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EVALUATION OF CALCIUM CHANNEL BLOCKER ACTIVITY OF NEW DIHYDROPYRIDINE DERIVATIVES USING 2D-QSAR AND THREE-DIMENSIONAL SIMILARITY

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ABSTRACT

Calcium channel blockers (CCBs) act as blockers of L-type calcium channels, inhibiting the movement of calcium ions across cell membranes, by means of calcium influx. Calcium channel blocking activity of 36 dihydropyridine derivatives (DHPs) was correlated with different parameters including hydrophobicity, molecular volume, number of hydrogen bond acceptor atoms, refractivity and number of rotatable bonds in five QSAR models. Significant fitted correlation coefficients and standard error of prediction were obtained, indicating that the established equations can be used to successfully predict the calcium channel blocking activity of DHPs. The results indicate that the contribution of molecular descriptors as the number of rotatable bonds, hydrophobicity and refractivity play a key role for the inhibitory activity of DHPs against the L-type calcium channels. Multilinear models allowed us to create a library of twenty one new DHPs, proposed by us as a source of compounds with more potent antiblocking activity towards L-type calcium channel.

Key Words:- hydrophobicity, refractivity, rotatable bonds, ROCS, combinatorial chemistry.

INTRODUCTION

Calcium channel blockers (CCBs) were thought to be a breakthrough in cardiac pharmacology with multiple therapeutic effects including real benefits upon atherosclerosis. Generally, CCBs act as blockers of slow calcium ion channels (so called L-type), inhibiting the movement of calcium ions across cell membranes, by means of calcium influx (McDonagh MS *et al.*, 2005). CCBs are chemically heterogeneous, being identified mainly three types of calcium antagonists: dihydropyridines, phenylalkylamines and benzothiazepines. Despite the variety of calcium channels, CCBs act quite exclusively on the L-type channels (Yousef WM *et al.*, 2005). The inhibition of the L-type channels at the level of vascular smooth muscles determines the vasodilatatory and antihypertensive properties of CCBs (Clunn GF *et al.*, 2010; Shukla N *et al.*, 2005) and also antioxidant properties of CCBs (Godfraind T, 2005) by direct scavenging effect or preservation of the superoxide dismutase activity. Other actions of CCBs consist in angiotensin and endothelin production reduction, and inhibition of lipid peroxide

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formation by different mechanisms (Matsumori A *et al.*, 2010; Eickelberg O *et al.*, 1999).

Among CCBs, DHPs show a superior rate of nephroprotection in terms of proteinuria decrease in patients with diabetic and/or hypertensive nephropathy (Yousef WM *et al.*, 2005). The explanation resides in the fact that DHPs produce a more consistent vasodilation of afferent arterioles. Cilnidipine, a unique CCB, expresses antisympathetic effects, beyond classical, nephroprotective and cardio protective effects. Because of its particular action mentioned above, cilnidipine could be the representative for the fourth generation of DHPs CCBs (Takahara A, 2009). Other new DHPs prolong the cardiac action potential duration (Garaliene V *et al.*, 2011) or act as beta-secretase inhibitors, being an attractive therapeutic target in Alzheimer's disease (Choi SJ *et al.*, 2010). Despite all these studies involving DHPs, the limited knowledge about DHPs mechanisms at calcium channels active site and the high cost of antihypertensive drugs synthesis are real obstacles for cardiovascular disease treatments.

One of the goals of the current study (Ebalunode JO *et al.*, 2011; Zuo Z *et al.*, 2005; Snyder RD and Hendry LB, 2005; Zhao X *et al.*, 2010) is to investigate the ability of molecular descriptors to correlate with calcium channels L-type blocking activity by means of 2D-QSAR equation. In our study we rely on the consideration that, basically, all QSAR methods presume that macroscopic properties are induced by the molecular structure and any change of molecular structure leads to the modification of these properties (Akamatsu M, 2002). Our study shows that steric (van der Waals volume), electronic (refractivity) and descriptors derived directly from atoms type (hydrophobicity, number of hydrogen bond acceptor atoms and the number of rotatable bonds) are critical for the inhibition of the L-type calcium channels. Thus, our results open a new perspective for the design of new 1,4-dihydropyridine derivatives with improved pharmacological properties that will supply more effective treatments for cardiovascular diseases.

Literature has documented few qualitative chemical structure – biological activity (SAR) studies regarding DHPs (Gupta S, 2006; Schleifer KJ, 1997; Gupta SP *et al.*, 2004), out of which several conclusions were formulated upon structural requirements of DHPs in order to be biologically active: (i) the substituents of phenyl ring at position 4 enhance the activity in the order *ortho*, *meta*, *para* (Gupta S, 2006); (ii) the 1,4-dihydropyridine ring and the presence of N1–H are essential (Gupta S, 2006); (iii) compounds revealed pronounced hydrophobic regions parallel to aromatic and aliphatic ring systems (Schleifer KJ, 1997); (iv) additional hydrophobic interactions may be postulated for bulky substituents in both CCBs (Schleifer KJ, 1997); (v) the molecular conformation, the relative orientation of the aryl ring with respect to pyrimidine ring, and some substituents capable to form hydrogen bonds with the receptor but less bulky in nature, and also high molar refractivity (Gupta SP *et al.*, 2004).

Supplementary, another aim of our study was to employ the three dimensional similarity search method ROCS (Rapid Overly of Chemical Structures) to demonstrate the similarity of the dataset molecules with respect to cilnidipine and another DHP derivative (compound 9e) that display the second highest activity against L-type calcium channel.

According to the similarity principle, the structurally similar molecules are prone to display equivalent biological activities (Johnson AM and Maggiora GM, 1990). Molecular shape, flexibility and pharmacophoric pattern are among the most important characteristics to determine the biological activities of chemical compounds. Tanimoto Combo, Shape Tanimoto, Combo and Color scores have been employed to estimate the similarity between all molecules included in the study and query compounds. In the following, we hope to detect the similar behavior among the compounds included in the current data set of thirty six 1,4-dihydropyridine derivatives. Given the importance of DHPs in cardiovascular pathologies, we consider that it is important to evaluate the biological activity of new DHPs derivatives against calcium channels. Considering that DHP 9e is the most selective drug candidate among the studied DHPs, a number of twenty one derivatives were generated and evaluated as L-type calcium channels inhibitors based on the 2D-QSAR models giving us the possibility to propose the newly derivatives as more effective calcium channels inhibitors.

