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Characterization of the Pharmacophore Properties of Novel Selective Estrogen Receptor Downregulators (SERDs)

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Abstract

Selective estrogen receptor (ER) downregulators (SERDs) reduce ER α protein levels as well as block ER activity, and therefore are promising therapeutic agents for the treatment of hormone refractory breast cancer. Starting with the triarylethylene acrylic acid SERD **4**, we have investigated how alterations in both the ligand core structure and the appended acrylic acid substituent affect SERD activity. The new ligands were based on high affinity, symmetrical cyclofenil or bicyclo[3.3.1]nonane core systems, and in these, the position of the carboxyl group was extended from the ligand core, either retaining the vinylic linkage of the substituent or replacing it with an ether linkage. Although most structural variants showed binding affinities for ER α and ER β higher than that of **4**, only the compounds preserving the acrylic acid side chain retained SERD activity, although they could possess varying core structures. Hence, the acrylic acid moiety of the ligand is crucial for SERD-like blockade of ER activities.

Keywords

Selective Estrogen Receptor Downregulators (SERDs); Selective Estrogen Receptor Modulators (SERMs); Estrogen Receptor alpha; endocrine resistance

Introduction

A novel class of compounds capable of modulating the level and activity of the estrogen receptor (ER) are termed selective estrogen receptor downregulators (SERDs). These compounds are mechanistically distinct from ER ligands such as 4-hydroxytamoxifen, which act as either agonists or antagonists, depending upon the target tissue, and are termed selective estrogen receptor modulators (SERMs).^{1, 2} Although certain SERMs are clinically useful for menopausal hormone replacement and for the prevention and treatment of breast cancer,³ there is a need for new ER ligands that are capable of overcoming acquired endocrine resistance in breast cancer. Current SERDs, such as ICI 182,780 (ICI, Figure 1),

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Supporting Information Available: Synthesis and spectroscopic characterization (9-12, 14-46), ¹H and ¹³C NMR spectra (9-12, 14-46), normal and reversed phase HPLC chromatograms (11, 15, 17, 22-26, 29, 31-36, 42-44, 46). This material is available free of charge via the Internet at <http://pubs.acs.org>.

have a more antagonistic profile than SERMs, and are capable of inhibiting the growth of tamoxifen-resistant breast cancer cells.⁴

SERDs are currently used in the treatment of metastatic breast cancer,^{4, 5} and their improved clinical activity is thought to derive from their ability to downregulate the ER protein as well as block ER action.⁶⁻⁹ ICI, however, has poor oral bioavailability,¹⁰ and a search for alternate drugs led to the identification of GW-5638 (Compound **1**, Figure 1), a SERD that shares the triarylethylene ligand core scaffold of tamoxifen but has the basic side chain replaced by an acrylic acid unit. Compound **1** has an agonist profile in bone, but acts as an antagonist in the breast,¹¹⁻¹³ and can inhibit the growth of tamoxifen-resistant breast tumors.⁹ GW-7604 (Compound **4**, Figure 1) is a hydroxylated analog of compound **1** with higher ER affinity,^{11, 12} analogous to 4-hydroxytamoxifen, being a high affinity analog and metabolite of tamoxifen.¹⁴

It has been proposed that the SERD activities of both **4** and ICI result from their distortion of the conformation of the ER ligand binding domain (LBD) so that hydrophobic surfaces become exposed, thereby promoting accelerated degradation of the ER protein.^{15, 16} The X-ray crystallographic structure of an ER α -LBD complex with a structural analog of ICI, shows the ligand bound in an inverted mode relative to estradiol, with the long 7 α -substituent projecting outward from the ligand binding pocket, displacing helix-12 of the LBD and occupying the coactivator binding groove.¹⁵ A recent structure of **4** with ER α shows that helix-12 is tilted from the normal SERM position through interaction of the carboxylic acid group of the ligand with the positive end of the helix-12 dipole.¹⁶ In both cases, fluorescent probes of exposed protein hydrophobic regions have revealed that these ER-SERD structures are more hydrophobic than are complexes with estrogen agonists (estradiol, DES) or SERMs (hydroxytamoxifen and raloxifene).¹⁶

Although the distortion of helix-12 in the ER α structure complexed with **4** suggests an important role for the carboxylic acid function of the acrylic acid side chain, it is not clear whether this function is positioned optimally or might be improved by presentation in an alternate manner, through other linkages, or at different distances from the ligand core. Similarly, it is not known whether the triarylethylene core structure is essential or might be replaced by alternate moieties with higher ER binding affinities. The hydroxy-triarylethylene (TAE) core of **4**, in particular, might be subject to cis-trans isomerization in solution, because it shares this structural feature with hydroxytamoxifen and diethylstilbestrol, both of which do undergo this isomerization in a facile manner.^{17, 18}

Therefore, to investigate to what extent the SERD activity of **4** is dependent on the hydroxytamoxifen-type core, the vinyl linkage, or the acrylic acid side chain, we prepared and evaluated the SERD activity of a new series of ER ligands having acidic side chains of varying length and linkages and built on different ligand core structures, cyclofenil (Compound **2**) and bicyclononane (Compound **3**), that are symmetrical and therefore do not exist as stereoisomers (Figures 1 and 2). From our initial series, we selected analogs having high binding affinity for ER α and ER β , and we further characterized their ability to alter the subcellular localization of ER α and to downregulate its protein levels and inhibit gene stimulation by estrogen (SERD activity). The two new compounds having the most favorable SERD-like activity retained the acrylic acid side chain, although they had different core structures.

This study expands our current understanding of the optimal pharmacophore requirements for SERD activity and their capabilities in regulating the activity of the estrogen receptor. It also highlighted the importance of the acrylic acid side chain of **4** for engendering SERD-

like activity, but revealed that alternate ligand core structures can be used in place of the tamoxifen-like triarylethylene core used in the GW series.

Results

Chemical Synthesis

The structures of the SERD **4** analog series that we prepared are summarized in Figure 2, and those studied in greater detail are shown in Table 1 and Table 2 (see compound numbers with underline). Some analogs (**42**, **43**, **44**) are based on a well known, high affinity non-steroidal estrogen, cyclofenil (**2**, Figure 1), but the majority (**11**, **12**, **15**, **17**, **22-26**, **29**, **32-36**, **46**) are based on bicyclononane (**3**, Figure 1), a related non-steroidal estrogen that has exceptionally high binding affinity for both ER α and ER β (Table 1a).¹⁹ Notably, neither of these symmetrical ligand cores have cis-trans isomers, as does the TAE-based compound (compound **4**, Figure 1). In both the cyclofenil and bicyclononane series (designated **CF** and **BN** for simplicity), we prepared the direct analog of **4** containing the acrylic acid substituent (**17**, **42**), as well as a series with carboxy-terminated alkoxy substituents (Figure 2, "Alkoxy Acids"). Because members of the bicyclononane series proved to have higher affinity, we prepared additional homologs of the acrylate-substituted system, having longer chain acids linked through a styryl function (Figure 2, "Styrene Acids"), as well as some individual variants, bis-carboxymethyl and a deoxy analog or members with vinylphosphonate or trifluoromethanesulfonyloxy groups (Figure 2, bottom).

The synthetic routes used to prepare these compounds are shown in Schemes 1-3. McMurry coupling was used to prepare the parent cyclofenil and bicyclononane systems (Compounds **7-9**) from their appropriate benzophenone precursors (Compounds **5** and **6**, Scheme 1). Members of the alkoxy ether series were prepared from the parent cyclofenil and bicyclononane ligands by Williamson ether synthesis using various ω -bromo esters, followed by ester hydrolysis (Compounds **32-36**, Scheme 2 and Compound **43**, Scheme 3). Good yields of mono substitution on the bisphenol parent ligands could be obtained in all cases, except with ethyl bromoacetate. In this case, the bisphenols were first protected as the mono-TBDMS ethers (Compounds **10**, **37**); mono alkylation followed by saponification/deprotection gave the desired mono alkoxymethyl ethers (Compounds **15**, **44**). The mono-TBDMS ethers were also used to prepare the monotrifluoromethane sulfonates (Compounds **11**, **38**), which were used in Suzuki coupling to prepare members of the styryl series (Compounds **17**, **22-25**, Scheme 2 and Compound **42**, Scheme 3). A complete description of the syntheses with experimental details and spectroscopic characterization is given in the Supporting Information section.

Biological Results

Estrogen Receptor Binding Assays and Structure-Affinity Relationships

The compounds were assayed for their binding affinity for human ER α and ER β in a radiometric competitive binding assay using [³H]estradiol as tracer and estradiol as the reference standard.^{20, 21} Binding affinities are expressed as relative binding affinity (RBA) values, with estradiol set at 100. Results are given in Table 1 (comparison of triarylethylene (**TAE**), cyclofenil (**CF**) and bicyclononane (**BN**) series) and Table 2 (extended **BN** styryl and alkoxy series).

The binding affinities of the parent phenols in the triarylethylene (**TAE**), cyclofenil (**CF**) and bicyclononane (**BN**) systems are presented in Table 1a. All three parent compounds have very high binding affinity for ER α and ER β , but the highest affinity is shown by the parent bicyclononane (**3**). As with hydroxytamoxifen,¹⁴ compound **4** exists as a cis-trans isomer mixture. After careful recrystallization, we were able to obtain only the Z isomer in

pure form (**4'**). The lower affinity of the pure Z isomer (Table 1a) suggests that the E isomer is predominant in binding to the ERs. This is consistent with the X-ray crystal structures of this compound,¹⁶ as well as binding affinity measurements and X-ray structures for hydroxytamoxifen and diethylstilbestrol, where the isomer with the corresponding trans-4-hydroxystilbene isomer is the active one.^{14, 17, 22}

In the acrylic acid series (Table 1), the affinity of the cyclofenil analog (**42**) is considerably lower than that of the GW compound (**4**); the bicyclononane analog (**17**), however, binds with comparable affinity. The binding affinities of two alkoxy analogs prepared in both the cyclofenil and bicyclononane series (compounds **44** and **43**, and compounds **15** and **34**, respectively) provided additional evidence for the higher affinity of members of the bicyclononane series; therefore, further synthetic efforts focused on this series. Also of note are the binding affinities of certain other bicyclononane analogs (Tables 1a and 1b, Figure 2, **bottom**) designed to test other side chain functional groups. Compounds in which the OH is missing (**46**) or substituted with a second alkoxy group (**12**) have poor binding affinities. The ester (**29**) has lower affinity than the corresponding acid **34**; the methyl phosphonate **26** binds less well than the corresponding carboxylate **17**, and the trifluoromethane sulfonate (**11**) binds only moderately well.

Binding affinities of the extended bicyclononane series of styryl and alkoxy analogs are given in Table 2. In both series and for both receptors, binding affinities (with but one exception) increase with increasing chain length, reaching affinities much greater than that of **4** and even rivaling that of estradiol. This is shown graphically in Figure 3. None of the compounds in either Table 1 or 2 showed significant selectivity for either ER α or ER β , although those in the cyclofenil series (Table 1a) tended to be more ER β selective, with the cyclofenil acrylic acid (**42**) being ca 5-fold in favor of ER β .

Western Immunoblotting

We selected ten compounds with good binding affinity for ER for further analysis of their SERD character (see underlined compound numbers in Tables 1 and 2). These compounds were the initial SERD compound (**4**), the direct analogs of **4** in both the cyclofenil (**42**) and bicyclononane (**17**) series, the high affinity analogs in the bicyclononane styryl (**24** and **25**) and alkoxy (**15**, **33**, **34**, and **36**) series, and the BN vinyl methyl phosphonate (**26**). The well established SERD ICI was also included for comparison.

MCF-7 breast cancer cells were treated with these compounds, and the ER α protein was extracted and analyzed by SDS-PAGE and Western immunoblotting. Because SERDs are known not only to lower ER levels in cells but also to affect the subcellular distribution of ER,⁶⁻⁸ we monitored ER levels in whole cell extracts (WC), and in cytoplasmic (C), nuclear (N), matrix (M), and insoluble (I) fractions after treatment with these various compounds. The procedures used to obtain these extracts are given in the Methods, and representative Western immunoblots are shown in Figure 4.

The well characterized SERD ICI markedly reduced ER α protein levels in the cytoplasmic fraction and did not raise ER α levels in the nuclear fraction; the slight elevation of ER α in the matrix and insoluble fractions is consistent with reports that this compound does redistribute ER α into the less soluble cell fractions (Figure 4A).⁶⁻⁸ The known SERD **4** also reduces cytoplasmic ER α protein, but ER α levels in the nuclear, matrix and insoluble fractions are elevated, consistent with what others have shown.²³ The bicyclononane analog (**17**) redistributed and downregulated of ER in a manner very similar to that of the SERD **4**, as did the cyclofenil analog (compound **42**), though the latter to a somewhat lesser degree (Figure 4A).

Surprisingly, although they have higher ER α binding affinities than the three compounds discussed above, none of the bicyclononane acrylate homologs (styryl compounds **24** and **25**) nor the carboxyalkoxy analogs (compounds **15**, **33**, **34**, and **36**) that we tested demonstrated substantial SERD-like activity (Figure 4A and B). While cytoplasmic ER α levels were somewhat lower, nuclear ER, and in some cases also matrix and insoluble fractions, showed substantially increased ER α levels. These compounds all lack the acrylic acid side chain moiety which is present in compounds **4**, **17**, and **42**.²⁴

Western immunoblot analysis of MCF-7 cells treated with ICI and compounds **4**, **42**, and **17**, alone or with E2, is shown in Figure 5. In the presence of E2, all of the SERDs tested were capable of lowering ER α protein levels. This downregulation was seen particularly in the whole cell lysate, the cytoplasmic and the nuclear fractions. The cyclofenil acrylic acid (**42**) showed lower activity (i.e., less ER protein reduction), especially in the cytoplasmic fraction. The sequestration of ER α protein in the insoluble fraction increased upon concomitant E2 treatment. This may represent ligand-dependent turnover of the receptor,²⁵ as it is well known that E2 enhances ER turnover.²⁶

Analysis of ER α Mediated Transcription Regulation

Transcriptional analysis of established ER α target genes was carried out on MCF-7 cells treated with the best three compounds in the absence or presence of E2. The RNA levels of three E2-upregulated genes (pS2, progesterone receptor, carbonic anhydrase 12) were in most cases reduced by the SERDs **4**, **17**, and **42** (Figure 6A). Compound **4** and **17** significantly blocked the E2-mediated stimulation of the pS2 and PR genes, but compound **42** was ineffective, consistent with its less effective downregulation of the ER protein. However, **42** did block the E2-mediated activation of the CA12 gene even more so than did compound **17**. Compound **4** was the most effective in blocking estrogen action on all three of these E2-stimulated genes.

The three SERDs were also effective in reversing estrogen action on two genes that are repressed by E2, namely, BLNK and ENC1 (Figure 6B).²⁷ Compounds **4** and **17** did, in fact, reduce the E2-mediated repression of BLNK and ENC1 gene expression (Figure 6B), increasing RNA levels for these genes relative to the E2-repressed state, but the repression was not fully reversed to control, basal level of expression.

Discussion

In this study, we have examined a range of compounds structurally related to the selective estrogen receptor downregulator (SERD), compound **4**. In all of these analogs, the isomerization-prone hydroxyl-triarylethylene ligand core of **4** was replaced by two symmetrical cyclic systems, cyclofenil and bicyclononane, that are known to engender high ligand binding affinity for both estrogen receptors, ER α and ER β . In addition, homologs were prepared in which the carboxylic acid group on the acrylic acid side chain was extended (keeping the styrene linkage) or analogs in which it was replaced by an ether-linked carboxyalkyl substituent. While in both analog series, extension of the acid function away from the ligand core increased ER binding affinity substantially, it did not result in compounds having SERD activity. In fact, the only compounds capable of reducing levels of ER α protein, bicyclononane **17** and cyclofenil **42**, were ones that retained the acrylic acid group present in **4**. Of the two new SERDs, **17** and **42**, the higher affinity bicyclononane compound (**17**) proved to be the better, and in most respects it closely replicated the activity of **4**. The marked dampening of ER action shown by compounds **4** and **17** indicates an obliteration of transactivation from both AF-1 and AF-2, a known effect of pure antagonist/SERD binding to the ER.²⁸⁻³⁰

Structural studies have suggested that the acrylic acid side chain of **4** down regulates ER α protein levels by interactions with specific amino acids, namely D351 in the ligand binding domain of the receptor.³¹ This charge-charge repulsion blocks the proper positioning of the critical helix 12,^{31, 32} preventing it from adopting either the folded-back orientation found in agonist structures or the extended conformation found in SERM structures, where helix 12 occludes the hydrophobic groove of the AF-2 function, blocking interaction with coregulators.¹⁶ With helix 12 thus mal-disposed, hydrophobic patches are exposed, and the receptor is thought to become a target for ubiquitination and processing by the ubiquitin-proteasome pathway.³³ A related mechanism is proposed for the activity of ICI-type SERDs,¹⁶ although in this case, helix-12 appears to be completely disordered,³⁴ with even greater exposure of hydrophobic regions for targeting ubiquitination.¹⁶

Remarkably, the SERD activity of the compounds in this series appears to be uniquely associated with the acrylic acid unit that is common to compound **4**, cyclofenil **42**, and bicyclononane **17**. Even replacing it with a carboxymethyl ether, which enables the carboxyl group to access the same space as it occupies in the acrylic acid, did not suffice to engender SERD activity. This suggests that the rigidity of the acrylate unit, in fact, might be essential for maintaining the critical charge-charge repulsion with D351. Thus, the higher binding affinity found when the carboxylic acid group is spaced further from the aromatic ring might indicate relief of this ligand carboxylate-ER α D351 coulombic repulsion, and it suggests that the critical interaction needed to distort the position of helix 12 and induce ER degradation—the very essence of SERD activity—comes with a cost in binding energy. Relevant to this point, others have found that maintaining the double bond but replacing the carboxylic acid group with a carboxamide or methyl ketone, which would maintain rigidity but remove the charge, also results in loss of SERD activity.²⁴

While activation of the ubiquitin-proteasome pathway is a plausible explanation for the downregulation of ER α protein, Wijayaratne and McDonnell did not find a large increase in ER α ubiquitination upon treatment with compound **4**.³⁵ There are, however, other reports implicating the proteasome-ubiquitin pathway in ER turnover. Duong et al. described a ternary complex between ER α , p53, and the oncogenic ubiquitin-ligase Mdm2 in MCF-7 cells.³⁶ Also, receptor downregulation is blocked by proteasome-inhibitor MG132 treatment.³⁷⁻³⁹ It has been suggested that the 26S proteasome is necessary for the cycling of ER α protein needed to maintain transcription of target genes.²⁵ These studies all suggest the involvement of the ubiquitin-proteasome pathway in the downregulation of ER α protein.

