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RESEARCH ARTICLE

QSAR and Molecular docking Studies of oxadiazole ligated Pyrrole derivatives as Enol ACP co Reductase Inhibitors

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Abstract:
In the present study, we have focused on use of molecular modeling techniques to design highly potent molecules that are able to predict biological activities of anti-tubercular compounds. It includes the methodologies in constructing main components of QSAR model, namely the methods for selection of informative descriptors, validating the model for antitubercular activity prediction. It includes the methodologies in constructing main components of QSAR model, namely the methods for selection of informative descriptors, validating the model for antitubercular activity prediction. Two dimensional (2D) and three dimensional(3D) QSAR studies were performed for correlating chemical composition Oxadiazole ligated pyrrole and receptor enol ACP co reductase inhibitory activity using Multiple Linear Regression (MLR) Analysis and k Nearest Neighbor Molecular Field Analysis(kNN MFA), respectively. Rigorously validated QSAR model is important to ensure that the model have acceptable predictive power. The developed QSAR models were found to be statistically significant with respect to training (r2> 0.7), cross-validation (q2> 0.7), and external validation (pred_r2> 0.8).QSAR model was developed on a series of compounds containing Oxadiazole ligated pyrrole pharmacophore to identify key structural fragments required around pyrrole pharmacophore for anti-tubercular activity.

Keywords: QSAR, drug design, molecular modelling, oxadiazole ligated pyrrole , Enol ACP co Reductase,

Introduction

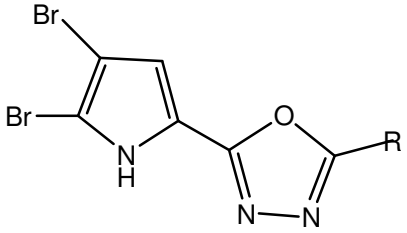
Tuberculosis caused by *Mycobacterium tuberculosis* is a pandemic disease and complicated due to its resistant strains like, multiple drug resistance(MDR) ,extensive drug resistance(XDR) as well as total drug resistance (TDR) reported in some parts of the world. [1-3]. Because of the unique characteristics, the mycobacterial cell wall is impermeable to a number of compounds and partly responsible for causing resistance to large number of drugs. The complexity of the cell wall is a challenge to the mycobacteria requiring specialized mechanisms for the cell division to occur. The inner compartment of the cell wall consists of peptidoglycan (PG), arabinogalactan (AG), and mycolic acids (MA) , which are covalently linked together to form a complex known as the MA-AG-PG complex. This complex extends from the plasma membrane in layers, starting with PG and ending with MAs**.** There are two main pathways for synthesizing MAs. The first is through a multienzyme complex known as fatty acid synthase (FAS) type I. The complex synthesizes MA de novo, generating fatty acids with the help of acyl coenzyme (Enol ACP co Reductase). The second is FAS type II, which further elongates the initial acylated product. Many of the drugs used as antimycobacterial agents target the MA-AG-PG complex. Various Pyrrole derivatives are reported to act as inhibitors of the enzyme, enol ACP Co reductase involved in fatty acid synthesis I(FAS I) of mycobacterium[4].

As a continuation of our ongoing work on antimycobacterial studies and to further explore the structural requirement for competitive inhibitors of Enol ACP co Reductase, we herein report the molecular modeling studies on a series of 20 pyrrole ligated oxadiazole ligated pyrrole compounds reported by Rajesh Rane et al[19]. Initially, two-dimensional (2D) and three-dimensional (3D) quantitative structure–activity relationship (QSAR) studies were carried out. New chemical entities (NCEs) were then designed using the information obtained from literature survey and results of 2D QSAR and 3D QSAR studies[5]

2.Materials and Methods-

Data set:

A data set consisting of 20 substituted oxadiazole ligated pyrrole derivatives reported by Rajesh Rane et al. [19] for antimycobacterial activity was use for the molecular modeling studies. The minimum inhibitory activities (MIC) were converted into the corresponding pMIC (pMIC = -log(MIC)) values. The structures and antimycobacterial activity data are given in Table 1



Computational details: All the computational studies were carried out using the V-Life sciences,MOLECULAR DESIGN SUITE (MDS) version 3.5. Geometry optimization was carried out using the standard Merck molecular force field

2D QSAR Studies:

Experimental design for 2D QSAR:

Dataset of 20 molecules was divided into training and test set using random selection method. In an attempt to ensure robustness of the model and increase predictive ability of QSAR model they were subjected to randomization test. It was ensured that selected training and test sets satisfied the following criteria: (i) Representative points of the test set were close to those of the training set; (ii) Representative points of the training set were close to representative points of the test set; (iii) Training set had chemical and biological diversity. To further gain confidence that a training and test set have uniform representation of molecules; uni-column statistics were performed.

