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Molecular binding modes of diarylheptanoids from *Curcuma comosa* on the ER-β receptor

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Abstract

Curcuma comosa Roxb. is a medicinal plant that belongs to the Zingiberaceae family. The plant has been used in traditional Thai medicine for the treatment of postpartum uterine bleeding. The major compounds found in this plant are diarylheptanoids, which are reported to have estrogenic activity. The aim of the study was to understand the three-dimensional (3-D) aspects of diarylheptanoids from *C. comosa* on ER-β estrogenic receptor at the molecular level. The binding conformations of diarylheptanoid analogues with ER-β receptor were obtained by the AutoDock 4.2 program using the Lamarckian genetic algorithm (LGA) in conjunction with an empirical force field to calculate the complex binding free energy. From the analysis of docking results, diarylheptanoid analogues with higher activity have a hydroxyl group in ring C which can be modified by using the isosteres groups while the other phenyl ring have less polarity to fit into the hydrophobic pocket of the ER-β receptor. In addition, the heptyl chain needs some flexibility to allow the phenyl ring to adjust suitably into the receptor-binding pocket. Molecular modeling using AutoDock 4.2 was effectively applied to understand the binding conformation of diarylheptanoid analogues with ER-β receptor. Information of three-dimensional conformation can be applied in rational drug design for further searching the new highly activity ER-β receptor agonists.

Key Words: AutoDock, *Curcuma comosa*, Diarylheptanoids, ER-β receptor, Molecular modeling

Introduction

Curcuma comosa Roxb. (Zingiberaceae) is a medicinal plant, which has been used in traditional Thai medicine for the treatment of postpartum uterine bleeding. The hexane extract of the plant rhizome exhibits estrogenic-like activities such as an increase in uterine weight and cornification of the vaginal epithelium [1]. The diarylheptanoids were isolated from *C. comosa* and showed estrogenic-like activities [2]. This is a new class of compounds that have different three-dimension (3-D) structure when compared to 17β–estradiol or genistein. Estrogenic-like compounds or phytoestrogens are secondary metabolites that exhibit biological activities by binding to estrogen receptors (ER). This receptor belongs to the nuclear receptor superfamily of ligand-inducible transcription factors [3] that can be separated into two forms, ER-α and ER-β [4]. These two estrogen receptors have similar overall structures, displaying a high degree of amino acid conservation in the central DNA-binding domain at 97 % and moderate conservation in the ligand-binding domain at 60 %, respectively [5]. Therefore,

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ER-α and ER-β exhibit similar binding affinities with the same DNA response unit; they also demonstrate binding affinity with an array of endogenous, synthetic, and natural occurring estrogen *in vitro* [6]. The ER-α expression pattern is found mainly in the breast, uterus, cervix, vagina and several other target organs. Prior research has verified that estrogen can increase the proliferation rate of breast cancer cells by binding to the ER-α receptor [7, 8]. The ER-β receptor is found in the ovary, prostate, testis, spleen, lung, hypothalamus, and thymus [9]. Several more studies have indicated that the majority of ER-positive tumors contain both subtypes, but that some tumors contain only ER-β receptor and may have distinct clinical behaviors and responses [10]. *In vitro* studies have shown that the actions of ER-α and ER-β receptors are quite different at the level of gene expression in response to estrogen and anti-estrogen [11, 12].

In this research, molecular docking studies of active diarylheptanoids isolated from *C. comosa* [13] were achieved using AutoDock version 4.2 [14, 15].The binding modes, the orientation of ligand relative to the receptor or the conformation of the ligand and receptor when bound together, of diarylheptanoids were explored. The 3-D binding patterns were evaluated to understand how diarylheptanoids interact with ER-β receptor in order to explain their estrogenic activity. In addition, proposed isosteres derivatives were also studied. This information can be used in rational drug design to obtain high potential new lead compounds for ER-β receptor.

