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Molecular docking study of Xanthyl Chalcone derivatives as potential inhibitor agents against KIT tyrosine kinase and KIT kinase domain mutant D816H

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ABSTRACT

The mutation of D816H that occurs in the tyrosine kinase protein is responsible for gastrointestinal stromal tumors (GISTs). The mutation is commonly followed by the formation of a protein-resistant expression, thus new inhibitor agents are highly required in the near future. Molecular docking studies were carried out to evaluate the inhibition activity of six xanthyl chalcone derivatives to the wild and mutant types of KIT tyrosine kinase protein. The results showed that six xanthyl chalcone derivatives gave strong binding interactions with both tyrosine kinase proteins yielding on binding energy of −8.45 to −11.8 kcal mol−1, respectively. The molecular docking studies confirm that the binding interactions between the xanthyl chalcone and the amino acid residue were similar to those of sunitinib as the native ligand. Among all xanthyl chalcone derivatives, compound **X6** possessed the lowest free binding energy value. Thus, compound **X6** possibly has the highest inhibition activity toward wildtype and D816H mutant KIT protein. **X6** was observed successfully to bind the activated KIT tyrosine kinase active site with a low binding energy value of −11.22 kcal mol−1. Therefore, this compound could become a promising inhibition agent to treat GISTs.

INTRODUCTION

Cancer is a disease with a high mortality rate caused by uncontrolled cell proliferation activity. Protein resistance is a common effect of long-term cancer treatment, which worsens cancer metastases. Thus, it leads to the necessities of developing new anticancer drugs that will give a better response and could replace current medications. Systematic studies have found that receptor tyrosine kinases regulated a key signal to initiate cell growth and proliferation of signal transduction (Linnekin, 1999). The mutation in KIT tyrosine kinase was caused by the overexpression of oncogene activity and the absence of a stem cell factor involved in several human tumors, including gastrointestinal stromal tumors (GISTs), myeloid leukemia, germ cell tumors, small cell lung cancer, and mastocytosis (Kansal *et al*., 2010; Tosoni *et al*., 2004). GISTs are the most common mesenchymal tumors that potentially develop into benign or malignant cancer. Metastases are commonly spread to the liver, resulting in a low survival rate. Imatinib mesylate is well known as the first-line treatment to inhibit tumor growth for patients with GISTs. However, a long-term treatment using imatinib leads to drug resistance as reported by other studies (Schnittger *et al*., 2006; Zalcberg *et al*., 2005).

Sunitinib is currently the second-line treatment replacing imatinib mesylate, and it is effective in inhibiting the secondary mutation in the KIT tyrosine kinase. The main disadvantage of sunitinib is its poor inhibition activity toward advanced mutations in the activation loop of tyrosine kinase (D816H/V), which results in an increase in the population of molecules in the activated state (Gajiwala *et al*., 2009). The existence of D816H mutant protein affects the amount of protein target in the activated state, which leads to low efficacy of the drug in GIST patients (Garner *et al*., 2014). Over the years, several investigations have been conducted to evaluate new compounds and their ability to solve the above-mentioned issues; however, their activity is still unsatisfactory.

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Xanthone derivatives have been widely studied in medicinal chemistry as they have various biological activities, such as antimalarial (Amanatie *et al*., 2017), anti-inflammatory (Chen *et al*., 2017), antioxidant (Rohman *et al*., 2019), antituberculosis (Sudta *et al*., 2013), and anticancer (Shan *et al*., 2011) properties. Structural modifications of xanthone, by introducing new substituents to the xanthone ring, have been carried out to increase its pharmacological activity. The presence of different substituents has been reported to significantly affect the activity of xanthone derivatives as an antitumor agent (Miladiyah *et al*., 2018; Na, 2009; Su *et al*., 2011).

On the other hand, chalcone, a compound which belongs to the flavonoid family, had been reported to be effective for cancer treatment (Kong *et al*., 2005). Chalcone structure has been extensively investigated in the identification of new precursors for drug discovery. This compound possesses two aromatic rings connected by an *α,β*-unsaturated carbonyl moiety. It has been known to have broad biological properties owing to the presence of *α,β*-unsaturated carbonyl moiety (Xu *et al*., 2017).

Molecular docking has been considered as an efficient method for drug development. Chemical compounds modeling using molecular docking is not time-consuming, efficient, and effective as trial and errors in the wet laboratory could be minimized (Khaw *et al*., 2014). This method can predict the preferred orientation of molecules as well as their interactions, to find the best complex conformation (Forli *et al*., 2016). The results from molecular docking analysis could be used to explain the binding affinity or strength of association between the ligand and the protein receptor. The lowest binding energy value of molecular interaction is chosen as the most stable interactions between the ligand and the protein receptor (Zamri *et al*., 2016). The use of the molecular docking method is prospective to predict the inhibition activity of KIT tyrosine kinase.

