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Molecular in Silico Test of the Interaction Between Keloid Cell Protein TGF – β1 (PDB ID: 3TZM) and Lycopene Compounds

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Abstrak

Keloids are pathological scars, characterized histologically by excessive aggregation of type I collagen and fibroblasts in the reticular dermis inflammation. Scar tissue (keloid) is one problem the most frustrating in wound healing. Clinically, keloids are defined as a scar that invades adjacent healthy tissue and seldom experience regression along time. Keloids can be said to be a disorder of the healing wound, which form growth excessive from network scar, exceed limit wound, and beyond the normal time (can be years) wound healing process. In this research conducted development drug anti - keloids in silicon from compound secondary metabolites Lycopene with target protein TGF-β1 with code PDB ID: 3TZM. Testing conducted with series process covers analysis of compounds and proteins,physicochemical properties,ADME,RMSD value ≤ 2 Å, geometry optimization, 3D and 2D interactions between compounds and target proteins using DOCK 6. Based on results test in silicon, will be obtained compound docking scorequercetinwith the 3TZM ligand that t has the potential to be developed into one candidate drug anti keloids with score bond energy free (affinity) – 71,453, van derwals bond energy value (vdw) -71,990, ES energy value 0,536 and internal repulsive energy value 72,413.

Kata kunci: Keywords: antikeloid; Lycopein, TGF- β1, Insilico test, DOCK6

PRELIMINARY

Facial skin has an important role in interacting with people other,in a *Aesthetic*, if treated with good skin on the face will be build confidence in communicating Facial skin that has scars is usually caused by burns, collisions, acne, surgery, smallpox infections, and folliculitis which will trigger the formation of hypertrophic scars (scars) to become keloids. Moreover, if keloids/scars form on facial skin, especially the eye area (eyelids) will disturb in a manner appearance. Keloids is tumor dermal fibroproliferative benign which generated from traumatized skin and network scar abnormal (1).

In the country Nigeria on year 2009 which aim evaluate perception and psychological impact on patient's keloids, as much as 35.8% and 48.9% patient keloids give impact negative to interaction social in communicate (Olaitan, 2009). Prevalence keloid patients about 10% in Africa (Mari et al., 2015). The prevalence is lower in Asia, with a higher incidence annual keloid to be 0.15% among the general Taiwanese population (LM Sun et al., 2014). Cases of keloids in Indonesia, precisely in Surabaya, show a prevalence patient keloid in Poly Clinic Skin and Sex HOSPITAL Dr.Soetomo Surabaya is 1.4% (2013), 1.6% (2014), and 1.5% (2015) (2).

Secondary metabolite compounds produced by plants besides functioning to defend itself from environmental conditions that do not profitable, also beneficial in field treatment (3). There are many active compounds from natural ingredients such as the triterpenoid group of compounds believed to have anti-keloid and anti-inflammatory activity inflammation (4) (5) (6). class Flavonoids (bioflavonoids) is substance natural ones lowered from various plant and used for prevent formation scar that's bad (7). Compounds the like lycopene. Molecular docking has been widely used search for and design new potential drugs from ingredient natural (8). Based on activity biological fruit Grape and component metabolites secondary, study this aim for see and predict candidate drug which originated from compound active lycopene to activity inhibition protein TGF -β1 (pdb id:3tzm) in silicon (9)(10).

METHOD STUDY

Ingredient

The material used consists of three structures dimensions from TGF-β1 Target Protein (pdb id:3tzm), downloaded through website Proteins Data Bank (GDP) https ://w ww.rcsb.org/ with ID code 3CCZ, structure from lycopene can be made in marvinskechts software.

