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Ionotropic Glutamate Receptor Subtype 6: Docking and Site Moiety Map Analysis Study

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ABSTRACT

In an attempt to discover more potent ligands, we present here a docking study for ionotropic glutamate receptor subtype 6 which is important in many neurodegenerative diseases. Total 97 compounds including a small series of commercially available compounds are characterized in iGluR6 binding. Z-scores were employed to recognize hits for this receptor. Twenty eight compounds were shown to have excellent binding affinity on this target. A detailed in silico study showed the strong interactions of compound no. 2h and 13515655 with iGluR6. Total four anchors were found. They involve three hydrogen bonding interactions and one vander waal interaction.

Key words: Ionotrropic glutamate receptor 6, Binding affinity, Hydrogen bonding, Vander waal interaction.

INTRODUCTION

Glutamic acid is the major excitatory neurotransmitter in the mammalian central nervous system. Besides its physiological functions, it is involved in many neuropathologies. The excitotoxicity of glutamate is well established in ischemia, convulsions, and epilepsy. Glutamate is also implicated in neurodegenerative diseases such as Huntington's and Parkinson's diseases, in drug withdrawal symptoms, in pain and in psychiatric disorders such as anxiety and schizophrenia. Thus, glutamate receptors are excellent therapeutic targets [1]. Glutamate receptors are subdivided into ionotropic (iGluRs) and metabotropic (mGluRs) receptors. The ionotropic receptors mediate fast synaptic transmission through ligand-gated ion channels while metabotropic receptors are G protein coupled and have a modulatory role in the CNS [2, 3].

The mGluRs are divided into three groups according to their sequence similarity, transduction mechanism, and pharmacological profile. Group 1 receptors (mGlu1,5R) activate phospholipase C, while group 2 (mGlu2,3R) and group 3 (mGlu4,6,7,8R) inhibit adenylyl cyclase when expressed in heterologous system [4, 8]. Based on ligand affinity studies, the iGluRs have been further divided into three groups: The 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionic acid (AMPA) receptors (comprising the subtypes iGluR1-4), the (-)-(a)-kainic acid (KA) receptors (comprising subtypes iGluR5-7 and KA1,2) and the N-methyl-D-aspartic acid (NMDA) receptors (comprising subtypes NR1, 2A-D, 3A-C) [3].

Kainic acid and other kainoids such as domoic acid are highly neurotoxic, and their patterns of neurotoxicity and resulting CNS dysfunction have been well characterized. These and other findings suggest important potential therapeutic uses of kainate antagonists including the treatment of epilepsy, pain, and acute and chronic

neurodegeneration. When presynaptic kainate receptors are activated they inhibit the release of glutamate. Thus, highly selective agonists for these receptors, if not neurotoxic, may also have important therapeutic potential [5, 6]. Among the ionotropic glutamate receptors, the pharmacology of kainate receptors is the least understood primarily because of the limited number of selective ligands, agonists and antagonists, available for pharmacological studies [7].

Certainly, increased knowledge of the structural features governing iGluR binding affinity and selectivity could play a major role in the discovery and development of new ligands with enhanced potency and selectivity.

Keeping above facts in mind, we present herein, the docking study of some reported and commercially available compounds and binding site mapping of ionotropic Glutamate receptor subtype 6.

