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Designing allosteric modulators for active conformational state of m-glutamate G-protein coupled receptors

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Abstract:
G-protein coupled receptors (GPCRs) are found to be attractive drug targets for the treatment of various neuronal diseases. Allosteric modulators have their role in enhancing or suppressing the effect of glutamate on mGluRs. Structure of mGluR1 was generated with the help of Modeller software by considering human B2-adrenergic GPCR protein as template. Structure of various already known drug molecules were used for similarity search in the ZINC database and a large number of similar molecules were obtained, than filtering of these molecules were done by applying drug features. Molecules were screened by Molegro Virtual Docking program and numbers of novel molecules were generated by using LigBuilder software. Finally 16 novel drug candidates were selected, which were showing better results than the seed molecule and previously known modulators. These results will help in designing and synthesis of better drugs against diseases like Epilepsy and Parkinson's.

Key words: GPCRs, allosteric ligands, homology, modulators, drug features.

Background:
GPCRs are the large protein families that are found only in eukaryotes. They are the membrane spanning proteins that traverse the membrane several times **[1].** There are a variety of ligands that can activate these receptors like hormones, pheromones, neurotransmitters and many others. GPCRs are now a day's used as potent targets in the process of many modern drug developments. GPCRs play important role in causing many diseases like neuronal dysfunction including seizures, Parkinson's disease, night blindness and epilepsy **[2].** Antagonists of mGluRs may exert anticonvulsant effect in contrast to soman-induced seizures in rats challenged with soman **[3].** Glutamate plays a very crucial role of excitatory neurotransmitter in brain. mGluRs are a member of GPCR super family. Metabotropic receptors are a type of GPCRs as their activity involves in a series of intracellular events which involves the activation of G-protein and often signal transduction pathway. Glutamate which is an amino acid acts as a neurotransmitter **[4].** Swanson C. J. *et al* indicated that mGluR are interesting new targets to treat human's anxiety and stress disorders **[5].** Among known eight classes of mGluRs the most extensively studied receptor is the metabotropic glutamate receptor mGluR1 because of their pharmacological, physiological, and anatomical as well as biochemical characteristics **[1].** There are two states of GPCR proteins i.e. active state and inactive states, and these states maintains equilibrium with each other and can switch over to each other on sensitization of any ligand or external signals**[1].** Active structure of mGluR1 was modeled by using human B2-adrenergic G protein-coupled receptor as template **[6].**

Materials and Methods:
Obtaining template sequence from PDB
mGluR proteins previously used the structure of Rhodopsin protein as a template to model the structure of other GPCRs as stated by Melherbe *et al* **[4].** In this work human B2-adrenergic

G protein-coupled receptor protein was selected as template strand because it has similar active sites and activation mechanism of mGluR protein **[6].** The structure of human B2-adrenergic G protein-coupled receptor was taken from PDB database having the PDB ID 2RH1 in the activated state and the structure was generated for mGlur1 its sequence was obtained from NCBI,GenBank **[7, 8].**

Modelling and Evaluation

Homology model of mGlur1 protein was generated by using Modeller 9v7 version and finally structures were evaluated by using Procheck program **[9, 10].**

Similarity searching on ZINC database

ZINC database was used to obtain similar molecules on the basis of the structures of previously known ligands, similar molecules screened based on drug likeliness parameters and also based on Lipinski's rule of 5 **[11].**

Pharmacophore generation

The pharmacophore structure was generated by extracting the information about the common interacting atoms among 15 already known ligand molecules with the help of Pharmagist online server **[12, 13].**

Docking and interaction study

The Molegro docking program was used for analyzing the binding between receptor and ligand molecules to study the existing interaction between the receptor and the ligand molecule Ligplot was used **[14, 15].**

