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CORRELATIONS BETWEEN THE LIPOPHILICITY AND THE INHIBITORY ACTIVITY OF DIFFERENT SUBSTITUTED BENZIMIDAZOLES

2-Amino and 2-methylbenzimidazole derivatives were tested in vitro for their inhibitory activity against the bacteria Bacillus cereus. The minimum inhibitory concentration (MIC) was determined for all compounds. The lipophilicity descriptors were calculated by using CS Chem-Office Software, version 7.0. The stepwise regression method was used to derive the most significant model as a calibration model for predicting the antibacterial activity of this class of compounds. A complete regression analysis resorting to linear and quadratic relationships was made. Theoretical models were validated by leaving one out (LOO) technique, as well as by the calculation of statistical parameters for the established models. The best QSAR model for the prediction of an inhibitory activity of the investigated series of benzimidazoles was developed. A high agreement between the experimental and predicted inhibitory values was obtained. The results indicated that the antibacterial activity could be modeled using the lipophilicity descriptor.

Key words: benzimidazole; antibacterial activity; quantitative structure-activity relationship; lipophilicity; in vitro studies; Bacillus cereus.

The benzimidazole functional group plays important roles in numerous bioactive compounds. The literature indicated that the benzimidazole nucleus is an essential part of many clinically useful chemotherapeutic agents. Currently, benzimidazole derivatives are the subject of sustained interest due to the vast range of their potential activities. Biologically active benzimidazoles have been known for a long time and they can act as bacteriostats or bactericides [1-15]. For example, thiabendazol, triclabendazole and mebendazole are effective anthelmintic agents [16], and are, as well as lansoprazole and omeprazole, used as antiulcer agents [17,18]. Moreover, compounds containing a benzimidazole ring were found to have antifungal [19-21], antitubercular [22], antioxidant [23,24], antiallergic [25,26], and antiparasitic [27] activities.

Although a variety of benzimidazole derivatives are known, the development of new and convenient strategies to synthesize more biological active benzimidazoles is of considerable interest. The quantitative structure-activity relationship (QSAR) study is a useful tool for a rational search of bioactive molecules. The success of QSAR method is the possibility to estimate the characteristics of new chemical compounds without the need to synthesize and test them. This analysis represents an attempt to relate structural descriptors of compounds with their physicochemical properties and biological activities. It is widely used for the prediction of physicochemical properties in chemical, pharmaceutical, and environmental spheres. This method includes the data collection, the molecular descriptor selection, the correlation model development, and finally the model evaluation. QSAR studies have a predictive ability and simultaneously provide a deeper insight into the mechanism of drug receptor interactions [28,29].

In this context, the aim of the present work was to investigate the activity of different substituted benzimidazoles against Gram-positive bacteria *Bacillus cereus* and to study the quantitative effect of lipophilicity on an antibacterial activity. The central objective of the study was to select the most significant QSAR model which links the structure of these compounds with their inhibitory activity.

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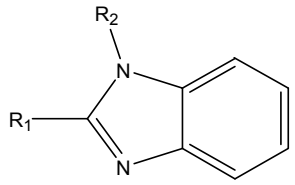
EXPERIMENTAL

Material and methods

The structures of the benzimidazoles tested in this study are presented in Table 1. All the compounds, except **1** and **8** were synthesized by the general procedure described by Vlaović [30]. 2-Aminobenzimidazole (**1**) and 2-methylbenzimidazole (**8**) were of analytical reagent grade, commercially available.

Table 1. The structures of the compounds studied



Compound	R_1	R_2
1	NH_2	H
2	NH_2	C_6H_5-CH_2
3	NH_2	4-CH_3-C_6H_4-CH_2
4	NH_2	4-Cl-C_6H_4-CH_2
5	NH_2	C_6H_5-CO
6	NH_2	4-CH_3-C_6H_4-CO
7	NH_2	4-Cl-C_6H_4-CO
8	CH_3	H
9	CH_3	C_6H_5-CH_2
10	CH_3	4-CH_3-C_6H_4-CH_2
11	CH_3	4-Cl-C_6H_4-CH_2
12	CH_3	C_6H_5-CO
13	CH_3	4-CH_3-C_6H_4-CO
14	CH_3	4-Cl-C_6H_4-CO

Antibacterial investigations

All the benzimidazole derivatives were evaluated for their *in vitro* growth inhibitory activity against Gram-positive bacteria *Bacillus cereus* (ATCC 10876).

Antibacterial activities of the compounds were tested by the disc-diffusion method under standard conditions using Mueller-Hinton agar medium as described by NCCLS [31]. The investigated isolate of bacteria was seeded in the tubes with nutrient broth (NB). A volume of 1 cm^3 of seeded NB was taken and homogenized in tubes with 9 cm^3 of melted (45 °C) nutrient agar (NA). The homogeneous suspension was poured out into Petri dishes. The discs of filter paper (diameter 5 mm) were ranged on cool medium. After cooling on formed solid medium, 2×10^{-5} dm^3 of the investigated compounds (γ = 1000 μg/ml) were placed with a micropipette. After the incubation in a thermostat at 37 °C for 24 h, inhibition (sterile) zone diameters (including disc) were measured (in mm). An inhibition zone diameter over 8 mm indicates that the tested compound is active against microorganisms. Every test was done in three replications.

