Synthesis, Cytotoxic Evaluation and Molecular Docking of Bromo-Substituted 1,3,6-Trihydroxyxanthone as Protein Tyrosine Kinase Inhibitor

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A series of bromo-substituted hydroxyxanthone were synthesized using bromine in glacial acetic acid as a solvent, producing compounds 3 and 5 with 82.1% and 84.2% yield, respectively. *In vitro* analyses of these two compounds using MMT assay against murine leukemia P388 cell line resulted in IC_{50} of 2,550 and 3,455 μg/mL and selectivity index of 43.21 and 74.40, respectively. These results indicated that the bromo-substituted hydroxyxanthone compounds could potentially be developed as selective and sensitive anti-cancer agents. Furthermore, through the molecular docking conducted using Discovery Studio, compound 3 exhibited interactions between amino acid residues and protein tyrosine kinase (1T46.pdb). This article provides supporting data that xanthone derivatives can be used as drugs for cancer chemotherapy through the mechanism of how this compound can inhibit protein tyrosine kinase.

Key words: Cytotoxic; xanthone; synthesis; docking; MMT assay

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Xanthone is a class of oxygen-containing heterocyclic planar compounds with anticancer activities [1,2]. These biological activities of xanthone are based on their tricyclic scaffold and the nature and/or position of their substituents. Among the substituents that could attach to these compounds are hydroxy, methoxy, phenyl, alkoxy, and halogens. Halogens, such as bromine, are one of the most important substituents often found as an active ingredient in most cancer chemotherapy drugs. Based on the study conducted by Pai *et al*. (2016) on flavanol, it showed that the addition of bromine and chlorine resulted in the increase in the anticancer activity of the compound against Hela and V79 cancer cells [3]. A similar trend was also found with Flavone-4-oximes tested against MCF-7 and Hep-G2 cells [4].

Additionally, previous QSAR analyses revealed that xanthone derivatives with halogen groups, such as bromo or chloro, are active against HepG2 cancer cell line, i.e. at positions 4, 5, and 7 of the xanthone compounds. Also, their cytotoxicity towards HepG2 cancer cell line (IC_{50} value of 0.001-0.484 μM) [5] was lower compared with doxorubicin (IC_{50} value of 3.83 μM). However, xanthones with halogen substituents are not usually available from isolation considering the fact that they are secondary plant metabolites which can be obtained naturally from higher plant families and fungi and bacteria kingdoms. In addition, majority of these compounds have been found in just two families of higher plants formed through mixed biosynthesis pathway, i.e acetate then shikimate pathway [6], through condensation, cyclization, oxidation, and dehydration [7,8]. Hence, synthesis of bromo-substituted hydroxyxanthones was carried out in an attempt to produce xanthone derivatives with halogen substituents, especially for bromo groups in a hydroxyxanthone compound.

Various reagents and reaction conditions have been developed and reported for the bromination of aromatic systems, with the use of different brominating agents, such as $Br_2/SbF_3/HF$ for phenol [9], $NBS/H_2SO_4/CF_3COOH$ [10], NBS/NaOH [11], NBS/NH_4OAc [12], HBr/tert-BuOH, HBr/H_2O_2, and

HBr/DMSO [13], Br_2/C_2-symmetric diphenyl pyrrolidine catalyst [14], $NH4Br/H_2O_2$ in CH_3COOH [15], Br_2/SO_2Cl_2 over microporous catalyst [16], 3-methylimidazolium tribromide [17], NaBr/Oxone®, KBr/Oxone®, KBr/H_2O_2/Oxone® [18], Br_2/tetra butyl ammonium peroxydisulfate [19], quaternary ammonium tribromide [20], and quinolinium bromochromate in glacial acetic acid [21]. However, with respect to the safety side of the reactions, the use of NBS is better than others. Most reactions with NBS are performed to attach bromine in the open chain rings. Furthermore, electrophilic aromatic substitution with bromine is advantageous in terms of regioselectivity, as well as reaction yields. This was proven in a study conducted by Choi and Ma (2007), involving the bromination of 5.6-dimethoxy-, 5,6-dihydroxy-, 5,6-difluoro indan-1-one, and indan-1-one using bromine (Br_2), which resulted in bromo-substituents on aromatic rings, however this did not occur with the use of NBS, KBr, NH_4Br, and pyridinium bromochromate [22].

This study attempted to find a simple and high yield method of brominating 1,3,6-trihydroxy xanthone. The reaction conditions studied involved the use of Br_2 with acetic acid as a solvent, conducted at room temperature considering the fact that hydroxyxanthone with hydroxyl group reacts with bromine without catalyst at room temperature.

Also, the molecular docking studies of hydroxyxanthone derivatives as inhibitors against C-kit Protein Tyrosine Kinase (1T46.pdb) (PTK) as reported by Yuanita *et al*. (2019) [23] were carried out using Discovery Studio 3.1® software package from Accelrys Inc., San Diego, USA.

