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A Quantitative Structure-Activity Relationship Study, Compound Development, Pharmacophore Feature, and Molecular Docking of Pyrazolo-[3,4-d]-Pyrimidine Derivatives as Mer Tyrosine Kinase Inhibitor

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Abstract: Objective: Mer Tyrosine Kinase is ectopically expressed in T and Bcells of Acute Lymphoblastic Leukemia(ALL) patient, but is not expressed in normal human T and B cells at any stage of its development. Therefore Mer Tyrosine Kinase can be a treatment target ALL with a good selectivity. Phosphorylation inhibition of Mer receptor by signal transduction inhibitor decreases cell proliferation and increases apoptosis, there by suppressing the development of leukemia cells. Pirazolo-[3,4-*d*]-pyrimidines are a new generation of drugs that act as inhibitors of Mer tyrosine kinase. The purposes of the present research are to determine descriptors that influence the inhibitory activity on Mer receptor tyrosine kinase, to determine the ligands pharmacopores features and receptors which play important roles i nligand-receptors binding and to study model and free energy value of pirazolo-[3,4-d]-pyrimidines with Mer interactions. **Methods**: Modeling and optimization geometry was carried out using HyperChem® software. Molecules structure were geometrically optimized using Ab initio method. Predictors values were computed using MOE® and statistical calculationsof QSARequations was carried out using SPSS®. The selected equation was determined by the best statistical criteria, such as r^2, Pearson correlations, and q^2 *Leave One Out* validation. Determination of pharmacophores features used optimized model structure using 'Pharmacophore Query ditor' in the MOE software. The study Molecular docking used 'Simulations Dock' where the scoring values were calculated using the London dG approach. **Conclusion:** The most important descriptors were mr, vol_vdw, ASA_H, log S and LUMOenergy. Ligands pharmacophores features were composed of a proton donor, a proton acceptor, one cations and proton donors, and aromatic. Distance (6.92 Å) between cation and proton donors features with aromatic group play importantrole as Mer inhibitors. Receptor pharmocophore features were composed of a proton acceptor (Met 674), three proton donors (Pro 672, Arg727 and Asn728) and one anion (Asp 678), which is important in the binding with ligand features pharmacopore. All of pirazolo-[3,4-d]-pyrimidines derivates had good docking score where as compound 40 had the best scoring -12.7584 kcal/mol.

Keywords : acute lymphoblastic leukemia, pyrazolo-[3,4-*d*]-pyrimidine, Mer, QSAR, pharmacophore features.

Introduction

Leukemia is a rare disease, but the incidence of death is quite high. In 2008, leukemia cases for every100.000 populations in developed countries is 9,1 for men and 6,0 for women with death rate 53% for men and 48% for women of the total patients. As for the developing countries, leukemia cases for every 100.000 populations is 4,5 for men and 3,6 for women with death rate 82% for men and 80% for women of the total patients. There are 5,8 (2.8%) of 206 cancer patients of leukemia incidence for every 100.000 populations[1].

Generally, leukemia is divided into two categories, these are acute and chronic. It is based on the difference in cell origin and maturation of cell line, clinical presentation, rapid progression of untreated disease,and response to the therapy. Although many varieties of leukemia exist, and can be distinguished by the affected cell type, four major categories are recognized. These are acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), cronic lymphositic leukemia (CLL), and cronic myleoid leukemia (CML). Acute leukemia is the most common cancer in children andthe leading cause of cancer-related deaths in patients younger than 35 years old[2].

Mer is a member of the receptor tyrosine kinase family known as TAM (Tyro3/Axl/Mer) family. This family has a unique sequence of the kinase domain and certainly has the potential to become a selectively target because the difference of other kinase families. Mer is not found in T and B cells of human and mice at each stage of lymphocite development, but found in relatively large amounts in T cells ALL and B cells ALL samples as a results of E2A-PBX1 translocation which drastically produce Mer-RNA. This ectopic expression has been identified as products of tumor cell survival in ALL cells and cause potential chemical resistance of ALL.

Like the most receptor tyrosine kinase (RTK) groups, Mer present in cell membrane (trans-membrane) which connects extracellular environment to the cytoplasm and nucleus. TAM receptor serves as signaling receptor in the regulation of macrophage clearance of apoptotic cells, platelet aggregation and differentiation of natural killer (NK) cells.

ALL is one of the most common malignant cancer in children. Treatment with chemotherapy is still causing toxicity problems related with short-term and long-term. Therefore, new compounds with low toxicity are needed. Mer tyrosine kinase receptor which expressed ectopically in the ALL cell samples. Inhibition of Mer expression reduces pro-survival signal, chemo sensitivity increases, thus delay the progression of leukemia cells. Mer tyrosine kinase inhibitor is an excellent candidate as a target for the treatment of leukemia. Development pyrazolo pyrimidine compounds demonstrated success as a new strategy in the treatment of ALL.

Liu, J et al (2012)[3] have conducted a study of SAR (Structure Activity Relationship) pyrazolo-[3,4-d]-pyrimidine derivatives to the enzyme Mer Tyrosine Kinase (MERTK) inhibitory activity based on inhibition constants of ATP using microfluidic capillary electrophoresis (MCE). Jing Liu also has elucidated the co-crystal structure of Mer in complex with 43 derivatives compound of pyrazolo-[3,4-d]-pyrimidine and determined at a resolution 2.69 Å with its binding mode. From these data we made QSAR studies on pyrazolo-[3,4-d]-pyrimidine derivatives. These QSAR results will be applied to predict some pyrazolo-[3,4-d]-pyrimidine derivatives. The prediction results are expected to be useful in determining the activity of compounds which will be carried out the synthesis and subsequent testing. Moreover, we also determined the pharmacophore features which play roles in ligand binding to the receptor. The purpose of this study was to obtain QSAR models and pharmacophore features of pyrazolo-[3,4-d]-pyrimidine derivatives which play roles in ligand-receptor binding.

