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Rational Design of Antifungal 1,2,4-triazole derivatives by 2D-QSAR Study

Rajesh D. Hunashal , Mahesh B. Palkar

***Abstract*- 2D-QSAR studies were performed on a set of 35 analogs of 1,2,4-triazole using V-Life Molecular Design Suite (MDS 3.5) QSAR plus module by using Multiple Linear Regression (MLR) and Partial Least Square (PLS) regression methods against fungal strain *Aspergillus niger* (ATCC 6275). MLR and PLS have shown a very promising antifungal activity prediction results against *A.niger*. QSAR models were (MLR and PLS) generated by a training set of 25 molecules with correlation coefficient (r^2) of 0.7632, 0.7666, and F test of 16.1183, 22.9938 respectively. In the selected descriptors, alignment independent descriptors such as T_N_Cl_5, T_N_O_4, T_C_O_1, T_O_O_3 and G_C_O_1 were the most important descriptors in predicting antifungal activity.**

***Index Terms*— Antifungal activity; 1,2,4-Triazole; 2D QSAR; Multiple Linear Regression (MLR); Partial Least Square (PLS) Regression;.**

I. INTRODUCTION

During the past two decades, the fungal infection complication have been recognised as a major cause of morbidity and mortality in immunocompromised patients including those suffering from tuberculosis, infected with HIV-1, organ-transplant patients, diabetic patients and those undergoing cancer chemotherapy. Increased incidence of fungal infections also follows the frequent use of antibacterial and cytotoxic drugs [1]. Some antifungal drugs are either highly toxic (e.g., amphotericin B, AMB) or increasingly ineffective due to appearance of resistant strains, limited spectrum of activity, tissue distribution, central nervous system (CNS) penetration, or high cost [2].

In fact, azole resistance is a major concern in long-course treatment of AIDS patients. The causes of resistance are generally associated with mutations in lanosterol 14α-demethylase that reduce azole binding and decreased intracellular drug accumulation due to increased expression of efflux pump genes. Moreover, long-term treatments may also cause hepatotoxicity, as azole derivatives can also interact to P450 enzymes from mammalian cytochromes [3]. Prompted by these observations, we incorporate azole nucleus with various heterocyclic moiety.

In view of above fact mortality from fungal infections is still unacceptably high. Thus the development of new and effective antifungal agents against life-threatening systemic mycoses is an urgent need. Thus in order to improve antifungal potency and selectivity, efforts has been made to our reported synthesized new classes of antifungal agents or modify the structures of so far effective azole molecule [4-6]. Indeed, several two or three -dimensional quantitative structure-activity relationship studies (2D or 3D QSAR) have been reported for different datasets of azole derivatives [7-12]. The purpose of using QSAR-Descriptors is to calculate the properties of molecules that serve as numerical descriptions or characterizations of molecules in other calculations such as diversity analysis or combinatorial library design. Using such an approach one could predict the activities of newly designed compounds before a decision is being made whether these compounds should be really synthesized and tested. One could not, however, confirm that the compounds we synthesised would always possess good inhibitory activity to fungal organism, even as experimental assessments of inhibitory activity of these compounds are time-consuming and expensive. Consequently, it is of interest to develop a prediction method for biological activities before the synthesis.

The aim of this work was to develop a predictive QSAR model [13,14], which will applicable to diverse sets of molecules and would aid in search for the novel fungal inhibitors from a diverse chemical space.

II. COMPUTATIONAL METHODS

Chemical Data

A series of 35 molecules belonging to 1,2,4-triazole derivatives as *Aspergillus niger* (ATCC 6275), inhibitors were taken from the literature and used [4-6]. The 2D-QSAR models were generated using a training set of 25 molecules. The observed and predicted biological activities of the training and test set molecules are presented in Table 1. Predictive power of the resulting models was evaluated by a test set of 10 molecules with uniformly distributed biological activities. The observed selection of test set molecules was made by considering the fact that test set molecules represents a range of biological activity similar to the training set.

Data Set

All computational work was performed on Apple workstation (8-core processor) using Vlife MDS QSAR plus software developed by Vlife Sciences Technologies Pvt Ltd, Pune, India, on windows XP operating system . All the compounds were drawn in Chem DBS using fragment database and then subjected to energy minimization using batch energy minimization method. Conformational search were carried out by systemic conformational search method.

Biological Activities

The negative logarithm of the measured $PMIC_{50}$ (μM) against *Aspergillus niger*, as $PMIC_{50}$ [$PMIC_{50}$ = –log ($PMIC_{50}$ X

Dr. Rajesh D. Hunashal, Department of Pharmacology, Karnataka Institute of Medical Sciences, Vidyanagar, Hubballi-580021, Karnataka, India

Dr. Mahesh B. Palkar, Department of Pharmaceutical Chemistry, K.L.E.U. College of Pharmacy, Vidyanagar, Hubballi-580031, Karnataka. India.

