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Understanding the Antifungal Activity of Terbinafine Analogues Using Quantitative Structure–Activity Relationship (QSAR) Models

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Abstract—Terbinafine and its analogues, which are a major class of non-azole antifungal agents, are known to act by inhibition of squalene epoxidase enzyme in fungal cells. We have performed a quantitative structure–activity relationship (QSAR) study on a series of 92 molecules using different types of physicochemical descriptors. Inhibitors were divided into five classes depending upon chemical structure. QSAR models were generated for correlation between antifungal activity against *Candida albicans* using genetic function approximation (GFA) technique. Equations were evaluated using internal as well as external test set predictions. Models generated for all these classes show that steric properties and conformational rigidity of side chains play an important role for the activity. The present QSAR analysis agrees with the results of the previously reported CoMFA study. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Squalene epoxidase (SE) (EC 1.14.99.7) enzyme present in fungal and mammalian cell systems is important in ergosterol biosynthesis.[1] SE is involved in the conversion of squalene to squalene 2,3-epoxide, which is subsequently converted into lanosterol and then into ergosterol. SE, which is a membrane bound enzyme, has been proposed as a target for the action of the allylamine class of antifungal agents. Naftifine, terbinafine and similar molecules exhibit antimycotic activity with widespread clinical applications.[2] The antifungal activity of allylamines has been attributed exclusively to the inhibition of the SE enzyme system in the ergosterol biosynthetic pathway.

The SE enzyme system requires molecular oxygen, nicotinamide adenine dinucleotide phosphate (NADP) and flavin adenine dinucleotide (FAD) for its activity.[3] Terbinafine and its analogues have been found to be potent inhibitors of fungal SE and their inhibitory potency is well correlated with antifungal activity. Kinetic studies have shown that terbinafine and analogues do not act as substrate analogues and show non-competitive kinetics.[4,5] It has been hypothesized that these molecules interact with the lipid binding site or nonspecific site on the enzyme system. These are proposed to inhibit a total enzyme system rather than the specific site on the enzyme.

There are different chemical classes of SE inhibitors developed over the years.[6–10] These classes include terbinafine analogues, benzylamine analogues, homopropargyl amines and carbon atom analogues. These have been tested against a variety of fungal pathogenic organisms. Important structural features include a fused naphthalene or fused benzothiophene ring system and double–triple bonded functionality 'eneyne' functionality. In certain classes such as benzyl amines and propargyl amines this 'eneyne' system is replaced by a benzyl or propargyl system. Design of new molecules in this class is purely based upon the structural information derived from existing inhibitors. In order to study and deduce the correlation between structure and biological activity of these molecules, we reported a 3D-QSAR study on 92 molecules using comparative molecular field analysis (CoMFA) technique.[11] Initial analysis resulted in low predictive and correlative properties. The original dataset was re-classified into amines and non-amines. The dataset was modified by exclusion of non-amines (13 carbon atom analogues). Analysis of this modified dataset resulted in improvement in correlative and predictive properties.

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CoMFA technique is based upon calculation of steric and electrostatic properties of the molecules and in some cases, this could limit its applicability to different types of biological activity data. This is particularly true in cases where cell based in vivo activity data such as MIC, ED_{50}, MIC_{90} is used. In such cases, a combination of CoMFA fields and other parameters have proved to be more useful than CoMFA fields alone. This has been observed in the case of analysis of triazines for anticoccidial activity, where use of log k' resulted in better predictivity.[12] Additional factors contributing to in vivo activity can be understood through the use of physicochemical parameters as lipophilicity and other molecular properties. Descriptors of physicochemical properties such as molecular lipophilicity potential (MLP) have been used as an additional regressor in CoMFA analysis of imidazoline receptor ligands.[13] For antipicornavirus compounds, log*P* has been observed to contribute significantly to the activity.[14] Analysis of antibacterial activity of macrolide compounds has resulted in identification of physicochemical descriptors such as log*P*, log *D*, CMR, p*K*a and HPLC capacity factor for deriving correlation with in vitro (MIC) and in vivo activity.[15] Recently, there has been an increasing trend towards the use of various types of physicochemical descriptors for explaining the variation for in vivo activity data. In a study involving prediction of chemicals binding to an estrogen receptor, descriptors generated from holographic QSAR (HQSAR) and CODESSA were used.[16] Descriptors involving three-dimensional properties such as van der Waals volume and excluded volume were used in the study of steroids binding to corticosteroid-binding globulin.[17] Various quantum-chemical and quantum-mechanical descriptors are being applied for quantitative structure–activity relationship and quantitative structure–pharmacokinetic relationship studies involving complex biological phenomena.[18] New molecular representations such as field descriptors, topological descriptors, fragment descriptors and molecular holograms have been developed.[19] These various types of descriptors have made prediction of pharmacokinetic properties such as absorption and metabolism possible. Molecular surface areas were used in the prediction of the absorption of β-blocking agents.[20] Biological activity of terbinafine and its analogues is based upon cell based assays, i.e., minimum inhibitory concentration. Application of CoMFA technique to handle MIC-cell based activity data has indicated some limitations and some extrapolation was necessary for explaining the antifungal activity of terbinafine analogues.[11] In the present study we have derived QSAR models for SE inhibitors using different molecular descriptors to make better predictions for cell based activity data. In a similar study on a particular class of terbinafine analogues using different descriptors such as atomic charges and, van der Waals' volume, differentiating structural requirements were deduced for activity against different fungal organisms.[21]

We have used genetic function approximation (GFA) technique to generate different QSAR models from various descriptors generated using Cerius2 molecular modeling software. GFA technique was used since it generates a population of equations rather than one single equation for correlation between biological activity and physicochemical properties. GFA developed by Rogers involved combination of Friedman's multivariate adaptive regresssion splines (MARS) algorithm with Holland's genetic algorithm to evolve a population of equations that best fit the training set data.[22] This is done as follows

i) An initial population of equations is generated by random choice of descriptors. The fitness of each equation is scored by lack-of-fit (LOF) measure

$$\mathrm{LOF} = \frac{\mathrm{LSE}}{\{1 - [(c + d^{*}p)/m]\}^{2}}$$

where LSE is least square error, c is the number of basis functions in the models, d is the smoothing parameter which controls the number of terms in the equation and p is the number of features contained in all terms of the models.

ii) Pairs from the population of equation are chosen at random and 'crossovers' are performed and progeny equations are generated.

iii) The fitness of each progeny equation is assessed by LOF measure.

iv) If the fitness of the new progeny equation is better, then it is preserved.

