# *In-silico,* Antioxidant and Antiepileptic Effect of N(2,3-methylenedioxy-4benzoyloxy-phenthylamine)-3,4-dimethyl-1, propanoamide Derivatives

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**Summary:** Epilepsy is a severe neurological illness that affects millions of people the globally and is characterized by unpredicted and intermittent seizures.This research aimed to investigate the antiepileptic and antioxidant properties of papaverine derivatives using in-silico, in-vitro, and in-vivo methods.Epileptic seizure was induced in Swiss albino mice of either gender by administering PTZ (pentylenetetrazol). The antioxidant potential of test compounds was computed using previously published DPPH assay methods with minor modifications, while *In-silico* experiments were conducted using Auto-dock Vina (1.5.6) software and post dock analysis was completed using Discovery Studio Visualizer. The results showed that both compounds have strong antioxidant potential, with a noticeable change in color when compared to ascorbic acid as a control, and very low mortality when anti-epileptic potential was observed. The development of seizures was greatly delayed at first, but after 30 minutes of PTZ, they were completely gone. Both synthesized derivatives also comply the "Lipinski's rule of 5", which states that after structural alterations, extensive investigations, and trials, the chemical products would be evaluated for epilepsy management in the future *In-silico* investigations demonstrated that ligands with sufficient hydrogen bonds, pi-pi bonds, and Vander-Waals forces have a suitable propensity to engage with the binding pocket of selected protein targets. The current investigation of papaverine derivatives and their binding affinities against gamma aminobutyric acid (GABA) protein may have a vital function in epilepsy aetiology, according to the results of the studies. H1 and H2 were further verified in vivo for their anticonvulsant and antioxidant therapeutic potential. This could lead to more research into neurological problems.

**Keywords:** Papaverine derivatives, Docking analysis, DPPH assay, Anti-epileptic assay.

## Introduction

Heterocyclic compounds in nature exhibit numerous pharmacological actions; as a result, their use as biologically active medicines, agrochemicals, and functional materials has grown in importance. Several compounds have been produced thus far and tested for antimicrobial, anti-inflammatory, analgesic, anti-epileptic, and other properties. Epilepsy is a serious neurological illness characterized by unpredicted and intermittent seizures that affect people all over the world. Seizure is often described as a physical and behavioral handicap that results from an episode of disturbed electrical activity in the brain. According to surveys and reports, epilepsy is a globally prevalent condition that affects over 50 million people and contributes considerably to rising morbidity and mortality rates.[1] 5-hydroxytryptamine (5-HT) receptors can be found throughout the peripheral and central nervous systems. Several subtypes of 5-HT receptors have been implicated in the pathophysiology of various antiepileptic medicines that generate antiepileptic effect by upregulating extracellular levels of serotonin, according to recent research.[2]In addition, a number of 5-HT receptor subtypes, including 5-HT3, 5-HT1B, and 5-HT1A, have been connected to epilepsy. [3]. Activation of 5-HT3 has been shown to have antiepileptic properties in a number of behavioral pharmacology experiments. However, the fundamental process is not fully known. In vivo models, pentylenetetrazol (PTZ), a receptor antagonist of gamma aminobutyric acid (GABA), is used to induce seizures.[4, 5] So the induction of epileptic seizures by PTZ mainly decrease the GABA levels.[6] It has hence resulted that the prominent feature of PTZ-induced seizure is to render the levels of GABA proteins.The 5-HT3 receptor is expressed in GABAergic neurons in the cortex and hippocampus, according to earlier studies.[7] In addition, utilizing in vivo animal models, antagonists of the 5-HT3 receptor were found to alter the GABA-generated current. [6, 8]. As a result of the aforementioned information, it is possible to conclude that GABAergic neurons are linked to the actions of the 5-HT3 receptor in seizure regulation. Multiple medications have been discovered to have antiepileptic properties and are being utilized in clinical practice as potential therapeutic agents; several are undergoing clinical trials, and a large number of novel compounds being investigated as antiepileptics are currently undergoing preliminary screening.