The minimum inhibitory concentration (*MIC*) was obtained by the agar dilution method according to the guidelines established by the NCCLS standard M7-A5 [32]. The *MIC* of tested benzimidazoles is defined as the lowest concentration of the compound at which no growth of the strain was observed in a specified period of 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 37 °C for 20-24 h. A control, using DMF without any test compound, was included. There was no inhibitory activity in the wells containing only DMF. The *MIC* values of the benzimidazoles tested were obtained as μg/ml. For further QSAR analyses, negative logarithms of molar *MIC*s (log $(1/c_{MIC})$) were used. In order to classify the antibacterial activity we established comparisons with antibacterial agents currently employed in a therapeutic treatment. The *MIC*s were compared with standard discs of ampicillin and gentamicin which were screened under similar conditions as reference drugs.

Molecular modeling and log *P* calculations

Molecular modeling studies were performed using CS Chem-Office Software version 7.0 (Cambridge software) running on a P-III processor [33]. All molecules were constructed by using Chem Draw Ultra 7.0 and saved as template structures. 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. The Austin Model-1 (AM-1) method was used for re-optimization until the *RMS* gradient attained the value smaller than 0.0001 kcal/mol using MOPAC. The lowest energy structure was used for each molecule to calculate *C*log *P* values by using ChemDraw Ultra 7.0 (Table 2).

Table 2. Data of lipophilicity parameters

Compound	*C*log *P*
1	0.99
2	2.96
3	3.44
4	3.52

Table 2. Continued

Compound	*C*log *P*
5	2.84
6	3.32
7	3.39
8	1.48
9	3.45
10	3.94
11	4.01
12	3.33
13	3.81
14	3.89

Statistical methods

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

RESULTS AND DISCUSSION

The values of antibacterial activity of the benzimidazole derivatives against the tested Gram-positive bacteria are summarized in Table 3. The screening results reveal that reported compounds expressed the inhibitory activity against *Bacillus cereus*. Consequently, the compounds with high log ($1/c_{MIC}$) are the best antibacterials.

generation as they belong to a different structural series. The inhibitory activity data determined as μg/ml were first transformed to negative logarithms of molar *MIC*s (log ($1/c_{MIC}$)), which was used as a dependent variable in the QSAR study. The lipophilicity parameters were used as independent variables and were correlated with the antibacterial activity. An attempt has been made to find a structural requirement for the inhibition of Gram-positive *B. cereus* using the QSAR Hansch approach on benzimidazole derivatives. To obtain the quantitative effects of the lipophilicity parameter of benzimidazole derivatives on their antibacterial activity, QSAR analysis with log *P* was operated.

Usually, lipophilicity parameters are linearly related to pharmacological activity (*MIC*s), but in a more general case this relationship is not linear [35]. Therefore, a regression analysis was made resorting to linear and quadratic. The statistical quality of the generated models, as depicted, is determined by statistical measures: correlation coefficient (*r*), the standard error of estimation (*s*), and *F*-test (Fisher's value) for statistical significance [36-38]. The correlation coefficient (or coefficient of a multiple determination) is a relative measure of fit by the regression equation. Correspondingly, it represents the part of the variation in the observed data that is explained by the regression. The correlation coefficient values closer to 1.0 represent a

Table 3. Antibacterial screening summary

Compound	log ($1/c_{MIC}$(exp))	log ($1/c_{MIC}$(predicted))	Residuals
1	3.425	3.377	0.048
2	4.854	4.990	-0.136
3	4.579	4.443	0.136
4	4.615	4.416	0.198
5	4.88	5.069	-0.189
6	4.604	4.614	-0.010
7	4.638	4.517	0.121
8	4.325	4.358	-0.033
9	4.551	4.427	0.123
10	3.277	3.381	-0.105
11	3.313	3.315	-0.002
12	4.577	4.601	-0.024
13	3.602	3.770	-0.168
14	3.637	3.596	0.041
Ampicillin	4.446	-	-
Gentamicin	5.787	-	-

In an effort to determine the role of lipophilicity on the inhibitory activity, QSAR studies of title compounds were performed. A set of benzimidazoles consisting of 14 compounds was used for model generation. Reference drugs were not included in the model

better fit of the regression. Standard deviation is measured by the error mean square, which expresses the variation of the residuals or the variation about the regression line. Thus, the standard deviation is an absolute measure of quality of fit and should have a low

value for the regression to be significant. The *F*-test reflects the ratio of the variance explained by the model and the variance due to the error in regression. High values of the *F*-test indicate that the model is statistically significant. It is observed that fitting equations improve when resorting to second order polynomials (Eq. (1)).

$$\log 1/c_{MIC} = -0.799\,C\log P^2 + 3.976\,C\log P + 0.224;$$
$$r = 0.971;\ s = 0.024;\ F = 92.16 \quad (1)$$

For the estimation of the quality with regard to a predictive ability of the best model (Eq. (1)), the cross-validation statistical technique has been applied (Table 4).

