International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:10, No:4, 2016

Quantitative Structure Activity Relationship and Insilco Docking of Substituted 1,3,4-Oxadiazole Derivatives as Potential Glucosamine-6-Phosphate Synthase Inhibitors

Suman Bala, Sunil Kamboj, Vipin Saini

***Abstract*—**Quantitative Structure Activity Relationship (QSAR) analysis has been developed to relate antifungal activity of novel substituted 1,3,4-oxadiazole against *Candida albicans* and *Aspergillus niger* using computer assisted multiple regression analysis. The study has shown the better relationship between antifungal activities with respect to various descriptors established by multiple regression analysis. The analysis has shown statistically significant correlation with R^2 values 0.932 and 0.782 against *Candida albicans* and *Aspergillus niger* respectively. These derivatives were further subjected to molecular docking studies to investigate the interactions between the target compounds and amino acid residues present in the active site of glucosamine-6-phosphate synthase. All the synthesized compounds have better docking score as compared to standard fluconazole. Our results could be used for the further design as well as development of optimal and potential antifungal agents.

***Keywords*—**1,3,4-Oxadiazole, QSAR, Multiple linear regression, Docking, Glucosamine-6-Phosphate Synthase.

I. Introduction

IN the present scenario, treatment of infectious diseases is very challenging due to development of resistance towards most of the antimicrobial agents. Various side effects are associated with the use of antimicrobials such as irritation, toxicity, resistance, hypersensitivity etc. So, there is urgency in the area of microbiological research for the development of better, safe and effective antifungal and antibacterial agents [1], [2].

1,3,4-Oxadiazole substituted derivatives are found to be having diverse pharmacological activities such as anti-inflammatory [3], [4], antimicrobial [1], antiviral [5], analgesic [6], anti-mycobacterial [7], anticonvulsant [8], etc. So, it was planned to synthesize a novel series of 1,3,4-oxadiazole derivatives and further subjected for antifungal activity.

Nowadays, Glucosamine-6-Phosphate synthase has emerged out as a novel potential target for the development of potent antifungal agents [9]. The structure of this enzyme consists of two domains, N-terminal and C-terminal [10], [11]. G-6-P synthase catalyzes the rate-limiting step i.e. conversion of fructose-6-P to glucosamine-6-P in chitin biosynthesis pathway [12], a major fungal cell wall component [13]. Its potential has been proposed as a promising target for development of novel, potent and safe antifungal agent. The enzyme exhibits absolute specificity for L-glutamine as an amino donor and for D fructose-6-P as an acceptor substrate. The substrates bind to the enzyme in an ordered fashion and binding of D-Fru-6-P precedes that of L-Gln [14], [15].

Computational chemistry has developed as an important contributor to rational drug design. In the present paper, QSAR analysis using multiple linear regression [16] and molecular docking studies was carried out on substituted 1,3,4-oxadiazole analogues to develop a relationship between the physicochemical parameters and antifungal activity as potential glucosamine-6-Phosphate synthase [17].

We have developed the following QSAR models for substituted 1,3,4-oxadiazoles using multi linear regression method.

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II. Materials and Methods

QSAR (with multiple linear regressions) and docking studies were performed using software Analyse- it version 3.0 and Molegro Virtual Docker 5.0.0. respectively [18], [19].