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However, due to the risky side effects, more investigation is needed. Anti-epileptic medicines (AEDs) such as phenobarbital and benzodiazepines are commonly prescribed by doctors; both drugs raise the intracellular concentration of chloride ions via the GABA-A receptor. The duration of chloride channel opening is increased by phenobarbital, whereas the frequency of opening is increased by benzodiazepines. GABA transaminase (an enzyme that leads to the degradation of GABA neurotransmitters) is inhibited by vigabatrin, which raises the cellular level of GABA (having inhibitory potential). Based on how they work, AEDs are split into two categories:1) Drugs that increase inhibition (phenobarbital, benzodiazepines, vigabatrin, tigabine, gabapentin) and 2) Drugs that decrease excitation (phenytoin, carbamazepine, lamotrigine, felbamate, topiramate, ethosuximide, ketamine, Mg++).[9] The procedure of bischler – Napicralski isoquinoline synthesis can be used to make papaverine, an alkaloid, from vanillin. Dehydrogenation of tetrahydro papaverine can also be used to make papaverine from opium poppy (THP)[10]. The above-mentioned reaction is stereo specific, as it is solely related with the L-(S)- isomer, and the substrate specificity is undefined. The phospholipid hydrolysis mediated by thrombin in dog platelets is blocked by papaverine. Anti-inflammatory and anti-aggregate properties are found in papaverine. Papaverine causes discrete circular strips in the beef coronary artery, which relax in a dose-dependent manner. Papaverine has a smooth muscle relaxant effect due to its suppression of phosphodiesterase (PDE).[6] Furthermore, reversible suppression of morphogenesis of cultured mouse salivary glands is possible without compromising tissue integrity.[11] The simple condensation procedure can be used to produce papaverine in vitro. In this process, homoveratric acid and serine methyl ester HCl are condensed under specific conditions, followed by the elimination method to obtain N-acyl enamine. We may be able to obtain a large overall yield at the end of this process, and the quickest synthesis methods for these alkaloids have been commercially published.[10]

In recent research, we used in-silico techniques and animal testing with mice to investigate the anticonvulsant and antioxidant capabilities of certain papaverine compounds. Figs 1 and 2 show the structures of papaverine and related derivatives, which were created using Chem Sketch 2015 2.5.

**Data And Materials**

Both the compounds were synthesized by the given reported procedure with slight modifications [12].

Male and female adult albino mice were collected, and they were maintained in the animal room at Riphah International University in Islamabad, Pakistan, in a temperature-controlled setting (22–25°C). The normal diet and unlimited access to water were provided to the treated mice.All studies were conducted in accordance with the stringent guidelines established by the Commission on Life Sciences University, National Research Council (1996), and Institute of Laboratory Animal Resources. The Riphah Institute of Pharmaceutical Sciences' Ethical Committee gave its seal of approval to all procedures (Reference No. REC/RIPS/2016/10).Roche Pharmaceuticals provided the diazepam (Valium) 10mg/2ml injectable (Karachi). PTZ, DMSO, and normal saline were purchased from Sigma Aldrich Co. LLC in the United States. Analytical-grade chemicals were utilized throughout.

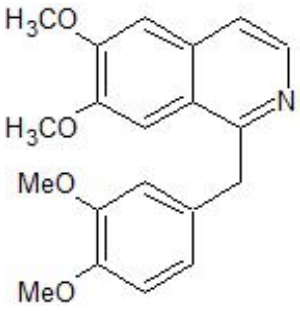
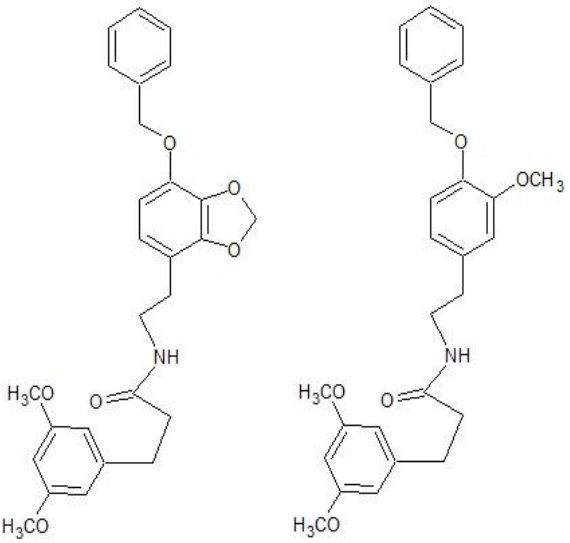



Fig 1: Chemical structure of the papaverine.




