IJAPB International Journal of Advances in Pharmacy and Biotechnology

Journal homepage: http://ijapbjournal.com/

Research Article

Synthesis of Piperonal based Dihydropyrimidinones and evaluation for possible Anticonvulsant and Antibacterial activities

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ARTICLE INFO

Article history:
Received 11 Feb 2020
Received in revised form 24 Feb 2020
Accepted 27 Feb 2020
doi.org/10.38111/ijapb.20200601002

Keywords:
Piperona, Dihydropyrimidinones, Anticonvulsant, Antibacterial activities

ABSTRACT

A new series of piperonal based DHPMs substituted diaryl urea derivatives were synthesized and their anticonvulsant effects on the activity and antibacterial were evaluated. Maximal electroshock (MES) induced convulsion and strychnine induced convulsion models were used for the study. There was a significant ($p<0.05$) decrease in the duration of tonic hind limb extension at both the doses of test compounds in MES model. Compared with the control group, treatment with test compounds had no significant effect on onset and duration of convulsions in the strychnine induced seizure model. Anti-convulsant studies with test drug showed a significant protection in a dose dependent manner. The antibacterial activity of the test compounds was assayed systematically against four different strains of bacteria. It was observed that few compounds were shown better inhibitory activities when compared to the standard drug Streptomycin..

1. Introduction

Epilepsy is currently one of the most common disorders of the brain, affecting about 50 million individuals worldwide of different cerebral disorders of the CNS[1]. This syndrome is characterized by the periodic and unpredictable occurrence of epileptic seizures that are caused by abnormal discharges of cerebral neurons. Epilepsy is not a disease, but a syndrome, excessive and hyper synchronous discharges of large number of neurons. Abnormal electrical activity during a seizure can be detected by EEG (Electroencephalography) recording from electrodes distributed over the surface of the scalp. Different types of epilepsy are usually distinguished by the type of the seizures and area of the brain affected[2]. In the absence of a specific etiologic understanding in any of the epilepsies or epileptic syndromes, approaches to drug therapy of epilepsy must be directed at the control of the symptoms, that is, the suppression of seizures. Currently, all available drugs are anticonvulsant (*i.e.* antiseizure) rather than antiepileptic[3]. The goal of therapy with an anticonvulsant agent is to have the patient seizure free without interfering with normal brain function. Thus, the selection of an anticonvulsant agent is based primarily on its efficacy for specific types of seizures and epilepsy. Although seizure control is generally good in most patients, a significant proportion of patients with epilepsy suffer from intractable or drug resistant epilepsy, despite early treatment and an optimum daily dosage of an adequate anticonvulsant agent. There is thus a need for new drugs with a greater benefit as related to side effects and tolerability, even at the expense of efficacy, when compared to the existing antiepileptic agents[4].

Pyrimidine, also known as m-diazine (or) 1, 3-diazine isolated in 1899, is the most important member of all the diazines as this ring system occurs widely in living organisms[5]. It is the parent substance of large group of heterocyclic compounds and plays a vital role in many biologic processes, as being found in nucleic acids, several vitamins, co-enzymes and purines. Pyrimidine itself is not found in nature but substituted pyrimidines and compounds containing the pyrimidine ring system are widely distributed in nature[6,7]. Piperonal is a naturally occurring compound found in block locust (*Robinia pseudoacacia*), a tree which is majorly used for timber but also for paper pulp and fuel. Piperonal is a derivative of safrole, a naturally occurring aromatics obtained from botanical sources such as *cinnamomum petrophilum* and *sassafras albidum*. In view of the general observation, piperonal derivatives were found to be associated with diverse

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pharmacological activities, such as antimicrobial, anticancer, antiviral, analgesic, antipyretic, anti-inflammatory activities etc[8-10]. In the present investigation, synthesis and evaluation of new O-Mannich bases of piperonal derivatives and evaluated for their anticonvulsant activity using 4-amino pyridine induced method and their antimicrobial activity.

