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# Anticonvulsant Activity of Some Mannich bases of 2-Aminothiophenes having Indole-3-Carboxaldehyde against Strychnine, Picrotoxin, Lithium-pilocarpine-induced Seizures

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**ABSTRACT**

*Anticonvulsant activity of some mannich bases of 2-aminothiophenes having indole-3-carboxaldehyde in mice was assessed using Strychnine, Picrotoxin animal models. The lithium-pilocarpine model of status epileptics was also used to assess the anticonvulsant activity in rats. Compound II have shown good activity, compound I, III, IV, V have shown moderate activity against the standard drugs. The dose of 100mg/kg afforded protection to all animals.*

**Keywords:** 2-aminothiophenes, mannich bases, strychnine, Picrotoxin, lithium –pilocarpine

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## INTRODUCTION

Thiophenes are known to possess biological activities like Central nervous system depressant activity[1], Anti-inflammatory[2], Antimicrobial[3], Antitumor[4], Cytoprotectant[5], Antioxidant[6], Antinociceptive[7], Antitubercular[8], Local anesthetic agents[9], Antifungal[10]. In our previous work we reported synthesis and anticonvulsant of 2-aminothiophenes against MES, PTZ-Induced seizures [11], so the present work was undertaken to screen anticonvulsant activity against strychnine, Picrotoxin, lithium-pilocarpine-induced seizures.

## MATERIALS AND METHODS

**Animals:** Male Albino Swiss mice weighing 22-25 g and male Albino Wistar rats weighing 125-150 g were selected for present study obtained from National institute of Nutrition, Hyderabad.The institutional animal ethics permission was obtained (1217/a/08/CPCSEA) before starting the experiments on the animals. Animals were housed in groups of 6-8 cage at a temperature $25^{o}$ $\pm 1^{o}$ C and relative humidity of 45-55%.A 12:12 dark: light cycle were followed during the experiments .Animals had free access to food and water however, food but not water was withdrawn 8 h before and during the experiments.

**Acute oral toxicity studies**

Thirty Albino mice & rats of both sexes were randomly divided to five groups consisting of the control and test treated groups. Mice & rats were fasted for 12 h and various doses of test compounds were administered orally to test groups. The mice & rats were closely observed for toxic symptoms and behavioral changes for the first 2h after test compounds administration and mortality recorded within 24 h.The lethal dose that killed 50% of mice and rats was estimated after 24 h.

**Strychnine-induced seizures**

Mice were divided into five groups each containing six animals. Group I received 10mL normal saline per kg body weight i.p, Group II received Diazepam 5mg/kg body weight(i.p),Group III received were treated with test compounds at dose 100 mg/kg body weight i.p. Thirty minutes later, mice in all the groups received 1mg/kg body weight strychnine. Any group that did not convulsive within 30 min after strychnine administration was considered protected, abolition of tonic extensor jerks of hind limbs was considered as an indicator that the testing drug could prevent strychnine induced convulsion [12].

**Picrotoxin-Induced Seizures**

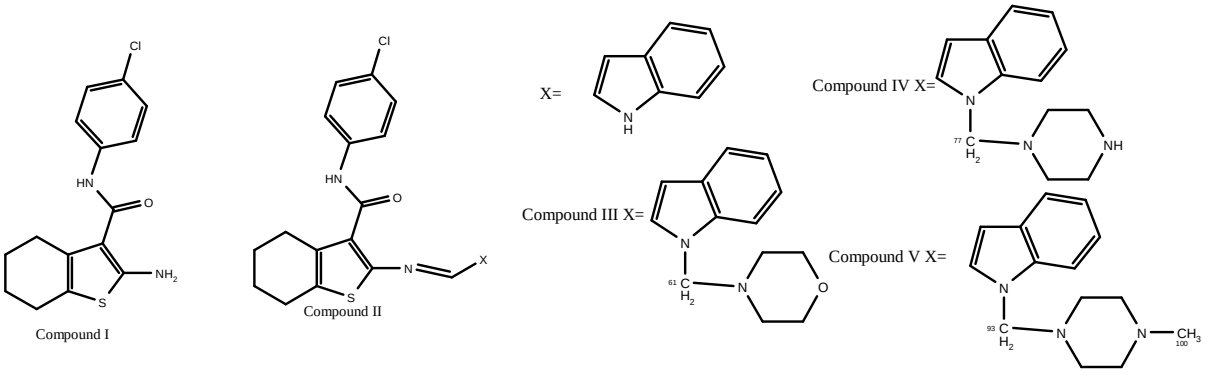
Mice were randomly allotted to the different control and test groups. Picrotoxin (5mg/kg, i.p) was used to induce seizures. Procedure of strychnine induced convulsions was carried out for screening.

