Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3489–3494

The discovery of tetrahydrofluorenones as a new class of estrogen receptor β-subtype selective ligands

R. R. Wilkening,[a,*] R. W. Ratcliffe,[a] E. C. Tynebor,[a] K. J. Wildonger,[a] A. K. Fried,[a] M. L. Hammond,[a] R. T. Mosley,[a] P. M. D. Fitzgerald,[a] N. Sharma,[a] B. M. McKeever,[a] S. Nilsson,[c] M. Carlquist,[c] A. Thorsell,[c] L. Locco,[b] R. Katz,[b] K. Frisch,[b] E. T. Birzin,[b] H. A. Wilkinson,[b] S. Mitra,[b] S. Cai,[b] E. C. Hayes,[b] J. M. Schaeffer[b] and S. P. Rohrer[b]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Molecular Endocrinology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[c]*Karo Bio AB, Novum, S-14157 Huddinge, Sweden*

Received 6 March 2006; revised 29 March 2006; accepted 30 March 2006
Available online 2 May 2006

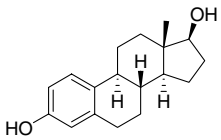
Abstract—Synthesis and derivatization of a series of substituted tetrahydrofluorenone analogs giving potent, ERβ subtype selective ligands are described. Several analogs possessing ERβ binding affinities comparable to 17β-estradiol but with greater than 75-fold selectivity over ERα are reported.

The estrogen receptor ER is comprised of two subtypes, ERα[1,2] and ERβ,[3,4] which bind 17β-estradiol with similar affinity and mediate the effects of estrogen throughout the body. The effects of the non-selective ligand 17β-estradiol have been studied extensively during hormone replacement therapy (HRT) and have produced unexpected and controversial results. The beneficial effects of HRT include the prevention of osteoporosis, amelioration of hot flashes,[5] and reduction in the risk of colorectal cancer in menopausal women. The adverse effects, as observed in the womens health initiative[6] (WHI), include a slight elevation in the incidence of invasive breast cancer, as well as an increase in the risk of coronary heart disease, pulmonary embolism, and stroke in menopausal women. The clinical usefulness of HRT, and the safety concerns raised by the WHI, provides a compelling case to delineate the physiological functions mediated by these two ER receptors.

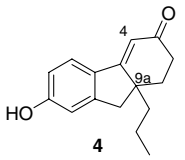
Studies on the tissue distribution of the two estrogen receptors show that they are widely and to a large extent differentially expressed in humans.[7,8] For instance, ERβ is largely expressed in the lung, prostate, and brain, while ERα is predominant in the uterus and breast. This observation suggests that the biological roles of ERα and ERβ receptors might be tissue specific and that an ER subtype selective ligand might produce a biological response which is different than the non-selective ligand 17β-estradiol.

These findings prompted an intensive search in both academia and the pharmaceutical industry for ER subtype selective agonists and/or antagonists, to help elucidate the pharmacological importance of these receptors. The recent identification of selective ERα[7,9,10] and ERβ[11–18] ligands has indeed provided some insight into the physiological role of the ER receptors. In this paper, we describe the synthesis and structure–activity relationship (SAR) of a series of tetrahydrofluorenone derivatives leading to the identification of potent ERβ subtype selective analogs.

The tetrahydrofluorenone derivative **4**[19] was identified as an ERβ-selective ligand during a high-throughput



17β-Estradiol



4

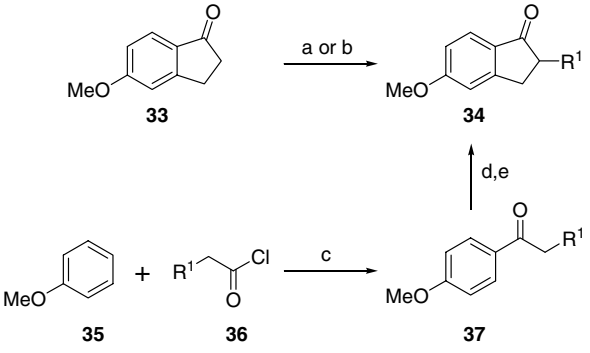
Keywords: Estrogen receptor ligands; Tetrahydrofluorenone.
* Corresponding author. Tel.: +1 732 594 5729; fax: +1 732 594 9556; e-mail: robert_wilkening@merck.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.03.098

