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Identification and Ligand-based Virtual screening of 1, 4-Dihydropyridine Analogues as Novel Calcium Channel Blockers

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Abstract: *High altitude pulmonary edema (HAPE) is a non-cardiogenic pulmonary edema which typically occurs in lowlanders who ascend rapidly without prior acclimatization to altitudes greater than 2500-3000 m. It mainly occurs due to exaggerated hypoxic pulmonary vasoconstriction and elevated pulmonary artery pressure. Previous studies have reported that Calcium channel Blockers (CCBs) can minimize HAPE. Calcium channels display remarkable sensitivity to Calcium channel blockers (CCBs), such as Dihydropyridines (DHPs). This study was undertaken to carry out docking simulations and investigate the binding affinity of 28 new ligands designed through side chain modifications of 6 FDA approved existing DHPs. A 3D model of L-type Voltage-gated Calcium channel (CaV) 4MS2 was used as receptor. Molecular operating environment (MOE 2008.10) was used for docking studies of the 28 trial ligands into the 3D model 4MS2. Interestingly, 3 (7^{th}, 20^{th}, and 25^{th}) out of 28 ligands showed higher affinity as compared with the S-scores of the 6 approved DHPs. 25^{th} showed the highest negative S-Score followed by 20^{th} and next by 7^{th}. These ligands may open a possibility to prevent diseases such as HAPE and pulmonary hypertension and may be potent drug candidates.*

Keywords: *Pulmonary hypertension, Calcium channel blockers, Dihydropyridines, Pulmonary edema.*

1. INTRODUCTION

Rapid ascent to high altitude (>2500 m) without prior acclimatization leads to the development of a potential life-threatening complication known as high-altitude pulmonary edema (HAPE) [1]. HAPE is characterized by exaggerated pulmonary hypertension and an unnatural increase in pulmonary arterial pressure that results in transvascular leakage of capillary fluid to alveoli's [2]. Calcium channels are transmembrane proteins found on the surface of cells and specific organelles for the transport of calcium ions. Various studies have shown that during HAPE, voltage gated calcium channels are upregulated. Voltage-gated calcium channels (CaV) act by responding to membrane depolarization and mediating calcium influx which significantly contributes in regulating a number of intracellular processes such as contraction, secretion, neurotransmission, and gene expression [3]. CaVs display remarkable sensitivity to Dihydropyridines (DHPs), for example, Nifedipine, Amlodipine, Clevidipine, Felodipine, Nimodipine, Nitrendipine, etc., a type of highly selective calcium channel blockers (CCBs) which blocks the transport of calcium ions through the calcium channels. DHPs act primarily on vascular smooth muscle cells by binding directly to inactive voltage-gated L-type calcium channels and stabilizing their inactive conformation [4]. A study reported that minimizing pulmonary hypertension using a DHP, nifedipine, prevented HAPE on rapid ascent to 4559 m [5]. Generally, if a drug molecule shows binding affinity to receptors other than the one it is specifically designed for, it is likely to show side effects. So, specificity to the target protein or receptor is an important aspect. Adverse effects have always confined the application of a drug and that preventive medicines for HAPE are currently limited in their applications. Nifedipine was found to cause peripheral edema; tadalafil was observed to be associated with headache and anorexia; and dexamethasone is quite familiar with mood disorder and hyperglycemia [6]. Ankle edema is a common adverse effect of amlodipine. Cilnidipine, a newer antihypertensive approved for the treatment of essential hypertension was launched as an acceptable alternative for patients with

amlodipine-induced edema [7]. Common side effects of CCBs include dizziness, drowsiness, nausea, slurred speech, swelling in hand, ankles, feet, pounding heartbeat and chest pain [11]. Therefore, the aim of this investigation was to design more efficient calcium channel blockers with higher binding affinity, higher bioavailability, longer half-life, minimum toxicity and lesser side effects.

2. METHODS

In this study, an effort has been made to virtually screen out potential lead molecules that could binds with voltage gated L-type calcium channel. Towards this end we have used molecular docking algorithm implemented in MOE (Molecular Operating Environment) software package.

