e - ISSN - 2348 - 2168
Print ISSN - 2348 - 215X

Acta Biomedica Scientia

Journal homepage: www.mcmed.us/journal/abs

STRUCTURE PREDICTION AND INSILICO DESIGNING OF DRUGS FOR THE INHIBITION OF EPH A10 TYROSINE KINASE RECEPTOR PROTEIN

Manish Devgan*

Faculty of Pharmacy, R.P. Educational Trust Group of Institutions, Bastara, Karnal-132001, Haryana, India.

Article Info

Received 29/12/2015
Revised 16/01/2016
Accepted 22/01/2016

Keywords :-
Breast cancer, Tyrosine kinase, Eph receptor, Docking, Homology modelling.

ABSTRACT

Eph/Ephrin genes are profusely expressed in all adult organs. Various studies have indicated that Eph receptors are often over-expressed in malignant cancer. In this work, a theoretical model of Eph A10 receptor protein was generated using the concepts of homology modeling and loop modeling. The resulting model was validated with Ramachandran plot analysis. The ligands generated with the help of Drug bank and Zinc data base were docked against Eph A10 receptor protein using AutoDock Vina in PyRx 0.8. The structure of compound DB07255[N^4-(5-chloro-1,3-benzodioxol-4-yl)-N^2-(3-morpholin-4-ylphenyl)pyrimidine-2,4 diamine] with least binding energy (-8.3 Kcal/mol) was varied by using ACD/ChemSketch 8.0 and the docking was done for the resulting 20 new ligands. The study revealed that the ligand 4, N^2-[3-(1,3-benzodioxol-4-yl)phenyl]-N^4-(5-chloro-1,3-benzo dioxol-4-yl)pyrimidine-2,4-diamine has the maximum probability to bind with Eph A10 receptor protein which might arrest the over-expression of Eph A10 receptor protein, making the management of breast cancer more efficient.

INTRODUCTION

The Erythroprotein-producing hepatoma amplified sequence (Eph) receptor tyrosine kinase family is the largest family of tyrosine kinases. The family is further partitioned into class A and class B based on sequence homology and binding affinity for ephrin ligands. Presently in mammals nine type-A (Eph A1-A8, Eph A10) and five type-B (Eph B1-B4, Eph B6) molecules have been identified [1]. Eph/Ephrin genes are extensively expressed in all adult organs with explicit organ-site-specific system:

Eph A6, Eph A8 and Eph B1 were very eminent in brain and testis. Eph A7 was abundant in kidney vasculature. Eph A3 was up regulated in hepatocellular carcinoma. Eph A8 was down-regulated in colon cancer and Eph A1/ Eph A8 was down-regulated in glioblastomas [2]. Eph family receptors play crucial roles in physiological development such as neural development and glucose homeostasis [3]. It was observed that Eph receptors are often over-expressed in malignant cancer. In animal models it was contemplated that diminutions in Eph receptor level were effective in tumour inhibition. Thus, wide ranges of therapeutic strategies have been developed for cancer treatment. These avenues include activating monoclonal antibodies against Eph receptors, ligands- or activating antibody-cytotoxin conjugates, SiRNA, antagonistic peptides, small molecular inhibition and immunotherapy [4].

The gene encoding Eph A10 is located on chromosome 1p34 and expression study showed that Eph A10 mRNA is primarily expressed in testis [5]. In one study, it was seen that Eph A10 was over expressed in breast cancer cell lines as well as in prostate and colon cancer cell lines [6]. Another study showed that Eph A10 expression at both the gene and protein level in clinical

Corresponding Author

Manish Devgan
Email: - manishdevgan12@gmail.com

breast cancer tissues is appreciably linked with lymph node metastasis as well as stage progression [5, 6]. In one study, proteomics-based analysis using two-dimensional differential in-gel electrophoresis, the expression of Eph A10 receptor, TRAIL-R2 and cytokeratin 8 in breast cancer tissues were successfully validated [7]. Another study suggested that inhibition of Eph A10 signalling may be a novel therapeutic opportunity for management of breast cancer including triple negative breast cancers (TNBCs) which are currently not treated with molecularly targeted agents. Here it was seen that tumour growth was significantly curbed by administration of an anti- Eph A10 monoclonal antibody in a xenograft mouse model [8]. Another study reported that a diabody, an antibody derivative binding two different target molecules, recognizing both Eph A10 and CD3 could have a range of potential applications in cancer therapy [9].

