"Design, Synthesis and Characterization of Novel Phenobarbitone derivatives for CNS activity by In Silico model"

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ABSTRACT
This abstract presents an in silico study, the synthesis, and pharmacological evaluation of a new series of Phenobarbitone derivatives. Phenobarbitone exhibits sedative–hypnotic and anticonvulsant properties and is widely used in the treatment of seizures in both humans and animals. Phenobarbitone is one of the most widely used anticonvulsant agents but is also used in CNS disorders. In this work, an initial **in silico** study was performed to screen the designed derivatives. The compounds demonstrating the highest binding affinity and favorable docking scores—specifically compounds**. 3-[2-(2-nitro phenyl amino)acetyl]-5-ethyl-5 phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ1),2-[2-(4-bromophenoxy)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ2), and 3-[2-(4-chloro anilino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ3)**—were selected for laboratory synthesis. The targeted high-affinity compounds (**IQ, 1, 2, and 3**) were synthesized using Phenobarbitone as the starting material. Initially, the target molecules were obtained through chloroacetylation of Phenobarbitone followed by condensation with various 2-nitro aniline,4-chloro aniline and 4-bromo phenol . The progress of all reactions was monitored by thin-layer chromatography (TLC). The final compounds were purified through recrystallization and characterized by spectroscopic techniques. Pharmacological evaluation for antianxiety and skeletal muscle relaxant activities was performed on albino rats. Most of the synthesized derivatives demonstrated mild to moderate activity, exhibiting pharmacological profiles like known CNS-active agents. This study highlights the potential of Phenobarbitone derivatives as promising leads for the development of safer and more effective CNS therapeutic agents.

Keywords: Phenobarbitone, Aniline, phenol, In Silico study,Molecular docking Anxiolytic activity, Skeletal Muscles Relaxant activity, CNS agents.

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Introduction

Although phenobarbitone skeleton is mostly used to treat convulsions, it has also been shown to be effective in treating a number of other conditions, including cardiac arrhythmias, microbial infections, CNS problems, etc. Many people use this medication as an anticonvulsant. [1]. For the treatment of epilepsy and other related conditions, it is important and necessary. [2]. In pharmacology, barbituric acids and their derivatives are chemical compounds that are employed as hypnotics, sedatives, antihypertensive medications, anesthetics, anticancer, anticonvulsants, antioxidants, antifungal agents, and antibacterial agents. [3]. Phenobarbital quickly emerged as the "king of the barbiturates," both in hospital and outpatient settings, due to its longer-lasting pharmacologic activity than that of its predecessor. According to its Controlled Substances Act classification, phenobarbital has a limited potential for abuse and is regarded as a sedative rather than a hypnotic. [4]. A benzodiazepine derivative with hypnotic effects is chlordiazepoxide (L1 H).The most commonly used anticonvulsant in the world is phenobarbital (L3 H), a barbiturate. [5]. BZDs have strong anticonvulsant effects in many different animal types of seizures. [6].. Phenobarbital, carbamazepine, and phenytoin all have a urea action near phenyl rings. [7-8]. Seizures and epilepsy can be caused by a variety of etiologies, such as genetic abnormalities and metabolic disorders, traumatic brain damage, brain tumors, infections of the central nervous system, alcoholism, hypoglycemia, and drug abuse.Currently available medications for the treatment of various seizures include barbiturates (Phenobarbital, pentobarbital), benzodiazepines (clonazepam, diazepam), carbamazepine, oxcarbazepine, ethosuximide, gabapentin, lamotrigine, levetiracetam, midazolam, pregabalin, phenytoin, tiagabine, topiramate, valproate, vigabatrin, zonisamide, and propofol. [9]. Many alkyl

