0013-7227/99/$03.00/0
Endocrinology

Vol. 140, No. 2
Printed in U.S.A.

Novel Ligands that Function as Selective Estrogens or Antiestrogens for Estrogen Receptor-α or Estrogen Receptor-β*

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ABSTRACT

We report on the identification of novel, nonsteroidal ligands that show pronounced subtype-selective differences in ligand binding and transcriptional potency or efficacy for the two estrogen receptor (ER) subtypes, ERα and ERβ. An aryl-substituted pyrazole is an ERα potency-selective agonist, showing higher binding affinity for ERα and 120-fold higher potency in stimulation of ERα *vs.* ERβ in transactivation assays in cells. A tetrahydrochrysene (THC) has a 4-fold preferential binding affinity for ERβ; it is an agonist on ERα, but a complete antagonist on ERβ. Intriguingly, the antagonist activity of THC is associated with the R,R-enantiomer (R,R-THC). The S,S-enantiomer (S,S-THC) is an agonist on both ERα and ERβ but has a 20-fold lower affinity for ERβ than R,R-THC. This difference in binding affinity accounts for the full ERβ antagonist activity of the THC racemate (a 1:1 mixture of R,R-THC and S,S-THC). These compounds should be useful in probing the conformational changes in these two ERs that are evoked by agonists and antagonists, and in evaluating the distinct roles that ERβ and ERα may play in the diverse target tissues in which estrogens act. (*Endocrinology* **140:** 800–804, 1999)

AS A MEDIATOR of the actions of estrogenic hormones, the estrogen receptor (ER) plays a central role in regulating a diverse array of normal physiological processes involved in the development and function of the reproductive system, as well as in many other aspects of health (cardiovascular, bone density, etc.), and estrogen pharmaceuticals have been developed to regulate these processes or their pathological counterparts, including infertility, breast cancer, and osteoporosis. The recent discovery of a second estrogen receptor, named estrogen receptor-β (ERβ) to distinguish it from the classical ER (now named ERα), has opened new possibilities by which estrogen pharmaceuticals might exert tissue- and cell-selective activity (1–3). These ER subtypes have significantly different primary sequences in their ligand binding and transactivation domains, with ERα and ERβ showing 56% amino acid identity in their hormone binding domain/activation function-2 region and only *ca.* 20% homology in their A/B domain/activation function-1 region. This suggests that these ER subtypes might bind some ligands with different affinity and that these ligands might also have different agonist or antagonist character mediated by the two receptors. Because ERα and ERβ have some overlapping but also some different tissue distributions (2–4), differences in ligand interaction or activity with the two ERs could translate into important differences in their biological actions at the tissue level.

To identify compounds that might have ER subtype-selective activity, we have examined some structurally diverse ER ligands and report on the identification of novel, nonsteroidal ligands that show pronounced subtype-selective differences in binding affinity and in transcriptional potency or efficacy. Notably, one is an ERα potency-selective agonist, and another is an agonist on ERα but a complete antagonist on ERβ. These compounds should be useful in structural studies on ER agonist and antagonist complexes, and in evaluating the distinct roles that ERβ and ERα may play in the diverse target tissues in which estrogens act.

Received August 3, 1998.

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* Support of this work through grants from the National Institutes of Health (PHS 5R37 CA18119 and 5R37 DK-15556) is gratefully acknowledged.

