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Anticonvulsant and Convulsant effects of Indole derivatives against Chemical Models of Epilepsy

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Abstract: Indole is a benzopyrrole in which the benzene and pyrrole rings are fused through the 2-and 3-positions of the pyrrole nucleus. The indole derivatives are very much used as anticonvulsant agents. In the same context, several indole derivatives were prepared by the reaction between indole-3-carboxaldehyde and various p-substituted phenylsemicarbazides, in the presence of glacial acetic acid. The Mannich bases of above synthesized compounds were prepared by using formaldehyde & various secondary amines. All the prepared derivatives were screened against different chemical induced convulsion models such as isoniazid, thiosemicarbazide & 4-aminopyridine respectively, for their possible convulsant and anticonvulsant activity. The derivatives were also evaluated for their sedative-hypnotic properties using phenobarbitone induced sleeping model & also for their neurotoxicity. The result showed that some of the compounds were very much active against different convulsant models, proving their different mode of actions in the course of epileptic seizures.

Keywords: (Indole, 4-arylsemicarbazone, Isoniazid, Thiosemicarbazide, 4- aminopyridine, Anticonvulsant activity).

Introduction

Epilepsy is a physical condition that occurs when there is a sudden, brief change in how the brain works & characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor, or autonomic phenomenon with or without loss of consciousness. In developed countries the incidence of epilepsy is around 50/100 000/year. In recourse poor countries, the incidence is likely to be higher [1]. Around 80-85% of epileptic patients may be provided with the help of conventional antiepileptic drugs & many newer drugs which possesses more than one mechanisms of action.

A chemical model of epilepsy is based on the application of, or withdrawal from, chemical substances with consequent appearance of epileptic sympatomatology [2]. Different chemical models of epilepsy, which mimic different clinical seizure types, dealing with different mechanisms and acute versus chronic epileptic phenomena, were used to screen the synthesized 4-arylsemicarbazone derivatives of indole. The thiosemicarbazide & other hydrazides are very much used to induce convulsion & has been demonstrated in various earlier publications & reviews

(Parks *et al* [3]1952, Dieke [4] 1949 & Reilly *et al* [5]). Isoniazid can precipitate convulsions in patients with seizure disorders. The compound is regarded as a GABA-synthesis inhibitor. INH lowers GABA level and the activity of glutamate decarboxylase (GAD)[6]. Thiosemicarbazide can precipitate convulsions by inhibiting the GABA synthesis via cofactor antagonism through impairment of the synthesis or coenzyme action of pyridoxal phosphate [7-11]. The K+ channel antagonist 4-aminopyridine is a powerful convulsant in animals and in man. The drug readily penetrates the blood-brain barrier and is believed to induce seizure activity by enhancing spontaneous and evoked neurotransmitter release [12-15]. Thus we screened the synthesized derivatives using three different chemical induced convulsion models such as isoniazid, thiosemicarbazide & 4-aminopyridine respectively, for their possible convulsant & anticonvulsant activities to show that the same drug can work with different mode of action at different receptors or ion channels. The semicarbazones of the indole served as open chain bioisosteres to desmethyl Diazepam & Bromazepam respectively and thus assumed to act as anticonvulsants (Figure 1).

