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Original Article

INSILICO EVALUATION OF ANTISMOKING ACTIVITY OF ERYTHRININE: A COMPUTATIONAL COMPARISON AGAINST CYTISINE

KAMALRAJ RAJI[a], VIJEYAANANDHI MUTHUKUMAR[b*]

[a,b]Research Scholar, Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Vels University (VISTAS), Chennai, Tamilnadu, India
Email: hodpchemistry@velsuniv.ac.in

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ABSTRACT

Objectives: Erythrinine is a new ring-C-oxygenated *Erythrina* alkaloid from plant sources *Erythrina Indica*. The aim of this study is to investigate the smoking cessation activity of Erythrinine and its analogues using *in silico* computational approach.

Methods: Quantitative structure-affinity relationship approach has been employed to determine the antismoking activity of Erythrinine and its analogues. Multiple molecular docking analyses was carried out using Auto dock 4.2 to determine the inhibitory constants of designed compounds. Multiple linear regression analyses using SPSS 17.0 were carried out to build quantitative structure-affinity relationship model. Computation of descriptors was done using Molecular Operating Environment. All molecular visualizations were done using Pymol.

Results: The inhibitory constants of Erythrinine and Cytisine were found to be 5.95 and 30.23µM with negative binding energies of 7.13 and 6.17kcal/Mol respectively. A quantitative structure-affinity relationship model was built with a correlation coefficient of 0.96. Compound 12, 11, 8 and 7 were found to be highly active with inhibitory constants in the range of nanomolar concentrations.

Conclusion: A quantitative structure-affinity relationship model with a strong correlation coefficient of 0.96 endorses that Erythrinine is having a strong affinity with α4β2 nACh receptors as Cytisine. Hence, the comparative study concluded that Erythrinine may use as an antismoking agent.

Keywords: Erythrinine, Cytisine, Docking studies and smoking session.

INTRODUCTION

Tobacco use is the single most important preventable health risk with an annual death of 4, 80,000 across the globe. Besides affecting various systems and causing diverse diseases such as malignancy, chronic obstructive pulmonary disease, coronary artery disease, stroke, peripheral vascular disease, and peptic ulcer, using tobacco with and without smoke remain the major cause of mortality [1]. Thus pharmacological and non-pharmacological modalities of smoking cessation play important roles in both increasing the life expectancy and increasing the health-related quality of life [2]. The current existing options for tobacco smoking cessation are limited. The FDA has approved nicotine replacement preparations such as nicotine gum, transdermal nicotine patches, nicotine nasal spray, nicotine inhaler and sustained release bupropion hydrochloride as first line agents for smoking cessation therapy. The prescription medications clonidine and nortriptyline hydrochloride are used as second-line agents. However, lack of FDA approval for smoking cessation and associated potential adverse effects limit the use of these agents for smoking cessation therapy [3].

A common feature is the sparse knowledge of their pharmacology, in part due to a lack of selective receptor subtype ligands. The discovery of more selective nACh receptor agonists and antagonists is a prerequisite for an adequate understanding of CNS nicotinic function and for the development of useful agents potentially useful in the management of a broad range of mental conditions [11]. Pharmacological treatments such as nicotine-replacement therapy have been shown to help smokers stop smoking. Using a medication that does not contain nicotine, such as antidepressant have several reasons. Smokers are more likely to have a history of major depression than nonsmokers [4, 5]. Nicotine may act as an antidepressant in some smokers. [6, 7] the development of a depressed affect or depression after smoking cessation may lead to relapse. [8-10] hence a constant need for safer and effective novel molecules for smoking cessation therapy exists. Cytisine (cy) is an alkaloid with a strongly restricted conformation and well-established nicotinic agonist pharmacology, exhibiting low nanomolar affinity at α4β2 nACh receptors and low micromolar affinity at the α7 subtype, which has been used as a template to infer active conformations of flexible nicotinic drugs [12]. In the human body, Cytisine plays the role of nicotine substitution substance, and reduces the period of the interaction of the received nicotine with relevant nicotine receptors, i.e. the specific substance Cytisine "substitutes" nicotine, acting on the same receptors, thus preventing the appearance of abstinence syndrome. This results in a gradual reduction and suspension of the psychic and physical dependency to nicotine in smokers. Cytisine possesses a structure and mechanism of action that are similar to those of nicotine, but it has much lower toxicity. Cytisine manifests one of the best results to date for the group of preparations, intended for tobacco smoking therapy. *Cytisus laborinum L* the source plant aka Golder Rain acacia. It is very well tolerated, and when applied in therapeutic doses, allows a gradual giving up of smoking without any side effects. Erythrinine, a new ring-C-oxygenated *Erythrina* alkaloid,

