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In silico Activity of Potential Compounds Derived from Sargassum sp at P_2Y_{12} Purinoceptor

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Abstract

Sargassum had potential compounds as source of natural medicine. To explore compounds from natural product, in silico approach was effective way for screening. The aims of this study was to investigate the potential of compounds derived from Sargassum sp as platelet antiaggregation using molecular docking. SWISS-MODEL was used to construct the model of P_2Y_{12} purinoceptor. Docking method was prior validated by redocking of native ligand, five active compounds, and 250 decoys using Molegro Virtual Docker and Discovery Studio for visualization. RMSD and enrichment factor of docking process were 0.36 and 2.6 respectively. Among the tested compounds, isoketochabrolic acid, linoleic acid, and alginate were more active than ticlopidine and clopidogrel as comparative compounds. VAL190 and TYR105 were the amino acid residu of the receptor that involved in protein-active ligand interaction.

Keywords: homology modeling, molecular docking, P_2Y_{12} purinoceptor, Sargassum compounds

INTRODUCTION

Sargassum was one of Phaeophyceae class brown seaweed. It consists of about 400 species. Some of them are *S. tenerrimum*, *S. micracanthum*, *S. thunbergii*, *S. wightii*, *S. cristaefolium*, *S. muticum*, *S. binderi*, *S. polycystum*, dan *S. horneri*. Sargassum extracts had various biological activity such as antiinflamation, antihyperlipidemia, antioxidant, and antithrombotic. Antithrombotic was the activity that closely with cardiovascular disease. Some of the Sargassum content that had been identified are fucoidan [1] [2][3][4], alginate [5], phlorotannin [6] [7], fucoxanthin [6], sargachromanol [8], sargaquinoic acid [9][10][11][12], sargahydroquinoic acid [13][10][12] linoleic acid [14], fucosterol [15][16], and isoketochabrolic acid [17].

In order to explore compound from natural product with antithrombotic activity, in silico approach was effective way for screening activity. P_2Y_{12} receptor is the most clinical drug target for inhibition of platelet aggregation [18] [19]. Purinoceptor P_2Y_{12}, a member of the P_2Y purinergic GPCR family stimulated ADP, is a major player in platelet aggregation and granule secretion and supports the formation of thrombus. There were three crystal structures of human purinoceptor P_2Y_{12}. All of them were mutant protein (http://www.rscb.org/pdb). In order to obtained the wildtype of protein, it need homology modeling. Homology modeling is an in silico method that predicts the tertiary structure of a query amino acid sequence based on a homologous experimentally determined template structure [20]. Homology modeling for preparation of P_2Y_{12} receptor based on G-protein coupled receptor *Meleagris gallopavo* b1 adrenergic receptor had reported which shares a higher sequence similarity with human P_2Y_{12} and contains a ligand binding site [19]. In silico methods to get the tertiary structure using human P_2Y_{12} receptor as template is the solution to get the wildtype protein. Molecular docking study of the compound derived from Sargassum using protein that generated by homology modeling hasn't mentioned before. Here we are report the homology modeling to built wildtype protein using SWISS-MODEL webserver (https://swissmodel.expasy.org) and applied to investigate the potential of compounds in Sargassum sp as platelet antiaggregation.

MATERIAL AND METHODS

Hardware, software and webserver: Desktop-NKU6719 personal computer, Intel® Core ™ i5-9400 CPU 2.90 GHz 2.90 GHz, RAM 16.0 GB, Graphic Card NVIDIA GeForce GT 1030; Sonny Vaio laptop, Intel® CoreTM i5-2450M CPU@2.50GHz 2.50 GHz, Random Access Memory (RAM) 4 gigabyte, Graphic Card, Microsoft Windows 10 Pro 64-bit. Both of them were internet connected. Molegro Virtual Docker (MVD) 6.0 (CLC Bio Company) and Discovery Studio Visualizer v19.1.0.18287 (Dassault Systemes Biovia Corp), SWISS-MODEL (https://swissmodel.expasy.org) to built protein, ChemBioDraw Profesional 16.0, ChemBio3D Profesional 16.0 (PerkinElmer Inc., Chambridge, MA, USA). PubChem (pubchem.ncbi.nlm.nih.gov), PROCHECK https://services.mbi.ucla.edu/PROCHECK, A Directory of Useful Decoys (DUD) situs http://dude.docking.org/generate

Material. Amino acid sequence of human P_2Y_{12} purinoceptor in FASTA format from UniProtKB, clopidogrel, prasugrel, ticlopidine, AZD1283, ARL66096 structures from PubChem, 250 decoys generated from DUD-E [21], and potential compounds from Sargassum. Test set and decoys were prepared as 3D structure, and multiconformer database construction was built.

