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2D & 3D QSAR AND DRUG DESIGNING OF PHENOTHIAZINE DERIVATIVES AS POTENT ANTITUBERCULAR AGENTS

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ABSTRACT

In search of newer and potent antitubercular agents, a series of phenothiazine derivatives were subjected to 2D and 3D quantitative structure-activity relationship (QSAR) analyses. Statistically significant models were generated, and the most robust model for 2D QSAR was obtained using partial least square regression method coupled with stepwise forward-backward method using V-Life Molecular Design Suite software version 3.5. The physicochemical descriptors, viz., slogp, estate descriptors like Saa CHE index and Chiv2, contribute significantly to the biological activity. About 20 QSAR models were generated, among which 2 significant models were finally selected on the basis of various statistical parameters such as squared correlation co-efficient (r^2), and cross-validated square correlation co-efficient (q^2). The statistical values of the 2 significant models (model 1, model 2) are r^2(0.9444, 0.9437) and q^2(0.8454, 0.8374). The descriptors showed by QSAR study can be used further for study and designing of new compounds. Consequently, this study may prove to be helpful in development and optimization of existing antitubercular activity of this class of compounds.

KEYWORDS: Antitubercular, Mycobacterium *tuberculosis*, Partial Least Square, Phenothiazine Derivatives, QSAR, Type II NADH.

INTRODUCTION

Tuberculosis (TB) is the disease caused by *Mycobacterium tuberculosis* that infects approximately two billion people. The World Health Organization estimates that about a total of 1.77 million people died from TB due to the lack of inability to afford proper health care.[1] Overcrowding and ill-nourishment of poor people living in large cities leads to a high incidence of the disease due to the ease at which the infection can be transferred. This

situation contributes to the accelerated speed at which TB spreads in underdeveloped countries. TB has become a serious worldwide problem, infecting in synergy with human immunodeficiency virus (HIV) infection.[2] There is also an alarming increase in cases of TB caused by Multi drug-resistant strains of *Mycobacterium tuberculosis* due in part to inadequate drug therapy as a result of incorrectly selected medications or suboptimal drug dosing.[3] Keeping in view of the above statistics, WHO declared TB as a global health emergency and aimed at saving 14 million lives between 2006 and 2015.[4]

TB is difficult to treat due to residence of bacteria within the macrophages and its unusual cell wall barrier. Moreover, multi-drug resistant strains of TB (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) have emerged recently.[5] Thus, there is a need for new drugs targeting enzymes essential to mycobacterial survival. One such target is type II NADH: menaquinone dehydrogenase (ndh-2). By inhibiting ndh-2, the electron transport chain in *Mycobacterium tuberculosis* becomes blocked and shuts down. ndh-2 is the only NADH dehydrogenase enzyme expressed in *Mycobacterium tuberculosis* and is thus vital to its survival.

Mycobacterium tuberculosis is an obligate aerobe that is capable of long-term persistence under conditions of low oxygen tension. Analysis of the *Mycobacterium tuberculosis* genome predicts the existence of a branched aerobic respiratory chain terminating in a cytochrome *bd* system and a cytochrome aa_3 system. Both chains can be initiated with type II NADH: Menaquinone Oxidoreductase. A biochemical characterization of the aerobic respiratory chains from Mtb and show that phenothiazine analogs specifically inhibit NADH: Menaquinone Oxidoreductase activity. Type-II NADH-*Menaquinone Oxidoreductase* (NDH-2) is an essential respiratory enzyme of the pathogenic bacterium *Mycobacterium tuberculosis* that plays a vital role in its growth.[6-10]

In the present research work, a series of phenothiazine derivatives were subjected to 2D quantitative structure-activity relationship (QSAR) analyses, in search of newer and potent antitubercular agents. Statistically significant models were generated, and the most robust model for 2D QSAR was obtained using partial least square regression method coupled with stepwise forward-backward method using V-Life Molecular Design Suite software version 3.5.

QSAR study

All the 2D descriptors were calculated for QSAR analysis using Vlife MDS 3.5 software. Thermodynamic parameters describe free energy change during drug receptor complex formation. Spatial parameters are the quantified steric features of drug molecules required for its complimentary fit with receptor. Electronic parameters describe weak non-covalent bonding between drug molecules and receptor. Partial least square regression method is used to generate QSAR equation. For variable selection, stepwise forward-backward method was used.

Criteria for selection of model

n = number of molecules (> 20 molecules)

K = number of descriptors in a model (statistically n/5 descriptors in a model)

df = degree of freedom (n-k-1) (higher is better)

r^2 = coefficient of determination (> 0.7)

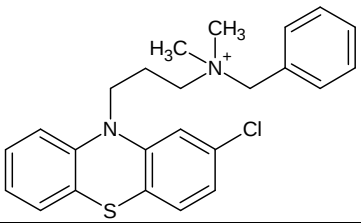
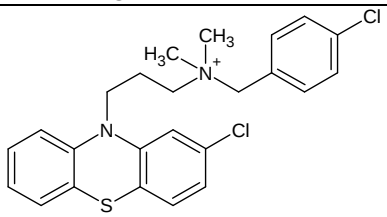
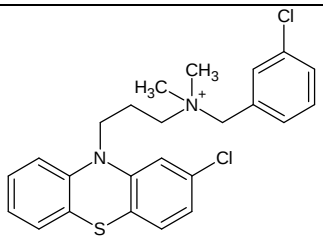
q^2 = cross-validated r^2 (>0.5)

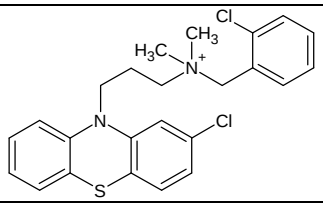
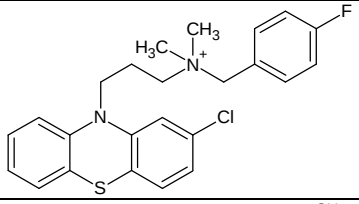
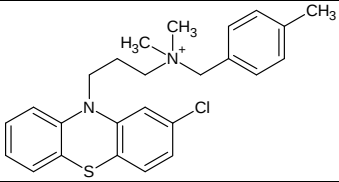
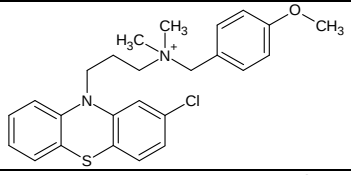
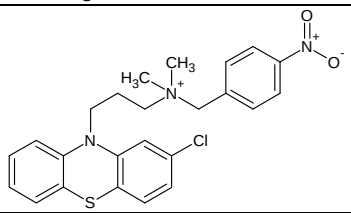
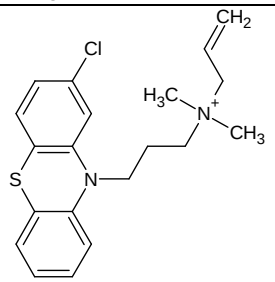
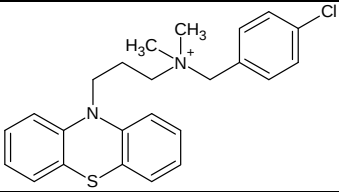
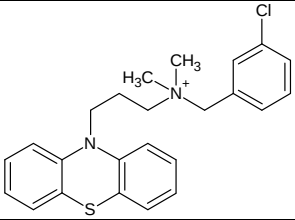
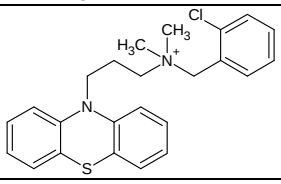
pred_r^2 = r^2 for external test set (>0.5)

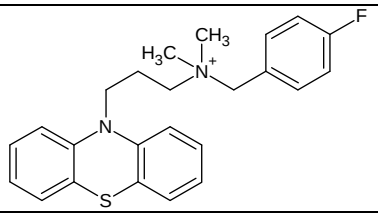
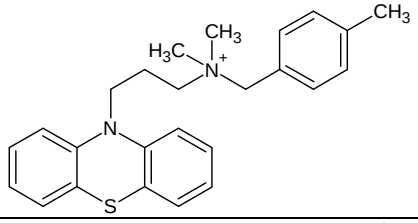
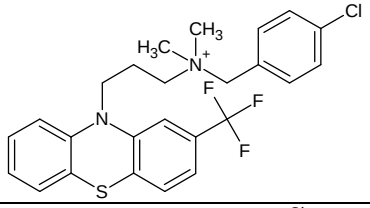
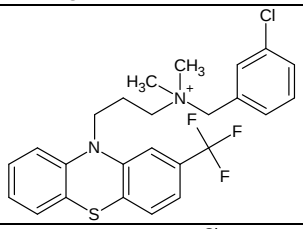
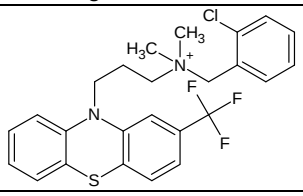
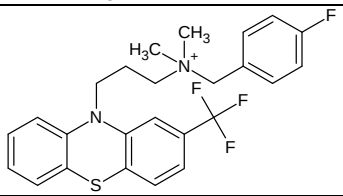
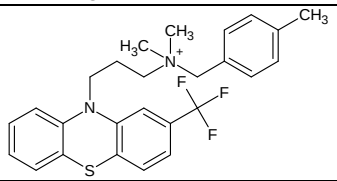
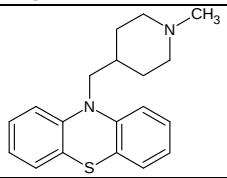
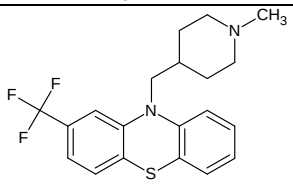
SEE = standard error of estimate (smaller is better)

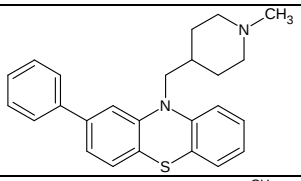
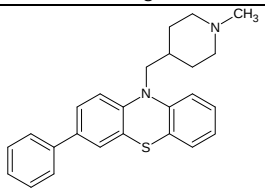
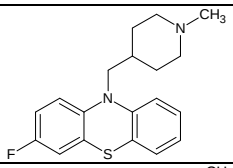
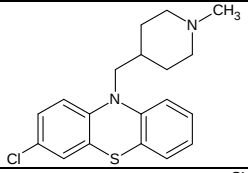
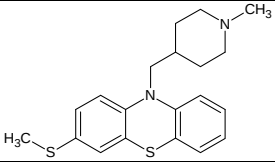
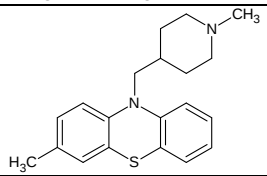
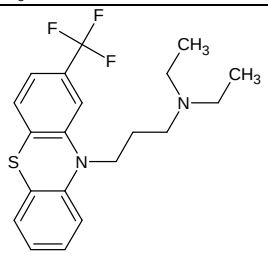
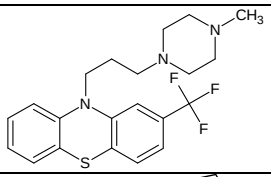
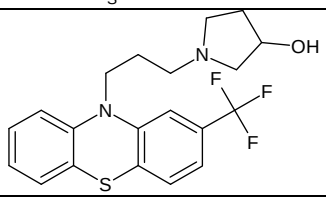
F-test = F-test for statistical significance of the model (higher is better, for same set of descriptors and compounds).