As others have seen, SERDs such as ICI not only reduce ER α protein levels overall, but also redistribute it from the most soluble cellular compartments, the cytoplasm and nucleus, to less soluble compartments, the matrix and insoluble fractions. Presumably, at these locations it cannot function in its role as a ligand-modulated transcription factor. In fact, the movement of ER into these compartments might be a consequence of its ubiquitination, as others have noted.⁴⁰⁻⁴² Our observations of such downregulations and alterations in intracellular distribution of ER after treatment with these new SERDs are consistent with their prevention of estrogen-regulated gene expression and highlight the importance of the acrylic acid side chain in enabling SERD activity.

Conclusion

Compounds capable of suppressing estrogen action by reducing estrogen receptor levels appear to have particular promise for novel endocrine therapies in breast cancer. It is hoped that they might not succumb to the development of acquired resistance that typically follows treatment with SERMs, and this appears to be the case in model systems.⁴³ Our study has demonstrated that other ligand core structures, in addition to the triarylethylene ligand core

in traditional SERMs like tamoxifen and in the original GW SERD (**4**), can be used to produce SERD compounds (namely, the cyclofenil compound **42** and the bicyclononane compound **17**) that are still very effective at moderating the activity of ER. Our biological analyses of these novel SERDs underscore the importance of the acrylic side chain in effecting the downregulation of ER α and the suppression of ER-regulated gene expression.

Experimental

Synthesis Materials and Methods

See Supporting Information for details on synthesis, spectroscopic characterizations, and purity determination by normal and reversed phase HPLC analysis. All compounds assayed were >95% pure in both HPLC systems.

Biological Materials and Methods

Compounds

E2 and ICI 182,780 (ICI) were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Culture

The human breast cancer MCF-7 cell line was maintained in culture as previously described.⁴⁴ For Western analysis, cells were steroid depleted in phenol red-free media supplemented with 5% charcoal-dextran-treated calf serum for five days prior to the indicated ligand treatment. Media was changed on day 2 of culture, and cells were then treated with ligands. For real-time PCR gene expression analysis, cells were steroid depleted in phenol red-free media supplemented with 5% charcoal-dextran-treated calf serum for seven days prior to the indicated ligand treatment. Media was changed on d 2 and 4 of culture, and cells were then treated with ligands.

Cellular Fractionation

MCF-7 cells were plated on 10 cm² plates and grown to ca. 80% confluency. Cells were treated with either vehicle (0.1% EtOH) or 1 μ M SERDs for 16 h \pm 1 nM E2 for 4 h and harvested by scraping in 100 μ L of buffer. Cellular fractionation was carried out as previously described.^{23, 45} Whole cell lysates were obtained by harvesting in 100 μ L of whole cell lysis buffer [50 mM Tris (pH 8), 1 mM EDTA, 150 mM NaCl, 1% IGEPAL, 1% SDS, 5% glycerol]. Protein concentrations of the whole cell lysates, cytoplasmic and nuclear extracts were determined by BCA Protein Assay (Pierce, Rockford, IL).

SDS-PAGE and Western Analysis

20 μ g of whole cell lysate and 40 μ g of cytoplasmic and nuclear extracts were loaded on Tris-HCl SDS-PAGE gels (Bio-Rad, Hercules, CA). Equivalent fraction volumes of the detergent-extractable nuclear matrix and insoluble fractions were loaded. Proteins were transferred to nitrocellulose membranes (Pall Corporation, Pensacola, FL). Membranes were blocked in Odyssey Blocking Buffer (Li-Cor Biosciences, Lincoln, NE). Overnight primary antibody (α -F10 [Santa Cruz Biotechnology, Santa Cruz, CA] and α -Sp1 [Upstate/Millipore, Bedford, MA] at 1:1000 dilution) and secondary antibody (Li-Cor Biosciences, Lincoln, NE at 1:15,000 dilution) incubations were done in the presence of 0.1% Tween 20. PBST (PBS buffer with Tween) was used to wash off unbound antibody, and subsequent PBS washes were performed to limit the cross-reactivity of Tween 20 with the LiCor infrared imaging system.

Total RNA Isolation and Real-Time PCR Analysis

MCF-7 cells were plated in six-well plates and grown to ca. 80% confluency. Cells were pretreated with 1 μ M SERDs or 0.1% EtOH vehicle for 1h, and then treated with SERDs alone or concomitantly with 1 nM E2 for 16h. Total RNA was extracted, reverse transcribed, and analyzed via real-time PCR as previously described.⁴⁴ Primers used are as follows: ENC1 forward (f), GGCCTCCCCCTCAGTCTCT; ENC1 reverse (r), GCACTCACTACTGCGGCGT. The primers used for pS2, progesterone receptor, CA12, and BLNK were those previously described.^{27, 44, 46, 47} Statistical analysis was performed with repeated measures one-way ANOVA using the Tukey's t-test.

Estrogen Receptor Binding Affinity Assays

Relative binding affinities were determined by a competitive radiometric binding assay, as previously described,^{20, 21} using 10 nM [³H]estradiol as tracer (GE Healthcare, Piscataway, NJ), and purified full-length human ER α and ER β (PanVera/Invitrogen, Carlsbad, CA). Incubations were for 18-24 h at 0 °C, then the receptor-ligand complexes were absorbed onto hydroxyapatite (BioRad, Hercules, CA) and unbound ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values, with the RBA of estradiol set to 100. The values given are the average \pm range or SD of two or more independent determinations. Estradiol binds to ER α with a K_d of 0.2 nM and to ER β with a K_d of 0.5 nM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Katzenellenbogen BS, Katzenellenbogen JA. Estrogen receptor transcription and transactivation: Estrogen receptor alpha and estrogen receptor beta: regulation by selective estrogen receptor modulators and importance in breast cancer. *Breast Cancer Res* 2000;2:335–344. [PubMed: 11250726]
2. Grese TA, Dodge JA. Selective estrogen receptor modulators (SERMs). *Curr Pharm Des* 1998;4:71–92. [PubMed: 10197034]
3. Eng-Wong J, Zujewski JA. Raloxifene and its role in breast cancer prevention. *Expert Rev Anticancer Ther* 2004;4:523–532. [PubMed: 15270657]
4. Chia S, Gradishar W. Fulvestrant: expanding the endocrine treatment options for patients with hormone receptor-positive advanced breast cancer. *Breast* 2008;17(Suppl 3):S16–21. [PubMed: 18353647]
5. Vergote I, Abram P. Fulvestrant, a new treatment option for advanced breast cancer: tolerability versus existing agents. *Ann Oncol* 2006;17:200–204. [PubMed: 16251200]
6. Dauvois S, Danielian PS, White R, Parker MG. Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. *Proc Natl Acad Sci U S A* 1992;89:4037–4041. [PubMed: 1570330]
7. Dauvois S, White R, Parker MG. The antiestrogen ICI 182780 disrupts estrogen receptor nucleocytoplasmic shuttling. *J Cell Sci* 1993;106(Pt 4):1377–1388. [PubMed: 8126115]
8. Lupien M, Jeyakumar M, Hebert E, Hilmi K, Cotnoir-White D, Loch C, Auger A, Dayan G, Pinard GA, Wurtz JM, Moras D, Katzenellenbogen J, Mader S. Raloxifene and ICI182,780 increase

- estrogen receptor- α association with a nuclear compartment via overlapping sets of hydrophobic amino acids in activation function 2 helix 12. *Mol Endocrinol* 2007;21:797–816. [PubMed: 17299137]
9. Connor CE, Norris JD, Broadwater G, Willson TM, Gottardis MM, Dewhirst MW, McDonnell DP. Circumventing tamoxifen resistance in breast cancers using antiestrogens that induce unique conformational changes in the estrogen receptor. *Cancer Res* 2001;61:2917–2922. [PubMed: 11306468]
 10. Yoneya T, Taniguchi K, Tsunenari T, Saito H, Kanbe Y, Morikawa K, Yamada-Okabe H. Identification of a novel, orally bioavailable estrogen receptor downregulator. *Anticancer Drugs* 2005;16:751–756. [PubMed: 16027525]
 11. Willson TM, Henke BR, Momtahan TM, Charifson PS, Batchelor KW, Lubahn DB, Moore LB, Oliver BB, Sauls HR, Triantafyllou JA, Wolfe SG, Baer PG. 3-[4-(1,2-Diphenylbut-1-enyl)phenyl]acrylic acid: a non-steroidal estrogen with functional selectivity for bone over uterus in rats. *J Med Chem* 1994;37:1550–1552. [PubMed: 8201587]
 12. Willson TM, Norris JD, Wagner BL, Asplin I, Baer P, Brown HR, Jones SA, Henke B, Sauls H, Wolfe S, Morris DC, McDonnell DP. Dissection of the molecular mechanism of action of GW5638, a novel estrogen receptor ligand, provides insights into the role of estrogen receptor in bone. *Endocrinology* 1997;138:3901–3911. [PubMed: 9275080]
 13. McDonnell DP. The Molecular Pharmacology of SERMs. *Trends Endocrinol Metab* 1999;10:301–311. [PubMed: 10481160]
 14. Robertson DW, Katzenellenbogen JA, Long DJ, Rorke EA, Katzenellenbogen BS. Tamoxifen antiestrogens. A comparison of the activity, pharmacokinetics, and metabolic activation of the cis and trans isomers of tamoxifen. *J Steroid Biochem* 1982;16:1–13. [PubMed: 7062732]
 15. Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, Ljunggren J, Gustafsson JA, Carlquist M. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *Embo J* 1999;18:4608–4618. [PubMed: 10469641]
 16. Wu YL, Yang X, Ren Z, McDonnell DP, Norris JD, Willson TM, Greene GL. Structural basis for an unexpected mode of SERM-mediated ER antagonism. *Mol Cell* 2005;18:413–424. [PubMed: 15893725]
 17. Katzenellenbogen JA, Carlson KE, Katzenellenbogen BS. Facile geometric isomerization of phenolic non-steroidal estrogens and antiestrogens: limitations to the interpretation of experiments characterizing the activity of individual isomers. *J Steroid Biochem* 1985;22:589–596. [PubMed: 4010284]
 18. Airy SC, Sinsheimer JE. Isomerization of trans-diethylstilbestrol to pseudo-diethylstilbestrol. *Steroids* 1981;38:593–603. [PubMed: 7324087]
 19. Muthyala RS, Sheng S, Carlson KE, Katzenellenbogen BS, Katzenellenbogen JA. Bridged bicyclic cores containing a 1,1-diarylethylene motif are high-affinity subtype-selective ligands for the estrogen receptor. *J Med Chem* 2003;46:1589–1602. [PubMed: 12699377]
 20. Carlson KE, Choi I, Gee A, Katzenellenbogen BS, Katzenellenbogen JA. Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open pocket conformation is required for ligand interaction. *Biochemistry* 1997;36:14897–14905. [PubMed: 9398213]
 21. Katzenellenbogen JA, Johnson HJ Jr, Myers HN. Photoaffinity labels for estrogen binding proteins of rat uterus. *Biochemistry* 1973;12:4085–4092. [PubMed: 4745660]
 22. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* 1998;95:927–937. [PubMed: 9875847]
 23. Wittmann BM, Sherk A, McDonnell DP. Definition of functionally important mechanistic differences among selective estrogen receptor down-regulators. *Cancer Res* 2007;67:9549–9560. [PubMed: 17909066]
 24. Fan M, Rickert EL, Chen L, Aftab SA, Nephew KP, Weatherman RV. Characterization of molecular and structural determinants of selective estrogen receptor downregulators. *Breast Cancer Res Treat* 2007;103:37–44. [PubMed: 17033922]

25. Lonard DM, Nawaz Z, Smith CL, O'Malley BW. The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation. *Mol Cell* 2000;5:939–948. [PubMed: 10911988]
26. Eckert RL, Mullick A, Rorke EA, Katzenellenbogen BS. Estrogen receptor synthesis and turnover in MCF-7 breast cancer cells measured by a density shift technique. *Endocrinology* 1984;114:629–637. [PubMed: 6690295]
27. Frasor J, Stossi F, Danes JM, Komm B, Lyttle CR, Katzenellenbogen BS. Selective estrogen receptor modulators: discrimination of agonistic versus antagonistic activities by gene expression profiling in breast cancer cells. *Cancer Res* 2004;64:1522–1533. [PubMed: 14973112]
28. McDonnell DP, Norris JD. Connections and regulation of the human estrogen receptor. *Science* 2002;296:1642–1644. [PubMed: 12040178]
29. Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J Biol Chem* 2001;276:36869–36872. [PubMed: 11459850]
30. Johnston SR. Endocrinology and hormone therapy in breast cancer: selective oestrogen receptor modulators and downregulators for breast cancer - have they lost their way? *Breast Cancer Res* 2005;7:119–130. [PubMed: 15987442]
31. O'Regan RM, Jordan VC. The evolution of tamoxifen therapy in breast cancer: selective oestrogen-receptor modulators and downregulators. *Lancet Oncol* 2002;3:207–214. [PubMed: 12067682]
32. Bentrem D, Dardes R, Liu H, MacGregor-Schafer J, Zapf J, Jordan V. Molecular mechanism of action at estrogen receptor alpha of a new clinically relevant antiestrogen (GW7604) related to tamoxifen. *Endocrinology* 2001;142:838–846. [PubMed: 11159857]
33. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 2002;82:373–428. [PubMed: 11917093]
34. Pike AC, Brzozowski AM, Walton J, Hubbard RE, Thorsell AG, Li YL, Gustafsson JA, Carlquist M. Structural insights into the mode of action of a pure antiestrogen. *Structure* 2001;9:145–153. [PubMed: 11250199]
35. Wijayaratne AL, McDonnell DP. The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. *J Biol Chem* 2001;276:35684–35692. [PubMed: 11473106]
36. Duong V, Boulle N, Daujat S, Chauvet J, Bonnet S, Neel H, Cavailles V. Differential regulation of estrogen receptor alpha turnover and transactivation by Mdm2 and stress-inducing agents. *Cancer Res* 2007;67:5513–5521. [PubMed: 17545634]
37. Nawaz Z, Lonard DM, Dennis AP, Smith CL, O'Malley BW. Proteasome-dependent degradation of the human estrogen receptor. *Proc Natl Acad Sci U S A* 1999;96:1858–1862. [PubMed: 10051559]
38. Alarid ET, Bakopoulos N, Solodin N. Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. *Mol Endocrinol* 1999;13:1522–1534. [PubMed: 10478843]
39. Laios I, Journe F, Nonclercq D, Vidal DS, Toillon RA, Laurent G, Leclercq G. Role of the proteasome in the regulation of estrogen receptor alpha turnover and function in MCF-7 breast carcinoma cells. *J Steroid Biochem Mol Biol* 2005;94:347–359. [PubMed: 15857754]
40. Stenoien DL, Patel K, Mancini MG, Dutertre M, Smith CL, O'Malley BW, Mancini MA. FRAP reveals that mobility of oestrogen receptor-alpha is ligand- and proteasome-dependent. *Nat Cell Biol* 2001;3:15–23. [PubMed: 11146621]
41. Stenoien DL, Mancini MG, Patel K, Allegretto EA, Smith CL, Mancini MA. Subnuclear trafficking of estrogen receptor-alpha and steroid receptor coactivator-1. *Mol Endocrinol* 2000;14:518–534. [PubMed: 10770489]
42. Reid G, Hubner MR, Metivier R, Brand H, Denger S, Manu D, Beaudouin J, Ellenberg J, Gannon F. Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling. *Mol Cell* 2003;11:695–707. [PubMed: 12667452]
43. Osborne CK, Coronado-Heinsohn EB, Hilsenbeck SG, McCue BL, Wakeling AE, McClelland RA, Manning DL, Nicholson RI. Comparison of the effects of a pure steroidal antiestrogen with those

- of tamoxifen in a model of human breast cancer. *J Natl Cancer Inst* 1995;87:746–750. [PubMed: 7563152]
44. Frasar J, Danes JM, Komm B, Chang KC, Lyttle CR, Katzenellenbogen BS. Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. *Endocrinology* 2003;144:4562–4574. [PubMed: 12959972]
45. Coutts AS, Davie JR, Dotzlaw H, Murphy LC. Estrogen regulation of nuclear matrix-intermediate filament proteins in human breast cancer cells. *J Cell Biochem* 1996;63:174–184. [PubMed: 8913869]
46. Stossi F, Likhite VS, Katzenellenbogen JA, Katzenellenbogen BS. Estrogen-occupied estrogen receptor represses cyclin G2 gene expression and recruits a repressor complex at the cyclin G2 promoter. *J Biol Chem* 2006;281:16272–16278. [PubMed: 16608856]
47. Barnett DH, Sheng S, Charn TH, Waheed A, Sly WS, Lin CY, Liu ET, Katzenellenbogen BS. Estrogen receptor regulation of carbonic anhydrase XII through a distal enhancer in breast cancer. *Cancer Res* 2008;68:3505–3515. [PubMed: 18451179]

Abbreviation List

BN	bicyclononane
CF	cyclofenil
DES	diethylstilbestrol
E2	estradiol
ER	estrogen receptor
SERD	selective estrogen receptor downregulators
SERM	selective estrogen receptor modulators
TAE	triarylethylene

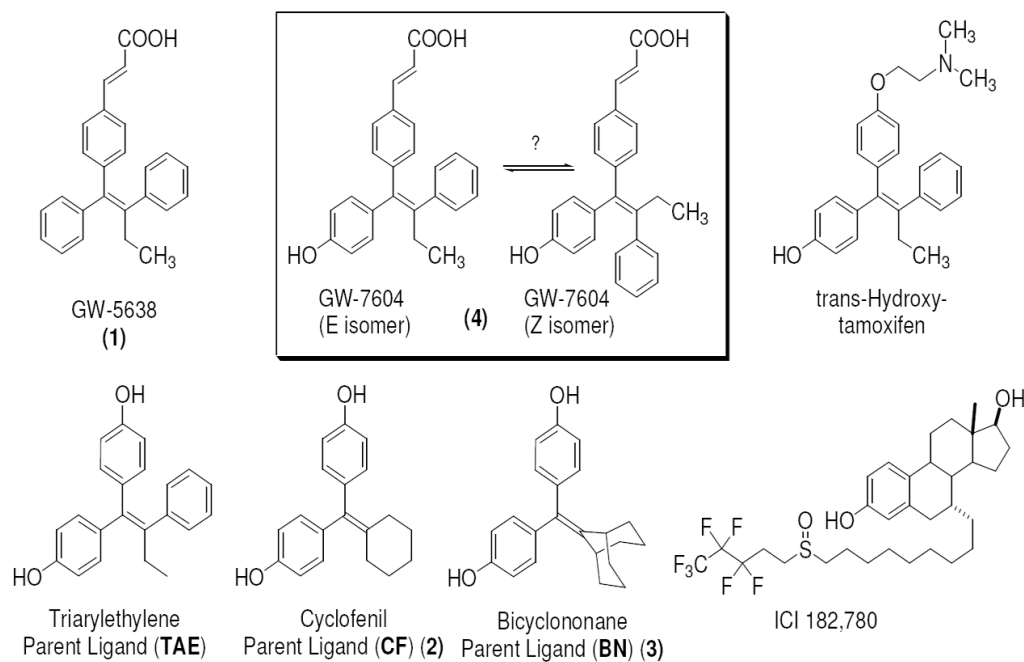


Figure 1. The Selective Estrogen Receptor Downregulators (SERDs) and Modulators (SERMs), and Symmetrical Ligands

The SERD GW-5638 (1), and its hydroxylated metabolite, GW-7604 (4), can potentially undergo cis-trans isomerization (boxed structures, see text). The GW compounds as well as *trans*-hydroxytamoxifen, share a triarylethylene (TAE) structural core. The cyclic-core ligands, cyclofenil (CF, 2) and bicyclononane (BN, 3), are symmetrical and thus do not exist as cis-trans isomers. ICI is a known SERD.

