In Silico Molecular Docking Analysis of Breast Cancer Therapy Using Zerumbone Derivatives

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Abstract. Cancer has high prevalence to cause a mortality in the world in case of breast cancer, although numerous medical treatment have been developed. Cancer treatment and therapy failures are frequently caused by drug resistance and toxicity. Therefore, efforts to develop and discover renewed cancer drugs still have an enormous attention to improve effectiveness of bioavailability. Potential of natural products as an anti-cancer has been reported, especially zerumbone through antiproliferation, antiapoptotic, and antimetastatic mechanism. Zerumbone, a phytochemical sesquiterpenoid with cyclic ketone as a main building block, isolated from *Zingiber zerumber* Smith (lempuyang wangi). A number of derivates of zerumbone have been reported using in silico method to obtain potential candidate of breast cancer. Focus of our research to predict derivatives of zerumbone with the best pharmacokinetic properties and the best interaction with main breast cancer targets. Further, molecular docking with autodock were conducted to predict the interaction with breast cancer target. Our protein target docking are EP300 (E1A Binding Protein P300) and HER2 (Human Epidermal Growth Factor Receptor 2) that roles to accelerate breast cancer cells growth. Twenty one zerumbone derivatives have been determined for molecular docking in silico study. Assesment of binding energy showed that five zerumbone derivatives have higher interaction with EP300 compared to zerumbone (-5,01 kcal/mol). In addition, more than half of examined zerumbone derivatives have higher stability over HER2 than zerumbone (-5,92 kcal/mol) based on calculated binding affinity.

Keywords: Zerumbone, Breast Cancer, In Silico, Molecular Docking.

1 Introduction

Breast cancer is a global health problem and affects women (and sometimes men) in all parts of the world. According to the World Health Organization (WHO), breast cancer is the most common cancer among women worldwide, with an estimated 2.3 million new cases and 685,000 deaths in 2020 [1,2]. Currently, natural compounds are being explored as potential alternatives for cancer treatment. Zerumbone is a natural bioactive compound that is found in the rhizomes of certain plants, particularly in Zingiberaceae

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family, such as the tropical plant Zingiber zerumbet (also known as lempuyang wangi). Zerumbone has been studied for its potential medicinal properties, particularly for its anti-inflammatory, antioxidant, and anticancer effects [3]. Several studies have conducted molecular docking analysis to explore the potential anti-breast cancer activity of zerumbone by investigating its binding affinity with various molecular targets, including proteins and enzymes involved in breast cancer progression. Zerumbone with estrogen receptor alpha (ERα) showed that zerumbone had a higher binding affinity than tamoxifen, a well-known ERα antagonist [4]. Another study performed molecular docking analysis of zerumbone with HER2 kinase and showed that zerumbone could bind to the active site of HER2 kinase, which is overexpressed in approximately 25% of breast cancer cases [5]. EP300 has been identified as a potential target for cancer therapy, as it is frequently mutated or overexpressed in several types of cancer. Inhibition of EP300 has been shown to have anti-cancer effects by suppressing tumor growth and inducing apoptosis [6].

2 Materials and Method

2.1 Tools, software, website

Swissadme (http://swissadme.ch/index.php), Protein Data Bank (https://www.rcsb.org/), ChemSketch ACD2021, Gaussview 5.0, Avogadro, Autodock 1.5.6, Discovery Studio 2017.

2.2 Selection of pharmacokinetic properties of zerumbone derivatives

Zerumbon derivatives were modelled on swissadme website and pharmacokinetic parameter was calculated using menu on the website. Lipinski's rules of five which are molecular weight, logP, hydrogen bond donor number, hydrogen bond acceptor, and surface area were listed.

2.3 Validation of 3D protein

Estrogen and progesteron receptor were retrieved from pdb website with the 3D structure of which are estrogen receptor (PDB ID: EP300, resolution 2.03 A), progesteron receptor (PDB ID: HER2, resolution 2.41 A). All the proteins obtained from Protein Data Bank contained water molecules and the original ligands. Each of the protein were validated using cocrystalled natural ligand data from pdb to obtain the rmsd ≤2.

