Quantitative structural–activity relationship (QSAR) study for antimycobacterial activities of pyrazine containing thiazoline and thiazolidinone derivatives

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A set of 70 compounds pyrazine containing thiazoline and thiazolidinone derivatives against antimycobacterial activities. was subjected to 2D studies using various combination of descriptors.2D QSAR studies through Multiple Linear Regression (MLR) and Partial Least Statistical (PLS) led to five statistically significant models for antimycobacterial activities (all with r^2>0.90, F>> tabulated value, and chance correlation <0.001) having acceptable statistical quality and predictive potential as indicated by the value of cross validated squared coefficient (q^2>0.80). Alignment independent descriptors (H-Donor Count, T_Cl_Cl_6, H-AcceptorCount, XlogP, XKHydrophobicArea, T_T_O_2, Polarizability AHC, Polar Surface Area (Polar Surface Area excluding P and S), were found to have significant correlation with biological activity.

(Received February 12 2010; accepted March 12, 2010)

*Keywords***:** QSAR, Pyrazine, Antimycobacterial activities

1. Introduction

Quantitative drug design embraces two major activities, the quantitative description of the structural differences among series of chemical compounds of biological interest, and the formulation of "QSAR" useful in the design of new and better therapeutic agents [1] QSAR is a mathematical relationship between a biological activity of a molecular system and its geometric and chemical characteristics.QSAR attempts to find consistent relationship between biological activity and molecular properties, so that these "rules" can be used to evaluate the activity of new compounds 3D models are more easily interpretable than 2D descriptor or fingerprint-based QSAR models, making it easier to suggest new compounds for synthesis. It should also be possible to make connections from such activity models to structure-based design, either to add more information to overlays for the construction of a pharmacophore mode or to use a pharmacophore to assist in the refinement of protein homology models [2]. Micro-organisms are proven causative agents for several disorders. An infectious disease is one in which pathogens triumph over the host immunity [3]. After penetrating the defense mechanism micro-organism cause damage to the host[4] and faith of an infection, without medication or due to resistance strain, is either the death of the host or the establishment of mutual adaptation between the host and parasite. Antimicrobial resistance refers to micro organism that has developed the ability to inactive, exclude or blocks the inhibitory or lethal antimicrobialagents[5,6] .Tuberculosis (abbreviated as TB for *tubercle bacillus*) is a common and often deadly infectious disease caused *Mycobacterium tuberculosis*[7] and few of its strains are multidrug resistance. Other mode of medications is either slow acting or not a validated process. Chemotherapy is the keystone in the management of all types of infections in man.

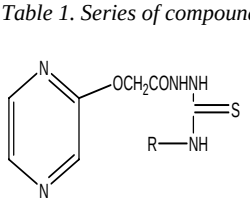
2. Experimental

It is always attempted to prepare a potent agent with broad spectrum activity, lesser side-effects. Pyrazine ring is important for antimycobacterial activity. In addition, many thiazolidine derivatives exhibited wide variety of biological activity, such as anti-microbial, antihistaminic, anti-inflammatory, antihypertensive etc. Hence, it was found worthful to work with an objective to develop more potent antimycobacterial agent by QSAR analysis of Pyrazine containing thiazoline and thiazolidinone derivatives.

2.1 Data set

The selected series[8] of Pyrazine containing thiazoline and thiazolidinone derivatives as antimycobacterial agent having 70 compounds with well defined biological activity and fulfil the criteria of indirect drug design. The biological activity data (IC_{50} in μm) were converted to negative logarithmic dose (pIC_{50}) for quantitative structure activity analysis. The general structure of these analogues is shown in Table 1 list the structural features and anti-cancer activity of the respective compounds under study. The biological data were converted to logarithmic scale (pIC_{50}) in mathematical operation mode of software to reduce skewness of data set and then used for subsequent QSAR analysis as dependent variables.

Table 1. Series of compounds of 2- substituted halogenobenzimidazoles with IC_{50} and P_{IC50} values.