The alkaloidal components of eight *Erythrina plants (Leguminosae), E. arborescence Roxb., E. orientalis (L.) Murr, E. crysta-galli Linn, E. crysta-galli (L.) cv. Maruba Deiko H. Murata, E. x bidwilli Lindl, E. poeppigiana (Walp) O. F. Cook, E. glauca Willd, and E. indica L.*, which is having a similar structure of the Cytisine. [12, 13] Computer-aided structure-based drug design, molecular docking is frequently used to predict the putative geometry of a protein-ligand complex. The success of this computational methodology can be rapidly verified by parallel crystallography efforts. In addition, docking is often used in conjunction with scoring functions to predict binding affinities of ligands in virtual screening experiments and in studying structure-activity relationships to prioritize synthesis of the new compound [14-16]. The aim of the study is to design to be simplified analogues of Aromatic Erythrinine with the aim of obtaining subtype-selective antagonists for the nAChRs and thereby probe the potential of using these natural products as scaffolds for further ligand optimization. Erythrinine have been investigated for the nicotinic agonist pharmacology, exhibiting low nanomolar affinity at α4β2 nACh receptors using the computer-aided structure-based drug design. Accuracy and precision of the Erythrinine was compared with the

Cytisine and finalized by evaluating the pharmacological properties of the initial lead compound at monoamine transporters and investigate to use in possible smoking cession-agent.

MATERIALS AND METHODS

Retrieval of crystal structure of human nicotinic acetylcholinergic receptor (nAchR)

The three-dimensional crystal structure of human nAchR was retrieved from the 3D repository for macromolecules, the protein data bank (PDB id: 4UXU). The primary structure of the retrieved 3D macromolecule was cross-validated with its FASTA sequence from the PubMed protein database (ID: NP_001243502.1).

Validation of protein confirmation and stability

The dihedral angles phi and psi in the protein structure were used as a measure of its confirmation. The Ramachandran plot predicts the confirmation and stability of a protein in terms of allowed and disallowed region.

Dataset preparation

A quantitative structure active relationship study is generally carried to predict the biological activity of unknown structures using the known biological activity structurally similar scaffolds. Biological activity is generally defined in terms of half minimal inhibitory concentration (IC50) and inhibitory constant (kI). Since no studies have been done previously to evaluate the biological activity of Erythrinine and hence prior IC50's did not exist for the same. Hence, the inhibitory constant of 10 Erythrinine analogues obtained by multiple molecular docking analyses was used to predict the biological activity of 20 newly designed compounds.

Active site prediction

The active site of the target protein was predicted using FT site server. The server uses multiple molecular probes to determine the active sites of the protein.

Molecular docking analysis

Molecular docking analysis was carried out using Autodock 4.2 from the Scripps research institute, USA. The target was prepared prior to docking analysis. Polar hydrogen atoms were added to the protein. The deletion of the both water molecule and inorganic charges were done to avoid error. Gasteiger charges were computed and added to the macromolecule. Lamarckian Genetic Algorithm was applied for ligand docking. The grid size was set to 54, 48 and 62 along the X-, Y- and Z-axis to recognize the binding site. Spacing was set as 0.381E. The lowest binding energy conformers were selected out of 20 different conformers for each docking simulation and resultant data was further analyzed. Other miscellaneous parameters were assigned to the default values obtained from the AutoDock 4.2 program. Ligands were similarly prepared followed by detection of torsion root. Predicted active site residues were used for grid generation using the Autogrid program. Docking analysis was used to yield inhibitory constants.

