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Rational design and optimization of selenophenes with basic side chains as novel potent selective estrogen receptor modulators (SERMs) for breast cancer therapy†

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To increase the diversity of estrogen receptor (ER) ligands having novel structures and activities, series of selenophene derivatives with a basic side chain (BSC) were synthesized and their biological activity as subtype-selective antagonists for the ER was explored. Compared with the selenophenes without a BSC, most compounds showed an increase in binding affinity, and several compounds displayed enhanced antagonist potency and antiproliferative activity. Especially, compound **16c** exhibited excellent transcriptional activity for ER α (IC₅₀ = 13 nM) which made this compound the most potent antagonist for ER α of the whole series and is 66-fold better than the best selenophene compound without a BSC. Moreover, several compounds showed values of IC₅₀ better than that of 4-hydroxytamoxifen in breast cancer MCF-7 cells. The modeling study indicated that the basic side chain might contribute to their increased antagonist potency and antiproliferative activity. These new ligands have the potential to be further developed as novel agents to improve therapeutics that target the estrogen receptor.

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1. Introduction

Selenium (Se) is an important nutritional trace element involved in different physiological functions and possesses anti-oxidative, antitumor and chemopreventive properties. Dietary Se has been inversely associated with the risk of cancer, and substantial evidence shows that selenium has a significant influence on the incidence of many cancers.¹ Epidemiological studies reveal that a low Se status may contribute to the etiology of different diseases, for example, viral infections, reproductive deficiencies, loss of immunocompetence, thyroid and cardiovascular diseases, and pancreatitis.² Selenium is present in more than twenty five human selenoproteins, and most of them have been involved in anti-oxidant defence systems and cancer prevention, *etc.*^{3,4} The anti-carcinogenic potential of Se has also been reported in geographical studies during the past

40 years.⁵ A variety of selenium-containing compounds with diverse chemical structures are known to inhibit cell proliferation *in vitro*, such as inorganic selenium salts,^{6–12} selenoamino acids,⁵ methylselenocyanate,^{12,13} as well as benzyl and phenyl selenium derivatives.¹⁴ In particular, many diarylselenides possess anticancer, antitumor, antiviral, antimicrobial, and antioxidant properties.^{15–17} Various biologically active selenaheterocycles such as ebselen have been discovered in recent years.¹⁸ Furthermore, anticancer mechanisms of MSeA have been hypothesized to involve estrogen receptor (ER) stress signal mediators and apoptosis.^{19,20}

As has been known, estrogens are important regulators of many physiological functions related to the reproductive and non reproductive tissues in both women and men.^{21,22} The effects of estrogens are mediated *via* two estrogen receptor subtypes, ER α and ER β ,²³ which are ligand-regulated transcription factors that regulate many physiological and pathological processes. The estrogen receptors have different tissue distributions and significant differences in their ligand binding preferences.^{24,25} While estrogens are necessarily beneficial in some tissues, including the reproductive,²⁶ skeletal,²⁷ cardiovascular,²⁸ and central nervous systems,²⁹ inappropriate or over-expression of ER is associated with a number of endocrine disorders. For example, the estrogen receptors play a predominant role in breast cancer growth because of the pro-proliferative effect.³⁰ Thus, developing ER subtype-selective ligands with tissue- and

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gene-selective biological activities is a critical clinical objective. In order to discover “ideal” selective estrogen receptor modulators (SERMs), extensive investigation has been made to increase the chemical diversity of these compounds, especially the non-steroidal ones. Apart from the preserved peripheral substituents, *e.g.* phenols, simple alkyl groups, and polar phenyl substituents, a wide variety of heterocyclic cores have been explored,³¹ from the five-membered ring heterocycles to six-membered ring heterocycles as well as fused heterocycles. Some examples of the five-membered ring heterocycles are presented below (Fig. 1), including furan (1),³¹ imidazole (2),^{32,33} propyl pyrazole triol (3),^{34–36} thiophene (4),³⁷ selenophene (5),³⁸ raloxifene (RAL), *etc.* Because the activity profiles of the current SERMs, *e.g.* tamoxifen, are not ideal and resistance to their effectiveness as anti-tumor agents can develop with time (Fig. 1), there has been interest in finding new SERMs that might prove more effective as hormonal or therapeutic agents.

As part of our long-term interest in the development of ligands for the ERs having novel structures and activities,^{39,40} we have recently described a new series of analogues based on the selenophene scaffold that showed good binding affinity to ERs. The 2,5-bis(2-fluoro-4-hydroxyphenyl) selenophene **8c** showed the highest relative binding affinity (RBA, estradiol = 100) of 24.3 for ER β . In transcription assays, most of the selenophenes exhibited partial to full agonist activity for both ER subtypes, but several compounds displayed a range of ER α or ER β antagonistic activities.³⁸ A few selenophenes exhibited antiproliferative activity comparable to that of 4OHT in breast cancer MCF-7 cells. Interestingly, from the modeling study of the complex of ER α -**8c**, it is observed that one of the phenolic groups of **8c** interacts with helix 11 through H-bonds and does not further destabilize helix 12; rather, it mimics the role of the D-ring phenol of E₂ and stabilizes helix 12 in the agonist conformation.

Therapeutic targeting of the estrogen receptor has traditionally involved direct disruption of the surface coactivator binding sites by a basic side chain (BSC) that is a characteristic structural feature of SERMs, such as tamoxifen and raloxifene (Fig. 1). The nature and spatial orientation of the basic

Fig. 1 Examples of the five-membered ring heterocycles (1–5) as ER ligands and structures of known SERMs raloxifene and 4-hydroxytamoxifen.

side chains in SERMs can influence their tissue selectivity and affect the balance of desired and undesired activities.⁴¹ Thus, we wondered whether the introduction of basic side chains into a selenophene-based core structure (*e.g.*, a pyrrolidine or piperidine side chain) might provide ER ligands with interesting antagonistic activities (Fig. 2). Herein, we introduced two different types of aminoethoxy moieties into the selenophene core system, placing these at different positions of the phenolic groups. We expected that these selenophene-core derivatives could act as models with improved biological activity for the development of novel estrogen receptor ligands. It was proved that several compounds (*e.g.* **16c**) showed values of IC₅₀ better than that of 4-hydroxytamoxifen in breast cancer MCF-7 cells.

2. Results and discussion

2.1. Chemical synthesis

Three series of novel selenophene derivatives, as depicted in Scheme 1, were prepared from selenophenes according to the synthetic procedures established in our laboratory (described in the Experimental section).

In the synthesis of compounds **9a–g** (Scheme 1A), key intermediates **7a–g** were obtained using the Suzuki cross-coupling reaction of aryl boronic acids with 2,5-disubstituted selenophene **6a**.^{38,42} Then, compounds **7a–g** were treated with boron tribromide affording the corresponding 2,5-disubstituted diphenolic selenophenes **8a–g**. Finally, the target products **9a–g** were obtained from corresponding phenols and chloroethyl pyrrolidine or piperidine by Williamson ether synthesis under microwave conditions. This step produced a mixture of mono-alkylated products **9a–g** (yield 60–70%) and was accompanied by about 30–40% of dialkylated by-products (Scheme 1A).

Fig. 2 Rationale for design of novel potent selenophenes with a BSC for breast cancer therapy.

Scheme 1 Synthesis of substituted selenophene derivatives. Reagents and conditions: (a) [Pd] catalyst, Na₂CO₃, toluene/water (1:1), reflux, 24 h; (b) BBr₃, CH₂Cl₂, -20 °C to rt, 4 h; (c) *N*-(2-chloroethyl)pyrrolidine or *N*-(2-chloroethyl)piperidine, KOH, K₂CO₃, DMF, microwave, 120 °C, 45 min; (d) pyridine hydrochloride, 190 °C, 3 h.

In the synthesis of 3,4-disubstituted selenophene derivatives 12a–i (Scheme 1B), 10a–i served as the key intermediates, which were prepared by treating 3,4-dibromoselenophene 6b³⁸ with aryl boronic acids by using Pd(OAc)₂/PPh₃ as the catalyst. Subsequent ether cleavage of 10a–i by pyridine hydrochloride yielded the intermediates 11a–i. Finally, treatment of intermediates 11a–i with chloroethyl pyrrolidine or piperidine afforded the desired products 12a–i in good yields.

