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Research Paper

Docking, QSAR and CoMFA Studies on Arecoline Analogues as Muscarinic Acetylcholine Receptor (mAChR) M_1 Agonists

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Muscarinic Acetylcholine Receptor (mAChR) is one of the important receptors in medicinal chemistry belonging to the family of G-protein-coupled receptor (GPCR). In the present study, 3D quantitative structure activity relationship (3D QSAR) studies involving comparative molecular field analysis (CoFMA) were performed on arecoline derivatives which were reported as inhibitors of M_1 mAChR [1-4]. The CoMFA model provided the most significant correlation of steric and electrostatic fields with biological activities. The information rendered by 3D QSAR model affords valuable clues to optimize the lead and design new potential inhibitors for M1 mAChR.

Key Words: Muscarinic; Alzheimer; CoMFA; Arecoline; Docking

Introduction

Muscarine and Nicotine are the first compounds to indicate that receptor selectivity was possible, but they have undesirable side effects resulting from their interactions with other receptors. In the search for a potent drug, it is important to gain selectivity for one class of receptor over another (for example the acetylcholine receptor in preference to a noradrenaline receptor), and selectivity between receptor types (for example, muscarinic receptor in preference to a nicotinic receptor). It is also preferable to gain selectivity for particular subtypes of receptor [5]. For example, not every muscarinic receptor is the same throughout the body. At present, four types of muscarinic receptors are known, named M1–M4 [6] and five subtypes of muscarinic receptors have been cloned and designated m1–m5 [7,8].

Activation of M1 mAChR by agonists modulates learning and memory [9, 10], protects cells from oxidative stress and mitochondrial impairment [11], blocks caspase activation in neurons [12], increases non-amyloidogenic processing of β-amyloid precursor protein, which are responsible for the formation of plaques in brains of Alzheimer's patients [13, 14], reduces the Aβ-peptide production [15-17], reduces the Aβ hyperphosphorylation [18, 19] and inducerepressive postsynaptic signals [20, 21]. Identifying M1 selective muscarinic agonists, which are capable of crossing the blood-brain barrier, is the subject of active research for pharmacological application [22].

An alkaloid from betel nut, arecoline, has been demonstrated as an active muscarinic agonist [23]. But it lacks M1 receptor selectivity, and stimulates M2 and M3 muscarinic receptor subtypes [23]. Substitution of the ester functional group of arecoline with either 3-alkoxy-1,2,5-thiadiazole [22] or 3-alkyl-1,2,4-oxadiazole [24] has produced potent muscarinic

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agonists. However, the systematic removal of a heteroatom in the 3-methyl-1,2,4-oxadiazole giving oxazoles and furans caused reduced affinity [24]. Although the exact receptor structure (M1 mAChR) is unknown, it is possible to infer the receptor properties by finding important commonalities of the arecoline analogues by Comparative Molecular Field Analysis (CoMFA) method which proves to be appropriate for such problems and hence used in this study.

The CoMFA methodology is a 3D QSAR technique that facilitates design and interprets activities of small molecules [25, 26]. This is based on shape complementarity and non-covalent interactions of the ligand and receptor [27]. QSAR study was carried out for designing better inhibitors as well as to understand inhibition effects of the designed compounds. A 3D-QSAR method, CoMFA is used to examine the QSAR for these compounds. Partial least square (PLS) [28] was used to correlate activity data from lattice field data sets. Many such QSAR studies succeeded in identifying drug candidates [29-31]. In the present study, we carried out CoMFA calculation to examine the correlation between the observed biological activity and the structures of arecoline derivatives [1-4] (Fig. 1) in order to gain a better understanding of functional groups responsible for activity and to further modify the functional groups in order to design more potent molecules.

Experimental Section

Datasets

The results of these reported inhibitory activities were used as dependent variables in this study. The IC50 values were converted to the corresponding pIC50 (–logIC50) and used as dependent variables in CoMFA analysis. The dataset includes *N*-aryl sulfonamide substituted 3-morpholino arecoline derivatives (A), *N*-aryl thioureas substituted 3-morpholino arecoline derivatives (B), *N*-aryl carboxamide substituted 3-morpholino arecoline derivatives (C) and *N*-alkyl/aryl substituted thiazolidinone arecoline analogues (D), respectively (Table 1). The structures of representative compounds from each dataset (A, B, C & D) are shown in Fig. 1.