Uni-Column Statistics: The maximum and minimum value in training and test set were compared in a way that[6]

1. The maximum value of pIC50 of test set should be less than or equal to maximum value of pIC50 of training set.
2. The minimum value of pIC50 of test set should be higher than or equal to minimum value of pIC50 of training set.

This observation showed that test set was interpolative and derived within the minimum—maximum range of training set.

The mean and standard deviation pIC50 values of sets of training and test provide insights to relative difference of mean and point density distribution of two sets.

1. Mean in test set were found to be higher than mean in training set indicating that presence of relatively more active molecules as compared to inactive ones.
2. Higher standard deviation in training set indicates wide distribution of activity of molecules as compared to test set molecules.

Correlation Matrix-

In the present study we have considered the correlation between descriptor with activity as well as their inter-correlation i.e. descriptor-descriptor correlation[6]. We have considered only those descriptors which shown either direct or indirect correlation with activity by more than 0.30 and inter correlation less than 0.8 generated for the selected series of compound is shown in Table 3.

After applying MLR method, descriptor were generated viz. chiV3Cluster, XKAverage , T_O_O_5 , RotatableBondCount, SdsCHE-index

PMIC = + 6.6224 (chiV3Cluster) - 3.1570 (XKAverage) + 1.6748 (T_O_O_5) - 0.2851 (RotatableBondCount) + 0.0873 (SdsCHE-index)

By looking at the results we came to know that this descriptors satisfies all evaluation parameters. The above mentioned descriptors showed highest correlation with activity (as shown in correlation matrix) and also shown proper distribution of data points. To increase the predictive power we tried different combinations of selected descriptors by keeping T_O_O_5 as constant descriptor.

On the basis of these statistical parameters viz. $r^2 > 0.7$, cross-validated r^2 .i.e. $q^2 > 0.5$and parameter to assess external validation i.e. pred_$r^2 > 0.8$; the generated regression equation of model was used for further studies.

3D QSAR MODEL –Training and test set was selected by random selection in the range 80%.Then regression was done by SA-KNN(Simulated Annealing k Nearest Neighbor Molecular Field Analysis)Method. Model showed good predictivity. The points which contribute to SA KNN-MFA model are displayed in the q^2, pred $_r^2$, and K values of model B (SA kNN MFA) were found to be 0.5124, 0.7166 and 2 respectively

Model validation:

Internal validation:

Internal validation was carried out using leave-one-out (q^2,LOO) method. To calculate q^2, each molecule in the training set was sequentially removed, the model was refitted using same descriptors and the biological activity of the removed molecules were predicted using the refit model. This attempt was made to calculate robustness of QSAR model. All cross-validation studies were performed by considering the fact that a value of q^2 is > 0.5.

External validation:

External validation of generated models was carried out by predicting the activity of test set of compounds. The pred_r ^{2} value is calculated as follows

$$Pred\_r^2 = 1 - \frac{\sum(y_i-\hat{y}_i)^2}{\sum(y_i-\hat{y}_{mean})^2} \quad \text{------- (1)}$$

Where y_i , $\hat{y}_i$ are the actual and predicted activity of the *i*th molecule in the test set, respectively, and y_{mean} is the average activity of all molecules in the training set.

Randomization test:

This is a most popular tool used by researchers to prevent from chance correlation. In this method, keeping the x-variable intact, a repeated permutation of response variable is done. After each permutation r^2 and q^2 is recorded. If in each case the r^2 and q^2 gives very low value than original data, then we can say with some confidence that original QSAR model is real and not generated by chance[19].