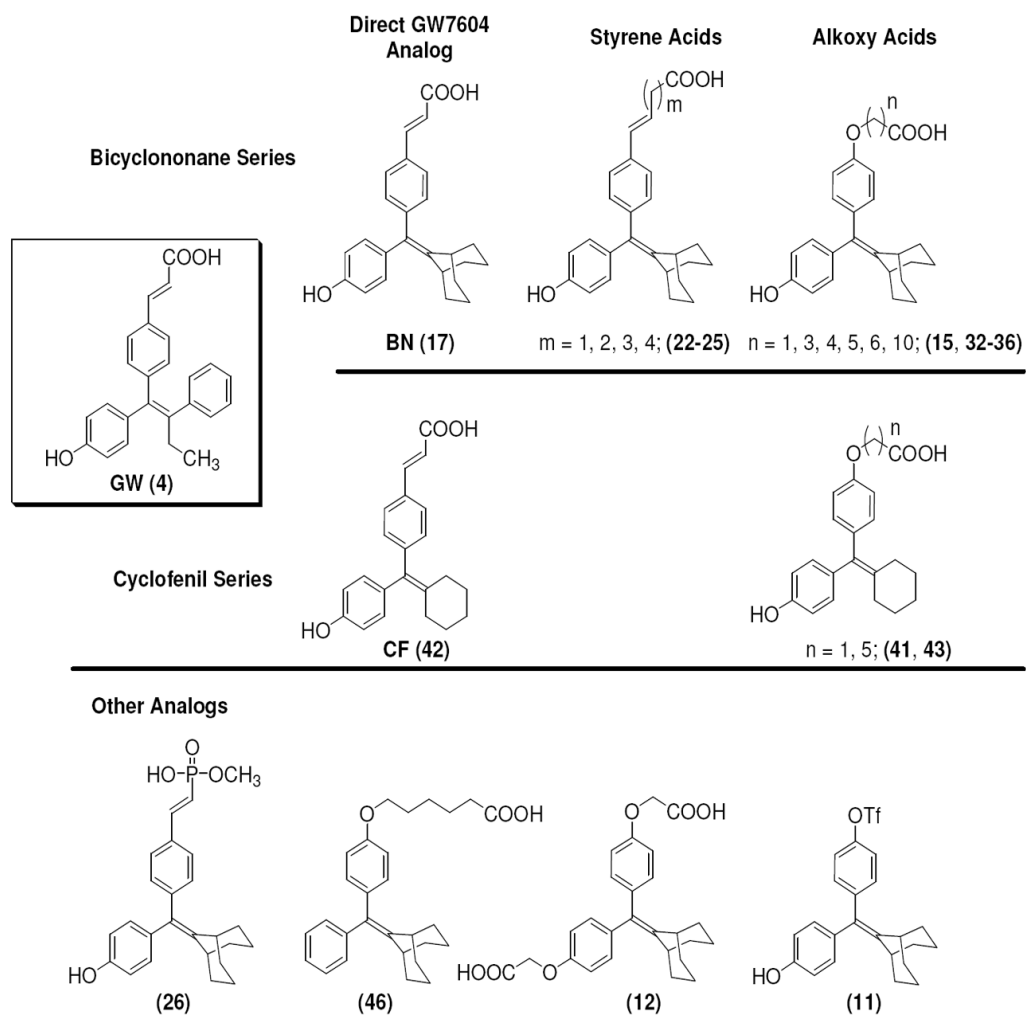


Figure 2. Series of SERD Compound 4 Analogs, Based on Cyclofenil (CF) and Bicyclononane (BN) and Other Analogs

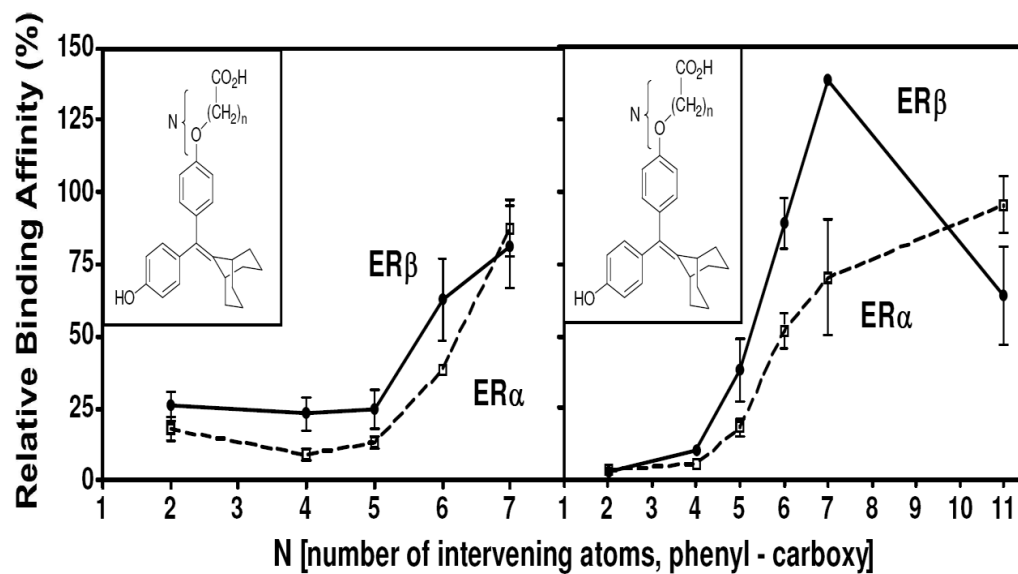


Figure 3. Dependence of Ligand Relative Binding Affinity on Acid Chain Length
N represents the number of intervening atoms between the phenyl and the carboxyl moieties, irrespective of whether they are carbon or oxygen.

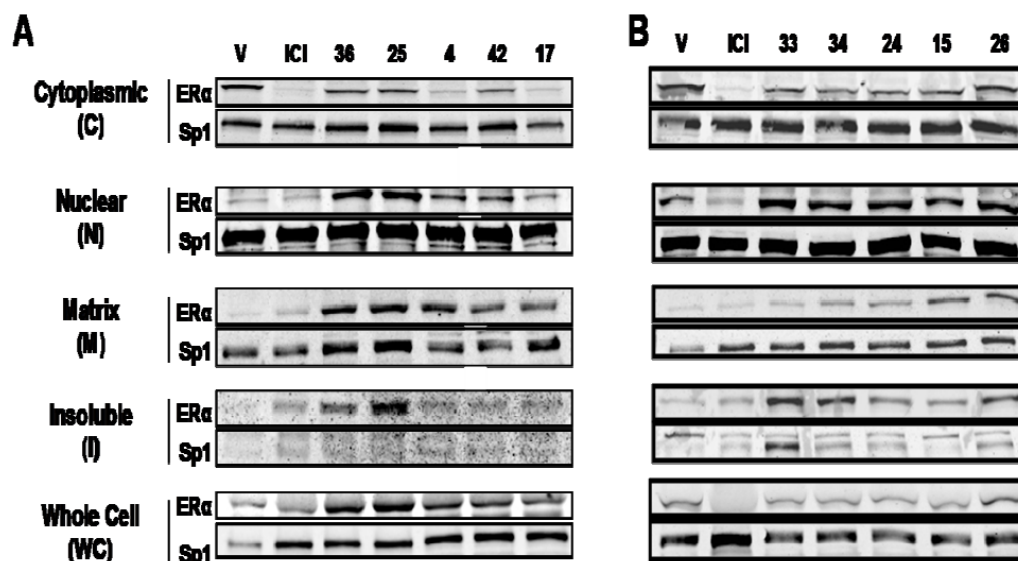


Figure 4. Subcellular Distribution and Downregulation of ER α Protein by ICI and compounds 36, 25, 4, 42, 17 (Panel A) and 33, 34, 24, 15, and 26 (Panel B)
MCF-7 cells were treated with 1 μ M compounds for 16h, and the level of cellular ER α in the indicated subcellular fraction and in whole cell extracts was measured by Western immunoblotting. Sp1 was monitored as an internal reference for gel loading.

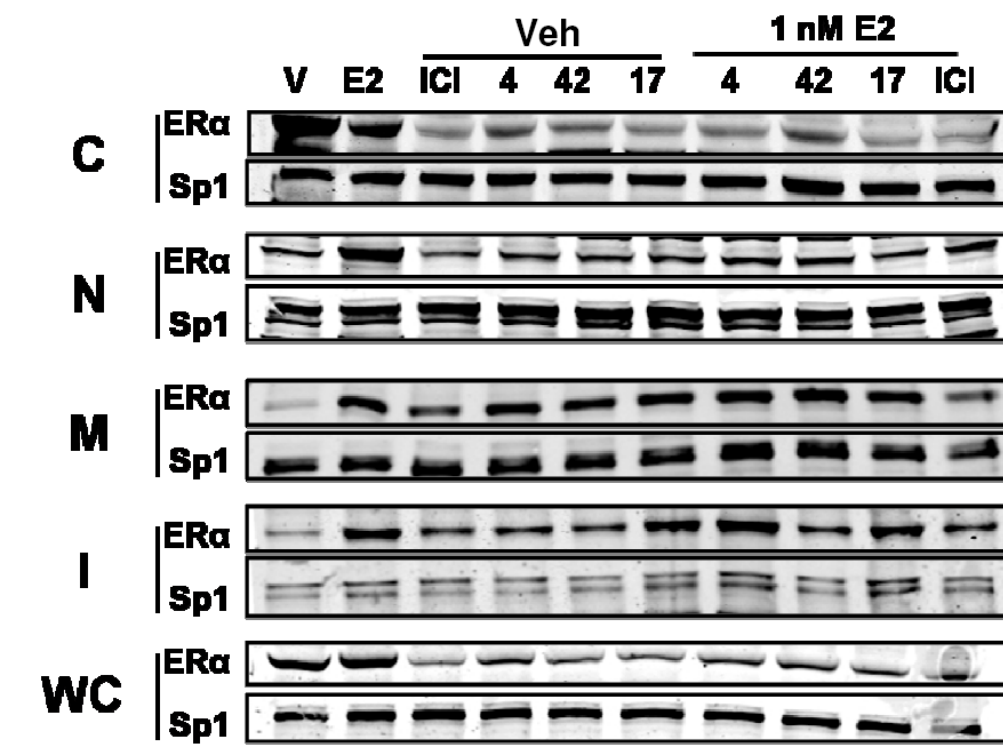


Figure 5. Western Immunoblot Analysis of ER α Protein Level and Subcellular Distribution with Compound and Estradiol Treatment

MCF-7 cells were treated with 1 μ M compound for 16h and then \pm 1 nM estradiol for 4h prior to cell fractionation and monitoring of ER α by Western immunoblots; leftmost two lanes show vehicle or E2 treatment for 20 h. Sp1 was monitored as an internal reference for gel loading.

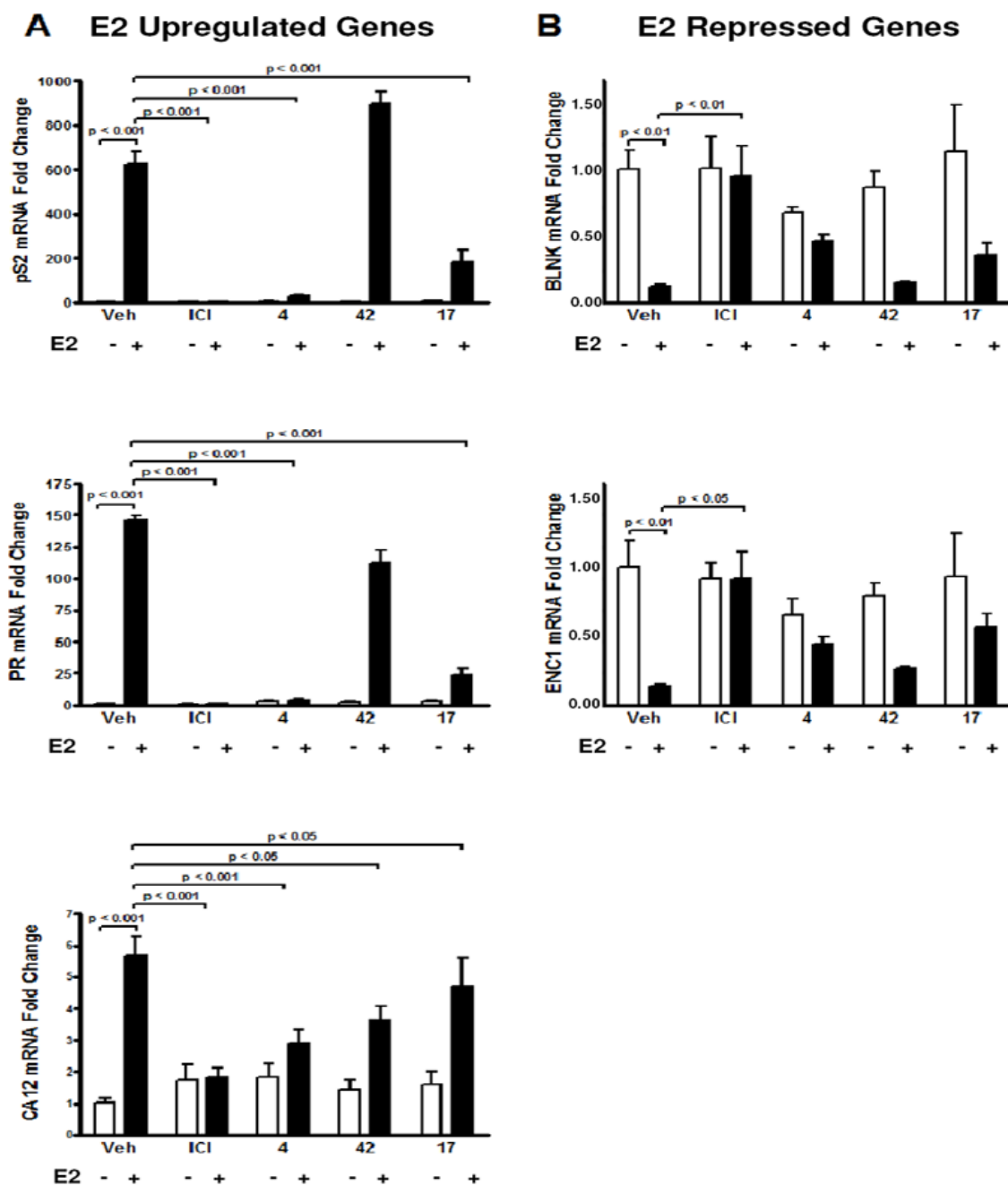
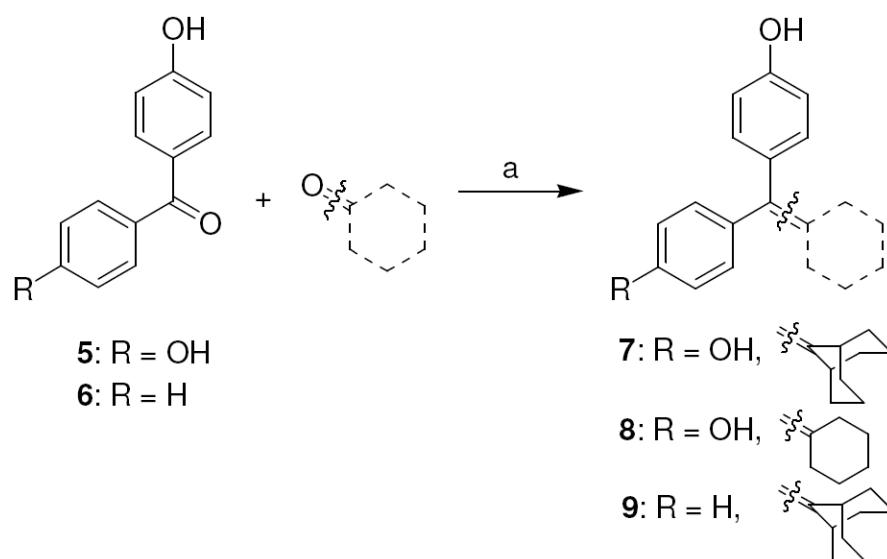
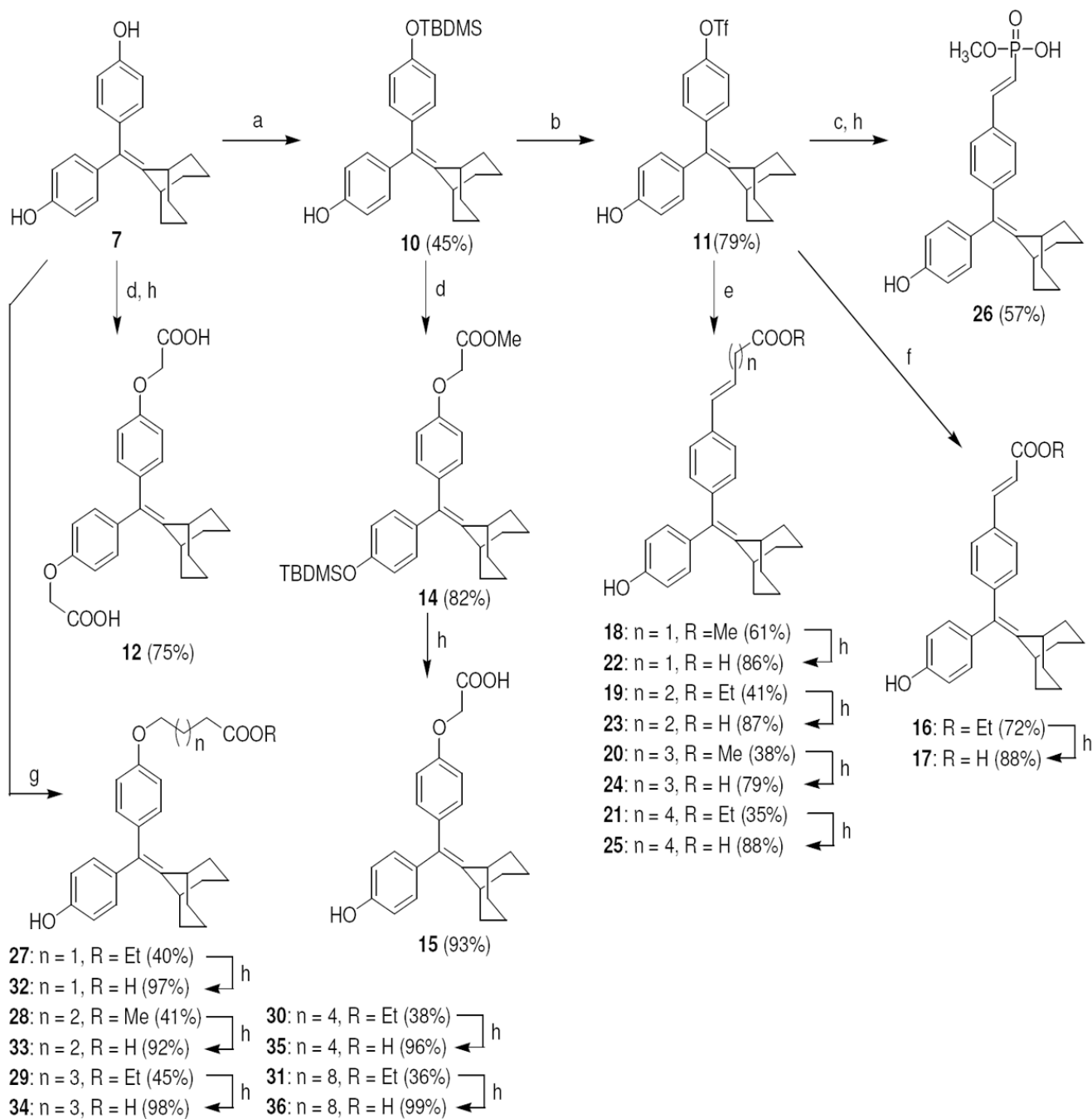


