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Estrogen receptor β-subtype selective tetrahydrofluorenones: Use of a fused pyrazole as a phenol bioisostere

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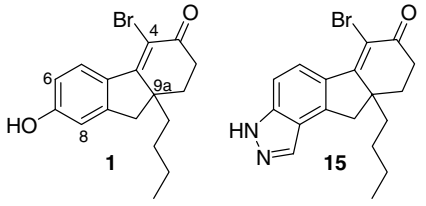
Abstract—Synthesis of a series of fused pyrazole tetrahydrofluorenone analogs which are potent, ERβ subtype selective ligands is described. Analogs possessing subnanomolar ERβ binding, greater than 100-fold ERβ-selectivity, and oral bioavailability are reported.

The physiological effects of estrogen are mediated through the estrogen receptor subtypes ERα[1,2] and ERβ.[3,4] While our understanding of the complex role that these receptors play in the body is incomplete, it is clear that decreasing levels of 17β-estradiol during peri-menopause are associated with bone loss, cognitive impairment, and hot flashes.[5] Hormone replacement therapy (HRT) provides relief from these symptoms but does so with an increased risk of breast and uterine cancer as well as an increase in the incidence of coronary heart disease and stroke.[6]

The observation that 17β-estradiol binds ERα and ERβ receptors with equal affinity, coupled with the differential distribution of these receptors throughout the body,[7,8] provides an intriguing possibility that tissues can be targeted with receptor selective ligands. For example, ERβ is expressed in the lung, prostate, and brain but is not the predominant receptor in the uterus or breast tissues. Consequently, a β-selective ligand could specifically target tissues which express ERβ while minimizing the risks associated with the proliferative effects of 17β-estradiol in tissues, such as the breast and uterus, which predominately express ERα.

The identification of an ER subtype selective ligand is further complicated by the structural similarity of these receptors. In particular, the binding pockets of ERα and ERβ were observed to differ by only two amino acid residues.[4] In the ERβ binding pocket, Met336 was replaced by Leu384 and Ile373 was replaced by Met421. In spite of the subtle changes in receptor isoforms, selective ERα[7,9,10] and ERβ[11–18] ligands have been described in the literature. We recently reported a new class of potent, ERβ selective phenolic tetrahydrofluorenone analogs which are exemplified by analog **1**.[19] In this paper, we will describe modifications to **1** aimed at improving the ERβ binding, β-selectivity, and pharmacokinetic properties (PK) of these compounds.



Keywords: Estrogen; Selective; Biosostere; Tetrahydrofluorenone.
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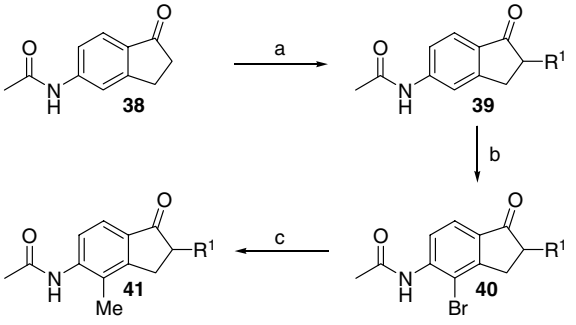
Analog **1** displayed poor oral bioavailability and high plasma clearance rates in rats. The poor PK was, presumably, the result of first-pass glucuronidation of the phenol group followed by rapid elimination from the rat. Several approaches aimed at improving the PK of these analogs were investigated. They included the synthesis of a series of 6- and 8-substituted analogs in an attempt to sterically minimize glucuronidation of the phenol, the synthesis of prodrug derivatives of the phenol, and replacement of the phenol by a fused pyrazole ring.[20] The results from the first approach, that substitution at the 8-position was found to be preferable to substitution at the 6-position, helped define regioisomer[21] **15** as the target for the third approach.[22] The syntheses of the pyrazole analogs **2**–**37** follow the procedures described in Schemes 1–4.

