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Pharmacophore Based Atomic QSAR Study of Novel Quinaxaline 1, 4-di-N-oxides as Selective Non-cytotoxic Anti-tubercular Agents

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Tuberculosis persists to be a lethal disease globally and potential danger due to emergence of multidrug and extremely drug resistant (MDR/XDR) tuberculosis making conventional therapies ineffective. These motivated the researcher to search a novel leads against multi and extensively resistant strains of tuberculosis. Pharmacophore development is a crucial step to design and search new leads, in this study the structural insight of Quinaxaline 1, 4-N-Oxide derivatives and there selective non-cytotoxic activity against *Mycobacterium tuberculosis* has been analyzed to develop a pharmacophore based atomic quantitative structure activity relationship (QSAR) model. The 3D QSAR model exhibited statistically significant results like R^2=0.881, Q^2=0.72 Pearson-R= 0.82, and R^2_{pred} =0.7. The following pharmacophore may be used for further virtual screening studies for rational drug development against tuberculosis. The statistically significant model thus developed indicates the QSAR model can be useful to design novel drugs against multidrug resistance tuberculosis.

Keyword: Pharmacophore alignment, multiple drug resistant species, QSAR, *Mycobacterium tuberculosis,* selective inhibitor, external validation.

1. Introduction

Tuberculosis turns out to be a disease with quite a negative impact globally. World Health Organization (WHO) reports of 14 million prevalent cases of TB. Amongst the 9.4 million incident cases, 0.38 million deaths were among HIV positive people [1]. According to WHO definition MDR-TB multidrug-resistant tuberculosis is defined as TB caused by strains of *Mycobacterium tuberculosis* that are resistant to at least isoniazid and rifampicin and extensively drug-resistant tuberculosis as resistance to a fluoroquinolone and at least one second-line injectable agent (amikacin, kanamycin and/or capreomycin) added to the list of drug resistance in MDR-TB. The amplified prevalence of the disease in immuno-challenged patients, requiring lengthy therapeutic period as a result of resistance development, earmarks the urgency for new therapeutic agents to extend the range of effectiveness in TB treatment options [2-4].

It is an apprehension that in 2008; 440,000 cases of MDR-TB emerged globally. The most awful thing is 5.4% of the MDR-TB cases were extensively drug resistant tuberculosis XDR-TB[3-6] .It is however alarming as it suggests that XDR-TB is arising on a regular basis and is by now disseminated[7].Thus there is an urgent need for an antitubercular drug with significant inhibitory effect against M/XDR-TB [8-10].

Quinaxaline 1, 4-di-N-Oxides derivatives were reported of selective activity against multiple drug resistant (MDR) strains of *Mycobacterium tuberculosis* [11]. The compounds have been reported of bactericidal activity with activity on non-replicating anaerobes, both in-vitro and in-vivo, promises its eligibility as an antitubercular drug which may lead to shortened therapy [11-13]. Moreover its bactericidal activity on PA-824

resistant species (a bioreduced nitroimidazole undergoing clinical trials) signifies of a new pathway of action which might be responsible for their activity against the resistant species [12-13].

In our persistent persuasion towards pharmacophore development, we initiate this study to get a structural insight of Quinaxaline 1, 4-N-Oxide derivatives and there selective activity against *Mycobacterium tuberculosis*. For the development of a pharmacophore based QSAR model we have used the ones reported by *Vicente et al.(*2008) [11] having IC_{50} ranging from 0.36 to 57.5 (nM). There were about 70 molecules that were published and the variance in activity distribution makes the data set unique for pharmacophore based quantitative structure activity relationship (QSAR) model development. For computational purpose Phase module of molecular modeling software of Schrodinger LLC, Maestro 9.1 has been used [14-15]. The ligand-based pharmacophore model has been externally validated to get statistically significant values like R^2=0.881, Q^2=0.72, Pearson-R= 0.82, and R^2pred =0.7. The apt values of the statistical parameters promise its predictive feasibility and thus a model for the development of novel anti-tubercular molecule.

2.Materials And Methods.

