Structure-Guided Optimization of Estrogen Receptor Binding Affinity and Antagonist Potency of Pyrazolopyrimidines with Basic Side Chains

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2,3-Diarylpyrazolo[1,5-*a*]pyrimidines are estrogen receptor (ER) antagonists of modest potency that we have described previously. Guided by the crystal structure of an ER−ligand complex that we have obtained with one of these compounds, we prepared analogs that contain a basic side chain at the 2- or 3-aryl group and quickly found one that, according to the structure-based prediction, shows an increase in binding affinity and antagonist potency and a loss of residual agonist activity.

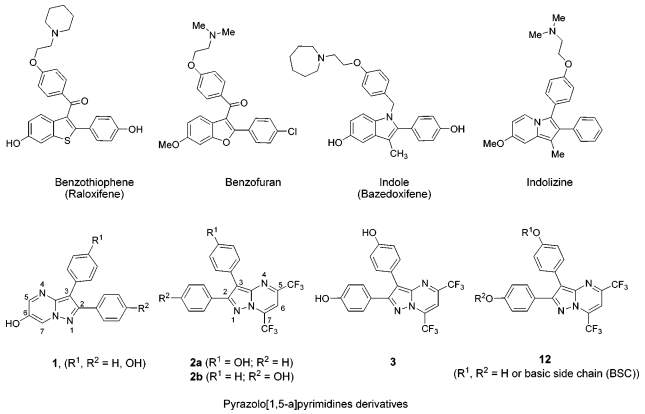
Introduction

The estrogen receptors (ERs[a]) have emerged as attractive therapeutic targets for a variety of diseases, including osteoporosis and breast cancer. Estrogens act through two ER subtypes, ERα and ER*β*,[1] which have different tissue distributions and significant differences in their ligand-binding preferences.[2] Estrogens can have remarkable tissue-selective effects, and this has led to the development of compounds termed selective ER modulators (SERMs), which function principally as estrogen agonists in some tissues (bone, brain and the cardiovascular system), but as antagonists in others (uterus and breast).[3,4]

The ERs can bind a variety of steroidal and nonsteroidal ligands, and the search for better SERMs has driven efforts to increase the chemical diversity of these compounds, especially the nonsteroidal ones. Frequent examples are heterocycles,[5−8] including fused bicyclic systems such as benzothiophenes,[9] indoles,[10] benzofurans,[11] indazoles,[12] and indolizines[13] (Scheme 1), as well as recently reported benzoxazole-,[14] benzothiazole,[15] and quinoline-based,[16] ER*β*-selective ligands. The majority of these fused heterocycles contain only a single heteroatom; so, to extend the structural scope of ER ligands, we explored fused bicyclic systems that contain multiple heteroatoms. This led us to synthesize ER ligands having indazole[12] and pyrazolo[1,5-*a*]pyrimidine[17,18] core structures (Scheme 1).

In preparing the pyrazolo[1,5-*a*]pyrimidines, our original intent was to have a hydroxy group on the pyrimidine ring function as the A-ring mimic of estradiol, as in the 6-hydroxy analogs **1**. These compounds, however, had very low ER binding affinity (Table 1, entry 1).[17] Thus, we took an alternative approach, substituting the pyrazolo[1,5-*a*]pyrimidine core with

Scheme 1. Fused Heterocyclics and Pyrazolo[1,5-α]pyrimidines Systems and Analogs as ER Ligands



p-hydroxyphenyl rings at the 2 and 3 positions,[18] one of which we imagined might function as a mimic of the phenolic A-ring of estradiol. Such a "pendant phenol" orientation is thought to be adopted by other fused heterocyclic systems[19,20] and is illustrated in certain crystal structures, at least in ER*β*.[21] We also changed the substituents at the 5 and 7 positions to vary the hydrophobicity and size of the system.

On this basis, we developed a series of 44 pyrazolo[1,5-*a*]-pyrimidine core ER ligands, the best of which, the bisphenol analog **3**, still exhibited only modest binding affinity for ERα and ER*β* and no ER subtype selectivity, with the relative binding affinities (RBA) values (measured on a sample newly synthesized for this study) being 0.77% and 1.0% for ERα and ER*β*, respectively (RBA[estradiol] = 100%; Table 1, entry 4), comparable to values determined previously.[18] The monophenolic analogs, compounds **2a** and **2b**, showed even lower affinities (Table 1, entries 2 and 3).[18] These compounds profiled as ER functional antagonists, with some showing modest potency preference for ER*β* and low, residual ERα agonist activity (e.g., see compound **3**, Figure 2C).[18] As was the case in our prior work with members of the pyrazole triphenols series,[7,22] it was difficult, based on comparisons of the binding affinities and transcriptional potencies of the monophenols (**2a**,**b**) with the bisphenol (**3**), to ascertain whether the C-2 phenol or the C-3 phenol was functioning as the estradiol A-ring mimic.