Materials and Methods

Structure preparation and modeling software. The ER-β receptor structure was obtained from the protein data bank (PDB ID: 2YJD) [16]. The isolated diarylheptanoid

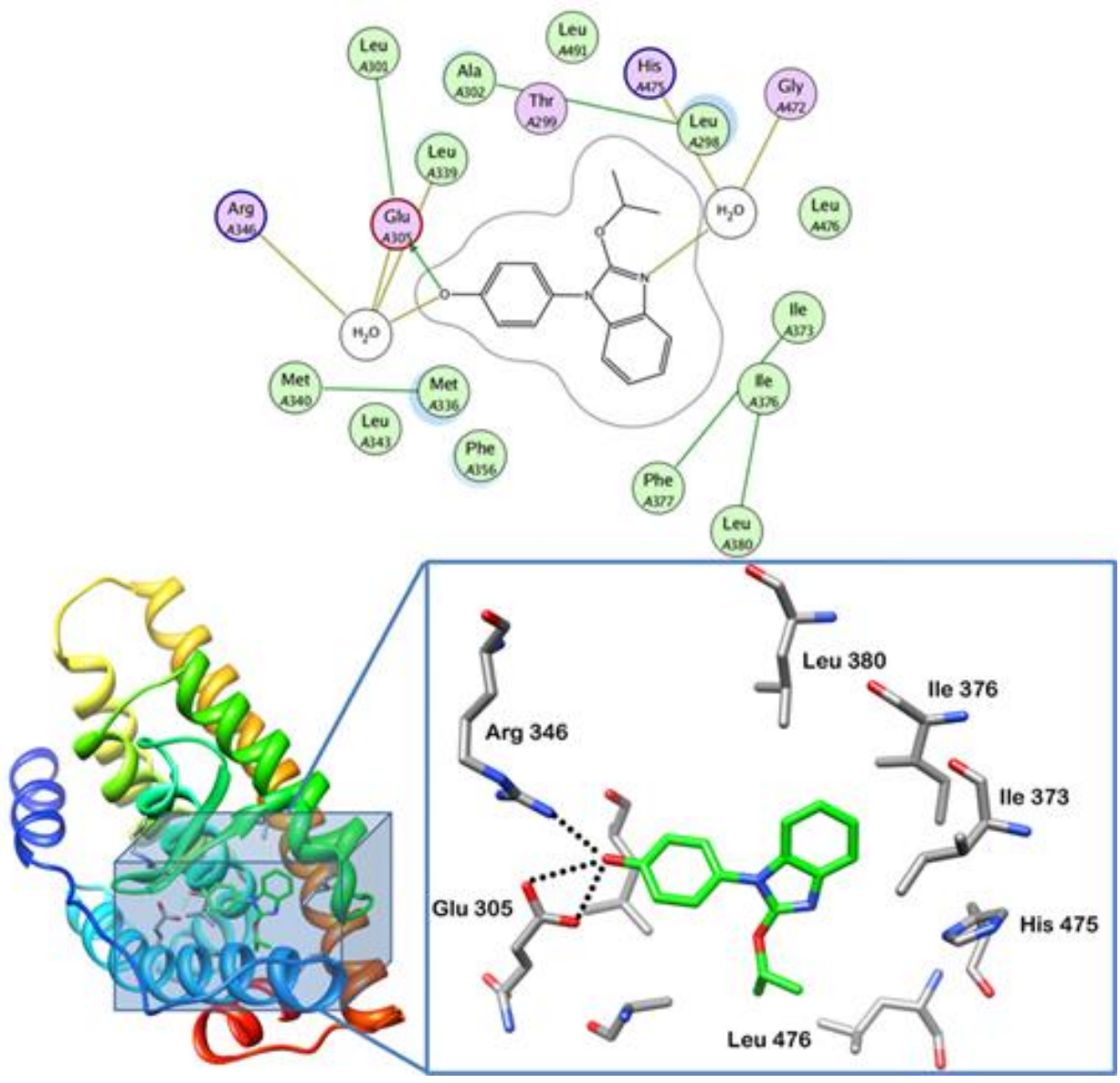


Figure 1 Binding conformation of the 2YJD ligand in the ER-β crystal structure

structures and estrogenic-activity data were obtained from the publication of Suksamrarn A. and a colleague [13]. The data contained the relative transcription activation activity of the diaryheptanoids on ER-β receptor and Bcl-xL genes in the HeLa cell line. Genistein and 17β–estradiol were used as the positive control compounds.

Computational tools were used to construct the ligands. The ISIS Draw program was employed to draw the 2D structures of the ligands, which were then optimised by the Gaussian 09 program using DFT (density functional theory) with the B3LYP functional at 6-31g (d) basis set [17]. The DS Visualizer and Chimera 1.6 programs [18] were used to perform all figures.

Docking studies. The ER-β crystal structure (PDB ID: 2YJD) was used as a starting complex structure and, in particular, hydrogens were added to the protein structure. Binding conformation of the diarylheptanoid analogues with the ER-β receptor were obtained by the AutoDock 4.2 program using the Lamarckian genetic algorithm (LGA) in conjunction with an empirical force field to calculate the free energy of the binding. Kollman-all-atom charges were assigned to the receptor whereas Gasteiger-Hückel charges were assigned to all the ligands. For the AutoDock runs, all the defaults were used except during longer runs, which were set at 100 runs with 60 X 60 X 60 $Å^3$ dimensions of 0.375, Å grid spacing covered the binding site of the ligands. For the dockings, the receptor was rigid and the ligands were flexible in the grid box within the centre of the receptor. Two water molecules were kept in the pocket to demonstrate the important role of hydrogen bonding. The conditions were applied throughout the docking simulations which reproduced co-crystals bound to the ER-β receptor that had a root mean square deviation (RMSD) value of 0.29 Å. The estimated free binding energy using these docking conditions for the 2YJD ligand binding to the ER-β receptor was -9.56 kcal/mol.