Materials and Methods

Chemicals, materials, and plasmid constructions

Cell culture media were purchased from Gibco BRL (Grand Island, NY). Calf serum was from Hyclone Laboratories, Inc. (Logan, UT) and FCS was from Atlanta Biologicals (Atlanta, GA). ^{14}C-Chloramphenicol (50–60 Ci/mmol) and [^{3}H]E_2 were from DuPont NEN Research Products (Boston, MA). The expression vector for human ERα (pCMV5-hER) was constructed previously as described (5). The expression vector pCMV5-ERβ was constructed by inserting the full length complementary DNA encoding human ERβ (530 residues, pNGV1-ERβ (2) and including the additional 53 N-terminal amino acids as in GenBank accession number AF 051427, kindly provided by Sietse Mosselman of Organon, Oss, The Netherlands), into the *Bam*HI site of pCMV5. The estrogen responsive reporter plasmids were $(ERE)_3$-pS2-CAT, constructed as described previously (6), $(ERE)_2$-TATA-CAT (5), C3-Ti-LUC, kindly provided by Donald McDonnell of Duke University, which contains −1030 to +58 of the human complement C3 promoter fused to the firefly luciferase reporter gene (7), and lactoferrin ERE-tk-CAT, kindly provided by Suzanne Fuqua of the University of Texas at San Antonio, which contains two copies of the nonconsensus lactoferrin ERE fused to the thymidine kinase promoter and CAT reporter gene. The plasmid

pCH110 (Pharmacia, Piscataway, NJ) or pCMVβ (CLONTECH Laboratories, Inc., Palo Alto, CA) which contains the β-galactosidase gene, was used as an internal control for transfection efficiency. The synthesis of compound 2 has been described (8, 9). The synthesis of compounds 1, 3, and 4 will be described elsewhere (Fink, B. E., M. J. Meyers, and J.A. Katzenellenbogen, in preparation).

Cell culture and transient transfections

Human endometrial cancer (HEC-1) cells, Chinese hamster ovary (CHO) cells and HeLa cells were maintained in culture and transfected as described (5, 10, 11). Transfection of HEC-1 cells in 60-mm dishes used 0.4 ml of a calcium phosphate precipitate containing 0.5 μg of pCMVβGal as internal control, 2 μg of the reporter gene plasmid, 100 ng of ER expression vector, and carrier DNA to a total of 5 μg DNA. CAT or luciferase activity, normalized for the internal control β-galactosidase activity, was assayed as described (10, 11).

Ligand binding assays

Ligand binding affinities were determined by competitive radiometric binding assays using 10 nM [^{3}H]E_2 as tracer, purified preparations of ERα (amino acids 304–554) and ERβ (256–505) ligand binding domains expressed in *Escherichia coli*, and hydroxylapatite to adsorb bound receptor-ligand complex, as described previously (12). Incubations were done at 0 C for 18 h.

Results

Compounds investigated

The structures of the compounds selected for analysis are shown in Fig. 1. Compound 1 (pyrazole) is a triaryl-substituted pyrazole, which is a novel nonsteroidal ER ligand. Compound 2 (THC) is a *cis*-diethyl-substituted tetrahydrochrysene that was prepared as a racemate in the development of fluorescent ER ligands (8, 9). Compounds 3 (R,R-THC) and 4 (S,S-THC) are the R,R-and S,S-enantiomers of compound 2, prepared by an enantioselective synthesis.

Transcriptional activation with ER subtype-selective ligands

Figure 2 shows the transcriptional activity of the pyrazole (compound 1) assayed in human endometrial cancer (HEC-1) cells with ERα and ERβ. Cells were transfected with an expression plasmid for ERα or ERβ together with an estrogen-responsive reporter gene construct ((ERE)$_3$-pS2-CAT), and were treated with increasing concentrations of the pyrazole, or with estradiol (E_2) for comparison. The pyrazole behaved as an ERα potency-selective agonist, showing a 120-fold higher potency in activating transcription via ERα than via ERβ. Similar ERα potency-selective character was observed with this compound in transcription assays in other cell types [Chinese hamster ovarian (CHO) cells, and HeLa cells], using the estrogen-responsive pS2 promoter, as well as the simple TATA promoter and the complement C3 promoter (data not presented).

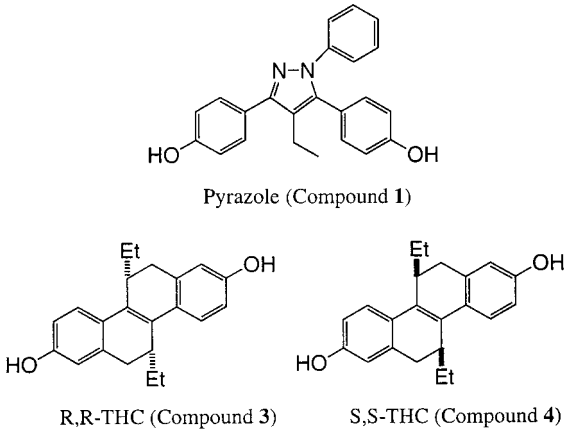

FIG. 1. Structures of the compounds used in this study. THC (a racemate, compound 2) is a 1:1 mixture of the R, R-THC and S, S-THC.

