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A CHEMOMETRIC APPROACH FOR PREDICTION OF ANTIFUNGAL ACTIVITY OF SOME BENZOXAZOLE DERIVATIVES AGAINST *Candida albicans*

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The purpose of the article is to promote and facilitate prediction of antifungal activity of the investigated series of benzoxazoles against Candida albicans. The clinical importance of this investigation is to simplify design of new antifungal agents against the fungi which can cause serious illnesses in humans. Quantitative structure activity relationship analysis was applied on nineteen benzoxazole derivatives. A multiple linear regression (MLR) procedure was used to model the relationships between the molecular descriptors and the antifungal activity of benzoxazole derivatives. Two mathematical models have been developed as a calibration models for predicting the inhibitory activity of this class of compounds against Candida albicans. The quality of the models was validated by the leave-one- out technique, as well as by the calculation of statistical parameters for the established model.

KEY WORDS: chemometricc, antifungals, benzoxazole derivatives, *Candida albicans*, molecular descriptors

INTRODUCTION

Predictions of antimicrobial properties of molecules based on their structure are the fundamental and most interesting objectives of chemistry. The conception that there exists a close relationship between bulk properties of compounds and their molecular structure is quite rooted in chemistry. This idea allows one to provide a clear connection between the macroscopic and the microscopic properties of matter, and thus has been firmly established as one of the central foundations of chemistry. Therefore, it is the basic aim of chemistry to attempt to identify these assumed relationships between chemical structure and physico-chemical properties and then to quantify them.

Benzoxazoles and their derivatives are well known to the chemists, mainly because of the broad spectrum of the antimicrobial properties exhibited by this class of compounds (1-12). Interest in the chemistry, synthesis and microbiology of this pharmacophore

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continues to be fuelled by their biological properties such as antifungal, antitubercular, antioxidant, antiallergic, and antiparasitic. It is also well known that these molecules are present in a variety of antitumoural, anthelmintic and herbicidal agents (1-12).

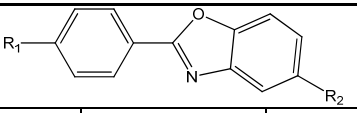
A large number of research studies are needed to analyze the pharmacophore present in these compounds using the Three Dimensional QSAR (quantitative structure-activity relationship) methods. The physicochemical properties predicted from structure are helpful in the search for new molecules of similar or increased biological activity. QSAR studies enable the investigators to establish reliable quantitative relationships, to derive a QSAR model, and predict the activity of novel molecules prior to their synthesis. These studies reduce the trial-and-error element in the design of compounds by establishing mathematical relationships between physical, chemical, biological, or environmental activities of interest and measurable or computable physicochemical, electronic, topological, or stereochemical parameters. The 3D-QSAR methodology has been successfully used to generate models for various chemotherapeutic agents (13-20).

In view of above and in continuation of our studies on QSAR analyses (21-29), the aim of this investigation was to study the quantitative effect of the structure on antifungal activity of some benzoxazole derivatives against *Candida albicans*.

EXPERIMENTAL

The structures of the benzoxazoles investigated in this study are presented in Table 1.

Table 1. The structures of the compounds studied



Compound	R_1	R_2
1	$N(CH_3)_2$	CH_3
2	CH_3	CH_3
3	C_2H_5	CH_3
4	OCH_3	CH_3
5	F	CH_3
6	$NHCOCH_3$	CH_3
7	$NHCH_3$	CH_3
8	$N(CH_3)_2$	CH_3
9	C_2H_5	Cl
10	$NHCOCH_3$	Cl
11	NH CH_3	Cl
12	Cl	Cl
13	NO_2	Cl
14	H	H
15	$C(CH_3)_3$	H
16	NH_2	H
17	$NHCH_3$	H
18	C_2H_5	NH_2
19	F	NH_2

The results of antifungal activity against *Candida albicans* (MTCC 183) for all the benzoxazole derivatives were taken from the literature (30). Minimum inhibitory concentration (MIC) of tested benzoxazoles is defined as the lowest concentration of the compound at which no growth of the strain. The negative logarithms of molar MICs ($\log 1/c_{MIC}$) were determined and used for further calculations.

Molecular Modeling

The molecular modeling study was performed using HyperChem 7.5 software (HyperCube Inc, Version 7.5) running on P-III processor (31). HyperChem includes a model builder that turns a rough 2Dsketch of a molecule into 3D. The created 3-D models were cleaned up and subjected to energy minimization using molecular mechanics (MM_2). The minimization is executed until the root mean square (RMS) gradient value reaches a value smaller than 0.1kcal/molÅ. The Austin Model-1 (AM-1) method was used for re-optimization until the RMS gradient attains a value smaller than 0.0001kcal/molÅ using MOPAC. The lowest energy structure was used for each molecule to calculate molecular descriptors.

Generation of the Descriptors

The numerical descriptors for each compound in the data set were calculated using the software HyperChem (31), Dragon (32) and CS Chem Office Software version 7.0 (33). Since there was a 78 different descriptors for each compound (electronic, constitutional, hydrophobic, and topological), Pearson's correlation matrix was used as a qualitative model, in order to select the suitable descriptors for MLR analysis. One way to avoid data redundancy is to exclude descriptors that are highly intercorrelated with each other before performing statistical analysis.

