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QSAR modeling of benzoxazole derivatives as antimicrobial agents

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ABSTRACT

A quantitative structure activity relationship study on a series of 5 (or 6)-nitro/amino-2-(substituted phenyl/benzyl)-benzoxazole analogues were made using combination of various thermodynamic, electronic and spatial descriptors. Several statistical expressions were developed using stepwise multiple liner regression analysis. The best model was validated by leave-one-out method of cross-validation. The study revealed that the thermodynamic property, i.e., Standard Gibbs free energy contributed positively, Electronic property like Electronic energy contributed positively and HOMO energy and Repulsion energy contributed negatively. The study suggested that substitution of group at R_1 position on benzoxazole ring with electron withdrawing group favourable for the antibacterial activity. The quantitative structure activity relationship study provides important structural insights in designing of potent antibacterial agents.

Keywords: Benzoxazoles, antimicrobial activity, quantitative structure activity relationship (QSAR).

INTRODUCTION

The dramatically rising prevalence of multidrug-resistant microbial infection in the past few decades has become a serious health care problem. In particular, the emergence of multidrug-resistant strains of gram-positive bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant *Enterococcus* is a problem of ever increasing significance [1-3]. In order to prevent this serious medical problem, the elaboration of the new types of the previously known drugs is a very actual task. The benzoxazoles have been the aim of many researchers for many years because they constitute an important class of heterocyclic compounds exhibiting substantial chemotherapeutic activities [4].

The benzoxazoles derivatives exhibited antibacterial, antifungal, antimycobacterial, antitumoral, HIV-1 reverse transcriptase, and topoisomerase I inhibitory activity [5-12].

With the continuing development of clinical drug resistance among bacteria and the advent of resistance to the recently released agents quinupristin, dalfopristin and linezolid, the need for new, effective agents to treat multidrug- resistant Gram-positive infections remains important. Since the early 1990s, the epidemiology of pathogenic bacteria isolated from hospital infections has shifted from Gram-negative organisms, with the majority of nosocomial infections now caused by Gram-positive isolates. Increasingly, nosocomial pathogens are resistant to first-line antimicrobial agents, with 34% of *staphylococcus aureus* clinical isolates in the US, 26% of *S. aureus* isolates in Europe and 45% of *S. aureus* isolates in the western pacific, resistant to methicillin. Similarly, the incidence of vancomycin-resistant among US enterococcal bloodstream isolates has now reached ~ 20%, with the frequency of penicillin-non-susceptibility in US pneumococci at 34% [1].

Secondary metabolite formation (i.e., natural products), by microbes, is believed to be a Darwinian type response mechanism to environmental pressures. Some of these secondary metabolites are the basis for the widely used antibacterials (e.g., carbapenems, cephalosporins, macrolides, monobactams and penicillins)and antifungal agents(e.g., amphotericin B, nystatin). The introduction of these therapeutic agents has contributed significantly to reduce morbidity and deaths due to infection diseases. Ironically, as the pharmaceutical industry has created newer antibacterials and antifungals, the biological targets of these drugs have evolved mechanisms to overcome the effects of these potent drugs [2].

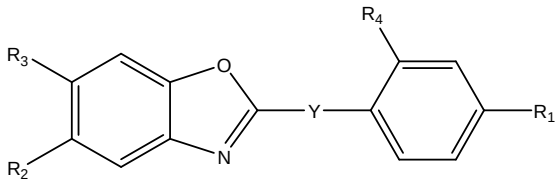
A benzoxazole derivative, Calcimycin, is a carboxylic polyether antibiotic from a strain of *Streptomyces chartreuses* (NRRL 3882). It was found to be very active against Gram-positive bacteria including some *Bacillus* and *Micrococcus* strains. Two calcimycin analogues, routiennocin and cezomycin which are 3-hydroxy-11,15-desmethyl and 3-demethylamino derivatives of it, respectively, were found to be highly active against *Bacillus cereus*, *Bacillus negaterium*, *Micrococcus luteus* and *streptomyces rimosus*. Additionally, frankamide, that is 11-demethyl cezomycin, showed activity against *Bacillus subtillis*, *Staphylococcus aureus*, *Entercoccus faecalis* and against several plant pathogenic fungal strains [6].

