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ARTICLE

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 antagonist 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 ERα (IC_{50} = 13 nM) which made this compound the most potent antagonist for ERα of the whole series and is 66-fold better than the best selenophene compound without a BSC. Moreover, several compounds showed the values of IC_{50} 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.

Introduction

Selenium (Se) is an important nutritional trace element involved in different physiological functions, such as antioxidative, 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 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 last 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,[6-11] 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 we all 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, ERα and ERβ,[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 benefit in some tissues, including reproductive system,[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 proproliferative effect.[30] Thus, developing ER subtype-selective ligands with tissue- and 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 a lesser extent six-membered ring heterocycles as well as fused heterocycles. Some examples of the five-membered ring heterocycles are presented below (Figure 1), including furan **1**,[31]

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imidazole **2**,[32, 33] propyl pyrazole triol **3**,[34-36] thiophene **4**,[37] selenophene **5**[38] and raloxifene (RAL) *etc*. Because activity profiles of the current SERMs e.g. tamoxifen are not ideal and resistance to their effectiveness as antitumor agents can develop with time (Figure 1), there has been interest in finding new SERMs that might prove more effective as hormonal or therapeutic agents.

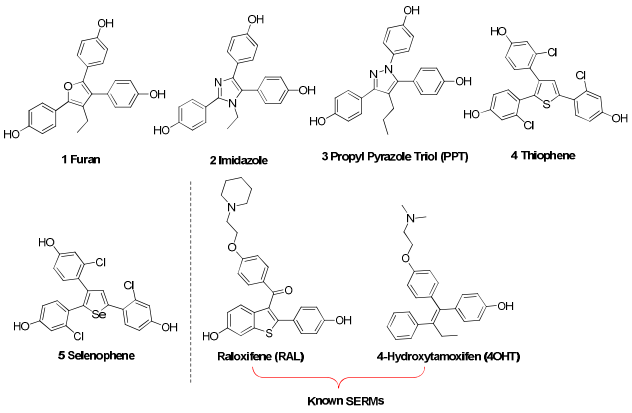

Figure 1. Examples of the five-membered ring heterocycles **1**-**5** as ER ligands, structures of known SERMs Raloxifene and 4-Hydroxytamoxifen.

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 selenohene scaffold that showed good binding affinity to ERs. The 2,5-bis(2-fluoro-4-hydroxylphenyl)selenophene **8c** showed the highest relative binding affinity (RBA, Estradiol = 100) of 24.3 for ERβ. In transcription assays, most of selenophenes exhibited partial to full agonist activity for both ER subtypes, but several compounds displayed a range of ERα or ERβ 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 ERα-**8c**, it is observed that one of phenolic group 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 E_2 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 (Figure 1). The nature and spatial orientation of the basic 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 selenophene-based core structure (e.g., a pyrrolidine or piperidine side chain) might provide ER ligands with interesting antagonistic activities (Figure 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 the value of IC_{50} better than that of 4-hydroxytamoxifen in breast cancer MCF-7 cells.

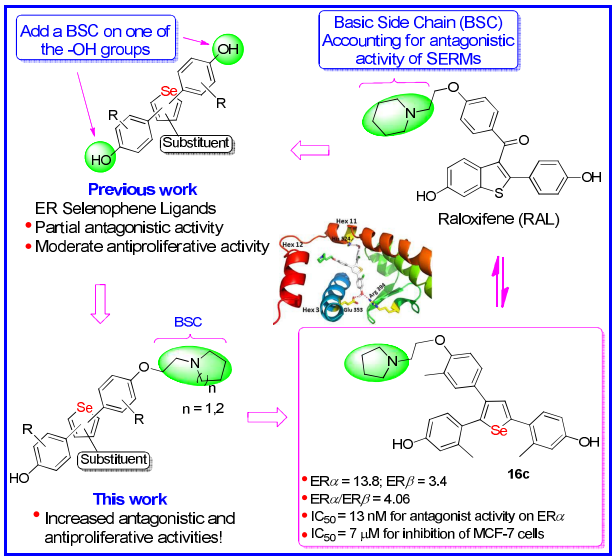

Figure 2. Rational for design of novel potent selenophenes with a BSC for breast cancer therapy.

2. Results and Discussion

2.1. Chemical Synthesis

Three series of novel selenophene derivatives, as depicted in **Schemes 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 monoalkylated products **9a**-**g** (yield 60-70%) and was accompanied by about 30-40% of dialkylated byproducts (**Scheme 1A**).

