

Design, synthesis and evaluation of 1, 2, 4-triazole derivatives as antifungal.***P. B. Choudhari, R. P Dhavale, S. D. Jadhav, D. V. Mahuli, M. S. Bhatia.**

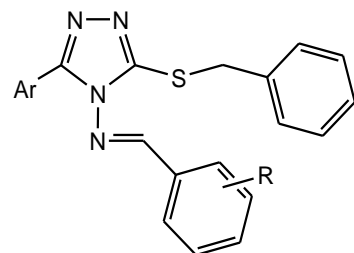
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Abstract

A series of 1, 2, 4-triazoles was designed, synthesized and screened for antifungal activity against strain of *Aspergillus niger*. The design of the triazole compounds was based on docking studies performed on *Lanosterol 14 α -demethylase* an important enzyme required for the synthesis of ergosterol. The three-dimensional QSAR studies of 1, 2, 4-triazole for analysis of the structural requirements for antifungal activity using Vlife MDS 3.5 has been carried out. The negative logarithm of activity (MICs) of the compounds against resistant *Aspergillus niger* exhibited a strong correlation with the selected 3D molecular descriptors of the triazole analogues. The present findings suggest that the triazole framework is an attractive template for optimization of targeted antifungal activity to achieve better potency and a wider spectrum of activity.

Key Words*Aspergillus niger*, 3D QSAR, antifungal.**Introduction**

There has been an increase in the numbers of immuno compromised hosts with pan-epidemic fungal infections. Various enzymes are involved in the fungal cell growth which could be possible targets for effective treatment of fungal infections. New agents with high specificity for such targets could produce selective antifungal agents. Employing triazole nucleus which is an attractive basic core which is required for various activities like anticancer, antimicrobial, and anti-inflammatory¹⁻⁷. We have attempted to characterize the electronic and steric properties of some triazole derivatives that influence its antifungal activity. Thus, we herein report the design, synthesis, and evaluation of 1, 2, 4 triazole derivatives as a novel antifungal agents.

**Material and methods**

The molecules which are utilized in present study are synthesized using the procedure from our previous published work^{8,9}.

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Biological Activity:

The synthesized derivatives were screened for activity against *Aspergillus niger* (NCIM-945), The cup plate agar diffusion method was used for activity; MIC was calculated using serial dilution method. The tested compounds were dissolved in distilled water to get a solution of 1000, 500, 250, 125, 62, 31.5 mg/ml. Distilled water was used as control. Commercial fluconazole (100mg/ml) was also tested under similar conditions for comparison. The synthesized compounds and the standard were then screened for activity. Standard was used in concentration of 100mg/ml.

Ligand Preparation

The structure of substituted 1, 2, 4- triazole derivatives was used as the template to built the molecules in the dataset in Vlife MDS 3.5. The ligand geometries were optimized by energy minimization using MMFF94 forcefield and Gasteiger-Marsili charges for the atoms, till a gradient of 0.001 kcal/mol/Å^o was reached, maintaining the template structure rigid during the minimization.

Molecular alignment

The molecules of the dataset were aligned by the atom-fit technique, using atoms common with the structure of 1, 2, 4- triazole derivatives. The most active molecule was selected as a template for

alignment of the molecules. The alignment of all the molecules on the template is shown in figure.no.1.

Descriptor Calculation

Like many 3D QSAR methods, a suitable alignment of given set of molecules was performed using the Vlife MDS 3.5 Engine. This was followed by generation of a common rectangular grid around the molecules. The hydrophilic, steric and electrostatic interaction energies are computed at the lattice points of the grid using a methyl probe of charge +1. These interaction energy values are considered for relationship generation and utilized as descriptors to decide nearness between molecules. The term descriptor is utilized in the following discussion to indicate field values at the lattice points.

Data Set

The dataset was divided into a training set (30 molecules) and a test set (6 molecules) on the basis of chemical and biological diversity using the random selection method for generation of the training and test set data, pMIC was used for the present QSAR study.

Results and Discussion

In the present study, 30 molecules were used in the training set to derive QSAR models with the number of field grid points being not more than seven per model. To evaluate the predictive ability of generated 3D-QSAR models, a test set of six molecules with regularly distributed biological activities was used. A prerequisite for QSAR study is a congeneric series of molecules, all having the same mechanistic profile with similar functional properties.

