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In Silico Study on the Effect of Heliannuol A, B, C, D, E Compounds of Sunflower (*Helianthus annuus* L.) on Dual PI3K/mTOR (5OQ4) Enzyme

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ABSTRACT

Heliannuol is a sesquiterpene that has a benzoxepine ring, oxepin. Many derivatives of benzoxepine compounds show anticancer activity by inhibiting the phosphoinositide 3-kinase *(PI3K)* enzyme. These enzymes play a role in cell proliferation and growth. The study aims to predict the physicochemical properties using Lipinski's Rule of Five parameters on phosphoinositide 3- kinase (*PI3K/Mtor*; PDB 5OQ4) enzyme and the toxicity of *Heliannuol A, B, C, D, E* compounds. The process uses the pkCSM online tool. The validation of receptor 5OQ4 is done using the value parameter *RMSD* < 2 (*Å*). Protox online tool dan pkCSM online tool is employed to predict the toxicity using parameter LD_{50}, skin sensitization, Ames toxicity, hepatotoxicity, and toxicity class. The interaction of ligan and enzyme is tested using *Molegro Virtual Docker 6.0.* Heliannoul A, B, C, D, E compounds fulfill Lipinski's Rule of Five. The receptor 5OQ4 is known valid using the value of *RMSD* 0,923 (*Å*). Heliannuol A, B, C, D, E compounds inhibit Dual PI3K / mTOR enzyme less than Bimiralisib. As a result of the toxicity test of compounds Helliannouls A, B, C, E, and Bimiralisib compounds are included in class 4, while Helliannouls D compounds are included in class 5.
Keywords: *in-silico; Heliannuols; PI3K/mTOR; glioblastoma*

INTRODUCTION

Brain cancer covers about 85-90% of all central nervous system cancers. The incidence rate for malignant brain cancer worldwide based on world standard population rates is 3.4 per 100,000 population. The mortality rate is 4.25 per 100,000 population per year. Understanding the molecular principles and signaling pathways involved in glioblastoma is essential for the development of more effective therapies. (AnZhenyi *et al*., 2018; (Pedoman Nasional Pelayanan Kedokteran Tumor Otak, 2017).

The *phosphoinositide 3-kinase* signaling pathway have a central role in the regulation of signal transduction, mediating various biological processes, including cell proliferation, apoptosis, metabolism, motility, and angiogenesis in glioblastoma. Excessive activation of the PI3K or Akt pathway provides rapid growth, tumor development, and multi-drug resistance to glioblastoma cells (Zhao *et al*., 2017).

Heliannuols is a new group of phenolic sesquiterpenes isolated from sunflower cultivars (*Helianthus annuus*). The chemical structure of *Heliannuols* has a benzoxepine ring in which various benzoxepine derivatives have shown activity towards various types of cancer. The benzoxepine derivative mechanism inhibits the enzyme *phosphoinositide* 3- kinase (PI3K) (Hefron *et al*., 2011; Kuntala *et al*., 2017).

The insilico test is a method through simulation carried out with computer media. In silico test was conducted by tethering the molecule (molecular docking) Selected drug candidates to the receptor. *In Silico* testing has the advantage of efficiently finding new drugs using a modeling method that is a simulation with the help of computational technology (Pelkonen *et al*., 2011). Prediction of drug activity at the receptors can be performed using in silico test.

This research was carried out because, in the development of cancer drugs, it was needed to predict anticancer activity, toxicity, and physicochemical properties, research of *Heliannuol A, B, C, D, E* compounds contained in sunflower plants (*Helianthus annus* L.) towards *phosphatidylinositol 3-kinase*s receptors.

METHODOLOGY

Materials

Tools: Chem Bio Draw Ultra Version 12 (CambridgeSoft), Avogadro, Molegro Virtual Docker 6.0, protox online tool, pkCSM online tool.
Ingredients: Dual PI3K / mTOR enzyme (5OQ4), protein subtype: gamma isoform subunit, Native Ligand: 5- (4,6-dimorpholin-4-yl-1,3,5-triazine-2-yl) - 4- (trifluoromethyl) pyridin-2-amine or

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bimiralisib (PQR309), resolution: 2.70 Å; 2-dimensional and 3-dimensional structures of *Heliannuol* A, B, C, D, E, and *Bimiralisib* compounds along with the SMILE code.

