Design and Synthesis of Aryl Diphenolic Azoles as Potent and Selective Estrogen Receptor-β Ligands

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New diphenolic azoles as highly selective estrogen receptor-β agonists are reported. The more potent and selective analogues of these series have comparable binding affinities for ERβ as the natural ligand 17β-estradiol but are >100-fold selective over ERα. Our design strategy not only followed a traditional SAR approach but also was supported by X-ray structures of ERβ cocrystallized with various ligands as well as molecular modeling studies. These strategies enabled us to take advantage of a single conservative residue substitution in the ligand-binding pocket, ERα Met_{421} → ERβ Ile_{373}, to optimize ERβ selectivity. The 7-position-substituted benzoxazoles (Table 5) were the most selective ligands of both azole series, with ERB-041 (**117**) being >200-fold selective for ERβ. The majority of ERβ selective agonists tested that were at least ~50-fold selective displayed a consistent in vivo profile: they were inactive in several models of classic estrogen action (uterotrophic, osteopenia, and vasomotor instability models) and yet were active in the HLA-B27 transgenic rat model of inflammatory bowel disease. These data suggest that ERβ-selective agonists are devoid of classic estrogenic effects and may offer a novel therapy to treat certain inflammatory conditions.

Introduction

Estrogens play an essential role in the growth, development, and homeostasis of a diverse range of tissues.[1] Estrogens exert their physiological role via estrogen receptors (ER), which function as ligand-activated transcriptional regulators.[2] A number of marketed products target estrogen receptors, such as oral contraceptives (e.g., 17α-ethynyl estradiol), hormone therapy agents (e.g., 17β-estradiol, conjugated equine estrogens), and breast cancer therapeutics (e.g., tamoxifen, fulvestrant).

The first discovered ER, now called ERα, was cloned in 1986[3] and was believed to mediate the effects of estrogens solely. However, in 1996, Gustafsson and coworkers discovered a second estrogen receptor during a search for novel nuclear receptors in a rat prostate cDNA library and named it ERβ.[4] The discovery of ERβ has caused considerable excitement within the scientific community and has provided the motivation to identify its physiological role in mediating estrogen action. Because the two ER isoforms exhibit overlapping but distinct tissue distribution patterns,[5] it appeared likely that an ERβ-selective ligand would exhibit a pharmacological profile that is different from that of nonselective estrogens such as 17β-estradiol. The fact that ERβ is widely expressed but not the dominant estrogen receptor in the uterus or breast tissues makes it a very attractive drug target.

Both estrogen receptors have distinct domains that are critical to transactivation, DNA binding, and hormone binding. ERα and ERβ have modest overall sequence identity, differing primarily in their N-terminus domains, with the sequences more conserved at the DNA (95% identity) and ligand-binding domains (LBD) (58% identity).[6] Despite the modest sequence identity, the overall structural differences in the ligand-binding pocket are rather small. The X-ray structure of the human ERβ LBD complexed to genistein[6] showed that there are two subtle amino acid differences in close proximity to the bound ligand: ERα Leu_{384} is replaced by ERβ Met_{336}, and ERα Met_{421} is replaced by ERβ Ile_{373}. Considering this small change in the ligand-binding cavity, it is not surprising that 17β-estradiol displays a similar affinity for both receptors.

Despite a large body of mapping studies, in vitro characterization studies, and the creation of knockout mice, the physiological role of ERβ has remained unclear until recently,[7] when the availability of selective ligands helped further the investigation of the physiological function of ERβ. ERβ-selective agonist ERB-041 (compound **117**, Table 5) was used to demonstrate that this receptor may be a useful target for inflammation,[7]

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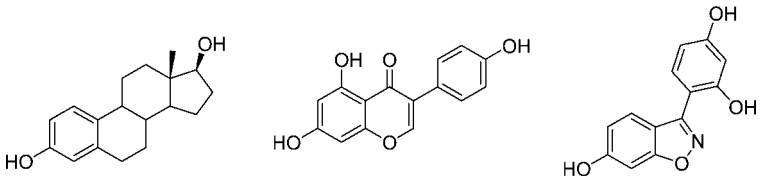
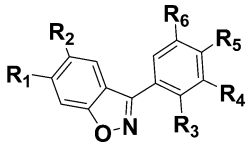


Figure 1.

Table 1. Phenyl Benzisoxazoles



compd	R_1	R_2	R_3	R_4	R_5	R_6	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
3	OH	H	OH	H	OH	H	3.5 ± 1.3	24 ± 8	8
34	H	H	OH	H	OH	H	718 ± 428	6100	8
35	OH	H	OH	H	H	H	254 ± 20	3000	12
36	OH	H	H	H	OH	H	54 ± 26	815 ± 207	15
37	H	OH	OH	H	OH	H	138 ± 75	4068 ± 1498	29
38	H	OH	H	H	OH	H	46 ± 21	819 ± 383	18
39	OH	H	H	Cl	OH	H	10 ± 2	273 ± 125	27
40	OH	H	CN	H	OH	H	12 ± 8	264 ± 53	23
41	OH	H	H	Br	OH	H	12 ± 1	183 ± 16	16
42	OH	H	H	F	OH	H	8 ± 2	161 ± 66	19
43	OH	H	H	Me	OH	H	31 ± 1	431 ± 30	14
44	OH	H	Me	H	OH	H	37 ± 6	475 ± 200	13
45	OH	H	propyl	H	OH	H	20	50	2
46	OH	H	H	propyl	OH	H	25 ± 14	210 ± 14	8
47	OH	H	OH	Me	OH	H	33 ± 21	511 ± 123	18
48	OH	H	CH_2CN	H	OH	H	96	717	7
49	OH	H	CH=NOH	H	OH	H	383	2390	6
50	OH	H	OH	H	OH	Br	1.8 ± 0.1	30 ± 16	17
51	OH	H	OH	H	OH	Cl	2 ± 0.1	33 ± 8	15
52	OH	H	OH	H	OH	Me	5 ± 0.1	65 ± 8	14
53	OH	H	ethyl	H	OH	propyl	3.6	13	4
54	OH	H	propyl	H	OH	propyl	3.1	22	7
1				17β-estradiol			3.6 ± 1.6	3.2 ± 1.0	1
2				genistein			10 ± 4	395 ± 181	41

[a] IC_{50} values are the means of at least two experiments ±SD (performed in triplicate, determined from eight concentrations). Values without SD are for a single determination only.

whereas other studies utilizing the ERα-selective agonist propylpyrazole triol (PPT) showed that many classical estrogenic effects are mediated primarily by ERα.[8]

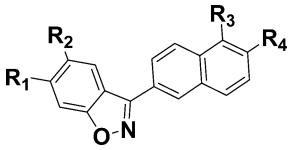
Several groups have investigated a variety of nonsteroidal scaffolds mimicking either the dihydroxyl arrangement of the nonselective estradiol or the moderately ERβ-selective phytoestrogen genistein as potential selective ERβ ligands. Although these studies have produced excellent potent ligands for both estrogen receptors, the generation of highly ERβ-selective ligands has proved to be quite challenging. Naturally occurring phytoestrogens and several modified analogues were reported to possess modest selectivity for the ERβ receptor (10−40-fold),[9] and one series of genistein analogues was claimed to exhibit impressive binding selectivity.[10] Diarylpropylnitriles (DPN),[11,12] biphenyl compounds,[9,13] and benzothiazoles/benzoxazoles[14,15] exhibited as much as ~70-fold selectivity for ERβ, whereas other scaffolds, for example, tetrahydrochrysenes (THC),[16−18] aryl benzothiophenes,[19] isoxazoles,[20,21] benzimidazoles,[22] triazines,[23] benzoxazines,[24] and tetrahydrofluorenones,[25] have been reported to be on the order of 10−40-fold ERβ selective.

In this paper, we report the design and synthesis of potent and selective ERβ ligands initially based on diphenolic benzisoxazole (**3)** (Figure 1). Compound **3** was identified through a competitive radioligand binding assay screen of our in-house sample collection. It had excellent potency for ERβ with an IC_{50} value of 3.5 nM, but it was minimally selective (8-fold) for ERβ (Table 1). The development of our ligands followed a traditional SAR approach and was supported by X-ray structures of ERβ cocrystallized with various ligands and molecular modeling studies to expedite the discovery of selective ligands. The finer details of these crystallographic and modeling studies will be reported elsewhere.[26] We have primarily concentrated our efforts on the α face of the ERβ LBD binding pocket, utilizing only one of the amino acid differences (ERα Met_{421} → ERβ Ile_{373}) observed when comparing the binding pocket residues of the two ER isoforms.

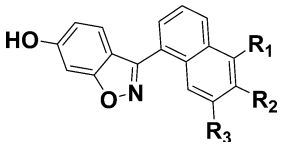
Chemistry

Compounds shown in Tables 1−5 were synthesized according to general synthetic procedures (Schemes 1−5). The benzisoxazoles in Tables 1 and 2 were

Table 2. Naphthyl Benzisoxazoles



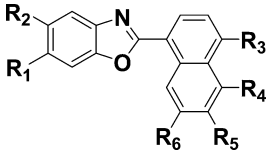
compd	R_1	R_2	R_3	R_4	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
55	OH	H	H	OH	1.4 ± 0.3	8 ± 4	6
56	OH	H	OH	H	20 ± 12	24 ± 12	1
57	OH	H	Br	OH	87 ± 13	360 ± 5	4
58	H	OH	H	OH	112	463	4



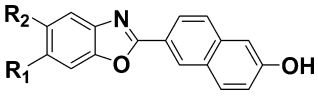
compd	R_1	R_2	R_3	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
59	H	OH	H	17	49	3
60	OH	H	H	1.5	6	4
61	H	H	OH	879 ± 499	1727 ± 45	2

[a] IC_{50} values are the means of at least two experiments ±SD (performed in triplicate, determined from eight concentrations). Values without SD are for a single determination only.

Table 3. Naphthyl Benzoxazoles



compd	R_1	R_2	R_3	R_4	R_5	R_6	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
62	OH	H	H	OH	H	H	5 ± 2	117 ± 26	23
63	OH	H	H	H	OH	H	3 ± 0.1	35 ± 5	12
64	OH	H	OH	H	H	H	1530	1900	1
65	OH	H	H	H	H	H	1050	1880	2
66	OH	H	H	Br	OH	H	134	373	3
67	OH	F	H	H	OH	H	46	434	10
68	H	OH	H	H	OH	H	15 ± 7	153 ± 30	10
69	OH	H	H	OH	H	Me	6 ± 5	163 ± 25	26

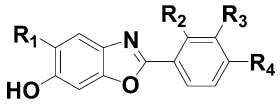


compd	R_1	R_2	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
70	H	OH	521 ± 369	3800 ± 3649	7
71	OH	H	533	780	2

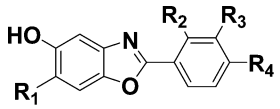
[a] IC_{50} values are the means of at least two experiments ±SD (performed in triplicate, determined from eight concentrations). Values without SD are for a single determination only.

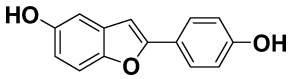
prepared via the general routes depicted in Schemes 1−3. In Scheme 1, two synthetic routes were used to produce benzisoxazole **8b**, using a common intermediate, **6**. Arylbromide **4** was first treated with *n*-butyllithium and then with an appropriately substituted benzaldehyde **5** to produce the addition product, which was oxidized with either manganese dioxide or chromic acid to afford benzophenone **6**. In route a, acetone oxime was treated with potassium *tert*-butoxide, and then benzophenone **6** was added to the mixture to produce oxime **7**. The cyclization of **7** to benzisoxazole **8a** was accomplished under acidic conditions with 5% hydrochloric acid in acetonitrile. The demethylation of **8a** with either boron tribromide or a mixture of hydriodic acid, acetic anhydride, and acetic acid afforded benzisoxazole **8b**. In route b, benzisoxazole **8b** was obtained via a more direct approach, where benzophenone **6** was treated with hydroxylamine and sodium hydride in *N*,*N*-dimethylformamide to produce **8a**, which upon treatment with boron tribromide afforded **8b**. In Scheme 2, benzophenone **11a** was prepared from benzoyl chloride **9** and 1,4-dimethoxybenzene **10** in the presence of aluminum chloride and 1,2-dichloroethane. The demethylation of **11a** with pyridine hydrochloride at high temperatures (200 °C) produced **11b**. Benzophenone **11b** was converted to benzisoxazole **13** in two steps. First, oxime formation (**12**) was accomplished with hydroxylamine in ethanol, and second, dehydration of **12** with diethylazodicarboxylate and triphenyl phosphine furnished benzisoxazole **13**. Dialkyl analogues **53** and **54** (Table 1) were prepared according to Scheme 3. The treatment of **14a** (prepared from the corresponding phenol and chloromethyl methyl ether/NaH) first with *tert*-butyllithium and second with an appropriately substituted benzaldehyde **14b** produced alcohol **15**. The dehydroxylation of **15** with triethylsilane in the presence of trifluoroacetic acid furnished phenol **16a**. The protection of phenol **16a** with iodomethane in the presence of sodium hydride afforded the corresponding anisole **16b**, which upon treatment with *N*-bromosuccinimide in acetonitrile produced bromide **17**. The conversion of **17** to benzisoxazole **19** was accomplished according to Scheme 1. The benzoxazoles of Tables 3 and 4 were prepared according to Scheme 4. Dimethoxyaniline **20** was treated with either benzoyl or naphthoyl chloride **21** in the presence of triethylamine to produce amide **22**, which was converted to benzoxazole **23** upon treatment with pyridine hydrochloride at high temperatures (200 °C). Bromo analogue **66** (Table 3) was prepared from **63** upon treatment with bromine in acetic acid. The 7-position-substituted benzoxazoles (Table 5) were prepared according to Scheme 5. Nitrophenol **24** was first brominated with Br_2/NaOAc in acetic acid and then reduced with H_2/Ra−Ni in EtOAc to afford aniline **25b**. The coupling of **25b** with appropriately substituted benzoyl chloride **26** in the presence of pyridine produced amide ester **27**. The conversion of **27** to benzoxazole **28** was accomplished under acidic conditions (*p*-toluenesulfonic acid) at high temperature (150 °C). The demethylation of **28** with boron tribromide afforded the diphenolic benzoxazole **29**. The palladium-catalyzed cross-coupling reaction[27,28] of benzoxazole **29** with alkyl stannates or aryl boronic acids produced benzoxazoles **30a** and **30b**. 2-Fluorovinyl analogue **31a** was prepared from **30a** (R_2 = vinyl) by the initial formation of the 1,2-bromofluoroethane adduct of the vinyl group with hydrogen fluoride−pyridine and 1,3-dibromo-5,5-dimethyl hydantoin in sulfolane and subsequent hydrogen bromide elimination with DBU.[29] 2-Bromovinyl analogue **31b** was prepared in three steps from **28** upon vinylation of the 7-position of the benzoxazole nucleus, boron tribromide treatment (resulted in demethylation of the methoxy groups and the bromination of the vinyl group, affording1,2-dibromoethane), and hydrogen bro-

Table 4. 5- and 6-Hyxdroxy-2-Phenyl Benzoxazoles



compd	R_1	R_2	R_3	R_4	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
72	H	OH	H	OH	3 ± 1	82 ± 180	26
73	H	H	H	OH	50 ± 15	902 ± 444	18
74	H	OH	OH	H	181 ± 97	2353 ± 536	13
75	H	H	OH	OH	105 ± 25	2410 ± 523	20
76	H	H	F	OH	39 ± 10	8430 ± 168	22
77	H	H	Cl	OH	703	5000	7
78	Cl	H	H	OH	157 ± 11	2765 ± 7	18
79	H	H	CMe_3	OH	1600	5000	3
80	H	H	O−*n*-C_4H_9	OH	3660	6240	2



compd	R_1	R_2	R_3	R_4	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
81	H	H	H	OH	49 ± 14	1227 ± 533	25
82	H	H	F	OH	66 ± 37	1570 ± 537	24
83	H	H	Cl	OH	239 ± 15	5280 ± 1131	22
84	H	Cl	H	OH	59 ± 62	139 ± 42	2
85	H	OH	H	OH	25	190	8
86	Cl	H	H	OH	16 ± 5	464 ± 86	30
87	Cl	H	F	OH	64 ± 11	1813 ± 206	29
88	Br	H	F	OH	42 ± 10	1210 ± 289	29
89	H	OH	OH	H	963 ± 194	5110	5
90					6 ± 2.4	176 ± 76	29

[a] IC_{50} values are the means of at least two experiments ±SD (performed in triplicate, determined from eight concentrations). Values without SD are for a single determination only.