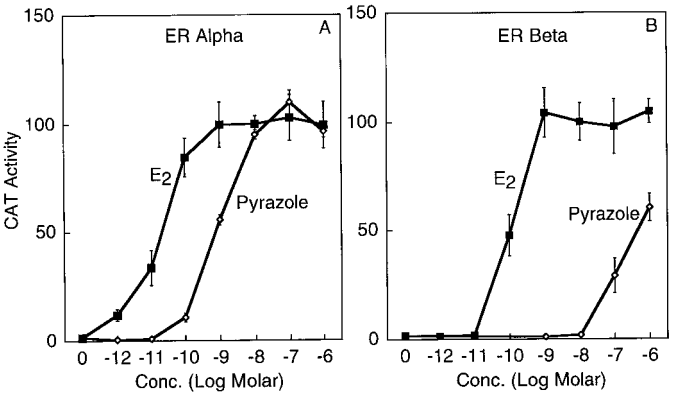

FIG. 2. Transcription activation by ERα and ERβ in response to the pyrazole (compound 1). Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ERα (A) or ERβ (B) and an (ERE)$_3$-pS2-CAT reporter gene and were treated with the indicated concentrations of estradiol (E_2) or the pyrazole for 24 h. CAT activity was normalized for β-galactosidase activity from an internal control plasmid. Values are the mean ± SD for three or more separate experiments, and are expressed as a percent of the ERα or ERβ response with 10 nM E_2. For some values, *error bars* are too small to be visible.

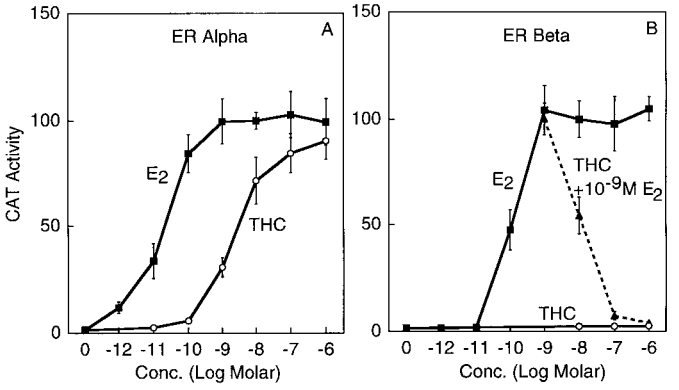

FIG. 3. Transcription activation by ERα and ERβ in response to the THC (compound 2). Transfection assays were conducted in HEC-1 cells using the (ERE)$_3$-pS2-CAT reporter as described in the legend of Fig. 2. B, The antagonist activity of THC on ERβ was assayed in the presence of 1 nM E_2.

The transcriptional activity of compound 2 (THC) is shown in Fig. 3. Of interest, compound 2 (THC) was a full ERα agonist, but showed no transcriptional activation of ERβ, and it fully suppressed E_2-stimulated transcriptional activity via ERβ. Thus, this compound acts through ERα as an agonist, but through ERβ as an antagonist. The potency of THC for ERα stimulation is approximately 200-fold less than E_2 (Fig. 3A). Even at very high concentrations of THC,

the compound showed no stimulation of ERβ (Fig. 3B), and a 100-fold excess of THC fully suppressed the E_2 stimulation of ERβ, with a 10-fold excess giving 50% suppression (Fig. 3B). Thus, the THC appears to be more potent as an ERβ antagonist than it is as an ERα agonist, which may be explained by its preferential binding affinity for ERβ (see below, Table 1).

