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DESIGN OF QUINOLINE-CHALCONE HYBRIDS DERIVATIVES AS ANTICANCER USING QSAR AND MOLECULAR DOCKING STUDIES

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ABSTRACT

Cancer is a disease caused by abnormal growth of cells that has infected many people worldwide. This reason encourages many scientists to develop medicinal compounds for cancer therapy. One type of drug that has the potential to be developed is based on the quinone-chalcone structure. A method that is highly effective for drug development is the quantitative structure-activity relationship (QSAR) method. In this study, the QSAR problem modeling was developed for quinone-chalcone derivative compounds that have been synthesized previously. Some parameters (descriptors) used in this study were obtained from quantum mechanical modeling with AM1 and ZINDO/S semiempirical methods. The relationship between descriptors and anticancer activity (pIC50) was obtained using the Multiple Linear Regression (MLR) method, so that the QSAR model equation $\text{pIC50} = -6.39 + 0.17(\text{binding}) - 1.87(\text{LUMO}) - 9.29(\text{qC2}) + 9.67(\text{qC1}) - 41.02(\text{qC5}) - 9.86(\text{qC14}) + 6.94(\text{qC18})$ was obtained. This equation has a value of R = 0.998 with Sig = 0.017. Therefore, the equation is valid, and it represents the relationship between descriptors and pIC50. Based on the QSAR equation, four new compound designs were proposed and tested using molecular docking. The results from the pIC50 prediction and molecular docking showed that compound design -12 has the best potential as an anticancer compound.

Keywords: cancer, QSAR, quinone-chalcone, molecular docking

ABSTRAK

Kanker merupakan penyakit yang disebabkan oleh pertumbuhan sel abnormal yang telah menginfeksi banyak orang di seluruh dunia. Alasan tersebut mendorong banyak ilmuwan untuk mengembangkan senyawa obat untuk terapi kanker. Salah satu jenis obat yang berpotensi untuk dikembangkan adalah yang berbasis pada struktur kuinon-kalkon. Salah satu metode yang sangat efektif untuk pengembangan obat adalah metode *quantitative structure-activity relationship* (QSAR). Dalam penelitian ini, pemodelan masalah QSAR dikembangkan untuk senyawa turunan kuinon-kalkon yang telah disintesis sebelumnya. Beberapa parameter (deskriptor) yang digunakan dalam penelitian ini diperoleh dari pemodelan mekanika kuantum dengan metode semiempiris AM1 dan ZINDO/S. Hubungan antara deskriptor dengan aktivitas antikanker (pIC50) diperoleh dengan menggunakan metode *Multiple Linear Regression* (MLR), sehingga diperoleh persamaan model QSAR $\text{pIC50} = -6{,}39 + 0{,}17(\text{binding}) - 1{,}87(\text{LUMO}) - 9{,}29(\text{qC2}) + 9{,}67(\text{qC1}) - 41{,}02(\text{qC5}) - 9{,}86(\text{qC14}) + 6{,}94(\text{qC18})$.
Persamaan ini memiliki nilai R = 0,998 dengan Sig = 0,017. Oleh karena itu, persamaan tersebut valid dan menggambarkan hubungan antara deskriptor dengan pIC50. Berdasarkan persamaan QSAR, empat desain senyawa baru diusulkan dan diuji menggunakan molecular docking. Hasil prediksi pIC50 dan *molecular docking* menunjukkan bahwa desain senyawa -12 memiliki potensi terbaik sebagai senyawa antikanker.

Kata kunci: kanker, QSAR, kuinon-kalkon, *molecular docking*

INTRODUCTION

Cancer is a health disorder caused by abnormal and potentially invasive cell growth. Based on data from WHO in 2020, new cases of cancer reached 10 million cases. Based on data compiled by WHO, in 2020, the newest cancer cases include breast cancer (2.26 million cases), lung cancer (2.21 million cases), colon and rectal cancer (1.41. cases), skin cancer (1.2 million cases) and stomach cancer (1.09 cases). The highest mortality rate is lung cancer with 1.8 million death cases (Ferlay J et al., 2020; World Health Organization, 2022). In Indonesia, in 2020, there were 396,314 new cancer cases with 234,511 deaths. Women are

a group with a high risk of developing cancer, such as breast cancer as many as 65,858 cases, and cervical cancer 36,633, while in men there were 25,943 lung cancer sufferers (Tim Website Dinkes 2023).

