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DEVELOPMENT OF COMPETITIVE POTENT DRUG TARGET TO FACTOR VIII IN HAEMOPHILIA

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ABSTRACT

Hemophilia A is a hereditary bleeding disorder caused by a lack of blood clotting factor VIII.It is caused by hundreds of different mutations and manifests itself in clinical conditions of varying severity [8]. This study is an attempt to design such drug which controls the blood clotting during injury and to maintain the biological function of coagulation factor VIII. . By using several tools drug library is created and they are tested for their ADMET and TOPKAT properties. The qualifying drug is selected for docking with receptor molecule 2R7E. It gives good binding site towards active site of receptor which is used to stop bleeding when a blood vessel is broken. This studies may give paves a new way for better treatment for hemophilia.

Key words:-Pharmacophore, docking, minimization, coagulation factor**,** Lipinski rule.

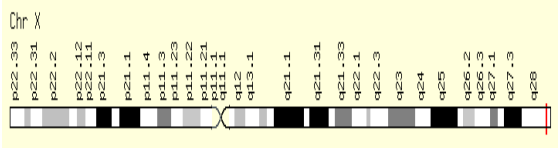
INTRODUCTION

Haemophilia is a group of hereditary genetic disorders that impair the body's ability to control blood clotting or coagulation, which is used to stop bleeding when a blood vessel is broken Persons with haemophilia may bleed for a longer time than others after an injury or accident[11]. They also may bleed internally, especially in the joints (knees, ankles, and elbows).Babies born with haemophilia are missing or have a low level of a protein needed for normal blood clotting or blood coagulation[2]. The protein is called coagulation **Factor XIII**. [3]. the major types of this condition are hemophilia A (also known as classic hemophilia) they are caused by mutations in different genes. These genes are located on the X chromosome.

Coagulation factor VIII

Recommended name for this protein is **Coagulation factor VIII** and *Alternative* name **Antihemophilic factor, Procoagulant component.** (www.uniprot.org)

This protein circulates in the bloodstream in an inactive form, bound to another molecule called von Willebrand factor, until an injury that damages blood vessels occurs. In response to injury, coagulation factor VIII is activated and separates from von Willebrand factor. The active protein interacts with another coagulation factor called factor IX. This interaction sets off a chain of additional chemical reactions that form a blood clot. Coagulation factor VIII is made chiefly by cells in the liver.



F8 Gene in genomic location: bands according to Ensembl, locations according to Geneloc

But the Mutations in the F8 gene lead to the production of an abnormal version of coagulation factor VIII or reduce the amount of this protein[10]. The altered or missing protein cannot participate effectively in the blood clotting process. As a result, blood clots cannot form properly in response to injury. These problems with blood clotting lead to excessive bleeding that can be difficult to control. Some mutations, such as the large inversion described above, almost completely eliminate the activity of coagulation factor VIII and result in severe hemophilia. Other mutations reduce but do not eliminate the protein's activity, resulting in mild or moderate hemophilia.
The length of protein sequence is 2351 amino acid. The main function of this protein is to attach carbohydrate-binding domain to the cell surface, present in eukaryotes and assumed to have horizontally transferred to eubacterial genomes

Metabolic pathway

This gene encodes coagulation factor VIII, which participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca+2 and phospholipids, converts factor X to the activated form Xa. This gene produces two alternatively spliced transcripts. Transcript variant 1 encodes a large glycoprotein, isoform a, which circulates in plasma and associates with von Willebrand factor in a noncovalent complex. This protein undergoes multiple cleavage events. Transcript variant 2 encodes a putative small protein, isoform b, which consists primarily of the phospholipids binding domain of factor VIIIc. This binding domain is essential for coagulant activity. Defects in this gene results in hemophilia, a common recessive X-linked coagulation disorder.(www.genome.jp/kegg/pathway.)

MATERIALS AND METHODS

Bioinformatics represents a new, growing area of science that uses computational approaches to answer biological questions. The potential of such an approach is beginning to change the fundamental way in which basic science is done, helping to more efficiently guide experimental design in the laboratory. With the explosion of sequence and structural information available to researchers, the field of bioinformatics is playing an increasingly large role in the study of fundamental biomedical problems.

