

Computer-Aid Molecular Docking Technology in Cereal Mycotoxin Analysis

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Abstract: Computer-aid molecular docking is a simulative process that receptors and ligands recognize each other through energy matching and geometric matching. It is widely used in bioactive compounds simulative screening and preliminary exploring the bioactivity and toxicity of molecular, which plays an important guiding role in toxicity and bioactivity study of molecular entities. In our study, we used the computer-aid molecular docking software-discovery studio 3.1 client to test the mechanism of aflatoxins such as aflatoxin B1, B2, M1, M2, G1, G2 and the results of our experiment help to illustrate the pathway of aflatoxin's toxication. We also used this technology to test the preliminary toxicity of zearalenone (ZEN) and its two degradation products: α -zearalenol (α -ZOL) and β -zearalenol (β -ZOL), which indicates that these three products possessed significant estrogenic activity. The order of the estrogenic activity is: α -zearalenol > zearalenone > β -zearalenol.

Key words: Computer-aid molecular docking, aflatoxin, zearalenone, toxicity.

1. Introduction

Over the last few decades, computer-aid molecular docking technology has grown significantly in the development of new drug molecules. As a powerful technique, it relieves the tension in drug discovery such as time-consuming, high-cost and low success rates. Additionally, with rapid development of biological structures and computer technology, this technology is widely used in mycotoxin toxicity pathway research [1, 2].

Using direct docking methods or virtual high-throughput screening (HTS), affinity of molecules to targets can be estimated based on compounds' conformation and complementarity with residues in binding site. Through analysis of binding free energies, further filtering and optimization of possible molecules subsequently follow, a limited number of lead molecules are selected for *in vitro*

bioactivity tests. Automatic docking is aimed at the determination of the optimal position and orientation of molecule in binding pocket of particular protein target [3, 4]. Quality of protein-ligand interactions is usually qualified by ligand efficiency (LE) and average binding energy per non-hydrogen atom of the ligand. While virtual high-throughput screening is performed to evaluate libraries of molecules for binding affinity to the protein target, this HTS strategy can shortlist compounds that are most likely to bind to the selected target with the highest affinity. A plethora of software has been developed for molecular docking including Dock, GOLD, and AutoDock, etc. In addition, other docking strategies such as flexible ligand docking, fragment docking and fragment growing have been used in high-throughput docking campaigns [5, 6].

In our research, we used the computer-aid molecular docking software-discovery studio 3.1 client to test the mechanism of aflatoxins and illustrate the pathway of aflatoxin's toxication. We also used

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this technology to test the preliminary toxicity of zearalenone and its two degradation products: α -zearalenol and β -zearalenol, which indicates that these three products possessed significant estrogenic activity.

2. Materials and Methods

The 2D structure of aflatoxin B1 and oltipraz was generated by ChemDraw Ultra 12.0 (Cambridge Soft, Cambridge, MA, USA). A homology model of protein receptor was constructed from crystal structure of PDB: obtained from the RCSB protein Data Bank.

Water molecules were removed and H atoms were added to the structure; 3D structures of the compounds were generated and optimized by the Discovery Studio 2.1 package (Accelrys, San Diego, CA, USA). The receptor-grid files were carried out using a grid-receptor generation program using default settings after ensuring that the ligands and the protein are in correct form. The GOLD program in the Discovery Studio software was used to perform the docking simulations, which allows full flexibility of the ligand.

The structures of the aflatoxins, zearalenone, α -zearalenol and β -zearalenol were drawn in Chem3D with standard lengths and angles. The Gasteiger-Huckel charge, with a distance-dependent

dielectric function, and AM1 docking calculations were applied for the minimization of the molecules. To modify the structure of receptor, missing atoms, bonds, and contacts were checked, hydrogen atoms were added to the enzyme structure, and water molecules were removed. Intercalation models were optimized using the Chemistry at HARvard Macromolecular Mechanics (CHARMM) force field with the added parameters. After performing the docking simulation, the scores of the docked conformers were ranked and the best binding modes in the cavity were picked out.

3. Results and Discussion

3.1 Aflatoxin Toxicity Analysis

Aflatoxins are among the most potent natural hepato-carcinogenic products, which are produced mainly by the fungi *Aspergillus flavus* and *A. parasiticus*. Nowadays scientist has separated and identified twelve aflatoxins analogues including aflatoxin B1, B2, G1, M1, P1, Q1, H1, GM, B2a and aflatoxicol (Fig. 1). The basic structures of aflatoxins are dihydrofuran and coumarin and aflatoxin B1 (AFTB1), which is the analogue of dihydrofuran oxynaphthalene, and contains two furan ring (the basic toxic structure) and one coumarin [7, 8].

