Sains Malaysiana 52(10)(2023): 2855-2867
http://doi.org/10.17576/jsm-2023-5210-10

Revealing the Potency of 1,3,5-Trisubstituted Pyrazoline as Antimalaria through Combination of *in silico* Studies

(Mendedahkan Potensi Pirazolin 1,3,5-Tritertukarganti sebagai Antimalaria melalui Gabungan Kajian *in silico*)

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Received: 31 May 2023/Accepted: 14 September 2023

ABSTRACT

The potency of 1,3,5-trisubstituted pyrazoline as an antimalarial agent has been studied through quantitative structure-activity relationship, molecular docking, and molecular dynamics simulation as a combination of *in silico* studies. The study commenced by applying quantitative structure-activity relationship (QSAR) to 25 derivative compounds using 3D-descriptor. The genetic algorithm and multiple linear regression analysis were used to construct the QSAR model, which resulting an equation that has $R_{training}$ as 0.8100 and $R_{test\ set}$ as 0.9222. Descriptors involved in the QSAR equation are TDB4 m, TDB8s, RDF30e, and RDF552, all of which belong to the group of 3D autocorrelation and RDF. This result is in line with the principal component analysis, which shows that both group descriptors represent whole 3D descriptors. Molecular docking analysis is conducted to study the interaction between pyrazoline derivatives and the falcipain-2 enzyme. Interactions between compound **14** and falcipain-2 is describing by hydrogen bond against Glu14 amino acid residue, more pi-stacking interaction, and van der Waals. Chloroquine as a positive control also presented one hydrogen bond with Gly83, pi-sulfur against Cys42, and van der Waals. The stability of the ligand–enzyme interaction is evaluated by molecular dynamics simulation, and after 100 ns simulations, the root mean square deviation results show that compound **14** and chloroquine have a stable interaction with the falcipain-2 enzyme. Overall, this research provides the insight of 1,3,5-trisubstitued pyrazoline compounds as antimalaria by giving a QSAR equation and used to design a better falcipain-2 inhibitors.

Keywords: Antimalaria; molecular docking; molecular dynamics simulation; QSAR; 1,3,5-trisubstituted pyrazoline

ABSTRAK

Potensi pirazolin 1,3,5-tritertukarganti sebagai agen antimalaria telah dikaji melalui hubungan struktur-aktiviti kuantitatif, dok molekul dan simulasi dinamik molekul sebagai gabungan kajian *in silico*. Kajian dimulakan dengan menggunakan hubungan struktur-aktiviti kuantitatif (QSAR) kepada 25 sebatian terbitan menggunakan petunjuk 3D.

Algoritma genetik dan analisis linear berbilang telah digunakan untuk membina model QSAR yang menghasilkan persamaan yang mempunyai $R_{training}$ sebagai 0.8100 dan $R_{test\ set}$ sebagai 0.9222. Petunjuk yang terlibat dalam persamaan QSAR ialah TDB4 m, TDB8s, RDF30e dan RDF552 yang kesemuanya tergolong dalam kumpulan autokorelasi 3D dan RDF. Keputusan ini adalah selaras dengan analisis komponen utama yang menunjukkan bahawa kedua-dua petunjuk kumpulan mewakili keseluruhan petunjuk 3D. Analisis dok molekul dijalankan untuk mengkaji interaksi antara terbitan pirazolin dan enzim falcipain-2. Interaksi antara sebatian **14** dan falcipain-2 diterangkan dengan ikatan hidrogen terhadap residu asid amino Glu14, lebih banyak interaksi susun pi dan van der Waals. Klorokuin sebagai kawalan positif juga membentangkan suatu ikatan hidrogen dengan Gly83, pi-sulfur terhadap Cys42 dan van der Waals. Kestabilan interaksi ligan-enzim dinilai oleh simulasi dinamik molekul dan selepas simulasi 100 ns, hasil sisihan kuasa dua punca purata menunjukkan bahawa sebatian **14** dan klorokuin mempunyai interaksi yang stabil dengan enzim falcipain-2. Secara keseluruhannya, penyelidikan ini memberikan gambaran tentang sebatian pirazolin 1,3,5-tritertukarganti sebagai antimalaria dengan memberikan persamaan QSAR dan digunakan untuk mereka bentuk perencat falcipain-2 yang lebih baik.