Figure 6. Impact of Different Compounds on ER α Mediated Transcriptional Activity
MCF-7 cells were treated with 1 μ M compounds for 16h and then with vehicle (0.1% ethanol) or 1 nM estradiol for 4h. Real-time PCR analysis was performed for E2-stimulated ER α -target genes (**Panel A**) or E2-repressed ER α -target genes (**Panel B**), as a measure of compound ability to antagonize estradiol action.

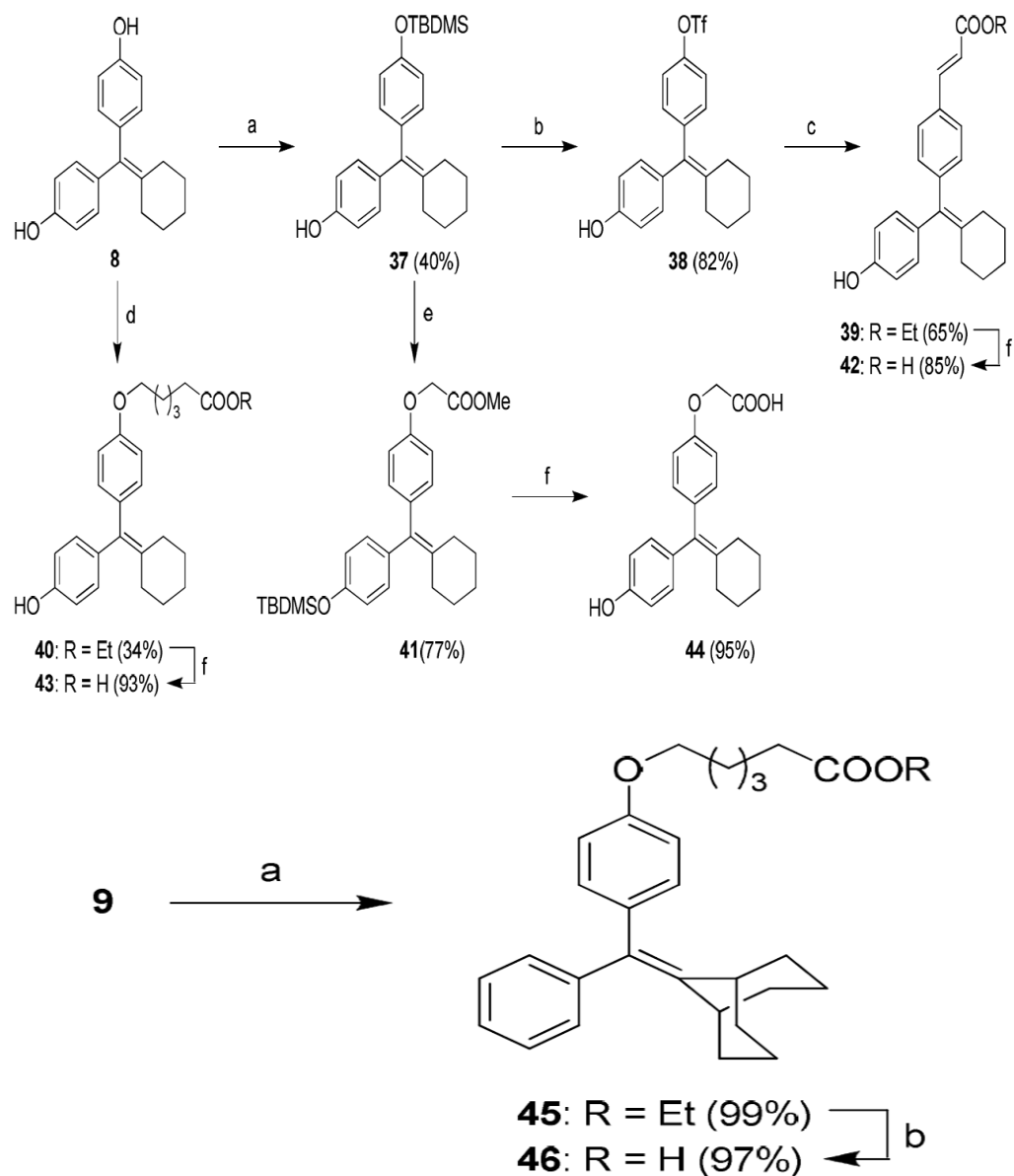


Scheme 1. Synthesis of Cyclofenil and Bicyclononane Analogs
Reagents: (a) TiCl_4 , Zn, THF, reflux, 10 h.



Scheme 2. Synthesis of Acid Substituted Cyclononane Analogs

Reagents: (a) TBDMSCl, imidazole, room temp, THF, 24 h; (b) i) TiF_2O , triethylamine, 0 °C to room temp, 8 h, ii) TBAF, rt, 15 min; (c) dimethyl vinylphosphonate, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, triethylamine, DMF, 120 °C, 24 h; (d) $\text{BrCH}_2\text{COOCH}_3$, Cs_2CO_3 , CH_3CN , room temp, 24 h; (e) $\text{CH}_2=\text{CH}(\text{CH}_2)_n\text{COOR}$, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, triethylamine, DMF, 120 °C, 24 h; (f) ethyl acrylate, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, triethylamine, DMF, 120 °C, 24 h; (g) $\text{BrCH}_2(\text{CH}_2)_n\text{CH}_2\text{COOR}$, Cs_2CO_3 or K_2CO_3 , CH_3CN , room temp, 24 h; (h) 2 N KOH, MeOH, room temp, 24 h.

**Scheme 3.**

a. Synthesis of Acid Substituted Cyclofenil Analogs. Reagents: (a) TBDMSCl, imidazole, room temp, THF, 24 h; (b) i) TiF_2O , triethylamine, 0 °C to room temp, 8 h, ii) TBAF, rt, 15 min; (c) ethyl acrylate, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, triethylamine, DMF, 120 °C, 24 h; (d) ethyl 6-bromohexanoate, Cs_2CO_3 , CH_3CN , room temp, 24 h; (e) $\text{BrCH}_2\text{COOCH}_3$, Cs_2CO_3 , CH_3CN , room temp, 24 h; (f) 2 N KOH, MeOH, room temp, 24 h.

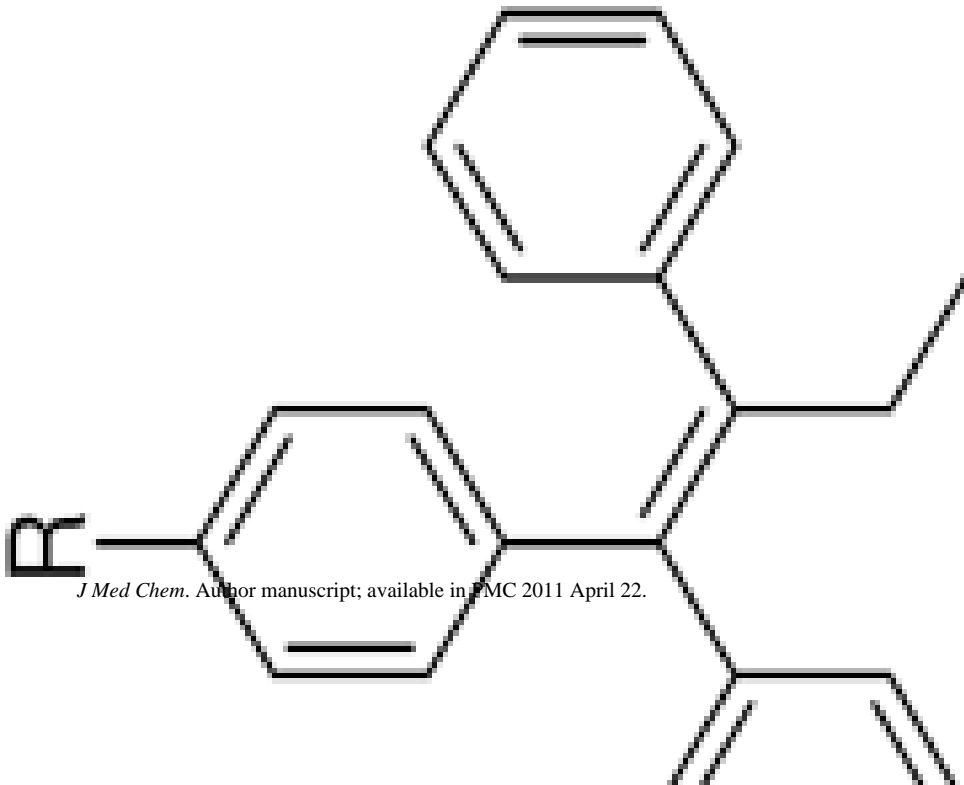
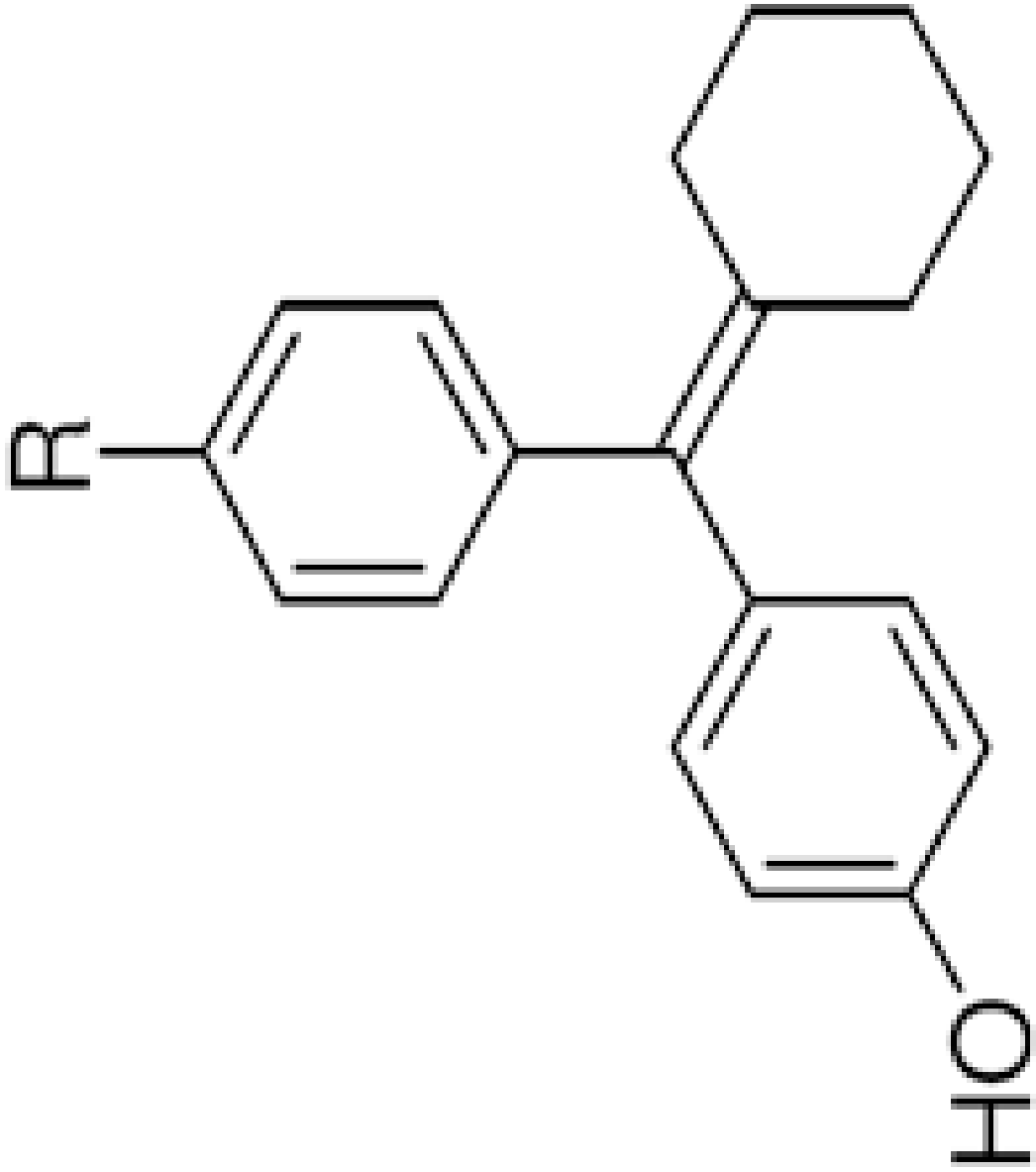
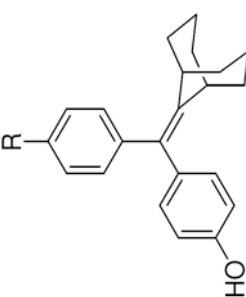
b. Synthesis of the Deoxy Analog (46) of Bicyclononane Compound 34 Reagents: (a) ethyl 6-bromohexanoate, Cs_2CO_3 , CH_3CN , room temp, 24 h; (b) 2 N KOH, MeOH, room temp, 24 h.

