Published in final edited form as:
J Med Chem. 2013 April 25; 56(8): 3346–3366. doi:10.1021/jm400157e.

Thiophene-Core Estrogen Receptor Ligands Having Superagonist Activity

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Abstract

To probe the importance of the heterocyclic core of estrogen receptor (ER) ligands, we prepared a series of thiophene-core ligands by Suzuki cross-coupling of aryl boronic acids with bromo-thiophenes, and we assessed their receptor binding and cell biological activities. The disposition of the phenol substituents on the thiophene core, at alternate or adjacent sites, and the nature of substituents on these phenols all contribute to binding affinity and subtype selectivity. Most of the bis(hydroxyphenyl)-thiophenes were ERβ selective, whereas the tris(hydroxyphenyl)-thiophenes were ERα selective; analogous furan-core compounds generally have lower affinity and less selectivity. Some diarylthiophenes show distinct superagonist activity in reporter gene assays, giving maximal activities 2–3 times that of estradiol, and modeling suggests that these ligands have a different interaction with a hydrogen-bonding residue in helix-11. Ligand-core modification may be a new strategy for developing ER ligands whose selectivity is based on having transcriptional activity greater than that of estradiol.

INTRODUCTION

The beneficial physiological actions of estrogens on development and functional maintenance of many target tissues (reproductive system, bone,[1] vasculature,[2] and brain,[3] etc.[4]) are well recognized, as are the pathological roles that they play in promoting breast and uterine cancers.[5–8] This spectrum of effects in diverse tissues provides an intriguing opportunity for the creation of tissue-selective estrogens that on balance have net desirable vs. undesirable activities, such as ones that would provide bone and cardiovascular protection without stimulation of breast or uterus. The challenge of creating such selective estrogens has, over time, been approached in different ways.

The actions of estrogens are mediated by two members of the Nuclear Receptor superfamily,[9] the estrogen receptor (ER) subtypes, ERα and the more recently identified

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Supporting Information Available: HPLC results, HPLC spectra for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

ERβ.[10] These subtypes have distinct tissue distributions (ERα mainly in the female reproductive system and ERβ mainly in prostate, colon, cardiovascular and central nervous systems[11]), and even when both ER subtypes are present in the same cells, estrogen action through these receptors leads to differential gene regulation, resulting in distinct physiological outcomes.[12, 13] Thus, much effort has been made to develop ER subtype-selective ligands having tissue- and gene-selective biological activities. A major focus has been on obtaining ERβ-selective compounds, because this ER subtype is considered more likely to mediate desirable estrogen action without the increased risk of cancer from stimulation of the breast and uterus.[14–18] Despite the similarity of their ligand-binding pockets (LBPs),[19–21] considerable success has been achieved in obtaining ER ligands having *affinity* or *potency* preferences for one or the other ER subtype (Figure 1),[14] but thus far preferential *efficacy* or *intrinsic activity* has been only in favor of ERα.[22] The medical utility of ERβ subtype-selective estrogens, however, is less clear.[18]

Prior to the discovery of ERβ, efforts to obtain tissue-selective estrogens focused on compounds, termed SERMs (for selective estrogen receptor modulators), that showed different levels of partial agonist/antagonist activity (i.e., intrinsic activity) in different target tissues. The selectivity of SERMs was believed to arise from their differential engagement of coregulator proteins in a target cell- and gene-specific manner,[23] and compounds such as tamoxifen (Figure 1), as well as raloxifene, lasofoxifene, and basedoxifene, were found to be largely agonistic in bone and antagonistic in breast, but having varying activity in the uterus.

In the development of both SERMs and subtype-selective ligands, extensive investigation has been made of non-steroidal compounds having heterocyclic cores. In fact, while the peripheral substituents—typically phenols, simple alkyl groups, and basic and polar phenyl substituents—have generally remained the same, a wide variety of heterocyclic cores has been explored. We and others have examined ER ligand cores consisting of five-membered ring heterocycles[5, 24–37] and to a lesser extent six-membered ring heterocycles.[38] Some examples of the 5-membered ring heterocycle series are presented below (Figure 2).

We noted that the highest affinity ligands had 5-membered heterocyclic cores of low polarity (furan > pyrazole > imidazole), presumably reflecting both the penalty for desolvation to remove the ligand from water to bind to ER, as well as an intolerance of the receptor ligand binding pockets for core units having large dipole moments.[22] Also, in the pyrazole and furan series, we mapped quite extensively the requirements for high ERα binding affinity, and concluded that tri and even tetra substitution was required.[24, 30] Thiophenes, by contrast, have been relatively unexplored, and the only two prepared in our prior studies had lower levels of substitution and quite low affinities for ER.[30] Hartmann and co-workers have reported on certain disubstituted thiophenes as inhibitors of 17β-hydroxysteroid dehydrogenase type1 (17β-HSD1), but they also showed very low ER binding affinity,[39, 40] consistent with our prior observations.

In connection with recent work on a series of very different ER ligands, based on an inherently three-dimensional 7-thia-bicyclo[2.2.1]hept-5-ene-7-oxide core, we prepared a series of *3,4*-disubstituted thiophene precursors. These thiophenes were used as diene components in an *in situ* oxidative Diels-Alder reaction with various dienophiles to give the sulfoxide-bridged bicyclic system.[41] As part of that work, we tested the binding affinity of some of the diaryl-substituted thiophene Diels-Alder diene precursors (**4a–d**), and we were pleased to find that they showed significant ER binding affinities, in some cases with modest ERβ selectivity.

To pursue this lead further, we have now extended our investigation of thiophene-core ER ligands by making systematic modifications of the number, nature, and orientation of

substituents on these ligands, examining their binding affinity and cellular potencies for ERα and ERβ; some congeneric furan-core ligands were also prepared for comparison. We have been able to map structure-activity relationships (SARs) for these compounds, and most intriguingly, we have identified among these thiophene-core ligands, compounds that have striking superagonist activity. Superagonism, which is intrinsic activity greater than that of a paradigmatic agonist, in this case the endogenous hormone estradiol (E_2), has only scant precedent in the nuclear receptor field (see Discussion). The enhanced intrinsic activity of ligands showing superagonism might provide a novel mechanism for the development of estrogens having desirable selective activity that is complementary to the reduced intrinsic activity of SERMs.[42] In addition, molecular modeling of these novel ligands complexed to ER offers a framework for understanding the structural basis for their superagonist activity, and might reveal a general strategy for obtaining superagonists for other members of the nuclear receptor superfamily.

RESULTS

(We have deviated somewhat from the IUPAC rules in specifying the locant positions of the hydroxyl, methyl, and halogen substituents on the phenyl groups attached to the core heterocycle (i.e., thiophene or furan): Strict application of IUPAC rules is awkward because it causes locant positions on the phenyl groups to change when the nature of the substituent changes from –OMe (the thiophene or furan is the root) to –OH (the phenol becomes the root). Thus, to facilitate comparisons across compound series and discuss structure-activity relationships in a more understandable way, we have opted to use consistently the heterocyclic core (thiophene or furan) as the root name, regardless of the nature of the substituents on the phenyl groups. We also display these locant positions clearly in indicator structures in the schemes and tables. In addition, for clarity, the locant positions at which the aryl groups are attached to the thiophene or furan core rings are given in *italic* typeface.)

Chemical Synthesis

Thiophenes—In the synthesis of compounds **2a–m** (Scheme 1), key intermediates **1a–m** were obtained by a one-step double Suzuki cross-coupling reaction.[43, 44] Compounds **1a–d** and **1g–k** were easily obtained using $Pd(OAc)_2/PPh_3$ as catalyst. This reaction failed, however, with compounds **1e** and **1f**, and attempts to employ the two-step Suzuki coupling using $Pd(OAc)_2/PPh_3$ also gave very low overall yields of **1e** and **1f**, accompanied by a large amount of homo coupled diaryl byproducts. In a brief catalyst screen, we found that while $Pd_2(dba)_2$, $Pd(PPh_3)Cl_2$, and $Pd(CN)_2Cl_2$ did not give improved yields, $Pd(dppf)Cl_2$ gave the products **1e** and **1f** in 45% and 62% yields, respectively, with little by products (Scheme 1A). We presume that this improvement is due to the increased rate of reductive elimination resulting from the increased bite angle of the dppf ligand.[45] Unsymmetrical compounds **1l** and **1m** were obtained by a two-step Suzuki coupling (Scheme 1B). In the first step, 1 equivalent of commercially available 2,5-dibromothiophene reacted with 2 equivalents of aryl boronic acid under standard conditions to give the monosubstituted thiophene **19**, and a second cross-coupling with 2 equivalents of aryl boronic acid gave the intermediates **1l–m**. Finally, treatment of the compounds **1a–m** with boron tribromide gave the final 2,5-disubstituted diphenolic thiophenes **2a–m** in good yields.

In the synthesis of *3,4-, 2,4-, 2,3*-disubstituted thiophene derivatives **4a–f, 6a–b** and **8a–b** (Scheme 2), we used $Pd(OAc)_2/PPh_3$ for the preparation of **3a–d** but again turned to $Pd(dppf)Cl_2$ as catalyst for the preparation of compounds **3e–f, 5a–b** and **7a–b**, which were obtained in moderate to good yields from the appropriate dibromothiophene precursor. Ether cleavage gave final products **4a–f, 6a–b** and **8a–b** in 60–80% yields.

To investigate the effect of a third substituent on the thiophene core, we synthesized a series of trisubstituted thiophene compounds **10a–d, 12a–b** and **14a–c** (Scheme 3). *3*-Methyl-*2,5*-dibromothiophene (**20**) was first synthesized by bromination of *3*-methylthiophene using NBS.[46] A one-step Suzuki coupling with Pd(dppf)Cl_2 afforded **9a–d**, which gave the products **10a–d** in good yield after ether cleavage (Scheme 3A). 3-Phenyl bisphenol thiophenes **12a–b** were obtained by demethylation of **11a–b**, prepared by two successive cross-coupling reactions (Scheme 3B). In the first step, 1 equivalent of commercially available *2,3,5*-tribromothiophene was reacted with 4 equivalents of aryl boronic acid using standard conditions, and the resulting *2,5*-bis-substituted thiophenes **21** and **22** were subsequently submitted to a second cross-coupling reaction with 2 equivalents of phenyl boronic acid to yield the intermediates **11a–b**.

In our previous work on furan-core ER ligands,[30] we found that triaryl, especially trisphenol, furans showed higher binding affinity and subtype selectivity than the corresponding bisphenol analogues. Thus, we also wanted to introduce a third phenol ring into the thiophene-core compounds. Key intermediates **13a–c** were prepared using 1 equivalent of *2,3,5*-tribromothiophene with 5 equivalents of aryl boronic acid using standard conditions; ether cleavage then gave compounds **14a–c** in very good yields (Scheme 3C).

Furans—For comparison with selected thiophenes, we prepared their furan-core counterparts (**16a–d** and **18a–d**, Scheme 4). *2,5*-Dibromofuran (**23**) was synthesized by bromination of furan using DMF as solvent with dropwise addition of Br_2 (Scheme 4A).[47] *3,4*-Dibromofuran (**24**) was synthesized by oxidation of commercially available (*E*)-2,3-dibromo-2-butane-1,4-diol using chromosulfuric acid (Scheme 4B).[48] Because *3,4*-dibromofuran decomposes under the reaction conditions, it was continuously removed from the reaction mixture by steam distillation and stored below 0 °C as a white solid. We first used Suzuki coupling reaction conditions similar to those used for the thiophenes, but we failed to get to corresponding furans. Considering the lower aromaticity of furan compared to thiophene, we then switched to Pd(PPh_3)$_4$ as Pd(0) catalyst.[49] The dibromofurans were coupled with 5 equivalents of the aryl boronic acid and then deprotected with boron tribromide to give the final products **16a–d** and **18a–d** in moderate to good yields.

The identity of all final compounds were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectroscopy (see experimental section).

Biological Results

(See comment on nomenclature at the start of the Results Section)

Relative Binding Affinities—The relative binding affinities were determined by a competitive radiometric receptor-binding assay and are reported in Tables 1–3. These affinities are presented as relative binding affinity (RBA) values, where estradiol has an affinity of 100. For reference, the K_D of estradiol is 0.2 nM for ERα and 0.5 nM for ERβ. (The affinities for a subset of compounds representing the different modes of diaryl core substitution are compared schematically in Figure 3.)

A number of the bis(hydroxyphenyl)thiophenes (Table 1) show moderate levels of ERβ selectivity, which is a general characteristic of rather slender ligands.[14] In terms of absolute affinity, however, substituents on the phenol rings have major effects on their binding affinity and selectivity. Among the *2,5*-bisphenol thiophenes (Series **2**), those substituted at C2 (*meta* to the phenol) bound better than the corresponding non-substituted compound **2a**. The highest affinity ERβ ligand, *2,5*-bis(2-fluoro-4-hydroxyphenyl)thiophene (**2e**), binds to ERβ with an affinity of 33 and a 16-fold ERβ binding affinity preference (Table 1, entry 5).

The highest affinity ERα ligand in the Series **2** was the 2-chloro analogue **2f**, with an RBA of 6.7, but it still showed ERβ selectivity (RBA of 10 for ERβ, Table 1, entry 6).

The thiophenes having substituents at the C3 position (i.e., *ortho* to the phenol) all show dramatically decreased binding affinities compared to the corresponding ones substituted at C2 (i.e., *meta* to the phenol). Their affinities are even lower than that of the corresponding unsubstituted compound **2a** (Table 1, entry 1), with the tetra *ortho* methyl compound **2d** binding particularly poorly (Table 1, entry 4). This is very likely due to steric occlusion of the hydrogen bonding capacity of the phenols by *ortho* substitution, which is known to greatly reduce affinity in both steroidal and other non-steroidal estrogens.[50]

The position of the hydroxyl group is also of great importance, and when this group is moved from the *para* to the *meta* position of the phenyl ring (e.g., **2i–k**), binding affinities are remarkably lowered (Table 1, entries 9–11), again consistent with known trends in other ligand series. Unsymmetrically substituted compounds (e.g., **2l** and **2m**) also had somewhat lower binding affinities than their corresponding symmetrical counterparts **2e** and **2f** (Table 1, entries 5 and 6).

Like of *2,5*-disubstituted compounds (Series 2), similar trends were also observed for the *3,4*-disubstituted (Series **4**), *2,4*-disubstituted (Series **6**), and *2,3*-disubstituted compounds (Series **8**). The highest affinities were those substituted at C2, *meta* to the phenol, and the lowest, those with C3 (ortho) substitution or no substitution. Chlorine substitution gave the highest affinity for both ERs (**4f** and **6b**, Table 1, entries 19 and 21). Other comparisons among the disubstituted thiophenes (and furans) can be viewed more easily in Figure 3 (see below).

To investigate the influence of an additional substituent on the thiophene core, trisubstituted thiophenes **10a–d, 12a–b** and **14a–c** were prepared (Table 2). Introduction of a methyl group at the *3*-position of the thiophene core (Table 2, **10a–d**) did not cause a significant change in RBA but appeared to cause a decrease in selectivity for ERβ (**10a** vs. **2a, 10b** vs. **2b, 10c** vs. **2e, 10d** vs. **2f**). When the methyl group was replaced by a phenyl group, as with compounds **12a–b**, there was a further decrease in binding for ERβ (RBA of ERβ **12a** 2.1 vs. **2e** 33, **12b** 3.0 vs. **2f** 10), and when the third substituent is replaced by a phenol moiety, the subtype selectivity was actually reversed. Thus, compounds **14a–c** show ERα-selectivity, with **14c** having an affinity for ERα of 86 and a selectivity of 14, which is similar to that reported earlier for the triphenolic furans and pyrazoles.[24, 30] This reversal of ER subtype binding preference as additional substituents are added to the heterocycle core likely reflects the fact that the ligand-binding pocket of ERα is larger than in ERβ.[24, 30] Also, because members of the tris-phenol series (Series **14**) bound better than the phenyl bis-phenols (Series **12**), we inferred that the third phenolic hydroxyl group might be involved in a hydrogen bond with another residue in the ligand binding pocket, most likely T347 in ERα. Similar enhancement in binding with a third phenolic hydroxyl group can be found in the furan and pyrazole systems that we have previously investigated.[24, 30]

Finally, to compare the affinity of thiophene-core ligands with furan-core ligands, we prepared a limited series of bis(hydroxylphenyl)furans (**16a–d** and **18a–d**, Table 3). Gratifyingly, most of these disubstituted furans retained the ERβ selectivity of the corresponding thiophenes. The best furan, compound **16c**, had an RBA of 6.9 and 32 for ERα and ERβ, respectively, giving a 4.7-fold ERβ selectivity (Table 3, entry 3). Where direct comparisons can be made, however, the furans (except for compound **16c**) have lower affinity and poorer selectivity than their corresponding thiophenes. This might be due to the higher electron negativity of the oxygen atom, making the furan core more polar than the

thiophene core, and/or the longer length of the C-S vs. C-O bond, which somewhat alters the geometric disposition of the substituents.

Global comparisons of structure-affinity relationships among the disubstituted thiophenes and furans can better be made by reference to Figure 3, which displays an overlay of the two core substitution patterns that unite congeneric (positionally isomeric) compounds, an "alternate" pattern, with the phenols attached to non-adjacent positions (*2,5*- and *2,4*-patterns of substitution), and an "adjacent" pattern, with phenols on neighboring positions (*2,3*- and *3,4*-patterns of substitution). RBA values for ERα and [ERβ] are shown to the right. In all cases, the importance of C2 substitution on enhancing affinity is evident. This has been noted in our prior work on ER ligands with bicyclic cores[41, 52, 53] and in a recent report by Gust and colleagues on pyrroles.[29] Beyond this, the halogens, Cl and F, bind better than the proteo and Me analogs, with Cl being better than F in most cases. Curiously, despite their considerably different shapes, no clear pattern emerges in comparing the alternate vs. adjacent substitution patterns (upper vs. lower table), the two types of alternate or adjacent substitutions (blue vs. red in the thiophene tables), nor are there systemic differences between the furans and the congeneric thiophenes (right vs. left). In addition to the comparisons illustrated in this figure, other trends noted above were the increased affinity and ERα preference that comes from adding a third substituent, especially a phenol, onto the thiophene and furan cores.