EXPERIMENTAL SECTION

Materials

The materials used in this experimental study included resorcinol, phloroglucinol, Eaton's reagent (CH_3SO_3H /P_2O_5), Br_2, and glacial acetic acid. All these chemicals were of high grade purchased from Merck and used without any further purification, except Eaton's reagent which was purchased from Sigma Aldrich.

Instrumentation

Infrared spectra were obtained using Shimadzu-Prestige 21 spectrophotometer. ^{1}H-NMR spectra were recorded at 500 MHz with a JEOL Agilent and at 500 MHz with a JEOL JNM-ECA spectrophotometer, using TMS as an internal reference. Mass spectra were measured on Shimadzu QP-2010 MS spectrophotometer.

Procedure

Synthesis of 1, 3, 6-trihydroxyxanthone (2)

All reagents and conditions of synthesis are as shown in Figure 1. Compound **2** was prepared based on the method of Yuanita *et al*. (2018, 2019) [24, 23], with

modifications using the method of Lim *et al*. (2012) [25]. FTIR (KBr, ν; cm-1): 3410 (OH), 1612 (C=O), 1512 (C-C aromatic), 1242 (C-O). ^{1}H-NMR (CD3OD; 500 MHz) δ (ppm): 8.04-8.02 (1H, d, J=8.75 Hz), 6.95-6.92 (1H, dd, J=8,75 Hz and J=2.1 Hz), 6.84 (1H, s, J=2.1 Hz), 6.36 (1H, s, J=2.1Hz), 6.21-6.22 (1H, s, J=2 Hz). MS (EI) m/z: 244 (M+), 215(M-29), 187 (M-57).

Synthesis of 4, 5-dibromo-1, 3, 6-trihydroxyxanthone (3)

Compound 3 was prepared by placing 0.244 gram (1 mmol) of compound 2 in 2 mL of glacial acetic acid in a three-necked round bottom flask. Then, 0.2 ml (1 mmol) of bromine was gradually added to the flask and the mixture was stirred for 2 hours at room temperature. Next, the mixture was diluted with 200 mL of distilled water, after which it was extracted with dichloromethane, added with a saturated NaCl solution. The organic layer was collected and dried with anhydrous $MgSO_4$. Then, the filtrate was evaporated to form compound 3 as a red solid substance with 75.5% yield. FTIR (KBr, ν; cm-1): 3410 (OH), 1612 (C=O), 1512 (C-C aromatic), 1242 (C-O). 688 and 848 (o- and p- directing). ^{1}H-NMR (DMSO; 500 MHz) δ (ppm): 8.10-8.12 (1H, d, J=8.45 Hz), 7.05-7.06 (1H, dd, J=8.45,1.5 Hz), 7.02-7.03 (1H, d, J=1.05 Hz). ^{13}C-NMR (DMSO; 125 MHz) δ (ppm: 102.6 (C-2, C-H), 109.55 (C-4, C-Br),104.21(C-4a,C-H), 104,89 (C-5, C-Br), 104.99 (C-7,C-H), 126.24 (C-9a, C-8, C-H), 127.96 (C-10a,C-5a, C-H), 154 (C-3, C-OH), 156 (C-6,C-OH), 165(C-1,C-OH), 180 (C=O). MS (EI) m/z: 402 (M+), 322 (M-80), 242 (M-80-80).

Synthesis of 7-bromo 1, 3, 6-trihydroxyxanthone (5)

A mixture of 0.06 mol of compound 4, 0.06 mol of phloroglucinol, 50 mL of chloroform, and 10 mL of Eaton's reagent was refluxed for 3 hours. The mixture was cooled to room temperature through the addition of cool water. This mixture was then stirred and extracted with chloroform. Also, the organic layer was added with 10% $NaHCO_3$ and washed with water. Next, the solution was dried with anhydrous Na_2SO_4 and concentrated. Finally, the crude product was purified by recrystallization in ethanol and water, thereby resulting in compound 5, a yellow solid with 84.2% yield%. ^{1}H -NMR (DMSO; 500 MHz) δ (ppm):

8.11-8.12 (1H, d, J=8.7 Hz), 7.06 (1H, s), 7.04 (1H, s), 7.03 (1H, s). ^{13}C-NMR (DMSO; 125 MHz) δ (ppm: 96.4 (C-2, C-H), 134 (C-8, C-H),94.2(C-4,C-H), 107,1 (C-5, C-H), 104.99 (C-7,C-H), 115 (C-9a; C-1a), 156 (C-10a,C-5a), 163 (C-3, C-OH), 157 (C-6,C-OH), 163(C-1,C-OH), 179 (C=O). MS (EI) m/z: 322 (M+), 242(M-80).