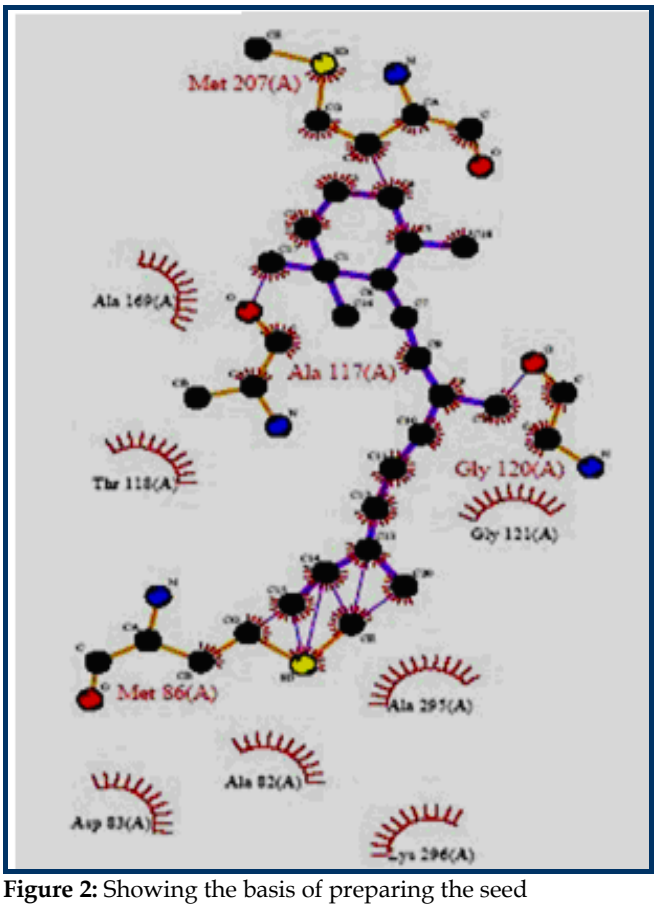


Figure 2: Showing the basis of preparing the seed

Designing Novel drug like molecules

The de novo method of ligand designing process was done using the Ligbuilder software **(Figure 2)** in which Pocket, Grow and Process modules have been used to develop novel drug candidates **[16, 17].**

Results

The Ramachandran plot in case of active mGluR1 protein was showing that 90.5% residues were falling in the core region, 8.8% of the total residues in the allowed region and 0.7% in the generously allowed region. By analyzing Naveena *et al*., 2008 work allosteric modulators were used and developed pharmacophore **(Figure 3**), based on this pharmacophore Zinc database has been screened **(Figure 1**) and finally 200 molecules obtained **[1].**

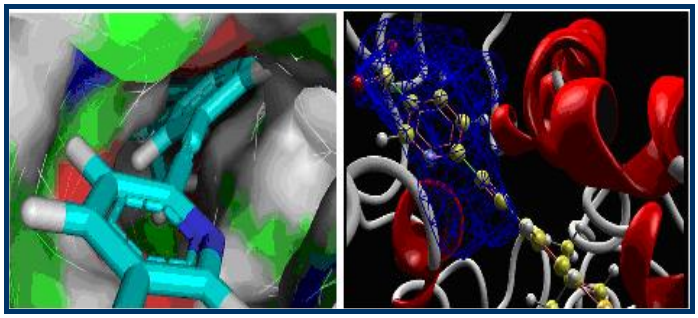


Figure 1: Showing the docked position of ligand (ID-ZINC24890) in the cavity in two different forms**.**

On the basis of docking energies 12 molecules were selected. In the other method de novo ligand designing done and results into 200 novel molecules, from this finally 16 potent molecules were screened based on binding energies and other parameters **Table 3 (see supplementary material).** After docking the molecules obtained by de novo and similarity search method both are showing better results than the previously known drug molecules **Table 2a & 2b (see supplementary material)**.Total energy of 10 best ligands obtained by both de novo method and similarity search method were compared **(Figure 4),** it shows ligands generated by de novo designing method have greater energy values and they may be a good potential drug candidates.

