



***In-vivo* Anticonvulsant and *In-vitro* Antitubercular Activity of methyl Indole Derivative of some 6-aryl-4, 5-Dihydropyridazin-3(2H)-ones and their Expected Anticonvulsant Mechanisms**

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Abstract

Methyl indole derivative of some 6-aryl-4,5-dihydropyridazin-3(2H)-ones (3a-e) were synthesized by Mannich reaction and evaluated as anticonvulsant against MES (50mA, for 2sec), INH (250mg/kg), scPTZ (80mg/kg) and STR (3mg/kg) induced convulsion methods at 50 mg/kg dose level. All compounds 3a-e were also evaluated as antitubercular agent against *M. tuberculosis* H37Rv by MABA method. All compounds 3a-e showed anticonvulsant activities against MES, INH and scPTZ-induced convulsions methods but no compounds were active against STR-induced method. Compound 3b, compound 3d and 3e showed highest protection against MES, INH and scPTZ-induced convulsion models respectively. Compound 3d showed highest antitubercular activity, with 12.5µg/ml MIC value. Previous data reported that the pyridazinones are the important biological compounds which possess almost all types of biological activities. Due to this reason, we synthesized some pyridazinone compounds and characterized them by spectral analysis. Results indicated that title compounds showed moderate to good anticonvulsant activities but less antitubercular activity.

Keywords : Anticonvulsant, antitubercular; biological active, heterocyclic, pyridazinone, synthetic.

1. Introduction

Pyridazinones are important heterocyclic compounds which possess almost all types of biological activities such as antimicrobial, antifungal, antiviral [1-5], antifeedant, herbicidal [6,7], antiinflammatory, analgesic and antipyretic [8-14], antidepressant, antianxiety [15-17], antihypertensive, antiplatelets, car-

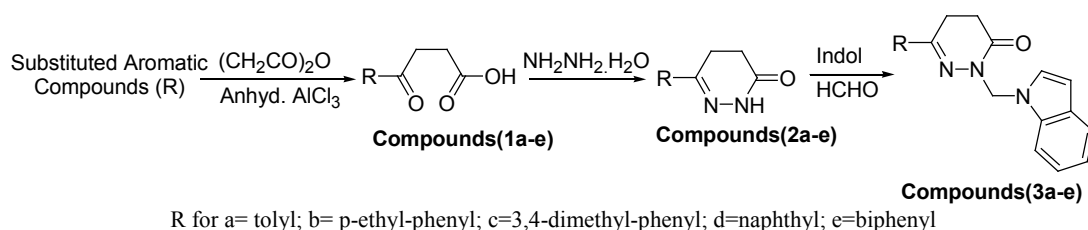
diotonic, antidiabetic [18-24], diuretics, anticancer, antiasthmatic, antiallergy and other anticipated activities [25-31]. In particular, a large number of pyridazinone derivatives are also used as intermediates for drugs and agrochemicals. Some pyridazinone compounds like indolidan, bemoradan, primobendan, levosimendan (antihypertensive), minaprine (antidepressant), emorfazone (antiinflammatory), and azanrinone (cardiotonic), are used in the clinical marketed drugs [32].

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and it is the primary cause of mortality in the world. The emergence of AIDS, decline of socioeconomic standards and a reduced emphasis on TB control programs contribute to the disease's resurgence. Multidrug and

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Cite this article as: Asif M, Singh A, Bilkanti L. *In-vivo* anticonvulsant and *in-vitro* antitubercular activity of Methyl Indole derivative of some 6-aryl-4, 5-Dihydropyridazin-3(2H)-ones their expected anticonvulsant mechanisms, *Iranian Journal of Medical Sciences*, 2013, 9 (1), 67-80.



Scheme 1. General synthesis route of the title compounds (3a-e).

extensively (MDR-TB & XDR-TB) drug resistant of *M. tuberculosis* strains to anti-TB agents is an increasing problem worldwide. In spite of severe toxicity on repeated dosing of first line drugs. It is still considered to be a first line drug for chemotherapy of TB [33,34].

Epilepsy affects about 0.5% of the world's population. The seizure is caused by an asynchronous high-frequency discharge of a group of neurons, starting locally and spreading to a varying extent to affect other parts of the brain. Two common forms of epilepsy are the tonic-clonic fit (grand mal) and the absence seizure (petit mal). In absence seizures, the discharge is regular and oscillatory. Partial seizures affect localized brain regions, and the attack may involve mainly motor, sensory or behavioral phenomena. Generalized seizures affect the whole brain. Status epilepticus is a life-threatening condition in which seizure activity is uninterrupted. Many animal models have been devised, including electrically and chemically induced seizures, production of local chemical damage and kindling. These provide good prediction of antiepileptic drug effects in humans. It may be associated with enhanced excitatory amino acid transmission, impaired inhibitory transmission, or abnormal electrical properties of the affected cells. The glutamate content in areas surrounding an epileptic focus may be increased. Repeated epileptic discharge can cause neuronal death. Current drug therapy is effective in 70-80% of patients [35-38].