or aryl-barbiturates have sedative, hypnotic, anticonvulsant, and antihypertensive properties. [10]. Many neurological disorders, including epilepsy, Parkinson's disease, anxiety, migraines, Alzheimer's disease, and depression, are largely caused by a decrease in GABA levels in the brain. Additionally, glutamate is the most abundant amino acid in the brain and a significant excitatory neurotransmitter. [11]. The currently utilized drugs are effective, yet there is a significant demand for the creation of new hypnotics that have fewer side effects.[12]. An anticonvulsant drug functions by engaging with multiple distinct targets. Consequently, the assessment of anticonvulsant medications primarily involves in vivo screening tests using various seizure models designed to explore the different mechanisms of action of potential drug compounds.Some of the drugs that are presently employed to manage epilepsy include phenobarbitone, phenytoin, lamotrigine, carbamazepine, and rufinamide. [13]. Phenobarbital, a BA derivative introduced in 1912, is the oldest anti-seizure medication still widely used. Phenobarbital is used to treat all seizure types except for absence seizures. In the treatment of partial starting seizures, phenobarbitals are more effective than carbamazepine. Carbamazepine is a more effective treatment for generalized tonic-clonic seizures compared to phenobarbital[14]. Phenytoin is among the most commonly used drugs for treating epilepsy. However, due to its limited solubility in water, both as a sodium salt and free acid, it is difficult to provide to patients, and it has rarely been satisfactory. Phenytoin requires a pH of 10 to 12 to stay dissolved, so it is given orally as sodium salt in a highly alkaline solution. A significant drawback of this form of dosing is that its alkaline nature often causes stomach discomfort.Although phenytoin can be given intramuscularly, it often precipitates at the injection site, leading to variable levels of the drug in the bloodstream. In addition, the absorption of phenytoin administered intramuscularly occurs at a slow pace.Phenytoin possesses anti-seizure properties while not typically leading to central nervous system depression..[15]. Phenobarbital (5-ethyl-5-phenylbarbituric acid), developed in 1912, was revolutionary due to its sedative-hypnotic and anticonvulsant properties, making it an effective treatment for epilepsy[16]. Anticonvulsant medications currently used in clinical practice are linked to a variety of adverse side effects[17]. The oldest antiseizure medication can effectively treat partial seizures and generalized tonic-clonic seizures. Phenobarbitone, commonly referred to as primidone, is now considered a last-resort medication and is unlikely to be used in the initial stages of treatment [18]. The total occurrence of intolerable adverse effects that required the withdrawal of the randomized drug was 10%. When it comes to the specific medications, phenobarbitone (22%) was withdrawn more frequently than phenytoin (3%), carbamazepine (11%), and sodium valproate (5%)[19]. In contrast to older children, neonatal seizures often do not respond to standard medications such as phenobarbitone and phenytoin.. In addition, employing the well-established phenobarbitone is linked to both short-term (such as sedation, hypotension, respiratory depression, etc.) and long-term negative effects. Animal studies have linked phenobarbitone use to hastened neuronal apoptosis in the immature brain[20-21]. Antiepileptic drugs that are conventional have been in use for a long time. Their effectiveness and therapeutic uses are satisfactory. Their negative effects are better known than those of the new antiepileptic drugs. The currently used conventional antiepileptic medications include phenytoin, ethosuximide, carbamazepine, valproic acid, and phenobarbital.[22].

The shortcomings of traditional antiepileptic drugs necessitate the development of new AEDs that offer improved safety, reduced toxicity, and greater efficacy for patients who are difficult to control[23]. Predicting crystalline structure based solely on atomic connectivity within a molecule has posed a significant challenge for the modeling community, and this pursuit has driven the creation of more precise methods for modeling molecular crystals. A molecule's ability to take on multiple crystal structures is known as polymorphism.[24]. Predicting crystalline structure based solely on atomic connectivity within a molecule has posed a significant challenge for the modeling community, and this pursuit has driven the creation of more precise methods for modeling molecular crystals. A molecule's ability to take on multiple crystal structures is known as polymorphism.Barbituric acids and their derivatives are organic compounds utilized in pharmacology for a variety of purposes, including as hypnotics, sedatives, antihypertensives, anesthetics, anticancer agents, anticonvulsants, antioxidants, antifungals, antibacterials, and Alpha-glycosidase enzyme inhibitors[25].

