

## IN SILICO STUDIES OF NOVEL 2-(2-(4-ARYLOXYBENZYLIDENE)-HYDRAZINYL)-BENZOTHAZOLE DERIVATIVES AS ANTI-TUBERCULAR AGENTS: 3D QSAR STUDY

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### Abstract

Novel 2-(2-(4-Aryloxybenzylidene)-Hydrazinyl)-Benzothiazole derivatives were studied using molecular modeling techniques including 3D-QSAR (three-dimensional quantitative structure–activity relationship), CoMFA, CoMSIA, and molecular docking. In the present study 3D-QSAR for 25 derivatives possessing a wide variety of bioactivity against mycobacterium tuberculosis H37Rv strain. The results of the CoMFA model has  $q^2 = 0.767$  and  $r^2 = 0.938$ , the best CoMSIA model has  $q^2 = 0.806$  and  $r^2 = 0.945$ . The models were graphically interpreted by using contour plots, which gave more accuracy into the structural requirements for increasing the biological activity of compounds and proved a strong basis for future rational drug design of more active inhibitors for tuberculosis. The results indicated the steric, electrostatic, and hydrophobic, H-acceptor fields play key roles in models. The results of the CoMSIA model show better results than CoMFA model. Molecular docking shows the binding relationship of the ligand and the receptor protein.

### Keywords:

3D QSAR, CoMFA, CoMSIA, Molecular Docking, Benzothiazole derivatives.

## INTRODUCTION

Tuberculosis is a chronic infectious disease caused by mycobacteria, including Mycobacterium tuberculosis etc., every year more than 8 million people are suffering with tuberculosis [1]. Around 52,000 deaths per week [2]. Tuberculosis is one of the leading causes of death throughout the universe. Tuberculosis is an airborne disease transmitted person to person when a person with developed tuberculosis coughs, speaks or sneezes. The risk of transmission is related with number of factors such as the intimacy of contact, immune status of the potential host, infectiousness of the source case and the bacillary load inhaled [3]. Tuberculosis is difficult to treat due to habitation of bacteria within the macrophages and its unusual cell wall barrier. Despite various attempts to develop new structural model in the search for more active antimicrobials, benzothiazole still continue as one of the most functional class of compounds against microbes [4-8] and therefore, are valuable substructures for further molecular assessment. Benzothiazole derivatives have attracted continuing interest because of their different biological activities viz., anti-tubercular [9-11], anti-inflammatory [12], anti-helmintic[13], anti-malarial[14], anti-diabetic[15], analgesic[16], anti-tumour [17-20]. The aim of current study is to analyze a correlation between the biological activity of molecules used in training set and their three dimensional structure. The importance of steric and electrostatic field characteristics is revealed by aligning structurally similar analogues using pharmacophoric features as structural superimposition guides.

CoMFA (Comparative molecular field analysis) [21] and CoMSIA (Comparative Molecular Similarity Indices Analysis) [22] has emerged as a very important methods in ligand based drug design strategies. Comparative molecular field analysis and comparative molecular similarity indices analysis has a combination of molecular

descriptors, statistical analysis and graphical representation of results. Molecular structures are described with molecular interaction energies as steric fields and electrostatic fields surrounding the molecules, the statistics is computed by PLS [23] regression analysis and the output is displayed as contour maps superimposed on the molecules. The methodology of comparative molecular field analysis predicts that a suitable sampling of steric and electrostatic fields surround a set of forty four aligned molecules provides all the information necessary for understanding their biological properties. The CoMSIA methodology assumes that a suitable sampling of hydrophobic, hydrogen bond donor and hydrogen bond acceptor along with steric and electrostatic fields.

CoMFA is usually employed to increase the binding affinity of the model. When used in a comparative investigation on the same series of compounds acting on multiple targets, such methodology is valuable for identifying the structural basis of the observed quantitative differences in the pharmacotoxicological properties. We developed the 3D QSAR CoMFA and CoMSIA models on mycobacterium tuberculosis H37Rv inhibitors in the expectation of getting a perfect model that would account for the quantitative differences in biological activity seen in this series and to capitalize upon the insights to design ligands with strong inhibitory potency and selectivity.

