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CORRELATION OF 5, 6, 7, 8-TETRAHYDROACRIDINE BASED SCAFFOLD INHIBITORY AGENTS FOR ALZHEIMER DISEASE.

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and one of the most common causes of dementia in the elderly. Acetylcholine sterase (AChe) inhibitors are the main drugs used in the treatment of AD. In this work, docking studies have been performed in order to understand the interaction between 5, 6, 7, 8-tetrahydroacridine inhibitor and AChe. The increase observed in the calculated binding affinities between inhibitor and AChe, reflect the experimental inhibitory activity expressed in terms of the half maximal inhibitory concentration (IC_{50} = 0.002 to 13.0 µm) of the above inhibitor. The AM1 and PM3 semi-empirical methods are used to estimate the predictive power of final QSAR equations. QSAR and molecular docking studies indicated that, 2-chloro, 9-amine derivative of 5, 6, 7, 8-tetrahydroacridine showed the highest percentage of concentration and can become a potential lead for treating Alzheimer's disease.

KEYWORDS: Inhibitor, 5, 6, 7, 8-tetrahydroacridine derivatives, QSAR, Semi-empirical methods, Regression analysis, DOCKING, AChe.

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1. INTRODUCTION

Alzheimer's is the most progressive and fatal brain disease, which attributes to the loss of memory and influences the learning process in the affected individuals. Alzheimer's disease (AD) affected individuals were characterized by the presence of senile plaques and neurofibrillary tangles in their brain, the loss of memory is directly correlated with the abridged cholinergic neuro-transmission, caused by the pronounced diminish in the levels of the neuro transmitter [1-2]. ACh abnormal activity of human acetyl-cholinesterase (hAChe), the enzyme responsible for hydrolysis of ACh, was attributed to the reduced level of ACh. Treating AD was achieved through inhibiting the anomalous action of ACh by various specific ACh inhibitors such as

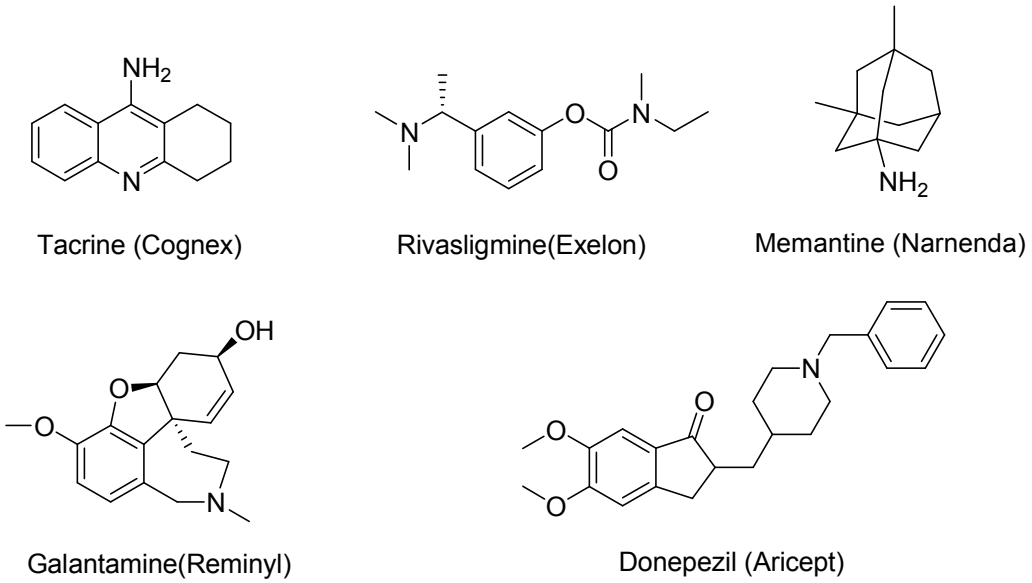


Figure1
Marketed drugs for the treatment of Alzheimer's disease.

5, 6, 7, 8-tetrahydroacridine ($C_{13}H_{13}N$) is too basic heterocyclic compound. It is a raw material used for the production of dyes and some valuable drugs. Many 5, 6, 7, 8-tetrahydroacridines such as proflavine also have antiseptic properties. 5, 6, 7, 8-tetrahydroacridine and related derivatives bind to DNA and RNA through intercolation mode [3--5]. Acridine orange (3, 6 dimethylaminoacridine) is a nucleic acid-selective metachromatic stain useful for cell cycle determination. Complex of the anti-alzheimer drug 5, 6, 7, 8-tetrahydroacridine with acetyl cholinsterase centrally active cholinesterase inhibitor that has been used to counter the effects of muscle relaxants as a respiratory stimulant and in the treatment of Alzheimer's disease and other central nervous system disorders.

2. MATERIALS AND METHODS

Bioassay

In the present investigation bioassay of 19 derivatives of 5, 6, 7, 8-tetrahydroacridine found in the literature whose IC_{50} values are known. The criteria for selection of molecules are

1) against same target.
2) which have available biological activity data.

3) molecules possessing 5, 6, 7, 8-tetrahydroacridine scaffold.
19 molecules were selected based on the above set criteria. More than 15 molecules showed IC_{50} below 1_Micro_molar concentration. These were further supported by docking studies using GOLD and Argus Lab 4.0.1 Software.

Structural Skeleton of 5, 6, 7, 8-tetrahydroacridine

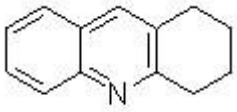
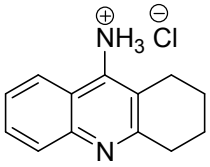
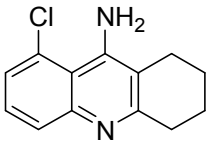
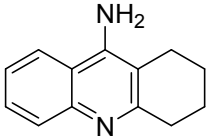
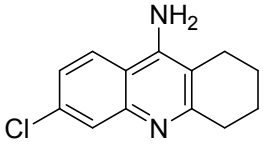
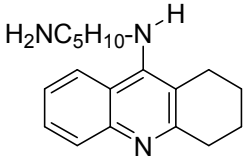
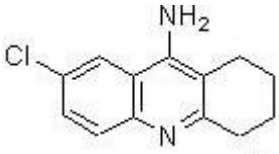
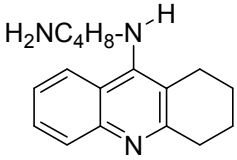
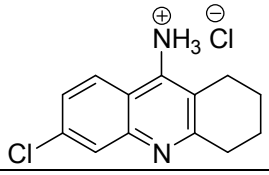
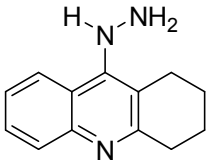
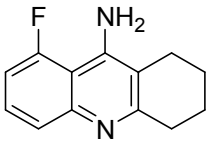
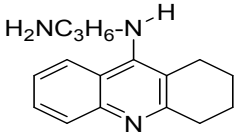
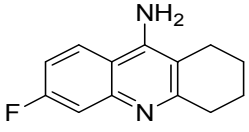
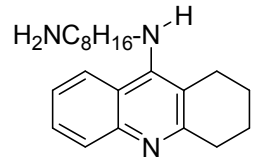
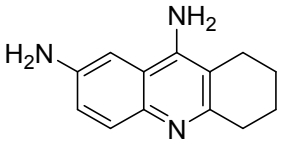
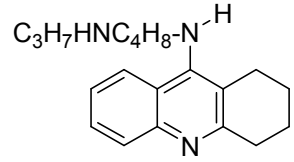
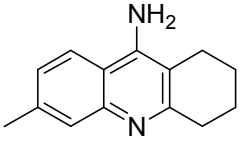
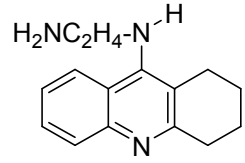
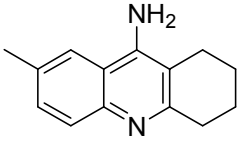
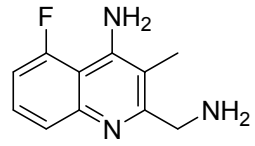


Figure 2
5, 6, 7, 8-tetrahydroacridine.

Table 1
Derivatives of 5, 6, 7, 8-tetrahydroacridine with IC50 values.

COMPOUND	STRUCTURE	IC_{50}(µm)	COMPOUND	STRUCTURE	IC_{50}(µm)
1		0.205	11		0.004
2		0.05	12		0.002
3		0.003	13		0.04
4		0.023	14		0.005
5		13	15		0.078
6		0.069	16		0.087

7		0.003	17		3.8
8		0.023	18		0.1
9		0.054	19		8.1
10		1.9			

3. COMPUTATIONAL CALCULATIONS

Molecular Structure Building

A series of 19 compounds tested for inhibitory activity were selected for the present study from pubchem[6], and Hyperchem software[7] were used in modeling studies. The molecules were generated and the geometry optimization was performed using molecular modeling programme (Force Field).

3.1 Data Set and Validation of QSAR Models

QSAR technique was applied to the 5, 6, 7, 8-tetrahydroacridine analogues that were varied at the positions of different substituent's from structure to structure shown in Table 1. The appropriate descriptors or parameters for the identified compounds viz; vertical ionization potentials (IPV's), electron affinity (EA), electro negativity (χ), hardness (η), softness (S), electrophilicity index (ω), partition coefficient (LOGP), polarisability (POL) and hydration energy (HE) were used as independent variables for deciding in AChe inhibitory activity.

