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MODELING AND DOCKING STUDIES OF 4-AMINOBUTYRATE AMINOTRANSFERASE FOR HUNTINGTON'S DISEASE

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disease and 4-aminobutanoic acid (GABA) is an inhibitory neurotransmitter in mammalian nervous system which regulates muscle tone of the body. GABA acts at inhibitory synapses in the brain by binding to specific transmembrane receptors in the plasma membrane of both pre- and postsynaptic neuronal processes. γ-Aminobutyrate aminotransferase (GABA-AT) degrades the inhibitory neurotransmitter GABA. GABA-AT, a pyridoxal-dependent enzyme, is a target for antiepileptic and several other serious neuroactive drugs including drugs for Huntington's disease. Hence, its selective inhibition raises concentrations of GABA in brain. Defects in GABA-T cause accumulation of beta-alanine and gamma aminobutyric acid in plasma and spinal fluid, as well as accumulation of homocarnosine in spinal fluid. Symptoms include hyperreflexia, hypotonia, lethargia, macrosomia, mental retardation, and siezures. Therefore, GABA-AT is the preferential choice for inhibition to increase the concentration of GABA in brain. Therefore, an attempt was made to obtain the suitable inhibitors of GABA-AT by *de novo* creation of structurally flattering lead molecules which were further validated by docking analysis with GABA-AT protein. The screening of these results revealed that (2 S)- 3 - [(3aR, 4S, 6R, 7aS) - 6 - methyloctahydro- 1 H - inden - 4 - yl] -2- (propanoylamino)propanoic acid was found as the best fit over Lipinski's rule of five and other ADME parameters.

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KEYWORDS

GABA-AT, Huntington's disease, Homology Modeling, ligand-receptor interaction

INTRODUCTION

Huntington's disease (HD) is a rare neurodegenerative disorder that progressively destroys the mental capacity and motor control of patients.[1] Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by an expansion in the CAG trinuleotide repeats in the Huntingtin (Htt) gene present on the Chromosome 4p in *Homo sapiens*. Translation of these expanded CAG repeats gives polyglutamine (polyQ) in protein, which causes aberrant functions and aggregate formation of mutant Htt (mHtt). Normally in Htt gene, number of CAG repeats remains 10 to 35. But when number of repeats exceeds 35, it becomes HD. Individuals having HD have number of CAG repeats 36 to 120. As the Htt gene passes generation to generation, the number of CAG repeats tends to increase. As the number of repeats increases, chances of development of symptoms become greater. HD can be characterized by chorea, psychiatric disturbances, and dementia.

Toxic function of mHtt may noticeable and produce the HD pathology via multiple cellular changes[2,3]. When posttranslational modification of mHtt occurs, then cleavage of protein leaves behind shorter fragments. Those fragments can be constituted of parts of the polyglutamine expansion[2]. Polar glutamine interacts with other proteins when it is present over abundantly in Htt proteins. Thus, hydrogen bonding between Htt molecules strands forms protein aggregate rather than folding and forming functional proteins[3]. With time, accumulated aggregates interfere with neuron functioning as protein aggregation occurs to form inclusion bodies within the cells[2, 4]. Transmission of neurotransmitters stops mechanically, because of ceased movement of neurotransmitter vesicles through the cytoskeleton due to the excess protein aggregates clumping together at dendrites and axons. Over time, it results in very less availability of neurotransmitters in signaling processes[3]. Therefore, it is necessary to increase the availability of neurotransmitters in brain.

γ-Aminobutyrate aminotransferase (GABA-AT), a pyridoxal-dependent enzyme, is a target for antiepileptic and several other serious neuroactive drugs including drugs for Huntington's disease[5]. GABA-AT degrades the inhibitory neurotransmitter 4-aminobutanoic acid (GABA). This converts GABA and 2-oxoglutarate into succinic semialdehyde and glutamate. In humans, GABA is directly responsible for the regulation of muscle tone[6]. If GABA-AT is not present here then GABA degradation can be prevented. So if we can inhibit GABA-AT, we can increase GABA concentration in brain. Therefore, GABA-AT is the preferential choice for inhibition to increase the concentration of GABA in brain.

Although the genetic basis of the disease has been identified, the mechanisms behind the cellular pathogenesis are still not clear and as a result no candidate drugs with the potential for disease modification have been found clinically until now. Since there is no effective treatment for HD, the main aim of this study is to computationally design a molecule which can be a potential drug for treatment of this disease.

