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Molecular Docking Studies of Glycyrrhizic Acid (GA), Glycyrrhetic Acid (GE) and Glabridin (GLA) with Estrogen Receptors (ERs)

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Treating post-menopausal symptoms with hormone replacement therapy is associated with various health risks. Alternatively, licorice root is often used to relief post-menopausal symptoms in traditional medicine. The bioactive componentss of licorice root, such as GA, GE and GLA, have been previously studied *in vitro* for their estrogenic effects. In this study, they were subjected to molecular docking study to further acknowledge their estrogenicity. GA and GE showed positive binding energy in the molecular docking study, suggesting that they could not bind nor activate ERs. Intermolecular interaction analysis identified multiple sites of unfavorable steric bumps, indicating pentacyclic structure and methyl side groups of GA and GE were detrimental to formation of energy favorable positions in ligand binding cavity of ERs. On the other hand, GLA could be docked into the ligand binding cavity of ERs, though with comparatively higher binding energy to that of 17â-estradiol (E_2). It was observed that the rotatable 1,3-benzenediol of GLA is essential for GLA-ERs binding. Furthermore, oxidation of the methyl side groups of GLA might improve the binding affinity. In conclusion, GLA, not GA and GE, is a partial ERs agonist and could be further modified to design novel semi-synthetic post-menopausal drugs.

Keywords: Molecular docking, Estrogen receptor (ER), GLA, Glycyrrhizic acid (GA), Glycyrrhetinic acid (GE).

Estrogen is important for developing and maintaining the normal female reproductive and sexual functions in women. These endocrinal effects are modulated via activation of estrogen receptors (ERs) and successive signaling pathways or gene transcriptional activities[1]. Post-menopausal women have reduced female sex hormones and thus often suffer from symptoms such as hot flashes, night sweats, osteoporosis, and so on. Hormone replacement therapy (HRT) is the standard treatment to control these post-menopausal symptoms and diseases for women[2]. Despite the beneficial effects, the use of endogenous estrogen and progesterone in HRT was associated with the increased risks of stroke, venous thrombosis, and ovarian cancer[3]. Hence, it is significant to continuously seek for alternative therapies for menopause symptoms and post-menopause conditions.

Glycyrrhiza glabra, commonly known as licorice, is a traditional herb found in subtropical regions and custom to treat several kinds of diseases, including menopausal symptoms[4-5]. The

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extracts from its root contain various types of active compounds that exert many medicinal benefits[6-8]. Among the active compounds found in licorice root extracts are GA, GE and GLA (Figure 1).

GA and GE had been reported to possess a wide range of biological effects, such as antioxidant, anti-inflammatory, anti-viral, anti-allergic, anti-hepatotoxic and anti-ulcer[9-11]. Literature review shows that GA and GE might not be estrogenic. For example, Hillerns *et al*. (2005) reported that GA and GE were unable to displace E_2 even at high concentrations (500 µM)[12]. In another previous study however, GA was reported to bind to estrogen receptors but at minimal level[13]. GLA is a phytoestrogen, and it shares several structural features with E_2. To be specific, both possess 3 phenanthrene shape fused rings and contain an aromatic ring substituted with hydroxyl group[14]. GLA was reported to exert estrogenic effects yet at a relatively lower level compared to that of E_2[15].

To date, there are no studies that reason how GLA showed lower estrogenic effects to ERs compared to E_2 even though they share several common features. There are also very little insights on GA and GE binding to ERs. Therefore, in this study, molecular docking studies were conducted to acknowledge the binding mode and intermolecular interactions of GLA, GA and GE with ERs as well as to compare with that of E_2 and subsequently associating the findings with ERs agonistic binding.

METHODOLOGY

Molecular docking and intermolecular analysis were driven by Dell Inspiron 3443 laptop computer with the following specifications: Windows 10 Pro, Intel ® Core ™ i7-5500U CPU at 2.40 GHz, 8 GB RAM, NVIDIA GeForce 840M graphics card.

Preparation of Macromolecules and Ligands

The crystallographic structure of ER-á and -â (PDB ID: 1ERE and 3OLS) were downloaded from RCSB Protein Data Bank (www.rcsb.org). Both PDB structures were bound with E_2 at respective ligand binding domain. The 2D structures of GA, GE and GLA (PubChem CID: 14982, 10114 and 124052 respectively) were downloaded from the PubChem Compound Database.

Using AutoDockTools (ADT) 1.5.6, the water molecules and E_2 were removed and the macromolecules (ERs) were converted to Autodock PDQBT format where Gasteiger charges were added to each atom, non-polar hydrogens merged followed by re-distribution of atomic partial charges and determination of atom types.

