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In Silico Study of Monascus sp. Pigment Derivatives as Anticardiovascular Candidate

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Abstract

Background: Cardiovascular disease is the leading cause of death in the world. The therapeutic activity of Monascus sp. pigment can act as an anticardiovascular agent. Research on Monascus sp. pigment is rapidly developing, including the discovery of new pigments, the methods used, and their identification. Currently, there are 57 dyestuff compounds that have been successfully isolated from Monascus molds. So, researchers conducted an in-silico study of Monascus sp.

Objective: To determine whether it can have better interactions and activities as an anticardiovascular medicine candidate.

Method: PAK1 is used as a receptor for anticardiovascular drugs. 57 test compounds were carried out for ligand preparation and application of Lipinski's rule of five by using MarvinSketch software, ADME prediction and toxicity testing using PreADMET, the docking process using Autodock tools, and visualization using Discovery Studio.

Results: The results of the docking analysis are seen from the values of binding affinity consecutively. compound R3 (-8.74 kcal/mol), red shandong (-8.16 kcal/mol), and monaphilol (-8.14 kcal/mol) are lower than the comparison compound bisoprolol (-6.44 kcal/mol), which shows that the three compounds have better interactions than the comparison compounds.

Conclusion: Derivative compounds from Monascus sp. Pigment are predicted to have better interactions and can be used as anticardiovascular medicine candidates.

Keywords: Monascus sp., pigment, anticardiovascular, in silico, PAK1, ADME, and toxicity

1. Introduction

The cardiovascular system is a very important system in the body because cells and tissues can work properly with the supply of oxygen and blood. Cardiovascular disease is a disease that affects the heart and blood vessels. Some people experience this disease. Common diseases include coronary heart disease, stroke, heart failure, and hypertension (Aisyah, 2014).

Coronary Heart Disease (CHD) is a major health problem that often occurs in developed and developing countries. There are various factors that can cause this disease, so multifactorial prevention is needed. Prevention is pursued wherever possible by controlling risk factors because they play an important role in primary and secondary prevention (Farahdika & Azam, 2015). Heart disease is one of the main causes of problems in the world and the number one cause of death. In 2015, there were more than 17 million people in the world who died from heart and blood vessel disease, or about 31% of all deaths in the world, around 8.7 million were due to coronary heart disease. More than 75% of heart and vascular disease occurs in low- to moderate-income developing countries (WHO, 2015).

The therapeutic activity of the red pigment depends on the presence of several bioactive metabolites such as monascopyridines, xanthomonadin, monascumic acid, ascorbic acid, polyphenols, and monacolin, which act as anticardiovascular agents. Monascus sp. is a type of mold

that is used for rice fermentation to produce red rice (Red Mold Rice) or Angkak. For a long time, Angkak has been used as food in Asia and is used in traditional medicine with various bioactive compounds, including monacolin, which has the potential to be used as a nutraceutical (Mostafa & Abbady, 2014; Nguyen *et al.*, 2017).

Research on the pigments of Monascus sp. has developed rapidly, including the discovery of new pigments, methods, and methods of identification. Until now, there have been 57 dyestuff compounds that have been isolated from Monascus molds (Yuliana *et al.*, 2017). However, the study of its activities and safety is relatively limited. The *in-silico* toxicology method can provide a preliminary overview and identify the toxicity of a compound or a selection of potential drug compounds that will be developed into new drug candidates. The in silico test is a complement to i*n vitro* and *in vivo* tests that can streamline the use animals, reduce cost and save time (Purnomo, 2013).

The laboratory test of one of the pigments of Monascus sp. has proven to have anticardiovascular activity and has successfully isolated the color pigment from Monascus sp., so further research is needed regarding other pigments from Monascus sp., which can be a solution to finding new drug candidate compounds that have anti-cardiovascular effects. So, the study with the title "In Silico Study of pigment derivative compounds from Monascus sp. as an Anticardiovascular candidate" is carried out to predict the active compound of the Monascus sp. Pigment and its derivatives as an anticardiovascular agent and the interaction that accompanies the molecule complex with the receptor on the target cell (Yuliana *et al.*, 2017).

2. Methodology

2.1. Tool

The tools that are used in this research are computer hardware and software. These tools include a personal computer with Intel(R) Celeron(R) N4000 specification between 1.10 GHz (CPUs), 1.1GHZ and 4.96 GB of RAM, and the software used in this research is MarvinSketch, Autodoc, Molegro Molecular Viewer, and web-based programs such as, PdbSum and PreADMET.

