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Deconstructing estradiol: Removal of B-ring generates compounds which are potent and subtype-selective estrogen receptor agonists

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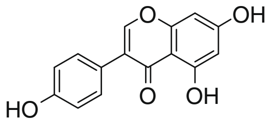
ABSTRACT

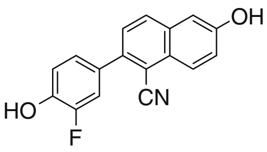
Estradiol and related estrogens have been widely used as supplements to relieve menopausal symptoms, but they lead to an increased risk of breast and endometrial cancer. Here we report the synthesis of a new family of compounds where we have removed the B-ring from the steroid ABCD structure, and functionalized the A-ring. These A-CD compounds show a preferential affinity for the estrogen receptor subtype ERβ. Some show binding affinities which are greater than estradiol. The presence of electron-withdrawing substituents on the A-ring should reduce the tendency of these compounds to form carcinogenic metabolites, so they might lead to a safer approach to hormone replacement therapy.

The recent discovery that estrogens bind to two estrogen receptor subtypes, ERα and ERβ, has resulted in major efforts to develop ligands that are selective agonists for either subtype.[1–3] Such compounds might have considerable potential for the treatment of a number of symptoms and/or diseases associated with estrogen deficiency, including hot flashes, osteoporosis and cardiovascular problems.[3] Also, the suggestion that ERα is proliferative whereas ERβ is antiproliferative may be relevant in the treatment of breast cancer.[4]

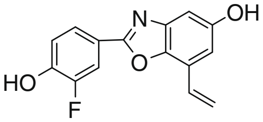
In the search for selective agonists, several non-steroidal families of compounds have been developed, inspired by the structure of genistein, **1**. Considerable success has been achieved in this area for structures such as WAY 202196, **2** and ERB-041, **3**.[5,6] These compounds show not only strong binding but also excellent selectivity; the ERβ/ERα binding affinity ratios for these structures are: **1** (41); **2** (78); and **3** (226). However, the highly ERβ-selective compounds appear to be devoid of classical estrogenic activity. For example, they do not stimulate an increase in uterine weight or promote the growth of estrogen-dependent MCF-7 breast cancer cells, nor do they protect against osteoporosis or hot flashes. However, these compounds are of interest in the treatment of inflammatory bowel disease.[7] The value of genistein for the treatment of hot flashes has not been unambiguously established, although soy products (containing genistein) are commonly used for this purpose.[8] In hormone replacement therapy (HRT) a desirable therapeutic target would be the suppression of menopausal symptoms without increasing the risk of breast or uterine cancer or vascular problems.



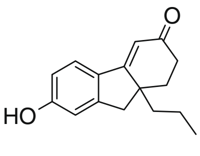
1, genistein



2, WAY 202196



3, ERB-041



4,

Following the genistein lead, consider the simple structures 1–4. The common motif in 1–3 is two aromatic ring systems, each carrying a hydroxyl group, and connected by a bond which allows

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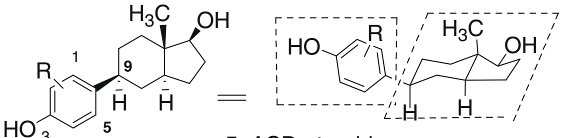
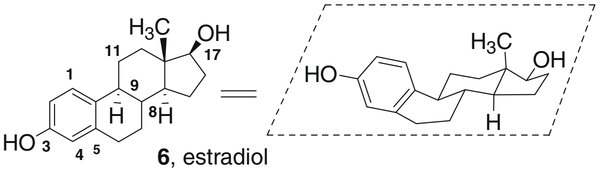
conjugation between the systems. As in 2 and 3, one of the aromatic rings can be a naphthalene or a bicyclic aromatic heterocycle such as a benzoxazole. Many variations of this structure have been reported (see Ref. 9 for a review). Exceptions to the above are the fused-ring systems such as tetrahydrofluorene 4.[10,11]

What has not been thoroughly explored is the idea of maintaining the essential structure, connectivity and spacing of estradiol, while increasing the flexibility. In principle, this should allow the ligand to more fully explore the active site in the receptor and thus have the potential to increase the binding affinity or subtype selectivity. To investigate these possibilities, we performed some computational studies on A-CD and AB-D ring systems. The A-CD system has the additional advantage (relative to ABCD steroids) that the unsubstituted positions 1, 2, 4, and 5 in the A-ring could be more easily functionalized with a variety of substituents, some of which may increase the binding affinity, selectivity, and/or prevent formation of undesirable metabolites, especially carcinogenic ortho-quinones. The relationship between the parent estradiol and its A-CD derivative is shown below. The terminology 'deconstructing estradiol' seems appropriate since we have retained the same stereochemistry in the derivative.