## COMPUTATIONAL STUDIES

### Dataset

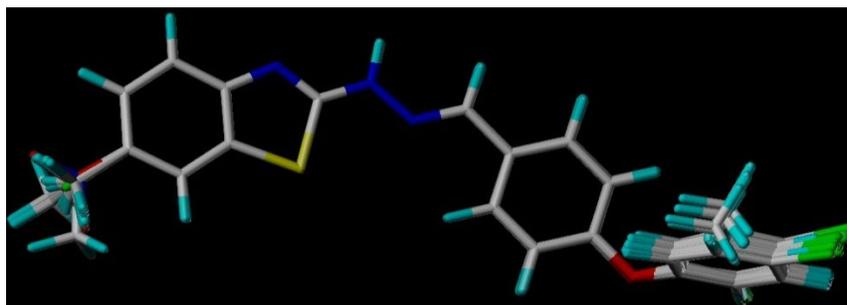
The biological activity data reported as  $IC_{50}$  for inhibition of Novel 2-(2-(4-Aryloxybenzylidene)-Hydrazinyl)-Benzothiazole as Anti-Tubercular agents was used for the present study. Structures of all the Benzothiazole derivatives are given in table 1. Biological activities were reported in  $IC_{50}$  values were then converted into the corresponding  $pIC_{50}$  using the formula  $pIC_{50} = -\log IC_{50}$  [24]. The logarithmic transformation helps to obtain a symmetrically distributed data which is apt for the PLS regression analysis and it is important because log-dose response curve is linear about its middle region.

### Molecular Modeling

All molecular modeling studies were performed using the molecular modeling package SYBYL6.7 [25] installed on a Silicon Graphics Work station. All the molecules were sketched and minimized by using Gasteiger-Huckel charges. Taken tripos force field and 0.005 kcal/mol cutoff value was used. Later performed geometry optimization using MOPAC interfaced with Sybyl. MMOK, ESP, NOMM was used during geometry optimization and MOPAC partial charges were computed.

### Molecular Alignment

Alignment plays very important role in 3D QSAR studies. One of the important parameters wherein 3D-QSAR studies are based on that a geometric parallelism should exist between the modeled structures and that of the bioactive conformation of the molecule. It indicates spatial alignment of compounds under study as one of the major sensitive and influential factors in obtaining robustness and meaningful models. In the present study the MOPAC (molecular orbital package) geometry optimized structures were aligned on the template by the routine ALIGN DATABASE option in SYBYL employing the divide and conquer strategy as follows: molecules that have benzothiazole moiety was used as the maximum common substructure for alignment. Finally these alignment were combined for subsequent CoMFA and CoMSIA study. Figure 1 shows the aligned molecules.



**Fig.1** The conformations of 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)-benzothiazole derivatives superimposed on template

### CoMFA Interaction Energies

COMFA steric and electrostatic fields were calculated at each lattice intersection of a regularly spaced grid of 2.0. The Van der Waals potential and Coulombic terms, these two represent steric and electrostatic fields respectively, and were calculated using the standard Tripos's force field. A distance dependent dielectric constant of 1.00 was used. A  $sp^3$  hybridized carbon atom with +1 charge served as probe atom to calculate steric and electrostatic fields. +30.0 kcal/mol steric and electrostatic contributions were truncated [26]. Cross-validation analysis was performed using leave-one-out method. The cross-validated  $q^2$  value that resulted in optimum number of components five and lowest standard error of estimate was taken and also same weights for CoMFA were allocated to steric and electrostatic fields using CoMFA standard scaling option. To speed up the analysis column filtering value of 2.00 kcal/mol was used for the cross-validation. Further, final analysis was performed to evaluate non cross-validated  $r^2$  using the optimum number of components obtained from the leave one out cross validation analysis. To assess the robustness and statistical confidence we performed bootstrapping analysis by taking 100 runs.

### CoMSIA

In the present study, we analyze the nature of Benzothiazole derivatives using 3D-QSAR (Three-dimensional quantitative structure–activity relationship) analysis. (CoMSIA) Comparative molecular similarity indices analysis was used. In CoMSIA, ligand affinity changes are directly related to changes in molecular properties. CoMSIA method describes the intermolecular interactions (steric, electrostatic, hydrophobic, hydrogen bond donor and acceptor) present at the molecular binding site. The method has been used to study the ligand–protein interactions before and has proved to be of good predictivity.

