ANALISIS HKSA SENYAWA TURUNAN 4-FENOKSIPIRIMIDIN-5-KARBOKSIAMIDA SEBAGAI ANTIDIABETIK MENGGUNAKAN METODE AM1

QSAR Analysis Of 4-Phenoxypyrimidine-5-Carboxyamide Derivatives As Antidiabetic Compounds Using Semiempirical AM1 Method

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ABSTRAK

Penelitian bertujuan untuk memodelkan senyawa turunan 4-fenoksipirimidin-5-karboksamida dan menentukan persamaan HKSA yang dihasilkan. Penelitian ini menggunakan metode semiempirik AM1 karena tingkat ketelitiannya cukup tinggi dan waktu perhitungannya relatif cepat. Metode statistika yang digunakan untuk pemilihan deskriptor yang berhubungan dengan aktivitas biologis senyawanya adalah analisis *multilinear regression* (MLR) karena jumlah deskriptornya relatif banyak. Pemodelan dalam penelitian ini menghasilkan satu model persamaan dengan rincian sebagai berikut:
$-\log EC_{50} = -47{,}95 + (71{,}322 \times qCl7) + (15{,}357 \times qC10) + (-169{,}512 \times qC11) + (1{,}955 \times qC23) + (0{,}493 \times E_H) + (-0{,}612 \times \log P) + (-0{,}017 \times \alpha) + (146{,}598 \times grad) + (0{,}018 \times \Delta H_f)$
$n = 14$; $r = 1{,}000$; $r^2 = 1{,}000$; *adjusted* $r^2 = 1{,}000$; PRESS = 8,706.

Kata Kunci: 4-fenoksipirimidin-5-karboksamida, AM1, HKSA.

ABSTRACT

A QSAR-based molecular modelling of 4-phenoxypirimidine-5-carboxamide derivatives has been carried out to determine the appropriate equations from semiempirical method among AM1 in forecasting bioactivity equations. Beside high accuracy, such method takes shorter time consuming when running. In order to analyse the data resulted by the computational approach, a statistical evaluation through multilinear regression analysis was performed due to the numerous data for the descriptors. As the result, there is a model proposed by using AM1 which are considered as the proper model:
$-\log EC_{50} = -47.95 + (71.322 \times qCl7) + (15.357 \times qC10) + (-169.512 \times qC11) + (1.955 \times qC23) + (0.493 \times E_H) + (-0.612 \times \log P) + (-0.017 \times \alpha) + (146.598 \times grad) + (0.018 \times \Delta H_f)$
$n = 14$; $r = 1.000$; $r^2 = 1.000$; *adjusted* $r^2 = 1.000$; $PRESS = 8.706$

Keywords*: 4-phenoxypirimidine-5-carboxamide, AM1, QSAR.*

INTRODUCTION

Research and development of drug compounds especially for deadly disorders through a laboratory-based design usually takes time and high cost as well that needs 10–15 years and US$ 800 million to complete a project (Patrick, 2013). Economically, this approach might not be efficient since the demand of powerful drugs against the diseases elevate relatively. Consequently, such trends became a serious problem in the past. However, in the 1960s, Hansch and Fujita succcessfully developed an innovative method, namely computational chemistry, combining quantitative approach and stucture and activity relationship (QSAR) of medicinal compounds (Bachrach, 2007; and Thomas,

2003). The method could not only overcome the problem but also avoid potential errors when designing a new drug molecule run.

Computational chemistry is a branch of chemistry that employs quantum mechanics equation expressed in programming code to study the characteristics and dynamics of molecules (Cramer, 2004). Due to the advanced ability to describe them beyond the conventional manner, it could be applied in designing such drug molecules which always relies on chemotherapic index. The variable, initially introduced by Paul Ehrlich in the beginning of 20^{th} century, is called structure–activity relationship (SAR). SAR has been considered as a trustworthy guidance for synthesising and evaluating new medicines (Gaurav, 2012; and Hevener, 2008).