MATERIALS AND METHODS

A. Dataset

The pharmacological activity expressed as half inhibitory concentration (IC_{50}) of cilnidipine analogs were compiled from literature (Yamamoto T *et al.*, 2006; Yamamoto T *et al.*, 2008). Although these values originate from different literature sources, they are determined in the same laboratory.

IC_{50} activity originally determined as micromolar values was converted to pIC_{50} by taking Log ($1/IC_{50}$) and used as dependent variable in all QSAR models developed in this study. The data set was selected according to the following criteria: the range of observed inhibitory activities (IC_{50}) from 0.0011μ to 10μ and significant variety of the substituents on 1,4-dyhydropiridine scaffold.

Name, chemical structures and the corresponding observed pIC_{50} of calcium channels blockers are given. The scaffolds of DHP 9a-j, 12; DHPs 7a-m, DHPs 7, 13a-h, DHPs 11, 12a-c and 2 are presented in Figure 1.

METHODOLOGY

Molecular modeling of calcium channel blockers and minimum energy calculation

The three dimensional structures of the data set compounds were obtained using the HyperChem software package. In the first step, the 2D structures of calcium channel blockers were obtained using the module Build followed by the addition of hydrogen atoms and automated conversion of 2D structures into 3D structures. In our study, the conformation with minimum potential energy was obtained using AM1 semi-empirical method, conjugate gradient method, with Polak-Ribiere algorithm and a convergence limit of 0.05kcal/Åmol.

An important objective of the current investigation was to predict the activity of the new calcium channel blocking derivatives, obtained by combinatorial chemistry, starting from the compound 9e (Yamamoto T *et al.*, 2008). In our attempt to design new compounds we generated new electrostatic contacts by adding halogens, amino, hydroxyl, and ester groups and secondly increasing the number of hydrophobic contacts by specific aliphatic groups at position R1 on 1,4-dyhydropyridine ring and at position R2 on chlorophenyl ring. Molecular modeling and minimum energy calculation of twenty one new 1,4-dyhydropyridine derivatives was performed under the above described protocol. During energy minimization, the specific groups mentioned above were allowed to move freely.

Descriptors calculations

Hereby, the independent variables as van der Walls volume (VOL), refractivity (R), hydrophobicity as log P (w/o) and also the number of total acceptor atoms and the number of rotatable bonds were obtained with HyperChem and FILTER software from OpenEye package. In HyperChem the descriptors are evaluated in the following manner: the steric descriptor van der Walls volume is calculated considering the probe radius of 1.4 Å, the molar refractivity A is a measure of the total polarizability of a molecule and is dependent on the temperature, the refractive index, and pressure.

In FILTER, XLog P (o/w) was evaluated as the octanol/water partition coefficient (including implicit hydrogens) of atomic contribution model that calculates hydrophobicity from the given structure. The number of acceptor atoms and the number of rotatable bonds were evaluated and stored in specific files.

QSAR methodology

Initially in the 2D-QSAR models, the descriptors included in this study were considered as individual independent variables or in various combinations to predict the calcium channels blocking activity. To avoid the redundancy of independent variables and to ensure the absence of chance correlation, the calculation of inter-correlation matrix of the independent variables was performed following Pearson correlation method (Table 1). In the final statistical analysis were kept only those descriptors that meet the non-redundancy criteria. A sufficient number of descriptors in order to allow an accurate validation of QSAR model (R^2 higher than 0.6, standard error of prediction SEE less than 0.5) were considered.

Statistical calculations

Multiple linear regression analysis (MLRA) was used in order to generate the correlation models that relate the structural features to the IC_{50} values and also to calculate the fitted correlation coefficient R^2, standard error of estimate SEE and Fisher test (Table 2). A reliable equation for quantitative structure activity relationship should possess a high correlation coefficient, low standard error of estimate (SEE) and the lowest possible number of variables.

Training and test sets

The generation of consistent statistical models depends on the quality of both training and test sets in terms of structural diversity and biological activity distribution. From the original data set of thirty six calcium channels blockers twenty seven compounds were selected for the training set and implicitly for model construction and the remaining nine compounds were designated for the test set. The compounds included in the training set have been randomly chosen giving both chemical and biological diversity in the training and test sets (Table 3). The composition of training and test sets was kept constant in all 2D-QSAR models. A cluster analysis confirmed that the composition of both training and test sets was chosen in a representative manner for whole data set (Table 3).

ROCS

Conformer generation

Conformational search was carried out with OMEGA, version 2.2.2 from OpenEye package. A maximum of 200 conformations were generated for each ligand employing the default values for root mean square cutoff distance (0.8 Å) and energy window (10 kcal/mol). In order to build the conformers, OMEGA first divide the

molecule into fragments and subsequently reconstruct the structural conformers by assembling the lowest energy fragments. The output conformations should pass the energy test in order to be retained. The conformationally expanded database of 1,4-dihydropyridine compounds was subjected to 3D similarity search.

3D Similarity calculation

ROCS accounts for three-dimensional similarity by executing shape/volume overly of a query compound with dataset compounds. ROCS are built on solid-body optimization algorithm that widens the overlap between the query and database molecules. Gaussian functions that mirror the hard-sphere volumes are employed to portray the shape of the molecules. This algorithm leads to fast and accurate volume overlapping by fluctuation of their reciprocal orientations. Tanimoto and Tversky similarity functions evaluate the correspondence between the pair of molecules when the volume superposition is complete. When generating the shape of a molecule ROCS takes into consideration only the heavy atoms and omits the hydrogen atoms. The most common score reported by ROCS is Shape Tanimoto that is calculated as follows (eq. 1):

$$ST = \frac{V_C}{V_A + V_B - V_C} \quad (1)$$

where, V_C =common volume between A and B molecules, V_A =query volume(A), V_B = database molecule volume(B) (24).

