International Journal of Drug Discovery, ISSN: 0975–4423, Volume 1, Issue 1, 2009, pp-1-9

Synthesis and QSAR analysis of 5-substituted (arylmethylene) pyridin-2-amine derivatives as potential antibacterials

Bhatia M.S.*, Mulani A.K., Choudhari P.B., Ingale K.B., Bhatia N.M.

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of pharmacy,Near chitranagri, Kolhapur.416013 , Phone No. (0231) 2638392, +919822172940, E mail: manish_bhatia13@yahoo.co.in , drmsb13@yahoo.com

Abstract- A series of 5-substituted (arylmethylene) pyridin-2-amine were synthesized by condensing various 5-substituted pyridyl-2-amines with various aromatic aldehydes. The structures of newly synthesized compounds were characterized by spectral and elemental analysis. All the compounds were screened for their antibacterial activities. The QSAR studies of were performed on MOE 2006.08 software. QSAR equation reveled that selected electronic, steric and liphophillic parameters have correlation with antibacterial activity. Best equations were selected on basis of correlation coefficient (r2) and predicitivity of equation. The frequent appearance of Log P and SMR terms in the QSAR equations is indicative of lipophilic and steric parameters are the prerequisites for molecules to exhibit activity against bacteria.

Keywords: QSAR, antibacterial, pyridin-2-amine, imines

INTRODUCTION

Pyridine derivatives continue to attract great interest due to the wide variety of interesting biological activities observed for these compounds, such as anticancer, analgesic, antimicrobial, and antidepressant, activities[1-4]. In view of these reports, the synthesis of a new series of 5-substituted pyridine-2-amine derivatives is now reported. Several compounds were screened for their antifungal activity. Fungi have been identified as causative agents of human diseases earlier than bacteria. In spite of earlier beginnings, the study of pathogenic fungi received only scant attention in comparison with study of other pathogens. in various immuno comprised diseases like HIV. Therefore, it was necessary to identify potent pharmacophores for antibacterial activity in order to develop new antibacterial agents. Many studies have been carried out on various rings such as triazoles, pyrazoles, oxadiazoles, and imidazoles to develop new antibacterial agents. Hence, there is a need to analyze the correlation present in between antibacterial activity and physico-chemical parameters using the Quantitative Structure Activity Relationship (QSAR) methods. Quantitative structure-activity relationship (QSAR) enables the investigators to establish reliable quantitative structure-activity and structure-property relationships to derive an in silico QSAR model to predict the activity of novel molecules prior to their synthesis. In order to study and deduce a correlation between structure and biological activity of 5-substituted-N-(arylmethylene) pyridin-2-amine derivatives as antibacterial agents, we have developed QSAR models. Together with these models derived it revealed the significance of some steric, electrostatic, hydrophobic parameters with biological activity. Structural variations in the molecular fields of particular regions in the space can be studied and QSAR models can be used to give an insight in the design of potent antibacterial agents.

Experimental

Twenty-five different 5-substituted (arylmethylene) derivatives of pyridin-2-amine were synthesized (scheme I) and evaluated for their antibacterial activity. The starting material 2-amino-5-bromo pyridine (**1**) and 2-amino-3-nitro-5-bromo pyridine (**2**) were prepared by bromination of 2-aminopyridine and subsequent nitration as per the literature procedure .The bromo derivatives **(I, II)** underwent nucleophilic substitution reaction with substituted phenols to give corresponding 5-aryloxy pyridine-2-amine derivatives **(III)**. Further all the 5-aryloxy and 5-bromo derivatives were condensed with aldehydes to give corresponding imines. Table 1 shows the substitutions at he aryl methylene group. The IR spectra were recorded on Jasco FT/IR 4100 spectrophotometer. ^{1}H NMR spectra were recorded on Varian NMR 400 MHz spectrometer using $CDCl_3$ as a solvent and TMS as internal standard [5-6].

