# Exploring potential drugs against Scrapie disease and Creutzfeldt-Jakob disease using molecular docking techniques

A Project Thesis Submitted in Partial Fulfillment of The Requirement for

The Degree of

**BACHELOR OF TECHNOLOGY**

In

**BIOTECHNOLOGY**


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# CERTIFICATE

This is to certify that the project report title "**Exploring potential drugs against Scrapie disease and Creutzfeldt-Jakob disease using molecular docking techniques**" submitted by **SIDDHANT MOHANTY** (110BT0606) in the partial fulfillment of the requirement for the degree of the B.Tech in Biotechnology Engineering in Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision.

To the best of my knowledge the matter embodied in the report has not been submitted to any other Institute/University for the award of any Degree or Diploma.

N Sarkar.

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# ACKNOWLEDGEMENT

I would like to take this opportunity to extent my hearty gratitude to my guide and advisors Dr. Nandini Sarkar, Assistant Professor and Dr. Subhankar Paul, Associate Professor; Department of Biotechnology and Medical Engineering, National Institute of Technology-Rourkela, Odisha-769008, whose constant guidance and encouragement made the completion of my B.Tech thesis possible.

I also thank National Institute of Technology, Rourkela, for permitting me to utilize the facilities in its laboratories to carry out my experiment.

I would also like to thank Karthic Krishnan, M.Tech for giving me constant support with all the necessary things involve in this whole project.

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# ABSTRACT

Scrapie is a lethal, degenerative sickness that influences the sensory systems of sheep and goats. Creutzfeldt-Jakob infection is a degenerative neurological issue that is serious and constantly lethal in human. Both the maladies are brought about by a protein called prion. The transformation of the α-helical, cell isoform of the prion protein ($Prp^{c}$) to the insoluble, β-sheet rich irresistible, infection creating isoform ($PrP^{sc}$) is the key occasion in prion illness. I have discovered two prion proteins on which the inhibitors could be docked. They are 1XYU (sheep) and 1FO7(human). I have additionally searched for different inhibitors which might be utilized to tie with the dynamic locales of the two proteins. Some of them are tannin, quinacrine, astemizole, terfenadine, lovastatin, amantadine, acyclovir, clonazepam, pentosan polysulfate, perk inhibitors, rivastigmine,etc.

**KEYWORDS: Prion, 1XYU, tannin, quinacrine, 1FO7, acyclovir, clonazepam, perk inhibitors, rivastigmine.**

# CHAPTER -1

# INTRODUCTION

# 1.1 INTRODUCTION

**Scrapie** is a deadly, degenerative disease which attacks the nervous system of sheep and goats. It is a type of Transmissible Spongiform Encephalopathies (TSEs), also related to Bovine Spongiform Encephalopathies (BSEs or "mad cow disease") and chronic wasting disease of deer. A protein named PRION causes Spongiform Encephalopathies which include Scrapie disease.

The disease apparently causes an itching sensation in animals. The traits of this disease are:

- Excessive lip smacking
- Altered gaits
- Convulsive collapse

It is transmissible and infectious among similar animals. So the best way to contain it is to quarantine or destroy those affected. However it tends to continue in flocks and can also arise apparently spontaneously in flocks that have not previously had cases of the disease. It usually affects sheep around 3 to 5 years of age. This appears due to transmission at the time of birth or from contact with placental tissues.

Scrapie is not found to be infecting humans. The mechanism of transmission between animals and other aspects of the biology of the disease are poorly understood and these are active areas of research. Scrapie is associated with modification of normal Prion protein to a misfolded abnormal protein, which accumulates in amyloid plaques in lymphoreticular and nervous tissues.

**CREUTZFELDT-JAKOB DISEASE** is a degenerative neurological disorder which is not curable and has a high mortal rate. It is also known as the human version of mad cow disease even though classic CJD can't be compared to bovine spongiform encephalopathy. The disease is caused by a protein named Prion. Prions are misfolded proteins which replicates by the conversion of properly folded counterparts in their hosts, to the same misfolded structure they possess. The disease leads to rapid neurodegeneration, causing the brain tissue to develop holes and eventually forming a sponge like texture.

**SYMPTOMS:** The initial symptoms of CJD are rapidly progressive dementia, causing memory loss, hallucinations and personality changes. Other symptoms include depression, obsessive-compulsive symptoms, paranoia, anxiety and psychosis, which are accompanied by problems with speech impairment, jerky movements, balance and coordination disfunction. Most victims are observed to die within six months of the appearance of initial symptoms, mainly due to impaired coughing reflexes caused by pneumonia. The symptoms of CJD are caused by the progressive death of brain's nerve cells, which is associated with the growth of abnormal Prion proteins forming amyloids. When brain tissue (of a patient suffering from CJD) is observed under microscope, many tiny holes are visible due to the death of nerve cells. The structure of the tissue is more of a sponge like structure. The word "spongiform" in TSE refers to the sponge like appearance of brain tissue.

# 1.2 CAUSE OF THE DISEASES

**PRION PROTEIN:** Prions are small proteinaceous particles that resist inactivation by procedures known to modify nucleic acids. The abnormal prion protein that forms in animals with Scrapie is resistant to denaturing agents, heat and proteinase K digestion. The abnormal Prion protein does not fulfill Koch's postulates and it has been postulated that abnormal Prion protein is a pathological response to infection rather than the causal agent. Variations in the prion protein gene result in differing susceptibilities or resistance to Prion and thus to natural and experimental Scrapie[16].

- In sheep, all except one of the 15 known Prion protein genotypes are known to have some susceptibility to classical Scrapie and some are known to be also susceptible to BSE. The only exception is the homozygous ARR genotype
- In goat, Prion protein is expressed from a single gene, which appears>99% homologous to that of sheep, but goats do not share the Scrapie modulation polymorphisms present in the sheep. Analysis of the caprine Prion protein gene has revealed several different alleles. Four prion protein variants have been described in British goats, three of them are goat specific with single amino acid changes at codon 142, 143, 240. The amino acid changes associated with Scrapie susceptibility of sheep, i.e. Val at codon 136 have not been described in goats[18].

The Prion that is believed to cause Creutzfeldt-Jakob exhibits at least two stable conformations. One, the native state, is water soluble and present in healthy cells. The other conformational state is relatively water insoluble and readily forms protein aggregates. The CJD Prions are dangerous because it promotes refolding of

native proteins into the diseased state. The number of misfolded protein molecules will increase exponentially and the process leads to a large quantity of insoluble protein in affected cells. This mass of misfolded proteins disrupts cell function and causes cell death. Mutations in the gene for the Prion protein can cause a misfolding of the dominantly alpha-helical regions into beta-pleated sheets. This change in conformation disables the ability of the protein to undergo digestion.

# 1.3 LITERATURE REVIEW

The change of the α-helical, cell isoform of the prion protein (Prpc) to the insoluble, β-sheet-rich, irresistible, illness-creating isoform (Prpsc) is the key occasion in prion illnesses. In a prior study, a few manifestations of Prp were changed over into a fibrillar state by utilizing an as a part of vitro change framework comprising of low centralizations of SDS and 250 mm Nacl. Here, we portray the structure of the fibril antecedent state, that is, the solvent state under fibrillization conditions.. Exponential, seed-improved development might be attained in homogeneous result, which could be upgraded by sonication. From these information, we propose an unthinking model of fibrillization, including the vicinity of a few halfway structures.[1]

Requested protein collection in the cerebrum is a sign of Alzheimer's illness and scrapie. The illness-particular amyloid fibrils involve basically a solitary protein, amyloid beta, in Alzheimer's infection, and the prion protein in scrapie. These proteins might be prompted to structure totals in vitro that are unclear from cerebrum-determined fibrils. Hence, much exertion has been put resources into the improvement of in vitro model frameworks to study the subtle elements of the total methodologies and the impacts of endogenous atoms that have been involved in malady Moreover, amyloid shaping could be seeded by a preformed fibril. The physiological results of this system are examined.[2]

Location of Prpsc protein and Prp heredity of the transmission of BSE to sheep and goats, with the impacts of the transmission of common scrapie from a cerebrum homogenate from a solitary sheep. After intracerebral and oral immunizations there were similitudes in the clinical signs because of the two wellsprings of contamination, yet there were contrasts in pathology at the end phase of sickness and in the genotypes of the sheep which succumbed to the

difficulties. The hatching time of BSE was connected with the sheep Prp codon 171 genotype, yet the common scrapie source, notwithstanding affecting sickness just in known defenseless genotypes, demonstrated no reasonable affiliation with Prp genotype.[3][4]