Diabetes melitus (DM) is a deficiency of metabolism system signed by the increase of blood glucose level (hyperglychemia) causing some fatal complications (Abuhammad and Taha, 2015). The disease which has been estimated approximately 90% cases of DM as type 2 DM (T2DM) could be caused by the resistance of insulin in peripheral artery and the scarce secretion of insulin from pancreas (Shivangee *et al.*, 2015). Specifically, the failure of GLP-1 (glucagone-like peptide-1) secretion might lead to the decrease of the insulin production. It acts as vital component in both stimulation of insulin and glucagone secretion (Herbert *et al.*, 2010). Therefore, a strategy to improve the number of the hormone could be considered as an effective way to manage T2DM.

In order to find out not only an antidiabetic agent but also an antiobesity one, activation of the TGR5 might be taken into account as the most interesting choice recently (Duan *et al.*, 2013; and Chen *et al.*, 2011). Naturally, both oleanolic acid and betulinic acid can enhance the production of the hormone whereas the further study of the side effects is urgent issue (Herbert *et al.*, 2010; Gertzen *et al.*, 2011; and Pols *et al.*, 2013). Although some semisynthetic drugs derived from bile acid compounds may also be applied for the purpose, the oral treatment in a high dosage can lead to the damage of liver (Colliva, 2013; Duboc, Taché, and Hofmann, 2014).

The disadvantages of the both types of medicine encourage reseachers to develop novel synthetic compounds which could be proposed as candidates of the new agent. One of them is 4-phenoxypyrimidine-5-carboxamide derivatives that have been empirically demonstrated to be able to improve homeostatic metabolism, stimulate pancreatic insulin secretion, and show antiinflamation activity through *in vitro* assay (Herbert *et al.*, 2010; and Duan *et al.*, 2013). However, further investigation is essential to develop the more sophisticated compounds. Accordingly, this study aims to evaluate the compounds theoretically by modelling to determine a proper equation providing a mathematical blueprint to predict new derivatives with higher activity.

METHOD

The study took data from Shivangee *et al.* (2015) that provide all structures of 4-phenoxypyrimidine-5-carboxamide derivatives. The bioactivity data was taken from Duan *et al.* (2013).

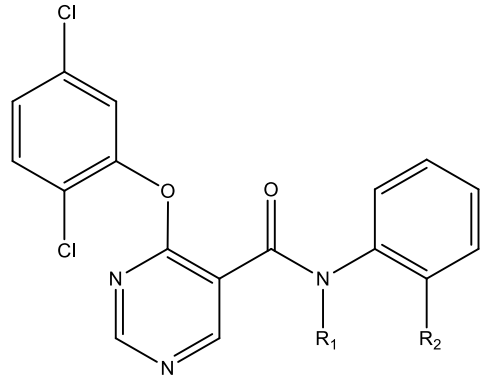

Figure 1. The main skeleton of 4-phenoxypyrimidine-5-carboxamide molecule I

Table 1. The list of substituents and activity of 4-phenoxypyrimidine-5-carboxamide derivatives I

No	R1	R2	EC_{50} ($\times 10^{-6}$ M)	-log EC_{50}
1	$-CH_3$	$N(CH_3)_2$	3.711	5.431
2	$-CH_3$	$-CH_3$	0.535	6.272
3	$-CH_3$	H	3.151	5.502
4	$-CH_3$	$-OCH_3$	0.160	6.796
5	$-CH_2CH_3$	$-CH_3$	4.886	5.311

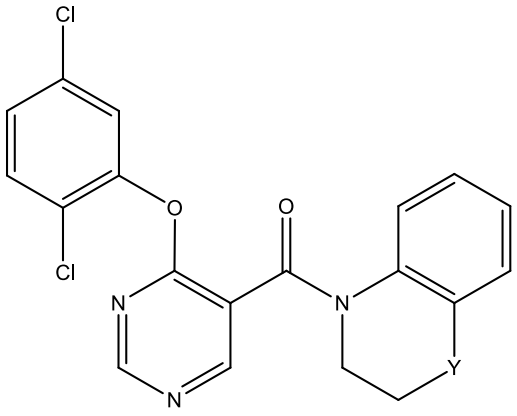

Figure 2. The main skeleton of 4-phenoxypyrimidine-5-carboxamide molecule II

Table 2. The list of substituents and activity of 4-phenoxypyrimidine-5-carboxamide derivatives II