$H_1$: N(2, 3-methylenedioxy-4benzoyloxy-phenthylamine)-3,4-dimethyl-1, propanoamide
$H_2$: N(2, 3-methylenedioxy-4benzoyloxy-phenthylamine-3,methoxy)-3,4-dimethyl-1, propanoamide

Fig. 2: Chemical structures of papaverine derivatives.

*In-silico* studies

Molecular docking investigations were carried out using PyRx and the Auto Dock Vina program[8, 9]. In order to calculate the free energy values and constant for binding of test compounds, the affinity of the best-docked posture of the ligand-target complex was determined using binding energy values (Kcal/mol).[13]. The target protein's 3D structure was retrieved from the Protein Data Bank's online database (http://www.rcsb.org/pdb/home/home.doGamma amino butyric acid (GABA), which has the PDB ID 6A9X and is known to be involved in the pathophysiology of epilepsy, was chosen as the target protein. The Biovia Software, Discovery Studio Visualiser 2016, was then used to remove any ligands and water molecules that had previously been linked to the protein target.The 2D-structures of test synthesized derivatives (papaverine derivatives) were downloaded from pub chem (online data base) (https://pubchem.ncbi.nlm.nih.gov/search/) which was then transFig to PDB form by using the software of Open Babel JUI. PDB files of test compounds and protein targets were processed through Auto Dock Vina (Version 1.5.6 Sep_17_14) to generate PDBQT files. Then PDBQT files of test compounds and protein target were uploaded in PyRx and docked to obtain the best docked pose of test compounds. Binding affinities of each of the test compounds were determined in Kcal/mol. Post dock analysis was performed by using the software Discovery Studio visualiser for calculating the number of hydrogen bonds (conventional and non-conventional), number of π-π bonds and multiple amino acid residues amino acid residues tangled in the development of hydrogen bond, π-π bond and other hydrophobic bonds. The interaction of protein target with test compounds involves the following amino acids: alanine (ALA), asparagine (ASN), arginine (ARG), aspartic acid (ASP), cysteine (CYS), glutamine (GLN), glutamic acid (GLU), glycine (GLY), histidine (HIS), leucine (LEU), lysine (LYS), serine (SER), threonine (THR), tryptophan (TRP), tyrosine (TYR), valine (VAL) and phenylalanine (PHE). This is illustrated in the 2-D images of ligand and protein complex showing interactions.

*Anti-epileptic activity*

The PTZ injection (90 mg/kg, i.p.) was given to all the male mice after they were randomly separated into 5 groups (n = 5). The latency to the initial generalized myoclonic seizures as well as the length and onset of tonic-clonic seizures were all observed in all animals for up to 30 minutes following the injection. Seizures were seen and evaluated by a neutral researcher who was blind to all information. Additionally, behavioral changes were seen and kept an eye on for 30 min after PTZ (90 mg/kg, i.p.) injection. The behavior of the animal during a seizure was categorized as follows[14]: Stages include: stage #0, giving no response at all; stage #1, feeding; stage #2, myoclonic seizures; stage #3, forelimb clonus; stage #4, turning onto the side; stage #5, generalized clonic seizures; and stage #5, turning onto the back. The latencies to the onset of myoclonic and tonic-clonic seizures, as well as the length of tonic-clonic seizures, were noted for a period of 30 minutes following the PTZ injection [15]. At the conclusion of the experiment, when all of the treated mice's vital signs had failed, they were humanely put to death by CO2 inhalation. Every effort was taken to reduce suffering, including minimizing the number of animals used, the duration of the experiments, avoiding painful operations, and death.

*Anti-oxidant activity*

Anti-oxidant activity of synthesized derivatives was performed by using 2,2-diphenyl-1-picrylhydrazide (DPPH) assay with slight changes (Molyneux, 2004). The stock solution of synthesized compounds and ascorbic acid of 1 mg/mL (1000 µg/mL) concentration was prepared. Moreover, The concentrations of the samples were made in a series of dilutions (700 µg/mL, 300 µg/mL, 100 µg/mL, 10 µg/mL, 3 µg/mL, 1 µg/mL). In methanol, a 1mM DPPH solution was prepared. 1 mL of test compounds was withdrawn from the dilution and placed in a separate test tube, while 3 mL of previously prepared DPPH solution was mixed in the test tubes to form a total volume of 4 mL. Each test tube was stored at ambient temperature and wrapped with aluminum foil. The color change from purple to yellow indicates the ability of produced chemicals to scavenge free radicals and oxidize them. Finally, the absorbance was measured using a UV spectrophotometer set to 517 nm. The formula for calculating percent inhibition was as follows: [15].

