Bioorganic & Medicinal Chemistry Letters 24 (2014) 3841–3844

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and receptor binding in *trans*-CD ring-fused A-CD estrogens: Comparison with the *cis*-fused isomers

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ARTICLE INFO

Article history:
Received 10 March 2014
Revised 19 June 2014
Accepted 20 June 2014
Available online 27 June 2014

Keywords:
Estrogen receptor
ERα
ERβ
β-Selectivity
Ligand synthesis

ABSTRACT

Ligands which selectively activate only one of the estrogen receptors, ERα or ERβ, are current pharmaceutical targets. Previously, we have reported on substituted *cis* A-CD ligands in which the B-ring of the steroidal structure has been removed and *cis* refers the stereochemistry of the CD ring junction as compared to *trans* in estradiol. These compounds often showed good potency and selectivity for ERβ. Here we report the synthesis and binding affinities for a similar series of *trans* A-CD ligands, and compare them to the *cis*-series. Counterintuitively, *trans* A-CD ligands, which are structurally more closely related to the natural ligand estradiol, show weaker binding and less β-selectivity than their *cis*-counterparts.

Current studies of the mechanism of disease initiation in breast cancer and several other cancers have focused on the pathway starting from the natural estrogen 17β-estradiol (**E2**), which by P450 aromatic hydroxylation can form the catechol estrogen and potentially the corresponding *ortho*-quinones.[1] Of various possible metabolites, the 3,4-quinone has been shown to intercalate into DNA and undergo Michael addition with DNA bases, ultimately forming depurinating adducts. DNA repair then leads to a high mutation rate and tumor initiation.[2] Once initiated, activation of the estrogen receptor ERα by endogenous ligands such as **E2** has been demonstrated to promote proliferation of the cancer cells.[3] On the other hand, in some experiments activation of the receptor ERβ may suppress cancer cell proliferation,[4] hence the current intense interest in developing ERβ-selective ligands.[5]

We have recently reported the synthesis of a series of estrogen agonists represented by structure **1** shown in Figure 1, in which the A ring and C rings are connected by a single bond,[6–8] which we called A-CD estrogens. These compounds, especially those having electronegative substituents at C5, showed high affinity binding to the estrogen receptors (ERs), which for ERβ was in some cases even stronger than the naturally occurring ligand **E2**, which has the highest affinity of the natural ligands. We also observed some, and at times considerable, enhancement of ERβ- receptor binding selectivity. In general, the compounds which showed this type of binding selectivity also acted as strong β-selective agonists; such compounds are denoted "super-agonists" when they exceed the maximum level of transcription activation by **E2**.[9] In our initial report,[6] we presented these compounds as having the general structure **2** (see Fig. 1), where the CD ring junction is *trans* as in **E2**. Subsequently,[7,8] we corrected the structure assignment and showed that the compounds we had prepared actually had the *cis*-CD ring structure, as shown below in the parent A-CD compound for series **1** (see Scheme 2).

We now present our work on the preparation of a series of compounds of structural type **2** which have the *trans*-CD ring junction. We compare their binding affinity to both estrogen receptors with the corresponding *cis*-compounds in series **1**. One might expect that retaining the geometry of the natural ligand at the CD interface would lead to optimum binding of the A-CD system. However, we will show that the unintended synthesis of compounds with structure **1** rather than **2** turns out to have been quite fortuitous, since the A-CD compounds that most closely resemble **E2** generally bind less strongly and with lower selectivity to ERβ than those in the *cis*-series **1**. As before, for convenience we have chosen the

Abbreviations: RBA, relative binding affinity; ER, estrogen receptor; **E2**, 17β-estradiol.

