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ORIGINAL ARTICLE

Pharmacophore modeling and 3D QSAR studies of aryl amine derivatives as potential lumazine synthase inhibitors

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Abstract Design and discovery of novel antifungal compounds is the need of time, more than ever before due to the unavailability of effective antifungal therapy to treat resistant fungal infections. Due to morphological and functional similarities of fungi both with plant cell and human cell, the search for effective targets leading to specificity of antifungal drug action becomes all that more difficult. For the design of novel antifungal agents, it is necessary to comprehend the life cycle, morphology, metabolic pathways, etc. of fungi scientifically and systematically to identify critical targets for antifungal drug design. Fungi specific riboflavin metabolism involves lumazine synthase catalyzed synthesis of 6,7-dimethyl-8-D-ribityl lumazine which is converted to riboflavin by a riboflavin synthase. Therefore lumazine synthase has been targeted for the design of newer antifungal agents. The pharmacophore modeling and 3D QSAR studies were carried out on 32 N-substituted aryl amine derivatives as fungal lumazine synthase inhibitors. The selected model of 3D QSAR showed positive correlation of electronic descriptors with antifungal activity while steric and hydrophobic descriptors showed negative correlation with antifungal activity. The resulting model exhibited good q^2 and r^2 values up to 0.9109 and 0.845 respectively.

1. Introduction

In recent years the fungal infections have become a major cause of death in patients suffering from disease like AIDS (Morgunova et al., 2007). The main classes of antifungal drugs which are in market now are having problems like high risk of toxicity, development of resistance and pharmacokinetic deficiencies (Cushman, 1998; Graybill, 1996). A detailed study of biochemical constituents of fungal cell wall, cytoplasmic membrane and DNA and protein synthesis of fungal cell, signal transduction

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pathways and virulence factors was taken into consideration for the new potential targets for antifungal development (Cushman, 2005; Leonor, 2003; Urbina, 2000). Fungi can synthesize their own riboflavin, so design, synthesis and screening of inhibitors of lumazine synthase have been targeted to develop effective antifungals. 3D QSAR studies correlate the physiochemical properties in the form of 3D descriptors with biological activity which can be utilized to optimize the molecules with better biological activity (Bhatia et al.,2009a,b; 2010a,b) We have already reported the design of novel N-substituted aryl amine derivatives as potential inhibitors of lumazine synthase (Bhatia, 2010c). In continuation of our previous work, we are reporting the 3D QSAR and pharmacophore modeling of N-substituted aryl amine derivatives.

2. Materials and methods

2.1. Three dimensional QSAR analysis

2.1.1. Ligand preparation

The structure of the C–N bond was used as the template to build the molecules in the dataset in Vlife MDS 3.5 (Bhatia et al., 2009a,b; 2010a,b). The ligand geometries were optimized by energy minimization using MMFF94 force field and Gasteiger-Marsili charges for the atoms, till a gradient of 0.001 kcal/mol/Å was reached, maintaining the template structure rigid during the minimization.

2.1.2. Molecular alignment

The molecules of the dataset were aligned by the atom-fit technique, using atoms common to the structure of 4-fluoro-N-[1-(4-chlorophenyl) but-3-en-1-yl] aniline. The virtually most active lead was selected as a template for alignment of the molecules. The alignment of all the molecules on the template is shown in Fig. 1.

2.1.3. Descriptor calculation

Like many 3D QSAR methods, a suitable alignment of a 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. For getting reliable results, dataset having typically 5 times as many data points (molecules) as independent variables (descriptors) are required, so the five descriptors were selected in the final equation from calculated 2000 descriptors.

2.1.4. Data set

The dataset was divided into a training set (27 molecules, Table 1) and a test set (05 molecules, Table 1) on the basis of chemical and biological diversity using the random selection method for generation of the training and test set data. The molar Inhibitory concentration (pMIC) values for antifungal activity were used for the present 3D QSAR study.