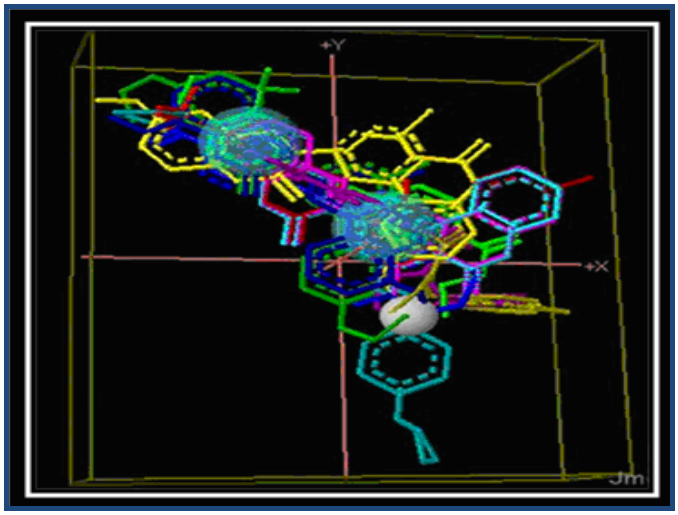


Figure 3: Pharmacophore molecule generated

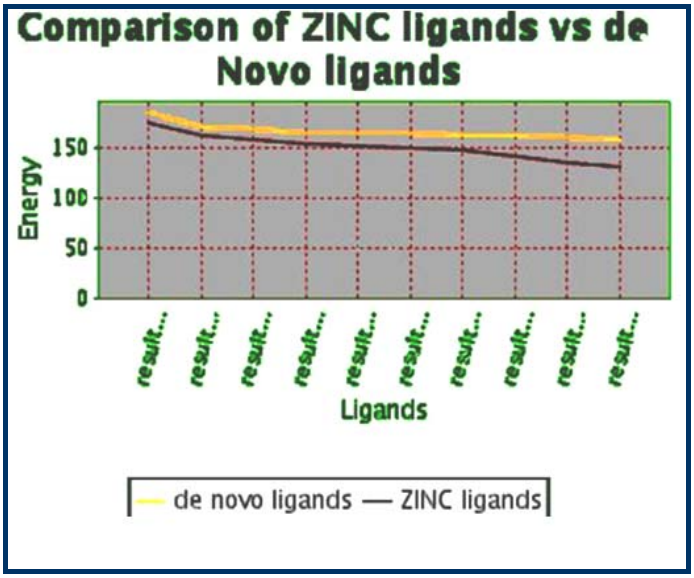


Figure 4: Comparison of the energy values between ligands generated by de novo method and ligands obtained by similarity search method by pharmacophore based on ZINC database.

The total energy value of the pivot molecule was found to be -132.576; it has been compared by the ligands generated by the de novo method. The graph shows that **(Figure 5)** the energy comparison of the drug like molecules generated by LigBuilder and the energy of pivot molecule **(Figure 6)** i.e. -132.576, it is observed the de novo method based developed ligands are better than the pivot molecule. The residues involved in the close interaction (Glycine 120, Alanine 117, Methionine 207 and Methionine 86 while the residues that are distantly interacted with the ligands are Asp 83, Lys 296, Ala 83, Thr 118 and Ala 169) were obtained by Ligplot analysis and a pivot molecule (ZINC ID: [00] ZINC24890) was selected based on pharmacophoric characters. A graph has been generated by comparing the energy values of 10 best previously known ligands and the ligands generated by de novo method.