MATERIALS AND METHODS

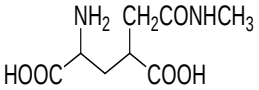
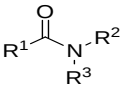
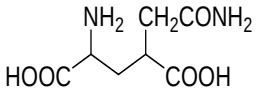
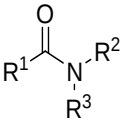
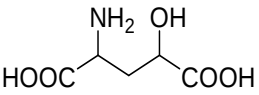
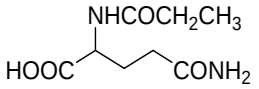
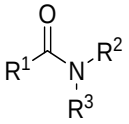
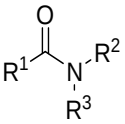
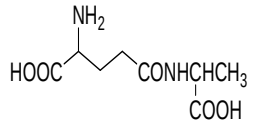
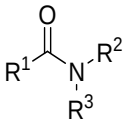
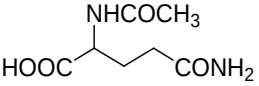
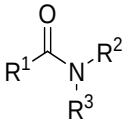
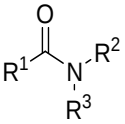
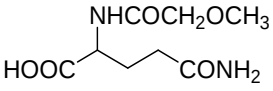
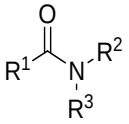
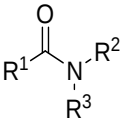
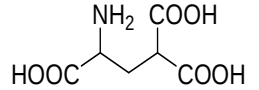
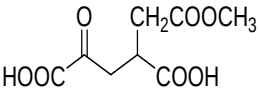
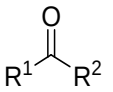
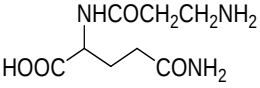
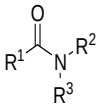
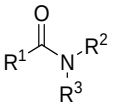
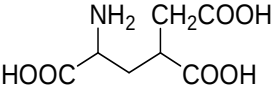
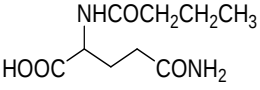
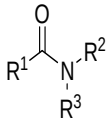
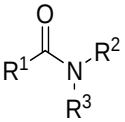
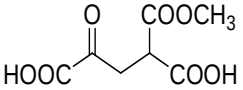
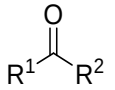
The scope of virtual screening is to enrich a set of molecules extracted from a database with active compounds by weeding out those that are likely to be inactive, prior in vitro assays [8]. We performed an automated docking study in order to find most active ligands for iGluR6, using igemdock software. The Generic evolutionary method in the igemdock4.2.3 was used for docking experiments.

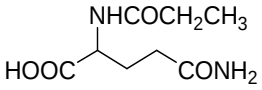
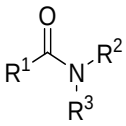
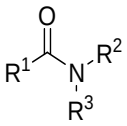
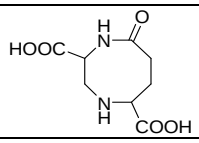
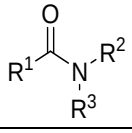
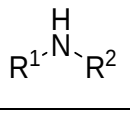
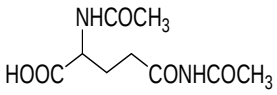
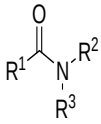
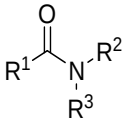
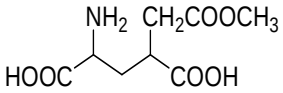
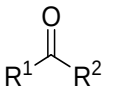
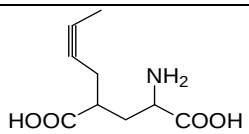
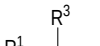
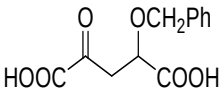
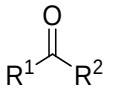
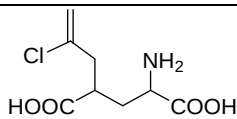
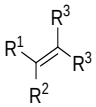
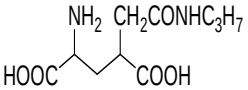
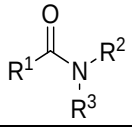
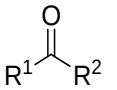
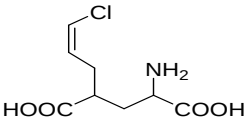
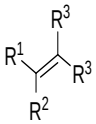
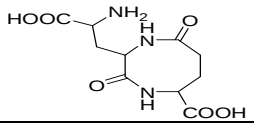
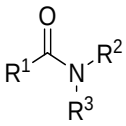
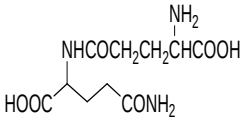
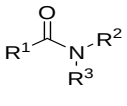
To access the virtual screening program, the docking accuracy of the igemdock for the ionotropic glutamate receptor 6 was evaluated by docking the co-crystallized ligand quisqualic acid (QUS) into the binding site. The protein structure was taken from Protein data bank (1S9T) [9]. The docked conformation of QUS was compared with co crystallized QUS conformation in crystallized protein iGluR6 based on the root mean square deviation (rmsd). Igemdock was used to perform virtual screening on iGluR6 using screening set consisting of 97 compounds (known and unknown ligands). The unknown ligands were taken from Zinc database using glutamic acid as a structure for search. Known ligands were taken from published work [9, 10]. The numbering of compounds taken is retained as it is. Structure of the known ligands were prepared using chem. office 8.0.4 and saved in mdl.mol files after energy minimization with MM2 method. Structures of unknown compounds taken from Zinc were splitted using Zinc split. A population size of 300 with 70 generations and 3 solutions were used in docking accuracy setting. Values of scoring functions were taken as default.