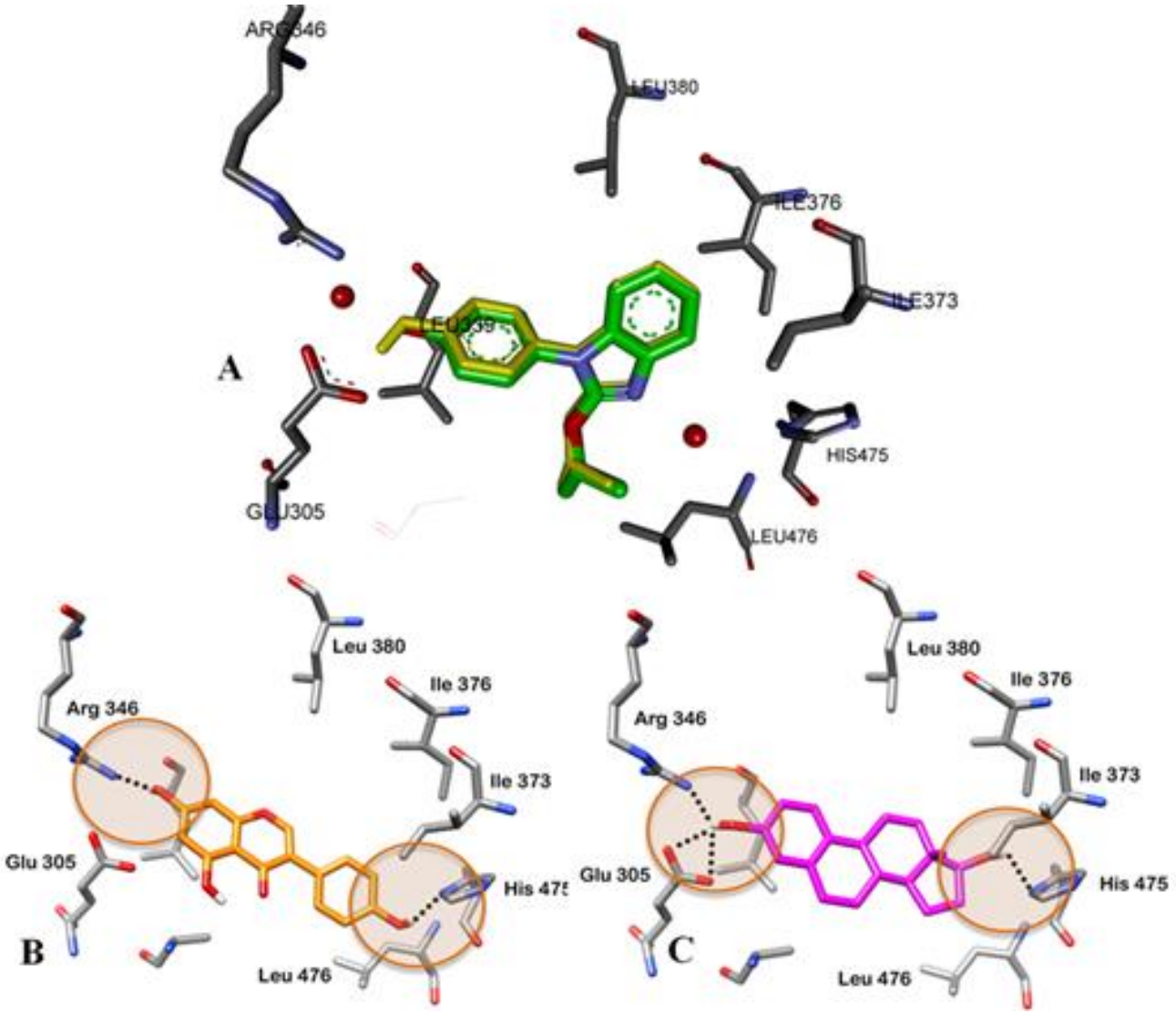


Figure 2 (A) Comparison of the 2YJD ligand re-docking and docking with the overlay structure (B) Genistein docking results with key residues (C) Estradiol docking results with key residues

Finally, the docked complexes of inhibitor- receptor were selected according to the criteria of the binding energy and the geometrical matching quality. All amino acid residues within a 5.0 Å radius of the ligand atoms were considered and analysed for their activity contributions

Results and Discussion

The binding orientation of the 2YJD ligand, [4-(2-propan-2-yloxybenzimidazol-1-yl)phenol] [16], was used as a reference structure to show the key interactions within the binding pocket of the ER-β receptor, as shown in Figure 1. Water molecules are routinely included in molecular docking methods and protocols because of their important role in mediating ligand protein interactions [19]. The two water molecules, which co-crystals bound to the ER-β receptor, were kept in the pocket to demonstrate the crucial role hydrogen bonding. For genistein and 17β-estradiol, there was hydrogen bonding between the hydroxyl groups of each compound and His475 of the receptor, while the 2YJD ligand showed no interaction at this part. This means that the 2YJD ligand interacts with the ER-β receptor in a different orientation to the classical genistein and the 17-estradiol orientations. The docking results from genistein, 2YJD ligand, and 17β-estradiol revealed that the main requirements of the interactions between the receptor and the ligand came from the phenolic ring and the hydrophilic amino acid residues (Glu305, Arg346) in the ER-β receptor, as shown in figure 2.

