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3D- Comparative Modelling, Structure Comparison & Docking Studies for AVPR2 Protein with Some Anti-Diabetic Plant Compounds from North East India

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ABSTRACT: Herbal application of different plant parts has long been used as anti-diabetic agent by rural people since time immemorial in North East India. This unexplored paradise of India is geographically rich in medicinal plants diversity, where more than two hundred plant species are documented for their potential use in diabetic treatment by various ethnic and aboriginal communities. Presently thousand of small drug-like compounds have been elucidated from those reported plant species by many researchers. It has been revealed that Arginine Vessopressin Protein Receptor 2 (AVPR2) plays an important role in water homeostasis normally but certain mutation of AVRP2 leads to Nephrogenic Diabetes Insipidus (NDI) in human. However, the experimental 3D structure of this protein has not yet been established. Herein, an attempt has been made to predict an ideal homology based 3D model of this protein and docking studies against some of anti-diabetic compounds from those plants. The best 3D model was found with highest sequence identity and least RMSD of 0.1414. The Molecular Docking and ADME results suggest two potential agents as mutant AVPR2 protein inhibitor for future NDI therapeutics.

KEYWORDS: Anti-diabetic; AVPR2; Diabetes Insipidus; Homology Model; Docking; ADME; Inhibitor.

INTRODUCTION

The Nephrogenic Diabetes Insipidus (NDI), a kidney disorder, which can be either inherited or acquired, is characterized by the excretion of large volumes of dilute urine and resistance to the antidiuretic action of vasopressin. It was suggested that there are more than 180 types of mutations of X-linked recessive manner in Arginine Vasopressin Receptor 2 (AVPR2) protein responsible to Nephrogenic Diabetes Insipidus. The Arginine Vessopressin hormone (AVP) binds to AVPR2 on the basolateral plasma membrane of renal collecting duct especially principal cells, thereby inducing apical plasma membrane insertion of aquaporin-2 leading to increased water uptake and urine concentration [1-3].

It is necessary to understand the structural aspects of the receptor protein AVPR2 in order to know functions and to raise broad-spectrum diuretic inhibitors. It was found that though there are lots of studies done for the protein AVPR2 about its molecular and genetical character, the experimental 3D structure of the protein is still lacking [4]. Herein, we try to generate some theoretical models of the protein and correspondingly to acquire the best model of the protein by a comparative study among different models, on basis of their energy value, quality factor, RamaChandan plot [5] analysis and RMSD[6] calculation.

The literature studies reflected that, the treatment of the nephrogenic diabetes insipidus form represents a much larger challenge as no single treatment module has been shown to be effective and no randomised controlled trials are reported [7]. Hence it becomes very necessary to find some potential inhibitors against this NDI. In this current study, we have adopted a motivative approach to find out a potential anti-diabetic plant compounds from diverse group of medicinal plants traditionally used in North-East part of India. The North East India is one of the major biodiversity hot-spot within Indo-Burma mega biodiversity hot zone endowed with more than 7000 endemic plants species areas where lots of traditional importance medicinal plants [8]. In this communication we reported details of the target protein modelling. We have screened plant compounds reported from biodiversity rich North East India and docked them with our predicted protein model.

METHODOLOGY

3D Model Prediction and Structure Comparison

The amino acid sequence of human AVPR2 was obtained from NCBI, protein database having UniProtKB accession no P30518.1. Next the template structure protein for the Avpr2 protein sequence was detected using PDB-BLAST. Thereafter 3D models were generated using MODELER 9v8 [9], SWISS MODEL [10], M4T [11], I-TASSER [12] and LOMET [13]. A python script based method, MODELER 9v8, could generate a number of models and the best model was chosen based on the DOPE score [14] and GA341 score [15].

Consequently, structure evaluation and validation was performed for all the generated protein models based on model quality, RamaChandan Plot[5], energy scores using ERRAT-2[16], Q-Mean [17], ProCheck [18], Anolea [19], dDFIRE [20] and RAMPAGE [5] online Servers. The superimposition step was also performed with the Molsoft-ICM Browser [21] software to calculate RMSD [6] value between protein structures of model proteins to their related template protein.

Dataset preparation of traditional anti-diuretic plant compounds

The dataset of plant compounds was prepared by extensive literature review on anti-diabetic plants, specifically used in North-East India [22-26]. A list of fifteen anti-diabetic plants was prepared and eighty four reported compounds were chosen for computational analysis. Some of the available 3D structures were collected from PubChem (http://pubchem.ncbi.nlm.nih.gov) database, and others were sketched in ChemBioDraw Ultra 12.0 [27]. All those structures were cleaned-up and checked for any valence error. The drawn 2D structures were subsequently converted into 3D representation and calculated their minimized energy considering MM2 force field using ChemBio3D Ultra 12.0 and structures were saved in .mol format for subsequent docking purpose. The present published AVPR2 inhibitors namely Vasopressin and Desmopressin (http://www.drugbank.ca) were also retrieved from PubChem for Comparative studies.