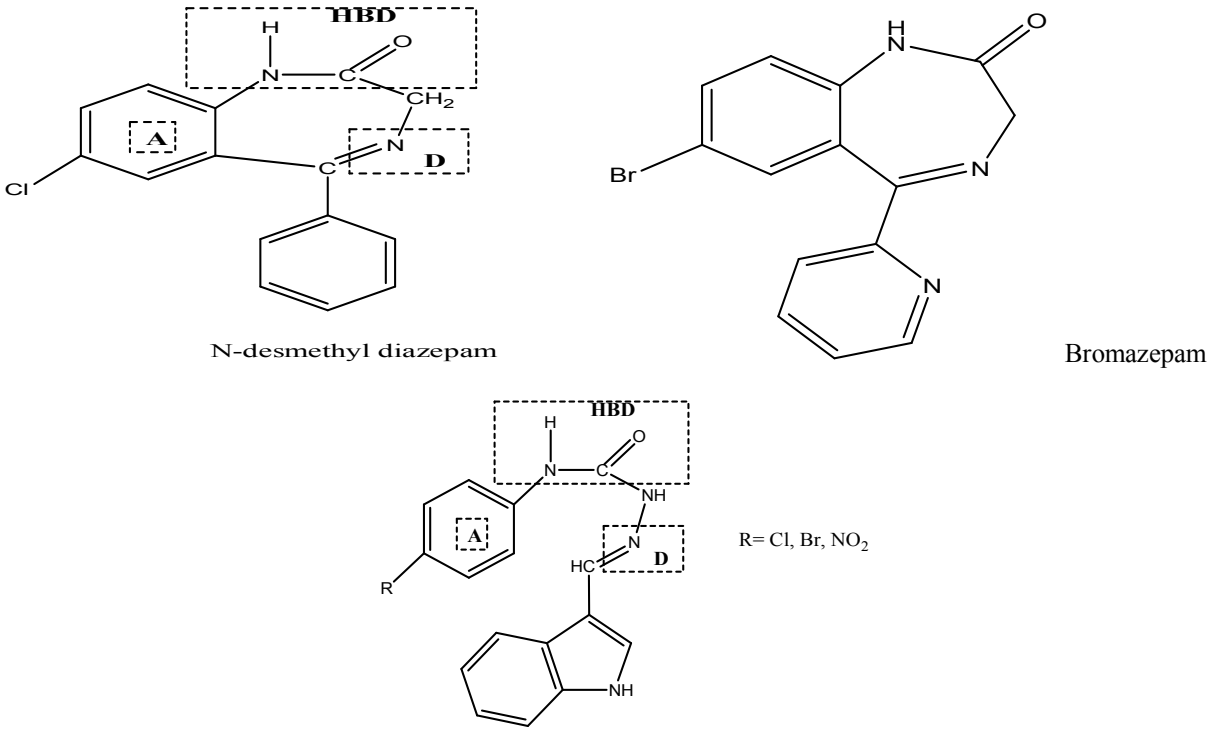
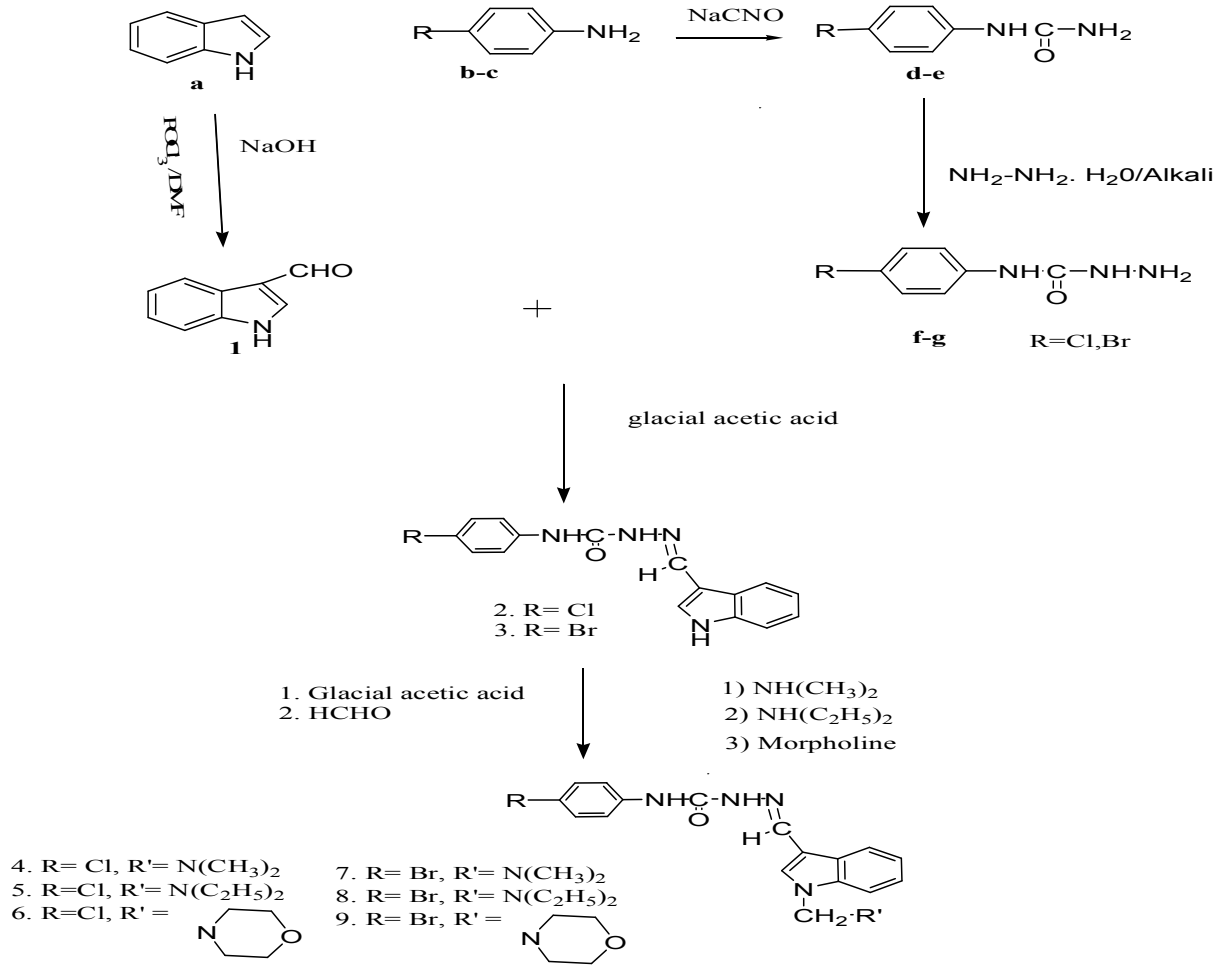