Throughout the time between contamination and the appearance of the clinical side effects, moment measures of Prpsc imitate by change of host Prpc, producing a lot of Prpsc totals in the brains of unhealthy people. We intended to recreate this occasion in vitro. Here we report a methodology including cyclic enhancement of protein misfolding that permits a fast change of vast overabundance Prpc into a protease-safe, Prpsc-like structure in the vicinity of moment amounts of Prpsc layout. In this technique, adroitly closely resembling polymerase chain response cycling, totals shaped when Prpsc is brooded with Prpc are upset by sonication to create numerous littler units for the proceeded development of new Prpsc. After cyclic intensification more than 97% of the protease-safe Prp show in the specimen relates to recently changed over protein.[5]

In late studies, the amyloid manifestation of recombinant prion protein (Prp) enveloping buildups, processed in vitro actuated transmissible prion infection in mice. These studies indicated that dissimilar to "traditional" Prpsc handled in vivo, the amyloid fibrils produced in vitro were more proteinase-K touchy. Here we show that the amyloid structure holds a proteinase K-safe center made just out of buildups. The PK-safe parts of the amyloid structure are like those saw upon PK assimilation of a minor subpopulation of Prpsc as of late distinguished in patients with sporadic Creutzfeldt-Jakob ailment (CJD). Surprisingly, this center is sufficient for multiplying toward oneself movement in vitro and jam a β-sheet-rich fibrillar structure. Full-length recombinant Prp, on the other hand, creates two subpopulations of amyloid in vitro: One is like the minor subpopulation of Prpsc, and the other to established.[6].

We distinguish porphyrins and phthalocyanines as inhibitors of Prp-res aggregation. The most intense of these tetrapyrroles had Ic50 qualities of 0.5–1 μm in scrapie-contaminated mouse neuroblastoma (Scnb) cell societies. Hindrance was seen without impacts on protein biosynthesis when all is said in done or Prp-sen biosynthesis specifically. Tetrapyrroles likewise repressed Prp-res structuring in a without phone response made overwhelmingly out of hamster Prp-res and Prp-sen. Inhibitors were found around phthalocyanines, deuteroporphyrins IX, and meso-substituted porphines; cases included mixes holding anionic, impartial protic, and cationic fringe substituents and different metals.[7][8]

Ox-like spongiform encephalopathy (BSE) and its human proportional, variant Creutzfeldt–jakob infection (vcjd), are created by the same strain of irresistible operator, which is like, however unique from, >20 strains of their sheep scrapie homologue. A finer understanding of the sub-atomic strain determinants could be gotten from cells in monoculture than from entire creature studies where diverse cell focusing on is normally a strain-related characteristic. Despite the fact that a couple of cell sorts could be contaminated with distinctive strains, the phenotypes of the new strains have not been mulled over.[9]

Prion maladies, including scrapie, are hopeless neurodegenerative issue. A few mixes can postpone illness after a fringe scrapie vaccination, however few are powerful against cutting edge infection. Here, we tried different related porphyrins, yet just Fe(iii)meso-tetra(4-sulfonatophenyl)porphine infused into mouse brains after intracerebral scrapie immunization generously expanded survival times.[8]

We indicate that rabbit Prp-sen does not structure Prp-res in murine tissue society cells tirelessly tainted with the mouse-adjusted scrapie operator. Dissimilar to other TSE species hindrances that have been considered, discriminating amino corrosive deposits that restrain Prp-res framing are spotted

all around the rabbit Prp arrangement. Our outcomes recommend that the safety of rabbits to contamination by the TSE executor is because of numerous rabbit Prp-particular amino corrosive buildups that bring about a Prp structure that is unable to refold to the anomalous isoform connected with infection.[10]

**MECHANISM:** The transformation of the α-helical, cell isoform of the prion protein ($PrP^{c}$) to the insoluble, β-sheet rich irresistible, malady creating isoform ($PrP^{sc}$) is the key occasion in prion infection. In a prior study, a few types of $PrP^{c}$ changing over to $PrP^{sc}$ causes Prion replication.[1][17][19]

Several unthinking models have been proposed for this change of adaptation, which are

•heterodimer model • the cooperative model •the model of seeded polymerization

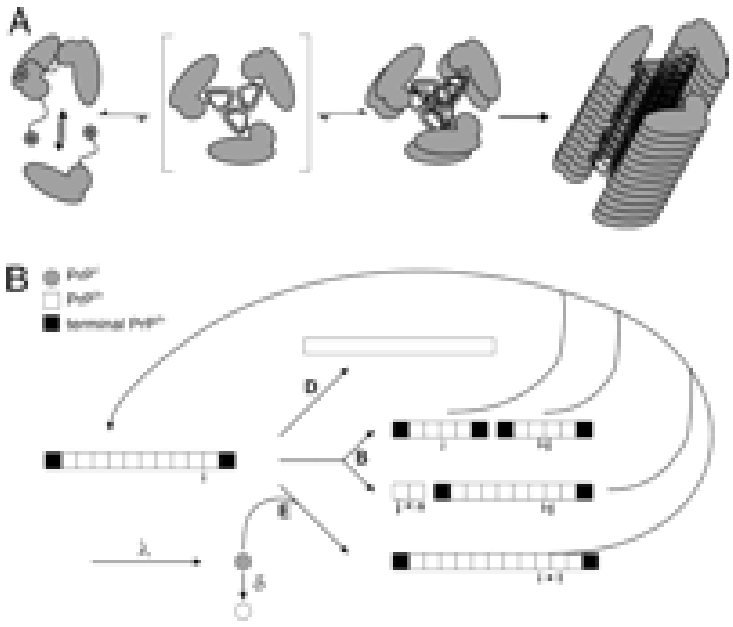



**Figure 1.1:Mechanism :** (A)A proposed model depicts the preamyloid state in a monomer dimer equilibrium, stationary state of trimer, stable nucleus of two trimers and growing fibril.(B)Reaction scheme of seeded fibrillization. D=degradation, E=seed dependent growth, B=breakage of fibrils, λ= synthesis, δ= degradation of monomeric PrP

# CHAPTER 2

# SOURCES OF PRION PROTEIN

# 2.1 *Ovis aries*

We go to the site www.rcsb.org and look for Prion protein and keeping scientific classification as *Ovis aries*. At that point we get a rundown of PDB Ids. From the schedule I haphazardly pick a protein which is:

**1XYU**: The NMR structures if the recombinant cell type of the Prion proteins ($PrP^c$) of the feline, canine and pig and of two polymorphic types of prion protein from sheep are exhibited. In these species, $PrP^c$ comprises of N-terminal adaptably amplified tail with roughly 100 amino corrosive deposits and a C-terminal globular area of more or less 100 buildups with three alpha-helices and a short antiparallel beta-sheet. In spite of the fact that this worldwide structural engineering corresponds with at one time reported murine, Syrian hamster, ox-like and human $PrP^c$ structures, there are nearby contrasts between the globular space of diverse species. Since the five recently decided $PrP^c$ structure start from species with broadly distinctive transmissible spongiform encephalopathy records, the present information demonstrate long ago uncharacterized conceivable associations between neighborhood offers in $PrP^c$ 3-D structures and powerlessness of diverse mammalian species to transmissible spongiform encephalopathy.

**RELATED STRUCTURES:**

- 1XYJ
- 1XYK
- 1XYQ
- 1Y2S

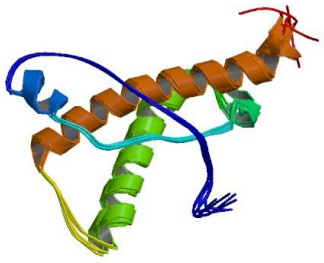


**Figure 2.1:** 1XYU

## 2.2 *Homo sapiens*

**1FO7** Human prion protein mutant E200k Fragment 90-231 prion engendering in TSE s includes the transformation of cell protein $PrP^{c}$ into a pathogenic conformer $PrP^{sc}$. Heredity types of the sickness are connected to particular transformations in the gene coding for the prion protein. To increase understanding into the atomic premise of these issue, the result structure of the familial CJD identified with E 200k variant of human prion protein was dictated by multi-dimensional atomic attractive thunder spectroscopy. Exceptionally, separated from minor contrasts in adaptable districts, the spine tertiary structure of the E200k variant is almost indistinguishable to that reported for the wild sort human prion protein. The main real result of the transformation is the bother of surface electrostatic potential. The present structural information positively propose that protein surface locates prompting variations from the norm in the collaboration of prion protein with auxillary proteins/chaperones or cell layers ought to be viewed as key parts of a spontaneous $PrP^{c} \rightarrow PrP^{sc}$ change in the E200k type of genetic prion ailment.[14]

**RELATED STRUCTURES**:

**1FKC:** It assumes a part in neuronal improvement and synaptic pliancy. It may be needed for neuronal myelin sheath support. May assume a part in iron uptake and iron homeostasis. Dissolvable oligomers are harmful to cosmopolitan neuroblastoma cells and actuate apoptosis (in vitro). Acquaintanceship with Gpc1 (by means of its heparin sulfate chains) targets PRNP to lipid pontoons. Likewise gives $Cu^{2+}$ or $Zn^{2+}$ to the ascorbate-intervened Gpc1 deaminase debasement of its heparin sulfate side chains.