Methods

Ligand Preparation and Receptors

Receptor preparation was done through downloading on the protein data bank website https://www.rcsb.org/structure/5OQ4. Ligand preparation was done by drawing ligands in 2D and obtained the SMILE code used Chem Bio Draw Ultra version 12. Then 3D was changed using Avogadro. Geometry optimization using Avogadro with the MMFF94 method.

Physicochemical Test

The Physicochemical prediction was carried out using Lipinski parameter 5 was molecular weight, the logarithm of octanol/water partition coefficient, number of bonds between rotating atoms, Hydrogen Bond Acceptors, Hydrogen Bond Donors, and Polar Surface Activity by entering SMILE code into the online tool pkCSM.

Determination of Cavity

The determination of the cavity was used to detect the location of the ligand with 5OQ4 receptors. The selection was made by looking at the native sites of the ligands that interact with the 5OQ4 receptor. A cavity at the receptor is shown in green.

Method Validation

The validation parameter seen was the value of RMSD (Root Mean Square Deviation) through backing natives with receptors. Validation was done by re-docking between native ligands and 5OQ4 receptors.

Docking *Heliannuols* A, B, C, D, E, and *Bimiralisib*at the 5OQ4 receptor

Docking *Heliannuols* A, B, C, D, E, and *Bimiralisib* were performed used Molegro Virtual Docker 6.0. RS value (rerank score) was the bond energy needed to form bonds between ligands and receptors so that the activity of a compound can be predicted (Zaidan *et al*., 2019).

Toxicity Test

Prediction of compound toxicity based on skin sensitization, Ames toxicity, Hepatotoxicity, LD50 using the online Protox tool, and online pkcsm. The toxicity class is based on the Globally Harmonized System.

RESULT AND DISCUSSION

This research aims to predict physicochemical properties with the legal parameters of five Lipinski, activity towards the *phosphoinositide 3-kinase* enzyme (PI3K/ mTOR; PDB 5OQ4), and the toxicity of the compounds *Heliannuols* A, B, C, D, E.

Ligand Preparation and Receptors

The Dual PI3K / mTOR (5OQ4) enzyme was used as a receptor protein and the ligand used is the compound *Heliannuol* A, B, C, D, E with the comparative compound *Bimiralisib*. The results of two-dimensional images are shown in figure 1. The process of geometry optimization aims to minimize energy to obtain the most stable structure (Susanti *et al*., 2018). The results of the geometry optimization of *Heliannuol* A, B, C, D, E, and *Bimiralisib* compounds are shown in table I.

Physicochemical Test

The physicochemical test aims to predict the absorption properties of the compounds *Heliannuols* A, B, C, D, E, *Bimiralisib*. Compounds have low absorption if there are more than five donor H-bonds (expressed as the number of OH and NH), molecular weights more than 500, Log P is more than 5, and there are more than 10 H-bond acceptors (expressed as the number of Ns and Os) (Lipinski *et al*., 2011). Prediction results of physicochemical properties can be seen in table II.

The results of the analysis in table II that the compounds *Heliannuols* A, B, C, D, E, comparative compounds (*Bimiralisib*) meet the five Lipinski legal requirements, the compounds are predicted to be easily absorbed. They have good permeability, so it requires further testing related to distribution, metabolism, and excretion in a manner *in silico*.

Determination of Cavity

Native sites of binding ligands have potential as active holes so that they can be used for docking ligands from the compound *Heliannoul* A. B, C, D, E, *Bimiralisib*. The cavity at the receptor is shown in green. figure 2 A (A) shows the five cavities that may interact with the 5OQ4 receptor. The five cavities were selected by looking at the places of native ligands that interact with receptors 5OQ4. The results of cavity determination can be seen in figure 2. The results of the selection obtained one cavity, namely volume 452.608, with a surface area of 1149.44.