2.2. Material

The materials used were the PAK1 receptor in the form of PDB files, which were the results of the identification of receptors for anti-cardiovascular products that were downloaded from http://www.rscb.org, and 57 dyestuff compounds isolated from azhapilone derivatives from the mold Monascus sp. which is listed in Table 1.

2.3. Method

2.3.1 Ligand preparation

Ligands were drawn using MarvinSketch software version 5.2.5.1, which further optimized the geometry and protonation at pH 7.4. The next process is geometry optimization to get a stable structure with the minimum potential energy, and then the results are saved in pdb format for docking process using mol2 format (Prasetia, 2011).

2.3.2 Drug Scan

Drug observations Conducted on dyestuffs that are derived from azhapilone from Monascus sp. The analysis was carried out considering Lipinski's rule of five and the oral bioavailability of the ligand. The parameters used were <500 g mol molecular weight, <5 lipophilicity, <5 hydrogen bond donors, <10 hydrogen bond acceptors, and a refractory molarity between 40-130 (Lipinski *et al.*, 1997; Lipinski *et al.*, 2001).

2.3.3 ADME study

The preADMET program is accessed at http://preadmet.bmdrc.org/. The structure of each compound is converted to molfile (*.mol). The preADMET program will automatically calculate the predicted absorption for Caco-2 cells, HIA (Human Intestinal Absorption), and bound plasma proteins (Nursamsiar *et al.*, 2016).

2.3.4 Toxicity test

The toxicity test was carried out on the Monascus sp. pigment compound. This process uses the preADMET program at http://preadmet.bmdrc.org/ and can be classified based on their toxicity (Raies & Bajic, 2016; Ruswanto, 2015).

2.3.5 Receptor analysis

Anticardiovascular receptors were analyzed using a web-based program called PDBsum (www.ebi.ac.uk/pdbsum/). Enter the 5IME code as the PDB receptor code, and then the profile data of the receptor will appear. Then download it from the Protein Data Bank at www.rcsb.org (Raies & Bajic, 2016; Ruswanto, 2015).

2.3.6 Docking validation

Docking method validation is done using Autodoc software. This validation is carried out on valid ligands and docking results. The parameter used is the Root Mean Square Deviation (RMSD) parameter. The docking method is said to be valid if it has an RMSD value <2. This validation process will compare the position of the original ligand on the tested receptor against the same ligand position (copy ligand) when the copy ligand is docked. At this stage, it is carried out in the

absence of water to determine the effect of the presence of water on the docking process. The presence of water will block the bond between the ligand and the receptor because water can form hydrogen bonds with the receptor (Raies & Bajic, 2016; Ruswanto, 2015).

2.3.7 Docking of sample ligands and visualization of interactions with target proteins

Docking is done with Autodock software, and then the same grid box is set in the validation process. The docking results were selected for ligands and proteins with the lowest binding affinity value and then stored in the pdb format (Meiyanto, 2012).

3. Result and discussion

3.1. Drug scan

Based on the data from the drug scan results in Table I, it shows that there are 12 compounds that do not meet Lipinski's rule of five: Isolate MPs 1, Isolate MPs 2, Monaspyridine B, N-gluscosyl rubropuctamine, N-gluscosyl monascorubramine, N-glutoryl rubropuctamine, N-glutoryl monascuorubramine, Red derivate 3, Red derivate 4, Red derivate 7, Red derivate 8, Y3. Meanwhile, 45 other compounds meet the requirement of Lipinski's rule of five; those are FK 17-P2B2, Monankarin AB, Monankarin CB, Monankarin E, Monankarin F, Monaphilones A, Monaphilones B, Monaphilones C, Monapurones A, Monapurones B, Monapurones C, Monarubrin (Y, Bf), Monascusone A , Monascusone B, Monascuspiloin, Monashexenon, Purpureus One, Robropuctin, Xantomonascin A, Xantomonascin B, Yellow II, Monaphilol A, Monaphilol B, Monaphilol C, Monaphilol D, Monasfluor A, Monasfluor B, Coumpound R3, Glycil-rubamin 3 , Isolate MPs 4, Monaspyridine A, Monaspyridine C, Monaspyridine D, New red pigment, Red derivate 1, Red derivate 2, Red derivate 5, Red derivate 6, Red Shandong 1, Red Shandong 2, Unnamed, PPV, Monascuskaodione A, Monascuskaodione B. Compounds that meet the Lipinski rules are presumed to have good bioavailability. However, not all compounds have good activity according to Lipinski's rules (Santoso *et al.*, 2016).