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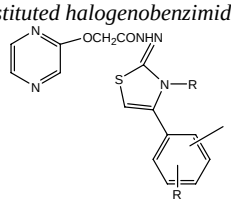

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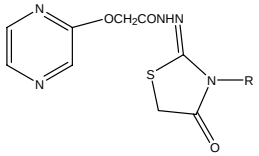

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Compound	R	R^1	MIC	-PMIC
4	4-Chloro-2-nitrophenyl	-	52	4.284
5	4-Chlorophenyl	-	62	4.208
6	2,4-Cholrophenyl	-	57	4.244
7	4-Ethoxyphenyl	-	85	4.071
8	n-Butyl	-	45	4.347
9	Isopropyl	-	56	4.252
10	t-Butyl	-	51	4.292
11	4-Chloro-2-nitrophenyl	4-Bromo	1.01	6
12	4-Chloro-2-nitrophenyl	4-Chloro	0.3	6.523
13	4-Chloro-2-nitrophenyl	4-fluro	6.1	5.215
14	4-Chloro-2-nitrophenyl	4-Methoxy	2.7	5.569
15	4-Chloro-2-nitrophenyl	4-Hydroxy	4.0	5.398
16	4-Chloro-2-nitrophenyl	2,4-Dichloro	3.6	5.444
17	4-Chloro-2-nitrophenyl	-	5.2	5.284
18	4-Chloro-2-nitrophenyl	4-Methoxy	4.5	5.347
19	4-Chlorophenyl	4-Bromo	10.7	4.971
20	4-Chlorophenyl	4-Chloro	11.5	4.939
21	4-Chlorophenyl	4-fluro	12.7	4.896
22	4-Chlorophenyl	4-Methoxy	11.6	4.936
23	4-Chlorophenyl	4-Hydroxy	12.4	4.907
24	4-Chlorophenyl	2,4-Dichloro	10.8	4.966
25	4-Chlorophenyl	-	12.5	4.903
26	4-Chlorophenyl	4-Methoxy	12.1	4.917
27	2,4-Dichlorophenyl	4-Bromo	10.5	4.979
28	2,4-Dichlorophenyl	4-Chloro	11.9	4.924
29	2,4-Dichlorophenyl	4-fluro	12.2	4.914
30	2,4-Dichlorophenyl	4-Methoxy	9.6	5.018
31	2,4-Dichlorophenyl	4-Hydroxy	10.8	4.966
32	2,4-Dichlorophenyl	2,4-Dichloro	10.6	4.975
33	2,4-Dichlorophenyl	-	12.4	4.907
34	2,4-	4-Methoxy	11.5	4.939

Compound	R	R^1	MIC	-PMIC
	Dichlorophenyl			
35	4-Ethoxyphenyl	4-Bromo	8.9	5.051
36	4-Ethoxyphenyl	4-Chloro	8.7	5.06
37	4-Ethoxyphenyl	4-fluro	10.2	4.991
38	4-Ethoxyphenyl	4-Methoxy	8.7	5.06
39	4-Ethoxyphenyl	4-Hydroxy	10.4	4.983
40	4-Ethoxyphenyl	2,4-Dichloro	1.5	5.824
41	4-Ethoxyphenyl	-	3.5	5.456
42	4-Ethoxyphenyl	4-Methoxy	3.1	5.509
43	n-Butyl	4-Bromo	7.4	5.131
44	n-Butyl	4-Chloro	7.6	5.119
45	n-Butyl	4-fluro	7.6	5.119
46	n-Butyl	4-Methoxy	6.6	5.18
47	n-Butyl	4-Hydroxy	7.9	5.102
48	n-Butyl	2,4-Dichloro	7.2	5.143
49	n-Butyl	-	9.2	5.036
50	n-Butyl	4-Methoxy	8.7	5.06
51	Isopropyl	4-Bromo	8.1	5.091
52	Isopropyl	4-Chloro	8.7	5.06
53	Isopropyl	4-fluro	8.5	5.071
54	Isopropyl	4-Methoxy	7.5	5.125
55	Isopropyl	4-Hydroxy	8.4	5.076
56	Isopropyl	2,4-Dichloro	8.2	5.086
57	Isopropyl	-	10	5
58	Isopropyl	4-Methoxy	9.5	5.022
59	t-Butyl	4-Bromo	6.3	5.201
60	t-Butyl	4-Chloro	7.2	5.143
61	t-Butyl	4-fluro	6.9	5.161
62	t-Butyl	4-Methoxy	5.8	5.237
63	t-Butyl	4-Hydroxy	7.1	5.149
64	t-Butyl	2,4-Dichloro	6.6	5.18
65	t-Butyl	-	8.7	5.06
66	t-Butyl	4-Methoxy	8.2	5.086
68	4-Chloro-2-nitrophenyl	-	85	4.071
69	4-Chlorophenyl	-	102	3.991
70	2,4-Dichlorophenyl	-	148	3.83
71	4-Ethoxyphenyl	-	169	3.772
72	n-Butyl	-	156	3.807
73	Isopropyl	-	191	3.719
74	t-Butyl	-	128	3.893

Table 2. Actual and predicted activities of training and test set compounds in statistically significant models.

