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Article

Pharmacophore Modeling, Molecular Docking, and ADMET Approach for Identification of Anti-Cancer Agents Targeting the C-Jun N-Terminal Kinase (JNK) Protein

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Abstract. One of the most prevalent cancers in Indonesia is breast cancer, based on Indonesia's pathological-based registration. Breast cancer is a complex, heterogeneous disease classified into hormone-receptor-positive, human epidermal growth factor receptor-2 overexpressing (HER2+) and triple-negative breast cancer (TNBC) based on histological features. Patients with HR+, HER2- Early Breast Cancer (EBC) do not experience recurrence or recurrence for a long time with currently available standard therapy [11]. However, up to 30% of patients with high-risk clinical and/or pathological features may experience a relapse in the first few years. This results in the need for research and development regarding updates in medicine both in terms of treatment and targets and drug compounds used. The c-Jun N-terminal kinase (JNK) protein functions in signaling and influences the apoptotic pathway as well as cancer cell survival. In this study, an insilico screening experiment of inhibitory compounds was carried out on the JNK protein receptor target by screening compounds and molecular docking of compounds for breast cancer therapy.Two novel herbal compounds, Mangostin and ent-Copalyl Dyphospate, have the potential to be turned into medicines that may cause apoptosis through JNK protein targets according to an in-silico-based molecular simulation technique.

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1. Intoduction

Breast cancer is a most common cancer in the world, It is caused by of malignancy breast tissue that originates from the ductal epithelium or lobules [1-2]. Based on Pathological Registration in Indonesia, breast cancer is one of the most common types of cancer in Indonesia with a relative frequency of 18.6% in women and breast cancer is the most prevalent cancer [3-5]. Breast cancers differ in their clinical behavior and treatment responses due to the heterogeneity of their morphologic and biological characteristics. Based on the molecular and pathological type of breast cancer, it is classified based on the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), histological grade, and multigene prognostic tests [6-7].

Breast cancer is a complex, heterogeneous disease classified into hormone-receptor-positive, human epidermal growth factor receptor-2 overexpressing (HER2+) and triple-negative breast cancer (TNBC) based on histological features [8-10]. Endocrine therapy, the mainstay of treatment for hormone-responsive breast cancer involves use of selective estrogen receptor modulators (SERMs), selective estrogen receptor downregulation (SERDs) and aromatase inhibitors (AIs). Agents that target estrogen receptor (ER) and HER2 such as tamoxifen and trastuzumab have been the most extensively used therapeutics for breast cancer [11-12]. Patients with HR+, HER2- Early Breast Cancer (EBC) do not experience recurrence or recurrence for a long time with currently available standard therapy [13]. However, up to 30% of patients with high-risk clinical or pathological features may experience a relapse in the first few years. This results in the need for research and development regarding updates in medicine both in terms of treatment and targets and drug compounds used [11]. In addition to the need for new methods and targets in treatment. Crosstalk between ER and other signaling networks as well as epigenetic mechanisms have been envisaged to contribute to endocrine therapy resistance. HER2, a complex, heterogeneous, aggressive form of breast cancer in which the cells express targeted hormone is refractory to therapy [14-16].

Several molecular targets are being explored to target HER2 including androgen receptor, epidermal growth factor receptor (EGFR), poly(ADP-ribose) polymerase (PARP), and vascular endothelial growth factor (VEGF). Receptors, protein tyrosine kinases, phosphatases, proteases, PI3K/Akt signaling pathway, microRNAs (miRs) and long noncoding RNAs (lncRNAs) are potential therapeutic targets [17-18]. Inflammatory cytokines, thermal shock, oxidative stress, osmotic stress, and UV irradiation are a few of the stimuli that might activate the JNK pathway. When JNK is activated, it phosphorylates several proteins on particular serine and threonine residues that are followed immediately by a proline residue. This regulates a range of cellular functions, such as cellular proliferation, differentiation, survival, and apoptosis. JNK plays a dual role in regulating the ratio of apoptosis to proliferation, and the result of JNK activation is influenced by the cellular environment and the particular stimuli. The JNK signaling pathway has been linked to a variety of pathophysiological illnesses, including cancer, diabetes, autoimmune diseases, and neurodegenerative diseases. Because of this, JNK is a therapeutic target for many disorders [19-20].