3.2. ADMET study

This is a quantitative test using the preADMET program. In general, the ADME process aims to determine when the drug enters the body and the drug is absorbed (absorption), then spread to all body tissues through the blood (distribution), then metabolized in certain organs, especially the heart (metabolism), and then the metabolic results are released from the body (excretion). Based on the results of the ADME test using the web-based PreADMET program in Table 2, the pigment derivative compounds of Monascus sp. had a moderate permeability value, which is in the range of

4-70%; only FK 17-P2B2 (0.993%) compounds have low permeability. The absorption process in the human intestine is in the range of 70-100% which is a good range (Nursamsiar *et al.*, 2016). The protein binding in the blood of the Isolate MPs3 compound, Isolate MPs4, N-glutaryl monascorubramine, Monaspyridine A, Monaspyridine B, Monaspyridine D, Red derivate 1, Red derivate 5, Monascuskaodione B, Monaphilones A, Monaphilones B, Monarubrin (Y, Bf), Purpures one, Robropuctin, Yellow II, Xantomonascin A, Xantomunascin B, Monaphilol A, Monaphilol B, Monasfluor A, Monasfluor B have high values of > 90%, indicating strong bonds with plasma proteins in the body.

Based on the data in Table 2, the toxicity test used the Ames test parameter to determine that most of the compounds are non-mutagenic, which means that they do not cause changes in genetics (DNA or RNA) either at the level of the gene or chromosome sequences that can cause cancer or are also known as carcinogens.

Table 1. Drug scan test results in accordance with Lipinski's the rule of five

No	Compound Name	Parameter				
		Molecular Weight (<500)	**Lipofility (<5)**	**Hydrogen Bond Donor (<5)**	**Hydrogen Bond Acceptor (<10)**	**Refractory Molars (40-130)**
		Red Pigment				
1	*Coumpound R3*	374.4275	2.05	1	8	101.41
2	*Glycyl-rubropuntamin*	413.4636	3.33	1	10	114.09
3	***Isolate MPs 1***	**510.5821**	2.85	5	**13**	**152.26**
4	***Isolate MPs 2***	**538.6352**	3.74	5	**13**	**161.46**
5	*Isolate MPs 3*	439.5009	4.08	1	10	123.54
6	*Isolate MPs 4*	439.5439	4.32	1	8	126.87
7	*Monaspyridine A*	355.4275	4.23	0	7	98.81
8	***Monaspyridine B***	383.4807	**5.12**	0	7	108.01
9	*Monaspyridine C*	357.4434	3.90	1	7	98.88
10	*Monaspyridine D*	343.4599	4.61	1	6	100.56
11	***N-glucoscyl rubroputamine***	**557.6320**	2.65	4	12	149.92
12	***N-glucoscyl monascurobamine***	**585.6851**	3.54	4	**12**	**159.12**
13	***N-glutaryl monascorubramine***	**511.5635**	4.30	2	**13**	**138.82**
14	***N-glutaryl rubropuctamine***	483.5104	3.41	2	**13**	129.62
15	*New red pigment*	375.4587	1.16	3	7	103.91
16	*Red derivate 1*	453.5274	4.65	1	10	128.03
17	*Red derivate 2*	425.4743	3.76	1	10	118.83
18	***Red derivate 3***	497.5369	4.01	2	**13**	**134.07**
19	***Red derivate 4***	469.4838	3.12	2	**13**	124.87
20	*Red derivate 5*	453.3274	4.65	1	10	128.03
21	*Red derivate 6*	425.4743	3.76	1	10	118.83
22	***Red derivate 7***	497.5369	4.01	2	**13**	**134.07**
23	***Red derivate 8***	469.4838	3.12	2	**13**	124.87