We will return to this type of comparative analysis where we discuss the results of the cellular activity of these compounds and when we present examples of docking representative members into the ERα ligand-binding pocket and examine ligand volumes, geometries, and conformations.

Transcriptional Activation Assays—The thiophene- and furan-core compounds that have significant affinity for ERα or ERβ were characterized for activation of an estrogen response element (ERE)-driven luciferase reporter in HepG2 liver cells cotransfected with ERα or ERβ; this is considered to be a versatile cell system for characterizing the efficacy of ER ligands.[54] Dose-response curves were generated, and when full curves were evident, potency (EC_{50}) values and efficacy (Eff, % of E_2) values were determined. The results are summarized in Table 4, and dose-response curves for representative ligands, are shown in Figure 4.

Alternate-Position Aryl-Disubstituted Thiophenes (2,5-diaryl Series 2 and 2,4-diaryl Series 6): The thiophenes in these two regioisomeric series are congeneric except for the position of the sulfur atom in the thiophene ring. In the reporter gene assays, the Series **2** (*2,5*-bisphenol-substituted) thiophenes mostly profiled as superagonists on both ERα and ERβ, showing efficacies in ERβ 2–3 times greater than that of estradiol (e.g., see compound **2l**, Figure 4B). To test whether the superagonist activity was cell-type specific, compounds were also tested at a single, high dose (10 μM) for activation of the ERE-luciferase reporter in Ishikawa endometrial cells that contain native ERα, and here again most of the Series **2** tested profiled as superagonists (Table 4).

Although these compounds have affinities in the low nanomolar range, most of their potencies in the HepG2 assays were in the micromolar range, reflecting the fact that cellular potency involves the affinities of the receptor both for the ligand and for the recruited coregulators that provide the essential signal for activation of transcription.[55] In addition, the liver-derived HepG2 cells are known to metabolize small molecules, which sometimes reduces their cellular potency.[5657]

With respect to the effects of substitutions on the phenolic groups, the 2-methyl analog **2b** displayed lower potency than **2a**, despite its higher affinity. Thus, the lack of superagonist activity for **2b** in the HepG2 cells may reflect the fact that maximal activity (i.e., saturation) was not reached at the doses we tested. While the 2-Cl substitution in **2f** had little effect on activity, the 2-F containing analog **2e** displayed even greater super-agonist activity. The other alternate-position thiophene regioisomers, Series **6**, differ only in the location of the sulfur atom in the thiophene core from Series **2**. Nevertheless, the superagonist activity for the 2-F compound **6a** was attenuated with respect to the Series **2** regioisomers, with the 2-Cl substituted **6b** having only a full agonist profile. Further interpretation of the structural basis of superagonism is presented in the Discussion.

The congeneric *2,5*-disubstituted furan Series **16** displayed less agonist activity, but this may reflect the lower potency and lack of curve saturation at the highest dose tested (10 μM). Again, these differences in potency were not related to affinity, as was the case with the corresponding compounds in Series **2**.

Adjacent-Position Aryl-Disubstituted Thiophenes (3,4-diaryl Series 4 and 2,3-diaryl 8) and Trisubstituted Thiophenes: Thiophenes in these series contain substitutions on adjacent positions on the thiophene, drastically changing the overall shape of these ligands compared to those of the alternate-position ligand. Also, compared to the planar ground-state conformation of the alternate-position ligands, steric interaction of the adjacent-disposed aryl groups also causes substantial (ca. 30°) twisting of the phenols out of the plane of the thiophene ring. These compounds displayed variable partial to full agonist activity profiles, with little evidence of superagonist activity. Surprisingly, **4e** and **4f** displayed >25 fold better EC_{50} values on ERα and ERβ than the corresponding **8a** and **8b**, despite affinity differences of only ~2 fold.

The effects of adding a third substituent onto the core were very dramatic. For the methyl-substituted Series **10**, the efficacies were similar to Series **2**, but with substantial increases in potency for ERα. The phenyl **12** or phenol **14** series as well displayed selective activation of ERα. In fact, compounds **12b** and **14c** (Figure 4A and **D**) displayed low nM potency for activating ERα. All of these changes in potency reflect the trends in binding affinity. Notably, while **12b** had no activity on ERβ up to 10 μM, **14a** and **14c** displayed inverse agonist activity on ERβ (Figure 4C and D), representing to the best of our knowledge first-in-class ligands. Even the full antagonist ICI 182,780 (Fulvestrant) does not have inverse agonist activity on ERβ (Figure 4C, black line).

A summary of efficacy values for the alternate and adjacent di-substituted thiophenes and furans are presented in Figure 5, according to the same layout as were the binding affinities (Figure 3); efficacy values above 120 are noted in boldface. The predominance of superagonism in the alternate compared to the adjacent mode thiophenes, and in the thiophenes compared to the furans is very evident. To ascertain that the effects of these compounds are ER mediated, we confirmed their activity in a mammalian 2 hybrid assay of ER-coactivator binding and also by the induction of a canonical ER target gene, GREB1 (See supporting information). A proposed structural model for superagonist activity is presented in the Discussion.

Discussion

The X-ray structures of many ER-ligand complexes show a pocket volume that is considerably larger than the ligand volume, with a ligand such as E_2 appearing to be “suspended” by close contacts and hydrogen bonding at its ends (the A and D rings) but having relatively few close contacts at its middle (B and C rings). The ER can form a variety

of pocket shapes upon binding structurally different ligands, and these shapes relate to the overall conformation of the ER-ligand complex, defining its interaction with coregulators, and ultimately determining its biological output. To understand more fully the connections between ligand shape, ligand-binding pocket shape, and biological output of the ER, we have examined the respective contributions that the *core* vs. the *peripheral substituents* of an ER ligand makes to its affinity, potency, efficacy, and ER-subtype selectivity in a number of systems.

Reviving Thiophene-Core Ligands

In this work, we have extended our examination of ER ligands having 5-membered ring heterocyclic cores such as isoxazoles,[58] imidazoles,[58] pyrazoles,[23, 41] and furans,[30] to thiophenes. Previously, we found that these 5-membered, as well as some related 6-membered ring heterocyclic cores, afforded high affinity when the core had low polarity (or a small dipole moment) and when there were a sufficient number of peripheral substituents. Larger ligands showed distinct preferential affinity and potency for ERα and smaller ligands for ERβ. However, our initial efforts to prepare thiophene-core ligands from the furan 1,4-diones precursors in the presence of thiolating agents were largely thwarted by the strong tendency of the highly substituted diones to form the furans in preference to the thiophenes.[30]

Our interest in the thiophenes was revived when we prepared some 3,4-diarylthiophenes to use as dienes for the preparation of 7-thia-bicyclo[2.2.1]hept-5-ene-7-oxides through a Diels-Alder reaction (using their corresponding monoxides).[41] This alternative approach of preparing the di- and tri-substituted thiophenes from di- and tribromothiophene precursors using palladium-mediated cross coupling with aryl boronic acids, described here, works very well, and it enabled us to prepare the larger series of thiophenes (as well as related furans) that are examined herein.

Structural Trends in Binding Affinities and Estrogen Receptor Subtype Selectivity in the Thiophenes and Furans

From the binding data in Tables 1–3 and the summary of the diaryl congeners (Figure 3), we can make some novel observations and note a number of familiar trends.

With earlier biaryl-substituted heterocycle-core ER ligands, we generally used simple 4-hydroxyphenyl (phenol) substituents and enhanced affinities by adding a third phenol and even a fourth (alkyl) group. Here, we have enhanced affinities by adding halogens (F, Cl) and small alkyl groups (Me) at C3, meta to the phenolic OH. The boost in affinity can be quite remarkable (>100 fold on ERα for compounds **2f** vs. **2a**, and >10 fold for many others) and presumably arises from a more complete filling of the ligand-binding pocket, such that high affinity binding can be obtained even with thiophenes and furans containing only two substituted phenols. By contrast, as has long been known in many ligand series, substitution at C3, *ortho* to the phenol, greatly reduces affinity, presumably by interfering with critical hydrogen bonding.[2050] It is of note that in a series of B-seco or A-CD steroidal estrogens studied earlier, a beneficial effect of *meta* but not ortho substitution was also found.[59, 60] Similar observations of the beneficial effect of *meta* substitution were made with a number of our bicyclo[2.2.1]heptane core ligands,[41, 52, 53] and have been noted recently by Gust and colleagues in a series of pyrroles.[29] Thus, filling voids in ligand volume without interfering with critical receptor interactions is an emerging strategy to generate high affinity ER ligands. It was also surprising that in the bisaryl-substituted thiophenes and furans, there are no great differences in the affinity between members of the alternate-position vs. adjacent-position series, despite marked differences in ligand length and shape, as well as phenol-heterocycle core torsional angles.

Other general trends are well known. The ERβ preference of smaller ligands (bisphenols or methyl-bisphenols) vs. larger ligands (trisphenols or phenyl bisphenols) is known from the pyrazoles and furans,[2430] and doubtless reflects the generally smaller pockets that form around ERβ ligands.[21] Bisphenol C3-substituted ligands have high affinity and high ERβ selectivity; the best bisphenolic compound is **2e**, with an ERβ affinity of 33 and selectivity of 16; the best tris(hydroxyphenyl) thiophene is compound **14c**, with an ERα affinity of 85 and selectivity of 14. Also, where they can be directly compared (**2a/16a, 2b/16b, 2e/16c, 2f/16d, 4a/18a, 4b/18b, 4e/16c**, and **4f/18d**), the thiophenes generally bind somewhat better than the furans (though not by a large factor), perhaps because the thiophene core is less polar than the furan core. This trend was also evident in comparisons of the more polar core imidazoles with the corresponding pyrazoles.[24, 58] The greater electron density and higher polarizability of the sulfur atom as well as the geometric changes due to the longer C-S bond vs. C-O bond could also be factors that enhance the affinity of the thiophenes (see below).

Models for Thiophenes Binding in the Estrogen Receptor α Ligand Binding Pocket and a Possible Model for Superagonism

The most unusual finding in this study is the pronounced superagonism shown by a number of the alternate-substituted thiophenes. Ligands exhibiting superagonistic activity have been described in the nuclear receptor field. Gustafsson and others have reported that some phytoestrogens gave a somewhat higher reporter gene maximal transcriptional output than estradiol, especially on ERβ.[61–64] Because the maximum transcriptional output from ERβ at saturating concentrations of both expression plasmid and E_2 ligand is only about 25–40% of that from ERα,[62] one could speculate that other ligands might be able to drive ERβ into a yet more active conformation. Also, there is evidence that steroidal ligands other than E_2, notably 3β-adiol [65] and Δ^5-3β-adiol,[66] are likely the endogenous ligands for ERβ in some tissue contexts. Nevertheless, we find that certain of the thiophenes can drive superagonism from ERα as well as from ERβ; so, superagonism is not exclusively the domain of ERβ.

We have used molecular modeling to examine potential modes of interaction of the thiophenes with ER and to explore what might be the molecular basis for superagonism, which is seen predominantly with the alternate-position thiophenes. Crystal structures of ERα-ligand complexes show that the A-ring phenolic hydroxyl of estradiol has hydrogen bonding interactions with helix 3 residues Glu-353, Arg-394, and an ordered water molecule, while the D-ring hydroxyl hydrogen bonds with the helix 11 residue, His-524 (Figure 7A). This stabilizes helices 3 and 11, and allows helix 12 to dock across them. In this so-called active conformation, helix 12 forms one side of a hydrophobic groove on the surface where transcriptional coactivators bind. Hydrogen bonding to helix 3 via an A-ring mimetic has been seen in every ligand-bound ER crystal structure to date, while hydrogen bonding to helix 11 is more variable.

Molecular modeling of complexes with ERα suggests that thiophenes in the two alternate-substituted series bind in a different manner compared to a typical steroidal agonist. It seems reasonable to assume that the thiophenes in the *2,5*- and *2,4*-alternate-substituted series (**2** and **6**) would bind to ER in a congruent fashion. The only expected difference would be in the placement of the thiophene sulfur atom towards opposite sides of the pocket, where it might engage in different interactions with residues in the ligand-binding pocket. However, there seem to be no pronounced differences between the *2,5*- and *2,4*-bisphenolic thiophenes and furans in terms of affinity and intrinsic activity, except the more pronounced superagonism exhibited by the thiophenes compared to the furans. The most likely polar interactions for the alternate-pattern bisphenols, then are with Glu-353 and Arg-394 on one end and His-524 on the other (See Figure 7). While it is a speculation, superagonism might be the consequence of the increased O-O distance between the two phenols in the thiophenes

compared to other ligands. This distance is 10.9 Å in E_2 and 11.8 Å in diethylstilbestrol; however, in both alternate thiophene bisphenols it is 12.8 Å, that is, 1–2 Å longer. The O-O distance in the thiophenes is also somewhat longer than in the alternate-position furan bisphenols, due to the greater length of the C-S bonds (1.47 Å) vs. the C-O bonds (1.24 Å) in the heterocyclic core. (This bond length difference also affects to a lesser degree, the display angle of the two phenols on the heterocyclic core.) Thus, the alternate-position bisphenol thiophenes might be too long to form adequate hydrogen bonds with His-524.

We recently published on a series of oxabicyclic compounds, exemplified by OBHS, where the side group extends between helices 8 and 11 (Figure 7B).[52] Modeling predicts that the thiophene **2e** will bind similarly between helices 8 and 11 (Figure 7C), but this does not explain their superagonist activity. We have found that ligand-specific “mis-positioning” of helix 11 can interfere with the agonist conformation of helix 12;[52] so, we suggest that the superagonist activity may stem from “improved” positioning of helix 11 that stabilizes helix 12 in the agonist conformation to an even greater degree than does E_2. This issue is being examined further by X-ray crystallography.

The binding of thiophenes with adjacent phenols (Series **4** and **8**) was modeled with compound **4e**, based on the assumption that the two hydroxyls will form hydrogen bonds similar to the two phenols in OBHS by wrapping around helix 3. In this model (Figure 7D), the ligand makes no contacts with helix 11, which would be a novel form of binding for an ER ligand, but one likely utilized by members of the cyclofenil class of ligands. Changing the position of the sulfur (i.e., *2,3-* vs. *3,4-* substitution) could provide subtle shifts in helix 3, which comprises part of the coactivator-binding site on the external surface. The adjacent phenol thiophenes do not show superagonism, and from this model, there is no apparent reason why they should. All of these issues are being examined further by X-ray crystallography.

Conclusion

In this paper, we present the synthesis of a novel series of thiophenes and an investigation of their ER binding and biological activity. Ligands of this series can be easily prepared by Suzuki coupling, an approach that may prove convenient for further library synthesis. These compounds show an interesting binding affinity pattern: Small substituents placed meta to the phenols cause marked enhancements of binding affinity, and in general, most of the bis(hydroxylphenyl) thiophenes were selective for ERβ, whereas the tris(hydroxyphenyl) thiophenes were selective for ERα. Reporter gene assays reflect these trends, but also reveal that a number of the alternate-pattern thiophene bisphenols have marked superagonistic activity, more so than the adjacent-pattern bisphenols or the corresponding furans from either series. Molecular modeling suggests that the 1–2 Å increased O-O distance in the alternate-mode thiophenes might be responsible for this superagonism. Further studies of the cellular and *in vivo* biological activities of key members of this new class of ER ligands, as well as X-ray crystallographic analyses, which are underway, will be reported in due course.

The generation of this new series of ER ligands provides important insight into the diversity of structures that can function as ligands for both ER subtype, and our findings may open new strategies for the design of both ERα- and ERβ-selective ligands. They also illustrate that changes to the ligand core structure, though it is remote from close contacts with residues in the ligand-binding pocket, can have important effects on activity, in this case increased efficacy leading to superagonism. When better understood at the level of molecular interactions, superagonism itself might prove to be a new mode for developing ER ligands having selective activities.[42]

Experimental section

General

Starting materials were purchased from Aldrich, Acros, Aladin-reagent, Alfa-Asar and were used without purification. Tetrahydrofuran was freshly distilled from sodium/benzophenone under Argon. 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 Biospin AV400 (400 MHz) 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. The purity of all compounds for biological testing was determined by HPLC method (see Supporting Information), confirming >95% purity.

General Procedure for Suzuki Coupling—Under Ar atmosphere, a mixture of bromothiophene (1 equiv), arylboronic acid (2 equivalents for mono-substituted, 4 equivalents for di-substituted, 5 equivalents for tri-substituted thiophenes), Pd catalyst, sodium carbonate (2 equiv.) in an oxygen-free toluene/water (1:1) solution was stirred at 120 °C for 24 hour. After 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 $MgSO_4$/Na_2SO_4, then filtered and concentrated on vacuum. The product was purified by column chromatography (CC).

General Procedure for Ether Cleavage—Under argon atmosphere, To a solution of methoxyphenyl derivative (1 equiv.) in dry dichloromethane at −20 °C, boron tribromide (3 equivalents per methoxy function) was added dropwise. The reaction mixture was stirred at room temperature. After 4h, water was added to quench the reaction, and ethyl acetate was used to extract the aqueous layer. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$/Na_2SO_4, then filtered and concentrated on vacuum. The product was purified by column chromatography (CC).

2,5-Bis(4-methoxyphenyl)thiophene (1a)—Compound **1a** was prepared by reaction of 2,5-dibromothiophene and (4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 94–98 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.51 (m, 4H), 7.15 (s, 2H), 6.95 – 6.89 (m, 4H), 3.84 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.12, 144.51,126.84, 122.90, 114.31, 55.38.

2,5-Bis(4-methoxy-2-methylphenyl)thiophene (1b)—Compound **1b** was prepared by reaction of 2,5-dibromothiophene and (4-methoxy-2-methylphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 72–74 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, J = 8.4 Hz, 2H), 6.86 (s, 2H), 6.72 (d, J = 2.5 Hz, 2H), 6.68 (dd, J = 8.4, 2.6 Hz, 2H), 3.72 (s, 6H), 2.37 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.11, 142.33, 137.55, 131.49, 126.87, 126.04, 116.24, 111.29, 55.29, 21.56.

2,5-Bis(4-methoxy-3-methylphenyl)thiophene (1c)—Compound **1c** was prepared by reaction of 2,5-dibromothiophene and (4-methoxy-3-methylphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 78–81 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.4 Hz, 2H), 6.89 (s, 2H), 6.72 (d, *J* = 2.5 Hz, 2H), 6.58 (dd, *J* = 8.4, 2.6 Hz, 2H), 3.82 (s, 6H), 2.29 (s, 6H)..

