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Novel Anti-leukemia Calanone Compounds by Quantitative Structure-Activity Relationship AM1 Semiempirical Method

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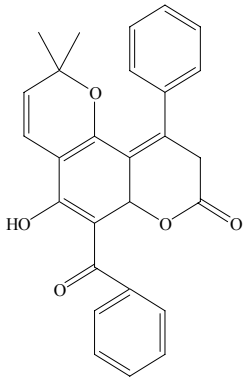
***Abstract*—**Quantitative Structure-Activity Relationship (QSAR) approach for discovering novel more active Calanone derivative as anti-leukemia compound has been conducted. There are 6 experimental activities of Calanone compounds against leukemia cell L1210 that are used as material of the research. Calculation of theoretical predictors (independent variables) was performed by AM1 semiempirical method. The QSAR equation is determined by Principle Component Regression (PCR) analysis, with Log IC_{50} as dependent variable and the independent variables are atomic net charges, dipole moment (μ), and coefficient partition of n-octanol/water (Log P). Three novel Calanone derivatives that obtained by this research have higher activity against leukemia cell L1210 than pure Calanone.

***Keywords*—**AM1 semiempirical calculation, Calanone, Principle Component Regression, QSAR approach.

I. Introduction

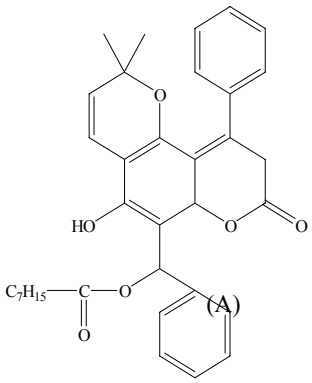
COMPUTATIONAL chemistry is a new field of study in chemistry which used the computer as a tool to generate data from a model of chemical system [1]. Quantitative Structure-Activity Relationship (QSAR) analysis, one of computational chemistry applications can make the discovery of new drug compound become more efficient. It can reduce "trial and error" synthesis, the usage of chemicals and also the cost of drug production [2]-[4].

Calanone is a natural product compound that has activity against leukemia cell L1210. In previous study, some derivatives of this compound were synthesized in order to increase the activity [3]. But only one derivative from that previous work has higher activity than pure Calanone. The previous work was done without QSAR approach. In the next work an effort to guide the synthesis of new more active Calanone derivative is needed. QSAR approach can be applied to guide structural modification of pure Calanone. It has two crucial problems. They are selecting computational chemistry calculation method and statistical analysis. Because of the large number of atom in Calanone structure and computer processor capability, the AM1 semiempirical method is chosen [2]. The lack of experimental data becomes a reason to use Principle Component Analysis (PCA) as statistical analysis. By using PCA it is possible to use more than 6 theoretical predictors of each compound [5].



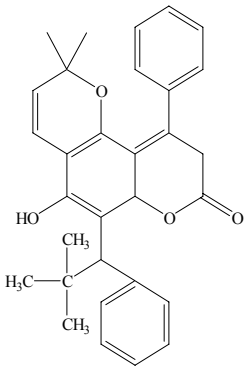
a. Kalanon

Calanone



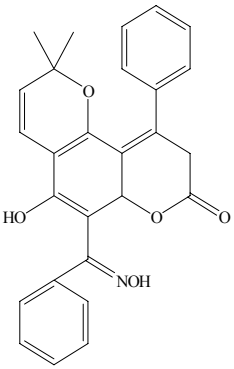
b. Kalaniloktanoat

Calanyl Octanoic



c. Isobutil kalanon

Isobuthyl Calanone



d. Oksim kalanon

Oxym Calanone

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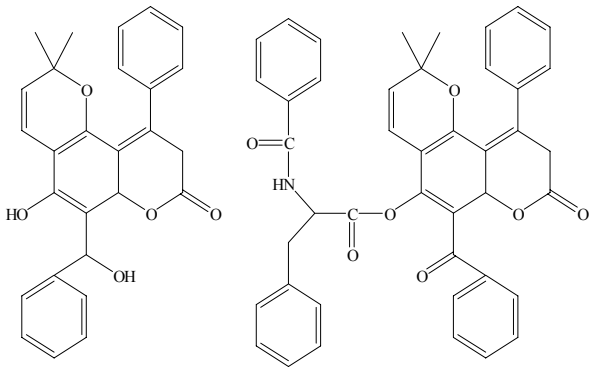

Calanol Benzoilphenylalanine Ester Calanone

Fig. 1 Molecular structure and experimental anti-leukemia activity of Calanone compounds (μg/mL); A = 59.4; B = 75.2; C = 105.5; D = 83.6; E = 70.0 and F = 52.5 [3].