During our literature survey, it is observed that various pyridazinone derivatives possess anti-TB [39-42] and anticonvulsant activity

[43-45]. Considering the 6-aryl-pyridazin-3(2H)-one residue as the pharmacophoric group for these activities, so we have synthesized some 6-aryl-2-(indol-1-ylmethyl)-dihydropyridazin-3(2H)-one derivatives and evaluated them for antitubercular agents against *M. tuberculosis* H37Rv by using Microplate Alamar Blue Assay (MABA) method and for anticonvulsant activities, by using maximum electro shock (MES), isoniazid (INH), pentylenetetrazole (scPTZ) and strychnine sulphate (STR) induced convulsion methods.

2. Materials and Methods

2.1. Experimental Protocols

2.1.1. Chemistry

All title compounds (3a-e) were synthesized according to Scheme 1. The Friedel-Craft acylation of appropriate aromatic hydrocarbon with succinic anhydride in presence of anhydrous aluminium chloride to form aryl propionic acids (1a-e). The compounds 1a-e on hydrazinolysis gave the 6-aryl-pyridazinones (2a-e). The compounds 2a-e react with indole (a cyclic secondary amine) and formaldehyde to get the final 6-aryl-2-(indol-1-ylmethyl)-4,5-dihydropyridazin-3(2H)-ones (3a-e) by Mannich reaction. Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) plates (silica gel G) which were visualized by exposing to iodine vapors and UV light. The FT-IR spectra were recorded on Bio-rad FTS-135 spectrophotometer using KBr pellets; ν_{max}

values are given in cm^{-1} . ^1H NMR spectra were recorded on Bruker Spectrospin DPX 300 MHz using CDCl_3 as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) scale and coupling constants (J values) are expressed in Hz.

2.1.2. General Procedure for the Synthesis of Aroyl-propionic Acids (1a-e)

Suspending anhydrous aluminum chloride (0.15 mol) in dry toluene (50 mL) under anhydrous conditions, the mixture was refluxed on a water bath. Succinic anhydride (0.10 mol) was then added to the reaction mixture in small portions with continuous stirring. Stirring and heating were continued for 6 h. The reaction mixture was left overnight at room temperature and then made acidic by addition of an ice cold solution of hydrochloric acid (2.5% v/v). The mixture was concentrated to a small volume by heating on a water bath. The separated precipitate was filtered. It was purified by dissolving in 5% w/v sodium bicarbonate solution, followed by extraction with ether. The aqueous layer on acidification with dilute hydrochloric acid gave tolyl-propionic acid (1a). It was crystallized from aqueous ethanol to give a compound. All the remaining acids (1b-e) were synthesized from respective aromatic hydrocarbon by analogous procedure with minor modification in temperature of reaction and use of nitrobenzene as a solvent and characterized on the basis of spectral data as per earlier reported procedure [41,42].

Toloyl propionic acid (1a)
ethylbenzoyl propionic acid (1b)
dimethyl benzoyl propionic acid (1c)
Naphthoyl propionic acid (1d)
Phenylbenzoyl propionic acid (1e)

2.1.3. General Procedure for the Synthesis of 6-aryl-4,5-Dihydropyridazin-3(2H)-one (2a-e)

To a solution of tolyl propionic acid (1a) (0.1 mol) in methanol (30 mL), hydrazine hydrate (1 mL) and sodium acetate (0.5 g) were

added and the mixture was refluxed for 6 h. After completion of the reaction, methanol was distilled off and the content was poured into cold water. The solid compound (1a) that separated out was filtered and crystallized from methanol. The appropriate substituted aroyl-propionic acids were reacted with hydrazine hydrate to get corresponding pyridazinone (2b-c) and characterized on the basis of spectral data as per earlier reported procedure [41,42].

Tolyl-pyridazinone (2a)
(4-ethylbenzyl)-pyridazinone (2b)
Naphthoyl-pyridazinone (2c)
(3,4-Dimethyl benzyl)-pyridazinone (2d)
Phenylbenzoyl-pyridazinone (2e)

2.1.4. General Procedure for the Preparation of 6-aryl-2-(1H-indol-1-yl-methyl)-4,5-Dihydropyridazin-3(2H)-one (3a-e)

To a solution of compounds (2a-e) (0.001 mol) in absolute ethanol (30 mL), formaldehyde (37–41%) (1.5 mL) and cyclic secondary amine (0.001 mol) were added and the contents refluxed for 24 h. After completion of the reaction, ethanol was distilled off and the residue poured into crushed ice and kept in refrigerator for overnight to separate out the compounds (3a-e). The solid which separated out, was filtered and recrystallized from ethanol [46].

2.1.4.1. 2-Indol-1-yl-methyl)-6-p-tolyl-4,5-Dihydro-Pyridazin-3(2H)-one (3a)

Yield: 44%; m.p. 105–107 °C; Molecular formula: $\text{C}_{20}\text{H}_{19}\text{N}_3\text{O}$; Molecular weight: 317.4; IR (KBr) ν_{max} (cm^{-1}): 2995 (CH), 1680 (C=O), 1570 (C=N). ^1H NMR (CDCl_3) δ (ppm): 2.36 (s, 3H, CH₃), 2.64 (t, $J = 7.6$, 2H, C-CH₂), 2.98 (t, $J = 7.6$, 2H, CH₂-CO), 5.36 (s, 2H, -N-CH₂-N-), 7.46–7.78 (m, 10H, Ar-H); Elemental Analysis (calculated/found): C (75.69/75.46), H (6.03/5.88), N (13.24/13.12); MS (m/z): 317/318(M⁺/M⁺⁺¹).