METHODS AND MATERIALS

Homology modeling

The first step towards successful *in silico* drug designing is 3D structure modeling. The FASTA[7] sequence of the target GABA-AT was obtained from protein database of NCBI (Accession Number AAA74449.1 and Gene Id 602705). The protein consists of 500 amino acids. Subsequently, the template 3Q8N selected with protein BLAST[8] of target sequence and the target sequence were aligned using MODELLER 9v8[9] and thus, construction of rough 3D models (10 models) was done. The constructed models were evaluated for geometry, stereochemistry checks and energy distribution on the basis of DOPE score and with PROCHECK[9] (Ramachandran plot). Best rough 3D model structure containing 83.4% residues in the core region of the Ramachandran plot was then processed for refining. For model refinement loop modeling and energy minimization was done with SPDBV[10] and model was again checked with PROCHECK (Ramachandran plot). (Figure 1)

Active site prediction

Active inhibitory site identification was done with the help of Ligsite^{csc}[12]. Ligsite^{csc} used the concept of surface-solvent-events and involved surface residues conservation degree. The binding site prediction was based on 1.5 Å grid space, probe radius 5 Å and number of binding site to 3. Largest site was chosen as ligands binds with more affinity in ~80% cases[12]. Largest binding site residues are HIS 72, TYR97 and ILE100 in the protein model. These residues were captivatingly near to the binding site in the protein model.

Ligand Designing

Ligbuilder 1.2[14] was used to form ligands (10 ligands) according to inhibitory site (HIS72, TYR97, ILE100) in model. We took acetic acid and its derivatives as seed molecules. Ligand structures were drawn by CHEMSKETCH software[15] and afterward converted into the .pdb format from .mol format by using OPENBABEL[15]. The rigid docking based on the potential distributions of the TYR97 was done by HEX 6.3[17] for protein model and seed molecule. Seed molecule could be matured in the available binding site after setting up the position of TYR97. In the GROW module of Ligbuilder, number of generations was set to 10 and number of molecules per generation was set by default to 3000. Generated molecules were filtered through PROCESS module in Ligbuilder and 10 final molecules (Table 3) were selected appropriate with parameters (Table 5).

Molecular Docking

For studying ligand-receptor interactions AUTODOCK 4.2/ADT[18] program was used. For inhibitory site direction, grid encompassing was used. The Autodock program went through pre-calculated grids of affinity potentials with a variety of search algorithms and combined a rapid energy evaluation to find suitable binding positions[18]. The search results were on the basis of the Lamarckian genetic algorithm and for analysis, binding energy was used. Then each ligand was processed in docking experiment with 10 simulations using Autodock and ranked according to increasing binding energy. All ligands were compared with each other on the basis of binding energy and other factors (Table 4). Finally, ligand 5 was selected with minimum binding energy -7.1 kcal/mol (Table 4). Molecular properties of the ligands docked were calculated by Molinspiration server (http://www.molinspiration.com) for all molecules (Table 3) for the ligand 5,**(2S)-3-[(3aR,4S,6R,7aS)-6-methyloctahydro-1H-inden-4-yl]-2-propanoylamino)propanoic acid** and results showed that molecule is having properties identical to be a drug.

Visualization

Visualization of the docked structure was performed on PyMol molecular graphics program, a comprehensive software package for rendering and animating 3D-structures. This software produced high quality three dimensional images of small molecules, proteins and nucleic acids. The protein atoms were shown in cartoon model, while the ligand is shown in the stick model.

RESULTS

Model evaluation

Since there was no structure predicted for the GABA-AT protein, homology modeling was used to generate 3D protein models of the desired protein i.e. GABA-AT using a template sequence with PDB code 3Q8N. The constructed 3D models were checked for DOPE score and Ramachandran plot respectively with Modeller 9v8 and PROCHECK. Results are shown in Table 1. Model No. 7 was chosen as the best model with parameters like Ramachandran plot (83.4 core, 12.5 allowed, 2.7 generously allowed, 1.4 disallowed) and DOPE score (-45846.371094). Model refinement with SPDBV was done by loop modeling and energy minimization. Figure 1 shows the Ramachandran plot analysis of the model after refinement. Model structure prepared for visualization by Pymol is shown in Figure 2. Protein structure is having 500 amino acids and contains 10 β-sheets with 15 α-helices.