In our previous work on furan or thiophene derived ER ligands,³⁷ triphenol furans and thiophenes were proved to be more effective than the corresponding bisphenol analogues with higher binding affinity and subtype selectivity. Particularly, a few triaryl selenophenes exhibited substantial anti-proliferative activity in breast cancer MCF-7 cells.³⁸ Thus, we wanted to introduce a basic side chain into the triphenol selenophene scaffold, as such selenophenes 16a–c were obtained by demethylation of 15a–c, which were prepared through Suzuki cross-coupling reactions followed by installa-

tion of a BSC (Scheme 1C). In the first step, 1 equiv. of 2,3,5-tribromoselenophene 6c³⁸ was reacted with 4 equiv. of aryl boronic acid under standard conditions, and the resulting 2,5-bis-substituted selenophenes 13 were subsequently submitted to a cross-coupling reaction with 2 equiv. of phenyl boronic acid to yield the intermediates 14a–c. Installation of the two types of basic side chains in the free phenolic positions in 14a–c was effected by a Williamson reaction, and the remaining methyl group was then selectively cleaved with boron tribromide, leaving the basic side chain unaffected. By this approach, we prepared the analogues containing the two types of basic side chains at the *para* position of the C-3 phenyl group, 16a–16c.

2.2. Relative binding affinities

The binding affinities of the selenophene compounds for both ER α and ER β were determined by a competitive fluorometric receptor-binding assay and are summarized in Table 1. These affinities are presented as relative binding affinity (RBA) values, where estradiol (E₂) has an affinity of 100%.

As a global observation, it is noteworthy that the position of basic side chains in the phenyl ring of selenophene derivatives has very significant effects on the binding affinity of conjugates. In general, most of the compounds in series I (except 9c and 9f) that possess the basic side chain in the 2-phenol moiety exhibit better binding affinity for ER α . In contrast, the vast majority of the members of series II (apart from 12b and 12c) with the basic side chain located in the 3-phenol moiety display moderate levels of ER β selectivity. The compound that shows the highest binding affinity for ER β and moderate ER subtype selectivity of all of the ligands is 9f, which possesses a basic side chain on the 2-phenol unit. The RBA values of this compound are 4.59 and 23.6 for ER α and ER β , respectively; and it has an ER α /ER β selectivity as low as 0.19 (Table 1, entry 6). Compared to the parent compound 2,5-bis(2-chloro-4-hydroxyphenyl)-selenophene³⁸ (RBA values were 6.11 for ER α and 12.7 for ER β ; α/β was 0.48), 9f still retained high binding affinity for ER β and displayed higher selectivity. The compound that has the highest ER α /ER β selectivity is 12h (ER α /ER β ratio < 0.1), which also shows a high RBA value for ER β (23.3). As we expected, the halogens Cl and F bind better than the other ligands, with Cl being better than F in most cases (9e, 9f, and 12g–i).

Compared to the diphenolic selenophene compounds previously reported by our group, the introduction of the basic side chains can alter the ER subtype selectivity through changing the substitution position. Moreover, introduction of a substituent in the phenol rings has significant effects on the binding affinity and selectivity. Among the compounds of series I, the nonsubstituted compound 9a showed a good binding affinity (12.4 and 1.02 for ER α and ER β , respectively). When the substitution occurred at the *meta* position of the phenol ring (9b–9g), moderate affinity, ranging from 0.1 to 23.6, was observed. Interestingly, most of the compounds in series I show better

Table 1 Relative binding affinity (RBA) of compounds **9a–g**, **12a–i**, **16a–c** for ER α and ER β ^a

Entry	Compound	Core	R	<i>n</i>	ER α ^b	ER β ^b	α/β
1	9a		H	1	12.4 ± 0.42	1.02 ± 0.08	12.2
2	9b		2-Me	1	1.96 ± 0.11	0.58 ± 0.07	3.37
3	9c		2-Me	2	<0.1	0.62 ± 0.08	<0.1
4	9d		2-F	1	0.76 ± 0.01	<0.1	>10
5	9e		2-F	2	6.89 ± 1.02	0.63 ± 0.09	10.9
6	9f		2-Cl	1	4.59 ± 0.35	23.6 ± 0.49	0.19
7	9g		2-Cl	2	<0.1	<0.1	—
8	12a		H	1	2.06 ± 0.09	5.92 ± 0.28	0.35
9	12b		H	2	7.58 ± 0.11	2.35 ± 0.04	3.3
10	12c		2-Me	1	1.65 ± 0.03	0.46 ± 0.08	3.6
11	12d		2-Me	2	0.56 ± 0.06	10.2 ± 0.17	0.05
12	12e		3-Me	1	2.03 ± 0.23	5.92 ± 0.14	0.34
13	12f		3-Me	2	<0.1	<0.1	—
14	12g		3-F	2	4.82 ± 0.32	15.3 ± 0.29	0.32
15	12h		2-Cl	1	<0.1	23.3 ± 0.06	<0.1
16	12i		2-Cl	2	5.38 ± 0.08	18.4 ± 0.31	0.29
17	16a		H	1	3.14 ± 0.31	16.7 ± 0.10	0.18
18	16d		H	2	4.59 ± 0.35	<0.1	>10
19	16c		2-Me	1	13.8 ± 0.27	3.4 ± 0.29	4.06

^a To simplify comparisons of the compounds in related series, we designated locant positions of the substituents on the phenyl groups with respect to the selenophene core; locant positions on the selenophene core itself are given by numbers in italics. ^b Relative Binding Affinity (RBA) values are determined by competitive fluorometric binding assays and are expressed as $IC_{50}^{estradiol}/IC_{50}^{compound} \times 100 \pm \text{the range}$ (RBA, estradiol = 100%). In these assays, the K_d value of estradiol is 3.1 nM for ER α and 3.4 nM for ER β , respectively. For details, see the Experimental section.

selectivity for ER α , and when basic side chains were introduced at the C-3 position of the selenophene ring (series II), most of the compounds show good selectivity for ER β (Table 1, entries 8–16). For the disubstituted selenophene derivatives, when the substitution occurred at 3- and 4-positions (**12a–12i**), lower binding affinities were observed. A similar trend was also observed for furan and thiophene derivatives.³⁸ Nevertheless, it should be pointed out that these compounds also show a moderate affinity for ER β . Moreover, the binding affinities and selectivities for ER β dramatically changed when the substitution was moved from the C-2 position to the C-3 position (**12c–12f**, entries 10–13). For comparison, trisubstituted selenophene analogues with a basic side chain were also prepared (series III). With the exception of **16b** with undetectable ER β binding affinity of less than 0.1, all showed good binding affinity for both ER α and ER β (entries 17–19). The desired compound showing the highest binding affinity for ER α was **16c** (13.8 for ER α), which also displayed a moderate ER subtype selectivity.

Overall, we found that the compounds synthesized with *N*-(2-chloroethyl)pyrrolidine might display a better binding affinity for ER than those obtained with *N*-(2-chloroethyl)piperidine.

Compared with our previous work, it was interesting to find that most of the biaryl substituted selenophene compounds with basic side chains exhibited an equivalent or better binding affinity for ER than the corresponding parent selenophenes except **9b** (Table 2).

2.3. Transcription activation assays

The effects of these selenophene derivatives on ER transcriptional activity were determined using an ER responsive luciferase reporter gene. HEK 293 cells were seeded in 24-cell plates at a concentration of 2×10^6 cells per plates and allowed to settle for 24 hours. Then the cells were transfected with a widely used $3 \times ERE$ -luciferase reporter and an ER α or ER β expression plasmid for agonist activity (% efficacy) and potency (EC_{50}) determinations. These cells were stimulated with increasing concentrations of 17 β -estradiol (E_2) or these compounds. For antagonist mode assays (% efficacy and IC_{50}), cells were stimulated with a combination of estradiol (10 nM) and an increasing concentration of the various selenophene compounds. The luciferase activity was measured the next day. These results are summarized in Table 3, and dose-response curves for representative samples are shown in Fig. 3.