2. Materials and Methods

2.1. Chemistry

All the chemicals were obtained from SD fine chemicals Ltd, Ranbaxy chemicals Ltd and Qualigens chemicals Ltd and the solvents were of laboratory grade. Each reaction of every step was monitored by TLC by using appropriate solvent system, which was selected by trial and error method. Precoated TLC plates (silica gel GF254) were obtained from E. Merk. All the synthesized compounds were purified by recrystallization. Melting points were noted on open capillary and they were uncorrected.

2.2. Biological Evaluation

Acute Toxicity Study and Dose Selection of Drug: Acute Toxicity study for the test compounds (3a, 3b, 3f) was carried out on mice according to OECD guidelines. Three mice were fasted overnight and maintained with water ad libitum. Each animal received single dose of test compound (300 mg/kg, 0.1%. CMC). After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hr and 24 hr to detect changes in the autonomic or behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and then monitored for any mortality for the following 14 days. No, mortality or any other autonomic or behavioral responses such as tremors, convulsion, salivation, diarrhea, lethargy, sleep and/or coma were observed during first 14 days.

2.3. Experimental Animals:

All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for ;;the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee. Mice were obtained from Mahaveer enterprises, Hyderabad, of weighing 25-30 gm were housed in groups of 6. Mice were maintained at standard laboratory conditions. All mice were fed with semi purified basal diet and demineralised drinking water ad libitum. Mice were maintained at 22 ± 1ºC with 60% relative humidity, and kept under 12 h light:dark cycle. The animals were allowed to acclimatize to laboratory conditions prior to experimentation. All experiments were conducted during the light period of 12 hours of the day/night cycle.

Table 3: Grouping of the animals for Anticonvulsant Activity

Group (n =6)	Treatment
I (Control)	Normal saline 10ml/kg
II (Standard)	Phenytoin 30 mg/kg
III (Test 1)	Test compound (3a) 50 mg/kg
IV (Test 2)	Test compound (3b) 100 mg/kg
V (Test 3)	Test compound (3f) 100 mg/kg

3. Experimental

3.1. Synthesis of chalcones:

A mixture of 22 gm of sodium hydroxide in 200 ml of water and 100 gm (122.5 M) of rectified spirit in a 500 ml bolt head flask was provided with a mechanical stirrer. The flask was immersed in a bath of crushed ice, 52 gm (0.43 M) of freshly distilled different aromatic acetophenone derivatives was added while stirring and then 46 gm (44 ml, 0.43 M) of piperonal was added (See Figure 1). The temperature of the mixture was kept at about 0-5ºC and stirred vigorously until the mixture was so thick that stirring is no longer effective (2-3 hr). Stirrer was removed and the reaction mixture was kept in an ice chest or refrigerator overnight. Thus, obtained product was filtered and washed with cold water until the washings were neutral to litmus and then with 20ml of ice-cold rectified spirit and dried. It was purified by recrystallization from ethanol to give a pure compound[11-13].

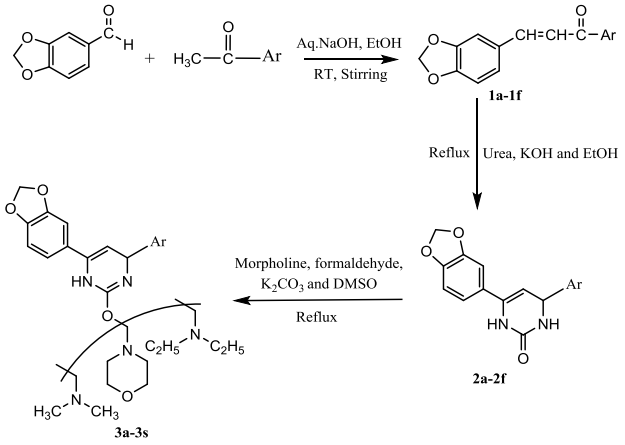
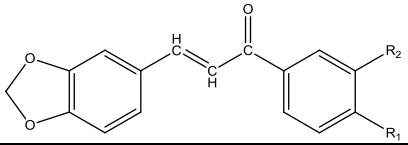