In this study, an investigation on the binding energy and inhibition activity of xanthyl chalcone derivatives against KIT tyrosine kinase protein has been carried out to investigate its inhibition activity toward GISTs. An *in silico* study was conducted to determine the xanthyl chalcone derivatives' activity as an initial stage of the novel compound development before synthesis and biological analysis were carried out. In this report, molecular docking had been employed to study xanthyl chalcone derivatives' antitumor activity mechanism toward KIT tyrosine kinase protein active site.

MATERIALS AND METHODS

Chemistry

Six xanthyl chalcone derivatives were designed using Chemdraw Professional 17.1 and their chemical structure is shown in Figure 1.

Methods

Molecular docking was carried out on wildtype KIT tyrosine kinase protein (PDB ID: 3G0E), KIT kinase domain D816H mutant (PDB ID: 3G0F), and activated KIT tyrosine kinase protein (PDB ID: 6ITV) which were obtained from the Protein Data Bank (www.rcsb.org). The three-dimensional (3D) structure of xanthyl chalcone derivatives (Fig. 1) was drawn using GaussView 5.0.8 and optimized using Gaussian 09W with DFT/B3LYP method using 3–21G basis set. Native ligands and proteins' preparation were conducted using Chimera. Native ligands and proteins were saved in the .pdb format separately. The 3D grid box with 60 Å grid size (*x,y,z*) and spacing of 0.375 Å was created on the macromolecule coordinates. The grid maps were generated to represent the intact ligand in the actual docking target site. The ligand position was flexibly maintained and the protein was kept in its rigid form throughout the procedures. The free binding energy (ΔG) and dissociation constant (Kd) of the xanthyl chalcone derivatives and native ligand interacted with protein target were calculated using AutoDock 4.2. The root mean square deviation (RMSD) value was used as a parameter of the success of docking analysis, that is, when the RMSD value was less than 2 Å (Huey *et al*., 2007). One hundred independent docking conformations were set for each analysis. Discovery Studio Visualizer 2019 was used to visualize the interaction between ligand and macromolecule from the docking results.

RESULTS AND DISCUSSION

Molecular docking analysis

Molecular docking was conducted with two types of KIT proteins, which are KIT tyrosine kinase protein and KIT kinase domain mutant D816H, whose structure and RMSD values are shown in Figure 2 and Table 1, respectively. The investigation on xanthyl chalcone derivatives' interaction as ligand and binding affinities with the protein binding site was conducted to study the possible interactions of xanthyl chalcone derivatives as KIT tyrosine kinase inhibitor agents. Xanthyl chalcone derivatives were designed through a chemical modification by combining the xanthone structure and phenyl vinyl ketone group that represented chalcone. In this study, the effect of the phenyl vinyl ketone group (R_1, R_2, or R_3) position and iodine group addition (R_4) in xanthone nuclei to its inhibition activity toward KIT tyrosine kinase and KIT mutant D816H was observed.

The native ligand (sunitinib) was docked into inactivated KIT tyrosine kinase protein (Fig. 3a) and KIT mutant D816H (Fig. 3(b)) to identify the binding site of the protein. Redocking sunitinib was successfully carried out with an RMSD value of less than 2 Å (0.77 Å for KIT tyrosine kinase and 0.67 Å for KIT

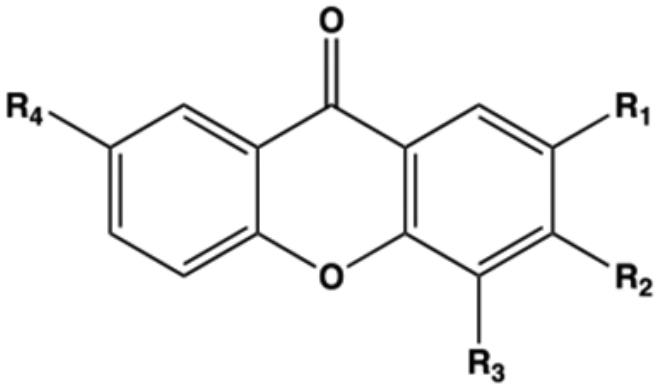


Figure 1. The used xanthylchalcone derivatives in the molecular docking analysis. X1: R_1=X (R_2=R_3=R_4=H); X2: R_3=X (R_1=R_2=R_4=H); X3: R_2=X (R_1=R_3=R_4=H); X4: R_1=X (R_2=R_3=H, R_4=I); X5: R_3=X (R_1=R_2=H, R_4=I); X6: R_2=X (R_1=R_3=H, R_4=I); X: -CH=CH-CO-C_6H_5.

mutant D816H). Redocking was needed to validate the binding position and also to adjust the parameters for docking estimation (Cosconati *et al*., 2010). Sunitinib interaction with the protein was observed from the presence of hydrogen bond and hydrophobic interaction between the native ligand and the amino acid residues as listed in Tables 2 and 3.