2.1 Data Set and Biological Activity.

Quinaxaline 1, 4-di-N-oxide compounds from the literature by E.Vicente et al was taken [11]. The molecules were selected considering there IC_{50} values to be precise and not in range. The insoluble compounds were discarded. The biological activities taken for study were converted to pIC_{50} values, using Gaussian statistics, to get a more significant figure for visualizing bioactivity. Thus 43 Quinaxaline 1, 4-di-N-oxide molecules were finally selected, out of which random 70% compounds were taken as training set and the rest of the compounds were used as a test set. All the 2D molecular structures were developed using ChemDraw Ultra 8.0 and then were transformed into 3D structures. Energy minimization was done using OPLS 2005 force field of Ligprep module [16]. The pH of ionization of the molecules was taken to be neutral. For the development of the pharmacophore model these ligands were imported in PHASE [15]. Conformation generation is an important step in PHASE algorithm; conformations were generated using Configen taking GB/SA solvent model [17]. About 1000 conformers were generated per structure and ensuring 50 step minimization. The minimized conformers were filtered using a relative energy parameter limitation of 10 kcal mol^{-1} and a minimum atom deviation of 1.00 Å. If there was any conformer higher than this limit, or is redundant its incessant disposition was ensured. Thus we successfully incorporated only the lowest energy non-redundant conformers of a ligand in the process of pharmacophore model development. A couple of conformer was defined as identical if the relative distance between them is below
1.00 Å [18].

2.2 Creating Pharmacophore Sites and Common pharmacophore hypothesis generation.

According to the pIC_{50} values the molecules were divided into actives and inactives setting the maximum and minimum values in the activity threshold window of PHASE. Pharmacophore sites of a ligand are represented in the 3D space by a set of points. These points coincide with various chemical characteristics with type, location and directionality, which facilitate non covalent bonding with the receptor sites. The pharmacophore features like hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic/Non-polar group (H), negatively ionizable (N), positively ionizable (P) and aromatic ring (R) present in the PHASE were used to create the pharmacophore sites for the energy calculated ligands. The following features were assigned using SMART queries [19]. Tree based partition algorithm is used by PHASE for detection of common pharmacophore from a set of variants taking maximum tree depth 3. To find common pharmacophore PHASE algorithm use an exhaustive analysis of k-point pharmacophore match picked from the conformations of a set of active ligands on the basis of inter site distances, and then find all spatial arrangements of

pharmacophore features those are common to at least 8 out of 10 active ligands. Pharmacophores thus generated have matches across different set of actives eliminating the chance of its exclusiveness towards a small subset of ligands. The different pharmacophore hypothesis produced were further examined by using a scoring function so that it produced the best alignment of the ligands which are active yet also incorporating the features from the inactives to make the model more versatile [20-21].

2.3 Scoring Pharmacophore Hypotheses According to Actives and Inactive Ligands.

The pharmacophore hypotheses were scored pertaining to the active ligands. To ensure that no inappropriate pharmacophore is inside the survived pharmacophore models least squares site-to-site alignment is considered. Now the scoring of the pharmacophore hypotheses was done in relation to the information from the active ligands considering various geometric and heuristic factors. The alignment to a reference pharmacophore is considered according to RMSD of the site points and the average cosine of the vectors keeping their tolerance 1.2 Å and 0.5 respectively was set. To preferentially get the reference ligand from the most active set the ones scoring the upper 10 % was considered for score calculation. For further refinement volume scoring is also done in order to measure quantitatively of how each non-reference ligand is superimposing with the reference ligand, in account of Vander Waals models of the structures and taking into account all heavy atoms of the active ligands. Here the cutoff for volume scoring was kept at 1.00 for the non reference pharmacophores. To ensure the lowest energy ligands for better binding to be incorporated in the best pharmacophore the relative conformational energy of the reference ligand was constrained to 0.000. Thus we generated the survival active scores for the pharmacophore hypotheses. A ligand can be inactive due to number of ways but for successful implementation of only those characters which are important for good binding we need to incorporate the knowledge why the inactive molecules are inactive. This would make our pharmacophore models a better one having ability to distinguish between an active and an inactive molecule. This score inactive is calculated with the help of fitness score which is assessed with the same constraints as that of score active. A good hypothesis has a low fitness score multiplied by a user adjustable factor which was set to default mode.

2.4 Atomic 3D-QSAR Model Generation.

We generated atom based 3D QSAR model of pharmacophore hypotheses. We choose to build the atom based 3D QSAR model as our data set ligands showed quite very good alignment as it consisted of a large variety of derivatives of a parent molecule. It's also true that atom based 3D QSAR model provides more chemical significance than pharmacophore based 3D QSAR model can give which only depends upon pharmacophore sites for alignment to the hypothesis selected. This is because of the fact that atom based 3D QSAR takes the total molecule and facts like probable steric hindrance with the receptor site can be taken into account while building a model with this atom based approach.