2.1.4.2. 6-(4-Ethyl-Phenyl-2-indol-1-yl-methyl)-4,5-Dihydro-Pyridazin-3(2H)-one (3b)

Yield: 50%; m.p. 140–142 °C; Molecular formula: C₂₁H₂₁N₃O; Molecular weight: 331.4; IR (KBr) y_{max} (cm⁻¹): 2985 (CH), 1690 (C=O). ¹H NMR (CDCl₃) δ (ppm): 1.2 (q, 3H, CH₃), 2.5 (t, 2H, CH₂), 2.66 (t, J = 7.5, 2H, C–CH₂), 3.0 (t, J=7.5, 2H, CH₂–CO), 5.3 (s, 2H, –N–CH₂–N–), 6.51–7.73 (m, 10 H, Ar–H); Elemental Analysis (calculated/found): C (76.11/66.92), H (6.39/6.12), N (12.68/12.51) MS (m/z): 331/332(M+/M++1).

2.1.4.3. 6-(3,4-Dimethyl-Phenyl)-2-Indol-1-yl-Methyl-4,5-Dihydro-Pyridazin-3(2H)-One (3c)

Yield: 42%; m.p. 125–127 °C; Molecular formula: C₂₁H₂₁N₃O; Molecular weight: 331.4; IR (KBr) y_{max} (cm⁻¹): 3001 (CH), 1680 (C=O), 1600 (C=N); ¹H NMR (CDCl₃) δ (ppm): 2.35 (s, 6H, 2xCH₃), 2.60 (t, J = 7.8, 2H, C–CH₂), 2.96 (t, J = 7.8, 2H, CH₂–CO), 5.28 (s, 2H, –N–CH₂–N–), 6.68–7.78 (m, 9H, Ar–H); Elemental Analysis (calculated/found): C (67.11/66.90), H (6.39/6.14), N (12.68/12.50); MS (m/z): 331/332(M+/M++1).

2.1.4.4. 2-Indol-1-yl-Methyl-6-Naphthalen-2-yl-4,5-Dihydro-2H-Pyridazin-3-One (3d)

Yield: 50%; m.p. 125–172 °C; Molecular formula: C₂₃H₁₉N₃O; Molecular weight: 353.4; IR (KBr) y_{max} (cm⁻¹): 2995 (CH), 1695 (C=O). ¹H NMR (CDCl₃) δ (ppm): 2.66 (t, J = 7.5, 2H, C–CH₂), 3.0 (t, J=7.5, 2H, CH₂–CO), 5.3 (s, 2H, –N–CH₂–N–), 6.48–8.21 (m, 13 H, Ar–H); MS (m/z): 353/354 (M+/M++1)

2.1.4.5. 6-Biphenyl-4-yl-2-Indol-1-yl-Methyl-4,5-Dihydro-2H-Pyridazin-3(2H)-One (3e)

Yield: 60%; m.p. 150–152 °C; Molecular formula: C₂₅H₂₁N₃O; Molecular weight: 379.2; IR (KBr) y_{max} (cm⁻¹): 2998 (CH), 1680 (C=O), 1602 (C=N); ¹H NMR (CDCl₃) δ (ppm): 2.63 (t, J = 7.5, 2H, C–

CH₂), 3.02 (t, J = 7.5, 2H, CH₂–CO), 5.02 (s, 2H, –N–CH₂–N–), 7.12–7.76 (m, 15H, Ar–H); Elemental Analysis (calculated/found): C (79.13/78.86), H (5.58/5.32), N(11.07/10.86); MS (m/z): 379/380(M+/M++1)

2.2. Pharmacology

2.2.1. Experimental Animals

Swiss albino mice weighing 25-30 g were maintained under controlled conditions of light (12 hr) and temperature 25±1°C in the animal house of Department of Pharmacy, GRD (PG) IMT, Dehradun, India, two weeks prior to the experiment for acclimatization. Animals had access to food and water ad libitum. All pharmacological activities were carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) norms (Regn No: 1145/a/07/CPCSEA), after obtaining the approval from the Institutional Animal Ethics Committee of Department of Pharmacy, GRD (PG) IMT, Dehradun, India.

2.2.2. Anticonvulsant Activity:

Male albino mice (25-30 gm) were used to test the drugs (synthesized pyridazinone compounds). The MES method was used for inducing seizures (Model: Techno Electro Convulso-meter). The MES induced convulsions in animal represent grand mal (tonic-clonic) type of epilepsy in chemical induced seizures methods, INH, scPTZ and strychnine (STR). The chemo induced convulsions in animal mainly represent petit mal (absence seizures) type of epilepsy. The chemo convulsions were divided into three phases (i) onset of convulsions (ii) duration of convulsions and (iii) Recovery or Death. Suspension of test compounds was prepared in 0.5% carboxyl methyl cellulose (CMC) and was injected i.p. at dose level 50 mg/kg body weight. Reduction in the extensor phase in mice was used to evaluation of anticonvulsant activity in MES model, in chemical models, onset and duration of convulsions was used to evaluation of anticonvulsant ac-

tivity. Phenytoin sodium (25mg/kg), sodium valproate (50mg/kg) and diazepam (4mg/kg) were used as reference drugs [47].