Superior treatment options are needed to prevent early relapse and the development of metastases for this group of patients [21-22]. The c-Jun N-terminal kinase (JNK) protein functions in signaling and influences the apoptotic pathway as well as cancer cell survival [12]. Emerging evidence suggests that JNK promotes tumor development and is involved in a variety of cancers, including human pancreatic, lung, and breast cancer. In-silico drug design consist of theoretical and computational approaches can be used to identify novel hits or leads against selected biological active macromolecules. Nowadays, computer aided drug design (CADD) approach like pharmacophore modeling, virtual screening and molecular docking approaches are widely used to discover, develop, and analyze drugs and similar biologically active molecules. In this study, an insilico screening experiment of inhibitory compounds was carried out on the JNK protein receptor target by screening compounds and molecular docking of compounds for breast cancer therapy [11, 23-24].

2. Experimental Section

The experimental section based on firoz, dkk method [25], this workflow describes the steps in screening Hit Compound and finding the active site in the target protein.

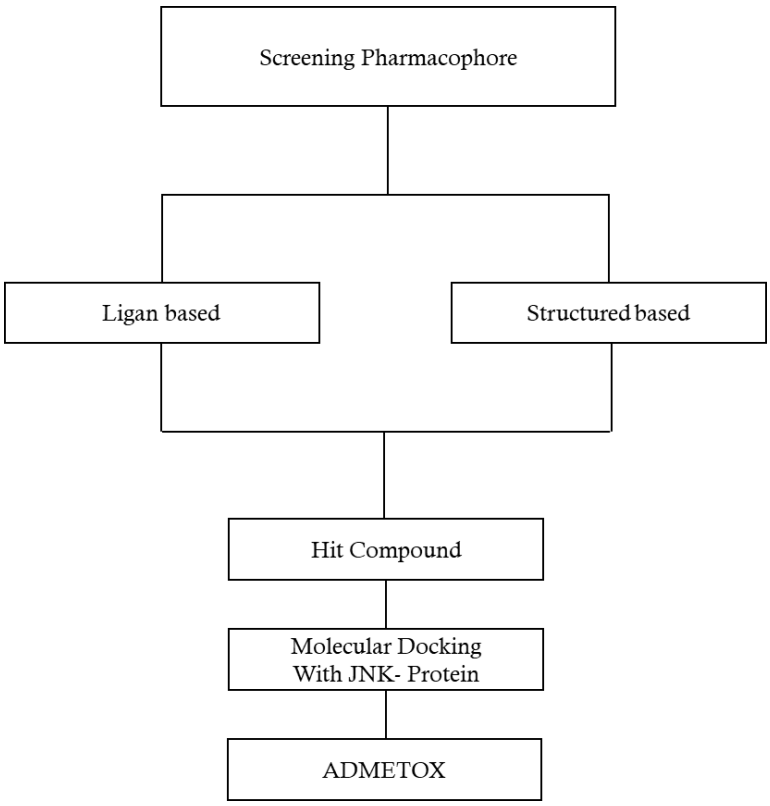


Figure 1. Pharmacophore modeling and molecular docking JNK-Protein workflow

2.1. Materials and Methods

2.1.1 Structure and Ligand-Based Pharmacophore

The HerbalDb Compound Library, a library of herbal compounds from Indonesia from the FMUI Department Pharmacy, was used as the compound database. The database is downloaded from the website's server (http://herbaldb.farmasi.ui.ac.id/v3) to retrieve the data. Using an algorithm and the Liganscout 4.3 software, a pharmacophore model-based ligand screening was produced. Using decoy chemicals supplied from the Useful Decoys Database (DUDe) online site, the final model was additionally cross screened. The ROC curve and AUC values utilized for model validation were acquired from screening results based on the pharmacophore model [25-26].