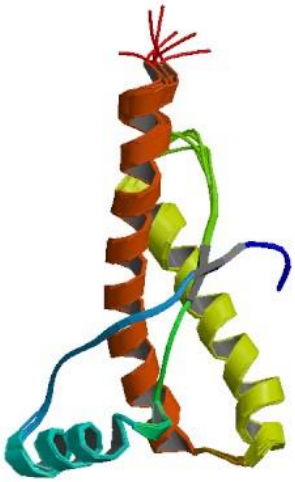


**Figure 2.2:**1FO7

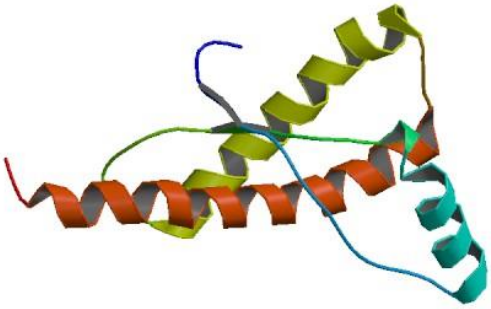


**Figure 2.3:** 1FKC

# CHAPTER 3

# MOLECULAR DOCKING

# 3.1 IMPORTANT ASCPECTS OF MOLECULAR DOCKING

Molecular docking is a broadly utilized computational instrument for the investigation of sub-atomic distinguishment, which intends to anticipate the coupling mode and tying partiality of a complex shaped by two or more constituent atoms with known structures.

Goals:

- to check whether two atoms collaborate with one another( (fitting introduction).
- whether introduction that boosts collaboration or minimizes absolute vitality of the complex.

**TYPES OF MOLECULAR DOCKING:**

- receptor - -LIGAND
- enzyme - -SUBSTRATE
- protein - -PROTEIN
- protein- -DNA/RNA

PROTEIN-PROTEIN DOCKING:

- both atoms are inflexible
- steric imperatives to cutoff pursuit space and inspect energetics of tying conformities.

PROTEIN-LIGAND DOCKING:

- flexible ligand, unbending receptor
- search space much bigger
- either diminish adaptable ligand to inflexible pieces joined by one or more pivots or pursuit the conformational space utilizing atomic progress.

FACTORS AFFECTING MOLECULAR DOCKING:

- intermolecular strengths—covalent powers
- bond lengths
- bond edges
- dihedral edges
- electrostatic strengths, dipole-dipole collaborations

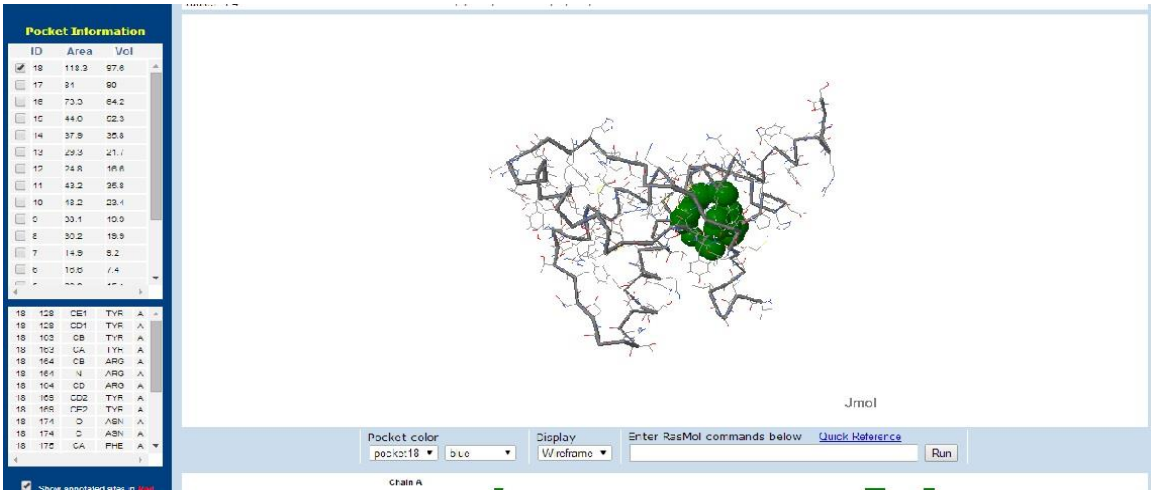
# 3.2 ACTIVE SITE PREDICTION

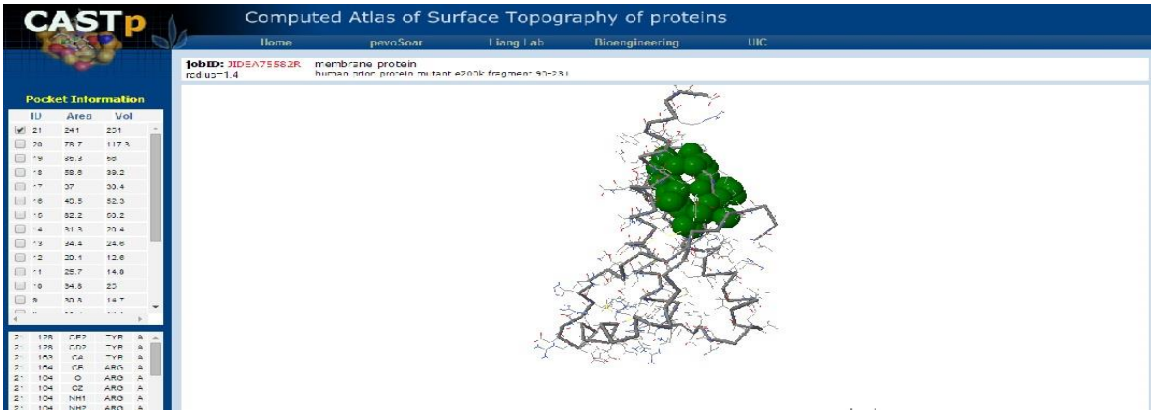
Use of Q-site finder → we open the site www.modelling.leeds.ac.uk/qsitefinder. We then hunt down obliged tying destinations of proteins and DNAs are frequently connected with structural cavities and pockets. After that from the right hand side we pick the rundown of dynamic destinations which are found as outcome.

- We can also find the active site of Protein by going to the site http://sts-fw.bioengr.uic.edu/castp/calculation.php . There we can upload or .pdb files and predict the active binfing site.

The active sites of 1XYU and 1FO7 are:

**Figure 3.0:** Active sites of 1XYU and 1FO7 as predicted in CASTP



# 3.3 STEPS INVOLVED IN DOCKING

- First we download the protein files from the site www.rcsb.org .
- Then we download the inhibitors from www.drugbank.ca in **.mol** format.
- Next we open the file in Chimera (version 1.8) software and convert the protein files as well as the inhibitors into .**pdb**.
- Then we open the .pdb file in Autodock tools (version 1.5.6) software.
- Next we add hydrogen to the molecule (polar only).
- Next we go to grid→macromolecule→choose→save as .**pdbqt** file
- Next we click on grid again and then click on grid box.
- Then we set the desired values in grid box .
- Next for the ligand we click on ligand→input→open→open the inhibitors.pdb file.
- Next we go to ligand→torsion tree→choose torsions→done.
- Next we click ligand→torsion tree→detect root.
- Next we save the file by ligand→output→save as .**pdbqt**.
- Next we create a folder name "config" and put all the .**pdbqt** files in it.
- In the config folder we create two new text files by the name conf.txt and log.txt
- In conf.txt we write:

  receptor = receptor.pdbqt

  ligand = ligand.pdbqt

  out = all.pdbqt

center_x = ____

center_y = ____

center_z = ____

size_x = ____

size_y = ____

size_z = ____

- Then we enter the desired values.
- Then we open cmd and run autodock vina by typing
  C:\config_folder_path>"C:\Program Files\The Scripps Research Institute\Vina\vina.exe" –config conf.txt –log log.txt
  And then we press enter
- We get the output stored in log.txt file.

