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COMPUTER AIDED DESIGN OF 1, 2, 3, 4,-TETRAHYDROPYRIMIDINE DERIVATIVES CONTAINING CARBAMATES AND CARBAMIDES: AS SELECTIVE CALCIUM CHANNEL BLOCKERS

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ABSTRACT

The curtius rearrangement of Ethyl - 1 - (2 – azido – 2 - oxoethyl) – 6 – methyl – 2 – oxo – 4 – (3 – substituted) - 1, 2, 3, 4 – tetrahydropyrimidine -5 – carboxylate prepared from the readily accessible 4 - (1 – substituted) – 5 - ethoxy carbonyl – 6 - methyl] - 3, 4 – dihydropyrimidine – 2 (1H) – one was investigated in presence of ethanol, substituted phenols and substituted amines. The resulting carbamates and carbamides were has been made to correlate the calcium channel blocking activity to the physicochemical descriptors through QSAR studies. kNN- MFA and other advanced methods of analysis were opted for a set of twelve derivatives to figure out the correlationship between steric and electrostatic descriptors. In 3D-QSAR, statistically significant model 2 was obtained using simulated annealing algorithm, with the corresponding q2 and pred_r2 values 0.5141and 0.1109 respectively, showing internal and external predictivities 51% and 11 % respectively. The ten descriptors which contributed significantly to the model were E_945, S_764, S_897, E_774, E_898, S_973, E_383, E_319, S_832, E_546. The models were found to show good statistical and predictive significance which can further be used for guiding ligand modification for the development of potential calcium channel blocking agents.

KEY WORDS: 3D QSAR, calcium channel blockers, 1,2,3,4-tetrahydropyrimidine,

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INTRODUCTION

Dihydropyridines are the largest and most studied class of organic calcium channel blockers[1]. In addition to their proven clinical utility in cardiovascular medicine. Dihydropyrimidine calcium entry blockers are clinically effective cardiovascular agents and have been extensively studied to elucidate the molecular and conformational requirements for their interaction at the receptor level[2]. Ion channel blockers are attractive drug target for an expending range of therapeutic indications and voltage dependent calcium channels plays important roles in critical biological processes[3,4]. Selective calcium channel blockers in particular are now emerging as prospective therapeutics for the treatment of antihypertensive agents. DHPMs may lead to other beneficial effects such as regression of left ventricular pressure and vascular hypertrophy, renal protection, weak anti-platelet, anti-ischemic and anti- atherogenic activity4-7. The term cardio protection refers to techniques used to prevent or delay the development of myocardial injury, particularly during ischemia. Ischemia and reperfusion produce profound effects on the function of molecules involved in the control of calcium homeostasis, leading to increased free cystolic Ca2+ concentration. Calcium overload is one of the crucial alterations responsible for ischemia and reperfusion injury. Calcium overload can trigger several injurious mechanisms. Many ATP-consuming enzymes require Ca2+ for activity, so that calcium overload increases ATP consumption and exacerbates the unbalance between energy supply and demand, which is the metabolic hallmark of ischemia. by considering all facts , we have used computational approach to understand the mechanism of interactions and binding affinity between receptor and drug molecules[5-7]. In present study molecular docking studies were carried out to explore the binding mechanism of 1,2,3,4-tetrahydropyrimidine derivatives to receptor. The redefined models of 1,2,3,4-tetrahydropyrimidine derivatives were obtained after minimization using Vlife 4.0 MDS. The stable model protein ITEL from protein data bank (PDB) was further used for batch docking of 1,2,3,4-tetrahydropyrimidine.

COMPUTATIONAL DETAILS

The structures of the 12 substituted aldehydes analogues are given in table 1. The biological activities were converted into the corresponding –log (IC_{50}) values. The observed calcium channel blocking activities for the training set and test set are given in table 3 and 4, respectively. The various 3D-QSAR studies were carried out using Phase while the docking studies were carried out respectively[8].