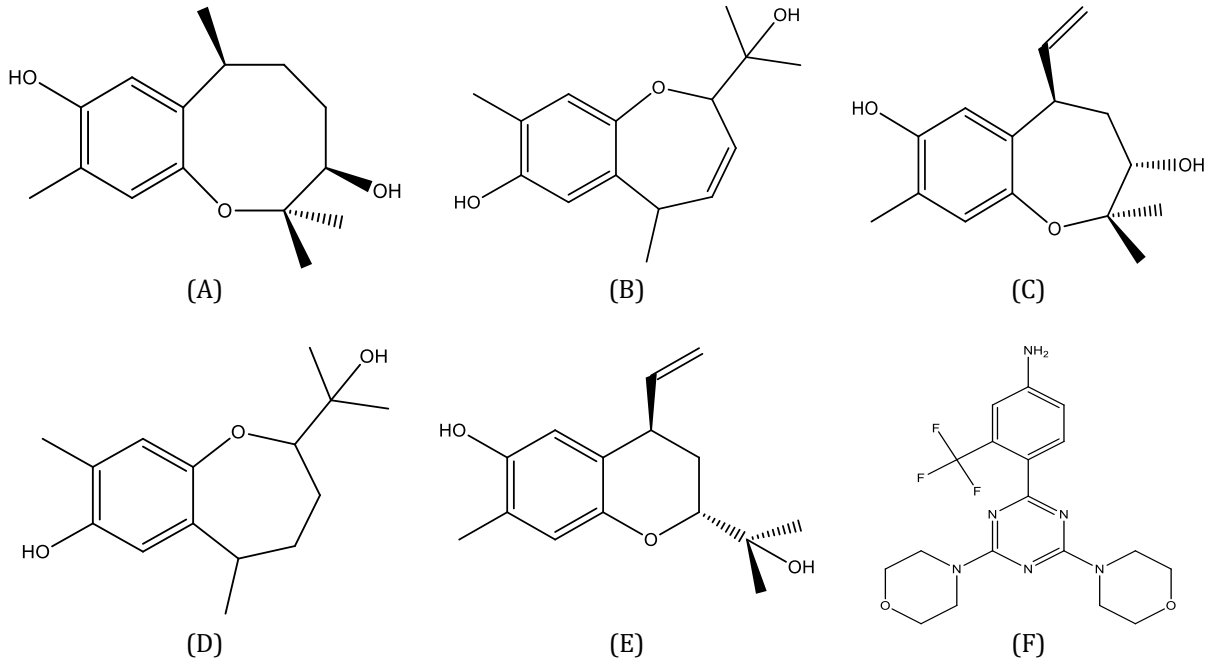


Figure 1**:** Two-dimensional structure of Heliannuols A (A); Two-dimensional structure of Heliannuols B (B); Two-dimensional structure of Heliannuols C (C); Two-dimensional structure of Heliannuols D (D); Two-dimensional structure of Heliannuols E (E); Two-dimensional structure Bimiralisib dimension (F).

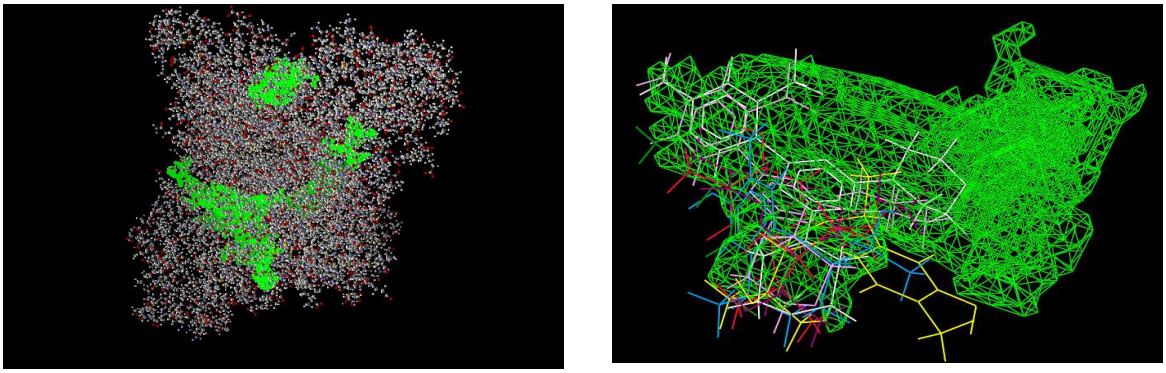
Table I. Results of determining the minimum energy of *Heliannuols* compounds with MMFF94 in Avogadro applications

