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RESEARCH ARTICLE

In-silico Studies on Phthalimide GABA Analogs for Anticonvulsant Activity Against Sodium Channel and GABA-AT

Rajan K. Kurmi*, Reema Sinha, Anurag Agrawal, Snigdha Srivastava

ABSTRACT

Introduction

GABA is a type of inhibitory neurotransmitter; it blocks or inhibits certain nerve transmission. Our nervous system is calmed by GABA, thus preventing excessive fear or anxiety is a simple and rapid approach to raise and maintain healthy mental health. Some medical conditions associated with a change in the level of GABA are anxiety and mood disorders, schizophrenia, autism spectrum disorder, depression, epilepsy, and seizures. The aim of this study to increase the activity of GABA which can be enhanced by the incorporation of anthracene, phthalimide, benzene, and thalidomide.

Materials and methods

Insilico Docking study was carried out by using Auto Dock 4.0 against GABA-AT protein and the binding energy was found out to be -7.61kcal/mol. GABA-Phthalimide substitution, derivatives were designed as they possess a similar degree of anticonvulsant potency due to their phenytoin-like profile. The phthalimide pharmacophores interaction with voltage dependent sodium channels in neurons was investigated.

Results and conclusion

This suggests the substitution of GABA as a possible lead compound. Therefore, in the present study, N, N-phthaloyl GABA combine with aniline a Noval compound 4-(1,3-dioxoisoindolin-2-yl)-N-phenylbutanamide is designed with improved anticonvulsant properties.

Keywords: GABA, Neurotransmitter, Sodium channel, epilepsy, Docking

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INTRODUCTION

The 4-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter regulating neuronal activity in the mammalian central nervous system (CNS) and variety of physiological functions. [1–5] Based on data, GABA ergic synapses make up 20–50% of all CNS synapses depending on the region of the brain, and their concentration ranges from 200–1000 times higher than that of other neurotransmitters.[6–8] Furthermore, Huntington's, and Parkinson's disorders have all been linked to a GABA shortage. Enhancing the activity of inhibitory GABA neurons may have a significant role in reducing the frequency and severity of many seizures, according to several current ideas concerning the underlying processes of epilepsy.[9,10] Anticonvulsants work by potentiating inhibitory mechanisms, mostly the GABA system, by influencing inhibitory synaptic processes, and by suppressing excessive neuronal firing, they inhibit excitatory mechanisms, the glutamate system and ionic channels (modulation of membrane cation conductance via sodium, calcium or potassium channels).[11] GABA mediates the inhibitory processes, which helps to play a significant role in the genesis and management of epilepsy. Recent research has shown that regulating the activity of voltage-gated calcium channels may be a component of the inhibitory effect of GABA mediated by low-affinity receptors.[12]

It is generally known that the aetiology of numerous CNS illnesses in humans, including anxiety, pain, and epilepsy, involves attenuation of GABAergic neurotransmission. Low GABA levels may also be a factor in mania and depression.[13] As a result, during the past 25 years, research on treatments in the aforementioned fields has exploded, with significant attention being paid to the different possible pharmacological methods for improving GABAergic function in humans.[14] One of the effective treatment techniques is direct agonism of GABA receptors.[15,16], the prevention of GABA's enzymatic breakdown.[17], and the inhibition of the uptake of GABA into neuronal and glial cell bodies.[18,19] In the case of epilepsy, it has been demonstrated that convulsions can happen when the quantity of GABA in the brain drops below a crucial threshold and that seizures are

stopped when GABA is directly administering to the brain.[20–22] However, GABA does not pass the blood-brain barrier, a defence mechanism that keeps xenobiotics out of the brain. As a result, GABA is ineffective as an anticonvulsant.[23]

MATERIALS AND METHODS

Docking

AutoDock4.2 was used for docking, and grid box values for each ligand's ideal grid were determined by both trial and earlier research.[24–26] Grid maps with a point spacing of 0.375 and a dimension of 60×60×60 was built.[25] To carry out the molecular docking investigations, the Lamarckian Genetic Algorithm (LGA), regarded as one of the finest docking techniques accessible in AutoDock was used. The docking log (dlg) files were processed using the AutoDock tools, the graphical user interface of Autodock. Based on the binding energy, the docked conformations of each ligand were grouped into clusters, and the top-ranked conformations underwent visual inspection. Using Auto Dock Tools, hydrogen bonding and hydrophobic interactions between docked potent drugs and macromolecules were investigated (version 4.2).

