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Research Article

Modelling and Docking studies of Endothelin receptors (PAH Inducing proteins) with Bosenten Monohydrate

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ARTICLE INFO

ABSTRACT

Pulmonary arterial hypertension (PAH) is a serious disease of pulmonary arteries. When Pulmonary Arterial Hypertension (PAH) develops, blood flow through the pulmonary arteries is restricted and the right side of the heart is put under increasing strain to pump blood through to the lungs. The different changes in the blood vessels raise the pressure and increase the resistance to blood flow through the lungs, making it harder for the heart to pump blood through the lungs. Over time, due to the additional strain, the heart begins to work less effectively and eventually, the right heart can fail (this is known as 'right ventricular failure'). Pulmonary arterial hypertension (PAH) is relatively rare. It is estimated that there are about 30-50 people with PAH for every million of the population. While Pulmonary Arterial Hypertension (PAH) can occur at any age, it is more common in women in their childbearing years and the average age of diagnosis is 36. The exact cause of pulmonary arterial hypertension (PAH) remains unknown. In recent years research has focused particularly on the role of three specific substances that are produced in these cells: prostacyclin, nitric oxide and endothelin. These three agents work together to help the blood to flow smoothly through the heart and lungs and it is thought that an imbalance in levels of one or more of these substances contributes to the development of conditions such as Pulmonary Arterial Hypertension (PAH). This leads to the progressive changes in the vessels and the subsequent rise in pressure. This area of research has led to the development of specific treatments for Pulmonary Arterial Hypertension (PAH).

Keywords: Pulmonary arterial hypertension (PAH), heart, lungs, blood vessels and endothelin

INTRODUCTION

Endothelin is produced by the endothelium and is essential for a number of functions, including the regulation of normal blood flow. However, it has been shown that people with Pulmonary Arterial Hypertension (PAH) produce too much endothelin.[1, 2] Excessive levels of endothelin can have a number of detrimental effects in the body including:

- Thickening and scarring of tissue and blood vessels (Fibrosis)
- Enlargement or increase in the number of cells in the vessel wall, which can lead to thickening and obstruction of the blood vessels
- Inflammation
- Narrowing of the blood vessels (Vasoconstriction)

Once it is released from the endothelium, endothelin binds to specific receptors in a similar way to a key fitting into a lock. There are two types of Endothelin receptors, ET_A and ET_B, and each type has a slightly

different action.[3] A class of drugs, endothelin receptor antagonists (ERAs) aim is to reduce the impact of PAH by helping to protect against the damaging effects of excessive endothelin.

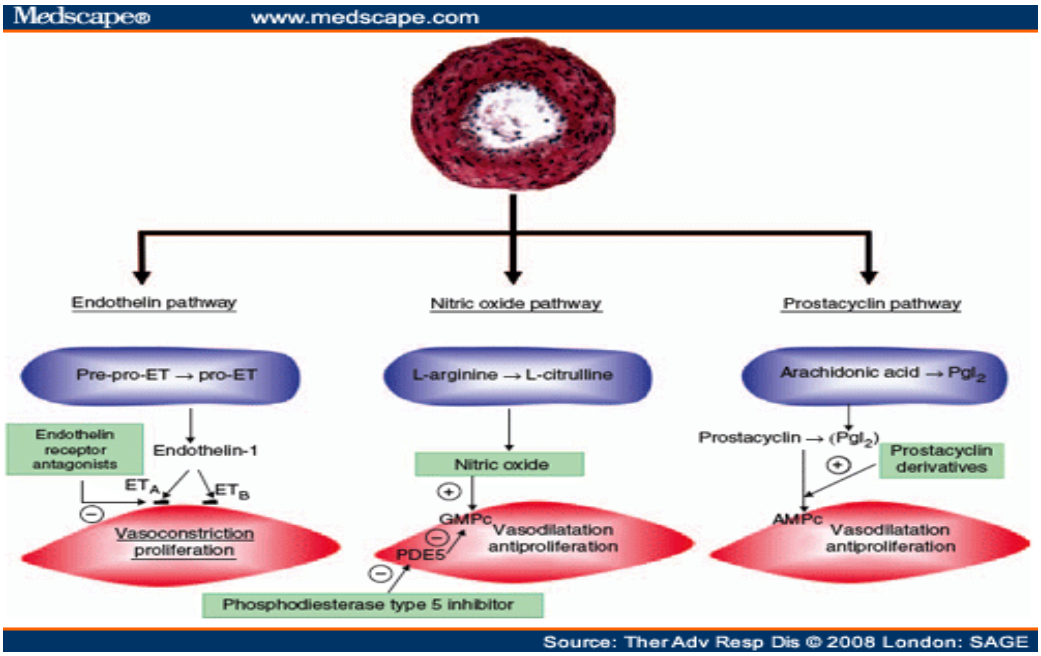

Fig 1: The Endothelin, Nitric Oxide and Prostacycline involved in blood flow through blood vessels.

Endothelin receptors:

There are two distinct receptors for the endothelin family of peptides, endothelin receptor A (ETA) and endothelin receptor B (ETB). The endothelin receptors belong to the family of receptors connected to guanine nucleotide-binding (G) proteins. The two receptors have unique locations and binding affinities for the endothelin peptides. The ETA receptors are expressed on pulmonary vascular smooth muscle cells, whereas ETB receptors are located on both pulmonary vascular endothelial cells and smooth muscle cells.[4]

Endothelin receptor antagonist

Endothelin receptor antagonism has emerged as an important therapeutic strategy in pulmonary arterial hypertension (PAH). Laboratory and clinical investigations have clearly shown that endothelin (ET)-1 is over expressed in several forms of pulmonary vascular disease and likely plays a significant pathogenetic role in the development and progression of pulmonary vasculopathy.