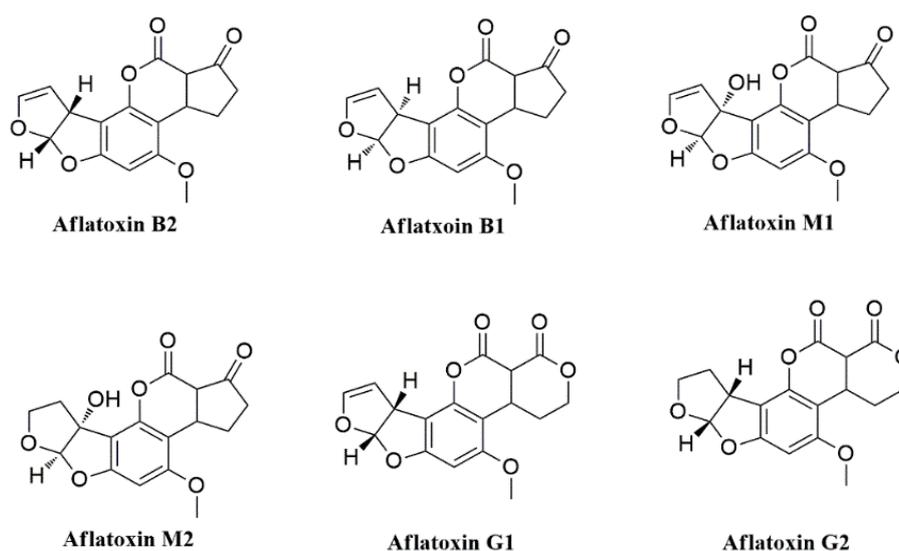


Fig. 1 The chemical structures of aflatoxins metabolic products.

3.1.1 The Interaction between AFTB1 and Receptor Proteins

In our research, we chose some kinds of proteins which play significant roles in cell apoptosis, estrogen metabolism, immunosuppression and digestive system function as the potential targets of the toxic pathway of aflatoxin B1, including Caspase-1, cell division protein kinase 2, serine/threonine protein kinase chk1, progesterone receptor, androgen receptor, estrogen receptor, alpha-thrombin, prostaglandin g/h synthase 2, estradiol 17-beta-dehydrogenase 1, macrophage migration inhibitory factor and estrogen sulfotransferase. We made AFTB1 molecular docked with the above proteins by molecular docking software, the results were shown in Table 1. As the results showed, estrogen sulfotransferase was proved to be the best dock receptor of AFTB1 and the scores were 130.22 and -10.9013 by Libdock and CDOCKER, respectively.

3.1.2 The Interaction between AFTB1 and Estrogen Sulfotransferase

We made AFTB1 docked with estrogen sulfotransferase, and the docking result was shown in Fig. 2. AFTB1 could perfectly dock into the formed cavity of estrogen sulfotransferase protein and there was formed cation- π interaction between the benzene ring and arginine residue (ARG B:129). The carbonyl group in coumarin formed hydrophobic interactions

with tyrosine residue (TYR B:192) and the oxygen atom in furan ring formed hydrophobic interactions with arginine residue (ARG B:256).

3.1.3 The Interaction between AFTB2 and Estrogen Sulfotransferase

We made AFTB2 docked with estrogen sulfotransferase, and the docking result was shown in Fig. 3. AFTB2 could perfectly dock into the formed cavity of estrogen sulfotransferase protein and there was formed cation- π interaction between the benzene ring and arginine residue (ARG B:129). The carbonyl group in coumarin formed hydrophobic interactions with arginine residue (ARG B:256), allysine residue (LYS B:257) and glycine residue (GLY B:258).

3.1.4 The Interaction between AFTB M1 and Estrogen Sulfotransferase

We made AFTB M1 docked with estrogen sulfotransferase, and the docking result was shown in Fig. 4. AFTB M1 could perfectly dock into the formed cavity of estrogen sulfotransferase protein and there was formed cation- π interaction between the benzene ring and arginine residue (ARG B:129). The phenolic hydroxy group in furan ring formed hydrophobic interactions with arginine residue (ARG B:256), allysine residue (LYS B:257) and glycine residue (GLY B:258). The oxygen atom in furan ring formed hydrophobic interactions with arginine residue (ARG B:256).