No	Y	EC_{50} ($\times 10^{-6}$ M)	-log EC_{50}
6	$-CH_2$	0.156	6.807
7	$-NC_2H_5$	0.020	7.699
8	$-NCH_2CH_2CH_3$	0.030	7.523
9	$-NCH(CH_3)_2$	0.710	6.149
10	$-NCH_2CH{=}CH_2$	0.030	7.523
11	$-NCH(CH_2)_2$	0.003	8.538
12	$-N(CH_2)_3CH_3$	0.590	6.229
13	$-NCH(CH_2)_3$	0.023	7.638
14	S	0.164	6.785

Devices

The study used personal computer with AMD Quad-Core E2-7110 (1.8GHz) processor, OS Windows 2016, RAM 4096 MB DDR2, and hard disk 500 GB. The software were ChemDraw Pro 12 for drawing 2D structures, Hyperchem 8.0 for optimisation of the 3D molecules; and SPSS 17 for analysis of QSAR models.

Procedures

Generally, the QSAR modelling was carried out through a multisteps-process (Pranowo, 2011). The steps are:

a. Validation of the method to ensure that the modelling of the stuctures would be run by the appropriate method.
b. Preparation of the fitting molecules via stucture optimisation by using the validated method.

c. Determination of electronic and molecular descriptors which are responsible to the bioactivity of the molecules via QSAR analysis by using multiple linear regression (MLR) approach.
d. Verification of the mathematics model via cross-checking validation between the fitting molecules and the testing molecules.

Geometrical optimisation

As the fitting series, there were 10 compounds derived from 4-phenoxypyrimidine-5-carboxamide taken randomly (no 2, 3, 4, 6, 7, 8, 10, 12, 13, and 14) from Table 1 and 2. All structures were drawn in 2D format on ChemDraw Pro 12 then stored in *.sdf* file type. The 2D structures were exported into Hyperchem 8.0 in order to convert them into 3D format.

The 3D form was created by operating model build to organise the molecules reaching the actual configuration. After that, geometrical optimisation was performed to find out the best comformation. The limit of convergency, gradient limit of energy change per position change, was 0.001 kcal/Å. The limit of iteracy was 32767. This maximum limit was applied to set an optimum performance of calculation. The optimisation of method was conjugate gradient with Polak Ribiere algoritma.

QSAR analysis by using electronic and molecular parameters

Furthermore, the independent variables were atomic nett charge (q), log P, bipolar moment (μ), hidration energy (E_H), molecular polarisability (α), total energy (E_T), bonding energy (E_b), isolated atomic energy ($E_{at.is}$), electronic energy (E_e), core-core interaction (E_{int}), surface are (SA), volume (V), molar refractivity (RM), molecular mass (MW), and formation heat (ΔH_f). However, the dependent variable was -log EC_{50}.

In order to determine the best QSAR equation model, the research followed this procedure (Asmara, Mudasir, and Siswanta, 2017):

a. Four testing molecules were selected randomly from fourteen derivatives of 4-phenoxypyrimidine-5-carboxamide. The testing series were used for cross validation towards QSAR equation obtained from statistical analysis of descriptors of 10 fitting molecules.
b. QSAR equations were produced by screening the driving independent variables through MLR analysis with backward method in SPSS.
c. The best QSAR equation were proposed after analising the statistical parameters such as the value of r, r^2, adjusted r^2, and PRESS (Predicted Residual Sum of Square).
d. The selected QSAR equation as the best one then was validated by ploting the actual values of -log EC_{50} and predictive values of -log EC_{50} obtained from the four testing molecules in a graph.

RESULT AND DISCUSSION

Parameterisation Of The Semiempirical Method

Basically, methods in computational chemistry have been classified into two main types: molecular mechanics (MM) and quantum mechanics (QM). Moreover, the last one consists of *ab initio* and semiempirical method. Pranowo (2003) argues that MM is an empirical method ignoring electron of atoms explicitly. As a result, such method is not able to explain chemical phenomena in which electrons drive those mainly. On the other hand, this approach is a proper choice for calculating chemical processes driven by force fields in macromolecules such as: polysaccharides, polypeptides, and the other polymers. In contrary, an appropriate method for analysing smaller compound, like 4-phenoxypyrimi-dine-5-carboxamide derivatives, is the QM based method.

