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Original Article

HISTONE ACETYLTRANSFERASE P300/CBP-ASSOCIATED FACTOR INHIBITION BY QUERCETIN AS ANTICANCER DRUG CANDIDATE WITH *IN SILICO* AND *IN VITRO* APPROACH

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ABSTRACT

Objective: The objective of this research was to show quercetin potency to inhibit histone acetyltransferase p300/CBP-associated factor (HAT PCAF) activity. Molecular docking study was used to show inhibition model of quercetin towards HAT PCAF and the kinetic study was used to give the information about inhibition constant (Ki) of quercetin.

Methods: Molecular docking simulations between HAT and quercetin were performed using AutoDock Vina, and the results were scored based on its Gibbs free energy change (ΔG) (the most negative ΔG). The kinetic assay of HAT PCAF inhibition by quercetin used fluorometry methods to measure enzyme inhibition by quercetin.

Results: Molecular docking showed that quercetin could inhibit HAT PCAF through binding to acetyl-CoA that involved glutamine 525 (Gln525) and cysteine 574 (Cys574) on chain A, and Cys574 and Gln581 on chain B of HAT PCAF. Quercetin also binds to histone active site on HAT PCAF through aspartic acid 610 (Asp610). The kinetic study results showed that quercetin could inhibit histone acetylation based on the fluorescence intensity. Analysis by Dixon plot showed that quercetin competes with histone. Therefore, it had competitive inhibition. Its Ki value of 9.575 μM. Kinetic study showed the same result as molecular docking study that quercetin had potency as an HAT PCAF inhibitor.

Conclusion: The result of this research showed that quercetin had the potency to inhibit HAT PCAF through competition with HAT PCAF substrates. Quercetin could interact with the HAT PCAF active site, thus, lower the HAT PCAF activity.

Keywords: Histone acetyltransferase, Quercetin, PCAF, Epigenetic drug, Docking

INTRODUCTION

Histone acetylation is one of the epigenetic modifications, and the effects are very important for development, differentiation, and cellular processes of an organism [8]. Histone acetylation catalysis by HAT through transfer acetyl group from acetyl-CoA to lysine ε-amino group on histone causes chromatin structure into a relaxation form that leads to recruitment of transcription factors, resulting in activation of DNA transcription. This activation affects the level of gene expression, mitosis, meiosis, cell differentiation, and DNA replication [1, 2, 7, 14]. Errors that occur during these modifications are particularly vulnerable as one of the causes of cancer, inflammation, and viral infections [7].

HAT has different types, but p300/CBP and PCAF are the HATs are found in human [2, 17]. HAT's substrates could be histone or nonhistone proteins, such as p53 and *nuclear factor-kappa beta* (NF- κ) [21]. Hyperacetylation on histone can cause an increase in cell proliferation, and acetylation on p53 can inactivate tumor suppressor thus p53 cannot repair DNA or inhibit apoptosis [20]. Those are some factors that are associated with cancer manifestation. HAT as transcription cofactor has an important role in transcription regulation through transcription factors binding. Interference in this enzyme can lead to several diseases, such as cancer and neurodegenerative. Therefore, HAT PCAF can be used as an anticancer target.

Epigenetic drugs development is very rapid and is intended as an alternative cancer therapy. Epigenetic drugs that have been accepted by the Food and Drug Administration (FDA) are Dacogen and Vizada to inhibit DNA methylation, and Vorinostat and Romidepsin as a histone deacetylase (HDAC) inhibitor [3], whereas histone acetyltransferase inhibitor, is still in the pre-clinical stage [16]. HAT inhibitors are in the development stage either natural or synthetic products, such as curcumin, garcinol, isothiazolone, anacardic acid, C646, H3-CoA-20, and Lys-20. The problem with the development of these inhibitors is the permeability of the cell or low specificity [2, 7, 11, 13]. Therefore, the development of small inhibitors with a cell permeability and high specificity against HAT PCAF needs further study.

Natural products from plants that showed potential as HAT inhibitors are quercetin, a common flavonoid found in plants. Quercetin has antioxidant effects, furthermore, it can inhibit macrophage proliferation, and nuclear factor κB (NF-κB) [5]. Quercetin inhibits transcription factor, NF-κB, recruitment at the chromatin of proinflammatory genes [23]. Quercetin also has potential to inhibit HAT p300 in breast cancer [25].

