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In Silico Prediction of *Heliannuol* A, B, C, D, and E Compounds on Estrogen Receptor β Agonists

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Abstract

Heliannuols has a benzoxepine ring that produces anticancer activity by the inhibition mechanism of phosphoinositide 3 kinases (PI3K). *Heliannuols* are a compound that can be found in the leaves of sunflower (*Helianthus annuus* L.). The purpose of this study is to predict interactions, toxicity, physicochemical, and pharmacokinetics of *Heliannuol* A, B, C, D, and E based *in silico* as candidate anticancer drugs. Estrogen receptor beta (ERß) is a new potential therapy for glioma with an antiproliferative effect. Ligands agonist ERß have the potential activity to inhibit the proliferation of glioma cells and the discovery of this ligand has opened new therapy through the ERß to prolong survival in cancer patients. Prediction of physicochemical properties based on Lipinski rules and penetrate in the blood-brain barrier. Receptor validation shows that 2I0G(A) has a smaller RMSD value than 2I0G(B), receptor validation is valid if the RMSD value less than 2. The result of molecular docking shows that *Heliannuols* comply with Lipinski rules and have low toxicity. *Heliannuols* also have a similar amino acid with comparison drug (Erteberel), but the rerank score of Erteberel still lower than *Heliannuols*.

Keywords: *Helianthus annuus, Heliannuols, estrogen receptor ß (ERß), in silico, toxicity.*

INTRODUCTION

Sunflower plant (*Helianthus annuus* L.) has many chemical compounds, especially in the leaves of this plant containing monoterpene, diterpene, alkaloids, phenols, and lactone sesquiterpenes (Ceccarini, 2004). *Heliannuols* are one of the sesquiterpenes which has a benzoxepine ring and anticancer activity produced by the inhibition mechanism of phosphoinositide 3 kinases (PI3K) (Ghantous, *et al*., 2010; Hefron, *et al*., 2011; Kuntala, *et al*., 2017; Ren, *et al*., 2003). *Heliannuol* A, B, C, D, and E were obtained from sunflower leaves that had been immersed in water for 24 h and extracted with chloroform (CH2Cl2) then observed using HPLC with hexane and ethyl acetate solvents (Macias, *et al*., 1994; Macias, *et al*., 2000; Macias, *et al*., 2002). The new anticancer treatment therapy involves estrogen receptor beta-agonists (ERβ) by the pathway of inhibiting cancer growth and anticancer therapy with estrogen receptors can reduce glioma cell proliferation (Sareddy, *et al*., 2012; Sareddy and Vadlamudi, 2015).

ERβ has quite a different function from ERα, ERβ has antiproliferative action and

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overexpression of ERβ can reduce proliferation in cancer cells (Strom, *et al*., 2004; Hartman, *et al*., 2009; Nilsson, *et al*., 2011). This research focuses on estrogen receptor beta agonists because it has the potential as an antitumor therapy agent by activating beta subtypes and preventing proliferative effects on cancer. Ligands that are included as estrogen receptor beta-agonists that have the potential activity to inhibit the proliferation of glioma cells are DPN (Diarylpropionitrile), MFF101, Liquiri-tigenin, and LY500307 (comparison drug (Erteberel)) (Sareddy, *et al*., 2012; Sareddy and Vadlamudi, 2015). Erteberel is one of the agonists ERβ that has high potential in reducing glioma cell proliferation and it can penetrate the blood-brain barrier (Sareddy, *et al*., 2016). The discovery of a specific agonists ERβ provided naturally has opened the development of new therapies through the estrogen receptor beta to prolong survival in cancer patients (Sareddy and Vadlamudi, 2015).

Based on the research by Sareddy, *et al*. (2012) show that ERβ pathway activation is a potential therapeutic target for glioma because ERβ agonists are under clinical trials and are well tolerated with fewer side effects. The use of ERβ as a therapeutic agent can be extended to clinical use and is predicted to be a new class of drugs for treating glioma. Future studies examining the mechanism of ERβ in cancer progression will be useful for maximizing treatment using ERβ natural ligands (Sareddy and Vadlamudi, 2015). The purpose of this study was to determine the activity of *Heliannuols* on estrogen receptor beta and the lack of information on estrogen receptor beta-agonists as therapeutic agents in cancer patients, encouraging researchers to compile this study.

MATERIALS AND METHODS

Software

This research uses Chem Bio Draw Ultra 12.0 application, Avogadro, pkCSM online tool, Protox II online tool (accessed on January 2020), and Molegro Virtual Docker 6 (Lisensi expires on: January 01, 2099).