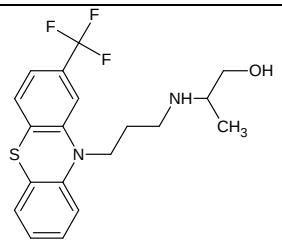
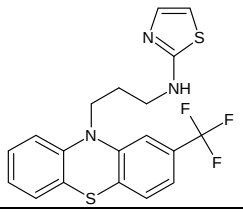
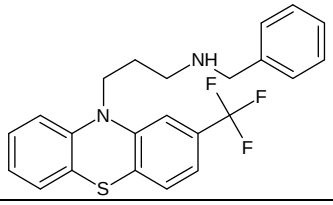
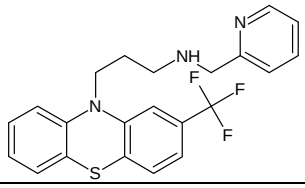
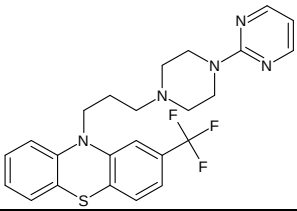
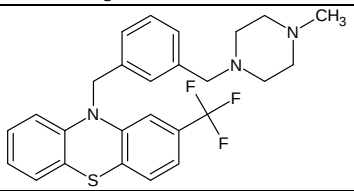
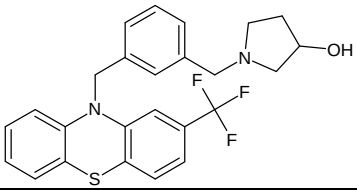
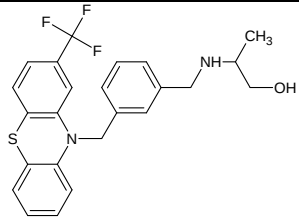
RESULTS AND DISCUSSION

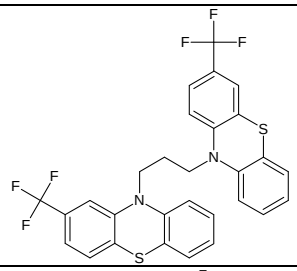
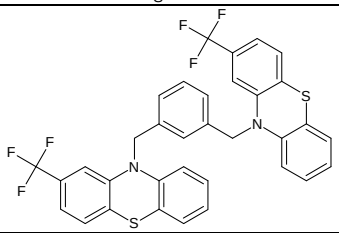
S.NO	STRUCTURE	MIC	-log MIC
PR01.		7.33	-0.86510
PR02.		6.06	-0.78247
PR03.		4.5	-0.65321

PR04.		5.6	-0.74819
PR05.		7.6	-0.88081
PR06.		4.7	-0.67210
PR07.		8.5	-0.92942
PR08.		12.3	-1.08991
PR09.		30.6	-1.48572
PR10.		9.31	-0.96895
PR11.		7.5	-0.87506
PR12.		14.3	-1.15534

PR13.		14.6	-1.16435
PR14.		9.9	-0.99563
PR15.		3.81	-0.58092
PR16.		3.8	-0.57978
PR17.		7.3	-0.86332
PR18.		6.4	-0.80618
PR19.		6.8	-0.83251
PR20.		17	-1.23045
PR21.		7.2	-0.85733

PR22.		4.5	-0.65321
PR23.		2.1	-0.32222
PR24.		10.8	-1.03342
PR25.		14.3	-1.15534
PR26.		11.6	-1.06446
PR27.		7.2	-0.85733
PR28.		15	-1.17609
PR29.		7.6	-0.088081
PR30.		11	-1.04139

PR31.		4.6	-0.66276
PR32.		14	-1.14613
PR33.		4.2	-0.62325
PR34.		20	-1.30103
PR35.		16	-1.20412
PR36.		15	-1.17609
PR37.		8.4	-0.92428
PR38.		6.4	-0.80618

PR39.		2.3	-0.36173
PR40.		2.0	-0.30103

P1 TO P36 WERE USED FOR 2D AND 3D QSAR BECAUSE THEY HAVE ACTIVITY IN FULL INTEGER NUMBER.

P37 TO P40 CAN NOT BE USED BECAUSE QSAR CAN NOT BE DONE OF STRUCTURES WHICH HAVE ACTIVITY IN DECIMAL.

OPTIMIZATION OF 3D STRUCTURE

Optimization of the 3D structures was done by using MMFF (Merck Molecular Force Field) method and the results obtained after Optimization are summarized in the Table 01.

Table 01: Energy Optimization of 36 Compounds (Phenothiazine Derivatives) as Antitubercular Agents.

CODE	Initial Energy	Final Energy	Residual	% Optimization	No. of Cycles
PR01	392.5	388.1	4.4	1.1	106
PR02	395.4	389.1	6.3	1.5	131
PR03	397.0	99.9	297.1	74.8	1580
PR04	394.5	103.4	291.1	73.7	1529
PR05	380.8	100.4	280.4	73.1	1441
PR06	381.3	102.6	278.7	73.0	1556
PR07	401.7	110.2	291.5	72.5	1578
PR08	422.3	120.1	302.2	71.5	1752
PR09	424.9	80.1	344.8	81.1	1106
PR10	398.2	97.3	300.9	75.5	2234
PR11	456.1	96.8	359.3	78.7	2241
PR12	414.5	100.0	314.5	75.8	1227
PR13	418.3	96.6	321.7	76.9	2050
PR14	402.9	107.0	295.9	73.4	2023
PR15	397.7	112.4	285.3	71.7	1687
PR16	403.5	111.5	292	72.3	1692
PR17	417.3	111.0	306.3	73.4	1327

PR18	392.5	111.3	281.2	71.6	1958
PR19	407.9	113.8	294.1	72.1	1602
PR20	154.3	78.7	75.6	48.9	1568
PR21	166.2	88.0	78.2	47.0	1474
PR22	194.9	101.7	93.2	47.8	1563
PR23	191.2	101.5	89.7	46.9	1611
PR24	136.1	76.4	59.7	43.8	1205
PR25	152.6	76.2	76.4	50.5	1445
PR26	157.8	80.3	77.5	49.1	1616
PR27	157.4	80.7	76.7	48.7	1415
PR28	124.5	77.4	47.1	37.8	1737
PR29	166.3	114.9	51.4	30.9	1845
PR30	428.2	174.4	253.8	59.2	1803
PR31	209.5	143.9	65.6	31.3	1754
PR32	95.7	69.2	26.5	27.9	1381
PR33	224.8	89.5	135.3	60.1	1837
PR34	226.2	94.9	131.3	58.0	1432
PR35	152.3	96.1	56.2	36.9	1923
PR36	307.1	131.0	176.1	57.3	2425

The Structure PR01 has Least Percentage of Optimization and Structure PR09 has highest Percentage of Optimization. In PR01 the Semi Polar Benzene group (unsubstituted) provided least energy change in the Structure PR01 and in PR09 the Presence of alkene group provides hindrance in the movement of the structure and thus PR09 has highest change in energy and have highest Percentage of Optimization.

2D QSAR MODELS

The 2D QSAR of the 36 Structures (Phenothiazine Derivatives) as Antitubercular Agents was done by Using PLS (Partial Least Square) method and the Result obtained of Model 01 and Model 02 is summarized below.

MODEL 01

STATISTICS

The regression on the 36 compounds was applied by using PLS method and the values obtained are tabulated in Table 02.

MODEL 01 TEST SET: 1, 3, 5, 13, 18, 24, 28.

Table 02: Statistical Values of Model 01.

Model 01	
r^2	0.7501
q^2	0.6547
r^2se	0.1434
q^2se	0.1685
Pred_r^2	0.6901
Pred_r^2 se	0.1085
OC	1
n(no. of training set)	27
Degree of Freedom	25
F-Test	75.0232

The values obtained in Model 01 were above the Standard Values for the Stated Parameters. The value of r^2 (0.7501) and the standard value of r^2>0.7, q^2 (0.6547) and the standard value of q^2>0.5, pred_r^2 (0.6901) and the standard value is pred_r^2>0.5 and all the errors were within the limit and below 0.3. Thus Model 01 has all the standards fulfilled for the perfect QSAR equation.

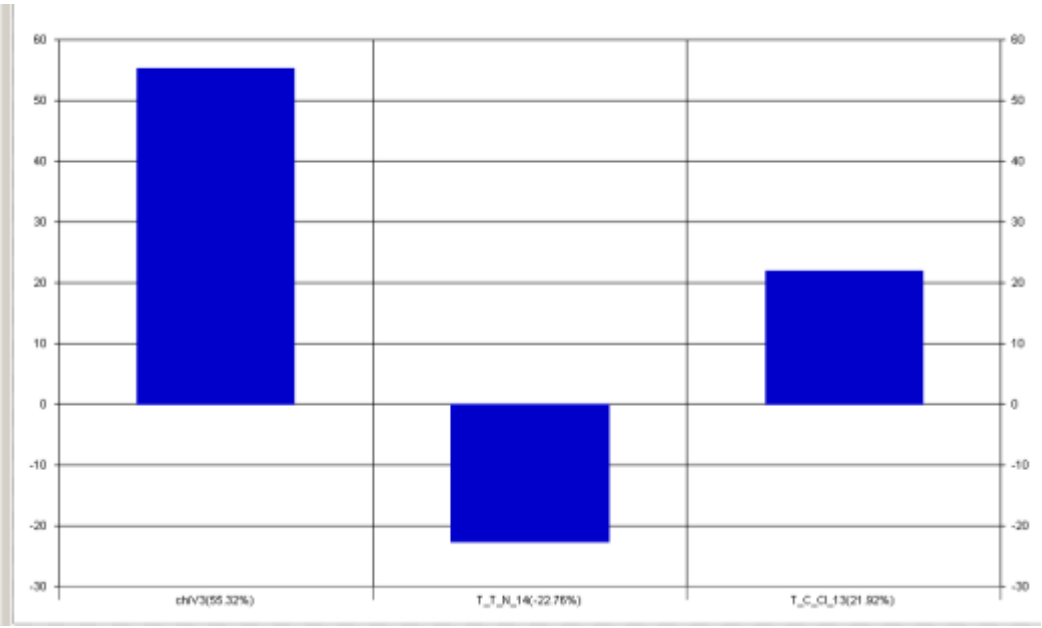
EQUATION

MODEL 01

pMIC=+0.2480chiV3 - 0.0651 T_T_N_14 + 0.0584 T_C_Cl_13 - 2.6345.