MATERIALS AND MATHODOLOGY

1.In Silico Study

Virtual molecular docking studies were used to investigate the inhibitory potential of produced compounds . Virtual screening was performed using Molegro Virtual Docker (MVD), which was installed on an Intel Core i7 9700k processor with 120 GB SSD, 1 TB hard drive, and an NVIDA GeForce GTX 1050 graphics card (Fahad et al.,2022) Molecular docking studies were carried out to evaluate the

binding affinity of the synthesized phenobarbitone derivatives against the selected target protein, the human gamma-aminobutyric acid receptor (GABA(A) receptor, PDB ID: 6HUP). The docking results were analyzed based on MolDock score, Rerank score, and hydrogen bonding interactions to determine the stability and binding potential of the synthesized compounds within the receptor binding pocket.

Table 1 : Molecular docking scores, rerank scores, and hydrogen bonding interactions of synthesized phenobarbitone derivatives against GABA(A) receptor (PDB ID: 6HUP)

Cpd.code	Mol.Doc. Score	Rerank Score	HBond
COMP	-96.1731	-56.9424	-1.46907
LIGAND	-102.715	-75.2631	-0.0984889
SUB1	-114.421	-74.177	-0.596227
SUB2	-112.987	-70.1944	-2.19597
SUB3	-121.181	-72.0001	-0.985849
SUB4	-111.492	-63.6411	-2.23051
SUB5	-105.448	-50.1584	-0.613646
SUB6	-118.103	-74.4353	-2.85282
SUB7	-113.282	-65.6473	-2.48595
SUB8	-119.163	-56.6462	-2.05488
SUB9	-124.941	-76.4459	-2.60834
SUB10	-119.695	-77.6058	-2.6057
SUB11	-104.2	-47.9806	-4.94757

2.Synthesis:

Step-1:General Procedure for the Synthesis of 1-(2-chloroacetyl)-5-ethyl-5-phenylpyrimidine2,4,6(1H,3H,5H)-trione(B)

Procedure: 0.1mole of Phenobarbitone as dissolved in 50 ml of DMF in 250 ml of round bottom flask and the mixture were cooled in an ice bath to get the temperature 0-5 o C. To this cooled solution 7.9 ml (0.1mol) of chloroacetylchloride was added drop by drop and the mixture was stirred continuously for about 6-8 hrs. After completion of reaction the mixture was allowed to stand at room temp for about 1 hr and then it was poured into 100-150 ml of cold water and kept overnight for precipitation. The product was collected by vacuum filtration. The crude residue was recrystallized in ethanol. The reaction is depicted in Fig. (**1**)

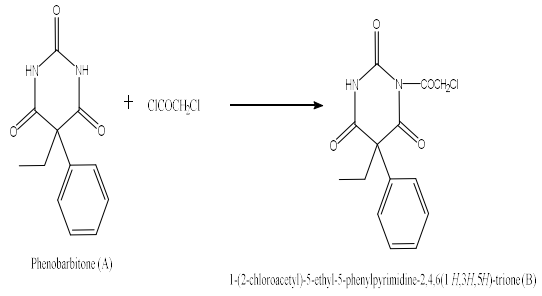


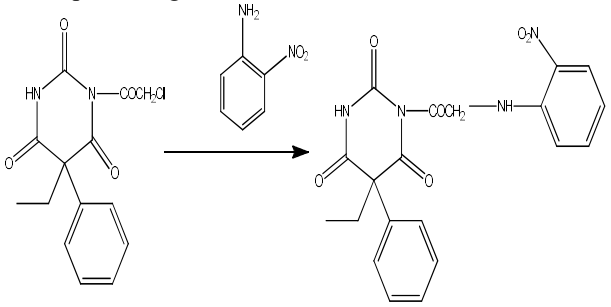
Fig.(1).Step-1 Representing synthesis of 1-(2-chloroacetyl)-5-ethyl-5-phenylpyrimidine2,4,6(1H,3H,5H)-trione(B)