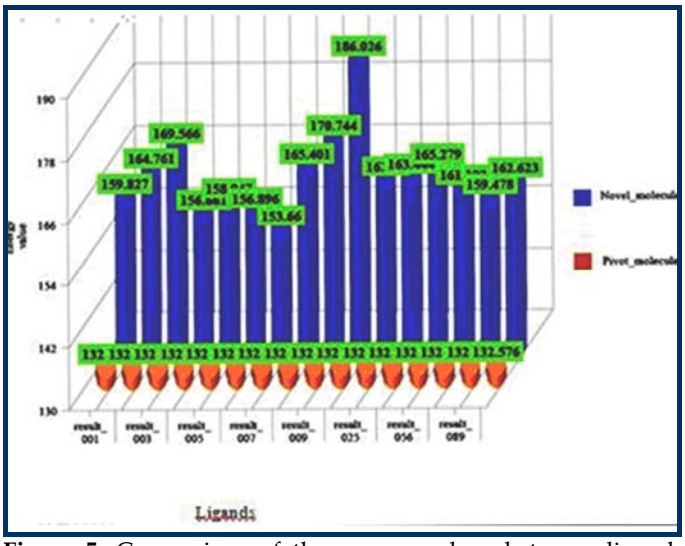
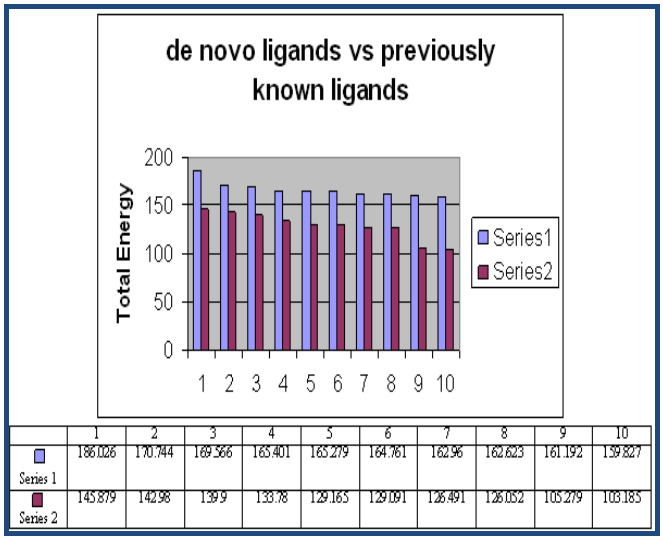


Figure 5: Comparison of the energy values between ligands generated by de novo method and the pivot molecule (ZINC ID: [00] ZINC24890).



	1	2	3	4	5	6	7	8	9	10
Series 1	186.026	170.744	169.566	165.401	165.279	164.761	162.96	162.623	161.192	159.827
Series 2	145.879	142.98	139.9	133.78	129.165	129.091	126.491	126.052	105.279	103.185

Figure 6: Comparison of the energy values between ligands generated by de novo method and the pivot molecule. Series one indicates energy of molecule generated by de novo and series two indicates energy of molecule generated by pivote molecule. The blue color represents the de novo ligands while red one indicates already known ligands.

Discussion:

Our goal was to generate suitable drug candidate which can be used for the treatment of neural dysfunction diseases. We got insight from previous work of Melherbe *et al* **[4]** who used structure of Rhodopsin protein as a template to model the structure of other GPCRs. Human B2-adrenergic GPCR template because it has similar active sites and activation mechanism of mGluR protein **[6].** Human B2-adrenergic G protein-coupled receptor structure was taken from PDB in the activated state and the structure was generated for mGlur1 **[7, 8].** Homology model of mGlur1 protein was generated and finally structures were evaluated **[9, 10].** ZINC database was used to obtain similar molecules on the basis of the structures of previously known ligands, similar molecules screened based on drug likeliness parameters and also based on Lipinski's rule of 5 **[11].** Pharmacophore structure was generated by extracting the information about the common interacting atoms among 15 already known ligand molecules **[12, 13].** Analyzed the binding between receptor and ligand molecule **[14].**The de novo method of ligand designing process was also performed to develop novel drug candidates **[16].** It was found de novo based drugs showed better binding than pivot molecule.

Conclusion:

However the ligands generated through our method are showing better binding to mGluR glutamate receptor protein as compared to previous work. Hence they may be better modulators for the activity of mGluR1 protein. They may help to treat various neural dysfunctions like Epilepsy and Parkinson's disease, but these results may be proved by experimental evaluation.

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Supplementary material:

Table 1: Comparison of various parameters of ligand molecules obtained by Pharmacophore based similarity searching using ZINC database.