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[a] Abbreviations: ER, estrogen receptor; SERMs, selective estrogen receptor modulators; RBA, relative binding affinity; HEC-1, human endometrial cancer cells; THF, tetrahydrofuran; TLC, thin-layer chromatography.

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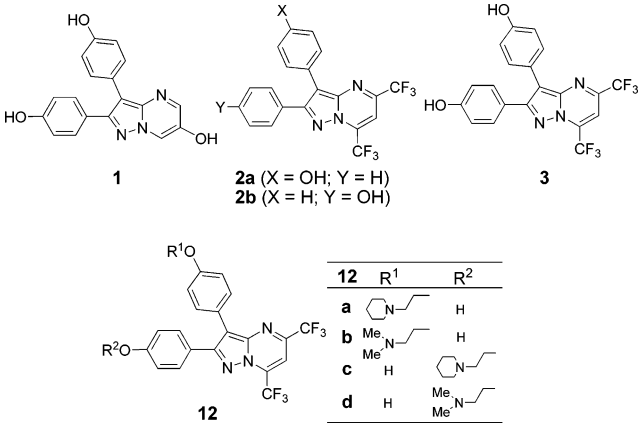
Table 1. ERα and ERβ Binding Affinities of Pyrazolo[1,5-*a*]pyrimidine Derivatives



		ligand binding					
		RBA[a] (%)			K_i[b] (nM)		
entry	cmpd	ERα	ERβ	α/β ratio[c]	ERα	ERβ	α/β ratio[c]
1	**1**	0.006 ± 0.002	0.033 ± 0.001	0.18	3333	1515	0.45
2	**2a**	0.009 ± 0.001	0.36 ± 0.01	0.025	2222	139	0.06
3	**2b**	0.39 ± 0.03	0.41 ± 0.09	1	51	122	2.5
4	**3**	0.77 ± 0.04	1.04 ± 0.09	0.7	26	48	1.8
5	**12a**	2.8 ± 0.44	0.70 ± 0.20	4	7	71	10
6	**12b**	0.42 ± 0.07	0.28 ± 0.06	1.5	47	178	3.8
7	**12c**	0.075 ± 0.01	0.26 ± 0.01	0.29	266	192	0.72
8	**12d**	0.049 ± 0.004	0.32 ± 0.04	0.15	408	156	0.38

[a] RBA values are $IC_{50}^{estradiol}/IC_{50}^{compound} \times 100$. The K_d for estradiol is 0.2 nM (ERα) and 0.5 nM (ERβ).[27] [b] K_i values of each compound for each receptor were obtained from the RBA values by the formula $K_i = (100/RBA) \times K_d$. [c] In each case, α/β ratios represent the affinity preference for ERα vs ERβ; the α/β ratios calculated from the K_i values are 2.5-fold higher than those calculated from the RBA values, because estradiol binds to ERα 2.5-fold better than to ERα (see K_d values noted in table footnote a).

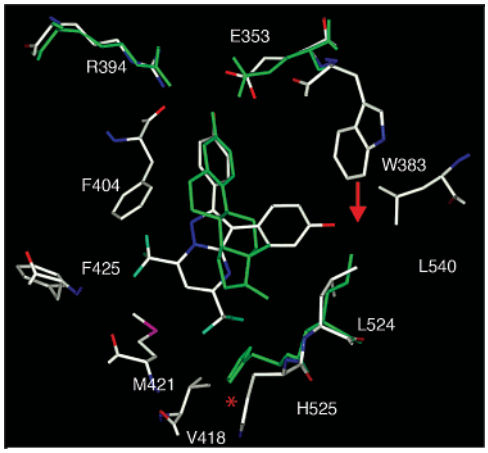


Figure 1. Comparison of compound **3** versus estradiol in complex with ERα, as determined by X-ray crystallography. The estradiol/ERα ligand binding pocket is colored green and shown superimposed on the compound **3**/ERα structure (atom colors). The red arrow denotes the interaction of the C-3 phenol with helix 12 Leu540, while the red asterisk highlights the ligand associated positioning of helix 11 His524.

