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# Phenylalkylamines as calcium channel blockers

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**Abstract.** In this study we present *ab initio* Hartree Fock molecular orbital calculations with complete geometry optimizations on some phenylalkylamines (PAAs) that are clinically used as antiarrhythmic drugs. Pharmacophoric features of PAAs have been derived. An explanation of potency regulation in PAAs has been suggested based on ion capturing vs. ion holding by the drug. Ion capturing by the drug is always electrostatically highly favourable but has to be analysed in terms of conformational changes required and physiological accessibility of the situation. Our results also seem to offer an explanation for inhibitory effect of $Ca^{2+}$ ion concentration on binding affinity of PAAs.

**Keywords.** Phenylalkylamines; Hartree Fock; ion holding capacity; substituent effect; intermolecular interaction.

## 1. Introduction

Voltage-gated $Ca^{2+}$ channels are important pharmacological target for treatment of cardiovascular diseases.[1] Commonly used drugs fall into three distinct classes: Phenylalkylamines (PAA), Benzothiazepines (BTZ) and Dihydropyridines (DHPs).[2] Block of L-type $Ca^{2+}$ channels in cardiac and smooth muscles by verapamil and related phenylalkylamines is an important therapy for hypertension, cardiac arrhythmias and angina pectoris.[3] Phenylalkylamines block voltage gated $Ca^{2+}$ Channels by binding within the intracellular mouth of the ion conducting pore.[4] The levorotatory (–) enantiomers of PAAs were found to be more potent than the dextrorotatory (+) enantiomers.[5] The Phenylalkylamines: Devapamil, Verapamil and Gallopamil cause voltage- and use-dependent block of L-type $Ca^{2+}$ channels and differ from each other only in the number of methoxy groups on each of their two terminal phenyl rings (figure 1).[6]

Devapamil (D888) contains one meta methoxy group at the inside of the aromatic ring of the phenethyl part, whereas verapamil contains two methoxy group in meta and para positions. Devapamil blocks the channel with affinity much higher than that of verapamil.[6] These phenylalkylamines have been widely used in clinical medicine and in experimental biology to block $Ca^{2+}$ inflow into cells across the plasma membrane.[7] The conformational features of these drugs have been studied. The neutral form of gallopamil was characterized by a unique conformation whereas the protonated form was suggested to exist in different conformations with great mobility of the torsional angles and of the ionized site of the molecule.[8] Previous studies suggest that verapamil adopts a relatively compact structure.[9] Verapamil can adopt three structural forms (or conformational shapes): extended, folded and half folded.[10] The folded conformation is stabilized by non-bonded interactions between the two dimethoxy aryl rings situated on the opposite ends of verapamil and is suggested to be the most stable conformation of the isolated drug. Molecular simulations for the free drug with the crystal structure of verapamil as the starting point have also been performed.[11] Solution structure of verapamil in deuterated DMSO using one-dimensional $^{1}H$ and $^{13}C$ NMR data[12] has been analysed.

Verapamil gains access to its binding site from the cytoplasm[2] and seems to inhibit the central pore by physical occupancy. Electrophysiological data have indicated that the binding domain for PAA is located on the intracellular side of the membrane in cardiac myocyte.[13] The PAA binding pocket is composed of at least seven amino acid residues based on the alanine scanning mutagenesis results on Devapamil. Three amino acid residues in segment IVS6-Tyr