Table 1 AFTB1 targets predicted by Libdock and CDOCKER.

Protein	PDB	Libdock score	CDOCKER score
Caspase-1	3D6F	82.8002	/
Cell division protein kinase 2	1HCK	97.2643	-0.549952
Serine/threonine-protein kinase chk1	1IA8	100.060	/
Progesterone receptor	1A28	98.6966	/
Androgen receptor	5DIU	113.528	-20.9024
Estrogen receptor	1QKM	110.000	-22.1885
Alpha-thrombin	1ABI	82.2093	/
Prostaglandin g/h synthase 2	1PD2	117.653	/
Estradiol 17-beta-dehydrogenase 1	1BHS	98.6731	/
Macrophage migration inhibitory factor	1MFI	90.8723	-968.253
Estrogen sulfotransferase	1HY3	130.022	-10.9013
Sex hormone-binding globulin	1D2S	111.558	-16.2633

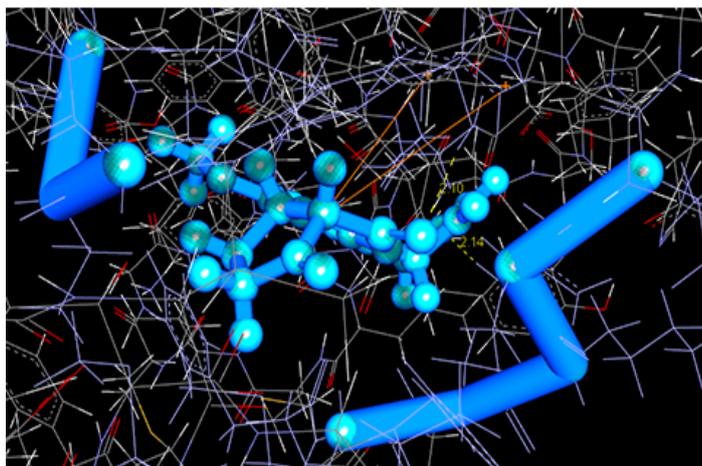


Fig. 2 The interaction between AFTB1 and estrogen sulfotransferase.

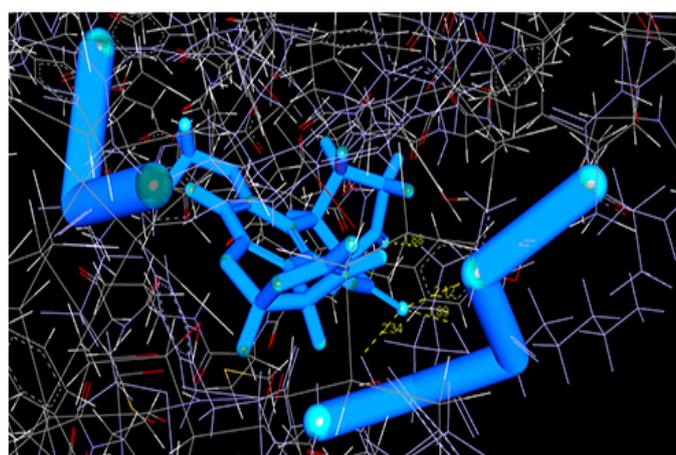


Fig. 3 The interaction between AFTB2 and estrogen sulfotransferase.

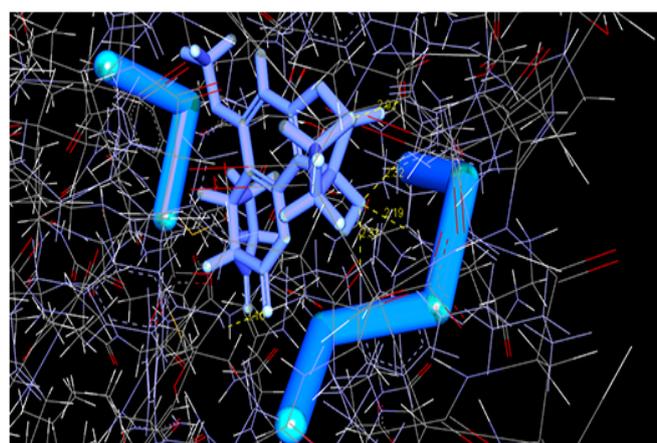


Fig. 4 The interaction between AFTB M1 and estrogen sulfotransferase.

