Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 1605–1611
Bioorganic & Medicinal Chemistry

First pharmacophoric hypothesis for T-type calcium channel blockers

Munikumar Reddy Doddareddy, Hee Kyung Jung, Jae Yeol Lee, Yong Sup Lee, Yong Seo Cho, Hun Yeong Koh and Ae Nim Pae*

Biochemicals Research Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul, 130–650, South Korea

Received 3 January 2004; accepted 26 January 2004

Abstract—A three-dimensional pharmacophore model was developed for T-type calcium channel blockers in order to map common structural features of highly active compounds by using CATALYST program. In the absence of three dimensional structure based information like binding mode and unavailability of more number of specific T-type calcium channel blockers, this hypothesis which consists of three hydrophobic regions, one hydrogen bond acceptor and one positive ionizable regions will act as a valuable tool in designing new ligands. Further more after the withdrawal of mibefradil, the first marketed T-type calcium channel blocker, due to the drug–drug interactions, there is an urgent need for more work in this interest.

1. Introduction

Calcium channel blockers (CCBs) are widely used for the treatment of patients with stable angina pectoris, hypertension and variant angina.[1–3] CCBs are a heterogeneous group of drugs that inhibit inward calcium channel current to a variable degree in different tissues including the vascular smooth muscle, myocardium, and sinus and atrioventricular (AV) nodes.[1,2] First generation CCBs such as nifidepine, diltiazem and verapamil all block L-type calcium channels and are classified as dihydropyridine (e.g., nifedipine) or non-DHP agents such as phenylalkylamines (e.g., verapamil) and benzothiazepines (e.g., diltiazem). The second and third generations CCBs are either slow-release or long-acting formulations of the first generation CCBs, examples such as amlodipine or felodipine. But most of the CCBs used in the therapy of hypertension and angina pectoris feature to some extent unwanted effects such as negative inotropism, atrioventricular blockade or neurohormonal activation,[1–3] which often limit their therapeutic use. In addition to conventional L-type calcium channels, there are T-type calcium channels, which play important role in the initial depolarization of sinus and AV nodes.[4,5] Blockade of these channels slows the sinus rate and prolongs AV nodal conduction, in addition to causing vasodilation, without adverse negative inotropic or positive chronotropic cardiac actions.[6,7] Most important example of the selective T-type calcium channel blockers is mibefradil, which was introduced to the market in 1997, then abruptly withdrawn. It was approved for use in hypertension and angina and marketed as the first selective T-type calcium channel blocker.[8] Indeed, depending on the cell type, mibefradil blocks T-type calcium channels 10–30 times more potently than L-type calcium channels.[9,10] In addition, mibefradil is highly tissue selective, relaxing smooth muscle without inducing reflex tachycardia, or having much effect on cardiac chronotropy or inotropy.[8,11] However, pharmacokinetic interactions[4] with other drugs metabolized by cytochromes P-450 3A4 and 2D6 (antihistamines, such as astemizole) and postmarketing data showing an increase in mortality in the elderly who were also taking β-blockers and DHP-type CCBs and reports of rhabdomyolysis with concomitant simvastatin therapy,[12,13] eventually led to the withdrawal[14,15] of mibefradil from the clinic.

So as a first step towards finding a novel T-type calcium channel blockers devoid of adverse effects like that on cytochrome P-450 3A4, we generated 3D pharmacophore by using 8 highly active compounds including mibefradil. We made an attempt to identify the hypothetical 3D ligand-based pharmacophore model by using common feature hypothesis generation approach

Keywords: Pharmacophore; Hypothesis; T-type calcium channel.
* Corresponding author. Tel.: +82-2958-5185; fax: +82-2958-5189; e-mail: anpae@kist.re.kr

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.01.034

(HipHop) implemented in CATALYST[16] program. In particular, HipHop algorithm finds common feature pharmacophore models among a set of highly active compounds thus carrying out a 'qualitative model' without the use of activity data, which represents the essential 3D arrangement of functional groups common to a set of molecules for interacting with a specific biological target. This approach is the most appropriate for our ligands as the 'quantitative' hypothesis generation method (HypoGen) requires compounds with 3–4 order difference in activity, whereas we got maximum 2 order difference in a focused synthetic library of about 600 compounds.

