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Utilization of Marine Organisms as Potential Anticancer Agents Using the *in silico* Method of Ulithiacyclamide and Patellamide C, E, F

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ABSTRACT

The objective of this work was to conduct *in silico* study of secondary metabolites derived from symbiont microbe of tunicate *Lissoclinum patella* to obtain the anticancer potential of patellamide C, E, F, and ulithiacyclamide. Molecular docking method using Autodock in PyRx 9.5 version, as well as WAY2DRUG PASS analysis were applied to measure binding affinity of the ligands to their receptor proteins and to predict their bioactivities. Among the four ligands, patellamide E exhibited the strongest binding affinity to human epidermal growth factor receptor 2 (HER2) protein scored –9.4 kcal mol^{-1}, having higher score than doxorubicin –8.9 kcal mol^{-1}, but lower score than TAK258 as the control –9.8 kcal mol^{-1}. While patellamide F and ulithiacyclamide reached –9.3 kcal mol^{-1} as the highest binding affinity to human epidermal growth factor (EGF) protein compared to erlotinib score of –6.9 kcal mol^{-1} and also to doxorubicin binding score of –9.1 kcal mol^{-1}. Analysis using PASS prediction resulted in probability of action of 0.845, 0.822, 0.884, and –0.728 for patellamide C, E, F, and ulithiacyclamide, respectively, and revealed their high anticancer potential activity based on their probability to be active (Pa) scores above 0.7.

INTRODUCTION

Marine organisms had become famous for their bioactive secondary metabolites. More than 10.000 compounds have been reported, with around, 1.000 compounds reported from ascidian. There are two compounds namely ecteinascidin 743 and dehydrodidemnin B; they have successfully been developed as drugs and used clinically for cancer treatment **(Fayette *et al.*, 2006; Jimenez *et al.*, 2019)**. Compounds derived from ascidian especially from patellamides group and its derivatives are known as compounds with anticancer activity **(McDonald & Ireland, 1992; Williams & Jacobs, 1993)**. Therefore, it is important to study their pharmaceuticals benefit not only for human health but also for environmental values. Revealing their pharmaceuticals potentials can also make human understand the urge of the marine environment conservation as the original habitat.

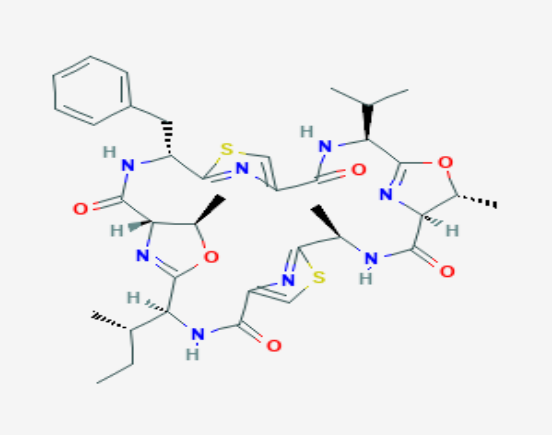
Compounds from patellamides classes derived from tunicate *Lissoclinum patella* have shown high cytotoxic activities **(McDonald & Ireland, 1992)** as well as multidrug resistance blockers

(Williams & Jacobs, 1993). Patellamide C, E, F and its profound analog ulithiacyclamide is a cyclic peptide group with unique thiazole and oxazoline amino acids **(Ireland & Scheuer, 1980; McDonald & Ireland, 1992; Rashid *et al.*, 1995)**. Several bioactive compounds have been isolated from the Didemnidae family of tunicates, which are didemnin, bistramide, trunkamide, patellazole, and patellamide **(Schmidt *et al.*, 2012)**. Although they are rich in pharmaceutical value, according to several reported publications, these bioactive compounds are not produced by the tunicates themselves, but by the symbiotic microbes associated with them **(Donia *et al.*, 2008; Donia *et al.*, 2011)**. Molecular docking is a tool that can be used for prediction as well as confirmation, which would describe the best-fit orientation of a ligand to a particular protein of interest. In modern drug designing, molecular docking is routinely used to understand drug-receptor interaction. It provides useful information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule **(Vijesh *et al.*, 2013)**. The aim of this study was to conduct *in silico* and anticancer screening using molecular docking as well as bioactivity prediction using PASS Server software of patellamide C, E. F and ulithiacyclamide isolated from symbiont microbe of *Lissoclinum patella* from Manado Bay waters.