In our study we have calculated Z-score to check significance of the model. Following formula was used for the same [19-22].

$$Zscore = \frac{(q^2_{org}-q^2_a)}{q^2_{std}} \quad \text{- - - - - [2]}$$

Where q^2_{org} is the q^2 value calculated for the actual data set, q^2_a is the average q^2 , and q^2 std is the standard deviation of q^2 , calculated for various iterations using different randomized data sets.

3.Result and Discussion-

Interpretation of 2D QSAR:

The present QSAR model reveals that Baumann's alignment independent descriptor has major contribution in explaining variation in activity. Descriptors T_X_Y_Z can be defined as total count of fragments formed with atom types X and Y separated by topological distance of Z bonds [18].

The careful observation of descriptors in model suggest that T_O_O_5 is an indicator variable which positively contributes for QSAR equation up to 30% and signifies importance of Oxygen group at R_1 position of ring is most influential for ENR inhibitory activity. The descriptors like sds CHE index indicates number of –CH group connected with one double bond and one single bond .Also chiV3 cluster signifies valence molecular connectivity index of 3 rd order cluster. Other descriptors XKAverage, RotatableBondCount, which is inversely proportional to activity shows that Average hydrophobicity value and rotatable bond count may be detrimental for biological activity

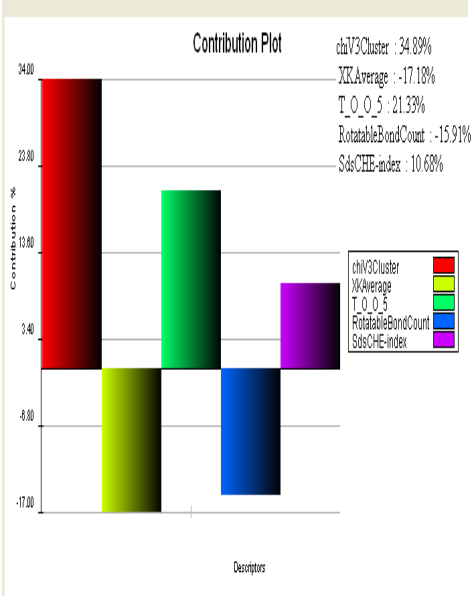


Figure 1 (a) Contribution plot of selected descriptors

Interpretation of 3D QSAR model:

3D-QSAR was used to optimize the electrostatic, steric and hydrophobic requirements around oxadiazole ligated pyrrole pharmacophore. 3D data points were generated that contributed to SA kNN–MFA 3D-QSAR model. The data points generated by 3D QSAR are shown in **Figure 2**. The property values for the generated data points helped us for the design of potent NCEs. The ranges of data point values were based on the variation of the field values at the chosen points using the most active molecule and its nearest neighbour set. Negative and positive values in electrostatic data points indicated the requirement of negative and positive electrostatic potential respectively for enhancing the biological activity of Pyrrole ligated oxadiazole derivatives. Points generated in SA kNN–MFA 3D-QSAR model are S_1048, H_457,E_348, E_235 i.e. steric, hydrophobic and electronic data points at lattice points 1048, 457, 348 and 235,respectively. Negative steric value indicates the less steric groups are required to increase activity. The positive and negative values in electrostatic field descriptors indicated the requirement of electro positive and electro negative

electrostatic potential respectively for enhancing the biological activity of oxadiazole ligated pyrrole pharmacophore derivatives.

4. Design of new chemical entities (NCEs) containing pyrrole ligated oxadiazole pharmacophore:

The information obtained from 2D and 3D QSAR studies had helped a lot in optimizing Pyrrole ligated oxadiazole pharmacophore and for design of NCEs having potent anti-tubercular activity. Substitution pattern around pyrrole ligaed oxadiazole pharmacophore was used for the design of NCEs using CombiLib tool of vLife MDS software.. Designed compounds were subjected to Lipinski's screen [20] to ensure drug like pharmacokinetic profile of the designed compounds in order to improve their bioavailability **(Table 6)**. The following parameters were used as Lipinski's filters(Values in parenthesis indicate ideal requirements)

1. Number of Hydrogen Bond Acceptor (A) (<10)
2. Number of Hydrogen Bond donor (D) (<5)
3. Number of Rotatable Bond (R) (<10)
4. XlogP (X) (<5)
5. Molecular weight (W) (<500 g/mol)
6. Polar surface area (S) is (<140 Å)

5.**Docking studies**

The molecular docking tool, Glide (Schrödinger, LLC, New York) software was used for studying binding modes of the designed compounds in to the binding pocket of Enol ACP co Reductase enzyme(ENR). GLIDE was found to produce least number of inaccurate poses and 85% of GLIDEs binding models had an RMSD of 1.4 Å or less from native co-crystallized structures[26]. These studies helped to sort out the designed compounds with good binding affinity against ENR enzyme. The docking score in terms of G-Score (GLIDE Score), results of docking studies of designed compounds of Oxadiazole ligated pyrrole series are presented in Table 7.