Quantitative structure activity relationship

Multiple linear regression analyses were used to develop logistic QSAR models. 3D-QSAR model was built by Multiple Linear Regression (MLR) Method. The compounds were divided into training set and 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.

RESULTS

The chemical structures of Erythrinine and Cytisine are shown in fig. 1.

Protein confirmation and stability

The total amino acids in the protein were 420 out of which 419 (99.8%) were in allowed region and 404 (96.2%) residues were in favored region (98%) as determined Molprobity analysis. The glutamine residue at position 101 was found to be an outlier. The Ramachandran Plot is as shown in fig. 4.

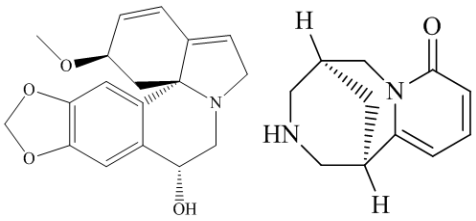


Fig. 1: Structure of erythrinine and cytosine

3D QSAR model

The biological activity of a molecule is a function of its structure and physicochemical properties. These compounds were divided into training set and validation set. 3D-QSAR was performed with the training set by Multiple Linear Regression Analysis. Thus, pKI of 10 compounds with 298 descriptors were subjected to MLR analysis. 4 models were built by stepwise MLR with a median correlation coefficient of 0.68 (Q1-Q8 0.38-0.76). The model with r^2 value of 0.96 consisting of four was selected for prediction of biological activity. The equation of the selected model is given below:

pKI=-0.012(Peoe_Vsa_Hyd)+0.124(Smr_Vsa0)-5.397(Bcut_Smr_1)+0.8(Q_Vsa_Fpneg)-4.615.

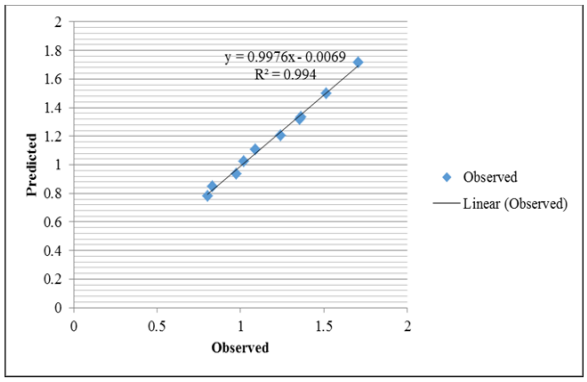


Fig. 2: Scatter plot for predicted vs observed pKI values. A correlation coefficient of (r^2) 0.95 was observed suggestive of a model good quality

Molecular docking analysis

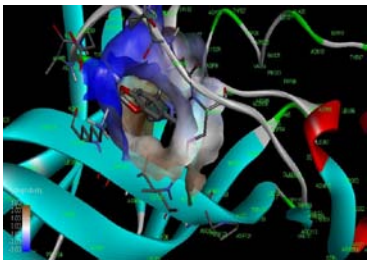
Both Erythrinine and Cytisine were individually docked to the predefined active site of human nicotinic acetylcholinergic receptor. The results of molecular docking analysis are shown in table 1**.** Results of multiple molecular docking analyses were used for logistic regression model building; however they are not shown here, considering space constraints.

The structure of the Erythrinine and Cytisine are showed in fig. 1. The docking activity of the Erythrinine docked to the active site of human nAchR and Cytisine docked to the active site of human nAchR were compared in the fig. 3. Conformation of Erythrinine Bound to nAchR–Interactions and Cytisine Bound to nAchR are shown in Yellow Lines for better understanding.

