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Research Article

3DQSAR Studies of *N*-[Alkyl or Un/substituted phenyl]-2-(3-oxo-3,4-dihydro-2*H*-benzo[b] [1,4] thiazin-2-yl)acetamide

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Abstract

3DQSAR studies were performed on a series of 1,4-benzothiaine derivatives to investigate the structural requirements of antifungal compounds. Multiple linear regression (MLR) methodology coupled with feature forward selection method was applied to derive QSAR models which were further validated for statistical significance and predictive ability by internal and external validation. The statistically significant best 3D-QSAR model having the correlation coefficient r^2= 0.9172 was selected for further study. The model was further validated by means of crossed squared correlation coefficient q^2 =0.8223 and pred_r^2 =0.5960, which show that the model has good predictive ability. QSAR investigations it could be concluded that steric (molecular shape analysis descriptors) properties of 1,4-Benzothiazine derivatives contribute significantly to the antifungal activity and need of bulky substitution at para or meta position of phenyl ring.

1. INTRODUCTION

Structural features of 10*H* Phenothiazines, 4*H*-1,4-Benzothiazines,[1-3] possess a broad spectrum of biological and pharmacological properties due to presence of a fold along the nitrogen and sulfur axis, which is considered to be responsible as one of the structural features to impart their activities. During the past two decades, the frequency of invasive and systemic fungal infections has increased dramatically in the population with altered immunity.[4-5] Today available therapy in treating fungal infections can suffer from adverse effect like drug related toxicity, hazardous drug–drug interactions, non-optimal pharmacokinetics and development of drug resistance.[6] Fungal infections remain a significant cause of morbidity and mortality, specially in immunocompromised patient where the incidence of life threatening fungal infections has risen dramatically.[7]

The most common antifungal agents used in clinic are azoles (such as fluconazole, ketoconazole and itraconazole),[8] polyenes (such as amphotericin B)[9] and nystatin,[10] echinocandins (such as caspofungin and micafungin)[11] and allylamines (such as naftifine and terbinafine).[12] Among these, azoles are widely used in antifungal chemotherapy.

With the aim of developing a new class of antifungal drugs embracing certain characteristic structural features for effective drug–receptor interaction, 3DQSAR model was derived and develop for synthesis of new compounds by applying physiochemical parameters with multiple linear regression (MLR) method. To the best of our knowledge, till date such QSAR study has not been reported on benzothiazine as antifungal as discussed in this article. This inspired us to undertake this work. This study is aimed to elucidate the structural features of benzothiazine derivatives required for antifungal activity and to obtain predictive 3D-QSAR models to guide the rational synthesis of novel antifungal drug.

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2. MATERIALS AND METHODS

The 3DQSAR studies were performed using the Molecular Design Suite (VLife MDS software package, version 3.5; from VLife Sciences, Pune, India), on a DELL PC with a Pentium IV processor and a Windows XP operating system. Structures were sketched using the 2D draw application and converted to 3D structures (VLife MDS 3.5, 2010).

2.1 Biological Activity Dataset for QSAR Analysis

The antifungal activity [MIC (µmol)] data against four fungal species (*Candida albicans, Trichophyton rubrum, Epidermophyton floccosum* and *Malassazia furfur*) of substituted 1,4-Benzothiazine derivatives were taken from the reported work,[13] the result showed in table 1. The total 23 set of compounds was divided into a training set (18 compounds) for generating 3D QSAR models and a test set (5 compounds) for validating the quality of the models. Selection of the training set and test set molecules was done on the basis of structural diversity and a wide range of activity such that the test-set molecules represent a range of biological activity similar to that of the training set; thus, the test set is truly representative of the training set. The biological activity values [MIC] reported in micromolar units(µM) were converted to their microgram units(µgm) and then further to negative logarithmic scale and subsequently used as the dependent variable for the QSAR analysis.

2.2 Computational Details

The structures of all 23 compounds were drawn in 2DDrawApp (MDS 3.5 2010). The 2D structures were converted to 3D structures by sending them to MDS. Each compound was energy minimized and batch optimized by using Merck Molecular Force Field (MMFF) and charges.[14]

Table 1: Pharmacology activity data of BTA series derivatives

Sr. No.	Compound Code	*C. albicans*	*E. floccosum*	*T. rubrum*	*M. furfur*
1	BTA-4	0.250	0.125	0.250	0.125
2	BTA-8	0.125	0.125	0.125	0.250
3	BTA-5	0.125	0.125	0.125	0.125
4	BTA-47	0.0625	0.0625	0.0625	0.125
5	BTA-20	0.0625	0.0625	0.125	0.125
6	BTA-43	0.0312	0.0625	0.125	0.125
7	BTA-64	0.250	0.250	0.250	0.250
8	BTA-35	0.125	0.125	0.125	0.0625
9	BTA-25	0.125	0.250	0.0625	0.125
10	BTA-66	0.250	0.250	0.125	0.250
11	BTA-69	0.125	0.125	0.125	0.250
12	BTA-63	0.250	0.125	0.250	0.250
13	BTA-57	0.250	0.250	0.125	0.250
14	BTA-58	0.250	0.250	0.125	0.125
15	BTA-56	0.0625	0.0625	0.0312	0.0312
16	BTA-65	0.250	0.250	0.250	0.250
17	BTA-59	0.125	0.125	0.250	0.250
18	BTA-60	0.250	0.250	0.250	0.125
19	BTA-61	0.250	0.0625	0.125	0.250
20	BTA-67	0.250	0.250	0.125	0.125
21	BTA-68	0.125	0.250	0.125	0.125
22	BTA-24	0.0625	0.0625	0.0625	0.0625
23	BTA-70	0.125	0.125	0.250	0.250
24	Ketaconazole (std)	0.0312	0.0312	0.0312	0.0312

All MIC value in µmol/ml
**Each result represents the average of triplicate reading.*
E. Floccusom= Epidermophyton. floccusom; M. ruburum = Microsporum. rubrum: M. furfur = Malassazia furfur