Studies about quercetin as HAT p300 inhibitor have been done [23], whereas ~~a~~ study about quercetin potential as an inhibitor against HAT PCAF has not been done. In addition, inhibition kinetics of quercetin against HAT PCAF is still unknown. This study predicts an interaction model between quercetin and HAT PCAF *in silico* by using molecular docking technique. HAT inhibition kinetic assay by quercetin (*in vitro*) was carried out after the molecular docking *in silico*. This kinetic inhibition assay provides information about the inhibition type and the inhibition constant value which is also a parameter to be considered for determining quercetin potential in inhibiting HAT PCAF activity.

MATERIALS AND METHODS

Materials

All the reagents were from Sigma-Aldrich, except HAT buffer pH 7.5 (Cayman Chemical Company) unless stated otherwise.

Materials used in the molecular docking were two-dimension of quercetin structure and three-dimension of HAT PCAF structure (code: 1CM0) obtained from http://www.rscb.org/pdb. Kinetic inhibition assay used HAT PCAF human recombinant, purity >95% by SDS-PAGE were expressed in *Escherichia coli*, quercetin with purification 95% by HPLC, histone-3 (H3) peptide purification 90%, acetyl-CoA with purification 93% by HPLC, Coenzyme A, and CPM as probe, all the materials obtained from Sigma-Aldrich, while buffers HAT (5x) used

consists of HEPES buffer, and Triton-X, and ultrapure water obtained from Cayman. This assay also used dimethyl sulfoxide (DMSO) as a solvent and isopropanol to stop the reaction.

Molecular docking simulation of HAT and quercetin

Molecular docking simulation used was molecular docking methods adapted from previous studies [24]. The crystal structure of PCAF was downloaded from Protein Data Bank (code 1CM0). Quercetin was drawn using Chemaxon Marvin Sketch and prepared using Open Babel GUI (to convert pdb format to pdbqt). Quercetin availability was analyzed by Lipinski rule. Molecular docking simulations were performed using AutoDock Vina. Molecular docking results were scored based on its ΔG to obtain the best value (the most negative ΔG). Molecular interaction such as hydrogen bond, hydrophobic interaction, and the bond was at a distance from the result of AutoVina docking visualized by using Ligplot+1.5.4.

HAT inhibition kinetic assay

HAT inhibition kinetic assay procedures were adapted from previously described methods [13]. All of the reagents were from Sigma-Aldrich unless stated otherwise. The final concentrations of each reagents were 10 and 15 µM histone 3 peptide, 2 µM acetyl-CoA, 0, 10, 20, 30, 40, 50 µM quercetin, and 5 nM PCAF. The HAT inhibition assay was carried out at 30 °C for 10 min in 1x HAT buffer pH 7.5 (Cayman Chemical Company) that consisted of HEPES (free acid), triton X-100, and water. The reaction was stopped with 10 µl isopropanol. Hereafter, 50 µM CPM as the probe was added to the reaction, followed by incubation for the next 10 min. Total volume for this reaction was 100 µl. Relative fluorescence unit (RFU) was measured by Fluostar Omega with 392 nm and 482 nm for excitation and emission wavelength, respectively. This assay was carried out in duplo.

Inhibition kinetic analysis

The histone final concentrations and reaction rate were measured from its relative fluorescence units (RFU). The high RFU indicated high HAT activity and vice versa. The measurable RFU plotted to a linear equation of the standard curve. Dixon plot was used to for HAT inhibition analysis. The histone final concentrations and reaction rate were plotted to Dixon plot to obtain the inhibition constant value and the inhibition type of quercetin against HAT.

RESULTS AND DISCUSSION

Quercetin characteristics

Chemical substances that will serve as drug candidates need to fulfill some physicochemical properties including absorption, distribution, metabolism, excretion, and toxicity [26]. Absorption is the initial parameters that need to be considered in drug development; it is associated with the bioavailability. The first screening of drug candidates related to its absorption characteristic can be done with *in silico* methods if it fulfilled Lipinski rule. Chemical components of drug candidates are membrane permeable and easily absorbed by the body if it meets the following criteria: (1) molecular mass less than 500 g/mol; (2) chemical component lipophilicity expressed as logarithm of the partition coefficient between water and 1-octanol (log*P*) less than 5; (3) less than 5 hydrogen bond donors; (4) less than 10 hydrogen bond acceptors [15]. The analysis of quercetin characteristics result indicates that quercetin has quite a good bioavailability because it meets all the parameters of Lipinski rules (table 1).