Compound	Minimum Energy (Kcal/mol)			Average (Kcal/mol), ± SD
	Replication I	Replication II	Replication III	
Heliannuols A	75,835	75,835	75,834	75,835 ± 0,0003
Heliannuols B	76,461	76,462	76,460	76,461 ± 0,0010
Heliannuols C	67,480	67,480	67,480	67,480± 0,0001
Heliannuols D	63,585	63,585	63,585	63,585± 0,0000
Heliannuols E	54,188	54,188	54,188	54,188 ± 0,0000
Bimiralisib	67,265	67,265	67,265	67,265 ± 0,0000

Table II. Results of the determination of physicochemical properties and prediction of toxicity to the compounds *Heliannuols* A, B, C, D, E, *Bimiralisib*

Compound	Parameters of the Five Lipinski Laws					PSA (A2)	Application of the Five Lipinski Laws
	BM (g/mol)	Log P	Torsion	HBA	HBD		
Heliannuols A	250.338	3.11622	0	3	2	108.412	Yes
Heliannuols B	248.322	2.89222	1	3	2	107.722	Yes
Heliannuols C	248.322	2.89222	1	3	2	107.722	Yes
Heliannuols D	250.338	3.11622	1	3	2	108.412	Yes
Heliannuols E	248.322	2.89222	2	3	2	107.722	Yes
Bimiralisib	410.4	1.8128	3	8	1	165.004	Yes

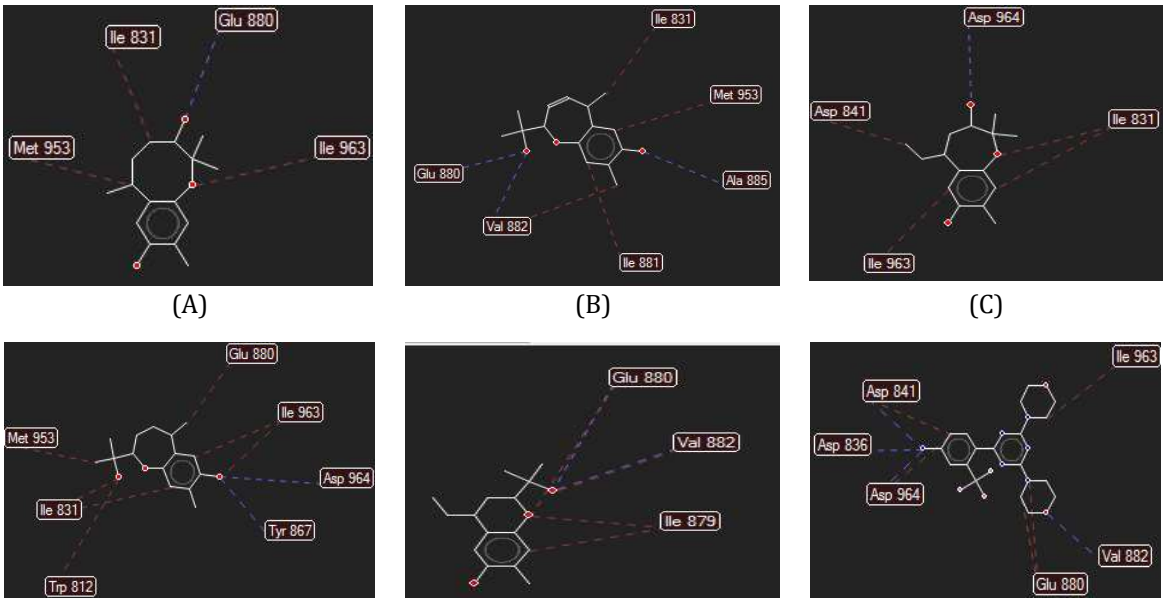
Note: BM (Molecular weight) <500; Log P (The logarithm of octanol/water partition coefficient <5; Torsion (H-bonding that can rotate); HBD (donor H-bonds) <5; HBA (H-bond acceptors) <10; PSA (Polar Surface Activity)