The PHASE algorithm uses a very flexible approach for the development of 3D QSAR model. It considers a rectangular grid of 1 Å grid distance in a 3D space. Thus it creates cubes of said dimension in the 3D space. The atoms of the molecules which are considered as overlapping Vander Waal spheres fall inside these cubes depending on the volume of the atomic spheres. These occupied cube spaces are termed as volume bits [15, 19]. A volume bit is allocated for each different class of atom that occupies a cube. There are six atom classes two hydrogen bond acceptor (A), one positively ionizable (P) and two aromatic ring (R) used for classifying the atom characteristics. The total number of volume bits consigned to a specified cube is based on how many training set molecules occupy that cube. A single cube may represent the occupation by one or various atoms or sites, and even those from the same molecule or may be from unlike molecules of the training set. Thus A molecule may be

represented by a binary string concurrent to the occupied cubes, and also the various types of atomic sites that exist in those cubes.

To create an atom based QSAR model, these volume bits which encodes the geometrics and chemical characteristics of the molecule are regarded as independent variables in PLS (Partial Least square) regression analysis. For generating a predictive QSAR model we have to select 6 number of PLS factor. The maximum PLS factor that can be taken is N/5 [22] where N is the number of ligands present in the training set.

3. Results and Discussion

The aim of the study was to elucidate the 3D structural features of clinically significant anti-tubercular agents crucial for effective therapy against M/XDR TB, by generating 3D pharmacophore and to quantify the structural features of Quinaxaline 1, 4-N-Oxide derivatives essential in biological activity by generating atom based 3D QSAR model. For the pharmacophore modeling and QSAR studies we have used Phase module of Schrodinger molecular modeling suite. Hence we have used conformation that suggested by the hypothesis for generating 3D QSAR model that identifies overall aspects of molecular structure that govern the activity.

3.1 Pharmacophore Hypothesis

For the generation of pharmacophore model we have considered 46 compounds. We used minimum sites 4 and maximum sites 5 to have optimum combination of sites or features common to the active compounds. The molecules were classed out into actives and in-actives based on activity threshold for identifying the pharmacophore features considering the highest active molecule. The pharmacophore hypotheses were developed and their survival, survivals minus inactive, post hoc score and various other factors were optimized to choose the perfect hypothesis for further QSAR development. On the basis of scoring and re-scoring of active and inactive molecules AHHRR.2 hypothesis was selected for aligning the molecules (Fig.1). These pharmacophore models should also discriminate between active and inactive molecules. It is true that the hypothesis incomplete if it lacks either a critical site that explains the binding or information on what prevents inactive from binding the therapeutic target. To identify the pharmacophore models with more active and less inactive features among these models, were mapped to inactive compounds and scored. If inactives score well, the hypothesis could be invalid because it does not discriminate between actives and inactives. Therefore adjusted survival score was calculated by subtracting the inactive score from survival score. All the molecules showed good alignment with plausible fitness score ranging from 3.00 (for highest active) to 1.84 (for lowest active). The three dimensional QSAR analysis was carried out based on the pharmacophore alignment. The scoring algorithm includes contributions from the alignment of site points and vectors, volume overlap, selectivity, number of ligands matched, relative conformational energy, and bioactivity. The scores of all the hypotheses thus generated displays (Table 1) the rationality in selection of the hypothesis above mentioned.

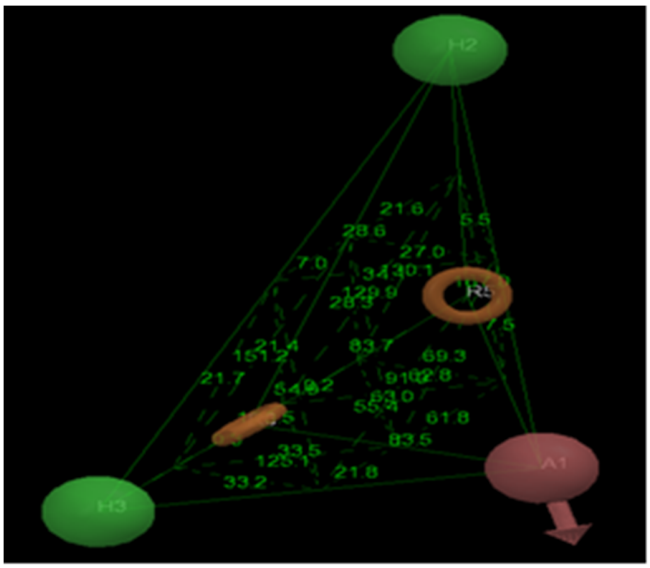

Fig 1: Pharmacophore Features.

