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RESEARCH ARTICLE

Common SAR Derived from Linear and Non-linear QSAR Studies on AChE Inhibitors used in the Treatment of Alzheimer's Disease

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Abstract: ***Background*:** Deficits in cholinergic neurotransmission due to the degeneration of cholinergic neurons in the brain are believed to be one of the major causes of the memory impairments associated with AD. Targeting acetyl cholinesterase (AChE) surfaced as a potential therapeutic target in the treatment of Alzheimer's disease. The present study is pursued to develop quantitative structure activity relationship (QSAR) models to determine chemical descriptors responsible for AChE activity.

***Methods*:** Two different sets of AChE inhibitors, dataset-I (30 compounds) and dataset-II (20 compounds) were investigated through MLR aided linear and SVM aided non-linear QSAR models.

***Results*:** The obtained QSAR models were found statistically fit, stable and predictive on validation scales. These QSAR models were further investigated for their common structure-activity relationship in terms of overlapping molecular descriptors selection. Atomic mass weighted 3D Morse descriptors (MATS5m) and Radial Distribution Function (RDF045m) descriptors were found in common SAR for both the datasets. Electronegativity weighted (MATS5e, HATSe, and Mor17e) descriptors have also been identified in regulative roles towards endpoint values of dataset-I and dataset-II.

***Conclusion*:** The common SAR identified in these linear and non-linear QSAR models could be utilized to design novel inhibitors of AChE with improved biological activity.

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1. INTRODUCTION

Alzheimer's disease (AD) is one of the most dreaded forms of progressive neurodegenerative disorder of the modern era. It is associated with cognitive, functional and behavioral impairments which affect the brain regions that control thought, memory and language leading to a devastating status affecting predominantly elderly people. Alzheimer's disease (AD) and other forms of dementia are growing public health problems in developing countries, whose aging population is increasing rapidly [1]. It is estimated that by the year 2020, approximately 70% of the world's population aged 60 and above will suffer with Alzheimer's in developing countries [2-5].

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However, the precise cause of AD at the molecular grounds remains enigmatic, deficits in cholinergic neurotransmission due to the degeneration of cholinergic neurons in the brain are believed to be one of the major causes of the memory impairments associated with AD [6-8]. During the course of the normal aging process, concentrations of ACh tend to decrease, resulting in the sporadic lapses of short-term memory that elderly individuals tend to experience from time to time [9]. Since cholinergic transmission is prominently involved in human memory system, AChE is a potential pharmacological target for treatment of AD.

Although there is no complete cure for Alzheimer's, there are two drugs presently marketed which inhibit acetyl cholinesterase - the enzyme which inactivates AChE at the synapse. Tetrohydroaminoacridine (THA), marketed in 1993, under the name Tacrine or Cognex, was the first Alzheimer's drug to be approved by the USFDA and has been shown to improve memory and language deficits in early stages of the disease [10]. A second drug, Donepezil was approved by the FDA in 1996, marketed under the name Aricept that targets

only ACh in the brain and allows for the same improvements in cognitive function as THA [11]. Although these drugs helps to ease some of the symptoms of the disease, nevertheless have narrow therapeutic window and suffer side effects like lack of the drug's substrate specificity [12-14]. In the view of the given problem, the scientific team around the globe put forth active compounds targeting AchE anticipating for better management of Alzheimer's disease.

The present study focuses on building linear MLR and non-linear Gaussian kernel aided SVM QSAR models from two datasets which structurally belong to different scaffold and are derived from two different schemes of synthesis. The synthesis of derivatives was aimed to explore new candidates which can inhibit AChE more efficiently towards AD treatment. Few molecules among newly synthesized derivatives appeared promising when evaluated *in vitro* and effectively inhibited AChE. We aim to derive common structure-activity relationship from two different datasets through QSAR models which could assist to understand the overlapping structural features towards minimum chemical structure requirement to inhibit AChE in AD treatment.

2. MATERIALS AND METHODS

2.1. Selection of Compound Dataset

The first dataset includes 1,2,3,4-tetrahydroisoquinoline derivative (30 molecules) synthesized by N. Toda and colleagues [15]. The second dataset involved Galantamine, Tacrine and 18 coumarin–tacrine hybrids synthesized and evaluated by Qi Sun *et al*. [16]. These two sets of compounds were subjected to MLR (Linear) and SVM (Non-linear) QSAR studies to derive individual QSAR models for each set and finally extract common chemical structure features responsible for structure-activity relationship (SAR) with reference to their action on AchE as a therapeutic target.

