Research Article

Molecular Properties and Insilico Neuroprotective Activity of Eugenol Against Glutamate Metabotrophic Receptors

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ABSTRACT

Metabotrophic glutamate receptors were expressed abnormal in the neurodegenerative diseases. The negative regulation of the abnormal expression induce glutamate excitotoxicity has connected to interminable neurodegenerative issues for example, amyotrophic parallel sclerosis, multiple sclerosis and Parkinson's disease. The molecular target metabrophic glutamate receptors were modulated and regulated by the potent medicinal compounds. Eugenol is a medicinal value rich compound found in the buds and leaves of clove (Syzygium aromaticum (L.) Merrill and Perry), which has been accounted to have antioxidant, anticancer and neuroprotective activity. In this present study the Eugenol compound was evaluated for the neuroprotective activity through molecular docking and molecular property prediction. The eugenol compound was optimized and prepared as ligand molecule using ligprep tool. Similarly the glutamate receptors were prepared for molecular docking through auto dock tool. Prelimarily, the eugenol was evaluated for the molecular properties and drug likeliness sore. The duglikeliness score -0.60 of eugenol indicated the highest score and confirmed as potent drug like compound. Totally ten glutamate receptors were chosen and docked with eugenol ligand molecule. Among the receptors 2e4y, 3ks9, 2wjw and 3lmk were significantly inhibited by eugenol. 2e4y and 3ks9 achieved the best docking frequency (-28.01and -32.90) with atomic contact energy.

Keywords: Eugenol, Glutamate receptors, Druglikeliness, Docking, Neuroprotective activity.

INTRODUCTION

Plants are rich wellspring of bioactive parts that have attractive medical advantages and are traditionally useful for various ailments. Eugenol (4-allyl-2-methoxy phenol), is a phenolic compound found in the leaves, buds of clove (*Syzygium aromaticum* (L.) Merrill and Perry[1], essential oils and is a noteworthy constituent of Ocimum, Cinnamon, and Clove oils. Principally, eugenol is isolated from the clove buds of *Eugenia aromatica*, *E. caryophyllata* having a place with family Mytraceae indigenous to the Molluca Islands, and which are likewise developed in different parts of Indonesia, Zanzibar, Madagascar, and Ceylon. Eugenol is a fascinating compound inspite of its wide range of exercises like pain relieving, calming, antifungal, antibacterial and antihypertensive movement[2]. As of late, numerous pharmacological and restorative activities of eugenol have been explored particularly where traditional medications are ineffectual in the treatment of infection.

Eugenol separated from the fringe activities additionally acts at focal level. In sensory system, eugenol is acts as neuroprotective against excitotoxicity, ischemia and amyloid-β peptide, restrains the conduction of activity potential in sciatic nerves and enhances neuronal and vascular intricacies in exploratory diabetes[3-7]. Eugenol has been reported to stretch bradykinin and kallikrein incited sedation[8]. Old Chinese pharmacopeias uncovered that most natural cures demonstrated for Alzheimer illness (AD or indications reminiscent of AD) contains the organic Rhizoma acori graminei which is rich in eugenol[9]. Alzheimer's ailment (AD) is an unending and dynamic neurodegenerative issue which was initially portrayed in 1907 by Alois Alzheimer and is described by a dynamic loss of neurons and neurotransmitters with the nearness of substantial quantities of extracellular amyloid plaques. It extremely influences psychological capacity and other behavioral angles, for example, official capacity and dialect aptitudes. Both extracellular and intracellular aggregation of Aβ starts a course of occasions including synaptic and neuriticharm, microglial and astrocytic initiation (provocative reaction), modified neuronal ionichomeostasis, oxidative harms, changes of kinases/phosphatases exercises, development of NFTs and at long last cell demise. Eugenol shields neuronal cells from NMDA-incited excitotoxic and oxidative damage[4, 10]. However, no *in silico* reports were accessible on the eugenol molecular mechanism on neuroprotection and modulation. The present study investigates on the molecular interaction, inhibition of eugenol on metabotrophic glutamate receptors.

