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Synthesis of Triphenylethylene Bisphenols as Aromatase Inhibitors That Also Modulate Estrogen Receptors

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Supporting Information



ABSTRACT: A series of triphenylethylene bisphenol analogues of the selective estrogen receptor modulator (SERM) tamoxifen were synthesized and evaluated for their abilities to inhibit aromatase, bind to estrogen receptor α (ER- α) and estrogen receptor β (ER- β), and antagonize the activity of β -estradiol in MCF-7 human breast cancer cells. The long-range goal has been to create dual aromatase inhibitor (AI)/selective estrogen receptor modulators (SERMs). The hypothesis is that in normal tissue the estrogenic SERM activity of a dual AI/SERM could attenuate the undesired effects stemming from global estrogen depletion caused by the AI activity of a dual AI/SERM, while in breast cancer tissue the antiestrogenic SERM activity of a dual AI/SERM could attenuate the antiproliferative effect. The potent aromatase inhibitory activities and high ER- α and ER- β binding affinities of several of the resulting analogues, together with the facts that they antagonize β -estradiol in a functional assay in MCF-7 human breast cancer treatment.

■ INTRODUCTION

Aromatase (also known as CYP19) is a member of the general class of cytochrome P450 enzymes. It catalyzes the conversion of androgens to estrogens, which is a crucial step in the biosynthesis of estrogens in the human body.¹ Aromatase inhibitors (AIs) have been widely used for treatment of hormone-receptor-positive breast cancer in postmenopausal women. Currently, three AIs [letrozole (1), anastrozole (2), and exemestane (3), Figure 1] have been approved by the FDA. Comparative clinical trials involving postmenopausal women with breast cancer have demonstrated that AIs are superior to the selective estrogen receptor modulator (SERM) tamoxifen (4) (Figure 1), which, as with other SERMs, blocks estrogen receptors in breast cancer tissue while stimulating them in a variety of normal tissues.²⁻⁶ In the 5-year ATAC (arimidex, tamoxifen, alone or in combination) trial, the use of anastrozole alone resulted in a 13% improvement of diseasefree survival, 21% reduction in the time-to-recurrence, 42% reduction in occurrence of contralateral breast cancer, and 14%

reduction in risk of distant metastasis when compared to tamoxifen alone.⁴ The use of AIs is also reported to cause fewer vaginal bleeding events, thromboembolic events, and endometrial cancer occurrences than tamoxifen.^{4–6} However, the use of AIs is associated with serious side effects. Since AIs nonselectively deplete estrogen in the whole body, they lead to severe musculoskeletal pain, reduction of bone density, and an increased frequency of bone fractures and cardiovascular events.^{7–11} According to the 5-year ATAC trial, anastrozole treatment led to a higher incidence of bone fractures (11% vs 7.7%) and arthralgia (35.6% vs 29.4%) than tamoxifen.⁴ The increased musculoskeletal pain caused by AIs negatively impacts patient compliance. Reported AI discontinuation rates attributed to severe musculoskeletal symptoms range from 13% to 52%.^{12–14} Nonadherence rates are also high.^{15,16} For example, observations from three data sets indicate that

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Figure 1. Structures of the aromatase inhibitors letrozole, anastrozole, exemestane, and the selective estrogen receptor modulator tamoxifen.



Figure 2. Structures and biological activities of (E,Z)-norendoxifen, Z-norendoxifen, E-norendoxifen, and 4'-hydroxynorendoxifen.

only 62–79% of women adhere (take anastrazole more than 80% of the days during the treatment period) after 3 years.¹⁶

One possible approach to improve the efficacy and decrease the side effects associated with AIs is to build SERM activity into them. The estrogenic activity of a dual AI/SERM due to binding to and stimulation of estrogen receptors (ERs) in noncancerous musculoskeletal tissue could counteract some of the negative effects of the dual AI/SERM that result from global estrogen loss due to aromatase inhibition. On the other hand, the antagonistic blockade of ERs in breast cancer cells by a dual AI/SERM might act synergistically with the decrease in estrogen concentration due to aromatase inhibition, assuming that the inhibition of estrogen production is not totally complete. In other words, as with the SERMs, the ER agonist effect of a dual AI/SERM would be beneficial in normal, noncancerous musculoskeletal tissue relative to an AI alone by decreasing the side effects that result from estrogen depletion, while the ER antagonist effects of a dual AI/SERM would be beneficial in breast cancer cells by blocking the effect of residual estrogen resulting from incomplete aromatase inhibition. In fact, according to Brodie et al., a combination of the aromatase inhibitor letrozole and the estrogen receptor antagonist/downregulator fulvestrant was more effective than either letrozole or fulvestrant alone in suppressing breast tumor growth and in delaying the development of tumor resistance.^{17–19} In that case, the delay in development of resistance was thought to result from down-regulation of the ER by fulvestrant and an associated down-regulation of signaling proteins that play a role in the maintenance of hormonal resistance.¹⁹ Meanwhile, it is also possible that the estrogenic component of the SERM activity of a dual AI/SERM agents could stimulate estrogen receptors in noncancerous musculoskeletal tissues and ameliorate the side effects caused by estrogen depletion of conventional AIs (e.g., osteoporosis, musculoskeletal pain, and bone fractures). In fact, according to the ATAC trail, a combination of anastrozole with tamoxifen resulted in fewer fractures than when anastrozole was used alone.²⁰ As expected,

no pharmacological significance.²²

the combination resulted in more fractures than tamoxifen

alone, indicating that the fractures were the result of the AI and not the SERM.²⁰ For these reasons, dual AI/SERM agents

could possibly have superior efficacy and decreased side effects

compared to conventional AIs alone. However, the combina-

tion of anastrozole with tamoxifen resulted in a greater

incidence of endometrial cancer (0.3%) than anastrozole

alone (0.1%), and in this regard it may be better to combine

raloxifene-type SERM activity than tamoxifen-type SERM

activity with an AI (different SERMs produce unique spectra

of agonist activities in different normal tissues because they

have different affinities for ER- α and ER- β , which are expressed to different extents in various tissues, and the consequences of

binding to ER- α and ER- β are different).²¹ Although the ATAC

trial showed no therapeutic advantage of a specific tamoxifen

plus anastrozole combination with regard to anticancer

activity,²⁰ this result should not be generalized to all SERM

plus AI combinations or to a hypothetical dual AI/SERM agent.

Of note, the serum concentration of anastrozole, a relatively

weak aromatase inhibitor, was 27% lower in the combination

arm of the ATAC trial, although it was claimed that this was of

norendoxifen (5) was reported in 2013.²⁴ Biological testing

results confirmed the aromatase inhibitory activity of (E,Z)-

norendoxifen and further established high affinity for both ER-

 α and ER- β (Figure 2), establishing (*E*,*Z*)-norendoxifen as the

first substance with potential dual AI and ER binding

structure-based drug design approach. This led to the discovery

Norendoxifen is a metabolite of tamoxifen, and it is also a potent aromatase inhibitor.²³ The synthesis of (E,Z)-



			HO		ОН	
				Aromatase	ER-a	ER-β
Cpd	R_1	R ₂	R ₃	(IC ₅₀ , nM)	(EC50, nM)	(EC ₅₀ , nM)
7	-CH ₂ CH ₃	-H	-H	24900 ± 1400	80% competition	307 ± 106
11	-CH ₂ CN	-H	-H	12800 ± 2000	56% competition	49% competition
12		-H	-H	4.77 ± 0.38	27.3 ± 5.2	40.9 ± 12.1
13	N N	-H	-H	137 ± 6	12% competition	33% competition
17a	-CH ₂ CN	-OH	-H	15200 ± 300	0% competition	3% competition
18a	X N N	-OH	-H	60.0 ± 4.1	97.8 ± 42.3	73.6 ± 29.9
19a		-OH	-H	3030 ± 150	45% competition	40% competition
20a	N N	-OH	-H	12500±400	943 ± 285	1080 ± 100
17b	-CH ₂ CN	-OH	-F	17200 ± 2200	0% competition	0% competition
18b	N N	-OH	-F	94.4 ± 3.5	85.2 ± 14.2	56.3 ± 17.8
19b		-OH	-F	2980 ± 50	2% competition	0% competition
20b		-OH	-F	14200 ± 1100	0% competition	0% competition
25	-CH ₂ CN	$-\mathrm{NH}_2$	-H	36.3 ± 0.8	0% competition	0% competition
26	N N	$-\mathrm{NH}_2$	-H	5.24 ± 0.41	1830 ± 910	296 ± 154
27	N N N	$-\mathrm{NH}_2$	-H	104 ± 9	0% competition	2% competition
28		-NH ₂	-H	17.7 ± 1.2	13% competition	36% competition
32	N N	-H	-OH	60.4 ± 1.7	0% competition	29% competition
35	× N N	-H	-NH ₂	439 ± 16	60% competition	71% competition

^aThe values are mean values of at least three experiments. Percent ER competition was determined at the concentration of 100 μ M for each compound. EC₅₀ values were determined only for compounds that displayed >90% competition.



Figure 3. Design strategy for triphenylethylene bisphenol analogues.

of 4'-hydroxynorendoxifen (6), which has elevated potency against aromatase and higher affinity for ER- α and ER- β . It is also a more potent antagonist of estradiol-stimulated progesterone receptor mRNA expression in MCF-7 cells compared to norendoxifen.²⁶

Like 4-hydroxytamoxifen, most norendoxifen analogues undergo facile E/Z isomerization in solution.²⁴ Although the detailed mechanism is still controversial, the E/Z isomerization is considered to be facilitated by the presence of a phenolic hydroxyl group in one of the para positions.²⁷ The rate of isomerization speeds up as the number of para phenolic hydroxyl group increases, and the rate is also dependent on the solvent and temperature.²⁸ The E/Z isomerization makes the preparation of pure E and Z isomers of norendoxifen analogues difficult. Moreover, since isomerization happens both in stock solutions and during biological testing, it also influences the accuracy of the biological testing results for pure *E* and *Z* isomers. To develop more promising norendoxifen analogues for treatment of breast cancer, it is important to prepare analogues as pure *E* and *Z* isomers. A mixture of *E* and *Z* isomers would complicate the pharmacological profiles and limit the use of the drugs because the *E* and *Z* isomers would be expected to have different biological activities against aromatase, ERs, and other CYPs. This argument is in fact supported by results derived from testing tamoxifen, which document different relative binding affinities of the two isomers for the ER, as well as different agonist vs antagonist properties as monitored by uterine weight.²⁹ Furthermore, the isomerization of (*Z*)-4-hydroxytamoxifen to its less active isomer is thought to contribute to tamoxifen resistance.³⁰

To overcome the problem presented by E/Z isomerization, a series of triphenylethylene bisphenol analogues were designed



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^{*a*}Reagents and conditions: (a) 4,4'-dihydroxybenzophenone, Zn, TiCl₄, THF; (b) NaH, MOMCl, THF; (c) NBS, CCl₄; (d) KCN, THF, H₂O; (e) methanol, HCl; (f) NaH, imidazole, THF; (g) NaH, 1,2,4-triazole, THF.

