Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 62 - 72

Research Article Bioinformatics

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

HOMOLOGY MODELING, ACTIVE SITE PREDICTION AND TARGETING THE ANTI EPILEPTIC ACTIVITY THROUGH MOLECULAR DOCKING TECHNIQUES ON LAFORIN

R. CAROLINE NIRMALA[1,2] AND V.K.GOPALAKRISHNAN [1*]

[1*] *Department of Biochemistry and Bioinformatics, Karpagam University, Coimbatore, India.*
[2] *Department of Bioinformatics, CMS College of Science and Commerce, Coimbatore, India*

ABSTRACT

Epilepsy is a second common neurological disorders characterized by repeated seizures. This is a genetic disorder caused by mutation in Laforin, encoded by the *EPM2A*gene. Laforin is a protein mutated in patients with Lafora disease. This contains a dual specificity phosphatase domain (DSP) and a carbohydrate binding module subtype 20 (CBM20). To develop antiepileptic drugs, understanding the characteristics of the protein responsible is essential. Hence EPM2A coding protein model has been built using Discovery Studio 3.5 and validated. The active site has been predicted for this model and analyzed. Molecular docking studies have been performed for this model. To carry out docking studies compounds having similar structure of Carbamazepen and the key amino acid residues involved in ligand binding have been determined from public databases. Totally 71 compounds has been screened and after conducting toxicity studies 5 compounds 2_4_-bis (N_ - (4-methylphenyl) ureido) toluene, carboxyphenylureido phenyl, N- 3-((2-methylphenyl)amino carbonyl amino)-2-naphthoyl, 1-Phenyl-3-(2-carboxy-phenyl)-urea and 2_6_- bis (N - (4 – methylphenyl) ureido) toluene has been selected as potent target candidate drugs for Lafora disease.

KEYWORDS: **Laforin, EPM2A, Homology modeling, docking**

V.K.GOPALAKRISHNAN
Department of Biochemistry and Bioinformatics,
Karpagam University, Coimbatore, India.

Corresponding author

INTRODUCTION

Epilepsy is a chronic disorder characterized by recurrent seizures or convulsions[1]. Lafora disease is a brain disorder in which a person has repeated seizures or convulsions over time. This disease is an autosomal recessive type of Progressive myoclonus epilepsy caused by mutations in the EPM2A gene[2]. Lafora disease is one of the common PMEs. Symptoms of Lafora disease begin to manifest themselves in children from 10 to 17 years old[3]. Males and females are equally affected. This disease is caused by mutations[4] in one of two genes, EPM2A and EPM2B. EPM2A codes for the dual specificity phosphatase (DSP) protein Laforin. It consists of a carbohydrate binding domain (CBM-20)[5]. This EPM2A gene is present on chromosome 6 in humans.[6] The EPM2A gene provides instructions for making a protein called Laforin. This protein is active in cells throughout the body, and it plays an important role in the survival of nerve cells in the brain. To carry out these functions, Laforin interacts with several other proteins, including malin. These proteins are part of complex networks that transmit chemical signals and break down the abnormal proteins.[7] Laforin also act as a tumor suppressor protein, which means that it keeps cells from growing and dividing in an uncontrolled way. Laforin play a critical role in regulating the production of a glycogen which is a major source of stored energy in the body. The body stores this sugar in the liver and muscles, breaking it down when it is needed for the metabolism.[8] About 50 mutations in the *EPM2A* gene have been identified in people with Lafora progressive myoclonus epilepsy. These mutations change aminoacids in the Laforin protein. Other mutations delete or insert genetic material in the *EPM2A* gene. Almost all mutations in this gene prevent cells from producing any Laforin or lead to the production of a nonfunctional version of the protein.[9] Hence Laforin serves as a Potential target for drug design. This study focuses on studying the structural characterization of Laforin by predicting the 3D structure. To derive better inhibitors for Progressive myoclonus epilepsythe pharmacophoric features of an existing drug Carbamazepene[10] has been modified and ligands were prepared and docking studies has been carried out.