5.1 G-score

The scoring function of GLIDE docking program is presented in the G-score form. The G-score indicates the binding affinity of the designed compound to the receptor/enzyme. The G-score of
the standard compound Isoniazid was found to be -7.500947. The G-score of the designed NCEs 1 and 11 was found to be -7.099278 and -7.09647 respectively. The close analysis of these results suggests that the designed NCEs have comparable G-score with the standard compound.

5.2. H-Bond interactions

H-bond is one of the most widely used parameter for the evaluation of the docking results, as it is an influential parameter in the activity of the drug compound. The number of H-bond interactions in the standard compounds was compared with that of designed NCEs. The number of H-bond in the standard compound Isoniazid was found. The no. of H-bond contact for the designed compounds 8,9,10,11 and 12 were found to be 1, 1, 1, 1and 1 respectively.

5.3. Contacts

The contacts are represented in the form of Vender Waals (vdw) Interaction.
_ Good vdw interactions.
_ Bad vdw interactions.
_ Ugly vdw interactions.
It was found that all designed compound has more number of good vdw interactions, less number of bad vdw and ugly contacts when compared with the standard Isoniazid. But Gscore for these molecules were less. In conclusion Gscore and H-bond interactions, number of good, bad and ugly vdw contact decide the possible binding affinity and in turn potency of the designed NCEs.

KA11 showed the H-bond interaction with Lys 165 residue (Fig. 4). The NO atom On benzene ring of pyrrole ligated oxadiazole nucleus showed the H-bond interaction with NH group of Lys 165 (2.139 Å).

Conclusion:

In this study aim was focused on devlopment of the potential compound containing pyrrole ligated oxadiazole analogue for anti-TB activity by developing NCE's using QSAR studies. The main outcome is generated through 2D QSAR is we got basic idea regarding nucleus and information about the structural confamation of oxadiazole and pyrrole link through descriptor like T_O_O_5 and sdsCHE index. Then 3D QSAR gave information about nature of substituents like E_235 (0.1534) electron withdrawing group are required at 4^{th} and 5^{th} position of pyrrole and S_1048(0.0014) it indicates less steric group at meta, para, ortha position of benzene ring is necessary, E_348(-4.5178) explains electron withdrawing group at 4 position of benzene and finaly H_457(0.5940) more hydrophobic group at para position of benzene ring increases antimycobacterial activity Finally all compound designed through 2D and 3D QSAR are subjected to lipinsky rule,which gives information about highly potent compound. The result of 2D and 3D QSAR studies using different interpretation approaches have yielded detailed insights of proper working in the research area,and way of thinking. we have discussed the best practices for developing robust and externally predictive QSAR model and successful application of QSAR studies for development of NCE's for anti-TB activity.activities and statistical significance with different training and test sets which provides confidence

