In silico Analysis of Binding Interaction of Antiepileptic Drugs with GABA Receptor Associated Protein of *Caenorhabditis elegans*

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ABSTRACT

Malfunctioning of synaptic vesicle in the absence of some essential regulatory proteins causes a significant decrease in the level of neurotransmitters like gamma-amino butyric acid (GABA) leading to epilepsy. In the present work, GABA receptor associated protein (GABARAP), being the major inhibitory neurotransmitter receptor from *C. elegans*, is investigated for its efficacy with various antiepileptic drugs. These drugs interact with the binding site of GABA receptor and regain the normal level of the inhibitory neurotransmitter, GABA. These inhibitors play a crucial role in controlling the expression of the neurotransmitter receptor at the postsynaptic membrane. Our results shows that out of the various drugs docked at the binding site, Vigabatrin and Pregablin came up with the highest score thereby proving them to be effective in the treatment of the neurological disorder.

General Terms

Epilepsy, Drug, Docking, Drug efficacy,

Keywords

Antiepileptic drugs, GABA(A) receptor, GABARAP, *C. elegans*

1. INTRODUCTION

Epilepsy is a common chronic central nervous system disorder characterized by repeated malicious seizures, which is an enormous disruption of electrical communication between neurons in the brain, leading to the temporary release of excessive energy in a coordinated form [1-2]. Neurons communicate with each other by firing electrical impulses that travel along the axon, and stimulate the release of neurotransmitters which flow across the synaptic cleft to the dendrites of the receiving cell.

About 50 million people worldwide are suffering from this disease with almost 90% belonging to the developing countries [3]. The disease is more common among young children and people above the age of 65 years however, central nervous system infections may occur at any age. Epilepsy is a group of syndromes with divergent symptoms but all involving episodic abnormal electrical activity in the brain. A constant higher level of excitory neurotransmitters, or very few inhibitory ones, increases the occurrence of a seizure. Some recent medications relate directly to this process and are designed to increase the level of inhibitory neurotransmitters, especially gamma-amino butyric acid (GABA) [4], or to decrease the amount of the excitatory ones like glutamate. In 2003, vigabatrin was approved in Mexico for the treatment of epilepsy. Vigabatrin is an irreversible decreasing calcium entry, and inhibiting the presynaptic release of other transmitters.

inhibitor of Gamma-AminoButyric Acid-Transaminase (GABA-T), the enzyme responsible for the catabolism of GABA, which increases the level of GABA at the site of synapses. Tiagabine (an anticonvulsant) is used in combination with other medications to treat partial seizures.

The different receptors acted upon by antiepileptic drugs are NMDA Receptor, AMPA-kainate Receptor, Glutamate Receptor, Nicotinic acetylcholine Receptor, GABA Receptor.

GABA receptors are the major classes of ionotropic neurotransmitter receptor made up of five protein subunits arranged in a circle to form a pore, or channel, that remains closed until its specific ligand (in this case, GABA) binds to the recognition site. This pentameric structure comprises of two alpha, two beta and one gamma subunit. GABA(A) receptors are the major inhibitory neurotransmitter receptors. These are chloride ion channels, activated by the binding of the neurotransmitter GABA. Drugs that bind to GABA(A) receptors and modulate their activity, such as the benzodiazepines, offer both medical and economic potential. In the present work, GABA associated protein (GABARAP) from *C. elegans* was taken as a drug target for docking as it acts as a receptor by controlling the neurotransmitter receptor expression at the postsynaptic membrane.

2. *Caenorhabditis elegans* AS A MODEL

The nematode *Caenorhabditis elegans* gives an opportunity to study the disease in a simple animal with a defined nervous system consisting of 302 neurons in contrast to the 100 billion neurons of the human. It is unique among animal models, as the anatomy and connectivity of its nervous system has been determined from electron micrographs and refined by pharmacological assays.