Figure 1: Scheme for the synthesis of desired compounds

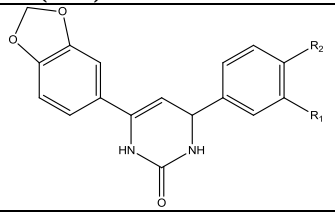
Table 1: Physicochemical data of synthesized Chalcones (1a-1f):



Code	R_1	R_2	Mol. Formula	Mol. Wt.	M.P (ºC)	% Y	*R_f
1a	H	H	$C_{16}H_{12}O_3$	252	103-105	68	0.7
1b	CH_3	H	$C_{17}H_{14}O_3$	266	118-120	72	0.6
1c	Br	H	$C_{16}H_{11}BrO_3$	331	116-118	87	0.7
1d	NH_2	H	$C_{16}H_{13}NO_3$	267	112-114	69	0.4
1e	OH	H	$C_{16}H_{12}O_4$	268	107-109	55	0.7
1f	H	NO_2	$C_{16}H_{11}NO_5$	297	131-133	68	0.6

*(Hexane: Ethyl acetate - 3: 2)

Table 2: Physicochemical data of synthesized of the Dihydropyrimidines **(2a-2f)**



Code	R_1	Mol. Formula	Mol. Wt.	M.P (^{0}C)	% Y	*R_f
2a	H	$C_{17}H_{14}N_2O_3$	294	78-81	72	0.61
2b	CH_3	$C_{18}H_{16}N_2O_3$	308	83-85	75	0.68
2c	Br	$C_{17}H_{13}BrN_2O_3$	373	96-98	68	0.72
2d	NH_2	$C_{17}H_{15}N_3O_3$	303	121-123	74	0.75
2e	OH	$C_{17}H_{14}N_2O_4$	310.3	145-148	65	0.52
2f	H	$C_{18}H_{16}N_2O_3$	308.3	129-131	71	0.65

*(Hexane: Ethyl acetate - 3:2); R_2 = 2a-2e = H; 2f = NO_2

3.2. General procedure for the synthesis of 4,6-diaryl-1,4-dihydropyrimidin -2- one:

A mixture of 0.01 M of chalcone, 0.01 M of urea and potassium hydroxide (1 gm) in 20ml ethanol was heated under reflux for 6 hr (Figure 1). The reaction was monitored by TLC. After completion of the reaction, the contents were cooled to room temperature and poured into ice cold water (50 ml) while stirring. The solid thus separated was filtered, washed with portions of cold water and dried. It was purified by recrystallization from ethanol to give a pure compound[14-18].

3.3. General procedure for synthesis of O- Mannich bases of 3, 4-dihydropyrimidine-2-one:

Dihydropyrimidine (II, 0.005 M) was dissolved in DMSO (25 ml) in a conical flask, and stirred with 37% HCHO (0.01 M) then added anhydrous potassium carbonate (1.0g), appropriate secondary amine (0.005 M) & continued the stirring magnetically for about 2 hr (Figure 1). The reaction mixture was then heated under reflux for about 5 hr and then kept at refrigerator for 48h, filtered the separated product, washed with small portions of cold water and dried. The crude product was purified by recrystallization from Pet. Ether: Chloroform (1:1 mixture)[19-21].

4-(((6-(benzo[d][1,3]dioxol-5-yl)-4-phenyl-1,4-dihydropyrimidin-2-yl)-oxy)methyl)morpholine (3a): Mol. Formula: $C_{22}H_{25}N_3O_4$, Mol. Weight: 395.45, Solubility: Chloroform, DMSO, MP (ºC): 92-94, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 5.93 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 396 (M+-1) m/z.

4-(((6-(benzo[d][1,3]dioxol-5-yl)-4-(p-tolyl)-1,4-dihydropyrimidin-2-yl) oxy)methyl)morpholine (3b): Mol. Formula: $C_{23}H_{27}N_3O_7$, Mol. Weight: 409.48, Solubility: Chloroform, DMSO, MP (ºC): 78-80, IR (KBr): 3460 (N-H), 3157 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 410 (M+-1) m/z.