Interpretation of 3D QSAR Model A

The model A describes the structural features optimum for the antifungal activity. The steric and electrostatic fields were calculated using the MMFF94 force field and Gasteiger-Marsili charges. A training set of 30 molecules, and a test set of 6 molecules were used as described earlier. The model was selected on basis of r^2 , q^2 , $\text{pred } r^2$, F and values. The r^2 value for model A was 0.9011. The F test and p significance values were considered for the selection of model. The lattice points that were found optimum for the antifungal activity after the QSAR study are shown in figure 2. The contribution of lattice points S_259 E_239 S_222 S_1120 E_546 which are the electronic and steric interaction fields (blue and green lattice points respectively) at lattice

points 259, 239, 222, 1120, 546 imply that these lattice points are indeed significant for the structure-activity relationship. The positive contribution of the fields E_239 indicates that the addition of group having electrostatic interaction at lattice point 239 (Blue lattice point in fig. 2) is required for amplified antifungal activity and negative contribution to the activity of steric interactions at fields S_259, S_222, S_1120 (Green lattice point in fig. 2) are need to be reduced.

Interpretation of 3D QSAR Model B

The r^2 value for model B was 0.8914. The F test and p significance values were considered for the selection of model. The lattice points that were found optimum for the antifungal activity after the QSAR study are shown in figure 3. The contribution of lattice points E_518 E_545 S_249 S_681 H_546 which are the electronic, steric and hydrophobic interaction fields (Blue, Green and yellow lattice points respectively) at lattice points 518, 545, 249, 681, 546 imply that these lattice points are indeed significant for the structure-activity relationship. The positive contribution of the fields E_518 and S_681 indicates that the addition of groups having electrostatic interaction at lattice point 518 and steric interaction at lattice point 681 (Blue lattice point and Green lattice point in fig. 3) are required for amplified activity and groups having steric, electrostatic and hydrophobic interaction at lattice points 545, 249, 546 (Green and Blue lattice points in figure 3) which contribute negatively to the activity also need to be taken into account and need to be reduced.

Interpretation of 3D QSAR Model C

In model C the correlation coefficient (r^2) was found to be 0.8711. The lattice points that were found optimum for the activity after the QSAR study are shown in figure 4. The contribution of lattice points S_259 S_1031 E_546 S_465 S_1120 which are the electronic and steric interaction fields (blue and green points respectively) at lattice points 259, 1031, 546, 465, 1120 imply that these lattice points are indeed significant for the structure-activity relationship. The positive contribution of the fields S_1031, S_465 indicates that the addition of groups having steric interaction at lattice point 1031, 465 (Green lattice point in fig. 4) are required for amplified antifungal activity. Groups having steric and electrostatic interaction at lattice points 259, 1120, 546 (Green and Blue lattice points in fig. 4)

which contribute negatively to the activity also need to be taken into account and need to be reduced.

Conclusion

All the synthesized 1, 2, 4 - triazole molecules were found to be active against fungi. These molecules have been used to build and test the 3D-QSAR which can be used to predict the antifungal potential of other such molecules. Amongst the three 3D-QSAR models presented the model A is best not only in terms of the correlation coefficients (r^2 and predicted r^2) but it also has very few outliers. We suggest that this model could further enable rapid discovery of modulators of this enzyme for therapeutic use. Activities of most of the molecules obtained through prior screening employing docking analysis produced good antifungal activity and these results suggest a potential mode of action mediated by *Lanosterol 14 α -demethylase* inhibition. Therefore the selected target enzyme is appropriate target for design of novel inhibitors that could lead to development of effective antifungal agents.

Acknowledgement

The authors are thankful to Dr. H. N. More, Principal, Bharati Vidyapeeth College of Pharmacy, Kolhapur for providing facilities to carry out the work.

