a white solid (3.13 g, 92% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79–7.64 (m, 4H), 7.42–7.35 (m, 2H), 7.18–7.14 (m, 1H), 3.85 (s, 3H).

3-bromo-2-(4-fluorophenyl)-6-methoxybenzo[b]thiophene 1-oxide (**10**)

Trifluoacetic acid (10 mL) was added dropwise to a solution of 3-bromo-2-(4-fluorophenyl)-6-methoxybenzo [b]thiophene (2.4 g, 7 mmol) in 10 mL of anhydrous CH_2Cl_2. After the mixture was stirred for 5 min, H_2O_2 (1.0 mL, 7 mmol, 30% aqueous solution) was added dropwise, and the resulting mixture was stirred for 2 h at room temperature. Sodium bisulfite (0.3 g) was added into the solution followed by 5 mL of water. The mixture was stirred vigorously for 15 min and then concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ aqueous solution. Combined organic layer was then dried over anhydrous Na_2SO_4 and concentrated in vacuo to give 2.1 g (84% yield) of the desired compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83–7.79 (m, 3H), 7.63–7.60 (m, 1H), 7.46–7.39 (m, 2H), 7.33–7.31 (m, 1H), 3.90 (s, 3H).

Tert-butyl (2-(4-((2-(4-fluorophenyl)-6-methoxy-1-oxidobenzo [b]thiophen-3-yl)oxy)phenoxy)ethyl)carbamate (**11**)

To a solution of 3-bromo-2-(4-fluorophenyl)-6-methoxybenzo [b]thiophene 1-oxide (2 g, 5.7 mmol) dissolved in 15 mL DMF, $C_{S2}CO_3$ (3.7 g, 11.4 mmol) and *tert*-butyl (2-(4-hydroxyphenoxy) ethyl)carbamate (1.44 g, 5.7 mmol) were added. The solution was

stirred at 50 °C for 3 h and quenched with NH_4Cl aqueous solution, extracted with CH_2Cl_2 and collected organic layer was washed with 10% LiCl aqueous solution. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness to obtain the crude product which was purified by column chromatography (SiO_2, n-hexane: ethyl acetate = 10:1–5:1) to give title compound as white solid (2.49 g, 82% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71–7.66 (m, 2H), 7.45–7.44 (m, 1H), 7.02–6.83 (m, 6H), 6.78–6.67 (m, 2H), 3.92–3.88 (m, 2H), 3.47–3.41 (m, 2H), 1.39 (s, 9H).

Tert-butyl (2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)carbamate (**12**)

To a solution of *tert*-butyl (2-(4-((2-(4-fluorophenyl)-6-methoxy-1-oxidobenzo [b]thiophen-3-yl)oxy)phenoxy)ethyl) carbamate (2 g, 3.81 mmol) dissolved in 10 mL THF, $LiAlH_4$ (289.6 mg, 7.62 mmol) was added at 0 °C under N_2 protected. After 30 min, the reaction was quenched with NH_4Cl aqueous solution and then extracted with CH_2Cl_2, the combined organic layer was collected and concentrated to dryness, purified by column chromatography (SiO_2, n-hexane: ethyl acetate = 10 : 1–5 : 1) to obtain the desired compound (1.7 g, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74–7.66 (m, 2H), 7.26–7.23 (m, 2H), 7.09–6.97 (m, 2H), 6.88–6.86 (m, 3H), 6.80–6.75 (m, 2H), 3.94 (t, J = 5.1 Hz, 2H), 3.86 (s, 3H), 3.52–3.47 (m, 2H), 1.44 (s, 9H).

2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl) oxy)phenoxy)ethan-1-amine (**13**)

Tert-butyl (2-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy)ethyl)carbamate (2 g, 3.9 mmol) was dissolved in 10 mL hydrochloric acid solution (1 M in MeOH). The mixture was stirred at rt for 1 h and then concentrated in vacuo to remove most of solvent, the mixture was poured into 10 mL water and adjust pH to 7–8, then extracted with CH_2Cl_2 three times (30 mL each). The collected organic layer was concentrated to dryness and used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74–7.66 (m, 2H), 7.25 (d, J = 3.0 Hz, 2H), 7.09–7.00 (m, 2H), 6.93–6.83 (m, 3H), 6.82–6.74 (m, 2H), 3.91 (t, J = 5.1 Hz, 2H), 3.04 (t, J = 5.1 Hz, 2H).

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-N-methylbut-2-enamide (**14a**)

2-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl) oxy)phenoxy)ethan-1-amine (500 mg, 1.22 mmol) and DIPEA (1.57 g, 12.2 mmol) were added into 5 mL DMF, (E)-4-bromo-N-methylbut-2-enamide (192 mg, 1.09 mmol) was added. The solution was stirred at rt for18 h and then poured into CH_2Cl_2. The organic layer was washed with 10% LiCl aqueous solution, followed by concentrated to dryness which was purified by column chromatography (SiO_2, CH_2Cl_2: MeOH = 20 : 1–10 : 1) to obtain the desired compound (380 mg, 62% yield) as white solid. ^{1}H NMR (300 MHz, MeOD) δ 8.70–8.78 (m, 2H), 8.04–8.79 (m, 2H), 7.93–7.84 (m, 4H), 7.79 (d, J = 9.1 Hz, 2H), 6.99 (d, J = 15.4 Hz, 1H), 5.02 (t, J = 5.0 Hz, 2H), 4.48–4.43 (m, 2H), 4.01 (t, J = 4.9 Hz, 2H), 3.87 (d, J = 4.8 Hz, 3H).

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-N,N-dimethylbut-2-enamide (**14b**)

Compound **14b** was synthesized using the similar method to **14a** (53% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75–7.66 (m, 2H), 7.28–7.25 (m, 2H), 7.06–7.04 (m, 2H), 6.94–6.83 (m, 4H), 6.81–6.73 (m, 2H), 6.49–6.44 (m, 1H), 4.03–4.00 (m, 2H), 3.86 (s, 3H), 3.51–3.49 (m, 2H), 3.06 (s, 3H), 3.02–2.99 (m, 5H).

(E)-1-(azetidin-1-yl)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)but-2-en-1-one (**14c**)

Compound **14c** was synthesized using the similar method to **14a** (49% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75–7.71 (m, 2H), 7.29 (d, J = 2.1 Hz, 2H), 7.08–7.05 (m, 2H), 6.97–6.80 (m, 6H), 6.14–6.12 (m, 1H), 4.24 (d, J = 8.4 Hz, 2H), 4.11–4.09 (m, 4H), 3.91 (s, 3H), 3.61–3.57 (m, 2H), 3.11 (t, J = 4.9 Hz, 2H), 2.34–2.28 (m, 2H).