The ligands (E_2, GA, GE and GLA) were loaded to ADT 1.5.6 viewer, which then automatically computed Gasteiger charges and mapped the atom types. The root and torsion number were determined and subsequently the ligands were converted to Autodock PDBQT format.

Setting up Grid Parameter File and Docking Parameter File

Using ADT 1.5.6, the map types were set by selecting the respective E_2 from ER-á and -â as chosen ligand. The grid boxes were centered on E_2 and the grid box size was set to 40 points at x, y, and z dimensions. The respective coordinates of E_2 from ER-á and -â were noted and were used to setup the grid box location for subsequent molecular docking of GA, GE and GLA with ERs. The grid box sizes for GE and GLA were set identically to that of E_2 whereas the grid box for GA were set to 56 points at x, y, and z dimensions to accommodate the bigger ligand. The grid parameter files for respective ligands were generated and saved.

Next, ER-á and -â were set as rigid macromolecule while E_2, GLA, GA, and GE were set as ligands respectively. The docking search parameters were set to genetic algorithm with 100 number of runs and the rest left with default settings. The respective Lamarckian Genetic Algorithm docking parameter files for each ligands were generated.

Molecular Docking

Prior to molecular docking, AutoGrid 4 was run to calculate the interaction energies grid maps for respective ligand atom types. Autodock 4 was executed and molecular docking simulations were performed thrice for the respective ligands with ERs. The lowest binding energy complexes of the respective ligands with ERs were viewed and extracted using ADT 1.5.6. The binding mode and intermolecular interactions of GLA, GA and GE were then determined and compared to that of E_2 using Discovery Studio 4.1 Client and PoseView (Protein

Plus Server, Center for Bioinformatics, University of Hamburg).

RESULTS AND DISCUSSION

Molecular Docking

The outcome of molecular docking was analyzed and summarized in Table 1 and Figure 2-3. It was observed that E_2 could be docked into the ligand binding cavity of ERs. The triplicates of 100 run molecular docking of E_2 with ERs yielded identical conformations throughout (as shown in the histogram summary of Figure 2 and Table 1). The mean lowest binding energy of E_2 docked with ER-á and -â are -10.47 ± 0.00 kcal/mol and -10.920 ± 0.000 kcal/mol respectively. It was also determined that the root mean square deviation (RMSD; average distance between the atoms) of lowest binding energy conformations of molecular docked E_2 with ERs and the original crystallographic data

