Research Article

Study of Quinazoline Derivative Compound as Anticancer on EGFR^{WT} Protein using Quantitative Structure-Activity Relationship (QSAR)

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ABSTRACT

Analysis of quantitative structure-activity relationship of quinazoline compound had been carried out. Some of quinazoline derivative structures and activities were taken from literature. Electronic and molecular parameters were calculated by using DFT (B3LYP/6-31G) method. Multi-linearity regression analysis was employed to determine QSAR equation model. The result of the analysis is QSAR equation, described as follows:

Log IC_{50} = -0.896 + 6.476qC12 + (-33.215)qC19 + (-49.783)HOMO + 0.005Volume + (-0.473)Log P + (-0.007)Mass

(n = 34, R^2 = 0.951, PRESS = 0.602, SD = 0.282, χ^2 = 0.220)

This equation was utilized to design and predict a new compound containing a more effective bioactivity as an anti-cancer. From the analysis, we found two new compounds with IC_{50} = 1.820 nM) and IC_{50} = 1.585 nM on EGFR^{WT} protein. These compounds have met the Lipinski's rule, claiming them as fine drug molecules with a good adsorption system.

Keywords: Quinazoline, protein EGFR^{wt}, QSAR, DFT (B3LYP/6-31G).

INTRODUCTION

Quinazoline derivatives compounds have been frequently used as the basic compound in developing anti-cancer drug molecules, especially as a lung anti-cancer.[1,2] Different researches have synthesized quinazoline derivative compound and directly tested it in cancerous cells and the protein; such as EFGR (Epidermal Growth Factor Receptor) protein, showing inhibitor properties. The first generation inhibitors were erlonitib[3], the second generation afanitib[4] and the third one rociletinib[5]. All of these inhibitors were developed based on quinazoline compound. Protein EFGR^{WT} was hired since in the lung cancer. Protein EGFR were able to be the target protein in inhibition growth of cancer.[6] This protein has responsibility in tumour growth and progression, including proliferation, angiogenesis, metastasis, and inhibition of apoptosis. Targeting on EGFR using small molecules such as gefinitib (Iressa) has been a strategy for inhibition of EGFR tyrosine kinase activity. These small molecules will hinder ATP binding to the tyrosine kinase domain of the receptor, then preventing tyrosine kinase activity and autophosphorylation. Finally it will block signal transductions from the EGFR.[7] QSAR analysis was performed as an attempt to find a consistent relationship among variants of some molecular properties and biological activities from some series of compounds.[8] From statistical analysis, a new equations could be applied to design new chemical compound.

MATERIALS AND METHODS

Data Set

A set of 34 quinazoline derivatives compound with their IC_{50} values for anticancer activity was taken from previous research[9] as seen in Table 1. All of calculation in this research was done by using a PC with Intel Core i7 4720HQ 3.6 GHz which has RAM 4 GB. Software which used were Gaussian 09 W[10] and Gauss View 5.0.8.

Procedure

Computational Methods

Compound **b1** with lowest IC_{50} value was optimized using the density function of B3LYP, BPV86, B3PW91, and LSDA. ^{1}H-NMR chemical shift obtained from the DFT calculation was compared with experimental values. The density function resulted more accurate the chemical shift value was chosen to be used in descriptor calculation for QSAR analysis.

A set of 5 compounds was used for testing QSAR equation models, and remaining 29 compounds were grouped to be training set.

Electronic and molecular properties were taken into account in the equation model using multi linear regression analysis. Statistical parameters were applied in training set to find the best equation model. Statistical parameters which conducted in training set are value of R, R^2, and comparison F_{calc}/F_{table}. A good QSAR model has R^2 value higher than 0.6.[11] A model can be regarded as a good one if the difference between value of R and R^2 was not more than 0.3, indicating the number of involved descriptors in the model are accepted.[12]

The best equation model was validated using test set to obtain a reliable equation model. Then, the reliable equation model was used to design new compound with

low IC_{50} value. Validation using test set called external validation.

Statistical parameters used in this validation are value of R^2, SD, PRESS, and chi-squared (χ^2). A good statistical model if it has R^2 higher than 0.6 and chi-square (χ^2) less than 0.5.[12]

RESULTS AND DISCUSSION

In this study we use DFT method and do variation in density function to choose the best function to describe the molecular wave function.