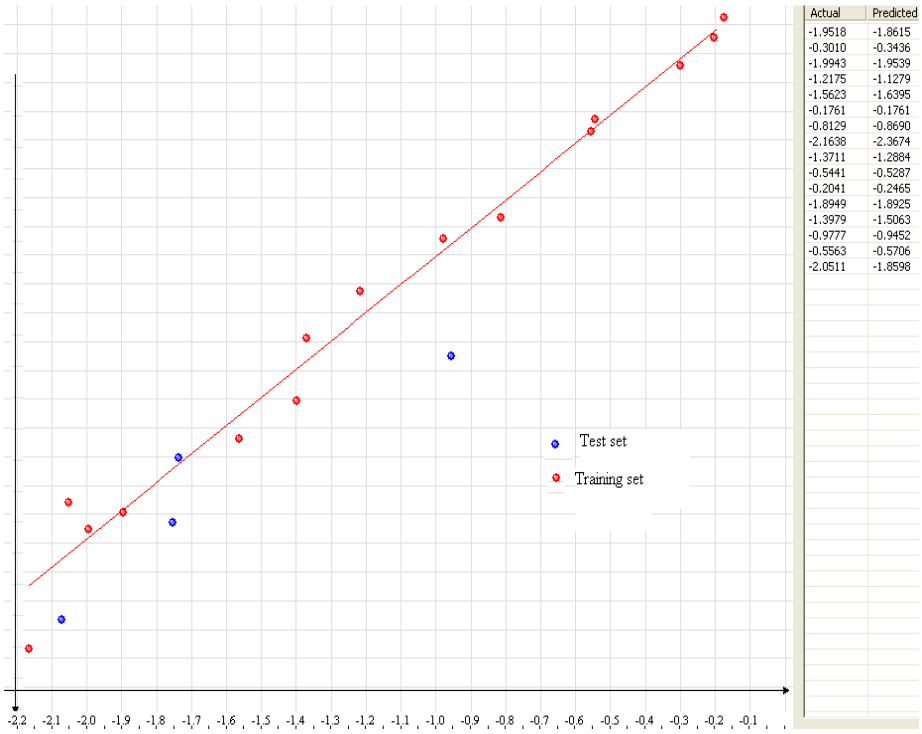
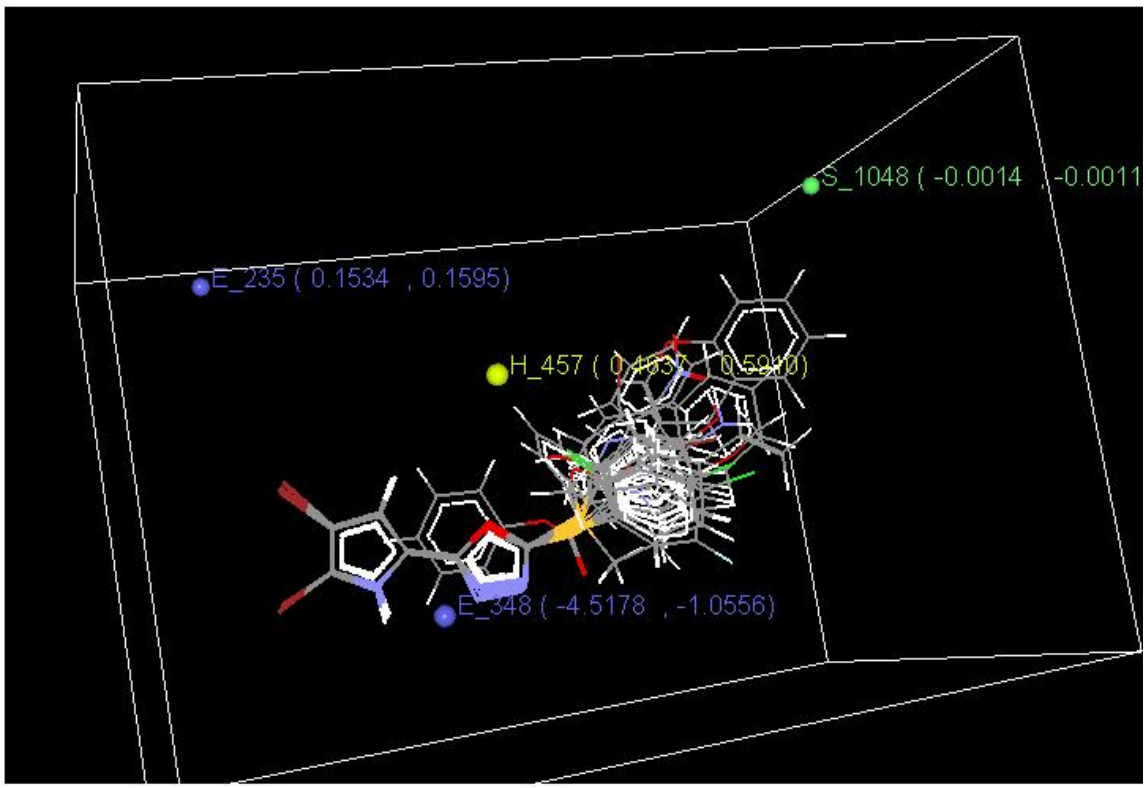


Figure 1 (b) Plot of Actual versus predicted Activity

Figure 2: Common template data points generated using kNN–MFA method (3D- QSAR) in 3D rectangular grid showing contributions of electrostatic,hydrophobic and steric functional groups for significant antitubercular activity.