Tool Device soft and hard

Tools which used on this research is Laptop ASUS TUF FX506HEB 17R5BTO 17 11600H 16 GB 512 SSD RTX3050TI 4GB W10+ OHS 15.6 FHD 144HZ RGB BLK with Intel CORE i7, Windows 10 64-Bit Operation System. Device soft which used is Marvin Sketch version 16.9.12, DOCK6, chimera 1.16, Avogadro, Discovery studio 2021, ChemBio version 19.0.0.22, OECD QSAR Toolbox version 4.3 and Switzerland Target Predictions.

PROCEDURE

predictions NaturePhysicochemistry: Lipinski RO5

S ecompound active ingredient lycopene conducted modeling molecule compound test 2d and 3d with using software such as ChemDraw and Ligand Scout. Furthermore, the prediction of physicochemical properties is carried out online onpageSwissADME: (http://www.swissadme.ch/index.php). Results which there is seen and determined compound test which in accordance with condition Lipinski Rule of Five.

predictions ADMET

On page (https://preadmet.qsarhub.com/) chosen order "ADME" or "Toxicity" on corner right on page web, then the structure of the test compound is drawn and submitted if already done. Results prediction ADME or toxicity downloaded in format. pdf.

preparation Ligand and Receptors

Ligand in format pdb opened with use DOCK6 was prepared with add hydrogen atoms, merge nonpolar, and add charge Gasteiger. Files are saved in the form pdbqt (Protein Data Bank, Partial Charge (Q), & Atom Type (T)). Meanwhile for receptor, downloaded in advance in pdb format on site Proteins Data Bank (https ://w ww.rcsb.org/) with code proteins 3CCZ. preparation which conducted that is add atom hydrogen polar and payload Kollman. Receptors saved in shape. pdbqt.

Validation Method Molecular Docking

validity is known with method re-docking it returns native ligand on proteins target which has prepared. Method said valid if score roots Means Square Deviation (RMSD) obtained ≤ 2Å (19). For coordinates grid box results obtained in the form of a large grid box volume used that is 40 x 40 x 40 points with distance between the point that is 1.08967 Å.

Docking Ligand Test on Proteins Target

The docking method is carried out by tethering each ligand on receptor 3 TZM with coordinate belay results validation in a manner consecutive for x,y,z that is 80476, -2,731,

-8,617. DOCK 6 used on simulation docking of the test ligand and the comparison ligand against the receptor 3 TZM.

Analysis Data and Visualization Docking

Results docking (.dlg) could seen use notepad. Determination conformation complex results docking conducted by choosing a conformation with a bond free energy value smallest. Visualization complex conducted with use software BIOVIA Discovery Studio 2017.

RESULTS AND DISCUSSION

Identified lycopene compounds nature its physicochemistry for see potency developed into medicinal preparations. Physicochemical parameters from compound which tested could adjusted to rule lipinski. There are 5 rules, if compound test have heavy molecule more from 500 Yes, so compound the will difficult penetrate membrane cell. Score logs P which more big from5 showing that compound the the more characteristic lipophilic and very tightly bound to the membrane difficult to recognize the target enzyme and also toxic. However, the log P value is too small or negative too not too good because compound will be difficult for penetrate lipid bilayer membrane. Hydrogen bond donors and acceptors is magnitude capacity bond hydrogen. If capacity hydrogen the more tall, so energy which needed for process absorption the more tall also. kindly general, Lipinski rules of five (RO5) this show solubility Certain compounds penetrate the membrane by diffusion passive (10). Compound test said Fulfill requirements for formed preparations orally if no more from one violation to rule Lipinski (11).

Table 1: Results of lycopene compounds

Compound name	BM (molecular weight) < 500Da)	Log P (<5)	absorption	Log Kp (-2.5)	surface areas	Hydrogen Bonds	
						Donors (<5)	Acceptor (<10)
Lycopene	536.9	15.6	-6,289	- 2,735	248,007	0	0

Based on Table 1 of the compounds tested, lycopene compounds do not meet the requirements of Lipinski RO5 and fulfill condition making preparations orally. However, research on the development of keloid drugs can be made in transdermal preparations with due regard to Log Kp (skin permeability) and skin sensitization. Research more carry on could conducted to determine the pharmacokinetic profile of the compound the active.

