http://dx.doi.org/10.5012/bkcs.2013.34.9.2707

N-Phenyl-3-Pyridin-2-yl Imino Derivatives as Vascular Smooth Muscle Relaxants: Potential Phosphodiesterase V Inhibitors

Prafulla Choudhari,* Manish Bhatia, and Neela Bhatia

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Kolhapur
**E-mail: prafulla.choudhari@bharatividyapeeth.edu, praffula12@rediffmail.com, praffula12@gmail.com*
Received May 21, 2013, Accepted June 19, 2013

The present communication deals with development of ten novel *N*-Phenyl-3-Pyridin-2-yl imino derivatives as vascular smooth muscle relaxants. The derivatives were prepared and optimized using pocket modelling and pharmacophore modelling. The 4 hydroxy substituted derivatives are showed potent activity comparable to the sildenafil.

Key Words : Goat aorta, Binding pocket, Phosphodiesterase V, Pharmacophore

Introduction

Phosphodiesterase are enzymes which regulate the cellular concentration of cAMP and cGMP. In human body there are 21 PDE genes which are classified into the 11 families. Due to their involvement in various important biological functions the Phosphodiesterase is emerged as promising target for the treatment of various human diseases particularly the CVS disorders.[1-5] PDE 5 inhibitors have potentials for treatment of heart diseases as they can act as vascular smooth muscle relaxants. Due to discovery of molecules like sildenafil the development of PDE5 inhibitor is main focused research area for many pharmaceutical companies and researchers.[6-11] Advances in structural biology and computational chemistry resulted in increased speed of drug discovery. Pharmacophore modelling is an advanced method which is utilized to identify the structural features which are important for the biological activity. Binding pocket analysis involves analysis of binding pocket using electrostatic potential which can give relative orientation of important amino acids in the binding pocket and size and shape of the binding pocket. Integration of binding pocket modelling and pharmacophore modeling can result in design of potent drug candidates. Here we report the design of some vascular smooth muscle relaxants with potential to be act as PDE5 inhibitors using integration of binding pocket modelling and pharmacophore modeling.

Experimental

The dataset in the present study was taken from the previously reported paper.[12]

Pocket Modeling.[13] The pocket modeling was carried out in proviz module of Vlife MDS 4.3 software. The PDB (2H42) of Phosphodiesterase V was downloaded from the protein data base. The water molecules were removed and hydrogen was added. The energy of the protein was minimized using MMFF and the residues at the periphery of the active pocket at a distance of less than 5 Å from the native ligand surface were marked. The marked pocket of the protein was used for optimizing structural requirements of designed molecules and further studies.

Docking Studies. To explore the interactions of the compounds, we carried out binding simulations using biopredicta module of Vlife MDS 4.3 suite. We used the crystal structure of the Phosphodiesterase V PDB ID 2H42, to perform docking simulations and potential H-bonding, π-stacking, VDW, Hydrophobic interactions with the designed compounds were recorded. The structure of 3,4-thiadiazol-2-yl]-3-[(5-nitropyridin-2-yl)imino]butanamide was used as the template to built the molecules in the dataset in builder module of Vlife MDS 4.3. Prior to docking studies a systematic conformational search was performed to obtain the low energy conformations of the ligands. The low energy conformations thus obtained, were optimized till they reached rms gradient energy of 0.001 kcal/mol Å. In order to define ligand–receptor interactions, docking of all the low energy conformations within a range of 5 kcal/mol Å from the lowest energy conformation of each molecule.

Molecular Alignment.[13] The molecules of the dataset were aligned by the template based technique, using sildenafil as a template for alignment of the molecules.

Pharmacophore Modeling.[13] This Pharmacophore modeling was carried out in the mol sign module of Vlife MDS 4.3 software. Series of designed inhibitors were first aligned on the sildenafil. A pharmacophore model is a set of three-dimensional features that are necessary for bioactive ligands. The minimum number of pharmacophore features generated for an alignment is taken 4 and tolerance is kept to 10 Å. The Max Distance Allowed between two features is placed to 10 Å.

Smooth Muscle Relaxant Activity. All the synthesized compounds were tested in vitro for their pulmonary vein relaxant activity. Pulmonary veins and arteries of adult goat of either sex were brought from a local slaughterhouse. The media used to carry the muscle was ice-cold krebs–henseleit solution. These were cut into spiral strips and were used within 12–24 h. These strips were mounted in 15 mL

isolated organ baths, containing krebs–henseleit solution, mixed with 95% O_2 and 5% CO_2 at 37 °C. The strip was allowed to equilibrate for 2 h under a resting load of 2 g. Relaxation of muscle strip was recorded for each drug using force transducer multichannel physiograph (BIOPAC MP35 SYSTEM). The title compounds were compared with Sildenafil, a standard drug used for relaxation.

