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Quantitative Structure Activity Relationships Studies on Benzimidazole Derivatives as antibacterial agents against *Escherichia coli*

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ABSTRACT: In a continuing effort to develop novel Benzimidazole endowed with better pharmacological profiles. A series of Benzimidazole derivatives were designed on the basis of previously developed QSARs. These drugs offer novel mechanisms of action and expanded spectrums of activity over traditional treatment option. However, with these new agents comes the need for increased awareness of the potential interactions and toxicities associated with these drugs. The best models for different antibacterial agents against *Escherichia coli* were first validated by leave-one-out cross validation procedure. It was revealed that topological, physicochemical and indicator parameters were found to have overall significant correlationship with antibacterial activity against *Escherichia coli* and these studies provide an insight to design new molecules.

Key Words: QSAR, Hansch Approach, Antibacterial Activity

1 INTRODUCTION

Medicinal chemists today are facing a serious challenge because of the increased cost and enormous amount of time taken to discover a new drug, and also because of rigorous competition amongst different pharmaceutical companies. Thereby, importance of Quantitative Structure Activity Relationships (QSAR) and molecular modeling is increasing nowadays. Target specific drug discovery is the need of the hour. Techniques evolved in the post genomic era have given us an opportunity to accelerate discovery process by looking at many cellular processes simultaneously. Advances in computational power, algorithms and modern database mining techniques are accelerating the discovery in science even more.

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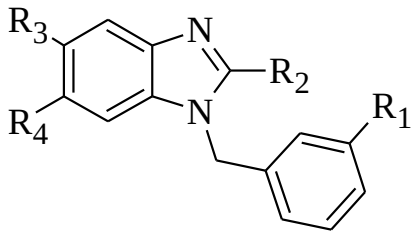
Drug development is the process not only of finding and producing therapeutically useful pharmaceuticals and of turning them into high-quality formulations of usable, effective and safe medicines, but also of delivering valuable, reliable and trustworthy information about appropriate doses and dosing intervals and about likely effects and side-effects of these treatments.

The conception that there exists a close relationship between bulk properties and their molecular structure is quit rooted in chemistry. The basic tenet of chemistry to identify these assumed relationships between molecular structure and physiochemical properties and then to quantify them. QSAR approach including multivariate data analysis in combination with statistical design, has been extensively employed. Quantitative structure activity relationship (QSAR) studies are useful tools in the rational search for bioactive molecules. The main success of the QSAR method is the possibility to estimate the characteristics of new chemical compounds without the need to synthesize and test them. The prediction of physicochemical properties in the chemical, pharmaceutical and environmental spheres is widely used. QSAR studies have predictive ability which provide deeper insight into mechanism of drug receptor interactions.[1-2] In recent years, the number of life-threatening infections caused by multidrug-resistant Gram-positive and Gram-negative bacteria has reached an alarming level in many countries around the world. The contribution of simple organic acids in prevention of bacterial infections[3] directed us to search for new antibacterial compounds.

Benzimidazole and its derivatives are history of long chemical family used as antimicrobial agents against the wide spectrum of microorganism with its synthetic utility and broad range of pharmacological effects. Several thousands of benzimidazole analogs have been synthesized and screened for pharmacological activity. They are of wide interest because of their diverse biological activity and clinical applications. The benzimidazole nucleus is an important heterocyclic ring, and interest in the chemistry with their different activities as they can act as bacteriostats or bactericides, as well as fungicides[4-8], and they are present in numerous, antiparasitic, antitumoral and antiviral drugs[9-10]. Also, some of them exhibit appreciable antiprotozoal activity[11]. They are also used to treat microsporodial and cryptosporodial infections, which can cause lethal diarrhea in patients treated with immunosuppressive drugs, or infected with HIV [12-14].

In View of the above and in continuation of our studies on the inhibitory activities of benzimidazole derivatives, as well as on correlation of molecular properties with the activity.[15-19] The objective of this investigation was to study the usefulness of QSAR in the prediction of antibacterial activity/ properties of benzimidazole molecules against Gram negative bacteria *Escherichia coli* based on their structure represents one of the fundamental basis of theoretical chemistry. Multiple linear regression (MLR) models have been developed as a mathematical equation which can relate chemical structure to the inhibitory activity.[20-26] The structures of the benzimidazoles tested in this study are presesented in Table 1.