2.2. Full search multiple linear regression method

Relationship between independent and dependent variables (3D fields and biological activities, respectively) was determined

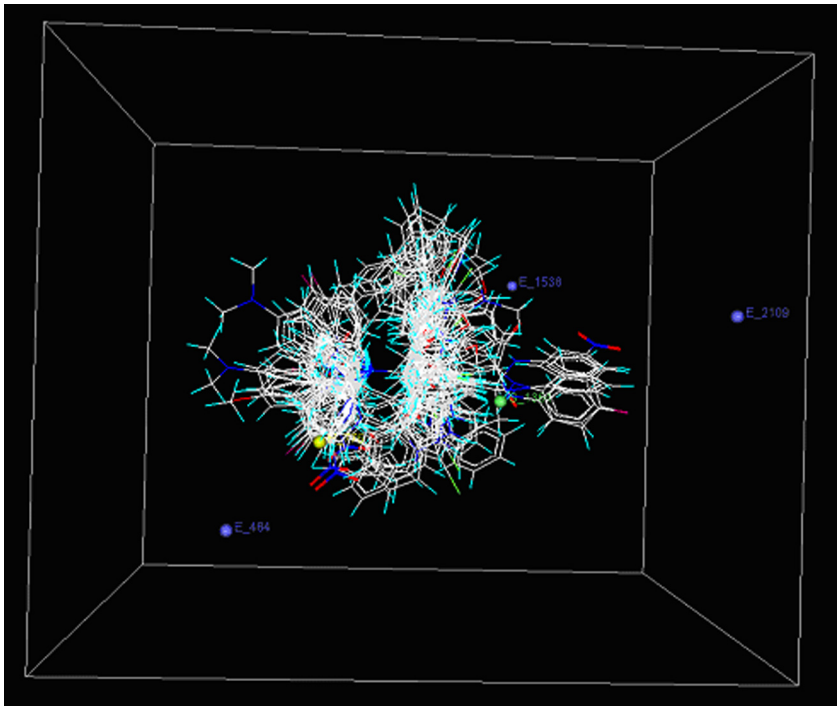
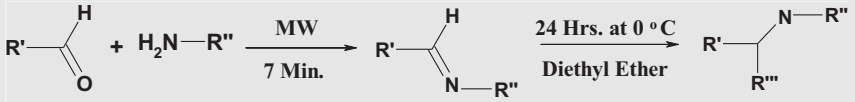


Figure 1 Field points for selected QSAR models. The figure indicates the field points for selected QSAR models, that is steric (green), hydrophobic (yellow), electric (blue) features which are required for activity.

Table 1 Synthesized molecules utilized in QSAR study.



Aromatic Aldehyde 1 | **Aromatic Amine 2** | **3** | **N - Substituted Aryl Amine Derivatives 4**

Sr. No.	R′	R″	R‴
1	4-Dimethylamino benzaldehyde	2-Amino pyridine	Allyl bromide
2	4-Dimethyl amino benzaldehyde	3-Nitro aniline	Allyl bromide
3	4-Dimethyl amino benzaldehyde	4-Fluoro aniline	Allyl bromide
4	4-Dimethyl amino benzaldehyde	1-Naphthyl amine	Allyl bromide
5	4-Methoxy benzaldehyde	2-Amino pyridine	Allyl bromide
6	4-Methoxy benzaldehyde	3-Nitro aniline	Allyl bromide
7	4-Methoxy benzaldehyde	4-Fluro aniline	Allyl bromide
8	4-Methoxy benzaldehyde	1-Naphthyl amine	Allyl bromide
9	4-Chloro benzaldehyde	2-Amino pyridine	Allyl bromide
10	4-Chloro benzaldehyde	3-Nitro aniline	Allyl bromide
11	4-Chloro benzaldehyde	4-Fluro aniline	Allyl bromide
12	4-Chloro benzaldehyde	1-Naphthyl amine	Allyl bromide
13	Indole-3-carboxyaldehyde	2-Amino pyridine	Allyl bromide
14	Indole-3-carboxyaldehyde	3-Nitro aniline	Allyl bromide
15	Indole-3-carboxyaldehyde	4-Fluro aniline	Allyl bromide
16	Indole-3-carboxyaldehyde	1-Naphthyl amine	Allyl bromide
17	4-Dimethyl amino benzaldehyde	2-Amino pyridine	Cinnamoyl chloride
18	4-Dimethyl amino benzaldehyde	3-Nitro aniline	Cinnamoyl chloride
19	4-Dimethyl amino benzaldehyde	4-Fluro aniline	Cinnamoyl chloride
20	4-Dimethyl amino benzaldehyde	1-Naphthyl amine	Cinnamoyl chloride
21	4-Methoxy benzaldehyde	2-Amino pyridine	Cinnamoyl chloride
22	4-Methoxy benzaldehyde	3-Nitro aniline	Cinnamoyl chloride
23	4-Methoxy benzaldehyde	4-Fluro aniline	Cinnamoyl chloride
24	4-Methoxy benzaldehyde	1-Naphthyl amine	Cinnamoyl chloride
25	4-Chloro benzaldehyde	2-Amino pyridine	Cinnamoyl chloride
26	4-Chloro benzaldehyde	3-Nitro aniline	Cinnamoyl chloride
27	4-Chloro benzaldehyde	4-Fluro aniline	Cinnamoyl chloride
28	4-Chloro benzaldehyde	1-Naphthyl amine	Cinnamoyl chloride
29	Indole-3-carboxyaldehyde	2-Amino pyridine	Cinnamoyl chloride
30	Indole-3-carboxyaldehyde	3-Nitro aniline	Cinnamoyl chloride
31	Indole-3-carboxyaldehyde	4-Fluro aniline	Cinnamoyl chloride
32	Indole-3-carboxyaldehyde	1-Naphthyl amine	Cinnamoyl chloride