Because the enantiomers in a racemate (a 1:1 mixture of enantiomers) often have different biological activities (13), we synthesized the individual R,R- and S,S-enantiomers of the THC and examined their separate activities through ERα and ERβ. Intriguingly, as shown in Fig. 4, the ERβ selective antagonist activity of THC resides completely in the R,R-enantiomer of THC (compound 3). Although R,R-THC was an agonist on ERα, it showed no stimulation of ERβ, and it fully suppressed E_2 activation of ERβ. By contrast, the S,S-enantiomer (compound 4), which was an agonist on ERα, was also a nearly full agonist on ERβ. It is of note that the R,R-enantiomer of THC gave a profile for suppression of E_2 stimulation through ERβ similar to that of the THC racemate, which is consistent with the much higher affinity of the R,R-THC enantiomer for ERβ than that of the S,S-enantiomer (see below, Table 1).

The activity of compounds 2–4 shown in Figs. 3 and 4 was also observed in all other cell and promoter contexts examined. One example is presented in Fig. 5, using the complement C3 promoter, an estrogen-responsive promoter which contains nonconsensus EREs, and another is shown in Fig. 6 using the nonconsensus lactoferrin estrogen-responsive element and the heterologous thymidine kinase promoter. THC is again seen to be an ERβ selective antagonist ligand. In addition, pyrazole is seen to be an ERα potency-selective agonist ligand (Fig. 6). Other cell and promoter contexts examined but not shown, which gave similar transactivation profiles to those presented, included CHO cells and a simple promoter with consensus EREs (2ERE-TATA-CAT) and HEC-1 cells with nonconsensus EREs from the pS2 and cathepsin D genes linked to the thymidine kinase promoter.

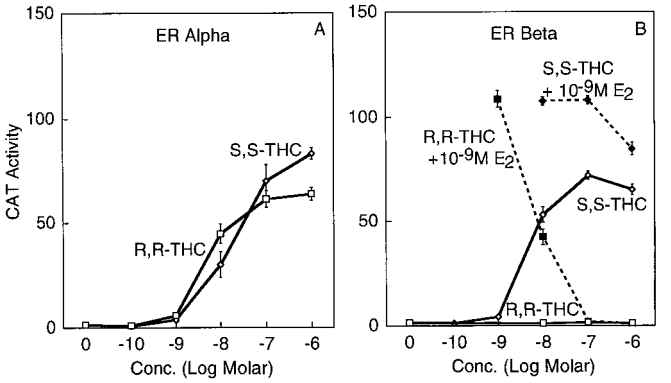

FIG. 4. Transcription activation by ERα and ERβ in response to the R, R-THC (compound 3) and the S, S-THC (compound 4). Transfection assays were conducted in HEC-1 cells using the $(ERE)_3$-pS2-CAT reporter as described in the legend of Fig. 2. B, The antagonist activity of both compounds on ERβ was assayed in the presence of 1 nM E_2.

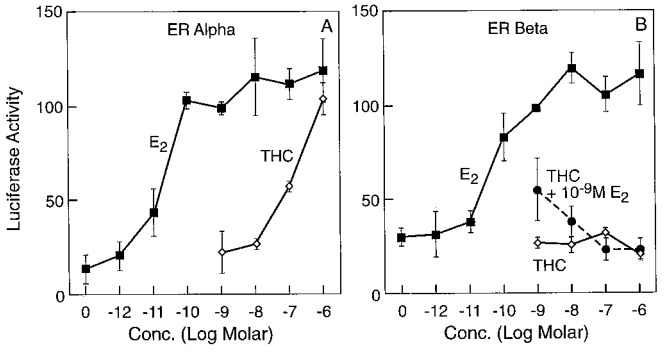

FIG. 5. Transcription activation by ERα and ERβ in response to the THC (compound 2) through the complement C3 promoter. Transfection assays were conducted in HEC-1 cells as described in the legend of Fig. 2, except that the complement C3 promoter was used (−1030 to +58 of C3 promoter fused to the luciferase reporter gene). B, The antagonist activity of THC on ERβ was assayed in the presence of 1 nM E_2. Luciferase activity was normalized for β-galactosidase activity from a cotransfected internal control plasmid. Values are the mean ± SD from three separate experiments and are expressed as a percent of the ERα or ERβ response with 1 nM E_2.