Kata kunci: Antimalaria; dok molekul; QSAR; pirazolina 1,3,5-tritertukarganti; simulasi dinamik molekul

Introduction

Malaria is a disease caused by infection with a parasite transmitted by Anopheles mosquitoes and can cause death (Latifah, Subarnas & Chaerunnisaa 2020). This disease is commonly found in tropical and subtropical regions (Ugwu et al. 2020), such as Saharan Africa, Asia, and Latin America (Gogoi et al. 2020). Prevention and treatment of malaria are carried out in various ways, such as terminating the mosquito breeding cycle with insecticides, using mosquito nets and repellents, and using antimalarial drugs for those who are infected by the disease. However, malaria parasites have developed resistance to almost all antimalarial drugs on the market, such as chloroquine (White 2004) and its derivatives (quinine, amodiaquine, primaquine, mefloquine, and halofantrine), as well as other drugs such as antifolate compounds (pyrimethamine, proguanil, and dapsone), and hydroxy-1,4-naphthoquinone (Winstanley 2001), which has caused this disease to become a major global health problem. This encourages researchers and scientists to continue to look for alternative treatments to overcome the problem of resistance (Gogoi et al. 2020).

One of the antimalarial drug candidates currently being looked at and developed by researchers is pyrazoline and its derivatives (Wanare et al. 2010). Pyrazole is a promising antimalarial drug candidate (Himangini et al. 2018), which primarily inhibits hemozoin (Pandey et al. 2016). Pyrazole-pyrazoline substituted with benzene sulphonamide is active against chloroquine-sensitive (3D7) and CQ-resistant (RKL-9) strains of *Plasmodium falciparum* with an EC_{50} value of 2 μM (Himangini et al. 2018). Several compounds of oxazoline–pyrazoline hybrids were successfully synthesized by Pandey et al. (2016) through the condensation reaction of substituted oxazoline-based chalcones (3a-m) and substituted hydrazine in methanol, exhibiting promising *in vitro* antimalarial activity for chloroquine-sensitive CQS (3D7) and chloroquine-resistant CQR (RKL9) strains. One of the pyrazoline compounds (IC_{50} 0.322 mg/mL) exhibited significant *in vivo* antimalarial potential against a *Plasmodium berghei* mouse model with a 4i–hematin (1:1 stoichiometry) complex, which suggests that heme may be one possible target for these hybrid compounds.

Another type of pyrazole currently being developed as an antimalarial drug is 1,3,5-trisubstituted pyrazoline. This compound can be synthesized by the reflux method using the precursor chalcones and nicotinic acid hydrazide in methanol (Mishra et al. 2017). Research on 1,3,5-trisubstituted pyrazoline compounds *in vitro* carried out on *P. falciparum* strains that were sensitive to chloroquine (MRC-02) and resistant to chloroquine (RKL9) at nanomolar concentrations showed activity inhibiting the formation of β-hematin (BHIA50). The antimalarial mechanism of this compound is the same as that of chloroquine, which involves inhibition of the formation of hemozoin. *In vivo*, some of these compounds showed better antimalarial activity against resistant *P. falciparum* strains than chloroquine (Acharya et al. 2010). This finding showed the potential of pyrazoline derivative as a candidate of antimalarial drug. In 2006, Chimenti et al. studied the potential of 1,3,5-trisbustituted pyrazoline as highly selective monoamine oxidase inhibitor by using 3D-QSAR analysis. The other study conducted by George et al. (2020) synthesized some of

1,3,5-trisubstituted pyrazoline as a breast cancer and did molecular docking as their *in silico* study and resulted that the synthesized compound had a better docking score than erlotinib as positive control. Recently, QSAR analysis on tri-substituted pyrazolines was conducted to evaluate their potential as phosphodiesterase 5 (PDE5) (Nandi et al. 2021). However, *in silico* analysis of 1,3,5-trisubstituted pyrazoline especially Quantitative Structure Activity Relationship (QSAR) as antimalarial had never been conducted.

Currently, recent developments in drug design use *in silico* analysis before *in vitro* or *in vivo* stages. The process of finding candidate antimalarial drug compounds can be carried out either *in vitro*, *in vivo* or *in silico* (Gemma et al. 2007). The use of *in silico* methods can be computerized with the principle of allocating a variety of complex structures and mechanisms for predicting or investigating biological activity (Pornputtapong et al. 2020) The *in silico* method is a solution to the high demand for medicinal compounds and conventional research processes, which are expensive and take a long time (Mandal, Moudgil & Mandal 2009). Quantitative Structure-Activity Relationship (QSAR) is a computational method in predicting the relation between compound structure and their activity as a mathematic equation. This method could be used to design a new and better activity of a compound (Rasyid et al. 2023). The recent QSAR development is attempting the 3D descriptor to construct the equation (Banjare et al. 2023). QSAR analysis combine with molecular docking analysis gave a better insight about relation of structure-activity and mechanism of action from a bioactive compound (Laskar, Mazumder & Talukdar 2023).

