Pharmacophore Screening and Docking Studies of Glutamate Receptor with Some CNS Acting Phytocompounds from Selected Ayurvedic Medicinal Plants

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Abstract

The receptor GRM3, a G protein which is among the major excitatory neurotransmitter in the central nervous system, is taken for this study. Again, in the present study phyto-compounds from Ayurvedic Medicinal plants are used. The active components of the plants are taken and pharmacophore modeling is performed using known GRM3 receptors. Based on the pharmacophore modeling results, ADME and docking is done for the selected phytocompounds against GRM3 receptor. Based on virtual screening, shortlisted ligands selected were Bacopaside A.

Keywords*: GRM3, modeling, Ramachandran plot, pharmacophore, docking, ADME*

1. Introduction

Various medicinal plants are part & parcel of major populations of India & other South East Asian countries for the management and different therapeutic benefits of different neuro-degenerative diseases. The binding affinity of specific phytochemicals with the gene-products (i.e., specific proteins) of the above disorders using bioinformatic softwares can prove effective for future drug discovery using these phytochemicals. Metabotropic glutamate receptor 3 (a G-protein coupled receptor) is a protein that in humans is encoded by the GRM3 gene (Palmada & Centelles, 1998; Egan et. al., 2004). Ligand binding to it causes a conformational change that triggers signaling via guanine nucleotide-binding proteins (the G proteins) and modulates the activity of down-stream effectors. The protein signaling inhibits adenylate cyclase activity. G proteins including the glutamate receptors are the major excitatory neurotransmitter in the central nervous system and activates both ionotropic and metabotropic glutamate receptors. Glutamatergic neurotransmission is involved in most aspects of normal brain function and can be perturbed in many neuropathologic conditions; imbalances in glutamatergic function have been implicated in neuronal death following ischemia, in hypoglycemia or anoxia, in epilepsy, and in neurodegenerative disorders. G proteins can be involved in the stimulation of phospholipase C, the presynaptic inhibition of glutamate release, the closure of cation channels in retinal on bipolar cells, and the modulation of adenylate cyclase. *GRM3* is targetable by several drugs that have been used in previous trials of schizophrenia and other anxiety disorders; the agonist, antagonist and allosteric modulator drugs of *GRM3* can now be explored as new treatments for mental illness and this might become the first example of personalized medicine based on genetics for psychiatric disorders (Palmada & Centelles, 1998; Egan et. al., 2004).. In the current research phytocompounds from herbs like *Convolvulus pluricaulis, Morus alba, Bacopa monnieri, Vitex negundo, Picrorhiza kurrooa, Azadirachta indica, Coffea arabica, Sutherlandia frutescens/Bougainvillea spectabilis, Phyllanthus emblica* etc. are selected and virtually screened against GRM3 receptor.

2. Methodology

The three dimensional structure (3D) of the GRM3 receptor was modeled using modeler software (Sali & Blundell, 1993). The GRM3 receptor's amino acid sequence was downloaded from GeneBank database; its homologous templates were selected by BLAST. The receptor and their corresponding templates were submitted

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to modeler software to model their 3d structure. Using Rampage ramachandran plot server (this stereochemical check was applied to verify if the φ and ψ dihedral angles were in available regions of the Ramachandran plot) (Laskoswki et. al., 1993) the models generated by modeler were analyzed and the best model is selected.

The 3d structures of the above phyto-compounds were downloaded from PubChem, a database of chemical molecules maintained by the NCBI and various other online databases.

Structure-based pharmacophore (e-pharmacophores) was selected by mining the regular features of the three-dimensional structure of GRM3 receptor interacting with the known ligands. Pharmacophores were selected in the 3D structure of the GRM3 receptor at the interaction sites with the known ligands (Schrödinger Suite 2010; Taha et. al., 2008; Singh et. al., 2012).

Using Molinspiration server the ADME properties of the selected ligands was determined (Ertl et. al., 2000; Lipinski et. al., 1997; Veber et. al., 2002). Molinspiration offers calculation of various molecular properties needed in QSAR and drug design (Ertl et. al., 2000; Lipinski et. al., 1997; Veber et. al., 2002).

Docking was performed by PATCHDOCK server by selecting the best protein model with the ligands selected by ADME studies to get the docked structure (Duhovny et. al., 2002; Schneidman et. al., 2005).

3. Results & Discussions

3.1 Homology Modelling and Model verification

The amino acid sequences of GRM3 receptor was downloaded from NCBI (Table 1). Their homologous templates were selected by BLAST (Table 1).

