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In-silico study of arylalkylamine-n-acetyltransferase enzyme to regulate circadian rhythmicity

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Abstract:
Circadian Rhythmicity is present in the sleeping and breeding patterns of animals, including human beings and also related with brain wave activity, hormone production, cell regeneration and other biological activities. Melatonin is thought to play important roles in regulating circadian rhytmicity of the animals. Arylalkylamine-N-acetyltransferase (AANAT) is an enzyme which is responsible for the melatonin metabolism. In this study AANAT enzyme is targeted for the control of sleeping sickness and other irregular circadian rhythmicity by regulating the melatonin formation. AANAT protein 3D-structure was modeled, followed by loop modeling, refinement through energy minimization processes by molecular dynamics simulation and validation. Analysis of the Ramachandran plot shows 90.9% amino acids falls in the allowed region. The modeled protein was docked with N-Acetyl Serotonin. Combinatorial library was generated by using N-Acetyl Serotonin as a reference molecule and molecules having 80% similarity to N-Acetyl Serotonin was selected from Zinc database. These molecules were virtually screened by MOLEGRO virtual docker and top 5 molecules were selected and docked by using AutoDock**.** The AutoDock result shows that the ZINC01587152 molecule is having best interactions with the receptor protein. On the basis of this study we can suggest that the ZINC01587152 molecule is the best ligand against AANAT enzyme. It may be further synthesized and tested for sleep related disorders.

Keywords: Melatonin, AANAT, N-Acetyl Serotonin, Circadian Rhythmicity, Sleeping Sickness.

Background:
Circadian rhythm is an internally driven, approximately 24-hour cycle of physiological, biochemical or behavioral processes of an organism. It is visceral and adjusted by environmental factors such as daylight. Disturbance in circadian rhythm can cause serious health problems in humans like sleeping sickness, mood disorders etc. Regular circadian rhythm is depends upon balanced formation of melatonin and conversion of melatonin from serotonin is governed by an enzyme AANAT **[1].** It is already reported that the enzyme AANAT would be a good target for the treatment of irregular circadian rhythmicity and related diseases like sleeping sickness, mood disorders etc **[2].** Human AANAT gene spans 2.5 kb, contains four exons, and is located at chromosome 17q25. The open reading frame encodes a 23.2- kDa protein that is 80% identical to sheep and rat AANAT. The AANAT is highly abundant in the pineal gland and is expressed at lower levels in the retina and in the retinoblastoma cell line **[2].** The important regulatory role of AANAT has made it of central interest in research of melatonin biochemistry and neurochemical signal transduction and because it may lead to a better understanding of the role of melatonin in human physiology **[3, 4].** Studies in the rat indicates that expression of the AANAT gene is regulated by circadian clock in the suprachiasmatic nucleus (SCN), which drives rhythmic activity of the pineal gland via neural circuit that not passes through central and peripheral structures **[5, 6].** The neurotransmitter that regulates the abundance of AANAT mRNA is noradrenalin, which acts through a cyclic AMP mechanism **[2].** As a hormone noradrenaline affects several parts of the brain, which controls attention and responses **[7].**

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More specific analysis of the AANAT may reveal mutations that could explain the large individual-to-individual variations in serum melatonin levels among human **[8-13].** In this study we have done *insilico* analysis of AANAT enzyme's inhibitors by molecular modeling, molecular dynamics simulation, virtual screening and docking.