A distinct feature of GFA is that it produces a population of models. GFA models provide some useful additional information such as relevance of a particular descriptor in the model and activity prediction. GFA has been applied to a set of ellipticine analogues.[23] These analogues were divided into different groups depending upon chemical structure and analysis was performed for different types of anticancer activities. GFA technique has also been applied for the analysis of antimycin analogues with antifilarial activity and analysis of aceylcholinesterase inhibitors. Recently, GFA was used for binding affinity predictions of ligands using free energy force field descriptor terms as in the case of inhibitors of glycogen phosphorylase[24] and peptidomimetic renin inhibitors.[25,26] GFA has also been used for the QSAR analysis of steroids, dopamine β-hydroxylase inhibitors[27] and anticancer agents.[28] An interesting application of GFA is in the QSAR studies on acetylcholinesterase inhibitors which has resulted in discovery of a new molecule, E2020, for treatment of Alzheimer's disease.[29]

Combination of robust statistical technique GFA coupled with use of different types of descriptors would result in better prediction of biological activity for antifungals. In this paper, we present QSAR analysis of fungal SE inhibitors for antifungal activity against *Candida albicans*.

Results

Ninety-two molecules were divided into five classes depending upon chemical nature.

a. Terbinafine analogues—naphthalene class
b. Terbinafine analogues—benzothiophene class
c. Benzyl amine analogues
d. Carbon atom analogues
e. Propargyl amine analogues

Molecules in each class were divided into training set and test set. Biological activity was used in terms of log (1/MIC) where MIC is minimum inhibitory concentration against *C. albicans* in mM/L. Although some of the molecules used in the study contain more than seven rotatable bonds, the bonds connecting the aromatic ring and the side chain are important in determining the overall conformation and shape of the molecules. During conformational search these bonds were taken into consideration. In order to obtain sterically accessible conformations within optimum computational time, we have used a random sampling technique for conformational search. Conformation obtained from this technique was found to be similar to the conformation derived in our previous 3D-QSAR study.[11] Conformational search using random sampling was performed during molecular shape analysis (MSA) technique. All the molecules used in the study were subjected to conformational search and lowest energy conformers were aligned using MSA technique. Molecules in a particular chemical class were superimposed on the lowest energy conformer of the molecule with highest biological activity in that chemical class. This alignment resulted in orientation of the molecule in such a way that the naphthalene or benzothiophene ring is parallel to the z-axis; side chain along the *x*-axis and *N*-methyl group is along the *y*-axis.

GFA technique was used in each case for generating QSAR models. It was observed that in each case, 20,000 crossovers result in optimum internal and external predictivity as assessed from the LOF value, cross-validated r^2 value and variable usage graphs. Thus the number of crossovers has been set to 20,000 for all the classes.

For each chemical class, the number of terms in each QSAR model equation was decided depending upon the number of molecules in the training set. Smoothness value (d) of 1.0 was used during GFA equation generation.

All statistically significant equations for each chemical class have been presented in Tables 8 and 9. The term 'BA' in these equations represents biological activity expressed as log (1/MIC).

Terbinafine analogues—naphthalene class

This class consisted of 21 training set and seven test set molecules. Various types of descriptors were calculated for each molecule and QSAR equations were generated using GFA technique. GFA was performed with 20,000 crossovers and a smoothness value equal to one. A set of QSAR equations were evaluated for their predictive ability. Observation of the variable usage graph indicated that the terms dipole_Z, Hf, Alog*P* and dipole_X contribute more significantly than all other descriptors. The best equation from the set of equations was selected on the basis of predictivity, variables and LOF value and other statistical parameters such as *F* value. Eqs (1) and (2) (Table 8) show better internal predictivity and also result in better predictions for test set molecules. Dipole moment, Hf (heat of formation) and Alog*P* (atom based partition coefficient) terms contribute to these equations and explain about 70% variance in the activity. Three variable terms in the equation show low correlation among themselves indicating low probability of chance correlation. Eq (2) with better predictive r^2 value (0.550) is proposed as the QSAR model describing antifungal activity for this class.

Terbinafine analogues—benzothiophene class

The benzothiophene class is characterized by the presence of sulfur atom in the ring. The presence of benzothiophene ring instead of naphthalene ring seems to impart better activity against *C. albicans*. The training set consisted of 15 molecules and the test set of seven molecules. QSAR models were generated and the equations were then analyzed on the basis of cross-validated r^2, LOF values and variable usage graph. This resulted in the identification of three top equations (eqs (3)–(5)) in Table 8. These equations exhibited moderate internal predictivities. One main important observation is the occurrence of 'Apol' (atomic polarisability) as a common descriptor in all these equations. These three equations were then analyzed for their external prediction power. All these equations exhibited low predictive power. Eq (3) with better predictive r^2 value of 0.342 is proposed as the best equation describing the model for this class.

Benzyl amine analogues

Benzyl amine class is characterized by the presence of large, bulky benzylamino side chain attached to naphthalene or benzothiophene ring systems. Out of a total of 14 molecules, the training set consisted of 10 molecules and the test set of four molecules.

GFA runs using non-MSA based parameters resulted in a series of equations with principal moment of inertia (PMI) and RotlBonds (number of rotatable bonds) as significant variables. These equations were then analyzed on the basis of cross-validated r^2, predictivity and LOF values. This analysis resulted in the identification of two equations as the most significant eqs (6) and (7) in Table 8. The equations and the variable terms in these equations clearly indicate the importance of shape and structure based factors playing an important role in biological activity for this class of inhibitors. QSAR model generation was performed with the inclusion of MSA based descriptors. Inclusion of MSA based parameters clearly showed the improvement in the predictivity both in qualitative and quantitative terms. Detailed analysis of equations for cross-validated r^2, predictivity and variables in the equations resulted in identification of three equations eqs (8)–(10) as statistically most significant. These equations indicate that the shape based parameters—ShapeRMS, COSV (common overlap steric volume) and NCOSV (non-common overlap steric volume) are contributing significantly to activity. The QSAR eq (8) with better overall predictivity is considered for describing the activity of benzyl amines.

Propargyl amines

The class of propargylamines is characterized by the absence of structurally rigid double bond functionality in the side chain. Differences in the length of the side chain is another important factor which would affect the interaction of these inhibitors at the enzyme site. A total of 15 molecules were considered separately as 10 training set and five test set molecules. QSAR models were initially generated using different descriptors for activity against *C. albicans.* These equations showed the presence of COSV as an important descriptor contributing to the activity. The equations so generated were analyzed for their statistical performances. This resulted in the identification of three top equations—eqs (13)–(15) for this class. All these equations exhibit moderate internal predictivity (cross-validated r^2). The equations were then subjected to the analysis of test set predictions. Two equations—eq (13) and (15) showed better predictive power. Since the predictive r^2 values of both the equations are similar, their ability to predict and handle training set data was used as criteria for the selection of the best equation representing the whole class. Eq (15) showed only one outlier while eq (13) had shown two outliers for training set. Thus eq (15) containing PMI-mag and COSV terms is described as the best equation for this class.