Nevertheless the major function of ROCS is to evaluate the shape; the inclusion of chemical functionality aimed at enhancing the pharmacological insight is also possible. The chemistry types implemented in ROCS or the "so called" color force field includes hydrogen-bond donors, hydrogen-bond acceptors, hydrophobes, anions, cations, and rings (24). Hence, ROCS is qualified to detect similar compounds in terms of shape and pharmacophoric features. ImplicitMillsDean color force field implemented in ROCS includes a pKa model that assumes the ionic state of molecules at pH=7.

RESULTS

QSAR evaluation

In our study we have generated five QSAR models, each of them containing different combination of molecular descriptors so that the optimum values of statistical parameters fitted correlation coefficient, R^2, and standard error of estimate SEE were obtained.

The correlation coefficients and standard errors of predictions were superior when the following combination of descriptors are meet: (i) number of rotatable bonds, log P and refractivity (R^2=0.820, SEE=0.418); (ii) number of hydrogen bond acceptor atoms, volume and refractivity (R^2 = 0.814, SEE = 0.488), (iii) number of rotatable bonds and log P (R^2 = 0.813, SEE = 0.478); (iv) number of hydrogen bond acceptor atoms (R^2 = 0.781, SEE = 0.506); (v) number of rotatable bond (R^2 = 0.772, SEE = 0.517). Hence, these descriptors are the most appropriate to model the biological activity. The descriptors selected in the QSAR models are simple and straightforward in the sense that they are essential factors influencing biological activity.

In the QSAR models developed a consistent variation trend of the selected descriptors with biological activity was observed. Molecular conformation is of special importance for biological activity of a ligand since the last depends upon the conformation of the ligand-receptor complex (Bradbury SP, 1998). In the developed QSAR equations, the number of rotatable bonds vary inverse proportionally with biological activity. Generally, this fact have been observed since higher molecular flexibility is associated with promiscuous activity and always rational drug discovery experiments take into account flexibility and impose a maximum cut-off value.

The binding of ligands to receptors is believed to involve interactions by which shapes or volumes of both receptor and ligand are changed upon the ligand–receptor complex is formed (Bradbury SP, 1998). In the equation 1 the descriptor volume has a small positive influence since van der Waals interactions that stabilize the ligand-receptor complex depends upon molecular volume. To some extent a larger volume will not be beneficial since steric clash with the receptor can occur in extremis and too large ligands cannot fit into the receptor binding site. Hydrophobicity is another crucial factor for biological activity contributing to the stabilization of ligand-receptor complex and according to Lipinski lower or higher lipophilicity can compromise biological activity.

The number of hydrogen bond acceptor atoms is another crucial factor that defines biologically active compounds (Lipinski CA, 2004). In the QSAR models 1 and 4 this descriptor vary inverse proportionally with biological activity; the most active compounds, i.e. 9e, 9a, 9b, 12b have three hydrogen bond acceptor atoms, while the distribution of values of this descriptor is between 3-5 and lower activity compounds possess 4-5 hydrogen bond acceptor atoms. It seems that the most active compounds involve maximum three hydrogen bond acceptor atoms that are situated at favorable positions to maximize the interaction with the receptor.

The external predictive ability of the 2D-QSAR models derived using the twenty seven training set

molecules was assessed by predicting pIC_{50} values for nine test set molecules which were not used to develop the model. The values for predicted pIC_{50} of the calcium channel blockers belonging to training and test sets and also the residual values (the differences between $pIC_{50\ observed}$ and $pIC_{50\ predicted}$) of the most significant QSAR models were presented (Table 3).

The data presented in Table 3 are supported by the correlation between observed and predicted inhibitory activities when the QSAR models 5 and 4 (Figure 2) are considered.

The best correlations between observed and calculated pIC_{50} for the calcium channel blockers included in QSAR model 5 in which the considered descriptors simultaneously share the number of rotatable bond, logP and refractivity seem to be for 13g (residual value = 0.00), 13a (residual value = -0.02) and 7i (residual value = 0.04).

In the QSAR model 4, when the contributions of the number of acceptor atoms, volume and refractivity were considered, good correlations between observed and predicted pIC_{50} were obtained for the compound 13b (residual value = -0.02), 13f (residual value = -0.05) and also 13g (residual value =0.08). When descriptors such as number of rotatable bond and logP are taken into account, the best correlations between observed and calculated inhibitory activity for the calcium blockers were noticed for 13a (residual value = 0.01), 7g (residual value = 0.02) and 13g (residual value = 0.04). Furthermore, the prediction power of the QSAR models proposed in our study is confirmed by the squared correlation coefficient, R^2 (0.772-0.820) between the predicted and observed pIC_{50} of calcium channel blockers which display acceptable values.

Table 1. The intercorrelation matrix of independent variables

	No of acceptor atoms	LogP	Vol	Ref	Rotatable bond
No of acceptor atoms	1.000				
LogP	0.019	1.000			
Vol	0.797	0.511	1.000		
Ref	0.665	0.489	0.914	1.000	
Rotatable bond	0.902	0.316	0.902	0.833	1.000

In this respect, statistically relevant QSAR models included:
QSAR model 1, pIC50 = constant + c_1* No of acceptor atoms
QSAR model 2, pIC50 = constant + c_2* Rotatable bond
QSAR model 3, pIC50 = constant + c_3* Rotatable bond + c_4* logP
QSAR model 4, pIC50 = constant + c_5* No of acceptor atoms + c_6*volume + c_7* refractivity
QSAR model 5, pIC50 = constant + c_8* Rotatable bond +c_9*logP+c_{10}*refractivity

Table 2. The statistical results of the 2D-QSAR models

Descriptors	Model	R^{2*}	SEE †	F ‡	QSAR coefficients
No of acceptor atoms	1	0.781	0.506	89.37	constant = 10.189 c_1 = -0.823
Rotatable bond	2	0.772	0.517	84.57	constant = 11.864 c_2 = -0.583
Rotatable bond logP	3	0.813	0.478	52.20	constant = 10.489 c_3 = -0.628 c_4 = 0.326
No of acceptor atoms Volume Refractivity	4	0.814	0.488	33.44	constant = 13.087 c_5 =-0.697 c_6 = 0.002 c_7 = -0.033
Rotatable bond logP Refractivity	5	0.820	0.418	34.588	constant = 11.770 c_8 = -0.554 c_9 = 0.383 c_{10} = -0.017