Step-2: General Procedure for the Synthesis of substituted Aniline and Phenol (IQ1-IQ3)

Step-2.1:Method for the Synthesis of substituted 3-[2-(2-nitro phenyl amino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ1)

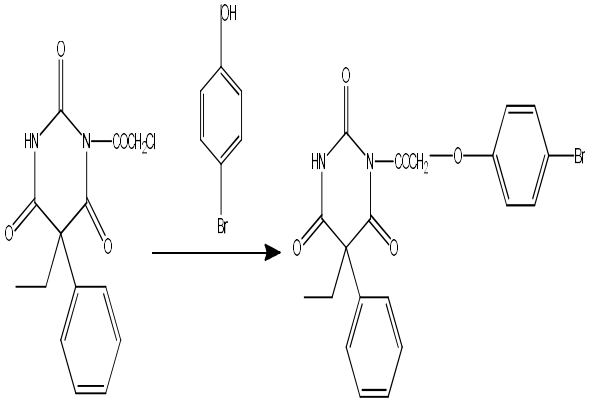
Procedure: 0.1 mole of 1-(2-Chloroacetyl)-5-ethyl-5-Phenylpyrimidine-2,4,6(1H,3H,5H)-trione(B) will

dissolve in 30 ml of DMF in a 100 ml round bottom flask, 0.374 g (0.002 mole) of anhydrous potassium carbonate (K2CO3) catalytic amount of 0.332 gm (KI) . 9.1ml (0.1 mole) of appropriate 2-nitro aniline will add into above solution. The above mixture will stirr at 50-55 o C for 24-26 hrs. After completion of reaction, the reaction mixture pour in ice cold water and kept overnight.



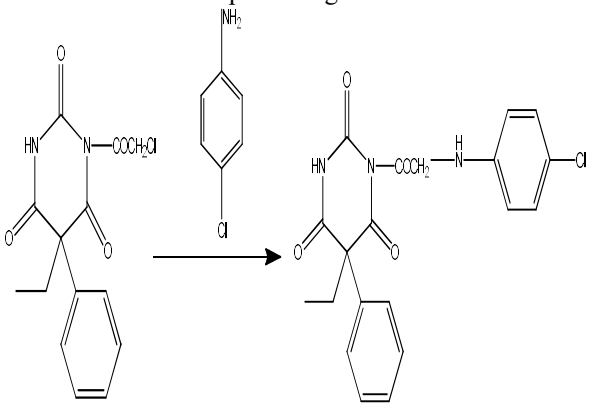
3-[2-(2-nitro phenyl amino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (IQ1)
Fig. (2)Step-2.1 Representing synthesis of 3-[2-(2-nitro phenyl amino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ1)

Step-2.2.Method for Synthesis of [2-(4-bromophenoxy)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ2)
Procedure: 0.1 mole of 1-(2-Chloroacetyl)-5-ethyl-5-Phenylpyrimidine-2,4,6(1H,3H,5H)-trione(B) will dissolve in 30 ml of DMF in a 100 ml round bottom flask, 0.374 g (0.002 mole) of anhydrous potassium carbonate (K2CO3) catalytic amount of 0.332 gm (KI) . 9.1ml (0.1 mole) of appropriate 4-bromo phenol will add into above solution. The above mixture will stirr at 50-55 o C for 24-26 hrs. After completion of reaction, the reaction mixture pour in ice cold water and kept overnight.



[2-(4-bromophenoxy)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ2)
Fig.(3)Step-2.2:Representing the synthesis of [2-(4-bromophenoxy)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ2)

Step-2.3: Method for Synthesis of 3-[2-(4-chloro anilino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ3)
Procedure: 0.1 mole of 1-(2-Chloroacetyl)-5-ethyl-5-Phenylpyrimidine-2,4,6(1H,3H,5H)-trione(B) will dissolve in 30 ml of DMF in a 100 ml round bottom flask, 0.374 g (0.002 mole) of anhydrous potassium carbonate (K2CO3) catalytic amount of 0.332 gm (KI) . 9.1ml (0.1 mole) of appropriate 4-chloro aniline will add into above solution. The above mixture will stirr at 50-55 o C for 24-26 hrs. After completion of reaction, the reaction mixture pour in ice cold water and kept overnight.