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 **6b**[38] with aryl boronic acids by using $Pd(OAc)_2/PPh_3$ 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. In particularly, a few triaryl

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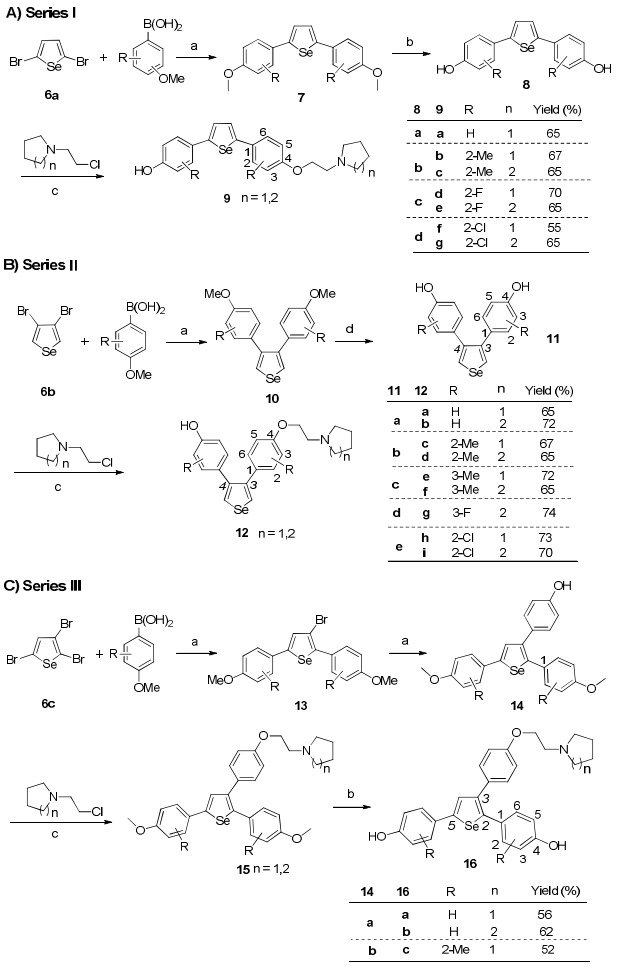
selenophenes exhibited substantial antiproliferative activity in breast cancer MCF-7 cells.[38] Thus, we wanted to introduce a basic side chain into the triphenol selenophene scaffold, as such selenophenes **16a**-**c** were obtained by demethylation of **15a**-**c**, which were prepared through Suzuki cross-coupling reactions followed by installation of a BSC (**Scheme 1C**). In the first step, 1 equiv of 2,3,5-tribromoselenophene **6c**[38] 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 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**.



8	9	R	n	Yield (%)
a	a	H	1	65
b	b	2-Me	1	67
	c	2-Me	2	65
c	d	2-F	1	70
	e	2-F	2	65
d	f	2-Cl	1	55
	g	2-Cl	2	65

11	12	R	n	Yield (%)
a	a	H	1	65
	b	H	2	72
b	c	2-Me	1	67
	d	2-Me	2	65
c	e	3-Me	1	72
	f	3-Me	2	65
d	g	3-F	2	74
e	h	2-Cl	1	73
	i	2-Cl	2	70

14	16	R	n	Yield (%)
a	a	H	1	56
	b	H	2	62
b	c	2-Me	1	52

Scheme 1. Synthesis of substituted selenophene derivatives. Reagents and conditions: (a) [Pd] catalyst, Na_2CO_3, toluene/water (1:1), reflux, 24 h; (b) BBr_3, CH_2Cl_2, -20 °C to rt, 4 h; (c) *N*-(2-chloroethyl)pyrrolidine or *N*-(2-chloroethyl)piperidine, KOH, K_2CO_3, DMF, microwave, 120 °C, 45 min; (d) Pyridine hydrochloride, 190 °C, 3 h.

2.2. Relative Binding Affinities.

The binding affinities of selenophene compounds for both ERα and ERβ 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 (E_2) 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 ERα. On the contrary, the vast majority of *series II* (apart from **12b** and **12c**) that located the basic side chain in the 3-phenol moiety display moderate levels of ERβ selectivity. The compound that shows the highest binding affinity for ERβ and moderate ER subtype selectivity of all of 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 ERα and ERβ, respectively; and it has an ERα/ERβ selectivity as low as 0.19 (**Table 1**, entry 6). Compared to the parent compound 2,5-bis(2-chloro-4-hydroxylphenyl)-selenophene[38] (RBA values were 6.11 for ERα and 12.7 for ERβ; α/β was 0.48), **9f** still retained high binding affinity for ERβ and displayed higher selectivity. The compound that has the highest ERα/ERβ selectivity is **12h** (ERα/ERβ ratio < 0.1), which also shows high RBA value for ERβ (23.3). As we expected, these 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 substitution position. Moreover, introduction of substituent in the phenol rings, has significant effects on the binding affinity and selectivity. Among the *series I*, the nonsubstituted compound **9a** showed a good binding affinity (12.4 and 1.02 for ERα and ERβ respectively). When the substitution occurred at *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 selectivity for ERα, and when basic side chains were introduced at C-3 position of the selenophene ring (*series II*), most of the compounds show good selectivity for ERβ (**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. 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 ERβ. Moreover, the binding affinities and selectivities for ERβ dramatically changed when the substitution was moved from C-2 position to C-3 position (**12c**-**12f**, entries 10-13). For comparison, trisubstituted selenophene analogues with basic side chain were also prepared (*series III*), except **16b** with undetectable ERβ binding affinity of less than 0.1, all showed good binding affinity for both ERα and ERβ (entries 17-19). The desired compound showing

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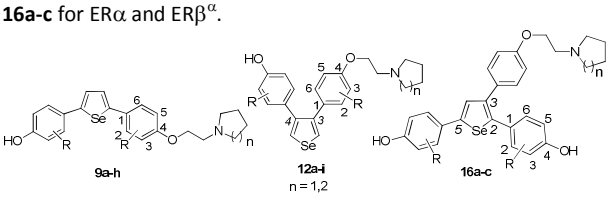
highest binding affinity for ERα was **16c** (13.8 for ERα), which also displayed a moderate ER subtype selectivity.