Producing novel Calanone derivatives by QSAR approach is conducted by several steps such as: modeling the three dimensional (3D) structure of Calanone and its derivatives, calculation theoretical predictors, constructing the QSAR equation, designing and synthesis new Calanone compound, and finally the determination of anti-leukemia activity. This paper presents the influence of QSAR approach that can make the synthesis some new compounds become more focus.

II. Method

Materials and apparatus

This research consists of theoretical and experimental studies. Theoretical study or QSAR analysis used six data of experimental anti-leukemia activity of Calanone derivatives (see Fig. 1) to produce: the QSAR equation and the model of novel Calanone derivatives. Theoretical study was conducted on 1 personal computer (PC), Intel Pentium IV 2.66 GHz processor, 1 GB RAM, and 40 GB Harddisk Drive. Calculating electronic and lipophylic predictor was conducted by Hyperchem ver 6.0 program. It has been installed in the PC of Austria-Indonesia Centre for Computational Chemistry (AIC), Chemistry Department, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Experimental study is synthesis the novel compound based on QSAR recommendation. It was conducted at Chemistry Department, Faculty of Science and Engineering, Universitas Jenderal Soedirman, Purwokerto, Indonesia. The pure Calanone compound is obtained from Natural Product Laboratory, Chemistry Research Center, The Indonesian Institute of Sciences (LIPI), Serpong, Indonesia.

In the case of atomic charges calculation, only the elements in the main structure of Calanone was used as predictors (see Fig. 2). Additional parameters of computational calculation are RHF spin pairing, lowest state and 0.001 kcal/mole of RMS gradient.

Molecular modeling of Calanone derivatives

Each compound was drawn into a three dimensional (3D) model of compound. Then, AM1 semiempirical calculation was chosen for each model.

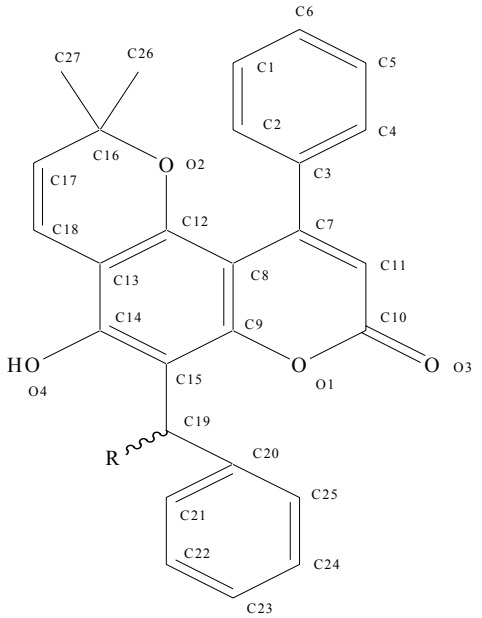

Fig. 2 Numbering of atoms in the main structure of Calanone that used in atomic charge calculation

TABLE I PREDICTORS OF CALANONE COMPOUNDS THAT USED TO PRODUCE QSAR EQUATION AND HOW TO OBTAIN THEM

Predictor	Symbol	Unit	How to obtain
Atomic net charges of the compound	Q	Coulomb	Optimizing the structure of the compound by AM1 semiempirical calculation
Dipole moment	μ	Debye	Optimizing the structure of the compound by AM1 semiempirical calculation
Partition coefficient	log P	-	QSAR Properties calculation

Constructing QSAR equation and designing new compound

Determination of QSAR equation was started by PCA to the theoretical predictors until we get the latent variables. After that, Principle Component Regression (PCR) was conducted to the results of PCA. The anti-leukemia activity is dependent variable and latent variables as independent variables. By the statistical parameters, such as correlation coefficient, standard error, the selected QSAR equation was chosen. Below is the form of QSAR equation.

$$A = C + b_1.x_1 + b_2.x_2 + b_3.x_3 + \ldots\ldots.$$

Where: A = the anti-leukemia activity of the compound
C = a constant
$x_n = n^{th}$ latent variable
b_n = a coefficient of n^{th} latent variable

Then the designing of new Calanone derivative was carried out by following some of consideration such as: which is the new compound that has higher activity? and the ease of synthesis procedure for making it. By this consideration, the more active new Calanone compound can be produced.