$$\textit{Percentage scavenging activity} = \frac{\textit{Absorbance of control} - \textit{Absorbance of sample}}{\textit{Absorbance of control}} \times 100$$

*Statistical analysis*

SEM was used to analyze the data (Standard Error of Mean). A two-way analysis of variance (ANOVA) was used to assess the significance of the data, and a post-hoc Tukey's test was employed to confirm the results. If $P < 0.05$, it was statistically significant. The statistical program Graph Pad Prism 6 was used for graph construction, statistical analysis, and evaluation.

## Results and Discussion

*Docking evaluation*

Binding energy values (kcal/mol) collected from docking study results were used to estimate the binding affinity of test substances,[16] compound H1 and H2 represented in table 1 & 2 respectively. The tables 3 and 4, respectively, show the number of conventional non-conventional hydrogen bonds, pi-pi bonds, and amino acid binding residues used in the formation of hydrogen bonds, pi-pi bonds, and other types of hydrophobic bonds for a specific protein target that may be linked to the development and pathophysiology of epileptic seizures, as shown in the 2-D interaction images of ligand H1 and H2. Fig 3 -6 also displays binding pockets for both ligands, 9 different conformations of best pose/binding modes for each of the test compounds with a specific target, and 2D and 3D pictures of the ligand-protein interaction with specific protein targets.

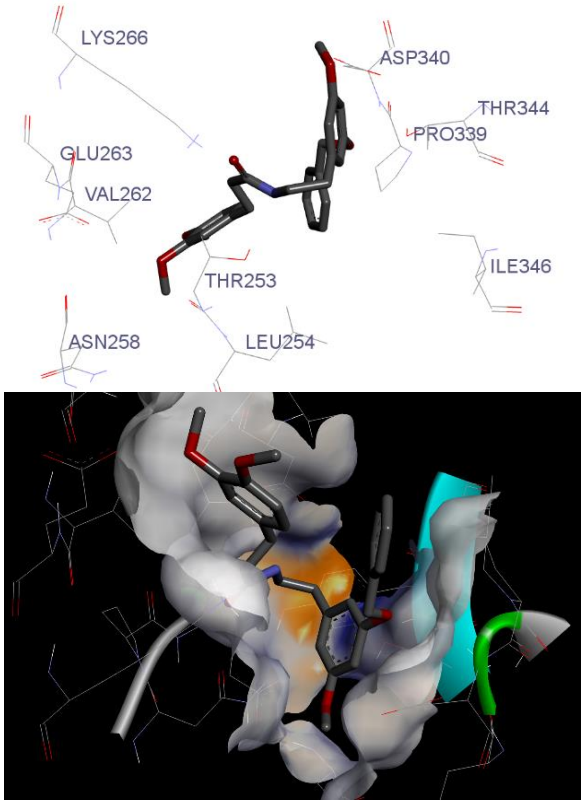



Fig. 3: Illustrates the binding pocket and 3D image of ligand H1 evaluated through Biovia Discovery Studio 2016.

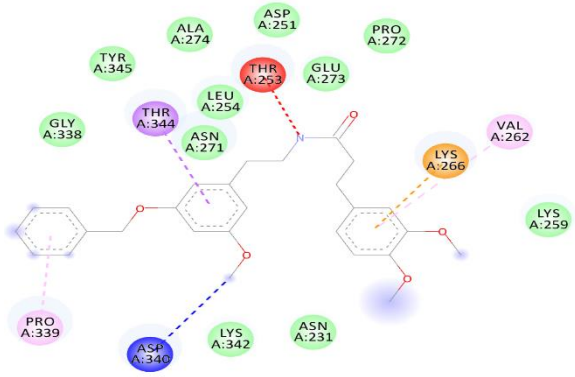



Fig. 4: Illustrates the 2 dimentional interaction of ligand H1 with gamma amino butyric acid (GABA) evaluated through Biovia Discovery Studio 2016.