The interesting activities are seen in compounds of series I, 2,5-disubstituted selenophenes **9a–g**. These ligands displayed a wide range of activities at both ER α and ER β , most of which are potent and highly efficacious ER α agonists and ER β antagonists, respectively. Compound **9a** with a pyrrolidinyl side chain was a weak agonist of ER α and antagonist of ER β with a nanomolar to micromolar IC_{50} range; however, addition of a methyl group to **9a** converted it from being an agonist to an antagonist of ER α and a strong

Table 2 A direct comparison of RBA values to ER α and ER β for the various biaryl selenophene compounds with a BSC in this work and the corresponding ones without a BSC studied previously³⁸

Entries: 1-4

Entries: 5-8

This work			Biaryl selenophenes (without BSC)			
Entry	R	Compound	RBA		RBA	
			ER α	ER β	ER α	ER β
1	H	9a	12.4 $\pm$ 0.42	1.02 $\pm$ 0.08	0.61 $\pm$ 0.034	2.87 $\pm$ 0.20
2	2-Me	9b	1.96 $\pm$ 0.11	0.58 $\pm$ 0.07	5.60 $\pm$ 0.43	11.1 $\pm$ 0.73
3	2-F	9c	6.89 $\pm$ 1.02	0.63 $\pm$ 0.09	5.90 $\pm$ 0.90	24.3 $\pm$ 0.52
4	2-Cl	9f	4.59 $\pm$ 0.35	23.6 $\pm$ 0.49	6.11 $\pm$ 0.05	12.7 $\pm$ 3.66
5	H	12a	2.06 $\pm$ 0.09	5.92 $\pm$ 0.28	0.32 $\pm$ 0.04	2.01 $\pm$ 0.11
6	2-Me	12d	0.56 $\pm$ 0.06	10.02 $\pm$ 0.17	0.27 $\pm$ 0.06	6.70 $\pm$ 0.49
7	3-Me	12e	2.03 $\pm$ 0.23	5.92 $\pm$ 0.14	0.71 $\pm$ 0.09	0.62 $\pm$ 0.12
8	3-F	12g	4.32 $\pm$ 0.32	15.3 $\pm$ 0.29	0.58 $\pm$ 0.14	1.56 $\pm$ 0.23

Table 3 Effects of selenophene-core compounds on the transcriptional activities of estrogen receptors α and β and antiproliferative activity in MCF-7 cells^a

Agonist mode ^b								Antagonist mode ^{c,d,e}				MCF-7
Entry	Cmpd	R	n	ER α		ER β		ER α		ER β		IC ₅₀ ^e (μ M)
				EC ₅₀ (μ M)	Eff (% E ₂)	EC ₅₀ (μ M)	Eff (% E ₂)	IC ₅₀ (μ M)	Eff ^c (% E ₂)	IC ₅₀ (μ M)	Eff (% E ₂)	
1	9a	H	1	0.219	15.3 $\pm$ 4.8	—	—	1.213	71.5 $\pm$ 16.7	1.266	83.9 $\pm$ 7.7	5.77 $\pm$ 0.06
2	9b	2-Me	1	—	—	0.24	72.3 $\pm$ 12.5	0.874	78.3 $\pm$ 27.8	0.146	4.6 $\pm$ 15	53.8 $\pm$ 4.24
3	9c	2-Me	2	2.369	11.2 $\pm$ 17.2	—	—	0.041	52.3 $\pm$ 7.7	1.235	78.3 $\pm$ 29.4	25.3 $\pm$ 0.55
4	9d	2-F	1	0.136	86.9 $\pm$ 26.6	—	—	—	—	1.120	92.1 $\pm$ 10.9	>100
5	9e	2-F	2	0.204	68.4 $\pm$ 13.5	—	—	—	—	1.945	75.4 $\pm$ 4.2	>100
6	9f	2-Cl	1	—	—	—	—	0.415	69.7 $\pm$ 21.9	0.299	49.4 $\pm$ 11.1	7.81 $\pm$ 0.13
7	9g	2-Cl	2	0.911	19.9 $\pm$ 5.0	—	—	—	—	2.96	24.9 $\pm$ 14.4	>100
8	12a	H	1	1.003	45.9 $\pm$ 6.3	—	—	—	—	0.102	46.7 $\pm$ 12.3	>100
9	12b	H	2	—	—	1.147	53.5 $\pm$ 11	1.589	89.2 $\pm$ 8.3	0.451	22.8 $\pm$ 8.9	8.13 $\pm$ 0.46
10	12c	2-Me	1	—	—	—	—	0.179	21.4 $\pm$ 1.7	0.043	66.7 $\pm$ 3.6	16.6 $\pm$ 1.34
11	12d	2-Me	2	0.158	23.6 $\pm$ 19.7	—	—	0.237	84.6 $\pm$ 21.3	1.335	43.4 $\pm$ 10.2	15.8 $\pm$ 0.98
12	12e	3-Me	1	0.116	36.5 $\pm$ 4.9	—	20.8 $\pm$ 3	0.836	87.4 $\pm$ 5.6	0.005	34.1 $\pm$ 5.8	12.8 $\pm$ 2.40
13	12f	3-Me	2	0.721	30.3 $\pm$ 12.2	—	—	—	—	0.415	30.3 $\pm$ 12.2	>100
14	12g	3-F	2	—	—	—	—	0.069	15.8 $\pm$ 2.6	0.54	45.0 $\pm$ 1.2	15.6 $\pm$ 2.41
15	12h	2-Cl	1	—	—	—	—	0.769	38.5 $\pm$ 16.4	1.159	68.1 $\pm$ 19.6	10.9 $\pm$ 1.12
16	12i	2-Cl	2	—	—	—	—	0.413	77.8 $\pm$ 25.7	0.331	37.7 $\pm$ 11.0	14.1 $\pm$ 2.27
17	16a	H	1	—	—	—	—	0.974	56.2 $\pm$ 9.4	0.475	49.2 $\pm$ 9.9	4.95 $\pm$ 0.26
18	16b	H	2	1.831	9.2 $\pm$ 3.3	—	—	0.779	91.2 $\pm$ 10.4	5.391	33.8 $\pm$ 14.2	7.04 $\pm$ 0.49
19	16c	2-Me	1	—	—	—	—	0.013	24.8 $\pm$ 4.5	0.016	170.0 $\pm$ 1.0	7.05 $\pm$ 0.82
20	4OHT	—	—	0.008	35 $\pm$ 3	—	−1 $\pm$ 1	0.003	35 $\pm$ 3	0.001	−20 $\pm$ 2	15.6 $\pm$ 1.77

^a Luciferase activity was measured in HEK293T cells transfected with a 3 $\times$ ERE-driven luciferase reporter and expression vectors encoding ER α or ER β and treated in triplicate with increasing doses (up to 10^{−5} M) of the compounds. ^b EC₅₀ and standard deviation (mean $\pm$ SD), shown as a percentage of 10^{−8} M 17 β -estradiol (E₂), were determined. ^c IC₅₀ and standard deviation (mean $\pm$ SD) were determined in the percentage of 10^{−8} M 17 β -estradiol (E₂) on ER α or ER β . ^d ERs have considerable basal activity in HEK293T cells; compounds with inverse agonist activity are given negative efficacy values. Omitted EC₅₀ or IC₅₀ values were too high to be determined accurately; omitted Eff (% E₂) values were too low to be accurately determined. ^e IC₅₀ values are an average of at least three independent experiments $\pm$ standard deviation (mean $\pm$ SD).

Fig. 3 Illustrative dose–response curves for the ER α antagonist effects of 4OHT, and two selenophene-core compounds **12g** and **16c**. Efficacy values are the mean $\pm$ SD from three experiments. For details, see the Experimental section.

antagonist of ER β (**9b**, IC₅₀ = 0.24 and 0.146 μ M for ER α and ER β , respectively). Compared with **9b**, compound **9c** prepared with *N*-(2-chloroethyl) piperidine didn't have obvious influence on the activity (Table 3, entries 2 and 3). Moreover, replacing the methyl group (**9b**) with a halogen group also had significant effects on the transcriptional activity of the ER subtypes. The fluorine-substituted compounds **9d** and **9e** profiled as ER α agonists, being about 4 to 6-fold more potent than **9a**, whereas the chloro analogues **9f** and **9g** had little effect on ER α (Table 3, entries 4, 5 and 6, 7). However, when the halogen group was introduced into these compounds, the antagonistic activity for ER β significantly increased (Table 3, entries 4 and 6).

Comparisons of the position of basic side chains in the selenophene core indicate that 3,4-disubstituted compounds (series II) had decreased ER α binding affinity (Table 1, **12a–i**), which also demonstrate decreased efficacy as ER α agonists in general (Table 3). Interestingly, these 3,4-disubstituted ligands show improved ER β binding affinity and also display increased potency as ER β antagonists, whereas **12b** profiled as a moderate ER β agonist with a μ M range IC₅₀ but had low efficacy. When the methyl group was changed from the C2- to C3-position (**12e**, **12f**), these compounds profiled as antagonists for ER β , and were more efficacious than **12c** and **12d** (Table 3, entries 12 vs. 10; 13 vs. 11). It was remarkable that **12e** could act as a potent ER β antagonist with a nanomolar IC₅₀ value (5 nM).