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-1-(pyrrolidin-1-yl)but-2-en-1-one (**14d**)

Compound **14d** was synthesized using the similar method to **14a** (41% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75–7.66 (m, 2H), 7.26–7.21 (m, 2H), 7.08–6.99 (m, 2H), 6.98–6.91 (m, 1H), 6.90–6.84 (m, 3H), 6.82–6.75 (m, 2H), 6.31–6.28 (m, 1H), 4.01 (t, J = 5.0 Hz, 2H), 3.86 (s, 3H), 3.54–3.46 (m, 6H), 3.00 (t, J = 5.0 Hz, 2H), 2.01–1.85 (m, 4H).

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-1-morpholinobut-2-en-1-one (**14e**)

Compound **14e** was synthesized using the similar method to **14a** (39% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79–7.70 (m, 2H), 7.30 (d, J = 2.0 Hz, 2H), 7.12–7.04 (m, 2H), 7.03–6.95 (m, 1H), 6.95–6.90 (m, 3H), 6.86–6.79 (m, 2H), 6.49–6.43 (m, 1H), 4.06–4.01 (m, 2H), 3.75–3.69 (m, 6H), 3.62–3.59 (m, 2H), 3.55–3.49 (m, 2H), 3.09–3.02 (m, 2H).

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-1-(piperidin-1-yl)but-2-en-1-one (**14f**)

Compound **14f** was synthesized using the similar method to **14a** (51% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77–7.63 (m, 2H), 7.30–7.19 (m, 2H), 7.05–7.02 (m, 2H), 6.93–6.74 (m, 6H), 6.56–6.43 (m, 1H), 4.11–3.94 (m, 2H), 3.87 (s, 3H), 3.59–3.52 (m, 2H), 1.64–1.51 (m, 8H), 0.87–8.81 (m, 4H).

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-N-methylbut-2-enamide (**15a**)

To a solution of ((E)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-N-methylbut-2-enamide (300 mg, 0.59 mmol) dissolved in 10 mL anhydrous CH_2Cl_2 at 0 °C under N_2 protected, BBr_3 (2.36 mL, 1 M in CH_2Cl_2) was added. The solution was stirred at 0 °C for 1 h and then quenched with MeOH. The mixture was concentrated to dryness and purified by column chromatography (SiO_2, CH_2Cl_2: MeOH = 30 : 1–15 : 1) to obtain the desired compound as white solid in 81% yield, mp:164–166 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.74–7.69 (m, 2H), 7.24–7.14 (m, 2H), 7.11–7.03 (m, 2H), 6.85–6.73 (m, 6H), 6.11–6.05 (m, 1H), 4.00 (t, J = 5.2 Hz, 2H), 3.45–3.41 (m, 2H), 2.95 (t, J = 5.2 Hz, 2H), 2.79 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 168.63, 164.64, 162.19, 157.39, 155.49, 153.11, 142.08, 141.29, 138.55, 130.27, 128.16, 125.98, 125.37, 123.35, 117.33, 116.66, 116.46, 115.76, 108.80, 68.28, 50.65, 26.32. MS ESI m/z 493.3 $[M + H]^+$.

*(E)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-N,N-dimethylbut-2-enamide (**15b**)*

Compound **15b** was prepared from (E)-4-((2-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) ethyl)amino)-N,N-dimethylbut-2-enamide similarly as the procedure for **15a.** Desired compound **15b** was obtained as white solid in 47% yield, mp: 172–174 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.72–7.63 (m, 1H), 7.20–7.12 (m, 2H), 7.07–6.98 (m, 2H), 6.82–6.72 (m, 6H), 6.57–6.54 (m, 1H), 3.98–3.94-3.89 (m, 2H), 3.49–3.43 (m, 2H), 3.06 (s, 3H), 2.95–2.89 (m, 5H). ^{13}C NMR (101 MHz, MeOD) δ 168.52, 164.60, 162.15, 157.41, 155.50, 153.04, 143.72, 142.07, 138.54, 130.26, 130.18, 128.13, 125.35, 123.37, 122.60, 117.33, 116.68, 116.64, 116.46, 115.79, 108.83, 68.40, 50.92, 37.77, 35.99. MS ESI m/z 507.3 $[M + H]^+$.

(E)-1-(azetidin-1-yl)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)but-2-en-1-one (**15c**)

Compound **15c** was prepared from (E)-1-(azetidin-1-yl)-4-((2-

(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy) phenoxy)ethyl)amino)but-2-en-1-one similarly as the procedure for **15a**. Desired compound **15c** was obtained as white solid in 81% yield, mp: 182–184 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.74–7.68 (m, 2H), 7.20–7.11 (m, 2H), 7.11–7.01 (m, 2H), 6.88–6.74 (m, 6H), 6.18–6.11 (m, 1H), 4.29–4.24 (m, 2H), 4.10–3.93 (m, 4H), 3.47–3.45 (m, 2H), 2.95 (t, J = 5.2 Hz, 2H), 2.35–2.24 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ 167.35, 165.03, 161.75, 157.42, 155.50, 153.06, 143.13, 142.09, 138.55, 130.28, 130.17, 128.14, 125.37, 123.38, 120.56, 117.36, 116.73, 116.66, 116.44, 115.80, 108.85, 68.38, 51.51, 50.75, 16.05. MS ESI m/z 519.2 $[M + H]^+$.

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-1-(pyrrolidin-1-yl)but-2-en-1-one (**15d**)

Compound **15d** was prepared from (E)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo [b]thiophen-3-yl)oxy)phenoxy) ethyl)amino)-1-morpholinobut-2-en-1-one similarly as the procedure for **15a**. Desired compound **15d** was obtained as white solid in 66% yield, mp: 168–170 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.72–7.67 (m, 2H), 7.21–7.12 (m, 2H), 7.09–7.02 (m, 2H), 6.90–6.73 (m, 6H), 6.55–6.45 (m, 1H), 4.08 (t, J = 5.1 Hz, 2H), 3.65–3.63 (m, 2H), 3.55 (t, J = 6.6 Hz, 2H), 3.49–3.43 (m, 2H), 3.14 (t, J = 5.1 Hz, 2 H), 2.01–1.82 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 164.44, 163.27, 160.81, 156.07, 153.77, 151.94, 140.66, 138.22, 137.19, 128.91, 128.83, 126.74, 124.84, 124.04, 121.95, 116.03, 115.40, 115.34, 115.12, 114.44, 107.47, 65.61, 46.57, 48.69, 45.79, 25.52, 23.80. MS ESI m/z 533.3 $[M + H]^+$.

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-1-morpholinobut-2-en-1-one (***15e***)

Compound **15e** was prepared from (E)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo [b]thiophen-3-yl)oxy)phenoxy) ethyl)amino)-1-(piperidin-1-yl)but-2-en-1-one (546 mg, 1 mmol) similarly as the procedure for **15a.** Desired compound **15e** was obtained as white solid in 83% yield, mp: 182–184 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.74–7.64 (m, 2H), 7.19–7.14 (m, 2H), 7.09–6.99 (m, 2H), 6.86–6.72 (m, 6H), 6.59–6.52 (m, 1H), 3.99–3.97 (m, 2H), 3.65–3.6 (m, 8H), 3.48–3.44 (m, 2H),2.96–2.91 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 175.54, 167.12, 164.68, 157.43, 155.57, 153.11, 143.49, 142.12, 138.57, 130.30, 130.22, 128.17, 125.37, 123.35, 122.89, 117.35, 116.70, 116.48, 115.77, 108.78, 68.45, 50.98, 47.40, 27.77, 25.47. MS ESI m/z 549.3 $[M + H]^+$.