Three-dimensional (3D) protein structure furnish crucial understanding of the molecular basis of protein function which makes the structure based design of drugs possible. The experimental methods of determination of protein structure, such as X-ray crystallography, NMR spectroscopy etc. takes a lot of time and is not successful with all proteins. Homology modelling is one of the approaches to theoretical structure prediction. It predicts the 3D structure of a given protein sequence based essentially on its sequence resemblance to one or more proteins of known structure. The homology modelling method consists 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 accomplished [10-12].

In this study, the structure of Eph A10 receptor protein was designed by using homology modelling. The docking of the ligands was done in order to predict the binding affinity of the small drug molecule with the target protein which in turn will lead to hampered activity and decline in Eph A10 over expression in breast cancer.

MATERIALS AND METHODS

The hardware used for calculating molecular modelling includes a personal computer with Intel (R) Core (TM) i3 CPU processor, Windows 7 Home Premium 32-bit operating system having RAM of 2.00 GB.

Sequence alignment

Fast alignment (FASTA)

The FASTA format is a text based format for representing either nucleotide sequences or peptide sequences, in which nucleotides or amino acids are represented using single letter codes. A sequence in FASTA format begins with a single line description, followed by lines of sequence data. The description line is distinguished from the sequence data by a greater-than (“>”) symbol in the first column [13]. The FASTA sequence of Eph A10 was acquired from the website of National Centre for Biotechnology Information [14].

Basic Local Alignment Search Tool (BLAST)

The BLAST is an algorithm for comparing primary biological sequence information, such as the amino acid sequence of different proteins or the nucleotides of DNA sequences [15]. Using the FASTA sequence, the standard protein BLAST was performed on the NCBI. The protein data bank proteins data base was chosen and the BLAST-P was performed [16].

Three Dimensional Position-Specific Scoring Matrix (3D-PSSM)

The 3D-PSSM is a fast web based method for protein fold recognition using 1D and 3D sequence profiles coupled with secondary structure and salvation potential information. The FASTA sequence was submitted to 3D-PSSM for fold recognition [17].

Protein Homology/Analogy Recognition Engine (Phyre)

Phyre is a web based service for protein structure prediction. Phyre is among the most popular methods for protein structure prediction [18]. The FASTA sequence was submitted to Phyre for amino acid sequence prediction [19].

Templates Preparation

The data obtained from combined BLAST, 3D-PSSM and Phyre was subjected to RCSB protein data bank. The templates were selected on the basis of their resolution (Å) and R-value. All the above templates were submitted by X-ray crystallography method in PDB.

Molecular Modelling

Homology modelling of Eph A10 was done by using EasyModeller. EasyModeller is a graphical user interface to Modeller program. It is a standalone tool in windows platform with Modeller and Python preinstalled [20, 21]. The Swiss-Pdb viewer was installed from the respective site which is an application that provides a user friendly interface allowing analyzing several proteins at the same time [22].

Structure Prediction

The six templates were submitted to the EasyModeller and were aligned. The Discreet Optimized Protein Energy (DOPE) score is a statistical tool to assess homology models in protein structure prediction. The model with the minimum score can be chosen as the best possible structure.

Validation of Predicted Model

The validation of all the five models was performed by submitting the PDB files to PDBsum [23]. The PDBsum is a pictorial database that provides an at-a-

glance overview of the contents of each 3D structure deposited in the Protein Data Bank. It shows the molecule(s) that make up the structure (*i.e.*, protein chains, DNA, ligands and metal ions) and schematic diagrams of their interactions [24]. The Ramachandran plot validated the result. The residues in the most favoured region are at maximum and those in the generously allowed and disallowed regions are at minimum.

Loop Modelling

The loop regions in the given protein usually contribute to active and binding sites. Thus, loops generally regulate the functional specificity of a given protein framework [25]. The co-ordinate file in PDB format was submitted for loop optimization to ModLoop, *i.e.*, Modelling of Loops in Protein Structures. ModLoop is a web server for automated modelling of loops in protein structures [26]. The resulting co-ordinate file was sent back by e-mail. This structure was validated by using PDBsum. The process of loop modelling and subsequent validation was continued until an optimized structured model of protein was obtained.

2.4 Ligand Generation

The Drug bank is an exclusive bioinformatics/ chemin formatics resource that amalgamates comprehensive drug data with thorough drug target information [27]. The Drug Bank [28] was used online; the FASTA sequence of the target protein was entered. The similar structures corresponding to the individual drug were also saved. The Zinc Data base was utilized to incorporate into the data the structures showing similarity up to 50 % [29].