Previous research[13] has compared ^{1}H-NMR chemical shift obtained between DFT (Density Function Theory), Hartree-Fock (HF) and Austin Model 1 (AM1) semi empirical methods with experimental data, showing that DFT gave best correlation value (r = 0.932), while HF and AM1 method only 0.897 and 0.846, respectively.

In this research, based on ^{1}H-NMR chemical shift calculation, it can be seen that DFT method using B3LYP density function provides a better result in comparison with experiment data (See Table 2) than other density functions, showing the highest R^2 (0.9344) and the lowest SD values (0.6415).

This result suits with previous study[14] which said that B3LYP density function gave a high accuracy for organic compounds.

Stable quinazoline derivative compounds were obtained from optimizing their structure using DFT B3LYP method.

In compound **c6**, we found that atomic charge of phenyl carbon (C-3) directly bonded to fluor has positive partial charges (0.303), due to the high electronegativity on fluor.

Meanwhile, in compound **c8**, atomic charge of phenyl carbon (C-3) in the same position bonded to chlor has negative partial charge (-0,227). Structure of compound c6 and c8 can be seen in Figure 1.

The different partial charge sign of C-3 atom reflect the smaller electronegativity of chlor in comparison with fluor. Result of geometrical optimization shows that changing substituent affect to different atomic charge.

Therefore, atomic charges are necessary to be regarded as one of parameters in modelling QSAR properties.

Development Model

Four models with their statistical parameters are provided in Table 3.

There is always log P descriptor in resulted models signifying descriptor influences in the activities of quinazoline compounds because lipophilicity is a key of physicochemical characteristics of a certain compound. It has good agreement with previous study[15] said that lipophilicity parameter has role in determining ADMET process and compatibility from a candidate compound to be a drug molecule.

Validation of QSAR model could be done using training set to establish the good predictive models.

Statistical parameters utilized in this validation are value of R, R^2, and comparison value of $F_{calc/table}$ (See Table 3).

From table 3, the four models have R and R^2 values are higher than 0.6 and the ratio between F_{calc} and F_{table} is more than 1.

It is regarded that all of models has 95% significance levels.

Those models fill all of these statistic parameters. Thus, all of models fulfil all of statistical parameters, then external validations could be conducted.

Model Validation

External validation is a statistical method to ensure that QSAR model has a good productiveness.

A set of compound as test set is used in this method to evaluate the QSAR model.

Statistical parameters used in external validations are the values of R^2, SD, PRESS, and χ^2.

External validation results can be seen in Table 4.

Based on the data, only model 4 which has value of R^2 higher than 0.6 which means there is a linear correlation among the parameters with anti-cancer activity values.

Values of SD and PRESS from model 4 also have the lowest value.

Hence, the best model was model 4 which can be used to design a new quinazoline compound.

From MLR analysis on model 4, the QSAR equation resulted as follows:

Log IC_{50} = -0.896 + 6.476 qC12 + (-33.215) qC19 + (-49.783) HOMO + 0.005 Volume + (-0.473) Log P + (-0.007) Mass

This equation model is then used to design new quinazoline compound as lung anti-cancer.

Design New Compound

Designing new compound is based on substituent changes with some other functional group.

These changes are aimed to produce new compounds with better activities.

Some crucial things to concern in designing compounds are the ease of synthetic processes and existences of the materials.

Changing a substituent can be done by paying attention on isosteres characteristics owned by the functional group.

Halogen group could be replaced with CN and CF_3 as alternative electron-withdrawing groups.[16]

By employing QSAR equation resulted by model 4, two compound which have smaller IC_{50} values (1.820 nM and 1.585 nM) than the former compound (IC_{50} = 2 nM) can be designed.

Structures of designed compounds can be seen in Figure 2.