2. Materials and methods

Eight molecules (No 1–8, Fig. 1) including mibefradil[8] are selected for the generation of pharmacophore model, representing the most interesting compounds among three chemical classes of compounds,[17] which were synthesized by us. The biological activities (IC_{50} data) on HEK 293 cell with stabilized α1G T-type calcium channel were shown in Table 1.

All structures were generated using 2D/3D editor sketcher in catalyst 4.8 software package and are minimized to the closest local minimum using the CHARMm-like force field implemented in the program.[18] The chirality of mibefradil is known and assigned S as reported in the literature. But regarding the asymmetric centers of **1a–c** compounds, as no experimental data on the biologically relevant conformations of these molecules is available, it was arbitrarily decided to assign 'undefined' chirality, allowing the pharmacophore model procedure to choose which configuration of the asymmetric carbon atoms is the most appropriate. A stochastic research coupled to a poling method[19] was applied to generate conformers for each compound by using 'Best conformer generation' option with 20 kcal/mol energy cutoff (20 kcal/mol maximum compared to the most stable conformer).

Table 1. The biological activities of the compounds

No	Compound	IC_{50} (μM)
1	**1a**	0.2
2	**1b**	0.25
3	Mibefradil	0.84
4	**1c**	0.9
5	**2a**	1.02
6	**2b**	1.53
7	**2c**	2.02
8	**2d**	2.04

2.1. Pharmacophore model generation

In hypothesis generation, on the basis of the atom types in the molecules, the following chemical functions were selected in the feature dictionary of the catalyst: hydrogen bond acceptor, positive ionizable and hydrophobic groups. The default HBA of the feature dictionary which recognizes N, O, and S as hydrogen bond acceptors was modified to include F also as HBA, as some of the active compounds contain trifluoromethyl groups in positions where others contain O or N as hydrogen bond acceptors based on electronegativity differences, F is also thought to act as hydrogen bond acceptor.

In generating hypothesis, we followed two strategies.

2.1.1. Strategy 1. In strategy I, hypothesis generation was done by assuming that 'all compounds are equally important and all contain important features', this is achieved by putting 2 in principal number which ensures that all of the chemical features in the compound will be considered in building hypothesis space and 0 in maximum omitting features column which forces mapping