Molecular Docking

Virtual screening, sometimes called *in-silico* screening, is a new branch of medicinal chemistry that represents a fast and cost effective tool for computationally screening database in search for the novel drug leads [30]. Molecular docking is a crucial tool in structural molecular biology and computer assisted drug design. The objective of ligand-protein docking is to anticipate the cardinal binding mode(s) of a ligand with a protein known three dimensional structures. Successful docking methods explore high- dimensional spaces productively and utilize a scoring function that unerringly ranks candidate dockings [31].

Both the macromolecule and ligands were prepared for docking with the help of PyMol and chemBio3D computer software respectively [32,33]. The molecular docking of these drugs was done against Eph A10 receptor protein using AutoDock Vina in PyRx 0.8 [34]. The grid dimensions were maximized and the parameters used were:

Centre coordinates:

Dimensions (Å):

X = 50.3412 X = 148.4417

Y = 79.0240 Y = 79.9271

Z = 87.9180 Z = 127.3250

The best compound was selected on the basis of the binding energy/binding affinity (Kcal/mol) and the root mean square deviation (upper bound and lower bound).

Ligand Designing and Docking

The selected ligand was used to design 20 new molecules with the help of ACD/ChemSketch 8.0 freeware. The Lipinski's rule of five was used as reference to decide the theoretical capability of the drugs. These sketched structures were then subjected to energy minimization by using ChemBio3D as done before. The molecular docking of these 20 sketched molecules vis-à-vis the selected parent ligand was done against the Eph A10 receptor protein by using AutoDock Vina in PyRx 0.8, the coordinates and dimensions remaining same as before.

RESULTS AND DISCUSSION

Template Generation

FASTA sequence of Eph A10 protein was retrieved from the website of NCBI. The GenBank No. is AAH67734.1 and gi no. is 45709950. The BLAST was performed on the NCBI and 34 hits were recorded as shown in Figure 1.

The FASTA sequence was put through the 3D-PSSM and Phyre for prediction of protein structure. The results attained were connected and ranked in the descending order of % ID as shown in Table 1. The six templates (3NRU, 4LOP, 4ET7, 3CKH, 2WO1, 3GXU) were selected on the basis of their ID %, resolution (≤ 3 Å) and the R-value (≤ 0.5).

Homology Modelling

The six models were generated with the help of EasyModeller and their DOPE score was obtained (Table 2). Models with the lowest DOPE assessment score, or with the highest GA341 assessment score have the most stable minimized energy. The model number 2 was selected on these bases for further analysis.

Validation

The models were further validated by Ramachandran plot, by submitting the files to PDBsum. The model number 2 was endorsed as the residues in most favoured region are 78.2 % (Table 3).

Loop modelling

The PDB file format of model number 2 was submitted for loop optimization to ModLoop (https://modbase.compbio.ucsf.edu/modloop/) and the structure was validated by using PDBsum. The model was validated as it had maximum percentage of residues in most favoured region (97.9 %) and no residue in generously allowed as well as disallowed regions (Figure

2). The model of Eph A10 receptor protein (Figure 3) was successfully submitted to Protein model data base (http://bioinformatics.cineca.it/PMDB/) bearing the PMDB ID: PM0080377.

Ligand Generation and Docking

About 50 drugs like compounds downloaded from The Drug Bank and Zinc Data Base were docked against Eph A10 protein using AutoDock Vina in PyRx 0.8. The results (Table 4) showed that the lowest binding energy (-8.3 Kcal/mol) with Eph A10 protein is of ligand **DB07255** [N^4-(5-chloro-1,3-benzodioxol-4-yl)-N^2-(3-morpholin-4-ylphenyl) pyrimidine-2,4-diamine] (Figure 4). This suggested that the compound can be a promising ligand for the target Eph A10 protein.

Ligand Designing and Docking

The structural variation was done in the molecule DB07255 and 20 new compounds were designed with the help of ACD/ChemSketch 8.0. The virtual screening of these compounds was done against Eph A10 protein using AutoDock Vina in PyRx 0.8. The results (Table 5) indicated that out of all these compounds, Ligand4, N^2-[3-(1,3-benzodioxol-4-yl)phenyl]-N^4-(5-chloro-1,3-benzodioxol -4-yl)pyrimidine-2,4-diamine, possesses the minimum binding energy (-8.7 Kcal/mol) (Figure 5), which is greater than that of compound DB07255, others ligands like, ligand6 ($C_{21}H_{19}Cl_2N_5O_3$), ligand7 ($C_{23}H_{17}Cl_2N_5O_2$) and ligand15 ($C_{23}H_{24}ClN_5O_3$) have binding energy comparable to ligand DB07255.