4-(((6-(benzo[d][1,3]dioxol-5-yl)-4-(4-bromophenyl)-1,4-dihydropyrimidin-2-yl)oxy)methyl) morpholine (3c): Mol. Formula: $C_{22}H_{24}BrN_3O_4$, Mol. Weight: 474.35, Solubility: Chloroform, DMSO, MP (ºC): 92-94, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 476 (M+-1) m/z.

4-(6-(benzo[d][1,3]dioxol-5-yl)-2-(morpholinomethoxy)-1,4-dihydropyrimidin-4-yl)aniline (3d): Mol. Formula: $C_{22}H_{26}N_2O_4$, Mol. Weight: 410.47, Solubility: Chloroform, DMSO, MP (ºC): 96-98, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 411 (M+-1) m/z.

4-(6-(benzo[d][1,3]dioxol-5-yl)-2-(morpholinomethoxy)-1,4-dihydropyrimidin-4-yl)phenol (3e): Mol. Formula: $C_{22}H_{25}N_3O_5$, Mol. Weight: 411.45, Solubility: Chloroform, DMSO, MP (ºC): 148-150, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 412 (M+-1) m/z.

4-(((6-(benzo[d][1,3]dioxol-5-yl)-4-(3-nitrophenyl)-1,4-dihydropyrimidin-2-yl)oxy)methyl) morpholine (3f): Mol. Formula: $C_{22}H_{24}N_4O_6$, Mol. Weight: 440.45, Solubility: Chloroform, DMSO, MP (ºC): 92-94, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 441 (M+-1) m/z.

1-((6-(benzo[d][1,3]dioxol-5-yl)-4-phenyl-1,4-dihydropyrimidin-2-yl)oxy)-N,N-dimethylmethanamine (3g): Mol. Formula: $C_{20}H_{23}N_3O_3$, Mol. Weight: 353.41, Solubility: Chloroform, DMSO, MP (ºC): 91-93, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 354 (M+-1) m/z.

1-((6-(benzo[d][1,3]dioxol-5-yl)-4-(p-tolyl)-1,4-dihydropyrimidin-2-yl)oxy)-N,N-dimethylmethanamine (3h): Mol. Formula: $C_{21}H_{25}N_3O_3$, Mol. Weight: 367.44, Solubility: Chloroform, DMSO, MP (ºC): 82-84, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 368 (M+-1) m/z.

1-((6-(benzo[d][1,3]dioxol-5-yl)-4-(4-bromophenyl)-1,4-dihydropyrimidin-2-yl)oxy)-N,N-dimethylmethanamine (3i): Mol. Formula: $C_{23}H_{24}BrNO_4$, Mol. Weight: 430.33, Solubility: Chloroform, DMSO, MP (ºC): 92-94, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 431 (M+-1) m/z.

4-(6-(benzo[d][1,3]dioxol-5-yl)-2-((dimethylamino)methoxy)-1,4-dihydropyrimidin-4-yl)aniline (3j): Mol. Formula: $C_{20}H_{24}N_4O_3$, Mol. Weight: 368.43, Solubility: Chloroform, DMSO, MP (ºC): 108-110, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 369 (M+-1) m/z.

4-(6-(benzo[d][1,3]dioxol-5-yl)-2-((dimethylamino)methoxy)-1,4-dihydropyrimidin-4-yl)phenol (3k): Mol. Formula: $C_{22}H_{25}N_3O_5$, Mol. Weight: 426.45, Solubility: Chloroform, DMSO, MP (ºC): 161-163, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 427 (M+-1) m/z.

1-((6-(benzo[d][1,3]dioxol-5-yl)-4-(3-nitrophenyl)-1,4-dihydropyrimidin-2-yl)oxy)-N,N-dimethylmethanamine (3l): Mol. Formula: $C_{20}H_{22}N_4O_5$, Mol. Weight: 398.41, Solubility: Chloroform, DMSO, MP (ºC): 214-219, IR

(KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 399 (M+-1) m/z.