3.1.5 The Interaction between AFTB M2 and Estrogen Sulfotransferase

We made AFTB M2 docked with estrogen sulfotransferase, and the docking result was shown in

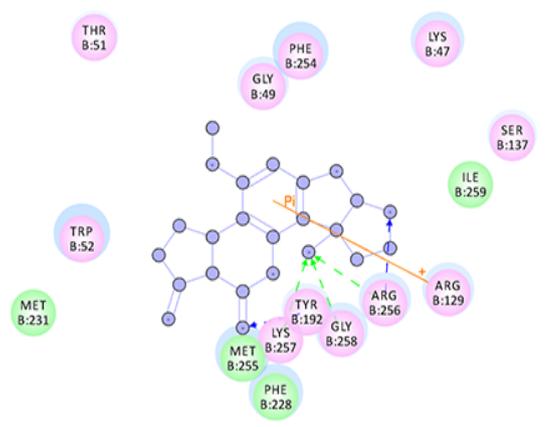
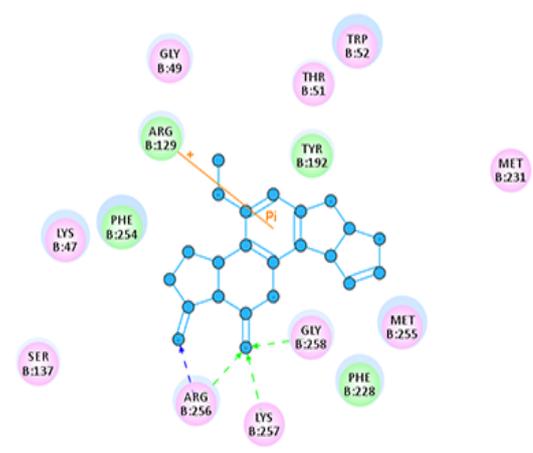
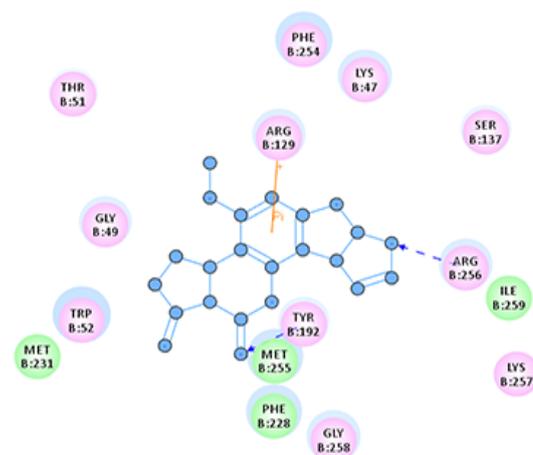


Fig. 5. There was formed cation- π interaction between the benzene ring and arginine residue (ARG B:129). The phenolic hydroxy group in furan ring formed hydrophobic interactions with arginine residue (ARG

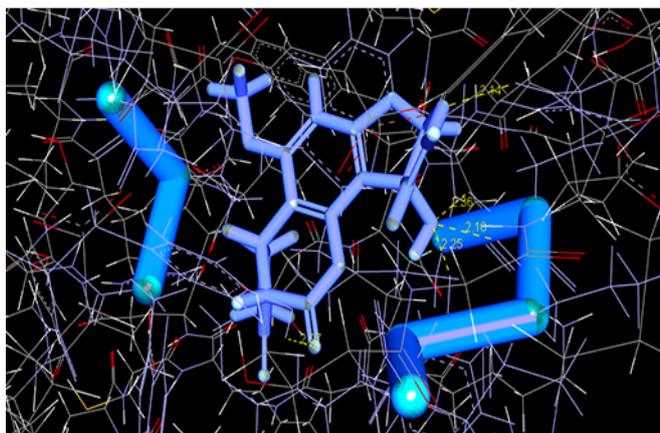


Fig. 5 The interaction between AFTB M2 and estrogen sulfotransferase.