Table 1: Pharmacophore Hypothese

ID	Survival	Survival –inactive	Post-hoc	Site	Vector	Volume	Selectivity	Activity	Inactive
AHHRR.2	3.656	1.212	3.656	0.81	0.996	0.846	1.936	6.462	2.444
AHHRR.16	3.642	1.191	3.642	0.79	0.999	0.854	1.928	6.462	2.451
AHHRR.18	3.64	1.181	3.639	0.79	0.997	0.849	1.964	6.462	2.459
AHHRR.3	3.628	1.16	3.628	0.79	0.996	0.845	1.936	6.799	2.467
AHHRR.19	3.614	1.14	3.614	0.77	0.997	0.85	1.964	6.799	2.474
AHHRR.15	3.609	1.141	3.609	0.76	0.999	0.848	1.928	6.799	2.468
AHHRR.20	3.559	1.16	3.559	0.72	0.996	0.845	1.959	6	2.399
AHHRR.1	3.063	1.081	3.063	0.4	0.983	0.685	1.955	7.273	1.982

3.2 Atomic 3D QSAR Model

The atom based 3D QSAR visualize 3D characteristics of the ligands (atoms or pharmacophores) as that contribute positively or negatively to activity. The QSAR model display 3D characteristics (see figure 2(a), 2(b)) as cubes that represent the model and colored according to the sign of their coefficient values, which by default is blue for positive coefficients and red for negative coefficients. Positive coefficients indicate an increase in activity, negative coefficients a decrease. The visualization of the coefficients is useful to identify characteristics of ligand structures that tend to increase or to decrease activity. This might give a clue to what functional groups are desirable or undesirable at certain positions in a molecule.

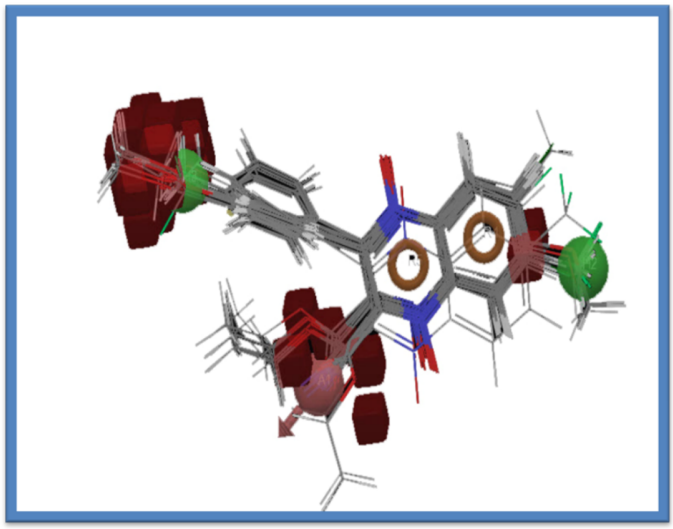

Fig 2(a): Pharmacophore Model showing negative activities.

The molecule 21 showed highest anti-tubercular activity. The ligands showed very good structural alignment (see fig 3) in the 3D space indicating the similarity of their 3D characteristics specially its topological properties considered by the topological descriptors, hence their chemical similarity may also be extrapolated from the following alignment diagram due to the superimposition of various derivatives attached to its parent structure which may be favorable or less favorable for bioactivity. The following

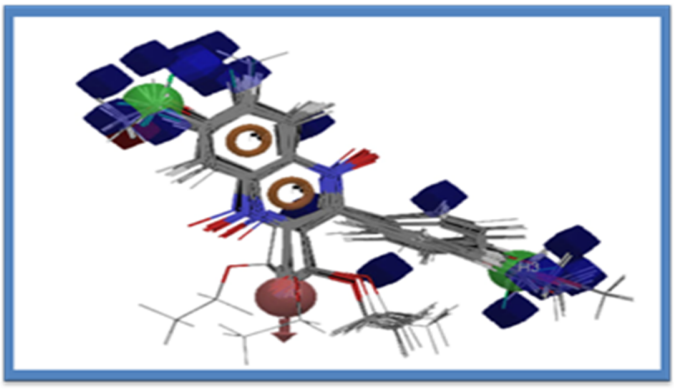

Fig 2(b): Pharmacophore features showing positive contribution.