2.2.2.1. Maximum Electroshock (MES) Induced Seizures Method:

The MES induced seizures test was performed on mice (25-30 g). The mice were injected with test drug (3a-e) 50 mg/kg i.p. After 30 min, electro shock was applied through the ear pinna passing current of 50 mA for 0.2 sec. The MES convulsions were divided into five phases (i) Flexion (ii) Extensor (iii) Clonus (iv) Stupor and (v) Recovery or Death. Abolition of the hind limb tonic extension spasm was recorded as the anticonvulsant activity.

2.2.2.2. Isoniazid (INH) Induced Seizures Method:

The test was performed on mice (25-30 g). The mice were injected with test drug (3a-e) 50 mg/kg i.p. After 30 min, INH injection of dose 250 mg/kg was given i.p. The sequence of seizure latency, no. of seizures and % protection were studied. Animals exhibiting these seizures pattern were detected [42-45].

2.2.2.3. Pentylentetrazole (PTZ) Induced Seizures Method:

The test was performed on mice (25-30 g). The mice were injected with test drug (3a-e) 50 mg/kg i.p. After 30 min, PTZ injection of dose 80 mg/kg was given subcutaneous (s.c). The sequence of seizure latency, no. of seizures and % protection were studied. Animals exhibiting these seizures pattern were detected [42-45].

2.2.2.4. Strychnine (STR) Induced Convulsions:

For the evaluation of anticonvulsant activity against strychnine induced convulsions, the same procedure was adopted as mentioned in PTZ induced convulsions. Instead, STR (3 mg/kg i.p) was used for inducing convulsions.

2.2.2.5. Neurotoxicity Screening:

Minimal motor impairment was measured in mice by the Rota rod (Techno, India) test. The mice were trained to stay on an accelerating Rota rod of diameter 3.2 cm that rotates at 10 revolutions per min. Previously trained Albino mice were given test compounds i.p. in doses of 25, 50 and 100 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least one min. in each of the three trials.

2.2.3. Anti-TB Activity by Using Microplate Alamar Blue Dye (MABA) Test:

The anti mycobacterial activity of synthetic compounds (3a-e) were assessed against *M. tuberculosis* using MABA method. This methodology non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile de-ionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations of compounds 3a-e were tested at 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink [48].

2.2.4. Statistical Evaluation

All the values were reported as mean \pm S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall p-Value was found statistically significant ($p < 0.05$), further comparisons among groups were made according to Tukey's test.

Table 1. Anticonvulsant activities of 6-aryl-4,5-dihydropyridazin-3(2H)-ones against MES-induced convulsions method.

Compounds	Flexion (in sec)	Extensor (in sec)	Clonus (in sec)	Stupor (in sec)	Recovery (in sec)	Recovery (%)
Control	6.84±0.10	16.17±0.50	16.48±0.29	29.3±1.66	225.4±9.61	00
3a	8.25±0.85 ^{γ3}	6.0±1.15 ^c	12±1.0 ^c	18.5±3.5 ^{γ3}	49.2±11.77 ^c	100
3b	5.6±1.43 ^{β3}	3.5±0.5 ^{γc}	6.0±2.0 ^c	16±2.0	39.6±11.03 ^c	100
3c	12.4±0.81 ^{γ3c}	4.6±0.50 ^c	7.5±0.50 ^c	11.5±5.14	34.6±7.45 ^{βc}	100
3d	11.6±0.50 ^{γ3c}	3.8±0.37 ^{αc}	6.5±1.5 ^c	11±2.0 ¹	36.8±4.17 ^{γc}	100
3e	10.4±0.50 ^{γ5c}	4.2±0.37 ^c	7.0±1.0 ^c	10.5±1.5	39.4±6.64 ^{γc}	100
Phenytoin sod	3.12±0.20	3.3±0.56	6.65±0.51	14.22±0.82	25.0±2.1	100
Sod valproate	3.64±0.82	5.26±0.68	7.56±1.24	18.83±2.43	26±3.31	80

Standard drug: Phenytoin sodium (25mg/kg) and Sodium valproate (50mg/kg), Tested drugs: synthesized compounds 11-15 (50mg/kg), Control group: 0.5 % Carboxyl methyl cellulose (CMC) in distilled water; n=5 (No. of animals in each group); *Value represents mean ± S.E.M

$ap < 0.05$, $bp < 0.01$ and $cp < 0.001$ when compared to control group.

$ap < 0.05$, $βp < 0.01$ and $γp < 0.001$ when compared to standard drug sodium valproate

$1p < 0.05$, $2p < 0.01$ and $3p < 0.001$ when compared to standard drug phenytoin sodium

3. Result and Discussion

3.1. Chemistry

Spectral data (1H NMR, IR) of all the synthesized title compounds (3a-e) were in full agreement with the proposed structures. In general, for example compound (3a) IR spectra (KBr) revealed ν_{max} 2995 (CH),

1680 (C=O), 1570 (C=N) cm^{-1} , respectively.

