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In Silico Studies: Virtual Screening Of The Compound Of Sea Fan (*Gorgonia Mariae*) As Antiasthmatic

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ABSTRACT

Sterol is a secondary metabolites for a group of steroids that can treat a disease caused by synergistic effect between compound of metabolite with polyvalent activity. Sea fan (*G. mariae*) has a major compound (sterol) that has been used by the society of Maluku, Indonesia as an asthma substance but needs research done because it has not been reported widely and only empirical data from the society. For that, virtual screening is a necessary using the *in silico* method as a first step in determining the effectiveness and predicting the value of free energy bonding (ΔG), inhibition contents (Ki), interaction of amino acid residue using Autodock Tools 4.2, Lipinski Rule of Five and pre-ADMET in determining absorption, distribution and toxicity. The result showed that 24-methyl cholesterol had a value of ΔG (-9.98 kcal/mol) and Ki (48.26 nM), where the value of ΔG were smaller than salmeterol as a comparative drug. Prediction of oral drug candidate is a not eligible because the of Log P should not be >5 (Log P = 7.635). The value of absorption (HIA = 100% ; Caco-2 = 51.404 nm sec^{-1}) and toxicity (there are no risk of mutagenic and carcinogenic). Thus, compound of the 24-methyl cholesterol from *G. mariae* are potentially as asthma medications but cannot be administered oral.

Keyword: Asthma, *in* silico, sea fan (*G. marie*), sterol.

INTRODUCTION

Traditional medicine is derived from knowledge, skills and practices based on the theory, beliefs and experience of a local culture, can be explained or not, used for health care as well as prevention, diagnosis, repair or treatment of physical and mental illness[1]. The main aim of natural substance based treatment is to use from nature that has been used by local society in generations and keep the health safe without side effects when consumed.

The society of Maluku, Indonesia often use materials from nature to treat diseases, one is the use of a sea fan (*G. Mariae*) as antiasthmatic. Based on the result of the study, it has been reported that *G. mariae* has a major compound that the sterol identified consist of cholesterol, 24-methyl cholesterol, 24-methyl-22-dehydrocholesterol, gorgosterol, 23-demethyl gorgosterol, 4,24-dimethyl dehydrocholestanol and 4,24-dimethyl cholestanol [2].

Sterol are a secondary metabolites of a steroid group compound that a important role in treating a disease caused by the synergism effect between secondary metabolite compound with polyvalent activity so that it may be possible to cope various diseases[3]. Based on the research results from Reo [4] it has been reported that the secondary metabolites contained in gorgonia, *Paramuricea clavata* more steroids contained in all fractions of the solvent (methanol, ethyl acetate and n-hexane) are used both on the skin and axial. The steroid has antiinflammatory activity so as not to trigger the occurrence of asthma by inhibiting the release of cytokom IL-2, IL-4 and IL-6, the movement of leukocytes as well as induced of lipocortin[5].

Asthma is a chronic disease of pneumonia that is not infectious and heterogeneous with inflammation of the respiratory tract characterized by a wheezing history, chest tightness, shortness of breath and cough[6].The medications used for asthma include a drug of β-agonist synthesis as a highly effective bronchodilator for asthma therapy. Salbutamol, terbutalin and fenoterol are asthma medications that have Duration of Action shorter known as Short Actingβ$_2$-Agonist (SABA), while salmeterol and formoterol have Duration of Action long called Long Acting β$_2$-Agonist (LABA). SABA is known as an early therapeutic treatment of asthma before acute asthma [7].

The use of people's perceived synthesis drugs is very expensive and has side effects, so they often use the drug from natural ingredients namely*G. mariae*. For that research is necessary much less is not reported by the results of his research. This research is the first step to know the effectiveness of components in *G. mariae* as one of asthma drugs with predicting of absorption, distribution and toxicity by conducting virtual screening using in-Silico method.

Material dan Methods

Material

Hardware : Laptops with spesificationsprocessor INTEL(R) Celeron (R) CPU N2840 @ 2.16GHz (2 CPUs), ~2.2GHz and memory 2048MB RAM.

