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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP OF SOME 1-BENZYL BENZIMIDAZOLE DERIVATIVES AS ANTIFUNGAL AGENTS

Sanja O. Podunavac-Kuzmanović , Dijana J. Barna, Dragoljub D. Cvetković

In the present study, the antifungal activity of some 1-benzylbenzimidazole derivatives against yeast Saccharomyces cerevisiae was investigated. The tested benzimidazoles displayed in vitro antifungal activity and minimum inhibitory concentration (MIC) was determined for all the compounds. Quantitative structure-activity relationship (QSAR) has been used to study the relationships between the antifungal activity and lipophilicity parameter, logP, calculated by using CS Chem-Office Software version 7.0. The results are discussed on the basis of statistical data. The best QSAR model for prediction of antifungal activity of the investigated series of benzimidazoles was developed. High agreement between experimental and predicted inhibitory values was obtained. The results of this study indicate that the lipophilicity parameter has a significant effect on antifungal activity of this class of compounds, which simplify design of new biologically active molecules.

KEY WORDS: 1-Benzylbenzimidazole, lipophilicity, antifungal activity, quantitative structure-activity relationship, *in vitro* studies.

INTRODUCTION

Benzimidazoles are a group of molecules which have shown potential for application in a variety of pharmacological targets. They are of wide interest because of their diverse biological activity and clinical applications. Biologically active benzimidazoles have been known for a long time and they can act as bacteriostats or bactericides, as well as fungicides (1-9). This ring system was proved to be very important as it is involved in numerous antiparasitic, antitumoral and antiviral drugs (10,11). It is also well known that these molecules are present in a variety of antioxidant and antiallergic agents (12-14). Many derivatives of benzimidazole show antiparasitic (15) and antiprotozoal (16) activities. In recent years, benzimidazole derivatives have attracted particular interest due to their anticancer activity or as potential *in vitro* anti-HIV agents (17-18).

Dr. Sanja O. Podunavac-Kuzmanović, Assoc. Prof., Faculty of Technology, 21000 Novi Sad, Bul. Cara Lazara 1, Serbia, M.Sc. Dijana Barna, Chief of Laboratory, Institute of Public Health, 24000 Subotica, Zmaj Jovina 30, Serbia, Mr. Dragoljub D. Cvetković, Ass., Faculty of Technology, 21000 Novi Sad, Bul. Cara Lazara 1, Serbia

A large number of research studies are needed to analyze the pharmacophore present in these compounds using the Three Dimensional QSAR methods (19-20). The understanding of the role of chemical structure in biological activity is very important. Predictions of biological and physicochemical properties of molecules from their structures are the fundamental and one of the most interesting objectives of chemistry. 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 structure-activity 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 parameters such as physicochemical, electronic, topological, or stereochemistry. 3D-QSAR methodology has been successfully used to generate models for various chemotherapeutic agents.

Octanol-water partition coefficient, referred to as log*P*, is a frequently used parameter in organic synthetic chemistry (21-22). It is a quantitative descriptor of lipophilicity, one of the key determinants of pharmacokinetic properties. Log*P* is commonly used in QSAR studies and drug design, since this property is related to drug absorption, metabolism, bioavailability, and toxicity. This parameter is also used in many environmental studies to determine the environmental fate of chemicals. By knowing exact values for this parameter, it is possible to predict the inhibitory activity of a drug.

In this context, the aim of the present study was to investigate the activity of different substituted 1-benyzlbenzimidazoles against yeast *Saccharomyces cerevisiae* and study the quantitative effect of lipophilicity on antifungal activity. The main objective was to establish a quantitative lipophilicity-inhibitory activity relationships and derive a high-quality model which would link the lipophilicity of these compounds with their inhibitory activity.

EXPERIMENTAL

Molecules

The structures of the benzimidazoles tested in this study are presented in Table 1. The investigated compounds were synthesized by a procedure described earlier (23).

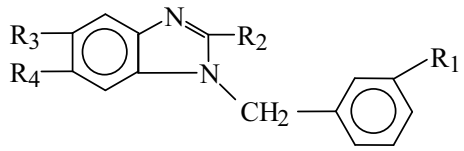
Antifungal activity

All the benzimidazole derivatives were tested for their *in vitr*o growth inhibition activity against yeast *Saccharomyces cerevisiae* (ATCC 24860). For the evaluation of the antifungal activities of the samples, agar disc diffusion method was used as described by NCCLS (24).

The strains were grown on Sabouraud Dextrose slants for 24 hours at 25 ^{o}C and checked for purity. After incubation the cells were washed from the surface of agar and suspended in a sterile phisyological solution. The number of cells in 1 cm^3 of suspension for inoculation, measured by Mc Farland nephelometer, was $1 \cdot 10^7$ cfu cm^{-3}. The 1 cm^3 of this suspensions was homogenized with 9 cm^3 of melted (45^{o}C) Sabouraud Dextrose

Agar and poured into Petri dishes. On the surface of the agar the 6 mm diameter sterile paper discs (Hi Media, Mumbai, India) were put and impregnated with 10^{-3} cm^3 of samples.

