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SYNTHESIS, CHARACTERIZATION AND MOLECULAR DOCKING OF CHLORO-SUBSTITUTED HYDROXYXANTHONE DERIVATIVES

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Abstract. Xanthone compounds are of great interest due to their wide range of biological applications. Xanthone compounds can be obtained by structural modification of the substituent on the xanthone rings through various reactions. In this study, the chloro-substituted hydroxyxanthones (**4a-c**) were prepared by cyclodehydration of acid derivatives and substituted phenol in the presence of Eaton reagent to afford **3a-c**, followed by halogenation step to electrophilic substitution of chlorine in a moderate yield. The *in vitro* anticancer activity study on various cell lines showed that there was an enhanced activity of compounds **4a-c** in comparison to **3a-c**. It has been shown that compounds **4a-c** have the best anticancer activity only toward P388 murine leukaemia cells with IC_{50} of 3.27, 1.809 and 0.18 μg/mL, respectively. The results revealed that the chloro functional group increases the anticancer activity of the hydroxyxanthone derivatives. As for the selectivity index, the number was increased from a range of 0.88-843 (**3a-c**) to 3.33-9199.67 (**4a-c**). This result indicates that the hydroxyxanthone derivatives (**4a-c**) have potential to be developed into chemotherapy agents due to their higher sensitivity and selectivity. Molecular docking studies showed that there was a binding interaction between **4c** and the amino acid residues such as Asp810, Cys809, Ile789, His790, and Leu644 of protein tyrosine kinase receptor (PDB ID: 1T46).

Keywords: chlorination, chloro-substituted hydroxyxanthone, derivative, anticancer, molecular docking.

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Introduction

Xanthone compounds possess significant biological properties, including analgesic [1], antioxidant [2], antibacterial [3], anti-tuberculosis [4], antifungal [5], anti-inflammatory [6], and also anticancer [7-8]. A wide range of biological activities of xanthone compounds can be obtained by structural modification of the substituent on the xanthone rings through various reactions. Previous studies reported by Su, Q.G., *et al.* showed that the position of the OH groups, as well as the number of OH groups attached to the xanthone scaffold can determine the anticancer activity (IC_{50} value) [9]. Comparison of the anticancer activity of 1,6-dihydroxyxanthone and 1,3-dihydroxyxanthone showed different IC_{50} values of 40.45 and 71.36 μM, respectively. Likewise, the increased number of OH group attached to the xanthone compounds, such as 1-hydroxyxanthone and 1,3,6,8-tetrahydroxyxanthone, showed a significant difference in IC_{50} values of 85.32 and 9.18 mM [9]. Another research study reported that there was attested an increasing cytotoxic activity of a series of chloro and bromo flavanols against HeLa and V79 cell lines [10] and also on halogenated flavone-4-oximes derivatives against MCF-7 and Hep-G2 [11]. Halohydrin (bromo and chloro) xanthones were also reported to be the most effective inhibitors of Topo II [12]. The previous QSAR analysis studies showed that the addition of halogen groups such as chloro and bromo could increase the anticancer activity of the xanthones substituted chloro and bromo compounds in the range of 0.001–0.484 μM against HepG2 cell line [13].

Chlorination of aromatic compounds can be carried out through various reagents and

conditions such as Cl_2 for nitrophenol [14], H_2O_2−HCl for arenes, alkenes, and alkyne [15], hydrocarbon and naphtol [16], methyl phenol [17], $SnCl_4$/$Pb(OAc)_4$ [18], $InCl_3$/NaClO [19], For high selectivity and safety regulation purposes, N-chlorosuccinimide (NCS) is commonly used as chlorination reagent [20-26], which more recently was used along with NaCl/*p*TsOH in a water system [27]. According to the literature study, chloro-substituted hydroxyxanthone compounds have not been reported yet. Therefore, this study aimed to synthesize and develop novel chloro-hydroxyxanthone compounds and to investigate the *in vitro* anticancer activity towards various cell lines. A molecular docking study has also been reported to validate the mechanism of drug interaction with the binding site of an active compound. Molecular docking has become a focus of attention in recent years for its successful application in finding candidates for new drugs from some complex compounds, such as prenylated xanthone [28]; 1,2-dihydroxy-6-methoxyxanthone-8-O-*β*-D-xylopyranosyl [29]; hydroxyxanthone for malaria activity [30]. Thus, additionally this study presents molecular docking of hydroxyxanthone derivatives into inhibitor C-kit protein tyrosine kinase (PTK) (PDB ID: 1T46) using Discovery Studio 3.1® software package (Accelrys, Inc., San Diego, USA). Inhibitors that block the activity of PTK and its activated unregulated signalling pathways can provide a useful basis for designing new drug candidates because drugs as PTK inhibitors such as imatinib have severe toxic effects including cardiac toxicity. Thus, the development of newer drug molecules with lower toxicity and better oral bioavailability requires special novel compounds based on xanthone derivatives.

Experimental

Chemistry

All reagents were purchased from Sigma-Aldrich, Acros, and Merck with high grade of purity and used without any further purification. All solvents used in the synthesis were of analysis and synthesis grade. The solvents used in spectroscopic measurements were of spectroscopic grade.

The *melting point* of the synthesized compounds was determined by Electrothermal 9100 with a temperature gradient of 10 °C/min.