2.4 Preparation of ligan

Derivat zerumbon which has passed the Lipinski`s rule of five were built using Chemsketch ACDLab2021, optimized using Gaussview 5.0 with Density Functional-Theory (DFT) calculation and saved in pdb format using Avogadro. The prepared ligand of zerumbone derivatives was used as input for Autodock 1.5.6. receptor (PDB ID: HER2, resolution 2.41 A). All the proteins obtained from Protein Data Bank contained

water molecules and the original ligands. Each of the protein were validated using co-crystalled natural ligand data from pdb to obtain the rmsd ≤2.

2.5 Molecular docking

Derivat zerumbon was evaluated with further tests. They were interacted with estrogen, progesteron receptor using Autodock 1.5.6. A grid of 32, 20, and 36 points in x, y, and z directions was built with a grid spacing of 0.375 Å for EP300. Also grid 32, 30, 26 for HER2 with grid spacing 0.375 A. Molecular docking study was performed by using Lamarckian genetic algorithm. The standard docking procedure was adopted for a rigid protein and a flexible ligand (100 independent runs per ligand). Substrat-enzyme Gibss energy was calculated and compared to natural ligand with the same mechanism.

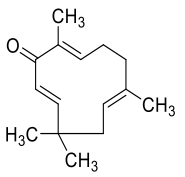
2.6 Analysis of binding affinity

For interaction analysis, the Discovery Studio Visualizer Software was used to study the binding modes of synthesized compounds with the target proteins.

3 Results and Discussion

Zerumbone is a natural sesquiterpene found in the rhizomes of the subtropical ginger plant Zingiber zerumbet [3]. Several studies have investigated the potential anticancer activity of zerumbone against breast cancer. Previous research has evaluated the anti-proliferative activity of zerumbone on breast cancer cells. The study found that zerumbone inhibited the growth of breast cancer cells in a dose-dependent manner by inducing apoptosis and cell cycle arrest. Girisa et.al reported that zerumbone downregulated the expression of HER2 and its downstream signaling molecules in breast cancer cells, suggesting that the anticancer activity of zerumbone may be mediated by the inhibition of HER2 signaling [7]. Another study found that zerumbone inhibited the activity of EP300 in a dose-dependent manner and alsosuppressed the acetylation of histone H3 and H4, resulting in the downregulation of certain oncogenes and the upregulation of tumor suppressor genes in breast cancer cells [3].