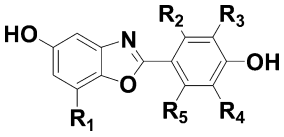
mide elimination by DBU. Methoxy analogue **30c** was prepared by the displacement of the bromine of **28** with NaOMe in the presence of CuBr. Ethynyl **32a**, cyano **32b**, and alkyl analogues **32c** were prepared from **28** via a palladium-catalyzed cross-coupling reaction using ethynyl(trimethyl)silane, $Zn(CN)_2$, or alkyl zinc chlorides (Rieke reaction),[30] respectively, and subsequent demethylation with BBr_3. Cyano analogue **32b** was also prepared by the displacement of the bromine of **29** with CuCN. Metal halogen exchange of **28** with *n*-BuLi followed by acetone addition and subsequent treatment with pyridine hydrochloride at high temperature (200 °C) produced benzoxazole **32e.** The reduction of **32e** with H_2/Pd−C furnished isopropyl analogue **32f**. 7-Lithiated benzoxazole **28** was also treated with various electrophiles (i.e., EtI, PhMeNCHO, CN−CO_2R_4) to produce analogue **32d**. Compounds **100**−**102** (Table 5) were successively prepared by standard synthetic protocols from **103** upon reduction ($NaBH_4$, MeOH), bromination (BBr_3, CH_2Cl_2), and nitrile formation (KCN, 18-C-6, DMF). Bromo analogues **135** and **136** (Table 5) were prepared from **117** upon bromination with bromine in acetic acid, whereas bromo analogues **137** and **138** were prepared from **91** upon treatment with *N*-bromosuccinimide in acetonitrile.

Results and Discussion

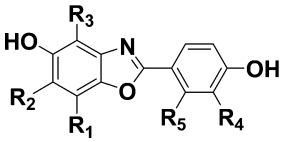
The primary screening assay for the program was a competitive radioligand binding assay,[31] which was used to determine the relative binding affinity (IC_{50}) of compounds for the human ERα and ERβ LBD. Selected compounds were also evaluated using mouse and rat LBDs as well as full-length human receptors. These data are presented in Tables 1−7. As expected in these assays, radioinert 17β-estradiol bound equally well to ERα and ERβ.

Benzisoxazole Analogues. High-throughput screening-hit benzisoxazole **3** (Figure 1) was successfully cocrystallized with ERβ (Figure 2a). As shown in Figure 2a and discussed above, there are only two conservative amino acid differences among the residues closest to the ligand, ERβ Met_{336} → ERα Leu_{384} and ERβ Ile_{373} → ERα Met_{421}. Benzisoxazole **3** occupies the ligand-binding cavity in an orientation where the hydroxyl group of the benzisoxazole nucleus interacts with the receptor via a hydrogen-bonding network involving the side chains of Glu_{305} and Arg_{346} and a buried water molecule, whereas the 4′-hydroxyl group of the resorcinol nucleus extends to the distal end of the cavity making a hydrogen-bond interaction with His_{475}. Both of these hydroxyl groups are important to the binding affinity of the compound because the elimination of either hydroxyl group (examples **34**, **35**; Table 1) proved to be detrimental to the compound's potency. However, the benzisoxazole hydroxyl group appears to be more important to the ligand binding than the 4′-hydroxyl group of the resorcinol, given that **34** was 3× less potent than **35**. This is consistent with the placement of **3** in the electron

Table 5. 7-Substituted 2-Phenyl Benzoxazoles



compd	R_1	R_2	R_3	R_4	R_5	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
91	OMe	H	H	H	H	59 ± 19	2557 ± 1618	43
92	Br	H	H	H	H	2 ± 1	155 ± 47	68
93	Br	H	H	F	H	3 ± 1	260 ± 93	81
94	Br	H	H	CF_3	H	166	1870	11
95	Br	H	H	H	F	1.4 ± 0.4	47 ± 12	32
96	Br	H	H	H	CH_3	1.4 ±1.0	44 ± 12	32
97	CN	H	H	H	H	6 ± 2	411 ± 131	72
98	CN	H	H	F	H	26 ± 11	1435 ± 92	56
99	CN	H	H	H	F	2.4 ± 0	138 ± 5	57
100	CH_2Br	H	H	H	H	40 ± 29	2975 ± 2298	74
101	CH_2CN	H	H	H	H	1040 ± 1408	>5000	>5
102	CH_2OH	H	H	H	H	1340	*b*	
103	CHO	H	H	H	H	59 ± 22	2638 ± 519	45
104	CO_2Me	H	H	H	H	356	>5000	>14
105	CO_2Et	H	H	H	H	190 ± 88	7827 ± 3427	41
106	$CONH_2$	H	H	H	H	95 ± 40	9620	101
107	CO_2H	H	H	H	H	>5000	>5000	
108	ethyl	H	H	H	H	13 ± 6	537 ± 81	40
109	propyl	H	H	H	H	11 ± 6	390 ± 25	37
110	isopropyl	H	H	H	H	82	1200	15
111	butyl	H	H	H	H	79	498	6
112	ethynyl	H	H	H	H	15 ± 3	481 ± 133	33
113	allyl	H	H	H	H	13 ± 5	727 ± 356	55
114	allyl	H	H	F	H	14 ± 10	1100 ± 544	78
115	allyl	H	H	H	F	3 ± 1	98 ± 38	39
116	vinyl	H	H	H	H	3.5 ± 1.7	447 ± 226	129
117 (ERB-041)	vinyl	H	H	F	H	5.0 ± 4	1216 ± 688	226
118	2-F-vinyl	H	H	F	H	3.2 ± 2.2	376 ± 201	116
119	2-Me-vinyl	H	H	H	H	142 ± 92	775 ± 208	6
120	2-Br-vinyl	H	H	H	H	45	462	10
121	2-Br-vinyl	H	H	H	F	16	196	12
122	3-Me-vinyl	H	H	H	F	23 ± 6	539 ± 179	23
123	vinyl	H	H	H	F	1.9 ± 0.6	227 ± 107	123
124	vinyl	H	H	F	F	3.7 ± 0.6	474 ± 211	127
125	vinyl	F	H	H	F	2.2 ± 1.1	249 ± 76	115
126	vinyl	H	F	H	F	66 ± 23	4040	62
127	vinyl	H	F	F	H	201 ± 125	>10 000	>50
128	vinyl	H	H	H	CH_3	27 ± 30	1116 ± 1556	41
129	phenyl	H	H	H	H	235	1300	6
130	2-furyl	H	H	H	H	135	809	6
131	2-furyl	H	H	F	H	313	1980	6
132	2-thienyl	H	H	H	H	97	1030	11
133	2-thiazole	H	H	H	H	366	1340	4
134	cyclopentane	H	H	H	H	102	1010	10

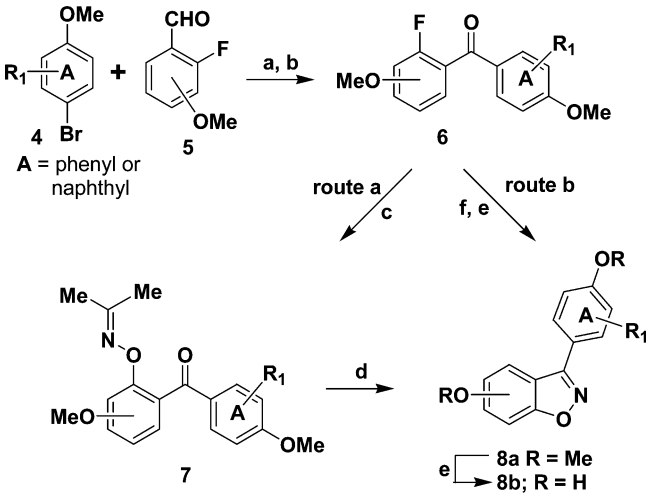


compd	R_1	R_2	R_3	R_4	R_5	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
135	vinyl	H	Br	F	H	25 ± 13	1036 ± 488	41
136	vinyl	Br	Br	F	H	155	803	5
137	OMe	H	Br	F	H	52 ± 30	2668 ± 1172	52
138	OMe	Br	Br	F	H	64	559	9

[a] IC_{50} values are the means of at least two experiments ±SD (performed in triplicate, determined from eight concentrations). Values without SD are for a single determination only. [b] not tested.

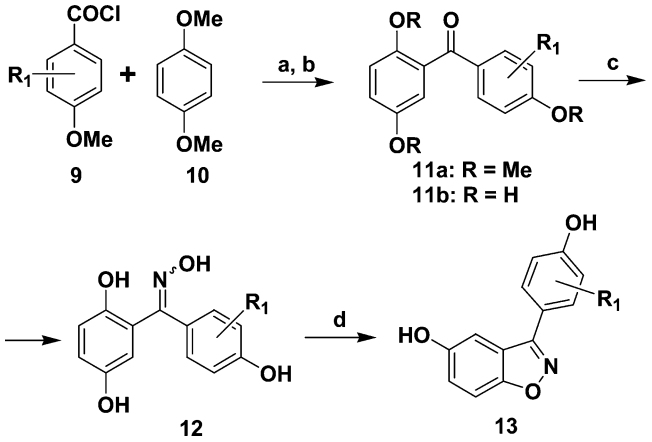
density shown in Figure 2b, with the benzisoxazole hydroxyl mimicking the A-ring hydroxyl of 17β-estradiol[32] as described above. The elimination of the 2′-hydroxyl of the resorcinol also affected the potency of **3** but to a lesser extent (**3** vs **36**). This hydroxyl appears to participate in an intramolecular hydrogen-bonding interaction with the nitrogen of the benzisoxazole nucleus, rendering planarity to the molecule. This intramolecular hydrogen bond is thus likely to act in a manner similar to that of genistein, improving the

Scheme 1[a]



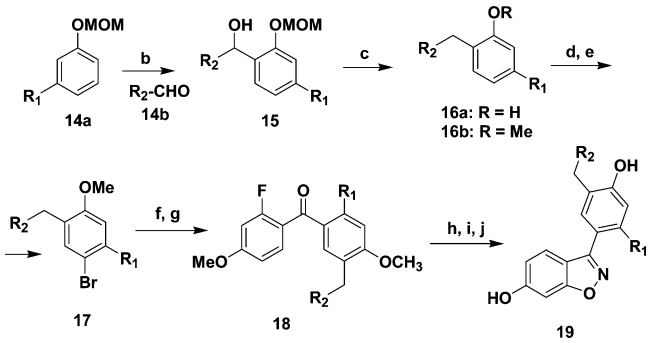
[a] Reagents: (a) *n*-BuLi, THF; (b) MnO_2 or CrO_3, H_2SO_4; (c) acetone oxime, *tert*-BuOK; (d) HCl, MeCN; (e) BBr_3 or HI, AcOH, Ac_2O; (f) NaH, $NH_2OH{\cdot}HCl$.

Scheme 2[a]



[a] Reagents: (a) $AlCl_3$, $ClCH_2CH_2Cl$; (b) pyridine·HCl; (c) $NH_2OH{\cdot}HCl$, EtOH; (d) diethylazodicarboxylate, PPh_3, THF.

Scheme 3[a]

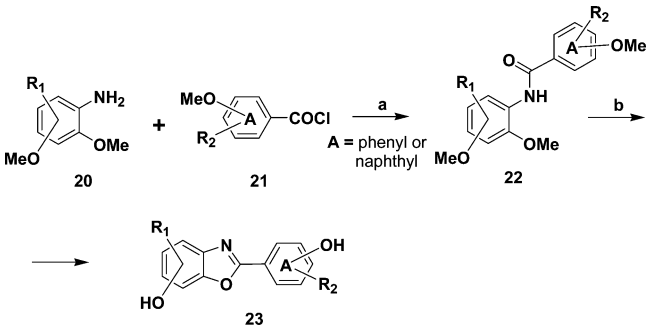


[a] Reagents: (a) MOM−Cl, Et_3N; (b) *t*-BuLi, R_2CHO; (c) Et_3SiH, TFA; (d) NaH, MeI; (e) *N*-bromosuccimide, CH_2Cl_2; (f) *n*-BuLi, 2-F, 4-OMe-benzaldehyde, THF; (g) MnO_2, $CHCl_3$; (h) acetone oxime, *tert*-BuOK; (i) HCl, MeCN; (j) BBr_3, CH_2Cl_2.

potency by increasing the effective lipophilicity. Regioisomeric 5-hydroxy analogues **37** and **38** were more selective for ERβ but less potent.

The examination of the ERβ complex with **3** suggested that the introduction of groups at positions 2′ and 3′of the resorcinol nucleus can be directed toward the α face of the ERβ LBD binding pocket, exploiting the ERα

Scheme 4[a]



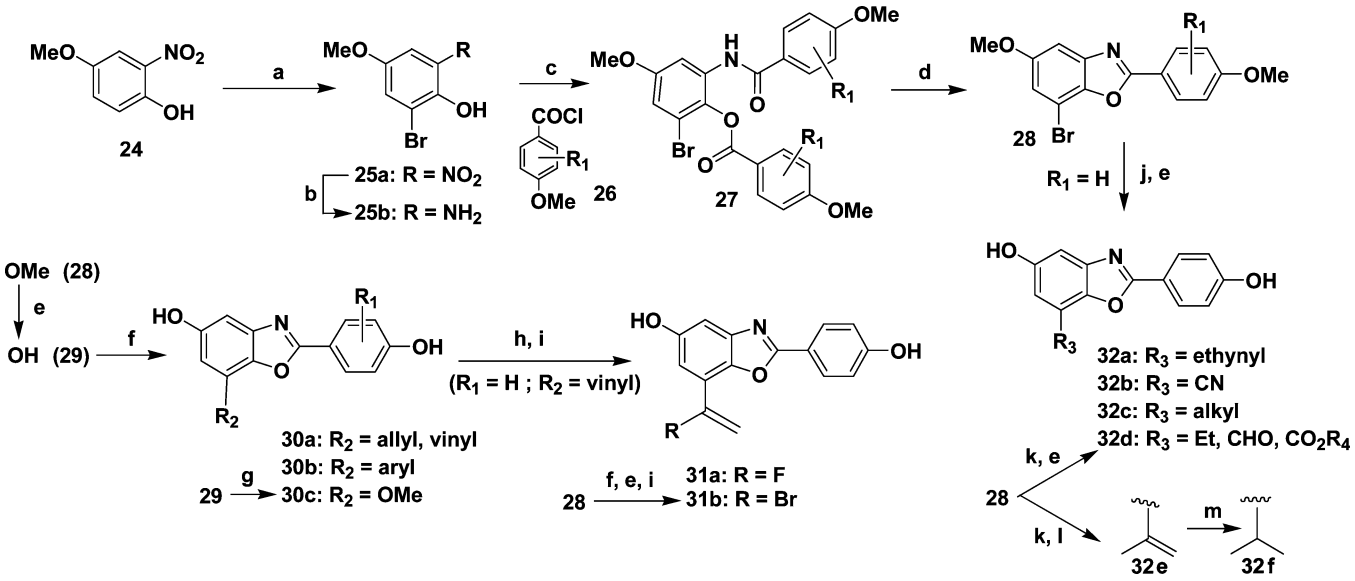
[a] Reagents: (a) Et_3N, CH_2Cl_2; (b) pyridine−HCl, 200 °C.

Met_{421} → ERβ Ile_{373} residue substitution to achieve greater ERβ selectivity. To confirm this hypothesis, we prepared 3′-chloro analogue **39** and 2′-cyano analogue **40** (their fit to the pocket was confirmed by docking calculations), and both were found to be more selective than **3** (27- and 23-fold selective for ERβ, respectively). These improvements in selectivity are the result of an ∼11-fold decrease in affinity for ERα versus an ∼3-fold decrease in affinity for ERβ in both cases. In comparison, various other groups (entries **41**−**49**) exhibited similar or reduced ERβ potency and, in most cases, smaller improvements in selectivity compared to **3**. The introduction of similar groups at the 5′-position of the resorcinol nucleus in compounds **50**−**52** resulted in compounds similar to **3** in potency but only about 2-fold more selective. Disubstituted alkyl analogues **53** and **54** were similar to **3**.

Because the A−C ring hydroxyl−hydroxyl distance of **3** is only 10.6 Å compared to the 12-Å distance for genistein, we attempted to extend this distance by replacing the phenyl with a naphthalene to gain more optimal hydrogen-bonding interactions at both ends of the cavity. In addition, because the majority of the ERβ ligand-binding pocket is hydrophobic in nature, by increasing the ligand size and lipophilicity it was quite reasonable to expect an enhancement in ligand affinity. In fact, bulkier naphthyl analogue **55** (Table 2) had an IC_{50} value of 1.4 nM for ERβ. However, this compound was only 8-fold selective for ERβ. 5′-Hydroxyl analogue **56** was 15-fold weaker than **55**, whereas bromo analogue **57** and 5-hydroxyl benzisoxazole **58** were almost 60-fold weaker than **55**. Regioisomeric 1′-naphthalene benzoxazole **59** (Table 2) was 10× less potent than **58**, whereas 5′-hydroxyl analogue **60** was similar to **55**. 7′-Hydroxyl analogue **61** was about 600-fold weaker than **60**. Even though both the phenyl and naphthyl benzisoxazoles have produced ligands with excellent affinity for the ERβ receptor, the selectivity against the ERα receptor was only modest (10−30-fold).