The xanthyl chalcone derivatives (compounds **X1**–**X6**) were docked into protein binding pocket similar to the sunitinib position. The results were proven by the presence of hydrogen bonding of xanthyl chalcones to Cys673 amino acid residue of KIT/D816H mutant KIT tyrosine kinase protein that is similar to sunitinib–proteins interaction. Cys673 is an amino acid residue of KIT tyrosine kinase located in the hinge region of the kinase domain. Xanthyl chalcone also interacted with Lys623, an amino acid residue that linked phosphates from the adenosine diphosphate (ADP) molecule in KIT active form (Mol *et al*., 2003). The binding energy, dissociation constant (Kd), and intermolecular interaction of xanthyl chalcone derivatives with KIT tyrosine kinase protein and KIT mutant D816H are summarized in Tables 2 and 3, respectively.

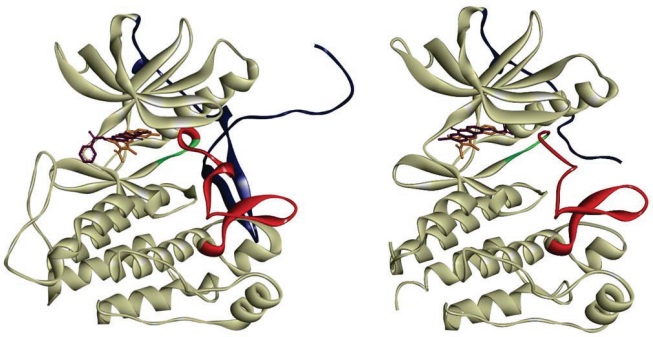


Figure 2. (a) Wildtype KIT tyrosine kinase–sunitinib–**X6** structure and (b) KIT tyrosine kinase mutant D816H–sunitinib–**X6** structure. The JM domain, A-loop, and DFG-motif are shown in blue, red, and green, respectively.

Table 1. PDB ID protein and RMSD value of redocking results.

Pathology of cancer cells	Drug target mechanism	PDB ID	RMSD (Å)
Tumor promoting GIST	Tyrosine kinase domain inhibitor	3G0E	0.77
Secondary mutations of GIST	Tyrosine kinase domain secondary mutation D816H inhibitor	3G0F	0.67

Docking results of Xanthyl Chalcone derivatives interaction with wildtype (wt)KIT tyrosine kinase protein

All the six xanthyl chalcone derivatives showed a similar binding interaction between the compound and amino acid residues (Cys673, Leu595, Cys809, Leu799, Val603, Lys593, Phe811, Tyr672, Ala814, and Ala621) as sunitinib (Fig. 4a). The molecule was docked and generated into 100 possible ligand conformations. The conformations were grouped based on conformation similarity. Then, the final predicted ligand-binding pose was chosen according to the binding site similarity of the ligand to the native ligand interaction with the protein target and also through the most preferred binding energy from the group (Nguyen *et al*., 2020). The negative score of binding energy indicated the compound's potential as an inhibitor for protein yielding in higher possibilities for this compound to be the best candidate for being the novel drug compound (Dolatkhah *et al*., 2017). Besides binding energy and intermolecular interaction, the inhibition constant (Ki) value of the ligand is also an important parameter. Ki value indicated the rate of ligand inhibition to the protein target that described how potent an inhibitor hinders protein activity. The lower Ki value, which represented the ligand interaction with the protein, is more preferred (Heh *et al*., 2013). Ki value was assumed to be the same as the Kd value in the docking calculation (Fukunishi *et al*., 2017). These parameters are taken into consideration when proposing the best candidate ligand pose from docking.

Xanthyl chalcones were successfully docked at the site of the kinase domain. The binding modes were stabilized by hydrophobic interactions between aromatic rings, as well as hydrogen bonds of oxygen from the xanthone ring and carbonyl group of chalcone moieties (Fig. 5a). Hydrogen bonding and hydrophobic interactions played significant roles in the drug–protein study. Drug modification was made by adding a substituent that increased the number of hydrogen bonds and hydrophobic region to increase binding affinity and ligand efficacy as a drug (Freitas and Schapira, 2017).

The results showed that the binding energies of **X1–X6** were lower than that of sunitinib in its interaction with wtKIT

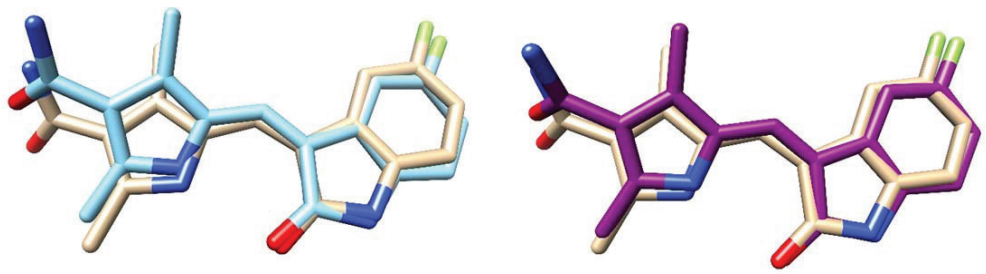


Figure 3. The X-ray crystal structure of native ligand (light brown) and redocking result of sunitinib to (a) KIT tyrosine kinase protein (light blue) and (b) KIT kinase mutant D816H (dark purple).