10^{-6})] was used as dependent variable, thus correlating the data linear to the free energy change. Since some compounds exhibited insignificant/no inhibition, such compounds were excluded from the present study. The zone of inhibition and minimum inhibitory concentration (MIC) values were obtained by agar-dilution method against *Aspergillus niger* using Muller-Hinton agar (MHA) medium [15-17]. The $PMIC_{50}$ values of reference compounds were checked to ensure that no difference occurred between different groups. The $pMIC_{50}$ values of the molecules under study spanned a wide range from 2 to 7.

Molecular Descriptors

Various 2D descriptors (a total of 208) like element counts, molecular weight, molecular refractivity, log *P*, topological index, Baumann alignment independent topological descriptors *etc*., were calculated using VlifeMDS software**.** The preprocessing of the independent variables (i.e., descriptors) was done by removing invariable (constant column) and cross-correlated descriptors (with $r^2 = 0.7632$) which resulted in total 156 and 162 descriptors for MLR and PLS respectively to be used for QSAR analysis.

Selection of Training and Test Set

The dataset of 35 molecules was divided into training and test set by Sphere Exclusion (SE) method for MLR, PCR and PLS model with $pMIC_{50}$ activity field as dependent variable and various 2D descriptors calculated for the molecules as independent variables.

III. RESULT AND DISCUSSION

Training set of 28 and 11 of test set of 1,2,4-triazole having different substitution, were employed. Following statistical measure was used to correlate biological activity and molecular descriptors; n, number of molecules; k, number of descriptors in a model; df ,degree of freedom; r^2 ,coefficient of determination; q^2 , cross validated r^2; pred_r^2 , r^2 for external test set; pred_r^2se , coefficient of correlation of predicted data set;

Multiple Linear Regression (MLR) Analysis

After 2D QSAR study by Multiple Linear Regression method using forward-backward stepwise variable selection method, the final QSAR equation was developed having 4 variables as follows.

pMIC = -2.1379 (T_N_Cl_5)–0.08772 (T_N_O_4)-0.4580 (T_C_O_1) + 1.9135 (T_O_O_3)

Model 1 (MLR) has a correlation coefficient (r^2) of 0.7632, significant cross validated correlation coefficient (q^2) of 0.4434, F test of 16.1183, r^2 se of 0.4733, q^2 se of 0.7256 and degree of freedom (df) 20. The randomization test suggests that the developed model have a probability of less than 1% that the model is generated by chance. The equation of MLR model explains 76% ($r^2 = 0.76$) of the total variants in the training set as well as it has internal (q^2) and external (pred_r^2) predictive ability of 44% and 20% respectively. The observed and predicted $pMIC_{50}$ along with residual values are shown in Table 1**.** Statistical data is shown in Table 2**.** The plot of observed vs. Predicted activity is shown in Figure 1**.** The descriptors which contribute for the pharmacological action are shown in Figure 2.

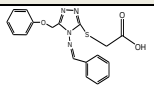
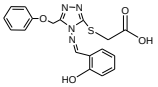
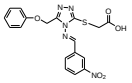
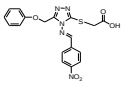
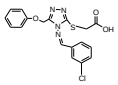
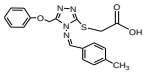
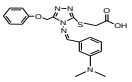
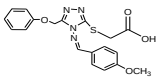
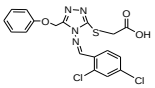
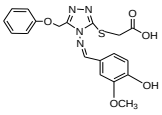
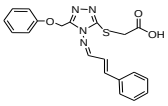
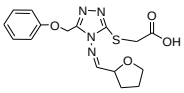
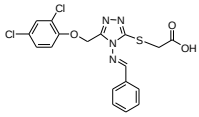
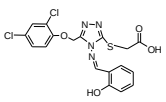
Partial Least Squares (PLS) Analysis