statistically using regression analysis. The quality of fit for a regression equation was assessed using various statistical parameters like r^2, q^2 (F test). Models showing q^2 below 0.6 were discarded. The selected models for antifungal activity are shown in Table 1.

2.3. Activity prediction

To systematically assess a QSAR model, a reliable validation is required. Usually, a QSAR model is evaluated by the predictive results for the given dataset. Selected models having r^2 above 0.7 were checked for their external predictivity. The observed and the predicted values for antifungal activity are shown in Table 2.

2.4. Pharmacophore modeling

This pharmacophore modeling was carried out in the mol sign module of Vlife MDS 3.5 software. A series of lumazine synthase inhibitors was first aligned on the active molecule. Alignment of small organic molecules is one of the important tasks in drug design and bioactivity prediction. A pharmacophore model is a set of three dimensional features that are necessary for bioactive ligands. Thus, it makes logical sense to align molecules based on features that are responsible for bioactivity, the number indicates the minimum number of pharmacophore features generated for an alignment is taken as 4 and the tolerance is kept at 10 Å. The max distance allowed between two features is placed at 10 Å.

3. Results and discussion

3.1. Results

To obtain the effects of the structural parameters of the N-substituted aryl amine derivatives on antifungal activity, 3D QSAR analysis was performed by using various molecular descriptors which were correlated with the negative logarithm

Table 2 Observed and predicted activity by QSAR equation along with residuals.

Compound code	Observed activity	Predicted activity	Residuals
Training set			
1	4.328	4.378	−0.05
10	4.387	4.382	0.005
11	4.301	4.308	−0.007
12	4.387	4.347	0.04
13	4.319	4.396	−0.077
4	4.387	4.412	−0.025
15	4.553	4.53	0.023
16	4.301	4.29	0.011
18	4.509	4.488	0.021
19	4.357	4.772	−0.415
2	4.398	4.34	0.058
21	4.638	4.701	−0.063
22	4.398	4.423	−0.025
23	4.469	4.451	0.018
24	4.276	4.325	−0.049
25	4.444	4.506	−0.062
26	4.678	4.603	0.075
28	4.409	4.701	−0.292
3	4.553	4.451	0.102
30	4.284	4.325	−0.041
31	4.469	4.506	−0.037
32	4.409	4.603	−0.194
4	4.481	4.449	0.032
5	4.31	4.34	−0.03
6	4.352	4.178	0.174
7	4.337	4.385	−0.048
8	4.292	4.269	0.023
Test set			
17	4.509	4.793	−0.284
20	4.77	4.264	0.506
27	4.244	4.617	−0.373
29	4.155	4.423	−0.268
9	4.398	4.541	−0.143