Table 1. The structures of the compounds studied



Compound	R_1	R_2	R_3	R_4
I-CH_3	CH_3	H	CH_3	CH_3
I-Cl	Cl	H	CH_3	CH_3
I-F	F	H	CH_3	CH_3
I-OCH_3	OCH_3	H	CH_3	CH_3
II-CH_3	CH_3	NH_2	H	H
II-Cl	Cl	NH_2	H	H
II-F	F	NH_2	H	H
II-OCH_3	OCH_3	NH_2	H	H
III-CH_3	CH_3	NH_2	CH_3	CH_3
III-Cl	Cl	NH_2	CH_3	CH_3
III-F	F	NH_2	CH_3	CH_3
III-OCH_3	OCH_3	NH_2	CH_3	CH_3

The plates were incubated for 24-47 hours at 25 ^oC, and the diameter of the resulting inhibition zone (including the disc) was measured (in mm). The evaluation of antifungal activities of samples was carried out in three repetitions.

Minimum inhibitory concentration (MIC) was determined by the agar dilution method according to guidelines established by the NCCLS standard M7-A5 (25). The MIC of tested benzimidazoles is defined as the lowest concentration of the compound at which no growth of the strain is observed in time and under specified experimental conditions. Stock solutions of the compounds were prepared in dimethylformamide (DMF). Further dilutions were performed with distilled water. The inoculated plates were then incubated at 35^oC for 16-20 h. A control (using DMF without any test compound) was included for each organism. It was determined that the solvent had no activity against any of the test microorganisms. The negative logarithms of molar MICs ($\log 1/c_{MIC}$) were determined and used for further calculations.

Molecular Modelling and logP Calculations

Molecular modelling studies were performed by using CS Chem-Office Software version 7.0 (Cambridge software) running on a P-III processor (26). All molecules were constructed by using Chem Draw Ultra 7.0 and saved as the template structure. For every compound, the template structure was suitably changed considering its structural features, copied to Chem 3D 7.0 to create a 3-D model and, finally, the model was cleaned up and subjected to energy minimization using molecular mechanics (MM_2). The minimization was executed until the root mean square (RMS) gradient value reached a value smaller than 0.1 kcal/mol·A. The Austin Model-1 (AM-1) method was used for re-optimization until the RMS gradient attained a value smaller than 0.0001 kcal/mol·A using MOPAC. The lowest energy structure was used for each molecule to calculate lipophilicity parameters (Table 2).

Table 2. Data of the lipophilicity parameters used in this study and the experimental and predicted values of $\log 1/c_{MIC}$

Compound	Lipophilicity	Antifungal activity		
	log*P*	Log1/c_{MIC} exper.	log1/c_{MIC} predict.	Residuals
I-CH_3	4.24	4.604	4.595	0.009
I-Cl	4.31	4.638	4.612	0.026
I-F	3.91	4.611	4.657	-0.046
I-OCH_3	3.63	4.330	4.268	0.062
II-CH_3	3.44	3.971	4.003	-0.032
II-Cl	3.52	4.316	4.276	0.040
II-F	3.11	3.685	3.757	-0.072
II-OCH_3	2.83	3.405	3.368	0.037
III-CH_3	4.42	4.629	4.631	-0.002
III-Cl	4.49	4.662	4.638	0.024
III-F	4.09	4.335	4.343	-0.008
III-OCH_3	3.80	4.354	4.390	-0.036

Statistical Methods

The complete regression analysis was carried out by PASS 2005, GESS 2006, NCSS Statistical Softwares (27).

RESULTS AND DISCUSSION

The results of antifungal studies of 1-benzylbenzimidazoles against *Saccharomyces cerevisiae* are summarized in Table 2. As indicated, all the compounds show noteworthy antifungal activities against the tested yeast. Consequently, compounds with high $\log 1/c_{MIC}$ (or low MIC) are the best antifungals.

In this study, the $\log 1/c_{MIC}$ values were correlated against log*P* parameters calculated by using CS Chem-Office Software. Usually, the lipophilicity parameters are linearly related to the biological activity (MICs), but in a more general case this relationship is not linear.

Therefore, a complete regression analysis resorting to linear, quadratic and cubic relationships (Table 3) was made. It is apparent, from the correlation coefficients (r), that fitting equation improves when resorting to higher order (second or third) polynomials.

The statistical quality of the regression equations was justified by the parameters such as correlation coefficient (r), square of adjusted correlation coefficient (r^2_{adj}), standard error of estimate (s), square of cross-validation coefficient (Q^2) and probability factor related to F-ratio. Statistical data presented in Table 3 indicate that very good nonlinear equations were obtained.

