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Pharmacophore Modelling, Quantitative Structure Activity Relationship (QSAR) and Docking Studies of Pyrimidine Analogs as Potential Calcium Channel Blockers

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ABSTRACT. The present communication deals with the Pharmacophore modeling, 3D QSAR and docking analysis on series of Pyrimidine derivatives as potential calcium channel blockers. The computational studies showed hydrogen bond donor, hydrogen bond acceptor, and hydrophobic group are important features for calcium channel blocking activity. These studies showed that Pyrimidine scaffold can be utilized for designing of novel calcium channels blockers for CVS disorders.

Key words: Pyrimidine, Calcium channel blocker, QSAR

INTRODUCTION

Calcium ions plays a vital role in the cardiovascular system, they impart their action by acting on the calcium channels. The entry of calcium inside the cell can increase the vascular tone as well as heart rate and ultimately the blood pressure. The currently approved calcium channel blockers binds with L-type calcium channels located on the vascular smooth muscle, cardiac myocytes, and cardiac nodal tissue. These channels are responsible for regulating the influx of calcium into muscle cells,[1–3] which in turn stimulates smooth muscle contraction and cardiac contraction. Therefore, by blocking calcium entry into the cell, will cause vascular smooth muscle relaxation (vasodilation), decreased myocardial force generation decreased heart rate (negative chronotropy), and decreased conduction velocity within the heart (negative dromotropy) at the AV node. The 1,4-dihydropyridine (DHP) class of calcium channel blockers are widely utilized in the treatment of cardiovascular diseases such as hypertension, angina pectoris and other spastic smooth muscle disorders.[4–6] The SAR of calcium channel blockers signifies the presence of ester linkage and electron withdrawing group like nitro and carbonyl groups. The Pyrimidine nucleus has been showing the various pharmacological activities like calcium channel blockers, anti cancer, antimicrobial, antiviral. In this communication an attempt is made to design and develop novel calcium channel blockers based on the Pyrimidine scaffold and identification of structure requirement of these molecules in the form of 3D descriptors and Pharmacophoric features for optimization of these ligands.

EXPERIMENTAL PROTOCOLS

Synthesis of Training Set (01–24)

All the molecules under study were taken from our previously published work.[7]

Pharmacological Screening

Smooth muscle relaxant activity

The synthesized compounds 1–24 were tested in vitro for their pulmonary vein relaxant activity. Pulmonary veins and arteries of adult goat of either sex were brought from a local slaughterhouse. The Media used to carry the muscle was ice-cold Krebs–Henseleit solution. These were cut into spiral strips and were used within 12–24 h. These strips were mounted in 15 ml isolated organ baths, containing Krebs–Henseleit solution, mixed with 95% O_2 and 5% CO_2 at 37 °C. The strip was allowed to equilibrate for 2 h under a resting load of 2 g. Relaxation of muscle strip was recorded for each drug using force transducer multichannel physiograph (BIOPAC MP35 SYSTEM). The title compounds were compared with nifedipine, a standard drug used for relaxation. Alcohol was used as control.

Ligand Preparation:

The structure of 5-(ethoxycarbonyl)-6-methyl-2-oxo-2, 3-dihydropyrimidine was used as the template to built the molecules in the dataset in builder module of Vlife MDS 3.5. The ligand geometries were optimized by energy minimization using MMFF94 forcefield and Gasteiger-Marsili charges for the atoms, till a gradient of 0.001 kcal/mol/Å was reached, maintaining the template structure rigid during the minimization.

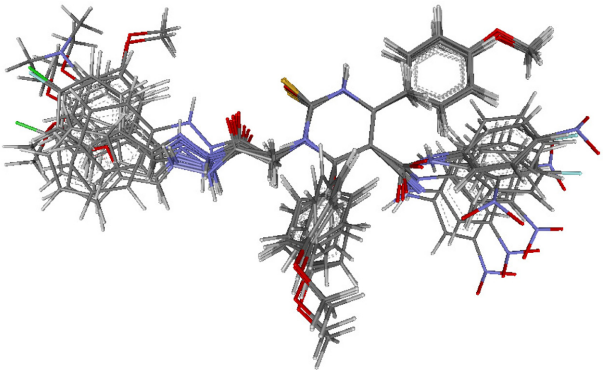


***Fig.* 1.** Figure showing alignment of molecules used in study.

Molecular alignment:

The molecules of the dataset were aligned by the template based technique, using the most active molecule as a template for alignment of the molecules. The alignment of all the molecules on the template is shown in *Fig*. 1.