Oral Endothelin Antagonists

Oral endothelin receptor antagonists (ERAs) have been shown to improve pulmonary hemodynamics, exercise capacity, functional status, and clinical outcome in several randomized placebo-controlled trials. These are some of Oral Endothelin receptor antagonists.

1. Bosentan
2. Darusentan
3. Sitaxentan
4. Ambrisentan

Bosentan, a dual-receptor antagonist, is approved by the U.S. Food and Drug Administration for class III and IV patients with PAH, based on to phase III trials. In addition to its efficacy as sole therapy, bosentan may have a role as part of a combination of drugs such as a prostanoid or sildenafil. The selective endothelin receptor-A antagonist's sitaxsentan and ambrisentan are currently under investigation.[5]

BOSENTAN

Bosentan is the first of a new drug class, an endothelin receptor antagonist. TRACLEER (bosentan) belongs to a class of highly substituted pyrimidine derivatives, with no chiral centers. It is designated chemically as 4 – tert - butyl – N - [6 - (2-hydroxy - ethoxy) – 5 -(2 – methoxy - phenoxy) - [2,2] - bipyrimidin-4-yl] - benzene sulfonamide monohydrate and has the following structural formula.[6] :

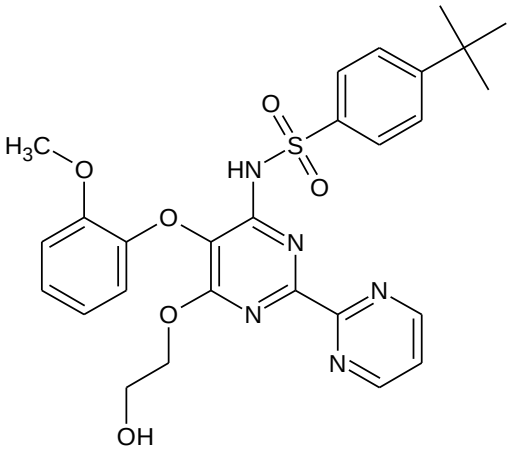

Fig 2 : Structure of Bosentan.

Bosentan has a molecular weight of 569.64 and a molecular formula of $C_{27}H_{29}N_5O_6S•H_2O$. Bosentan is a white to yellowish powder. It is poorly soluble in water (1.0 mg/100 mL) and in aqueous solutions at low pH (0.1 mg/100 mL at pH 1.1 and 4.0; 0.2 mg/100 mL at pH 5.0). Solubility increases at higher pH values (43 mg/100 mL at pH 7.5). [7]

Mechanism of action

Bosentan is a competitive antagonist of endothelin-1 at the endothelin-A (ET-A) and endothelin-B (ET-B) receptors. Under normal conditions, endothelin-1 binding of ET-A or ET-B receptors causes pulmonary vasoconstriction. By blocking this interaction, bosentan decreases pulmonary vascular resistance. Bosentan has a slightly higher affinity for ET-A than ET-B.[8]

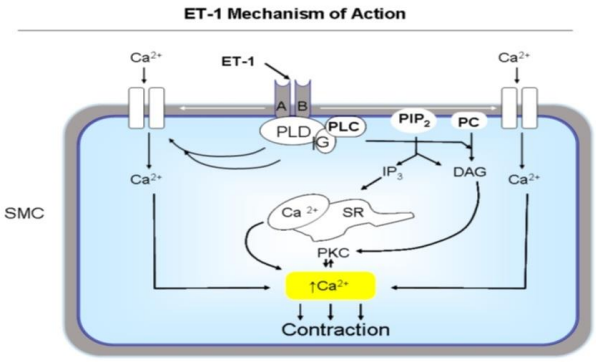

Fig 3: Endothelin-1(ET A ET B) receptor Antagonists mechanism

IMPORTANCE OF WORK

Researchers and clinicians are constantly faced with new pathophysiological concepts, new drugs and new therapeutic frontiers.

Pulmonary hypertension is a syndrome that encompasses a broad range of diseases and conditions affecting hundreds of thousands of patients worldwide .

Several classes of drugs are now available for treatment of PAH. These drugs have a substantial impact on the survival of patients with PAH.[9]

Two substances, bosentan and sitaxsentan, have already been approved in USA and Europe and a third compound, ambrisentan, is expected to be available soon.

The importance of this work was to identify the role and the mechanism of action of Bosentan on Endothelin receptors. Although the results suggest that Bosentan inhibits endothelial receptors. There is no evidence as to how Bosentan prevents the action of endothelial receptors. In order to gain insight in to problem, to explore the interactions to the active site.[10]

A study of docking of Bosentan in to Endothelin receptors is here by carried out in order to explore the interaction of Bosentan and its structural analogs with the active site of endothelin receptor ET_A and ET_B .

AIM AND OBJECTIVE OF STUDY

A) The Aim of this present work is to generate the 3D models for Endothelin A, Endothelin B receptor Proteins and docking of Bosentan with ET_A, and ET_B.