Table 1

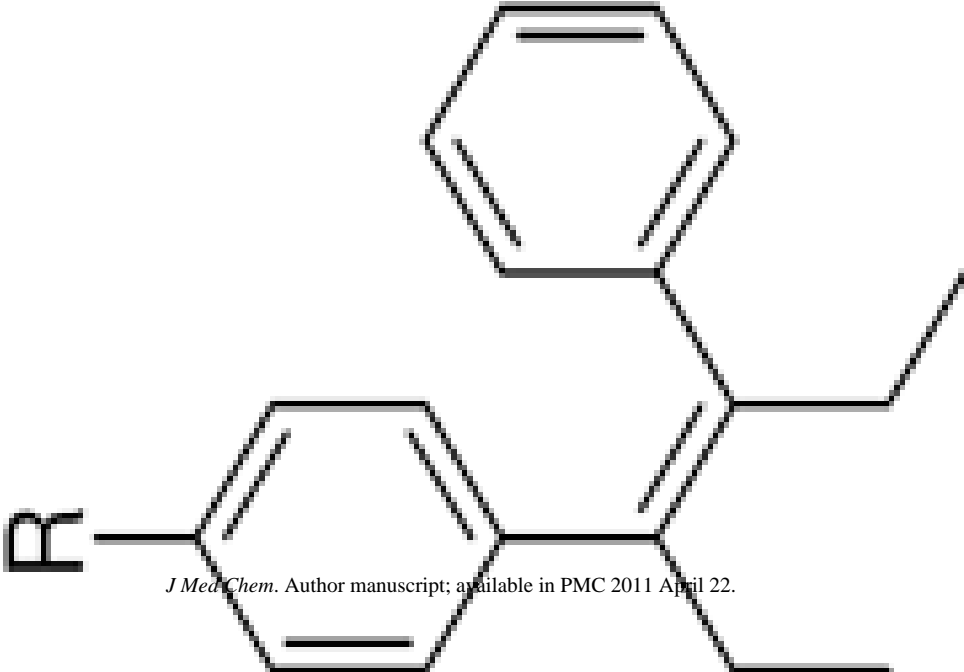
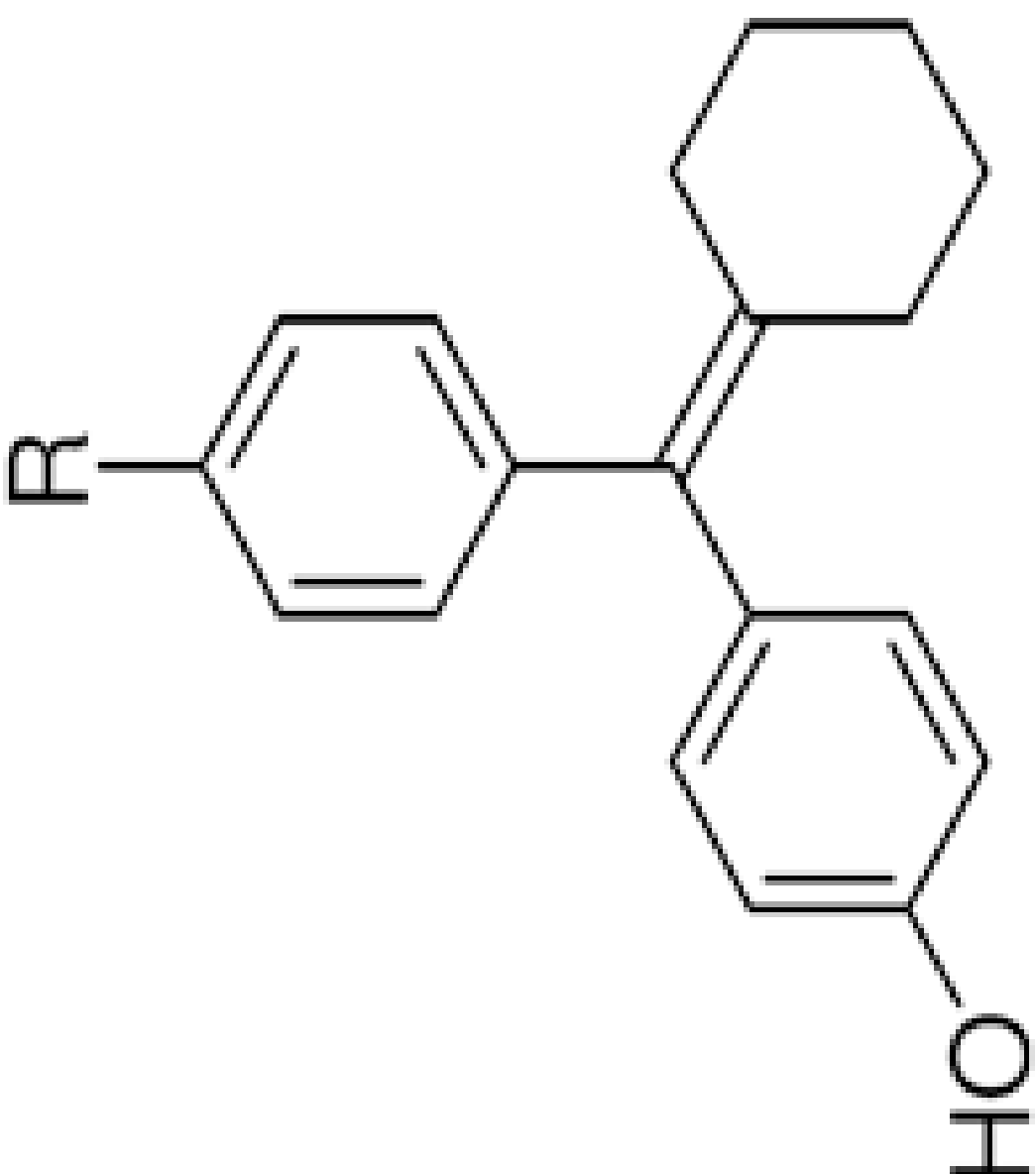
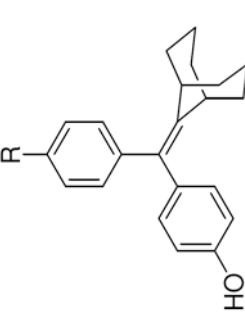
Estrogen Receptor Binding Assay

presented in the top part of (Table 1a).

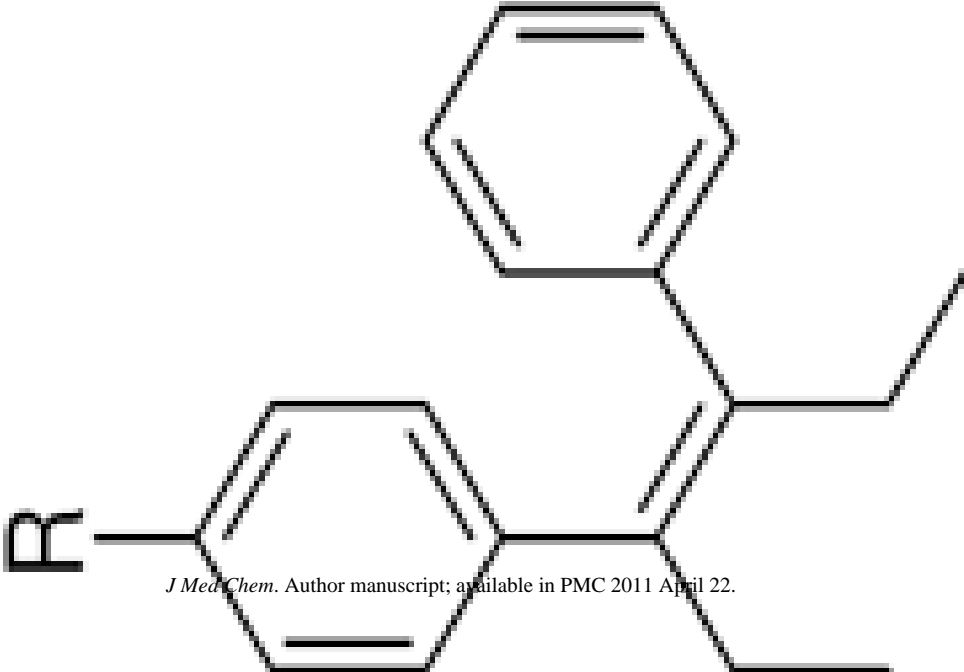
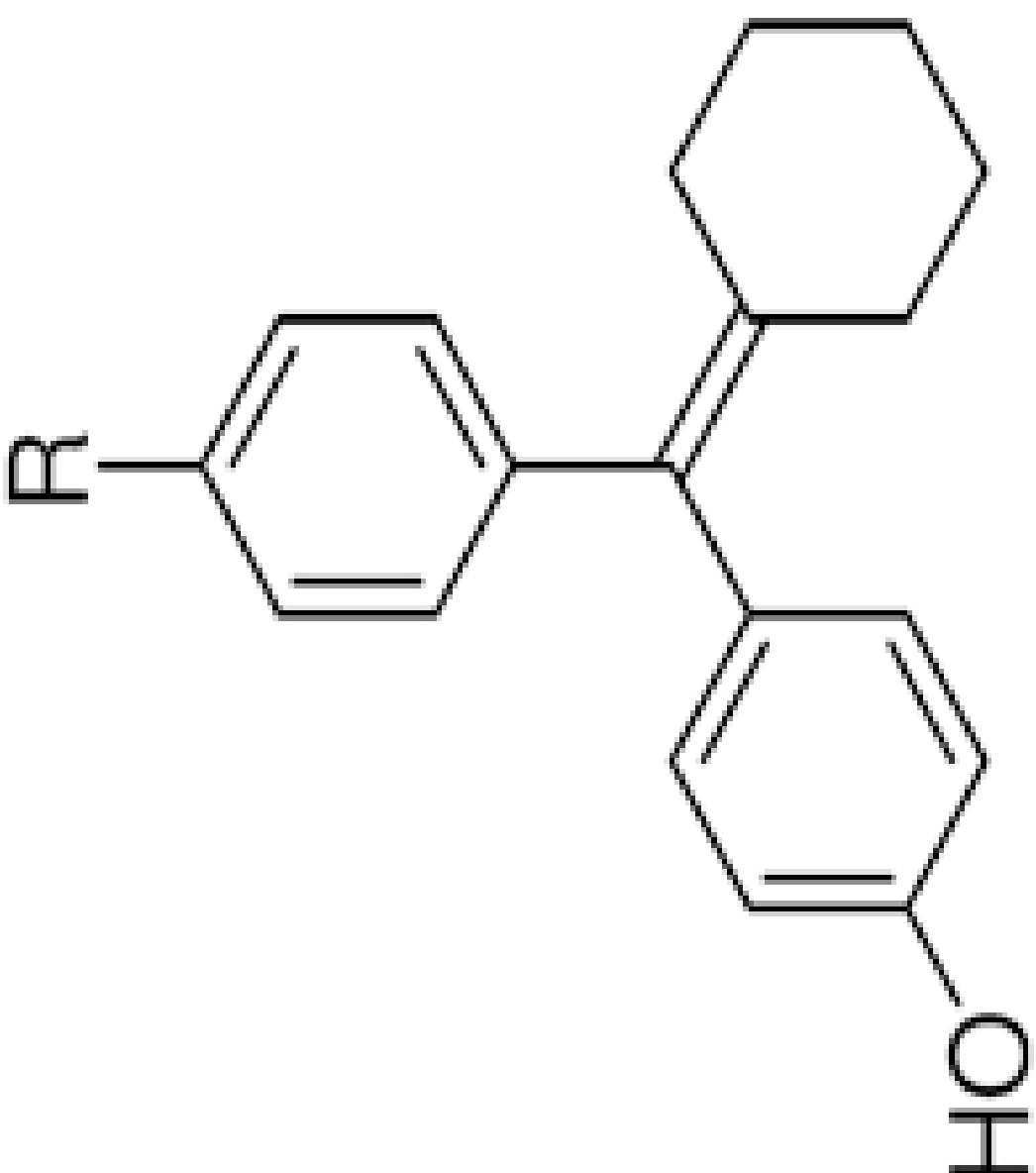
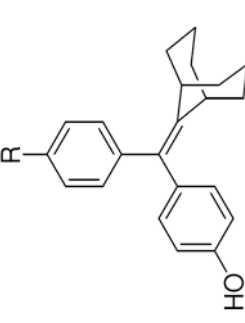
a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)
		
RBA ^a ER _α	RBA ^a ER _α	RBA ^a ER _α
377 ± 106	68 ± 18	513 ± 17
RBA ^a ER _β	RBA ^a ER _β	RBA ^a ER _β
238 ± 48	334 ± 54	301 ± 54
β/α ^b	β/α ^b	β/α ^b
0.6	4.9	0.6
lignad	lignad	lignad
CF, 2	BN, 3	BN, 3
TAE	CF, 2	BN, 3

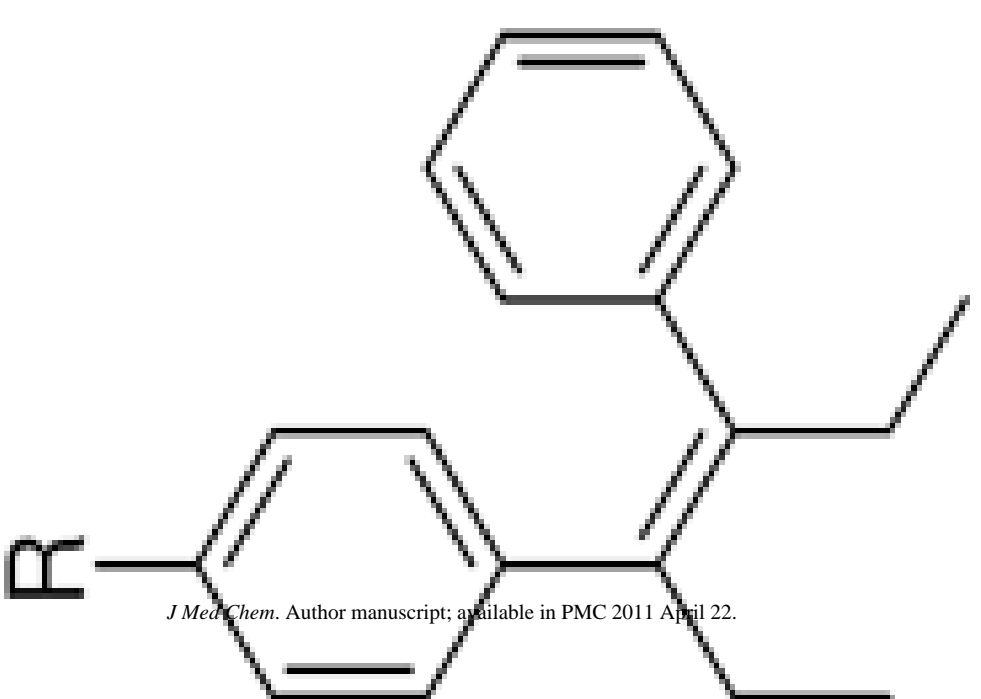
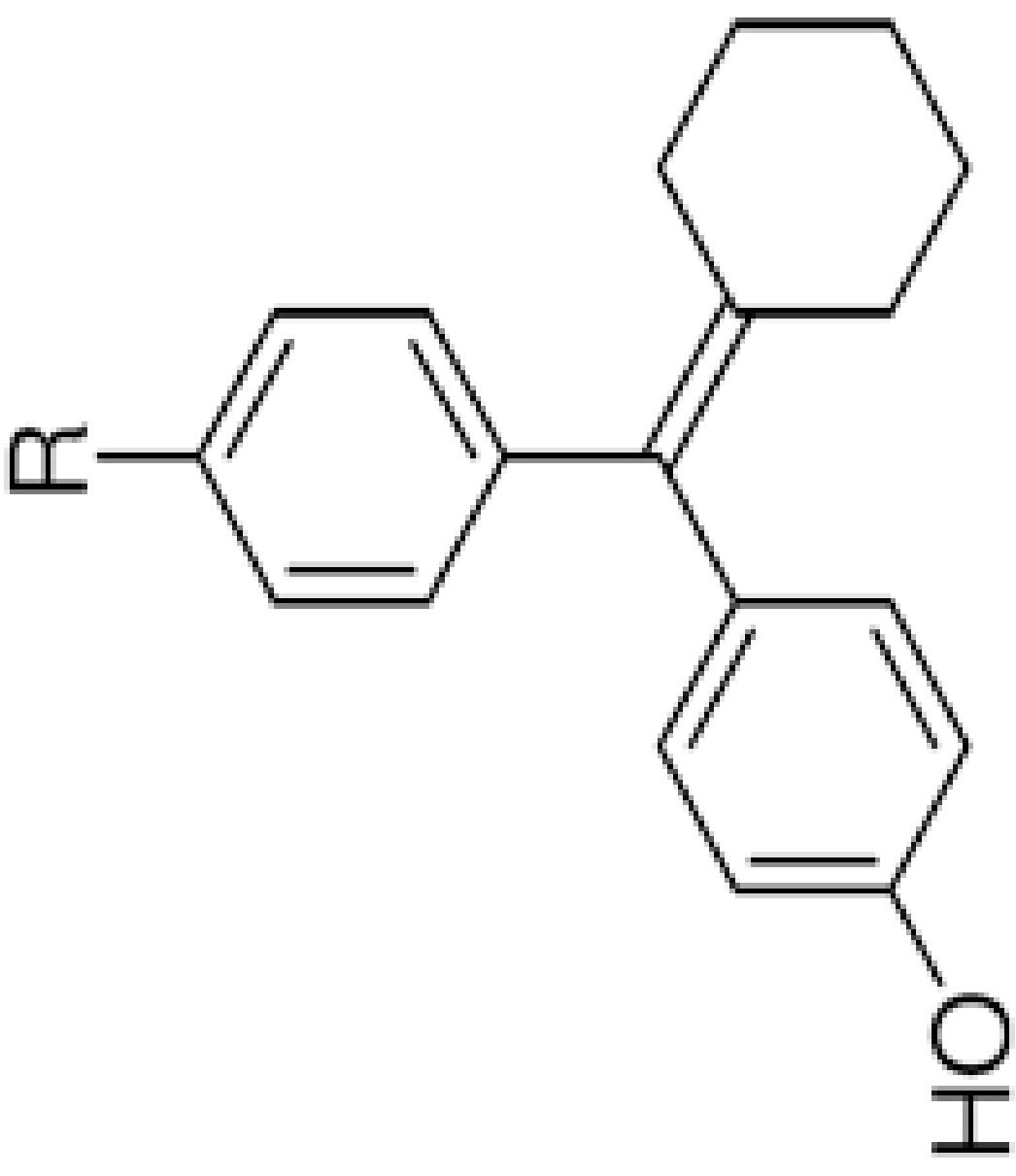
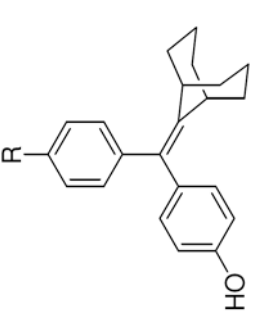
a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	lignad	RBA ^a ERα	RBA ^a ERβ	β/α ^b	lignad	RBA ^a ERα	RBA ^a ERβ	β/α ^b	RBA ^a ERα	RBA ^a ERβ	β/α ^b
			4 ^{c,d}	20.3 ± 6	28.3 ± 7	1.4	4 ^c	2.1 ± 5	11.3 ± 3	5.4	17.7 ± 4	25.9 ± 5	1.5
4 ^c	--	--	--	3.6 ± 1	4.2 ± 0.6	1.2	--	--	--	--	--	--	--

a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	ligand	RBA ^a ERα	RBA ^a ERβ	β/α ^b	ligand	RBA ^a ERα	RBA ^a ERβ	β/α ^b	ligand	RBA ^a ERα	RBA ^a ERβ	β/α ^b
				--	--	--	44 ^c	0.63 ± 0.1	1.9 ± 0.6	3.0	15 ^c	3.6 ± 1	3.1 ± 0.3	0.9

a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	ligand	RBA ^a ER α	RBA ^a ER β	β/α^b	ligand	RBA ^a ER α	RBA ^a ER β	β/α^b	ligand	RBA ^a ER α	RBA ^a ER β	β/α^b
														

J Med Chem. Author manuscript; available in PMC 2011 April 22.