3.2. CHEMICAL DESCRIPTORS

3.2.1. SEMI EMPIRICAL METHODS

Quantum chemical calculations at the DFT/RB3LYP/631G* (restricted B3LYP), RHF/6-31G* (restricted Hartree-Fock), AM1 and PM3 semi empirical theory levels, are employed for full optimization of the selected neutral compounds [8-10]. The geometrical structures of the radicals studied are optimized independently from the neutral molecules prior to the calculation of energies, treated as open shell systems [11-14]. All calculations are performed by using the program of Hyperchem software Inc. The calculated vertical ionization potential (IPV's) and electron affinity (EA) are corrected for zero-point energy, assuming a negligible error and thus saving computer-time. The IPV are calculated as the energy differences between a radical cation and the respective neutral molecule; IPV (E_{cation} – $E_{neutral}$) $_{DFT}$ and Koopmans's theorem (IPV = -ε $_{HOMO}$). The EA are computed as the energy differences between a neutral form and the anion molecule; EA=$E_{neutral}$ – E_{anion}. The AM1 reactivity descriptors are obtained from Eqs. (1) & (2) [15-17].

3.3. Correlation Analysis

The window version software SPSS [18] was used in the regression analysis. A relation between biological activity, expressed as Log ($1/IC_{50)}$, and the physicochemical parameters and QSAR was analyzed statistically by fitting the data to correlation equations consisting of

various combinations of these parameters. The statistical optimization was used to propose the best correlation model. The matrix correlation uses the Pearson product moment correlation to measure the degree of linear relationship between two variables. The coefficient assumes a value between -1 and +1. If one variable tends to increase the other decreases, the correlation coefficient is negative. Conversely, if the two variables tend to increase together the correlation coefficient is positive. We obtained the correlation matrix between inhibitory activity and respective calculated properties for nineteen 5, 6, 7, 8-tetrahydroacridine derivatives. The more relevant regression models were selected: The correlation coefficient (R), the Fisher ratio values (F) and the standard deviations(s),standard error estimate (SEE), percentage of effective variable(%EV) and R^2adjusted ($AdjR^2$). The best equation was also tested for their predictive power using a cross- validation procedure .The cross-validation is a practical and reliable method for testing this significance. In principle, the so-called "leave-one-out" approach consists in developing a number of models with one sample omitted at the time [19]. After developing each model, the omitted data is predicted and the differences between actual and predicted reduction potential (y) values are calculated .The sum of squares of these differences is computed and finally the performance of the model (its predictive ability) is given by PRESS (Predictive Sum of Squares) and S_{PRESS} (Standard deviation of cross validation**).** The predictive ability of the model was also quantified in terms of the Q^2.

3.4. Docking Studies and Validation

GOLD and Argus lab 4.0.1 are Molecular Docking software**.** This helps in computational virtual screening to find the lead compounds. Molecular docking started with Fischer's lock and key theory, where, every receptor has its unique ligand to catalyze the reaction [20-22]. Now-a-days docking is frequently to predict the binding orientations of small molecules of drug candidates to their protein targets in order to predict the affinity of the small molecules. The GOLD Score was calculated by defining the site using the list of atom numbers and retaining all the other default parameters. The 3D structure of AChe was retrieved from Protein Data Bank (PDB ID 1QTI) [23] with an X-ray resolution of $2A^O$. Docking poses were obtained by applying Gold score, fitness functions available for scoring. All the results reported in the present paper are referred to the ChemScore and GOLD fitness functions. These complexes were prepared for docking studies by adding hydrogen atoms, removing water molecules and co-crystallized inhibitors and refined by using the DeepView/SwissPdbViewer3.7(SP5)(Guex N, Peitsch MC).

Swiss Model and the Swiss Pdb-Viewer

DeepView/Swiss PdbViewer3.7 (SP5) Enzyme-inhibitor interactions within a radius equal to 10Å centered on reported bound inhibitors were taken into account. As a conclusive part of docking we expect, generated results should yield RMSD values below 1.5 Å. Successful docking has been performed for the selected set of 5, 6, 7, 8-tetrahydroacridine s inhibitors and their corresponding Chemscore with their RMSD have been produced in the Table 2.

Table 2
Energy, ChemScore and gold fitness values of the docked ligands.

Compound	Activity(IC_{50} in µm)	GOLD Software		ArgusLab(Energy Values)
		CHEMSCORE	FITNESS	
1	0.205	34.95	45.12	-10.2991
2	0.05	34.00	47.761	-10.3031
3	0.003	38.78	-1032.58	0
4	0.023	37.44	12.48	-10.221
5	13	34.07	-209.75	-10.9152
6	0.069	40.85	22.66	-10.3651
7	0.003	40.74	-179.78	0
8	0.023	26.84	-395.25	-10.9786
9	0.054	33.72	-19.94	0
10	1.9	28.28	4.54	0
11	0.004	35.76	49.74	-10.7032
12	0.002	35.59	42.63	-10.9478
13	0.04	36.10	47.67	-10.5404
14	0.005	39.47	31.76	-9.72585
15	0.078	33.99	45.36	-10.4386
16	0.087	34.13	40.23	-10.1563
17	3.8	33.96	48.19	-8.85014
18	0.1	34.75	-0.88	-10.6019
19	8.1	36.16	-45.75	-10.5482

4. RESULTS AND DISCUSSIONS

4.1. Simple linear regression model

The biological activity data and the physicochemical properties IPV, IP, EA, EI, EN, Hardness, Softness, LOGP, HE and POL of the 5, 6, 7, 8-tetrahydroacridine derivatives are given in Table 3. The data from this table was subjected to regression analysis [24]. The Correlation matrices were generated with 19 analogs (Table 4). The term close to 1 indicates high co-linearity, while the value below 0.5 indicates that no co-linearity exist between more than the two parameters. The perusal of correlation matrix (Table 5) indicates that Softness and HE are the predicted parameters from AM1 method. POL and HE were found to be explainable variable from regression methods backward, forward, removed and stepwise.

Predicted Activity = (0.178*HE) + (0.142*POL) ----------→ (1)
N=19; R=0.967; R^2 =0.936; AdjR^2=0.928; %EV = 93.6; SEE = 0.88969; F= 123.651; Q=1.0868;

In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. Eq.1 shows that the values of %EV are less and to improve its value, outliers were sought and eliminated. After the elimination of the outlier (2, 3, 5, 8, 11, 12, 17 and 19), a second model was developed. Overall, there is an increase in R and %EV (93.6-100) values, and a decrease in SEE (0.88969-0.05969).

Predicted Activity= (-1.287*EA) + (-0.662*Hardness) + (-6.688*Softness) + (0.994*EI) +
(-0.494*HE) (0.835*LOGP) + (0.049*POL) ----------→ (2)

N=11; R=1.000; R^2=0.999; AdjR^2=1.000; %EV=99.99; SEE=0.05969; F=4868.272; Q=16.7532; Eq.2 is an improved model since it explains the biological activity to the extent of 99.99%. In this way, the

predictive molecular descriptors EA, Hardness, Softness, EI, LOGP, POL and HE were considered as variables. In an attempt to investigate the predictive potential of proposed models, the cross-validation parameters (q^2_{cv} and PRESS) were calculated and used. The predictive power of the equations was confirmed by cross-validation method where, compounds are deleted one after another and prediction of the activity of the deleted compound is made based on QSAR model. The cross-validation evaluates the validity of a model by how well it predicts the data rather than how well it fits the data. The cross-validation parameter, q^2_{cv}, is mentioned in the respective equations (Table 6).

$$q^2_{cv} = \frac{(SD-PRESS)}{SD}$$

Where the PRESS (predictive residual sum of squares) and SD (standard deviation) values are obtained as

$$PRESS = \sum (property_{observed} - property_{predicted})^2.$$

$$SD = \sum (property_{observed} - property_{mean})^2.$$

The PRESS, SD, q^2_{cv} values for the nineteen 5, 6, 7, 8-tetrahydroacridine derivatives (AM1 method) is given by

PRESS=13.4453, SD=23.25385, q^2_{cv}=0.421803.

The PRESS, SD, q^2_{cv} values for the eleven 5, 6, 7, 8-tetrahydroacridine derivatives (AM1 method) is given by

PRESS=0.0157, SD=5.391964, q^2_{cv}=0.997088.

From the above observations, AM1 method gave a good q^2_{cv}values, which should be always smaller than %EV. A model is considered to be significant when q^2_{cv}>0.3.

Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that predicted when the compound is omitted from the fitting process, also supports the predictive ability of Eq.2. Its value decreases from Eq.1.

The quality factor Q, is defined as the ratio of regression constants (R) to the standard error estimation (SEE), i.e. Q = R/SEE. This indicates that the higher the value of R, and the lower the value of SEE, the higher is the magnitude of Q and the better will be the correlation. In present case, Q increases from 1.0868 to 16.7532 (Eq. 1 & 2).

4.2. *Docking Analysis*

The compounds were then docked in to protein active site using docking software [25]. The ChemScore and GOLD fitness of two docking software's are presented in Table 2. The binding energies obtained in Argus Lab ranged from -8.85 to -10.97kJ/mol. The results of CCDC GOLD can be analyzed both in terms of energy values ranging from 26.84 to 40.85 and -0.88 to 49.74. The docking simulation of the most active 5, 6, 7, 8-tetrahydroacridine derivatives 19 towards AChe (PDB ID 1QTI) showed that the enzyme–inhibitor complex was stabilized by hydrophobic interactions occurring between the aromatic moieties of the ligand and lipophilic residues of the binding site [26]. The compounds 4, 6, 7, 13 and 15 were oriented towards the hydrophobic region lined by TYR130, ASP72, ARG289, PHE290 and HIS440. Result of docking studies has proved that the molecule numbered 13 shows Chemscore, Gold fitness and RMSD values as 36.10, 47.67 and 1.5 Å respectively (Table2).The molecule 13 has been reported with appreciable IC_{50} values of 0.04μM. All the poses of molecule 13 (chosen as best in docking studies) and its interactions in the active pocket of AChe have been illustrated in Figure 4.