All the controlling activities in the vertebrate brain depend on the balance between excitatory and inhibitory neurotransmission. The most abundant inhibitory neurotransmitter in the brain is GABA whose normal functioning requires specialized proteins such as biosynthetic enzymes, transporters and receptors. Any changes in these proteins lead to specific imbalance of GABA neurotransmission thereby causing the disease. For example, mutations in the a1 and g2 GABA receptor subunits can cause ancestral forms of epilepsy [5-7]. The vesicular GABA transporter (VGAT) was first discovered in *C. elegans* and this sequence was used to identify the mammalian homolog [8, 9]. GABA is formed within GABAergic axon terminals and released into the synapse, where it acts at any of the two types of receptor: GABA(A); controlling chloride entry into the cell, and GABA(B); increasing potassium conductance,

GABA is rapidly removed by uptake into both glia and presynaptic nerve terminals and then catabolized by GABA

transaminase [10]. GABA(A) receptor associated protein (GABARAP), Golgi-specific DHHC zinc finger protein (GODZ) , phospholipase C-related, catalytically inactive proteins (PRIP-1 and PRIP-2), Plic-1, radixin, HAP1, GABA(A) receptor interacting factor-1 (GRIF-1) and brefeldin A-inhibited GDP/GTP exchange factor 2 (BIG2) are the different proteins playing a crucial role in modulating the activities of GABA (A) receptors [11]. A single GABA(A) receptor, encoded by the unc-49 gene which encodes three GABA(A) receptor subunits under the control of a single promoter, functions at the inhibitory neuromuscular junction in *C. elegans* [12-13]. The *C. elegans* GABA(A) receptor was modulated to identify a new site of action for drugs which is an important strategy to treat neurological disorders [14].

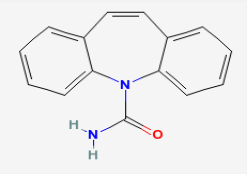
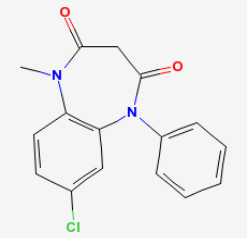
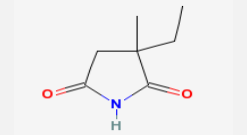
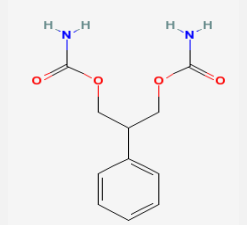
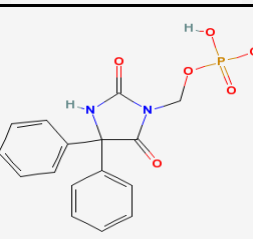
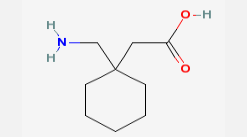
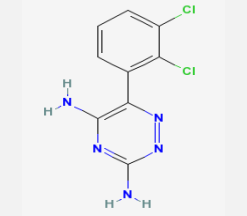
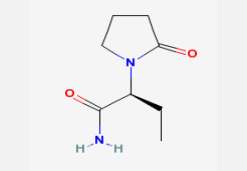
3. MATERIALS AND METHOD