In silico prediction of physicochemical, ADME and toxicity properties

In order to reduce the need for labour intensive experimental testing and literature searches, the software developed by the Advanced Chemistry Development Inc. was used to predict and compare the physicochemical, ADME and toxicity properties of both ligand DB07255 and ligand 4 [35]. The requisite information was also taken from ACD/ChemSketch 8.0. The results as shown in Table 6 clearly indicate that both DB07255 and ligand 4 are comparable in almost all aspects.

Table 1. Generation of templates using 3D-PSSM, Phyre and RCSB protein data bank

S.No.	Template/Accession No	ID %	Resolution(Å)	R-Value (Obs)
1	3NRU	73	2.3	0.237
2	4LOP	66	2.05	0.150
3	4ET7	64	2.60	0.212
4	3CKH	64	2.80	0.234
5	2WO1	63	1.85	0.189
6	3GXU	63	2.50	0.273
7	4M4P	59	2.08	0.190
8	4BK4	59	3.65	0.351
9	4BKF	59	4.65	0.329
10	3SKJ	58	2.5	0.219
11	3C8X	57	1.95	0.166
12	3P1I	57	2.10	0.187
13	2QBX	57	2.30	0.198
14	1NUK	56	2.90	0.206

Table 2. DOPE score of the six possible models of EphA10 receptor protein

S.No.	Query File Name	Molpdf	DOPE score	GA341 score
1	B99990001.pdb	14267.15723	-22011.37305	0.94206
2	B99990002.pdb	14677.58984	-21376.87891	0.99382
3	B99990003.pdb	14280.81543	-21512.02344	0.94128
4	B99990004.pdb	14651.00391	-21696.64063	0.90443
5	B99990005.pdb	14554.23535	-21584.33984	0.68864
6	B99990006.pdb	14090.59180	-21442.31836	0.92241

Table 3. Ramachandran plot statistics of the six models

Models	Residues in most favored region	Residues in additional allowed region	Residues in generously allowed regions	Residues in disallowed regions
B99990001.pdb	77.4	17.3	3.7	1.6
B99990002.pdb	78.2	17.3	2.9	1.6
B99990003.pdb	77.0	18.5	3.7	0.8

B99990004.pdb	73.7	19.8	5.3	1.2
B99990005.pdb	72.4	20.6	3.3	3.7
B99990006.pdb	77.0	18.1	2.5	2.5

Table 4. The docking results of ligand generated using Drug Bank and Zinc Data Base against Eph A10 protein as target

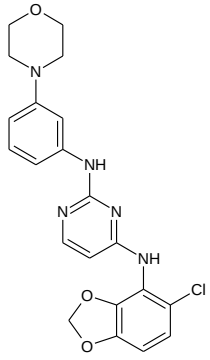
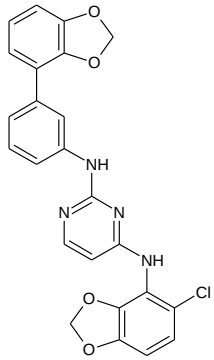
S.No	Accession No.	Binding Affinity (Kcal/mol)	RMSD/UB	RMSD/LB	S.No	Accession No.	Binding Affinity (Kcal/mol)	RMSD/UB	RMSD /LB
1	DB01254	-7.2	0	0	26	DB03478	-7.0	0	0
2	DB00131	-7.3	0	0	27	DB03755	-7.1	0	0
3	DB00171	-7.5	0	0	28	DB03909	-6.9	0	0
4	DB00317	-6.3	0	0	29	DB04366	-7.3	0	0
5	DB00398	-7.3	0	0	30	DB04395	-6.8	0	0
6	DB00530	-6.4	0	0	31	DB04497	-7.4	0	0
7	DB01660	-7.1	0	0	32	DB04554	-5.9	0	0
8	DB01690	-8.1	0	0	33	DB05465	-6.9	0	0
9	DB01717	-6.3	0	0	34	DB06616	-7.1	0	0
10	DB01774	-7.0	0	0	35	DB06991	-6.4	0	0
11	DB01812	-7.1	0	0	36	DB07249	-7.5	0	0
12	DB01829	-7.0	0	0	37	DB07250	-7.3	0	0
13	DB01842	-7.1	0	0	38	DB07251	-6.7	0	0
14	DB01860	-7.4	0	0	39	DB07252	-6.9	0	0
15	DB02059	-7.7	0	0	40	DB07254	-7.6	0	0
16	DB02082	-6.3	0	0	41	**DB07255**	**-8.3**	**0**	**0**
17	DB02098	-7.1	0	0	42	DB07256	-7.7	0	0
18	DB02363	-7.6	0	0	43	DB07268	-7.0	0	0
19	DB02527	-7.2	0	0	44	DB07750	-6.8	0	0
20	DB02623	-7.0	0	0	45	DB07755	-6.7	0	0
21	DB02738	-7.1	0	0	46	DB07831	-7.1	0	0
22	DB02930	-6.9	0	0	47	DB07970	-6.9	0	0
23	DB03222	-7.2	0	0	48	DB08043	-6.9	0	0
24	DB03230	-6.9	0	0	49	DB08764	-7.1	0	0
25	DB03365	-6.6	0	0	50	DB08896	-7.5	0	0