TABLE 1. Binding affinity of compounds for ERα and ERβ

Compound	Relative binding affinity[a] [K_i][b]	
	ERα	ERβ
Estradiol (E_2)	100 [0.30 nM]	100 [0.90 nM]
Pyrazole (Cpd 1)	60 ± 16 [0.54 nM]	18 ± 4 [5.1 nM]
THC (Cpd 2)	2.5 ± 0.7 [13 nM]	25 ± 15 [3.6 nM]
R,R-THC (Cpd 3)	3.6 ± 1.3 [9.0 nM]	25 ± 6 [3.6 nM]
S,S-THC (Cpd 4)	0.83 ± 0.2 [39 nM]	1.3 ± 0.2 [70 nM]
Hydroxytamoxifen	149 ± 24 [0.22 nM]	62 ± 8 [1.5 nM]
ICI182,780	32 ± 14 [1.0 nM]	25 ± 0 [3.6 nM]

[a] Competitive radiometric binding assays were done using 10 nM [^{3}H] E_2 as tracer and purified ligand binding domains of ERα and ERβ expressed in *E. coli*, as described (12) and are expressed relative to E_2, which is set at 100.

[b] K_i values for compounds 1–4, and for hydroxytamoxifen and ICI182,780 are calculated using the Cheng-Prusoff relationship (14) from their IC_{50} values and the K_d values for [^{3}H]E_2 with ERα (0.30 nM) and ERβ (0.90 nM), determined after 18 h at 0C, following the protocol in Ref 12.

Binding affinities of ER subtype-selective ligands

The relative binding affinities and K_i values of compounds 1–4 for ERα and ERβ are shown in Table 1. For comparison, the values are also given for the antiestrogens hydroxytamoxifen and ICI182,780. The pyrazole compound, which was an ERα potency-selective agonist (Fig. 2), showed higher affinity for ERα, as might have been predicted. The 9-fold difference in receptor binding affinity, however, does not account fully for the 120-fold difference in transactivation potency on the two ER subtypes.

The binding affinities of the three THC compounds reflect closely their ER subtype selective potencies. The R,R-enantiomer (compound 3) has a 3-fold preferential binding for ERβ, consistent with the higher potency of this compound as an ERβ antagonist than as an ERα agonist. The S,S-enantiomer (compound 4) has lower overall affinity, showing little preference for either subtype, which matches its equivalent potency as an agonist on both ERα and ERβ. The relative affinities of compounds 3 and 4 for the two ERs are nicely

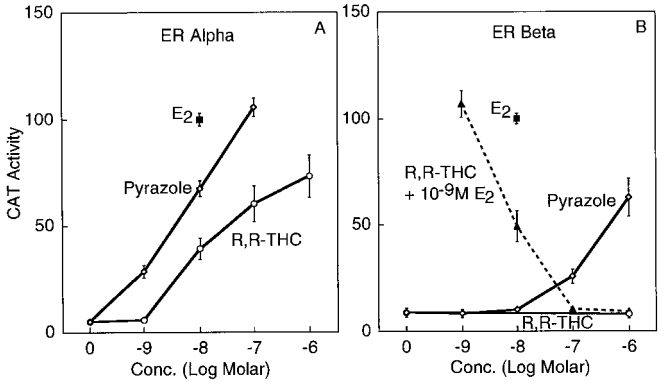

FIG. 6. Transcription activation by ERα and ERβ in response to the pyrazole (compound 1) and R,R-THC (compound 3) monitored in transfection assays in HEC-1 cells using the lactoferrin nonconsensus ERE fused to the thymidine kinase promoter and CAT reporter gene. CAT activity was normalized for β-galactosidase activity from a cotransfected internal control plasmid. Values are the mean ± SD from three experiments and are expressed as a percent of the ERα or ERβ response with 10 nM E_2.

reflected in the racemate (compound 2), whose binding is simply the average of that of the two enantiomers. The two well known antiestrogens, hydroxytamoxifen and ICI182,780, show somewhat higher affinities for ERα than for ERβ.