Our interest in preparing more potent, fully antagonistic analogs in the pyrazolo[1,5-*a*]pyrimidine series was piqued when we obtained a crystal structure for compound **3** complexed to ERα. Because the nature and spatial orientation of the basic side chains in SERMs relative to their central core structure are known to influence their tissue selectivity and to affect the

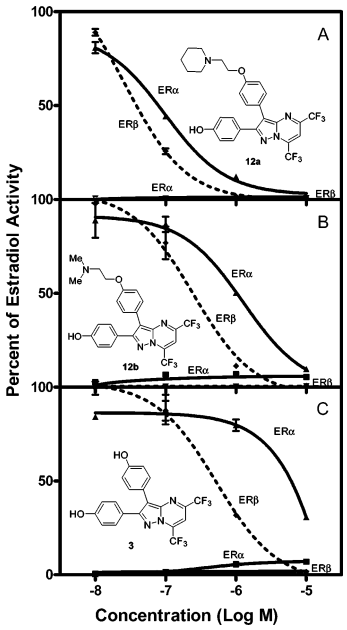
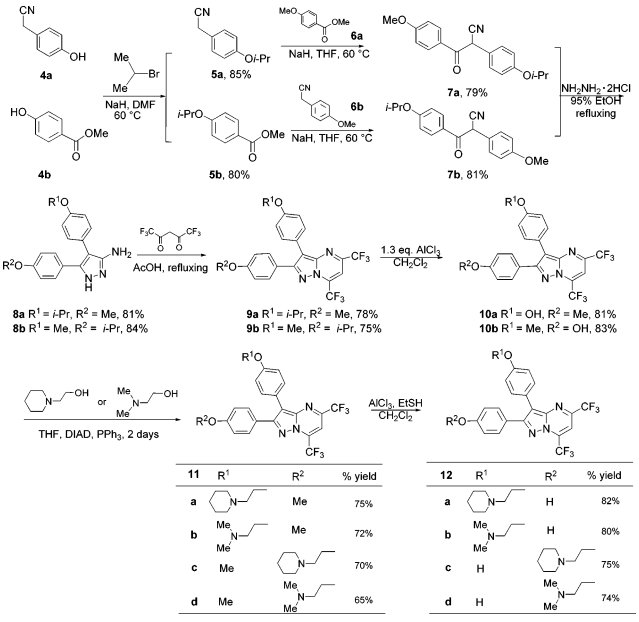


Figure 2. Transcription activation through ERα (shown as solid lines) and ERβ (shown as dotted lines) of compounds **3**, **12a**, and **12b**. HEC-1 were transfected with expression vectors for ERα or ERβ and the estrogen responsive gene 2xERE-pS2-Luc and were incubated with the indicated ligand for 24 h. Agonist assays are the upper curves, and antagonist assays (done in the presence of 1 nM estradiol, E2) are the lower lines. Values are the mean ± SD of two or more experiments, expressed as a percent of the activity of ERα and ERβ with 10^{-9} M E2, which is set at 100%. The following IC_{50} values are estimated from the antagonist profiles: compound **3**, ERα 6 μM, ERβ 600 nM; compound **12b**, ERα 1 μM, ERβ 250 nM; and compound **12a**, ERα 90 nM, ERβ 40 nM.

Scheme 2. Synthesis of Pyrazolo[1,5-*a*]pyrimidine Derivatives Containing a Basic Side Chain



balance of desired and undesired activities, we introduced two different types of aminoethoxy moieties into the pyrazolo[1,5-*a*]pyrimidine core system, placing these at two positions. Thus, the compounds we made (**12a**−**d**) contained either a 2-dimethylaminoethoxy or a 2-*N*-piperidinylethoxy group, which was positioned on either the C-2 or the C-3 phenolic hydroxyl group (Scheme 2). From the crystal structure, as discussed below, we expected that the C-3-substituted systems (**12a**,**b**)

would show improved affinity and potency; the other regioisomeric compounds (**12c**,**d**) were prepared to challenge this expectation.

Results and Discussion

Synthesis. The synthesis of these systems is illustrated in Scheme 2. The main feature of these syntheses is the selective protection of one of the hydroxyl groups on the *para* position of the C-2 or C-3 phenyl group on the pyrazolo[1,5-*a*]pyrimidine core systems (**9a**) with the easily removable isopropyl moiety, starting from an appropriate precursor aryl acetonitrile **4a** or methyl benzoate **4b**. A Claisen-type condensation between an appropriate pair of these two components (**5a** + **6a** or **5b** + **6b**) gave the 2,3-diaryl-3-oxopropionitriles **7a** and **7b**, respectively, in high yield. Further condensation of these α-cyanoketones with hydrazine dihydrochloride went smoothly and afforded the key 3-aminopyrazole intermediates **8a**,**b**. The pyrazolo[1,5-*a*]pyrimidine cores **9a**,**b** were assembled by condensing the aminopyrazoles **8a**,**b** with hexafluoro-acetylacetone. The regioisomeric monophenol derivatives **10a** and **10b** were obtained by selective cleavage of the isopropyl ether with $AlCl_3$ in dichloromethane at room temperature, which left the aryl methyl ether intact.[23] Installation of the two types of basic side chains in the free phenolic positions in **10a** and **10b** was effected by a Mitsunobu reaction, and the remaining methyl ether group was then selectively cleaved with $AlCl_3$−EtSH, leaving the basic side chain unaffected. By this approach, we prepared the analogs containing the two types of basic side chains at the *para* position of either the C-2 or C-3 phenyl groups, **12a**−**12d**.