Table 5. The docking of ligands (ChemSketch) against Eph A 10 as target protein.

S.No.	Ligand No. / (Mol. Formula)	Binding Affinity (kcal/mol)	RMSD/UB	RMSD/LB
1	Ligand1 ($C_{23}H_{17}ClN_4O_2$)	-7.9	0	0
2	Ligand2 ($C_{23}H_{16}Cl_2N_4O_2$)	-7.7	0	0
3	Ligand3 ($C_{23}H_{15}Cl_3N_4O_2$)	-8.0	0	0
4	**Ligand4 ($C_{24}H_{17}ClN_4O_4$)**	-8.7	0	0
5	Ligand5 ($C_{22}H_{20}ClN_5O_5$)	-8.2	0	0
6	Ligand6 ($C_{21}H_{19}Cl_2N_5O_3$)	-8.4	0	0
7	Ligand7 ($C_{23}H_{17}Cl_2N_5O_2$)	-8.4	0	0
8	Ligand8 ($C_{22}H_{20}ClN_5O_5$)	-8.1	0	0
9	Ligand9 ($C_{24}H_{17}ClN_4O_4$)	-8.0	0	0
10	Ligand10 ($C_{21}H_{21}ClN_6O_3$)	-7.6	0	0
11	Ligand11 ($C_{23}H_{22}ClN_3O_3$)	-6.9	0	0
12	Ligand12 ($C_{23}H_{21}Cl_2N_2O_3$)	-6.7	0	0
13	Ligand13 ($C_{23}H_{20}Cl_3N_3O_3$)	-7.5	0	0
14	Ligand14 ($C_{23}H_{26}ClN_7O_3$)	-7.4	0	0
15	Ligand15 ($C_{23}H_{24}ClN_5O_3$)	-8.3	0	0
16	Ligand16 ($C_{22}H_{19}Cl_4N_3O$)	-7.1	0	0
17	Ligand17 ($C_{20}H_{21}N_5O$)	-8.0	0	0

18	Ligand18 ($C_{20}H_{20}ClN_5O$)	-8.0	0	0
19	Ligand19 ($C_{20}H_{22}ClN_7O$)	-7.7	0	0
20	Ligand20 ($C_{21}H_{21}ClN_6O_3$)	-7.9	0	0

Table 6. Predictive physicochemical, ADME and toxicity properties of ligand DB07255 and ligand 4

S.No	Properties	Ligand DB07255	Ligand 4
1	Molecular Formula	$C_{21}H_{20}ClN_5O_3$	$C_{24}H_{17}ClN_4O_4$
2	Formula Weight	425.8682	460.86918
3	IUPAC Name	N^4-(5-chloro-1,3-benzodioxol-4-yl)-N^2-(3-morpholin-4-ylphenyl)pyrimidine-2,4-diamine	N^2-[3-(1,3-benzodioxol-4-yl)phenyl]-N^4-(5-chloro-1,3-benzodioxol-4-yl)pyrimidine-2,4-diamine
4	Structure		
5	Molar refractivity	114.05±0.3 cm^3	122.99±0.3 cm^3
6	Molar Volume	294.4±3.0 cm^3	307.5±3.0 cm^3
7	Parachor	857.6±6.0 cm^3	902.2±4.0 cm^3
8	Index of refraction	1.702±0.02	1.731±0.02
9	Surface tension	72.0±3.0 dynes/cm	74±3.0 dynes/cm
10	Density	1.446±0.06 g/cm^3	1.498±0.06 g/cm^3
11	Polarizability	45.21±0.51-24 cm^3	48.75±0.51-24 cm^3
12	Monoisotopic mass	425.125467 Da	460.093833 Da
13	Nominal mass	425 Da	460 Da
14	Average mass	425.875562 Da	460.877327 Da
15	Boiling point	622.61±65.0℃ (at 760 mmHg)	656.22±65.0℃ (at 760 mmHg)
16	LogP	4.34	5.03
17	pKa (Base)	7.1±0.8	6.7±0.8
18	Solubility (S_w)	0.022 mg/ml	0.0026 mg/ml
19	Oral bioavailability	30% - 70%	30% - 70%
20	Active transport	PepT1: not transported ASBT: not transported	PepT1: not transported ASBT: not transported
21	Absorption rate	K_a = 0.051 min^{-1}	K_a = 0.05 min^{-1}
22	Blood brain barrier transport parameters: i) Rate of brain penetration, LogPS ii) Extent of brain penetration, LogPB iii) Brain/plasma equilibration rate, Log(PS*fu, brain)	 -1.4 -0.03 -3.2	 -1.3 -0.41 -3.4
23	LogBB	-0.17	-0
24	LogPS	-1.4	-1.3
25	PPB (Plasma binding)	98.61%	99.7%
26	Volume of distribution	4.18 L/Kg	3.78 L/Kg