Software : Operating system windows 8.1 Single Language with Bing 64-bit (6.3, build 9600), Chem3D Pro 12.0, ChemDraw Ultra 12.0, Discovery Studio 2016 Client®, AutoDock Tools 4.2, Pre-ADMET and Lipinski Rule of Five.

Test compounds: 1-(5-azanyl-4~{H}-1,2,4-triazol-3-yl)-~{N}-[2-(4-bromophenyl)ethyl]-~{N}-(2-methylpropyl)piperidin-4-amine as a natural ligands, the test compound components are used from the 7 sea fan compounds (*G. mariae*) andsalmeterolis used as a comparative drug.

Receptor : Data of the 3D crystal receptor structures used for molecular docking analysis obtained from the Protein Data Bank (PDB) obtained from the sitehttp://www.rscb.org/pdb/[8, 9].Receptors used to predict activity as Antiasma are called chitotriosidase-1 receptors with 5NRA PDB code.

Methods

Protein preparation

Receptor preparations of 5NRA used as asthma receptors obtained from PDB (http://www.rscb.org/pdb/).Receptors are then visualized using programs Discovery Studio 2016 Client®. In the program, the downloaded receptors are done by eliminating water molecules and natural ligands. The results obtained are pure of receptors and stored in PDB format (.pdb).

Ligand preparation

The ligand preparation of the test compound is a1-(5-azanyl-4~{H}-1,2,4-triazol-3-yl)-~{N}-[2-(4-bromophenyl)ethyl]-~{N}-(2-methylpropyl)piperidin-4-

amine as native ligands obtained from GDP, sea fan (*G.mariae*) as test ligandas well as salmeterol as comparative drug obtained from the sitehttps://pubchem.ncbi.nlm.nih.gov/[10]and is also drawn manually using theChemDraw Ultra 12.0 dan Chem3D Pro 12.0if no chemical structure is found.Then the structure is conditioned with a body pH condition that ranges between 7.35 – 7.45 andsaved in the format (.pdb).

Method validation

For validation method (ligands) used a congenital compound of receptors/proteins (ligands preference) that is re-docking to the receptors. The value viewed is*Root Mean Square Deviation* (RMSD), Where the RMSD value is≤2.00 Å used as standard molecular tethering values.

Docking test compound with receptor

The Docking is done using the Autodock4 software, (run-autodock) by tether between the ligands and the receptors. Then edit the cmd by deleting the directory so that it is in the column only cmd (D: / Autodock / Autodock4 –p dock.dpf –l dock.dlg &) and then click to processed.

Moleculer tethering analysis and visualization

The result of docking calculations can be seen in the output in notepad format. The determination of the docking confirmation result of the test compound is done by choosing the configuration of the ligands that have the lowest bonding energy (best pose). The position and orientation of the ligands are in macromolecules as well as amino acids that are bound on the ligand to be visualized using Discovery Studio 2016 Client®.

Pre-ADMET

The ADMET Parameter is calculated using the preADMET® program accessed through the sitehttp://preadmet.bmdrc.kr/adme/[11]. Chemical structure of compounds depicted or uploaded in the format files Mol (*.mol). The Program automatically calculates the predicted values of the selected parameters, including the permeability of the cellhuman colon adenocarcinoma(Caco-2), Human Intestinal Absorption(HIA), Plasma Protein Binding (PPB), mutagenic and carcinogenic.

Results andDiscussion

Protein and Ligand

The method of computation with an *in silico* approach is an experimental method that uses computer software by learning the physicochemical properties of the structure of chemical compounds[12]. At present, the computational chemical approach (*in silico*) is used to maximize and facilitate the study of absorption, distribution, metabolism, excretion and toxicity from the discovery of new medicinal substances before testing *in-vitro* and *in-vivo*[13].