Synthesis new Calanone compounds

The new derivative of Calanone based on QSAR recommendation are gemdiol Calanone, 2,4-dinitrophenyl hydrazone Calanone and 2,4,6-trinitrophenyl hydrazone Calanone. They were synthesized by certain procedure. Gemdiol Calanone was made by adding 424 mg pure Calanone into 40 mL glacial acetic acid and 551.68 mg $Pb(OAc)_2$, then reflux 6 hours at 55 ^{0}C. After the brown solution was obtained, purify the solution by column chromatography. The static phase is gel silica and the mobile phase are n-hexane and ethyl acetate in ratio 7 : 2 (v/v).

Synthesis of 2,4-dinitrophenylhydrazone Calanone was conducted by reacting 424 mg of Calanone and 237.7 mg 2,4-dinitrophenylhydrazone. The solvent for this reaction was a mixture of 1 mL glacial acetic acid and 20 mL ethanol. Then reflux the mixture for 8 hours at 78 ^{0}C. Purification the product of reaction was conducted by re-crystalization method with a mixture of chloroform and n-hexane in ratio 2:1 as a solvent.

The 2,4,6-trinitrophenylhydrazone Calanone was made by reacting 424 mg of Calanone and 328 mg of 2,4,6-trinitrophenylhydrazone and ethanol as a solvent, then reflux 4 hours at 78 ^{0}C. Purification the product of reaction was conducted by re-crystalization method with a mixture dichlormethane and n-hexane in ratio 2:1 as a solvent.

All the novel compounds were characterized by spot at Thin Layer Chromatography (TLC), melting point determination, FTIR and LC-MS. After that, the anti-leukemia activity was determined against leukemia L1210 cell.

The activity of new compound was determined against the L1210 leukemia cell. This step was conducted at The Nuclear Energy Research Center of Indonesia, Serpong, Indonesia.

III. RESULTS AND DISCUSSION

Generating predictor data and its representation

Modeling the chemical system into three-dimensional structure (3D) is important. It was done for representing the real chemical system. The detail checking to 3D structure of the model is also important. If the structure is wrong, the calculation will get wrong results. Modeling chemical system is one of important things besides choosing calculation method in computational chemistry study [1]. The result from modeling step was a 3D coordinate of the compound. This coordinate was applied for calculating predictors.

Predictors were generated by optimization of Calanone structure based on AM1 semiempirical calculation. The advantages of using AM1 semiempirical method are not long time consuming in calculation, electronic structure data can be obtained and suitable for big structure organic compound such as Calanone [2]. The AM1 semiempirical calculation method is already applied in QSAR analysis of antibacterial Fluoroquinolone compounds and gives a good compromising between the quality QSAR equation and the need of computer capability [3]. The predictors were the physicochemical properties which influence the anti-leukemia activity of Calanone compounds (see Table II).

Table II shows some theoretical properties of Calanone compounds. Atomic net charges and dipole moment describes the electronic properties, where partition coefficient is solubility property of compounds. In the atomic net charges value, the most significant difference was occurred in the 19th Carbon in the Calanone structure (C_{19}). The atomic charge was significantly different because of the difference of functional group in each Calanone compound. For instance, atomic net charges of C_{19} in oxym Calanone and isobuthyl Calanone are -0.005 and -0.002 Coulomb, respectively. These are happened because the functional group on their C_{19} can push the electron density to the C_{19}, such as alkyl functional group [6].

In contrast, other compounds have positive value of C_{19} atomic charge. They have functional groups that pull the electron density from C_{19}. All of predictors were applied for constructing the QSAR equation. These results can be used as a strategy to vary the C_{19} atomic charge. If we want to make C_{19} become more negative, we can change the carbonyl into alkyl functional group.

Dipole moment values were also different among the compounds. The highest value was happened in Benzoylphenylalanine ester Calanone. It has a lot of electronegative atom in C_{19}'s functional group. Dipole moment value is influenced by the difference of electronegativity among the atoms and geometry certain functional group in the structure [6].

