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*Research article*

# MODELLING AND DRUG DESIGNING OF GLUTAMATE RECEPTOR INVOLVED IN ALZHEIMER'S DISEASE

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ABSTRACT: Glutamate receptors dysfunction plays an important role in the pathogenesis and disturbance which is probably a secondary phenomenon to other neurochemical, genetic or metabolic changes, and essential to the development of Alzheimer Disease. Glutamate receptors are synaptic receptors, which are located on the membranes of neuronal cells. Glutamate is used to assemble proteins and also it is abundant in many areas of the body, but it also functions as a neurotransmitter and is particularly abundant in the nervous system. In this work we have modeled a three dimensional structure for Glutamate [NMDA] receptor subunit using MODELLER7V7 software with 2RC7 (Crystal Structure of the NR3A Ligand Binding Core Complex with Glycine) as template. With the aid of Molecular dynamics and Molecular simulations studies it was identified that the generated structure was reliable. This structure was used to identify better inhibitor using docking studies. The drug derivatives were docked to the Glutamate receptor structure into the active site containing residues such as ASP21, LEU30, TYR31, HIS59, and MET60. Among the 21 derivatives 14 were docked and 3rd drug derivative showed better docking energy than the others. Our experimental studies can be further used to develop a better drug for Alzheimer disease.

**Key words:** Alzheimer disease, Glutamate receptors, Homology Modelling, Drug designing, Molecular simulations

## INTRODUCTION

Glutamate receptors consist of two major classes which are ionotropic (iGluR) and metabotropic glutamate receptor (mGluR). The iGluRs are cation-specific ion channels and, are subdivided into three groups by their specific agonists, namely N-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainic acid (KA). mGluRs are a family of G-protein-coupled and can be divided to three groups, I,II and III, according to their signal transduction pathways, pharmacology and sequence homology. While the expression level and localization of iGluR have been extensively studied in Alzheimer's disease (AD), the regulation of iGluR expression is still controversial (Lee et al. 2002a). It is estimated that over 5 million people live with Alzheimer's disease in the USA, and it is predicted that by the year 2025 there will be an average 50% increase in patients with AD (Hebert, L.E., 2004). AD is a leading cause of dementia in the aging population (Ashford, J.W. 2004). Patients with AD experience symptoms including cognitive alterations, memory loss and behavioral changes (Katzman, R. 1986 and Budson, A.E. 2005). The dementia in AD is associated with neuro degeneration that is characterized initially by synaptic injury (Terry, R, 1994 and DeKosky, S, 1990) followed by neuronal loss (Terry, R., 1981). This is accompanied by astrogliosis (Beach, T., 1989), microglial cell proliferation (Rogers, J.,198 and Trojanowski, J.Q 2000) and the presence of neurofibrillary tangles composed of dystrophic neurites and hyperphosphorylated tau (Terry, R.1994, Trojanowski, J.Q. and Lee, V.M. 2000, Lee, V.M., 2001, Iqbal, K, 2002, Crews, L., 2010).

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More recent studies have uncovered evidence, suggesting that another component to the neurodegenerative process in AD might include the possibility of interference with the process of adult neurogenesis in the hippocampus (Boekhoorn, K.2006 and Li, B., 2008). Indeed, while some authors report that the NMDA glutamate receptor subunit NR1 is markedly increased in vulnerable neurons of AD (Ikonomovic et al. 1999), other reports indicate that there is a reduction of NMDA receptors in AD (Hynd et al. 2001, Sze et al. 2001, Ulas and Cotman 1997), or no difference between AD and age-matched controls (Bi and Sze 2002, Panegyres et al.2002, Thorns et al. 1997, Wakabayashi et al. 1999). Despite this disparity, it is important to note that the distribution of NMDA receptors does, however, correlate with the predilection for neurofibrillary tangles and neuritic plaques in hippocampal subfields (Geddes and Cotman 1986). Although it is not clear whether NMDA expression is decreased in AD, it should be noted that elective decrease of NMDA receptors may affect the memory dysfunction in AD. For example, a recent study clearly showed NMDA receptors play a pivotal role in memory formation (Clayton et al. 2002, Nakazawa et al. 2002) and, therefore, it is plausible that alterations of NMDA receptors may be responsible for the decreased memory function that is clinically evident in patient with AD. Indeed, memory impairments are evident when NMDA antagonists are injected into different brain structures in animal experiments (Castellano et al.2001) and glutamate levels in cerebrospinal fluid (CSF) and tissue are decreased in AD (Hyman et al. 1987, Kuiper et al. 2000). Thus, it is likely that NMDA receptors may contribute significantly to the pathophysiology in AD *via* degeneration of synaptic activity rather than cell death *via* excitotoxicity.