In the present work, we describe the QSAR employing multivariable regression analysis (MRA) in order to investigate the quantitative effect between the various physicochemical parameters of benzoxazole derivative (fig. 1) on their antibacterial activity against Gram-positive bacteria *Bacillus subtilis* ATCC 6633.

MATERIALS AND METHODS

The **Table1** shows the structural features of bezoxazole derivatives along with their biological activities (MIC µg/ml) reported by T. Ertan *et al* [5]. and descriptors included in final QSAR model:

Table 1: Structure, Antimicrobial Activities of Compounds and descriptors used in QSAR model:



Comp no	Substitutions						Structural descriptors			
	R1	**R2**	**R3**	**R4**	**Y**	**MIC**	**Homo**	**NRE**	**G**	**ElcE**
1.	$C(CH_3)_3$	H	NO_2	-	-	50	-8.99	20394	292.09	-24161
2.	F	H	NO_2	-	-	50	-9.180	15647	60.76	-19264
3.	C_2H_5	H	NO_2	-	-	50	-8.034	17364	272.41	-20797
4.	C_2H_5	NO_2	H	-	-	50	-8.034	17364	272.41	-20797
5.	F	NO_2	H	-	-	50	-9.267	15499	60.76	19116
6.	Br	H	NO_2	-	CH_2	25	-9.807	16998	278.31	-20638
7.	F	H	NO_2	-	CH_2	12.5	-9.788	17263	69.18	-21034
8.	F	NO_2	H	-	CH_2	12.5	-9.687	17250	69.18	-21022
9.	CH_3	NO_2	H	-	CH_2	50	-9.482	17220	272.41	-20676
10.	$C(CH_3)_3$	H	NH_2	-	-	25	-8.051	17750	437.2	-20907
11.	F	H	NH_2	-	-	50	-8.168	13078	205.87	-16083
12.	Br	H	NH_2	-	-	25	-8.228	12901	415	-15775
13.	C_2H_5	H	NH_2	-	-	50	-8.161	14446	417.52	-17291
14.	H	NH_2	H	-	-	12.5	-8.242	11730	410.31	-14264
15.	F	NH_2	H	-	-	25	-8.323	13066	205.87	-16027
16.	F	H	NH_2	-	CH_2	50	-8.285	14606	214.29	-17768
17.	F	NH_2	H	-	CH_2	50	-8.358	14628	214.20	-17789
18.	H	CH_3	-	H	-	12.5	-8.910	11694	352.28	-14163
19.	Cl	CH_3	-	H	-	12.5	-8.992	12903	330.72	-15732
20.	Br	CH_3	-	H	-	12.5	-9.028	12850	356.97	-15658
21.	NO_2	CH_3	-	H	-	6.25	-9.404	15608	263.99	-18980
22.	Cl	-	CH_3	H	-	12.5	-8.885	12929	330.72	-15759
23.	Br	-	CH_3	H	-	12.5	-8.932	12856	356.97	-15665
24.	H	CH_3	-	Cl	-	25	-8.951	13226	330.72	-16055
25.	H	CH_3	-	OCH_3	-	25	-8.899	15203	246.07	-18148
26.	H	CH_3	-	F	-	25	-8.941	13739	147.84	-16380
27.	H	CH_3	-	NO_2	-	25	-9.241	16489	263.99	-19788
28.	Cl	CH_3	-	Cl	-	25	-9.029	14527	309.16	-17716
29.	H	-	CH_3	Cl	-	25	-8.847	13242	330.16	-16071
30.	H	-	CH_3	OCH_3	-	25	-8.793	15213	246.07	-18158
31.	H	-	CH_3	F	-	25	-8.843	13420	147.84	-16360
32.	Cl	-	CH_3	Cl	-	25	-8.927	14541	309.16	-17730
33.	CH_3	-	CH_3	CH_3	-	50	-8.721	14848	349.86	-17629