B) The Objective of this study is to identify which receptor is having more binding energy towards the Bosentan drug, in Pulmonary Arterial Hypertension.

METHODOLOGY

Blast

BLAST searches for high scoring sequence alignments between the query sequence and sequences in the database using a heuristic approach that approximates the Smith-Waterman algorithm. The exhaustive Smith-Waterman approach is too slow for searching large genomic databases such as GenBank. Therefore, the BLAST algorithm uses a heuristic approach that is slightly less accurate than Smith-Waterman but over 50 times faster. The speed and relatively good accuracy of BLAST are the key technical innovation of the BLAST programs and arguably why the tool is the most popular bioinformatics search tool.[11]

Homology modeling

All homology-modeling methods consist of the following four steps:
(i) Template selection
(ii) Target template alignment
(iii) Model building and
(iv) Evaluation
These steps can be iteratively repeated, until a satisfying model structure is achieved. Several different techniques for model building have been developed. The SWISS-MODEL server approach can be described as rigid fragment assembly is outlined briefly.

DOCKING

Methodology

GOLD (Genetic Optimization of Ligand Docking) is a docking program, based on genetic algorithm which is used for docking of Bosentan derivative compounds. Along with Bosentan the derivatives are docked to the active site of the Endothelin receptors and BSEP Protein. The interaction of these derivatives with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0 A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the ligand binding site in the Endothelial B receptor was defined within a 10 A° radius with the centroid as CE atom of ASP81. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5A° RMSD. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied. The best and most energetically favourable conformation of each ligand was selected.[12]
Gold Score fitness function:
Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond);
2. Protein-ligand vander Waals energy (external vdw);
3. Ligand internal vander Waals energy (internal vdw) ;
4. Ligand intramolecular hydrogen bond energy (internal- H- bond).
The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

Gold Score = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Fitness = S (hb_ext) + 1.3750 * S (vdw_ext) + S (hb_int) + 1.0000 * S (int)

S (int) = S (vdw_int) + S (tors)
Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intermolecular strain in the ligand.[13]

RESULTS AND DISCUSSION

Homology Modeling of Endothelin A receptor domain:

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only two-reference proteins, including 2VT4 A (Chain A Structure of Turkey Beta -1 Adrenergic Receptor with Stabilizing Mutations And Bound Cyanopindolol Protein) has a high level of sequence identity and the identity of the reference protein with the Endothelin A receptor (Rhodopsin-like GPCR super family-like) domain are 41% Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment.

CLUSTAL 2.0.12 multiple sequence alignment

```
Endothelin-A            ----------GNATLLRIIYQNKCMRNGPNALIASLALGDLIYVVIDLPINV 42
2VT4          WEAGMSLLMALVVLLIVAGNVLVIAAIGSTQRLQTLTNLFITSLACADLVVGLLVVPFGA 60
**. ::  * ..: ::. .* :*:*** .**:  :: :*:..

Endothelin-A          FKLLAGRWPFDHNDFGVFLCKLFPFLQKSSVGITVLNLCALSVDRYRAVASWSRVQGIGI
102
2VT4                  TLVVRGTWLWGS-----FLCELWTSLDVLCVTASIETLCVIAIDRYLAITSPFRYQSLMT 115
:: * * :.     ***:*:. *: .*  :: .**.:::*** *::*  * *.:

Endothelin-A          PLVTAIEIVSIWILSFILAIPEAIGFVMVPFEYRGEQHKTCMLNATSKFMEFYQDVKDWW
162
2VT4             RARAKVIICTVWAISALVSF-----LPIMMHWWRDEDPQALKCYQDPGCCDFVTNR---- 166
: : * ::* :* ::::    : :: . :*.*: ::     .  :*  :

Endothelin-A          LFGFYFCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRREVAKTVFCLVVIFALC
222
2VT4                    ----AYAIASSIISFYIPLLIMIFVALR---VYREAKEQIREHK-ALKTLGIIMGVFTLC 218
:.:.   ::: .*:  :: *  :   .*:::::: . **: :: :*:**

Endothelin-A  WFPLHLSRILKKTVYNEMDKNRCELLSFLLLMDYIGINLATMNSCINP---IAL----- 273
2VT4          WLPFFLVNIVN--VFNRDLVPDWLFVAFNWLG-YANSAMNPIIYCRSPDFRKAFKRLLA 274
*:*:.* .*:: *:*.     :::* *  * . : .: * .*   *:
```

Fig 4: Sequence Alignment of Endothelin A Receptor with 2VT4 Clustal w server that was submitted to modeler, the conserved regions are indicated by*

In the following study, we have chosen 2VT4 A as a reference structure for modeling Endothelin A domain. Coordinates from the reference protein (2VT4 A) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. In the modeler we will get a 20 PDB out of which we select a least energy .The energy unit will be in kilo joule .All side chains of the model protein were set by rotamers.