Target and Template Selection

The target of this research is the estrogen receptor beta 2I0G from Protein Data Bank (https://www.rcsb.org). The test compounds of this research are *Heliannuol* A, B, C, D, and E where the SMILES code from Chem Bio Draw 12.0 and the Erteberel SMILES code from PubChem.

Prediction of Physicochemical, Pharmacokinetic, and Toxicity Properties

Prediction of physicochemical properties using the pkCSM online tool and the Protox II online tool by entering the SMILES code of the compound. The prediction of physicochemical properties is based on Lipinski's rules and the ability of compounds to penetrate the brain barrier membrane. Lipinski's rules use to evaluate compounds that have pharmacological activity with predictable physical and chemical properties as drug candidates for humans. While the prediction of pharmacokinetic properties using the online pkCSM tool is based on the absorption, distribution, metabolism, and excretion parameters. Toxicity prediction using the pkCSM online tool and the Protox II online tool based on several parameters and toxicity classes according to the Globally Harmonized System (GHS).

Molecular Docking

Molecular docking using Molegro Virtual Docker 6.0 to know the interaction between the test compound and the receptor. 2D structures of *Heliannuol* A, B, C, D, and E were drawn using Chem Draw Ultra 12.0 and to know the SMILES code of the test compounds then 3D structures were made using Avogadro and energy minimization was performed. Validation of molecular docking ERβ (2I0G) using Molegro Virtual Docker 6.0 and a docking simulation process was also performed. Validation of molecular docking result is the Root

Mean Square Deviation (RMSD), the docking simulation process can be run if the RMSD value was less than 2Å. The parameters used in docking simulation include rerank score, bond distance, and type of interaction.

RESULTS

Prediction of Physicochemical Properties and Toxicity

Prediction of physicochemical properties using Lipinski rules of five with several parameters includes molecular weight, the partition coefficient of octanol/water (log P), Hydrogen Bond Donors (HBD), and Hydrogen Bond Acceptors (HBA). Topological Polar Surface Area (TPSA) also a parameter to determine compound can penetrate the brain barrier membrane (Kelder, *et al*., 1999). The physicochemical test was used to predict that the compound has good permeability, high absorption, and can penetrate the blood-brain barrier. The results of physicochemical properties, the ability of compounds to penetrate the brain barrier membrane, and toxicity are shown in Table 1.

The results of physicochemical prediction show that all compounds comply with Lipinski rules, it can be predicted that all compound easy to absorption and has good permeability. The result of TPSA showed that all compounds have a TPSA value of less than 80, it can be predicted that all compounds could penetrate the brain barrier membrane (Hughes, 2008). *Heliannuol* A, B, and C are in toxicity class 4 with Lethal Dose (LD_{50}) 300-2000 mg/kg. *Heliannuol* D and Erteberel are in toxicity class 5 with LD_{50} 2000-5000 mg/kg. *Heliannuol* A, B, C, D, E, and Erteberel were predicted to be non-toxic in the AMES Mutagenic test, not toxic in the Hepatotoxicity test, and did not cause skin irritation in the Skin Sensitization test.

Prediction of Pharmacokinetic Properties

Prediction of pharmacokinetic properties is based on the prediction of absorption, distribution, metabolism, and excretion (ADME) using the pkCSM online tool. The results of the prediction of pharmacokinetic properties are shown in Table 2.

Table 1. Prediction of physicochemical and toxicity.

Compound	Physicochemical Properties						Toxicity					Lipinski Rules of Five
	MW*	Log P*	HBA*	HBD*	Torsion*	TPSA**	AMES Mutagenic*	Hepatotoxicity*	Skin Sensitization*	LD_{50}**	Class of Toxicity**	
Heliannuol A	250.338	3.11622	3	2	0	49.69	No	No	No	860	4	Yes
Heliannuol B	248.322	2.8922	3	2	1	49.69	No	No	No	482	4	Yes
Heliannuol C	248.322	2.89222	3	2	1	49.69	No	No	No	500	4	Yes
Heliannuol D	250.338	3.1162	3	2	1	49.69	No	No	No	2148	5	Yes
Heliannuol E	248.322	2.89222	3	2	2	49.69	No	No	No	500	4	Yes
Erteberel	282.339	4.1152	3	2	1	49.69	No	No	No	5000	5	Yes

Description: *pkCSM online tool; **Protox II online tool.