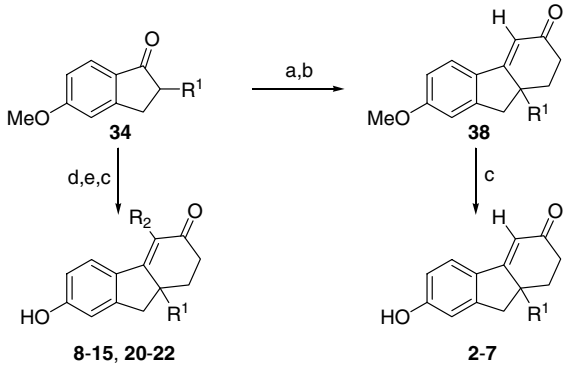
screening of Merck's sample collection. The ERβ binding (IC_{50} 218 nM) and β-selectivity (12-fold) of lead compound **4** prompted an investigation into the SAR of this class of compounds.

The syntheses of the racemic tetrahydrofluorenone analogs **1**–**32** began with the preparation of the intermediate 2-substituted indanones **34**, as described in Scheme 1. Reaction of **33** with an alkyl halide and base provided a low yield of **34** owing to the propensity of **33** to undergo dialkylation. Consequently, a one-pot reductive alkylation[20] of indanone **33** with an aldehyde[21] was developed. This procedure provided **34** in high yield without evidence of dialkylated products. In a third method,[19] the indanone **34** was prepared by Friedel–Crafts acylation of **35** with acid chloride **36**, to provide the *para*-substituted anisole **37**. Reaction of **37** with formaldehyde and cyclization of the aldol products in sulfuric acid gave **34**.

Scheme 2 describes the formation of the racemic tetrahydrofluorenone platform using the Robinson annulation reaction. Reaction of **34** with methyl vinyl ketone (MVK) and cyclization of the addition product with pyrrolidine/acetic acid gave **38**. Deprotection of the methyl ether with boron tribromide provided analogs **2**–**7**. Reaction of **34** with the appropriate alkyl vinyl ketone and cyclization of the addition product under strongly acidic conditions provided analogs **8**–**15** and **20**–**22** after boron tribromide deprotection of the methyl ether.



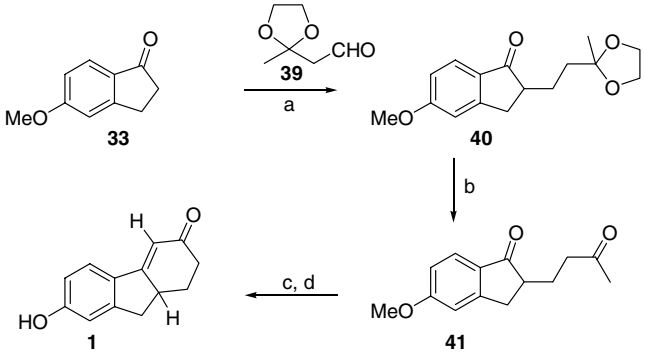
Scheme 1. Reagents: (a) NaH, R^1I, DMF, R^1 is Et, *i*-Pr, Bu, pentyl; (b) H_2, RCHO, KOH or NaOH, 10% Pd/C, EtOH, R^1 is Pr, Bu, *i*-Bu, Bn, 2-hydroxyethyl; (c) $AlCl_3$, CH_2Cl_2, R^1 is Me, Ph; (d) HCHO, K_2CO_3, MeOH; (e) H_2SO_4.