QSAR MODEL BUILDING

The in vitro biological data of a series of 12 calcium channel blockers was used for this study. 6 molecules were used as training set and 6 molecules were used as test set. The ligands of training set were used for generating QSAR model.

STRUCTURE BASED STUDIES

Docking studies of the title compounds was done on Vlife 4.0 MDS using grid docking method for calcium channel blocking activity. 2D structures of the compounds were built and then converted into the 3D with the help of software. The 3D structures were then energetically minimized up to the RMS gradient of using molecular force field. The active site for docking was defined as all atoms within 5 Å radius using biopredicta tools of software, open docking and then batch grid docking. Batch docking shows browsing of receptor and ligand. Results generated were saved in output file. Molecules saved in output file as a docked ligand format with proper conformation and to check binding interactions. Those molecules shows good interaction with receptor are further used for predicting the activity and calculated for their IC_{50}[9].

STEPS UNDERTAKEN IN DOCKING STUDY

Step I- Ligand Preparation

The structure of 3-substituted-aminophenyl-4-hydroxy-coumarin derivatives (Table 1) was used as the template to build the molecules in the dataset in V Life MDS 4.0. Hydrogen bond interaction was calculated and the ligand geometries were optimized by energy minimization using MMFF force field charges for the atoms, till a gradient of 0.001 kcal/mol/A° was reached, maintaining the template structure rigid during the minimization (Table 2). Diagrams of hydrogen bond interaction of selected derivatives have been mentioned in figure 1.

Step II- Data set for Analysis

The calcium channel blocking activity of 12 compounds was measured with IC50 values in vitro (50-100 microM). These inhibitors along with conformational energy were shown in Table 3 & 4.

Step III- Preparation of the docking file

Dihydropyridine-sensitive L-type, calcium channel beta-2 subunit (1T3L) is strongly associated with calcium channel blocking activity. On this basis, we selected 1T3L as a biological target for docking study of synthesized compounds. The crystal structure of 1T3L was obtained from the Protein Data Bank (www.rcsb.org/pdb). The optimized receptor was then saved as mol file and used for docking simulation. The 2D structure of the compounds were built and then converted into the 3D with the help of VLife MDS 4.0 software[10]. The 3D structures were then energetically minimized upto the RMS gradient of 0.01 using Merck Molecular Force Field (MMFF). Conformers of all the synthesized ligands were selected and were then energetically minimized upto the RMS gradient of 0.01 and then saved in a separate folder. The active site selection was done by choosing the cavity having maximum hydrophobic surface area. Docking simulation was done by GA docking method. All the conformers were virtually docked at the defined cavity of the receptor. The parameters fixed for docking simulation was like this number of placements: 30, rotation angle: 30°, exhaustive method, scoring function: dock score. By rotation angle, the ligand gets rotated for different poses. By placements, the method will check all the 30 possible placements into the active site pocket and results out few best placements out of 30. For each ligand, all the conformers with their best placements and their dock score will be saved in output folder. The method also highlights the best placement of best conformer of one particular ligand which is having best (minimum) dock score. In the results of docking, we have listed only best conformers and its dock score for each ligand in Table 2. After docking simulation, the best docked conformer of each ligand and receptor were merged and aggregated by defining the radius of 5 Å. The receptor complexes were then energetically minimized along with the docked ligand. Stepwise aggregation was done first with hydrogen, second side chains and finally the backbone of receptor. The optimized complexes were then checked for various interaction of ligand with receptor like hydrogen bonding, hydrophobic bonding and Vander Waal's interaction[11]. Docking profile of previously synthesized compounds are shown in figure 1

