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# Molecular Modelling Of Phentolamine for Cardiovascular Disease

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**ABSTRACT:** Molecular modeling includes wide range of molecular graphics and computational chemistry techniques used to build, display, manipulate, stimulate and analyze molecular structures. By calculating binding affinity the highest binding affinity molecule of new analog of phentolamine is identified. Phentolamine is the active ingredient in the drug Regitime. Its acts as a long lasting adrenergic alpha antagonist, antihypertensive drug which expand blood vessels and reduce blood pressure. Phentolamine blocks alpha adrenergic receptors which lead to muscle relaxation and widening of blood vessels. This widening of blood vessels results in lowering of blood pressure. Phentolamine control episodes of hypertension and sweating that occur with a disease called pheochromocytoma. Phentolamine is used in diagnosis of pheochromocytoma and to control paroxysmal hypertension during pheochromocytomectomy.

**Key words**: phentolamine; binding affinity, alpha adrenergic receptor; pheochromocytoma; blood pressure

## Introduction

Molecular modeling [1-3] is a general term which covers a wide range of molecular graphics and computational chemistry [4] techniques used to build, display, manipulate, simulate, and analyze molecular structures, and to calculate properties of these structures. By calculating binding affinity the highest binding affinity molecule [5] of new analog of phentolamine is identified. Phentolamine is marketed under the trade name Regitine, Regitin and several others is a member of the drug class α-adrenergic blocker used for muscle relaxation and widening of blood vessels. Phentolamine control episodes of hypertension [6 7] and sweating that occur with a disease called pheochromocytoma. It is a long lasting adrenergic, α-receptor blocking agent. Phentolamine produces its therapeutic actions by blocking alpha receptors, leading to muscle relaxation and widening of blood vessels. This widening of blood vessels results in lowering of blood pressure.

Currently Phentolamine injections are marketed by Novartis 5mg/vial for injection. The drug acts adrenergic alpha antagonist antihypertensive agent. Its an α adrenergic blocker as a competitive α-adrenergic antagonist, phentolamine binds to α-1 and α-2 receptors resulting in a decrease in peripheral vascular resistance and vasodilatation. This agent also may block 5-hydroxytryptamine (5-HT) receptors and stimulate release of histamine from mast cells. Phentolamine is used in the diagnosis of pheochromocytoma and to control or prevent paroxysmal hypertension [8] immediately during pheochromocytomectomy. Phentolamine lower the risk of death from cardiovascular disorder and has several additional benefits. It s injected to control hypertensive emergencies like pheochromocytoma. Specially phentolamine has diagnostic and therapeutic roles in complex regional pain syndrome.

**Materials and methods**

**Software:**

**Hyperchem*:***

HyperChem [9] is a versatile molecular modeler and editor and a powerful computational package. It offers many types of molecular and quantum mechanics calculations. For optimization of small molecules in solution and protein complex the intra molecular energies of ligand-solvent and ligand protein will be calculated using molecular mechanics calculations of Hyperchem software enlisted in figure 1.

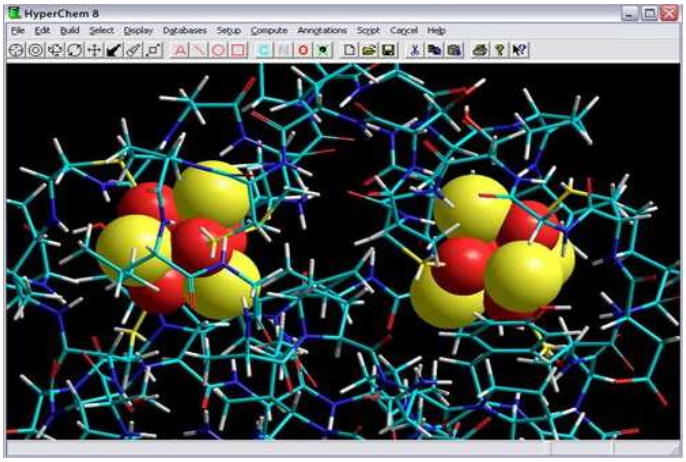


**Figure 1.** Hyperchem8

HyperChem includes these functions:

1. Drawing molecules from atoms and converting them to three-dimensional (3D) models)
2. Constructing proteins and nucleic acids from standard residues.Using molecules from other sources; for example, Brookhaven Protein Data Bank (PDB) files.
3. Rearranging molecules by, for example, rotating and translating them changing display conditions, including stereo viewing, rendering models, and structural labels

4. Setting up and directing chemical calculations, including molecular dynamics, by various molecular mechanical or *abinitio or DFT or semi-empirical quantum mechanics methods*
5. Determination of isotope effects in vibrational analysis calcu-lations for semi-empirical and *ab initio SCF methods.*
6. Graphing the results of chemical calculations
7. Solvating molecules in a periodic box..