Procedure for Synthesis of 5-aryloxy pyridin-2-amine derivatives (III)

Various substituted phenols and potassium hydroxide were placed in a round bottom flask and the mixture was heated to 130-140 ^{0}C until all the alkali has dissolved. Then copper catalyst and 2-amino-5-bromopyridine or 2-amino-5-bromo-3-nitropyridine were added to the flask. The flask was then fitted with a mechanical stirrer, thermometer, and a reflux condenser, and another portion of 2-amino-5-bromopyridine or 2-amino-5-bromo-3-nitropyridine was added. The mixture is again heated as before until a second spontaneous reaction begins. The dark colored reaction mixture was then poured into ice water containing sodium hydroxide and stirred well to remove excess phenol. The crude product is allowed to settle. The product is filtered on a Buchner funnel, washed water, and pressed as free from water as possible and dried in air. Recrystallization from chloroform gives pure product

Procedure for Synthesis of 5-substituted (arylmethylene) pyridin-2-amine derivatives (1-25)

The amines (I, II, III) were triturated with substituted aldehydes in equal ratio in presence of 0.1 M sodium hydroxide solution. The reaction mixture was neutralized using 0.1 M HCL solution. Further the reaction mixture was filtered, washed with water and air dried. The crude product was recrystallised using mixture of solvent Chloroform: Cyclohexane: Ethanol (4:4:2) to give the pure product. I. R. (KBr) cm^{-1} 1265.07(Ar-ether), 1627.63 (CH=N), 3066.26 (C-H stretching), 1487.33 (NO_2 stretching).^{1}H NMR (DMSO)δ 3.4 (s, H_2O); 6.13 (s, 1H, Pyri); 6.3 (d, 2H, arom); 6.7(d, 2H, arom); 7.49-7.67 (m, 5H, arom); 7.91(s, 1H, Pyri); 9.63(s, 1H, CH=N)

Antibacterial Activity

The synthesized compounds were screened for antibacterial activity against *E. coli (NCIM-27350), P. aeruginosa (NCIM-501), S. aureus and B. subtilis (NCIM-2197).*The cup plate agar diffusion method was used for antibacterial activity [7]; MIC was calculated using serial dilution method. The tested compounds were dissolved in N, N-dimethylformamide (DMF) to get a solution of 1000, 750, 500, 250, 125, 62, 31.5 mg/ml. The inhibition zones were measured in millimeters at the end of an incubation period of 48 h at 28 ºC. DMF was used as control. Commercial antibacterial ciprofloxacin was also tested under similar conditions for comparison.

QSAR Analysis

Data Set

The builder module of the Vlife MDS was used to generate molecular models of series of 5-substituted (arylmethylene) pyridin-2-amine derivatives. They were then energy-minimized using the Merck Molecular Force Field (MMFF). The charge equilibration method was used to assign atomic partial charges to each of the compounds. Activity values for the QSAR equation were obtained using the negative logarithm of Minimum Inhibitory concentration. The physicochemical properties of each compound were specified using 252 descriptors, which delineate liphophillic, conformational, electronic, spatial, structural, thermodynamic and quantum mechanical information [8].

Full Search Multiple Linear Regression Method

A relationship between independent and dependent variables (physicochemical descriptors and biological activities, respectively) were determined statistically using regression analysis. Linear regression is achieved by fitting a best-fit straight line to the data using the least squares method. Descriptors that are included in a reasonable QSAR equation should exhibit low inter-correlation and thus, behave as independent variables. The inter-correlation between descriptors was used for selecting descriptors for equation and the quality of fit for a regression equation was assessed relative to its correlation coefficient and standard deviation. The F value represents the level of statistical significance of the regression. The predictive quality of a regression model can be evaluated using the leave-one-out cross-validation procedure (XR^2). For a regression model, r^2 was used to describe the fitness of data and fitness is considered to improve as r^2 approaches 1. The sum of the squared deviations of dependent variables (SD) The cross-validated correlation coefficient (XR^2) is defined as (1 – PRESS/SD)1/2 and it used to evaluate the predictive power of the QSAR equations that were generated. Each molecule was eliminated from the training set and cross-validated XR^2 was calculated using the predicted values for the missing molecule. Given that, the full search method performs an exhaustive examination all possible descriptor combinations, there is little concern that important descriptors might be missed and this method enables identification of the QSAR equation with the best correlations. The program determines inter-correlation between descriptors and those combinations containing high inter-descriptor inter-correlation were discarded. QSAR equations that have correlation coefficient which equal or exceed a preset value are reported. We specified 0.5 and 0.65 as the inter-correlation and correlation coefficient cutoff values, respectively.