Cytotoxic Evaluation

The MTT assay method was used in analyzing the xanthones' (compounds 3 and 5) *in vitro* anticancer activity. This was performed by following the method of Mossmann T [26]. To carry out the analysis, murine leukemia P388 cell line [ex. HSRRB Lot Number: 113098 seed (JCRB0017)] was used. The initial cell line was obtained from Natural Organic Chemical Laboratory of Natural Material, Chemical Department of Institut Teknologi Bandung.

Molecular Docking

In this research, the molecular docking studies of compound **3** activities in inhibiting protein tyrosine kinase (PTK) were performed. This is one of the common strategies used in treating proliferative diseases, including cancer, i.e the inhibition of PTK, since it works on the cell survival and proliferation stage. Also, the activities of compound 3 were compared with that of STI571 ligand (imatinib) as a native ligand of C-kit receptor Protein-Tyrosine Kinase (code: 1T46.pdb). In addition, docking simulations have been conducted in previous works (23; 27-28) under the receptor-ligand interaction section using the Discovery Studio 3.1 from Accelrys Inc., San Diego, CA, USA. CHIMERA 1.9 and ChemOffice®2015 softwares were also used to study the molecular modeling of the compound.

RESULTS AND DISCUSSION

Synthesis

For the first pathway, xanthone building block 2 was synthesized based on the method of Grover, Shah, and Shah, as reported by Yuanita *et al*. [23,24] to produce 1,3,6-tryhydroxyxanthone, as shown in Figure 1.

Synthesis of 4,5-dibromo-1,3,6-trihydroxyxanthone (3)

In this study, brominating was carried out using Br_2 in glacial acetic acid. Firstly, glacial acetic acid was reacted and then followed by the addition of bromine, which functioned as an electrophile. Compound 3 possessed 3 hydroxyl groups, which were electron-donating substituents. This allowed non-bonding lone pair electrons of oxygen to have higher-energy HOMO than low-energy bonding electrons in the benzene ring, which created partial negative charge rings and led to substitutions at ortho and para positions [29]. This was due to the fact that electron density is mainly located at the ortho and para carbon atoms on the π system of hydroxyxanthone, which means that no substitution would occur in meta position towards hydroxyl group, as shown by the mechanism of electrophilic substitution in Figure 2. Also, the bromination reaction carried out at room temperature caused the brominated substitution to be a direct substitution rather than a reaction with the formation of bromine radicals.

To clearly understand the structure, IR spectrum was used. Substitutions at -o and -p of the aromatic were represented by fingerprint vibrations at 680-800 cm^{-1}. The presence of bromo substitutions was evident from absorption at 688 and 848 cm^{-1}, which was different from the IR spectrum of compound 2. In addition, MS spectrum was used in studying the structure of compound 2. The molecular mass of 402 was indicated as the molecular ion, as well as the base peak. This differed from that of compound 3 of the molecular mass of 244, due to the presence of two bromine atoms. This was an indication that the substitutions of the two bromo atoms had been successful.

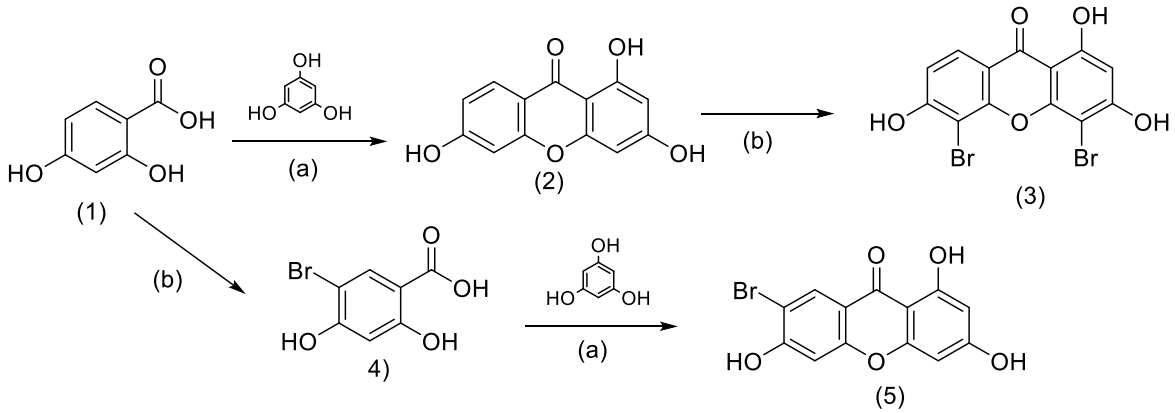

Figure 1. Reagents and conditions of synthesis: (a) Eaton's reagent, reflux, 80°C, 3 h; (b) Br_2/CH_3CO_2H, 2 h, r.t.