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http://dx.doi.org/10.1016/j.bmcl.2014.06.066
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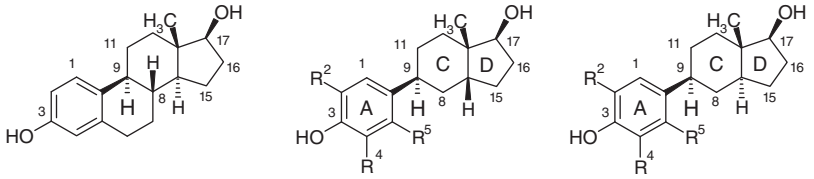


trans 17β-estradiol, **E2** *cis* A-CD, series **1** *trans* A-CD, series **2**

Figure 1. Stereochemical relationship between the natural estrogen **E2**, the A-CD estrogens, *cis*-series **1** and the *trans*-series **2**. The *cis* and *trans* designations refer to the stereochemistry at the CD ring junction.

numbering in these compounds to correspond to the same numbering scheme as in **E2** (see Fig. 1).

The *trans*-CD ring junction in the structures for series **2** was installed in one of two ways.[10] In the first approach (Scheme 1), the CD ring ketone **4** having the *trans*-ring junction was prepared following the method of Micheli et al.[11] This ketone was then reacted with the lithio-derivative of a suitably protected ring A (moiety **5)** to give the alcohol **6**. Dehydration, with concomitant removal of the 3-phenol and 17-alcohol protecting groups, generally MEM-ethers, gave exclusively the alkene **7.** The typical overall yield over these two steps was in the 80–90% range. Simple hydrogenation with Pd/C as catalyst afforded quantitatively a mixture of isomers at C9 from which the major desired isomer **2** was isolated by preparative HPLC. The structure of the parent compound in the *trans*-series was verified by an X-Ray structure determination, shown in Figure 2 for **2a**, the *trans* A-CD parent compound.

The ^{1}H NMR spectrum of **2a** showed the C13 methyl group at 0.83 ppm; this compared with 1.05 ppm for the *cis*-CD-fused derivative **1**. This difference was consistent for all other pairs of compounds described herein. ^{13}C NMR also showed significant differences for the C17, which helped subsequently to verify whether either the *trans*- or the *cis*-CD ring juncture was present in derivatives of **1** and **2**.

Alternatively, reaction of **5** with the MEM-protected enone **8** yielded the allylic alcohol **9**. Acid-catalyzed dehydration with concomitant deprotection afforded the diene **9** (Scheme 2) in excellent yield. Hydrogenation with a variety of common catalysts, including Pd/C, gave mainly the compound **2**, as shown by ^{1}H NMR examination of the crude reaction product. Small amounts of the three other possible stereoisomers were also formed. The formation of **2** as the major product in the hydrogenation of **10** indicates that the C14–C15 double bond is hydrogenated first, with preferential delivery of hydrogen from the less hindered side, to give the C8–C11 alkene **11** having the *trans*-CD ring junction. Subsequent hydrogenation gives, as expected, mainly the isomer **2** with the (S) configuration at C9. In contrast, hydrogenation of the *cis* CD-junctioned alkene **12** gives a close to a 1:1 mixture of stereoisomers at C9.

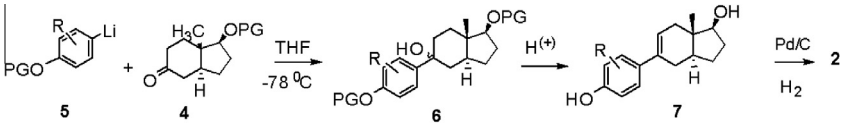
Either of the above sequences or slight variations thereof were used to prepare a series of 10 derivatives carrying a variety of substituents in the A-ring.[10] For example, the 5-hydroxy derivative **2i** was obtained in three steps by first converting 1,3-dibenzyloxy-4-bromobenzene into its lithio-derivative and reaction of this species with the enone **8**. Purification of the reaction mixture via silica gel chromatography (Scheme 3) resulted in formation of the diene **11**, which was hydrogenated to give a mixture of compounds from which **2i** was obtained after reverse phase HPLC purification.