Table 1. Relative Binding Affinity (RBA) of compounds **9a-g**, **12a-i**, **16a-c** for ERα and ERβ[α].



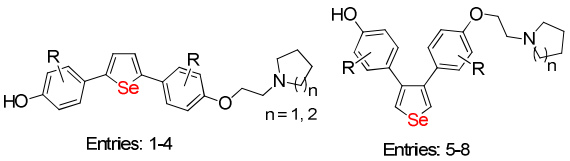
Entry	compound	Core	R	n	ERα[b]	ERβ[b]	α/β
1	**9a**		H	1	12.4 ± 0.42	1.02 ± 0.08	12.2
2	**9b**		2-Me	1	1.96 ± 0.11	0.58 ± 0.07	3.37
3	**9c**		2-Me	2	<0.1	0.62 ± 0.08	<0.1
4	**9d**		2-F	1	0.76 ± 0.01	<0.1	>10
5	**9e**		2-F	2	6.89 ± 1.02	0.63 ± 0.09	10.9
6	**9f**		2-Cl	1	4.59 ± 0.35	23.6 ± 0.49	0.19
7	**9g**		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**		2-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	0.48
18	**16b**		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]For simplify comparisons 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 $IC_{50}^{estradiol}$ / $IC_{50}^{compound}$ × 100 ± the range (RBA, estradiol = 100%). In these assays, the K_d value of estradiol is 3.1 nM for ERα and 3.4 nM for ERβ, respectively. For details, see Experimental Section.

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

Table 2. A direct comparison of RBA values to ERα and ERβ for the various biaryl selenophene compounds with a BSC in this work and the corresponding ones without a BSC studied previously.[38]



	This Work				Biaryl Selenophenes (without BSC)	
Entry	R	compd	RBA ERα	RBA ERβ	RBA ERα	RBA ERβ
1	H	**9a**	12.4 ± 0.42	1.02 ± 0.08	0.61 ± 0.034	2.87 ± 0.20
2	2-Me	**9b**	1.96 ± 0.11	0.58 ± 0.07	5.60 ± 0.43	11.1 ± 0.73
3	2-F	**9e**	6.89 ± 1.02	0.63 ± 0.09	5.90 ± 0.90	24.3 ± 0.52
4	2-Cl	**9f**	4.59 ± 0.35	23.6 ± 0.49	6.11 ± 0.05	12.7 ± 3.66
5	H	**12a**	2.06 ± 0.09	5.92 ± 0.28	0.32 ± 0.04	2.01 ± 0.11
6	2-Me	**12d**	0.56 ± 0.06	10.02 ± 0.17	0.27 ± 0.06	6.70 ± 0.49
7	3-Me	**12e**	2.03 ± 0.23	5.92 ± 0.14	0.71 ± 0.09	0.62 ± 0.12
8	2-F	**12g**	4.82 ± 0.32	15.3 ± 0.29	0.89 ± 0.32	1.95 ± 0.58

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/plates and allowed 24 hours to settle. Then the cells were transfected with a widely used 3 × ERE-luciferase reporter and an ERα or ERβ expression plasmid for agonist activity (% efficacy) and potency (EC_{50}) determinations. These cells were stimulated with increasing concentrations of 17β-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 next day. These results are summarized in **Table 3**, and dose-response curves for representative samples are shown in **Figure 3**.

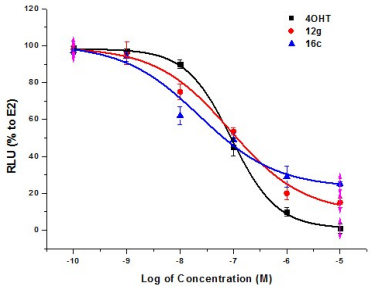


Figure 3. Illustrative dose-response curves for the ERα antagonist effects of 4TOH, and two selenophene-core compounds **12g** and **16c**. Efficacy values are the mean ± SD from three experiments. For details, see the Experimental Section.

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 ERα and ERβ, most of which show potent and highly efficacious ERα agonists and ERβ antagonists respectively. Compound **9a** with pyrrolidinyl side chain was a weak agonist of ERα and antagonist of ERβ with nanomolar to micromolar range IC_{50}, however, addition of a methyl group to **9a** converted it from agonist into antagonist for ERα and a strong antagonist for

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ERβ (**9b**, IC_{50} = 0.24 and 0.146 μM for ERα and ERβ, respectively). Compared with **9b**, compound **9c** prepared by *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 ERα agonist, being about 4 to 6-fold more potent than **9b**, whereas the chloro analogues **9f** and **9g** had little effect on ERα (**Table 3**, entries 4, 5 and 6, 7). However, when the halogen group was introduced into these compounds, the antagonistic activity for ERβ significantly increased (**Table 3**, entries 4 and 6).