# 3.4 INHIBITORS USED FOR DOCKING

Given below are the Pymol visualization of various inhibitors which are used in the docking process of 1XYU and 1FO7:

Fig 3.1:QUINACRINE (DB01103)

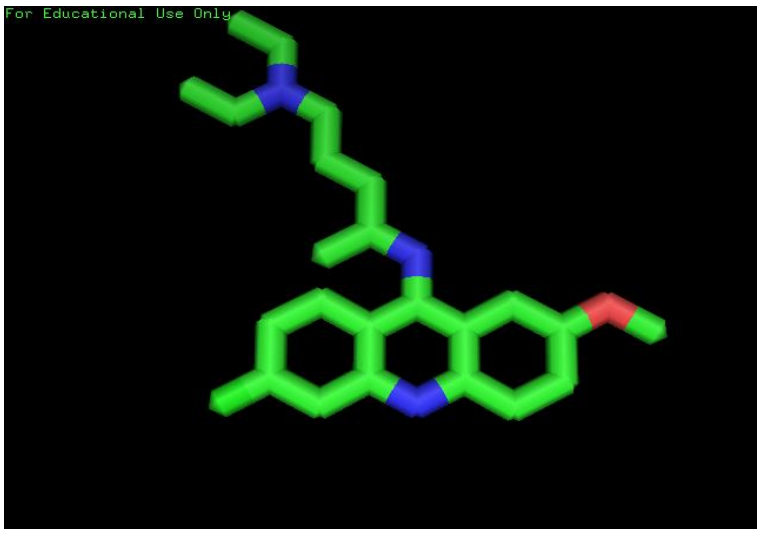



Fig 3.2: ASTEMIZOLE (DB00637)

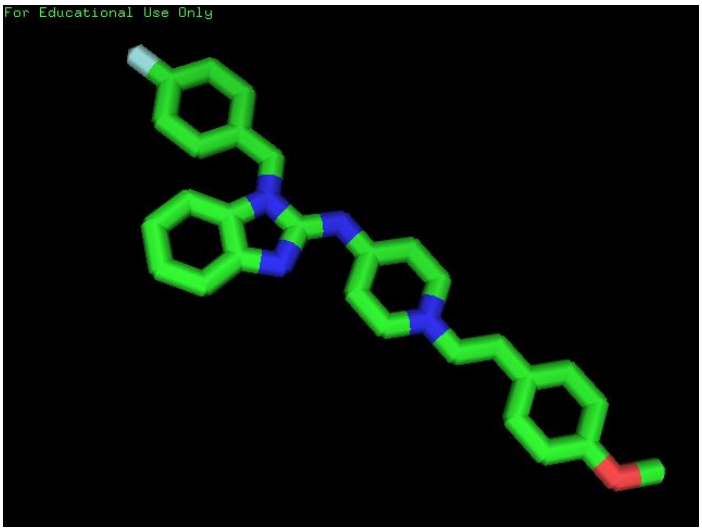



Fig 3.3: ACYCLOVIR(DB00787)

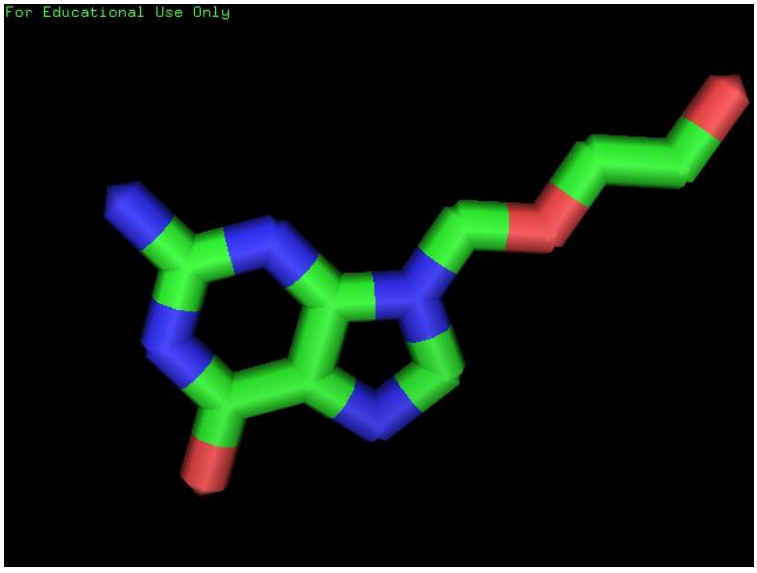



Fig 3.4 TERFENADINE (DB00342)

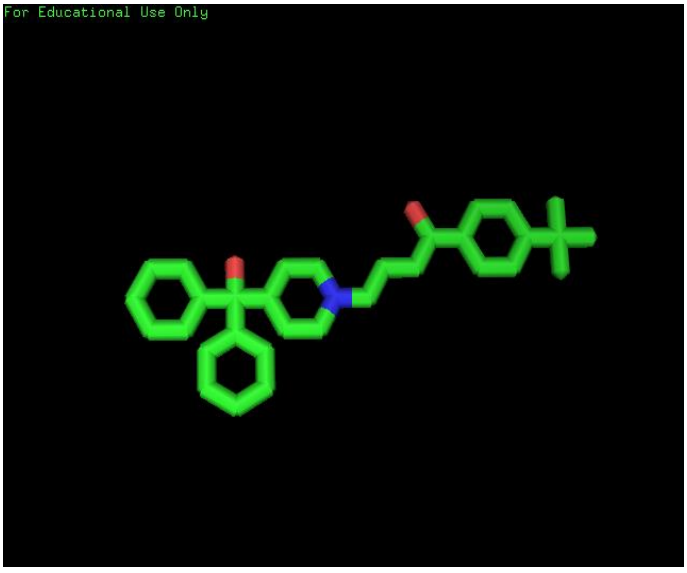


Fig 3.5:LOVASTATIN (DB00227)

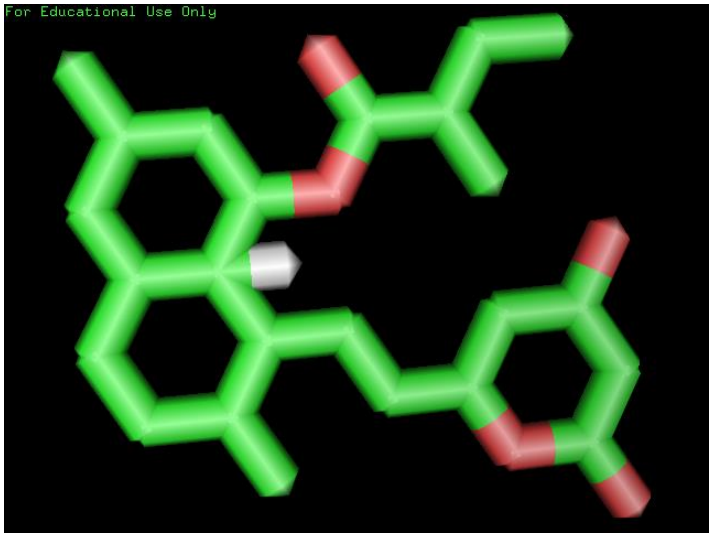



Fig 3.6: AMANTADINE (DB00915)

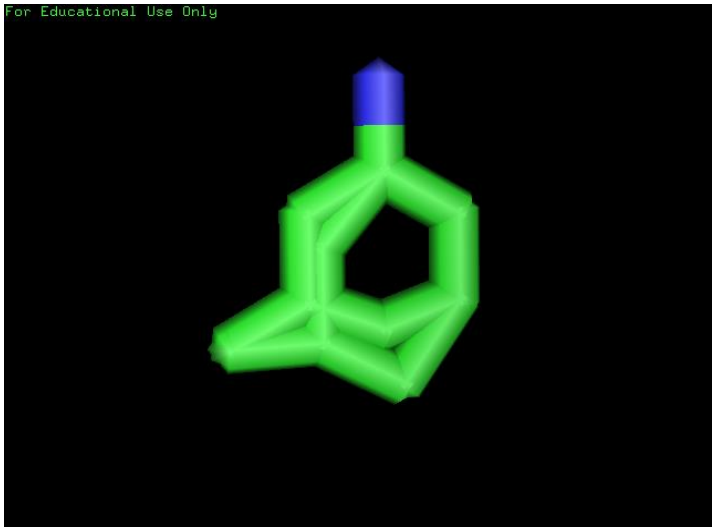



Fig 3.7:CLONAZEPAM (DB01068)