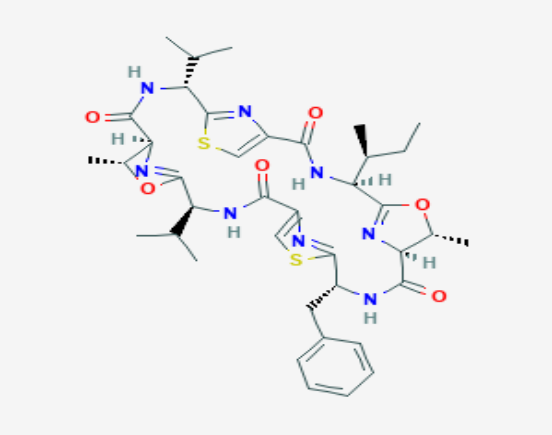
MATERIALS AND METHODS

A set of personal computer with Intel (R) Core (TM) i3-7100U CPU @2,40 GHz, RAM 4 GB and 500GB hardisk was used as the hardware. Several softwares were used such as Autodock Vina in PyRx 9.5 to predict the binding affinity with specific molecular docking (The Scripps Research Institute), PyMol Molecular Graphic System 1.7.4 (DeLano Scientific LLC) for molecular visualization, LigPlot for ligand protein interaction visualization, and Open Babel GUI 2.4.1 for converting chemical objects from one file format to another needed.

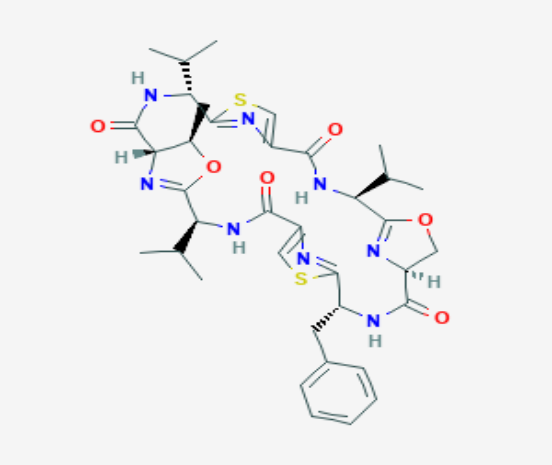
Targeted proteins as the macromolecules were downloaded from data bank on Protein Data Bank (PDB) http://www.rcsb.org. Human epidermal growth factor (EGF) (PDB ID 1M17) with its control [6,7-BIS(2-Methoxy-Ethoxy) Quinazoline E-4-YL]-(3-Ethynylphenyl)amine, Erlotinib; human epidermal growth factor receptor 2 (HER2) (PDB ID 3RCD) with its control, TAK-285; human estrogen receptor alpha (ESRA) (PDB ID 3ERT) with its control, 4-hydroxytamoxifen; as well as doxorubicin as the comparison drug (PUBCHEM ID 31703). The ligands were patellamide C, E, F, and ulithiacyclamide. These were also downloaded in PDB file format (.pdb). The structures of the ligands are shown in Fig. (1).



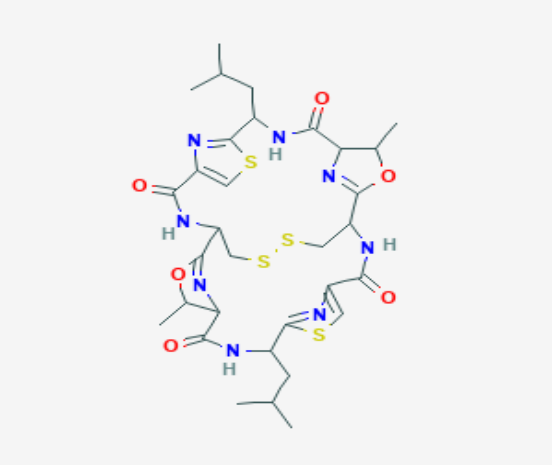
Patellamide C (PubChem CID: 9767970)



Patellamide E (PubChem CID: 44576033)



Patellamide F (PubChem CID: 392480)



Ulithiacyclamide (PubChem CID: 5633)

Fig. 1. Structure of patellamide C, E, F and ulithiacyclamide (downloaded from PubChem Comp. https://www.ncbi.nlm.nih.gov/)

Molecular docking of patellamide C, E, F and ulithiacyclamide to target proteins

Prior to docking, there must be a preparation steps for ligands and macromolecules to be set for proper docking processes. The chemical structures of the ligands were downloaded from PubChem Compound (www. pubchem.ncbi.nlm.nih.gov/compound) to obtain their 2D structures then converted to 3D structure using Open Babel. All the heteroatoms were removed from the macromolecules to make complex receptor be free of any ligand before docking. The Graphical User Interface program AutoDock tools was used to prepare, run, and analyze the docking simulations. Docking was performed to obtain the binding affinity of ligands and macromolecules. For this, human EGF, human HER2, dan human ESRA were chosen because those were laboratorialy tested and bound to their controlled compounds based on crystallographic experiments. Patellamide C, E, F and ulithiacyclamide were docked to the macromolecules using Autodock Vina Tools in PyRx 9.5 version. AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked as it stores the potential energy arising from the interaction with macromolecule.

