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Exploration of the Bicyclo[3.3.1]nonane System as a Template for the Development of New Ligands for the Estrogen Receptor

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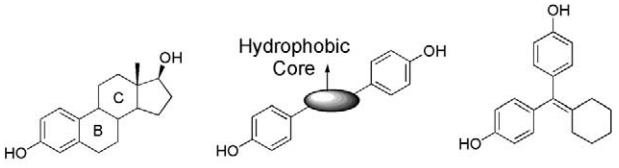
Abstract—Three novel structural motifs based on a bicyclo [3.3.1]nonane template were examined as new ligands for estrogen receptor (ER). Type III compounds emerged as the most promising leads for developing high-affinity ER ligands, but they showed little selectivity for either ER subtype. Type II compounds, on the other hand, despite their lower affinity, exhibited significant ERβ binding selectivity.

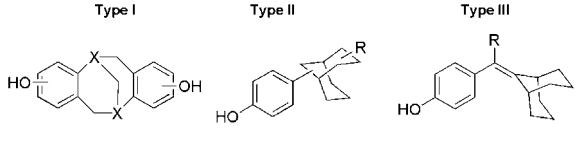
The estrogen receptor (ER) is a ligand-dependent transcription factor whose native hormonal ligand is estradiol (E_2). The actions of estrogens are mediated through the ER of which there are two subtypes, ERα and ERβ.[1,2] Based on crystallographic and ligand analogue studies,[3–7] we now have a reasonable understanding of the structural requirements for a ligand to bind to ER: The ER pharmacophore basically consists of (1) an A-ring phenol, (2) a second hydroxyl group or phenol placed approximately 11 Å from the A-ring phenol to mimic the 17β-hydroxy group of E_2, and (3) a central structurally variable hydrophobic core which mimics the B and C rings of estradiol.[8]



Estradiol (E_2) ER Pharmacophore Cyclofenil

As part of our long-term interest in ER ligands, we undertook an exploratory study aimed at preparing new ligands wherein the central hydrophobic core had overall a more *three-dimensional* topology. This design strategy was based on an examination of the binding pockets of both ERα and ERβ that shows there is substantial unoccupied space above and below the plane of the ligand, particularly near the middle of the ligand (i.e., near the B and C rings of E_2).[3] By incorporating a hydrophobic bicyclic core, we hoped to exploit this unfilled space in the ER binding pocket, and thereby, potentially, to gain binding affinity or ER subtype selectivity.

A brief survey of the various available bicyclic cores suggested that a bicyclo[3.3.1]nonane system would be an appropriate three-dimensional hydrophobic core element, because its overall dimensions and carbon atom count closely match those of the B and C rings of E_2. In addition, we were encouraged by the fact that several synthetic routes are available to prepare bicyclo[3.3.1]nonanes.[9] Therefore, we proposed the three different structural motifs shown below, each containing a bicyclo[3.3.1]nonane core, as possible E_2 mimics, and we have conducted a limited structure–activity relationship (SAR) study of each type.



Type I ligands, inspired by Troger's base,[10] consist of a central bicyclo[3.3.1]nonane core fused to phenol groups. In this design, we reasoned that the periphery of the bridged bicyclic core would mimic the dimensions of the B and C rings of estradiol, whereas the bridging methylene group would provide steric bulk above (or