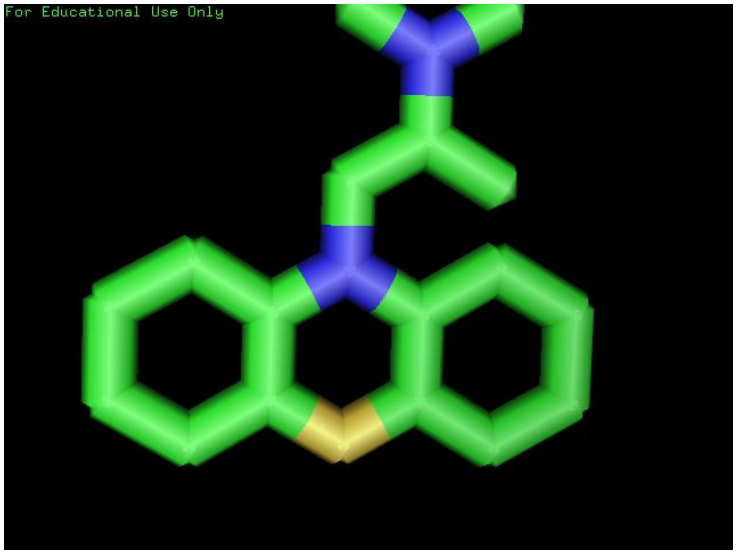



Fig 3.8: PENTOSAN POLYSULFATE (DB00686)

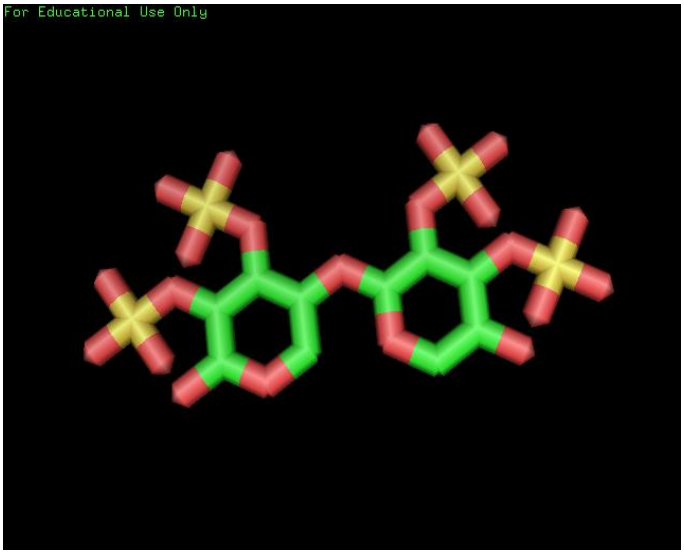


Fig 3.9:PROMETHAZINE (DB01069)

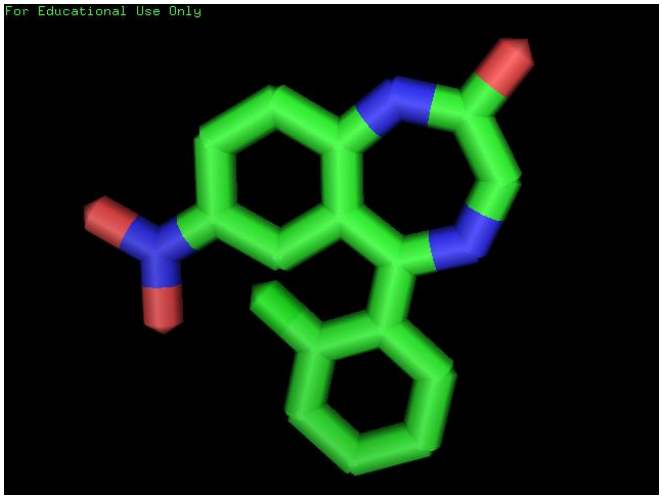



Fig 3.10: PERK INHIBITORS

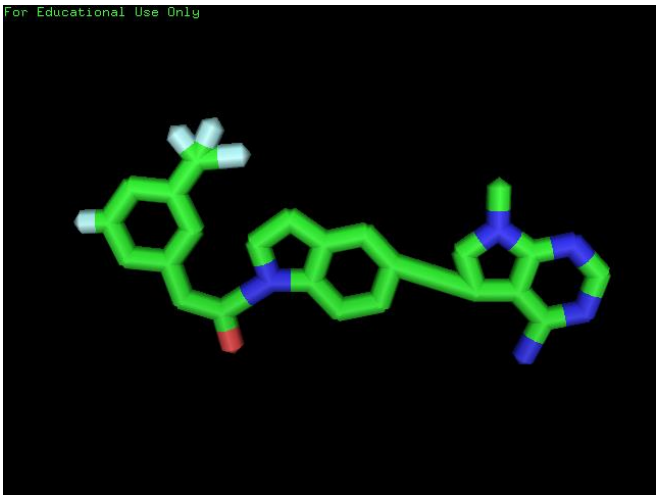



Fig 3.11/:2-(2-benzimidazolyl)-5-[4 -(2-imidazolino)phenyl]furan dihydrochloride (DB00772)

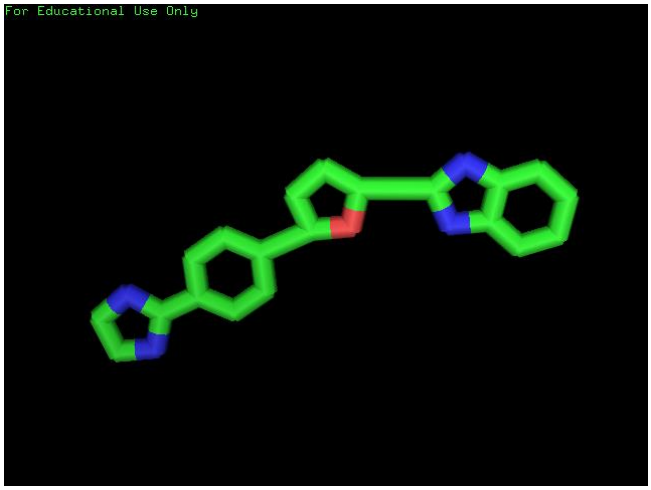



Fig 3.12 GALANTAMINE(DB00674)

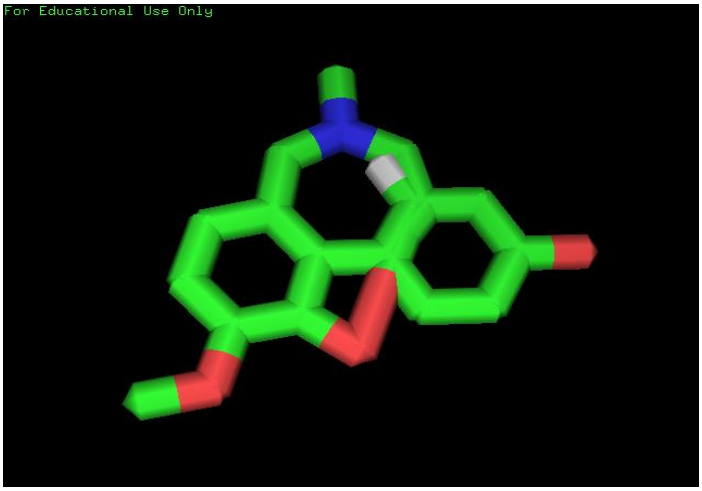


Fig 3.13:RIVASTIGMINE (DB00989)

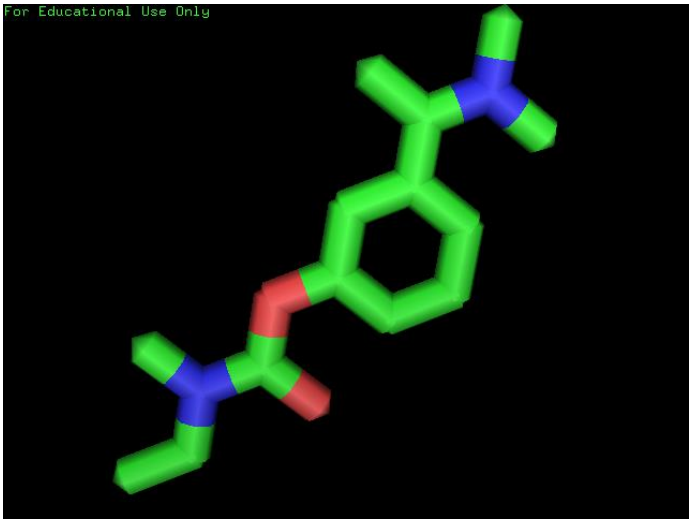



Fig 3.14:MEMANTINE (DB 01043)