Table 1 GRM3 receptor with its GenBank accession number and homologous templates

Receptor	Accession Number	Homologous templates	Query coverage	Identity
GRM3	XP_011514390.1	4XARA	82%	99%
		3SM9A	77%	99%
		2E4UA	77%	97%

The amino acid sequences of the receptors along with their homologous templates were submitted to modeler software for the generation of the 3d structures of the receptors using the principles of homology modeling (Sali & Blundell, 1993). Modeler generated five models for each receptor. The 3d models generated by modeler of GRM3 (Table 2) are submitted to Rampage Ramachandran Plot server for model verification (Laskoswki et. al., 1993). The best 3d GRM3 (Figure 1, 2) model is selected.

Table 2 Ramachandran Plot analysis of GRM3 receptor's modeler generated models

	Number of residues in favoured region (~98.0% expected)	Number of residues in allowed region (~2.0% expected)	Number of residues in outlier region	
Model 1	493 (92.1%)	30 (5.6%)	12 (2.2%)	
Model 2	491 (91.8%)	33 (6.2%)	11 (2.1%)	
Model 3	496 (92.7%)	23 (4.3%)	23 (4.3%)	
Model 4	495 (92.5%)	25 (4.7%)	15 (2.8%)	
Model 5	499 (93.3%)	25 (4.7%)	11 (2.1%)	selected

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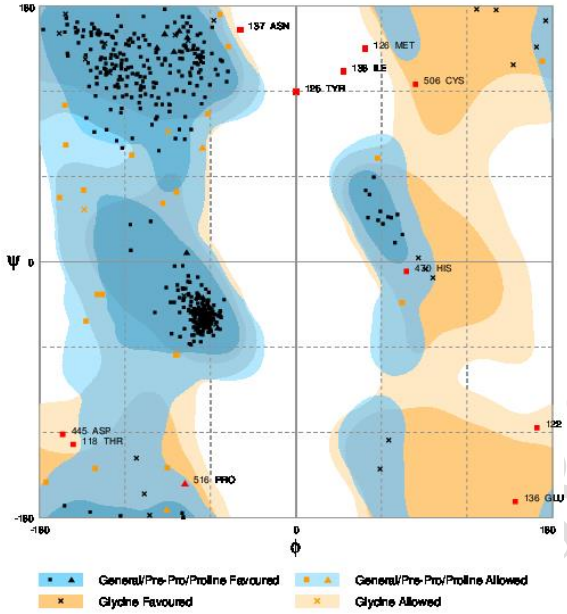


Figure 1 Ramachandran Plot analysis of GRM3 receptor model 5

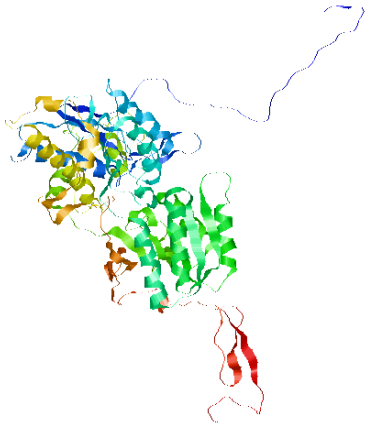
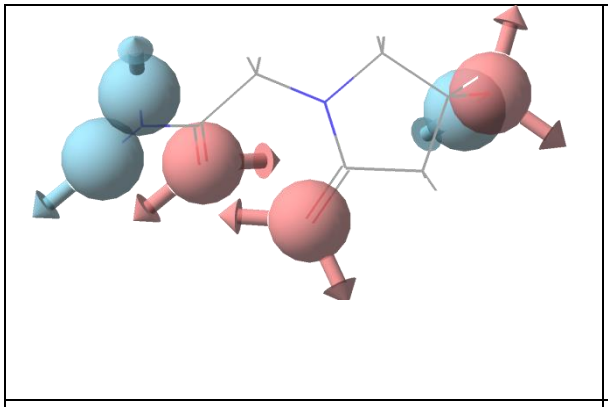


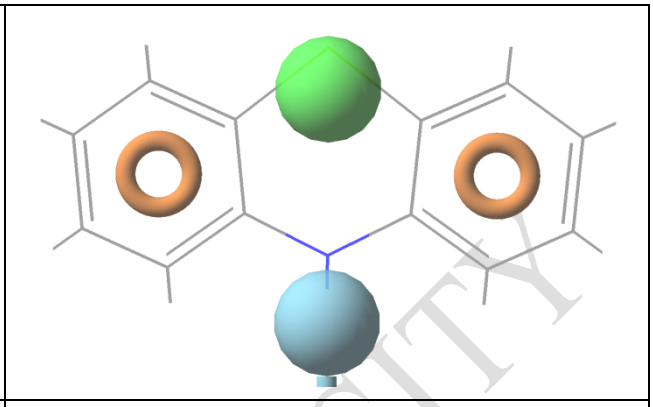
Figure 2 3d structure of GRM3 receptor model 5