One of the known therapeutic methods for cancer treatment is chemotherapy. However, chemotherapy has side effects and often affects healthy tissues and organs surrounding the cancer cells (Love et al. 2013). These reasons encourage researchers to discover other alternatives to cancer treatment, one of which is with nature. Some natural products are reported to have anticancer activity. Aliviyanti reported the cytotoxic effect of bajakah extract has potential active compound as breast cancer inhibitor (Aliviyanti et al. 2021). Polyphenols and bioactive compound present in rosemary extract also reported has activity as anticancer. Rosemary extracts contain high concentrations of polyphenols such as carnosic acid and rosmarinic acid, which may act as anticancer agents (Moore et al. 2016). However, the use of therapeutics derived from natural extracts has little selectivity. Therefore, several studies were developed to search for derivatives of natural materials that have high selectivity and activity as anticancers to increase the effectiveness of treatment.

Several researchers reported that derivatives of compounds such as xanthone, quinone, chalcone, or hybrid compounds have been successfully developed as anticancer agents. One of the potential natural products, chalcones, is a potential compound that can be developed as an anticancer agent. Several studies have reported chalcone-derived compounds have promising anticancer activity. Hou's group successfully synthesized ten new steroidal-chalcone hybrid compounds that can inhibit cancer in MDA-MB-231 cells related to breast cancer (Hou et al. 2020). Luo's group in 2021, also succeeded in synthesizing a hybrid compound of ligustrazine-chalcone which has potential as an anti-triple negative breast cancer (anti-TNBC) agent (Luo et al. 2021).

In addition, the α, β-unsaturated moieties in chalcone and quinoline have also attracted some researchers to maximize their potential as anticancer agents. This is because the α, β - unsaturated moieties of chalcone are good precursors to be used in synthesizing flavonoid-based compounds. Furthermore, chalcone is considered to have privileged structures in the process of developing chalcone-derived compounds (Ducki et al. 2009; Zhang et al. 2013; Li et al. 2017). On the other hand, quinone is unique in that the heteroatom ring has a nitrogen atom, so it can be expected to have good bioactivity. The presence of nitrogen atoms in quinones provides the possibility of the compound forming hydrogen bonds with target proteins and also can increase the solubility of the drug compound and drug absorption. Moreover, quinoline structure already developed a broadly anticancer drug treatment (Yakes et al. 2011; Álvarez et al. 2016). 3-phenyl quinolinyl-chalcones are one of the quinone-chalcone hybrid compounds successfully synthesized by Tseng's group. This compound shows promising potential as an anti-TNBC compound with an IC_{50} value of less than 1.05μM (Tseng et al. 2015).

The development of synthesizing new quinoline-chalcone-based compounds is still being developed for new drug development. One of the drug development methods is using quantitative structure-activity relationship (QSAR) analysis. QSAR analysis is a medium to connect the bioactivity of a compound with several physiochemical variables (descriptors) of the compound (Hari Narayana Moorthy et al. 2011; Su et al. 2011). The purpose of QSAR is to predict potential bioactivity quantitatively based on certain properties (descriptors) of a compound. The result of a QSAR study is a mathematical equation in which the physiochemical properties of a compound are the variables, and the potential bioactivity is the dependent variable. Based on the QSAR equation obtained, it can be recommended and developed a new compound design that is expected to have higher bioactivity (Syahri et al. 2020)

In this study, a QSAR study will be conducted for hybrid quinone-chalcone compounds successfully synthesized by (Mirzaei et al. 2020). Based on QSAR model obtained, then new compounds design is proposed, which expected to have better bioactivity potential as anticancer.