Figure 1: Pharmacophore model for substituted semicarbazones
A= Hydrophobic unit, HBD= hydrogen bonding domain, D= electron donor

Synthetic scheme



Experimental

The melting points were measured in open capillaries on Jindal melting point apparatus & were uncorrected. Yields were obtained after recrystallization. The IR spectra were recorded (KBr) on Jasco FT-IR 6100 spectrophotometer.^{1}H NMR were recorded on Bruker Avauce II 300MHz NMR spectrometer using DMSO-d6 as a solvent. Tetramethylsilane serves as internal standard in ^{1}H NMR. Elemental analysis was done on Vario EL-III analyzer. TLC was performed on silica gel G using chloroform: methanol (9:1) as a solvent system.

General Synthesis of 1-H-Indole-3-Carboxaldehyde (1)

The solution of indole **(a)** (6gm; 0.05mole) was prepared in dimethylformamide (DMSO) in a beaker. $POCl_3$ was added dropwise to DMSO taken in another beaker in ice bath, by keeping the temperature below $10C^0$. After the addition the reaction mixture was stirred for 0.5 hr. To The above formylation complex, the solution of indole was added.

During the addition, the temperature was maintained below $10C^0$. The temperature was raised to $35C^0$ & maintained for 1hr. The mixture was then cooled to $10C^0$ & made alkaline with NaOH solution. The resulting suspension was heated to $60C^0$ & cooled to room temperature. The product was filtered & washed with water and dried at room temperature. The TLC was performed on silica gel 60 using chloroform: methanol (9:1) as a solvent system [16].

General synthesis of 4-substituted aromatic ureas (d-e)

The synthesis was performed by using the well known procedure described by Pandeya *et al* [17]. Various 4-substituted anilines i.e. chloro & bromo anilines **(b-c)** (0.1 mol) were dissolved in 10ml of glacial acetic acid and to it 50 ml of water was added. To this solution an equimolar (0.1 mol)

quantity of sodium cyanate in 50 ml of warm water was mixed with stirring. The reaction mixture was kept for 30 minutes and crystals were collected, recrystallized from absolute ethanol.