ZINC ID	M. Wt. (dalton)	Total Energy(KJ/mol)	H. Bonding Energy(KJ/mol)	xLogP (KJ/mol)	B. affinity(KJ/mol)
ID -[00]ZINC1025445	372.421	-151.029	-0.0206	3.61	-116.398
ID-[01]ZINC1025451	371.421	-158.326	-0.339	3.61	-120.465
ID-[02]ZINC5553120	292.296	-117.324	-8.003	0.01	-101.15
ID-[03]ZINC636503	355.349	-132.789	-2.232	4.50	-113.391
ID-[04]ZINC753995	355.349	-134.974	-1.350	4.15	-108.543
ID-[05]ZINC856324	415.401	-153.856	-1.170	4.21	-126.366
ID-[06]ZINC856325	415.405	-152.931	-3.062	4.16	-122.806
ID- [07]ZINC856326	443.455	-175.99	-2.17	4.96	-33.027
ID-[08]ZINC2478323	387.347	-148.81	-5.322	3.14	-24.577
ID-[09]ZINC4217469	450.69	-63.444	-0.2227	4.08	-88.974
ID-[010]ZINC24890	322.409	-32.576	-1.086	3.42	-100.85
ID- [011]ZINC1542199	351.471	-41.799	-2.670	4.05	-11.568

Table 2(a) and 2(b): Previously known ligands and their docking energy (KJ/mol) values used to develop analogues **[2].**

Ligands	AMN082	PTEB	CPPHA	Fenobam	Ro01-6128	Ro67-7476	Ro67-4853	CPCCOEt
Energy value	-145.879	-142.98	-139.9	-133.78	-129.165	-129.091	-126.491	-126.052

Table 2(b)

Ligands	SIB-1757	SIB-1893	MTEP	5MPEP	MPEP	MPEP-gamma	PHCCC
Energy value	-103.186	-102.282	-102.314	-101.01	-99.897	-96.861	-105.279

Table 3: Comparison of various parameters of the drug analogues obtained by de novo method and their IUPAC names (by Marvin sketch).