RESULTS AND DISCUSSION

Docking of 4-Aminobutyric acid (GABA) was done with GABA-AT Protein in order to check the antiepileptic Properties of 4-Aminobutyric acid, GABA-AT is responsible for the inhibition of excitatory neuron, as epilepsy is a neurological disorder. The binding of GABA at GABA-AT protein (PDB ID 1OHV) gives a binding energy of -5.06kcal/mol with LYS B:329 with hydrogen bonding is an important amino acid responsible for Anti-epileptic property As shown in Table 1 and Figure 1, 2.

Table 1: Insilico (Docking of GABA at GABA-AT inhibitor)

PDB file used to dock	*Ligand*	*Binding Energy*	*Ligand (Binding residues)*
1OHV	GABA	-5.06	LYS B:329 GLS B:301 ILE B:267 SER B:269 PHE B:189

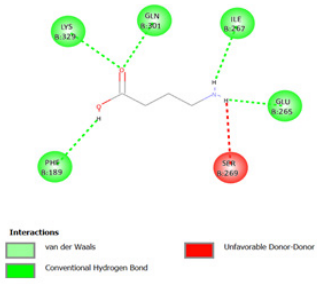

Figure 1: Docking of GABA at GABA-AT inhibitor

Insilico Docking of GABA with different analogs

After obtaining the docking score of 4-Aminobutyric acid with GABA-AT protein, 4-Aminobutyric acid is combined with different analogues in order to increase the biological activity and also to increase the lipophilicity of the drug. Some of the analogues which have been selected for docking are anthracene, phthalimide, cyclohexane, and thalidomide. The combination of Anthracene-GABA obtains the highest binding energy of -9.78kcal/mol and -7.61kcal/mol with pi bonding with LYA B:329 and Phthalimide-GABA obtains the binding energy of -7.61kcal/mol with hydrogen binding of LYA B:329 which is responsible for biological activity as shown in Table 2 and Figure 2.

Table 2: Docking of GABA with different analogs result

PDB file used to dock	*Ligand*	*Binding Energy*	*Ligand (Binding residues)*
1OHV	Anthracene + GABA	-9.78	LYS B:329 LEU A:355 ALA A:134 CYS A:138 ASN A:352 CYS B:135 SER B:137 THR A:353 ARG B:192
1OHV	Phthalimide + GABA	-7.61	LYS B:329 THR A:353 ARG B:192 TRP A:354 SER B:137 CYS B:135 GYL B:136
1OHV	Cyclohexane + GABA	-5.04	LYS B:329 PHE B:189 GLY B:136 THR A:353 SER B:137 VAL B:300
1OHV	Thalidomide + GABA	-8.09	LYS B:329 ARG B:192 CYS A:138 CYS B:135 ALA A:134 THR A:353

Base on the result of the docking, Phthalimide-GABA has been selected for future investigation. GABA cannot be entirely administered peripherally since it can only partially penetrate the blood-brain barrier (BBB) when administered at excessively high levels, which results in significant undesirable side effects.[27] Consequently, research over the past several decades targeted at successfully delivering GABA into the CNS has led to the identification of a variety of GABA analogues with better pharmacological activity.[28] particularly because several modified N-phenyl phthalimide compounds appeared to be effective at preventing convulsions.[29–33], The phthalimide pharmacophore has been added to GABA to increase lipophilicity.[34]