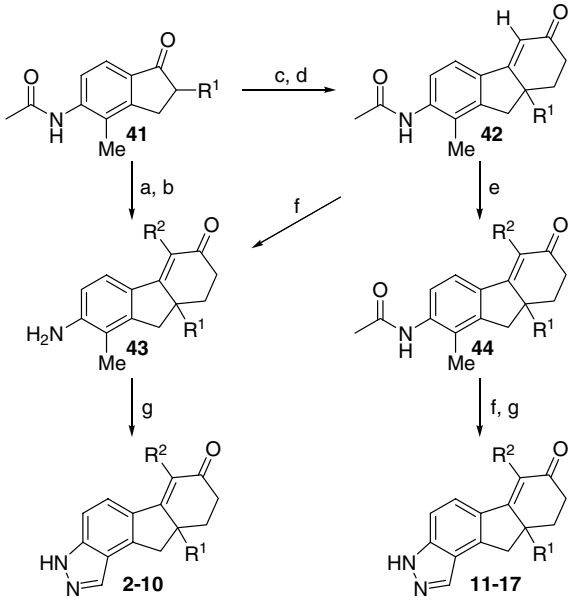
Preparation of the requisite 2-substituted indanone **41** is described in Scheme 1. Reductive alkylation of **38** provided the monoalkylated product **39** in high yield.[23] Bromination of **39** with NBS in dimethylformamide gave an approximately 85:15 mixture of the 4- and 6-regioisomers from which the 4-isomer **40** was obtained by crystallization from acetonitrile. Conversion of **40** into the 4-methyl indanone **41** was accomplished under Stille reaction conditions.

Scheme 2 describes the Robinson annulation of **41** with alkyl vinyl ketones and cyclization under acidic conditions to provide the racemic tetrahydrofluorenone platform. Michael reaction of **41** with methyl vinyl ketone and cyclization of the addition product with pyrrolidine/acetic acid gave analog **42**. The halogenation of the enone function of **42** provided the chloro and bromo derivatives **44**. The addition product of **41** and ethyl vinyl ketone or propyl vinyl ketone was cyclized in a mixture of acetic acid/6 N HCl, with loss of the acetyl group, to provide aniline **43**.

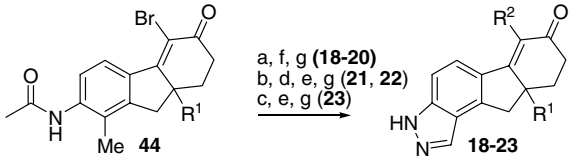
A one-pot nitrosation and concomitant cyclization[24] of anilines **43**, and **44** after basic hydrolysis of the acetyl group, with nitrosonium tetrafluoroborate, potassium acetate, and dibenzo-18-crown-6 provided the racemic pyrazole analogs **2**–**17**. Chiral resolution[25] of selected racemic pyrazole products gave enantiomers A and B.



Scheme 1. Reagents and conditions: (a) H_2, RCHO, KOH or NaOH, 10% Pd/C, EtOH; (b) NBS, DMF; (c) $SnMe_4$, $PdCl_2(PPh_3)_2$, PPh_3, LiCl, DMF.



Scheme 2. Reagents and conditions: (a) EVK or PVK, NaOMe, MeOH; (b) HOAc, 6 N HCl; (c) MVK, NaOMe, MeOH; (d) pyrrolidine, HOAc, toluene; (e) NCS, DMF or Br_2, $NaHCO_3$, CH_2Cl_2; (f) NaOMe, MeOH; (g) $NOBF_4$, KOAc, dibenzo-18-crown-6, CH_2Cl_2.