ADMET

On Table 2 showing profile absorption, distribution, metabolism, and toxicity of lycopene compounds. Score HIA showing many compounds active which absorbed in intestines. HIA is calculated from bioavailability and absorption is excreted in the urine, bile and feces (12). From results obtained 5 compound which absorbed with good, that is resveratrol, caffeic AC ID, ferulic AC ID, isorhamnetin and syringetin with HIA values of 70-100%. including into the category good. For parameter HIA itself there are 3 categories, namely 70-100% including categories good, 20-70% including category currently, and 0-20% including low category (13).

Table 2: ADMET Prediction Results

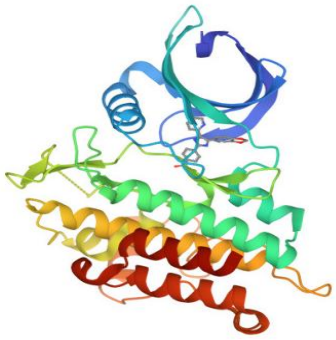
Properties	**Model Name**	**Predicted Value**	**units**
absorption	Water solubility	**-6,289**	Numeric (log mol/L)
absorption	Caco2 permeability	**1,273**	Numeric (log Pap in 10^{-6} cm/s)
absorption	Intestinal absorption (human)	**91025**	Numeric (% Absorbed)
absorption	Skin Permeability	**-2,737**	Numeric (log Kp)
absorption	P-glycoprotein substrates	**No**	Categorical (Yes/No)
absorption	P-glycoprotein I inhibitors	**No**	Categorical (Yes/No)
absorption	P-glycoprotein II inhibitors	**Yes**	Categorical (Yes/No)
Distributions	VDSs (human)	**-0.137**	Numeric (log L/kg)
Distributions	Faction unbound (human)	**0**	Numeric (Fu)
Distributions	BBB permeability	**1,049**	Numeric (log BB)
Distributions	CNS permeability	**-0.244**	Numeric (PS log)
metabolism	CYP2D6 substrates	**No**	Categorical (Yes/No)
metabolism	CYP3A4 substrates	**Yes**	Categorical (Yes/No)
metabolism	CYP1A2 inhibitors	**No**	Categorical (Yes/No)
metabolism	CYP2C19 inhibitors	**No**	Categorical (Yes/No)
metabolism	CYP2C9 inhibitors	**No**	Categorical (Yes/No)
metabolism	CYP2D6 inhibitors	**No**	Categorical (Yes/No)
metabolism	CYP3A4 inhibitors	**No**	Categorical (Yes/No)
Excretion	Total Clearances	**1949**	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrates	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**-0.613**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitors	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitors	**Yes**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**2,174**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**0.751**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**No**	Categorical (Yes/No)
Toxicity	Skin Sensitization	**No**	Categorical (Yes/No)
Toxicity	*T. Pyriformis* toxicity	**0.289**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**-4,868**	Numeric (log mM)

Cell Caco-2 is model in vitro for knowing transport drug through epithelium intestinal which originated from adenocarcinoma colon man which have track transportation double. Parameter this conducted for determine the permeability of active compounds in oral preparations in vitro. Score Cell Caco-2 >70 nm/sec showing that compound have permeability which tall (14). Results This shows that the lycopene compound has permeability which low with value of 1.273 nm /sec.

Drugs must be in a state that is not bound to plasma proteins in in body, so that could diffuse penetrate membrane cell and interact with target to have pharmacological activity. PPB value calculated to determine the number of active compounds that are bound with plasma proteins. From results obtained compound lycopene has a PPB value of >90% which shows these compounds bond strong with proteins plasma (14).