No	Compound Name	Parameter				
		Molecular Weight (<500)	**Lipofility (<5)**	**Hydrogen Bond Donor (<5)**	**Hydrogen Bond Acceptor (<10)**	**Refractory Molars (40-130)**
24	*Red Shandong 1*	303.3960	0.54	4	4	91.77
25	*Red Shandong 2*	331.4492	1.43	4	4	100.96
26	*Unnamed*	375.4587	1.16	3	7	103.91
	Red Purple					
27	*PP-V*	412.4556	3.26	3	9	125.22
	Colorless oil					
28	*Monascuskaodione A*	356.4123	3.38	0	7	100.67
29	*Monascuskaodione B*	384.3654	4.27	0	7	109.87
	Yellow Pigment					
30	*FK 17-P2B2*	236.2637	0.35	2	5	66.34
31	*Monankarin A-B*	358.3851	2.36	2	7	98.10
32	*Monankarin C-D*	372.4117	2.90	2	7	103.14
33	*Monankarin E*	344.3585	2.02	2	7	93.63
34	*Monankarin F*	356.4123	2.99	2	6	103.21
35	*Monaphilones A*	360.4871	4.16	1	6	106.90
36	*Monaphilones B*	322.4339	3.27	1	6	97.70
37	*Monaphilones C*	336.4657	4.14	1	7	95.95
38	*Monapurones A*	330.4180	2.98	1	6	97.87
39	*Monapurones B*	344.4446	3.93	0	5	101.83
40	*Monapurones C*	344.4446	3.93	0	5	101.83
41	*Monarubrin (Y.Bf)*	330.4180	3.13	1	6	97.95
42	*Monascusone A*	254.2790	0.99	3	6	67.08
43	*Monascusone B*	302.3218	1.64	0	7	82.07
44	*Monascuspiloin*	360.4440	3.11	1	6	101.32
45	*Monashexenoone*	320.4232	3.70	1	7	92.33
46	*Purpureus one*	390.5131	5.43	0	8	107.95
47	*Robropuctin*	358.4712	4.02	1	6	107.15
48	*Xantomonascin A*	388.4111	4.00	2	8	102.20
49	*Xantomonascin B*	414.4914	3.75	2	8	126.81
50	*Y3*	448.571	0.34	6	8	115.88
51	*Yellow II*	372.4547	4.31	1	7	116.32
	Orange Pigment					
52	*Monaphilol A*	384.4654	3.62	1	6	111.10
53	*Monaphilol B*	356.4123	2.73	1	6	101.89
54	*Monaphilol C*	440.5287	3.59	1	8	125.32
55	*Monaphilol D*	412.4755	2.70	1	8	116.12
	Blue Fluorescence Pigment					
56	*Monasfluor A*	354.4394	3.98	0	5	104.30
57	*Monasfluor B*	384.4654	4.27	0	7	109.87

Note: Text in bold does not meet the requirements.

Table 2. Prediction of toxicity and ADME result

No	Compound Name	Parameter			
		$CaCo_2$	**HIA**	**Plasma Protein Binding**	**Ames test**
	Red Pigment				
1	*Coumpound R3*	17.4163 (Medium)	95.677133 (Good)	73.231309 (Weakly Bonded)	Non mutagen