MATERIALS AND METHODS

Preparation of target molecules

Glutamate receptor proteins were chosen for docking studies. The crystal structures of the proteins are available in Protein Data Bank (http://www.rcsb.org/pdb) and the PDB ID for the receptors is (2E4U, 2E4V, 2E4W, 2E4X, 2E4Y, 2E4Z, 2WJW, 3KS9, 3LMK, 3MQ4) was mined and optimized for docking studies.

Ligand preparation

The identified Chemical compound namely Eugenol from *Syzygium aromaticum* clove Seed and these compound structure were retrieved from Pubchem structural database. Both of these compounds were prepared by Ligprep package. The ligand molecules were generated and the three dimensional optimizations were done and then saved as MOL file (a file format for holding information about the atoms, bonds, connectivity and coordinates of a molecule).

Active Site Prediction

Active site recoganization of Metabotrphic glutamate receptors proof of human estrogen receptor The catalytic sites of Metabotrphic glutamate receptors area and volume of binding pocket was was done with Computed Atlas of Surface Topography of Proteins (Castp) program (http://cast.engr.uic.edu)[11].

Molecular docking

In the present study the glutamate receptor proteins are docked with the eugenol ligand. The molecular docking was performed with AutoDock [4.2.1]. In order to analyze the effect of ligand association, all the water molecules and the hetero atoms have been removed from the target protein. All the hydrogen atoms were added to the protein as it is required for the electrostatics and then non polar hydrogen atoms were merged together.

RESULTS

The active compounds in many ayurvedhic drugs constituted compounds like eugenol was treated for neuroprotective role and neurodegenerative diseases. The eugenol Figure 1 was chosen as the ligand and appraised for the antagonist action against metabotropic glutamate receptors. The eugenol was first predicted for the quantitative structure activity relationship and drug like lines using Molinsipiration tool Figure 2. The eugenol structure was submitted in the molinspiration tools using jmol chemical structure drawer. The molecular properties and drug likeliness were shown in the Figure 3. The molecular properties such as moleuclar formula: $C_{10}H_{12}O_2$, molecular weight-164.08, number of Hydrogen bond-1, molecular log polarity- 2.61, molecular solubility -2.45, molecular polar surface area- 24.42, molecular volume 181.27 and no stereo centers. The drug likeliness score was found to be -0.60. The transport and recognization of the drugs is very essential for the target specific therapy. The target specific drug action can be assessed by the parameters such as G- protein coupled receptor (GPCR), ion channel modulator, nuclear receptor and protease inhibitor presented in the Figure 4a and b. The Bioproperty predictor predicts eugenol as the GPCR ligand -0.86, Ion channel modulator -0.36, Kinase inhibitor -1.14,Nuclear receptor ligand -0.78 , Protease inhibitor -1.29, Enzyme inhibitor -0.41. The *Insilico* docking of eugenol with metabotrophic glutamate receptors 2E4U, 2E4V, 2E4W, 2E4X, 2E4Y, 2E4Z, 2WJW, 3KS9, 3LMK, 3MQ4 were presented in the Figure 5a and b.The docking cycles and other parameters were used according to the Murris good self mentod and the docking score profile were presented as shown in the figure.

The active sites residues of the metabotrophic glutamate receptors were predicted by the active site prediction tool. The active site residues in 2E4U-Phe 207,208,Arg-206,Glu 518, 2E4V-Arg 206,Phe 208,Asn-209, 2E4W-Arg-206,Phe 208,Asn 209,Val 504, 2E4X-Arg 206,Phe 208,Asn 209,Val 504, 2E4Y-Arg 206,Phe 208,2E4Z-Pro 56,Gly-58,Lys-71,Asn 74,Ser-159,Ser-229, 2WJW-Asn-370,Glu-391,Val-392, 3KS9-Trp-110, Gly-163, Ser-164, 165, 186, Tyr-236, Asp-318,319,Ala-329,Gly 379, 3LMK- Arg 206,Phe 208Asn-445, Leu-455 and 3MQ4-Gly-158, Ala-180,Ser-181,Thr-182Ser-187.