Molecular Modeling and Alignment

The structures of the compounds were built using the sketcher tool provided in the modeling environment of SYBYL 7.3 software package (Tripos Inc., St. Louis, USA) running on Red Hat Enterprise Linux workstation. The molecules were subjected for energy minimization (geometry optimization) using

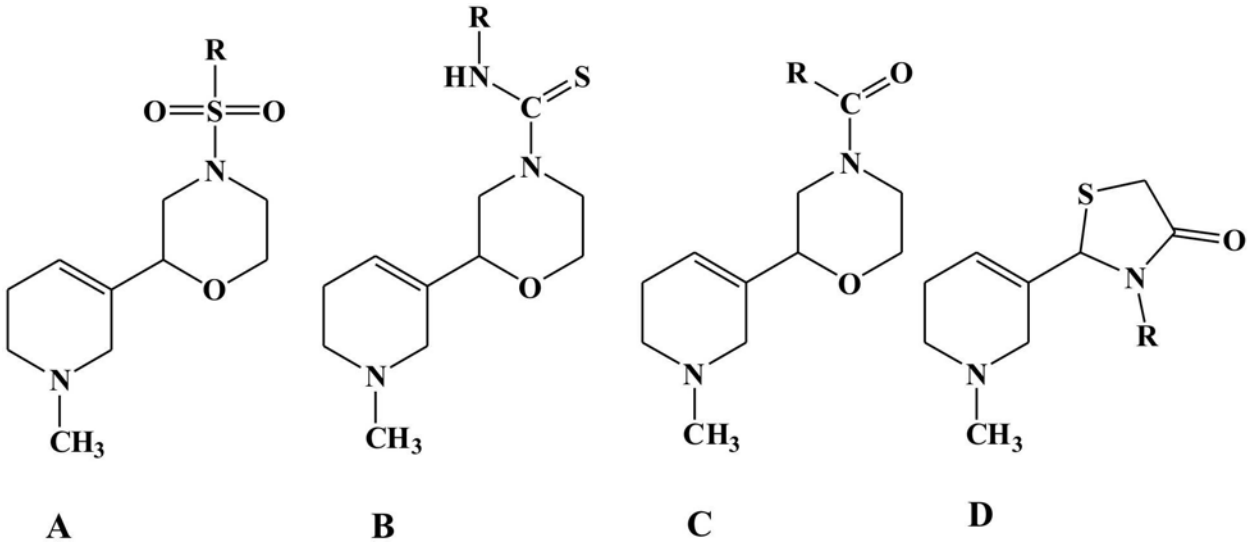

Fig. 1: Representative molecules from each data set (as listed in Table 1)

Table 1: Structures and biological activities of molecules used in the present study. A. *N*-aryl sulfonamide substituted 3-morpholino arecoline derivatives [1]; B. *N*-aryl thioureas substituted 3-morpholino arecoline derivatives [2]; C. *N*-aryl carboxamide substituted 3-morpholino arecoline derivatives [3]; D. *N*-alkyl/aryl substituted thiazolidinone arecoline analogues [4]

Arecoline derivatives	R	pIC50 (Experimental)	pIC50 (Predicted)	Residual
Arecoline	-	3.33724	2.71545	0.621789
A				
A1	4-methyl phenyl	4.21467	4.63802	–0.423353
A2	4-tert-butyl phenyl	4.72125	4.37621	0.345032
A3	4-chloro phenyl	6.03621	5.38495	0.651260
A4	2,5-dichloro phenyl	3.9393	4.23694	–0.297638
A5	phenyl	4.04576	4.54913	–0.503368
A6	2-nitro phenyl	3.50307	3.03184	0.471226
A7	3-nitro phenyl	3.6968	3.86211	–0.165311
A8	4-nitro phenyl	3.68194	3.75978	–0.077848
B				
B1	2-methoxy phenyl	3.44129	3.30346	0.137828
B2	3-methoxy phenyl	3.92082	4.14488	–0.224058
B3	4-methoxy phenyl	4.82391	4.8099	0.014012
B4	2-chloro phenyl	3.28819	2.95766	0.330532
B5	3-chloro phenyl	3.62709	3.75611	–0.129024
B6	4-chloro phenyl	5.92082	4.82204	1.098770
B7	2-fluoro phenyl	4.17393	4.49077	–0.316840
B8	4-fluoro phenyl	4.50864	4.726	–0.217357
B9	2,4-dichloro phenyl	3.95078	4.29915	–0.348368
B10	phenyl	3.81531	4.16081	–0.345499
C				
C1	phenyl	4.11919	4.33916	–0.219973
C2	4-methyl phenyl	5.79588	5.09286	0.703023
C3	4-chloro phenyl	5.39794	4.35463	1.04331
C4	4-tert-butyl phenyl	Nil	Nil	Nil
C5	2,4-dichloro phenyl	Nil	Nil	Nil
C6	2-nitro phenyl	3.20901	2.65103	0.557981
C7	3-nitro phenyl	3.84771	3.50502	0.342695
C8	4-nitro phenyl	3.66756	3.63663	0.030929
D				
D1	4-chloro phenyl	3.18509	3.19258	–0.007492
D2	benzyl	3.10292	3.09499	0.007937
D3	ethyl	3.17393	3.18728	–0.013357
D4	phenyl	3.05257	2.97818	0.074382
D5	hexyl	3.19928	3.25252	–0.053240
D6	butyl	4.16749	4.12537	0.042120
D7	isopropyl	3.23732	3.27223	–0.034909
D8	4-bromo phenyl	3.27084	3.27051	0.000326
D9	4-carboxy phenyl	4.13077	4.10725	0.023515
D10	4-(phenylamino) phenyl	4.31876	4.32128	–0.002516
D11	4-nitro phenyl	4.31876	4.34833	–0.029574
D12	4-methyl-2-nitro phenyl	3.20343	3.24279	–0.039369
D13	3-hydroxy phenyl	3.24949	3.21732	0.032176