Descriptor Calculation:

Like many 3D QSAR methods, a suitable alignment of given set of molecules was performed using the Vlife MDS 3.5 Engine. This was followed by generation of a common rectangular grid around the molecules. The hydrophilic, steric and electrostatic interaction energies are computed at the lattice points of the grid using a methyl probe of charge +1. These interaction energy values are considered for relationship generation and utilized as descriptors to decide nearness between molecules. The term descriptor is utilized in the following discussion to indicate field values at the lattice points.

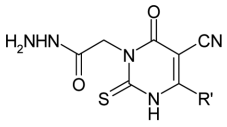
Data Set:

The dataset was divided into a training set and test set on the basis of chemical and biological diversity using the random selection method for generation of the training and test set data. The molar Inhibitory concentration ($_pED_{80}$) values for Smooth muscle relaxant were used for the present 3D-QSAR study.

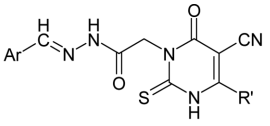
Full Search Multiple Linear Regression Method:

A relationship between independent and dependent variables (3D fields and biological activities, respectively) were determined statistically using regression analysis. Linear regression is achieved by fitting a best-fit straight line to the data using the least squares method. The quality of fit for a regression equation was assessed relative to its correlation coefficient and standard deviation. The F value represents the level of statistical significance of the regression. Quality of selected models was further ascertained to select the best model from cross-validated squared correlation coefficient (q^2). The selected models for the calcium channel blocker activity are shown in *Table* 2.

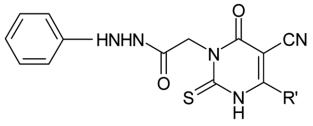
***Table* 1.** Table showing different substitutions performed in the pyrimidine ring



(AH1 or BH1)

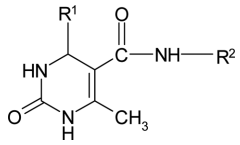


(AH3 - AH8, BH3 -BH6)



(AH2 or BH2)

Sr. No.	Comp code	R'	Ar
1	AH1	4-methoxy phenyl	–
2	AH2	4-methoxy phenyl	–
3	AH3	4-methoxy phenyl	4-chloro phenyl
4	AH4	4-methoxy phenyl	4-fluro phenyl
5	AH5	4-methoxy phenyl	3,5-dimethoxy phenyl
6	AH6	4-methoxy phenyl	furfural
7	AH7	4-methoxy phenyl	4-dimethylamino phenyl
8	AH8	4-methoxy phenyl	3,4,5-trimethoxy phenyl
9	BHI	Phenyl	–
10	BH2	phenyl	–
11	BH3	phenyl	4-chloro phenyl
12	BH4	phenyl	4-methoxy phenyl
13	BH5	phenyl	phenyl
14	BH6	phenyl	4-methyl phenyl



Sr. No.	Comp code	R^1	R^2
15	DA	phenyl	phenyl
16	DB	phenyl	2-nitrophenyl
17	DC	phenyl	3-nitrophenyl
18	DD	phenyl	4-nitrophenyl
19	DE	phenyl	4-fluro phenyl
20	DF	4-methoxy phenyl	phenyl
21	DG	4-methoxy phenyl	2-nitrophenyl
22	DH	4-methoxy phenyl	3-nitrophenyl
23	DI	4-methoxy phenyl	4-nitrophenyl
24	DJ	4-methoxy phenyl	4-fluro phenyl

Activity prediction:

To systematically assess a QSAR model, a reliable validation is required. Usually, a QSAR model is evaluated by the predictive results for the given dataset. Selected models having r^2 above 0.7 were checked for their exter-