This grid must surround the region of interest (active site) in the macromolecules. In the present study, the binding site was selected based on the amino acid residues, which were involved in binding macromolecules as obtained from PDB with ID 1M17, ID 3RCD, ID 31703 which would be considered as the best accurate active region as it is solved by experimental crystallographic data. The grid was set to 22,272; -0,784; 51,613 for EGF, 12,761; 1,798; 27,868 for HER2 and 31,090; -3,362; 24,612 for ESRA, for x, y and z, respectively. The analysis will indicate the lowest binding energy as good conformation. More negative the binding affinity score expressed in kcal mol^{-1} is between the ligands and the target protein, the stronger the binding, the stronger the bond between the ligandsand protein target.

Anticancer potential prediction of patellamide C, E. F and ulithiacyclamide

Patellamide C, E, F and ulithiacyclamide were also screened for their anticancer potential using WAY2DRUG PASS prediction which can be accessed on http://www.pharmaexpert.ru/passonline. PASS (prediction of activity spectra for substances) is a software product designed as a tool to evaluate the general biological potential of an organic drug-like molecule. PASS provides simultaneous predictions of many types of biological activities based on the structure of organic compounds. Thus, PASS can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing. In this study, we predicted antineoplastic and antimetastatic potential. The results were shown as Pa scores. Pa (probability "to be active") estimates the sub-class of active compounds (resembles the structures of molecules, which are the most typical in a sub-set of "actives" in PASS training set). If the Pa score of a compound was more than 0.7, it would indicate that the compound has high anticancer potential. If the Pa score is between 0.3 and 0.7, it will indicate that the compound has less anticancer potential and needs to be subjected to further test **(Filimonov *et al.*, 2014)**.

RESULTS AND DISCUSSION

Table (1) shows the docking results of ligands to receptors, including the standards, defined as the binding affinity score (kcal·mol^{-1}). The binding energy of a ligand to the enzyme catalytic site

is represented by the binding affinity score. Lower binding energy indicates a stronger interaction between the ligands and the target proteins and predicts that the interactions will be more stable.

Table 1. Docking binding affinity scores of ligands to macromolecules

Macromolecules	Ligand	Binding Affinity Score (kcal mol^{-1})	Remarks
EGF	Doxorubicin	–9.1	
	Ulithiacyclamide	–9.3	
	Patellamide C	–5.2	
	Patellamide E	–6.9	
	Patellamide F	–9.3	
	Erlotinib	–6.9	
HER2	Doxorubicin	–8.7	
	Ulithiacyclamide	–8.1	
	Patellamide C	–8.1	
	Patellamide E	–9.4	
	Patellamide F	–8.5	
	TAK285	–9.8	
ESRA	Doxorubicin	–6.2	
	Ulithiacyclamide	37.1	Positive
	Patellamide C	26.7	Positive
	Patellamide E	36.1	Positive
	Patellamide F	15.6	Positive
	4-Hydroxytamoxifen	–9.9	

The docking results of patellamide F and ulithiacyclamide to the target protein EGF showed the strongest binding affinity score of –9.3 kcal·mol^{-1} compared to the others, especially erlotinib (–6.9 kcal·mol^{-1}) as the control compound and doxorubicin (–9.1 kcal·mol^{-1}) as the comparison drug.

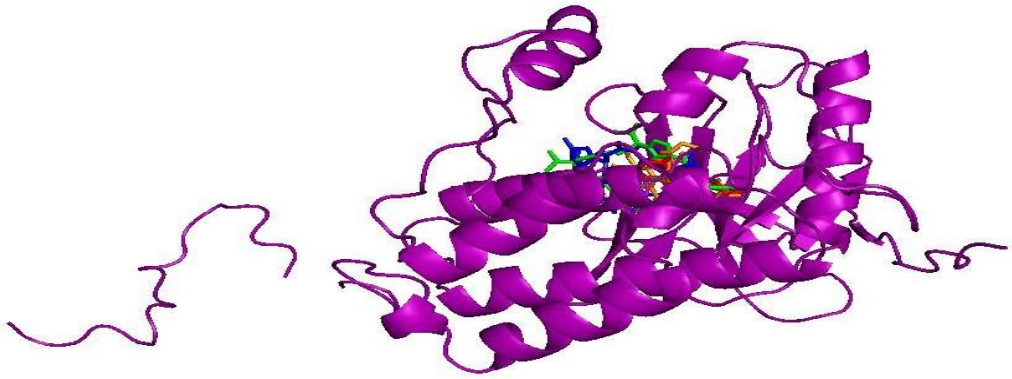


Fig. 2. PyMol visualization as a docking result of patellamide F (blue) and ulithiacyclamide (green) to EGF protein with controlled compound erlotinib (red), doxorubicin (orange).