* R^2-fitted correlation coefficient, † SEE-standard error of estimate, ‡ F-Fisher test

Table 3. Observed and predicted pIC50 / residual values of calcium channel blockers

Training set								
DHPs		**pIC_{50obs}**	**$pIC_{50\ pred}$**/res QSAR model5		**$pIC_{50\ pred}$**/res QSAR model4		**$pIC_{50\ pred}$**/res QSAR model3	
12a	R1,R2=Me	7.7	7.93	-0.23	7.83	-0.13	7.87	-0.17
12b	R1=MeR2=Et	8.15	7.48	0.67	7.72	0.43	7.39	0.76
12c	R1= $CH(OMe)_2$R2=Me	5.89	6.03	-0.14	6.15	-0.26	5.95	-0.06
13a	R= 2-Cl	6.15	6.17	-0.02	6.02	0.13	6.14	0.01
13b	R= 3-Cl	6	6.19	-0.19	6.02	-0.02	6.16	-0.16
13c	R= 4-Cl	5.85	6.17	-0.32	6.01	-0.16	6.14	-0.29
13d	R= 4-F	5.7	6.08	-0.38	6.12	-0.42	6.00	-0.30
13e	R= 4-CF3	5.49	5.73	-0.24	6.00	-0.51	5.63	-0.14
13f	R= 2-OMe	5.23	5.35	-0.12	5.28	-0.05	5.31	-0.08
13g	R= 3-OMe	5.36	5.36	0.00	5.28	0.08	5.32	0.04
13h	R= 4-OMe	6.27	5.35	0.92	5.28	0.99	5.31	0.96
7a	R=2-NO_2-Ph	7.51	7.09	0.42	7.78	-0.27	7.01	0.50
7c	R= Ph	8.5	7.77	0.73	7.96	0.54	7.66	0.84
7f	R=3-Me-Ph	7.12	7.82	-0.70	7.82	-0.70	7.77	-0.65
7g	R= 3-F-Ph	7.7	7.84	-0.14	7.96	-0.26	7.72	-0.02
7i	R= 3-Br-Ph	8	7.96	0.04	7.76	0.24	7.93	0.07
7h	R= 3-Cl-Ph	7.7	7.93	-0.23	7.83	-0.13	7.87	-0.17
7j	R= 3-I-Ph	7.89	8.03	-0.14	7.61	0.28	8.06	-0.17
7l	R=3-Thienyl	7.36	7.64	-0.28	7.93	-0.57	7.55	-0.19
9b	R= OC_3H_4-C_6H_4-Cl	8.24	8.08	0.16	7.70	0.54	8.06	0.18
9c	R= OC_3H_4-C_6H_4-Me	8.17	7.96	0.21	7.70	0.47	7.96	0.21
9d	R= OC_3H_4-C_5H_4N	6.49	7.50	-1.01	7.19	-0.70	7.46	-0.97
9e	R=$OC_2H_2(CH_3)$-CH-Ph	8.55	7.88	0.67	7.72	0.83	7.88	0.67
9f	R=OC_2H_2(O)-NH-Ph	7.3	7.34	-0.04	7.20	0.10	7.33	-0.03
9i	R=OC_3H_5(Ph)2	6.44	7.01	-0.57	7.21	-0.77	7.11	-0.67
9j	R=$OC_3H_5(C_6H_4N)_2$	5.8	6.12	-0.32	5.97	-0.17	6.28	-0.48
2	R=3-NO_2-Ph	7.34	6.81	0.53	7.22	0.12	7.02	0.32
Test								
11	R1= MeR2 = CN	5.7	7.68	-1.98	7.12	-1.42	7.02	0.32
7	R1 = MeR2= $CH(OMe)_2$	5.09	6.03	-0.94	6.14	-1.05	7.66	-1.96
7b	R=4-NO_2-Ph	5.8	7.09	-1.29	7.78	-1.98	5.95	-0.86
7d	R=3-CO_2Me-Ph	5.36	6.46	-1.10	6.97	-1.61	7.01	-1.21
7k	R=3-Pyridyl	6.72	7.34	-0.62	7.32	-0.60	6.40	-1.04
7m	R= 3-Furyl	6.89	7.43	-0.54	8.12	-1.23	7.26	-0.54
9a	R= OC_3H_4-C_6H_{11}	8.43	7.92	0.51	7.83	0.60	7.28	-0.39
9g	R=OCH_2-napth	7.85	9.13	-1.28	7.82	0.03	7.87	0.56
12	R=HNC_3H_5Ph	6.07	7.67	-1.60	7.80	-1.73	9.10	-1.25

QSAR model 5 - descriptors: rotatable bonds, logP and refractivity; QSAR model 4 - descriptors: number of hydrogen bond acceptor atoms, volume and refractivity; QSAR model 3 - descriptors rotatable bonds and logP.

Table 4. 3D Similarity coefficients calculated with ROCS

Name	Tanimoto Combo		Shape Tanimoto		Combo		Color	
Query	9e	ciln	9e	ciln	9e	ciln	9e	ciln
7	1.65	1.29	0.91	0.82	1.80	1.49	-8.96	-6.73
11	1.78	1.38	0.97	0.87	1.87	1.54	-8.96	-6.76