MATERIALS AND METHODS

Homology Model construction

From the literature the EPM2A was identified as a target protein in Laforin disease. The EPM2A sequence with Uniprot accession number O95278 was selected for Insilco analysis through homology modelling. Around 331 amino acids are present in the target sequence. The Discovery Studio v 3.5 was used for homology model construction (Accelrys, San Diego, USA). The homologous structures in the protein O95278 were searched through NCBI – PSI Blast (The National Center for Biotechnology Information). The parameters of the applied algorithm are (BLOSUM62; E-threshold, 10) using pdbaa server[11]. Two template structures were selected for the model building with PDB ID: 1D3C and 2PQ5. Multiple sequence alignment was carried out to identify the conserved regions by aligning the target with the template structure[12]. The aligned sequences were used for the model construction was built using “Build homology model” protocol in DS[13]. The protein model was generated by MODELER, which was originally developed by Sali (Sali et al., 1995)[14].

Protein simulation and validation

Modeled structure was refined by CHARMm force field (Brooks et al., 1983) in DS Modeling protocol, which provides powerful mechanics and dynamic protocols for studying the energetic and motion of molecules, from small ligands to multi-component physiological complexes. Accelrys CHARMm force field was used throughout the simulation studies. Constraint was applied to allow only binding

site and ligand to be flexible during the simulation. Potential energy of the modelled protein was analyzed before and after minimization by using calculate energy and minimization protocol respectively in DS 3.5. Parameters of minimization protocols were the smart minimizer algorithm with 200 maximum steps, 0.1 RMS gradient value and 0.0 energy changes value and electrostatic based on spherical cut off [15]. The stereochemistry quality of the structures were validated with PROCHECK[16] and Verify 3D. Quality factors for the protein models were calculated using ERRAT2.

Active site prediction

The active sites of the protein were predicted using DS 3.5, which is based on the receptor cavity method ("Eraser" algorithm) [17]. This study reveals the key residues in the target protein which are responsible for ligand binding, which are present in the active site or elsewhere.

Ligand identification and retrieval

The Synthetic compound Carbamazepen and the compounds having similar structures to Carbamazepen was obtained from chemicalize.org. Around 71 compounds with similar structures were obtained.

Ligand minimization

Ligand optimization was carried out using CHARMm and MMF force field by small molecules minimization protocol in DS 3.5[18]. Various ligand conformations were generated based on Bond energy, CHARM energy, dihedral energy, electrostatic energy, initial potential energy and initial RMS gradient values.

Evaluation of drug likeliness

The drug likeliness was evaluated using the Lipinski rule of 5 via Lipinski drug filter protocol using DS 3.5[19]. The cutoff values of the rule are- Molecular mass of the ligand less than 500Da, Hydrogen bond donors less than 5, Hydrogen bond Acceptors less than 10 and High lipophilicity (expressed by Log P less than 5).

ADME-Toxicity investigation

Lipophilicity plays a critical role in the drug discovery and design. Lipophilicity plays a key role in the determination of physicochemical property which has a crucial role in finding out the ADMET[20] (absorption, distribution, metabolism, excretion, and toxicity) and the over suitableness of drug candidates. ADMET studies give insight into the pharmacokinetic property of the ligand compounds. There is enhanced evidence to show that control of key physicochemical properties such as lipophilicity, within a defined range ameliorate the quality of compound. The studies of aqueous solubility, blood brain barrier level, CYP 2D6, Hepatoxicity and plasma protein binding levels were carried out.[21] Toxicity profile of the ligand molecules was predicted by using TOPKAT[22] which applies a range of robust, cross validated and Quantitative Structure-Toxicity Relationship (QSTR) models for assessing specific toxicological endpoints. The toxicity profile also includes NTP carcinogenicity, mutagenicity and developmental toxicity and skin irritation assessment.

Molecular docking

The Molecular docking studies were carried to investigate the binding affinities and interaction modes between the inhibitors and the target using BioSolveIT FlexX[23]. The active site of the modelled protein was loaded in the BioSolveIT FlexX. The active site amino acids were defined in the target molecule during the target preparation step of FlexX. A sphere of 10Å radius was defined as an active site. The screened 71 compounds were loaded in FlexX as docking library. The Protein Ligand clash was set to 2.9 Å and Intra Ligand clash was set to 0.6 in the docking. Maximum number of fragmentation and iterations were set to 200. The docked ligand-target complexes were analyzed carefully to identify the interactions and binding affinities. The docking score was

recorded and docking poses were saved for reference.