Experiment

Software

HyperChem® Release 8.0, Molecular Operating Environment (MOE 2009.10), SPSS Statistics 17.0.

Hardware

Processor: Intel® Core™ i5-3210M CPU @ 2.50 GHz 2.50 GHz; *RAM* : 4 GB; System type: 64 bit *Operating System*; Operating system : *Windows® 7 Professional.*

Procedures :

QSARs equation modeling

Modeling the compound structures is made by using HyperChem package. These compounds are pyrazolo-[3,4-d]-pyrimidine derivatives according to the research by Liu, J. (2012)[3], total of 14 compounds. Modeling consists of the selection of atoms, binding type, and the total charge of the test molecule. Three-dimensional (3D) structure of each compounds is saved in format extension *.hin

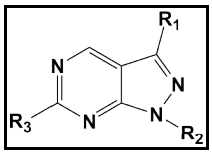


Figure 1. pyrazolo-[3,4-d]-pyrimidine structure compound with three substituen positions

The compound structures are optimized by HyperChem software using Ab initio methods with a minimal set basis parameters and convergence limit 10^{-5}. Files which have been optimized is saved in format extension *.mol. Calculation with Ab initio is more accurate when compared with semi-empirical because Ab initio solve all equations of quantum mechanics exactly and all the electrons are calculated[4].

14 compounds, which its descriptors value will be calculated, opened by MOE and combined in a single files with format extension *mdb. Descriptor values were calculated using the software MOE. Total of 13 physical chemical properties were calculated to represent hydrophobic parameters, electronic and steric, according to the QSAR models with Hansch approach.

The best equation models were searched using dependent variables Mer inhibitory activity (log $1/IC_{50}$) from the results of the experiment and the independent variables were used in the form of descriptor values. All variables were analyzed using multiple linear regression enter method. Results obtained in the form of QSAR equation with the value of statistical parameters such as value of r, $r^2$and F. The F value indicates significance of the relationship when compared with the F table. The F value is an indicator of numbers to indicate that relationship, which expressed by the obtained equation, is true or coincidence. To get the model with the highest r value, elimination of compounds which have the largest deviation based on Z value on MOE results were conducted, where the compound structure with a value Z > 2 are eliminated from statistical calculations.

Besides the statistical parameters, from the calculation results are also obtained constanta value and coefficient value of each independent variables which is involved in the resulting equation. Coefficient value which is obtained then used to calculate the theoretical inhibitory activity.

10 best equation models then cross-validated using Leave One Out method, each predicted compound is eliminated in the calculation of the linear regression analysis. q^2 value as a result of cross validation then calculate according to the following formula:

$$q^2 = 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2}$$

y_i = the actual activity
$\bar{y}$ = the average actual activity
$\hat{y}_i$ = the predicted activity of compound i

Beside that, the value of Pearson correlation also calculated, to evaluate whether the descriptors in the equation has a correlation with activity Log ($1/IC_{50}$). The selcted QSAR equation is an equation with the best value of statistical criteria and meet the criteria of validation that is $q^2 \geq 0.5$[5].

Compound Development

The aim of new compound design is to obtain compound which having a better activity and selectivity than previous compound. QSAR analysis results can be used as guidelines based on the physicochemical

properties that increases the activity. Compound 43 is parent compound because this compound have good activity and selectivity.

Design of new compounds can be done by considering the results of the analysis of the previous QSAR molecules, such as the parameters that affect the biological activity of the compound. Pharmacophore features also a consideration, where the ring pyrazolo- [3,4-d] -pyrimidine, amine on chain polar groups on R3 and R2 chain is maintained.

From the analysis of the experimental data presented in Table 2, it appears that an aryl group is preferred at R1 position and alkyl groups are preferred at R3 position**.** This is in accordance with the most influential descriptors, including the steric descriptors. In equation models of Mer inhibitors, the most influential descriptors are molar refractivity (mr). Besides mr, descriptors inhibitors that influence significantly is vdw_vol. The addition of bulky groups as a substituent will increase Mer activity. Electronic descriptors of Mer inhibitors has the opposite effect on the activity. The decrease of LUMO energy or the increase in the HOMO energy will increase inhibitory activity of Mer, so the addition of substituent groups donating electrons will increase the selectivity of Mer inhibition. In addition, some descriptors including relevant descriptors to protein-ligand interactions, such as electronic and steric descriptors.

Pharmacophore Feature Determination of Receptors and Ligands

Pharmacophore according to IUPAC is steric and electronic factors which are necessary to ensure the optimal molecular interactions with a specific biological target structure as inducers or inhibitors of biological response[6].

Pharmacophore features were made by considering the PLIF (Protein Ligand Interaction Features) model. Fingerprints of protein interactions with ligands were made by using 4 protein structures which were downloaded from RSCB PDB sites. The entire structure was then opened on the MOE window and aligned so only the chain which has the same structure will move together as one unit. In this way the protein-ligand complex can be aligned. Files were then saved as database and PLIF analyszes were conducted.