Several studies suggest that structural modifications of zerumbone can improve bioactivity by binding affinity with molecular targets related to breast cancer, and that molecular docking analysis can be a valuable tool for predicting the effects of such modifications [8]. In our research, SwissADME website used to predicting the pharmacokinetic of 33 zerumbone derivatives. SwissADME also provides additional information such as molecular weight, number of hydrogen bond donors and acceptors, and number of rotatable bonds, which are important parameters for drug-likeness evaluation. The molecules and pharmacokinetic result are showed in figure 1 and table 1.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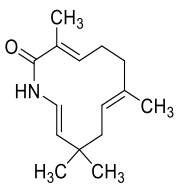
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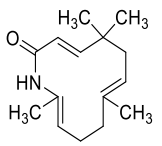
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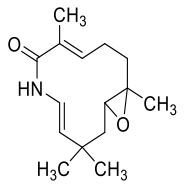
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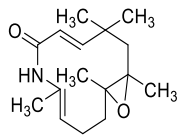
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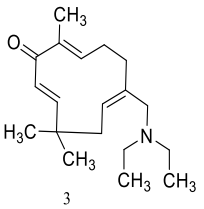
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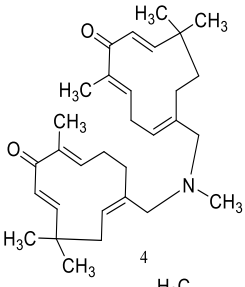
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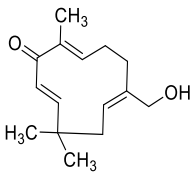
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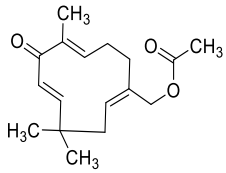
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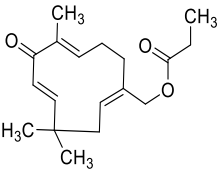
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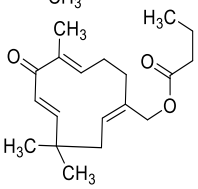
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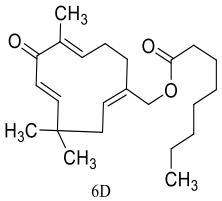
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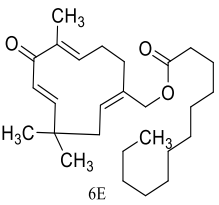
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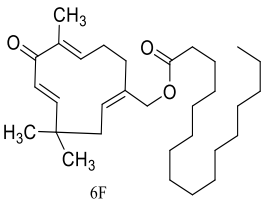
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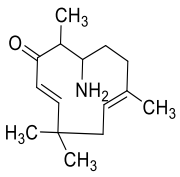
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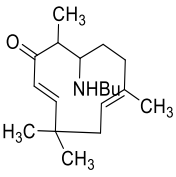
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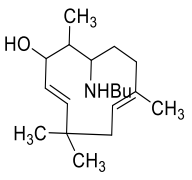
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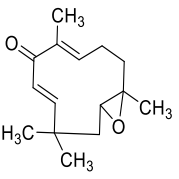
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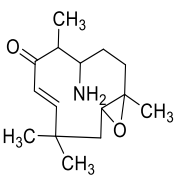
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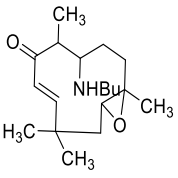
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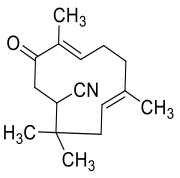
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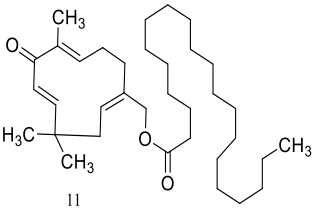
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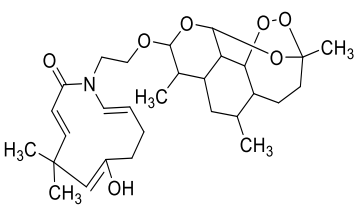
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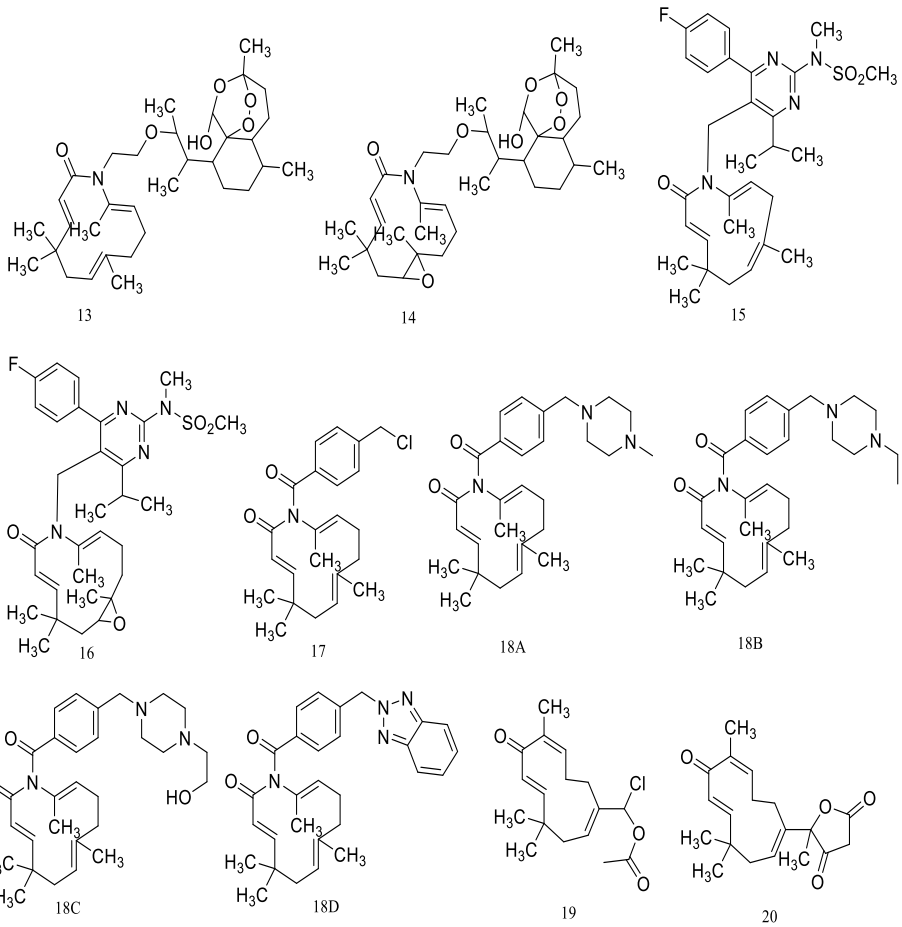


Fig 1. Zerumbone derivatives.