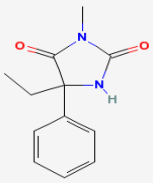
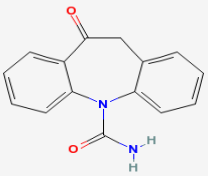
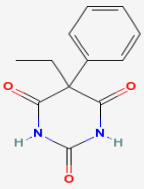
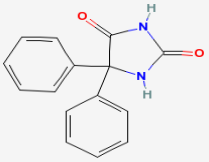
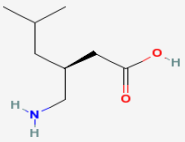
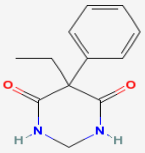
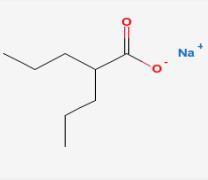
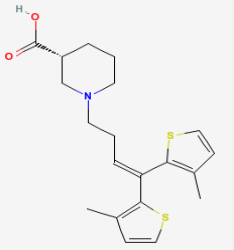
In the present work GABARAP was selected as the target for antiepileptic drugs. Mutation in GABARAP protein allows the binding of GABA receptor with gamma subunit thereby lowering the concentration of the receptor on postsynaptic membrane. This ceases the inhibition while enhances the excitatory activity leading to epilepsy [15]. The structure of GABA associated protein (GABARAP) was downloaded from RCSB (http://www.rcsb.org/pdb/). The Simplified Molecular Input Line Entry Specification (SMILES) notation of different antiepileptic drugs [16-32] was downloaded from pubchem compounds which were tested on *C. elegans* (http://www.wormbase.org) [33, 34].

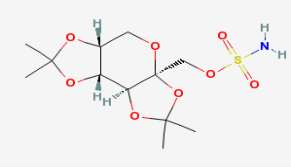
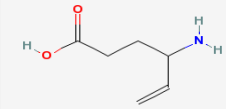
Docking was performed by applying appropriate force field on the target and the drugs through Accelrys Discovery studio ligand fit protocols. Ligand, with highest dock score, that binds to the binding site was selected and finally the amino acid residues to which the ligand binds was revealed.

4. RESULT

In total 18 compounds were selected from the pubchem compound database possessing antiepileptic properties and tested on *C. elegans* model system. These compounds are highlighted in Table 1.

S.No	Name of drugs	Molecular weight (g/mol)	Formula	IUPAC name	Structure
1.	Carbamazepine	236.269	$C_{15}H_{12}N_2O$	benzo[b][1]benzazepine -11-carboxamide	
2.	Colbazam	300.739	$C_{16}H_{13}ClN_2O_2$	10-chloro-6-methyl-2-phenyl-2,6-diazabicyclo[5.4.0]undeca-8,10,12-triene-3,5-dione	
3.	Ethosuximide	141.168	$C_7H_{11}NO_2$	3-ethyl-3-methyl-pyrrolidine-2,5-dione	
4.	Felbamate	238.24	$C_{11}H_{14}N_2O_4$	(3-carbamoyloxy-2-phenyl-propyl) carbamate	
5.	Fosphenytoin	362.274	$C_{16}H_{15}N_2O_6P$	(2,5-dioxo-4,4-diphenyl-imidazolidin-1-yl)methoxyphosphonic acid	
6	Gabapentin	171.237	$C_9H_{17}NO_2$	2-[1-(aminomethyl)cyclohexyl]acetic acid	
7.	Lamotrigine	256.091	$C_9H_7Cl_2N_5$	6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine	
8.	Levetiracetum	170.209	$C_8H_{14}N_2O_2$	(2S)-2-(2-oxopyrrolidin-1-yl)butanamide	

9.	ephen-ytoin	218.252	$C_{12}H_{14}N_2O_2$	5-ethyl-3-methyl-5-phenyl-imidazolidine-2,4-dione	
10.	Oxcarbazepine	252.268	$C_{15}H_{12}N_2O_2$	5-oxo-6H-benzo[b][1]benzazepine-11-carboxamide	
11.	Phenobarbital	232.235	$C_{12}H_{12}N_2O_3$	5-ethyl-5-phenyl-1,3-diazinane-2,4,6-trione	
12.	Phenytoin	252.268	$C_{15}H_{12}N_2O_2$	5,5-diphenylimidazolidine-2,4-dione	
13.	Pregablin	159.226	$C_8H_{17}NO_2$	(3S)-3-(aminomethyl)-5-methyl-hexanoic acid	
14.	Primidone	218.252	$C_{12}H_{14}N_2O_2$	5-ethyl-5-phenyl-1,3-diazinane-4,6-dione	
15.	Valproic acid	166.193	$C_8H_{15}NaO_2$	sodium 2-propylpentanoate	
16.	Tiagabine	375.55	$C_{20}H_{25}NO_2S_2$	(3R)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-enyl]piperidine-3-carboxylic acid	