**Table 1: Statistical analysis of Benzothiazole derivatives**

	CoMFA		CoMSIA	
$q^2$	0.767		0.806	
$r^2$	0.938		0.945	
SEE	0.131		0.129	
F value	42.490		36.901	
CV	0.787		0.817	
<b>Bootstrap</b>				
	Mean	Std.dev	Mean	Std.dev
SEE	0.091	0.064	0.091	0.068
$r^2$	0.968	0.017	0.970	0.017
<b>Field Contribution (%)</b>				
Steric	52.5		14.3	
Electrostatic	47.5		45.8	

Hydrophobic	-	28.7
Donor	-	0.00
Acceptor	-	11.2

### Partial Least Square (PLS)

The CoMFA and CoMSIA analyses were performed using the partial least square (PLS) method. PLS regression technique is useful in common cases where the number of descriptors is comparable to or greater than the number of compounds and / or there exist other factors leading to correlations between variables. Biological activity is used as dependent variable and descriptors as independent variable. The column filtering was set to 2.0 kcal/mol, to improve the signal-to-noise ratio.  $q^2$  (conventional  $r^2$ ) were performed by the Leave-One-Out (LOO) procedure, for the calculation of optimum number of components (N). The cross-validated  $r^2$  resulted in optimum number of components and lowest standard error of estimate was considered for further analysis. No-validation, cross-validation and finally bootstrapping analysis was performed to calculate conventional  $r^2$  using the optimum number of components. Bootstrapping analysis for 100 runs was performed.

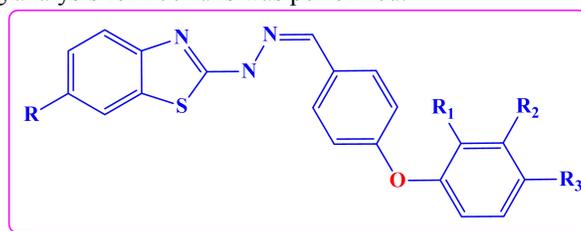


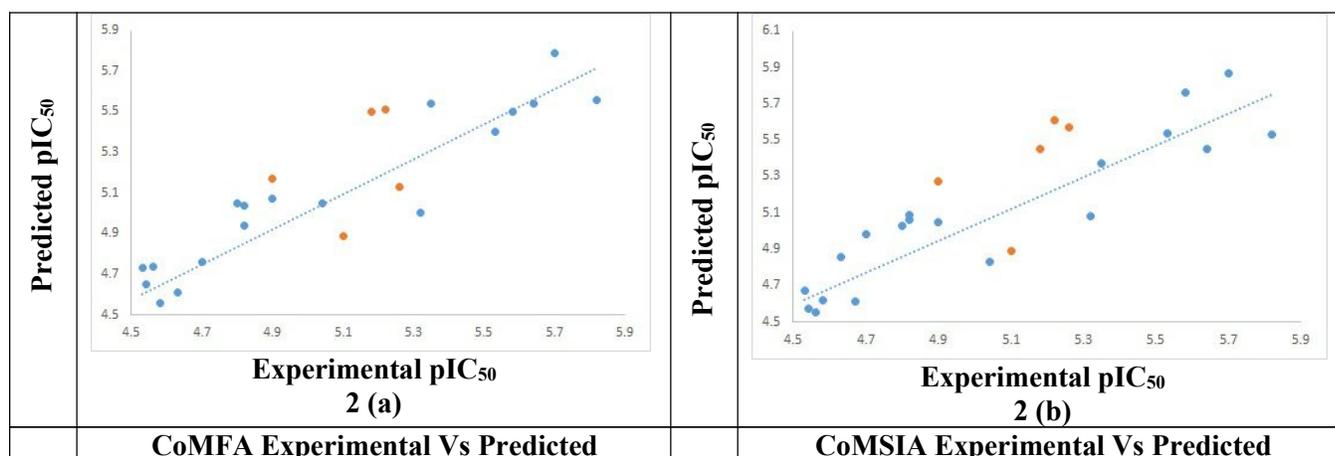
Table.2: Experimental and predicted residual values of 2-(2-(4-Aryloxybenzylidene)hydrazinyl)-benzothiazole used in training set.

