QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS ANALYSIS OF THE ANTICONVULSANT ACTIVITY OF ERYTHRININE

KAMALRAJ RAJI, VIJEYAANANDHI MUTHUKUMAR*

Research scholar, Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Vels University, Chennai, Tamil Nadu, India. Email: hodpchemistry@velsuniv.ac.in

ABSTRACT

Objective: The objective of this study is to determine the biological activity of Erythrinine against human nicotinic acetyl cholinergic receptor by quantitative structure-activity relationships (QSAR) and determine the anticonvulsant activity of the crude extract of *Erythrina indica* in mice.

Methods: Half minimal inhibitory concentration (IC50) of structurally similar analogs was identified from a literature. 298 2D and 3D descriptors were computed using molecular operating environment. The compounds were divided into training set and test set after principal component analysis. Multiple linear regression (MLR) analysis was carried out using International Business Machines - Statistical Package for the Social Sciences, version 17 (IBM SPSS 17). Molecular docking analysis was carried out using Autodock 4.2. The *in vitro* anticonvulsant activity of the crude extract was determined by administration of alcoholic extract to albino mice of either sex on pentylene tetrazole-induced seizures.

Results: Computed descriptors were used as independent variables. The model with high correlation coefficient was selected for the biological activity of Erythrinine (r^2=0.982). The biological activity of Erythrinine was found to be 0.62 µmol/L. Interactions of Erythrinine with the druggable domains of human nicotinic acetyl cholinergic receptor was elucidated by molecular docking which showed a binding energy of −7.12 kcal/mol and IC50 5.95 µM. When administered to the albino mouse of either sex, the crude extract offered significant protection of 71.4% against pentylenetetrazole-induced convulsions.

Conclusion: The study has determined the antiepileptic activity of Erythrinine to act through a novel mechanism of nicotinic acetyl cholinergic receptor inhibition (nAchR). Future studies have to be carried out to determine the activity of similar analogs and thereby identify molecules that act through this novel mechanism.

Keywords: Erythrinine, Quantitative structure-activity relationships, Multiple linear regression, Anticonvulsant.

INTRODUCTION

During the last 20 years quantitative structure-property relationships (QSPR) and QSAR models have gained extensive recognition in physical, organic, analytical, pharmaceutical and medicinal chemistry, biochemist, chemical engineering and technology, toxicology, and environmental sciences [1-9]. The success of the QSPR and QSAR approach can be explained by the insight offered into the structural determination of chemical properties and biological activities, and the possibility to estimate the properties of new chemical compounds without the need of synthesize and test them. These molecular design techniques, which significantly reduce the cost and time involved in obtaining compounds with desired properties, were applied a wide range of properties, such as melting and boiling temperature, molar heat capacity, standard Gibbs energy of formation, vaporization enthalpy, refractive index, density, aqueous solubility, 1-octano water partition coefficient, solvation free energy, receptor binding affinities, pharmacological activities, and enzyme inhibition constants.

The search for safe and more potent anticonvulsant remains a drug design priority, and a wide variety of compound has been synthesized for this purpose. Previous comparisons of the structural characteristics of the anticonvulsant drug have identified a common pattern defined by a nitrogen heteroatom system, at least, one carbonyl group, together with two or one phenyl group [10-12]. On the other hand, the role of hydrogen bond in the docking process has been largely discussed, although in spite of the efforts no correlation has been found [12].

QSAR studies have received widespread attention as a powerful tool to better direct the rational synthesis of new drugs with anticonvulsant activity [13-15].

QSAR methodology to elucidate the structure correlates of anticonvulsant activity in the series of 35 benzylacetamide derivatives using the method of partial least-squares regression in conjunction with leave-one-out (LOO) cross-validation, the influence of 31 topological, electronic, physicochemical, and structural molecular descriptors on anticonvulsant activity was already investigated [16,17]. In the present study, we aimed to develop QSAR equation for the anticonvulsant activity of a series of Erythrinine derivatives of Erythrina alkaloids. IC50 of 20 compounds was retrieved from the literature, and using 20 compounds the (pIC50) for the Erythrinine was derived for the anticonvulsant activity model and compared the high value of the correlation coefficient and proved the satisfactory results.