Table 3
Values obtained for the AM1 computational method.

Compound	IPV	EA	EN(μ)	Hardness(η)	Softness(S)	EI(ω)	ACT	HE	LOGP	POL(Aº3)
1	7.15	1.86	4.51	2.64	0.19	3.84	2.69	-3.06	1.93	23.84
2	7.8	1.21	4.51	3.29	0.15	3.08	3.3	-4.18	0.33	23.82
3	8.56	2.03	5.29	3.27	0.15	4.29	4.49	-6.21	0.85	34.35
4	8.02	1.38	4.7	3.32	0.15	3.32	3.63	-6.62	0.45	32.51
5	8.37	1.52	4.94	3.43	0.15	3.56	0.89	-8.86	0.48	25.17
6	8.08	1.32	4.7	3.38	0.15	3.27	3.16	-6.93	0	30.68
7	7.97	1.28	4.62	3.35	0.15	3.19	4.46	-5.11	2.04	39.86
8	11.46	4.51	7.99	3.48	0.14	9.17	3.63	-2.23	1.67	38.02
9	8.62	1.2	4.91	3.71	0.13	3.24	3.26	-7.01	0.05	28.84
10	9.29	1.11	5.2	4.09	0.12	3.3	1.72	-7.66	-1.79	22.19
11	11.03	4.5	7.76	3.26	0.15	9.22	4.35	-3.48	0.11	25.75
12	11.19	4.51	7.85	3.34	0.15	9.23	4.54	-3.83	0.11	25.75
13	11.18	4.54	7.86	3.32	0.15	9.3	3.4	-3.77	0.11	25.75
14	7.2	3.62	5.41	1.79	0.28	8.17	4.24	-1.85	1.71	30.71
15	11.29	4.67	7.98	3.31	0.15	9.62	3.11	-3.6	-0.27	23.73
16	11.33	4.62	7.98	3.36	0.15	9.48	3.06	-3.88	-0.27	23.73
17	11.04	4.69	7.86	3.18	0.16	9.73	1.42	-9.3	-1.39	25.17
18	10.99	4.26	7.63	3.37	0.15	8.64	3	-3	0.48	25.66
19	10.99	4.29	7.64	3.35	0.15	8.71	1.09	-2.92	0.48	25.66

IPV→Vertical Ionization Potential, *LOGP→Partition Coefficient,* ω→Electophilicity Index.

Table 4
Correlation matrix between the selected variables, by using AM1 method.

		IPV	EA	EN	η	S	EI	ACT	HE	LOGP	POL
IPV	Pearson Correlation	1	.846**	.963**	.358	-.394	.839**	-.105	.236	-.410	-.256
	Sig. (2-tailed)		.000	.000	.132	.095	.000	.668	.331	.081	.290
	N	19	19	19	19	19	19	19	19	19	19
EA	Pearson Correlation	.846**	1	.959**	-.195	.140	.997**	.046	.507*	-.115	-.215
	Sig. (2-tailed)	.000		.000	.425	.568	.000	.852	.027	.638	.377
	N	19	19	19	19	19	19	19	19	19	19
EN	Pearson Correlation	.963**	.959**	1	.092	-.139	.953**	-.033	.385	-.277	-.246
	Sig. (2-tailed)	.000	.000		.709	.571	.000	.894	.104	.252	.310
	N	19	19	19	19	19	19	19	19	19	19
η	Pearson Correlation	.358	-.195	.092	1	.970**	-.203	-.275	-.456*	-.551*	-.091
	Sig. (2-tailed)	.132	.425	.709		.000	.403	.255	.050	.015	.711
	N	19	19	19	19	19	19	19	19	19	19
S	Pearson Correlation	-.394	.140	-.139	-.970**	1	.164	.216	.398	.470*	.074
	Sig. (2-tailed)	.095	.568	.571	.000		.501	.374	.091	.042	.765
	N	19	19	19	19	19	19	19	19	19	19
EI	Pearson Correlation	.839**	.997**	.953**	-.203	.164	1	.045	.496*	-.143	-.221
	Sig. (2-tailed)	.000	.000	.000	.403	.501		.855	.031	.559	.363
	N	19	19	19	19	19	19	19	19	19	19
ACT	Pearson Correlation	-.105	.046	-.033	-.275	.216	.045	1	.440	.449	.523*
	Sig. (2-tailed)	.668	.852	.894	.255	.374	.855		.060	.054	.022
	N	19	19	19	19	19	19	19	19	19	19
HE	Pearson Correlation	.236	.507*	.385	-.456*	.398	.496*	.440	1	.543*	.054
	Sig. (2-tailed)	.331	.027	.104	.050	.091	.031	.060		.016	.826
	N	19	19	19	19	19	19	19	19	19	19
LOGPPP	Pearson Correlation	-.410	-.115	-.277	-.551*	.470*	-.143	.449	.543*	1	.615**
	Sig. (2-tailed)	.081	.638	.252	.015	.042	.559	.054	.016		.005
	N	19	19	19	19	19	19	19	19	19	19
POL	Pearson Correlation	-.256	-.215	-.246	-.091	.074	-.221	.523*	.054	.615**	1
	Sig. (2-tailed)	.290	.377	.310	.711	.765	.363	.022	.826	.005	
	N	19	19	19	19	19	19	19	19	19	19

IPV→Vertical Ionization Potential, *LOGP→Partition Coefficient,* ω→Electophilicity Index.

Table 5
Correlation matrix between the selected variables, by using AM1 method.

		IPV	EA	EN	η	S	EI	ACT	HE	LOGP	POL
IPV	Pearson Correlation	1	.744**	.938*	.434	.456	.735**	.294	.185	-.554	.535
	Sig. (2-tailed)		.009	.000	.183	.159	.010	.380	.587	.077	.090
	N	11	11	11	11	11	11	11	11	11	11
EA	Pearson Correlation	.744	1	.930*	-.279	.236	.996**	.081	.751	-.052	.443
	Sig. (2-tailed)	.009		.000	.406	.484	.000	.813	.008	.880	.173
	N	11	11	11	11	11	11	11	11	11	11
EN	Pearson Correlation	.938	.930**	1	.094	.129	.922**	.121	.492	-.333	.526
	Sig. (2-tailed)	.000	.000		.783	.706	.000	.723	.124	.317	.097
	N	11	11	11	11	11	11	11	11	11	11
H	Pearson Correlation	.434	-.279	.094	1	.974	-.286	.530	.748	-.726*	.169
	Sig. (2-tailed)	.183	.406	.783		.000	.393	.094	.008	.011	.619
	N	11	11	11	11	11	11	11	11	11	11
S	Pearson Correlation	.456	.236	-.129	.974*	1	.263	.482	.680	.624*	.155
	Sig. (2-tailed)	.159	.484	.706	.000		.435	.133	.021	.040	.649
	N	11	11	11	11	11	11	11	11	11	11
EI	Pearson Correlation	.735	.996**	.922*	-.286	.263	1	.089	.736	-.081	.436
	Sig. (2-tailed)	.010	.000	.000	.393	.435		.795	.010	.814	.180
	N	11	11	11	11	11	11	11	11	11	11
ACT	Pearson Correlation	.294	.081	-.121	-.530	.482	.089	1	.329	.720*	.816
	Sig. (2-tailed)	.380	.813	.723	.094	.133	.795		.324	.012	.002
	N	11	11	11	11	11	11	11	11	11	11
HE	Pearson Correlation	.185	.751**	.492	.748*	.680	.736**	.329	1	.545	.170
	Sig. (2-tailed)	.587	.008	.124	.008	.021	.010	.324		.083	.617
	N	11	11	11	11	11	11	11	11	11	11
LOGP	Pearson Correlation	.554	-.052	-.333	.726*	.624	-.081	.720	.545	1	.580
	Sig. (2-tailed)	.077	.880	.317	.011	.040	.814	.012	.083		.061
	N	11	11	11	11	11	11	11	11	11	11
POL	Pearson Correlation	.535	-.443	-.526	-.169	.155	-.436	.816	.170	.580	1
	Sig. (2-tailed)	.090	.173	.097	.619	.649	.180	.002	.617	.061	
	N	11	11	11	11	11	11	11	11	11	11

IPV→Vertical Ionization Potential, *LOGP→Partition Coefficient,* ω→Electophilicity Index.

Table 6
Observed Activity and Predicted Activity values of 5, 6, 7, 8-tetrahydroacridine derivatives by using AM1 equations.