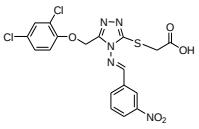
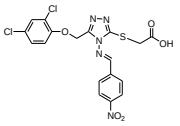
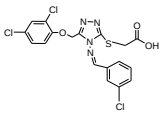
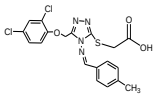
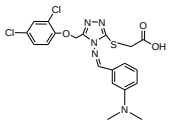
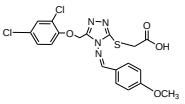
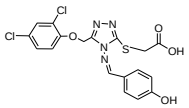
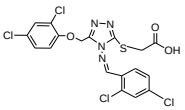
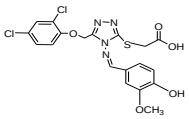
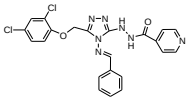
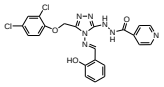
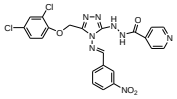
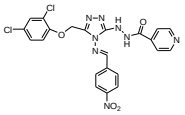
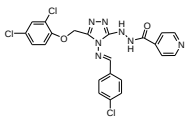
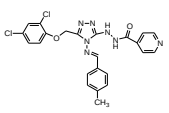
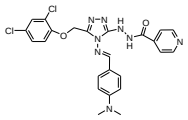
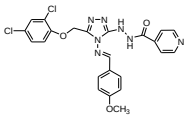
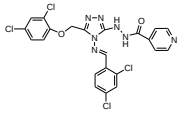
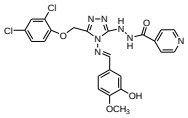
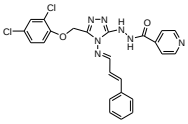
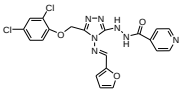
PLS Analysis is having following QSAR equation with 4 variables.

pMIC = –2.1835 (T_N_Cl_5)–0.8437 (T_N_O_4)–0.3385 (G_C_O_1)+1.7695 (T_O_O_3)

Model 2 (PLS) The PLS Analysis gave correlation coefficient (r^2) of 0.7666, significant cross validated correlation coefficient (q2) of 0.4332, F test of 22.9938 and degree of freedom 21. The randomization test suggests that the developed model have a probability of less than 1% that the model is generated by chance. Statistical data is shown in Table 2. The plot of observed vs. predicted activity is shown in Figure 3**.**The descriptors which contribute for the pharmacological action are shown in Figure 4.

Table 1: Structure, Experimental and Predicted Activity of 1,2,4-Triazoles Used in Training and Test Set Using MLR.

Code	Compound	MIC (µg/mL)	pMIC		Residual
			Exp.	Pred.	
1		4	6.602	7.724	-1.122
2[T]		8	6.903	5.473	1.430
3		16	7.204	6.808	-0.396
4		8	6.903	6.389	0.514
5		0.50	5.698	6.808	-1.110
6[T]		2	6.301	7.267	-0.966
7[T]		16	7.204	5.015	2.189
8		2	6.301	6.808	-0.507
9[T]		0.50	5.698	7.266	-1.568
10[T]		64	7.806	6.808	0.998
11		16	7.204	6.808	0.396
12[T]		32	7.505	8.264	-0.759
13		4	6.602	6.808	-0.206
14		1	7.000	7.724	-0.724

15		16	7.204	6.808	0.396
16		8	6.903	7.266	-0.363
17		0.25	5.397	5.586	-0.189
18		2	6.301	7.266	-0.965
19		16	7.204	5.931	1.273
20 T		2	6.301	5.586	0.715
21		1	7.000	7.266	-0.266
22		0.25	5.397	7.266	-1.869
23 T		1	7.000	6.808	0.808
24		16	7.204	7.266	062
25		0.25	5.397	6.350	-0.953
26		16	7.204	5.128	2.076
27 T		4	6.602	7.724	-1.122
28		128	8.107	7.806	0.301
29 T		0.25	5.397	8.264	-2.867
30		64	7.806	7.266	0.540
31		0.25	5.397	5.473	-0.076
32		0.25	5.397	6.808	-1.411
33		64	7.806	6.808	0.998
34		64	7.806	5.586	2.220
35		0.25	5.397	5.892	-0.495
	Fluconazole	1	7.000		

Expt. = Experimental activity, Pred. = Predicted activity; a = Compound concentration in micro mole required to inhibit growth by 50%; b = –Log ($PMIC_{50}$ X 10^{-6}): Training data set developed using MLR; T = Test Set, Test organism= *Aspergillus niger* (ATCC 6275).

Table 2: Statistical parameters of MLR and PLS

Parameters	MLR	PLS
n	25	25
df	20	21
r^2	0.7632	0.7666
q^2	0.4434	0.4332
F test	16.1183	22.9938
r^2 se	0.4733	0.4585
q^2 se	0.7256	0.7146
pred_r^2	1.8003	1.6766
pred_r^2se	1.2818	1.2531

MLR = Multiple Linear Regression, PLS = Partial Least Squares, n = number of molecules of training set, Df =degree of freedom, r2 = coefficient of determination, q2 = cross validated r2, pred_r2 = r2 for external test set, pred_r2se = coefficient of correlation of predicted data set.

