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Study of drug likeness of praziquantel derivatives for the inhibition of thioredoxin peroxidase and aspartic protease in *Opisthorchis viverrini* by Molecular Docking Method

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Abstract

This research aimed to study the effectiveness of eight praziquantel derivatives such as derivatives substituted with $-CH_2(CH_3)_2$ in R_1 position (ligand G03_R1), $-CH_2CH_2OCH_3$ in R_1-R_5 positions (ligands G05_R1, G05_R2, G05_R3, G05_R4 and G05_R5) and $-CH(NHCH_3)(COOCH_3)$ in R_3 and R_4 positions (ligands G17_R3 and G17_R4) as a drug for the inhibition of thioredoxin peroxidase and aspartic protease in *Opisthorchis viverrini* by molecular docking method. It was found that most derivatives could better interact with amino acids in the active site of both types of enzymes than praziquantel**.** The ligand G03_R1 interaction with both types of enzymes was similar to praziquantel**.** No interaction of ligands G17_R3 and G17_R4 in the active site of thioredoxin peroxidase was found but they could best interact in the active site of aspartic protease. The ligand G05_R3 could interact with both types of enzymes, and best interact with thioredoxin peroxidase. The interaction energy value of -31.79 was obtained. $-CH_2CH_2OCH_3$ group in ligand G05_R3 bound with amino acids Ser148 and Glu151 in the active site by hydrophobic interaction and the O1 and O33 atoms of this ligand could form hydrogen bonds with the amino acids Ile140 (N) and Thr139 (OG1). Furthermore, the interaction of ligand G05_R3 with aspartic protease was similar to ligands G17_R3 and G17_R4 . The interaction energy value of -39.83 was obtained. Therefore, it was concluded that the derivative, most suitable for development as a drug for inhibiting thioredoxin peroxidase and aspartic protease, was the ligand G05_R3, a PZQ derivative substituted with $-CH_2CH_2OCH_3$ in R3 position.

Keywords: praziquantel derivatives, thioredoxin peroxidase, aspartic protease, *Opisthorchis viverrini*, Molecular docking method

1. Introduction

The *Opisthorchis viverrini*, common name Southeast Asian liver fluke, is a trematode parasite from the family Opisthorchiidae that attacks the area of the bile duct. Infection is acquired when people ingest raw or undercooked fish. Infection with the parasite is called opisthorchiasis. *Opisthorchis viverrini* infection also predisposes the infected for cholangiocarcinoma, a cancer of the gall bladder and its ducts.

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The *Opisthorchis viverrini* (together with *Clonorchis sinensis* and *Opisthorchis felineus*) is one of the three most medically important species in the family Opisthorchiidae. In fact *Opisthorchis viverrini* and *Clonorchis sinensis* are capable of causing cancer in humans, and are classified by the International Agency for Research on Cancer as a group 1 biological carcinogen in 2009 [1]. Praziquantel (PZQ) is the drug most commonly used to cure the *Opisthorchis viverrini* [2, 3]. However, in the case of drug resistance of *Opisthorchis viverrini*, this causes the decreased cure efficiency of PZQ. Therefore, the invention of a new drug used to supplement or substitute PZQ especially in the case of drug resistance of *Opisthorchis viverrini* is very necessary.

Presently, computational chemistry has had an increasingly active role in the design of medicine molecules for the treatment of various diseases. Research experiments are frequently limited by the instruments used for analysis. Conditions for experiments need to be controlled, such as temperature, pressure and concentration. Many chemicals are also potentially hazardous to researchers, and chemicals used in research are often quite expensive. The cost of research waste treatment is high, and many animal lives are used in experiments. Therefore, computational chemistry has been used as an instrument to design the molecular structure of drugs effectively and to calculate the structural properties that affect the binding between a drug and enzymes associated with a disease. These data are often essential to the drug design. Many drugs, including most of the drugs currently used for HIV treatment, were invented by computational chemistry. The molecular structure is dramatically important for study and research in pharmaceutical chemistry. This is because chemists can understand physical properties such as boiling point, melting point, and chemical properties such as reaction reactivity, selectivity. Such properties are fundamentally important data for chemists to use in understanding the mechanisms of chemical reactions associated with the interaction of a drug's molecular structure with an enzyme. Furthermore, it also can help chemists design new molecules that have desired structural and chemical properties [4].

Recently, Nusai *et al*. [5] reported the design of molecular structures of PZQ derivatives using PZQ as the molecular model and calculation of ADMET property of PZQ and all PZQ derivatives such as absorbtion, distribution, metabolism, excretion and toxicity by using the ADMET Descriptors in Discovery Studio Client version 2.5 program (DS 2.5). They reported that there were eight PZQ derivatives having drug likeness property [5]. However, such data are not enough to conclude that all eight PZQ derivatives are possible to be a drug for curing the *Opisthorchis viverrini*. We further researched about the *Opisthorchis viverrini*, we found that the aspartic protease is a major enzyme in its digestive system. An inflammation is a response of the host's immune system when host infects the *Opisthorchis viverrini*. Host inflammatory cells secrete the oxidized substances to destroy the *Opisthorchis viverrini*. Then, the *Opisthorchis viverrini* produces thioredoxin peroxidase to destroy these oxidized substances [6]. Accordingly, this research aimed to study the effectiveness of eight PZQ derivatives such as derivatives substituted with $-CH_2(CH_3)_2$ in R_1 position (ligand G03_R1), $-CH_2CH_2OCH_3$ in R_1–R_5 positions (ligands G05_R1, G05_R2, G05_R3, G05_R4 and G05_R5) and $-CH(NHCH_3)(COOCH_3)$ in R_3 and R_4 positions (ligands G17_R3 and G17_R4) as a drug for the inhibition of thioredoxin peroxidase and aspartic protease in *Opisthorchis viverrini* by Molecular docking method.

2. Materials and Methods

2.1 Used software

Discovery Studio Client version 2.5 (DS 2.5) program [7], Open Babel version 2.3.2 program [8] and LigPlot+ version 1.4.5 program [9] were used.