In the nuclear magnetic resonance spectra (1H NMR) (CDCl₃) δ (ppm): 2.36 (s, 3H, CH₃), 2.64 (t, = 7.6, 2H, C-CH₂) methylene group in pyridazinone ring at 5 position, 2.98 (t, J = 7.6, 2H, CH₂-CO) methylene group in pyridazinone ring at 4-position, 5.36 (s, 2H, -N-CH₂-N-) for methylene group bind

Table 2. Anticonvulsant activities of 6-aryl-4,5-dihydro pyridazin-3(2H)-one against INH- induced convulsions method.

Compounds	Onset of convulsion	No. of convulsion	Recovery (%)
Control	26.16±0.094	2.2±0.20	00
3a	39±3.92 ^{γb###}	1.8±0.37	100
3b	37.6±2.56 ^{γb###}	2.2±0.20	100
3c	52.2±3.82 ^{γc###}	2.2±0.20	100
3d	57.8±2.83 ^{γc###}	2.4±0.24	100
3e	34.6±3.23 ^{γ###}	1.6±0.24	100
Sodium valproate	130.2 ± 2.15	1.21±0.20	100
Phenytoin Sodium	--	--	40
Diazepam	110.2 ± 2.15	1.32±0.20	100

Standard drug: Phenytoin sodium (25mg/kg) and Sodium valproate (50mg/kg), Diazepam (4mg/kg) Tested drugs: 3a-e (50mg/kg), Control group: INH-250mg/kg+0.5% CMC in distilled water; n=5 (No. of animals in each group).

$ap < 0.05$, $bp < 0.01$ and $cp < 0.001$ when compared to control group.

$ap < 0.05$, $βp < 0.01$ and $γp < 0.001$ when compared to standard drug sodium valproate

$\#p < 0.05$, $###p < 0.01$ and $####p < 0.001$ when compared to standard drug Diazepam

Table 3. Anticonvulsant activities of 2-indol-1-yl-methyl derivatives of 6-aryl-4,5-dihydro pyridazin-3(2H)-one against scPTZ -induced convulsion method.

Compounds	Onset of convulsion (min)	No. of convulsion	Recovery (%)
Control	7.6±.74	1.8±0.44	00
3a	14.4±0.50 ^{γ3c}	1±0.00	80
3b	13.4±0.92 ^{γ3c}	1±0.00	80
3c	14.8±0.37 ^{γ3c}	1.2±0.44	100
3d	15.2±0.86 ^{γ3c}	1±0.00	80
3e	18±0.070 ^{γ3c}	1.2±0.44	80
Sodium valproate	68.2 ± 1.54	1.3±1.4	100
Phenytoin Sodium	--	--	60
Diazepam	--	--	100

Standard drug: Phenytoin sodium (25mg/kg), Sodium valproate (50mg/kg), Diazepam (4mg/kg) and tested compounds 3a-e (50mg/kg), Control group: scPTZ-80mg/kg+0.5 % CMC in distilled water; n=5 (No. of animals in each group).

$ap < 0.05$, $bp < 0.01$ and $cp < 0.001$ when compared to control group.

$ap < 0.05$, $βp < 0.01$ and $γp < 0.001$ when compared to standard drug sodium valproate.

with N atoms on both side, 7.46–7.78 (m, 10H, Ar–H) for aromatic rings. In the nuclear magnetic resonance spectra (¹H NMR) the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts and multiplicities. The other compounds are also identified in a similar manner.

3.2. Anticonvulsant Activities

The final compounds (3a-e) were evaluated as anticonvulsant by MES, INH, scPTZ and STR methods and as antitubercular, MABA method was used. The results of anticonvulsant activities were shown in table 1 (MES), table 2 (INH), table 3 (scPTZ) and table 4 (STR) induced convulsion models and

Table 4. Anticonvulsant activities of 6-aryl-4,5-dihydro pyridazin-3(2H)-one against STR-induced convulsions method.

Compounds	Onset of convulsion (min)	No. of convulsion	Recovery (%)
3a	2.54±0.30 ³	2.92±0.23 ³	00
3b	2.28±0.38 ³	2.84±0.15 ³	00
3c	2.18±0.35 ³	2.84±0.15 ³	00
3d	3.22±0.27* ³	3.44±0.16 ³	00
3e	3.48±0.073* ³	3.08±0.20 ³	00
Control	1.50 ±1.09	3.17±2.18	00
Diazepam	--	--	100
Sodium valproate	--	--	100
Phenytoin sodium	8.45±1.86	1.06±0.78	40

Standard drug: Phenytoin sodium (25mg/kg) and Sodium valproate (50mg/kg), Diazepam (4mg/kg) Tested drugs: 3a-e (50mg/kg), Control group: STR 3mg/kg+0.5% CMC in distilled water; n=5 (No. of animals in each group).

$ap < 0.05$, $bp < 0.01$ and $cp < 0.001$ when compared to control group.

$1p < 0.05$, $2p < 0.01$ and $3p < 0.001$ when compared to standard drug phenytoin

compared with reference drugs phenytoin sodium, sodium valproate and diazepam. The result of antitubercular activity was shown in table 5 (MABA) compared with reference drug INH, PZA and SM.