Lipophilicity is an important parameter in drug development. Lipophilicity has an impact on all part of the pharmacokinetic profile of oral drugs, such as solubility, absorption, the permeability of the cell, binding to plasma proteins, metabolism, and final excretion [9]. Lipophilicity optimum range, along with low molecular mass and narrow polar surface area greatly affects the level of chemical substances absorption in the intestine by passive diffusion. Lipophilicity plays an important role in regulating kinetics and dynamics aspects of drugs [17]. Drug's lipophilicity expressed in log *P* between 1-octanol and water (1-octanol/water). The partition coefficient is the concentration ratio of a component in a mixture of immiscible phases under an equilibrium condition. Quercetin coefficient partition value of 0.269279 (<5) meet the Lipinski rule. This value indicates that quercetin is easily absorbed by the body.

Molecular mass also affects the absorption rate of drugs in the body. The higher molecular mass associated with poor permeability through the intestine [15]. Quercetin molecular mass of 316 g/mol showed that quercetin eligible Lipinski rule, which is less than 500 g/mol so that quercetin is considered quite easy to be absorbed by the body.

Another Lipinski parameter is the number of proton donors and acceptors. The excessive number of hydrogen bond donors and acceptors that excess can interfere with drug permeability across the membrane bilayer [15]. The number of hydrogen bond donors and acceptors of quercetin respectively by 5 and 6 showed that quercetin also meet the parameter of Lipinski, therefore quercetin can be used to stimulate interaction with histone acetyltransferase. Quercetin meets all the Lipinski rules, consequently, it can be used as ligands in the fixation with the enzyme target.

Table 1: Quercetin characteristics

Characteristics	Quercetin
Molecular mass (<500 g/mol)	316 Da
Octanol-water partition coefficient (log*P*<5)	0.269279
Hydrogen bond receptor (<5)	5
Hydrogen bond acceptor (<10)	6

Gibbs free energy change (ΔG) HAT PCAF with quercetin

Molecular docking between HAT PCAF and quercetin used AutoDock Vina which gives information about ΔG from binding mode between the ligand and its receptor. The best model was chosen based on ΔG value because it showed conformation stability between HAT PCAF and quercetin. HAT PCAF and quercetin reaction could be possible if ΔG<0 that means complex formation reaction is spontaneous [19]. The ΔG value of this molecular docking was-7.5 kcal/mol for HAT PCAF chain A, and-8.0 kcal for HAT PCAF chain B (fig. 1). This value was lower than ΔG of HAT PCAF and CoA, its natural ligand, but not that significant. This suggested that HAT PCAF–quercetin bond was more stable and more spontaneous compared to HAT PCAF–CoA.

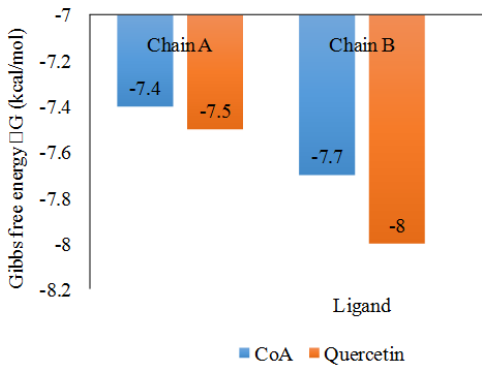

Fig. 1: ΔG of molecular docking between HAT PCAF with CoA and quercetin

Molecular docking between HAT PCAF and quercetin

The molecular docking study used HAT PCAF crystallography from a previous study [4]. CoA connected to HAT PCAF through some noncovalent interactions which include hydrogen bond, hydrophobic interactions, and van der Waals forces. The residues include Cys574, Valine 576 (Val576), Lysine 583 (Lys583), Glycine 584 (Gly584), Threonine 587 (Thr587) that interact through hydrogen bonding, while Gln525, Cys574, Alanine 575 (Ala575), Val576, Gln581, Gly586, Ala613, Tyrosine 616 (Tyr616), and Phenylalanine 617 (Phe617) interact through Van der Waals interactions. The other binding site was histone binding site to HAT PCAF. Glutamic acid 570 (Glu570) was a binding site for lysine of histone on HAT PCAF and stabilized by Asp610 [4]. Glu570 was surrounded by some hydrophobic residues,

such as Phe563, Phe568, Ile571, Val572, Leucine 606 (Leu606), Isoleucine 637 (Ile637), and Tyr640 that raised the *p*Ka of the glutamate side chain and thus facilitated proton extraction from the lysine substrate [4].