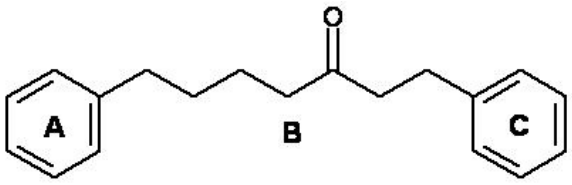


Figure 3 The core structure of a standard ligand from *Curcuma comosa* which composes of ring A, heptyl chain (B), and ring C

The results of the free energy binding from the genistein, 2YJD ligands, 17β-estradiol were shown in Table 1. For the active diarylheptanoids isolated from *C. comosa* [13], four high activity to ER-β receptor which were compound 2, 10, 15, and 16 were selected for this study and the free energy binding were shown in Table 2. The orientations of all the ligands were compared with the 2YJD ligands from the crystal structure. After evaluating the binding conformations, it was found that the diarylheptanoid structure was composed of three main parts, as shown in figure 3, it was also noted that the

Table 1 Binding energies and activities of reference compounds form AutoDock studies

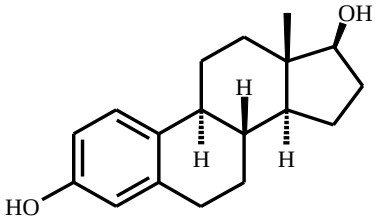
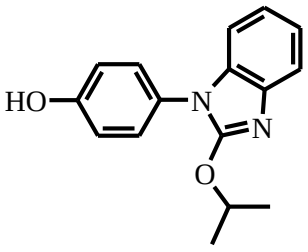
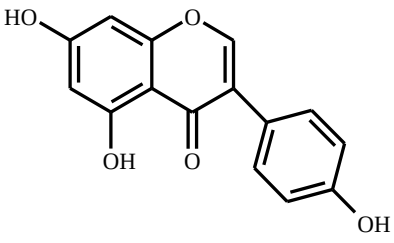
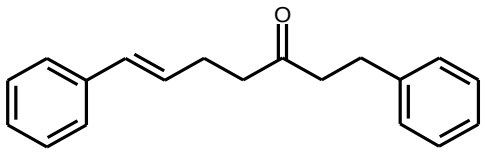
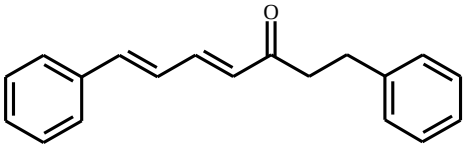
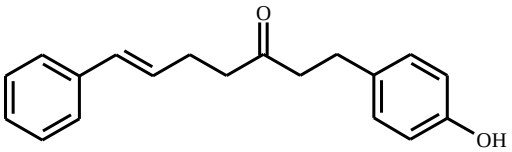
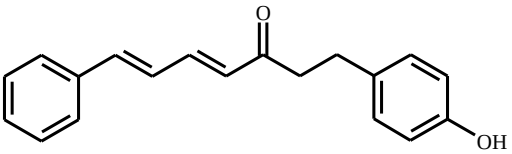
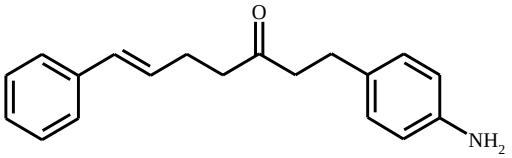
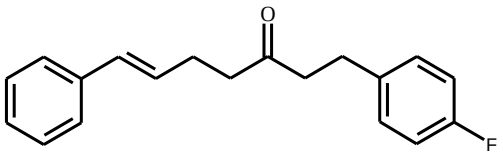
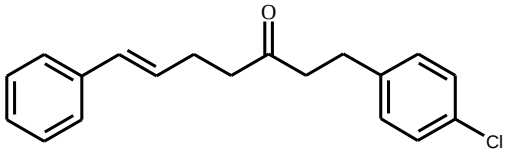
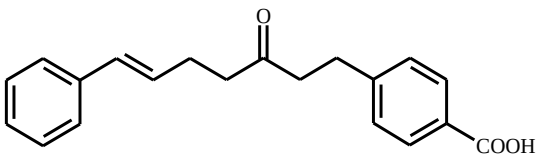
Compounds	2D structures	ER-β activity[13]	AutoDock kcal/mol
17β –estradiol		100	-9.29
2YJD ligand		-	-9.56
Genistein		75.51	-7.35