MATERIAL AND METHODS

Software and Hardware

Compound calculations and modeling are done using a laptop with Intel™ Core i7 7200U, 8 GB RAM, 250GB SSD, and 2 GB NVIDIA 930MX GPU. The software used includes the Windows 10 operating system, ChemBioDraw Ultra 14 drawing the compound structure, Avogadro for 3D modeling, and Orca for geometry optimization and electronic calculations.

The series of quinoline-chalcone hybrid compounds **1-10** were obtained from previously published research by Mirzae et al. 2020 (Mirzaei et al. 2020) with the structure depicted in **Figure 1**. The data set consisted of 10 quinoline-chalcone hybrid compounds with IC_{50} data available in **Table 1**. The inhibitory concentration of the compounds in µM was converted to M and transformed to the logarithmic scale of the reciprocal into pIC_{50} (-log IC_{50}), so that the resulting concentration would be proportional to the interaction energy of the compound with the receptor and reduce the skewness of the data set.

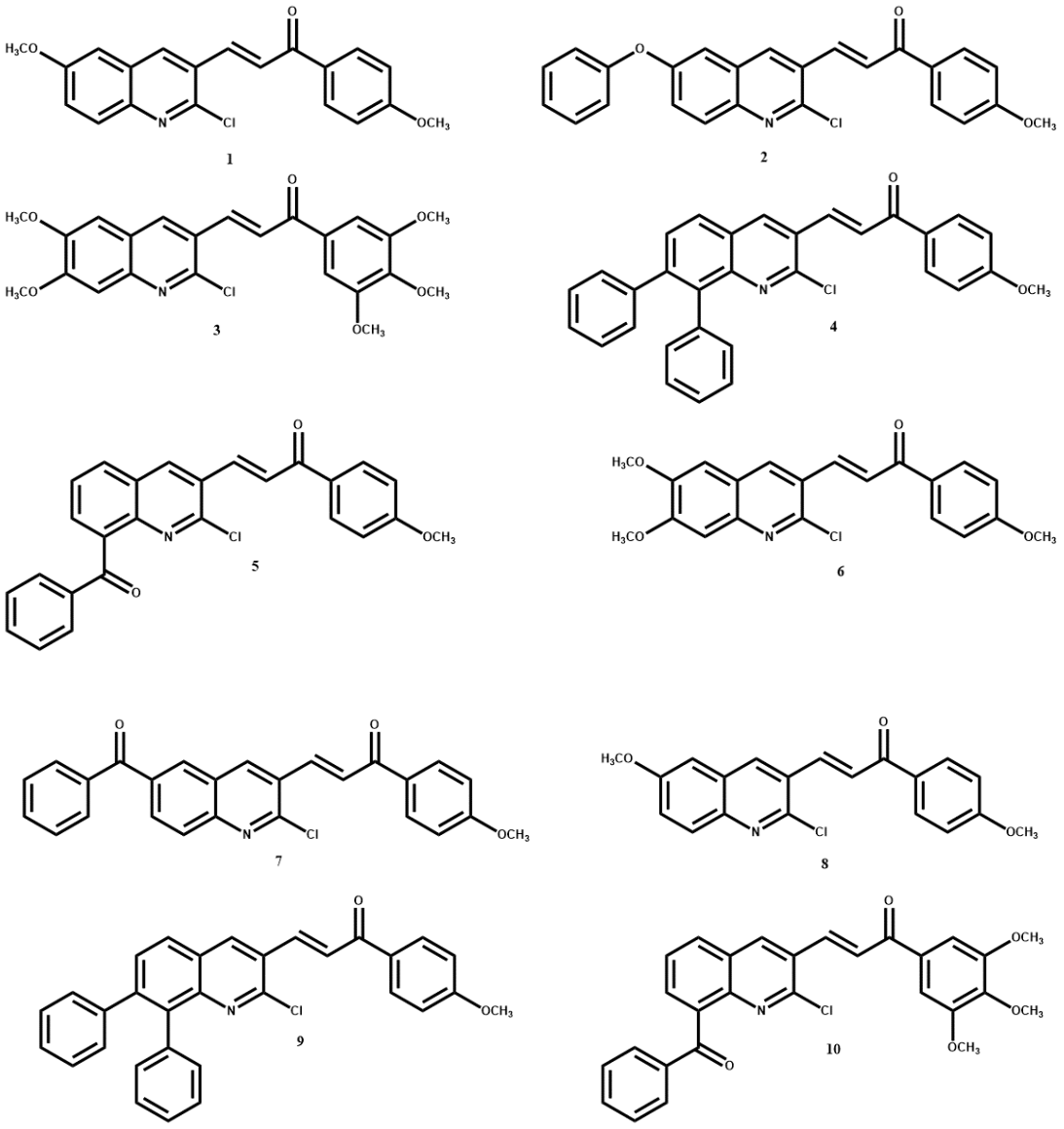

Figure 1. Quinoline-chalcone for QSAR study from Hou experiment.

Table 1. Anticancer activity for 10 structures for steroid-chalcone hybrids

Compound	IC_{50} (M)	pIC_{50}
1	0.00007271	1.138
2	0.000101	0.996
3	0.00002186	1.660
4	0.00003765	1.424
5	0.00001383	1.859
6	0.00001488	1.827
7	0.000003122	2.506
8	0.00005576	1.254
9	0.00002811	1.551
10	0.00000232	2.635

Methods

Geometry Optimization of Quinoline-Chalcone Derivatives

The structure of the quinoline-chalcone hybrid compound in this study was drawn using ChemBioDraw Ultra 14.0 and then converted into 3D using Avogadro. The 3D structure obtained was then optimized to get the optimum geometry using semiempirical method by Austin Model 1 (AM1). Electronic transition calculations to obtain HOMO-LUMO compounds were carried out using Orca with the ZINDO/S semiempirical method.

Determining Descriptor