The predicted catalytic sites of 10 metabotrphic glutamate receptors were utilized as the synergist locales for eugenol compound were characteristic utilized for docking studies presented in the Table 1. The effects of the docking interaction between the dynamic site deposits of target metabotrphic glutamate receptor proteins and eugenol ligand were presented in the Table 2. By investigating the docking associations, Eugenol was found to have the most noteworthy enactment vitality with receptors 2e4y, 3ks9, 2wjw and 3lmk. The 2e4y receptor showed the highest significant activity with the Ligand eugenol. The atomic contact energy, Ligand transformation and docking frequency was found to be -28.01, 2740 Score kcal/mol and -1.09 -1.17 -0.60 -7.34 12.66 68.26. Similarly the 3ks9 significantly attained -32.90, 3238 Score kcal/mol and -0.75 0.83 -0.89 -75.68 24.99 17.80. Further the above results could be valuable for recognizable proof and improvement of new preventive and remedial medication against Neurodegenerative diseases.

DISCUSSION

At present, different phytal activities including flavors and zest bioactive agents are broadly utilized as alternative therapeutic compounds as reciprocal expertise compounds in the control and management of neurodegenerative diseases[12, 13]. Human Consumption of natural product eugenol and other derived compounds has been used in the preparation various recepies and medicinal formulations. These bioactive compounds were exhibited to possess different nourishment

arrangements, antioxidant, anti-inflammatory properties and restorative definitions[14]. These zest dynamic standards have been shown to have fantastic cancer prevention agent, anticonvulsant and local anaesthetic, antistress, bacteriostatic and bactericidal and possess brain calming properties. Eugenol is constituted in the many ayurvedhic and siddha drugs especially the saraswatarishtam an ayurvedhic drug possess 80% of this compound[12]. Further, in cell models Eugenol is appeared to improve the activities of a few glutathione –related proteins, repress the movement of 5-lipoxygenase and shield essential neuronal cells from excitotoxic and oxidative harm[15-17]. The previous studies reported in perspective of Smith et al.[19] the principle objectives in drug discovery distinguishing the innovative small molecular scaffold exhibiting high binding affinity and selectivity for the target with a sensible retention, conveyance, digestion system, discharge and lethality (ADMET) profile, lead and/or drug resemblance. In the present study the eugenol compound was evaluated for the neuroprotective and modulatory activity through the insilico docking of metabotrphic glutamate receptros. There are plenty of research were carried out to screen the bioactive compound in *invivo* animal models. But none of the reports were not identified and hypothesized the mechanism of drug action on the molecular targets of neurodegenerative diseases and disorders. To our mind and from the literature, we found this is the first work emphasis the neuroprotective and modulatory activity of eugenol by *insilico* computational methods through docking. As indicated by[18], molecular docking keeps on holding awesome guarantee in the field of computational based molecular targeting which screens small lead molecules by orienting and scoring them in the catalytic site of receptor protein. The docking procedure includes the expectation of ligand affirmation and orientation (posturing) inside focused restricting site and their communication energies were utilized by the scoring capacities[19, 20].

Consequently the 10 glutamate receptors 2E4U, 2E4V, 2E4W, 2E4X, 2E4Y, 2E4Z, 2WJW, 3KS9, 3LMK, 3MQ4 were evaluated completely to hypothesize the lead binding catalytic sites for the mechanism of protective action and modulation. The above receptors were docked with eugenol Ligand and interesting findings attained from this study that the motif was found in all the glutamate receptors. The conserved amino acid sequences were found in all the receptors such as Arg[206], Phe[208] and Asn[209]. Hence the molecular targets of the active site residues were identified for targeting the neurodiseases. Finally the docking studies, suggests that eugenol significantly inhibits the active sites of metabotrophic glutamate receptors 2e4y, 3ks9, 2wjw and 3lmk.