Discussion

In this report, we describe novel ligands for ERα and ERβ, a pyrazole compound (compound 1) that is a 120-fold potency selective agonist for ERα, and THC compounds (THC and R,R-THC) that are agonists via ERα but full antagonists via ERβ. The latter two compounds differ from antiestrogenic compounds such as tamoxifen and raloxifene, that are full antagonists on ERβ but usually have only minimal agonist activity on ERα when assayed in systems similar to those used here (4, 10, 11, 15–17). For example, in HEC-1 cells hydroxytamoxifen has 25% the agonist activity of estradiol through ERα, but is a pure antagonist through ERβ (11, 15). By contrast, THC and R,R-THC are nearly full agonists on ERα.

Others have reported on compounds that show differences in their binding affinity for ERα and ERβ (4). ERα affinity selectivities up to 5 have been found for certain substituted estrogens, especially those with 17α substituents. The highest ERβ affinity selectivities of approximately 7 have been reported for certain nonsteroidal phytoestrogens, such as genistein. When the difference in relative binding affinity of the tracers used in these studies (16α -[^{125}I] iodoestradiol in Ref. 4 and [^{3}H] estradiol, this study) for ERα and ERβ is taken into consideration, THC and the R,R-enantiomer show equally high ERβ affinity selectivities.

The relative potency of R,R-THC (compound 3) in the ERα and ERβ transcription assays correlates rather well with its relative affinity in the ligand binding assays: It has a higher affinity for ERβ, which is consistent with its greater potency as an ERβ antagonist than as an ERα agonist. The S,S-enantiomer (compound 4) has both lower affinity and less selectivity in its binding to ERα and ERβ, which is consistent with its equivalent potency as an agonist on both ER subtypes. Interestingly, the racemate (compound 2), which consists of an equal mixture of the R,R- and S,S-enantiomers, behaves much the same as the R,R-enantiomer in both binding and transactivation assays. As expected, the affinity of the racemate for both ER subtypes is the average of that of the two enantiomers. Because the antagonistic R,R-enantiomer has a 20-fold higher affinity for ERβ compared with that of the agonistic S,S-enantiomer (Table 1), the antagonist character of the R,R-enantiomer dominates the agonist character of the S,S-enantiomer, so that the racemate is also a very effective antagonist on ERβ.

There is, however, not always a direct correlation between binding affinity and transcriptional potency. The pyrazole (compound 1) has only moderately lower affinity for ERα and only 5-fold lower affinity for ERβ than does E_2, yet its potency as an agonist on both subtypes is considerably less than that of E_2 (Fig. 2). Its higher affinity for ERα *vs.* ERβ, however, is reflected in its ERα agonist potency selectivity, although its 120-fold potency selectivity in the transcription assay is considerably greater than its 9-fold affinity selectivity. This suggests that factors beyond ligand-receptor interaction, such as receptor-coactivator interactions, are likely to be important determinants of transcriptional potency (18). In addition, the role of activation functions 1 and 2 (AF1 and AF2) in mediating the activity of the pyrazole and THC compounds as agonists and antagonists through ERα and ERβ remains an aspect of importance. This is a complex issue because we and others find that the importance of AF1 and AF2 in ERα and ERβ with estradiol and other ligands depends greatly on both the cell background and promoter context (11, 15, 19), as we observe also with compounds 1–4.

Recently reported crystal structures of ERα complexed with E_2 or the antiestrogen raloxifene reveal that the position of helix-12, the activation function 2 activation helix, is very different with these two ligands, being packed into the ligand binding pocket in the complex with agonist, but displaced and extended in the antagonist complex (20). This conformational change was suggested to be an important molecular determinant in mediating the agonist *vs.* antagonist activity of the bound ligand (20). In this regard, compound 3, the R,R-enantiomer of THC, should be of considerable interest in probing the conformational correlates of agonist *vs.* antagonist activity in the two ER subtypes, as one would predict that its complex with ERα would be in the agonist conformation, whereas its complex with ERβ would be in the antagonist conformation.