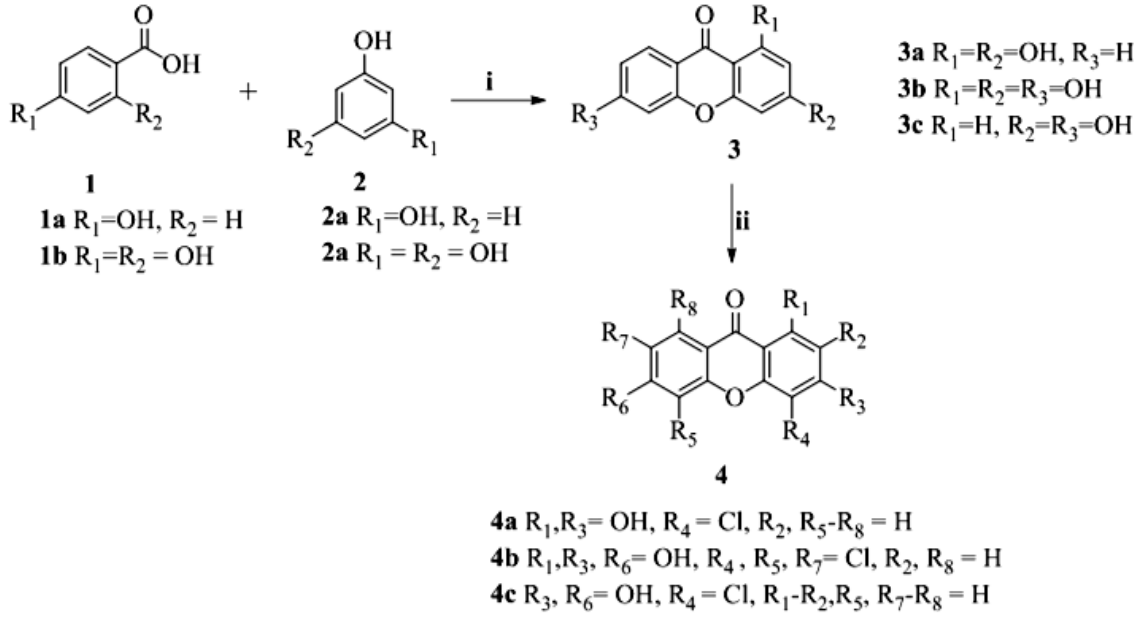
Infrared spectra were obtained on a Shimadzu FTIR-8201 PC spectrometer.

The *^1H and ^{13}C NMR spectra* were recorded on a JEOL 500 MHz Spectrometer with tetramethylsilane as an internal standard.

Mass spectroscopy spectra were recorded on Shimadzu-QP 2010S.

General procedure for synthesis of hydroxyxanthone compound (3a-c)

As show in Scheme 1, the synthesis of hydroxyxanthones **3a-c** were carried out by reacting the compound **1** (10.2 mmol) with phenol derivatives **2** (10 mmol) and Eaton's reagent (5 mL) in one pot reaction, according to the method described by Yuanita *et al.* [31].



Scheme 1. General procedure for synthesis of hydroxyxanthone compounds and their chloro-substituted derivatives. Reagents and conditions of synthesis:
***(i)* Eaton reagent, reflux 80ºC, 3 h;**
***(ii)* NCS/*p*-TsOH/NaCl/EtOH, 40ºC, 1 h.**

The mixture was heated under reflux at 80±3ºC for 3 h. After the completion of the reaction, the mixture was cooled to room temperature, poured into cold water, and stirred for 1 h. The precipitate formed was filtered and washed with water and 5% $NaHCO_3$, then dried over desiccator to afford the desired product.

1,3-dihydroxy-9H-xanthen-9-one (**3a**), red solid (85.5%), m.p.: 223-223.8°C. FTIR (KBr, *v*, cm^{-1}): 3448 (OH), 1612 (C=O), 1458 (C-C aromatic), 1296 (C-O-C). ^{1}H-NMR (CD_3OD; 500 MHz) *δ* (ppm): 6.20 (1H, d, *J*= 2.00 Hz and *J*= 1.50 Hz), 6.36 (1H, dd, *J*= 8.00 Hz and *J*= 3.75 Hz), 7.49 (1H, dd, *J*= 8.00 Hz and *J*= 3.75 Hz), 7.40 (1H, dd, *J*= 8.50 Hz and *J*= 1.5 Hz), 7.74 (1H, dd, *J*= 8.50 Hz and *J*= 1.50Hz), MS (EI) *m/z*: 228 (M^{+1}).

1,3,6-trihydroxy-9H-xanthen-9-one (**3b**), light yellow solid (81.96%), 322-323°C. FTIR (KBr, *v*, cm^{-1}): 3163 (OH), 1612 (C=O), 1465 (C-C aromatic), 1296 (C-O). ^{1}H-NMR (DMSO-d_6; 500 MHz) *δ* (ppm): 8.75 (1H, dd, *J*= 8.75 Hz and *J*= 2.10 Hz) 8.02- 8.04 (1H, dd, *J*= 8.75 Hz and *J*= 2.10 Hz), 6.84 (1H, s, *J*= 2.10 Hz), 6.36 (1H, s, *J*= 2.10 Hz), 6.20-6.22 (1H, s, *J*= 2.10 Hz). MS (EI) *m/z*: 244 (M^+).

3,6-dihydroxy-9H-xanthen-9-one (**3c**), reddish solid (70.15%), m.p.: 220-223°C. FTIR (KBr, *v*; cm^{-1}): 3248 (OH), 1620 (C=O), 1458 and 1512 (C-C aromatic), 1242 (C-O-C). ^{1}H-NMR (CD_3OD; 500 MHz) *δ* (ppm): 7.49 (2H, d, *J*= 8.7 Hz), 6.48 (2H, d, *J*= 8.7 Hz and 2.35 Hz), 6.43 (2H, d, *J*= 2.35 Hz). MS (EI) *m/z*: 228 (M^+).