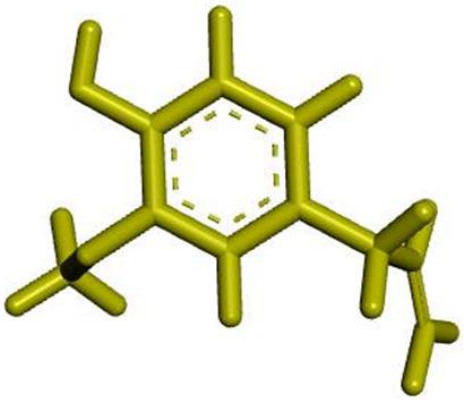
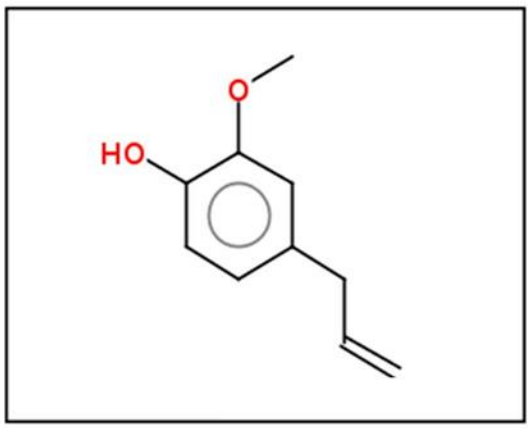


Figure 1: Three dimensional Entity of Eugenol



Molinspiration bioactivity score v2014.03

GPCR ligand	-0.86
Ion channel modulator	-0.36
Kinase inhibitor	-1.14
Nuclear receptor ligand	-0.78
Protease inhibitor	-1.29
Enzyme inhibitor	-0.41