Addition of a third substituent onto the selenophene core also has drastic effects on transcriptional activity. We prepared three compounds **16a–c** and these were potent and highly efficacious ER α and ER β antagonists, especially compound **16c**, which gave low IC₅₀ values of 0.013 and 0.016 μ M for ER α and ER β , respectively. This makes **16c** the most potent antagonist for ER α of the whole series, and the transcription activity of **16c** is 66-fold better than the best compound in selenophenes without a BSC.³⁸ The trend is consistent with the RBA of these compounds. Again, there was also a trend that most compounds with pyrrolidine side chains displayed better activities than those with piperidine side chains.

2.4. Antiproliferative activity on breast cancer cells

To evaluate the antiproliferative activity of this type of compounds, all selenophene-core compounds were screened against MCF-7, and the results are summarized in Table 3.

Overall, most of the selenophene derivatives were effective in inhibiting the MCF-7 breast cancer cells. Among them, six compounds, **9a**, **12h–i** and **16a–c**, exhibited potent antiproliferative activity with values of IC₅₀ better than that of 4-hydroxytamoxifen in breast cancer MCF-7 cells. Generally, the compounds that displayed potent and highly efficacious antiproliferative activity on MCF-7 breast cancer cells also showed moderate to good binding affinities for ER α and ER β ; meanwhile, two compounds, **9g** and **12f**, had low antiproliferative activity due to their unobvious RBA values (less than 1%). However, the compounds **9d**, **9e**, and **12a** possessing high binding affinities showed weak inhibition for MCF-7 cells, which suggested that the binding affinity and antiproliferative potency should be independent. Furthermore, introduction of the basic side chains in compounds demonstrated more promising antiproliferative activity.

In order to determine that the antiproliferative activity of these compounds arises from their antiestrogenicity on ER, the cells were incubated with 50 μ M of **9a**, **12b** and **16a** in the presence and absence of 10 nM E₂. As shown in Fig. 4, after incubation with different doses of ligands, relative cell activities decreased substantially. Exogenous estradiol could also restore a portion of the MCF-7 cells, which demonstrated that these selenophene derivatives inhibited the MCF-7 cells by interacting with ER. As such, those potent compounds in suppressing the proliferation of MCF-7 cell showed moderate to excellent antiproliferative activity, which might be attributed to their antagonistic potency on ER.

2.5. Structural analysis of the origin of the enhanced antagonist character of these ligands

Estradiol supports transcriptional activation of ER α and ER β by stabilizing helix 12 in a position where it forms a hydrophobic groove for binding transcriptional coactivators. The traditional

Fig. 4 Effect of E₂, **9a**, **12b** and **16a** on the proliferation of hormone-dependent breast cancer MCF-7 cells after 5 days of culture. Nontreated MCF-7 cells are used as the control set at 100%. Mean of three separate experiments $\pm$ range.

Fig. 5 Model of selenophene ligands **11c**, **12e** and **16c** bound to ER α and comparisons with raloxifene. (A) Computer-developed model of **11c** bound to the ER α (PDB: 1ERR). The phenolic hydroxyl group of **11c** forms hydrogen bonding interactions with residue Glu353 on helix 3 and residue Arg394 on helix 6, respectively. (B) Computer-developed model of **12e** bound to the ER α with the conserved H-bonds to Glu353 and Arg394 residues. The basic side chain of the **12e** is oriented toward helix 12. (C) Crystal structure of the ER α LBD in complex with raloxifene. Raloxifene forms H-bonds to the conserved Glu353, Arg394, Asp351, and His524 residues. The basic side chain displaces helix 12. (D) Computer-developed model of the ER α LBD in complex with **16c**. Consistent with the complex of raloxifene, the second phenol forms a hydrogen bond with the residue His524 on helix 11, and the basic side chain displaces helix 12.

SERMs or full antagonists have typically been developed by adding a bulky side group that directly obstructs the agonist position of helix 12, relocating it out of this position and thereby blocking the recruitment of transcriptional coactivators.⁴³

Consistent with this model, we find that **11c** (Fig. 5A) can similarly form a hydrogen bond with residue Glu353 on helix 3 and residue Arg394 on helix 6, respectively.⁴⁴ In contrast, the SERM with a close structure, raloxifene (Fig. 5C), has a bulky side chain that projects between helices 3 and 11, directly displacing helix 12 from its active conformation and destroying the transcriptional coactivator binding site; moreover, compound **12e** (Fig. 5B) mimics the binding orientation of raloxifene, with the BSC on one of the phenol groups directly interacting with any helix 12 residues, which is consistent with raloxifene, thus giving **12e** potent ER α antagonist activity. For triaryl substituted selenophene **16c**, the ligand is consistent with raloxifene: there are similar hydrogen bonds and direct displacement of helix 12 with a BSC to those of raloxifene and **12e**. The second phenolic hydroxy of **16c** also forms a hydrogen bond with the residue His524 on helix 11 (Fig. 5D), which makes **16c** the most potent antagonist for ER α of the whole series.

3. Conclusions

Therapeutic targeting of the estrogen receptor has traditionally involved direct disruption of the surface coactivator binding sites by a basic side chain that is a characteristic structural feature of SERMs, such as tamoxifen and raloxifene. In this study, we sequentially introduced different basic side chains into the selenophene scaffold to form new subtype-selective antagonists

for the estrogen receptor. Interestingly, most of the compounds displayed good binding affinity and increased antagonistic activity for both ERs in comparison with the parent selenophenes without a BSC. Several compounds showed values of IC₅₀ better than that of 4-hydroxytamoxifen in breast cancer MCF-7 cells. The preliminary mechanistic study indicated the antiproliferative activity arises from their antiestrogenicity; the modeling study showed that the introduction of a basic side chain had a significant effect on the antiproliferative activity of these compounds *via* directly interacting with helix 12. These new ligands could act as models for the development of novel agents to improve therapeutics that target the estrogen receptor.

4. Experimental section

4.1. Chemistry

4.1.1. General. Starting materials were purchased from Aldrich, Acros, Aladdin-Reagent, and Alfa-Aesar and were used without purification. Toluene was freshly distilled with sodium, and dichloromethane was distilled with anhydrous CaH₂. Glassware was oven-dried, assembled while hot, and cooled under an inert atmosphere. Unless otherwise noted, all reactions were conducted in an inert atmosphere. All reactions were performed under an Ar atmosphere unless otherwise specified. Reaction progress was monitored by analytical thin-layer chromatography (TLC). Visualization was achieved by UV light (254 nm).

¹H NMR and ¹³C NMR spectra were measured on a Bruker AVANCE III 400 (400 MHz, ¹H NMR; 101 MHz, ¹³C NMR) instrument. Chemical shifts are reported in ppm

(parts per million) and are referenced to either tetramethylsilane or the solvent. Melting points were determined on a X-4 Beijing Tech melting point apparatus, and the data were uncorrected.

General procedure for Suzuki coupling. Under an Ar atmosphere, a mixture of bromoselenophene (1 equiv.), arylboronic acid (3 equiv. for disubstituted, 4 equiv. for trisubstituted selenophenes), the Pd catalyst, sodium carbonate (2 equiv.) in an oxygen-free toluene/water (1:1) solution was stirred at 120 °C for 24 h, after which, the reaction mixture was cooled to room temperature. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and then filtered and concentrated under vacuum. The product was purified by column chromatography (CC).

General procedure for ether cleavage. Method A: under an Ar atmosphere, to a solution of the methoxyphenyl selenophene derivative (1 equiv.) in dry dichloromethane, boron tribromide (3 equiv. per methoxy function) was added dropwise at –20 °C. The mixture was allowed to stir for 4 h, and quenched with MeOH. The reaction mixture was poured into water, and extracted with ethyl acetate. The extracts were dried (Na₂SO₄) and evaporated. The residue was purified by silica gel column chromatography (CC).

Method B: a mixture of the methoxyphenyl selenophene derivative (1 equiv.) and pyridine hydrochloride (10 equiv.) was stirred at 190 °C for 3 h, after which, the reaction mixture was cooled to room temperature. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with dilute hydrochloric acid, dried over anhydrous Na₂SO₄ and then filtered and concentrated under vacuum. The product was purified by column chromatography (CC).