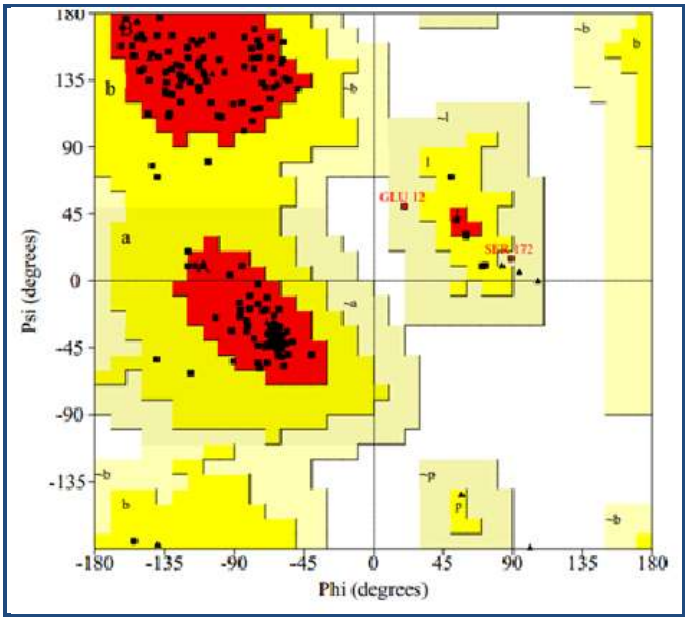

Figure 1: Ramachandran plot shows 90.9% residues found in allowed region

Methodology:

Preparation of Receptor Protein

Protein sequences for AANAT enzyme were retrieved from Uniprot (Q16613) (http://www.uniprot.org). PBLAST was conducted against the Protein data bank (PDB, http://www.rscb.org/pdb/) using query sequences to search for structural templates. The closest structural template of AANAT enzyme was found in Ovis Aries (Sheep) with a 78% sequence identity was chosen for structural modeling. Out of those 16 structures hits, the best protein (PDB ID: 1KUV) has been taken for modeling. After the modeling, loop modeling has been performed for minimizing the loop structure of modeled protein by SWISS-PDB Viewer. The quality of protein was checked by SAVES server and stereo chemical quality of protein was analyzed by Ramachandran Plot **(Figure 1).**

Model Protein Refinement by MDS

The molecular dynamic simulation (MDS) analysis was performed for the stability study of modeled AANAT enzyme. The protein was minimized and simulated by GROMACS 4.5.4. Optimized Potential for Liquid Simulations (OPLS) force field was used for the minimization. The minimization was carried out by Periodic Boundary Condition (PBC) in all directions and it involves 1000000 steps of steepest descent optimization. All the solvent atom and protein molecules were allowed to relax in MDS.

Virtual Screening for Ligand Prepration

Ligand N-Acetyl Serotonin was used as a reference molecule to generate ligand library from ZINC database. Virtual screening has been performed, with the receptor protein by using the Molegro. 269 molecules have shown 80% similarities with reference ligand, out of these top 10 molecules were selected on the basis of their MolDock scores **(Table 2).**

Molecular docking

The minimized structure of receptor protein obtained from MD simulation and top five ligand molecules were obtained from virtual screening have been taken for molecular docking by using AutoDock 4. The reference molecule N-Acetyl Serotonin was also docked with receptor protein. The other parameters were selected as default and number of poses for every ligand was set to 10. After both docking each protein ligand complex were observed and their interaction with amino acids were analyzed. The most energetically suitable conformation of ligand receptor complex was selected.