Results and Discussion

Pocket Modeling Studies.[13] The pocket modeling studies were carried out on the proviz module of Vlife MDS 4.3. Proviz is a module for evaluation and visualization of three-dimensional molecular properties including those derived from ab initio quantum mechanical wave function obtained through programs like GAMESS and Gaussian. The reported active site of Phosphodiesterase V was found to be composed of amino acids like tyrosine 664, alanine 823, leucine 864, phenylalanine 820, phenylalanine 786, alanine 783, valine 782, and tyrosine 612. These amino acids were marked from the whole protein in biopredicta module of Vlife MDS 4.3 and this isolated pocket was further analyzed in proviz module. The electrostatic potential and hydrophobic surface map of this isolated binding pocket indicated the shape of the cavity. The binding pocket modelling showed active site of phosphodiesterase V is u shaped so a complimentary u shaped ligand can fit in to the active site of PDE5, and will be act as a potent inhibitor. Keeping in this mind we utilized our previously designed set of molecules in which two aryl rings are separated by the amide bridge and having u shaped conformation. The electrostatic surface map also indicated the designed sets of molecules are having desired electrostatic potential for the interaction with PDE5. The brown region of the surface map indicates the electropositive region while the purple color indicated the electronegative region which is responsible for the hydrogen bond interactions with the receptor as shown in Figures 1 and Figure 2.

Biological Activity. The vascular smooth muscle relaxant activity of the designed inhibitors was carried out by using the goat aorta and almost all the designed inhibitors showed

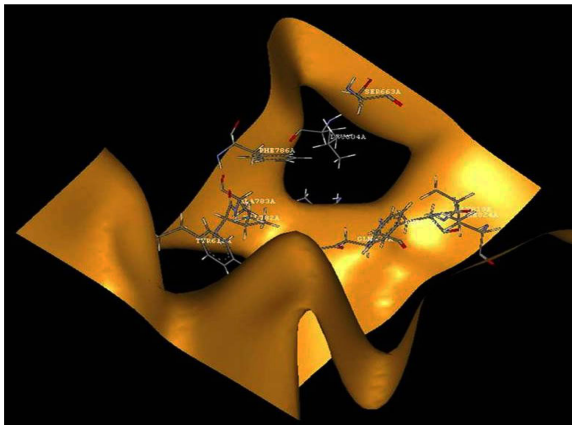


Figure 1. Figure showing binding pocket of PDE5.

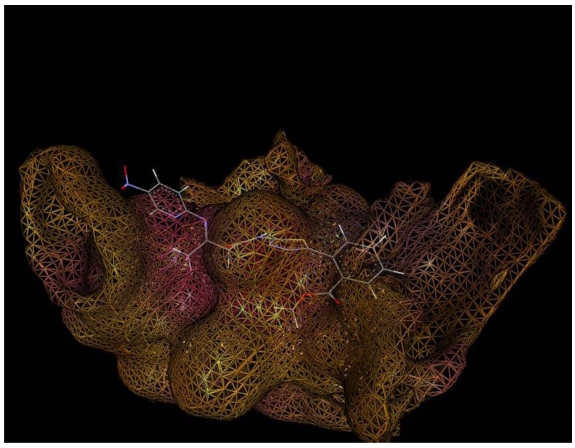
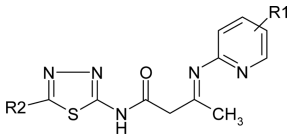


Figure 2. Figure showing surface map of designed inhibitor.

good-to-moderate vasodilatation activity. The activities of PB5 and PB10 are very good and comparable to the activity of Sidenafil, while PB1, PB2, PB4, PB7, PB8, and PB9 produced moderate activity as shown in Table 1 and Figure 3. The PB5 and PB10 are showed profound activity due to the introduction of hydroxy group which imparts the hydrogen bond donation capacity which resulted in to the hydrogen bond interaction with tyrosine 612 in the binding pocket of PDE5. Table 1 and Figure 3.

