3D QSAR and pharmacophore modeling studies on C-2 and C-4 substituted Quinazoline series as Anti-tubercular agents.

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ABSTRACT

Antitubercular drug discovery is a challenging strain in the recent era. Quinazolines are an important chemical compounds having diverse significant biological activities. Three dimensional Quantitative structure-activity relationships (3D QSAR) represent an attempt to correlate three dimensional structural features of compounds with biological activities. The pharmacophore identification and QSAR studies on C-2 and C-4 substituted quinazoline series was carried out by partial least-squares (PLS) method to identify the potential framework of the molecules responsible for the antitubercular activity. The QSAR models were developed considering training and test set approaches further validated for statistical significance and predictive ability by internal and external validation. The results of 3D QSAR model demonstrate the high degree of statistical importance and excellent predictive capability. The hydrogen bond accepter, hydrogen bond donor, positively charged, aromatic carbon, steric and hydrophobic parameter are the important features contributing towards the activity. The selected best QSAR model A has a training set of 20 molecules and test set of 6 molecules with correction coefficient of 0.9641.

Key words: Antitubercular activity, 3D QSAR, PLS analysis, Quinazoline.
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INTRODUCTION

Tuberculosis, a respiratory disorder caused by *Mycobacterium tuberculosis*. In recent decade the number more than 20 million new cases and 1.4 million deaths are reported due to the disease. Current TB treatment requires a combination of four drugs, isoniazid, rifampicin, pyrazinamide, ethambutol for two months followed by an additional four months of isoniazid and rifampicin [1, 2]. Due to the long term use of these drugs the problem of the TB become critical due to the emergence of multidrug resistant (MDR) and extensively drug-resistant (XDR) strains of *M.Tuberculosis*. Discover of the newer anti TB drug is need of time and these new drugs are required to shorten the duration of therapy and to treat drug-resistant strains[3]. Quinazoline derivatives have been reported possess various biological applications including antimicrobial, anti-convulsant, anti-cancer and antiviral activities [4]. Computing is used in various ways in drug discovery. Important examples include QSAR, artificial intelligence, and structure-based methods. Quanative Structure Activity Relationship (QSAR) studies and pharmacophore modelling are capable of providing information about the structural features accountable for biological [5]. These physicochemical descriptors, which include parameters to account for hydrophobicity, topology, electronic properties and steric effects, which are determined empirically or more recently by computational methods.. Activities used in QSAR include chemical measurements and biological assays [6].

The goal of QSAR is to transform searches for compounds with desired properties using chemical knowledge and experience into a mathematically quantified form. Thus, the QSAR approach accelerates the process of development of new molecules for use as drugs. In partial least squares regression is an extension of the multiple linear regression

models. In simplest form it, a linear model specifies the (linear) relationship between a dependent (response) variable Y, and a set of predictor variables X's. PLS regression, the X block of independent variables (descriptors) is correlated with the y vector (activities) in such a way that the projected coordinates. T, are good predictors of y. partial least squares regression is probably the least restrictive of the various multivariate extensions of the multiple linear regression model. We execute here our pharmacophore identification and 3D QSAR studies using PLS method on training set of quinazoline series as antitubercular activity by considering the steric and electrostatic influences [7,8].

MATERIALS AND METHODS

All computational studies were carried out using VLife MDS 4.3 suit installed in Dell inspiron 15 Laptop running on a 1.50 GHz Intel core i3 processor with 2GB RAM and 500 GB hard disk with Windows 8 operating system.

Experimental:
Biological activity Dataset
A dataset of 26 compounds was taken from the published antitubercular activity by J. Odingo *et.al*.,[9]. The general structures of molecules (Figure 1) and structures of all the compounds used in study are given in Table 1. The whole dataset was randomly divided into a training set of 20 compounds and a test set of 6 compounds (Table 1). The training set was used to construct 3D QSAR models based on steric and electrostatic fields that gives a deep insight into structure and activity correlations and the test set was used for the models validation. The molar inhibitory concentration (PMIC50) values were used for the present 3D QSAR study [10]. A relationship between independent and dependent variables three dimensional (3D) fields and biological activities respectively were determined statistically using PLS [11,12].
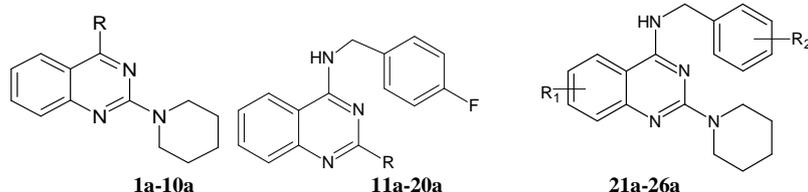