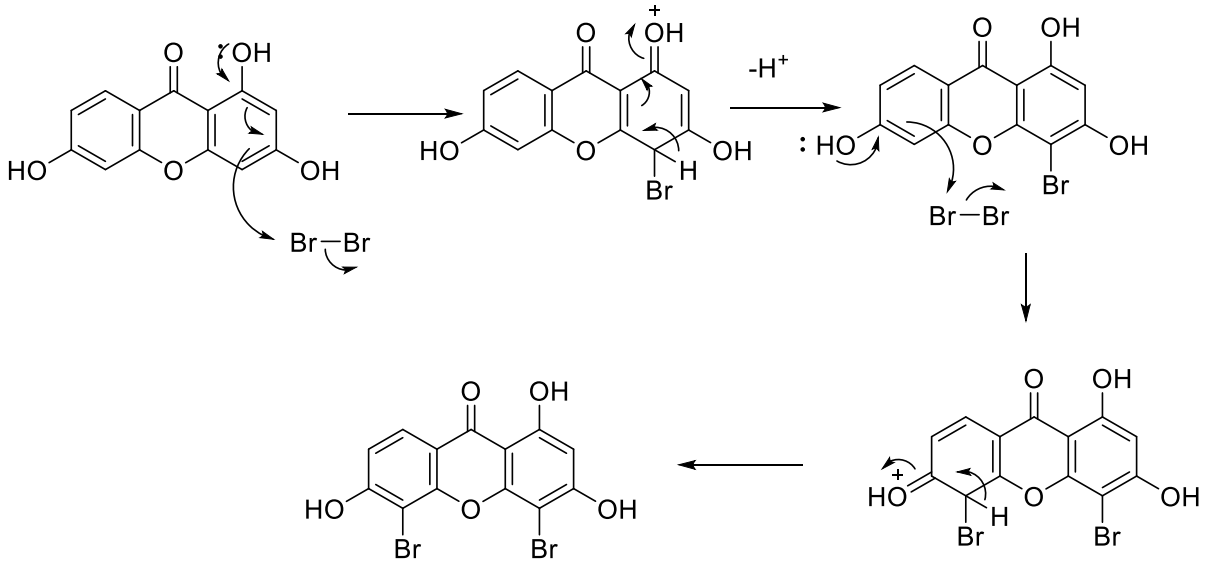

Figure 2. Proposed mechanism of bromination of 1,3,6-trihydroxyxanthone

Based on the position of the substitute-bromo in xanthone, the clear explanation of its structure was performed using ^{1}H-NMR, which gave compounds 2 and 3 different spectra. Additionally, in theory, the bromides were bonded at positions 4 and 5 of the xanthone ring, which was the o-position from positions 3-OH and 6-OH. Also, the ^{1}H-NMR spectrum showed that the product had 3 protons, which were not equivalent to each other. Signals δ_H 8.10-8.12 ppm (d, 1H, *J*=8.45 Hz), 7.05-7.06 ppm (1H, dd, *J*=8.45,15 Hz), and 7.02-7.03 ppm (1H, d, *J*=1.05 Hz). The spectrum was different from that of compound 2, which showed five proton signals in the aromatic region, δ_H 6.21 -6.22 (1H, d, *J* = 2.1 Hz), 6.36 (1H, d, *J* = 2.1 Hz), 6.84 (1H, d, *J* = 2.1 Hz), 6.95-6.92 (1H, dd, *J* = 8.75, 2.1 Hz), and 8.04 – 8.02 (1H, d, *J* = 8.75 Hz). The presence of a doublet at δ 6.90 was assigned to proton H-7, which was meta-coupled (*J* = 2.1 Hz) with proton H-5 and ortho-coupled (*J* = 8.75 Hz) with proton H-8. Based on FT-IR, ^{1}H-NMR and MS analyses, it could be concluded that compound 3 was successfully synthesized.

Synthesis of 7-bromo-1,3,6-trihydroxyxanthone (5)

Compound 1 had a carboxyl group and a hydroxyl group at the o-position as well, however they were of different directing groups. The existence of two different groups usually leads to the activation of an o-/p- directing group and the deactivation of the m-position. Hydroxyl group is a strong activator of o-/p-directing, hence the result of bromination of compound 1 would produce compound 4 in the form of a white solid substance with the melting point in the region of 188-189°C at 73.8% yield. To clearly explain the structure, compound 4 was analyzed by direct MS. Based on the results of the MS analysis, a molecular weight of 231 was obtained, which became the base peak, and this molecular mass shows the difference of 79 g/mol of bromo from the molecular mass of 2,4-dihydroxybenzoic acid, which is 154.

Table 1. Anticancer activity (IC_{50}) of P388 cell line and Selectivity Index (SI)

Compound	IC_{50}P388	IC_{50}VERO	SI
4,5-dibromo-1,3,6-trihydroxy-9H-xanthen-9-one **(3)**	2.550	110.21	43.21
7-bromo-1,3,6-trihydroxy-9H-xanthen-9-one **(5)**	3.455	257.06	74.40
Artonin E**	0.800		

* Determined as Origin 8 program.
** Standard

Also, the cyclodehydration of compound 4 with phloroglucinol in Eaton's reagent produced compound 5. The structural explanation of compound **5** was achieved through MS, NMR, and ^{13}C-NMR. The MS analysis result showed a base peak of 322 a.m.u and this was different from that of compound 2 by one atom of bromo with a molecular mass of 244. This indicated that only one bromide was substituted in the xanthone compound.