Activity prediction

To systematically assess a QSAR model, a reliable validation is required. Usually, a QSAR model is evaluated by the predictive results for the given dataset

Result and Discussion

The synthesis of the pyridine derivatives has been accomplished as shown in **Scheme I.** The products formed were confirmed by chromatographic and spectral data. The IR absorption band at 1612 cm^{-1} showed the presence of a conjugated imine (>C=N), while the presence of absorption band at 1234 cm^{-1} indicates presence of ether group. The ^{1}H NMR spectrum of compounds explained the presence of singlet of imine hydrogen at δ 9.63 (1H, CH=N). Biological activity data, MIC (Minimum Inhibitory Concentration) was converted to negative log dose in moles (pMIC) for QSAR analysis. The data of biological activity is given in Table 2. In order to derive a reasonable QSAR equation, the obtained equations were evaluated by the predictive results for the dataset. Removal of outliers improved the correlation coefficient of the QSAR equations. Statistical values indicated that equations with outliers

were unreliable (r^2 < 0.55). Following elimination of the outliers there was great improvement in the statistical value of the QSAR equations. The final QSAR equation was as follows:

pMIC *S. aureus* = 4.1101 - 0.0026 (ASA_P) - 0.00007 (pmiX) - 0.00247 (E) (Eqn 1)

n = 25, r^2 = 0.79742, SE= 0.13544, F = 29.842.

In case of antibacterial activity on *S. aureus* various statistically significant equations were obtained but the model (Eqn 2) showed good correlation. The equation was further subjected to confirm internal consistency, the cross validation coefficient (q^2 = 0.541) shows good antibacterial activity prediction. The plot of predicted pMIC and observed pMIC is shown in Fig. 1

pMIC *B. subtilis* = 1.91137 + 0.24510(rgyr)- 0.00981(E) + 0.00202(ASA_P) (Eqn 2)

n = 25, r^2 = 0.83923, SE= 0.04224, F = 19.243

For *B. subtilis,* correlation (R^2 = 0.83923) was identified (Eqn 3) as good model for activity against organism, with cross validation squared coefficient (0.55). The plot of predicted pMIC and observed pMIC is shown in Fig. 2

pMIC *E. coli* = 4.5978 - 0.62803(rgyr) - 0.3553(AM1_LUMO) + 0.18772(logP) (Eqn 3)

n = 25, r^2 = 0.82547 , SE= 0.10270 , F = 23.642.

The model obtained for *E coli* was found to be significant (R^2 = 0.82547) as seen in (Eqn 4), this was cross validated and the cross validation squared correlation coefficient (0.61) suggest good predictive ability of equation. The plot of predicted pMIC and observed pMIC is shown in Fig. 3

pMIC *P. aerugenosa* = 3.0570 - 0.0693(SlogP) - 0.0498(SMR)-.32414(AM1_LUMO) (Eqn 4)

n = 25, r^2 = 0.87264, SE= 0.05543, F = 29.112.

The model obtained for *P.aerugenosa* was found to be significant (R^2 = 0.87264) as seen in (Eqn 4), this was cross validated and the cross validation squared correlation coefficient (0.61) suggest good predictive ability of equation. The plot of predicted pMIC and observed pMIC is shown in Fig. 4

DISCUSSION

The given QSAR equation best describes antibacterial activity as confirmed by validation of the model judging internal, intercorrelation, and other statistical terms like the F value. The variables in the equation show low correlation among themselves, indicating a lesser probability of chance correlation.