Pharmacophore features were determined through three steps, create conformation databases by using a set of compounds which have been optimized, create Query pharmacophore by selecting annotation points based on protein-ligand binding from the results of PLIF analyze, then refinement Query structure which can be hit with the conformation of active compounds.

Molecular docking

Semi-rigid approach was used, where the protein structure is made of rigid while the ligand is flexible. This approach will provide the possibility of interaction in a various ligand conformations which allow to obtain the best result. The possibility number of conformation shapes depending on the number of the existing rotatable bonds[7].

Docking procedure consists of three steps, ligand preparation, protein preparation, and docking simulation. Ligands which have been optimized by Ab initio method in HyperChem software, were protonated to add hydrogen and partial charge by setting pH 7.4 and cutoff 10.0. Then files were saved in database (*.mdb).

Receptor structures were downloaded from RSCB.PDB site with 3TCP code in the format *.pdb/ent. Then water molecules are removed from the structure. Proteins then protonated with the same steps in the ligand preparation. Amino acids arginine, lysine, and histidine which have base groups will be ionizing at pH 7.4 to form a cationic environment. Acidic groups such as carboxylic acid side chains of aspartic and glutamic will be deprotonated to produce anionic groups of COO^- which can interact with cationic groups[8].

The protonated ligands and receptors are opened on the MOE window. Simulation panel docking is opened. In the panel, Placement arranged to Triangle Matcher, rescoring 1 using London dG, and refinement arranged to Force Field. The best docking position is selected based on proximity to the natural ligand structure and the lowest scoring.

Result and Discussion

QSAR study

QSAR studies using 14 compounds. The selection of these compounds was based on the similarity of the framework structure of these compounds, and Mer inhibitory activity was obtained fro the research of Liu, J et al (2012)[3]. Descriptor value of each compound was calculated with MOE. The compounds must have Z activity value ≤ 2, which means that IC_{50} is below 2 times the standard deviation.

The result of the calculation predictor obtained then were statistically analyzed multiple linear regression analysis with SPSS 17. These predictors were regressed against Mer inhibitory activity (Log $1/IC_{50}$, μM) as the dependent variable. Predictors which were positioned as independent variable in the regression analysis were combined with each other with a combination of 3-5 types of predictors.

Multiple linear regression results are then arranged and ranked based on the value of statistical parameters, such as correlation coefficient (r), regression coefficient (r^2), standard error (SE), and Fischer criterion (F). The best 10 equation model then cross-validated leave-one-out (LOO) by using criteria q^2. The model equation must meet the criteria $q^2 \geq 0{,}5$.

Examination of the predictor activities made by this model is compared with experimental activity showed no clear pattern if only the prediction activities of the molecules were examined. However, if the exammination includes the value of q^2, the pattern begins to develop. The value of q^2, in this context, is the difference in error that model is not appropriate. The values of q^2which were closer to one indicating that a smaller number of errors, and the values which were less than one indicating a greater quantity of the remaining errors. Negative values of q^2 showed a large discrepancy.

The selected QSAR model is the model with the best statistical criteria value. Table 5 presents a comparasion of 4 statistical criterion of equation models which have the value of regression coefficient r^2 and the highest LOO cross validation of q^2. Both of criteria equation model values are not much different so it is difficult to determine the best equation if only use these two criteria values. Therefore, it also need to determine the other statistical criteria, such as curve regression coefficient IC_{50} experiments with prediction and Pearson correlation value descriptors. Equation 1 was chosen as model for QSAR of Mer inhibitor because the value of curve regression coefficient IC_{50} experiments with prediction is higher than equation 3.

The best multiple linear regression contains five descriptors give strong correlation with the experimental result (r^2>0.98). These five descriptors were considered significant according to the Pearson correlation.

According to Liu, J. et al (2012)[3], an aryl group is selected in the position of R1 and alkyl group is selected in R3 position. This is in accordance with the most influential descriptors which inlude steric descriptor, although up to a certain volume, activity will decrease. The most influential descriptor is molar refraktivity (mr).

It seems most of the descriptors were include in relevant descriptors to protein-ligan interaction, such as electronic and steric descriptors. Descriptors in this model allows to interprete modification structure sistematically in order to develop SAR which will lead to a stronger and more spesific inhibitors.

Pharmacophore Feature Study

The purpose of the query pharmacophore preparation is to explain 3D structure features of pyrazolo-[3,4-d]-pyrimidin by using its derivatives which wereimportant for binding with receptor by producing pharmacophore and to measure structure feature of Mer which is important to biological activity by looking the residues of aino acids which play role in binding. For the preparation of pharmacophore by using Pharmacophore Query Editor and Protein-Ligand Interaction Fingerprint on MOE. Hypothetical pharmacophore which is resulted will also explain the binding of the ligand in the binding site or catalytic of receptor. Therefore, we use conformation which has been optimized and has the most stable structure.

The crystal structure of the Mer complex with ligand has long been studied. There are five compound structures which have been reported and can be downloaded from www.rscb.org site, but only four complex

structure which is bound to ligand with 3TCP, 2P0C, 3BRB, 3BPR codes, while protein structure without ligand is 2DBJ[3,9].

By using 4 complex ligand-protein structure above, it can be made fingerprint ligand-protein interaction by comparing how each ligand bind to the protein residues in the protein binding sites. This method is useful to summarize the interaction between ligands and proteins by using fingerprint schemes. Interaction such us hydrogen bonding, ionic interaction and contact surface are classified according to the origin of the residue, and built in fingerprint scheme which is a representation of a database of protein-ligand complexes. It is seen that Pro 672 (proton donor), Met 674 (proton acceptor), Asp 678 (ionic), Arg 727 (proton donor) and Asn 728 (proton donor).