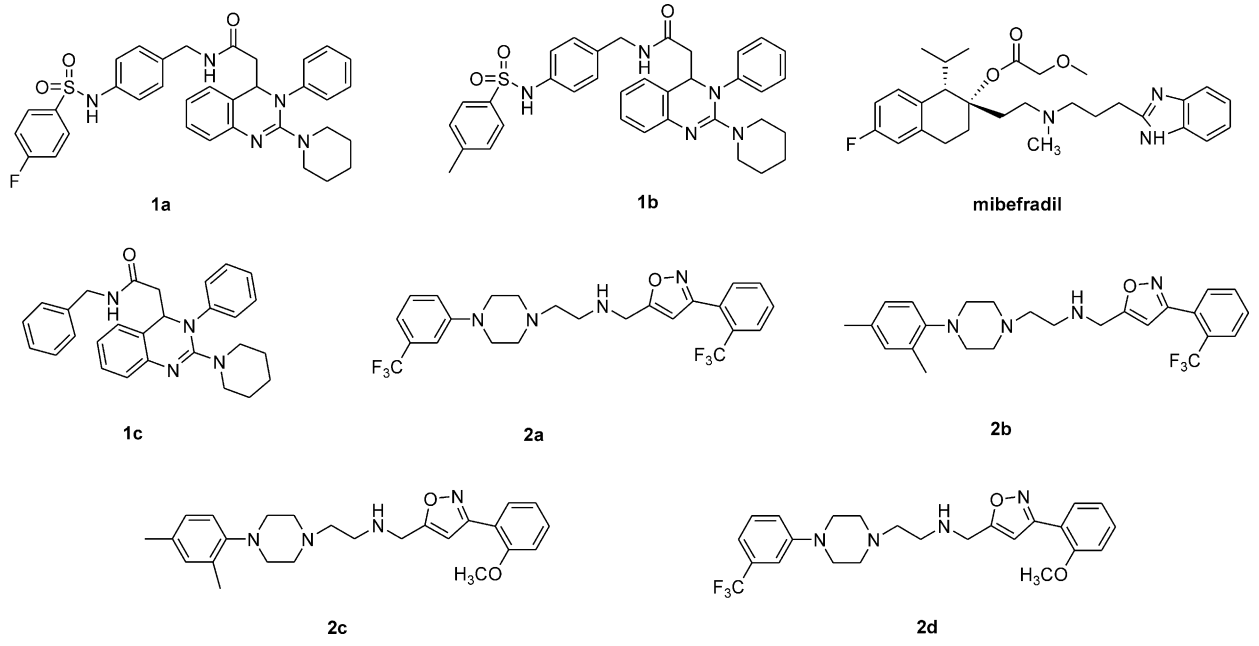


Figure 1. The compounds that were used for pharmacophore generation.

Table 2. Summary of hypothesis run (strategy I)

No.	Composition	Ranking score	Direct hit mask	Partial hit mask
1	PZZZH	107.242	11111111	00000000
2	PZZZH	107.242	11111111	00000000
3	PZZZH	107.219	11111111	00000000
4	PZZZH	107.219	11111111	00000000
5	PZZZH	106.804	11111111	00000000
6	PZZZH	105.055	11111111	00000000
7	PZZZH	105.055	11111111	00000000
8	PZZZH	104.964	11111111	00000000
9	PZZZH	104.718	11111111	00000000
10	PZZZH	104.500	11111111	00000000

P; Positive ionizable, Z; hydrophobic, H; hydrogen bond acceptor Direct hit mask indicates whether (1) or (0) not a training set molecule mapped every feature. Partial hit mask indicates whether (1) or (0) not a molecule mapped all but one feature.

of all features for all compounds. Except these, all other parameters were kept at default.

All the ten hypotheses generated using modified HBA feature contained three hydrophobic regions, one positive ionizable region and one hydrogen bond acceptor with ranking scores between 107.242 to 104.500 (Table 2). Higher ranking score, the less likely it is that the molecule in the training set fit the hypothesis by a chance correlation. This small range of ranking score and same features in all hypotheses suggests that the same five features are spatially arranged almost similar way in all hypotheses. Further more, when in catalyst some models exhibit the same value of ranking score, this means that their statistical relevance is identical and that the molecules in the training set have exactly the same probability to fit those hypotheses by a chance correlation. In our study this occurred between models 1 and 2, 3 and 4 and also for 6 and 7, which all appears like mirror images of each other.

The choice of best hypothesis was based on the criteria that of all the top ranking hypotheses, the hypothesis which maps to all important features of the active compounds and if possible to some extent show correlation between best fit values, conformational energies and actual activities of the training set. In the absence of activity data and any bias towards a particular compound, catalyst tries to generate hypothesis taking each compound as reference, in such way that it satisfies features of all other compounds also. From thousands to millions of potential hypotheses it gives top 10 ranking

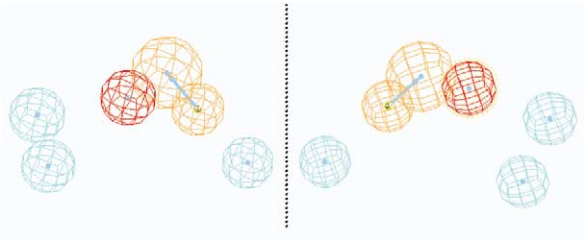


Figure 2. Hypothesis 1 and 2.

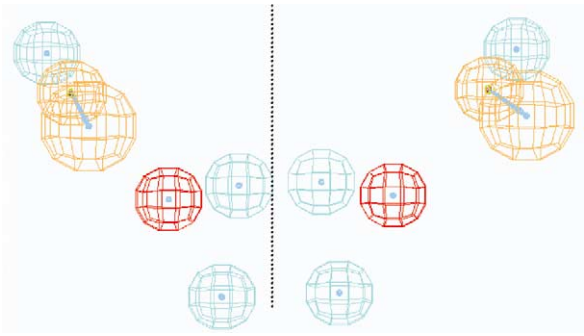


Figure 3. Hypothesis 3 and 4.

hypotheses as output. In our study the first two models (Fig. 2) though statistically best, their best fit values (Table 3) shows that they are biased towards majority, that is, four structurally similar compounds of **2** series of total 8 compound training set. The next two models 3 and 4 (Fig. 3) shows good correlation between best fit values and activities (Table 3) and fits best to the most active compounds. As our requirement is to select best possible 3D arrangement of chemical features of the most active compounds, at the same time satisfying the arrangement in all other compounds also, we selected both 3 and 4 as best because both are identically ranked and are almost like mirror images.