The biological activity data MIC (minimum inhibitory concentration in µg/ml) were converted to negative logarithmic dose in moles (pMIC) for QSAR analysis. The correlations were sought between inhibitory activity and various substituent constants at position R_1, R_2 and R_3 of molecule. The series was subjected to molecular modelling using CS Chem-Office 6.0 [15]. Structures of all the compounds were sketched using builder module of the programme. These structures were then subjected to energy minimization using force field molecular mechanics-2 (MM2) until the root mean square (RMS) gradient value became smaller than 0.1 kcal/mol. Å. Minimized molecules were subjected to re-optimization via MOPAC method until the RMS

gradient attained a value smaller than 0.0001 kcal/mol. Å. The descriptor values for all the molecules were calculated using "compute properties" module of program.

Table 2: The various descriptors calculated using chem.-office software

Steric descriptors	Thermodynamic descriptors	Electronic descriptors
Connolly accessible area (SAS)	Critical temperature (Tc)	Dipole (DPL)
Connolly molecular area (MS)	Ideal gas thermal capacity (Cp)	Electronic energy (ElcE)
Connolly solvent excluded volume (CSEV)	Critical pressure (Cp)	Highest occupied molecular orbital energy (HOMO)
Principal moment of inertia-X-component (Pmix)	Henry's law constant (H)	Lowest unoccupied molecular orbital energy (LUMO)
Principal moment of inertia-Y-component (Pmiy)	Bend energy (Eb)	Repulsion energy (NRE)
Principal moment of inertia-Z-component (Pmiz)	Heat of formation (Hf)	VDW-1,4-Energy (E14)
Molar refractivity (MR)	Total energy (Et)	Non-1,4-VDW Energy (Ev)
Ovality (Oval)	Partition coefficient (PC)	Dipole length (DPLL)
Balaban index (Blndx)	Critical volume (Vc)	Total energy (TotE)
Cluster count (ClsC)	Dipole-dipole energy (Ed)	
Diameter (Diam)	Log P	
Molecular topological index (Tlndx)	Stretch energy (Es)	
Sum of valence degrees (SVDe)	Torsion energy	
Total valence connectivity (TVCon)		

Stepwise multiple linear regression analysis method was used to perform QSAR analysis employing in-house VALSTAT programme [16]. The ± data within the parentheses are the error of regression coefficients associated with corresponding regression coefficients in regression equation. The best model was selected on the basis of various statistical parameters such as correlation coefficient (r), standard error of estimation (std), sequential Fischer test (F). Quality of the each model was estimated from the cross-validated squared correlation coefficient ($q^{2)}$. calculated root mean square error (S_{DEP}), chance statistics evaluated as the ratio of the equivalent regression equations to the total number of randomized sets; a chance value of 0.001 corresponds to 0.1% chance of fortuitous correlation and boot-strapping square correlation coefficient (r^2_{bs}), which confirm the robustness and applicability of QSAR equation.

RESULTS AND DISCUSSION

When data set was subjected to sequential multiple linear regression analysis, in order to develop QSAR between antimicrobial activity as dependent variables and substituent constants as independent variables, several equations were obtained. The statistically significant equations were considered as best model.