ID of molecules	IUPAC name of molecules	M. Wt. (Dalton)	Total Energy(KJ/mol)	H. Bonding Energy(KJ/mol)	xLogP	B.affinity (KJ/mol)
ID-result_001	(4S,6S,8E,12E,14E)-8-(1-carbamoyleth-1-en-1-yl)-4-[ethane-1,2-bis(ylium)-1-yl]-6-hydroxy-14-(prop-2-en-1-ylidene)heptadeca-8,11,12- triene-1,2,3,9,17-pentakis(ylium)	387.6	159.827	-5.1906	4.75	-57.807
ID- result_002	(4S,6S,8E,12E,14E)-8-(1-carbamoyleth-1-en-1-yl)-4-[ethane-1,2-bis(ylium)-1-yl]-6-hydroxy-14-(prop-3-en-1-ylidene)heptadeca-8,11,12-triene-1,2,3,9,17-pentakis(ylium)	387.5	-164.761	-5.26	4.76	-71.44
ID- result_003	(6R)-1-(ethan-2-ylium-1-yl)-2-[(1E,5E,8S)-8-hydroxy-6-[1-(methylcarbamoyl)eth-1-en-1-yl]dodeca-1,2,5-triene-5,11,12-tris(ylium)-1-yl]-4-methylidene-6-methyliumylcyclohex-2-en-1-ide	399.6	-169.566	-6.456	4.41	-40.10
ID- result_004	(5S,7E,11E,13E)-5-hydroxy-7-[1-(methylcarbamoyl)eth-1-en-1-yl]-13-(prop-2-en-1-ylidene)hexadeca-7,10,11-triene-1,2,8,16-tetrakis(ylium)	359.5	-156.661	-2.32	4.73	-121.532
ID- result_005	(4E,5E)-9-[(1E,2S,5R)-2-[(1Z)-buta-1,3-dien-1-ylium-1-yl]-5-[2-(carbamoylamino)-2-oxoethyl]cyclohexan-4-ylium-1-ylidene]-4-(prop-2-en-1-ylidene)nona-5,6-diene-1,9-bis(ylium)	396.6	-158.947	-0.557	4.70	-14.94
ID- result_006	(3E,7E,9E)-3-[(2R,4R)-2,4-dihydroxyhexyl]-9-(prop-2-en-1-ylidene)dodeca-1,3,6,7-tetraene-1,4,12-tris(ylium)	318.5	-156.896	-7.59	4.50	-107.004
ID- result_007	(5S,7E,11E,13E)-7-(1-carbamoyleth-1-en-1-yl)-5-hydroxy-13-(prop-2-en-1-ylidene)hexadeca-7,10,11-triene-1,2,8,16-tetrakis(ylium)	445.5	-153.66	-0.73	4.33	-54.69
ID- result_008	[(3E,5E,9E,14R,15E)-14-(2-oxoethyl)-4-(propan-3-ylium-1-yl)heptadeca-3,5,6,9,15-pentaene-9,10,12,15,16,17-exakis(ylium)-1-yl]urea	374.6	-165.401	-3.66	4.23	-130.615
ID- result_009	[(3E,5E,9E,14R,15E)-14-(2-oxoethyl)-4-(propan-3-ylium-1-yl)heptadeca-3,5,6,9,14-pentaene-9,10,12,15,16,17-hexakis(ylium)-1-yl]urea	374.6	-170.744	-4.833	4.32	-140.22
ID- result_010	(4E,6E,10E,12R,17R)-12-ethenyl-17,19-dihydroxy-6-methyl-13-oxo-5-(propan-3-ylium-1-yl)nonadeca-4,6,7,10-tetraene-1,10-bis(ylium)	392.6	-156.275	-9.49	4.56	-104.845
ID- result_016	(4E,5E,9E,11S)-12-[(4R)-4-hydroxycyclohex-1-en-2-ylium-1-yl]-11-(2-hydroxyethyl)-4-(3-oxopropylidene)trideca-5,6,9,12-tetraene-1,9,10-tris(ylium)	372.5	-186.026	-5.73	4.98	-110.593
ID- result_025	[(3E,5E,9E,14R,15E)-14-(2-oxoethyl)-4-(propan-3-ylium-1-yl)heptadeca-3,5,6,9,15-pentaene-9,10,12,15,17-pentakis(ylium)-1-yl]urea	374.6	-162.96	-3.348	4.32	-85.30
ID- result_056	(4E,6E,9E)-10-[(3S)-3-[(1S)-1,2-dicarboxyethyl]cyclopent-1-ene-2,4-bis(ylium)-1-yl]-5-(propan-3-ylium-1-yl)deca-1,4,6,8,9-pentaene-1,2,7-tris(ylium)	358.5	-165.279	-7.744	4.32	-104.75
ID- result_078	(4E,6E,9E)-10-[(3S)-3-[(1R)-1-carboxy-2-hydroxyethyl]cyclopent-1-ene-2,4-bis(ylium)-1-yl]-5-(propan-3-ylium-1-yl)deca-1,4,6,8,9-pentaene-1,2,7-tris(ylium)	330.5	-161.192	-5.190	5.01	-108.786
ID- result_101	[(3R,4E,6S,10E,12E)-11-methyl-6-(2-methylprop-2-en-1-yl)-12-(3-oxopropylidene)pentadeca-4,9,10-triene-1,7,15-tris(ylium)-3-yl]urea	388.6	-162.623	-2.74	4.05	-85.424
ID- result_089	(4E,6E,9E)-10-[(3S)-3-[(1R)-1-carboxy-2-hydroxyethyl]cyclopent-1-ene-2,4-bis(ylium)-1-yl]-5-(propan-3-ylium-1-yl)deca-1,4,6,8,9-pentaene-1,2,7-tris(ylium)	320.5	-159.478	-4.24	4.95	-93.99