**Lithium-Pilocarpine Induced seizures**

Albino rats were divided randomly into five groups each containing six animals. Status epileptics was induced by administration of pilocarpine (30 mg/kg, i.p) 24 hr after lithium sulphate ( 3 mEq/k.g,i.p).The effect of test compounds at dose 100 mg/kg.i.p was studied on the severity of the seizures. One group received only vehicle while the other group received diazepam (10 mg/kg).The severity of status epileptics was observed every 15 min till 90 min and thereafter every 30 min till 180 min, using the scoring system [13].No response-stage 0, fictive scratching-stage 1, tremors-stage 2, head nodding-3, forelimb clonus-stage-4, rearing and falling back stage-5.

**Statistical Analysis**: Data are represented as mean ± SEM.Graphpad prism 5.0 software is used to analyze the results by one-way analysis of variance(ANOVA) followed by Dunnett's test and the data of lithium-pilocarpine-induced seizures were analyzed using Kruskal-wallis ANNOVA followed by Dunnett's test. The differences were considered significant at 5%.

**Table 1: Effect of Mannich bases of thiophenes on Strychnine-Induced seizures in mice**




| Group | Treatment and route | Seizure onset in Sec | |
|---|---|---|---|
| | | Clonic (Mean ± SEM) | Tonic(Mean ± SEM) |
| I | Control-Normal saline ( 10 ml/kg,i.p) | 1.123 ± 0.003 | 1.417 ± 0.009 |
| II | Diazepam ( 10 mg/kg, i.p) | 6.478 ±0.001**** | 7.287 ±0.008**** |
| III | Compound I | 7.673± 0.002** | 7.893±0.004** |
| IV | Compound II | 4.808±0.001**** | 5.205±0.003** |
| V | Compound III | 5.607±0.002** | 6.005±0.003** |
| VI | Compound IV | 5.180±0.016*** | 6.195±0.002ǂ |
| VII | Compound V | 5.005±0.003** | 5.497±0.002** |

*n=6 Values are Mean ±SEM **** (P<0.0001),***/** (P <0.05), ǂ not significant, different compared to control*

**Table 2: Effect of Mannich bases of thiophenes on Picrotoxin-Induced seizures in mice**

| Group | Treatment and route | Seizure onset in Sec | |
|---|---|---|---|
| | | Clonic (Mean ± SEM) | Tonic(Mean ± SEM) |
| I | Control-Normal saline ( 10 ml/kg,i.p) | 5.033±0.02 | 5.450±0.05 |
| II | Diazepam ( 10 mg/kg, i.p) | 15.90±0.001**** | 17.79±0.006**** |
| III | Compound I | 14.83±0.04** | 15.92±0.04** |
| IV | Compound II | 10.21±0.04*** | 11.12±0.002** |
| V | Compound III | 11.42±0.01*** | 12.27±0.006** |
| VI | Compound IV | 13.80±0.004** | 14.95±0.002** |
| VII | Compound V | 12.61±0.004** | 14.95±0.002** |

*n=6 Values are Mean ±SEM **** (P<0.0001),***/** (P <0.05), different compared to control*