General procedure for synthesis of chloro-substituted hydroxyxanthone (4a-c)

The preparation of compounds **4a-c** (Scheme 1) was conducted according to the method of Mahaja, T. *et al*. [27], with the difference that the water solvent was replaced with ethanol. Ethanol (8 mL) was added to a finely crushed powder of xanthone (**3a-c**) (0.01 mol) in a 100 mL round-bottom flask with a magnetic stirring bar at room temperature. To the mixture, NaCl (0.015 mol), *p*-toluenesulphonic acid (*p*-TsOH) (0.01 mol), and *N*-chlorosuccinimide (NCS) (0.01 mol) were added while stirring at 40ºC. The reaction was monitored by thin layer chromatography. After the completion of the reaction, 5 mL of water was added to separate the precipitated mass, and the precipitate was filtered and dried in an oven to afford the compounds **4a-c** as powder products.

4-chloro-1,3-dihydroxy-9H-xanthen-9-one (**4a**) orange solid (81.5%), m.p.: 212.2-215.5°C, FTIR (KBr, *v*, cm^{-1}): 3448 (OH), 1612 (C=O), 1458 (C-C aromatic), 1296 (C-O-C), 887 (*o/p*-substituted chloro). ^{1}H-NMR (CD_3OD; 500 MHz) *δ* (ppm): 6.54 (1H, s), 7.55 (1H, d, *J*= 8 Hz), 7.67 (2H, t), 7.92 (2H, t), 8.25 (1H, d *J*= 8 Hz). ^{13}C-NMR (CD_3OD; 125 MHz) *δ* (ppm): 99.18; 99.24; 104.40; 118.77; 121.04; 121.04; 125.25; 126.45; 136.75; 153.97; 156.75’ 163.81; 181.42 (C=O). MS (EI) *m/z*: 262/264 (M^+).

4,5,7-trichloro-1,3,6-trihydroxy-9H-xanthen-9-one (**4b**) bright yellow solid (65.5%). FTIR (KBr, *v*, cm^{-1}): 3425 (OH), 1620 (C=O), 1465 (C-C aromatic), 1280 (C-O), 802, 840 (*o/p*-substituted chloro); ^{1}H-NMR (DMSO-d_6; 500 MHz) *δ* (ppm): 7.21–7.84 (1H, s), 6.55–6.64 (1H, s). ^{13}C-NMR (CD_3OD; 125 MHz) *δ* (ppm): 98.56; 100.80; 102.22;103.65; 113.48; 115.13; 115.47; 128.47; 151.92; 156.89; 157.99; 159.68; 165.45; 181.45 (C=O). MS (EI) *m/z*: 346 (M^+), 312 (M-34).

4-chloro-3,6-dihydroxy-9H-xanthen-9-one (**4c**) yellow solid (73.05%), m.p.: 201-203 °C. FTIR (KBr, *v*; cm^{-1}): 3248 (OH), 1620 (C=O), 1458 and 1512 (C-C aromatic), 1242 (C-O-C), 779 (*o/p*-substituted chloro). ^{1}H-NMR (CD_3OD; 500 MHz) *δ* (ppm): 7.5 (2H, d, *J*= 15.2 Hz), 6.49 (2H, s), 6.43 (2H, d, *J*= 16.5 Hz). ^{13}C-NMR (CD_3OD; 125 MHz) *δ* (ppm): 103.04; 104.95; 105.13;107.49; 108.43; 110.96; 112.48; 116.33; 128.81; 111.56; 133.55; 159.37; 161.16 (C=O). MS (EI) *m/z*: 262 (M^+).

In vitro anticancer activity assay

The *in vitro* anticancer activity of xanthone was carried out by MMT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay. The HepG2, HeLa, Vero and T47D cell lines used during the present study were obtained from Toxicology Laboratory, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia. The murine leukaemia P388 cell line [ex. HSRRB Lot Number: 113098 seed (JCRB0017)] was received from Natural Organic Chemical Laboratory of Nature Material, Chemical Department of the Bandung Institute of Technology, Indonesia.

Molecular docking

The inhibition of protein tyrosine kinase (PTK) has been shown to be one of the strategies in treating cancer as well as other proliferative diseases, as protein kinases are involved in the cell survival and proliferation stage. In this work, the protein crystal docking study of the active compound **4c** was performed to investigate its ability to inhibit PTK, while compared to the STI571 ligand (imatinib) as native ligand of C-kit receptor protein tyrosine kinase (PDB ID: 1T46). Docking simulations were performed in previous work [30] under the receptor–ligand interaction section in Discovery Studio 3.1 (Accelrys, Inc., San Diego, CA, USA). Other molecular modeling

software used throughout this study were CHIMERA 1.9 and ChemOffice®2015.

Results and discussion

Synthesis and characterization

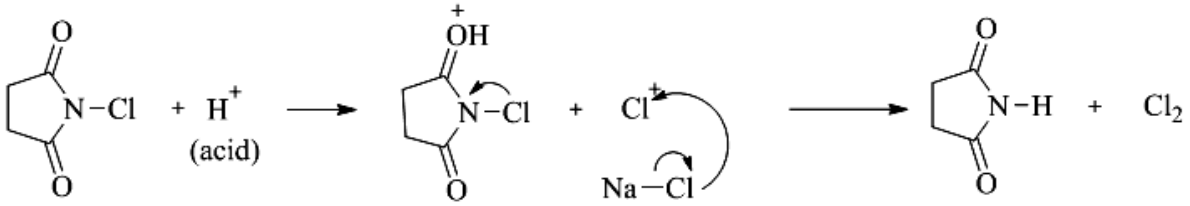
The prepared chloro-substituted hydroxyxanthones derivatives (**4a-c**) were afforded through a two-step reaction *i.e.* cyclodehydration of acid derivatives and substituted phenol in the presence of Eaton reagent to afforded **3a-c**, followed by halogenation step, electrophilic substitution of chlorine as show in Scheme 1.

