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

T.H. Sugara¹,², Jumina²,.*, E.N. Solikhah³ and H.D. Pranowo²

¹Departement of Pharmacy, Faculty of Health Sciences, Muhammadiyah University of Mataram, 83115, West Nusa Tenggara, Indonesia  
²Departement of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, 55281, Yogyakarta, Indonesia  
³Departement of Pharmacology and Therapy, Faculty of Medicine, Public Health, and Nursing. Universitas Gadjah Mada, 55281, Yogyakarta, Indonesia  

*Corresponding Author: jumina@ugm.ac.id

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 (0NR) 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.

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,³,⁴ hydroxyxanthone,⁶ 3,6-diamino carbonyl methoxy xanthone,⁷ 3-mangosteen,²⁰,²¹ β-mangosteen,²¹ γ-mangosteen,²¹ garcinone D,²¹ and gartanin.²¹ The xanthone derivatives enhance the apoptosis of the cancer cell by stimulating various caspase enzymes and increasing Bax protein.²⁰,²¹ Furthermore, xanthone derivatives also retard the cancer cell cycle through inhibition of various cyclines, Bcl-2 and nuclear factor-kappa B/NF-κB.²⁰,²¹

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.\textsuperscript{16-18} However, the anticancer activity of haloxanthones still needs to be developed because their molecular interaction with the targeted proteins is still unknown.

![Chemical Structure of Xanthone](image)

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.\textsuperscript{5} The data used were xanthone derivatives with IC\textsubscript{50} value ≤ 25 µg/mL for each cancer cell line. The IC\textsubscript{50} values were then converted to log IC\textsubscript{50}.

**Calculation of Molecular Descriptors**
\(^1\)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), \textit{ab initio}, and DFT (Density Functional Theory) methods. The obtained results were compared with the experimental \(^1\)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 \(F_{cal}/F_{tab}\). Furthermore, the best-selected model was used to calculated log IC\textsubscript{50} prediction of training and test set. The model was validated using criteria \(r^2\) prediction > 0.5.\textsuperscript{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\textsubscript{2}O 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.

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 $^1$H-NMR chemical shifts. It was found that the predicted $^1$H-NMR chemical shifts were close 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).

| 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| 4.66| 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 | 6.71 | 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, BM, 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, $F_{cal}/F_{tab}$. These parameters indicate the significance level of the model. Each of these parameters must meet criteria $r^2 > 0.6$, SEE < 0.3, and $F_{cal}/F_{tab} \geq 1$.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 $F_{cal}/F_{tab}$ 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.

$$\log (IC_{50} \text{ MCF-7}) = (7.85) + (100.54 \times \text{LUMO}) + (0.36 \times \text{MD}) - (3.02 \times \text{qC}_6) - (1.71 \times \text{qC}_4) - (7.98 \times \text{qC}_1)$$

$$\log (IC_{50} \text{ HepG2}) = -6.034 - (1.198 \times \text{qC}_7) + (9.274 \times \text{qC}_8a) - (1.887 \times \text{qC}_8) - (23.354 \times \text{qO}_{11})$$

Equation-1 showed that the anticancer activity of haloxanthones 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.](image)