Statistical Methods

The complete regression analysis was carried out by PASS 2005, GESS 2006, NCSS Statistical Softwares (34).

RESULTS AND DISCUSSION

The results of the antifungal studies of 19 benzoxazole derivatives against *Candida albicans* are summarized in Table 2. As is evident, all the compounds show noteworthy antifungal activities against the tested fungi. Consequently, the compounds with high $\log 1/c_{MIC}$ (or low MIC) are the best antifungals.

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Table 2. Data of the experimental and predicted values of $\log 1/c_{MIC}$

Compound	Antifungal activity		
	$\log 1/c_{MIC}$ exper.	$\log 1/c_{MIC}$ predict.	Residuals
1	4.005	4.015	0.010
2	3.950	3.955	-0.005
3	3.977	3.973	0.004
4	3.980	3.992	-0.012
5	3.958	3.964	-0.006
6	4.027	4.037	-0.010
7	3.979	3.961	0.018
8	4.004	3.998	0.006
9	4.013	3.996	0.017
10	4.059	4.053	-0.006
11	4.015	4.011	0.004
12	4.024	4.009	0.015
13	4.040	4.050	-0.010
14	3.892	3.909	-0.017
15	4.001	4.005	-0.004
16	3.924	3.924	0.000
17	3.952	3.955	-0.003
18	3.979	3.962	0.017
19	3.960	3.960	0.000

In order to identify the effect of the chemical structure on the inhibitory activity, QSAR studies of title compounds were performed. A set of benzioxazoles consisting of 19 molecules was used for multilinear regression model generation. An attempt has been made to find structural requirement for inhibition of *Candida albicans* using QSAR Hansch approach on benzoxazole derivatives. Different physicochemical, steric, electronic, and structural molecular descriptors were used as independent variables and were correlated with antifungal activity. From the QSAR study of the series of benzoxazoles, two best biparametric models were derived. Both the models include lipophilicity descriptor (log*P*). The specifications for the best-selected MLR models are shown in Table 3.

Table 3. Best MLR models for the prediction of antifungal activity

Model	Coefficient		n	r	S	F
1	Intercept	0.7685	19	0.9758	0.0767	128.6125
	logP	0.9314				
	MR	0.0198				
2	Intercept	0.0715	19	0.9759	0.0774	127.3176
	logP	0.8941				
	HE	0.0033				

But, only high correlation coefficient is not enough to select the equation as a model and hence various statistical approaches were used to confirm the robustness and practical applicability of the equations. The statistical validity of the resulting models, as given in Table 3, is determined by *r*, *s*, and F. It is noteworthy that all these equations were derived using the entire data set of compounds (n = 19) and no outliers were identified. The *F*-value presented in Table 3 is found statistically significant at 99% level since all the calculated *F* values are higher as compared to the tabulated values.

For the testing the quality of the predictive power of selected MLR models the LOO procedure was used (Table 4). The PRESS value above can be used to compute an r^2_{CV} statistic, called r^2 cross-validated, which reflects the prediction ability of the model. This is a good way to validate the prediction of a regression model without selecting another sample or splitting the data. In this study, the r^2_{adj} and r^2_{CV} are taken as a proof of the high predictive ability of the QSAR models. A high value of these statistical characteristic (> 0.5) is considered as a proof of the high predictive ability of the models. The adjustable correlation coefficient (r^2_{adj}) tells us the statistical significance of incorporated physicochemical descriptor in MLR. It takes into account the adjustment of the conventional correlation coefficient (r^2). PRESS is an acronym for prediction of the sum of squares. It is used to validate a regression model with regard to its predictability.

Table 4. Cross-validation parameters

Model	PRESS	SSY	PRESS/SSY	S_{PRESS}	r^2_{CV}	r^2_{adj}
1	0.1201	1.6764	0.0716	0.0795	0.9284	0.9345
2	0.1449	1.6764	0.0864	0.0873	0.9127	0.9330

Thus, the high value of LOO r^2_{CV} is the necessary condition for a model to have a high predictive power, but it is not a sufficient condition. The only way to estimate the true predictive power of a model is to test its ability to predict accurately the inhibitory activities of compounds.

In order to verify the predictive power of the developed model, the predicted $\log 1/c_{MIC}$ values of benzoxazole investigated were calculated by using models 1 and 2 and compared with the experimental values (Table 2). The data presented in Table 2 show that the observed and the estimated activities are very close to each other. The residual activity (the difference between experimentally observed log ($1/c_{MIC}$) and QSAR calculated log ($1/c_{MIC}$)) is less than or equal to 0.018. Further, Fig. 1 shows the plot of the linear regression of the predicted versus experimental values of the antifungal activity of the investigated benzoxazoles.

To investigate the existence of a systemic error in developing the QSAR models, the residuals of predicted values of inhibitory activity were plotted against the experimental values in Figure 2. The propagation of the residuals on both sides of zero indicates that no systemic error exists in the development of the regression models, as suggested by Jalali-Heravi and Kyani (35). It indicates that these models can be successfully applied to predict the antifungal activity of this class of molecules.