17.	Topiramate	339.363	$C_{12}H_{21}NO_8S$	(2,2,7,7-tetramethyltetrahydro-3aH-bis[1,3]dioxolo[4,5-b:4',5'-d]pyran-3a-yl)methyl sulfamate	
18.	Vigabatrin	129.157	$C_6H_{11}NO_2$	4-aminohex-5-enoic acid	

The functional/ binding sites of GABARAP protein were identified through Define and Edit binding site module of Accelrys Discovery Studio based on the cavity available on the surface of GABARAP protein. In total 2 binding cavities were identified on the GABARAP protein. Site 1 had a volume of 22.125 $Å^3$, while the volume of site 2 was 16.875 $Å^3$. Both the sites are shown in Figure 1.

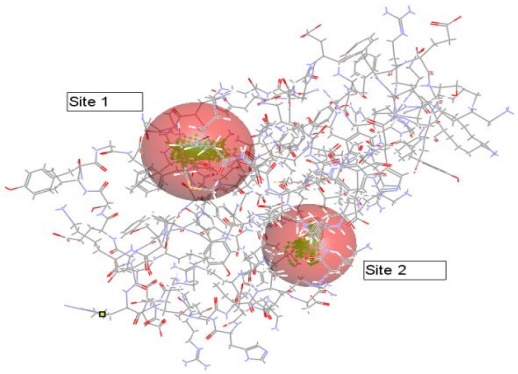


Figure 1 3D structure of GABARAP protein along with predicted binding sites is shown. Binding site points are highlighted as green color + signs.

The docking library of 18 compounds was designed by importing sdf files collected from pubchem database in a single structure file. All the sdf files were optimized for their best biological conformations by applying charmM force field. Figure 2 shows the virtual 3D library of 18 drug like molecule with probable antiepileptic property.

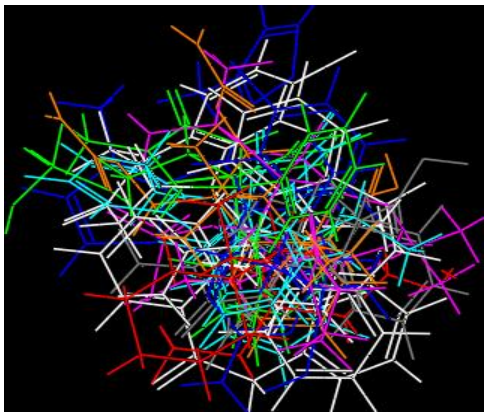


Figure 2 Virtual 3D library of 18 drug like molecules screened as potential binder for GABARAP protein

The interaction study of the GABARAP with virtual ligand library was performed using "LigandFit" protocol available in Accelrys discovery studio on the specified binding sites. Candidate ligand poses in the binding site were evaluated and prioritized according to the DockScore function based on forcefield approximation for internal energy of the ligand and the interaction energy of the ligand with the receptor as per the equation given below:

DockScore(forcefield) = - (ligand/receptor interaction energy + ligand internal energy)

For the docking between GABARAP and lignad library, we set the parameters as per our previously published study on the interaction of geraniol and limonene interaction with HMG-CoA reductase. The Energy Grid Force Field parameter was set to Dreiding, for computing ligand-protein interaction energy. The Energy Grid parameters control the grid bases docking used in the initial evaluation of the poses. In the Dreiding force field the Gasteiger charging method is employed to calculate the partial charges of ligands and proteins. The Energy Grid Extension from site was set to 5.0 Å. To reduce the complexity of the system, rigid docking was performed by setting the Conformation search Number of Monte Carlo Trial to "0". Total 10 poses of individual ligand was analyzed in the receptor cavity. Out of 18 drug tested, 4 were successfully docked in the binding site 1 of GABARAP. The ligand internal energy and docking score is mentioned in the Table 2.