As pointed out above, the isomeric compounds **1** and **2** were most readily distinguished by focusing on the quaternary methyl group. In all of the compounds described these methyl hydrogens absorbed in the 0.83–0.85 ppm range for the *trans*-compounds but more downfield at 1.09–1.11 ppm for the *cis*-isomers. In the ^{13}C spectrum this carbon was found near 12 ppm in the *trans*-compounds but again considerably more downfield near 20 ppm for the corresponding *cis*-isomers.[10,11] Many of the *trans* compounds were synthesized starting with the *trans* hydridanone **4**, so the *trans* CD stereochemistry is unambiguous. The others were prepared by hydrogenation of the diene **10** and finally several including the parent compound were prepared via both routes. In all cases the *trans* ACD compounds showed clear and consistent proton and carbon spectra differences, as described above.[12,13] For structural details, see Supplementary data.

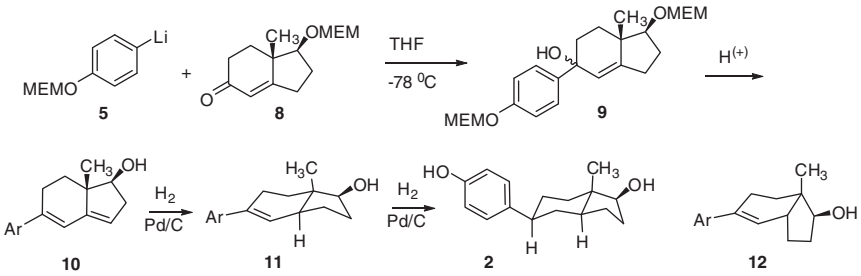
Binding affinities to the estrogen receptors ERα and ERβ are given as relative binding affinities (RBAs), where the RBA for estradiol on both ERs is 100. The RBA value and selectivity ratio RBA(ERβ)/RBA(ERα) for a series of 10 *cis* A-CD (series **1**) and 10 *trans* A-CD compounds (series **2**) containing saturated C-rings are given in Table 1. The binding affinity ratios RBA(*trans*)/RBA(*cis*) for both receptors are given in Table 2.

The values of the RBAs shown in Table 1 are correlated via a sigmoidal dependence with the transcription activation, or 'potency', of each ligand in its receptor,[8] so the RBAs are already useful predictors of potential drug activity. With four distinct data sets (*cis*- and *trans*-ligands in two receptors, ERα and ERβ) there are a number of interesting comparisons that can be made. First, consider the selectivity of *trans* versus *cis* ligands binding to ERα and ERβ. The β-selectivity of the parent *trans* compound **2a**, for example, is given by the β/α ratio = 10/2.38 = 4.2 (see Table 1), whereas for the *cis* compound **1a** it is 21.5/1.47 = 14.6, over three times larger. Looking at the selectivity ratios β/α for each series, the average value for the ligands in the *trans*-series is 3.7, whereas for the *cis*-series it is 9.1. Thus, the *cis*-series is much more β-selective than the *trans*-series.

It is also of interest to compare the magnitude of the RBAs of the *trans* versus the *cis* compounds in Table 1. For example, comparing the *trans*/*cis* RBA ratio for compound **c** (**2c**/**1c)** in ERα, the ratio is 4.22/27.3 = 0.15 (see Table 2); the *cis*-structure is much more strongly bound. Continuing in this way, the average ratio for *trans*/*cis* binding into ERα for the 10 ligands is 0.48, showing that the binding of the *cis*-compounds into ERα is, generally speaking,



Scheme 1.



Scheme 2.

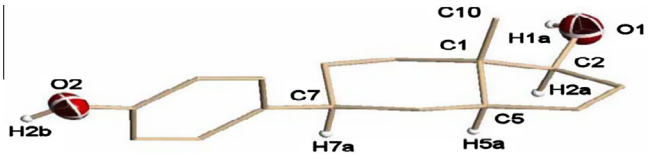


Figure 2. X-ray structure of compound **2a**, the *trans* A-CD parent compound. Cambridge Crystallographic Data Centre deposition no. is CCDC 1007460.

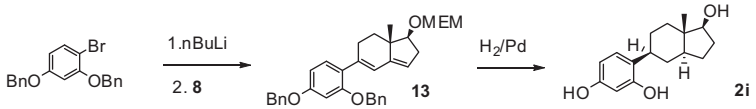
over twice as strong as that of the *trans*-compounds. Performing the same operations on binding to the ERβ receptor, the *trans*/*cis* ratio for compound **a** (**2a/1a**) is 10/21.5 = 0.47. The average for the whole series is only 0.20, so binding to the ERβ receptor favors the *cis*-structures by a factor of five.