Figure 2: Bioactivity Score prediction of Eugenol

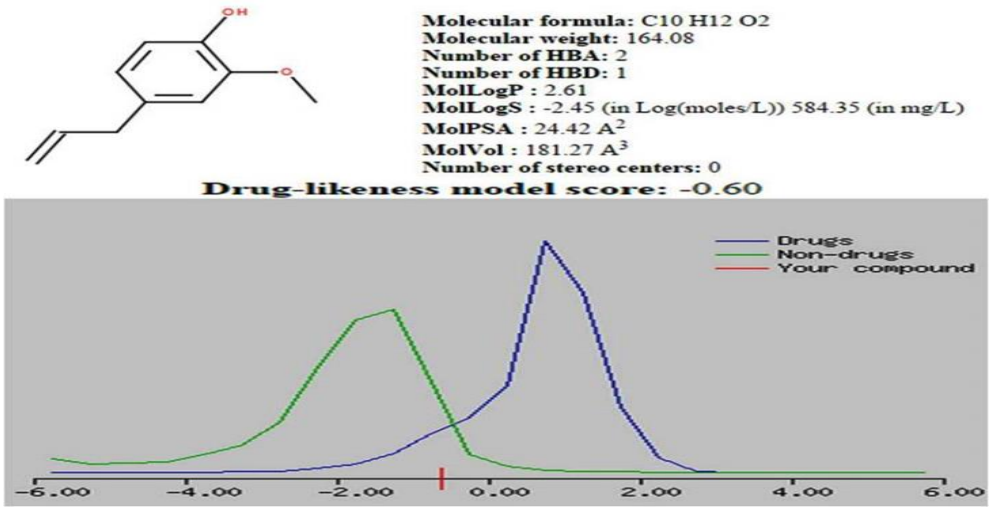


Figure 3: Molecular Properties and Druglikeliness score prediction of Eugenol

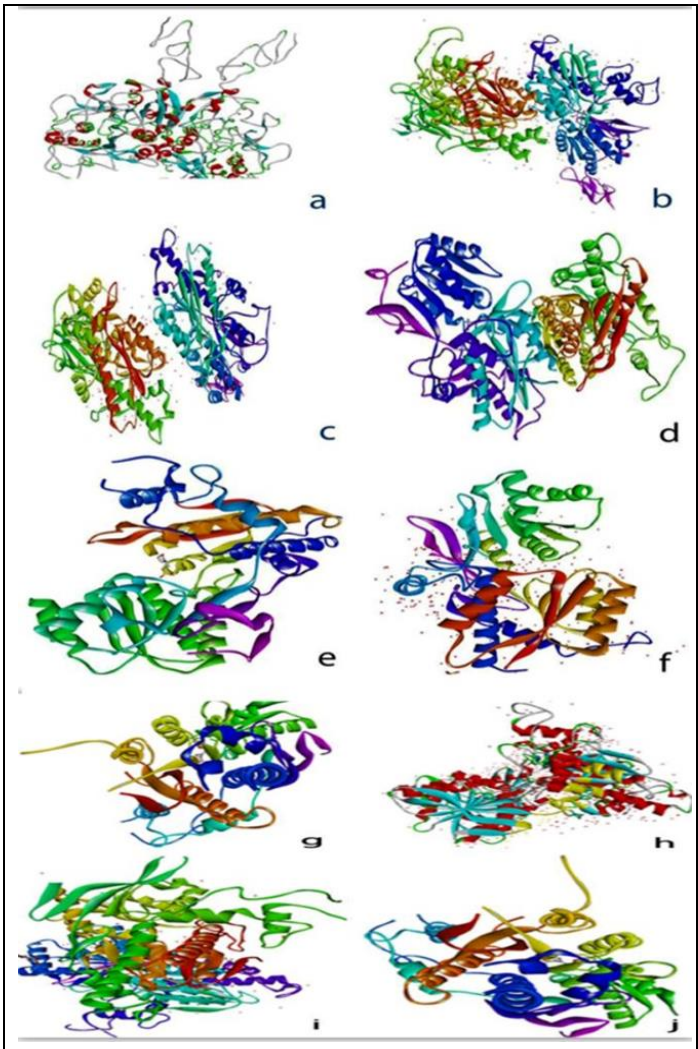


Figure 4: Three dimensional Entity of Glutamate receptors (a.2E4U, b.2E4V, c.2E4W, d.2E4X, e.2E4Y, f.2E4Z, g.2WJW, h. 3KS9, i.3LMK and j.3MQ4)

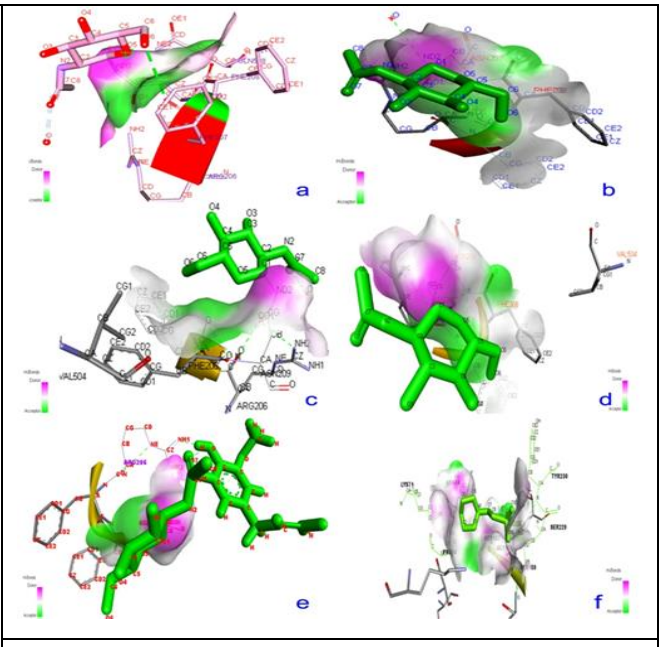


Figure 5a: Molecular interaction of eugenol with Glutamate receptors (a.2E4U, b.2E4V, c.2E4W, d.2E4X, e.2E4Y, f.2E4Z)