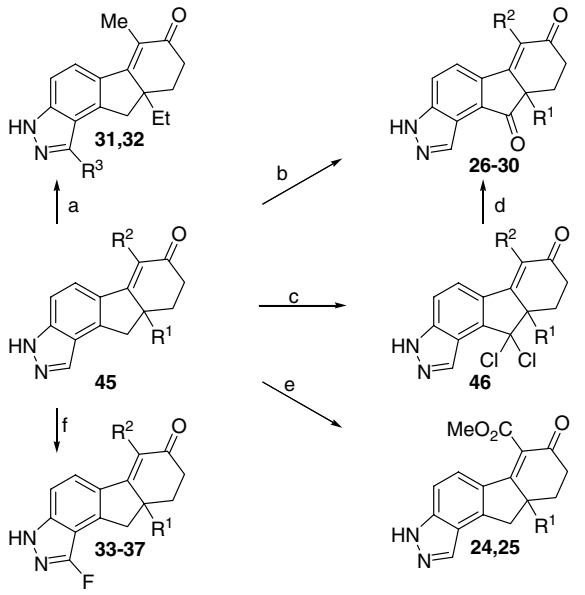


Scheme 3. Reagents and conditions: (a) $FSO_2CF_2CO_2CH_3$, CuI, DMF; (b) Me_3Sn(vinyl), $PdCl_2(PPh_3)_2$, toluene; (c) $C_6H_5B(OH)_2$, $PdCl_2(PPh_3)_2$, (d) CH_2N_2, Et_2O; (e) NaOMe, MeOH; (f) HCl, EtOH; (g) $NOBF_4$, KOAc, dibenzo-18-crown-6, CH_2Cl_2.

Derivatization of the bromo enone **44** is described in Scheme 3. Introduction of a trifluoromethylgroup was accomplished using copper(I) mediated chemistry.[26] Phenyl and vinyl groups were introduced using Stille chemistry. The vinyl group was then converted into a cyclopropyl ring by reaction with excess diazomethane in ether. Removal of the acetyl group was followed by pyrazole ring formation and chiral resolution to afford **18**–**23**.

Solvolysis of the trifluoromethyl group **45** under basic conditions gave the esters **24** and **25** (see Scheme 4). The reaction apparently proceeds via deprotonation of the pyrazole nitrogen followed by conjugative elimination of fluoride from the trifluoromethyl group. Methanol or water then adds to the vinyl fluoride intermediate and the process is repeated until the ester is formed. Interestingly, there is no evidence for the formation of the acid product which would be expected from the addition of two moles of water.

Halogenation of the pyrazolo tetrahydrofluorenone **45** was examined under a variety of reaction conditions



Scheme 4. Reagents and conditions: (a) NCS or Br_2, NaOH, EtOH; (b) (R^2 = Cl or Br), $K_2S_2O_8$, MeCN, H_2O; (c) (R^2 = CF_3 or CH_3), NCS, CH_2Cl_2; (d) silica gel or HCl, EtOH; (e) (R^2 = CF_3), NaOH, MeOH; (f) XeF_2,MeCN.

(see Scheme 4). Chlorination and bromination of **45** under basic conditions produced the anticipated pyrazole ring halogenated products **31** and **32**. However, when the chlorination was performed in a non-polar solvent and R^2 was a trifluoromethyl group, the reaction produced the benzylic dichloro analog **46**. This reaction proceeded rapidly at room temperature without the addition of a free radical initiator. The isolated dichloro product **46** was found to slowly hydrolyze on silica gel during purification or in dilute acid to give the ketones **29** and **30**. Extension of this reaction to analogs **45** in which R^2 was a methyl, chloro or bromo group gave a low yielding conversion to the dichloro intermediate, in the case of the methyl analog, and a complex mixture of products with the chloro and bromo derivatives. Alternatively, oxidation of the chloro and bromo analogs **45** with potassium persulfate in aqueous acetonitrile provided the ketones **27** and **28**.

Few examples of pyrazole ring fluorination have been described in the chemical literature and this target presented a synthetic challenge. After surveying a variety of fluorinating reagents, it was found that reaction of **45** with xenon difluoride provided a low but useful yield of analogs **33**–**37**. This procedure produced a complex mixture of products from which the desired product and a small amount of a mono-fluorinated benzylic product were isolated.

The estrogen receptor binding affinities[27] of racemic (A/B) and resolved (A or B) tetrahydrofluorenone analogs **1**–**37** are summarized in Table 1. Comparison of the ER binding SAR for the pyrazole and phenolic tetrahydrofluorenone analogs showed that, after correcting for enantiomeric purity, the pyrazole analogs displayed similar or slightly improved ERβ binding affinity and selectivity in comparison with the corresponding phenol analogs.[19] In both series, alkyl substitution of R^1 with ethyl, propyl, and butyl groups imparted the most favorable ERβ binding and β-selectivity. The large difference between the ERβ binding values of the phenolic enantiomers was also seen in the pyrazole series, where the inactive pyrazole enantiomer **6** exhibited less than 1% of the ER binding of the active enantiomer **5**.