Homology modeling to prepare protein. Human purinoceptor P_2Y_{12} amino acid sequence was obtained

from UniProtKB with ID Q9H244 (https://www.uniprot.org/uniprot). Homology modeling was constructed using SWISS-MODEL (https://swissmodel.expasy.org) with copying FASTA format and paste to the webserver. The step of homology modeling are template recognition and initial alignment, alignment correction, backbone generation, loop modeling, side-chain modeling, model optimization, and model validation [22][20]. The protein quality was assessed using PROCHECK webserver. The model then used for molecular docking.

Ligand preparation. 2D structure of ARL 66096 (CID 5311009), AZD 1283 (CID23649325), clopidogrel (CID 60606), ticlopidine (CID 5472); prasugrel (CID 6918456), and some compounds of Sargassum: alginate (CID 131704328), fucoidan (CID 92023653), laminaran (CID 4396), fucoxanthin (CID 5281239), sargachromanol (CID 10455044), sargaquinoic acid (CID 10202734), sargahydroquinoic acid (CID 101145056), linoleic acid (CID 5280450), fucosterol (CID 5281328), isoketochabrolic acid [17] were drawn using ChemBioDraw Professional 16.0. All of the above structures was made into a 3D form using the ChemBio3D Professional 16.0 program. The most stable form of the all stereochemical compounds can be done using this program by selecting MMFF94. After obtaining the most stable form, the structure was saved in the form of Sybyl.mol2 file, and used for the docking process [23].

Docking validation. Internal docking validation was conducted by redocking native ligand using MVD. 6AD (2-(methylsulfanyl)adenosine 5'-(trihydrogen diphosphate) was choosed as native ligand. This ligand was in the cavity with volume of 71.68 and surface of 262.4 at radius of 8 center: vector [13.05 -2.88 52.89]. Redocking of five poses with ten replications was runned in sixteen combinations of scoring function-algorithms. External validation was also done by docking simulation of AZD 1283, ARL 66096, clopidogrel, ticlopidine, prasugrel, and 250 decoys generated from DUD E(http://dude.docking.org). Validation result was evaluated based on Root Mean Square Deviation (RMSD) and the enrichment factor (EF).

Molecular docking and amino acid analysis. This step was conducted using MVD. Receptor model constructed from homology modeling was prepared by adding H, detection of position at the receptor, where the ligand will be bound (interact). These are cavities in the receptor structure. Things that need to be considered in this process are the selection of the docking compound and the cavity where the ligand will interact [24]. The parameters measured in the docking process are the energy values involved, in the form of MolDock Score, Rerank Score, Hbond, and RMSD values. To measure the strength of ligand-receptor binding, a parameter often used is the rerank score [23]. The docking process obtained rerank score as output data that showed the energy of the ligand in binding site to the protein target.

RESULT AND DISCUSSION

Homologi modeling. The ultimate goal of protein modeling is to predict a structure from its sequence with an accuracy that is comparable to the best results achieved experimentally. It took no more than two minutes after starting to built. SWISS-MODEL was the fully automated protein homology modeling server and had the fastest response time [25]. Human purinoceptor was obtained from UniProtKB with ID Q9H244 that had 342 residues. A total of 435 templates were found to match the target sequence and then was filtered a heuristic down to 50. Among fifty templates in this di web, the best model was generated as shown in Fig. 1.

The model then evaluated using PROCHECK webserver. Based Ramachandran plot this model had residu in most favoured region value of 96.3%. The model was ideal because it had the residues in most favoured region greater than 90%. It means the accuracy of the model can be compared to crystallographically [22] and Z-score analysis of the model for QMEAN, C-β, all atom, solvation and torsion were – 2.70; -0.83; - 0.04; 0.99; and 3.08 respectively.