Figure 4 shows the mapping of hypothesis 3 and 4 with the most active compound **1a**. It is observed that hypothesis 3 is mapping to *S*-isomer and hypothesis 4 to *R*-isomer indicating their parent reference conformer from which hypothesis is generated. In both cases best fits came from low energy conformers (6 kcal/mol), where hydrogen bond acceptor position is occupied by O of sulfonamide group, positive ionizable to double bonded N of fused tetrahydropyrimidine group, two hydrophobic regions are occupied by phenyl rings and one by piperidine moiety. However these models failed

Table 3. Best fit values of four statistically best hypothesis

No	Compd	Principal No.	Max omitting feature	Best fit values				IC_{50} (μM)
				hypo-1	hypo-2	hypo-3	hypo-4	
1	**1a**	2	0	2.46	2.382	4.98	4.98	0.2
2	**1b**	2	0	2.14	2.204	4.99	4.99	0.25
3	Mibefradil	2	0	3.001	2.966	3.185	3.552	0.84
4	**1c**	2	0	0.846	0.792	0.0137	0	0.9
5	**2a**	2	0	4.63	4.627	4.004	4.125	1.02
6	**2b**	2	0	4.93	4.92	3.633	3.739	1.53
7	**2c**	2	0	5	5	3.397	3.568	2.02
8	**2d**	2	0	4.68	4.67	3.039	3.42	2.04

to explain the role of ester side chain of mibefradil (Fig. 5), which is thought to be very important for activity. It is not clear whether the ester side chain plays role in the binding or in pharmacokinetics or in both and further more **1c** which has almost equal activity like that of mibefradil showed poor fit values of less than 1 (Table 3)

2.1.2. Strategy II. Strategy 2 involves hypothesis generation giving bias to the most active compounds and neglecting the compounds which showed poor fit values, this was achieved by putting 2 and 0 in principal and maximum omitting features columns respectively for the most active compounds, and 1, 1 in principal and maximum omitting features columns respectively of all other compounds. Compound **1c** was deleted from training set to get more reasonable results.

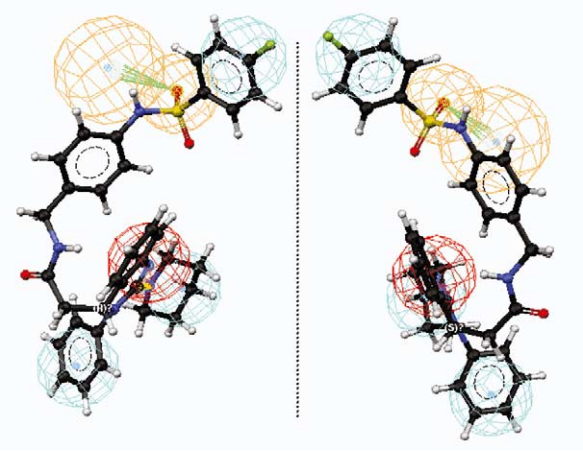


Figure 4. The compound **1a** mapping to hypothesis 3 and 4.

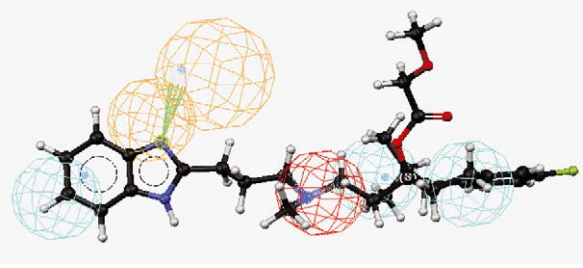


Figure 5. Mibefradil mapping to the hypothesis 3.