Another structural explanation conducted through ^{1}H-NMR showed that the product had 4 protons which were not equivalent to each other. The proton shift shows 8.11-8.12 (doublet, 1H), 7.06 (singlet, 1H), 7.04 (singlet, 1H), and 7.03 (singlet, 1H). Based on these, it could be concluded that compound 5 with bromide substitution was successfully formed.

Also, another derivative of xanthone could be synthesized from compound 4 and the method used was the same procedure. Generally, the mechanism of forming the xanthones was the same, but with a difference in the number of bromo substituted. The first pathway produced xanthone 3 with two bromides attached to the xanthone ring. However, the second pathway produced only one bromide attached to the xanthone ring.

Cytotoxic Evaluation

MMT assay is a colorimetric cytotoxic test used to determine the number of living cells based on discoloration in MTT solution, changing from yellow to purple. The intensity of this purple color is directly proportional to the amount of inactive cells. Also, the darker of the color, the higher of the absorbance value of the living cells [30]. Compounds **3** and **5** appeared to be potential anticancer compounds with excellent inhibition concentration (IC_{50}) values of 2.550 and 3.455 µg/mL, respectively, as shown in Table 1. The criterion of strong cytotoxicity for a crude extract, which was established by the U.S. National Cancer Institute, is $IC_{50} < 20$ µg/mL in the preliminary assay [29].

Based on the IC_{50} value, all series of xanthone derivatives have the potential to be developed into chemotherapy agents. This is due to the fact that synthesized compounds with $IC_{50} < 4$ µg/mL could be considered for evaluation as chemotherapy agents in pre-clinical studies using animals [31-32]. However, there is a need for further studies of cytotoxic activities of xanthones as chemotherapy agents. The cytotoxic anticancer drugs have multiple targets and modes of action, but a common motif is that they inflict damage on the cells to activate the intrinsic pathway of apoptosis. While different cell types may respond more or less readily to such treatment, the apoptotic pathway itself is functional in all cells, and it is usually not possible to limit the cytotoxic effect on specific cell types. Therefore, the presence of bromo substituents in compounds **3** and **5** could specifically inhibit the apoptosis pathway.

Furthermore, selectivity analysis was performed by comparing IC_{50} of each compound to those of normal cells (VERO cell line) and the tested murine leukemia P388 cell line. Theoretically, when a selectivity index (SI) value is greater than 3, it is an indication that the compound has a high selectivity toward certain cancer cells [33-34]. From this study, SI values of the xanthone derivatives, i.e, compounds **3** and **5,** are presented in Table 1. The results showed that the prepared bromo-substituted hydroxyxanthones **3** and **5** have the potential to be developed as anticancer compounds, based on their lower toxicity and higher sensitivity.

Molecular Docking

When tested against 1T46.PDB protein target using molecular docking, compound **3** showed a satisfying anticancer activity. The cDOCKER interaction energy obtained from the process was -30.82 kcal/mol, while the bond lengths between hydrogen bond and amino acids was around 1.99 – 4.83 Å. This means that energy of compound 3 was much lower compared to that of ST1571 ligands at -79.38 kcal/mol, and also the bond length of the ligands' hydrogen bonds was between 2.41 – 4.79 Å. Nonetheless, the *in vitro* anticancer activity assay of compound 3 showed that the compound is a great anti-cancer agent against P388 murine leukemia cell line. This could be explained by the fact that compound 3 and amino acids were involved in binding interactions similar to each co-crystallized ligand as shown in Figure 3.

The molecular binding studies conducted on compound 3 revealed it was in binding interactions with some of the amino acid residues, such as Asp810, Cys809, Ile789, His790, and Leu644 of 1T46.pdb protein. This same set of amino acid residues were also known to have binding interactions with co-crystallized ST1571 ligands. Consequently, compound 3 and co-crystallized STI571 ligands have similar binding pockets. Thus, the anticancer activity of the experimental results was proven using *in silico* molecular docking studies, specifically against P388 murine leukemia cell line.