Drug-likeness prediction

The prediction of drug likeness properties of these optimized compounds was performed using Mol-Soft ICM Browser [21] and ChemBioOffice [27]. The predicted properties are numbers of H-bond donor, acceptor, c*logP*, molecular weight and numbers of rotatable bonds. Then the PASS (Prediction of Activity Spectra for Substance) software [28] was used to predict the drug-likeness and toxicity properties.

Molecular dynamics studies

The molecular dynamic simulation was carried out for the model protein with and without the ligand complexes. For calculation of RMSD (Root mean square deviation) fluctuation and Potential Energy value of the model protein GROMACS 4.5.3[29] (Groingen Machine for Chemical Simulation) tool was used. GROMACS is a versatile package to perform molecular dynamics that simulates the Newtonian equations of motion for systems with hundreds to millions of particles. The model was subjected to molecular dynamics simulation in water surface for 20ns by using Gromos96 43a2 [30] force field and the flexible SPC water model. The initial structure was immersed in a periodic water box of cubic shape (0.9 nm thick).The system was neutralized with six CL-counterion. The solvated system was then subjected to further energy minimization (maximum number of steps: 1000) to remove steric conflicts between the protein and water molecules, using the steepest descent integrator. Convergence was achieved in the energy minimization when the maximum force was smaller than 1000 kJ mol−1 nm−1. The energy-minimized model was subjected to position-restrained MD under NPT conditions, keeping the number of particles (N), the system pressure (P) and the temperature (T) constant. This was carried out for 50,000 steps for a total of 100 ps. LINCS [31] constraints were performed for all bonds, keeping the whole protein molecule fixed and allowing only the water molecule to move to equilibrate with respect to the protein structure. The reference temperature for coupling (via v-rescale temperature coupling) was 300 K, and a pressure of 1 atm was maintained by the Parrinello–Rahman algorithm. The final MD calculations steps were carried out for 20ns using the particle mesh Ewald (PME) electrostatics method under NPT conditions. The results were analyzed using the standard software provided by the GROMACS package. After completion of the docking the simulation was again carried out for the dock complexes with 20 ns. Additional geometrical quantities that are routinely calculated from an MD simulation trajectory include the root mean square difference (RMSD) between two structures and RMS fluctuations (RMSF). The time trajectory of RMSD shows how a protein structure deviates from a reference structure as a function of time, while the time-averaged RMSF indicates the flexibility of different regions of a protein, which is related to the crystallographic B-factors.

Molecular Docking Studies

Docking was carried out to find the best binding interaction (hydrogen bonds and hydrophobic interactions) for each plant compounds at active site of modelled AVPR2 protein. MVD-Molegro Virtual Docker (2010.4.0.2) [32] based on new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm used to dock those plant compounds against the receptor model. The MVD has special features for detecting the cavities in the sense of active sites of the model protein. On MVD workspace the cavity of volume 12.288 $Å^3$ and surface of 52.48 $Å^2$ was detected and docking was performed with each of the plant compounds. On the basis of Re-rank score and Mol-Dock [32] score best poses were taken to view and annotate their H-bond interaction.

ADME prediction

ADME (Absorption, Distribution, Metabolism, Excretion) prediction was performed using online sever based program Pre-ADMET [33]. Pre ADME like properties HIA (Human Intestinal Absorption), MDCK (Madin Darby Canine Kidney) cell permeability, CACO-2 cell permeability, Skin permeability, Blood Brain Barrier permeability and Plasma Protein Binding values were calculated to find out the best possible NDI inhibitor agent.

RESULTS & DISCUSSION

Protein Model Generation and Verification

In this study, we have generated five protein models for finding the best one on the basis of model quality, Ramachandran Plot [5] and energy scores. The MODLER 9v8 generated four models (Table 1), where best model having Molpdf score: 1833.22449, DOPE score: -41481.84766 and GA341 score: 0.07279. Thereafter all the models, resulting from each method were selected for model assessment and validation. The model assessment was done by using the online servers as QMEAN, ERRAT-2 version, PROCHEK, RAMPAGE, DDFIRE and PSVS. These server results revealed the model quality scores, energy values, Ramachandran plot especially for allowed and disallowed regions (Table 2).