Compound no.	QSAR Set	Actual Activity	Predicated activity				
			Pred.1	Pred.2	Pred.3	Pred.4	Pred.5
4	training	4.284	4.38	4.35	4.22	4.29	4.15
5	training	4.208	4.35	4.35	4.22	4.26	4.08
6	training	4.244	4.31	4.27	4.24	4.22	4.19
7	training	4.071	4.10	3.89	3.87	4.11	4.23
8	training	4.347	4.15	4.11	4.11	3.99	4.09

Compound no.	QSAR Set	Actual Activity	Predicated activity				
			Pred.1	Pred.2	Pred.3	Pred.4	Pred.5
9	training	4.252	4.15	4.11	4.10	4.04	4.19
10	test	4.292	4.12	4.22	4.24	4.32	4.28
11	training	6	5.36	5.31	5.31	5.38	5.74
12	test	6.523	5.32	5.25	5.85	5.97	6.23
13	training	5.215	5.24	5.10	5.14	5.06	5.01
14	training	5.569	5.32	5.13	5.21	5.34	5.45
15	test	5.398	4.96	4.94	5.15	5.22	5.29
16	training	5.444	5.34	5.24	5.31	5.29	5.35
17	training	5.284	4.98	4.94	5.13	5.21	5.13
18	training	5.347	5.29	5.22	5.25	5.32	5.38
19	training	4.971	5.36	5.26	5.11	5.03	4.95
20	training	4.939	5.31	5.17	5.12	5.15	5.04
21	test	4.896	5.22	5.07	5.01	4.97	4.80
22	test	4.936	5.28	5.16	5.09	4.97	4.94
23	training	4.907	4.95	4.87	4.86	4.83	4.81
24	test	4.966	5.34	5.35	5.11	5.06	5.23
25	training	4.903	4.95	4.91	4.87	4.86	4.74
26	training	4.917	4.27	4.58	4.64	4.87	4.89
27	test	4.979	5.37	5.23	5.34	5.32	5.35
28	training	4.924	4.33	4.56	4.63	4.71	4.86
29	training	4.914	4.24	5.02	5.05	5.11	4.90
30	training	5.018	5.28	5.12	5.23	5.21	5.01
31	training	4.966	4.95	4.87	4.76	4.78	4.93
32	training	4.975	5.34	4.87	4.81	4.78	4.91
33	training	4.907	4.95	4.95	4.99	5.05	5.11
34	training	4.939	4.82	4.91	4.91	4.98	4.87
35	training	5.051	5.08	4.98	5.02	5.04	5.05
36	test	5.06	5.04	5.12	5.34	5.12	5.21
37	training	4.991	4.92	4.22	5.31	4.99	4.87
38	training	5.06	5.08	4.24	5.25	4.68	4.91
39	training	4.983	4.95	3.87	5.10	5.06	4.98
40	training	5.824	5.28	5.11	5.13	5.68	5.81
41	test	5.456	4.97	5.10	4.94	5.07	5.65
42	training	5.509	5.32	5.24	5.24	4.94	5.40
43	training	5.131	5.07	5.31	4.94	4.94	5.09
44	training	5.119	5.02	5.15	5.22	4.93	5.10
45	training	5.119	4.98	5.14	5.26	4.90	5.09
46	training	5.18	5.04	5.01	5.17	4.62	5.02
47	training	5.102	4.69	5.15	5.07	4.99	4.98.
48	training	5.143	5.04	5.31	5.16	4.65	4.86
49	training	5.036	4.92	5.13	4.87	4.98	5.01
50	training	5.06	5.03	5.15	5.31	4.44	5.11
51	training	5.091	5.09	5.11	5.25	5.06	5.06
52	test	5.06	5.07	5.12	5.10	5.11	5.24
53	test	5.071	5.06	5.01	5.13	5.15	5.03
54	test	5.125	4.99	5.09	4.94	5.25	5.15
55	training	5.076	4.68	4.99	5.24	4.80	5.01
56	training	5.086	5.06	5.26	4.94	4.87	5.15
57	training	5	4.68	4.82	5.22	5.28	5.31
58	test	5.022	5.07	5.11	5.26	4.95	5.13
59	training	5.201	5.07	4.98	5.11	5.15	5.17
60	training	5.143	4.94	4.94	5.07	4.95	5.11
61	test	5.161	4.93	4.92	5.16	4.82	5.19