Table-1: Binding affinity values of the binding modes of ligand H1.

| Mode | Affinity (Kcal/mol) | Distance from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -6.3 | 0.000 | 0.000 |
| 2 | -6.2 | 1.396 | 1.902 |
| 3 | -6.2 | 2.368 | 5.750 |
| 4 | -6.0 | 2.283 | 7.318 |
| 5 | -6.0 | 2.217 | 3.273 |
| 6 | -6.0 | 1.736 | 2.288 |
| 7 | -5.9 | 2.278 | 3.500 |
| 8 | -5.9 | 2.467 | 5.215 |
| 9 | -5.9 | 2.310 | 7.481 |

Table-2: Shows binding energy (kcal/mol) and post-dock analysis of the best conformational pose of ligand H1 with gamma amino butyric acid (GABA) receptor.

| Protein target | PDB ID | E-value (kcal/mol) | H bonds | Amino acids forming H bonds | Pi-Pi bonds | Amino acids forming Pi-Pi bonds | Amino acids forming other hydrophobic interactions |
|---|---|---|---|---|---|---|---|
| GABA | 6A9X | -6.3 | 01 | ASP A: 340 | 0 | _ | LYS A: 266, THR A: 344, VAL A: 262, PRO A: 339 |

Table-3: Binding affinity values of the different binding modes of ligand.

| Mode | Affinity (Kcal/mol) | Distance from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -6.9 | 0.000 | 0.000 |
| 2 | -6.8 | 2.494 | 3.783 |
| 3 | -6.7 | 2.605 | 5.283 |
| 4 | -6.7 | 1.257 | 2.098 |
| 5 | -6.7 | 1.971 | 3.306 |
| 6 | -6.7 | 3.810 | 5.782 |
| 7 | -6.6 | 1.772 | 2.611 |
| 8 | -6.6 | 2.238 | 3.450 |
| 9 | -6.5 | 1.216 | 1.715 |

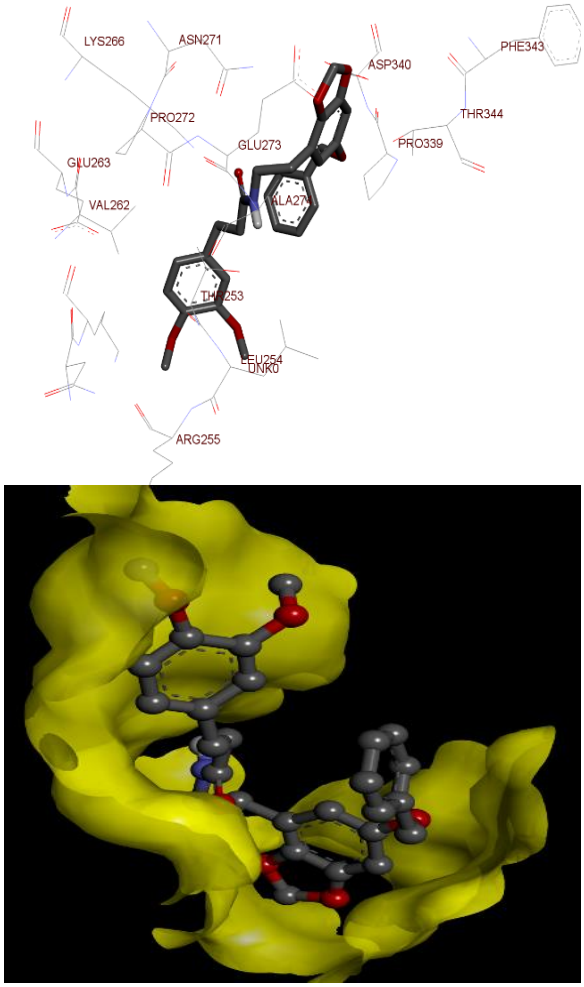



Fig. 5: Illustrates the binding pocket and 3D image of ligand H2 evaluated through Biovia Discovery Studio 2016.

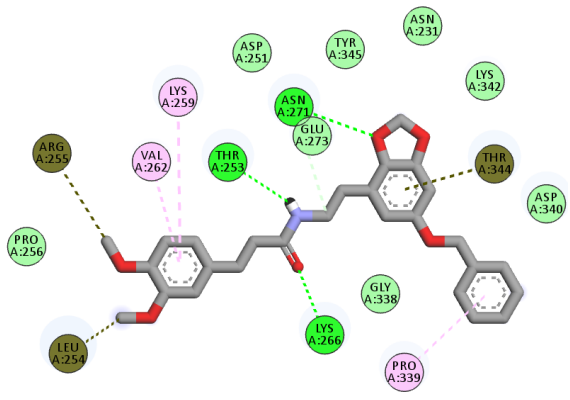



Fig. 6: Illustrates the 2 dimensional interaction of ligand H2 with gamma amino butyric acid (GABA) evaluated through Biovia Discovery Studio 2016.