The 1,3,5-trisubstitued pyrazoline compounds was docked into the active site of falcipain-2 enzyme. This enzyme has a role in the parasite's life cycle by involving in the hydrolysis of hemoglobin. This enzyme catalyzed the change of hemoglobin to be hemozoin. This change was needed to produce cell's food. The inhibition of the enzyme caused swelling and resulted in disrupted food manufacture. Then, the cell died caused by starvation (Himangini et al. 2018).

In this research, we conducted a combination of *in silico* studies through QSAR, molecular docking, and molecular dynamics simulation. This method gave an insight on designing a better bioactive compound through QSAR analysis, continued with molecular docking and molecular dynamics simulation to know the binding affinity and the stability of 1,3,5-trisubstituted pyrazoline against falcipain-2 enzyme.

Materials and Methods

Data collection and geometry optimization

A total of 25 derivative compounds of 1,3,5-trisubstituted pyrazoline were collected from two references shown in Figure 1 as the core structure of 1,3,5-trisubstituted pyrazoline and Table 1 which show their derivative compounds (Acharya et al. 2010; Mishra et al. 2017).

All compounds were tested as antimalaria agents against chloroquine-sensitive (MRC-02) strains. Those compounds were modeled and optimized using the DFT B3LYP 6-311G (d,p) method in the ORCA program (Nesse et al. 2012) and visualized using Avogadro software (Hanwell et al. 2012).

3D-descriptor calculation

The optimized structures of 1,3,5-trisubstituted pyrazoline were then subjected to calculation of the 3D-descriptors using the PaDEL descriptor resulting in 431 descriptors, which were used for further analysis using principal component analysis (PCA) and QSAR equation development (Yap 2011).

Principle component analysis (PCA)

To examine the distribution of 431 descriptors on 25 compounds, PCA was carried out with RStudio software using the FactoMineR package (Le, Josse & Husson 2008). Normalization of the data was conducted first to overcome the magnitude of variance and scale differences between descriptors. Two-dimension visualization was carried out using the factoextra package (Kassambara & Mundt 2016).

QSAR model development and validation

QSAR model development was started by normalizing the data and performing pre-treatment to ensure the development of a good QSAR model using software from the Drug Theoretics and Cheminformatics Laboratory (dtclab.web.com). The 431 descriptors from each compound were divided into two parts: the training set and the test set. The training set was used to develop the QSAR equation, and the test set was used to validate the QSAR model. The training set data was further analyzed using the BuildQSAR software (De Oliveira & Gaudio 2000) by applying a genetic algorithm and multilinear regression (Hemmateenejad et al. 2003). Three models were selected for further validation.

Validation of the QSAR model was done for internal and external validations. Internal validation included $R^2_{training}$, Q^2, and $Y_{randomization}$ and external validation included the $R^2_{test\ set}$, r^2_m, and leverage approach.

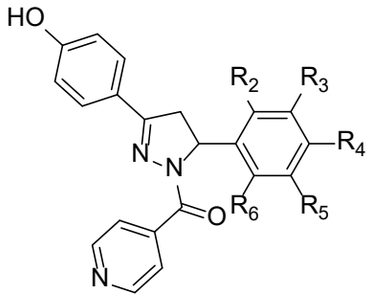

FIGURE 1. Core structure of 1,3,5-trisubstituted pyrazoline compound

TABLE 1. Structure and bioactivity value of 1,3,5-trisubstituted pyrazoline derivative compounds

Compound code	R_2	R_3	R_4	R_5	R_6	IC_{50} (μm)	References
1	C_2H_5	H	H	H	H	0.0810	
2	i-C_3H_7	H	H	H	H	0.1100	
3	CH_3	H	CH_3	H	CH_3	0.0940	
4	OCH_3	H	H	OCH_3	H	0.0400	
5	H	OCH_3	OCH_3	H	H	0.0300	
6	H	H	OCH_2CH_3	H	H	0.2140	
7	H	CH_3	OCH_3	H	H	0.0630	
8	H	H	Br	H	H	0.1410	(Mishra et al. 2017)
9	H	H	F	H	H	2.5000	
10	H	H	NO_2	H	H	1.5660	
11	H	H	OCH_3	H	H	0.0590	
12	H	Cl	H	H	H	0.2400	
13	H	H	i-C_3H_7	H	H	0.0340	
14	H	H	C_3H_7	H	H	0.0220	
15	H	H	Oi-C_3H_7	H	H	0.0380	
16	Cl	H	Cl	H	H	0.0375	
17	H	H	H	H	H	0.0304	
18	CH_3	H	H	H	H	0.0625	
19	H	H	CH_3	H	H	0.0555	
20	H	OCH_2CH_3	OCH_3	H	H	0.0755	(Acharya et al. 2010)
21	Br	H	H	H	H	0.0272	
22	H	OCH_3	OCH_3	OCH_3	H	0.0265	
23	OCH_3	H	H	Br	H	0.0495	
24	H	Br	H	H	H	0.0260	
25	H	NO_2	H	H	H	0.1065	