2.2.1 Molecular Modeling for 3 D-QSAR

2.2.1.1 Molecular Alignment

Proper alignment of molecules is the most critical step in the ligand based 3D-QSAR modeling method to obtain meaningful results.[15-16] Molecular alignment is useful for studying shape variation with respect to the base structure selected for alignment (VLife MDS 3.5, 2010). Energy-minimized and geometry optimized structures of molecules were aligned by the template-based method,[17] where a template structure used for alignment of a set of molecules. The template structure, i.e. 1,4-Benzothiazine ring, was used for the alignment by considering the common elements of the series as shown in Fig. 1. The reference molecule is chosen in such a way that it is the most active among the series of molecules considered. The reference molecule is the molecule on which the other molecules of the align dataset get aligned based on the chosen template.[18] Compound BTA-56 had very high inhibitory activities against all fungus which made it is a good lead molecule and therefore, was chosen as a reference molecule. After optimizing, the template structure and the reference molecule were used to superimpose all molecules from the series using the template alignment method to obtain optimal alignment between the molecular structures necessary for ligand–receptor interactions. This adjusts the geometry of the molecules such that their steric and electrostatic fields match the fields of the template molecule.[19] The superimposition of all molecules based on minimizing RMS deviation is shown in Fig. 2.

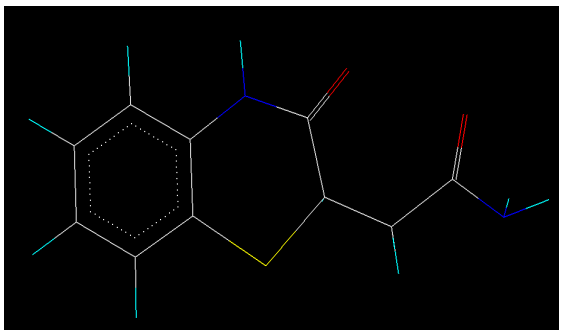


Fig. 1: 1, 4-Benzothiazine ring as template used for alignment of Benzothiazine derivatives

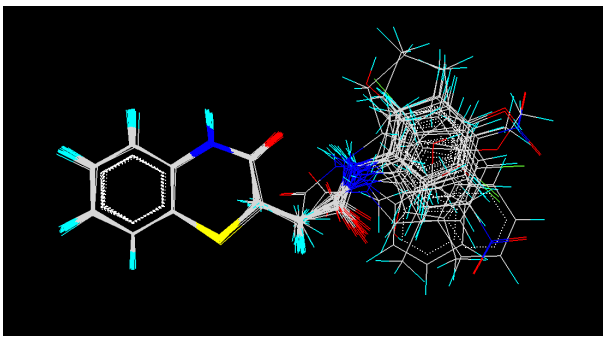


Fig. 2: 3D view of template based alignment of benzothiazine derivatives on the base template

2.2.1.2 Molecular Descriptors

MFA is a method for quantifying the interaction energy between a probe molecule and a set of aligned molecules in a rectangular grid box and can be useful in deriving 3DQSAR.[20] This approach is effective for the analysis of data sets where activity information is available but the structure of the receptor site is unknown. It attempts to postulate and represent the essential features of a receptor site from the aligned common features of the molecules that bind to it.[21] The aligned biologically active conformations of benzothiazine were used for the calculation of molecular fields. Molecular fields are the electrostatic, steric and hydrophobic

interaction energies which are used to formulate a relationship among electrostatic, steric and hydrophobic properties together with the biological activities of compounds. Descriptors were calculated using a sp^3 carbon probe atom with a van der Waals radius of 1.52 Å and a charge of +1.0 with default cut-off energy 30 kcal/mol to generate steric field, electrostatic and hydrophobic fields. Molecular descriptors such as steric, electrostatic and hydrophobic fields have been calculated using VLife MDS 3.5 software.[22] This is done by generating 3D rectangular grids around the molecule and calculating the interaction energy between the molecule and probe group placed at each grid point. Using Tripos force field, [23] steric, electrostatic and hydrophobic fields were computed at each grid point considering Gasteiger-Marsili charges.[24] A value of 1.0 was assigned to the distance-dependent dielectric constant. A total of 3568 three dimensional descriptors were produced before development of model using VLife MDS software. Prior to model development descriptors having zero values or same values were removed which resulted in more than total 2000 descriptors. These included electrostatic, steric and hydrophobic field descriptors for all the compounds in separate columns (Table 2). These interaction energy values at the grid point are considered for relationship generation using MLR method.

Table 2: List of descriptors with value to be used in the most significant 3DQSAR models of Benzothiazine derivatives