In this study, used protein receptors of 5NRA (Figure. 1)whereas previous research uses glucocorticoid receptors[14]using the *in silico* method and also to know the activity of antiasthma.
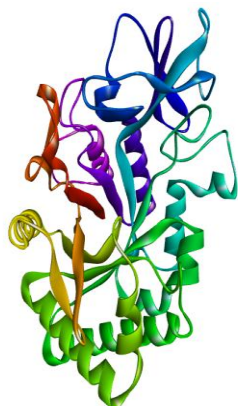
Figure 1. Protein of 5NRA (chitotriosidase-1 receptor)

The AutoDock Tools 4.2 program is used to determine the grid box on the area known as the active side of the protein.The determination of this grid box includes setting the location of the parameters box and specifying the size of the grid box using the distance (angstrom).In this protein of 5NRA, the result of the grid box obtained is the center x = 93.542; y = 32.275 and z = 22.365 with a grid point distance (Angstrom) is 0.375 Å(Figure. 2).
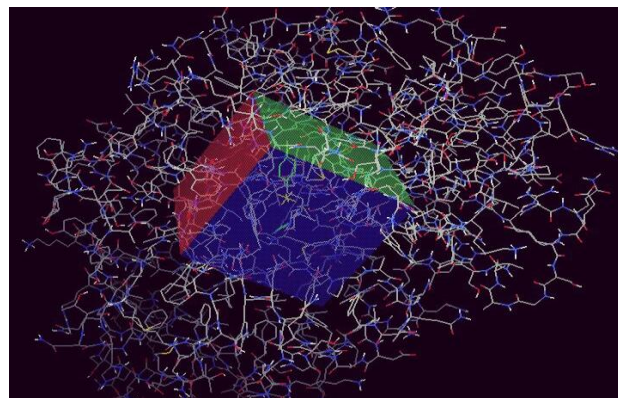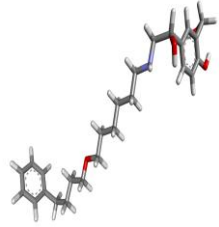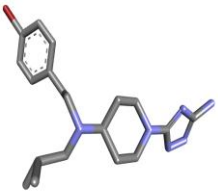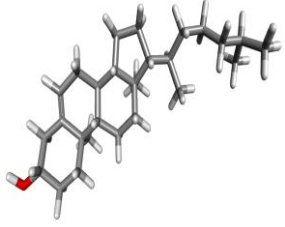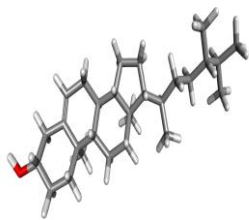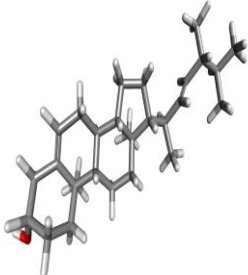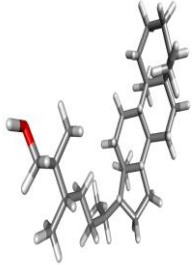

Figure: 2. Protein preparation with Autodock4

For the preparation of ligands, the component of the compound on the sea fan (*G. mariae*)has 7 test compounds consisting of cholesterol groups (cholesterol, 24-methyl cholesterol, 24-methyl-22-dehydrocholesterol), gorgosterol groups (gorgosterol, 23-demethyl gorgosterol) and cholestanol groups (4,24-dimethyl-22-dehydrocholestanol and 4,24-dimethyl cholestan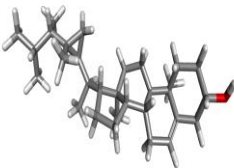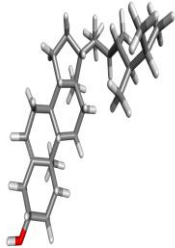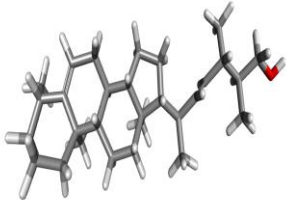ol) and use a comparison of the drug is a salmeterol. Then optimized by conditioned the structure of chemical compounds on the body with the pH 7.4 (range 7.35 – 7.45) can be seen in table 1.