In general, an increase in the steric size of the R^2 substituent produced an increase in ERβ potency. When R^1 was substituted with an ethyl group, the bromo **13** (2.2 nM), trifluoromethyl **19** (2.8 nM), and phenyl **23** (1.4 nM) analogs showed the most favorable ERβ binding affinities, while the methyl **5** (190-fold), bromo **13** (113-fold), and trifluoromethyl **19** (130-fold) analogs displayed the greatest ERβ selectivity. When R^1 was substituted with a butyl group, the ethyl **10** (1.1 nM) and trifluoromethyl **20** (0.8 nM) analogs possessed the most potent ERβ binding, while the trifluoromethyl **20** (220-fold) and methyl ester **25** (400-fold) analogs displayed the greatest ERβ selectivity.

In analogs where R^1 was substituted with a cyclopropyl group, the cyclopropylethyl analog **17** (1.6 nM) showed the most potent and ERβ selective binding affinity, although, it was found to be somewhat less selective than analog **15**. Analogs **21** and **22**, where R^2 was substituted with a cyclopropyl group, also displayed slightly reduced ERβ potencies when compared with analogs **5** and **8**.

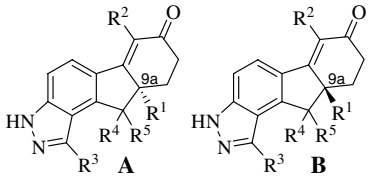
Oxidation of the benzylic position to give ketones **26**–**30** had essentially no effect on ERβ binding when compared with the parent analogs **5**, **11**, **13**, **19**, and **20**. However, a small but significant decrease in ERβ-selectivity was observed for these compounds.

A comparison of the analogs, in which the pyrazole ring was substituted with a halogen **31**–**37**, showed that decreasing steric size produced an increase in ERβ potency (F > Cl > Br). The fluorinated analogs **33**–**37** showed a 1–9-fold improvement over the parent analogs in ERβ binding and with the exception of **37**, essentially unchanged ERβ selectivity. Analog **35** (0.3 nM) showed an ERβ potency which was 4-fold better than 17β-estradiol but with greatly improved ERβ selectivity.

Compounds were also evaluated in a cell-based transactivation assay[28] utilizing HEK 293 cells which were stably cotransfected with human ERα or ERβ and the alkaline phosphatase reporter gene. The transcriptional activity of the tetrahydrofluorenone analogs was determined and compared as a percent response of 17β-estradiol. These data are summarized in Table 2.

Overall, the transactivation data tracked reasonably well with the ER binding data from Table 1. Several analogs displayed subnanomolar ERβ transactivation values with the fluoropyrazole **35** exhibiting the greatest ERβ potency (0.2 nM). The potent and selective EC_{50} values demonstrated that these analogs behaved as functional ERβ agonists.

Table 1. Human estrogen receptor binding affinities



Compound	R^1	R^2	R^3	$R^4 = R^5$	9a stereochemistry	ERα IC_{50} (nM)	ERβ IC_{50} (nM)	ERα/β
Estradiol	—	—	—	—	—	1.3	1.2	1.1
1	Bu	Br	—	H	A/B	141	1.8	76
2	Et	H	H	H	A/B	>10,000	618	>16
3	Bu	H	H	H	A/B	>10,000	283	>35
4	Me	Me	H	H	A/B	4410	69	64
5	Et	Me	H	H	A	1360	7.2	190
6	Et	Me	H	H	B	>10,000	4460	>2
7	Pr	Me	H	H	A/B	976	12.6	77
8	Bu	Me	H	H	A/B	1050	12	85
9	Et	Et	H	H	A/B	223	4.0	56
10	Bu	Et	H	H	A	93	1.1	83
11	Et	Cl	H	H	A	470	7.7	61
12	Me	Br	H	H	A/B	1990	26	77
13	Et	Br	H	H	A	249	2.2	113
14	Pr	Br	H	H	A/B	663	5.4	123
15	Bu	Br	H	H	A/B	1410	4.9	288
16	Cyclopropylmethyl	Br	H	H	A	230	4.0	58
17	Cyclopropylethyl	Br	H	H	A	229	1.6	145
18	Me	CF_3	H	H	A	1478	8.0	185
19	Et	CF_3	H	H	A	360	2.8	130
20	Bu	CF_3	H	H	A	176	0.8	220
21	Et	Cyclopropyl	H	H	A	470	7.7	61
22	Bu	Cyclopropyl	H	H	A/B	422	7.0	60
23	Et	Phenyl	H	H	A	46	1.4	37
24	Et	CO_2Me	H	H	A/B	>10,000	207	>48
25	Bu	CO_2Me	H	H	A	2080	5.2	400
26	Et	Me	H	O	A/B	624	31.8	20
27	Et	Cl	H	O	A	139	4.1	34
28	Et	Br	H	O	A/B	352	8.0	44
29	Et	CF_3	H	O	A	58	1.5	39
30	Bu	CF_3	H	O	A	40	0.9	43
31	Et	Me	Cl	H	A/B	2420	42.5	57
32	Et	Me	Br	H	A/B	5880	103	57
33	Et	Cl	F	H	A	82	1.1	72
34	Et	Br	F	H	A/B	197	1.6	124
35	Et	CF_3	F	H	A	50.9	0.3	170
36	Bu	Et	F	H	A	23.0	0.3	87
37	Bu	CF_3	F	H	A	49.2	1.0	49

