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RESEARCH PAPER

3D quantitatve structure activity relationship of tetrahydroimidazo [1,2-a] pyrimidine as antimicrobial agents

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ABSTRACT

A series 24 tetrahydroimidazo[1,2-a] pyrimidine derivatives were subjected to 3D quantitative structure activity relationship for antimicrobial activity. Partial least square methodology was utilized for the development of QSAR models. Six different QSAR models have been generated for antimicrobial efficiency of tetrahydroimidazo[1,2-a] pyrimidine derivatives against three human pathogens. QSAR models revealed positive influence of steric and electronic parameters on antimicrobial potential - of tetrahydroimidazo[1,2-a] pyrimidine derivatives.

Keywords: Antimicrobial, Pyrimidine, QSAR, Partial least square.

1. Introduction (1-8)

Pyrimidine, a two-nitrogen containing heterocyclic system, is very abundant in the nature. Pyrimidine is the core constituent of many important biomolecules like proteins and nucleic acids in living organism. Pyrimidine and condensed pyrimidine derivatives are important chemical scaffolds due to their prevalence in important biological process in human and microorganisms. Chemically pyrimidine is a cyclic amine, which can be considered as m-diazine. Pyrimidine derivatives were found to be showing variety of biological activity like antimicrobial, anticancer, anti-inflammatory, antifungal and antioxidant (1-10). Pyrimidine derivatives were active antimicrobial agents showing activity against Gram + and Gram – microorganism as well as fungal pathogens like *Candida albicans*. In recent decade, due to prevalence of resistant of microbes towards molecules in clinical use, this indeed results in generation of newer and novel antimicrobial agents. Quantitative structure activity relationship is the methodology which correlates biological properties of molecules with physicochemical parameters of chemical structures. QSAR methods are having great importance in modern pharmaceutical chemistry. Because they are directly correlating with biological activity with structural features of molecules (1, 2). QSAR analysis is carried out via various methodologies like 2D, 3D and GQSAR respectively. 3D QSAR is an advanced type of QSAR where binding potential

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of the molecules is correlated with bioactivity via utilization of regression analysis like PLS and PCA. Normally in 3D QSAR binding potential of the molecules is considered in terms of steric and electrostatic potential of the molecule. In 3D QSAR methodology, alignment of the molecules plays an important role because molecules under study should be aligned on a common template because for proper correlation of bioactivity and structural features of molecules alignment is important. QSAR methodology has been utilized by number of researcher for prediction of biological activity (11-13) In this manuscript, we have attempted to develop 3D QSAR equation for antimicrobial potency of 24 tetrahydroimidazo[1,2-a] pyrimidine derivatives reported by Verma *et al* Six different QSAR models were generated using Vlife MDS 4.3 running on PIV processor. QSAR models revealed importance of steric and electrostatic parameters in antimicrobial potential of pyrimidine derivatives.

2. Materials and Methods

2.1. Computational details

A dataset of 24 compounds was taken from the published tetrahydroimidazo[1,2-a] pyrimidine by Rani *et al* (14); their structures and their inhibitory activities are shown in Table 1. All the structures of tetrahydroimidazo[1,2-a] pyrimidine was drawn in 2D and converted into 3D structures using Vlife engine module of Vlife MDS 4.4. The energy minimization was carried out using Merck molecular force field (MMFF) and 0.001kcal/mol Å energy gradient was used with 10000 cycles and 0.01 as convergence criteria (rms gradient). Vlife MDS version 4.4 provided by Vlife Sciences Pvt. Ltd. Pune, India. All molecules of present dataset were aligned on common templet of pyrimidine using template based technique. Alignment of the molecules was shown in Figure 1. In 3D QSAR hydrophilic, steric and electrostatic descriptors in the form of interactions energies were calculated at the lattice points of the grid using methyl probe of charge +1. The hydrophilic, steric and electrostatic interaction energies of the molecules were calculated and expressed in terms of grid points which are correlated with biological activity as independent variables. The whole data set was randomly divided into a training set of 16 compounds and a test set 08 of compounds as shown in Table 2. Partial least square analysis was utilized for development of QSAR Models. Models generated were internally validated using test set molecules. QSAR model r^2 above 0.7 and predicted r^2 above 0.5 were selected for further analysis. The selected models are shown in Table 2 whereas observed and predicted activities of selected models are shown in Table 3.

2.2. Prediction of activity

To understand and thoroughly measure a QSAR model, proper validation is necessary. Commonly QSAR models are evaluated by the predicted activity of given dataset. Selected models of QSAR having r^2 above 0.7 were checked for external predictive activity which measured as pred_r^2. The predicted and observed activity values are shown in Table 3.

2.3. Validation of QSAR model (15, 16)

Validation of developed QSAR model is the main critical step in development of QSAR model. Validation is done by internal and external methods.