Crystal Structure. We crystallized compound **3** with the ERα ligand-binding domain and a leucine-rich peptide from the nuclear receptor interaction box 2 of the glucocorticoid receptor interacting protein 1 coactivator, known to interact with ERα (see data table in Supporting Information). The overall structure resembles the protein bound to full agonists, such as estradiol, with the ligand fully enclosed in a hydrophobic pocket, which is sealed by helix 12 Leu540. This structure (Figure 1) demonstrates that the C-2 phenol functions as the estradiol A-ring mimic. One notable feature of this structure is the relocation of helix 11 His524 by the C-5 substituted CF_3 group (Figure 1A, red asterisk). This positioning of His524 is also associated with a shift in the last two turns of helix 11, resulting in an approximately 1 Å displacement of the main chain of amino acids 528−530, compared to the position seen with full agonist ligands (not shown). This shift in helix 11 has been previously demonstrated to induce partial agonist or antagonist activity by destabilizing the helix 12 portion of the coactivator binding pocket,[24,25] and is consistent with the weak partial agonist activity of this compound, described below.

The structure also demonstrates that the C-3 phenol directly contacts helix 12 Leu540 (Figure 1, red arrow), extending roughly in the direction where SERMs typically project their basic 2-aminoethoxy-phenyl substituents (compare structures **2**, **3**, and **12** in the lower line of Scheme 1 with raloxifene in the upper line). Helix 12 also forms part of the coactivator binding site, and its ligand-induced positioning is the ultimate determinant of the recruitment of coactivators and the degree of associated transcriptional activity. SERMs such as tamoxifen or raloxifene contain an extended side chain that displaces helix 12 from its position as part of the coactivator binding site.[24,26] The crystal structure thus suggested that substitution of the C-3 phenol in compound **3** with the types of basic side chains typically found in SERMs would be sterically well tolerated by the ER and might, in fact, enhance both the affinity and antagonist character of these novel ligands.

Binding Affinity. The binding affinities of compounds **12a**−**d** for both ERα and ERβ were determined using a competitive radiometric assay and are reported in Table 1.[27] These affinities are discussed here as relative binding affinity (RBA) values, where estradiol has an affinity of 100% (K_i values, calculated from RBA values, are also given in Table 1; the α/β ratios shown for the two methods differ by a factor of 2.5, which represents the 2.5-fold higher affinity that the standard, estradiol, has for ERα vs ERβ).

The contribution of the basic side chain to the binding affinity of the pyrazolo[1,5-*a*]pyrimidines is significant, with both the position and the nature of the basic side chain proving to be very important. The compounds having the basic side chain at the *para* position of 3-aryl group (**12a**,**b**) showed good binding to ERα (Table 1, entries 5 and 6), with pyrazolo[1,5-*a*]-pyrimidine **12a** having the highest overall RBA for ERα and ERβ, 2.8% and 0.70%. The former value represents an increase in binding affinity for ERα that is about 4-fold compared to the parent compound **3**. By contrast, when the basic side chain was positioned at the *para* position of C-2 aryl group (**12c**,**d**), ERα binding affinity was markedly lower (entries 7 and 8).

The preferential binding of the C-3 aryl-substituted compound **12a** compared to the C-2 regioisomer **12c** is consistent with predictions derived from X-ray structure of the bisphenol **3**−ERα complex, which showed that the C-2 (4-hydroxyphenyl) group was in the A-ring binding pocket and, thus, was not a favorable site for substitution with the basic side chain. While not predicted, it is noteworthy that the nature of the basic side chain also has a significant effect on the binding affinity of the ligands. Thus, compared to the ligand **12a**, containing a piperidinylethoxy substituent, the dimethylaminoethoxy analog **12b** shows considerably lower binding affinity. Affinities of the new compounds for ERβ were not improved. This might arise from the fact that the ligand binding pocket in ERβ is, overall, smaller than that for ERα;[21] so, any increase in steric bulk (such as from the addition of a side chain), even when extending in a favorable direction, might reduce binding affinity.