Table 2. Binding energy and interaction of six xanthyl chalcone derivatives and the native ligand with KIT tyrosine kinase protein.

Compound	ΔG (kcal mol^{-1})	ΔΔG (kcal mol^{-1})	Kd (nM)	Hydrogen bond interaction	Other binding interactions with amino acid residue
X1	−10.20	−1.95	32.97	Lys593;Cys673	p-alkyl: Val603, Ala621, Val654, Met757, and Cys809 p-sigma or p-σ: Leu595;Leu799
X2	−8.79	−0.54	363.50	Cys673	p-alkyl: Ala621, Cys809 p-sigma or p-σ: Leu595, Val603, Gly676, and Leu799 p-sulfur: Asp677
X3	−9.85	−1.60	60.72	Lys593; Cys673	p-alkyl: Val603, Ala621, Val654, and Cys809 p-sigma or p-σ: Leu595;Leu799 p-sulfur: Met757 p- pstacked: Tyr672
X4	−10.70	−2.45	14.91	Lys593;Cys673	p-alkyl: Ala621, Lys623, Val654, Cys809, and Ala814 p-sulfur: Met757 p-sigma or p-σ: Leu595, Val603, and Leu799
X5	−9.90	−1.65	55.09	Cys673	p-alkyl: Ala621, Lys623, Val654, Cys809, and Ala814 p-: Leu595, Val603, and Leu799 p- pstacked: Tyr672
X6	−10.90	−2.65	9.98	Lys593;Cys673	p-alkyl: Val603, Ala621, Lys623, Val654, and Cys809 p-sigma or p-σ: Leu595;Leu799 p- p stacked: Tyr672 p-sulfur: Met757
Sunitinib	−8.25	–	889.60	Glu671,Cys673, and Asp677	p-sulfur: Cys809 p-sigma or p-σ: Val603 Carbon hydrogen bond: Lys623 Halogen (Fluorine): Asp810 p-alkyl:Leu595, Ala621, Val654, Leu799, Phe811, and Ala814

tyrosine kinase protein (Table 2). Xanthyl chalcones were predicted to have preferable inhibitory activity. The docking outcome disclosed that the phenyl vinyl ketone group at R_3 (**X2** and **X5**) with free binding energy values of −8.79 and −9.90 kcal mol^{-1} affected the xanthyl chalcone–wtKIT tyrosine kinase protein interaction. It was due to the presence of a bulky phenyl vinyl ketone group in the R_3 position from the main xanthone ring that caused the steric hindrance. The substitution of the xanthone ring with the halogen functional group increases the antitumor activity as shown from their free binding energy value. Compounds that contain iodine substituent (**X4**, **X5**, and **X6**) have a lower energy than compounds without iodine (**X1**, **X2**, and **X3**). Iodine-substituent existence in some compounds leads to hydrophobic interactions of the molecules with the kinase (Ibrahim *et al*., 2015). Hence, the compounds are predicted to have better inhibition activity.

Although it was confirmed from the results that compounds **X1**–**X6** had the same inhibitory activity as the native ligand, compound **X6** was found to be the best inhibitor for the KIT tyrosine kinase as this compound had the lowest binding energy value of −10.9 kcal mol^{-1}. The low binding value of **X6** indicated a stable interaction with the binding site of the protein. This result was preferable compared to the native ligand binding energy result of −8.25 kcal mol^{-1}. From the ΔΔG calculation (change in xanthyl chalcone-binding energy relative to sunitinib-binding energy) (Table 2), it was seen that the ΔΔG values of all xanthyl chalcones were negative (−0.54 to −2.65 kcal mol^{-1}), which means xanthyl chalcones' pose in the kinase domain site of wtKIT tyrosine kinase is more stable than sunitinib. The 3D and 2D docking results of **X6** compound against KIT kinase domain protein are shown in Figures 4a and 5a, respectively.

Docking results of Xanthyl Chalcone derivatives interaction with KIT kinase domain mutant D816H

The interaction between six xanthyl chalcone derivatives and protein KIT mutant D816H was observed. The study was carried out to resolve previous drug resistance to the KIT mutation that occurred in the D816 region due to GIST drugs. The change in the amino acid sequence was able to alter the structural characteristics of proteins (Shaik *et al*., 2019). This phenomenon would affect the ligand-binding affinity to the target binding pocket.