Table 2 Result of docking at receptor binding site no. 1 with ligand library by applying CHARMm force field

Drug Tested	Ligand Internal Energy	Docking Score	Pose No.
Ethoximide (M.Wt.-141.17)	2.945	**6.726**	**1**
		4.004	2
		1.965	3
		0.93	4
		0.521	5
Levetiracetam (M.Wt.-170.21)	-0.86	**13.59**	**1**
		10.887	**2**
Pregablin (M.Wt.-159.23)	7.668	16.834	1
		2.243	2
Vigabatrin (M.Wt.- 129.16)	2.094	**29.845**	**1**
		26.896	2
		16.732	3
		14.971	4
		7.723	5
		7.29	6

		7.094	7
		6.033	8
		5.225	9

All ligand poses are arranged with the decreasing docking score and the value in bold represents the highest dock score for a particular ligand docked.

Vigabatrin and Pregablin are the two drugs selected on the basis of high dock score. Vigabatrin showed best interaction at binding site 1 (Figure 1) with a dock score of 29.485 followed by Pregablin with dock score 16.834 (Figure 2). In the interaction of Vigabatrin, Glu100 was identified as the key amino acid residue in the binding site 1 of GABARAP to form the hydrogen bonds. In case of Pregablin, Met1 was involved in the hydrogen interaction.

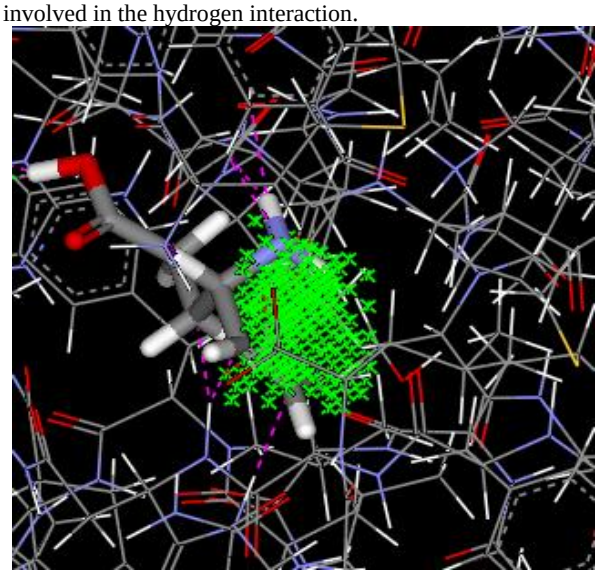


Figure 1 Binding of Vigabatrin at receptor site 1.

Hydrogen bond (green doted lines) was formed between Vigabatrin: H20- A: GLU100: OE2 while bumps (pink dotted lines) was observed between Vigbatrin: N4- A: PHE3: HE1; Vigabatrin: C6- A: ASN81: HN; Vigabatrin: H5-A: MET1: HT2; Vigabatrin: C1-A: ASN82: HN; Vigabatrin: H17-A: ASN81: HN; Vigabatrin: O10-A: GLU100: 0E2; Vigabatrin: H14-A: PHE3: HE1; Vigabatrin: H14-A: PHE3: CE1 and Vigabatrin: H20-A: GLU100: CD.