Carbon atom analogues

This class of inhibitors is different from other classes in that it lacks the presence of a tertiary amine group and hence would exhibit different physicochemical properties than other classes of compounds. The absence of a tertiary amine group makes the molecules in this class conformationally different. Side chains of these carbon analogues exhibit different orientation and flexibility of the side chain. QSAR analysis of this class is important because this class has shown significant deviations from the dataset in previous studies and exclusion of these molecules has resulted in improved predictivity of the models for rest of the dataset.[11]

Thirteen molecules belonging to this class were divided into training set (10 molecules) and test set (three molecules). Different types of descriptors were generated and analysis was performed using GFA technique. A series of equations was generated and analyzed for their statistical performance. Variable terms dipole moment, molar refractivity (MR) and principal moment of inertia (PMI) contribute significantly to the equations. Although the equations exhibited better internal predictivity, in the case of the test set activity these equations showed poor predictive power. None of the equations showed good predictive r^2 values. Analysis was then performed by inclusion of MSA based parameters and resulting set of equations was then analyzed. QSAR eq (11) can be described as the best equation for this class of molecules.

Discussion

Molecules belonging to the class of terbinafine analogues are known to act by inhibition of SE enzyme system. 3D-QSAR studies using CoMFA technique helped us to understand the requirements for activity against different pathogenic fungal organisms. Although CoMFA models developed initially exhibited low predictivity, the classification of molecules according to the chemical nature (amines and non-amines) resulted in better cross-validated r^2 and predictive r^2 values. Although CoMFA technique is known to generate highly predictive models, in some cases its applicability is limited because of the type of biological activity data. Cell based data ED_{50}, MIC, I_{50}, etc., are known to be influenced by the factors, than only molecular fields and in such cases use of different physicochemical parameters or descriptors can result in better predictions.

Taking into consideration these factors we have developed different models for different chemical classes of SE inhibitors.

Terbinafine analogues—naphthalene class

The equation describing the biological activity for this class is eq (2) containing thermodynamic parameters-Hf and Alog*P*, and electronic parameter—Dipole_X contributing significantly to the biological activity. Hf (heat of formation) is a thermodynamic parameter and accounts for the chemical stability of the molecule. Negative coefficient of this term in the equation means that the lower the value, the better the activity. This means that the biological activity is directly dependent upon chemical stability of the molecules in biochemical systems. Alog*P* is the partition coefficient calculated using the atom-based approach and represents the hydrophobicity of the molecules. Alog*P* is positively correlated with activity. This property assumes significance in the present case because of the fact that the molecules under study contain lipophilic groups (naphthalene ring) and further cell based biological activity data is involved. Dipole moment is an electronic property and is important in cases when dipole–dipole interactions are involved in drug action. Dipole_X describes the dipole moment along the *x*-axis i.e., perpendicular to the plane of the naphthalene ring. This dipole moment along x-axis is mainly due to electrons of the naphthalene ring and the side chain. This term in the QSAR equation is positively correlated and underlines the importance of dipole interactions in the action of these molecules. Thus the interaction between these electrons and corresponding amino acid residues on the enzyme plays an important role during inhibition. All the molecules in this class possess similar side chain i.e., typical of allylamine antifungals (‘eneyne’) and the rigidity imposed by this sidechain is also important for activity against *C. albicans*. This type of side-chain is highly rigid in conformation and this seems to be significant for activity. Although the equations generated do not contain the term reflecting this property but the presence of dipole_X and Alog*P* parameters reflecting electronic, entropic and solvation phenomenon do account for the significance of structural rigidity for better activity.

Terbinafine analogues—benzothiophene class

One main important observation is the occurrence of Apol (atomic polarisability) as common descriptor in all the significant equations (eq (3) to (5)) describing activity for this class. These three equations were then analyzed for their external prediction power. All these equations exhibited low predictive power. The best equation showed predictive r^2 value of 0.342. On the basis of these analyses, eq (3) describes the activity of this class of molecules.

The terms contributing to the activity include Vm—molecular volume—and PMI_Y-principal moment of inertia Y-component—are the spatial descriptors while Apol is an electronic descriptor. The presence of 'Apol' term is important because this term is unique for this class and this is due to the presence of a sulfur atom in the ring. Apol term in the QSAR models supports the biological activity data that these molecules are more active than corresponding naphthyl ring analogues. This implicates the possibility that there could be the presence of charge-transfer or electronic interaction between molecules and the enzyme responsible for activity. Two spatial descriptors are describing the non-covalent component of the antifungal action. Molecular volume and principal moment of inertia terms describe the distribution and orientation of the molecules reflecting that the conformation of the molecule is important for activity. The benzothiophene class of molecules has been found to exhibit better activity than other classes particularly naphthalene analogues. Application of GFA to this series has resulted in appearance of 'Apol' as a significant descriptor in QSAR model for this class. GFA has been successful in this case in that it indicates the presence of additional electronic interaction along with non-covalent component of the interaction. QSAR equation for benzothiophene class of molecules exhibits better conventional r^2 value ($=0.816$) and accounts for approximately 80% of variance in biological activity. Presence of 'Apol' indicates the importance of charge transfer/electronic interaction during enzyme inhibition. Electronic effect in this QSAR study was measured using semi-empirical calculations, which may be insufficient to model the electronic effects. More accurate calculations may help to obtain better predictions.

Benzyl amine analogues

Eq (8) with better predictivity is considered as the best equation for the benzyl amine class.

Two descriptors, namely ShapeRMS and COSV, significantly explain the variance in the biological activity to a greater extent. Thus it is hypothesized that the antifungal activity involves shape and steric properties of the molecules more than electronic properties. Requirement

Table 1. Descriptors used in the present study

Compound	Descriptor	Type	Description
1	DIFFV	MSA	Difference volume
2	COSV	MSA	Common overlap steric volume
3	Fo	MSA	Common overlap volume ratio
4	NCOSV	MSA	Non-common overlap steric volume
5	ShapeRMS	MSA	RMS to shape reference
6	SRVol	MSA	Volume of shape reference compound
7	Vm	Spatial	Molecular volume
8	Area	Spatial	Molecular surface area
9	Density	Spatial	Molecular density
10	RadOfGyr	Spatial	Radius of gyration
11	PMI-mag	Spatial	Principal moment of inertia
12	PMI_X	Spatial	Principal moment of inertia X-component
13	PMI_Y	Spatial	Principal moment of inertia Y-component
14	PMI_Z	Spatial	Principal moment of inertia Z-component
15	Charge	Electronic	Sum of partial charges
16	Apol	Electronic	Sum of atomic polarisabilities
17	Dipole-mag	Electronic	Dipole moment
18	Dipole-X	Electronic	Dipole moment—X-component
19	Dipole-Y	Electronic	Dipole moment—Y-component
20	Dipole-Z	Electronic	Dipole moment—Z-component
21	HOMO	Electronic	Highest occupied molecular orbital energy
22	LUMO	Electronic	Lowest unoccupied molecular orbital energy
23	Sr	Electronic	Superdelocalisability
24	MW	Structural	Molecular weight
25	RotlBonds	Structural	Number of rotatable bonds
26	HbondAcc	Structural	Number of hydrogen bond acceptors
27	HbondDon	Structural	Number of hydrogen bond donors
28	AlogP	Thermodynamic	Logarithm of partition coefficient
29	Fh_2o	Thermodynamic	Desolvation free energy for water
30	Foct	Thermodynamic	Desolvation free energy for octanol
31	Hf	Thermodynamic	Heat of formation
32	MolRef	Thermodynamic	Molar refractivity