Table 1: Summary table of models generated in MODELER 9v8

Modelled protein	Molpdf score	DOPE score	GA341 score
Avpr2.B99990001.pdb	1970.27588	-41043.54297	0.06583
Avpr2.B99990002.pdb	2073.59741	-40758.98438	0.08837
Avpr2.B99990003.pdb	1833.22449	-41481.84766	0.07279
Avpr2.B99990004.pdb	2062.28931	-41173.35938	0.12458

Table 2: Result showing the comparison of 3D models

Models by program	Template	Seq. Identity (%)	E-Value	QMEAN score	ERRAT Quality	dDFIRE Energy value	RMSD	Rampage result		
								FR	AR	DR
Swiss- Model (automated)	2J4XA	91	7E-11	0.318	81.597	-59.55	0.1414	84.1	13.6	2.3
MODELER-9v8	1U19A	19	0.0	0.322	69.318	-807.03	4.3035	91.3	5.7	3
M4T	2J4XA	91	7E-11	0.34	90.00	-62.55	0.6251	97.8	0	2.2
I-TASSER Model (automated)	2KS9A	21	3e-04	0.323	44.90	-815.53	4.0407	86.2	8.1	5.7
LOMET Model (automated)	2KS9A	21	3e-04	0.31	54.749	-808.06	9.6311	93.8	3.8	2.4

NB: M4T =Multiple Mapping Method with Multiple Templates, F.R= Favoured Region, A.R= Allotted Region, D.R= Disallowed Region

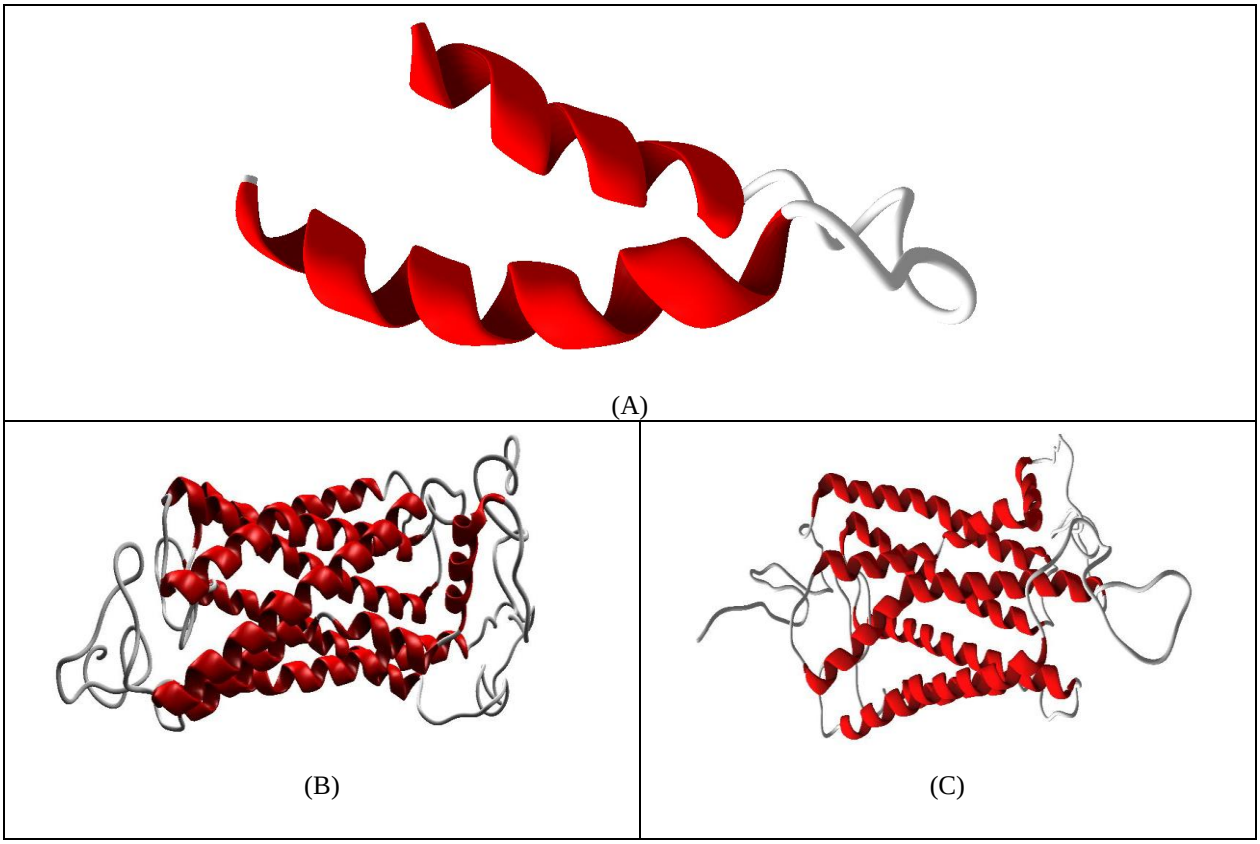


Fig 1-A: Model generated by using template 2XJ4A (SWISS-MODEL), B: Model generated by using template 1U19A (MODELER9v8), C: Model generated using template 2KS9A (I-TASSER)