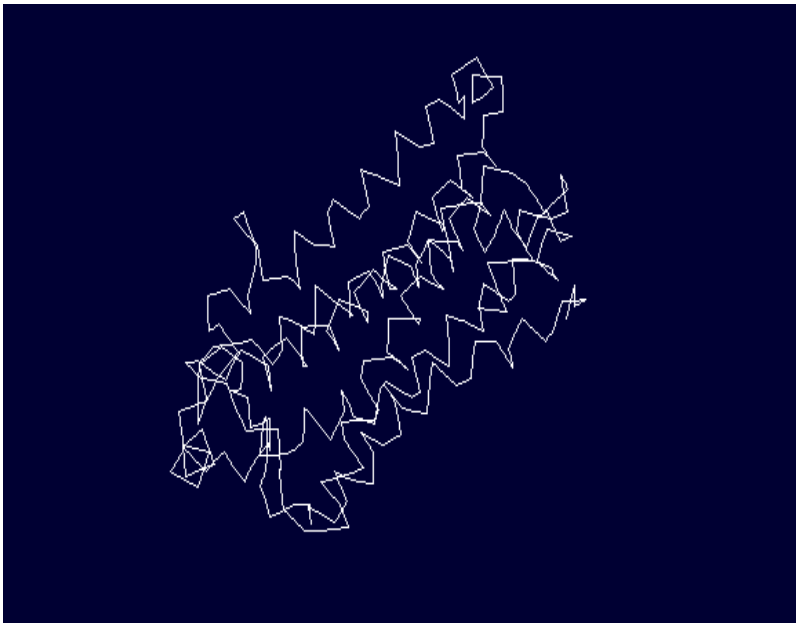

Fig 5: The final stable structure of the Endothelin A receptor obtained is shown in Figure

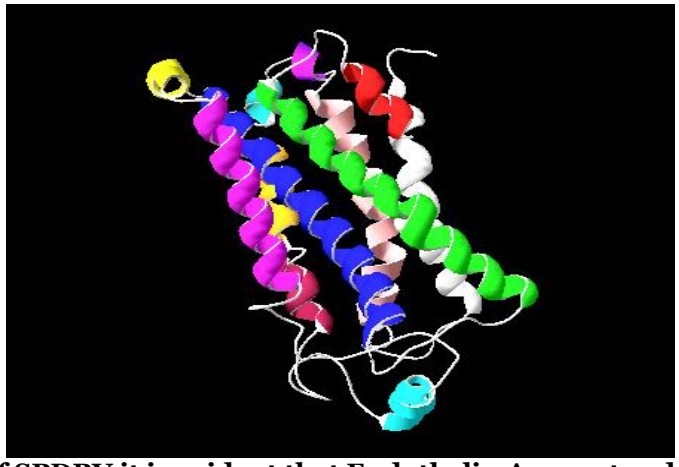

Fig 6: By the help of SPDBV it is evident that Endothelin A receptor domain has 9 helices shown in the Figure

The final structure was further checked by verify 3D graph and the results have been shown in Figure:7. The overall scores indicates acceptable protein environment.

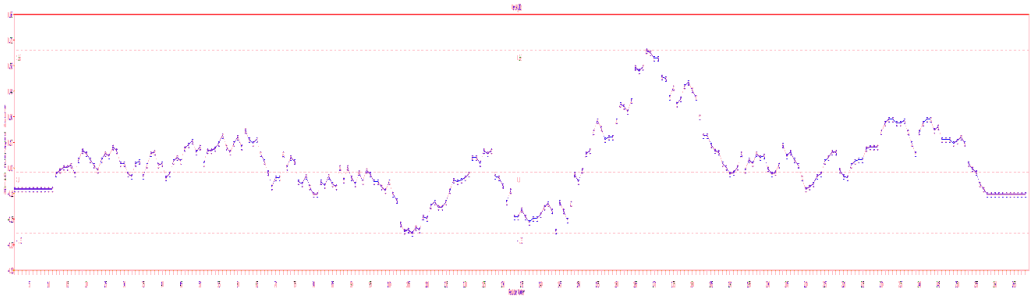

Fig 7: The 3D profiles verified results of Endothelin-A receptor model: overall quality score indicates residues are reasonably folded.

Validation of Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. The π and ψ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table 2. Altogether 50 % of the residues of Endothelin-A receptor model was in favoured and allowed regions. The overall PROCHECK G-factor of Endothelin A was –0.4 and verify3D environment profile was good.

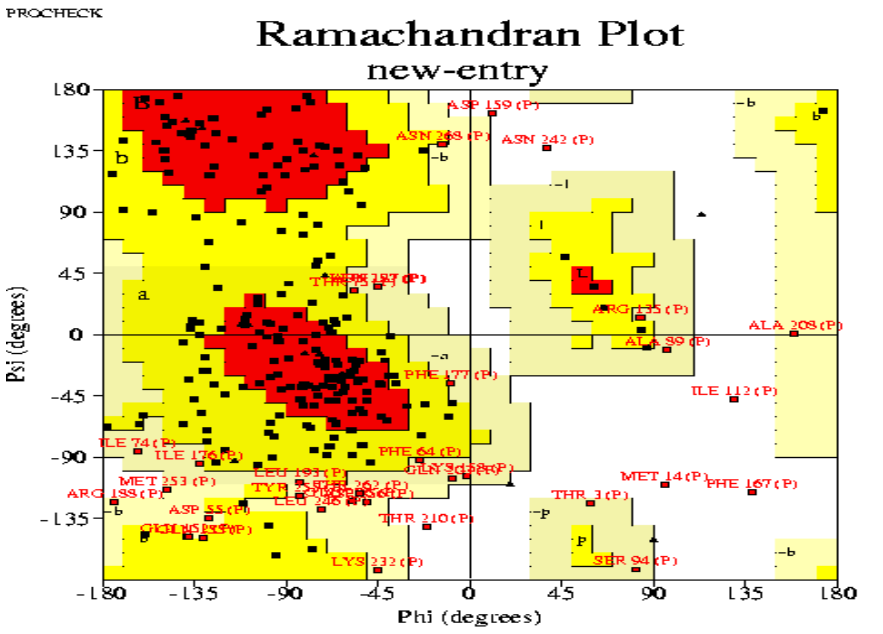

Fig 8: Ramachandran's map of Endothelin A domain using MODELLER software. The plot calculations on the 3D model were computed on the PROCHECK program.

