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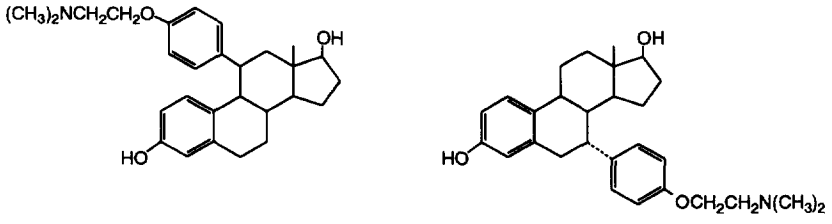
MOLECULAR MODELING STUDIES ON 11β-AMINOETHOXYPHENYL AND 7α-AMINOETHOXYPHENYL ESTRADIOLS. EVIDENCE SUGGESTING A COMMON HYDROPHOBIC POCKET IN ESTROGEN RECEPTOR.

Jacques H. Poupaert,[1] Didier M. Lambert,[1] Joseph Vamecq,[1] and Yusuf J. Abul-Hajj[2*]

[1]Ecole de Pharmacie, Université Catholique de Louvain, Brussels, Belgium
[2]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455, USA

Abstract. Molecular modeling studies were performed on 11β and 7α-aminoethoxyphenyl estradiols to determine whether these compounds may interact with the same receptor subsite. Energy minimization and molecular dynamics studies lend support to this hypothesis.

Based on structure-activity relationship studies, a hypothetical model for the interaction of the ligand with the estrogen receptor binding site has been described to delineate the structural features necessary to initiate or to inhibit the estrogenic response. The two functional groups of estradiol, the 3-OH and 17-OH, play an important role for binding to the estrogen receptor. Furthermore, the spatial configuration of the internal hydrocarbon skeleton provides the major binding energy from hydrocarbon interactions.[1] Thus, Leclercq and Heuson[2] proposed a model in which the estrogens (and probably most antiestrogens) interact with the receptor with a narrow and deep cleft. Moreover, these authors proposed a hydrophobic region around the 7α-position of estradiol which enhances estrogenic activity which they call the "estrogenic region." Additionally, they describe a different hydrophobic region around the 11β-position of estradiol called the "antiestrogenic region". The resulting model suggests the presence of two hydrophobic regions corresponding to the 7α- and 11β-positions of estradiol.



1 2

Figure 1. Structure of 11β-dimethylaminoethoxyphenyl estradiol (**1**) and 7α-dimethylaminoethoxyphenyl estradiol (**2**).

Recent studies in our laboratory on the synthesis of 7α- and 11β-dimethylaminoethoxyestradiol[3,4] gave compounds which had essentially similar binding affinity to the receptors. Studies by workers from Roussel-Uclaf showed that 11β-aminoethoxyphenyl estradiol (**1**) and 7α-aminoethoxyphenyl estradiol (**2**) (Figure 1) were found to have high and similar binding affinities to the estrogen receptor as well as having similar estrogenic and antiestrogenic activities to 4-hydroxytamoxifen.[5-7] The above studies along with previous studies by Zeelen and Bergink[1] and Raynaud[8] showed that substitution with the same hydrophobic groups at either the 7α- or 11β-positions leads to compounds with essentially the same binding affinities, suggesting that these analogs may interact with the same hydrophobic region on the receptor. Indeed, if one rotates **2** by 180°along the 3-OH, 17-OH axis, the 7α-substituent will be at almost the same position as the 11β-substituent in compound **1**. Table 1 shows the relative binding affinities for several 7α- and 11β-substituted estradiol.

Table 1. Relative binding affinity of 7α- and 11β-substituted estradiols.

Substitutent	RBA*	Reference
H	100	(1)
7α-CH_3	104	(1)
11β-CH_3	65	(1)
7α-C_2H_5	85	(16)
11β-C_2H_5	78	(1)
7α-CH_2OCH_3	28	(1)
11β-CH_2OCH_3	37	(1)
7α-CH_2-Ph	90	(16)
11β-CH_2Ph	77	(16)
7α-Ph-4'-OH	49	(17)
11β-Ph-4'-OH	40	(17)
7α-OCH_2CH_2-$N(CH_3)_2$	2.1	(16)
11β-OCH_2CH_2-$N(CH_3)_2$	1.6	(4)
7α-Ph-4'-OCH_2CH_2-$N(CH_3)_2$	86	(17)
11β-Ph-4'-OCH_2CH_2-$N(CH_3)_2$	130	(17)
7α-CH_3,11β–OCH_3	3	(15)

* RBA's were collected by different workers at different times. Studies in our laboratory were obtained using rat uterine issue as described in Ref 4.