Databases used:-

- NCBI (www.ncbi.nlm.nih.gov)
- PDB(www.pdb.org)
- Gene Cards (www.genecards.org)
- Pubchem(www.ncbi.nlm.nih.gov/pccompound)
- Drug bank (www.drugbank.ca)
- UNI-PROT(www.uniprot.org)

Actual method:-

The coagulation factor VIII which is the target protein of length 2351 amino acid was retrieved from NCBI database and it was validated from gene card. The sequence analysis of target protein was done with 10 model organism by using BLAST. The structure analyses of target protein was studied by using cph model3.0 Server, to retrieved the receptor molecule Then validation of target protein sequence was done by using various

confirmatory tools like ProDom, PfaM, CATH, Prosite, SOSUI etc. Receptor was minimized by using Accelyrs Discovery Studio 2.5. Active site of receptor is identified by using online tools like PDB SUM, Q SITE finder. Six ligands were designed with help of CHEMSKETCH TOOL and drug library was created. All the ligand compounds were passed through ADMET & TOPKAT filter in Accelyrs Discovery Studio2.5. Non toxic and non carcinogenic ligand compounds are selected for docking with receptor molecule by using Accelyrs Discovery Studio 2.5 and HEX6.3.

RESULT AND DISCUSSION

The structure analysis of target protein was studied by using CPH model 3.0Server. The protein databank identification number (PDB id) 2R7E was selected, having least E value i.e.0.0 and high score value i.e. is 1449 to retrieved the receptor molecule from PDB (www.pdb.org)

CPH MODEL 3.0Server

Searching for template ...

```
Round 0. Hits better than threshold:
0.000010:
entry: 2R7E chain: A score: 1449 E: 0.0
entry: 2R7E chain: B score: 1370 E: 0.0
entry: 3CDZ chain: B score: 1336 E: 0.0
entry: 3CDZ chain: A score: 1293 E: 0.0
entry: 1SDD chain: B score: 520 E: 1e-
146
entry: 2J5W chain: A score: 425 E: 1e-
118
entry: 1KCW chain: A score: 422 E: 1e-
117
entry: 1D7P chain: M score: 334 E: 9e-
91
entry: 3HNB chain: M score: 330 E: 1e-
89
```

Receptor Minimization

Receptor is prepared for minimization by removing water molecules, hetro atom and minimized by applying force field CHARMm in Accelyrs Discovery Studio 2.5. Active site of receptor is identified by using online tools like PDB SUM, Q SITE finder.

Thus minimization was obtained with constant energy **-91165.8864 kcal/mol** at conjugant gradient 1800 in fig:2

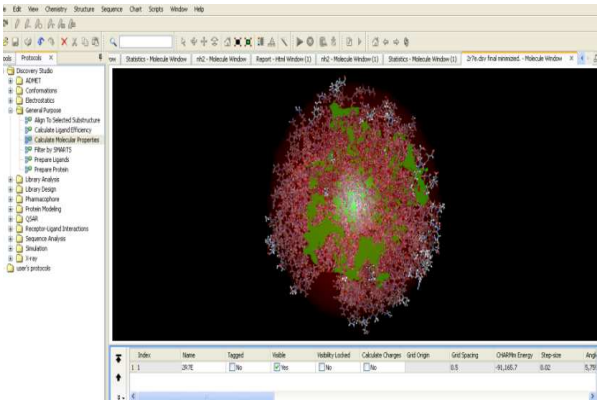


Fig: 2 Minimized receptor 2R7E

Drug library

Six ligands were designed by using scaffold with help of CHEMSKETCH TOOL and drug library was created. Then I evaluate the basic properties of all the ligand compounds as shown in table 1. After that, the ligand compounds are applied to the Lipinski rule of five for checking the drug likeliness property. Then all the six ligand were prepared and minimized one after another till the energy remained constant

Ligand (Drug) name	ALogP	Mol_Wt.	Num H Acpts	Num_H_ Donors	Num _RotaBonds	Num_ Rings	Mole._ FractiPolar Srf.area
2-amino-2-[(*trans*-4-carboxycyclohexyl) methyl]tniazan-2-ium (L.1)	-1.755	205.262	5	4	3	1	0.523
trans-4-ethylcyclohexanecarboxylic acid (L.2)	2.629	156.222	2	1	2	1	0.224
4-(2-carboxypropyl) cyclohexanecarboxylic acid (L.3)	1	2.27	214.25	4	2	4	0.34
[(*trans*-4-carboxycyclohexyl) methyl](difluoromethyl) fluoronium (L.4)	2.879	211.201	2	1	4	1	0.225
(2*S*)-2'-amino-5-(aminomethyl)-4'-hydroxy-1,1'-bi(cyclohexyl)-2-carboxylic acid (L.5)	1	0.684	144.168	3	2	1	0.394
trans-4-(hydrazinylmethyl) cyclohexanecarboxylic acid (L.6)	0.388	172.22	4	3	3	1	0.419