2.2 Studied PZQ derivatives

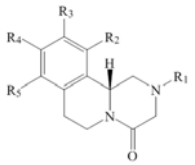


Figure 1. Substituted position of PZQ used as an original drug model [5]

The substituted position of PZQ used as an original drug model is shown in Figure 1. The three substituents such as $-CH_2(CH_3)_2$ (G03), $-CH_2CH_2OCH_3$ (G05) and $-CH(NHCH_3)(COOCH_3)$ (G17) were chosen and studied because these three substituents generated the PZQ derivatives that passed the examination of drug likeness property. This drug likeness property was studied by Nusai *et al*. [5]. The two and three dimensional molecular structures of eight PZQ derivatives are shown in Figure 2.

2.3 The creation of the molecular models of thioredoxin peroxidase and aspartic protease in *Opisthorchis viverrini* as the receptors

The three-dimensional structures of thioredoxin peroxidase and aspartic protease as the receptors were downloaded from the RCSB Protein Data Bank [10]. The various parameters and binding positions of ligands (PZQ and eight PZQ derivatives) at binding sites of receptors (thioredoxin peroxidase and aspartic protease in *Opisthorchis viverrini*) were specified by Discovery Studio Client version 3.0 (DS 3.0) program and CHARMm force field program.

2.4 To perform the molecular docking between the ligands and the receptors

The molecular docking between the ligands (PZQ and eight PZQ derivatives) with the receptors (thioredoxin peroxidase and aspartic protease in *Opisthorchis viverrini*) was performed by the Receptor-Ligand Interaction: Dock Ligand (CDOCKER) protocol in the Discovery Studio 3.0 Client program to consider the active sites from the cavities of two enzyme molecules as shown in Figure 3. Then, the parameters of molecular docking were specified as follows; top hits = 10, pose cluster radius = 0.5, random conformations = 10 and orientations to define = 10 and the CHARMm force field was used for the calculation. The orientation of ligands (PZQ and eight PZQ derivatives) in the active sites of receptors (thioredoxin peroxidase and aspartic protease in *Opisthorchis viverrini*) and the ligands-receptors interaction diagram were generated by LigPlot+ version 1.4.5 to study the binding efficiency of ligands with the two enzymes.

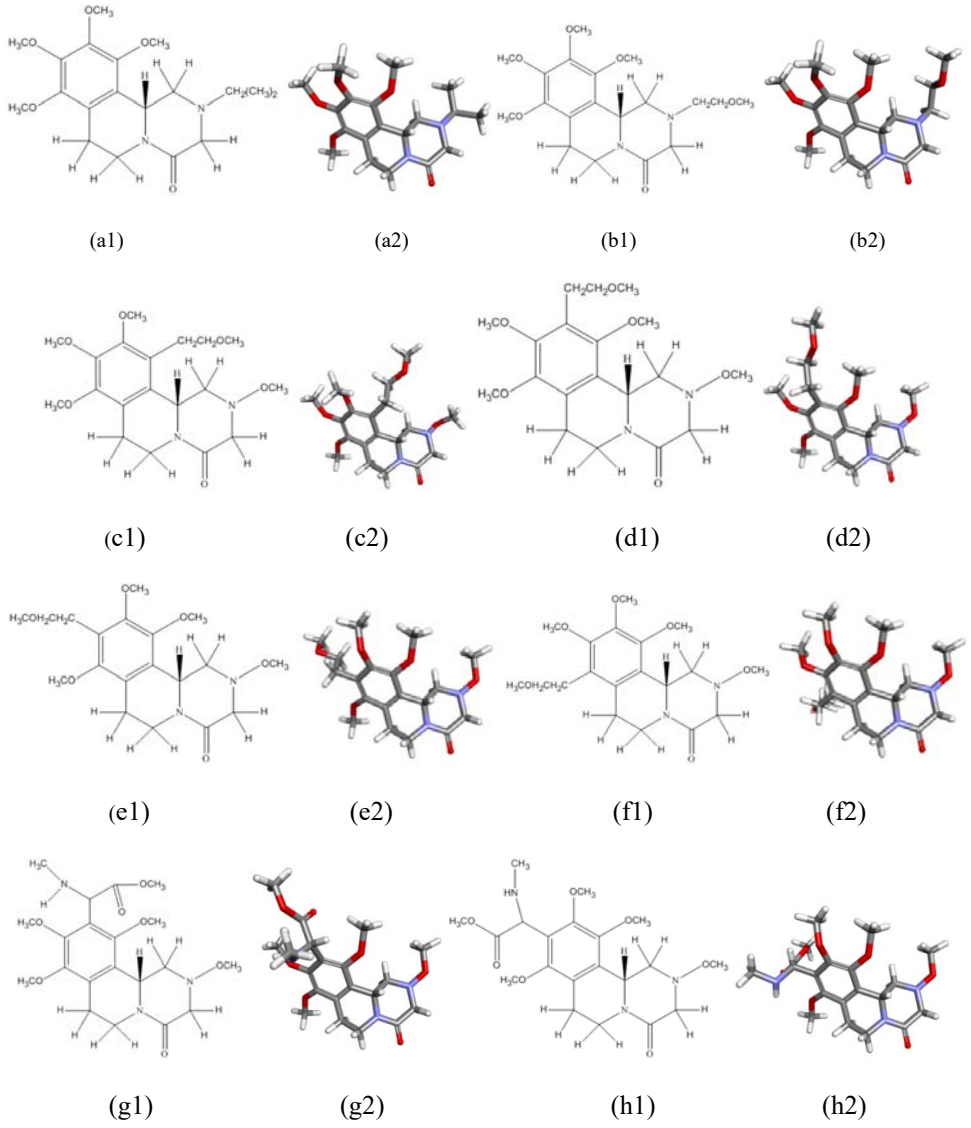


Figure 2. Molecular structures of eight PZQ derivatives. (a1,a2) ligand G03_R1;MW = 364.44 g/mol , (b1,b2) ligand G05_R1;MW = 380.44 g/mol,(c1,c2) ligand G05_R2;MW =380.44 g/mol, (d1,d2) ligand G05_R3;MW = 380.44 g/mol, (e1,e2) ligand G05_R4;MW = 380.44 g/mol, (f1,f2) ligand G05_R5;MW = 380.44 g/mol, (g1,g2) ligand G17_R3;MW = 423.46 g/mol, (h1,h2) ligand G17_R4;MW = 423.46 g/mol.