Simulated annealing

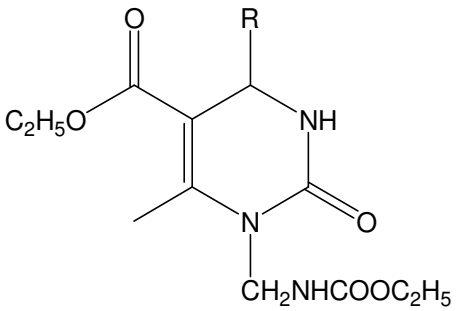
During this process of simulated annealing (SA), the system adopts all the low energy states which are most populated. Here, all the calculated descriptors remaining after removal of invariable columns were subjected to SA algorithm coupled with k-nearest neighbour (k-NN) methods for building a QSAR model based on the training set. Model evaluation is done next to validate the model both internally and externally so as to check the effectiveness and the predictive ability of the model. Internal validation is carried out using leave-one-out (q2, LOO) method. In this method, biological activity of each molecule is predicted once, eliminating it out of the system. For external validation, activity of each molecule in the test set was predicted using the model generated from the training set. Pred_r2 determines the

ability of the model to effectively predict the biological activity values for a external test set. Developed quantitative models were evaluated using following statistical measures: n, number of observations (molecules); k, number of variables (descriptors); Number of components; Number of nearest neighbors, number of k-nearest neighbor in the model; r2, coefficient of determination; q2, cross-validated r2 (by leave one out); pred_r2, r2 for external test set; F-test, pred_r2_se, standard error of external test set prediction. The r2 and q2 values are used as determining factors in checking the effectiveness of the model[12].

RESULTS AND DISCUSSION

3D QSAR models were obtained using all the three methods, SW, SA and GA. Model obtained using Stepwise Linear Regression showed good q2 and pred_r2 values but, since only a single descriptor contributed to the model, the model was unable to describe the exact structural requirements of the molecule. Next, model was developed using SA with kNN (Table 5) which led to statistically significant results. Reports of the statistically significant parameters of both the models. 3D data points were generated around the molecule (Figure 4, 5 & 6) which helps to define the requirements which would promote the calcium channel blocking activity. Ten parameters both electrostatic and steric, E_945, S_764, S_897, E_774, E_898, S_973, E_383, E_319, S_832, E_546 (Table 6) were found to show significant contributions to the model. This helps to generate the steric and electrostatic fields which correlate these parameters to the activity profile of the Pharmacophore. In 2D-QSAR, study unveils key structural requirements for calcium channel blocking activity. The analysis signifies the importance of various physicochemical and topological descriptors and their related contribution towards activity. This generated model can prove effective in accounting for the changes to be accommodated so as to obtain good biological activity results. On the other hand, the importance and utility of the new 3D-QSAR method discussed here has been established using the V-Life software. 3D-QSAR model which was generated by kNN-MFA using the simulated annealing (SA) method has been reported. This model has shown good internal as well as external predictivity values. This study shows how chemical features for a set of compounds along with their activities ranging over several orders of magnitudes can be used to generate QSAR equation that can successfully predict the activity.

Table 1
Structures of compounds used for 3D QSAR study



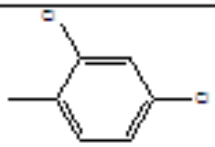
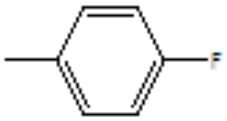
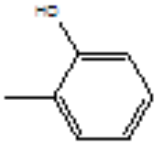
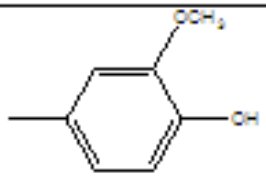
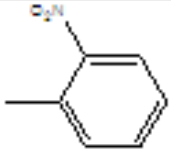
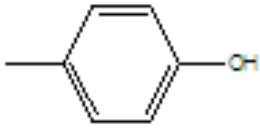
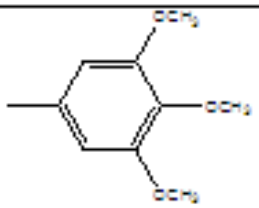
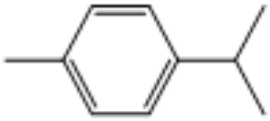
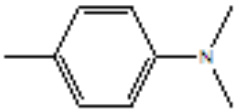
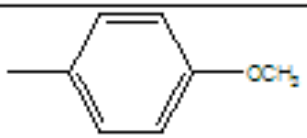
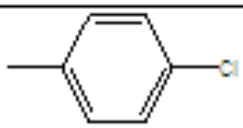
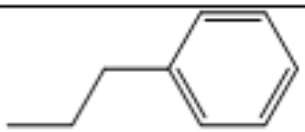
Compound	R	Compound	R
A		G	
B		H	
C		I	
D		J	
E		K	
F		L	