Pharmacophore query is made computational from three-dimensional structure model of leading molecule which is compound 43. This pattern is based on physical model and binding mechanism, so it sensitives to conformational changes. Better result can be obtained when supported by the data crystal or NMR structural[10].

Distance between aromatic ring of pyrazol (F4:Aro) with polar groups on the R2 chain (F3:Cat&Don) is 6.92 Å and it is important to be maintained[3]. Polar groups have a role to occupy catalytic enzyme site thus blocking ATP to be converted to ADP so the signal stops, while the aromatic groups (F4:Aro), proton donor groups (F1:Don) and proton accepton groups (F2:Acc) will occupy the binding site of [9]. This distance should be maintained during the design of new drug because change in binding conformation will give significance effect on compound of design result.

Then this pattern can be used to test the compounds of design result, is it active or not by looking the suitability between pharmacophore features which is exist in the compound of design result with pharmacophore query more quickly, or how many atoms or groups which is hits with pharmacophore query.

Molecular Docking Study

Before performing docking, molecule target must be prepared in advance. Molecules which have been downloaded from rscb site is displayed on MOE window. In order not to interfere with the docking process, water molecules should be removed, thus ensured that molecule which is interacted is test molecule as the ligand and target molecule. The next step is protonated, to add the atommic charge and hydrogen to molecule. Protein structure which is used is 3TCP structure. The next step is docking simulation of test compound against Mer. In the docking process, this test compound was tested in MOE 2009. Docking simulation process begins by identifying the binding site of protein Mer. Binding site will be automatically identified by using show pocket facility. Binding site was identified as the amino acid residues located at a distance of 5 Å from the natural ligand. Furthermore, with the docking simulation facilities, the test compounds as ligands are docked on Mer as receptor, and is directed at binding site which had previously been identified. Docking process use flexible ligand and rigid receptor by using London dG scoring method.

Docking method validation performed by redocking native ligand in binding site. The value of rmsd (root mean square deviation) obtained is 1.3226 which means the method has high validity as evidence the value of rmsd < 2, which means copy ligand position is similar to the original ligand position.

Generally, pyrazolo-[3,4-d]-pyrimidine binds to the receptor Mer by binding Asp 678, Arg 727, Asn 728 which is catalytic residue of Mer through a hydrogen and ionic bond. N atom of pyrimidine ring also binds with Met 674 and NH group of R3 chain binds with Pro 672 residue. Overall, when compared with the interaction with natural ligand such as ADP or compound 43, although it has different structure to the natural ligand.

The interaction which occurs between the compounds of pyrazolo-[3,4-d]-pyrimidine with Mer indicated by the value of docking score (S), the lower the value of S, the stronger interaction between both of compounds. Scoring function often do not work well in all classes of proteins[11,12]. The same case also occured in this study when modeling the inhibition of pyrazolo-[3,4-d]-pyrimidine against Mer receptor activity. The scoring value of compound number 40 is greater than compound 41 which have the best activity., but this does not mean low effciency at the level of selectivity and side effects, even otherwise has many benefits, such as avoiding drug resistance and toxicity.

Table 1. Descriptors list

No	Symbols on software	General symbols	Descriptors
1	AM1_dipole	μ	Dipole moment
2	AM1_E	E_{Tot}	The total energy
3	AM1_Eele	E_{Ele}	Electronic energy
4	AM1_HOMO	E_{HOMO}	HOMO energy
5	AM1_LUMO	E_{LUMO}	LUMO energy
6	AM1_HF	HF	Heat of formation
7	ASA_H	Å	Hydrophobic surface are
8	Glob	glob	Globularity
9	log P (o/w)	log P	Partition coefficient
10	log S	log S	Logarithm solubility in water
11	Mr	M	Molar refractivity
12	vol_vdw	Vw	Van Der Waals volume
13	Vol	vol	Molecular volume

Table 2. SAR of pyrazolo-[3,4-d]-pyrimidine as Mer inhibitor[3].

R1	• aryl > alkenyl > alkyl • Substitution of the *para* methoxy more active than the *meta* and *ortho* positions • Electron donating groups such as phenyl and pyridyl, may be substituted at the *para* position • Electron withdrawing groups decrease the activity, while the electron donor increases the activity
R2	• The distance between the rings pyrazol with polar groups on R2 is important, and substitution cyclohexylmethyl is preferred • isomer trans-4-aminocyclohexylmethyl more active than its cis isomer
R3	• Replacement of secondary amines with tertiary amines decreases the activity • Extension of the alkyl chain (C3-C5) to increase activity, while side chain alkyl and cycloalkyl groups are less favorable • The addition of polar groups on the alkyl chain dramatically lowering activity • Addition or substitution of the phenyl ring in the phenyl well tolerated • Addition of polar groups (electron-withdrawing) lowering activity (while if nonpolar groups will do otherwise)

Table 3. Activity Value and Z

Molecules	Log (1/IC_{50})Mer	Value of Z Mer
9	-0.3802	0.6603
10	-1.4472	1.4023
12	-0.9823	0.9618
13	0.0655	0.2185
14	0.6778	0.1561
15	0.6990	0.1951
16	1.2518	1.6842
17	0.7447	0.7226
39	2.7447	0.1989
40	3.1192	1.4721
41	3.6021	1.7069
42	3.8239	0.1422
43	2.5376	0.9484
44	2.5229	1.0788