5, ACD-steroid



6, estradiol

Docking studies were performed on the A-CD structure 5, retaining the same stereochemistry. Figure 1 shows that the important residues in the ligand binding site overlay almost perfectly between ERα and ERβ except at two points: where Met336 (in ERβ) has been switched for Leu384 (in ERα), and Ile373 (ERβ) for Met421 (ERα). There are the beginnings of a steric clash between the D-ring of the ligand and Met421 (see dotted line). Based on this observation, we hypothesized that there should be a natural preference of the A-CD ligand for binding to the ERβ-subtype. Both the relative binding affinity and transcription activation data strongly support this hypothesis.

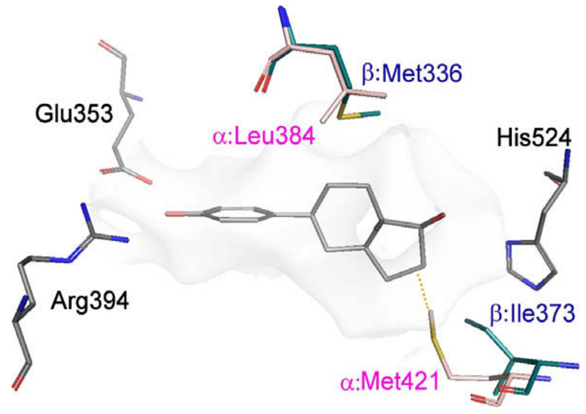
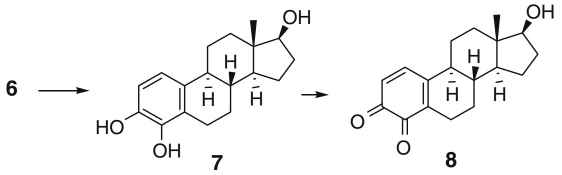


Figure 1. Important residues surrounding the active sites in ERα (pink) and ERβ (blue).

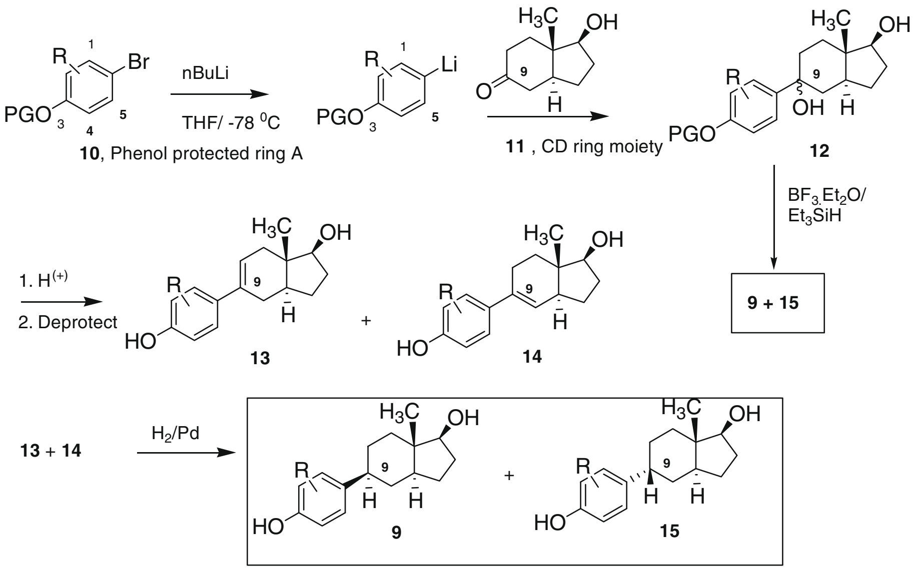
Here we report the synthesis, relative binding affinities (RBA) and transcriptional activation relative to estradiol (RTA) for a series of A-CD compounds **5–15**. We have retained the original steroid numbering system so as to show the relationship with estradiol. Our initial studies have focused on how varying substituents at the 2-, 4-, and 5-positions in the A-ring of **5** affect the strength and the selectivity of binding to the estrogen receptors. The substituents were chosen mainly from the electron-withdrawing groups (EWG) F and Cl. The choice of using EWG was made since our calculations have shown that such substituents may retard the possible formation of catechols and their oxidation products, the ortho-quinones.[12,13] This property is relevant to the potential use of these compounds in humans, because the quinones produced metabolically from estradiol (e.g., 4-hydroxyestradiol, **7**, and the derived 3,4-quinone **8**) have been implicated in estrogen-induced cancers.[14,15] For comparison, an electron-donating group, methyl, has also been used in several structures.