Table: 1- The Structure of the Compounds Studied



C.No.	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
13	Ampicillin			
14	Gentamicin			

2 Material and Methods

The selected descriptors was calculated from the data of benzimidazole derivatives and the regression analysis were carried out using NCSS (version 2007) to derive the QSAR equations. Biologically highest activity molecule 09 taken as a template molecules. A common substructure based alignment similarity score of molecules is in terms of their surface and electrostatic properties and hydrophobic effects on and near the surface of a molecule.

All the 1-benzylbenzimidazole derivatives were evaluated for their in vitro growth inhibitory activity against Gram negative bacteria and given in the form of Minimum Inhibitory Concentration (MIC) and their negative logarithms (log $1/c_{MIC}$) were directly taken from the work of Sanja O. Podunavac et. al.[13] used for further QSAR analysis.

In present study, **Table-1** represents the structure of different benzimidazoles derivatives while **Table-2** shows the inhibitory activity in the form of MIC and calculated topological descriptors such as winear indicx (W), connectivity index of zero and first order is considered χ^0 and χ_v^1 **Table-3** represents the correlation matrix between descriptors, **Table-4** shows cross- validation statistical parameters for all developed QSAR models and regression analysis with the help of statistical parameters are given in **Table-5** while in **Table-6** shows the Predicted, Observed antibacterial activity with residuals and **Figure-1** is the graph plotted between predicted and observed antibacterial activity of

benzimidazoles derivatives while **Figure-2** show graph plotted between the residual and observed activity and **Figure-3** is the graph plotted between VIF and K.

Table-2: Inhibitory Activity and Calculated Topological Descriptors of Benzimidazoles Derivatives

Compound No.	MIC	W	χ_0	χ^1_v
1	6.25	714	13.405	6.609
2	6.25	798	14.276	6.896
3	6.25	798	14.276	6.518
4	12.5	926	14.983	6.941
5	12.5	1365	17.646	8.455
6	12.5	3234	24.499	11.775
7	25	5974	29.361	15.024
8	50	714	13.405	6.676
9	6.25	714	13.405	6.298
10	6.25	834	14.113	6.722
11	12.5	599	12.535	6.002
12	12.5	599	12.535	6.069
13	12.5	706	13.242	6.122
14	0.78	706	13.242	6.114

3 RESULTS AND DISCUSSION

A set of 14 benzimidazoles consisting of 12 compounds was used for multilinear regression model generation using QSAR Hansch approach on benzimidazole derivatives. The correlation matrix studies performed between biological activity and alignment similarity score are presented in Table 3. The correlation between activity and similarity as indicated by values near to 1. The simple linear regression method performs a standard linear regression calculation for QSAR analysis[22]. This method is good for exploring simple relationships between structure and activity.

Table-3: Correlation Matrix between the Different Topological Descriptors

	MIC	W	χ^0	χ_v^1
MIC	1.0000			
W	0.2541	1.0000		
χ^0	0.2167	0.9771	1.0000	
χ_v^1	0.2599	0.9862	**0.9962**	1.0000

Using these above data **Table-3**, a correlation matrix was calculated to find the correlation as well as the collinearity between the descriptors. It is important for further analysis to develop a correlation matrix for the descriptors utilized and their correlations with the biological activities. A high interrelationship was observed between χ^0 and χ_v^1 **(r = 0.99)** as well as the low interrelationship was observed between MIC and χ^0 **(r=0.21).**

A perusal of **Table-3,** shows that all chosen descriptors are not well correlated with the antibacterial activity; meaning that in mono parametric regressions those properties are not appropriate to obtain statistically significant results. After performing regression analysis, we have adopted maximum-R^2 method, followed by stepwise regression analysis and the developed QSAR/QSPR models are given below

MIC = 3.9081+ 1.1986(±1.2853)χ_v^1

n = 14 R^2 = 0.0676 R^2A = 0.0000
F-Ratio = 0.8700 ……1

The developed QSAR model Eq-1 demonstrate the importance of valence connectivity index which is directly proportional to the antibacterial activity. The developed QSAR model Eq-1 is not statistically significant due to their low correlation coefficient between the descriptor and antibacterial activity (r = 0.26) , minimum adjusted regression coefficient and fisher value [23-26] to overcome it we added a descriptor to develop biparameteric QSAR model Eq-2 which is given below.

