QSAR AND MOLECULAR DOCKING APPROACHES FOR DEVELOPMENT OF HALOXANTHONES AS THE ANTICANCER AGENT AGAINST MCF-7 AND HepG2

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ABSTRACT

The development of haloxanthones as potential anticancer agents is critical since their derivatives have remarkable cytotoxicity against several cancer cell lines. This study aims to explore the anticancer activity of haloxanthones against liver cancer (HepG2) and Breast cancer (MCF-7) based on the Quantitative Structure-Activity Relationship (QSAR) and molecular docking approaches. Through the QSAR study, we found that the lowest unoccupied molecular orbital energy, dipole moment, and atomic charges on C1, C4, and C6 affect the anticancer activity of haloxanthones against MCF-7 cell line. Meanwhile, the atomic charges on C7, C8, C8a, and O11 of haloxanthones affect the anticancer activity against HepG2 cell line. The prediction of the anticancer activity of 26 haloxanthone derivatives showed that they belong to the strong category (IC₅₀ predictive <6.25 µg/mL) according to the QSAR study. On the other hand, we found that the geometric structure of haloxanthone was not much different compared with the native ligand (ONR) that was bound to the c-JNK protein (RMSD <2Å) on the molecular docking study. It was found that haloxanthones were able to interact with c-JNK protein through hydrogen bonds (MET111 and GLU109), alkyl/pi-alkyl (VAL40), and halogen interactions (MET108, ASP112), which is remarkable.

Keywords: QSAR, Molecular Docking, Haloxanthone, Anticancer, HepG2 and MCF-7.

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INTRODUCTION

In 2018, it was reported that cancer causes the death of 9.6 million people thus cancer was mentioned as the second most global fatal disease.¹ Breast cancer (MCF-7) and liver cancer (HepG2) are the most significant contributors to human death among the other cell lines. A high number of cancer cases is worsened since some cancer cell lines are resistant to standard drug compounds, such as 5-fluorouracil, cisplatin, cytarabine, and doxorubicin.² Various efforts are given by researchers to find new potential drug compounds for cancer therapy.

One of the potential drug compounds is xanthone derivative. The structure of xanthone is shown in Figure-1. It has been reported that xanthone derivatives are known to have good anticancer activity, such as haloxanthone,³⁻⁵ hydroxyxanthone,⁶ 3,6-diamino carbonyl methoxy xanthone,⁷ α-mangosteen,⁸⁻¹⁵ β-mangosteen,¹⁶ γ-mangosteen,¹⁵ garcinone D,¹⁷ and gartanin.¹⁸ The xanthone derivatives enhance the apoptosis of the cancer cell by stimulating various caspase enzymes and increasing Bax protein.⁸⁻¹⁰ Furthermore, xanthone derivatives also retard the cancer cell cycle through inhibition of various cyclines, Bcl-2 and nuclear factor-kappa B/NF-κB.^{8,19-21}

In the last recent years, anticancer activity studies of xanthones have been focused on the haloxanthone derivatives. Haloxanthone is a xanthone derivative with one or more halogen (F, Cl, Br, or I) substituents. It was reported that both chloro and bromo substituted xanthones have higher activity as an anticancer than

the unmodified xanthone itself.¹⁶⁻¹⁸ However, the anticancer activity of haloxanthones still needs to be developed because their molecular interaction with the targeted proteins is still unknown.

Fig.-1: The Chemical Structure of Xanthone

Therefore, this study is conducted to investigate the anticancer activity of haloxanthones through Quantitative Structure-Activity Relationship (QSAR) and molecular docking approaches. Both approaches play an essential role in discovering and developing potential drug compounds. QSAR, also known as structure-based drug design (SBDD), is a study of the suitability of molecular structure (microscopic) with its pharmacological (macroscopic/empirical) activity. Meanwhile, molecular docking, also known as ligand-based drug design (LBDD), studies the binding between the targeted protein with the drug candidate from a three-dimensional point of view.