the standard Tripos force field (Powell method and 0.05 kcal/(mol. Å) energy gradient convergence criteria) and electrostatic charge was assigned by the Gasteiger-Hückel method. Finally, the molecules were named and saved in appropriate databases. CoMFA results may be extremely sensitive to a number of factors such as alignment rules, overall orientation of the aligned compounds, lattice shifting step size and probe atom type [32]. The accuracy of prediction of CoMFA models and the reliability of the contour models depend strongly on the structural alignment of the molecules [32]. Thus, we applied molecular alignment to align all the molecules used in the present study.

CoMFA Modeling

CoMFA modeling studies were performed using the method followed by Ramasamy *et al*. [33]. The molecules were caged in a 3D lattice of 2.0 Å and CoMFA interaction fields were calculated by employing Lennard-Jones and Coulomb potentials. This set up mimics the pharmacophoric pattern and their non-covalent interaction with the receptor. The probe used to calculate CoMFA fields consisted of sp3 carbon atom with +1.0 charge. The steric and electrostatic energies were truncated at 30.0 kcal/mol. The field values were stored in SYBYL molecular spreadsheet for statistical analysis.

Partial Least Square Analysis

The regression analysis of CoMFA field energies was performed using the partial least squares (PLS) algorithm [33]. PLS regression analysis was performed alongwith the cross-validation option (leave-one-out (LOO) method). The column filtering value (σ) of 2.0 kcal/mol was assigned for cross-validated analysis. The optimal number of components was chosen from the highest cross validated r^2 values in the non-cross-validated analysis.

CoMFA Contour Maps

The CoMFA steric and electrostatic contour plots were the product of the associated standard deviation and the coefficient at each lattice point [34]. The CoMFA coefficients with respect to each sampled field point in the correlation equation were

graphically contoured as percentage contribution.

Docking

The homology modeled structure of M1 mAChR was used for the study. The protein was prepared by removing all water molecules and adding all hydrogen atoms using the Accelrys Discovery Studio ver 1.7 (Accelrys, Inc., San Diego.). The ligands were docked into the active site using the molecular docking software SYBYL ver 7.3 (Tripos, L.P.) Surflex-Dock (BioPharmics LLC.) with the default parameters. Both the proprietary software is licensed to Manipal Institute of Technology, Manipal University, India. Surflex-Dock is a program for calculating the docking modes of small molecules into protein-binding sites. In this study we have used ChemScore, a scoring function that is derived from regression against ligand-receptor binding free energies. In the docking process the active site was defined. For each ligand, 20 conformations were generated (40x20=800 conformations) and then docked into M1 mAChR.

Results and Discussion

A template model for human M1 mAChR has been scanned using InterProScan [35]. High resolution crystal structures of human B2 adrenergic receptor (2rh1A), cholesterol bound form of human beta2 adrenergic receptor (3d4sA), Turkey B1 adrenergic receptor (2vt4B), beside these templates, squid rhodopsin and bovine rhodopsin were also included as templates to build a model of M1 mAChR. The model was built by using an automated homology modeling server, Swiss Model [36].

Molecular modeling was carried out to overlay both active and inactive compounds of several analogues [1-4]. Three dimensional conformations for each arecoline analogues were modeled. The space occupied by each molecule was then analysed and compared with its affinity for the receptor. These molecules were subjected for energy minimization and geometry optimization using the standard Tripos force field, Gasteiger-Hückel charges, by conjugate gradient method to a convergence gradient before alignment. Optimized structures were aligned (shown in Fig. 2) and used for CoMFA.

CoMFA potential fields were calculated based on 2.0 Å lattice spacing with boundaries extended beyond 4 Å in all directions. Van der Waals potentials and Coulombic terms, which represent steric and electrostatic fields, respectively, were calculated using Tripos force field. The probe used to calculate the CoMFA fields (steric and electrostatic) consisted of sp3 hybridized carbon atom of van der Waals radius 1.52 Å with +1 charge. The steric and electrostaticfields were calculated at each lattice intersection using Lennard–Jones and Columbic electrostatic potentials.