The synthesis of compounds **3a-c** was carried out using a cyclodehydration reaction, as the name suggests, the cyclization of xanthone occurs followed by the dehydration reaction. The presence of phosphorus pentoxide in the Eaton reagent will absorb the water molecules obtained in the reaction, while the function of *p*-toluenesulphonic acid activates the acyl group from the acidic compound to form the carbocation which facilitates the addition of phenol compounds to form cyclic ether in the hydroxyxanthone compound. Meanwhile, synthesis of compounds **4a-c** was carried out using the chlorination reaction. Chlorination was performed using Cl_2 obtained from the reaction of NCS (*N*-chlorosuccinimide) with acids and Cl^-. The acid used was derived from the *p*-TsOH, which is a strong organic acid capable of reacting directly with the NCS compounds without causing their oxidation. By reaction mechanism *p*-TsOH acts as an acid by activating the carbonyl oxygen group from NCS and releasing Cl^- ion, subsequently reacting with Cl^- derived from the perfect ionized sodium chloride salt in solution, the Cl_2 formation mechanism is shown in Scheme 2.

^{1}H NMR data allowed observing the differences in the peaks of hydroxyxanthone compounds before and after chlorination. For compound **3a** were identified 5 proton peaks in chemical shits 6.2; 6.36; 7.49; 7.40 and 7.74 ppm while compound **4a** had three peaks that were identified in chemical shifts 6.65; 7.67 and 8.25. This difference showed that there has been a substitution of protons by chloro groups on aromatic rings. Additionally, there is a difference in the value of the chemical shift from shielding into deshielding in compounds **3a** and **4a** indicating the effect of chloro substitution on xanthone aromatic rings both on *ortho* and *para* positions. This has also been done to another compounds **3b-c** into **4b-c**, as listed in the experimental section.

Anticancer activity

The results of the study presented in this work showed that compounds **4a-c** and **3b-c** have moderate to very good anticancer activities for Murine leukaemia P388 cell line with the range of IC_{50} 5.75–0.18 μg/mL. Furthermore, the inhibition concentration of **4c** was much lower than for arconine E that is a standard compound (as seen in Table 1). Meanwhile, there were no significant cytotoxic activities of **4a-c** and **3b-c** attested towards other human cancer cell lines such as T47D, HeLa, and HepG2.



Scheme 2. Proposed reaction of chlorine formation from NCS.

Table 1

Anticancer activity against various cancer cell line (μg/mL).

Compound	*Anticancer activity (IC_{50})***				
	P388	*HepG2*	*T47D*	*HeLa*	*Vero*
1,3-dihydroxy-9H-xanthen-9-one (**3a**)	15.53	188.00	549.00	45.00	484.26
1,3,6-trihydroxy-9H-xanthen-9-one (**3b**)	5.75	192.00	88.20	233.00	2000.00
3,6-dihydroxy-9H-xanthen-9-one (**3c**)	2.37	188.60	170.20	37.00	2000.00
4-chloro-1,3-dihydroxy-9H-xanthen-9-one (**4a**)	3.27	54.00	73.40	86.11	286.48
4,5,7-trichloro-1,3,6-trihydroxy-9H-xanthen-9-one (**4b**)	1.81	91.00	485.70	87.34	2000.00
4-chloro-3,6-dihydroxy-9H-xanthen-9-one (**4c**)	0.18	175.00	143.40	168.14	1655.94
artonin E*	0.80				

* *Standard compound;*

** *Probit analysis (%) percentage of viability cell.*

Biological activities of xanthone derivatives are affected by the presence of substituents on their rings. The substituent could both improve and reduce the biological activities. Based on data in Table 1, it can be seen that the best anticancer activities of **4a-c** were found toward P388 Murine leukaemia cells. The activities could be categorized as very active with IC_{50} of the compounds **4a-c** being 3.27; 1.809 and 0.18 µg/mL respectively. Meanwhile, the anticancer activity of **3a-c** was found to be lower than **4a-c,** with IC_{50} 15.53, 5.75 and 2.37 µg/mL, respectively. Furthermore, there was a much lower activity of **4a-c** and also **3a-c** to the other cancer cells, *i.e.* HepG2, HeLa, than T47D (IC_{50}> 45 µg/mL). According to the criteria set by the National Cancer Institute, U.S.A., the values of IC_{50} less than 30 µg/mL are considered cytotoxic. This result proves that the presence of chloro groups enhances the anticancer activity of hydroxyxhantone compounds as seen in case of compounds **4a-c**.

There is always a possibility for a compound with a high cytotoxic effect against cancer cell to have cytotoxic effect on normal cells at the same time and *vice versa*. The calculated selectivity index of **3a-c** and **4a-c** are presented in Table 2. These results showed the average selectivity index (*SI*) value of compound **4a-c** was above 3 ranging from 3.33 to 9199.67. Therefore, the investigation of the cytotoxic effects for both normal and cancer cells was necessary in order to determine the selectivity of the compound expressed as *SI*. An *in vitro* selectivity assay was conducted by comparing the IC_{50} value of each compound to cancer cells with those of normal cells. A compound can be categorized as having high selectivity toward cancer cells if its *SI* value is higher than 3 [32]. In this study, the selectivity analysis was performed by comparing IC_{50} values obtained for each compound to normal cells (Vero cell line) and to the tested human cancer cell line. The obtained results indicated that the prepared chloro-substituted hydroxyxanthone **4a-c** have a potential to be developed as anticancer compounds, based on their lower toxicity and higher sensitivity, therefore chloro-substituted hydroxyxanthone could be recommended as active compounds for anticancer chemotherapy.