EXPERIMENTAL

Quantitative Structure-Activity Relationship (QSAR)

Experimental Data

The chemical structure and anticancer activity of xanthone derivatives (33 compounds) were taken from reported literature.⁵ The data used were xanthone derivatives with IC_{50} value $\leq 25 \mu\text{g/mL}$ for each cancer cell line. The IC_{50} values were then converted to $\log IC_{50}$.

Calculation of Molecular Descriptors

$^1\text{H-NMR}$ chemical shifts of compound 1 (1,3-dihydroxy-7-chloroxanthone) were calculated using the AM1 (Austin Model 1), PM3 (Parameterized Model Number 3), *ab initio*, and DFT (Density Functional Theory) methods. The obtained results were compared with the experimental $^1\text{H-NMR}$ chemical shifts value. All quantum mechanical calculation was conducted using Gaussian 09 (AIC Laboratory, Departement of Chemistry, Universitas Gadjah Mada). Meanwhile, the correlation models were evaluated by multiple linear regression analysis using SPSS® Release 23.0.0.

Validation QSAR Model

The best model was chosen based on some statistical parameters such as r , r^2 , adjusted R, Standard Estimation of Error (SEE), PRESS, and $F_{\text{cal}}/F_{\text{tab}}$. Furthermore, the best-selected model was used to calculate $\log IC_{50}$ prediction of training and test set. The model was validated using criteria r^2 prediction > 0.5 .²²

Molecular Docking Studies

Protein Target Preparation

Three-dimensional structure of c-Jun protein N-terminal kinase (c-JNK) was obtained from the world protein data bank with the code 4e73.pdb.id with 0NR as the native ligand. The residue (H_2O molecules and standard ligands) was removed with the Chimera® 1.10 program's help. The re-docking process was proceeded using the AutoDockTools 1.5.6 program's assistance to determine the interaction between native ligands and the targeted protein. The formed interactions were displayed using the Discovery studio® 3.1 software.

Ligand Preparation

Preparation of haloxanthone compounds as ligands was carried out by increasing the charge on each atom, making up the ligand, adding hydrogen atoms, and minimizing the energy. These processes were carried out using the Chimera® 1.10 software.

Docking Process

The docking process was first evaluated using standard ligands and had to get a Root Mean Square Deviation (RMSD) value less than 2Å. After the docking method is validated, then the haloxanthone derivatives are docked on the target protein. The docking process was carried out using the BIOVIA Discovery Studio® 3.1 software.

Analysis of Docking Results

The docking analysis stage was carried out using the Discovery studio® 4.5 visualizer software. The interactions between targeted protein and ligands could be found in the form of hydrogen- and pi-bonds, which are distinguished based on the color of the bonds formed. In the Discovery studio software, the binding energy between a protein and a ligand could be determined. Smaller binding energy reflects stronger interaction between the targeted protein and the ligand.²³

RESULTS AND DISCUSSION

QSAR Model of Haloxanthones for MCF-7 and HepG2 Cancer Cell Lines

The geometry structures of the xanthone derivatives were optimized using the Density Functional Theory (DFT) method with B3LYP/6-311G as a basis set. Afterward, the descriptor data were compiled from the Multiple Linear Regression (MLR) method to obtain a QSAR model. Optimization of 1,3-dihydroxyl-7-chloroxanthone with the DFT method produced a prediction of ¹H-NMR chemical shifts. It was found that the predicted ¹H-NMR chemical shifts were closed to the experimental data in which the DFT B3LYP/6-311G gave the smallest PRESS value (see Tabel-1). The MLR analysis with the backward method provides five QSAR models for MCF-7 cancer cell lines and six QSAR models for HepG2 cancer cell lines (see Table-2).