MATERIALS AND METHODS

Retrieval of IC50
The IC50 against the human neuronal nicotinic acetyl cholinergic receptor were retrieved from previous studies. A thorough review of the literature was conducted using the binding database for previous studies reporting IC50 values (Fig. 2).

Compound dataset creation and descriptor computation
IC50 of 20 compounds against human nicotinic acetyl cholinergic receptor was retrieved from the literature. Compounds with diverse

side chains but structurally similar molecular scaffold were used for QSAR analysis. The compounds were energy minimized under MMFF94× force field with an RMS gradient of 0.1. 298 2D, i3D, and ×3D molecular descriptors were calculated for the energy minimized structures using a molecular operating environment.

Principal component analysis (PCA)

PCA was carried out using MATLAB version 7.10.0. PCA was performed for sample selection prior to model building. Molecular descriptors and log IC50 (pIC50) were used as independent and dependent variables, respectively. Data were pre-processed by mean centering and cross-validation was carried out by LOO method. Significant outliers were removed from sample subset for further processing.

3D-QSAR model building

The 3d-qsar model was built by MLR Method. The compounds were divided into training set and validation set. Clustering was done by k-means agglomerative hierarchical cluster analysis method using three principal components. 10% of the selected samples were segregated as a validation set. The training set compounds were used for 3D-QSAR model building using SPSS statistics version 17.0. Various MLR models were built, and the model with a best correlation coefficient (r^2) was used for predicting the biological activity of designed compounds.

Protein homology modeling

The crystallographic structure human nicotinic acetyl cholinergic receptor was extracted from RCSB Protein Data Bank (www.rcsb.org) identifier (PDB ID: 4UXU). However, the entire protein was remodeled using the Swiss-model automated server since the structure was a biological assembly with various co-crystallized ligands. Swiss-model server was used to model the secondary structure of the protein. FASTA

sequences were retrieved from RCSB server to generate the templates. The automated mode was employed in Swiss-model server. This mode automatically selects suitable templates of the query sequence by Blast E-value limit. It has been reported that automated sequence alignment method allows reliable results when the target and templates shared around >50% identical residues (Fig. 1) [18].

Molecular docking analysis

Interactions of the designed compounds with the active site of human nicotinic acetyl cholinergic receptor were studied by molecular docking. Autodock 4.2 tool was used for molecular docking analysis. Both the receptor and ligands were prepared by addition of hydrogen's and gasteiger charges. A grid defining the active site was constructed before running the docking simulation. A genetic algorithm was adopted for conformer search while docking.

In vitro validation of anticonvulsant activity of crude extract

To validate the antiepileptic activity determined by QSAR analysis, *in vitro* anticonvulsant studies were carried out. It has to be noted that the QSAR model built and predicted biological activity are not validated and *in vitro* experimentation carried out is to be consider evidence to support the antiepileptic activity of the crude extract of *Erythrina indica.* Fresh leaves of the plant were collected and dried. After preliminary phytochemical analysis, the crude alcoholic extract was administered to albino mice (20-25 g) of either sex.