Pranowo (2003) describes *ab initio* as a method of QM that computes all electrons setting an atom out. Consequently, the method is time consuming when running whereas the result of calculation probably meet a high accuracy compared to the actual data. However, QM semiempirical method might be faster due to its distinctive approach: computing valence-shell electron only. Unfortunately, the result might be less accurate than the data given by the *ab initio*. Therefore, it is an essential consideration that the QM method should be parameterised by the *ab initio* calculation. This step is needed to choose the most suitable type of QM method in a computation-assisted project of molecule.

Recently, there are ten types of QM method: Extended Huckel, CNDO, INDO, MINDO3, MNDO, MNDO/d, AM1, PM3, ZINDO/1, and ZINDO/S. Among them, AM1 is in the lowest place of the misaccuracy order of physico-data such as binding length and binding angle compared to the data given by the *ab initio* for molecules containing aromatic rings including phenoxypyrimidine groups (Pranowo, 2003). Due to the evidence, the AM1 method was selected to undertake the analysis.

Molecular Optimisation By Using Semiempirical AM1 Method

Regarding the expectation of precise conformation in molecular and electronic structure computation, firstly, fourteen derivatives of 4-phenoxypyrimidine-5-carboxamide were optimised. This step was carried out with AM1 method provided by HyperChem 8.0. Moreover, the step also calculated the charge value of all of the atoms which could help the study to investigate the correlation between these parameters and the activity of the compounds.

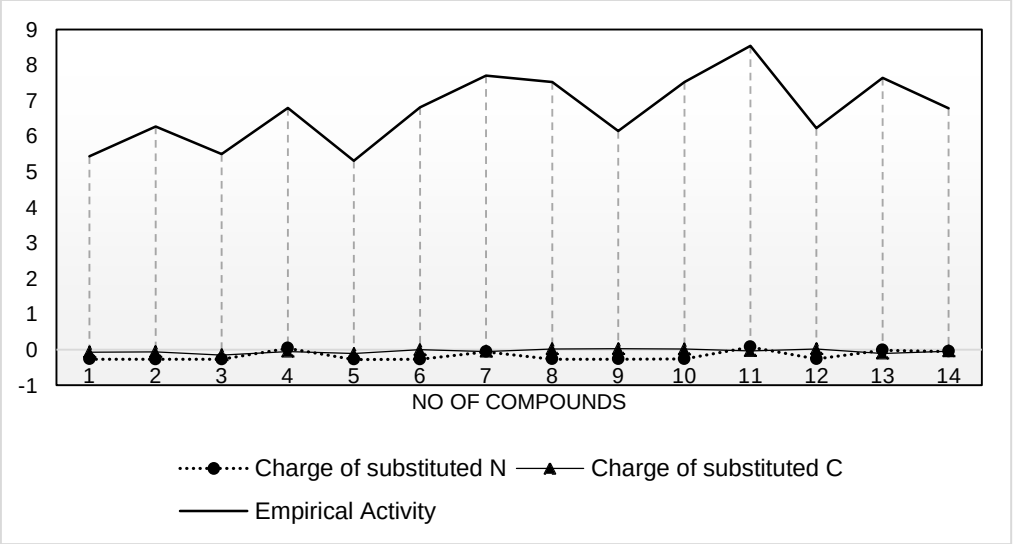

Figure 3. A graph for representing the effect of atomic charges of substituted–N and C of 4-phenoxypyrimidine-5-carboxamide derivatives towards their empirical values of –log EC_{50}

Figure 3 provides the charge value of both substituted atoms, atom N in the carboxamide group and C in the phenyl ring, in the main skeleton of the compounds. This illustration can be used to describe the effect of the type of substituent groups attached on the both atoms towards the bioactivity of the series.