CONTRIBUTION CHART

Chart 01: Contribution Chart of Model 01.



chiV3: 55.32%

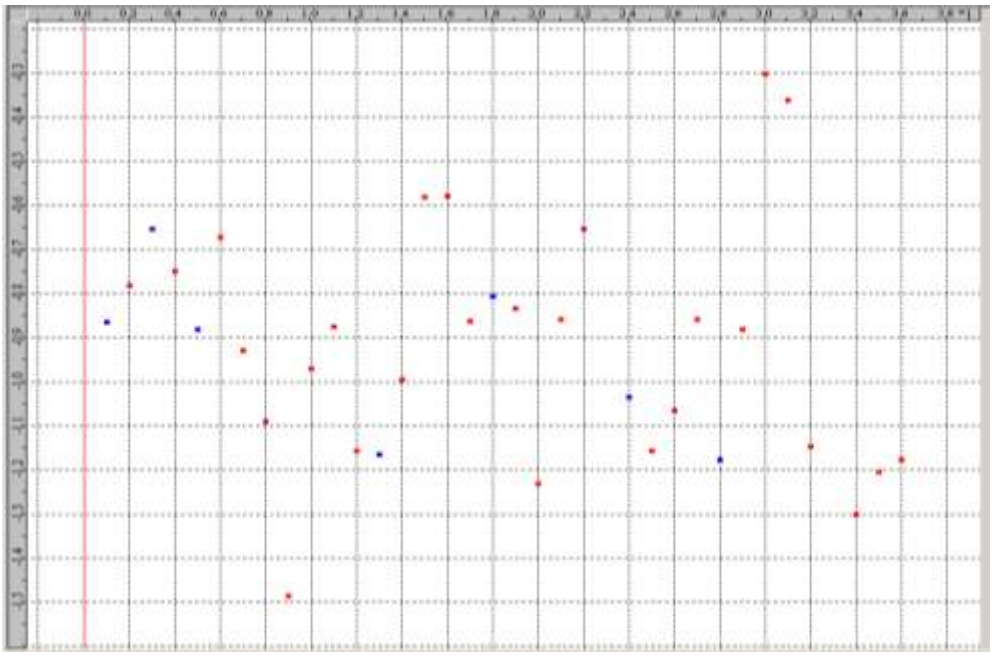
T_T_N_14: -22.76%

T_C_Cl_13: 21.92%

Contribution Chart of the Mode 01 shows that chiV3 with 55.32% has positive contribution in the model and it is directly correlated to the structures and on increasing the descriptor value the structures will correlate with much better values. T_T_N_14 with -22.76% has negative contribution in the model and it will decrease the values if its contribution is increased and also on decreasing its contribution it will give better values thus it signifies that the descriptor is not properly correlated with all the structures. T_C_Cl_13 with 21.92% has positive contribution in the model and on increasing its value it will correlate with better values thus chiV3, T_C_Cl_13 are the descriptors which are correlated positively with the structures and T_T_N_14 is the descriptor which is not correlated properly with the structures and have negative contribution.

ACTIVITY DISTRIBUTION PLOT

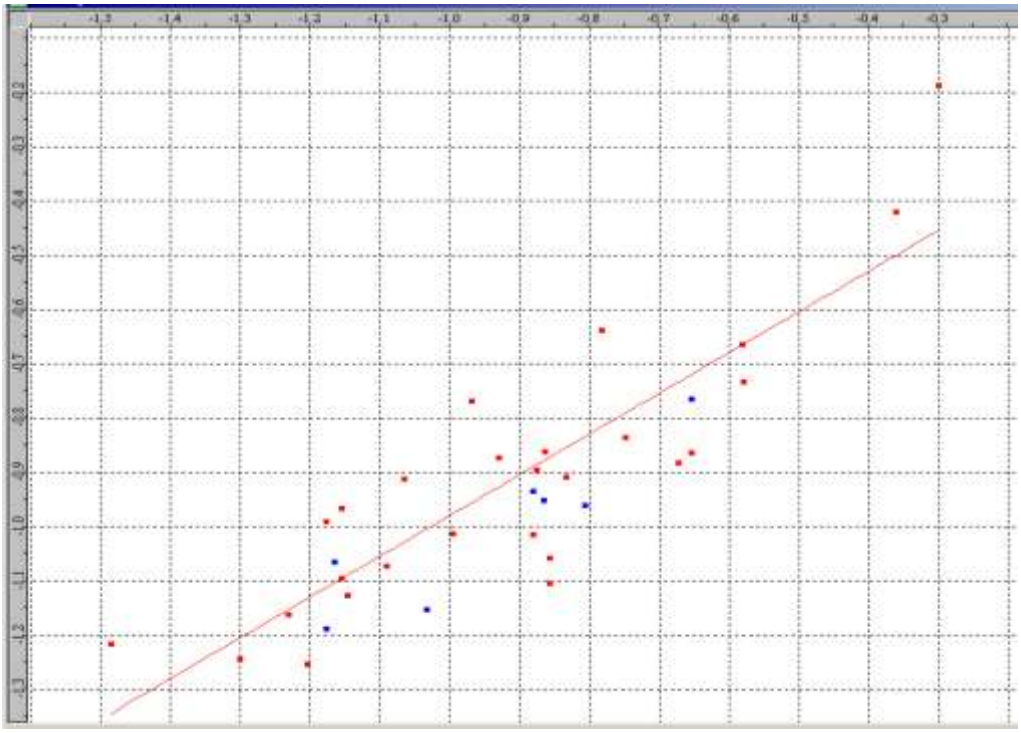
Plot 01: Activity Distribution Plot of Model 01(Blue colour {Test Set} Red Colour {Training Set})



From the Activity Distribution Plot of Model 01 it was seen that all the Test Set Structures were covered by the Training Set Structures and the Structures lying in the Periphery were not included in the Test set.

FITNESS PLOT

From the Fitness Plot of Model 01 it was seen that all the Structures lie within the Best Fit Line and Structure PR30 was always away from the Best Fit Line and all the Test Set was near to the Best Fit Line. There was no outlier in the fitness plot and all the Training Set was also near about to the Best Fit Line.



Graph 01: Fitness Plot of Model 01 [Training set (red spot) and Test set (blue spot)

CORRELATION MATRIX

The correlation matrix of the model shows that the descriptors that are generated in the model don't have strong correlation with each other and they have correlation below 0.5. If the descriptors have strong correlation with each other then they have the same meaning and use in the model and one of the either can be used and it will also not give perfect QSAR equation and will also hinder the entry of other descriptors in the model.

From the correlation of Model 01 shown in Table 03 it was seen that chiV3, T_T_N_14, T_C_Cl_13 were not strongly correlated with each other.

Table 03: Correlation Matrix of Model 01.

	chiV3	T_T_N_14	T_C_Cl_13
chiV3	1	0.111586	-0.01473
T_T_N_14	0.111586	1	-0.12975
T_C_Cl_13	-0.01473	-0.12975	1

UNI-COLUMN STATISTICS

Table 04: Uni-Column Statistics of Model 01.

Model	Column Name	Average	Maximum	Minimum	Std. Deviation	Sum
01	**Training**	-0.9166	-0.3010	-1.4857	0.2812	-24.7474
	Test	-0.9398	-0.6532	-1.1760	0.1932	-6.5789

From the Uni Column Statistics of the Model 01 shown in Table 04 it was seen that the Maximum of the Training Set should be higher than Test Set and Minimum of the Test Set should be higher than Training Set.

ACTUAL PREDICTION TABLE

The Actual Activity and the Predicted Activity along with the Residual of the Model 01 in the Table 05 show that all the Structures have the Prediction activity near about to the Actual activity and the Residual of the Structures was within the limit and in the range of double the value of r^2 se. Structure PR23 and PR33 have the Residual value greater than the double the value of r^2 se and they were in the Training set thus the two Structures were deleted from the Training set as on keeping them in the Training set they were having high residual and also the Statistical values were not good and the PR23 was always outlier with PR33 and they were giving pred_r2 in negative. On keeping them in Test Set they were also not giving satisfactory values thus PR23 and PR33 were deleted from the Training Set of Model 01. In PR23 and PR33 the Prediction of the activity was worst.

Table 05: Actual Activity along with Predicted Activity and Residual o Model 01.

CODE	MODEL 01		
	Actual Activity	Predicted Activity	Residual
PR01	**-0.8651**	**-0.95148**	**0.08638**
PR02	-0.7824	-0.63785	-0.14455
PR03	**-0.6532**	**-0.76488**	**0.111683**
PR04	-0.7481	-0.83456	0.086458
PR05	**-0.8808**	**-0.93401**	**0.053214**
PR06	-0.6721	-0.8826	0.210499
PR07	-0.9294	-0.87306	-0.05634
PR08	-1.0899	-1.07141	-0.01849
PR09	-1.4857	-1.21574	-0.26996
PR10	-0.9689	-0.76839	-0.20052
PR11	-0.875	-0.89542	0.02042
PR12	-1.1553	-0.9651	-0.1902
PR13	**-1.1643**	**-1.06455**	**-0.09975**
PR14	-0.9956	-1.01314	0.017536
PR15	-0.5809	-0.66399	0.083086
PR16	-0.5797	-0.7326	0.152901
PR17	-0.8633	-0.8607	-0.0026
PR18	**-0.8061**	**-0.96015**	**0.154054**
PR19	-0.8325	-0.90874	0.076238
PR20	-1.2304	-1.16129	-0.06911
PR21	-0.8573	-1.05689	0.199591
PR22	-0.6532	-0.86352	0.210324
PR23	-0.3222	-0.86783	0.545629
PR24	**-1.0334**	**-1.15178**	**0.118376**
PR25	-1.1553	-1.09348	-0.06182
PR26	-1.0644	-0.91113	-0.15327
PR27	-0.8573	-1.1038	0.246504
PR28	**-1.176**	**-1.18793**	**0.011925**
PR29	-0.8808	-1.01398	0.13318
PR30	-0.301	-0.18682	-0.11418
PR31	-0.3617	-0.42014	0.058442
PR32	-1.1461	-1.12659	-0.01951
PR33	-0.6232	-1.14906	0.525859
PR34	-1.301	-1.24272	-0.05828
PR35	-1.2041	-1.25316	0.049062
PR36	-1.176	-0.99061	-0.18539

The Structures in Bold indicate the Test Set of Model 01. The Structures PR23 and PR33 indicated in dark are deleted from the series.