RESULTS AND DISCUSSION

Homology modeling and validation

The amino acid sequence of our target protein was retrieved from Uniprot and the physicochemical properties of the protein were studied. Multiple sequence alignment was carried out to identify the conserved regions of the target protein by aligning with the template structures of 1D3C and 2PQ5. The results of multiple sequence alignment are shown in [Figure 1]. The sequence identity and similarity of EPM2A sequences were 39% and 56%, respectively. Five models were generated and the model showing the least DOPE (Discrete Optimized Protein Energy) score (-2.6217.009766) of the crystal structure of the templates, PDB ID (1D3C and 2PQ5) was saved for further loop refinement and validation. Loop refinement of the 5 models was done after they are built and energy refinement method gives best conformation to the model.[24] After the Loop refinement the newly built model that is refined with all its conserved regions should be now refined at the loop regions. Five Loop refinement models were generated and the least DOPE score (-27583.332031) was selected for further study [Table 1 and Figure 2]. Model validation was carried out using SAVS server. The percentage of residues in the most favorable regions of Ramachandran plot was 79.5% and of those lying in disallowed region was 2.2% remaining values are as shown in the [Figures 4].

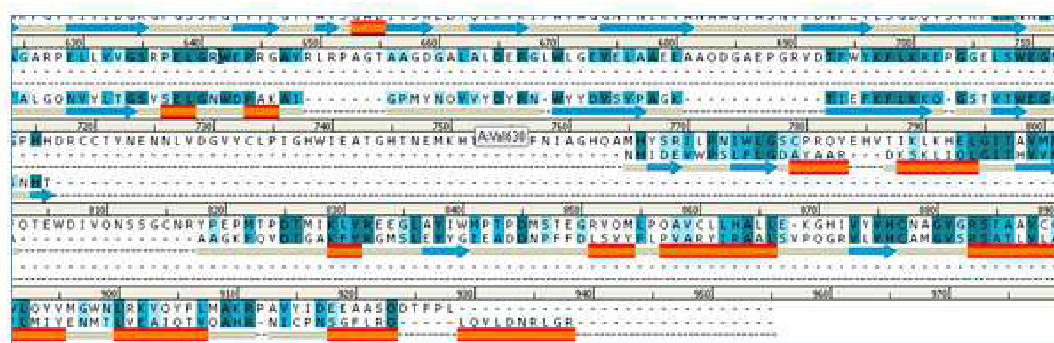

Figure 1
Multiple sequence alignment: the target (O95278) and template sequences aligned with Align2D in-built in Discovery Studio 3.5. Deep blue color shows conserved residue in all three sequences.

Table 1
Homology modeling result
DOPE (Discrete Optimized Protein Energy)
PDF (Probability Density Function Energy)

Loop Model Scores			
Name	**PDF Total Energy**	**PDF Physical Energy**	**DOPE Score**
EPM2A_HUMAN.M0009L0001	-4143.9341	-4549.781506	-27583.332031
EPM2A_HUMAN.M0010L0001	-3781.7864	-4160.005168	-26639.730469
EPM2A_HUMAN.M0004L0001	-3736.2175	-4218.40337	-26736.144531
EPM2A_HUMAN.M0007L0001	-3699.4883	-4256.056878	-27207.300781
EPM2A_HUMAN.M0006L0001	-3672.4077	-4198.00083	-26933.921875

Figure 2
Superimposition of modelled Protein (Blue color) over template ((1D3C, chain A) (Red), (2PQ5, Chain A) (Green)).