Our intention was that extension of the phenyl to a naphthalene would preserve the benzisoxazole as the A−B ring, allowing us to use naphthalene substituents to modulate selectivity. Although docking calculations suggested that this was a reasonable outcome, the larger size of **55** relative to that of genistein made it difficult to rank the order of the potential binding modes. Thus, to test the above hypothesis and to help validate our docking calculations, **55** was cocrystallized with ERβ. As can be seen in Figure 3, the naphthalene now acts as the A−B ring, and the only substitutable position in

Scheme 5[a]



[a] Reagents: (a) Br_2, NaOAC, ACOH; (b) H_2, Ra−Ni, THF; (c) aroyl-chloride (26), pyridine, CH_2Cl_2; (d) *p*-toluenesulfonic acid, *p*-xylene, 150 °C; (e) BBr_3, CH_2Cl_2; (f) R_2-stannyltributyl, $[P(o\text{-tolyl})_3]_2PdCl_2$, diethoxyethane or $R_2-B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene; (g) MeONa, CuBr, DMF; (h) pyridine·HF, 1,3-dibromo-5,5-dimethyl hydantoin, sulfolane; (i) DBU, CH_3CN; (j) ethynyl(trimethyl)silane, $Pd(PPh_3)_4$, CuI, Et_3N or $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF or R_3-ZnCl, $P(o\text{-tolyl})_3]_2PdCl_2$, THF; (k) *n*-BuLi, acetone or $CNCO_2-R_4$ or EtI or PhMeNCHO; (l) pyridine·HCl; (m) H_2, 10% Pd−C.

Table 6. Binding Affinity (IC_{50}) of Selected Compounds for Rat and Mouse ERβ and ERα LBD

	rat			mouse		
compd	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
36	36 ± 12	716 ± 76	19	13 ± 2.1	1089 ± 603	80
73	20 ± 4.9	821 ± 150	40	9.5 ± 2	1335 ± 550	140
81	22 ± 6	1295 ± 189	57	9.9 ± 2	1629 ± 521	164
92	1.2 ± 0.8	132 ± 17	109	1.7 ± 1	132 ± 6.9	78
93	2 ± 0.8	171 ± 17	85	2.7 ± 1.7	199 ± 71	74
97	4 ± 0.4	362 ± 35	89	4.3 ± 0.7	423 ± 29	98
103	29 ± 7.5	2252 ± 27	76	16.5 ± 5	2601 ± 565	158
117 (ERB-041)	3.14 ± 2.1	618 ± 72	197	3.7 ± 4.0	746 ± 303	200
17β-estradiol	1.7 ± 0.5	1.9 ± 0.4	1	2.3 ± 0.7	2.2 ± 0.5	1

[a] IC_{50} values are the means of at least two experiments ±SD (performed in triplicate, determined from eight concentrations).

Table 7. Binding Affinity (IC_{50}) of Selected Compounds for Human Full-Length ERα and ERβ

compd	ERβ IC_{50} (nM)[a]	ERα IC_{50} (nM)[a]	fold selectivity for ERβ
91	52 ± 16	4124 ± 423	79
92	2 ± 0.43	187 ± 63	94
93	4.4 ± 2.2	315 ± 54	71
97	5.3 ± 0.7	671 ± 258	127
98	18 ± 7	1970 ± 459	109
99	2.9 ± 0.9	253 ± 52	87
103	60 ± 3	3314 ± 892	55
106	93 ± 45	>10 000	>107
113	15 ± 7.3	1455 ± 170	99
117 (ERB-041)	2.8 ± 1.5	2572 ± 646	920
118	6.3 ± 1.4	690 ± 182	110
124	3.1 ± 1.4	412 ± 88	133
137	82 ± 9	4480 ± 1131	55
17β-estradiol	2.6 ± 0.8	3.5 ± 1.2	1

[a] IC_{50} values are the means of at least two experiments ±SD (performed in triplicate, determined from eight concentrations).

proximity to ERα Met_{421}/ERβ Ile_{373} is the 7-position of the benzisoxazole. Unfortunately, it appeared to us that substituents introduced at this position to enhance ERβ selectivity would also tend to have unfavorable interactions with His_{475}, thereby lowering ERβ affinity. Given this observation, we decided to turn our attention to more promising strategies. The observation that benzisoxazole can occupy either end of the cavity when comparing **3** (Figure 2) with **55** (Figure 3) is similar to what we report below for the benzoxazole series, and thus we defer an explanation for this behavior to the following section.

Benzoxazole Analogues. A logical progression in the SAR study was to explore the regioisomeric benzoxazoles rather than the benzisoxazoles. In addition, docking studies suggested that 2-phenyl- and 2-naphthyl-benzoxazoles would provide a greater opportunity to access ERα Met_{421}/ERβ Ile_{373}. Regioisomeric 6-hydroxylnaphthyl-benzoxazole **62** (Table 3) of benzisoxazole **60** showed a 3-fold decrease of ERβ potency but was found to be 6× more selective for ERβ. 6′-Hydroxyl analogue **63** was slightly more potent in ERβ but with weaker selectivity (12-fold) against ERα. 4′-Hydroxyl analogues **64** were 500-fold weaker than **62**. Halogen analogues **60** and **67** were also weaker, as was 5′-hydroxyl naphthyl-benzoxazole **68**.

Docking studies with **62**, later confirmed by cocrystallization with ERβ (Figure 4), suggested that substitu-

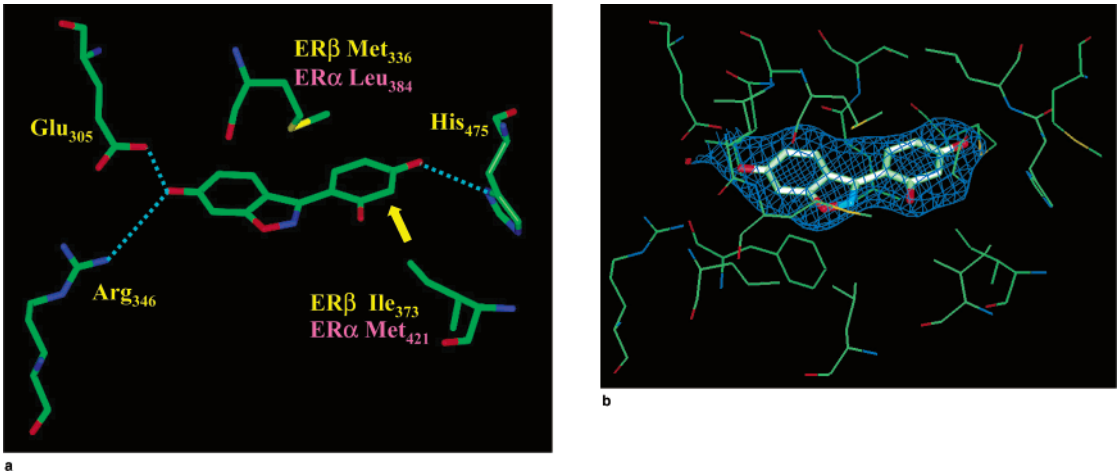


Figure 2. (a) Schematic representation of **3** cocrystallized with ERβ, showing key interactions as well as opportunities to improve ERβ selectivity. (b) Unbiased $2f_o-f_c$ map contoured at σ, showing the electron density for **3** complexed with ERβ.

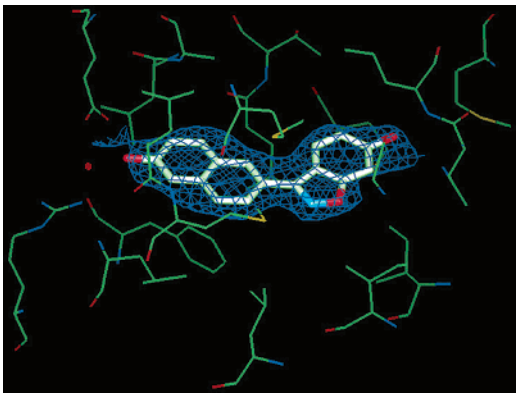


Figure 3. Unbiased $2f_o-f_c$ map contoured at σ, showing the electron density for **55** complexed with ERβ.

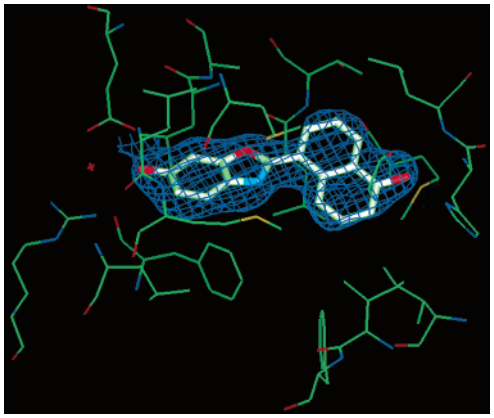


Figure 4. Unbiased $2f_o-f_c$ map contoured at σ, showing the electron density for **62** complexed with ERβ.

tion at the 7′-position of the naphthalene would provide the best access to ERα Met_{421}/ERβ Ile_{373} to enhance ERβ selectivity. This binding mode shown in Figure 4 is in contrast to the binding mode of naphthyl benzisoxazole **55**, where the naphthalene acts as the A−B ring and occupies the opposite end of the cavity. (See Figure 3 and Discussion below.) Even so, the naphthalene moiety of **62** already fills the pocket near ERα Met_{421}/ERβ Ile_{373} so well that there is minimal room left to explore the remainder of the pocket with functional groups. Unfortunately, although the more lipophilic 7′-methyl analogue **69** retained the ERβ potency of **62**, the ERβ selectivity did not improve significantly. Regioisomeric 2′-naphthalene benzoxazole analogues **70** and **71** were only weakly active (IC_{50} about 500 nM). Similar to the naphthyl-benzisoxazoles, the naphthyl-benzoxazole ligand affinities were also strongly dependent on the dihydroxyl substitution pattern. Small regiosomeric hydroxyl modifications resulted in wide range of potencies, consistent with the critical hydroxyl-mediated anchoring of dihydroxyl ligands to the ER's binding cavity shown in Figure 4.

Regioisomeric 6-hydroxyl benzoxazole **72** (Table 4), an analogue of benzisoxazole **3**, showed similar ERβ potency and increased ERβ selectivity (26-fold). The elimination of the 2′-hydroxyl of the resorcinol nucleus (**73**) resulted in the 15-fold decrease of ERβ potency, similar to that of benzisoxazole **36**. Several other hydroxyl regioisomers (**74** and **75**) as well as halogen-substituted analogues (**76**−**78**) were also found to have a weaker affinity for ERβ. Bulky substituents (**79** and **80**) next to the phenolic hydroxyl were detrimental to the potency. As one might expect, the regioisomeric 5-hydroxyl benzoxazoles (**81**−**89**) exhibited a similar SAR pattern to that of the 6-hydroxyl benzoxazoles.

The docking of benzoxazole **81** to the X-ray crystal structure of benzofuran **90**[26] (Table 4) bound to ERβ revealed a nearly perfect superimposition of these two structures (Figure 5). Interestingly, the phenol of **81** is predicted to act as the A ring, in contrast to the orientation of 2-naphthylbenzoxazole **62** (Figure 4), where the benzoxazole acts as the A ring. Similar changes in the binding mode are observed when comparing **81** to benzisoxazole **3** (Figure 2) and, as pointed out above, when comparing benzoxazole **62** to benzisoxazole **55** (Figure 3) and benzisoxazoles **3** and **55** with each other. Clearly, this is a somewhat general phenomenon, which deserves a brief explanation. These "flipping" effects appear to result primarily from interactions between the hydrophobic scaffold and the binding pocket as well as the relative geometric orientation of the hydroxyl groups, both of which in turn affect the way these hydroxyl groups are presented to key hydrogen-bonding residues Glu_{305}, Arg_{346}, and His_{475}. This is consistent with the fact that docking calculations, in conjunction with a molecular mechanics evaluation of the potential binding modes, are generally quite predic-

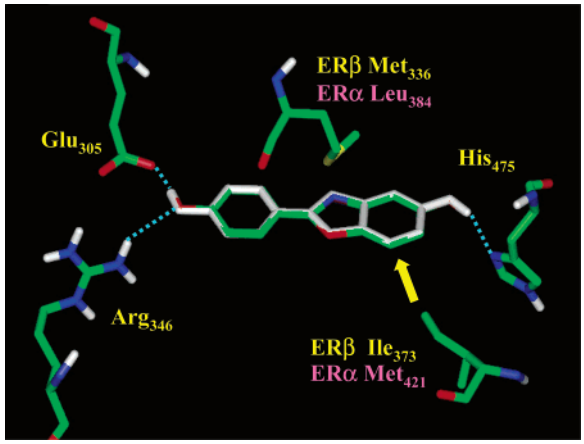


Figure 5. Compound **81** docked to the binding pocket of ERβ complexed with **90**.[26] Only key residues as well as both ligands are shown. Compound **90** is colored white. All other atoms are colored by atom type.

tive of the X-ray binding modes of these ERβ ligands. The docking calculations on ER tend to be less predictive mainly with larger ligands such as **55**, where the flexibility of the protein is more likely to play an important role in determining the true binding mode.

The examination of docked benzoxazole **81** and the X-ray binding mode of **90** revealed that substitutions at the 7-position offered an opportunity to improve the ERβ selectivity of the phenyl−benzoxazole scaffold by targeting the ERα Met_{421} → ERβ Ile_{373} residue substitution. In addition, there appeared to be more unoccupied space in the pocket compared to that of the naphthyl benzoxazoles. Therefore, we felt that the 7-position of the phenyl−benzoxazole scaffold would be ideal to explore with diverse functional groups to enhance the ERβ potency and selectivity.

The docking calculations were used to assist in the selection of functional groups. In addition, we hypothesized that electron-rich and/or chemically hard groups (nitrogen (e.g., nitrile), oxygen (e.g., carbonyl), halogens lighter than iodine, and alkenes) would have a greater likelihood of differentiating between ERα Met_{421} and ERβ Ile_{373} given the electronegative and polarizable nature of the methionine sulfur atom. A detailed justification of this hypothesis, as well as the optimization of the related phenyl−benzofuran scaffold 7-position, is beyond the scope of this paper and will be elaborated elsewhere.[26]

The introduction of a methoxy group (**91**, Table 5) at the 7-position of the benzoxazole nucleus resulted in a small improvement of the selectivity (43-fold) without affecting the ERβ potency. However, the introduction of a bromo substituent (**92**) resulted in a marked increase in ERβ potency (IC_{50} = 2 nM) and a significant improvement in ERβ selectivity (68-fold). The small fluorine group ortho to the A-ring hydroxyl (entry **93**) did not alter the potency or selectivity of the 7-bromo analogue, whereas the bulkier trifluoromethoxy group (entry **94**) noticeably decreased ligand binding. This finding is not surprising because this hydroxyl participates in key interactions with the Glu_{305} and Arg_{346} residues, which may have been adversely affected by the bulkier trifluoromethoxy group. Substitution at the meta position of phenol (F, CH_3) resulted in a small increase in potency and about a 2-fold loss of selectivity (entries **95** and **96**). 7-Cyano analogues **97** and **99** exhibited similar potency and selectivity to that of 7-bromo analogues **92** and **95**, whereas ortho-substituted fluoro analogue **98** maintained its selectivity but lost about 8-fold in ERβ potency (**98** vs **93**). Methyl bromide **100** showed similar selectivity to that of **92** but had a 20-fold reduction in potency, whereas acetonitrile **101** was weakly active in ERβ. The carbonyl class of substituents (**103**−**106**) proved to be much weaker for both ERs but displayed moderate to good levels of ERβ selectivity. Amide analogue **106** was about 100-fold selective for ERβ. The ethyl and propyl substituents (**108** and **109**) exhibited small decreases in potency and selectivity relative to **92**, whereas the bulkier isopropyl and butyl groups (**110**, **111**) had noticeably weaker binding affinity for ERβ. The ethynyl and allyl groups (**112**, **113**) were similar to the ethyl and propyl groups with respect to potency and selectivity. However, the incorporation of a vinyl group (**116**) increased the selectivity to >100-fold in favor of ERβ. Introducing fluorine ortho to the hydroxyl group produced **117** (ERB-041), which showed a somewhat greater selectivity (226-fold). 2-Fluorovinyl analogue **118** was equipotent to **117** but 2-fold less selective, whereas the bulkier 2-bromovinyl and 2-methylvinyl analogues (**119**−**122**) were substantially less potent and selective for ERβ. It is likely that unfavorable steric interactions between these bulkier groups and Ile_{373} are responsible for the decreased ligand affinity.

Some interesting findings were also observed with various fluorine analogues of **117**. Fluoro and difluro analogues **123**−**125** were about 2−3× more potent and 2× less selective than **117**, whereas difluoro analogues **126** and **127** were considerably less potent than **117**. Considering that fluoro substituents do not significantly alter the size of the molecule, electrostatic repulsion involving one of the fluorine groups is the likely reason for the loss of potency. Supporting evidence for this hypothesis is the fact that 2,6-difluoro analogue **127**, most likely to experience repulsion with the carboxylic acid of Glu_{305}, was the least potent analogue among them. For 2,5-difluoro analogue **126**, it is likely that the 5-fluoro analogue experiences somewhat weaker electrostatic repulsion with the carbonyl of Leu_{346}.