The base structure uses the JNK protein (4L7F) obtained from the protein data bank server (https://www.rcsb.org/). The best compound with the highest binding affinity (kcal/mol) was selected for structure-based pharmacophore modeling. The highest-scoring compounds in complexes with JNK proteins are used to interact with natural compounds. The results of screening compounds based on ligands and protein structures will produce compounds called hit compounds [27].

2.1.2 Molecular Docking

The hit compounds obtained from the pharmacophore screening were carried out by molecular tethering using software including AutoDock and AutoDock Vina with the Lamarckian genetic algorithm (LGA) as an assessment function. This study uses PyRx AutoDock Vina tools to carry out

molecular docking interactions. The resulting docking compounds with better binding affinity (kcal/mol) were extracted and visualized using BIOVA Discovery Studio Visualizer Tool 16.1. [28-29].

2.1.3 Analysis of the ADME-TOX

Evaluation of Absorption, Distribution, Metabolism, and Excretion (ADME) properties is one of the main criteria before developing a molecule into a drug. Previously, many drug candidates could not meet the demand for clinical trials, so computer-based prediction was important for the early stages of prediction. Physicochemical properties, hydrophobicity, lipophilicity, gastrointestinal environment, and the blood-brain barrier are directly affected by the ADME profile before the drug is excreted from the body via urine and feces [14]. The freely accessible Swiss-ADME server (http://www.swissadme.ch/) was used to evaluate ADME properties such as the solubility profile, GIT absorption, and bioavailability profile of the selected compounds [15].

3. Results and Discussion

3.1 Ligand-Based Pharmacophore

The herbal_db database is a curated collection of commercially available chemical compounds where we can get information on the molecular weights, chemical structures, and physical and chemical properties of biologically active macromolecules. It contains more than 230 million compounds that can be purchased in a 3D format to freely accessible websites, ready to be used for further analysis [16]. Screening results from the herbal_db database with the pharmacophore model were carried out using the screening perspective method as shown in Figure 2. Then the results of the compounds with the best-fit scores were obtained which could be used for further analysis in the form of docking and admetox simulations[30-32].

Model validation is needed to obtain authentic pharmacophore analysis and to evaluate the quality of molecular models. The structure- and ligand-based pharmacophore model produced in this study was validated prior to database screening to evaluate whether the selected model was able to distinguish the active compound from the test set. The pharmacophore model was validated using 20 active compounds as a test set and 1200 decoy compounds obtained from the Useful decoys Database (DUDe). From a total of 32 drug compounds that were considered to have gone through phase III, they were separated into 2 groups of test sets and training sets. Active test sets with constant inhibitor IC50 values were combined with test compounds and initial screening was run to validate the model. The performance of classification models such as AUC values and EF values of compounds is estimated from receiver operating characteristic (ROC) curves. In general, ROC is a probability graph that states the performance of a classification model that can provide an overview of the degree of separation, where AUC is used to describe a summary of model performance. Models with higher AUC values should have better predictability. AUC values range between 0 and 1, so a model whose prediction rate is 100% correct has an AUC value of 1. In our validation process, the initial enrichment factor (EF1%) is 5.1 with AUC (area below ROC) a very good value for the curve in the threshold of 1% is 0.83 , which proves that the pharmacophore model has the ability to distinguish the actual active substance from the training compound [33-35].