RESULTS AND DISCUSSION

Total 97 compounds were docked into the active site of iGluR6. Most of the docking program use energy based scoring methods which are often biased toward both the selection of high molecular weight compounds and charged polar compounds. These approaches generally cannot identify the key features (for example pharmacophore scores) that are essential to trigger or block the biological responses of the target protein. To solve this problem we have carried out site-moiety mapping to infer the key features that describe relationship between the moiety preferences and physicochemical properties of the binding site. Best poses of the docked compounds were taken in pdb file format and they were used for SiMMap analysis [11]. Active site of iGluR6 1S9T excluding QUS co-crystallized ligand was used in pdb file format. Then site moiety map analysis was performed. The binding affinities of compounds were estimated on the basis of relative binding scores [12]. In order to verify the results of the docking study, we calculated the Z-scores (Table-1)

Table 1. Site moiety map and Z-Scores of hits

Compd. No.	Structure	Rank	Score	H1	H2	H3	V1
2h		1	4.339	R−NH$_2$	R−COOH		Others
2g		2	4.325	R−NH$_2$	R−COOH		Others
2a		3	4.315	R−NH$_2$	R−COOH	R−OH	Others
13515655		4	4.304	R−COOH			Others
01529737		5	4.303	R−COOH		R−NH$_2$	Others
01731762		6	4.302	R−COOH			Others
19389831		7	4.302	R−COOH			Others
2c		8	4.301	R−NH$_2$	R−COOH	R−COOH	Others
1e		9	4.296	R−COOH	R−COOH		Others
40479001		10	4.291			R−NH$_2$	Others
2f		11	4.289	R−COOH	R−COOH	R−NH$_2$	Others
19395966		12	4.287		R−COOH		Others
1d		15	4.272	R−COOH		R−COOH	Lactone

19282023		16	4.271	R−COOH			Others
02384790		17	4.265	R−COOH			Lactams
32228277		18	4.264		R−COOH		Others
2e		19	4.26	$R-NH_2$	R−COOH		Others
36		20	4.252	R-COOH	R−COOH	$R-NH_2$	
1b		21	4.25			R−COOH	
50		22	4.25	R−COOH		R−COOH	
2i		23	4.249	R−COOH			Others
45		24	4.247	$R-NH_2$	R−COOH	R−COOH	
04544977		25	4.24	R−COOH		$R-NH_2$	Lactams
04544971		28	4.223	$R-NH_2$	R−COOH		Others

The Z-score possesses high predictive accuracy of affinity of ligand-receptor binding. The higher Z-score indicates the stronger binding affinity with the receptor subtype. The anchor candidates are found by identifying the pockets with significant interacting residues and moieties with Z-score ≥ 1.645. Compounds with Z-score ≥ 1.645 may be either inhibitors or activators of iGluR6. The anchor candidates with same interaction type and with distance < 3.5 A^0 are grouped into one anchor.