for conformational rigidity and proper orientation of the side chain is signified by ShapeRMS–rms deviation between the molecule and the reference molecule. The greater the conformational deviation of the individual molecule from the conformation of the reference molecule the lesser the activity. Thus the rigid conformation attained by the side-chain of the molecules appears to be a strict requirement for antifungal activity. Since the reference molecule possesses the highest biological activity, molecules that are similar in structure and conformation to the reference molecule are expected to exhibit better activity. COSV is the common volume between the molecule and reference molecule. The higher the value of COSV, the better the activity; that means the greater the similarity the better the activity.

Propargyl amines

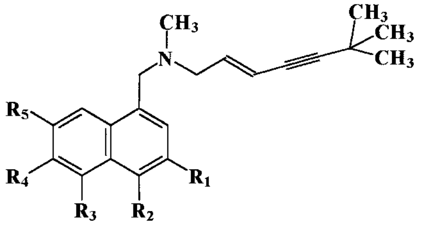
Equation (15) containing PMI_mag and COSV terms is described as the best equation for this class. Principal moment of inertia for any body describes the total mass of the body and its distribution. In the case of molecules, PMI-mag is a spatial descriptor describing orientation of the molecules and groups. In this case, the spatial orientation of the molecule (in other words, conformation of the side chain) appears to be determining the activity profile. Thus for all the molecules in this class, similar conformation of the side chain is essential for activity. Requirement of conformational rigidity is important since structurally rigid elements are absent in these molecules. COSV represents the common volume between individual molecule and reference molecule (highest activity molecule). COSV term is positively correlated with the activity indicating strict conformational requirements of the side-chain. Antifungal activity is determined by occupation of enzyme active site by the inhibitors and this inhibition appears to be non-covalent in nature. Antifungal activity is mainly controlled by steric and shape properties of the molecules with conformational rigidity playing an important role.

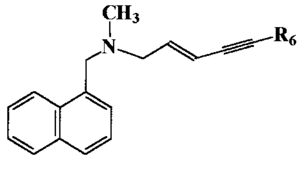
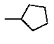
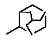
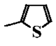
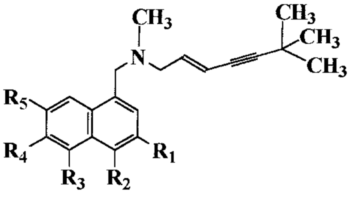
Carbon atom analogues

This class of inhibitors lacks the presence of a tertiary amine group and hence would exhibit different physicochemical properties than other four classes. Absence of tertiary amine group makes the molecules in this class conformationally different. The side chain of these carbon analogues exhibits different orientation and flexibility of the side chain. QSAR analysis of this class is important because this class has shown significant deviations in previous studies.[11]

Eqs (11) and (12) represent statistically significant equations. As evident from the equations COSV and PMI_X accounts for the significant variance in the biological activity. PMI_X represents the orientation or distribution of the total molecular mass in the three-dimensional space. This would mean that the similarity in the side-chains with respect to orientation is important for activity. COSV is the common volume between individual molecule and shape reference molecule. Since reference molecule exhibits the highest activity, molecules with

Table 2. Structures and biological activity of the molecules used in the present study—naphthalene class[a]



Molecule	Sructure	Obs. act.[b]	Pred. act.[c]
1	R_1 = -CN; $R_2 = R_3 = R_4 = R_5$ = -H	0.199	0.615
2	R_1 = -CH_3; $R_2 = R_3 = R_4 = R_5$ = -H	0.786	0.864
3	R_2 = -F; $R_1 = R_3 = R_4 = R_4$ = -H	1.093	1.268
4	R_2 = -CN; $R_1 = R_3 = R_4 = R_5$ = -H	0.199	0.655
5	R_3 = -F; $R_1 = R_2 = R_4 = R_5$ = -H	1.995	1.374
6	R_3 = -Br; $R_1 = R_2 = R_4 = R_5$ = -H	2.073	2.195
7	R_3 = -CH_3; $R_1 = R_2 = R_4 = R_5$ = -H	1.388	0.856
8	R_4 = -F; $R_1 = R_2 = R_3 = R_5$ = -H	1.093	1.364
9	R_5 = -Cl; $R_1 = R_2 = R_3 = R_4$ = -H	1.416	1.245
10	R_5 = -CH_3; $R_1 = R_2 = R_3 = R_4$ = -H	0.786	0.869
11	$R_3 = R_5$; = -F; $R_1 = R_2 = R_4$ = -H	2.322	1.859
			
12		0.482	0.752
13		0.568	1.002
14		0.502	0.119
15	-C(=CH_2)CH_3	0.440	0.314
16	-C(=CH_2)C(CH_3)$_3$	1.104	0.567
17	-C≡C–C(CH_3)$_3$	0.499	0.386
18	-C≡C–C(OH)(CH_3)$_2$	0.467	0.406
19	-C≡C–C(OCH_3)(CH_3)$_2$	0.488	0.552
20	-I	0.558	0.761
21	-C(CH_3)$_3$	1.067	0.715
28	-C_6H_5	0.493	0.397
			
22	R_1 = -F; $R_2 = R_3 = R_4 = R_5$ = -H	1.695	1.192
23	R_2 = -CH_3; $R_1 = R_3 = R_4 = R_5$ = -H	0.786	0.852
24	R_3 = -Cl; $R_1 = R_2 = R_4 = R_5$ = -H	2.320	1.399
25	R_3 = -CN; $R_1 = R_2 = R_4 = R_5$ = -H	0.199	0.701
26	R_4 = -CH_3; $R_1 = R_2 = R_3 = R_5$ = -H	1.087	0.866
27	R_5 = -F; $R_1 = R_2 = R_3 = R_4$ = -H	1.394	1.358

[a] Molecules **1**–**21** belong to training set while **22**–**28** belong to test set.

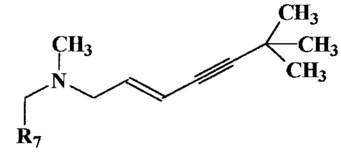
[b] Obs. act = observed biological activity is defined as log (1/MIC) where MIC is minimum inhibitory concentration against *Candida albicans* in mM/L units.