Scheme 2. Synthesis of Analogues 17-20^a



"Reagents and conditions: (a) NaH, PvCl, THF; (b) 4,4'-dihydroxybenzophenone, Zn, TiCl₄, THF; (c) NaH, MOMCl, THF; (d) NBS, CCl₄; (e) KCN, THF, H₂O; (f) methanol, HCl; (g) NaH, imidazole, THF; (h) KOH, THF, H₂O; (i) NaH, 1,2,4-triazole, THF.

by eliminating the aminoethoxy side chain of norendoxifen, thus resulting in two identical substituents on one end of the double bond and eliminating the possibility of E/Z isomers. This allowed a straightforward evaluation of the aromatase inhibitory activities, ER- α and ER-B binding affinities, and abilities of the compounds to antagonize β -estradiol-stimulated transcriptional activity in MCF-7 human breast cancer cells. The results of these studies will facilitate the development of a new generation of dual AI/SERM agents for breast cancer treatment.

RESULTS AND DISCUSSION

Design of Triphenylethylene Bisphenol Analogues. Compound 7 is a weak aromatase inhibitor, and it also shows moderate binding affinities to ER- α and ER- β (Table 1).²⁴ This substance is also a good ER antagonist without significant agonistic side effects in MCF-7 cells.³¹ On the basis of the structure of compound 7, the following structural modifications were explored to improve the potency (Figure 3): (1) incorporation of hydrogen bond donors (hydroxyl or amino groups) on the meta or para positions of the "A" ring; (2) introduction of iron-coordinating groups (nitrile, imidazole, or triazole groups) in the location of the ethyl group. Hydrogen bond donors on the "A" ring can be expected to form hydrogen bonds with aromatase and the ERs, while iron-coordinating groups could improve aromatase inhibitory activity by coordinating to the iron of aromatase.

Synthesis. A short and efficient synthetic route was established to prepare analogues with an iron-coordinating group in the location of the ethyl side chain (Scheme 1). The bisphenol 9 was first prepared by McMurry cross-coupling of acetophenone (8) with 4,4'-dihydroxybenzophenone as described.²⁵ The bisphenol 9 was treated with an excess of MOMCl to afford the diprotected product 10 in good yield. The protected intermediate 10 underwent a series of reactions, including bromination with NBS, alkylation of potassium cyanide, and deprotection of the MOM groups with HCl to afford the nitrile 11 in very good yield. The imidazole product 12 and triazole compound 13 were also obtained in good yield by treating 10 with similar sequential reactions, including



"Reagents and conditions: (a) Zn, TiCl₄, THF; (b) Boc₂O, dioxanes; (c) NBS, CCl₄; (d) KCN, H₂O, THF; (e) HCl, methanol; (f) NaH, imidazole, THF; (g) KOH, H₂O, THF; (h) NaH, 1,2,4-triazole, THF.





"Reagents and conditions: (a) NaH, PvCl, THF; (b) 4,4'-dihydroxybenzophenone, Zn, TiCl₄, THF; (c) NaH, MOMCl, THF; (d) NBS, CCl₄; (e) NaH, imidazole, THF; (f) KOH, H₂O, methanol; (g) HCl, methanol.

Scheme 5. Synthesis of Analogue 35^a



^{*a*}Reagents and conditions: (a) **22**, Zn, TiCl₄, THF; (b) Boc₂O, dioxanes; (c) NBS, CCl₄; (d) NaH, imidazole, THF; (e) KOH, H₂O, methanol; (f) HCl, methanol.

bromination with NBS, alkylation of imidazole or 1,2,4-triazole, and cleavage of the phenols.

Analogues 17a, 18a, 19a, and 20a were designed by incorporating a hydroxyl group on the "A" ring to probe the importance of a hydrogen bond donor in the para position. For analogues 17b, 18b, 19b, and 20b, a fluorine atom was introduced ortho to the "A" ring hydroxy group. The presence of the electronegative fluorine atom would increase the acidity of the hydroxy group and enable stronger hydrogen bonds to be formed. To prepare analogues 17-20, the corresponding hydroxylated acetophenones 14a,b were first protected with a pivaloyl group and the product was reacted with 4,4'dihydroxybenzophenone under the McMurry cross-coupling conditions to provide the bisphenols 15a,b (Scheme 2). The phenolic hydroxyl groups were protected by MOM groups to afford 16a,b. Compounds 16a,b were brominated with NBS, followed by alkylation of KCN, to install the nitrile group. Unexpectedly, the pivaloyl group was also cleaved under the alkylation reaction conditions. In the next step, the MOM protecting groups were removed with HCl to directly provide the products 17a,b. To prepare the imidazole products 18a,b, compounds 16a,b underwent a series of sequential reactions including bromination with NBS, alkylation of imidazole, deprotection of the pivaloyl group with KOH, and removal of the MOM groups under acidic conditions to afford 18a,b in good yield. Interestingly, subjection of 16a,b to a similar sequence of reactions incorporating 1,2,4-triazole instead of imidazole led to the production of two isomers in each case (i.e., 19a and 20a were obtained from 16a, and 19b and 20b were obtained from 16b) due to the presence of two nonequivalent nucleophilic nitrogens in the 1,2,4-triazole system vs only one for the imidazole case. Compounds 19a and 19b were isolated as the major products, and compounds 20a and 20b were the minor products.

In order to prepare analogues with an amino group in the para position of the "A" ring, 4-aminoacetophenone (21) was reacted with the diprotected 4,4'-dihydroxybenzophenone 22 under McMurry cross-coupling reaction conditions to afford 23

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(Scheme 3). The amino group was protected with a Boc group, and the product 24 was subjected to a series of sequential reactions, including bromination with NBS, alkylation of KCN (both pivaloyl groups were cleaved under these conditions), and removal of the Boc group with HCl to afford the product 25 in very good yield. The imidazole product 26 and triazole products 27 and 28 were also obtained by subjecting 24 to a similar set of reactions.

Analogue 32 was designed to probe the effect of introducing a hydroxyl group in the meta position of the "A" ring. The synthesis of 32 is outlined in Scheme 4. The phenolic hydroxyl group of 29 was first protected with a pivaloyl group. The product 30 reacted with 4,4'-dihydroxybenzophenone under McMurry cross-coupling reaction conditions, followed by protection of the phenolic hydroxyl groups with MOMCl, to afford 31. Compound 31 underwent bromination with NBS, alkylation of imidazole, and removal of the pivaloyl group and MOM groups to afford 32 in good yield.

To synthesize analogue **35** with a meta amino group in the "A" ring, 3-aminoacetophenone (**33**) was reacted with the diprotected 4,4′-dihydroxybenzophenone **22** under McMurry cross-coupling reaction conditions, followed by protection of the amino group with a Boc group to afford **34** (in Scheme 5). Then, compound **34** underwent bromination with NBS, alkylation with imidazole, and cleavage of the pivaloyl group and Boc group to provide **35** in good yield.

Biological Activities. The aromatase inhibitory activities and ER- α /ER- β binding affinities of the bisphenols are summarized in Table 1. Compound 11 with a nitrile side chain showed slightly improved aromatase inhibitory activity $(IC_{50} = 12\ 800\ nM)$ when compared with compound 7 $(IC_{50} =$ 24 900 nM), but it only displayed very weak binding affinity for both ER- α and ER- β . The imidazole compound 12 was the most potent aromatase inhibitor (IC₅₀ = 4.77 nM), and it also retained high binding affinities with both ER- α (EC₅₀ = 27.3 nM) and ER- β (EC₅₀ = 40.9 nM). Compound 13 with the triazole side chain was also a good aromatase inhibitor (IC_{50} = 137 nM) but had weak ER binding affinity. Similar structureactivity relationships were also observed for compound series 17a-20a and series 17b-20b. The nitrile compounds (17a and 17b) are weak aromatase inhibitors (IC₅₀ = 15200-17200nM), and they showed no binding affinity for ER- α and ER- β . The triazole compounds (19a,b and 20a,b) are moderate aromatase inhibitors (IC₅₀ = 2980-14200 nM), but they only showed weak binding affinities for ER- α (EC₅₀ \geq 943 nM) and ER- β (EC₅₀ \geq 1080 nM). The imidazole compounds (18a and **18b**) are very potent aromatase inhibitors (IC₅₀ = 60.0-94.4nM), and they also displayed good binding affinities for ER- α $(IC_{50} = 85.2-97.8 \text{ nM})$ and $ER-\beta$ $(IC_{50} = 56.3-73.6 \text{ nM})$. Compared with the "A" ring unsubstituted analogues 11-13, introducing a hydroxyl group in the para position of the "A" ring (analogues 17a-20a) unexpectedly resulted in moderate decreases in aromatase inhibitory activity or ER binding affinities. A comparison of series 17b-20b with series 17a-20a reveals that incorporating a fluorine atom ortho to the hydroxyl group produced minor effects on aromatase inhibitory activity and ER- α /ER- β binding affinity; except in the case of the two triazole systems, it significantly decreased ER- α /ER- β affinity. The introduction of an amino group in the para position of the "A" ring (analogues 25-28) produced minor effects on aromatase inhibitory activity, or in the case of the nitriles 11 vs 25, it increased the inhibitory activity dramatically $(IC_{50} \text{ of } 12 800 \text{ vs } 36.3 \text{ nM})$. However, the para amino group is

uniformly unfavorable for ER binding affinity. The imidazole **26** displayed much weaker binding affinities with ER- α (EC₅₀ = 1830 nM) and ER- β (EC₅₀ = 296 nM) compared with compound **12**, while compounds **25**, **27**, and **28** all have weak binding affinities with ER. Rotating the "A" ring para hydroxyl group to the meta position (**32** vs **18a**) did not influence aromatase inhibitory activity, but it decreased the binding affinities with ER- α and ER- β significantly. Rotating the "A" ring para amino group to the meta position (**35** vs **26**) decreased both aromatase inhibitory activity and ER binding affinities.