DESCRIPTOR SHEET

Descriptor Sheet of chiV3, T_T_N_14, T_C_Cl_13 descriptors that were generated in the Model 01 is tabulated in Table 06. The Descriptor Sheet signifies that chiV3 is the descriptor

that is directly correlated with the Structures and it has the highest value in PR30 which is the reported Potent Structure of the series and it has the lowest value in PR09 which is the Reported Worst Compound of the series. T_C_Cl_13 is the descriptor which also contributes and correlates with the Structures. T_T_N_14 has no strong correlation with the structures of the series.

Table 06: Descriptor Sheet of Model 01.

	chiV3	T_T_N_14	T_C_Cl_13
PR01	6.551704	0	1
PR02	6.874113	0	5
PR03	6.833005	0	3
PR04	7.023215	0	1
PR05	6.622137	0	1
PR06	6.829482	0	1
PR07	6.867934	0	1
PR08	6.856038	3	1
PR09	5.721622	0	0
PR10	6.583287	0	4
PR11	6.54218	0	2
PR12	6.73239	0	0
PR13	6.331311	0	0
PR14	6.538656	0	0
PR15	7.00297	0	4
PR16	6.963189	0	3
PR17	7.153399	0	0
PR18	6.75232	0	0
PR19	6.959665	0	0
PR20	5.941196	0	0
PR21	6.362205	0	0
PR22	7.142001	0	0
PR23	7.124639	0	0
PR24	5.979563	0	0
PR25	6.214661	0	0
PR26	6.950023	0	0
PR27	6.173019	0	0
PR28	5.833785	0	0
PR29	6.535255	0	0
PR30	9.870939	0	0
PR31	8.930032	0	0
PR32	6.081144	0	0
PR33	5.990518	0	0
PR34	5.612804	0	0
PR35	7.146635	6	0
PR36	7.417455	3	0

SIGNIFICANCE OF THE DESCRIPTORS GENERATED IN THE EQUATION OF MODEL 01.

- **chiV3:** This descriptor signifies atomic valence connectivity index (order 3).

- **T_T_N_14:** This Descriptor signifies that the Distance between any atom and nitrogen is of fourteen bonds.
- **T_C_Cl_13:** This Descriptor signifies that the Distance between carbon and chlorine is of thirteen bonds.

MODEL 02

STATISTICS

The regression on the 36 compounds was applied by using PLS method and the values obtained are tabulated in Table 07.

MODEL 02 TEST SET: 14, 19, 22, 25, 26, 27, 31.

Table 07: Statistical Values of Model 02.

Model 02	
r^2	0.7059
q^2	0.6197
r^2se	0.1453
q^2se	0.1652
Pred_r^2	0.6908
Pred_r^2 se	0.1607
OC	1
n(no. of training set)	27
Degree of Freedom	25
F-Test	59.9964

The values obtained in Model 02 were above the Standard Values for the Stated Parameters. The value of r^2 (0.7059) and the standard value of r^2>0.7, q^2 (0.6197) and the standard value of q^2>0.5, pred_r^2 (0.6908) and the standard value is pred_r^2>0.5 and all the errors were within the limit and below 0.3. Thus Model 02 has all the standards fulfilled for the perfect QSAR equation.

EQUATION

MODEL 02:

pMIC= + 0.2184 chiV3 + 0.0765 T_C_Cl_13 - 0.0425 T_T_N_1 -2.1717.

CONTRIBUTION CHART

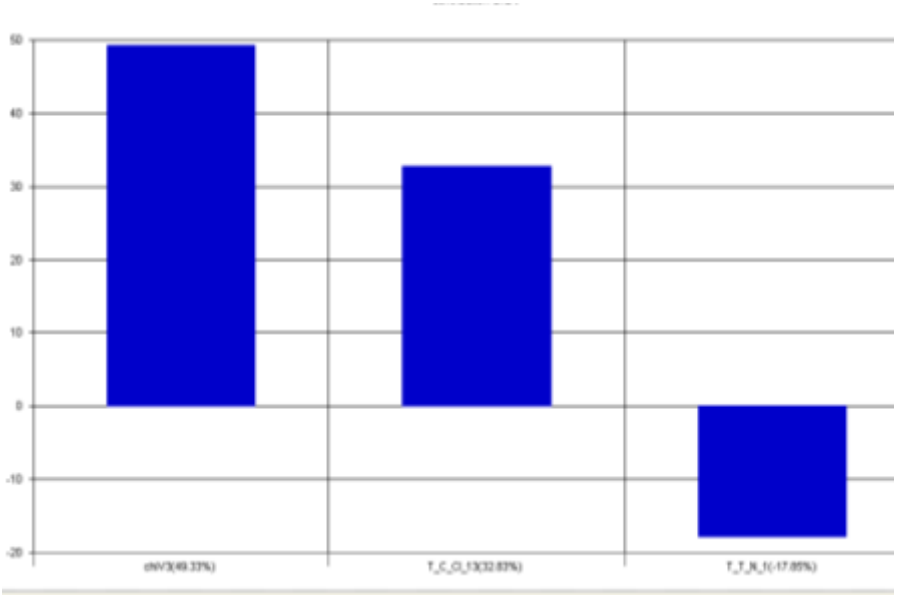


Chart 02: Contribution Chart of Mode 02.

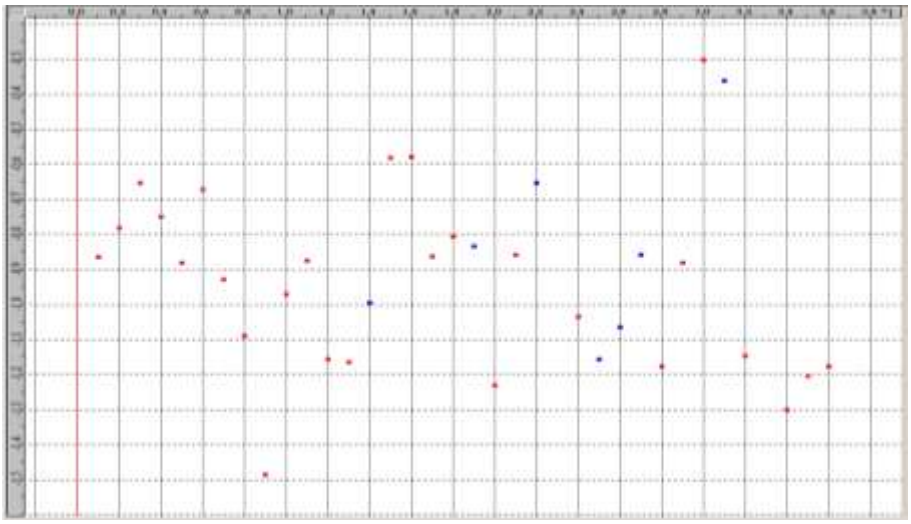
chiV3: 49.33%

T_T_N_1: -17.85%

T_C_Cl_13: 32.83%

Contribution Chart of the Mode 02 shows that chiV3 with 49.33% has positive contribution in the model and it is directly related to the structures and on increasing the descriptor value the structures will correlate with much better values. T_T_N_1 with -17.85% has negative contribution in the model and it will decrease the values if its contribution is increased and also on decreasing its contribution it will give better values thus it signifies that the descriptor is not properly correlated with all the structures. T_C_Cl_13 with 32.83% has positive contribution in the model and on increasing its value it will correlate with better values thus chiV3, T_C_Cl_13 are the descriptors which are correlated positively with the structures and T_T_N_1 is the descriptor which is not correlated properly with the structures and have negative contribution.

ACTIVITY DISTRIBUTION PLOT

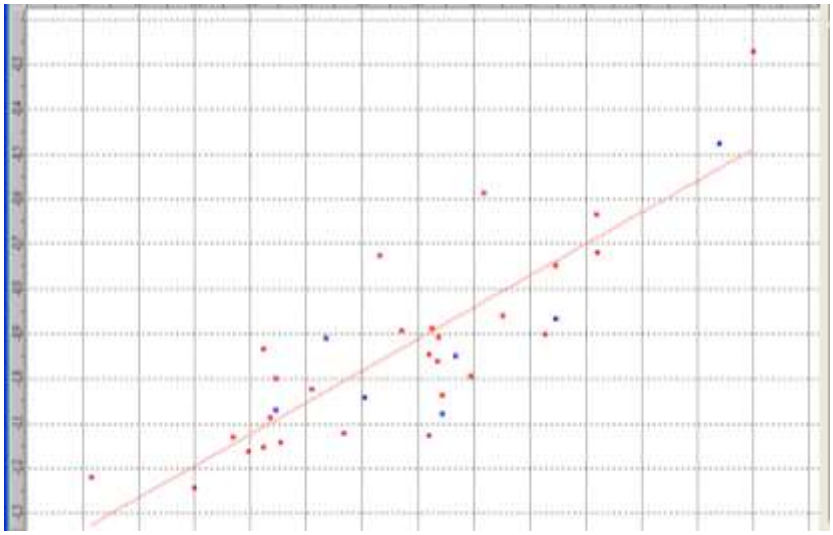


Plot 02: Activity Distribution Plot of Model 02(Blue colour {Test Set} Red Colour {Training Set})

From the Activity Distribution Plot of Model 02 it was seen that all the Test Set Structures were covered by the Training Set Structures and the Structures lying in the Periphery were not included in the Test Set.

FITNESS PLOT

From the Fitness Plot of Model 02 it was seen that all the Structures lie within the Best Fit Line and Structure PR30 was always away from the Best Fit Line and all the Test Set was near to the Best Fit Line. There was no outlier in the fitness plot and all the Training Set was also near about to the Best Fit Line.



Graph 03: Fitness Plot of Model 02 [Training set (red spot) and Test set (blue spot)].

CORRELATION MATRIX

The correlation matrix of the model shows that the descriptors that are generated in the model don't have strong correlation with each other and they have correlation below 0.5. If the descriptors have strong correlation with each other then they have the same meaning and use in the model and one of the either can be used and it will also not give perfect QSAR equation and will also hinder the entry of other descriptors in the model.

The Correlation Matrix of Model 02 shown in Table 08 shows that chiV3, T_T_N_1, T_C_Cl_13 were not strongly correlated with each other.