3.2.1. MES Model:

All 2-indol-1-yl-methyl derivatives of 6-aryl-4,5-dihydropyridazin-3(2H)-one (3a-

Other remaining compounds showed anticonvulsant activity compared to control group. Compound 3e and 3a reduced the no. of convulsions compared to control group but not less as reference drugs. All compounds 3a-e were less effective than standard drugs. The order of activity of the onset of convulsions was 3d>3c>3e>3a>3b (table 2).

Table 5. AntiTubercular activity of the synthesized compounds of 6-aryl-4, 5-dihydropyridazin-3(2H)-one against M. tuberculosis.

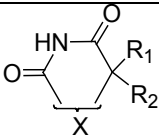
Compounds	MIC value ($\mu\text{g/ml}$)
3a	25
3b	25
3c	25
3d	12.5
3e	25
INH	3.125
PZA	3.125
SM	6.25

e) against MES-induced method, compound (3b) showed highest protection in extensor phase. Other remaining compounds also showed anticonvulsant activity. All compounds were less effective as reference drug

3.2.3. PTZ Model:

Compounds 3a-e against scPTZ-induced method, compound (3e) showed highest protection by increasing onset of convulsion. Other remaining compounds (3a-d) showed

Table 6. Structural features of Anticonvulsant drugs.

General structure	Antiepileptic Drugs	X=
	Barbiturates	-NH-C=O
	Hydantoin	-NH-
	Oxazolidinediones	-O-
	Succinimides	-CH ₂ -

phenytoin but all compounds except 3a were more effective than reference drug sodium valproate. The order of activity of compounds 3b > 3d > 3e > 3c > 3a were found (table 1).

3.2.2. INH Model:

Compounds (3a-e) against INH-induced method, compound 3d showed highest protection by increasing onset of convulsion.

anticonvulsant activity compared to control group. All compounds 3a-e reduced the no. of convulsion compared to control group and were less effective than standard drugs sodium valproate and diazepam. The order of activity was compound 3e>3d>3c>3a>3b (table 3).

3.2.4. STR Model:

Compounds 3a-e against STR-induced

method, compound 3e showed maximum onset of convulsions. Other remaining compounds were also increase onset of convulsions when compare with control group. All compounds were ineffective in STR model. The order of onset of convulsion activity was compound 3e>3d>3b>3a>3c (table 4).

3.2.5. Neurotoxicity:

All title compounds 3a-e were tested at 25, 50 and 100 mg/kg dose level on Rotarod apparatus. All compounds 3a-e did not showed neurotoxic effect up to 100mg/kg dose level because of the ability of the animal to maintain equilibrium on the rod for at least one min in each of the three trials.

3.3. Antitubercular Activity

All compounds 3a-e evaluated against MABA method, compound 3d showed maximum anti-TB activity with 12.5µg/ml MIC value. Other remaining compounds showed 25µg/ml MIC value when compare with reference drugs INH (3.12µg/ml), PZA (3.12µg/ml) and SM (6.25µg/ml). All compounds 3a-e showed weak antitubercular activity (table 5).

The investigations on chemical and biological behavior of pyridazinone derivatives have gained more importance in recent decades for its medicinal reasons. The current work described that pyridazine-3(2H)-ones were encouraging the research towards anticonvulsant and anti-TB activities. While considering all the title compounds (3a-e) together, substitution with 4-ethyl-phenyl and naphthyl group at 6-position of pyridazinone moiety showed high anticonvulsant activity than other aryl substituted (4-methyl-phenyl, 3,4-dimethylphenyl, biphenyl ring) compounds in MES model. Substitution with naphthyl and 3,4-methyl-phenyl group at 6-position of pyridazinone moiety showed high anticonvulsant activity than other aryl (like 4-methyl-phenyl, 4-ethyl-phenyl, biphenyl ring) pyridazinone compounds in INH model. Substitution with biphenyl and naphthyl group at 6 position of pyridazinone moiety showed higher anticonvulsant activity

than other aryl (like 4-methyl-phenyl, 4-ethyl-phenyl, 3,4-methyl-phenyl) pyridazinone compounds in PTZ model. All compounds showed significant anticonvulsant activities against MES, INH, PTZ-induced convulsions except STR induced convulsions. In antitubercular activity, compound having naphthyl substitution (3d) at 6-position on pyridazinone ring showed better response towards MABA model with MIC value is 12.5µg/ml than other aryl substituted (like 4-methyl-phenyl, 4-ethyl-phenyl, 3,4-dimethylphenyl, biphenyl ring) compounds (25µg/ml) in anti-TB activity against M. tuberculosis H37Rv. Therefore, it was concluded that 6-aryl 2-(1H-indol-1-ylmethyl)-4,5-dihydro-pyridazin-3(2H)-one derivatives can be further modified to exhibit better potency.

3.4. Structure Features and Biological Activities

The final compounds (3a-e) were evaluated for anticonvulsant by MES and INH, PTZ and STR induced convulsion methods as well anti-TB activities by MABA assay. Both activities were evaluated on same molecules because one of most commonly used antitubercular drug INH produced convulsion in higher dose level than used in TB therapy and also caused some serious adverse effects. Due these effects of INH and some other compounds were also evaluated against both convulsion and TB, both activities were exhibited by same molecules [33-36], so we synthesized and evaluated some pyridazinone compounds for antitubercular as well as anticonvulsant activities.