General procedure for the installation of basic side chains by Williamson ether synthesis. To a mixture of the phenolic selenophene derivative (1 equiv.) and *N*-(2-chloroethyl)-pyrrolidine or *N*-(2-chloroethyl)piperidine (1.1 equiv.) in DMF was added K₂CO₃ (4 equiv.) and KOH (4 equiv.). The reaction mixture was stirred for 45 min under microwave conditions. The reaction mixture was poured into water, and extracted with ethyl acetate. The extracts were dried (Na₂SO₄) and evaporated. The residue was purified by silica gel column chromatography (CC).

2-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenyl)-5-(4-hydroxyphenyl)selenophene 9a. Compound 9a was prepared with 2,5-bis(4-hydroxyphenyl)selenophene (8a) and *N*-(2-chloroethyl)-pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:1) gave the title compound as a yellow solid (65% yield; mp 89–92 °C); ¹H NMR (400 MHz, acetone-*d*₆) δ 7.97 (dd, *J* = 10.2, 2.8 Hz, 2H), 7.07 (d, *J* = 8.8 Hz, 2H), 7.01–6.95 (m, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 6.72 (t, *J* = 10.1 Hz, 2H), 4.15–4.04 (m, 2H), 2.90 (t, *J* = 5.9 Hz, 2H), 2.65 (t, *J* = 6.0 Hz, 4H), 1.82–1.71 (m, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 157.69, 156.76, 143.96, 143.58, 131.09, 130.26, 129.37, 129.29, 129.01, 127.71, 115.40, 114.48, 66.62, 54.65, 54.47, 23.54. HRMS (ESI) calcd. for C₂₂H₂₃NO₂Se [M + H]⁺, 414.0972; found 414.0976.

2-(2-Methyl-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-5-(2-methyl-4-hydroxyphenyl)selenophene 9b. Compound 9b was prepared with 2,5-bis(2-methyl-4-hydroxyphenyl)selenophene (8b) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:1) gave the title compound as a yellow solid (67% yield; mp 82–86 °C); ¹H NMR (400 MHz, acetone-*d*₆) δ 7.30 (d, *J* = 8.5 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.10 (dd, *J* = 7.9, 3.8 Hz, 2H), 6.85 (d, *J* = 2.4 Hz, 1H), 6.79–6.75 (m, 2H), 6.71 (dd, *J* = 8.3, 2.4 Hz, 1H), 4.14 (dd, *J* = 7.7, 4.2 Hz, 2H), 2.92–2.88 (m, 2H), 2.67–2.62 (m, 4H), 2.41 (s, 3H), 2.36 (s, 3H), 1.75 (dd, *J* = 6.8, 3.2 Hz, 4H). ¹³C NMR (101 MHz, acetone-*d*₆) δ 159.31, 158.18, 150.15, 149.24, 137.57, 137.50, 132.38, 132.28, 129.58, 129.27, 129.02, 128.09, 118.43, 117.69, 114.01, 112.81, 67.68, 55.49, 55.15, 24.20, 21.80, 21.70. HRMS (ESI) calcd. for C₂₄H₂₇NO₂Se [M + H]⁺, 442.1285; found 442.1286.

2-(2-Methyl-4-(2-(piperidin-1-yl)ethoxy)phenyl)-5-(2-methyl-4-hydroxyphenyl)selenophene 9c. Compound 9c was prepared with 2,5-bis(2-methyl-4-hydroxyphenyl)selenophene (8b) and *N*-(2-chloroethyl)piperidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:1) gave the title compound as a yellow solid (65% yield; mp 156–158 °C); ¹H NMR (400 MHz, acetone-*d*₆) δ 7.30 (t, *J* = 6.8 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 7.12 (q, *J* = 3.8 Hz, 2H), 6.86 (d, *J* = 2.5 Hz, 1H), 6.79 (dt, *J* = 5.5, 2.8 Hz, 2H), 6.72 (dd, *J* = 8.3, 2.6 Hz, 1H), 4.12 (s, 2H), 2.72 (t, *J* = 6.0 Hz, 2H), 2.50 (s, 4H), 2.42 (s, 3H), 2.38 (s, 3H), 1.56 (dt, *J* = 10.9, 5.6 Hz, 4H), 1.42 (dd, *J* = 11.1, 6.0 Hz, 2H). ¹³C NMR (101 MHz, acetone-*d*₆) δ 159.41, 158.01, 150.07, 149.31, 137.56, 136.73, 132.39, 132.26, 129.50, 129.26, 129.06, 128.23, 118.36, 117.70, 113.94, 112.86, 66.85, 58.69, 55.74, 26.76, 25.05, 21.77, 21.66. HRMS (ESI) calcd. for C₂₅H₂₉NO₂Se [M + H]⁺, 454.1450; found 454.1452.

2-(2-Fluoro-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-5-(2-fluoro-4-hydroxyphenyl)selenophene 9d. Compound 9d was prepared with 2,5-bis(2-fluoro-4-hydroxyphenyl)selenophene (8c) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:1) gave the title compound as a yellow solid (70% yield; mp 162–165 °C); ¹H NMR (400 MHz, CD₃OD) δ 7.65 (t, *J* = 8.7 Hz, 1H), 7.56 (dd, *J* = 5.5, 3.2 Hz, 2H), 7.52 (dd, *J* = 4.3, 1.6 Hz, 1H), 6.87 (dd, *J* = 8.0, 6.0 Hz, 2H), 6.65 (ddd, *J* = 15.5, 10.8, 2.5 Hz, 2H), 4.21 (t, *J* = 5.5 Hz, 2H), 3.05 (t, *J* = 5.4 Hz, 2H), 2.80 (t, *J* = 6.7 Hz, 4H), 1.95–1.85 (m, 4H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 159.33 (d, *J* = 247.1 Hz), 159.06 (d, *J* = 247.3 Hz), 141.32 (d, *J* = 29.0 Hz), 140.02 (d, *J* = 29.7 Hz), 129.17 (d, *J* = 12.1 Hz), 129.01 (d, *J* = 11.4 Hz), 127.35, 126.71, 116.05, 114.37, 113.07, 112.33, 103.67, 103.42, 103.04, 102.78, 67.84, 54.56, 54.42, 23.60. HRMS (ESI) calcd. for C₂₂H₂₁F₂NO₂Se [M + H]⁺, 450.0784; found 450.0781.

2-(2-Fluoro-4-(2-(piperidin-1-yl)ethoxy)phenyl)-5-(2-fluoro-4-hydroxyphenyl)selenophene 9e. Compound 9e was prepared with 2,5-bis(2-fluoro-4-hydroxyphenyl)selenophene (8c) and *N*-(2-chloroethyl)piperidine according to the general

procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:1) gave the title compound as a yellow solid (65% yield; mp 158–162 °C); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.38 (s, 1H), 7.76–7.68 (m, 1H), 7.68–7.58 (m, 2H), 6.99 (dd, J = 13.6, 2.5 Hz, 1H), 6.86 (dd, J = 8.8, 2.4 Hz, 2H), 6.75–6.64 (m, 2H), 4.11 (t, J = 5.9 Hz, 2H), 2.65 (t, J = 5.8 Hz, 2H), 2.43 (s, 4H), 1.50 (dt, J = 10.8, 5.5 Hz, 4H), 1.38 (d, J = 5.1 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 159.28 (d, J = 247.2 Hz), 159.05 (d, J = 249.1 Hz), 141.30 (d, J = 31.4 Hz), 140.06 (d, J = 32.3 Hz), 129.16 (d, J = 11.3 Hz), 128.96 (d, J = 12.1 Hz), 127.32, 126.72, 116.04, 114.44, 113.06, 112.34, 103.67, 103.42, 103.06, 102.80, 66.65, 57.60, 54.79, 25.96, 24.33. HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{23}\text{F}_2\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 464.0940; found 464.0948.

2-(2-Chloro-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-5-(2-chloro-4-hydroxyphenyl)selenophene 9f. Compound 9f was prepared with 2,5-bis(2-chloro-4-hydroxyphenyl)selenophene (8d) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:1) gave the title compound as a yellow solid (55% yield; mp 137–140 °C); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.56 (t, J = 7.0 Hz, 1H), 7.51–7.42 (m, 3H), 7.13 (d, J = 2.6 Hz, 1H), 6.98–6.93 (m, 2H), 6.82 (dt, J = 4.6, 2.3 Hz, 1H), 4.11 (t, J = 5.7 Hz, 2H), 2.81 (t, J = 5.8 Hz, 2H), 2.58–2.52 (m, 4H), 1.70–1.63 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 158.27, 157.95, 145.89, 144.80, 131.55, 131.36, 131.11, 130.92, 128.95, 128.53, 126.51, 124.72, 116.71, 115.84, 115.18, 114.37, 66.96, 53.90, 53.83, 23.05. HRMS (ESI) calcd. for $\text{C}_{22}\text{H}_{21}\text{Cl}_2\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 482.2830; found 482.2830.