Fig 1. Ligand-receptor complex generated from homology modeling.

Docking validation. Docking method validation was done by redocking between native ligands and the target. This step was assessed based on RMSD. RMSD is a parameter that describes how much the change in protein-ligand interactions before and after docking. The docking method is considered to be reliable valid if the RMSD value is less than 2 Angstroms [26], so that it could be used further for docking the test compound. This redocking procedure was conducted by trying 16 combinations of scoring and algorithm available in the MVD docking feature.

Total of 50 docking poses were generated for each combination and an evaluation was performed on one docking pose with the lowest score. The parameter evaluated was the lowest RMSD value for each docking pose. Based on the RMSD value, all of the 16 combinations produced RMSD value lower than 2 so that

it met the requirements (Table 1). Docking validation was also performed on the results of docking between five active set compounds and 250 decoys on 16 combinations of scoring functions and algorithms. From the rerank score of all active set and decoys poses, the enrichment factor was calculated. Enrichment factor is a measure obtained from the calculation of active set compounds found in the data set which is then compared with the ratio between active compounds and decoys from the database being tested. The results of the EF20% calculation for 16 combinations are presented in Table 1.
Based on enrichment factor, the highest value was 2.6 using combination of MolDock Score-Iterated Simplex, and be regarded as good [27]. Superimposed visualization of native ligand with docking pose which the RMSD value of 0.36 Å was shown in Fig. 2.

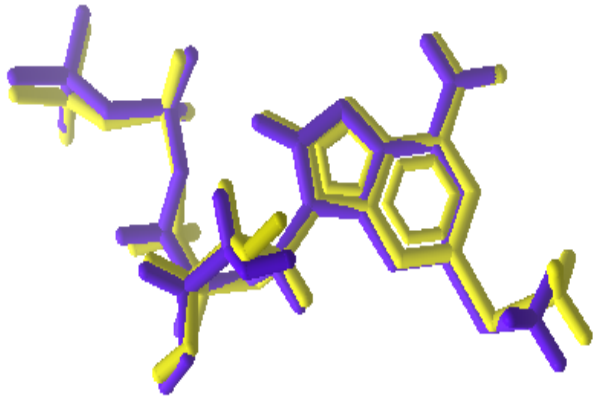


Fig 2. Superimposed of native ligand (yellow) with pose docking (blue)

Molecular docking of the compound derived from Sargassum.
Docking simulation of all compounds was performed using combination of MolDock Score - Iterated Simplex. Three thienopyridine derivative was used as comparative compounds. Clopidogrel, ticlopidine, and prasugrel are P_2Y_{12} purinoceptor antagonist that had been approved by FDA (http:www.drugbank.ca). The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information. The docking score was calculated based on Gibbs free energy where the smaller value means the greater affinity of this compound. This was expressed as rerank score.
Rerank score is value that correlated with the bond energy needed to form bonds with receptors, so that from that value can be predicted the activity of a compound. The lower rerank score, the more stable bond between ligands and receptors. The docking results of best ten compounds are presented in Fig 3.
Based on the rerank score, three compounds were more active than comparative compound. Isoketochabrolic had the best score. It was isolated from *Sargassum micracanthum* and has anti-inflamatory effect [17]. Alginate was abundance in Sargassum sp. Using docking studies, this compound has cyclooxygenase inhibitor activity [28]. Linoleic acid was one of *Sargassum horridum* constituent [5] and *Sargassum muticum* [29]. It was polyunsaturated fatty acid that good effect for human health [30].