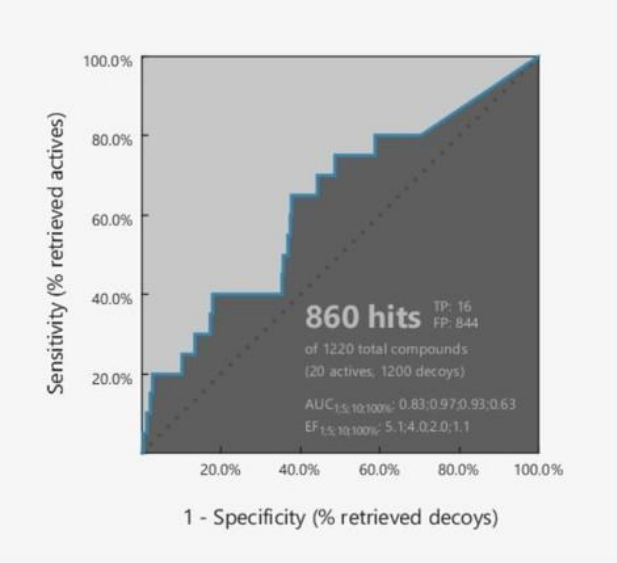


Figure 2. ROC curve model pharmacophore (Liganscout)

The results of the screening of ligand-based pharmacophore compounds with the specified pharmacophore model were then screened with the herbalDB database. Obtained 10 compounds that have features similar to the validation model.

3.2 Pharmacophore Structure Based

The validated protein structure can be downloaded from the protein database or homology modeling can be done to determine the 3D model of a protein. To identify the antagonists to the x-ray structure of protein desirability protein Jnk (PDB: 4L7F) crystals and a structure-based pharmacophore model for the enzymatic cavity was generated. The 3D and 2D structures are displayed as shown in the image obtained from the RSCB PDB webserver [36].

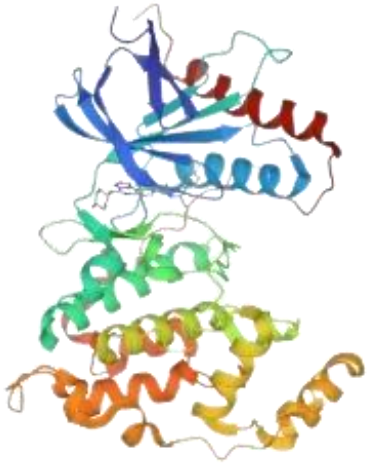
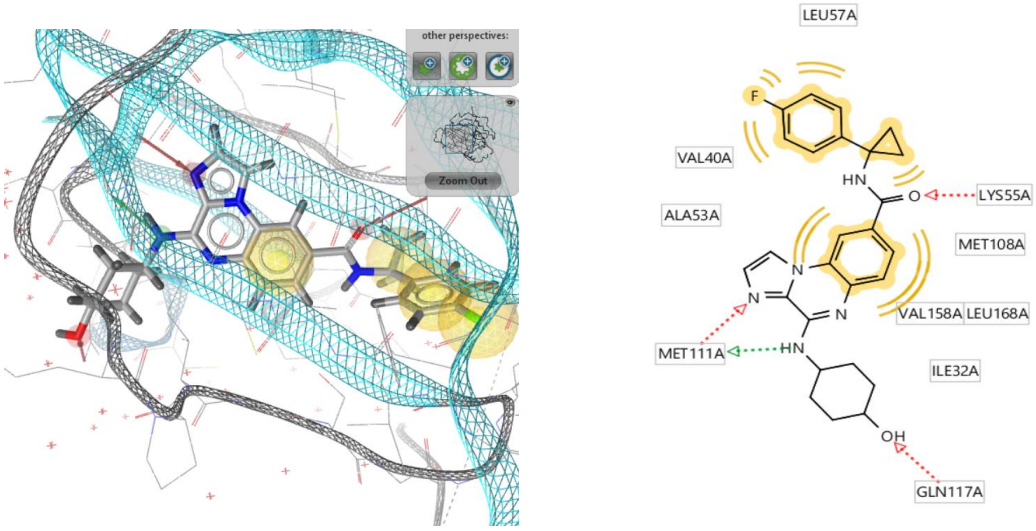


Figure 3. JNK protein structure (Pdb: www.rcsb.org/structure/4L7F)

The protein structure is known to have one chain with one native ligand. With the identification of the structure using the X-Ray method and a resolution of 1.55A, this is good because it is still below 2A which is the maximum resolution sharpness limit for proteins needed in molecular docking [37].