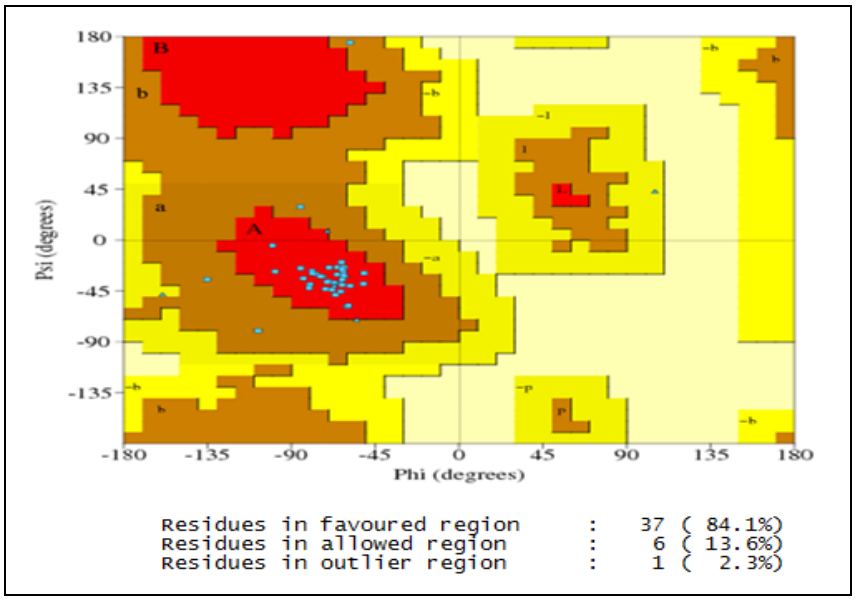


Fig 2: Ramachandran plot result of Swiss Model based protein model

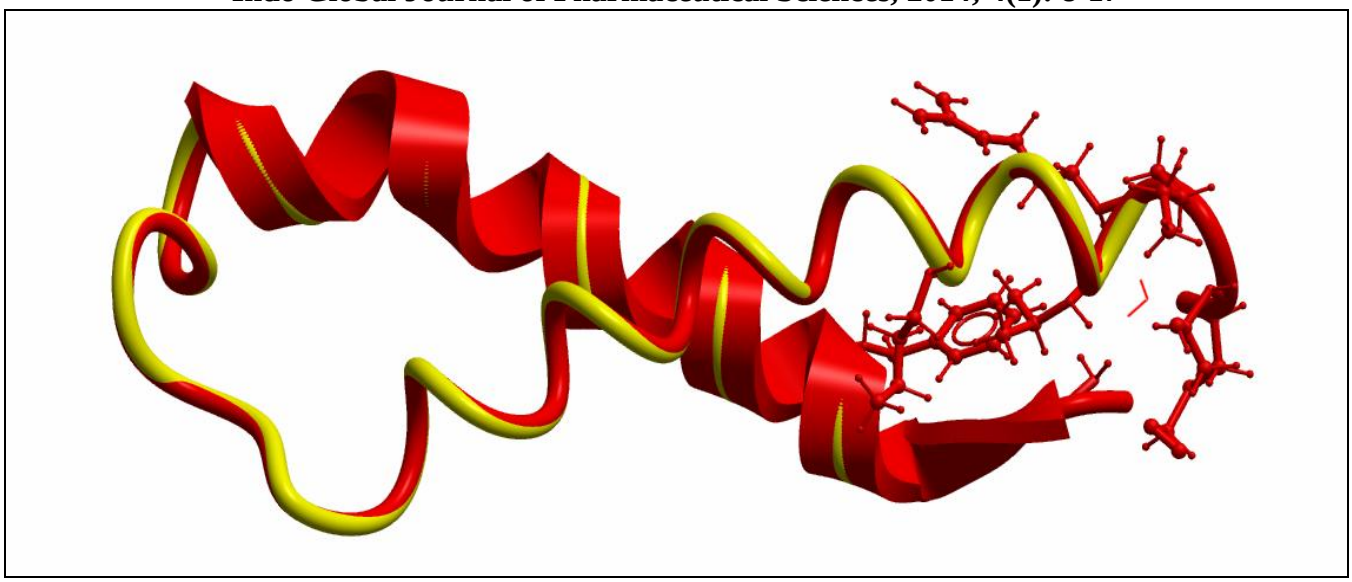


Fig 3: Superimposed structure of 2JX4 (Template) and model protein at RMSD= 0.1414

Structure comparison and model selection

The generated protein 3D model structures (Fig 1) were compared on the basis of their quality factor, energy value, allowed and disallowed regions in Ramachandran plot (Fig 2) and value of Root Mean Square Deviation (RMSD). The RMSD calculates the distance between the Cα atoms of the two protein backbone chain. For this purpose we performed the superimposition of protein structures against their template protein structure (Fig 3) by using the Mol Soft ICM Browser software.