Interpretation of Equation 1

Aryloxy substitutions on ring at imino carbon and the 2nd position were responsible for variation the in activity. The antibacterial activity against *S. aureus* according to QSAR equation 1 is dependant on polar solvent (water) accessible surface area, principle moment of inertia and potential energy. The (water) accessible surface area is the property of hydrophilicity. The property indicates affinity of compounds towards water. Compounds in the series show variable solubility values due to substitution on the 5th carbon of the pyridine ring. The ASA value has negative correlation with the activity; this indicates that the substituents which decrease solubility in water will lead to increased activity. The decrease in solubility in water or increase in the lipophillic nature of compounds will lead to increased penetration of the compounds across the cell wall. The principle moment of inertia is a steric parameter that is dependant on the spatial array of the aromatic ring in the synthesized compounds. The spatial arrangement also is necessary to study the interaction of the ligand with the receptor. The principle moment of inertia is negatively correlated with the activity against *S. aureus.* This indicates that the arrangement of the aromatic rings present on the pyridine ring should be closer for the activity to be maximum. Potential energy indicative of the reactivity of the particular compound towards the receptor. The potential energy of compounds is positively correlated with the activity. Increase in the potential energy of molecules might be responsible for the kinetics or dynamics of the compounds inside the fungal cell. Thus, molecules having high potential energy will require less energy of activation for interaction with the receptor target. Increase in activity may be achieved by substitutions favoring low solubility values and having a higher potential energy.

Interpretation of Equation 2

The equation 2 explains the variation in the activity of the 5-substituted (arylmethylene)pyridin-2-amine derivatives against *B. subtilis*. The activity is dependant on variables like E, ASA_P and Radius of Gyration. The radius of gyration and the polar solvent (water) accessible surface area are positively correlated with the activity against *B. subtilis*, while the potential energy is negatively correlated with the activity. This indicates that the molecules if have higher potential energy along with lower water accessible surface area the molecule is tend to have interactions with unworthy targets reducing the mortality of the compound.

Interpretation of Equation 3

The equation 2 explains the variation in the activity of the 5-substituted (arylmethylene)pyridin-2-amine derivatives against *E coli* The activity is dependant on variables like LUMO, Log P and Radius of Gyration. LUMO is a quantum descriptor; indicative of the reaction with the receptor site is nucleophilic. Log P values are indicative of lipophilic nature of a particular moiety, while the radius of gyration is, a steric descriptor. Negative correlation with LUMO values indicates interaction taking place at the target favors higher electron density site. The partition coefficient is quantitative measure of the lipophilic and hydrophilic properties of the compounds. The high value of partition coefficient indicates that the compounds favor the lipophilic phase. In QSAR equation the log P value is positively correlated, indicating that the compounds having high log P values will have more activity. The substitution at the 5^{th} position is attributed to difference in log P values. Substitutions favoring the lipophilic phase will be leading to increase in activity. The positive contribution of log P values may be due to fact that compounds require to penetrate the bacterial cell wall to interact with the actual target inside the cell

Interpretation of Equation 4

The dependant variables contributing for activity against *P.aerugenosa* according to equation 4 are LUMO, Log P and molar refractivity. Negative correlation with LUMO values indicates interaction taking place at the target favors higher electron density site. The partition coefficient is quantitative measure of the lipophilic and hydrophilic properties of the compounds. The high value of partition coefficient indicates that the compounds favor the lipophilic phase. In QSAR equation the log P value is positively correlated, indicating that the compounds having high log P values will have more activity.

REFERENCES:

[1] Amr A.G.; Mohamed A.M.; Mohamed S.F.; Abdel-Hafez N.A.; and Hammam A.G. (2006) *Biorganic Medicinal. Chemistry*,14,5481–5488.

[2] Bart A.; Jansen J.;Zwan J.V.; Dulk. H.; Brouwer. J.; and Reedijk J. (2001) *Journal of Medicinal. Chemistry*, 44,245-249.

[3] Shinkai H.; Ito T.; Iida T.; Kitao Y.;, Yamada H.; and Uchida I. (2000) *Journal of Medicinal. Chemistry*, 43, 4667-4677.