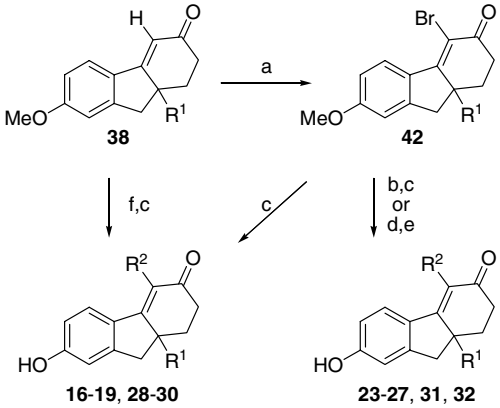
Scheme 2. Reagents: (a) MVK, DBU, THF; (b) pyrrolidine, HOAc, toluene; (c) BBr_3, CH_2Cl_2; (d) $R^2CH_2C(O)CH{=}CH_2$, DBU, THF, R^2 is Me, Et, Pr, Bu; (e) HOAc, 6 N HCl.

The synthesis of analog **1**, where R^1 is hydrogen, was complicated by the failure of the unsubstituted indanone **33** to alkylate with MVK. Instead, analog **1** was prepared as described in Scheme 3. Reductive alkylation of the indanone **33** with aldehyde[22] **39** provided **40**. Acidic hydrolysis of the ketal group gave the ketone **41**. Cyclization of **41** under basic conditions was followed by an aluminum chloride/ethanethiol deblock of the methyl ether to give **1**.

A variety of R^2 substituents were introduced as described in Scheme 4. Bromination of **38** provided the bromo enone intermediate **42**. Palladium-catalyzed reactions of **42** provided the 2-furyl (**23** and **31**), 2-thienyl **24**, and phenyl **25** analogs after deblocking of the methyl ether. Conversion of **42** into the cyano (**26** and **32**) and trifluoromethyl[23] **27** analogs was accomplished using copper(I)-mediated chemistry. The halogenated products **16**–**19** and **28**–**30** were also prepared as described below.



Scheme 3. Reagents: (a) **39**, H_2, KOH 10% Pd/C, EtOH; (b) 6 N HCl, THF; (c) NaOMe, MeOH; (d) $AlCl_3$, EtSH, CH_2Cl_2.



Scheme 4. Reagents: (a) Br_2, $NaHCO_3$, CH_2Cl_2; (b) 2-(Bu_3Sn)furan, $PdCl_2(PPh_3)_2$, toluene; or 2-(Bu_3Sn)thiophene, $PdCl_2(PPh_3)_2$, toluene; or $C_6H_5B(OH)_2$, $PdCl_2(PPh_3)_2$, Cs_2CO_3, DMF; (c) BBr_3, CH_2Cl_2; (d) CuCN, NMP; or $FSO_2CF_2CO_2CH_3$, CuI, DMF; (e) $C_5H_5N{\cdot}HCl$; (f) NCS, DMF; or I_2, $NaHCO_3$, CH_2Cl_2.

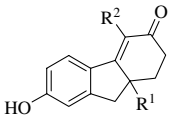
The estrogen receptor binding affinities[24] of the racemic tetrahydrofluorenone analogs **1**–**32** are summarized in Table 1. Our initial effort to evaluate the SAR of the tetrahydrofluorenone series focused on the R^1 substitution of lead compound **4**. We found that analogs **1**–**7**, where R^2 is hydrogen, displayed the most favorable ERβ binding when R^1 is an ethyl, propyl or butyl group. Smaller groups, that is, hydrogen and methyl, and larger groups, that is, pentyl and benzyl, had deleterious effects upon ERβ binding and selectivity. While the ethyl, propyl, and butyl analogs possessed similar ERβ binding, the ethyl and butyl analogs displayed increased β-selectivity relative to the propyl lead compound **4**.