The figure shows that compound 11 as the most active derivative consists of the nitrogen with the most positive number of charge. As the other examples, compound 4, 7, and 13 that contain the atom N with higher charge perform higher bioactivity as well while the charge of the atom C tends to be steady. On the other word, there is a consideration that positive charge of atom N in the carboxamide group might correlate with the high activity of the compounds.

According to the Table 1 and 2, all of the atoms N connected to C carbonyl bind strong electron-donating groups. The strength of the substituent effect could probably be recognised by the charge value of atomic centre of the substituents. Obviously, due to the process of donating electron, the values of them that are owned by the more active compounds are positive relatively (Table 3).

Interestingly, the substituent of compound 11, most active derivative, is not the strongest donating-electron group. In contrast, the activity of compound 8 which bind the most strongest substituent is much lower than compound 11. As a result, the electronic parameter is not the main factor of the compounds' activity. As shown in the Table 3, the substituent of compound 11 is more complex than the substituent in the compound 8. It means that molecular parameter such as sterical factor and the value of hidrophobicity should be taken into account.

Table 3. Charge list of the central atom of substituents bonded to N of carboxamide

Compound	Substituent of N-carbonyl	Charge of the central atom on the substituent
1	$-CH_3$	-0.1
2	$-CH_3$	-0.008
3	$-CH_3$	-0.04
4	$-CH_3$	0.029
5	$-CH_2CH_3$	-0.029
6	$-CH_2$	0.024
7	$-NC_2H_5$	0.077
8	$-NCH_2CH_2CH_3$	0.085
9	$-NCH(CH_3)_2$	-0.02
10	$-NCH_2CH{=}CH_2$	-0.019
11	$-NCH(CH_2)_2$	0.077
12	$-N(CH_2)_3CH_3$	-0.02
13	$-NCH(CH_2)_3$	0.059
14	S	-0.012

Therefore, statictical analysis is really essential to determine the driving factors in the activity of the compounds.

QSAR Analysis By Using Multilinear Regression Method

A statistic analysis, namely MLR method, was employed in this study because of the enormous number of descriptors. Furthermore, the method provided by SPSS 17 program for Windows was setted up in enter and backward mode. All electronic and molecular parameters were setted as independent variables while -log EC_{50} was placed on dependent variable column.

Regarding the statistic analysis, one candidate of QSAR model was successfully obtained. In detail, the equation can be written as:

$-\log EC_{50} = -47.95 + (71.322 \times qCl7) + (15.357 \times qC10) + (-169.512 \times qC11) + (1.955 \times qC23) + (0.493 \times E_H) + (-0.612 \times \log P) + (-0.017 \times \alpha) + (146.598 \times grad) + (0.018 \times \Delta H_f)$

n = 14; r = 1.000; r^2 = 1.000; adjusted r^2 = 1.000; PRESS = 8.706

The result of the optimisation of fitting compounds and the PRESS value can be seen in Table 4. The PRESS value indicates the gap between theoretical values of bioactivity of the compounds and the actual values. This small value (less than 10) shows that the equation could be considered as a reliable mathematic expression to represent the relationship between molecular structure and the activity. Moreover, the statistic test result including ANOVA and *t*-test could show the qualiy of the mode. The *F* significancy test result gives p value as 0.000 (<0.05). Meaningly, this equation can significantly describe the diversity of the dependent variables. In addition, based on *t*-*t*est output, all value of *p* for the descriptors is below 0.05. Therefore, each variable can also influence the bioactivity of the compounds significantly.

According to Table 4, the difference between predictive IC_{50} and the actual value from the literature of both compound 3 and 4 is greater than others.