(A) (B)

Figure 2. The results of cavity detection at the 5OQ4 receptor (A); Cavity that binds to the native ligand 5OQ4 receptor (cavity 2 (B))

Note Figure 2A : *Cavity* 1 Vol = 1339.39 Surface = 2714.88; *Cavity* 2 Vol = 452.608 Surface = 1149.44; *Cavity* 3 Vol = 285.696 Surface = 697.6; *Cavity* 4 Vol = 143.872 Surface = 559.36; *Cavity* 5 Vol = 73.216 Surface = 243.2
Note Figure 2B : Red: *Heliannuols* A; Yellow: *Heliannuols* B; Green: *Heliannuols* C; Blue: *Heliannuols* D; Purple: *Heliannuols* E; Pink: *Bimiralisib*



(A) (B) (C)

(D) (E) (F)

Figure 3. The results of two-dimensional interaction of hydrogen bonds (blue line) and steric bonds (red lines) *Heliannuols* A (A); *Heliannuols* B (B); *Heliannuols* C (C); *Heliannuols* D (D); *Heliannuols* E (E); *Bimiralisib*(F) compounds with the 5OQ4 receptor amino acid.

Table III. Method validation results

Ligand	3AW_1201			Average, ±SD
	Replication I	Replication II	Replication III	
Nilai RMSD	0,99	0,8	0,98	0,923 ; ± 0,1069
Rerank Score	-99	-99	-102	-100 ; ± 1,4930
Moldock Score	-113	-107	-118	-113 ; ± 5,1411

Note : RMSD : A deviation value that represents the ratio between the ligand receptor conformation in the simulation process and the initial ligand-receptor conformation (Dermawan *et al*., 2019).