[4] Klimesˇova V.; Svoboda M.; Waisser K.; Pour M.; and Kaustova J. (1999) *Farmaco*,54, 666–672.

[5] Morrison R.T.; Boyd R.E. Aldehydes and ketones, In, Organic Chemistry,New Delhi, 6th ed, Prentice-Hall of India Private Ltd., New Delhi(1996) 657-675.

[6] Loudon G.M.; Chemistry of aldehyde and ketones: carbonyl-addition reactions.In Organic chemistry, 4th Edition. Oxford University press New York (2002): 874-880

[7] Indian Pharmacopoeia, New Delhi. Vol-II. Controller of Publications. 1996: A-100.

[8] Pleiss M.A.; Unger S.H.; The design of test series and significance of QSAR relationships. In Comprehensive Medicinal chemistry.. vol-4. Elsevier New Delhi (2005) 561-589.

Scheme

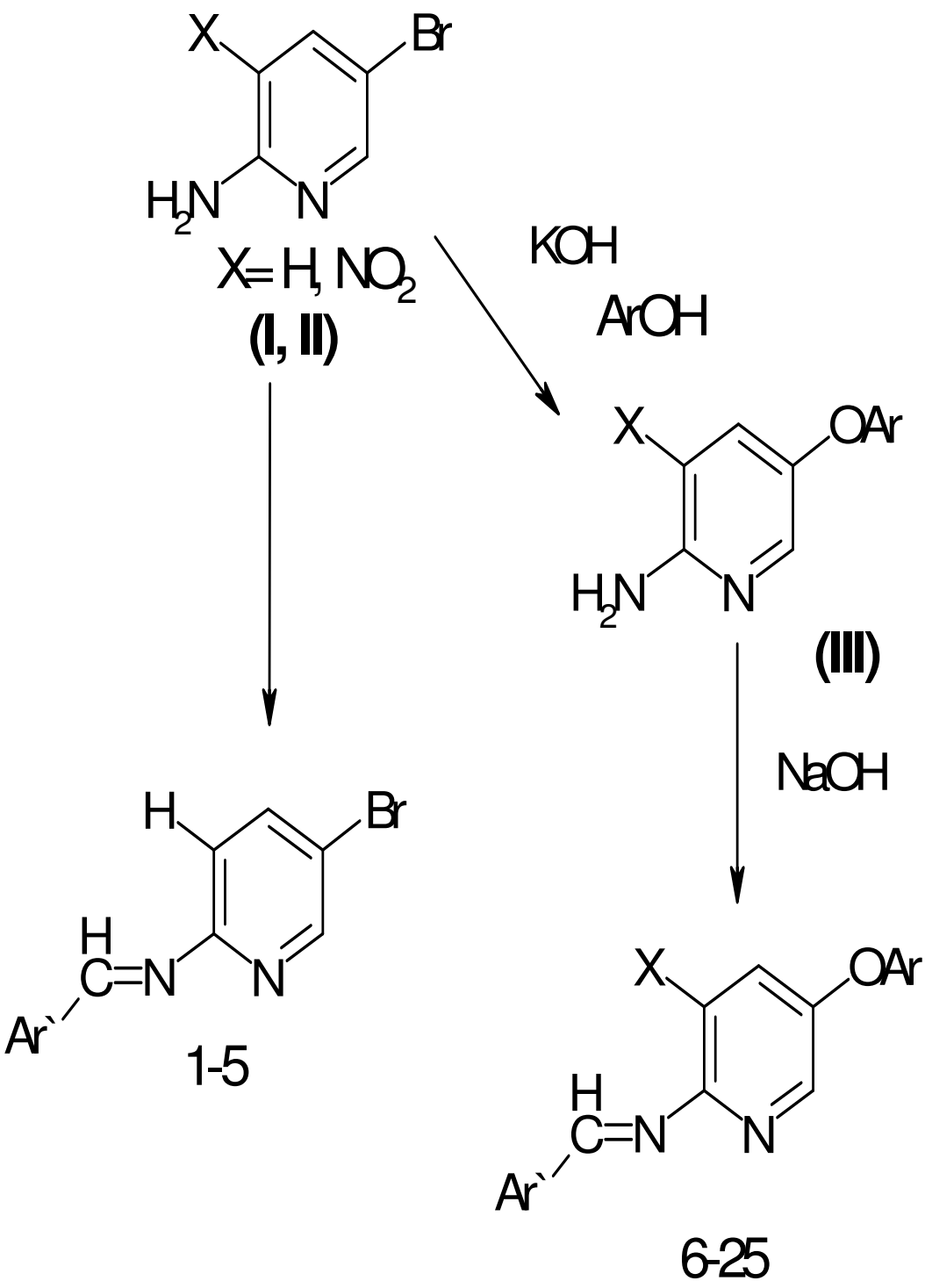
X
Br
H2N
N
X= H, NO2
(I, II)
KOH
ArOH
X
OAr
H2N
N
(III)
NaOH
H
Br
H
C=N
N
Ar`
1-5
X
OAr
H
C=N
N
Ar`
6-25