Model: 1

pMIC = G [0.0014(± 0.00045)] +ElcE [4.593e-005(± 1.854e-005)] -Homo [0.266(± 0.102)] + [5.061(± 0.895)]

n=21, r=0.931, r^2=0.868, std=0.087, F=37.268, r^2_{bs}=0.868 q^2=0.807, S_{PRESS}= 0.105, S_{DEP}= 0.094

The model 1 shows that thermodynamic parameter; Standard Gibbs free energy (G) contribute positively and electronic parameters; electronic energy (ElcE) and HOMO energy contribute positive and negative respectively towards the activity. The model has correlation coefficient (r) of 0.931. The model have significance level more than 99% as the value F=37.268 against tabulated value F=26.1, with a low standard deviation of estimation 0.087, demonstrate accuracy of the model. The robustness of model was shown by magnitude of the bootstrapping r^2, which was near to conventional r^2. The internal predictivity of model (q^2=0.807) was also good. The model once again favored by the least S_{PRESS} and S_{DEP} values. The observed, calculated and predicted activities (pMIC) for training set of model 1 is presented in **Table 3.**

TABLE: 3 Training set activity (model: 1)

Compound No.	Observed value	Calculated value	Predicted value
3	6.729	6.650	6.589
6	7.125	7.140	7.147
15	6.960	6.847	6.825
2	6.713	6.716	6.717
1	6.773	6.782	6.791
25	6.981	6.964	6.963
31	6.959	6.887	6.873
11	6.659	6.804	6.842
26	6.959	6.912	6.903
20	7.362	7.276	7.261
5	6.713	6.745	6.759
32	7.046	7.083	7.086
18	7.223	7.306	7.325
14	7.225	7.208	7.202
30	6.981	6.935	6.933
19	7.289	7.224	7.215
27	7.007	7.006	7.006
29	6.989	7.170	7.188
28	7.046	7.112	7.117
16	6.685	6.771	6.787
23	7.363	7.250	7.233

The **figure1** shows plot of observed versus calculated pMIC values for training set molecules and **figure 2** is plot of observed versus predicted pMIC values for same set (model 1)

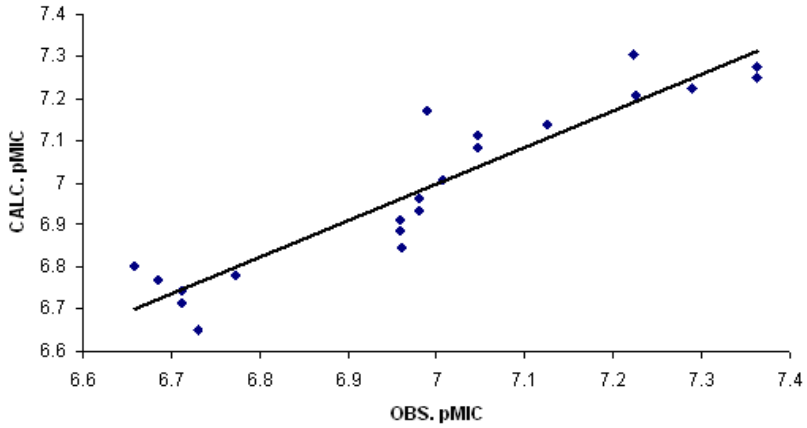


Fig. 1: Discrete Plot of training set between observed vs. calculated by leave-one-out cross-validation pMIC values. (model: 1)

Obs. pMIC: Observed pMIC, Cal. (LOO): calculated by leave-one-out cross-validation pMIC, y=0.868x+0.9226, r^2=0.868