The ligand binding capacity of selected 4L7F proteins was determined experimentally. An Overall expression can be regulated by binding of the inhibitor to the active site of the JNK protein. Because sometimes the ability of the proper inhibitor against any protein may be unreliable due to improper binding. Thus, determination of the active series of inhibitors should be checked for sufficient interactions to obtain more biological activity compared to the existing ones. LigandScout4.3 advanced important molecular design software was used to generate key chemical features based on the pharmacophore model [38-39]. In this process, 8 interaction features were obtained on the 417F protein which can interact with several amino acids [16]. As shown in the image, screening is carried out by removing the H2O group at the native ligand binding position with the protein. Some of the amino acids that have potential biological interactions with ligand compounds are shown in figure 4.



(a) (b)

Figure 4. (a) 3D structure 8 interaction features were obtained on the 417F, (b) 2D 8 interaction features were obtained on the 417F (LigandScout4.3)

Based on the results of screening with the herbal_db database, compounds were obtained that could bind to the amino acid position that interacted with the native ligand. Some of these compounds include:

Table 1. Screening compound from ligan and structure pharmacophore screening

Compound	
Dehydrosafynol.mol	Chlorogenic acid.mol
Safynol.mol	Violaxanthin.mol
beta-Citraurin.mol	Capsorubin.mol
Kuwanon T.mol	6-Shogaol.mol
Geraniol.mol	Octadienoyl-4-deoxyphorbol 13-acetate.mol
9-Ribosyl-cis-zeatin.mol	Dehydrosafynol.mol
ent-Copalyl diphosphate.mol	Anacyclin.mol
Mangostin.mol	Spilanthol.mol

From the two screening approaches, 17 compounds were taken to be followed by further molecular docking processes.

3.3 Molecular Docking

Molecular docking is an important part of the drug design process, which is carried out in studies to evaluate the binding ability of hit compounds to target proteins. The 4L7F monomer protein already has an active group which is the native ligand attachment site, the position of the ligand is then matched to the hits compound. The protein was prepared and the receptor lattice with a grid box of 60 x 60 x 60 size with coordinates (x, y, z) -4.963, 54.083, 4.844. Some of the hit's compounds that are suspected to have high pharmocophore fits and have a match with the receptor were selected and docked on the 4L7F protein and a binding energy value, RMSD and inhibition constant were listed. In the molecular docking process, it is also carried out by comparing the results of binding energy values, RMSD and inhibition constants with native ligands and also positive control compounds which are drug compounds that have been circulating in the market.

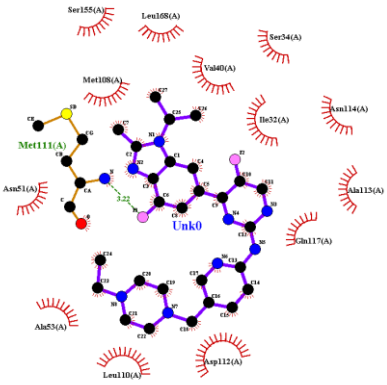
It is known that the top three compounds that have the best values of binding energy, RMSD and inhibition constant are ent-Copalyl diphosphate with binding energy -10.31, and inhibition constant of 27.63 nM (nanomolar) (Temperature = 298.15 K) and RMSD 52.147 A, this value is obtained at the 5th run position out of a total of 10 runs assigned to each run for all docking interactions for each compound, Capsorubin with a binding energy value of -11.59 inhibition constant 3.19 nM (nanomolar) (Temperature = 298.15 K) and RMSD value of 32.825 A on running to 3 out of 10 runs, Mangostin with an energy binding value of -10.35 , an inhibition constant of 25.70 nM (nanomolar) (Temperature = 298.15 K) and an RMSD value of 47.515 A in the 8th running, of the three compounds which had the lowest binding energy value, further analysis was carried out, namely analysis protein–ligand interactions [40-42].