Switching the R^2 substituent from hydrogen to a methyl group and examining the R^1 group in more detail produced analogs with greater than 10-fold improvement in ERβ binding. A comparison of derivatives **8**–**15** showed that the ethyl, propyl, butyl, and phenyl groups possessed the most favorable ER binding. Branching of the R^1 group close to the bridgehead, analog **11**, was not well tolerated, while substitution further along the alkyl chain was better tolerated, analog **13**. These branched analogs, however, displayed modest ERβ-selectivity when compared with their linear propyl **10** and butyl **12** counterparts. Finally, polar groups were observed to greatly reduce ER binding, as was seen with the 2-hydroxyethyl analog **14**.

In a series of analogs where R^2 is bromo **16**–**19**, we observed a 50- to 100-fold improvement in ERβ binding relative to the proteo analogs **2**–**5**. Analog **19** was found to have the most favorable ERβ binding affinity (1.8 nM) and selectivity (76-fold). This analog showed ERβ potency comparable to 17β-estradiol, but with greatly improved β-selectivity.

It was apparent that variation of the R^2 group had a more profound effect upon improving ER binding than

Table 1. Human estrogen receptor affinity and selectivity



Compound	R^1	R^2	IC_{50} (nM)		ERα/β
			ERα	ERβ	
17β-Estradiol	—	—	1.3	1.2	1.1
1	H	H	>10,000	1530	>7
2	Me	H	4245	936	5
3	Et	H	>10,000	374	>27
4	Pr	H	2530	218	12
5	Bu	H	6450	188	34
6	Pentyl	H	3740	945	4
7	Benzyl	H	>10,000	4220	>2
8	Me	Me	1190	63	19
9	Et	Me	1210	28	43
10	Pr	Me	455	11	26
11	*i*-Pr	Me	1420	123	12
12	Bu	Me	630	16	39
13	*i*-Bu	Me	195	20	10
14	2-Hydroxyethyl	Me	>10,000	>10,000	—
15	Phenyl	Me	481	15	32
16	Me	Br	883	13.4	66
17	Et	Br	319	4.5	71
18	Pr	Br	79	4.4	18
19	Bu	Br	141	1.8	76
20	Bu	Et	264	13	20
21	Bu	Pr	30	1.5	20
22	Bu	Bu	273	15	18
23	Bu	2-Furyl	196	3.1	63
24	Bu	2-Thienyl	70.5	1.6	45
25	Bu	Phenyl	41	1.2	34
26	Bu	CN	1650	38	43
27	Bu	CF_3	128	1.5	85
28	Bu	Cl	114	5.3	22
29	Bu	I	124	1.3	95
30	Phenyl	Br	113	2.5	45
31	Phenyl	2-Furyl	389	16	24
32	Phenyl	CN	880	32	28

did variation of the R^1 group. Consequently, a more extensive evaluation of R^2 substitution was initiated in a series where R^1 was fixed as either a butyl or phenyl group. In the series where R^1 was butyl, a comparison of the R^2-substituted analogs revealed that the methyl **12**, ethyl **20**, and butyl **22** analogs possessed similar ERβ binding. The propyl analog **21** possessed the most potent ERβ binding (1.5 nM), while the methyl analog **12** possessed the best ERβ-selectivity (39-fold). The cyano analog **26** showed a marginal improvement in ERβ-binding potency (5-fold) relative to the proteo analog **5**. In contrast, the heteroaryl and phenyl analogs **23**–**25**, and halogenated analogs **19** and **27**–**29** showed an approximately 60- to 160-fold and 35- to 145-fold improvement in ER binding when compared with the proteo analog **5**.

In the 9a-phenyl series, the bromo derivative **30** was found to be the most potent and β-selective analog. However, this analog exhibited slightly decreased binding affinity and β-selectivity compared with the butyl analog **19**. This trend was also seen with the furan derivative **31** which showed a significant decrease in binding and selectivity from the related butyl analog **23**. The cyano derivative **32**, however, exhibited similar potency and selectivity to the corresponding butyl analog **26**.