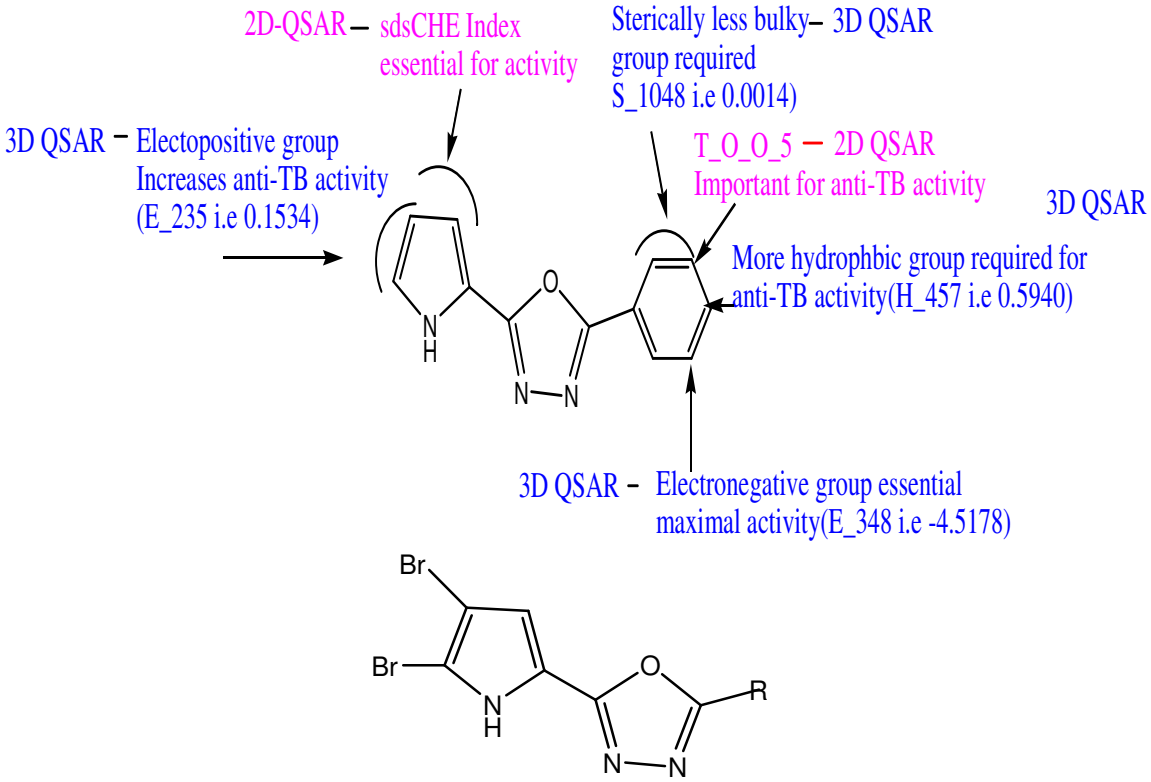
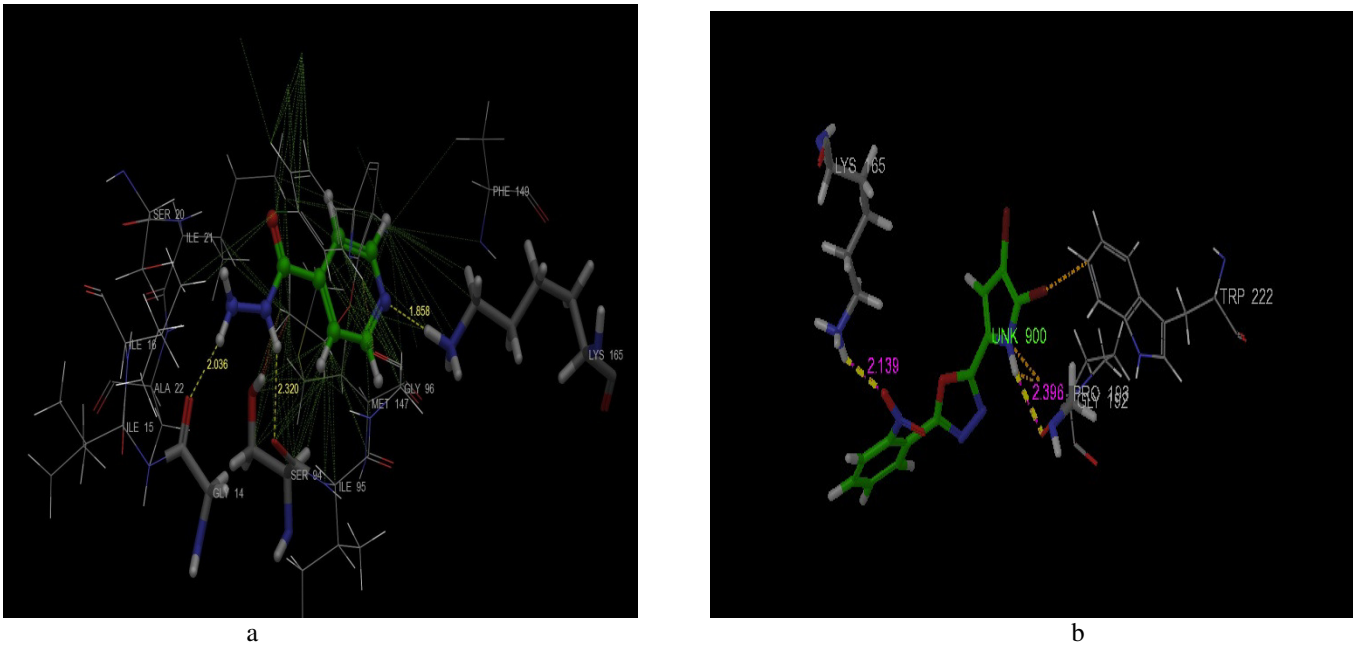