Table 08: Correlation Matrix of Model 02.

	T_T_N_1	chiV3	T_C_Cl_13
T_T_N_1	1	0.124719	-0.08802
chiV3	0.124719	1	0.095694
T_C_Cl_13	-0.08802	0.095694	1

UNI-COLUMN STATISTICS

Table 09: Uni-Column Statistics of Model 02.

Model	Column Name	Average	Maximum	Minimum	Std. Deviation	Sum
02	**Training**	-0.9410	-0.3010	-1.4857	0.2628	-25.4063
	Test	-0.8457	-0.3617	-1.1553	0.2701	-5.9200

From the Uni Column Statistics of the Model 02 shown in Table 09 it was seen that the Maximum of the Training Set should be higher than Test Set and Minimum of the Test Set should be higher than Training Set.

ACTUAL PREDICTION TABLE

The Actual Activity and the Predicted Activity along with the Residual of the Model 02 in the Table 10 show that all the Structures have the Prediction activity near about to the Actual activity and the Residual of the Structures was within the limit and in the range of double the value of r^2 se. Structure PR23 and PR33 have the Residual value greater than the double the value of r^2 se and they were in the Training set thus the two Structures were deleted from the Training set as on keeping them in the Training set they were having high residual and also the Statistical values were not good and the PR23 was always outlier with PR33 and they were giving pred_r2 in negative. On keeping them in Test Set they were also not giving satisfactory values thus PR23 and PR33 were deleted from the Training Set of Model 02. In PR23 and PR33 the Prediction of the activity was worst.

Table 10: Actual Activity along with Predicted Activity and Residual Model 02:

CODE	MODEL 02		
	Actual Activity	**Predicted Activity**	**Residual**
PR01	-0.8651	-0.96177	0.096666
PR02	-0.7824	-0.58537	-0.19703
PR03	-0.6532	-0.74734	0.094142
PR04	-0.7481	-0.8588	0.110702
PR05	-0.8808	-0.94639	0.065585
PR06	-0.6721	-0.90111	0.229007
PR07	-0.9294	-0.89271	-0.03669
PR08	-1.0899	-1.02272	-0.06718
PR09	-1.4857	-1.21953	-0.26617
PR10	-0.9689	-0.72538	-0.24353
PR11	-0.875	-0.88735	0.012347
PR12	-1.1553	-0.99881	-0.15649
PR13	-1.1643	-1.08639	-0.07791
PR14	**-0.9956**	**-1.04111**	**0.045513**
PR15	-0.5809	-0.63344	0.052539
PR16	-0.5797	-0.71891	0.139214
PR17	-0.8633	-0.90687	0.043571
PR18	-0.8061	-0.99446	0.188355
PR19	**-0.8325**	**-0.94918**	**0.116677**
PR20	-1.2304	-1.12911	-0.10129
PR21	-0.8573	-1.03717	0.179873
PR22	**-0.6532**	**-0.86689**	**0.213689**
PR23	-0.3222	-0.87068	0.54848
PR24	-1.0334	-1.12073	0.087331
PR25	**-1.1553**	**-1.06939**	**-0.08591**
PR26	**-1.0644**	**-0.90881**	**-0.15559**
PR27	**-0.8573**	**-1.07849**	**0.221186**
PR28	-1.176	-1.15256	-0.02344
PR29	-0.8808	-1.1268	0.245998
PR30	-0.301	-0.27097	-0.03003
PR31	**-0.3617**	**-0.47644**	**0.114737**
PR32	-1.1461	-1.14102	-0.00508
PR33	-0.6232	-1.07587	0.452667
PR34	-1.301	-1.24329	-0.05771
PR35	-1.2041	-1.16318	-0.04092
PR36	-1.176	-0.93415	-0.24185

The Structures in Bold indicate the Test Set of Model 02. The Structures PR23 and PR33 indicated in dark are deleted from the series.

DESCRIPTOR SHEET

Descriptor Sheet of chiV3, T_T_N_1, T_C_Cl_13 descriptors that were generated in the Model 02 is tabulated in Table 11. The Descriptor Sheet signifies that chiV3 is the descriptor

that is directly correlated with the Structures and it has the highest value in PR30 which is the reported Potent Structure of the series and it has the lowest value in PR09 which is the Reported Worst Compound of the series. T_C_Cl_13 is the descriptor which also contributes and correlates with the Structures. T_T_N_1 has highest value in PR35 and lowest value in PR33 which shows that the descriptor is not correlated with the structures of the series and gives negative contribution in the Model 02.

Table 11: Descriptor Sheet of Model 02.

	chiV3	T_C_Cl_13	T_T_N_1
PR01	6.551704	1	7
PR02	6.874113	5	7
PR03	6.833005	3	7
PR04	7.023215	1	7
PR05	6.622137	1	7
PR06	6.829482	1	7
PR07	6.867934	1	7
PR08	6.856038	1	10
PR09	5.721622	0	7
PR10	6.583287	4	7
PR11	6.54218	2	7
PR12	6.73239	0	7
PR13	6.331311	0	7
PR14	6.538656	0	7
PR15	7.00297	4	7
PR16	6.963189	3	7
PR17	7.153399	0	7
PR18	6.75232	0	7
PR19	6.959665	0	7
PR20	5.941196	0	6
PR21	6.362205	0	6
PR22	7.142001	0	6
PR23	7.124639	0	6
PR24	5.979563	0	6
PR25	6.214661	0	6
PR26	6.950023	0	6
PR27	6.173019	0	6
PR28	5.833785	0	6
PR29	6.535255	0	9
PR30	9.870939	0	6
PR31	8.930032	0	6
PR32	6.081144	0	7
PR33	5.990518	0	5
PR34	5.612804	0	7
PR35	7.146635	0	13
PR36	7.417455	0	9

SIGNIFICANCE OF THE DESCRIPTORS GENERATED IN THE EQUATION OF MODEL 02.

- **chiV3:** This descriptor signifies atomic valence connectivity index (order 3).
- **T_T_N_1:** This Descriptor signifies that the Distance between any atom and nitrogen is of one bond.
- **T_C_Cl_13:** This Descriptor signifies that the Distance between carbon and chlorine is of thirteen bonds.

INTERPRETATION OF 2D MODELS

- The descriptors that were generated in Model 01 and Model 02 had strong correlation with the activity.
- chiV3: 55.32%, T_T_N_14: -22.76%, T_C_Cl_13: 21.92%(Contribution of descriptors of Model 01)
- chiV3: 49.33%, T_T_N_1: -17.85%, T_C_Cl_13: 32.83% (Contribution of descriptors of Model 02
- chiV3 was important descriptor for the activity in Model 01 and Model 02 and it directly correlates with the structure and has positive contribution in the models and on increasing its contribution the values will also increase.
- T_T_N_14 and T_T_N_1 were the descriptors of Model 01 and Model 02 respectively and they had negative contribution in the model and they do not correlate with the structures.
- T_C_Cl_13 was the descriptor of Model 01 and Model 02 and it also correlates with the structures.
- The generated descriptors show thjat the steric effect is to be increased in order to increase the activity by increasing the chain length.

ALIGNMENT OF 3D STRUCTURES

For the generation of 3D Model Alignment of 3D structure is necessary and these align molecules were used for the generation of 3D descriptors. Alignment of the 3D optimized structures was done by Template Based Alignment method by taking 10-methyl-10*H*-phenothiazine as the template with potent structure PR30 as the reference structure and the alignment obtained was not satisfactory. Thus conformers were generated for the structures coding PR31 and PR36 as they were not giving better alignment with the reference structure (PR30).

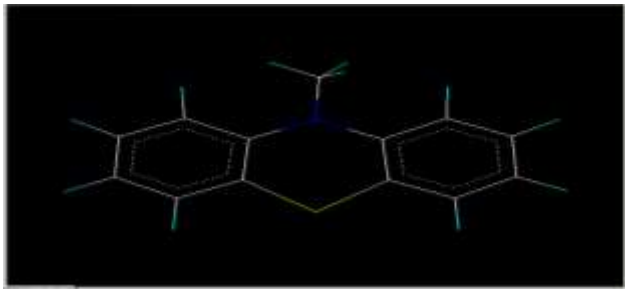


Figure 03: Template used for Alignment of 36 Structures.

Conformers of PR31 were generated and total 108 conformers were generated out of which Conformer PR31_C47 was used for alignment as it was of least energy and was giving better alignment with the reference Structure (PR30).

Conformers of PR36 were generated and total 13 conformers were generated out of which Conformer PR36_C9 was used for alignment as it was of least energy and was giving better alignment with the reference Structure (PR30).

Table 12: Conformers of PR31 and PR36.

CODE	No. of Conformers Generated	Conformer used for Alignment
PR31	108	PR31_C47
PR36	13	PR36_C9

Thus the 36 structures were aligned with the reference structure and the alignment values obtained is summarized in Table 13 and the alignment picture is also shown below.

Table 13: Alignment Results of 36 Structures.

CODE	Alignment Value	CODE	Alignment Value
PR01	0.012049	**PR19**	0.024398
PR02	0.011553	**PR20**	0.078255
PR03	0.012383	**PR21**	0.074180
PR04	0.011442	**PR22**	0.076664
PR05	0.011920	**PR23**	0.079444
PR06	0.011218	**PR24**	0.077318
PR07	0.011944	**PR25**	0.077428
PR08	0.011487	**PR26**	0.078429
PR09	0.017047	**PR27**	0.077582
PR10	0.012712	**PR28**	0.027857
PR11	0.012252	**PR29**	0.016862
PR12	0.012001	**PR30**	0.000000
PR13	0.012126	**PR31**	0.014615
PR14	0.073008	**PR32**	0.023865
PR15	0.025344	**PR33**	0.035024
PR16	0.025159	**PR34**	0.021388
PR17	0.015761	**PR35**	0.074048
PR18	0.025540	**PR36**	0.005886

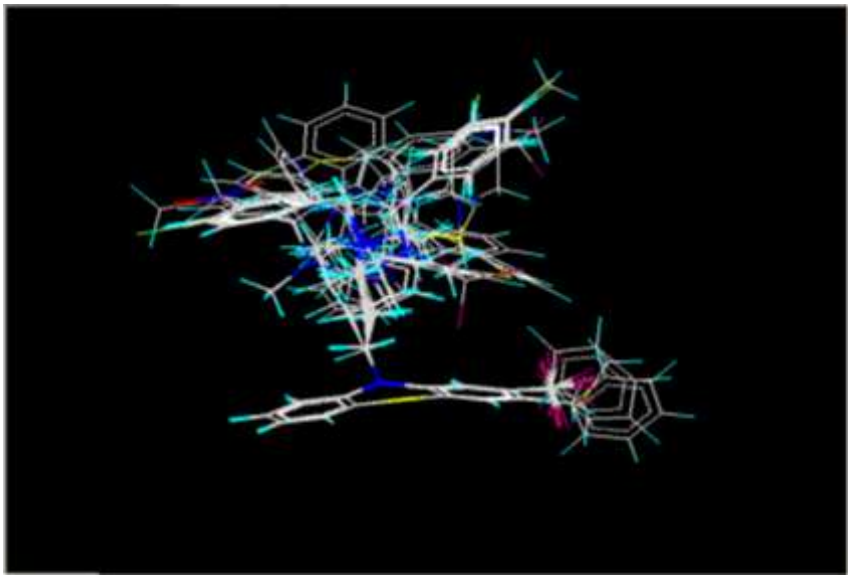


Figure 04: Alignment of 36 Structures (Phenothiazine Derivatives) by using Template Based Method.