Figure 1: Showing general structure of molecules used for QSAR study

Table 1: Showing derivatives under QSAR study with observed and predicted activity

Sr. No.	Compd Code	R-group	Observed Activity	Predicted Activity
1	1a	PhCH$_2$HN-	5.93	6.22
2	2a	4-FPhCH$_2$HN-	7.1	7.5
3	3a	4-CF$_3$PhCH$_2$HN-	6.3	7.0
4	4a	4-FPhCH$_2$O-	9.9	10.
5	5a	4-FPhCH$_2$S-	15	14
6	6a	MeHN-	10	11
7	7a	*i*PrHN-	11	11
8	8a	4-CF$_3$PhCH$_2$CH$_2$HN-	5	7
9	9a	4-FPhHN-	6	7
10	10a	H-	5	6
11	11a	H$_2$N-	5	6
12	12a	MeHN-	7.	7
13	13a	Me$_2$N-	6	7
14	14a	Pyrrolidin-1-yl	6	7
15	15a	Piperazinyl	5.8	5.7
16	16a	(Piperidin-1-yl)CH$_2$HN-	5.9	5.9
17	17a	H-	7.5	7.9
18	18a	Me-	4.3	3.2
19	19a	Ph-	5.4	5.6
20	20a	Cyclohexyl-	6.0	5.5
		R$_1$, R$_2$ Groups		
21	21a	H, Me	6.2	6.4
22	22a	Me, H	6.5	7.7
23	23a	H, Me$_2$N-	5.5	5.4
24	24a	Me, Br	5.9	6.7
25	25a	Cl , H	4.4	4.2
26	26a	H, NO$_2$,	6.3	5.4

Ligand Preparation
The structure of C-2 and C-4 substituted quinazoline was used as the template to built the molecules in the data set in builder module of VLife ^{TM} MDS and ligand   geometries were subsequently optimized to make the

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conformations having least potential energy. Energy minimizations were performed using Merck molecular force field (MMFF) [13].

Molecular alignment
Molecular alignment is a crucial step in 3D QSAR study to obtain meaningful results. This method is based on moving of molecules in 3D space, which is related to the conformational flexibility of molecules. The molecules of the data set were aligned by the template based technique, using quinazoline as a template for alignment of the molecules [14]. The aligned view of all the molecules on the template was shown in Figure 2.
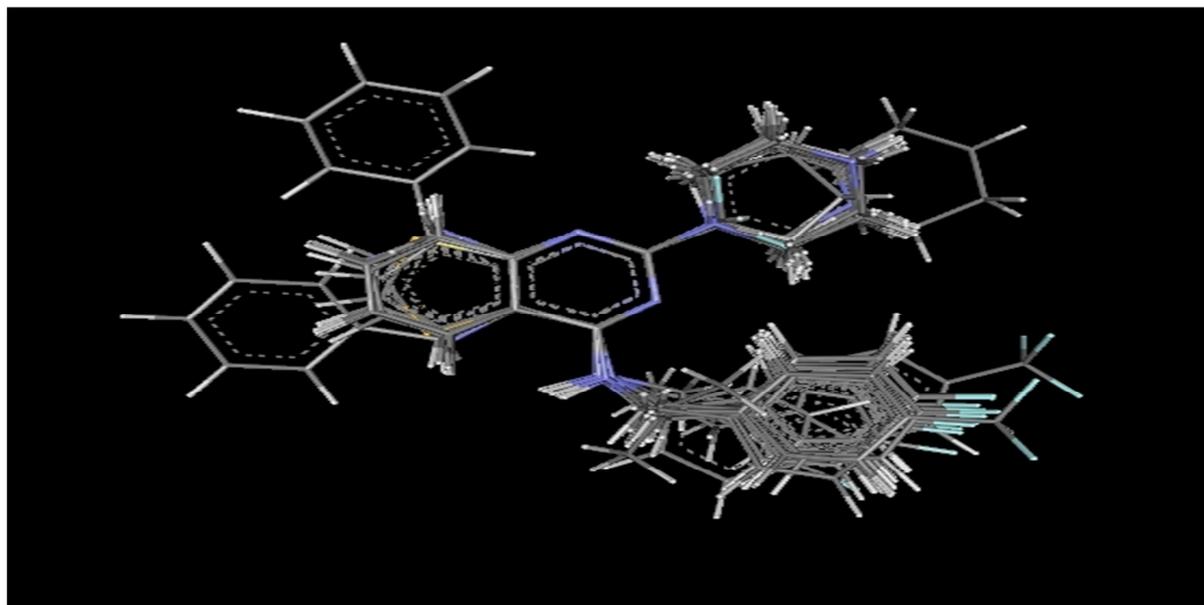