3-[2-(4-chloro anilino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (IQ3)
Fig. (4)Step-2.3 Representing synthesis of 3-[2-(4-chloro anilino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (IQ3)

3.Characterization of synthesized phenobarbitone derivatives

3.1. 3-[2-(2-nitro phenyl amino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ1)

Yellow to orange solids|crystals, **M.P** -185-210 ºC. **Molecular formula:** $C_{20}H_{18}N_4O_6$; IR (KBr, cm^{-1}): 3328 (str, N-H imide), 3219 (str, N-H anilino), 2918 (str, CH alip), 1734 (str, C=O imide), 1471 (str, C=C arom), 1295 (str, NO_2 sym), 1112 (str, C-N alip), 753 (str, C-H arom oop), 556 (str, ring def). **^{1}H NMR** (400 MHz; DMSO-d_6) δ ppm: 8.30 (s, 1H, Ar-$NHCH_2$); 7.97-7.93 (m, 1H, Ar-H ortho to NO_2); 7.71-6.59 (m, 10H, Ar-H); 3.36, 3.18 (s, 2H,

$COCH_2NH$); 2.15 (q, 2H, CH_2CH_3); 1.22-1.04 (t, 3H, CH_2CH_3); 11.0 (br s, 1H, N1-H, exchangeable). **^{13}C NMR** (100 MHz; DMSO-d_6) δ ppm: 168.5-165.0 (4C, C=O imide + amide); 148.3 (C, C-NO_2); 138.5-128.5 (16C, Ar-C); 65.8 (C, C5 quaternary); 52.4 (CH_2, $COCH_2NH$); 30.2 (CH_2, CH_2CH_3); 8.1 (CH_3, CH_2CH_3). **HRMS (ESI$^+$) m/z:** 411.3049 $[M+H]^+$, 433.2965 $[M+Na]^+$. Calcd for $C_{20}H_{18}N_4O_6$: C, 58.54%; H, 4.42%; N, 13.65%. Found: C, 58.51%; H, 4.39%; N, 13.62%.

3.2: [2-(4-bromophenoxy)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ2)

Off white crystalline solid,**M.P**-170-220 ^oC **Molecular formula:** $C_{20}H_{17}BrN_2O_5$; IR (KBr, cm^{-1}): 3306 (str, N-H imide), 3080 (str, C-H arom), 2975 (str, C-H alip), 1733 (str, C=O imide), 1662 (str, C=O amide), 1589 (str, C=C arom), 1248 (str, C-O-C ether), 1065 (str, C-Br), 755 (str, C-H arom oop). **^{1}H NMR** (400 MHz; DMSO-d_6) δ ppm: 11.2 (br s, 1H, N1-H, exchangeable); 7.52-7.28 (m, 9H, Ar-H phenyl + 4-bromophenoxy); 4.85 (s, 2H, $COCH_2O$); 2.18 (q, 2H, CH_2CH_3); 1.12 (t, 3H, CH_2CH_3). **^{13}C NMR** (100 MHz; DMSO-d_6) δ ppm: 168.2-165.1 (4C, C=O imide + amide); 156.4 (C, C-O ether); 138.9-128.3 (12C, Ar-C); 115.2, 113.8 (2C, C-Br & C-O ipso); 66.1 (C, C5 quaternary); 65.4 (CH_2, $COCH_2O$); 30.5 (CH_2, CH_2CH_3); 8.3 (CH_3, CH_2CH_3). LC-MS (ESI$^+$) m/z: 445.1 $[M+H]^+$, 447.1$[M+2+H]^+$ 1:1 ratio for Br isotope. Calcd for $C_{20}H_{17}BrN_2O_5$: C, 54.07%; H, 3.86%; N, 6.31%; Br, 17.98%. Found: C, 54.03%; H, 3.83%; N, 6.29%; Br, 17.95%.