A methyl group meta to the A-ring hydroxyl group (**128**) caused a 9-fold decrease in ERβ potency and 2-fold reduction in selectivity. Various aromatic and carbocyclic groups (**129**−**134**) were found to be 50−100-fold less potent and selective than **116**, most likely because of unfavorable steric interactions with the pocket. The introduction of a bromine group at the 4-postion of the benzoxazole nucleus of compounds **117** (7-vinyl) and **91** (7-methoxy) produced monobromo analogues **135** and **137**, respectively, causing a reduction in potency. Dibromovinyl analogue **136** exhibited an additional loss of ERβ potency, although analogous methoxy analogue **138** maintained its ERβ potency. Both dibromo analogues were 5−10× less ERβ selective than the monobromo parent compounds.

To confirm that functional groups at the 7-position were targeting the ERα Met_{421}/ERβ Ile_{373} pocket, we cocrystallized compound **117** (ERB-041) with human ERβ (Figure 6).[26] The binding mode of compound **117** is similar to what we predicted for parent compound

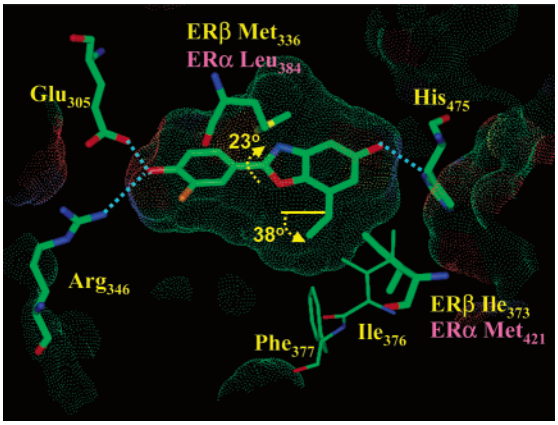


Figure 6. ERβ complexed with **117** (ERB-041) (colored by atom type). A Connolly surface is used to represent the shape of the binding site. Dihedral angles determining the bound conformation are indicated. As intended, the vinyl group sits in a groove consisting of Ile_{373}, Phe_{377}, and Ile_{376}, confirming that we have indeed targeted the ERα Met_{421} → ERβ Ile_{373} residue substitution

Table 8. Activity of Compounds in a Cell-Based Transcriptional Assay

	regulation of IGFBP-4 MRNA in SAOS-2 cells
compd (1uM)	% activity relative to 10 nM 17β-estradiol
91	130
93	100
95	117
98	122
99	100
105	83
108	86
109	100
112	117
114	158
116	117
117 (ERB-041)	120
118	120
122	131
123	120
124	117
125	81

81, where the phenolic hydroxyl of **117** interacts with the Glu_{305}−Arg_{346}−water triad through a hydrogen-bonding network, whereas the hydroxyl group of the benzoxazole nucleus extends to the distal end of the cavity making a hydrogen-bond interaction with His_{475}. The 2-phenol and 7-vinyl groups exhibit dihedral angles of 23 and 38°, respectively, relative to the benzoxazole plane. The 7-vinyl group extends into the ERα Met421/ERβ Ile373 pocket as intended and sits in a groove formed by Ile_{373}, Ile_{376}, and Phe_{377}. The vinyl CH acts as a "hinge" that directs the ethylene moiety into this relatively narrow groove and forces it to be in close proximity to ERα Met_{421}/ERβ Ile_{373}. We hypothesize that the substitution of ERβ Ile_{373} with ERα Met_{421} within this groove would lead to a combination of electrostatic and steric repulsion associated with the methionine side chain, leading to enhanced ERβ selectivity.[26] The crystallography studies also confirmed that helix 12 of ERβ maintains an agonist-like conformation when **117** is bound to the receptor, allowing for the binding of a nuclear receptor box coactivator peptide, consistent with the fact that **117** behaves as a full agonist on ERβ and ERα (see below).

Table 9. Effect of Compounds on Rat Uterine Weight

	uterine weight (mean mg ± SEM)
vehicle	30.5 ± 3.2
17α-ethynyl-17β-estradiol (EE; 0.06 μg/rat)	104.7 ± 5.4[a]
92 (2 mg/rat)	39.2 ± 0.7
92 + EE	95.9 ± 5.5[a]
93 (2 mg/rat)	38.3 ± 1.7
93 + EE	93.9 ± 5.9[a]
vehicle	21.4 ± 1.6
17α-ethynyl-17β-estradiol (EE; 0.06 μg/rat)	85.5 ± 3.1[a]
97 (2 mg/rat)	30.3 ± 1.5
97 + EE	76.6 ± 3.0[a]
117 (ERB-041) (2 mg/rat)	14.2 ± 1.1
117 (ERB-041) + EE	80.7 ± 5.3[a]

[a] Significantly >vehicle, $p < 0.05$

Table 10. Effect of Compounds on Mouse Uterine Weight

	uterine weight (mean mg ± SEM)
vehicle	13.7 ± 0.8
17β-estradiol	40.5 ± 5.8[a]
92 (50 mg/kg)	13.1 ± 0.8
81 (50 mg/kg)	13.7 ± 0.8
vehicle	9.6 ± 0.5
17β-estradiol	40 ± 2[a]
114 (50 mg/kg)	10.3 ± 0.7
vehicle	11.7 ± 0.5
17β-estradiol	41.9 ± 2.9[a]
117 (ERB-041) (50 mg/kg)	10.7 ± 0.9
117 (ERB-041) (100 mg/kg)	10.6 ± 0.3
vehicle	9.8 ± 1.2
17β-estradiol	42.9 ± 4.9[a]
124 (50 mg/kg)	9.0 ± 0.3
106 (50 mg/kg)	9.5 ± 0.6
118 (50 mg/kg)	9.8 ± 0.7
vehicle	10.3 ± 0.8
17β-estradiol	45.3 ± 1.9[a]
123 (50 mg/kg)	10.3 ± 0.4
vehicle	9.5 ± 0.3
17β-estradiol	46.7 ± 2.5[a]
113 (50 mg/kg)	10.0 ± 0.6
125 (50 mg/kg)	10.0 ± 0.9

[a] Significantly >vehicle, $p < 0.05$.

Selected compounds were evaluated in rat and mouse ERα/β LBD binding assays as well as human full-length ERα/β binding assays. In both rat and mouse LBD binding assays, the majority of the tested compounds (Table 6) exhibited similar potency and selectivity relative to the human LBD assays, with the exception of compounds **73**, **81**, and **103** that were about 4−7× more potent in the mouse LBD assay. In the human full-length binding assays, all tested compounds (Table 7) were similar to the LBD assays with respect to potency and selectivity, with the exception of compounds **97**, **98**, **113**, and **117**, which demonstrated somewhat higher selectivity relative to the human LBD assays for ERβ.

Biological Evaluation. Cell-Based Transcriptional Activity. Two assays were used during the program to determine whether compounds were ERβ agonists. Both assays used the human osteosarcoma cell line, SAOS-2, and these cells were engineered to overexpress ERβ via adenovirus infection. One assay measured increases in metallothionein-II mRNA,[33] and the other measured increases in insulinlike growth factor binding protein-4 (IGFBP-4) mRNA.[7] All compounds

Table 11. Evaluation of Bone Mineral Density in the Ovariectomized Rat

compd	total bone mineral density (mean mg/cm^3 ± SEM)	trabecular bone mineral density (mean mg/cm^3 ± SEM)
vehicle	543.49 ± 14.24	353.96 ± 13.46
17β-estradiol (2 μg/rat)	639.49 ± 14.47[a]	453.28 ± 24.93[a]
93 (10 mg/kg)	501.40 ± 11.97	312.34 ± 19.73
92 (10 mg/kg)	525.51 ± 7.93	287.56 ± 17.56
92 (10 mg/kg) + 17β-estradiol (2 μg/rat)	682.41 ± 24.01[a]	491.43 ± 36.43[a]
sham operated	685.28 ± 15.68[a]	510.96 ± 16.99[a]

[a] Significantly >vehicle, $p < 0.05$.

tested were essentially full agonists. (Representative examples from the IGFBP-4 assay are shown in Table 8.)

In Vivo Evaluation. We had two goals during the in vivo evaluation of our compounds. The first was to assess selectivity and/or classic estrogenic activity, and the second was to evaluate efficacy in a model of inflammation.

Rodent Uterotrophic Assays. The sexually immature rodent uterus is a classic estrogen target tissue and is used as a sensitive estrogenic bioassay. Nonselective estrogens, such as 17β-estradiol and 17α-ethynyl-17β-estradiol, increase organ weight in both rats and mice approximately 4-fold, and an ERα-selective ligand (propylpyrazole triol (PPT)) is as efficacious as these reference estrogens.[8] These data suggest that ERα activation is sufficient to elicit a full estrogenic response in the uterus (as measured by organ weight increase). For the rat assay, ERβ-selective compounds were administered for 3 days at a dose of 2 mg/rat/day, which is equivalent to 36−53 mg/kg when the typical growth of the animals is taken into account. For the mouse assay, ERβ-selective compounds were dosed for 4 days at 50 mg/kg (based on the initial weight of the mice). As shown in Tables 9 and 10, all ERβ-selective compounds tested were nonuterotrophic. Compounds **92**, **93**, **97**, **117** (ERB-041), and **124** were not antagonistic when tested in combination with 17α-ethynyl-17β-estradiol. Taken together, these data show that these compounds are functionally selective for ERβ in vivo and do not impact ERα activity. The in vivo selectivity of these compounds is striking in that even at very high doses no activation of ERα is seen. This finding, likely, cannot be explained by binding selectivity alone. It is well recognized that the receptor−ligand interaction is but the first step in receptor activation, and it is possible that although the compounds interact weakly with ERα they do not elicit the conformational changes required for dimerization and coactivator recruitment.

Rat Model of Osteopenia. After ovariectomy, rats lose bone mineral density (mass), which can be prevented by the administration of nonselective estrogens (e.g., 17β-estradiol), selective estrogen-receptor modulators (e.g., TSE-424),[34] or an ERα-selective ligand (PPT).[8] However, **92** and **93** had no effect on either total or trabecular bone mineral density (Table 11). Moreover, when **92** was combined with 17β-estradiol, no antagonistic effect was seen. Previously, we showed that **117** (ERB-041) was also inactive in this assay.[7] These data are consistent with the results from the uterotrophic assay above, suggesting that the estrogenic response in this model is mediated via ERα and that these ERβ-selective compounds do not impact ERα activity in vivo.

Table 12. Effect of Compounds on Rat Vasomotor Instability

	tail skin temperature change 15 min after naloxone injection (mean ± SEM)
vehicle	4.6 ± 0.8
17α-ethynyl-17β-estradiol (0.3 mg/kg)	2.1 ± 1.1[a]
propylpyrazole triol (PPT; 15 mg/kg)	2.0 ± 0.8[a]
92 (15 mg/kg)	5.3 ± 0.7
92+ PPT	1.9 ± 0.8[a]
97(15 mg/kg)	5.2 ± 0.7
97 + PPT	2.7 ± 1.1[b]

[a] Significantly <vehicle, $p < 0.02$. [b] Significantly <vehicle, $p < 0.07$.

Table 13. Effect of Compounds on Androgen-Induced Increase in Ventral Prostate Weight

	ventral prostate weight (mean mg ± SEM)
vehicle	10.7 ± 0.48
testosterone propionate (TP; 0.8 mg/kg)	119.4 ± 6.9
93 (20 mg/kg) + TP	116.5 ± 6.0[a]
117 (**ERB-041**; 20 mg/kg) + TP	131.8 ± 6.2[a]
flutamide (10 mg/kg) + TP	15.2 ± 1.3

[a] Not significantly <TP alone, $p > 0.05$.

Vasomotor Instability. At menopause, many women experience debilitating vasomotor instability, and nonselective estrogens (e.g., 17β-estradiol and conjugated equine estrogens) are indicated for relief. One animal model of vasomotor instability, where clinically active compounds are also active, is the morphine-addicted ovariectomized rat. In this model, acute withdrawal of morphine (using an opioid receptor antagonist) induces a rise in tail skin temperature.[37] Nonselective estrogens and an ERα-selective ligand (PPT) prevent this tail skin temperature increase in morphine-addicted rats upon acute withdrawal.[8] As shown in Table 12, **92** and **97** failed to blunt this "flush", although other studies have shown that they do cross the blood−brain barrier (data not shown). Again, these data are consistent with the uterine and osteopenia model results, illustrating a role for ERα in these endpoints and the fact that ERβ-selective ligands have no effect.

Antiandrogenic Assay. Because ERβ is highly expressed in the rat prostate and there have been suggestions that ERβ may influence prostate proliferation,[38] we sought to determine if ERβ-selective agonists were antiandrogenic. As shown in Table 13, **93** and **117** (ERB-041) did not decrease ventral prostate weight when given in combination with testosterone propionate, whereas the reference antiandrogen completely blocked the androgen-induced response. These data

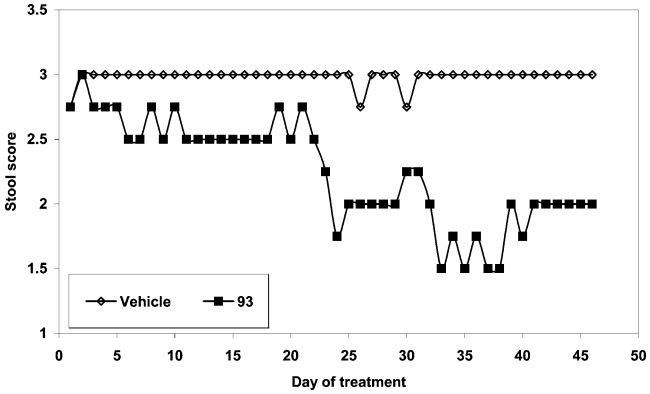


Figure 7. Male HLA-B27 transgenic rats were treated with daily oral doses of the test compound, and stool character was assessed daily (1 = normal; 3 = diarrhea). From days 1−18, the dose was 10 mg/kg, and from days 19−46, it was 20 mg/kg.

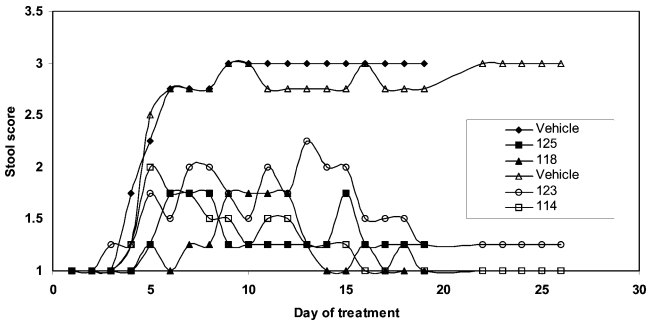


Figure 8. Male HLA-B27 transgenic rats were treated with daily oral doses of the test compound, and stool character was assessed daily (1 = normal; 3 = diarrhea). **123** was dosed at 10 mg/kg from days 1−14; the dose was then increased to 20 mg/kg. Other compounds were dosed at 10 mg/kg throughout the study.

suggest that in normal rats ERβ-selective agonists do not interfere with androgen-regulated prostatic growth responses.

HLA-B27 Transgenic Rat. HLA-B27 transgenic rats are used as models of both inflammatory bowel disease and arthritis. Previously, we showed that **117** (ERB-041) normalized stool character and improved colonic histology in this model.[7] As shown in Figures 7 and 8, other ERβ-selective agonists also possess this activity. In addition to improving stool scores, most compounds also improved colonic histology scores (Table 14). Finally, **93**, although modestly improving stool score but not colon histology, largely prevented arthropathy from developing in these rats (Figure 9). One possible explanation for the divergent activity on these two endpoints is that the arthropathy studies were prophylactic,

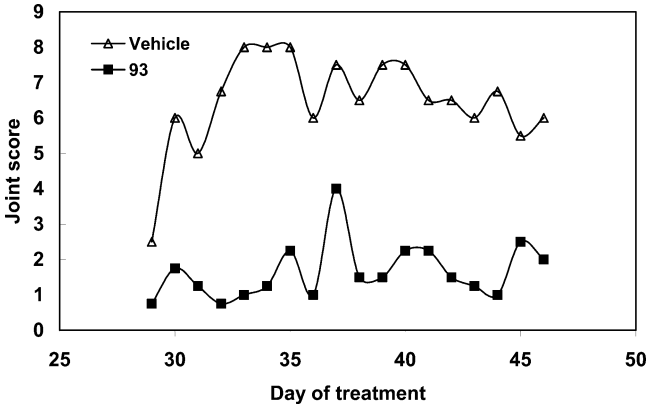


Figure 9. Rats from the study depicted in Figure 7 were scored for joint redness and swelling beginning 28 days after the first treatment.

whereas the colon studies were initiated when the rats were symptomatic. These data show that this class of compounds, although being devoid of classic estrogenic activity, does possess anti-inflammatory activity in this model.