Partial Least Square (PLS) analysis was employed to obtain correlation between the descriptors derived by CoMFA and pIC50 values. The minimum sigma (column filtering) was set to 2.0 kcal/mol to accelerate the regression analysis and to reduce the noise. Initially, leave-one-out (LOO) cross validation method was carried out to check the predictivity of the derived model and to determine the optimum number of components with minimum standard error of estimate. Lists of the experimental activities, estimated activities and residual values of the data set by CoMFA model are presented in Table 1. It is observed that the predictions made using CoMFA model matches with the experimental results. The conventional correlation coefficient (r^2) was used to measure the quality of the model. The CoMFA PLS yielded cross–validated correlation coefficient $r^2 > 0.5$ for all the analyzed arecoline derivatives. The conventional correlation coefficient (r^2) was found to be 0.721 ($y = 0.721x + 1.178$) for the *N*-aryl sulfonamide substituted 3-morpholino arecoline derivatives (A), 0.670 ($y = 0.670x + 1.364$) for *N*-aryl thioureas substituted 3-morpholino arecoline derivatives (B), 0.821($y = 0.808x + 0.364$) for *N*-aryl carboxamide substituted 3-morpholino arecoline derivatives (C) and 0.995 ($y = 0.995x + 0.017$) for *N*-alkyl/aryl substituted thiazolidinone arecoline analogues (D). It is noted that the correlation between the predicted pIC50 of the arecoline derivatives (D) is in very close agreement with the experimentally determined value. These correlation coefficients suggest that the model proposed is reliable and accurate. Fig. 3 shows the correlation between the experimental and predicted pIC50 values of data sets

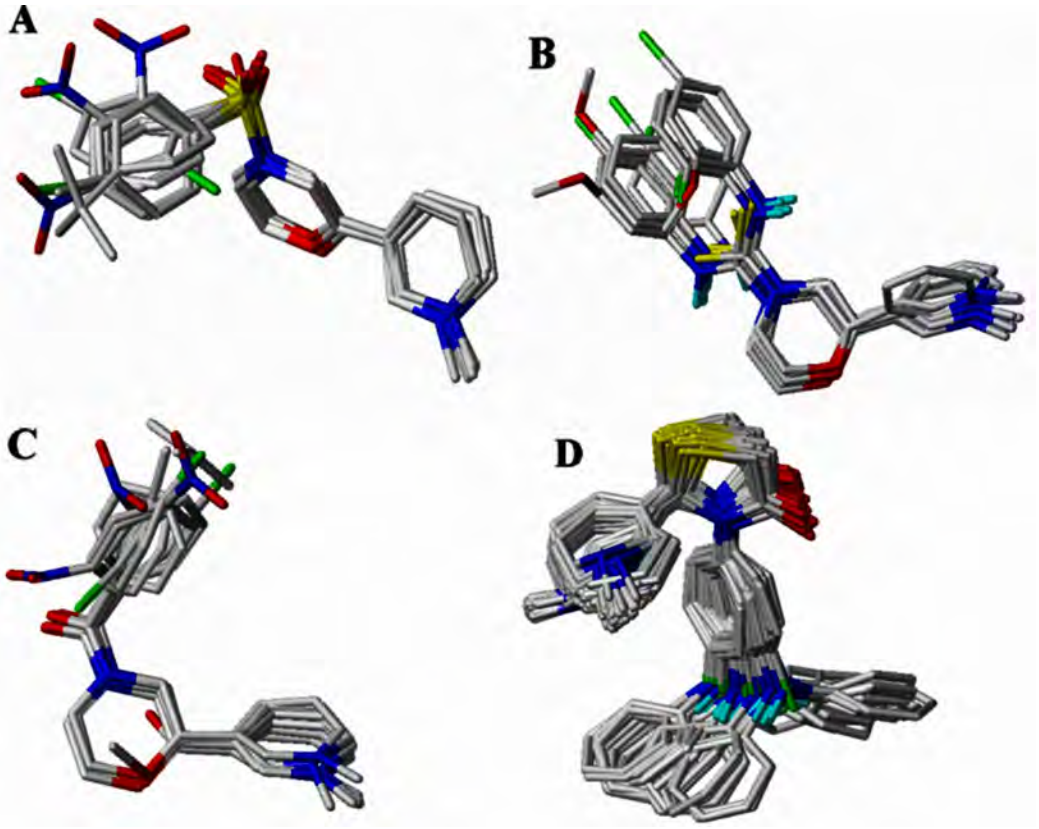

Fig. 2: The molecular alignments of (A), (B), (C) and (D) used for CoMFA

by CoMFA model.