RESULTS AND DISCUSSION

Protein homology modeling

The three dimensional structure of the protein was homology modeled using Swiss-model server. The server determines the three
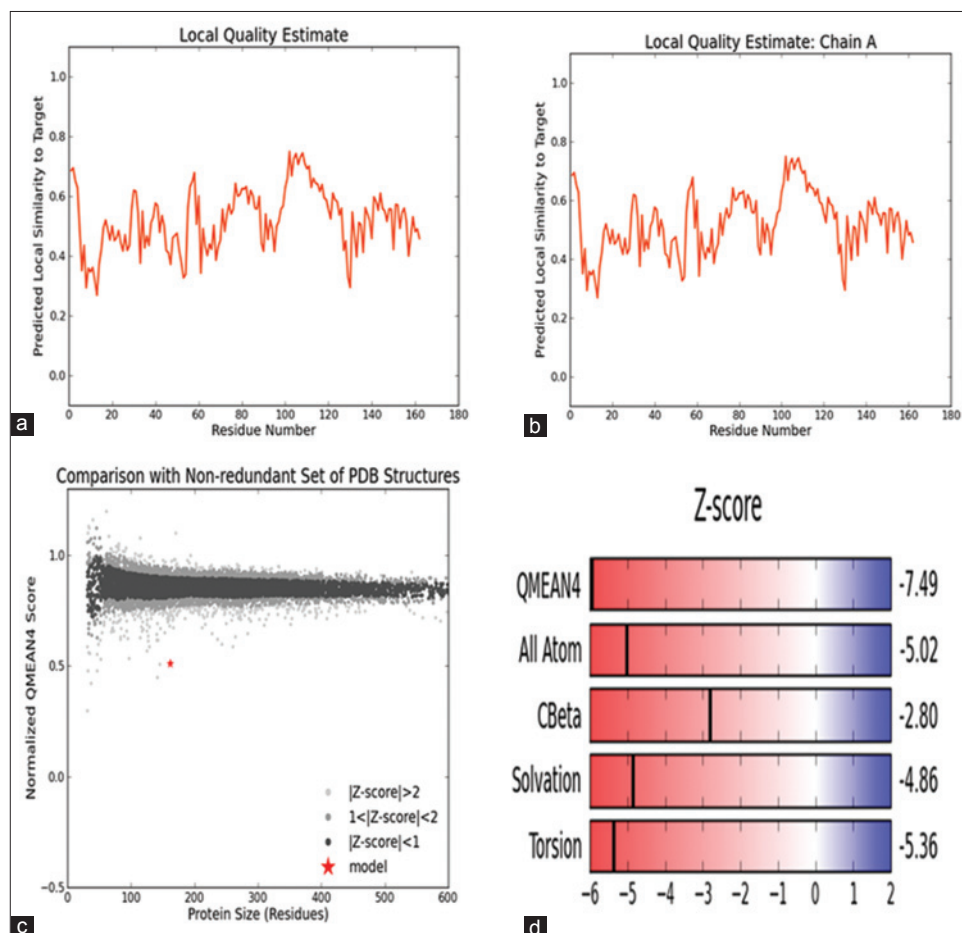

Fig. 1: (a-d) Local quality of estimation of the modeled target

dimensional structure of the target protein in four steps which include template selection, target template alignment, model building and evaluation [20,21]. The quality of the built homology model was assessed through qualitative model energy analysis (QMEAN) and Z-Score. The Z score provides an estimate of the absolute quality of a model by relating it to reference structures solved by X-ray crystallography. The QMEAN Z-score is an estimate of the "degree of nativeness" of the structural features observed in a model by describing the likelihood that a model is of comparable quality to high-resolution experimental structures.

PCA

PCA analysis showed the following compounds to be significant outliers: 147, 149,150, 152, and 153. A PCA model with three principal components was selected for identification of significant outliers within the compound samples considered. The eigenvalue of Cov (X) of the selected model was 6300000 with a percentage variance of 0.01 and a cumulative percentage variance of 100. The root mean square error of cross (RMSEC) and RMSEC-validation (RMSECV) were found to be 83.61 and 457076.7, respectively. RMSECV measures differences between the observed and predicted values by a model. In leave-one out method adopted for cross-validation, each sample is left out of the model formulation followed by its prediction. It is, therefore, a model's ability to predict new samples. RMSECV is generally defined by the following equation:

$$\text{RMSECV} = \sum_{i=1}^{n} \frac{(\hat{y}_i - y_i)^2}{n}$$

Where, $\hat{y}_i$ the predicted value is y_i the measured value and n is the number of measurements. Out of the 21 compounds subjected for PCA, compounds 9a and 9e were found to be significant outliers. Since the independent variables are only used for a PCA analysis, outliers are considered as molecules with different physico-chemical properties. Hence, the outliers were removed from further processing.