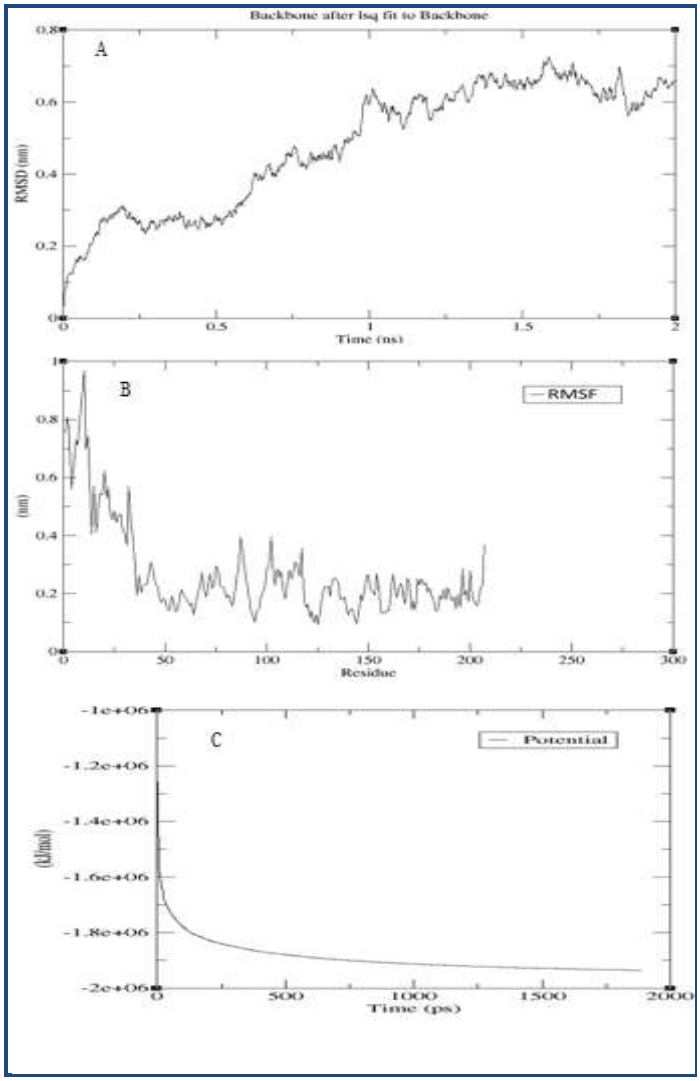

Figure 2: (A) RMSD of backbone atoms shows stable nature of model. **(B)** RMSF graph showing fluctuations in protein with respect to time**. (C)** Graph of potential energy shows the energetically stable conformation**.**

Results:

Modeling and simulation analysis

Closest structure AANAT enzyme of Sheep (*Ovis Aries*) with a 78% sequence identity has been taken for modeling. The stability of receptor protein has been verified by Ramachandran plot and it showed 90.9% residues lie in the allowed region

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(Figure 1). Molecular dynamic simulation was performed by GROMACS to analyze the stability of the homology models of the proteins and lowest energy conformation for each protein was chosen for further docking study. The RMSD value was raised from 0.2nm to 0.6nm and then remained quite stable in the subsequent simulation time from 1.25 ns onwards. Gradual decrease in potential energy of the model from -1.2e+06 kJ/mol to -1.9e+06 kJ/mol at around 1900ps which indicates that the model is energetically stable during MDS. RMSF graph also decreasing gradually from 0.800 to 0.235 **(Figure 2).**

Molecular Docking analysis

In docking studies strength of the drug and receptor interactions have been analyzed by scoring each possible interaction **(Table 1).** On the basis of earlier reported work motif A (LHALAVHRSFRQQGKGSVLL) and motif B (VPFYQRFGFHPAG) of AANAT protein have considered, hence we have included whole domain as an active site to dock with ligand. N-Acetyl Serotonin is selected as a reference ligand **[15, 16]** and ligand ZINC01587152 has shown lowest binding energy (-9.38 Kcal/Mol) with five hydrogen bonds **(Table 1) (Figure 3).** Above said active site has been predicted by CastP server also.

