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QSAR MODELING OF ANTIBACTERIAL ACTIVITY OF SOME BENZIMIDAZOLE DERIVATIVES

A quantitative structure-activity relationship (QSAR) study has been carried out for a training set of 12 benzimidazole derivatives to correlate and predict the antibacterial activity of studied compounds against Gram-negative bacteria Pseudomonas aeruginosa. Multiple linear regression was used to select the descriptors and to generate the best prediction model that relates the structural features to inhibitory activity. The predictivity of the model was estimated by cross-validation with the leave-one-out method. Our results suggest a QSAR model based on the following descriptors: parameter of lipophilicity (logP) and hydration energy (HE). Good agreement between experimental and predicted inhibitory values, obtained in the validation procedure, indicated the good quality of the generated QSAR model.

Key words: benzimidazole; molecular descriptors; quantitative structure-activity relationship; antibacterial activity; Pseudomonas aeruginosa.

Quantitative structure-activity relationship (QSAR) and quantitative structure-property relationship (QSPR) studies are undoubtedly of great importance in modern chemistry and biochemistry. To obtain a significant correlation, it is essential that appropriate descriptors be used, regardless of whether they are theoretical, empirical or derived from readily available experimental characteristics of structures. Many descriptors reflect simple molecular properties and can thus provide insight into the physicochemical nature of the activity/property under consideration [1-3].

Activities used in QSAR include chemical measurements and biological assays. For example, biological activity can be expressed quantitatively as in the concentration of a substance required to give a certain biological response. Additionally, when physicochemical properties of structures are expressed by numbers, one can find a quantitative structure-activity relationship between properties and structures. The mathematical expression can then be used to predict the biological response of other chemical structures. QSAR are currently being applied in many disciplines, such as drug design and environmental risk assessment. Using QSAR, an estimate of the activity of a chemical from its molecular structure can be obtained; QSAR offers the possibility for screening a large number of chemicals in a short time and at low cost [4-7].

The benzimidazole nucleus, which is a useful structure for further research and for development of new pharmaceutical molecules, has received much attention in the last decade. Because of their antimicrobial activities, new benzimidazoles have been synthesized and investigated for medical applications. The position and type of the substituents on the benzimidazole ring are responsible for the variety of biological activities. Many derivatives of benzimidazole are well known as antibacterial agents, as well as this class of compounds have been found to show antimicrobial activities against Gram-positive and Gram-negative bacteria, primarily because of the potential bio-activity of benzimidazole-based ligands [8-19]. So, the incorporation of the imidazole and benzimidazole nuclei is an important synthetic strategy in drug discovery.

Synthesis of benzimidazoles fused to another heterocyclic ring has also attracted wide spread attention due to their diverse application as antioxidant [20,21], antifungal [22], antitubercular [23], anticancer [24,25] and antiallergic drugs [26]. Various benzimidazoles are also effective inhibitors of the growth of HIV-virus [27,28].

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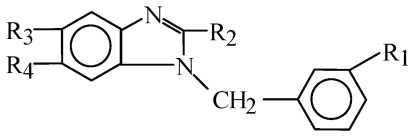
In view of the above and in continuation of our studies on inhibitory activities of benzimidazole derivatives [4-7], [11-18], as well as on correlation of molecular properties with activity, the objective of this investigation was to study the usefulness of QSAR in the prediction of the antibacterial activity of benzimidazole derivatives against Gram-negative bacteria *Pseudomonas aeruginosa.* The multiple linear regression (MLR) models have been developed as mathematical equations which relate chemical structure to the inhibitory activity.

EXPERIMENTAL

Material and methods

The investigated compounds (Table 1) were synthesized by procedure described earlier [29].

Table 1. Structural formulae of the compounds



Compound	R_1	R_2	R_3	R_4
1	CH_3	H	CH_3	CH_3
2	Cl	H	CH_3	CH_3
3	F	H	CH_3	CH_3
4	OCH_3	H	CH_3	CH_3
5	CH_3	NH_2	H	H
6	Cl	NH_2	H	H
7	F	NH_2	H	H
8	OCH_3	NH_2	H	H
9	CH_3	NH_2	CH_3	CH_3
10	Cl	NH_2	CH_3	CH_3
11	F	NH_2	CH_3	CH_3
12	OCH_3	NH_2	CH_3	CH_3

Antibacterial investigations

All the benzimidazole derivatives were evaluated for their in vitro growth inhibitory activity against bacteria *Pseudomonas aeruginosa* (ATCC 27853). Antibacterial activities of the compounds were tested by the disc-diffusion method under standard conditions using Mueller-Hinton agar medium as described by NCCLS [30].