No	Compound Name	Parameter			
		$CaCo_2$	HIA	Plasma Protein Binding	Ames test
2	*Glycyl-rubropuntamin*	20.9325 (Medium)	96.702970 (Good)	87.663371 (Weakly Bonded)	Non mutagen
3	***Isolate MPs 1***	**9.25845 (Medium)**	**74.407933 (Good)**	**64.976138 (Weakly Bonded)**	**Mutagen**
4	***Isolate MPs 2***	**8.92909 (Medium)**	**78.193552 (Good)**	**78.061242 (Weakly Bonded)**	**Mutagen**
5	***Isolate MPs 3***	**21.5677 (Medium)**	**98.309963 (Good)**	**90.416630 (Strongly Bonded)**	**Non mutagen**
6	***Isolate MPs 4***	**25.6923 (Medium)**	**99.303546 (Good)**	**92.314035 (Strongly Bonded)**	**Non mutagen**
7	*N-glucoscyl rubroputamine*	12.4451 (Medium)	85.412276 (Good)	65.185992 (Weakly Bonded)	Non mutagen
8	*N-glucoscyl monascurobamine*	12.5306 (Medium)	87.690078 (Good)	79.078783 (Weakly Bonded)	Non mutagen
9	***N-glutaryl monascorubramine***	**19.8389 (Medium)**	**92.538271 (Good)**	**90.486415 (Strongly Bonded)**	**Non mutagen**
10	*N-glutaryl rubropuctamine*	19.7598 (Medium)	90.311224 (Good)	88.136.616 (Weakly Bonded)	Non mutagen
11	*New red pigment*	17.9896 (Medium)	89.666473 (Good)	66.572743 (Weakly Bonded)	Non mutagen
12	***Monaspyridine A***	**26.367 (Medium)**	**98.750840 (Good)**	**91.953695 (Strongly Bonded)**	**Non mutagen**
13	***Monaspyridine B***	**32.7272 (Medium)**	**98.912525 (Good)**	**93.500167 (Strongly Bonded)**	**Non mutagen**
14	*Monaspyridine C*	22.7729 (Medium)	96.648798 (Good)	89.685299 (Weakly Bonded)	Non mutagen
15	***Monaspyridine D***	**22.9611 (Medium)**	**96.270412 (Good)**	**97.268350 (Strongly Bonded)**	**Non mutagen**
16	***Red derivate 1***	**22.1952 (Medium)**	**98.668455 (Good)**	**90.932647 (Strongly Bonded)**	**Non mutagen**
17	*Red derivate 2*	21.2765 (Medium)	97.891289 (Good)	88.641765 (Weakly Bonded)	Non mutagen
18	*Red derivate 3*	20.5128 (Medium)	91.491229 (Good)	89.736964 (Weakly Bonded)	Non mutagen
19	*Red derivate 4*	20.4423 (Medium)	88.981951 (Good)	86.838945 (Weakly Bonded)	Non mutagen
20	***Red derivate 5***	**22.1952 (Medium)**	**98.668455 (Good)**	**90.932647 (Strongly Bonded)**	**Non mutagen**
21	*Red derivate 6*	21.2765 (Medium)	97.891289 (Good)	88.641765 (Weakly Bonded)	Non mutagen
22	***Red derivate 7***	**20.5128 (Medium)**	**91.491229 (Good)**	**89.736964 (Weakly Bonded)**	**Mutagen**
23	***Red derivate 8***	**20.4423 (Medium)**	**88.981951 (Good)**	**86.838945 (Weakly Bonded)**	**Mutagen**
24	***Red Shandong 1***	**13.6843 (Medium)**	**85.719545 (Good)**	**71.398196 (Weakly Bonded)**	**Mutagen**
25	*Red Shandong 2*	14.2523 (Medium)	87.122330 (Good)	87.278357 (Weakly Bonded)	Non mutagen
26	*Unnamed*	17.9896 (Medium)	89.666473 (Good)	66.572743 (Weakly Bonded)	Non mutagen

No	Compound Name	Parameter			
		$CaCo_2$	HIA	Plasma Protein Binding	Ames test
		Red Purple			
27	***PP-V***	**4.01366 (Medium)**	**92.923736 (Good)**	**86.114618 (Weakly Bonded)**	**Mutagen**
		Colorless oil			
28	***Monascuskaodione A***	**28.3725 (Medium)**	**98.770329 (Good)**	**88.897997 (Weakly Bonded)**	**Mutagen**
29	***Monascuskaodione B***	**36.0846 (Medium)**	**98.771155 (Good)**	**92.381422 (Strongly Bonded)**	**Mutagen**
		Yellow Pigment			
30	***FK 17-P2B2***	**0.993 (Low)**	**90.432010 (Good)**	**56.076521 (Weakly Bonded)**	**Mutagen**
31	*Monankarin A-B*	21.4435 (Medium)	93.567317 (Good)	85.583078 (Weakly Bonded)	Non mutagen
32	*Monankarin C-D*	21.9971 (Medium)	93.909198 (Good)	87.093425 (Weakly Bonded)	Non mutagen
33	*Monankarin E*	20.8618 (Medium)	93.127449 (Good)	81.200843 (Weakly Bonded)	Non mutagen
34	*Monankarin F*	35.5122 (Medium)	93.789307 (Good)	88.543275 (Weakly Bonded)	Non mutagen
35	***Monaphilones A***	**46.4843 (Medium)**	**96.065559 (Good)**	**93.232878 (Strongly Bonded)**	**Non mutagen**
36	***Monaphilones B***	**40.8229 (Medium)**	**96.054536 (Good)**	**90.112573 (Strongly Bonded)**	**Non mutagen**
37	*Monaphilones C*	26.9554 (Medium)	95.760530 (Good)	86.135746 (Weakly Bonded)	Non mutagen
38	*Monapurones A*	26.4733 (Medium)	96.071604 (Good)	85.103277 (Weakly Bonded)	Non mutagen
39	***Monapurones B***	**44.272 (Medium)**	**97.697949 (Good)**	**88.300451 (Weakly Bonded)**	**Mutagen**
40	***Monapurones C***	**44.272 (Medium)**	**97.697949 (Good)**	**88.300451 (Weakly Bonded)**	**Mutagen**
41	***Monarubrin (Y.Bf)***	**40.5147 (Medium)**	**96.071613 (Good)**	**92.263384 (Strongly Bonded)**	**Non mutagen**
42	*Monascusone A*	19.3778 (Medium)	78.683369 (Good)	34.939799 (Weakly Bonded)	Non mutagen
43	***Monascusone B***	**22.9891 (Medium)**	**97.536574 (Good)**	**61.438550 (Weakly Bonded)**	**Mutagen**
44	***Monascuspiloin***	**30.4892 (Medium)**	**96.468903 (Good)**	**90.306522 (Strongly Bonded)**	**Non mutagen**
45	*Monashexenoone*	22.609 (Medium)	95.857284 (Good)	88.158433 (Weakly Bonded)	Non mutagen
46	***Purpureus one***	**31.1835 (Medium)**	**98.247814 (Good)**	**90.721848 (Strongly Bonded)**	**Non mutagen**
47	***Robropuctin***	**46.2928 (Medium)**	**96.050080 (Good)**	**95.896405 (Strongly Bonded)**	**Non mutagen**
48	***Y3***	**19.3732 (Medium)**	**50.125685 (Good)**	**67.649190 (Weakly Bonded)**	**Mutagen**
49	***Yellow II***	**34.2219 (Medium)**	**96.423561 (Good)**	**91.738989 (Strongly Bonded)**	**Mutagen**
50	***Xantomonascin A***	**19.6124**	**88.865206**	**96.842726**	**Mutagen**