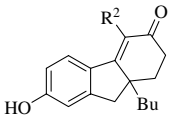
In addition to the ER binding affinity assay, compounds were evaluated in a cell-based transactivation assay[25] utilizing HEK 293 cells which were stably cotransfected with human ERβ and the alkaline phosphatase reporter gene. The transcriptional activity of a series of R^2-substituted tetrahydrofluorenone analogs was determined and compared as a percent response of 17β-estradiol. These data are summarized in Table 2. Generally, the potency of these derivatives tracked well with the ER binding values listed in Table 1 and demonstrated that these analogs behaved as functional ERβ agonists.

The percent agonist response for the butyl-substituted analogs varied between 11% and 112%. In the alkyl-substituted analogs **12** and **20**–**22**, an increase in chain length resulted in a decrease in the ERβ agonist response. This trend was also seen with the heteroaryl analogs **23**–**25**, where an increase in steric size produced a decrease in agonist response. In the three analogs **22**, **24**, and **25**, where the response decreased to approximately 50% or less, the ability of these analogs to antagonize estradiol's effects was tested. The butyl **22**, thienyl **24**, and phenyl **25** analogs were determined to effectively antagonize the transcriptional activation of estrogen in HEK 293 cells. The similar EC_{50} and IC_{50} of these analogs identified them as mixed agonist/antagonists.

Chiral HPLC resolution[26] of the racemic analogs **12** and **19** provided the *S*-**43**, -**45** and *R*-**44**, -**46** enantiomers, respectively. The ER binding values for the resolved enantiomers are listed in Table 3. It is apparent that essentially all the binding affinity of the racemates is derived from the *S*-enantiomers. The *R*-enantiomers are much less active with their binding values comprising 1% or less of the racemates' binding affinity.

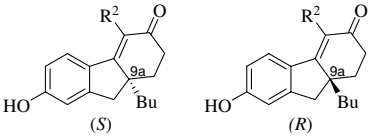
X-ray analysis[27] of the estrogen β-subtype receptor complexed with **45** provided evidence for the configurational assignments of the R^1 position and suggested an explanation for the observed ERβ-selectivity. The selectivity results from a number of subtle factors that include the planar nature of the tetrahydrofluorenone core and the positioning of an R^1 substituent orthogonal to that plane. The first factor is a putative stabilizing interaction between the planar/aromatic surface of the tricyclic core and Met336 of hERβ which is not possible with the analogous Leu384 in hERα (see Fig. 1). The Sδ and Cε of Met336 make better van der Waals contacts with the mostly planar tricyclic platform than is possible with either Cδ of Leu384 in hERα. This feature appears to be common to other planar ERβ-selective molecules including some phytoestrogens such as genistein[28] or other synthetic molecules including some benzisoxazoles,[15] for example. The second selectivity enhancing interaction is the putative favorable hydrophobic interaction between the *n*-butyl group at R^1 which protrudes orthogonally from the plane of the tricyclic core toward Ile373 in hERβ as depicted in Figure 1. We speculate that Ile373 in hERβ can nicely accommodate the pres-

Table 2. Transactivation in ERβ cotransfected HEK 293 cells



Compound	R^2	EC_{50} (nM)	% Estradiol agonism	IC_{50} (nM)
12	Me	20	112	—
20	Et	2	66	—
21	Pr	4	64	—
22	Bu	30	11	40
23	2-Furyl	5	80	—
24	2-Thienyl	4	52	9
25	Phenyl	5	41	2
26	CN	5	93	—
27	CF_3	40	81	—
28	Cl	11	78	—
19	Br	4	81	—
29	I	2	70	—