Determination of the selected QSAR equation begins with the selection of descriptors that will be used to predict the activity of quinoline-chalcone based anticancer compounds. The selected descriptors must represent several properties of the compound molecule. In this study, some of the descriptors was collected and analyzed. Some electronic properties were collected such as atomic charge, LUMO, HOMO, E_{gap}, dipole moment, and binding energy. Log P and log S were selected to represent lipophilic properties. Some of steric properties like molecular weight (M_w), molecular surface, and refractivity also collected. Atomic charge, LUMO, HOMO, E_{gap}, dipole moment was collected from Orca calculation using semiempirical method (ZINDO/S).

Build QSAR model through multiple linear regression (MRL) analysis

The QSAR model equation is obtained through the MLR method to determine the relationship between the descriptor that has been obtained with the compound's activity as an anticancer (pIC50 value). In this analysis, the multivariable linear regression method was used with the backward method through the SPSS 22.0 program. Several QSAR models will be obtained from MLR analysis. The selection of the best QSAR equation is based on several criteria such as $R^2 > 0.6$ value, PRESS value, and Fcal/Ftab comparison value. The QSAR equation obtained is then used as a guide in designing new quinone-chalcone compounds.

Design Proposed Compound and Molecular Docking

The proposed new compound was designed based on the QSAR equation obtained. The IC_{50} values of the proposed new anticancer compounds were calculated based on the QSAR equation. The bioactivity of the new compound as an anticancer was identified through molecular docking to determine its interaction with the target protein. In this study, molecular docking used VEGFR target protein whose crystal structure was uploaded through Protein Data Bank (ID: 2OH4) with a resolution of 2.05 A. Molecular docking was performed using Autodock Vina and analyzed the interaction through Biovia Discovery Studio (Trott and Olson 2009; Eberhardt et al. 2021).