Table-1: Predicted ¹H-NMR Chemical Shift of 1,3-dihydroxyl-7-chloroxanthone Using PM3, PM6, AM1, HF, and *ab initio* (DFT) Methods and its Comparison to The Experimental

Atom H number	¹ H-NMR experiment	PM3		PM6		AM1		HF		DFT B3LYP-6311G	
H1	12.52	2.98	91.01	4.18	69.56	3.57	80.10	3.32	84.64	4.38	66.26
H2	6.23	4.41	3.31	4.46	3.13	4.47	3.10	3.71	6.35	4.92	1.72
H4	6.39	5.98	0.17	5.78	0.37	6.01	0.14	5.36	1.06	6.35	0.00
H5	7.63	6.70	0.86	6.70	0.86	6.82	0.66	6.17	2.13	6.96	0.45
H6	7.87	6.86	1.02	6.82	1.10	6.92	0.90	6.50	1.88	7.05	0.67
H8	8.00	7.69	0.10	7.76	0.06	7.92	0.01	7.73	0.07	8.04	0.00
PRESS			96.47		75.09		84.91		96.13		69.10

Table-2: QSAR Model for MCF-7 and HepG2 Cancer Cell Lines Based on MLR Analysis With the Backward Method

Model	Descriptor	r	r ²	Adjusted R	SEE	F _{cal} /F _{tab}
MCF-7 Cancer Cell Lines						
1	qO11, qC6, qC8, qC3, Log P, LUMO, MD, qC4, qC1	0.97	0.94	0.88	0.17	4.29
2	qO11, qC6, qC8, qC3, LUMO, MD, qC4, qC1	0.96	0.93	0.87	0.18	4.75
3	qO11, qC6, qC8, LUMO, MD, qC4, qC1	0.95	0.91	0.85	0.19	4.67
4	qO11, qC6, LUMO, MD, qC4, qC1	0.95	0.89	0.84	0.20	4.96
5	qC6, LUMO, MD, qC4, qC1	0.94	0.88	0.83	0.21	5.46
HepG2 Cancer Cell Lines						
1	qO11, qC1, qC7, qC6, BM, qC8, Log P, qC8a, qC9	0.99	0.97	0.94	0.11	8.32
2	qO11, qC1, qC7, qC6, qC8, Log P, qC8a, qC9	0.99	0.97	0.94	0.10	11.04
3	qO11, qC1, qC7, qC6, qC8, qC8a, qC9	0.98	0.97	0.94	0.10	12.75
4	qO11, qC1, qC7, qC6, qC8, qC8a	0.98	0.96	0.94	0.10	14.54
5	qO11, qC7, qC6, qC8, qC8a	0.98	0.96	0.94	0.11	16.87
6	qO11, qC7, qC8, qC8a	0.97	0.95	0.93	0.11	17.61

Abbreviations: SEE, standard error of the estimate; qO11, the atomic charge at Oxygen number 11; qCn, the atomic charge at Carbon number n; LUMO, lowest unoccupied molecular orbital; MD, dipole moment.

The selection of QSAR models refers to several statistical parameters such as r^2 , adjusted R, SEE, $F_{\text{cal.}}/F_{\text{tab.}}$. These parameters indicate the significance level of the model. Each of these parameters must meet criteria $r^2 > 0.6$, $\text{SEE} < 0.3$, and $F_{\text{cal.}}/F_{\text{tab.}} \geq 1$.^{22,24} Table-1 shows that all QSAR models have met the specified statistical parameters' criteria. Models 5 and 6 were chosen as the best model for the MCF-7 and HepG2 cancer cell lines, respectively. The selection of models 5 and 6 refers to the highest SEE and $F_{\text{cal.}}/F_{\text{tab.}}$ values than the other models. The higher value of each statistical parameter indicates the higher validity of the used model to predict anticancer activity. Equations-1 and 2 show the coefficient values of both models 5 and 6, respectively.

$$\begin{aligned} \text{Log (IC}_{50} \text{ MCF-7)} = & (7.85) + (100.54 \times \text{LUMO}) + (0.36 \times \text{MD}) - (3.02 \times \text{qC}_6) - (1.71 \times \text{qC}_4) \\ & - (7.98 \times \text{qC}_1) \end{aligned} \quad (1)$$