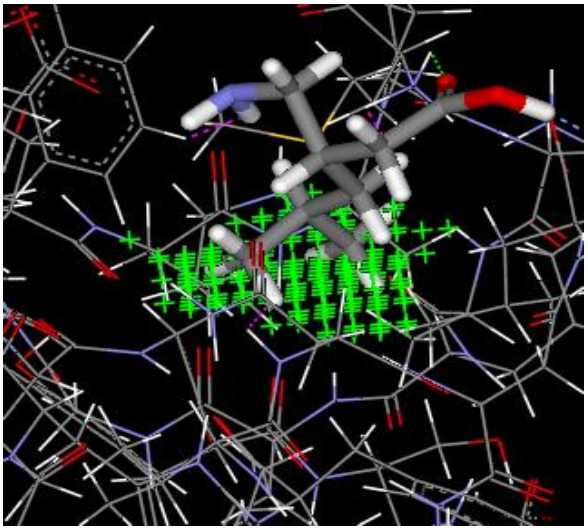


Figure 2 Binding of Pregablin at its receptor site 1.

Hydrogen bond (green doted lines) was formed between Pregablin: O10- A: MET1: HT3 while bumps (pink dotted lines) was observed between Pregablin: H28- A: PHE3: HE1; Pregablin: H20- A: MET1: HT2; Pregablin: H19- A: ASN81: HN

Bumps are generally considered as the von der Waals clashes between the interaction molecules. Thus to reduce the clashes, these docked structures were further optimized.

The similar analysis was performed on the binding site - 2 of GABARAP with the ligand virtual library created. Out of 18 molecules tested, only two were found to dock at the binding site 2. only Vigabatrin and Ethoximide showed positive interaction (Table 3). However, no such interaction was observed with Pregablin.

Table 3 Result of docking at receptor binding site no. 2 with ligand library

DRUG TESTED	LIGAND INTERNAL ENERGY	DOCKING SCORE	POSE_ NO.
Vigabatrin (M.Wt.-129.16)	2.094	**10.319**	**1**
		5.153	2
		4.549	3
Ethoximide (M.Wt.-141.17)	2.945	**4.82**	**1**
		0.74	2
		0.505	3

The values in bold represent the ligand poses with a highest dock score.

5. CONCLUSION

Epilepsy is the major neurodegenerative disease due to imbalance in excitatory and inhibitory type of neurotransmitters. Some medications can be taken daily in order to prevent seizures completely or just reduce the frequency of their occurrence. We worked on various drugs which were previously tested on *C. elegans* for epilepsy and are also beneficial to human. We identified one of the important target (GABRAP), for epilepsy and listed out various drugs that are used in the treatment of epilepsy.

We found two functional binding sites in GABARAP and performed docking between target and drugs where the H-bonds between drugs and target were discovered. We analyzed that vigabatrin binds to both the binding sites. In binding site 1, nine poses of vigabatrin were identified, out of which only two poses selected with high dock score. For the first pose dock score was found to be 29.845 while for second pose it was 26.896. In binding site 2, three poses of which only one has a high dock score of 10.319 suggest that binding site 1 gives a better docking result. From the result it is clear that Vigabatrin is the best drug identified with highest dock score for both binding site 1 and 2 out of the 18 drugs tested. The analysis is important to find out more suitable antiepileptic candidates with better binding affinity than vigabatrin. Vigabatrin dock score can serve as a bench mark to find other good drug candidates using *in silico* screening.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

[1] [No authors listed], (1993). Commission on Epidemiology and Prognosis, International League against Epilepsy. "Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis, International League against Epilepsy", Epilepsia, 34, 4, 592–6.

[2] Blume, W., Lüders, H., Mizrahi, E., Tassinari, C., van Emde Boas, W., Engel, J. (2001). "Glossary of descriptive terminology for ictal semiology: report of the ILAE task force on classification and terminology", Epilepsia, 42, 9, 1212–8.

[3] Amadou, G.D., Hanneke, M.de.B., Custodia, M., Leonid, P. and Harry, M. (2003). The global campaign against epilepsy in Africa,Acta Tropica,87, 149-159.