Compound	Observed Activity	Equation(1)		Equation (2)	
		Predicted	Residual	Predicted	Residual
1	2.69	2.84	0.15	2.7	0.01
2	3.3	2.64	-0.66	-	-
3	4.49	3.77	-0.72	-	-
4	3.63	3.44	-0.19	3.56	-0.07
5	0.89	2	1.11	-	-
6	3.16	3.12	-0.04	3.24	0.08
7	4.46	4.75	0.29	4.48	0.02
8	3.63	5	1.37	-	-
9	3.26	2.85	-0.41	3.27	0.01
10	1.72	1.79	0.07	1.72	0
11	4.35	3.04	-1.31	-	-
12	4.54	2.97	-1.57	-	-
13	3.4	2.99	-0.41	3.42	0.02
14	4.24	4.03	-0.21	4.25	0.01
15	3.11	2.73	-0.38	3.07	-0.04
16	3.06	2.68	-0.38	3.1	0.04
17	1.42	1.92	0.5	-	-
18	3	3.11	0.11	3.01	0.01
19	1.09	3.12	2.03	-	-

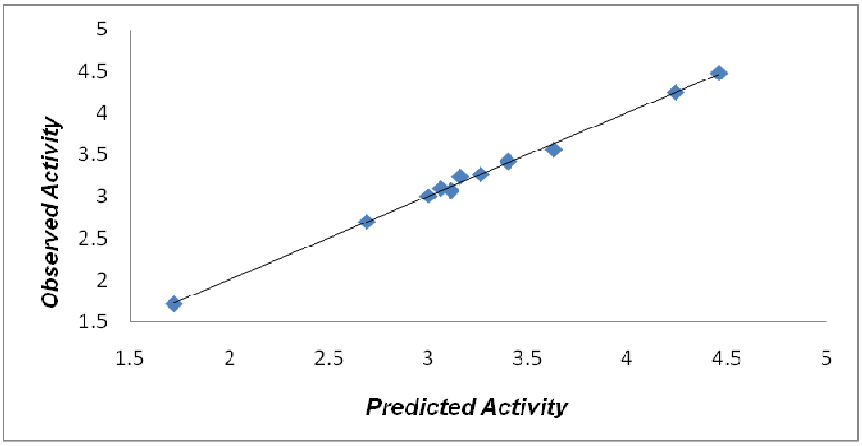


Figure 3
Plot of Observed Verses Predicted activity (AM1 Method)

The most active compounds docked successfully into the active site of the inhibited enzyme. Inhibitory activity of the most potent compounds was explained mostly by hydrophobic interactions. The compounds 1, 4, 6, 7, 9, 10, 13, 14, 15, 16 and 18 were found to present high antibacterial activity and significant inhibitory activity on AChe. The information rendered by QSAR models and the docking interactions may afford valuable clues to optimize the lead and design of new potential inhibitors. The order of the more effective and the higher activity of the remaining eleven 5, 6, 7, 8-tetrahydroacridine compounds 13, 15, 1, 16, 14, 6, 4, 10, 18, 9 and 7. Best pose of the more effective and the higher activity of the 5, 6, 7, 8-tetrahydroacridine compound 13 is as follows.

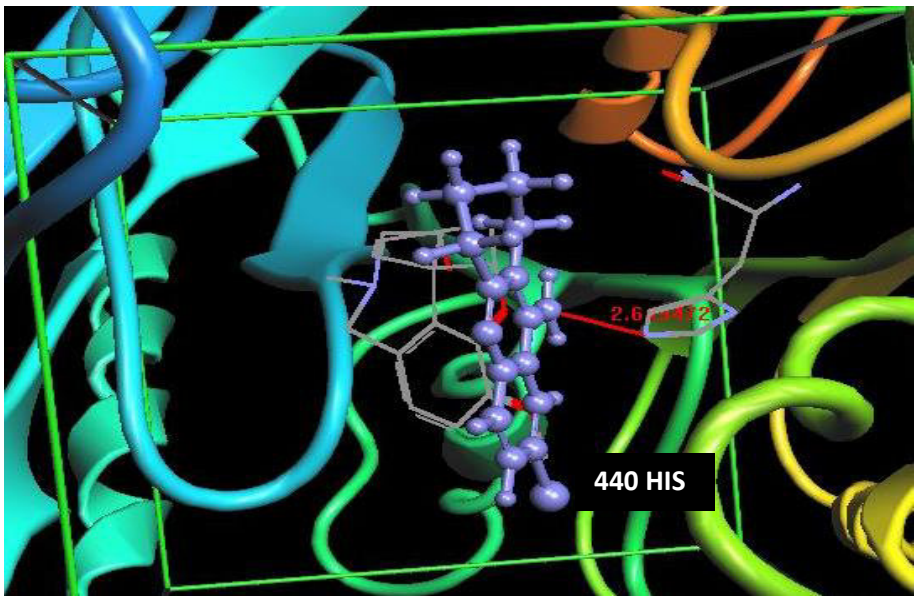


Figure 4
Best pose of molecule 13 and secondary structure of AChe (PDB ID 1QTI).

Table 7
Values obtained for the PM3 computational method.

Compound	IPV	EA	EN(μ)	Hardness(η)	Softness(S)	EI(ω)	ACT	HE	LOGP	POL(Aº3)
1	7.42	2.09	4.75	2.66	0.19	4.23	2.69	-3.2	1.93	23.84
2	7.97	1.33	4.65	3.32	0.15	3.26	3.3	-4.29	0.33	23.82
3	8.49	2.3	5.39	3.1	0.16	4.7	4.49	-6.37	0.85	34.35
4	8.26	1.53	4.89	3.36	0.15	3.56	3.63	-6.94	0.45	32.51
5	8.31	1.62	4.97	3.35	0.15	3.68	0.89	-8.76	0.48	25.17
6	8.13	1.48	4.8	3.33	0.15	3.47	3.16	-7.25	0	30.68
7	8.19	1.43	4.81	3.38	0.15	3.42	4.46	-5.28	2.04	39.86
8	10.92	4.15	7.54	3.39	0.15	8.39	3.63	-2.33	1.67	38.02
9	8.6	1.64	5.12	3.48	0.14	3.76	3.26	-7.25	0.05	28.84
10	5.51	1.71	3.61	1.9	0.26	3.42	1.72	-8.52	-1.79	22.19
11	10.39	4.01	7.2	3.19	0.16	8.12	4.35	-3.47	0.11	25.75
12	10.49	4.16	7.32	3.17	0.16	8.47	4.54	-3.97	0.11	25.75
13	10.47	4.16	7.32	3.16	0.16	8.48	3.4	-3.91	0.11	25.75
14	7.25	3.6	5.43	1.82	0.27	8.08	4.24	-1.85	1.71	30.71
15	10.52	4.18	7.35	3.17	0.16	8.51	3.11	-3.76	-0.27	23.73
16	10.65	4.26	7.45	3.19	0.16	8.7	3.06	-4.02	-0.27	23.73
17	10.5	4.48	7.49	3.01	0.17	9.32	1.42	-9.53	-1.39	25.17
18	10.38	3.96	7.17	3.21	0.16	8	3	-3.14	0.48	25.66
19	10.37	3.97	7.17	3.2	0.16	8.03	1.09	-3.06	0.48	25.66

IPV→Vertical Ionization Potential, *LOGP→Partition Coefficient,* ω→Electophilicity Index.

Table 8
Correlation matrix between the selected variables, by using PM3 method.

		IPV	EA	EN	η	S	EI	ACT	HE	LOGP	POL
IPV	Pearson Correlation	1	.799	.958**	.568	.603**	.792**	.073	.364	-.055	-.037
	Sig. (2-tailed)		.000	.000	.011	.006	.000	.767	.126	.822	.881
	N	19	19	19	19	19	19	19	19	19	19
EA	Pearson Correlation	.799**	1	.938**	.040	-.008	.996**	.027	.493*	-.145	-.226
	Sig. (2-tailed)	.000		.000	.871	.973	.000	.912	.032	.554	.352
	N	19	19	19	19	19	19	19	19	19	19
EN	Pearson Correlation	.958**	.938	1	.309	-.352	.932**	.055	.445	-.101	-.129
	Sig. (2-tailed)	.000	.000		.197	.139	.000	.823	.056	.681	.599
	N	19	19	19	19	19	19	19	19	19	19
H	Pearson Correlation	.568*	.040	.309	1	.991**	-.047	.084	-.070	.107	.249
	Sig. (2-tailed)	.011	.871	.197		.000	.850	.733	.776	.664	.305
	N	19	19	19	19	19	19	19	19	19	19
S	Pearson Correlation	.603**	.008	-.352	.991	1	.005	-.061	.056	-.092	-.188
	Sig. (2-tailed)	.006	.973	.139	.000		.985	.805	.819	.708	.440
	N	19	19	19	19	19	19	19	19	19	19
EI	Pearson Correlation	.792**	.996	.932**	.047	.005	1	.045	.496*	-.125	-.204
	Sig. (2-tailed)	.000	.000	.000	.850	.985		.854	.031	.610	.403
	N	19	19	19	19	19	19	19	19	19	19

ACT	Pearson Correlation	.073	.027	.055	.084	-.061	.045	1	.441	.449	.523*
	Sig. (2-tailed)	.767	.912	.823	.733	.805	.854		.059	.054	.022
	N	19	19	19	19	19	19	19	19	19	19
HE	Pearson Correlation	.364	.493	.445	.070	.056	.496*	.441	1	.571*	.063
	Sig. (2-tailed)	.126	.032	.056	.776	.819	.031	.059		.011	.798
	N	19	19	19	19	19	19	19	19	19	19
LOGP	Pearson Correlation	-.055	.145	-.101	.107	-.092	-.125	.449	.571*	1	.615**
	Sig. (2-tailed)	.822	.554	.681	.664	.708	.610	.054	.011		.005
	N	19	19	19	19	19	19	19	19	19	19
POL	Pearson Correlation	-.037	.226	-.129	.249	-.188	-.204	.523*	.063	.615**	1
	Sig. (2-tailed)	.881	.352	.599	.305	.440	.403	.022	.798	.005	
	N	19	19	19	19	19	19	19	19	19	19

IPV→Vertical Ionization Potential, *LOGP→Partition Coefficient,* ω→Electophilicity Index.

Table 9

Correlation matrix between the selected variables, by using PM3 method.