Xanthyl chalcones were successfully docked to the KIT mutant D816H in the same site as sunitinib (Fig. 4b). Compounds **X1**–**X6** (−8.45 to −11.8 kcal mol^{-1}) were observed to have a higher binding affinity than sunitinib (−8.01 kcal mol^{-1}) (Table 3). These results indicated that the xanthyl chalcone compounds were predicted to be more biologically active than the native ligands as a secondary mutation KIT tyrosine kinase D816H inhibitor. The ΔΔG calculation (Table 3) showed that the ΔΔG values of all xanthyl chalcones were negative (−0.44 to −3.79 kcal mol^{-1}),

Table 3. Binding energy and interaction of six xanthyl chalcone compounds and the native ligand with KIT kinase domain mutant D816H.

Compound	ΔG (kcal mol^{-1})	ΔΔG (kcal mol^{-1})	Kd (nM)	Hydrogen bond interaction	Other binding interactions with amino acid residue
X1	−10.30	−2.29	26.75	Lys623;Cys673	p-alkyl: Ala621, Val654, Leu799, and Cys809 p-p stacked: Tyr672 Carbon hydrogen bond: Phe811 p-anion: Glu640 p-sigma or p-σ: Leu595
X2	−10.10	−2.09	42.28	Lys623;Cys673	p-alkyl: Leu595, Ala621, Val654, and Ala814 p-sigma or p-σ: Val603 p-sulfur: Cys809
X3	−8.45	−0.44	886.71	Cys673	p-alkyl: Ala621 and Cys809 p-sigma or p-σ: Leu595, Val603, Gly676, and Leu799 p-p stacked: Phe811 Carbon hydrogen bond: Tyr672
X4	−10.70	−2.69	14.82	Lys623;Cys673	p-alkyl: Ala621, Val654, Leu799, and Cys809 p-p stacked: Tyr672 Carbon hydrogen bond: Phe811 p-anion: Glu640 p-sigma or p-σ: Leu595
X5	−11.80	−3.79	2.28	Lys623;Cys673	p-alkyl: Ala621, Leu644, Val654, and Ala814 p-sigma or p-σ: Leu595 and Val603 p-sulfur: Cys809 Carbon hydrogen bond: Phe811
X6	−10.70	−2.69	14.17	Lys623;Cys673	p-alkyl: Val603, Ala621, and Cys809 p-sigma or p-σ: Leu595 and Leu799 Carbon hydrogen bond: Phe811 p- p stacked: Tyr672 p-anion: Glu640
Sunitinib	−8.01	−	1,350	Glu671, Cys673, and Asp677	p-sulfur: Cys809 p-alkyl: Leu595, Ala621, Val654, and Leu799 Carbon hydrogen bond: Tyr672 p-sigma or p-σ: Val603 and Gly676 p- p: Phe811

which means xanthyl chalcones' pose in the kinase domain site of KIT mutant D816H protein was more stable than sunitinib.

The influence of iodine was also observed in the interaction between xanthyl chalcones and mutant proteins, where its existence in creased the binding affinity of xanthyl chalcone. Compounds **X4**, **X5**, and **X6** (−10.7, −11.8, and −10.7 kcal mol^{-1}, respectively), which contained iodine substituents at position R_4, had lower free binding energy values than compounds **X1**, **X2**, and **X3** (−10.3, −10.1, and −8.45 kcal mol^{-1}, respectively), which have no iodine substituents in the compound structures. Compound **X5** with phenyl vinyl ketone group at R_3 position was the most stable proposed compound with a free binding energy value of −11.8 kcal mol^{-1}. This result was dissimilar to **X5** interaction with KIT tyrosine kinase from previous docking results. KIT mutant D816H docking results showed that **X2** and **X5** binding energy values (−10.1 and −11.8 kcal mol^{-1}, resp.) were lower than **X2** and **X5** binding energy values (−8.79 and −9.90 kcal mol^{-1}, resp.) in the wtKIT tyrosine kinase. The phenomena were related to the change in the shape of wtKIT tyrosine kinase active sites due to D816 mutation into H816 (Chauvot de Beauchêne *et al*., 2014). The shape alteration made the interaction between **X2** and **X5** with KIT mutant D816H-binding site more stable than in wtKIT tyrosine kinase.

Another compound with a low free binding energy value is **X6**, with a binding energy value of −10.7 kcal mol^{-1}. The findings of previous docking analysis indicated that compound **X6** was predicted as a prominent inhibitor for the protein of KIT tyrosine kinase. This compound fitted as the best xanthyl chalcone compound that was predicted to actively inhibit both the wtKIT tyrosine kinase protein and the KIT mutant D816H protein. The 3D and 2D docking results of **X6** against KIT mutant D816H protein are shown in Figures 4b and 5b, respectively.