2.3.1. Internal validation

By means of leave-one-out (q^2, LOO) method internal validation is carried out. In this method, q^2 is calculated by removing every molecule from training set one after other, activity of removed molecules is predicted from remaining set of molecules. Following equation is used to calculate q^2.

$$\mathbf{q^2 = 1 - \Sigma (y_i - \ddot{y}_i)^2 \quad \Sigma (y_i - y_{mean})^2 \ldots\ldots\ldots\ldots\ldots\ldots (1)}$$

Where, ŷi and yi are the predicted and actual activities of i^{th} molecule, individually and y mean is an average activity of training set molecules.

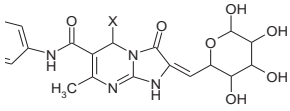
2.3.2. External validation

For external validation of QSAR model (pred_r^2) activity of every molecule from test set was predicted from the developed model of QSAR from training set molecules. Following equation is used to calculate pred_r^2.

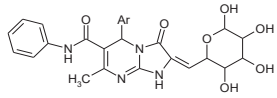
$$\mathbf{Pred\_r^2 = 1 - \Sigma (y_i - \ddot{y}_i)^2 \; \Sigma (y_i - y_{mean})^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)}$$

Where, ŷi and yi are the predicted and actual activities of the ith molecule, individually and y mean is an average activity of training set molecules.

Table 1. Structures of the molecules under study



1 and 24



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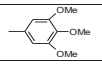
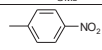
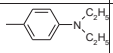
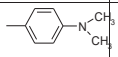
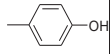
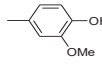
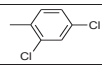
Compound	X	Ar	MIC µM/ml (SA)	MIC µM/ml (BS)	MIC µM/ml (EC)	Compound	X	Ar	MIC µM/ml (SA)	MIC µM/ml (BS)	MIC µM/ml (EC)
1		-----	2.1	2.1	2.1	13	-----		9.3	2.3	2.1
2	-----		4.1	2.1	2.1	14	-----		2.1	2.1	2.1
3	-----		2.2	2.2	2.2	15	-----		4.1	1.0	2.2
4	-----		4.3	4.3	8.6	16	-----		4.6	1.1	8.6
5	-----		9.0	1.1	9.0	17	-----		2.3	1.1	9.0
6	-----		2.2	2.2	2.27	18	-----		4.6	0.58	2.27
7	-----		2.2	1.1	2.2	19	-----		2.4	1.2	2.2
8	-----		2.3	2.3	2.3	20	-----		2.3	1.1	2.3
9	-----		2.2	2.2	2.2	21	-----		2.2	1.1	2.2
10	-----		2.1	2.1	2.1	22	-----		2.3	2.3	2.1
11	-----		2.3	1.1	2.3	23	-----		4.6	1.1	2.3
12	-----		2.1	2.1	2.1	24		-----	2.3	1.1	2.1

SA: ***S. aureus,*** **BC:** ***B. subtilis,*** **EC:** ***E. coli***

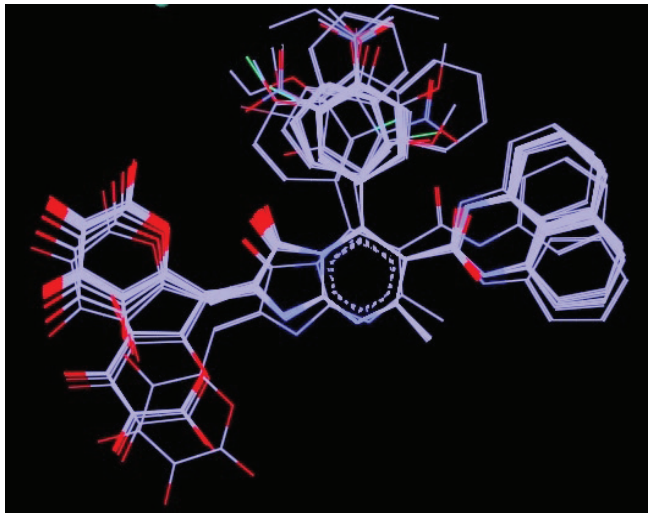


Figure 1. Alignment of molecules

Table 2. Selected PLS 3D QSAR equations along with statistical parameters employed for model selection.