References

1. Ravi TK, Rajkannan R. Indian journal of pharmaceutical sciences (2004) 66(3): 347- 48.
2. Amir M, Azam F, IJPS (2004) 66(3): 818-21.
3. Amir M, Oberoi A, Alam S. Indian J Chem. (1999), 38B:237-42.
4. Banachiewicz B, Banachiewicz J, Chodkowska A, Wojtowicz EJ, Mazur L. Eur J Med Chem (2004) 39(10):873-77.
5. Colanceska-Ragenovic K, Dimova V, Kakurinov V, Molnar DG, Buzarovska A. Molecules (2001) 6(10):815-24.
6. Metwally KA, Yaseen SH, Lashine EM, El-Fayomi HM, El-Sadek ME. Eur J Med Chem (2007) 42(2):152-60.
7. Mishra RK, Tiwari RK, Srivastav SK, Bahel SC. J Indian Chem Soc (1991), 8:110-15.
8. Parmar SS, Gupta AK, Singh HH, Gupta TK. J Med Chem (1972) 15(9):999-100.
9. Bhatia, M.S., Zarekar, B. E., Choudhari, P. B., Ingale, K. B., Bhatia, N. M.,. Med Chem Res DOI 10.1007/s00044-009-9283-8.
10. Bhatia, M.S., Ingale, K. B., Choudhari, P. B., Sawant, R. L., Patil, C. R.. Lat. Am. J. Pharm. (2009) 28 (6): 927-31.
11. Bhat M V, Ravindranathan M S and Rao G V J. *Org. Chem.* 1984, 49, 3170.



Fig.1: Figure showing the alignment of molecules.

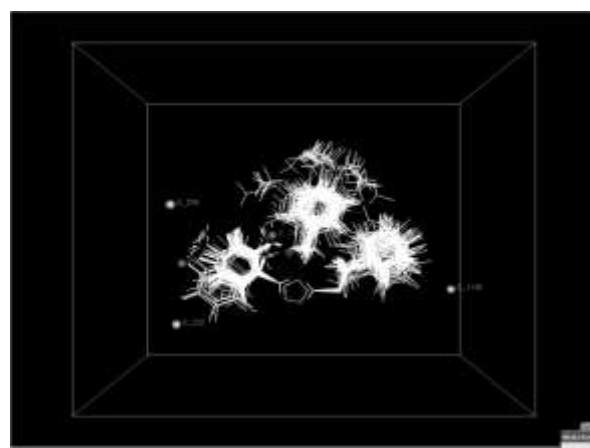


Fig. 2: Figure showing the field points for model A.

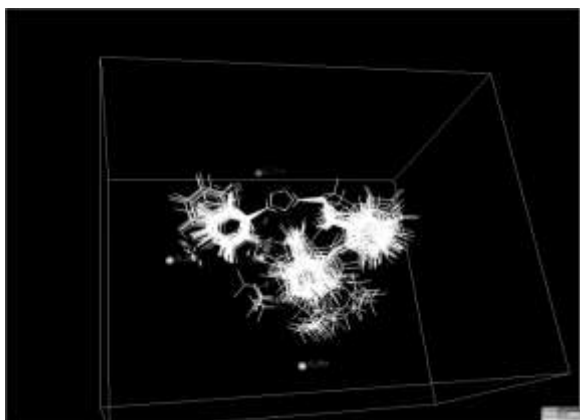


Fig. 3: Figure showing the field points for model B.

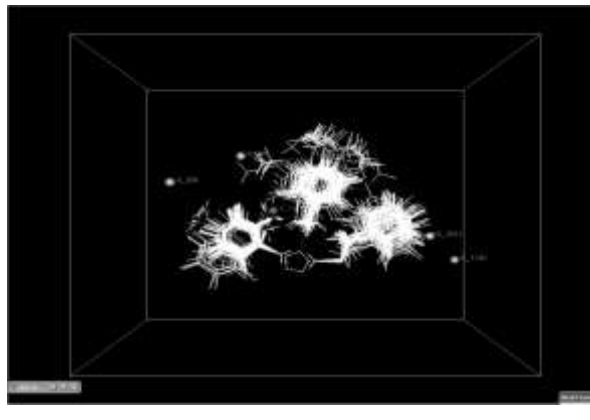


Fig. 4: Figure showing the field points for model C.