[4] Steiger, J.L. and Russek, S.J. (2004). "GABAA receptors: building the bridge between subunit mRNAs, their promoters, and cognate transcription factors", Pharmacology & Therapeutics, 101, 3, 259-281.

[5] Baulac, S. et al. (2001). First genetic evidence of GABAA receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene, Nat. Genet., 28, 46–48.

[6] Cossette, P. et al. (2002). Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy, Nat. Genet., 31, 184–189.

[7] Wallace, R.H. et al. (2001). Mutant GABAA receptor gamma2-subunit in childhood absence epilepsy and febrile seizures, Nat. Genet., 28, 49–52.

[8] McIntire, S.L. et al. (1997). Identification and characterization of the vesicular GABA transporter, Nature, 389, 870–876.

[9] Docherty, M. et al. (1985). Evidence for specific immunolysis of nerve terminals using antisera against choline acetyltransferase, glutamate decarboxylase, and tyrosine hydroxylase, Brain Res., 339, 105–113.

[10] Treiman, D.M. (2001). GABAergic mechanisms in epilepsy, Epilepsia, 42, 3, 8-12.

[11] Chen, Z.W. and Olsen, R.W.(2007). GABAA receptor associated proteins: a key factor regulating GABAA receptor function, J Neurochem., 100(2), 279-94.

[12] Bamber, B.A., Beg, A.A., Twyman, R.E., Jorgensen, E.M. (1999).The Caenorhabditis elegans unc-49 locus encodes multiple subunits of a heteromultimeric GABA receptor, J. Neurosci.,19,5348–5359.

[13] Richmond, J.E., Jorgensen, E.M. (1999). One GABA and two acetylcholine receptors function at the C. elegans neuromuscular junction, Nat. Neurosci., 2, 791–797.

[14] Wardell, B., Marik, P. S., Piper, D., Rutar, T., Jorgensen, E. M., Bamber, B. A.(2006). Residues in the first transmembrane domain of the Caenorhabditis elegans GABAA receptor confer sensitivity to the neurosteroid pregnenolone sulfate, British Journal of Pharmacology, 148, 162-172.

[15] Wang, H. and Olsen, R.W. (2000). Binding of the GABAA receptor-associated protein (GABARAP) to microtubules and microfilaments suggests involvement of the cytoskeleton in GABARAP-GABAA receptor interaction, J Neurochem, 75, 644eC655.

[16] Anhut, H., Ashman, P., Feuerstein, T.J., Sauermann, W., Saunders, M., Schmidt, B. (1994).Gabapentin (Neurontin) as add-on therapy in patients with partial seizures: a double-blind, placebo-controlled study. The International Gabapentin Study Group, Epilepsia, 35,795-801.

[17] Sivenius, J., Kalviainen, R., Ylinen, A., Riekkinen, P. (1991). Double blind study of gabapentin in the treatment of partial seizures, Epilepsia, 32, 539-42.

[18] [No authors listed], (1990). Gabapentin in partial epilepsy. UK Gabapentin Study Group, Lancet, 335, 1114-7.

[19] [No authors listed], (1993). Gabapentin as add-on therapy in refractory partial epilepsy: a double-blind, placebo-controlled, parallel-group study. US Gabapentin Study Group No 5, Neurology, 43, 2292-8.

[20] Matsuo, F., Bergen, D., Faught, E., Messenheimer, J.A., Dren, A.T., Rudd, G.D., et al., (1993). Placebo-controlled study of the efficacy and safety of lamotrigine in patients with partial seizures. US Lamotrigine Protocol 0.5 Clinical Trial Group, Neurology, 43, 2284-91.

[21] Binnie, C.D., Debets, R.M., Engelsman, M., Meijer, J.W., Meinardi, H., Overweg, J., et al.,(1989). Double blind crossover trial of lamotrigine (Lamictal) as adds on therapy in intractable epilepsy, Epilepsy Res, 4, 222-9.