***Table* 2.** Table Showing the selected QSAR equations along with statistical parameters employed for model selection

Model No.	QSAR model	N	r^2	q^2	F value	Pred r^2
A	pED_{80}=2.2508+0.2269(±0.0015) S_349+1.1525(±0.0650) S_338-0.0424(±0.0000) S_755+0.0437(±0.0000) S_844−0.1189(±0.0006) E_446−0.1033(±0.0029) S_360	24	0.9692	0.9299	224.15	0.8658

***Table* 3.** Table showing the observed and predicted activity by QSAR equations along with the residuals

Sr. No.	Observed activity	Predicted activity	Residuals
1	2.84	2.76	−0.14
2	1.37	1.36	0.15
3	2.13	1.90	−0.39
4	2.13	1.93	0.02
5	1.29	1.25	−0.11
6	1.95	2.86	0.44
7	0.89	1.06	−0.40
8	2.05	2.20	−0.26
9	1.18	1.03	−0.02
10	2.16	2.55	0.06
11	2.08	2.06	−0.38
12	2.18	2.29	0.18
13	2.19	1.75	−0.57
14	2.12	2.52	1.00
15	2.16	2.43	0.63
16	2.12	2.14	0.30
17	2.15	2.09	−0.31
18	2.25	2.63	−0.30
19	2.12	1.93	0.16
20	2.09	2.67	0.26
21	2.18	1.18	0.09
22	2.84	2.76	−0.14
23	1.37	1.36	0.15
24	2.13	1.90	−0.39

nal predictivity. The observed and the predicted values for calcium channel blocker activity are shown in *Table* 3.

Docking Studies:

Docking simulation was carried out to explore the inhibition mode for the molecules under study. We conducted docking simulation using Biopredicta module of V life MDS 3.5 using crystal structure of the L type calcium channels PDB ID 1T0J.

Pharmacophore modeling

Pharmacophore modeling was also carried out in Vlife MDS 3.5 using Mol sign module. A pharmacophore model is a set of three dimensional features that are necessary for bioactive ligands. Thus, it makes logical sense to align molecules based on features that are responsible for bioactivity, the number indicates the minimum number of pharmacophore features generated for an alignment is taken 4 and tolerance is kept to 10 Å. The max distance allowed between two features is kept to 10 Å. Pharmacophore identification is also carried out on the calcium channel blockers in clinical use (Nifedipine) to indentify the pharmacophoric features and compared with the molecules under study.

RESULTS AND DISCUSSION

In the present study, 24 molecules were used in the training set (*Table* 1) to derive QSAR models with the number of field grid points being not more than six per model. On successful runs of MLS, different sets of equations were generated and these equations were further analyzed statistically to select the best model. As shown in *Table* 2, two models were selected after screening various combinations of different descriptors.

Interpretation of QSAR Model

The model A which is generated through QSAR module of V life MDS 3.5 describes the optimum structural features that are required for the calcium channel blocker activity of pyrimidine analogs. The steric and electrostatic fields were calculated using the Tripos force field and Gasteiger-Marsili charges. A training set of 20 molecules, and a test set of 04 molecules. The model was selected on basis of r^2, q^2, pred r^2, F and p values. The r^2 value for model A was 0.9635 compared to that of model B 0.95930. The F test and p significance values were considered for the selection of model. Variations of steric and electrostatic properties in the structural features of the compounds in the data set led to an increase or decrease in binding affinities and selectivity's. The steric interaction fields are represented in green lattice points at S349, S338, S844 implies that the steric interaction along these lattice points are required to be addressed and interaction at the points like S349, S338, S844 are positively contributing are so the compounds which are having the bulky substituents at the aromatic ring can show the increased activity. In the pharmacophore optimizations study it is also clear that the when the chain length of carbon or any substituent which is imparting the hydrophobic character can lead potent calcium channel blocker. The steric interac-