It should also be pointed out that in the *cis* series **1**, the compounds such as **1c** (5-F) and **1e** (5-CF3), which showed the highest binding affinities, also had the lowest β/α selectivity, 5.0 and 2.3, respectively. In contrast, some ligands with more modest binding affinities to both receptors **1a** (parent) and **1i** (5-OH) showed the maximum selectivity for the ERβ to ERα ratio of ca. 15. The very low binding affinity of the 5-OCH_3 derivatives **1j** relative to those carrying CF_3, CH_3 and OH groups at this position was unexpected. For comparison, the binding affinity to ERβ of **1e** (5-CF_3) is about 1700 times greater than that of **1j**. A similar comparison for **2e** versus **2j** gives a value of close to 130. It should be possible to explain these large differences by examining the interaction of these substituents with receptor residues, work that we have already begun.

Table 2
Ratio of binding to ERα and ERβ for the *trans*-compounds **2** versus the *cis*-compounds **1**

Compound	ERα	ERβ
a(Parent, R = H)	1.62	0.47
b(4-F)	1.58	0.79
c(5-F)	0.15	0.10
d(5-CH_3)	0.17	0.04
e(5-CF_3)	0.054	0.024
f(4,5-DiF)	0.20	0.17
g(2,4,5-TriF)	0.16	0.056
h(2,4-DiF 5-CH_3)	0.11	0.042
i(5-OH)	0.14	0.038
j(5-OCH_3)	0.63	0.30
Average β/α ratio	0.48	0.20

Since the results presented above were so unexpected, we looked at two more series of compounds with the *trans*-, *cis*-CD ring fusion (**Series 7** and **Series 14**, Table 3). These were prepared during the course of our studies and are C9–C11 alkenes having the *trans* CD ring fusion (**Series 7**) and the *cis* fusion (**Series 14**). The compounds in **Series 7** were obtained as intermediates in the synthesis via Scheme 2; those in **Series 14** were formed in the preparation of the *cis* A-CD compounds.[10] As shown in Table 3, even



Scheme 3.

Table 1
Relative binding affinities for *cis*-series **1** and *trans*-series **2** to estrogen receptors ERα and ERβ, selectivity ratio for ERβ/ERα. Error bars in parentheses

Compound	*cis* A-CD structures (**1**)			*trans* A-CD structures (**2**)		
	RBA(α)	RBA(β)	β/α	RBA(α)	RBA(β)	β/α
a(H)	1.47(0.26)	21.5(4.6)	14.6	2.38(0.19)	10(1.3)	4.2
b(4-F)	1.04(0.09)	8.7(1.5)	8.4	1.68(0.15)	6.84(0.41)	4.1
c(5-F)	27.3(0.70)	135.5(7.3)	5	4.22(0.06)	13.6(0.35)	3.2
d(5-CH_3)	2.82(0.45)	33.6(6.2)	11.9	0.47(0.1)	1.3(0.3)	2.8
e(5-CF_3)	89.7(13.8)	205(23)	2.3	4.8(1.1)	4.9(0.1)	1
f(4,5-diF)	4.62(0.93)	42.8(5.5)	9.3	0.92(0.15)	7.3(1.8)	7.9
g(2,4,5-triF)	0.186(0.01)	1.73(0.02)	9.3	0.029(0.004)	0.097(0.025)	3.3
h (2,4-diF 5-CH_3)	0.75(0.20)	5.35(0.88)	7.1	0.08(0.007)	0.226(0.04)	2.8
i(5-OH)	0.26(0.07)	3.97(0.08)	15.3	0.037(0.003)	0.15(0.04)	4.1
j(5-OCH_3)	0.016(0.002)	0.123(0.03)	7.7	0.010(0.001)	0.037(0.008)	3.7
Estradiol (**E2**)				100	100	1
Average β/α ratio*			9.1			3.7

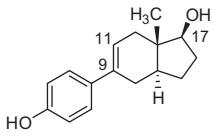
* **E2** is not included in the averages.