Figure 2: Alignment of molecules under study

Descriptor Calculation:
Molecular fields are the steric and electrostatic interaction energies which are used to formulate a relationship between steric and electrostatic properties together with the biological activities of compounds. The alignment of the molecules under study was carried out using VLife[TM]. The hydrophilic, steric and electrostatic fields are computed at each grid point considering MMFF charges [15-18]. Methyl probe of charge +1 were used for fields generation.

3D QSAR studies using partial least squares regression :
A relationship between independent and dependent variables (3D fields and biological activities, respectively) were determined statistically using regression analysis. Linear regression is achieved by fitting a best-fit straight line to the data using the least squares method. The quality of fit for a regression equation was assessed relative to its correlation coefficient and standard deviation. QSAR model was evaluated by the predictive results for the given data set. The F value represents the level of statistical significance of the regression. Quality of selected models was further ascertained to select the best model from cross-validated squared correlation coefficient (q^2). For a regression model, r^2 was used to describe the fitness of data and fitness is considered to improve as r^2 approaches 1. Thus models having correlation coefficient above 0.7 were used to check the external predictivity while the significance of the model was decided on the basis of F value. Models showing q2 below 0.6 were discarded. The selected models are shown in Table 2.

Table 2: Models A and B selected on the basis of r^2, q^2, pred r^2, F and p values showing the selected MLR QSAR equations along with statistical parameters

Model No.	QSAR model	N	r^2	q^2	F value	Pred r^2
A	PMIC50= 15.8415 -14.3088 E_240+2.7869 E_862-2.5748 S_660	26	0.98	0.96	404	0.82
B	PMIC50= 15.8415 -14.3088 E_240+2.7869 E_862-2.5748 S_660	26	0.91	0.89	310	0.81

Pharmacophore modeling
Pharmacophore modeling was carried out using the mol sign module of VLife MDS 4.3 software. Series of C-2 and C-4 substituted quinazoline were first aligned on the active molecule. The software was set to generate minimum 4 pharmacophoric features obtained keeping the tolerance limit at 10 A[o].

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RESULTS AND DISCUSSION

The total data set was randomly divided, 20 molecules were used in the training set (Table 1) to derive 3D QSAR models with the number of field grid points being not more than five per model. To evaluate the predictive ability of generated 3D QSAR models, and test set of 6 molecules with regularly distributed biological activities was used (Table 1). On successful run of PLS two models were selected they are shown in Table no 2.