a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b

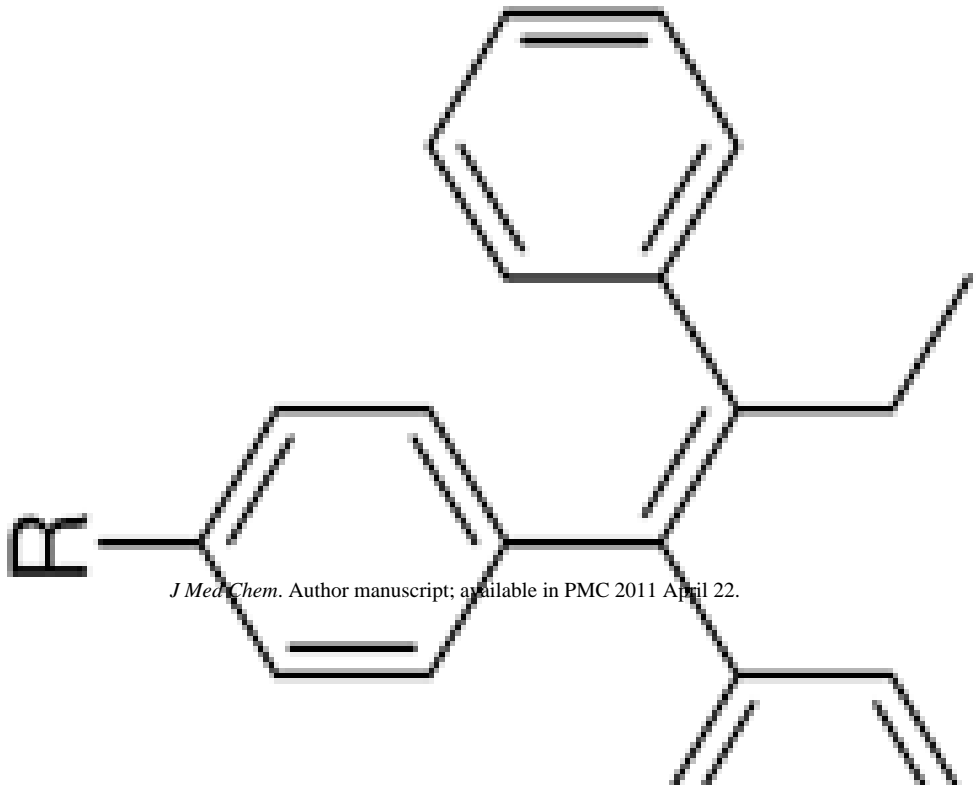
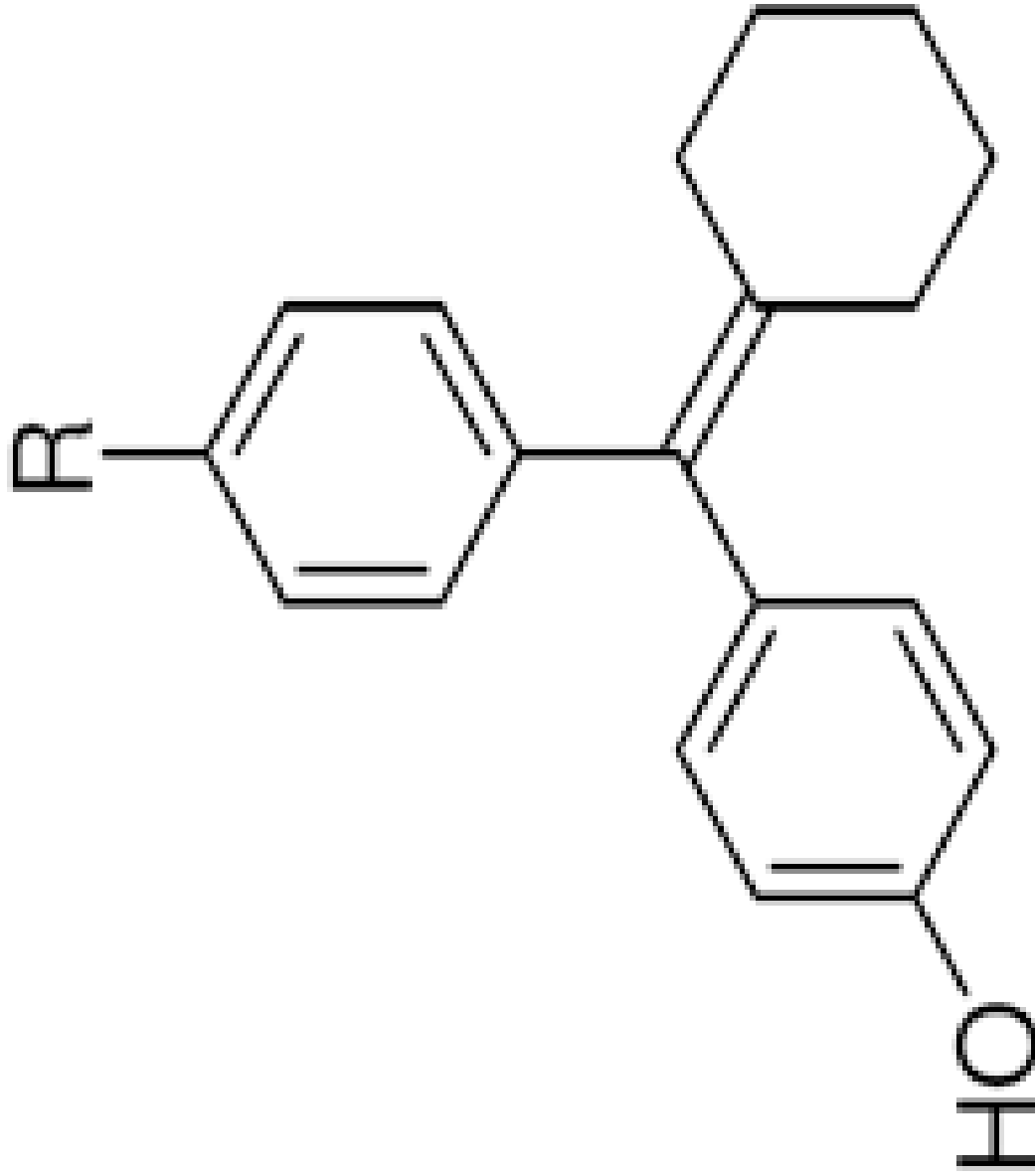
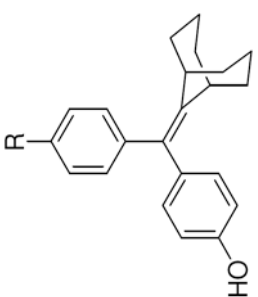
a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b

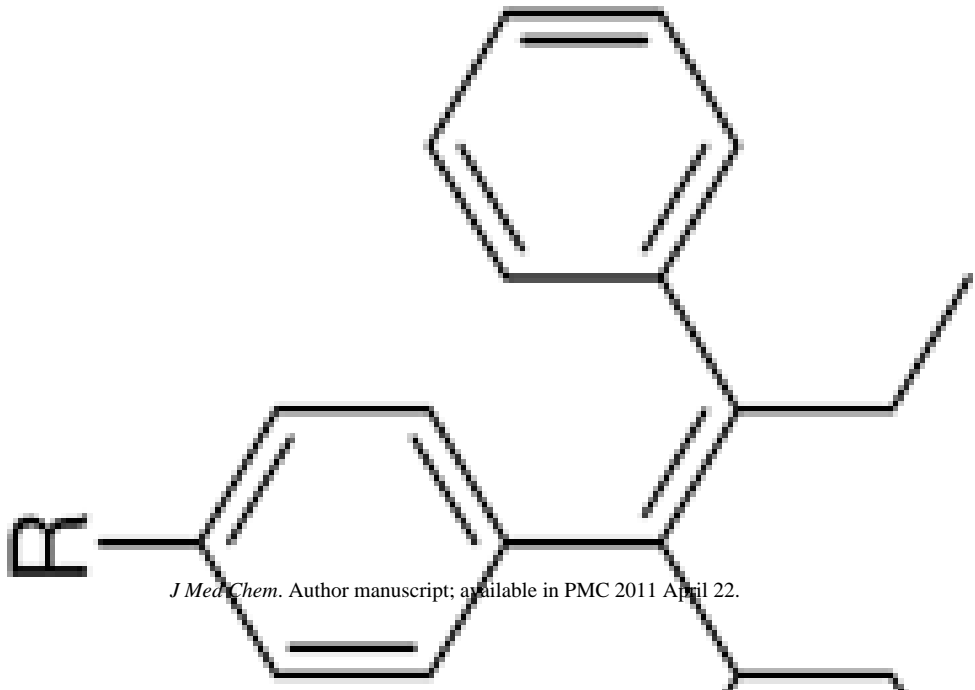
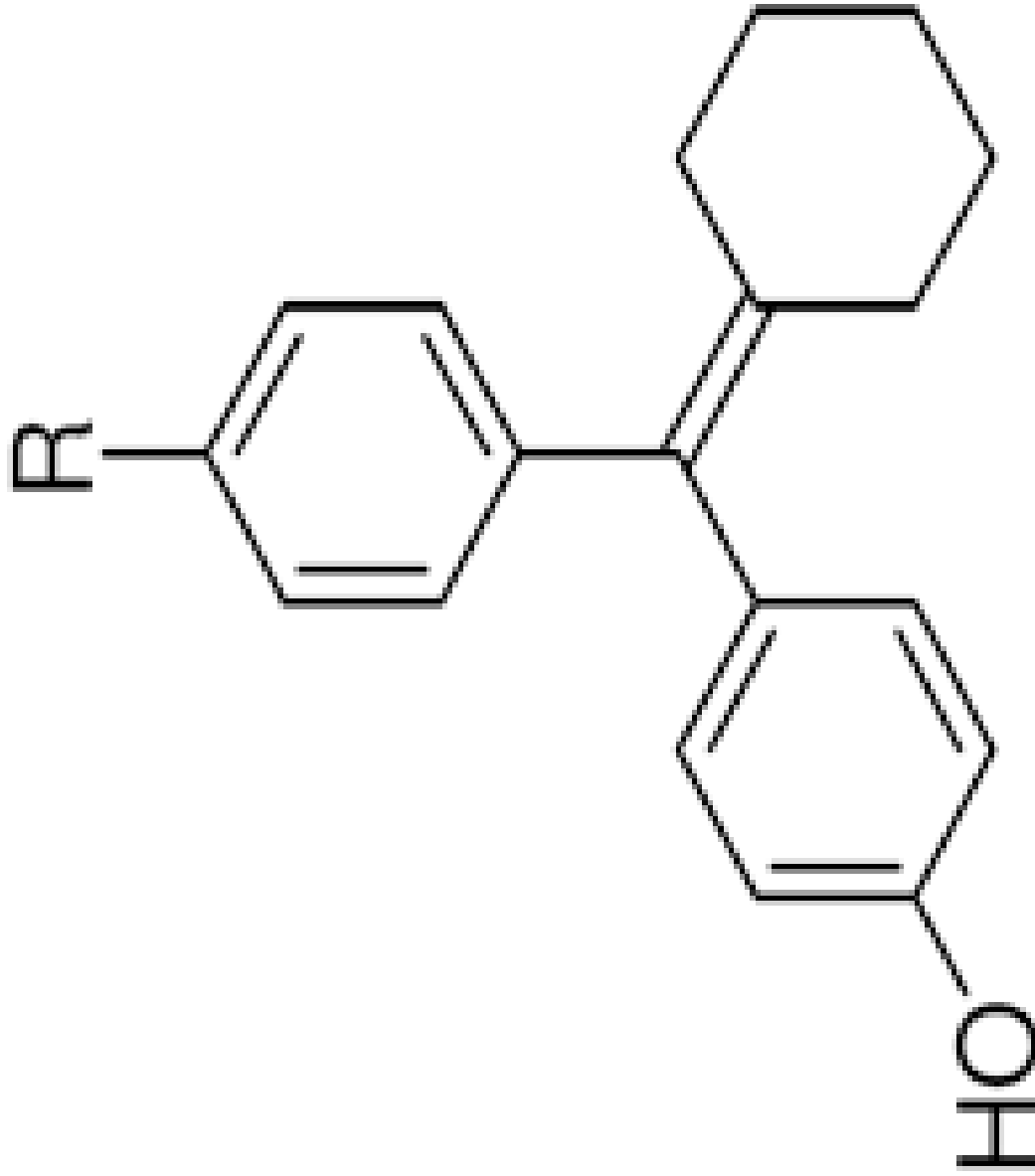
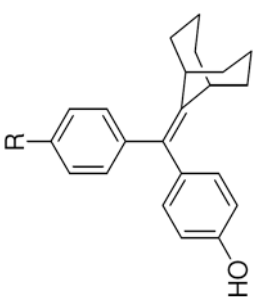
a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	ligand	RBA ^α ERα	RBA ^α ERβ	β/α ^b	ligand	RBA ^α ERα	RBA ^α ERβ	β/α ^b	ligand	RBA ^α ERα	RBA ^α ERβ	β/α ^b
<p><i>J Med Chem.</i> Author manuscript; available in PMC 2011 April 22.</p>			ligand	RBA ^α ERα	RBA ^α ERβ	β/α ^b	ligand	RBA ^α ERα	RBA ^α ERβ	β/α ^b	ligand	RBA ^α ERα	RBA ^α ERβ	β/α ^b

a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b
														

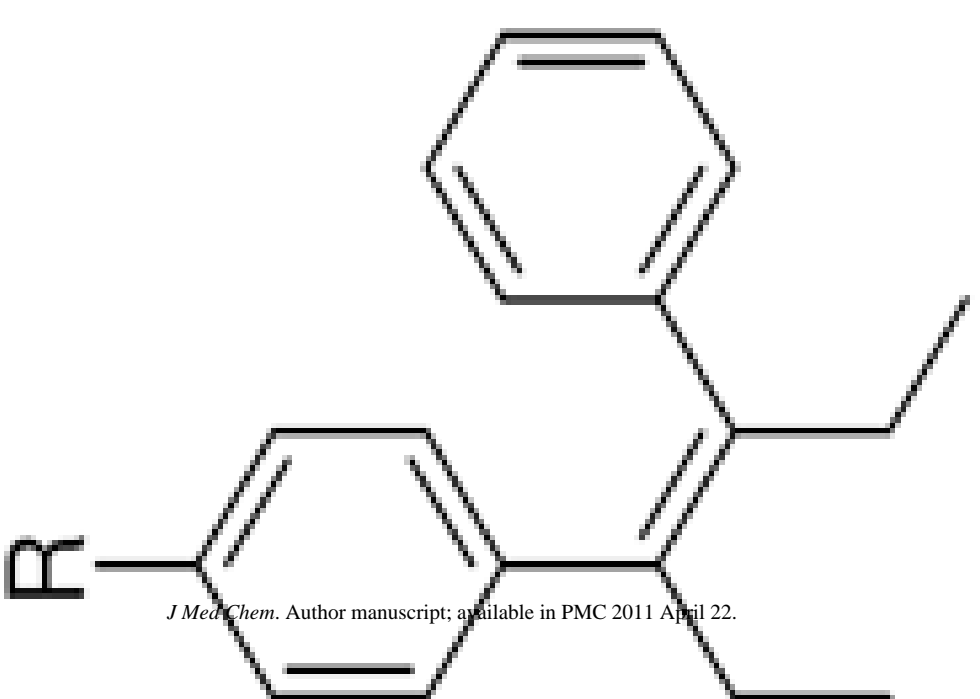
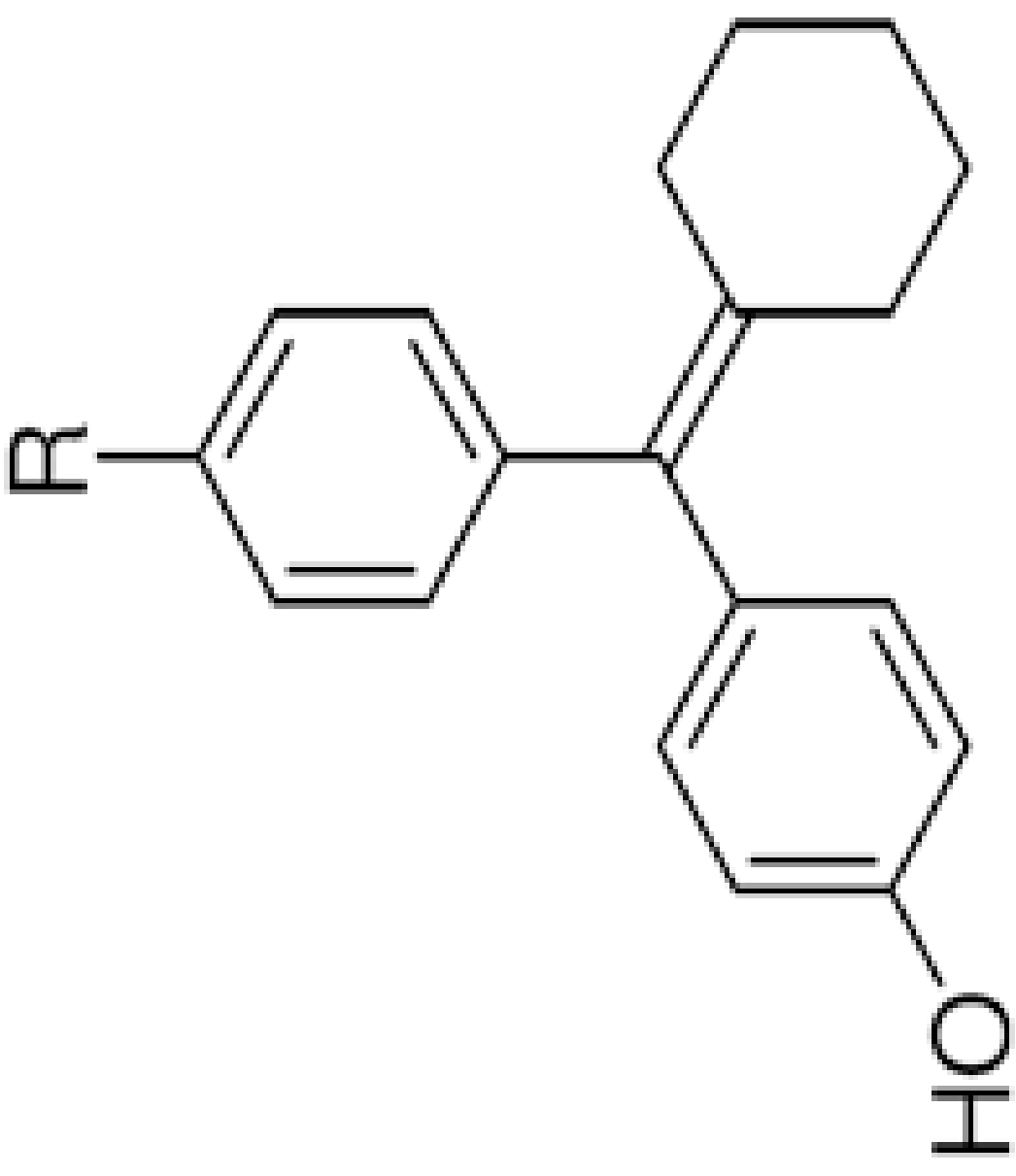
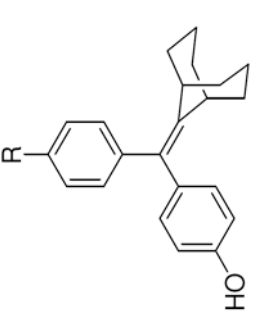
a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	lignad	RBA ^a ERα	RBA ^a ERβ	β/α ^b	lignad	RBA ^a ERα	RBA ^a ERβ	β/α ^b	RBA ^a ERβ	β/α ^b
				46	0.14 ± 0.02	0.30 ± 0.06						
			2.1									