N-(((6-(benzo[d][1,3]dioxol-5-yl)-4-phenyl-1,4-dihydropyrimidin-2-yl)oxy)methyl)-N-ethylethanamine (3m): Mol. Formula: $C_{22}H_{27}N_3O_3$, Mol. Weight: 381.47, Solubility: Chloroform, DMSO, MP (ºC): 97-99, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 382 (M+-1) m/z.

N-(((6-(benzo[d][1,3]dioxol-5-yl)-4-(p-tolyl)-1,4-dihydropyrimidin-2-yl)oxy)methyl)-N-ethylethanamine (3n): Mol. Formula: $C_{23}H_{29}N_3O_3$, Mol. Weight: 395.49, Solubility: Chloroform, DMSO, MP (ºC): 72-74, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 396 (M+-1) m/z.

N-(((6-(benzo[d][1,3]dioxol-5-yl)-4-(4-bromophenyl)-1,4-dihydropyrimidin-2-yl)oxy)methyl)-N-ethylethanamine (3p): Mol. Formula: $C_{22}H_{26}N_3O_3$, Mol. Weight: 460.36, Solubility: Chloroform, DMSO, MP (ºC): 89-90, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 461 (M+-1) m/z.

4-(6-(benzo[d][1,3]dioxol-5-yl)-2-((diethylamino)methoxy)-1,4-dihydropyrimidin-4-yl)aniline (3q): Mol. Formula: $C_{22}H_{28}N_4O_3$, Mol. Weight: 396.48, Solubility: Chloroform, DMSO, MP (ºC): 121-123, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 397 (M+-1) m/z.

4-(6-(benzo[d][1,3]dioxol-5-yl)-2-((diethylamino)methoxy)-1,4-dihydropyrimidin-4-yl)phenol (3r): Mol. Formula: $C_{22}H_{27}N_3O_4$, Mol. Weight: 397.47, Solubility: Chloroform, DMSO, MP (ºC): 110-112, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 398 (M+-1) m/z.

N-(((6-(benzo[d][1,3]dioxol-5-yl)-4-(3-nitrophenyl)-1,4-dihydropyrimidin-2-yl)oxy)methyl)-N-ethylethanamine (3s): Mol. Formula: $C_{22}H_{26}N_4O_5$, Mol. Weight: 426.47, Solubility: Chloroform, DMSO, MP (ºC): 214-219, IR (KBr): 3400 (N-H), 3057 (C-H), 1494 (C=C), 1362 (C-N), 1273 (C-O), cm^{-1}.1H NMR spectrum ($CDCl_3$, δ ppm) : 2.32 (s, 6H, -$N(CH_3)_2$), 3.8 (s, 3H, O-CH_3), 4.82 (s, 2H, -O-CH_2-N), 6.9-7.5 (m, 8 Ar-H), 8.0 (s, 1H, -NH), 6.7 (s, 1H, =C-H), 3.5 (d, 1H, =C-C-H). MS (ESIMS): 427 (M+-1) m/z.

3.4. Evaluation of Anticonvulsant Activity:

The method described by Yamaguchi and Rogawski (1992) was employed. Thirty mice were randomly divided into groups of six mice each (Table 3). The first group received normal saline (10 ml/kg body weight i.p.); the second, third and fourth groups were given 50, 100 and 100 mg/kg body weight i.p. of the test compounds, respectively, and the last group received 30 mg/kg body weight of phenytoin i.p. Thirty minutes later, mice in all the *groups* received 15 mg/kg body weight of 4-aminopyridine (4-AP) s.c. Ability of the extract/drug to protect the mice from lethality within a 30 minute observation period was considered as an indication of anticonvulsant activity[22].

3.5. Antibacterial activity:

The antibacterial activity of the test compounds was assayed systematically against four different strains of bacteria *i.e. Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* by agar diffusion method. Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar. The bacterial inhibition can be measured by two methods: one is serial dilution method and the other is diffusion method. Serial dilution method is not much useful for the qualitative detection tests. The method adopted in the present investigation was solid plate method. In this method, standard concentrations of test compounds were added to the nutrient agar medium, then standard bacterial inoculum. The test compounds were introduced into the nutrient agar by increasing concentrations and minimum inhibitory concentration was measured[23].