2,5-Bis(4-methoxy-3,5-dimethylphenyl)thiophene (1d)—Compound **1d** was prepared by reaction of 2,5-dibromothiophene and (4-methoxy-3,5-dimethylphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 99–101 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (s, 4H), 7.16 (s, 2H), 3.74 (s, 6H), 2.32 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.63, 142.91, 131.36, 130.04, 126.07, 123.35, 59.83, 16.20.

2,5-Bis(2-fluoro-4-methoxyphenyl)thiophene (1e)—Compound **1e** was prepared by reaction of 2,5-dibromothiophene and (2-fluoro-4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as yellow solid (mp 94–96 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (t, *J* = 8.9 Hz, 2H), 7.34 (s, 2H), 6.78 – 6.67 (m, 4H), 3.83 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.96, 158.47, 136.41, 129.07, 125.66, 114.84, 110.54, 102.30, 55.68.

2,5-Bis(2-chloro-4-methoxyphenyl)thiophene (1f)—Compound **1f** was prepared by reaction of 2,5-dibromothiophene and (2-chloro-4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as green solid (mp 107–110 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.7 Hz, 2H), 7.26 (s, 2H), 7.03 (d, *J* = 2.6 Hz, 2H), 6.86 (dd, *J* = 8.7, 2.6 Hz, 2H), 3.84 (s, 6H).

2,5-Bis(3-fluoro-4-methoxyphenyl)thiophene (1g)—Compound **1g** was prepared by reaction of 2,5-dibromothiophene and (3-fluoro-4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as green solid (mp 92–97 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.17 (s, 2H), 7.00 (d, *J* = 2.6 Hz, 2H), 3.95 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.46, 141.69, 127.95, 127.38, 124.96, 123.65, 122.95, 112.31, 56.29.

2,5-Bis(3-chloro-4-methoxyphenyl)thiophene (1h)—Compound **1h** was prepared by reaction of 2,5-dibromothiophene and (3-chloro-4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as green solid (mp 108–110°C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 2.3 Hz, 2H), 7.46 (dd, *J* = 8.5, 2.3 Hz, 2H), 7.16 (s, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 3.94 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.35, 142.37, 142.17, 138.80, 123.65, 121.47, 113.80, 113.26, 56.38.

2,5-Bis(2-fluoro-5-methoxyphenyl)thiophene (1i)—Compound **1i** was prepared by reaction of 2,5-dibromothiophene and (2-fluoro-5-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as yellow solid (mp 102–104 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (s, 2H), 7.06 (dd, *J* = 6.1, 3.0 Hz, 2H), 7.01 – 6.94 (m, 2H), 6.69 (dt, *J* = 8.9, 3.4 Hz, 2H), 3.74 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.82, 154.92, 152.51, 137.44, 127.16, 122.36, 117.04, 113.94, 55.84.

2,5-Bis(3-fluoro-5-methoxyphenyl)thiophene (1j)—Compound **1j** was prepared by reaction of 2,5-dibromothiophene and (3-fluoro-5-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as yellow solid (mp 89–90 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 2H), 6.84 (dd, *J* = 7.9, 1.9 Hz, 4H), 6.51 – 6.45 (m, 2H), 3.76 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.86, 157.43, 155.99, 137.63, 131.12, 119.54, 102.13, 99.89 (s, 3H), 99.66, 95.60, 50.40.

2,5-Bis(4-fluoro-3-methoxyphenyl)thiophene (1k)—Compound **1k** was prepared by reaction of 2,5-dibromothiophene and (4-fluoro-5-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as yellow solid (mp 105–108 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (m, 3H), 7.17 – 7.12 (m, 3H), 7.08 (dd, J = 10.7, 8.4 Hz, 2H), 3.96 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.40, 150.94, 147.91, 142.78, 130.87, 124.06, 118.29, 116.49, 56.35.

2-(2-Fluoro-4-methoxyphenyl)-5-(4-methoxy-2-methylphenyl)thiophene (1l)—Compound **1l** was prepared by reaction of compound **19** and (2-fluoro-4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (t, *J* = 8.9 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 2.5 Hz, 1H), 6.97 (d, *J* = 3.6 Hz, 1H), 6.82 (d, *J* = 2.4 Hz, 1H), 6.75 (ddd, *J* = 20.7, 10.9, 2.5 Hz, 3H), 3.83 (s, 6H), 2.45 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.92, 159.89, 159.20, 158.44, 142.34, 137.62, 136.51, 131.48, 129.08, 126.63, 125.17, 116.22, 115.03, 111.28, 110.51, 102.31, 102.05, 55.65, 55.28, 21.46.

2-(2-Chloro-4-methoxyphenyl)-5-(4-methoxy-2-methylphenyl)thiophene (1m)—Compound **1m** was prepared by reaction of compound **19** and (2-chloro-4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.6 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 3.6 Hz, 1H), 6.91 (d, *J* = 2.5 Hz, 1H), 6.86 (d, *J* = 3.6 Hz, 1H), 6.72 (dd, *J* = 8.8, 2.5 Hz, 2H), 6.66 (dd, *J* = 8.4, 2.5 Hz, 1H), 3.70 (d, *J* = 2.5 Hz, 6H), 2.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.22, 142.21, 138.25, 136.52, 131.78, 130.82, 130.45, 125.95, 125.58, 124.96, 124.71, 115.19, 114.59, 112.22, 110.23, 54.51, 54.18.

2,5-Bis-(4-hydroxyphenyl) thiophene (2a)—Compound **2a** was prepared by 2,5-bis-(4-methoxyphenyl) thiophene (**1a**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as white solid (mp 168–170 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.50 (s, 1H), 7.42 – 7.33 (m, 4H), 7.08 (s, 2H), 6.81 – 6.71 (m, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 158.18, 143.20, 127.51, 126.88, 123.52, 116.67. HRMS (MALDI/DHB) calcd for $C_{16}H_{12}O_2S$ ($M+H^+$) 268.0556, found 268.05525.

2,5-Bis(4-hydroxy-2-methylphenyl)thiophene (2b)—Compound **2b** was prepared by 2,5-bis(4-methoxy-2-methylphenyl)thiophene (**1b**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as white solid (mp 172–175 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.17 (t, *J* = 4.6 Hz, 2H), 6.92 (s, 2H), 6.74 (d, *J* = 12.5 Hz, 2H), 6.69 – 6.60 (m, 2H), 2.06 (d, *J* = 5.8 Hz, 6H). ^{13}C NMR (101 MHz, acetone-d_6) δ 144.04, 136.74, 132.19, 131.69, 128.41, 126.86, 118.76, 115.40, 21.00. HRMS (MALDI/DHB) calcd for $C_{18}H_{16}O_2S$ ($M+H^+$) 296.0875, found 296.08655.

2,5-Bis(4-hydroxy-3-methylphenyl)thiophene (2c)—Compound **2b** was prepared by 2,5-bis(4- methoxy-3-methylphenyl)thiophene (**1c**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp187–189 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38 (s, 2H), 6.95 (d, J = 1.2 Hz, 2H), 6.70 (dd, J = 8.2, 1.8 Hz, 2H), 6.64 (d, J = 8.2 Hz, 2H), 2.06 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 149.05, 142.28, 137.54, 131.46, 126.84, 125.99, 116.18, 111.24, 21.53. HRMS (MALDI/DHB) calcd for $C_{18}H_{16}O_2S$ (M +H$^+$) 296.0875, found 296.08655.

2,5-Bis(4-hydroxy-3,5-dimethylphenyl)thiophene (2d)—Compound **2d** was prepared by 2,5-bis-(4-methoxy-3,5-dimethylphenyl)thiophene (**1d**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 201–203 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.27 (s, 4H), 7.18 (s, 2H), 2.27 (s, 12H).^{13}C NMR (101 MHz, acetone-d_6) δ 153.99, 143.32, 126.96, 126.36, 125.41, 123.27, 16.71. HRMS (MALDI/DHB) calcd for $C_{20}H_{20}O_2S$ (M+H$^+$) 324.1184, found 324.11785.

2,5-Bis(2-fluoro-4-hydroxyphenyl)thiophene (2e)—Compound **2e** was prepared by 2,5-bis(2-fluoro-4-methoxyphenyl)thiophene (**1e**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 178–180 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.59 (t, J = 8.9 Hz, 2H), 7.37 (s, 2H), 6.76 (ddd, J = 15.3, 10.8, 2.4 Hz, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 161.72, 159.29, 137.16, 130.04, 126.03, 114.17, 113.13, 104.20. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2F_2S$ (M+H$^+$) 304.0369, found 304.03641.

2,5-Bis(2-chloro-4-hydroxyphenyl)thiophene (2f)—Compound **2f** was prepared by 2,5-bis(2-chloro-4-methoxyphenyl)thiophene (**1f**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 194–196 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.50 (d, J = 8.5 Hz, 2H), 7.28 (s, 2H), 7.04 (d, J = 2.5 Hz, 2H), 6.91 (dd, J = 8.5, 2.5 Hz, 2H).^{13}C NMR (101 MHz, acetone-d_6) δ 141.00, 133.08, 133.06, 127.75, 124.89, 117.89, 117.81, 115.81, 115.73. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2Cl_2S$ (M+H$^+$) 335.9788, found 335.97731.

2,5-Bis(3-fluoro-4-hydroxyphenyl)thiophene (2g)—Compound **2g** was prepared by 2,5-Bis(3-fluoro-4-methoxyphenyl)thiophene (**1g**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as green solid (mp 187–190 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.47 – 7.37 (m, 1H), 7.37 – 7.31 (m, 1H), 7.28 (d, J = 8.3 Hz, 1H), 7.05 (t, J = 8.8 Hz, 1H). ^{13}C NMR (101 MHz, acetone-d_6) δ 133.04, 127.60, 124.87, 123.08, 122.51, 118.89, 114.81, 113.50. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2F_2S$ (M+H$^+$) 304.0361, found 304.03641.

2,5-Bis(3-chloro-4-hydroxyphenyl)thiophene (2h)—Compound **2h** was prepared by 2,5-Bis(3- chloro-4-methoxyphenyl)thiophene (**1h**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as green solid (mp 192–195 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (s, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.39 (s, 2H), 7.02 (d, J = 8.7 Hz, 2H).^{13}C NMR (101 MHz, DMSO-d_6) δ 141.03, 138.97, 126.75, 126.48, 125.55, 124.53, 120.76, 117.50. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2Cl_2S$ (M+H$^+$) 335.9774, found 335.97731.

2,5-Bis(2-fluoro-5-hydroxyphenyl)thiophene (2i)—Compound **2i** was prepared by 2,5-bis(2-fluoro-5-methoxyphenyl)thiophene (**1i**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as yellow solid (mp 176–179 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.39 (s, 2H), 7.07 (dd, J = 6.1, 2.8 Hz, 2H), 7.01 – 6.90 (m, 2H), 6.78 – 6.61 (m, 2H). ^{13}C NMR (101 MHz, acetone-d_6) δ 154.81, 152.50, 138.16, 127.77, 122.81, 117.92, 117.68, 114.74. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2F_2S$ (M+H$^+$) 304.0361, found 304.03641.

2,5-Bis(3-fluoro-5-hyroxyphenyl)thiophene (2j)—Compound **2j** was prepared by 2,5-bis(3-fluoro-5-methoxyphenyl)thiophene (**1j**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as yellow solid (mp 212–214 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (s, 2H), 7.01 (d, J = 9.9 Hz, 2H), 6.90 (s, 2H), 6.55 (d, J = 10.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.57, 162.16, 159.35, 141.79, 135.74, 125.81, 108.29, 102.82, 102.59, 102.04, 101.81. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2F_2S$ (M+H$^+$) 304.0361, found 304.03641.

2,5-Bis(4-fluoro-3-hydroxyphenyl)thiophene (2k)—Compound **2k** was prepared by 2,5-bis(4-fluoro-3-methoxyphenyl)thiophene **(1k)** and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as yellow solid (mp 169–172 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.74 (s, 2H), 7.16 (s, 4H), 6.98 (d, J = 5.3 Hz, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 153.33, 150.93, 146.15, 143.19, 131.96, 125.22, 117.99, 115.71. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2F_2S$ (M+H$^+$) 304.0361, found 304.03641.

2-(2-Fluoro-4-hydroxyphenyl)-5-(4-hydroxy-2-methylphenyl)thiophene (2l)—Compound **2l** was prepared by 2-(2-fluoro-4-methoxyphenyl)-5-(4-methoxy-2-methylphenyl) thiophene (**1l**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as yellow solid (mp 151–153 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.57 (t, J = 8.9 Hz, 1H), 7.34 (d, J = 3.7 Hz, 1H), 7.27 (d, J = 8.3 Hz, 1H), 7.02 (d, J = 3.7 Hz, 1H), 6.84 – 6.68 (m, 4H), 2.38 (s, 3H). ^{13}C NMR (101 MHz, acetone-d_6) δ 161.67, 159.21, 158.11, 143.23, 138.12, 137.10, 132.17, 130.05, 127.22, 126.67 – 125.17, 118.32, 113.89, 113.04, 104.25, 104.00, 21.42. HRMS (MALDI/DHB) calcd for $C_{17}H_{13}O_2FS$ (M+H$^+$) 300.0607, found 300.06148.

2-(2-Chloro-4-hydroxyphenyl)-5-(4-hydroxy-2-methylphenyl)thiophene (2m)—Compound **2m** was prepared by 2-(2-chloro-4-methoxyphenyl)-5-(4-methoxy-2-methylphenyl) thiophene (**1m**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 167–169 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.49 (d, J = 8.5 Hz, 1H), 7.27 (dd, J = 7.4, 6.2 Hz, 2H), 7.02 (d, J = 3.5 Hz, 2H), 6.90 (dd, J = 8.5, 2.5 Hz, 1H), 6.81 (d, J = 1.7 Hz, 1H), 6.75 (d, J = 8.3 Hz, 1H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, acetone-d_6) δ 158.65, 158.19, 144.22, 139.94, 138.12, 133.02, 132.20, 127.74, 126.74, 125.99, 125.17, 124.44, 118.36, 117.83, 115.73, 113.93, 21.44. HRMS (MALDI/DHB) calcd for $C_{17}H_{13}O_2ClS$ (M + H$^+$) 316.0325, found 316.03193.

3,4-Bis(4-methoxyphenyl)thiophene (3a)—Compound **3a** was prepared by reaction of 3,4- dibromothiophene and (4-methoxyphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 105–106 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.23 (s, 2H), 7.12 (d, *J* = 8.8 Hz, 4H), 6.80 (d, *J* = 8.8 Hz, 4H), 3.80 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.57, 141.32, 130.10, 129.22, 123.08, 113.57, 55.22.

3,4-Bis(4-methoxy-2-methylphenyl)thiophene (3b)—Compound **3b** was prepared by reaction of 3,4-dibromothiophene and (4-methoxy-2-methylphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 72–76 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (s, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 6.64–6.58 (m, 4H), 3.75 (s, 6H), 2.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.46, 141.98, 137.67, 131.61, 128.98, 123.26, 115.26, 110.62, 55.08, 20.52.

3,4-Bis(4-methoxy-3-methylphenyl)thiophene (3c)—Compound **3c** was prepared by reaction of 3,4-dibromothiophene and (4-methoxy-3-methylphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 77–78 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 2H), 7.05 (s, 2H), 6.93 (dd, *J* = 8.4, 2.1 Hz, 2H), 6.69 (d, *J* = 8.4 Hz, 2H), 3.81 (s, 6H), 2.16 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.56, 139.23, 129.02, 125.14, 124.46, 120.58, 107.17, 53.00, 13.98.

3,4-Bis(4-methoxy-3,5-dimethylphenyl)thiophene (3d)—Compound **3d** was prepared by reaction of 3,4-dibromothiophene and (4-methoxy-3,5-dimethylphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 92–96 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (s, 2H), 6.76 (s, 4H), 3.64 (s, 6H), 2.12 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.01, 141.45, 132.13, 130.21, 129.49, 123.08, 59.76, 16.01.

3,4-Bis(2-fluoro-4-methoxyphenyl)thiophene (3e)—Compound **3e** was prepared by reaction of 3,4-dibromothiophene and (2-fluoro-4-methoxyphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 88–90 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (s, 2H), 6.94 (t, *J* = 8.7 Hz, 2H), 6.54–6.47 (m, 4H), 3.71 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.47, 159.01, 135.52, 131.57, 124.49, 109.65, 101.81, 101.55, 55.49.

3,4-Bis(2-chloro-4-methoxyphenyl)thiophene (3f)—Compound **3f** was prepared by reaction of 3,4-dibromothiophene and (2-chloro-4-methoxyphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 121–125 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (s, 2H), 6.92 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 2.6 Hz, 2H), 6.59 (dd, *J* = 8.6, 2.6 Hz, 2H), 3.69 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.15, 139.18, 134.06, 132.37, 127.60, 124.54, 114.66, 112.49, 55.43.

3,4-Bis(4-hydroxyphenyl)thiophene (4a)—Compound **4a** was prepared by 3,4-bis(4-methoxyphenyl) thiophene (**3a**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as white solid (mp 198–201 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.49 (s, 2H), 7.33 (s, 2H), 7.03 (d, *J* = 8.2 Hz, 4H), 6.75 (d, *J* = 8.1 Hz, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 157.45, 142.46, 130.87, 129.04, 123.63, 115.86. HRMS (MALDI/DHB) calcd for $C_{16}H_{12}O_2S$ ($M+H^+$) 268.0563, found 268.05525.

3,4-Bis(4-hydroxy-2-methylphenyl)thiophene (4b)—Compound **4b** was prepared by 3,4-bis(4- methoxy-2-methylphenyl)thiophene (**3b**) and boron tribromide according to

general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as white solid (mp 214–215 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 2H), 7.41 (s, 2H), 6.80 (d, J = 8.2 Hz, 2H), 6.57 (s, 2H), 6.50 (d, J = 8.2 Hz, 2H), 1.98 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.09, 141.68, 136.75, 131.19, 126.97, 123.24, 116.38, 112.20, 20.00. HRMS (MALDI/DHB) calcd for $C_{18}H_{16}O_2S$ (M +H^+) 296.0875, found 296.08655.