Table 2 Binding energies and activities of reference compounds form AutoDock studies

Compounds	2D structures	ER-β activity[13]	AutoDock kcal/mol
Comosa 2		85.45	-9.06
Comosa 10		33.72	-8.37
Comosa 15		90.56	-9.13
Comosa 16		87.75	-8.67
Comosa15-NH2		-	-9.17
Comosa15-F		-	-9.35
Comosa15-Cl		-	-9.36
Comosa15-COOH		-	-9.03

structure demonstrated different roles when binding with the ER-β receptor. The first part was the phenyl ring (A), which was placed into the hydrophobic pocket surrounded by isoleucine and leucine residues (Ile373, Ile376, Leu380), as shown in figures 4 and 5. The second part was the phenyl ring (C), which contained some polar functional groups that were suitable for making hydrogen bonds interact with hydrophilic residues such as Glu305 and Arg346, as shown in figures 4 and 5.

One of the possible reasons for compound 15 showing higher activity close to 17β-estradiol than the other diarylheptanoids in *C. comosa* is the hydrogen bonding interaction with the hydrophilic residues Glu305 and Arg346. The hydroxyl group on the phenyl ring can be substituted with isosteres groups such as F and Cl. The modeling results indicated that the fluorine derivative showed good binding energy with the ER-β receptor and slightly higher energy than the hydroxyl derivative. This must come from the high electronegativity of the halogen atoms that make the hydrogen bonding stronger than the hydroxyl group. Other substituents such as NH_2 and COOH were also used; however, the binding results showed no significant difference from the hydroxyl derivative.

Therefore, the poses of the high potency ligands (compound 15, compound 15-F, compound 15-Cl) could suggest that a phenyl ring with small and high electronegativity substituted groups is required. In skeleton B, flexibility is necessary and the unconjugated ketone (compound 2) showed higher activity and better binding energy than the conjugated ketone (compound 10). Furthermore, the unconjugated ketone (compound 15) with a hydroxyl group in ring C showed different ligand poses from the conjugated ketone (compound 16) by preferring to turn the ring C point to Arg346, while the compound 16 turned the ring C point to the other side of the ER-β binding pocket. When compound 15 and its derivative were overlaid with the 2YJD ligands, the aromatic rings laid over the aromatic rings of the 2YJD ligands, which indicate that chain lengths with flexibility are also the crucial points for adjusting into the binding site.

Conclusion

This study focuses on the three-dimension conformation of active diarylheptanoids because diarylheptanoids is a new class of estrogenic compounds