As was the case in the phenolic series,[19] an increase in the steric size of the R^2 substituent produced a decrease in the ERβ agonist response. Analogs that showed a moderate percent agonist response were then tested for their ability to antagonize estradiol's transcriptional effects. The cyclopropyl **21** and phenyl **23** analogs were observed to weakly antagonize the effects of estradiol (526 and 428 nM, respectively) and these analogs were identified as partial agonists/antagonists.

A summary of rat pharmacokinetic (PK) data[29] is listed in Table 3. In contrast with phenolic analog **1**, all pyrazole derivatives showed oral bioavailability in the rat (21–74%). In the ethyl series, the pair of resolved enantiomers showed relatively good PK with the inactive enantiomer **6** displaying a slight advantage in AUC and plasma clearance rates over the active enantiomer **5**. In the halogenated analogs, the trifluoromethyl analog **19** displayed improved PK over the chloro **11** and bromo **13** analogs. A comparison of the trifluoromethyl analogs **19** and **20** showed ethyl substitution to be preferable to butyl substitution.

In addition to minimizing glucuronidation using a fused pyrazole group, an attempt was made to improve PK by minimizing the potential for oxidative metabolism. Potential metabolic sites on the tetrahydrofluorenone were protected from oxidative metabolism with the introduction of a fluoro, cyclopropyl, or keto group. Unfortunately, the PK data for analogs containing a

Table 2. Transactivation in ERα and ERβ cotransfected HEK 293 cells

Example	ERα EC_{50} (nM)	% Estradiol agonism	ERβ EC_{50} (nM)	% Estradiol agonism
2	>1000	37	76	84
5	104	78	2.1	91
6	1100	7	935	49
9	59	67	1.7	77
11	152	42	1.5	70
13	65	50	0.7	74
17	61	70	0.9	76
19	50	66	0.6	79
21	>1000	13	5.8	69
23	72	37	0.8	54
24	1170	33	210	61
28	49	65	1.5	85
29	10.4	90	0.4	86
34	33.1	71	0.8	81
35	8.3	75	0.2	67

Table 3. Pharmacokinetics in female Sprague–Dawley rats at an intravenous dosing of 1 mg/kg and oral dosing of 2 mg/kg

Example	IV-AUC (μM h)	Clp (mL/min/kg)	$T_{1/2}$ (h)	*F*%
1	0.5	102	1.5	0
5	1.5	44	1.1	51
6	2.0	31	0.8	44
11	0.9	72	0.5	47
13	0.7	74	0.7	64
19	1.5	36	0.7	74
17	0.8	55	1.2	51
21	1.0	58	0.9	55
29	3.2	15	1.2	62
35	1.2	42	1.9	27
20	0.9	64	0.9	25
30	0.7	69	1.8	21

cyclopropyl or a fluoro group were not improved relative to related unsubstituted derivatives. For instance, the rat PK of the cyclopropyl analogs **17** and **21** were not significantly different from that of the methyl **5** or halogenated analogs **11** and **13**. Likewise, the fluoro analog **35** displayed similar rat PK to that of the parent analog **19**.