A. Biological Data

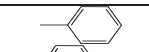
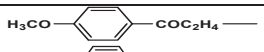
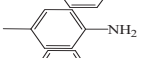
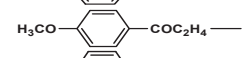
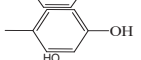
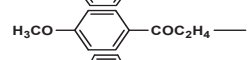
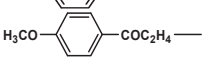
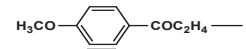
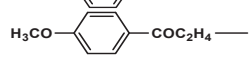
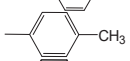
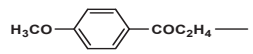
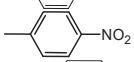
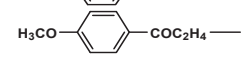
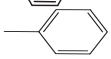
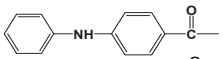
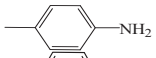
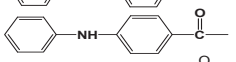
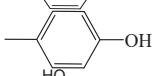
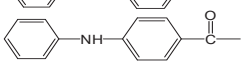
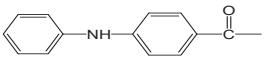
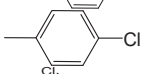
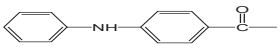
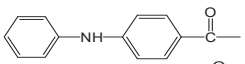
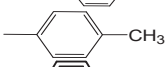
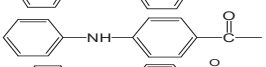
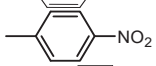
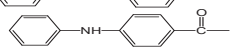
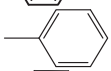
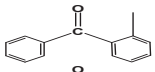
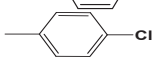
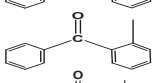
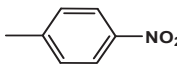
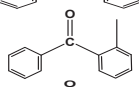
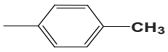
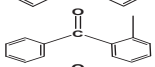
The antifungal activities used in the present studies were expressed as pMIC50; logarithm of a reciprocal concentration for 50% inhibition where MIC50 is minimum inhibitory concentration of the compounds producing 50% reduction in the effect caused by fungus (*Candida albicans* and *Aspergillus niger*) is stated as a mean of at least two experiments for 24 compounds.

B. Optimization of Molecular Structure and Descriptors Calculation

Molecular structures of 24 test compounds (Table I) are drawn in Chem Draw version 10. The physicochemical parameters are computed using Chem 3D Ultra version 10 after energy minimization to minimum RMS Gradient of 0.100 kcal/mole Å by MOPAC software package. A most stable structure for each compound was generated and used for calculating various physicochemical parameters like lipophilic, steric and electronic values of parameters. In the present study, the calculated descriptors were octanol-water

partition coefficient (LogP) [20], connolly solvent accessible surface area (SAS) [21], [22], molar refractivity (MR) [23], ovality [24], molecular surface area (MSA) [25] and molecular weight (MW) [26]. Selected physicochemical parameters are given in Table II.

TABLE I
BASIC STRUCTURE OF TEST COMPOUNDS

Com	R	R′	MIC *C. a*	MIC *A. n*
1			50	100
2			25	200
3			200	50
4			200	100
5			200	50
6			12.5	25
7			25	25
8			50	200
9			200	50
10			100	25
11			-	200
12			100	200
13			400	-
14			200	50
15			100	50
16			200	200
17			50	50
18			12.5	10
19			25	10
20			50	50
21			100	10
22			25	25
23			50	100
24			100	200

R and R′ groups represent the synthesized 1,3,4-oxadiazole derivatives along with their MIC values

C. a: Candida albicans, A. n: Aspergillus niger

C. QSAR Models Development and Validation

In QSAR equation, n is the number of test compounds; R^2, squared correlation coefficient; R^2adj, coefficient of determination; F, Fischer statistics for statistical significance; S, standard error of estimate; and biological activity; Press, sum of the squared prediction errors; Q^2=cross validated correlation coefficient. The calculated antifungal activity was computed using QSAR models. The formulae used to calculate the cross-validated correlation coefficient Q^2 and are given below:

$$\text{Press} = \Sigma(\text{Ycalculated} - \text{Yobserved})^2 \quad (1)$$

$$\text{Total} = \Sigma(\text{Yobserved} - \text{Ymean})^2 \quad (2)$$

$$Q^2 = 1 - \Sigma(\text{Ycalculated} - \text{Yobserved})^2 / \Sigma(\text{Yobserved} - \text{Ymean})^2 \quad (3)$$

where, Ycalculated, Yobserved and Ymean are the calculated, observed and mean values of activity respectively.