Preparation of Nutrient Agar medium: Media was prepared by dissolving required quantities of beef extract, sodium chloride, peptone in distilled water and pH was adjusted to 7.0-7.2. Then required quantity of agar was added and allowed to dissolve by heating on water bath. The prepared media was sterilized by autoclaving at 15 lb/in² for about 20 minutes. The entire test compounds equivalents to concentration of 10, 20, 30, up to 100µg/ml were prepared by dissolving in DMSO. Sterilized media was cooled to 40ºC and add the prepared concentrations of sample to each tube mix thoroughly and poured into the Petri plates. The plates were left at room temperature to allow solidification of the media. All these procedures were conducted aseptically in laminar air flow workstation. After solidification of the medium, the plate was divided into 4 equal parts on the outer surface of the plate with glass marker and labeled for each organism, respectively. 24 hr bacterial cultures were streaked on the specified area by inoculation loop, respectively. Incubation of the petri plates was done at 37±1ºC for 24h. The growth/inhibition of bacteria was observed after 24 h. simultaneously controls were maintained employing methanol to observe the solvent effects. MIC Values (µg/ml) for the synthesized Compounds against the tested bacterial species were depicted in tables.

4. Results and Discussion

4.1 Anticonvulsant activity

The oral median lethal dose (LD50) of the test compounds 3a, 3b & 3f were found to be 500,1000,1000 mg/kg body weight in mice respectively. The drugs did protect the animals from 4-Aminopyridine induced seizures nor did it affect the latency of the seizures.

Table 4: Effect of test compounds on 4AP induced seizures in mice

Treatment	Mean onset of seizure (min)	Quantal protection	% Protection
Control	4.8 ± 0.43	0/6	0
Phenytoin	22.87 ± 3.21***	6/6	100
3a	9.86 ± 0.96*	0/6	0
3b	15.54 ± 2.32***	3/6	50
3f	11.45 ± 1.76**	1/6	16.66

Table showing the mean onset of seizures, the results are analysed by using one-way ANOVA followed by Dunnets test, ***= p< 0.001; **=p<0.01; *=p<0.05.

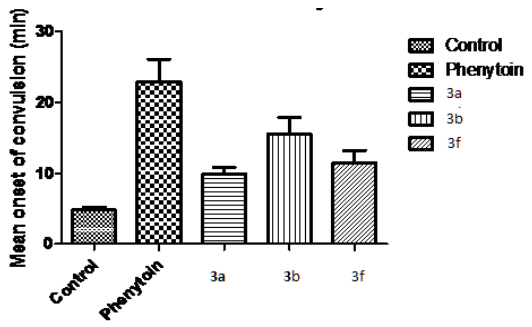


Figure 2: Anticonvulsant activity of the synthesized DHPMs

4-Aminopyridine is a known potassium channel blocker (Yamaguchi and Rogawski, 1992). The presence of anticonvulsant activity against 4-AP induced seizures suggests that the test drugs may have activity against potassium channels. The result of the investigation suggests that the test compounds does possess significant anticonvulsant property in mice, and this supports the ethnomedical use of the plant in the treatment of epilepsy. From our findings, the synthesized drugs may be valuable for the treatment of petitmal generalized seizures (absence or myoclonic).

4.2 Antibacterial activity

The antibacterial activity was performed for the synthesized new O-Mannich bases of 4,6-di aryl dihydropyrimidines (**3a-r**) exhibited mild to moderate antibacterial activity against the test organisms employed in the present investigation. However, the degree of inhibition varied with the test compound and the test bacterium. It could be observed from the results of the present investigation that compared to the standard drug Streptomycin. From the results it was found that the compounds **3g, 3h, 3m** shown more potent activity against *Escherichia coli*. Compound **3g,** showed high activity against *Proteus vulgaris.* Compound **3q** showed high activity against *pseudomonas aerugenosa.* Compounds3a,3n,3q showed high activity against *Staphylococcus aureus.* Among all synthesized compounds, **3q** shown more potent activity than the standard against *pseudomonas aerugenosa*