2.2. Calculation of Molecular Descriptor & Preparation of QSAR Model

The structures of compounds from each series were drawn and optimized in Chem Axon - Marvin Sketch version 5.6.0.2 [17]. The descriptors were calculated using an online web server E-Dragon (version 5.4) [18-20]. More than 2000 descriptors belonging to various classes were further analyzed by data analysis package of Graphpad Prism 6 for MLR analysis and for non-linear SVM analysis, GIST server was employed [21]. All the generated descriptors were further screened to establish valid models. In the process, constant and missing set of descriptors which were considered insignificant in statistical analysis were pruned (pruning parameters; standard deviation ≤ 0, and missing values greater than equal to 1) [22]. Pearson's correlation coefficient was used to establish correlation between molecular descriptors and biological responses (endpoints). Odds of redundancy in regression models were thoroughly inspected and removed using correlation matrix [23]. Forward selection wrappers were used to generate MLR [24] and non-linear QSAR [22] models. The SAR models thus obtained were validated using internal validation statistics like cross validated regression analysis, mean and maximum absolute error calculations.

3. RESULTS AND DISCUSSION

3.1. Statistical Fitness and Stability of QSAR Models

The present QSAR studies are an attempt to extract common SAR from two different sets of synthesized compounds tested against human AChE targeted in Alzheimer's disease. Two statistical methods, Multiple Linear Regression (MLR) and Support Vector Machine (SVM), have been used to achieve QSAR models. Gaussian Kernel function was used to obtain non-linear QSAR models in SVM.

Table **1** provided below represents selected descriptors in multivariable models respectively for dataset-I and dataset-II. QSAR models obtained from forward selection method show uni-variable to tetra-variable models separately for dataset-I and dataset-II. The variable selection has been achieved under thumb-rule criteria which states that maximum descriptor variables cannot exceed 1/5 of total samples (compounds) in dataset. Dataset-I includes 30 molecules allows to exceed till hexa-variable model, though we decided to limit our models to tetra-variable model as it has gained necessary statistical fitness thereby. Wherein dataset-II includes 20 molecules and allows selecting tetra-variable models. For a comparative study of QSAR models in either of datasets, we limited variables to tetra-variable models.

Statistical fitness of QSAR models obtained can be clearly understood from numerical magnitudes of R^2, R^2_A, S.E. and R^2_{CV} (N-Fold) produced in Table **1**. Dataset-I with tetra-variable models, (Linear (MLR) R^2=0.8961 and Non-linear (SVM) R^2=0.9307)) approves acceptable statistical fitness and stability (R^2_{CV}=0.8462, 0.734) of QSAR models. Similarly dataset-II with tetra-variable (Linear, R^2=0.9057 and Non-linear, R^2=0.9307) is found fit and stable (R^2_{CV} =0.8401, 0.9061) in terms of statistical fitness. Maximum and mean absolute errors are diagnosed under the limits. Descriptors selected in forward selection method fall in similar group of indices which also points out similar structural properties underlying the structure-activity relationship.

3.2. Descriptor Sensitivity of Linear QSAR Models

The aim of including two datasets of different chemical origin but targeting the same therapeutic target (AChE) is to study and extract common structure-activity information underlying in QSAR models. In order to achieve comparative SAR, we decided to compare the descriptors sets derived from linear (MLR aided) QSAR models. The contribution of a descriptor (X) in regression equation can be understood when it is multiplied by its coefficient (M). Keeping intercept as constant, we obtained products of descriptors magnitudes multiplied with their coefficients *e.g*. M1X1, M2X2, M3X3 and M4X4. We have plotted a comparative graph taking Y (Activity) and M1X1, M2X2, M3X3 and M4X4.

Graph (Fig. **1**) is comparative contribution of descriptors in regression equation. The magnitude scales on the graph shows activity magnitude scale with positive and negative contribution of molecular descriptors selected thereby in regression equation. For dataset-I, Fig. **1** illustrates PW3 molecular descriptor as major contributor in regulation of pIC_{50} values and so as in linear QSAR models. Activity

Table 1. Statistical fitness of QSAR models obtained for two datasets.