Table 4. Summary of hypothesis run (strategy II)

No.	Composition	Ranking score	Direct hit mask	Partial hit mask
1	PZZZHH	116.838	1111111	0000000
2	PZZZHH	116.184	0111111	1000000
3	PZZZHH	116.184	0111111	1000000
4	PZZZHH	115.503	1111111	0000000
5	PZZZHH	115.503	1111111	0000000
6	PZZZZHH	114.148	0111111	1000000
7	PZZZHH	113.372	1111111	0000000
8	PZZZZH	112.922	0111111	1000000
9	PZZZZH	112.922	0111111	1000000
10	PZZZHH	112.808	1111111	0000000

P; positive ionizable, Z; hydrophobic, H; hydrogen bond acceptor Direct hit mask indicates whether (1) or (0) not a training set molecule mapped every feature. Partial hit mask indicates whether (1) or (0) not a molecule mapped all but one feature.

Ten hypotheses are obtained using the default parameters of the catalyst; all except the 6th one contained six features (Table 4) with ranking scores between 116.8 and 112.8. The statistically best hypothesis (Fig. 6) contained 3 hydrophobic groups, 2 hydrogen bond acceptor groups and one positive ionizable group.

Figure 7 shows the mapping of statistically best hypothesis with the most active compound **1a**. The

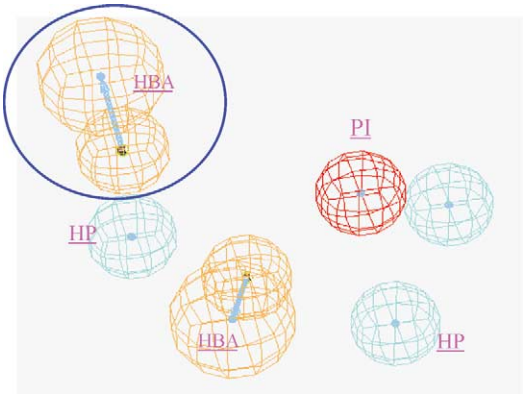


Figure 6. Statistically best 6-feature hypothesis (strategy II).

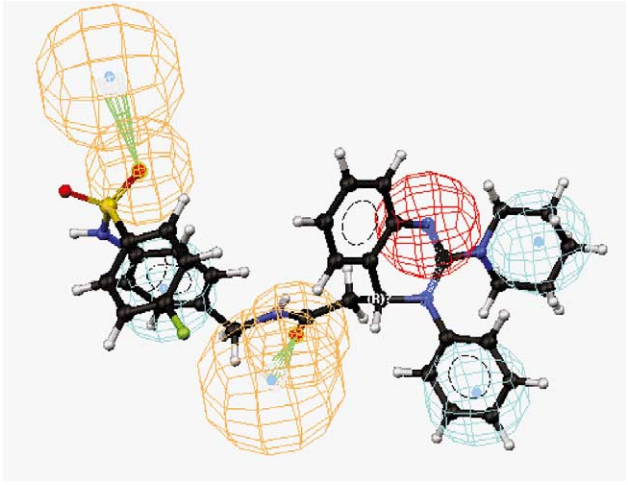


Figure 7. Compound **1a** mapping to the best hypothesis.

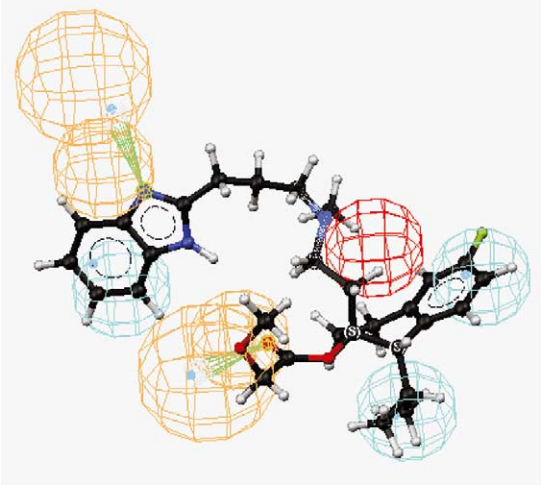


Figure 8. Mibefradil mapping to the best hypothesis.