These compounds should also be useful as pharmacological probes to elucidate the biological roles played by ERβ *vs.* ERα. For example, the ERβ-selective antagonist (the R,R-THC, compound 3) could be used to identify ERβ-mediated activities in both wild-type and ERα-knockout animals by their selective suppression by this ligand. Likewise, the ERα potency-selective agonist (pyrazole, compound 1), at an appropriate dose, could be used to selectively activate ERα in cells or tissues that contain both receptor subtypes.

Acknowledgments

We thank Kathryn E. Carlson for assistance with some of the assays.

References

1. **Katzenellenbogen BS, Korach KS** 1997 Editorial: a new actor in the estrogen receptor drama-enter ERβ. Endocrinology 138:861–862
2. **Mosselmen S, Polman J, Dijkema R** 1996 ERβ: identification and characterization of a novel human estrogen receptor. FEBS Lett 392:49–53
3. **Kuiper GJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA** 1996 Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925–5930
4. **Kuiper GJM, Carlsson B, Grandien J, Enmark E, Haggblad J, Nilsson S, Gustafsson JA** 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138:863–870
5. **Wrenn CK, Katzenellenbogen BS** 1993 Structure-function analysis of the hormone binding domain of the human estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast. J Biol Chem 268:24089–24098
6. **Kraus WL, McInerney EM, Katzenellenbogen BS** 1995 Ligand-dependent, transcriptionally productive association of the amino- and carboxyl-terminal regions of a steroid hormone nuclear receptor. Proc Natl Acad Sci USA 92:12314–12318
7. **Norris JD, Fan D, Wagner BL, McDonnell DP** 1996 Identification of the sequences within the human complement 3 promoter required for estrogen responsiveness provides insight into the mechanism of tamoxifen mixed agonist activity. Mol Endocrinol 10:1605–1616
8. **Hwang KJ, O'Neil JP, Katzenellenbogen JA** 1992 5,6,11,12-tetrahydrochrysenes: synthesis of rigid stilbene systems designed to be fluorescent ligands for the estrogen receptor. J Org Chem 57:1262–1271
9. **Hwang KJ, Carlson KE, Anstead GM, Katzenellenbogen JA** 1992 Donor-acceptor tetrahydrochrysenes, inherently fluorescent, high affinity ligands for the estrogen receptor: binding and fluorescence characteristics, and fluorometric assay for receptor. Biochemistry 31:11536–11545
10. **Montano MM, Müller V, Trobaugh A, Katzenellenbogen BS** 1995 The carboxyl-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists. Mol Endocrinol 9:814–825
11. **McInerney EM, Katzenellenbogen BS** 1996 Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation. J Biol Chem 271:24172–24178
12. **Carlson KE, Choi I, Gee A, Katzenellenbogen BS, Katzenellenbogen JA** 1997 Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open-pocket conformation is required for ligand interaction. Biochemistry 36:14897–14905
13. **Bentley R** 1995 From optical activity in quartz to chiral drugs: molecular handedness in biology and medicine. Perspect Biol Med 38:188–229
14. **Cheng YC, Prusoff WH** 1973 Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108
15. **McInerney EM, Weis KE, Sun J, Mosselman S, Katzenellenbogen BS** 1998 Transcription activation by the human estrogen receptor subtype β (ERβ) studied with ERβ and ERα receptor chimeras. Endocrinology 139:4513–4522
16. **Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, Giguere V** 1997 Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor β. Mol Endocrinol 11:353–365
17. **Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J, Nilsson S** 1998 Differential response of estrogen receptor α and estrogen receptor β to partial estrogen agonists/antagonists. Mol Pharmacol 54:105–112
18. **Katzenellenbogen JA, O'Malley BW, Katzenellenbogen BS** 1996 Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. Mol Endocrinol 10:119–131
19. **Tzukerman MT, Esty A, Santiso-Mere D, Danielian P, Parker MG, Stein RB, Pike JW, McDonnell DP** 1994 Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. Mol Endocrinol 8:21–30
20. **Brzozowski AM, Pike ACW, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson J-A, Carlquist M** 1997 Molecular basis of agonism and antagonism in the estrogen receptor. Nature 389:753–758