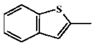
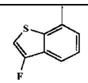
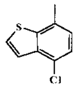
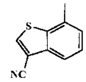
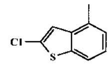
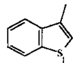
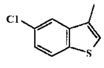
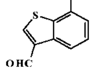
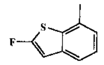
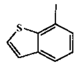
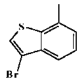
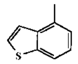
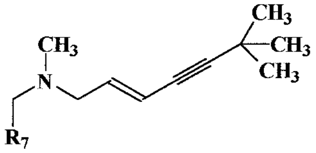
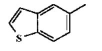
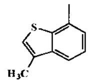
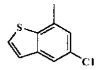
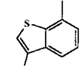
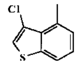
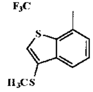
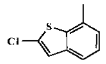
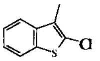
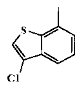
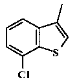
[c] Pred. act. = predicted biological activity calculated using eq (2) in Table 8.

structure and conformation similar to that of the reference molecule (molecule 89) would exhibit similar activity. This common volume would be significant in terms of occupation at the enzyme binding site and COSV represents the volume available for inhibition. Similar orientation of the side chains in all the molecules is required for exhibiting activity. Another important aspect of this class is that the activity data are approximate and this would result in chance correlation.

Development of different QSAR models for different chemical classes was performed using various physicochemical descriptors and GFA technique. One of the main reasons for using GFA was that it would generate a population of equations incorporating activity and descriptors for the series. One of the important observations of this study is that for all the classes of squalene SE, shape and steric properties seem to play a major role in antifungal activity. In the case of benzyl amines, propargyl amines and carbon atom analogues COSV appears to contribute significantly to the top QSAR models developed for these classes. COSV-shape based descriptor describing the common volume between the individual molecule and the shape reference molecule appears to contribute significantly to activity. Since the shape reference molecule is the one with highest biological activity, molecules that are structurally and conformationally similar to the reference molecule are expected to exhibit better activity. Presence of COSV indicates the importance of conformational rigidity of the side chain and its correct orientation at the enzyme active site for better activity.

Table 3. Structures and biological activity of the molecules used in the present study—benzothiophene class[a]



Molecule	R7	Obs. act[b]	Pred. act.[c]	Molecule	R7	Obs. act.	Pred. act.
29		0.172	−0.059	**35**		2.596	1.677
30		0.220	0.147	**36**		2.031	2.135
31		1.440	1.360	**37**		0.473	0.873
32		1.442	1.206	**38**		1.717	1.355
33		1.101	1.351	**39**		1.075	0.770
34		2.673	2.925	**40**		0.774	1.067
							
41		0.172	0.429	**46**		2.016	2.379
42		0.220	0.478	**47**		2.086	2.275
43		0.872	1.193	**48**		1.740	2.113
44		0.220	1.345	**49**		0.220	1.096
45		2.618	1.521	**50**		1.123	1.731

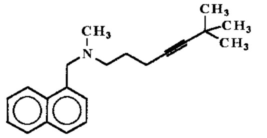
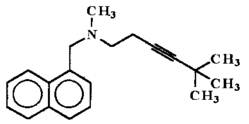
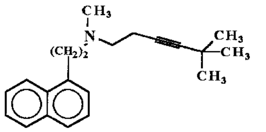
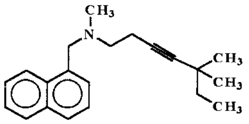
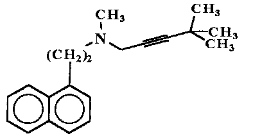
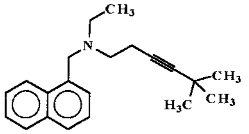
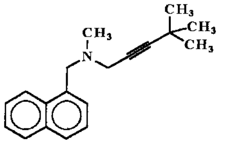
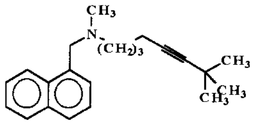
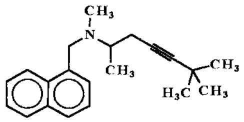
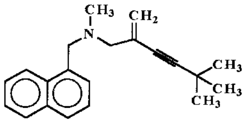
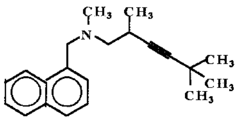
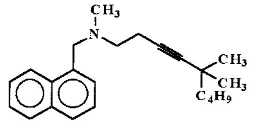
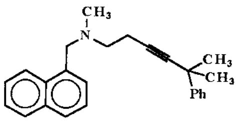
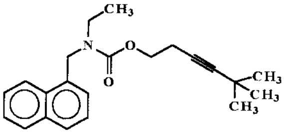
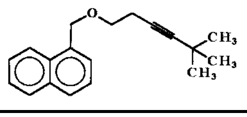
[a] Molecules **29**–**43** belong to training set while **44**–**50** belong to test set.

[b] Obs. act = observed biological activity is defined as log (1/MIC) where MIC is minimum inhibitory concentration against *C. albicans* in mM/L units.

[c] Pred. Act. = Predicted biological activity calculated using eq. (3) in Table 8.

The QSAR model developed for naphthalene class of terbinafine analogues includes thermodynamic and electronic parameters contributing significantly to the biological activity. For these inhibitors, Dipole_X i.e., dipole moment along the *x*-axis (perpendicular to fused ring system) seems to contribute significantly. The contribution to dipole moment is mainly from naphthyl ring electrons and electrons of the side chain. Thus the dipole interactions are important for better antifungal activity. Hf represents the chemical stability and reactivity of the molecules. At this point no information is available about chemical reactivity of the molecules with the target enzyme. However usage of Hf in the QSAR model actually indicates the chemical stability which means the conformational stability. Since no descriptor indicative of the energy difference between receptor binding conformation and low energy conformation is available, GFA uses Hf to model such effects in present QSAR study. Alog*P* is a thermodynamic parameter-represents hydrophobic character of the molecules. Alog*P* also accounts for solvation and entropic properties of the molecules. Descriptors Dipole_X and Alog*P* underline the importance of conformational rigidity of the side chain in these molecules. All the molecules in this class contain a double and triple bonded 'eneyne' system. This type of side chain imposes rigidity in the molecule and it is essential for better activity although terms describing this property do not appear in the QSAR equation. Analysis of benzothiophene analogues has resulted in

Table 4. Structures and biological activity of the molecules used in present study-propargyl amines[a]

Molecule	Structure	Obs. act.[b]	Pred. act.[c]	Molecule	Structure	Obs. act.[b]	Pred. act.[c]
51		0.307	0.319	**59**		0.881	0.616
52		0.304	0.291	**60**		0.912	0.798
53		0.608	0.311	**61**		0.304	0.213
54		0.274	0.404	**62**		0.330	0.259
55		0.305	0.249	**63**		0.306	0.277
56		0.319	0.638	**64**		1.256	0.868
57		1.544	1.448	**65**		0.315	0.562
58		0.264	0.642				

[a]Molecules **51**–**60** belong to training set while **61**–**65** belong to test set.