CoMFA contour maps were generated to visualize the information content of the derived 3D-QSAR model. The contour plots are the representation of the lattice points and the difference in the molecular field values at lattice points is strongly connected with difference in the receptor binding affinity. Molecular fields define the nature of interaction energies of aligned molecules with a probe atom traversing across the lattice grid points surrounding the molecules. The steric interactions are represented by green and yellow coloured contours whereas electrostatic interactions are displayed as red and blue contours. Figs. 4 (A, B, C, D) show the contour maps derived from the CoMFA PLS model.

The QSAR tools were applied to find the best predictive model of the system, using cross-validation. Structure activity relationships were drawn from the CoMFA PLS model for all the derivatives of the series A, B, C and D. Among sulphonamide derivatives A(1-8) it is observed that the sulfonylated aromatic ring which is in the yellow contours indicating more bulky group will decrease the activity. Further it was observed that the most potent compound was A3 with chlorine group at the para position. The di-substituted chlorine compound A4 at ortho and meta positions reduces the affinity of M1 mAChR. Methyl group at para position A1 also exhibited considerably high affinity and potency for the receptor and showed the methyl group positioned in green contour. Substituting the methyl group with more bulky group will increase the activity as indicated by green contour. On the other hand, substitution of electron withdrawing group such as NO_2 on the aromatic ring, reduced the affinity and potency of the compounds for the M1 mAChR [1]. Among the derivatives A(1-8), those having electron withdrawing groups, NO_2 at meta position A7 showed considerable high affinity when compared to ortho position A6 and para position A8. The contour map of steric and electrostatic fields is shown in Fig. 4(A).