Table 3. Human estrogen receptor affinity and selectivity



Compound	R^2	9a	IC_{50} (nM)		ERα/β selectivity
			ERα	ERβ	
12	Me	*R*/*S*	630	16	39
43	Me	*S*	567	19	30
44	Me	*R*	>10,000	1810	>5
19	Br	*R*/*S*	141	1.8	76
45	Br	*S*	129	1.5	89
46	Br	*R*	4023	313	13

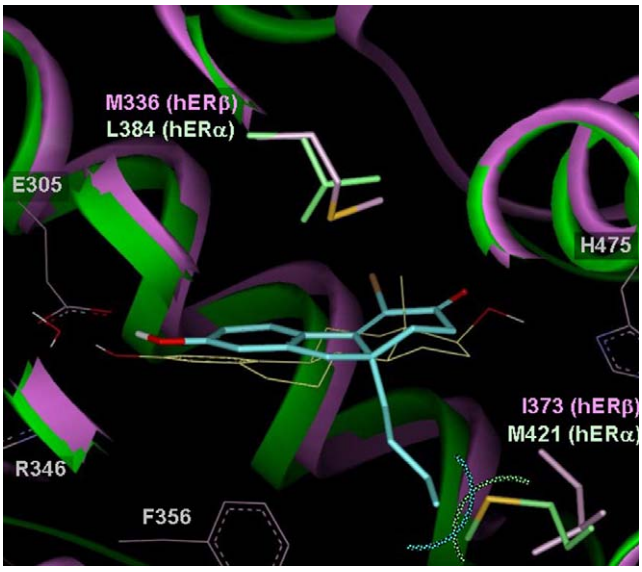


Figure 1. Superposition of the crystallographic complexes of compound **45** (cyan) with hERβ (purple) and 17β-estradiol (yellow) with hERα (green) (pdb entry: 1ERE) displaying the principal amino acids involved in ligand recognition. Unless otherwise indicated, residue numbering is that of hERβ.

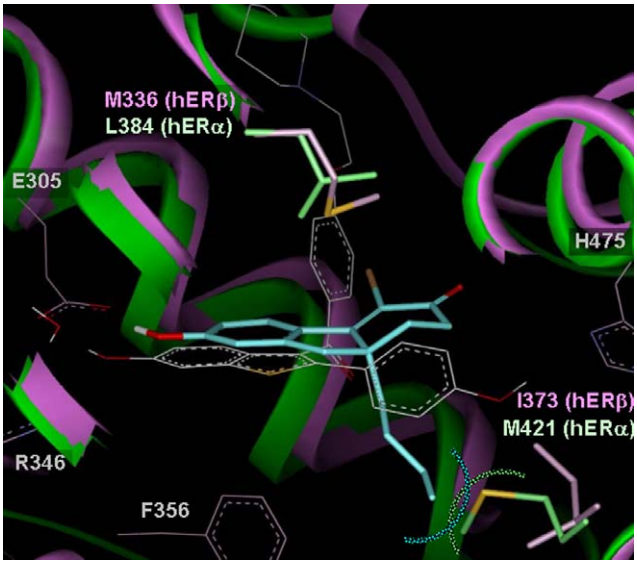


Figure 2. Superposition of the crystallographic complex of compound **45** (cyan) with hERβ (purple) and raloxifene (in white, extracted from pdb entry 1ERR) in the context of hERα (green) (pdb entry: 1ERE) displaying the principal amino acids involved in ligand recognition. Unless otherwise indicated, residue numbering is that of hERβ.

ence of the *n*-butyl moiety into space which is not available in hERα because the sidechain of the analogous Met421 fills this space. The residue differences in this region have been used to rationalize some of the hERβ-selectivity exhibited by certain 7-substituted 2-phenyl-benzofurans[16] and 1,3,5-substituted triazines.[13]

The loss of agonism coupled to a concomitant increase in the steric bulk of R^2 is reasonable in light of the superposition of raloxifene with compound **45** depicted in Figure 2. Clearly, the bromine of compound **45** appears to fill the same space occupied by the aryl group found in the antagonist sidechain of raloxifene. It could readily be conjectured then that as the groups replacing Br get larger that the antagonism should increase as well.