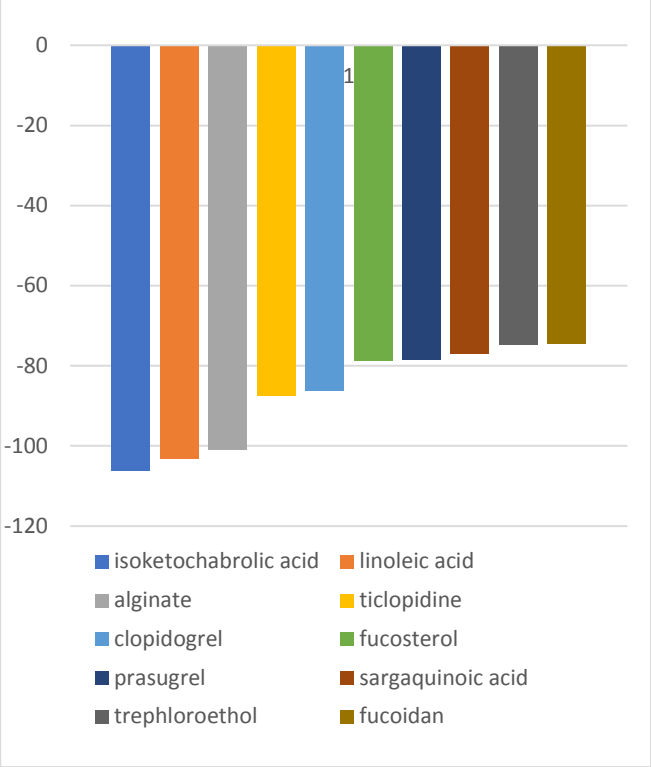


Fig 3. The rerank score of comparative structure and compounds from Sargassum

Ligand-protein interaction. Ligand-protein complex of five compound with best rerank score were shown in Fig.4. Fig 5. showed ligand-protein interaction after docking simulation of isoketochabrolic acid, linoleic acid, alginate, ticlopidine and clopidogrel after docking simulation. From ligand-protein visualization, amino acid residu that involved in ligand binding side could be determined. The results was shown in Table 2.
Table 2. showed that the five compound had the same bonding with amino acid residu in the binding pocket. These residu were TYR105 and VAL190. This was appropriate with AZD1283 that makes bonding with amino acid residu of TYR105, LEU155 and VAL190 in the binding pocket. AZD1283 was a P2Y12 purinoceptor antagonist [31]. So TYR105 and VAL190 residues at receptor have important role to interact with ligand. Beside TYR105 and VAL190, alginate in this docking simulation had bonding with LEU155 also. ARG256 was amino acid residu that interacts with isoketochabrolic acid, linoleic acid, and alginate. Linoleic acid, alginate, ticlopidine, and clopidogrel hadthe same interaction with HIS187. Amino acid CYC175 was residu in binding site that had interaction with isoketochabrolic acid, alginate, ticlopidine and clopidogrel.

