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Molecular simulation of interaction between estrogen receptor and selective estrogen receptor modulators

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KEY WORDS estrogen receptors; molecular models; structure-activity relationship; molecular conformation

ABSTRACT

AIM: To study the mechanism of interaction between a series of potent racemic selective estrogen receptor modulators (SERM) and estrogen receptors (ER). **METHODS**: Active conformations of these conformationally restricted raloxifene analogues in binding pocket were determined by molecular mechanics. The interactive energies between ligand and receptor were calculated by docking program. **RESULTS**: Both *R* and *S* configurations of these SERM were accommodated by the binding pocket of ER. The hydroxy group of compounds forms hydrogen bonds with amino acid residues of ER and the phenolic group mimics the A-ring of estradiol. The most potential compounds were those with two hydroxy groups and accommodated by binding pocket in *S* configuration with phenolic group at C(16) imitating A-ring of estradiol. **CONCLUSION**: Chiral center conferred little effect on the binding affinity of these conformationally restricted raloxifene analogues. The hydroxy group(s) play(s) a critical role to the orientation of compounds in active pocket of ER and the binding between ligand and receptor.

INTRODUCTION

In females, estrogens have beneficial effects on the skeletal, cardiovascular, and central nervous systems. Most of these estrogenic responses are mediated by estrogen receptors (ER) which are members of the nuclear receptor superfamily of ligand-dependent transcription factors. Selective estrogen receptor modulators (SERM) such as tamoxifene[1] and raloxifene (Ral)[2] (Fig 1) have been studied to exhibit potential effects in reducing a patient's risk of breast carcinoma and preventing osteoporosis. Although the intriguing biology of estrogen in its diverse target cells is determined by many factors[3] such as the ER subtype involved, the hormone-responsive gene promoter, coactivators and corepressors, the structure of ligands, and their interaction with ER function as the basement of transcriptional activities.

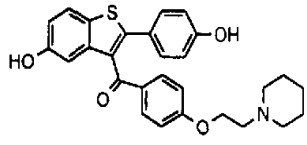

Fig 1. The structure of raloxifene.

It is well documented by X-ray crystallography[4] that with its rigid scaffold, estrogen binds to ER binding pocket with a definite orientation, the 3-hydroxy group on the A-ring forming hydrogen bonds to Glu 353, Arg 394 and a water molecule (Fig 2). This paper is to verify the significance of the presence of hydroxy group(s) in a

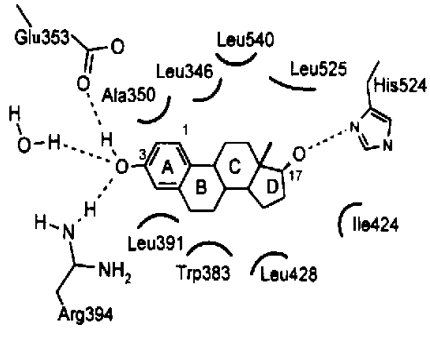

Fig 2. Schematic representation of the interaction made by estradiol within binding cavity. Residues that interact with the ligand are shown in the mode of 2D-projection. Those that make direct hydrogen bonds are depicted in broken lines between the interacting atoms.

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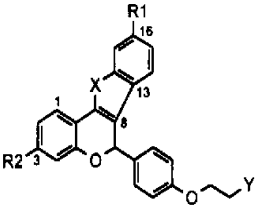
series of conformationally restricted Ral analogues (Tab 1) for the complex activation. These ER modulators are reported to be potent both in ER binding and inhibition of MCF-7 cell proliferation[5]. However, the orientation of these compounds in binding pocket can not be determined since they are racemic and the crystal structures for ER-ligand complex are not available. Herein by means of molecular simulation, the orientations and binding modes of racemic Ral analogues in the ER binding pocket are investigated, and the role of hydroxy group(s) for the binding and disposition of ligands is explored.

METHODS

All modeling work was carried out with the SYBYL6.5 software package, run on Silicon Graphic Iris O2 workstation with default setting values except specially stated. Because the raloxifene analogues are racemates and have a hydroxy group at C-3 or C-16 or two groups at both positions, each phenolic ring in principle could correspond to A-ring of estradiol. Thus four orientations could be adopted (Fig 3) in the binding pocket of ER, two of them with *R* form, otherswith *S* form. First the

Tab 1. The structure and docking energy of 12 compounds.