Compounds	S_205	S_567	S_609	S_667	E_689	E_690	S_392	S_559
BTA-4	-0.35298	-0.63683	-0.12519	1	-1	-0.5337	-0.2927	-0.03081
BTA-8	-0.36559	0.478404	0.695717	1	0.307654	-0.19861	-0.30063	-0.02585
BTA-5	-0.18426	1	-0.11433	1	1	0.67811	-0.29455	-0.0367
BTA-47	-0.34717	0.403819	-0.46246	1	-1	-0.95078	-0.27772	-0.04433
BTA-20	-0.34467	-0.39056	-0.50744	1	-1	-0.76849	-0.27076	-0.03981
BTA-43	-0.20481	1	-0.12244	1	-0.48258	-0.37265	-0.26762	-0.02365
BTA-64	-0.36939	1	-0.04709	1	-0.13266	-0.17063	-0.29427	-0.02041
BTA-35	-0.26238	-0.31211	-0.29959	1	-0.6783	-0.33686	-0.2966	-0.03793
BTA-25	-0.27182	1	-0.17864	1	0.142447	0.065206	-0.29749	-0.13426
BTA-66	-0.36578	1	-0.22365	1	0.126349	-0.17004	-0.27373	-0.16198
BTA-69	-0.26118	1	-0.29305	1	0.431529	0.162868	-0.27585	-0.03962
BTA-63	-0.34785	-0.47086	-0.21513	0.32790	0.418243	0.138998	-0.28015	-0.01934
BTA-57	-0.26675	1	-0.30809	1	-0.23546	-0.42448	-0.28086	-0.04422
BTA-58	-0.34261	1	-0.2321	1	-1	-0.97894	-0.26902	-0.05935
BTA-56	-0.33695	1	-0.49656	1	-1	-1	-0.27152	-0.05338
BTA-65	-0.35364	1	-0.16446	1	1	0.489838	-0.28282	-0.17103
BTA-59	-0.23437	1	-0.58386	1	0.308508	-0.04946	-0.30187	-0.06424
BTA-60	-0.27211	0.790469	-0.2963	1	0.76837	0.29139	-0.27802	-0.032
BTA-61	-0.27211	0.790469	-0.2963	1	0.76837	0.29139	-0.27802	-0.032
BTA-67	-0.36787	0.097327	-0.43245	1	1	0.574755	-0.27431	-0.04302
BTA-68	-0.34855	1	-0.43509	1	1	0.562876	-0.27952	-0.24051
BTA-24	-0.19407	1	-0.13141	1	-0.33191	-0.12154	-0.26668	-0.29965
BTA-70	-0.36651	-0.51723	-0.38615	0.79080	0.54004	0.045596	-0.27515	-0.03183

Table 2: (*continued*)

Compounds	E_316	S_71	S_462	S_857	E_396	E_476	E_658	S_94
BTA-4	0.905829	-0.00657	-0.02457	-0.25221	1	1	1	-0.01343
BTA-8	-0.92718	-0.0067	-0.0248	-0.17153	1	1	-1	-0.01343
BTA-5	1	-0.00626	-0.02646	-0.1557	1	1	-0.68976	-0.01372
BTA-47	1	-0.00669	-0.02415	-0.52046	1	1	-1	-0.01367
BTA-20	1	-0.00664	-0.02415	-0.40783	1	1	0.522089	-0.0137
BTA-43	-0.05443	-0.00669	-0.02377	-0.15035	1	0.584955	-1	-0.01364
BTA-64	-1	-0.00646	-0.0249	-0.06126	1	1	0.881817	-0.01319
BTA-35	-1	-0.00644	-0.02508	1	-1	1	-0.04748	-0.01376
BTA-25	-0.82643	-0.00648	-0.02586	-0.46879	1	1	1	-0.01399
BTA-66	1	-0.00644	-0.02546	-0.45333	1	1	1	-0.01379
BTA-69	0.164879	-0.00671	-0.02404	-0.45486	1	-1	-0.30331	-0.01378
BTA-63	1	-0.00663	-0.02363	-0.06014	1	1	-0.37695	-0.01344
BTA-57	-0.51223	-0.00668	-0.02408	-0.51556	1	-0.7359	-1	-0.01381
BTA-58	1	-0.00659	-0.02448	-0.43973	1	1	1	-0.01376
BTA-56	1	-0.00664	-0.02425	-0.60408	1	-0.79829	-0.06796	-0.01388
BTA-65	-1	-0.00658	-0.0258	-0.37265	1	1	1	-0.01384
BTA-59	-1	-0.00646	-0.02497	-0.26644	1	1	1	-0.01389
BTA-60	-0.03292	-0.00669	-0.02432	-0.44869	1	-0.6731	-0.31359	-0.01374
BTA-61	-0.03292	-0.00669	-0.02432	-0.44869	1	-0.6731	-0.31359	-0.01374
BTA-67	1	-0.0064	-0.02534	-0.34585	1	1	0.176547	-0.01366
BTA-68	1	-0.00667	-0.0245	-0.36698	1	1	-1	-0.01386
BTA-24	1	-0.00652	-0.02421	-0.39849	1	1	1	-0.01381
BTA-70	1	-0.00647	-0.02467	-0.09903	1	1	-0.09289	-0.01358

2.2.1.3 Division of a Dataset into Training and Test Sets

The random selection method was adopted for division of training and test data sets in order to assess the similarity of the distribution pattern of the compounds in the generated sets, statistical parameters (with respect to the biological activity) i.e. mean, maximum, minimum and standard deviation were calculated for the training and test sets. For selection of training and test sets, we were ensured that the compounds have uniform spread (training and test) in terms of both activity and chemical space. Random selection method resulted in the selection of 5 compounds as the test set for validating the quality of the models and the remaining 18 compounds as the training set for generating 3D-QSAR models (Table.3). The test was used to ascertain the predictive power of the model.

2.2.1.4 Forward Stepwise as Feature (variable) Selection Method

Chance correlations and multi-collinearity are two major problems often encountered when attempting to find generalized QSAR models for use in drug design. Feature selection is a key step in QSAR analysis. An integral aspect of any model building exercise is the selection of an appropriate set of features with low complexity and good predictive accuracy. This process forms the basis of a

technique known as feature selection or variable selection. Among several search algorithms, stepwise (SW), genetic algorithm (GA) and simulated annealing (SA) based feature selection procedures are most popular for building QSAR models and can explain the situation more effectively.[25] In SW forward variable selection algorithm, the search procedure begins with developing a trial model step by step with a single independent variable and to each step, independent variables are added one at a time, examining the fit of the model by using the MLR procedure. Thus, the model is repeatedly altered from the previous one by adding or removing a predictor variable in accordance with the 'stepping criteria' (in this case, F= 4 for inclusion for the forward selection method). The method continues until there is no more significant variable remaining outside the model. In the selected equations, the cross-correlation limit was set at 0.5, the number of variables at 4 and the term selection criteria at r^2. An F value was specified to evaluate the significance of a variable. The variance cut-off was set at 0.0, and scaling as none.