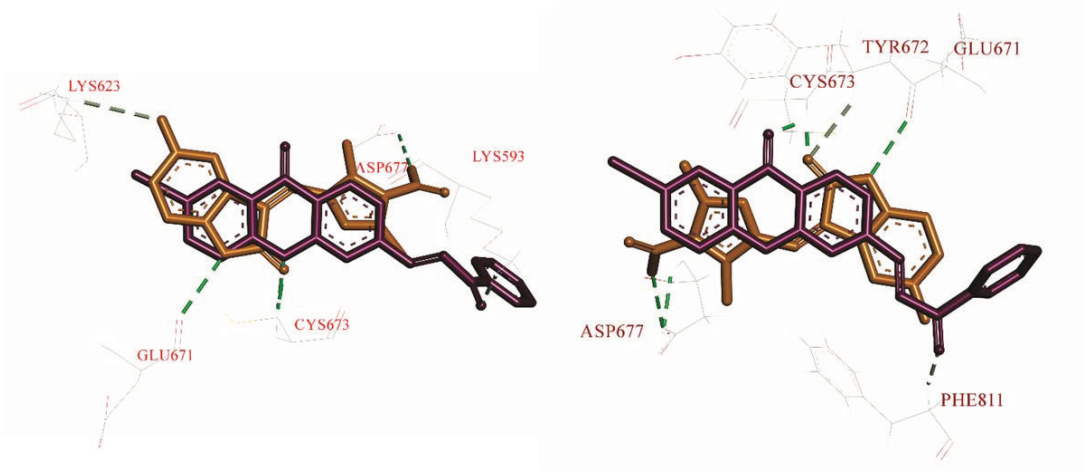


Figure 4. 3D docking results of **X6** (magenta) in (a) KIT tyrosine kinase and (b) KIT kinase domain mutant D816H, overlapped with sunitinib (orange) position in the proteins, and hydrogen bonding interaction (green) of the ligands with the amino acid residues of the proteins.

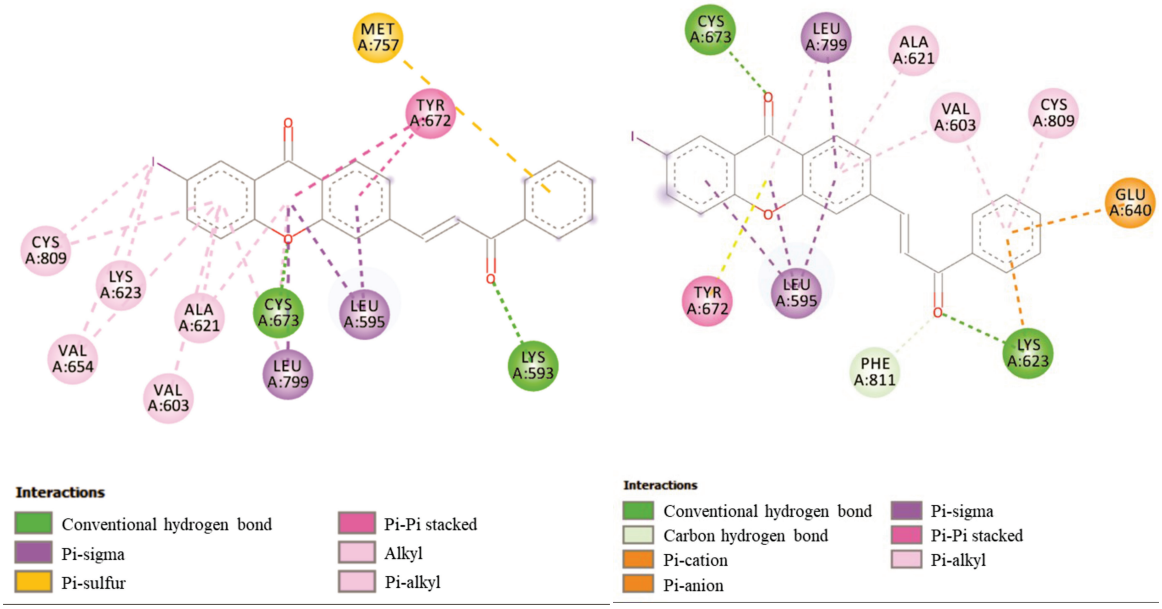
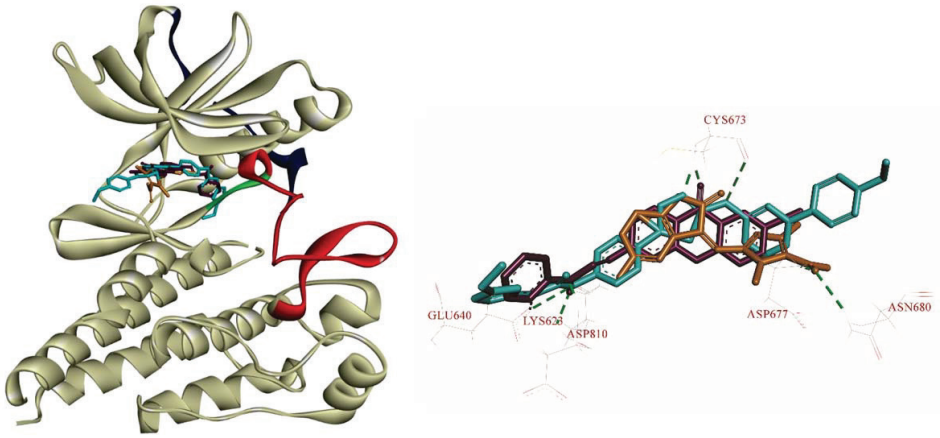


Figure 5. (a) 2D docking results of **X6** compound against KIT tyrosine kinase protein and (b) 2D docking results of **X6** compound against KIT kinase domain mutant D816H.