	X	Y	R_1	R_2	ER[1] RBA	$\Delta G^{2)}$ *R*-C(3)	$\Delta G^{2)}$ *S*-C(3)	$\Delta G^{2)}$ *R*-C(16)	$\Delta G^{2)}$ *S*-C(16)
1	O	-N⬡	OH	OH	0.07	-70.2	27.0	1.8	-73.4
2	S	-N⬡	OH	OH	0.23	-67.3	64.0	-71.5	-92.0
3	S	-N⬡	H	OH	0.03	-57.9	78.7	63.7	-43.9
4	S	-N⬡	OH	H	0.04	-42.4	107.6	-60.7	-78.5
5	S	-N⬡	H	H	0.01	-31.2	-33.9	-22.8	-39.2
6	S	-N⬡	OMe	OMe	0.01	105.6	53.4	7.9	7.4
7	S	NMe_2	OH	OH	0.16	-71.9	-54.6	-69.9	-72.7
8	S	NEt_2	OH	OH	0.29	-70.4	-59.4	-77.8	-88.6
9	S	-N⬡	OH	OH	0.21	-72.6	-52.6	-40.9	-83.5
10	S	-N⬡O	OH	OH	0.28	-70.2	30.2	-27.6	-83.2
11	S	-N⬡	OH	OH	0.28	-68.5	30.2	-28.1	-82.3
12	-C=C-	-N⬡	OH	OH	0.22	-61.7	6.2	188.2	-74.6

[1)]RBA=relative binding affinity by competition with [^{3}H]-17β-estradiol. [2)]Docking energy (kcal·mol^{-1}).

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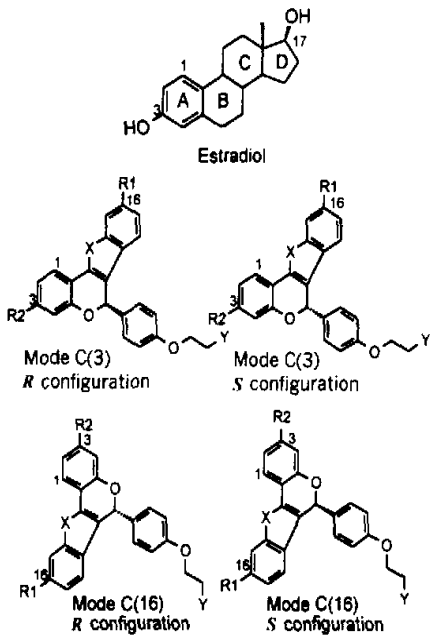

Fig 3. Potential binding pattern relative to estradiol.

starting conformations of compounds were generated by simulated annealing, among the resulting lowest-energy conformers those having best superimposition with Ral in Ral-ERα complex crystal structure were used for the simulation studies. Each compound in four orientations was prepositioned to the binding pocket, then Ral was extracted and all amino acid residues in ERα were defined as an aggregate. The conformations of compounds were optimized using minimization and keeping the aggregate static. After convergence terminated, the interactive energy between each compound and ERα was determined by docking program in SYBYL6.5 (Tab 1). In the whole process, MMFF94 force field was selected and MMFF94 charge was calculated.

RESULTS AND DISCUSSION

As shown in Tab 1, most of the investigated molecules contain two phenolic groups. Comparing the docking results of compounds, it is convinced that one specific orientation in each kind of configuration possesses a low interactive energy and high affinity with ER, this indicates configurations may have little effect on the activity of the ligands and enantiomers may not reduce binding force. The results suggest that for *S*-form the phenolic group at C(16) preferentially locates at the position of ER binding site, where A-ring of estradiol occupies, since the interactive energy in this way is much lower than that in *S*-C(3) form. However, the data of Tab 1 can not determine the orientation of *R* configurations. Comparing energies of the four orientations [*S*-C(3), *S*-C(16), *R*-C(3), *R*-C(16)], most of *S*-C(16) compounds possess prevailed binding pattern, as documented from the statistic parameters by regression analyses of energies *vs* lgRBA in Tab 2.

Tab 2. The results of regression analysis of energies *vs* lgRBA for four modes.

Orientations	R^{2} [1]	SE[2]	*F* test
R-C(3)	0.499	37.2	9.98
S-C(3)	0.12	59.4	1.37
R-C(16)	0.012	77.9	0.12
S-C(16)	0.738	15.3	28.16

[1] R^2 stands for the relative coefficient of the linear regression analysis.
[2] SE represents the standard error of the linear regression analysis.

It is well known that the alteration in free energy during the interaction between ligand and receptor (ΔG) is correlated with the binding constant (K_i) as expressed

Tab 3. The calculated and predicted values of free energy change as well as the residuals from Eq 1.

No	Calculated value	Predicted value	Residual	No	Calculated value	Predicted value	Residual
1	73.4	62.0	11.4	7	72.7	77.8	-5.1
2	92.0	84.7	7.3	8	88.6	89.1	-0.5
3	43.9	45.9	-2.0	9	83.5	82.9	0.4
4	78.5	51.4	27.1	10	83.2	88.4	-5.2
5	39.2	25.0	14.2	11	82.3	88.4	-6.1
6	-7.4	25.0	-32.4	12	74.6	83.8	-9.2

in the equation: $\Delta G = -2.303RT \lg K_i$. Theoretically and ideally the free energies of a series of ligands are linearly related to the binding constants. Equation 1 and Fig 4 account for the significant correlation. However, the calculation was carried out in a vacuum condition, the entropy penalty and solvent effect, which also contribute to the receptor-ligand interaction, being ignored andkept constant. Nonetheless the simplified operation explains the structure-activity relationship to an extent.

$$\Delta G = -43.8(\pm 8.3)\ \lg RBA - 112.7(\pm 9.7) \quad (1)$$
$$(R^2 = 0.738,\ SE = 15.3,\ F = 28.16)$$

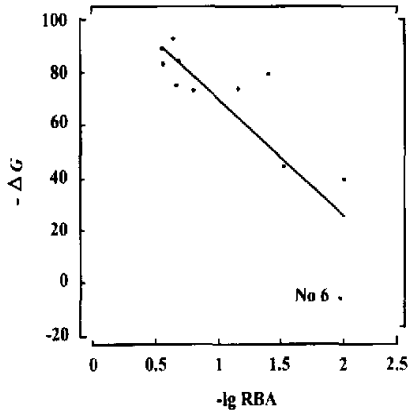

Fig 4. The correlation of relative binding affinity (RBA) and the docking free energy in terms of *S*-C(16) mode.