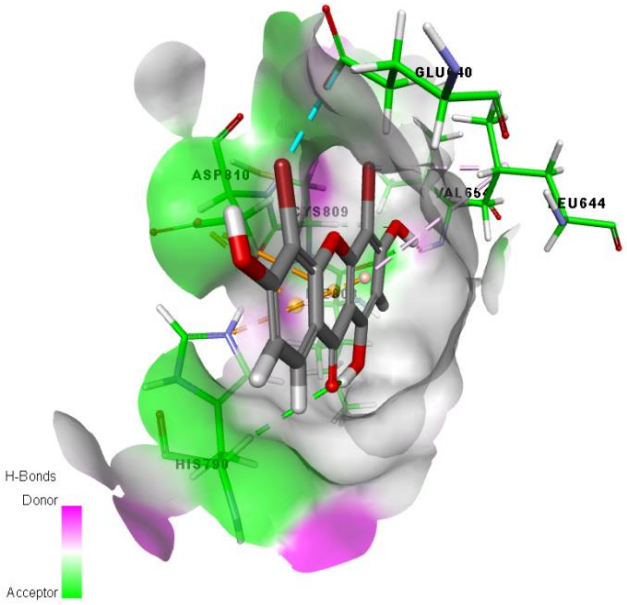

Figure 3. 3D-predicted binding mode from docking simulation of compound **3** into the active site of c-kit Protein Tyrosine Kinase (1T46.pdb)

The molecular docking in this study was used to predict and comprehend the active site mechanism and the fitness of active compounds or ligands with the residues of the amino acid target protein. The fitness between compounds and amino acid residues can be seen from the way they interact with each other. When the interaction produces low energy, the ligand-protein bond that exists is more stable. The energy is known as cDOCKER energy in the Discovery Studio® software. Table 3 shows that the binding interactions of compound 3 with amino acid residues, namely Asp810, Cys809, Ile789, His790, and Leu644 of 1T46.pdb protein were similar with those of STI-571 C-kit protein tyrosine kinase (IT46.pdb). This similarity was also found when other compounds were tested against cancer cells with amino acid residues. For instance, a study conducted by Shrestha *et al*. (2008) [35] revealed a binding interaction of deazaflavin-cholestane with Asp680, Lys593, Leu595, Asp677, Val603, Gly676, Leu799, Tyr672, Ala621, and Phe811. The latest compound was tested against some cancer cells such as A549, HepG2, HCT116, CCRF-HSB-2, MCF7, and KB tumor cells. It was discovered that compound 3 works as an anti-cancer agent by inhibiting protein tyrosine kinase.

Generally, the stability of ligands in molecular docking is characterized by the low cDOCKER energy and how short the bond length formed. From Table 3, the cDOCKER energy for compound 3 was 30.82 kcal/mol, with bond lengths of 1.99 – 4.83 Å, which were lower and shorter compared to that of STI571 ligand of 79.38 kcal/mol and bond length of 2.41 – 4.79 Å [23]. Also, lower than Artonine E, a known active compound that has been shown to have several activities including strong cytotoxic effects on 11 types of tumor cells and in the mechanism Artonin E has higher affinity for FAT10 protein, where FAT10 gene is involved in cell-cycle regulation and modulator of tumor genesis [36] and induced apoptosis and cell cycle arrest at the S phase [37]. Based on the binding activity that is indicated the compound involved the cell cycles by a regulatory protein. One of the regulatory proteins is protein tyrosine kinase (PTK). In addition, the comparison of compound 3 energy was made with the energy of 2-amino-4-phenyl-5-methyl thiazol complex with some metals such as Cu, Zn, Ni, and Co (<20 kcal/mol) [38] and the energy of deazaflavin derivative (<18 kcal/mol) [39]. These energy data show that compound 3 has a high potential to be used as an anti-cancer compound as it was more stable compared to other tested compounds for the low energy it possessed.

CONCLUSION

Compounds **3** and **5** tested *in vitro* have been proven to have excellent inhibition and high selectivity index against P388 murine leukemia cells. Also, *in silico* studies showed that STI571 (1T46.pdb) protein and compound **3** have a binding interaction. This is an indication that an active chemotherapy site is available on compound **3**, which makes it act as an inhibitor of protein tyrosine kinase (PTK), an enzyme responsible in controlling and regulating phosphorylation and various cellular functions.

Table 3. Energy, bond length of hydrogen bond and binding interaction of compound 3

Compound	cDOCKER Energy (kcal/mol)	Binding Interaction (amino acid residue)	Hydrogen bond length (Å)
3	-30.82	His790	4.83
		Cys809	2.59
		Leu644	2.39
		Ile789	1.99
		Asp810	4.11
Artonine E	143.76	His 790	3.85
		Cys809	5.30
		Asp810	2.50
		Val654	2.56
		Leu644	5.28

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REFERENCES

1. Aisha, A. F. A., Abu-Salah, K. M., Ismail, Z., Majid, A. M. A. A (2012) In Vitro and In Vivo Anti-Colon Cancer Effects of Garcinia Mangostana Xanthones Extract. *BMC Compl. Alter. Med.*, **12**, 104.

2. Matsumoto, K., Akao, Y., Kobayashi, E., Ohguchi, K, Ito, T., Tanaka, T., Linuma, M., Nozawa, Y. (2003) Induction of Apoptosis by Xanthones from Mangosteen Human Leukemia Cell Lines. *J. Nat. Prod.*, **66**, 1124–1127.