[b]Obs. act = observed biological activity is defined as log (1/MIC) where MIC is minimum inhibitory concentration against *Candida albicans* in mM/L units.

[c]Pred. act. = predicted biological activity calculated using eq (15) in Table 8.

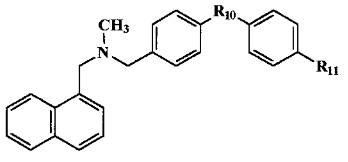
the identification of new property 'Apol' contributing to the activity. Spatial descriptors—PMI_Y, Vm—and electronic descriptor—Apol—constitute the model for biological activity against *C. albicans*. Presence of Apol term supports the data that the benzothiophene analogues show better activity than their corresponding naphthalene derivatives. Since antifungal activity of SE inhibitors is due to inhibition of the enzyme system, presence of charge transfer or electronic interactions during enzyme inhibition appear significant.

Analysis of benzyl amines class show that the shape related descriptors are optimal for activity and Shape RMS and COSV account for the 80% variance in the antifungal activity. The equations containing these two descriptors show better internal and external (test set) predictivity. Thus for these molecules, activity appears to be dependent on shape properties of the molecules. For better inhibition and activity, the molecules must be similar to the reference molecule. ShapeRMS (rms deviation between each molecule and shape reference molecule) term seems to represent the unique side chain in these molecules. Terminal phenyl ring and its orientation are important with respect to activity. The phenyl ring orientation similar to the conformation of the 'eneyne' side chain in terbinafine series is essential for activity. The molecules showing any deviation from this conformation appear to exhibit less activity.

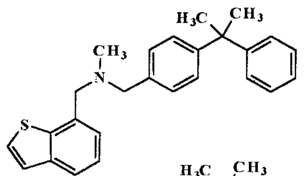
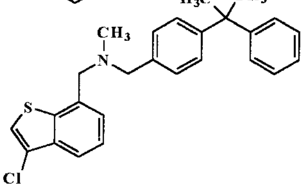
In the class of carbon atom analogues, the equations describing the activity exhibit better statistics but, the activity data being approximate, the probability of chance correlation is higher compared to other classes. COSV and PMI_X are the two descriptors constituting the model explaining the activity. COSV-shape related descriptor while PMI_X signifies the principal moment of inertia of the molecule along *x*-axis. Thus the orientation of the side chain along *x*-axis is significant for better activity. Statistical instability of the equations generated for this class supports the fact that the carbon atom analogues behave differently than other classes (being non-amines) and must be treated separately from other classes.

In the case of propargyl amines, the structurally rigid side chain typical of terbinafine analogues is absent and the variable number of linker atoms make these molecules conformationally flexible. QSAR analysis of these molecules indicates that principal moment of inertia (PMI_mag) and COSV descriptors account for 70% of the variance in biological activity. Presence of both the terms in the equation indicate the importance of

Table 5. Structures and biological activity of the molecules used in the present study—benzyl amines[a]

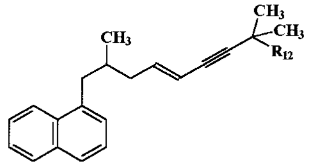


Molecule	R$_{10}$	R$_{11}$	Obs. act.[b]	Pred. act.[c]
66	-CH=CH-	-H	0.362	0.227
67	-C(=O)-	-H	0.349	0.147
68	-C(=CH$_2$)-	-H	0.365	0.336
69	-CH$_2$-	-H	0.639	0.613
70	-C((CH$_3$)$_2$)-	-H	2.484	2.224
71	-C((CH$_3$)$_2$)-	-CH$_3$	1.904	2.543
72	-C(CH$_2$CH$_2$)-	-H	2.180	1.531
73	-N(COCH$_3$)-	-H	0.381	0.874
74	-N(CH$_2$CH$_3$)-	-H	0.391	0.953
76	-(bond)-	-H	0.326	0.428
77	-O-	-H	0.341	−0.052
78	-NH-	-H	0.352	0.116

Molecule	Structure	Obs. act	Pred. act
75		2.770	2.377
79		2.502	2.074

[a] Molecules **66**–**75** belong to training set while **76**–**79** belong to test set.
[b] Obs. Act = Observed biological activity is defined as log (1/MIC) where MIC is minimum inhibitory concentration against *C. albicans* in mM/L units.
[c] Pred. act. = predicted biological activity calculated using eq (8) in Table 8.

Table 6. Structures and biological activity of the molecules used in the present study-carbon atom analogues[a]



Molecule	R$_{12}$	Obs. Act.[b]	Pred. Act.[c]
80	-COOCH$_3$	0.643	0.615
81	-COOH	0.626	0.613
82	-CH$_2$OH	0.622	0.659
83	-CONH$_2$	0.624	0.635
84	-CN	0.598	0.573
85	-OH	0.601	0.598
86	-NH$_2$	0.603	0.629
87	-NHCOCH$_3$	0.951	0.956
88	-N(C$_6$H$_5$)(CH$_3$)	0.709	1.392
89	-N((CH$_3$)$_3$)	1.269	1.261
90	-CONHCH$_3$	0.648	0.633
91	-CH$_2$NH$_2$	0.928	0.767
92	-N((CH$_3$)$_2$)	0.943	0.903

[a] Molecules **80**–**89** belong to training set while **90**–**92** belong to test set.
[b] Obs. act = observed biological activity is defined as log (1/MIC) where MIC is minimum inhibitory concentration against *C. albicans* in mM/L units.
[c] Pred. act. = predicted biological activity calculated using eq (11) in Table 8.

conformational rigidity of the side chain and this is significant since structurally rigid elements are absent in these molecules.

Table 7. Observed and predicted biological activities along with residuals for the test set molecules used in the present study

Molecule	Obs. act.[a]	Pred. act.[b]	Residuals[c]
22	1.695	1.192	0.503
23	0.786	0.852	−0.066
24	2.320	1.399	0.921
25	0.199	0.701	−0.502
26	1.087	0.866	0.221
27	1.394	1.358	0.036
28	0.493	0.397	0.096
44	0.220	1.345	−1.125
45	2.618	1.521	1.097
46	2.016	2.379	−0.363
47	2.086	2.275	−0.189
48	1.740	2.113	−0.373
49	0.220	1.096	−0.876
50	1.123	1.731	−0.608
61	0.304	0.213	0.091
62	0.330	0.259	0.071
63	0.306	0.277	0.029
64	1.256	0.868	0.388
65	0.315	0.562	−0.247
76	0.326	0.428	−0.102
77	0.341	−0.052	0.393
78	0.352	0.116	0.236
79	2.502	2.074	0.428
90	0.648	0.633	0.015
91	0.928	0.767	0.161
92	0.943	0.903	0.040

[a] Obs. act = observed biological activity is defined as log (1/MIC) where MIC is minimum inhibitory concentration against *C. albicans* in mM/L units.