Table:2 Plot Statistics

Residues in most favored region [A, B, L]	118	50.0%
Residues in additional allowed regions [a, b, l, p]	85	36.0%
Residues in generously allowed regions [~a, ~b, ~l, ~p]	27	11.1%
Residues in disallowed regions	6	2.5%
Number of non-glycine and non-praline residues	236	100.0%
Number of end-residues (excl, Gly and Pro)	10	
Number of Glycine residues (shown as triangle)	12	
Number of Proline residues	10	

Total Number of residues	268	

Docking of Bosentan with the active site of Endothelin A receptor

Docking of the Bosentan given in Figure 9 with Endothelin A receptor protein was performed using GOLD 3.0.1, which is based on genetic algorithm (Cambridge Crystallographic Data Center, Cambridge, United Kingdom). The docking procedure includes several steps. First, the protein-ligand complex is generated using the GOLD package without constraints between the ligand and the specific amino acids of the pocket. The algorithm exhaustively searches the entire rotational and translational space of the ligand with respect to the receptors. The flexibility of the ligand is given by dihedral angle variations. The various solutions evaluated by a score, which is equivalent to the absolute value of the total energy of the ligand in the protein environment. We used Bosentan showed affinity towards Endothelin A with total Gold score fitness 36.81Kcal/mol. It is observed that Bosentan was located at center of active site and is stabilized by hydrogen bonding interactions as shown in fig 9.

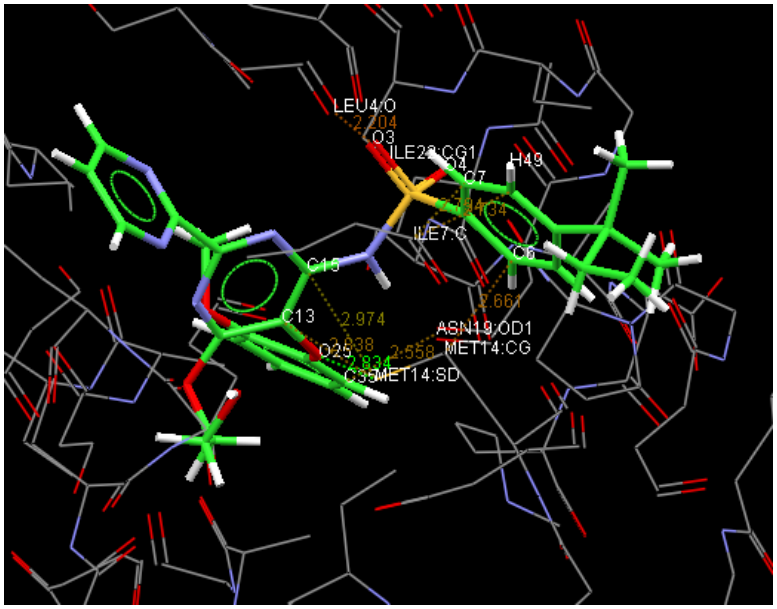

Fig 9: **Bosentan docked with domain Endothelin A receptor**

Table1: Hydrogen bonds along with their distances of Bosentan and active site residues of **Endothelin -A.**

Endothelin A Residue	atom	Bosentan atom	bond length (Å)
MET14	**SD**	**O25**	**2.834**

The docking of Bosentan into the active site of Endothelin A was carried out using the GOLD software and the docking evaluations were made on the basis of Gold fitness functions.

Gold Score fitness function: One hydrogen bond was observed when the molecule Bosentan was docked into the active site of Endothelin A, see Fig 16 and Tab 3. The best RMSD was found to be 1.5 Å. The Binding energy value of Bosentan to the Endothelin A receptor is 36.81 kcal/mol.

ENDOTHELIN – B RECEPTOR

Homology Modeling of Endothelin –B

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only two-reference proteins, including 3C9M A (Chain A, Structure of a mutant bovine Rhodopsin in hexagonal crystal form protein) has a high level of sequence identity and the identity of the reference protein with the Endothelin – B Rhodopsin-like GPCR super family - like domain are 22%. Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment.[14]