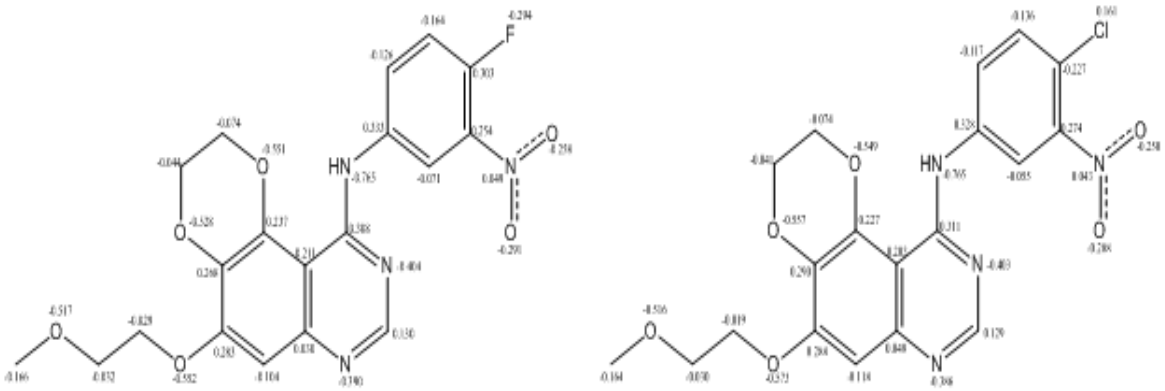
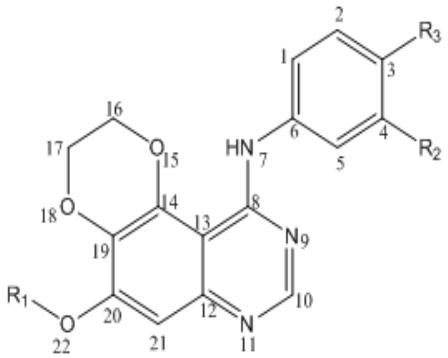
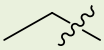
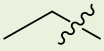
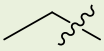
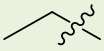
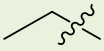
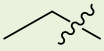
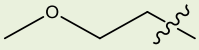
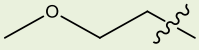
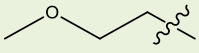
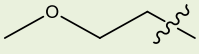
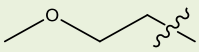
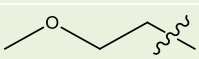


Figure 1: Structure of compound c6 and c8 calculated using DFT B3LYP/6-31G

Table 1: Core structure and Anticancer Activity of Quinazoline Derivative Compound



Compound	R_1	R_2	R_3	log IC_{50} (nM)
a1	Me	Cl	F	1.35
a2	Me	ethynyl	H	1.04
b1		Cl	F	0.30
b2		ethynyl	H	0.96
b3		MeO-	H	0.64
b4		Cl	Me	0.83
b5		NO_2	H	0.99
b6		H	Me	0.56
c1		Cl	F	1.12
c2		ethynyl	H	1.32
c3		H	F	2.30
c4		MeO^-	H	2.20
c6		NO_2	F	1.64
c7		NO_2	Me	1.87

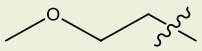
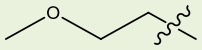
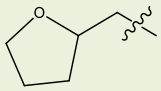
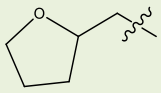
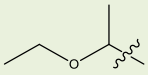
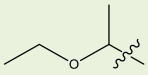
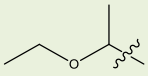
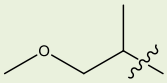
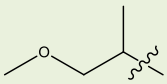
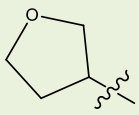
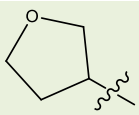
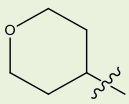
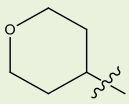
c8		NO_2	Cl	1.56
c9		CN	F	2.59
d1		Cl	F	1.38
d2		ethynyl	H	1.44
e2		NO_2	F	1.92
e3		NO_2	Me	2.27
e4		NO_2	Cl	2.01
e5		CN	F	2.50
e6		CN	Me	2.62
e7		Cl	F	1.46
e8		ethynyl	H	1.37
f2		NO_2	F	2.37
f3		NO_2	Me	2.56
f4		CN	F	2.55
g1		Cl	F	2.39
g2		ethynyl	H	2.68
h1		Cl	F	1.31
h2		ethynyl	H	1.22
i1		Cl	F	1.77
i2		ethynyl	H	1.76