The result from the prediction of pharmacokinetic properties shows ADME of the compound, in this research shows that all compound is predicted to have good intestinal absorption with the value is more than 80% (Chander, *et al*., 2017). According to the pkCSM online tool page website (http://biosig.uni-melb.edu.au/pkcsm/theory), the compound has a high skin permeability if the Log value of Kp>-2.5 cm/h, and all compound are predicted have high skin permeability. Caco2 permeability is good if the Papp value>0.90 cm/s and all compounds are predicted to have good permeability. *Heliannuol* C and E do not include as Pgp substrates, then they are not predicted to be

Table 2. Prediction of pharmacokinetic properties.

Prediction Category		Result					
		Heliannuol A	*Heliannuol* B	*Heliannuol* C	*Heliannuol* D	*Heliannuol* E	Erteberel
Absorption	Absorption in intestine (%)	90.904	91.276	91.826**	90.8	91.759+	93.474*
	Skin Permeability (Log Kp cm/h)	-3.243	-3.192	-3.285+	-3.159	-3.35**	-2.751
	Caco-2 Permeability(Log Ppap in 10^{-6} cm/s)	1.283+	1.3	1.242	1.312**	1.248	1.248
	P-glycoprotein substrate	Yes	Yes	No++	Yes	No++	Yes
	P-glycoprotein I inhibitor	No	No	No	No	No	No
	P-glycoprotein II inhibitor	No	No	No	No	No	No
Distribution	Vdss (Log L/kg)	0.092	0.156	0.082	0.166**	0.135+	0.378*
	BBB Permeability (Log BB)	0.295**	-0.024	0.233+	-0.023	0.221	-0.062
	CNS Permeability (Log PS)	-2.769	-2.793	-1.942**	-2.787	-2.042	-1.745*
Metabolism	CYP2D6 substrate	No	No	No	No	No	No
	CYP3A4 substrate	No	No	No	No	No	Yes++
	CYP1A2 inhibitor	No	No	No	No	No	Yes++
	CYP2C19 inhibitor	No	No	No	No	No	Yes++
	CYP2C9 inhibitor	No	No	No	No	No	Yes++
	CYP2D6 inhibitor	No	No	No	No	No	No
	CYP3A4 inhibitor	No	No	No	No	No	Yes++
Excretion	Total Clearance (Log ml/min/kg)	0.947	1.009+	1.085**	0.946	1.08**	0.005
	Renal OCT2 substrate	No	No	No	No	No	No

Description: Vdss (Volume of Distribution at Steady State); BBB (Blood Brain Barrier); CNS (Central Nervous System); CYP2D6 (Cytochrome P2D); CYP3A4 (Cytochrome 3A4); CYP1A2 (Cytochrome 1A2); CYP2C19 (Cytochrome 2C19); CYP2C9 (Cytochrome 2C9); Renal OCT2 (Renal Organic Cation Transporter 2). *The highest value of comparative drugs; **The highest value of the first test compound; +The second highest value of the test compound; ++Different result from other.

removed from the target cell (Chakraborty and Ramakrishnan, 2016). *Heliannuol* A, B, C, D, E, and Erteberel are not included as Pgp I and II inhibitors, it predicts that the compound did not inhibit the work of the Pgp substrate (Robert and Jarry, 2003). The distribution volume of the compound is declared low if the Log Vdss value <-0.15 and high if the Log Vdss value >0.45, all compound has good distribution volume. Compounds with log BB>0.3 can penetrate the brain barrier membrane while compounds with log BB<-1 cannot be well distributed in the brain. Compounds with Log PS>-2 are considered to be able to penetrate CNS while Log PS<-3 are considered unable to pene-

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Table 3. Validation of molecular docking.

Receptor	RMSD (Å)			Average±SD (Å)
	Replication I	Replication II	Replication III	
2I0G (A)	0.4429	0.7535	0.7794	0.6586±0.1872
2I0G (B)	0.5947	0.8566	0.8296	0.7603±0.1440

trate CNS. All compound is predicted to penetrate the brain barrier and central nervous system. *Heliannuol* A, B, C, D, E are predicted not to be part of the CYP substrate or inhibitor then the compound is predicted to be metabolized without the help of cytochrome enzymes and does not inhibit the work of CYP 450. Whereas Erteberel is predicted to include CYP3A4 substrate and inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2C9, and CYP3A4. *Heliannuol* C is predicted to have a faster excretion process than other compounds because if the CLTOT value is higher then the excretion is faster (Hardjono, *et al*., 2018). *Heliannuol* A, B, C, D, E, and Erteberel are predicted to not include Organic Cation Transporter 2 (OCT2) substrate then the compound is predicted to be excreted without OCT2 substrate, OCT plays an important role in cationic drugs (Koepsell, 2015).