3.4.1. Pyridazinones as Anticonvulsants:

A series of substituted pyridazinones revealed varying degrees of activity against various animal models such as MES, scPTZ, STR etc induced seizures. Research indicated that the presence of at least one aryl group, one or two electron donor atoms and/or an NH group in a special spatial arrangement is necessary for anticonvulsant activity [49,50]. The pyridazinones agrees with this feature. In order to explore the activity associated

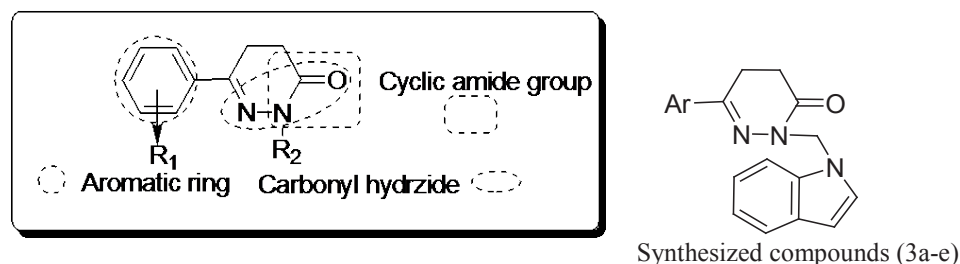


Figure 1. Concept of pyridazinones as anticonvulsant agent.

with the presence of an amide group, cyclic/acyclic, is present in most anticonvulsants. The common structural feature of antiepileptic drugs appeared to be a nitrogen hetero atomic system. It has been proposed that for activity, higher is the hydrophobic parameter π (π) of the substituent on phenyl ring, more potent anticonvulsant. Compounds with an electron withdrawing substituent on the phenyl ring exhibited appreciable anticonvulsant activity. Substituting the hydrogen (from -NH in pyridazinones) with methyl and acetyl group enhanced the lipophilicity of the compounds [33-36]. The lipophilic drugs must pass through blood brain barrier (BBB) and reach to its receptors in the central nervous system (CNS) (figure 1).

3.4.2. Amide Derivatives as Anticonvulsant (Acyclic/Cyclic) Derivatives

In the early days, acyclic amide and cyclic amide demonstrated potent anticonvulsant activity. Many of them are still preferred

drugs for the treatment of epilepsy. Phenytoin, fosphenytoin (water soluble prodrug) are used for the treatment of epileptic seizures. A succinimide derivative ethosuximide is considered the first choice drug for treating absence seizures. Phenobarbital is a barbiturate and is the most widely used anticonvulsant worldwide. Primidone is used for generalized tonic-clonic and complex seizures (table 6 and figure 2).

3.4.3. Pyridazinones as Antitubercular Agents

Several pyridazinones have been possessing anti-TB activity. Modification of the drug molecules has resulted in clinically effective drugs for TB. A series of pyridazinones revealed varying degrees of activity against *M. tuberculosis* infections [33, 34]. Almost all pyridazinones exhibited moderate levels of anti-TB activity. Recently, more works have been done on pyridazinones to afford

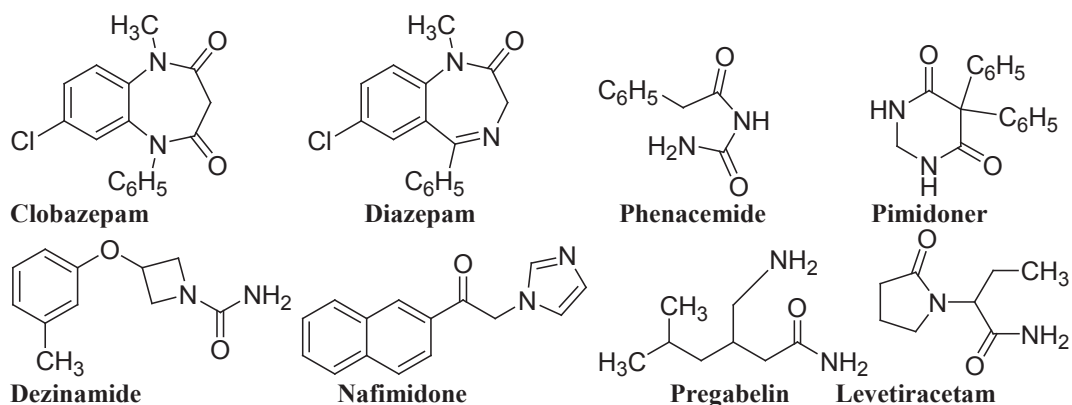


Figure 2. Some anticonvulsant drugs with different structures.

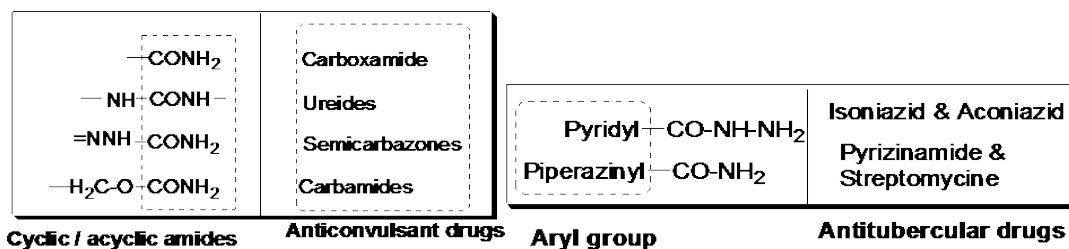


Figure 3. Concept of pyridazinones as anticonvulsants and as antitubercular agents.

significant molecule to protection against TB (figure 3).