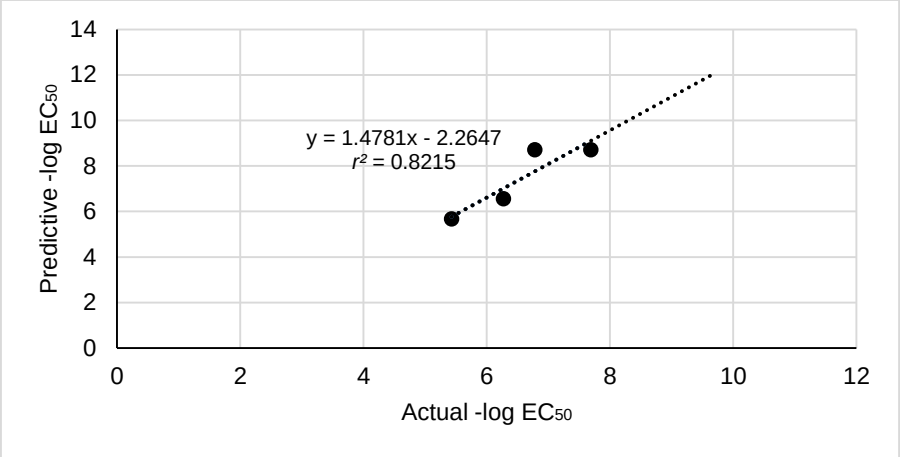

Figure 4. Graph for determining the validity of the QSAR equation

By focusing on the type of R2 substituent linked to the phenyl group, the cause could be explained as follows. Both compounds bond strong electron donors as the R2 while the other derivatives are substituted by the weaker type. On the other words, this method has calculated accurately for compounds having deactivator substituents. Based on Duan *et al.* (2012), the compounds tend to be more active when the phenyl ring bind electron withdrawing groups. Thus, this approach find that the 4-phenoxypyrimidine-5-carboxamide derivatives binding deactivator substituents on the phenyl group show a quite similariry of value between theoretical activity and the empirical value. The validity level of the equation has to be determined because the equation needs to be accurate where higher validity appears to be higher accuracy. It can be seen from the value of determinant (r^2) of a graph showing the relationship between actual -log EC_{50} and predictive -log EC_{50} of the testing compounds. Figure 4 illustrates the graph and the r^2 as well.

The curve shown in the figure above indicates the r^2 of the equation which is more than 0.5. It means that this equation meets the requirement as a statistically valid model. Therefore, the equation could be considered as the appropriate model for expressing the relationship between the structure of 4-phenoxypyrimidine-5-carboxamide derivatives and their activity. **CONCLUSION**

This study could be considered to describes that semiempirical AM1 method is appropriate for electronic and molecular parameters-based modelling of 4-phenoxypyrimidine-5-carboxamide derivatives. It produced one equation model which is valid for predicting the bioactivity of the series. Further research is essential to evaluate the model for designing theoretically novel derivatives.

Table 4. Data of optimisation output of driving descriptors and the PRESS value from fitting molecules

Molecule	Atomic charges				E_H	log P	α	grad	ΔH_f	-log EC_{50}		Δ -log EC_{50}	PRESS
	Cl7	C10	C11	C23						Predictive	Actual		
3	-0.006	0.213	-0.215	-0.112	-5.72	-0.07	37.94	0.093	67	2.655	5.501	2.846	
4	0.034	0.188	-0.210	-0.131	-4.91	-1.06	40.41	0.099	50	5.740	6.796	1.055	
5	0.035	0.221	-0.233	-0.124	-4.89	0.43	41.61	0.071	56	5.281	5.311	0.030	
6	0.033	0.179	-0.226	-0.110	-5.36	0	40.83	0.097	37	6.824	6.807	-0.017	
8	0.034	0.182	-0.228	-0.165	-4.22	0.92	46.11	0.098	47	7.547	7.522	-0.025	8.709
9	0.034	0.182	-0.228	-0.168	-4.75	-0.44	45.85	0.085	51	6.157	6.149	-0.008	
10	0.036	0.180	-0.225	-0.160	-6.48	-0.45	45.66	0.099	79	7.556	7.523	-0.033	
11	0.036	0.180	-0.224	-0.160	-5.13	-0.8	45.08	0.101	89	8.573	8.538	-0.035	
12	0.036	0.182	-0.225	-0.166	-4.29	0.01	47.69	0.089	40	6.244	6.229	-0.015	
13	0.031	0.177	-0.230	-0.123	-5.04	-0.4	46.91	0.100	69	8.485	7.638	-0.847	

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