C.No	R	R1	R2	R3	pIC50	CoMFA		CoMSIA	
						Predicted	Residual	Predicted	Residual
3	H	H	H	Cl	5.53	5.40	0.13	5.54	-0.01
4	H	Cl	H	Cl	5.58	5.50	0.08	5.76	-0.18
7	Cl	Cl	H	H	5.70	5.79	-0.09	5.87	-0.17
8	Cl	Cl	H	Cl	5.64	5.54	0.10	5.45	0.19
9	Cl	Cl	H	Cl	5.82	5.56	0.26	5.53	0.29
10	Cl	H	CH <sub>3</sub>	Cl	5.35	5.54	-0.19	5.37	-0.02
11	CH <sub>3</sub>	H	H	H	5.04	5.05	-0.01	4.83	0.21
12	CH <sub>3</sub>	Cl	H	H	5.32	5.00	0.32	5.08	0.24
13	CH <sub>3</sub>	H	H	Cl	4.82	5.04	-0.22	5.09	-0.27
14	CH <sub>3</sub>	Cl	H	Cl	4.90	5.07	-0.17	5.05	-0.15
15	CH <sub>3</sub>	H	CH <sub>3</sub>	Cl	4.82	4.94	-0.12	5.06	-0.24
16	OCH <sub>3</sub>	H	H	H	4.80	5.05	-0.25	5.03	-0.23
17	OCH <sub>3</sub>	Cl	H	H	4.70	4.76	-0.06	4.98	-0.28
19	OCH <sub>3</sub>	Cl	H	Cl	4.56	4.74	-0.18	4.55	0.01
20	OCH <sub>3</sub>	H	CH <sub>3</sub>	Cl	4.57	4.39	0.18	4.26	0.31
21	NO <sub>2</sub>	H	H	H	4.54	4.65	-0.11	4.57	-0.03
22	NO <sub>2</sub>	Cl	H	H	4.53	4.73	-0.20	4.67	-0.14
23	NO <sub>2</sub>	H	H	Cl	4.58	4.56	0.02	4.62	-0.04
24	NO <sub>2</sub>	Cl	H	Cl	4.63	4.61	0.02	4.86	-0.23

25	NO <sub>2</sub>	H	CH <sub>3</sub>	Cl	4.67	4.34	0.33	4.61	0.06
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**Table.3: Experimental and predicted residual values of 2-(2-(4-Aryloxybenzylidene)hydrazinyl)-benzothiazole used in test set.**

C.No	R	R1	R2	R3	pIC <sub>50</sub>	CoMFA		CoMSIA	
						Predicted	Residual	Predicted	Residual
1	H	H	H	H	4.90	5.17	-0.27	5.27	-0.37
2	H		H	H	5.22	5.51	-0.29	5.61	-0.39
5	H	H	CH <sub>3</sub>	Cl	5.18	5.50	-0.32	5.45	-0.27
6	Cl	H	H	H	5.26	5.13	0.13	5.57	-0.31
18	OCH <sub>3</sub>	H	H	Cl	5.10	4.89	0.21	4.89	0.21



**Fig.2 the plots of experimental versus predicted pIC<sub>50</sub>**

## RESULTS & DISCUSSION

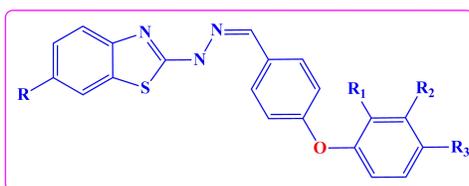
The 3D-QSAR CoMFA and CoMSIA studies were carried out using Benzothiazole derivatives, which are reported IC<sub>50</sub> values on mycobacterium tuberculosis H37RV. 25 molecules were taken for the present study. All the 25 compounds were partitioned into a test set of five and a training set of 20 compounds as 1:3 ratio (1 percent in test set is and 3 percent in training set) were selected randomly. The ambiguity of ligand-receptor interactions in general, statistically robust models were obtained from the CoMFA and CoMSIA models. Training set and test set Experimental and predicted activities are given in figure 2 (a) and 2 (b)

The CoMFA and CoMSIA PLS analysis is summarized in Table 1. The cross-validated correlation co-efficient is used as a measure of goodness of prediction whereas the non-cross-validated conventional correlation co-efficient indicates goodness of fit of a QSAR model. F-value indicates for the degree of statistical confidence. A cross-

validated correlation co-efficient  $q^2$  of 0.767 was obtained using 5 as optimum number of components and 2.0 kcal/mol column filtering was used for the present model. The  $r^2_{cv}$  obtained indicates a good internal predictive ability of the models. The developed models also exhibited a good non-cross validated correlation co-efficient  $r^2$  of 0.938. The Test set compounds are used to assess the external predictive capabilities of QSAR models. 5 compounds were selected in test set randomly were set-aside during model development. Further, a bootstrapping analysis was done for 100 runs. The  $r^2_{bs}$  value obtained 0.968 of bootstrapping by 100 runs which further supports the statistical validity of the model. The contributions of steric to electrostatic fields were found to be 52.5% for steric and 47.5% for electrostatic. Steric contribution slightly is more compared to electrostatic contribution.