a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

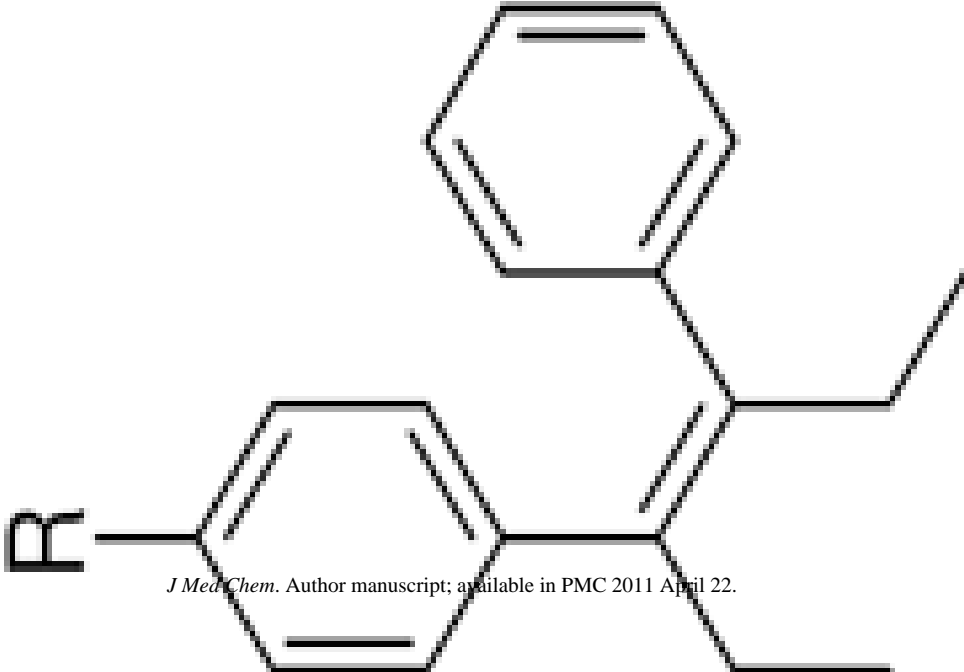
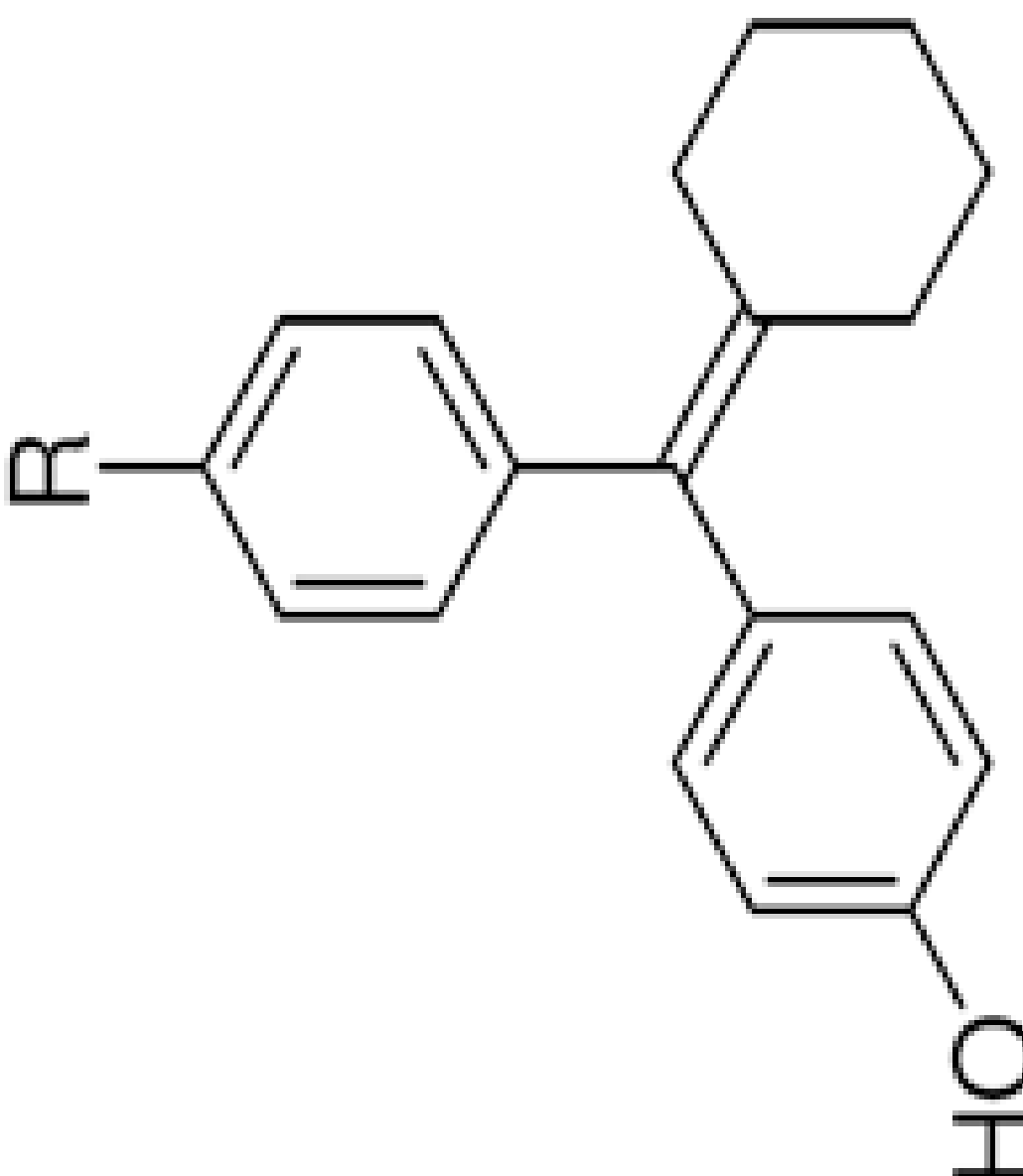
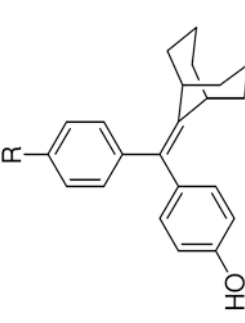
Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	ER α	ER β	RBA ^a	β/α^b	lignad	ER α	ER β	RBA ^a	β/α^b	ER α	ER β	RBA ^a	β/α^b
<p><i>J Med Chem.</i> Author manuscript; available in PMC 2011 April 22.</p>			12	0.26 ± 0.05	0.40 ± 0.05	1.5									

a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	lignad	RBA ^a ER α	RBA ^a ER β	β/α^b	lignad	RBA ^a ER α	RBA ^a ER β	β/α^b	lignad	RBA ^a ER α	RBA ^a ER β	β/α^b
				29	15.2	30.8	2.0							

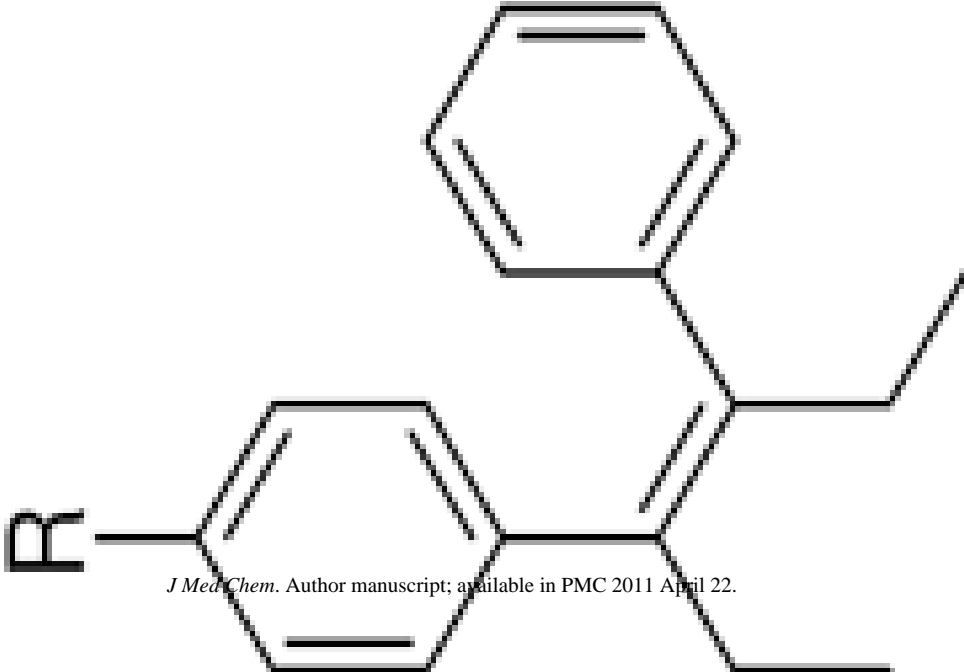
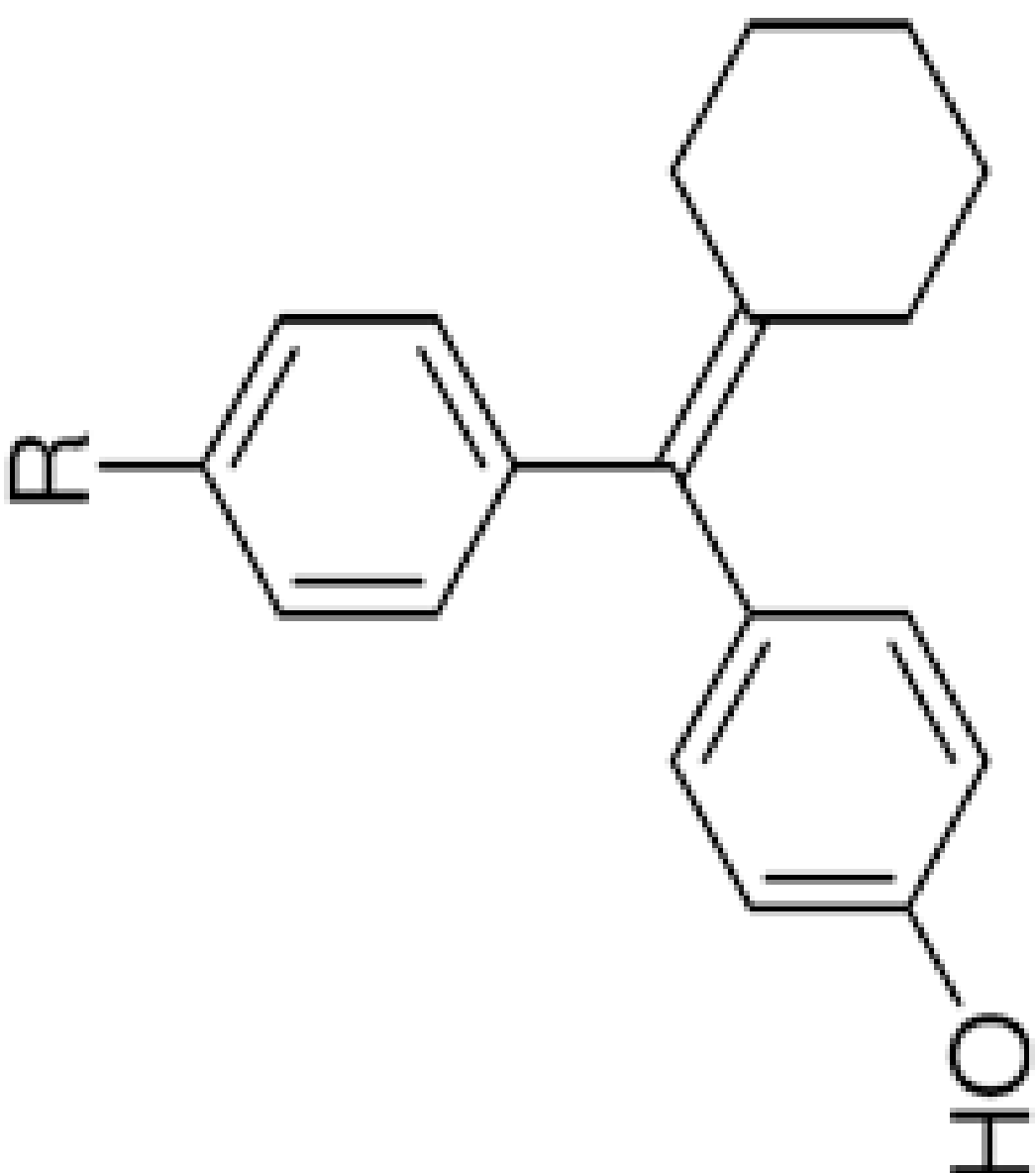
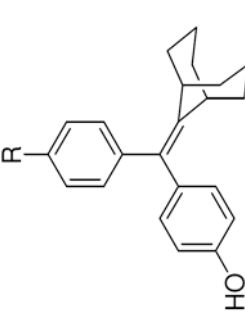
J Med Chem. Author manuscript; available in PMC 2011 April 22.

a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	lignad	RBA ^a ERα	RBA ^a ERβ	β/α ^b	lignad	RBA ^a ERα	RBA ^a ERβ	β/α ^b	lignad	RBA ^a ERα	RBA ^a ERβ	β/α ^b
				26 ^c	7.5 ± 1	7.7 ± 0.7	1.0							

J Med Chem. Author manuscript; available in PMC 2011 April 22.

a. Structure and Binding Affinity of Basic TAE, CF, and BN Systems

Triarylethylene (TAE)	Cyclofenil (CN)	Bicyclononane (BN)	ligand	RBA ^a ERβ	RBA ^a ERα	β/α ^b	ligand	RBA ^a ERβ	RBA ^a ERα	β/α ^b	RBA ^a ERβ	RBA ^a ERα	β/α ^b
			11	4.2 ± 1	8.9 ± 0.7	2.1							

^aRelative binding affinity (RBA) values are determined by competitive radiometric binding assays and are expressed as $IC_{50}[\text{estradiol}] / IC_{50}[\text{compound}] \times 100$ (RBA, estradiol = 100). In these assays the K_d for estradiol is 0.2 nM for ER α and 0.5 nM for ER β .

^bFor each value, the β/α ratio is calculated such that the ratio is >1 for compounds having higher affinity on ER β than on ER α .

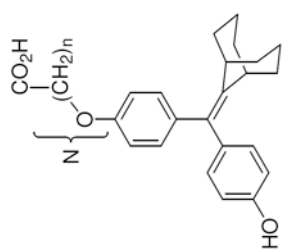
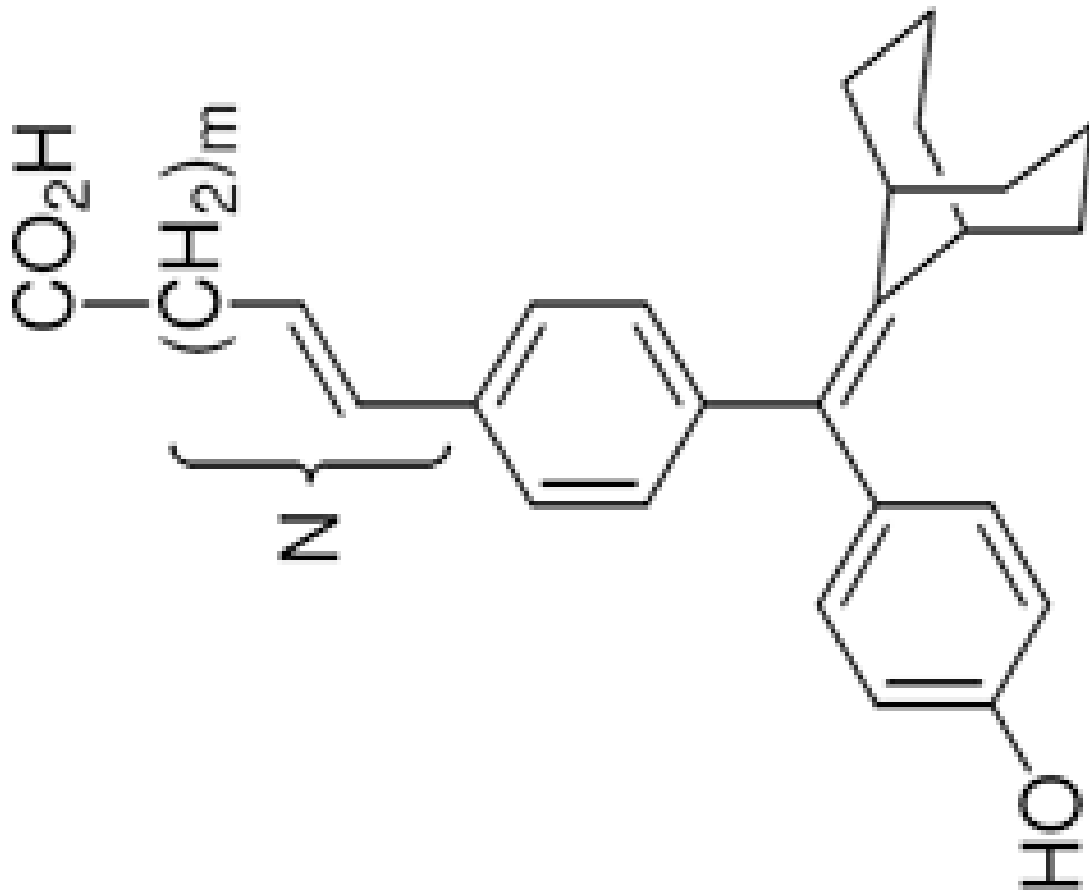
^cCompounds selected for further studies are indicated by a bold underline.

^dThis compound, GW-7604 (**4**), exists as a mixture of E and Z isomers. See Figure 1.

Table 2
Structure and Binding Affinity of the Extended Series of Styryl and Alkoxy Bicyclononanes

The ER α and ER β binding affinity of both the styryl and alkoxy bicyclononane series was determined and expressed as outlined in the legend of Table 1.