3. Pai, A., Kumar, D. V., Jayashree, B. S. (2016) Synthesis, Characterization, Antibacterial and Anticancer Evaluation of Some Novel Flavone-3-ols. *Asian J. Pharm. Sci.*, 187–188.

4. Jayashree, B. S., Gurushyam, S., Pai A. (2016) Synthesis, Characterization, Antioxidant and Anticancer Evaluation of Some Novel Flavone-4-oximes. *Asian J. Pharm. Sci.*, 185–186.

5. Yuanita, E., Pranowo, H. D., Jumina, Mustofa (2016) Study on Anticancer Activity of Xanthones Derivatives Using Quantitative Structure-Activity Relationship (QSAR). *Asian .J Pharm.Clin. Res.*, **9(2)**, 180–185.

6. Masters, K. S. and Brsee, S. (2012) Xanthones from fungi, lichens, and bacteria: the natural products. *Chem. Rev.*, **112**, 3717−3776.

7. Bennett, G. J., Lee, H. H., Nagaratnam, P. D. (1990) Biosynthesis of Mangostin. Part 1: The origin of the xanthone skeleton. *J. Chem*. Soc., *Perkin Trans. 1*., **1(10)**, 2671–2676.

8. Dewick, P. M. (1998) The biosynthesis of shikimate metabolites. *Nat. Prod. Rep.*, **15(1)**, 17–58.

9. Jacquesy, J., Jouannetaud, M., Makani, S. (1980) Meta-Bromination of Phenols in Superacids. *J. Chem. Soc. Commun.*, 110–111.

10. Duan, J., Zhang, L. H., Dolbier, W. R., Jr, A. (1999) Convenient New Methods for The Bromination of Deactivated Aromatic Compounds. *Synlett.*, 1245–1246.

11. Auerbach, J., Weissman, S. A., Blacklock, T. J. (1993) Bromosuccinimide/Dibromodimethyl

hydantoinin Aqueous Base: A Practical Method for The Bromination of Activated Benzoic Acids. *Tetrahedron Lett.*, **34**, 931–934.

12. Tanemura, K., Suzuki, T., Nishida, Y., Satsumabayashi, K., Horaguchi, T. (2003) Halogenation of aromatic compounds by N-chloro, N-bromo, and N-iodosuccinimide. *Chem. Lett.*, **32**, 932–933.

13. Barhate, N. B., Gajare, A. S., Wakharkar, R. D., Bedekar, A. V. (1998) Simple and Efficient Chlorination and Bromination of Aromatic Compounds Using Aqueous TBHP (or H_2O_2) and a HydrohalicAcid. *Tetrahedron Lett.*, **39**, 6349–6350.

14. Bertelsen, S., Halland, N., Bachmann, S, Marigo, M., Braunton, A., Jorgensen, K. A. (2005) Organo catalytic Asymmetric α-bromination of Aldehydes and Ketones. *Chem. Commun*., 4821–4823.

15. Mohan, K. V. V. K., Narender, N., Srinivasu, P., Kulkarni, S. J., Ragavan, K. V. (2004) Novel Bromination Method for Aniline and Anisole Using NH_4Br/H_2O_2 in CH_3COOH. *Synth. Commun.*, **34**, 2143–2152.

16. Gnaim, J. M. and Sheldon, R. A. (2005) Regioselective Bromination of Aromatic Compounds with Br_2/SO_2Cl_2 Over Microporous Catalysts. *Tetrahedron Lett.*, **46**, 4465–4468.

17. Chiappe, C., Leandri, E., Pieraccini, D. (2004) Highly Efficient Bromination of Aromatic Compounds Using 3-Methylimidazolium Tribromideas Reagent/Solvent. *Chem. Commun.*, 2536–2537.

18. Narender, N., Sriniva, P., Ramakrishna, M., Kilkarmi, S. J., Reghaven, K. V. (2002) An Efficient andRegioselectiveOxybrominationof Aromatic Compounds Using Potassium Bromide and Oxone®. *Synth. Commun.*, **32**, 2313–2318.

19. Doyle, M. P., Van Lente, M. A., Mowat, R., Fobare, W. F. (1980) Alkyl Nitrite-Metal Halide Deamination Reactions. 7. Synthetic Coupling Of ElectrophilicBrominationwith Substitutive Deamination. *J. Org. Chem.*, **45**, 2570–2575.

20. Park, M. Y., Yang, S. G., Jadhav, V., Kim, Y. H. (2004) Practical and Regioselective Brominations of Aromatic Compounds Using Tetrabutyl ammonium Peroxydisulfate. *Tetrahedron Lett.*, **45**, 4887–4890.

21. Ozgun, B., Degirmenbasi, N. (1996) QuinoliumBromochromate—A New Reagent For Bromination and Oxidation. *Synth. Commun.*, **26**, 3601–3601.