Compound no.	QSAR Set	Actual Activity	Predicated activity				
			Pred.1	Pred.2	Pred.3	Pred.4	Pred.5
62	test	5.237	4.90	5.38	4.87	5.08	5.21
63	test	5.149	4.62	5.17	5.31	5.18	5.09
64	training	5.18	4.99	5.06	5.25	4.92	4.99
65	training	5.06	4.65	5.34	5.10	5.06	5.01
66	training	5.086	4.98	5.22	5.13	5.15	5.15
68	test	4.071	4.44	4.29	4.94	5.28	5.31
69	training	3.991	3.56	3.81	4.24	3.97	3.87
70	test	3.83	3.71	3.72	3.94	4.32	4.11
71	training	3.772	3,65	3.63	4.22	3.87	3.71
72	training	3.807	3.85	3.75	4.26	3.72	3.78
73	training	3.719	3.80	3.97	4.17	3.68	3.67
74	training	3.893	3.87	3.97	4.07	4.15	3.85

2.2 Molecular modelling

The molecular modeling studies were performed using MDS 3.0, supplied by V Life science [9]. The structure of each compound was drawn in 2D apply mode of software and export in 3D mode for create 3D model. Energy minimization was performed of each model using Merk Molecular Force Field (MMFF). Complete geometry optimization was performed taking the most extended conformations as starting geometries. The basis of energy minimization is that the drug binds to effectors/receptor in the most stable form i.e. minimum energy state form. The relationship between biological activities and various descriptors (Physiochemical and alignment-independent) were established by sequential multiple regression analysis (MLR) using MDS 3.0, in order to obtain QSAR models. The MDS 3.0 program was employed for the calculation of different quantum chemical descriptors including heat of formation, dipole moment, local charges, and different topological [10], elemental count including Bromine count, fluorine count, Path count and constitutional descriptors for each molecule. Chemical parameters including molar volume (V), molecular surface area (SA), hydrophobicity (log P), hydrogen acceptor count (HAC), hydration energy (HE) and molecular polarizability (MP) were also calculated by using software. The various descriptors selected for 2D QSAR were vdWSurfaceArea (van der Waals surface area of the molecule), –vePotential Surface Area (total van der Waals surface area with negative electrostatic potential of the molecule), +vePotentialSurfaceArea (total van der Waals surface area with positive electrostatic potential of the molecule) dipole moment, YcompDipole (y component of the dipole moment), element count, slogP, path count, cluster, distance based topological indices, connectivity index, hydrophobic and hydrophilic areas like SA Most Hydrophilic (Most hydrophilic value on the vdW surface by Audry Method using Slogp), SAMostHydrophobic Hydrophilic Distance (distance between most hydrophobic and hydrophilic point on the vdW surface by Audry Method using Slogp), SAHydrophilicArea (vdW surface descriptor showing hydrophilic surface area by Audry Method using SlogP) and SKMostHydrophilic (Most hydrophilic value on the vdW surface by Kellog Method using Slogp), radius of gyration, Wiener's index, moment of inertia, semi- empirical descriptors, HOMO (Highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbital), heat of formation and ionization potential. Besides these all alignment independent descriptors were also calculated. The hydrophobic descriptors govern the movement of a drug molecule across the biological membranes in order to interact with the receptor by vander Waals binding forces whereas both electronic and steric descriptors influence the affinity of a drug molecule necessary for proper drug- receptor interaction. The optimal training and test sets were generated by either random selection method or the sphere exclusion algorithm. A commonly used ratio of training to validation objects (test set), which was also adopted in this work, is 70%: 30% [9]. However, rational splitting was accomplished by applying a sphere-exclusion type algorithm [11-15]. In classical sphere-exclusion algorithm the molecules are selected whose similarities with each of the other selected molecules are not higher than a defined threshold. Each selected molecule generates a hyper-sphere around itself, so that any molecule inside the sphere is excluded from the selection in the train set and driven toward the test set. The number of compounds selected and the diversity among them can be determined by adjusting the radius of the sphere (R).