Transcriptional Activities in MCF-7 Human Breast Cancer Cells. To investigate the effects of ligand binding on ER-mediated transcriptional activities, the triphenylethylene bisphenols were tested for their abilities to antagonize β estradiol (E2) in a functional assay. Four compounds (12, 18a, 18b, and 26) were selected for this test because of their high binding affinities to both ER- α and ER- β . In this commonly used assay, the progesterone receptor (PGR) mRNA expression is used for assessing estrogenic or antiestrogenic activity in MCF-7 human breast cancer cells.³² As shown in Figure 4, E2 (10 nM) was able to significantly increase the PGR



Figure 4. Abilities of compounds **12, 18a, 18b, 26**, endoxifen, and (E,Z)-norendoxifen $(1 \ \mu M)$ to antagonize β -estradiol (E2, 10 nM)-stimulated progesterone receptor (PGR) mRNA expression in MCF-7 cells.

mRNA expression compared to the control, which contained only 0.1% methanol (vehicle). The PGR mRNA expression level with 10 nM E2 stimulation alone was set as 100%, and the antiestrogenic effects of the compounds were monitored by the reduction of stimulated mRNA levels. Endoxifen (positive control) can antagonize the PGR mRNA expression in the presence of 10 nM E2 to 10%, which is consistent with the published result.³² (*E*,*Z*)-Norendoxifen can also antagonize the stimulatory effects of E2, as PGR mRNA expression level was reduced to 33% as we previously reported.²⁵ All of the tested triphenylethylene bisphenol analogues (**12**, **18a**, **18b**, and **26**) were able to antagonize the ER-stimulated PGR mRNA expression to the levels of 26–31%, regardless of their different binding affinities with estrogen receptors. The antiestrogenic effects of the tested compounds as monitored by the reduction



Figure 5. (a) The hypothetical binding mode of **12** (green) in the active site of aromatase (PDB code 3s79)³³ overlapped with *E*-norendoxifen (pink). (b) The hypothetical binding mode of **12** (green) in the active site of ER- α (PDB code 3ert)³⁴ overlapped with *Z*-norendoxifen (pink).



Figure 6. Hypothetical binding mode of compound **36** (cyan) in the active site of aromatase (PDB code 3s79)³³ overlapped with *E*-norendoxifen (pink), and a comparison of aromatase inhibitory activities of compounds 7, 5, 12, and 36.

of estradiol-stimulated mRNA levels were weaker than endoxifen but very similar to (E,Z)-norendoxifen.

Molecular Modeling. Molecular docking studies were performed in order to investigate the possible binding mode of the triphenylethylene bisphenol analogues with aromatase and ER- α . Compound 12 was docked into the active site of aromatase (PDB code 3s79)33 with GOLD 3.0. The high potency of 12 compared with 7 (>5000-fold improvement in aromatase inhibitory activity) indicates imidazole-iron bonding. Therefore, a distance constraint (1.5-3.5 Å) was imposed between the imidazole nitrogen and iron during docking. The best docking pose of 12 (shown in Figure 5a) was overlapped with the hypothetical binding mode of E-norendoxifen (E-5) that was previously reported (see Supporting Information for more detailed molecular modeling results, including stereoviews, hydrogen bond angles, and distances calculated between hydrogens and hydrogen bond acceptors).²⁴ The binding mode of 12 is in general very similar to that of E-norendoxifen, a

result that is not surprising since the imidazole fragment was installed in 12 in a location for iron binding based on the structure calculated for the E-norendoxifen-aromatase complex. The imidazole group faces toward the heme and coordinates with the iron. One of the phenolic hydroxy groups forms a hydrogen bond with the backbone carbonyl group of Leu372, which is similar to E-norendoxifen binding. A notable difference between 12 and E-norendoxifen can be observed in their interaction with Ser478 and Asp309. For E-norendoxifen, a hydrogen bond was observed between the ether oxygen and the side chain hydroxyl group Ser478. For compound 12, because of the size of the imidazole group, the whole molecule moves "up" (further away from the heme) when compared with E-norendoxifen. Due to this move, the other phenolic hydroxy group moves away from Ser478 and approaches Asp309 with the formation of a hydrogen bond with the backbone carbonyl group of Asp309.

To explore the binding mode with ER- α , compound 12 was docked into the active site of ER- α (PDB code 3ert)³⁴ with GOLD 3.0. The best docking pose of 12 (shown in Figure 5b) was overlapped with the published hypothetical binding mode of *Z*-norendoxifen (*Z*-5). According to the docking results, the binding mode of 12 is nearly identical to that of *Z*norendoxifen. The imidazole group is situated in the ethyl binding pocket surround by Met421, Met388, and Leu428. One of the phenolic hydroxy groups forms bifurcated hydrogen bonds with Arg394 and Glu353. The other phenolic hydroxy group projects toward the outside of the ligand binding pocket and forms a hydrogen bond with Thr347.

Our previous testing results showed that the aminoethoxyl side chain of (E,Z)-norendoxifen (5) is favorable for aromatase inhibitory activity (>240-fold increase compared with 7, Figure 6).²⁴ In this report, the imidazole group of compound **12** is also demonstrated to be optimal for aromatase inhibitory activity (>5000-fold increase compared with 7). Unfortunately, combining the aminoethoxyl side chain and imidazole group in one molecule (compound 36) did not result in a more potent aromatase inhibitor than 12.²⁶ The failure of combining optimal substitutions to afford the most potent compound can be attributed to the hypothetical imidazole-induced binding mode movement as noted for compound 12. The hypothetical binding mode of compound 36 overlapped with the hypothetical binding mode of E-norendoxifen (E-5) is shown in Figure 6. Similar to compound 12, the imidazole nitrogen of 36 coordinates with the iron. Because of the size of the imidazole group, the whole molecule 36 also moves "up" (further away from the heme) when compared with E-norendoxifen. This movement pushes the ether oxygen away from Ser478 (distance 3.7 Å vs 3.3 Å of E-norendoxifen) and weakens the hydrogen bond between the ether oxygen and the side chain of Ser478. This movement also pushes the aminoethoxy side chain away from Asp309, resulting in the loss of the salt bridge interaction (between the protonated amino group and the carboxyl group of Asp309) and hydrogen bond (between the protonated amino group and the backbone carbonyl group of Asp309). Therefore, the aminoethoxy side chain of compound 36 cannot contribute positively to the aromatase inhibitory activity.

CONCLUSION

A series of triphenylethylene bisphenol analogues were designed and synthesized by eliminating the aminoethoxy side chain of (E,Z)-norendoxifen. The biological testing results showed that an imidazole group in the location of the ethyl group was optimal for both aromatase inhibitory activity and ER binding affinities. The imidazole compounds 12, 18a, 18b, and 26 displayed superior aromatase inhibitory activity compared with (E,Z)-norendoxifen, while 12 also showed ER binding affinities comparable with (E,Z)-norendoxifen and 18a and 18b were slightly less active. These four imidazoles also act as antagonists in the ER transcriptional assays in MCF-7 breast cancer cells with activity similar to (E,Z)-norendoxifen. The possible binding modes of the most potent compound 12 with aromatase and ER- α were also investigated by molecular modeling, and the results were used to rationalize the observed activity trends. Since these triphenylethylene bisphenol analogues have no possibility for the E/Z isomerization problems encountered with the norendoxifen analogues that were previously published, they introduce a superior class of compounds that can be developed toward the goal of obtaining the rapeutically useful dual AI/SERM agents. Any future biological work with these compounds should provide a detailed profile of their agonistic and antagonistic activities in various organs and tissues in animal models. The facts that compounds **12**, **18a**, and **18b** have high binding affinity to ER- α and ER- β and are analogues of the SERM tamoxifen argue in favor of the possibility of them being estrogen antagonists in breast tissue and estrogenic agonists in other tissues.

EXPERIMENTAL SECTION

General. Melting points were determined using capillary tubes with a Mel-Temp apparatus and are uncorrected. The nuclear magnetic resonance (¹H and ¹³C NMR) spectra were recorded using a Bruker ARX300 spectrometer (300 MHz) with a QNP probe or a Bruker DRX-2 spectrometer (500 MHz) with a BBO probe. High-resolution mass spectra were recorded on a double-focusing sector mass spectrometer with magnetic and electrostatic mass analyzers. The purities of biologically important compounds were determined by HPLC or elemental analyses. For elemental analyses, the observed percentages differ less than 0.40% from the calculated values. For HPLC, the major peak accounted for \geq 95% of the combined total peak area when monitored by a UV detector at 254 nm. The HPLC analyses were performed on a Waters 1525 binary HPLC pump/ Waters 2487 dual λ absorbance detector system using a 5 μ m C18 reversed phase column. The cytochrome P450 (CYP) inhibitor screening kit for aromatase (CYP19) was purchased from BD Biosciences (San Jose, CA). Estrogen receptor α and β competitor assay kits were purchased from Invitrogen (Carlsbad, CA).

General Procedure for the McMurry Cross-Coupling Reaction. Zinc powder (653 mg, 10 mmol) was suspended in dry THF (8 mL), the mixture was cooled to 0 °C, and then TiCl₄ (0.55 mL, 5 mmol) was added dropwise under argon. When the addition was complete, the mixture was warmed to room temperature and then heated at reflux for 2 h. After cooling down, a solution of the corresponding benzophenone (1 mmol) and ketone (3 mmol) in dry THF (8 mL) was added, and the mixture was heated at reflux in the dark for 3 h. After being cooled to room temperature, THF was evaporated. The residue was dissolved in saturated NH₄Cl aqueous solution (20 mL) and extracted with ethyl acetate (20 mL × 4). The organic layers were combined, dried over Na₂SO₄, concentrated in vacuo, and further purified by silica gel column chromatography to provide the McMurry cross-coupling product.