12	1.79	1.37	0.98	0.87	1.88	1.53	-8.95	-6.63
12a	1.88	1.43	0.98	0.88	1.88	1.55	-8.96	-6.73
12b	1.78	1.38	0.97	0.87	1.87	1.54	-8.96	-6.73
12c	1.64	1.28	0.91	0.81	1.80	1.48	-8.92	-6.69
13a	1.62	1.28	0.88	0.81	1.78	1.48	-8.97	-6.75
13b	1.59	1.28	0.84	0.81	1.74	1.49	-8.96	-6.73
13c	1.64	1.28	0.90	0.81	1.79	1.48	-8.96	-6.73
13d	1.64	1.28	0.90	0.81	1.79	1.48	-8.96	-6.73
13e	1.57	1.23	0.83	0.76	1.72	1.43	-8.96	-6.73
13f	1.55	1.24	0.86	0.80	1.76	1.47	-8.95	-6.73
13g	1.54	1.24	0.85	0.80	1.75	1.47	-8.96	-6.73
13h	1.56	1.24	0.88	0.80	1.77	1.47	-8.96	-6.73
7a	1.54	1.23	0.88	0.74	1.72	1.43	-8.41	-6.98
7b	1.65	1.29	0.90	0.81	1.80	1.49	-8.96	-6.87
7c	1.84	1.36	0.95	0.82	1.84	1.49	-8.96	-6.73
7d	1.74	1.49	0.93	0.89	1.83	1.64	-8.96	-7.50
7e	1.64	1.55	0.96	0.91	1.85	1.77	-8.96	-8.66
7f	1.87	1.43	0.98	0.88	1.88	1.55	-8.96	-6.73
7g	1.87	1.42	0.98	0.87	1.88	1.54	-8.96	-6.69
7h	1.88	1.43	0.98	0.88	1.88	1.55	-8.96	-6.73
7i	1.79	1.38	0.98	0.88	1.88	1.55	-8.96	-6.73
7j	1.77	1.38	0.95	0.88	1.85	1.55	-8.96	-6.73
7k	1.06	1.03	0.63	0.60	1.23	1.20	-6.03	-6.01
7l	1.12	1.03	0.65	0.58	1.26	1.17	-6.03	-5.92
7m	1.08	1.01	0.65	0.59	1.25	1.18	-6.03	-5.96
9a	1.70	1.30	0.91	0.79	1.75	1.43	-8.41	-6.41
9b	1.86	1.42	0.97	0.87	1.87	1.54	-8.96	-6.73
9c	1.86	1.42	0.97	0.87	1.87	1.54	-8.96	-6.73
9e	2.00	1.35	1.00	0.85	2.00	1.51	-10.0	-6.67
9f	1.46	1.21	0.81	0.79	1.64	1.41	-8.28	-6.14
9g	1.54	1.21	0.82	0.78	1.70	1.41	-8.76	-6.30
9i	1.54	1.15	0.79	0.68	1.65	1.32	-8.58	-6.37
9j	1.45	1.10	0.81	0.72	1.67	1.33	-8.63	-6.05
2	1.70	1.62	0.96	0.91	1.85	1.78	-8.96	-8.71
ciln *	1.41	2.00	0.87	1.00	1.57	2.00	-6.95	-10.0

* ciln-cilnidipine

Table 5. Predicted pIC_{50} of the new 1,4-dihydropyridine derivatives and their residual values

Derivative 9e	**R1**	**R2**	**pIC_{50} (QSAR model 5)**		**pIC_{50} (QSAR model 4)**		**pIC_{50} (QSAR model 3)**	
				res		res		res
Derivative 1	O=COMe	H	7.34	-1.21	8.30	-0.25	7.34	-1.21
Derivative 2	O=COEt	H	6.84	-1.71	8.18	-0.37	6.82	-1.73
Derivative 3	$O=COC_2H_3$	H	6.82	-1.73	8.21	-0.34	6.79	-1.76
Derivative 4	O=COiPr	H	6.94	-1.61	8.06	-0.49	6.97	-1.58
Derivative 5	Me	H	8.70	0.15	9.17	0.62	8.71	0.16
Derivative 6	Et	H	8.24	-0.31	9.04	0.49	8.23	-0.32
Derivative 7	iPr	H	8.31	-0.24	8.92	0.37	8.36	-0.19
Derivative 8	C2H3	H	8.27	-0.28	9.02	0.47	8.25	-0.3
Derivative 9	OH	H	8.61	0.06	8.53	-0.02	8.60	0.05

Derivative 10	OMe	H	8.08	-0.47	8.40	-0.15	8.06	-0.49
Derivative 11	NH_2	H	8.44	-0.11	9.17	0.62	8.48	-0.07
Derivative 12	F	H	8.81	0.26	9.26	0.71	8.75	0.2
Derivative 13	Cl	H	8.90	0.35	9.13	0.58	8.89	0.34
Derivative 14	Br	H	8.93	0.38	9.05	0.5	8.96	0.41
Derivative 15	O=COH	Me	7.90	-0.65	7.59	-0.96	7.97	-0.58
Derivative 16	O=COH	F	7.93	-0.62	7.72	-0.83	7.93	-0.62
Derivative 17	Me	F	8.75	0.2	9.16	0.61	8.76	0.21
Derivative 18	Me	Me	8.72	0.17	9.03	0.48	8.80	0.25
Derivative 19	Me	OH	8.55	0.00	8.41	-0.14	8.61	0.06
Derivative 20	Me	NH_2	8.36	-0.19	9.02	0.47	8.49	-0.06
Derivative 21	Me	OMe	8.01	-0.54	8.29	-0.26	8.07	-0.48

Fig 1. The structures of 1,4-dihydropyridines (a) DHPs 9a-j, 12; (b) DHPs 7a-m; (c) DHPs 7, 13a-h; (d) DHPs 11, 12a-c, and 2