Table.1 drug library with there molecular properties

ADMET & TOPKAT

All the ligand compounds were passed through the ADMET/TOPKAT filters to discard the toxic and carcinogenic compounds in Accelyrs Discovery Studio2.5 ADMET have many different

parameters which give us information about the toxicity and TOPKAT gives us the information about the carcinogenicity of compounds. Non toxic and non carcinogenic ligand compounds L.4 and L.5 were further proceed for docking.

Ligand/drug name	Admet_adsopt n_lvl	Admet_solubilty	Admet_solubilty_lvl	Admet hepatoxity	Admet_hepatoxity prob	Admet_ppb level	Admet_alogp98
L.1	1	0.364	4	0	0.052	0	-1.755
L.2	0	-1.378	4	0	0.092	0	0.776
L.3	0	-2.122	3	0	0.046	0	2.27
L.4	0	-1.545	4	0	0.079	0	1.049
L.5	0	-2.512	3	0	0.072	0	2.629
L.6	0	-1.473	4	0	0.112	0	-0.137

Table.2 shows the ADMET analysis

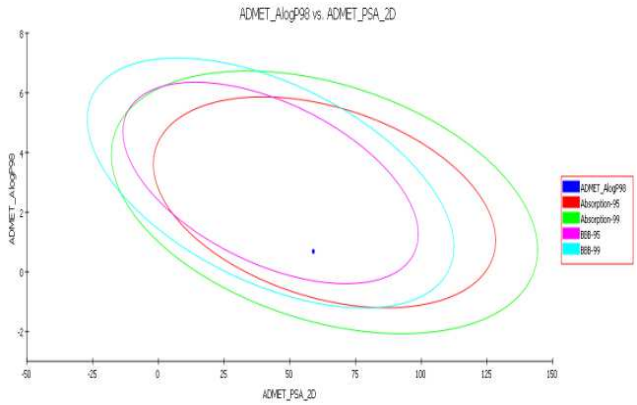


Fig.3 ADMET graph

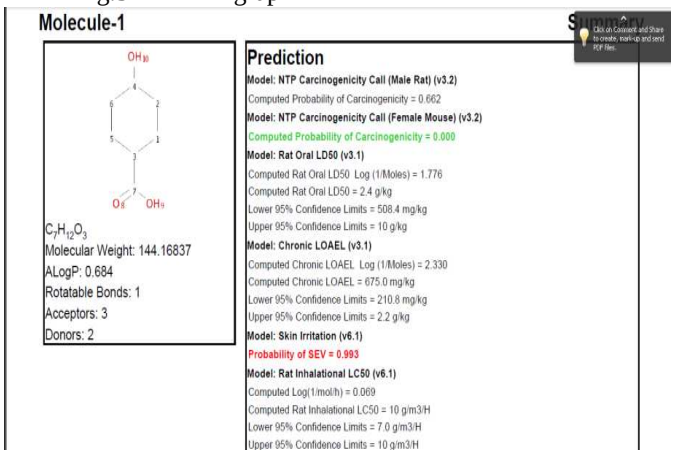


Fig.4 showing toxicity analysis

Docking in Accelyrs Discovery Studio 2.5

Accelyrs Discovery Studio 2.5 is used for predicting the receptor-ligand interactions. The process of docking involves a conformational search for a compound which complements a target binding site, with the aim of identifying the best matching binding pose. Here, the ligand compound L.5 gives best 10 binding pose with least Cdocker energy i.e.**-112.46** fig.5