Table-4: Shows binding energy (kcal/mol) and post-dock analysis of the best conformational pose of ligand H2 with gamma amino butyric acid (GABA) receptor.

| Protein target | PDB ID | E-value (kcal/mol) | H bonds | Amino acidsforming H bonds | Pi-Pi bonds | Amino acidsformingPi-Pi bonds | Amino acids forming other hydrophobic interactions |
|---|---|---|---|---|---|---|---|
| GABA | 6A9X | -6.9 | 03 | ASN A: 271, THR A: 253, LYS A: 266 | 0 | _ | LYS A: 259, THR A: 344, VAL A: 262, PRO A: 339, ARG A: 255, LEU A: 254 |

*Effect on PTZ-Induced Seizures*

H1 delayed the onset of myoclonic jerks generated by PTZ (90 mg/Kg), at a dose of 10 mg/kg, the frequency and length of tonic-clonic seizures, as well as mortality, were reduced as shown in Fig 9. Time of commencement of myoclonic jerks, time of onset of tonic-clonic seizures, and the length of tonic-clonis seizures were all shorter in the normal saline (10 mL/kg) group as 30.45 ± 2.5, 42.67 ± 1.82 and 40.6 ± 2.52 seconds (sec.) respectively. H1 (10 mg/kg) prolonged the time of onset of myoclonic jerks and tonic-clonic seizures to 60.00 ± 2.12 ($P$ < 0.001 vs. saline group) and 90.00 ± 2.5 second respectively ($P$ <0.001vs. saline group), at the same time the duration of tonic-clonic seizures decreased to 30 ± 2.12 sec. In the H2 (20 mg/kg) treated group, the time of onset of myoclonic jerks and tonic-clonic seizures increased to 80.20 ± 3.4 and 130 ± 2.7 sec. respectively (P < 0.001 vs. saline group), but the duration of tonic-clonic seizures get reduced to 20.00 ± 3.95 sec. ($P$ < 0.001 vs. saline group). In diazepam (1 mg/kg) treated group, onset times of myoclonic jerks and tonic-clonic seizures increased to 78.60 ± 2.5 and 125.8 ± 4.6 sec. respectively ($P$ <0.001 vs. normal saline group), while the duration of tonic-clonic seizures reduced to 15.60 ± 2.56 sec. (P < 0.001 vs. saline group). The group treated with nrmal saline (10 mL/kg) exhibited a 100% rate of mortality. However, H1and H2 at doses of 10 mg/kg and 20 mg/kg respectively reduced the mortality rate to 60% ($P$ < 0.01 vs. saline group) and 80% ($P$ < 0.001 vs. saline group) respectively. In the group treated with Diazepam (1 mg/kg) the rate of mortality remained 0% ($P$ < 0.001 vs. normal saline group) was observed as demonstrated in Table-5.

Table-5: Effect of H1, H2 and diazepam on pentylenetetrazol(PTZ)-induced seizures on the mortality rate in mice. Mortality (%) = (number of mice dead after convulsion/total number of mice used) × 100, n=5. ***P* < 0.01, ****P* < 0.001 vs. the normal saline group.

| Groups | Mortality (%) |
|---|---|
| Saline (10 mL/Kg) + PTZ (90 mg/Kg) | 100.00 |
| H1 (10 mg/Kg) + PTZ (90 mg/Kg) | 80.00** |
| H2 (20 mg/Kg) + PTZ (90 mg/Kg) | 60.00*** |
| Diazepam (1 mg/Kg) + PTZ (90 mg/Kg) | 0.00*** |