3.3: 3-[2-(4-chloro anilino)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ3)

Whitw to pale yellow crystalline solids\powder**,M.P**-180-215 ^oC **Molecular formula:** $C_{20}H_{18}ClN_3O_4$; **IR (KBr, cm^{-1}):** 3327 (str, N-H amide), 3247 (str, N-H), 2935 (str, C-H alip), 1733 (str, C=O ester), 1712 (str, C=O amide/ketone), 1688 (str, C=O conjugated), 1628 (str, C=C arom), 1527 (str, N-H bend), 1218 (str, C-O ester), 1097 (str, C-N), 875, 801 (str, C-H arom oop), 657, 591 (str, C-Cl). **^{1}H NMR** (400 MHz; DMSO-d_6) δ ppm: 8.31 (s, 1H, heteroaromatic H); 7.75 (d, 1H, Ar-H ortho to Cl); 7.46 (d, 1H, Ar-H); 7.43 (t, 1H, Ar-H); 7.36 (t, 1H, Ar-H); 4.43 (m, 1H, CH-N/O); 4.09 (m, 1H, CH-N/O); 3.31 (m, 2H, CH_2-N/O); 2.96 (m, 1H, CH_2-α to C=O); 2.79 (m, 1H, CH_2-α to C=O); 2.69 (m, 1H, CH_2); 2.60 (m, 1H, CH_2); 2.17 (s, 3H, CH_3); 2.06 (s, 3H, CH_3); 1.04 (t, 3H, CH_2CH_3). **^{13}C NMR** (100 MHz; DMSO-d_6) δ ppm: 172.8, 168.4 (2C, C=O ester + amide); 159.2, 155.6 (2C, C=N heteroarom); 138.9, 134.2, 131.5, 129.8, 128.3, 127.1, 126.4, 124.0, 122.7, 120.5 (10C, Ar-C); 58.2, 54.7 (2C, CH-N/O); 42.1, 38.5, 33.9, 29.4 (4C, CH_2); 22.3, 18.7, 14.2 (3C, CH_3). **MS (ESI$^+$) m/z:** 402.0 $[M+H]^+$, 404.0 $[M+H+2]^+$. **Calcd for $C_{20}H_{18}ClN_3O_4$:** C, 60.08%; H, 4.51%; N, 10.52%; Cl, 8.87%. Found: C, 60.05%; H, 4.48%; N, 10.50%; Cl, 8.85%.

4. PHARMACOLOGICAL EVALUATION:

The anxiolytic activity was performed by Elevated plus maze method and skeletal muscle relaxant activity was carried out by Rotarod method in Albino Rat (Parle et al., 2010, Kulkarni et al., 1996; Sinoriya et al., 2011). Prior permission of the Animal Ethics Committee was obtained and all experiments were conducted according to the approved protocol. Diazepam was employed as a standard (positive control). Statistical analysis of the results in the test group was done by comparison with the results in the control group employing one way ANOVA. Level of significance was fixed at $p<0.05$.

4.1. Anxiolytic activity (Elevated plus maze method):

Albino rat, weighing –100-150g each, was selected from the stock colony maintained in the central animal facility with free access to food and water. Animals were maintained in an air-conditioned room. The room was maintained at 25 ± 2 ^{0}C with natural daytime. Concentration of each compound (25 mg/kg) was used in the form of freshly prepared suspensions in 0.5% CMC. All solutions were prepared freshly on test days and given orally in a volume of 0.2 ml/kg 100-150g body weight of rat. The experimental animals were treated with diazepam (2 mg/ kg, n = 3 i.p), or the test compounds (25 mg/kg) 60 min before evaluation in the maze. The control group was given saline with0.5% CMC. Elevated plus maze apparatus consisted of two open (16 x 5 cm^2) and two closed arms (16 x 5 x 12 cm^3) facing each other with an open roof (Moser et al., 1989; Pellow et al., 1985; Rabbani et al., 2004). The entire maze is elevated to a height of 25 cm. In the test group, mice were individually examined in 5 min sessions in this apparatus. Each mouse was placed in the central platform facing one open arm.