In an effort to further substantiate our understanding of the interaction between estrogens and antiestrogens with the estrogen receptor, molecular modeling studies of compounds **1** and **2** were carried out[9] and the results are the subject of this communication.

Carbon-skeletons of both **1** and **2** were found rather rigid during the molecular dynamics study, with only the phenoxyethylamino residues showing some mobility. A superposition view of the lowest conformers of **1** and **2** (absolute minima) is shown in Figure 2. The interdistances between the phenolic oxygens, ether oxygens, alcoholic oxygens and nitrogens were found to be 0.49 Å, 1.89 Å, 1.87 Å and 1.32 Å, respectively. According to the molecular dynamics studies performed here, it appears that **1** and **2** explore the same volume at physiological temperatures as the dynamics provide conformers where the side chains are confined within a distance of less than 3 Å allowing for a potential common site for **1** and **2** to bind in a deep and narrow cleft of the estrogen receptor. Recent studies on a series of tamoxifen analogs led Robertson *et al*[13] to conclude that the optimal interactions of the side chain with the receptor are unlikely to be ionic but rather hydrogen bonding between the side chain and amino acids of the receptor. It is noteworthy, however, that calculations for both protonated and nonprotonated **1** and **2** were found to be quite similar and could support this hypothesis.[9]

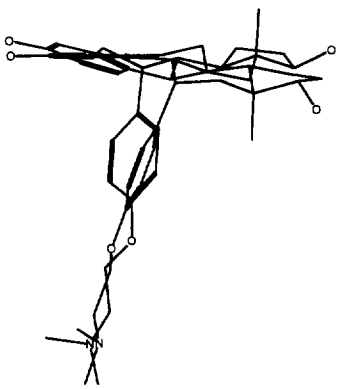


Figure 2. Superposition of **1** and **2**

In conclusion, the results obtained from previous biological studies,[1-8] as well as those reported in this investigation, suggest that the 7α- and 11β-substituents occupy the same hydrophobic pocket. Indeed, we propose that the estrogen receptor has only one hydrophobic pocket corresponding to either the 7α- or 11β-region of estradiol which can accomodate the side chains for estrogenic and antiestrogenic compounds in estradiol and triphenylethylenes. These results are supported by the recent studies of Wakeling[14] and Teutsch[6,7] on the 7α- and 11β-substituted estradiols, respectively. Furthermore, support for this model is obtained from studies by Raynaud *et al*[15] who showed that the combined effect of both 7α- and 11β-substituents in estradiol leads to compounds that have poor affinity to the estrogen receptor. Current studies are underway to synthesize several 7α-,11β-disubstituted estradiol analogs to test this hypothesis.

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9. The coordinates of **1** and **2** were intially generated using Chem 3D Plus (3.0 version, Cambridge Scientific Computing, Cambridge, MA) and energy-minimized by Allinger's MM2 force field calculational method as implmented in the 3.0 version.[10-12] The resulting structures of **1** and **2** provided two sets of cartesian coordinates which were transferred to Mopac 5.0, a molecular orbital software package, and were again energy-minimized. The final energies were 40.2 and 39.4 kcal/mol for **1** and **2**, respectively. The low-energy conformers were then studied in the molecular dynamics module of Chem 3D plus and were not modified. Dielectric constant was 80. Molecular dynamics studies were performed using a target temperature of 320°K along with an evolution of 30 psec for amine bases and 45 psec for the protonated species, by dynamic steps of 1 fsec. The heating/cooling rate was 3 kcal/mol/atom/psec. Potential energy figures were collected every psec during the periods of thermal equilibrium within a range of ±20°K of the target temperature and averaged to provide the final value of potential energy. The potential energies obtained were 113.6 ±1.4 and 115.1 ± 0.6 kcal/mol for non-protonated **1** and **2**, respectively; and 109.4 ± 1.2 and 111.9 ± 0.7 kcal/mol for the corresponding protonated species, respectively. This increment of energy relative to the energy-minimized structure can be easily found in the contribution of local hydrophobic forces. To delineate the conformational space explored by the side-chains, the conformers obtained after dynamics were energy-minimized. The lowest energy conformers of **1** and **2** were superimposed to provide Figure 2. The steric energies obtained were 38.2 and 39.7 kcal/mol for the free bases, and 33.7 and 36.5 kcal/mol for the protonated species, respectively. Conformations where the T shapes of the side-chains were distorted were also found, however, in these cases, an increase of 20 kcal/mol was observed in the potential energies.
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18. Graphic was obtained by rotating **2** by 180° along the 3-OH, 17-OH axis, followed by overlapping carbons at 2,3,4,17 in **1** over carbons 4,3,2,17 in **2**, respectively.

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