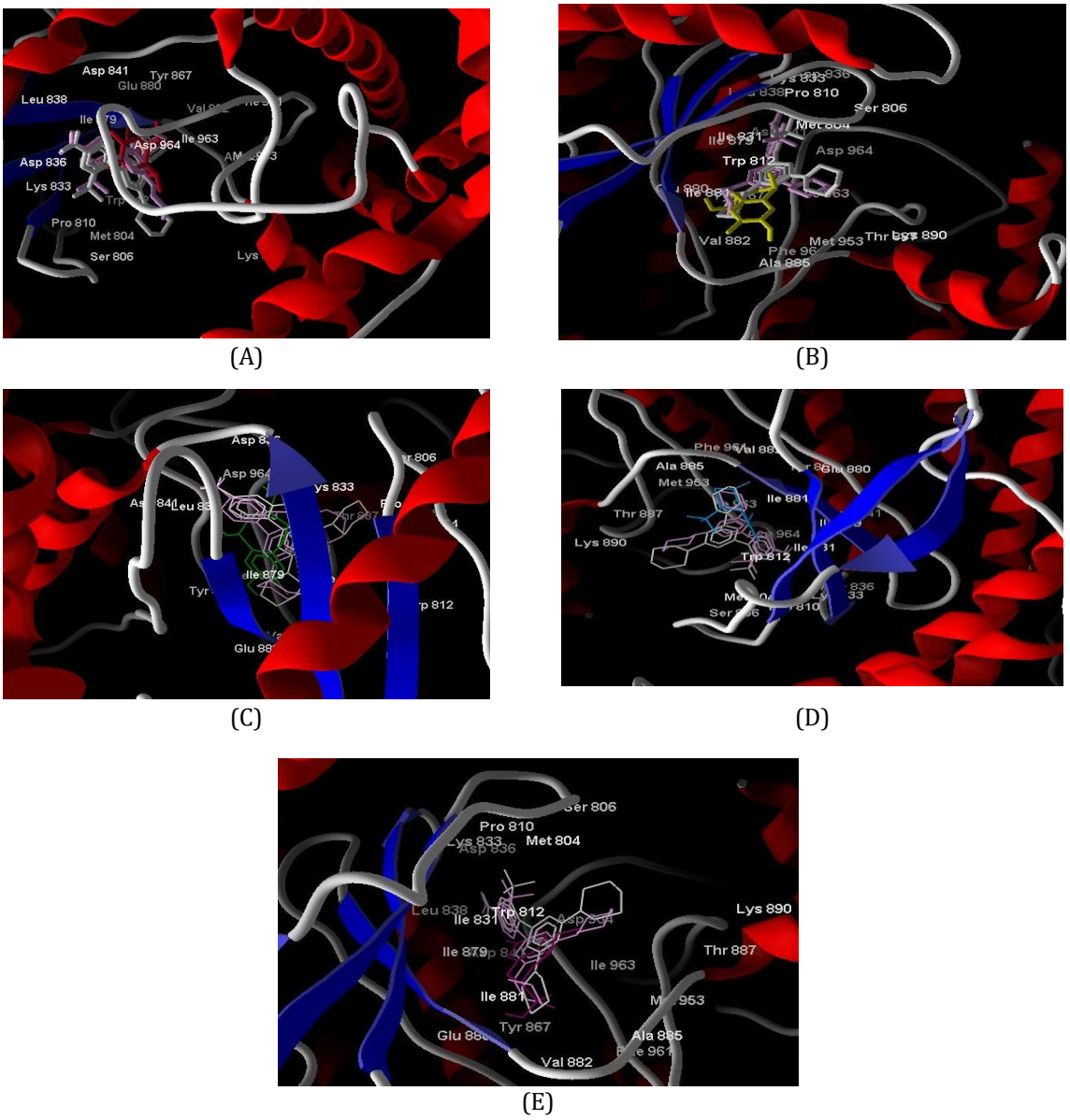


Figure 4. Three-dimensional form of interaction between Heliannuols A (A); Heliannuols B (B); Heliannuols C (C); Heliannuols D (D); Heliannuols E (E); and Bimiralisib compounds with the amino acid receptor 5OQ4.

Note : Red: Heliannuols A; Yellow: Heliannuols B; Green: Heliannuols C; Blue: Heliannuols D; Purple: Heliannuols E; Pink: Bimiralisib.

Method Validation

The validation of the method was replicated three times by re-docking the native ligand with the hole (cavity 2) of the 5OQ4 target protein receptor. Based on the results of re-docking, the average RMSD value is 0.923 Å, which indicates that the docking method meets the validity criteria, so that the parameter can be used for docking the test compound (Muttaqin *et al*., 2019). The results of research validation can be seen in Table III.

Molecular Docking and Amino Acid Interaction

The 5OQ4 receptor docking analysis (an enzyme of Dual PI3K /mTOR) of *Heliannuols* A, B, C, D, E compounds are obtained. The lowest average *rerank score* was -69,000 Kcal/mol in *Heliannuol* E test compound so that it has the highest activity compared to *Heliannuols* compounds A, B, C, D. Bond energy shows the amount of energy needed to form bonds between ligands and receptors. The smaller the bond energy

Table V. Results of Interactions with Amino Acids

Compound	Hydrogen Bonding		Steric Bonding	
	Amino Acid (Distance (Å))	Ligand Group	Amino Acid (Distance (Å))	Ligand Group
Heliannuols A	Glu 880 (3,00)	OH	Ile 963 (3,12)	O
			Ile 831 (3,00)	C
			Met 953 (3,05)	C
Heliannuols B	Val 882 (2,73)	OH	Met 953 (3,08)	C
	Glu 880 (2,69)	OH	Ile 831(2,96)	C
	Ala 885(2,60)	OH	Ile 831(3,97)	C
			Val 882(2,96)	C
Heliannuols C	Asp 964 (3,10)	OH	Asp 841(3,03)	C
			Ile 831(3,04)	O
			Ile 831(2,94)	C
			Ile 963(3,08)	C
Heliannuols D	Tyr 867 (3,07)	OH	Glu 880(2,77)	C
	Asp 964(3,10)	OH	Ile 963(2,90)	C
			Ile 963 (3,19)	OH
			Met 953(3,13)	C
			Trp 812(3,04)	OH
			Ile 831(3,03)	OH
			Ile 831(2,88)	C
Heliannuols E	Glu 880 (3,10)	OH	Glu 880 (3,14)	O
	Val 882 (3,09)	OH	Val 882(3,10)	OH
			Ile 879(3,12)	O
			Ile 879(2,91)	C
Bimiralisib	Asp 836 (2,61)	NH2	Ile 963 (3,14)	C
	Val 882 (3,12)	O	Glu 880(3,18)	N
	Asp 841 (3,42)	NH2	Glu 880(3,08)	C
	Asp 964 (3,10)	NH2	Asp 964(3,16)	C
			Asp 841(3,13)	C