The reaction mechanism of histone acetylation by HAT PCAF was preceded by the ternary complex formation between HAT PCAF, acetyl-CoA, and histone. The ε-amino group of Lys14 directly attacks the carbonyl of acetyl-CoA, transferring the acetyl moiety to the histone as acceptor peptide. The release of the product in the order, acetylated is the first, follow by CoA [22]. Therefore, development of HAT PCAF inhibitors can be done based on the acetylation mechanism, that is inhibitor bound to CoA and histone binding site in order the both substrates cannot interact with HAT PCAF, thus acetylation histone cannot occur. The other molecules that could interact through noncovalent interactions with the substrates binding site on HAT PCAF could have a potential as HAT inhibitors.

Molecular docking predicted binding mode between HAT PCAF amino acid residues and quercetin. Chain A of HAT PCAF interacted with quercetin through hydrogen bond that involved Gln525, Cys574, Tyr608, and Asp610 (fig. 2). This binding mode had similarities with CoA binding site on HAT PCAF, those were Cys574 and Gln525. Hydroxyl groups of quercetin formed hydrogen bonds with an amine group (-NH_2) and thiol group (-SH) of Cys574 with the bond distances were 4.55 and 4.25 Å, respectively. Quercetin also formed a hydrogen bond with the hydroxyl group of Gln525 with bond distance is 4.60 Å. Bond distances between quercetin and Cys574 were relatively weak compared to CoA binds to Cys574, but quercetin interaction with Gln525 was stronger than CoA to Gln525 because quercetin interacted through hydrogen bond, meanwhile CoA interacted through van der, Waals. Quercetin interaction was also stabilized by the hydrophobic interaction that involved the same amino acid residues of CoA binding sites, which were Ala575, Ala613, Tyr616, and Phe617. This indicated that quercetin could have the potentiality to compete with CoA. Another hydrogen bond was formed between quercetin and Asp610 which was an amino acid that was involved in the binding of histone at PCAF thus alleged that quercetin was also bound to the histone binding site, and this indicated that quercetin also could have competed with histone. This molecular docking predicted that quercetin could be the competitive inhibitor for both CoA and histone on chain A HAT PCAF.

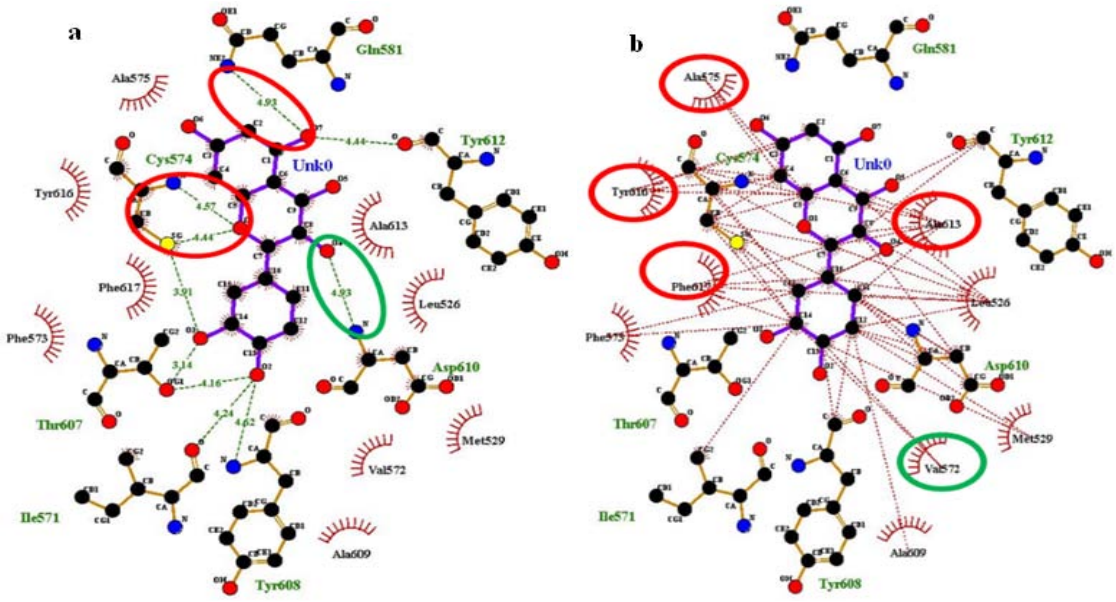

Fig. 2: Chain A HAT PCAF interaction with quercetin a) hydrogen bond; b) hydrophobic interaction. The red circle showed the amino acids involved in the CoA binding, meanwhile, the green circle showed the amino acids involved in the histone binding