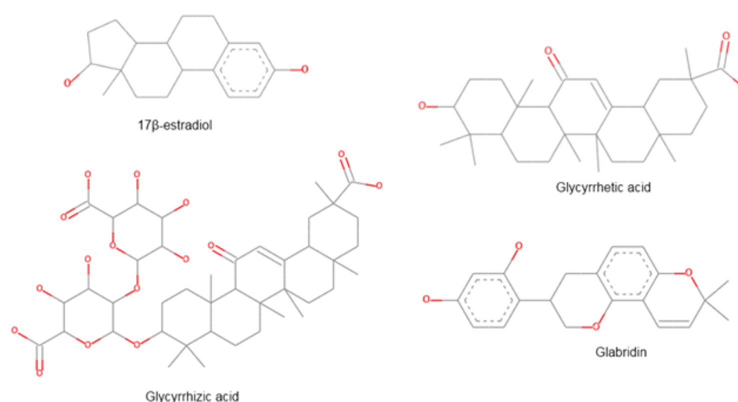

Fig. 1. Chemical structures of E_2, GA, GE and GLA

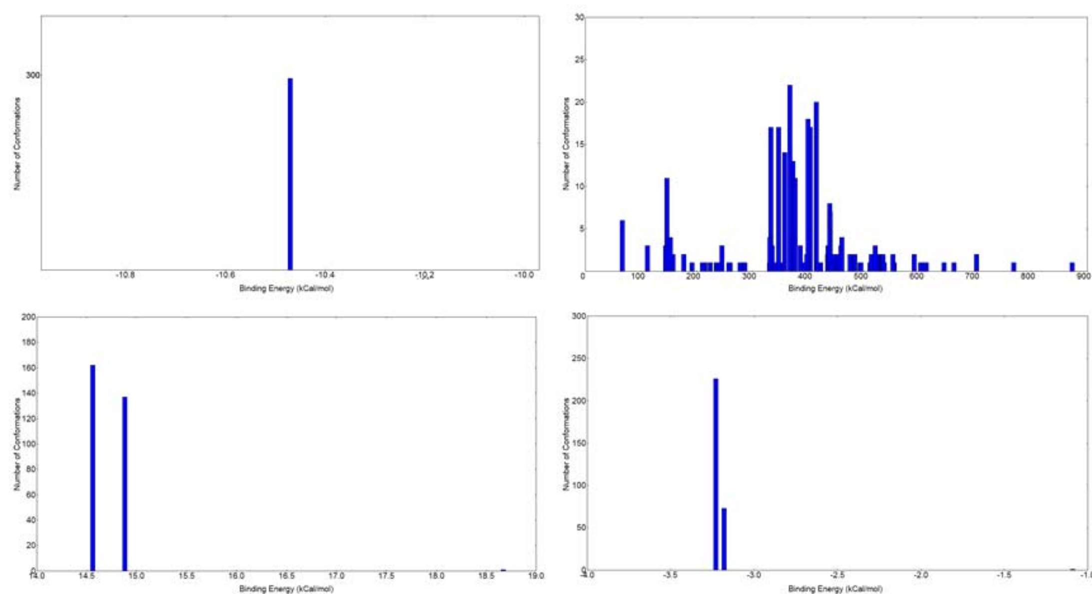

Fig. 2. Histogram plots showing clustering summary of E_2 (upper left), GA (upper right), GE (lower left) and GLA (lower right) molecular docked with ER-á at RMS tolerance = 2.0; n = 300

were 0.6297 Å and 0.3432 Å respectively. As the RMSD values were less than 2 Å, therefore the molecular dockings in this study were generally accepted as repeatable and reliable.

In this study, GA and GE were calculated to have mean lowest binding energy of 332.30 ± 1.2349 kcal/mol and 14.57 ± 0.008165 kcal/mol respectively in the molecular docking to ER-á. Similar trend was observed for that of ER-â, which the mean lowest binding energy were 330.550 ± 6.765 kcal/mol and 24.147 ± 0.015 kcal/mol respectively. These observations suggested that GA and GE could not bind to nor activate ERs, which corresponded to the findings by Nishihara *et al.* (2000) and Hillerns *et al*. (2005), and opposed to the results reported by Fujisawa *et al*. (2000)[12-13, 16].

On the other hand, GLA was able to be docked into the ligand binding cavity of ERs, but with relatively higher mean lowest binding energy (-3.21 ± 0.01633 kcal/mol and -3.083 ± 0.006 kcal/mol respectively). Simons *et al*. (2011) reported

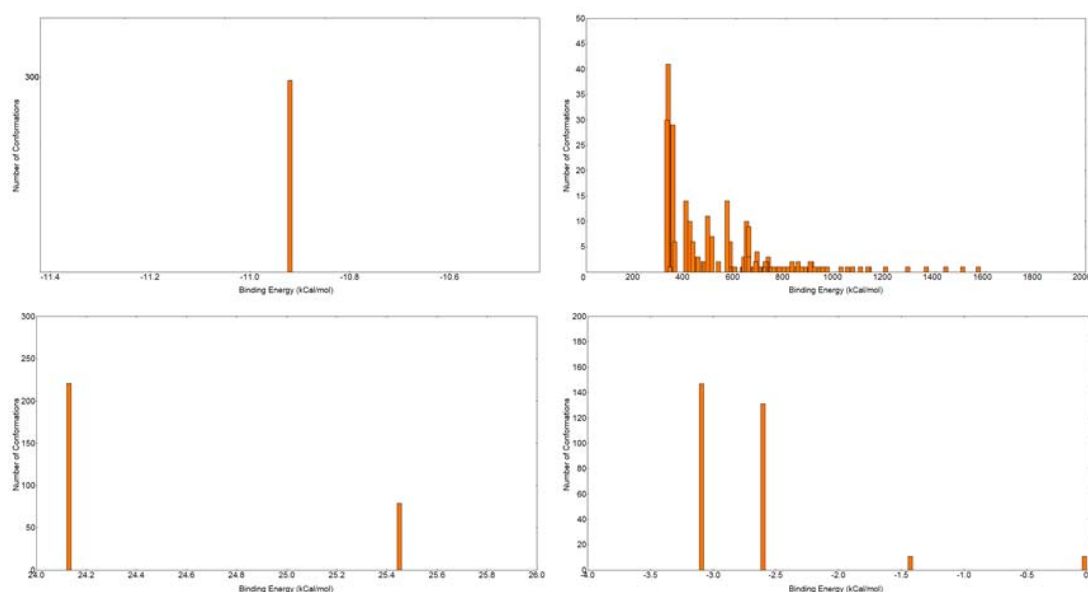

Fig. 3. Histogram plots showing clustering summary of E_2 (upper left), GA (upper right), GE (lower left) and GLA (lower right) molecular docked with ER-â at RMS tolerance = 2.0; n = 300