Table 3: Data set for 3DQSAR models

Compounds	Model I	Model II	Model II	Model IV
BTA-4	Training	Training	Training	Training
BTA-8	Test	Test	Test	Test
BTA-5	Training	Training	Training	Training
BTA-47	Test	Training	Test	Test
BTA-20	Training	Training	Training	Training
BTA-43	Test	Training	Test	Test
BTA-64	Training	Training	Training	Training
BTA-35	Training	Training	Training	Training
BTA-25	Training	Training	Training	Training
BTA-66	Test	Training	Test	Test
BTA-69	Test	Test	Test	Test
BTA-63	Training	Training	Training	Training
BTA-57	Training	Test	Training	Training
BTA-58	Training	Training	Training	Training
BTA-56	Training	Training	Training	Training
BTA-65	Training	Test	Training	Training
BTA-59	Training	Training	Training	Training
BTA-60	Training	Training	Training	Training
BTA-61	Training	Test	Training	Training
BTA-67	Training	Training	Training	Training
BTA-68	Training	Training	Training	Training
BTA-24	Training	Training	Training	Training
BTA-70	Training	Training	Training	Training

2.2.2 MLR Methodology for Building QSAR Models

2.2.2.1 Model Validation and Evaluation

This is done to test the internal stability and predictive ability of the QSAR models.

2.2.2.2 Internal and External validations

Internal validation was carried out using leave-one-out (q^2, LOO) method. For calculating q^2, each molecule in the training set was eliminated once and the activity of the eliminated molecule was predicted by using the model developed by the remaining molecules. The cross-validated coefficient, q^2, was calculated using Eq. (1).

$$q^2 = 1 - \frac{\Sigma(y_i - \hat{y}_i)^2}{\Sigma(y_i - y_{mean})^2} \quad \text{---------- (1)}$$

Where yi and yi are the actual and the predicted activity of the i^{th} molecule in the training set, respectively, and y_{mean} is the average activity of all molecules in the training set. However, a high q^2 value does not necessarily give a suitable representation of the real predictive power of the model for antifungal activity. So, an external validation is also carried out in this study. The external predictive power of the model is assessed by predicting pMIC value of five test set molecules, which are not included in the QSAR model development. The predictive ability of the selected model is also confirmed by pred_r^2. For external validation, the activity of each molecule in the test set was predicted using the model developed by the training set. The pred_r^2 value is calculated as follows (Eq. 2)

$$pred\_r2 = 1 - \frac{\Sigma(y_i - \hat{y}_i)^2}{\Sigma(y_i - y_{mean})^2} \quad \text{---------- (2)}$$

Where yi and yi are the actual and the 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. Both summations are over all molecules in the test set. Thus the pred_r^2 value is indicative of the predictive power of the current MLR model based on the external test set.

2.2.2.3 Evaluation of the Quantitative Model

The developed 3D-QSAR model was evaluated using the following statistical measures: N, number of observations (molecules) in the training set; q^2, cross-validated r^2 (by leave one out) which is a relative measure of quality of fit; pred_r^2, r^2 for external test set; q^2se, standard error of cross-validation and pred_r^2se, standard error of external test set prediction. However, a QSAR model is considered to be predictive, if the following conditions are satisfied: $q^2 > 0.6$ and pred_$r^2 > 0.5$.[26] The low standard error of pred_r^2se and q^2se shows absolute quality of fitness of the model. The high pred_r^2 and low pred_r^2se show high predictive ability of the model. The q^2 and pred_r^2 values were used as deciding factors in selecting the optimal models.

3. RESULTS AND DISCUSSION

The biological data of 1,4-benzothiazine derivatives for 3DQSAR studies was taken from literature. The importance and utility of the new 3D QSAR method discussed has been established by applying it to known sets of molecules as mention in table no. 4. The biological activity consider as dependent variable and all the calculated descriptors were considered as independent variable. In 3D QSAR analysis, significant methods MLR analysis, were applied to generate four models: Models I, II, III and IV, respectively, from these models, one of them were having good q^2 and pred_r^2 values, one of which was selected having good internal and external predictivity. Selecting training and test set was by random selection method. The QSAR models developed by MLR include both the electrostatic, steric and hydrophobic descriptors along with their range to indicate their importance for interaction in molecular field. Models IV 3D QSAR was good model amongs the all model. QSAR investigations of the substituted benzothiazine derivatives resulted in several QSAR equations. Some statistically significant 3D QSAR models were chosen for discussion.

3.1 Model I (Activity against *C. albicans*)

pMIC = -0.1647(±0.0054)S_567 + 0.1814(±0.0251)S_690 + 0.4733(±0.1303)S_205 + 0.1575(±0.0535)S_667 + 0.7435

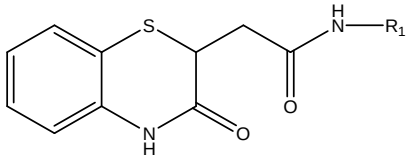
Statistics

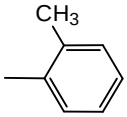
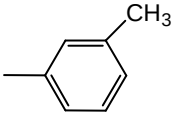
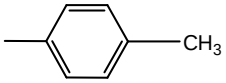
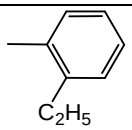
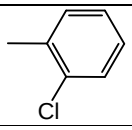
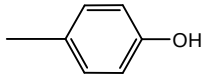
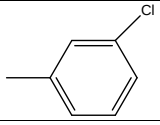
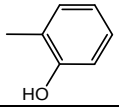
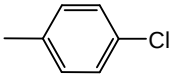
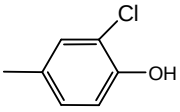
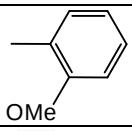
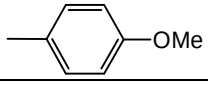
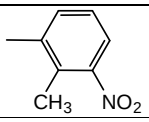
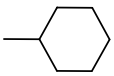
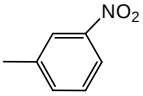
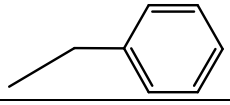
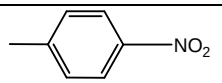
n = 18, Degree of freedom = 13, r^2 = 0.9172, q^2 = 0.8223, F test = 36.0169, r^2 se = 0.0327, q^2 se = 0.0479, pred_r^2 = 0.5960, pred_r^2se = 0.4931