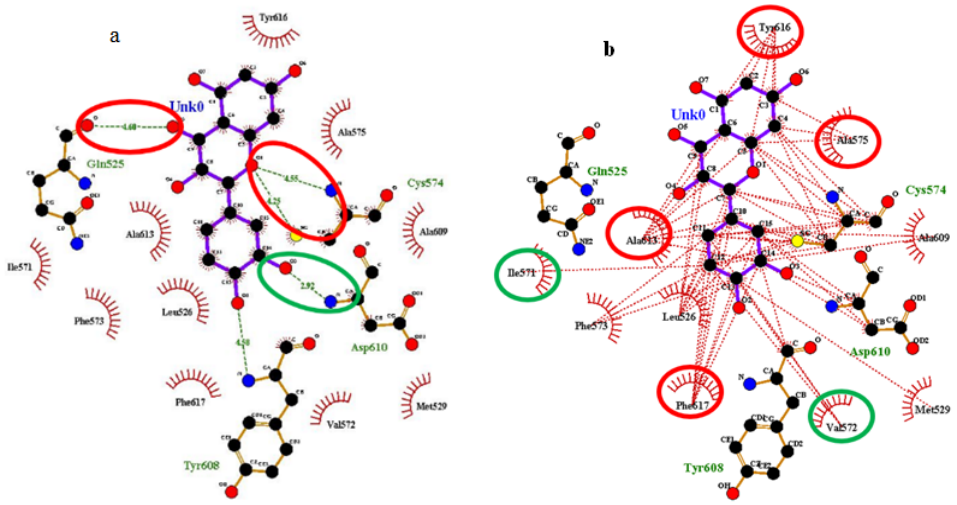

Fig. 3: Chain B HAT PCAF interaction with quercetin a) hydrogen bond; b) hydrophobic interaction. The red circle shows the amino acids involved in the CoA binding, meanwhile, the green circle shows the amino acids involved in the histone binding

The hydrogen bond between quercetin and CoA binding sites also occurred at chain B HAT PCAF through Cys574 and Gln581 (fig. 3). Hydroxyl group from quercetin bound with amide group (-NH_2) and a thiol group (-SH) of Cys574 with bond distances were 4.44 and 4.57 Å, respectively. Quercetin also interacted with CoA binding sites through hydrophobic interaction that involved Ala575, Ala613, Tyr616, and Phe617. Quercetin on chain B HAT PCAF also bound to histone binding site, Asp610, and that was stabilized by Val572. The molecular interaction that occurred between quercetin and chain B HAT PCAF indicated that quercetin could be the competitive inhibitor for both CoA and histone on chain B HAT PCAF, the same as it happened with chain A. The kinetic inhibition assay in this study would give more information. The *in vitro* assay for this study was carried out with HAT PCAF inhibition kinetic assay. The other HAT PCAF amino acids that interacted with quercetin are shown in table 2.

Table 2: Interaction between HAT PCAF and quercetin with radius<5 Å

HAT PCAF chain	Hydrogen bond distance (Å)	Amino acids	Functional groups on HAT PCAF	Functional groups ligand	Hydrophobic interaction
A	4.60	Gln525	-COOH	-OH	Leu526, Met529, Ile571, Val572, Phe573, Ala575, Ala609, Ala613, Tyr616, Phe617
	4.25	Cys574	-SH	-OH	
	4.55	Cys574	-NH_2	-OH	
	4.58	Tyr608	-NH_2	-OH	
	2.92	Asp610	-NH_2	-OH	
B	4.24	Ile571	-COOH	-OH	Leu526, Met529, Val572, Phe573, Ala575, Ala609,
	4.44	Cys574	-NH_2	-OH	
	4.57	Cys574	-SH	-OH	
	4.93	Gln581	-OH	-OH	