The investigated isolate of bacteria was seeded in the tubes with nutrient broth (NB). 1 cm^3 of seeded NB was taken and homogenized in tubes with 9 cm^3 of melted (45 °C) nutrient agar (NA). The homogenous suspension was poured out in 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 were placed by micropipette. After incubation of 24 h in thermostat at 25-27 °C, inhibition (sterile) zone diameters (including disc) were measured (in mm). The inhibition zone diameter over 8 mm indicates the tested compound is active against microorganism. Every test was done in three replications.

Minimum inhibitory concentration (MIC) was performed by the agar dilution method according to guidelines established by the NCCLS standard M7-A5 [31].

The MIC of tested benzimidazoles is defined as the lowest concentration of the compound at which no growth of the strain as observed in a 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 concentration range of the compounds tested was between 6.25-125 μg/ml. The inoculated plates were than incubated at 35 °C for 16-20 h. A control using DMF without any test compound was included for each organism. There was no inhibitory activity in the wells containing only DMF. The MIC values of the benzimidazoles tested were obtained as μg/ml. In order to classify the antibacterial activity, we established comparisons with antibacterial agents currently employed in therapeutic treatment. The MICs were compared with Ampicillin and Gentamicin which were screened under similar conditions as reference drugs.

Molecular modelling

All molecular modeling studies were performed by using HyperChem 7.5 software (HyperCube Inc., Version 7.5) running on P-III processor [32]. HyperChem includes a model builder that turns a rough 2D sketch of a molecule into 3D. The created 3D models were cleaned up and subjected to energy minimization using molecular mechanics (MM2). The minimization is executed until the root mean square (RMS) gradient value reaches 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 attains a value smaller than 0.0001 kcal/mol·A using MOPAC. The lowest energy structure was used for each molecule to calculate molecular descriptors.

Descriptors generation

The numerical descriptors are responsible for encoding important features of the structure of the molecules and can be categorized as electronic, geometric, hydrophobic, and topological characters. Descriptors were calculated for each compound in the data set, using the software HyperChem [32], Dragon [33], and CS Chem Office Software version 7.0 [34].

Since there was a large number of descriptors for each compound, Pearson's correlation matrix was used as a qualitative model, in order to select the suitable descriptors for MLR analysis. The eight descriptors which were showing maximum correlation with inhibitory activity were chosen for further evaluation. The values of descriptors selected for MLR model are presented in Table 2 (molar refractivity (MR), polarizability (P), molar volume (MV), hydration energy (HE), total energy (TE), surface area grid (SAG), and partition coefficient (log *P*)).

Statistical methods

The complete regression analysis was carried out by PASS 2005, GESS 2006, NCSS Statistical software [35].

RESULTS AND DISCUSSION

The substituted benzimidazoles were first evaluated for *in vitro* antibacterial activity against Gram-negative bacteria *Pseudomonas aeruginosa*. The values of MIC are summarized in Table 3. The screening results reveal that all the compounds exhibited in vitro activity against the tested strain.

In an effort to determine the role of structural features, QSAR study was undertaken. A set of benzimidazoles consisting of 12 molecules was used for multilinear regression model generation.

The reference drugs were not included in model generation as they belong to a different structural series. Different physicochemical, steric, electronic, and structural molecular descriptors were used as independent variables and were correlated with antibacterial activity.

Developing a general model requires a diverse set of data, and thereby, a large number of descriptors have to be considered. Descriptors are numerical values that encode different structural features of the molecules. Selection of a set of appropriate descriptors from a large number of them requires a method,

Table 2. Values of molecular descriptors used in the regression analysis

Compound	MR	P	MV	HE	TE	DM	SAG	log*P*
1	87.25	30.78	811.60	-1.00	27.17	3.98	490.32	4.24
2	87.69	30.87	804.81	-1.86	26.87	3.974	429.54	4.31
3	83.10	28.85	777.39	-2.23	27.02	3.976	477.14	3.91
4	89.34	31.42	841.79	-3.68	28.92	3.978	507.17	3.63
5	81.56	28.46	744.54	-6.39	26.35	4.464	458.41	3.44
6	81.99	28.55	736.31	-7.16	26.03	4.427	450.08	3.52
7	77.40	26.53	704.51	-7.31	26.07	4.428	433.17	3.12
8	83.65	29.1	771.05	-8.95	27.94	4.429	470.97	2.83
9	90.12	32.13	844.29	-4.26	27.67	4.428	503.71	4.42
10	89.24	32.22	833.57	-5.38	27.18	4.409	498.16	4.49
11	85.97	30.2	802.16	-5.21	27.46	4.410	480.77	4.09
12	92.27	32.77	871.99	-6.83	29.38	4.406	517.74	3.80