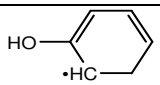
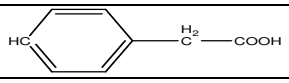
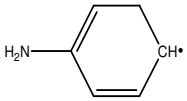
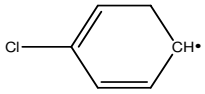
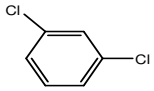
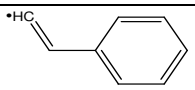
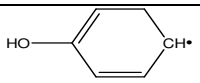
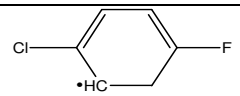
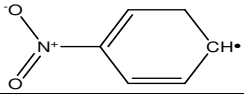
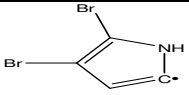
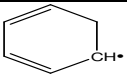
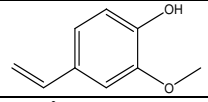
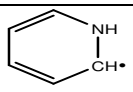
Figure 3: Pharmacophoric requirements around pyrrole ligated oxadiazole derivatives.



a b

Figure 4:docking interaction of (a)isoniazid with enol ACP co Reductase and (b) interaction of compound 11

Table 1 Selected series PMIC=-logMIC

Compound No.	**R**	**MIC(µg/ml)**	**pMIC**
5a		36.50	-1.56229
5b		145.80	-2.16376
5c		16.50	-1.21748
5d		9.50	-0.97772
5e	-4OCH_3	56.50	-1.75205
5f		1.60	-0.20412
5g		78.50	-1.89487
5h		25.00	-1.39794
5i		6.50	-0.81291
*5j		9.00	-0.95424
5k		3.50	-0.54407
5l		98.70	-1.99432
5m		112.50	-2.05115
5n		3.50	-0.5563

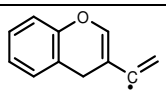
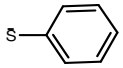
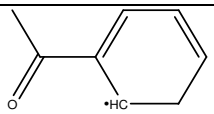
5o		2.00	-0.30103
*6	SH-	54.50	-1.7364
7a	S-CH_3	89.50	-1.95182
*7b	S-C_2H_5	118.00	-2.07188
7c		23.50	-1.37107
7d		1.50	-0.17609

Table 2: Uni-Column statistics for training set and test set.