		IPV	EA	EN	η	S	EI	ACT	HE	LOGP	POL
IPV	Pearson Correlation	1	.711	.945	.651	-.674	.703	.216	.446	-.020	-.157
	Sig. (2-tailed)		.014	.000	.030	.023	.016	.523	.169	.953	.644
	N	11	11	11	11	11	11	11	11	11	11
EA	Pearson Correlation	.711	1	.902	-.072	.034	.994	.030	.741	-.085	-.495
	Sig. (2-tailed)	.014		.000	.834	.921	.000	.931	.009	.804	.121
	N	11	11	11	11	11	11	11	11	11	11
EN	Pearson Correlation	.945	.902	1	.365	-.397	.895	.146	.619	-.052	-.328
	Sig. (2-tailed)	.000	.000		.269	.227	.000	.667	.042	.879	.325
	N	11	11	11	11	11	11	11	11	11	11
η	Pearson Correlation	.651	-.072	.365	1	-.992	-.077	.275	-.167	.063	.312
	Sig. (2-tailed)	.030	.834	.269		.000	.823	.414	.623	.854	.351
	N	11	11	11	11	11	11	11	11	11	11
S	Pearson Correlation	.674	.034	-.397	-.992	1	.048	-.220	.116	-.077	-.240
	Sig. (2-tailed)	.023	.921	.227	.000		.889	.515	.735	.822	.477
	N	11	11	11	11	11	11	11	11	11	11
EI	Pearson Correlation	.703	.994	.895	-.077	.048	1	.118	.762	-.033	-.424
	Sig. (2-tailed)	.016	.000	.000	.823	.889		.729	.006	.923	.194
	N	11	11	11	11	11	11	11	11	11	11
ACT	Pearson Correlation	.216	.030	.146	.275	-.220	.118	1	.374	.720	.816
	Sig. (2-tailed)	.523	.931	.667	.414	.515	.729		.257	.012	.002
	N	11	11	11	11	11	11	11	11	11	11
HE	Pearson Correlation	.446	.741	.619	-.167	.116	.762	.374	1	.575	-.131
	Sig. (2-tailed)	.169	.009	.042	.623	.735	.006	.257		.064	.702
	N	11	11	11	11	11	11	11	11	11	11
LOGP	Pearson Correlation	.020	-.085	-.052	.063	-.077	-.033	.720	.575	1	.580
	Sig. (2-tailed)	.953	.804	.879	.854	.822	.923	.012	.064		.061
	N	11	11	11	11	11	11	11	11	11	11
POL	Pearson Correlation	.157	-.495	-.328	.312	-.240	-.424	.816	-.131	.580	1
	Sig. (2-tailed)	.644	.121	.325	.351	.477	.194	.002	.702	.061	
	N	11	11	11	11	11	11	11	11	11	11

IPV→Vertical Ionization Potential, *LOGP→Partition Coefficient,* ω→Electophilicity Index.

Table 10
Observed Activity and Predicted Activity values of 5, 6, 7, 8-tetrahydroacridine derivatives by using PM3 Equations.

Compound	Observed Activity	Equation(3)		Equation(4)	
		Predicted	Residual	Predicted	Residual
1	2.69	2.84	0.15	2.66	-0.03
2	3.3	2.65	-0.65	-	-
3	4.49	3.79	-0.7	-	-
4	3.63	3.44	-0.19	3.55	-0.08
5	0.89	2.08	1.19	-	-
6	3.16	3.12	-0.04	3.32	0.16
7	4.46	4.76	0.3	4.45	-0.01
8	3.63	5	1.37	-	-
9	3.26	2.86	-0.4	3.2	-0.06
10	1.72	1.7	-0.02	1.72	0
11	4.35	3.07	-1.28	-	-
12	4.54	2.98	-1.56	-	-
13	3.4	2.99	-0.41	3.27	-0.13
14	4.24	4.05	-0.19	4.25	0.01
15	3.11	2.73	-0.38	3.04	-0.07
16	3.06	2.69	-0.37	3.08	0.02
17	1.42	1.95	0.53	-	-
18	3	3.11	0.11	3.19	0.19
19	1.09	3.12	2.03	-	-

From the correlation matrix table, it reveals POL and HE are found to be explainable variables from regression methods backward, forward, removed and stepwise. A diparametric QSAR equation with POL and HE was generated in PM3 method also.

Predicted Activity = (0.170*HE) + (0.142*POL) ----------→ (3)
N=19; R=0.967; R^2=0.935; AdjR^2=0.928; %EV=93.5; SEE =0.89183; F=123.016; Q=1.0842;

Eq.3 shows that the values of %EV is less and to improve its value, outliers were sought and eliminated, In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. After the elimination of the outlier (2, 3, 5, 8, 11, 12, 17 and 19), a second model was developed.

Predicted Activity = (-1.612*EA) + (-1.700*Softness) + (0.939*EI) + (0.140*LOGP) +
(0.088*POL) ---------- →(4)

N=11; R=1.000; R^2 =0.999; AdjR^2=0.999; %EV = 99.9; SEE=0.12754; F=1492.084; Q=7.8406;

The PRESS, SD, q^2_{cv} values for the nineteen 5, 6, 7, 8-tetrahydroacridine derivatives (PM3 method) is given by

PRESS=13.4875, SD=23.25385, q^2_{cv}=0.419989.

The PRESS, SD, q^2_{cv} values for the eleven 5, 6, 7, 8-tetrahydroacridine derivatives (PM3 method) is given by

PRESS=0.095, SD=5.391964, q^2_{cv}=0.982381.

From the above observations PM3 method given a good q^2_{cv} value i.e. q^2_{cv}>0.3 and Q value increases from 1.0842 to 7.8406 **(**Eqs. 3 & 4).

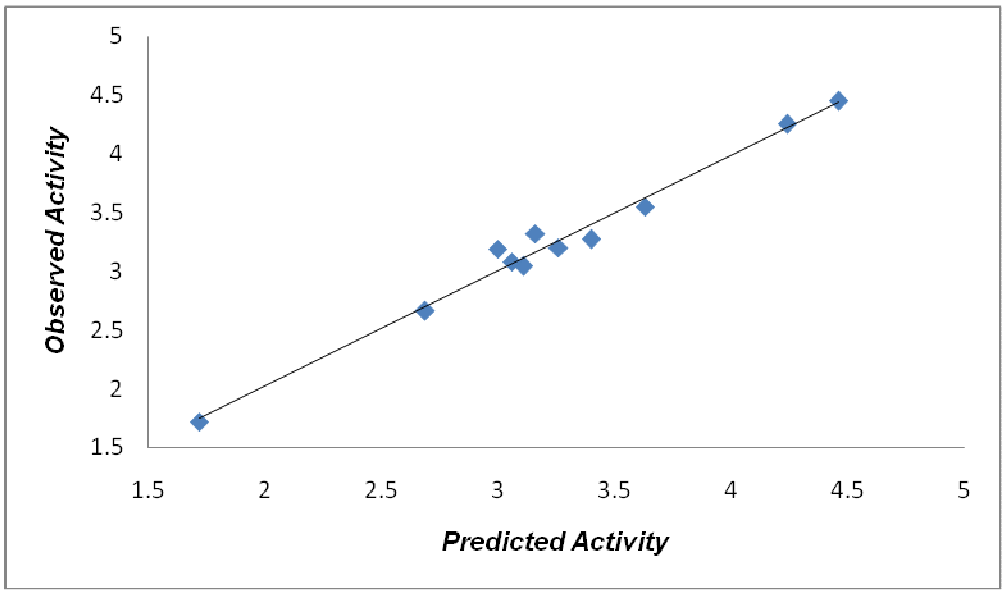


Figure 5
Plot of Observed Verses Predicted activity (PM3 Method).

Best pose of the more effective and the higher activity of the remaining ten 5, 6, 7, 8-tetrahydroacridine compounds 15, 1, 16, 14, 6, 4, 10, 18, 9 and 7 is as follows.

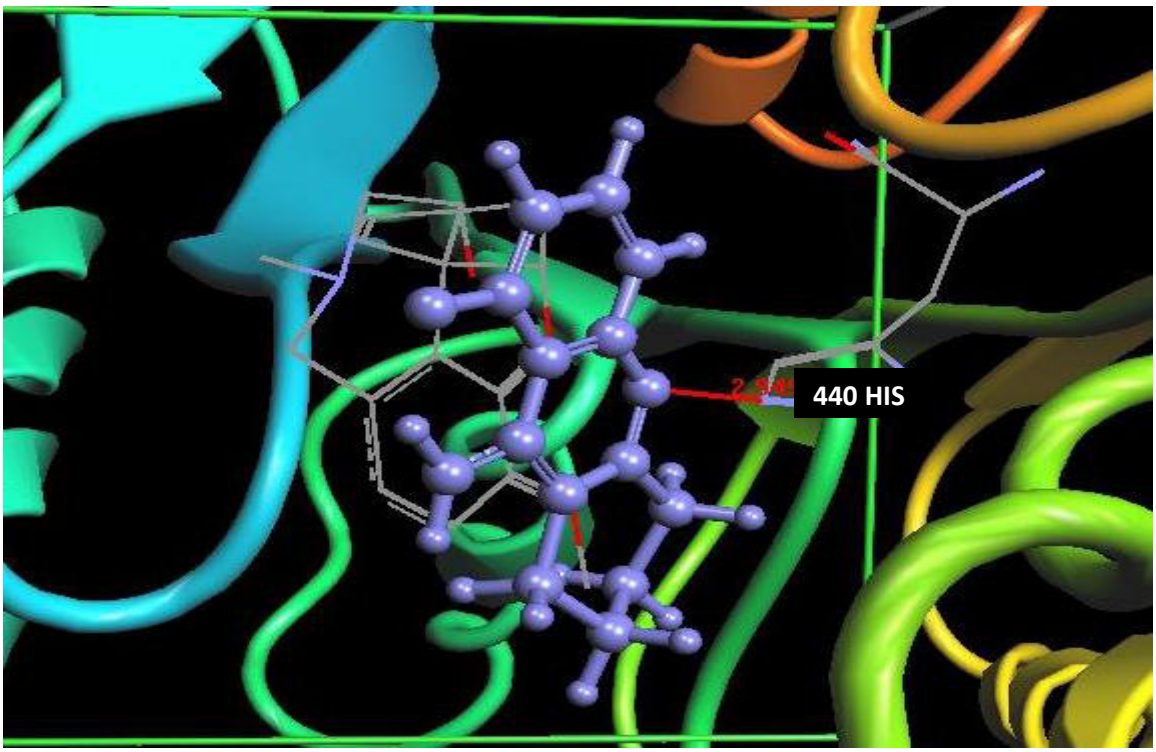


Figure 6
Best pose of molecule 15 and secondary structure of AChe (PDB ID 1QTI).