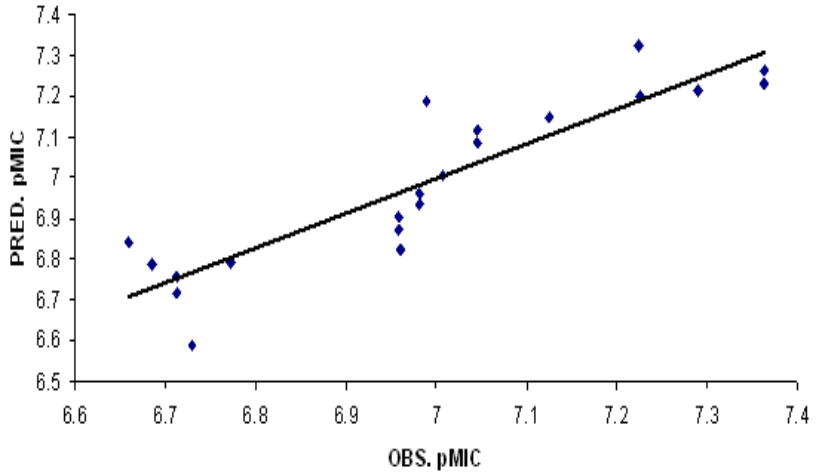


Fig. 2: Discrete Plot of training set between observed vs. predicted by leave-one-out cross-validation pMIC values. (model: 1)

Obs. pMIC: Observed pMIC, Pred. (LOO): calculated by leave-one-out cross-validation pMIC, y = 0.8516x + 1.0363, r^2 = 0.8101

The predicted activities for test set molecules are presented in **Table 4.**

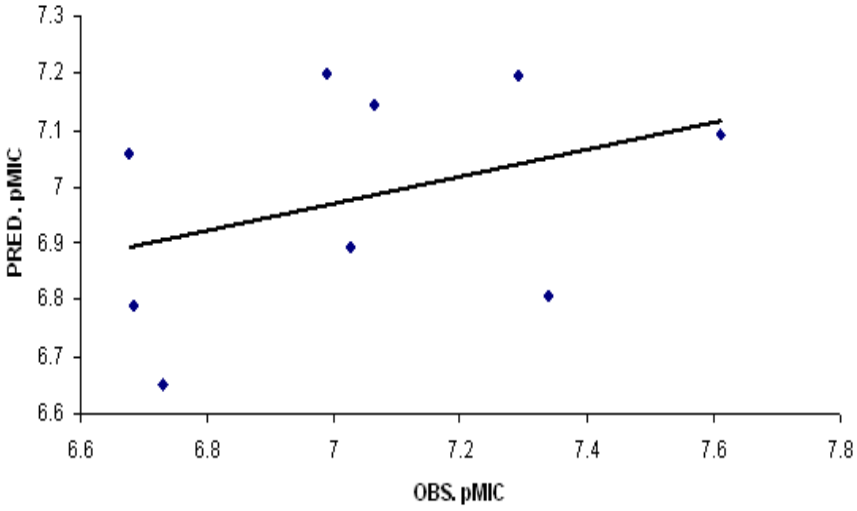


Fig. 3: Discrete Plot of test set between observed vs. predicted by leave-one-out cross-validation pMIC values. (model: 1)

TABLE: 4 Test set activity (model: 1)

Compound No.	Observed activity	Predicted value
12	7.063	7.142
13	6.678	7.059
24	6.989	7.198
22	7.290	7.195
21	7.609	7.090
7	7.338	6.808
10	7.028	6.892
17	6.685	6.790
4	6.730	6.650

The applicability of model 1 in predicting activities of external molecules is shown by a plot of observed versus predicted pMIC values for test set in **Figure 3.**
Obs. pMIC: Observed pMIC, Pred. (LOO): calculated by leave-one-out cross-validation pMIC,
$y = 0.2409x + 5.283$, $r^2 = 0.1506$

Model: 2
pMIC =G [0.00152(± 0.000457)] -Homo [0.262(± 0.103)] -NRE [5.160e-005(± 2.133e-005)] + [5.028(± 0.907)]
n=21, r=0.929, r^2=0.864, std=0.088, F=36.04, r^2_{bs}=0.865, q^2=0.804, S_{PRESS}= 0.106, S_{DEP}= 0.094

TABLE: 5 Training set activity (model: 2)