Table1. Component of compounds on the comparative drug, native ligand and test compounds

Compounds	Molecular Formula	Structure
Salmeterol	$C_{25}H_{37}NO_4$	
1-(5-azanyl-4~{H}-1,2,4-triazol-3-yl)-~{N}-[2-(4 bromophenyl)ethyl]-~{N}-(2-methylpropyl)piperidin-4-amine	$C_{19}H_{29}BrN_6$	
Cholesterol	$C_{27}H_{46}O$	

24-methyl cholesterol	$C_{28}H_{48}0$	
24-methyl-22-dehydrocholesterol	$C_{28}H_{47}O$	
Gorgosterol	$C_{30}H_{50}O$	
23-demethyl gorgosterol	$C_{29}H_{48}0$	
4,24-dimethyl-22-dehydrocholestanol	$C_{29}H_{50}O$	
4,24-dimethyl cholestanol	$C_{29}H_{52}O$	

Method Validation

The analysis used to evaluate the validation result is by looking at the RMSD value and the binding location. The parameters used are considered valid, if the RMSD value obtained ≤ 2.00 Å means the position of copy ligands after the superimposed will be more closely with the native ligand. In addition, it is also influenced by the protein resolution and receptor modelling we used[15]. In this study obtained the validation result for the RMSD value of 0.627 Å, this indicates that it qualifies as determined so that the parameters can be used to conduct a docking simulation of test compound and comparative drug.

Docking Interactionof Test Compounds and Visualization

In this study, 7 compounds contained on the *G. marie* as well as the drug as comparative performed tethering or docking with chitotriosidase-1 receptors using Autodock Tools 4.2 software that dimensions grid box 40×40×40. The result is the interaction of the test compound component with the amino acid residue indicating the binding to the place of the active side of the chitotriosidase-1 receptor. In addition, the obtained free energy bond (ΔG) and inhibitory constant (Ki), seen in table 2.

Table: 2. Docking interaction on the comparative drug, native ligand and test compounds

| Compounds	Interactions with Amino Acid Residues		Ki (nM)	(ΔG) (Kcal/mol)
	Hidrogen Bonds	**Van der Waals (hydrofobic)**		
Salmeterol	• Conventional hydrogen bond(**ARG 269 ; TYR 141 ; GLU 140**) • Carbon hydrogen bond (**ASP 213 ; TYR 267**)	TRP 358 ; TYR 212 ; MET 210 ; TRP 99 ; GLU 297 ; THR 295 ; ALA 302 ; LEU 301 ; TYR 190 ; GLN 145	846.22	-8.28
1-(5-azanyl-4~{H}-1,2,4-triazol-3-yl)-~{N}-[2-(4 bromophenyl)ethyl]-~{N}-(2-methylpropyl)piperidin-4-amine	• Conventional hydrogen bond(**TYR 212**) • Carbon hydrogen bond (**TYR 27**)	ASP 213 ; PHE 58 ; ARG 269 ; ASN 208 ; GLU 297 ; THR 295 ; LEU 301 ; ALA 302	1.60	-12.00
Cholesterol	• Conventional hydrogen bond(**GLU 297**) • Carbon hydrogen bond(**THR 295**)	GLY 98 ; ASP 138 ; PHE 58 ; GLU 140 ; ASP 213 ; TRP 31 ; LEU 362 ; ARG 35	3.01	-11.62
24-methyl cholesterol	• Conventional hydrogen bond(**GLU 297**) • Carbon hydrogen bond (**no**)	ASP 213 ; GLU 140 ; GLY 98 ; ASP 138; ARG 35 ; THR 295	48.26	-9.98
24-methyl-22-dehydrocholesterol	• Conventional hydrogen bond(**no**) • Carbon hydrogen bond (**no**)	ASP 213 ; GLU 297 ; GLU 140 ; GLY 98 ; ASP 138 ; THR 295 ; ARG 35 ; TRP 31 ; ALA 302	0.86434	-12.36
Gorgosterol	• Conventional hydrogen bond(**THR 295**) • Carbon hydrogen bond (**no**)	TRP 31 ; ARG 35 ; GLU 297 ; ASP 213 ; ASP 138 ; GLU 140	68.43	-9.77
23-demethyl gorgosterol	• Conventional hydrogen bond(**ARG 35**) • Carbon hydrogen bond (**no**)	ASP 213 ; GLU 140; GLY 98 ; ASP 138 ; TRP 31 ; THR 295 ; GLU 297	1.74	-11.95