## MATERIALS AND METHODS

### 3D model building:

The MODELLER software was used to build the initial model of Glutamate receptor. The first step is searching a number of related sequences to find a related protein as a template by the BLAST program. The high sequence identity between Glutamate receptor **(**Q8TCU5**)** and the reference protein 2RC7 is 60%, which allowed for rather straight forward sequence alignment. In the second step, the backbone coordinates of the residues in Glutamate receptor were generated with the MODELLER software. The structurally conserved regions (SCRs) were determined by multiple sequence alignment, which is based on the Needleman and Wunsch Algorithm, and the coordinates of SCRs in Glutamate receptor were generated by copying from 2RC7. The structure having the least modeller objective function, obtained from the modeller was improved by molecular dynamics and equilibration methods using NAMD 2.5 software (Kale, *et al*, 1999) using CHARMM27 force field for lipids and proteins along with the TIP3P model for water. The energy of the structure was minimized with 10,000 steps. A cutoff of 12 Å (switching function starting at 10 Å) for van der Waals interactions was assumed. Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model was improved.

The final structure obtained was checked by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) and environment profile using ERRAT graph (Structure Evaluation server). This model was used for the identification of active site and for docking of the substrate with the protein.

### Binding-site analysis:

The Bindig-site of Glutamate receptor was identified using CASTP server. A new program, CAST, for automatically locating and measuring protein binding pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory.

### Docking of derivatives to Glutamate protein

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows as partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of the P-gp. The interaction of these compounds with the active site residues are thoroughly studied using molecular mechanics calculations. During docking, the default algorithm speed was selected and the ligand binding site was defined within a 10 $A^0$ radius. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied.

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### Gold Score fitness function

Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3. Ligand internal vander Waals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

## RESULTS AND DISCUSSION

### Homology modeling of Glutamate receptor:

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only the 2RC7 which has a high level of sequence identity with the Glutamate receptor domain. Structurally conserved regions (SCRs) for the model and the template were determined by sequence alignment and the SCRs were determined as shown by Fig.1.

In the following study, we have chosen 2RC7 as a reference structure for modeling Glutamate receptor domain. Coordinates from the reference protein (2RC7) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. In the modeller we will get a 20 PDB out of which we select a least energy. The energy unit will be in kilo joule. All side chains of the model protein were set by rotamers. The final stable structure of the Glutamate receptor protein obtained is shown in Figure 2. By the help of SPDBV it is evident that Glutamate receptor domain has 4 helices and 2 sheets.

The final structure was further checked by verify3D graph and the results have been shown in Figure 3: The overall scores indicates acceptable protein environment.

**Table 1: % of residue falling in the core region of the Ramachandran's plot**

| | |
|---|---|
| % of residue in most favored regions | 84.4 |
| % of residue in the additionally allowed zones | 13.9 |
| % of residue in the generously regions | 1.1 |
| % of residue in disallowed regions | 0.6 |
| % of non-glycine and non-proline residues | 100.0 |

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```
domain      ----------------------------------------------------VPIK 4
template    KLHLRVVTLIEHPFVFTREVDDEGLCPAGQLCLDPMTNDSSMLDRLFSSLHSSNDTVPIK
60
                                                ****

domain
FKKCCYGYCIDLLEKIAEDMNFDFDLYIVGDGKYGAWKNGHWTGLVGDLLRGTAHMAVTS 64
template
FKKCCYGYCIDLLEQLAEDMNFDFDLYIVGDGKYGAWKNGHWTGLVGDLLSGTANMAVTS 120
          **************..************************************ ***.*****
domain      FSINTARSQVIDFTSPFFSTSLGILVRTRDTAAPIGAFMWPLHWTMWLGIFVALHITAVF
124
template    FSINTARSQVIDFTSPFFSTSLGILVRTRGT--------------------------- 151
          ****************************.*
domain      LTLYEWKSPFGLTPKGRNRSKVFSFSSALNICYALLFGRTVAIKPPKCWTGRFLMNLWAI
184
template    ------------------------------------------------------