Transcriptional Activity and Molecular Modeling. Compounds **12a** and **12b** and parent compound **3** were assayed for their transcriptional activity through ERα and ERβ by reporter gene cotransfection assays in human endometrial cancer cells (HEC-1; Figure 2).[28] All of the compounds are antagonists on both ERα and ERβ (IC_{50} values are given in Figure 2 legend). Most interesting was the activity of the C-3 substituted piperidinyl analog **12a**, which showed considerably enhanced antagonist potency compared to parent compound **3** on both ERs, about 70-fold on ERα and 15-fold on ERβ. The other C-3 substituted compound **12b** also showed enhanced ERα antagonist potency (ca. 6-fold), but a lesser change in ERβ antagonist potency (only ca. 2-fold), compared to the parent compound (**3**). The most potent compound (**12a**) also lacked the residual ERα partial agonist efficacy of the parent compound (**3**).

The improved binding affinity and antagonist activity of the C-3-substituted analog **12a** can be rationalized by the X-ray crystal structure of the complex of compound **3** with ERα. The aromatic ring at the C-3 position is oriented in such a direction that addition of the basic amine side chain would avoid steric clashes and would place the amine function in a position to form the salt bridge with Asp351, the residue with which amine function in the basic side chains of hydroxytamoxifen and raloxifene are known to interact.[26,29] Such an interaction would

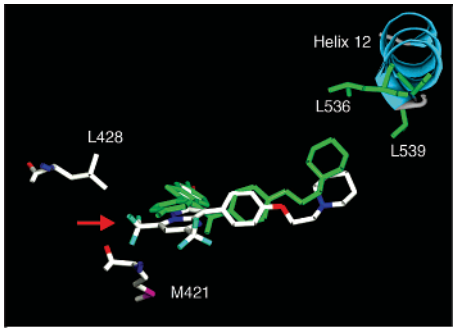


Figure 3. Model of compound **12a** ERα (atom colors) compared to raloxifene ERα (green).

enforce the antagonist conformation of ER and, thereby, enhance ligand antagonist character, as appears to be the case with compound **12a**. This compound bears the piperidinylethoxyl side chain on the C-3 phenol, which leaves the C-2 phenol free to function as the estradiol A-ring mimic. As expected, the C-2/C-3 regioisomer, compound **12c**, is unable to reverse the roles of the two phenols and, hence, suffers from very low binding affinity.

To understand why the piperidinylethoxyl side group provides a significant increase in affinity and antagonist potency compared to the dimethylamino group (compounds **12a**,**b**), we generated molecular models of these compounds based on the X-ray structure we determined for compound **3**. A comparison of the previously published tamoxifen[26] and raloxifene-bound ER structures[29] shows that they differ in that the piperidine group of raloxifene forms direct, van der Waals contacts with helix 12, whereas the dimethylamino group in tamoxifen does not contact helix 12. Thus, these SERMs are thought to differ in how they control the dynamics of helix 12 and the associated recruitment of transcriptional coregulator proteins.[30]

In our structure, the C-3 phenyl group of compound **3** is oriented in a slightly different manner than the corresponding group in the raloxifene−ER structure (Figure 3). We generated a model of compound **3** bound to ER in the antagonist conformation by superimposing our structure with the raloxifene/ER complex. We then introduced by molecular modeling the piperidine or dimethylamino groups onto the C-3 phenol in compound **3** by superimposing the tamoxifen or raloxifene compounds, using the respective phenols that correspond to the C-3 phenol, thereby generating models of compounds **12a** and **12b**. These models showed that the basic side chains are optimally positioned to form an electrostatic interaction with Asp351, but are not able to directly contact helix 12. We then asked whether a rotation of the core molecule in these models might allow the basic side chains in compounds **12a** and **12b** to contact helix 12. The rotation of the molecule is limited by the C-7 CF_3 group, which lies in a pocket between Leu428 and Met421. In this position, the CF_3 group is located 3.3 Å from Leu426 and 3.8 Å from Met421, which allows it to rotate the 10−15 degrees necessary for the piperidine group to form stabilizing contacts with helix 12. These models suggest that the higher affinity of compound **12a** relative to **12b** is associated with a direct interaction that only the larger piperidine group can make with helix 12.