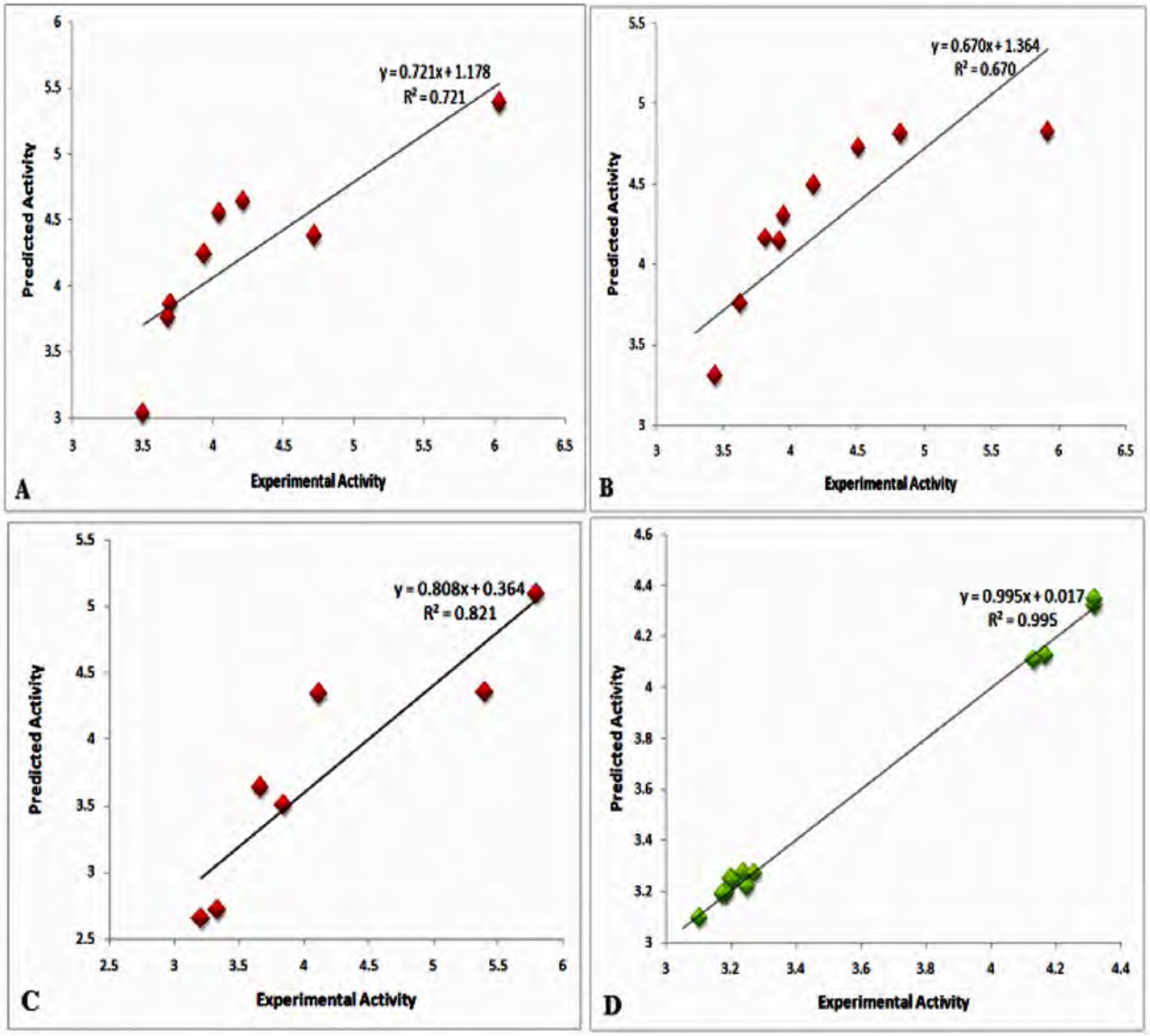

Fig. 3: The graphs A, B, C & D show the plot of pIC50 experimental (X-axis) versus pIC50 predicted (Y-axis). Compounds in the upper right corner are the most active

Among derivatives of the series B, compound B6 with chlorine at the para position of the aryl group attached to the nitrogen of thiourea is found to be more potent. Substitution of methoxy group (B3), at para position of the aryl group showed that the methoxy group is positioned in green contour, indicating the increase in the activity. However, when chloro and methoxy groups are substituted at meta position (B5 and B2, respectively) they showed moderate affinity, and when they are substituted at ortho position (B4 and B1, respectively) showed least or no affinity, respectively, for M1 mAChR *in vitro* [2]. Introduction of fluoro group at para position (B8) showed good affinity for the M1 receptor, whereas when introduced at ortho position (B7) it decreases the affinity of compound for the receptor *in vitro*. However, disubstituted chlorine (B9) at ortho and para positions showed only moderate affinity for the receptor. Compound B10 with benzene (without any substitution on it) ring substituted on the nitrogen of thiourea showed average affinity towards M1 mAChR *in vitro* [2]. The contour map of steric and

electrostatic fields is shown in Fig. 4(B).

Among the derivatives of derivatives in the series C, compound C2 with methyl group at the para position of the aryl group attached to the carboxamide of morpholino arecoline showed that three fourth of the molecule is positioned in green contour, indicate the increase in the activity. Substitution of chlorine group at para position as in C3 also showed good affinity towards M1 mAChR *in vitro* [3]. However, disubstituted chlorine at 2 and 5 positions (C5) and also tertiary butyl substituted derivative (C4) at the para position of the aryl group attached to carboxamide of morpholino arecolines, showed no affinity for M1 mAChR *in vitro* [3]. On the other hand substitution of nitro group at the different positions of the aryl group attached to the carboxamide of morpholino arecolines C(6-8), reduced the affinity and potency of the compounds for M1 mAChR. Among the derivatives having electron withdrawing NO_2 group at the aryl group, NO_2 at meta position, the compound C7 showed considerable high affinity when compared with the compound C8 at para position, while NO_2 at ortho position, the compound C6 showed least affinity. Compound C1 having phenyl group without any