Table 2

Dock score of benzaldehyde substituted 1,2.3.4-tetrahydropyrimidines

Sr.No	Molecule Name	Dock Score
A	2,4-Dichloro Benzaldehyde._3D.mol2	**-4.237663**
B	2- Hydroxy Benzaldehyde._3D.mol2	-3.670146
C	2-Nitro Benzaldehyde._3D.mol2	**-3.919744**
D	3,4,5-Trimethoxy Benzaldehyde._3D.mol2	-3.230161
E	4-(N,N-Dimethylamine) Benzaldehyde_3D.mol2	**-4.026305**
F	4-Chlorobenzaldehyde._3D.mol2	-3.953336
G	4-Fluorobenzaldehyde._3D.mol2	-3.801322
H	4-Hydroxy 3- Methoxy Benzaldehyde._3D.mol2	-3.431987
I	4-Hydroxy Benzaldehyde._3D.mol2	-3.871687
J	4-Isopropyl Benzaldehyde._3D.mol2	**-3.954107**
K	4-Methoxy Benzaldehyde._3D.mol2	-3.388676
L	Cinnamaldehyde_3D.mol2	-3.881204

Table 3
Experimental –Log (Ic50) and Corresponding Model Predicted Values (Training set)

Compound	(R)	Actual IC50	Predicted IC50	Fitness Score
A	2,4-Dichloro Benzaldehyde	1.65	2.03	-0.38
B	2-Hydroxy Benzaldehyde	2.48	2.22	0.26
C	2-Nitro Benzaldehyde	8.13	3.17	4.96
D	3,4,5-Trimethoxy Benzaldehyde	1.3	1.31	-0.01
E	4-(N,N dimethylamino) Benzaldehyde	1.31	1.30	0.01
F	4-Chloro Benzaldehyde	1.07	1.80	-0.73

Table 4
Experimental –Log (Ic50) And Corresponding Model Predicted Values (Test set)

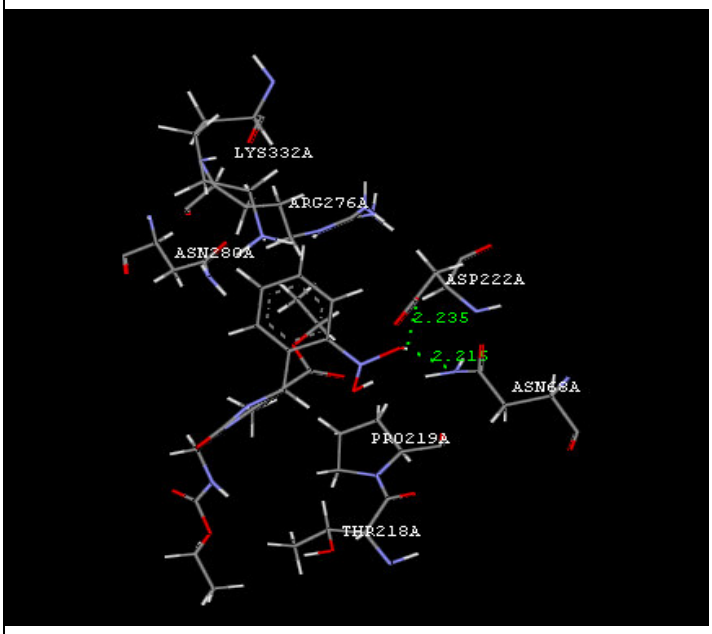
Compound	(R)	Actual IC50	Predicted IC50	Fitness Score
G	4-Fluro Benzaldehyde	1.40	1.79	0.39
H	4-Hydroxy 3-methoxy Benzaldehyde	3.92	2.46	1.46
I	4-Hydroxy Benzaldehyde	8.93	1.69	7.24
J	4-Isopropyl Benzaldehyde	1.96	1.67	0.29
K	4-Methoxy Benzaldehyde	0.51	1.69	-1.1
L	Cinnamaldehyde	0.49	1.30	-0.81