Conclusions

In this study, we show how the X-ray structure of an ER−ligand complex can serve as a useful guide that enabled us to enhance the affinity, potency, and antagonist character of SERMs of the pyrazolo[1,5-*a*]pyrimidine core class by directing the site for introducing basic side chains that are characteristic of SERMs. We evaluated the ERα and ERβ binding affinities and the transcriptional potency and efficacy of four of these derivatives, and we observed that when the basic side chain is properly positioned (on the C-3 phenol, as suggested by the X-ray structure) and has the appropriate structure (2-*N*-piperidinyl-ethoxy), it can significantly enhance affinity and potency, with compound **12a**, the best of our series, having about a 4-fold increase in ERα binding affinity, a loss of residual agonist activity, and up to a 70-fold increase in antagonist potency compared to that of the parent compound **3**.

The rapid, structure-guided optimization of antagonist character and potency that we experienced here in the pyrazolo-[1,5-*a*]pyrimidine series can be contrasted with our experiences in an earlier study in which we endeavored to convert an ERα agonist ligand of the pyrazole-triphenol class (propylpyrazole triol) into an ERα-selective antagonist by adding a basic side chain.[31] Although ultimately successful, without the guidance of an X-ray structure, we had to prepare all four possible regioisomers and several analogs[31−33] to find the one (methyl-piperidinopyrazole) that showed good binding and effective ERα antagonism.[32,33] By contrast, the results in this study illustrate the value of X-ray structures of ER−ligand complexes in guiding an efficient optimization of the pharmacological characteristics of an ER ligand.

Experimental Section

General Procedure for the Installation of Basic Side Chain (BSC) by a Mitsunobu Reaction (11). To a solution of corresponding monophenols **10**, triphenylphosphine (5 equiv), and corresponding amino alcohols (5 equiv) in dry THF was added diisopropyl diazodicarboxylate (5 equiv). The resulting solution was stirred at room temperature under N_2 for 2 days and concentrated under reduced pressure. The residue was chromatographed over a silica gel column eluted with 30−50% EtOAc/hexane to afford the product.

2-(4-Methoxyphenyl)-3-(4-(2-piperidin-1-yl-ethoxy)-phenyl)-5,7-bis(trifluoromethyl)pyrazole-[1,5-*a*]pyrimidine (11a). Recrystallized from ethyl acetate/hexane to give a yellow solid (75% yield; mp 149−150 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ1.43 (m, 2H), 1.59 (m, 4H), 2.46 (m, 4H), 2.81 (t, 2H, J = 6.0 Hz), 3.82 (s, 3H), 4.14 (t, 2H, J = 6.0 Hz), 6.90 (d, 2H, J = 8.5 Hz), 6.96 (d, 2H, J = 8.5 Hz), 7.39 (s, 1H), 7.41 (d, 2H, J = 8.5 Hz), 7.60 (d, 2H, J = 8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.42, 26.14, 55.28, 55.52, 58.18, 66.17, 102.15, 112.18, 114.29, 115.03, 119.91 (q, J = 274.8 Hz), 121.31 (q, J = 274.8 Hz), 122.42, 124.36, 130.75, 131.39, 135.31 (q, J = 37.5 Hz), 145.21 (q, J = 37.5 Hz), 146.59, 156.57, 158.79. 160.82; LRMS *m*/*z* 564.2; HRMS (EI) calcd for $C_{28}H_{26}F_6N_4O_2$, 564.1960 (M^+); found, 564.1964.

General Procedure for the Synthesis of Compounds 12. The pyrazolo[1,5-*a*]pyrimidine **11** (0.5 mmol) was dissolved in 10 mL of dichloromethane, and to the resulting solution was added $AlCl_3$ (10 equiv) and EtSH (5 equiv). The solution was stirred overnight and was quenched by careful addition of MeOH, and 10 mL of water was added. The solution was then extracted (3 × 50 mL) with ethyl acetate, and the organic layers were combined. The combined organic layers were washed (2 × 25 mL) with a saturated NaCl solution and dried with Na_2SO_4. The ethyl acetate was removed by rotary evaporation, and the crude product was purified by flash chromatography (40−60% EtOAc/hexanes), and further purification was effected using preparative TLC.