4-(1,1-Bis(4-hydroxyphenyl)prop-1-en-2-yl)phenyl Pivalate (15a). A suspension of 14a (678 mg, 4.98 mmol) and NaH (206 mg, 95%, 8.15 mmol) in dry THF (5 mL) was stirred under argon for 10 min, and then pivaloyl chloride (0.90 mL, 7.31 mmol) was added. The solution was stirred at room temperature for 3 h and quenched with water (2 mL). The solvent was evaporated, and the residue was dissolved with water (15 mL) and extracted with ethyl acetate (15 mL \times 4). The organic layers were combined, dried over Na₂SO₄, and concentrated. The concentrated product was reacted with 4,4'dihydroxylbenzophenone (1.98 g, 9.24 mmol) according to the general McMurry cross-coupling reaction procedure. The product was purified by silica gel column chromatography (2:1 hexanes-ethyl acetate) to afford the product 15a as a white solid (1.55 g, 77%): mp 138–140 °C. ¹H NMR (300 MHz, methanol- d_4 and CDCl₃) δ 7.11– 7.08 (m, 2 H), 7.03-7.00 (m, 2 H), 6.80-6.74 (m, 4 H), 6.69-6.66 (m, 2 H), 6.48-6.45 (m, 2 H), 2.07 (s, 3 H), 1.29 (s, 9 H); ¹³C NMR (75 MHz, methanol-*d*₄ and CDCl₃) δ 179.3, 156.7, 156.0, 150.1, 143.7, 140.7, 136.6, 136.3, 134.0, 133.5, 132.6, 131.7, 122.1, 116.1, 115.6, 40.4, 28.3, 24.5; ESIMS m/z (relative intensity) 425 (MNa⁺, 100); HRESIMS m/z calcd for C₂₆H₂₇O₄ (MH⁺) 403.1909, found 403.1916. 4-(1,1-Bis(4-hydroxyphenyl)prop-1-en-2-yl)-2-fluorophenyl

4-(1,1-Bis(4-nycroxypnenyi)prop-1-en-2-yi)-2-nuoropnenyi Pivalate (15b). A suspension of **14b** (187 mg, 1.21 mmol) and NaH (70.7 mg, 95%, 2.42 mmol) in dry THF (5 mL) was stirred at room temperature under argon. Pivaloyl chloride (0.22 mL, 1.8 mmol) was added dropwise. The mixture was stirred at room temperature for 3 h and quenched with water (2 mL). The THF was evaporated, and the residue was dissolved with 20% K_2CO_3 solution (20 mL) and extracted with ethyl acetate (15 mL × 3). The organic layers were combined and dried over Na₂SO₄ and concentrated. The residue was combined with 4 4'-dihydroxybenzophenone (0.80 g, 3.73 mmol) and reacted according to the general McMurry cross-coupling reaction procedure. The product was further purified by silica gel column chromatography (7:3 hexanes–ethyl acetate) to afford the product **15b** as yellow oil (348 mg, 68%). ¹H NMR (300 MHz, CDCl₃) δ 7.07–7.04 (m, 2 H), 7.04–6.84 (m, 3 H), 6.79–6.76 (m, 2 H), 6.74–6.71 (m, 2 H), 6.52–6.49 (m, 2 H), 2.09 (s, 3 H), 1.35 (s, 9 H); EIMS *m/z* (relative intensity) 420 (M⁺, 16), 57 (100); negative ion HRESIMS *m/z* calcd for C₂₆H₂₄FO₄ (M – H⁺)⁻ 419.1659, found 419.1665.

(2-(4-Aminophenyl)prop-1-ene-1,1-diyl)bis(4,1-phenylene) Bis(2,2-dimethylpropanoate) (23). Zinc powder (1.12 g, 17.1 mmol) was suspended in dry THF (10 mL), and the mixture was cooled to 0 °C. TiCl₄ (0.8 mL, 7.2 mmol) was added dropwise under argon. When the addition was complete, the mixture was warmed to room temperature and then heated at reflux for 2 h. After cooling down, a solution of 22³⁵ (501 mg, 1.31 mmol) and 21 (175 mg, 1.29 mmol) in dry THF (10 mL) was added, and the mixture was heated at reflux in the dark for 3 h. After the reaction mixture was cooled to room temperature, THF was carefully evaporated. The residue was dissolved with saturated ammonium chloride aqueous solution (20 mL) and extracted with ethyl acetate (20 mL \times 4). The organic layers were combined, dried over Na2SO4, concentrated in vacuo, and further purified by silica gel column chromatography, eluting with 3:1 dichloromethane-hexanes, followed by 2:1 hexanes-ethyl acetate, to afford the product 23 as yellow solid (284 mg, 45%): mp 208-210 °C. ¹H NMR (300 MHz, CDCl₃) δ 7.23-7.20 (m, 2 H), 7.05-7.02 (m, 2 H), 6.95-6.92 (m, 2 H), 6.91-6.88 (m, 2 H), 6.76-6.73 (m, 2 H), 6.51-6.48 (m, 2 H), 2.10 (s, 3 H), 1.37 (s, 9 H), 1.31 (s, 9 H); ¹³C NMR (75 MHz, CDCl₃) δ 177.1, 176.9, 149.5, 148.8, 144.8, 141.1, 140.7, 136.2, 136.1, 133.6, 131.8, 131.1, 130.3, 121.0, 120.4, 114.6, 39.1, 39.0, 27.1, 27.0, 23.3; ESIMS m/z (relative intensity) 508 (MNa⁺, 100); HRESIMS m/z calcd for $C_{31}H_{36}NO_4$ (MH⁺) 486.2645, found 486.2648.

General Procedure for the Preparation of 10 and 16a,b. A solution of bisphenol (9 or 15a,b, 0.502 mmol) and NaH (53.0 mg, 95%, 2.10 mmol) in dry THF (5 mL) was stirred under argon for 10 min, and then methyl chloromethyl ether (0.16 mL, 2.10 mmol) was added. The mixture was stirred at room temperature for 3 h and quenched with saturated aqueous NaHCO₃ solution (2 mL). The solvent was evaporated, and the residue was dissolved in saturated aqueous NaHCO₃ solution (15 mL) and extracted with ethyl acetate (15 mL × 4). The organic layers were combined, dried over Na₂SO₄, and concentrated in vacuo to provide the crude product 10 or 16a,b.

4,4'-(2-Phenylprop-1-ene-1,1-diyl)bis((methoxymethoxy)benzene) (10). The crude product was purified by silica gel column chromatography (9:1 hexanes-ethyl acetate) to provide the pure product **10** as a colorless oil (69%). ¹H NMR (300 MHz, CDCl₃) δ 7.18–7.07 (m, 7 H), 7.04–7.00 (m, 2 H), 6.82–6.79 (m, 2 H), 6.71– 6.68 (m, 2 H), 5.21 (s, 2 H), 5.07 (s, 2 H), 3.52 (s, 3 H), 3.43 (s, 3 H), 2.14 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 155.9, 155.2, 144.3, 138.2, 137.3, 136.9, 134.7, 132.0, 131.2, 129.3, 127.9, 126.0, 115.7, 115.0, 94.4, 94.3, 56.0, 55.9, 23.4; ESIMS *m*/*z* (relative intensity) 413 (MNa⁺, 100); HRESIMS *m*/*z* calcd for C₂₅H₂₆O₄Na (MNa⁺) 413.1729, found 413.1731.

4-(1,1-Bis(4-(methoxymethoxy)phenyl)prop-1-en-2-yl)phenyl Pivalate (16a). The crude product was purified by silica gel column chromatography (85:15 hexanes-ethyl acetate) to provide the pure product **16a** as a colorless oil (81%). ¹H NMR (300 MHz, CDCl₃) δ 7.16-7.13 (m, 4 H), 7.02-6.99 (m, 2 H), 6.88-6.85 (m, 2 H), 6.82-6.79 (m, 2 H), 6.73-6.69 (m, 2 H), 5.19 (s, 2 H), 5.08 (s, 2 H), 3.51 (s, 3 H), 3.43 (s, 3 H), 2.12 (s, 3 H), 1.34 (s, 9 H); ¹³C NMR (75 MHz, CDCl₃) δ 177.0, 155.9, 155.3, 149.1, 141.6, 138.5, 137.2, 136.7, 133.8, 132.0, 131.1, 130.2, 120.8, 115.7, 115.2, 94.4, 56.0, 39.0, 27.1, 23.4; ESIMS m/z (relative intensity) 513 (MNa⁺, 100); HRESIMS m/z calcd for C₃₀H₃₄O₆Na (MNa⁺) 513.2253, found 513.2272. **4-(1,1-Bis(4-(methoxymethoxy)phenyl)prop-1-en-2-yl)-2-fluorophenyl Pivalate (16b).** The crude product was purified by silica gel column chromatography (85:15 hexanes–ethyl acetate) to provide the product **16b** as a colorless oil (194 mg, 49%). ¹H NMR (300 MHz, CDCl₃) δ 7.15–7.11 (m, 2 H), 7.02–6.99 (m, 2 H), 6.97–6.87 (m, 3 H), 6.82–6.79 (m, 2 H), 6.74–6.71 (m, 2 H), 5.19 (s, 2 H), 5.08 (s, 2 H), 3.50 (s, 3 H), 3.43 (s, 3 H), 2.10 (s, 3 H), 1.36 (s, 9 H); MALDIMS *m/z* (relative intensity) 508 (M⁺, 80), 379 (100); HRESIMS *m/z* calcd for C₃₀H₃₃FO₆Na (MNa⁺) 531.2159, found 531.2143.

General Procedure for the Preparation of 11, 17a,b, and 25. A solution of 10 or 16a,b or 24 (0.169 mmol) and Nbromosuccinimide (30.8 mg, 0.173 mmol) in CCl₄ (5 mL) was heated at reflux under argon for 3 h. After cooling down, the solid was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in THF (4.5 mL), and a solution of KCN (46.2 mg, 0.709 mmol) in water (1.5 mL) was added. The mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was dissolved with water (10 mL) and extracted with ethyl acetate (10 mL \times 3). The organic layers were combined, dried over Na₂SO₄, and concentrated in vacuo. The residue was dissolved with methanol (4.5 mL), and concentrated HCl (1 mL) was added. The solution was stirred at room temperature overnight. The solvent was removed, and the residue was dissolved with water (10 mL). neutralized with NaHCO₃, and extracted with ethyl acetate (10 mL \times 3). The organic layers were combined, dried over Na_2SO_4 , and concentrated in vacuo to provide the crude product 11 or 17a,b or 25.