2.3 Statistical analysis

Models were generated by using three significant statistical methods, namely, partial least square analysis, multiple regressions, and principle component analysis. The cross-validation analysis was performed using the leave-one-out method. In the selected equations, the cross-correlation limit was set at 0.5, the number of variables at 10, and the term selection criteria at r^2. An F value was specified to evaluate the significance of a variable. The higher the F value, the more stringent was the significance level: F test ''in'' as 4 and F test ''out'' as 3.99. The variance cutoff was set at 0, and scaling was auto scaling

in which the number of random iterations was set at 100.The following statistical parameters were considered for comparison of the generated QSAR models: correlation coefficient (r), squared correlation coefficient (r2), predictive r2 for external test set (pred r2) for external validation, and Fischer's (F).The predicted r2 (pred_r2) value was calculated using Eq. 1, where yi and y^i are the actual and predicted activities of the i^{th} molecule in the test set, respectively, and y mean is the average activity of all molecules in the training set. Both summations are over all molecules in the test set. The pred_r^2 value indicates the predictive power of the current model for the external test set as follows

$$pred\_r^2 = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - y_{mean})^2} \quad (1)$$

Internal validation was carried out using leave-one-out (q2, LOO) method. For calculating q2, each molecule in the training set was eliminated once and the activity of the eliminated molecule was predicted by using the model developed by the remaining molecules. The q2 was calculated using the equation which describes the internal stability of a model:

$$q^2 = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - y_{mean})^2} \quad (2)$$

where y_i, and $\hat{y}_i$ are the actual and predicted activity of the $_i$th molecule in the training set, respectively, and y_{mean} is the average activity of all molecules in the training set.

3. Results and discussion

Biological activity data and various physico-chemical parameters were taken as dependent and independent variables and correlations were established using PLS method. When the compounds were subjected to under goes PLS method to developed QSAR models by using step wise forward-backward variable selection mode, four QSAR models.

Log_{10}(IC_50) = 2.431 H-Donor Count- 4.1233 polarizabilityAHC- 0.0197 T_T_O_2 - + 1.7067 **(Model 1)**

Optimum Components = 5, Degrees of Freedom =21, n = 53, r^2= 0.8217, q2= 0.659, F test = 64.73 r2 se = 0.4321, q2 se = 0.541, pred_r^2 = 0.7214, SEE = 0.044, SECV= 0.312, SEP=0.152, best_ran_r^2=0.395, best_ran_q^2= 0.5641 Zscore_ran_r2 =0.283, Zscore_ran_q2= 0.132, α_ran_r^2 =<0.0001, α_ran_q^2 = <0.001

Log_{10}(IC_50) = + 2.6838 H-AcceptorCount + 0.6315 T_Cl_Cl_6 + 0.8013 XKHydrophobicArea +0.5364 +0.694 XlogP **(Model 2)**

Optimum Components = 4, Degrees of Freedom = 21, n = 53, r^2= 0.7783 q2= 0.6328, F test = 44.63, r2 se = 0.4421, q2 se = 0.5490, pred_r^2 = 0.7251, SEE = 0.176, SECV= 0.215, SEP=0.138, best_ran_r^2= 0.172, best_ran_q^2 = 0.318 Zscore_ran_r2 =0.305, Zscore_ran_q2= 0.052, α_ran_r^2 =<0.0001, α_ran_q^2 = <0.001

Model –2 shows good squared correlation coefficient (r^2) of 0.7783 explains 78.83% variance in biological activity. This model also indicates statistical significance >99.9% with F values F = 44.63. Which shows the good internal prediction power of this model. The graph of observed vs. predicted biological activities for the training and the test molecules is shown in Figs. 1-3.