3.2 Structure-Based Pharmacophore

Pharmacophore (Suite 2010; Taha et. al.; 2008, Singh et. al., 2012) sites were created in the GRM3 receptor (model 5) using the known ligands viz., Oxiracetam (Marchi et. al., 1990) and Piracetam (Lencz & Malhotra, 2015). The phytocompounds were separately screened for common phores against the known ligands Oxiracetam and Piracetam as given in Table 3. The above ligands are established ligands for glutamate receptor. Based on the pharmacophore site information (Figure 3) in the receptor, the unknown ligands in Table 3 were screened.



Oxiracetam



Piracetam

Figure 3 Pharmacophore features of Oxiracetam and Piracetam

Table 3 Pharmacophore analysis of phytocompounds

Sl.No.	Phytocompounds	Plant Name	Fitness score
Oxiracetam			
1	20-Oxodotriacontanol	*Convolvulus pluricaulis*	1.204752
2	1-Deoxynojirimycin	*Morus alba*	1.075382
3	Bacopaside II	*Bacopa monnieri*	1.040053
4	Beta-Glucogallin	*Phyllanthus emblica*	1.021548
5	Deacylgymnemic Acid.1	*Gymnema sylvestre*	1.033705
6	Eclalbasaponin I.1	*Eclipta alba*	1.137095
7	Glycyrrihizin ammonical hydrate	*Glycyrrhiza glabra*	1.182755
8	Gymnemagenin	*Gymnema sylvestre*	0.802587
9	Negundoside	*Vitex negundo*	0.963057
10	Picroside I	*Picrorhiza kurrooa*	1.351833
11	Picroside II	*Picrorhiza kurrooa*	1.186592
12	Quercetin dihydrate	*Azadirachta indica*	1.004929
13	Rutin	*Ruta graveolens*	1.178817
14	Trigoneoside IVA	*Trigonella foenum-graecum*	1.0429
Piracetam			
15	Vicine	*Momordica charantia*	1.351266
16	Agnuside	*Vitex negundo*	1.315252
17	Arjunetin	*Terminalia arjuna*	1.193699
18	Arjungenin	*Terminalia arjuna*	0.979138
19	Asiatic acid	*Centella asiatica*	0.882453
20	Bacopaside A	*Bacopa monnieri*	1.36562

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21	Catechin 5-O-gallate	*Acacia nilotica*	1.099072
22	Chebulagic acid	*Terminalia chebula*	1.077451
23	Chebulinic acid	*Terminalia chebula*	1.048981
24	Chlorogenic Acid.1	*Coffea Arabica*	1.25777
25	D-Pinitol.1	*Sutherlandia frutescens/ Bougainvillea spectabilis*	1.599359
26	Epicatechin-3-gallate	*Camellia sinensis*	1.098369
27	Epigallocatechin 3-gallate	*Camellia sinensis*	1.110839
28	Gallic Acid	*Phyllanthus emblica*	1.235741

3.3 ADME screening

ADME screening (Ertl et. al., 2000; Lipinski et. al., 1997; Veber et. al., 2002) was performed with molinspiration server for the compounds in Table 3. Molinspiration generated the following output (Table 5) for the phytocompounds.