Model No.	QSAR model	N	r^2	q^2	F value	Pred r^2
A (S. aureus)	MIC= 6.9222+-1.0592 H_1040-0.0555E_968+7.0538 S_705+0.2022 S_1337	24	0.93	0.81	32	0.58
B (S. aureus)	MIC=0.1297+-0.0217E_959 -1.7540S_1056-0.2299E_1337-0.0079E_617	24	0.73	0.56	26	0.59
C (B. subtilis)	MIC=36.5597+1.0712 S_616+19.1912S_1273+0.5639 S_850+89.7640 H_572	24	0.93	0.85	62	0.68
D *(B. subtilis)*	MIC=44.7666+1.3047 E_626-6.2354 E_1247 -33.9430 S_644-0.1753 S_959	24	0.78	0.65	25	0.58
E (*E. coli)*	MIC= 3.9279+3.0702 S_517+5.2189 H_581-0.6357E_1488-0.0127 S_635	24	0.98	0.81	82	0.78
F(*E. coli)*	MIC = 2.9963+ 2.9963S_527-1.7061 S_1065-0.0515E_400+0.5281 H_581	24	0.95	0.83	82	0.78

3. Results and Discussion;

To establish the structural requirement of condensed pyrimidine derivatives for antimicrobial efficiency 3D QSAR model has been generated. Two different models for each microorganism were generated and selected on the basis of their predictive significance and correlation coefficient.

Interpretation of 3D QSAR Model A and B:

3D QSAR models were generated using different set of test and training set molecules as shown in table 3 for correlation of antimicrobial activity of pyrimidine derivatives against *S. aureus*. Model A and B were selected on the basis of values of r^2, q^2, pred r^2, F (degree of freedom). A was found to be most significant model generated for correlation of antimicrobial activity of pyrimidine derivatives against S. aureus. Correlation coefficient of model A was found to be 0.93 while F test was found to be 32. H_1040 is the hydrophilic interaction at the lattice point 1040, negative contribution of this interaction energy indicates substitution of lipophilic groups on pyrimidine rings will potentiate the antimicrobial potential. Steric interactions at lattice point 705 and 1337 are positively contributing towards antimicrobial activity which also signifies substitution of bulkier groups like aromatic rings or higher carbon containing groups will potentiate the antimicrobial activity of pyrimidine derivatives. Electrostatic interactions at lattice point E_968 is negatively contributing which indicates substitution of electron withdrawing groups will increase the antimicrobial activity. Molecule no 10 and 12 are halogen substituted derivatives having potent activity against the *S. aureus* which validates the results of developed QSAR model A. For Model B correlation coefficient was found to be 0.73 while pred_r^2 0.59. Electrostatic interaction at lattice points E_959, E_1337 and E_617 are negatively contributing to activity of molecules. Which favors substitution of electron withdrawing groups will increase the antimicrobial activity. Steric interaction at lattice point S_1056 is also negative contributed in activity of molecules, which signifies substitution of smaller groups might increase the antimicrobial activity. The contribution plot with grid points and correlation plot of model A and B are shown in fig. 2, 3 and 4, 5 respectively.