Table 1. Pharmacokinetic properties of zerombone derivatives.

Mole-cules	MW (g/mol)	MlogP	tPSA	nHBA	nHBD	nRB	Violation from lipinski rule	BBB Permanent	GI Absorp-tion
Zer	218.33	3.37	17.07	1	0	0	No	Yes	High
1A	233.35	2.85	29.10	1	1	0	No	Yes	High
1B	233.35	2.85	29.10	1	1	0	No	Yes	High
2A	249.35	2.06	41.63	2	1	0	No	Yes	High
2B	263.38	2.06	41.63	2	1	0	No	Yes	High
3	303.48	3.64	20.31	2	0	5	No	Yes	High
4	463.69	3.64	37.38	3	0	4	No	Yes	High

5	234.33	2.45	37.30	2	1	1	No	Yes	High
6A	276.37	2.45	43.47	3	0	3	No	Yes	High
6B	290.40	3.25	43.37	3	0	5	No	Yes	High
6C	304.42	3.25	43.37	3	0	5	No	Yes	High
6D	360.53	4.14	43.37	3	0	9	No	Yes	High
6E	416.64	4.96	43.37	3	0	13	Yes	No	Low
6F	472.74	5.74	43.37	3	0	17	Yes	No	Low
7A	235.37	2.54	43.09	2	1	0	No	Yes	High
7B	291.47	3.50	29.10	2	1	4	No	Yes	High
8	293.49	3.59	32.26	2	2	4	No	Yes	High
9A	234.33	2.54	29.60	2	0	0	No	Yes	High
9B	251.36	1.75	55.62	3	1	0	No	Yes	High
9C	323.51	2.94	41.63	3	1	4	No	Yes	High
10	245.36	2.69	40.86	2	0	0	No	Yes	High
11	528.85	6.48	43.37	3	0	21	Yes	Yes	High
12	559.78	6.30	77.46	6	1	0	Yes	No	-
13	529.71	5.89	66.46	6	0	0	Yes	No	High
14	589.80	3.20	78.99	7	0	4	Yes	No	Low
15	568.75	3.76	91.85	6	0	6	Yes	No	Low
16	584.75	3.86	128.09	7	0	6	Yes	No	Low
17	385.93	3.86	37.38	2	0	3	No	Yes	Low
18	449.63	3.86	43.86	4	0	4	Yes	No	Low
19A	463.65	3.86	34.86	4	0	5	Yes	No	Low
19B	479.65	3.09	64.09	5	1	6	-	-	-
19C	468.59	5.68	68.09	4	0	4	-	-	-
19D	310.82	3.02	43.37	3	0	3	No	Yes	High
20	316.39	2.32	60.44	4	0	1	No	Yes	High

Note: MW : molecular weigh, nHBA :number of hydrogen bond acceptors, nHBD : number of hydrogen bond donors, tPSA: topological polar surface area.

There are 21 zerumbone subordinates were qualified through the Lipinski's rule of five such as the molecular weight (MW), the number of hydrogen bond acceptors (nHBA), number of hydrogen bond donor (nHBD), the number of rotatable bonds (nRB), and topological polar surface area. Assesment of ADMET properties are important to determine the bioavailability, pharmacokinetics, and safety for the developmet of drug candidate. ADMET stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity, and it refers to the study of how drugs are processed by the body. The results of the ADMET study showed that there are 21 molecules were passed the Lipinski`s rule of five as the character of drug molecule, previously. Good

pharmakocinetic properties and that several of new molecules passed the druglikeness criteria [9,10].