HAT inhibition kinetics

Study of inhibitor potency to inhibit its target enzyme is done by inhibition kinetics assay. This assay gives information about Ki and type of inhibition. The information can be used to understand the interaction between inhibitor and its target enzyme in drug development. Inhibition kinetics of HAT PCAF by quercetin showed that quercetin inhibited HAT PCAF based on fluorescence intensity. The product of HAT PCAF reaction decreased by increasing quercetin concentrations increased. The result of Dixon plot analysis showed that the inhibition type of HAT PCAF by quercetin was competitive inhibition (fig. 4), with Ki values of 9.575 µM. Inhibition Competitive inhibition indicated that quercetin could bind to CoA's binding site that could inhibit HAT PCAF activity [6]. Ki value indicated quercetin affinity against HAT PCAF, the smaller the Ki value, the higher the quercetin affinity for HAT PCAF, and vice versa [6]. The Ki value can be used as a quantitative comparison between quercetin affinity with histone and other inhibitors [6]. Affinity comparative of quercetin and histone could be compared based on the Ki value of quercetin with the K_M value of histone which indicated its affinity for HAT PCAF [6]. Based on the comparison, quercetin affinity was slightly higher than histone, so that it can compete with histone on HAT PCAF. The same result was shown by molecular docking of quercetin and showed by molecular docking of quercetin and HAT PCAF.

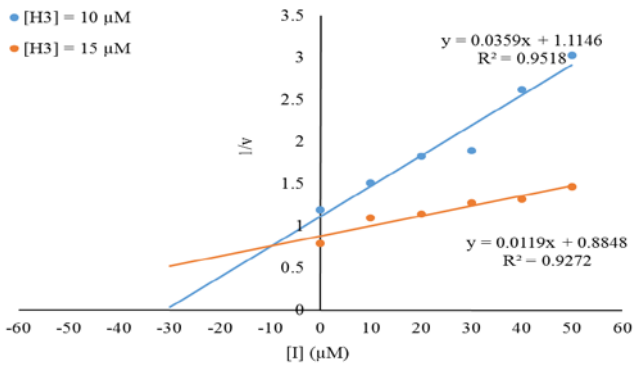

Fig. 4: Dixon plot of product formation rate (1/v) as a function of inhibitory concentration [I]

HAT inhibition assay used quercetin as inhibitor and histone as a substrate in various concentrations, while CoA as a substrate in fixed concentration. The results of HAT inhibition assay showed that quercetin could inhibit HAT activity. HAT activity inhibition in this study was observed from the CoA concentration which was a product of HAT enzymatic reaction. CoA concentration was equivalent to the formation of acetylated histones [10]. Therefore, the low concentration of CoA showed that quercetin could inhibit histone acetylation reaction that catalyzed by HAT PCAF.

The results of HAT inhibition assay by Dixon plot provided information about the type of HAT PCAF inhibition by quercetin. Inhibition type was observed from the intersection of the two lines of linear inhibition (fig. 4). The intersection of the lines above the x-axis (inhibitor concentration) indicates that inhibition of this enzyme is a competitive inhibition [12]. Quercetin as competitive inhibitor bound to the histone active site and inhibited H3 as HAT PCAF's substrate to its binding site. This was leading to inhibition of acetyl transfer acetyl from acetyl-CoA to histone; thereby inhibit the formation of acetylated histones.

Another information that we could get from this kinetics study was the value of inhibition constant (Ki) which was the concentration of inhibitor that was required decreasing decreasing a half of maximum reaction rate. Hence, the lower the Ki value was, the lower the inhibitor concentration required to inhibit enzyme activity, and vice versa. Ki values could also be used to predict the interaction between inhibitor and enzyme. Ki value that was lower than plasma concentration showed that the interaction could occur, whereas if Ki was higher than the plasma concentration, the interaction could not occur [12]. Ki values for quercetin against HAT PCAF were 9.575 µM.

CONCLUSION

This study described quercetin potency as HAT PCAF inhibitor with *in silico* and *in vitro* approach. Quercetin binding mode on HAT PCAF used molecular docking. Quercetin bound to CoA active site through Gln525 and Cys574 on chain A, and through Cys574 and Gln581 on chain B HAT PCAF. Quercetin also bound to histone active site Asp610, Ile571, and Val572. The most important part of quercetin in binding HAT PCAF is a hydroxyl group. The result of molecular docking showed that quercetin could compete with both CoA and histone. Quercetin interaction with HAT PCAF is slightly more stable than the interaction between CoA and HAT PCAF based on ΔG value. The same indication happened to kinetics inhibition (*in vitro*). Quercetin against HAT PCAF affinity (Ki = 9.575 µM) is hihger the affinity of histone on HAT PCAF (K_M = 10 µM), but the affinity was not significantly different. Dixon plot analysis also showed that quercetin competed with histone to inhibit HAT PCAF activity.

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CONFLICT OF INTERESTS

There is no conflict of interest.

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