Interpretation of 3D QSAR Model

The optimum structural properties of quinazoline analogs for antitubercular activity were obtained in the form of the 3D descriptors of model A. The model was selected on basis of r^2, q^2, pred r^2, F and p values. The r^2 value for model A was 0.9806 compared to that of model B 0.9105. Model A shows the first model which is selected on the basis of statistical coefficient like r2 (0.9806) and Pred r2 (0.8205). The F test and p significance values were considered for the selection of model. Electrostatic interactions at E_240 , E_862 important features responsible for the biological activity of the molecules. E_240 is contributing negatively towards the biological activity indicatives substitution of the electron withdrawing groups on the quinazoline nucleus will potentiate the antimycobacterial potential of the compound, while the another electrostatic descriptor at E_862 grid point is positively contributing towards the activity which indicates that the substitution of electron releasing groups on the halogen substituted aromatic ring will be increasing the antimycobacterial activity of the molecules. The steric interactions at S_660 is negatively contributing implies that the steric interaction along these lattice points are required to be decreased via substitution of smaller groups (Figure 3).
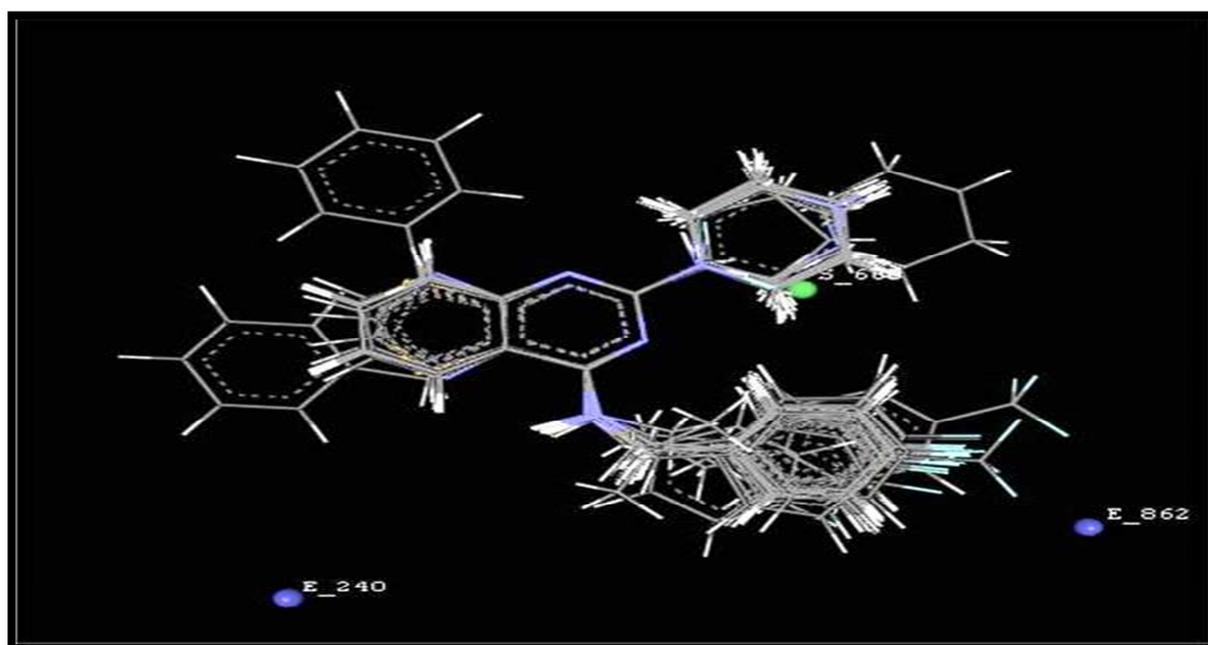

Figure 3: Grid point of Model A

In another selected model B, 3D grid E_162 and S_590 are contributing negatively which indicates importance of substitution of electron withdrawing and hydrophilic groups, will potentiate the biological potential of the synthesized derivatives (Figure 4).
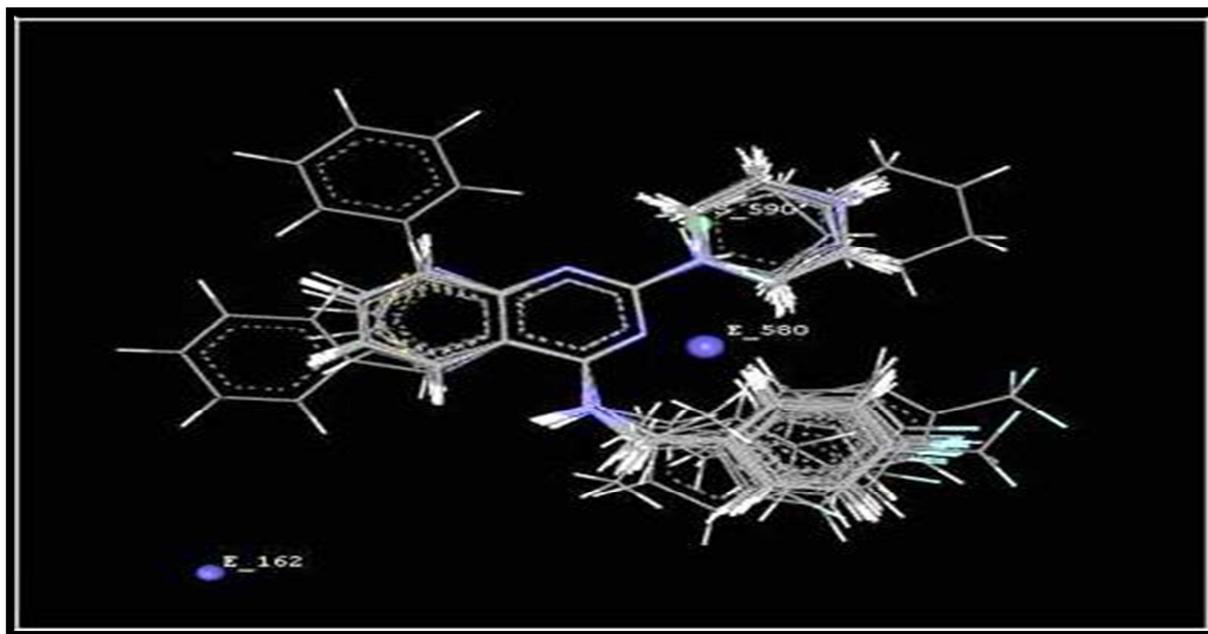
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Figure 4: Grid point of Model B