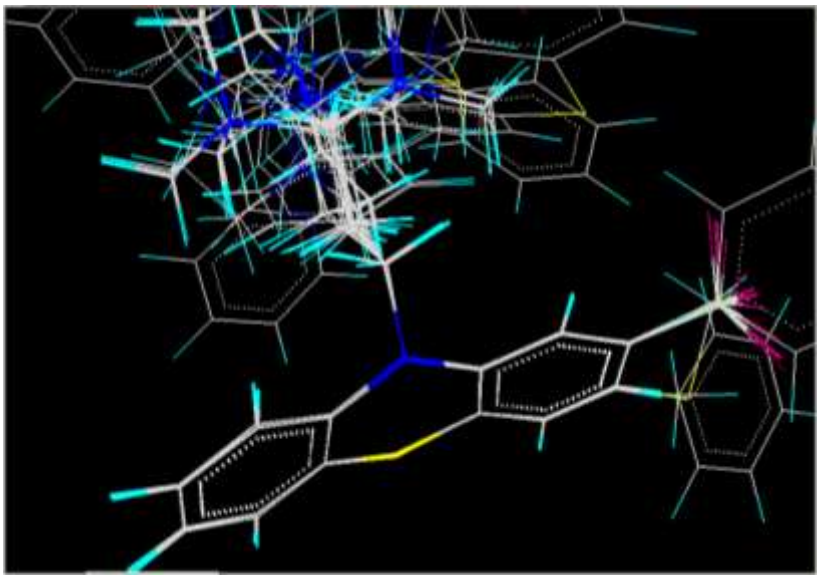


Figure 05: Magnification view of Alignment of Phenothiazine by using Template Based Method.

3D QSAR MODEL

The 3D QSAR of the 36 Structures (Phenothiazine Derivatives) as Antitubercular Agents was done by using kNN (k Nearest Neighbour) method. The Test Set of the Model 01 (2D Model) was used for the generation of 3D QSAR equation and the statistical values obtained are tabulated in Table 14.

Test Set of 3D Model: 1, 3, 5, 13, 18, 24, 28

STATISTICAL EVALUATION OF 3D MODEL

Table 14: Statistical values of 3D Model.

3D Model	
q^2	0.5847
q^2se	0.1806
Pred_r^2	0.6311
Pred_r^2 se	0.1184
K Nearest Neighbour	2
n(no. of training set)	27
Degree of Freedom	23

CALCULATION OF 3D DESCRIPTORS

The steric, electrostatic and hydrophobic descriptors were used to generate the descriptor sheet. The descriptors that were calculated depend on unaligned sites of the 3D structures. The descriptor sheet of the 3D model is tabulated in Table 15.

Table 15: Descriptor sheet of 36 Structures of 3D Model.

CODE	**S_525**	**S_668**	**S_688**
PE01	30	30	-0.02623
PR02	30	30	-0.0264
PR03	30	30	-0.02634
PR04	30	30	-0.02656
PR05	30	30	-0.02646
PR06	30	30	-0.02637
PR07	30	30	-0.02666
PR08	30	30	-0.02641
PR09	-0.19806	30	-0.03549
PR10	-0.13965	11.37143	-0.03596
PR11	-0.14034	11.64419	-0.04037
PR12	-0.14527	14.06277	-0.0471
PR13	-0.14622	15.27113	-0.03559
PR14	-0.06844	-0.44076	26.00713
PR15	-0.16608	28.78375	-0.02944
PR16	-0.16411	26.56279	-0.03233
PR17	-0.23277	30	-0.37791
PR18	-0.16492	27.01925	-0.02981
PR19	-0.16379	26.00332	-0.0308
PR20	-0.18907	30	-0.03885
PR21	-0.18475	30	-0.03976
PR22	-0.1848	30	-0.03934
PR23	-0.18768	30	-0.03912
PR24	-0.18902	30	-0.03902
PR25	-0.18837	30	-0.03892
PR26	-0.18712	30	-0.03905

PR27	-0.18698	30	-0.03904
PR28	-0.11598	-0.69053	-0.06095
PR29	-0.13278	23.3694	-0.25677
PR30	-0.16765	5.872454	-0.4501
PR31	-0.25781	3.917909	-0.02922
PR32	-0.07521	-0.54124	-0.05527
PR33	-0.08217	-0.56336	-0.01842
PR34	-0.07166	-0.46614	-0.03717
PR35	-0.36105	30	-0.25566
PR36	-0.06667	-0.52281	-0.05356

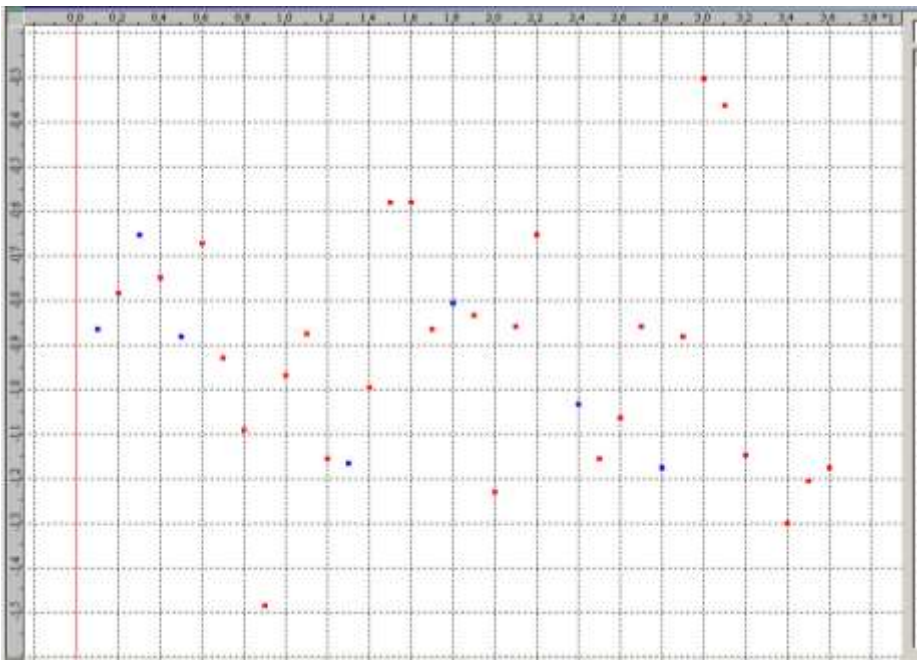
UNI-COLUMN STATISTICS

From the Uni Column Statistics of the 3D Model shown in Table 16 it was seen that the Maximum of the Training Set should be higher than Test Set and Minimum of the Test Set should be higher than Training Set.

Table 16: Uni Column of 3D Model.

Model	Column Name	Average	Maximum	Minimum	Std. Deviation	Sum
01	**Training**	-0.9166	-0.3010	-1.4857	0.2812	-24.7474
	Test	-0.9398	-0.6532	-1.1760	0.1932	-6.5789

ACTIVITY DISTRIBUTION PLOT



Plot 03: Activity Distribution Plot of 3d Model (Red Colour {Training Set} Blue Colour {Test Set})

From the Activity Distribution Plot of Model 01 it was seen that all the Test Set Structures were covered by the Training Set Structures and the Structures lying in the Periphery were not included in the Test set.

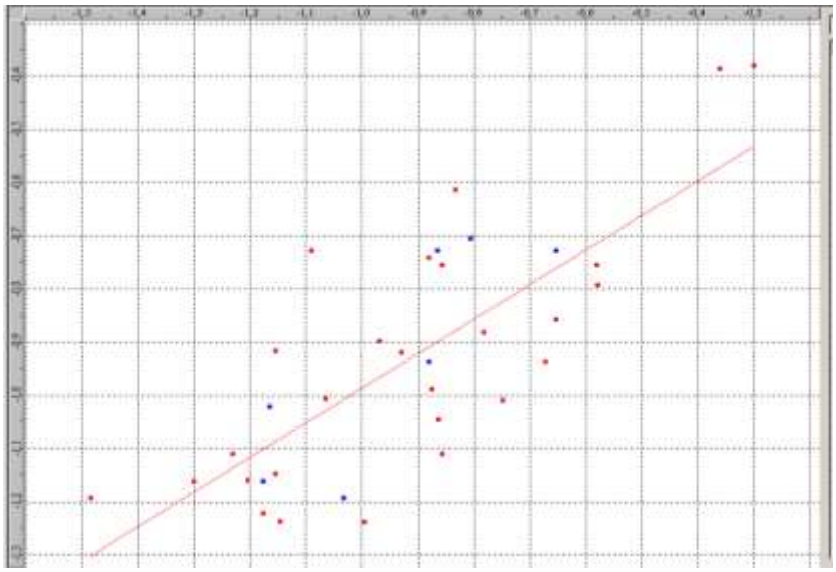
CORRELATION MATRIX

Table 17: Correlation Matrix of 3D Model.

	S_688	S_668	S_525
S_688	1	-0.35164	-0.08641
S_668	-0.35164	1	0.352409
S_525	-0.08641	0.352409	1

From the Correlation of 3D Model shown in Table 17 it was seen that no descriptor was correlated with each other.

FITNESS PLOT



Graph 03: Fitness Plot of 3D Model [Training set (red spot) and Test set (blue spot)]

From the fitness plot it was seen that all the test set structures were near to the best fit line but some structures of the training deviated from the best fit line and were far from the best fit line.

ACTUAL PREDICTED ACTIVITY OF 3D MODEL

The Actual Activity along with Predicted Activity and Residual is given in Table 18. In the 3D Model PR23 and PR33 were deleted from the Training Set as they were giving higher residual value and also when they were included in the Test or Training set they were not giving satisfactory values of the stated parameters. Thus they were deleted from the series

and they were at the Periphery in the Activity Plot and they were always outlier in the fitness plot.