Comparisons of the position of basic side chains in selenophene core indicate that 3,4-disubstituted compounds (*series II*) with decreased ERα binding affinity (**Table 1**, **12a**-**i**), which also demonstrate decreased efficacy as ERα agonists in general (**Table 3**). Interestingly, these 3, 4-disubstituted ligands show improved ERβ binding affinity, also display increased potency as ERβ antagonist, whereas **12b** profiled as the moderate ERβ agonist with μM range IC_{50} but had low efficacy. When the methyl group was changed from the C2- to C3-position (**12e**, **12f**), these compounds profiled as antagonist on ERβ, and were more efficacious compared to **12c** and **12d** (**Table 3**, entries 12 vs 10; 13 vs 11). It was remarkable that **12e** could act as a potent ERβ antagonist with a nanomolar IC_{50} value (5 nM).

Addition of a third substituent onto the selenophene core also having drastic effects on transcriptional activity. We prepared three compounds **16a**-**c** that showed potent and highly efficacious ERα and ERβ antagonists, especially for compound **16c**, which gave the low IC_{50} values of 0.013 and 0.016 μM for ERα and ERβ, respectively. This makes **16c** the most potent antagonist for ERα 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 keeps 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 antiproliferative activity of this type of compounds, all selenophene-core compounds were screened against MCF-7, and the results were summarized in **Table 3**.

Overall, most of 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 the value of IC_{50} 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 ERα and ERβ; meanwhile, those 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.

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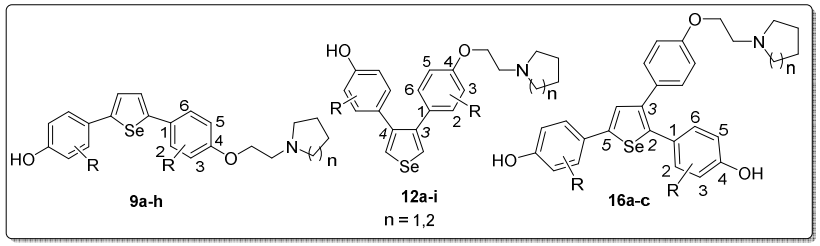
Table 3. Effects of selenophene-core compounds on the transcriptional activities of Estrogen Receptors α and β and antiproliferative activity in MCF-7 cells [a].



		Agonist Mode[b]				Antagonist Mode[c, d, e]				MCF-7
		R n	ERα		ERβ		ERα		ERβ	
Entry	cmpd		EC_{50} (μM)	Eff (%E_2)	EC_{50} (μM)	Eff (%E_2)	IC_{50} (μM)	Eff (%E_2)[c]	IC_{50} (μM)	Eff (%E_2)	IC_{50} (μM)[e]
1	**9a**	H 1	0.219	15.3 ± 4.8	-	-	1.213	71.5 ± 16.7	1.266	83.9 ± 7.7	5.77 ± 0.06
2	**9b**	2-Me 1	-	-	0.24	72.3 ± 12.5	0.874	78.3 ± 27.8	0.146	4.6 ± 15	53.8± 4.24
3	**9c**	2-Me 2	2.369	11.2 ± 17.2	-	-	0.041	52.3 ± 7.7	1.235	78.3 ± 29.4	25.3 ± 0.55
4	**9d**	2-F 1	0.136	86.9 ± 26.6	-	-	-	-	1.120	92.1 ± 10.9	>100
5	**9e**	2-F 2	0.204	68.4 ± 13.5	-	-	-	-	1.945	75.4 ± 4.2	>100
6	**9f**	2-Cl 1	-	-	-	-	0.415	69.7 ± 21.9	0.299	49.4 ± 11.1	7.81 ± 0.13
7	**9g**	2-Cl 2	0.911	19.9 ± 5.0	-	-	-	-	2.96	24.9 ± 14.4	>100
8	**12a**	H 1	1.003	45.9 ± 6.3	-	-	-	-	0.102	46.7 ± 12.3	>100
9	**12b**	H 2	-	-	1.147	53.5 ± 11	1.589	89.2 ± 8.3	0.451	22.8 ± 8.9	8.13 ± 0.46
10	**12c**	2-Me 1	-	-	-	-	0.179	21.4 ± 1.7	0.043	66.7 ± 3.6	16.6 ± 1.34