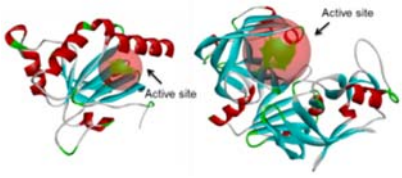


Figure 3.The active sites of thioredoxin peroxidase (Left) and aspartic protease (Right)

3. Results and Discussion

3.1 The creation of two enzyme structures

This research studied two enzymes whose structures were downloaded from the RCSB Protein Data Bank. The two enzymes studied were thioredoxin peroxidase (PDB code: 3ZL5) and aspartic protease (PDB code: 3FNU). The most complete one-chain structures of the two enzymes used as the representatives to perform the molecular docking are shown in Figure 4.

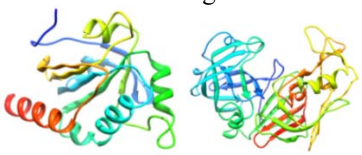
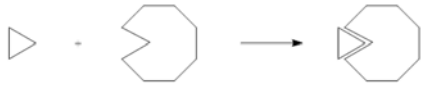


Figure 4. The structures of thioredoxin peroxidase (Left) and aspartic protease (Right)

3.2 The result of molecular docking between thioredoxin peroxidase, aspartic protease and PZQ derivatives

The molecular docking is a method to predict the orientation form and the binding between the ligand and receptor by Monte Carlo simulation (A ligand is a small organic molecule expected to be a drug while a receptor is enzyme or protein that causes the disease). The appropriate orientation form and position of the ligand provide the lowest total energy. The ligands are usually in the active site where ligand best interects the amino groups of enzyme. The active site is a position that affects the function of the enzyme. The binding diagram between ligand and receptor is shown in Figure 5. When the ligand successfully binds with the enzyme, this causes effective inhibition of the enzyme as well.



Ligand **Receptor (enzyme)** **Complex**

Figure 5. The binding diagram between ligand and receptor (enzyme)

The orientation position of ligand bound with the enzyme in the active site was obtained from the molecular docking. The interaction energy (E_{int}) was used to evaluate the binding efficiency between ligand and enzyme. When the interaction energy value was strongly negative, it indicated that the ligands were effective in inhibiting enzymes.

3.2.1 The interaction between eight PZQ derivatives and thioredoxin peroxidase

The result of molecular docking between PZQ and thioredoxin peroxidase showed that PZQ could locate in the active site cavity partly (See Tables 1 and 2 for consideration). The main interaction between PZQ and amino acid of enzyme involved hydrophobic interaction which was the interaction including various attractions such as electrostatic force and Van der Waals forces. Consideration of the orientation of PZQ and the interaction that occurred found that the atoms in the benzene ring interacted mainly with amino acids in the active site by hydrophobic interaction. PZQ oriented the benzene ring toward amino acids Thr139, Asn141, Ser148 and Glu151, which were the main amino acids located in the active site of thioredoxin peroxidase. The results of molecular docking of eight PZQ derivatives showed that ligand G03_R1 (substituted with $-CH_2(CH_3)_2$ in R_1 position) and ligands G05_R1, G05_R2, G05_R3, G05_R4 and G05_R5 (substituted with $-CH_2CH_2OCH_3$ in R_1–R_5 positions) could bind with the amino group in the active site of thioredoxin peroxidase. The binding direction of these ligands turned the molecule side with less

steric effect toward the active site. For example, ligand G03_R1, $-CH_2(CH_3)_2$ group would enter to bind with amino acids Glu151, SER148 and Arg147 by hydrophobic interaction and the hydrogen bond was occurred between O17 atom of ligand G03_R1 with OG1 atom of amino acid Thr139 (Denoted Thr139(OG1)) of enzyme as well. This caused ligand G03_R1 could more bind with enzyme. Similarly, ligand G05_R1, $-CH_2CH_2OCH_3$ group would enter to bind with amino acids Leu126, Arg147 and Glu151 in the active site and the hydrogen bond was occurred between N15 atom of ligand with amino acid Thr139(OG1). However, no difference of the obtained interaction energy (E_{int}) of ligands G03_R1 and G05_R1 compared with ligand PZQ (E_{int} = –26.54 kcal/mol). In the case of ligands G05_R2, G05_R3, G05_R4 and G05_R5, it was found that the position of substituents directly affected the orientation of the ligand in active site. $-CH_2CH_2OCH_3$ group of ligands G05_R2, G05_R3 and G05_R5 would enter to bind with amino acids SER148 and Glu151 in the active site by hydrophobic interaction. This is because $-CH_2CH_2OCH_3$ group is a long hydrocarbon chain that can be one factor that enables binding to amino acids in the active site with the narrow cavity of thioredoxin peroxidase. The case of ligand G05_R4, no interaction between $-CH_2CH_2OCH_3$ group and amino acids in the active site was found because the surrounding area is $-OCH_3$ in other R position to make this side of ligand is too bulky. This ligand turned into the opposite interaction with the amino acids in the active side instead. Ligand G05_R3, $-CH_2CH_2OCH_3$ group in this ligand bound with amino acids SER148 and Glu151 in the active site by hydrophobic interaction and the O1 and O33 atoms of this ligand could form hydrogen bonds with the amino acids Ile140 (N) and Thr139 (OG1) as well, which results in efficient ligand binding to the enzyme increases. Considering the interaction energy values of two ligands G05_R2 (E_{int} = -28.00 kcal / mol) and G05_R3 (E_{int} = -31.79 kcal / mol) showed that the interaction energy values of both ligands were higher than the interaction energy value of PZQ. Ligands G17_R3 and G17_R4, it was found that no interaction between these two ligands and amino acids in the active site of thioredoxin peroxidase was found. This is because G17 ($-CH(NHCH_3)(COOCH_3)$ group) is too sterics, it cannot enter to bind with the amino acids in the active site that is small and narrow of this enzyme. Therefore, the optimum molecules to be tested to inhibit the thioredoxin peroxidase were the derivatives of PZQ substituted in position of R_2 and R_3 by $-CH_2CH_2OCH_3$ only.