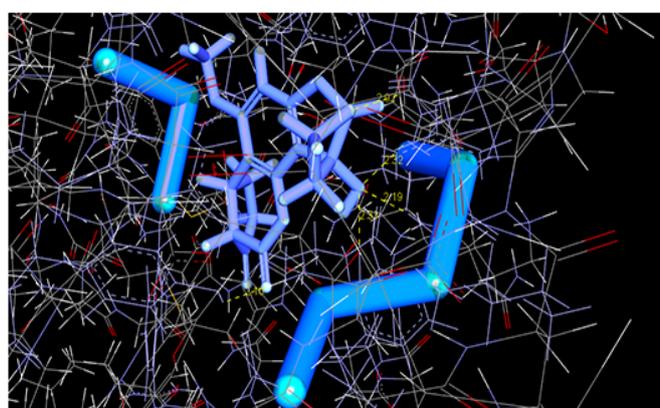
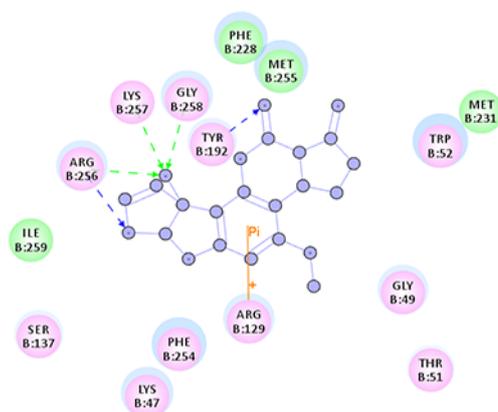
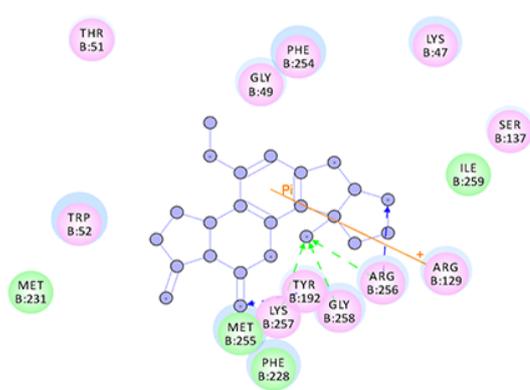


Fig. 6 The interaction between AFTB G1 and estrogen sulfotransferase.



B:256), lysosine residue (TYR B:192) and glycine residue (GLY B:258). The oxygen atom in furan ring formed hydrophobic interactions with arginine residue (ARG B:256), the carbonyl group in coumarin formed hydrophobic interactions with tyrosine residue (TYR B:192).

3.1.6 The Interaction between AFTB G1 and Estrogen Sulfotransferase

We made AFTB G1 docked with estrogen sulfotransferase, and the docking result was shown in Fig. 6. There was formed cation- π interaction between the benzene ring and arginine residue (ARG B:129). The inside carbonyl group in coumarin formed hydrophobic interactions with arginine residue (ARG B:256), allysine residue (LYS B:257) and glycine residue (GLY B:258). The outside carbonyl group in coumarin formed hydrophobic interactions with allysine residue (LYS B:47). The oxygen atom in furan ring

formed hydrophobic interactions with arginine residue (ARG B:256).

3.1.7 The Interaction between AFTB G2 and Estrogen Sulfotransferase

We made AFTB G2 docked with estrogen sulfotransferase, and the docking result was shown in Fig. 7. There was no formed cation- π interaction between the benzene ring and any residue. The inside carbonyl group in coumarin formed hydrophobic interactions with arginine residue (ARG B:256), allysine residue (LYS B:257) and glycine residue (GLY B:258). The outside carbonyl group in coumarin formed hydrophobic interactions with allysine residue (LYS B:47).

3.1.8 The Binding Energy and Docking Score between Ligand and Receptors

The results of binding energy and docking score between aflatoxin B1, B2, M1, M2, G1, G2 and estrogen sulfotransferase were shown in Table 2. The