MIC = 22.7011-13.5819 (±7.0174) χ^0 + 26.9698(±13.3656)χ_v^1

n = 14 R^2 = 0.3044 R^2A = 0.1780
F-Ratio = 2.407 …….2

QSAR model Eq-2 demonstrated the importance of zero order connectivity index and first order valence connectivity index with respect to antibacterial activity. According to QSAR model Eq-2 Zero order connectivity index is inversely proportional while first order connectivity index is directly proportional to the antibacterial activity it means that as the value of zero order connectivity decreases and first order valence connectivity increases the antibacterial activity increases but statistically the correlation coefficient between the descriptor is highly correlated which is about (r = 0.98) and the correlation between the descriptors and the antibacterial activity is very low (r = 0.55) indicate that the developed model have not statistically significant. The rule of thumb not allowed us for further addition of descriptors on a close look there is five serious outlier which generally lowers the statistics of developed

model on removing it the developed QSAR model Eq-3 is given below.

After Deletion of Outlier Compound No.04, 08, 09, 10 and 11

MIC = -4.04571-2.69874(±1.9276) χ^0 + 7.18508(±3.6355)χ_v^1

n = 09 R^2 = 0.9121 R^2A = 0.8828
F-Ratio = 31.121 …….3

The QSAR Model Eq-3 is highly statistically significant model. This model demonstrated the importance of zero order connectivity index and first order valence connectivity index in which first one is inversely proportional while later one is directly proportional with the antibacterial activity. The cross validated correlation coefficient is pretty close to the correlation coefficient, that suggests a good predictive ability of the best linear model. It can be easily observed that our linear regression equation is better in terms of stability and predictive ability with a lower difference $R^2 - R^2_{cv}$.

Table-4.4.4 Cross Validation Statistical parameters

Model	n	PRESS	SSY	PRESS/SSY	R^2_{cv}	R^2_{adj}	S_{press}
1	14	2203.175	127.815	17.23	0.0000	0.0000	12.544
2	14	2677.075	575.892	04.64	0.0000	0.1780	13.828
3	09	83.7865	351.845	**00.23**	**0.7828**	**0.8828**	**03.051**

The high R^2_{cv} value is indicative of its reliability in predicting the inhibitory activity. But, the only way to estimate the true predictive power of a model is to test their ability to predict accurately the biological activities of compounds. In order to verify the predictive power of the developed model, predicted MIC value of benzimidazoles investigated were calculated by using QSAR model Eq-3 and compared with the experimental values. The regression statistical analysis is given below.

Table-5: Regression Statistical Analysis Benzimidazoles derivatives

Model No.	Para. Used	Ai(1----5)	Intercept	Se	R^2	Q = r/Se	F-Ratio
1	χ_v^1	A_1=1.1986(±1.2853)	3.9081	12.1237	0.0676	0.021	0.870
2	χ^0 χ_v^1	A_1=-13.5819(±7.0174) A_2=26.9699(±13.3656)	22.7012	10.9367	0.3044	0.050	2.407
3	χ^0 χ_v^1	A_1=-2.6987(±1.9276) A_2=7.1851(±3.6355)	-4.0457	2.3775	0.9121	0.401	31.121

Based on the magnitude of residue, a close agreement between the observed and calculated inhibitory activity is found. Further, the plot of predicted MIC values against observed MIC values also proves the superiority of the model expressed by Eq-3 The results of antibacterial studies of benzimidazole

are summarized in given below **Table-6**.

In order to investigate the existence of a systemic error in developing the QSAR models, the residuals of the predicted activity were plotted against the observed activity value (Figure-2). The propagation of the residuals on the both sides of the zero axis indicates that no systemic error in the development of regression models exists.

The trend may be explained by the existence of a variance inflation factor (VIF). The VIF value was calculated for $1/1-r^2$, where r^2 is the squared multiple correlation coefficient of one parameter effect on the remaining parameter. VIF values greater than 5 indicate the presence of unacceptably large multicolinearity between the parameters in the correlation. The VIF value of Eq.3 is closer to 5 and may be responsible for the marginal increase in its r value.

Table-6: Antibacterial Screening Summary

Com.	Actual	Predicted	Residual
1	6.25	7.264	-1.014
2	6.25	6.975	-0.725
3	6.25	4.259	1.991
4	12.5	9.082	3.418
5	12.5	14.442	-1.942
6	25	24.665	0.335
7	6.25	5.029	1.221
8	6.25	6.165	0.085
9	0.78	4.147	-3.367