2-(2-Chloro-4-(2-(piperidin-1-yl)ethoxy)phenyl)-5-(2-chloro-4-hydroxyphenyl)selenophene 9g. Compound 9g was prepared with 2,5-bis(2-fluoro-4-hydroxyphenyl)selenophene (8d) and *N*-(2-chloroethyl)piperidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:1) gave the title compound as a yellow solid (65% yield; mp 92–95 °C); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.59 (d, J = 8.7 Hz, 1H), 7.54–7.45 (m, 3H), 7.16 (d, J = 2.5 Hz, 1H), 7.01–6.94 (m, 2H), 6.83 (dt, J = 11.9, 5.9 Hz, 1H), 4.15–4.04 (m, 2H), 2.65 (t, J = 5.8 Hz, 2H), 2.42 (s, 4H), 1.48 (dd, J = 10.8, 5.4 Hz, 4H), 1.37 (d, J = 4.8 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 158.40, 157.91, 145.84, 144.83, 131.60, 131.36, 131.09, 130.91, 128.97, 128.58, 126.43, 124.74, 116.68, 115.87, 115.18, 114.50, 66.10, 57.13, 54.28, 25.48, 23.84. HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{23}\text{Cl}_2\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 496.0349; found 496.0341.

3,4-Bis(2-chloro-4-methoxyphenyl)selenophene 10e.

Compound 10e was prepared with 3,4-dibromoselenophene (6b) and 2-chloro-4-methoxyphenylboronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether:ethyl acetate = 98:2) gave the title compound as a yellow solid; ^1H NMR (400 MHz, CDCl_3) δ 7.71 (s, 2H), 7.09 (t, J = 14.5 Hz, 2H), 6.74 (dt, J = 17.4, 14.8 Hz, 2H), 6.67–6.56 (m, 2H), 3.77–3.63 (m, 6H).

3,4-Bis(2-chloro-4-hydroxyphenyl)selenophene 11e.

Compound 11e was prepared with 3,4-bis(2-chloro-4-methoxyphenyl)selenophene (10e) using boron tribromide

according to the general procedure for ether cleavage. Purification by CC (petroleum ether:ethyl acetate = 4:1) gave the title compound as a yellow solid; ^1H NMR (400 MHz, acetone- d_6) δ 8.00 (d, J = 4.9 Hz, 2H), 7.01–6.93 (m, 2H), 6.82 (d, J = 2.5 Hz, 2H), 6.71–6.63 (m, 2H).

3-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenyl)-4-(4-hydroxyphenyl)selenophene 12a. Compound 12a was prepared with 3,4-bis(4-hydroxyphenyl)selenophene (11a) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (65% yield; mp 89–92 °C); ^1H NMR (400 MHz, acetone- d_6) δ 7.96 (dd, J = 10.2, 2.8 Hz, 2H), 7.06 (d, J = 8.8 Hz, 2H), 7.00–6.94 (m, 2H), 6.81 (d, J = 8.8 Hz, 2H), 6.71 (t, J = 10.1 Hz, 2H), 4.14–4.03 (m, 2H), 2.89 (t, J = 5.9 Hz, 2H), 2.64 (t, J = 6.0 Hz, 4H), 1.81–1.70 (m, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 157.62, 156.69, 143.89, 143.51, 131.02, 130.19, 129.30, 129.22, 128.94, 127.44, 115.33, 114.41, 66.55, 54.58, 54.40, 23.47. HRMS (ESI) calcd. for $\text{C}_{22}\text{H}_{23}\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 414.0972; found 414.0970.

3-(4-(2-(Piperidin-1-yl)ethoxy)phenyl)-4-(4-hydroxyphenyl)selenophene 12b. Compound 12b was prepared with 3,4-bis(4-hydroxyphenyl)selenophene (11a) and *N*-(2-chloroethyl)piperidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (72% yield; mp 159–163 °C); ^1H NMR (400 MHz, acetone- d_6) δ 8.01–7.90 (m, 1H), 7.98–7.93 (m, 1H), 7.08–7.04 (m, 1H), 7.09–6.92 (m, 2H), 6.99–6.94 (m, 1H), 6.83–6.78 (m, 1H), 6.75 (ddd, J = 11.3, 6.7, 2.3 Hz, 2H), 6.74–6.70 (m, 1H), 4.06 (t, J = 6.0 Hz, 2H), 2.72–2.66 (m, 2H), 2.48 (s, 4H), 1.54 (dt, J = 10.9, 5.5 Hz, 4H), 1.41 (d, J = 2.5 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 157.70, 156.63, 143.83, 143.47, 130.84, 130.13, 130.10, 129.18, 129.16, 128.85, 115.27, 114.36, 65.84, 57.80, 54.79, 25.94, 24.31. HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{25}\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 428.1129; found 428.1120.

3-(2-Methyl-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4-(2-methyl-4-hydroxyphenyl)selenophene 12c. Compound 12c was prepared with 3,4-bis(2-methyl-4-hydroxyphenyl)selenophene (11b) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (67% yield; mp 97–101 °C); ^1H NMR (400 MHz, acetone- d_6) δ 7.90 (dd, J = 7.9, 2.8 Hz, 2H), 6.98 (dd, J = 11.8, 1.2 Hz, 2H), 6.86 (dd, J = 8.4, 1.8 Hz, 1H), 6.70 (dt, J = 18.3, 8.3 Hz, 3H), 4.09 (t, J = 5.8 Hz, 2H), 2.93 (t, J = 5.7 Hz, 2H), 2.67 (s, 4H), 2.11 (d, J = 4.7 Hz, 6H), 1.78–1.73 (m, 4H). ^{13}C NMR (101 MHz, acetone- d_6) δ 159.22, 158.08, 150.06, 149.15, 137.47, 137.41, 132.29, 132.19, 129.49, 129.17, 128.92, 128.00, 118.33, 117.60, 113.92, 112.71, 55.40, 55.06, 29.84, 24.11, 21.71, 21.60. HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{27}\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 442.1285; found 442.1283.

3-(2-Methyl-4-(2-(piperidin-1-yl)ethoxy)phenyl)-4-(2-methyl-4-hydroxyphenyl)selenophene 12d. Compound 12d was prepared with 3,4-bis(2-methyl-4-hydroxyphenyl)selenophene (11b) and *N*-(2-chloroethyl)piperidine according to the general

procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (55% yield; mp 91–95 °C); ^1H NMR (400 MHz, acetone- d_6) δ 7.93 (d, J = 2.8 Hz, 1H), 7.91 (d, J = 2.8 Hz, 1H), 7.00 (d, J = 1.6 Hz, 1H), 6.97 (s, 1H), 6.86 (dd, J = 8.4, 2.0 Hz, 1H), 6.75 (d, J = 8.4 Hz, 1H), 6.70 (d, J = 2.0 Hz, 2H), 4.15 (t, J = 5.7 Hz, 2H), 2.94–2.87 (m, 2H), 2.70 (s, 4H), 2.16–2.08 (m, 6H), 1.65–1.60 (m, 4H), 1.49–1.42 (m, 2H). ^{13}C NMR (101 MHz, acetone- d_6) δ 159.38, 158.17, 150.11, 149.25, 137.56, 137.52, 132.38, 132.26, 129.51, 129.27, 129.04, 128.11, 118.39, 117.67, 113.98, 112.86, 66.74, 58.58, 55.65, 26.66, 24.96, 21.74, 21.64. HRMS (ESI) calcd. for $\text{C}_{25}\text{H}_{29}\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 454.1450; found 454.1455.