2.1. Comparison of 6 FDA Approved DHPS

Six FDA approved DHPs namely, Nifedipine (DrugBank ID-DB01115), Amlodipine (DB00381), Nitrendipine (DB01054), Nisoldipine (DB00401), Nimodipine (DB00393), Felodipine (DB01023) were compared and chosen for their overall effectiveness and safety over other DHPs available so far (Table 1). These 6 structures were used as template for modification and development of new DHP ligands in the study. Their 2D structures were prepared using ACD/ chemsketch **(**Table 2).

2.2. Preparation of Ligands

Side chain modifications of the above-mentioned 6 FDA approved DHP yielded a ligand library of 28 new ligands. The 2D structures of the ligands were converted into 3D using 3D optimization tool in ACD/Chemsketch. The 3D optimized structures were saved as MDL Molfiles [V2000]. IUPAC names of ligands were obtained using ACD/Chemsketch. These structures were imported into MOE and energy minimized applying MMFF94x force field with an energy gradient of 0.05.

2.3. Preparation of Receptor Protein

The crystal structure of voltage gated L-type calcium channel, PDB_ID 4MS2 (resolution Å) was used as the target protein. All the water molecules were removed and hydrogen atoms were added using the molecular building of MOE. The structure was energy minimized successively with force field OPLS_2005 and energy gradient of 0.001.

2.4. Prediction of Binding Site

Ligand binding sites of voltage gated L-type calcium channel were predicted using MOE Site Finder which follows an energy based method for the prediction of such binding sites [14]. An individual ligand was selected by selecting the atoms of the ligand in the Sequence Editor tool and then the surface of the respective active site was computed using Surface and Maps tool in MOE.

2.5. Molecular Docking

Molecular docking is a computational method that can be used to explain the interactions of ligands with the receptor [15]. Docking simulation was carried out using MOE-Dock. The default parameters of MOE-Dock program were used to find the correct conformations of the ligands and to obtain minimum energy structures. Ligands were allowed to be flexible (Rotation of bonds were allowed) in the docking algorithm. At the end of docking, the best conformations of the ligands were analyzed for their binding interactions. The parameters used for docking were: Triangle Matcher placement with timeout (300 Sec) and number of Return poses = 1000, Rescoring1: London dG, Retain=30, forcefield refinement with a final gradient of 0.01 with a maximum of 500 Iterations. The docking of the 28 CCBs into the receptor was simulated. Each docking simulation was evaluated based on the S-value, which indicates the interaction energy between the ligand and the 4MS2 ligand binding site [17].

3. RESULTS AND DISCUSSION

High Altitude Pulmonary Edema (HAPE) occurs as a result of rapid ascent to high altitude (>2500 m) without prior acclimatization. During HAPE, the expression of voltage gated calcium channels increases manifold. Calcium channels are highly sensitive DHP binding targets. Calcium channels are involved in vasoconstriction which occurs in response to hypoxia prevalent at high altitudes (Figure1). If a ligand is designed in such a way that it can directly block such important sites on calcium channels, it may open the possibility to prevent HAPE by inhibiting vasoconstriction. There is an urgent need to design more efficient CCBs with higher binding affinity, higher bioavailability,

longer half-life, minimum toxicity and lesser side effects. The present study was undertaken to investigate and carry out a docking simulation of 28 DHP ligands bearing structural similarities with 6 FDA approved DHPs into the 3D model of Calcium channel '4MS2'. Each docking simulation was evaluated based on the S-value, which indicates the interaction energy between the ligand and the 4MS2 ligand binding site [17]. At the end of docking, the best conformations of the ligands were analyzed for their binding interactions. Out of 28 ligands, 3 ligands (7^{th}, 20^{th} and 25^{th}) showed higher affinity for the receptor than the 6 FDA approved drugs chosen in the study.