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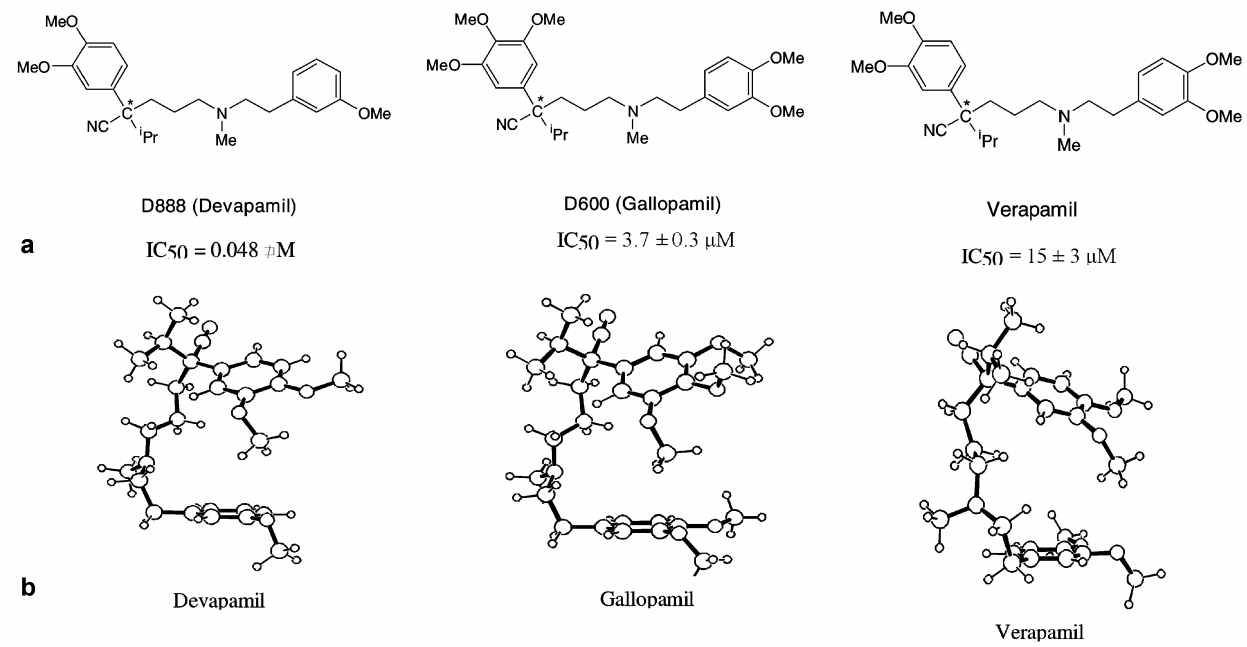


**Figure 1.** (**a**) Phenylalkylamines (indicates chiral center*). (**b**) Optimized conformations of phenylalkylamines.

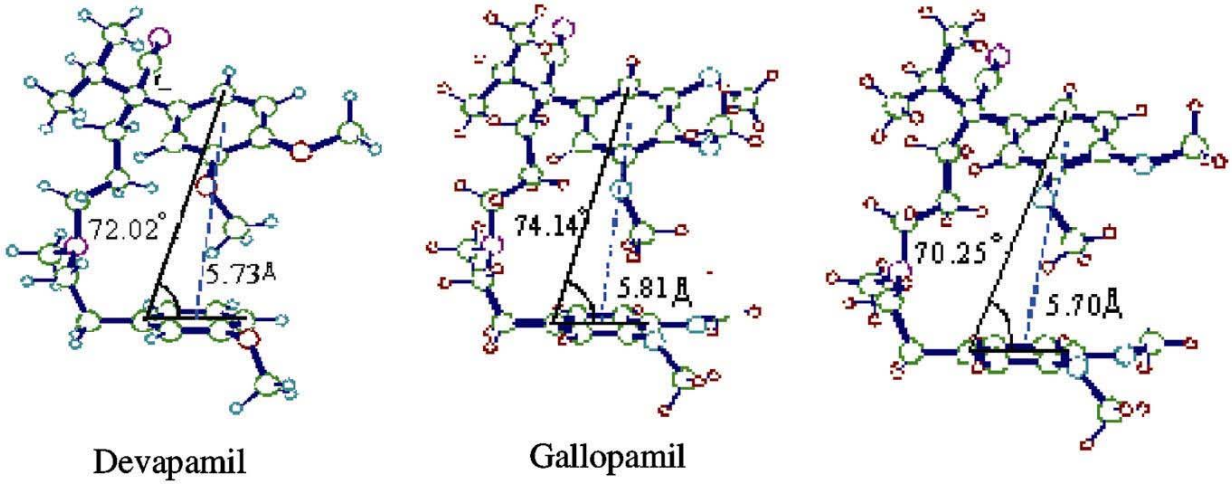


**Figure 2.** Pharmacophoric features of phenylalkylamines (black lines indicate angle required between the two aryl rings and dashed blue line indicates required distance between two aryl rings).