Molecular docking

Molecular docking of compound **4c** showed that there is an interaction against protein tyrosine kinase target that indicated that compound has a good anticancer activity. The molecular docking process generated cDOCKER interaction energy of -31.06 kcal/mol and the distance of hydrogen bonds between atoms of the compounds and amino acids was ranging from 2.06–5.18 Å. These results showed that the energy produced by each co-crystallized ST1571 ligand (-79.38 kcal/mol) was much lower than the energy of compound **4c** while the distance of hydrogen bonds of the ligands was also shorter, ranging from 2.41–4.79 Å. Nevertheless, based on an *in vitro* anticancer activity assay, **4c** could be categorized as an excellent anticancer toward P388 murine leukaemia cell line. This result may be caused by the binding interaction between **4c** and the amino acid residues, which showed similar binding interaction from each co-crystallized ligand (Figure 1).

Molecular docking studies revealed the binding interaction of **4c** with amino acid residues such as Asp810, Cys809, Ile789, His790, and Leu644 of protein tyrosine kinase receptor (PDB ID: 1T46). Meanwhile, co-crystallized STI571 ligands showed binding interaction with Asp810, Cys809, Ile789, His790, and Leu644 (Table 3). This result means the binding pocket formed by compound **4c** was similar to the co-crystallized STI571 ligands. Therefore, it can be concluded that the anticancer activity from the experimental result could be proved through *in silico* molecular docking studies, especially toward P388 murine leukaemia cell line.

Table 2

Selectivity index toward various cell line.

Compound	*Selectivity index (SI)**			
	HepG2	*T47D*	*HeLa*	*P388*
1,3-dihydroxy-9H-xanthen-9-one (**3a**)	2.58	0.88	10.76	31.18
1,3,6-trihydroxy-9H-xanthen-9-one (**3b**)	10.42	22.68	8.58	347.83
3,6-dihydroxy-9H-xanthen-9-one (**3c**)	10.60	11.75	54.05	843.88
4-chloro-1,3-dihydroxy-9H-xanthen-9-one (**4a**)	5.31	3.90	3.33	87.61
4,5,7-trichloro-1,3,6-trihydroxy-9H-xanthen-9-one (**4b**)	9.46	11.55	9.85	9199.67
4-chloro-3,6-dihydroxy-9H-xanthen-9-one (**4c**)	21.98	4.12	22.90	1104.97

* *$SI = (IC_{50}$ Vero Cell)/(IC_{50} cancer cell line).*

Table 3

Energy, hydrogen bonds distance and binding interaction of 4c and co-crystallized STI571 ligands.

Compound	*cDOCKER energy (kcal/mol)*	*Binding interaction (amino acid residue)*	*Hydrogen bond distance (Å)*
4c	-31.06	Asp810	4.54
		Cys809	2.37
		Ile789	2.06
		His790	5.18
		Leu644	5.04
STI571	-79.38	Asp810	2.81
		Cys809	2.41
		Ile789	2.65
		His790	2.64
		Leu644	2.81

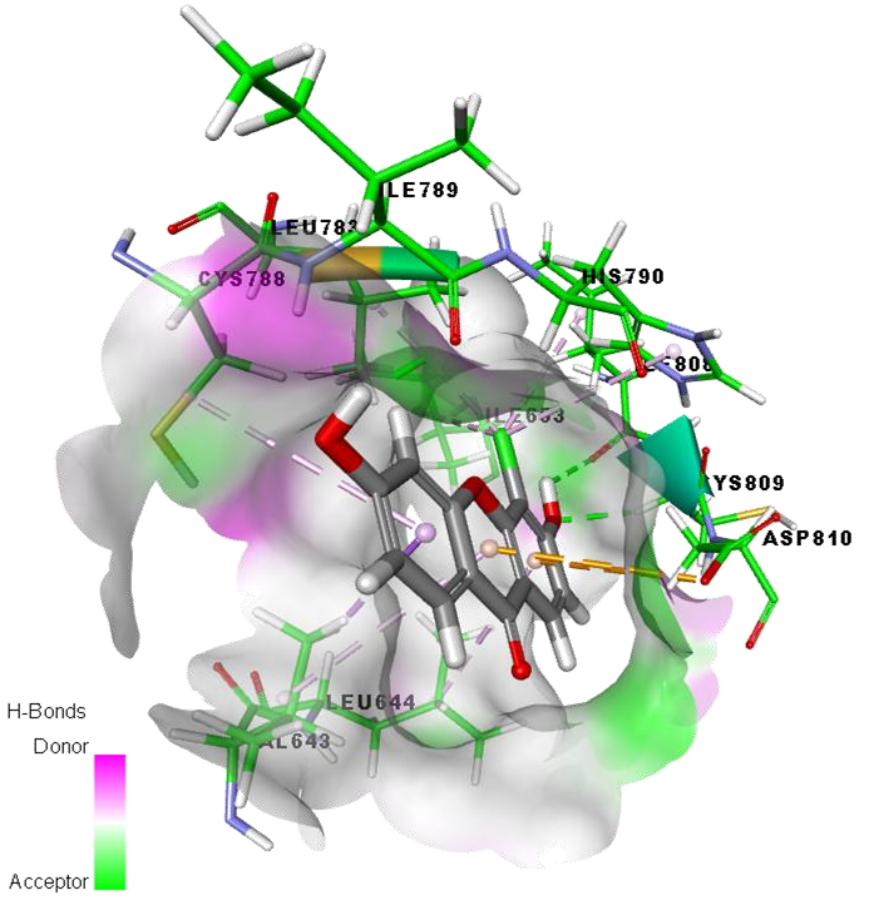


Figure 1. The 3D predicted binding mode from docking simulation of 4c compound into the active site of c-kit protein tyrosine kinase (PDB ID: 1T46).