N [number of atoms: phenyl-carboxyl]



Alkoxy Bicyclononane

Styryl Bicyclononane

N

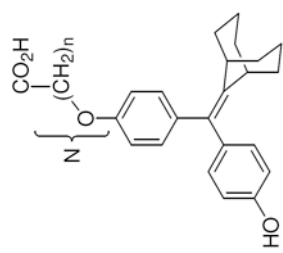
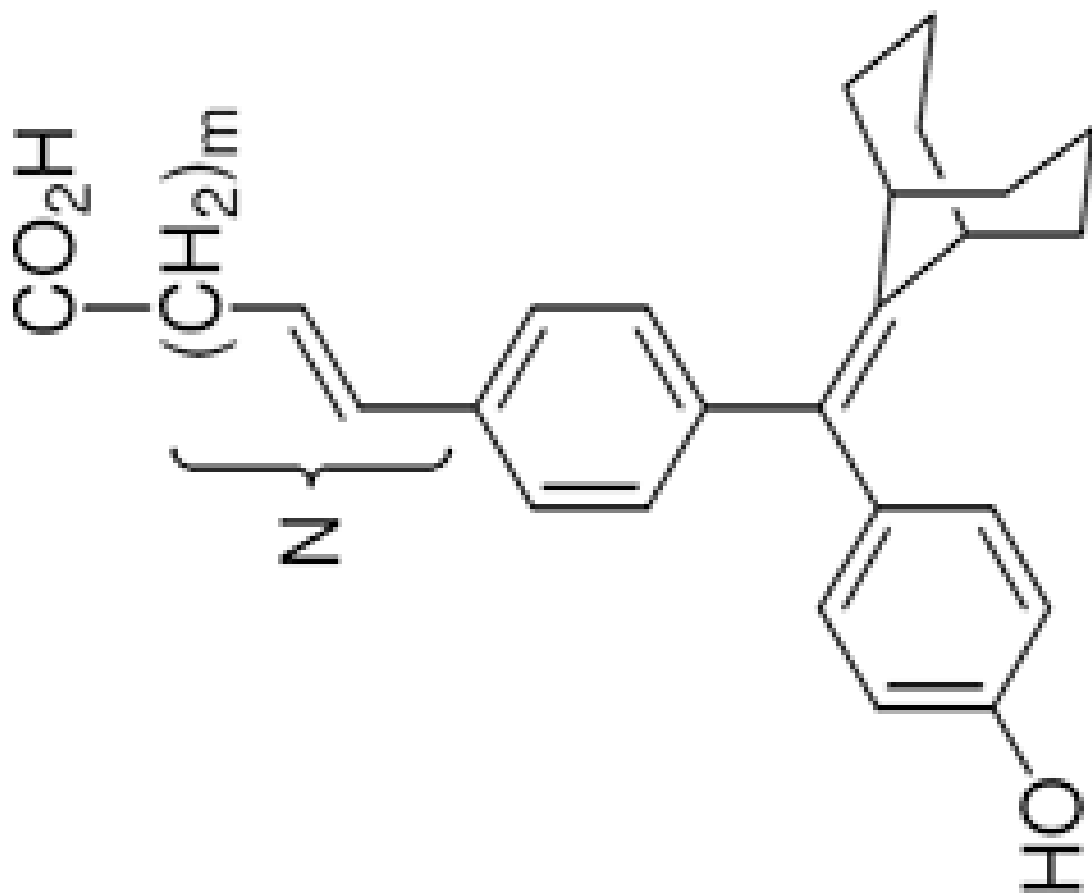
lignad

RBA α ED β RBA α ED β $\beta/0^b$

lignad

RBA α ED β RBA α ED β $\beta/0^b$

N [number of atoms: phenyl-carboxyl]

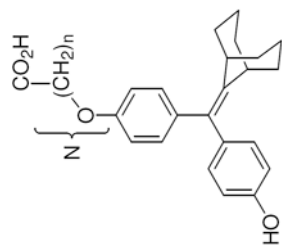
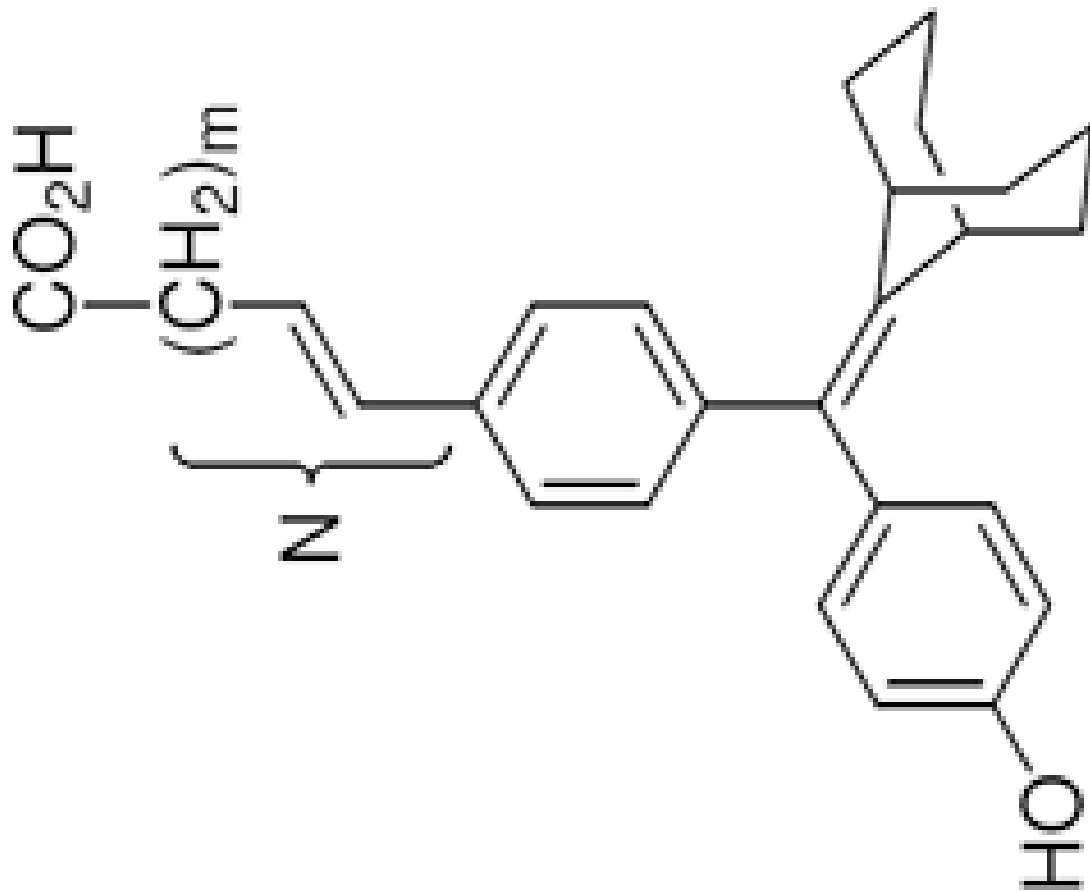


Alkoxy Bicyclononane

Styryl Bicyclononane

N	lignad	RBA ^a ER ^a	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^a	RBA ^a ER ^β	β/α ^b
1	22	0.0+2	23.1+6	2.6	32	5.6+1	10.5+1	1.9

N [number of atoms: phenyl-carboxyl]

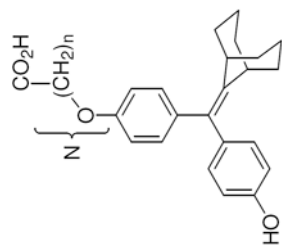
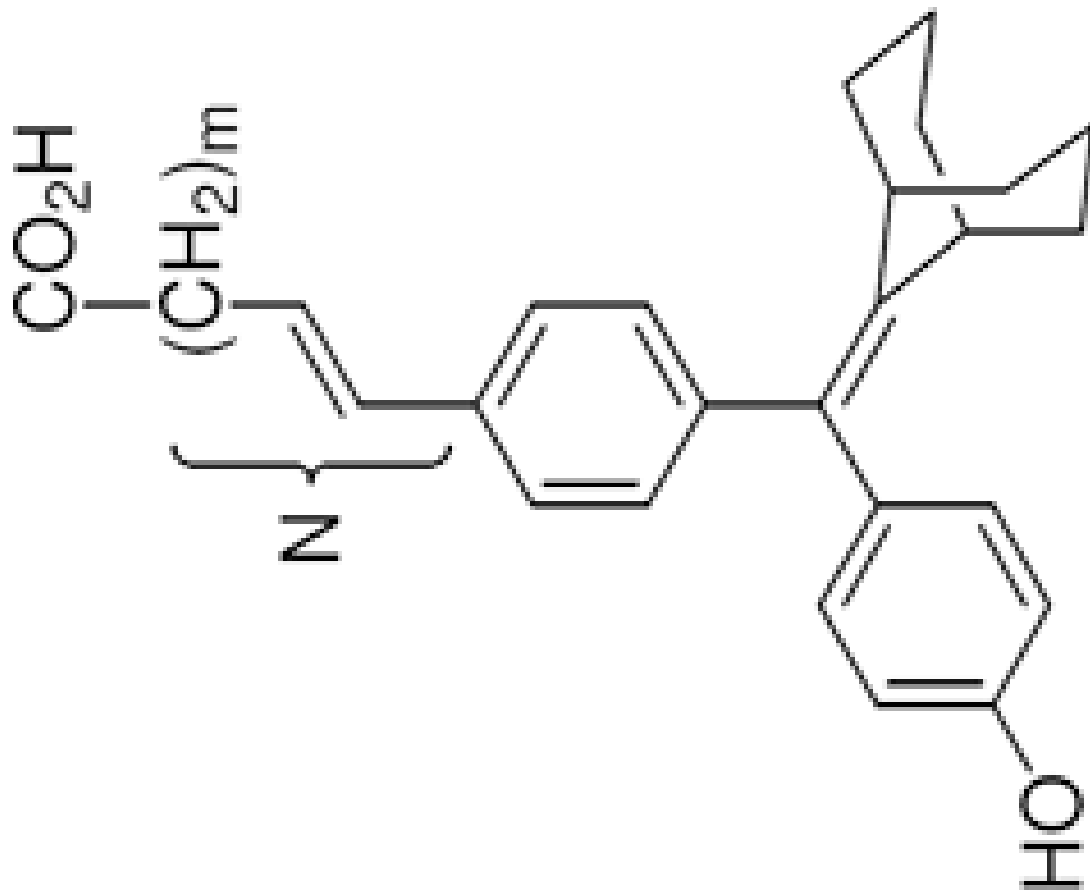


Alkoxy Bicyclononane

Styryl Bicyclononane

N	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b
5	23	13.1+2	24.6+7	1.9	23	18.3+3	38.3+11	2.1

N [number of atoms: phenyl-carboxyl]

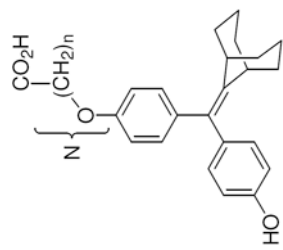
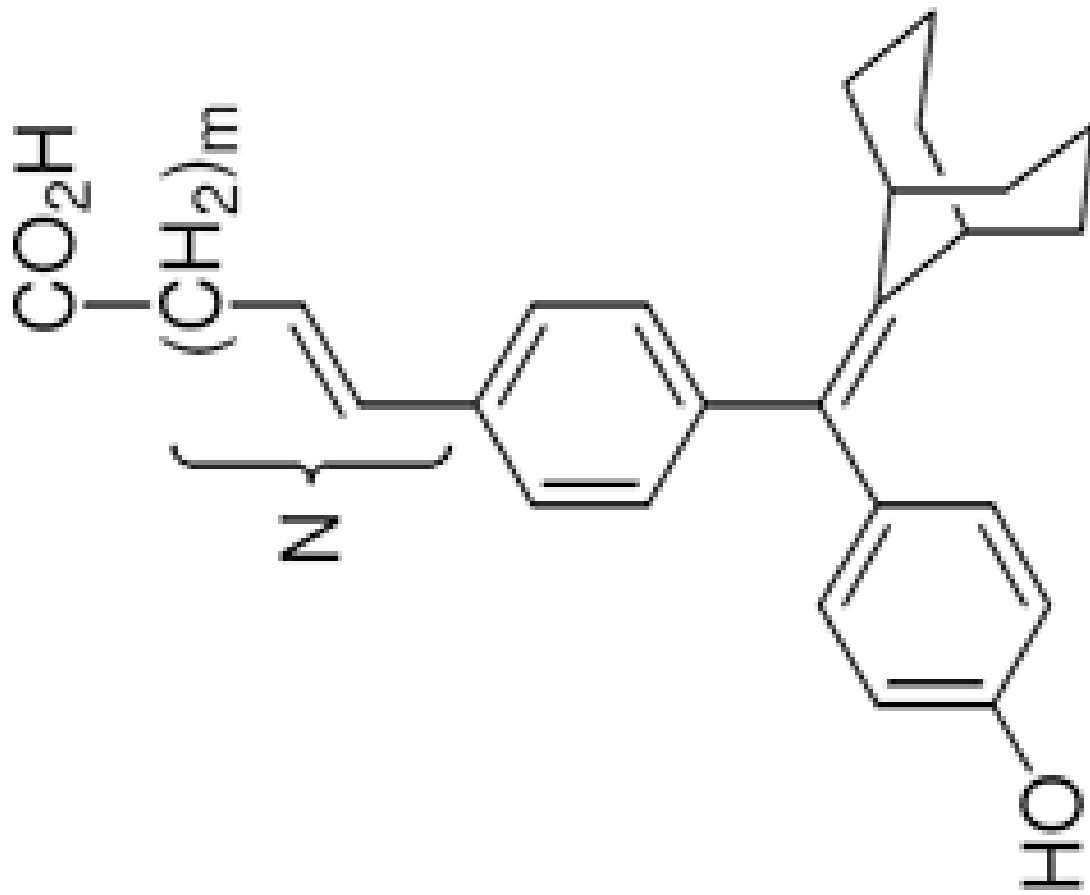


Alkoxy Bicyclononane

Styryl Bicyclononane

N	lignad	RBA ^a ER ^a	RBA ^a ER ^β	β/α ^β	lignad	RBA ^a ER ^a	RBA ^a ER ^β	β/α ^β
6	5.0	38.5+0.2	62.8+1.4	1.6	5.0	52.0+6	80.1+9	1.7

N [number of atoms: phenyl]-carboxyl]

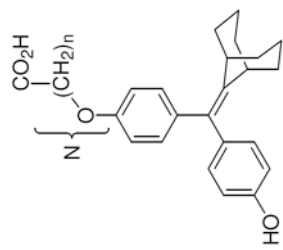
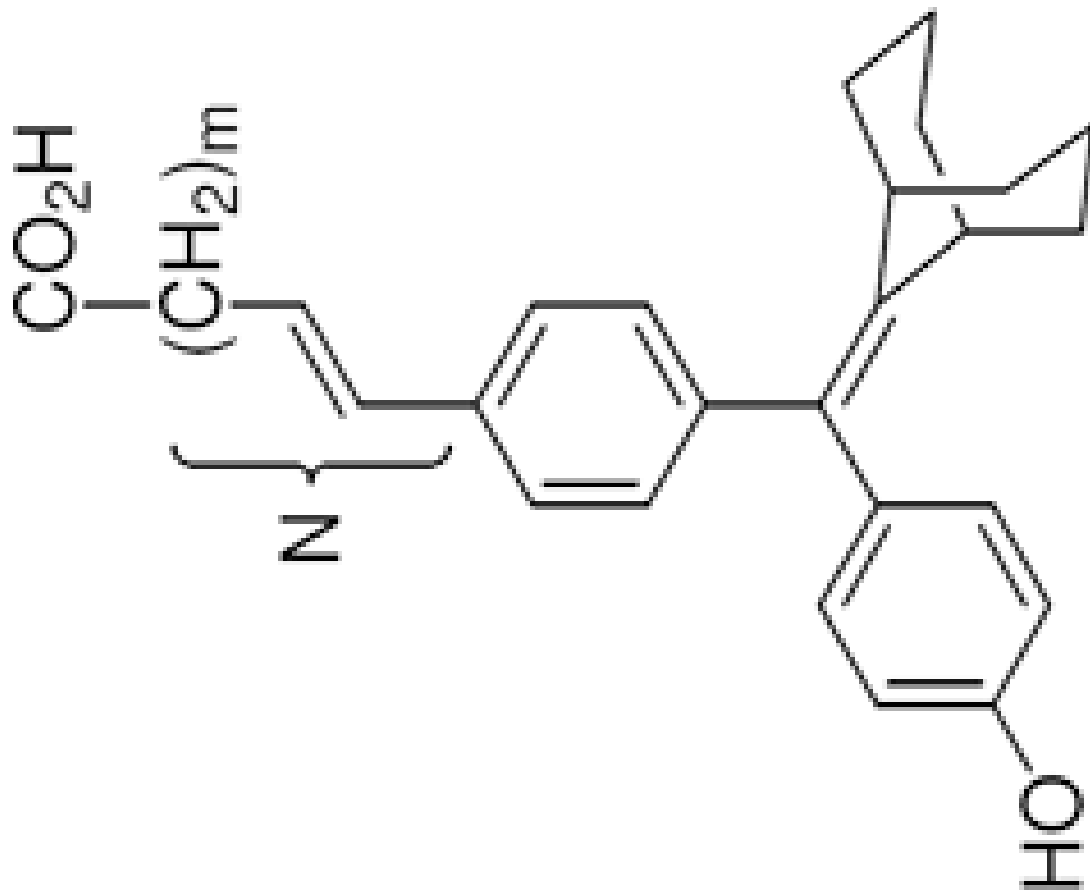


Alkoxy Bicyclononane

Styryl Bicyclononane

N	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^α	RBA ^a ER ^β	β/α ^b
7	35	87.4 + 10	81.1 + 14	0.93	35	70.4 + 20	139 + 0	2.0

N [number of atoms: phenyl-carboxyl]



Alkoxy Bicyclononane

Styryl Bicyclononane

N	lignad	RBA ^a ER ^a	RBA ^a ER ^β	β/α ^b	lignad	RBA ^a ER ^a	RBA ^a ER ^β	β/α ^b

^aRelative binding affinity (RBA) values are determined by competitive radiometric binding assays and are expressed as $IC_{50}^{[estradiol]} / IC_{50}^{[compound]} \times 100$ (RBA, estradiol = 100). In these assays the K_d for estradiol is 0.2 nM for ER α and 0.5 nM for ER β .

^bFor each value, the β/α ratio is calculated such that the ratio is >1 for compounds having higher affinity on ER β than on ER α .

^cCompounds selected for further studies are indicated by a bold underline.