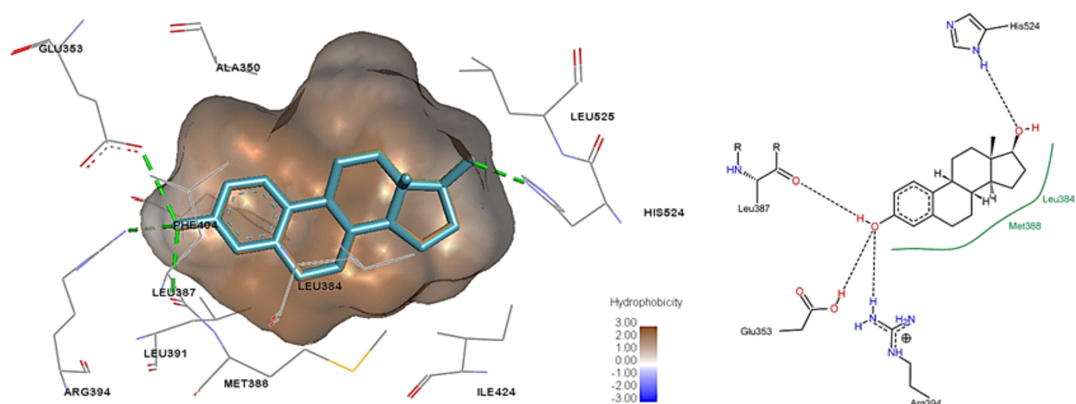

Fig. 4. Illustration of intermolecular interaction (left: 3D and right: 2D) between lowest binding energy conformations of E_2 in triplicates and ER-á. Hydrogen bonds are shown in green and black dotted lines; hydrophobic interactions are shown as surfaces and smooth contour lines

Fig. 5. Illustration of intermolecular interaction (left: 3D and right: 2D) between lowest binding energy conformations of E_2 in triplicates and ER-â. Hydrogen bonds are shown in green and black dotted lines; hydrophobic interactions are shown as surfaces and smooth contour lines

Fig. 6. Illustration of intermolecular interaction (left: 3D and right: 2D) between lowest binding energy conformations of GA in triplicates and ER-á. Hydrogen bonds are shown in green and black dotted lines; hydrophobic interactions are shown as surfaces and smooth contour lines; steric bumps are shown as red wire spheres

Fig. 7. Illustration of intermolecular interaction (left: 3D and right: 2D) between lowest binding energy conformations of GA in triplicates and ER-â. Hydrogen bonds are shown in green and black dotted lines; hydrophobic interactions are shown as surfaces and smooth contour lines; steric bumps are shown as red wire spheres

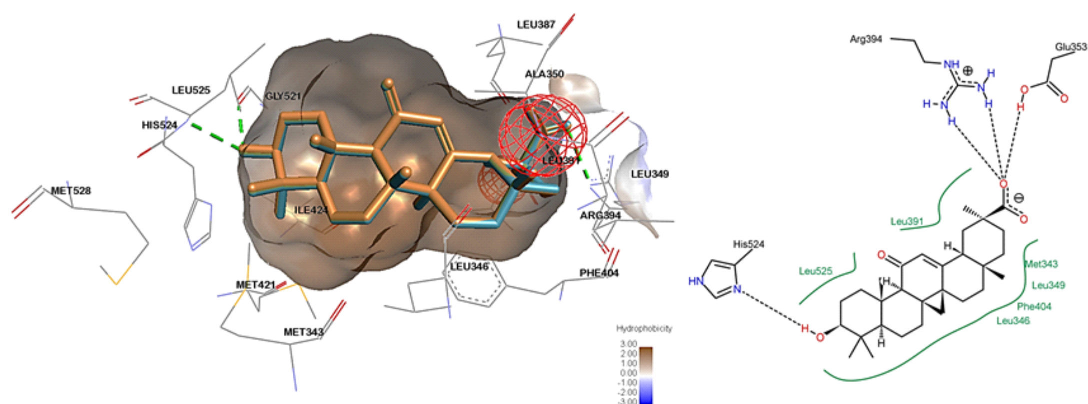

Fig. 8. Illustration of intermolecular interaction (left: 3D and right: 2D) between lowest binding energy conformations of GE in triplicates and ER-á. Hydrogen bonds are shown in green and black dotted lines; hydrophobic interactions are shown as surfaces and smooth contour lines; steric bumps are shown as red wire spheres