3.1. Binding Interactions of 7th, 20th and 25th Ligands with 4MS2 Calcium Channel

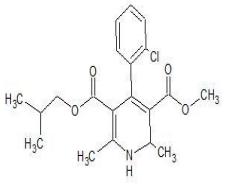
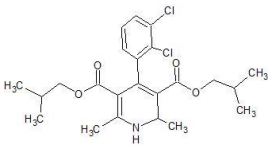
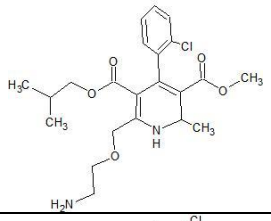
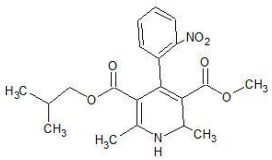
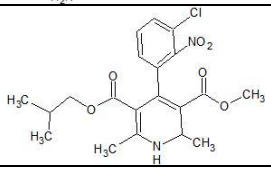
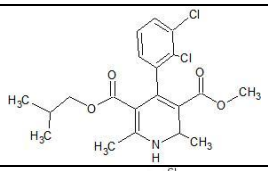
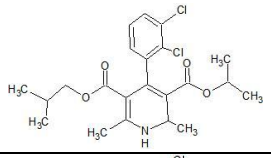
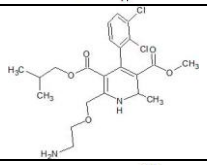
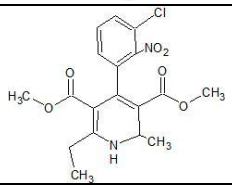
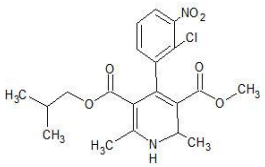
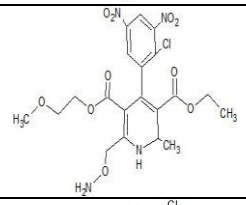
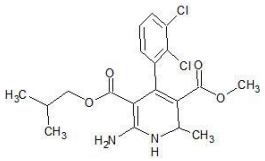
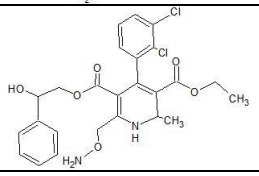
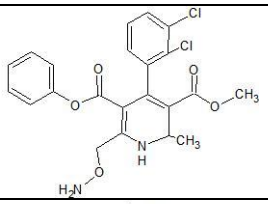
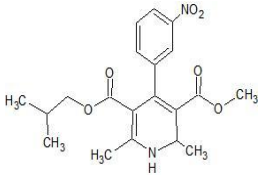
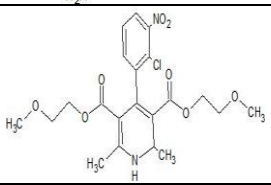
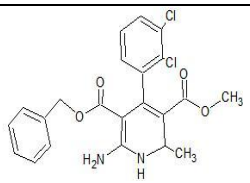
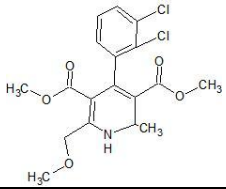
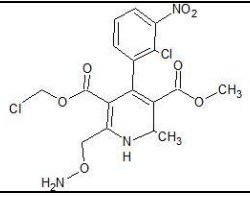
The most active compounds were 7^{th}, 20^{th} and 25^{th}. It is clear from [Table 4] that these compounds were firmly bound into the binding cavity of 4MS2 making interactions with the residues Thr1046, Thr1091, Arg1099, Arg1102, Gly1042, Glu1032, and Met1038. Thr1046 interacts with the oxygen double bonded to carboxylic acid group in the propan-2-yl acetate side chain attached at C-3 of the DHP structure (Figure 2. This side chain was found to be significantly interacting with an amino acid residue in the binding site of calcium channel. Another side chain which displayed important interactions in all the three cases was the side chain at C-6 position of the parent DHP structure. Interaction diagram of 25^{th} ligand illustrates that Arg 1099 interacts with the oxygen in the side chain attached at C-6. The parent DHP structure showed minimal flexibility as far as rotation along C4-C12 bond is concerned. The parent DHP structure expressed rigidness during conformational changes. Side chain modification with Cloro- and the nitro- groups at C-13 and C-14 and vice-versa lacked any importance whatsoever but their removal might change the overall interactions and MOE docking S-Score. 7^{th}, 20^{th} and 25^{th} ligands showed more negative S-Scores than those from the template FDA approved drugs studied in this investigation (Table 3 and 4). The 25^{th} showed the highest negative S-score of -19.6883 followed by 20^{th} (-19.4577) and next by 7^{th} (-18.2531).