The lower energy produced by the protein-ligand interaction indicates that the ligand-protein bond is more stable. According to Table 3, it can be seen that compound **4c** has a similar interaction with the interaction of STI-571 c-kit protein kinase (PDB ID: 1T46) and amino acid residues. The similarity was observed from the existence of the binding interaction between the compound and amino acid residues such as Asp810, Cys809, Ile789, His790, and Leu644 of PDB ID: 1T46 protein. The planar xanthone ring compound **4c** was involved in an electrostatic surface interaction. The interaction was also similar with the interaction between amino acid residues and other compounds tested against cancer cells. Shrestha, A.R. *et al.* have performed docking simulation of deazaflavin compound and found the binding interaction with amino acid residues

of Leu595, Tyr 672, Leu799, Glu640, Asp810, and Phe811 [33]. Meanwhile, it has also been reported that 2-deoxo-2-methylamino-5-deazaflavin possesses binding interaction with Asp810 and Glu640 amino acid [33]. Quinoxaline has been found to interact with Cys673, Thr670 and Ile789 amino acid [34]. Furthermore, the hybrid compound of deazaflavin-cholestane showed interaction with Asp680, Lys593, Leu595, Asp677, Val603, Gly676, Leu799, Tyr672, Ala621, and Phe811[33], which was performed on various cancer cells *i.e.* HepG2, MCF7, A549, HCT116, CCRF-HSB-2 and KB tumour cells. These results indicate that compound **4c** possess anticancer activities with active site or chemotherapy mechanism by inhibiting protein tyrosine kinase.

In the molecular docking study, the ligand stability is also acquired from the low cDOCKER energy and the short length of the bonds formed. Based on Table 3, the cDOCKER energy of compound **4c** was -31.06 kcal/mol and the bond length was 2.06–5.18 Å. On the other hand, the cDOCKER energy of ligand STI571 was -79.38 kcal/mol with hydrogen bond distance of 2.41–2.81 Å. The low interaction energy and the short bond length indicate the formation of the ligand-protein bond, showing that the native ligand is better than compound **4c**. The energy produced by compound **4c** was also compared with the interaction energy of 2-amino-4-phenyl-5-methyltiazole complex with some metals, including Ni, Zn, Cu, and Co (<20 kcal/mol) [35] and also with deazaflavin derivative which was less than 18 kcal/mol [34]. This result means that the interaction of compound **4c** was more stable and it has potential as an anticancer agent when compared to the other compounds interaction with the same protein and ligand, which cause the hydroxy moieties of hydroxyxanthone to give surface interaction through hydrogen bond formation. Thus, theoretically, the effectiveness of compound **4c** as an anticancer drug is better than the previously reported compound.

Conclusions

Chloro-substituted hydroxyxanthones **4a-c** were prepared by cyclodehydration of acid derivatives and substituted phenol in the presence of Eaton reagent to afforded **3a-c**, followed by halogenations step to electrophilic substitution of chlorine in a moderate yield using *N*-chlorosuccinimide with NaCl/*p*TsOH in ethanol system, where the compounds formed were characterized based on spectroscopy data (IR, NMR and MS). The *in vitro* study of compounds **4a-c** showed an excellent inhibition and a higher selectivity index against P388 murine leukaemia cells compared with T47D, HeLa, and HepG2 cell line. The *in silico* analysis of anticancer activity showed that there was a binding interaction between **4c** compound and STI571 with the protein tyrosine kinase (PDB ID: 1T46). This result indicated that the compound has an active chemotherapy site as an inhibitor of protein tyrosin kinase, which controls the phosphorylation and regulates various cellular functions.

Acknowledgments

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References

1. Cui, J.; Hu, W.; Cai, Z.; Liu, Y.; Li, S.; Tao, W.; Xiang, H. New medicinal properties of mangostins: analgesic activity and pharmacological characterization of active ingredients from the fruit hull of *Garcinia mangostana* L. Pharmacology Biochemistry and Behavior, 2010, 95(2), pp. 166–172. DOI: https://doi.org/10.1016/j.pbb.2009.12.021
2. Proença, C.; Albuquerque, H.M.T.; Ribeiro, D.; Freitas, M.; Santos, C.M.M.; Silva, A.M.S.; Fernandes, E. Novel chromone and xanthone derivatives: Synthesis and ROS/RNS scavenging activities. European Journal of Medicinal Chemistry, 2016, 115, pp. 381-392. DOI: http://doi.org/10.1016/j.ejmech.2016.03.043
3. Suksamrarn, S.; Suwannapoch, N.; Phakhodee, W.; Thanuhiranlert, J.; Ratananukul, P.; Chimnoi, N.; Suksamrarn, A. Antimycobacterial activity of prenylated xanthones from the fruits of *Garcinia mangostana*. Chemical and Pharmaceutical Bulletin. 2003, 51(7), pp. 857–859. DOI: https://doi.org/10.1248/cpb.51.857
4. Sakagami,Y.; Iinuma, M.; Piyasena, K.G.N.P.; Dharmaratne, H.R.W. Antibacterial activity of *α*-mangostin against vancomycin resistant enterococci (VRE) and synergism with antibiotics. Phytomedicine, 2005, 12(3), pp. 203–208. DOI: https://doi.org/10.1016/j.phymed.2003.09.012
5. Kaomongkolgit, R.; Jamdee, K.; Chaisomboon, N. Antifungal activity of alpha-mangostin against *Candida albicans*. Journal of Oral Science, 2009, 51(3), pp. 401–406. DOI: https://doi.org/10.2334/josnusd.51.401
6. Chen, L.-G.; Yang, L.-L.; Wang, C.-C. Anti-inflammatory activity of mangostins from