4,4-Bis(4-hydroxyphenyl)-3-phenylbut-3-enenitrile (11). The crude product was purified by silica gel column chromatography (2:1 hexanes-ethyl acetate) to provide the pure product **11** as an orange solid (61.7 mg, 51%): mp 198–200 °C. ¹H NMR (300 MHz, methanol- d_4 and CDCl₃) δ 7.19–7.10 (m, 5 H), 7.09–7.05 (m, 2 H), 6.83–6.79 (m, 2 H), 6.69–6.65 (m, 2 H), 6.47–6.43 (m, 2 H), 3.50 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4 and CDCl₃) δ 157.9, 157.0, 145.9, 141.5, 134.7, 134.3, 133.3, 132.1, 130.9, 129.7, 128.5, 127.2, 120.0, 116.8, 115.8, 26.8; EIMS *m*/*z* (relative intensity) 327 (M⁺, 100); HREIMS *m*/*z* calcd for C₂₂H₁₇NO₂·CH₃OH: C, 76.86; H, 5.89; N, 3.90. Found: C, 76.88; H, 5.57; N, 3.93.

3,4,4-Tris(4-hydroxyphenyl)but-3-enenitrile (17a). The crude product **17a** was purified by silica gel column chromatography (1:1 hexanes-ethyl acetate) to provide the pure product as red foam (83%). ¹H NMR (300 MHz, methanol- d_4) δ 7.07–7.00 (m, 4 H), 6.84–6.80 (m, 2 H), 6.73–6.69 (m, 2 H), 6.66–6.62 (m, 2 H), 6.50–6.46 (m, 2 H), 3.53 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4) δ 158.2, 157.6, 157.2, 144.5, 135.1, 134.8, 133.1, 132.7, 132.0, 131.8, 127.7, 120.1, 116.4, 116.2, 115.4, 25.8; ESIMS *m*/*z* (relative intensity) 366 (MNa⁺, 100); HRESIMS *m*/*z* calcd for C₂₂H₁₇NO₃Na (MNa⁺) 366.1106, found 366.1120. HPLC purity 99.8% (C-18 reverse phase, methanol- H_2 O, 90:10).

3-(3-Fluoro-4-hydroxyphenyl)-4,4-bis(4-hydroxyphenyl)but-3-enenitrile (17b). The crude product was purified by silica gel column chromatography (1:1 hexanes-ethyl acetate) to provide the pure product 17b as white solid (70%): mp 180–183 °C. ¹H NMR (300 MHz, methanol- d_4) δ 7.07–7.04 (m, 2 H), 6.91–6.87 (m, 1 H), 6.84–6.79 (m, 3 H), 6.78–6.75 (m, 1 H), 6.73–6.70 (m, 2 H), 6.53–6.49 (m, 2 H), 3.55 (s, 2 H); EIMS *m*/*z* (relative intensity) 361 (M⁺, 100); negative ion HRESIMS *m*/*z* calcd for C₂₂H₁₅FNO₃ (M – H⁺)⁻ 360.1036, found 360.1033. HPLC purity 99.3% (C-18 reverse phase, methanol–H₂O, 90:10).

3-(4-Aminophenyl)-4,4-bis(4-hydroxyphenyl)but-3-enenitrile (25). The crude product **25** was purified by silica gel column chromatography (1:1 hexanes-ethyl acetate) to provide the pure product **25** as light yellow solid (70%): mp 235–238 °C. ¹H NMR (300 MHz, methanol- d_4) δ 7.06–7.03 (m, 2 H), 6.96–6.93 (m, 2 H), 6.82–6.79 (m, 2 H), 6.72–6.69 (m, 2 H), 6.59–6.56 (m, 2 H), 6.47–6.44 (m, 2 H), 3.52 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4) δ 158.1, 157.1, 147.9, 143.9, 135.3, 135.0, 133.1, 131.8, 131.6, 131.1, 128.0, 120.2, 116.4, 116.3, 115.3, 25.7; EIMS *m*/*z* (relative intensity) 342 (M⁺, 100); HRESIMS *m*/*z* calcd for C₂₂H₁₉N₂O₂ (MH⁺)

343.1447, found 343.1446. HPLC purity 97.8% (C-18 reverse phase, methanol $-H_2O$, 90:10).

General Procedure for the Preparation of 12 and 13. A solution of 10 (186 mg, 0.476 mmol) and N-bromosuccinimide (81.7 mg, 0.459 mmol) in CCl_4 (5 mL) was heated at reflux under argon for 3 h. After cooling down, the solid was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in dry THF (8 mL), and the solution was added to a solution of imidazole or 1,2,4-triazole (1.07 mmol) and NaH (30.1 mg, 1.25 mmol) in THF (4 mL) at 0 °C. The mixture was warmed to room temperature and stirred under argon overnight. The reaction was quenched with saturated NH₄Cl aqueous solution (1 mL). The solvent was evaporated, and the residue was dissolved with saturated NH4Cl aqueous solution (25 mL) and extracted with ethyl acetate (25 mL × 4). The organic layers were combined, dried over Na₂SO₄, and concentrated in vacuo. The residue was dissolved with methanol (5 mL), concentrated HCl (0.4 mL), and heated at reflux for 0.5 h. The solvent was removed, and the residue was neutralized with saturated NaHCO₃ solution (15 mL) and extracted with ethyl acetate (15 mL × 4). The organic layers were combined, dried over Na₂SO₄, concentrated in vacuo, and further purified by silica gel column chromatography (95:5 dichloromethane-methanol) to provide the product 12 or 13.

4,4'-(3-(1*H***-Imidazol-1-yl)-2-phenylprop-1-ene-1,1-diyl)diphenol (12).** The purified product was dissolved in DMSO (1 mL) and then diluted with water (10 mL). The solid was collected by filtration and dried in vacuo to provide the product **12** as a yellow solid (150 mg, 89%): mp 225–227 °C. ¹H NMR (300 MHz, DMSO- d_6) δ 7.41 (s, 1 H), 7.10–6.99 (m, 8 H), 6.79–6.76 (m, 3 H), 6.69–6.66 (m, 2 H), 6.44–6.41 (m, 2 H), 4.89 (s, 2 H); ¹³C NMR (75 MHz, DMSO- d_6) δ 157.6, 156.8, 144.8, 140.8, 133.6, 132.7, 132.3, 131.0, 130.4, 128.8, 127.4, 119.9, 116.2, 115.3, 51.1; ESIMS *m*/*z* (relative intensity) 369 (MH⁺, 33), 301 (100); HRESIMS *m*/*z* calcd for C₂₄H₂₀N₂O₂·1.3H₂O: C, 73.56; H, 5.81; N, 7.15. Found: C, 73.47; H, 5.83; N, 6.90.

4,4'-(2-Phenyl-3-(1*H***-1,2,4-triazol-1-yl)prop-1-ene-1,1-diyl)diphenol (13).** Yellow solid (54 mg, 54%): mp 143–145 °C. ¹H NMR (300 MHz, methanol- d_4 and CDCl₃) δ 7.98 (s, 1 H), 7.90 (s, 1 H), 7.19–7.15 (m, 2 H), 7.08–7.02 (m, 5 H), 6.80–6.77 (m, 2 H), 6.73–6.69 (m, 2 H), 6.46–6.43 (m, 2 H), 5.20 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4 and CDCl₃) δ 158.0, 157.1, 147.3, 140.9, 134.5, 133.4, 132.1, 130.8, 129.7, 128.2, 116.6, 115.7, 56.0; EIMS *m*/*z* (relative intensity) 369 (M⁺, 53), 300 (100); HREIMS *m*/*z* calcd for C₂₃H₁₉N₃O₂ (M⁺) 369.1472, found 369.1484. Anal. Calcd for C₂₃H₁₉N₃O₂·1.6CH₃OH: C, 70.23; H, 6.09; N, 9.99. Found: C, 70.48; H, 5.70; N, 9.59.

General Procedure for the Preparation of 18a,b and 26. A solution of 16a,b or 24 (0.127 mmol) and N-bromosuccinimide (24.8 mg, 0.139 mmol) in CCl_4 (5 mL) was heated at reflux under argon for 3 h. After cooling down, the solid was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in dry THF (5 mL), and the solution was added to a solution of imidazole (35.4 mg, 0.52 mmol) and NaH (27.1 mg, 95%, 1.07 mmol) in THF (3 mL). The mixture was stirred at room temperature overnight. The reaction was quenched with saturated NH₄Cl aqueous solution (1 mL). The solvent was evaporated, and the residue was dissolved with saturated NH₄Cl aqueous solution (10 mL) and extracted with ethyl acetate (10 mL \times 3). The organic layers were combined, dried over Na2SO4, and concentrated in vacuo. The product was dissolved in THF (2.5 mL), and 2 N KOH solution (2.5 mL) was added. The mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was dissolved with saturated NH4Cl aqueous solution (15 mL) and extracted with ethyl acetate (10 mL \times 3). The organic layers were combined, dried over Na_2SO_4 , concentrated in vacuo, and further purified by silica gel column chromatography (95:5 dichloromethane-methanol). The purified product was dissolved with methanol (4.5 mL), and concentrated HCl (1 mL) was added. The mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was dissolved with water (10 mL), neutralized with NaHCO₃, and extracted with ethyl acetate (10 mL \times 3). The organic layers were combined, dried over Na₂SO₄, concentrated in vacuo, and further purified by silica gel column chromatography (9:1 dichloromethane–methanol) to provide the product **18a,b** or **26**.

4,4',4"-(**3**-(1*H*-Imidazol-1-yl)prop-1-ene-1,1,2-triyl)triphenol (18a). Red glass (58%). ¹H NMR (300 MHz, methanol- d_4) δ 7.35 (s, 1 H), 7.06–7.03 (m, 2 H), 6.91 (s, 1 H), 6.88–6.79 (m, 5 H), 6.77– 6.73 (m, 2 H), 6.58–6.54 (m, 2 H), 6.49–6.45 (m, 2 H), 4.88 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4) δ 158.0, 157.3, 157.1, 145.2, 138.3, 134.9, 133.0, 132.4, 132.1, 131.7, 128.6, 120.5, 116.4, 116.1, 115.4, 52.2; negative ion ESIMS *m*/*z* (relative intensity) 383 [(M – H⁺)⁻, 8], 315 (100); negative ion HREIMS *m*/*z* calcd for C₂₄H₁₉N₂O₃ (M – H⁺)⁻ 383.1396, found 383.1398. HPLC purity 97.6% (C-18 reverse phase, methanol-H₂O, 90:10).