4. FIGURES AND TABLES

Table1. *Comparison of 6 FDA approved Dihydropyridines*

Sl.no	Criteria	Nifedipine	Nitrendipine	Amlodipine	Felodipine	Nisoldipine	Nimodipine
1.	**Formula**	$C_{17}H_{18}N_2O_6$	$C_{18}H_{20}N_2O_6$	$C_{20}H_{25}ClN_2O_5$	$C_{18}H_{19}Cl_2NO_4$	$C_{20}H_{24}N_2O_6$	$C_{21}H_{26}N_2O_7$
2.	**Mol. Wt.**	346.35 g/mol	360.36 g/mol	408.87 g/mol	384.26 g/mol	388.41 g/mol	418.44 g/mol
3.	**Dosage**	Orally	20 mg/day Orally	5-10 mg/day, Orally	5-10 mg/day, Orally	Orally	60mg/4 hrs, intravenously Orally
4.	**Bioavailability**	45-56%	NA	64-90%	15-20%	NA	100% intravenous 13% oral
5.	**Protein binding**	92-98%	99%	97.5%	99%	99%	95%
6.	**Metabolism**	Gastrointestinal, Hepatic	Hepatic	Hepatic via Cyt P450 3A4	Hepatic via Cyt P450 3A4	Hepatic	Hepatic via Cyt P450 3A4
7.	**Half-life**	2 hrs	12-24 hrs	30-50 hrs	17 -37 hrs	7-12 hrs	2-9 hrs
8.	**Excretion**	60-80% via urine. 20% via bile/feces	Renal	Renal	Renal	Renal	Feces and Urine
9.	**Side effects**	Dizziness , Drowsiness, nausea, drop in blood pressure, Slurred speech, and weakness.	Peripheral edema	Swelling in hand, ankles, feet. Pounding heart beat, Fluttering in your chest, Chest pain	Swelling in hand, ankles, feet. Pounding heart beat, Fluttering in your chest, Chest pain	Peripheral edema	Peripheral edema
10	**Diseases**	Hypertension & Angina pectoris	Hypertension, chronic stable angina pectoris, & Prinzmetal's variant angina	Angina pectoris , Hypertension, Coronary artery disease.	Hypertension	Hypertension	Hypertension

Table2. *MOE S-scores of the new DHP ligands after docking with 4MS2 protein*

Sl. no.	DHP Structure	S-Score (Kcal/mol)	Sl. No.	DHP structure	S-score (Kcal/mol)
1.		**-14.9655**	**2.**		**-14.6319**
3.		**-12.8477**	**4.**		**-15.8841**
5.		**-13.9592**	**6.**		**-15.8776**
7.		**-18.2531**	**8.**		**-14.4025**
8.		**-16.2047**	**10.**		**-14.9864**
11.		**-16.4543**	**12.**		**-12.2044**
13		**-16.5830**	**14.**		**-8.7097**
15		**-17.8074**	**16.**		**-15.7903**
17.		**-16.1601**	**18.**		**-15.2318**
19.		**-13.1195**	**20.**		**-19.4577**