domain      FCMFCLSTYTANLAAVMVGEKIYEELSGIHDPKLHHPSQGFRFGTVRESSAEDYVRQSFP
244
template    ----------------------ELSGIHDPKLHHPSQGFRFGTVRESSAEDYVRQSFP 187
                     ************************************
domain
EMHEYMRRYNVPATPDGVEYLKNDPEKLDAFIMDKALLDYEVSIDADCKLLTVGKPFAIE 304
template
EMHEYMRRYNVPATPDGVQYLKNDPEKLDAFIMDKALLDYEVSIDADCKLLTVGKPFAIE 247
          ******************.*********************************************
domain      GYGIGLPPNSPLTANISELISQYKSHGFMDMLHDKWYRV 343
template    GYGIGLPPNSPLTSNISELISQYKSHGFMDVLHDKWY-- 284
          ************.*****************.******
```

**Fig 1: Alignment of Glutamate receptor with 2RC7**

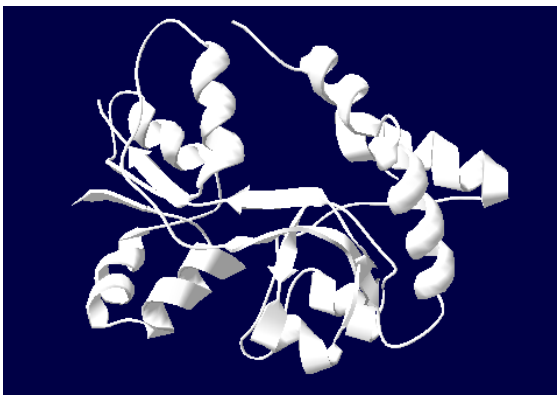

**Figure 2: Modeller result- 3D structure of Glutamate**

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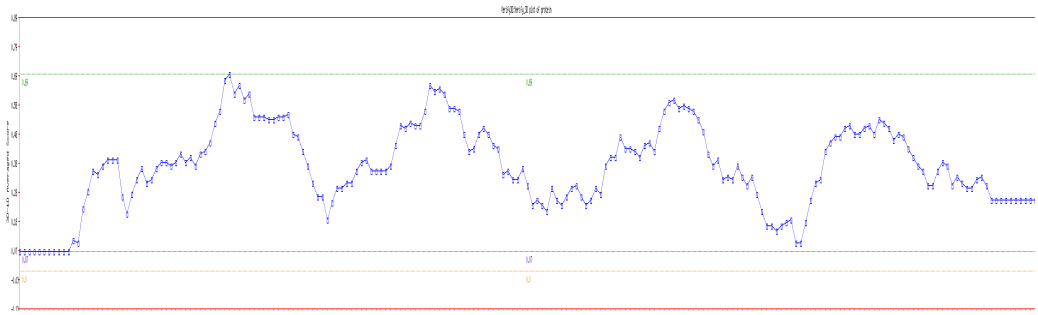

**Figure 3:The 3D profiles verified results of Glutamate receptor model; overall quality score indicates residues are reasonably folded.**

## Validation of Glutamate receptor Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program (Figure 4). The distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table 1. The RMSD (Root Mean Square deviation) deviation for covalent bonds and covalent angles relative to the standard dictionary of GLUTAMATE [NMDA] RECEPTOR SUBUNIT 3A was -5.27 and -0.55 Å. Altogether 99.4 % of the residues of GLUTAMATE [NMDA] RECEPTOR SUBUNIT 3A **(Q8TCU5)** was in favored and allowed regions. The overall PROCHECK G-factor of GLUTAMATE [NMDA] RECEPTOR SUBUNIT 3A was – 2.32 and verify3D environment profile was good.

## Superimposition of 2RC7 with Glutamate receptor domain

The structural superimposition of 2RC7 template and Glutamate receptor is shown in Figure 5. The weighted root mean square deviation of trace between the template and final refined models 0.72A°. This final refined model was used for the identification of active site and for docking of the substrate with the domain Glutamate receptor.

## Active site Identification of Glutamate receptor domain

After the final model was built, the possible binding sites of Glutamate receptor was searched based on the structural comparison of template and the model build and also with CASTP server and was shown in Figure 6. Since, Glutamate receptor from Human and the 2RC7 are well conserved in both sequence and structure; their biological function should be identical. Infact from the structure-structure comparison of template, final refined model of Glutamate receptor domain using SPDBV program and was shown in Figure3. It was found that secondary structures are highly conserved and the residues, PHE5, LYS7, CYS9, TYR10, GLY11,TYR12, CYS13, ILE14, PHE28, LEU30, TYR31, ALA61, VAL62, THR63, PHE82, VAL230, LEU281.