Equation-1 showed that the anticancer activity of haloxanthenes against the MCF-7 cancer cell line was determined by LUMO energy, dipole moment, the atomic charge on C1, C4, and C6. The high coefficient value of LUMO energy (100.54) indicates that even a slight change in this descriptor will significantly affect the anticancer activity against the MCF-7 cell line. Meanwhile, the negative atomic charge coefficients value indicates that the increasing descriptor amount will decrease the IC_{50} value (expected). Plot validation between the predictive and experimental IC_{50} values is shown in Fig.-2. It was found that the predictive IC_{50} value was not much different from the experimental IC_{50} value in both training and test data set as seen from the R^2 values of 0.86 and 0.89, respectively. The QSAR equation is valid when $R^2 > 0.5$.²² The high R^2 value indicates that equation 1 could predict well the anticancer activity of Haloxanthone derivatives.

Fig.-2: The Plot of Predicted and Experimental IC_{50} Values for MCF-7 Cell Line for (a) Training and (b) Test Set.

$$\text{Log (IC}_{50} \text{ HepG2)} = -6.034 - (1.198 \times \text{qC}_7) + (9.274 \times \text{qC}_{8a}) - (1.887 \times \text{qC}_8) - (23.354 \times \text{qO}_{11}) \quad (2)$$

Equation-2 shows that the anticancer activity of haloxanthenes against the HepG2 cell line is affected by the atomic charges on C7, C8, C8a, and O11. The negative value on the atomic charge coefficients of C7, C8, and O11 indicated that the anticancer activity gets higher when the charge of these atoms increased. The anticancer activity would be higher when the atomic charge on the C8a decreased. Equation 2 also showed that the anticancer activity of haloxanthenes against HepG2 cell line is concentrating around the oxygen atom of the carbonyl group and the nearest carbon atom in ring A of the xanthone backbone. Meanwhile, descriptors that determine the anticancer activity against MCF-7 cell line spread in all aromatic rings (A, B, and C) of the xanthone.^{3,4,27}

Figure-3 shows the validation plot between predictive and experimental IC_{50} values. The predictive IC_{50} value was not much different from the experimental IC_{50} value with R^2 values of 0.94 (training set) and 0.93 (test set), respectively. Therefore, the prepared QSAR model is entirely valid and can be used to predict the anticancer activity of haloxanthenes against the HepG2 cancer cells line.

Fig.-3: The Plot of Predicted and Experimental IC_{50} Values Against HepG2 Cell Line for (a) Training and (b) Test Set.

Predictive IC_{50} Values of Haloxanthones against MCF-7 and HepG2 Cell Lines

The predictive IC_{50} value of 87 haloxanthones derivatives (25 fluoroxanthone, 27 chloroxanthone, 18 bromoxanthone, and 17 iodoxanthone) was calculated using equations 1 and 2. Table-3 shows the five compounds with the lowest predictive IC_{50} values against the MCF-7 cancer cell line in each of the haloxanthone groups. Whereas Table-4 presents the lowest predictive IC_{50} value against the HepG2 cancer cell line.

Table-3: Potential Anticancer Agents in Each Haloxanthone Group Against MCF-7 Cancer Cell Line According to The Predicted IC_{50} Values