2-(4-Hydroxyphenyl)-3-(4-(2-piperidin-1-yl-ethoxy)-phenyl)-5,7-bis(trifluoromethyl)pyrazole-[1,5-*a*]pyrimidine (12a). Purified by preparative TLC (50% EtOAc/hexane) to give a yellow solid (82% yield) that was recrystallized from ethyl acetate (mp 147−149 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (m, 2H), 1.70 (m, 4H), 2.66 (m, 4H), 2.90 (t, 2H, J = 5.5 Hz), 4.18 (t, 2H, J = 5.5 Hz), 4.59 (br s, 1H, OH), 6.77 (d, 2H, J = 8.8 Hz), 6.82 (d, 2H, J

= 8.8 Hz), 7.35 (s, 1H), 7.39 (d, 2H, J = 8.8 Hz), 7.53 (d, 2H, J = 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.07, 25.17, 54.95, 58.15, 64.40, 101.64, 114.70, 116.27, 117.33 (q, J = 274.9 Hz), 119.36 (q, J = 274.9 Hz), 121.52, 123.00, 128.73, 130.92, 131.19, 132.27, 135.42 (q, J = 37.8 Hz), 144.49 (q, J = 37.8 Hz), 149.47, 158.33, 158.56; HRMS (ESI) calcd for $C_{27}H_{24}F_6N_4O_2H$, 551.1882 ($M + H^+$); found, 551.1882.

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Supporting Information Available: Experimental description for ER binding affinity assay, gene transcriptional activity assay, protein purification and X-ray crystallography, molecular modeling, and characterization of compounds **10a**,**b**, **11b**−**d**, and **12b**−**d**; HPLC results and chromatograms for compounds **12a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kuiper, G. G.; Enmark, E.; Pelto-Huikko, M.; Nilsson, S.; Gustafsson, J. A. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5925−5930.

(2) Pettersson, K.; Gustafsson, J. A. Role of estrogen receptor beta in estrogen action. *Annu. Rev. Physiol.* **2001**, *63*, 165−192.

(3) Park, W. C.; Jordan, V. C. Selective estrogen receptor modulators (SERMS) and their roles in breast cancer prevention. *Trends Mol. Med.* **2002**, *8*, 82−88.

(4) Sato, M.; Grese, T. A.; Dodge, J. A.; Bryant, H. U.; Turner, C. H. Emerging therapies for the prevention or treatment of postmenopausal osteoporosis. *J. Med. Chem.* **1999**, *42*, 1−24.

(5) Fink, B. E.; Mortensen, D. S.; Stauffer, S. R.; Aron, Z. D.; Katzenellenbogen, J. A. Novel structural templates for estrogen-receptor ligands and prospects for combinatorial synthesis of estrogens. *Chem. Biol.* **1999**, *6*, 205−219.

(6) Mortensen, D. S.; Rodriguez, A. L.; Carlson, K. E.; Sun, J.; Katzenellenbogen, B. S.; et al. Synthesis and biological evaluation of a novel series of furans: ligands selective for estrogen receptor alpha. *J. Med. Chem.* **2001**, *44*, 3838−3848.

(7) Stauffer, S. R.; Huang, Y.; Coletta, C. J.; Tedesco, R.; Katzenellenbogen, J. A. Estrogen pyrazoles: defining the pyrazole core structure and the orientation of substituents in the ligand binding pocket of the estrogen receptor. *Bioorg. Med. Chem.* **2001**, *9*, 141−150.

(8) Zhou, H. B.; Comninos, J. S.; Stossi, F.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Synthesis and evaluation of estrogen receptor ligands with bridged oxabicyclic cores containing a diarylethylene motif: estrogen antagonists of unusual structure. *J. Med. Chem.* **2005**, *48*, 7261−7274.

(9) Palkowitz, A. D.; Glasebrook, A. L.; Thrasher, K. J.; Hauser, K. L.; Short, L. L.; et al. Discovery and synthesis of [6-hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-hydroxyphenyl)]benzo[b]thiophene: a novel, highly potent, selective estrogen receptor modulator. *J. Med. Chem.* **1997**, *40*, 1407−1416.

(10) Gruber, C.; Gruber, D. Bazedoxifene (Wyeth). *Curr. Opin. Invest. Drugs* **2004**, *5*, 1086−1093.

(11) Teo, C. C.; Kon, O. L.; Sim, K. Y.; Ng, S. C. Synthesis of 2-(*p*-chlorobenzyl)-3-aryl-6-methoxybenzofurans as selective ligands for antiestrogen-binding sites. Effects on cell proliferation and cholesterol synthesis. *J. Med. Chem.* **1992**, *35*, 1330−1339.

(12) De Angelis, M.; Stossi, F.; Carlson, K. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Indazole estrogens: highly selective ligands for the estrogen receptor beta. *J. Med. Chem.* **2005**, *48*, 1132−1144.

(13) Jorgensen, A. S.; Jacobsen, P.; Christiansen, L. B.; Bury, P. S.; Kanstrup, A.; et al. Synthesis and estrogen receptor binding affinities of novel pyrrolo[2,1,5-*cd*]indolizine derivatives. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2383−2386.