27	P-gp substrate probability	0.65	0.67
28	P-gp inhibitor probability	0.29	0.22
29	AMES test	+0.29	+0.19
30	Genotoxicity Hazards	No hazardous fragments have been found	No hazardous fragments have been found
31	hERG inhibitor probability	0.99	1
32	LC50 In species Pimephales promelas	0.026 mg/L	0.013 mg/L
33	LD50 (mg/Kg): Species/route of adminstration: Mouse/intraperitoneal Mouse/oral Mouse/intraveous Mouse/subcutaneous Rat/peritoneal Rat/oral	 620 650 44 100 110 340	 510 520 32 82 110 130
34	Toxicity category 2 and 3 probability	77%	79%

Fig 1. Distribution of 34 BLAST hits on the query sequence

(query Id: gi|45709950|gb|AAH67734.1| in pdb protein database and the program is BLASTP 2.2.31+.

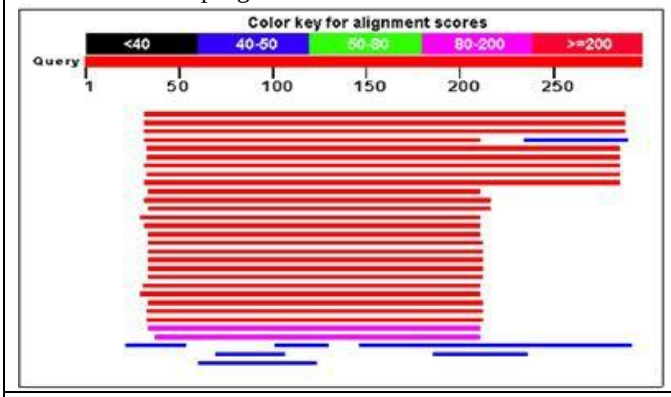


Fig 2. Ramachandran plot for optimized model of Eph A10 receptor protein

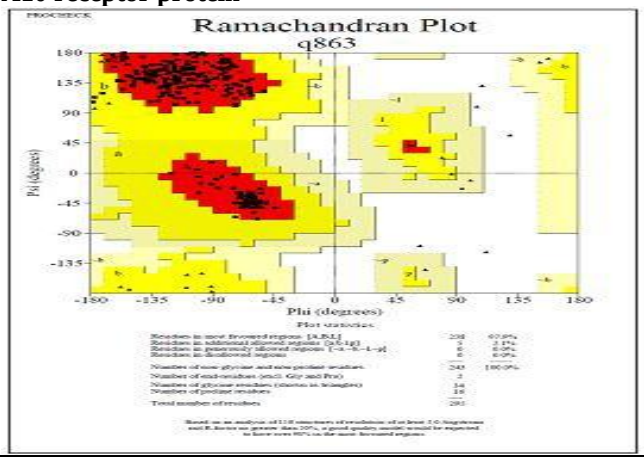


Fig 3. Optimized model of Eph A10 receptor protein

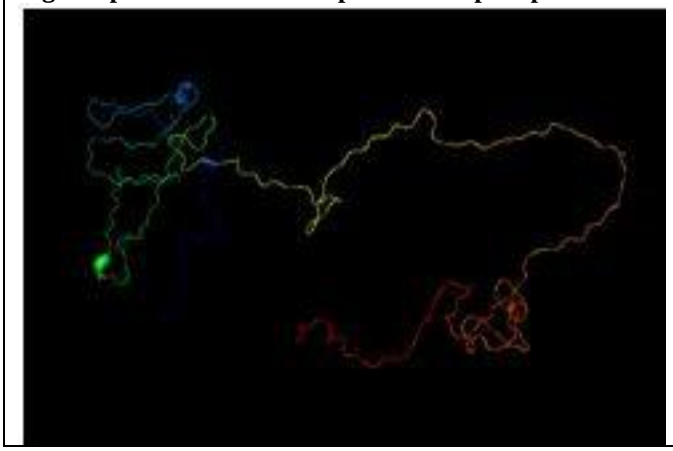


Fig 4. Docking of ligand07255 with Eph A10 receptor protein

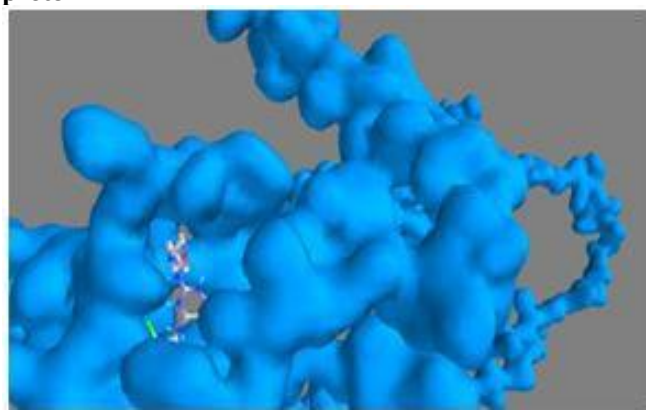