RESULTS AND DISCUSSION

Determination of QSAR model

The results of MLR analysis and statistical parameters to find the QSAR model is available in **Table 3**. Based on statistical calculations through MLR with the backward method, 4 QSAR equation models were obtained with different R2 values. Four QSAR models in full are available in Table 2. In the table, QSAR equation models 2, 3, and 4 have Sig values < 0.05, so it can be said that the variables used in this equation significantly affect the value of y (pIC_{50}). The QSAR 1 equation model has the best R2 value compared to the other QSAR model equations. However, the resulting Sig value is >0.05, so it can be said that the variables in the QSAR 1 model do not affect the y (pIC_{50}) value simultaneously.

Table 3. QSAR model from MLR method and statistical parameters

Model	Variable	R	R^2	F	Sig	PRESS
1	Dipole, binding, LUMO, qC2, qC1, qC5, qC14, qC18	0.998	0.996	28.06	0.145	-
2	Binding, LUMO, qC2, qC1, qC5, qC14, qC18	0.998	0.995	57.50	0.017	0.013
3	Binding, LUMO, qC2, qC1, qC5, qC18	0.992	0.984	31.75	0.008	0.042
4	LUMO, qC2, qC1, qC5, qC18	0.998	0.977	34.17	0.002	0.061

Each QSAR model that is obtained and meets the criteria for R2, sig, and F values, then continues with the validation process. Model 1 was not included in the validation process because the sig value obtained was > 0.05. The validation process of the QSAR equation is done by calculating the PRESS value of each QSAR model. The best equation is the one with the smallest PRESS value. PRESS values and other statistical parameter results are available in Table 3. Based on the calculated PRESS value, model 2 has the smallest value compared to the PRESS value of models 3 and 4. So based on the value of other statistical parameters, model 2 is used in the next QSAR study to design new anticancer compounds. The QSAR model 2 is $pIC50 = -6.39 + 0.17(binding) - 1.87(LUMO) - 9.29(qC2) + 9.67(qC1) - 41.02(qC5) - 9.86(qC14) + 6.94(qC18)$

Design novel quinone-chalcone hybrid as anticancer

Based on model 2, independent variables that influence the anticancer activity is binding energy, LUMO, and charge in carbon atom (C2, C1, C5, C14, and C18). To design new anticancer compounds, several variables in the QSAR 2 model equation must be considered. Good anticancer activity means having a high pIC50 value. To increase the pIC50 value, several variables that have positive constant values must be increased such as binding energy, charge on C1, and charge on C18. Meanwhile, variables with negative values mean that these variables can reduce the value of pIC50. In addition to considering variables in QSAR, previous experimental results are also taken into consideration in determining novel compounds.

Based on these considerations, three novel compounds were designed and are expected to have potential as new anticancer compounds. In previous research results, it was shown that the presence of benzoyl groups in quinone-chalcone hybrid compounds had the best anticancer potential. Based on the QSAR equation obtained, the pIC value can be increased by decreasing the LUMO value of the compound (LUMO is getting negative). The LUMO energy level can be lowered by adding several electron-withdrawing groups such as nitro (NO_2), halide, or carbonyl groups. However, the addition of EWG groups can also reduce the reactivity of the compound so that it is less favorable in the synthesis process. Therefore, the number of EWG groups needs to be considered. The charge on some desired carbon atoms can be adjusted by adding several groups, either right on the carbon atom or on the carbon atom. Based on that, this study proposed three novel quinone-chalcone hybrids that are shown in **Figure 2**.