Table 5. Best fit values allowing missing one of the feature

No	Compd	Principal value	Max. Omit. feat	Best fit values	
				Hypo-1[a]	Hypo-1[b]
1	**1a**	2	0	5.999	5.999
2	**1b**	2	0	5.72	5.72
3	Mibefradil	1	1	1.895	4.28
4	**2a**	1	1	4.22	4.5
5	**2b**	1	1	4.27	4.54
6	**2c**	1	1	2.42	4.56
7	**2d**	1	1	2.5	4.51

[a] Best fit values with max.omit.feat value as 0.
[b] Best fit values with max.omit.feat value as 1.

hydrogen bond acceptor positions are occupied one by O of sulfonamide group and other by O of amide group, positive ionizable to N of fused tetrahydropyrimidine group and three hydrophobic regions to two phenyl groups and one piperidine group like before. This model involves ester side chain of mibefradil in the hydrogen bond forming (Fig. 8). Table 5 shows the best-fit values of other compounds with selected hypothesis.

3. Results and discussion

By using 8 structurally different molecules, total 20 hypotheses are generated by using common feature hypothesis generation approach implemented in CATALYST. For this two strategies are followed, first one assumes that 'all compounds are important and all contain important features, differences in activities is related to the differences in other relevant factors like conformational energies, but not due to the absence of any important feature required for binding'. In contrast, the hypothesis generation in second strategy is done by giving bias to the most active compounds assuming that they contain all important features and others may or may not be. The hypothesis 3 and 4 of the first strategy (Fig. 3) are statistically identical and low energy conformers of the active compounds mapped best to them.

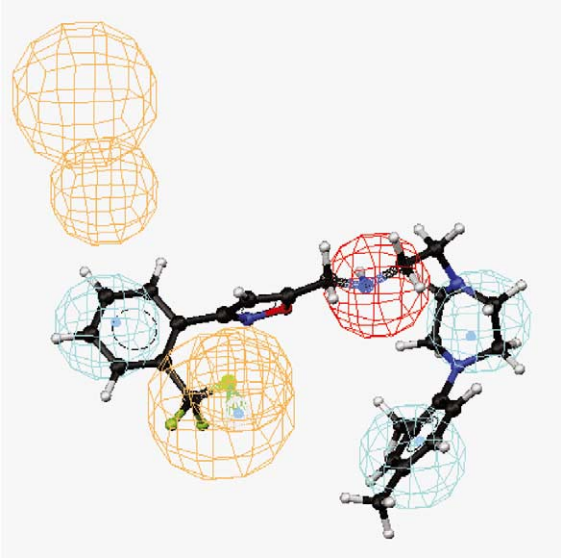


Figure 9. One hydrogen bond acceptor is not mapping.

However both models failed to account for ester side chain of mibefradil, in fact of all the 100 mappings not even single mapping involved ester side chain in either hydrogen bond formation or in hydrophobic interactions, further more these two models gave poor fit values for **1c** which has almost equal activity to that of mibefradil.

The statistically best hypothesis (Fig. 6) obtained from the second strategy is more satisfactory in the sense that it involves ester side chain of mibefradil in the hydrogen bond formation. Compare fit analysis of all compounds with maximum omitting features value as 1 (Table 5) showed that all compounds except the two compounds **1a** and **1b** left out one of the hydrogen bond acceptor group (Fig. 9) indicating that, it may not be essential, but may be the reason for the additional activity of **1a** and **1b**. Removing that feature and subsequent compare fit analysis of **1c** (poor fit with earlier hypotheses) gave a best-fit value of 4.85 that too in a low energy conformation (7.9 kcal/mol). Figure 10 shows the mapping of **1c** with the modified hypothesis and Table 6 shows best-fit values and conformational energies of all the training set molecules with this modified 5-feature hypothesis.

Table 6 also includes best-fit values and conformational energies of **2** series, when HBA position is occupied by acceptor group on aromatic ring, and also of mibefradil, when HBA position is occupied by O of ester side chain. The low energies in that column of the Table 6 clearly show that they are most favored positions for acceptor group. Though for all compounds of **2** series, best fitting

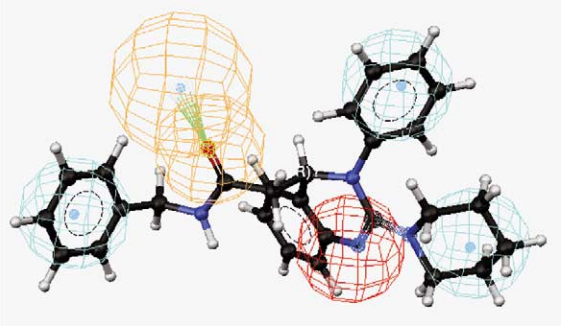


Figure 10. The mapping of compound **1c** to modified hypothesis.