The descriptors that get selected in a given model are the field points of steric nature at particular locations in a common grid around reported set of molecules. The model selection criterion is the value of q^2, the internal predictive ability of the model, and that of pred_r^2, the ability of the model to predict the activity of external test set. As the cross-validated correlation coefficient (q^2) is used to measure the reliability of prediction, the correlation coefficient suggests that our model is reliable and accurate. The descriptors S_567, S_690, S_205 and S_667 are the steric field energy of interactions between probe (CH3) and compounds at their corresponding spatial grid points 567, 690, 205 and 667. Only the steric field is contributed, the contribution chart of selected descriptors were represented in Fig. 5. It is evident that the predicted activities of all the compounds in the test set are in good agreement with their corresponding experimental activities and optimal fit is obtained (Fig no. 4). The contribution plot of steric field interactions indicates relative regions of the local fields (steric)

around the aligned molecules, leading to activity variation in the model. The green-colored balls (fig no.6) specify the positions of the steric descriptors and the descriptors with positive or negative coefficients show a region where bulky substituent is favored or disfavored, respectively. From 3D-QSAR model it is observed that steric descriptors like S_690, S_205 and S_667 with positive coefficient signifying positive range of steric descriptors indicate that positive steric potential is favorable for activity and bulky substituent is favorable in that region. The robustness of the QSAR model for experimental training sets was examined by comparing this model to those derived for sphere exclusion dataset.

Table 4: Structures of 1, 4-Benzothiazine derivatives used for QSAR analysis.



Compounds	R_1	Compounds	R_1
BTA-4	$-CH_3$	BTA-57	
BTA-8	$-C(CH_3)_3$	BTA-58	
BTA-5	$-CH_2CH_3$	BTA-56	
BTA-47	$-CH_2CH_2CH_2CH_3$	BTA-65	
BTA-20	$-CH_2CH_2OH$	BTA-59	
BTA-43		BTA-60	
BTA-64		BTA-61	
BTA-35		BTA-67	
BTA-25	$-CH(CH_3)CH_2OH$	BTA-68	
BTA-66		BTA-24	
BTA-69		BTA-70	
BTA-63			

The correlation matrix between the physico-chemical parameters and the biological activity is presented in Table 5 which shows good correlation of selected parameters with biological activity. The above model is validated by predicting the biological activities of the test molecules, as indicated in Table 6.

Table 5: Correlation matrix between physico-chemical descriptors present in 3D-QSAR model I

	S_205	S_567	S_609	S_667	5:Score
S_205	1.000000	0.269919	-0.124835	0.137081	4
S_567	0.269919	1.000000	0.013542	0.416815	4
S-609	-0.124835	0.013542	1.000000	-0.023751	4
S_667	0.137081	0.416815	-0.023751	1.000000	4

Table 6: Observed and predicted activity by 3DQSAR equations along with the residuals

Compound	For model I (3DQSAR) *C. albicans*			For model II (3DQSAR) *E. Floccusom*		
	observed	predicted	residuals	observed	predicted	residuals
BTA20	0.8192	0.816	0.0032	0.8192	0.8077	0.0115
BTA24	0.782	0.7753	0.0067	0.782	0.7596	0.0224
BTA25	0.6543	0.6283	0.026	0.5467	0.5393	0.0074
BTA35	0.6103	0.5862	0.0241	0.6103	0.6718	-0.0615
BTA43	1.009	0.7101	0.2989	0.7735	0.692	0.0815
BTA47	0.8065	0.6171	0.1894	0.8065	0.8071	-0.0006
BTA4	0.5647	0.5529	0.0118	0.6804	0.6876	-0.0072
BTA56	0.775	0.7738	0.0012	0.7752	0.773	0.0022
BTA57	0.5285	0.5752	-0.0467	0.5285	0.5841	-0.0556
BTA58	0.5285	0.5226	0.0059	0.5285	0.5442	-0.0157
BTA59	0.618	0.5595	0.0585	0.618	0.6058	0.0122
BTA5	0.669	0.669	0	0.669	0.7359	-0.0669
BTA60	0.5211	0.5541	-0.033	0.5211	0.5151	0.006
BTA61	0.5211	0.532	-0.0109	0.7593	0.7613	-0.002
BTA63	0.5173	0.4867	0.0306	0.6127	0.5628	0.0499
BTA64	0.5277	0.5391	-0.0114	0.5277	0.5785	-0.0508
BTA65	0.5232	0.5194	0.0038	0.5232	0.5241	-0.0009
BTA66	0.5127	0.5882	-0.0755	0.5127	0.543	-0.0303
BTA67	0.5225	0.5882	-0.0657	0.5225	0.5188	0.0037
BTA68	0.62	0.6323	-0.0123	0.5225	0.543	-0.0205
BTA69	0.6127	0.4924	0.1203	0.6127	0.6507	-0.038
BTA70	0.6285	0.6206	0.0079	0.6285	0.5748	0.0537
BTA8	0.6489	0.7096	-0.0607	0.6489	0.5911	0.0578

Table 6: (*continued*)