No	Compound Name	Parameter			
		$CaCo_2$	HIA	Plasma Protein Binding	Ames test
		(Medium)	**(Good)**	**(Strongly Bonded)**	
51	***Xantomonascin B***	**26.5245 (Medium)**	**94.538788 (Good)**	**94.764093 (Strongly Bonded)**	**Mutagen**
	Orange Pigment				
52	***Monaphilol A***	**36.7103 (Medium)**	**96.501044 (Good)**	**95.427557 (Strongly Bonded)**	**Mutagen**
53	***Monaphilol B***	**29.408 (Medium)**	**96.568439 (Good)**	**91.926288 (Strongly Bonded)**	**Mutagen**
54	*Monaphilol C*	34.038 (Medium)	97.366571 (Good)	89.549401 (Weakly Bonded)	Non mutagen
55	*Monaphilol D*	27.7997 (Medium)	97.311031 (Good)	83.639250 (Weakly Bonded)	Non mutagen
	Blue Fluorescence Pigment				
56	***Monasfluor A***	**49.808 (Medium)**	**97.649968 (Good)**	**92.794086 (Strongly Bonded)**	**Mutagen**
57	***Monasfluor B***	**36.0846 (Medium)**	**98.771155 (Good)**	**92.381422 (Strongly Bonded)**	**Mutagen**

Note: Text in bold does not meet the requirements.

Table 3. Analysis of anticardiovascular receptors

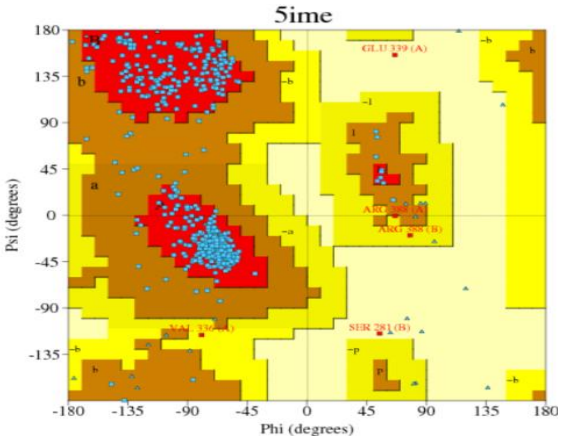
No	Receptor Name	Receptor Code	Grid box			RMSD
			X	Y	Z	
1	Phospholipase	1TGM	11.516	14.32	3.35	2.28
2	Phospholipase A2	10XR	46.406	32.446	7.672	5.59
3	Myotoxin II	6MQF	11.404	-71.575	55.805	4.80
4	Lactoperoxidase	2QQT	3.367	4.056	29.937	6.29
5	**PAK1**	**5IME**	**18.472**	**-16.384**	**11.213**	**0.75**

Note: Text in bold is the best receptor.