Table 5
Statistical parameters for 3D QSAR (kNN Method (Simulated Annealing)

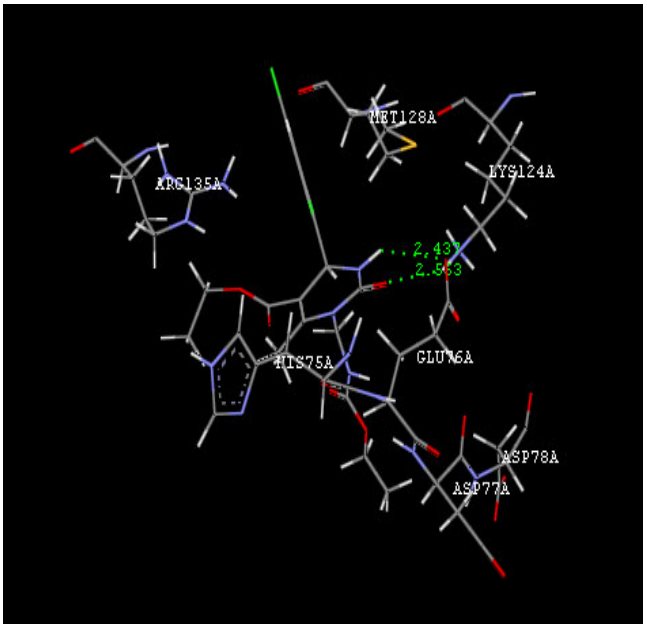
Parameter	Reading
k Nearest Neighbour (kNN)	2
n	6
Degree of freedom	-5
q2	0.5141
q2_se	0.6931
Predr2	0.1109
pred_r2se	3.9922

Table 6
Descriptors involved in SA studies

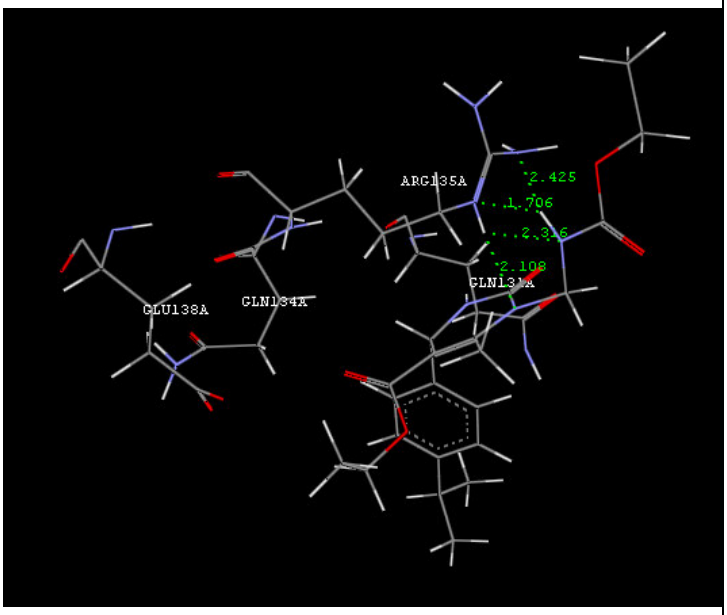
Descriptors	Range	
E_945	0.0566	0.0475
S_764	0.3492	2.2903
S_897	0.0220	0.0134
E_774	3.8370	0.3605
E_898	0.2903	0.2839
S_973	0.1085	0.0279
E_383	0.0802	0.2196
E_319	0.0764	0.3860
S_832	0.0178	0.0107
E_546	0.6775	0.4462