(14) Harris, H. A.; Albert, L. M.; Leathurby, Y.; Malamas, M. S.; Mewshaw, R. E.; et al. Evaluation of an estrogen receptor-beta agonist in animal models of human disease. *Endocrinology* **2003**, *144*, 4241−4249.

(15) Barlaam, B.; Bernstein, P.; Dantzman, C.; Warwick, P. Preparation of benzoxazoles and benzothiazoles as selective ligands for human b-estrogen receptor. PCT Int. Appl. (Astrazeneca AB, Sweden) WO, 2002; p 71.

(16) Vu, A. T.; Cohn, S. T.; Manas, E. S.; Harris, H. A.; Mewshaw, R. E. ERβ ligands. Part 4: Synthesis and structure−activity relationships of a series of 2-phenylquinoline derivatives. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4520−4525.

(17) Compton, D. R.; Carlson, K. E.; Katzenellenbogen, J. A. Pyrazolo-[1,5-*a*]pyrimidines as estrogen receptor ligands: defining the orientation of a novel heterocyclic core. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5681−5684.

(18) Compton, D. R.; Sheng, S.; Carlson, K. E.; Rebacz, N. A.; Lee, I. Y.; et al. Pyrazolo[1,5-*a*]pyrimidines: estrogen receptor ligands possessing estrogen receptor beta antagonist activity. *J. Med. Chem.* **2004**, *47*, 5872−5893.

(19) Kohno, H.; Bocchinfuso, W. P.; Gandini, O.; Curtis, S. W.; Korach, K. S. Mutational analysis of the estrogen receptor ligand-binding domain: influence of ligand structure and stereochemistry on transactivation. *J. Mol. Endocrinol.* **1996**, *16*, 277−285.

(20) Anstead, G. M.; Peterson, C. S.; Katzenellenbogen, J. A. Hydroxylated 2,3-diarylindenes: synthesis, estrogen receptor binding affinity, and binding orientation considerations. *J. Steroid Biochem.* **1989**, *33*, 877−887.

(21) Pike, A. C.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G.; et al. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *EMBO J.* **1999**, *18*, 4608−4618.

(22) Stauffer, S. R.; Coletta, C. J.; Tedesco, R.; Nishiguchi, G.; Carlson, K.; et al. Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists. *J. Med. Chem.* **2000**, *43*, 4934−4947.

(23) Banwell, M. G.; Flynn, B. L.; Stewart, S. G. Selective cleavage of isopropyl aryl ethers by aluminum trichloride. *J. Org. Chem.* **1998**, *63*, 9139−9144.

(24) Nettles, K. W.; Greene, G. L. Ligand control of coregulator recruitment to nuclear receptors. *Annu. Rev. Physiol.* **2005**, *67*, 309−333.

(25) Shiau, A. K.; Barstad, D.; Radek, J. T.; Meyers, M. J.; Nettles, K. W.; et al. Structural characterization of a subtype-selective ligand reveals a novel mode of estrogen receptor antagonism. *Nat. Struct. Biol.* **2002**, *9*, 359−364.

(26) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; et al. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **1998**, *95*, 927−937.

(27) Carlson, K. E.; Choi, I.; Gee, A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open pocket conformation is required for ligand interaction. *Biochemistry* **1997**, *36*, 14897−14905.

(28) Sun, J.; Meyers, M. J.; Fink, B. E.; Rajendran, R.; Katzenellenbogen, J. A.; et al. Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-alpha or estrogen receptor-beta. *Endocrinology* **1999**, *140*, 800−804.

(29) Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; et al. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **1997**, *389*, 753−758.

(30) Webb, P.; Nguyen, P.; Kushner, P. J. Differential SERM effects on corepressor binding dictate ERα activity in vivo. *J. Biol. Chem.* **2003**, *278*, 6912−6920.

(31) Stauffer, S. R.; Huang, Y. R.; Aron, Z. D.; Coletta, C. J.; Sun, J.; et al. Triarylpyrazoles with basic side chains: development of pyrazole-based estrogen receptor antagonists. *Bioorg. Med. Chem.* **2001**, *9*, 151−161.

(32) Huang, Y. R.; Katzenellenbogen, J. A. Regioselective synthesis of 1,3,5-triaryl-4-alkylpyrazoles: novel ligands for the estrogen receptor. *Org. Lett.* **2000**, *2*, 2833−2836.

(33) Sun, J.; Huang, Y. R.; Harrington, W. R.; Sheng, S.; Katzenellenbogen, J. A.; et al. Antagonists selective for estrogen receptor alpha. *Endocrinology* **2002**, *143*, 941−947.

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