Table 4.The combination of descriptors with a value of statistical criteria and validationleave one out (q^2)

No	Predictors	r^2	F	q^2
1	vdw_vol, AM1_LUMO, LogS, ASA_H,mr	0.9893	148.5	0.9664
2	vdw_vol, AM1_LUMO, AM1_HF, AM1_HOMO, AM1_E	0.9880	131.6	0.9468
3	vdw_vol, AM1_LUMO, LogP, AM1_HOMO, mr	0.9869	120.7	0.9699
4	vdw_vol, LogS, glob, ASA_H, mr	0.9856	109.5	0.9503
5	vol, AM1_LUMO, AM1_HF, AM1_HOMO, AM1_E	0.9854	107.9	0.9602
6	vol, AM1_LUMO, LogP, AM1_HOMO, mr	0.9852	106.8	0.9636
7	vdw_vol, AM1_LUMO, glob, LogS, mr	0.9850	104.8	0.9628
8	vdw_vol, AM1_LUMO, AM1_HF, AM1_HOMO, AM1_Eele	0.9846	102.4	0.9570
9	vdw_vol, AM1_LUMO, glob, LogP, mr	0.9844	101.1	0.9411
10	vol, AM1_LUMO, AM1_HF, AM1_HOMO, AM1_Eele	0.9841	99.3	0.9306

Table 5. Comparison of similarities between the model equation with the best statistical criteria

Model	A	B	C	D
1*♦	0.9893	0.9664	1 S, 4 SS	0.9767
3**	0.9869	0.9699	1 S, 4 SS	0.8841

A : r^2 equation model,
B : q^2 cross validation leave one out,
C : Pearson Correlation Descriptor Significance,
D : $r^2 IC_{50}$ Experiment with Prediction curve,
S : Significance,
SS : Very Significance.
* Equation model with the highest r^2 value,
** Equation model with the highest cross validation LOO value,
♦The chosen equation model

Table 6. Pearson Correlation Descriptor against Log IC_{50}

Equation 1(with the best regression coefficient (r^2) value)					
Pearson Correla-tion	AM1_LUMO	ASA_H	LogS	mr	vdw_vol
Log $1/IC_{50}$	-0.607[a]	0.93[b]	-0.88[b]	0.945[b]	0.941[b]
Signifi-cance	0.0208	1.48×10^{-6}	3.28×10^{-5}	3.69×10^{-7}	5.32×10^{-7}
* significance in level 0.05			1 descriptor		
** significance in level 0.01			4 descriptors		
Equation 3 (with the best cross validation Leave One Out (q^2) value)					
Pearson Correla-tion	AM1_HOMO	AM1_LUMO	LogP	mr	Vdw_vol
Log $1/IC_{50}$	0.716[b]	-0.609[a]	0.786[b]	0.945[b]	0.941[b]
Signifi-cance	3.96×10^{-3}	2.08×10^{-2}	8.63×10^{-4}	3.70×10^{-7}	5.32×10^{-7}
[a]significance in level0.05			1 descriptor		
[b] significance in level 0.01			4 descriptors		

Table 7. IC50 experiment and prediction valueof equation 1

Comp ound	IC_{50} Mer (µM)	
	Experiment	Prediction
9	2.4	1.00010
10	28	38.67920
12	9.6	7.46410
13	0.86	0.93549
14	0.21	0.30756
15	0.2	0.29708
16	0.056	0.07767
17	0.18	0.13323
39	0.0018	0.00209
40	0.00076	0.00074
41	0.00025	0.00048
42	0.00015	0.00011
43	0.0029	0.00247
44	0.003	0.00204

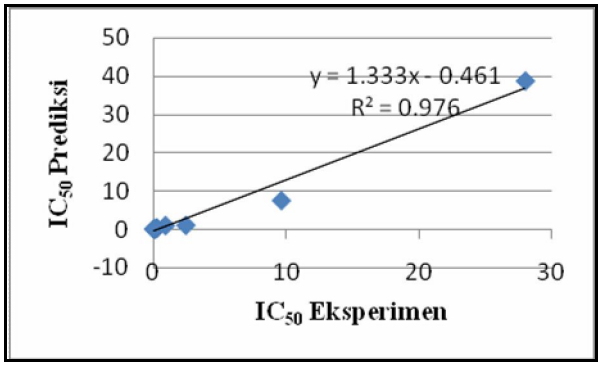
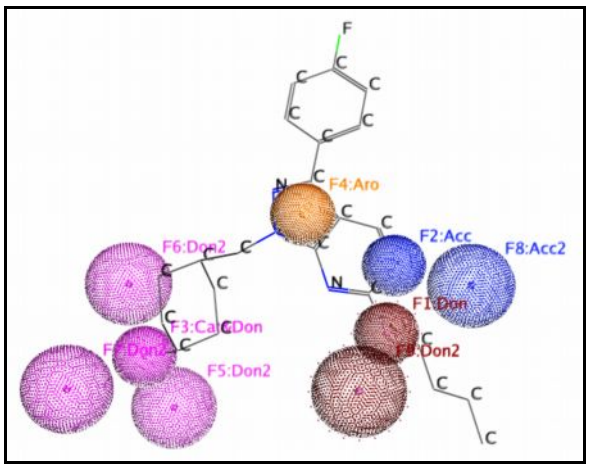


Figure 2. Experiment and prediction curve of IC_{50} Mer inhibitor



Don: Proton Donor, **Acc**: Proton Acceptor, **Cat&Don**: Cation and Proton Donor, **Aro**: Aromatic Ring, **Don2**: Projection Proton Donor, **Acc2**: Projection Proton Acceptor.