Table 1. Interaction between PZQ and eight PZQ derivatives with thioredoxin peroxidase

Ligand	E_{int} (kcal/mol)	H-bond	Hydrophobic interaction
PZQ	–26.54	–	Thr139 : PZQ(C7,C8,C9,C10,C11,C12) Asn141 : PZQ (C14,C15,C17) Val145 : PZQ (O22) Gly146 : PZQ (C3) Arg147 : PZQ (C2,C3,C18) Ser148 : PZQ (C18,C19) Glu151 : PZQ (C7,C8)
G03_R1	–26.13	Thr139 (OG1) : G03_R1(O17) Distance: 2.74 Å	Thr139 : G03_R1 (C9,C10) Asn141 : G03_R1 (C3,C4,C8,C46) Val145 : G03_R1 (C38,C46) Arg147 : G03_R1 (C51) Ser148 : G03_R1 (C51) Glu151 : G03_R1 (C1,C2,C10)
G05_R1	–26.31	Thr139 (OG1) : G05_R1 (N15) Distance: 2.89 Å	Leu126 : G05_R1 (C52) Gln137 : G05_R1 (C9,C10,O17) Thr139 : G05_R1 (C1,C2,C9,C10,N15) Ile140 : G05_R1 (C46) Asn141 : G05_R1 (C38) Arg147 : G05_R1 (C52) Ser148 : G05_R1 (C52) Glu151 : G05_R1 (C1,C2,C10,O31) Thr152 : G05_R1 (C52)

Table 1. Interaction between PZQ and eight PZQ derivatives with thioredoxin peroxidase (cont.)

Ligand	E_{int} (kcal/mol)	H-bond	Hydrophobic interaction
G05_R2	–28.00	-	Thr139 : G05_R2 (C9,C10,C11) Asn141 : G05_R2 (C46) Val145 : G05_R2 (C38,C46,O18,O29) Ser148 : G05_R2 (C2,C34) Glu151 : G05_R2 (C9,C10,N15) Thr152 : G05_R2 (C2) Leu155 : G05_R2 (C10)
G05_R3	–31.79	Thr139 (OG1) : G05_R3 (O33) Distance: 3.05 Å Ile140 (N) : G05_R3 (O1) Distance: 3.06 Å	Ile138 : G05_R3 (C2) Thr139 : G05_R3(C11,C13,C29,C38,O1,O33) Ile140 : G05_R3 (C9,C10,C11) Asn141 : G05_R3 (C4,C5,C7,C8,C42) Val145 : G05_R3 (C42) Gly146 : G05_R3 (C42) Ser148 : G05_R3 (C52) Glu151 : G05_R3 (C29,C38,O41)
G05_R4	–24.56	–	Gln137 : G05_R4 (C9,O17) Thr139 : G05_R4 (C9,C10) Ile140 : G05_R4 (C52) Asn141 : G05_R4 (C34) Gly146 : G05_R4 (C34) Arg147 : G05_R4 (O1) Ser148 : G05_R4 (C2) Glu151 : G05_R4 (C6,C9,C10,N15,N16) Thr152 : G05_R4 (C2) Leu155 : G05_R4 (O17)
G05_R5	–23.84	-	Gln137 : G05_R5 (C46) Thr139 : G05_R5 (C46,O18) Ser148 : G05_R5 (C42,C52) Glu151 : G05_R5 (C3,C13,C14,C34,C46,C52,O45) Thr152 : G05_R5 (C52) Leu155 : G05_R5 (C46)
G17_R3	No interaction		
G17_R4	No interaction		

Table 2. Orientation of ligand in the active site and the occurred interaction between atom of ligand and amino acid in the active site of thioredoxin peroxidase obtained by Ligplot+ program (Dotted line is the occurrence of hydrogen bond, atoms of the ligand with a small lines showing the hydrophobic interaction with an amino acid that is in the direction of a small lines)

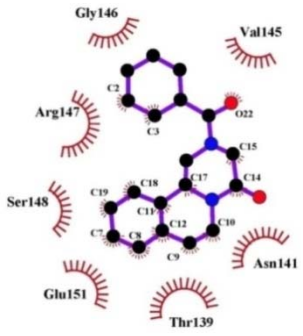
Ligand	Orientation of ligand in the active site	Occurred interaction
PZQ		

Table 2. Orientation of ligand in the active site and the occurred interaction between atom of ligand and amino acid in the active site of thioredoxin peroxidase obtained by Ligplot+ program (Dotted line is the occurrence of hydrogen bond, atoms of the ligand with a small lines showing the hydrophobic interaction with an amino acid that is in the direction of a small lines) (cont.)