3D QSAR model

The compounds were divided into training set and validation set prior to regression analysis. Logistic linear regression models were built for QSAR analysis using computed descriptors as independent variables and IC50 (pIC50) as dependent variables. Out of the 21 compounds used or QSAR model building, 14 compounds were used as training set, whereas 5 compounds were used as a validation set. A total of 19 compounds were used for the regression model. Four QSAR models were built as follows:

pIC50 = 209.9 (Kier A3) – 306.98

pIC50 = 206.99 (Kier A3) + 23.77 (VSurf_IW) – 327.21

pIC50 = 237.42 (Kier A3) + 30.0 (VSurf_IW) - 1.846 (SLogp_VSA7) – 316.98

pIC50 = 215.67 (Kier A3) + 35.18 (VSurf_IW) – 2.074 (SLogp_VSA7) – 15.52 (VSurf_HB) – 181.12

Where, Kier A3 is the third kappa shape index, Vsurf_IW is the hydrophilic integy moment, SLogp_VSA7 is the Sum of vi such that Li is in 0.25, 0.30. Vi is the van der Waals surface area, whereas Li is the contribution to logP(o/w) for atom i as calculated in the SLogP descriptor. VSurf_HB is the H-bond donor capacity.

Model 4 was selected for prediction of biological activity on the basis of the correlation coefficient (r^2=0.982) of Erythrinine and newly designed compounds. The selected model has four components. The correlation plot of predicted and observed pIC50 values of training set compounds is as shown in Fig. 3.
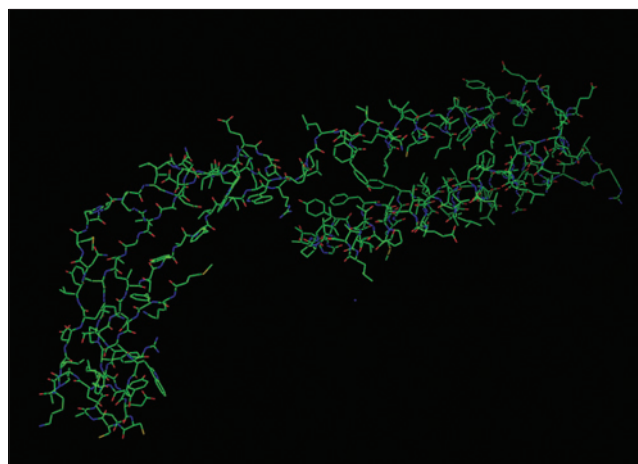

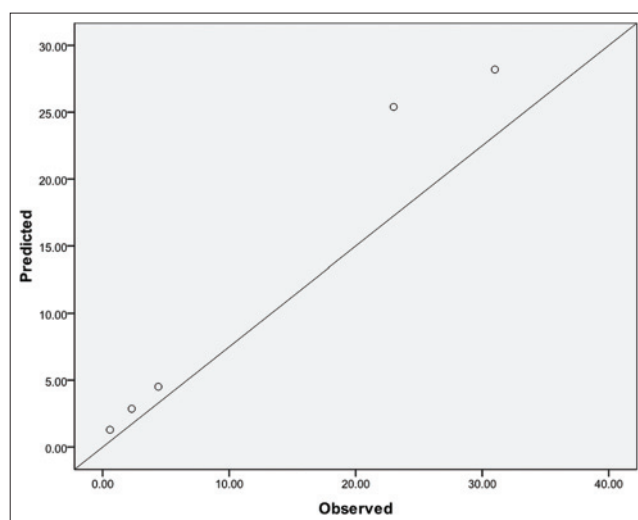**Fig. 2: Structure of the modeled neuronal nicotinic acetyl cholinergic receptor**