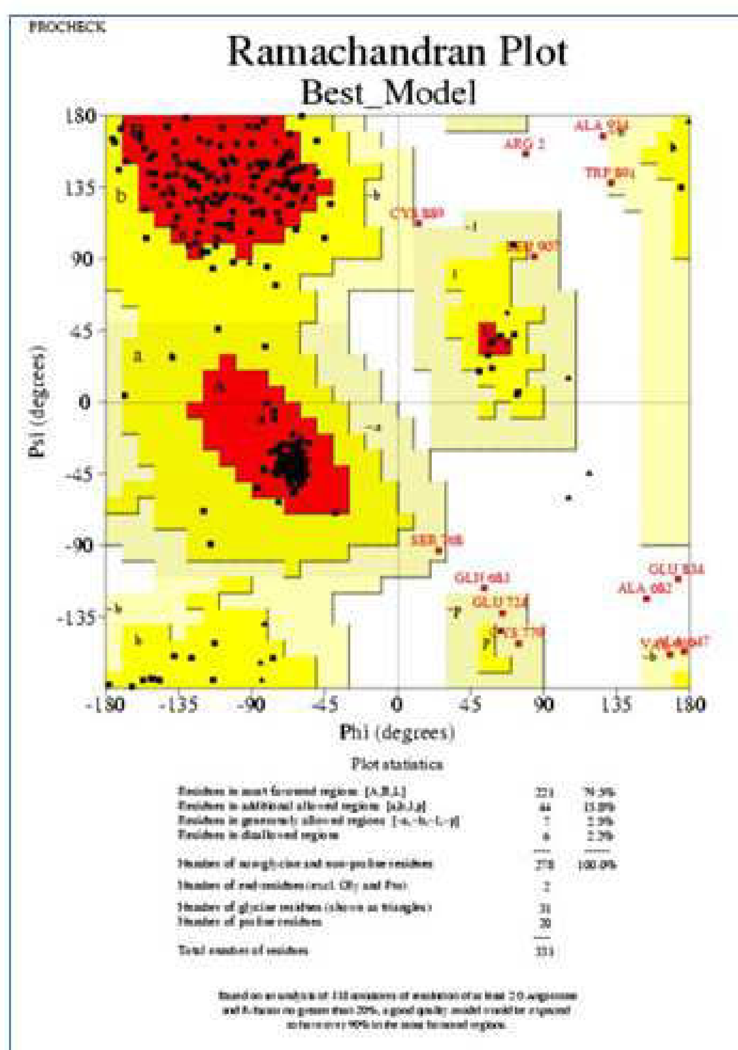

Figure 4
Ramachandran's Map of Modelled protein by PROCHECK program

This article can be downloaded from www.ijpbs.net

Protein simulation and Active site prediction

Modeled protein potential energy was analyzed before and after minimization by using calculate energy and minimization protocol respectively in DS 3.5 and it was found to be 19898.05374 Kcal/mol and -18280.05355 Kcal/mol respectively [Table 2 and Figure 3]. Based on the receptor cavity method we identified 13 active sites in the modeled structure. Based on the size of the volume, we selected the first active site for the further study and the details of the active site residues and the binding site are; the area of active site 479.5 volume, XYZ coordinates -38.834 A , 15.363 A and -1.933 A [Figure 5].

Table 2
Energy values of modelled protein before and after Minimization

Modelled Protein	Forcefield	Potential Energy (kcal/mol)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	RMS Gradient (kcal/(mol x A)
Before Minimization	CHARMm	19898.05374	25591.23409	-9453.47273	1377.99046
After minimization	CHARMm	-18280.05355	-1939.18195	-19427.83275	1377.99046

Figure 3
Modeled protein visualized using Discovery studio 3.5

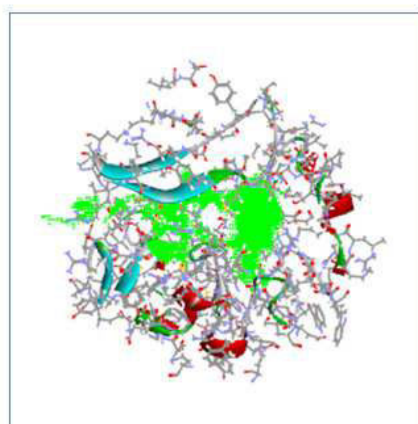

Figure 5
Active site of modeled protein by Discovery studio 3.5
Note: green color indicates binding area of the active site of modeled protein.

This article can be downloaded from www.ijpbs.net

Ligand retrieval and minimization
The 71 synthetic compounds having similar structure of Carbamazepen were retrieved from public databases such as Chemicalize.org. All the selected compounds passed the Lipinski rule of 5. The energy minimizations of the filtered ligands were performed using DS 3.5.