3-(3-Methyl-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4-(3-methyl-4-hydroxyphenyl)selenophene 12e. Compound 12e was prepared with 3,4-bis(3-methyl-4-hydroxyphenyl)selenophene (11c) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (72% yield; mp 84–88 °C); ^1H NMR (400 MHz, CDCl_3) δ 7.73 (d, J = 4.8 Hz, 2H), 7.73 (d, J = 4.8 Hz, 1H), 6.89–6.76 (m, 1H), 6.52 (dd, J = 8.1, 5.4 Hz, 2H), 6.48–6.42 (m, 2H), 4.01 (t, J = 5.6 Hz, 2H), 2.96 (t, J = 5.6 Hz, 2H), 2.76 (d, J = 20.0 Hz, 4H), 2.01 (s, 3H), 1.96 (s, 3H), 1.84 (s, 4H). ^{13}C NMR (101 MHz, acetone- d_6) δ 156.86, 155.31, 145.22, 144.92, 132.27, 132.02, 131.61, 130.62, 128.48, 128.32, 128.27, 128.25, 126.49, 124.54, 114.88, 111.19, 68.03, 55.53, 55.26, 24.22, 16.55, 16.31. HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{27}\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 442.1285; found 442.1283.

3-(3-Methyl-4-(2-(piperidin-1-yl)ethoxy)phenyl)-4-(3-methyl-4-hydroxyphenyl)selenophene 12f. Compound 12f was prepared with 3,4-bis(3-methyl-4-hydroxyphenyl)selenophene (11c) and *N*-(2-chloroethyl)piperidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (65% yield; mp 85–89 °C); ^1H NMR (400 MHz, acetone- d_6) δ 7.92 (dd, J = 9.9, 2.8 Hz, 2H), 7.02–6.95 (m, 2H), 6.86 (dd, J = 8.4, 2.0 Hz, 1H), 6.75 (d, J = 8.4 Hz, 1H), 6.70 (d, J = 2.0 Hz, 2H), 4.15 (t, J = 5.7 Hz, 2H), 2.92 (dd, J = 10.9, 5.3 Hz, 2H), 2.70 (s, 4H), 2.15–2.08 (m, 6H), 1.62 (dt, J = 11.0, 5.6 Hz, 4H), 1.49–1.39 (m, 2H). ^{13}C NMR (101 MHz, acetone- d_6) δ 159.39, 158.19, 150.12, 149.26, 137.58, 137.53, 132.39, 132.27, 129.52, 129.28, 129.06, 128.12, 118.41, 117.68, 113.99, 112.87, 66.75, 58.59, 55.66, 26.67, 24.97, 21.75, 21.65. HRMS (ESI) calcd. for $\text{C}_{25}\text{H}_{29}\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 454.1450; found 454.1448.

3-(3-Fluoro-4-(2-(piperidin-1-yl)ethoxy)phenyl)-4-(3-fluoro-4-hydroxyphenyl)selenophene 12g. Compound 12g was prepared with 3,4-bis(3-fluoro-4-hydroxyphenyl)selenophene (11d) and *N*-(2-chloroethyl)piperidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (74% yield; mp 89–93 °C); ^1H NMR (400 MHz, DMSO- d_6) δ 7.63 (t, J = 9.1 Hz, 1H), 7.59–7.55 (m, 2H), 7.54–7.51 (m, 1H), 6.90 (dd, J = 13.6, 2.5 Hz, 1H), 6.78 (dd, J = 8.8, 2.4 Hz, 1H), 6.65–6.58 (m, 2H), 4.03 (t, J =

5.9 Hz, 2H), 2.57 (t, J = 5.8 Hz, 2H), 2.34 (s, 4H), 1.41 (dt, J = 10.8, 5.5 Hz, 4H), 1.30 (d, J = 5.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 159.22 (d, J = 241.9 Hz), 158.99 (d, J = 233.1 Hz), 141.24 (d, J = 25.6 Hz), 140.00 (d, J = 27.4 Hz), 129.10 (d, J = 14.0 Hz), 128.90 (d, J = 15.9 Hz), 127.26, 126.66, 115.98, 114.38, 113.00, 112.28, 103.61, 103.36, 103.00, 102.74, 66.59, 57.54, 54.73, 25.90, 24.27. HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{23}\text{F}_2\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 462.0948; found 462.0942.

3-(2-Chloro-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4-(2-chloro-4-hydroxyphenyl)selenophene 12h. Compound 12h was prepared with 3,4-bis(2-chloro-4-hydroxyphenyl)selenophene (11e) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (73% yield; mp 79–83 °C); ^1H NMR (400 MHz, CD_3OD) δ 7.82 (dt, J = 8.3, 4.1 Hz, 2H), 6.93 (dd, J = 8.4, 3.3 Hz, 1H), 6.83 (ddd, J = 6.4, 5.5, 1.7 Hz, 1H), 6.66 (dd, J = 8.6, 2.6 Hz, 2H), 6.59 (t, J = 2.3 Hz, 1H), 6.44 (dd, J = 8.4, 2.5 Hz, 1H), 4.13–4.08 (m, 2H), 3.31–3.27 (m, 2H), 3.12 (dd, J = 12.4, 5.9 Hz, 4H), 1.90 (td, J = 6.8, 2.9 Hz, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 158.00, 157.36, 141.15, 140.89, 132.98, 132.60, 132.36, 132.30, 130.16, 130.03, 128.76, 126.85, 115.66, 114.69, 113.84, 113.02, 66.83, 59.73, 53.89, 23.06. HRMS (ESI) calcd. for $\text{C}_{22}\text{H}_{21}\text{Cl}_2\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 482.0193; found 482.0190.

3-(2-Chloro-4-(2-(piperidin-1-yl)ethoxy)phenyl)-4-(2-chloro-4-hydroxyphenyl)selenophene 12i. Compound 12i was prepared with 3,4-bis(2-chloro-4-hydroxyphenyl)selenophene (11e) and *N*-(2-chloroethyl)piperidine according to the general procedure for Williamson ether synthesis. Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (70% yield; mp 109–113 °C); ^1H NMR (400 MHz, CD_3OD) δ 7.93–7.89 (m, 2H), 7.03–6.99 (m, 1H), 6.91 (dt, J = 6.6, 3.3 Hz, 2H), 6.71 (dd, J = 6.3, 4.2 Hz, 2H), 6.54 (dd, J = 8.4, 2.3 Hz, 1H), 4.12 (t, J = 5.3 Hz, 2H), 2.97 (t, J = 5.2 Hz, 2H), 2.76 (s, 4H), 1.68 (dd, J = 10.9, 5.4 Hz, 4H), 1.53 (d, J = 4.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 158.06, 157.30, 141.14, 140.90, 132.97, 132.60, 132.36, 132.26, 130.16, 130.03, 128.72, 126.90, 115.65, 114.75, 113.83, 113.08, 65.81, 57.22, 54.30, 25.47, 23.85. HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{23}\text{Cl}_2\text{NO}_2\text{Se}$ $[\text{M} + \text{H}]^+$, 496.0349; found 496.0346.

3-(4-Hydroxyphenyl)-2,5-bis(4-methoxyphenyl)selenophene 14a. Compound 14a was prepared with 3-bromo-2,5-bis(4-methoxyphenyl)selenophene (13a) and 4-hydroxyphenylboronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether:ethyl acetate = 30:1) gave the title compound as a yellow solid; ^1H NMR (400 MHz, CDCl_3) δ 7.51–7.48 (m, 1H), 7.51–7.48 (m, 1H), 7.42–7.40 (m, 1H), 7.22–7.16 (m, 4H), 6.93–6.89 (m, 2H), 6.81–6.72 (m, 4H), 3.84 (s, 3H), 3.79 (s, 3H).

3-(4-Hydroxyphenyl)-2,5-bis(2-methyl-4-methoxyphenyl)selenophene 14b. Compound 14b was prepared with 3-bromo-2,5-bis(2-methyl-4-methoxyphenyl)selenophene (13b) and 4-hydroxyphenylboronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether:ethyl acetate = 30:1) gave the title compound as a yellow

solid; ^1H NMR (400 MHz, CDCl_3) δ 7.48–7.33 (m, 3H), 7.21–7.16 (m, 2H), 6.88 (d, J = 2.6 Hz, 1H), 6.86–6.73 (m, 5H), 3.86–3.84 (t, J = 8.9 Hz, 6H), 2.53 (d, J = 8.1 Hz, 3H), 2.08 (d, J = 13.6 Hz, 3H).