4,4'-(**2**-(**3**-Fluoro-4-hydroxyphenyl)-**3**-(1*H*-imidazol-1-yl)prop-1-ene-1,1-diyl)diphenol (18b). Orange foam (49%). ¹H NMR (300 MHz, methanol- d_4) δ 7.41 (s, 1 H), 7.08–7.05 (m, 2 H), 6.97 (s, 1 H), 6.85 (s, 1 H), 6.82–6.79 (s, 2 H), 6.77–6.74 (m, 2 H), 6.73–6.65 (m, 3 H), 6.51–6.48 (m, 2 H), 4.92 (s, 2 H); ESIMS *m*/*z* (relative intensity) 403 (MH⁺, 13), 335 (100); HRESIMS *m*/*z* calcd for C₂₄H₂₀FN₂O₃ (MH⁺) 403.1458, found 403.1459. HPLC purity 98.9% (C-18 reverse phase, methanol–H₂O, 90:10).

4,4'-(**2**-(**4**-Aminophenyl)-**3**-(**1***H*-imidazol-**1**-yl)prop-**1**-ene-**1,1-diyl)diphenol (26).** Yellow foam (59%). ¹H NMR (300 MHz, methanol- d_4) δ 7.40 (s, 1 H), 7.05–7.01 (m, 2 H), 6.94 (s, 1 H), 6.84–6.73 (m, 7 H), 6.52–6.49 (m, 2 H), 6.47–6.43 (m, 2 H), 4.90 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4) δ 158.0, 157.1, 147.6, 144.8, 138.2, 135.1, 133.2, 133.0, 131.7, 130.8, 128.3, 120.6, 116.3, 115.3, 52.2; ESIMS *m*/*z* (relative intensity) 384 (MH⁺, 18), 316 (100); HRESIMS *m*/*z* calcd for C₂₄H₂₂N₃O₂ (MH⁺) 384.1712, found 384.1725. HPLC purity 99.4% (C-18 reverse phase, methanol–H₂O, 90:10), 99.8% (C-18 reverse phase, methanol–H₂O, 85:15).

General Procedure for the Preparation of 19a,b, 20a,b, 27, and 28. A solution of 16a,b or 24 (0.201 mmol) and Nbromosuccinimide (35.8 mg, 0.201 mmol) in CCl₄ (5 mL) was heated at reflux under argon for 3 h. After cooling down, the solid was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in dry THF (6 mL) and the solution was added to a solution of 1,2,4-triazole (42.6 mg, 0.62 mmol) and NaH (32 mg, 95%, 1.27 mmol) in THF (3 mL). The mixture was stirred at room temperature overnight. The reaction was quenched with saturated NH₄Cl aqueous solution (1 mL). The solvent was evaporated, and the residue was dissolved in saturated ammonium chloride aqueous solution (10 mL) and extracted with ethyl acetate (10 mL \times 3). The organic layers were combined, dried over Na2SO4, and concentrated in vacuo. The product was dissolved in THF (2.5 mL), and 2 N KOH solution (2.5 mL) was added. The mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was dissolved with saturated aqueous ammonium chloride solution (15 mL) and extracted with ethyl acetate (10 mL \times 3). The organic layers were combined, dried over Na2SO4, concentrated in vacuo, and further purified by silica gel column chromatography (95:5 dichloromethanemethanol). The purified product was dissolved in methanol (4.5 mL), and concentrated HCl (1 mL) was added. The mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was dissolved in water (10 mL), neutralized with NaHCO₃, and extracted with ethyl acetate (10 mL \times 3). The organic layers were combined, dried over Na2SO4, concentrated in vacuo, and further purified by silica gel column chromatography (9:1 dichloromethanemethanol) to first provide 19a,b or 27 and then 20a,b or 28.

4,4',4"-(**3**-(1 \hat{H} -**1**,**2**,**4**-**Triazol-1-yl**)**prop-1-ene-1**,**1**,**2**-**triyl**)**triphenol** (**19a**). Yellow oil (93.1 mg, 60%). ¹H NMR (300 MHz, methanol- d_4) δ 8.00 (s, 1 H), 7.87 (s, 1 H), 7.28–7.25 (m, 2 H), 6.90–6.87 (m, 2 H), 6.81–6.74 (m, 4 H), 6.55–6.52 (m, 2 H), 6.49– 6.46 (m, 2 H), 5.18 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4) δ 158.0, 157.3, 157.1, 151.8, 145.3, 135.2, 134.8, 133.2, 132.2, 132.1, 131.9, 116.2, 116.1, 115.4, 55.0; negative ion ESIMS m/z (relative intensity) 384 [(M – H⁺)⁻, 5], 315 (100); negative ion HREIMS m/z calcd for $C_{23}H_{18}N_3O_3$ [(M – H⁺)⁻] 384.1348, found 384.1353. HPLC purity 98.8% (C-18 reverse phase, methanol-H₂O, 90:10).

4,4',4"-(**3**-(**4***H*-**1**,**2**,**4**-**Triazol-4**-**yl**)**prop-1-ene-1**,**1**,**2**-**triyl**)**triphenol (20a).** Yellow oil (32.5 mg, 21%). ¹H NMR (300 MHz, methanol-*d*₄) δ 8.23 (s, 2 H), 7.08–7.05 (m, 2 H), 6.95–6.92 (m, 2 H), 6.83–6.80 (m, 2 H), 6.78–6.75 (m, 2 H), 6.60–6.57 (m, 2 H), 6.48–6.45 (m, 2 H), 5.03 (s, 2 H); ¹³C NMR (75 MHz, methanol-*d*₄) δ 158.2, 157.7, 157.4, 146.0, 144.5, 134.7, 134.5, 133.0, 132.1, 131.8, 131.5, 116.5, 116.4, 115.4, 50.8; negative ion ESIMS *m/z* (relative intensity) 384 [(M – H⁺)⁻, 3], 315 (100); negative ion HREIMS *m/z* calcd for C₂₃H₁₈N₃O₃ (M – H⁺)⁻ 384.1348, found 384.1359. HPLC purity 96.1% (C-18 reverse phase, methanol-H₂O, 90:10).

4,4'-(2-(3-Fluoro-4-hydroxyphenyl)-3-(1H-1,2,4-triazol-1-yl)prop-1-ene-1,1-diyl)diphenol (19b). Orange foam (37%). ¹H NMR (300 MHz, methanol- d_4) δ 8.07 (s, 1 H), 7.90 (s, 1 H), 7.29–7.26 (s, 2 H), 6.83–6.61 (m, 7 H), 6.52–6.47 (m, 2 H), 5.20 (s, 2 H); ESIMS *m/z* (relative intensity) 426 (MNa⁺, 100); HRESIMS *m/z* calcd for C₂₃H₁₈FN₃O₃Na (MNa⁺) 426.1230, found 426.1243. HPLC purity 99.6% (C-18 reverse phase, methanol–H₂O, 90:10).

4,4'-(2-(3-Fluoro-4-hydroxyphenyl)-3-(4H-1,2,4-triazol-4-yl)prop-1-ene-1,1-diyl)diphenol (20b). Yellow foam (21%). ¹H NMR (300 MHz, methanol- d_4) δ 8.31 (s, 2 H), 7.11–7.06 (m, 2 H), 6.86–6.66 (m, 7 H), 6.53–6.49 (m, 2 H), 5.06 (s, 2 H); ESIMS *m/z* (relative intensity) 426 (MNa⁺, 100); HRESIMS *m/z* calcd for C₂₃H₁₈FN₃O₃Na (MNa⁺) 426.1230, found 426.1244. HPLC purity 98.0% (C-18 reverse phase, methanol–H₂O, 90:10).

4,4'-(2-(4-Aminophenyl)-3-(1*H***-1,2,4-triazol-1-yl)prop-1-ene-1,1-diyl)diphenol (27).** Orange foam (24%). ¹H NMR (300 MHz, methanol- d_4) δ 8.02 (s, 1 H), 7.87 (s, 1 H), 7.26–7.23 (m, 2 H), 6.85–6.82 (m, 2 H), 6.79–6.73 (m, 4 H), 6.50–6.43 (m, 4 H), 5.19 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4) δ 158.0, 157.1, 151.7, 147.5, 145.2, 144.8, 135.4, 135.0, 133.1, 132.1, 131.7, 130.3, 116.3, 116.1, 115.3, 54.9; ESIMS *m*/*z* (relative intensity) 407 (MNa⁺, 100); HRESIMS *m*/*z* calcd for C₂₃H₂₀N₄O₂Na (MNa⁺) 407.1484, found 407.1490. HPLC purity 97.6% (C-18 reverse phase, methanol-H₂O, 90:10).

4,4'-(**2**-(**4**-Aminophenyl)-**3**-(**4***H*-**1,2**,**4**-triazol-**4**-yl)prop-1-ene-**1,1-diyl)diphenol (28).** Yellow foam (20%). ¹H NMR (300 MHz, methanol- d_4) δ 8.24 (s, 2 H), 7.06–7.03 (m, 2 H), 6.89–6.86 (m, 2 H), 6.83–6.80 (m, 2 H), 6.79–6.76 (m, 2 H), 6.54–6.51 (m, 2 H), 6.47–6.44 (m, 2 H), 5.03 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4) δ 158.2, 157.3, 148.0, 145.4, 144.5, 134.9, 134.7, 133.0, 132.0, 131.7, 131.5, 129.7, 116.5, 116.4, 115.3, 50.8; ESIMS *m*/*z* (relative intensity) 407 (MNa⁺, 100); HRESIMS *m*/*z* calcd for C₂₃H₂₀N₄O₂Na (MNa⁺) 407.1484, found 407.1498. HPLC purity 99.2% (C-18 reverse phase, methanol–H₂O, 90:10).