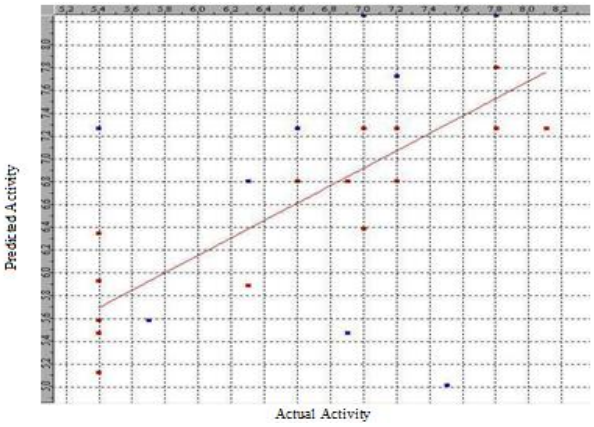


Fig.1 Graph of Actual vs. Predicted activities for training and test set molecules from the Multiple Linear Regression model. (A) Training set (Red dots) (B) Test Set (Blue dots).

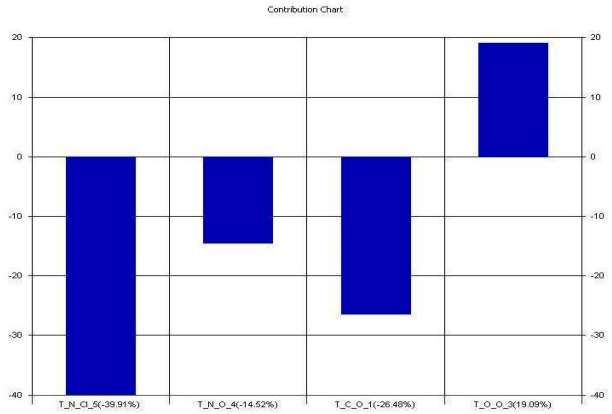


Fig. 2: Plot of percentage contribution of each descriptor in developed MLR model explaining variation in the activity.

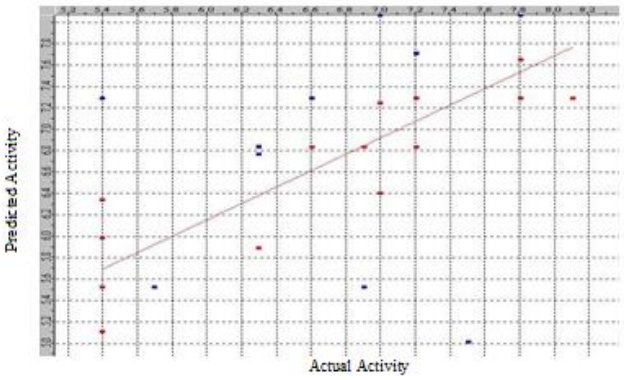


Fig. 3: Graph of Actual vs. Predicted activities for training and test set molecules by Partial Least Square model. (A) Training set (Red dots) (B) Test Set (Blue dots).

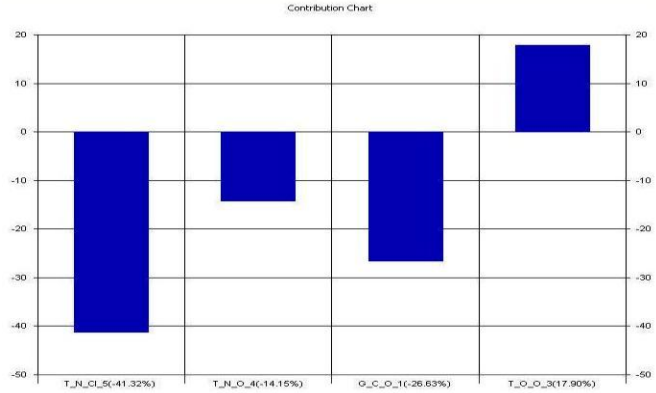


Fig. 4: Plot of percentage contribution of each descriptor in developed PLS model explaining variation in the activity.

IV. Conclusion

In conclusion, the model developed to predict the structural features of 1,2,4-triazole derivatives to display antifungal activity against *Aspergillus niger*, reveals useful information about the structural features requirement for the molecule. In this QSAR analysis, Partial Least Squares (PLS) method is giving very significant results. The results revealed that the alignment independent (AI) descriptors have greatly contributed for the variation in the biological activity of compounds. The results obtained from QSAR study consider not only wide range of structures, but also various physico-chemical interactions involved in enzyme inhibitor complex. The present study is more versatile than the earlier reported methods. The QSAR results obtained are in agreement with the observed SAR of 1,2,4-triazole derivatives studied. Hence the model proposed in this work is useful and can be employed to design novel 1,2,4-triazole derivatives as promising anti-fungal agents.

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