Table 1: Table showing substitutions on the pyridine ring

Comp. code	Ar	X	Ar`	Comp. Code	Ar	X	Ar`
1	-	H	Phenyl	14	3-Nitro phenyl	NO_2	3,4,5-trimethoxy phenyl
2	-	H	4-Chloro phenyl	15	3-Nitro phenyl	NO_2	3-Indolyl
3	-	H	N,N Dimethyl amino phenyl	16	2-Nitro phenyl	H	Phenyl
4	-	H	3,4,5-trimethyl phenyl	17	2-Nitro phenyl	H	4-Chloro phenyl
5	-	H	3-Indolyl	18	2-Nitro phenyl	H	N,N Dimethyl amino phenyl
6	4-Nitro phenyl	NO_2	Phenyl	19	2-Nitro phenyl	H	3,4,5-trimethyl phenyl
7	4-Nitro phenyl	NO_2	4-Chloro phenyl	20	2-Nitro phenyl	H	3-Indolyl
8	4-Nitro phenyl	NO_2	N,N Dimethyl amino phenyl	21	2,4,6-tri nitro phenyl	H	Phenyl
9	4-Nitro phenyl	NO_2	3,4,5-trimethyl phenyl	22	2,4,6-tri nitro phenyl	H	4-Chloro phenyl
10	4-Nitro phenyl	NO_2	3-Indolyl	23	2,4,6-tri nitro phenyl	H	N,N Dimethyl amino phenyl
11	3-Nitro phenyl	NO_2	Phenyl	24	2,4,6-tri nitro phenyl	H	3,4,5-trimethyl phenyl
12	3-Nitro phenyl	NO_2	4-Chloro phenyl	25	2,4,6-tri nitro phenyl	H	3-Indolyl
13	3-Nitro phenyl	NO_2	N,N Dimethyl amino phenyl				

Table 2: Table showing pMIC values, predicted values and residuals for QSAR models