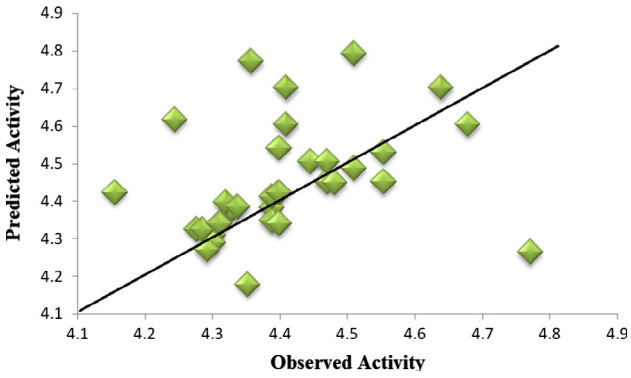


Figure 2 Correlation plot between observed activity and predicted activity. The figure indicates the correlation plot between predicted activity and observed activity which shows the significance of QSAR equation in predicting the activity.

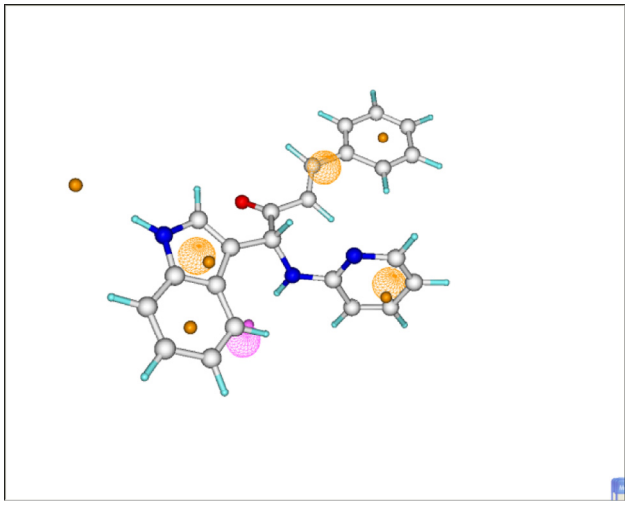


Figure 3 Pharmacophore for synthesized molecules. The figure indicates the pharmacophoric features like hydrogen bond donor (magenta) and hydrophobic (orange) which are required for activity.

of MIC. The QSAR analysis generated various models out of which only one was selected based on correlation coefficient and different statistical parameters and are given in Table 3. The square of correlation coefficient (r^2 = 0.9109), square of correlation coefficient for cross-validation (q^2 = 0.8451) and the F test (42.9425) were employed to judge the validation of regression equation observed against *Candida albicans*. The electronic descriptors which measured as 0.0921 E_1538 and 0.0249 E_464 (blue) contribute positively for anti-fungal activity while the electronic descriptor measured as 0.0159 E_2109, steric descriptor measured as 0.0055 S_1364 (green) and hydrophobic descriptor measured as 0.3286 H_776 (yellow) contribute negatively for anti-fungal activity as shown in Figs. 1 and 2.

3.2. Pharmacophore modeling study

A set of pharmacophore hypothesis was generated using the mole sign module of Vlife 3.5. Each hypothesis contains the four features like hydrogen bond donor, hydrogen bond acceptor, hydrophobic, and aliphatic that are common. The pharmacophore models were validated by using the pharmacophoric search for reported lumazine synthase inhibitors. The structures of the reported lumazine synthase which are in clinical use are used to predict the pharmacophore model for them and these features are compared with the training set and designed molecules to validate the results as shown in Fig. 3. The results of the pharmacophoric search can give the following results. The important features that are required for the lumazine synthase inhibitory activity are the hydrogen bond donor (buff)

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

QSAR model	N	r^2	q^2	F value	Predicted r^2
pMIC = 4.7903 − 0.0159 E_2109−0.0055 S_1364−0.3286 H_776 + 0.0921 E_1538 + 0.0249 E_464.	32	0.9109	0.8451	42.942	0.8650

and hydrophobic interaction groups (magenta). The introduction of groups which are fascinating these parameters may increase the lumazine synthase inhibitory activity.

4. Conclusion

The 3D QSAR statistical model described in this work shows both good internal and external consistencies. By 3D QSAR studies of steric and electrostatic contour maps we can conclude that electronegative groups surrounding the N-substituent are required for antifungal activity. In addition, the favorable steric contours suggest that aromatic bulky groups at the pyridine ring moiety may increase ligand potency. The 3D QSAR models should be useful for the design of new structurally related potential lumazine synthase inhibitors.

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