**GOLD**

A genetic algorithm (GA) for protein-ligand docking An easy to use interface with interactive docking set-up via *Hermes* enlisted in figure 2. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular Docking is a computational method to find out binding modes of ligands to their receptors rapidly. Molecular interactions play the key role in all biological reactions. Most of the biological reactions get triggered by binding of a small molecular ligand to their receptor, which is usually a protein. Even most of the drugs exert their pharmacological reactions depend only upon their successful binding to their receptor's active site inside the body thus either mimicking or mitigating the effect of natural ligand's binding to the receptor. Hence the understanding of mode of binding of ligands to their receptors will be crucial in successful design of more efficient drugs. Experimental methods to identify these binding modes are more expensive and time consuming.

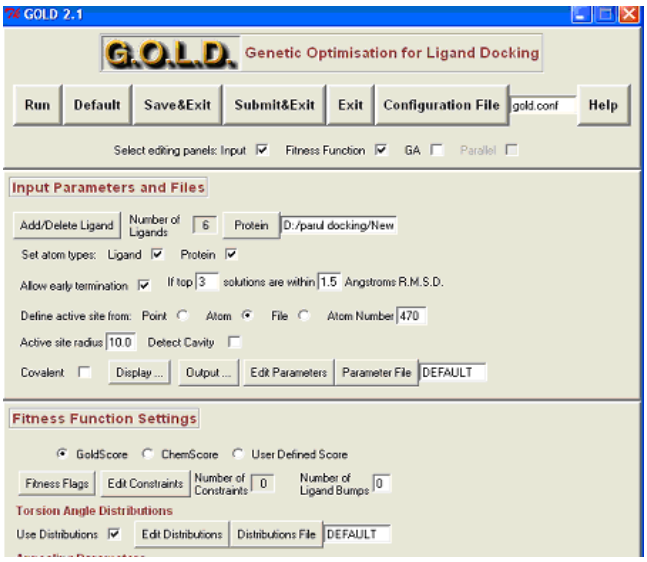


Figure 2. Gold Soft Ware

**GOLD FEATURES:**

- A genetic algorithm (GA) for protein-ligand docking.
- An easy to use interface with interactive docking set-up via *Hermes*.
- A comprehensive docking set-up wizard.
- Full ligand flexibility.
- Partial protein flexibility, including protein side chain and backbone flexibility for up to ten user-defined residues.
- Energy functions partly based on conformational and non-bonded contact information from the CSD.
- A variety of constraint options.
- Automatic consideration of cavity bound water molecules.
- Improved handling and control of metal coordination geometries.
- Improved parameterisation for kinases and heme-containing proteins.
- Options for generating diverse solutions, based on RMSD.
- Automatic derivation of GA settings for particular ligands.
- A choice of GoldScore [9], ChemScore or Astex Statistical Potential (ASP) scoring functions.
- Extensive options for customising or implementing new scoring functions through a Scoring Function Application Programming Interface, allowing users to modify the GOLD scoring-function mechanism in order to either: implement their own scoring function or enhance existing scoring functions; customise docking output.
- A ChemScore Receptor Depth Scaling (RDS) rescore option so that the score attributed to hydrogen bonds is scaled depending on the depth in the binding pocket.
- Links between GOLD and GoldMine, enabling ligands to be received from or sent to a GoldMine database before or after having been docked in GOLD respectively. GOLD's genetic algorithm parameters are optimised for virtual screening applications. GOLD is optimised for parallel execution on processor networks; a distributed version of GOLD is available for use on commercial PC GRID systems.

**3) Open Eye scientific software:**

OpenEye Scientific Software develops large-scale modeling applications and toolkits. Primarily geared towards drug discovery and design, areas of application include structure generation, docking, shape comparison, electrostatics, chemical informatics and visualization. The software is designed for scientific rigor, as well as speed, scalability and platform independence.Ligand –solvent interactions (Inter-solvent) (intra-solventPB) [10]. For optimization of small molecules in solution, the electrostatic part of molecule-solvent interactions will be calculated using Poisson-Boltzmann model of Open Eye scientific software.