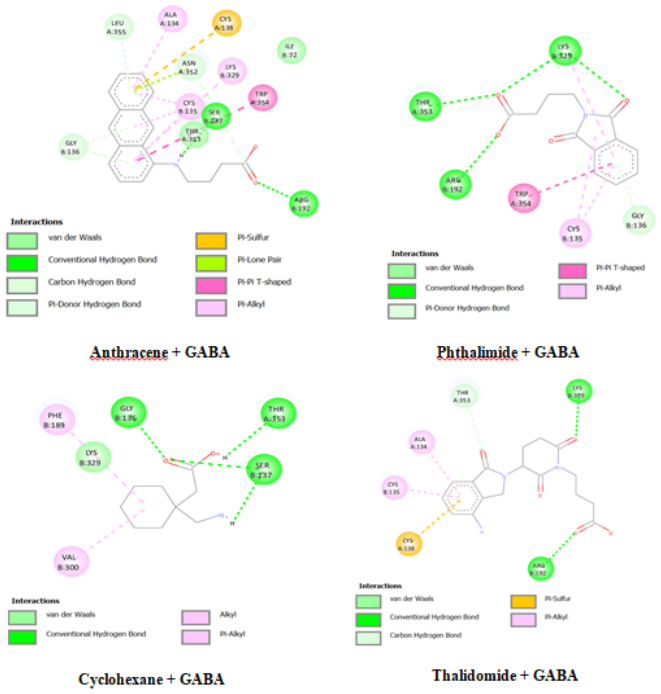

Figure 2: (2D result of combination of phthalimide)

A sodium channel in epilepsy

The sodium channel is one of the best targets in the treatment of epilepsy. The phthalimide pharmacophore is one of the new ligands that blocks sodium channels.J. V. Ragavendran and Maryam studied the anticonvulsant properties of N, N-phthaloyl GABA derivatives, which were developed and assessed as anticonvulsant medicines based on the structure-activity correlations of derivatives of thalidomide. Iman investigated Phthalimide pharmacophore as sodium channel antagonist which was created and evaluated as anticonvulsant agents based on the structure-activity relationships of derivatives .Vamecq et al. has found N-phenyl phthalimide compound as rigidized alternatives to amelotolide. They developed the 4-amino-N-(2-6-dimethyl phenyl) phthalimide model and phthalimide pharmacophore without the 4-amino group in the phthaloyl moiety.[35] N-phenyl phthalimide derivatives have a profile that is similar to that of phenytoin, and the interaction with voltage-dependent sodium channels in neurons was examined in the batrachotoxin affinity experiment. In the maximum electroshock seizure (MES) test, they are extremely strong, but inert in the subcutaneous pentylenetetrazol (ScMet) test.[36]

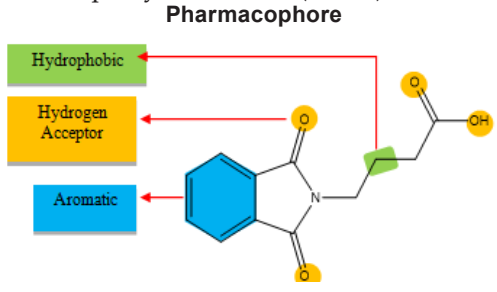

Figure 3: Pharmacophore of N, N-phthaloyl GABA

Molecular modeling

The chemical structures of inhibitors, shown in Tabel-3 were designed using ChemDraw 15.1 software. Energy minima conformers of the desired compounds were performed through the MM2 energy minima method using ChemDraw 3D software. By using the Pharmacophore of N, N-phthaloyl GABA a Noval compound 4-(1,3-dioxoisoindolin-2-yl)-N-phenyl butanamide butanamide Figure 1 has been designed by the addition of aniline. Exhibits strong binding energy of -7.19 kcal/mol Shown in Table 3. and Figure3, 4.

Table 3: *Insilico* (study of Noval Derivatives of N,N-phthaloyl GABA on sodium channel)

PDB file used to dock	*Ligand*	*Binding Energy*	*Ligand (Binding residues)*
2kaV	4-(1,3-dioxoisoindolin-2-yl)-N-phenylbutanamide	-7.19	SER A:1869 LEU A:1866 LEU A:1790 GLU A:1788 GLU A:1868 PRO A:1789

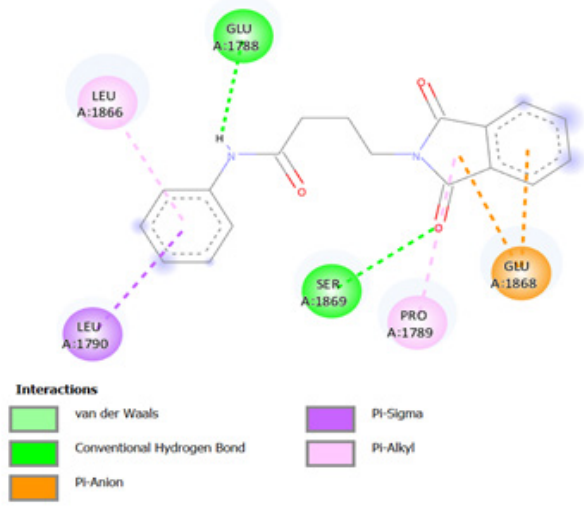

Figure 4: (2D result of Novel Derivatives of N,N-phthaloyl GABA dock with Sodium channel