3,4-Bis(4-hydroxy-3-methylphenyl)thiophene (4c)—Compound **4c** was prepared by 3,4-bis(4- methoxy-3-methylphenyl)thiophene (**3c**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as white solid (mp 183–184 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.38 (s, 1H), 7.30 (s, 2H), 7.01 (s, 2H), 6.78 (d, J = 8.1 Hz, 2H), 6.70 (d, J = 8.2 Hz, 2H), 2.13 (s, 6H). ^{13}C NMR (101 MHz, acetone-d_6) δ 155.40, 142.64, 132.15, 129.07, 128.18, 124.67, 123.35, 115.02, 16.23. HRMS (MALDI/DHB) calcd for $C_{18}H_{16}O_2S$ (M +H^+) 296.0875, found 296.08655.

3,4-Bis(4-hydroxy-3,5-dimethylphenyl)thiophene (4d)—Compound **4d** was prepared by 3,4-bis(4- methoxy-3,5-dimethylphenyl)thiophene (**3d**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 193–194 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.29 (s, 2H), 6.82 (s, 4H), 2.14 (s, 12H). HRMS (MALDI/DHB) calcd for $C_{20}H_{20}O_2S$ (M+H^+) 324.1184, found 324.11785.

3,4-Bis(2-fluoro-4-hydroxyphenyl)thiophene (4e)—Compound **4e** was prepared by 3,4-bis(2-fluoro-4-methoxyphenyl)thiophene (**3e**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 4:1) gave the title compound as white solid (mp 207–209 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.91 (s, 1H), 7.45 (s, 2H), 6.97 (t, J = 8.6 Hz, 2H), 6.57 (ddd, J = 14.1, 10.0, 2.4 Hz, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 162.32, 159.09, 136.91, 132.62, 125.16, 116.35, 111.98, 103.59. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2F_2S$ (M+H^+) 304.0378, found 304.03641.

3,4-Bis(2-chloro-4-hydroxyphenyl)thiophene (4f)—Compound **4f** was prepared by 3,4-bis(2-chloro-4-methoxyphenyl)thiophene (**3f**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 4:1) gave the title compound as white solid (mp 179–183 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.42 (s, 2H), 6.98 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 2.5 Hz, 2H), 6.69 (dd, J = 8.4, 2.5 Hz, 2H). ^{13}C NMR (101 MHz, acetone-d_6) δ 158.20, 140.43, 134.40, 133.54, 127.38, 125.26, 116.85, 114.65. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2Cl_2S$ (M+H^+) 335.9861, found 335.98513.

2,4-Bis(2-fluoro-4-methoxyphenyl)thiophene (5a)—Compound **5a** was prepared by reaction of 2,4- dibromothiophene and (2-fluro-4-methoxyphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 88–90 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.34 (m, 4H), 6.68 – 6.57 (m, 4H), 3.72 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.64, 161.01, 160.06, 159.17, 158.52, 137.12, 136.01, 129.78, 129.30, 125.32, 121.45, 116.33, 114.82, 110.55, 110.27, 102.30, 55.64, 55.59.

2,4-Bis(2-chloro-4-methoxyphenyl)thiophene (5b)—Compound **5b** was prepared by reaction of 2,4-dibromothiophene and (2-chloro-4-methoxyphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl

acetate = 98:2) gave the title compound as white solid (mp 135–138 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 5.0, 3.6 Hz, 2H), 7.38 (dd, *J* = 5.0, 3.6 Hz, 2H), 7.02 (dd, *J* = 4.3, 2.6 Hz, 2H), 6.85 (ddd, *J* = 8.7, 2.6, 0.6 Hz, 2H), 3.83 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.50, 159.26, 139.40, 139.02, 133.04, 132.93, 132.06, 131.64, 128.93, 127.81, 125.57, 123.51, 115.57, 115.34, 113.29, 113.15, 55.64, 55.61.

2,4-Bis(2-fluoro-4-hydroxyphenyl)thiophene (6a)—Compound **6a** was prepared by 2,4-bis (2-fluoro-4-methoxyphenyl)thiophene (**5a**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 190–194 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.26 (s, 1H), 9.12 (s, 1H), 7.62 – 7.60 (m, 4H), 6.75 – 6.73 (m, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 161.79, 159.99, 159.33, 139.61, 138.21, 137.06, 131.00, 130.37, 125.81, 121.85, 114.25, 113.08, 112.74, 110.89, 110.09, 103.99. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2F_2S$ ($M+H^+$) 304.0373, found 304.03641.

2,4-Bis(2-chloro-4-hydroxyphenyl)thiophene (6b)—Compound **6b** was prepared by 2,4-bis(2-chloro-4-methoxyphenyl)thiophene (**5b**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 163–165 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.56 – 7.47 (m, 3H), 7.41 (d, *J* = 8.5 Hz, 1H), 7.03 (dd, *J* = 11.6, 2.5 Hz, 2H), 6.90 (td, *J* = 8.3, 2.5 Hz, 2H). ^{13}C NMR (101 MHz, acetone-d_6) δ 158.83, 158.72, 158.45, 158.34, 140.33, 140.08, 133.15, 132.88, 129.55, 127.36, 125.07, 124.20, 117.85, 117.55, 115.64, 115.48. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2Cl_2S$ ($M+H^+$) 335.9784, found 335.97731.

2,3-Bis(2-fluoro-4-methoxyphenyl)thiophene (7a)—Compound **7a** was prepared by reaction of 2,3-dibromothiophene and (2-fluoro-4-methoxyphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 76–78 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 5.2 Hz, 1H), 7.11 – 7.04 (m, 2H), 6.95 (t, *J* = 8.5 Hz, 1H), 6.58 – 6.49 (m, 4H), 3.72 (s, 3H), 3.71 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.79, 159.15, 137.60, 136.71, 135.01, 133.94, 133.58, 132.40, 129.86, 127.62, 125.01, 124.18, 114.80, 112.72, 55.46.

2,3-Bis(2-chloro-4-methoxyphenyl)thiophene (7b)—Compound **7b** was prepared by reaction of 2,3-dibromothiophene and (2-chloro-4-methoxyphenyl)boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 115–117 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 5.2 Hz, 1H), 7.08 (dd, *J* = 6.9, 1.6 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 1H), 6.83 (dd, *J* = 11.1, 2.6 Hz, 2H), 6.63 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.57 (dd, *J* = 8.6, 2.6 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.42, 140.20, 132.90, 131.97, 127.02, 125.52, 115.61, 113.30, 55.63.

2,3-Bis(2-fluoro-4-hydroxyphenyl)thiophene (8a)—Compound **8a** was prepared by 2,3-bis (2-fluoro-4-methoxyphenyl)thiophene (**7a**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 158–162 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.54 (d, *J* = 5.2 Hz, 1H), 7.15 (dd, *J* = 5.2, 1.7 Hz, 1H), 7.09 (t, *J* = 8.6 Hz, 1H), 6.98 (t, *J* = 8.5 Hz, 1H), 6.67 – 6.54 (m, 4H). ^{13}C NMR (101 MHz, acetone-d6) 166.73, 162.32, 160.05, 134.63, 133.66, 133.40, 132.91, 132.53, 131.23, 130.84, 130.16, 128.90, 125.51, 112.42, 103.96. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2F_2S$ ($M+H^+$) 304.0377, found 304.03641.

2,3-Bis(2-chloro-4-hydroxyphenyl)thiophene (8b)—Compound **8b** was prepared by 2,3-bis (2-chloro-4-methoxyphenyl)thiophene (**7b**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as white solid (mp 160–163 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.16 (s, 1H), 8.98 (s, 1H), 7.53 (d, J = 5.2 Hz, 1H), 7.19 – 7.09 (m, 2H), 6.98 – 6.86 (m, 3H), 6.74 (dd, J = 8.4, 2.4 Hz, 1H), 6.66 (dd, J = 8.4, 2.4 Hz, 1H). ^{13}C NMR (101 MHz, acetone-d_6) δ159.09, 158.33, 135.31, 134.71, 134.29, 133.47, 130.69, 125.10, 124.58, 117.30,116.86, 114.95. HRMS (MALDI/DHB) calcd for $C_{16}H_{10}O_2Cl_2S$ (M+H$^+$) 335.9769, found 335.97731.

2,5-bis(4-methoxyphenyl)-3-methylthiophene (9a)—Compound **9a** was prepared by reaction of 3-methyl-2,5-dibromothiophene and 4-methoxy-phenyl boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 117–120 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.41 (m, 2H), 7.36 – 7.31 (m, 2H), 6.94 (s, 1H), 6.89 – 6.85 (m, 2H), 6.84 – 6.80 (m, 2H), 3.76 (s, 3H), 3.74 (s, 3H), 2.22 (s, 3H).

2,5-bis(4-methoxy-2-methylphenyl)-3-methylthiophene (9b)—Compound **9b** was prepared by reaction of 3-methyl-2,5-dibromothiophene and (2-methyl-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 112–114 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 6.78 – 6.66 (m, 5H), 3.77 (s, 3H), 3.75 (s, 3H), 2.39 (s, 3H), 2.18 (s, 3H), 1.97 (s, 3H).

2,5-Bis(2-fluoro-4-methoxyphenyl)-3-methylthiophene (9c)—Compound **9c** was prepared by reaction of 3-methyl-2,5-dibromothiophene and (2-fluoro-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 90–94 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (t, J = 8.9 Hz, 1H), 7.30 (t, J = 8.7 Hz, 1H), 7.19 (d, J = 1.1 Hz, 1H), 6.78 – 6.64 (m, 4H), 3.80 (d, J = 8.5 Hz, 6H), 2.18 (d, J = 1.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.64, 161.04, 159.94, 159.17, 158.46, 136.06, 132.61, 130.08, 129.02, 128.38, 114.86, 114.11, 110.49, 110.05, 102.08, 55.64, 55.62, 14.89.

2,5-Bis(2-chloro-4-methoxyphenyl)-3-methylthiophene (9d)—Compound **9d** was prepared by reaction of 3-methyl-2,5-dibromothiophene and (2-chloro-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 122–124 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 8.7 Hz, 1H), 7.32 (d, J = 8.5 Hz, 1H), 7.13 (s, 1H), 7.03 (d, J = 2.6 Hz, 1H), 7.00 (d, J = 2.6 Hz, 1H), 6.85 (dd, J = 8.1, 2.6 Hz, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 2.11 (s, 3H).

2,5-Bis(4-hydroxyphenyl)-3-methylthiophene (10a)—Compound **10a** was prepared by 2,5-bis (4-methoxyphenyl)-3-methylthiophene (**9a**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as white solid (mp 160–163 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.69 (s, 1H), 8.67 (s, 1H), 7.52 – 7.46 (m, 2H), 7.38 – 7.32 (m, 2H), 7.10 (s, 1H), 6.89 (dt, J = 4.8, 2.9 Hz, 4H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, acetone-d_6) δ 157.79, 141.78, 136.80, 133.86, 130.76, 128.23, 116.77, 15.25. HRMS (MALDI/DHB) calcd for $C_{17}H_{14}O_2S$ (M+H$^+$) 282.1027, found 282.10260.

2,5-Bis(4-hydroxy-2-methylphenyl)-3-methylthiophene (10b)—Compound **10b** was prepared by 2,5-bis (4-methoxy-2-methylphenyl)-3-methylthiophene (**9b**) and boron

tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate =3:1) gave the title compound as brown solid (mp 158–163 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.68 (d, J = 9.1 Hz, 1H), 8.46 (s, 1H), 7.53–7.32 (m, 4H), 7.10 (s, 1H), 6.99–6.84 (m, 4H), 2.40 (s, 3H), 2.18 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (101 MHz, acetone-d_6) δ 158.34, 157.77, 141.05, 139.24, 135.80, 137.90, 34.94, 133.15, 132.04, 129.23, 126.42, 124.59, 15.59, 117.66, 117.27, 113.97, 113.63, 29.91, 20.29, 14.49, 14.58. HRMS (MALDI/DHB) calcd for $C_{19}H_{18}O_2S$ ($M+H^+$) 310.1027, found 310.10220.

2,5-Bis(2-fluoro-4-hydroxyphenyl)-3-methylthiophene (10c)—Compound **10c** was prepared by 2,5-bis(2-fluoro-4-methoxyphenyl)-3-methylthiophene (**9c**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 162–166 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.20 (d, J = 9.8 Hz, 2H), 7.55 (t, J = 8.9 Hz, 1H), 7.27 (dd, J = 19.0, 10.4 Hz, 2H), 6.84–6.67 (m, 4H), 2.16 (s, 3H). ^{13}C NMR (101 MHz, acetone-d_6) δ 162.46, 161.70, 160.00, 159.43, 159.07, 136.57, 136.31, 133.56, 130.86, 129.97, 128.71, 114.22, 113.53, 112.59, 104.42, 103.46, 14.89. HRMS (MALDI/DHB) calcd for $C_{17}H_{12}O_2SF_2$ ($M+H^+$) 318.0527, found 318.05206.

2,5-Bis(2-chloro-4-hydroxyphenyl)-3-methylthiophene (10d)—Compound **10d** was prepared by 2,5-bis(2-chloro-4-methoxyphenyl)-3-methylthiophene (**9d**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as yellow solid (mp 112–116 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.13 (s, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.16 (s, 1H), 7.03 (dd, J = 11.0, 2.5 Hz, 2H), 6.90 (ddd, J = 8.7, 6.5, 2.5 Hz, 2H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, acetone-d_6) δ 159.34, 158.60, 139.44, 136.23, 135.54, 135.26, 134.49, 133.32, 132.89, 130.22, 125.08, 124.40, 117.89, 117.25, 115.76, 115.25, 14.775. HRMS (MALDI/DHB) calcd for $C_{17}H_{12}O_2SCl_2$ ($M+H^+$) 349.9938, found 349.99296.

2,5-Bis(2-fluoro-4-methoxyphenyl)-3-phenylthiophene (11a)—Compound **11a** was prepared by reaction of 3-bromo-2,5-bis(2-fluoro-4-methoxyphenyl)thiophene (**21**) and phenylboronic acid according to the general procedure for Suzuki coupling using Pd(dppf)Cl_2. Purification by CC (petroleum ether: ethyl acetate = 95:5) gave the title compound as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (t, J = 8.9 Hz, 1H), 7.38 (s, 1H), 7.25 – 7.09 (m, 6H), 6.67 (d, J = 7.1 Hz, 2H), 6.55 (t, J = 9.7 Hz, 2H), 3.76 (s, 3H), 3.72 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.91, 159.22, 157.52, 139.37, 135.70, 131.79, 128.03, 127.31, 126.38, 125.84, 109.54, 109.09, 101.22, 101.11, 101.13, 54.58.

2,5-Bis(2-chloro-4-methoxyphenyl)-3-phenylthiophene (11b)—Compound **11b** was prepared by reaction of 3-bromo-2,5-bis(2-chloro-4-methoxyphenyl)thiophene (**22**) and phenylboronic acid according to the general procedure for Suzuki coupling using Pd(dppf)Cl_2. Purification by CC (petroleum ether: ethyl acetate = 95:5) gave the title compound as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 8.6 Hz, 1H), 7.34 (s, 1H), 7.15 (td, J = 12.6, 6.4 Hz, 6H), 6.94 (d, J = 2.5 Hz, 1H), 6.87 (d, J = 2.4 Hz, 1H), 6.76 (dd, J = 8.6, 2.5 Hz, 1H), 6.67 (dd, J = 8.6, 2.4 Hz, 1H), 3.73 (s, 3H), 3.70 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.13, 159.58, 140.12, 139.53, 136.55, 135.31, 134.83, 133.78, 132.92, 131.93, 128.60, 128.34, 127.06, 126.81, 125.37, 115.77, 115.23, 113.37, 113.01, 55.62.

2,5-Bis(2-fluoro-4-hydroxyphenyl)-3-phenylthiophene (12a)—Compound **12a** was 2,5-bis(2-fluoro-4-methoxyphenyl)-3-phenylthiophene (**11a**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 2:1) gave the title compound as green solid (mp 142–145 °C). ^{1}H NMR (400

MHz, acetone-d_6) δ 9.22 (s, 1H), 7.67 (t, J = 8.9 Hz, 1H), 7.53 (s, 1H), 7.36 – 7.16 (m, 6H), 6.83 – 6.67 (m, 3H), 6.62 (dd, J = 11.6, 2.4 Hz, 1H). ^{13}C NMR (101 MHz, acetone-d_6) δ 159.19, 158.81, 158.32, 139.33, 138.51, 137.36, 135.25, 134.74, 134.27, 133.51, 132.95, 130.34, 127.25, 124.77, 124.33, 117.96, 117.16, 115.86, 115.02. HRMS (MALDI/DHB) calcd for $C_{22}H_{14}O_2SF_2$ ($M+H^+$) 380.0676, found 380.06771.

2,5-Bis(2-chloro-4-hydroxyphenyl)-3-phenylthiophene (12b)—Compound **12b** was 2,5-bis(2- chloro-4-methoxyphenyl)-3-phenylthiophene (**11b**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 2:1) gave the title compound as yellow solid (mp 152–154 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.58 (d, J = 8.5 Hz, 1H), 7.48 (s, 1H), 7.32 – 7.21 (m, 6H), 7.06 (d, J = 2.4 Hz, 1H), 6.97 (d, J = 2.4 Hz, 1H), 6.92 (dd, J = 8.7, 2.4 Hz, 1H), 6.84 (dd, J = 8.4, 2.4 Hz, 1H). ^{13}C NMR (101 MHz, acetone-d_6) δ158.83, 140.77, 135.61, 134.79, 133.17,132.89, 129.29,128.85, 127.72, 124.63, 117.87, 117.31, 115.78, 115.34. HRMS (MALDI/DHB) calcd for $C_{22}H_{14}O_2SCl_2$ ($M+H^+$) 412.0103, found 412.00861.

2,3,5-Tris(4-methoxyphenyl)thiophene (13a)—Compound **13a** was prepared by reaction of 2,3,5-tribromothiophene and 4-methoxy-phenyl boronic acid according to the general procedure for Suzuki coupling using Pd(dppf)Cl_2. Purification by CC (petroleum ether: ethyl acetate = 95:5) gave the title compound as red solid (mp 142–145 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 8.7 Hz, 2H), 7.18 (dd, J = 8.1, 2.1 Hz, 2H), 7.13 (s, 1H), 6.85 (d, J = 8.7 Hz, 2H), 6.75 (dd, J = 11.4, 8.7 Hz, 4H), 3.77 (s, 3H), 3.74 (s, 3H), 3.73 (s, 3H).