Table 1. Table showing observed activity of designed molecules



Sr no	R^1	R^2	IC_{50} (nM)
PB1	H	4-$NH_2C_6H_4$	46.06
PB2	H	2-$OCOCH_3$ C_6H_4	55.39
PB3	H	4-$NO_2C_6H_4$	59.65
PB4	H	C_2H_2 C_6H_4	1.06
PB5	H	4-OHC_6H_4	3.61
PB6	5-NO_2	4-$NH_2C_6H_4$	79.67
PB7	5-NO_2	2-$OCOCH_3$ C_6H_4	91.01
PB8	5-NO_2	4-$NO_2C_6H_4$	9.76
PB9	5-NO_2	$C_2H_2C_6H_4$	23.6
PB10	5-NO_2	4-OHC_6H_4	4.133
Std		Sidenafil	1.433

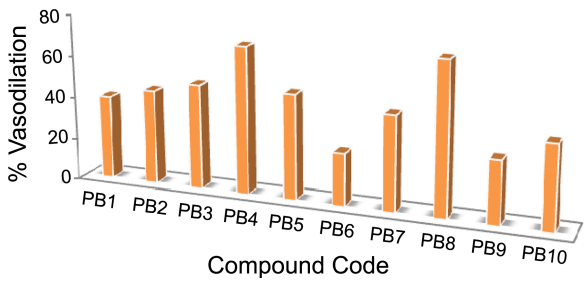


Figure 3. Figure showing % Vasodilatation of designed inhibitors.

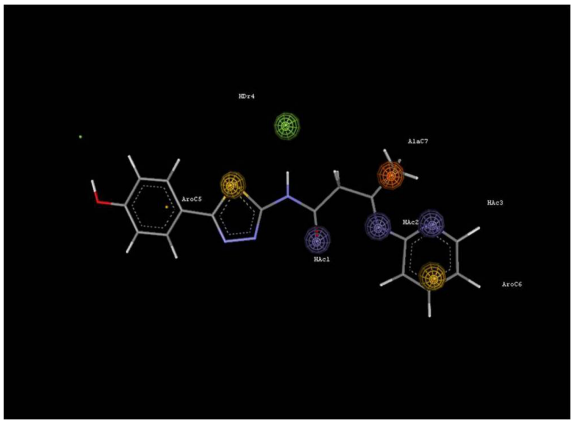


Figure 4. Figure showing pharmacophore features of designed molecules.

Pharmacophore Identification Studies using Vlife MDS 4.3. A set of pharmacophore hypothesis was generated using the mol sign module of V life MDS 4.3 on the reported inhibitors of Phosphodiesterase V. Each hypothesis was found to contain common features like hydrogen bond donor, hydrogen bond acceptor, and aromatic (Golden color). Results of pharmacophore identification studies revealed that the hydrogen bond donor (green Color), hydrogen bond acceptor (blue color), aromatic features (golden color), aliphatic (brown color) as shown in Figure 4 are important for the activity. The pharmacophoric features of the designed inhibitors were also compared with the sildenafil. The all the ten molecules are align with sildenafil structure and pharmacophoric features are calculated which indicates the designed set of molecules are having hydrogen bond donor (green Color), hydrogen bond acceptor (blue color), aromatic features (golden color), aliphatic (brown color) features in common, due to this features similarity the designed set of molecules are showed similar binding potential to that of sildenafil (yellow color) as shown in Figure 5.

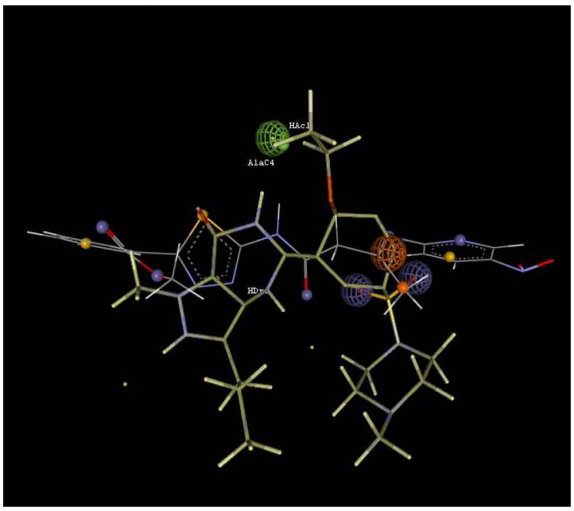


Figure 5. Figure showing pharmacophore features of designed molecules and sildenafil.