Equation-2 shows that the anticancer activity of haloxanthones 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 haloxanthones 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 haloxanthones against the HepG2 cancer cells line.
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. (µg/mL) |
|-------------------------|------------------|------|---------------|---------------|---------------------|-------------------------|
| Xanthone Structure      | 196.21           | -0.08| 2.95          | -0.12         | -0.17              | -0.08                   | 2.03                     | 21.12                   |
| **FLUORO-XANTHONE**     |                  |      |               |               |                     |                         |
| 1FX                     | 248.19           | -0.09| 5.39          | 0.34          | 0.15               | 0.31                    | -2.72                    | 0.0005                  |
| 2FX                     | 246.19           | -0.09| 2.85          | 0.06          | 0.26               | 0.12                    | -2.1                     | 0.002                   |
| 3FX                     | 246.19           | -0.1 | 3.86          | -0.1          | -0.14              | 0.26                    | -1.99                    | 0.003                   |
| 4FX                     | 262.19           | -0.1 | 2.07          | 0.31          | 0.27               | -0.07                   | -1.78                    | 0.004                   |
| 5FX                     | 246.19           | -0.09| 2.91          | -0.12         | -0.13              | 0.25                    | -1.73                    | 0.005                   |
| **CHLORO-XANTHONE**     |                  |      |               |               |                     |                         |
| 1CX                     | 281.09           | -0.1 | 6.81          | -0.1          | -0.15              | 0.47                    | -3.48                    | 0.0001                  |
| 2CX                     | 281.09           | -0.09| 5.03          | -0.02         | -0.16              | 0.4                     | -2.62                    | 0.0007                  |
| 3CX                     | 281.09           | -0.09| 5.16          | 0.08          | -0.16              | 0.24                    | -1.47                    | 0.01                    |
| 4CX                     | 262.65           | -0.08| 2.83          | 0.28          | -0.43              | 0.24                    | -1.14                    | 0.02                    |
| 5CX                     | 281.09           | -0.09| 4.28          | -0.02         | -0.43              | 0.25                    | -1.14                    | 0.02                    |
| **BROMO-XANTHONE**      |                  |      |               |               |                     |                         |
| 1BX                     | 291.1            | -0.08| 5.29          | -0.07         | -0.17              | 0.23                    | 0.09                     | 0.36                    |
| 2BX                     | 307.1            | -0.09| 3.86          | 0.31          | -0.49              | -0.06                   | 0.34                     | 0.67                    |
| 3BX                     | 291.1            | -0.08| 4.62          | -0.1          | -0.16              | 0.23                    | 0.41                     | 0.75                    |
The presence of hydroxyl and halogen groups at C1 and C2 would significantly increase 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 increase the atomic charge on the C1 atom. This can be seen in the fluoroxanthone compounds, such as doxorubicin, rifampin, and 5-fluorouracil.

Table-3 shows that 20 haloxanthone compounds have strong anticancer activity against the MCF-7 cell line. Almost all of them had predictive IC₅₀ values below 1 μg/mL, except for compounds 4BX (1.01 µg/mL) and 5BX (1.40 μg/mL). The Council of Scientific and Industrial Research (CSIR) classifies the cytotoxic activity of a compound into four categories based on the IC₅₀ value, including inactive (IC₅₀ > 50 µg/mL), weak (15-50 µg/mL), moderate (6.25-15 µg/mL), and strong/potent (< 6.25 µg/mL). Overall, fluoroxanthone compounds have better anticancer activity than other haloxanthone derivatives. However, the lowest predictive IC₅₀ value was obtained for 1-hydroxy-2,8-dichloroxanthone (1CX), which was 0.0001 μg/mL. This value is much lower when compared to the experimental IC₅₀ value of several standard drug compounds, such as doxorubicin, rifampin, and 5-fluorouracil.²⁵

A low predictive IC₅₀ 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 IC₅₀ values

| Compounds       | Molecular Weight | Atomic Charge | Log IC₅₀ pred. | IC₅₀ pred. (µg/mL) |
|-----------------|-----------------|---------------|----------------|-------------------|
|                 | Xanthone Structure | qC7 | qC8a | qC8 | qO11 |        |                |
| FLUORO-XANTHONE |                 | 196.21        | -0.15 | -0.12 | -0.08 | -0.37  | 1.76    | 11.35  |
| 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   |
haloxanthone derivatives to assess their interactions with the targeted protein. Based on QSAR studies, 26 haloxanthone compounds have potential anticancer activities against MCF-7 (lower activity) than fluoro-xanthone. 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 impact on decreasing 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.
### 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 growth\(^\text{26}\). 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                         |                   |

Molecular Docking Studies of Haloxanthones against c-JNK Protein

| Compound | RMSD (Å) | Free binding energy (kcal/mol) | Binding interaction |
|----------|----------|-------------------------------|-------------------|
| 1BX      | 0.36     | 1.72                          |                   |
| 7FX      | 1.91     | 0.04                          |                   |
| 14FX     | 1.59     | 0.56                          |                   |
| 5BX      | 1.40     | 1.27                          |                   |
| 4BX      | 1.01     | 2.44                          |                   |
| 5IX      | 0.99     | 2.51                          |                   |
| 6IX      | 1.43     | 2.08                          |                   |
| 13CX     | 3.11     | 1.48                          |                   |
| 15FX     | 1.13     | 5.92                          |                   |
| 6IX      | 5.94     | 1.82                          |                   |
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 walls 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.\(^\text{26}\)

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).\(^\text{23}\) 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 IC\(_{50}\) values less than 6.25 \(\mu\)g/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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