Fig. 3: Correlation plot of predicted and observed pIC50 values of training set compounds (r2 = 0.982, y = 0.9454 xs + 0.8714)
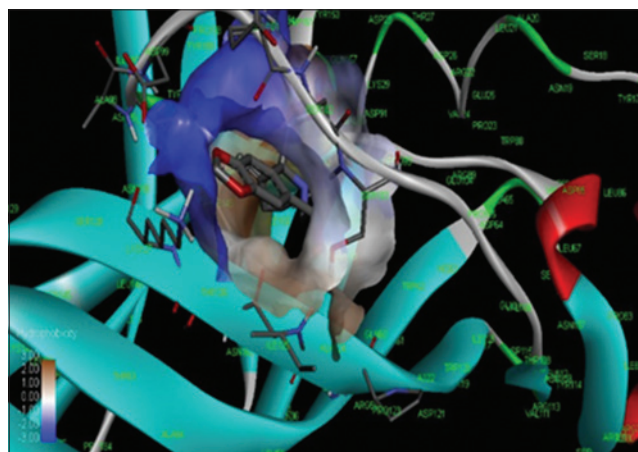

Fig. 4: Molecular docking analysis

Erythrinine biological activity prediction: The IC50 of Erythrinine for antiepileptic property was predicted applying the computed descriptors of Erythrinine to the built model. The pIC50 of Erythrinine was found to be 0.62 µM.

Molecular docking analysis

Molecular docking analysis were performed between the Erythrinine compound and active site of Human Nicotinic acetyl cholinergic receptors (Fig. 4) using Auto dock 4.2 tool. Ligand Interaction map (Fig. 5) and the obtained outcome of the docking results are tabulated in Table 1.
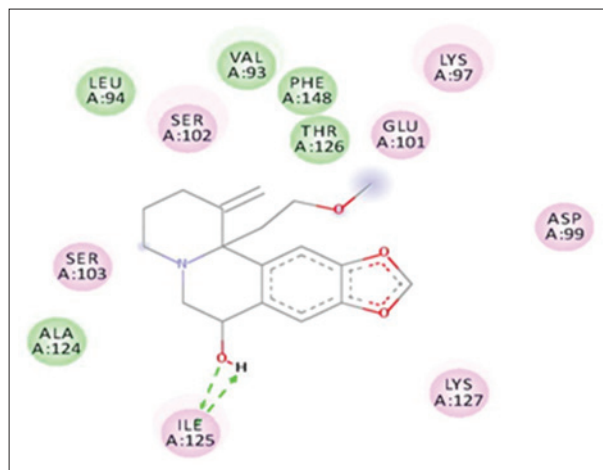
In vitro evaluation of anticonvulsant activity

The effect of alcohol extract on pentylenetetrazole (PTZ)-induced convulsion in mice

The extract (500 mg/kg p.o.) significantly ($p<0.05$) increased the threshold of PTZ-induced convulsion in mice compared with the control group (Fig. 6). At 500 mg/kg p.o., the extract produced significant protection (71.4%) against PTZ-induced convulsion in mice (Table 2).
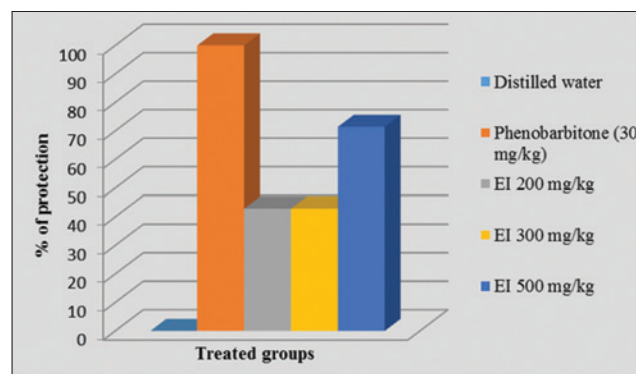