CLUSTAL 2.0.12 multiple sequence alignment

```
endo-B                                  ----------------------------------------------------GNSTLLR 7
3C9M       MCGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFLTLY 60
*   *

endo-B        IIYKNKCMRNGPNILIASLALGDLLHIVIDIPINVYKLLAEDWPFGAEMCKLVPFIQKAS 67
3C9M      VTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLG 120
:  ::* :*.  * :: .**:.**: :. .:. .:*. *   : **.  *:*  *: . .

endo-B        VGITVLSLCALSIDRYRAVASWSRIKGIGVPKWTAVEIVLIWVVSVVLAVPEAIGFDIIT 127
3C9M     GEIALWSLVVLAIERYVVVCKPMSNFRFG-ENHAIMGVAFTWVMALACAAPPLVGWSRYI 179
*:: ** .*:*:** .*..     :* : : : :.: **::: *.* :*:.

endo-B   MDYKGSYLRICLLHPVQKTAFMQFYKTAKDWWLFSFYFCLPLAITAFFYTLMTCEMLRKK187
3C9M           PEGMQCSCGIDYYTPHEETNNESFV-----IYMFVVHFIIPLIVIFFCYGQLVFTVK-EA 233
:  .  *   * ::*  .*      ::* .:* :** :  * *  :. : :

endo-B    SGMQIALNDHLKQRREVAKTVFCLVLVFALCWLPLHLSRILKLTLYNQNDPNRCELLSFL247
3C9M      AAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAG-----VAFYIFTHQGSCFGPIFM 288
:. *      * .:**:: *: :*:.* :****       :::*  .. . *    *:

endo-B     LVLDYIGINMASLNSCINPIALYLVSKRFKNCFKSCLCCWCQSFEEKQSLEEKQSCLKF307
3C9M       TIPAFFAKTSAVYN----PVIYIMMNKQFRNCMVTTLCCGKN------------------ 326
:  ::. . *  *    *:  ::.*:*:**: : *** :

endo-B       ANDHGYDNFRSSNKYSSS 325
3C9M         ------------------
```

Fig10 : Sequence Alignment of Endothelin B receptor with 3C9M A clustalw server that was submitted to modeler, the conserved regions are indicated by*

In the following study, we have chosen 3C9M A as a reference structure for modeling Endothelin- B Rhodopsin-like GPCR super family domain. Coordinates from the reference protein (3C9M A) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. In the modeler we will get a 20 PDB out of which we select a least energy .The energy unit will be in kilo joule .All side chains of the model protein were set by rotamers.

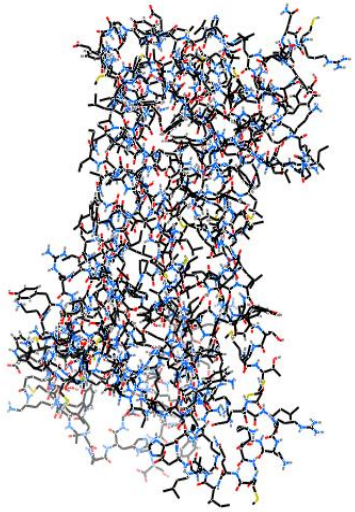

Fig 11: The final stable structure of the Endothelin B protein

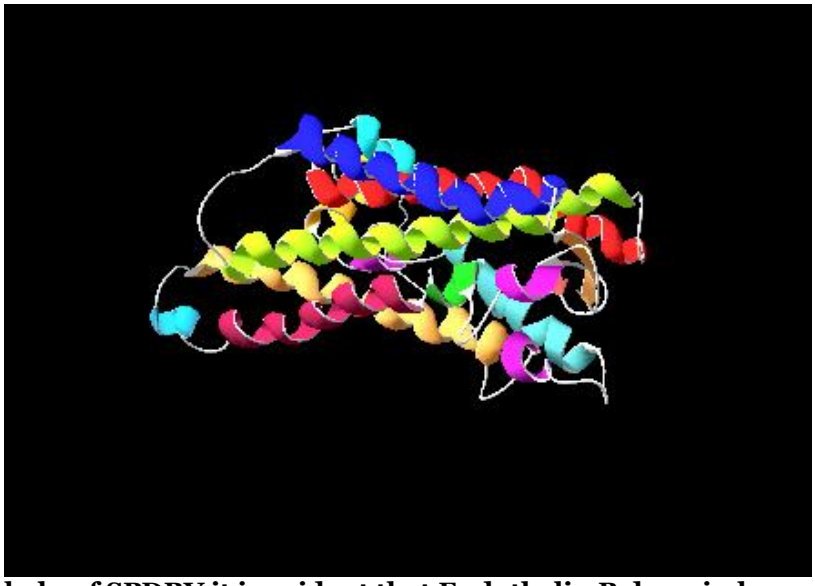

Fig 12 : By the help of SPDBV it is evident that Endothelin B domain has 13 helices and 2 sheets.

The final structure was further checked by verify 3D graph and the results have been shown in Figure13. The overall scores indicates acceptable protein environment.

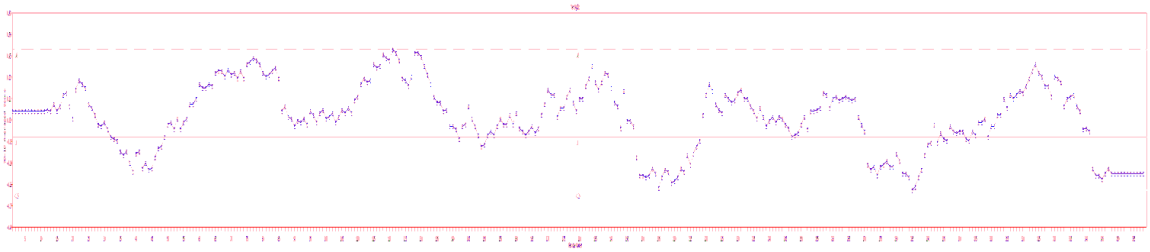

Fig 13 : The 3D profiles verified results of Endothelin-B receptor model: overall quality score indicates residues are reasonably folded.