SZYBKI optimizes molecular structures with the Merck Molecular Force Field, either with or without solvent effect, to yield quality 3D molecular structures [11] for use as input to other programs. Since the chemistry of molecular interactions is a matter of shape and electrostatics, it is impossible to consider either without reasonable 3D molecular structures. SZYBKI also refines portions of a protein structure and optimize ligands within a protein active site, making it useful in conjunction with docking programs is enlisted in figure 3.

**METHODOLOGY**

The method used for modeling a new drug for develop the phentolamine analogs taking phentolamine as ligand and D2 Dopamaine receptor as target includes the following steps:

- Drawing chemical structure of the ligand using hyper software
- Converting it to 3d structures
- Measuring bond distances , bond angles and torsion angles

- Performing single point calculations, geometry optimization by setting the
- Molecular mechanics to AMBER force field and measuring the optimized values.

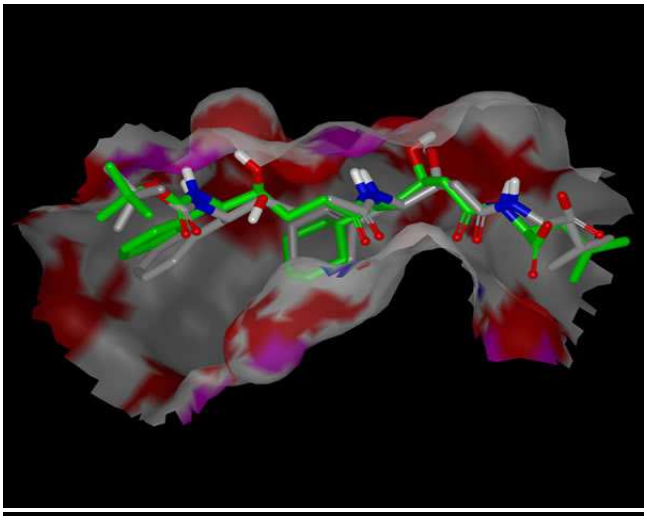

**Figure** 3. In situ optimization of a ligand with SZYBKI

- Confirming the behavior of the molecule by molecular dynamics and energy by monte carlo simulation.
- Assessing QSAR properties like charge, surface area, volume, log p, hydration energy, polarizability, refractivity, mass of the molecule.
- Solvating the molecule by creating a periodic box such that the activity of the molecule can be calculated in changed temperature.
- Performing the geometry optimization for solvated molecule using force field AMBER and calculating the energies obtained along with number of cycles required.
- The solvated molecule is now made devoid of water molecule and periodic box.
- Optimization is now performed for energy calculated solvated molecule in both MM+ and AMBER force fields .The calculated energy values of X1 and X2 are represented in table 1 and 2
- These values are denoted by X.
- Chemical structure of ligand molecule is then changed by causing variation in its R group ; as such 9 new different molecules have been designed.
- The same procedure is followed for all molecules. The protein into which the ligand molecule is fit is considered and the same Protein is used for the rest 9 molecules to check the optimization energy that is obtained using AMBER as force field , when fit into the protein.
- The ligand derivatives after undergoing optimization along with protein are Made devoid of protein and the optimization values are calculated using the Force fields MM+ and AMBER .
- These calculated energy values are denoted as 'Y1' and represented in the table 3.
- The optimized ligand molecule and its derivatives are converted to PBD
- Format along with the selected protein and docking is performed using GOLD software.
- The best ranking of the considered molecules are obtained and the fitness Energy of each molecule is noted down.
- The fitness energy is obtained. This fitness energy is denoted as 'y2 'and represented in table 5.
- Binding free energy of the molecules is calculated by the formula

Y= Y1+Y2; Z=Y-X; Z=Zfinal –Zintial

These values are represented in Table 5.

Once potential drugs have been identified by the methods described above, other molecular modeling techniques may then be applied. For example, Geometry optimization may be used to relax the structures and to identify low energy orientations of drugs in receptor sites. Molecular dynamics may assist in exploring the energy landscape and free energy [12] simulations can be used to compute the relative binding free energies [13 14] of a series of Putative drugs. The relative binding free energy values are represented in Table 6.