Table 1
Analysis of generated models with PROCHECK and MODELER 9v8

Model No.	PROCHECK (Ramachandran Plot), %				DOPE Score
	Core	*Allowed*	*Generously Allowed*	*Disallowed*	
1.	82.7	13.7	2.1	1.6	-45035.575625
2.	80.2	14.4	3.4	2.1	-46194.191406
3.	81.1	13.9	2.1	3.0	-45582.722656
4.	84.1	12.3	2.1	1.6	-46239.929688
5.	81.5	13.7	2.3	2.5	-45920.804688
6.	82.5	12.5	2.1	3.0	-46705.718750
7.	**83.4**	**12.5**	**2.7**	**1.4**	**-45846.371094**
8.	82.7	12.5	2.7	2.1	-45731.289062
9.	81.8	13.2	3.2	1.8	-45341.214844
10.	81.5	14.6	2.1	1.8	-46228.628906

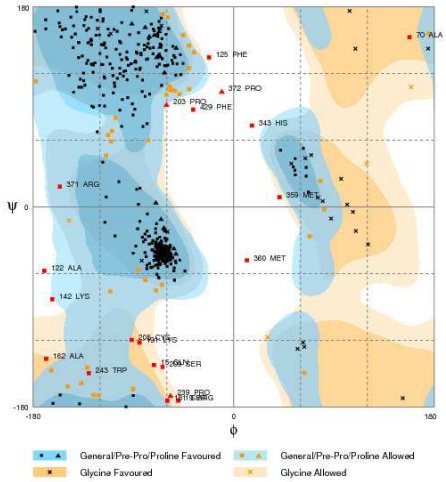

Figure 1

Ramachandran plot analysis of selected model after refinement of the model

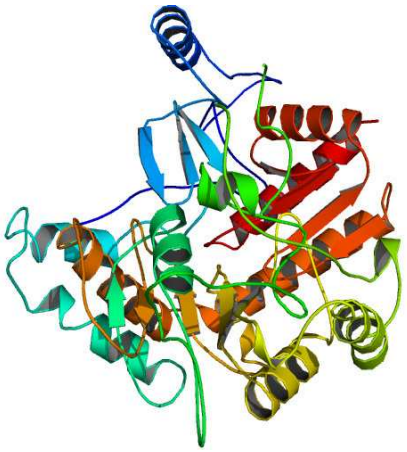

Figure 2

Cartoon model of 4-Aminobutyrate aminotransferase.

Details of inhibitory sites/pockets present in the protein model are given in Table 2. Pocket chosen for ligand building was PKT 258.

Table 2

Binding pockets in the protein found by Ligsite^{csc}

Sr. No.	Pocket Name	Residues
1.	PKT 258	HIS72, TYR97, ILE100
2.	PKT 34	LYS153, VAL352
3.	PKT 21	GLY331, GLY332, GLY333, PHE338, LYS358, MET359, MET360, VAL395, ILE399

Selection of Potential Inhibitors

GABA-AT is a 500 amino acid long protein and its structure is not known yet so domains are not known in the protein. From literature studies, it is evident that if active site of a protein is blocked then protein becomes inactive. So according to the active site (PKT 248) ligands (10 ligands) formed with the help of Ligbuilder 1.2. Ligands are shown in Figure 3. According to the minimum chemical criteria (Table 3) 200 out of the initial 3000 ligands were satisfactorily screened by using de novo drug design approach. Then these ligands were clustered into 10 different groups according to their structural similarity. Subsequently, based on binding affinity to protein model, molecules from each group were chosen. Details of all generated ligands are given in the Table 4.

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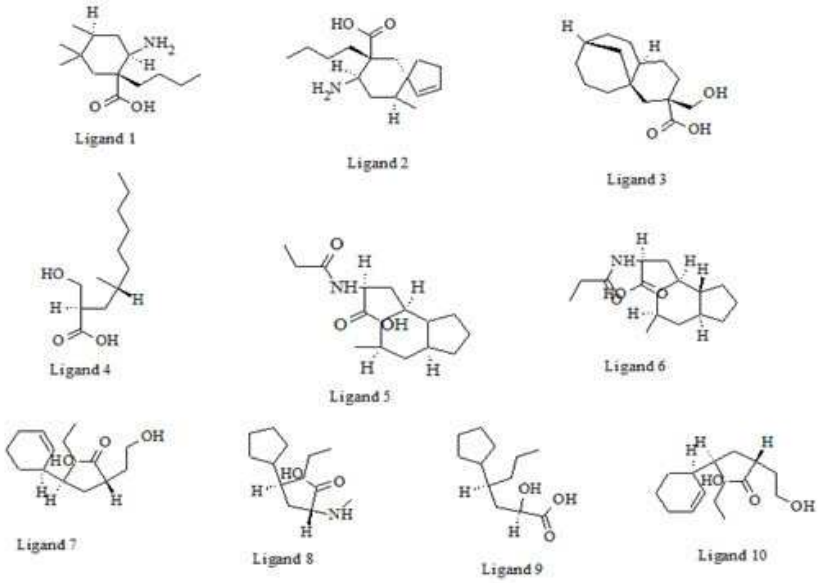