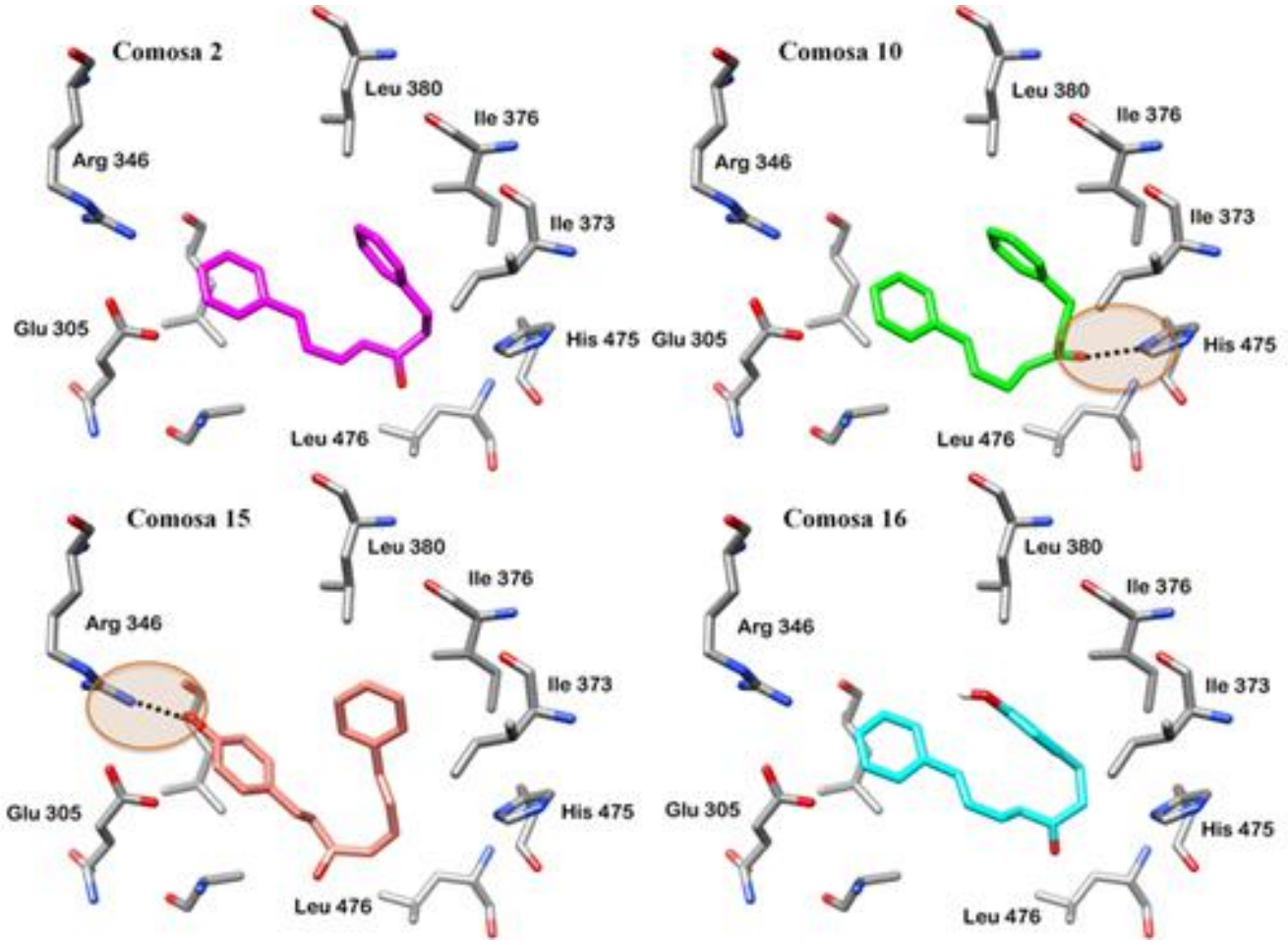


Figure 4 The binding conformation of compounds from *Curcuma comosa* in ER-β receptor