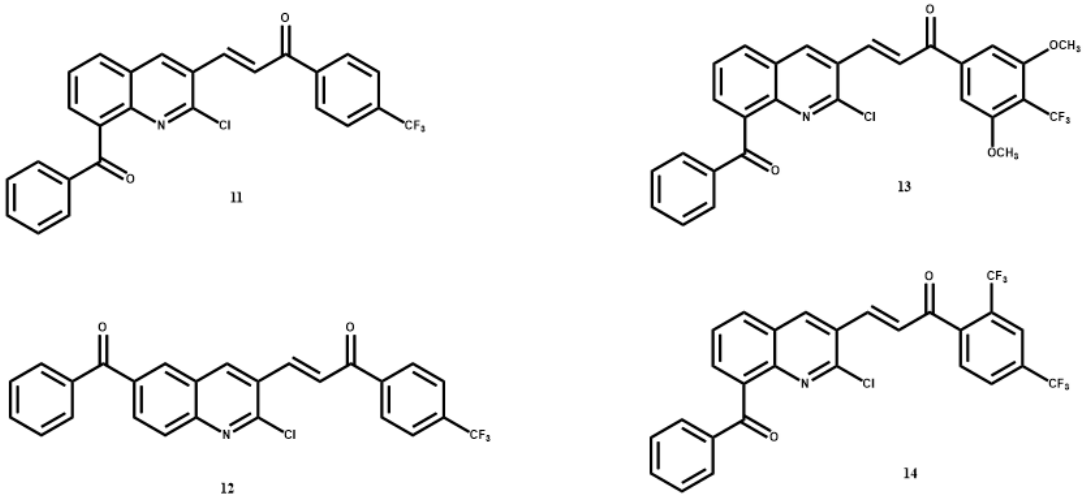

Figure 2. Design new quinone-chalcone hybrid

Table 4. pIC_{50} prediction

Ligand	pIC50*
Native	-
5	4.86
10	5.63
11	5.97
12	6.67
13	3.01
14	3.45

Figure 2 shows the design of new drug compounds with new EWG groups, namely trifluoro methyl, and CF_3. All new compounds are then predicted for their anticancer activity through QSAR model 2 calculations. Based on QSAR calculations, high anticancer activity is possessed by compounds 11, 12 and 14. This is in accordance with the prediction that the introduction of EWG groups on the benzene ring can reduce the LUMO level of the compound. Compounds 13 have lower activity compared to the other three new compounds. In compound 13, the addition of (-CF_3) groups does not help to reduce the LUMO value because of the two alkoxy groups that can play a role in increasing the LUMO value of the compound, so the pIC_{50} prediction becomes small.

Table 5. Docking results and ligand-protein interaction

Ligand	Binding Energy	Hydrogen bond	Hydrophobic interaction
Native	-11.5	Asp1044	Leu887, Ile890, Ile1042, Val896, Leu1017, His1024, Cys1043, Ile886, Val914, Ala864, Glu915, Asn921, Gly920, Phe916, Leu838, Phe1045, Leu1033, Val 846, Val897, Glu883
7	-10.1	Cys1043	Val914, Glu883, Leu887, Asp1044. Ile890, Ile886, Val897, Ala864, Lys866, Leu838, Phe916, Cys917, Leu1033, Gly920, Phe1045, Val846, Val914
12	-10.4	Cys917	Arg1049, Leu838, Leu1047, Phe1045, Asp1044, Cys1043, Val846, Lys886, Val914, Ala864, Glu915, Leu1033, Gly920, Phe916, Glu848, Lys918

Molecular docking was performed to support the results of the QSAR equation used. It was performed by tethering ligands with the highest anticancer activity performance **12** to the target protein vascular endothelial factor receptor (VEGFR). In this study, molecular docking has been validated using the redocking method. The native ligand of protein was extracted from the crystal structure and docked back to the binding site. The docking methods is valid if the root mean square deviation (RMSD) is ≤ 2 Å (Ferdian et al. 2021). The grid size used in this study is $40 \times 52 \times 40$ with center coordinate X = 3.173; Y = 33.766; Z = 17.175. The RMSD got from this method is 0.219 Å.

The results of molecular docking are binding energy and bonding interaction between ligand and protein. Binding energy is related to the stability of interaction between protein and ligand. More negative binding energy means more stable interaction. The molecular docking results are available in **Table 5**. Observation through molecular docking shows that compound **12** can form a similar interaction between ligand and amino acid residue. The similarity of interaction with amino acid residues suggests that the ligands have similar mechanisms of inhibition of target proteins. Moreover, compound **12** has a more negative binding energy value than compound **7**, which means compound **12** can interact with the binding site more stable than compound **7**. Therefore, it can be predicted that compounds **12** has good anticancer activity, either from QSAR or molecular docking studies.