ADME toxicity
The ADME (Absorption, Distribution, Excretion, and Metabolism) properties of the compounds are depicted in [Table 3]. These results show that the Lead compounds (1-5) possess good pharmacokinetic properties and it satisfies all the parameters to be taken over as a good drug. The toxicity profile of the compounds is shown in [Table 4].

Table 3
Comparison of the ADME values of Ligands

S. No	Descriptor	A	B	C	D	E
1	ADME.2D.FPSA	85.842	81.794	60.222	77.522	85.842
2	A LOG P98	4.674	0.678	3.676	0.678	4.674
3	AQ SOI	- 4.976	-2.038	-4.809	-1.874	-4.987
4	AQ SOI LEV	2	3	2	4	2
5	BBB LEV	4	3	1	3	4
6	BBB LOG LEV	-1.239	-1.239	0.029	-1.171	-1.171
7	CYP 2D6	-9.01612	-5.72577	-5.63689	-7.74269	-7.38912
8	CYP PROB	0.000813728	0.0124292	9.94E-07	0.0155768	0.00488746
9	HEPATOX	-0.0630897	0.457976	-1.31879	0.963913	-0.846976
10	HEPATOX PROB	0.302913	0.0135334	0.000155419	0.227244	0.201531
11	PPB LEV	5.00833	0.411036	5.42727	5.09854	4.81132
12	PPB LOG	0.9958	0.584359	0.406982	0.696877	0.999316

Table 4
Toxicity analysis using TOPKAT.
Note: 0- Negative result, 1: Positive result
NTP (National Toxicology Program)

Compound	NTP Carcinogenicity Call (Male Mouse) (v3.2)	NTP Carcinogenicity Call (Female Mouse) (v3.2)	Developmental Toxicity Potential (DTP) (v3.1)	Skin Irritation (v6.1)	Ames Mutagenicity (v3.1)
A	0.000	0.824	0.000	0.015	0.000
B	0.000	0.298	0.000	0.868	0.000
C	0.000	1.000	0.000	0.994	1.000
D	0.000	0.001	0.000	1.000	0.000
F	0.000	0.034	0.000	0.681	0.000

Note: (A) 2_4_-bis (N_ - (4-methylphenyl) ureido) toluene,
(B) Carboxyphenyl ureido phenyl,
(C) N- 3-((2-methylphenyl)amino carbonyl amino)-2-naphthoyl,
(D) 1-Phenyl-3-(2-carboxy-phenyl)-urea
(F) 2_6_- bis (N - (4 – methylphenyl) ureido) toluene

Docking studies
Molecular docking studies were performed using Flex X. The results of interaction between modelled protein with the compounds *2_4_-bis (N_ - (4-methylphenyl) ureido) toluene, carboxyphenyl ureido phenyl, N- 3-((2-methylphenyl) amino carbonyl amino)-2-naphthoyl, 1-Phenyl-3-(2-carboxy-phenyl)-urea and 2_6_- bis (N - (4 – methylphenyl) ureido) toluene* are shown in Fig 5. The green dot lines denote the hydrogen (H) bonds. All the amino acid residues which involved in molecular interactions are displayed as lines and the ligands are displayed as ball and sticks. The

This article can be downloaded from www.ijpbs.net

observed results of the drug-receptor interaction for the compounds are tabulated in [Table 5 and Figure 6]. The results show that a good interaction occurs between the protein and the Ligand. *2_4_-bis (N_ - (4-methylphenyl) ureido) toluene* showed more binding capacity than the other compounds.