Solubility property is described by Log P value. The highest Log P value is occurred in Calanyloctanoic (see Table II). It is ester compound with long hydrocarbon chain that can contribute non polar property to whole structure [6]. Hydrocarbon compounds have non polar characteristic and easily dissolved in non polar solvents such as n-hexane and carbon tetrachloride [6].

Increasing the number of predictor can represent the property of molecule in more detail. The previous research showed that using many predictors could obtain the good QSAR equation. Predictors that used in this research are too much. In contrast, the experimental data are too less. To solve this problem, PCR as regression calculation method was applied. Before PCR calculation, PCA was conducted to predictor data in order to produce principle components. The PCA result shows that first 4 principle components (t) can be applied in regression analysis. They had more than 95% of cumulative value. Below are the PCA results and the calculation of 1st until 4th latent variables for each Calanone compound (see Table III and IV).

The first 4 latent variables are applied because they can represent over 95% the variation in all predictors [7]. So, the latent variables are the representative data of all theoretical predictors. All of data in Table 4 are applied by PCR method as regression analysis for constructing the QSAR equation.

Selecting the best QSAR equation

Selecting the QSAR equation was conducted by evaluation the statistical parameters such as r (correlation coefficient), r^2 (determination coefficient), F (variance analysis), SE (standard error) and $PRESS$ (Prediction Residual Sum of Squares). The QSAR equation from this research was:

$$\text{Log } IC_{50} = 2.8055 - 0.1135\ t_1 + 0.2678\ t_2 - 0.5696\ t_3$$

This equation has some characteristics such as n (the number of data) = 6, $r = 0.991$, $r^2 = 0.982$, $SE = 0.0193$, and $F_{calc}/F_{table} = 1.987$ and $PRESS = 7.49\ 10^{-4}$. The symbol $t_1 - t_3$ are latent variables.

This QSAR equation has good correlation (r value) between activities and latent variables. It can explain over 98% variation of data (see r^2 value). Small error is also showed in this QSAR equation (see SE value). It is also accepted at 95% confidence level because it has F_{calc}/F_{table} more than 1.00. Finally, good quality is showed by this QSAR equation. It has good accuracy for prediction the activity of new Calanone compound (see the small $PRESS$ value). These good predictive characteristics are the same as equation that produced from previous research [8].

Designing new compounds

There are 3 factors that used for choosing the new Calanone derivative candidates. They are theoretical activity from QSAR equation calculation, the possibility to be synthesized and the ease to get the reactant [9]. Based on predictive calculation some new compounds which may have better anti-leukemia activity are gemdiol Calanone (1), 2,4-dinitrophenylhydrazone Calanone (2) and 2,4,6-trinitrophenylhydrazone Calanone (3) (see Fig. 3).

This three candidates guide synthesis procedures become more focus. All the synthesis works will start by using pure Calanone that obtained from Chemistry Research Center, The Indonesian Institute of Sciences (LIPI), Serpong, Indonesia. Synthesis of candidates also becomes an evaluation to predictive quality of the QSAR equation.

Synthesis and anti-leukemia activity of new compound

All recommended compounds can be synthesized by specific reactions [9]. The reaction product was characterized by some techniques such as melting point, FTIR and LC-MS in order to prove the compound is suitable to the target. Besides melting point, the spectroscopic characterizations also show the good agreement to the structure of new compounds [9].

After the characterization steps the anti-leukemia activity of new compounds were done by using leukemia L1210 cell. The theoretical activities (prediction) are not the same as the experimental activity (factual). But the QSAR approach can guide the synthesis new compound become more efficient. All of the new compounds have higher activity than pure Calanone. The QSAR equation from this research has good predictive capability (Table VI).

IV. CONCLUSION

QSAR analysis can be conducted to the less experimental data by PCR method as regression analysis. AM1 semiempirical calculation has good compromising between the accuracy and the capability of computer processor. More study is needed in order to increase the level theory of calculation method in obtaining predictor data and update the QSAR equation by the new experimental activities.