Table 2: Comparison of calculated and experimental ^{1}H-NMR chemical shift of compound b1

Number of Proton	Experiment	Proton signal	B3LYP	BPV86	B3PW91	LSDA
H-27	10.4800	s, 1H	8.8726	9.2183	8.9838	9.8799
H-29	8.7300	s, 1H	8.1907	8.3853	8.1610	8.3046
H-28	7.9500	dd, 1H	7.9192	8.3853	8.0131	9.1230
H-26	7.6500	dd, 1H	5.4990	5.6982	5.4671	5.5431
H-40	7.5400	t, 1H	5.9357	6.1274	5.9073	5.9819
H-34	6.9900	s, 1H	6.4197	6.6236	6.3870	6.4392
H-30, H-31	4.6000	dd, 2H	3.5386	3.8109	3.5139	3.7354
H-32, H-33	4.4400	dd, 2H	3.4316	3.6778	3.3992	3.5779
H-35, H-36	4.2400	q, 2H	3.7404	4.0391	3.7099	4.0963
H-37, H-38, H-39	1.4300	t, 3H	0.6692	0.9119	0.6399	0.8722
R^2			0.9344	0.9316	0.9311	0.8975
SD			0.6415	0.6837	0.6491	0.8621

Table 3: Some model calculation using MLR analysis

Model	Descriptors	R	R^2	$F_{calc/table}$
1	qC3, qC12, qC19, LUMO, Log P, Mass	0.873	0.701	3.5343
2	qC6, qC19, HOMO, Volume, Log P, Mass	0.818	0.669	3.0459
3	qC13, qC16, qC19, qO22, HOMO, Log P	0.846	0.715	3.7818
4	qC12, qC19, HOMO, Volume, Log P, Mass	0.797	0.635	2.3966

Table 4: Values of external validation for each model

Model	Tested Compound	R^2	SD	Press	χ2
1	g2, e6, c6, c8, e7	-0.5480	0.8277	2.8609	1.7247
2	g1, f2, c8, e7, a1	0.4754	0.5397	2.7755	14.6383
3	i2, c6, c8, b4, b3	0.4492	0.7967	3.2579	2.6324
4	f4, f2, c8, e7, a2	0.9510	0.2818	0.6020	0.2199

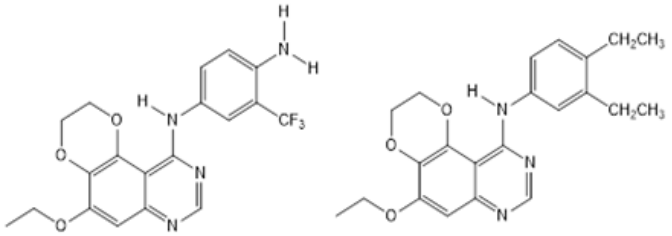


Figure 2: Structure of two design compound of quinazoline derivative

Table 5: Psychochemical characteristics of two design compound

No.	Name of structure Compound	Log P	Mr	Donor Hydrogen bond	Acceptor Hydrogen bond	Log IC_{50} (nM)
1	N1-(5-ethoxy-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-yl)-3-(trifluoromethyl)benzene-1,4-diamine	2.54	406.26	3	7	0.27
2	N-(3,4-diethylphenyl)-5-ethoxy-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine	4.34	379.46	1	6	0.20

The electronic and molecular parameters of those two compounds are measured to obtain IC_{50} value.

In designing a drug, it is necessary to pay attention on drug absorption processes in the body.

These processes can be observed by analysing psychochemical characteristics of a molecule. Results of some molecular parameter measurements displaying psychochemical characteristics of drug molecules calculated with DFT (B3LYP/6-31G) method can be seen in Table 5. From table 5, Log P values of both compounds are not more than 5, the relative molecular mass are under 500, and numbers of hydrogen bond donors and acceptors have fulfilled Lipinski's rules[17]. Those above compounds also have smaller log IC_{50} values, proving that their activities are better as pulmonary anti-cancer.

CONCLUSION

DFT (B3LYP/6-31G) is the best method to calculate electronic and molecular parameters at QSAR analysis for quinazoline derivative compounds.

Model 4 has utilized successfully to predict two new bioactive compounds with their activities that met the Lipinski's rule.

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