Compounds		Molecular Weight	LUMO	Dipole Moment	Atomic Charge			Log IC_{50} pred.	IC_{50} pred. ($\mu\text{g/mL}$)
					qC6	qC4	qC1		
Xanthone Structure		196.21	-0.08	2.95	-0.12	-0.17	-0.08	2.03	21.12
FLUORO-XANTHONE									
1FX	2-hydroxyl-1,6-difluoroxanthone	248.19	-0.09	5.39	0.34	-0.15	0.31	-2.72	0.0005
2FX	2,6-dihydroxyl-4-fluoroxanthone	246.19	-0.09	2.85	0.06	0.26	0.12	-2.1	0.002
3FX	2,8-dihydroxyl-1-fluoroxanthone	246.19	-0.1	3.86	-0.1	-0.14	0.26	-1.99	0.003
4FX	2,6,8-trihydroxyl-4-fluoroxanthone	262.19	-0.1	2.07	0.31	0.27	-0.07	-1.78	0.004
5FX	2,6-dihydroxyl-1-fluoroxanthone	246.19	-0.09	2.91	-0.12	-0.13	0.25	-1.73	0.005
CHLORO-XANTHONE									
1CX	1-hydroxyl-2,8-dichloroxanthone	281.09	-0.1	6.81	-0.1	-0.15	0.47	-3.48	0.0001
2CX	1-hydroxyl-2,7-dichloroxanthone	281.09	-0.09	5.03	-0.02	-0.16	0.4	-2.62	0.0007
3CX	1-hydroxyl-5,7-dichloroxanthone	281.09	-0.09	5.16	0.08	-0.16	0.24	-1.47	0.01
4CX	1,6-dihydroxyl-4-chloroxanthone	262.65	-0.08	2.83	0.28	-0.43	0.24	-1.14	0.02
5CX	1-hydroxyl-4,7-dichloroxanthone	281.09	-0.09	4.28	-0.02	-0.43	0.25	-1.14	0.02
BROMO-XANTHONE									
1BX	1-hydroxyl-7-bromoxanthone	291.1	-0.08	5.29	-0.07	-0.17	0.23	0.09	0.36
2BX	6,8-dihydroxyl-4-bromoxanthone	307.1	-0.09	3.86	0.31	-0.49	-0.06	0.34	0.67
3BX	1-hydroxyl-8-bromoxanthone	291.1	-0.08	4.62	-0.1	-0.16	0.23	0.41	0.75

4BX	1,3-dihydroxyl-7-bromoxanthone	307.1	-0.08	5.43	-0.07	-0.18	0.24	0.52	1.01
5BX	1-hydroxyl-6-bromoxanthone	291.1	-0.08	4.08	-0.37	-0.16	0.23	0.68	1.4
IODO-XANTHONE									
1IX	1,8-dihydroxyl-2-iodoxanthone	354.1	-0.09	4.52	-0.1	-0.16	0.24	-0.73	0.07
2IX	1-hydroxyl-6-iodoxanthone	338.1	-0.08	3.94	-0.14	-0.17	0.23	-0.12	0.26
3IX	1,8-dihydroxyl-7-iodoxanthone	354.1	-0.09	6.64	-0.1	-0.16	0.24	-0.06	0.31
4IX	1,3-dihydroxyl-5-iodoxanthone	354.1	-0.08	3.44	-0.12	-0.17	0.24	0.12	0.46
5IX	1-hydroxyl-7-iodoxanthone	338.1	-0.08	5.06	-0.11	-0.17	0.23	0.18	0.51

Abbreviations: LUMO, lowest unoccupied molecular orbital.

Table-3 shows that 20 haloxanthone compounds have strong anticancer activity against the MCF-7 cell line. Almost all of them had predictive IC_{50} value below 1 $\mu\text{g/mL}$, except for compounds **4BX** (1.01 $\mu\text{g/mL}$) and **5BX** (1.40 $\mu\text{g/mL}$). The Council of Scientific and Industrial Research (CSIR) classifies the cytotoxic activity of a compound into four categories based on the IC_{50} value, including inactive ($IC_{50} > 50 \mu\text{g/mL}$), weak (15-50 $\mu\text{g/mL}$), moderate (6.25-15 $\mu\text{g/mL}$), and strong/potent ($< 6.25 \mu\text{g/mL}$). Overall, fluoro-xanthone compounds have better anticancer activity than other haloxanthone derivatives. However, the lowest predictive IC_{50} value was obtained for 1-hydroxy-2,8-dichloroxanthone (**1CX**), which was 0.0001 $\mu\text{g/mL}$. This value is much lower when compared to the experimental IC_{50} value of several standard drug compounds, such as doxorubicin, rifampin, and 5-fluorouracil.²⁵

A low predictive IC_{50} value for compounds **1CX** is due to the significant change in LUMO energy and the atomic charge on the C1 atom. LUMO energy and atomic charge on C1 are two factors that influence anticancer activity based on the QSAR model. The addition of two Cl atoms at positions C2 and C8 can reduce the LUMO energy by -0.02 (compared to the xanthone structure). Also, the presence of hydroxyl group at the C1 atom can increase the atomic charge at that position by 0.55.