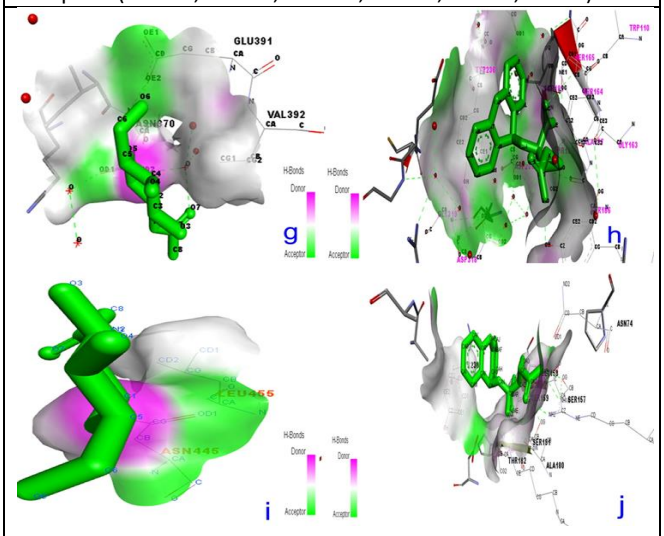


Figure 5b: Molecular interaction of eugenol with Glutamate receptors (g.2WJW, h. 3KS9, i.3LMK and j.3MQ4)

Table 1: Catalytic Site prediction of Metabotrophic Glutamate Receptors

S.No	Glutamate Receptors	Catalytic Amino acid residues
1	2E4U	Phe 207,208,Arg-206,Glu 518
2	2E4V	Arg 206,Phe 208,Asn-209
3	2E4W	Arg-206,Phe 208,Asn 209,Val 504
4	2E4X	Arg 206,Phe 208,Asn 209,Val 504
5	2E4Y	Arg 206,Phe 208
6	2E4Z	Pro 56,Gly-58,Lys-71,Asn 74,Ser-159,Ser-229
7	2WJW	Asn-370,Glu-391,Val-392
8	3KS9	Trp-110, Gly-163, Ser-164, 165, 186, Tyr-236, Asp-318,319,Ala-329,Gly 379
9	3LMK	Arg 206,Phe 208Asn-445, Leu-455
10	3MQ4	Gly-158, Ala-180,Ser-181,Thr-182Ser-187

Table 2: Molecular Interaction and Docking scores of Eugenol –Glutamate receptors

S.No.	Eugenol with Glutamate Receptors	Score kcal/mol	Area	Atomic Contact Energy (ACE)	Ligand Transformation
1.	2E4U	3780	429.50	23.00	-0.74465 -1.39370 -2.06089 25.53879 6.43612 48.63786
2.	2E4V	2732	293.00	-88.82	0.13 -0.38 -0.26 -3.45 1.47 -9.77
3.	2E4W	3598	415.50	-17.00	-0.41 0.08 -2.37 29.75 -0.84 0.23
4.	2E4X	3440	372.40	-111.28	-0.83 -0.75 0.31 16.36 -19.86 49.08
5.	2E4Y	2740	291.80	-28.01	-1.09 -1.17 -0.60 -7.34 12.66 68.26
6.	2e4z	3116	352.80	-178.78	-0.41 0.18 -0.93 35.08 11.39 -17.24
7.	2WJW	2732	364.50	-57.39	-2.85 -1.54 1.23 -11.08 -36.16 -21.89
8.	3LMK	3566	402.20	-64.26	-0.69 -0.22 -1.32 15.69 -11.19 -31.08
9.	3MQ4	3588	405.50	-112.96	-1.11 0.33 0.46 -11.26 -41.40 -7.75
10.	4S47	3238	336.70	-32.90	-0.75 0.83 -0.89 -75.68 24.99 17.80

CONCLUSION

The significance of lead molecules from different traditional medicines and their utilization upgrades the protein–ligand association ponders through *in-silico*. From the present investigation, we infer that the eugenol ligand was taken for the docking study which indicated that great interaction and inhibitory impact with the molecular targets of neural disorders through 10 metabotrophic glutamate receptors. Consequently we can foresee that four receptors 2e4y, 3ks9, 2wjw and 3lmk were pronouncedly inhibited by eugenol lead molecule. Therefore, we conclude, the eugenol can be strongly recommended and promoting as chemotherapy drugs for neuroprotection.

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