Table 2. Molecular docking results from 17 compund, native ligand and positive control with protein (JNK): Binding energy, RMSD and constanta inhibition value

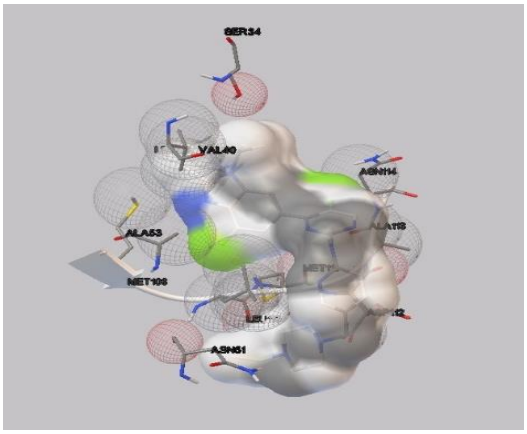
No	Compound	Binding energy (kcal/mol)	Constant inhibition [Temperature = 298.15 K]	RMSD
1	Native Ligan	-12.09	1.37 nM	0.77 A
2	Dehydrosafynol.mol -	-5.28	135.90 uM	52.840 A
3	Safynol.mol	-5.33	124.43 uM	49.230 A
4	beta-Citraurin.mol	-10.43	22.71 nM	46.089 A
5	Kuwanon T.mol	-10.25	30.54 nM	53.130 A
6	Geraniol.mol	-5.37	115.58 uM	53.111 A
7	9-Ribosyl-cis-zeatin.mol	-6.51	16.98 uM	53.228 A
8	ent-Copalyl diphosphate.mol	-10.31	27.63 nM	52.147 A
9	Mangostin.mol	-10.35	25.70 nM	47.515 A
10	Chlorogenic acid.mol	-7.77	2.01 uM	48.565 A
11	Violaxanthin.mol	-10.70	14.32 nM	52.384 A
12	Capsorubin.mol	-11.59	3.19 nM	32.825 A
13	6-Shogaol.mol	-7.20	5.29 uM	49.738 A
14	Deoxyphorbol3-acetate.mol	-9.74	72.54 nM	46.474 A
15	Dehydrosafynol.mol	-5.31	128.76 uM	52.547 A
16	Anacyclin.mol	-7.19	5.33 uM	41.034 A
17	Spilanthol.mol	-7.40	3.75 uM	47.794 A
18	Gallocatechin-epicatechin.mol	-8.27	873.88 nM	48.507 A
19	Pollenitin.mol	-7.91	1.59 uM	54.630 A
20	Kontrol (Abemaciclib)	-10.87	10.77 nM	54.775 A

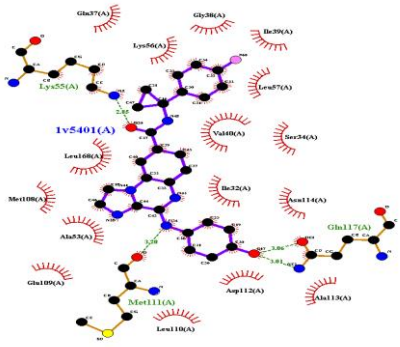
Based on analysis of protein ligand interactions from the top 3 compounds, native ligand and positive control. There are several amino acids which are always the sites of interaction for each compound, such as met111(A), leu110(A), and ile32(A). Compounds that have similar interactions with native ligands and positive control are ent-Copalyl diphosphate because they have the same hydrogen bonds with native ligands at the amino acid position lys55(A) and interact with van der Walls interactions with amino acids met108, met111, leu168, val40, ile32, ser34, ala113, leu110 and ala53.