Model 3

Log_{10}(IC_50) = 4.1243 - 0.9396 chi5chain- 0.1747 chi2 - 0.0062 SA Hydrophilic Area

Model 4

Log_{10}(IC_50) = 3.6416 - 0.9874 Iodine Count - 0.1511 RotatableBondCount

Model 5

Log_{10} (IC$_{50}$) = 4.3480 - 0.7860 slogp -0.0324 4 Path Count -0.0063 polarizabilityAHP

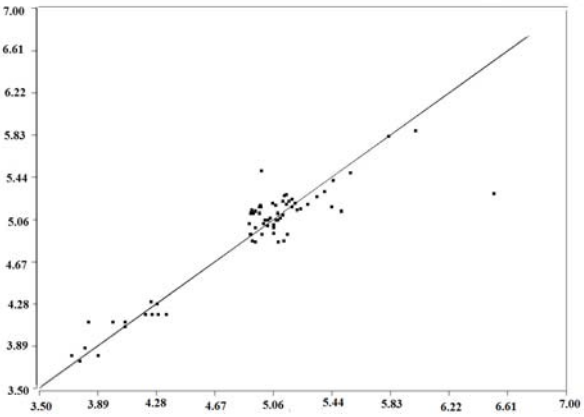

Fig. 1. Plot of predicated activity Vs reported activity for all compounds.

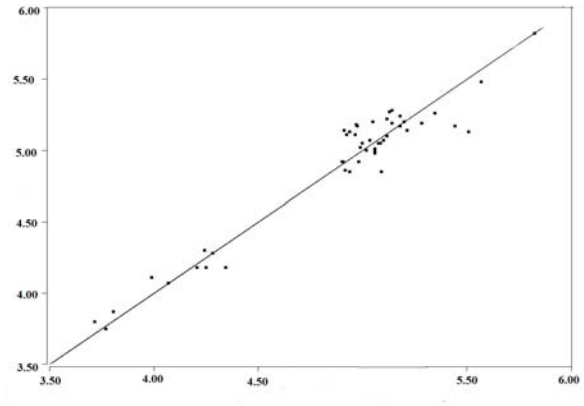

Fig. 2. Plot of predicated activity Vs reported activity for test set compounds

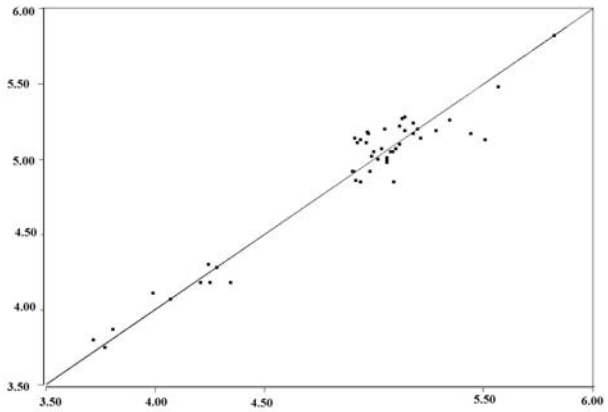

Fig. 3. Plot of predicated activity Vs reported activity for training set compounds.

The above QSAR models indicate the effects of the different types of descriptors on the antimycobacteial activity of the studied Pyrazine containing thiazoline and thiazolidinone of derivatives. Here model 3, 4, 5 A unified QSAR model 3 (MLR method) with high statistical quality (r^2 = 0.8103, F=38.43, Pred_r^2= 0.6951 and q^2=0.6429) was obtained from the pool of all type of descriptors. This equation contains chi5chain (signifies the number of chi5 atoms in a compound), and SA Hydrophilic Area (vdw surface area showing hydrophilic area). QSAR model 4 (PLS method) with statistical quality (r^2 = 0.76, F= 66.41, Pred_r^2= 0.6241 and q^2=0.731) was obtained which contains Iodine Count and Rotatable Bond Count descriptors where as model 5 (PCR method) with statistical quality (r^2 = 0.7902, F= 62.41, Pred_r^2= 0.7263 and q^2=0.6614) was obtained from the pool of all type of descriptors. The model 1 shows overall significance level better than 99% as the calculated F value exceed the tabulated $F_{(4, 32\ \alpha 0.001)}$ = 4.51 and higher q^2 value (0.82) and pred_r^2 (0.69) reflects good predictive potential of the model where as the model 4 shows overall significance level better than 98% as the calculated F value exceed the tabulated $F_{(5, 3\ \alpha 0.001)}$ = 12.51 and q^2 (0.6943) value and pred_r^2 (0.7121). All these models were screened on the basis of q^2 and pred_r^2 and the intercept to best fit line therefore model 2 is the best model.

Acknowledgements

The authors are thankful to Vlife Science Technologies Pvt. Ltd, 1, Akshay 50, Anand Park, Aundh, Pune, India to provide trial version of software.

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