Figure 3. Pharmacophore Query

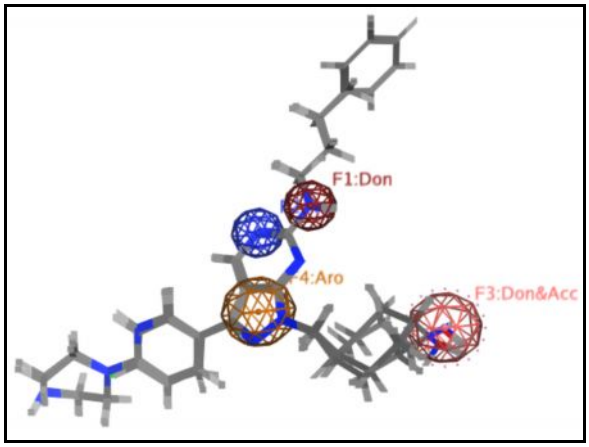


Figure 4. The alignment of the molecular structure

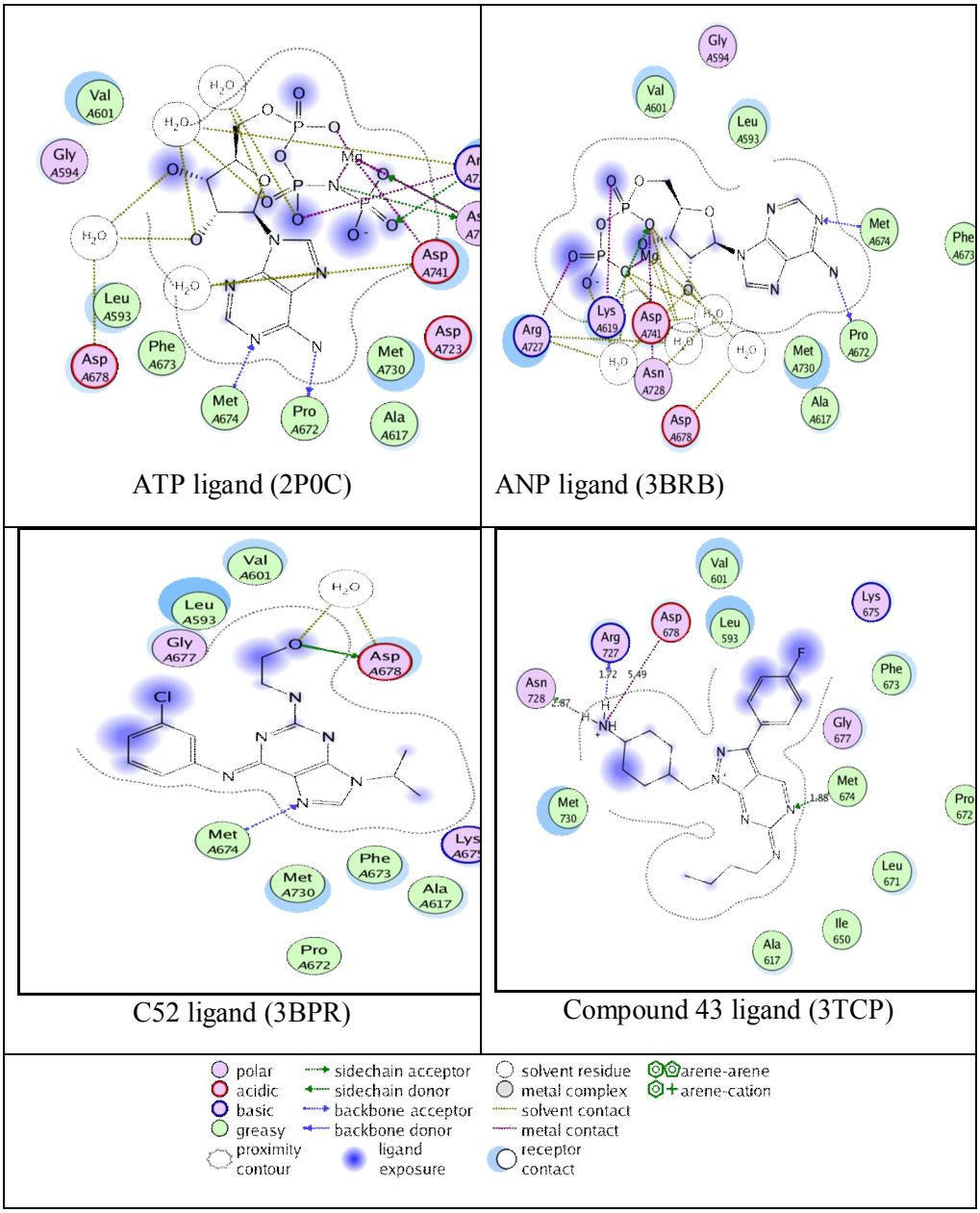


Figure 5. Binding model of ligands on Mer

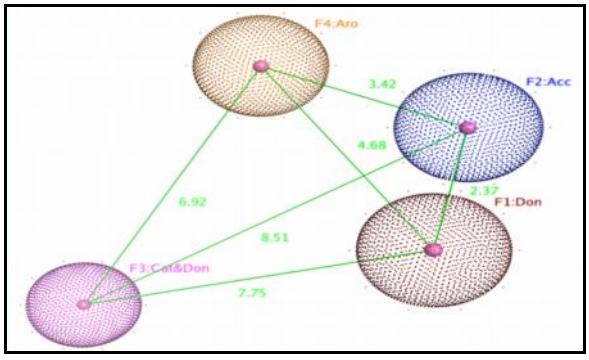


Figure 6.The distance between the features of pharmacophore query

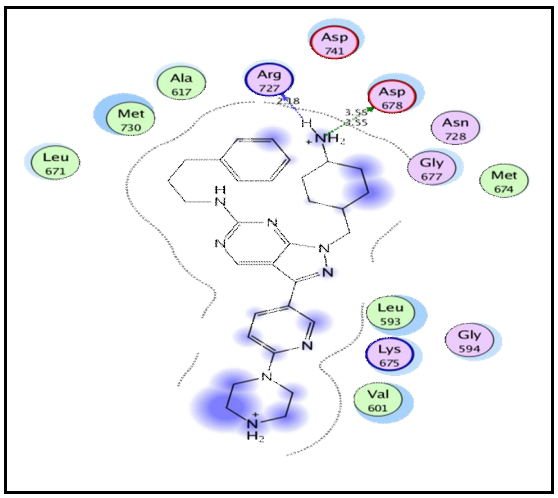


Figure 7. Model binding of docking result of compound 40

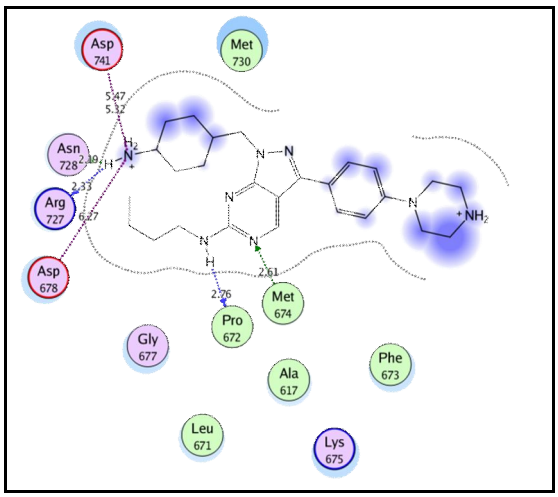


Figure 8. Model binding of docking result of compound 41

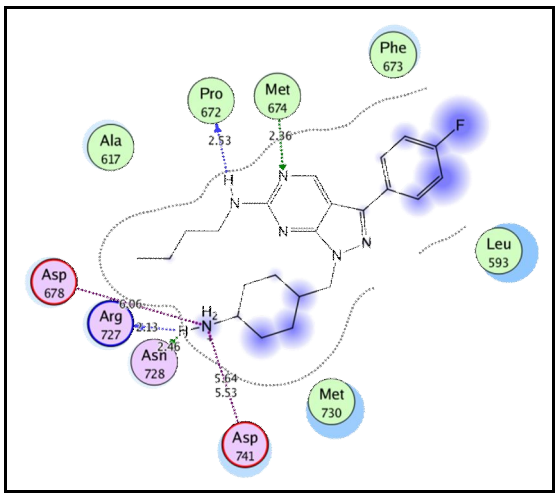


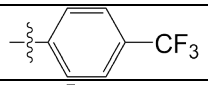
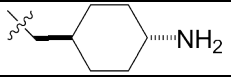
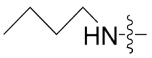
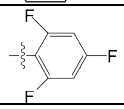
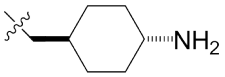
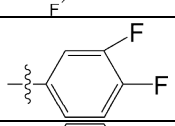
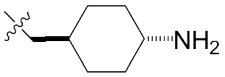
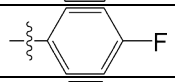
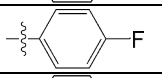