The presence of hydroxyl and halogen groups at C1 and C2 would significantly increase the atomic charge on C1. This can be seen in the **1FX**, **3FX**, **5FX**, **9FX**, **13FX**, **1CX**, and **2CX** compounds. The addition of fluoro group at C4 and C6 positions can also increase the atomic charge on each position (see compounds **1FX**, **2FX**, and **4FX**). However, the addition of other halogen atoms (Cl, Br, and I) in this position decreases the value of the atomic charge as shown in compounds **4CX**, **2BX**, and **1IX** compounds. The atomic charge at C4 and C6 can be leveled by adding Cl, Br, or I groups in the ortho position to C4 and C6.

Table-4: Potential anticancer agents in each haloxanthone group against HepG2 cancer cell line according to the predicted IC_{50} values

Compounds		Molecular Weight	Atomic Charge				Log IC ₅₀ pred.	IC ₅₀ pred. (µg/mL)
			qC7	qC8a	qC8	qO11		
	Xanthone Structure	196.21	-0.15	-0.12	-0.08	-0.37	1.76	11.35
FLUORO-XANTHONE								
6FX	1-hydroxyl-8-fluoroxanthone	230.20	-0.19	-0.16	0.31	-0.30	-0.90	0.03
7FX	1,3-dihydroxyl-8-fluoroxanthone	246.19	-0.19	-0.16	0.31	-0.30	-0.75	0.04
8FX	2-hydroxyl-1,8-difluoroxanthone	248.19	-0.19	-0.15	0.32	-0.30	-0.70	0.05
9FX	1-hydroxyl-7-fluoroxanthone	230.20	0.32	-0.14	-0.14	-0.33	0.22	0.38
10FX	3-hydroxyl-8-fluoroxanthone	230.20	-0.19	-0.14	0.32	-0.34	0.22	0.38
CHLORO-XANTHONE								
2CX	1-hydroxyl-2,7-dichloroxanthone	281.09	-0.32	-0.16	0.03	-0.32	0.34	0.62
6CX	1-hydroxyl-7-chloroxanthone	246.65	-0.32	-0.16	0.03	-0.33	0.49	0.76
3CX	1-hydroxyl-5,7-dichloroxanthone	281.09	-0.32	-0.15	0.04	-0.32	0.44	0.77

7CX	1-hydroxyl-6-chloroxanthone	246.65	-0.05	-0.14	-0.09	-0.33	0.62	1.02
8CX	1,3-dihydroxyl-7-chloroxanthone	262.65	-0.32	-0.16	0.02	-0.33	0.62	1.11
BROMO-XANTHONE								
5BX	1-hydroxyl-6-bromoxanthone	291.10	-0.09	-0.14	-0.08	-0.33	0.64	1.27
1BX	1-hydroxyl-7-bromoxanthone	291.10	-0.40	-0.15	-0.03	-0.33	0.77	1.72
6BX	1,3-dihydroxyl-6-bromoxanthone	307.10	-0.09	-0.14	-0.08	-0.34	0.77	1.82
7BX	6,8-dihydroxyl-1-bromoxanthone	307.10	0.26	-0.11	-0.10	-0.35	0.87	2.27
4BX	1,3-dihydroxyl-7-bromoxanthone	307.10	-0.39	-0.15	-0.03	-0.33	0.90	2.44
IODO-XANTHONE								
2IX	1-hydroxyl-7-Iodoxanthone	338.10	-0.16	-0.14	-0.08	-0.33	0.64	1.47
1IX	1-hydroxyl-6-Iodoxanthone	338.10	-0.13	-0.14	-0.08	-0.33	0.71	1.74
3IX	8-hydroxyl-2-Iodoxanthone	338.10	-0.15	-0.07	0.23	-0.33	0.77	2.00
6IX	1,3-dihydroxyl-7-Iodoxanthone	354.10	-0.15	-0.14	-0.08	-0.33	0.77	2.08
7IX	8-hydroxyl-3-Iodoxanthone	338.10	-0.15	-0.06	0.23	-0.33	0.84	2.32