Figure 3
Ligbuilder generated ligands

Table 3
Chemical criteria used for PROCESS module in LigBuilder

Property	Value
Maximal Molecular weight	500
Minimal Molecular weight	50
Maximal LogP	5.0
Minimal LogP	-5.0
Maximal Donor Atoms	5.0
Minimal Donor Atoms	2.0
Maximal Acceptor Atoms	5.0
Minimal Acceptor Atoms	2.0
Number of molecules satisfied the criteria	200

Table 4
Ligbuilder generated ligands

Sr. No.	Molecular Formula	Molecular Weight	IUPAC Name
1	$C_{14}H_{27}NO_2$	241.375	(1R,2R,4S)-2-amino-1-butyl-4,5,5-trimethylcyclohexanecarboxylic acid
2	$C_{16}H_{27}NO_2$	265.397	(5S,7R,8R,10S)-8-amino-7-butyl-10-methylspiro[4.5]dec-1-ene-7-carboxylic acid
3	$C_{15}H_{24}O_3$	252.354	(1R,3S,6S,9R)-3-(hydroxymethyl)tricyclo[7.3.1.01,6]tridecane-3-carboxylic acid
4	$C_{12}H_{24}O_3$	216.321	(2R,4R)-2-(hydroxymethyl)-4-methyldecanoic acid
5	**$C_{16}H_{27}NO_3$**	**281.396**	**(2S)-3-[(3aR,4S,6R,7aS)-6-methyloctahydro-1H-inden-4-yl]-2-(propanoylamino)propanoic acid**
6	$C_{15}H_{26}O_3$	254.37	(2S,4S)-4-[(1R)-cyclohex-2-en-1-yl]-2-(2-hydroxyethyl)heptanoic acid
7	$C_{15}H_{26}O_3$	254.37	(2S,4S)-4-[(1R)-cyclohex-2-en-1-yl]-2-(2-hydroxyethyl)heptanoic acid
8	$C_{13}H_{25}NO_2$	227.343	(2R,4S)-4-cyclopentyl-2-(methylamino)heptanoic acid
9	$C_{12}H_{22}O_3$	214.301	(2R,4S)-4-cyclopentyl-2-hydroxyheptanoic acid
10	$C_{15}H_{26}O_3$	254.365	(2S,4S)-4-[(1R)-cyclohex-2-en-1-yl]-2-(2-hydroxyethyl)heptanoic acid

Drug ability assessment of Inhibitors

Further, screening of all 10 molecules for Lipinski's rule of five[19] was done. The molecules, which violate this rule, are considered to be poor absorbents. Luckily, any molecule out of all 10 molecules did not violate the rule. An ideal drug molecule should be having a molecular weight of less than 500, total number of hydrogen bond should not exceed 5, miLogP value and should be less than 5 and the sum of N and O should not be more than 10. Details of the Lipinski's rule of five for each ligand are given in the Table 5.

Table 5
Molecular properties of ligand molecules by MolInspiration

Ligand	miLogP	MW	H acceptor	H donor	Violations
1	2.742	241.375	3	3	0
2	2.576	265.397	3	3	0
3	2.245	2.245	3	2	0
4	3.267	216.321	3	2	0
5	1.512	281.396	4	2	0
6	1.512	254.37	4	2	0

7	3.196	254.37	3	2	0
8	1.736	227.348	3	2	0
9	2.775	214.305	3	2	0
10	3.196	254.37	3	2	0

Interaction of potential inhibitor with protein model:

The ligand molecule 5, (2S)-3-[(3aR,4S,6R,7aS)-6-methyloctahydro-1H-inden-4-yl]-2-(propanoylamino)propanoic acid (Figure 3) is present in the cavity of GABA-AT (Figure 4) and is showing close interactions with the residues present in its active site, especially HIS72, TYR97 and ILE100. In addition to vander wall interaction, HIS72 and TYR97 are forming strong hydrogen bond to the ligand. (Figure 5 and 6) Surface diagram clearly represents that the ligand occupies the internal part of cavity and specifically binds with 4-Aminobutyrate aminotransferase with appreciable affinity (Figure 4).