Table 6. Low energy conformations mapping to the important groups in the modified pharmacophore deleting one feature

No	Compd	Bestfit	Conf. cnergy kcal/mol	Bestfit	Conf energy kcal/mol
1	**1a**	5	12.42	4.9[a]	9.7[a]
2	**1b**	4.91	10.59	4.75[a]	7.23[a]
3	Mibefradil	4.28	14.66	3.88[b]	11.95[b]
4	**1c**	4.85	7.9	4.85	7.9
5	**2a**	4.5	16.78	4.37[c]	4.32[c]
6	**2b**	4.54	14.07	4.21[c]	7.68[c]
7	**2c**	4.56	18.36	3.78[c]	9.20[c]
8	**2d**	4.51	7.93	3.92[c]	8.90[c]

[a] Low energy conformers of energy lessthan 10 *K*cal/mol.
[b] HBA mapping to ester side chain.
[c] HBA mapping to acceptor on aromatic ring.

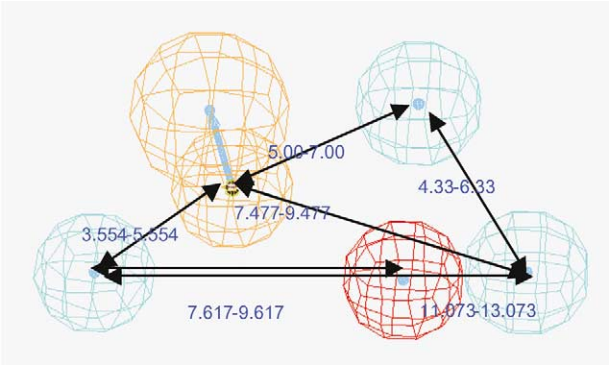


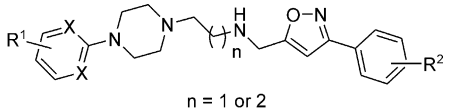
Figure 11. 5-feature pharmacophore with essential features. HBA, Hydrogen bond acceptor, HP, hydrophobic, PI, positive ionizable.

conformers mapped nitrogen of isoxazole as HBA, lower energy conformers favored acceptor at second position of the aromatic ring and that is what we expected from our knowledge of small molecule T-type calcium channel blockers (the compound **2** series), where all the molecules contain isoxazole ring but only very few that too with acceptor at position 2 showed good activity. From the above observations we propose a 5 feature hypothetical 3D pharmacophore containing three hydrophobic, one positive ionizable and one hydrogen bond acceptor regions as essential features with distance tolerances as shown in the Figure 11. The additional HBA present in 6 feature hypothesis (Fig. 6) may be accounting for additional activity of highly active compounds **1a** and **1b.**

3.1. External test set

Table 7 shows external test set of 10 compounds with IC_{50} values. Compounds **3**, **4**, and **5** showed less activity because of hydrogen bond acceptor position being away from favorable 2-position, compounds **6** and **7** showed less activity may be due to presence of less hydrophobic methoxy groups, where according to pharmacophore we need hydrophobic rich groups, this is also true for compound **8** which has pyrimidine group in the position where active compounds contain hydrophobic substituted phenyl groups. Higher activity of compound **9** compared to compound **10** which has one chain length more can be explained from the fact that former required low energy conformer of 2.61 kcal/mol, whereas later required high energy conformer of more than 14 kcal/mol to map the same pharmacophore, this is also true for compounds **11** and **12** which has low activity when compared to their homologues used in test set (**2a** and **b**).