Table 18: Actual Prediction Table of 3D Model.

CODE	Actual Activity	Predicted Activity	Residual
PR01	-0.8651	-0.72725	-0.13785
PR02	-0.7824	-0.881	0.098601
PR03	-0.6532	-0.72725	0.07405
PR04	-0.7481	-1.00965	0.26155
PR05	-0.8808	-0.93615	0.05535
PR06	-0.6721	-0.93615	0.264049
PR07	-0.9294	-0.91899	-0.01041
PR08	-1.0899	-0.72725	-0.36265
PR09	-1.4857	-1.19286	-0.29284
PR10	-0.9689	-0.89792	-0.07098
PR11	-0.875	-0.98842	0.113421
PR12	-1.1553	-0.91559	-0.23971
PR13	-1.1643	-1.0207	-0.1436
PR14	-0.9956	-1.2385	0.2429
PR15	-0.5809	-0.75525	0.17435
PR16	-0.5797	-0.79235	0.212654
PR17	-0.8633	-1.04492	0.18162
PR18	-0.8061	-0.70494	-0.10116
PR19	-0.8325	-0.61301	-0.21949
PR20	-1.2304	-1.10988	-0.12052
PR21	-0.8573	-0.75515	-0.10215
PR22	-0.6532	-0.8573	0.2041
PR23	-0.3222	-	-
PR24	-1.0334	-1.19285	0.15945
PR25	-1.1553	-1.14742	-0.00788
PR26	-1.0644	-1.00622	-0.05818
PR27	-0.8573	-1.10982	0.25252
PR28	-1.176	-1.16105	-0.01495
PR29	-0.8808	-0.74046	-0.14034
PR30	-0.301	-0.37927	0.078271
PR31	-0.3617	-0.3852	0.023498
PR32	-1.1461	-1.23672	0.09062
PR33	-0.6232	-	-
PR34	-1.301	-1.16119	-0.13981
PR35	-1.2041	-1.15946	-0.04464
PR36	-1.176	-1.22205	0.04605

SHOW POINTS

The show point parameters provide the information regarding the site where the structural modification has to be done. Figure 06 shows the descriptors that have been generated on the Lead (PR02).

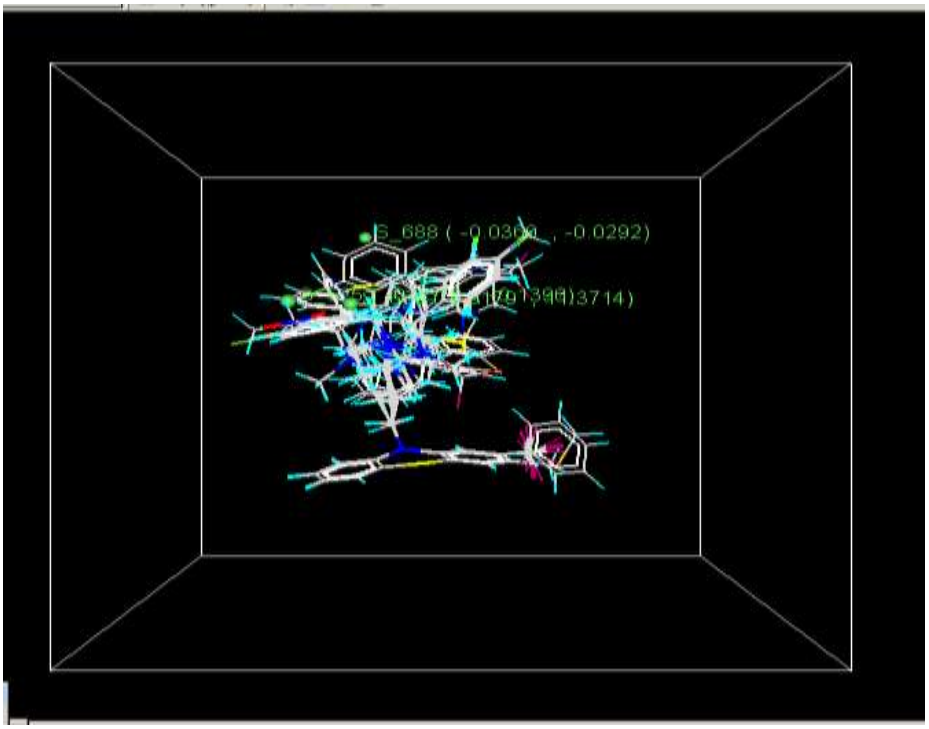


Figure 06: Site of alteration on Phenothiazine derivatives.

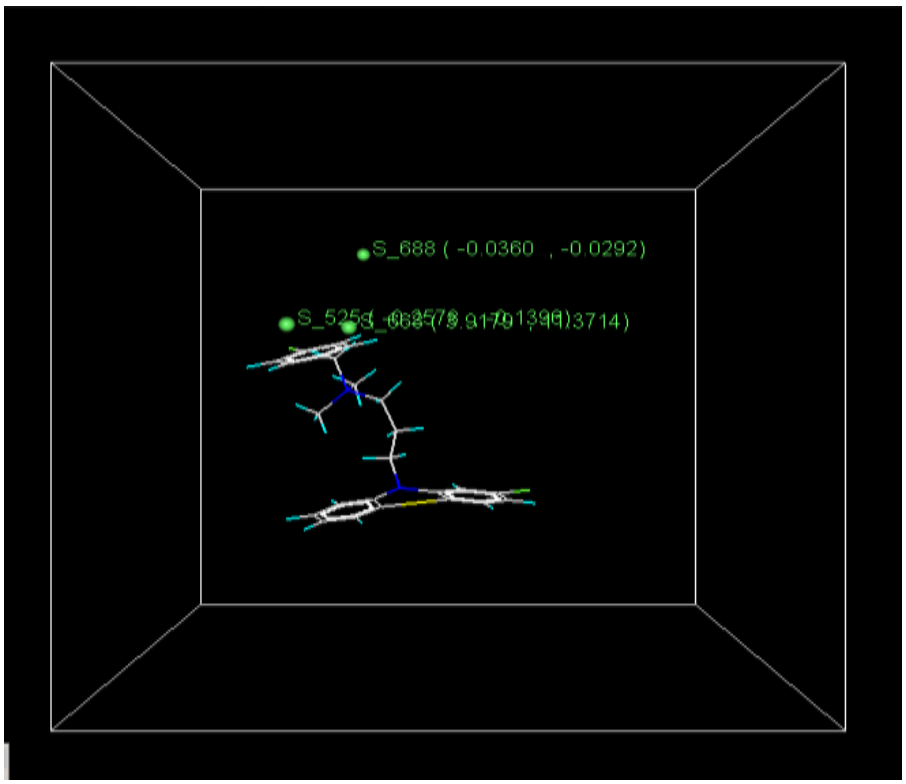


Figure 07: Site of alteration on PR02 (Lead).

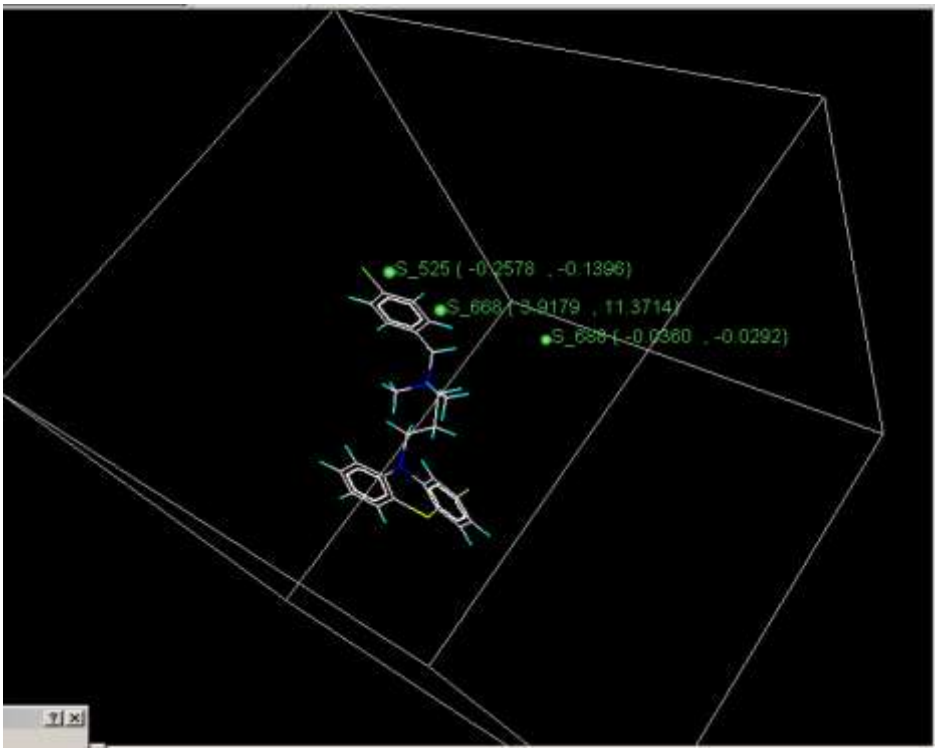


Figure 08: Magnified view of the Lead (PR02).

INTERPRETATION OF 3D MODEL

- The descriptors that were generated in 3D QSAR were tabulated in table 15. Only three descriptors have strong correlation with the activity.
- The descriptors that were generated were all steric descriptors. The sign of such descriptors provides knowledge of substituent's that has to be made on the structure for increasing the biological activity.
- The 3D QSAR model suggests point of alteration on PR02.
- In the 3D QSAR the suggestion provided is to increase the steric negative potential where as in 2D the descriptors provided give the information of increasing the steric effect by increasing the chain length.
- Thus 2D and 3D Models Validated.

DESIGNING

Designing of new compounds from the existing Phenothiazine moiety as antitubercular was done by taking PR02 as lead compound suggested by 3D QSAR Model.

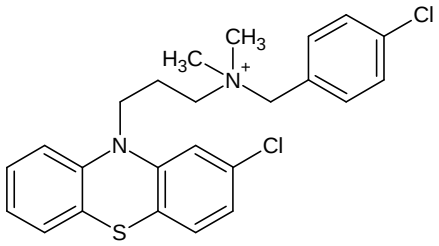


Figure 04: Basic Structure of Lead (PR02).