The optimum CoMSIA model was derived with the combination of steric, electrostatic, hydrophobic, H-bond donor and H-bond acceptor field contribution using Gasteiger-Hückel charge with 2.0 Å grid space. Leave one out analysis gave the cross-validated  $q^2$  of 0.806 with 6 components and column filtering was set to 1.0 kcal/mol. Non-cross-validated PLS analysis resulted in a correlation coefficient  $r^2$  of 0.945,  $F= 36.901$ , with an standard error of estimate 0.129. Later we performed bootstrapping analyses 100 runs to evaluate the robustness and statistical confidence of the final models ( $r^2$  bootstrapping = 0.970, StdDev= 0.017). Statistical results obtained from the developed model verified the predictive ability of the model (Table 1). The predictive ability of the developed CoMSIA model was assessed by the test set (five molecules), were excluded during model generation. Predicted, experimental, residual values of all inhibitors used in test set and training set are shown in Table 2 and 3.

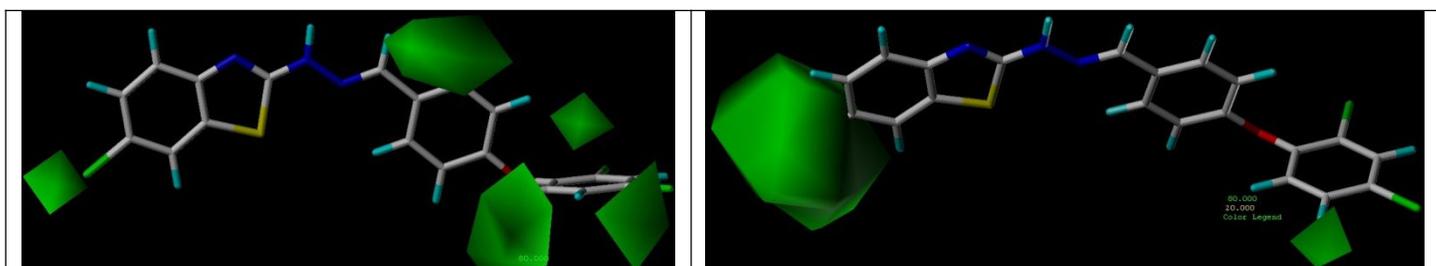
## CONTOUR ANALYSIS



### Steric Contour Maps

In SYBYL, steric interactions are present by green and yellow contours. Green contour maps represent sterically favoured regions where more bulky substituent's are expected to increase the biological activity, whereas yellow contour maps represent less sterically favoured regions where less bulky substituent are expected to increase biological activity.

The CoMFA and CoMSIA steric contour maps of 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)-benzothiazole derivatives have shown in Fig.3 (a) and 3 (b). The CoMFA steric contour map exhibited only green contours. Four large green contours around the 2-(4-Aryloxybenzylidene) and a large green contour near to benzothiazole position and CoMSIA contour plot showed two green contours. A large green contour near to benzothiazole ring  $R^1$  position and small contour present at  $R^4$  position of 4-Aryloxybenzylidene indicates more favourable regions where the bulky substitutions are expected to enhance the inhibitor potency.



a) CoMFA

b) CoMSIA

Figure 3: The CoMFA and CoMSIA steric contour maps of 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)-benzothiazole derivatives

**Electrostatic Contour Maps**

CoMFA and CoMSIA electrostatic contour maps of 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)-benzothiazole derivatives for most active compound displayed in Fig.4 (a) and 4 (b). CoMFA and CoMSIA electrostatic contour map showed only blue contours. The blue contour present at near R<sup>1</sup> position of benzothiazole and in CoMSIA a large blue contour present at near R<sup>1</sup> position of benzothiazole reveals favourable positions where the positively charged groups increase the activity.