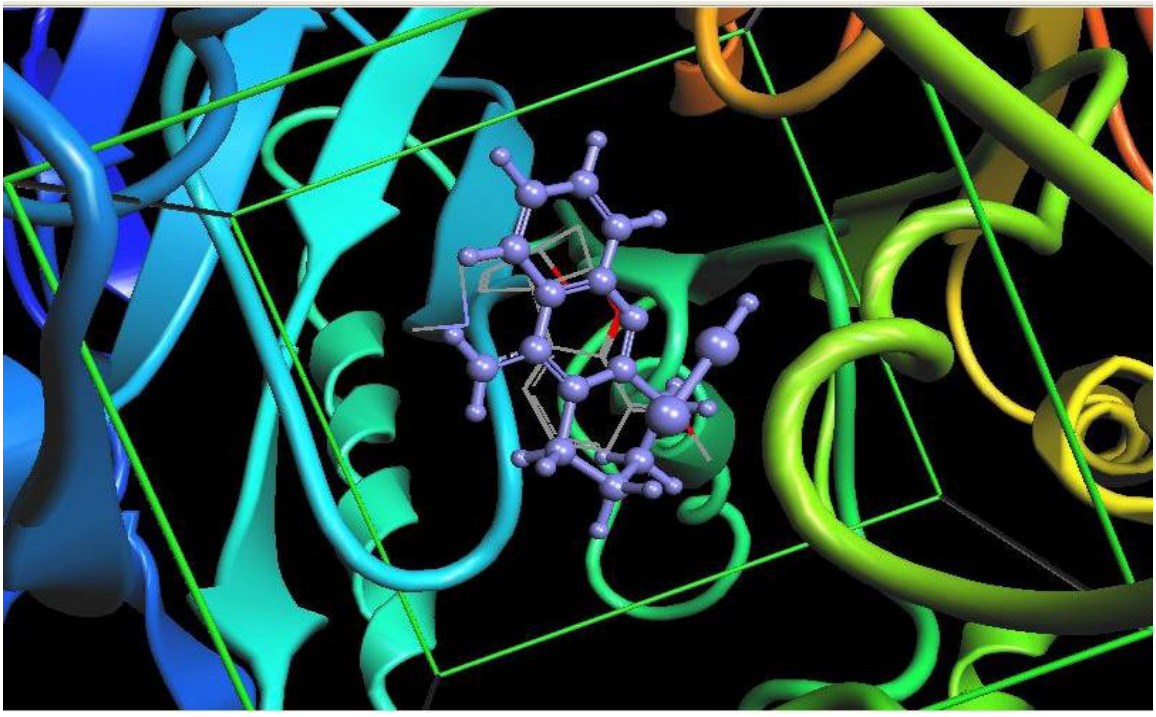


Figure 7
Best pose of molecule 1 and secondary structure of AChe (PDB ID 1QTI).

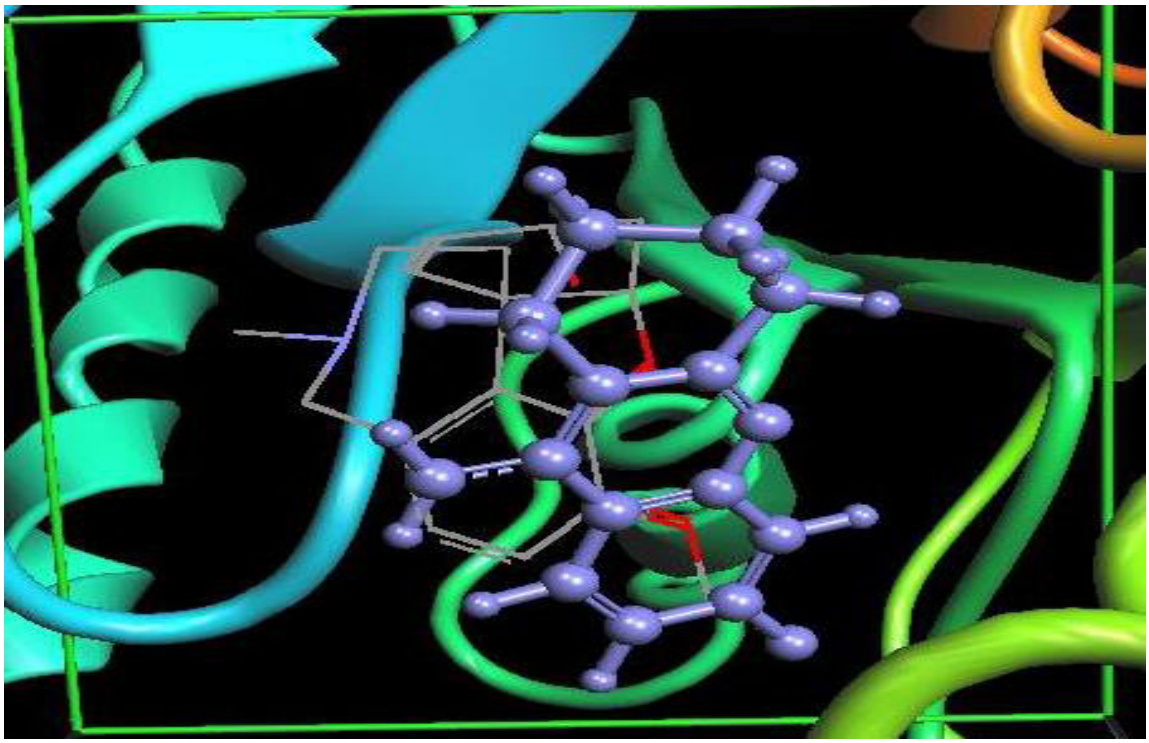


Figure 8
Best pose of molecule 16 and secondary structure of AChe (PDB ID 1QTI).

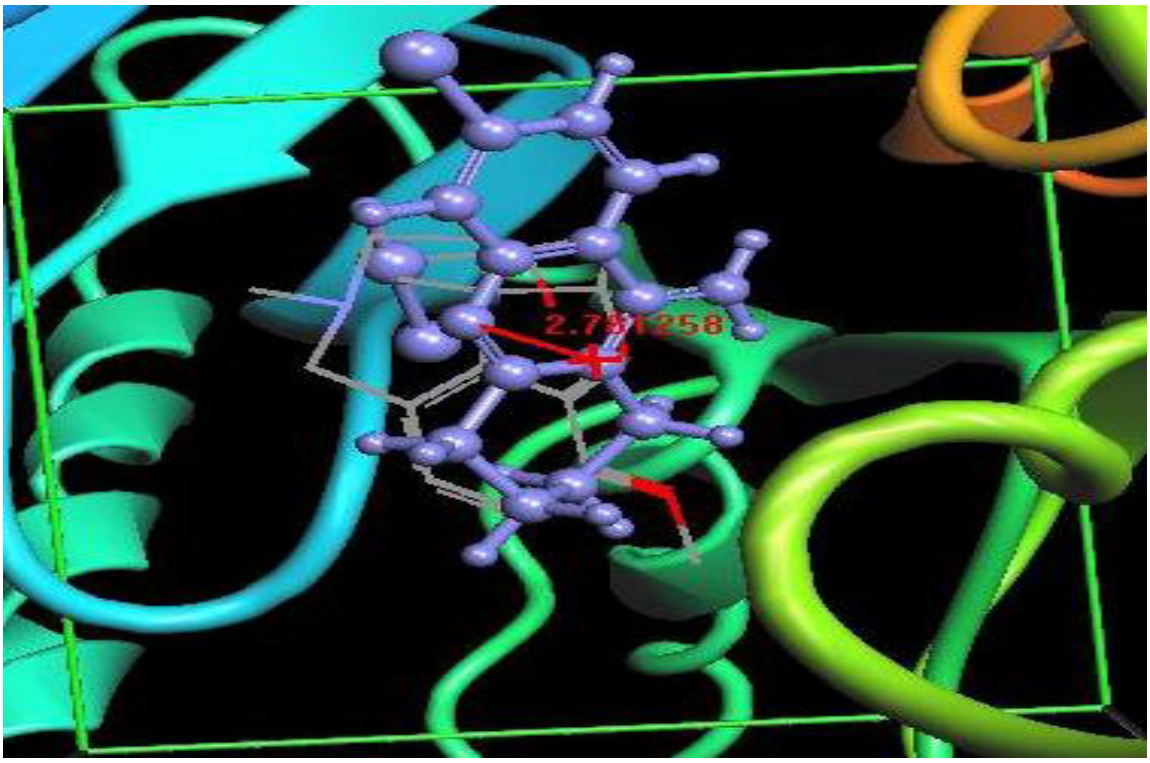


Figure 9
Best pose of molecule 14 and secondary structure of AChe (PDB ID 1QTI).