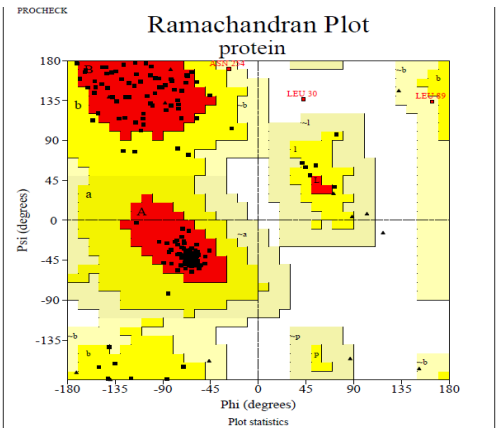


**Figure 4: Ramachandran Plot**

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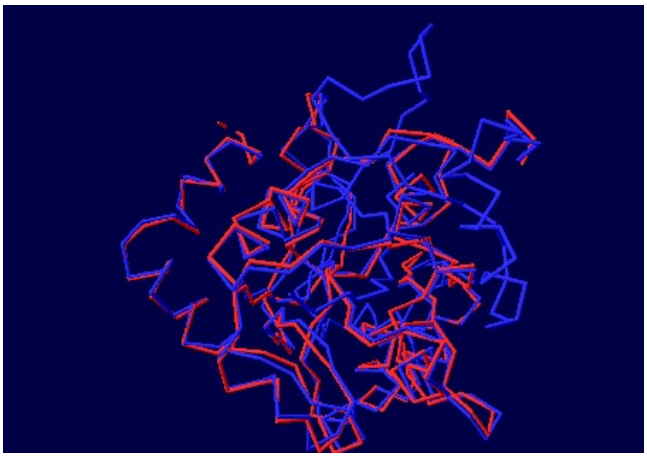

**Figure 5: superimposition of C alpha trace of Glutamate receptor (represented in red color) and 2RC7 (represented in blue color).**

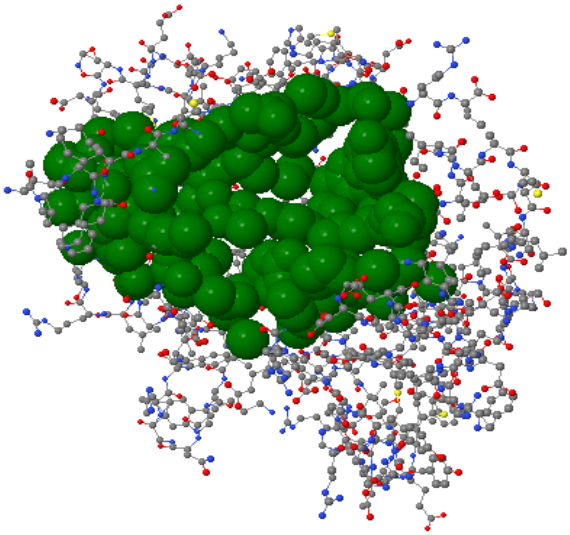

**Figure 6: Active site of Glutamate receptor**

## Docking of inhibitors with the active site of Glutamate receptor :

Docking of the inhibitors with Glutamate receptors was performed using GOLD 3.0.1, which is based on genetic algorithimn (Figure 7). This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a cocrystalized ligand by 4A.

This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with SILVER. To this set, the substrate corresponding to the modeled protein were added. Docking of inhibitors with the active site of protein.

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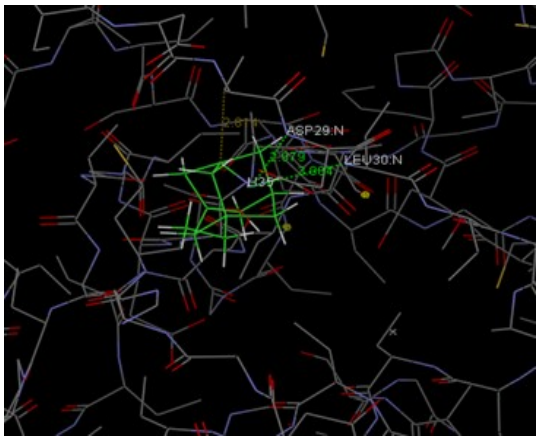


**Figure 7: Docking of Molecule3 with Glutamate receptor**

## CONCLUSION

In this work, we have amplified and constructed a 3D model of Glutamate receptor domain, from Human using the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by ERRAT & PROCHECK program, and the results show that this model is reliable. The stable structure is further used for docking of substrate with the derivatives of Memantine. Docking results indicate that conserved amino-acid residues in Glutamate receptor main play an important role in maintaining a functional conformation and are directly involved in donor substrate binding. The interaction between the domain and the inhibitors proposed in this study are useful for understanding the potential mechanism of domain and the inhibitor binding. As is well known, hydrogen bonds play important role for the structure and function of biological molecules. In this study it was found that PHE5, LYS7, CYS9, TYR10, GLY11, TYR12, CYS13, ILE14, PHE28, LEU30, TYR31, ALA61, VAL62, THR63, PHE82, VAL230, LEU281 of Glutamate receptor are important for strong hydrogen bonding interaction with the inhibitors. To the best of our knowledge ASP21, LEU30, TYR31, HIS59, and MET60 are conserved in this domain and may be important for structural integrity or maintaining the hydrophobicity of the inhibitor-binding pocket. The molecule3 showed best docking results with target protein.

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