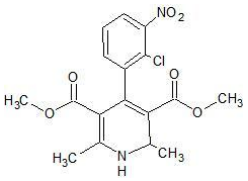
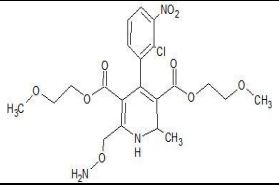
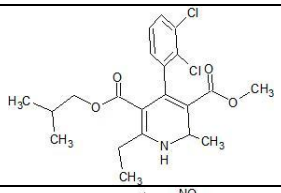
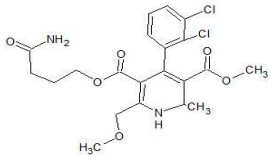
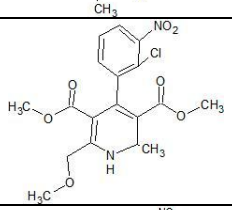
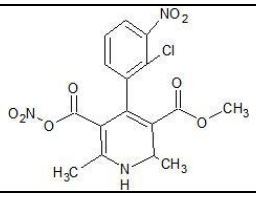
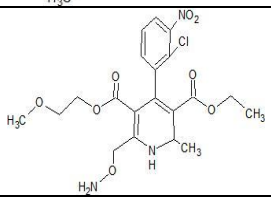
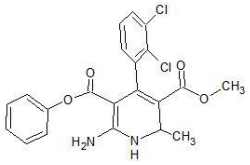
21.		**-12.5730**	**22.**		**-17.5531**
23.		**-15.2180**	**24.**		**-17.6421**
25.		**-19.6883**	**26.**		**-13.9874**
27.		**-15.1862**	**28.**		**-16.1034**

Table3. *MOE S-scores of the best ligands in the study*

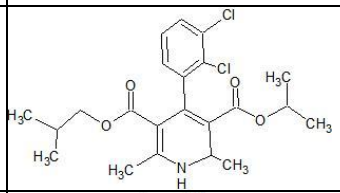
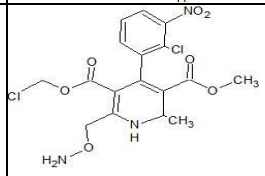
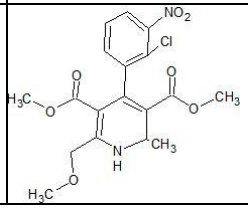
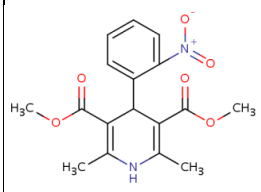
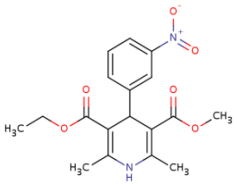
Sl.no.	DHP Structure	Predicted Chemical Name [12]	Mol wt.	S-Score(K cal/mol)
7		5-(2-methylpropyl) 3-propan-2-yl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,2-dihydropyridine-3,5-dicarboxylate	440.36008	-18.2531
20		5-(chloromethyl) 3-methyl(2R)-6-[(aminooxy)methyl]-4-(2-chloro-3-nitrophenyl)-2-methyl-1,2-dihydropyridine-3,5-dicarboxylate	446.23878	-19.4577
25		dimethyl (2R)-4-(2-chloro-3-nitrophenyl)-6-(methoxymethyl)-2-methyl-1,2-dihydropyridine-3,5-dicarboxylate	410.80566	-19.6883

Table4. *Docking interaction energy of the 6 FDA approved DHPs against 4MS2.pdb*

Sl.no.	DHP	Structure	S-Score (K cal/mol)	Sl.no	DHP	Structure	S-Score (Kcal/mol)
1.	Nifedipine		-15.0456	2.	Nitrendipine		-16.2726