(E)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)-1-(piperidin-1-yl)but-2-en-1-one (**15f**)

Compound **15f** was prepared from (E)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo [b]thiophen-3-yl)oxy)phenoxy) ethyl)amino)-1-(piperidin-1-yl)but-2-en-1-one (546 mg, 1 mmol) similarly as the procedure for **15a.** Desired compound **15f** was obtained as white solid in 63% yield, mp: 169–173 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.76–7.67 (m, 2H), 7.21–7.02 (m, 4H), 6.87–6.68 (m, 6H), 6.61–6.55 (m, 1H), 4.00 (t, J = 5.2 Hz, 2H), 3.58–3.51 (m, 4H), 3.47–3.44 (m, 2H), 2.94 (t, J = 5.2 Hz, 2H), 1.68–1.62 (m, 2H), 1.58–1.50 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 167.32, 164.64, 157.41, 155.53, 153.08, 144.51, 142.08, 138.55, 130.28, 130.22, 128.14, 125.37, 123.35, 122.01, 117.35, 116.70, 116.48, 115.77, 108.78, 68.45, 67.69, 50.98, 47.40, 43.62 MS ESI m/z 547.2 $[M + H]^+$.

1-(azetidin-1-yl)-4-((2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)amino)butan-1-one(**16**)

To a solution of **15c** (100 mg, 0.19 mmol) dissolved in 5 mL MeOH, catalytic amount of 10% Pd/C was added. The solution was stirred at rt under hydrogen protected. After completion, resulting solution was filtered to remove Pd/C, and filtrate was concentrated to dryness and purified by chromatography (SiO_2, CH_2Cl_2: MeOH = 20 : 1–25 : 1) to obtain the desired compound (90 mg, 92% yield) as a white solid, mp: 171–174 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.75–7.66 (m, 2H), 7.18–7.13 (m, 2H), 7.10–7.07 (m, 2H), 6.85–6.82 (m, 4H), 6.78–7.74 (m, 1H), 4.24–4.15 (m, 2H), 4.04–3.91 (m, 4H), 2.92 (t, J = 5.2 Hz, 2H), 2.65 (t, J = 7.3 Hz, 2H), 2.26–2.21 (m, 2H), 2.17–2.13 (m, 2H), 1.79–1.75 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 175.02, 164.63, 162.18, 158.53, 155.61, 153.13, 142.19, 138.64, 130.24, 130.16, 127.70, 123.26, 117.34, 116.67, 116.45, 116.24, 109.02, 68.40, 51.55, 49.81, 30.77, 29.55, 25.51, 15.80. MS ESI m/z 543.3 $[M + Na]^+$.

Tert-butyl 3-(4-((2-(4-fluorophenyl)-6-methoxy-1-oxidobenzo [b]thiophen-3-yl)oxy)phenoxy)azetidine-1-carboxylate (**17**)

To a solution of 3-bromo-2-(4-fluorophenyl)-6-methoxybenzo [b]thiophene 1-oxide **10** (2 g, 5.7 mmol) dissolved in 15 mL DMF, $C_{S2}CO_3$ (3.7 g, 11.4 mmol) and *tert*-butyl 3-(4-hydroxyphenoxy) azetidine-1-carboxylate **4** (1.51 g, 5.7 mmol) were added. The solution was stirred at 50 °C for 3 h and quenched with NH_4Cl aqueous solution. The mixture was extracted with CH_2Cl_2, and collected organic layer was washed with 10% LiCl aqueous solution, dried and concentrated to dryness to obtain the crude product which was purified by column chromatography (SiO_2, n-hexane: ethyl acetate = 5 : 1–3 : 1) to give title compound as white solid (2.1 g, 68% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75–7.65 (m, 2H), 7.12–6.84 (m, 6H), 6.62 (d, J = 9 Hz, 2H), 4.82–4.75 (m, 1H), 4.30–4.21 (m, 2H), 3.98–3.94 (m, 5H), 1.44 (s, 9H).

Tert-butyl 3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidine-1-carboxylate (**18**)

To a solution of *tert*-butyl 3-(4-((2-(4-fluorophenyl)-6-methoxy-1-oxidobenzo [b]thiophen-3-yl)oxy)phenoxy)azetidine-1-carboxylate (2 g, 3.72 mmol) dissolved in 10 mL THF, $LiAlH_4$ (289.6 mg, 7.62 mmol) was added at 0 °C under N_2 protected. After 30 min, the reaction was quenched with NH_4Cl aqueous solution and then extracted with CH_2Cl_2. The combined organic layer was collected and concentrated to dryness, purified by column chromatography (SiO_2, n-hexane: ethyl acetate = 5 : 1–3 : 1) to obtain the desired compound (1.1 g, 57% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74–7.65 (m, 2H), 7.26–7.21 (m, 2H), 7.04 (t, J = 8.7 Hz, 2H), 6.88–6.82 (m, 3H), 6.67–6.56 (m, 2H), 4.77–4.74 (m, 1H), 4.26–4.23 (m, 2H), 3.99–3.95 (m, 2H), 3.86 (s, 3H), 1.44 (s, 9H).

3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl) oxy)phenoxy)azetidine (**19**)

Tert-butyl 3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy)azetidine-1-carboxylate (2 g, 3.84 mmol) was dissolved in 10 mL hydrochloric acid solution (1 M in MeOH). The mixture was stirred at rt for 1 h and then concentrated in vacuo to remove most of solvent, the mixture was poured into 10 mL water and adjust pH to 7–8, then extracted with CH_2Cl_2 three times (30 mL each). The collected organic layer was concentrated to dryness and used in the next step without further purification.

(E)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-N-methylbut-2-enamide (**20a**)

3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl) oxy)phenoxy)azetidine (513 mg, 1.22 mmol) and DIPEA (1.57g, 12.2 mmol) were added into 5 mL DMF, (E)-4-bromo-N-methylbut-2-enamide (192 mg, 1.09 mmol) was added. The solution was stirred at rt for 18 h and then partitioned between water and ethyl acetate. The organic layer was washed with 10% LiCl aqueous solution, followed by concentrated to dryness to obtain the crude product which was purified by column chromatography (SiO_2, CH_2Cl_2: MeOH = 20 : 1–10 : 1). Desired compound was obtained as white solid in 53% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69–7.62 (m, 2H), 7.24–7.18 (m, 2H), 7.05–6.95 (m, 2H), 6.86–6.79 (m, 3H), 6.65–6.57 (m, 2H), 6.07–5.85 (m, 2H), 4.66–4.61 (m, 1H), 3.83 (s, 3H), 3.80–3.73 (m, 2H), 3.23–3.19 (m, 2H), 3.13–3.04 (m, 2H), 2.82–2.81 (m, 3H).