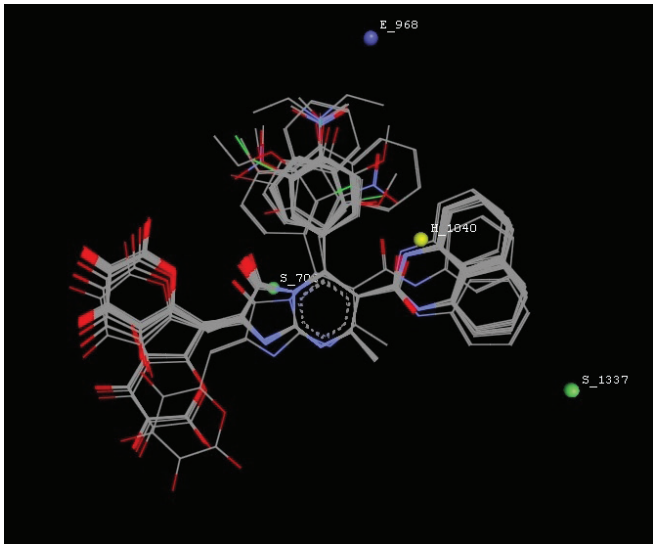


Figure 2. Grid points of QSAR model A

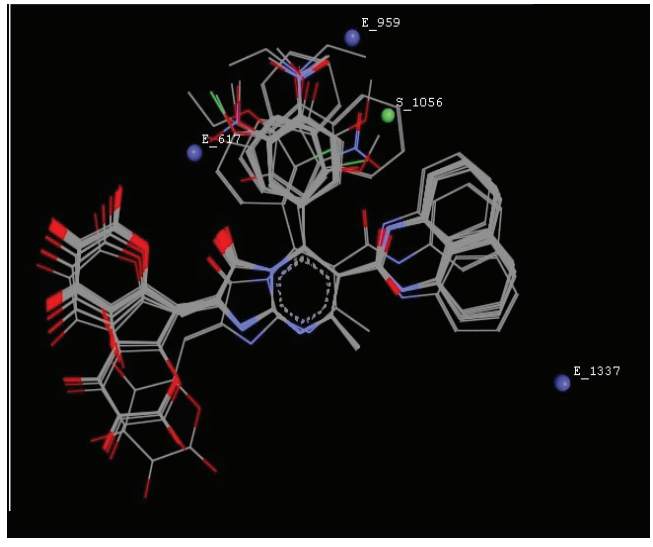


Figure 3. Grid points of QSAR model B

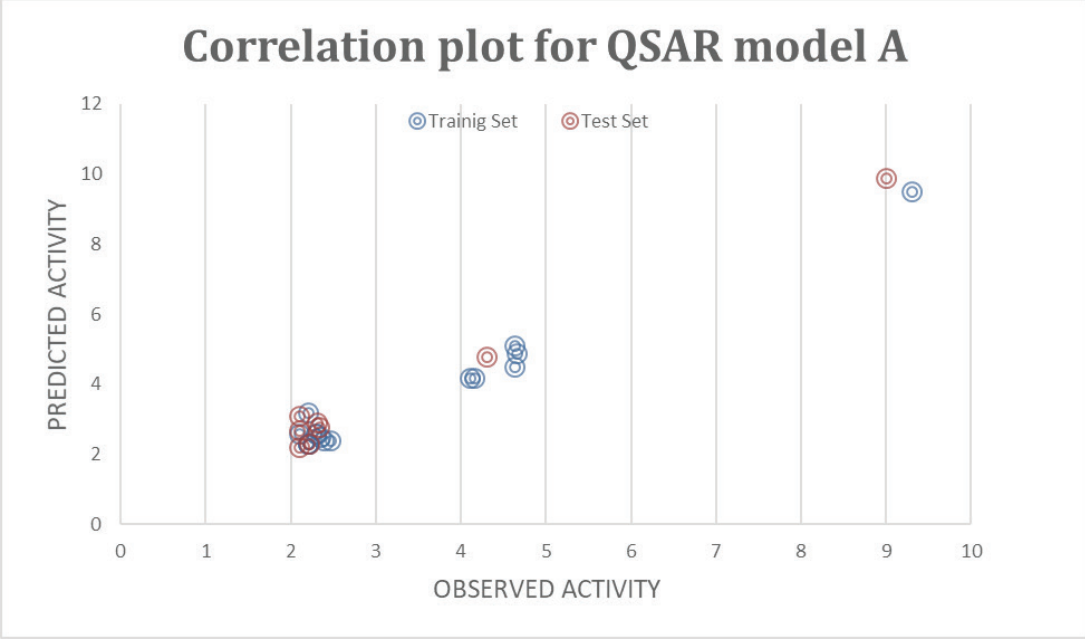


Figure 4. Correlation plot for QSAR model A

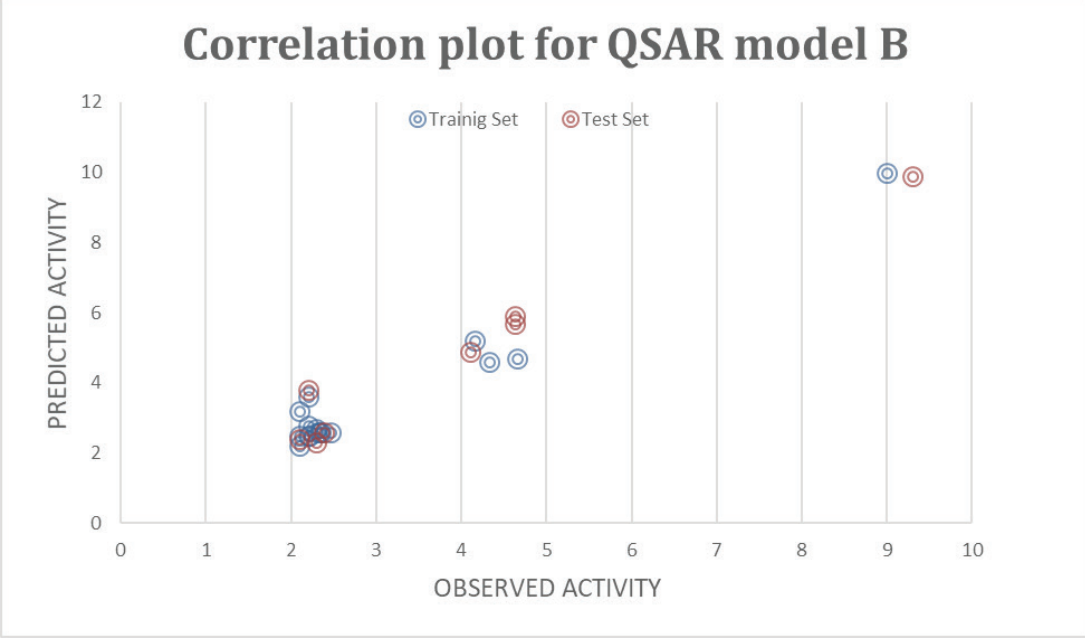


Figure 5. Correlation plot for QSAR model B

Interpretation of 3D QSAR Model C and D:

Similarly, for correlation of antimicrobial activity of pyrimidine derivatives against *B. subtilis* various QSAR models were generated using different set of training and test set of molecules. Model C was the most significant model generated for correlation of antimicrobial activity of pyrimidine derivatives against *B. subtilis.* Correlation coefficient of model C was found to be 0.93 while F test was found to be 62. H_572 is the hydrophilic interaction at the lattice point 572, positive contribution of this interaction energy indicates substitution of polar groups like hydroxyl groups on pyrimidine rings will potentiate the antimicrobial potential against *B. subtilis*. Activity of derivatives 15 and 20 against *B. subtilis* which is in argument with with developed QSAR model C. Steric interactions at lattice point S_616, S_1273 and S_850 are positively contributing towards antimicrobial activity which also signifies substitution of bulkier groups will potentiate the antimicrobial activity of pyrimidine derivatives. Correlation coefficient r^2 for model D was found to be 0.78, pred_r^2 0.58 and F test value was found to be 25. Electrostatic interaction at lattice point E_626 is contributed positively while at lattice point E_1247 contributed negatively which favors substitution of electron withdrawing groups will increase the antimicrobial activity. Negative contribution of steric interaction points at lattice points S_644 and S_959 favors substitution of smaller groups might increase the antimicrobial activity. Grid points and correlation plots of generated model C and D are shown in fig. 6, 7 and 8, 9 respectively.