TABLE II
VALUES OF SELECTED DESCRIPTORS USED IN THE MULTILINEAR REGRESSION ANALYSIS

Com	Physicochemical Parameters					
	LogP	SAS	MR	Ovality	MSA	MW
1	2.99	565.61	88.58	1.5621	298.14	308.34
2	2.19	578.425	93.28	1.574	306.2	323.35
3	2.61	573.584	90.27	1.6	303.02	324.34
4	2.61	571.141	90.27	1.6	302.35	324.34
5	3.55	590.062	93.38	1.5809	313.21	342.78
6	3.56	587.07	93.38	1.6	312.46	342.78
7	3.48	597.333	93.62	1.5918	317.41	322.36
9	4.54	596.6	101.1	1.6031	317.11	341.37
10	3.74	582.457	105.89	1.5735	324.48	356.39
11	4.15	576.296	102.88	1.5662	321.19	357.37
12	4.15	592.365	102.88	1.5715	320.18	357.37
13	5.09	617.51	105.99	1.6113	330.59	375.82
14	5.09	598.701	105.99	1.5746	328.6	375.81
15	5.02	629.02	106.24	1.6301	337.2	355.39
17	4.4787	572.71	98.9	1.5798	306.45	326.35
18	5.0369	596.623	103.71	1.5975	321.3	360.8
20	4.9658	604.563	103.94	1.6085	325.7	340.38
21	3.9628	626.902	107.06	1.6265	339.2	372.38
22	4.0892	580.924	100.59	1.5876	311.39	342.35
24	4.0892	576.924	100.59	1.581	310.43	342.35

D. Molecular Docking Studies

In the present study we used MVD Version 2011.5.0.0 software for docking studies. The molecules were built using Chem Sketch 10.0. The resulting structures were saved in Mol 2.0 format. The synthesized compounds were subjected to molecular docking studies for the inhibition of the enzyme glucosamine-6-phosphate synthase (GlcN-6-P) which is a new target for the antifungal compounds. Target compounds docked into active site of GlcN-6-P (PDB 1JXA) [27], [28]. 1JXA is a 3 chains structure of sequences from *E. coli*.

III. Results

A. QSAR Studies

The best model obtained from multiple linear regression analysis was (4) and (5). The observed and predicted activity of *Candida albicans* and *Aspergillus niger* was given in Table III.

QSAR Models for antifungal activity against *Candida albicans* and *Aspergillus niger* are given as under:

Candida albicans

$$PMIC_{50} = -11.32 + 0.2246(\text{Log P}) + 0.00976\,(\text{SAS}) - 0.05562(\text{MR}) + 11.42\,(\text{Ovality}) - 0.05816(\text{MSA}) + 0.03539(\text{MW})$$

N = 14, R^2 = 0.9322, R^2_{adj} = 0.875, q^2 = 0.9320, Spress= 0.0967, F=16.11, Standard Error of Estimate = 0.117, p-value=0.0009.

Aspergillus niger

$$PMIC_{50} = 7.885 - 0.4617\,(\text{Log P}) + 0.0156\,(\text{SAS}) + 0.1136\,(\text{MR}) - 6.2\,(\text{Ovality}) - 0.04824\,(\text{MSA}) + 0.00037(\text{MW}) + 7.885$$

N = 14, R^2 = 0.782, q^2 = 0.780, R^2_{adj} = 0.594, press= 0.266, F=4.17, Standard Error of Estimate = 0.194, p value = 0.0417.

B. Molecular Docking Studies

The interaction between target compounds (Fig. 2) and standard drugs with PDB 1jxa has shown for targeting the enzyme glucosamine-6-phosphate synthase. Ligand receptor interactions in terms of dock score (binding energy) of target compounds and standards were observed in Table IV.

TABLE III
Observed and Predicted Antifungal Activity of Synthesized 1,3,4-Oxadiazole Derivatives against *Candida albicans* and *Aspergillus niger*

Com	*Candida albicans*			*Aspergillus niger*		
	Obs.	Cal.	Residuals	Obs.	Cal.	Residuals
1	1.3979	1.3565	0.04138	1.6989	1.4907	0.20821
2	-	-	-	2	2.1381	-0.1382
3	2	1.9702	0.02975	1.3979	1.5189	-0.121
4	2	1.9853	0.01463	1.6989	1.5129	0.18603
5	2	2.0112	-0.0112	1.3979	1.3306	0.06733
6	-	-	-	1.0969	1.1968	-0.0999
7	1.0969	1.2105	-0.1136	1.0969	1.2263	-0.1295
9	2	1.8447	0.15528	1.3979	1.5267	-0.1289
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	1.6989	1.6436	0.05536	2	1.8964	0.10352
13	2.3	2.4290	-0.129	-	-	-
14	2	1.9418	0.05816	1.3979	1.4967	-0.0988
15	1.6989	1.6191	0.07987	1.3979	1.2665	0.13144
17	1.3979	1.5424	-0.1445	1.3979	1.5833	-0.1854
18	-	-	-	-	-	-
20	1.3979	1.3871	0.01082	1.3979	1.3295	0.06841
21	1.6989	1.7592	-0.0603	-	-	-
22	-	-	-	-	-	-
24	1.6989	1.7505	-0.0516	2	1.8279	0.17209