Dataset	QSAR Model	Descriptors	Variables	R^2	Max. Abs. Error	Mean Abs. Error.	R^2_{cv} (N-FOLD)
Dataset-I	Linear (MLR)	MATS5m	1	0.3668	0.6719	0.2825	0.3208
		MATS5m, RDF045m	2	0.6588	0.6199	0.2137	0.5802
		MATS5m, RDF045m, PW3	3	0.8091	0.4844	0.147	0.7476
		MATS5m, RDF045m, PW3, Mor17e	4	0.8961	0.2774	0.1221	0.8462
	Non-linear (SVM)	MATS5m	1	0.5351	1.0065	0.1966	0.4388
		MATS5m, MATS5e	2	0.8123	0.5296	0.1164	0.651
		MATS5m, MATS5e, HATSe	3	0.8575	0.4638	0.1006	0.728
		MATS5m, MATS5e, HATSe, SdCH2.2.Count	4	0.9304	0.4640	0.0796	0.7354
Dataset-II	Linear (MLR)	HATS1v	1	0.406	0.2579	0.1252	0.261
		HATS1v, Mor04m	2	0.7994	0.1646	0.0701	0.6933
		HATS1v, Mor04m, GATS4e	3	0.8624	0.1534	0.0551	0.7998
		HATS1v, Mor04m, GATS4e, G1v	4	0.9057	0.1200	0.0438	0.8401
	Non-linear (SVM)	p2p2-1C	1	0.4321	0.3703	0.0936	0.5197
		p2p2-1C, Mor04m	2	0.9405	0.0999	0.0292	0.882
		p2p2-1C, Mor04m, BELv6	3	0.9466	0.0938	0.0283	0.9018
		p2p2-1C, Mor04m, BELv6, RDF130m	4	0.9370	0.1188	0.0245	0.9061

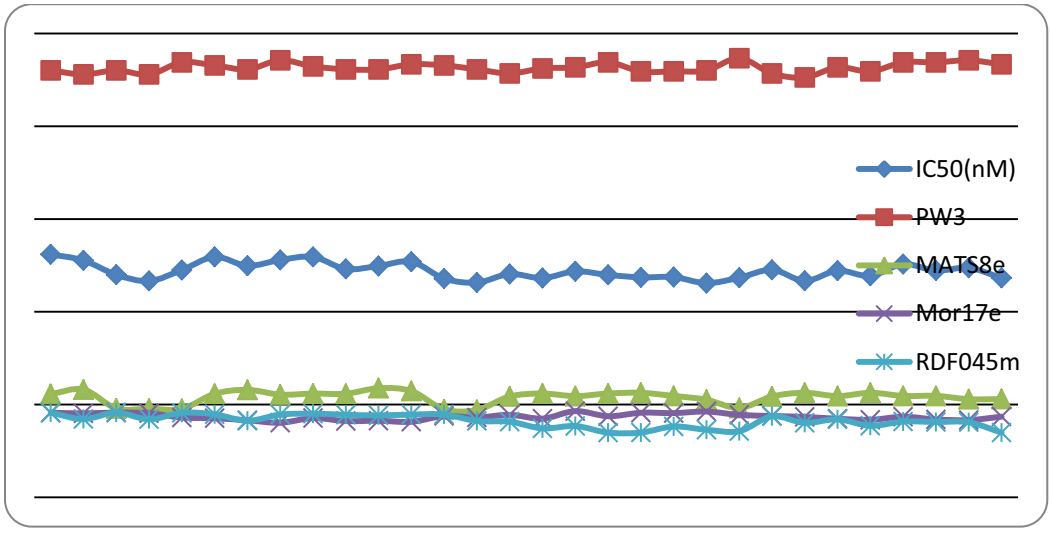

Fig. (1). Descriptor sensitivity in linear (MLR aided) QSAR models for dataset-I (30 molecules).

scales (pIC_{50}) lies intermediate to PW3 magnitudes and magnitudes of other three descriptors (MATS8e, Mor17e, RDF045m). It can also be understood from the graph below that the increase in magnitudes of PW3 descriptor *via* change in chemical structures of compounds in dataset-I would bring favorable changes in pIC_{50} values. This fact can be utilized to design new molecules in the same series.