The ability of the drug to penetrate the blood-brain barrier (Blood Brain Barrier) is one of the important parameters for reduce effect side as well as toxicity and also increase activity pharmacological drug (15). From results It was found that lycopene compounds can penetrate the blood barrier brain well, seen from the value of Log BB> 0.33 of 1,049 (16). From results It was found that lycopene was excreted not through the kidneys. Not toxic to the liver and can be developed into topical or transdermal preparations.

Ligands and receptors



Picture 1. Structure three dimensions from receptor TGF-β1 with code GDP 3TZM which has prepared

Testing next on preparation receptor and ligand in the series of molecular docking tests performed. 3 TZM is receptor from TGF-β1 which is a cell that forms keloid disease. modelling 3 TZM this uses method X-Ray Diffractionin Homo sapiens with a resolution value of 1.70 Å. The ligand will form a complex with 3 TZM receptors namely SB431542 ($C_{22}H_{16}N_4O_3$) which is ligand naturally. However, on process this receptor and ligand experience the will prepared use BIOVIA Discovery Studio 2017 to separate between the receptor and its natural ligand, as well as to remove the water molecules that form the complex with receptor (Picture 1). TGF-β1 is a scar tissue-forming protein that is produced in the process of cell proliferation when wound healing occurs. In this phase, fibroblast cells will produce TGF-β1 continuously to form keloids (17).

In molecular docking there are several parameters can be known. The validation results are in the form of grid box coordinates and score roots Means Square Deviation (RMSD). Score RMSD showing comparison location conformation ligand before docking and after docking, so the smaller the RMSD value obtained then it shows that position ligand the more accurate and approach position initially (Agistia et al., 2013). RMSD value requirements on something validation fulfilled when value < 2.0 Å (19). Score RMSD obtained from the method validation results of 1. 08967 Å. Thing this Fulfill requirements from score RMSD which has required. Thing the could prove with do overlays between natural ligands that have not been through docking process with re-docking natural metals (Fig 2) seen second ligand the coincide one same other which signify that position ligand after through process r e docking return approach to position at first. Thing This shows that the 3 TZM receptors can be used for the docking process with other originating ligands of the active compounds Lycopene.

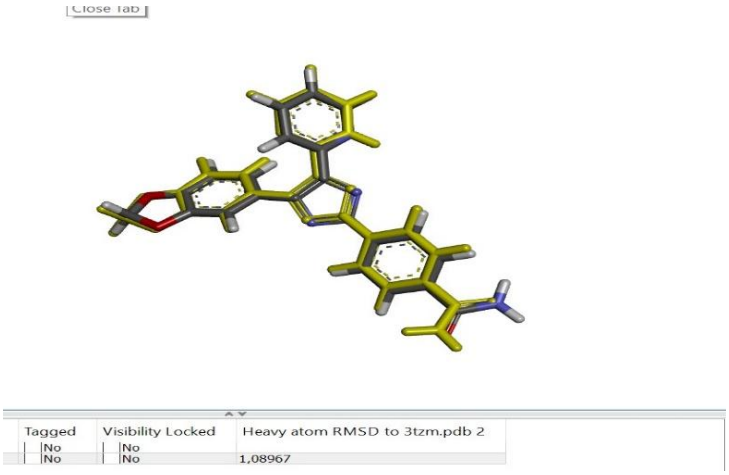


Figure 2 : Overlays Among ligand before and after through process docking (validation) with RMSD value < 2 Å: 1.08967 Å.

Table 3. Docking score values

Compound name	Grid_Scores	Grid_vdw_energy	Grid_es_energy	Internal_energy_repulsive
Lycopene	-71,453	-71,990	0.536	72,413

The more negative score bond energy which obtained signify that complex which formed Among 3TZM receptors with active compounds derived from lycopene more stable. Can be seen in table 3. Energy bond show strength binding (affinity) between the test ligand and the target protein. Score which the more negative show compounds the have interaction form style pull between atom which the more big whereas style reject between atom Becomes the more minimum so that conformation compound which obtained more stable (21). Score energy bond which low show complex ligand-protein which stable form.Lowest binding free energy (i.e., docking score best) and the inhibition constant denotes the affinity the highest ligand/protein (23).