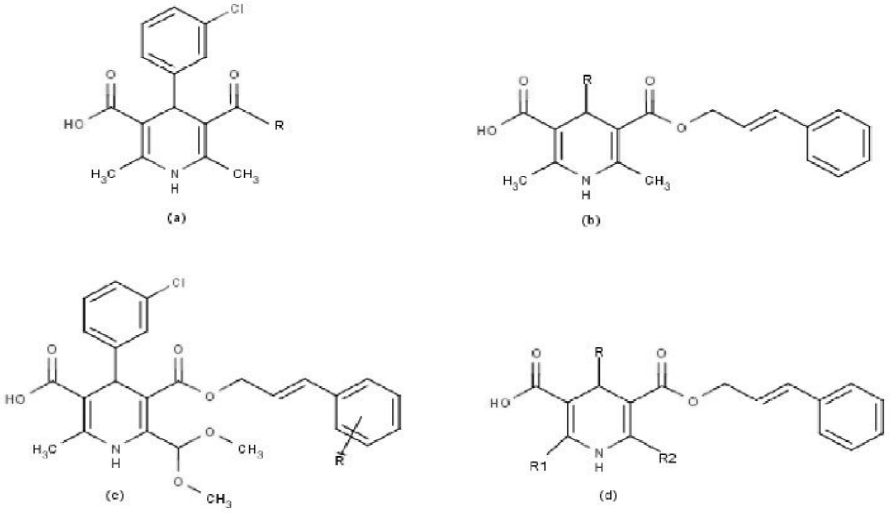


Fig 2. Correlation between predicted and observed pIC50 of calcium channel blockers - QSAR model 5 (a) and 4 (b)

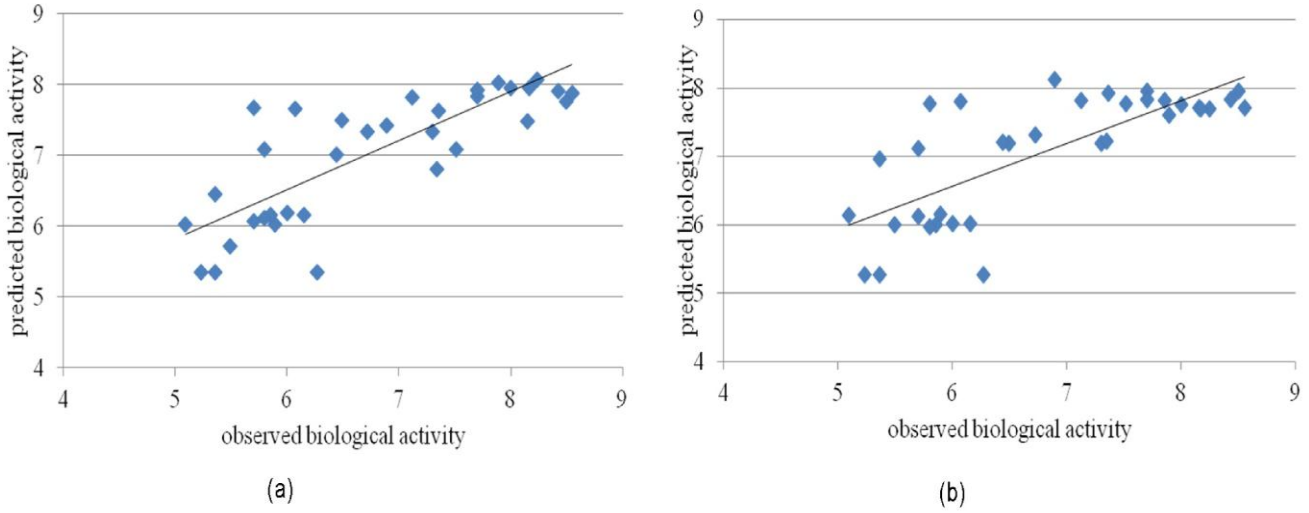


Fig 3. The overly of 1,4-dihydropyridine derivatives over the lowest energy conformer of: (a) cilnidipine, (b) compound 9e

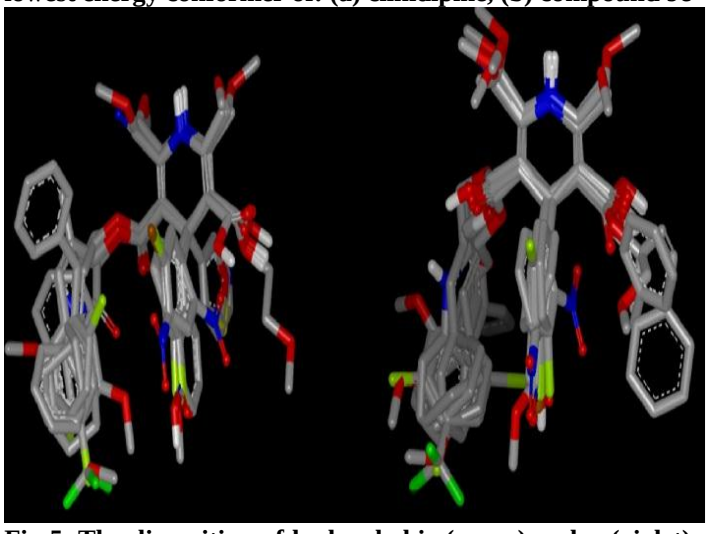


Fig. 4. The scaffold of novel 1,4-dihydropyridine derivatives

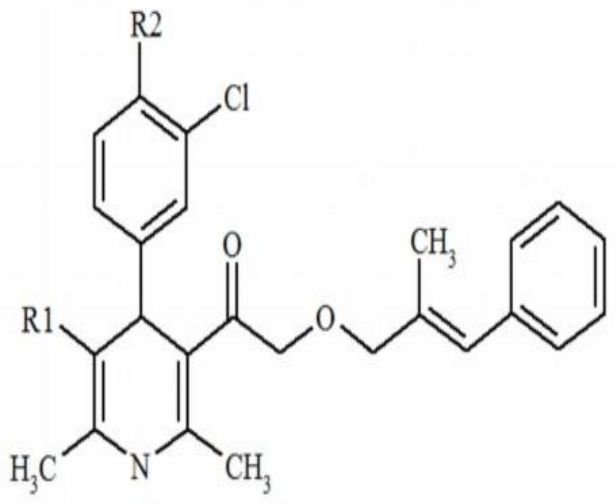
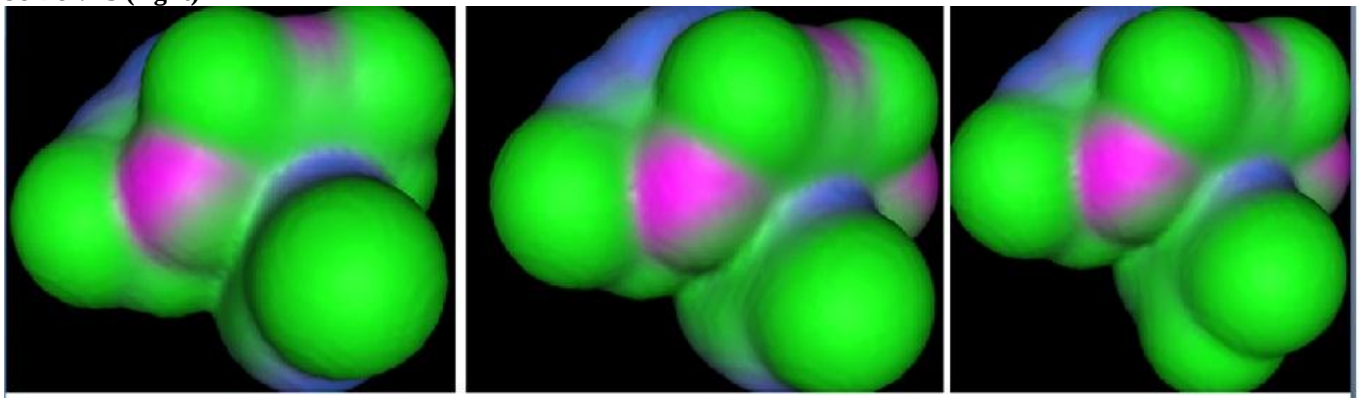