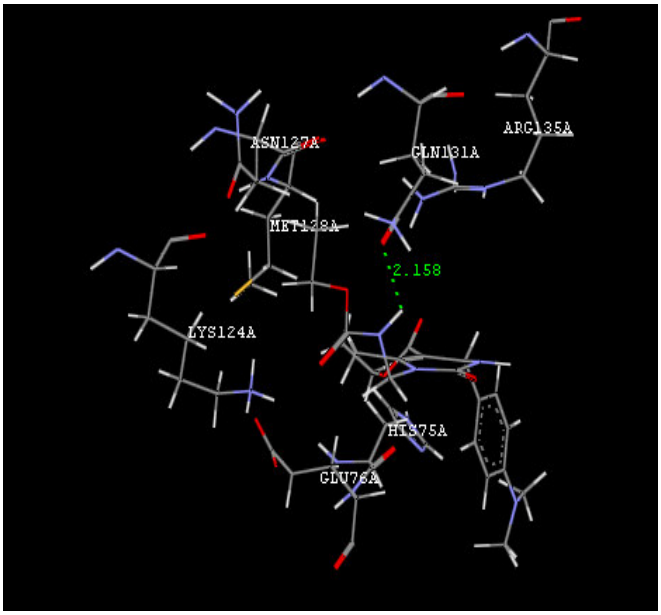
2-Nitrobenzaldehyde



2,4-Dichlorobenzaldehyde



4-Isoprobenzaldehyde



4-(n,n-dimethylamine) benzaldehyde

Figure1
Docking profile of previously synthesized compounds

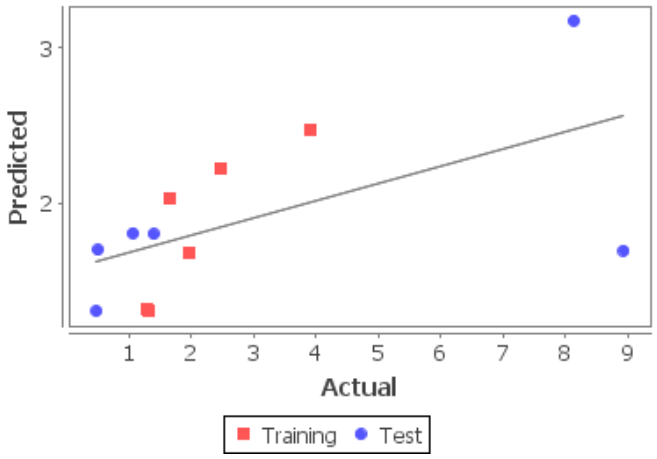


Figure 2
Experimental and Predicted Activity Values of IC_{50}Training and Test Set