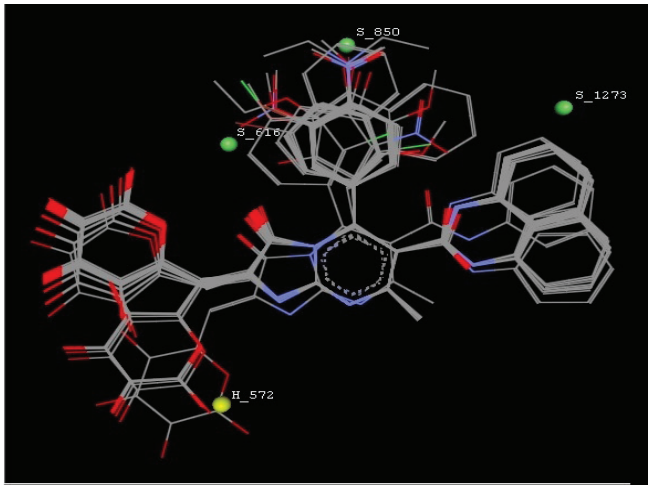


Figure 6. Grid points of QSAR model C

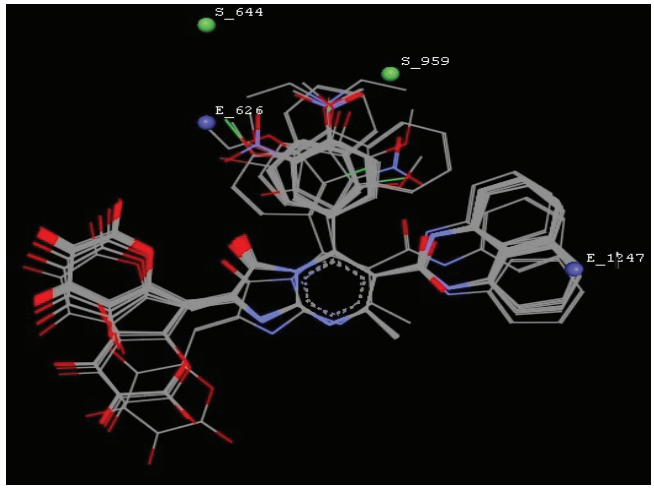


Figure 7. Grid points of QSAR model D

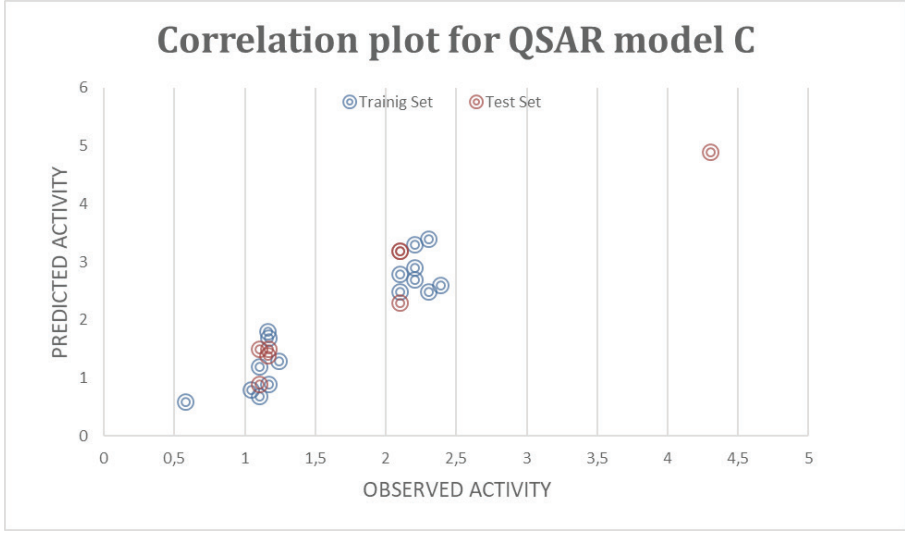


Figure 8. Correlation plot for QSAR model C

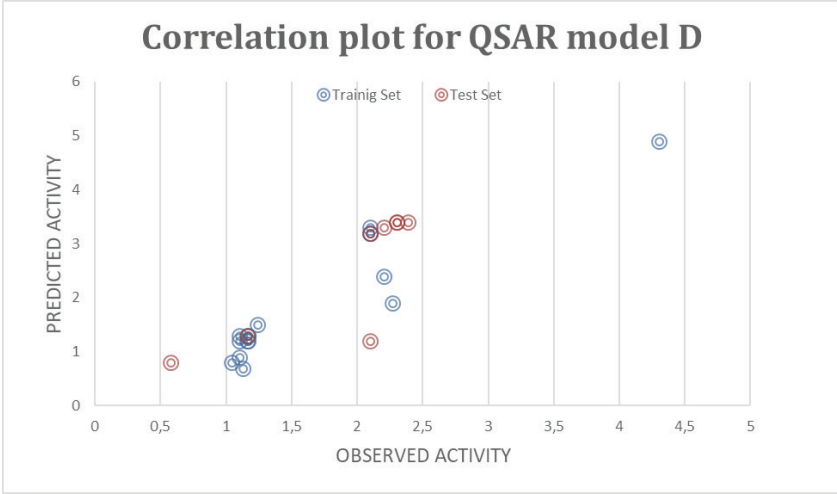


Figure No. 9. Correlation plot for QSAR model D

Interpretation of 3D QSAR Model E and F:

Likewise, for correlation of antimicrobial activity of pyrimidine derivatives against *E. coli,* various models were generated using different test and training set molecules as shown in table 3. Model E was found to be most significant model generated for correlation of antimicrobial activity of pyrimidine derivatives against *E. coli.* Correlation coefficient (r^2) of model E was found to be 0.98 while F test was found to be 82. Steric interactions fields at S_517 is positively contributing which indicates substitution of bulkier groups might increase the antimicrobial activity. H_581 is the hydrophilic interaction at the lattice point 581, positive contribution of this interaction energy indicates substitution of polar groups like hydroxyl groups on pyrimidine rings will potentiate the antimicrobial potential against *E. coli*. Steric interactions at lattice point S_635 is negatively contributing which indicates substitution of smaller groups increases antimicrobial activity. Negative contribution of electrostatic interaction at lattice point E_1488 favors substitution of electron withdrawing groups which will increase the antimicrobial activity. Model F having correlation coefficient r^2 0.95, F value 82 and pred_r^2 was found to be 0.78. Steric interaction at lattice point S_527 is positively contributing to activity whereas, at S_1065 is negatively contributing which means substitution of smaller groups increases antimicrobial activity. Negative contribution of electrostatic interaction at lattice point E_400 indicates substitution of electron withdrawing groups will increase the antimicrobial activity. Hydrophilic interaction at lattice point H_581 is positively contributing which indicates substitution of polar groups like hydroxyl groups on pyrimidine rings will potentiate the antimicrobial potential against *E. coli*. The contribution plot with grid points and correlation plot of model E and F shown in fig. 10, 11 and 12, 13 respectively.