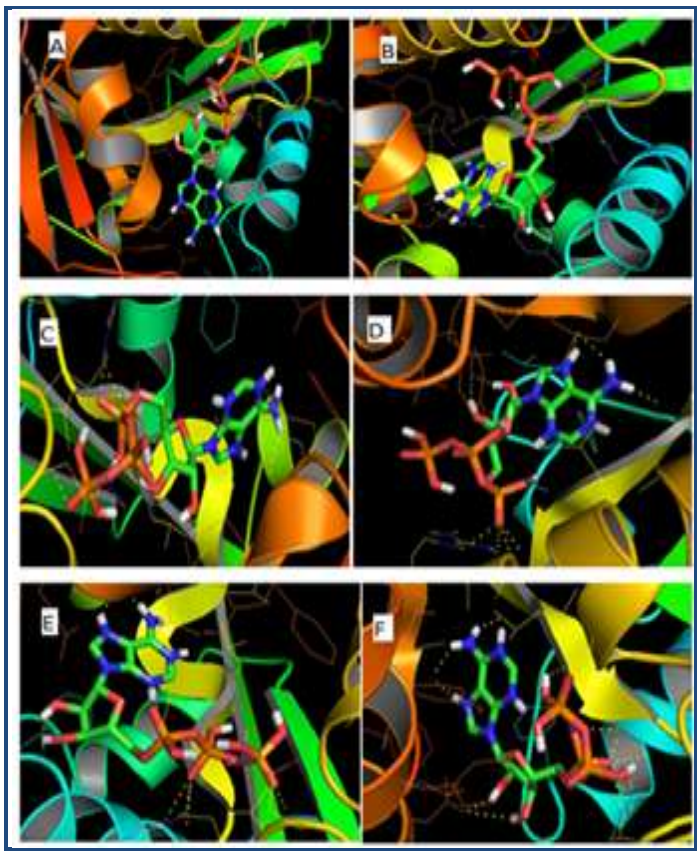

Figure 3: Showing interaction of ligands (A, B, C, D, E, and F) with receptor protein.

Discussion:

Melatonin has been proven to play an important role in regulation of sleep wake cycle, seasonal breeding patterns, and it depends upon environmental changes in dark-light patterns **[1, 2].** An AANAT enzyme activates acetyl transfer from acetyl-CoA from serotonin, gives N-acetyl serotonin, which acts as the messenger of melatonin. Thus it is proved to be a key enzyme involved in daily oscillation of melatonin **[14].** In this study, homology modeling has been used to model the AANAT protein by loop modeling. The molecular dynamic simulation showed that model protein was stable throughout the total time scale of the 2000ps and the potential energy was also decreased. Ligand N-Acetyl Serotonin was used to form a combinatorial library from ZINC database. On the selected database virtual screening was performed by using Molegro **[15].** Top five molecules obtained after performing virtual screening and these molecules were docked individually with AANAT protein by AutoDock. Reference ligand molecule was also docked with AANAT protein and results were compared and found that out of top five molecules, ZINC01587152 N-[2-(5-Hydroxy-1H-indol-3-yl)ethy1]-3-(4-hydroxyphenyl)-2-propenamide has showed least binding energy (-9.38 Kcal/Mol). On the basis of this result, we can suggest that ZINC01587152 molecule may be a putative drug to cure sleep and mood disorders **[15-16].**

Conclusion:

The AANAT is responsible for conversion of serotonin to melatonin and it is observed that it regulates wide variety of sleep disorders. 3D structure AANAT has been modeled and their energy minimization has been performed to bring optimized state later evaluated with Ramachandran Plot. Virtual screening of AANAT protein active site was performed against ZINC data based and ranked on the basis of their MolDock score. After screening top five ligands were selected and docked on above said protein by using AUTODOCK software. Finally docking studies showed better interaction between ZINC01587152 molecule and AANAT with least binding energy when compared to all the molecules including reference molecule. We can conclude that ZINC01587152 molecule has been proven insilico as a potential drug and may be further tested experimentally in the treatment of sleep and related disorders.

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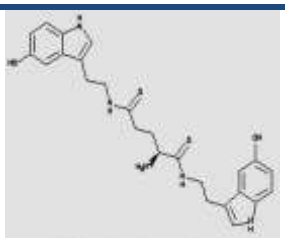
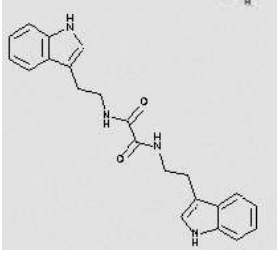
Supplementary material:

Table 1: Docking results of protein with different ligands

S. No.	Ligand	Binding energy	Ligand efficiency	Electrostatic energy	Hydrogen bond interaction
1	DB04275 (Reference ligand) [N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]acetamide]	-8.82	-0.28	-1.14	UNK1:H43: PHE130: O UNK1:H45: LEU124: O GLN132:HN : UNK1:O21 GLY134:HN : UNK1:O25 TYR168:HH : UNK1:O31 VAL126:HN : UNK1:O16 ARG131:HE : UNK1:016
2	ZINC3156178 *(2S)-2-amino-N,N'-bis[2-(5-hydroxy-1H-indol-3-yl)ethyl]pentanediamide*	-4.54	-0.15	-0.76	UNK1:H36: MET159:O ARG131: HE: UNK1:O16 UNK1: H45: ALA55:O
3	ZINC01755233 *N,N'-bis[2-(1H-indol-3-yl)ethyl]oxamide*	-8.13	-0.26	-1.06	GLN132:HN : UNK1:O25 VAL126:HN : UNK1:O16 UNK1:H45:LEU124:O ARG131:HE : UNK1:O16
4	ZINC12296993 *2-((1H-indol-4-yl)oxy)-N-(2-(5-methoxy-1H-indol-3-yl)ethyl)acetamide*	-5.82	-0.19	-0.88	VAL126:HN : UNK1:O16 LEU124:HN : UNK1:N35 UNK1:H36: HIS122:O UNK1:H45 : GLU161:OE1
5	ZINC00754630 *N-[2-(5-benzoxy-1H-indol-3-yl)ethyl]-2-(1H-indol-3-yl)acetamide*	-7.83	-0.25	-1.09	GLN132:HN : UNK1:O25 VAL126:HN : UNK1:O20 ARG131:HE : UNK1:O20 UNK1:H37: MET159:O UNK1:H45: ALA55:O UNK1:H4:MET159:O
6	ZINC01587152 *N-[2-(5-Hydroxy-1H-indol-3-yl)ethyl]-3-(4-hydroxyphenyl)-2-propenamide*	-9.38	-0.3	-1.26	UNK1:H36: MET159:O ARG131:HE : UNK1:O16 UNK1:H46 : GLU161:OE1 UNK1:H37: HIS122:O VAL126:HN : UNK1:O16

Table 2: Virtual screening results

S.No	Name of molecule	3D Structure	Mol Dock Score	Re-rank score	Hydrogen bond energy
1	**Molecule_ZINC31561738** *(2S)-2-amino-N,N'-bis[2-(5-hydroxy-1H-indol-3-yl)ethyl]pentanediamide*		-160.37	-126.427	-15.233
2	**Molecule_ZINC01755233** *N,N'-bis[2-(1H-indol-3-yl)ethyl]oxamide*		-160.24	-123.725	-6.08047

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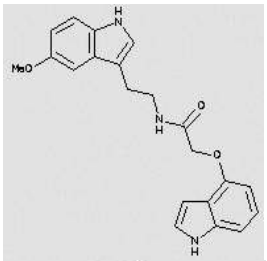
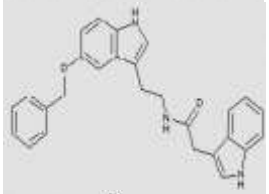
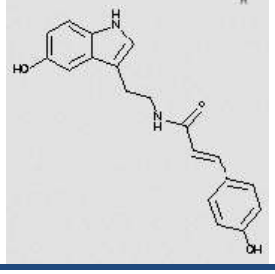
3	**Molecule_ZINC12296993** *2-((1H-indol-4-yl)oxy)-N-(2-(5-methoxy-1H-indol-3-yl)ethyl)acetamide*		-157.669	-102.463	-6.76432
4	**Molecule_ZINC00754630** *N-[2-(5-benzoxy-1H-indol-3-yl)ethyl]-2-(1H-indol-3-yl)acetamide*		-155.117	-34.7812	-1.92005
5	**Molecule_ZINC01587152** *N-[2-(5-Hydroxy-1H-indol-3-yl)ethyl]-3-(4-hydroxyphenyl)-2-propenamide*		153.177	119.882	-10.3396

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