1463, Ala 1467 and Ile 1470- are required for high affinity because their mutations reduced the affinity 6- to 12-folds. Met 1464 has also been included as a residue contributing to high affinity PAA interactions.[14]

Previous studies also show that in the $Ca^{2+}$ bound form, two verapamil molecules are arranged with a 2-fold symmetry such that the two methoxy oxygens from each molecule act as ligand to the cation.[9] A three-dimensional model of phenylalkylamine receptor was suggested which incorporates two nucleophilic areas of the $Ca^{2+}$ channel. Gallopamil binds to this area by amine function and via coordinated $Ca^{2+}$. BS Zhorov gave the hypothesis of ternary complex formation between the ligands of calcium channel, their receptors and $Ca^{2+}$.[15] In order to fully understand the

nature of the drug-$Ca^{2+}$ complex, Zhorov and Ananthanarayanan presented a systematic analysis of the protonated and neutral forms of verapamil and one of its potent analogues gallopamil.[16] The geometrical criteria involved the pre-disposition of the oxygen and nitrogen atoms of the drug molecule to form bi- tri- and tetradentate complexes with $Ca^{2+}$. They used these criteria to demonstrate that both verapamil and gallopamil have several low energy structural patterns that are pre-disposed for bi- and polydentate chelation of $Ca^{2+}$. Recent experimental evidence by Hockerman *et al* indicates that the $Ca^{2+}$ ions may be held by selectivity filter glutamates.[17] Experimental studies by Galizzi and coworkers indicate an inhibitory effect of $Ca^{2+}$ on the binding of PAAs to the receptor. There is also evidence that suggests, there could be a concentration dependence of the $Ca^{2+}$ effect.[18] Data using a fluorescent probe that interacts at the PAA site reveal that increase in $Ca^{2+}$ concentration results in a lowering of the binding affinity for these drugs.[19] At $Ca^{2+}$ concentrations >30 $\mu$M phenylalkylamine binding was inhibited and potency was much lowered ($IC_{50}$ raised to >300 $\mu$M).

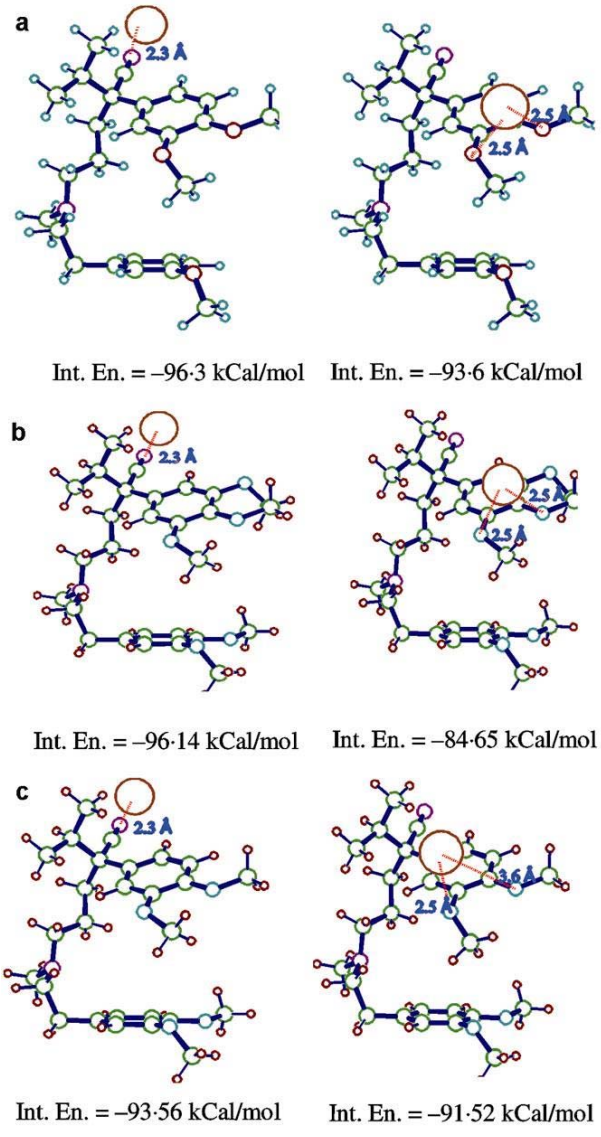


**Figure 3.** Ion holding sites in phenylalkylamines and corresponding ion holding capacity. (**a**) Devapamil; (**b**) Gallopamil and (**c**) Verapamil.