- compound not included in QSAR model development

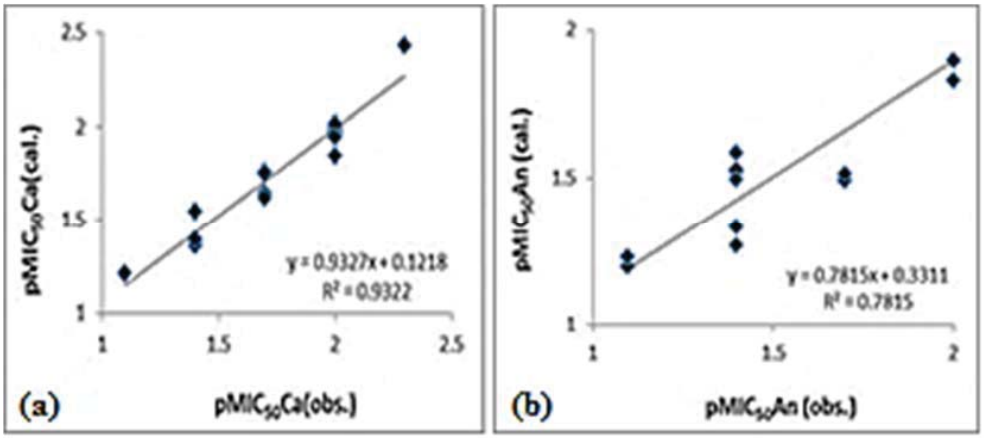


Fig. 1 Plot of calculated $pMIC_{50}$ values against observed $pMIC_{50}$ for QSAR model for (a) *Candida albicans*, (b) *Aspergillus niger*

IV. Discussion

A. QSAR Studies

The QSAR results showed the dependence of the antifungal activity on the structural and physicochemical features of 1,3,4-oxadiazole. Two QSAR models were generated by multilinear regression analysis for *Candida albicans* and *Aspergillus niger*. In both models R^2, $Q^2$and R^2adj values are good which is considered as a proof of high predictive power of QSAR models. Log P was an effective factor of fungicidal activities which is an indicator of compound hydrophobicity and solubility. The inclusion of an oxadiazole moiety in the synthesized compounds also showed high lipophilicity, hypothesizing that this lipophilicity could facilitate passage of these compounds through the fungal membrane.