Then other compounds that also have interactions similar to native ligands and positive control compounds are mangostin, mangostin has hydrogen bonds with Asn114 and Gln117 which are amino acids that also interact van der Wals with control compounds and native ligands, several other amino acids also interact by van der wals interactions with mangostin compounds and also into amino acids that interact with control compounds and native ligands such as: met108, met111, leu168, val40, ile32, ser34, ala113, leu110 ala53, and glu09. Whereas casporubin interacts with van der Walls interactions with several other amino acids and only a few are the same as the native ligand and its control compounds. These protein-ligand interactions were visualized in 2D and 3D using Marvin Sketch and Autodock Vina.

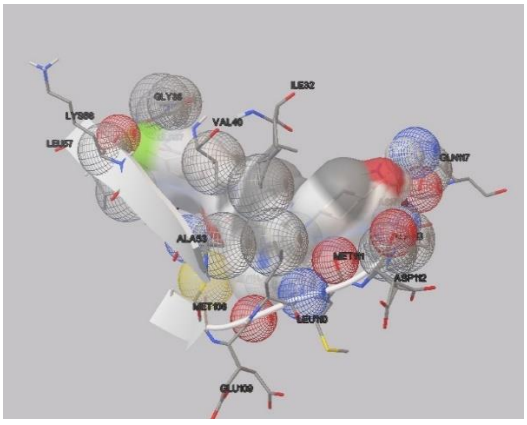


Abemaciclib_kompleks





kompleks_native_ligan



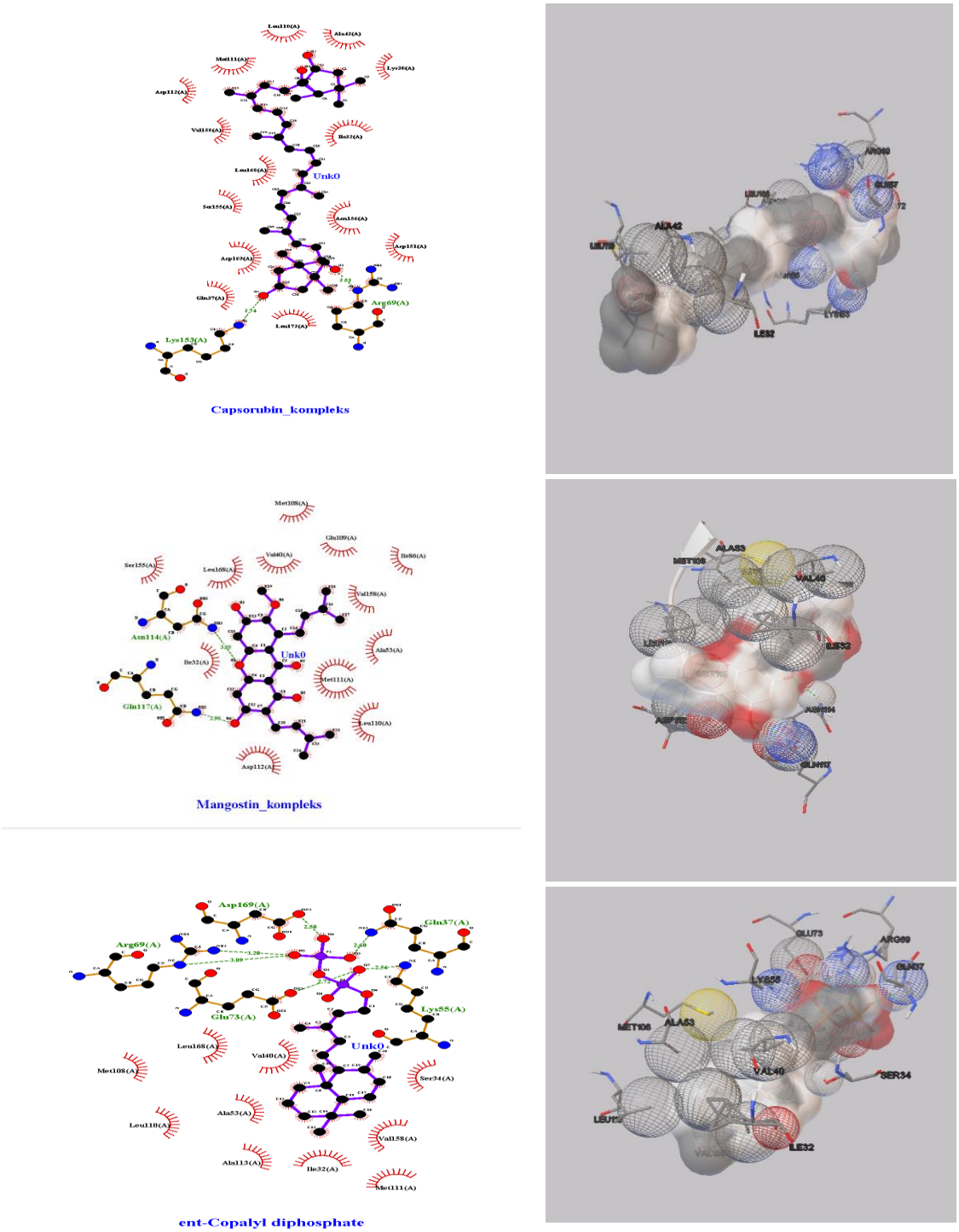


Figure 5. Interaction visualization control (Abemaciclib), native ligan, Capsorubin, Mangostin, ent Copalyl Dyphosphate with protein 4L7F (BIOVIA)

3.4 Adme-Tox

Analysis of drug-like properties was carried out using the SwissADME program, Absorption, distribution, metabolism, excretion, and toxicity profile predictions were carried out using Swiss-ADME server. Analysis of drug-like properties and ADMET prediction of the compounds capsorubin, mangostin, and ent-copalyl diphosphate was carried out by entering the SMILES list or the Simplified Molecular Input Line Entry Specification.

Table 3. Admetox result from swiss ADME server

Ligans	Druglikeness					Pharmacokinetics					Toxicities		
	MW(g/mol)	HBA	HBD	LogP	GI Abs	Inhibitor CYP					AMES	Carcinogenesis	AOT
						CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4			