2,3,5-Tris(2-fluoro-4-methoxyphenyl)thiophene (13b)—Compound **13b** was prepared by reaction of 2,3,5-tribromothiophene and (2-fluoro-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling using Pd(dppf)Cl_2. Purification by CC (petroleum ether: ethyl acetate = 95:5) gave the title compound as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (dd, J = 9.3, 4.1 Hz, 1H), 7.34 (s, 1H), 7.10 (t, J = 8.7 Hz, 1H), 7.01 (t, J = 8.8 Hz, 1H), 6.71 – 6.61 (m, 2H), 6.54 (td, J = 10.0, 2.5 Hz, 4H), 3.76 (s, 3H), 3.72 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.52, 161.01, 160.66, 160.05, 159.05, 158.53, 136.25, 133.87, 132.29, 131.61, 129.08, 127.92, 116.81, 114.57, 114.08, 110.57, 109.96, 102.31, 101.85, 55.80 – 55.40.

2,3,5-Tris(2-chloro-4-methoxyphenyl)thiophene (13c)—Compound **13c** was prepared by reaction of 2,3,5-tribromothiophene and (2-chloro-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling using Pd(dppf)Cl_2. Purification by CC (petroleum ether: ethyl acetate = 95:5) gave the title compound as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 8.7 Hz, 1H), 7.37 (s, 1H), 7.19 (d, J = 8.6 Hz, 1H), 7.05 – 6.98 (m, 2H), 6.91 (dd, J = 11.9, 2.5 Hz, 2H), 6.85 (dd, J = 8.7, 2.6 Hz, 1H), 6.70 (dd, J = 8.6, 2.5 Hz, 1H), 6.64 (dd, J = 8.6, 2.5 Hz, 1H), 3.82 (s, 3H), 3.76 (d, J = 3.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.84, 159.44, 159.22, 138.59, 137.42, 136.63, 134.95, 133.97, 133.62, 132.84, 132.44, 131.82, 129.69, 127.76, 125.42, 124.94, 115.71, 114.94, 113.34, 112.74, 55.71.

2,3,5-Tris(4-hydroxyphenyl)thiophene (14a)—Compound **14a** was prepared by 2,3,5-tris (4-methoxyphenyl)thiophene (**13a**) and boron tribromide according to the general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 2:1) gave the title compound as brown solid (mp 285–288 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.84 (s, 1H), 8.69 (s, 1H), 8.62 (s, 1H), 7.60 – 7.54 (m, 2H), 7.15 – 7.05 (m, 2H), 7.01 – 6.95 (m, 2H), 6.91 – 6.85 (m, 2H), 6.78 – 6.73 (m, 2H). ^{13}C NMR (101 MHz, acetone-d_6) δ 158.17, 138.99, 136.74, 136.43, 132.74, 131.35, 130.77, 128.07, 125.90, 125.39, 125.30,

116.66, 116.44, 116.19, 115.62, 111.18. HRMS (MALDI/DHB) calcd for $C_{22}H_{16}O_3S$ (M $+H^+$) 360.0819, found 360.08147.

2,3,5-Tris(2-fluoro-4-hydroxyphenyl)thiophene (14b)—Compound **14b** was prepared by 2,3,5-tris(2-fluoro-4-methoxyphenyl)thiophene (**13b**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 2:1) gave the title compound as brown solid (mp 232–238 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.21 (s, 1H), 9.14 (s, 1H), 8.98 (s, 1H), 7.63 (t, J = 8.9 Hz, 1H), 7.41 (s, 1H), 7.14 (t, J = 8.6 Hz, 1H), 7.05 (t, J = 8.7 Hz, 1H), 6.77 (ddd, J = 15.3, 10.7, 2.3 Hz, 2H), 6.70 – 6.57 (m, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 162.36, 159.92, 158.23, 136.85, 135.35, 135.01, 134.97, 133.32, 132.58, 130.04, 128.31, 125,57, 124.45, 120.12, 113.10, 112.56, 112.50, 112.33, 104.28, 104.04. HRMS (MALDI/DHB) calcd for $C_{22}H_{13}O_3SF_3$ (M $+H^+$) 414.0541, found 414.05320.

2,3,5-Tris(2-chloro-4-hydroxyphenyl)thiophene (14c)—Compound **14c** was prepared by 2,3,5-tris(2-chloro-4-methoxyphenyl)thiophene (**13c**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 2:1) gave the title compound as yellow solid (mp 185–187 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.17 (s, 1H), 9.09 (s, 1H), 8.91 (s, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.36 (s, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.05 (d, J = 2.5 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.95 – 6.89 (m, 3H), 6.77 (dd, J = 8.4, 2.4 Hz, 1H), 6.68 (dd, J = 8.4, 2.4 Hz, 1H). ^{13}C NMR (101 MHz, acetone-d_6) δ 159.19, 159.07, 158.81, 158.44, 158.32, 138.51, 135.25, 134.27, 132.95, 127.25, 124.33, 118.00, 117.91, 117.25, 117.16, 117.07, 115.90, 115.86, 115.16, 115.07, 114.97, 114.88. HRMS (MALDI/DHB) calcd for $C_{22}H_{13}O_3SCl_3$ (M+H^+) 461.9662, found 461.96455.

2,5-Bis(4-methoxyphenyl)furan (15a)—Compound **15a** was prepared by reaction of 2,5-dibromofuran and 4-methoxy-phenyl boronic acid according to the general procedure for Suzuki coupling using $Pd(PPh)_4$. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 113–114 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.8 Hz, 4H), 6.86 (t, J = 5.8 Hz, 4H), 6.50 (s, 2H), 3.77 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.9, 152.8, 125.0, 124.0, 114.1, 105.6, 55.3.

2,5-Bis(4-methoxy-2-methylphenyl)furan (15b)—Compound **15b** was prepared by reaction of 2,5-dibromofuran and (4-methoxy-2-methylphenyl) boronic acid according to the general procedure for Suzuki coupling using $Pd(PPh)_4$. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 106–108 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 8.4 Hz, 2H), 6.74 (d, J = 9.6 Hz, 4H), 6.45 (s, 2H), 3.76 (s, 2H), 2.46 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.73, 152.21, 136.15, 128.32, 123.43, 116.50, 111.48, 108.98, 55.26, 22.21.

2,5-Bis(2-fluoro-4-methoxyphenyl)furan (15c)—Compound **15c** was prepared by reaction of 2,5-dibromofuran and (2-fluoro-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling using $Pd(PPh)_4$. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 95–97 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (t, J = 8.8 Hz, 2H), 6.74 – 6.65 (m, 2H), 6.61 (dd, J = 12.9, 2.4 Hz, 2H), 3.74 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.66, 159.79, 158.17, 146.90, 126.53, 112.06, 110.37, 102.19, 101.94, 55.63.

2,5-Bis(2-chloro-4-methoxyphenyl)furan (15d)—Compound **15d** was prepared by reaction of 3,4-dibromofuran and (2-chloro-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling using $Pd(PPh)_4$. Purification by CC (petroleum

ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 112–114 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.8 Hz, 2H), 7.07 (s, 2H), 7.00 (d, *J* = 2.6 Hz, 2H), 6.90 (dd, *J* = 8.8, 2.6 Hz, 2H), 3.84 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.99, 149.23, 131.01, 128.84, 122.17, 115.72, 113.46, 111.26, 55.59.

2,5-Bis(4-hydroxyphenyl)furan (16a)—Compound **16a** was prepared by 2,5-bis(4-methoxyphenyl)furan (**15a**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as brown solid (mp 176–177 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.64 (s, 2H), 7.64 (d, *J* = 8.6 Hz, 4H), 6.90 (d, *J* = 8.7 Hz, 4H), 6.69 (s, 2H). ^{13}C NMR (101 MHz, acetone-d_6) δ 159.18, 145.20, 128.61, 126.88, 124.32, 116.68. HRMS (MALDI/DHB) calcd for $C_{16}H_{12}O_3$ ($M+H^+$) 252.0783, found 252.07810.

2,5-Bis(4-hydroxy-2-methylphenyl)furan (16b)—Compound **16b** was prepared by 2,5-Bis(4- methoxy-2-methylphenyl)furan (**15b**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 4:1) gave the title compound as brown solid (mp 184–187 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 6.86 – 6.78 (m, 4H), 6.65 (d, *J* = 2.2 Hz, 2H), 6.55 (dd, *J* = 8.4, 2.4 Hz, 2H), 2.10 (s, 6H). ^{13}C NMR (101 MHz, acetone-d_6) δ 159.04, 146.02, 138.60, 132.18, 128.93, 123.75, 117.98, 113.29, 20.40. HRMS (MALDI/DHB) calcd for $C_{18}H_{16}O_3$ ($M+H^+$) 280.1091, found 280.10940.

2,5-Bis(2-fluoro-4-hydroxyphenyl)furan (16c)—Compound **16c** was prepared by 2,5-bis(2-fluoro-4-methoxyphenyl)furan (**15c**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 4:1) gave the title compound as brown solid (mp 219–222 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.16 (s, 1H), 7.81 (t, *J* = 8.8 Hz, 2H), 6.81 (dd, *J* = 8.6, 2.3 Hz, 2H), 6.75 (dd, *J* = 5.8, 2.5 Hz, 3H), 6.71 (d, *J* = 2.3 Hz, 1H). ^{13}C NMR (101 MHz, acetone-d_6) δ 161.52, 159.43, 147.91, 127.66, 112.87, 111.38, 110.53, 104.23, 103.99. HRMS (MALDI/DHB) calcd for $C_{16}H_{16}O_3F_2$ ($M+H^+$) 288.0595, found 288.05925.

2,5-Bis(2-chloro-4-hydroxyphenyl)furan (16d)—Compound **16d** was prepared by 2,5-bis(2-chloro-4-methoxyphenyl)furan (**15d**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as yellow solid (mp 194–198 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.70 (d, *J* = 8.7 Hz, 2H), 6.91 (s, 2H), 6.88 (d, *J* = 2.5 Hz, 2H), 6.80 (dd, *J* = 8.7, 2.5 Hz, 2H). ^{13}C NMR (101 MHz, acetone-d_6) δ 158.36, 150.23, 131.34, 130.08, 121.62, 118.03, 115.77, 111.54. HRMS (MALDI/DHB) calcd for $C_{16}H_{16}O_3Cl_2$ ($M+H^+$) 320.0003, found 320.00015.

3,4-Bis(4-methoxyphenyl)furan (17a)—Compound **17a** was prepared by reaction of 3,4- dibromofuran and 4-methoxy-phenyl boronic acid according to the general procedure for Suzuki coupling using $Pd(PPh)_4$. Purification by CC (petroleum ether: ethyl acetate =98:2) gave the title compound as white solid (mp 144–148 °C).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 0H), 7.08 (d, *J* = 8.8 Hz, 1H), 6.76 (d, *J* = 8.8 Hz, 1H), 3.73 (s, 6H).

3,4-Bis(4-methoxy-2-methylphenyl)furan (17b)—Compound **17b** was prepared by reaction of 3,4- dibromofuran and (4-methoxy-2-methylphenyl) boronic acid according to the general procedure for Suzuki coupling using $Pd(PPh)_4$. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 102–106 °C).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 1.7 Hz, 1H), 7.33 (d, *J* = 1.7 Hz, 1H), 7.13 (d, *J* =

8.4 Hz, 1H), 6.82 (d, *J* = 2.4 Hz, 2H), 6.77 (dd, *J* = 8.4, 2.5 Hz, 2H), 3.82 (s, 6H), 2.24 (s, 5H).

3,4-Bis(2-fluoro-4-methoxyphenyl)furan (17c)—Compound **17c** was prepared by reaction of 3,4-dibromofuran and (2-fluoro-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling using $Pd(PPh)_4$. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 117–119 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.75 (s, 2H), 7.10 (t, *J* = 8.8 Hz, 2H), 6.78 – 6.68 (m, 4H), 3.82 (s, 6H). ^{13}C NMR (101 MHz, acetone-d_6) δ 162.57, 161.43, 160.13, 142.53, 132.02, 120.55, 110.92, 102.63, 102.37, 56.00.

3,4-Bis(2-chloro-4-methoxyphenyl)furan (17d)—Compound **17d** was prepared by reaction of 3,4-dibromofuran and (2-chloro-4-methoxy-phenyl) boronic acid according to the general procedure for Suzuki coupling using $Pd(PPh)_4$. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as white solid (mp 137–140 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.84 (d, *J* = 8.8 Hz, 2H), 7.07 (s, 2H), 7.00 (d, *J* = 2.6 Hz, 2H), 6.90 (dd, *J* = 8.8, 2.6 Hz, 2H), 3.84 (s, 6H). ^{13}C NMR (101 MHz, acetone-d_6) δ 160.47, 140.17, 134.63, 133.47, 128.47, 125.58, 115.38, 113.44, 55.91.

4,4'-(furan-3,4-diyl)diphenol (18a)—Compound **18a** was prepared by 3,4-Bis(4-methoxyphenyl) furan (**17a**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate =3:1) gave the title compound as brown solid (mp 172–174 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.63 (s, 2H), 7.10 – 7.05 (m, 4H), 6.78 (dd, *J* = 11.2, 4.5 Hz, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 157.53, 140.98, 130.45, 126.60, 124.21, 116.06. HRMS (MALDI/DHB) calcd for $C_{16}H_{12}O_3$ (M +H^+) 252.0782, found 252.07810.

3,4-Bis(4-hydroxy-2-methylphenyl)furan (18b)—Compound **18b** was prepared by 3,4-bis (4-methoxy-2-methylphenyl)furan (**17b**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate =3:1) gave the title compound as brown solid (mp 170 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.28 (s, 2H), 7.53 (s, 2H), 6.88 (d, *J* = 8.3 Hz, 2H), 6.64 (d, *J* = 2.3 Hz, 2H), 6.57 (dd, *J* = 8.2, 2.5 Hz, 2H), 2.00 (s, 6H). ^{13}C NMR (101 MHz, acetone-d_6) δ 157.52, 141.30, 138.59, 132.41, 127.12, 124.25, 117.68, 113.31, 20.67. HRMS (MALDI/DHB) calcd for $C_{18}H_{16}O_3$ (M+H^+) 280.1091, found 280.10940.

3,4-Bis(2-fluoro-4-hydroxyphenyl)furan (18c)—Compound **18c** was prepared by 3,4-bis(2-fluoro-4-methoxyphenyl)furan (**17c**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as gray solid (mp 209–211 °C). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.03 (s, 1H), 7.71 (s, 2H), 7.00 (t, *J* = 8.6 Hz, 2H), 6.63 – 6.58 (m, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 162.09, 159.70, 137.53, 130.41, 126.40, 114.54, 113.54, 104.61. HRMS (MALDI/DHB) calcd for $C_{16}H_{16}O_3F_2$ (M+H^+) 288.0595, found 288.05925.

3,4-Bis(2-chloro-4-hydroxyphenyl)furan (18d)—Compound **18d** was prepared by 3,4-bis(2-chloro-4-methoxyphenyl)furan (**17d**) and boron tribromide according to general procedure for ether cleavage. Purification by CC (petroleum ether: ethyl acetate = 3:1) gave the title compound as gray solid (mp 179–183 °C).^{1}H NMR (400 MHz, acetone-d_6) δ 7.68 (s, 2H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 2.5 Hz, 2H), 6.72 (dd, *J* = 8.4, 2.4 Hz, 2H). ^{13}C NMR (101 MHz, acetone-d_6) δ 160.47, 140.17, 134.63, 133.47, 128.47, 125.58, 115.38, 113.44. HRMS (MALDI/DHB) calcd for $C_{16}H_{16}O_3Cl_2$ (M+H^+) 320.0003, found 320.00015.

2-Bromo-5-(4-methoxy-2-methylphenyl)thiophene (19)—Compound **19** was prepared by reaction of 2,5-dibromofuran and (4-methoxy-2-methylphenyl) boronic acid according to the general procedure for Suzuki coupling using $Pd(OAc)_2$. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 3.7 Hz, 1H), 6.73 (m, 3H), 3.78 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.57, 144.81, 137.75, 133.82, 131.56, 129.96, 126.39, 125.98, 116.28, 111.38, 55.32, 21.28.

2,5-Dibromo-3-methylthiophene (20)—Compound **20** was prepared by bromination of 3-methyl thiophene using NBS. Distillation gave the title compound as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (s, 1H), 2.14 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.07, 131.90, 110.23, 108.47, 15.23.

3-Bromo-2,5-bis(2-fluoro-4-methoxyphenyl)thiophene (21)—Compound **21** was prepared by reaction of 2,3,5-dibromofuran and (2-fluoro-4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.33 (m, 2H), 7.23 (s, 1H), 6.73 – 6.60 (m, 4H), 3.77 (s, 3H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.51, 161.07, 160.62, 159.18, 158.58, 132.65, 128.79, 128.18, 110.70, 110.06, 102.37, 102.13, 101.90, 55.68.

3-Bromo-2,5-bis(2-chloro-4-methoxyphenyl)thiophene (22)—Compound **21** was prepared by reaction of 2,3,5-dibromofuran and (2-chloro-4-methoxyphenyl) boronic acid according to the general procedure for Suzuki coupling. Purification by CC (petroleum ether: ethyl acetate = 98:2) gave the title compound as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, J = 8.7 Hz, 1H), 7.27 (d, J = 8.6 Hz, 1H), 7.15 (s, 1H), 6.93 (dd, J = 13.3, 2.5 Hz, 2H), 6.76 (ddd, J = 12.4, 8.6, 2.6 Hz, 2H), 3.74 (s, 3H), 3.72 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.63, 159.87, 140.52, 135.28, 133.48, 132.94, 131.68, 129.42, 124.35, 123.61, 115.79, 115.18, 113.39, 112.81, 110.66, 55.63.

2,5-Dibromofuran (23)—Compound **23** was prepared by bromination of furan using Br_2 as brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 121.88, 114.24, 77.36, 77.04, 76.72.

3,4-Dibromofuran (24)—Compound **24** was prepared through oxidation of (*E*)-2,3-dibromo-2-3-butane-1,4-diol using chromosulfuric acid by steam distillation as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.60, 103.97.