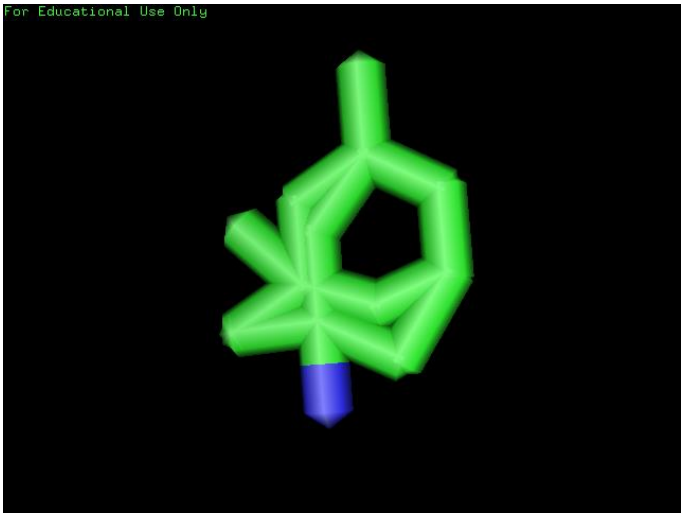



Fig 3.15:TACRINE (DB00382)

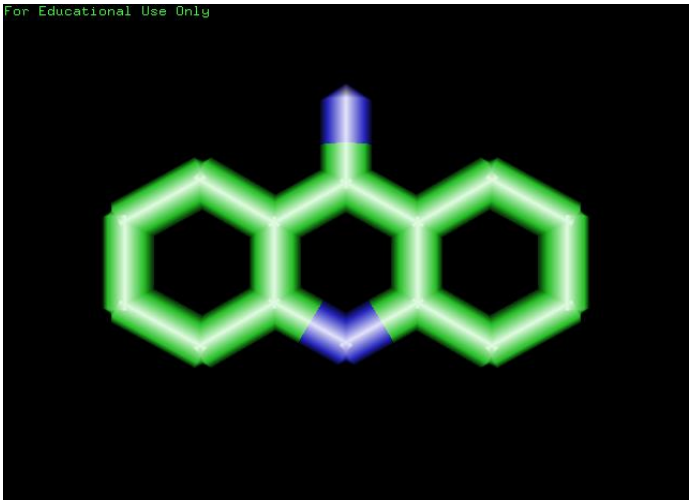



Fig 3.16:PORPHYRIN Fe(iii) (DB01710)

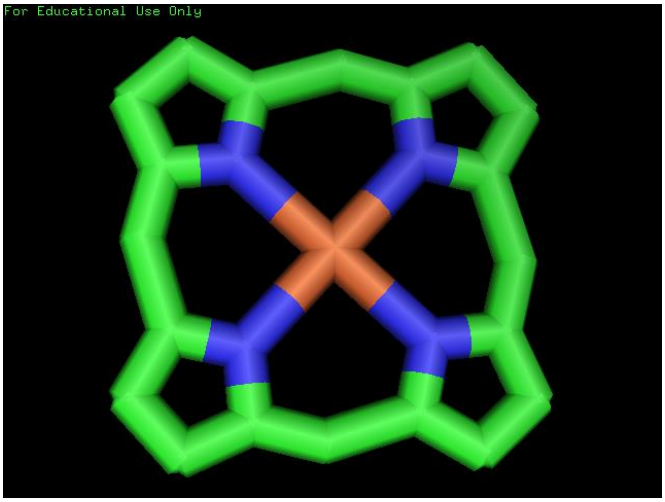
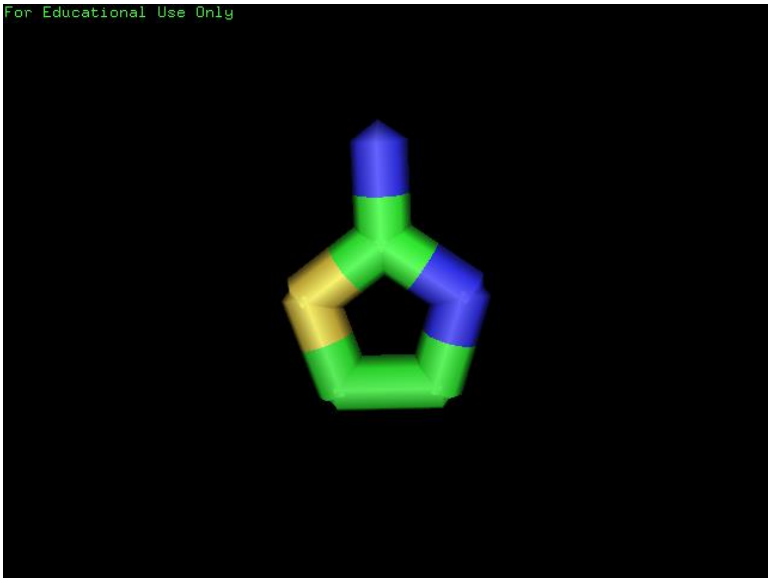
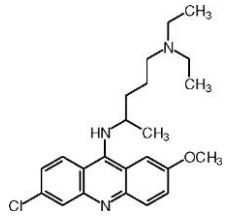
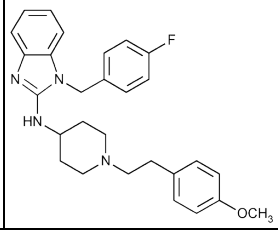
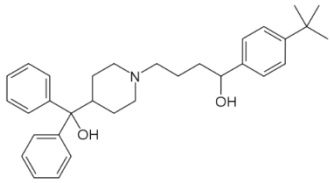
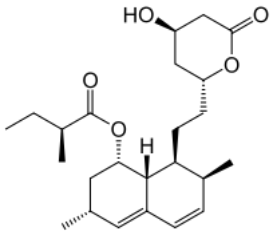


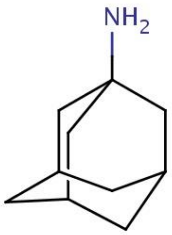
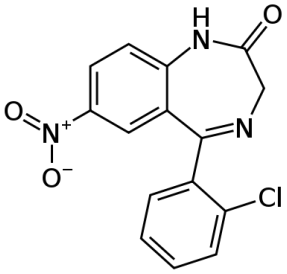
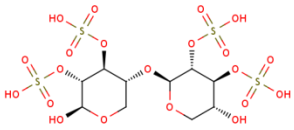
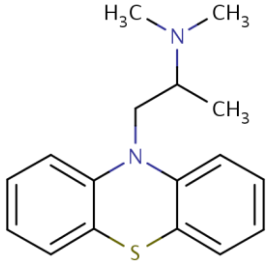
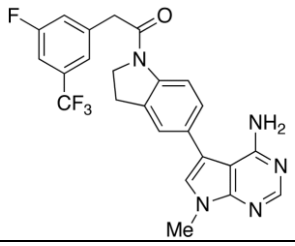
Fig 3.17:AMINOTHIAZOLINE (DB02335)

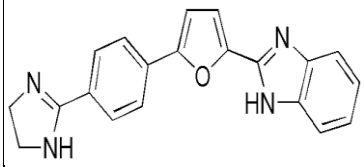
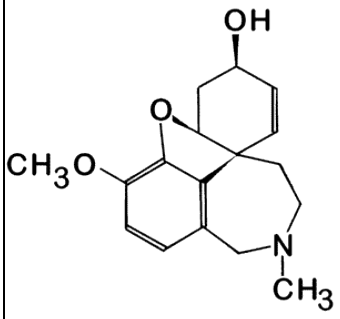
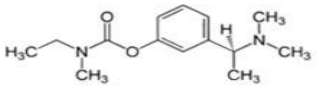
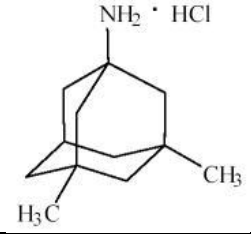
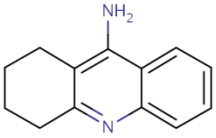


# 3.5 DOCKING RESULTS

**Table A:** Binding energy results obtained on docking (1XYU) with several inhibitors.

| SL NO. | Name of the compound | Drug bank ID | Structure | Binding Energy (Kcal/mol) |
|---|---|---|---|---|
| 1 | **QUINACRINE** | (DB01103) |  | -9.4 |
| 2 | **ASTEMIZOLE** | DB00637 |  | -7.1 |
| 3 | **TERFENADINE** | DB00342 |  | -9.6 |
| 4 | **LOVASTATIN** | DB00227 |  | -11.9 |

| | | | | |
|---|---|---|---|---|
| 5 | **AMANTADINE** | DB00915 |  | -7.6 |
| 6 | **CLONAZEPAM** | DB01068 |  | -9.7 |
| 7 | **PENTOSAN POLYSULFATE** | DB00686 |  | -11.4 |
| 8 | **PROMETHAZINE** | DB01069 |  | -9.6 |
| 9 | **PERK INHIBITORS** | |  | -12.9 |

| | | | | |
|---|---|---|---|---|
| 10 | **2-(2-benzimidazolyl)-5-[4-(2-imidazolino)phenyl]furan dihydrochloride** | DB00778 |  | -8.5 |
| 11 | **GALANTAMINE** | DB00674 |  | -8.3 |
| 12 | **RIVASTIGMINE** | DB00989 |  | -9.5 |
| 13 | **MEMANTINE** | DB01043 |  | -8.2 |
| 14 | **TACRINE** | DB00382 |  | -8.5 |