Fig. **2** illustrates comparative contribution of molecular descriptors in regulating activity (pIC_{50}) for dataset-II. As a peculiar observation, the three molecular descriptors (HATS1, Mor04m and G1v) have contributed with their positive magnitudes and therefore increase in their magnitudes would bring an increase in pIC_{50} values. GAT54e contributed with negative magnitudes and therefore must possess an inverse

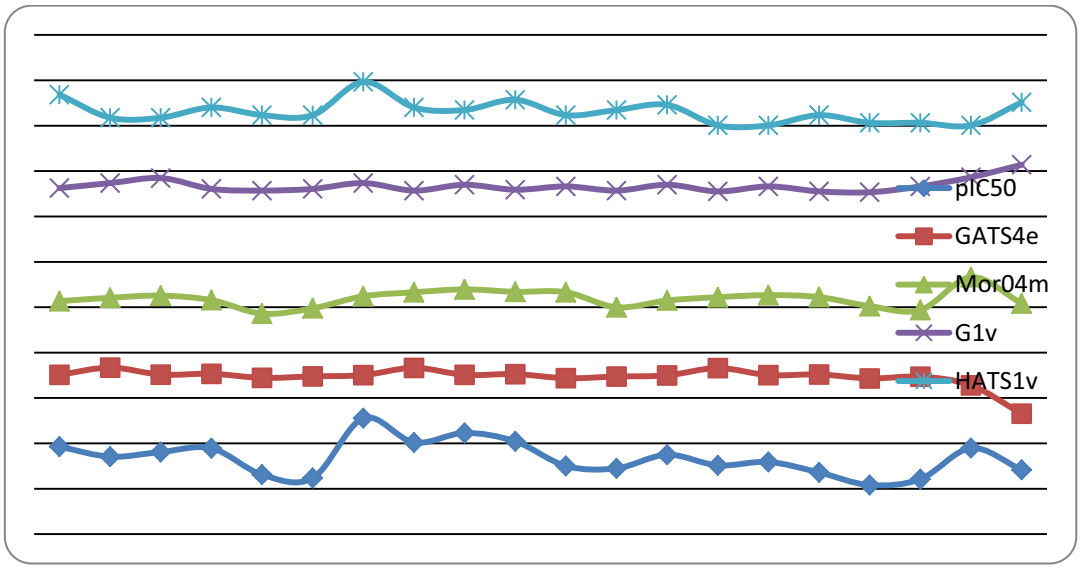

Fig. (2). Descriptor sensitivity in linear (MLR aided) QSAR models for dataset-II (20 molecules).

impact of activity regulation. Descriptors sensitivity could be an important tool in at least linear QSAR models to evaluate and validate the corresponding contribution of molecular descriptors.

3.3. Predictability of QSAR Models

Linear and non-linear QSAR models achieved for dataset-I and dataset-II were found statistically fit and stable. To evaluate the predictive powers of linear (MLR) and non-linear (SVM), pIC_{50} values have been predicted and correlated with their corresponding experimental activities. Regression equations of tetra-variable models for dataset-I and dataset-II have been provided below. 89% confidence has been observed in dataset-I with small standard error values (0.15) wherein 90% confidence was observed in dataset-II with even smaller standard error (0.06). Moving onto F-stat values of dataset-I (F=53.89) and dataset-II (F=36.07) confirm the significance of statistical models from their application point of view.

Dataset-I

Tetra-variable QSAR model

$$pIC_{50} = - 9.795 + 54.420[PW3] + 2.066[MATS8e] + 0.625[Mor17e] - 0.121[RDF045m]$$

$N = 30$ $R^2 = 0.89$ S.E. = 0.15 F=53.89

Dataset-II

Tetra-variable QSAR model

$$pIC_{50} = - 4.4792 - 0.9200[GATS4e] - 0.2596 [Mor04m] + 28.2186 [HATS1v] + 9.1796[G1v]$$

$N = 20$ $R^2 = 0.9057$ S.E. = 0.0649 F=36.037

Graphical correlation of predictive powers of QSAR models has been provided below. A straight indication received from graph confirms that SVM aided non-linear QSAR models are statistically fit and more predictive in case of dataset-I and dataset-II. Fig. (**3A**) represents graphical correlation of experimental pIC_{50} and their predicted values from linear QSAR models for dataset-I derived from tetra-variable models. Fig. (**3B**) represents graphical respective values of pIC_{50} from SVM aided non-linear QSAR models. The similar aftermath in predictive powers can be observed for dataset-II from tetra-variable models. Fig. (**4A**) and (**4B**) presents graphical correlation of experimental and predicted pIC_{50} values of dataset-II.

CONCLUSION

Comparative study of MLR aided linear and SVM aided non-linear QSAR models reveals the overlapping SAR required to inhibit AChE targeted in the treatment of Alzheimer's disease. Structure-activity relationship derived from the two different datasets is found to be predominantly regulated by 3D Morse descriptors, which can be clearly observed in all the linear and non-linear QSAR models achieved in present study. Most of the selected 3D Morse descriptors are weighted by their atomic masses which confirms the fact that the distribution of atomic masses in

Table 2. Molecules of dataset-I and dataset-II with their respective experimental pIC_{50} values, predicted pIC_{50} values using tetra-variable models achieved in linear (MLR) and non-linear (SVM) QSAR models.