Table 3. Antibacterial screening summary

Compound	log ($1/c_{MIC}$) (experimental)	log ($1/c_{MIC}$) (predicted)	Residual
1	4.602	4.669	-0.067
2	4.637	4.672	-0.035
3	4.609	4.494	0.115
4	4.328	4.332	-0.004
5	4.278	4.169	0.109
6	4.314	4.179	0.135
7	3.981	4.008	-0.027
8	3.704	3.837	-0.133
9	4.627	4.643	-0.016
10	4.659	4.637	0.022
11	4.333	4.476	-0.143
12	4.352	4.305	0.047
Ampicillin	4.446	-	-
Gentamicin	5.787	-	-

which is able to discriminate between the parameters. Pearson's correlation matrix has been performed on all descriptors by using NCSS Statistical Software. The analysis of the matrix revealed 8 descriptors for the development of MLR model (Table 2).

Mathematical models were formed by a stepwise addition of terms. A deletion process was then employed where each variable in the model was held out in turn and using the remaining parameters models were generated. Each descriptor was chosen as input for the software package of NCSS and then the stepwise addition method implemented in the software was used to choose the descriptors contributing to the antibacterial activity of benzimidazole derivatives.

The partition coefficient (log *P*) tends to correlate with antibacterial activity exclusively and the best monoparametric model was found to be the following:

$$\log 1/c_{MIC} = 0.518 \log P + 2.391$$

$$n = 12;\ r = 0.932;\ s = 0.085;\ F = 66.43 \quad (1)$$

Addition of *HE* as an additional parameter to log *P*, increased the correlation coefficient from 0.932 to 0.951 (Eq. (2)):

$$\log 1/c_{MIC} = 0.415 \log P + 0.031 HE + 2.940$$

$$n = 12;\ r = 0.951;\ s = 0.010;\ F = 42.21 \quad (2)$$

It should be noted that the addition of other parameters to log *P* and *HE* does not significantly improve the correlation coefficients.

From both above presented models, it can be concluded that the strong influence of the lipophilicity, log *P*, is important for the antibacterial activity and this parameter is usually related to pharmacological activity [36,37]. This evidence was clearly described in lipid theory advanced by Meyer and Overton. According to this theory, log *P* is a measure of hydrophobicity which is important for the penetration and distribution of the drug, but also for the interaction of drug with receptors. Therefore, it can be suggested that lipophilic properties have to be checked for designing of potent antibacterial agents as they are deciding factors for its activity.

For the testing of the validity of the predictive power of selected MLR model (Eq. (2)) the leave-one-out technique (LOO technique) was used. The developed model was validated by the calculation of the following statistical parameters: predicted residual sum of squares (*PRESS*), total sum of squares deviation (*SSY*), cross-validated correlation coefficient (r^2_{CV}), and adjusted correlation coefficient (r^2_{adj}) (Table 4).

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. Thus, in view of this, model 2 is statistically significant. Further, for a reasonable QSAR model, the *PRESS*/*SSY* ratio should be lesser than 0.4. The data presented in Table 4 indicate that for the developed model this ratio is 0.1746. The high value of r^2_{CV} and r^2_{adj} are the essential criteria for qualifying the QSAR model 2 as the best one.

Table 4. Cross-validation parameters

Model	*PRESS*	*SSY*	*PRESS*/*SSY*	S_{PRESS}	r^2_{CV}	r^2_{adj}
2	0.1644	0.9417	0.1746	0.1170	0.8254	0.8822

However, the only way to estimate the true predictive power of the developed model, the predicted log ($1/c_{MIC}$) values of the investigated benzimidazoles were calculated using model 2. The data presented in Table 3 show that the observed and the estimated activities, by using the model 2, are very close to each other. It indicates the good predictability of the established model 2. Figure 1 shows the plots of linear regression of predicted versus experimental values of the antibacterial activity of benzimidazoles here investigated.