Docking results of Xanthyl Chalcone derivatives interaction with activated KIT tyrosine kinase protein

Although the initial activation of the intracellular is unknown, KIT autoactivation can occur when the activation loop (A-loop) space is free from the juxtamembrane. This condition made the A-loop to allow the phosphorylation of tyrosine residue in the protein (Mol *et al*., 2004). Considering D816H mutation confirmed to accelerate the change of protein to its active form than the wildtype conformation (Gajiwala *et al*., 2009); this study was conducted to find out the predicted ligand ability to interact with activated KIT tyrosine kinase (Fig. 6a).

The result showed that xanthyl chalcone and sunitinib were successfully docked to the activated KIT tyrosine kinase in the similar site as the native ligand (Fig. 6b). Compounds **X1**–**X6** (−8.43 to −11.22 kcal mol^{-1}) were observed to have a higher binding affinity than sunitinib (−8.41 kcal mol^{-1}) (Table 4). The 1-(5-ethyl-isoxazol-3-yl)-3-3(4-{2-[6-(4-ethylpiperazin-1-yl) pyryimidin-4-ylamino]-thiazol-5-yl}phenyl)urea as the native ligand formed four hydrogen bonds to the protein target (Fig. 6b). The inhibition mechanism of the native ligand in the activated KIT tyrosine was confirmed by lead rearrangements of the protein structure that switched the active form back to the inactive form through a sequence of conformational changes (Wu *et al*., 2019). As the best predicted ligand from the xanthyl chalcones, **X6** could bind to the activated KIT tyrosine kinase with a similar mechanism as the native ligand. **X6** formed a hydrogen bond interaction with Cys673 and Lys623 (Fig. 7a). The interaction was confirmed as a similar interaction as the native ligand. However, sunitinib



(a) (b)

Figure 6. (a) Activated KIT tyrosine kinase structure with 1-(5-ethyl-isoxazol-3-yl)-3-3(4-{2-[6-(4-ethylpiperazin-1-yl)pyryimidin-4-ylamino]-thiazol-5-yl}phenyl) urea, sunitinib, and **X6** molecules. The JM domain, A-loop, and DFG-motif are shown in blue, red, and green, respectively. (b) 3D docking of 1-(5-ethyl-isoxazol-3-yl)-3-3(4-{2-[6-(4-ethylpiperazin-1-yl)pyryimidin-4-ylamino]-thiazol-5-yl}phenyl)urea ligand (cyan), sunitinib (orange), and **X6** (magenta).

Table 4. Binding energy and interaction of 1-(5-ethyl-isoxazol-3-yl)-3-3(4-{2-[6-(4-ethylpiperazin-1-yl)pyryimidin-4-ylamino]-thiazol-5-yl}phenyl) urea ligand, **X6**, and sunitinib against activated KIT tyrosine kinase.

Compound	ΔG (kcal mol^{-1})	RMSD	Kd (nM)	Hydrogen bond interaction
1-(5-Ethyl-isoxazol-3-yl)-3-3(4-{2-[6-(4-ethylpiperazin-1-yl)pyryimidin-4-ylamino]-thiazol-5-yl}phenyl)urea	−11.98	1.00	1.66	Lys623, Glu604, Cys673, and Asp810
X6	−11.22	1.49	6.00	Lys623 and Cys673
Sunitinib	−8.41	0.94	685.00	Asp677 and Asn680