Validation of Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. The π and ψ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table 2. Altogether 86.7% of the residues of Endothelin-B receptor was in favored and allowed regions. The overall PROCHECK G-factor of Endothelin B was 0.98 and verify3D environment profile was good.[15]

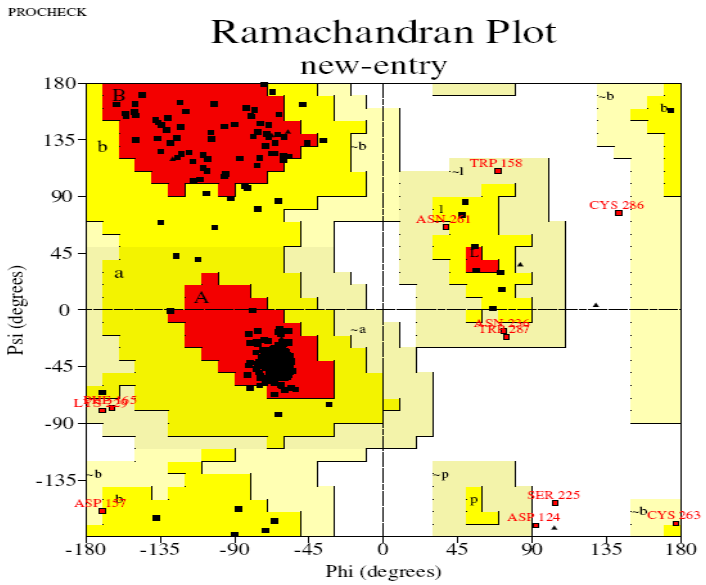

Fig14: Ramachandran's map of Endothelin B domain using MODELLER software. The plot calculations on the 3D model were computed on the PROCHECK program

Table: 2

Plot Statistics

Residues in most favored region [A, B, L]	261	86.7%
Residues in additional allowed regions [a, b, l, p]	29	9.6%
Residues in generously allowed regions [~a, ~b, ~l, ~p]	8	2.7%
Residues in disallowed regions	3	1.0%
---	---	
Number of non-glycine and non-praline residues	301	100%
Number of end-residues (excl, Gly and Pro)	1	
Number of Glycine residues (shown as triangle)	12	
Number of Proline residues	11	
--Total Number of residues	325	

Docking of Bosentan with the active site of Endothelin B receptor

Docking of the Bosentan given in Figure 25 with Endothelin B receptor protein was performed using GOLD 3.0.1, which is based on genetic algorithm (Cambridge Crystallographic Data Center, Cambridge, United Kingdom). The docking procedure includes several steps. First, the protein-ligand complex is generated using the GOLD package without constraints between the ligand and the specific amino acids of the pocket. The algorithm exhaustively searches the entire rotational and translational space of the ligand with respect to the receptors. The flexibility of the ligand is given by dihedral angle variations. The various solutions evaluated by a score, which is equivalent to the absolute value of the total energy of the ligand in the protein environment. We used Bosentan showed affinity towards Endothelin B with total Gold score fitness 52.19Kcal/mol. It is observed that Bosentan was located at center of active site and is stabilized by hydrogen bonding interactions as shown in fig 15

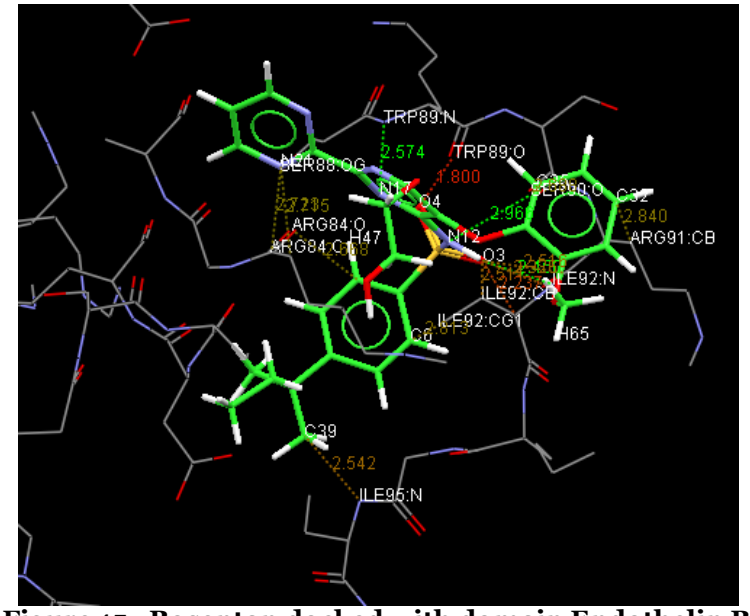

Figure 15: **Bosentan docked with domain Endothelin B**

Table 3: Hydrogen bonds along with their distances of Bosentan and active site residues of Endothelin B.

Endothelin B Residue	atom	Bosentan atom	bond length (Å)
ILE92	**N**	**O3**	**2.450**
SER90	**O**	**N12**	**2.966**
TRP89	**N**	**NI7**	**2.574**
TRP89	**O**	**O4**	**1.800**

The docking of Bosentan into the active site of Endothelin B was carried out using the GOLD software and the docking evaluations were made on the basis of Gold fitness functions.

Gold Score fitness function: A total of 4 hydrogen bonds were observed when the molecule Bosentan was docked into the active site of Endothelin B, see Fig 15 and Tab 3. The best RMSD was found to be 1.6 Å. The Binding energy value of Bosentan to the Endothelin B receptor is 52.19 kcal/mol.