Table-4 shows that 20 haloxanthone compounds have strong anticancer activity against HepG2 cells line with predictive IC₅₀ value of 0.03-2.44 µg/mL. Overall, the predictive IC₅₀ value of haloxanthones against the HepG2 cell line was still higher than the predictive IC₅₀ value against the MCF-7 cell line. This indicates that the anticancer activity of haloxanthones tends to be higher against MCF-7 than the HepG2 cell lines. The fluoro-xanthone group has better anticancer activity than the other haloxanthone groups (Chloro-, Bromo- and Iodo-xanthone). The addition of both fluoro and hydroxyl groups at positions C1 and C8 caused an increment in the atomic charge of O11 and C8, and a decrement in the atomic charge of C8a. That has an impact on decreasing the predictive IC₅₀ values for **6FX**, **7FX**, and **8FX** compounds. The addition of chloro-/bromo- group at C1 or C8 atoms affected the charge decrement on the C8 and O11 atoms, which causes an increase in the predictive IC₅₀ values. The increment of the C8 and O11 atomic charges occurs when the chloro- or bromo- groups are located at the C7 position. However, the addition can also cause an unexpected decrement in the atomic charge of the C7 atom as shown in **2CX**, **6CX**, **8CX**, **1BX**, and **4BX** compounds. This factor then causes the predictive IC₅₀ value of chloro- and bromo-xanthone to be higher (lower activity) than fluoro-xanthone.

Based on QSAR studies, 26 haloxanthone compounds have potential anticancer activities against MCF-7 and HepG2 cell lines. These compounds have a predictive IC₅₀ value of less than 6.25 µg/mL based on calculations using the obtained QSAR model (equations 1 and 2). The prediction of the IC₅₀ value of the 26 haloxanthone derivatives is shown in Table-5. Molecular docking studies were carried out on five haloxanthone derivatives to assess their interactions with the targeted protein.

Table-5: Predictive IC₅₀ value of 26 haloxanthone derivatives against MCF-7 and HepG2 cancer cells line

Compounds		IC ₅₀ Predictive (µg/mL)	
		MCF-7	HepG2
8FX	2-hydroxyl-1,8-difluoroxanthone	0.025	0.05
11FX	2-hydroxyl-1,7-difluoroxanthone	0.04	0.44
2CX	1-hydroxyl-2,7-dichloroxanthone	0.0007	0.62
3CX	1-hydroxyl-5,7-dichloroxanthone	0.01	0.77
12FX	1-hydroxyl-6-fluoroxanthone	0.014	1.01
6CX	1-hydroxyl-7-chloroxanthone	0.21	0.76
1FX	2-hydroxyl-1,6-difluoroxanthone	0.0005	1.16
9CX	1-hydroxyl-5-chloroxanthone	0.02	1.14
9FX	1-hydroxyl-7-fluoroxanthone	0.57	0.38
13FX	2-hydroxyl-1-fluoroxanthone	0.08	1.36
6FX	1-hydroxyl-8-fluoroxanthone	1.15	0.03
10CX	1,3-dihydroxyl-5-chloroxanthone	0.13	1.65
11CX	1-hydroxyl-6-chloroxanthone	0.64	1.02
12CX	1,3-dihydroxyl-7-chloroxanthone	0.63	1.11
1IX	1-hydroxyl-6-Iodoxanthone	0.26	1.74
2IX	1-hydroxyl-7-Iodoxanthone	0.51	1.47