11	**12d**	2-Me	2	0.158	23.6 ± 19.7	-	-	0.237	84.6 ± 21.3	1.335	43.4 ± 10.2	15.8 ± 0.98
12	**12e**	3-Me	1	0.116	36.5 ± 4.9	-	20.8 ± 3	0.836	87.4 ± 5.6	0.005	34.1 ± 5.8	12.8 ± 2.40
13	**12f**	3-Me	2	0.721	30.3 ± 12.2	-	-	-	-	0.415	30.3 ± 12.2	>100
14	**12g**	2-F	2	-	-	-	-	0.069	15.8 ± 2.6	0.54	45.0 ± 1.2	15.6 ± 2.41
15	**12h**	2-Cl	1	-	-	-	-	0.769	38.5 ± 16.4	1.159	68.1 ± 19.6	10.9 ± 1.12
16	**12i**	2-Cl	2	-	-	-	-	0.413	77.8 ± 25.7	0.331	37.7 ± 11.0	14.1 ± 2.27
17	**16a**	H	1	-	-	-	-	0.974	56.2 ± 9.4	0.475	49.2 ± 9.9	4.95 ± 0.26
18	**16b**	H	2	1.831	9.2 ± 3.3	-	-	0.779	91.2 ± 10.4	5.391	33.8 ± 14.2	7.04 ± 0.49
19	**16c**	2-Me	1	-	-	-	-	0.013	24.8 ± 4.5	0.016	170.0 ± 1.0	7.05 ± 0.82
20	**4OHT**			0.008	35 ± 3	-	-1 ± 1	0.003	35 ± 3	0.001	-20 ± 2	15.6 ± 1.77

[a]Luciferase activity was measured in HEK293T cells transfected with 3 × ERE-driven luciferase reporter and expression vectors encoding ERα or ERβ and treated in triplicate with increasing doses (up to 10^{-5} M) of the compounds. [b]EC_{50} and standard deviation (mean ± SD), shown as a percentage of 10^{-8} M 17β-estradiol (E_2), were determined. [c]IC_{50} and standard deviation (mean ± SD) were determined in the percentage of 10^{-8} M 17β-estradiol (E_2) on ERα or ERβ. [d]ERs have considerable basal activity in HEK293T cells; compounds with inverse agonist activity are given negative efficacy values. Omitted EC_{50} or IC_{50} values were too high to be determined accurately; Omitted Eff (%E2) values were too low to be accurately determined. [e]IC_{50} values are an average of at least three independent experiments ± standard deviation (mean ± SD).

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In order to determine that the antiproliferative activity of these compounds arises from their antiestrogenicity on ER, the cells were incubated with 50 μM of **9a**, **12b** and **16a** in the presence and absence of 10 nM E_2. As shown in **Figure 4**, after incubation with different doses of ligands, relative cell activities decreased substantially. Exogenous estradiol could also restore part 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.

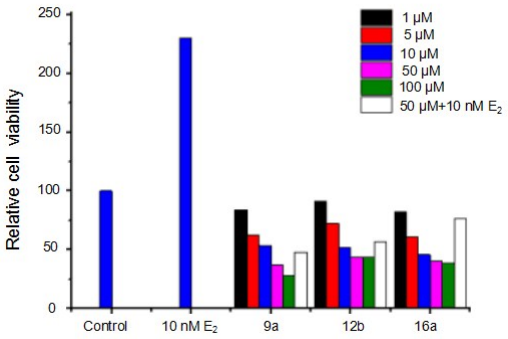

Figure 4. Effect of E_2, **9a**, **12b** and **16a** on the proliferation of hormone-dependent breast cancer MCF-7 cells after 5 days of culture. Nontreated MCF-7 cells are used as the control set at 100 %. Mean of three separate experiments ± range.

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

Estradiol supports transcriptional activation of ERα and ERβ by stabilizing helix 12 in a position where it forms a hydrophobic groove for binding transcriptional coactivators. The traditional 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** (**Figure 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 (**Figure 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, for compound **12e** (**Figure 5B**) mimics the binding orientation of raloxifene, with the BSC on one of the phenol group directly interacting with any helix 12 residues, which is consistent with raloxifene, thus giving **12e** potent ERα antagonist activity. For triaryl substituted selenophene **16c**, the ligand is consistent with raloxifene: the similar hydrogen bonds and directly displacing helix 12 with BSC to those of raloxifene and **12e**, the second phenolic hydroxy of **16c** also forms hydrogen bond with the residue His524 on helix 11 (**Figure 5D**), which makes the **16c** the most potent antagonist for ERα of the whole series.