In summary, starting from lead compound **4**, tetrahydrofluorenone analogs with low nanomolar affinity for ERβ and greater than 75-fold selectivity over ERα were identified. Furthermore, compounds of this class were determined to be functional agonists of ERβ in a cell-based transactivation assay. The new ERβ agonists described herein, particularly **19**, should prove to be valuable pharmacological tools for elucidating the physiological role of ERβ. Further investigation of the tetrahydrofluorenone class of ERβ agonists will be the subject of forthcoming publications from these laboratories.

Acknowledgments

I thank Drs. Mark Greenlee and Timothy Blizzard for their many useful suggestions during the preparation of the manuscript.

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. *Science* **1986**, *231*, 1150.
3. Kuiper, G. G. J. M.; Gustafuson, 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. Clin. 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. D.; Rohrer, S. A.; 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. D.; Rohrer, S. A.; 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. Cragoe, E. J.; Woltersdorf, O. W.; Gould, N. P.; Pietruszkiewicz, A. M.; Ziegler, C.; Sakurai, Y.; Stokker, G. E.; Anderson, P. S.; Bourke, R. S.; Kimelberg, H. K.; Nelson, L. R.; Barron, K. D.; Rose, J. R.; Szarowski, D.; Popp, A. J.; Waldman, J. B. *J. Med. Chem.* **1986**, *29*, 825.
20. International Publication WO 01/82923.
21. All aldehydes were commercially available with the exception of glycoaldehyde which was formed in situ from glyoxaldehyde dimer during the reductive alkylation of **33**.
22. Still, I. W.; Yian, S. *Tetrahedron Lett.* **1987**, *28*, 2489.
23. Chen, Q.-Y.; Wu, S.-W. *J. Chem. Soc. Commun.* **1989**, 705.
24. 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 **19**, the majority of analogs were tested once in the ER binding assay. The analogs were tested in duplicate in the transactivation assay (Table 2).
25. Barkhem, T.; Carlsson, B.; Nilsson, Y.; Enmark, E.; Gustafsson, J.-A.; Nilsson, S. *Mol. Pharmacol.* **1998**, *54*, 105.
26. The chiral resolution of the methyl ether precursor of **19** was typical of the tetrahydrofluorenone series. Chiralcel OJ column (Daicel) 10% ethanol/heptane, retention time 10.4 and 11.7 min.
27. A complex of ERβ with **45** was prepared by displacing the ligand binding domain of ERβ (residues 260–500) bound to a weakly absorbing affinity column by elution with **19**. Although the elution was with the racemic mixture **19**, only the active enantiomer **45** bound to the protein and displaced it from the affinity column. The complex of **45** with the ligand binding domain of ERβ (residues 260–500) was crystallized by vapor diffusion, using a precipitant containing 1.8 M NaCl, 6% PEG 6000, 3% isopropanol, and 50 mM Tris buffer, pH 7.2. Data were measured at beamline 17-ID of the Advanced Photon Source. The crystal had the symmetry of space group $P6_122$, with cell dimensions $a = b = 64.53$, $c = 251.16$ Å. The data were processed with program X-GEN, which yielded an *R*-merge of 0.078 for the data from ∞ to 2.20 Å. The structure was refined using program SHELXL, with final values for *R*-work and *R*-free of 0.227 and 0.323 for the data from 10.0 to 2.20 Å resolution. Coordinates and structures factors have been deposited with the Protein Data Bank (2GIU).
28. Kuiper, G.; Lemmen, J. G.; Carlsson, B.; Corton, J. C.; Safe, H.; Saag, P. T.; Burg, B.; Gustafsson, J. *Endocrinology* **1998**, *139*, 4252.