MOLECULAR DOCKING ANALYSIS

To evaluate the inhibition mechanism, molecular docking was attempted on the complex of falcipain-2 enzyme (PDB ID: 3BPF) (Himangini et al. 2018). Determination of the docked compound was based on the drug likeness evaluation using Lipinski rules. All compounds were analyzed for their ADME properties using SWISSADME web program (www.swissadme.ch). The selected compound was compound 14 due to good ADME results and has the lowest IC_{50} value. The positive control was chloroquine. The docking stage was started in the Chimera software to prepare the protein and ligand structures using *Dockprep* tools. Docking protocols were set in the box grid size of 50 × 50 × 50 Å, with a spacing of 0.375 Å in the AutoDock 4 and AutoDock Tools programs (Morris et al. 2009). The Lamarckian algorithm was used and set to produce 10 conformations. The visualization of the conformation was done in the Discovery Studio Visualizer (Dassault Systèmes 2019).

MOLECULAR DYNAMICS SIMULATION PROTOCOLS

The complex stability of the best conformation result in the molecular docking stage was further analyzed using molecular dynamics simulation in the YASARA Structure software (YASARA Bioscience GmBH, Vienna, Austria) (Land & Humble 2018). The force field used is Amber14 in the periodic boundary condition (Wang et al. 2004). The complex was set at a temperature of 310 K and a pH of 7.4. TIP3P solvent (Mark & Nissson 2001) and counterions (Na^+, Cl^-) were added to neutralize the system. Then, the system was run for 100 ns at a timestep of 0.25 fs. Trajectory data was collected every 25 ps and used to analyze the root mean square deviation (RMSD), root mean square fluctuation (RMSF), and the radius of gyration.

RESULTS AND DISCUSSION

PRINCIPLE COMPONENT ANALYSIS (PCA)

PCA is performed by grouping the descriptors according to their categories. By using two main components, we describe the descriptor (Figure 2). The description of the descriptor can be seen by using two main components with a total variance of 87.5%, which suggests that two PCA variables have represented 87.5% of the 25 compounds. It is clear that several of the descriptors overlap. The charged partial surface area and WHIM descriptor groups have similar areas. These descriptors

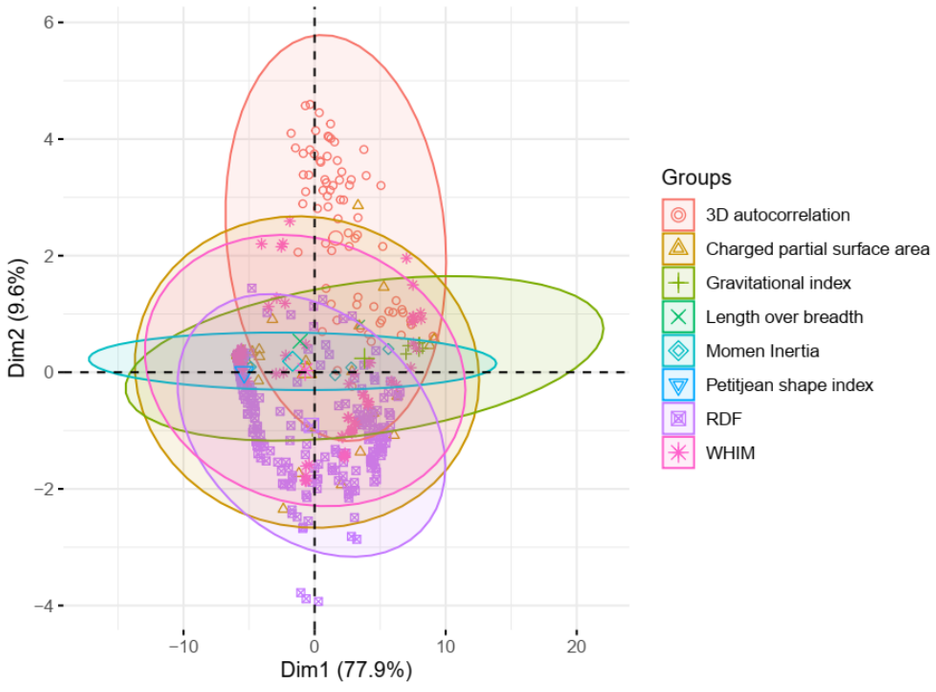

FIGURE 2. PCA result analysis of the 3D descriptors of 1,3,5-trisubstitued pyrazoline

have identical values and are correlated among the 25 substances. Moreover, several descriptors overlap with one another. For instance, the gravitational index descriptor overlaps with seven other descriptor groups. This demonstrates that the properties of the gravitational descriptors must not be linked with one another according to the linear regression assumptions. This description demonstrates the need for more descriptor screening to produce appropriate linear regression model results.