In contrast, the ethyl substituted ketone **29** showed a greater than 2-fold improvement in AUC and plasma clearance over the parent compound **19**. This analog displayed the best PK in this class of compounds and suggested that the benzylic position was a site of oxidative metabolism. The butyl substituted ketone **30**, however, showed a similar PK profile to the parent analog **20** and reinforced the conclusion that substitution of the R^1 group with an ethyl group was preferable to substitution with a butyl group.

In summary, we have shown that a fused pyrazole is a suitable phenol group replacement in the previously described tetrahydrofluorenone class of ERβ agonists. The pyrazole analogs were selective ERβ agonists in a cell-based transactivation assay and showed a marked improvement in their PK properties over the phenol tetrahydrofluorenones. Several analogs, such as **19**, **29**, and **35**, have shown a promising combination of ERβ potency, selectivity, and rat pharmacokinetic properties. Further modification of the tetrahydrofluorenone class of ERβ agonists will be the subject of forthcoming publications from these laboratories.

References and notes

1. Green, S.; Walter, P.; Kumar, V.; Krust, A.; Bornert, J. M.; Argos, P.; Chambon, P. *Nature* **1986**, *320*, 134.
2. Green, S.; Gilna, P.; Waterfield, M.; Baker, A.; Hort, Y.; Shine, J. *J. Sci.* **1986**, *231*, 1150.
3. Kuiper, G. G. J. M.; Gustafsson, J. -A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5925.
4. Mosselman, S.; Polman, J.; Dijkema, R. *FEBS Lett.* **1996**, *393*, 49.
5. Stearns, V.; Ullmer, L.; Lopez, J.; Smith, Y.; Isaacs, C.; Hayes, D. *Lancet* **2002**, *360*, 1851.
6. Women's Health Initiative. *JAMA* **2002**, *288*, 321.;
7. Harris, H. A.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Endocrinology* **2002**, *143*, 4172.
8. Enmark, E.; Pelto-Huikko, M.; Grandien, K.; Lagercrantz, S.; Lagercrantz, J.; Fried, G.; Nordenskjold, M.; Gustafsson, J.-A. *J. Endocrinol. Metab.* **1997**, *82*, 4258.
9. Kim, S.; Wu, J. Y.; Birzin, E. T.; Frisch, K.; Chan, W.; Pai, L. Y.; Yang, Y. T.; Mosley, R. T.; Fitzgerald, P. M. D.; Sharma, N.; Dahllund, J.; Thorsell, A. G.; DiNinno, F.; Rohrer, S. P.; Hammond, M. L. *J. Med. Chem.* **2004**, *47*, 2171.
10. Kim, S.; Wu, J. Y.; Chen, H. Y.; Birzin, E. T.; Chan, W.; Yang, Y. T.; Colwell, L.; Li, S.; Dahllund, J.; DiNinno, F.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2741.
11. Meyers, M. J.; Sun, J.; Carlson, K. E.; Marriner, G. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 4230.
12. Schopfer, U.; Schoeftter, P.; Bischoff, S. F.; Nozulak, J.; Feuerbach, D.; Floersheim, P. *J. Med. Chem.* **2002**, *45*, 1399.
13. Henke, B. R.; Consler, T. G.; Go, N.; Hale, R. L.; Hohman, D. R.; Jones, S. A.; Lu, A. T.; Moore, L. B.;