The plot of observed versus predicted activities (Model A) for data test was represented in (Figure 5), it is evident that the predicted and observed activities of data set will be in good agreement.
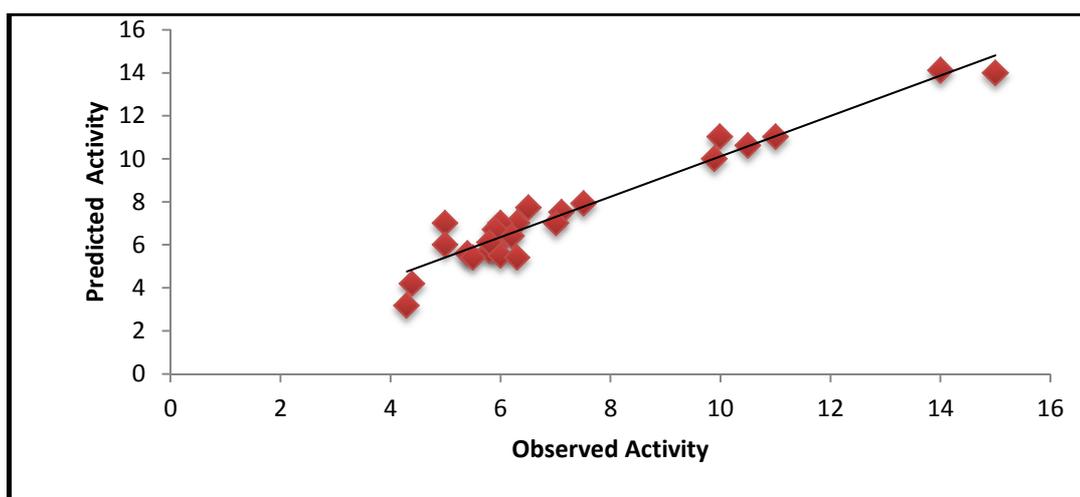

Figure 5: Correlation Plot of QSAR Model A

Pharmacophore Identification Studies Using V Life MDS 4.3
A set of pharmacophore hypothesis was generated using the mole sign module of V life MDS 4.3 on the reported antitubercular activity. Each hypothesis was found to contain common features like aromatic, hydrogen bond donor, hydrogen bond acceptor and positive charge. The pharmacophore hypothesis generated in VLife MDS 4.3 indicated the significance of presence of five aromatic features for the antitubercular activity; these features are contributed by the C-2 and C-4 substituted quinazoline nucleus, which are separated by 1.2 A$^{\circ}$. The positive ionizable is also important feature for antitubercular activity. In present data set the phenyl ring is contributing pi bonding, while the hydrogen bond donation is contributed by the substitution on the aromatic rings.

CONCLUSION

In this work we have made an attempt to identify the structural features of C-2 and C-4 substituted quinazoline series for antitubercular activity. Model derived from this investigation having good predictive ability and pharmacophore modelling give compounds with high potency and improved ADMET, which could aid new antitubercular leads prior to their synthesis. Results of QSAR studies clearly indicated that substitution of

hydrophilic groups and electron withdrawing groups will increase antitubercular activity of the quinazoline derivatives. Morever the 3D QSAR and pharmacophore model can be utilized as a quick and accurate tool to help out discovery of new antitubercular drugs by database screening and/ or lead optimization method.

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