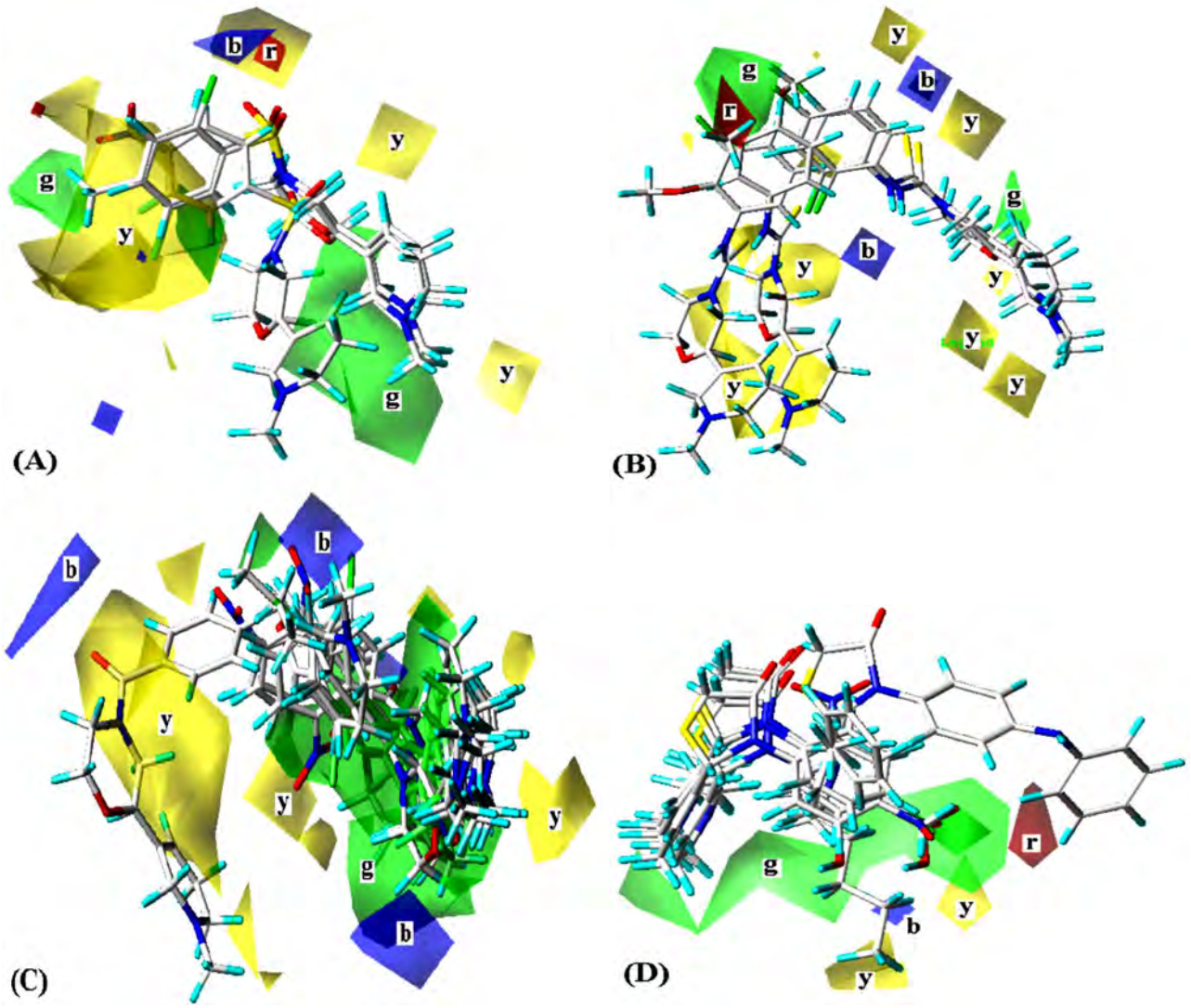

Fig. 4: CoMFA contour plots show steric and electrostatic field distributions for arecoline derivatives (A, B, C, D). Green (g) contour maps for sterically favoured areas and sterically disfavoured areas in yellow (y). Positive potential favoured areas in blue (b), negative potential favoured areas in red (r)

substitution on it, attached to the carboxamide of morpholino arecoline showed moderate affinity to M1 mAChR *in vitro* [3]. The contour map of steric and electrostatic fields is shown in Fig. 4(C).

CoMFA contour maps for D series compounds revealed that D6, D9, D10, and D11 showed greater affinity and potency towards M1 mAChR. The remaining derivatives did not show any agonistic activity [4]. The most potent compound D10 which is in the red contour area, indicates that electronegative substituents are favourable for interactions with the receptor. Among the compounds with alkyl chain as substituent, D3 having ethyl group did not show any agonistic activity. But replacement of the same with isopropyl chain or n-butyl chain increases the activity. Interestingly, further increase of the length of the alkyl chain by another two methylene groups (hexyl, compound D5) activity decreases drastically. This is clearly substantiated by the yellow contour area at the bottom, indicating more bulky group will decrease the activity. This suggests that, alkyl group with four carbon atoms may be the optimum length for the binding affinity. The contour map of steric and electrostatic fields is shown in Fig. 4(D).

As a check on authenticity of the receptor model, substituted analogues of arecoline (A, B, C & D) were modelled and a 3D-QSAR model derived. Each structure was docked into the model receptor and the receptor-ligand complex minimized. Affinity values for the structures used in the analysis were calculated and a graph of predicted affinity versus actual affinity showed a good relationship. The binding cavity of the M1 mAChR model is composed of Asp105, Tyr106, Tyr381, Asn382, Tyr404, Cys407 and Tyr408. Docking studies of the substituted analogues of arecoline [1-4] was carried out. All the substituted analogues of arecoline (A, B, C & D) were fitted exactly within an aromatic cage of amino acids in the binding pocket. The preponderance of aromatic residues is consistent with the composition of the binding sites of the nicotinic receptor [37] and acetylcholinesterase [38], and in accordance with the theoretical studies of Ma and Dougherty [39].

Docking studies revealed that the most potent compounds of the substituted analogues of arecoline (A, B, C & D) were A3, B6, C2 and D10, respectively. These results were in perfect agreement with the *in vitro* affinity assay [1-4]. Among the potent compounds, *N*-aryl substituted thiazolidinone arecoline analogue D10, being the most promising. The potency was perfectly correlated with the docking analysis *in silico*. The amine group of the compound D10 was interacting with OH group of Tyr404 and OD1 & OD2 of Asp105 by triple hydrogen bonds in the binding cavity. The head group of the compound D10 is located within an aromatic cage composed of Tyr106, Phe197, Tyr198, Trp378, Tyr404 and Trp405 (Fig. 5). These results were in agreement with what had been expected from the proposed receptor binding model. From these results, it is inferred that the 3D QSAR model generated can be successfully expanded to predict the activity of structurally diverse compounds, which will be used in designing new chemical entities and predicting their activity.