Comp	*S. Aureus* (pMIC)			*B. subtilis* (pMIC)			*E coli* (pMIC)			*P.aerugenosa* (pMIC)		
	Obs	Cal	Res	Obs	Cal	Res	Obs	Cal	Res	Obs	Cal	Res
1	3.59	3.75	-0.15	2.61	2.62	-0.01	3.11	3.07	0.04	2.78	2.78	0.00
2	3.67	3.66	0.01	2.7	2.77	-0.03	2.83	2.86	-0.02	2.77	2.75	0.01
3	3.47	3.50	-0.02	2.78	2.74	0.04	2.48	2.50	-0.02	2.60	2.64	-0.03
4	3.68	3.65	0.03	2.68	2.66	0.02	2.78	2.89	-0.10	2.63	2.62	0.01
5	3.47	3.58	-0.10	2.47	2.52	-0.04	2.87	2.73	0.14	2.77	2.72	0.05
6	3.26	3.23	0.02	2.60	2.56	0.04	2.86	2.94	-0.07	2.86	2.80	0.05
7	3.20	3.12	0.04	2.72	2.68	0.04	2.60	2.76	-0.16	2.71	2.76	-0.05
8	2.81	2.99	-0.18	2.61	2.64	-0.03	2.21	2.44	-0.23	2.64	2.67	-0.03
9	3.21	3.12	0.08	2.60	2.57	0.03	2.81	2.79	0.01	2.73	2.65	0.08
10	3.20	3.04	0.16	2.90	2.82	0.07	2.60	2.65	-0.04	2.77	2.71	0.06
11	3.16	3.19	-0.03	2.56	2.60	-0.04	2.86	2.88	-0.02	2.68	2.77	-0.09
12	3.07	3.10	-0.09	2.68	2.73	-0.04	2.60	2.69	-0.09	2.71	2.73	-0.02
13	3.07	2.96	0.04	2.68	2.67	0.01	2.61	2.42	0.18	2.61	2.64	-0.03
14	3.07	3.08	-0.07	2.61	2.61	-0.00	2.91	2.76	0.14	2.60	2.63	-0.02
15	3.20	3.01	0.18	2.93	2.85	0.07	2.60	2.64	-0.03	2.60	2.69	-0.09
16	3.40	3.41	-0.00	2.50	2.52	-0.02	2.80	2.78	0.01	2.62	2.65	-0.03
17	3.35	3.30	0.05	2.71	2.67	0.04	2.55	2.55	-0.00	2.60	2.61	-0.00
18	2.95	3.14	-0.18	2.61	2.63	-0.01	2.36	2.24	0.11	2.62	2.54	0.08
19	3.46	3.29	0.16	2.55	2.55	0.00	2.55	2.61	-0.05	2.55	2.51	0.04
20	3.45	3.21	0.024	2.75	2.80	-0.04	2.55	2.48	0.07	2.55	2.58	-0.03
21	3.28	3.01	0.26	2.61	2.56	0.04	3.21	3.21	0.00	3.14	3.05	0.08
22	2.78	3.90	-0.12	2.71	2.71	0.00	2.98	2.98	-0.03	2.90	3.01	-0.11
23	2.78	2.73	0.04	2.65	2.68	-0.02	2.65	2.70	-0.04	2.95	2.96	-0.00
24	2.65	2.88	-0.23	2.61	2.60	0.00	3.25	3.04	0.21	2.95	2.92	0.03
25	2.65	2.81	-0.15	2.75	2.85	-0.09	2.95	2.93	0.02	3.08	2.99	0.06

Obs: Observed pMIC values Cal: predicted pMIC values Res: Residuals.

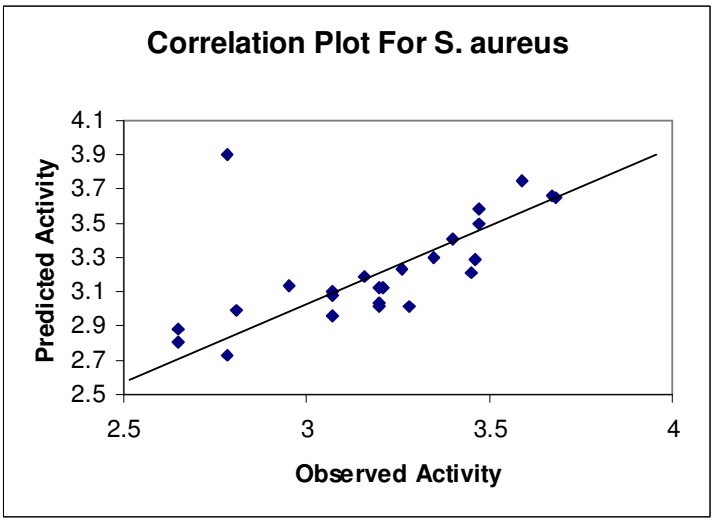

Fig. 1. Plot showing correlation between predicted and observed biological activity against *S. aureus*