Table 5
Ligand-Protein interaction with docking scores

S.NO	COMPOUND NAME	LEAD-IT					
		LEAD-IT SCORE	H-BOND	AMINO ACID	AMINO ACID ATOM	LIGAND ATOM	H-BOND LENGTH
1	2_4_-bis(N_-(4-methylphenyl)ureido)toluene_001	-34.0762	6	ILE916	O_	O15	2.64973 A°
				ILE916	HN_	O15	1.57632 A°
				ASP917	OD1_	H35	1.62561 A°
				ASP917	OD1_	H36	1.56749 A°
				SER941	O_	H38	1.76863 A°
				SER941	O_	H39	1.8452 A°
2	carboxyphenyl_ureido_phenyl_001	-29.7048	9	ARG651	HH12	O18	2.00887 A°
				ARG651	HH22	O19	2.47099 A°
				GLU666	O_	O3	1.95203 A°
				PRO688	O_	O19	1.9905 A°
				GLY689	O_	O19	2.63131 A°
				ARG690	HH21	O3	2.00557 A°
				TYR722	HH_	O19	2.13808 A°
				THR721	O_	H20	2.18412 A°
				THR721	OG1_	H21	2.24136 A°
3	N-_3-(__(2-methylphenyl)amino_carbonyl_amino)-2-naphthoyl_001	-29.6006	4	ARG641	HE_	O19	2.11479 A°
				ARG641	HH21	O19	2.1392 A°
				LEU639	O_	H27	2.00137 A°
				LEU639	O_	H28	2.15647 A°
4	1-Phenyl-3-(2-carboxy-phenyl)-urea_001	-29.2388	10	ILE916	O_	O1	2.67937 A°
				ILE916	O_	O3	2.89673 A°
				ILE916	O_	O8	2.63957 A°
				ILE916	HN_	O8	1.63917 A°
				GLU918	HN_	O1	2.15193 A°
				GLN930	OE1_	O1	3.19196 A°
				GLN930	HE22	O1	1.89077 A°
				GLN930	HE22	O3	1.88293 A°
				SER941	O_	H20	1.49525 A°
				SER941	O_	H21	1.81082 A°
5	2_6_-bis(N_-(4-methylphenyl)ureido)toluene_001	-27.5299	5	ALA913	HN_	O8	1.97206 A°
				ILE916	O_	O17	2.85611 A°
				ILE916	HN_	O17	1.53192 A°
				SER941	O_	H40	1.78725 A°
				SER941	O_	H41	1.65403 A°