3-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenyl)-2,5-bis(4-hydroxyphenyl)selenophene 16a. Compound 16a was obtained from the demethylation of compound 15a using boron tribromide; the latter was prepared with 3-(4-hydroxyphenyl)-2,5-bis(4-methoxyphenyl)selenophene (14a) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (dichloromethane:ethyl acetate = 2:3) gave the title compound 16a as a yellow solid (56% yield; mp 105–109 °C); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.79 (d, J = 23.2 Hz, 2H), 7.57–7.47 (m, 3H), 7.31–7.27 (m, 2H), 7.10–7.07 (m, 4H), 6.99 (d, J = 8.8 Hz, 1H), 6.85 (t, J = 8.0 Hz, 1H), 6.78–6.71 (m, 2H), 4.36 (t, J = 4.9 Hz, 2H), 3.53 (d, J = 19.2 Hz, 2H), 3.33 (d, J = 3.5 Hz, 4H), 1.97 (d, J = 3.5 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 157.93, 157.40, 156.97, 147.39, 141.56, 139.30, 130.91, 130.48, 130.43, 127.99, 127.42, 127.14, 126.96, 116.32, 116.01, 114.98, 63.93, 54.19, 53.30, 23.09. HRMS (ESI) calcd. for $\text{C}_{28}\text{H}_{27}\text{NO}_3\text{Se}$ $[\text{M} + \text{H}]^+$, 506.1234; found 506.1231.

3-(4-(2-(Piperidin-1-yl)ethoxy)phenyl)-2,5-bis(4-hydroxyphenyl)selenophene 16b. Compound 16b was obtained from the demethylation of compound 15b using boron tribromide; the latter was prepared with 3-(4-hydroxyphenyl)-2,5-bis(4-methoxyphenyl)selenophene (14a) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (dichloromethane:ethyl acetate = 2:3) gave the title compound 16b as a yellow solid (62% yield; mp 142–146 °C); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.52–7.42 (m, 3H), 7.21 (d, J = 8.7 Hz, 2H), 7.04 (d, J = 8.6 Hz, 2H), 6.84 (dd, J = 19.9, 8.7 Hz, 2H), 6.70 (d, J = 8.6 Hz, 2H), 4.09 (t, J = 5.6 Hz, 2H), 2.80 (t, J = 5.3 Hz, 2H), 2.55 (d, J = 19.8 Hz, 4H), 1.54 (dt, J = 10.8, 5.5 Hz, 4H), 1.40 (d, J = 4.4 Hz, 2H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 157.92, 157.62, 157.36, 147.33, 141.43, 139.39, 130.41, 130.34, 128.15, 128.03, 127.40, 127.21, 127.06, 116.32, 115.97, 114.80, 65.28, 57.39, 54.54, 25.41, 23.86. HRMS (ESI) calcd. for $\text{C}_{29}\text{H}_{29}\text{NO}_3\text{Se}$ $[\text{M} + \text{H}]^+$, 520.1391; found 520.1398.

3-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenyl)-2,5-bis(2-methyl-4-hydroxyphenyl)selenophene 16c. Compound 16c was obtained from the demethylation of compound 15c using boron tribromide; the latter was prepared with 3-(4-hydroxyphenyl)-2,5-bis(4-methoxyphenyl)selenophene (14b) and *N*-(2-chloroethyl)pyrrolidine according to the general procedure for Williamson ether synthesis. Purification by CC (dichloromethane:ethyl acetate = 2:3) gave the title compound 16c as a yellow solid (52% yield; mp 89–93 °C); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.78 (s, 1H), 9.63 (s, 1H), 7.47 (d, J = 2.0 Hz, 1H), 7.33 (d, J = 1.9 Hz, 1H), 7.28 (dd, J = 8.2, 2.3 Hz, 1H), 7.24–7.12 (m, 2H), 6.96 (d, J = 1.8 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 6.73 (dd, J = 8.3, 2.2 Hz, 1H), 6.63 (d, J = 8.4 Hz, 1H), 4.45–4.33 (m, 2H), 3.50 (d, J = 38.9 Hz, 2H), 3.38 (s, 4H), 2.17 (s, 3H), 2.01 (d, J = 11.8 Hz, 3H), 1.93 (d, J = 4.9 Hz, 4H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 156.40, 155.93, 153.82, 145.42, 141.29, 137.38, 135.30, 132.37,

131.94, 131.86, 131.66, 128.17, 127.54, 125.84, 125.46, 124.78, 124.65, 115.17, 115.03, 113.62, 110.61, 110.42, 65.65, 54.98, 53.45, 23.12, 16.46, 16.43. HRMS (ESI) calcd. for $\text{C}_{30}\text{H}_{31}\text{NO}_3\text{Se}$ $[\text{M} + \text{H}]^+$, 534.1537; found 534.1539.

4.2. Gene clone and protein purification

Human ER α or ER β ligand binding domain (LBD) genes were amplified by PCR from plasmid pVP-16-ER α and pVP-16-ER β . The PCR product was cloned into a plasmid, and PGEx-KG *E. coli* BL21 (DE3) was used for the overexpression of ER-LBD. The cells were induced by IPTG (10 μM) for 2 h, then the cells were harvested, frozen, and thawed in phosphate-buffered saline (PBS), containing 1 mM EDTA and 1 mM DTT. After being ultrasonicated in an icy bath, the supernatant was loaded into a column of GSH-resin. The collection was dialyzed in ice buffer for 4 h. After being checked by a combination of sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blotting, the protein was prepared as a 10 mM stock in potassium phosphate and stored at -80°C .⁴⁵

4.3. Estrogen receptor binding affinity

Relative binding affinities were determined by a competitive fluorometric binding assay as previously described. Briefly, 40 nM of a fluorescence tracer (coumestrol, Sigma-Aldrich, MO) and 0.8 μM purified human ER α or ER β ligand binding domain (LBD) were diluted in 100 mM potassium phosphate buffer (pH 7.4), containing 100 $\mu\text{g mL}^{-1}$ bovine gamma globulin (Sigma-Aldrich, MO). Incubations were for 2 h at room temperature (25 °C). Fluorescence polarization values were then measured. The binding affinities are expressed as relative binding affinity (RBA) values with the RBA of 17 β -estradiol set to 100%. The values given are the average $\pm$ range of two independent determinations. IC₅₀ values were calculated according to equations described previously.⁴⁶

4.4. Gene transcriptional activity

The human embryonic kidney cell line HEK 293T was maintained in Dulbecco's Minimum Essential Medium (DMEM) (Gibco by Invitrogen Corp., CA) with 10% fetal bovine serum (FBS) (Hylcone by Thermo Scientific, UT). Cells were plated in phenol red-free DMEM with 10% FBS. HEK 293T cells were transfected with 25 μL of the mixture per well, containing 300 ng of the 3 $\times$ ERE-luciferase reporter, 100 ng of the ER α or ER β expression vector, 125 mM calcium chloride (GuoYao, China) and 12.5 μL 2 $\times$ HBS. On the next day, the cells were treated with increasing doses of ER ligands diluted in phenol red-free DMEM with 10% FBS. After 24 h, luciferase activity was measured using a Dual-Luciferase Reporter Assay System (Promega, MI) according to the manufacturer's protocol.

4.5. Cell culture and cell viability assay

The human breast cancer cell line MCF-7 was obtained from ATCC. Cells were maintained in DMEM with 10% FBS. For all experiments, cells were grown in 96-well microtiter plates (Nest

Biotech Co., China) with appropriate ligand triplicates for 72 h. MTT colormetric tests (Biosharp, China) were employed to determine cell viability per manufacturer instructions. IC₅₀ values were calculated according to the following equation using Origin software: $Y = 100\% \text{ inhibition} + (0\% \text{ inhibition} - 100\% \text{ inhibition}) / (1 + 10^{[(\log \text{IC}_{50} - X) \times \text{Hillslope}]})$, where Y = fluorescence value, X = log[inhibitor].⁴⁶

4.6. Molecular modeling

Crystal structures of ER LBD in complex with raloxifene were downloaded from the Protein Data Bank (PDB ID: 3ERR). Compounds **11c**, **12e** and **16c** were docked into the three-dimensional structure of ER α LBD with AutoDock software (version 4.2).^{44,45} Crystallographic coordinates of **11c**, **12e** and **16c** were created by Biochemoffice. The crystal structure of ER α LBD (PDB ID: 3ERD) was obtained from the PDB, and all water molecules were removed. Preparations of all ligands and the protein were performed with AutoDockTools (ADT). A docking cube with edges of 60 Å, 60 Å, and 58 Å in the X , Y , and Z dimensions, respectively (a grid spacing of 0.375 Å), which encompassed the whole active site, was used throughout the docking. On the basis of the Lamarckian genetic algorithm (LGA), 80 runs were performed for each ligand with 500 individuals in the population.^{47,48} The figures were prepared using PyMOL.

Conflict of interest

The authors declare no competing interests.

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