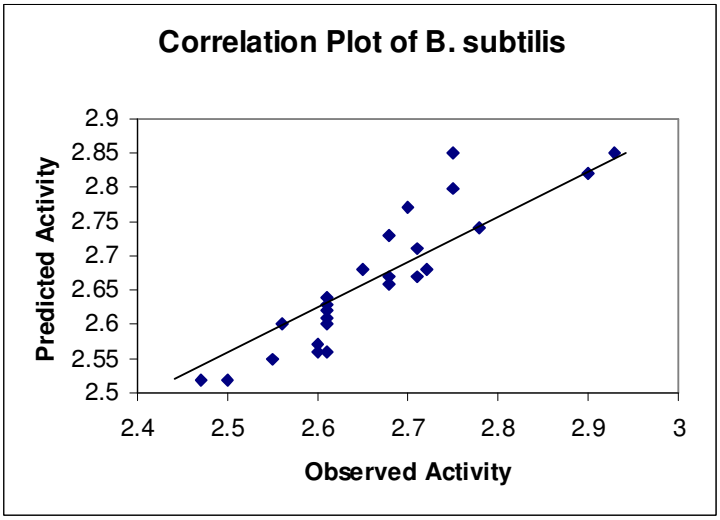

Fig. 2. Plot showing correlation between predicted and observed biological activity against *B. subtilis*

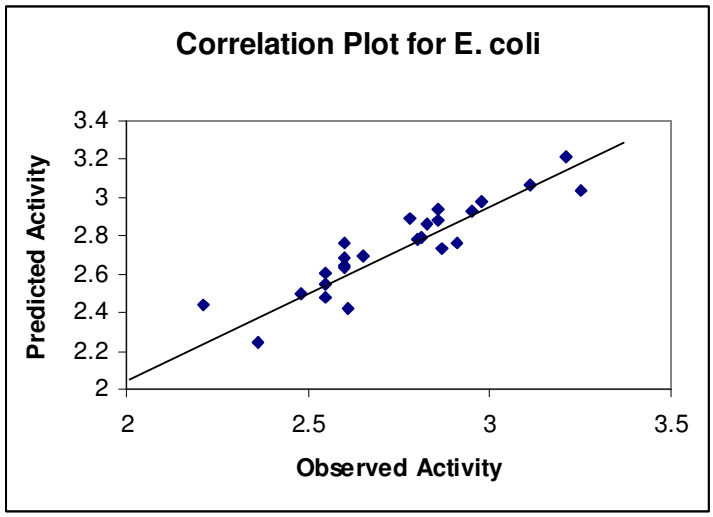

Fig. 3. Plot showing correlation between predicted and observed biological activity against *E. coli*

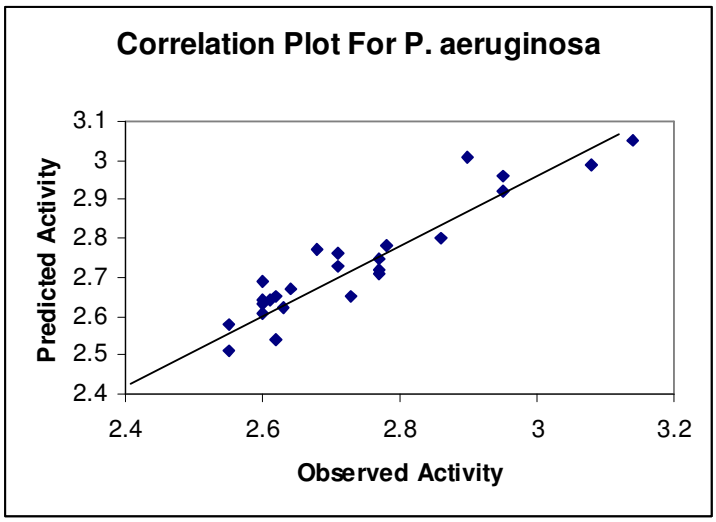

Fig. 4. Plot showing correlation between predicted and observed biological activity against *P. aeruginosa*