The three-dimensional protein-ligand interaction is mapped by calculating the distance (Figure 5) by the PyMOL. (It is further evident that ligand 5 is showing hydrogen bonds with GLN99, MET359, TYR97 and hydrophobic interactions with Glu298 and HIS72

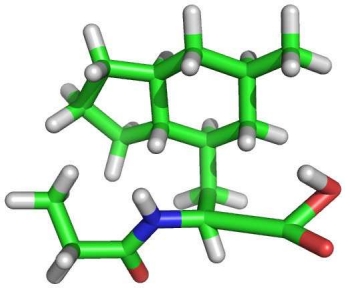

Figure 3
Chemical structure of molecule5 (2S)-3-[(3aR,4S,6R,7aS)-6-methyloctahydro-1H-inden-4-yl]-2-(propanoylamino)propanoic acid and its representation in stick model.

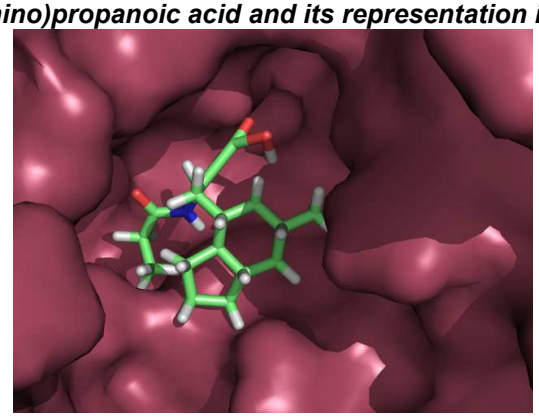

Figure 4
Stick model of Ligand 5 in the cavity of GABA-AT

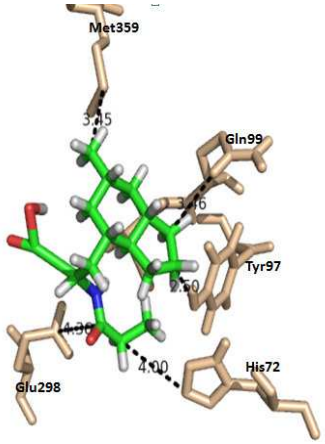

Figure 5
3D representations of interaction between residues docking complex between GABA-AT and Ligand 5

Drug ability assessment of Inhibitors

We have further screened the all 10 molecules for Lipinski's rule of five which states that poor absorption or permeation are more likely when a ligand molecules violates Lipinski's rule of 5 Interestingly, ligand 5 has well qualified in Lipinski's filter at Molinspiration Server. (http://www.molinspiration.com) (Table3 and 5)

DISCUSSION

In order to find out an effective treatment for Huntington's disease, many efforts have been made by different groups of people. But till now, there is no effective drug for Huntington's disease. For the same reason, we have made some efforts to find out a potential drug model for HD. It was found earlier that GABA-AT was a potential drug target for HD. But structure of this protein in humans is still unknown. For that, we have done homology modeling using a template sequence alignment with our protein sequence. Results of this homology modeling provided us with 10 models those can be the GABA-AT protein structure. Among all these models, we have to select one model. For that purpose, we did a brief analysis of these models with Ramachandran plot and DOPE score of these models. Ramachandran plot shows the number of residues according to the stability in protein. More number of residues in disallowed region gives instability to the protein structure. To make a protein stable to configuration, it is necessary to remain all the amino acid residues in allowed region in the Ramachandran plot. In the selected model, least number of amino acids was present in disallowed region and the DOPE score was considerable relative to other models. After structure refinement, the model number 7 was considerable as GABA-AT protein structure with 1.4% disallowed amino acid residues.

Ligbuilder generated ligands was docked with the protein model and results had shown that the ligand 5 was the best suited to be a drug for HD. Minimum binding energy with protein molecule shows that this ligand is having the maximum affinity to bind with the target protein. For blocking the active site of a protein, it is mandatory for a ligand to have strong interactions with that protein. Interactions of the selected ligand with the GABA-AT protein molecule is also significantly very good than other ligand molecules. This ligand is also satisfying the Lipinski's rule of five which means it is having good adsorbent properties within the body. Later it is analysed by LigPlot that the

residues in the pocket are also showing good binding affinity with the ligand 5.

Here, we have shown the use of de novo drug designing approach for potential design of inhibitor. GABA-AT plays a potential role in Huntington's disease. Therefore, the structure of GABA-AT is important begin the strategies for the design of compounds that block itsactivities and provide a basis for drug development for therapeutic intervention in Huntington's diseases. Our designed ligands are presumably getting insight for blocking the activity of GABA-AT.

CONCLUSIONS

This work provides a better insight for the structural based inhibition of the GABA by GABA-AT. Currently, there are no effective treatments for the growing global problem of Huntington's disease. Our designed ligand provides a platform to design an efficient and selective ligand for GABA-AT. However, further optimization may help to synthesize more potent ligand that many be considered as a drug molecule for further consideration in future studies.

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