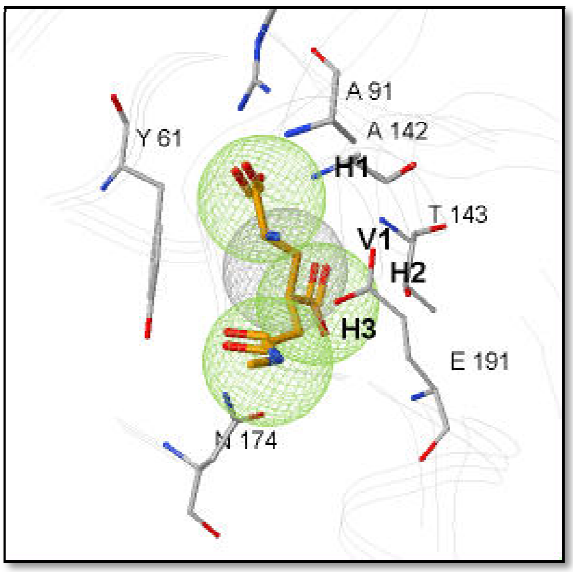


Figure 1 Anchors along with highest ranking compound (2h)

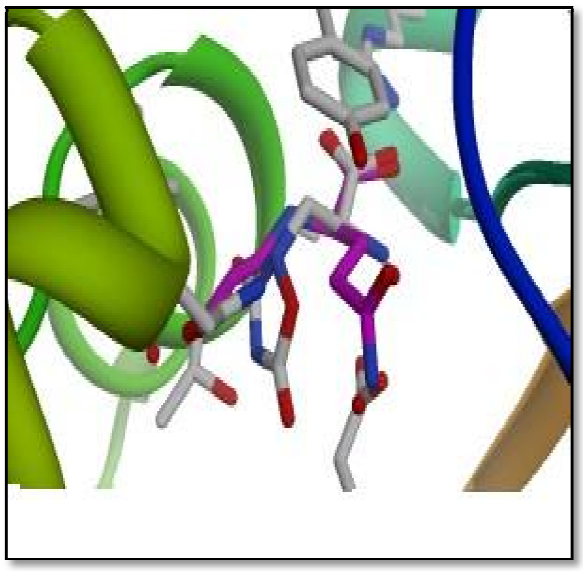


Figure 2 Docked conformation of one of the ligands

According to SiM Map analysis, the ligands were found to form four anchors H1, H2, H3 and V1 (Table-1 and Fig-1). It can be seen from Table-2 that the H1 anchor constitutes the pocket, containing residues alanine 91, arginine 96 and alanine 142. The moieties present in this anchor are carboxylic acid, amines, substituted amides, secondary amines and others. The pocket for the H2 anchor, contain threonine 143, asparagine 174 and glutamic acid 191 as key amino acid residues. The moieties present in this anchor are carboxylic acid, substituted amides, ketones, esters, lactams and others. In the similar manner the H3 anchor constitutes the pocket containing residues tyrosine 61, asparagines 174 and glutamic acid 191. The moieties are carboxylic acid, substituted amides, primary amines, ketones, esters and others. And the pocket for V1 anchor has the residues, tyrosine 61, alanine 142 and glutamic acid 191. Heterocyclic moieties, alkenes, lactones, lactams, alkynes and others are present in this anchor.

Anchors along with highest ranking compound are shown in Figure 1. The docked conformation of one of the ligands in the surface model of protein is shown in Figure 2. The representation of interactions essential for activity of 1S9T is shown in Figure 3.

On the basis of these interactions, we found that the total energy for the hydrogen bonding interaction in anchor H1 (for compound 2h) is -15.8Kcal/mol which shows strong binding. Amino group of 2h participated in this hydrogen bonding interactions (H1) with main chain of alanine 91, side chain of arginine 96 and main chain of alanine 142. Respective energies for these interactions are -3.5, -8.8 and -3.5Kcal/mol. The carboxylic acid group of 2h compound forms hydrogen bonds in anchor H2 with side chains of threonine 143, asparagines 174 and glutamic acid 191. Binding energies for these bonds are -7.9, -0.1, -0.1Kcal/mol and total energy for H2 anchor is -8.9Kcal/mol. In the similar manner H3 anchor have the substituted amide moiety which is involved in hydrogen bonding with side chains of tyrosine 61, asparagines 174 and glutamic acid 191. Binding energies with these amino acids in H3 anchor are -2.5, -3.5, -3.5Kcal/mol and total is -9.5Kcal/mol. In 2h the V1 anchor constitutes the side chain of tyrosine 61, main chain of alanine 142 and side chain of glutamic acid 191 with binding energies -5.3, -2.5 and -2.3Kcal/mol. Total energy for V1 anchor is -10.1Kcal/mol and moiety involved is propyl chain.