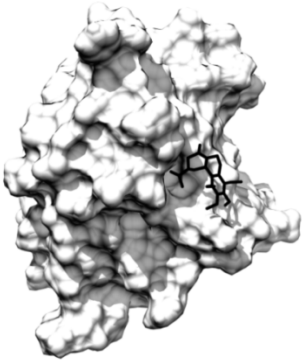
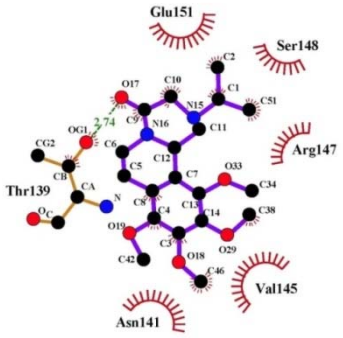
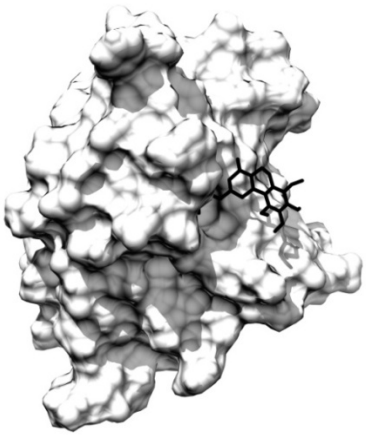
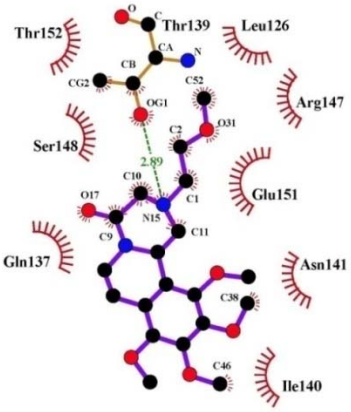
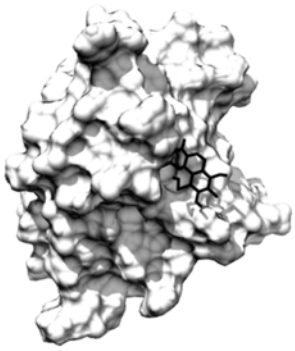
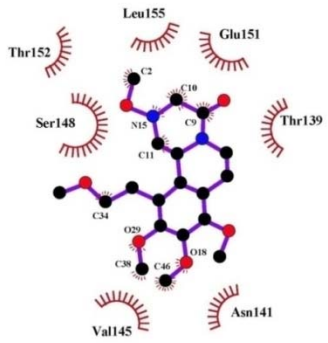
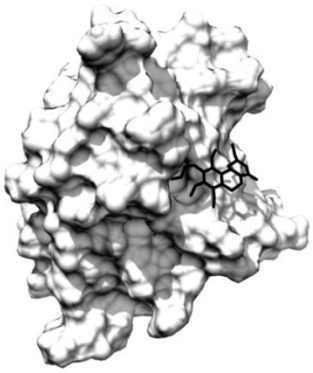
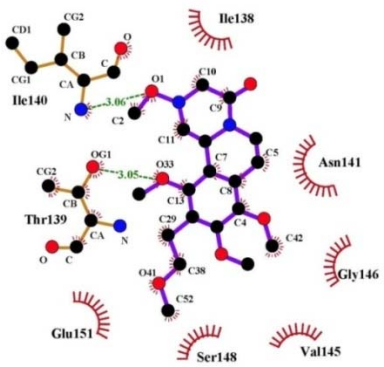
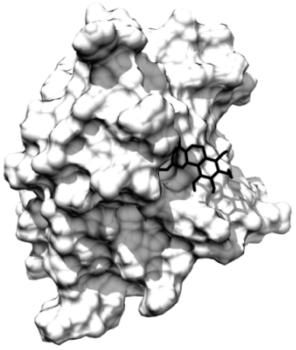
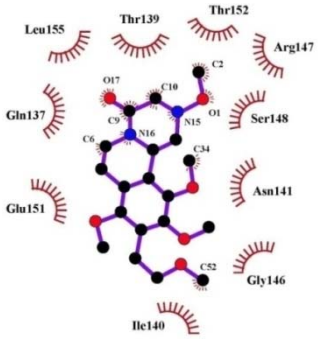
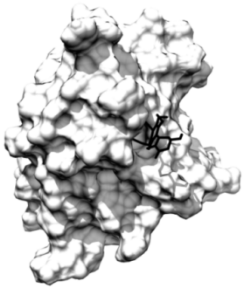
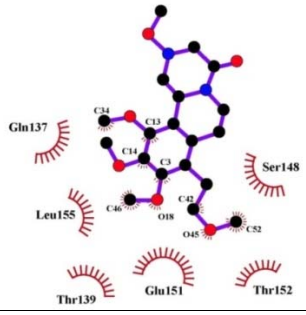
Ligand	Orientation of ligand in the active site	Occurred interaction
G03_R1		
G05_R1		
G05_R2		

Table 2. Orientation of ligand in the active site and the occurred interaction between atom of ligand and amino acid in the active site of thioredoxin peroxidase obtained by Ligplot+ program (Dotted line is the occurrence of hydrogen bond, atoms of the ligand with a small lines showing the hydrophobic interaction with an amino acid that is in the direction of a small lines) (cont.)

Ligand	Orientation of ligand in the active site	Occurred interaction
G05_R3		
G05_R4		
G05_R5		

3.2.2 The interaction between PZQ derivatives and aspartic protease

The result of molecular docking between PZQ, eight PZQ derivatives and aspartic protease is shown in Tables 3 and 4. Considering the orientation of PZQ that was an original ligand model found that all PZQ molecule could locate in the active site of thioredoxin peroxidase. Due to the cavity size of active site of aspartic protease is larger than of thioredoxin peroxidase. The main interaction between PZQ and amino acid in the active site of aspartic protease involved hydrophobic interaction. Especially, the carbon atoms in the benzene ring of the ligand could

interact well with the amino acid His3 2 by hydrophobic interaction. When calculating the all occurred interactions energy found that the amino acids in active site of aspartic protease interact with PZQ better than the amino acids in active site of thioredoxin peroxidase with interaction energy of -32.32 kcal / mol. This indicated that PZQ could inhibit aspartic protease better than thioredoxin peroxidase. The result of molecular docking between eight PZQ derivatives and aspartic protease showed that all eight PZQ derivatives could locate in the active site cavity of this enzyme and interact with the amino acids in the active site of this enzyme better than thioredoxin peroxidase (considering the obtained interaction energy value between eight PZQ derivatives and the aspartic protease compared to thioredoxin peroxidase), although all eight PZQ derivatives could not create the hydrogen bonds with the amino acids in the active site. Additionally, the interaction energy values of eight PZQ derivatives were higher than of PZQ, this indicated that eight PZQ derivatives had the ability to inhibit the aspartic protease better PZQ.