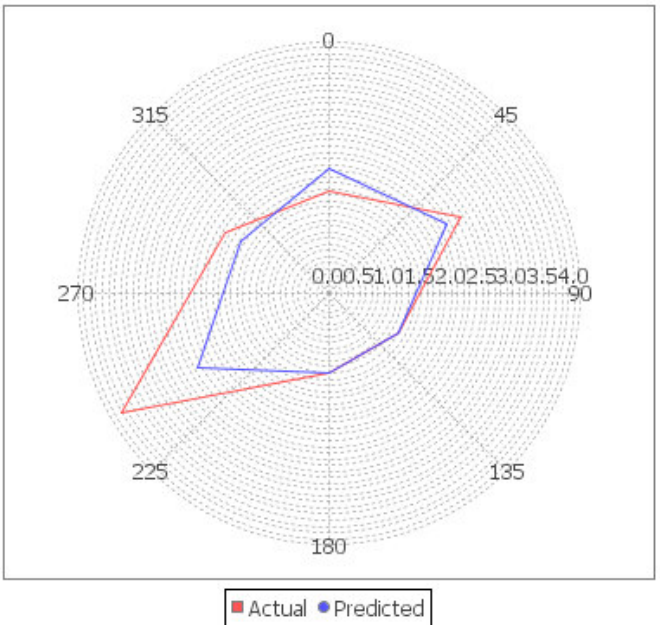


Figure 3
Template based Alignment (Training set)

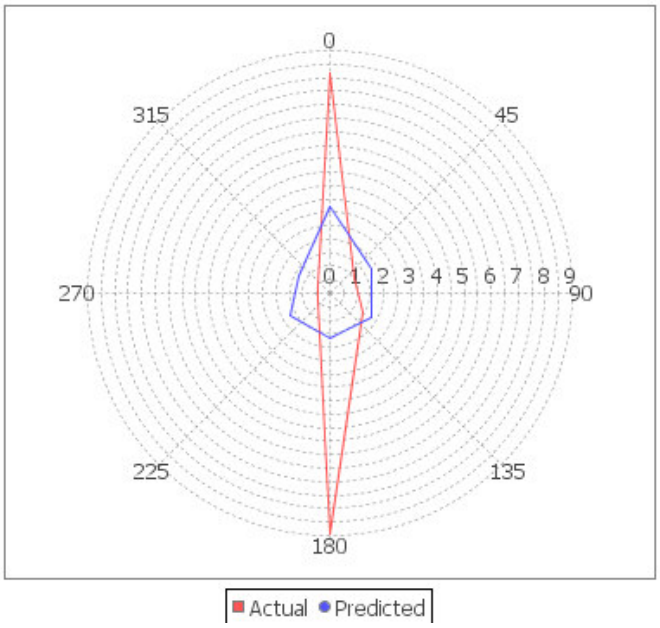


Figure 4
Template based Alignment (Test set)