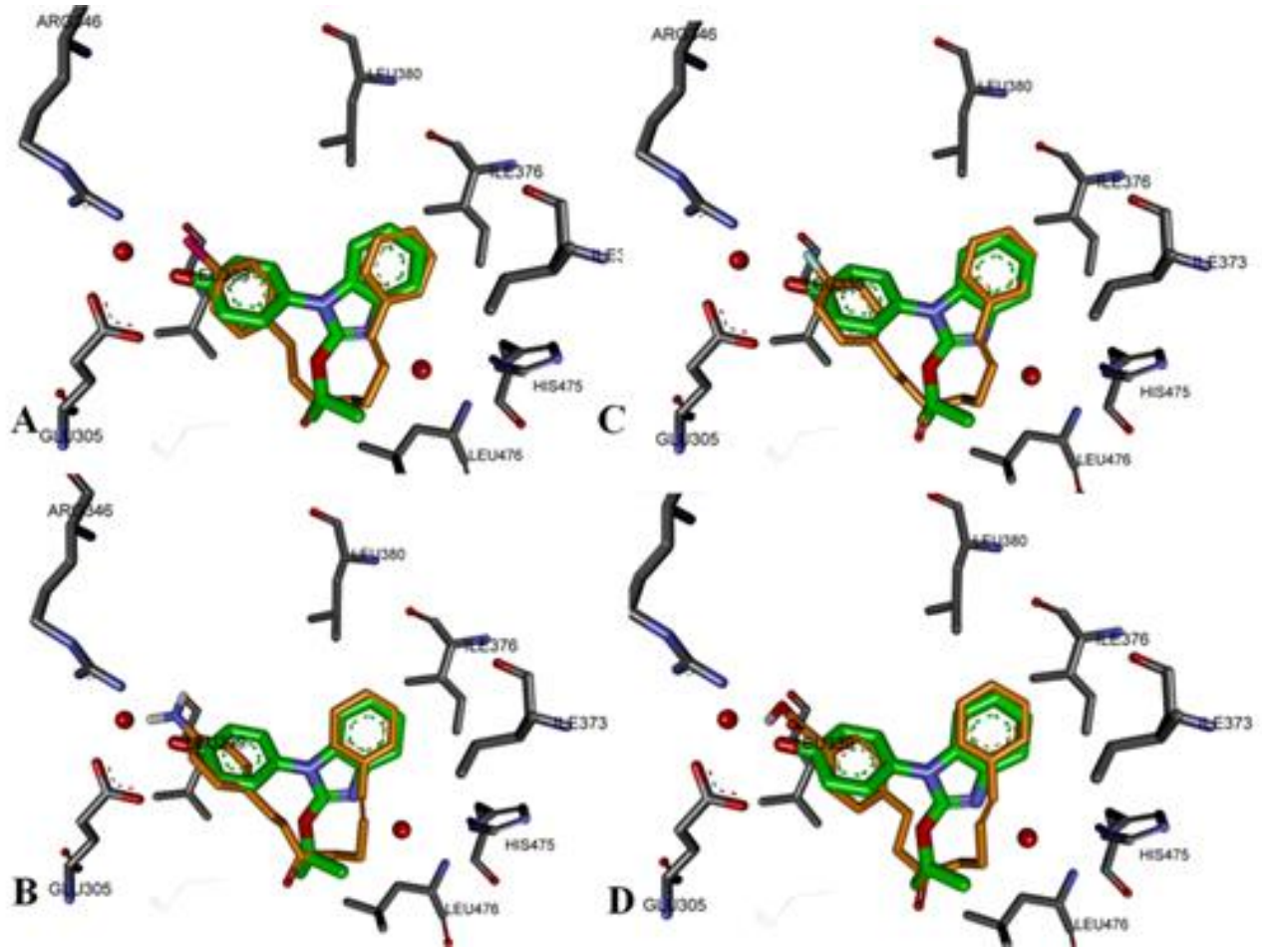


Figure 5 The binding conformation of comosa 15 compound derivatives (A) –Cl , (B)-NH_2 (C) –F (D) –COOH in ER-β receptor

that have different three-dimension structure when compared to 17β–estradiol or genistein. The docking results show that the greater activity diarylheptanoids come from the phenolic group of ring C, which can be modified into isosteres groups. The other phenyl ring prefers less polarity to fit into the hydrophobic pocket of the ER-β receptor. The heptyl chain needs some flexibility that allows the phenyl ring to fine-tune into the pocket. This information is suitable for extension the modification of the ligands such as new type of aryl rings, new tether connecting two aryl rings using rational drug design. The active diarylheptanoids are the promising lead compounds for ER-b (B) receptor research and development, as they, e.g. compound 2, are likely to selectively bind to ER-b (B) receptor which is up-regulated in estrogen responsive breast cancer cells (MCF-7) [20].

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