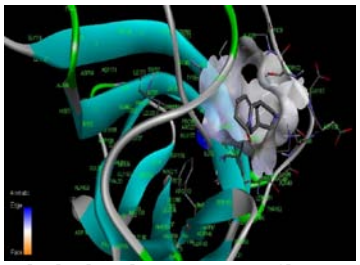
The Ligand Interaction Map of Erythrinine and Cytisine are shown in fig. 4. The oxygen atom in Erythrinine interacts with GLU101 through backbone electron donation whereas the hydroxyl group interacts with ILE125 by acting as a side chain acceptor. In contrast, Glu101 was found to interact with the hydrogen atom in Cytisine through backbone electron donation.

Table 1: Molecular docking analysis of erythrinine and cytisine with the human nicotinic acetylcholinergic receptor

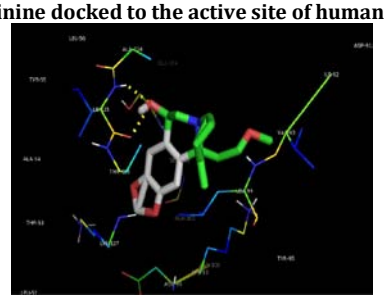
Compound	ΔG	kI (μM)	Intermolecular Energy	Internal Energy	Torsional Energy	Unbound Extended Energy	Hydrogen Bonding Interactions	Interacting Residues	Desolvation Energy
Erythrinine	-7.13	5.95	-7.73	0.13	0.6	0.13	1	ILE 125	-7.48
Cytisine	-6.17	30.23	-6.17	0.0	0.0	0.0	ABSENT	NA	-5.69



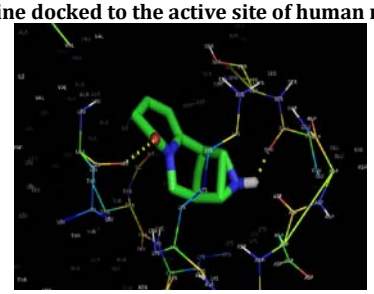
Erythrinine docked to the active site of human nAchR



Cytisine docked to the active site of human nAchR



Conformation of erythrinine bound to nAchR–interactions are shown in yellow lines



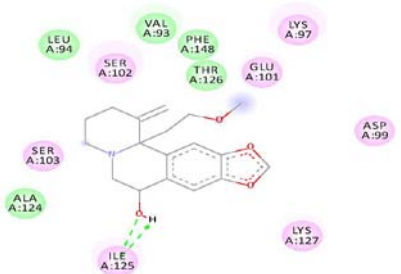
Conformation of cytisine bound to nAchR–interactions are shown in yellow lines

Fig. 3: Erythrinine and Cytisine Docked to active site and Its interaction to nAchR

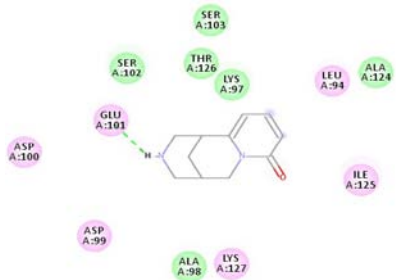
Table 2: Predicted biological activity of erythrinine analogues