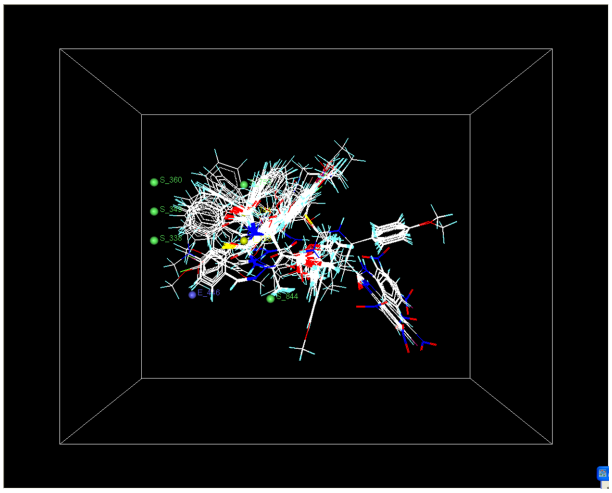


***Fig.* 2.** Figure showing the field points used in the QSAR model.

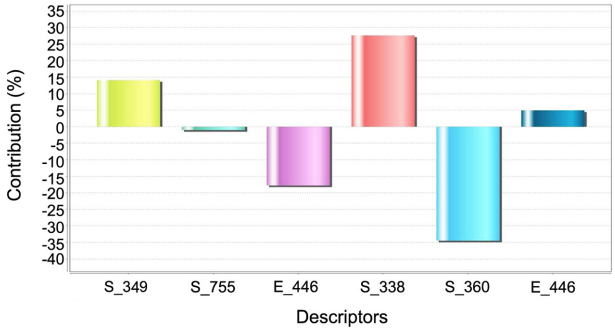


***Fig.* 3.** Contribution plot for descriptors in Model A.

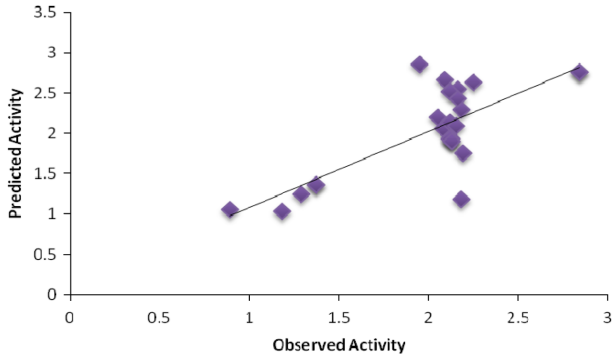


***Fig.* 4.** Correlation plot of observed activity and predicted activity.

tion along lattice point S360 contributing negatively so they need to be reduced. The electrostatic interaction at the lattice point E446 which is contributing negatively, so substitution of electron withdrawing groups can increase the activity (*Figs.* 2, 3, 4).