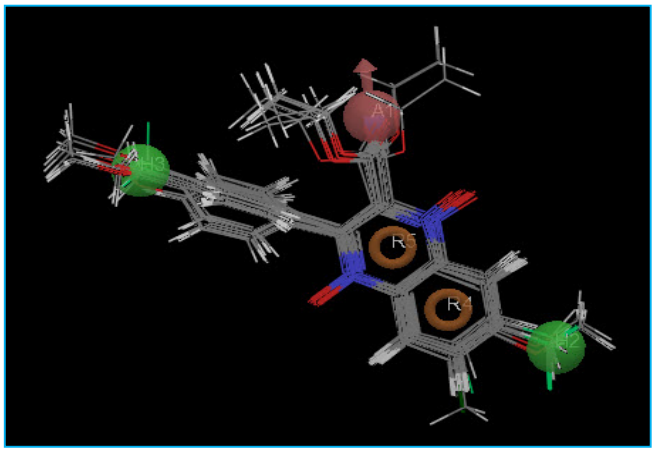

Fig 3: Overall alignment of all ligands in the dataset.

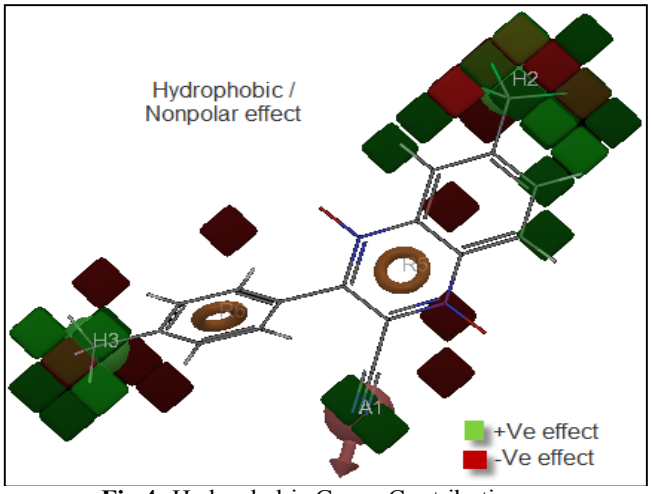

Fig 4: Hydrophobic Group Contribution.

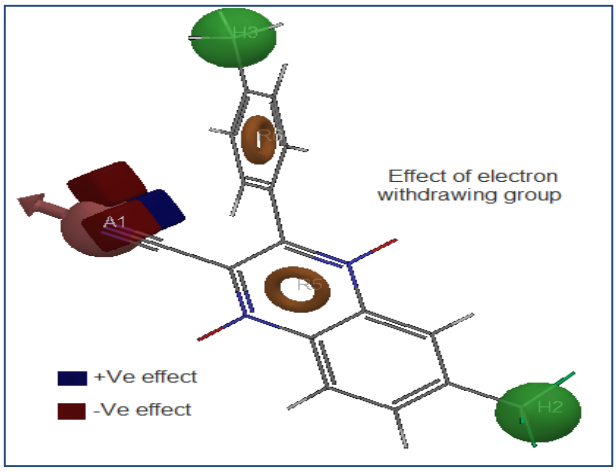

Fig 5: Electron withdrawing group contribution.

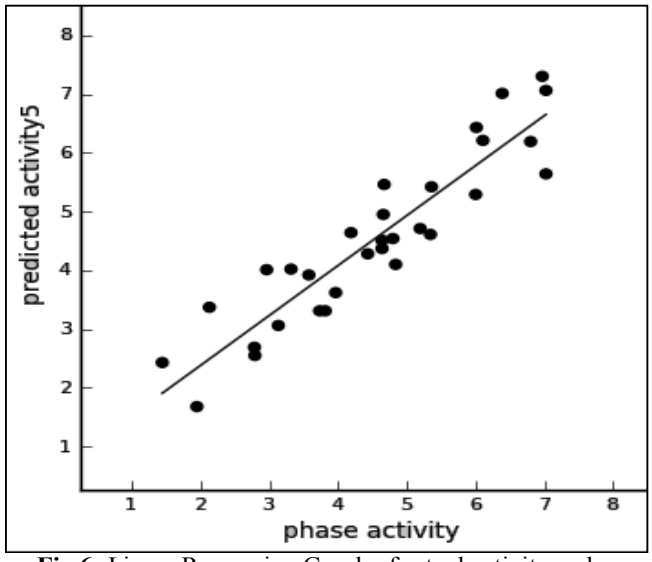

Fig 6: Linear Regression Graph of actual activity and predicted activity.