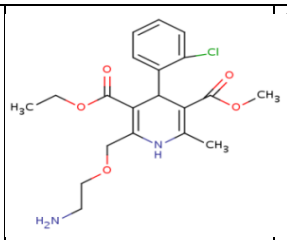
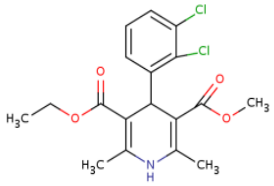
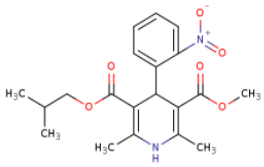
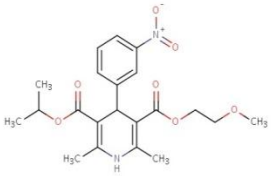
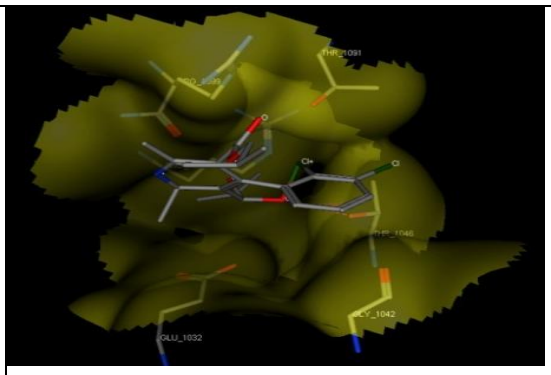
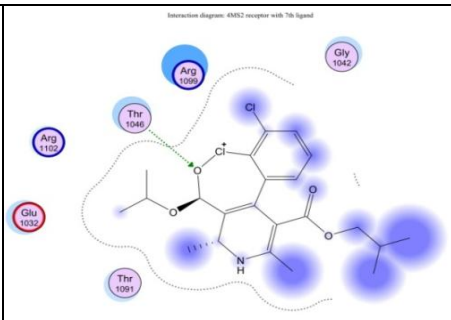
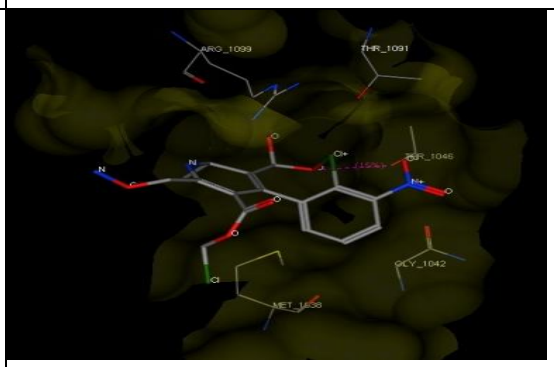
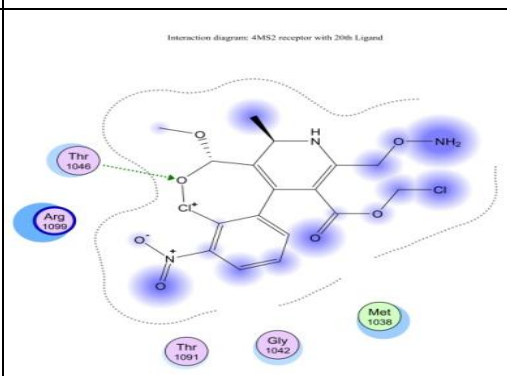
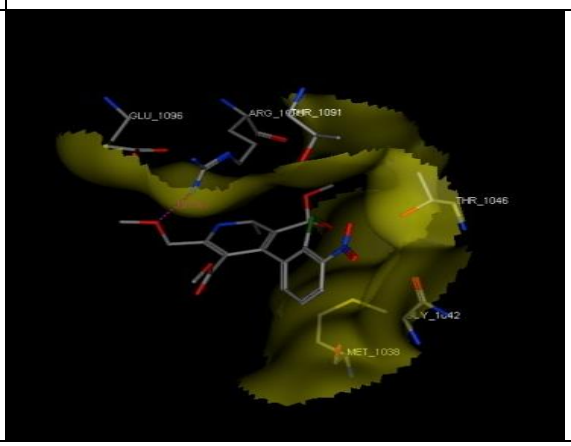
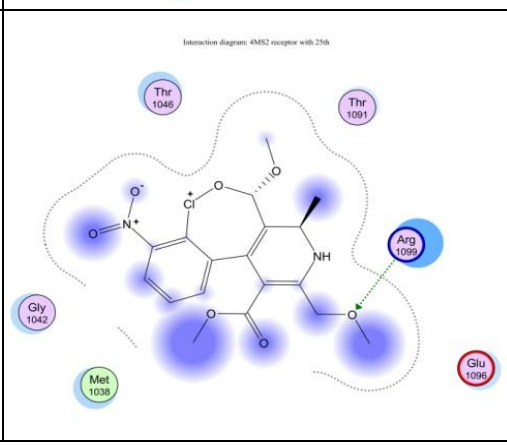
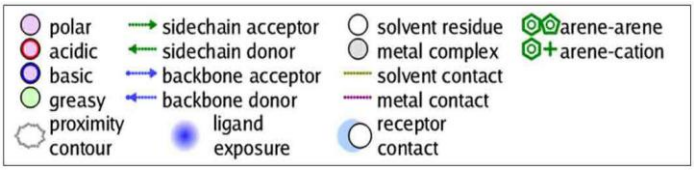
3.	**Amlodipine**		**-3.2397**	**4.**	**Felodipine**		**-13.7798**
5.	**Nisoldipine**		**-5.9080**	**6.**	**Nimodipine**		**-15.7288**