Inhibition constants compared straight with amount dose which needed for induce pharmacological activity. The smaller the constant inhibition, the smaller the number of doses required.

On study this compound active lycopene which have energy bond free gibs (affinity) that is -71,453 .The van der wals energy is -71,990, the electrostatic energy is 0.536 and the internal repulsive energy is 72,413. From the docking score, lycopene can be used as a keloid drug transdermally which has a fairly strong affinity.

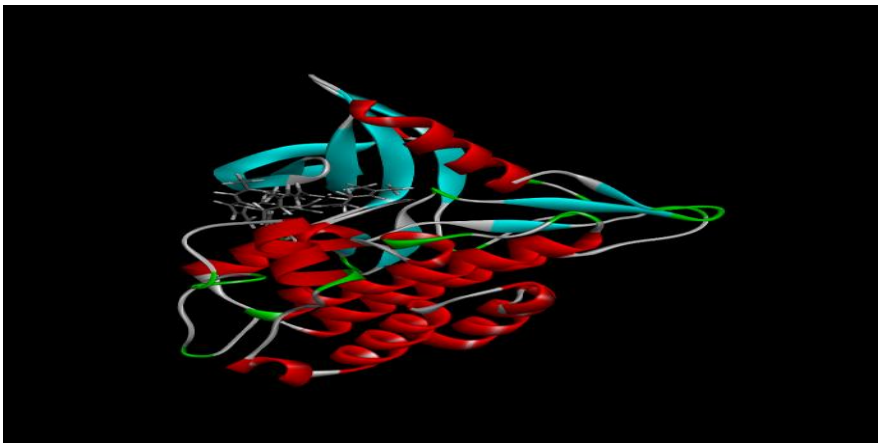


Figure 3: 3D docking results of lycopene compounds with pdbid : 3TZM

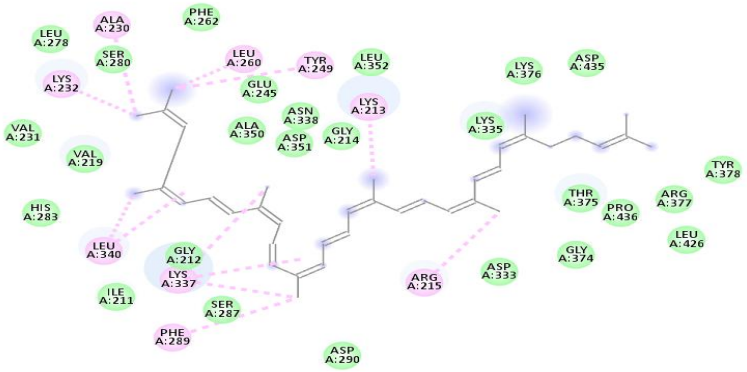


Figure 4: 3D docking results of lycopene compounds with pdbid : 3TZM

Parameter important other which considered for stabilize the intermolecular interactions of the target protein with the ligand is a hydrogen bond (H) (24). Basically, analysis interaction ligand-protein disclose that molecule- this molecule occupies the same binding site as substrate normal in domain catalytic and could form bond hydrogen, interaction electrostatic, and hydrophobic interactions with residues at the binding sites human TGF-β1 (25).Interaction Among ligand experience SB431542 with receptor 3 TZM got seen on Picture 4. Studies docking Lycopene with 3 TZM receptors exhibiting binding interactions with key residue. Docking analysis that has been done shows that there are three kinds of bonds that occur between the ligand and the 3TZM amino acid residue, namely the weak Vander Wals bond can be seen in the light green color. Where the van der wals bond is a bond that is weak and breaks easily so it is not given a line. The chemical bonds that occur between amino acid residues are purple alkyl bonds and pink pi alkyl bonds.

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