Table 5: Antibacterial activity and MIC (µg/ml) of DHPMs

Code	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*S. aureus*
3a	70	60	70	50
3b	80	70	60	80
3c	60	60	70	70
3d	70	60	80	80
3e	80	80	50	60
3f	60	70	70	90
3g	50	50	80	80
3h	50	60	90	80
3i	80	80	60	70
3j	60	50	80	90
3k	70	90	60	80
3l	70	70	50	70
3m	50	60	70	80
3n	80	80	60	50
3p	90	90	50	70
3q	70	70	40	50
3r	80	70	50	90
3s	50	60	60	60
Streptomycin	40	30	20	30

5. Conclusion

In the present investigation, piperonal based DHPMs were synthesized as per the established scheme and were characterized. They were subjected for acute toxicity studies for the determination of toxic dose in the mice models. Later they were evaluated for anticonvulsant activity *in vivo* and antibacterial activity *in vitro*. The results were found to be promising, among the synthesized compounds, **3a**, **3b** and **3f** were shown good anticonvulsant activity in the tested animals. From the results it was found that the compounds **3g**, **3h**, **3m** shown more potent activity against *Escherichia coli*. Compound **3g**, showed high activity against *Proteus vulgaris.* Compound **3q** showed high activity against *Pseudomonas aerugenosa*. Compounds **3a**, **3n**, **3q** showed high activity against *Staphylococcus aureus*. Among all synthesized compounds, **3q** shown more potent activity than the standard against *pseudomonas aerugenosa*.

Acknowledgements

The Authors wanted to thank the principal and management of Vishwambhara Educational Society, Warangal for their consistent support for the smooth functioning of the project.

Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

References

1. Sander, J. W. (2003). The epidemiology of epilepsy revisited. Current opinion in neurology, 16(2), 165-170.
2. Devinsky, O., Hesdorffer, D. C., Thurman, D. J., Lhatoo, S., & Richerson, G. (2016). Sudden unexpected death in epilepsy: epidemiology, mechanisms, and prevention. The Lancet Neurology, 15(10), 1075-1088.
3. Harden, C. L. (2002). The co-morbidity of depression and epilepsy: epidemiology, etiology, and treatment. Neurology, 59(6 suppl 4), S48-S55.
4. Banerjee, P. N., Filippi, D., & Hauser, W. A. (2009). The descriptive epidemiology of epilepsy—a review. Epilepsy research, 85(1), 31-45.
5. R Alavala, R., Kulandaivelu, U., Bonagiri, P., Boyapati, S., Jayaprakash, V., & T Subramaniam, A. (2015). Synthesis and Antiviral Activity of Dihydropyrimidines-Ciprofloxacin Mannich bases Against Various Viral Strains. Anti-Infective Agents, 13(2), 154-165.
6. Kaur, R., Chaudhary, S., Kumar, K., Gupta, M. K., & Rawal, R. K. (2017). Recent synthetic and medicinal perspectives of dihydropyrimidinones: A review. European journal of medicinal chemistry, 132, 108-134.
7. Alizadeh, A., & Rostamnia, S. (2010). Adducts of diketene, alcohols, and aldehydes: useful building blocks for 3, 4-dihydropyrimidinones and 1, 4-dihydropyridines. Synthesis, 2010(23), 4057-4060.
8. Upadhyay, P., Yadav, A. K., Panjwani, D., & Sachan, N. (2015). Anti-convulsant property of synthesized compound dihydropyrimidinone-5 in laboratory animals. Asian J Pharm Clin Res, 8(5), 146-149.
9. Aukunuru, J., Eedula, K., Pasham, V., Katla, V., & Reddy, S. K. (2009). Synthesis of novel piperonal derivatives and evaluation of their anticonvulsant activity using a nanoparticular formulation. Int. J. Pharm. Sci. Nanotechnol, 2, 435-442.