**Table 1:** Energy Calculations of Solvated Molecules

| Molecule | Intra Energy ($X_1$) |
|---|---|
| R =$CH_3$ | 18.821615 |
| R=OH | 18.426790 |
| R=$CCL_3$ | 18.824514 |
| R=$CF_3$ | 18.823942 |
| R=$CH_2OH$ | 19.437881 |
| R=$CCL_2OH$ | 27.717173 |
| R=Br | 18.324652 |

**TABLE 2 :-**Poission-Boltzman Solvent Mode Software: Open eye (SZYBKI TOOL)

| MOLECULE | psolvent ($X_2$) |
|---|---|
| R=CH3 | 27.64 |
| R=OH | 29.04 |
| R=$CCL_3$ | 30.34 |
| R=$CF_3$ | 32.64 |
| R=$CH_2OH$ | 30.01 |
| R=$CCL_2OH$ | 36.36 |
| R=Br | 34.04 |

**TABLE 3 :-** Protein –Ligand Interaction:-

| MOLECULE | INTRA ENERGY($y_1$) |
|---|---|
| R=CH3 | 19.243849 |
| R=OH | 11.054326 |
| R=$CCL_3$ | 20.886642 |
| R=$CF_3$ | 19.209364 |
| R=$CH_2OH$ | 18.409996 |
| R=$CCL_2OH$ | 20.428774 |
| R=Br | 19.066078 |

**TABLE 4 :-** Docking

| MOLECULE | INTRA ENERGY($y_2$) |
|---|---|
| R=CH3 | -46.98 |
| R=OH | -34.56 |
| R=$CCL_3$ | -31.43 |
| R=$CF_3$ | -39.62 |
| R=$CH_2OH$ | -50.97 |
| R=$CCL_2OH$ | -42.4 |
| R=Br | -34.85 |

**TABLE 5 :-** BINDING FREE ENERGY CALCULATIONS:

| MOLECULE | SOLVENT($x_1+x_2$=X) | PROTEIN($y_1+y_2$=Y) |
|---|---|---|
| R=CH3 | 46.461615 | -27.736151 |
| R=OH | 47.46679 | -23.505674 |
| R=$CCL_3$ | 49.164514 | -10.543358 |
| R=$CF_3$ | 51.463942 | -20.760636 |
| R=$CH_2OH$ | 49.447881 | -32.560004 |
| R=$CCL_2OH$ | 64.077173 | -21.971226 |
| R=Br | 52.364652 | -15.783922 |

where as :

Y=Y1+Y2;X=X1+X2; Y1=INTRA;Y2=DOCKING; X1=SOLVENT;X2=OPEN EYE VALUE;Y-X=Z

| MOL | X1 | X2 | Y1 | Y2 | X1+X2=X | Y1+Y2=Y | Y-X=Z |
|---|---|---|---|---|---|---|---|
| Mol 1 | 18.821615 | 27.64 | 19.243849 | -46.98 | 46.461615 | -27.736151 | -74.197766 |
| Mol 2 | 18.426790 | 29.04 | 11.054326 | -34.56 | 47.46679 | -23.505674 | -70972464 |
| Mol 3 | 18.824514 | 30.34 | 20.886642 | -31.43 | 49.164514 | -10.543358 | -59.70782 |
| Mol 4 | 18.823942 | 32.64 | 19.209364 | -39.62 | 51.463942 | -20.760636 | -72.224578 |
| Mol 5 | 19.437881 | 30.01 | 18.409996 | -50.97 | 49.447881 | -32.560004 | -82.007884 |
| Mol 6 | 27.717173 | 36.36 | 20.428774 | -42.4 | 64.077173 | -21.971226 | -86.048399 |
| *Mol 7* | 18.324652 | 34.04 | 19.066078 | -34.85 | 52.364652 | -15.783922 | *-68.148574* |

Binding Free Energy Calculations:

**TABLE 6 :- Relative Binding Free Energy**

| MOLECULE | RELATIVE BINDING REE ENERGY |
|---|---|
| R=OH | -3.225302 |
| R=$CCL_3$ | -14.489946 |
| R=$CF_3$ | -1.973188 |
| R=$CH_2OH$ | +7.810118 |
| R=$CCL_2OH$ | +11.850633 |
| R=Br | -6 |