Compounds	Predicted Pki	Peoe_Vsa_Hyd	Smr_Vsa0	Bcut_Smr_1	Q_Vsa_Fpneg
com_1	1.285306264	296.89053	67.861771	-0.1942066	0
com_2	3.685077455	254.19179	80.724098	-0.24839555	0
com_3	0.109832414	284.7399	56.861881	-0.20211932	0
com_4	0.25954891	274.57578	56.861881	-0.20726052	0
com_5	0.417220912	259.65903	56.861881	-0.20330851	0
com_6	0.086956672	285.73563	56.861881	-0.20009468	0
com_7	-0.049114907	297.81662	56.861881	-0.2017438	0
com_8	-0.09150026	301.18179	56.861881	-0.20137261	0
com_9	3.40339969	271.42981	80.724098	-0.23453201	0
com_10	4.333036209	291.9856	91.723984	-0.19975716	0
com_11	-0.196280385	302.01337	56.861881	-0.18380708	0
com_12	-0.292741211	310.96136	56.861881	-0.18582951	0
com_13	0.282951285	284.35535	56.861881	-0.23334116	0
com_14	3.622315183	263.97134	80.724098	-0.25851086	0
com_15	0.598474259	306.6701	67.861771	-0.088689245	0
com_16	4.14023541	301.76517	91.723984	-0.18577792	0
com_17	3.358855075	281.20938	80.724098	-0.24802288	0
com_18	5.865277294	295.54553	105.03357	-0.18578117	0
com_19	1.484381485	259.54086	63.947495	-0.23027033	0.052016817
com_20	0.7894896	279.39166	56.861881	-0.31616014	0

Compound 12, 11, 8 and 7 were found to be highly active with inhibitory constants in the range of nanomolar concentrations.