Molecular docking were conducted to predict the most energetically favorable binding mode of the small molecule (ligand) within the receptor's active site of breast cancer, typically a protein [11]. Our target investigation for the determination of putative binding mode are the amino acid residues of EP300 protein and HER2 receptor. After the docking process, the protein-ligand with different zerumbone derivatives was analyzed to investigate the nature of the interaction. The highest score binding energy (kcal/mol) from the AutoDock dlg output file was taken as the response for each run. The highest docking binding energies of the derivatives were obtained at Table 2. Binding free energy As molecular docking only measures the geometric fit of ligands at the active site of a protein in static conditions.

Table 2. Binding energy of each zerumbone derivatives with EP300 and HER2 receptor.

Molecules	Binding Energy with EP300 (kcal/mol)	Binding Energy with HER2 (kcal/mol)	Molecules	Binding Energy with EP300 (kcal/mol)	Binding Energy with HER2 (kcal/mol)
Natural ligand	-6.38	-8.23			-8.28
Zer	-5.01	-5.92	6D	-5.07	-5.98
1A	-5.1	-7.04	7A	-4.72	-6.64
1B	-4.5	6.12	7B	-3.70	-3.91
2A	-4.92	-5.48	8	-3.84	-3.91
2B	-5.09	-5.99	9A	-4.72	-7.57
3	0.22	0.31	9B	-4.72	-5.67
4	-0.94	-6.25	9C	1.10	-
5	0.44	-6.25	10	2.47	-6.83
6A	0.03	-7.01	17	-0.13	19.25
6B	-6.19	-6.90	19	0.31	-7.18
6C	-5.99	-7.20	20	-0.17	-7.25

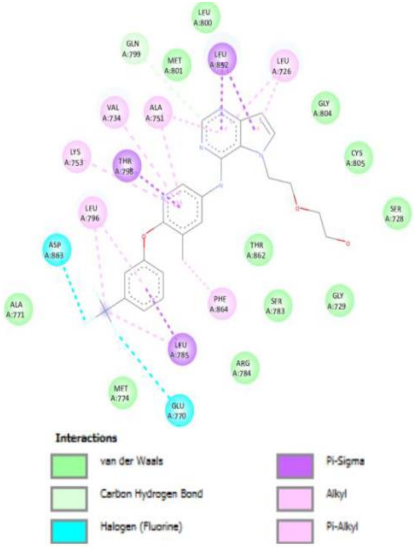
Analysis of the docking results (Table 2) reveals that almost zerumbone derivatives have weaker interaction than natural ligand. Assesment of binding energy showed that five zerumbone derivatives have higher

interaction with EP300 compared to zerumbone (-5,01 kcal/mol). In addition, more than half of examined zerumbone derivatives have higher stability over HER2 than zerumbone (-5,92 kcal/mol) based on calculated binding affinity. Derivate 6B showed the lowest binding energy for EP300 and derivate 6D showed the lowest binding energy for HER2, both of them indicate the best candidate for breast cancer therapy.

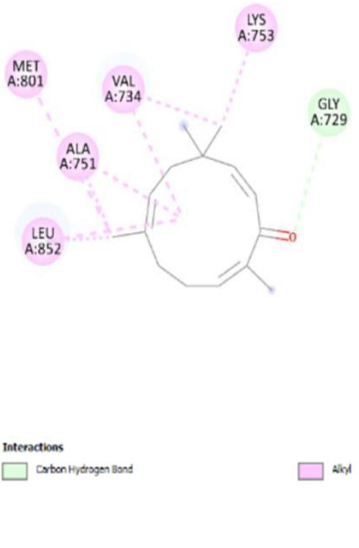
Binding analysis of natural ligand, zerumbone, derivative 6B and derivative 6D as the most potential zerumbon derivat was done using Discovery Studio (Figure 2 and Figure 3). Based on HER2 receptor environment, natural ligand and derivative

6D showed that van der Waals is dominant interaction. On the other hand, EP300 protein environment, natural ligand showed that alkyl is dominant interaction and derivative 6B showed that van der waals is dominant interaction. Interestingly, there is conventional hydrogen bond between EP300 and derivative 6B through asparagine (ASN).