The low interaction energies and significant correlation of orientation according to *S*-C(16) mode take the location of the hydroxy group at C(16) for granted, which conveys an important effect on the binding, indicating that the role of C(16)-OH corresponds to that of the 3-OH of estradiol in the binding.

Scrutinizing the residuals calculated from equation 1, it was found that compound 6 with two methoxy groups possesses the largest residual, which can be explained by the steric hindrance of bulky methoxy groups in the molecular modeling, because of no change in ER conformation during the dock operation.

The strong binding affinity of the compounds with two hydroxy groups at C(3) and C(16) was able to be explained by alternative orientations during the binding process, which was documented by the high interaction energies in terms of *S*-C(16) and *R*-C(3) dispositions.

The similar binding potency of No 3 and 4 may be explained that while compound 4 orients in mode C(16), compound 3 in mode C(3), the respective hydroxy group was in a position to mimic the A-ring of estradiol, the 3-hydroxy group of which is believed to make a major contribution to ligand-binding affinity[16].

A pair of enantiomers with optional accommodation to ER are also dependent on the character of the binding pocket, where the side chain with basic nitrogen atom enters. Obviously, C(3) of *R*-configuration and C(16) of *S*-form are preferential to bind the active pocket where A-ring of estrogen is occupied and simultaneously the side chain of the congeners optimally contacts the surface of amino acid residues in binding pocket. Fig 5 illustrates the binding modes of compound 3 and 4 in the binding pocket.

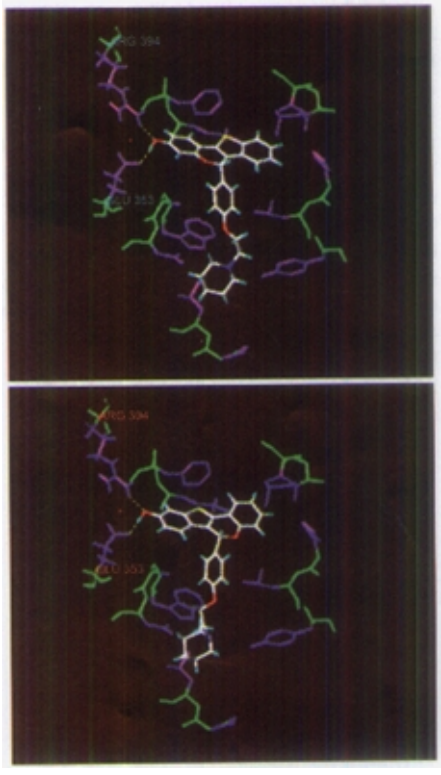

Fig 5. The binding modes of compound 3 (upper) and 4 (below) according to *R*-C(3) and *S*-C(16) respectively. The hydroxy group at C(3) of compound 3 and at C(16) of compound 4 both forms hydrogen bonds with Glu 353 and Arg 394, depicted in broken yellow line. The side chain in *R* configuration of compound 3 and *S* form of compound 4 locates in the same pocket and both side chains exhibit good interactions with the amino acid residues. This is one of the reasons that compound 3 orients in *R*-C(3) and compound 4 in *S*-C(16) with the best free energies.

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In summary, racemization may not lead to the decrease in activity of the conformationally restricted Ral analogues, since both *R*- and *S*-forms can be accommodated by the binding pocket. The significance of two hydroxy groups of the studied compounds lies not only in the reduction of total free energy of complex system but also in the increase of possibility that both phenolic groups can mimic A-ring of estrodiol.

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雌激素受体和选择性雌激素受体调节剂相互作用的分子模拟

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关键词 雌激素受体; 分子模型; 构效关系; 分子构象

目的: 研究一类高活性消旋化的雌激素受体调节剂与其受体间的相互作用机制. **方法**: 分子力学优化的方法获得这类刚性的 raloxifene 类似物的活性构象, 受体与配基间的相互作用能用分子对接程序计算. **结果**: 化合物的 *R* 和 *S* 构型均可进入雌激素受体的结合腔. 其羟基直接与受体氨基酸形成氢键, 其酚环相当于雌激素的 A 环. 活性最好的配基带有两个羟基, 并且模拟雌激素 A 环为含 16 位羟基的酚环的 *S* 构型化合物. **结论**: 消旋化对该类化合物的活性影响不大. 羟基对于化合物在活性口袋中的取向及与受体的结合有着重要意义.

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