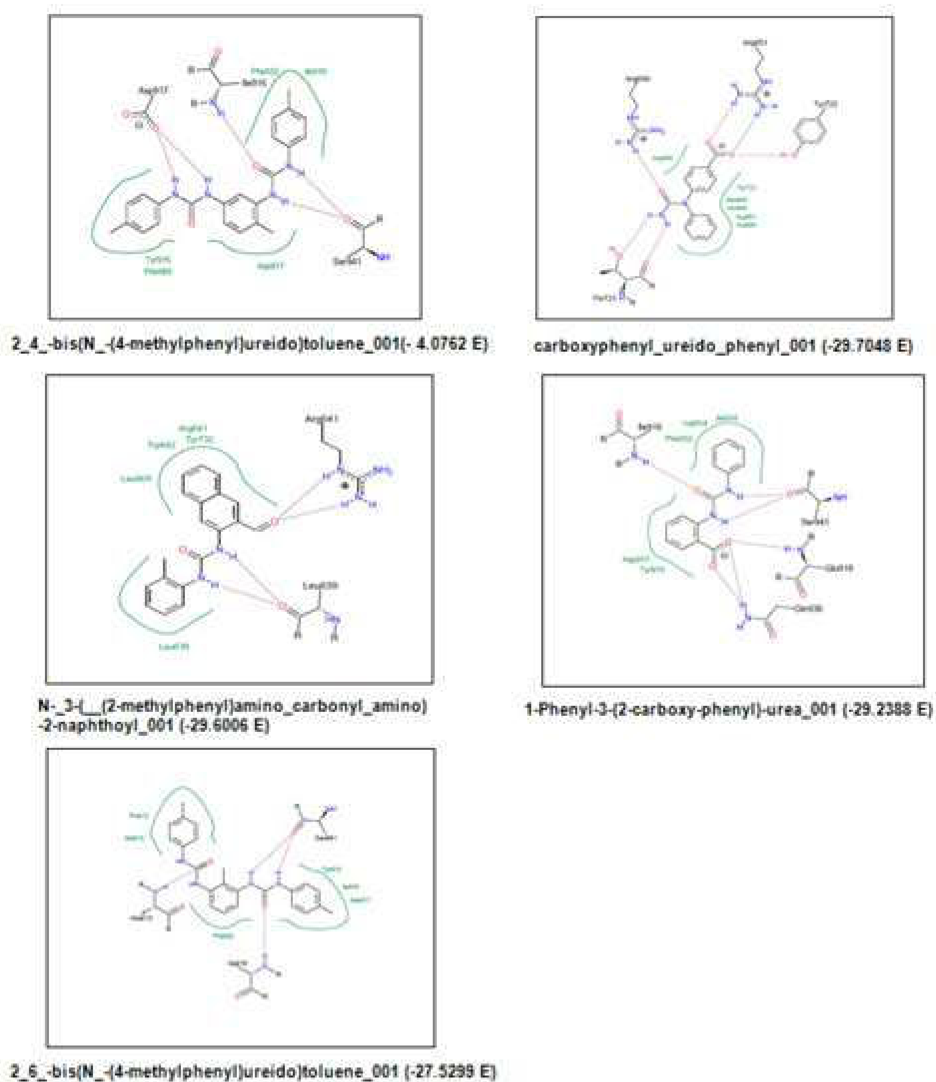

Figure 6
The docking poses of the top 5 candidates for Lafora disease

CONCLUSION

Computer aided drug designing and molecular docking analysis is one of the highly effective methodologies in creating and analyzing new candidate drug molecules. The EPM2A model has been built using Discovery Studio 3.5 and validated for stereo chemical and amino acid environment quality using appropriate programs. The active site architecture has been analyzed by docking studies with compounds having similar structure of carbamazepen and the key amino acid residues involved in ligand binding have been determined. Total 71 compounds from public databases have been screened by docking study of EPM2A. Further toxicity analysis was performed to confirm the carcinogenicity, toxicity and skin irritation using mouse models. After proceeding concisely 5 compounds, *2_4_-bis (N_ - (4-methylphenyl) ureido) toluene, carboxyphenyl ureido phenyl, N- 3-((2-methylphenyl) amino carbonyl amino)-2-naphthoyl, 1-Phenyl-3-(2-carboxy-phenyl)-urea and 2_6_- bis (N - (4 – methylphenyl) ureido) toluene* were selected as potent target

candidate drugs for Lafora disease. Out of the top five compounds selected *2_4_-bis (N_ - (4-methylphenyl) ureido) toluene* is not having any adverse propensities such as carcinogenicity, toxicity, mutageneicity and skin irritation. The LEAD-IT score of first compound is significantly promising compared to the rest of the four compounds, which indicates the potent drugable property of the lead molecule. Using a combination of homology modeling, virtual screening, and molecular docking, we successfully identified putative novel EPM2A activators, which can be further evaluated by *in vitro* and *in vivo* biological tests.

ACKNOWLEDGEMENT

The authors acknowledge Mr. Vivek Chandramohan, Assistant Professor, Department of Biotechnology, Siddaganga Institute of Technology, Tumkur, for providing software support for Bioinformatics Research.