The *pKa* pf PAAs have been reported to be about 9·04.[20] However, it is accepted that some portion of PAA's still remains in the unprotonated form in equilibrium that could diffuse through cell membrane and bind to intracellular site.[2a] The $IC_{50}$ values of PAAs are shown in figure 1.[21]

The present study concentrates on effect of substituent on $Ca^{2+}$ holding capacity of PAAs. A past study has suggested that the order of potency amongst (–)-isomers varies as follows: Devapamil greater than gallopamil greater than verapamil.[22] The (+)-isomers exert quantitatively similar effects but at orders of magnitude (10–200 times) higher concentrations.

## 2. Methodology

*Ab initio* HF molecular orbital calculations have been performed on various phenylalkylamines (PAAs) using 6-31G basis set.[23,24] Complete geometry optimizations have been performed using Berny optimization method utilizing steepest descent technique.[25,26] The obstruction of $Ca^{2+}$ ion flow by the drugs has been investigated by calculating intermolecular interaction energies using supermolecule approach that is, interaction energy $\Delta E_{int} = E_{complex} - (E_A + E_B)$ energy of isolated fragments. All possible sites of $Ca^{2+}$ ion interaction were explored by bringing in ion from all directions. Most favourable interactions were noted. Ion was not allowed to be covalently bonded or captured. The ion channel in presence of the drug only regulates ion flow, i.e. obstructed ion flow. It does not stop ion flow otherwise it will be fatal for the patient. This indicates that $Ca^{2+}$ ion should be loosely held (by non-bonded interaction). Conformational relaxation of the drug in presence of the ion has also been considered (this was not taken care of in our earlier calculations[27]). The relative capacity of the drugs to hold the $Ca^{2+}$ ion is given by intermolecular interaction energies as mentioned above and in our earlier study on verapamil.[27] Intermolecular interaction

energies have been analysed in terms of mechanistic aspects and potency regulation.

## 3. Results and discussion

The optimized conformations of the isolated unprotonated form of drugs are shown in figure 1b. In our earlier study[27] we have reported on the $Ca^{2+}$ ion holding capacity of verapamil. Our earlier study, was an attempt to understand how to explore ion flow interruption by these drugs. In this study we have tried to understand potency regulation in phenylalkylamines and correlate it with effect of substituent on $Ca^{2+}$ ion holding capacity of the drug. Figure 2 shows the characteristic conformational features of each drug and there from extracted pharmacophoric features. The required disposition of the two aryl rings is at an angle between 70–75°. The distance between the two aryl rings has to be maintained between 5·5–6·0 Å. Possible sites for $Ca^{2+}$ ion interaction and corresponding interaction energies are shown in figure 3. As expected, the possible site for holding the $Ca^{2+}$ ion in all the cases are either the cyano group on the chiral centre or the methoxy substituents (the methoxy substituent on the lower aryl were also considered but showed comparably lesser holding capacity). All the sites for holding ion in the drug when considered separately, i.e. one at a time show comparable holding capacities with minor differences due to conformational aspects.

To mimic the real physiological conditions in our *ab initio* calculations as closely as possible we have allowed for complete relaxation of the drug in presence of the ion. Results obtained are shown in figure 4. In Devapamil we observe that the drug has not relaxed conformationally much. Calcium ion places itself in a position where it can interact with all the possible sites simultaneously to achieve maximum electrostatic interaction. Ion in this position would also be held with comparable strength to other sites. Gallopamil has the maximum number of methoxy substituents (probably too many methoxy substituents were introduced trying to enhance $Ca^{2+}$ ion holding power of the drug). But, the drug only shows moderate activity. Drug shows huge conformational change trying to permanently capture the ion. The electrostatic interaction is so strong that the drug would permanently capture/hold the ion not letting it go. But the fact that moderate activity is observed proves that the conformational relaxation in this case is not physiologically attainable i.e., *inaccessible*, if at all, accessed then only in a small percentage of drug molecules. Results after conformational relaxation in verapamil are also shown in figure 4. Verapamil shows very little re-organization basically in the methoxy substituents to allow $Ca^{2+}$ ion to probe inside the molecule to maximize electrostatic interactions. The situation is easily accessed as not much recoganization of the molecule is required. However, the gain in electrostatic interaction is so much that the ion is permanently captured though not covalently bonded. This is reflected in verapamil's low potency indicating that a large percentage of drug molecules are probably 'wasted' after permanently capturing the ion. Our predictions are also indirectly supported by the observance of concentration dependence of $Ca^{2+}$ ions on the inhibitory effect of $Ca^{2+}$ ions on PAA binding. If the $Ca^{2+}$ concentration is high PAAs have