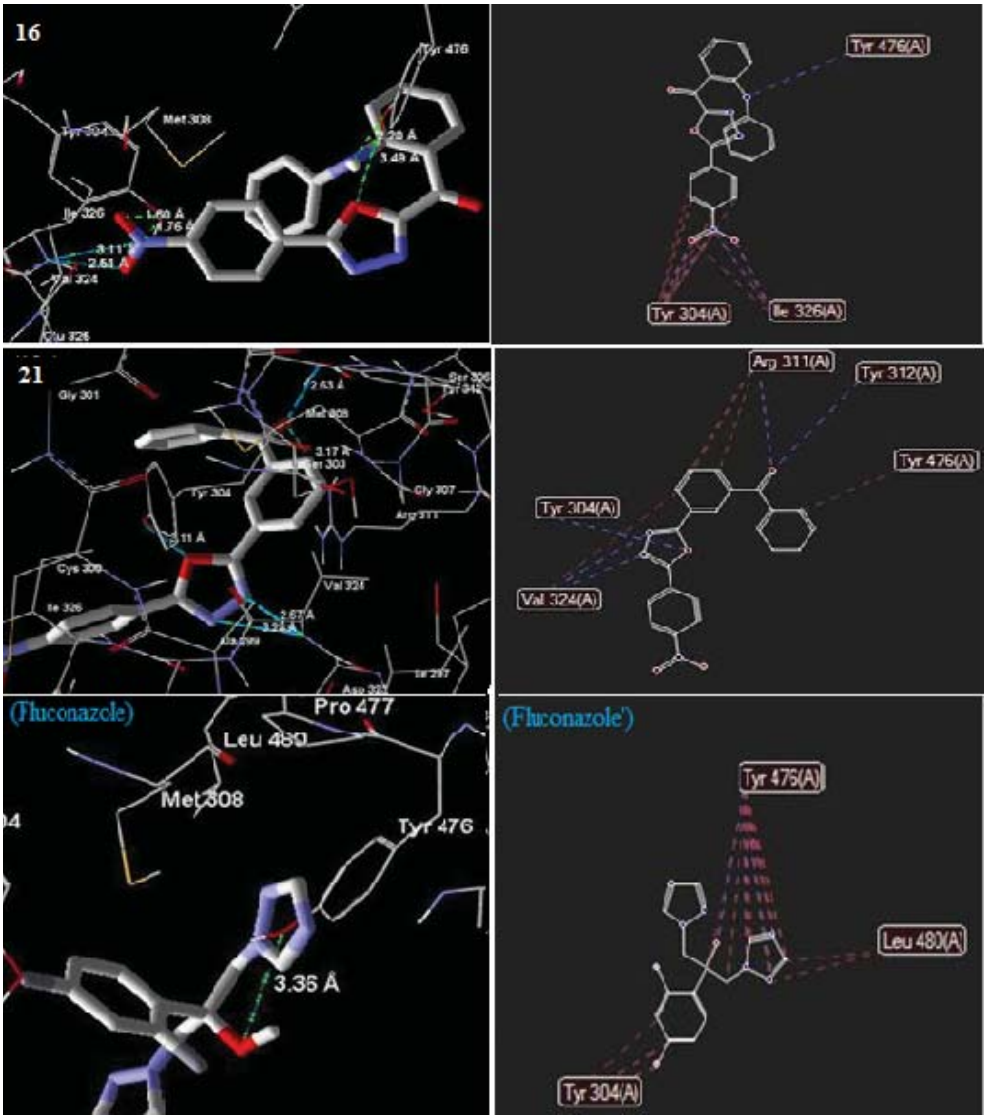


Fig. 2 Binding modes of compound 16, 21 and fluconazole (docking view) and 16', 21' and fluconazole as interaction view) with GlcN-6-P synthase, where blue/green lines and red lines represent hydrogen bonding and favourable steric interactions respectively

TABLE IV
LIGAND-RECEPTOR INTERACTION OF ANTIFUNGAL COMPOUNDS

Code	Docking Score	Distance (Å)	Amino acids	Group involved
8	-108.476	3.05	Tyr 476	-N- of oxadiazole ring
		3.57	Tyr 476	-O- of oxadiazole ring
16	-114.364	3.49	Tyr 476	-O- of oxadiazole ring
		2.20	Tyr 476	-NH- of n-Phenyl group
		1.76	Tyr 304	-N- of nitro group
		1.60	Tyr 304	O_1 of nitro group
		3.11	Ile 326	-N-of nitro group
		2.51	Ile 326	O_2 of nitro group
21	-116.246	2.97	Tyr 304	-O- of oxadiazole ring
		3.28	Val 324	-N- of oxadiazole ring
		2.81	Val 324	-N- of oxadiazole ring
FLZ	-89.687	3.36	Tyr 476	OH

FLZ: Fluconazole

B. Molecular Docking Studies

Hydrogen bonding (polar-polar interaction) is an important binding force in drug receptor interaction because drug receptor interaction is basically an exchange of the hydrogen bond between a drug molecule surrounded by water and receptor, since many drugs contain hydroxyl, amino, carboxyl and carbonyl groups, they can form hydrogen bonds with receptors or enzymes. The results of docking study of antifungal compounds depicted the interaction with Tyr 476 (8 and 16) and Tyr 304 (16 and 21). Standard drugs showed interaction with Tyr 476. The docking score of all the synthesized compounds was more than standard drugs. Tyr 476 and Tyr 304 were found to be common interacted amino acid for both synthesized and standard compounds.

V. Conclusion

The high predictive power of generated QSAR models was confirmed from values of R^2 and R^2adj.for *Candida albicans* and *Aspergillus niger* which was found to be higher than 0.5. Compound 21 was found to be most potent antifungal compound with good docking score, i.e. -116.246. These results could be used as guidelines for the study of the mechanism of action and further design and development of better fungicides.

Acknowledgment

Authors are thankful to M. M. College of Pharmacy, M. M. University, Mullana, Ambala for providing support and facility to carry out research.

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