[b] Pred. act. = predicted biological activity calculated using best equation for each class.

[c] Residuals calculated as difference between observed and predicted activity.

Analysis of 92 fungal squalene epoxidase inhibitors for activity against *C. albicans* has resulted in generation of different QSAR models for different chemical classes. This analysis is based on the fact that the antifungal activity of these compounds results from inhibition of the SE enzyme system. At this stage it would be better to compare the results of previously performed 3D-QSAR study with the models generated in this study.[11] CoMFA contour maps generated for activity against *C. albicans* showed the presence of positive charge favorable blue region around the naphthalene or benzothiophene ring systems indicating the significance of sulfur atom for activity. Thus the inhibition at enzyme active site level involves the electronic or charge interaction component. This fact is supported by the presence of electronic descriptors—'Apol'—in the QSAR models for benzothiophene class. Another important finding of the CoMFA study was the presence of large areas of steric interactions around the side chain of the inhibitors. QSAR models generated indicate the fact that the shape and volume related properties of the molecules are important for activity and together form a non-covalent component for antifungal activity. Another common factor between the two studies is that in both cases carbon atom analogues have been observed to be outliers so that their removal in CoMFA resulted in improvement in the predictivity.

Hypothetical mechanism of drug–enzyme interaction

Generation of QSAR models for different chemical classes of SE inhibitors has resulted in greater understanding about the mechanism of enzyme inhibition at molecular level. Although different types of descriptors (properties) contribute to QSAR models for different chemical classes, the most important of these appear to be the proper conformation and steric properties of the molecules. QSAR models developed for the naphthalene

Table 8. QSAR equations generated using genetic function approximation for the training set of the molecules in each class.[a]

No.	Equation	LOF	r^2	*F*-value	$r^2_{\text{predictive}}$
Terbinafine analogues—naphthalene class ($n=21$)					
1	BA = −0.856 −0.0043 (Hf) −0.112 (Dipole_Z) + 0.349 (Alog*P*)	0.186	0.758	16.663	0.401
2	BA = 0.484 + 0.0710 (Dipole_X) −0.0077 (Hf) + 0.192 (Alog*P*)	0.214	0.721	13.763	0.550
Terbinafine analogues—benzothiophene class ($n=15$)					
3	BA = −23.57 + 0.127 (Vm) −0.00457 (PMI-Y) −0.000816 (Apol)	0.342	0.816	16.298	0.342
4	BA = 18.21 −0.000922 (Apol) −0.0037 (PMI-mag) + 0.779 (DIFFV)	0.359	0.807	15.308	0.310
5	BA = −2.045 −0.00046 (Apol) + 1.0046 (RotlBonds) + 0.0125 (PMI_X)	0.487	0.738	10.315	0.276
Benzyl amines class ($n=10$)					
6	BA = 2.490 + 0.0148 (PMI_X) −0.884 (RotlBonds)	0.652	0.748	10.415	0.647
7	BA = 0.234 −0.00186 (PMI_mag) + 0.0142 (PMI_X)	0.767	0.704	8.328	0.751
8	BA = −8.152 + 1.637 (ShapeRMS) + 0.0295 (COSV)	0.464	0.821	16.031	0.896
9	BA = −0.527 + 0.510 (Alog*P*) −0.0177 (NCOSV)	0.643	0.752	10.601	0.711
10	BA = −2.801 + 0.0171 (COSV) −0.484 (HbondAcc)	0.662	0.745	10.209	0.638
Carbon atom analogues ($n=10$)					
11	BA = −1.888 + 0.00559 (PMI_X) + 0.005704 (COSV)	0.001	0.991	336.97	0.707
12	BA = −1.485 + 0.00781 (COSV)	0.019	0.757	21.779	0.413
Propargyl amines class ($n=10$)					
13	BA = −2.338 + 0.0208 (MolRef) + 0.00463 (COSV)	0.095	0.788	12.996	0.701
14	BA = −1.688 + 0.005811 (COSV) −0.175 (RotlBonds)	0.096	0.785	12.805	0.648
15	BA = −0.728 + 0.00036 (PMI_mag) + 0.00495 (COSV)	0.124	0.725	9.216	0.694

[a] BA in all the equations represent log (1/MIC) where MIC is in terms of mM/L.

Table 9. QSAR equations generated using multiple linear regression for the training set of the molecules in each class[a]

No.	Equation	sd[b]	r^2	*F*-value
Terbinafine analogues-naphthalene class ($n = 21$)				
1	BA = −0.856 −0.0043 (0.002) Hf −0.112 (0.038) Dipole_Z + 0.349 (0.119) Alog*P*	0.337	0.758	16.663
2	BA = 0.484 + 0.0710 (0.038) Dipole_X−0.0077 (0.002) Hf + 0.192 (0.168) Alog*P*	0.362	0.721	13.763
Terbinafine analogues- benzothiophene class ($n = 15$)				
3	BA = −23.57 + 0.127 (0.019) Vm −0.00457 (0.001) PMI_Y −0.000816 Apol	0.409	0.816	16.298
4	BA = 18.21 −0.00922 Apol −0.0037 (0.001) PMI_mag + 0.142 (0.022) DIFFV	0.420	0.807	15.308
5	BA = −2.045 −0.00046 Apol + 1.0046 (0.374) RotlBonds + 0.0125 (0.003) PMI_X	0.489	0.738	10.315
Benzyl amines class ($n = 10$)				
6	BA = 2.490 + 0.0148 (0.004) PMI_X −0.884 (0.460) RotlBonds	0.579	0.748	10.415
7	BA = 0.234 −0.00186 (0.001) PMI_mag + 0.0142 (0.004) PMI_X	0.628	0.704	8.328
8	BA = −8.152 + 1.637 (0.604) ShapeRMS + 0.0295 (0.006) COSV	0.489	0.821	16.031
9	BA = −0.527 + 0.510 (0.280) Alog*P* −0.0177 (0.005) NCOSV	0.575	0.752	10.601
10	BA = −2.801 + 0.0171 (0.004) COSV −0.484 (0.276) HbondAcc	0.583	0.745	10.209
Carbon atom analogues ($n = 10$)				
11	BA = −1.888 + 0.00036 (0.0002) PMI-X + 0.00658 (0.002) COSV	0.145	0.658	6.747
12	BA = −1.232 + 0.00684 (0.002) COSV	0.136	0.656	15.243
Propargyl amines class ($n = 10$)				
13	BA = −2.338 + 0.0208 (0.013) MolRef + 0.00463 (0.002) COSV	0.221	0.788	12.996
14	BA = −1.688 + 0.005811 (0.001) COSV −0.175 (0.108) RotlBonds	0.223	0.785	12.805
15	BA = −0.728 + 0.00036 (PMI_mag) + 0.00495 (0.002) COSV	0.252	0.725	9.216

[a] BA in all the equations represents log (1/MIC) where MIC is in terms of mM/L.