Compound	For model III (3DQSAR) *T. rubrum*			For model IV (3DQSAR) *M. furfur*		
	observed	predicted	residuals	observed	predicted	residuals
BTA20	0.6571	0.6618	-0.0047	0.6571	0.6271	0.03
BTA24	0.782	0.7826	-0.0006	0.782	0.7629	0.0191
BTA25	0.8148	0.835	-0.0202	0.6543	0.6823	-0.028
BTA35	0.6103	0.5785	0.0318	0.7477	0.7124	0.0353
BTA43	0.6274	0.6183	0.0091	0.6274	0.6038	0.0236
BTA47	0.8065	0.689	0.1175	0.6489	0.7	-0.0511
BTA4	0.5647	0.6219	-0.0572	0.6804	0.6834	-0.003
BTA56	1.0118	0.9749	0.0369	0.0118	0.0118	0
BTA57	0.6285	0.6046	0.0239	0.5285	0.5132	0.0153
BTA58	0.6285	0.6007	0.0278	0.6285	0.554	0.0745
BTA59	0.5211	0.5674	-0.0463	0.5211	0.555	-0.0339
BTA5	0.669	0.627	0.042	0.669	0.7173	-0.0483
BTA60	0.618	0.5641	0.0539	0.618	0.6087	0.0093
BTA61	0.5211	0.5191	0.002	0.5211	0.5597	-0.0386
BTA63	0.5173	0.4856	0.0317	0.5173	0.5273	-0.01
BTA64	0.5277	0.5105	0.0172	0.5232	0.5343	-0.0111
BTA65	0.5232	0.5423	-0.0191	0.5232	0.5343	-0.0111
BTA66	0.6062	0.6007	0.0055	0.5127	0.5783	-0.0656
BTA67	0.62	0.6007	0.0193	0.62	0.6359	-0.0159
BTA68	0.62	0.6247	-0.0047	0.62	0.5783	0.0417
BTA69	0.6127	0.6372	-0.0245	0.5173	0.6746	-0.1573
BTA70	0.5285	0.5605	-0.032	0.5285	0.5491	-0.0206
BTA8	0.6489	0.7094	-0.0605	0.5429	0.6695	-0.1266

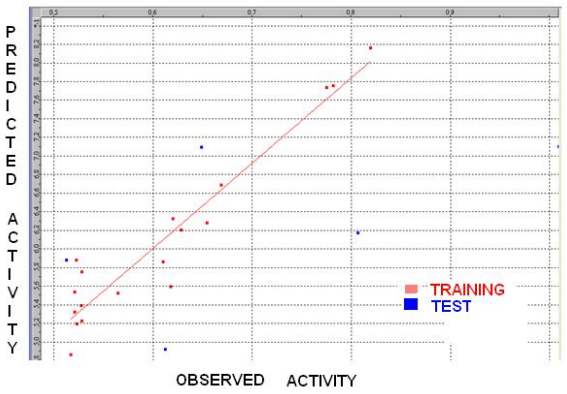


Fig. 3: Fitness plot between observed activity Vs predicated activity for model I

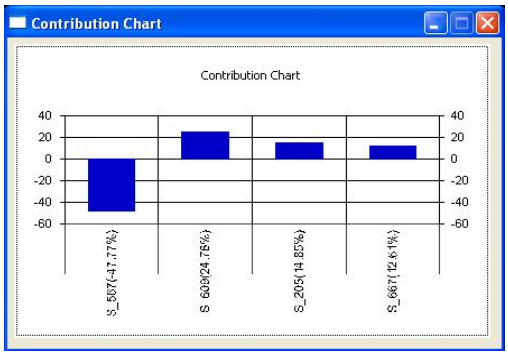


Fig. 4: Contribution charts of the descriptors for model I

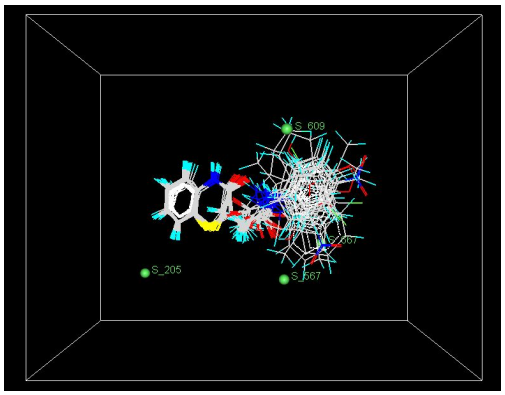


Fig. 5: 3D view of aligned molecule with contribution of descriptors (model I)

3.2 Model II-3D QSAR (*E. floccosum*)

pMIC = -0.3738(±0.0014)E_689 + 0.2266(±0.0010)S_599 + 0.3842(±0.0028)E_690 + 3.8137(±0.8468)S_392 + 1.8653

Statistics

n = 18, Degree of freedom = 13, r^2 = 0.9169, q^2 = 0.7577, F test = 35.8477, r^2 se = 0.0379, q^2 se = 0.0648, pred_r^2 = 0.5861, pred_r^2se = 0.4475

Model II are resulted from the antifungal activity against *E. floccosum*. Model II with coefficient of determination r^2= 0.9169 which is capable of explaining 91.69% of variance in the observed activity values. As the cross-validated correlation coefficient (q^2) is used as a measure of reliability of prediction, the correlation coefficient suggests that our model is reliable and accurate. S_599 and S_392 are steric descriptors contribute to model II (Fig.8). A data set of compounds containing five molecules was selected as the test set from the original data of 23 compounds for the validation experiments. The q^2 value obtained (0.7577) was a significant value of current MLR model. Predictive power of the model is 58.61% (external validation). The correlation matrix between the physico-chemical parameters and the biological activity is presented in Table 7. Forward method of variable selection indicating its crucial role in predicting antifungal activtity and signifying positive range of steric descriptors indicate that positive steric potential is favorable for activity and bulky substituent is preferred in that region. The steric effect, as shown with green color (Fig.9) around the phenyl ring at para position, implies about the preferred bulky group substitution to produce higher antifungal activity. Electrostatic descriptor with positive coefficient (E_690) around para position of the phenyl ring corroborates that electropositive (electron-withdrawing) group is preferred at 4-position of phenyl ring. The good correlation find between plot observed Vs predicted activity show in fig.7.The above model is validated by predicting the biological activities of the test molecules, as indicated in Table 6.