Fig 5. The disposition of hydrophobic (green), polar (violet) and hydrophilic (blue) fields of 9e Der. 12 (left), 9e, and 9e Der. 15 (right)



DISCUSSION

Results provided by ROCS for three-dimensional similarity algorithm are presented in Table 4, and Figure 3. In the absence of crystallographic structure were used the lowest energy conformer of two query molecules the highest active compound cilnidipine and compound 9e the second highest active derivative. Five compounds display Tanimoto Combo scores calculated with respect to cilnidipine below 1.2 (9i, 9j, 7k, 7l, 7m) and from these, three compounds whose phenyl substituent at position 4 is replaced by heterocycles show values below 1.1 (7k, 7l, 7m). These compounds are the least similar with respect to cilnidipine as resulted from Shape and Color Taniomoto scores. Thirteen compounds have Shape Tanimoto values below 0.8 for which the shape similarity with the query is not considered important. The lowest value (0.585) is registered for the compound 7m. This means that the alteration of substituents produced significant changes regarding the shape/volume of the compounds with respect to the parent compound cilnidipine that reflects the lower activity of this compound in comparison to the rest compounds. The Color Tanimoto score experience also a dramatic variation being as low as 0.379 in the case of compound 9j. In this case, the superposition of pharmacophoric features of the cilnidipine and compound 9j shows a different trend with respect to hydrogen bond donor and acceptor abilities. Combo score is higher than the usual similarity limit value (1.2) for the most of compounds, exception molecules 7l and 7m. With respect to cilnidipine, the compound 9e that exhibit the highest affinity towards L-type calcium channel is situated somehow intermediary regarding shape and pharmacophoric features. The compound 7 which have been designated as a good drug candidate by the authors

(Yamamoto T *et al.*, 2011) show close similarity towards compound 9e. The derivative 13f that present interesting inhibition properties is situated in the second half of the descending ordered list (Yamamoto T *et al.*, 2011). That means the structural distortion with respect to the parent compound cilnidipine is in this case pregnant. The most similar compound in terms of shape and pharmacophoric features with cilnidipine is the compound 2 because only the substituents at position 3 were altered (i.e. OCH_2CH_2OMe in cilnidipine is replaced by OH in compound 2). On the other side, this change reduces the affinity toward L-type Ca channel approximately by forty times. It seems that this substituent can be directly involved in the interaction with the receptor and OCH_2CH_2OMe group provide a better contact. In some situations, small structural differences as in the case of the compounds 12a and 12b where methyl substituent at position 6 is replaced by ethyl determine the increase of biological activity by approximately three times (Yamamoto T *et al.*, 2008), while for the compounds 7 and 13a the substitution of hydrogen with Cl on the phenyl ring of cynamyl unit produce the increase of activity by eleven times (Yamamoto T *et al.*, 2008). The ligand-based methods are not sensitive to small differences in ligand structure that lead to noticeable differences in biological activity. The low structural variance does not affect significantly certain properties of the ligand and the similarity algorithm cannot account explicitly for these small changes.

In the case of the results obtained against compound 9e the similarity functions take higher values than those calculated for cilnidipine. The compounds synthesized (Yamamoto T *et al.*, 2006; Yamamoto T *et al.*, 2008; Yamamoto T *et al.*, 2011) are more similar with compound 9e than cilnidipine. However, there are two compounds (7m and 7k) that have Tanimoto Combo score below 1.1. Regarding Shape score the values are significantly higher than in the case of cilnidipine for nineteen compounds (>0.9). There are four compounds showing Shape Tanimoto below 0.8 (7l, 7m, 7k, 9i) from which three compounds (7l, 7m, 7k) display values below 0.7. In the case of the compound 9i the six atom ring of the ester substituent at position 5 is replaced by diphenylmethane, a bulky hydrophobic group that change dramatically the molecular volume/shape. However, within this subseries thirteen compounds have Tanimoto Color score higher than 0.8, while in the case of three compounds (7l, 7m, 7k) the scores are below 0.5. Combo score is higher than 1.8 for sixteen compounds, whereas the lowest value appear in the case of 7k (1.233). Cilnidipine occupy the thirty fourth position showing lower values with respect to the rest of compounds, apart from the compounds 7l, 7m, and 7k.