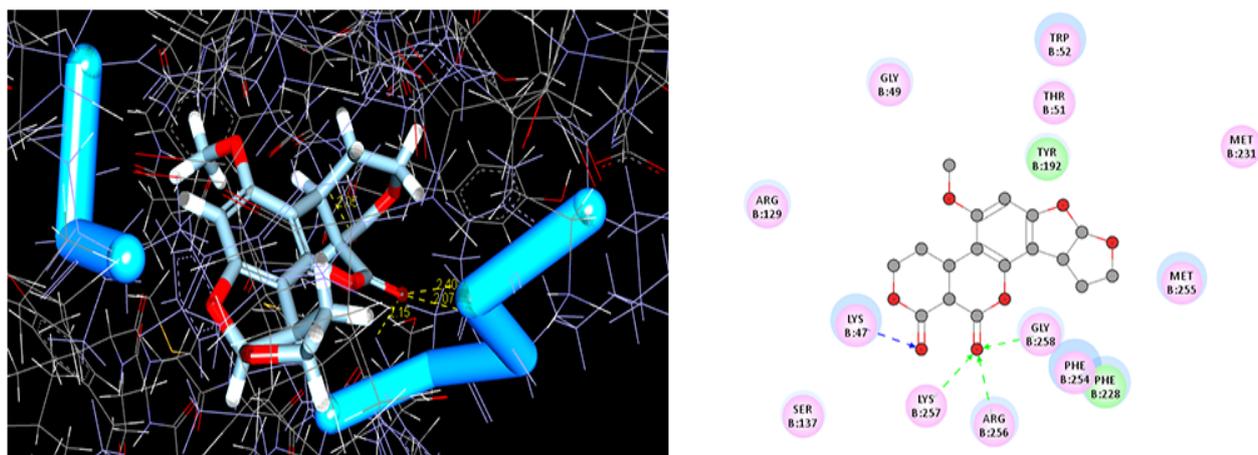


Fig. 7 The interaction between AFTB G2 and estrogen sulfotransferase.

Table 2 The binding energy and docking score between ligand and receptors.

Ligand	Binding energy	Number
Aflatoxin M2	-8.21584	10
Aflatoxin M1	-8.5654	10
Aflatoxin B1	-10.9013	10
Aflatoxin G2	-14.1769	10
Aflatoxin G1	-19.5298	10
Aflatoxin B2	-20.2356	10

binding energy between AFTB1, AFTB2, AFTB M1, AFTB M2, AFTB G1, AFTB G2 with estrogen sulfotransferase was -10.9013 kcal/mol, -20.2356 kcal/mol, -8.5654 kcal/mol, -8.2156 kcal/mol, -19.5298 kcal/mol, -14.1768 kcal/mol. AFTB M1 was proved to be the best binding ligand with estrogen sulfotransferase receptor.

3.2 Estrogenic Effect in Zearalenone

Zearalenone (6-(10-hydroxy-6-oxo-trans-1-undecenyl)-resorcylic acid lactone, abbreviated as ZEN) is a is produced by *Fusarium roseum* and is usually isolated from moldy corn [9]. Zeranol, a synthetic tetrahydro-derivative of ZEN, has been used as a growth promoter for food-producing animals [10]. Earlier studies have shown that ZEN and ZOL have strong estrogenic effects, and each of them was reported to have a similar dose-response curve pattern in stimulating uterine weight gains in neonatal rats or immature mice [11]. The most common ZOLs are α -ZOL and β -ZOL, which are shown in Fig. 8.

3.2.1 The Interaction between ZEN and α -estrogen Receptor Protein

In order to prove the estrogen effect of ZEN in molecular stage, we used the molecular docking software to simulate the binding situation between ZEN and α -estrogen receptor protein, as Fig. 9 showed, ZEN can perfectly dock into the cavity of estrogen receptor protein crystal structure, and the hydroxy group of benzene ring formed strong hydrophobic interactions with contiguous alanine residue (ALA350) and glutamic acid residue (GLU353), which strengthen the binding ability between the ligands and the receptors.

3.2.2 The Interaction between ZEN and β -estrogen Receptor Protein

We used the molecular docking software to simulate the binding situation between ZEN and β -estrogen receptor protein, as Fig. 10 showed, the 3-hydroxy group in benzene ring of ZEN formed strong hydrophobic interactions with contiguous histidine residue (HIS475) and glycine residue (GLY472),

which strengthen the binding ability between the ligands and the receptors.

3.2.3 The Interaction between ZOL and Estrogen Receptor Protein

We have proved that ZEN molecule had favorable binding ability with α -estrogen receptor protein and β -estrogen receptor protein, which demonstrated that ZEN possessed strong estrogen effect. In the following study, we would prove whether ZOL had the similar estrogen effect.