Fig. 5: Ligand interaction map

Table 1: Molecular docking analysis of Erythrinine with human nicotinic acetyl cholinergic receptor

Parameter	Predicted values
Binding energy	−7.13
Inhibitory constant	5.95 μM
Intermolecular energy	−7.73
Internal energy	0.13
Torsional energy	0.6
Unbound extended energy	0.13
Hydrogen bonding interactions	Present-1
Interacting residues	ILE 125
Vanderwaal's hydrogen bond desolvation energy	−7.48

Fig. 6: Effect of alcoholic extract of *Erythrina indica* on pentylenetetrazole-induced seizures in mice
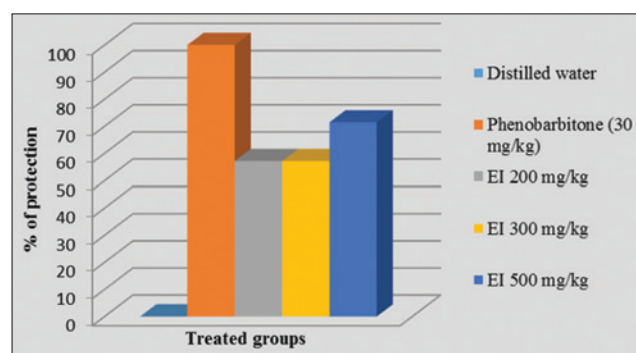

Fig. 7: Effect of Alcoholic extract of *Erythrina indica* on MES-induced convulsion in mice

Table 2: Anticonvulsant activity of alcoholic extract of *Erythrina indica* against PTZ-induced convulsions

Treatment	Onset of convulsion (seconds)	Number convulsed/ number used	Mortality (%)	Protection (%)
Distilled water	43.26±1.20	7/7	100	0
Phenobarbitone (30 mg/kg)		0/7	0	100
Erythrina indica 200 mg/kg	127.99±2.01*	4/7	57.142857	42.85714286
Erythrina indica 300 mg/kg	128.00±2.31*	4/7	57.142857	42.85714286
Erythrina indica 500 mg/kg	135.55±1.40*	2/7	28.571429	71.42857143

Results are expressed as mean±SEM and as % mortality and protection (n=7). *$p<0.05$ compared with control (one-way ANOVA). Statistically significant difference was observed between the treatment groups, SEM: Standard error of mean

Table 3: Anticonvulsant activity of alcoholic extract of *Erythrina indica* against MES-induced convulsion in mice

Treatment	Onset of convulsion (seconds)	Number convulsed/ number used	Mortality (%)	Protection (%)
Distilled water	9.68±0.3	7/7	100	0
Phenobarbitone (30 mg/kg)	NA	0/7	0	100
Erythrina indica 200 mg/kg	25.71±0.70*	3/7	42.857143	57.14285714
Erythrina indica 300 mg/kg	25.95±0.52*	3/7	42.857143	57.14285714
Erythrina indica 500 mg/kg	27.97±0.47*	2/7	28.571429	71.42857143

Results are expressed as mean±SEM and as % mortality and protection (n=7). *$p<0.05$ compared with control (one-way ANOVA). Statistically significant difference was observed between the treatment groups, SEM: Standard error of mean

The effect of alcohol extract on MES-induced convulsion in mice

The extract (500 mg/kg p.o.) significantly ($p < 0.05$) increased the threshold of MES-induced convulsions in mice compared with the control group (Fig. 7). At 500 mg/kg p.o., the extract produced (71.4%) protection in mice (Table 3).