Structure comparison gave us the best model which was generated by the Swiss-Model based protein model having greatest sequence identity, good quality and the lowest RMSD value of 0.1414 (Table 2). Finally this model was selected for further studies.

Table 2: Result showing the comparison of 3D models

Compound retrieval and property prediction

The anti-diabetic plant compounds were retrieved from NCBI PubChem database and some compounds were drawn by using drawing softwares. A total of eighty four compounds were listed from fifteen different anti-diabetic plants (Table 3) from North East India. The retrieved compounds were kept in Structured Data File format and used for further analysis of drug likeness and property calculation.

Table 3: Plants selected for studies

Sl No	PLANTS	LOCAL NAME	FAMILY	COMPOUNDS REPORTED
1	*Adhatoda vasica Nees[22]*	Bogabahok	Acanthaceae	2
2	*Aegle marmelos correa[25,34]*	Bel	Rutaceae	19
3	*Albizzia procera Benth[22,23]*	Koroi	Mimosaceae	1
4	*Andrograhis paniculata Nees [22,25]*	Kalmegh	Acanthaceae	18
5	*Centella asiatica Linn.[25]*	Bormanimuni	Apiaceae	3
6	*Citrus aurantifolia Linn[22,24,26]*	Nemu	Rutaceae	15
7	*Euphorbia hirta Linn.[22,26]*	Gakhirotibon	Euphorbiaceae	2
8	*Ficus racemosa[36]*	Dimoru	Moraceae	5
9	*Ipomoea aquatic Forsk[22,25,26]*	Kolmou	Convolvulaceae	1
10	*Mangifera indica Linn[22,25]*	Aam	Anacardiaceae	3
11	*Mimosa pudica Linn.[22,24]*	Nilazibon	Mimosaceae	1
12	*Moringa oleifera Linn.[22,26]*	Sogina	Moringaceae	4
13	*Oxalis corniculata Linn.[23,26]*	Tangasi	Oxalidaceae	1
14	*Phyllanthus niruri Linn.[22,24]*	Bomamlokhi	Euphorbiaceae	3
15	*Solanum tuberosum Linn.[25,26]*	Alu	Solanaceae	6

Docking Results

In docking procedure cavities were detected first in MVD. In our study MVD provided only one cavity for model AVPR2 having volume 12.288 Å and surface value 52.48 Å. After completion of docking procedure different energy values and H-bond interaction (Fig 4) has been observed. Out of 84 compounds 7 compounds exhibited better binding affinity to model AVPR2 as compared with standard (Table 4).

ADME Result

The selected compounds were subjected for ADME (Absorption, Distribution, Metabolism, and Excretion) prediction by Pre-ADMET. The ADME results (Table 5) show that out of all the seven compounds two compounds Marmesiline and Caffeic acid have greater absorption, distribution, permeability etc.

Table 4: Docking results of some selected compounds

PLANT NAME	Compound Name or ID	MolDock Score	Rerank Score	H Bond Score	H bond Interaction
Aegle marmelos correa	Marmesiline [37]	-129.040	-98.306	-10.335	7
Centella asiatica Linn.	Asiaticoside [38]	-95.494	-50.234	-8.302	7
	α-hederin [39]	-79.436	-23.491	-9.449	7
Andrograhis paniculata Nees	caffecic acid [40]	-95.981	-44.430	-5.595	7
	Andrographidin [41]	-65.695	-49.993	-2.609	6
	Neoadrenographolide [42]	-80.867	-47.714	-8.965	7
Mimosa pudica	Turgorin[42]	-108.620	-90.849	-5.595	10