Table VI. Toxicity results of *Heliannuols* and *Bimiralisib* compounds

Compounds	Toxicity				
	LD50 (mg/kg)*	Test AMES**	Hepatotoxic **	Skin Sensitivity **	Toxicity Class *
Heliannuols A	860	No	No	No	4
Heliannuols B	482	No	No	No	4
Heliannuols C	500	No	No	No	4
Heliannuols D	2148	No	No	No	5
Heliannuols E	500	No	No	No	4
Bimiralisib	2000	No	Yes	No	4

Note : * Using *Protox II Online Tool;* ** Using *pkCSM Online Tool*

means, the more stable the bond is. The more stable the ligand binds with the receptor, it can be predicted that the activity will also be more significant (Hardjono, 2016).

The bonds formed between the groups in the ligand with the receptors are essential in determining the affinity formed. The OH group bond with the receptor causes the *rerank score* of *Heliannuols* A, B, C, D, E to be more excellent, and the bonding groups F, N, and O on the *Bimiralisib* causes the value of the score to be smaller, so it has a greater affinity. It is because in *Bimiralisib* there are more bonds with groups that have large electronegativity so that they have a stronger hydrogen bond compared to *Heliannuols* A, B, C, D, E. The results of ligand interactions with amino acids in the 5OQ4 receptor are shown in and in table V and figure 2.

Figure 3 shows the interaction of hydrogen bonds in Aspartic 964 amino acid residues in

Bimiralsib which binds to NH2 groups also occur in *Heliannuol* C, D compounds that bind to OH ligand groups, but do not occur in *Heliannuol* A and E. Compounds. At the same time, hydrogen bonds with acid residues Amino Valin 882 in *Bimiralisib* also occur in *Heliannuols* B and E. The hydrogen bond occurs when a bond between an H atom has a partial positive charge and another electronegative atom and has a pair of free electrons with complete octets such as O, N, and F (F, Siswandono, 2016).

Whereas steric interactions in *Bimiralisib* occur in amino acids Isoleucine 963, Glutamate 880, Aspartate 841 which also binds to *Heliannuol* A, C, D, E. Compounds Steric bonds are involved in the interaction of benzene rings with the plane of the receptor plane and in the interaction of the hydrocarbon chain with macromolecules or receptors (Siswandono, 2016).

Research Bianchi *et al*., 2004 explained that glioblastoma growth is associated with increased concentrations of choline, GABA, isoleucine, leucine, lysine, phenylalanine, taurine, tyrosine, and valine. In grade III and grade IV tumors in concentrations of extracellular phenylalanine, isoleucine, tyrosine, valine, and lysine, all are increased compared to normal tissue.

Toxicity Test

The next *In Silico* research was to predict the compounds *Heliannuol* A, B, C, D, E, *Bimiralisib*. Toxicity prediction results are shown in Table VI, obtained LD50 values of *Heliannuol* D compound (toxicity class 5 (2000 <LD50 ≤ 5000 mg/kg)) greater than *Heliannuol* A, B, C, E and comparative compounds (*Bimiralisib*) with class toxicity 4 (300 <LD50 ≤ 200 mg/kg) so it is predicted that the toxicity of the compound *Heliannuol* D is low.

CONCLUSION

Helliannouls A, B, C, D, E compounds are predicted to have good absorption properties and to inhibit the Dual PI3K/mTOR enzyme is predicted not to be greater than *Bimiralisib In Silico* with a *Bimiralisib* score of -99.668 Kcal/mol. The toxicity of the compound *Helliannouls* A, B, C, E, and the compound *Bimiralisib* belong to class 4 while the compound *Helliannouls* D belongs to class 5.

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