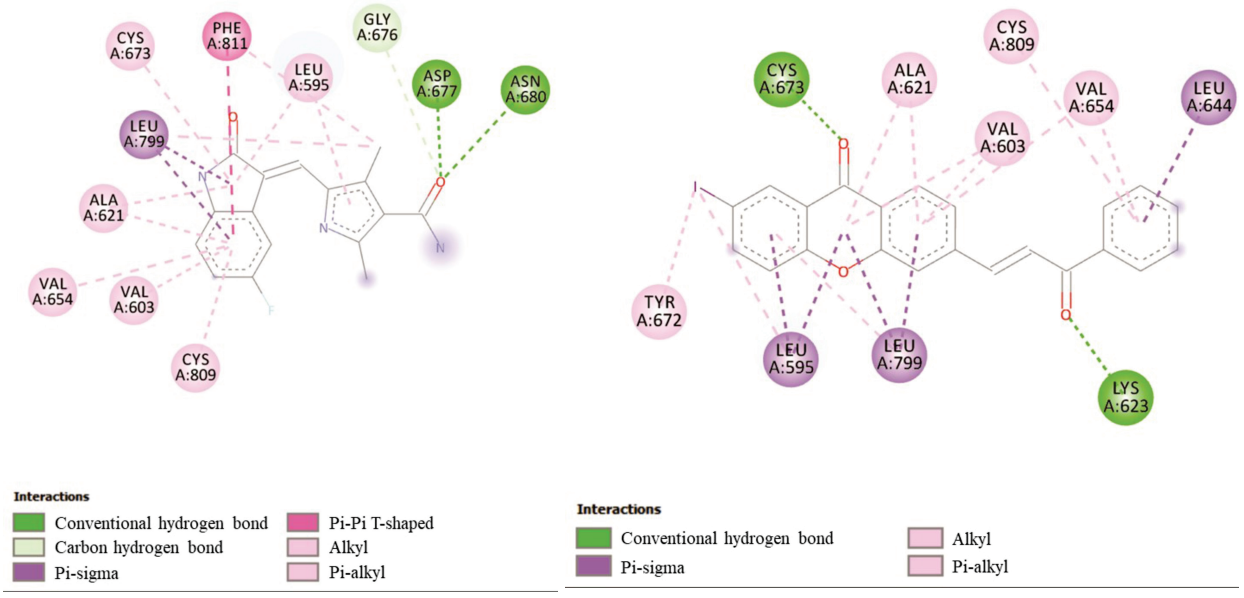


Figure 7. (a) 2D docking results of sunitinib and (b) **X6** compound against activated KIT tyrosine kinase protein.

interaction with the protein was found to be slightly different from the native ligand interaction. Sunitinib formed hydrogen bonds with Asp677 and Asn680, which were not found in the native ligand interaction (Fig. 7b). Sunitinib is a type II inhibitor that binds to the hinge region and the DFG-out motif of KIT tyrosine kinase. The DFG-out structure is indicated with the Phe811 of the Asp-Phe-Gly (DFG) region located near the binding pocket of adenosine triphosphate (ATP). The change in protein structure from inactive to active made sunitinib's interaction weaken (Gajiwala *et al*., 2009).

Based on the docking results, it was discovered that **X6** posed in the proteins (inactive KIT tyrosine kinase, KIT D816H mutant tyrosine kinase, and activated KIT tyrosine kinase) was predicted to have a high stability interaction in the binding sites. Hence, **X6** was chosen as the best predicted inhibitor from the xanthyl chalcones. **X6** showed effective interaction with the activated and inactivated KIT tyrosine kinase. The stability of **X6** interaction with the two forms of the KIT could minimize the effect of phosphorylation that happens in Y823. Y823 is the only phosphor-acceptor site on the A-loop that is known to affect the sunitinib-inactive KIT tyrosine kinase complex. As the last phosphorylation step happened in the protein, pY823 changed the protein structure from being autoinhibited to its active state. This condition reduced the stability of sunitinib to bind the KIT protein (DiNitto *et al*., 2010). Moreover, the mechanism of inhibition of **X6** to the three above-mentioned proteins needs confirmation from molecular dynamics for simulation of molecular movements in the protein and also needs to be proven by *in vitro* and *in vivo* experimental studies toward GISTs cell tumor.

CONCLUSION

The molecular docking study toward wtKIT tyrosine kinase and KIT mutant D816H proteins resulted in the finding that six xanthyl chalcone derivatives had better inhibition activity compared to sunitinib as the native ligand. The result showed that the six compounds interacted with proteins having binding energy ranging from −8.79 to −10.9 kcal mol^{-1} for a wildtype of KIT kinase domain protein and −8.45 to −11.8 kcal mol^{-1} for the mutant D816H of the KIT kinase domain, respectively. The molecular docking study revealed that the binding interactions between amino acid residues and xanthyl chalcone were similar to those of sunitinib. Among the evaluated xanthyl chalcone derivatives, compound **X6** exhibited the lowest binding energy values of −10.9 and −10.7 kcal mol^{-1} for a wild and mutant D816H type of KIT kinase, respectively. Moreover, it was found that the presence of iodine in the xanthone ring stabilized its conformation and provided hydrophobic interactions with amino acid residues of protein KIT. **X6** also showed effective interaction with the activated KIT tyrosine kinase protein (−11.22 kcal mol^{-1}) relative to sunitinib (−8.41 kcal mol^{-1}). **X6** was successfully docked into the 1-(5-ethyl-isoxazol-3-yl)-3-3(4-{2-[6-(4-ethylpiperazin-1-yl) pyryimidin-4-ylamino]-thiazol-5-yl}phenyl)urea-activated KIT tyrosine kinase binding site. Based on the docking results, it can be summarized that **X6** posed in the proteins (wtKIT tyrosine kinase, KIT D816H mutant tyrosine kinase, and activated KIT tyrosine kinase) was predicted to have a high stability interaction in the binding sites. In conclusion, compound **X6** was the most prospective candidate for cancer medication; however, *in vitro* and *in vivo* studies are needed to further confirm its ability.

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CONFLICT OF INTEREST

Authors declared that they have no conflicts of interest.

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