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TABLE II THEORETICAL PREDICTORS OF CALANONE AND ITS DERIVATIVES THAT HAVE SIGNIFICANT DIFFERENCE VALUE IN EACH COMPOUND BY AM1 SEMIEMPIRICAL CALCULATION

Compound	qC_5	qC_{15}	qC_{19}	qC_{20}	qC_{21}	μ	log P
Calanone	-0.138	-0.305	0.342	-0.136	-0.066	5.856	0.43
Calanol	-0.136	-0.228	0.121	-0.124	-0.107	4.066	0.42
Calanyloctanoic	-0.135	-0.276	0.127	-0.100	-0.115	5.558	2.32
Oxym Calanone	-0.135	-0.194	-0.005	-0.084	-0.110	5.258	1.08
Benzoylphenylalanine ester Calanone	-0.138	-0.230	0.126	-0.121	-0.127	8.789	1.24
Isobutylcalanone	-0.134	-0.191	-0.002	-0.077	-0.149	4.962	1.84

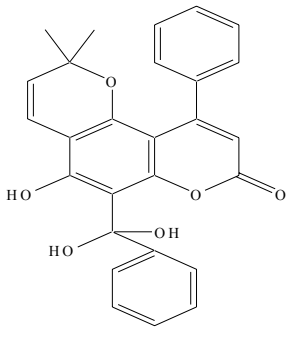
qCn = atomic net charge of n^{th} atom; μ = dipole moment; log P = partition coefficient

TABLE III CUMULATIVE VALUE OF PRINCIPLE COMPONENTS THAT PRODUCED BY STATISTICAL PCA AGAINST CALANONE'S PREDICTOR DATA

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	16.7	50.5	50.5
2	8.9	26.9	77.4
3	4.0	12.2	89.6
4	2.1	6.3	95.9
5	1.3	4.1	100

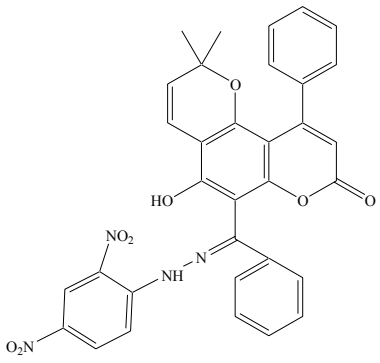
TABLE IV FOUR LATENT VARIABLES AND ACTIVITY DATA OF CALANONE AND ITS DERIVATIVES AS ANTI-LEUKEMIA COMPOUNDS THAT ARE USED TO PRODUCE QSAR EQUATION BY PRINCIPLE COMPONENT REGRESSION

Compound	t_1	t_2	t_3	t_4	t_5	log IC_{50}
Calanone	3.5833	4.1100	3.0538	1.1619	-0.2133	1.7738
Calanol	2.3393	2.7112	2.4796	0.7910	-0.2004	1.8451
Calanyloctanoic	1.9821	4.3197	3.2661	2.4024	-0.9870	1.8762
Oxym Calanone	2.3717	3.9066	2.8880	1.5401	-0.3421	1.9222
Benzoylphenylalanin e ester Calanone	3.9638	6.8895	4.2145	2.3246	-0.3011	1.7959
Isobutylcalanone	1.6918	3.8990	2.8962	1.9611	-0.7145	2.0233



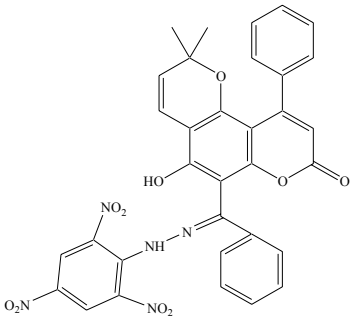
Senyawa Prediksi 1

(1)



Senyawa Prediksi 2

(2)



Senyawa Prediksi 3

(3)

Fig. 3 Molecular structure of new Calanone derivatives that are recommended by QSAR analysis based on AM1 semiempirical calculation

TABLE VI THEORETICAL AND EXPERIMENTAL ACTIVITIES OF PURE CALANONE AND ITS NEW DERIVATIVES. NOVEL CALANONE DERIVATIVES ARE SYNTHESIZED BASED ON QSAR RECOMMENDATION

Compound	Anti-leukemia activity (μg/mL)	
	Theoretical	Experimental
Calanone	-	59.4
Gemdiol Calanone	57.78	52.04
2,4-dinitrophenylhydrazone Calanone	30.94	47.09
2,4,6-trinitrophenylhydrazone Calanone	18.96	47.69