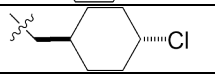
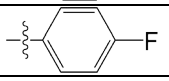
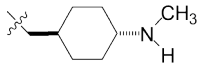
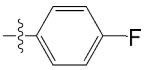
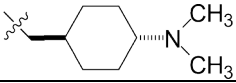
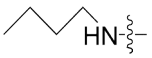
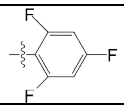
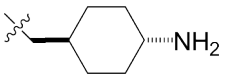
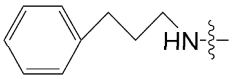
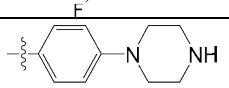
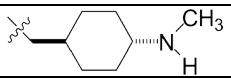
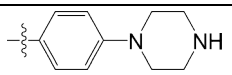
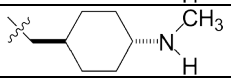
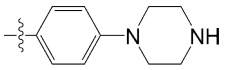
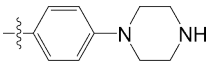
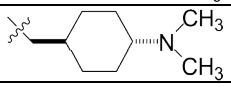
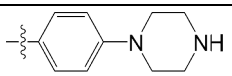
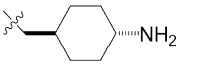
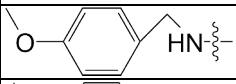
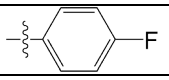
Figure 9. Model binding of docking result of compound 43

Table 8. Results of pyrazolo-[3,4-d]-pyrimidine derivatives compound docking against Mer