Estrogen Receptor Binding Affinity—Relative binding affinities were determined by a competitive radiometric binding assay as previously described,[51, 67] using 2 nM [^{3}H]estradiol as tracer ([2,4,6,7-^{3}H]-estra-1,3,5(10)-triene-3,17β-diol, 70–115 Ci/mmol, Perkin Elmer, Waltham, MA), and purified full-length human ERα and ERβ were purchased from PanVera/Invitrogen (Carlsbad, CA). Incubations were for 18–24 h at 0 °C. Hydroxyapatite (BioRad, Hercules, CA) was used to absorb the receptor-ligand complexes, and free ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values with the RBA of estradiol set to 100%. The values given are the average ± range or SD of two to three independent determinations. Estradiol binds to ERα with a K_d of 0.2 nM and to ERβ with a K_d of 0.5 nM; these values were determined by Scatchard analysis using the binding assay protocol described previously.[51]

Gene Transcriptional Activity—Assays were performed as previously described.[52] HepG2 and Ishikawa cells cultured in Dulbecco's minimum essential medium (DMEM) (Cellgro by Mediatech, Inc. Manassas, VA) supplemented with 10% fetal bovine serum (FBS) (Hyclone by Thermo Scientific, South Logan, UT), and 1% non-essential amino acids (Cellgro), Penicillin-Streptomycin-Neomycin antibiotic mixture and Glutamax (Gibco by Invitrogen Corp. Carlsbad, CA), were maintained at 37 °C and 5% CO_2. HepG2 cells were transfected with 10 μg of 3X ERE-luciferase reporter plus 1.6 μg of ERα or ERβ expression vector per 10 cm dish using FugeneHD reagent (Roche Applied Sciences, Indianapolis IN). The next day, the cells were transferred to phenol red-free growth media supplemented with 10% charcoal-dextran sulfate-stripped FBS at a density of 20,000 cells/well, incubated in 384-well plates overnight at 37 °C and 5% CO_2, and stimulated with various concentrations of compounds in triplicate. Ishikawa cells grown in phenol red-free growth media were transfected with 10 ug of 3X ERE-luciferase reporter per 10 cm dish using FugeneHD reagent. The next day, cells were plated at a density of 20,000 cells/well, incubated in 384-well plates overnight at 37 °C and 5% CO_2, and treated with compounds dissolved in DMSO using a robotic pin-tool dispenser. Compounds were assayed in dose curves ranging from 10 μM to 100 pM for ERE luciferase assays in HepG2 cells and at a single dose of 10 μM for ERE luciferase assays in Ishikawa cells. Luciferase activity was measured after 24 hours using BriteLite reagent (Perkin-Elmer Inc., Shelton, CT) according to manufacturer's protocol. Raw data measured as relative light units were normalized for each plate using the average of DMSO treated samples as 0% and the average of top of the E2 curve as 100%.

Molecular Modeling—The thiophenes **2e** or **4e** were superpositioned using[68] COOT with OBHS in the OBHS-bound ER crystal structure[52] so that the A-ring mimetic formed the canonical hydrogen-bonding pattern with R394 and E353. For **2e** the other phenol was superposed with the phenyl-sulfonate substituent of OBHS, and for **4e**, the second phenol was positioned to hydrogen bond with T347. The models were transferred to CCP4MG for presentation.[69]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by grants from NSFC (20872116, 81172935, 91017005), the Program for New Century Excellent Talents in University (NCET-10-0625), Key Project of Ministry of Education of China (313040), the Research Fund for the Doctoral Program of Higher Education of China (20100141110021) and the Fundamental Research Funds for the Central Universities. Research support from the National Institutes of Health (PHS 5R37 DK015556 to J.A.K. and R01 DK077085 to K.W.N.) is gratefully acknowledged. S.S. is supported by the Frenchman's Creek Women for Cancer Research. We are grateful to Teresa Martin to help in the binding assays.

Abbreviations

ER	estrogen receptor
CC	column chromatography
DCM	dicholoromethane
E_2	estradiol
HEC-1	human endometrial cancer cells
HepG2	human hepatoma cells
LBD	ligand-binding domain

LBP	ligand-binding pocket
RBA	relative binding affinity
SAR	structure-activity relationship
THF	tetrahydrofuran
TLC	thin layer chromatography

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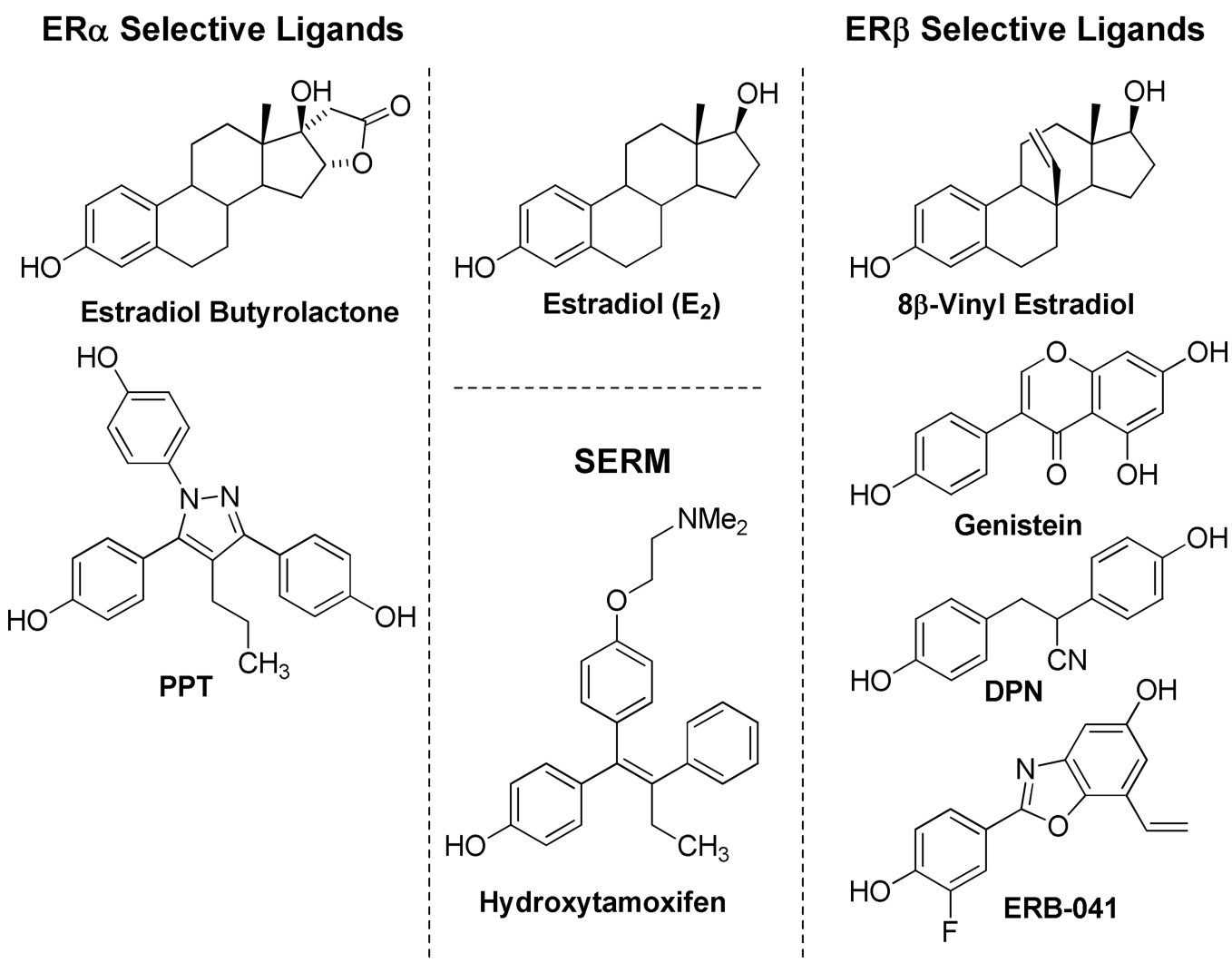


Figure 1.
Estradiol and examples of some ERα (left) and ERβ-selective (right) ligands and a SERM

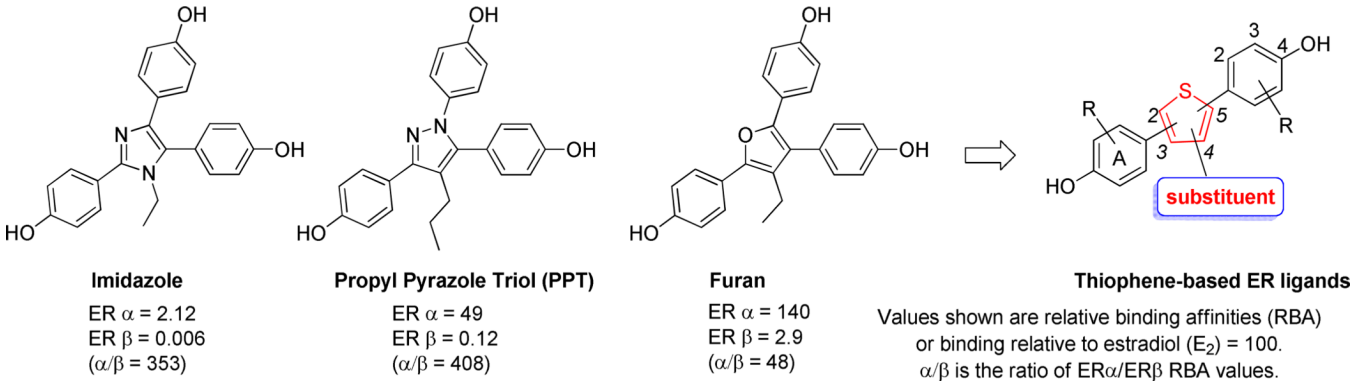
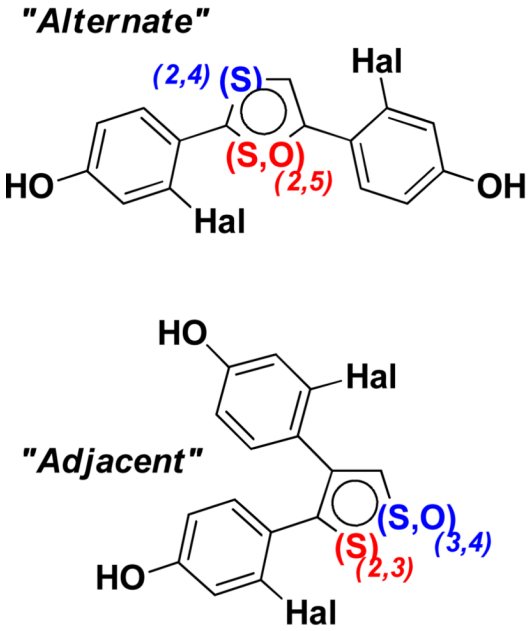


Figure 2.

Structure and binding affinity data of the most promising pyrazole, furan and imidazole and the title thiophene compounds. Binding, determined by competitive radiometric binding assays, is expressed as Relative Binding Affinity (RBA) values, with estradiol (E_2) as 100. α/β is the ratio of ERα RBA to ERβ RBA



	Thiophenes		Furans
	RBA ERα [ERβ]		
Hal	(2,5)	(2,4)	(2,5)
H	0.04 [0.79]	-- --	0.1 [0.34]
Me	1.4 [1.7]	-- --	0.8 [0.24]
F	2 [33]	1 [10]	7 [32]
Cl	7 [10]	4 [26]	3 [7]

	RBA ERα [ERβ]		
Hal	(2,3)	(3,4)	(3,4)
H	-- --	0.57 [1.7]	0.22 [0.69]
Me	-- ---	2.2 [4.9]	0.79 [2.3]
F	1 [3]	3 [9]	1 [5]
Cl	7 [8]	13 [18]	3 [5]

Figure 3.

Comparisons of ERα and ERβ binding affinities of congeneric bisphenolic thiophene and furan-core ER ligands. In each table, the RBA values are given for binding to ERα or [ERβ] for the various biaryl thiophenes and furans we have studied. The top tables are for the alternate-substituted systems and the bottom tables for the adjacent-substituted systems. The disposition of the aryl groups with respect to the heteroatom (S or O) is indicated by the numbers in parentheses, which are color coded to enable reference to be made between the structures and the appropriate columns in the tables.

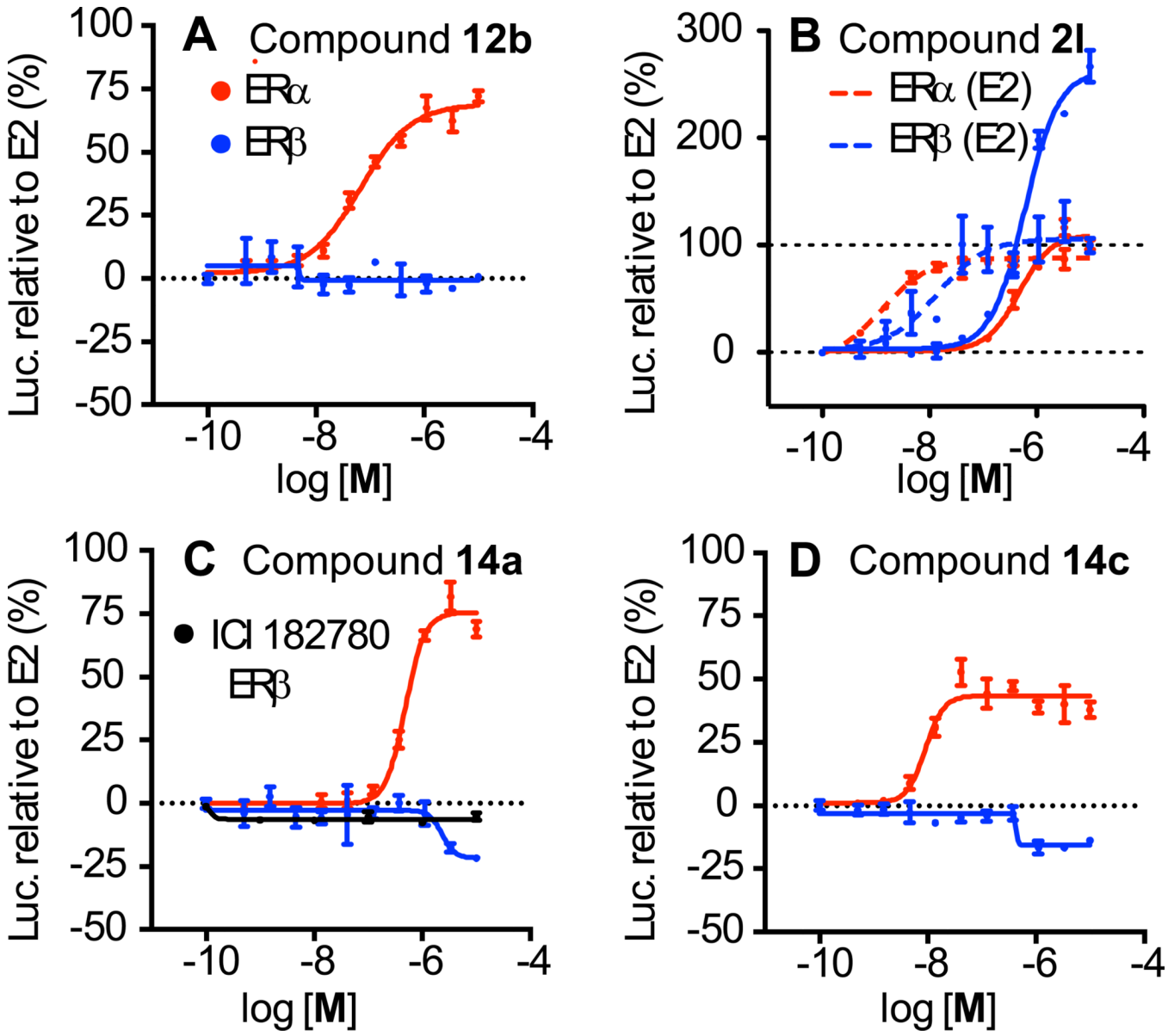
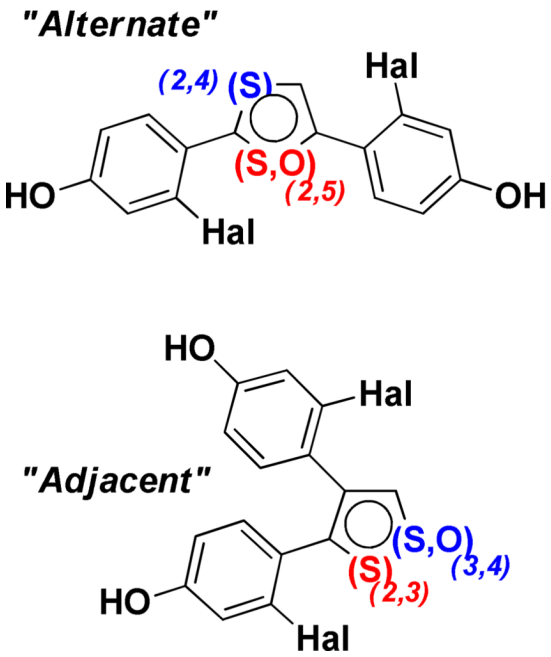


Figure 4. Activity profile of ERα selective compounds

HepG2 cells were transfected with 3xERE-luc and expression plasmids for ERα or ERβ, and then dispensed in 384-well plates. The next day cells were treated with the indicated compounds for 24 hr and assayed for luciferase activity. Compound with ERα (red solid line), with ERβ (blue solid line); estradiol with ERα (red dashed line, panel B only), with ERβ (blue dashed line, panel B only); ICI with ERβ (black line, panel C only)



	Thiophenes		Furans
	%Eff. ERα [ERβ]		
Hal	(2,5)	(2,4)	(2,5)
H	**125 [198]**	-- --	62 [94]
Me	78 [89]	-- --	36 [8]
F	**181 [336]**	**123 [177]**	**154 [159]**
Cl	111 **[169]**	87 [117]	82 [106]

	%Eff. ERα [ERβ]		
Hal	(2,3)	(3,4)	(3,4)
H	-- --	69 [73]	81 [37]
Me	-- ---	97 [69]	87 [56]
F	69 [33]	79 [29]	73 [85]
Cl	82 [16]	90 [57]	86 [54]

Figure 5.