Validation of Molecular Docking System

RMSD is a parameter for receptor validation. The validation of molecular docking process is carried out by docking between the native ligand and the receptor, if the results obtained are less than 2, it can be predicted that poses receptor with the native ligand is valid (Ruswanto, 2015). Validation of molecular docking result are shown in Table 3.

Validation of molecular docking result in table 3 show that the receptor has two ligands and the 2I0G(A) ligand has a smaller RMSD value than the 2I0G(B) ligand.

Docking Molecular and Interaction of Amino Acid

The docking simulation process uses several parameters including the MolDock Score, Rerank Score, and H bond. The results of

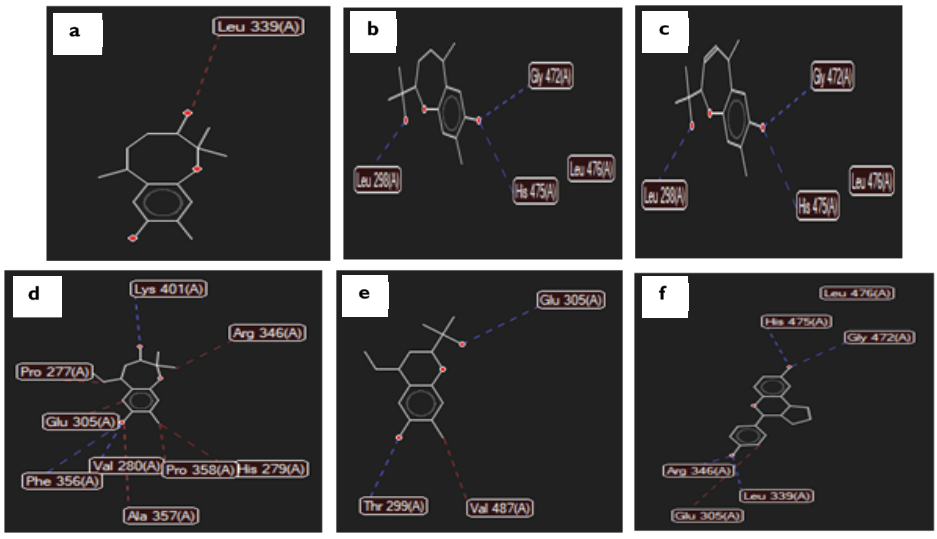

Figure 1. Interaction of amino acids between (a) *Heliannuol* A, (b) *Heliannuol* B, (c) *Heliannuol* C, (d) *Heliannuol* D, (e) *Heliannuol* E, (f) Erteberel on ERß with red *color* as steric bond and blue *color* as hydrogen bond.

Table 4. Interaction of amino acid and rerank score.

Compound	Hidrogen Bond and Distance (Å)	Steric Bond and Distance (Å)	*Rerank Score* (kcal/mol)
Heliannuol A	-	Leu 339 (3.09)*	-69.988
Heliannuol B	Gly 472 (2.61) His 475 (3.32)* Leu 298 (2.79)	-	-72.313
Heliannuol C	Lys 401 (3.00) Val 280 (3.10) Phe 356 (2.98)	Arg 346 (2.70)* Pro 358 (2.89) Pro 277 (2.79) His 279 (2,99) Ala 357 (3.15) Glu 305 (2,93)*	-78.532
Heliannuol D	Gly 472 (2.60) His 475 (3.18)* Leu 298 (2.84)	-	-70.814
Heliannuol E	Glu 305 (2.60)* Thr 299 (3.11)	Val 487 (3.12)	-80.245
Erteberel	His 475 (2.69)* Gly 472 (3.37) Leu 339 (3.10) Arg 346 (2.82)* Glu 305 (3.29)*	Glu 305 (2.99)*	-97.857

Description: *Amino Acid of *Heliannuols* that same with Erteberel.

molecular docking are showed by the interaction of amino acids and rerank scores are shown in Figure 1 and Table 4.

In Figure 1 and Table 4 are show that *Heliannuol* A has a steric bond with the amino acid Leu 339, *Heliannuol* B has a hydrogen bond Gly 472, Leu 298, His 475, *Heliannuol* C has a hydrogen bond Lys 401, Val 280, Phe 356, and a steric bond Arg 346, Pro 358, Pro 277, His 279, Ala 357, Glu 305. *Heliannuol* D has hydrogen bonds Gly 472, His 475, Leu 298, *Heliannuol* E has hydrogen bonds Glu 305, Thr 299, and steric bonds Val 487. Whereas Erteberel has bonds hydrogen His 475, Gly 472, Leu 339, Arg 346, Glu 305 and steric bonds Glu 305.