Absorption, Distribution, Metabolism and Excretion (ADMET)

Physicochemical parameters of 4-(1,3-dioxoisoindolin-2-yl)-N-phenyl butanamide were predicted using Swiss ADME online Web service Figure 5. ChemDraw software, specifically ChemDraw Pro, version 15.1, was used to create two-dimensional structures. With the use of an online tool, Swiss ADME, a streamlined molecular input line entry system for 4-(1,3-dioxoisoindolin-2-yl)-N-phenyl butanamide was created. For the study, the derivatives' physicochemical characteristics lipophilicity, and solubility were taken into account. The bioavailability score, Ghose's, and Veber's rules, as well as Lipinski's rule of five (ROF), were utilised to establish the physicochemical parameters cut-off values. Molecular

characteristics including molecular weight (MW), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), log P (lipophilicity log), log S (aqueous solubility), topological polar surface area (TPSA), MW, nRot (number of rotatable bonds), and MR (molar refractivity) were assessed for drug-likeliness. Additionally, *in silico* data were produced for the main human cytochrome P450 (CYP) isoforms implicated in drug metabolism, including CYP2C9, CYP2D6, and CYP3A4. to understand the excretion of substances 4-(1,3-dioxoisoindolin-2-yl)-N-phenylbutanamide. The safety profile of the derivatives is one of the main variables in medication attrition. Using pharmacokinetic analysis, we examine some of the primary toxicity endpoint for this medication. Additionally, safety factors such as hERG liability (inhibition of dofetilide binding), LD50, hepatotoxicity, skin sensitization, and cellular toxicity were assessed.

After the cut-off values established by Lipinski's ROF, bioavailability score, Ghose's rules, and Veber's rules were taken into consideration for the pharmacokinetic evaluation of 4-(1,3-dioxoisoindolin-2-yl)-N-phenyl butanamide.

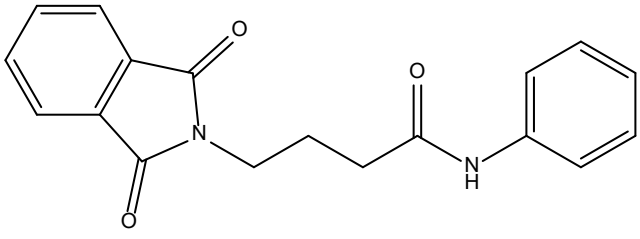

Figure 5: 4-(1,3-dioxoisoindolin-2-yl)-N-phenyl butanamide

Table 4: Important computed ADMET properties

Molecular weight	***308.33 g/mol***
Num. rotatable bonds	6
Num. H-bond acceptors	3
Num. H-bond donors	1
TPSA	66.48 Å²
Log *S* (Ali)	-3.70
Log $P_{o/w}$ (MLOGP)	2.24

Table 5: Computed safety end points

CYP2D6 inhibitor	yes
CYP3A4 inhibitor	No
Predicted oral Toxicity	LD50 1600mg/kg
Hepatotoxicity	0.73

CONCLUSION

Docking experiments of 4-(1,3-dioxoisoindolin-2-yl)-N-phenyl butanamide analogues were performed. Docking analyses showed that the majority of chemicals primarily connected with domain II-S6 of NaV1.2 by forming a hydrogen bond and also had additional hydrophobic interactions with domain I, II, III, and IV in the channel's inner pore in Table 4. The carbonyl group's oxygen has a significant impact on the hydrogen-binding interactions with the OH of Pro A:1789 in N-phenyl phthalimide derivatives. The hydrophobic interaction between the hydrophobic pocket of the receptor and the N-aryl portion of the phthalimide pharmacophore involves residues. Leu A:1790. There is barely any interaction between The Ser A: 1869 of domain III and the Aryl portion of phthalimide.

The docking study's findings suggest that the phthalimide's phenyl ring should be kept since it is essential to the drug's interaction with the receptor. They also suggest that an electronegative group can be added to the N-aryl portion at meta position to boost potency. The outcomes of this research are currently being utilized to create novel compounds with improved anticonvulsant properties. Table 5.

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