(E)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-N,N-dimethylbut-2-enamide (**20b**)

Compound **20b** was prepared using the method similar to the preparation of **20a.** Desired compound **20b** was obtained as white solid in 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71–7.63 (m, 2H), 7.23–7.21 (m, 2H), 7.05–6.97 (m, 2H), 6.89–6.81 (m, 3H), 6.74–6.58 (m, 1H), 6.67–6.58 (m, 2H), 6.37–6.24 (m, 1H), 4.70 (m, 1H), 3.84 (s, 3H), 3.83–3.77 (m, 2H), 3.28–3.23 (m, 2H), 3.15–3.08 (m, 2H), 3.05 (s, 3H), 2.98 (s, 3H).

(E)-1-(azetidin-1-yl)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)but-2-en-1-one (**20c**)

Compound **20c** was synthesized using the method similar to **20a** (51% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73–7.63 (m, 2H), 7.24–7.21 (m, 2H), 7.06–6.97 (m, 2H), 6.90–6.72 (m, 4H), 6.66–6.57 (m, 2H), 5.96–5.91 (m, 1H), 4.69–4.61 (m, 1H), 4.20–4.18 (m, 2H), 4.07–4.02 (m, 2H), 3.85 (s, 3H), 3.80–3.71 (m, 2H), 3.28–3.26 (m, 2H), 3.15–3.05 (m, 2H), 2.27–2.25 (m, 2H).

(E)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-1-(pyrrolidin-1-yl)but-2-en-1-one (**20d**)

Compound **20d** was synthesized using the method similar to **20a** (44% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.62 (m, 2H), 7.23–7.21 (m, 2H), 7.02–7.69 (m, 2H), 6.89–6.73 (m, 4H), 6.66–6.58 (m, 2H), 6.23–6.21 (m, 1H), 4.70–4.64 (m, 1H), 3.85 (s, 5H), 3.50–3.47 (m, 4H), 3.37–3.25 (m, 2H), 3.12–3.09 (m, 2H), 1.93–1.89 (m, 2H), 1.84–1.79 (m, 2H).

(E)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-1-morpholinobut-2-en-1-one (**20e**)

Compound **20e** was synthesized from 3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) azetidine using the method similar to **20a** (47% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79–7.68 (m, 2H), 7.29–7.25 (m, 2H), 7.12–7.03 (m, 2H), 6.95–6.78 (m, 4H), 6.74–6.64 (m, 2H), 6.40–6.37 (m, 1H), 4.75–4.71 (m, 1H), 3.91–3.85 (m, 3H), 3.86–3.83 (m, 2H), 3.72 (s, 6H), 3.60 (s, 2H), 3.35–3.29 (m, 2H), 3.18–3.16 (m, 2H).

(E)-4-(3-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-N-methylbut-2-enamide (**21a**)

To a solution of (E)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-N-methylbut-2-enamide (518 mg, 1 mmol) dissolved in 10 mL anhydrous CH_2Cl_2 at 0 °C under N_2 protected, BBr_3 (4 mL, 1 M in CH_2Cl_2) was added. The solution was stirred at 0 °C for 1h and then quenched with MeOH. The mixture was concentrated to dryness and purified by column chromatography (SiO_2, CH_2Cl_2: MeOH = 30 : 1–15 : 1) to obtain the desired compound as white solid in 83% yield, mp: >200 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.71–7.67 (m, 2H), 7.20–7.03 (m, 4H), 6.86–6.69 (m, 5H), 6.67–6.58 (m, 1H), 6.16–6.11 (m, 1H), 4.82–4.81 (m, 1H), 4.05–4.00 (m, 2H), 3.56–3.47 (m, 4H), 2.78 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 167.92, 164.53, 162.08, 157.27, 153.46, 153.10, 141.79, 138.45, 136.53, 130.20, 128.43, 127.99, 125.44, 123.20, 117.48, 116.95, 116.65, 116.43, 115.76, 108.80, 67.82, 61.84, 59.05, 26.42. MS ESI m/z 505.2 $[M + H]^+$.

*(E)-4-(3-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-N,N-dimethylbut-2-enamide (**21b**)*

Compound **21b** was prepared from (E)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) azetidin-1-yl)-N,N-dimethylbut-2-enamide (532 mg, 1 mmol) similarly as the procedure for **21a**. Desired compound **21a** was obtained as white solid in 77% yield, mp: 171–173 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.70–7.62 (m, 2H), 7.20–7.11 (m, 2H), 7.06–7.00 (m, 2H), 6.82–6.74 (m, 3H), 6.69–6.56 (m, 3H), 6.52–6.47 (m, 1H), 4.71–4.69 (m, 1H), 3.81–3.71 (m, 2H), 3.30–3.29 (m, 2H), 3.21–3.16 (m, 2H), 3.08 (s, 3H), 2.96 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 168.36, 157.44, 153.63, 153.40, 140.99, 138.57, 130.30, 130.22, 128.12, 125.47, 123.30, 123.81, 117.50, 116.85, 116.72, 116.50, 115.79, 108.79, 68.24, 62.12, 60.75, 37.82, 35.99, 30.76. MS ESI m/z 519.3 $[M + H]^+$.

(E)-1-(azetidin-1-yl)-4-(3-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)but-2-en-1-one (**21c**)

Compound **21c** was prepared from (E)-1-(azetidin-1-yl)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy) phenoxy)azetidin-1-yl)but-2-en-1-one (544 mg, 1 mmol) similarly as the procedure for **21a.** Desired compound **21c** was obtained as white solid in 79% yield, mp: 174–176 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.75–7.64 (m, 2H), 7.24–7.11 (m, 2H), 7.11–7.01 (m, 2H), 6.88–6.77 (m, 3H), 6.75–6.56 (m, 3H), 6.08–6.06 (m, 1H), 4.87–4.75 (m, 1H), 4.31–4.28 (m, 1H), 4.07–4.04 (m, 1H), 3.87–3.82 (m, 2H), 3.48–3.44 (m, 1H), 3.41–3.33 (m, 4H), 3.29–3.28 (m, 1H), 2.38–1.99 (m, 2H). MS ESI m/z 531.2 $[M + H]^+$.