3.3. Analysis of anticardiovascular receptors

Receptor preparation in this study was done by downloading anti-cardiovascular receptors from the Protein Data Bank (https://www.rcsb.org/). There are 5 anticardiovascular receptors with the codes 1TGM, 10XR, 6MQF, 2QQT, and 5IME. Due to its best RSMD value, only 5IME was taken out of the five other compounds. The PAK1 receptor with code 5IME is then downloaded in .pdb format (Table 3).

The Ramachandran Plot analysis shows that the 5IME receptor has a stable structure because 91.2% of the residues are in the most preferred area and only 0.4% are in the least preferred area. It can be said that the structural quality of the protein is good if the residue in the disallowed region (the unwanted area) is smaller than 15% and the amino acid residue in the most favored region is greater than 50% (Amelia, 2013).



PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	444	91.2%
Additional allowed regions	[a,b,l,p]	38	7.8%
Generously allowed regions	[~a,~b,~l,~p]	3	0.6%
Disallowed regions	[XX]	2	0.4%*
Non-glycine and non-proline residues		487	100.0%
End-residues (excl. Gly and Pro)		12	
Glycine residues		35	
Proline residues		26	
Total number of residues		560	

Figure 1. 5IME Ramachandran plot

3.4. Docking validation

Based on the validation results (Table 4), the RMSD value for the 5IME code PAK1 GDP receptor is 0.75, and the result is ≤2. Grid box X = 18.472, Y = -16.384, and Z = 11.213. So, from the results of the validation above, the PAK1 receptor with the 5IME code met the validation criteria for the docking method so that it could be used on the sample ligand. The validation process can compare the position of the original ligand (brown) to the PAK1 receptors with the 5IME code tested against the same ligand position (copy ligand) when the copy ligand is docked. At this stage, it is carried out without water to determine the effect of the presence of water at the docking process stage (Figure 2). The presence of water can block the bonds between ligands and receptors because water can form hydrogen bonds with receptors (Pebriana *et al.*, 2012).

Table 4. Docking validation results

PDB Code	Grid Box			RMSD
	X	Y	Z	
5IME	18.472	-16.384	11.213	0.75

Docking is done using Autodock. The receptors of the validation results are entered into the software, and then natural ligands are used for the validation process. The grid box is then arranged. The use of the grid box in the docking process is the same as the grid box used for natural ligands. So that the test ligand can interact with the area inside the receptor. The grid box center used is (X= 18.472, Y= -16.384 and Z= 11.213). The results are the binding affinity value (Raies & Bajic, 2016; Ruswanto, 2015).

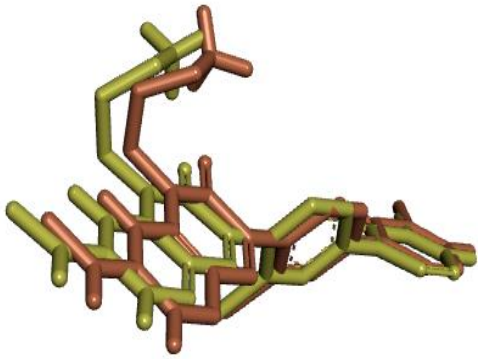


Figure 2. Visualization of the docking validation results (original ligand: brown, copy ligand: green)

3.5. Docking of sample ligands and visualization of interactions with target proteins

3.5. Analysis of docking results

The docking result between the ligand and the target protein will produce various conformations of the tested compound. The binding energy must be considered in the docking results. The best conformation can be seen through the binding energy (ΔG) which has units of kcal/mol. The binding energy describes the strength of the bond that occurs between the ligand and the target protein. The bond energy has a relationship with the inhibition constant. The smaller the inhibition constant, the smaller the bond energy. So, it is known that the smaller the bond energy, the more preferred the interaction between ligands and enzymes (Meiyanto, 2012). The results of each conformation between the ligands and the target protein can be seen in Table 5.

The data in Table 5 shows that all the sample ligands have the ability to bind to the target protein. According to the results of the analysis above, the average of the sample ligand has a smaller binding affinity than the comparison compound (Bisoprolol). This shows that the sample compound modified with the derivative compound from Monascus sp. can inhibit the target protein well and is more stable than the comparison compound. Bisoprolol is a drug belonging to the group of β-blockers, a class of medicines used primarily in cardiovascular diseases (Sabidó *et al.*, 2019).