The compound no. 13515655 also has the four anchors with same amino residues as reported for compound 2h. Moiety types are shown in Table 2 and binding energies for this compound are -3.5, -9.7 and -3.5Kcal/mol (anchor H1) for respective pocket residues. And total energy for H1 is -16.7Kcal/mol. Lower value of energy shows the stronger binding in this anchor. For H2 anchor the total energy is -6.2Kcal/mol and energies for key residues are -6.2, -0.1 and -0.1Kcal/mol. In the similar manner total energies are calculated for the above compound in H3 and V1 anchors respectively. The energy for hydrogen bonding (H3) interaction with pocket residues are -2.5,-0.1 and -3.5Kcal/mol. Vander waal interactions (V1) have the binding energies -5.0, -3.2 and -2.3Kcal/mol for respective

amino residues shown in Table 1. Compounds with Z-score more than four are shown in Table-2. The compounds with Z-score < 4 are not shown in Table-2 due to space consideration. Out of 97 compounds 28 compounds have Z-score more than 4.

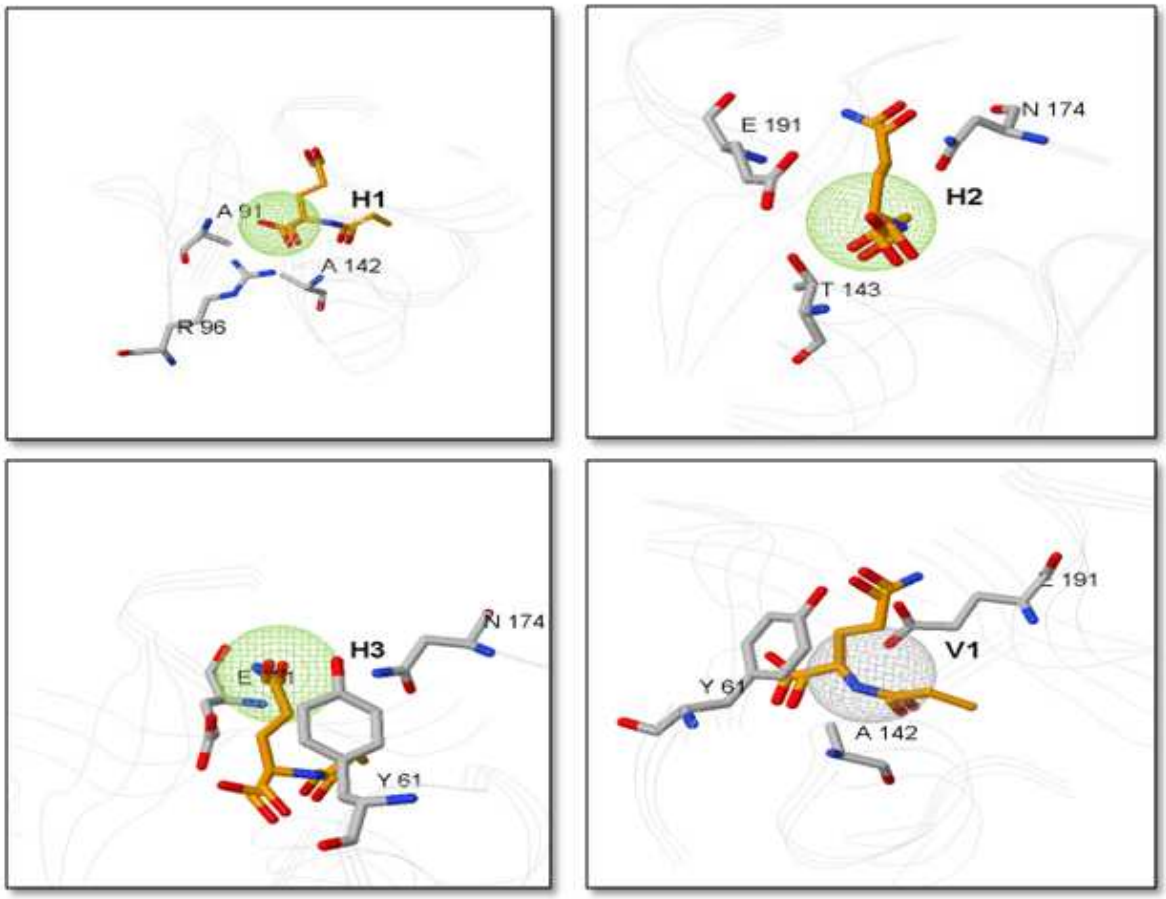
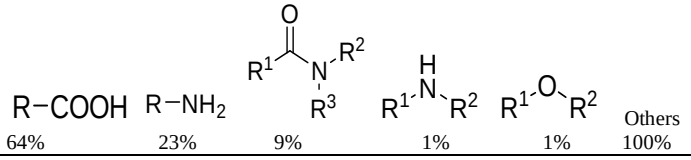
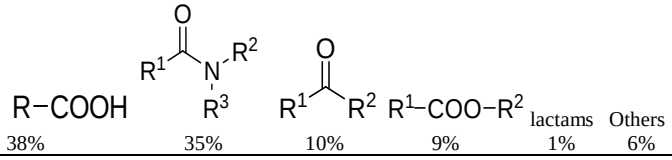
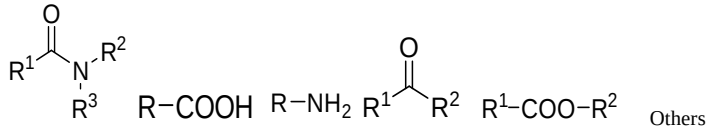
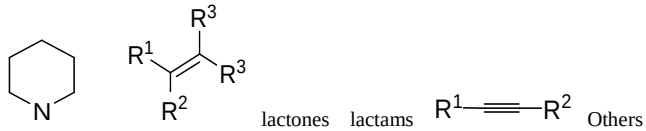