The highest values of Combo score are encountered for the compounds 12a and 7h. The structural changes in the case of compound 7h are minimal since only methyl and cinnamyl substituents were replaced, while in the case of compound 12 methyl substituents at position 2, 6 were altered. The compound 7h has been considered a good starting compound (Yamamoto T *et al.*, 2011) and after the optimization of cinnamyl moiety they obtained a compound with desired properties. As they pursued for a compound that have good affinities for both Ca channels, the compound most similar to the compound 9e proved to have similar activity profile for L-type Ca channel (Yamamoto T *et al.*, 2011). In conclusion, the compounds included in the current dataset are more similar with the compound 9e than cilnidipine regarding shape/volume and pharmacophoric features. However, the similarity values are heterogeneous indicating a significant structural variation, otherwise necessary for an appropriate diversity set designated for QSAR modeling. Any of the structural differences underlined by the 3D similarity evaluation method can be key factors for the interaction of 1,4-dihydropyridine derivatives with individual amino acid residues within its binding site, since they display an activity range of 3.32 logarithmic units. One of the reasons that explain the lower statistical parameters obtained in QSAR analysis is the low variation of the substituents at position 3 of 1,4-dihydropyridine unit.

Prediction of novel 1,4-dihydropyridine derivatives activity

Following our design strategy presented in the Methodology section, when the descriptors the number of rotatable bonds, logP, and refractivity are considered simultaneously, seven novel derivatives designed (Figure 4): 9e-derivative5 (residual value = 0.15), 9e-derivative12 (residual value = 0.26), 9e-derivative13 (residual value = 0.35), 9e-derivative14 (residual value = 0.38), 9e-derivative17 (residual value = 0.20) and 9e-derivative18 (residual value = 0.17) recorded a pIC_{50} values in accord with our goal (Table 5).

In contrast, the increase of pIC_{50} in 9e-derivative 3 (residual value = -1.73) and 9e-derivative2 (residual value = -1.71) recorded in the same 2D-QSAR model were not significant. Unexpectedly, in the QSAR model 4 discussed in our study, more than half of 9e derivatives were predicted to display increased blocking activity against calcium channel type L. Critical increasing of pIC_{50} values were recorded for 9e-derivative12 (residual value = 0.71), 9e-derivative5 (residual value = 0.62) and 9e-derivative11 (residual value = 0.62). Similar with QSAR model 5, in QSAR model 3 seven derivatives

recorded a higher pIC_{50} value in comparison with parent compound (e.g. 9e-derivative13, residual value = 0.34), 9e-derivative14, residual value = 0.41).

Following the strategy to identify the pharmacophore with possible superior activity than 9e against L-type calcium channel, we noticed that the replacement of a voluminous group (carboxyl) with smaller groups (methyl, ethyl, halogens) could increase the inhibitory activity. The increase of log P values has the same beneficial effect upon pIC50.

The replacement of the substituents of biologically active compounds affects several properties including size, conformation, inductive and mesomeric effects, polarizability, hydrogen bonding capacity and hydrophobicity. In this case the aim behind structural changes is to obtain new compounds with improved biological activity or selectivity towards Ca ion channel type L.

The new generated derivatives show lower similarities in terms of volume/shape and pharmacophore with respect to cilnidipine but closer to 9e derivative – the parent compound. Concerning the derivatives with higher predicted activity than the compound 9e, they show enhanced volume/shape and pharmacophore similarity. Similarity indices for the compounds with higher predicted activity than compound 9e registered the following variations: Tanimoto Combo 1.598-1.160, Shape Tanimoto 0.964-0.617, Combo score 1.662-1.215. Derivatives 7 and 6 are the closest to 9e regarding volume/shape, whereas the derivatives 1, 10, 15, 16 showed higher pharmacophoric similarity. Derivative 9 is close to 9e concerning its predicted activity as well as three-dimensional similarity. The rest of the compounds showing better activity than the compound 9e display a wide range of similarity values. For the compounds having activities comparable to 9e, there is no clear correlation between shape/volume and pharmacophore similarity aspects. The compounds displaying lower activities (derivatives 1, 2, 3) registered close similarity coefficients. The structural changes introduced led to significant alterations of pharmacophoric properties i.e. for the compound predicted most active the COOH group was replaced with an alkyl groups that is not bioisoster. Most likely this compound could interact in a different way with the biological receptor than the compound 9e does. Chlorine is the most benefic substituent introduced at position 4 of the 1,4-dihydropyridine. Hydrophobic substituents such as Cl, Me at position 4 of the phenyl substituent at position 4 of the 1,4-dihydropyridine unit are beneficial.

Comparing the variation of log P, the number of rotatable bonds and refractivity descriptors for the derivatives 12 and 15 of the compound 9e according to the QSAR equation, it can be observed that the increase of log P values (9e-derivative 12= 6.22, 9e- derivative 15= 5.77) induces the increase of the inhibitory activity (Figure 5), while the increase of the number of rotatable bonds (rotatable bond 9e-12=6, rotatable bond 9e-15=7) and refractivity (refractivity 9e-derivative 12=118.59, refractivity 9e- derivative 15= 129.60) produce the decrease of the affinity to the calcium channel binding site.

CONCLUSION

In this paper we have reported 2D-QSAR studies upon a series of 3thirty six DHP derivatives tested as calcium channels blockers and also upon twenty one new DHP derivatives. In our study the molecular descriptors ~~as~~ number of rotatable bonds and hydrogen acceptor atoms, hydrophobicity, molecular volume and refractivity were considered to predict the inhibitory activity of DHPs. Significant fitted correlation coefficients R^2 and low standard error of prediction (SEE) were obtained for simultaneous presence of (i) number of rotatable bond, logP and refractivity**,** (ii) number of hydrogen acceptor atoms**,** volume and refractivity; (iii) number of rotatable bond, logP; (iv) number of rotatable bond and (v) number of hydrogen acceptor atoms. The modulation of physicochemical properties, particularly volume, log P and the number of hydrogen bond acceptor atoms may be very useful for the design of new calcium channel blocker drugs. Considering the QSAR equations obtained in this study, a set of twenty one new derivatives of DHP starting from compound 9e was designed and their inhibitory activity was predicted against calcium channel type L, according to a certain biological need. It was noticed that the rotatable bond, small volume and high log P increase significantly the biological activity pIC_{50}, permitting new insights into the structure–activity relationship of these pharmacologically important compounds. The structural differences in terms of shape and pharmacophoric properties may include crucial factors influencing the interaction of 1,4-dihydropyridine derivatives with binding site residues. The proposed derivatives of 1,4-dihydropyridines may represent valuable molecular tools for future drug development.

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