Conclusion

In conclusion, the 3D QSAR using CoMFA method has been successfully applied to a set of synthesized arecoline derivatives [1-4]. The contour plots provide many useful insights into relationships between structural features and inhibitory activity and also give a picture of the main chemical features responsible for the good inhibitory activity. The results from this study will be helpful to design new candidate compounds with potent inhibitory activity.

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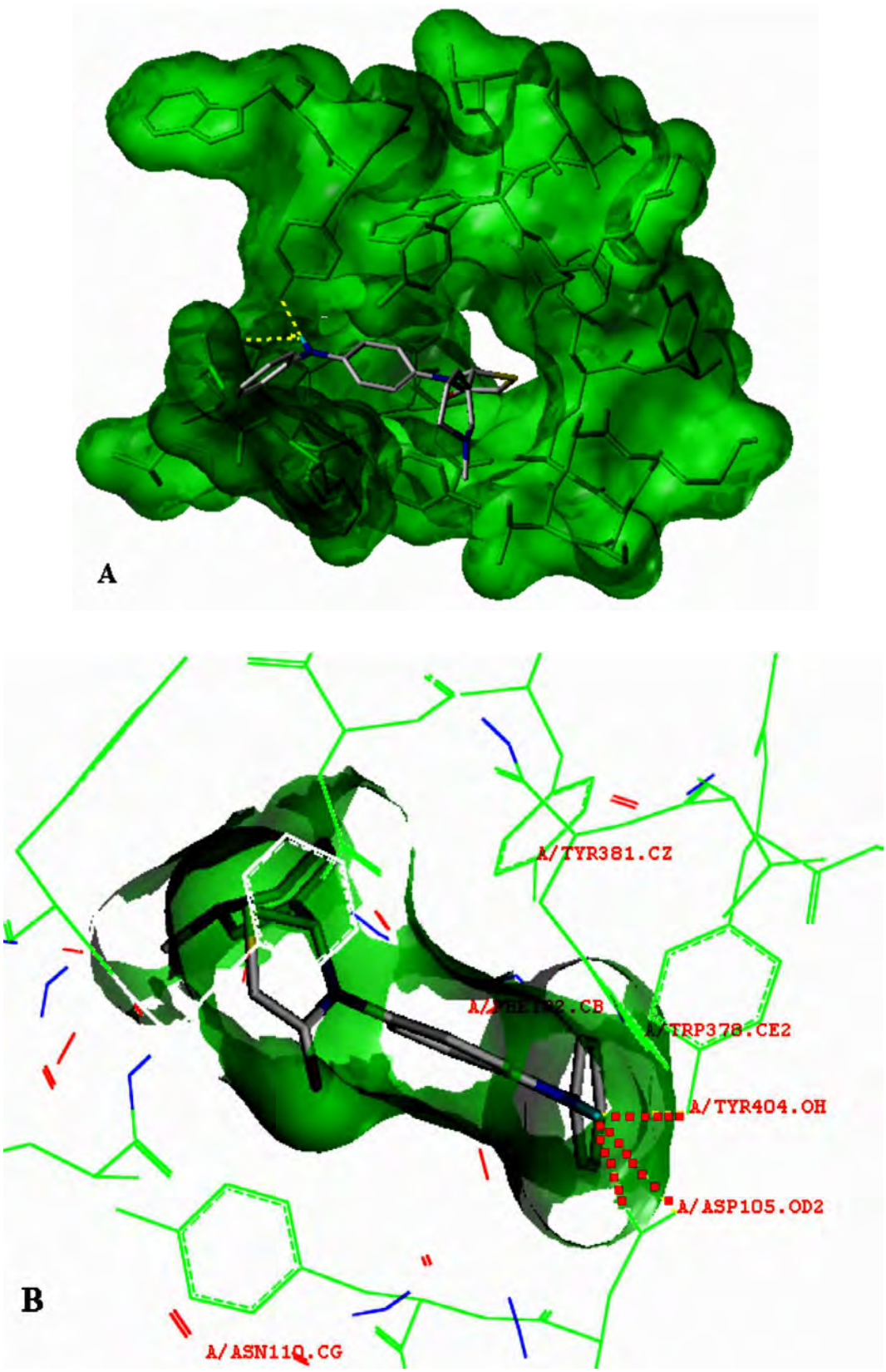

Fig. 5A: The head group of the compound D10 is in the aromatic cage of the modelled structure. B: D10 and its interactions with M_1 mAChR

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