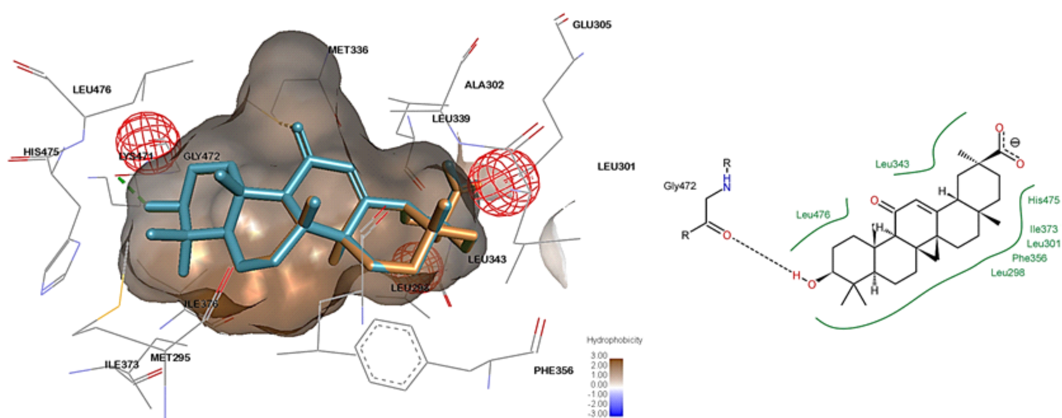

Fig. 9. Illustration of intermolecular interaction (left: 3D and right: 2D) between lowest binding energy conformations of GE in triplicates and ER-â. Hydrogen bonds are shown in green and black dotted lines; hydrophobic interactions are shown as surfaces and smooth contour lines; steric bumps are shown as red wire spheres

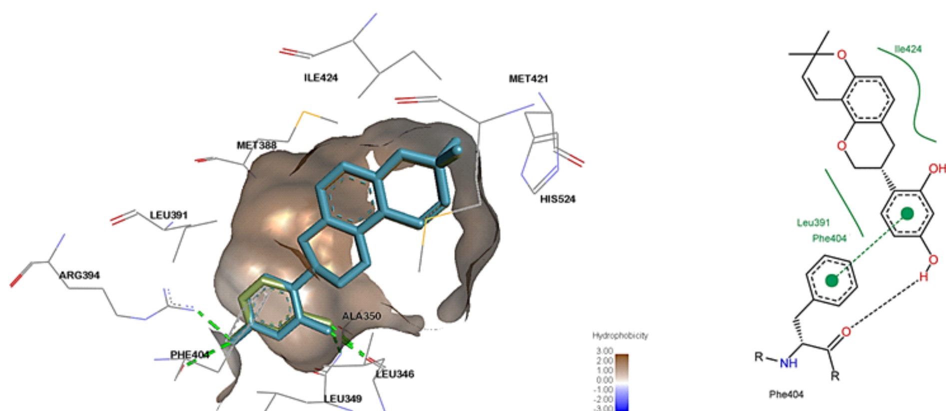

Fig. 10. Illustration of intermolecular interaction (left: 3D and right: 2D) between lowest binding energy conformations of GLA in triplicates and ER-á. Hydrogen bonds are shown in green and black dotted lines; hydrophobic interactions are shown as surfaces and smooth contour lines

that GLA displayed ERá-selective antagonism[17], which contrasted with the findings in this study. Nevertheless, Powers and Setzer (2015) reported that GLA could docked with ERs, with higher selectivity to ER-â[18]. Poh *et al*. (2015) also reported that GLA exert relatively lower estrogenic effects compared to E_2 in ligand binding assay[15]. These findings corresponded with the molecular docking results in this study, though it is to note that GLA did not portray selectivity to any ER subtypes significantly as reported by Powers and Setzer (2015)[18]. The molecular docking results suggested that GLA could be a partial agonist instead of a full agonist to ERs as partial agonists tend to divert less binding energy than full agonists into effective conformational changes[19-20].

Intermolecular Interaction Analysis

Table 2-5 shows the intermolecular interaction analysis summary of E_2, GA, GE and GLA with ERs. Previous study had demonstrated that GLU353, ARG394 and HIS524 of ER-á or GLU305, ARG346 and HIS475 of ER-â are the 3 important residues that form hydrogen bonds with E_2[21]. In this study, hydrogen bonding between E_2 and these residues were also observed (Figure 4-5). However, an additional hydrogen bond between LEU387 of ER-á and LEU339 of ER-â with E_2 was also observed in this study. This is most likely due to the fact that the molecular docking was run without water molecules. When water molecules were incorporated in the intermolecular interaction analysis using the original PDB information, a water molecule were found between LEU387 of ER-á and LEU339 of ER-â, and that water molecule formed hydrogen bond with E_2 instead. It is also worth to note that the amino acid residues that contributed to hydrophobic interactions in this study such as ALA350, LEU384, LEU391, PHE404, ILE424 and LEU525, were also reported by Wang *et al*. (2013) in their studies[21]. This further supported that our molecular docking study is reliable accurate.