Even though practically all descriptor groups are mutually exclusive, two are not, namely the 3D autocorrelation group and the RDF group. These two groups tend to measure different elements but can reflect practically all of the existing descriptors. As a result, the descriptors in these two groups have the potential to be selected as the variable selection stage in the regression model.

DEVELOPMENT OF THE QSAR MODEL

Data division was conducted using the Kennard–Stone algorithm (Kennard & Stone 1969), which resulted in 80% training set data, and the remaining test set data. The development of the QSAR model was carried out several times, and three QSAR models with R values of >0.8 were selected. Table 2 shows the selected QSAR model, which was validated and chosen to be the best QSAR model. Validation of the QSAR model through internal validation was done using several statistical parameters, such as least squares fit (R^2), cross-validation (Q^2), and bootstrapping and scrambling (Y-randomization).

There are some of minimum standard parameters to produce good QSAR models such as square correlation coefficient (R^2) should be bigger than 0.6 as well as the R value greater equals to 0.8. The difference of R^2 and Q^2 should be less than 0.3 to give a good predictability. Value of R^2 and Q^2 from randomization stage should lower than the real R^2 and Q^2 (Veerasamy et al. 2011).

Based on the minimum standard, all QSAR models fulfill the minimum parameters of R and R^2, but only Model 2 and Model 3 have a difference of R^2 and Q^2 lower than 0.3. The results of the Y-randomization test are shown in Table 3. Randomization was done five times and showed that only Model 2 gave an R^2 and Q^2 value lower than the original value. By this internal validation, Model 2 is the better QSAR model, but an external validation is still needed.

The statistical external validation parameters used in this study are the $R^2_{\text{test set}}$ and r_m^2. A good model is generated if the $R^2_{\text{test set}}$ value is >0.6 or if the r_m^2 value is >0.5. The external validation result showed that all models fulfilled the statistical parameters, but Model 2 presented the greatest r_m^2 value.

TABLE 2. QSAR models from the BuildQSAR program

No	Parameters	Model 1	Model 2	Model 3
1	Descriptors	TDB4 m, MOMI-X, RDF90u, RDF70s	TDB8 m, TDB8s, RDF30e, RDF55e	TDB8u, TDB10s, RDF55e, Te
2	$N_{\text{int,training set}}$	20	20	20
3	R	0.8100	0.8100	0.8360
4	p	0.002	0.002	0.001
5	Q^2	0.324	0.384	0.449
6	R^2–Q^2	0.3321	0.2721	0.2499
7	SPress	0.410	0.496	0.465
8	SDEP	0.364	0.441	0.413
9	$R_{\text{test set}}$	0.9743	0.9222	0.9056
10	$R^2_{\text{test set}}$	0.9492	0.8505	0.8204
11	r_m^2	0.4269	0.5352	0.5197

TABLE 3. Y-randomization results of each original models

Y-randomization test Model 1				Y-randomization test Model 2				Y-randomization test Model 3			
Model	R	R^2	Q^2	Model	R	R^2	Q^2	Model	R	R^2	Q^2
Original	0.8100	0.6555	0.3245	Original	0.8100	0.6561	0.3837	Original	0.8360	0.6989	0.4487
Random 1	0.7680	0.5898	0.2970	Random 1	0.6980	0.4872	0.2180	Random 1	0.6830	0.4665	0.0210
Random 2	0.7600	0.5776	0.1450	Random 2	0.6590	0.4343	n.p	Random 2	0.7180	0.5155	0.0120
Random 3	0.7100	0.5041	0.1630	Random 3	0.6570	0.4316	n.p	Random 3	0.7310	0.5344	0.3480
Random 4	0.8910	0.7939	0.6820	Random 4	0.6410	0.4109	n.p	Random 4	0.7040	0.4956	n.p
Random 5	0.8860	0.7850	0.6660	Random 5	0.6990	0.4886	0.1620	Random 3	0.8220	0.6757	0.3640

Due to the internal and external validation results, Model 2 was chosen as the best QSAR model. The QSAR equation is:
Log IC_{50} = −0.4883 × TDB4 m + 4.2103 × TDB8s +1.4521 × RDF30e - 3.3199

× RDF55e −2.3872

The descriptors involved in the QSAR model are TDB8 m, TDB8s, RDF30e, and RDF55e. The four descriptors come from two groups of descriptors, namely 3D autocorrelation and RDF. These results are appropriate with PCA, which shows that both descriptor groups represent the other descriptor.