(E)-4-(3-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-1-(pyrrolidin-1-yl)but-2-en-1-one (**21d**)

Compound **21d** was prepared from (E)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) azetidin-1-yl)-1-(pyrrolidin-1-yl)but-2-en-1-one (558 mg, 1 mmol) similarly as the procedure for **21a**. Desired compound **21d** was obtained as white solid in 69% yield, mp: 168–170 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.72–7.63 (m, 2H), 7.20–7.10 (m, 2H), 7.06–7.00 (m, 2H), 6.85–6.72 (m, 3H), 6.69–6.60 (m, 3H), 6.36–6.30 (m, 1H), 4.69–4.67 (m, 1H), 3.77–3.72 (m, 2H), 3.53 (t, J = 6.7 Hz, 2H), 3.44 (t, J = 6.8 Hz, 2H), 3.31–3.29 (m, 2H), 3.20–3.13 (m, 2H), 1.99–1.83 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.16, 160.26, 156.28, 152.09, 151.20, 140.37, 139.74, 136.58, 128.93, 125.89, 123.52, 123.24, 122.06, 116.34, 116.24, 116.02, 115.71, 115.22, 108.09, 66.63, 60.79, 59.55, 46.01, 45.48, 25.66, 23.86. MS ESI m/z 545.3 $[M+H]^+$.

(E)-4-(3-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-1-morpholinobut-2-en-1-one(**21e**)

Compound **21e** was prepared from (E)-4-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) azetidin-1-yl)-1-morpholinobut-2-en-1-one (574 mg, 1 mmol) similarly as the procedure for **21a.** Desired compound **21e** was obtained as white solid in 69% yield, mp: 145–150 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.71–7.67 (m, 2H), 7.19–7.13 (m, 2H),7.08–7.02 (m, 2H), 6.83–6.75 (m, 3H), 6.70–6.62 (m, 3H), 6.53–6.47 (m, 1H), 4.73–4.70 (m, 1H), 3.79–3.74 (m, 2H), 3.65–3.61 (m, 4H), 3.61–3.58 (m, 4H), 3.35–3.30 (m, 2H), 3.23–3.14 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 167.10, 164.63, 157.41, 153.59, 153.31, 141.95, 138.54, 130.27, 130.19, 128.09, 125.46, 123.33, 123.03, 117.49, 116.80, 116.71, 116.50, 115.82, 108.84, 68.22, 62.10, 60.84, 47.43, 43.62. MS ESI m/z 561.3 $[M + H]^+$.

N-(2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)but-3-enamide (**22a**)

To a solution of **13** (409 mg, 1 mmol) dissolved in 10 mL CH_2Cl_2, DIPEA (154 mg, 1.2 mmol) and acryloyl chloride (108 mg, 1.2 mmol) were added at 0 °C under N_2 protected. The solution was stirred at 0 °C for 2 h then quenched with 10 mL water. The mixture was extracted CH_2Cl_2 (10 mL x 2), combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness, used in the next step without further purification (83% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74–7.65 (m, 2H), 7.25 (d, J = 2.6 Hz, 2H), 7.11–6.99 (m, 2H), 6.88–6.82 (m, 3H), 6.82–6.74 (m, 2H), 6.37–6.30 (m, 1H), 6.17–6.06 (m, 1H), 5.68–5.64 (m, 1H), 4.14–4.97 (m, 2H), 3.87 (s, 3H), 3.74–3.72 (m, 2H).

N-(2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)methacrylamide (**22b**)

Compound **22b** was prepared using the method similar to the synthesis of **22a**.^{1}H NMR (300 MHz, MeOD) δ 7.74–7.65 (m, 2H), 7.22–7.00 (m, 4H), 6.88–6.71 (m, 5H), 5.67–5.63 (m, 1H), 5.35–5.31 (m, 1H), 3.99–3.91 (m, 2H), 3.57–3.52 (m, 2H), 1.91 (d, J = 1.3 Hz, 3H).

(E)-N-(2-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)but-2-enamide (**22c**)

Compound **22c** was prepared using the method similar to the synthesis of **22a**.^{1}H NMR (300 MHz, MeOD) δ 7.73–7.61 (m, 2H), 7.24–7.10 (m, 2H), 7.02–7.68 (m, 2H), 6.85–6.71 (m, 6H), 5.94–5.89 (m, 1H), 3.93–3.89 (m, 2H), 3.54–3.49 (m, 2H), 1.82–1.79 (m, 3H).

N-(2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)acrylamide (**23a**)

To a solution of **22a** (463 mg, 1 mmol) dissolved in 10 mL anhydrous CH_2Cl_2 at 0 °C under N_2 protected, BBr_3 (4 mL, 1 M in CH_2Cl_2) was added. The solution was stirred at 0 °C for 1h and then quenched with MeOH. The resulting mixture was concentrated to dryness and purified by column chromatography (SiO_2, CH_2Cl_2: MeOH = 30:1–15 : 1) to obtain the desired compound as white solid (320 mg, 69% yield), mp: 171–173 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.71–7.64 (m, 2H), 7.18–6.98 (m, 4H), 6.79–6.75 (m, 5H), 6.28–6.17 (m, 2H), 5.65–5.61 (m, 1H), 3.95 (t, J = 5.4 Hz, 2H), 3.57 (t, J = 5.4 Hz, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.31, 164.57, 162.12, 157.28, 155.38, 153.05, 142.03, 138.52, 131.78, 130.23, 128.15, 126.92, 125.37, 123.35, 117.30, 116.63, 116.41, 115.71, 108.79, 67.85, 40.16. MS ESI m/z 472.1 $[M + Na]^+$.

N-(2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)methacrylamide (**23b**)

Compound **23b** was prepared from 1-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) azetidin-1-yl)-2-methylprop-2-en-1-one (489 mg, 1 mmol) similarly as the procedure for **23a.** Desired compound **23b** was obtained as white solid in 64% yield, mp: 174–176 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.73–7.65 (m, 2H), 7.21–7.02 (m, 4H), 6.83–6.76 (m, 5H), 5.68–5.67 (m, 1H), 5.36–5.33 (m, 1H), 3.99 (t, J = 5.7 Hz, 2H), 3.57 (t, J = 5.7 Hz, 2H), 1.91 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 170.11, 163.28, 160.83, 156.00, 154.19, 151.17, 140.71, 139.77, 137.17, 128.90, 126.79, 124.00, 121.98, 119.26, 115.93, 115.34, 115.08, 114.36, 107.38, 66.42, 38.98, 17.37. MS ESI m/z 486.2 $[M + Na]^+$.

*(E)-N-(2-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)ethyl)but-2-enamide(**23c**)*

Compound **23c** was prepared from (E)-1-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) azetidin-1-yl)but-2-en-1-one (489 mg, 1 mmol) similarly as the procedure for **23a**. Desired compound **23c** was obtained as white solid in 61% yield, mp: 157–159 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.73–7.62 (m, 2H), 7.23–7.09 (m, 2H), 7.05–7.00 (m, 2H), 6.84–6.72 (m, 6H), 5.97–5.90 (m, 1H), 3.93 (t, J = 5.4 Hz, 2H), 3.54 (t, J = 5.4 Hz, 2H), 1.83–1.80 (m, 3H). ^{13}C NMR (101 MHz, MeOD) δ 168.76, 164.49, 162.03, 157.21, 155.31, 152.97, 141.98, 141.96, 138.48, 130.18, 128.13, 125.80, 125.36, 123.35, 117.27, 116.59, 116.57, 115.69, 108.81, 67.90, 40.02, 17.84. MS ESI m/z 486.2 $[M + Na]^+$.