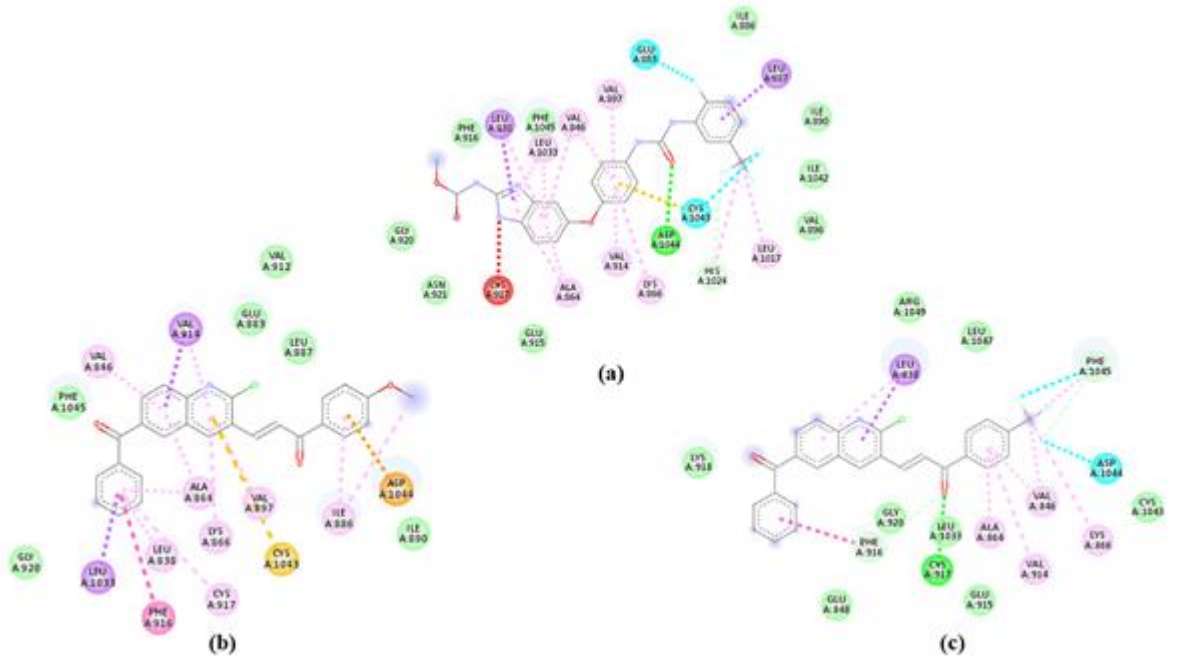

Figure 3. Interaction of ligand with protein (a) native ligand (b) compound **7**, and (c) compound **12**

CONCLUSIONS

The anticancer activity value of quinone-chalcone hybrid compounds has a quantitative relationship to various physicochemical properties of the compound, Based on the results of QSAR studies, it shows that quinone-chalcone compounds **1-10** as anticancer produce equation models $\mathrm{pIC50} = -6.39 + 0.17(\mathrm{binding}) - 1.87(\mathrm{LUMO}) - 9.29(\mathrm{qC2}) + 9.67(\mathrm{qC1}) - 41.02(\mathrm{qC5}) - 9.86(\mathrm{qC14}) + 6.94(\mathrm{qC18})$. Descriptors that affect anticancer activity in this study include the value of binding energy, LUMO, and atomic charge on C1, C2, C5, C14, and C18. Modification of the group based on the QSAR equation resulted in the proposed compounds **11-**

14. Based on the QSAR equation, the predicted pIC_{50} are 5.97, 6.67, 3.01, and 3.45 for compounds **11, 12, 13** and **14**. A compound with higher pIC_{50}, compound **12**, was conducted molecular docking. The results of molecular docking show that compound **12** has a similar binding interaction with compound **7** and has more negative binding energy than compound **7**

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