For the estimation of the quality with regard to predictive ability of this model, the cross-validation statistical technique has been applied. This is the most common validation technique, where a number of modified data sets are created by deleting, in each case, one or smaller group of objects from the data in such a way that each object is taken away once and only once. For each reduced data set, the model is calculated, and responses for the deleted objects are predicted from the model.

The simplest and most general cross-validation procedure is the leave-one-out technique (LOO technique) (28). This method uses cross-validated fewer parameters: PRESS (predicted residual sum of squares), SSY (total sum of squares deviation), Q^2 and r^2_{adj}. PRESS is an important cross-validation parameter as it is a good approximation of the real predictive error of the models. Its value being less than SSY points out that the model predicts better than chance and can be considered statistically significant. The present models have PRESS<<SSY. From the PRESS and SSY, Q^2 can be easily calculated:

$$Q^2 = 1 - \text{PRESS/SSY} \qquad [1]$$

The high Q^2 value observed for quadratic QSAR model is indicative of its reliability in prediction of inhibitory activity. For the verification of the predictive power of the developed model, predicted $\log 1/c_{MIC}$ values of benzimidazoles investigated were calculated by using quadratic equation and compared with the experimental values (Table 2). Based on the magnitude of residue, there is a high agreement between the observed and calculated inhibitory activity. All the presented results suggest dependence of the lipophilicity parameters on biological behaviour of benzimidazoles investigated. By knowing exact values of these parameters, we can accurately predict inhibitory activity.

The derived relationship between lipophilicity parameters and inhibitory activity can be used for estimating the antifungal activity of other benzimidazoles. The results illustrate that the regression technique is adequate to create fine QSAR model for predicting the inhibitory activity of different compounds, which is useful for drug design and medicinal chemistry.

Table 3. Statistical data calculated for the relationship between $\log 1/c_{MIC}$ and logP values

Equation	*a*	*b*	*c*	*d*	*r*	r^2_{adj}	*s*	Q^2	*F*
$\log 1/c_{MIC}=a\ \log P+b$	0.722	1.539	-	-	*0.930*	*0.852*	0.157	*0.799*	*64.549*
$\log 1/c_{MIC}= a\log P^2+b\log P+c$	-0.418	3.827	*-4.111*	-	*0.968*	*0.922*	0.114	*0.898*	*66.068*
$\log 1/c_{MIC}= a\ \log P^3 + b\log P^2 + c\log P+d$	-0.011	-0.239	*3.375*	*-3.574*	*0.968*	*0.912*	0.121	*0.789*	*39.157*

CONCLUSIONS

QSAR analysis was performed to estimate the quantitative effects of the lipophilicity parameters, log*P*, of the different substituted 1-benzylbenzimidazoles on their antifungal activity against *Saccharomyces cerevisiae*. log*P* values were calculated for each molecule, and three high-quality mathematical models relating the inhibitory activity, $\log 1/c_{MIC}$, and log*P* were defined. For the estimation of the predictive ability of these models, the cross-validation statistical technique was applied. Comparison of the linear, quadratic and cubic relationships showed that the quadratic equation was the most appropriate for prediction of antifungal activity of the investigated class of molecules. It is concluded that the lipophilicity parameter is a convenient quantity for modelling inhibition for the present set of benzimidazole derivatives. The developed QSAR mathematical model is used to predict inhibitory activity of the benzimidazoles investigated, and a high agreement between experimental and predicted values was obtained. It indicates that this model can be successfully applied to predict the antifungal activity of this class of molecules.

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КВАНТИТАТИВНА ЗАВИСНОСТ ИЗМЕЂУ СТРУКТУРЕ И АНТИФУНГАЛНЕ АКТИВНОСТИ НЕКИХ ДЕРИВАТА 1-БЕНЗИЛБЕНЗИМИДАЗОЛА

Сања О. Подунавац-Кузмановић, Дијана Ј. Барна, Драгољуб Д. Цветковић

У овом раду испитана је антифунгална активност неких деривата 1-бензилбензимидазола на квасац *Saccharomyces cerevisiae.* Испитивани бензимидазоли *in vitro* показују антифунгалну активност и за сва једињења је одређена минимална инхибиторна концентрација. Применом QSAR (quantitative structure-activity relationship) анализе испитане су зависности између антифунгалне активности и параметара липофилности, log*P*, који су израчунати применом CS Chem-Office 7.0 програмског пакета. Резултати су продискутовани на основу статистичких података. Развијен је математички модел за предвиђање антифунгалне активности у оквиру испитиване серије бензимидазола. Добијено је веома добро слагање између експериментално одређених и предвиђених вредности инхибиторних активности. Резултати ових испитивања показују да параметар липофилности има значајан утицај на антифунгалну активност испитиване класе једињења, што олакшава дизајнирање нових биолошки активних молекула.

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