The numbers of entries into open and closed arms and the time spent in open arms were recorded during a 5 min period. The percentage of number of entries into open arms [(open/open +closed) x 100] was calculated for each mouse. The results of EPM have been summarized in tables 2 and figure 5 .In analogs bearing **3-[2-(4-bromo phenoxy)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione)(IQ2):** has shown the highest antianxiety.

.**Table 2: Antianxiety activity of the target compounds (IQ1-3) by Elevated plus maze method.**

Compound code	Open Arm		
	Spent time	**Number of entries**	**% Number of Entries**
IQ1	56.73±1.60	7.86±0.35	46.37
IQ2	74.66±4.75	3.28±0.67	28.10
IQ3	70.93±2.42	3.65±0.33	36.20
Diazepam	91.83±2.31	10.89±0.74	63.42
Vehicle	42.12±3.40	2.84±0.90	20.22

Data represent the mean ± SEM; n= 5; P< 0.05 compared with vehicle

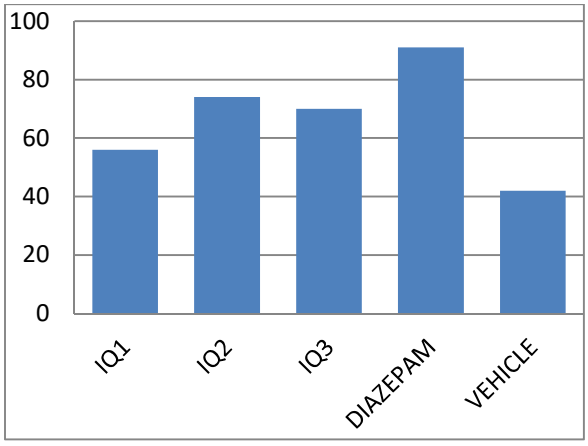


Figure- 5 : Antianxiety activity of the target compounds (IQ1-3) by Elevated plus maze method

4.2. Skeletal muscle relaxant activity (Rotarod method):

Skeletal muscle relaxant activity of target compounds was carried out by Rotarod method. Rat were placed on a horizontal wooden rod rotating at a speed of 25 rpm. The rat capable of remaining on the top for 1 min or more, in three successive trials were selected for the study. The selected animals were divided into 9 groups (n= 9). The stock solutions of all the test samples and standard were prepared by suspending in 0.5% CMCs. CMC (0.5%, Ml/kg) and diazepam (2 mg/kg, i.p.) were given to group control and standard. Test sample 100 mg/kg (i.p.) was administered into test groups. Each group of animals was then placed on the rod at an interval of 30 min. The animals that failed more than once on the Rotarod for 1 min were considered as passed the test. The results of skeletal muscle relaxant activity have been summarized in tables 3 and figure 6. In analogs bearing **3-[2-(4-bromo phenoxy)acetyl]-5-ethyl-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione)(IQ2), 3-[2-(4-chloroanilino)acetyl]5ethyl5phenylpyrimidine-2,4,6(1H,3H,5H)-trione(IQ3)** has shown the highest muscle relaxant activity than other analogs.

Table 3: Skeletal muscle relaxant activity of target compounds (IQ1-3) by Rotarod method

Compd. code	**Dose**	**Rotarod test**
IQ1	25 mg/kg	58.12±1.31
IQ2	25 mg/kg	72.51±2.92
IQ3	25 mg/kg	70.18±1.93
Diazepam	2mg/kg	38.20±1.43
Vehicle	10ml/kg	84.12±1.97

Data represent the mean ± SEM; n= 5; P< 0.05 compared with vehicle.