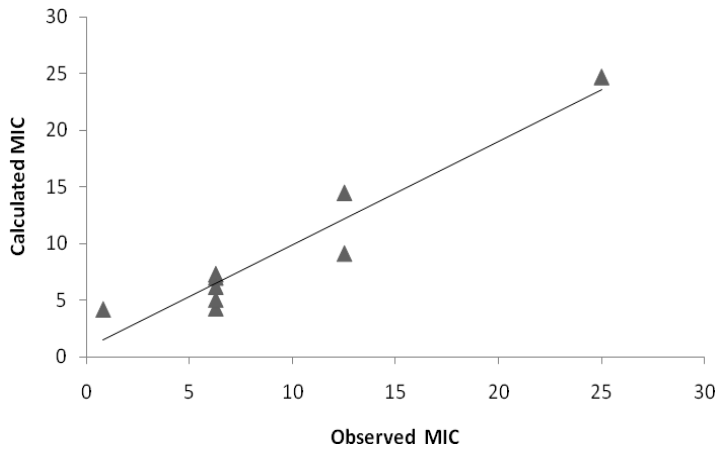


Figure-1: Plots of Predicted V/S experimentally observed inhibitory activity of benzimidazole against *Escherichia coli*

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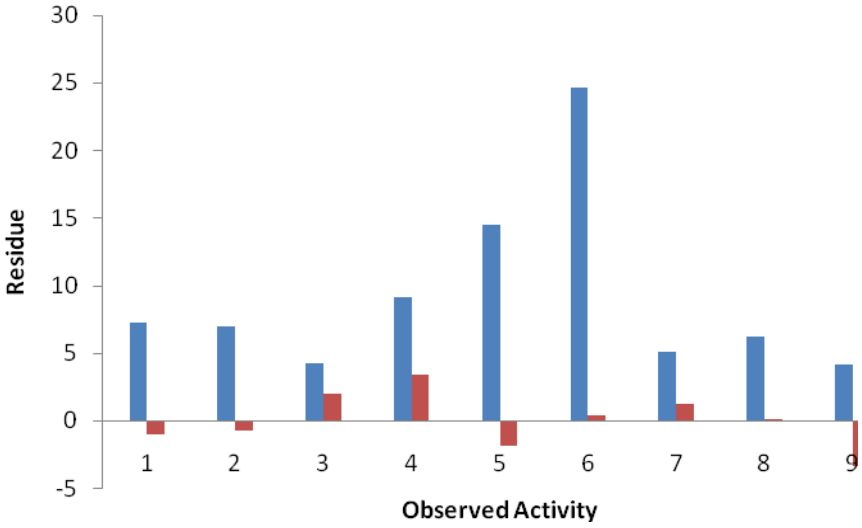

Figure-2: Plots of residual value against the experimentally observed activity benzimidazole against *Escherichia coli*

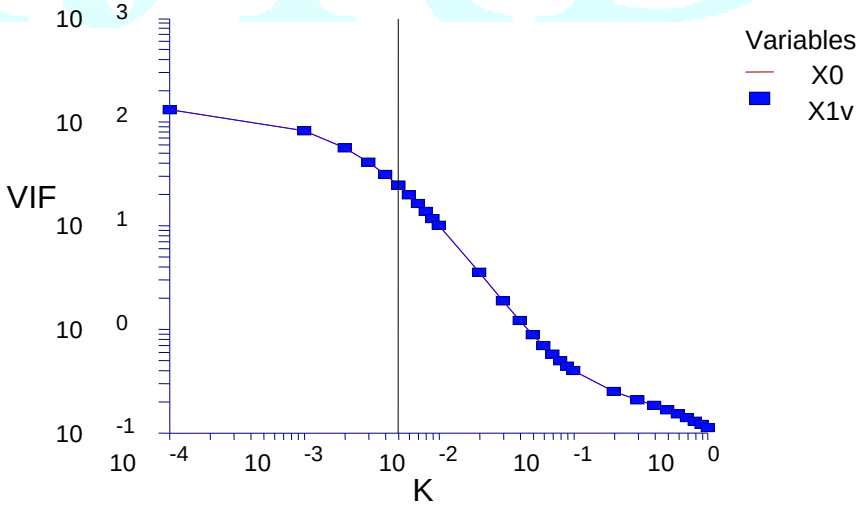

Figure-3: Plot between VIF and K

4 CONCLUSION

From the results and discussion made above, we conclude that the benzimidazole derivatives were more active against *Escherichia*

coli . The results of the QSAR study give rise to QSAR models with good predictive ability for antibacterial activity of benzimidazole derivatives. Linear regression for the total data set of 14 compounds in the present study with antibacterial activity demonstrated that the zero order connectivity index and first order valence connectivity index appears to be the governing factors for the antibacterial potency of synthesized benzimidazole derivatives.

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