statement is proved in case molecule number 16, 34 and 43, in those molecules due to the attachment of some bulky substituent to the parent structure resulted in decrease of activity. Whereas incorporation of hydrophobic group like ethyl or propyl substitution in the position of H2 and H3 was substantial for biological activity. This can be clearly seen in case of ligands 3, 13 and 15.In the said the molecules the activity increased perpetually due to attachment of ethyl and propyl groups in strategic positions which are crucial to bioactivity. While, in the same compound substitution of amino group adjacent to R6 phenyl ring is favorable for anti tubercular action where as substitution adjacent to alkyl side chain with –NH functionality in case of ligands 11, 27 and 33 resulted in reduction of biological activity .The green cubes in the around the ligand aligned to the pharmacophore model (see Fig 4) shows positive contribution if hydrophobic groups are attached.

Substitution of slightly electron withdrawing group like -CONHR, -CONR2 may favor the biological activity. Strong electron withdrawing group like $-NO_2$, -CN, -CHO,-COOH, $-SO_3H$, $-S^+R2$, $-N^+R3$ etc may reduce the biological activity (Fig 5).

For generating an atom based 3D QSAR hypothesis, we have used a dataset of 46 compounds having anti-tubercular activity. The

alignment generated by the best pharmacophore hypothesis AHHRR.2 was used for QSAR model generation. A model at PLS factor 6 with good statistics and predictive ability was generated from the dataset. The number of PLS factor included in model development is 6 as incremental increase in the statistical significance and predictive efficiency was observed for each incremental increase in the incorporated PLS factors up to 6. The model express variance exhibited by Quinaxaline 1, 4-N-Oxide derivatives, which is near to one and signifying a close agreement of fitting points on the regression line for the observed and PHASE predicted activity which can be visualized from the graphical representation through scatter plot.(Fig. 6.)

A 3D-QSAR analysis has been performed successfully on the series of Quinaxaline 1, 4-N-Oxide derivatives to inhibit the growth of resistant anaerobic tuberculosis strains. The value of F (18.9) indicates a statistically significant regression model, which is also supported by the small value of the variance ratio (P), an indication of a high degree of confidence. Further small values of standard deviation (0.745) of the regression and Root-Mean-Square Error (RMSE= 0.8253) makes an obvious implication that the data used for model generation are best for the QSAR analysis. Validity of the model can be expressed by cross validated correlation coefficient (q2 = 0.8253) that is obtained by leave one out (LOO) or leave N out method. The q2 is more reliable and robust statistical parameter than r2 because it is obtained by external validation method by dividing the dataset into training and test set (Fig.3) presents good alignment of the active ligands and negligible scattered alignment of inactive ligands to the developed pharmacophore model. The external validation of the QSAR model was done to ensure the statistical significance , thus $R^2_{predicted}$=0.7 further reinstates the statistical robustness of the model.

The statistically significant results (Table.2.) thus quantify the relation between the chemical characteristics to the biological activities of the ligands. Though the mode of action is not very clear to us but the inhibitory result and its further validation by the QSAR result with good predictivity indicates the thermodynamic and quantum stability of the unknown biochemical reaction responsible for the facilitation or direct action in the inhibition of the resistant strains. This also indicates the presence of new biological chemistry of the organism and a novel pathway of inhibition.

The chemical insight of the bioactive molecules makes us more strategically favorable positioned in using combinatorial synthesis of new and better active compounds against the target with the help of core hopping. This ensures the tremendous potential of anti-tubercular research as the data set contains molecules at clinical trials.

Table 2: Statistical results of the QSAR model.

ID	PLS Factor	SD	R^2	F	P	Stability	RMSE	Q^2	Pearson-R
AHHRR.2	6	0.145	0.9253	180.9	5.158e-008	0.614	0.2394	0.75	0.8183

4.Conclusion

The pharmacophore based 3D QSAR model thus generated from the new quinoxaline derivatives can provide intricate structural knowledge about a new class of anti-tubercular derivatives which may provide a leverage in the anti tubercular drug research. The pharmacophore model can help us in virtual screening of finding new potent ligands against multidrug resistant tuberculosis leading to shorter and better therapy. However, mechanism of action should be studied which is not yet known. Chemoinformatic analysis of the ligands can provide important insight towards this novel set of ligands.

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