22. Choi, T. and Ma, E. (2007) Simple and Regioselective Bromination of 5,6-Disubstitutedindan-1-Ones with Br_2 Under Acidic and Basic Conditions. *Molecules*, **12**, 74–85.

23. Yuanita, E., Pranowo, H. D., Mustofa, Swasono, R. T., Syahri, J., Jumina (2019) Synthesis, Characterization and Molecular Docking Chloro-Substituted Hydroxyxanthone Derivatives. *Chem. J. Mold.*, **14(1)**, 68–76.

24. Yuanita, E., Pranowo, H. D., Siswanta, D., Swasono, R. T., Mustofa, Zulkarnain, A. K., Syahri, J., Jumina (2018) One-Pot Synthesis, Antioxidant Activity and Toxicity Evaluation of Some Hydroxyxanthones. *Ch&ChT*, **12(3)**, 290–295.

25. Lim, C. K., Tho, L. Y., Lim, L. M., Shah, S. A. A. and Weber, J. F. F. (2012) Synthesis of 1,3,6-Trioxygenated Prenylated Xanthone Derivatives as Potential Antitumor Agents. *Lett. Org. Chem.*, **9(8)**, 549–555.

26. Mosmann, T. (1984) Rapid Colorimetric Assay for Cellular Growth and survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol Methods*., **65**, 55–63.

27. Syahri, J., Yuanita, E., Nurohmah, B. A., Wathon, M. H., Syafri, R., Armunanto, R., Purwono, B. (2017) Xanthone as Antimalarial: QSAR analysis, synthesis, molecular docking and *in-vitro* antimalarial evaluation. *Orient. J. Chem.*, **33(1)**, 29–40.

28. Syahri, J., Rullah, K., Armunanto, R., Yuanita, E., Nurohmah, B. A., Mohd Aluwi, M. F. F., Wai, L. K., Purwono, B. (2017) Synthesis, biological evaluation, QSAR analysis, and molecular docking of chalcone derivatives for antimalarial activity. *Asian. Pac. J. Trop. Dis.*, **7(1)**, 9−14.

29. Clayden, J., Greeeves, N., Warren, S. (2001) *Organic Chemistry, Second Edition, Oxford University Press*, 479–481.

30. Suffnessn, M. and Pezzuto, J. M. (1990) Assays related to cancer drug discovery, in Methods in Plant Biochemistry: Assays for Bioa. *Academic Press, London, UK*.

31. Tan, G., Gyllenhal, C., Soejarto, D. D. (2006) Biodiversity as a source of anticancer drugs. *Curr. drug targets*., **7**, 265–277.

32. Likhitwitayawuid, K., Angerhofer, C. K., Cordell, G. A., Pezzuto, J. M., Ruangrungsi, N. (1993) Cytotoxic and antimalarial bisbenzyliso quinoline alkaloids from *Stephaniaerecta*. *J. Nat. Prod.*, **56(1)**, 30–38.

33. Prayong, P., Barusrux, S., Weeraapreeyakul, N. (2008) Cytotoxic activity screening of some indigenous Thai Plants. *Fitoterapia*, **79**, 598–601.

34. Mahavorasirikul, W., Viyanant, V., Chaijaroenkul, W., Itharat, A., Na-Bangchang, K. (2010) Cytotoxic activity of Thai Medicinal Plants against human cholangiocarcinoma, laryngeal and Hepatocarcinoma cell in vitro. *BMC Compl. Alt. Med.*, **10**, 55.

35. Shrestha, A. R., Shindo, T., Ashida, N., Nagamatsu, T. (2008) Synthesis, biological active molecular design, and molecular docking study of novel deazaflavin–cholestane hybrid compounds. *Bioorg. Med. Chem.*, **16(18)**, 8685–8696.

36. Chaturvedi, I. (2015) A Molecular Docking study to find Natural Inhibitor Against FAT10 Protein for curing Hepatic Carcinoma. *Journal Of Science*, **1(2)**.

37. Rahman, M. A., Ramli, F., Karimian, H., Dehghan, F., Nordib, N., Ali, H. M., Mohan, S., Hashim, N. M. (2016) Artonin E Induces Apoptosis via Mitochondrial Dysregulation in SKOV-3 Ovarian Cancer Cells. *PLOS ONE*, **11(3)**.

38. Abd-Elzaher, M. M., Labib, A. A., Mousa, H. A., Moustafa, S. A., Ali, M. M., El-Rashedy, A. A. (2016) Synthesis, anticancer activity and molecular docking study of Schiff base complexes containing thiazole moiety. *Beni-Suef University Journal of Basic and Applied Science*, **5(1)**, 85–96.

39. Shrestha, A. R., Ali, H. I., Ashida, N., Nagamatsu, T. (2008) Antitumor studies. Part 5: Synthesis, antitumor activity, and docking molecule study of 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins. *Biorg. Med. Chem.*, **16**, 9161–9170.