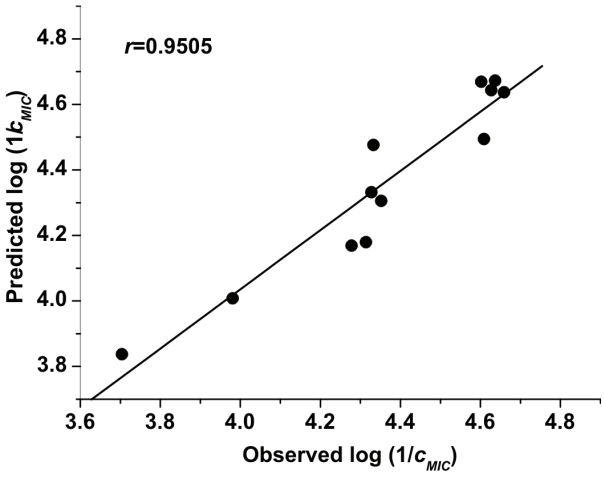


Figure 1. Plot of predicted versus experimentally observed antibacterial activity against Pseudomonas aeruginosa.

To investigate the existence of a systematic error in developing the QSAR models, the residuals of predicted values of the inhibitory activity were plotted against the experimental values, as shown in Figure 2. The propagation of the residuals on both sides of zero indicates that no systemic error exists, as suggested by Jalali-Heravi and Kyani [38]. It indicates that these models can be successfully applied to predict the antibacterial activity of this class of molecules.

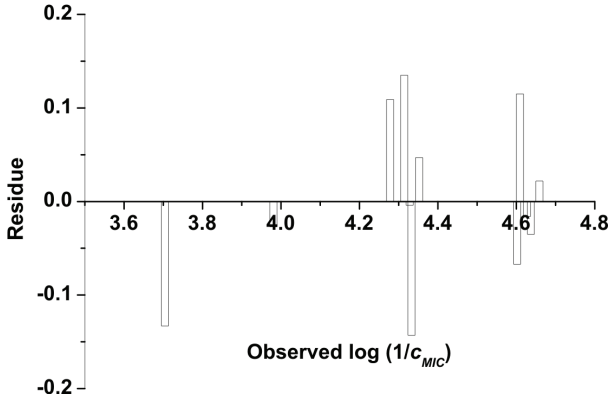


Figure 2. Plot of the residual values against the experimentally observed log (1/c_{MIC}) values.

CONCLUSIONS

From the above presented results, we conclude that the 1-benzylbenzimidazole derivatives are effective in vitro against the Gram-negative bacteria *Pseudomonas aeruginosa.* Molecular modeling and QSAR analysis were performed to find the quantitative effects of the molecular structure of the compounds on their antibacterial activity. An accurate mathematical model was developed for predicting the inhibitory activity of some benzimidazole derivatives. The validity of the model has been established by the determination of suitable statistical parameters. The established model was used to predict the inhibitory activity of the benzimidazoles investigated and close agreement between experimental and predicted values was obtained. The low residual activity and high cross-validated r^2 values (r^2_{CV}) obtained suggests a good predictive ability of the developed QSAR model. It indicates the antibacterial activity of series of 1-benzylbenzimidazole derivatives can be successfully modeled using various molecular descriptors. It can be concluded that the strong influence of the lipophilicity, log *P*, is important for the antibacterial activity and this parameter is usually related to inhibitory activity.

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NAUČNI RAD

QSAR MODELOVANJE ANTIBAKTERIJSKE AKTIVNOSTI NEKIH DERIVATA BENZIMIDAZOLA

Analiza kvantitativne zavisnosti struktura-aktivnost (QSAR) izvedena je na seriji od 12 derivata benzimidazola u cilju predviđanja antibakterijske aktivnosti ispitivanih jedinjenja prema Gram-negativnoj bakteriji Pseudomonas aeruginosa. Za odabir deskriptora i za postavljanje modela koji povezuje strukturu sa inhibitornom aktivnošću korišćena je metoda višestruke linearne regresije. Na osnovu dobijenih rezultata definisan je model zasnovan na lipofilnosti (log P) i energiji hidratacije (HE). Prediktivna sposobnost modela potvrđena je LOO (leave one out) tehnikom. Dobro slaganje između eksperimentalne i teoretski dobijene inhibitorne aktivnosti ukazuje na dobar kvalitet definisanog QSAR modela.

Ključne reči: benzimidazol; molekulski deskriptori; kvantitativna zavisnost struktura-aktivnost; antibakterijska aktivnost; Pseudomonas aeruginosa.