Comparisons of ERα and ERβ intrinsic activities (Eff % of E_2) of congeneric bisphenolic thiophene and furan-core ER ligands. Maximal activities above 120 are indicated in boldface. In each table, the %Eff. values are given for the reporter gene assays of the various biaryl thiophenes and furans we have studied with ERα or [ERβ]. The top tables are for the alternate-substituted systems and the bottom tables for the adjacent-substituted systems. The disposition of the aryl groups with respect to the heteroatom (S or O) is indicated by the numbers in parentheses, which are color coded to enable reference to be made between the structures and the appropriate columns in the tables.

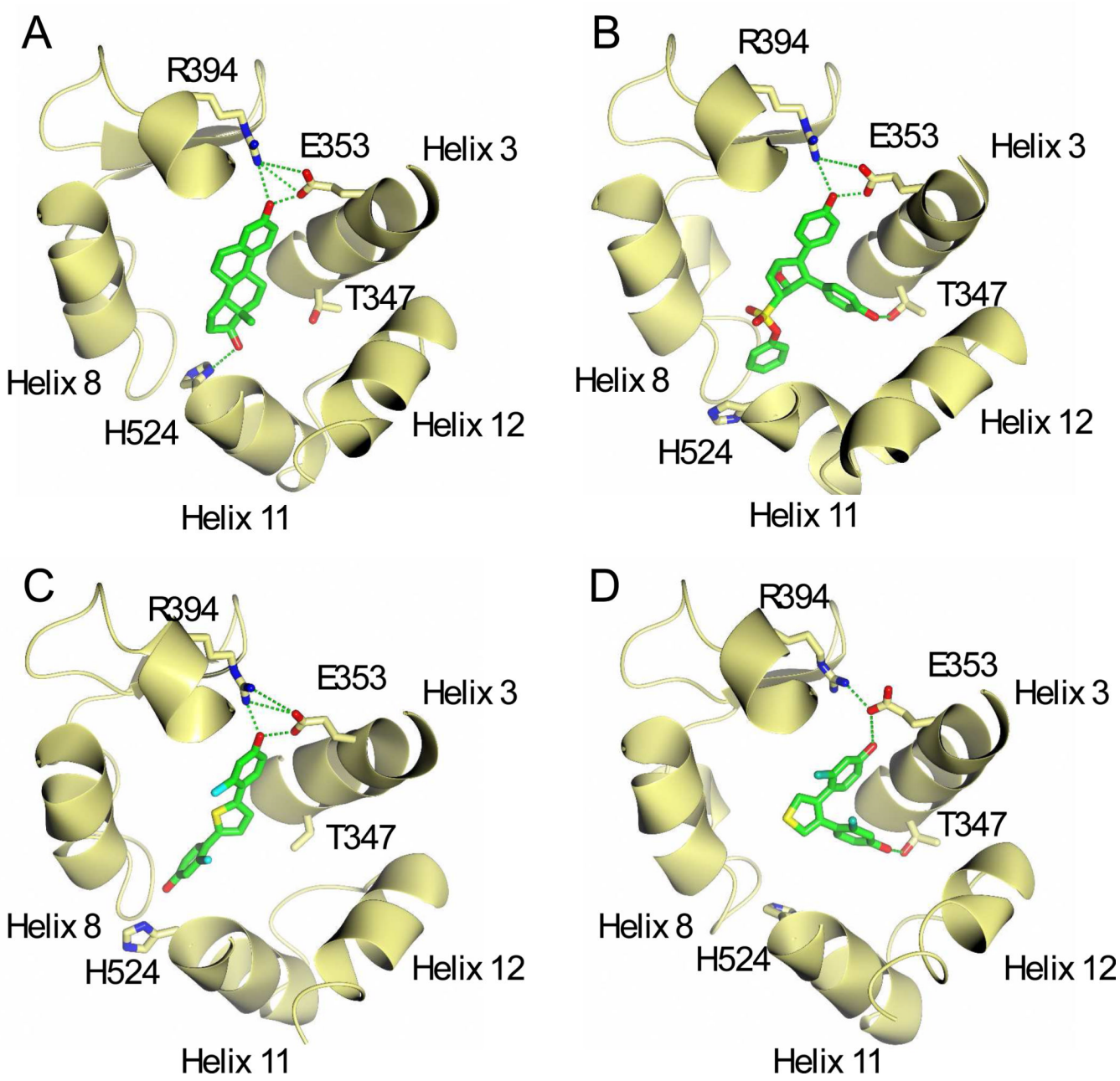
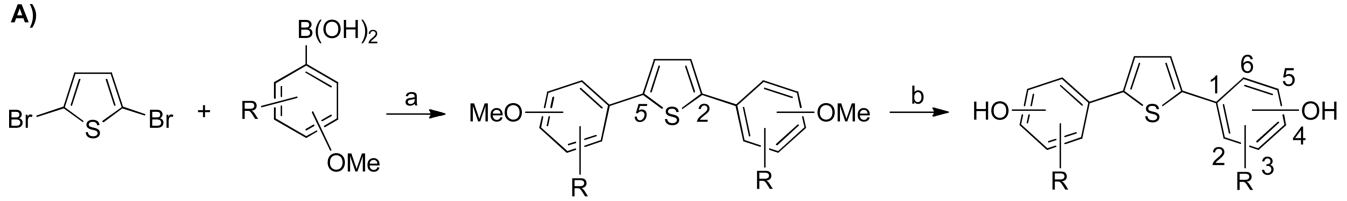


Figure 7. Model of thiophene ligands bound to ERα and comparisons with estradiol and OBHS

A portion of the ligand binding pocket is rendered as a ribbon, with selected amino acids shown as sticks. (**A**) Structure of E_2 bound ERα. The A ring of E_2 forms hydrogen bonds with E353 and R394 while the D ring bonds to H524 (PDB ID: 1ERE). (**B**) Structure of Oxabicyclic heptane sulfonate (OBHS) bound ERα.[52] OBHS H-bonds to both the conserved R394/E353 pair, and also T347 on helix 3. The phenyl sulfonate binds extends between helices 8 and 11. (**C**) Model of **2e** bound to ERα with the conserved H-bonding to R394/E353, and the other phenol extending between helices 8 and 11. (**D**) Model of **4e** with H-bonds to E353 and T347.

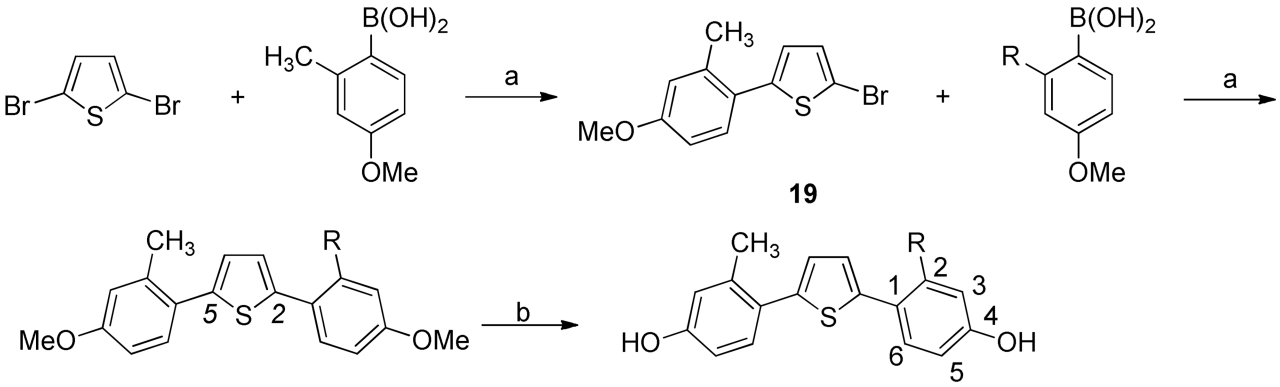
A)



1	R	OMe	Yield (%)
a	H	4-OMe	62
b	2-Me	4-OMe	68
c	3-Me	4-OMe	73
d	3,5-diMe	4-OMe	56
e	2-F	4-OMe	45
f	2-Cl	4-OMe	62
g	3-F	4-OMe	58
h	3-Cl	4-OMe	64
i	2-F	5-OMe	56
j	3-F	5-OMe	68
k	4-F	5-OMe	65

2	R	OH	Yield (%)
a	H	4-OH	75
b	2-Me	4-OH	78
c	3-Me	4-OH	82
d	3,5-diMe	4-OH	68
e	2-F	4-OH	64
f	2-Cl	4-OH	72
g	3-F	4-OH	76
h	3-Cl	4-OH	74
i	2-F	5-OH	78
j	3-F	5-OH	80
k	4-F	5-OH	88

B)



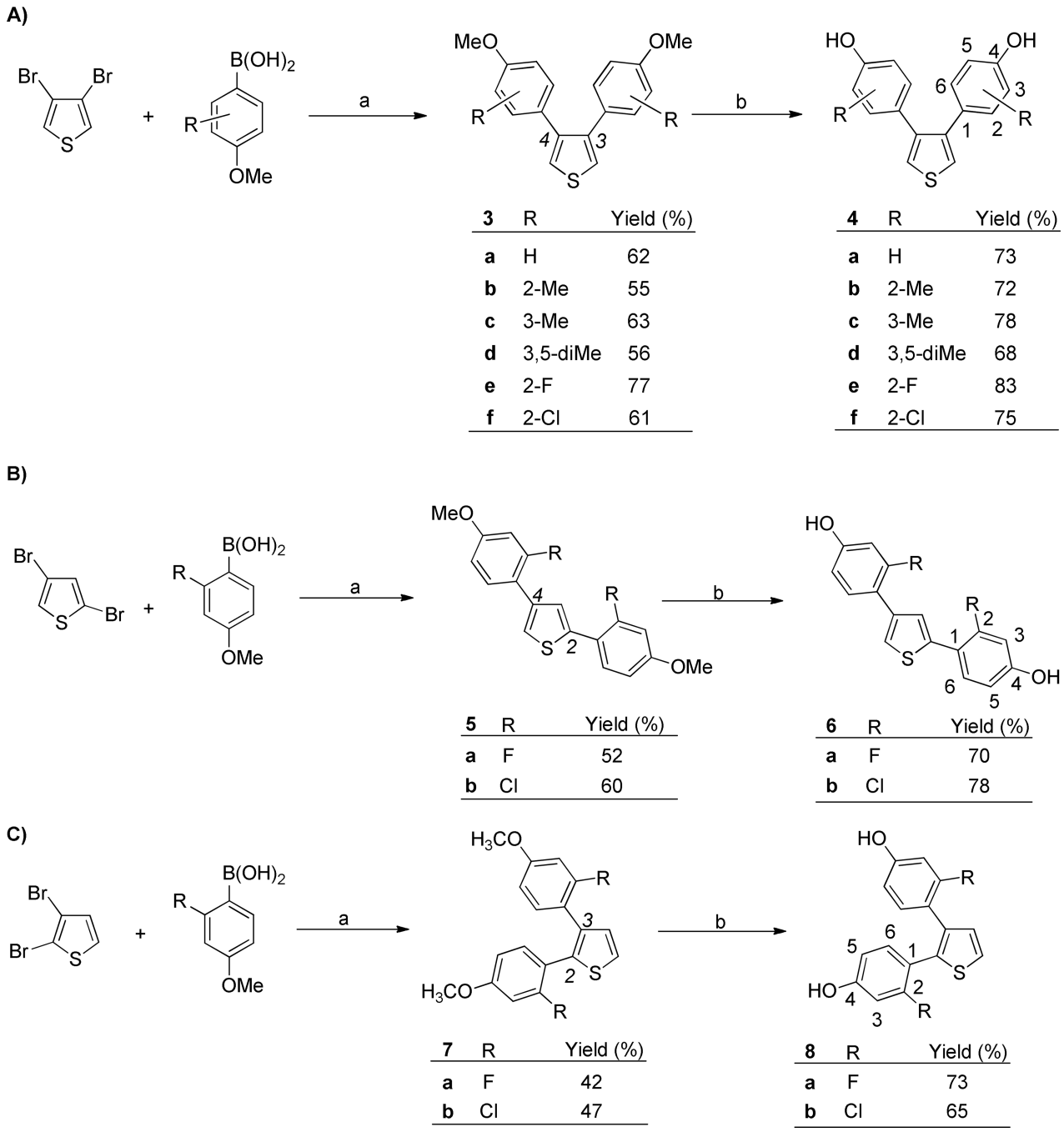
1	R	Yield (%)
l	F	60
m	Cl	58

2	R	Yield (%)
l	F	66
m	Cl	68

Scheme 1.

Synthesis of 2,5-substituted series compounds **2a–m**.a

Reagents and conditions: (a) [Pd] catalyst, Na_2CO_3, toluene/water (1:1), reflux, 24h; (b) BBr_3, CH_2Cl_2, −20 °C to r.t., 4h. [a] To simplify comparisons between compounds in closely related series, we designate locant positions of the substituents on the phenyl groups with respect to the thiophene core; locant positions on the thiophene core itself are given by numbers in italics.



3	R	Yield (%)
a	H	62
b	2-Me	55
c	3-Me	63
d	3,5-diMe	56
e	2-F	77
f	2-Cl	61

4	R	Yield (%)
a	H	73
b	2-Me	72
c	3-Me	78
d	3,5-diMe	68
e	2-F	83
f	2-Cl	75

5	R	Yield (%)
a	F	52
b	Cl	60

6	R	Yield (%)
a	F	70
b	Cl	78

7	R	Yield (%)
a	F	42
b	Cl	47

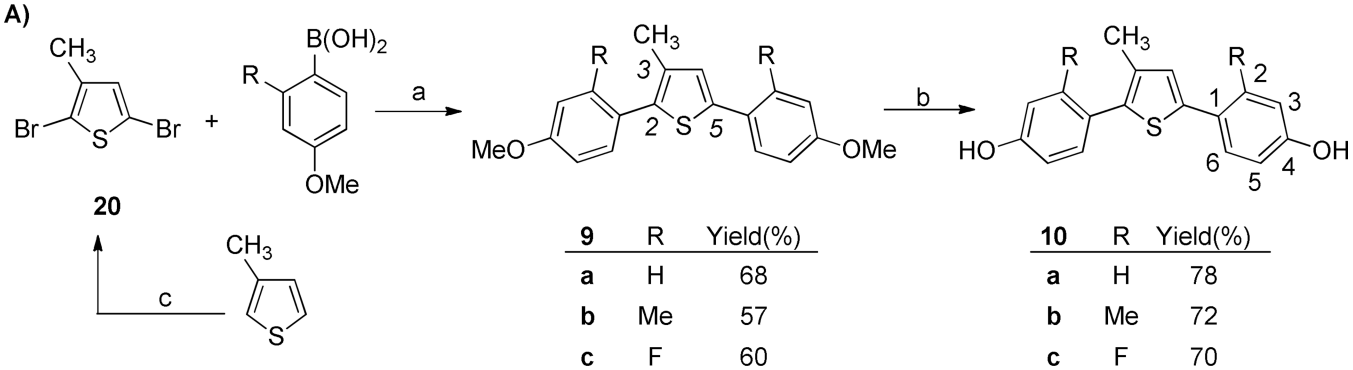
8	R	Yield (%)
a	F	73
b	Cl	65

Scheme 2.

Synthesis of *3,4-, 2,4-, 2,3*-substituted thiophenes **4a–f, 6a–b** and **8a–b**. a

Reagents and conditions: (a) [Pd] catalyst, Na_2CO_3, toluene/water (1:1), reflux, 24h; (b) BBr_3, CH_2Cl_2, −20 °C to r.t., 4h. [a] See footnote *a* in Scheme 1.

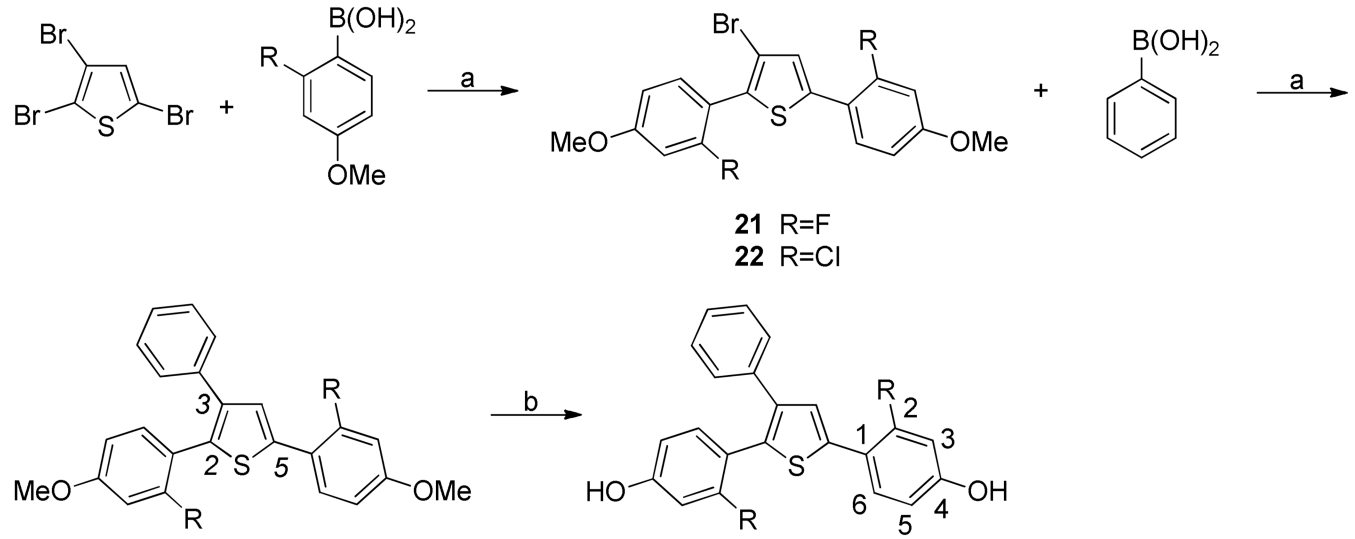
A)