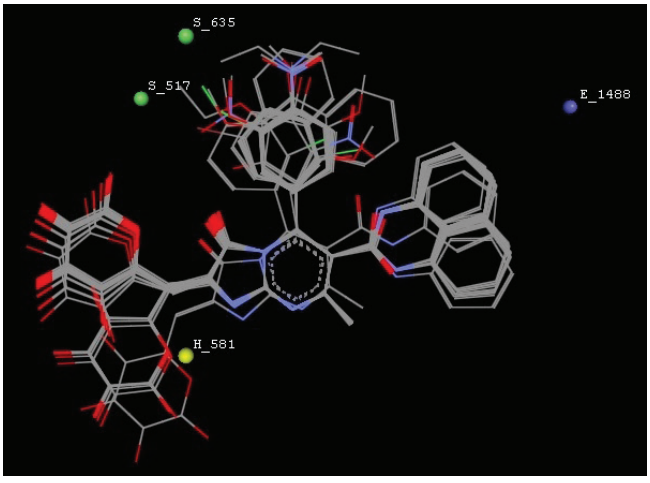


Figure 10. Grid points of QSAR model E

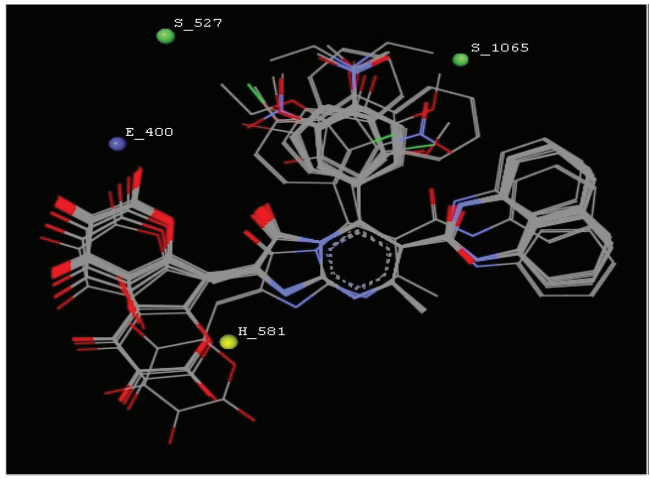


Figure 11. Grid points of QSAR model F

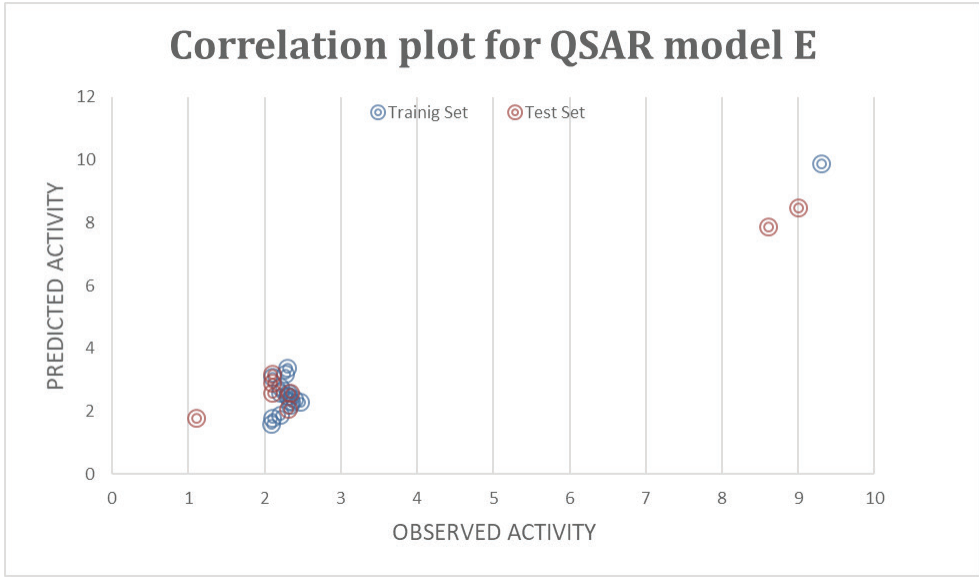


Figure 12. Correlation plot for QSAR model E

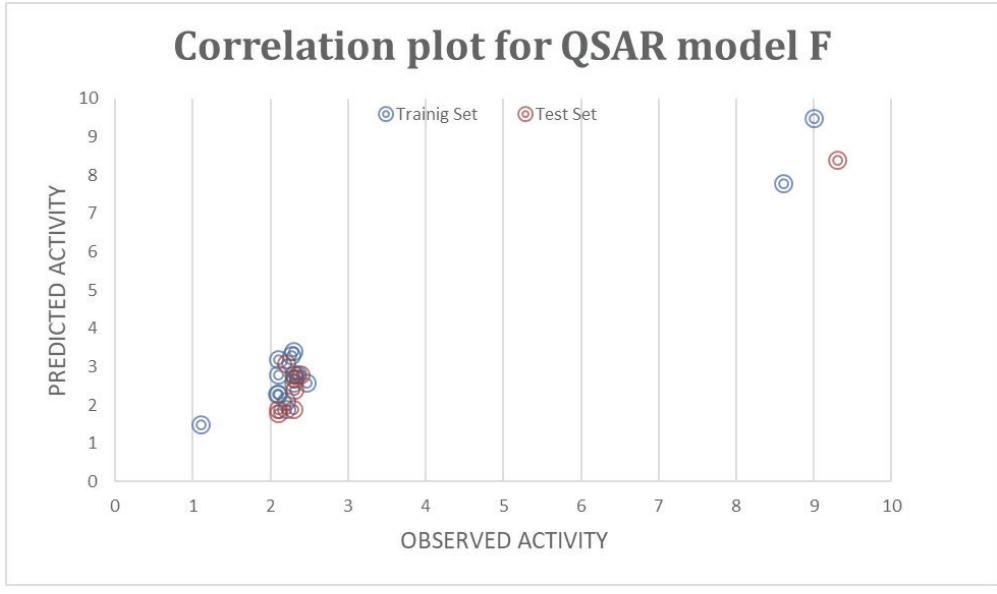


Figure 13. Correlation plot for QSAR model F

4. Conclusion:

In the present study, 3D QSAR analysis on 24 pyrimidine derivatives for antimicrobial activity was carried out. Six different QSAR models were generated by using different set of test and training set of molecules for establishment of correlation of molecular properties with biological activity of molecules. Out of these developed models, model A, C and E were found to be the most significant models to correlate molecular properties with bioactivity of molecules against *S. aureus, B. subtilis* and *E. coli* respectively. Results of QSAR models revealed importance of steric and electronic parameters in the antimicrobial activity of molecules. Developed QSAR models can be utilized for design and development of novel pyrimidine derivatives having antimicrobial activity.

Table 3. Observed and predicted activity (MIC= μM/ml) of selected PLS 3D QSAR equations

Sr. No	A		C		E		Sr. No.	B		D		F	
	OA	PA	OA	PA	OA	PA		OA	PA	OA	PA	OA	PA
1.#	2.1	3.1	2.1	3.2	2.1	3.2	**1.**	2.1	3.2	2.1	3.2	2.1	2.8
2.	4.1	4.2	2.1	2.8	2.1	3.1	**2.#**	4.1	4.9	2.1	1.2	2.1	1.9
3.	2.2	3.2	2.2	3.3	2.2	1.9	**3.#**	2.2	3.8	2.2	3.3	2.2	3.1