1-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)prop-2-en-1-one (**24a**)

To a solution of **19** (421 mg, 1 mmol) dissolved in 10 mL CH_2Cl_2, DIPEA (154 mg, 1.2 mmol) and acryloyl chloride (108 mg, 1.2 mmol) were added at 0 °C under N_2 protected. The solution was stirred at 0 °C for 2h then quenched with 10 mL water. Resulting mixture was extracted with CH_2Cl_2 (10 mL X 2), combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness, used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78–7.70 (m, 2H), 7.29 (d, J = 2.6 Hz, 2H), 7.15–7.03 (m, 2H), 6.92–6.89 (m, 3H), 6.86–6.77 (m, 2H), 6.35–6.31 (m, 1H), 6.14–6.12 (m, 1H), 5.70–5.64 (m, 1H), 4.14–3.97 (m, 2H), 3.91 (s, 3H), 3.76–3.74 (m, 2H).

1-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)-2-methylprop-2-en-1-one *(**24b**)*

Compound **24b** was obtained with the similar method to the synthesis of **24a** (41% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77–7.70 (m, 2H), 7.30–7.27 (m, 2H), 7.13–7.04 (m, 2H), 6.93–6.89 (m, 3H), 6.71–6.63 (m, 2H), 5.41–5.39 (m, 2H), 4.89–4.85 (m, 1H), 4.50–4.45 (m, 2H), 4.33–4.08 (m, 2H), 3.91 (s, 3H), 1.97 (t, J = 1.3 Hz, 3H).

(E)-1-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)but-2-en-1-one *(**24c**)*

Compound **24c** was obtained with the similar method to the synthesis of **24a** (54% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77–7.70 (m, 2H), 7.29 (d, J = 2.9 Hz, 2H), 7.12–7.05 (m, 2H), 6.98–6.87 (m, 4H), 6.71–6.64 (m, 2H), 5.90–5.85 (m, 1H), 4.90–4.85 (m, 1H), 4.62–4.37 (m, 2H), 4.29–4.05 (m, 2H), 3.91 (s, 3H), 1.90–1.87 (m, 3H).

1-(3-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl) oxy)phenoxy)azetidin-1-yl)prop-2-en-1-one (**25a**)

To a solution of 1-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)prop-2-en-1-one (500 mg, 1.07 mmol) dissolved in 10 mL anhydrous CH_2Cl_2 at 0 °C under N_2 protected, BBr_3 (4.28 mL, 1 M in CH_2Cl_2) was added. The solution was stirred at 0 °C for 1h and then quenched with MeOH. The mixture was concentrated to dryness and purified by column chromatography (SiO_2, CH_2Cl_2: MeOH = 30 : 1–15 : 1) to obtain the desired compound as white solid in 78% yield, mp: 168–172 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.78–7.66 (m, 2H), 7.23–7.02 (m, 4H), 6.91–6.69 (m, 5H), 6.40–6.19 (m, 2H), 5.73–5.69 (m, 1H), 4.97–4.92 (m, 1H), 4.69–4.63 (m, 1H), 4.40 (d, J = 1.6 Hz, 1H), 4.27–4.18 (m, 1H), 4.04–3.93 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.70, 162.71, 160.26, 156.27, 151.65, 140.29, 136.59, 128.94, 126.92, 126.78, 125.84, 123.57, 122.05, 116.45, 116.23, 116.01, 115.89, 115.23, 108.10, 65.79, 56.90, 54.61. MS ESI m/z 486.2 $[M + Na]^+$.

1-(3-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl) oxy)phenoxy)azetidin-1-yl)-2 methylprop-2-en-1-one(**25b)**

Compound **25b** was prepared from 1-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) azetidin-1-yl)-2-methylprop-2-en-1-one (489 mg, 1 mmol) similarly as the procedure for **25a.** Desired compound **25b** was obtained as white solid in 72% yield, mp: >200 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.72–7.71 (m, 2H), 7.21–7.04 (m, 4H), 6.89–6.70 (m, 5H), 5.48–5.35 (m, 2H), 4.96–4.95 (m, 1H), 4.63–4.61 (m, 1H), 4.39–4.37 (m, 1H), 4,25–4.23 (m, 1H), 3.96–3.94 (m, 1H), 1.90 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.00, 156.29, 151.69, 151.50, 140.30, 137.78, 136.59, 128.95, 128.87, 125.84, 123.56, 122.05, 119.89, 116.46, 116.25, 116.03, 115.85, 115.25, 108.10, 66.16, 59.44, 19.15. MS ESI m/z 498.2 $[M + Na]^+$.

*(E)-1-(3-(4-((2-(4-fluorophenyl)-6-hydroxybenzo[b]thiophen-3-yl)oxy)phenoxy)azetidin-1-yl)but-2-en-1-one(**25c**)*

Compound **25c** was prepared from (E)-1-(3-(4-((2-(4-fluorophenyl)-6-methoxybenzo [b]thiophen-3-yl)oxy)phenoxy) azetidin-1-yl)but-2-en-1-one similarly as the procedure for **25a**. Desired compound **25c** was obtained as white solid in 78% yield, mp: 174–178 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.74–7.67 (m, 2H), 7.21–7.02 (m, 4H), 6.90–6.70 (m, 6H), 6.07–5.97 (m, 1H), 5.00–4.92 (m, 1H), 4.65–4.56 (m, 1H), 4.40–4.37 (m, 1H), 4.20–4.18 (m, 1H), 3.97–3.92 (m, 1H), 1.89–1.86 (m, 3H). ^{13}C NMR (101 MHz, MeOD) δ 166.82, 162.93, 156.32, 152.27, 151.84, 141.61, 140.55, 137.21, 128.91, 128.83, 126.59, 121.87, 119.64, 116.21, 115.48, 115.34, 115.12, 114.53, 107.46, 65.64, 58.67, 54.75, 29.37, 16.69. MS ESI m/z 498.2 $[M + Na]^+$.

4.2. Biological evaluation

4.2.1. Cell culture

MCF-7 and Ishikawa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, KeyGEN Biotech), containing 10% fetal bovine serum (FBS, GIBCO), 100U/ml Penicillin and 100 mg/mL Streptomycin, at 37 °C under 5% CO_2 condition in humidified atmosphere. MDA-MB-231 cells were maintained in Leibovitz's L-15 media supplemented with 10% FBS.

4.2.2. Cytotoxicity assay

Cytotoxicity assay of analogs on tumor cells was determined by 2-(2-Methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazoliumsodiumsalt (CCK-8) assay. MCF-7 (4000 cells per well) were seeded into 96-well plates in 100 μL of 10% FBS containing DMEM media and incubated for 24 h. DMSO stocks synthesized analogs were diluted in culture media (2% FBS containing DMEM) to obtain a series of concentrations (100, 50, 25, 12.5, 6.25 and 3.125 μM). The final concentration of DMSO in the treatment medium was 0.1% for all wells. After 48 h of treatment, CCK-8 solution was added to each well (0.5 mg/mL) and incubated at 37 °C for 2 h. The optical density was determined at the wavelength of 450 nm. For each time point, we have performed triplicate data, and mean cell viability was calculated. IC_{50} values were calculated using GraphPad Prism 8 Software.