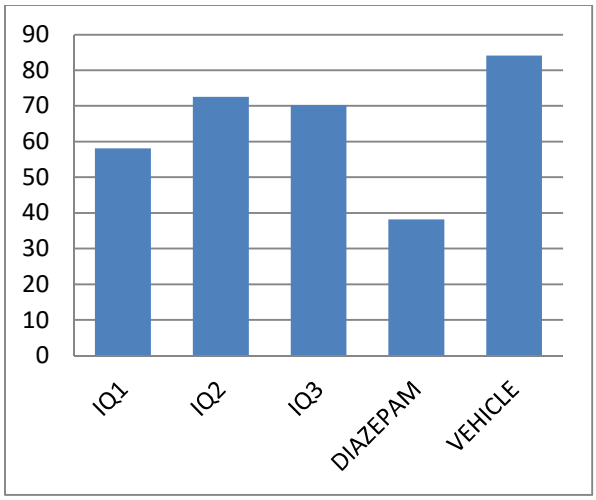


Figure 6: Skeletal muscle relaxant activity of target compounds (IQ1-3) by Rotarod method

Conclusion:

In this study, a novel series of Phenobarbitone derivatives was successfully designed, chemically synthesized, and pharmacologically validated to explore safer central nervous system (CNS) therapeutic agents (p. 1). Initial *in silico* molecular docking analysis against the human GABA A receptor{PDB\ ID:\ 6HUP} served as an effective virtual screening tool, identifying targeted compounds **IQ1**, **IQ2**, and **IQ3** as high-affinity candidates with stable receptor-ligand interaction profiles.Synthetic scheme 1 depicted the synthesis method for Phenobarbitone derivatives. The target compounds (Sub1-Sub11) were synthesized by reacting **Phenobarbitone (A)** with chloroacetyl chloride in DMSO to produce **1-(2-chloroacetyl)-5-ethyl-5-phenylpyrimidine2,4,6(1H,3H,5H)-trione(B))**, which was then reacted with various substituted 2-nitro aniline,4-chloro aniline and 4-bromo phenol to yield the product (IQ1-IQ3).The characteristic absorption bands and proton signals corresponding to the functional groups present in the target molecules were observed in the FT-IR and ^{1}H-NMR spectra of the synthesized compounds IQ1, IQ2, and IQ3.IR spectra of all three compounds displayed prominent bands in the following regions: 3328-3247 cm^{-1} for N-H stretching of amide/imide groups, 3080-2918 cm^{-1} for aromatic and aliphatic C-H stretching, 1734-1688 cm^{-1} for carbonyl C=O groups of ester/amide/ketone, 1628-1471 cm^{-1} for C=C aromatic stretching, 1295 cm^{-1} for the NO2 group in IQ1, 1218-1097 cm^{-1} for C-O/C-N stretching, and 875-556 cm^{-1} for C-H out-of-plane bending and C-Cl stretching in IQ3.^{1}H-NMR spectra obtained using a Bruker DRX-300 spectrophotometer displayed multiplet signals in the δ 7.0-7.97 ppm range indicative of aromatic protons, a singlet at δ 11.0-10.85 ppm for exchangeable amide/imide NH, a singlet at δ 9.24-8.30 ppm for Ar-NHCH2, a singlet at δ 3.45-3.27 ppm for CH2CO protons, and aliphatic signals at δ 4.43-1.04 ppm for CH-N/O and CH2CH3 groups. The proposed structures were corroborated by ^{13}C-NMR spectra obtained on a Bruker 100 MHz device, as well as by elemental analysis performed using a Shimadzu mass spectrometer and a Perkin Elmer 2400 CHN analyzer. All synthesized compounds were assessed for CNS activities, including antianxiety, skeletal muscle relaxant, and anticonvulsant effects.Most of the derivatives showed promising activity, with compound IQ3, which has a chloro substitution on the phenyl ring, demonstrating superior CNS activity compared to IQ1, IQ2, and the standard drug Diazepam. Similarity studies indicated a mild to moderate correlation with the standard drug. The physicochemical parameters, pharmacokinetic and toxicity studies of IQ1-IQ3 were consistent with the standard and suggested their drug-likeness and ability to cross the blood-brain barrier, indicating their potential as CNS active agents.

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