[b] sd is the standard deviation of the regression equation.

class of terbinafine analogues include thermodynamic and electronic properties as significant descriptors. Presence of dipole_X (dipole moment along x-axis) indicates the presence of electronic/π-π interaction between the molecules and active site residues. Although there is no direct involvement of conformational term but the presence of Alog*P*, Hf and dipole_X does underline the importance of conformational rigidity. This rigidity seems to be present as such in this class of molecules due to double and triple bonded systems.

The QSAR model for benzothiophene class indicates the significance of spatial descriptors—PMI_Y and Vm—for better activity. Thus the conformational and steric properties seem to play an important role contributing to the noncovalent component of the enzyme inhibition. Another important aspect is the presence of 'Apol' term. Apol (atomic polarisabilities) signifies the presence of charge transfer interaction between the benzothiophene ring (sulphur atom) and active site residues. This QSAR model supports the hypothesis that the benzothiophene class of molecules shows better activity than other classes particularly naphthalene ring analogues.

In the case of benzyl amines, propargyl amines and carbon atom analogues, shape related properties (molecular shape analysis descriptors) contribute significantly to activity. QSAR model for benzyl amines show the presence of MSA descriptors—ShapeRMS and COSV—as significant descriptors. Enzyme inhibition appears to be non-covalent in nature and depends upon the orientation of the side chain and volume occupied at the active site.

Carbon atom analogues show large variations in model generation. This class differs from other classes in that it lacks a tertiary amine group and this results in less activity for the molecules from this class. PMI-mag (principal moment of inertia) and COSV—common overlap steric volume—contribute significantly to the model. PMI_mag term indicates the orientation and conformational rigidity of the molecules being important for activity. The same is true for the case of propargyl amines where the presence of PMI_X term indicates that the flexible side chains must orient along the x-axis in proper conformation perpendicular to the fused ring system during interaction with enzyme.

In summary, QSAR models developed for different chemical classes indicate that the inhibition of SE enzyme is non-covalent in nature and depends upon the shape, steric and conformational properties of the molecules. The benzothiophene class of molecules could involve charge transfer or electronic interaction giving these molecules better activity than other classes.

The results from this study are significant as it involves handling of cell based activity data (MIC) with better activity predictions. Results from both these studies are complementary and can be used as a guide for developing molecules with better activity.

Conclusions

QSAR analysis of 92 molecules for their activity against *C. albicans* was performed using various physicochemical descriptors and GFA technique. The molecules were divided into five classes according to chemical structures. Separate analysis was performed for each chemical class and QSAR equations were generated. These equations were then analyzed for their statistical significance and making test set predictions. The results indicate that for naphthalene derivatives, the electronic and thermodynamic parameters appear to contribute significantly for biological activity. In the case of benzyl amines, the shape related properties ShapeRMS and COSV (common overlap steric volume) make significant contributions towards the activity. For two other classes—propargyl amines and carbon atom analogues PMI_X (moment of inertia) and COSV descriptors are involved in the QSAR models. One common property from the analysis of these classes is that the rigidity and orientation of the side chain is important for the activity. This is also true in the case of molecules where the side chains are either flexible (lack of rigid elements) or exhibit different orientation. Comparison of the results with the CoMFA study indicates that the information generated from these studies regarding antifungal activity is similar. Although the present study exhibits additional advantage over other studies, the results from both the studies are similar and can be used together for the design of molecules with better antifungal activity.

Experimental

Molecules

Ninety-two molecules belonging to different chemical classes were used in the study. These molecules have been reported as inhibitors of fungal SE enzyme. These molecules were divided into different chemical classes as terbinafine derivatives (naphthalene and benzothiophene class), benzylbenzyl amines, propargyl amines and carbon atom analogues. The molecules in each class were then divided into training set and test set. Structures of different molecules belonging to various chemical classes along with observed and predicted biological activity values are presented in Table 2–6.

Biological activity

Biological activity of these molecules in terms of minimum inhibitory concentration (MIC) in mg/L against various fungal pathogens has been reported.[6–10] MIC values against *C. albicans* were used in the present study. These MIC values were used as log (1/MIC) with MIC in terms of mM/L.

Molecular modeling

All molecular modeling studies were carried out using Cerius2 (version 3.5) running on Silicon Graphics O2 R5000 workstation.[30] All the molecules were imported within Cerius2. Partial charges were assigned using the charge equilibration method within Cerius2.[31] All the molecules were minimized until root mean square deviation 0.01 kcal/mol Å is achieved and used in the study.

Calculation of descriptors

Different types of descriptors were calculated for each molecule in the study table using default settings within Cerius2. These descriptors included electronic descriptors, spatial descriptors, structural descriptors, thermodynamic descriptors and molecular shape analysis (MSA) descriptors. A complete list of descriptors used in the study is given in Table 1.

MSA descriptors[32]

MSA descriptors were calculated using MSA module within Cerius2. Conformational analysis on all the molecules was performed using random sampling search with maximum number of conformers set equal to 10. Lowest energy conformer of the molecule with highest biological activity was used as reference for calculation of MSA descriptors. Molecules **11**, **35**, **57**, **75** and **89** were used as reference molecules.

Generation of QSAR models

QSAR analysis is responsible for generation of relationship between physicochemical properties and biological activity. In the present study, QSAR model generation was performed by GFA technique. GFA was performed using 20,000 crossovers, smoothness value of 1.00 and other default settings for each chemical class. GFA was asked to consider, predetermined number of terms in the equation depending upon the number of molecules in the training set. The set of equations generated for each chemical class was evaluated on the following basis:

a. LOF measure
b. variable terms in the equation
c. predictivity of the equation (predictive r^2 value)

Cross-validated r^2 values (r^2_{cv}) were calculated using the cross-validation test option in statistical tools supported within Cerius2.

Predictivity of the equations

Predictivity of each QSAR equation was assessed by predicting the activities of the test set molecules and these predictions were expressed as predictive r^2 values.

$$r^2_{\text{predictive}} = \frac{\text{SD-PRESS}}{\text{SD}}$$

where SD is the sum of squared deviation between biological activities of test set molecules and mean activity of the training set and PRESS is the sum of squared deviations between predicted and actual activities of the test set. Observed and predicted biological activities of test molecules alongwith residuals have been presented in Table 7.

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