| 4,24-dimethyl-22-dehydrocholestanol | • Conventional hydrogen bond(**GLU 140**)

• Carbon hydrogen bond (**no**) | GLY 98 ; ASP 213 ; ASP 138 ; PHE 58 ; MET 210 ; TRP 31 ; ARG 35 ; THR 295 ; GLU 297 | 1.89 | -11.90 |
| 4,24-dimethyl cholestanol | • Conventional hydrogen bond(**GLU 140**)

• Carbon hydrogen bond (**ASP 138**) | TYR 27 ; ALA 143 ; MET 210 ; TYR 212 ; ASP 213 ; GLY 98 ; PHE 58 ; THR 295 ; ARG 35 ; GLU 297 | 0.80987 | -12.40 |

The docking results from table 2. Shows the value of free energy bonds and inhibition constants that can be potentially as asthma drugs there are compounds 24-methyl cholesterol, where the value of ΔG (-9.98 Kcal/mol) and Ki (48.26 nM). This value is then compared to the salmeterol as a comparative drug, where the value of ΔG (-8.28 Kcal/mol) and Ki (846.22 nM). This suggests that 24-methyl gorgosterol is potentially a candidate for antiasthma drug because it has a smaller ΔG value compared to the salmeterol even though insignificant in the value of Ki, the meaning of the value of ΔG 24-methyl cholesterol can serve as an inhibitory constant of 48.26 nM while salmeterol although slightly large the value of the ΔG but can inhibit with the amount of inhibition constants are very significant as of 846.22 nM. In addition can also be seen interaction of amino acid residue with hydrogen bonds as well as Van der Waals on 24-methyl cholesterol and salmeterol shown in Figure 3.
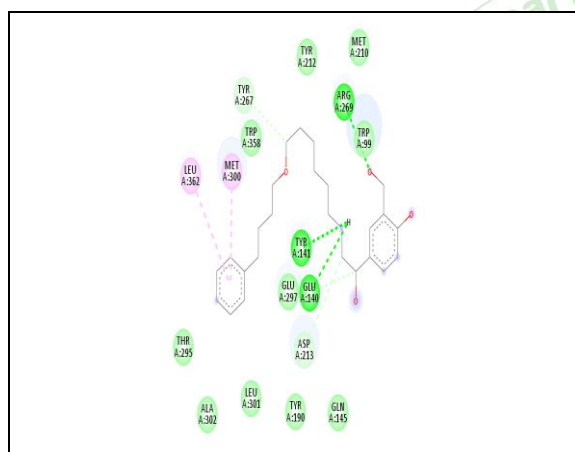
(3.a)
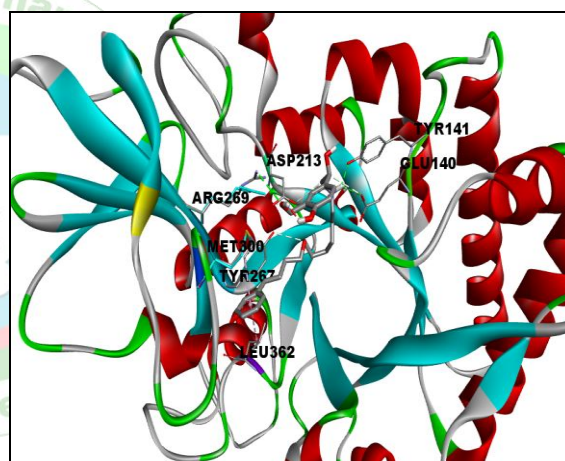
(3.b)
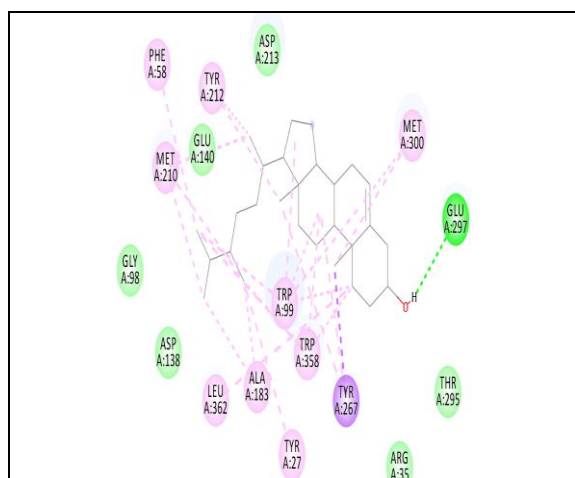
(3.c)
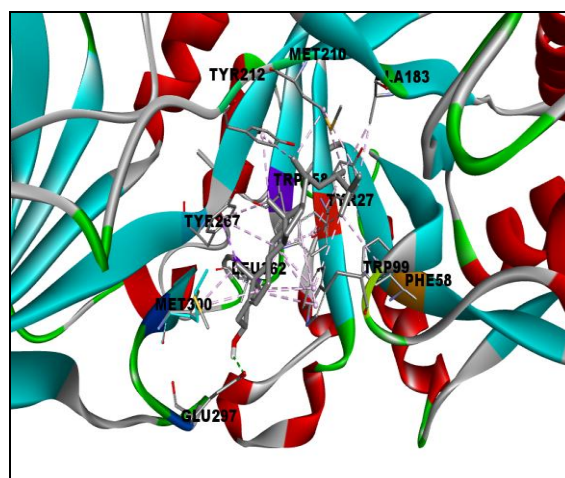
(3.d)