4.2.3. Real-time polymerase chain reaction (RT-PCR)

Real-time polymerase chain reaction (RT-PCR) RNA samples were reverse transcribed to cDNA and the PCR reactions were performed using TaKaRa SYBR Green Master Mix carried out in StepOnePlusTM Real-Time PCR instrument. The program for amplification was 1 cycle of 95 °C for 10 min followed by 40 cycles of 95 °C for 5 s, 60 °C for 60 s, and 95 °C for 15 s. The PCR results were normalized to GAPDH expression and were quantified by the ΔΔCT method.

4.2.4. Molecular docking

The crystal structure of $ER\alpha^{Y537S}$ in complex with antagonist H3B-5942 (PDB ID: 6CHW) was downloaded from Protein Data Bank (http://www.pdb.org). The molecular modeling was accomplished with Schrodinger 10.2/Glide docking protocol. Compounds were drawn with ChemBioDraw 14.0 and visually presented binding model is typical sample of the highest scored conformations.

4.2.5. Western blotting assay

MCF-7 cells were cultured in growth medium (DMEM/F12 phenol red-free media (Gibco) supplemented with 10% serum 72 h prior to plating cells. MCF-7 cells treated with indicated compounds were lysed in RIPA Lysis Buffer (Solarbio). After determination of protein concentration by BCA assay (Beyotime), equal amounts of total protein were electrophoresed through 10% SDS-polyacrylamide gels. The separated protein bands were transferred onto PVDF membranes and blotted against different antibodies as indicated. The human estrogen receptor α antibody (ab32063) and horseradish peroxidase-conjugated anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (ab181602) were purchased from Abcam, Inc., Cambridge, MA. The relative mean intensity of target proteins was expressed after normalization to the intensity of GAPDH bands from individual repeats.

4.2.6. Immunofluorescence experiment

MCF-7 cells were grown on glass slides, and then treated with a concentration of 5 μM indicated drug for 24 h. Then the culture solution was washed away with PBS, and the cells were fixed with pre-cooled acetone. Further treatment was performed according to the standard immunofluorescence procedures. The nucleus stained by DAPI is blue under ultraviolet excitation, and the positive expression is red light labeled with fluorescein.

4.2.7. ERα binding affinity assay

The recombinant ERα, fluorescent estradiol ligand and tested compounds (10 mM, dissolved in DMSO) were diluted to the set concentrations according to the protocol of ER Alpha Competitor Assay Kit, Green (A15882) using ES2 Screening Buffer. The concentrations of these three samples were 150 nM, 9.0 nM and 2 μM, respectively. The diluted recombinant ERα (5 μl), tested compounds (10 μl) and fluorescent estradiol (5 μl) were added to 394-well plate (Corning, NO.4514) orderly and the mixture system was reacted for 2 h at room temperature in darkness. The estradiol (1 μM) was set as a positive control group and the same volume buffer was set as negative control group. After 2 h, the polarization values were read by microplate reader (SpectraMax Paradigm) and were utilized to calculated inhibition ratio of the tested compounds.

4.2.8. Cellular apoptosis study

Apoptosis of MCF-7 cells was detected using a flow cytometric assay. Briefly, cells were seeded in 6-well plates and incubated overnight. The following day, cells were treated with different concentrations of **15c** for 48h. The cells and supernatants were harvested and washed twice with cold PBS and then resuspended in 100 μL1 × binding buffer. 5 μL FITC Annexin V and 5 μL PI were added into each tube and the cells were then gently vortexed and incubated for 15 min at 25 °C in the dark. 400 μL of 1 × binding buffer was then added to each tube. The stained cells were analyzed using a flow cytometer.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Project Program of Jiangsu Key Laboratory of Drug Design and Optimization at China Pharmaceutical University, and the Program of "Double-top-class" University Project (CPU2018PZQ02; CPU2018GY07).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113543.

References

[1] N. Bentzon, M. Düring, B.B. Rasmussen, H. Mouridsen, N. Kroman, Prognostic effect of estrogen receptor status across age in primary breast cancer, Int. J. Canc. 122 (5) (2008) 1089–1094, https://doi.org/10.1002/ijc.22892.
[2] D.V. Spicer, M.C. Pike, Breast cancer prevention through modulation of endogenous hormones[J], Breast Canc. Res. Treat. 28 (2) (1993) 179–193, https://doi.org/10.1007/BF00666430.
[3] E. Ferrazzi, G. Cartei, P. De Besi, et al., Tamoxifen in disseminated breast cancer [J], Tumori Journal 63 (5) (1977) 463–468, https://doi.org/10.1177/030089167706300507.
[4] K.I. Pritchard, J. Rolski, Z. Papai, et al., Results of a phase II study comparing three dosing regimens of fulvestrant in postmenopausal women with advanced breast cancer (FINDER2)[J], Breast Canc. Res. Treat. 123 (2) (2010) 453–461, https://doi.org/10.1007/s10549-010-1022-9.
[5] G.S. Tria, T. Abrams, J. Baird, et al., Discovery of LSZ102, a potent, orally bioavailable selective estrogen receptor degrader (SERD) for the treatment of estrogen receptor positive breast cancer[J], J. Med. Chem. 61 (7) (2018) 2837–2864, https://doi.org/10.1021/acs.jmedchem.7b01682.