Table5. *3D docking pose & 2D pictures of docked conformations of the most active ligands.*

7		
20		
25		
MOE Reference for interactions: 		

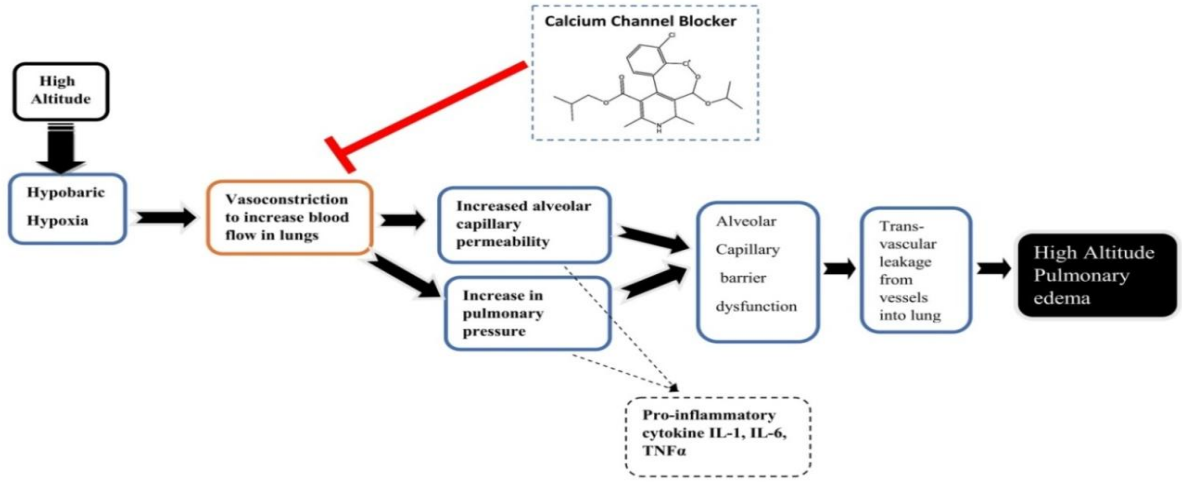


Figure1. *Dihydropyridine calcium channel blockers prevent vasoconstriction and minimize the occurence of HAPE.*

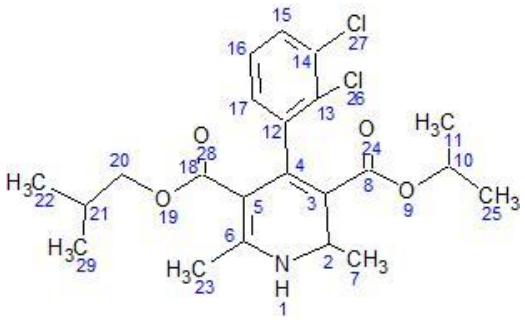


Figure2. *Numbering of atoms in 7th DHP ligand*

5. CONCLUSION

Through *in silico* docking simulation of selected DHPs with the 4MS2 calcium channel, binding potential was estimated. The relative potencies of these compounds were ranked in the order of 25th> 20th > 7th. So these ligands may open a possibility to prevent pulmonary hypertension and high altitude complications like HAPE and may be potential drug candidates.

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