Summary

In this report, we have described the detailed exploration of diphenolic benzisoxazole/benzoxazole scaffolds that led to the discovery of highly potent and selective agonists for ERβ. The most potent and selective analogues of this series have binding affinities of 1−5 nM at ERβ and selectivities relative to ERα of >100-fold.

X-ray cocrystal structures of structurally diverse ligands complexed with ERβ, in conjunction with docking calculations, were used to study a single, conservative residue substitution in the ligand-binding pocket, ERα $Met_{421} \rightarrow$ ERβ Ile_{373}, to optimize ERβ selectivity. The majority of ERβ-selective agonists tested that are at least ~50-fold selective display consistent in vivo profiles: they are inactive in several models of classic estrogen action and yet are active in a model of inflammation. These data suggest that this class of compounds may have utility in treating chronic inflammatory disorders such as inflammatory bowel disease while lacking the undesirable side effects of nonselective estrogen agonists.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes on a Mel-Temp-II apparatus and were reported uncorrected. ^{1}H NMR spectra were determined in the cited solvent on a Varian Unity or Varian Inova (400-MHz) instrument with tetramethylsilane as an internal standard. Chemical shifts are given in ppm, and coupling constants are in hertz.

Table 14. Colonic Histology Scores from HLA-B27 Transgenic Rats (Mean ± SD)

	ulceration (0−2)	inflammation (0−3)	lesion depth (0−2)	fibrosis (0−2)	total score
vehicle	1.92 ± 0.14	2.92 ± 0.14	1.50 ± 0.43	0.50 ± 0.50	6.83 ± 1.18
125	0.33 ± 0.14[a]	0.75 ± 0.25[a]	0.25 ± 0.25[a]	0	1.33 ± 0.63[a]
118	0.58 ± 0.38[a]	1.42 ± 0.29[a]	0.33 ± 0.14[a]	0.50 ± 0.00	3.19 ± 0.64[a]
vehicle	1.19 ± 0.69	2.38 ± 0.32	1.0 ± 0.54	0.94 ± 0.75	5.50 ± 2.1
93	0.81 ± 0.47	2.06 ± 0.43	0.75 ± 0.50	0.56 ± 0.32	4.19 ± 1.74
vehicle	1.44 ± 0.66	2.88 ± 0.14	1.56 ± 0.63	1.06 ± 0.32	6.94 ± 1.51
123	0.44 ± 0.24[a]	1.50 ± 0.35[a]	0.44 ± 0.24[a]	0.31 ± 0.13[a]	2.69 ± 0.52[a]
114	0.75 ± 0.46[a]	1.81 ± 0.13[a]	0.63 ± 0.32[a]	0.31 ± 0.32[a]	3.50 ± 1.10[a]

[a] Significantly <vehicle, $p < 0.05$

Splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. The infrared spectra were recorded on an AVATAR 360 Nicolet spectrophotometer as KBr pellets or as solutions in chloroform. Mass spectra were recorded on a Micromass LCT, Waters spectrometer. Elemental analyses (C, H, N) were performed on a Perkin-Elmer 240 analyzer, and all compounds are within ±0.4% of theory unless otherwise indicated. All products, unless otherwise noted, were purified by "flash chromatography" using 220−400 mesh silica gel. Thin-layer chromatography was done on silica gel 60 F-254 (0.25-mm thickness) plates. Visualization was accomplished with UV light and/or 10% phosphomolybdic acid in ethanol. The hydration was determined by Karl Fischer titration using Mitsubishi moisture meter model CA-05. Unless otherwise noted, all materials were obtained commercially and used without further purification. All reactions were carried out under an atmosphere of dry argon or nitrogen.

Representative synthetic protocols of the isoxazoles shown in Schemes 1−3 are described below. Both the phenylisoxazoles and naphthylisoxazoles were prepared in substantially the same manner from appropriately substituted starting material.

Atomic coordinates have been deposited in the Protein Data Bank (www.rcsb.org) with accession codes 1U3Q, 1U3R, and 1U3S for ERF complexed with compounds **3**, **55**, and **62**, respectively. Coordinates for ERβ complexed with **117** (**ERB-041**) have been submitted in conjunction with ref 26, with accession code 1X7B.

Route a. (2-Fluoro-4-methoxyphenyl)(4-methoxy-2-methylphenyl)methanone (6; R_1 = 2-Me; 2-F, 4-OMe-Ph Analog). *n*-Butyllithium (2.5 M, 44.0 mL) was added dropwise to a cold (−78 °C) solution of 1-bromo-4-methoxy-2-methylbenzene (22.0 g, 109.4 mmol) and THF (150 mL). The reaction mixture was stirred at −78 °C for 3 h, and then 2-fluoro-4-methoxybenzaldehyde (16.8 g, 109.4 mmol) in THF (10 mL) was added. The mixture was allowed to warm to 0 °C, was stirred for 10 min, and then was quenched with aqueous ammonium chloride. The mixture was poured into water, acidified with HCl (2 N), and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 3:1) gave (2-fluoro-4-methoxyphenyl)(4-methoxy-2-methylphenyl)-methanol as a yellow oil (23.6 g, 78% yield). The product was dissolved in acetone (150 mL) and cooled to 10 °C, and the Jones reagent (79.7 mL) was added dropwise. The reaction was stirred for 1 h, poured into water, and extracted with ethyl ether. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 3:1) gave a yellow oil (23.6 g, 78% yield): mp 88−90 °C; MS *m/e* 275 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.37 (s, 3H), 3.8 (s, 3H), 3.83 (s, 3H), 6.81 (dd, J = 8.5, 2.58 Hz, 1H), 6.87−6.92 (m, 3H), 7.28 (dd, J = 8.53, 1.0, Hz, 1H), 7.47 (m, 1H). Anal. ($C_{16}H_{15}FO_3$) C, H.

6-Methoxy-3-(4-methoxy-2-methylphenyl)-1,2-benzisoxazole (8a; R = Me, R_1 = 2-Me). Sodium hydride (60% in mineral oil, 1.46 g, 36.5 mmol) was added portionwise to a cold (0 °C) mixture of hydroxylamine hydrochloride (1.52 g, 21.9 mmol) and DMF (20 mL). After stirring for 20 min, (2-fluoro-4-methoxyphenyl)(4-methoxy-2-methylphenyl)methanone (2.0 g, 7.3 mmol) in DMF (5 mL) was added to the reaction mixture. The new mixture was stirred at 80 °C for 3 h, cooled to room temperature, and added to water. The mixture was extracted with EtOAc, and the organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 3:1) gave a white solid (1.61 g, 82% yield): mp 126−128 °C; MS *m/e* 270 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.35 (s, 3H), 3.82 (s, 3H), 3.88 (s, 3H), 6.92−7.02 (m, 4H), 7.36 (d, J = 1.98 Hz, 1H), 7.51 (m, 2H). Anal. ($C_{16}H_{15}NO_3$) C, H, N.

3-(4-Hydroxy-2-methylphenyl)-1,2-benzisoxazol-6-ol (8b; R = H; R_1 = 2-Me;) Hydriodic acid (57% w/w aq, 10 mL) was added to a mixture of 6-methoxy-3-(4-methoxy-2-methylphenyl)-1,2-benzisoxazole (1.0 g, 3.7 mmol), acetic acid (10 mL), and acetic anhydride (5 mL). The reaction mixture was stirred at 160 °C for 2 h and then cooled to room temperature, poured into water, and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 1:1) gave a white solid (0.62 g, 70% yield): mp 229−231 °C; MS *m/e* 242 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.26 (s, 3H), 6.74−6.8 (m, 2H), 6.84 (dd, J = 8.73, 1.98 Hz, 1H), 6.99 (d, J = 1.78 Hz, 1H), 7.35 (d, J = 8.33 Hz), 7.45 (d, J = 8.53 Hz, 1H), 9.79 (s, 1H), 10.36 (s, 1H). Anal. ($C_{14}H_{11}NO_3 \cdot 0.1H_2O$) C, H, N.

Route b. Acetone *O*-[5-methoxy-2-(6-methoxy-2-naphthoyl)phenyl]oxime (7; R_1 = H, A = 6-Methoxy-2-naphthalene). Potassium *tert*-butoxide (1.2 g, 10.6 mmol) was added to a solution of acetone oxime (0.78 g, 10.6 mmol) and THF (15 mL). After stirring for 1 h, (2-fluoro-4-methoxyphenyl)(6-methoxy-2-naphthyl)methanone (3.0 g, 9.7 mmol) in THF (3 mL) was added. The reaction mixture was refluxed for 3 h and then cooled to room temperature, poured into aqueous ammonium chloride, and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 3:1) gave a brown solid (3.35 g, 95% yield): mp 117−118 °C; MS *m/e* 364 (M^+); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.1 (s, 3H), 1.75 (s, 3H), 3.84 (s, 3H), 3.89 (s, 3H), 6.74 (dd, J = 8.54, 2.44 HZ, 1H), 7.0 (d, J = 2.19, 1H), 7.2 (dd, J = 8.79, 2.44 Hz, 1H), 7.39 (d, J = 2.44 Hz, 1H), 7.46 (d, J = 8.54 Hz, 1H), 7.74 (dd, J = 8.54, 1.7 Hz, 1H), 7.86 (d, J = 8.78 Hz, 1H), 7.96 (d, J = 9.0 Hz, 1H), 8.12 (d, J = 1.46 Hz, 1H). Anal. ($C_{22}H_{21}NO_4$) C, H, N.

6-Methoxy-3-(6-methoxy-2-naphthyl)-1,2-benzisoxazole (8a; R_1 = H, R = Me, A = 6-Methoxy-2-naphthalene). A mixture of acetone *O*-[5-methoxy-2-(6-methoxy-2-naphthoyl)-phenyl]oxime (3.0 g, 8.26 mmol), 5% HCl (30 mL), and EtOH (30 mL) was refluxed for 2 h. The mixture was poured into water and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 2:1) gave a yellow solid (2.2 g, 87% yield): mp 141−143 °C; MS *m/e* 306 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.90 (s, 3H), 3.91 s, 3H), 7.01 (dd, J = 8.78, 2.19 Hz, 1H), 7.26 (dd, J = 9.02, 2.68 Hz, 1H), 7.43 (d, J = 2.19 Hz, 1H), 8.02 (m, 2H), 8.1 (d, J = 9.02 Hz, 1H), 8.17 (d, J = 8.78 Hz, 1H), 8.55 (s, 1H). Anal. ($C_{19}H_{15}NO_3$) C, H, N.

(2,5-Dihydroxyphenyl)(4-hydroxyphenyl)methanone (11b; R_1 = H). To a cooled solution (0 °C) of 4-methoxybenzoyl chloride (5.1 g, 30 mmol) and 1,4-dimethoxy benzene (4.1 g, 30 mmol) in dichloroethane (100 mL) was added aluminum chloride (4.0 g, 30 mmol), and the reaction mixture was stirred at room temperature for 1 h. The reaction was then quenched with 2 N HCl, and the organic layer was separated and dried over $MgSO_4$. The organic layer was concentrated to give an oil, which was purified by column chromatography (20% EtOAc/hexanes) to give an oil (4.7 g) that was mixed with pyridine hydrochloride (20 g) and heated to 200 °C for 1h. The reaction was then cooled, diluted with 2 N HCl, and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give an oil residue. The crude product was crystallized from CH_2Cl_2 to produce a tan solid (3.1 g, 78% yield): MS *m/e* 229 $(M - H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.67 (m, 1H), 6.86−6.75 (m, 4H), 7.61 (d, J = 8.6 Hz, 2H), 9.03 (s, 1H), 10.37 (s, 1H), 9.64 (s, 1H). Anal. ($C_{13}H_{10}O_4$) C, H.

3-(4-Hydroxyphenyl)-1,2-benzisoxazol-5-ol (13; R_1 = H). A mixture of (2,5-dihydroxyphenyl)(4-hydroxyphenyl)methanone (1.0 g, 4.3 mmol), hydroxylamine hydrochloride (2.0 g, 29 mmol), pyridine (5 mL), and EtOH (30 mL) was heated to reflux. After stirring for 4 h, the reaction was cooled, poured into 2 N HCl, and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give a yellow foam (0.92 g, 87% yield). The product, (2,5-dihydroxyphenyl)(4-hydroxyphenyl)methanone oxime (0.9 g, 3.7 mmol), was taken in THF (25 mL), and triphenylphosphine (1.5 g, 5.6 mmol) was added, followed by the dropwise addition of diethylazodicarboxylate (0.9 mL, 5.6 mmol). After 30 min, the reaction was filtered and concentrated, and the resulting oil was purified by column chromatography (30% EtOAc/hexanes) to give a solid. The solid was triturated with CH_2Cl_2 and filtered to give

a solid (0.04 g): mp 266−268°C; MS *m/e* 228 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.93 (dd, J = 8.7 Hz, 2.4 Hz, 1H), 6.95 (d, J = 8.7 Hz, 2H), 7.02 (d, J = 2.3 Hz, 1H), 7.50 (d, J = 8.7 Hz, 1H), 8.00 (d, J = 8.7 Hz, 2H), 10.30 (s, 1H), 10.32 (s, 1H). Anal. ($C_{13}H_{19}NO_3 \cdot 0.25H_2O$) C, H, N.

1-(Methoxymethoxy)-3-propylbenzene (14; R_1 = Propyl). To a solution of 3-hydroxybenzaldehyde (11.0 g, 90.1 mmol) in DMF (110 mL) at 0 °C was added methoxymethyl chloride (7.5 mL, 99.1 mmol) followed by NaH (99.1 mmol, 3.96 g, 60% dispersion in oil) in three portions. The mixture was stirred at 0 °C for 15 min and then at room temperature for 15 h. The reaction was quenched with water, and more water was added until all the precipitate dissolved. The solution was extracted in ether (2 × 150 mL) with water (50 mL) and then with saturated Na_2CO_3 (100 mL) and finally with water (100 mL). The crude product was used in the next reaction. To a suspension of (ethyl)triphenylphosphonium bromide (36.8 g, 99.1 mmol, dried at 90 °C under high vacuum overnight) in THF (250 mL) at 0 °C was added *n*-BuLi (39.6 mL, 99.1 mmol, 2.5 M in hexanes) over 5 min. The resulting dark-burgundy mixture was stirred at 0 °C for 10 min and then at room temperature for 30 min. It was then recooled to 0 °C, and a solution of the above crude 3-methoxymethoxybenzaldehyde in THF (15 mL) was added slowly. The resulting medium-brown mixture was stirred at 0 °C for 2 h and then at room temperature for 2 h. THF was removed in vacuo, and the crude material was dissolved in DMF (100 mL). Extraction in hexanes (4 × 100 mL) with 50% saturated brine (100 mL) eliminated triphenylphosphonium oxide into the aqueous layer. Flash chromatography with silica gel (gradient to 6% ether/hexanes) produced 1-(methoxymethoxy)-3-(1-propenyl)-benzene [(E)/(Z) ≈ 1:1] as a colorless liquid (13.2 g, 82% yield for two steps). A suspension of the produced 1-(methoxymethoxy)-3-(1-propenyl)benzene (13.2 g, 74.1 mmol), $NaHCO_3$ (1.24 g, 14.8 mmol), and 10% palladium on carbon (3.3 g) in EtOAc (75 mL) and EtOH (19 mL) was stirred at room temperature for 20 h with a hydrogen balloon attached. The round-bottom flask was purged with nitrogen, and the mixture was filtered through celite to furnish 1-(methoxymethoxy)-3-propylbenzene as a colorless liquid (13.4 g, 100% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (t, J = 7.4 Hz, 3H), 1.64 (m, 2H), 2.56 (t, J = 7.7 Hz, 2H), 3.48 (s, 3H), 5.17 (s, 2H), 6.80−6.90 (m, 3H), 7.19 (m, 1H). Anal. ($C_{11}H_{10}O_2$) C, H.

(5-Bromo-2,4-dimethoxyphenyl)(2-fluoro-4-methoxyphenyl)methanone (6; R_1 = 2′-OMe, 2-Fluoro-4-methoxyphenyl; 5′-Br Analog). To a solution of (2,4-dimethoxyphenyl)(2-fluoro-4-methoxyphenyl)methanone (**6**; R_1 = 2′-OMe, 2-fluoro-4-methoxyphenyl analogue; 300 mg, 1.03 mmol) in chloroform (7 mL) at 0 °C was added a solution of bromine in chloroform (1.03 mmol, 0.53 mL from a stock solution of 0.10 mL bromine in 0.90 mL chloroform) dropwise. The dark-purple solution was stirred at 0 °C for 15 min and then extracted in CH_2Cl_2 (2 × 30 mL) with 50% saturated Na_2SO_3 (2 × 20 mL) followed by 50% saturated brine (20 mL). Flash chromatography with silica gel (gradient to 35% EtOAc/hexanes) provided the title compound as a light-purple solid (380 mg, 100% yield). A portion of this solid was triturated with ethyl ether to give a white solid: mp 114−115 °C; MS *m/e* 369/371 $(M + H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.72 (s, 3H), 3.86 (s, 3H), 3.96 (s, 3 H), 6.46 (s, 1 H), 6.56 (dd, 1H, J = 12.5, 2.2 Hz), 6.74 (dd, 1H, J = 8.7, 1.8 Hz), 7.67 (t, 1H, J = 8.6 Hz), 7.74 (s, 1H). Anal. ($C_{16}H_{14}BrFO_4$) C, H.