Fang *et al*. (2001) had suggested 3 main structural criteria for ligands to bind to ERs: 1) an aromatic ring attached with a hydroxyl group; 2) a second hydroxyl group; 3) hydrophobic core structure[22]. Structuraly, GA and GE is pentacyclic triterpenoids with hydroxyl and carboxyl side groups, which fits part of the criteria, theoretically might be able to be docked into the ligand binding cavity of ERs. However, intermolecular interaction analysis of GA and GE docked with ERs showed multiple sites of unfavorable steric bumps (Figure 6-9). The presence of steric bumps indicated that the pentacyclic structure and methyl side groups of GA and GE are too rigid and bulky to facilitate energy favorable position in the ligand binding cavity of ERs. Indeed, the general ERs agonist consist of maximum of tetracyclic structure (as indicated by search hits at The Binding Database; results not shown). Moreover, the molecular size of ligands is an important factor to fit into the binding pocket of estrogen receptors, as suggested by Armstrong (2011)[23]. Therefore, even though a long list of hydrogen bonds and hydrophobic

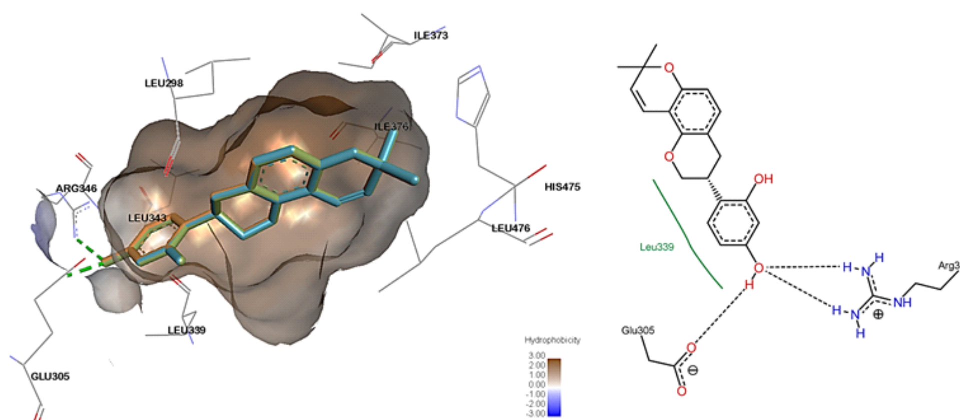

Fig. 11. Illustration of intermolecular interaction (left: 3D and right: 2D) between lowest binding energy conformations of GLA in triplicates and ER-â. Hydrogen bonds are shown in green and black dotted lines; hydrophobic interactions are shown as surfaces and smooth contour lines

interactions were formed with one or more of the three important amino acid residues, the steric hindrance masked the favorable interactions. It is therefore concluded that GA and GE are also not practical for further chemical modification as ligands for ERs.

GLA has been reported to share some similarity on molecular structures with E_2, which both of them contain aromatic ring that substituted with hydroxyl group[14]. In general, GLA also fits the structural criteria suggested by Fang (2001), except lacking the second hydroxyl group. Certainly, GLA was able to be docked into the ligand binding cavity of ERs in this study. As shown in Figure 10 and 11, it was observed that GLA formed hydrogen bond with 1 or 2 of the 3 important amino acid residues (ARG394 of ER-á; GLU305 and ARG346 of ER-â). The lacking of 1 or 2 hydrogen bonds could be the reason why GLA-ERs showed relatively higher binding energies compared to that of E_2-ERs. It is important to note that GLA also formed hydrogen bonds with LEU346, ALA350 and PHE404 in addition to ARG394 of ER-á in this study. Even though these hydrogen bonds were generally shorter and supposing stronger than those of E_2-ER-á[24], the binding energy of GLA-ER-á was still relatively higher. This suggested that van der Waals forces

Table 1. Molecular docking summary of E_2, GA, GE and GLA with ERs

Protein	Ligand	Mean Lowest Binding Energy (kcal/mol)	Mean RMSD (Å)	Mean Number in Cluster
		Between lowest binding conformations (triplicates)		
ER-α	E_2	-10.470 ± 0.000	0.000 ± 0.000	100.000 ± 0.000
	GA	332.300 ± 1.235	0.473 ± 0.081	37.667 ± 12.662
	GE	14.570 ± 0.008	0.066 ± 0.031	93.333 ± 4.509
	GLA	-3.210 ± 0.016	0.202 ± 0.152	75.333 ± 1.528
ER-β	E_2	-10.920 ± 0.000	0.000 ± 0.000	100.000 ± 0.000
	GA	330.550 ± 6.765	0.474 ± 0.156	46.333 ± 3.786
	GE	24.147 ± 0.015	0.048 ± 0.029	73.667 ± 4.041
	GLA	-3.083 ± 0.006	0.022 ± 0.008	96.333 ± 3.055