Nowadays, QSAR analysis towards pyrazoline derivatives has been conducted for several activities. Hammoudan and Chafi (2023) have conducted a QSAR analysis of pyrazoline derivatives as carbonic anhydrase inhibitors. Through internal and external validation, the QSAR model resulted has higher R^2, R^2_{test} and Q^2_{cv} values ($R^2 = 0.79$, $R^2_{test} = 0.95$, $Q^2_{cv} = 0.64$). The others QSAR analysis also attempted as inhibitors of Mycobacterium tuberculosis strain H37Rv by Venkatesan et al. (2023). The model resulted by using combination of machine learning in Python program language and found that the model score has R^2 value of 0.5901. QSAR models resulted for several bioactivity could be useful to design more active compounds of pyrazoline.

MOLECULAR DOCKING ANALYSIS

Molecular docking analysis was conducted on the complex 1,3,5-trisubstituted pyrazoline and the positive control (chloroquine) against falcipain-2 enzyme. Drug likeness analysis was conducted before the docking stage to select one of derivative compounds to be use as ligand in molecular docking. Table 4 shows the result of drug likeness analysis through SWISSADME program and found that all compounds meet the Lipinski rules indicating that they have good ADME profile when use as a drug. Compound **14** was selected as ligand to be docked with falcipain-2 enzyme because its lowest IC_{50} value comparing with the others molecule.

TABLE 4. Drug likeness prediction result using Lipinski rules

Compounds Code	Lipinski Rules				Druglikeness (Yes/No)
	Molecular Weight	Num. H-bond Acceptor	Num. H-bond Donor	Log P	
1	371.43	4	1	2.94	Yes
2	385.46	4	1	3.15	Yes
3	385.46	4	1	3.15	Yes
4	403.43	6	1	1.86	Yes
5	403.43	6	1	1.86	Yes
6	449.50	5	1	3.21	Yes
7	387.43	5	1	2.39	Yes
8	422.27	4	1	3.10	Yes
9	361.37	5	1	2.88	Yes
10	388.38	6	1	1.52	Yes
11	373.40	5	1	2.18	Yes
12	377.82	4	1	2.99	Yes
13	385.46	4	1	3.15	Yes
14	385.46	4	1	3.15	Yes
15	401.46	5	1	2.60	Yes
16	412.27	4	1	3.47	Yes
17	343.38	4	1	2.50	Yes
18	357.41	4	1	2.72	Yes
19	357.41	4	1	2.72	Yes
20	417.46	6	1	2.07	Yes
21	422.27	4	1	3.10	Yes
22	433.46	7	1	1.28	Yes
23	452.30	5	1	2.77	Yes
24	422.27	4	1	3.10	Yes
25	388.38	6	1	1.52	Yes

The ligands were docked into the active site of the falcipain-2 enzyme (PDB ID:3BPF). Molecular docking analysis showed binding energy and the interaction between ligands and the enzyme. Compound **14** has a lower binding energy of about −7.84 kcal/mol than chloroquine, which has −7.11 kcal/mol. The lower binding energy is due to the stronger interaction between ligands and the enzyme. Figure 3 shows the interaction between both ligands and falcipain-2. Ligand 14 has one hydrogen bond against the Glu14 amino acid residue, more pi-stacking interactions, and van der Waals. Chloroquine also presented one hydrogen bond with Gly83, pi-sulfur against Cys42, and van der Waals. Both ligands showed similar amino acid interactions with the cocrystal ligands of the falcipain-2 and epoxysuccinate complex. Epoxysuccinate and the inhibitor falcipain-2 have four hydrogen bonds against three amino acid residues: Gly83, Gln36, and His174 (Himangini et al. 2018). The similar interaction showed that both ligands have occupied the catalytic site of the enzyme and indicated the potential activity as falcipain-2 inhibitor.

MOLECULAR DYNAMICS SIMULATION

A molecular dynamic simulation has been conducted with a 100 ns simulation time. These simulations are dedicated to the complex of ligand 14 and chloroquine as a positive control. Those ligands were docked into the active site of the falcipain-2 enzyme, and they were chosen due to their binding energy, which is lower than the other tested ligand in molecular docking analysis.