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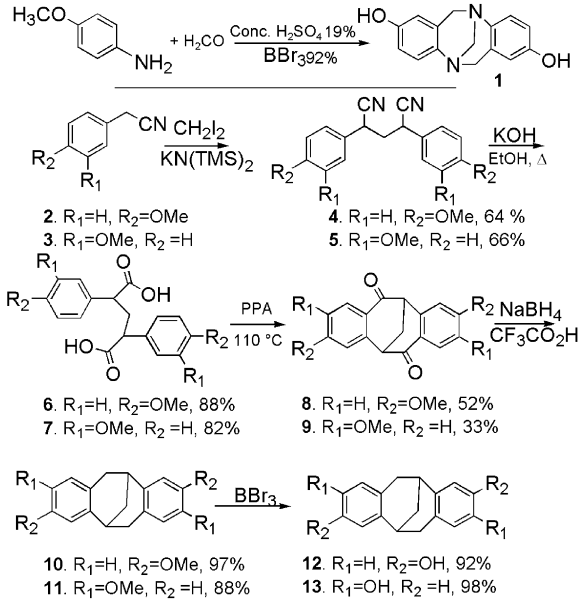
below) the rest of the ligand. The structural motifs in the remaining two types of ligands are such that the phenols are attached to the bicyclic core at various positions, either directly (Type II), or by an sp^2-hybridized carbon (Type III). The latter motif, with two phenols attached to the bridgehead as a diarylmethylidine unit, was inspired by the high affinity ER ligand, cyclofenil.

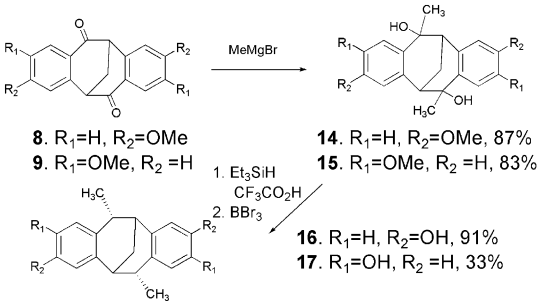
The Troger's base analogue, compound **1**, with bridgehead nitrogen atoms and a bicyclo[3.3.1]nonane core, was synthesized from 4-methoxyaniline as described[10] and was deprotected with BBr_3. Type I ligands with bridgehead carbon atoms were synthesized by the general route shown in Scheme 1 using methodology described earlier.[11]

In the first step, a substituted phenylacetonitrile was alkylated with diiodomethane to give a mixture of *meso* and DL-diphenyl glutaronitriles (**4** and **5**). This mixture was hydrolyzed to furnish a diasteromeric mixture of diphenylglutaric acids (**6** and **7**), which were subsequently subjected to a double intramolecular Friedel–Crafts acylation using polyphosphoric acid or methanesulfonic acid/P_2O_5,[12] furnishing the cyclized products **8** and **9**. These latter compounds could be further transformed into additional analogues, as shown in Scheme 2, using the keto groups as reactive handles.

For example, methyl substituents on the periphery of the bicyclic core could be introduced by addition of methyl magnesium bromide to the bis-keto compounds **8** and **9**. This was followed by deoxygenation using a triethylsilane–trifluoroacetic acid mixture.[13] The *endo* stereochemistry of the methyl groups in **16** and **17** was confirmed by X-ray crystallography. The exclusive formation of the *endo* isomers can be rationalized by assuming that the initial addition of the Grignard reagent occurs from the less hindered *exo* face. In the



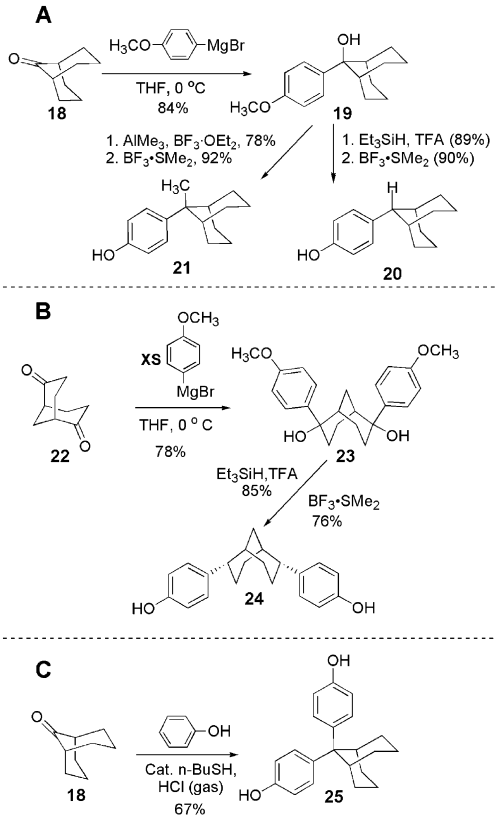
Scheme 1.