3.4.4. Amide and Carbonyl Hydrazide Derivatives as Antitubercular Derivatives

In the early days, carbonyl hydrazide and amides demonstrated potent anti-TB activity. Many of them are still preferred drugs for the treatment of TB. Isoniazid (INH) and aconiazid is a carbonyl hydrazide derivative of pyridine, and pyrizinamide (PZA) is an amide derivative of piperazine (diazine) and other amide compounds such as streptomycin (SM) derivatives (a carbohydrate) are used for the treatment of TB. Other compounds like ethionamide and prothionamide are also considered because both compounds having thioamide group in place of amide group present in PZA (act as bioisosters), the drug for treating TB [33,34]. Phenobarbital is a barbiturate and is the most widely used anticonvulsant worldwide. Primidone is used for generalized tonic-clonic and complex seizures (figure 4).

3.5. Possible Mechanisms

The preclinical discovery and develop-

ment of a new chemical entity for the treatment of epilepsy rely heavily on the use of predictable animal models. At the present time, there are three in vivo models that are routinely used by most antiepileptic drugs discovery programs. They include the MES, scPTZ, INH, and STR models. From these, the MES and scPTZ seizure models represent the two animal seizure models, which are most widely used in the search for new AEDs. All compounds were showed anticonvulsant activities against MES, INH and PTZ but no compound was effective against STR induced convulsions. The MES test is known to be sensitive to sodium (Na^+) channel inhibitors (e.g. phenytoin), which suggested that compounds 3a-e may inhibit voltage-gated ion channels (particularly Na^+ channels). The INH (a GABA synthesis inhibitor) and PTZ (a GABA antagonist) test are known to be sensitive to modulate γ -amino butyric acid (GABA), which suggested that compounds 3a-e may inhibit the GABA concentration in the brain. The STR (Glycine antagonist) test is known to be sensitive to antagonized glycine, which suggested that compounds 3a-e may not inhibit the glycine

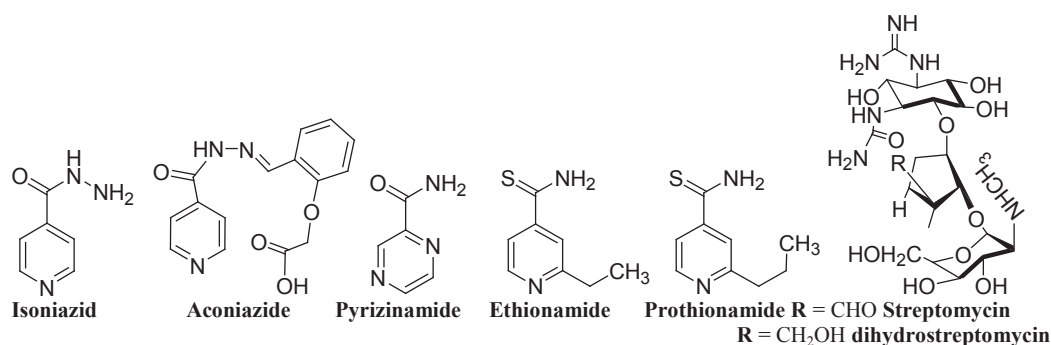


Figure 4. Some anticonvulsant drugs with different structures.

concentration in the brain. These results reported that all title compounds 3a-e showed voltage-gated ion channels (mainly Na⁺-channel) inhibitors and GABA inhibitors. Further investigations is needed to evaluate the effects of the anticonvulsant activity in several other animal models for the estimation of neurotransmitters to speculate about the exact possible mechanism of these anticonvulsant compounds.

4. Conclusion

It is observed that various pyridazinone derivatives possess anti-TB and anticonvulsant activities. In various biological activities 6-aryl-3(2H)-pyridazinone residue as the pharmacophoric groups, these finding encourage for the synthesis of some pyridazinone derivatives and to evaluate them for anticonvulsant and anti-TB activities. In the present investigation, a series of 6-aryl 2-(1H-indol-1-ylmethyl)-4,5-dihydropyridazin-3(2H)-one derivatives (3a-e) were synthesized and evaluated as anticonvulsant activities against MES, INH and PTZ induced in animal models and in-vitro anti-tubercular activity against *M. tuberculosis* H37Rv by MABA method. All compounds showed moderate to good anticonvulsant activities and weak antitubercular activity.

Acknowledgements

The authors are thankful to GRD (PG) Institute of Management & Technology, Dehradun, India, Sophisticated Analytical Instrument Facilities, Central Drug Research Institute, Lucknow, Uttar Pradesh, India for providing financial as well as technical support and facilities to carry out this work as well as to Department of Microbiology, Maratha Mandal NGH Institute of Dental Sciences and Research Centre, Belgaum, India for antitubercular activity.

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