Table 4: Docking results of Receptors with Bosentan

S.NO	Drug Targets (or) (Receptor Protein)	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	Total Gold Fitness with Bosentan (kcal/mol)
1	Endothelin-A	0.00	46.65	0.00	-27.33	36.81
2	Endothelin-B	0.00	53.38	0.00	-21.20	52.19

Order of Binding energies of Receptor Proteins with the drug of Bosentan
Endothelin B > Endothelin A .

CONCLUSION

In this work, we have constructed 3D models of Endothelin A, and Endothelin B receptor domain, from human using the MODELLER software and obtained refined models after energy minimization. The final refined model was further assessed by ERRAT & PROCHECK program, and the results show that this model is reliable. The stable structure is further used for docking with Bosentan and it's derivatives. Docking results indicate that conserved amino-acid residues in Endothelin A and B receptor proteins may mainly play an important role in maintaining a functional conformation and are directly involved in donor substrate binding. The interaction between the domain and the inhibitors proposed in this study are useful for understanding the potential mechanism of domain and the inhibitor binding. As is well known, hydrogen bonds play an important role for the structure and function of biological molecules. In this study it was found that MET14, of Endothelin A, TRP89, SER90 and ILE92 of Endothelin B, are important for strong hydrogen bonding interactions with the inhibitor. To the best of our knowledge MET14 of Endo-A, SER90, TRP89, ILE92 of Endo-B are conserved in these domains and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket. Among 2 proteins docked to Bosentan, Endothelin B receptor has higher binding energy than other protein. The binding energies of the two receptors are shown in table.4. Endothelin B receptor showed highest Binding energy than other proteins with 4 hydrogen bonds.
The stable structure of Endothelin B is further used for docking with Bosentan and its derivatives. Endothelin B is important for strong hydrogen bonding interaction with the inhibitors. According to our investigations from the docking results Bosentan can act as better Endothelin antagonist. This can be used for further investigation studies.

REFFERENCES

1. Clapp LH, Finney P, Turcato S (2002). Differential effects of stable prostacyclin analogs on smooth muscle proliferation and cyclic AMP generation in human Pulmonary artery. Am J Resp Cell Mol Biol;2: 194-201.
2. Yoshibayashi M, Nishioka K, Nakao K (1991). Plasma endothelin concentrations in patients with pulmonary hypertension associated with congenital heart defects. Evidence for increased production of endothelin in pulmonary circulation. Circulation; 84:2280-5
3. Levin ER, Epstein FH (1995). Mechanisms of diseases: endothelins. N Engl J Med; 333: 356-63.
4. Rubin, L.J., et al.,(2002) Bosentan therapy for Pulmonary arterial hypertension.N Engl J Med,. 346(12):P.896-903.
5. Richard N. Channick, MD,* Olivier Sitbon, MD,† Robyn J. Barst, MD,‡ Alessandra Manes, MD,§ Lewis J. Rubin, MD* (2004). Endothelin Receptor Antagonists in Pulmonary Arterial Hypertension. Journal of the American College of Cardiology Vol. 43, No. 12 Suppl S. Published by Elsevier Inc.
6. TRENTON D. NAUSER, M.D., and STEVEN W. STITES, M.D. (2001) Diagnosis and Treatment of Pulmonary Hypertension. AMERICAN FAMILY PHYSICIAN. Vol. 63, NO.9
7. Rubin, L.J., et al.,(2002) Bosentan therapy for Pulmonary arterial hypertension.N Engl J Med,. 346(12):P.896-903.
8. De Sherbrooke 280S Clinical Science (2002). Endothelin B receptors located on the endothelium provide cardiovascular protection in the hamster.**103**, Department of Pharmacology, IPS, Medical School, Universite!, 3001 12th Avenue North.
9. Kari E RobertsIoana R Preston Pulmonary., Safety and tolerability of bosentan in the management of pulmonary arterial hypertension. review,Critical Care and Sleep Medicine, Tufts Medical Center,Boston, Massachusetts, USA.
10. Broadway BH. Heart 1997;77:299-301, Division of Pediatric Cardiology,Columbia University College of Physicians and Surgeons,, Treatment of primary pulmonary hypertension with continuous intravenous prostacyclin. New York, NY 10032, USA.
11. THE LANCET .Vol 363. May1,(2004) Portopulmonary hypertension and hepato pulmonary syndrome.
12. Hossein-Ardeschir Ghofrani, MD, Ralph Schermuly, PhD, Norbert Weissmann, PhD, Robert Voswinckel, MD, Henning Gall, MD, Werner Seeger, MD and Friedrich Grimminger, MD, PhD. Medical Clinic II/V, (2009), Drug Interactions in Pulmonary Arterial Hypertension and Their Implications. © T O U C H B R I E F I N G S, *University Hospital Giessen and Marburg GmbH*.

13. Humbert M. Eur Respir J. (2007) The burden of pulmonary hypertension. ; 30: 1–2.
14. M.M. Hoeper* and A.T. Dinh-Xuan, Eur Respir J. (2008) Pulmonary hypertension: basic concepts and practical management; 31: 236–237 DOI:10.1183/09031936.00134407, Copyright_ERS Journals Ltd.
15. Mano Y, Usui T, Kamimura H. (2007) Effects of bosentan, an endothelin receptor antagonist, on bile salt export pump and multidrug resistance-associated protein 2. Biopharm Drug Dispos. Jan;28(1):13-8.