Docking Results

Molecular docking

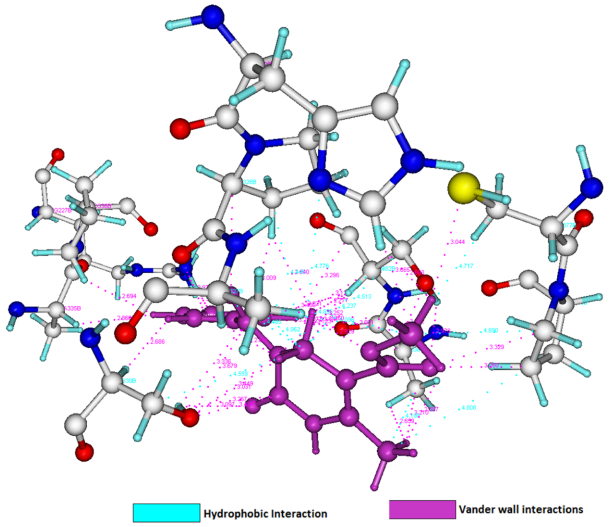


***Fig.* 5.** Figure showing the top posed docked molecule in the active site L type Calcium channel.

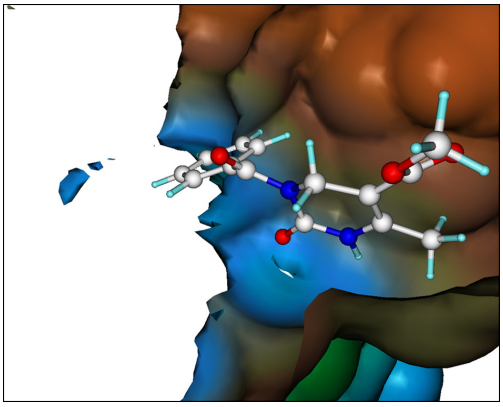


***Fig.* 6.** Figure showing the top posed docked molecule in the active site L type Calcium channel.

To gain insight into the molecular determinants that modulate the inhibitory activity of these compounds, molecular docking simulations for the synthesized compounds to L type calcium channel were performed using the biopredicta program in Vlife MDS 3.5 software based on the Xray crystal structure of L type calcium channels PDB ID 1T0J. The docking and subsequent scoring were performed using the default parameters of the biopredicta program. *Figs.* 5, 6 demonstrated that all the molecules under study have a nice fit in the active-site of L type calcium channel. The methyl and methoxy substituent's are making hydrophobic interaction Pro326, Ala327, Glu381, Ser382 while the phenyl and pyrimidine moieties also displays Vander wall interactions with HIS325, PRO326, ALA327 SER330 CYS377.

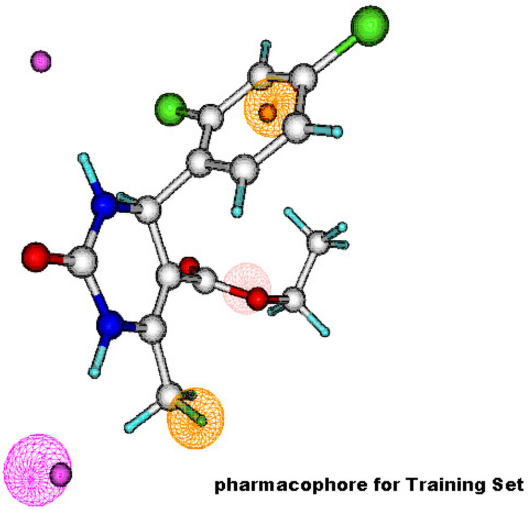


***Fig.* 7.** Figure showing pharmacophore for training set.

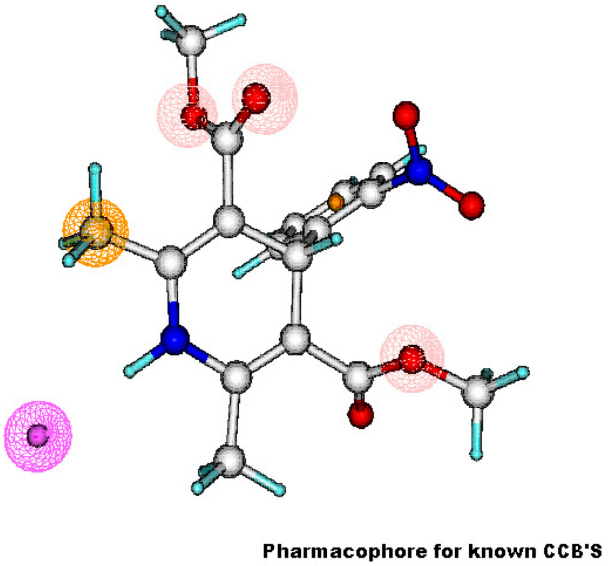


***Fig.* 8.** Figure showing pharmacophore for nifedipine.

Pharmacophore modelling studies

A set of pharmacophore hypothesis was generated using the mole sign module of Vlife 3.5. Each of hypothesis contain the four features like Hydrogen bond donor, Hydrogen bond acceptor, Hydrophobic, Aliphatic were common. The pharmacophore models were validated by using the pharmacophoric search for reported 1,4 dihydropyridines as calcium channel blockers. The structures of 15 calcium channel blockers which are in clinical use are used to predict the pharmacophore model for them and these features are compaired with the training set and designed molecules to validate the results as shown in *Figs.* 7, 8.

CONCLUSION

In conclusion, a computational approach along with the QSAR and docking analysis was employed to identify molecular structural features required for an effective calcium channel blocker, in an aim to discover drugs to prevent and cure cardiovascular diseases. A highly predictive pharmacophore model was generated based on 24 training set compounds, which consists of Hydrogen bond donor, Hydrogen bond acceptor, and hydrophobic group. Thus, our pharmacophore model should be helpful in identifying novel calcium channel blocker with improved activity as well as desired physiological properties.

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