Table 2. Intermolecular interaction analysis of E_2 molecular docked ERs

ER-α Interaction Residues/Atoms	ER-α Type	Triplicates 1	2	3	ER-β Interaction Residues/Atoms	ER-β Type	Triplicates 1	2	3
GLU353:OE2 - E_2:O3	HBond	R	R	R	GLU305:OE2 - E_2:O3	HBond	R	R	R
LEU387:O - E_2:O3	HBond	R	R	R	LEU339:O - E_2:O3	HBond	R	R	R
ARG394:NH2 - E_2:O3	HBond	R	R	R	ARG346:NH2 - E_2:O3	HBond	R	R	R
HIS524:ND1 - E_2:O17	HBond	R	R	R	HIS475:ND1 - E_2:O17	HBond	R	R	R
ALA350 - E_2	HY	R	R	R	LEU298 - E_2	HY	R	R	R
LEU384 - E_2	HY	R	R	R	ALA302 - E_2	HY	R	R	R
LEU387 - E_2	HY	R	R	R	MET336 - E_2	HY	R	R	R
MET388 - E_2	HY	R	R	R	LEU339 - E_2	HY	R	R	R
LEU391- E_2	HY	R	R	R	MET340 - E_2	HY	R	£	£
PHE404 - E_2	HY	R	R	R	LEU343 - E_2	HY	R	R	R
ILE424 - E_2	HY	R	R	R	PHE356 - E_2	HY	R	R	R
LEU525 - E_2	HY	R	R	R	ILE373 - E_2	HY	R	R	R
					ILE376 - E_2	HY	R	R	R
					LEU476 - E_2	HY	R	R	R

and desolvation energy could play a more critical role in the binding of GLA-ERá.

Another significant point to note is that the aromatic ring attached with hydroxyl group (1, 3-benzenediol) of GLA is rotatable. This is favorable as it provides flexibility to GLA so it could pose to better energy favorable conformation, such as that shown in Figure 11. It also gives more freedom to chemical modification and design of GLA analogues as ER agonists. The methyl side groups at the tricyclic structure is also ideal to be oxidized to form that extra hydroxyl groups that could facilitate better ERs binding as suggested by Fang *et al.* (2001). Therefore, it is suggested that GLA could be subjected to further chemical modifications and design novel semi-synthetic post-menopausal drugs.

CONCLUSION

In conclusion, GA and GE could not bind to ERs possibly due to the rigid and bulky pentacyclic ring structure and methyl side groups. GLA was observed to exert partial estrogenic activity towards ERs and there is potential to further chemically modify and optimize the estrogenicity.

Table 3. Intermolecular interaction analysis of GA molecular docked with ERs

Interaction Residues/Atoms	ER-α Type	Triplicates			Interaction Residues/Atoms	ER-β Type	Triplicates		
		1	2	3			1	2	3
PRO325:O - GA:O59	HBond	R	£	R	MET295:CA - GA:O61	HBond	£	£	R
ILE326:CA - GA:O45	HBond	R	R	R	PHE356:O - GA:O35	HBond	R	R	R
LEU327:O - GA:O46	HBond	£	R	R	PRO358:CD - GA:O35	HBond	R	R	R
MET357:N - GA:O61	HBond	£	R	£	PHE377:N - GA:O48	HBond	R	R	R
LEU387:O - GA:O55	HBond	R	R	R	GLY472:O - GA:O45	HBond	R	R	R
GLY390:CA - GA:O55	HBond	R	R	R	HIS475 - GA:O46	HBond	R	R	R
ARG394:NH1 - GA:O55	HBond	R	R	£	LEU476:N - GA:O45	HBond	R	R	R
PRO406:CD - GA:O60	HBond	£	R	R	MET295:SD - GA:O59	S-Bond	R	£	£
HIS524:CE1 - GA:O34	HBond	£	£	R	MET336:SD - GA:O55	S-Bond	£	£	R
MET343 - GA	HY	R	R	R	VAL280 - GA	HY	R	R	R
LEU346 - GA	HY	R	£	R	LEU301 - GA	HY	R	R	R
LEU349 - GA	HY	R	R	R	MET336 - GA	HY	R	R	R
ALA350 - GA	HY	R	R	£	LEU339 - GA	HY	R	R	R
LEU384 - GA	HY	R	R	R	MET340 - GA	HY	R	R	R
LEU387 - GA	HY	R	R	R	LEU343 - GA	HY	R	R	R
MET388 - GA	HY	R	R	R	ARG346 - GA	HY	R	R	R
LEU391 - GA	HY	R	R	R	LEU380 - GA	HY	R	R	R
PHE404 - GA	HY	R	R	R	HIS279 - GA	StericB	£	R	R
MET421 - GA	HY	R	R	R	VAL280 - GA	StericB	R	R	R
ILE424 - GA	HY	R	R	R	LEU298 - GA	StericB	£	R	£
LEU428 - GA	HY	R	£	R	THR299 - GA	StericB	£	£	R
HIS524 - GA	HY	R	R	R	LEU301 - GA	StericB	R	R	R
LEU525 - GA	HY	R	R	R	GLU305 - GA	StericB	R	R	R
LEU327 - GA	StericB	R	£	£	LEU339 - GA	StericB	R	R	R
LEU349 - GA	StericB	£	R	£	ARG346 - GA	StericB	R	R	R
GLU353 - GA	StericB	R	R	R	PHE356 - GA	StericB	R	R	R
ARG394 - GA	StericB	R	R	R	ILE373 - GA	StericB	R	R	R
ALA405 - GA	StericB	R	R	R					
HIS524 - GA	StericB	R	R	R					
LEU525 - GA	StericB	R	R	R					