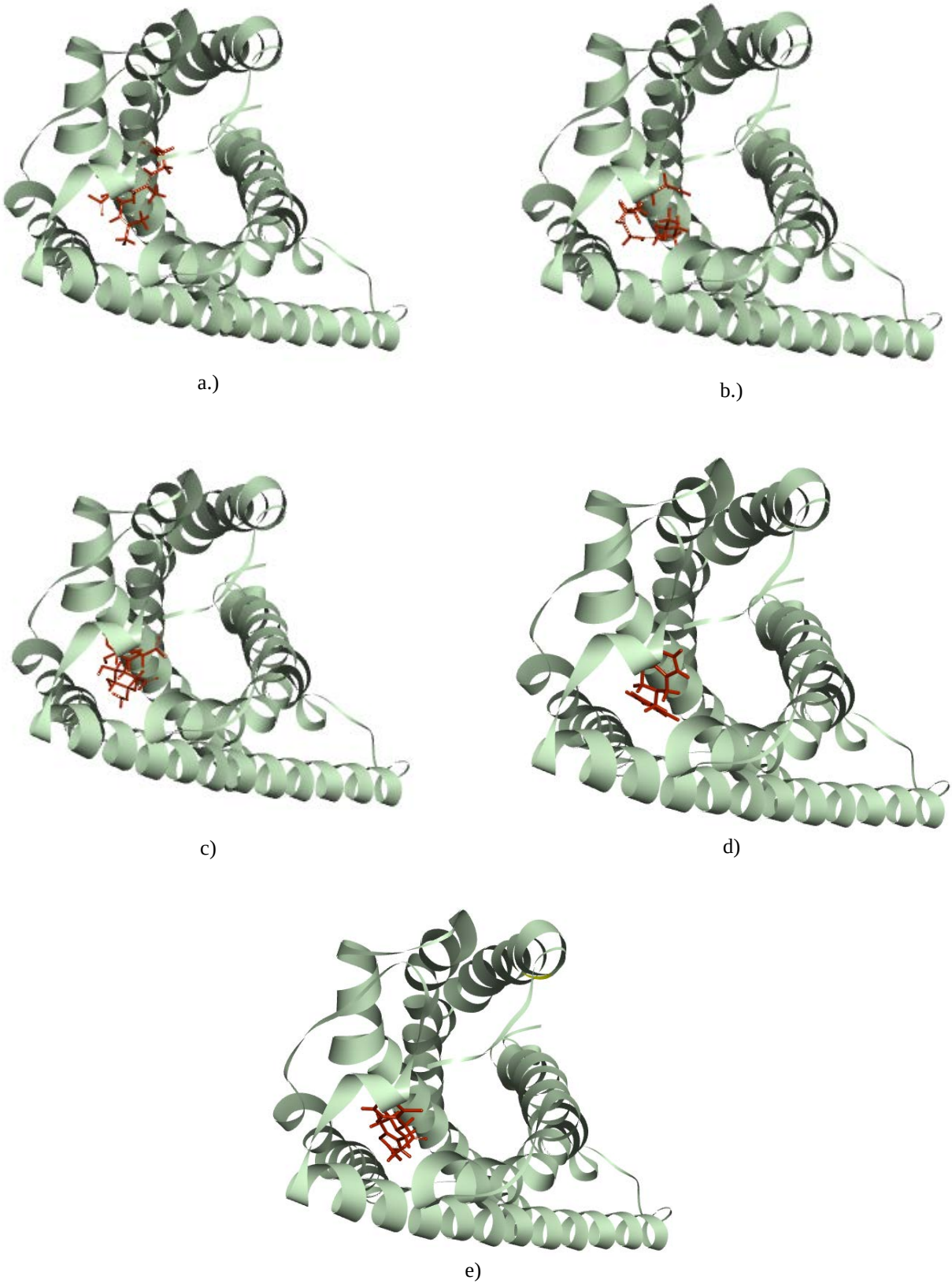
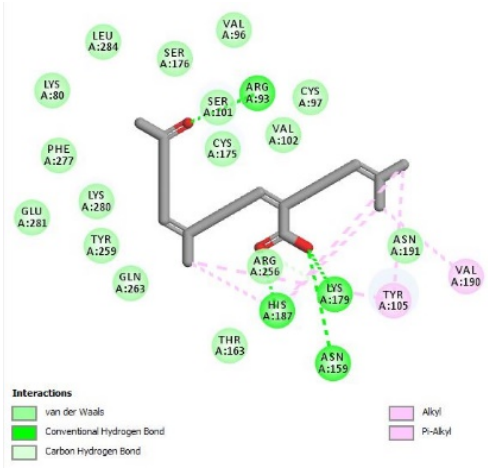
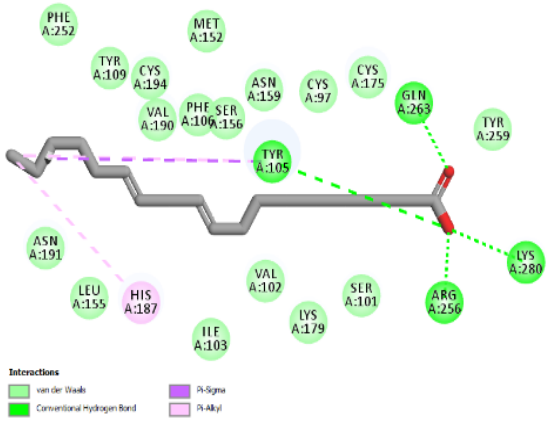


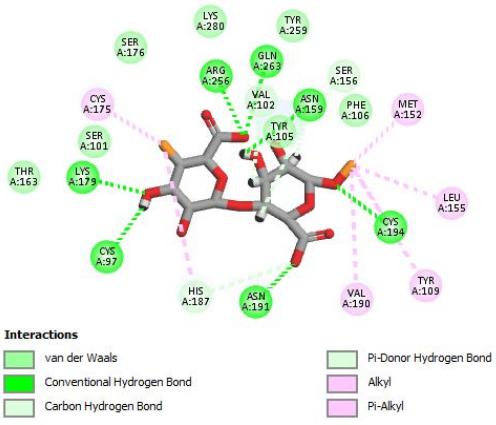
Fig.4. The model in complex with test compunds a) isochetochabrolic acid; b) linoleic acid; c) alginate; d) ticlopidine; e) clopidogrel