Docking result of patellamide E to targeted protein HER2 has resulted in the strongest binding affinity, –9.4 kcal mol^{-1}, though it was not as high as TAK285 –9.8 kcal mol^{-1} as the control compound, but higher than that of doxorubicin, –8.7 kcal mol^{-1}, as the comparison drug.

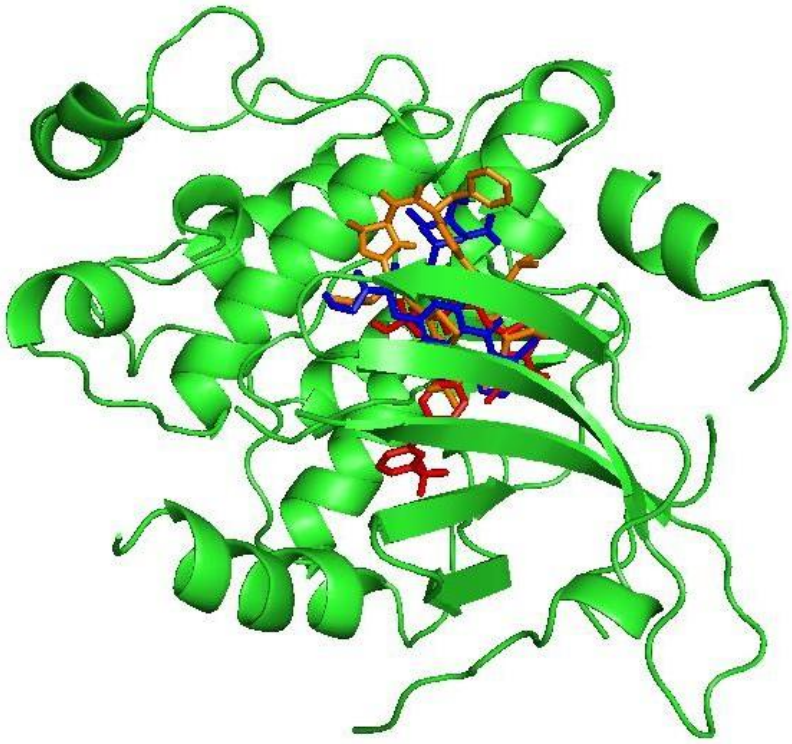


Fig. 3. PyMol visualization as a docking result of patellamide E (blue), HER2 protein with controlled compound TAK285 (red), doxorubicin (orange)