Table 5. Interaction of ligands with tested target proteins

No	Compounds Name	Binding affinity	Hydrogen Bond	Amino Acid
1	Comparison compound (Bisoprolol)	-6.44	LEU347 MET319	LEU347, MET319, SER351, GLY350, ALA348, LYS538, THR406, TYR330, ILE316. VAL342, PHE408, LYS299, ASP507. ALA297. GLU345, VAL328, LEV396, MET344, TYR346
2	***Coumpound R3***	**-8.74**	**LEU347 THR406 ASP407**	**LEU347, THR406, ASP407, LYS299, VAL343, ILE298, VAL284, ALA297, TYR346, LEU396, MET344, GLU315, TYR330, MET319, ILE316**
3	***Red Shandong 2***	**-8.16**	**LEU347**	**LEU347, THR406, GLY350, ALA297, VAL284,**

			THR406	**LYS299, MET319, TYR330, ILE316, VAL342, ILE298, GLU314, MET344, ASP407, GLU345, VAL328, LEU396, TYR346**
4	***Monaphilol C***	**-8.14**	**LEU347 LYS199**	**LEU347, LYS299, TYR346, LEU396, GLU345, VAL328, ALA297, THR406, MET344, ASP402, ILE316, GLU315, VAL342, MET319, VAL284, GLY350**
5	*Monankarin A-B*	-7.81	ASP407 LYS299 LEU347	ASP407, LYS299, LEU347, MET344, VAL342, ILE298, GLU345, VAL328, ALA297, TYR346, LEU396, GLY350, SER351, ASP393, ASN394
6	*Monankarin F*	-7.79	GLU315 VAL342	GLU315, VAL342, ASP393, THR406, ASP407, PHE408, MET319, MET344, LYS299, ILE298, VAL284, ALA297, LEU396, ASN394
7	*Unnamed*	-7.77	LEU347	LEU347, VAL342, THR406, ALA297, LEU396, VAL328, GLU345, MET344, TYR346, VAL284, ILE298, ASP407, LYS299, GLY350, ILE276
8	*Monaphilol D*	-7.76	LYS298 GLU315	LYS298, GLU315, ASP393, MET344, ASP407, PHE408, ILE316, TYR330, MET319, VAL342, MET301, GLU349, VAL284, ALA297, VAL328, LEU396, THR406
9	*Monaspyridine C*	-7.66	LEU347 GLU345	LEU347, GLU345, GLY279, GLN278, ILE276, LEU396, TYR346, VAL328, ALA297, MET344, GLU315, VAL342, LYS299, ILE298, VAL284, GLN278, GLY279
10	*Monankarin C-D*	-7.63	-	GLU315, ALA297, ILE276, LEU347, TYR346, VAL328, GLU345, GLY350, SER351, LEU396, VAL284, LYS299, ASP407, ILE298, VAL342, MET344
11	*Monashexenoone*	-7.60	LEU347 LYS299 ASP407 THR406	LEU347, LYS299, ASP407, THR406, GLY350, GLU345, LEU396, VAL328, GLU315, MET301, VAL342, PHE408, MET319, ILE276, TYR346, VAL284, ALA297

Note: Text in bold is the best binding affinity score

4. Conclusion

From all the stages and the results that have been obtained from this research, the following conclusions can be drawn:

1. Based on the results of Drugscan testing of 57 Monascus sp. pigment compounds, 12 compounds do not meet Lipisnki's rule of five. Meanwhile, 45 other compounds met the requirements.
2. Based on the toxicity test results and the results of the ADME study of 57 Monascus sp. pigment compounds, 34 compounds did not meet the parameters, while 23 other compounds did.
3. Based on the results of the docking analysis of the 10 best compounds from Drugscan, the ADME and Toxicity studies seen from the binding affinity value, respectively, show that there were 3 best compounds, compound R3, red Shandong 2, and Monaphilol C, which were -8.74, -

8.16, and -8.14 kcal/mol lower than the comparison compound (-6.44 kcal/mol), which means that the three compounds have better interactions than the comparison compound.

4. Pigment derivative compounds from Monascus sp. are predicted to have better interactions and can be used as anticardiovascular drug candidates: compound R3, red Shandong 2, and monaphilol C.

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