The simplest and most general cross-validation procedure is the leave-one-out technique (LOO technique). This method uses cross-validated fewer parameters: *PRESS* (predicted residual sum of squares), *SSY* (total sum of squares deviation), r^2_{CV} 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*, r^2_{CV} can be easily calculated:

$$r^2_{CV} = 1 - PRESS/SSY \quad (2)$$

Table 4. Cross-validation parameters (Eq. (1))

PRESS	*SSY*	*PRESS*/*SSY*	r^2_{CV}	r^2_{adj}
0.4064	4.7554	0.0855	0.9145	0.9334

The high r^2_{CV} value is indicative of its reliability in predicting the inhibitory activity.

To confirm the predictive power of a model, the inhibitory activity of 14 molecules included in the study was calculated by model 1, and the values are presented in Table 3. The data presented in Table 3 show that the observed and the estimated activities are very close to each other. The residual activity (difference between experimentally observed log ($1/c_{MIC}$) and QSAR calculated log ($1/c_{MIC}$) is less than equal to 0.198. Further, the plot of predicted log ($1/c_{MIC}$) values against the observed log ($1/c_{MIC}$) values also proves the superiority of the model expressed by Eq. (1) (Figure 1).

The analysis of the results indicates that the antibacterial activity exhibited by tested compounds is governed by the lipophilicity parameter, that is, log *P*. It can be concluded that a strong influence of the partition coefficient, log *P*, is important for the antibacterial activity and this parameter is usually related to the pharmacological activity. This evidence was clearly described in a lipid theory advanced by Meyer and Overton [39,40]. According to this theory, log *P* is the measure of hydrophobicity which is important for the penetration and distribution of the drug, but also for the interaction of the drug with receptors.

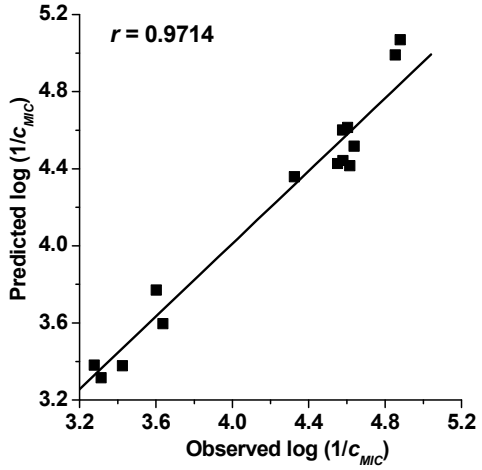


Figure 1. Plots of the predicted versus experimentally observed inhibitory activity against Bacillus cereus.

In order to investigate the existence of a systemic error in developing the QSAR models, the residuals of predicted log ($1/c_{MIC}$) were plotted against the observed log ($1/c_{MIC}$) values (Figure 2). The propagation of the residuals on both sides of the zero axis indicates that no systemic error in the development of regression models exists, as suggested by Jalali-Heravi and Kyani [41].

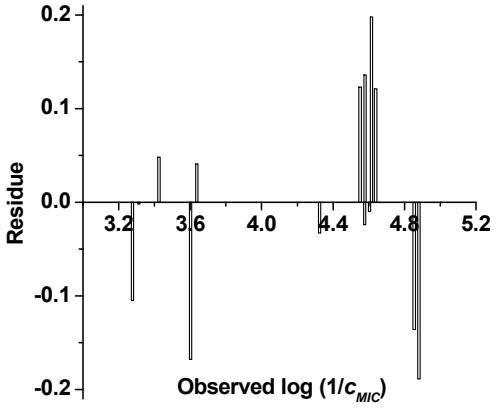


Figure 2. Plot of the residual values against the experimentally observed log $1/c_{MIC}$ values

This analysis represents an attempt to relate only lipophilicity descriptors of benzimidazole derivatives with their inhibitory activities. However, there are large number of descriptors which could be correlated

with an antibacterial activity. In the next step of our investigations, we focused our efforts on QSAR analysis of the above mentioned derivatives using a combination of various physicochemical, steric, electronic and structural molecular descriptors.

CONCLUSIONS

From the results and discussion made above, we conclude that 2-amino- and 2-methylbenzimidazole derivatives are effective *in vitro* against the Gram-positive bacteria *Bacillus cereus*. QSAR analysis was performed to estimate the quantitative effects of the lipophilicity parameter, log *P*, of different substituted 2-amino and 2-methylbenzimidazole derivatives on their inhibitory activity. log *P* values were calculated for each molecule, and a high-quality mathematical model relating the antimicrobial activity, log ($1/c_{MIC}$), and log *P* was defined. The comparison of linear and quadratic relationships showed that the quadratic equation was more appropriate for the prediction of the antibacterial activity of the investigated class of molecules. The validity of the models has been established by the determination of suitable statistical parameters. From the established QSAR, the inhibitory activity of the benzimidazoles investigated was calculated and a close agreement between experimental and predicted values was obtained. The low residual activity and high cross-validated r^2 values (r^2_{CV}) observed indicated the predictive ability of the developed QSAR models.

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