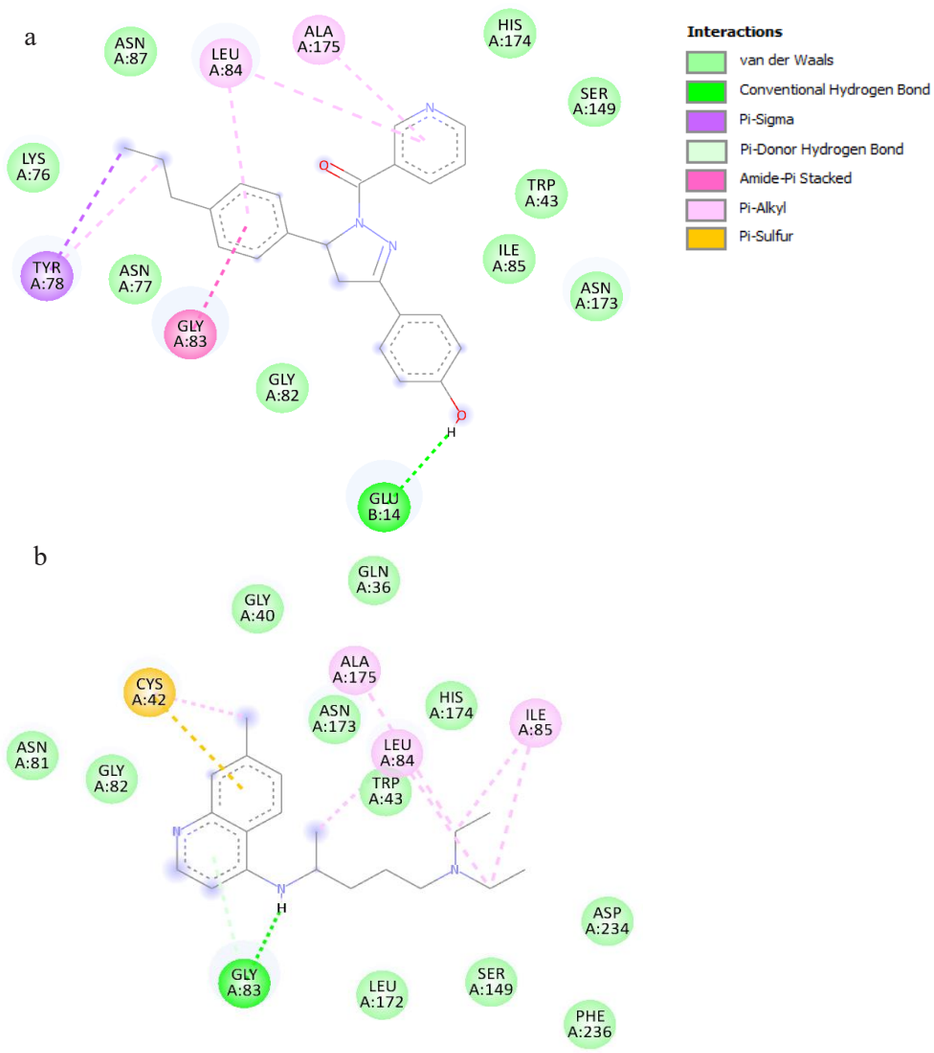

FIGURE 3. 2D-interactions of (a) compound 14 and (b) chloroquine against falcipain-2 enzyme

The radius of gyration is a parameter that describes the equilibrium conformation of a whole system. The lowest value showed the folded condition of the protein structure, and the highest value described the unfolded structure. We can see in Figure 4 that both ligands do not contribute to the folding effect of the protein complex.

This data is also confirmed in the RMSD graph (Figure 5). The RMSD analysis showed that both ligands provide stability to the protein. The high RMSD value is due to the protein structure, which has a high RMSD from the start. This is due to the low stability of the protein structure. Meanwhile, in Figure 6, the RMSF results showed that the positive control ligand (CHL) has better stability than ligand 14. However, the two ligands show the same fluctuation pattern, indicating that the two ligands do not have a folding effect on the protein structure.