Table 3. Interaction between PZQ, PZQ derivatives and aspartic protease

Ligand	E_{int} (kcal/mol)	H-bond	Hydrophobic interaction
PZQ	–32.32	–	Leu30 : PZQ (C18,C19) His32 : PZQ (C7,C8,C11,C12,C18,C19) Trp39 : PZQ (C15,O22) Leu73 : PZQ (O23) Ile80 : PZQ (O22) Ile107 : PZQ (C13) Phe109 : PZQ (C6, C13) Tyr112 : PZQ (C2,C13) Val120 : PZQ (C1,C3,C4,C16,N20)
G03_R1	–35.16	–	Leu30 : G03_R1 (C34,O29,O33) Ser35 : G03_R1 (C2) Trp39 : G03_R1 (C2) Ile107 : G03_R1 (C42) Phe109 : G03_R1 (C5,C6,C42,O19) Phe111 : G03_R1 (C14,C34,C38,O29) Tyr112 : G03_R1 (C46,O18) Val117 : G03_R1 (C38) Gly119 : G03_R1 (C46) Val120 : G03_R1(C3,C13,C14,C38,C46,O29,O33)
G05_R1	–37.24	–	Ser35 : G05_R1 (C38) Trp39 : G05_R1 (C34,C38,O29) Leu73 : G05_R1 (C3,C4,C8,C13,C14,C34,C46) Ile80 : G05_R1 (C34) Phe109 : G05_R1 (C6,C9,C10,C12,N15,N16,O17) Phe111 : G05_R1 (C2,C10,O31) Tyr112 : G05_R1 (O31) Val117 : G05_R1 (C52) Val120 : G05_R1 (C2,C38,C52,O33)
G05_R2	–36.82	–	His32 : G05_R2 (C34,C52,O37) Trp39 : G05_R2 (O17) Leu73 : G05_R2 (C5,C6,C42) Ser75 : G05_R2 (C42,C46) Ala77 : G05_R2 (C46) Ile80 : G05_R2 (O17) Met104 : G05_R2 (O17) Phe109A : G05_R2 (C4,C5,C8,O19) Phe111 : G05_R2 (C2,O1) Tyr112 : G05_R2 (C2,O1) Val117 : G05_R2 (C2) Val120 : G05_R2 (C2,C9,C10,C12,N15,N16)

Table 3. Interaction between PZQ, PZQ derivatives and aspartic protease (cont.)

Ligand	E_{int} (kcal/mol)	H-bond	Hydrophobic interaction
G05_R3	–39.83	–	Leu30 : G03_R3 (C42) His32 : G03_R3 (C42,O18) Ser35 : G03_R3 (C38,C52,O41) Trp39 : G03_R3 (C2,C34,C52) Leu73 : G03_R3 (C34,O33) Ile80 : G03_R3 (O1) Met104 : G03_R3 (C2,O1) Ile107 : G03_R3 (C10) Phe109 : G03_R3 (C6,C9,O17,N16) Phe111 : G03_R3 (C5,C6) Tyr112 : G03_R3 (O17) Gly119 : G03_R3 (C2) Val120 : G03_R3 (C2,C11,C52)
G05_R4	–36.57	–	Trp39 : G05_R4 (O17) Leu73 : G05_R4 (C5,C42) Ser75 : G05_R4 (C46,C52) Ala77 : G05_R4 (C46,O47) Ile80 : G05_R4 (O17) Met104 : G05_R4 (O17) Phe109A : G05_R4 (C3,C4,C8,C46,O19) Phe111 : G05_R4 (C2,C34,O1,O33) Tyr112 : G05_R4 (C2,C10,N15) Val117 : G05_R4 (C2) Val120 : G05_R4 (C9,N16,O1,O17)
G05_R5	–36.40	–	His32 : G05_R5 (C42,C52,O18) Ser35 : G05_R5 (C38) Trp39 : G05_R5 (C38) Leu73 : G05_R5 (C34) Phe109 : G05_R5 (C6,C9,O17) Phe111 : G05_R5 (C2,C9,O17) Tyr112 : G05_R5 (C2,C10,O1,O17) Gly119 : G05_R5 (O1) Val120 : G05_R5 (C11,C38,O1,O33) Asp215 : G05_R5 (C52) Ala217 : G05_R5 (C52,O45)
G17_R3	–40.56	–	His32 : G17_R3 (C5,C6,C9,C10,N16,O17) Ser35 : G17_R3 (C40) Trp39 : G17_R3 (C40,C44) Ile80 : G17_R3 (C44) Ile107 : G17_R3 (C56) Phe109 : G17_R3 (C34,C56) Phe111 : G17_R3 (C49,O33,O39) Val117 : G17_R3 (C49) Val120 : G17_R3(C3,C14,C38,C40,C49,C56,O18,O39, Ala217 : O48) G17_R3 (C2,C10,O1)
G17_R4	–40.61	–	His32 : G17_R4 (C2,C11,N15) Ser35 : G17_R4 (C9,C10,N15,O17) Trp39 : G17_R4 (C5,C6) Leu73 : G17_R4 (C6) Ile80 : G17_R4 (C42) Ile107 : G17_R4 (C53) Phe109 : G17_R4 (C34,C53,N46,O29) Phe111 : G17_R4 (C49,O47) Tyr112 : G17_R4 (C53,O50) Val117 : G17_R4 (C49) Val120 : G17_R4 (C4,C5,C8,C42,C48,C49,O19,O47,O50)

When considering the interaction between eight PZQ derivatives and aspartic protease, it was found that the interaction energy depended on the type and position of substituents. -CH($NHCH_3$)($COOCH_3$) group provided the highest interaction energy such as ligands G17_R3 and G17_R4. The orientation direction of these two ligands would rotate the CH ($NHCH_3$)($COOCH_3$) group into the active site. The OCH_3 groups in nearby R position would interact with the amino acids Phe109, Phe111, Val117, Val120 and Ile107 by hydrophobic interaction. These amino acids were the main amino acids in the active site of aspartic protease. No exact orientation direction was obtained for -CH_2 (CH_3) $_2$ substituent (ligand G03_R1) and -$CH_2CH_2OCH_3$ substituent (ligands G05_R1, G05_R2, G05_R3, G05_R4 and G05_R5) . The substituent always did not rotate into the active site cavity. The occurred main interaction involved hydrophobic interaction that occurred from either various substituents and from -OCH_3 in other substituted positions. In group of ligands G03 and G05 as mentioned above, it was found that ligand G05_R3 provided the highest interaction energy value of –39. 83 kcal/ mol which this interaction energy value was high near with the -CH($NHCH_3$)($COOCH_3$) substituent. This indicated that the inhibition efficiency of ligand G05_R3 to aspartic protease is similar to ligands G17_R3 and G17_R4.