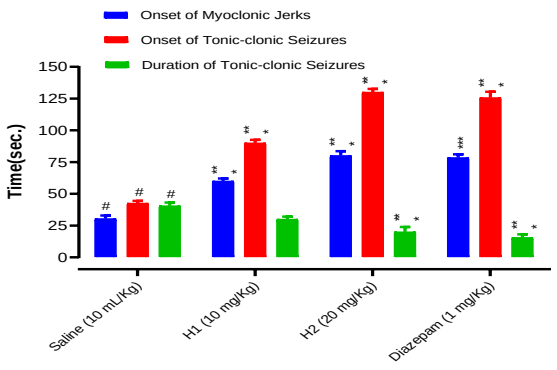



Fig. 7: Bar-graph showing effects of H1, H2 and diazepam on the time of onset of pentylenetetrazol-induced tonic-clonic seizures, myoclonic jerks, and the time span of the tonic-clonic seizures in treated mice. Symbol *** or ### show significant difference at p<0.001, while * and ** show significant difference at p< 0.05 and p< 0.01 respectively. Symbol * represents the significant difference relative to control group and the symbol # shows the significant difference relative to disease group Data expressed as mean ± SEM (n=5). two-way ANOVA followed by post-hoc Tukey's test.

*Antioxidant Results*

Fig 10 shows the dependence of all obtained results on the concentration of 2,2-diphenyl-1-picrylhydrazide (DPPH) free radical scavenging assay of test substances H1, H2, and ascorbic acid.In ascorbic acid group, percent free radical scavenging at concentrations of 1 µg/mL, 3 µg/mL, 10 µg/mL, 100 µg/mL, 300 µg/mL, 700 µg/mL and 1000 µg/mL was 32.91 ± 2.59, 36.63 ± 2.38, 39.53 ± 2.8, 42.97 ±2.68, 60.04 ± 2.11, 78.54 ± 3.32, 91.13 ± 3.58 % respectively. H1 at similar range of concentrations, 1 µg/mL, 3 µg/mL, 10 µg/mL, 100 µg/mL, 300 µg/mL, 700 µg/mL and 1000 µg/mL showed percent free radical scavenging of 10.66 ± 1.8, 19.2 ± 1.88, 24.55 ± 4.6, 30.3 ± 1.9, 55.78 ± 3.61, 56.22 ± 3.77, 60.9 ± 2.70 (*P* < 0.001 vs. ascorbic acid group) percent respectively. Whereas percent free radical scavenging mediated by H2 at similar range of concentrations, 1 µg/mL, 3 µg/mL, 10 µg/mL, 100 µg/mL, 300 µg/mL, 700 µg/mL and 1000 µg/mL was 18 ± 2.12, 20.71 ± 3.88, 38.32 ± 2.78, 40.55 ± 4.6, 62.14 ± 1.78, 65.73 ± 2.68, 68.77 ± 2.23 (*P* < 0.001 vs. ascorbic acid group) percent respectively.

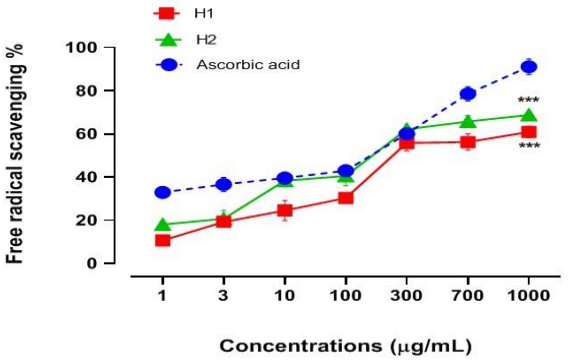



Fig. 8: Concentration response curve for using 2,2-diphenyl-1-picrylhydrazide (DPPH) free radical scavenging assay of H1, H2 and ascorbic acid. Data expressed as mean ± SEM (n=4). **P* < 0.05, ****P* < 0.001 vs. ascorbic acid group, two-way ANOVA followed by post-hoc Tukey's test.

Molecular docking is a technique used in drug research that involves structural characterization, drug specificity testing, and determining the binding affinity of molecules for certain protein targets [7]. In this investigation, the software Auto Dock Vina was used in conjunction with PyRx to perform molecular docking. [8]. It is carried out by using a specific gradient optimization approach to increase the precision and accuracy of predictions about the binding affinity of the test drug.[9]. Although hydrogen bonding is the most commonly utilised criterion for determining ligand-protein complex formation, [15]. The π–π bond, on the other hand, plays a similar role in ligand-protein complex stability as the hydrogen bond.[14]. Even though the absence of π–π bonds has no structural implications for the active binding site, it reduces the rate constant of the interaction between ligand and protein target by a factor of twenty to thirty.[17]. Other interactions, such as hydrophobic bonding, are also important in increasing the binding affinity of ligand molecules for certain protein receptors [18]. In the present investigation, binding energy values (kcal/mol), the number of hydrogen bonds, the number of π-π bonds, and binding residues of hydrogen bonds, as well as the hydrophobic interactions with the possible protein targets, were utilized to assess the affinity of ligands