Dataset 1 (30 Compounds)				Dataset 2 (20 Compounds)			
I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
1	8.097	7.904	7.905	1a	-1.537	-1.501	-1.635
2	7.770	7.548	7.335	1b	-1.646	-1.584	-1.645
3	6.996	7.139	7.005	1c	-1.596	-1.583	-1.596
4	6.660	6.513	6.595	1d	-1.554	-1.630	-1.689
5	7.252	7.271	7.034	1e	-1.845	-1.927	-1.850
6	7.959	7.802	7.952	1f	-1.881	-1.833	-1.880
7	7.481	7.338	7.494	1g	-1.223	-1.255	-1.225
8	7.796	7.777	7.804	1h	-1.490	-1.496	-1.488
9	7.959	7.799	7.972	1i	-1.386	-1.506	-1.422
10	7.310	7.413	7.302	1j	-1.479	-1.466	-1.433
11	7.469	7.692	7.464	1k	-1.749	-1.648	-1.671
12	7.699	7.800	7.694	1l	-1.775	-1.785	-1.777
13	6.793	7.070	7.018	1m	-1.625	-1.577	-1.627
14	6.577	6.397	6.642	1n	-1.742	-1.760	-1.775
15	7.036	7.008	7.184	1o	-1.705	-1.759	-1.717
16	6.815	6.871	6.828	1p	-1.820	-1.716	-1.757
17	7.180	7.322	7.177	1q	-1.960	-1.954	-1.958
18	6.987	7.100	6.913	1r	-1.893	-1.912	-1.895
19	6.857	6.843	6.876	Galath-mine	-1.553	-1.574	-1.553
20	6.870	6.979	6.883	Tacrine	-1.792	-1.783	-1.794
21	6.545	6.739	6.553	-	-	-	-
22	6.836	6.691	6.849	-	-	-	-
23	7.260	7.237	7.205	-	-	-	-
24	6.668	6.812	6.818	-	-	-	-
25	7.215	7.273	7.206	-	-	-	-
26	6.936	6.825	6.927	-	-	-	-
27	7.569	7.544	7.562	-	-	-	-
28	7.222	7.370	7.217	-	-	-	-
29	7.367	7.256	7.360	-	-	-	-
30	6.824	6.670	6.821	-	-	-	-

The legends used in table headings have been produced at the bottom of the Table **2**.
I: Molecules of Dataset I (30 Molecules)
II: Experimental Activity (pIC_{50} values)
III: Predicted Activity (pIC_{50} values) using Tetra-Variable model derived from MLR aided Linear QSAR models.
IV Predicted Activity (pIC_{50} values) using Tetra-Variable model derived from SVM (Gaussian function) aided Non-Linear QSAR models.
V: Molecules of Dataset II (20 Molecules)
VI: Experimental Activity (pIC_{50} values)
VII: Predicted Activity (pIC_{50} values) using Tetra-Variable model derived from MLR aided Linear QSAR models.
VIII: Predicted Activity (pIC_{50} values) using Tetra-Variable model derived from SVM (Gaussian function) aided Non-Linear QSAR models.