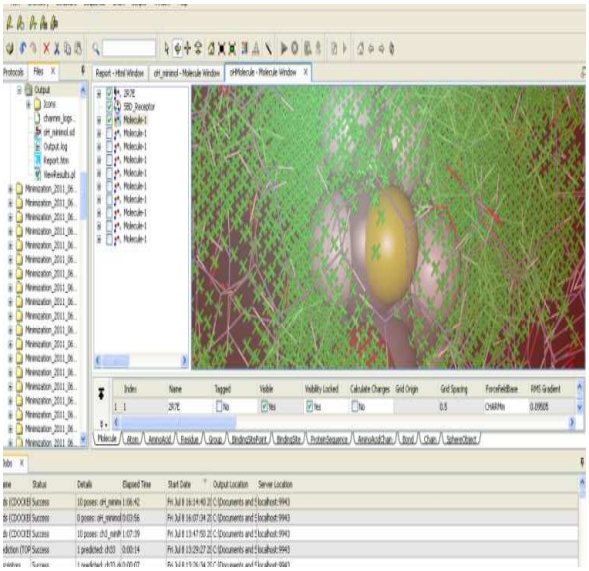


Fig.5 docking of 2R7E with L.5

Ligand Drug name	Cdocker energy	Cdocker interation energy	Pose. numbr	intial RMS gradient	intial RMS gradient
L.5	3.39401	20.4911	1	TRUE	21.1837
L.5	2.26973	18.2477	2	TRUE	21.1837
L.5	-0.171182	18.2673	3	TRUE	21.1837
L.5	-2.74988	13.8432	4	TRUE	21.1837
L.5	-6.96833	9.66913	5	TRUE	21.1837
L.5	-11.9764	8.68126	6	TRUE	21.1837
L.5	-12.1634	7.77918	7	TRUE	21.1837
L.5	-12.1964	7.81978	8	TRUE	21.1837
L.5	-12.2697	7.82694	9	TRUE	21.1837
L.5	**-112.46**	**-49.5444**	**10**	**TRUE**	**21.1837**

Table.3 docking result.

Docking in HEX 6.3

Hex docking is done to performed pharmacophore and to calculate the E-mini and E-max value.
E-mini = -157.46, E-max= -131.34.

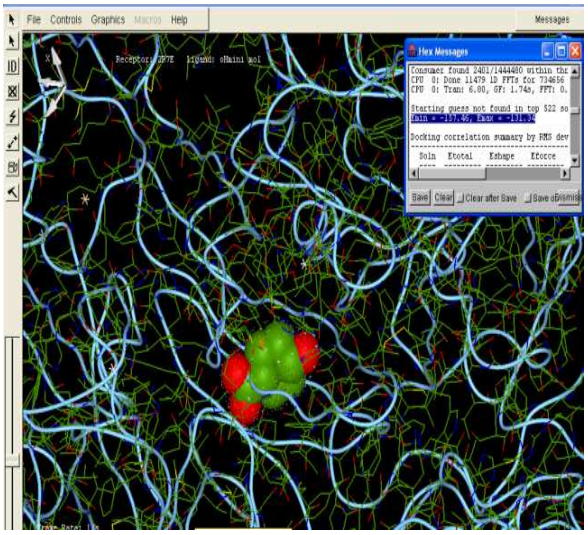


Fig.6 docking in HEX 6.3

Ligandscout

Ligandscout is used to study the pharmacophore of protein-ligand complex. In the result red color shows ionic properties, and binding sites which are attached to arginine and histidine

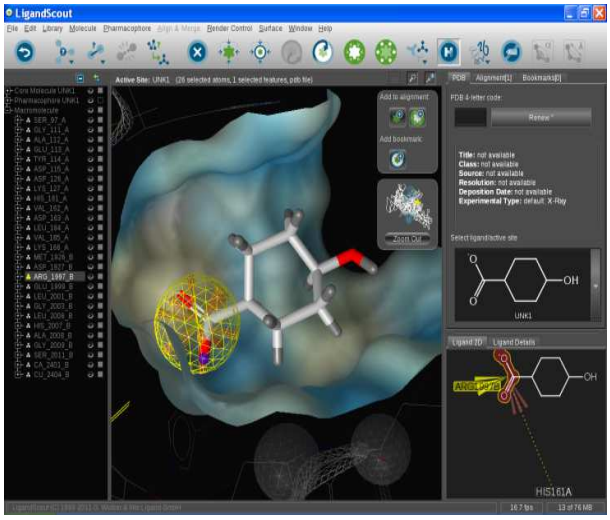


Fig .7 Pharmacophore analysis

CONCLUSION

The receptor-ligand interactions play a significant role in molecular docking and drug designing. The receptor 2R7E interacts with the ligand compound **(2*S*)-2'-amino-5-(aminomethyl)-4'-hydroxy-1,1'-bi(cyclohexyl)-2-carboxylic acid** effectively with the best **minimum energy -112.46.**, which may be more potent then the approved drug

REFERENCES

1.) Basilico F, Nardini I, Mori F, Brambilla E, Benazzi L, De Palma A, Rosti E, Farina C, Mauri P. Characterization of factor VIII pharmaceutical preparations by means of MudPIT proteomic approach. J Pharm Biomed Anal. 2010 Sep 21;53(1):50-7. Epub 2010 Mar 27.