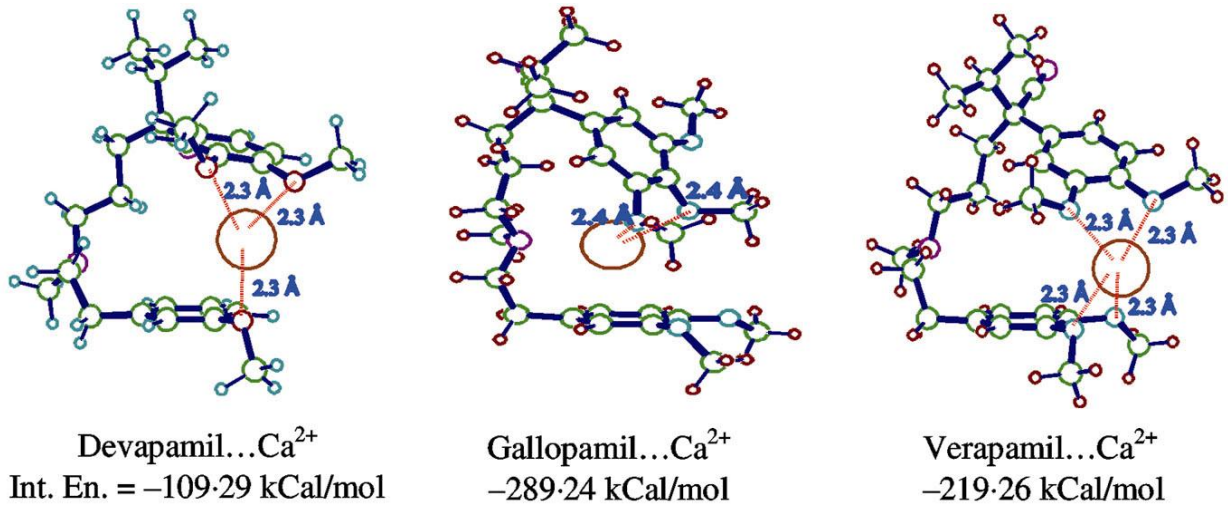


**Figure 4.** Ion holding by the drug after allowing complete conformational relaxation.

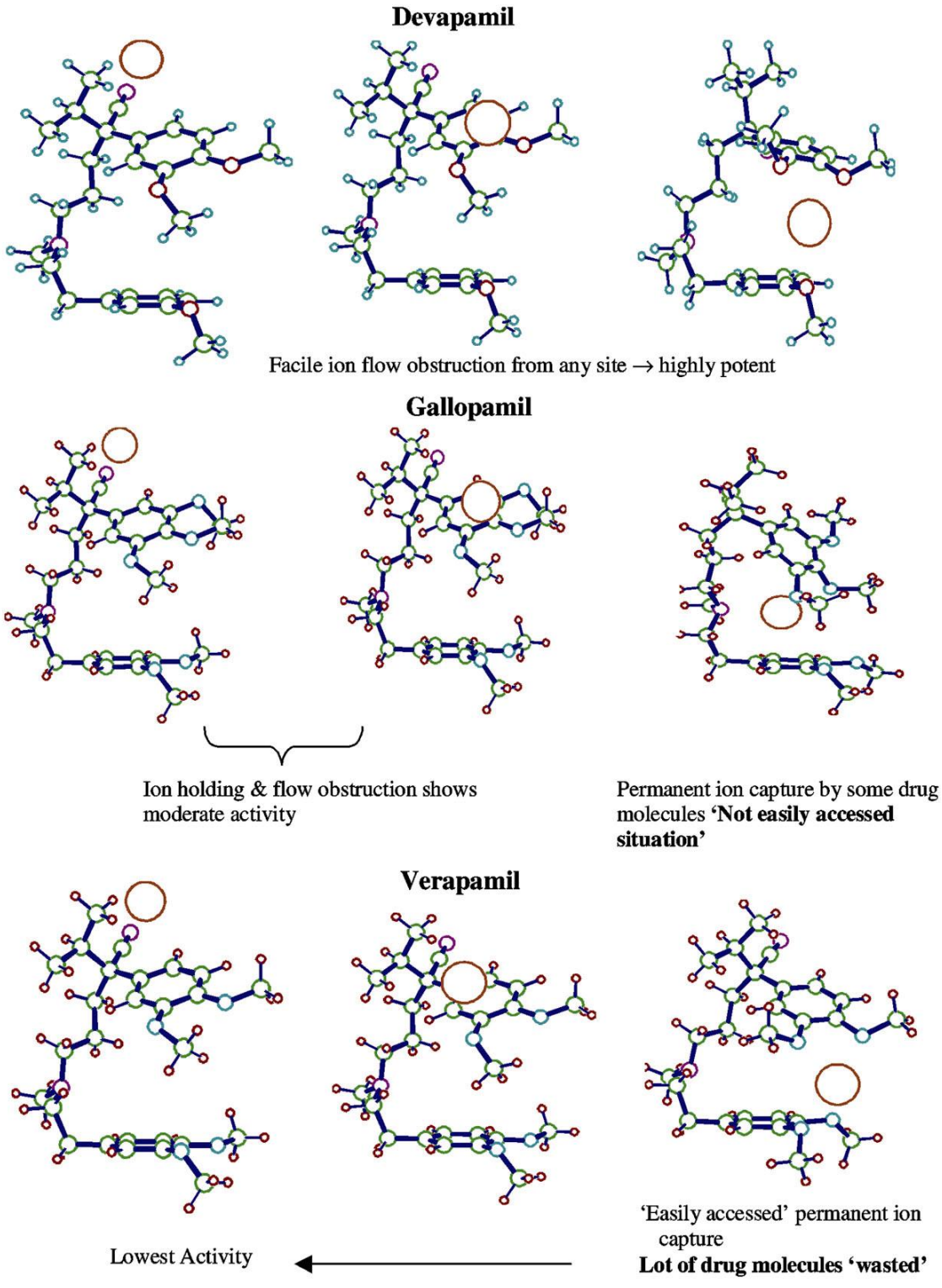


**Figure 5.** Mechanistic implications.

more probability of first capturing $Ca^{2+}$ ion and being wasted as compared to binding to the receptor.

The mechanistic implications of these drugs as discussed above are concluded in figure 5. Based on

our accurate intermolecular interaction calculations we suggest here a possible explanation of observed potency variation in these drugs. In Devapamil as shown in figure 5 all situations can be considered as occurring in equilibrium i.e. facile ion flow obstruction from any site (but not permanent obstruction of channel activity) leads to devapamil's high potency. Gallopamil has a situation that can lead to permanent ion capture but does not seem to be physiologically easily accessed. If at all attained it is only in very few drug molecules. Ion holding from other sites reassures its moderate activity.

In verapamil again all sites of ion holding can be easily accessed. One of the easily accessed sites in this case leads to strong electrostatic interaction where the ion may end up being permanently captured. In that situation lot of drug molecules would be 'wasted' after permanently capturing an ion, as evidenced through its low potency.

In this discussion we have to remember that phenylalkylamines can work as antiarrhythmic drugs in an optimum fashion only when they temporarily obstruct ion flow. Permanent obstruction is dangerous and reduces the medicinal power or potency of the drug.

## 4. Conclusions

Pharmacophoric features of phenylalkylamines have been derived based on conformational studies. Derived features can be used for virtual screening of various chemical compounds. Drug ion interaction energies indicate all possible sites from where the drug can 'hold' the ion. Potency of some phenylalkylamines has been explained in light of the fact that the drug is required to temporarily obstruct ion flow not permanently. Allowing conformational relaxation of drug in presence of the ion leads to a situation in Gallopamil where the ion seems to be 'captured' but the moderate activity actually observed indicates that probably only few drug molecule are 'wasted'. In verapamil results indicate that the ion can easily be so strongly held that a number of drug molecules are seemingly 'wasted' thus explaining its lowest activity. Potency has to be explained in terms of 'physiological accessibility' of the situation and acceptability in terms of mechanistic implications.

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