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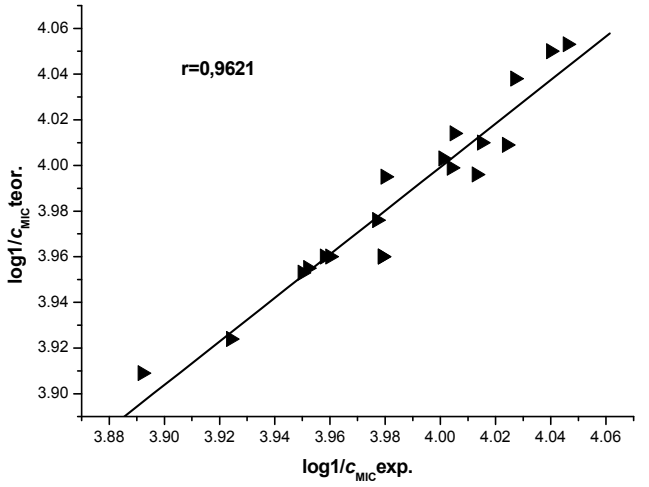

Figure 1. Plot of the predicted vs. the experimentally observed antifungal activity against *Candida albicans*

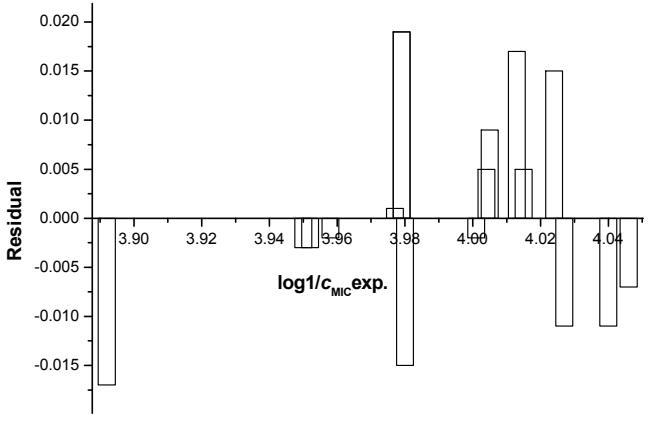

Figure 2. Plot of the residual values against the experimentally observed log1/c_{MIC} values

The positive contribution of log*P* in both the proposed equations thus suggests its significant participation in the inhibitory activity. The results clearly indicate that the compounds with higher lipophilicity values exhibited increased inhibitory action on the growth of the tested fungi. The other descriptors, *MR* and *HE,* were effective if combined with log*P*. Both the descriptors are the indicators of lipophilicity/hydrophobicity. They may be related to the binding between drug and receptor because the polarity is an essential factor to bind active site of the receptor molecule.

The results indicate the possibility of applying the chemometric techniques for a successful prediction of antifungal activity of the investigated series of benzoxazoles against *Candida albicans.* The results illustrate that the MLR technique is appropriate to create fine QSAR models for predicting the inhibitory activity of different compounds, and that is useful for drug design and medicinal chemistry.

CONCLUSIONS

From the results discussed above, it can be concluded that the different substituted benzoxazole derivatives showed *in vitro* considerable inhibitory activity against *Candida albicans.* Molecular modeling and QSAR analysis were performed to find the quantitative effects of the molecular structure of the compounds on their antifungal activity. Various physicochemical parameters, especially partition coefficient, molar refractivity and hydration energy can be used successfully for modeling antifungal activity of benzoxazoles. Two best QSAR mathematical models are used to predict inhibitory activity of the investigated benzoxazoles, and close agreement between experimental and predicted values was obtained. The low residual activity and high cross-validated r^2 values (r^2_{CV}) observed indicate the predictive ability of the developed QSAR models. This means that these models can be successfully applied to predict the antifungal activity of this class of molecules.

Acknowledgements

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ХЕМОМЕТРИЈСКИ ПРИСТУП У ПРЕДВИЂАЊУ АНТИФУНГАЛНЕ АКТИВНОСТИ НЕКИХ ДЕРИВАТА БЕНЗОКСАЗОЛА ПРЕМА *Candida albicans*

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Циљ овог рада је предвиђање антифунгалне активности испитиване серије бензоксазола према *Candida albicans*. Клинички значај ових испитивања је поједноставити дизајнирање нових антифунгалних агенаса који су узрочници многих озбиљних обољења код људи. QSAR (quantitative structure-activity relationship) анализа изведена је на деветнаест деривата бензимидазола. Вишеструка линеарна регресија коришћена је за моделовање зависности између молекулских дескриптора и антифунгалне активности деривата бензоксазола. Дефинисана су два математичка модела за предвиђање инхибиторне активности ове групе једињења према *Candida albicans*. Квалитет модела потврђен је LOO (leave one out) техником, као и израчунавањем статистичких параметара за постављене моделе.

Кључне речи: хемометрија, антифунгална активност, деривати бензоксазола, *Candida albicans*, молекулски дескриптори.

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