Scheme 2.

subsequent deoxygenation step, hydride donation from triethylsilane again occurs from the *exo* face, thus leading to an *endo* stereochemistry for the two methyl groups on the periphery of the bicyclic core. It is important to note that all chiral ligands described in this study were obtained and used as racemic mixtures.

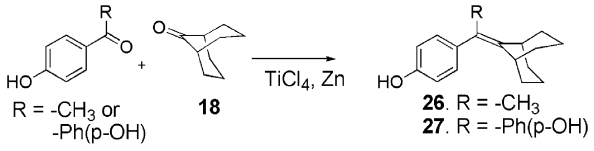
The synthesis of Type II ligands was accomplished starting from commercially available bicyclic ketones **18** and **22**, using the synthetic transformations shown in Scheme 3. A methyl group at C-9 (compound **21**) was introduced by nucleophilic substitution on tertiary benzylic alcohol **2** with trimethylaluminum, used in conjunction with boron trifluoride etherate.[14] The two phenyl groups in **24** were also determined to be *endo*, which can be rationalized in the same manner as for compounds **16** and **17** above. The introduction of the second phenol group at C-9 (compound **25**) was accomplished using established methods.[15]



Scheme 3.

Type III ligands were synthesized by crossed McMurry coupling, as shown in Scheme 4. The latter reaction was particularly efficient when a diaryl ketone was one of the reactants.[16]

Following the synthesis of different ligands, we examined their binding to both ERα and ERβ. The binding affinities of all the ligands prepared in this study are given in Table 1 and are expressed as relative binding affinity (RBA) values, where estradiol has an affinity of 100%.

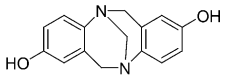
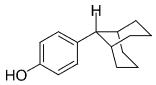
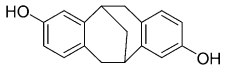
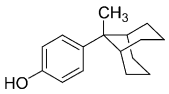
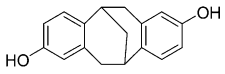
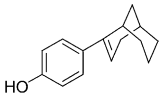
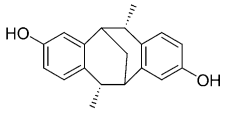
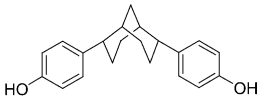
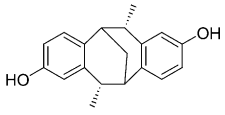
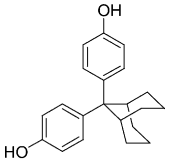
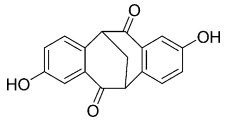
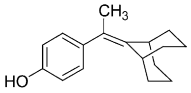
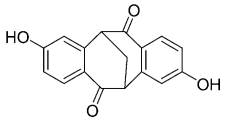
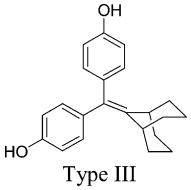


Scheme 4.

The binding affinity of the Troger's base analogue (compound **1**) to the estrogen receptor was below the limits of our assay. Initially, it was not clear whether the low binding affinity of **1** was due to its highly concave nature or to the presence of the bridgehead nitrogen atoms. To examine this, we synthesized the carbocyclic analogues in which the bridgehead nitrogen atoms were replaced by carbon atoms (compounds **12** and **13**).

The significantly increased binding affinity of these latter compounds suggests that the polar bridgehead nitrogen atoms in **1** are responsible for its immeasurably low binding affinity.