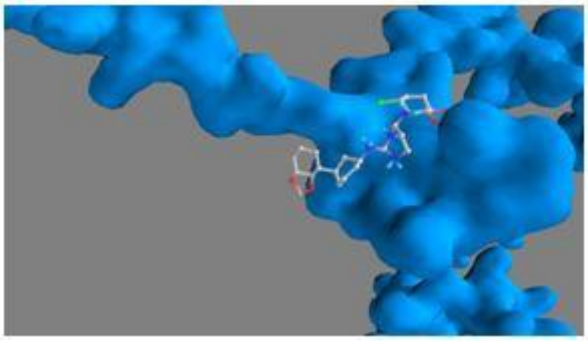


Fig 5. Docking of ligand4 with Eph A10 receptor protein



CONCLUSION

The model of Eph A10 receptor protein was created by using the concepts of homology and loop modelling. The model was validated by the Ramachandran plot. Various ligands were identified using Drug bank and Zinc data base. The molecular docking done against Eph A10 receptor protein of these ligands using AutoDock Vina in PyRx 0.8 identified DB07255 with minimum binding energy (-8.3 Kcal/mol). The structure of this compound was varied by using ACD/ChemSketch 8.0 and then docking was done against the target protein. This study suggested that the ligand 4 bears the minimum binding energy (-8.7 Kcal/mol) with the target protein and thus has the maximum probability to bind. The usage of this compound in particular and other compounds, *i.e.*, ligand 6, 7 and 15 might result in restriction of the over-expression of Eph A10 receptor protein, leading to a more efficient management of the breast cancer.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