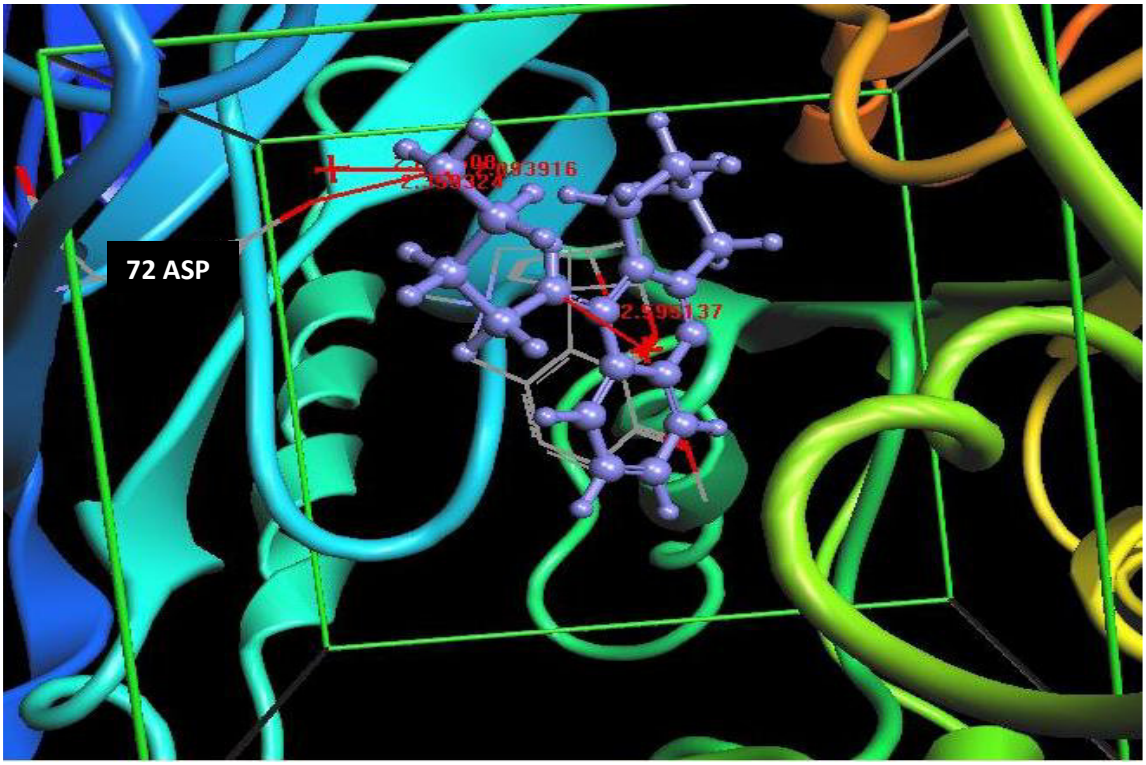


Figure 10
Best pose of molecule 6 and secondary structure of AChe (PDB ID 1QTI).

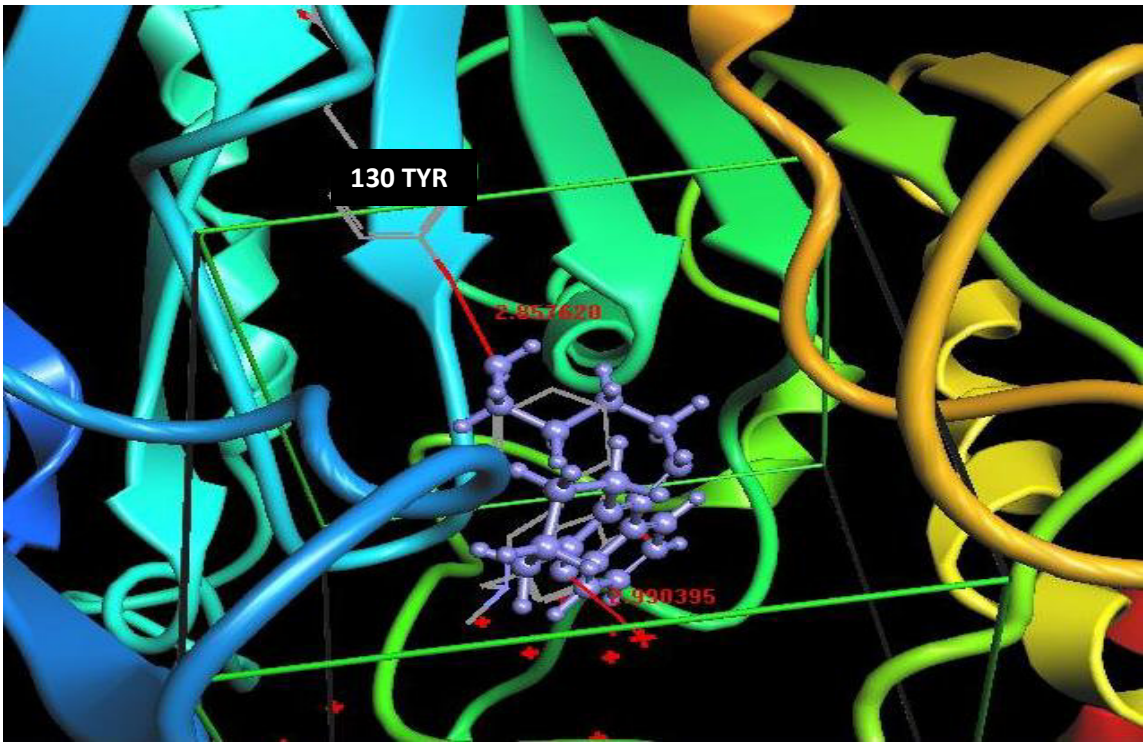


Figure 11
Best pose of molecule 4 and secondary structure of AChe (PDB ID 1QTI).

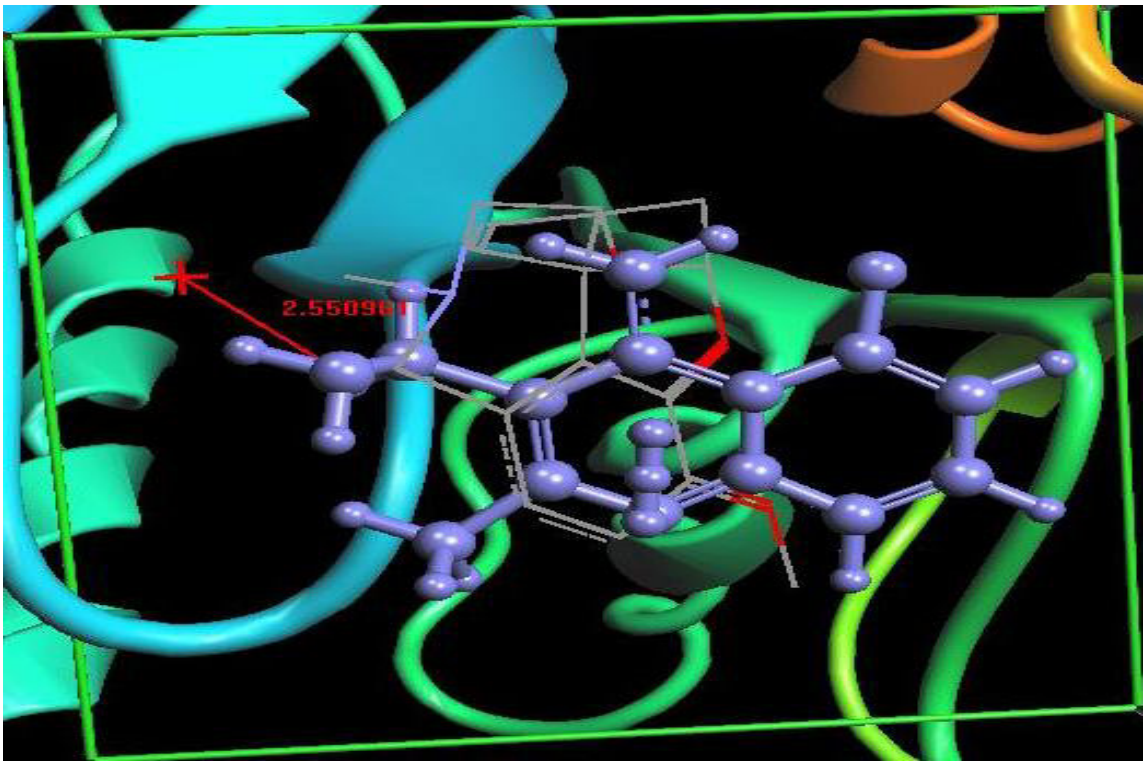


Figure 12
Best pose of molecule 10 and secondary structure of AChe (PDB ID 1QTI).