9	R	Yield(%)
a	H	68
b	Me	57
c	F	60
d	Cl	56

10	R	Yield(%)
a	H	78
b	Me	72
c	F	70
d	Cl	74

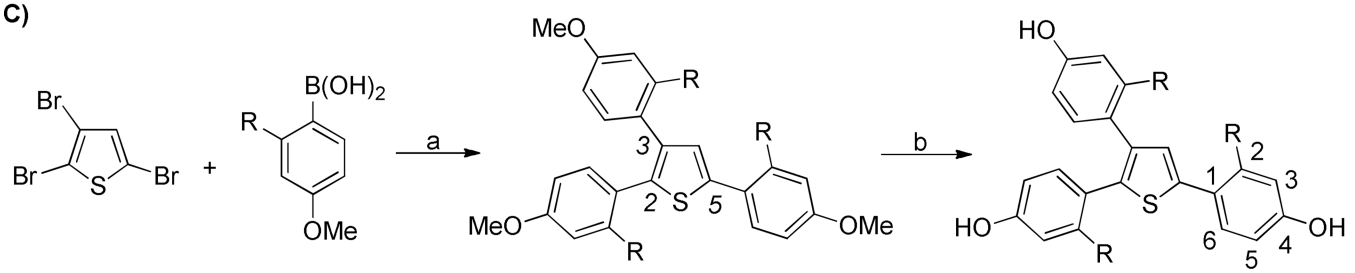
B)



11	R	Yield(%)
a	F	40
b	Cl	42

12	R	Yield(%)
a	F	76
b	Cl	72

C)



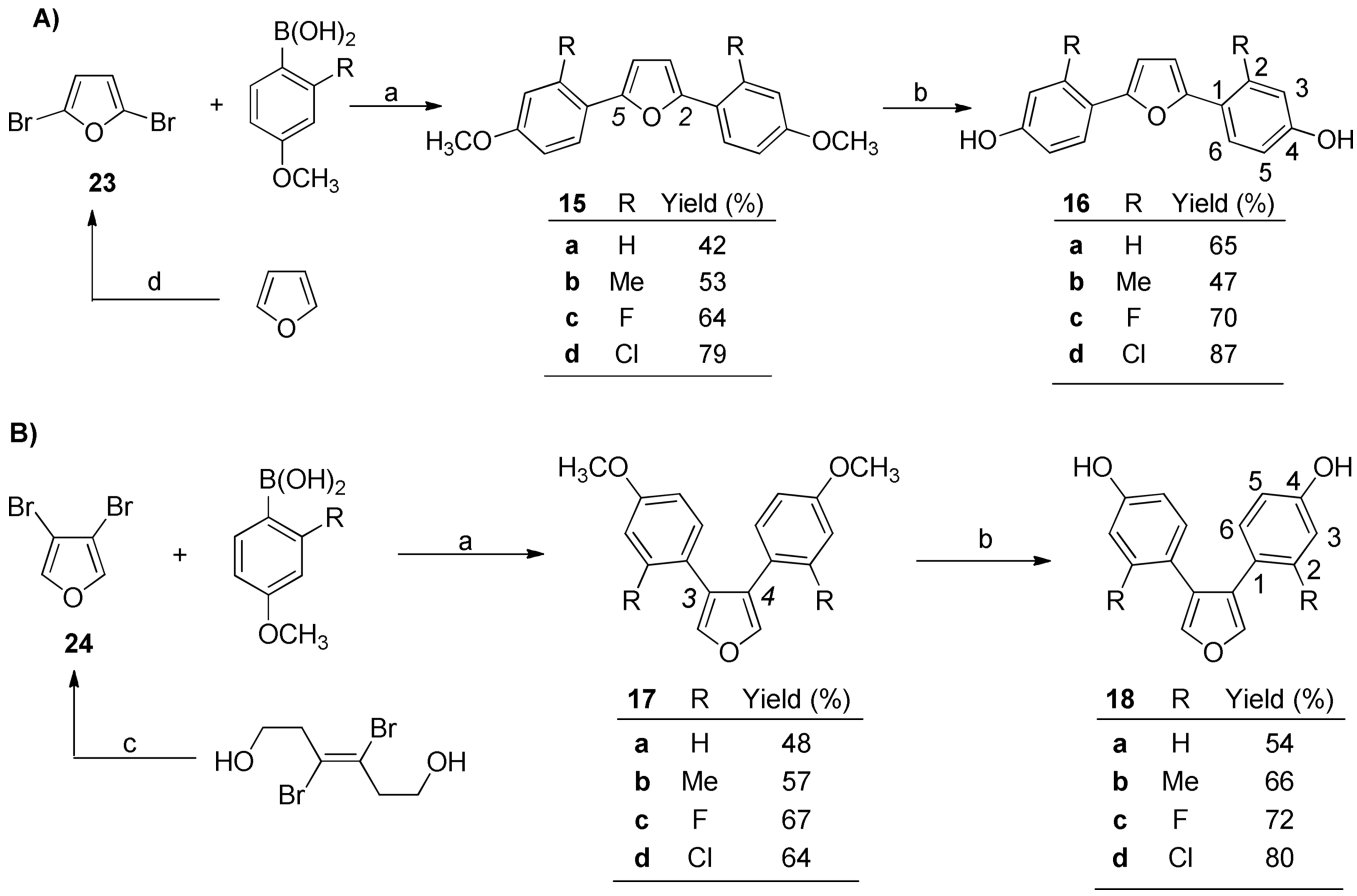
13	R	Yield(%)
a	H	65
b	F	23
c	Cl	20

14	R	Yield(%)
a	H	85
b	F	83
c	Cl	80

Scheme 3.

Synthesis of tri-substituted compounds **10a–d, 12a–b** and **14a–c**. a

Reagents and conditions: (a) [Pd] catalyst, Na_2CO_3, toluene/water (1:1), reflux, 24h; (b) BBr_3, CH_2Cl_2, −20 °C to r.t., 4h; (c) NBS, THF/AcOH (1:1), r. t., 12h. [a] See footnote *a* in Scheme 1.



15	R	Yield (%)
a	H	42
b	Me	53
c	F	64
d	Cl	79

16	R	Yield (%)
a	H	65
b	Me	47
c	F	70
d	Cl	87

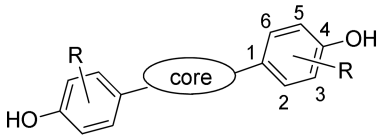
Scheme 4.

Synthesis of corresponding furan-core compounds **16a–d** and **18a–d**.a

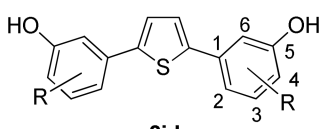
Reagents and conditions: (a) Na_2CO_3, THF/water (1:1), $Pd(PPh_3)_4$, reflux, 12h; (b)BBr_3, CH_2Cl_2, −20 °C to r.t., 4h; (c) $K_2Cr_2O_7$, H_2SO_4, H_2O, steam distillation; (d) Br_2, DMF, 30–35 °C, overnight. [a] See footnote *a* in Scheme 1.

Table 1

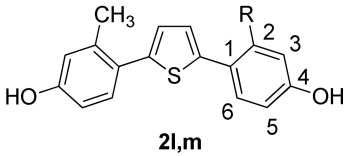
ERα and ERβ relative binding affinity (RBAs) of compounds **2a–m, 4a–f, 6a–b** and **8a–b**.[a]



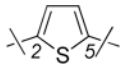
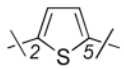
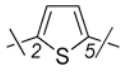
2a-h, 4a-f, 6a,b, 8a,b

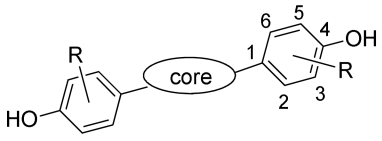


2i-k

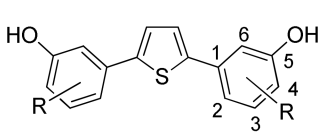


2l,m

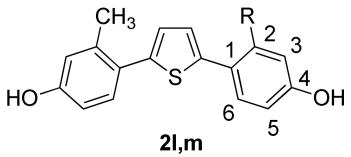
	2a–h, 4a–f, 6a,b, 8a,b			**2i–k**	**2l,m**	
entry	**cmpd**	**core**	**R**	**ERα**[b]	**ERβ**[b]	**β/α**
1	**2a**		H	0.040 ± 0.004	0.79 ± 0.11	19.8
2	**2b**		2-Me	1.43 ± 0.19	1.74 ± 0.26	1.2
3	**2c**		3-Me	0.004 ± 0.001	0.002	0.5
4	**2d**		3,5-diMe	0.003 ± 0.001	0.005 ± 0.001	1.7
5	**2e**		2-F	2.03 ± 0.19	33.1 ± 5.8	16
6	**2f**		2-Cl	6.7 ± 1.3	10.0 ± 2.4	1.5
7	**2g**		3-F	0.013 ± 0.004	0.142 ± 0.004	11
8	**2h**		3-Cl	0.009 ± 0.001	0.036	4.0
9	**2i**		2-F	0.008 ± 0.002	0.066 ± 0.066	8.3
10	**2j**		3-F	0.009 ± 0.006	0.041 ± 0.004	0.4
11	**2k**		4-F	~0.001	0.010 ± 0.002	10
12	**2l**		F	0.97 ± 0.01	0.91 ± 0.26	0.94
13	**2m**		Cl	4.29 ± 0.64	5.89 ± 0.21	1.4
14	**4a**		H	0.57 ± 0.12	1.69 ± 0.45	2.9
15	**4b**		2-Me	2.16 ± 0.54	4.9 ± 1.3	2.25
16	**4c**		3-Me	0.830	0.297	0.36
17	**4d**		3,5-diMe	0.039 ± 0.003	0.004 ± 0.001	0.10
18	**4e**		2-F	3.03 ± 0.85	9.1 ± 2.2	3.0
19	**4f**		2-Cl	13.07 ± 1.1	17.5 ± 2.1	1.3



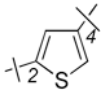
2a-h, 4a-f, 6a,b, 8a,b



2i-k



2l,m

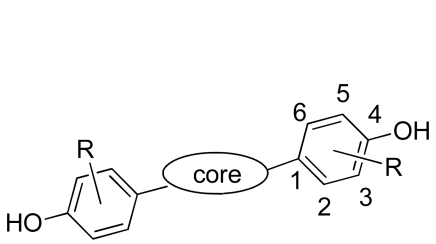
2a–h, 4a–f, 6a,b, 8a,b			**2i–k**		**2l,m**
entry	**cmpd**	**core**	**R**	**ERα**[b]	**ERβ**[b]	**β/α**
20	**6a**		2-F	1.22 ± 0.32	10.4 ± 1.2	8.5
21	**6b**		2-Cl	4.21 ± 0.54	25.6 ± 5.7	6.1
22	**8a**		2-F	1.22 ± 0.35	2.54 ± 0.76	2.1
23	**8b**		2-Cl	6.50 ± 0.61	7.73 ± 2.1	1.2

[a] To simplify comparisons between compounds in closely related series, we designate locant positions of the substituents on the phenyl groups with respect to the thiophene core; locant positions within the thiophene core itself are given by numbers in italics.

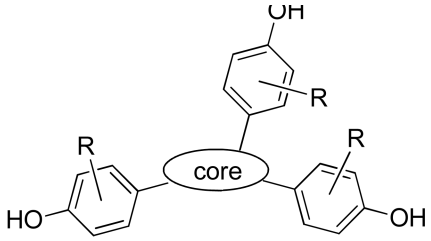
[b] Relative binding affinity (RBA) values are determined by competitive radiometric binding assays and are expressed as $IC_{50}^{estradiol}/IC_{50}^{compound} \times 100 \pm$ the range or standard deviation (RBA, estradiol = 100%). In these assays, the K_D for estradiol is 0.2 nM on ERα and 0.5 nM on ERβ.[51] For details, see the Experimental Section.

Table 2

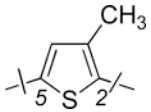
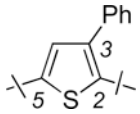
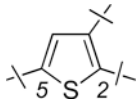
ERα and ERβ relative binding affinity (RBAs) of compounds **10a–d, 12a–b** and **14a–c**.[a]



10a-d, **12a**,**b**



14a-c

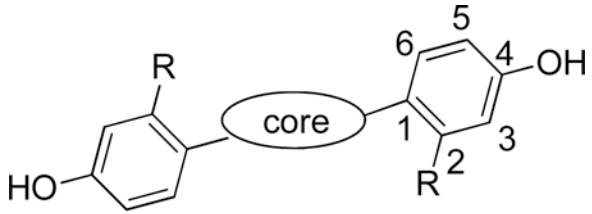
	10a–d, 12a,b			**14a–c**		
entry	**cmpd.**	**core**	**R**	**ERα**[b]	**ERβ**[b]	**β/α**
1	**10a**		H	0.95 ± 0.16	1.6 ± 0.2	1.7
2	**10b**		2-Me	5.15 ± 0.44	4.8 ± 0.6	0.92
3	**10c**		2-F	9.3 ± 1.1	20.6 ± 4.1	2.2
4	**10d**		2-Cl	10.4 ± 1.2	5.41 ± 0.81	0.52
5	**12a**		2-F	1.88 ± 0.28	2.02 ± 0.57	1.1
6	**12b**		2-Cl	3.65 ± 0.41	3.01 ± 0.74	0.83
7	**14a**		H	1.79 ± 0.23	0.47 ± 0.07	0.26
8	**14b**		2-F	6.35 ± 0.26	0.98 ± 0.02	0.15
9	**14c**		2-Cl	85.7 ± 2.5	6.0 ± 1.6	0.07

[a] See footnote *a* in Table 1.

[b] See footnote *b* in Table 1.

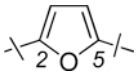
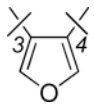
Table 3

ERα and ERβ relative binding affinity (RBAs) of furan-core compounds **16a–d** and **18a–b**.



16a-d, 18a-d

16a–d, 18a–d

entry	cmpd.	core	R	ERα[b]	ERβ[b]	β/α
1	**16a**		H	0.082 ± 0.007	0.34 ± 0.10	4.2
2	**16b**		Me	0.78 ± 0.12	0.24 ± 0.02	0.31
3	**16c**		F	6.92 ± 0.73	32.2 ± 1.0	4.7
4	**16d**		Cl	2.73 ± 0.41	6.9 ± 1.7	2.5
5	**18a**		H	0.22 ± 0.06	0.69 ± 0.07	3.1
6	**18b**		Me	0.79 ± 0.21	2.28 ± 0.34	2.9
7	**18c**		F	1.19 ± 0.20	5.41 ± 0.49	4.5
8	**18d**		Cl	2.99 ± 0.70	5.44 ± 0.19	1.8

[a] See footnote *a* in Table 1.

[b] See footnote *b* in Table 1.

Table 4

Potency and Intrinsic Activity of Thiophene and Furan Core Ligands[a]

				HepG2				Ishikawa
				ERα		ERβ		ERα
cmpd	core		R	EC_{50} (μM)	Eff (% E_2)	EC_{50} (μM)	Eff (% E_2)	Eff (% E_2)
THIOPHENES								
2a	*2,5-*	4-OH	H	2.35	**125 ± 4**	1.54	**198 ± 4**	111 ± 32
2b		4-OH	2-Me	-	78 ± 4	6.60	87 ± 15	**148 ± 23**
2e		4-OH	2-F	-	**181 ± 14**	-	**336 ± 63**	**315 ± 125**
2f		4-OH	2-Cl	2.18	111 ± 5	1.94	**169 ± 18**	**169 ± 14**
2l		4-OH	2-Me, 2'-F	0.47	117 ± 8	0.61	**277 ± 15**	8 ± 5
2m		4-OH	2,Me, 2'-Cl	1.91	78 ± 3	1.92	111 ± 7	102 ± 7
4a	*3,4-*		H	2.25	69 ± 4	1.27	73 ± 2	46 ± 4
4b			2-Me	3.15	97 ± 11	1.87	67 ± 4	**120 ± 14**
4c			3-Me	-	73 ± 5	-	70 ± 4	4 ± 0
4e			2-F	0.12	79 ± 3	0.780	29 ± 4	63 ± 5
4f			2-Cl	0.045	90 ± 7	0.203	57 ± 3	90 ± 3
6a	*2,4-*		2-F	1.06	**123 ± 6**	1.34	**177 ± 17**	**201 ± 13**
6b			2-Cl	0.563	87 ± 5	0.660	117 ± 2	81 ± 5
8a	*2,3-*		2-F	65.9	69 ± 3	-	33 ± 10	67 ± 6
8b			2-Cl	1.16	82 ± 8	5.70	116 ± 14	114 ± 6
10a	*2,5-*	*3*-Me	H	1.52	116 ± 5	1.23	**200 ± 10**	**225 ± 27**
10b		*3*-Me	2-Me	0.439	70 ± 5	2.46	106 ± 23	56 ± 4
10c		*3*-Me	2-F	0.447	**149 ± 1**	10.8	**284 ± 44**	**260 ± 53**
10d		*3*-Me	2-Cl	0.101	82 ± 1	0.295	113 ± 4	72 ± 8
12a	*2,5-*	*3*-Ph	2-F	0.615	70 ± 1	-	39 ± 11	91 ± 15
12b		*3*-Ph	2-Cl	0.066	72 ± 2	-	1 ± 1	92 ± 4

			HepG2				Ishikawa
			ERα		ERβ		ERα
cmpd	**core**	**R**	**EC_{50} (μM)**	**Eff (% E_2)**	**EC_{50} (μM)**	**Eff (% E_2)**	**Eff (% E_2)**
14a	*2,3,5-*	H	0.485	82 ± 6	2.23	−22 ± 1	**145 ± 9**
14b		2-F	0.387	84 ± 5	-	−1 ± 4	63 ± 2
14c		2-Cl	0.009	52 ± 5	-	−13 ± 2	35 ± 1
FURANS							
16a	*2,5-*	H	-	62 ± 3	-	94 ± 26	91 ± 21
16b		Me	3.79	36 ± 4	-	8 ± 6	58 ± 8
16c		F	-	**154 ± 7**	-	**159 ± 13**	61 ± 48
16d		Cl	3.89	82 ± 7	-	106 ± 3	118 ± 11
18a	*3,4-*	H	10.6	81 ± 2	-	37 ± 5	82 ± 25
18b		Me	0.944	97 ± 5	5.11	56 ± 3	104 ± 6
18c		F	3.64	73 ± 2	-	85 ± 16	85 ± 11
18d		Cl	0.266	86 ± 6	17.5	54 ± 4	116 ± 9

[a] See footnote a in Table 1.

Values indicating pronounced superagonism (i.e., Eff values ≥120) are indicated in boldface type.

J Med Chem. Author manuscript; available in PMC 2014 April 25.