The synthesis of the A-CD compounds (Scheme 1) involves coupling of a suitably protected bromophenol, **10** (ring A), with the CD-ring moiety **11**, prepared in enantiomerically pure form starting with 2-methyl-1,3-cyclopentanedione following the Hajos–Parrish procedure (Scheme 1).[16] The coupling can be done by using somewhat more than two equivalents of lithiated **10** per equivalent of **11,** or one equivalent of the lithiated intermediate if the OH of **11** is also protected with a base-stable group such as tBDMS or MOM. The initial coupling product **12** was obtained as a mixture of isomers at C9, separable via silica gel chromatography. The assignment of the stereochemistry of the less polar isomer as 'natural', that is, having the aryl group in the equatorial position relative to ring C, was made by comparing its NMR with those of compounds **5** and **15**. Acid-catalyzed dehydration of either of the individual isomers of **12** resulted in a mixture of unsaturated compounds **13** and **14**. Hydrogenation of the mixture of these unsaturated compounds afforded the desired compound **9** and the epimeric derivatives **15** in almost equal amounts. Alternatively, the mixture of the coupling products **12** was reduced to a mixture of **9** and **15** using triethylsilane and $BF_3 \cdot Et_2O$.[17]

The 'natural' isomers **9** have the aryl derivative in an equatorial position relative to the six-membered ring C, and thus the C9 hydrogen shows both axial–axial and axial–equatorial spin–spin coupling in the ^1H NMR (9 and 3.2 Hz, respectively). Once the assignment was secured for the parent structures **9a** and **15a**, it was noted that the chemical shift for H9 in the beta isomer in all of the examples was consistently at higher field. The ^{13}C NMR signal for C17 in both the 9-hydroxy and the hydrogenated isomers was even more diagnostic, appearing at 80–82 ppm for all of the derivatives of **9** and between 72 and 74 ppm for the compounds **15** that carry the aryl group in the axial position relative to ring C.

The RBAs were determined by competition between tritium-labeled estradiol and increasing concentration of the ligand under investigation.[18] Displacement of the radioactivity indicates that the ligand shows binding to the receptor, and this is quantified to give the relative binding affinity (RBA) with estradiol set equal to 100% for both receptors. Transcriptional activation capacity of some ligands was determined using COS-7 cells transfected with ERE-luciferase reporter plasmid and the ERα or ERβ.[19] Transcrip-



Scheme 1. Synthesis of ACD-estrogens.

tion activation at a concentration of 10 nM was compared to estradiol at the same concentration, giving the relative transcriptional activation (RTA) for each receptor.