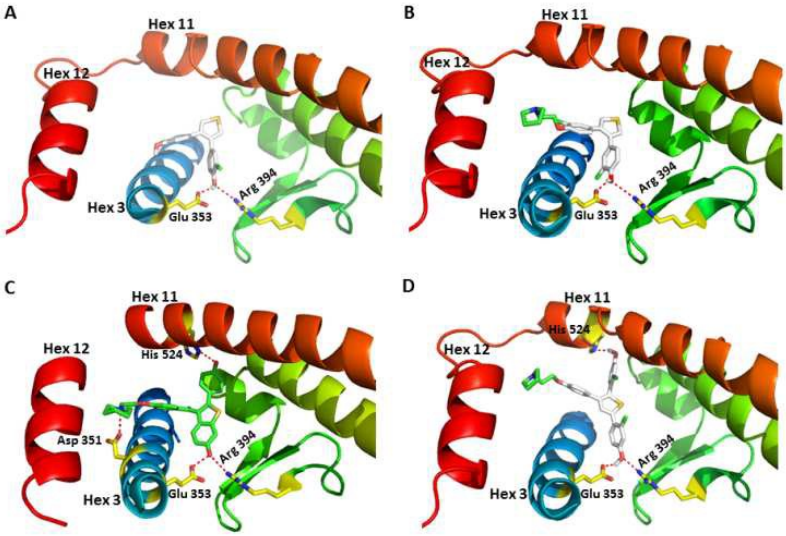

Figure 5. Model of selenophene ligands **11c**, **12e** and **16c** bound to ERα and comparisons with raloxifene. (A) Computer-developed model of **11c** bound to the ERα (PDB: 1ERR). The phenolic hydroxyl group of **11c** forms hydrogen bonding interactions with residue Glu353 on helix 3 and residue Arg394 on helix 6 respectively. (B) Computer-developed model of **12e** bound to the ERα with the conserved H-bonds to Glu353 and Arg394 residues. The basic side chain of the **6e** is oriented toward helix 12. (C) Crystal structure of the ERα 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 ERα 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.

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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 the value of IC_{50} 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 basic side chain had a significant effect on 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, Aladin-reagent, and Alfa-Asar and were used without purification. Toluene was freshly distilled from sodium, and dichloromethane was distilled from anhydrous CaH_2. 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).

^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AVANCE III 400 (400 MHz, ^{1}H NMR; 101 MHz, ^{13}C 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 Ar atmosphere, a mixture of bromoselenophene (1 equiv), arylboronic acid (3 equiv for disubstituted, 4 equiv for trisubstituted selenophenes), 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 Na_2SO_4 and then filtered and concentrated in vacuum. The product was purified by column chromatography (CC).

General procedure for ether cleavage. Method A: under Ar atmosphere, to a solution of 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 (Na_2SO_4) and evaporated. The residue was purified by silica gel column chromatography (CC).

Method B: a mixture of methoxyphenyl selenophene derivative (1 equiv) and pyridine hydrochloride (10 equiv) was stirred at 190 ^{o}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 Na_2SO_4 and then filtered and concentrated in vacuum. The product was purified by column chromatography (CC).

General procedure for the installation of Basic Side Chain by Williamson ether synthesis. To a mixture of phenolic selenophene derivative (1 equiv) and *N*-(2-chloroethyl)pyrrolidine or *N*-(2-chloroethyl)piperidine (1.1 equiv) in DMF was added K_2CO_3 (4 equiv) and KOH (4 equiv). The reaction mixture was stirred for 45 mins under microwave conditions. The reaction mixture was poured into water, and extracted with ethyl acetate. The extracts were dried (Na_2SO_4) and evaporated. The residue was purified by silica gel column chromatography (CC).

2-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenyl)-5-(4-hydroxylphenyl)-selenophene 9a. Compound **9a** was prepared by 2,5-bis(4-hydroxylphenyl)selenophene (**8a**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H 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). ^{13}C 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 $C_{22}H_{23}NO_2Se$ [M + H]$^+$, 414.0972; found 414.0976.

2-(2-Methyl-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-5-(2-methyl-4-hydroxylphenyl)selenophene 9b. Compound **9b** was prepared by 2,5-bis(2-methyl-4-hydroxylphenyl)selenophene (**8b**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H 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). ^{13}C 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 $C_{24}H_{27}NO_2Se$ [M + H]$^+$, 442.1285; found 442.1286.

2-(2-Methyl-4-(2-(piperidin-1-yl)ethoxy)phenyl)-5-(2-methyl-4-hydroxylphenyl)selenophene 9c. Compound **9c** was prepared by 2,5-bis(2-methyl-4-hydroxylphenyl)selenophene (**8b**) and *N*-(2-chloroethyl)piperidine according to general procedure for

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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); ^{1}H 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). ^{13}C 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 $C_{25}H_{29}NO_2Se$ [M + H]$^+$, 454.1450; found 454.1452.

2-(2-Fluoro-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-5-(2-fluoro-4-hydroxylphenyl)selenophene 9d. Compound **9d** was prepared by 2,5-bis(2-fluoro-4-hydroxylphenyl)selenophene (**8c**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C 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 $C_{22}H_{21}F_2NO_2Se$ [M + H]$^+$, 450.0784; found 450.0781.

2-(2-Fluoro-4-(2-(piperidin-1-yl)ethoxy)phenyl)-5-(2-fluoro-4-hydroxylphenyl)selenophene 9e. Compound **9e** was prepared by 2,5-bis(2-fluoro-4-hydroxylphenyl)selenophene (**8c**) and *N*-(2-chloroethyl)piperidine according to 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); ^{1}H NMR (400 MHz, 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, 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 $C_{23}H_{23}F_2NO_2Se$ [M + H]$^+$, 464.0940; found 464.0948.

2-(2-chloro-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-5-(2-chloro-4-hydroxylphenyl)selenophene 9f. Compound **9f** was prepared by 2,5-bis(2-chloro-4-hydroxylphenyl)selenophene (**8d**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H NMR (400 MHz, 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, 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 $C_{22}H_{21}Cl_2NO_2Se$ [M + H]$^+$, 482.2830; found 482.2830.