Garcinia mangostana. Food and Chemical Toxicology, 2008, 46(2), pp. 688–693. DOI: https://doi.org/10.1016/j.fct.2007.09.096
7. Aisha, A.F.A.; Abu-Salah, K.M.; Ismail, Z.; Majid, A.M.S.A. *In vitro* and *in vivo* anti-colon cancer effects of *Garcinia mangostana* xanthones extract. BMC Complementary and Alternative Medicine, 2012, 12, pp. 104–114. DOI: https://doi.org/10.1186/1472-6882-12-104
8. Matsumoto, K.; Akao Y.; Kobayashi, E.; Ohguchi, K.; Ito, T.; Tanaka, T.; Iinuma, M.; Nozawa, Y. Induction of apoptosis by xanthones from mangosteen in human leukemia cell lines. Journal of Natural Products, 2003, 66(8), pp. 1124–1127. DOI: 10.1021/np020546u
9. Su, Q.G.; Liu, Y.; Cai, Y.C.; Sun, Y.L.; Wang, B.; Xian, L.J. Anti-tumour effects of xanthone derivatives and the possible mechanisms of action. Investigational New Drugs, 2011, 29(6), pp. 1230–1240. DOI: https://doi.org/10.1007/s10637-010-9468-5
10. Fei, X.; Jo, M.; Lee, B.; Han, S.B.; Lee, K.; Jung, J. K.; Seo, S.Y.; Kwak, Y.S. Synthesis of xanthone derivatives based on α-mangostin and their biological evaluation for anti-cancer agents. Bioorganic & Medicinal Chemistry Letters, 2014, 24(9), pp. 2062–2065. DOI: https://doi.org/10.1016/j.bmcl.2014.03.047
11. Pai, A.; Kumar, D.V.; Jayashree, B.S. P-071 - Synthesis, characterization, antibacterial and anticancer evaluation of some novel flavone-3-ols. Asian Journal of Pharmaceutical Sciences, 2016, 11(1), pp. 187–188. DOI: https://doi.org/10.1016/j.ajps.2015.11.044
12. Jayashree, B.S.; Gurushyam, S.; Pai, A. Synthesis, characterization, antioxidant and anticancer evaluation of some novel flavone-4-oximes. Asian Journal of Pharmaceutical Sciences, 2016, 11(1), pp. 185–186. DOI: https://doi.org/10.1016/j.ajps.2015.11.045
13. Yuanita, E.; Pranowo, H.D.; Jumina, J.; Mustofa, M. Design of hydroxy xanthones derivatives as anticancer using quantitative structure-activity relationship. Asian Journal of Pharmaceutical and Clinical Research, 2016, 9(2), pp. 180–185. https://innovareacademics.in/journals/index.php/ajpcr/article/view/10341
14. Woo, S.; Jung, J.; Lee, C.; Kwon, Y.; Na, Y. Synthesis of new xanthone analogues and their biological activity test – cytotoxicity, topoisomerase II inhibition, and DNA cross-linking study. Bioorganic & Medicinal Chemistry Letters, 2007, 17(5), pp. 1163–1166. DOI: https://doi.org/10.1016/j.bmcl.2006.12.030
15. Desmurs, J.R.; Jouve, I. Chlorination of nitrophenols. U.S. Patent 4827047A, 1989. https://patents.google.com/patent/US4827047
16. Barhate, N.B.; Gajare, A.S.; Wakharkar, R.D.; Bedekar, A.V. Simple and practical halogenation of arenes, alkenes, and alkynes with hydrohalic acid/H_2O_2 (or TBHP). Tetrahedron, 1999, 55(36), pp. 111127–11142. DOI: https://doi.org/10.1016/S0040-4020(99)00628-6
17. Vyas, P.V.; Bhatt, A.K; Ramachandraiah, G.; Bedekar, A.V. Environmentally benign chlorination and bromination of aromatic amines, hydrocarbons and naphthols. Tetrahedron Letter, 2003, 44(21), pp. 4085–4088. DOI: http://doi.org/10.1016/S0040-4039(03)00834-7
18. Mukhopadhyay, S.; Mukhopadhyaya, J.K.; Ponde, D.E.; Cohen, S.; Kurkalli, B.G.S. Kinetics and process parameter studies in oxidative chlorination of 4-methylphenol under phase-transfer conditions. Organic Process Research & Development, 2000, 4(6), pp. 509–512. DOI: 10.1021/op0000273
19. Muathen, H.A. Mild chlorination of aromatic compounds with tin(IV) chloride and lead tetraacetate. Tetrahedron, 1996, 52(26), pp. 8863–8866. DOI: https://doi.org/10.1016/0040-4020(96)00433-4
20. Yu, G; Mason, H.J.; Wu, X.; Endo, M.; Douglas, J.; Macor, J.E. A mild and efficient method for aromatic chlorination of electron-rich arylalkyl amines. Tetrahedron Letters, 2001, 42(19), pp. 3247–3249. DOI: https://doi.org/10.1016/S0040-4039(01)00430-0
21. Jerzy, G.; Slawomir, Z. Oxidative chlorination of acetanilides by metal chlorides - Hydrogen peroxide in acid−aqueous medium systems. Synthetic Communications, 1997, 27(18), pp. 3291–3299. DOI: https://doi.org/10.1080/00397919708004189
22. Tamemura, K.; Suzuki, T.; Nishida, Y.; Satsumabayashi, K.; Horaguchi, T. Halogenation of aromatic compounds by *N*-chloro-, *N*-bromo-, and *N*-iodosuccinimide. Chemistry Letters, 2003, 32(10), pp. 932–933. DOI: https://doi.org/10.1246/cl.2003.932
23. Bovonsombat, P.; Ali, R.; Khan, C.; Leykajarakul, J.; Pla-on, K.; Aphimanchindakul, S.; Pungcharoenpong, N.; Timsuea, N.; Arunrat, A.; Punpongjareorn, N. Facile p-toluenesulfonic acid-promoted *para*-selective monobromination and chlorination of phenol and analogues. Tetrahedron, 2010, 66(34), pp. 6928–6935. DOI: https://doi.org/10.1016/j.tet.2010.06.041
24. Pravst, I.; Zupan, M.; Stavber, S. Halogenation of ketones with N-halosuccinimides under solvent-free reaction conditions. Tetrahedron, 2008, 64(22), pp. 5191–5199. DOI: https://doi.org/10.1016/j.tet.2008.03.048
25. Qiu, D.; Mo, F.; Zheng, Z.; Zhang, Y.; Wang, J. Gold(III)-catalyzed halogenation of aromatic boronates with N-halosuccinimides. Organic Letters, 2010, 12(23), pp. 5474–5477. DOI: 10.1021/ol102350v
26. Wang, C.; Tunge, J. Selenocatalytic *α*-halogenation. Chemical Communications, 2004, 0(23), pp. 2694–2695. DOI: 10.1039/B410576A
27. Mahajan, T.; Kumar, L.; Dwivedi, K.; Agarwal, D.D. Efficient and facile chlorination of