Table 3
Relative binding affinities for *cis*-series **14** and *trans*-series **7** to estrogen receptors ERα and ERβ, and selectivity ratio for ERβ/ERα

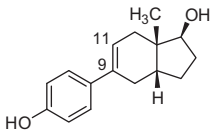
Compound	*cis* A-CD structures (**14**)			*trans* A-CD structures (**7**)		
	RBA(α)	RBA(β)	β/α	RBA(α)	RBA(β)	β/α
a(H)	0.39(0.04)	6.8(0.6)	17	2.38(0.19)	17.3(2.4)	4.7
b(5-F)	4.5*(0.8)	49*(7)	11*	21.7(0.4)	38(11)	1.7
c(5-Cl)	60(8)	118(4)	2	9.7(2.2)	14.1(3.8)	1.5
d(5-CF_3)	122(11)	174(8)	1.4	—	—	—
e(5-OCH_3)	0.004(0.001)	0.022(0.004)	5.5	0.006(0.001)	0.029(0.002)	4.8
f(2,4,5-triF)	—	—	—	0.099(0.03)	0.076(0.007)	0.8
g (2,5-DiF)	0.44(0.02)	2.7(0.5)	6.1			
h(4-F,5-Cl)	50(6)	149(9)	3			
i(4,5-DiCl))	3.0(0.8)	55(9)	18.3			
j(2,4-DiF 5-Cl)	3.35(0.06)	6(2)	1.8			
Average β/α ratio			6.6			2.7

* Determined for a ~1:1 mixture of 8–9 and 9–11 alkenes.

though relatively few pairs are currently available, the trends discussed above are also seen for these sets of isomers. Thus, the average β/α selectivity ratio for the *cis*-series **7** (6.6) is higher than that for the *trans*-series **14** (2.7) by a factor of 2.4; this is identical to the ratio for the *cis*-series **1** and *trans*-series **2** (factor of 2.4). Since the alkene series show no special advantages over the alkanes and is more likely to be reactive in vivo, and since the *cis*-series **1** shows improved β-selectivity and binding affinity compared to the *trans*-series **2**, future drug development using these compounds will be focused on the *cis*-series **1**.



***trans*-series 7**



***cis*-series 14**

We have begun a series of modeling studies aimed at understanding the trends in reactivity described above.[8,14] In future work we will focus on understanding the origins of the ERβ-selectivity described in this paper, as well as the preference for the *cis*- rather than the *trans*-geometry for the binding affinity in the receptors ERα and ERβ.

Acknowledgments

We thank the Canadian Breast Cancer Foundation (Ontario Region) for grants to J.S.W. and T.D. which supported this research. Support from the US National Institutes of Health (PHS 5R01 DK015556 to J. A.K.) is also gratefully acknowledged.

Supplementary data

Supplementary data (spectra of the *trans*-compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.066.

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12. The preparation of the A ring precursor bromides is described in Ref. [8].
13. All new compounds were characterized by high field ^{1}H and ^{13}C NMR and HREIMs. For example, for the 5-OH derivative, **2i**: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.11 (s, 1H, OH) 8.00 (s,1H, OH), 6.97 (d, *J* = 8.3 Hz, 1H), 6.36 (d, *J* = 2.4 Hz, 1H), 6.20 (dd, *J* = 8.3, 2.4 Hz, 1H) 3.69–3.61 (m, 2H, H17 and O-H)), 2.88–2.74 (m, 1H), 2.05–1.89 (m, 1H), 1.84 (dt, *J* = 12.5, 3.2 Hz, 1H), 1.74–1.27 (m, 8H), 1.17(dt, 12.7, 4.7 Hz, 1H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, acetone-d_6 δ 157.7, 157/1, 129.3, 126.3, 108.3, 104.4, 82.8, 47.5, 44.4, 39.4, 39.1, 34.0, 31.9, 30.2, 27.3, 11.9. HRMS calc'd for $C_{16}H_{22}O_3$: 262.1569. Found: 262.1570.
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