2-(2-Chloro-4-(2-(piperidin-1-yl)ethoxy)phenyl)-5-(2-chloro-4-hydroxylphenyl)selenophene 9g. Compound **9g** was prepared by 2,5-bis(2-fluoro-4-hydroxylphenyl)selenophene (**8d**) and *N*-(2-chloroethyl)piperidine according to 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); ^{1}H NMR (400 MHz, 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, 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 $C_{23}H_{23}Cl_2NO_2Se$ [M + H]$^+$, 496.0349; found 496.0341.

3,4-Bis(2-chloro-4-methoxylphenyl)selenophene 10e. Compound **10e** was prepared by 3,4-dibromoselenophene (**6b**) and 2-chloro-4-methoxylphenylboronic acid according to general procedure for Suzuki coupling. Purification by CC (petroleum ether:ethyl acetate = 98:2) gave the title compound as a yellow solid; ^{1}H 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-hydroxylphenyl)selenophene 11e. Compound **11e** was prepared by 3,4-bis(2-chloro-4-methoxylphenyl)selenophene (**10e**) using boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether:ethyl acetate = 4:1) gave the title compound as a yellow solid; ^{1}H 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-hydroxylphenyl)-selenophene 12a. Compound **12a** was prepared by 3,4-bis(4-hydroxylphenyl)selenophene (**11a**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H 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, 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 $C_{22}H_{23}NO_2Se$ [M + H]$^+$, 414.0972; found 414.0970.

3-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenyl)-4-(4-hydroxylphenyl)-selenophene 12b. Compound **12b** was prepared by 3,4-bis(4-hydroxylphenyl)selenophene (**11a**) and *N*-(2-chloroethyl)piperidine according to general procedure for Williamson ether synthesis.

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Purification by CC (petroleum ether:ethyl acetate = 1:2) gave the title compound as a yellow solid (72% yield; mp 159-163 °C); ^{1}H 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, 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 $C_{23}H_{25}NO_2Se$ [M + H]$^+$, 428.1129; found 428.1120.

3-(2-Methyl-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4-(2-methyl-4-hydroxylphenyl)selenophene 12c. Compound **12c** was prepared by 3,4-bis(2-methyl-4-hydroxylphenyl)selenophene (**11b**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H 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 $C_{24}H_{27}NO_2Se$ [M + H]$^+$, 442.1285; found 442.1283.

3-(2-Methyl-4-(2-(piperidin-1-yl)ethoxy)phenyl)-4-(2-methyl-4-hydroxylphenyl)selenophene 12d. Compound **12d** was prepared by 3,4-bis(2-methyl-4-hydroxylphenyl)selenophene (**11b**) and *N*-(2-chloroethyl)piperidine according to 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); ^{1}H 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 $C_{25}H_{29}NO_2Se$ [M + H]$^+$, 454.1450; found 454.1455.

3-(3-Methyl-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4-(3-methyl-4-hydroxylphenyl)selenophene 12e. Compound **12e** was prepared by 3,4-bis(3-methyl-4-hydroxylphenyl)selenophene (**11c**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H 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 $C_{24}H_{27}NO_2Se$ [M + H]$^+$, 442.1285; found 442.1283.

3-(3-Methyl-4-(2-(piperidin-1-yl)ethoxy)phenyl)-4-(3-methyl-4-hydroxylphenyl)selenophene 12f. Compound **12f** was prepared by 3,4-bis(3-methyl-4-hydroxylphenyl)selenophene (**11c**) and *N*-(2-chloroethyl)piperidine according to 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); ^{1}H 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 $C_{25}H_{29}NO_2Se$ [M + H]$^+$, 454.1450; found 454.1448.

3-(3-Fluoro-4-(2-(piperidin-1-yl)ethoxy)phenyl)-4-(3-fluoro-4-hydroxylphenyl)selenophene 12g. Compound **12g** was prepared by 3,4-bis(3-fluoro-4-hydroxylphenyl)selenophene (**11d**) and *N*-(2-chloroethyl)piperidine according to 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); ^{1}H 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 $C_{23}H_{23}F_2NO_2Se$ [M + H]$^+$, 462.0948; found 462.0942.

3-(2-chloro-4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-4-(2-chloro-4-hydroxylphenyl)selenophene 12h. Compound **12h** was prepared by 3,4-bis(2-chloro-4-hydroxylphenyl)selenophene (**11e**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H 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,

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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 $C_{22}H_{21}Cl_2NO_2Se$ $[M + H]^+$, 482.0193; found 482.0190.

3-(2-Chloro-4-(2-(piperidin-1-yl)ethoxy)phenyl)-4-(2-chloro-4-hydroxylphenyl)selenophene 12i. Compound **12i** was prepared by 3,4-bis(2-chloro-4-hydroxylphenyl)selenophene (**11e**) and *N*-(2-chloroethyl)piperidine according to 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); ^{1}H 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 $C_{23}H_{23}Cl_2NO_2Se$ $[M + H]^+$, 496.0349; found 496.0346.