Capsorubicin	600.87	4	2	8.33	Low	No	No	No	No	Yes	-	-	III
Mangostin	410.46	6	3	4.64	High	No	No	Yes	No	No	+	-	III
ent–Copalyl Dyphosphate	450.44	8	6	3.50	Low	No	No	No	No	No	-	-	III

Analysis of drug-like properties resulted in a score of compound properties against the Lipinski rule which included the molecular weight of the compound, the value of the log P partition coefficient, the number of hydrogen bond donors, and the number of hydrogen bond acceptors. ADMET profile prediction shows various profiles of absorption, distribution, metabolism, excretion, and toxicity. The prediction of these properties includes absorption, distribution, metabolism, excretion, and toxicity [6]. In several journals, it is known that Alpha-Mangostin can downregulate the c-JUN N-terminal kinase (JNK) pathway thereby inhibiting the ROS-mediated apoptotic pathway [43]. Mangostin regulates downstream effectors of the PI3K/AKT signaling pathway by decreasing RXRα/tRXRα. Mangostin can trigger PARP cleavage and induce apoptosis, and inhibit the development of breast cancer metastasis [44-45].

4. Conclusion

Two novel herbal compounds, Mangostin and ent-Copalyl Dyphospate, have the potential to be turned into medicines that may cause apoptosis through JNK protein targets according to an in-silico-based molecular simulation technique. In comparison to native ligands and their positive control, compounds were chosen based on the strength of their interactions with proteins and binding energies. This molecule conforms with the Lipinski rule criteria based on the in-silico toxicity test, and it has low toxicity based on the pharmacokinetic and toxicity testing.

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