Table 4. Orientation of ligand in the active site and the occurred interaction between atom of ligand and amino acid in the active site of aspartic protease obtained by Ligplot + program (Dotted line is the occurrence of hydrogen bond, atoms of the ligand with a small lines showing the hydrophobic interaction with an amino acid that is in the direction of a small lines)

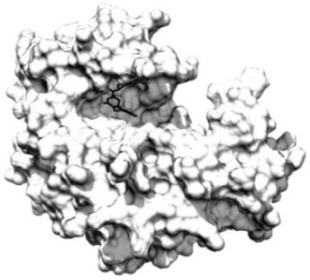
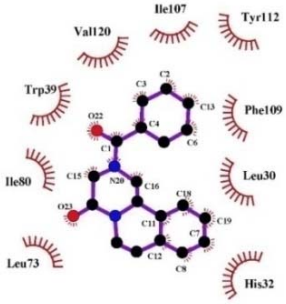
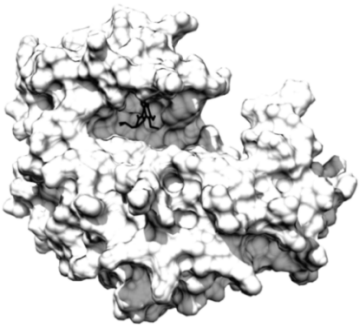
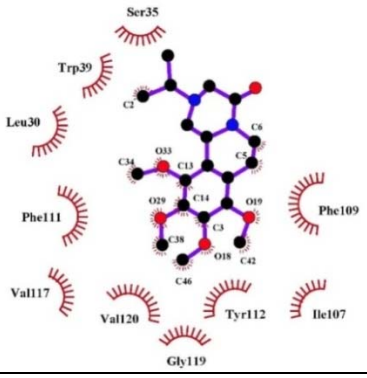
Ligand	Orientation of ligand in the active site	Occurred interaction
PZQ		
G03_R1		

Table 4. Orientation of ligand in the active site and the occurred interaction between atom of ligand and amino acid in the active site of aspartic protease obtained by Ligplot + program (Dotted line is the occurrence of hydrogen bond, atoms of the ligand with a small lines showing the hydrophobic interaction with an amino acid that is in the direction of a small lines) (cont.)

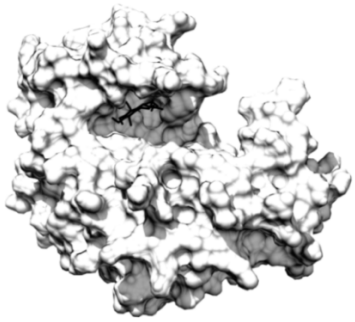
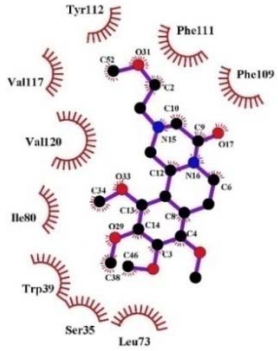
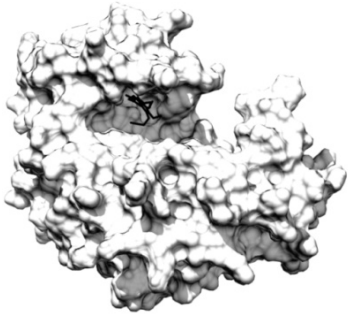
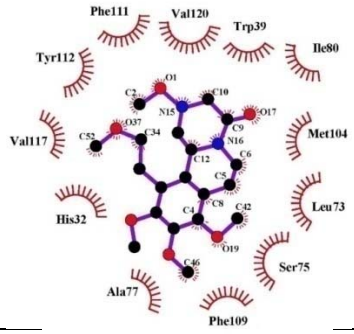
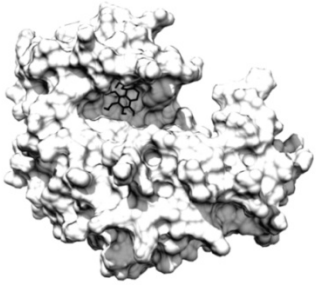
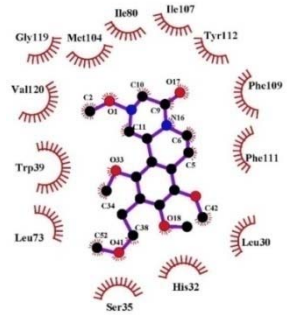
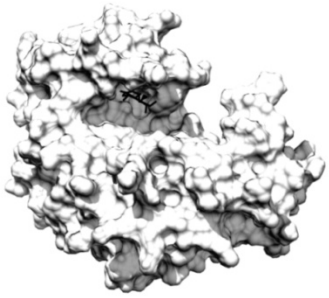
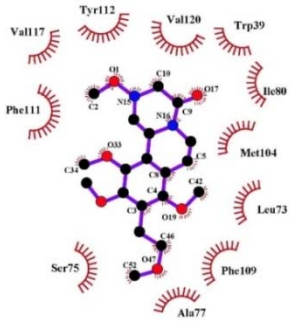
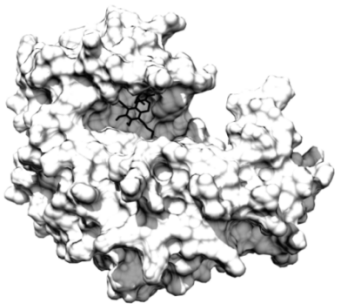
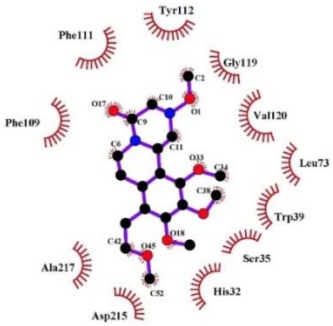
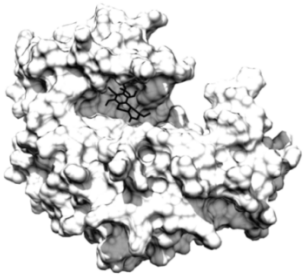
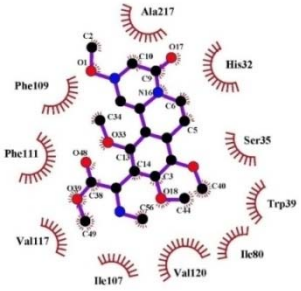
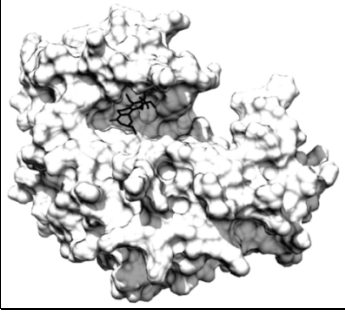
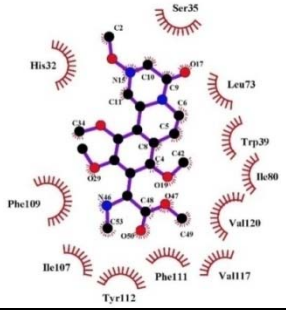
Ligand	Orientation of ligand in the active site	Occurred interaction
G05_R1		
G05_R2		
G05_R3		
G05_R4		