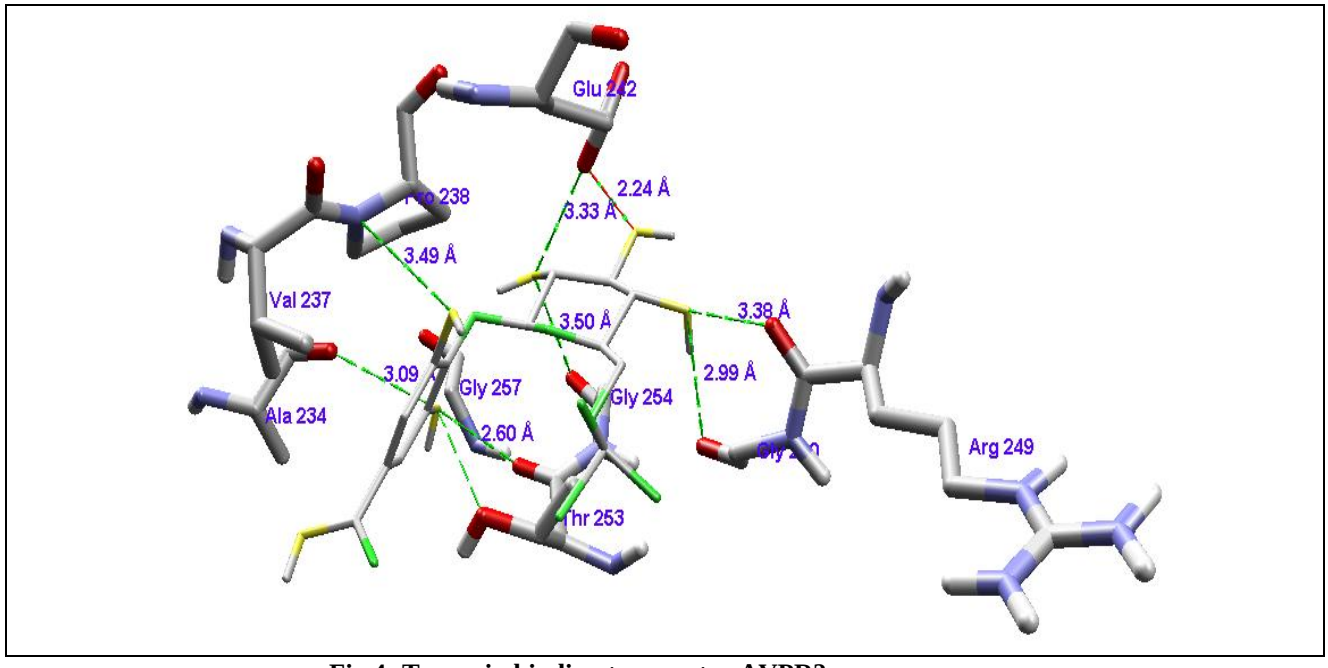


Fig 4: Turgorin binding to receptor AVPR2

Table 5: ADME results of selected compounds

COMPOUNDS	HIA	Caco2	MDCK	PPB	BBB
Marmesiline	92.212	17.345	164.668	89.003	0.996
α-hederin	59.912	20.672	0.043	87.677	0.091
Asiaticoside	40.044	19.776	0.043	41.017	0.029
neoadrenographolide	81.330	18.118	0.245	93.373	0.125
Andrographidin A	77.284	5.819	0.416	62.807	0.025
Caffeic acid	82.301	21.107	109.433	40.29	0.497
Turgorin	75.967	10.232	4.034	38.229	0.277

NB: HIA=Human Intestinal Absorption, Caco2= Human Colon Adenocarcinoma cells, MDCK= Madin-Darby Canine Kidney cell, PPB=Plasma Protein Binding, BBB=Blood Brain Barier penetration

Molecular Dynamics Simulation analysis

The GROMOS96 43a1 force field of the GROMACS 4.0 package is used for the molecular dynamics simulation of the protein AVPR2 with or without ligand complexes. Firstly the generated protein model of AVPR2 is simulated at 20ns where different snapshots were taken for RMSD, RMFS plots along with the energy values. After completion both energy and root mean square deviation plots were derived from the respective trajectory file by XMGRACE [34]. The RMSD fluctuation plot shows the C-alpha backbone deviation during the simulation process at 300K temperature and 5ns, which is within the range of 0.15-0.5 nanometre. This shows an increase in RMSD and the plot reaches equilibrium and oscillates around an average value after 8000 ps simulation time and almost remains equilibrium up to 20000 ps (Fig: 5). These values in the RMSD plot confirms the stability of the model protein and the whole system in equilibrium. The Avpr2-Marmesiline complex is more stable than the AVPR2-Caffeic acid ligand complex (Fig: 6).