The results of these experiments are summarized in Table 1, which shows the pattern of ring substitution according to Figure 2, the RBA for each receptor, and several entries for the RTA. As expected, the natural isomers **9** show consistently much greater binding affinity for both receptors than the non-natural isomers **15**. For brevity, only the RBA results for **15a**, the parent compound having the non-natural stereochemistry, are shown in Table 1; in general, none of the RBAs for non-natural isomers exceeded 1%. The RBA of **9a** for ERβ was 21.5%, with a 14:1 selectivity over ERα. From the RTA values **9a** demonstrated very strong ERβ agonist activity of 164%, and very weak (possibly negligible) ERα activity of 4.3%. Taken together with the RBA values, one can conclude that *the unsubstituted natural isomer* ***9a*** *is behaving essentially as a highly selective ERβ agonist.*

Substituents in the 4-position consistently lowered the binding affinity. Thus, for the small F-atom at C4 (**9b**), the RBA for ERβ decreased from 21.5% to 8.7%, while the RBA for ERα dropped also. This loss in binding affinity on 4-substitution appears to be amplified in the transcription assay, where now **9b** is only a weak agonist for ERβ. Fluorine substituents at both positions 2 and 4, as in **9l**, resulted in a further strong reduction in binding. The RBAs for the 4-methyl derivative **9c** were not measured because those for the precursor compounds were already very low.

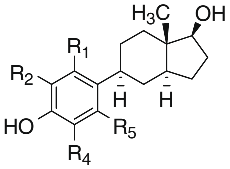


Figure 2. Pattern of substituents for A-CD ring system.

Having established that substitutions at positions 2 and 4 on the A-ring decrease the binding affinity how can it be increased? Computer modeling showed that there was some room around C5. Three derivatives were prepared, **9d, 9e**, and **9f**. The RBA was higher than that found for the parent compound **9a**, irrespective of the type of substituent and in **9e** and **9f** exceeded that of estradiol itself for ERβ. The transcriptional activation induced by the 5-F derivative **9e** shows that this compound, and presumably also the 5-Cl compound, is both an ERα and ERβ agonist.

A series of polyfluorinated ring A derivatives were also prepared to determine whether the detrimental effect of substituents

Table 1
Pattern of substituents, relative binding affinity (RBA) and relative transcription activation (RTA) of A-CD estrogen derivatives

Compound	Ring A:R1 = H			RBA (estradiol = 100)			RTA (estradiol = 100)	
	R2	R4	R5	ERα	ERβ	β/α	ERα	ERβ
9a	H	H	H	1.5	21.5	14	4.3	164
9b	H	F	H	1.0	8.7	8.7	−8.3	14
9c	H	CH_3	H	—	—	—	—	—
9d	H	H	CH_3	2.8	33.6	12	−9.7	149
9e	H	H	F	27	135	5.0	44	146
9f	H	H	Cl	49	168	3.4	—	—
9i	H	F	F	4.6	43	9.3	—	—
9j	F	H	F	0.38	3.3	8.7	—	—
9k	F	F	F	0.19	1.73	9.1	—	—
9l	F	F	H	0.04	0.28	7.0	—	—
13c, **14c**[a]	H	CH_3	H	0.05	0.02	0.5	—	—
15a	H	H	H	0.06	0.59	9.8	—	—

Compounds **9a**–**9l** all have natural stereochemistry (same as estradiol).

[a] Ring C, unsaturated.

at C2 and/or C4 on binding to the receptor could be overcome by the favorable effect of a substituent at C5. Analysis of the binding affinities for the structures **9i**–**9k** indicates that binding is most decreased by even the small fluorine substituent at position 2. Thus, using the binding data for the 5-F derivative **9e** as a marker, placing a second fluorine at C4, **9i**, reduces affinity for ERβ by a factor of 3, compared to a more than 40-fold reduction observed for **9j** where the second fluorine is at C2. The addition of a third F at C2 generates **9k** and is accompanied by an almost 25-fold reduction in RBA for ERβ. The corresponding 2,4 dichloro derivative (data not shown) showed very little binding to either receptor.

In conclusion, we have shown that by deconstructing the estradiol ABCD steroid ring structure into one having only the A-CD rings, there are several interesting consequences for binding to ERα and ERβ. First, there is a natural preference for the ERβ-subtype. Second, it is relatively easy to synthesize compounds with a variety of A-ring substituents. Third, positions 2 and 4 on A are deactivating, but position 5 is strongly activating. By varying the substituents, we have identified two pure ERβ agonists (**9a**, **9d**), which are more potent than estradiol. Other structures are agonists for both receptors. Fourth, calculations show that EWG-substituted phenols should retard the rate of formation of carcinogenic metabolites. Thus, these novel compounds may have potential for use in HRT.

Acknowledgments

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