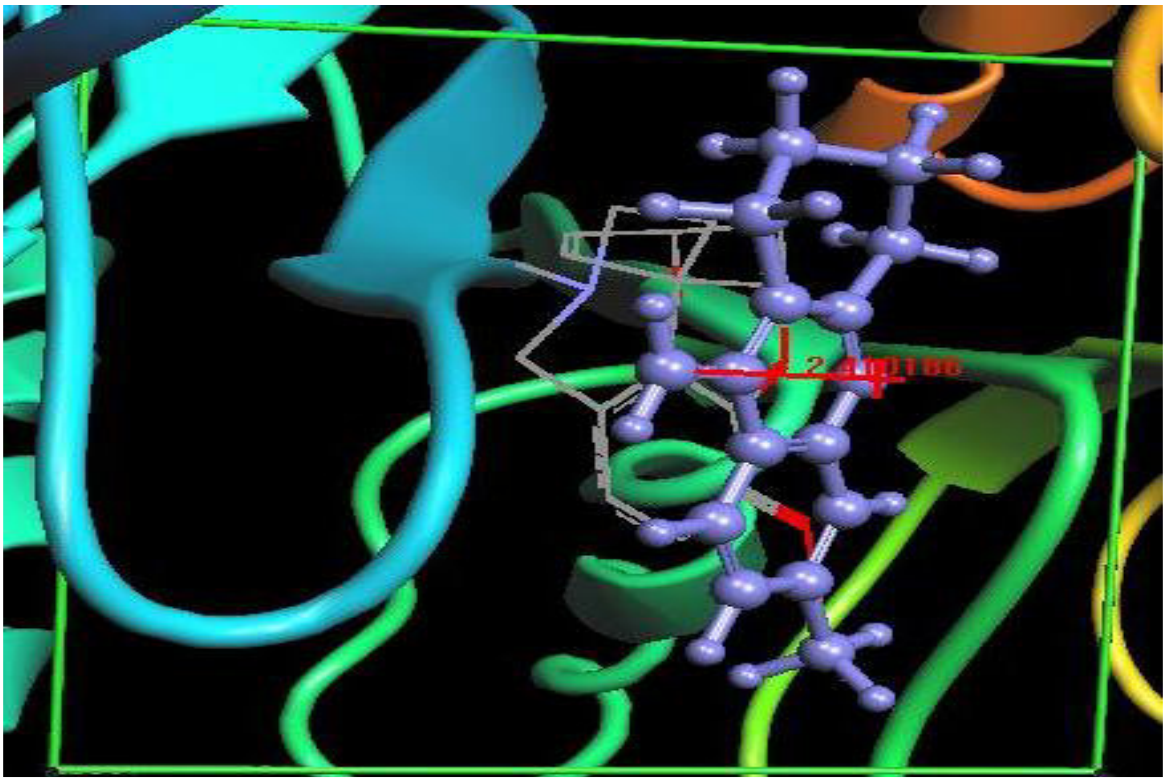


Figure 13
Best pose of molecule 18 and secondary structure of AChe (PDB ID 1QTI).

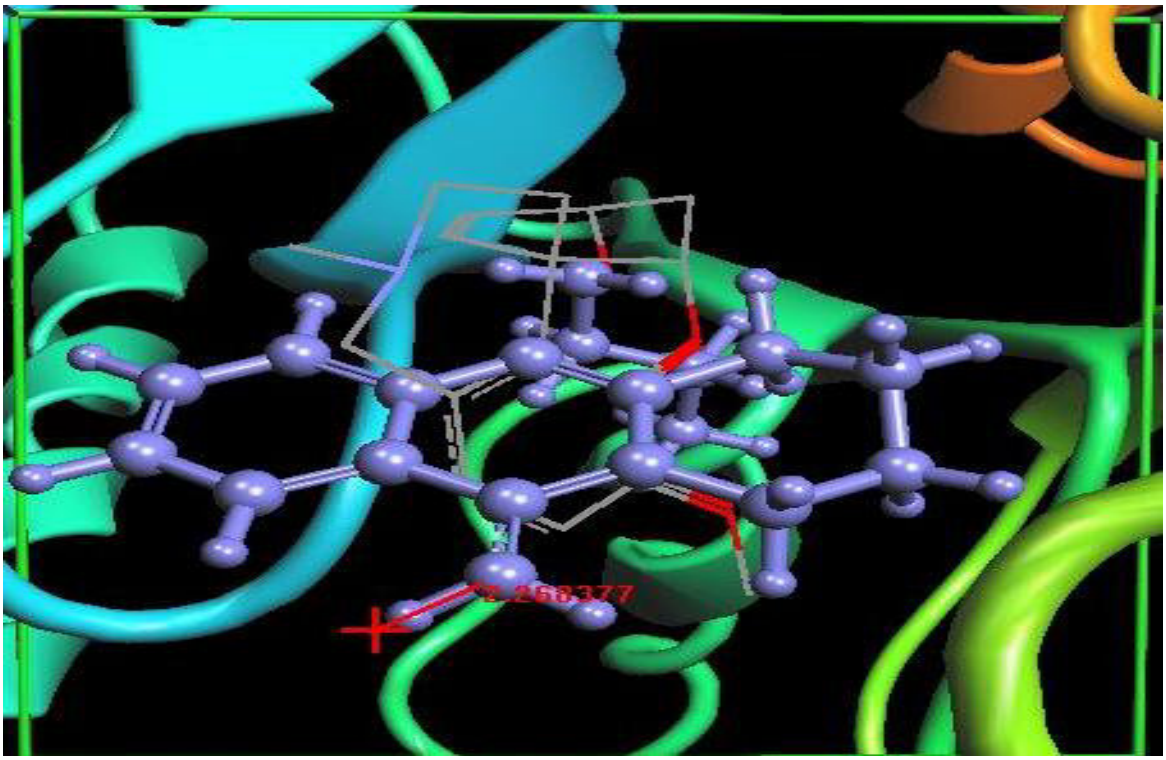


Figure 14
Best pose of molecule 9 and secondary structure of AChe (PDB ID 1QTI).

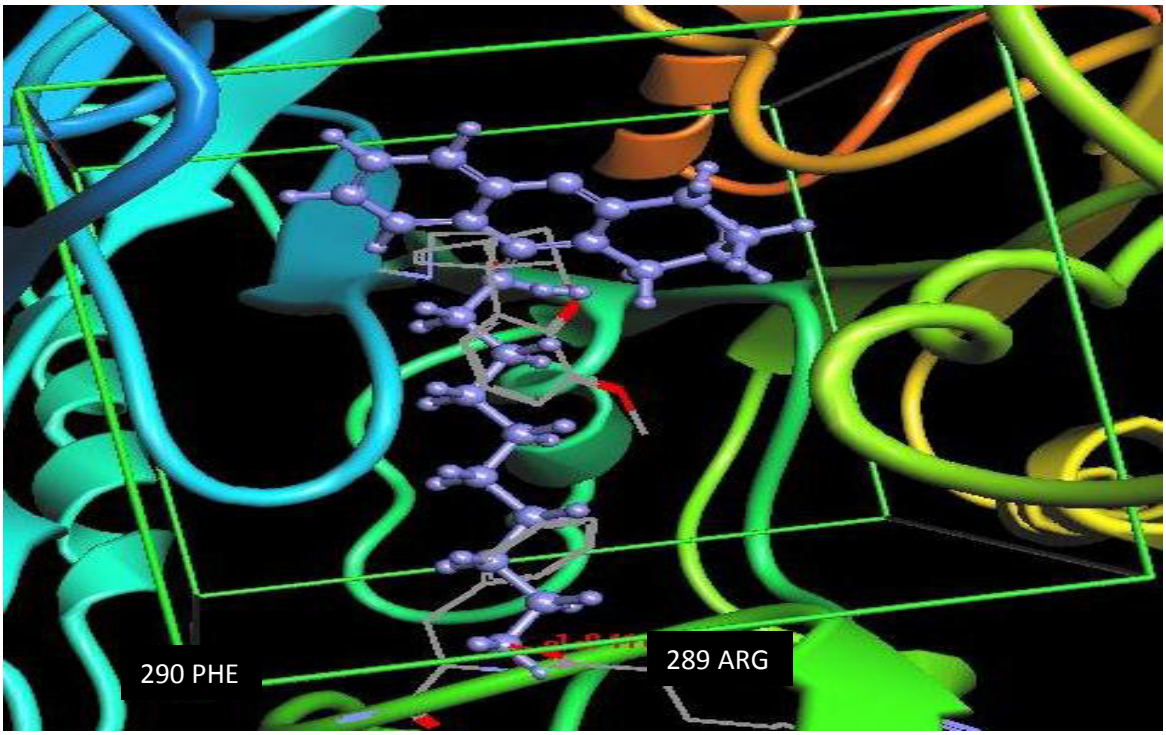


Figure 15
Best pose of molecule 7 and secondary structure of AChe (PDB ID 1QTI).

5. CONCLUSION

Quantitative structure–activity relationship studies (QSAR) and molecular docking were performed on nineteen 5, 6, 7, 8-tetrahydroacridine AChe inhibitors to find out the structural relationship with the activity. The best predictive AM1 model resulted in cross-validated R^2 value of 1.000, AdjR^2 value of 1.000 and standard error of estimate 0.05969 (AM_1). Similarly the best predictive PM3 model was derived with R^2 of 0.999, AdjR^2 of 0.999 and standard error of estimate of 0.12754, comprising softness, hydration energy, hydrophobic and hydrogen bond donor fields. These models were able to predict the activity of test set molecules efficiently (best molecules 1, 4, 6, 7, 9, 10, 13, 14, 15, 16 and 18) within an acceptable error range. GOLD and Argus lab docking software were employed to dock the inhibitors into the active site of AChe and these docking studies revealed the vital interactions and binding conformation of the inhibitors. Therefore, the present study showed that the QSAR studies and the docking approach of 5, 6, 7, 8-tetrahydroacridine derivatives as AChe inhibitors can be successfully modeled using the parametric equations. The Eq.2 from AM1, semi empirical calculations reveal EA, Hardness, Softness, EI, LOGP, POL and HE cause the inhibitory activity. Higher values of EA, Hardness, Softness, EI, LOGP, POL and HE were responsible for higher inhibitory activity nature for AChe enzyme. The linear dependence of inhibitory nature on Softness and HE were evident from Figure 3.

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