Table 4 ADME studies

	miLogP	TPSA	natoms	MW	nON	nOHNH	nrotb	volume	***nviolations***
Bacopaside II	2.36	276.15	65	929.11	18	10	10	847.65	***3***
Eclalbasaponin I	2.40	236.06	56	796.99	14	9	7	743.43	***3***
Quercetin dihydrate	1.68	131.35	22	302.24	7	5	1	240.08	***0***
Arjunetin	2.93	177.13	46	650.85	10	7	5	619.56	***2***
Asiatic acid	4.70	97.98	35	488.71	5	4	2	487.79	***0***
Epicatechin-3-gallate	2.54	177.13	32	442.38	10	7	4	359.55	***1***
20-Oxodotriacontanol	-5.49	336.47	47	691.63	21	13	10	579.84	***3***
1-Deoxynojirimycin	-2.40	92.94	11	163.17	5	5	1	147.18	***0***
Beta-Glucogallin	-1.48	177.13	23	332.26	10	7	4	267.22	***1***
Deacylgymnemic Acid	1.10	217.59	48	682.85	12	9	5	635.65	***3***
Glycyrrihizin ammonical hydrate	3.23	243.53	58	828.99	16	8	10	762.34	***3***
Gymnemagenin	2.92	121.37	36	506.72	6	6	2	501.35	***2***
Negundoside	0.05	192.45	35	496.46	12	6	7	419.04	***2***
Picroside I	0.03	167.68	35	492.48	11	5	8	417.89	***1***
Picroside II	-1.05	197.14	36	512.46	13	6	8	424.04	***3***
Rutin	-1.06	269.43	43	610.52	16	10	6	496.07	***3***
Trigoneoside IVA	-1.22	366.30	74	1065.21	23	14	15	957.50	***3***
Vicine	-3.25	197.18	21	304.26	11	9	3	246.57	***2***
Agnuside	-0.30	175.38	33	466.44	11	6	7	394.43	***2***
arjungenin	3.72	118.21	36	504.71	6	5	2	495.49	***1***
Bacopaside A	-1.88	140.35	24	352.28	9	2	5	268.76	***0***
Catechin 5-O-gallate	1.99	177.13	32	442.38	10	7	4	359.55	***1***
Chebulagic acid	0.07	447.10	68	954.66	27	13	5	723.14	***3***
Chebulinic acid	0.40	447.10	68	956.68	27	13	12	733.98	***3***
Chlorogenic Acid	-0.45	164.74	25	354.31	9	6	5	296.27	***1***

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	miLogP	TPSA	natoms	MW	nON	nOHNH	nrotb	volume	***nviolations***
D-Pinitol	-1.99	110.37	13	194.18	6	5	1	168.39	***0***
Epigallocatechin 3-gallate	2.25	197.36	33	458.38	11	8	4	367.57	***2***
Gallic Acid	0.59	97.98	12	170.12	5	4	1	135.10	***0***

Legends: LogP: (octanol/water partition coefficient); TPSA: Molecular Polar Surface Area; natoms: number of atoms; MW: Molecular weight; nON: Number of ON; nOHNH: number of OHNH; volume: Molecular Volume, nrotb: Number of Rotatable Bonds; nviolations: number of violations.

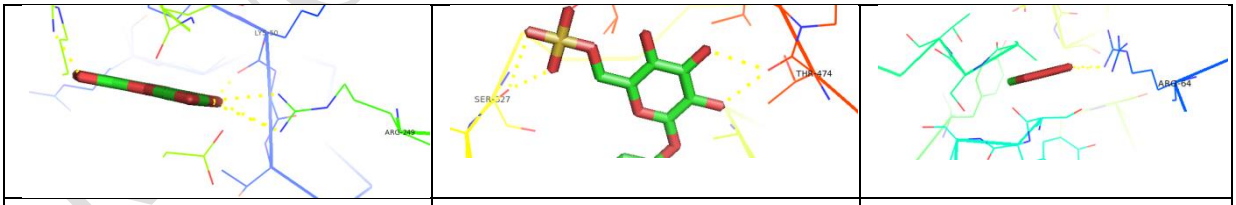
Phytocompounds having nviolations 0 were selected for further docking studies with GRM3 receptor Model 5.

3.4 Molecular Docking

GRM3 receptor (model 5) was docked with the phytocompounds having nvioloations 0 in Table 4 using PATCHDOCK server (Duhovny et. al., 2002; Schneidman et. al., 2005). It was seen that GRM3 receptor docks with the phytocompounds (Table 5, Figure 4).

Table 5 Docking results

Compound Name	Docking Score	No. of Interactions	Interacting Amino Acids	Docking
Quercetin dehydrate	2654	5	ARG 282 ARG 249 LYS 50	YES
Asiatic acid				NO
1-Deoxynojirimycin				NO
Bacopaside A	4642	4	SER 327 THR 474	YES
D-Pinitol	2218	2	ARG 64	YES
Gallic Acid				NO



Quercetin dehydrate | Bacopaside A | D-Pinitol

Figure 4 Docking studies of phytocompounds with GRM3 receptor with the interacting amino acids

Phytocompound Bacopaside A were selected as per docking studies since it has the best docking score & has a good number interactions with the GRM3 receptor.

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4. Conclusion

As per Rampage Ramachandran Plot analysis, Model 5 of GRM3 receptor is selected as the best model. Further, by ADME studies followed by virtual screening it is seen that phytocompound Bacopaside A [the main bioactive constituents of the plant responsible for the cognitive effects (Ramasamy S, et al., 2015)] from *Bacopa monnieri* can be successfully used as ligand for GRM3 receptor.

Further *in-vitro* receptor binding studies can be performed on the above selected receptor with the selected phytocompound to establish the efficacy of Bacopaside A as potential ligand for GRM3 receptor.

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