We used the molecular docking software to

simulate the binding situation between α -ZOL, β -ZOL and α -estrogen receptor protein, β -estrogen receptor protein. From Fig. 11, it was proved that α -ZOL can perfectly dock into the cavity of estrogen receptor protein crystal structure, 3-hydroxy group in benzene ring of α -ZOL formed strong hydrophobic interactions with contiguous arginine residue (ARG394) in α -estrogen receptor protein and glycine residue (GLY472) in β -estrogen receptor protein; aliphatic hydroxyl group in aliphatic chain formed strong

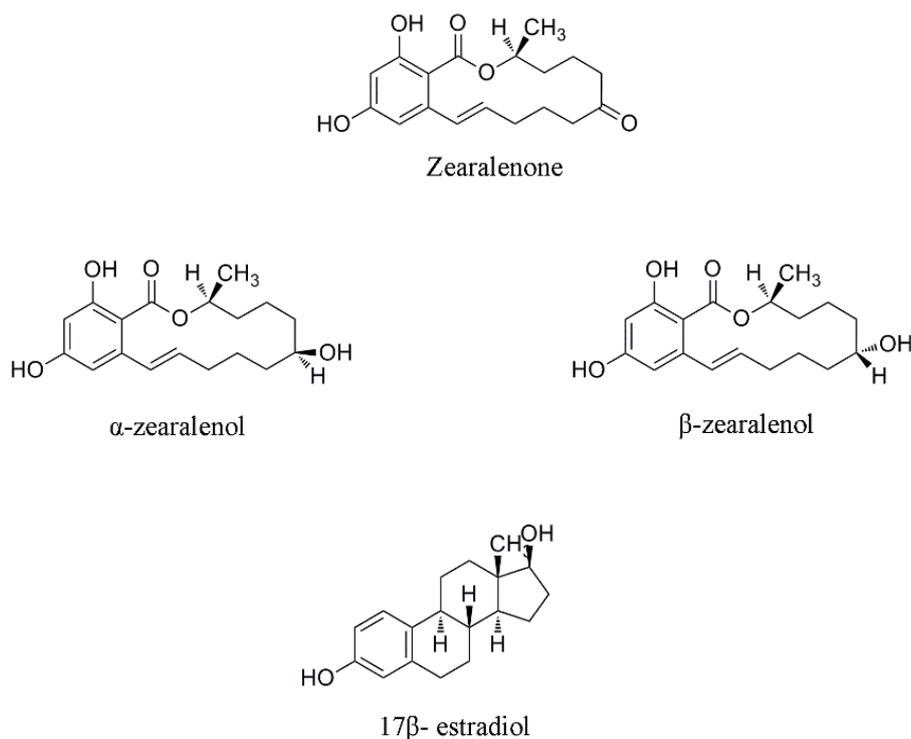


Fig. 8 Chemical structures of ZEN, α -ZOL, β -ZOL and β -estriol.