Table 1. Relative binding affinity (RBA) of bicyclic non-steroidal analogues for estrogen receptors α and β. The RBA values were determined in a competitive radiometric binding assay described earlier[a,17,18]

No.	Compound	ERα	ERβ	β/α	No.	Compound	ERα	ERβ	β/α
1	 Type I	<0.005	<0.005	—	**20**	 Type II	0.13±0.03	1.33±0.26	10
12	 Type I	0.033±0.005	0.072±0.004	2	**21**	 Type II	0.32±0	8.90±0	28
13	 Type I	0.22±0.011	0.77±0.15	3.5	**30**	 Type II	0.39±0.006	1.81±0.15	4.6
16	 Type I	0.007±0.0	0.025±0.003	3.6	**24**	 Type II	0.44±0.02	2.9±0.2	6.6
17	 Type I	0.004	0.011	2.7	**25**	 Type II	0.31±0.02	0.25±0.02	0.8
28	 Type I	0.006±0.001	0.021±0.006	3.5	**26**	 Type III	1.71±0.45	4.58±1.3	2.7
29	 Type I	0.005±0.001	0.022±0.004	4.4	**27**	 Type III	513±17	301±54	0.59

[a]The RBA of estradiol is 100, values represent the average±range or SD of 2–3 independent determinations.

For Type I ligands, it is intriguing to note that the binding affinity depends on the position of the bridge. For compound **13**, in which the bridge is *para* to the hydroxyl group, the binding affinity is higher than in compound **12**, in which the bridge is *meta* to the phenolic hydroxyl group. Another feature that is readily apparent from the SAR study for Type I ligands is that ER does not seem to tolerate any substituent, electron donating or withdrawing, on the periphery of the bicyclic core. For example, note the significantly reduced binding affinities of both **16** and **28**, compared to **12** and **13**, respectively.

Compounds containing phenols directly attached to the bicyclic core (Type II ligands) exhibit higher affinity toward ER compared to Type I ligands. Presumably, this is in part due to their increased flexibility. What is noteworthy, however, is that some of the Type II ligands (**20** and **21**) have significant ERβ affinity selectivity. Monophenol **20** exhibits a 15-fold selectivity for ERβ which is further increased by the introduction of a methyl group at C-9 as in **21**. The ERβ binding selectivity of **21** is comparable to some of the recently reported triazines.[19] Bisphenols **24** and **25** have lower binding affinities and exhibit little or no binding selectivity for ERβ. Preliminary data shows that compound **21** does not exhibit substantial selectivity for ERβ in cell based transfection assays, either in terms of efficacy or potency (S. Sheng and B. S. Katzenellenbogen, unpublished). During the preparation of this manuscript, we became aware of another report pertaining to the synthesis of non-steroidal ligands having bicyclo[3.3.1]-nonane cores.[20] However, the binding affinities and the ERβ, selectivities of the ligands described in that report are lower than those of compound **21**.

Of the various ligands investigated in this study, type III ligands exhibit the highest affinity for ER. Remarkably, unlike a similar substitution in the type II series, the replacement of a methyl group in **26** with another phenol leads to a very high affinity ligand (compound **27**), having an RBA of 500% of that of E_2. In fact, this binding affinity is almost twice that of the well-known estrogen cyclofenil (RBA: ERα = 68 and ERβ = 334), which is similar in all respects to **27** except for the three carbon bridge. This indicates that for certain types of structures, the binding affinity of a ligand can be increased simply by an increase in the number of hydrophobic interactions through the introduction of a bicyclic core. Interestingly, Type III ligands derived from other three-dimensional cores having a larger or smaller number of carbon atoms showed reduced affinity for ER. We have conducted and reported elsewhere a detailed structure–activity study of Type III ligands.[21]

In conclusion, by examining three novel structural motifs as E_2 mimics, we identified Type III ligands as lead compounds for developing high-affinity ER ligands. Type II ligands show significant ERβ affinity selectivity. It remains to be seen whether this selectivity and affinity can be further enhanced by the addition of a polar functionality on the bicyclic core to mimic the distal ring-OH group of E_2. Studies in this direction will be the subject of future investigations and will be reported in due course.

Acknowledgements

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