Figure: 3. Docking result visualization from salmeterol of 2D (3.a) dan 3D (3.b) ; 24-methyl cholesterol of 2D (3.c) and 3D (3.d)

In order to estimate the solubility and permeability of a candidate for drug substances, a computational approach is required. The solubility and permeability of a compound playing an important role in developing drug development. This is done to prevent the failure of a drug caused by low absorption or permeation[13].

Based on the rules of Lipinski in the development and discovery of a candidate for drug substances use the oral, so it must fulfill five conditions known as "*Rule of Five*" encompasses amolecular mass less than 500 Dalton, high lipophilicity (expressed as Log P less than 5), less than 5 hydrogen bond donors,less than 10 hydrogen bond acceptorsas well asmolar refractivity should be between 40-130[16]can be seenin the table 3.

Table: 3 Result of compounds screening based on*Lipinski Rule of Five*

Compounds	BM	Log P	Hydrogen Donors	Hydrogen Acceptors	Molar Refractivity	Desription
Salmeterol	415	4.107	4	5	120.526	Meet
1-(5-azanyl-4~{H}-1,2,4-triazol-3-yl)-~{N}-[2-(4 bromophenyl)ethyl]-~{N}-(2-methylpropyl)piperidin-4-amine	391	0.071	0	6	89.551	Meet
Cholesterol	386	7.389	1	1	119.053	Not meet
24-methyl cholesterol	400	7.635	1	1	123.599	Not meet
24-methyl-22-dehydrocholesterol	398	7.411	1	1	123.506	Not meet
Gorgosterol	426	7.881	1	1	130.580	Not meet
23-demethyl gorgosterol	412	7.491	1	1	125.963	Not meet
4,24-dimethyl-22-dehydrocholestanol	412	7.658	1	1	128.075	Not meet
4,24-dimethyl cholestanol	416	7.962	1	1	128.193	Not meet

The screening results of a compound that has been docking then become the determination of solubility and permeability as candidate for drug substances using*Lipinski Rule of Five* [17]shown in table 3. that all test compounds from *G. mariae* do not meet the required average with a Log P value of more than 5, meaning it cannot be used as a candidate for the oral drug ingredient because it has low lipophilicity.