2.) Balak DM, Gouw SC, Plug I, Mauser-Bunschoten EP, Vriends AH, Van Diemen-Homan JE, Rosendaal FR, Van Der Bom JG. Prenatal diagnosis for haemophilia: a nationwide survey among female carriers in the Netherlands. 2012 Jan 18. doi: 10.1111/j.1365-2516.2011.02742.x.

3.) Catherine J. Rea, M.B., B.Chir, Jonathan H. Foley, Factor XIII in the Treatment of Hemophilia A N Engl J Med 2012; 366:281-283January 19, 2012

4.) Esquilin J, Takemoto C, Green N. Female factor IX deficiency due to maternally inherited X-inactivation. Clin Genet. 2012 Jan 10. doi: 10.1111/j.1399-0004.2012.01842.x

5.) Gouw SC, van den Berg HM, Oldenburg J, Astermark J, de Groot PG, Margaglione M, Thompson AR, van Heerde W, Boekhorst J, Miller CH, le Cessie S, van der Bom JG., F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis., Blood. 2012 Jan 30.

6.) Heim M, Steinbach T.. Update on the use of orthotics in hemophilia, Orthop Rev. 1988 Oct;17(10):975-8.

7.) Leissinger C, Gringeri A, Antmen B, Berntorp E, Biasoli C, Carpenter S, Cortesi P, Jo H, Kavakli K, Lassila R, Morfini M, Négrier C, Rocino A, Schramm W, Serban M, Uscatescu MV, Windyga J, Zülfikar B, Mantovani L.Anti-inhibitor coagulant complex prophylaxis in hemophilia with inhibitors. 2011 Dec 22;365(25):2441.

8.) Lin Y, Stanworth S, Birchall J, Doree C, Hyde C.,Recombinant factor VIIa for the prevention and treatment of bleeding in patients without haemophilia. Cochrane Database Syst Rev. 2011 Feb 16;(2):CD005011.

9.) Mannucci P M The Safety and Efficacy History of B-domainless Factor VIII. Journal of Coagulation Disorders, July 2010; 2(2): 7-9

10.) Margaritis P, Gene-based continuous expression of FVIIa for the treatment of hemophilia. 2012 Jan 1; 4:287-99.

11.) Reitter-Pfoertner S, Horvath B, Lechner K, Sunder-Plassmann R, Mannhalter C, Pabinger I. Inhibitor development in two patients with mild haemophilia A - spontaneous disappearance and no recurrence of the inhibitor after re-challenge. 2012 Jan 18.

12.) Rodriguez-Merchan EC, Jimenez-Yuste V, Aznar JA, Hedner U, Knobe K, Lee CA, Ljung R, Querol F, Santagostino E, Valentino LA, Caffarini A,Joint protection in haemophilia. 2011 Sep;17 Suppl 2:1-23. doi: 10.1111/j.1365-2516.2011.02615.x.

13.) Salek SZ, Benson GM, Elezović I, Krenn V, Ljung RC, Morfini M, Remor E, Santagostino E, Sørensen B. The need for speed in the management of haemophilia patients with inhibitors. 2011 Jan;17(1):95-102. doi: 10.1111/j.1365-2516.2010.02265.x.

14.) Visich, JE (Visich, JE); Zuckerman, LA (Zuckerman, LA); Butine, MD (Butine, MD); Gunewardena, KA (Gunewardena, KA); Wild, R (Wild, R); Morton, KM (Morton, KM); Reynolds, TC (Reynolds, TC) Safety and pharmacokinetics of recombinant factor XIII in healthy volunteers - A randomized, placebo-controlled, double-blind, multi-dose studyVolume: 94 Issue: 4 Pages: 802-807 DOI: 10.1160/TH05-04-0292 Published: OCT 2005