Compound	*Docking* Score Ld (S) (kkal/mol)	The number of hydrogen bonds		Bond distance (Å)	Amino acid residues bond	Binding groups
09	-9.5750	Hydrogen	3	2.11	Asp 678	HO of R2 ^{Don}
				2.16	Met 674	N pyrimidine core ^{Acc}
				2.56	Pro 672	HN of R3 ^{Don}
10	-9.2021	Ionic	1	5.52	Asp 678	NH_2^+ of R2 ^{Cat}
		Hydrogen	2	2.32	Met 674	N pyrimidine core ^{Acc}
				2.45	Pro 672	HN of R3 ^{Don}
12	-10.0051	Ionic	1	4.91	Asp 678	NH_2^+ of R2 ^{Cat}
		Hydrogen	3	2.01	Arg 727	H_2N^+ of R2 ^{Don}
				2.17	Met 674	N pyrimidine core ^{Acc}
				2.40	Pro 672	HN of R3 ^{Don}
13	-9.7344	Hydrogen	2	3.70	Arg 727	HO of R2 ^{Don}
				2.75	Met 674	N pyrimidine core ^{Acc}
14	-9.2363	-	-	-	-	-
15	-8.9855	Hydrogen	3	2.03	Asn 728	HO of R2 ^{Don}
				2.50	Met 674	N pyrimidine core ^{Acc}
				2.41	Pro 672	HN of R3 ^{Don}
16	-10.6099	Ionic	1	6.13	Asp 678	NH_3^+ of R2 ^{Cat}
		Hydrogen	4	2.40	Asn 728	H_3N^+ of R2 ^{Don}
				2.38	Arg 727	H_3N^+ of R2 ^{Don}
				2.20	Met 674	N pyrimidine core ^{Acc}
				2.15	Pro 672	HN of R3 ^{Don}
17	-10.1707	Ionic	1	3.82	Asp 678	NH_3^+ of R2 ^{Cat}
		Hydrogen	3	2.29	Arg 727	H_3N^+ of R2 ^{Don}
				2.21	Met 674	N pyrimidine core ^{Acc}
				2.28	Pro 672	HN of R3 ^{Don}
39	-11.6571	Ionic	1	6.29	Asp 678	NH_3^+ of R2 ^{Cat}
		Hydrogen	3	2.59	Arg 727	H_3N^+ of R2 ^{Don}
				2.31	Asn 728	H_3N^+ of R2 ^{Don}
				2.14	Lys 675	H_2N^+ of R1 ^{Don}
40	**-12.7584**	Ionic	1	3.55	Arg 727	H_3N^+ of R2 ^{Cat}
		Hydrogen	2	2.18	Asp 678	H_3N^+ of R2 ^{Don}
				3.15	Asp 678	H_3N^+ of R2 ^{Don}
41	**-11.9987**	Ionic	1	6.27 /5.32	Asp 678 /Asp 741	NH_3^+ of R2 ^{Cat}
		Hydrogen	4	2.33	Arg 727	H_3N^+ of R2 ^{Don}
				2.19	Asn 728	H_3N^+ of R2 ^{Don}
				2.61	Met 674	N pyrimidine core ^{Acc}
				2,76	Pro 672	HN of R3 ^{Don}
42	-11.2583	Ionic	1	3.99	Asp 678	NH_3^+ of R2 ^{Cat}
		Hydrogen	2	2.06	Arg 727	H_3N^+ of R2 ^{Don}
				2.67	Met 674	N pyrimidine core ^{Acc}
43	-10.5672	Ionic	1	6.06 /5.53	Asp 678 /Asp 741	NH_3^+ of R2 ^{Cat}
		Hydrogen	4	2.13	Arg 727	H_3N^+ of R2 ^{Don}
				2.46	Asn 728	H_3N^+ of R2 ^{Don}
				2.36	Met 674	N pyrimidine core ^{Acc}
				2.53	Pro 672	HN of R3 ^{Don}
44	-11.8373	Ionic	1	3.96	Asp 678	NH_3^+ of R2 ^{Cat}
		Hydrogen	2	200	Arg 727	H_3N^+ of R2 ^{Don}
				2.67	Met 674	N pyrimidine core ^{Acc}

^{Acc} : Proton acceptor, ^{Don} : Proton donor, ^{Cat}: Cation, Ld : London dG

Table 9. The calculation results of activity of new compounds and selectivity

No	R1	R2	R3	IC_{50} Mer (µM)
PP1.				0.000035
PP2.				0.000142
PP3.				0.000302
PP4.				0.00000888
PP5.				0.007356
PP6.				0.000597
PP7.				0.00000678
PP8.				0.000056
PP9.				0.000092
PP10.				0.000111
PP11.				0.000004
PP12.				0.00000188
PP13.				0.000820
PP14.				0.003528
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PP20.				0.023520
PP21.				0.044029
PP22.				1.692955
PP23.				0.141640
PP24.				0.001979

Conclusion

Quantitative structure activity relationship of pyrazolo-[3,4-*d*]-pyrimidin derivativecompoundsas Mer inhibitor shows that five predictors affect the activity of the compounds, as illustrated by the best QSAR equation:

Log $1/IC_{50}$= 1.731(±1.417) - 3.201(±0,984) AM1_LUMO-0.065(±0.012) ASA_H - 0.846(±0.144) LogS - 8.348(±1.262)mr +0.243(±0.036)vdw_vol

Amino acids which are important in Mer protein interaction with pirazolo-[3,4-*d*]-pirimidin compounds are Pro 672, Met 674, Asp 678, Arg 727 dan Asn 728 with fingerprint code (daDID). The query pharmacophore which play roles in ligand-receptor interaction have feature a proton donor group, proton acceptor group, cations and proton donor group, and aromatic group.

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