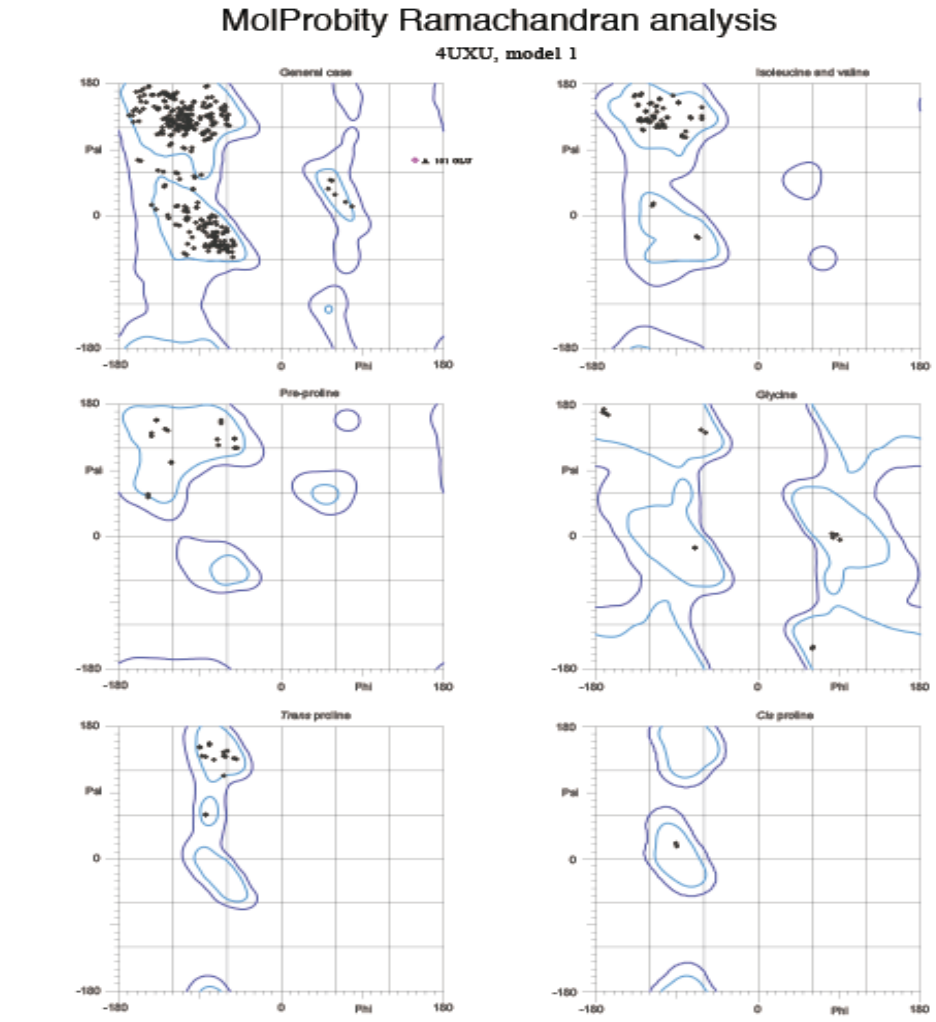


Ligand Interaction Map–Erythrinine



Ligand Interaction Map Cytisine

Fig. 4: Ligand interaction map of erythrinine and cytosine



96.2% (404/420) of all residues were in favored (98%) regions.
99.8% (419/420) of all residues were in allowed (>99.8%) regions.

There were 1 outliers (phi, psi):
A 101 GLU (149.9, 76.2)

Fig. 5: Molprobity Ramachandran analysis of 4UXU

DISCUSSION

A Classical quantitative structure-activity relationship study has to primary objectives: Development of common pharmacophore and prediction of the biological activity of unknown from the known. Therefore, retrieval of half minimal inhibitory concentrations (IC50) is an important aspect of setting dependent variables for a QSAR model. However, as far as smoking cessation is concerned, much extensive studies have not been carried out prior and hence, IC50 values are not available. Another important measure of biological activity is the inhibitory constant. It can be calculated using computational systems from the binding entropy of the system. Autodock 4.2 uses an empirical formula to calculate the inhibitory constant of small molecules against macromolecular targets. We exploited this phenomenon to build a biological activity model, not the conventional structure-activity relationship one, but a structure-affinity relationship one. i. e a multiple regression model was built with inhibitory constants derived from multiple docking analyses in place of half minimal inhibitory concentrations.

Ramachandran plot

Peptide bonds in a protein are incapable of rotation because of carbon-carbon double bonds. Hence, the other two bonds in the main chain are capable of rotation to provide a flexible planar confirmation. These bonds are called as phi and psi dihedral bonds. A Ramachandran plot measures the dihedral angles and defines them in terms of spatial geometries of allowed and favored regions as no two residues cannot spatially occupy in the same position. The total amino acids in the protein were 420 out of which 419 (99.8%) were in allowed region and 404 (96.2%) residues were in favored region (98%) as determined Molprobity analysis. The glutamine residue at position 101 was found to be an outlier. (fig. 5)

Molecular docking analyses

Autodock analyzes the interactions of small molecules with active sites of large peptide assemblies in terms of binding energy (ΔG), hydrogen bonding interactions, π-π interactions, and ligand conformation within the active site and root means square deviation (RMSD) of the active site residues. The free energy of binding is calculated using the empirical formula,

Binding energy (ΔG) = Intermolecular energy+Vanderwaal's hydrogen bond desolvation energy+Electrostatic energy+Total internal energy+Torsional energy–Unbound energy of the system. The similarity of docked structures is measured by computing the root mean square deviation and clusters are created based on the comparison of conformations and estimated RMSD values.

It was observed that oxygen atom in Erythrinine interacts with GLU101 through backbone electron donation whereas the hydroxyl group interacts with ILE125 by acting as a side chain acceptor. In contrast, Glu101 was found to interact with the hydrogen atom in Cytisine through backbone electron donation.

Cytisine was superior to nicotine-replacement therapy for smoking cessation among dependent

Smokers motivated to quit [17]. A quantitative structure-affinity relationship model with a strong correlation coefficient of 0.96 endorses that Erythrinine having a strong affinity with α4β2 nACh receptors as Cytisine it shows that Erythrinine is an effective smoking-cessation aid for use as a first-line treatment for tobacco dependence Hence the comparative study concluded that Erythrinine, derivatives of Erythrina and its analogues alkaloids may use as antismoking agent.

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CONFLICT OF INTERESTS

Declared none

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