Figure 3 Interactions essential for activity of 1S9T

Thus, from the molecular docking analysis, we are able to identify some compounds which may show stronger binding affinity to the iGluR6. The compound no. 2h is found to have highest score 4.339 (Table-2), which indicates the highest binding affinity for iGluR6. Besides 2h, compound no. 2g, 2a, 2c, 1e, 2f, 1d, 2e, 36, 1b, 50, 2i and 45 also have been reported in Table-2. The pharmacological properties of these known compounds were previously evaluated for binding affinities at native kainate receptors and published in Reference no.9 and 10. Eleven new commercially available compounds with their Z-scores and ranks are reported in Table-2. To our best knowledge, the pharmacological properties of these compounds have not been evaluated. So, these compounds can be better ligands for iGluR6 and can be tested in laboratory.

Table 2. Analysis of site-moiety map

Anchor	Pocket	Moiety (functional group)
H1 51 Compounds	A 91 R 96 A 142	 64% 23% 9% 1% 1% 100%
H2 54 Compounds	T 143 N 174 E 191	 38% 35% 10% 9% 1% 6%
H3 42 Compounds	Y 61 N 174 E 191	 25% 25% 17% 12% 9% 11%
V1 57 Compounds	Y 61 A 142 E 191	 Heterocyclic Moiety 19% 11% 8% 4% 3% 54%

CONCLUSION

In the above study, docking of known and unknown ligands for iGluR6 has been carried out. Furthermore, a small series of commercially available compounds are characterized in iGluR6 binding and some new ligands with potential binding affinity are discovered. Site moiety map analysis of iGluR6 and ligands shows four anchors namely H1, H2, H3 and V1. H1, H2, H3 are hydrogen bonding interactions and V1 is for vander waal interaction. Compound 2h showed maximum Z-score 4.339. Eleven new hits were found with Z-score > 4. Among these hits, the compound no. 13515655 has the highest score 4.304. The proposed compounds can be checked for their potential therapeutic efficacy and more new active molecules can be designed on the basis of this study. Thus, this study provides a strategy for the development of new ligands and biological insight of ionotropic glutamate receptor subtype 6 ligand binding models.

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