(5-Chloro-2,4-dimethoxyphenyl)(2-fluoro-4-methoxyphenyl)methanone (6; R_1 = 2′-OMe, 2-Fluoro-4-methoxyphenyl; 5′-Cl Analog). A solution of (2,4-dimethoxyphenyl)-(2-fluoro-4-methoxypaheyl)methanone (**6**; R_1 = 2′-OMe, 2-fluoro-4-methoxyphenyl analogue 300 mg, 1.03 mmol) and *N*-chlorosuccinimide (206 mg, 1.54 mmol) in CH_3CN (6 mL) was stirred at 70 °C for 20 h. Most of the solvent was removed in vacuo, and extraction was performed in ether (2 × 40 mL) with water (2 × 30 mL). Flash chromatography with silica gel (gradient to 35% EtOAc/hexanes) furnished the title compound as a light grayish-brown solid (328 mg, 98% yield). A portion of this solid was crystallized from 10% CH_2Cl_2/ethyl ether to give a light-orange solid: mp 128.5−130 °C; MS *m/e* 325/327 $(M + H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.73 (s, 3H), 3.87 (s, 3H), 3.98 (s, 3H), 6.48 (s, 1H), 6.56 (dd, 1H, J = 12.5, 2.4 Hz), 6.75 (dd, 1H, J = 8.7, 2.4 Hz), 7.59 (s, 1H), 7.68 (t, J = 8.6 Hz). Anal. ($C_{16}H_{14}ClFO_4$) C, H.

4-(6-Hydroxy-1,2-benzisoxazol-3-yl)-6-methylbenzene-1,3-diol (44). To a solution of 3-(5-bromo-2,4-dimethoxyphenyl)-6-methoxy-1,2-benzisoxazole (200 mg, 0.55 mmol) in THF (6 mL) at −78 °C was added *n*-BuLi (0.24 mL, 0.60 mmol, 2.5 M in hexanes) dropwise. The solution was stirred at −78 °C for 30 min, and then iodomethane (60 μL, 0.96 mmol) was added dropwise. The cooling bath was switched to an ice bath, and the solution was stirred at 0 °C for 2 h. The reaction was quenched with water, most of the solvent was removed in vacuo, and extraction was conducted in EtOAc (2 × 30 mL) with water (20 mL). Flash chromatography with silica gel (gradient to 25% EtOAc/hexanes) provided a mixture of the desired methylated product and the protonated side product (both compounds have the same R_f). The above mixture of compounds was treated with BBr_3 in CH_2Cl_2 to produce the title compound as a light-orange solid (114 mg, 81% yield for two steps): mp 195 °C; MS *m/e* 258 $(M + H)^+$; ^{1}H NMR (acetone-d_6, 300 MHz) δ 2.25 (s, 3H), 6.59 (s, 1H), 7.04 (dd, 1H, J = 8.5, 2.1 Hz), 7.09 (d, 1H, J = 1.8 Hz), 7.80 (s, 1H), 8.12 (d, 1H, J = 8.8 Hz), 8.88 (br s, 1H), 9.42 (br s, 1H), 9.52 (s, 1H). Anal. ($C_{14}H_{11}NO_4 \cdot 0.3H_2O$) C, H, N.

1-[2-(Methoxymethoxy)-4-propylphenyl]propan-1-ol (15; R_1 = Propyl, R_2 = Ethyl). To a solution of 1-(methoxymethoxy)-3-propylbenzene (14.0 g, 77.7 mmol) in pentane (80 mL) at 0 °C was added *tert*-BuLi (55 mL, 93.2 mmol, 1.7 M in pentane) slowly. The resulting suspension was stirred at 0 °C for 1.5 h, and then the precipitate was allowed to settle without stirring at 0 °C for 1.5 h. The supernatant was removed via a syringe, and THF (180 mL) was added. To the thick slurry at 0 °C was added propionaldehyde (11.2 mL, 155.4 mmol, ran through basic alumina) slowly. The resulting solution was stirred at 0 °C for 1 h and then at room temperature for 12 h. About half of the solvent was removed in vacuo, the reaction solution was quenched with water, and most of the remaining THF was removed in vacuo. Extraction in EtOAc (2 × 200 mL) with 1:1 saturated NH_4Cl/brine (100 mL) and flash chromatography with silica gel (gradient to 32% EtOAc/hexanes) provided the title compound as a colorless liquid (12.9 g, 70% yield): MS *m/e* 221 $(M + H - H_2O)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (t, 3H, J = 7.2 Hz), 0.96 (t, 3H, J = 7.2 Hz), 1.61 (m, 2H), 1.82 (m, 2H), 2.37 (broad s, 1H), 2.55 (t, 2H, J = 7.7 Hz), 3.50 (s, 3H), 4.82 (t, 1H, J = 6.6 Hz), 5.22 (s, 2H), 6.84 (d, 1H, J = 7.7 Hz), 6.91 (s, 1H), 7.23 (d, 1H, J = 7.7 Hz). Anal. ($C_{14}H_{22}O_3$) C, H.

2,5-Dipropylphenol (16a; R = H, R_1 = Propyl, R_2 = Ethyl). To a solution of 1-[2-(methoxymethoxy)-4-propylphenyl]propan-1-ol (12.7 g, 53.3 mmol) and triethylsilane (21.3 mL, 133 mmol) in CH_2Cl_2 (120 mL) at 0 °C was added trifluoroacetic acid (16.4 mL, 213 mmol) slowly with an outlet needle attached to the round-bottom flask. The solution was stirred at 0 °C for 5 min and then at room temperature for 14 h. Water (40 mL) was added, and the mixture was stirred at room temperature for 2 h. Extraction in CH_2Cl_2 (2 × 150 mL) with 50% saturated brine (5 × 100 mL) removed all of the trifluoroacetic acid. Flash chromatography with silica gel (gradient to 5% EtOAc/hexanes) furnished the title compound as a light-green liquid (3.60 g, 38% yield): MS *m/e* 179 $(M + H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, 3H, J = 7.3 Hz), 0.97 (t, 3H, J = 7.3 Hz), 1.62 (m, 4H), 2.52 (m, 4H), 4.58 (s, 1H), 6.60 (s, 1H), 6.69 (d, 1H, J = 7.6 Hz), 7.01 (d, 1H, J = 7.6 Hz). Anal. ($C_{12}H_{18}O$) C, H.

2-Methoxy-1,4-dipropylbenzene (16b; R = Me, R_1 = Propyl, R_2 = Ethyl). To a solution of 2,5-dipropylphenol (3.50 g, 19.6 mmol) and iodomethane (3.70 mL, 58.8 mmol) in DMF (65 mL) at 0 °C was added sodium hydride (1.60 g, 39.2 mmol, 60% dispersion in oil) in two portions. The resulting mixture was stirred at 0 °C for 10 min and then at room temperature for 14 h. After being quenched with water, the mixture was extracted in hexanes (3 × 100 mL) with 50% saturated brine

(50 mL). Flash chromatography with silica gel (100% hexanes) afforded the title compound as a colorless liquid (3.70 g, 97% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (two overlapping triplets, 6H), 1.60 (m, 4H), 2.55 (m, 4H), 3.81 (s, 3H), 6.66 (s, 1H), 6.70 (d, 1H, J = 7.5 Hz), 7.02 (d, 1H, J = 7.5 Hz). Anal. ($C_{15}H_{20}O$) C, H.

1-Bromo-4-methoxy-2,5-dipropylbenzene (17; R_1 = Propyl; R_2 = Ethyl). To a solution of 2-methoxy-1,4-dipropylbenzene (2.0 g, 10.4 mmol) in acetonitrile (30 mL) at room temperature, was added *N*-bromosuccinimide (1.85 g, 10.4 mmol) in three portions. The solution was stirred at 60 °C for 20 h, and then most of the solvent was removed in vacuo. Extraction in hexanes (2 × 100 mL) with 50% saturated brine (75 mL) and flash chromatography with silica gel (100% hexanes) provided the title compound as a colorless liquid (2.50 g, 89% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, 3H, J = 7.5 Hz), 0.99 (t, 3H, J = 7.6 Hz), 1.60 (m, 4H), 2.50 (t, 2H, J = 7.7 Hz), 2.65 (t, 2H, J = 7.8 Hz), 3.79 (s, 3H), 6.67 (s, 1H), 7.24 (s, 1H). Anal. ($C_{13}H_{19}BrO$) C, H.

Representative synthetic protocols of oxazoles shown in Schemes 4 and 5 are described below. Both the phenyloxazoles and naphthyloxazoles were prepared in substantially the same manner from the appropriately substituted starting material.

***N*-(2,5-Dimethoxyphenyl)-2,5-dimethoxybenzamide (22; R_1, R_2 = OMe).** A mixture of 2,5-dimethoxybenzoic acid (5.0 g, 27.5 mmol) and thionyl chloride (15 mL) was refluxed for 1 h. The volatiles were removed under vacuum. The residue was dissolved in THF (20 mL) and added to a cold (0 °C) solution of 2,5-dimethoxyaniline (4.6 g, 30.2 mmol), triethylamine (5 mL, 35.9 mmol), and THF (40 mL). The mixture was stirred for 30 min, poured into water, acidified with HCl (2 N), and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 2/1) gave a white solid (8.1 g, 93% yield): mp 121−123 °C; MS *m/e* 318 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.71 (s, 3H), 3.77 (s, 3H), 3.88 (s, 3H), 4.01 (s, 3H), 6.6 (dd, J = 8.78, 2.92 Hz, 1H), 7.0 (d, J = 8.78 Hz, 1H), 7.16 (dd, J = 9.0, 3.3.17 Hz, 1H), 7.23 (d, J = 9.02 Hz, 1H), 7.61 (d, J = 3.17 Hz, 1H), 8.17 (d, J = 3.17 Hz, 1H), 10.79 (s, 1H). Anal. ($C_{17}H_{19}NO_5$) C, H, N.

2-(5-Hydroxy-1,3-benzoxazol-2-yl)-benzene-1,4-diol (23; R_1, R_2 = OMe). A mixture of *N*-(2,5-dimethoxyphenyl)-2,5-dimethoxybenzamide (1.0 g, 3.1 mmol) and pyridine hydrochloride (2.0 g, 17.3 mmol) was stirred at 200 °C for 1 h. The mixture was cooled to room temperature, and HCl (10 mL, 2 N) was added. The mixture was then extracted with EtOAc, and the organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 2:1) gave a white solid (0.8 g, 76% yield): mp 309−311 °C; MS *m/e* 242 $(M - H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.85 (dd, J = 8.78, 2.44 Hz, 1H), 6.93 (d, J = 1.17 Hz, 1H), 7.1 (d, J = 2.44 Hz, 1H), 7.3 (d, J = 1.46 Hz, 1H), 7.6 (d, J = 8.78 Hz, 1H), 9.17 (br s, 1H), 9.65 (br s, 1H), 1068 (br s, 1H). Anal. ($C_{13}H_9NO_4$) C, H, N.

2-(5-Bromo-6-hydroxy-1-naphthyl)-1,3-benzoxazol-6-ol (66). Bromine (86 mg, 0.54 mmol) in acetic acid (2 mL) was added dropwise to solution of 2-(6-hydroxy-1-naphthyl)-1,3-benzoxazol-6-ol (0.15 g, 0.54 mmol) and acetic acid (9 mL). The reaction mixture was stirred for 2 h and poured into water, and the precipitated solid was filtered and dried. The product was recrystallized from acetone/EtOAc to give a yellow solid: mp 221−223 °C; MS *m/e* 354 $(M - H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.88 (dd, J = 8.73, 2.18 Hz, 1H), 7.13 (d, J = 1.98 Hz, 1H), 7.46 (d, J = 9.32 Hz, 1H), 7.66 (d, J = 8.73 Hz, 1H), 7.74 (t, J = 8.33 Hz, 1H), 8.21 (d, J = 6.74 Hz, 1H), 8.3 (d, J = 8.53 Hz, 1H), 9.3 (d, J = 9.52 Hz, 1H), 10.1 (br s, 1H), 10.9 (br s, 1H). Anal. ($C_{17}H_{10}BrNO_3 \cdot 1.4H_2O$) C, H, N.

2-Bromo-4-methoxy-6-nitrophenol (25a). Bromine (16.0 g, 100 mmol) in acetic acid (20 mL) was added at room temperature to a mixture of 4-methoxy-2-nitrophenol (16.9 g, 100 mmol), sodium acetate (16.4 g, 200 mmol), and acetic acid (100 mL). The mixture was stirred for 30 min at room temperature and at 70 °C for 2 h and was then poured into water (1.5 l) containing concentrated sulfuric acid (10 mL). The precipitated solid was filtered and crystallized from (chloroform/hexanes) to give 2-bromo-4-methoxy-6-nitrophenol as a brownish solid: mp 108−110 °C; MS *m/e* 246 $(M - H)^+$; ^{1}H NMR (DMSO-d_6) δ 3.76 (s, 3H), 7.48 (d, J = 2.9 Hz, 1H), 7.62 (d, J = 2.9 Hz, 1H), 10.5 (br s). Anal. ($C_7H_6BrNO_4$) C, H, N.

2-Amino-6-bromo-4-methoxyphenol (25b). Raney/Ni (2.5 g) was added to a solution of 2-bromo-4-methoxy-6-nitrophenol (8.8 g, 35.5 mmol) in THF (100 mL). The mixture was shaken in a Parr apparatus under hydrogen at 25 psi for 2.5 h. The reaction mixture was filtered through celite and concentrated under vacuum to give 2-amino-6-bromo-4-methoxyphenol as a gray solid (7.4 g, 96% yield): mp 95−97 °C; MS *m/e* 218 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 3.59 (s, 3H), 4.97 (br s, 2H), 6.2 (m, 2H), 8.1 (br s, 1H). Anal. ($C_7H_8BrNO_2$) C, H, N.

2-Bromo-6-[(3-fluoro-4-methoxybenzoyl)amino]-4-methoxyphenyl-3-fluoro-4-methoxybenzoate (27; R_1 = 3′-F). A mixture of 3-fluoro-4-methoxybenzoic acid (39.0 g, 229 mmol), thionyl chloride (100 mL), and *N*,*N*-dimethylformamide (0.2 mL) was refluxed for 1 h. The volatiles were removed under vacuum. The solids were taken in benzene (twice), and the volatiles were removed under vacuum. The residue was dissolved in CH_2Cl_2 (100 mL) and added to a cold (0 °C) mixture (mechanically stirred) of 2-amino-6-bromo-4-methoxyphenol (20.0 g, 91.7 mmol) and CH_2Cl_2 (150 mL). Anhydrous pyridine (37.0 mL, 468.5 mmol) was added dropwise to the new mixture. During the pyridine addition, a precipitate was formed. The mixture was stirred for 30 min, and then ethyl ether (250 mL) was added. The precipitated solids were filtered off and washed with ethyl ether. The solids were taken in water and stirred for 20 min and then filtered off and dried to give 2-bromo-6-[(3-fluoro-4-methoxybenzoyl)amino]-4-methoxyphenyl-3-fluoro-4-methoxybenzoate as an off-white solid (46.5 g, 97% yield): mp 184−186 °C; MS *m/e* 520 $(M-H)^+$; ^{1}H NMR (DMSO-d_6) δ 3.8 (s, 3H), 3.85 (s, 3H) 3.92 (s, 3H), 7.18−7.22 (m, 2H), 7.29 (d, J = 2.9 Hz, 1H), 7.33 (t, J = 8.5 Hz, 1H), 7.6−7.65 (m, 2H), 7.85−7.94 (m, 2H), 10.03 (s, 1H). Anal. ($C_{23}H_{18}BrF_2NO_6$) C, H, N.

7-Bromo-2-(3-fluoro-4-methoxyphenyl)-5-methoxy-1,3-benzoxazole (28; R_1 = 3′-F). A suspension of 2-bromo-6-[(3-fluoro-4-methoxybenzoyl)amino]-4-methoxyphenyl-3-fluoro-4-methoxybenzoate (46.0 g, 88.1 mmol), *p*-toluenesulfonic acid monohydrate (33.5 g, 177.2 mmol), and anhydrous *p*-xylene (1:l) was refluxed for 3 h with continuous water removal (Dean Stark trap). The initial suspension turned into a brown solution at refluxing temperature. The volatiles were removed under vacuum, and the solid residue was treated with NaOH (2 N, 300 mL). The solids then were filtered off and washed with ethyl ether and taken in ethyl ether (200 mL), stirred for 10 min, filtered off and dried to give 7-bromo-2-(3-fluoro-4-methoxyphenyl)-5-methoxy-1,3-benzoxazole as a tan solid (25.1 g, 81% yield, mp 175−177 °C). The ethyl ether layer was concentrated to 20 mL, and the precipitated solid was filtered off to give 2.5 g of additional product. MS *m/e* 352 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 3.8 (s, 3H) 3.95 (s, 3H), 7.24 (d, J = 2.3 Hz, 1H), 7.34 (d, J = 2.3 Hz, 1H), 7.4 (t, J = 8.5 Hz, 1H), 7.9−7.98 (m, 2H). Anal. ($C_{15}H_{11}BrFNO_3$) C, H, N.