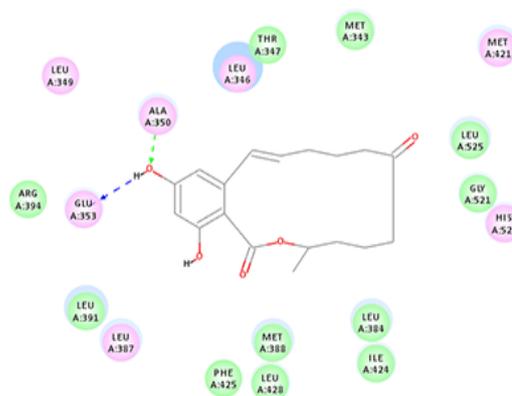
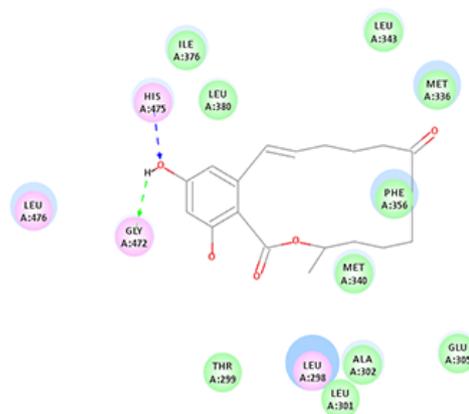
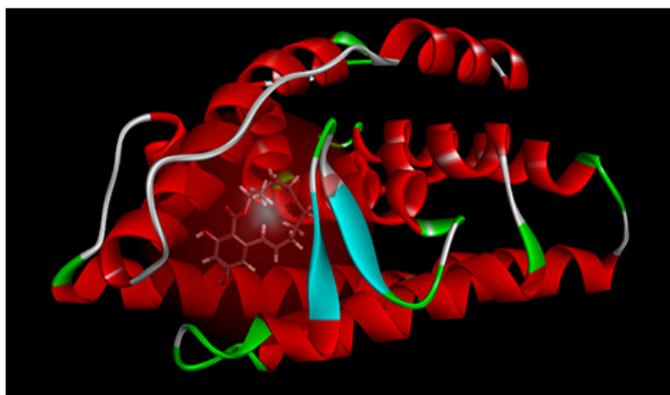
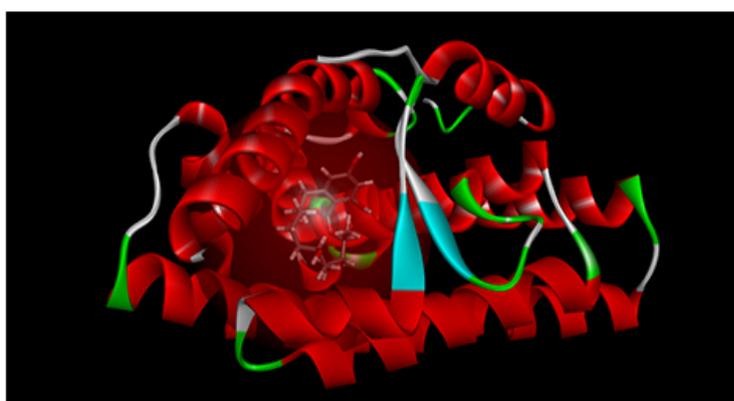
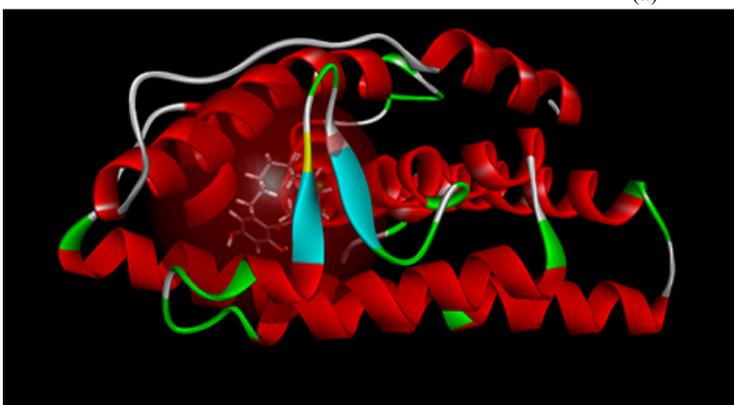
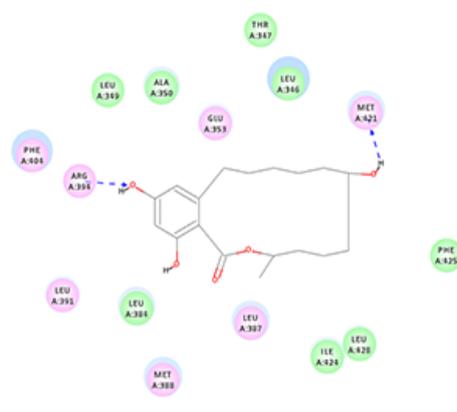
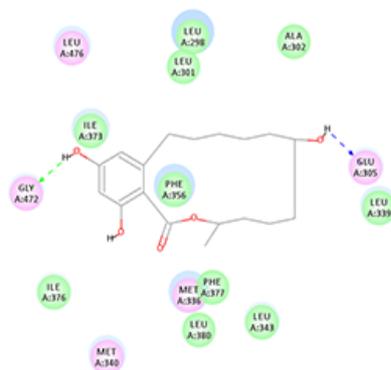


Fig. 9 The interaction between ZEN and α -estrogen receptor protein.Fig. 10 The interaction between ZEN and β -estrogen receptor protein.

(a)



(b)

Fig. 11 The interaction between α -ZOL and estrogen receptor protein.

hydrophobic interactions with methionine (MET421) in α -estrogen receptor protein and glutamic residue (GLU305) in β -estrogen receptor protein. The binding ability between β -ZOL and estrogen receptor protein was relatively weak and there only one existed hydrophobic interaction according to the docking results (Fig. 12).

3.2.4 The Binding Ability and Docking Score between Ligands and Receptor

The results of binding energy and docking score between 17 β -estradiol (positive control), ZEN, α -ZOL, β -ZOL and estrogen receptor protein were shown in Table 3. The order of the estrogenic activity is:

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