10. Kumar, S. (2013). A Review on Anticonvulsant Activity of 1, 3-Benzodioxole Ring System Based Compounds. International Journal of Pharmaceutical Sciences and Research, 4(9), 3296.
11. El ‐ Hashash, M. A., & El ‐ Badry, Y. A. (2011). Synthesis of a Novel Series of 2, 3 ‐ Disubstituted Quinazolin ‐ 4 (3H) ‐ ones as a Product of a Nucleophilic Attack at C (2) of the Corresponding 4H ‐ 3, 1 ‐ Benzoxazin ‐ 4 ‐ one. Helvetica Chimica Acta, 94(3), 389-396.
12. Boeck, P., Falcao, C. A. B., Leal, P. C., Yunes, R. A., Cechinel Filho, V., Torres-Santos, E. C., & Rossi-Bergmann, B. (2006). Synthesis of chalcone analogues with increased antileishmanial activity. Bioorganic & medicinal chemistry, 14(5), 1538-1545.
13. Hsieh, C. T., Hsieh, T. J., El-Shazly, M., Chuang, D. W., Tsai, Y. H., Yen, C. T., ... & Chang, F. R. (2012). Synthesis of chalcone derivatives as potential anti-diabetic agents. Bioorganic & medicinal chemistry letters, 22(12), 3912-3915.
14. Choudhary, A. N., & Juyal, V. (2011). Synthesis of chalcone and their derivatives as antimicrobial agents. International journal of pharmacy and pharmaceutical Sciences, 3(3), 125-128.
15. Shaikh, S., Shaikh, N. P., Salunke, S. D., & Baseer, M. A. (2015). Synthesis and Antimicrobial Activity of new 3, 4-Dihydropyrimidinones via Novel Chalcone Series. Heterocyclic Letters, 5(3), 443-449.
16. El-Hamouly, W. S., Amine, K. M., Tawfik, H. A., Dawood, D. H., & Moharam, M. E. (2011). Synthesis and antimicrobial activity of new 3, 4-dihydropyrimidinones. International Journal of Pharmaceutical Sciences and Research, 2(4), 1054.
17. Krishnakumar, V., Vindhya, N. G., Mandal, B. K., & Nawaz Khan, F. R. (2014). Green chemical approach: low-melting mixture as a green solvent for efficient Michael addition of homophthalimides with chalcones. Industrial & Engineering Chemistry Research, 53(26), 10814-10819.
18. Venkateshwarlu, K., Rao, G. C., Reddy, V. M., & Reddy, Y. N. (2014). Synthesis and in vitro and in vivo antitumor/anticancer activity of novel O-Mannich bases of 4, 6-diaryl-3, 4-dihydropyrimidine-2 (1H)-ones. Journal of the Iranian Chemical Society, 11(6), 1619-1627.
19. El-Hamouly, W. S., Amine, K. M., Tawfik, H. A., Dawood, D. H., & Moharam, M. E. (2011). Synthesis and antimicrobial activity of new 3, 4-dihydropyrimidinones. International Journal of Pharmaceutical Sciences and Research, 2(4), 1054.
20. Senthilkumar, R., Jothimanivannan, C., MKM, A. L., & Kiran, K. (2018). Synthesis, Characterization, In Silico prediction and Anti-microbial evaluation of 2-Substituted Benzimidazole Derived Mannich bases. International Journal of Research in Pharmaceutical Sciences, 9(2).
21. Upadhyay, P., Yadav, A. K., Panjwani, D., & Sachan, N. (2015). Anti-convulsant property of synthesized compound dihydropyrimidinone-5 in laboratory animals. Asian J Pharm Clin Res, 8(5), 146-149.
22. Viveka, S., Nagaraja, G. K., Shama, P., Basavarajaswamy, G., Rao, K. P., & Sreenivasa, M. Y. (2018). One pot synthesis of thiazolo [2, 3-b] dihydropyrimidinone possessing pyrazole moiety and evaluation of their anti-inflammatory and antimicrobial activities. Medicinal Chemistry Research, 27(1), 171-185.