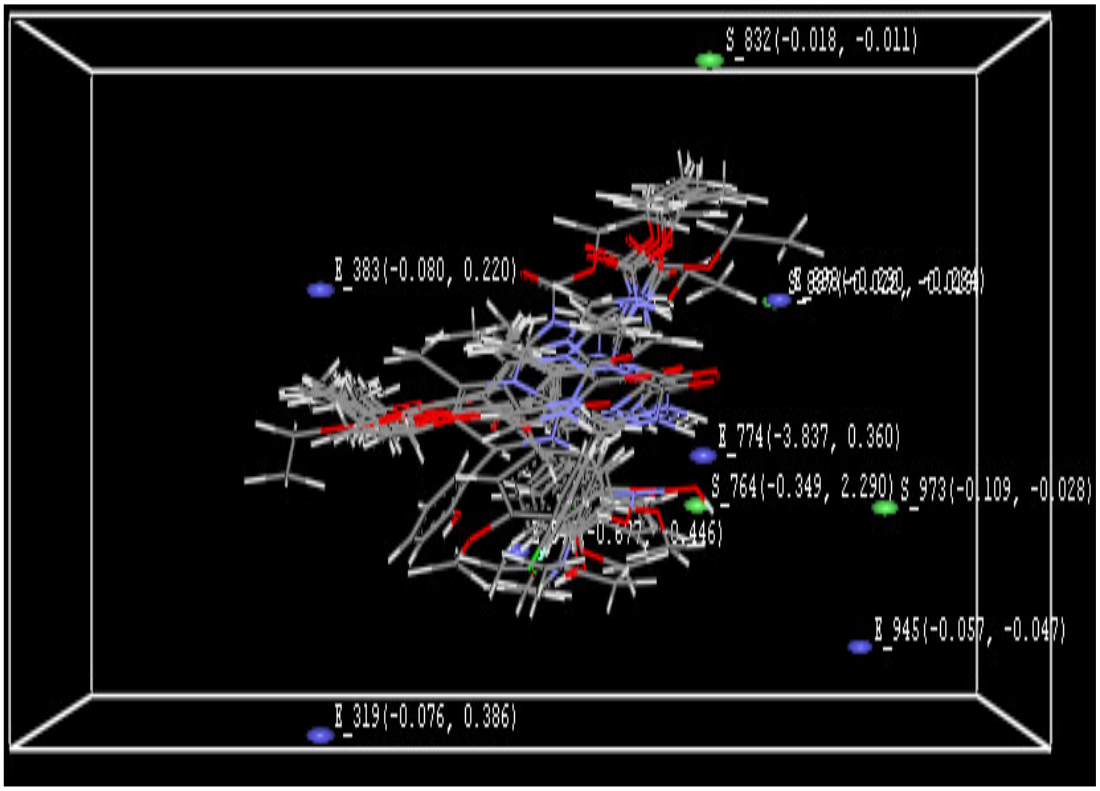


Figure 5
Showing electrostatic and steric descriptors (Showpoints diagram)

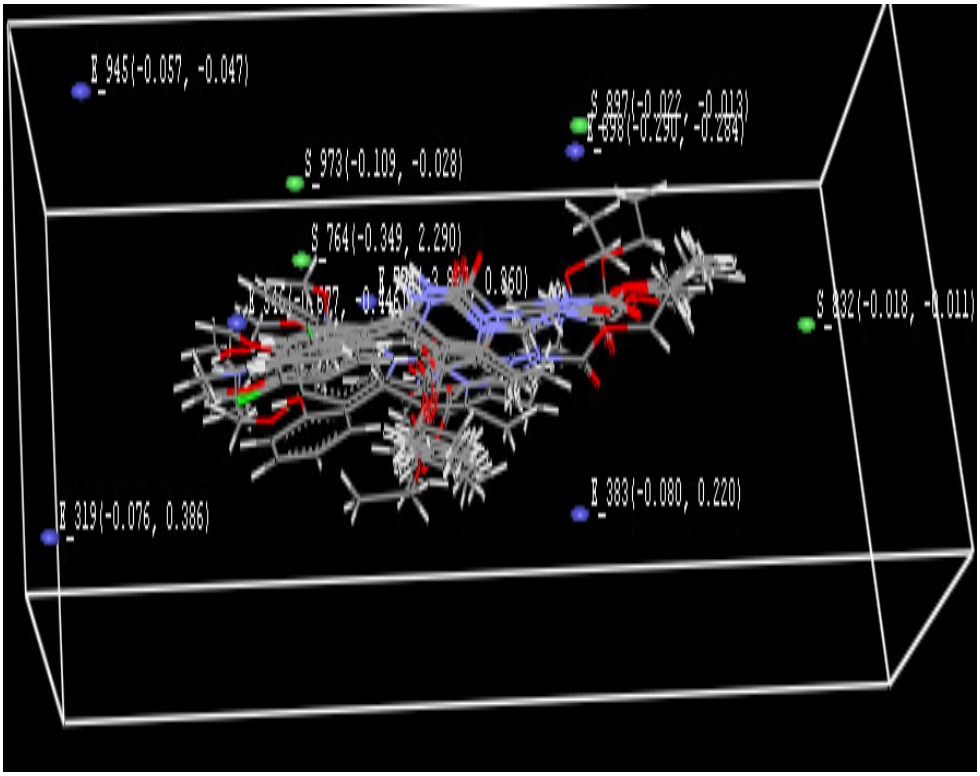


Figure 6
Showing electrostatic and steric descriptors (Showpoints diagram)

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