**Table 3: Effect of Mannich bases of thiophenes on lithium-pilocarpine- Induced seizures**

| Group | Treatment And route | Time after Pilocarpine in min | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 120 |
| I | Control (10ml/kg,,i.p) | 0.0±0.0 | 1.84±0.004 | 2.133±0.002 | 2.97 ±0.002 | 3.167±0.02 | 3.417±0.06 | 4.35±0.03 | 4.85±0.022 |
| II | Diazepam (10mg/Kg i.p) | 0.0±0.0 | 0.28±0.002** | 0.48±0.001** | 0.56±0.003** | 0.88±0.004** | 0.99±0.001**** | 1.16±0.02** | 0.76±0.004** |
| III | Compound I | 0.0±0.0 | `0.82±0.002** | 0.90±0.002** | 0.96±0.009** | 1.26±0.004** | 1.30±0.002 | 1.02±0.002* | 0.64±0.002** |
| IV | Compound II | 0.0±0.0 | 0.56±0.002** | 0.71±0.002** | 0.83±0.001** | 0.90±0.004** | 1.12±0.002** | 0.77±0.011** | 0.55±0.004** |
| V | Compound III | 0.0±0.0 | 0.97±0.005** | 1.25±0.022 | 1.497±0.002** | 1.94±0.001** | 1.03±0.002** | 0.85±0.003** | 0.75±0.002** |
| VI | Compound IV | 0.0±0.0 | 1.20±0.002** | 1.29±0.003 | 1.49±0.002* | 1.22±0.002* | 0.97±0.003** | 0.72±0.002* | 0.63±0.004** |
| VII | Compound V | 0.0±0.0 | 1.19±0.003** | 0.90±0.004** | 1.19±0.004 | 1.39±0.002 | 0.72±0.003** | 0.87±0.004** | 0.79±0.004** |

*n=6 in each group. The values are mean ±SEM of the scores indicating the severity of seizures*

## RESULTS

**Strychnine –induced seizures**: The test compounds at dose 100mg/kg, i.p against strychnine (4 mg/kg.i.p) showed a significant (P <0.05, Student's t test) and dose dependent prolongation of both clonic and tonic seizure latencies were observed compared with control mice. Compound II showed significant (P<0.0001) prevention against strychnine induced convulsion due to the presence of free indole ring. Compound I, III, IV, V have shown moderate activity against standard drug diazepam (Table-1).

**Picrotoxin –induced convulsions**: The test compounds at dose 100mg/kg, i.p like diazepam (10mg/kg, i.p), did not prevent seizure against Picrotoxin (4mg/kg, i.m)-induced convulsion test, but a significant (P<0.05, student's t test) and dose dependant prolongation of both clonic and tonic seizure latencies were observed compared with control. All the compounds have shown moderate activity against standard drugs (Table 2).

**Lithium-pilocarpine-Induced convulsions**: Rats treated with lithium and pilocarpine showed stage 4 seizures in all animals 75 min after pilocarpine. All the compounds showed moderate decrease in the intensity of stage 4 seizures and animals were normal in behavior after 180 min.

## DISCUSSION

2-aminothiophenes have been substitute with indole heterocyclic ring the presence of free NH group on indole have been substituted with morpholino,piperazine,N-methylpiperazine,The presence of these substituents against different anticonvulsant screening models have been observed. The presence of free NH group on the indole ring is essential to show activity against different models. The replacement of free NH group of the indole ring leads to decrease in the epileptic activity. Generally these drugs may inhibit seizures by regulating GABA-mediated synaptic inhibition through an action at distinct sites of the synapse. Lithium does not possess general proconvulsant activity in rats; its pretreatment provokes limbic seizures following administration of sub-convulsant doses of pilocarpine and other cholinergic agonists. The combined treatment with lithium and pilocarpine results in accumulation of acetylcholine and inositol monophosphate and reduction in cortical inosital that are about ten times greater than the effects obtained with either drugs alone.Phenobarbitone, sodium valproate, diazepam and trimethadione prevent limbic seizures induced in rats by pilocarpine, however Phenytoin and carbamazepine are ineffective but enhancement of sodium channel activation is observed. Strychnine directly acts by antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing spinal reflexes. Picrotoxin is a selective non-competitive

antagonist of gamma amino butyric acid at $GABA_A$ receptor which has been widely used in epilepsy. The proposed mechanism may involve both GABAergic and glycinergic inhibitory mechanisms. The exact mechanism of action of the drug may be done by assessing detailed biochemical studies and estimation of biogenic amines in the brain.

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