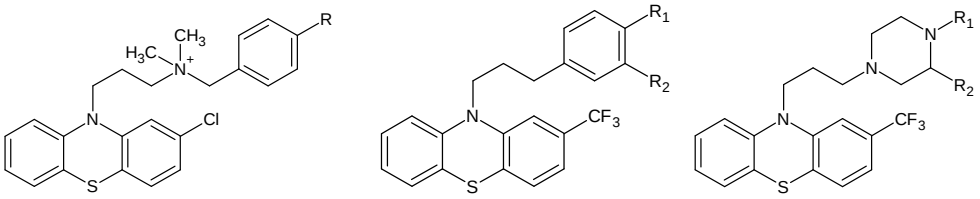
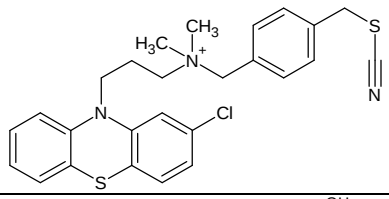
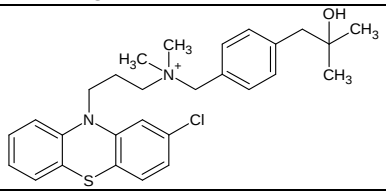
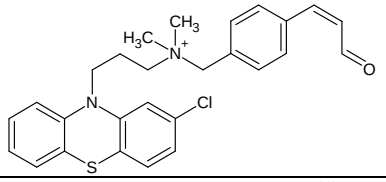
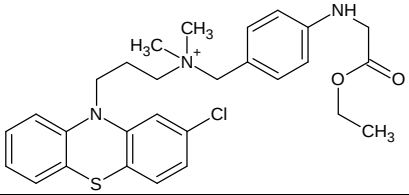
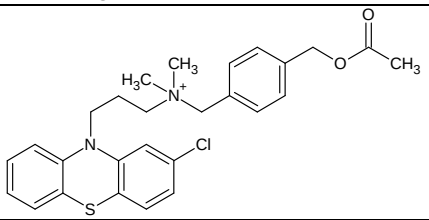


Figure 05: Basic Moiety used for Designing.

Table 19: Structures of Designed Compounds

CODE	STRUCTURE
DPR01	
DPR02	
DPR03	
DPR04	
DPR05	

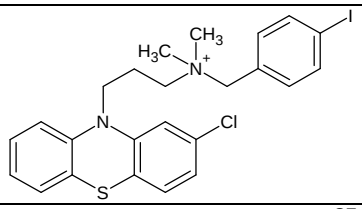
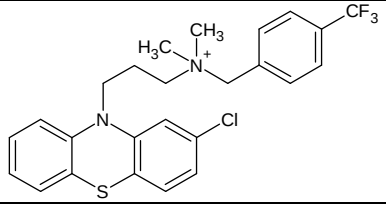
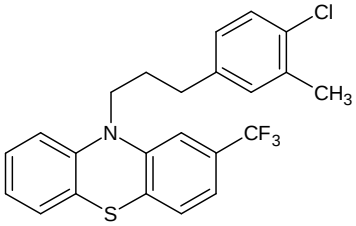
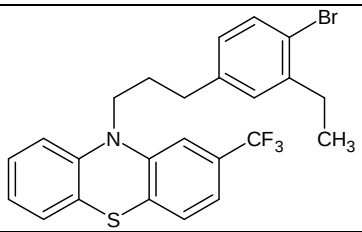
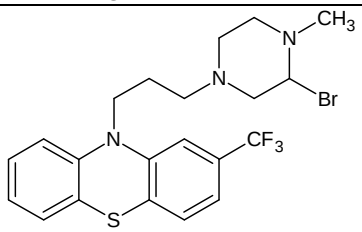
DPR06	
DPR07	
DPR08	
DPR09	
DPR10	

Table 20: Predicted Activity of Designed Compounds.

CODE	Predicted Activity (-log MIC)	MIC
DPR01	-0.78525	6.09
DPR02	-0.75525	5.69
DPR03	-0.75525	5.69
DPR04	-1.03317	10.79
DPR05	-0.33073	2.14
DPR06	-0.75525	5.69
DPR07	-1.22509	16.79
DPR08	-0.70525	5.07
DPR09	-0.75525	5.69
DPR10	-0.75525	5.69

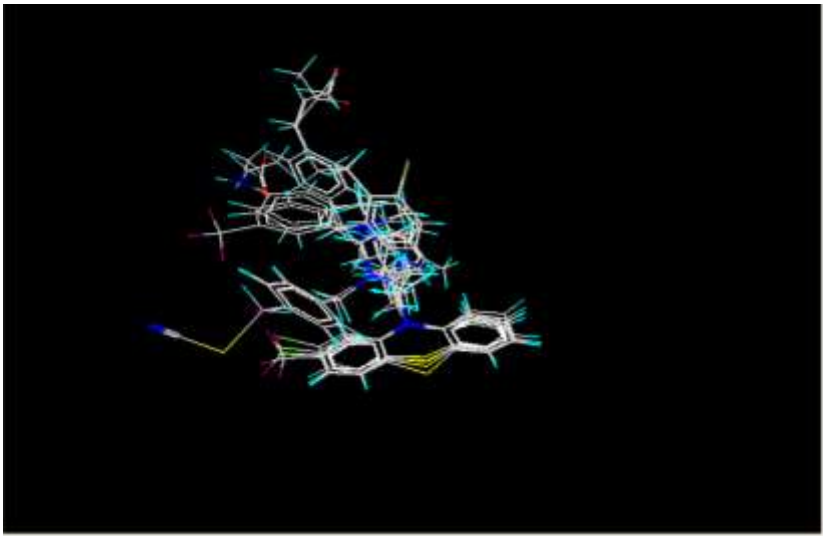


Figure 06: Alignment of Designed Compounds.

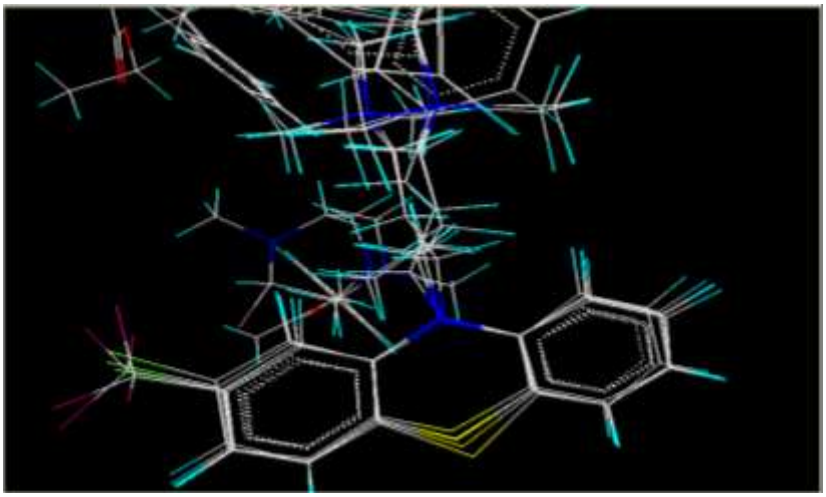


Figure 07: Magnification Picture of the Aligned Part of Designed Compounds.

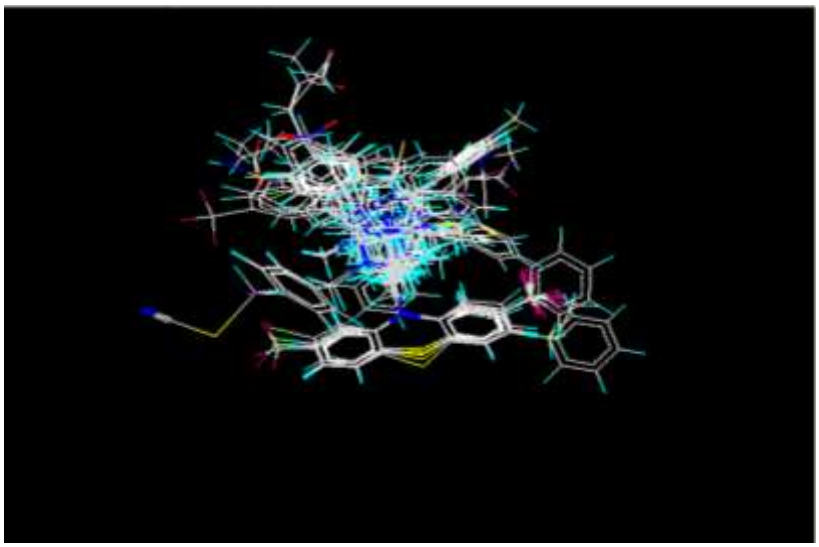


Figure 08: Alignment of Designed Compounds with 36 Compounds

Table 21: Alignment Value of Designed Compounds with 36 Compounds.

CODE	Alignment Value	CODE	Alignment Value	CODE	Alignment Value
PR01	0.012049	**PR19**	0.024398	**DPR01**	0.262196
PR02	0.011553	**PR20**	0.078255	**DPR02**	0.555154
PR03	0.012383	**PR21**	0.074180	**DPR03**	0.555287
PR04	0.011442	**PR22**	0.076664	**DPR04**	0.262107
PR05	0.011920	**PR23**	0.079444	**DPR05**	0.076147
PR06	0.011218	**PR24**	0.077318	**DPR06**	0.262073
PR07	0.011944	**PR25**	0.077428	**DPR07**	0.555315
PR08	0.011487	**PR26**	0.078429	**DPR08**	0.163684
PR09	0.017047	**PR27**	0.077582	**DPR09**	0.162158
PR10	0.012712	**PR28**	0.027857	**DPR10**	0.075420
PR11	0.012252	**PR29**	0.016862		
PR12	0.012001	**PR30**	0.000000		
PR13	0.012126	**PR31**	0.014615		
PR14	0.073008	**PR32**	0.023865		
PR15	0.025344	**PR33**	0.035024		
PR16	0.025159	**PR34**	0.021388		
PR17	0.015761	**PR35**	0.074048		
PR18	0.025540	**PR36**	0.005886		

CONCLUSION

- PR02 was used as Lead for designing new compounds.
- 10 compounds were designed from the lead.
- For designing alteration was done at the benzene ring, the protonated form of nitrogen was removed and pyrimidine ring was also substituted with functional groups.
- In the designed compounds DPR05 was the compound which was having activity near to the reported potent compound of the series.
- DPR05 was equipotent to PR30 of the reported series with MIC 2.14.
- DPR08 was found to be second equipotent compound to PR30 of the reported series with MIC 5.07.
- Due to the presence of small activity ratio in the biological activity of the reported series the designed compounds were giving same prediction thus only 10 compounds were designed.
- DPR07 was found to be worst among the designed compounds with MIC 16.79.

ACKNOWLEGEMENT

We are greatly thankful to all those persons who gave us their valuable support for carrying out our QSAR study on Phenothiazine for developing better analogues with enhanced anti-tubercular activity.

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