4.#	4.3	4.8	4.3	4.9	8.6	7.9	**4.**	4.33	4.6	4.3	4.9	8.6	7.8
5.#	9.0	9.9	1.1	0.9	9.0	8.5	**5.**	9.0	10	1.13	0.7	9.0	9.5
6.	2.2	2.3	2.2	2.9	2.27	3.2	**6.**	2.2	3.6	2.27	1.9	2.27	3.3
7.	2.2	2.3	1.1	0.7	2.2	2.8	**7.**	2.2	2.5	1.1	0.9	2.2	1.9
8.	2.3	2.6	2.3	2.5	2.3	3.4	**8.#**	2.3	2.3	2.3	3.4	2.3	1.9
9.	2.2	2.3	2.2	2.7	2.2	2.6	**9.**	2.2	2.8	2.2	2.4	2.2	2.1
10.#	2.1	2.7	2.1	2.3	2.1	2.9	**10.**	2.1	2.5	2.1	3.2	2.1	2.3
11.	2.3	2.6	1.1	1.2	2.3	2.5	**11.**	2.3	2.7	1.1	1.2	2.3	3.4
12.#	2.1	2.2	2.1	3.2	2.1	2.6	**12.**	2.1	2.2	2.1	3.3	2.1	3.2
13.	9.3	9.5	2.3	3.4	9.3	9.9	**13.#**	9.3	9.9	2.3	3.4	9.3	8.4
14.	2.1	2.6	2.1	2.5	2.1	1.8	**14.#**	2.1	2.4	2.1	3.2	2.1	1.8
15.	4.16	4.2	1.04	0.8	2.08	1.6	**15.**	4.16	5.2	1.04	0.8	2.08	2.3
16.	4.66	4.9	1.17	0.9	2.33	2.2	**16.**	4.66	4.7	1.17	1.3	2.3	2.8
17.#	2.34	2.8	1.17	1.5	2.34	2.6	**17.**	2.34	2.6	1.17	1.3	2.3	2.6
18.	4.63	5.1	0.58	0.6	2.31	2.5	**18.#**	4.63	5.7	0.58	0.8	2.31	2.7
19.	2.47	2.4	1.24	1.3	2.47	2.3	**19.**	2.47	2.6	1.24	1.5	2.47	2.6
20.#	2.31	2.9	1.16	1.4	2.31	2.1	**20.**	2.31	2.6	1.16	1.2	2.31	2.8
21.#	2.21	2.3	1.10	1.5	1.10	1.8	**21.**	2.21	2.5	1.10	1.3	1.10	1.5
22.	2.39	2.4	2.39	2.6	2.39	2.4	**22.#**	2.39	2.6	2.39	3.4	2.39	2.8
23.	4.63	4.5	1.16	1.8	2.31	2.6	**23.#**	4.63	5.9	1.16	1.3	2.31	2.4
24.	2.35	2.5	1.17	1.7	2.35	2.3	**24.**	2.35	2.6	1.17	1.2	2.35	2.8

OA: Observed Activity, PA: Predicted Activity, #: molecules under test set

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