Table 4. Orientation of ligand in the active site and the occurred interaction between atom of ligand and amino acid in the active site of aspartic protease obtained by Ligplot + program (Dotted line is the occurrence of hydrogen bond, atoms of the ligand with a small lines showing the hydrophobic interaction with an amino acid that is in the direction of a small lines) (cont.)

Ligand	Orientation of ligand in the active site	Occurred interaction
G05_R5		
G17_R3		
G17_R4		

The result of molecular docking between eight PZQ derivatives with thioredoxin peroxidase and aspartic protease is shown in Table 5. It was found that ligand G03_R1 could interact with both enzymes, but significantly no difference of interaction energy value of ligand G03_R1 compared with PZQ was obtained. Ligands G17_R3 and G17_R4 could not bind with thioredoxin peroxidase, but could best bind aspartic protease. However, the derivative that provided the interesting calculation results was ligand G05_R3. Ligand G05_R3 could interact with both enzymes, best interact with thioredoxin peroxidase and interact with aspartic protease similar to ligands G17_R3 and G17_R4 . Therefore, the derivative probable to be developed as a drug for inhibiting the thioredoxin peroxidase and aspartic protease was ligand G05_R3 (PZQ derivative substituted with $-CH_2CH_2OCH_3$ in R_3 position, as shown in Figure 6). Furthermore, other positions substituted with $-OCH_3$ group caused the G05_R3 molecule more interacted with the amino acids in active site of enzyme as well.

Table 5. Interaction energy of PZQ derivatives with thioredoxin peroxidase and aspartic protease

Ligand				E_{int} (kcal/mol)	
Substituent		Substituted position	Ligand Code	thioredoxin peroxidase	aspartic protease
–	–	–	PZQ	–26.54	–32.32
G03	$-CH_2(CH_3)_2$	R1	G03_R1	–26.13	–35.16
G05	$-CH_2CH_2OCH_3$	R1	G05_R1	–26.31	–37.24
		R2	G05_R2	–28.00	–36.82
		R3	G05_R3	–31.79	–39.83
		R4	G05_R4	–24.56	–36.57
		R5	G05_R5	–23.84	–36.40
G17	$-CH(NHCH_3)(COOCH_3)$	R3	G17_R3	–	–40.56
		R4	G17_R4	–	–40.61

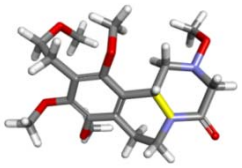


Figure 6. Molecular structure of PZQ derivative substituted with $-CH_2CH_2OCH_3$ in R_3 position (G05_R3)

4. Conclusions

This research used the molecular docking method to study the effectiveness of eight PZQ derivatives. The PZQ derivatives were substituted with $-CH_2(CH_3)_2$ in R_1 position (ligand G03_R1), $-CH_2CH_2OCH_3$ in R_1–R_5 positions (ligands G05_R1, G05_R2, G05_R3, G05_R4 and G05_R5) and $-CH(NHCH_3)(COOCH_3)$ in R_3 and R_4 positions (ligands G17_R3 and G17_R4) and were used as a drug for the inhibition of thioredoxin peroxidase and aspartic protease in *Opisthorchis viverrini*. Drug molecules (called ligands) are a derivative of PZQ, and this research compared eight derivatives with PZQ, the original drug. It was found that most ligands could interact with amino acids in the active sites of both types of enzymes better than PZQ. The ligand G03_R1 could interact with both enzymes similarly to PZQ. Ligands G17_R3 and G17_R4 did not interact with thioredoxin peroxidase, but these two ligands were found to have the strongest interactions with aspartic protease. The ligand G05_R3 interactions could occur with both types of enzymes. The interaction with the thioredoxin peroxidase was strongest and interaction with aspartic protease was similar to ligands G17_R3 and G17_R4. Therefore, it was concluded that the derivative most suitable for development as a drug for inhibiting thioredoxin peroxidase and aspartic protease is the ligand G05_R3, a PZQ derivative substituted with $-CH_2CH_2OCH_3$ in R3 position.

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