REFERENCES

1. Chan GJ, Zhao EM, Lohi XC, Scherer H, Minassian SW and Lafora BA. Progressive Myoclonus Epilepsy mutation database-EPM2A and NHLRC1 (EPM2B) genes. Human mutation, 26 (4): 397, (2005).
2. Machovic M., Svensson B, MacGregor EA and Janecek S. A new clan of CBM families based on bioinformatics of starch-binding domains from families CBM20 and CBM21. The FEBS journal, 272 (21): 5497-513, (2005) .
3. Blair MA, Abou-KhalAhmed SN and Spencer SS. An approach to the evaluation of a patient for seizures and epilepsy. WMJ : official publication of the State Medical Society of Wisconsin, 103 (1): 49-55 (2004).
4. Shahwan A, Farrell M and Delanty N. Progressive myoclonic epilepsies: a review of genetic and therapeutic aspects. Lancet neurology, 4 (4): 239-48 (2004).
5. Wolf NI, Bast T and Surtees R. Epilepsy in inborn errors of metabolism. Epileptic disorders : international epilepsy journal with videotape, 7 (2): 67-81 (2005).
6. Ianzano L, Zhanil B, Crunk A, Haines JL and Hedera P. A new locus for autosomal dominant generalized epilepsy associated with mild mental retardation on chromosome 3p. Epilepsia, 52 (5), 993-9 (2011).
7. Serratosa JM, Gomez-Garre P, Gallardo ME, Anta B, De Bernabe DB, Lindhout D, Augustijn PB, Tassinari CA, Malafosse RM, Topcu M, Grid D, Dravet C, Berkovic SF and de Cordoba SR. A novel protein tyrosine phosphatase gene is mutated in progressive myoclonus epilepsy of the Lafora type (EPM2). Human molecular genetics, 8 (2): 345-52 (1999).
8. Solaz-Fuster MC, Gimeno-Alcaniz JV, Ros S, Fernandez-Sanchez ME, Garcia-Fojeda B, Criado Garcia O, Vilchez D, Dominguez J, Garcia-Rocha M, Sanchez-Piris M, Aguado C, Knecht E, Serratosa J, Guinovart JJ, Sanz P and Rodriguez de Cordoba, S., Regulation of glycogen synthesis by the laforin-malin complex is modulated by the AMP-activated protein kinase pathway. Human molecular genetics, 17 (5): 667-78 (2008).
9. Ganesh S, Puri R, Singh S, Mittal S and Dubey D. Recent advances in the molecular basis of Lafora's progressive myoclonus epilepsy. Journal of human genetics, 51 (1): 1-8 (2006).
10. Anis S, Rizwan K, Jatin J and Tanu B. Formulation Parameters Characterization & Comparative Study Of Carbamazepine Tablets, International Journal of Pharma and Biosciences, 3(3): 32-43 (2012).
11. Berman HM, Westbrook J, Feng Z, Gilliland G., Bhat TN, Weissig H, Shindyalov I.N and Bourne PE, The

Protein Data Bank, Nucleic Acids Res, 28:235–242 (2000).
12. Wolf Y, Grishin N and Koonin E. Estimating the number of protein folds and families from complete genome data. Journal of Molecular Biology, 299: 897–905 (2009).
13. Smith SW, Overbeek R, Woese CR, Gilbert W and Gillevet PM. The Genetic data environment, an expandable GUI for multiple sequence analysis, Computer Applications in the Biosciences, 10: 671-675, (1994).
14. Browne WJ, North ACT, Phillips DC, Brew K, Vanaman TC and Hill RC, A possible three- dimensional structure of bovine-lactalbumin based on that of hen's egg-white lysozyme, Journal of Molecular Biology, 42: 65-86 (1969).
15. Laskowski RA, MacArthur MW, Moss DS, and Thornton JM, PROCHECK: a program to check the stereochemical quality of protein structures, Journal of Applied Crystals. 26: 283–291. (1993).
16. Fletcher R, Reeves CM, Function Minimization by Conjugate Gradients, Computer Journal, 7: 149- 154 (1964).
17. Venkatachalam CM, Jiang X, Oldfield T, Waldman M , Ligand Fit: a novel method for the shape-directed rapid docking of ligands to protein active sites, J. Mol. Graph. Model, 21, 289- 307 (2003)
18. Lipinski CA, Franco I, Dominy BW and Feeney PJ, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advance Drug Delivery Review, 23: 3–25 (1997).
19. Mayo SL, Olafson BD and Goddard WA. DREIDING: A Generic Force Field for Molecular Simulations. Journal of Physical Chemistry, 94: 8897-8909, (1990).
20. Egan WJ and Lauri G, Prediction of intestinal permeability. Advance Drug Delivery Review, 54: 273, (2002).
21. Gregory M and Bankil, *Insilico* ADME-TOX prediction: The more, the merrier, Current Drug Discovery. (2004).
22. Xia X, Maliski EG, Gallant P and Rogers D, Classification of kinase inhibitors using a Bayesian model, Journal of Medicinal Chemistry, 47: 4463-4470, (2004).
23. Rarey M, Kramer B, Lengauer T and Klebe G. A Fast Flexible Docking Method using an Incremental construct algorithm Journal of Molecular Biology, 261(3):470-489,(1996)
24. Fiser A and Sali. Mod. Loop: automated modeling of loops in protein structures, Bioinformatics, 19(18):2500-1 (2003).

This article can be downloaded from www.ijpbs.net