(2-(4-((tert-Butoxycarbonyl)amino)phenyl)prop-1-ene-1,1diyl)bis(4,1-phenylene) Bis(2,2-dimethylpropanoate) (24). A solution of 23 (284 mg, 0.585 mmol) and Boc₂O (207 mg, 0.948 mmol) in dry dioxane (10 mL) was heated at reflux under argon for 5 h. After cooling down, the solvent was evaporated, and the residue was dissolved with 10% K₂CO₃ solution (20 mL) and extracted with ethyl acetate (20 mL \times 3). The organic layers were combined, dried over Na₂SO₄, concentrated, and further purified by silica gel column chromatography (85:15 hexanes-ethyl acetate) to provide 24 as white solid (274 mg, 80%): mp 187–190 °C. ¹H NMR (300 MHz, CDCl₃) δ 7.23-7.20 (m, 2 H), 7.18-7.15 (m, 2 H), 7.06-7.02 (m, 4 H), 6.89-6.86 (m, 2 H), 6.75-6.72 (m, 2 H), 2.10 (s, 3 H), 1.50 (s, 9 H), 1.36 (s, 9 H), 1.30 (s, 9 H); ¹³C NMR (75 MHz, CDCl₃) δ 177.0, 176.9, 149.6, 149.0, 146.7, 140.7, 140.2, 138.1, 137.2, 136.6, 135.7, 131.8, 131.0, 129.8, 121.1, 120.4, 117.8, 80.4, 39.1, 39.0, 27.4, 27.1, 27.0, 23.3; EIMS m/z (relative intensity) 585 (M⁺, 0.6), 57 (100); HRESIMS m/z calcd for C36H43NO6Na (MNa⁺) 608.2988, found 608.3009.

3-Acetylphenyl Pivalate (30).³⁶ A solution of compound 29 (421 mg, 3.09 mmol) in dry THF (7 mL) was stirred under argon. NaH (111 mg, 95%, 4.39 mmol) was added portionwise. The solution was stirred for 30 min, and then trimethylacetyl chloride (0.6 mL, 4.87 mmol) was added dropwise. After stirring for 2 h, the reaction was quenched with H_2O (2 mL) and the solvent was evaporated. The

residue was dissolved with H₂O (20 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, dried over Na₂SO₄, concentrated in vacuo, and further purified by silica gel chromatography (4:1 hexanes–ethyl acetate) to provide **30** as pale yellow oil (541 mg, 95%). ¹H NMR (300 MHz, CDCl₃) δ 7.74 (d, *J* = 7.7 Hz, 1 H), 7.59 (s, 1 H), 7.40 (t, *J* = 7.9 Hz, 1 H), 7.21 (d, *J* = 8.1 Hz, 1 H), 2.53 (s, 3 H), 1.32 (s, 9 H).

3-(1,1-Bis(4-(methoxymethoxy)phenyl)prop-1-en-2-yl)phenyl Pivalate (31). The acetophenone 30 (1.47 g, 6.67 mmol) and 4,4'-dihydroxylbenzophenone (0.953 g, 4.4 mmol) were reacted according to the general McMurry cross-coupling reaction procedure. The product was purified by silica gel column chromatography (2:1 hexanes-ethyl acetate) to provide impure bisphenol intermediate which was dissolved in dry THF (20 mL) and treated with NaH (0.231 g, 95%, 9.14 mmol). The mixture was stirred 30 min under argon, and then chloromethyl methyl ether (2.0 mL, 9.0 mmol) was added dropwise. After stirring 3 h, the reaction was quenched with saturated NaHCO3 (10 mL) and the solvent was evaporated. The organic products were extracted from the aqueous phase using ethyl acetate $(3 \times 15 \text{ mL})$. The organic layers were combined, dried over Na₂SO₄, concentrated, and purified by silica gel column chromatography, eluting with 6:1 hexanes-ethyl acetate to provide product 31 as pale yellow oil (0.912 g, 43%). ¹H NMR (300 MHz, CDCl₃) δ 7.19-7.09 (m, 3 H), 7.06–7.00 (m, 2 H), 6.95 (dt, J = 7.7, 1.3 Hz, 1 H), 6.89 (t, J = 2.0 Hz, 1 H), 6.86-6.78 (m, 3 H), 6.76-6.71 (m, 2 H), 5.20 (s, 2 H), 5.08 (s, 2 H), 3.51 (s, 3 H), 3.43 (s, 3 H), 2.15 (s, 3 H), 1.34 (s, 9 H); ¹³C NMR (75 MHz, CDCl₃) δ 176.92, 155.92, 155.45, 150.75, 145.63, 138.87, 137.06, 136.52, 133.56, 131.88, 131.09, 128.66, 126.78, 122.19, 119.07, 115.71, 115.18, 94.40, 55.91, 38.97, 27.08, 23.17; ESIMS m/z (MNa⁺) 513; HRESIMS m/z calcd for C₃₀H₃₄O₆ (MNa⁺) 513.2253, found 513.2234.

4,4'-(2-(3-Hydroxyphenyl)-3-(1H-imidazol-1-yl)prop-1-ene-1,1-diyl)diphenol (32). A solution of 31 (0.68 g, 1.39 mmol) and Nbromosuccinimide (247 mg, 1.39 mmol) in CCl₄ (30 mL) was heated at reflux under argon for 2 h. After cooling down, the solid was filtered off, and the solvent was evaporated. The residue was dissolved in dry THF (10 mL) and added to a solution of NaH (67 mg, 95%, 2.78 mmol) and imidazole (143 mg, 2.1 mmol) in dry THF (10 mL). The mixture was stirred at room temperature overnight. The reaction was then quenched with saturated NH₄Cl (4 mL) solution, and the solvent was evaporated. The product was extracted from saturated NH₄Cl (15 mL) solution using ethyl acetate $(3 \times 15 \text{ mL})$. The organic layers were combined, dried over Na2SO4, and concentrated. The product was dissolved in methanol (5 mL) and treated with 2 N KOH to bring the pH above 12. After the reaction mixture was stirred overnight, it was quenched with saturated NH₄Cl (10 mL) solution and the solvents were evaporated. The product was extracted from saturated NH₄Cl (10 mL) solution using ethyl acetate (4 \times 10 mL). The organic layers were combined, dried over Na₂SO₄, and concentrated. The product was dissolved in methanol (10 mL) and treated with concentrated HCl (1 mL). After stirring overnight, the reaction was neutralized using NaHCO₃ and methanol was evaporated. Saturated NH₄Cl (10 mL) solution was added, and the product was extracted using ethyl acetate $(3 \times 10 \text{ mL})$. The organic layers were combined, washed with brine, dried over Na₂SO₄, concentrated, and further purified using silica gel column chromatography, eluting with 10:1 dichloromethanemethanol to provide 32 (103 mg, 21%) as white glass. ¹H NMR (300 MHz, methanol-d₄) δ 7.35 (s, 1 H), 7.09-7.04 (m, 2 H), 7.01-6.90 (m, 2 H), 6.86-6.74 (m, 5 H), 6.56 (dd, J = 2.0, 1.1 Hz, 1 H), 6.52 (dq, J = 4.2, 1.6 Hz, 2 H), 6.49–6.43 (m, 2 H), 4.89 (s, 2 H); ¹³C NMR (75 MHz, methanol-d₄) δ 160.80, 160.65, 159.84, 148.40, 145.46, 140.76, 137.12, 137.01, 135.68, 135.39, 134.14, 132.82, 131.11, 124.67, 123.00, 120.24, 118.87, 117.84, 117.40, 54.78; ESIMS m/z (relative intensity) 385 (MH⁺, 16), 317 (100); HRESIMS m/z cacld for C₂₄H₂₀N₂O₃ (MH⁺) 385.1552, found 385.1556. HPLC purity: 100% (C-18 reverse phase, methanol-H₂O, 90:10).

(2-(3-((*tert*-Butoxycarbonyl)amino)phenyl)prop-1-ene-1,1diyl)bis(4,1-phenylene) Bis(2,2-dimethylpropanoate) (34). The acetophenone 33 (0.201 g, 1.49 mmol) and benzophenone 22 (0.682 g, 1.78 mmol) were reacted according to the general McMurry crosscoupling reaction procedure. The product was purified by silica gel chromatography (4:1 hexanes-ethyl acetate) to provide the impure intermediate, which was then treated with di-*tert*-butyl dicarbonate (0.312 g, 1.43 mmol) in THF (20 mL). The reaction mixture stirred under argon for 36 h. The solvent was evaporated and the residue was purified by silica gel chromatography (4:1 hexanes-ethyl acetate) to provide **34** as cloudy oil (0.685 g, 79%). ¹H NMR (300 MHz, CDCl₃) δ 7.25–7.13 (m, 4 H), 7.11–7.02 (m, 3 H), 6.92–6.86 (m, 2 H), 6.78 (s, 1 H), 6.76–6.71 (m, 2 H), 6.42 (brs, 1 H), 2.12 (s, 3 H), 1.51 (s, 9 H), 1.37 (s, 9 H), 1.30 (s, 9 H); ¹³C NMR (75 MHz, CDCl₃) δ 177.01, 152.65, 149.69, 149.06, 144.51, 140.44, 139.98, 138.03, 137.55, 136.06, 131.65, 130.96, 128.56, 124.21, 121.09, 120.40, 119.08, 116.68, 80.31, 39.06, 28.31, 27.05, 23.42; HRESIMS *m/z* (relative intensity) calcd for C₃₆H₄₃NO₆ (MNa⁺) 608.2988, found 608.2999.