Compound No.	Observed value	Calculated value	Predicted value
3	6.730	6.654	6.589
6	7.125	7.147	7.147
15	6.960	6.850	6.825
2	6.713	6.716	6.717
1	6.773	6.782	6.791
25	6.981	6.964	6.963
31	6.959	6.887	6.873
11	6.659	6.805	6.843
26	6.959	6.912	6.903
20	7.363	7.276	7.261
5	6.713	6.745	6.759
32	7.046	7.083	7.086
18	7.224	7.306	7.325
14	7.226	7.209	7.202
30	6.981	6.935	6.933
19	7.290	7.224	7.216
27	7.007	7.006	7.006
29	6.988	7.170	7.188
28	7.046	7.111	7.116
16	6.685	6.771	6.787
23	7.363	7.250	7.233

The model 2 shows that thermodynamic parameter; Standard Gibbs free energy (G) contribute positively and electronic parameters; HOMO energy and repulsion energy (NRE) show negative contribution towards the activity. The model has correlation coefficient (r) of 0.929. The model have significance level more than 99% as the value F=36.04 against tabulated value F=26.1, with

a low standard deviation of estimation 0.088, demonstrate accuracy of the model. The robustness of model was shown by magnitude of the bootstrapping r^2, which was near to conventional r^2. The internal predictivity of model (q^2=0.804) was also good. The model once again favored by the least S_{PRESS} and S_{DEP} values. The observed, calculated and predicted activities (pMIC) for training set of model 2 is presented in **Table 5.**

The **figure 4** shows plot of observed versus calculated pMIC values for training set molecules and **figure 5** is plot of observed versus predicted pMIC values for same set (model 2)

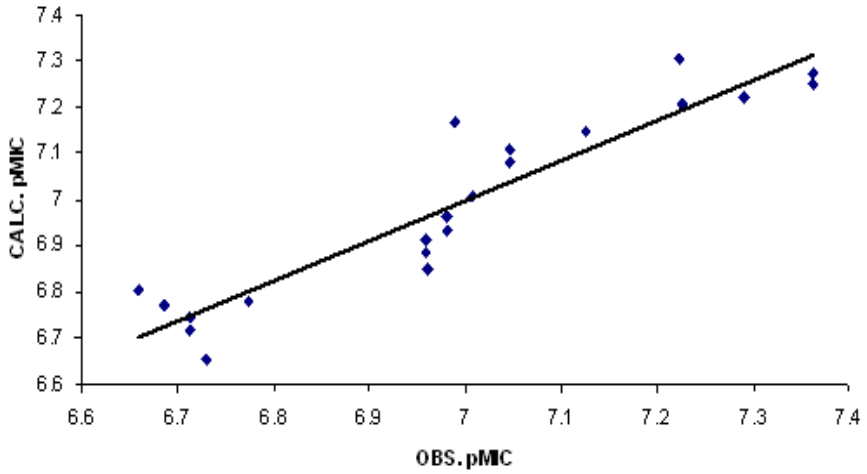


Fig. 4: Discrete Plot of training set between observed vs. calculated by leave-one-out cross-validation pMIC values. (model: 2)

Obs. pMIC: Observed pMIC, Cal. (LOO): calculated by leave-one-out cross-validation pMIC, y = 0.8679x + 0.9242, r^2 = 0.8692