Table 4. Intermolecular interaction analysis of GE molecular docked with ERs

ER-α Interaction Residues/Atoms	Type	Triplicates 1	2	3	ER-β Interaction Residues/Atoms	Type	Triplicates 1	2	3
ARG394:NH2 - GE:O4	HBond	R	R	R	LYS471:O - GE:O1	HBond	R	R	R
LEU525:N - GE:O1	HBond	R	R	R	MET336:SD - GE:O2	S-Bond	R	R	R
GLY521:O - GE:O1	HBond	R	R	£	MET295 - GE	HY	R	R	R
MET343 - GE	HY	R	R	R	LEU298 - GE	HY	R	R	R
LEU346 - GE	HY	R	R	R	LEU301 - GE	HY	R	R	R
LEU349 - GE	HY	R	R	R	ALA302 - GE	HY	R	R	R
ALA350 - GE	HY	R	R	R	MET336 - GE	HY	R	R	R
LEU387 - GE	HY	R	R	R	LEU339 - GE	HY	R	R	R
LEU391 - GE	HY	R	R	R	LEU343 - GE	HY	R	R	R
PHE404 - GE	HY	R	R	R	PHE356 - GE	HY	R	R	R
MET421 GE	HY	R	R	R	ILE373 - GE	HY	R	R	R
ILE424 - GE	HY	R	R	R	ILE376 - GE	HY	R	R	R
HIS524 - GE	HY	R	R	R	HIS475 - GE	HY	R	R	R
LEU525 - GE	HY	R	R	R	LEU476 - GE	HY	R	R	R
MET528 - GE	HY	R	R	R	GLU305:OE2 - GE:C27	StericB	R	R	R
ALA350 - GE	StericB	R	R	R	LEU343:CB - GE:C33	StericB	R	R	R
LEU391 - GE	StericB	R	R	R	GLY472:O - GE:C23	StericB	R	R	R

Table 5. Intermolecular interaction analysis of GLA molecular docked with ERs

ER-α Interaction Residues/Atoms	Type	Triplicates 1	2	3	ER-β Interaction Residues/Atoms	Type	Triplicates 1	2	3
LEU346:O - GLA:O3	HBond	R	R	R	ARG346:NH1 - GLA:O4	HBond	R	R	R
ALA350:N - GLA:O3	HBond	R	£	£	GLU305:OE1 - GLA:O4	HBond	R	R	R
ARG394:NH2 - GLA:O4	HBond	R	R	R	LEU298 - GLA	HY	R	R	R
PHE404:O - GLA:O4	HBond	R	R	R	LEU339 - GLA	HY	R	R	R
LEU346 - GLA	HY	R	R	R	LEU343 - GLA	HY	R	R	R
LEU349 - GLA	HY	R	R	R	ILE373 - GLA	HY	R	R	R
ALA350 - GLA	HY	R	£	R	ILE376 - GLA	HY	R	R	R
MET388 - GLA	HY	R	R	R	HIS475 - GLA	HY	R	R	R
LEU391 - GLA	HY	R	R	R	LEU476 - GLA	HY	R	R	R
PHE404 - GLA	HY	R	R	R					
MET421 - GLA	HY	R	R	R					
ILE424 - GLA	HY	R	R	R					
HIS524 - GLA	HY	R	R	R					

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