1. Kuang S, Bai H, Fang Z, Lopez G, Yang H, Tong W, Wang ZZ, Garcia-Manero G. (2010). Aberrant DNA methylation and epigenetic inactivation of Eph receptor tyrosine kinases and ephrin ligands in acute lymphoblastic leukemia. *Blood*, 115(12), 2412-19.
2. Hafner C, Schmitz G, Mever S, Bataille F, Hau P, Langmann T, Dietmaier W, Landthaler M, Vogt T. (2004). Differential gene expression of Eph receptors and epherins in benign human tissues and cancers. *Clin Chem*, 50(3), 490-99.
3. Nagano K, Kanasaki S, Yamashita T, Maeda Y, Inoue M, Higashisaka K, Yoshioka Y, Abe Y, Mukai Y, Kamada H, Tsutsumi Y, Tsunoda S. (2013). Expression of Eph receptor A10 is correlated with lymph node metastasis and stage progression in breast cancer patients. *Cancer Med*, 2(6), 972-77.
4. Vaught D, Brantley-Sieders DM, Chen J. (2008). Eph receptors in breast cancer: roles in tumour promotion and tumour suppression. *Breast Cancer Res*, 10(6), 72.
5. Aasheim HC, Patzpe S, Hjorthaug HS, Finne, EF. (2005). Characterization of a novel Eph receptor tyrosine kinase, EphA10, expressed in testis. *Biochim Biophys Acta*, 1723(1-3), 1-7.
6. Nagano K, Yamashita T, Inoue M, Higashisaka K, Yoshioka Y, Abe Y, Mukai Y, Kamada H, Tsutsumi Y, Tsunoda S. (2014). Eph receptor A10 has a potential as a target for a prostrate cancer therapy. *Biochem Biophys Res Commun*, 450(1), 545-49.
7. Imai S, Nagano K, Yoshida Y, Okamura T, Yamashita T, Abe Y, Yoshikawa T, Yoshioka Y, Kamada H, Mukai Y, Nakagawa S, Tsutsumi Y, Tsunoda S. (2011). Development of an antibody proteomics system using a phage antibody library for efficient screening of biomarker proteins. *Biomaterials*, 32(1), 162-69.
8. Nagano K, Maeda Y, Kanasaki S, Watanabe T, Yamashita T, Inoue M, Higashisaka K, Yoshioka Y, Abe Y, Mukai Y, Kamada H, Tsutsumi Y, Tsunoda S. (2014). Ephrin receptor A10 is a promising drug target potentially useful for breast cancers including triple negative breast cancers. *J Control Release*, 189, 72-79.
9. Kamada H, Taki S, Nagano K, Inoue M, Ando D, Mukai Y, Higashisaka K, Yoshioka Y, Tsutsumi Y, Tsunoda S. (2015). Generation and characterization of a bispecific diabody targeting both EPH receptor A10 and CD3. *Biochem Biophys Res Commun*, 456(4), 908-12.
10. Schwede T, Kopp J, Guex N, Peitsch, MC. (2003). Swiss-Model: an automated protein homology-modelling server. *Nucleic Acids Res*, 31(13), 3381-85.
11. Vyas VK, Ukawala RD, Ghate M, Chintha C. (2012). Homology modelling a fast tool for drug discovery: current perspectives. *Indian J Pharm Sci*, 74(1), 1-17.

12. Xiang Z. (2006). Advances in homology protein structure modelling. *Curr Protein Pept Sci.* 7(3), 217-27.
13. http://zhanglab.ccmb.med.umich.edu/FASTA/
14. http://www.ncbi.nlm.nih.gov/protein/45709950?report=fasta
15. http://en.wikipedia.org/wiki/BLAST
16. http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS=blastp&QUERY=AAH67734.1&LINK_LOC=protein&PAGE_TYPE=BlastSearch
17. http://www.sbg.bio.ic.ac.uk/~3dpssm/index2.html
18. https://en.wikipedia.org/wiki/Phyre/Phyre2
19. http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index
20. Bhushan KK. (2015). EasyModeller (Version 2.0). [Computer software]. Available from: http://www.sites.google.com/site/bioinformatikz (Accessed 08 April 2015).
21. Kuntal BK, Aparoy P, Reddanna P. (2010). EasyModeller: A graphical interface to MODELLER. BMC Res Notes, 3(1), 226.
22. DeepView-Swiss-PdbViewer. (2105). http://Spdbv.vital-it.ch/ (Accessed 06 April 2015).
23. PDBsum. (2015). https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/ GetPage.
24. Laskowski RA. (2009). PDBsum new things. Nucleic Acids Res, 37(1), D355-59.
25. Fiser A, Do RKG, Sali A. (2000). Modelling of loops in protein structures. *Protein Sci*, 9(9), 1753-73.
26. Fiser A, Sali A. (2003). ModLoop: automated modelling of loops in protein structures. *Bioinformatics*, 19(18), 2500-01.
27. Wishart DS, Knox C, Guo AC, Shrivastva S, Hassanali M, Stothard P, Chang Z, Woolsey J. (2006). DrugBank: a comprehensive resource for *in silico* drug discovery and exploration. *Nucleic Acids Res*, 34(1), D668-72.
28. The Metabolomics Innovation Centre. 2015. Drug Bank version 4.2. http://www.drugbank.ca.
29. Shoichet laboratory. 2015. ZINC. http://www.zinc.docking.org.
30. Vyas V, Jain A, Jain A, Gupta A. (2008). Virtual screening: a fast tool for drug design. *Sci Pharm*, 76(3), 333-60.
31. Morris GM, Lim-Wilby M. (2008). Molecular docking. *Methods Mol Biol*, 443, 365-82.
32. http://www.cambridgesoft.com/Ensemble_for_chemistry/chemBio3D/
33. Schrödinger. 2015. PyMol (Version 1.7.2). http://www.pymol.org (Accessed 05 May 2015).
34. Python Prescription. 2015. http://pyrx.sourceforge.net/ (Accessed 13 June 2015).
35. Advanced Chemistry Development Inc. 2015. http://ilab.acdlabs.com/ilab2/ (Accessed 23 September 2015).