Model-1	Average	Max	Min	Std.dev.	Sum
Training	-1.1986	-0.1761	-2.1638	0.7035	-19.1770
Test	-1.6286	-0.9542	-2.0719	0.4754	-6.5146

Descriptor	chiV3Cluster	XKAverage	T_O_O_5	RotatableBondCount	SdsCHE-index
chiV3Cluster	1	-0.4728	-0.61661	-0.63246	0.3423
XKAverage	-0.4728	1	0.71216	0.726289	0.444362
T_O_O_5	-0.61661	0.71216	1	0.729458	0.553539
RotatableBondCount	-0.63246	0.726289	0.729458	1	0.5
SdsCHE-index	0.3423	0.444362	0.553539	0.5	1
pMIC	0.9387	0.6475	-0.1257	0.2749	0.4148

Statistical Parameter	MLR	Contributing descriptors
n	16	chiV3Cluster
r^2	0.9827	XKAverage
r^2 se	0.1134	T_O_O_5
q^2	0.5754	RotatableBondCount
q^2 se	0.3615	SdsCHE-index
F test	113.3738	
pred_r^2	0.8392	
pred_r^2se	0.2757	

Table 3: Correlation matrix

Statistical Parameter	MLR	Contributing descriptors
n	16	chiV3Cluster
r^2	0.9827	XKAverage
r^2 se	0.1134	T_O_O_5
q^2	0.5754	RotatableBondCount
q^2 se	0.3615	SdsCHE-index
F test	113.3738	
pred_r^2	0.8392	
pred_r^2se	0.2757	

Table 4: statistical results of 2D QSAR generated by MLR

Statistical parameter	**SA-KNN MFA**
q2	0.5124
q2 se	0.3647
Pred_r2	0.7166
Pred_r2 se	0.4207
N	15
K nearest neighbour	2
Contributing descriptors	S_1048,H_457, E_348, E_235

Table 5: statistical results of 3D QSAR generated by SA kNN-MFA

Comp. no.	**Name of compound**	**R**	**Predicted activity**	**Lipinski Screen**	**Lipinski Score**
1	1	3-chlorophenyl	0.562388	ADRXWS	6
2	2	3-bromophenyl	-13.831	ADRXWS	6
3	3	3-iodophenyl	-0.45674	ADRXWS	6
4	4	4-methylphenyl	3.46978	ADRXWS	6
5	5	4-ethyl phenyl	-0.13015	ADRXWS	6
6	6	3-methylphenyl	-0.35629	ADRXWS	6
7	7	3-ethylphenyl	-1.5502	ADRXWS	6
8	8	3-methoxyphenyl	-0.04512	ADRXWS	6
9	9	4-methoxy phenyl	-0.04512	ADRXWS	6
10	10	2-methoxy phenyl	-0.21814	ADRXWS	6
11	11	2-nitro phenyl	-0.15777	ADRXWS	6
12	12	3-nitro phenyl	-0.35681	ADRXWS	6

Table 6 :Structures of designed NCEs along with predicted activity obtained by MLR equation generated by 2D-QSAR model.

Compound	G-Score	Good vdw	Bad vdw	ugly
1	-7.099278	224	0	0
2	-6.833546	226	0	0
3	-6.538829	239	3	0
4	-6.440214	220	7	0
5	-6.382636	214	2	0
6	-6.293452	197	3	0
7	-6.146766	235	5	0
8	-6.86602	238	9	0
9	-6.05158	242	7	0
10	-6.96048	246	8	0
11	-7.09647	196	3	0
12	-6.05158	207	1	1
Isoniazid	-7.500947	155	0	0

Table no 7:results of docking studies of designed compounds of Oxadiazole ligated pyrrole series

about robustness of generated model.Lipinski's rule and prediction of activity using MLR equation acts filter or screen for large library of designed compounds for selecting the best compound having better and predicted activity.All designed compounds shown good binding interaction with enol ACP co Reductase enzyme and carbonyl group of pyrrole ligated oxadiazole binds with Lys 165.Hence the compounds are active for the antitubercular activity

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