4,4'-(2-(3-Aminophenyl)-3-(1H-imidazol-1-yl)prop-1-ene-1,1-diyl)diphenol (35). A solution of 34 (0.341 g, 0.58 mmol) and N-bromosuccinimide (83 mg, 0.46 mmol) in CCl₄ (30 mL) was heated at reflux under argon for 2 h. After cooling down, the solid was filtered off, and the solvent was evaporated. The residue was dissolved in dry THF (10 mL) and added to a solution of NaH (16 mg, 95%, 0.63 mmol) and imidazole (39 mg, 0.58 mmol) in dry THF (15 mL). The mixture was stirred at room temperature overnight. The reaction was then quenched with saturated NH₄Cl (3 mL) solution, and the solvent was evaporated. The residue was dissolved in saturated NH₄Cl (10 mL), and the product was extracted using ethyl acetate (3×15) mL). The organic layers were combined, washed with brine (10 mL), dried over Na2SO4, and concentrated. The product was dissolved in methanol-THF (7:3, 10 mL) and treated with 1 N KOH (2 mL). After the reaction mixture stirred 1 h, it was quenched with saturated NH₄Cl (10 mL), and the solvent was evaporated. The product was extracted using ethyl acetate $(3 \times 15 \text{ mL})$, and the combined extract was dried over Na₂SO₄, concentrated, dissolved in methanol (8 mL), and the solution was treated with concentrated HCl (1.5 mL). After stirring 2 h at 50 °C, the reaction was neutralized using NaHCO3 and methanol was evaporated. Saturated NH₄Cl (10 mL) solution was added, and the product was extracted using ethyl acetate $(3 \times 15 \text{ mL})$. The organic layers were combined, washed with brine, dried over Na₂SO₄, concentrated, and purified using silica gel column chromatography, eluting with 5:1 dichloromethane-methanol, to provide 35 as white glass (55 mg, 25%). ¹H NMR (300 MHz, methanol-d₄) δ 7.36 (s, 1 H), 7.09-7.01 (m, 2 H), 6.97-6.88 (m, 2 H), 6.84 (dq, J = 9.4, 2.6 Hz, 3 H), 6.81-6.73 (m, 2 H), 6.51-6.55 (m, 2 H), 6.50–6.44 (m, 2 H), 6.41 (dt, J = 7.7, 1.3 Hz, 1 H), 4.89 (s, 2 H); ¹³C NMR (75 MHz, methanol- d_4) δ 158.13, 157.30, 148.54, 145.57, 142.46, 134.75, 134.59, 133.51, 132.90, 131.65, 130.01, 121.14, 117.94, 116.32, 115.26, 52.44; ESIMS m/z (relative intensity) 384 (MH⁺, 28), 316 (100); negative ion HRESIMS m/z calcd for $C_{24}H_{21}N_3O_2$ [(M – H⁺)⁻] 382.1555, found 382.1563. HPLC purity: 100% (C-18 reverse phase, methanol-H₂O, 90:10).

Molecular Docking of Compounds 12 and 36 in the Active Site of Aromatase. The structures of compounds 12 and 36 were constructed with Sybyl 7.1 software and energy minimized to 0.01 kcal/mol by the Powell method using Gasteiger–Huckel charges and the Tripos force field. The crystal structure of aromatase was obtained from the Protein Data Bank (PDB code 3s79),³³ and the natural ligand (androstenedione) and all crystal water molecules were removed. Compounds 12 and 36 were docked into the androstenedione binding pocket in aromatase using the GOLD 3.0 program. A distance constraint was added between the imidazole nitrogen of 12 or 36 and the iron to confine the distance within 1.5–3.5 Å during docking. The best docking solutions according to the GOLD fitness scores were selected.

Molecular Docking of Compound 12 in the Active Site of ER- α . The structure of compound 12 was constructed with Sybyl 7.1 software and energy minimized to 0.01 kcal/mol by the Powell method using Gasteiger–Huckel charges and the Tripos force field. The crystal structure of ER- α was obtained from the Protein Data Bank (PDB code 3ert),³⁴ and the natural ligand (4-hydroxy tamoxifen) and all crystal water molecules (except the water that forms bifurcated hydrogen bonds with Glu353 and Arg394) were removed. Compound 12 was docked into the ligand binding pocket of ER- α using the GOLD 3.0 program. The best docking solution according to GOLD fitness score was selected.

Inhibition of Recombinant Human Aromatase (CYP19) by Microsomal Incubations. The activity of recombinant aromatase (CYP19) was determined by measuring the conversion rate of the fluorometric substrate 7-methoxy-4-trifluoromethylcoumarin (MFC) to its fluorescent metabolite 7-hydroxytrifluoromethylcoumarin (HFC). Experimental procedures were consistent with the published methodology.³⁷ All of the incubations were performed using incubation times and protein concentrations that were within the linear range for reaction velocity. The fluorometric substrate, MFC, was dissolved in acetonitrile with the final concentration of 25 mM. All tested samples were dissolved in either methanol or DMSO. The sample solutions (2 μ L) were mixed well with 98 μ L of NADPHcofactor mix (16.25 µM NADP+, 825.14 µM MgCl₂, 825.14 µM glucose 6-phosphate, and 0.4 U/mL glucose 6-phosphate dehydrogenase) and were prewarmed for 10 min at 37 °C. Enzyme/substrate mix was prepared with fluorometric substrate, recombinant human aromatase (CYP19), and 0.1 M potassium phosphate buffer (pH 7.4). Reactions were initiated by adding enzyme/substrate mix (100 μ L) to bring the incubation volume to 200 μ L, and the mixture was incubated for 30 min. All the reactions were stopped by adding 0.1 M Tris base dissolved in acetonitrile (75 μ L). The amount of fluorescent product was determined immediately by measuring fluorescent response using a BioTek (Winooski, VT) Synergy 2 fluorometric plate reader. Excitation-emission wavelengths for MFC metabolite were 409 and 530 nm. The standard curve for MFC metabolite was constructed using the appropriate fluorescent metabolite standards. Quantification of samples was performed by applying the linear regression equation of the standard curve to the fluorescence response. The limit of quantification for the metabolites of MFC was 24.7 pmol with intra- and interassay coefficients of variation less than 10%. The rates of metabolite formation in the presence of the test inhibitors were compared with those in the control, in which the inhibitor was replaced with vehicle. The extent of enzyme inhibition was expressed as the percentage of remaining enzyme activity compared to the control. IC₅₀ values were determined as the inhibitor concentrations that brought about half reduction in enzyme activity by fitting all the data to a one-site competition equation using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA).

Binding Affinities for Recombinant Human ER- α and ER- β . The binding affinities of ER- α and ER- β were determined by measuring the change of polarization value when the fluorescent estrogen ligand, ES2, was displaced by the tested compounds. Experimental procedures were consistent with the protocol provided by Invitrogen. The fluorescent estrogen ligand, ES2, was provided in methanol/water (4:1, v/v) at a concentration of 1800 nM. Recombinant human ER- α and ER- β were provided in buffer (50 mM Bis-Tris propane, 400 mM KCl, 2 mM DTT, 1 mM EDTA, and 10% glycerol), at concentrations of 734 and 3800 nM, respectively. All tested samples were dissolved in either methanol or DMSO. The sample solutions (1 μ L) were mixed well with 49 μ L of ES2 screening buffer (100 mM potassium phosphate, 100 μ g/mL BGG, and 0.02% NaN₃). The ER- α /ES2 complex was prepared with the fluorescent estrogen ligand ES2, human recombinant ER and ES2 screening buffer at concentrations of 9 nM ES2 and 30 nM ER- α . The ER- β /ES2 complex was prepared with the fluorescent estrogen ligand ES2, human recombinant ER- β and ES2 screening buffer at concentrations of 9 nM ES2 and 20 nM ER- β . Reactions were initiated by adding ER/ ES2 complex (50 μ L) to bring the incubation volume to 100 μ L and incubated for 2 h avoiding light. The polarization value was determined by measuring fluorescent response using a BioTek (Winooski, VT) Synergy 2 fluorometric plate reader. Excitationemission wavelengths for fluorescence polarization were 485 and 530 nM. The polarization values in the presence of the test competitors were compared with those of the control, in which the competitor was replaced with vehicle. The extent of competition was expressed as the percentage of remaining polarization compared to the control. EC_{50} values were determined as the competitor concentrations that brought

about half reduction in polarization value by fitting all the data to a one-site competition equation using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA).

Cell Culture and Test Compound Treatment. Estrogen receptor-positive human breast carcinoma cell line (MCF-7 cells) were seeded at a density of 10^5 cells/well in six-well plates and maintained at 37 °C under a humidified atmosphere of 5% CO₂ and 95% air in minimum essential media (MEM) supplemented with 10% fetal bovine serum (FBS). Before the test compound treatments, the cells were preconditioned in charcoal-stripped FBS for 72 h to remove the estrogens from the growth medium containing 10% FBS. The cells were treated with vehicle (0.1% methanol) alone, 1 μ M test compound, or 1 μ M endoxifen (positive control) for 24 h in the presence of 10 nM β -estradiol (E2) dissolved in MEM supplemented with 10% charcoal-stripped FBS.

Ribonucleic Acid (RNA) Extraction and Concentration Measurement. The MCF-7 cells treated with test compounds or experimental controls for 24 h were harvested for progesterone receptor (PGR) messenger ribonucleic acid (mRNA) extraction. Before ribonucleic acid (RNA) extraction, genomic DNA was eliminated. RNA was extracted from approximately 3×10^5 cells by RNeasy Plus Mini Kit (Qiagen Inc., Valencia, CA, USA). The RNA concentration was measured using the Qubit RNA BR assay (Life Technologies Corp., Carlsbad, CA) for the Qubit 2.0 fluorometer (Life Technologies Corp., Carlsbad, CA). The RNA was stored at -80 °C before further use.

Complementary Deoxyribonucleic Acid (cDNA) Synthesis. Complementary DNA (cDNA) for the real-time quantitative polymerase chain reaction (PCR) assay was synthesized from DNase-treated total RNA using the QuantiTect reverse transcription kit (Qiagen Inc., Valencia, CA, USA).

Real-Time Quantitative Polymerase Chain Reaction (PCR) for cDNA. The cDNA was amplified with TaqMan Universal PCR Master Mix (Applied Biosystems Inc., Carlsbad, CA), and then PCR was performed in the QuantStudio 12K Flex Real-Time PCR System (Life Technologies Corp., Carlsbad, CA). Progesterone receptor gene (PGR, FAM, Hs01556702, Life Technologies Corp., Carlsbad, CA) was the target gene, while glyceraldehyde 3-phosphate dehydrogenase (GAPDH, VIC, Hs02758991, Life Technologies Corp., Carlsbad, CA) gene expression was quantified to normalize each sample. A total of 40 amplification cycles were performed. Quantitative values of amplification were obtained from the threshold cycle (Ct) defined as the cycle number at which the fluorescent signal is first recorded above the background as determined during the exponential phase of PCR rather than at the end point. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative mRNA expression, and the results were expressed as percentages of antagonism effects compared to E2stimulated PGR mRNA expression (considered as 100%). If amplification was not seen by 40 cycles, the measured RNA was considered to be undetectable.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmed-chem.5b01677.

Molecular models of compound 12 in the active sites of aromatase and ER- α , and associated tables listing hydrogen bond angles (PDF)

SMILES molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AI, aromatase inhibitor; SERM, selective estrogen receptor modulator; ATAC, arimidex, tamoxifen, alone or in combination; ER, estrogen receptor; FDA, Food and Drug Administration; MOMCl, methyl chloromethyl ether; NBS, *N*bromosuccinimide; CYP, cytochrome P450; E2, β -estradiol; PGR, progesterone receptor; PDB, Protein Data Bank

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