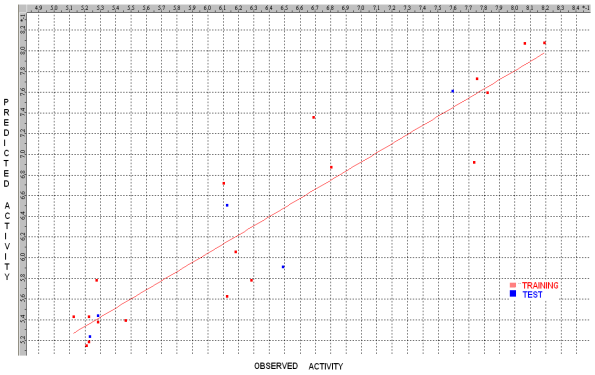


Fig. 7: Fitness plot between observed activity Vs predicated activity for model II

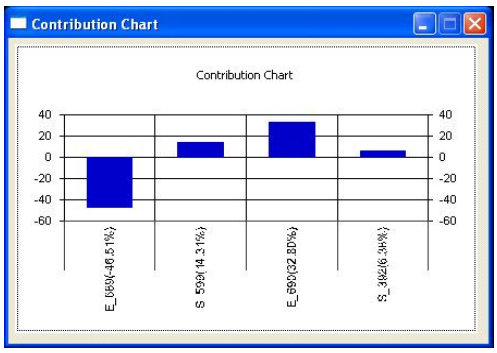


Fig. 8: Contribution charts of the descriptors for model II

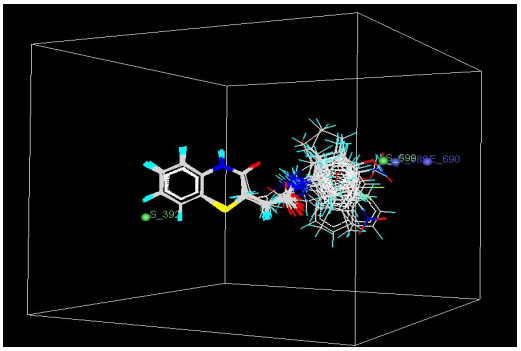


Fig. 9: 3D view of aligned molecule with contribution of descriptors (model II)

Table 7: Correlation matrix between physico-chemical descriptors present in 3D-QSAR model IIF

	E_689	E_690	S_392	S_559	5:Score
E_689	1.000000	0.963702	-0.201895	-0.378061	4
E_690	0.963702	1.000000	-0.250761	-0.332572	4
S_392	-0.201895	-0.250761	1.000000	-0.154724	4
S_559	-0.378061	-0.332572	-0.154724	1.000000	4

3.3 Model III-3D QSAR (*T. rubrum*)
pMIC = 0.4025(±0.0000)S_857 - 183.6630(±1.9221)S_462 - 916.5890(±57.4123)S_71 + 0.0659(±0.0000)E_316 - 9.8156

Statistics
n = 18, Degree of freedom = 13, r^2 = 0.9652, q^2 = 0.9129, F test = 90.2117, r^2 se = 0.0271, q^2 se = 0.0429, pred_r^2 = 0.5211, pred_r^2se = 0.4045

The descriptors that get selected in a given model are the field points of steric nature at particular locations in a common grid around reported set of molecules. According to this model pMIC is a function of independent variables and dependent variable are steric fields. The steric descriptor S_857 is positive are indicate that the bulky substituted is favorable at para position and S_462 is negative contribute to model are indicate that the substitution at ortho position is disfavorable for the activity. This model also indicates statistical significance > 99.9% with F values F= 90.2117. Cross-validated squared correlation coefficient of this model is q^2=0.9129, which shows good internal prediction power of this model with good external predictivity are 52 %. The contribution of electrostatic fields indicates that electric field is also important factor for the activity, contribution chart of selected descriptors are represented in Fig. no. 11. The correlation matrix between the physico-chemical parameters and the biological activity is presented in Table 8. The predicted activities of all the compounds in the test set are in good agreement with their corresponding experimental activities and fitness plot is obtained shown in fig.10. The 3D view of contributed descriptor around the molecule is given fig. 12.

Table 8: Correlation matrix between physico-chemical descriptors present in 3D-QSAR model III

	E_316	S_71	S_462	S_857	5:Score
E_316	1.000000	-0.032970	0.191469	-0.475944	4
S_71	-0.032970	1.000000	-0.804737	0.359050	4
S_462	0.191469	-0.804737	1.000000	-0.162765	4
S_857	-0.475944	0.359050	-0.162765	1.000000	4

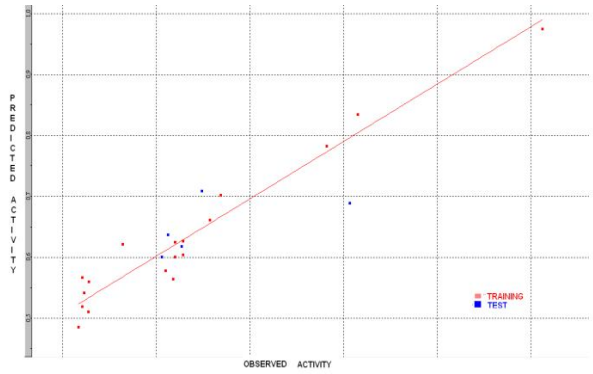


Fig. 10: Fitness plot between observed activity Vs predicated activity for model III