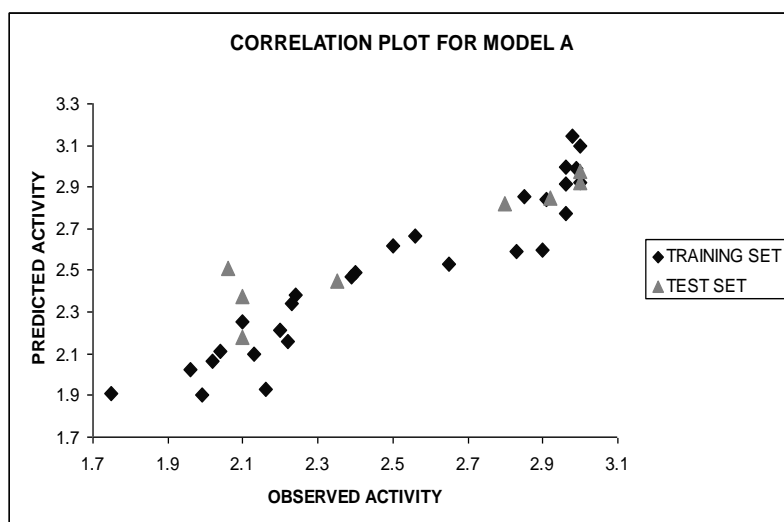


Fig. 5: Figure showing correlation plot for model A.

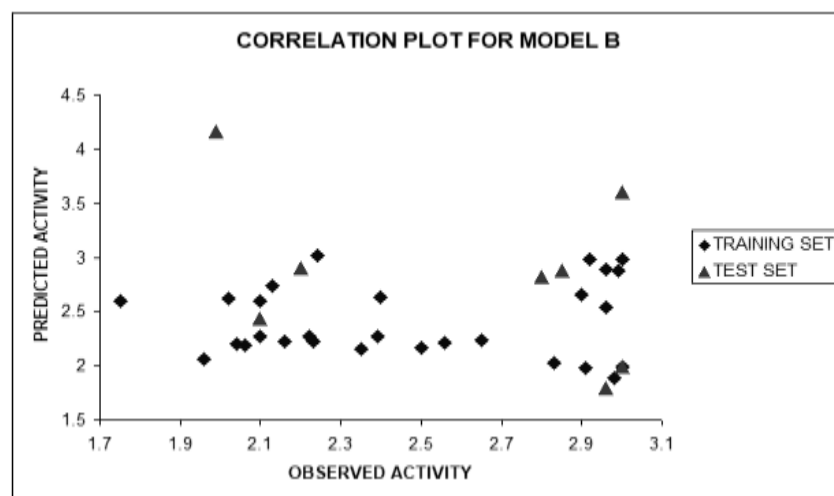


Fig. 6: Figure showing correlation plot for model B.