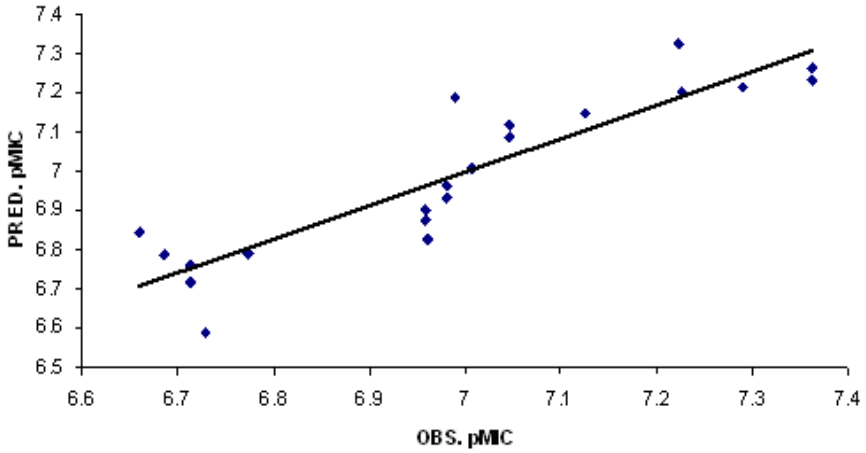


Fig. 5: Discrete Plot of training set between observed vs. predicted by leave-one-out cross-validation pMIC values. Eqn. 3.

Obs. pMIC: Observed pMIC, Cal. (LOO): calculated by leave-one-out cross-validation pMIC, y = 0.8516x + 1.0363, r^2 = 0.8101

The predicted activities for test set molecules are presented in **Table 6**.

TABLE: 6 Test set activity (model: 2)

Compound No.	Observed activity	Predicted value
12	7.063	7.153
13	6.678	7.060
24	6.989	7.197
22	7.290	7.195
21	7.609	7.091
7	7.338	6.809
10	7.028	6.891
17	6.685	6.792
4	6.730	6.654

The applicability of model 2 in predicting activities of external molecules is shown by a plot of observed versus predicted pMIC values for test set in **Figure 6.**

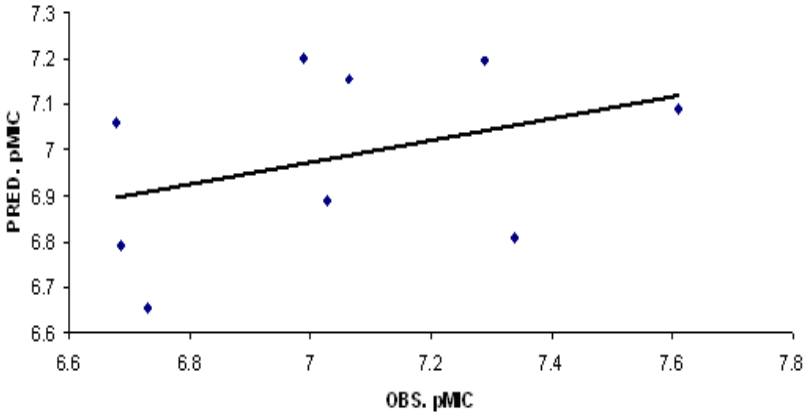


Fig. 6: Discrete Plot of test set between observed vs. predicted by leave-one-out cross-validation pMIC values. (model: 2)
Obs. pMIC: Observed pMIC, Cal. (LOO): calculated by leave-one-out cross-validation pMIC,
$y = 0.2393x + 5.2969$, $r^2 = 0.1483$

TABLE 7: Statistics of Significant Equations

Model No.	n	r^2	F	r^2_{bs}	Chance	S_{DEP}	S_{PRESS}	q^2
1	21	0.868	37.268	0.868	<0.001	0.094	0.105	0.807
2	21	0.864	36.04	0.865	<0.001	0.094	0.106	0.804

CONCLUSION

Ovality is the ratio of the molecular surface area to the minimum surface area. The minimum surface area is the surface area of a sphere having a volume equal to the solvent excluded volume of the molecule. The positive contribution of the ovality indicates the activity will be increase with bulky substituent.

HOMO is the highest occupied molecular orbital called frontier orbital and determines the way it interacts with other species. HOMO is the orbital that could act as an e- donor. Since it is

outermost (highest energy). The negative contribution of HOMO energy suggested that substitution of group at R_1 position on benzoxazole ring with electron withdrawing group favourable for the antibacterial activity in the concerned microbes

Electronic energy and repulsion energy are electronic descriptors. Electronic energy is defined as the total electronic energy at 298K, while the repulsion energy (Ev) is defined as the total core-core internuclear repulsion between atoms.

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