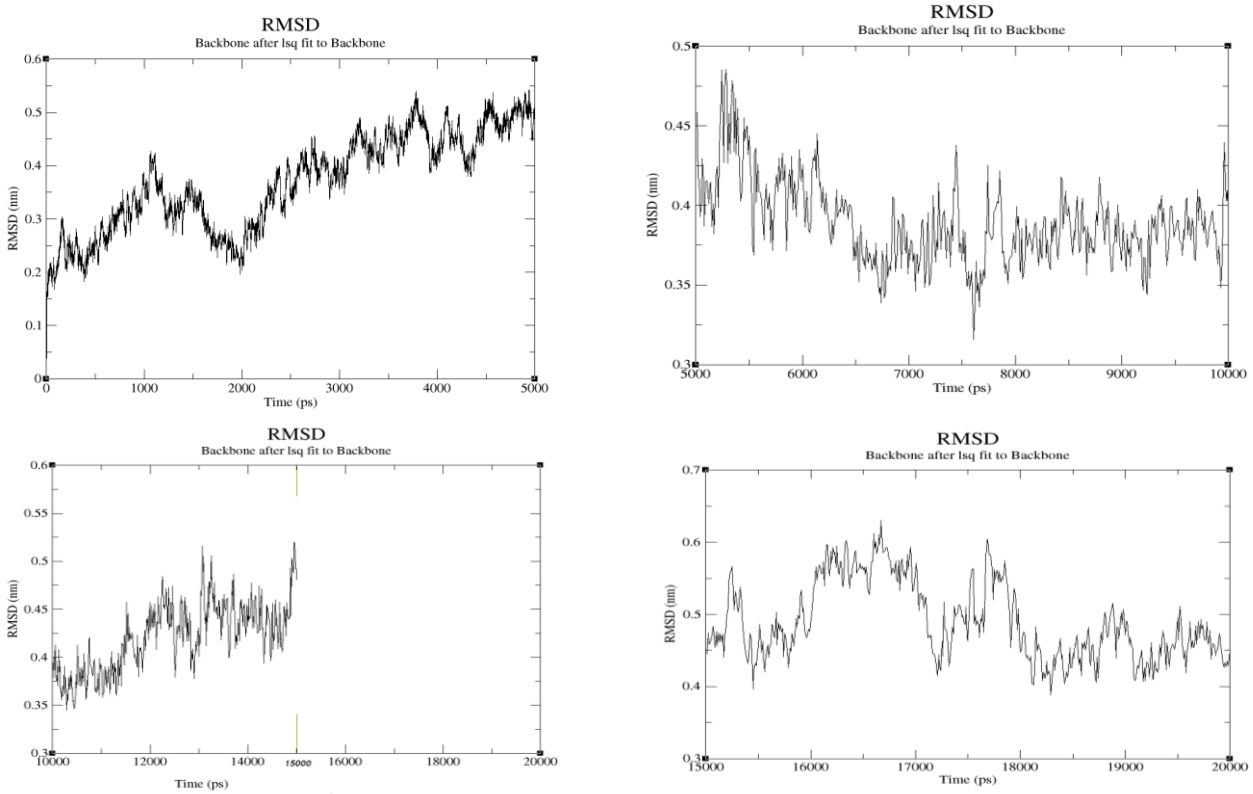
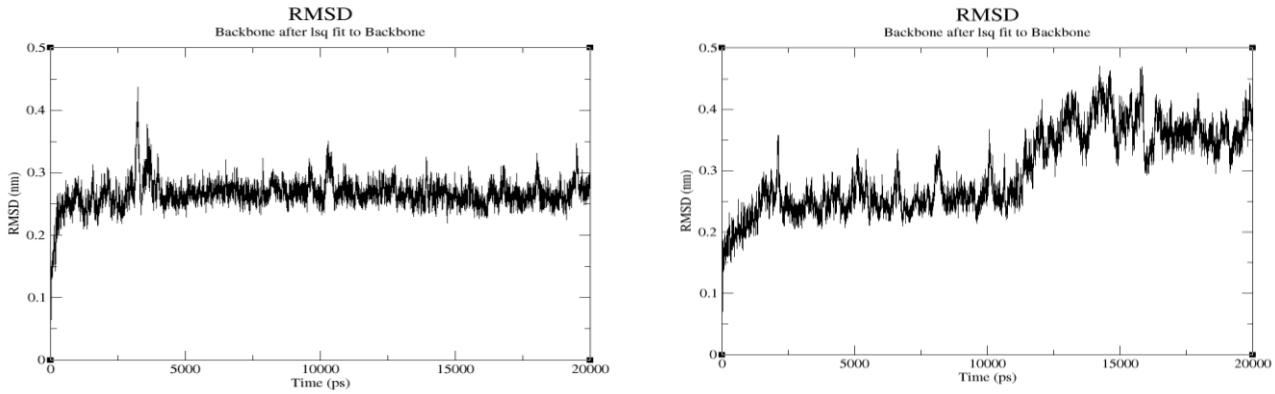


Figure 5: RMSD plot of AVPR2 protein at 0-20 ns MD simulation



(a) **(b)**

Figure 6: Root mean square deviation (RMSD) for (a) AVPR2-Marmesiline and (b) AVPR2-Caffeic acid complex during 20ns molecular dynamics (MD) simulation.