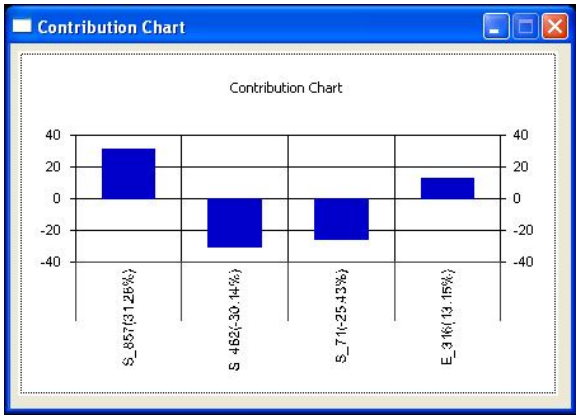


Fig. 11: Contribution charts of the descriptors for model III

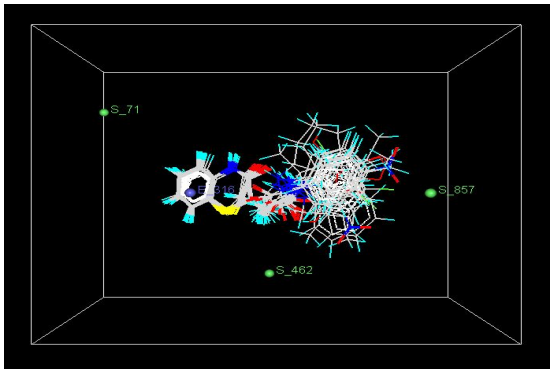


Fig. 12: 3D view of aligned molecule with contribution of descriptors (model III)

3.4 Model IV-3D QSAR (*M. furfur*)
pMIC = 0.3094(±0.0000)E_396 + 202.9650(±43.4077)S_94 - 0.0676(±0.0000)E_658 + 0.0444(±0.0000)E_476 + 3.0663

Statistics
n = 18, Degree of freedom = 13, r^2 = 0.9621, q^2 = 0.893, F test = 82.5554, r^2 se = 0.0362, q^2 se = 0.1775, pred_r^2 = 0.9286, pred_r^2se = 0.06099

In addition, this QSAR study allowed investigating influence of very simple and easy-to-compute descriptors in determining biological activities, which could shed light on the key factors that may aid in design of novel potent molecules. The model IV explains 96.21% variance in the observed activity values, r^2 is 0.9621, cross-validated correlation coefficient (q^2) of 0.893, (internal predectivity), F test of 82.5554 indicate overall 99.99% significance, r^2 for external test set (pred_r^2) 0.9286, coefficient of correlation of predicted data set (pred_r^2se) 0.06099. The presence of descriptor E_396 plays an important role in being directly proportional to the biological activity and highest contributed among the descriptor and E_658 is the electrostatic descriptor are positive coefficient indicate requirement of electropositive group that leads to better antifungal agent. The plots of observed vs. predicted values of pMIC and contribution chart are shown in Fig. no. 13 and 14 respectively. The predicted (LOO) activities of the compounds by the above model are shown in Table 6. The correlation matrix between the physico-chemical parameters and the biological activity is presented in Table 9.

Table 9: Correlation matrix between physico-chemical descriptors present in 3D-QSAR model IV

	E_396	E_476	E_658	S_94	5:Score
E_396	1.000000	-0.116941	0.028376	0.108910	4
E_476	-0.116941	1.000000	0.202878	0.298401	4
E_658	0.028376	0.202878	1.000000	0.002876	4
S_94	0.108910	0.298401	0.002876	1.000000	4

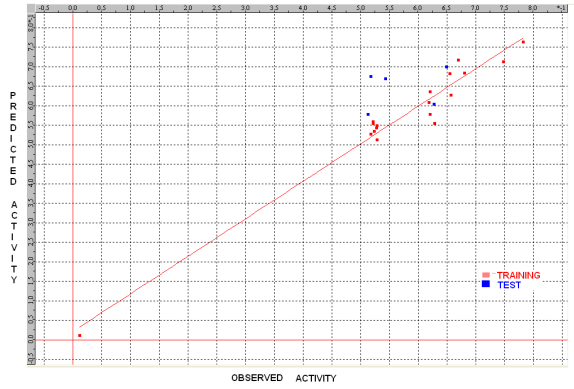


Fig. 13: Fitness plot between observed activity Vs predicated activity of training and test set compound for model IV

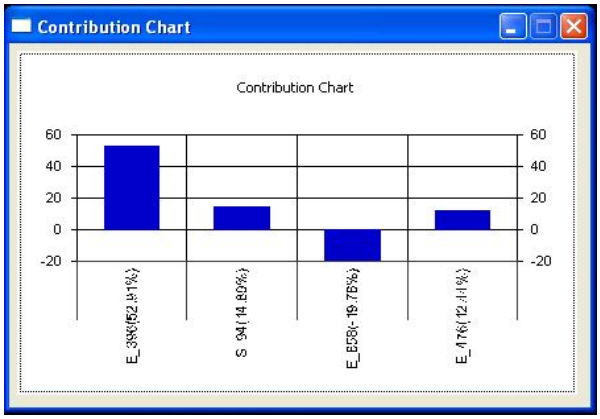


Fig. 14: Contribution charts of the descriptors for model IV

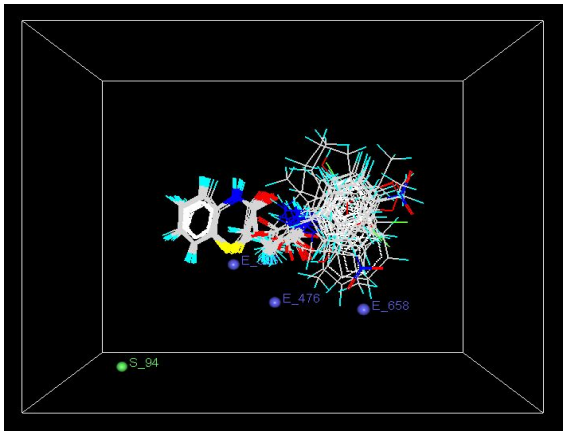


Fig. 15: 3D view of aligned molecule with contribution of descriptors (model IV)

4. CONCLUSION

3D QSAR models were statistically significant, thus from 3DQSAR investigations it could be concluded that steric properties of 1,4-Benzothiazine derivatives contribute significantly to the antifungal activity and need of bulky substitution at para or meta position of phenyl ring. The generated models were analyzed and validated for their statistical significance and external prediction power.

5. ACKNOWLEDGEMENT

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