industrially-important aromatic compounds using NaCl/*p*-TsOH/NCS in aqueous media. Industrial & Engineering Chemistry Research, 2012, 51(10), pp. 3881−3886.
DOI: 10.1021/ie201971j

28. Khaw, K.Y.; Choi, S.B.; Tan, S.C.; Wahab, H.A.; Chan, K.L.; Murugaiyah, V. Prenylated xanthones from mangosteen as promising cholinesterase inhibitors and their molecular docking studies. Phytomedicine, 2014, 21(11), pp. 1303–1309.
DOI: https://doi.org/10.1016/j.phymed.2014.06.017
29. Mahendran, G.; Vijayan, R. Neuropharmacological and molecular docking studies of xanthones from *Swertia corymbosa*. Journal of Receptors and Signal Transduction, 2018, 38(2), pp. 166-177. DOI: https://doi.org/10.1080/10799893.2018.1458875
30. Syahri, J.; Yuanita, E.; Nurohmah, B.A.; Wathon, M.H.; Syafri, R.; Armunanto, R.; Purwono, B. Xanthone as Antimalarial: QSAR analysis, synthesis, molecular docking and *in-vitro* antimalarial evaluation. Oriental Journal of Chemistry, 2017, 33(1), pp. 29–40.
DOI: http://dx.doi.org/10.13005/ojc/330104
31. Yuanita, E.; Pranowo, H.D.; Siswanta, D.; Swasono,, R.T.; Mustofa, ; Zulkarnain, A.K.; Syahri, J.; Jumina. One-pot synthesis, antioxidant activity and toxicity evaluation of some hydroxyxanthones. Chemistry and Chemical Technology, 2018, 12(3), pp. 290–295.
DOI: https://doi.org/10.23939/chcht12.03.290
32. Mahavorasirikul, W.; Viyanant, V.; Chaijaroenkul, W.; Itharat, A.; Na-Bangchang, K. Cytotoxic activity of Thai Medicinal Plants against human cholangiocarcinoma, laryngeal and Hepatocarcinoma cells *in vitro*. BMC Complementary and Alternative Medicine, 2010, 10, pp. 55–62.
DOI: https://doi.org/10.1186/1472-6882-10-55
33. Shrestha, A.R.; Shindo, T.; Ashida, N.; Nagamatsu, T. Synthesis, biological active molecular design, and molecular docking study of novel deazaflavin–cholestane hybrid compounds. Bioorganic & Medicinal Chemistry, 2008, 16(18), pp. 8685–8696.
DOI: https://doi.org/10.1016/j.bmc.2008.07.089
34. Galal, S.A.; Abdelsamie, A.S.; Tokuda, H.; Suzuki, N.; Lida, A.; El Hefnawi, M.M.; Ramadan, R.A.; Atta, M.H.E.; El Diwani, H.I. Part I: Synthesis, cancer chemopreventive activity and molecular docking study of novel quinoxaline derivatives. European Journal of Medicinal Chemistry, 2011, 46(1), pp. 327–340.
DOI: https://doi.org/10.1016/j.ejmech.2010.11.022
35. Abd-Elzaher, M.M.; Labib, A.A.; Mousa, H.A.; Moustafa, S.A.; Ali, M.M.; El-Rashedy, A.A. Synthesis, anticancer activity and molecular docking study of Schiff base complexes containing thiazole moiety. Beni-Suef University Journal of Basic and Applied Science, 2016, 5(1), pp. 85–96.
DOI: https://doi.org/10.1016/j.bjbas.2016.01.001