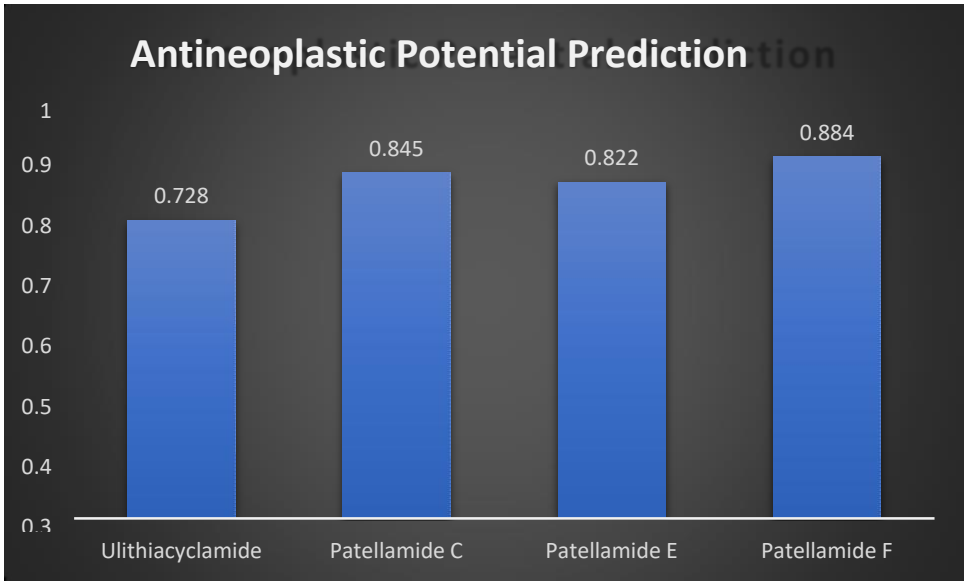


Fig. 4. Histogram of antineoplastic potential score from PASS prediction

Fig. (4) shows the antineoplastic potential scores of ulithiacyclamide, patellamide C, E, and F. Patellamide F possessed the highest Pa score, 0.884. Based on the probability to be active (Pa) score, patellamide F had the highest potential as an antineoplastic agent among the four tested compounds. Patellamide C and E, as well as ulithiacyclamide, also showed high antineoplastic potential, as their Pa scores exceeded 0.7.

Studies on the anticancer potential of patellamide compounds and their derivatives have been reported since the 1980s. The anticancer activity of ulithiacyclamide tested on L1210 murine leukemia cell cultures revealed that ulithiacyclamide is the most effective cyanobactin group, with an IC50 of 0.35 $\mu g \cdot mL^{-1}$ **(Ireland *et al.*, 1982)**, inhibiting cell growth through RNA inhibition and protein synthesis mechanisms **(Kohfuku *et al.*, 1989)**. The patellamide group, isolated from microbial symbionts of tunicates from Manado Bay, had its chemical composition studied by **Rumengan *et al.* (2016)**, followed by *in silico* bioactivity potential tests **(Rumengan *et al.*, 2021)**. These studies found that patellamide E and ulithiacyclamide were strongly bound to the protein receptor ER-β (estrogen receptor β) through computational simulation.

There are two types of ER in several tumors: ER-α, which induces proliferation, and ER-β, which is involved in cell aging and death. Among numerous target proteins, ER signaling is well-known as a promising target for acute leukemia **(Roma &**

Spagnuolo, 2020). ER-β signaling is also reported to be related to ovarian cancer **(Pinton *et al.*, 2018)**. A recent study by **Rumengan *et al.* (2021)** reported that ulithiacyclamide and patellamide E strongly bind to ER-β, providing important information for further experimental examination of both compounds in human breast cancer.

The present study indicated that patellamide E had the strongest binding affinity score to the target protein HER2, with a score higher than that of doxorubicin, an anticancer drug. Thus, patellamide E is highly likely to bind to HER2 as a target protein. HER2 (human epidermal growth factor receptor 2) is a membrane tyrosine kinase that can stimulate cell proliferation and survival and is a major driver of tumor development and progression in a subset of breast cancers. HER2 is amplified in about 15–20% of breast cancers **(Krishnamurti & Silverman, 2014)**. This finding is consistent with **Rumengan *et al.* (2021)**, who reported that patellamide E potentially strongly binds to the ER-β protein receptor.

The other two compounds, patellamide F and ulithiacyclamide, exhibited the strongest binding affinity to the EGF protein receptor. EGF (epidermal growth factor receptor; EGFR; ErbB-1; HER1 in humans) is a transmembrane protein that acts as a receptor for members of the epidermal growth factor family of extracellular protein ligands. EGFR is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/neu (ErbB-2), Her3 (ErbB-3), and Her4 (ErbB-4). In many cancer types, mutations affecting EGFR expression or activity can lead to cancer **(Herbst, 2004; Zhang *et al.*, 2007; Qiao *et al.*, 2021)**.

Our findings used different target proteins. The ligands employed in this study are bioactive compounds identified from *Prochloron*, a microbial symbiont in the tunicate *L. patella*. From an ecological point of view, **Hirose (2015)** stated that *Prochloron*, as a secondary metabolite-producing patellamide group and its derivatives, can produce MAAs (mycosporine-like amino acids), substances that can absorb UV light. Hence, conservation efforts for tunicates, particularly *L. patella* as the host of *Prochloron*, through habitat protection are necessary.

CONCLUSION

Patellamide E had the strongest binding affinity score (–9.4 kcal·mol^{-1}) to HER2, its target protein. This was higher than that of doxorubicin (–8.9 kcal·mol^{-1}), used as the comparison drug, but lower than TAK-285 (–9.8 kcal·mol^{-1}), the control compound. Patellamide F and ulithiacyclamide exhibited the strongest binding affinity to the EGF protein receptor (–9.3 kcal·mol^{-1}), compared to doxorubicin (–9.1 kcal·mol^{-1}) and erlotinib (–6.9 kcal·mol^{-1}) as the comparison drug and control compound, respectively. Patellamide

C, E, F, and ulithiacyclamide demonstrated high antineoplastic potential, with probability to be active (Pa) scores above 0.7.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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