Pre-ADMET

The design will be a new drug candidate should pay attention to absorption, distribution and toxicity so that the results obtained according to the clinical test,

the predicted parameters include the pharmacokinetic properties and prediction of toxicity. Parameters of the pharmacokinetic properties consist of absorption [(HIA) and Caco-2] and distribution (PPB), for the toxicity seen of mutagenic and carcinogenic.

HIA is the amount of biovailability and absorption from the ratio of excretion or cumulative excretion in urine, bile and feces whereas Caco-2 is widely used as an in-vitro model in predicting drug absorption in humans. The distribution parameters use PPB because it is closely related to the disposition of the drug in giving effect[18]. Results of pre-ADMET as shown in table 4.

Table: 4. Prediction results of Pre-ADMET

Compounds	Absorption		Distribution	Mutagenic	Carcinogenic
	HIA (%)	Caco-2 (nm sec^{-1})	PPB (%)		
Salmeterol	89.331	23.623	83.228	+	-
1-(5-azanyl-4~{H}-1,2,4-triazol-3-yl)-~{N}-[2-(4 bromophenyl)ethyl]-~{N}-(2-methylpropyl)piperidin-4-amine	92.016	30.359	100	+	+
Cholesterol	100	51.013	100	-	-
24-methyl cholesterol	100	51.404	100	-	-
24-methyl-22-dehydrocholesterol	100	51.360	100	-	+
Gorgosterol	100	50.894	100	-	+

23-demethyl gorgosterol	100	50.925	100	-	-
4,24-dimethyl-22-dehydrocholestanol	100	27.534	100	-	-
4,24-dimethyl cholestanol	100	27.632	100	+	+

According to the literature, the absorption rate range of% HIA has a value of 70 – 100% (well absorbed), 20 – 70% (moderately absorbed) and 0 – 20% (poorly absorbed). For Caco-2 (nm sec^{-1}) in-vitro on cell permeability has a value of > 70 nm sec^{-1} (higher permeability), 40 – 70 nm sec^{-1} (medium permeability) and < 4 nm sec^{-1} (low permeability)[13]. Pre-ADMET results as in table 3. indicates the absorption rate of% HIA with a value range of 100% in all compound components of *G. mariae*, which means very well absorbed in the intestines whereas Caco-2 ranges between 27 – 51 nm sec^{-1} which signify the medium permeability value when occurring transport of drugs through the intestinal epithelium of adenocarcinomas in human colon.

The distribution parameters are based on the plasma protein binding value, seen in table 4. that all compounds of the *G. mariae* are 100%. This indicates that the diffusion occurs through the plasma membrane and interacts with the pharmacological target[13].Human plasma contains 70% protein with albumin (Human Serum Albumin, HAS), α 1-glikoprotein (AGP)and lipoprotein as the main component[18].While the results of toxicity, all compounds are hardly mutagenic but are carcinogenic. Compounds that are mutagenic are harmful to human health because they directly have an impact that causes damage or mutation of the DNA.

CONCLUSION

The virtual screening results using a computational chemical approach with the *in silico* method on the 7 compounds of a sea fan (*G. mariae*) indicate that the 24-methyl cholesterol compound has a smaller free energy bond value than the salmeterol as comparative drug. However, predictions when administered oral do not meet the value of Log P asone of the requirements of*Lipinski Rule of Five*while the absorption of intestinal and cell permeability Caco-2 showed excellent results. For distribution based on the value of plasma protein binding, the predicted can diffuse through the plasma membrane and interact with the pharmacological target as well as the toxicity result of the 24-metil cholesterol compound is no risk of mutagenic and carcinogenic.

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