CONCLUSION

The study has determined the antiepileptic activity of Erythrinine to act through a novel mechanism of nicotinic acetyl cholinergic receptor inhibition. Future studies have to be carried out to determine the activity of similar analogs and thereby identify molecules that act through this novel mechanism.

ACKNOWLEDGMENT

The authors are thankful to Vels University (VISTAS) and its management for providing research facilities and encouragement.

REFERENCES

1. Hansch C, Fujita T. A new substituent constant, ~r, derived from partition coefficients. J Am Chem Soc 1964;86:1616-26.
2. Hansch C. A quantitative approach to biochemical structure-activity relationships. Acad Chem Res 1969;2:232-9.
3. Hansch C, Leo A. Exploring QSAR: Fundamentals and Applications in Chemistry and Biology. Washington, DC: American Chemical Society; 1995. p. 1.
4. Kubinyi H. QSAR and 3D QSAR in drug design Part 1: Methodology. Drug Discov Today 1997;2:457-67.
5. Cramer RD, Patterson DE, Bunce JD. Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. J Am Chem Soc 1988;110(18):5959-67.
6. Tarko L, Ivanciuc O. QSAR modeling of the anticonvulsant activity of phenylacetanilides with preclav (property evaluation by class variables). MATCDY 2001;44:201-14.
7. Kellogg GE, Semus SF, Abraham DJ. HINT: A new method of empirical hydrophobic field calculation for CoMFA. J Comput Aided Mol Des 1991;5(6):545-52.
8. Vanciuc DM, editor. 30 QSAR models. In: QSPR and QSAR Studies by Molecular Descriptors. Huntington, N.Y: Nova Science; 2001. p. 5-9.
9. Ivanciuc O, Ivanciuc T, Cabrol-Bass D. 3D quantitative structure activity relationships with CoRSA. Comparative receptor surface analysis. Application to calcium channel agonists. Analysis 2000;28:637-64.
10. Hemmateenejad B, Miri R, Tabarzad M, Jafarpour M, Zand F. Molecular modeling and QSAR analysis of the anticonvulsant activity of some N-phenyl-N'-(4-pyridinyl)-urea derivatives. J Mol Struct (Theochem) 2004;684:43-9.
11. Tassoa SM, Bruno-Blanch LE, Moon SC, Estiú GL. Pharmacophore searching and QSAR analysis in the design of anticonvulsant drugs. J Mol Struct (Theochem) 2000;504:229-40.
12. Carter MD, Stephenson VC, Weaver DF. Are anticonvulsants 'two thirds' of local anesthetics? A quantum pharmacology study. J Mol Struct (Theochem) 2003;638:57-62.
13. Garro Martinez JC, Duchowicz PR, Estrada MR, Zamarbide GN, Castro EA. Anticonvulsant activity of ringed enaminones: A QSAR study. QSAR Comb Sci 2009;28:1376-85.
14. Thareja S, Aggarwal S, Verma A, Bhardwaj TR, Kumar M. 3D QSAR studies on 1, 3, 4-thiadiazole derivatives: An approach to design novel anticonvulsants. Med Chem 2010;6(4):233-8.
15. Amnerkar ND, Bhusari KP. Synthesis, anticonvulsant activity and 3D-QSAR study of some prop-2-eneamido and 1-acetyl-pyrazolin derivatives of aminobenzothiazole. Eur J Med Chem 2010;45(1):149-59.
16. Jin AY, Kohn H, Béguin C, Andurkar SV, Stables JP, Weaver DF. A quantitative structure-activity relationship study for a-substituted acetamido-N-benzylacetamide derivatives – A novel anticonvulsant drug class. Can J Chem 2005;83:37-45.
17. Kohn H, Conley JD, Leander JD. Marked stereospecificity in a new class of anticonvulsants. Brain Res 1988;457(2):371-5.
18. Bickerton GR, Paolini GV, Besnard J, Muresan S, Hopkins AL. Quantifying the chemical beauty of drugs. Nat Chem 2012;4(2):90-8.