for binding with protein targets.As the post-docking study revealed,, H1 formed one hydrogen bond with GABA receptor involving amino acid ASP A: 340 whereas no π–π bonds were observed to be formed. However, a few hydrophobic interactions have been seen involving multiple amino acids such as LYS A: 266, THR A: 344, VAL A: 262, PRO A: 339. On the other hand, H2 was noticed to interact with GABA receptor with greater affinity as compared to H1since it formed 3 hydrogen bonds involving amino acids ASN A: 271, THR A: 253 and LYS A: 266. However, in case of π–π bonds, H1 followed a similar trend as that of H2 and formed no π–π bonds at all. As far as hydrophobic interactions are concerned, H2 formed multiple hydrophobic bonds involving amino acids LYS A: 259, THR A: 344, VAL A: 262, PRO A: 339, ARG A: 255, LEU A: 254. As a consequence of the post-dock analysis data, it is clear that both test drugs had strong binding affinity for the GABA receptor, and the order is H2 > H1 because compound H2 produced more hydrogen bonds than compound H1.

According to the results of molecular docking, GABA activating activity could be the mechanism responsible for H1 and H2's anticonvulsant impact. The GABA activating activity of H1 and H2, which was determined through post hoc analysis of computational analyses of both test compounds, was further authenticated using the PTZ-induced seizures in-vivo model. [19]. Previous research has suggested a connection between glycine receptors and the convulsant effect of PTZ.[20] H1 and H2 not only showed a significant reduction in the duration of tonic-clonic seizures and a lower death rate in animals, but they also postponed the onset of myoclonic and tonic-clonic seizures. This debate suggests that H2 (20 mg/Kg) had a stronger antiepileptic effect than H1 (10 mg/Kg), which had a weaker antiepileptic influence than diazepam. [21].

The antioxidant ability of papaverine derivatives H1 and H2 was determined using a DPPH free radical scavenging assay. Because of their hydrogen/proton donating capacity, which tends to stabilize the free radical DPPH, certain compounds exhibit antioxidant properties. As shown in Fig 10, the antioxidant potential of chosen compounds is in the following order: ascorbic acid > compound H2 > compound H1.

## Conclusion

Computational studies have shown that papaverine derivatives exhibit high binding affinities for a specific protein target, the GABA receptor, which is known to have a role in epileptic seizure aetiology. The produced's binding energies were good. In-vivo studies confirmed compound H1 and H2's therapeutic potential against GABA protein as an anti-oxidant and anticonvulsant drug, revealing their main function as a powerful inhibitor in PTZ-induced neuroinflammation. At last of this study, we claim that these derivatives may be further used against different neuroinflammatory diseases like Alzheimer and dementia.

## List of Abbriviations

| | | |
|---|---|---|
| PTZ | = | Pentylenetetrazol |
| DSV | = | Discovery Studio Visualizer |
| DPPH | = | 2,2-diphenyl-1-picrylhydrazide |
| GABA | = | Gamma-aminobutyric acid |
| THP | = | Tetrahydro papaverine |
| HT | = | Hydroxy tryptamine receptors |
| PDE | = | Phosphodiesterase |
| ADE | = | Antiepileptic drugs |
| HCl | = | Hydrochloric acid |
| DMSO | = | Dimethylsulphoxide |
| PDB | = | Protein data bank |
| ALA | = | Alanine |
| ASN | = | Asparagine |
| ARG | = | Arginine |
| ASP | = | Aspartic acid |
| CYS | = | Cysteine |
| GLN | = | Glutamine |
| GLU | = | Glutamic acid |
| GLY | = | Glycine |
| HIS | = | Histidine |
| LEU | = | Leucine |
| LYS | = | Lysine |
| SER | = | Serine |
| THR | = | Threonine |
| TRP | = | Tryptophan |
| TYR | = | Tyrosine |
| VAL | = | Valine |
| PHE | = | Phenylalanine |

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