[22] Jawad, S., Richens, A., Goodwin, G., Yuen, W.C. (1989). Controlled trial of lamotrigine (Lamictal) for refractory partial seizures, Epilepsia, 30, 356-63.

[23] Loiseau, P., Yuen, A.W., Duche, B., Menager, T., Arne Bes, M.C. (1990). A randomised double blind placebo controlled crossover adds on trial of lamotrigine in patients with treatment resistant partial seizures, Epilepsy Res, 7, 136-45.

[24] Messenheimer, J., Ramsay, R.E., Willmore, L.J., Leroy, R.F., Zielinski, J.J., Mattson, R., et al. (1994). Lamotrigine therapy for partial seizures: a multicenter, placebo-controlled, double-blind, cross-over trial, Epilepsia, 35, 113-21.

[25] Schapel, G.J., Beran, R.G., Vajda, F.J., Berkovic, S.F., Mashford, M.L., Dunagan, F.M., et al. (1993). Double blind, placebo controlled crossover study of lamotrigine in treatment resistant partial seizures, J Neurol Neurosurg Psychiatry, 56, 448-53.

[26] Smith, D., Baker, G., Davies, G., Dewey, M., Chadwick, D.W. (1993). Outcomes of add on treatment with lamotrigine in partial epilepsy, Epilepsia, 34, 312-22.

[27] Tassinari, C.A., Michelucci, R., Chauvel, P., Chodiewicz, J., Shorvon, S., Henriksen, O., et al. (1996). Double blind placebo controlled trial of topiramate (600 mg daily) for the treatment of refractory partial epilepsy, Epilepsia, 37, 763-8.

[28] Ben-Menachem, E., Henriksen, O., Dam, M., Schmidt, D. (1996). Double blind placebo controlled trial of topiramate as add-on treatment in patients with refractory partial epilepsy, Epilepsia, 36, 539-43.

[29] Faught, E., Wilder, B.J., Ramsay, E., Reife, R., Kramer, L., Pledger, G., et al. (1996). Topiramate controlled dose ranging trial in refractory partial epilepsy using 200, 400 and 600 mg daily dosages, Neurology, 46, 1684-90.

[30] Privitera, M., Fincham, R., Renry, J., Reife, R., Kramer, L., Pledger, G., et al. (1996). Topiramate placebo controlled trial in refractory partial epilepsy using 600, 800 and 1000 mg daily dosages, Neurology, 46, 1678-83.

[31] French, J.A., Mosier, M., Walker, S., Somerville, K., Sussman, N. (1996). A double-blind, placebo controlled study of vigabatrin 3g/day in patients with uncontrolled complex partial seizures, Neurology, 46, 54-61.

[32] Grunewald, R.A., Thompson, P.J., Corcoran, R., Corden, Z., Jackson, G.D., Duncan, J.S. (1994). Effects of vigabatrin on partial seizures and cognitive function, J Neurol Neurosurg Psychiatry, 57, 1057-63.

[33] Harris,T.W., Chen,N., Cunningham,F., Tello-Ruiz,M., Antoshechkin,I., Bastiani,C., Bieri,T., Blasiar,D., Bradnam,K., Chan,J. et al. (2004). WormBase: a multi-species resource for nematode biology and genomics, Nucleic Acids Res., 32 (Database issue), D411–D417.

[34] Harris,T.W., Lee,R., Schwarz,E., Bradnam,K., Lawson,D., Chen,W., Blasier,D., Kenny,E., Cunningham,F., Kishore,R. et al. (2003). WormBase: a cross-species database for comparative genomics, Nucleic Acids Res., 31, 133–137.

[35] Pattanayak, M., Seth, P.K., Smita, S., Gupta, S.K. (2009). Geraniol and Limonene interaction with 3-hydroxy-3-methylgluraryl-CoA (HMG-CoA) reductase for their role as cancer chemo-preventive agents., J Proteom and Bioinfo, 2(11), 466-474.