[6] F. Garner, M. Shomali, D. Paquin, et al., RAD1901: a novel, orally bioavailable selective estrogen receptor degrader that demonstrates antitumor activity in breast cancer xenograft models[J], Anti Canc. Drugs 26 (9) (2015) 948, https://doi.org/10.1097/CAD.0000000000000271.
[7] C. De Savi, R.H. Bradbury, A.A. Rabow, et al., Optimization of a novel binding motif to (E)-3-(3, 5-difluoro-4-((1 R, 3 R)-2-(2-fluoro-2-methylpropyl)-3-methyl-2, 3, 4, 9-tetrahydro-1 H-pyrido [3, 4-b] indol-1-yl) phenyl) acrylic Acid (AZD9496), a potent and orally bioavailable selective estrogen receptor downregulator and antagonist[J], J. Med. Chem. 58 (20) (2015) 8128–8140, https://doi.org/10.1021/acs.jmedchem.5b00984.
[8] A. Lai, M. Kahraman, S. Govek, et al., Identification of GDC-0810 (ARN-810), an orally bioavailable selective estrogen receptor degrader (SERD) that demonstrates robust activity in tamoxifen-resistant breast cancer xenografts[J], J. Med. Chem. 58 (12) (2015) 4888–4904, https://doi.org/10.1021/acs.jmedchem.5b00054.
[9] Early Breast Cancer Trialists' Collaborative Group, Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials[J], Lancet 378 (9793) (2011) 771–784, https://doi.org/10.1016/S0140-6736(11)60993-8.
[10] J.F.R. Robertson, Current role of endocrine therapy in the management of breast cancer[J], Breast Cancer 9 (4) (2002) 276–281, https://doi.org/10.1007/BF02967604.
[11] C.K. Osborne, V. Bardou, T.A. Hopp, et al., Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer[J], J. Natl. Cancer Inst. 95 (5) (2003) 353–361, https://doi.org/10.1093/jnci/95.5.35.
[12] G. Arpino, L. Wiechmann, C.K. Osborne, et al., Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance[J], Endocr. Rev. 29 (2) (2008) 217–233, https://doi.org/10.1210/er.2006-0045.
[13] S. Li, D. Shen, J. Shao, et al., Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts[J], Cell Rep. 4 (6) (2013) 1116–1130, https://doi.org/10.1016/j.celrep.2013.08.022.
[14] C.V. Segal, M. Dowsett, Estrogen receptor mutations in breast cancer-new focus on an old target[J], Clin. Canc. Res. 20 (7) (2014) 1724–1726, https://doi.org/10.1158/1078-0432.CCR-14-0067.
[15] S.W. Fanning, C.G. Mayne, V. Dharmarajan, et al., Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation[J], elife 5 (2016), e12792, https://doi.org/10.7554/eLife.12792.
[16] K.W. Nettles, J.B. Bruning, G. Gil, et al., NFκB selectivity of estrogen receptor ligands revealed by comparative crystallographic analyses[J], Nat. Chem. Biol. 4 (4) (2008) 241, https://doi.org/10.1038/nchembio.76.
[17] J.A. Katzenellenbogen, The 2010 philip S. Portoghese medicinal chemistry lectureship: addressing the “core issue” in the design of estrogen receptor ligands: award address[J], J. Med. Chem. 54 (15) (2011) 5271–5282, https://doi.org/10.1021/jm200801h.
[18] K.W. Harlow, D.N. Smith, J.A. Katzenellenbogen, et al., Identification of cysteine 530 as the covalent attachment site of an affinity-labeling estrogen (ketononestrol aziridine) and antiestrogen (tamoxifen aziridine) in the human estrogen receptor[J], J. Biol. Chem. 264 (29) (1989) 17476–17485, https://doi.org/10.1111/j.1471-4159.1991.tb06431.x.
[19] J.C. Reese, B.S. Katzenellenbogen, Mutagenesis of cysteines in the hormone binding domain of the human estrogen receptor. Alterations in binding and transcriptional activation by covalently and reversibly attaching ligands[J], J. Biol. Chem. 266 (17) (1991) 10880–10887, https://doi.org/10.1016/S0021-9258(18)99101-5.
[20] J.C. Reese, C.H. Wooge, B.S.J.M.E. Katzenellenbogen, Identification of two cysteines closely positioned in the ligand-binding pocket of the human estrogen receptor: roles in ligand binding and transcriptional activation 6 (1992) 2160–2166, https://doi.org/10.1210/mend.6.12.1491695.
[21] A.Y. Helena, S.K. Tian, A.E. Drilon, et al., Acquired resistance of EGFR-mutant lung cancer to a T790M-specific EGFR inhibitor: emergence of a third mutation (C797S) in the EGFR tyrosine kinase domain[J], JAMA oncology 1 (7) (2015) 982–984, https://doi.org/10.1001/jamaoncol.2015.1066.
[22] X. Puyang, C. Furman, G.Z. Zheng, et al., Discovery of selective estrogen receptor covalent antagonists for the treatment of ERαWT and ERαMUT breast cancer[J], Canc. Discov. 8 (9) (2018) 1176–1193, https://doi.org/10.1158/2159-8290.
[23] C. Furman, M.H. Hao, S. Prajapati, et al., Estrogen receptor covalent antagonists: the best is yet to come[J], Canc. Res. 79 (8) (2019) 1740–1745, 10.1158x`0008-5472.
[24] N. Rioux, S. Smith, M. Korpal, et al., Nonclinical pharmacokinetics and in vitro metabolism of H3B-6545, a novel selective ERα covalent antagonist (SERCA) [J], Canc. Chemother. Pharmacol. 83 (1) (2019) 151–160, https://doi.org/10.1007/s00280-018-3716-3.
[25] C.R. Overk, K.W. Peng, R.T. Asghodom, et al., Structure–activity relationships for a family of benzothiophene selective estrogen receptor modulators including raloxifene and arzoxifene[J], ChemMedChem: Chemistry Enabling Drug Discovery 2 (10) (2007) 1520–1526, https://doi.org/10.1002/cmdc.200700104.
[26] L.R. Jackson, K.L. Cheung, A.U. Buzdar, et al., Arzoxifene: the evidence for its development in the management of breast cancer[J], Core Evid. 2 (4) (2007) 251, https://doi.org/10.2147/ce.s7434.
[27] P. Y Maximov, M. Lee T, V. Craig Jordan, The discovery and development of selective estrogen receptor modulators (SERMs) for clinical practice[J], Curr. Clin. Pharmacol. 8 (2) (2013) 135–155, https://doi.org/10.2174/1574884711308020006.
[28] K.J. Andreano, S.E. Wardell, J.G. Baker, et al., G1T48, an oral selective estrogen receptor degrader, and the CDK4/6 inhibitor lerociclib inhibit tumor growth in animal models of endocrine-resistant breast cancer[J], Breast Canc. Res. Treat. 180 (3) (2020) 635–646, https://doi.org/10.1007/s10549-020-05575-9.
[29] H. Liu, J. Liu, R.B. Van Breemen, et al., Bioactivation of the selective estrogen receptor modulator desmethylated arzoxifene to quinoids: 4 ‘-Fluoro substitution prevents quinoid formation[J], Chem. Res. Toxicol. 18 (2) (2005) 162–173, https://doi.org/10.1021/tx049776u.
[30] C.D. Jones, M.G. Jevnikar, A.J. Pike, et al., Antiestrogens. 2. Structure-activity studies in a series of 3-aroyl-2-arylbenzo [b] thiophene derivatives leading to [6-hydroxy-2-(4-hydroxyphenyl) benzo [b] thien-3-yl]-[4-[2-(1-piperidinyl) ethoxy] phenyl] methanone hydrochloride (LY 156758), a remarkably effective estrogen antagonist with only minimal intrinsic estrogenicity[J], J. Med. Chem. 27 (8) (1984) 1057–1066, https://doi.org/10.1002/chin.198503198.
[31] Y. Anzai, C.F. Holinka, H. Kuramoto, et al., Stimulatory effects of 4-hydroxytamoxifen on proliferation of human endometrial adenocarcinoma cells (Ishikawa line)[J], Canc. Res. 49 (9) (1989) 2362–2365, https://doi.org/10.1016/0304-3835(89)90148-1.