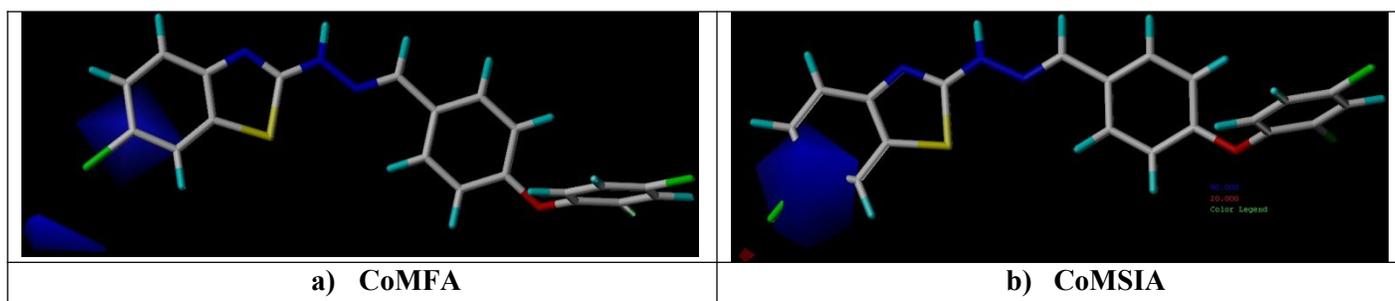
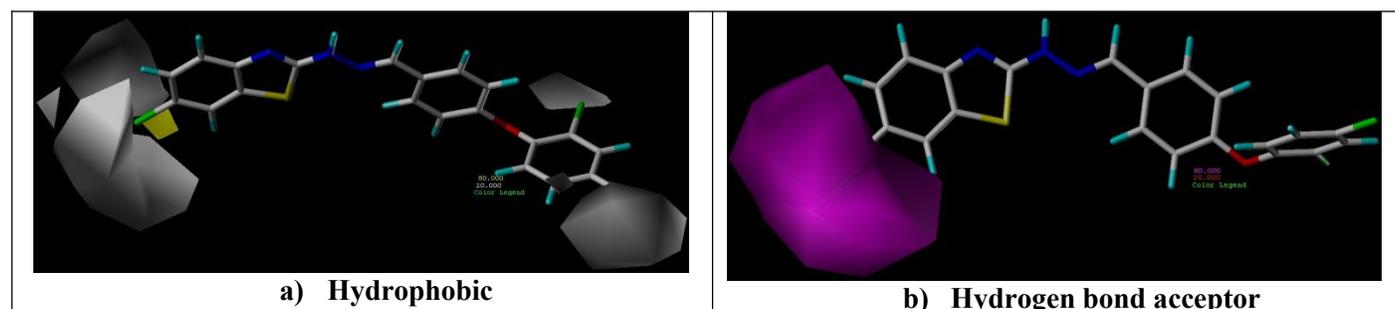


Fig.4 CoMFA and CoMSIA electrostatic contour maps of 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)-benzothiazole derivatives

**Hydrogen Bond Donor, Acceptor and Hydrophobic Contour Maps**

CoMSIA hydrophobic contour maps are represented by yellow and white colours. CoMSIA hydrophobic contour map shows two large white contours at nearer to R and R<sup>1</sup> position of 2-(4-Aryloxybenzylidene), R and R<sup>4</sup> positions of benzothiazole ring indicates disfavoured conformation of hydrophobic substitution. A small yellow contour present at R position of benzothiazole ring indicates favoured conformation where hydrophobic substitution will increase the activity. Figure 5(a) display the hydrophobic contour map of 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)-benzothiazole derivative.

Hydrogen bond acceptor contour maps of 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)-benzothiazole derivatives showed in Fig. 5 (b). Colour contours represent areas where hydrogen bonding correlates strongly with binding affinity. Magenta colour indicates hydrogen-bond acceptors favoured; and red colour indicates hydrogen-bond acceptors disfavoured. There is no Hydrogen bond donor for this model. Hydrogen bond acceptor explains the spacial arrangement of the favourable and disfavour able H-bond interactions to donor or acceptor groups of the target protein. Most active compound shows big sized magenta contour nearer to R position benzothiazole ring indicates hydrogen bond acceptors are favoured.



**Figure 5: Hydrophobic, Hydrogen bond donor and acceptor contour maps of 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)-benzothiazole derivatives**

## CONCLUSION

The utility of 2-(2-(4-aryloxybenzylidene)-hydrazinyl)-benzothiazole inhibitors in the treatment of tuberculosis diseases has not been fully explored yet. In the present study, a 3D-QSAR analyses have been performed on a series of 2-(2-(4-aryloxybenzylidene)-hydrazinyl)-benzothiazole derivatives in order to understand their anti tuberculosis activity. The CoMFA and CoMSIA methods were successfully employed for internal and external validation. Leave-one-out, no-validation, cross-validation and bootstrapping analysis were providing the most significant correlation between structural description and biological activity of the compound.

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