7-Bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol (29; R_1 = 3′-F). Boron tribromide (1 M in dichloromethane, 85.2 mL, 85.2 mmol) was added dropwise to a cold (−78 °C) suspension of 7-bromo-2-(3-fluoro-4-methoxyphenyl)-5-methoxy-1,3-benzoxazole (10.0 g, 28.4 mmol) and CH_2Cl_2 (50 mL). The mixture was allowed to warm to room temperature. During the warming up period, the suspension turned into a dark solution. The mixture was stirred at room temperature for 2 days and was then poured slowly into cold (0 °C) ethyl ether (1000 mL). Methyl alcohol (200 mL) was added slowly to the new mixture over a 20-min period. The mixture was then poured into water (1.5 L). The organic layer was washed three times with water and dried over $MgSO_4$. Evaporation and crystallization from acetone/ethyl ether/hexanes gave 7-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol as a white solid (8.6 g, 93% yield): mp 265−267 °C; MS *m/e* 332 $(M - H)^+$; ^{1}H NMR (DMSO-d_6) δ 7.0 (d, J = 2.1 Hz, 1H), 7.04

(d, $J = 2.1$ Hz, 1H), 7.15 (t, $J = 8.4$ Hz, 1H), 7.8−7.83 (m, 2H), 9.9 (br s, 1H), 10.9 (br s, 1H). Anal. ($C_{13}H_7BrFNO_3$) C, H, N

2-(3-Fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (30a; $R_1 = 3'F$; $R_2 =$ Vinyl). Dichlorobis(tri-*o*-tolylphosphine)palladium (II) (0.87 g, 1.1 mmol) was added to a mixture of 7-bromo-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol (7.16 g, 22.1 mmol), tributyl(vinyl)tin (10.5 g, 33.25 mmol), and ethylene glycol diethyl ether (65 mL). The reaction mixture was stirred at 115 °C for 48 h, cooled to room temperature, and treated with activated carbon. The mixture was filtered through $MgSO_4$ and concentrated. Purification by flash chromatography, on acidic silica gel (hexanes/EtOAc/CH_2Cl_2 1:1:1), gave a white solid (4.35 g, 72% yield): mp 250−252 °C; MS *m/e* 272 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 5.6 (d, $J = 11.7$ Hz, 1H), 6.2 (d, $J = 17.2$ Hz, 1H), 6.9−6.9 (m, 2H, vinyl), 6.96 (d, $J = 2.29$ Hz, 1H), 7.13 (t, $J = 8.5$ Hz, 1H), 7.8−7.9 (m, 2H), 9.5(br s, 1H), 10.8 (br s, 1H). Anal. ($C_{15}H_{10}FNO_3$) C, H, N.

5-Methoxy-2-(4-methoxyphenyl)-7-phenyl-1,3-benzoxazole (30b, $R_2 =$ Ph, $R_1 =$ H, Dimethoxy Intermediate). 7-Bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (200 mg, 0.60 mmol) and tetrakis(triphenylphosphine)palladium (63 mg, 0.03 mmol) were dissolved in toluene (5 mL) and stirred for 10 min at room temperature under a nitrogen atmosphere. Benzene boronic acid (110 mg, 0.90 mmol) was added followed by aqueous sodium carbonate (2 M, 1.5 mL) and ethanol (2 mL). The mixture was refluxed for 12 h, diluted with water, and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (20−40% EtOAc/petroleum ether) gave a light-pink solid: mp 92 °C; MS *m/e* 332 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.86 (s, 3H), 3.88 (s, 3H), 7.15 (d, $J = 9.0$ Hz, 2H), 7.2 (d, $J = 2.44$ Hz, 1H), 7.3 (d, $J = 2.44$ Hz, 1H), 7.46 (t, $J = 7.32$ Hz, 1H), 7.57 (t, $J = 7.63$ Hz, 2H), 7.98 (d, $J = 8.39$ Hz, 2H), 8.12 (d, $J = 9.0$ Hz, 2H). Anal. ($C_{21}H_{17}NO_3$) C, H, N.

2-(4-Hydroxyphenyl)-7-methoxy-1,3-benzoxazol-5-ol (30c; $R_2 =$ OMe). A mixture of 7-bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (100 mg, 0.33 mmol) and copper(I) bromide (56 mg, 0.39 mmol) in anhydrous *N*,*N*-dimethylformamide (1.5 mL) was stirred with freshly prepared sodium methoxide (15 wt % in methanol, 1 mL) and heated to 120 °C for 4 h. The mixture was cooled and diluted with HCl (1 N, 5 mL). Isolation of the crude product with ethyl acetate followed by flash chromatography (40−50% EtOAc/petroleum ether) gave the title compound as an off-white solid (50 mg, 60% yield): mp 225−228 °C; MS *m/e* 258 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 3.92 (s, 3H), 6.43 (s, 1H), 6.60 (s, 1H), 6.92 (d, $J = 8.5$ Hz, 2H), 7.95 (d, $J = 8.4$ Hz, 2H), 9.44 (br s, 1H), 10.2 (br s, 1H). Anal. ($C_{14}H_{11}NO_4 \cdot 0.75H_2O$) C, H, N.

2-(3-Fluoro-4-hydroxyphenyl)-7-(1-fluorovinyl)-1,3-benzoxazol-5-ol (31). Step a. Acetic anhydride (0.46 mL, 4.8 mmol) was added to a cold (10 °C) solution of 2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (0.5 g, 1.84 mmol), *N*,*N*-dimethylpyridin-4-amine (0.54 g, 4.43 mmol), and 1,4-dioxane (10 mL). The reaction mixture was allowed to warm to room temperature and stirred for 48 h. Water (50 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc and dried over $MgSO_4$. Evaporation and crystallization from EtOAc/hexane gave 4-[5-(acetyloxy)-1,3-benzoxazol-2-yl]- 7-vinyl-2-fluorophenyl acetate as an off-white solid (0.56 g, 86% yield): MS *m/e* 355 $(M)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.3 (s, 3H), 2.38 (s, 3H), 5.69 (d, $J = 11.45$ Hz, 1H), 6.38 (d, $J = 17.86$ Hz, 1H), 6.99 (m, 1H), 7.34 (d, $J = 1.98$ Hz, 1H), 7.54 (d, $J = 2.14$ Hz, 1H), 7.57 (t, $J = 8.24$ Hz, 1H), 8.13 (dd, $J = 8.4, 0.91$ Hz, 1H), 8.19 (dd, $J = 10.68, 1.22$ Hz, 1H). Anal. ($C_{19}H_{14}FNO_5$) C, H, N.

Step b. Hydrogen fluoride pyridine (1.14 mL) was added dropwise to a cold (0 °C) solution of 2-[4-(acetyloxy)-3-fluorophenyl]-7-vinyl-1,3-benzoxazol-5-yl acetate (0.25 g, 0.7 mmol) in sulfolane (3 mL). The reaction mixture was stirred for 5 min, and then 1,3-dibromo-5,5-dimethylimidazolidine-2,4-dione (120 mg) was added in one portion. The mixture was stirred at room temperature for 24 h, diluted with HCl (1 N), and extracted with EtOAc. The organic layer was dried over $MgSO_4$. Evaporation and purification by flash chromatography (CH_2Cl_2/isopropyl alcohol 0.3%) gave 7-(2-bromo-1-fluoroethyl)-2-(3-fluoro-4-hydroxyphenyl)-1,3-benzoxazol-5-ol as a white solid (0.25 g, mp 185−186 °C). The product was taken in acetonitrile (2 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (150 mg) was added. The reaction mixture was stirred for 24 h, poured into cold (0 °C) HCl (1 N, 10 mL), and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (20% EtOAc/hexanes) gave a white solid (160 mg): mp 213-214 °C): MS *m/e* 290 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.32 (dd, $J = 19.4, 3.88$ Hz, 1H), 5.69 (dd, $J = 53, 3.88$ Hz, 1H), 7.12 (m, 2H), 7.91 (m, 2H), 9.78 (s 1H), 10.84 (s, 1H). Anal. ($C_{15}H_9BrF_2NO_3 \cdot 0.3H_2O$) C, H, N.

7-Ethynyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (32a; $R_3 =$ Ethynyl). Tetrakis(triphenylphosphine)palladium (52 mg, 0.045 mmol) was added to a mixture of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (0.3 g, 0.9 mmol), copper(I) iodide (17.1 mg, 0.09 mmol), ethynyl(trimethyl)silane (0.2 g mg, 2 mmol), and triethylamine (12 mL). The mixture was stirred at 110 °C for 4 h, poured into aqueous ammonium chloride, and extracted with EtOAc/THF (1:1). The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 6:1) gave an off-white solid (0.27 g, 85% yield). The product was dissolved in CH_2Cl_2 (2 mL) and cooled to −78 °C, and boron tribromide (0.6 mL) was added dropwise. The mixture was allowed to warm to room temperature. After stirring for 18 h at room temperature, the mixture was slowly poured into cold (0 °C) ethyl ether (10 mL). Methyl alcohol (3 mL) was then slowly added to the mixture. The new mixture was washed with water (three times) and dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 3:1) gave a yellow solid (86 mg, 38% yield): mp 229−231 °C; MS *m/e* 252 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.57 (s, 1H), 6.84 (d, $J = 2.29$ Hz, 1H), 6.94 (d, $J = 8.55$ Hz, 2H), 7.09 (d, $J = 2.29$ Hz, 1H), 7.99 (d, $J = 8.7$ Hz, 2H), 9.69 (s, 1H), 10.3 (s, 1H). Anal. ($C_{15}H_9NO_3$) C, H, N.

5-Methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-carbonitrile (32b; $R_3 =$ CN, Dimethoxy Intermediate). A solution of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (200 mg, 0.60 mmol) in anhydrous *N*,*N*-dimethylformamide (1.5 mL) was stirred and heated to reflux under dry nitrogen with copper(I) cyanide (80 mg, 0.90 mmol) for 4 h. The mixture was cooled and poured into an excess of aqueous ethylenediaminetetraacetic acid. Isolation of the crude product gave the nitrile (164 mg, 98% yield) as tan needles from (30% EtOAc/petroleum ether): mp 180−183 °C; MS *m/e* 281 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.86 (s, 3H), 3.87 (s, 3H), 7.17 (d, $J = 8.85$ Hz, 2H), 7.51 (d, $J = 2.44$ Hz, 1H), 7.71 (d, $J = 2.44$ Hz, 1H), 8.13 (d, $J = 8.85$ Hz, 2H). Anal. ($C_{16}H_{12}N_2O_3 \cdot 0.2H_2O$) C, H, N.

2-(4-Hydroxyphenyl)-7-propyl-1,3-benzoxazol-5-ol (32c; $R_3 =$ Propyl). Tetrakis(triphenylphosphine)palladium (70 mg, 0.06 mmol) was added to a mixture of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (0.4 g, 1.2 mmol), bromo(propyl)zinc (0.5 M in THF, 3.6 mL, 1.8 mmol), and THF (4 mL). The mixture was stirred at room temperature for 48 h, poured into HCl (1 N), and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 6:1) gave an off-white solid (0.14 g). The product was dissolved in CH_2Cl_2 (2 mL) and cooled to −78 °C, and boron tribromide (0.35 mL) was added dropwise. The mixture was allowed to warm to room temperature. After stirring for 18 h at room temperature, the mixture was slowly poured into cold (0 °C) ethyl ether (10 mL). Methyl alcohol (3 mL) was then slowly added to the mixture. The new mixture was washed with water (three times) and dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 4:1) gave a white solid (90 mg, 27% yield): mp 110−112 °C; MS *m/e* 270 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 0.93 (t, $J = 7.52$ Hz, 3H), 1.73 (q, $J = 7.32$ Hz, 2H), 2.78 (t, $J = 7.32$ Hz, 2H), 6.6 (d, $J = 2.14$ Hz, 1H), 6.83 (d, $J = 2.29$ Hz, 1H), 6.94 (d, $J = 8.7$ Hz, 2H),

7.96 (d, J = 8.85 Hz, 2H), 9.3 (s, 1H), 10.23 (s, 1H). Anal. ($C_{16}H_{15}NO_3$) C, H, N.

7-Ethyl-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (32d: R_3 = Ethyl). Step a. *n*-Butyllithium (2.5 N, 0.43 mL, 1.08 mmol) was added dropwise to a cold (−78 °C) mixture of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (300 mg, 0.90 mmol) and THF (2 mL). The mixture was allowed to stir for 0.5 h. Iodoethane (0.14 mL, 1.8 mmol) was added dropwise to the mixture. The reaction mixture was allowed to warm to room temperature and was stirred for 2 h. The reaction was quenched with aqueous ammonium chloride, poured into water, and extracted with EtOAc. The organic extracts were washed with brine and dried over $MgSO_4$. Evaporation and flash chromatography (20% EtOAc/petroleum ether) gave (231 mg, 91% yield) 7-ethyl-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole as a light-brown solid: mp 85 °C; MS *m/e* 284 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.32 (t, J = 7.5 Hz), 2.88 (q, J = 7.5 Hz, 2H), 3.79 (s, 3H), 3.85 (s, 3H), 6.81 (s, 1H), 7.11 (s, 1H), 7.14 (d, J = 9.0 Hz, 2H), 8.10 (d, J = 9.0 Hz, 2H). Anal. ($C_{17}H_{17}NO_3 \cdot 0.2H_2O$) C, H, N.

Step b. Boron tribromide (0.31 mL, 3.2 mmol) was added dropwise to a cold (−78 °C) mixture of 7-ethyl-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (181 mg, 0.54 mmol) and dichloromethane (1.5 mL). The reaction mixture was allowed to come gradually to room temperature and was stirred for 1 h. The mixture was poured over ice and was extracted with EtOAc. The organic extracts were washed with brine and dried over $MgSO_4$. Evaporation and flash chromatography (30%-40% EtOAc/petroleum ether) gave of the product as a light-brown solid (136 mg, 98% yield): mp 110−115 °C; HRMS (ESI) $(M + H)^+$ anal. calcd for $C_{15}H_{13}NO_3$ 256.0966, found 256.0973; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.28 (t, J = 7.8 Hz, 3H), 2.82 (q, J = 7.5 Hz, 2H), 6.62 (s, 1H), 6.83 (s, 1H), 6.93 (d, J = 8.4 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H), 9.31 (br s, 1H), 10.2 (br s, 1H).

Ethyl 5-hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazole-7-carboxylate (32d; $R_3 = CO_2Et$). Step a. *tert*-Butyl(chloro)-dimethylsilane (3.39 g, 22.5 mmol) was added portionwise to a mixture of 7-bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (2.3 g, 7.5 mmol), imidazole (2.55 g, 37.5 mmol), *N*,*N*-dimethylpyridin-4-amine (0.15 g, 1.21 mmol), and DMF (300 mL). The mixture was stirred for 3 h, poured into water, and extracted with ethyl ether. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 50/1) gave 7-bromo-5-{[*tert*-butyl-(dimethyl)silyl]oxy}-2-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1,3-benzoxazole as a white solid (3.85 g, 96% yield): mp 90−91 °C; MS *m/e* 534 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.22 (s, 6H), 0.25 (s, 6H), 1.0 (s, 18H), 6.95 (d, J = 8.7 (Hz, 2H), 6.98 (d, J = 2.14 (Hz, 1H), 7.1 (d, J = 2.14 (Hz, 1H), 7.25 (s, 2H), 8.12 (d, J = 8.7 (Hz, 2H). Anal. ($C_{25}H_{36}BrNO_3Si_2$) C, H, N.

Step b. *n*-Butyllithium (2.5 M, 0.3 mL, 0.75 mmol) was added dropwise into a cold (0 °C) solution of 7-bromo-5-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(4-{[*tert*-butyl(dimethyl)silyl]oxy}-phenyl)-1,3-benzoxazole (0.4 g, 0.75 mmol) and THF (4 mL). The mixture was allowed to warm to 40 °C and was then stirred for 2 h. [(Cyanocarbonyl)oxy]ethane (84 mg) in THF (1 mL)] was added to the reaction mixture, and the reaction mixture was allowed to warm to 0 °C and was stirred for 1 h. The reaction was quenched with aqueous ammonium chloride, extracted with EtOAc, and dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/CH_2Cl_2/isopropyl alcohol 18:2:1) gave a colorless oil (340 mg). The product was dissolved in THF (3.5 mL) and treated with tetrabutylammonium fluoride (1 M in THF, 1.4 mL). The mixture was stirred for 30 min, poured into HCl (1 N), and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/CH_2Cl_2 /isopropyl alcohol 5:2:1) gave a white solid (119 mg, 53% yield): mp 305−307 °C; MS *m/e* 300 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.4 (t, J = 7.18 Hz, 3H), 4.4 (q, J = 7.02 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 7.29 (s, 2H), 8.0 (d, J = 8.85 (Hz, 2H), 9.92 (br s, 1H), 10.36 (br s, 1H). Anal. ($C_{16}H_{13}NO_5$) C, H, N.