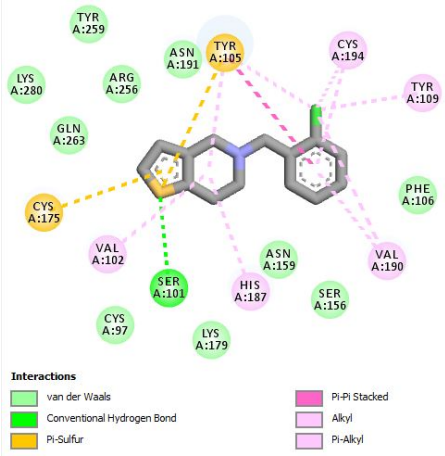
a)



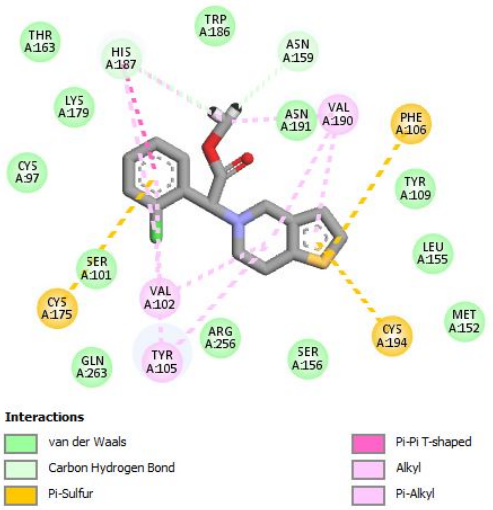
b)



c)



d)



e)

Fig. 5. The interaction of ligand and amino acid of receptor a) isoketochabrolic acid b)linoleic acid c) alginate d) ticlopidine d) clopidogrel

Table 1. Redocking results

Scoring function	Algorithm			
	MolDock Optimizer	MolDock SE	Iterated Simplex	GPU screening(CUDA)
	RMSD (Å); EF20%	RMSD (Å); EF20%	RMSD (Å); EF20%	RMSD (Å); EF20%
MolDock Score	0.67 ; 1.6	1.25 ; 0.16	0.36 ; 2.6	1.03 ; 0.2
MolDock Score (GRID)	0.49 ; 0.19	0.33 ; 0.8	0.42 ; 1.9	0.97 ; 1.3
PLANTS Score	1.48 ; 1	0.40 ; 0.8	1.40 ; 0.94	0.57 ; 0.4
PLANTS Score (GRID)	0.39 ; 0	0.32 ; 1.2	1.00 ; 1.2	0.76 ; 0.2

Table 2. Amino acids in the binding pocked receptor

No	Compounds	Rerank score	Amino Acid residu	Number of amino acid in the binding site
1	Isoketochabrolic acid	-106.176	**CYS175**; **ARG256**; LYS179 **ASN159**; **VAL190**; **TYR105** **VAL102**; ARG93	9
2	Linoleic acid	-103.198	LYS280; **ARG256**; GLN263 **VAL102**; **TYR105**; **VAL190** TYR109; **HIS187**; CYS194	5
3	Alginate	-100.97	ASN191; **HIS187**; CYS97 **LYS179**; **CYS175**; **TYR105** **ARG256**; GLN263; **ASN159** CYS194; **VAL190**; TYR109 **LEU155**; MET152	14
4	Ticlopidine	-87.53	**TYR105**; CYS194; TYR109; **VAL190**;**HIS187**;SER101; **VAL102**; **CYS175**	8
5	Clopidogrel	-86.28	**HIS187**; **ASN159**; **ASN191**; **VAL190**; PHE106; CYS194; **TYR105**; **VAL102**; **CYS175**	9

CONCLUSION

Homology modeling of P_2Y_{12} purinoceptor using SWISS-MODEL generated 3D structure model with good category in redocking validation. Isoketochabrolic acid was the most active Sargassum compound in receptor model**.** VAL190 and TYR 105 amino acid residu involved in active compound-binding side of receptor interaction.

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