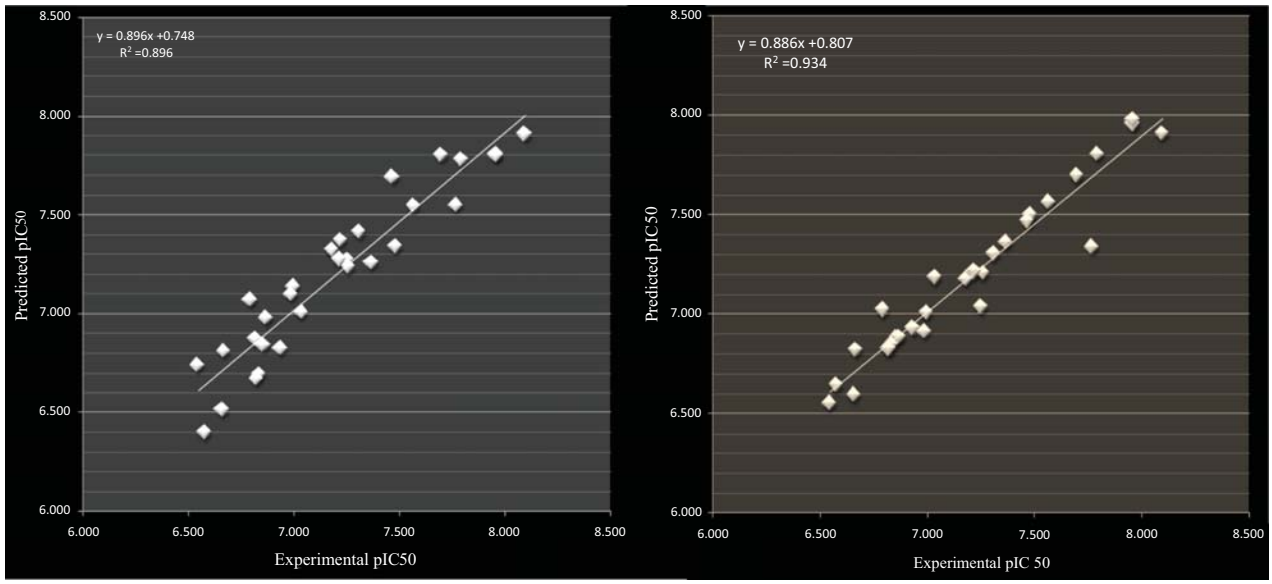

Fig. (3). (A) correlation of experimental and predicted pIC_{50} calculated from linear (MLR) aided tetra-variable model for dataset -I **(B)** correlation of experimental and predicted pIC_{50} calculated from non-linear (SVM) aided tetra-variable model for dataset-I.

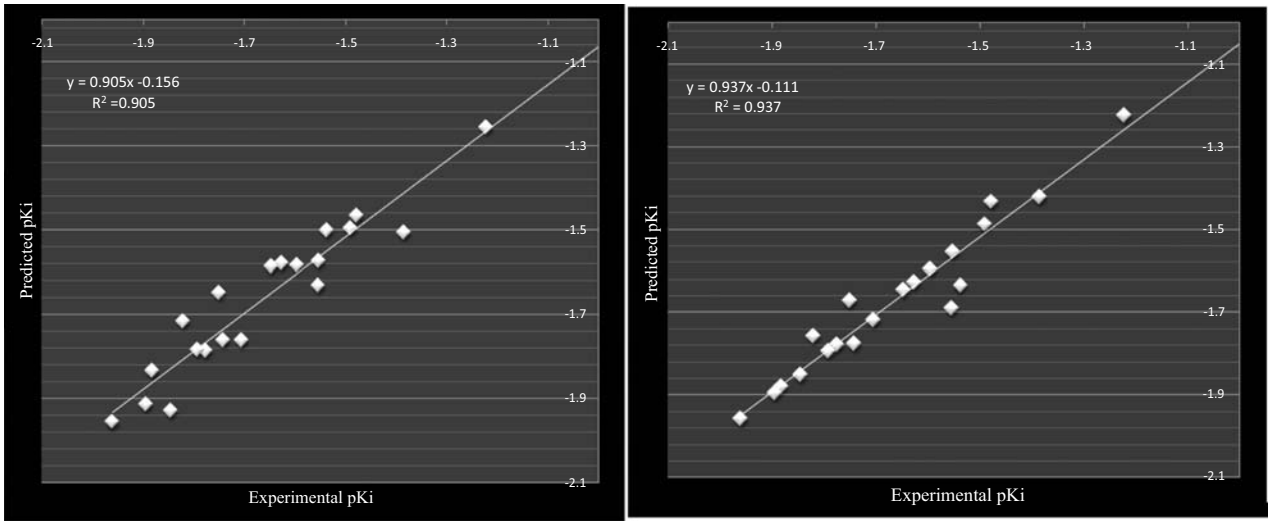

Fig. (4). (A) correlation of experimental and predicted pIC_{50} calculated from linear (MLR) aided tetra-variable model for dataset -II **(B)** correlation of experimental and predicted pIC_{50} calculated from non-linear (SVM) aided tetra-variable model for dataset-II.

three dimensional spaces is more crucial and not just as an additive structural property. Radial Distribution Function (RDFs) weighted by electronegativity of molecules has also been identified to regulate biological activity of AChE inhibitors. QSAR models have been found to be statistically fit and predictive. The descriptor sensitivity of linear QSAR models could be used to identify the dominant and recessive roles of molecular descriptors selected in multi-variable QSAR models. These overlapping structure-activity relationship can also be utilized to design and synthesize new set of inhibitors for AChE.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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