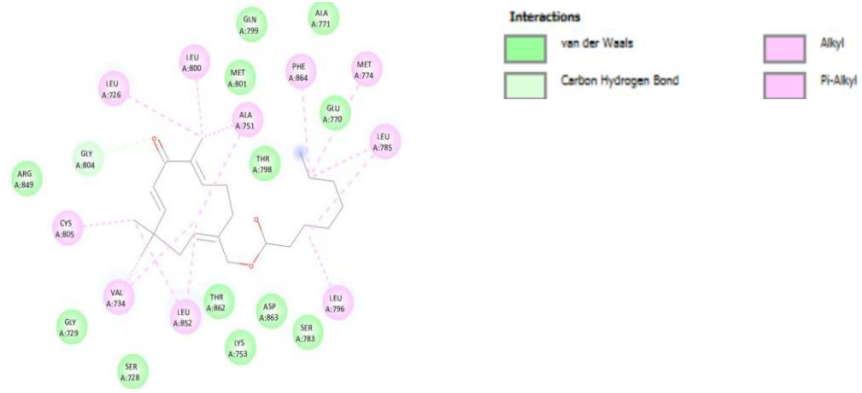
Hydrogen bonds are relatively stronger than van der Waals forces because they involve a partially positive hydrogen atom and a partially negative atom in another molecule, leading to a stronger electrostatic interaction between them. In biological systems, hydrogen bonds play a critical role in maintaining the stability and function of proteins, DNA, and other biomolecules [12].



Natural ligand HER-2



ZER-HER2



6D-HER2

Fig. 2. Binding of HER2 receptor with various ligan.

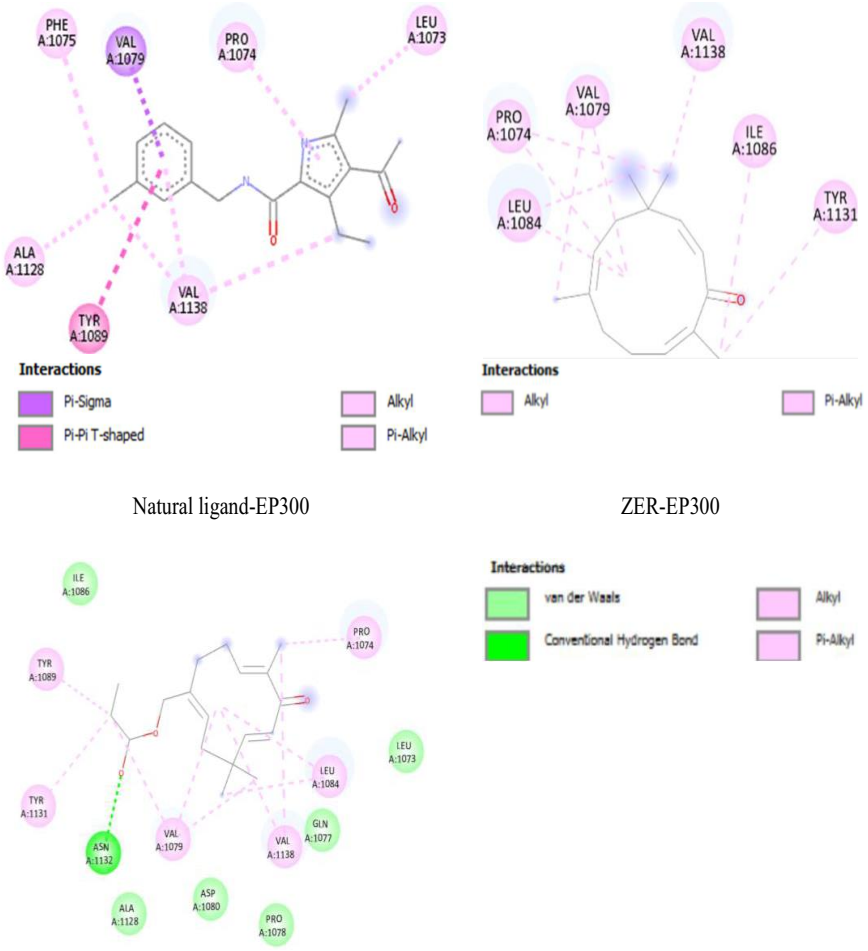


Fig. 3. Binding of EP300 protein with various ligan.

4 Conclusion

Derivate 6B showed the lowest binding energy for EP300 and derivate 6D showed the lowest binding energy for HER2, both of them indicate the best candidate for breast cancer therapy. Molecular dynamic analysis is needed to validate the efectiveness of the best zerumbone derivatives of molecular docking. Furthermore, synthesis, in vitro and in vivo test of the potential zerumbone derivative are crucial step for the development of breast cancer therapy.

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