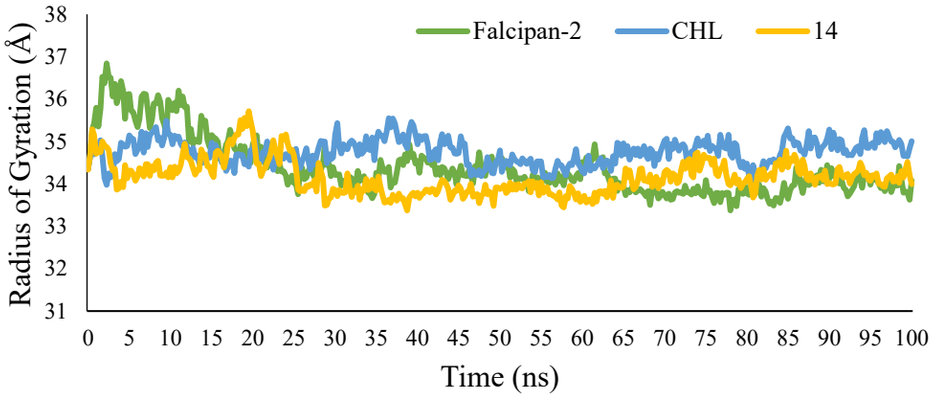

FIGURE 4. Radius of gyration plot between ligands and falcipain-2 enzyme

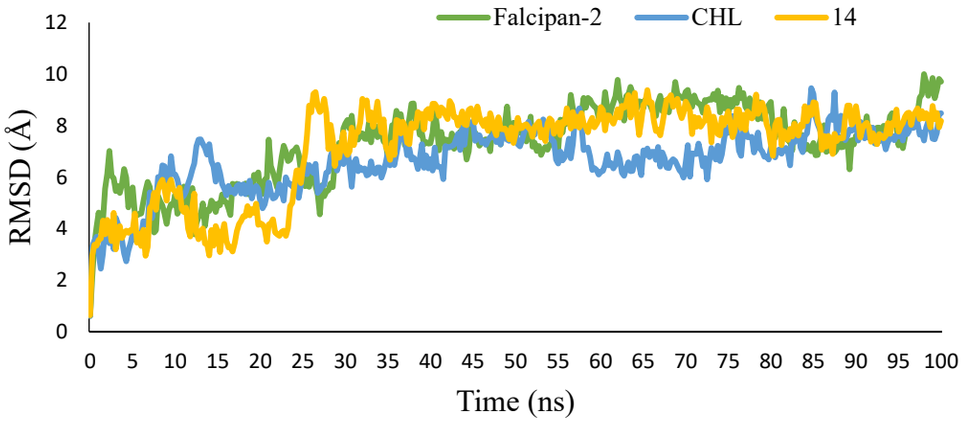

FIGURE 5. Root mean square deviation (RMSD) plot between ligands and falcipain-2 enzyme

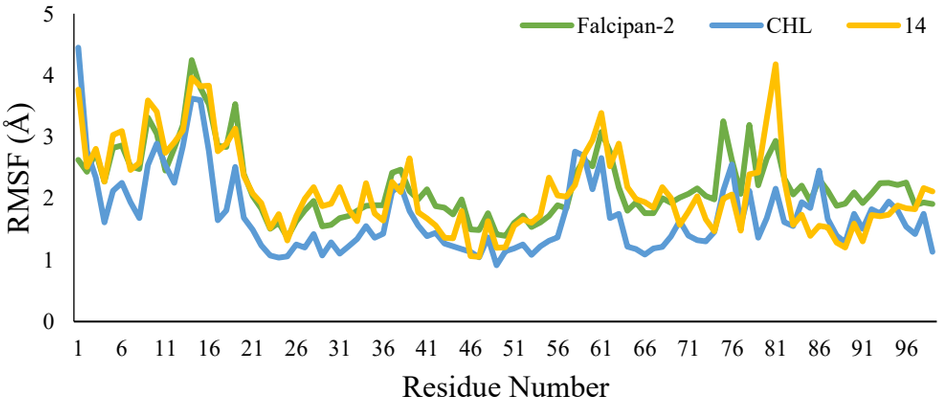

FIGURE 6. Root mean square deviation (RMSD) plot between ligands and falcipain-2 enzyme

CONCLUSIONS

A combination of *in silico* studies have been conducted on the 1,3,5-trisubstituted pyrazoline derivative compounds. A total of 25 compounds were used to construct the validated QSAR equation. Descriptors involved in the QSAR equation are TDB8m, TDB8s, RDF30e, and RDF55e, all of which belong to the 3D autocorrelation and RDF groups of descriptors. The molecular docking analysis of compound **14** against the falcipain-2 enzyme showed a lower binding energy than chloroquine as the positive control. To evaluate the stability of the enzyme−ligand complexes, molecular dynamics simulation was performed and showed that the addition of ligands could reduce the RMSD of single protein, indicating the potency of the ligand as an inhibitor of the falcipain-2 enzyme. Furthermore, the QSAR equation resulted could be utilized to design new compounds with remarkable activity and continued with synthesized stage as well as tested *in vitro* and *in vivo* stage.

ACKNOWLEDGEMENTS

The authors thank the Institute for Research and Community Service, Hasanuddin University, for the research grant (No. 00323/UN4.22/PT.01.03/2023) in the scheme of Fundamental Collaborative Research in 2023.

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