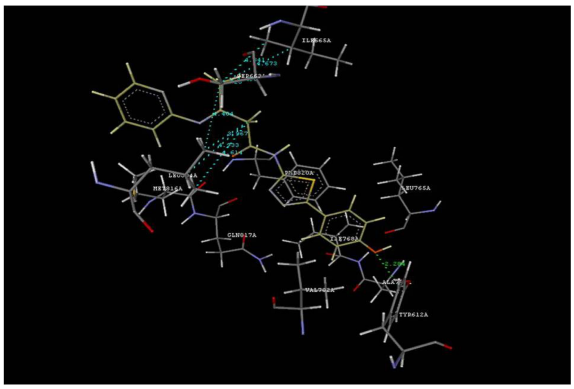


Figure 6. Figure showing interactions of designed molecule with PDE5.

Docking Analysis of Designed Inhibitors. To check the interaction potential of the designed inhibitors the docking analysis was performed on Phosphodiesterase V (PDB ID 2H24). PB8 having hydrogen bond donor, which found to show hydrogen bond interaction is observed at a distance of 2.284 Å with tyrosine 612 and aromatic feature of the inhibitor showed aromatic interaction with phenylalanine 820 at a distance 4.335 Å. The inhibitors showed Hydrophobic interactions with SER663, ILE665, LEU804 and Van der Waals interactions with amino acids like TYR612, SER663, ILE665, LEU765, ALA767, ILE768, VAL782, LEU804, MET816, GLN817, PHE820. Figure 6.

Conclusion

Structural insights for Phosphodiesterase-V inhibitors were obtained using pharmacophore modelling and pocket modelling studies with an aim to discover leads for the treatment and prevention of cardiovascular diseases. The 3,4-thiadiazol-2-yl]-3-[(5-nitropyridin-2-yl)imino]butanamide derivatives also showed good vascular smooth muscle relaxant activity in comparison to standard compounds.

Acknowledgments. The authors are thankful to Dr. H. N. More, Principal, Bharati Vidyapeeth College of Pharmacy, Kolhapur for providing facilities to carry out the work. And the publication cost of this paper was supported by the Korean Chemical Society.

References

1. Chen, G.; Wangb, H.; Robinson, H.; Cai, J.; Wana, Y.; Ke, H. *Biochem. Pharm*. **2008**, *751*, 1717.
2. Chen, C.; Chang, Y.; Bau, D.; Huang, H.; Tsai, F.; Tsai, C.; Chen, C. Y. *Acta Phar. Sini*. **2009**, *30*, 1186.
3. Guazzi, M. *Cir. Heart. Fail*. **2008**, *1*, 272.
4. Matsumoto, T.; Kobayashi, T.; Kamata, K. *J. Smooth Muscle Res*. **2003**, *39*, 67.
5. McAllister-Lucas, L. M.; Sonnenburg, W. K.; Kadlecek, A.; Seger, D.; Trong, H. L.; Colbran, J. L.; Thomas, M. K.; Walsh, K. A.; Francis, S. H.; Corbin, J. D.; Beavo, J. A. *J. Biol. Chem.* **1993**, *268*, 22863.
6. Palmer, M. J.; Bell, A. S.; Fox, D. N. A.; Brown, D. G. *Curr. Top.*

Med. Chem. **2007**, *7*, 405.
7. Reffelmann, T.; Kloner, R. A. *Cardio. Res*. **2009**, *83*, 204.
8. Rotella, D. P. Phosphodiesterase 5 inhibitors: current status and potential applications, *Drug dis*. **2002**, *1*, 674.
9. Sausbier, M.; Schubert, R.; Voigt, V.; Hirneiss, C.; Pfeifer, A.; Korth, M.; Kleppisch, T.; Ruth, P.; Hofmann, F. *Cir Res.* **2000**, *87*, 825.
10. Srivani, P.; Srinivas, E.; Raghu, R.; Narahari Sastry, G. *J. Mole. Graph. Mod*. **2007**, *26*, 378.
11. Tollefson, M. B.; Acker, B. A.; Jacobsen, E. J.; Hughes, R. O.; Walker, J. K.; Fox, D. N. A.; Palmer, M. J.; Freeman, S. K.; Yu, Y.; Bond, B. R. *Bio. Med. Chem. Lett*. **2010**, *20*, 3120.
12. Choudhari, P. B.; Bhatia, M. S.; Bhatia, N. M. *Med. Chem. Res*. **2013**, *22*, 976.
13. www.vlifesciences.com