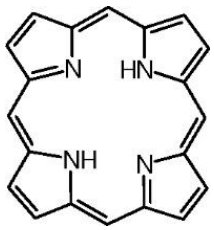
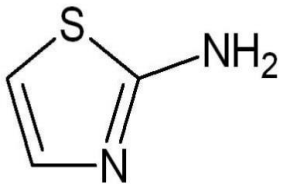
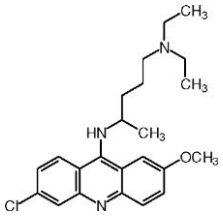
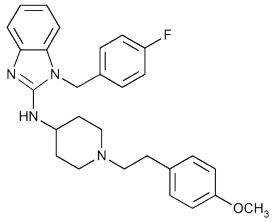
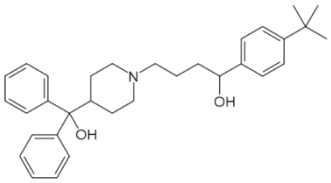
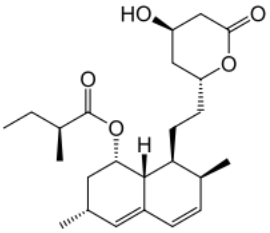
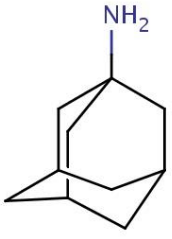
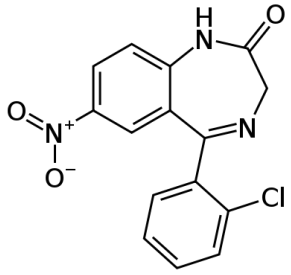
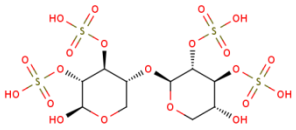
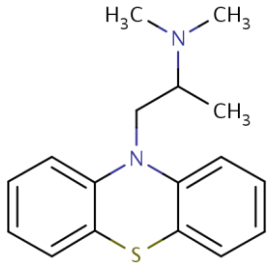
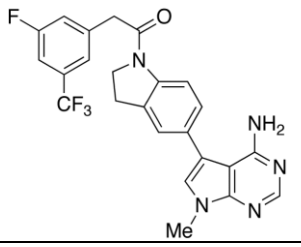
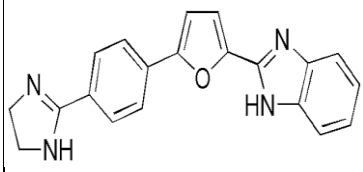
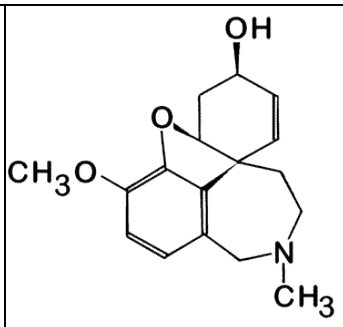
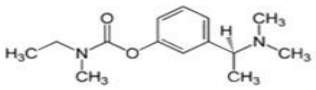
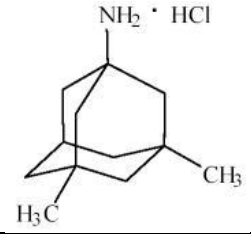
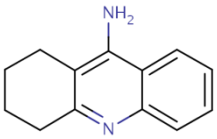
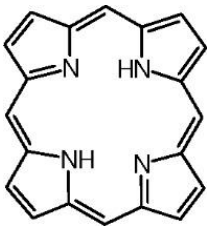
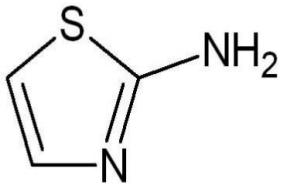
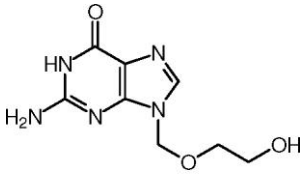
| | | | | |
|---|---|---|---|---|
| 15 | **PORPHYRIN Fe(iii)** | DB00382 |  | -6.9 |
| 16 | **AMINOTHIAZOLINE** | DB02335 |  | -2.7 |

**Table B:** Binding energy results obtained on docking (1FO7) with several inhibitors.

| **SL NO.** | **Name of the compound** | **Drug bank ID** | **Structure** | **Binding Energy (kcal/mol)** |
|---|---|---|---|---|
| 1 | **QUINACRINE** | (DB01103) |  | -8.6 |
| 2 | **ASTEMIZOLE** | DB00637 |  | -9.5 |
| 3 | **TERFENADINE** | DB00342 |  | -9.4 |
| 4 | **LOVASTATIN** | DB00227 |  | -9.3 |
| 5 | **AMANTADINE** | DB00915 |  | -5.5 |

| | | | | |
|---|---|---|---|---|
| 6 | **CLONAZEPAM** | DB01068 |  | -6.2 |
| 7 | **PENTOSAN POLYSULFATE** | DB00686 |  | -8.2 |
| 8 | **PROMETHAZINE** | DB01069 |  | -7.1 |
| 9 | **PERK INHIBITORS** | |  | -8.5 |
| 10 | **2-(2-benzimidazolyl)-5-[4-(2-imidazolino)phenyl]furan dihydrochloride** | DB00778 |  | -7.9 |

| | | | | |
|---|---|---|---|---|
| 11 | **GALANTAMINE** | DB00674 |  | -6.6 |
| 12 | **RIVASTIGMINE** | DB00989 |  | -8.7 |
| 13 | **MEMANTINE** | DB01043 |  | -6.5 |
| 14 | **TACRINE** | DB00382 |  | -6.4 |
| 15 | **PORPHYRIN Fe(iii)** | DB00382 |  | -6.7 |

| | | | | |
|---|---|---|---|---|
| 16 | **AMINOTHIAZOLINE** | DB02335 |  | -3.3 |
| 17 | **ACYCLOVIR** | DB00787 |  acyclovir | -5.6 |

# CHAPTER 4

# TOXICITY RESULTS

# 4.1 TOXICITY RESULTS:

We check the toxicity of the inhibitors by:

Select the required molecule in .pdb and open it in Ibabel. Convert the .pdb file into .mol in Ibabel and save it. Now Chembioserver website was opened.

o Open " Toxicity filtering "

o Select the "choose file" and choose the .mol file.

o Press " Proceed data " button below

o Download the toxicity results file.

The online server "Chembioserver" recognizes a list of organic toxic compounds. This server checks if the molecule contains any of organic toxic compounds. If it does not contain any of the organic toxic compounds, the result is PASS i.e Nontoxic. If it contains any toxic compounds, the result is FAIL i.e Toxic.

**Figure 4.1:** Website of ChemBioServer

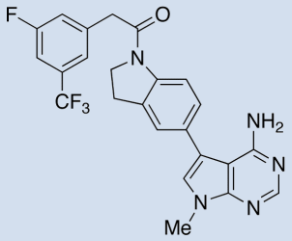
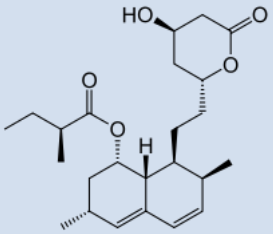
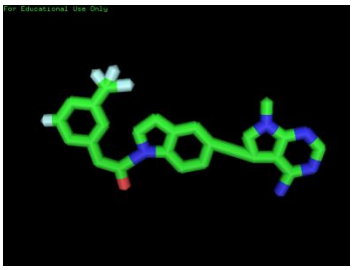
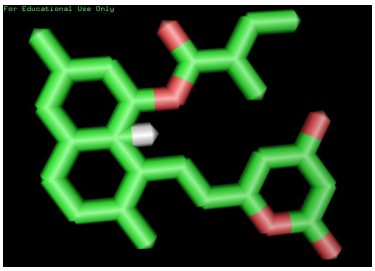
**Table C:** Toxicity results of inhibitors docked with 1XYU and 1FO7.