3-(4-hydroxylphenyl)-2,5-bis(4-methoxylphenyl)selenophene 14a. Compound **14a** was prepared by 3-bromo-2,5-bis(4-methoxylphenyl)selenophene (**13a**) and 4-hydroxylphenylboronic acid according to general procedure for Suzuki Coupling. Purification by CC (petroleum ether:ethyl acetate = 30:1) gave the title compound as a yellow solid; ^{1}H 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-hydroxylphenyl)-2,5-bis (2-methyl-4-methoxylphenyl)-selenophene 14b. Compound **14b** was prepared by 3-bromo-2,5-bis(2-methyl-4-methoxylphenyl)selenophene (**13b**) and 4-hydroxylphenylboronic acid according to general procedure for Suzuki Coupling. Purification by CC (petroleum ether:ethyl acetate = 30:1) gave the title compound as a yellow solid; ^{1}H 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-hydroxylphenyl)selenophene 16a. Compound **16a** was obtained from the demethylation of compound **15a** using boron tribromide, the latter was prepared by 3-(4-hydroxylphenyl)-2,5-bis(4-methoxylphenyl)selenophene (**14a**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H NMR (400 MHz, 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, 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 $C_{28}H_{27}NO_3Se$ $[M + H]^+$, 506.1234; found 506.1231.

3-(4-(2-(Piperidin-1-yl)ethoxy)phenyl)-2,5-bis (4-hydroxylphenyl)selenophene 16b. Compound **16b** was obtained from the demethylation of compound **15b** using boron tribromide, the latter was prepared by 3-(4-hydroxylphenyl)-2,5-bis(4-methoxylphenyl)selenophene (**14a**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H NMR (400 MHz, 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, 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 $C_{29}H_{29}NO_3Se$ $[M + H]^+$, 520.1391; found 520.1398.

3-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenyl)-2,5-bis (2-methyl-4-hydroxylphenyl)selenophene 16c. Compound **16c** was obtained from the demethylation of compound **15c** using boron tribromide, the latter was prepared by 3-(4-hydroxylphenyl)-2,5-bis(4-methoxylphenyl)selenophene (**14b**) and *N*-(2-chloroethyl)pyrrolidine according to 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); ^{1}H NMR (400 MHz, 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, 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 $C_{30}H_{31}NO_3Se$ $[M + H]^+$, 534.1537; found 534.1539.

4.2. Gene Clone and Protein Purification. Human ERα or ERβ ligand binding domain (LBD) genes were amplified by PCR from plasmid pVP-16-ERα and pVP-16-ERβ. The PCR product was cloned into plasmid, and PGEx-KG *E. coli* BL21 (DE3) used for the overexpression of ER-LBD. The cells were induced by IPTG (10 μM) for 2 h, then 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 applied to 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

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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 fluorescence tracer (coumestrol, Sigma-Aldrich, MO) and 0.8 μM purified human ERα or ERβ ligand binding domain (LBD) were diluted in 100 mM potassium phosphate buffer (pH 7.4), containing 100 μg/mL bovine gamma globulin (Sigma-Aldrich, MO). Incubations were for 2h 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 17β-estradiol set to 100%. The values given are the average ± range of two independent determinations. IC_{50} values were calculated according to equations described previously.[46]

4.4 Gene Transcriptional Activity. The human embryonic kidney cell lines, 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 mixture per well, containing 300 ng of 3 × ERE-luciferase reporter, 100 ng of ERα or ERβ expression vector, 125 mM calcium chloride (GuoYao, China) and 12.5 μL 2 × HBS. The next day, the cells were treated with increasing doses of ER ligands diluted in phenol red-free DMEM with 10% FBS. After 24h, luciferase activity was measured using 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 lines 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 triplicate for 72h. MTT colormetric tests (Biosharp, China) were employed to determine cell viability per manufacturer instructions. IC_{50} values were calculated according to the following equation using Origin software: Y = 100% inhibition + (0% inhibition - 100% inhibition)/ $(1 + 10^{[(LogIC50-X)\times Hillslope]})$, 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 ERα 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 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.

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Conflict of Interest

The authors declare no competing interests.

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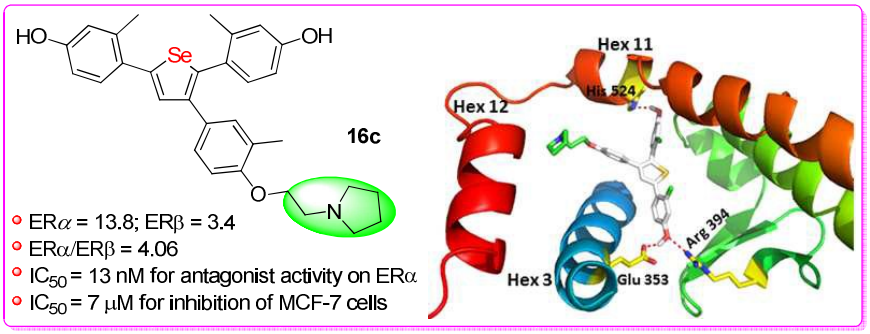

Selenophenes with basic side chains as novel estrogen receptor ligands showed excellent antagonist activity and better antiproliferative activity than that of 4-hydroxytamoxifen in breast cancer MCF-7 cells.