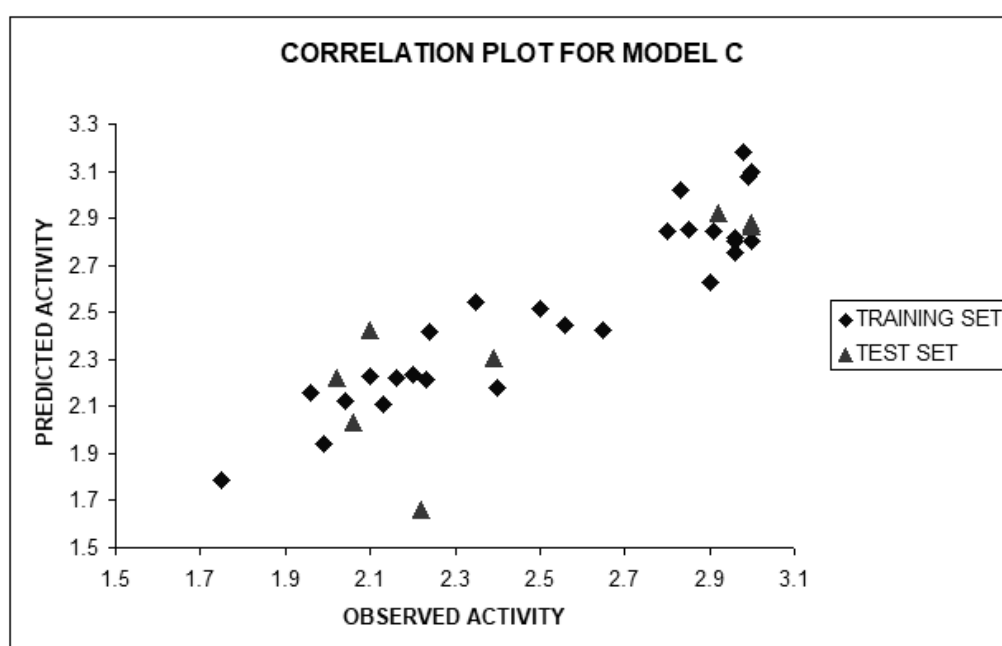


Fig.7: Figure showing correlation plot for model C.

Table 1: Different substituents which are utilized in study.

Sr. No.	Ar	R	Sr. No.	Ar	R
1.	C ₆ H ₅	H	19	4NH ₂ C ₆ H ₅	H
2.	C ₆ H ₅	4-Cl	20	4NH ₂ C ₆ H ₅	4-Cl
3.	C ₆ H ₅	2-OH	21	4NH ₂ C ₆ H ₅	2-OH
4.	C ₆ H ₅	4(N-CH ₃) ₂	22	4NH ₂ C ₆ H ₅	4(N-CH ₃) ₂
5.	C ₆ H ₅	4-OH	23	4NH ₂ C ₆ H ₅	4-OH
6.	C ₆ H ₅	3,4,5-OH	24	4NH ₂ C ₆ H ₅	3,4,5-OH
7.	2NH ₂ C ₆ H ₅	H	25	C ₆ H ₅ -CH ₂	H
8.	2NH ₂ C ₆ H ₅	4-Cl	26	C ₆ H ₅ -CH ₂	4-Cl
9.	2NH ₂ C ₆ H ₅	2-OH	27	C ₆ H ₅ -CH ₂	2-OH
10.	2NH ₂ C ₆ H ₅	4(N-CH ₃) ₂	28	C ₆ H ₅ -CH ₂	4(N-CH ₃) ₂
11.	2NH ₂ C ₆ H ₅	4-OH	29	C ₆ H ₅ -CH ₂	4-OH
12.	2NH ₂ C ₆ H ₅	3,4,5-OH	30	C ₆ H ₅ -CH ₂	3,4,5-OH
13.	2OH C ₆ H ₅	H	31	4C ₅ H ₅ N	H
14.	2OH C ₆ H ₅	4Cl	32	4C ₅ H ₅ N	4-Cl
15.	2OH C ₆ H ₅	2 OH	33	4C ₅ H ₅ N	2 -OH
16.	2OH C ₆ H ₅	4(N-CH ₃) ₂	34	4C ₅ H ₅ N	4(N-CH ₃) ₂
17.	2OH C ₆ H ₅	4-OH	35	4C ₅ H ₅ N	4-OH
18.	2OH C ₆ H ₅	3,4,5-OH	36	4C ₅ H ₅ N	3,4,5-OH

Table 2: Table showing the selected QSAR equations along with statistical parameters employed for model selection.

Model	Equation	r ²	q ²	F value	Predicted r ²
Model A	pMIC = 1.484 - 5.6835 S_259 + 0.0410 E_239 - 0.0067 S_222 - 1.7308 S_1120 - 0.0079 E_546	0.9011	0.8422	40.0937	0.7622
Model B	pMIC = 4.7421 + 0.0850 E_518 - 0.0087 E_545 - 0.0044 S_249 + 0.1316 S_681 - 0.7415 H_546 -	0.8914	0.8407	36.1072	0.6575
Model C	pMIC = 1.3339 - 5.7594 S_259 + 0.0031 S_1031 - 0.0142 E_546 + 0.0041 S_465 - 1.3422 S_1120	0.8711	0.7628	29.7427	0.6328

Table no 3: table showing the observed activity and predicted activity of molecules.