| **INHIBITOR** | **RESULT** | **LogP VALUE** |
|---|---|---|
| 2-(2-benzimidazolyl)-5-[4-(2-imidazolino)phenyl]furan dihydrochloride | **NON TOXIC** | **1.91** |
| LOVASTATIN | **NON TOXIC** | **3.79** |
| TERFENADINE | **NON TOXIC** | **7.18** |
| TACRINE | **NON TOXIC** | **2.92** |
| ASTEMIZOLE | **NON TOXIC** | **6.46** |
| GALANTAMINE | **TOXIC** | **1.69** |
| PENTOSAN POLYSULFATE | **NON TOXIC** | **-11.17** |
| ACYCLOVIR | **NON TOXIC** | **-1.67** |
| AMANTADINE | **NON TOXIC** | **1.92** |
| RIVASTIGMINE | **NON TOXIC** | **2.05** |
| MEMANTINE | **NON TOXIC** | **2.78** |
| CLONAZEPAM | **NON TOXIC** | **2.06** |
| PROMETHAZINE | **NON TOXIC** | **4.34** |
| QUINACRINE | **NON TOXIC** | **6.45** |
| PORPHYRIN Fe(iii) | **NON TOXIC** | **2.10** |
| AMINOTHIAZOLINE | **NON TOXIC** | **0.32** |
| PERK INHIBITORS | **NON TOXIC** | **4.11** |

# FINAL RESULTS

Two molecules Perk Inhibitors and Lovastatin have shown best binding results on docking with 1XYU and 1FO7.

**Table D:** Final results:

| | PERK INHIBITORS | LOVASTATIN |
|---|---|---|
| **CHEMICAL STRUCTURE** |  |  |
| **PYMOL VISUALIZED STRUCTURE** |  |  |
| **Docking result with 1XYU** | **-12.9 kcal/mol** | **-11.9 kcal/mol** |
| **Docking result with 1FO7** | **-8.5 kcal/mol** | **-9.3 kcal/mol** |
| **Toxicity** | **NON TOXIC** | **NON TOXIC** |
| **Partition Coeffecient (LogP) value** | **4.11** | **3.79** |
| **Molecular formula** | **C24 H20 F3 N5 O** | **C24 H36 O5** |
| **Molecular weight** | **451.16 g/mol** | **404.26 g/mol** |

## Output of docking LOVASTATIN with 1FO7 and 1XYU respectively

```
#                                                                      #
# O. Trott, A. J. Olson,                                               #
# AutoDock Vina: improving the speed and accuracy of docking           #
# with a new scoring function, efficient optimization and              #
# multithreading, Journal of Computational Chemistry 31 (2010)         #
# 455-461                                                              #
#                                                                      #
# DOI 10.1002/jcc.21334                                                #
#                                                                      #
# Please see http://vina.scripps.edu for more information.             #
########################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 100511008
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -9.3      0.000      0.000
   2        -8.9      1.833      4.393
   3        -8.7      1.474      4.402
   4        -8.7      2.412      4.738
   5        -8.7      1.705      3.446
   6        -8.5      1.657      3.615
   7        -8.5      1.545      3.007
   8        -8.3      1.580      2.792
   9        -8.3      2.474      3.223
```

```
#                                                                      #
# O. Trott, A. J. Olson,                                               #
# AutoDock Vina: improving the speed and accuracy of docking           #
# with a new scoring function, efficient optimization and              #
# multithreading, Journal of Computational Chemistry 31 (2010)         #
# 455-461                                                              #
#                                                                      #
# DOI 10.1002/jcc.21334                                                #
#                                                                      #
# Please see http://vina.scripps.edu for more information.             #
########################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1496544436
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -11.9      0.000      0.000
   2        -10.6      1.292      3.050
   3        -10.4      2.137      4.427
   4         -9.7      1.507      3.044
   5         -9.6      1.282      4.840
   6         -9.5      1.907      4.302
   7         -9.5      1.450      2.331
   8         -9.2      1.940      4.784
   9         -9.2      1.697      4.673
```

## Output of docking PERK INHIBITORS with 1XYU and 1FO7 respectively

```
########################################################################
# If you used AutoDock Vina in your work, please cite:                 #
#                                                                      #
# O. Trott, A. J. Olson,                                               #
# AutoDock Vina: improving the speed and accuracy of docking           #
# with a new scoring function, efficient optimization and              #
# multithreading, Journal of Computational Chemistry 31 (2010)         #
# 455-461                                                              #
#                                                                      #
# DOI 10.1002/jcc.21334                                                #
#                                                                      #
# Please see http://vina.scripps.edu for more information.             #
########################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1170543648
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -12.9      0.000      0.000
   2        -11.9      2.204      2.601
   3        -11.6      3.348      4.757
   4        -11.3      2.739      4.993
   5        -10.9      2.809      4.238
   6        -10.9      3.082      3.566
   7        -10.3      2.449      5.924
   8        -10.2      1.476      2.071
```

```
"                                                                      "
# O. Trott, A. J. Olson,                                               #
# AutoDock Vina: improving the speed and accuracy of docking           #
# with a new scoring function, efficient optimization and              #
# multithreading, Journal of Computational Chemistry 31 (2010)         #
# 455-461                                                              #
#                                                                      #
# DOI 10.1002/jcc.21334                                                #
#                                                                      #
# Please see http://vina.scripps.edu for more information.             #
########################################################################

Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -460384696
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -8.5      0.000      0.000
   2        -8.3      2.545      5.765
   3        -8.3      2.983      3.830
   4        -8.1      3.144      3.949
   5        -8.0      2.963      4.008
   6        -8.0      2.458      2.998
   7        -8.0      2.965      5.485
   8        -8.0      3.103      3.477
   9        -7.9      3.151      6.147
```

# DISCUSSION

Scrapie is a deadly, degenerative disease which attacks the nervous system of sheep and goats. It is a type of Transmissible Spongiform Encephalopathies (TSEs), also related to Bovine Spongiform Encephalopathies (BSEs or "mad cow disease") and chronic wasting disease of deer[11]. A protein named PRION causes Spongiform Encephalopathies which include Scrapie disease[15]. CREUTZFELDT-JAKOB DISEASE is a degenerative neurological disorder which is not curable and has a high mortal rate. It is also known as the human version of mad cow disease even though classic CJD can't be compared to bovine spongiform encephalopathy. The disease is also caused by a Prion protein. The transformation of the α-helical, cell isoform of the prion protein ($PrP^{c}$) to the insoluble, β-sheet rich irresistible, malady creating isoform ($PrP^{sc}$) is the key occasion in prion infection. In a prior study, a few types of $PrP^{c}$ changing over to $PrP^{sc}$ causes Prion replication. We then select two protein structures of Prion which are 1XYU and 1FO7 from Protein Data Bank and download them in .pdb format. We then search for various drugs which are already available in market for Scrapie disease and CJD. We also look for other potential drugs which could be useful in docking with the two proteins. We search various literatures in order to get information about various inhibitors and their role in inhibiting Prion protein replication. We found that some anti-malarial[19] and anti-cholesterol drugs from screening of several drugs are useful in the process of docking[12]. We also find the active sites of the two proteins by the help of CASTP calculator. We could also visualize the binding sites present in the protein by the help of CASTP. After docking the inhibitors with 1XYU and 1FO7, by the help of Autodock Vina 4.0, we found that inhibitors like Perk inhibitors, Lovastatin, Terfenadine, Quinacrine have shown better binding energy than others. After that we do online toxicity test on all the inhibitors and it

was found that Galantamine was toxic. We also calculate the Partition coefficient value of the inhibitors in order to predict the drug likeness.

# CONCLUSION

After docking various inhibitors with the proteins 1XYU and 1FO7, it was found that inhibitors like **Quinacrine, Terfenadine, Lovastatin, Pentosan Polysulfate, Promethazine, Perk Inhibitors, 2-(2-benzimidazolyl)-5-[4-(2-imidazolino)phenyl]furan dihydrochloride, Galantamine, Rivastigmine, Memantine** and **Tacrine** showed higher values of affinity when docked with 1XYU. After docking inhibitors with 1FO7, **Quinacrine, Astemizole, Terfenadine, Lovastatin, Pentosan Polysulfate, Perk Inhibitors** and **Rivastigmine** showed higher values of affinity. Toxicity tests of the inhibitors were done on all the inhibitors docked and all them (except **GALANTAMINE)** passed the toxicity test. Eventually it was found that the above inhibitors proved to be effective in the process of inhibiting the two Prion proteins 1XYU and 1FO7.

At present a huge amount of research is being done on this disease and day by day new inhibitors are found which are effective. Thus the future in doing research on this disease is quite approaching.

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