2-(4-Hydroxyphenyl)-7-isopropenyl-1,3-benzoxazol-5-ol (32e). *n*-Butyllithium (2.5 N, 0.72 mL, 1.80 mmol) was added dropwise to a cold (−78 °C) mixture of 7-bromo-5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole (500 mg, 1.49 mmol) and THF (5 mL). The mixture was allowed to stir for 0.5 h. Acetone (0.22 mL, 2.99 mmol) was added dropwise to the mixture. The reaction mixture was allowed to warm to room temperature and was stirred for 2 h. The reaction was quenched with aqueous ammonium chloride, poured into water, and extracted with EtOAc. The organic extracts were washed with brine and dried over $MgSO_4$. Evaporation and flash chromatography (20−40% EtOAc/petroleum ether) afforded a white solid (366 mg, 78% yield, mp 149 °C). A portion of the product (114 mg, 0.36 mmol) was added to a melt of pyridine hydrochloride (400 mg) heated to 190 °C, and the reaction was stirred for 2 h. The mixture was cooled to room temperature, dissolved in water, and extracted with EtOAc. The organic layers were combined and washed with HCl (1 N), water, and then brine and were dried over $MgSO_4$. Evaporation and purification by flash chromatography (50%−60% EtOAc/petroleum ether) gave the product (40 mg, 41% yield) as a light red-brown solid: mp 225−228 °C; MS *m/e* 268 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H), 5.45 (s, 1H), 5.89 (s, 1H), 6.81 (s, 1H), 6.94 (s, 1H), 6.95 (s, 2H), 7.98 (d, J = 8.9 Hz, 2H), 9.46 (br s, 1H), 10.3 (br s, 1H). Anal. ($C_{16}H_{13}NO_3 \cdot 0.5H_2O$) C, H, N.

2-(4-Hydroxyphenyl)-7-isopropyl-1,3-benzoxazol-5-ol (32f). 2-(4-Hydroxyphenyl)-7-isopropenyl-1,3-benzoxazol-5-ol (64 mg, 0.24 mmol) was dissolved in a mixture of EtOAc (5 mL) and absolute ethanol (5 mL) and placed under an inert atmosphere with argon. To this mixture was added 10% Pd−C (25 mg). The solution was hydrogenated on a Parr apparatus at 25 psi for 3 h. The solution was filtered through celite and rinsed with ethanol. The filtrate was concentrated, and the residue purified by flash chromatography (50% EtOAc/petroleum ether) to give the product (58 mg, 90% yield) as a tan solid: mp 200 °C; HRMS (ESI) $(M + H)^+$ anal. calcd $C_{16}H_{15}NO_3$ 270.1130, found 270.1119; ^{1}H NMR (DMSO-d_6) δ 1.33 (d, J = 7.0 Hz, 6H), 3.24 (m, 1H), 6.63 (s, 1H), 6.84 (s, 1H), 6.93 (d, J = 8.9 Hz, 2H), 7.97 (d, J = 8.7 Hz, 2H), 9.31 (br s, 1H), 10.2 (br s, 1H).

7-(Bromomethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (100). The title compound was prepared according to the above example from 5-methoxy-7-(hydroxymethyl)-2-(4-methoxyphenyl)-1,3-benzoxazole (200 mg) with prolonged stirring in the presence of boron tribromide (1 M in dichloromethane, 10 mL) and was obtained as a light-brown solid: mp 250−260 °C (dec); MS *m/e* 321 $(M + H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 4.89 (s, 2H), 6.85 (d, J = 2.29 Hz, 1H), 6.96 (d, J = 8.85 Hz, H), 6.99 (d, J = 2.4 Hz, 1H), 8.02 (d, J = 8.85 Hz, 2H), 9.58 (br s, 1H), 1.031 (br s, 1H). Anal. ($C_{14}H_{10}BrNO_3$) C, H, N.

[5-Hydroxy-2-(4-hydroxyphenyl)-1,3-benzoxazol-7-yl] acetonitrile (101). To a solution of 7-(bromomethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (122 mg, 0.40 mmol) in *N*,*N*-dimethylformamide (1.5 mL) was added 18-crown-6 ether (202 mg, 0.80 mmol) and potassium cyanide (131 mg, 2 mmol). The reaction mixture was allowed to stir for 2 h and was then poured into water and extracted with EtOAc. The organic extracts were washed with brine and dried over $MgSO_4$. Evaporation and flash chromatography (50%-60% EtOAc/petroleum ether) gave the product (80 mg, 75% yield) as a gray solid: mp 170−180 °C; MS *m/e* 265 $(M - H)^+$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 4.29 (s, 2S), 6.79 (d, J = 2.14 Hz, 1H), 6.96 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 2.16 (Hz, 1H), 7.99 (D, J = 8.7 Hz, 2H), 9.62 (s, 1H), 10.3 (s, 1H). Anal. ($C_{15}H_{10}N_2O_3 \cdot 1.5H_2O$) C, H, N.

5-Hydroxy-7-(hydroxymethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazole (102). Sodium borohydride (66.8 mg, 1.76 mmol) was added to a solution of 5-methoxy-2-(4-methoxyphenyl)-1,3-benzoxazole-7-carbaldehyde (250 mg, 0.88 mmol) in anhydrous MeOH (8 mL) at 0 °C. The reaction mixture was stirred for 30 min and then evaporated in vacuum. The residue was dissolved in diethyl ether and washed with water and

brine, dried over $MgSO_4$, and filtered. Evaporation and flash chromatography (50% EtOAc/petroleum ether) gave the product (210 mg, 83%), which was taken in dichloromethane (1.5 mL), cooled to −78 °C and treated with boron tribromide (0.25 mL, 2.7 mmol). The reaction mixture was allowed to come to room temperature gradually and was stirred for 1 h. The mixture was poured over ice and extracted with EtOAc. The organic extracts were washed with brine and dried over $MgSO_4$. Evaporation and flash chromatography (30−40% EtOAc/petroleum ether) gave the product (102 mg, 86% yield) as a light-brown solid: mp 282 °C (dec); MS *m/e* 258 $(M + H)^+$; ^1H NMR (DMSO-d_6, 400 MHz) δ 4.73 (d, $J = 5.65$ Hz, 2H), 5.34 (t, $J = 5.8$ Hz, 1H), 6.82 (d, $J = 2.29$ Hz, 1H), 6.88 (d, $J = 2.29$ Hz, 1H), 6.92 (d, $J = 8.85$ Hz, 2H), 7.97 (d, $J = 8.85$ Hz, 2H), 9.36 (s, 1H), 10.24 (s, 1H). Anal. ($C_{14}H_{11}NO_4 \cdot 0.5H_2O$) C, H, N.

7-(1-Bromovinyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (120). Step a. Boron tribromide (0.85 mL, 8.95 mmol) was added dropwise to a cold (−78 °C) mixture of 5-methoxy-2-(4-methoxyphenyl)-7-vinyl-1,3-benzoxazole (0.31 g, 1.12 mmol) and CH_2Cl_2 (4 mL). The mixture was allowed to warm to room temperature. After stirring for 18 h at room temperature, the mixture was slowly poured into cold (0 °C) ethyl ether (20 mL). Methyl alcohol (10 mL) was then slowly added to the mixture. The new mixture was washed with water (three times) and dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc 3:1) gave 7-(1,2-dibromoethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol as a light-yellow solid (0.27 g, 59% yield): mp 175−177 °C; MS *m/e* 412 $(M + H)^+$; ^1H NMR (DMSO-d_6, 400 MHz) δ 4.33 (m, 1H), 4.54 (t, $J = 9.85$ Hz, 1H), 5.78 (m, 1H), 6.93 (m, 3H), 7.03 (d, $J = 2.14$ Hz, 1H), 8.04 (m, 2H), 9.64 (s, 1H), 10.31 (s, 1H). Anal. ($C_{15}H_{11}Br_2NO_3$) C, H, N.

Step b. 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.25 g, 1.65 mmol) was added to a solution of 7-(1,2-dibromoethyl)-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (0.4 g, 0.96 mmol) and acetonitrile (4 mL). The reaction mixture was stirred for 24 h, poured into cold (0 °C) HCl (1 N, 10 mL), and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (CH_2Cl_2/hexanes/isopropyl alcohol 15:5:1) gave a white solid (185 mg, 58% yield): mp 228−230 °C; MS *m/e* 332 $(M + H)^+$; ^1H NMR (DMSO-d_6, 400 MHz) δ 6.26 (d, $J = 2.59$ (Hz, 1H), 6.84 (d, $J = 2.74$ Hz, 1H), 6.96 (d, $J = 8.7$ Hz, 2H), 7.01 (d, $J = 2.29$ Hz, 1H), 7.05 (d, $J = 2.29$ Hz, 1H), 8.01 (d, $J = 8.85$ Hz, 2H), 9.7 (s, 1H), 10.31 (s, 1H). Anal. ($C_{15}H_{10}BrNO_3$) C, H, N.

4-Bromo-2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (135) and 4,6-Dibromo-2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (136). *N*-Bromosuccinimide (0.49 g, 2.77 mmol) was added to a mixture of 2-(3-fluoro-4-hydroxyphenyl)-7-vinyl-1,3-benzoxazol-5-ol (0.75 g, 2.77 mmol) and acetonitrile (30 mL). The reaction mixture was stirred at room temperature for 16 h, poured into water, and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation and purification by flash chromatography (hexanes/EtOAc /CH_2Cl_2 2:1:1) gave **135** as a white solid (0.45 g): mp 226−228 °C; MS *m/e* 349 $(M + H)^+$; ^1H NMR (DMSO-d_6, 400 MHz) δ 5.65 (d, $J = 11.4$ Hz, 1H), 6.23 (d, $J = 17.7$ Hz, 1H), 6.87 (m, 1H), 7.0 (s, 1H), 7.15 (t, $J = 8.7$ Hz, 1H), 7.92 (m, 2H), 10.23 (s, 1H), 10.86 (s, 1H). Anal. ($C_{15}H_9BrFNO_3$) C, H, N. It also gave **136** as a white solid (0.18 g): mp 272−274 °C; MS *m/e* 428 $(M + H)^+$; ^1H NMR (DMSO-d_6, 400 MHz) δ 5.89 (d, $J = 12.5$ Hz, 1H), 6.23 (d, $J = 17.8$ Hz, 1H), 6.99 (m, 1H), 7.0 (s, 1H), 7.15 (t, $J = 8.55$ Hz, 1H), 7.89 (m, 2H), 10.22 (s, 1H), 10.92 (s, 1H). Anal. ($C_{15}H_8Br_2FNO_3$) C, H, N.

Biological Methods. Competitive Radioligand Binding Assay. This assay was performed as previously described.[31] Briefly, human ERα and ERβ ligand-binding domains were expressed in *Escherichia coli*, and a crude cell lysate of these cells was used in a solid-phase binding assay. The radioligand was [^{3}H]-17β-estradiol, a ligand known to bind equally well to ERα and ERβ.

Cell-Based Transcriptional Assays. The regulation of metallothionein-II[33] and IGFBP-4 mRNA[8] was monitored in SAOS-2 cells engineered with an adenovirus to express human ERβ. Briefly, cells were treated for 24 h with 1 μM test compound or 10 nM 17β-estradiol, and mRNA levels were measured by real-time quantitative RT-PCR.

Rat Uterotrophic Assay. This assay was performed as previously described.[8] Briefly, sexually immature rats (19 days old) were treated with the compound daily for 3 days and euthanized 24 h after the last dose. The vehicle was 50% DMSO/50% 1× Dulbecco's phosphate-buffered saline, and 0.2-mL doses were administered subcutaneously. The doses given are expressed in milligrams or micrograms per rat per day because the rats typically grow from 38 to 55 g during the course of the study. Thus, the ERβ-selective compound dose of 2 mg/rat translates into 36−53 mg/kg, and the ethynyl estradiol dose of 0.06 μg/rat ranges from 1.1 to 1.6 μg/kg.

Mouse Uterotrophic Assay. Sexually immature (18 d) wild type 129 Sv/Ev mice were either purchased from Taconic Farms (Germantown, NY) or obtained from our in-house ERβ knockout-mouse breeding colony.[35] Compounds were obtained from the Wyeth compound library, solubilized in 100% DMSO, and used in a vehicle of 5% DMSO/95% corn oil. Mice (six per group) were injected subcutaneously with 0.1 mL of solution for 4 days (aged 21−24 days) at a dose based on their weight at the start of the study. At necropsy, uteri were excised, trimmed of associated fat, drained of any internal fluid, and weighed. For all studies, the dose of 17β-estradiol was 50 mg/kg, which is a supramaximal dose.

Rat Vasomotor Instability Assay. This assay was performed as previously described.[37]

Antiandrogenic Assay. Immature male Sprague−Dawley rats, castrated by the vendor (Taconic Farms, NY) at 40−45 g body weight (~22 days old) were shipped 2 days after surgery and were acclimated for 5 days prior to the beginning of study. The animals were maintained on a diet of standard lab chow and water. Beginning 7 days after castration, ERB-041 was administered once daily for 10 consecutive days at 20 mg/kg, subcutaneously in a volume of 0.2 mL per rat in a vehicle of 50% DMSO/50% 1× Dulbecco's phosphate-buffered saline with a concurrent dose (0.8 mg/kg) of the reference androgen testosterone propionate. Flutamide was orally administered at 10 mg/kg as a positive antiandrogen control. Approximately 24 h after the final dosing, animals were euthanized by CO_2 asphyxiation. The ventral prostate was excised, cleaned of extraneous tissue, blotted on filter paper, and weighed.

HLA-B27 Transgenic Rat. This model was performed as previously described,[7] except that rats in the present study were 8−10 weeks of age at the start of the study.

X-ray Crystallography: Expression of ERβ LBD. Human ERβ cDNA was generated from human-testis RNA by RT-PCR and cloned into mammalian expression-vector pcDNA3. Amino acids 261−500 of the LBD were amplified from the cloned cDNA by PCR with the forward primer 5′-GAACCATGGACGACGCCCTGAGCCCCGAGCAGCTAGTG-3′ and the reverse primer 5′-GGACTCGAGTTAGTCGTCAAGCACGTGGGCATTCAGCATCTC-3′. The PCR fragment was inserted into *E. coli* expression-vector pET16b (Novagen) between the NcoI and XhoI restriction sites. The primers used encode three extra asp codons, one before the codon for D_{261} and two after L_{500}. The expressed LBD thus has the following sequence: MD-[D_{261}−L_{500}]DD. ERβ LBD was overexpressed from a high-density culture of *E. coli* BL21DE3 host cells (Stratagene) in a Biostat C-10 bioreactor (B. Braun Biotech). Cultures were induced with 1.0 mM IPTG final for 4 h at 37 °C. Cell pellets were quick frozen in liquid nitrogen prior to storage at −80 °C.

Purification of ERβ LBD. Harvested cells were lysed by two cycles of French press (SLM Instrument) at 20 000 psi in a buffer of 20 mM tris−Cl pH 7.5, 0.5 M NaCl, 5 mM DTT, and 1 mM EDTA (10 mL/g of cells). Lysate was clarified by centrifugation at 45 000*g* for 45 min at 4 °C and then applied to a Q Sepharose (Pharmacia) column. The flow through was then applied to a 5-mL estradiol−Sepharose fast flow column (PTI Research, Inc.) and washed with 300 mL of 10 mM tris−HCl, pH 7.5 containing 0.5 M NaCl and 1 mM EDTA (buffer

A). The column was then re-equilibrated with 50 mL of 10 mM tris−HCl, pH 7.5, 0.2 M NaCl and 1 mM EDTA (buffer B), and then the protein was carboxymethylated using 50 mL of buffer B containing 5 mM iodoacetic acid. The column was then washed with 500 mL of buffer A, followed by elution in buffer A containing 200 μM ligand. Finally, the eluate was concentrated by ultrafiltration and size-exclusion chromatography (Sephadex 200, Pharmacia) using the elution buffer containing 5 μM ligand. The purity was estimated to be ≥98% by SDS-PAGE. The excess amount of ligand was removed by passing the solution through a G-25 column (Pharmacia).

Docking Calculations. Docking calculations were performed using the QXP software package.[36] The QXP Monte Carlo docking algorithm mcdock was used to generate potential binding modes in the active site. In general, 1000 Monte Carlo steps was sufficient for the poses and their energy scores to converge. Constrained-residue flexibility was utilized to take subtle movements of the pocket into account when necessary. Visualization of X-ray structures and docking results was performed using the InsightII and Quanta software package (Accelrys, Inc., San Diego, CA).

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Supporting Information Available: X-ray crystallographic data (collection details, refinement statistics, related references) and analytical data of intermediates or final compounds not listed in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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