DISCUSSION

The purpose of this research was to predict interactions, toxicity, and physicochemical properties of *Heliannuol* A, B, C, D, and E compounds using *in silico* study as anticancer. Prediction of physicochemical properties and toxicity is obtained by submitting the compound SMILES code into the pkCSM online tool and the ability to penetrate the brain barrier is obtained from the Protox II online tool. *Heliannuol* A, B, C, D, and E can be predicted easily absorbed and have good permeability because have a molecular weight less than 500, log P values are less than 5, HBD expressed by the number of OH groups and NH is less than 5, and HBA which is expressed with some O and N atoms less than 10 (Lipinski, *et al*., 2001). All compounds have a TPSA value less than 80Å, it can be predicted that the compound can penetrate the brain barrier membrane (Hughes, 2008). *Heliannuol* A, B, and C are in toxicity class 4 which means fatal if ingested with LD_{50} 300-2000 mg/kg. *Heliannuol* D and Erteberel are in toxicity class 5 which means it might be dangerous if swallowed with LD_{50} 2000-5000 mg/kg (El-Din, *et al*., 2016). All compounds also were predicted to be non-toxic

in the AMES Mutagenic test, not toxic in the liver, and did not cause skin irritation. Pharmacokinetic properties are used to determine the absorption, distribution, metabolism, and excretion of the test compound. The result of pharmacokinetic shows that *Heliannuols* have high absorption and can penetrate the brain barrier membrane where these compounds are expected as therapy in glioma patients. Besides, *Heliannuol* C and E are not included in the Pgp substrate then these compounds are predicted not to be removed from the cell and are expected to increase the therapeutic effect of the compound. This prediction is expected to be used as an additional prediction regarding the effects of the compound in the body before proceeding to the *in vivo* and *in vitro* tests.

This research shows that the receptor has two active sides that bind with the native ligand is 2I0G(A) and 2I0G(B). Ligand 2I0G(A) has a smaller RMSD value than 2I0G(B), it can be predicted that the active side of the receptor for the docking simulation is 2I0G(A). The docking process can be performed if the receptor has RMSD value ≤2 and the RMSD value from ligand 2I0G(A) is 0.6586Å. Prediction of molecular docking between *Heliannuol* A, B, C, D, and E with estrogen receptors β (ERβ) show that *Heliannuol* E has the lowest energy with a rerank score of -69.988 kcal/mol and predicted to have the most stable bond among other *Heliannuols* and predicted have the greatest activity. And if the result of the rerank score (the value of bond energy) is lower (more negative), the bond between the ligand and receptor is more stable, and the more stable bond between the ligand and receptor than the activity is high (Hardjono, 2012).

The result of amino acid interaction from this research includes hydrogen and steric bond. *Heliannuols* have the same several amino acids with an amino acid of the Erteberel by hydrogen bonds. Hydrogen bonds are non-covalent bonds that play a role in the biological activity produced (Muchtaridi, *et al*., 2018; Wijaya, *et al*., 2003). Erteberel bind to amino acid His 475, Glu 305, and Arg 346 with hydrogen bonds, a compound that binds with that amino acid can be classified as an ERβ agonist. Estrogen receptor beta agonist bind to amino acid Glu 305, Arg 346, His 475 (Meegan and Lloyd, 2003). *Heliannuols* have the same amino acid with an amino acid of the Erteberel by hydrogen bond, but *Heliannuol* A doesn't bind to amino acid His 475, Glu 305, and Arg 346. From the result of this study, it can be predicted that *Heliannuol* B, C, D, E have the same function with the Erteberel as an ERβ agonist. Prediction of physicochemical, pharmacokinetic, and toxicity from *Heliannuols* are predicted to have high absorption, good permeability, can penetrate the blood-brain barrier, and toxicity in classes 4 and 5. The docking result of *Heliannuols* is not good than the Erteberel because Erteberel still has the lowest rerank score than *Heliannuols*.

CONCLUSION

The conclusion from the research shows that *Heliannuols* have the same amino acid with the Erteberel, then can be predicted that *Heliannuols* have the same effect on estrogen receptor beta. When looked at its physicochemical, pharmacokinetics, and toxicity, *Heliannuols* has high absorption, can penetrate the brain barrier membrane, has good permeability and toxicity is in grades 4 and 5. However, the rerank score of Erteberel is still lower than *Heliannuols*. And for further research, it is recommended that *Heliannuols* be tested on other receptors to produce a lower rerank score of test compound than comparison compound.

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