**PHENTOLAMINE**
**MOL-1**
DRUG NAME : PHENTOLAMINE
Chemical Formula: $C_{17}H_{19}N_3O$
Chemical IUPAC Name:3-[4,5-dihydro-1H-imidazol-2-ylme-thyl-(4- methylphenyl)amino]phenol
Average Molecular Weight 281.352

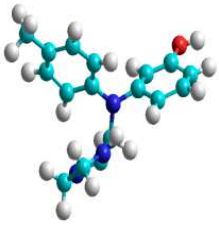

FIGURE A

Molecule 2 (R=OH)

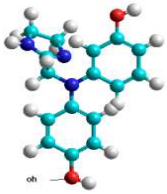

FIGURE B
Molecule - 3 (R=CCL3)

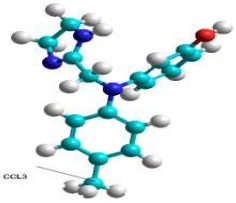

FIGURE C
Molecule 4 (R= Molecule CF3)
Molecule 5 (R=$CH_2$ OH)

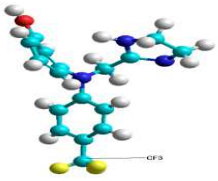

FIGURE D
Molecule (6) (R = $CL_2OH$)

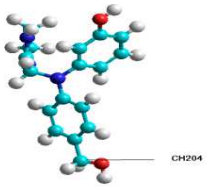

FIGURE E
MOLECULE(7) R= br

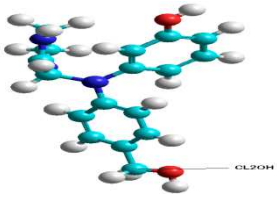

FIGURE F

**SOLVENT INTERACTIONS**
**Solvent Calculations**
A four-stage protocol was also established for energy minimization of the solvated inhibitor. These minimizations were carried out using periodic boundary conditions in all directions and in each stage involved 100 steps of steepest descent and 2000 steps of conjugate gradient Optimization. In the first stage of minimization, only the waters were minimized keeping the inhibitor (*i. e.*, the solute) fixed. In the second stage, only hydrogen in the system were allowed to relax. In this third stage, atoms common to the ligand in the crystal structure complex and the modified ligand were also fixed, while allowing the solvent and the modified group in the ligand to move during optimization. In the fourth stage of the solvent calculation, all water molecules and the solute (ligand) were allowed to relax.

**PROTEIN COMPLEX INTERACTIONS**
**Complex Calculations**
A four-stage protocol was set up for energy minimizations of the protein-inhibitor complex. Minimization at each stage was performed using 100 steps of steepest descent and 2000 steps of conjugate gradient algorithms for minimization. In the first stage, only the water molecules were minimized, keeping the inhibitor and the protein (in the complex calculation) fixed. The purpose of this step is to relieve any bad contacts involving water molecules in the initially solvated system. In the second stage, only hydrogen's in the system were allowed to relax. This step relaxes the hydrogen atoms prior to relaxing heavy atoms. It was performed because the hydrogen locations are not specified by the X-ray structure [15 16] and because adjustments in hydrogen atom locations are necessary to improve hydrogen bond geometries. The third stage was performed for all the modified ligand-protein complexes (*i.e.*, when the ligand is modified from the original ligand in an X-ray structure complex). In this third stage, all atoms of the protein were

fixed and atoms common to the ligand in the crystal structure complex and the modified ligand were also fixed, while allowing the modified group in the ligand and the solvent to move during optimization. This stage allows for the relaxation of the modified group with respect to the protein and establishes the preferred interactions (e.g., hydrogen bonds). In the fourth and final stage, all atoms of all residues within 25 Å of any atom from the center of the modified group (waters, protein atoms and the ligand) were allowed to relax.

**RESULTS & DISCUSSIONS**

One of the most common diseases found among the world population has been in steep rise, which is called the cardiovascular diseases. Cardiovascular diseases associated have affected more than one third of world's population. As these are found to be an associated one, its being a challenge to the medical field where all the new advance treatments are in vain. By the year 2030 it is being predicted that a world of cardiovascular generation would be evolved.

Drug designing, one of the hottest topics have found its new path to create a history in the field of medical sciences. Atrial fibrillation [17 18] & flutter, one of the arrhythmias which was common in old has been affecting the young presently. The challenge to find the lead compound has become a necessity.