Sr. no	Model A			Model B			Model C		
	Obs	Pre	Res	Obs	Pre	Res	Obs	Pre	Res
1.	2.65	2.5270	0.1229	2.65	2.2361	0.4138	2.65	2.4264	0.2235
2.	2.23	2.3437	-0.1137	2.23	2.2229	0.0070	2.23	2.2126	0.0173
3.	2.35	2.4466	-0.0966	2.35	2.1587	0.1912	2.35	2.5459	-0.1959
4.	2.50	2.6202	-0.1202	2.50	2.1658	0.3341	2.50	2.5176	-0.0176
5.	2.39	2.4665	-0.0765	2.39	2.2687	0.1212	2.39	2.3042	0.0857
6.	2.56	2.6638	-0.1038	2.56	2.2069	0.3530	2.56	2.4462	0.1137
7.	2.40	2.4880	-0.0880	2.40	2.6266	-0.2266	2.40	2.1792	0.2207
8.	2.96	2.7745	0.1854	2.96	2.8945	0.0654	2.96	2.8009	0.1590
9.	2.80	2.8219	-0.0219	2.80	2.8148	-0.0148	2.80	2.8479	-0.048
10.	2.20	2.2118	-0.0118	2.20	2.8994	-0.6994	2.20	2.2361	-0.0361
11.	2.10	2.3773	-0.2773	2.10	2.5973	-0.4973	2.10	2.2317	-0.1317
12.	2.90	2.5996	0.3003	2.90	2.6511	0.2488	2.90	2.6249	0.2751
13.	2.92	2.8470	0.0729	2.92	2.9812	-0.0612	2.92	2.9243	-0.0043
14.	2.85	2.8576	-0.0076	2.85	2.8750	-0.0250	2.85	2.8522	-0.0022
15.	3.00	2.9227	0.0772	3.00	3.5995	-0.5995	3.00	3.0944	-0.0944
16.	3.00	2.9775	0.0224	3.00	2.9874	0.0125	3.00	2.8643	0.1356
17.	2.96	2.9183	0.0416	2.96	2.5434	0.4165	2.96	2.7505	0.2094
18.	2.99	2.9861	0.0038	2.99	2.8796	0.1103	2.99	3.0787	-0.0887
19.	2.91	2.8396	0.0703	2.91	1.9804	0.9296	2.91	2.8468	0.0631
20.	2.98	3.1433	-0.1633	2.98	1.8896	1.0903	2.98	3.1781	-0.1981
21.	3.00	3.0972	-0.0972	3.00	1.987	1.0130	3.00	2.8813	0.1186
22.	3.00	2.9235	0.0764	3.00	1.9888	1.0111	3.00	2.8054	0.1945
23.	2.96	2.9980	-0.0380	2.96	1.7962	1.16379	2.96	2.8178	0.1421
24.	2.83	2.5889	0.2410	2.83	2.0289	0.8010	2.83	3.0190	-0.1890
25.	2.10	2.2504	-0.1504	2.10	2.2690	-0.1690	2.10	2.2253	-0.1253
26.	2.22	2.1592	0.0607	2.22	2.2756	-0.0556	2.22	1.6600	0.5599
27.	2.16	1.9312	0.2287	2.16	2.2184	-0.0584	2.16	2.2230	-0.0630
28.	2.06	2.5130	-0.4530	2.06	2.1883	-0.1283	2.06	2.0319	0.0280
29.	1.96	2.0231	-0.0631	1.96	2.0609	-0.1009	1.96	2.1591	-0.1991
30.	2.04	2.1145	-0.0746	2.04	2.1977	-0.1577	2.04	2.1239	-0.0839
31.	2.02	2.0638	-0.0438	2.02	2.6157	-0.5957	2.02	2.2233	-0.2033
32.	1.75	1.9088	-0.1588	1.75	2.5933	-0.8433	1.75	1.7882	-0.0382
33.	2.13	2.0957	0.0342	2.13	2.7360	-0.6060	2.13	2.1100	0.0199
34.	1.99	1.9035	0.0864	1.99	4.1562	-2.1662	1.99	1.9423	0.0476
35.	2.24	2.3815	-0.1415	2.24	3.0176	-0.7776	2.24	2.4146	-0.1746
36.	2.10	2.1825	-0.0825	2.10	2.4349	-0.3349	2.10	2.4243	-0.3243