Moore, J. T.; Orband-Miller, L. A.; Robinett, R. G.; Shearin, J.; Spearing, P. K.; Stewart, E. L.; Turnbull, P. S.; Weaver, S. L.; Williams, S. P.; Wisely, G. B.; Lambert, M. H. *J. Med. Chem.* **2002**, *45*, 5492.
14. Yang, W.; Wang, Y.; Ma, Z.; Golla, R.; Stouch, T.; Seethala, R.; Johnson, S.; Zhou, R.; Gungor, T.; Feyen, J. H. M.; Dickson, J. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2327.
15. Malamas, M. S.; Manas, E. S.; McDevitt, R. E.; Gunawan, I.; Xu, Z. B.; Collini, M. D.; Miller, C. P.; Dinh, T.; Henderson, R. A.; Keith, J. C.; Harris, H. A. *J. Med. Chem.* **2004**, *47*, 5021.
16. Collini, M. D.; Kaufman, D. H.; Manas, E. S.; Harris, H. A.; Henderson, R. A.; Xu, Z. B.; Unwalla, R. J.; Miller, C. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4925.
17. Vu, A. T.; Cohn, S. T.; Manas, E. S.; Harris, H. A.; Mewshaw, R. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4520.
18. Chesworth, R.; Wessel, M. D.; Heyden, L.; Mangano, M.; Zawistoski, M.; Gegnas, L.; Galluzzo, D.; Lefker, B.; Cameron, K. O.; Tickner, J.; Lu, B.; Castleberry, T. A.; Petersen, D. N.; Brault, A.; Perry, P.; Ng, O.; Owen, T. A.; Pan, L.; Ke, H. Z.; Brown, T. A.; Thompson, D. D.; DaSilva-Jardine, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5562.
19. Wilkening, R.; Ratcliffe, R.; Tynebor, E.; Wildonger, K.; Fried, A.; Hammond, M.; Mosley, R.; Fitzgerald, P.; Sharma, N.; McKeever, B.; Nilsson, S.; Carlquist, M.; Thorsell, A.; Locco, L.; Katx, R.; Frisch, K.; Birzin, E.; Wilkinson, H.; Mitra, S.; Cai, S.; Hayes, E.; Schaeffer, J.; Rohrer, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3489.
20. Phenol bioisosteres which utilize pyrazole and triazole moieties have been reported. Wright, J.; Tracy, G.; Kesten, S.; Boxer, P.; Serpa, K.; Meltzer, L.; Wise, L.; Espitia, S.; Konkoy, C.; Whittemore, E.; Woodward, R. *J. Med. Chem.* **2000**, *43*, 3408.
21. The 6,7-fused regioisomer was prepared in a related triazole series and was found to be significantly less potent than the analogous **15** isomer. R. Wilkening, unpublished results.
22. A detailed description of the synthesis and SAR of 6- and 8-substituted phenolic analogs, as well as, the investigation of a series of fused triazole tetrahydrofluorenone analogs will be the subject of forthcoming publications from these laboratories.
23. All aldehydes in this step were commercially available with the exception of cyclopropylmethylcarboxaldehyde which was prepared from cyclopropylethanol using a TPAP/NMO oxidation.
24. Bartsch, A. B.; Yang, I. *J. Heterocycl. Chem.* **1984**, *21*, 1063.
25. Chiral resolution was performed using a Daicel Chiralcel OJ semi-preparative column with varying ratios of ethanol and heptanes as the mobile phase. The stereochemistry of the more active enantiomer was assigned as 'A' (Table 1) by analogy with the phenol tetrahydrofluorenones (ref. 19). This assignment was confirmed in three cases (**5**, **10**, and **19**) by X-ray crystallography of ERβ complexed with the ligands (P. Fitzgerald unpublished results).
26. Chen, Q.-Y.; Wu, S.-W. *J. Chem. Soc. Commun.* **1989**, 705.
27. The IC_{50} values were generated in an estrogen receptor ligand binding assay. This scintillation proximity assay was conducted in NEN Basic Flash plates using tritiated estradiol and full-length recombinant human ERα and ERβ proteins with incubation times of 3–23 h. In our experience, this assay provides IC_{50} values that are reproducible to within a factor of 2–3. With the exception of 17β-estradiol and analog **1**, the majority of analogs were tested once in the ER binding assay. The analogs were tested in duplicate in the transactivation assay (Table 2).
28. Barkhem, T.; Carlsson, B.; Enmark, E.; Gustafsson, J.-A.; Nilsson, S. *Mol. Pharmacol.* **1998**, *54*, 105.
29. Female Sprague–Dawley rats were dosed at 1 mpk IV and 2 mpk PO using a 1.0 mg/mL solution of compound dissolved in EtOH/PEG400/H_2O (2:3:5). Plasma samples were mixed with acetonitrile, centrifuged, and analyzed by LC–MS/MS on an Applied Biosystems MDS SCIEX API 4000 tandem mass spectrometer/HPLC system.