The lead compound analysis starts with CADD, assisting to identify and to optimise the right compound. This technique helps in generating a suitable compound specific to the disease; thereby an effective treatment is achieved

The minimized structures for all the 9 ligands in the complex and solvated states were used for calculating the following energy variables [19 20].

$E_{bind}$ (intra) = $E_{com}$ (intra) - $E_{sol}$ (intra)

$E_{bind}$ (inter) = $E_{com}$ (inter) - $E_{sol}$ (inter)

Where, $E_{bind}$ (intra) and $E_{bind}$ (inter) are relative intra and intermolecular binding interaction energies of a ligand, respectively, and where $E_{com}$ (intra), $E_{com}$ (inter), $E_{sol}$(intra), and $E_{sol}$(inter) are intra and intermolecular interaction energies of a ligand in the complexed and solvated states, respectively and Relative differences in intra, intermolecular and total binding interaction energies for a pair of ligands.

**CONCLUSION:**

In this work, the binding modes of the putative/proposed inhibitors were obtained by carefully aligning them with the known crystal structures of inhibitors in the active site of the 1W6r. These inhibitors, which are shown in the above Figures, were then evaluated by performing minimization calculations both in solvent and in complex using the AMBER (Weiner SJ et al, 1984) force field. The technical details used for estimating relative binding affinities using energy components obtained from minimizations of each inhibitor, both in solvent as well as in complex phases, were explained by four stage protocol as described in the in methodology section. A comparison of the relative binding affinities for structurally similar Inhibitors to PHENTOLAMINE indicates that the molecular mechanics methods gave suitable analogues. These results clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis.The inhibitors Molecule 3 with the substiuents R=$CCL_3$(-14.489946) are identified as the most suitable analogues in the present study need to be further evaluated in laboratory.

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**References**

1. Hart, R. G. & Halperin, J. L. *Stroke* 32, 803-808, 2001.
2. Benjamin, E. J. et al.. The Framingham heart study. *Circulation* 98, 946-952, 1998.
3. Kurtz ID, Meng EC, and Shoichet BK.. Acc *Chem Res*; 27: 117-123, 1994.
4. Hubbard, S.J. & Thornton, J.M. '*ACCESS', Computer Program*. Department of Biochemistry and Molecular Biology, University College London, London, UK. 1993
5. Erion, M.D., van Poelje, P.D., and Reddy, M.R. *J. Am. Chem. Soc.*, 122. 6114-6115,2000.
6. Nattel, S. *Cardiovasc. Res.* 37, 567-577, 1998. |
7. Nattel, S. *Am. Heart J*. 130, 1094-1106, 1995.
8. Fenelon, G., Wijns, W., Andries, E. & Brugada, P. *Pacing Clin. Electrophysiol*. 19, 95-106, 1996.
9. Manoj Kumar Annamala, Krishna kishore Inampudi, Lalitha Guruprasad School of chemistry, University of Hyderabad. *Bioinformation*.; 1(9): 339–350, 2007
10. Erion, M.D., van Poelje, P.D., and Reddy, M.R. *J. Am. Chem. Soc.*, 122. 6114-6115, 2000.
11. Montgomery JA et al.. *J Med Chem*; 36: 55-69, 1993.
12. Merz KM and Kollman PA. *J Am Chem Soc*; 111: 5649-5658, 1989.
13. Reddy, MR.; Erion, M. D.; Agarwal, A. *Wiley*: 2, 17-304, 2000.
14. Reddy MR, M., Viswanadhan, V.N. and Weinstein, J.N. Proc. Natl. Acad. *Sci.* USA, 88 10287-10291, 1991
15. Picot D, Loll, and P.J. & GARAVITO, R.M.. *Adv Exp Med Biol* 400A: 107-111, 1997.
16. Picot D., Loll, P.J. & Garavito, R.M. *Nature* 367, 243-249, 1994.
17. Garrey, W. E. *Physiol. Rev*. 4, 215- 250, 1924.
18. Cox, J. L. & Ad, N. *Semin. Thorac. Cardiovasc. Surg*. 12, 68-73, 2000.
19. Reddy, M.R. Bacquet, R.J., Zichi, D., Matthews, D.A., Welsh, K.M., Jones, T.R., Freer, S. *J. Am. Chem. Soc.* 114, 10117-10122, 1992.
20. Reddy MR., Varney, M.D., Kalish, V., Viswanadhan, V.N. and Appelt, K. J. Med. Chem.,114. 10117-10122, 1994.