Table 7. Test compounds



n = 1 or 2

X = C or N

Compd	R^1	R^2	n	X	IC_{50}
3	4-Methyl	3,4-(1,3-Dioxolane)	1	C	7.62
4	4-Methyl	3,4-(1,4-dioxane)	1	C	7.49
5	4-Methyl	4-Benzyloxy	1	C	23.86
6	4-Methoxy	2-CF_3	1	C	11.20
7	4-Methoxy	2-Methoxy	1	C	33.48
8	—	2-Methoxy	1	N	8.24
9	4-Methyl	2-CF_3	1	C	2.51
10	4-Methyl	2-CF_3	2	C	4.75
11	2,4-Dimethyl	2-CF_3	2	C	4.17
12	2-CF_3	2-CF_3	2	C	2.71

5. Conclusion

The pharmacophore model described herein represents the first contribution to the rational design of T-type calcium channel blockers. This pharmacophore, which contained 3 hydrophobic regions, one positive ionizable region and one hydrogen bond acceptor region is further validated by using an external set of 10 compounds, whose successful explanation of structure activity relationships proves the efficiency of this approach in designing novel T-type calcium channel blockers. The most active compounds **1a** and **b** were satisfied with 6-feature hypothesis (Fig. 6), which has additional HBA feature and this may be the reason for their high activity, thus both 5 feature and 6-feature hypotheses can be used for virtual screening to get insight for new scaffolds design.

Acknowledgements

This work was supported by the Korea Ministry of Science and Technology.

References and notes

1. Opie, L.H; Frishman, W.H; Thadani, U. Calcium channel antagonists (calcium entry blockers). In: Opie, L.H, editor. *Drugs for the Heart. 4th ed.* Philadelphia: WB Saunders; **1994** p 50.
2. Abernethy, D. R.; Schwartz, J. B. *N. Engl. J. Med.* **1999**, *341*, 1447.
3. Thadani, U. *Curr. Opin. Cardiol.* **1999**, *14*, 349.
4. Asirvatham, S.; Sebastian, C.; Thadani, U. *Drug Safety* **1998**, *19*, 23.
5. Triggle, D. J. *J. Hypertens.* **1997**, *15*, 9.
6. Portegies, M. C.; Schmitt, R.; Kraaij, C. J.; Braat, S. H. J. G.; Gassner, A.; Hagemeijer, F.; Ponzenel, H.; Prager, G.; Viersma, J. W.; VanderWall, E. E.; Kleinbloesem, C. H.; Lie, K. I. *J. Cardiovasc. Pharmacol.* **1991**, *18*, 746.
7. Cremers, B.; Flesch, M.; Sudkamp, M.; Bohm, M. *J. Cardiovasc. Pharmacol.* **1997**, *29*, 692.
8. Ertel, S. I.; Ertel, E. A.; Clozel, J. P. *Cardiovasc. Drugs Ther.* **1997**, *11*, 723.
9. Mishra, S. K.; Hermsmeyer, K. *Circ. Res.* **1994**, *75*, 144.
10. Benardeau, A.; Ertel, E. *Selective block of myocardial T-type calcium channels by mibefradil: A comparison with the 1,4-dihydropyridine,amlodipine*, Clozel, J. P., Nargeot, J. Tsien, R. W. (Eds.). pp 386–394, Adis international chester, UK 1997.
11. Kobrin, I.; Bieska, G.; Charlon, V.; Lindberg, E.; Pordy, R. *Cardiology* **1998**, *89*, 23.

12. Schmassmann-Suhijar, D.; Bullingham, R.; Gasser, R.; Schmutz, J.; Haefeli, W. E. *Lancet* **1998**, *351*, 1929.
13. Mullins, M. E.; Horowitz, B. Z.; Linden, D. H.; Smith, G. W.; Norton, R. L.; Stump, J. *JAMA* **1998**, *280*, 157.
14. SoRelle, R. *Circulation* **1998**, *98*, 831.
15. Reimer, K. A.; Califf, R. M. *Circulation* **1999**, *99*, 198.
16. CATALYST version 4.8 software; ACCELRYS Inc:2001.
17. (a) Compounds **1a**–**1c**; *Bioorg. Med. Chem.* manuscript in preparation. (b) Compounds **2a**–**2d**; *Bioorg. Med. Chem. Lett.*, submitted for publication.
18. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
19. Smellie, A.; Teig, S. L.; Towbin, P. *J. Comput. Chem.* **1995**, *16*, 171.