1BX	1-hydroxyl-7-bromoxanthone	0.36	1.72
7FX	1,3-dihydroxyl-8-fluoroxanthone	1.91	0.04
14FX	1,3-dihydroxyl-7-fluoroxanthone	1.59	0.56
5BX	1-hydroxyl-6-bromoxanthone	1.40	1.27
4BX	1,3-dihydroxyl-7-bromoxanthone	1.01	2.44
5IX	1,3-dihydroxyl-6-Iodoxanthone	0.99	2.51
6IX	1,3-dihydroxyl-7-Iodoxanthone	1.43	2.08
13CX	1,3-dihydroxyl-6-chloroxanthone	3.11	1.48
15FX	4-hydroxyl-3-fluoroxanthone	1.13	5.92
6BX	1,3-dihydroxyl-6-bromoxanthone	5.94	1.82

Molecular Docking Studies of Haloxanthones against c-JNK Protein

The molecular docking study aims to determine the interactions between ligands and amino acid residues on c-JNK proteins that play a role in inhibiting cancer cell growth²⁶. Molecular docking studies were performed using the three-dimensional structure of the c-JNK protein from the www.worldwide protein databank with code 4e73. Table-6 shows the value of RMSD, free binding energy, and the interaction between ligands and amino acid residues due to molecular docking studies.

Table-6: The RMSD and free binding energy values, and interactions between ligands and amino acid residues on the c-JNK protein

Compound	RMSD (Å)	Free binding energy (kcal/mol)	Binding interaction
0NR	0.81	-8.54
2CX	1.41	-6.73
3CX	1.06	-6.51

10CX	0.94	-6.16	
5BX	1.19	-7.02	
4BX	1.05	-3.73	
Information:			
	van der Waals		Pi-Sigma
	Conventional Hydrogen Bond		Pi-Sulfur
	Carbon Hydrogen Bond		Alkyl
	Unfavorable Positive-Positive		Pi-Alkyl
	Unfavorable Donor-Donor		Halogen (Cl, Br, I)

Table-6 shows that free binding energy between the native ligands 0NR and c-JNK protein was 8.54 kcal/mol with several types of interactions formed (bonding and non-bonding). Types of interactions include van der Waals interactions (GLU109, LEU110, GLY33, SER34, ASN114, ALA113, and ASP112), hydrogen bonds (MET111), hydrogen-carbon bonds (SER115), pi-sigma (ILE32, LEU168, VAL40), alkyl and pi-alkyl (ALA53, Ile86, VAL158, and ALA42), as well as unfavorable positive-positive and donors (LYS30). Several types of interactions are very influential on the c-JNK protein activity.²⁶

Table-5 also shows that the docking of five chloro- and bromo-xanthone derivatives to the c-JNK protein produces an RMSD value of less than 2Å. It is indicated that all compounds' conformation is not much different from the native ligand (0NR).²³ The free binding energy produced by the **2CX**, **3CX**, **10CX**, **5BX**, and **4BX** compounds against the c-JNK protein was -6.73, -6.51, -6.16, -7.02, and -3.73 kcal/mol, respectively. Overall, the resulting free binding energy value was lower than that of the native 0NR ligand, which is remarkable. However, the number of hydrogen bonds produced by **2CX** and **10CX** compounds is higher than 0NR ligands. The **2CX** compound produces three hydrogen bonds to the amino acid residues MET111, LEU110, and GLU109. Meanwhile, the **10CX** compound produces two hydrogen bonds to the amino acid residues of MET111 and GLU109.

CONCLUSION

Twenty-six haloxanthone derivatives were found to give potential anticancer activity against MCF-7 and HepG2 cell lines with predictive IC₅₀ values less than 6.25 µg/mL. The LUMO energy, dipole moment, and atomic charge at C1, C4, and C6 determine the anticancer activity of the haloxanthones against the MCF-7 cancer cell line. Meanwhile, the anticancer activity of haloxanthones against the HepG2 cell line depends on the atomic charge at C7, C8, C8a, and O11. Haloxanthones affected the activity of the c-JNK protein through the formation of hydrogen bonding interactions with MET111 and GLU109, alkyl/pi-alkyl with VAL40, and halogen interactions with MET108 and ASP112.

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