Local protein mobility was analyzed by calculating the time-averaged root mean square fluctuation (RMSF) values of the protein and with it complexes, which were plotted against residue numbers. RMSF indicates the flexibility of different regions of a protein, which can be related to crystallographic structure. In our study it was found that the model protein has very less fluctuation (Fig: 7) than the AVPR2-ligand complexes. On the other hand the AVPR2-Marmasilin dock complex (Fig: 8) is showing more flexibility. During the simulation process the protein did not show any significant change in its secondary structural topology. However the Val1 deformed to generate coil in the simulated protein unlike the helix in its non simulated protein structure.

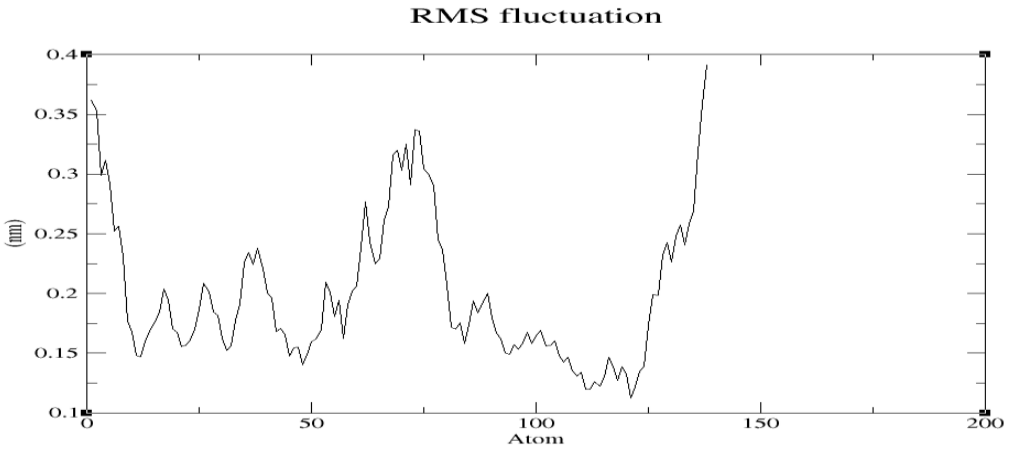


Figure 7: Root mean square fluctuations (RMSF) AVPR2 protein during 20 ns molecular dynamics (MD) simulation

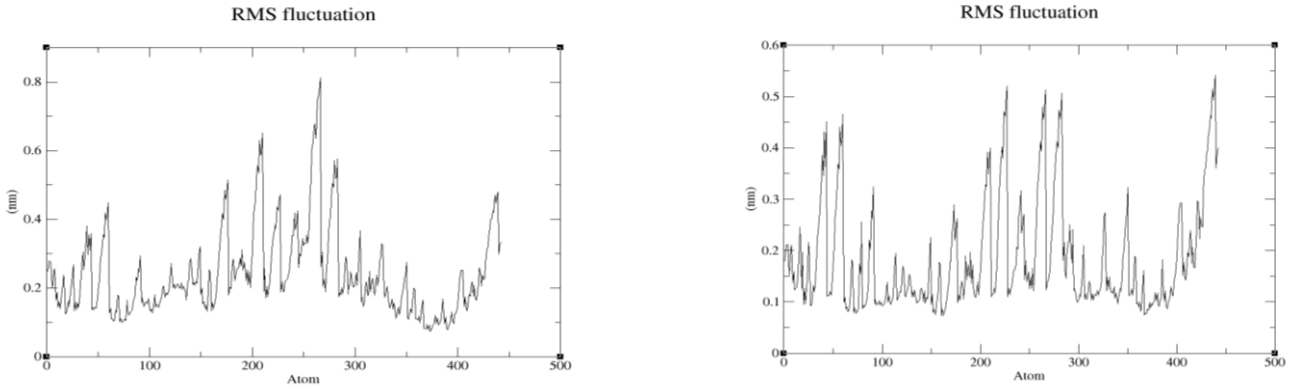


Figure 8: RMSF plot of (a) AVPR2-Caffeic acid and (b) AVPR2-Marmesiline complex during 20 ns MD simulation

CONCLUSION

In this study, we have employed advance molecular docking algorithm to identify potential drug-like compound for treating Nephrogenic Diabetes Insipidus (NDI). *In-silico* findings indicated two compounds namely Marmesiline and Caffeic acid which could be promising anti-diabetic insipidus agents having greater binding affinities to the receptor protein in comparison with the present NDI drug available in literature. These two small compounds were reported from *Aegle marmelos correa* and *Andrograhis paniculata Nees* accordingly. Hence, this study has widened the scope of developing these 2 compounds as future NDI therapeutics.

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