Synthesis of 4-substituted arylsemicarbazides (f-g)
To a solution of substituted ureas (d-e) (0.01 mol) in 100 ml of ethanol, an equimolar quantity (0.01mol) of hydrazine hydrate was added. The reaction mixture was made alkaline by adding NaOH and refluxed for 1.5h and cooled in ice. The product was filtered and recrystallized from ethanol.

General synthesis of indole semicarbazone derivatives (2-3)
1-H-indole-3-carboxaldehyde (1) (0.01 mol), was taken into a round bottom flask & to it added an equimolar quantity (0.01mol) of p-substituted arylsemicarbazides (f-g). The reaction mixture was refluxed in glacial acetic acid for about 3hr. The resultant compound was allowed to stand for 30min. & then cooled in ice. The product was filtered and recrystallized with ethanol.

General synthesis of Mannich bases of indole semicarbazone derivatives (4-9)
The Mannich bases of indole arylsemicarbazones (0.01 mole) were prepared by refluxing it with an equimolar amount of formaldehyde, and various secondary amines such as dimethylamine, diethylamine & morpholine respectively, in the presence of glacial acetic acid for 3h. The resultant products were cooled at room temperature, filtered & recrystallized with ethanol.

1-H-indole-3-carboxaldehyde (1)
Mol. Formula C_9H_7NO, Mol. Wt. 145.157, Rf value 0.88, m.p. 193-195°C, % yield 81, IR(KBr): 3460(NH),1720(C=O),1540(C=C aromatic ring), 3050(=C-H ring stretch), 2750CM^{-1}(-C-H stretch). ^{1}HNMR(DMSO-d6,δ):9.93($_S$,1H,CHO), 10.13 ($_S$,1H,,NH),8.35($_S$,1H,H^2), 7.21(2H,,H^5&H^6), 8.15($_S$,1H,H^4),7.54($_S$,1H,H^7).
Elemental Analysis (%) Cal. C(74.47%), H(4.86%), N(9.65%)
Found C(74.44%),H(4.81%),N(9.59%).

(2E)-N-(4-chlorophenyl)-2-(1H-indol-3-ylmethylidene)hydrazinecarboxamide (2)
Mol. Formula $C_{16}H_{13}ClN_4O$, Mol. Wt. 312.75, Rf value 0.77, m.p. 240-243°C, % yield 72%, IR(KBr): 3460(NH),3350(Ar-NH), 1610(C=N),1680(C=O),820(Ar-H),3280(-CONH)
^{1}HNMR(DMSO-d6, δ):9.1(s,1H,=NNH), 6.0(s,1H,CONH),7.5(s,1H,CH=N), 10.1(1H,s,NH),7.2-7.5(m,4H,CH, phenyl),7.1-7.6(m, 5H, 3-indole)
Elemental Analysis: Cal. C(61.64%)H(4.19%)N(17.91%). Found C(61.66%)H(4.22%) N(17.97%).

(2E)-N-(4-bromophenyl)-2-(1H-indol-3-ylmethylidene)hydrazinecarboxamide (3)
Mol. Formula $C_{16}H_{13}BrN_4O$, Mol. Wt. 356.03, Rf value 0.82, m.p. 272-275°C, % yield 83%, IR(KBr):3480(NH),3360(Ar-NH),1618(C=N),1700(C=O),825(Ar-H),3260(-CONH) ,^{1}HNMR(DMSO-d6,δ):9.2(s,1H,=NNH),6.2(s,1H,CONH),7.3(s,1H,CH=N), 9.8(1H,s,NH),7.4-7.5(m,4H,CH, phenyl),7.3-7.8(m, 5H, 3-indole),
Elemental Analysis: Cal. C(53.80%)H(3.67%)N(15.68%),
Found, C(53.84%)H(3.69%)N(15.59%)

(2E)-N-(4-chlorophenyl)-2-({1-[(dimethylamino) methyl]-1H-indol-3yl}methylidene)hydrazine carboxamide (4)
Mol. Formula $C_{19}H_{20}ClN_5O$, Mol. Wt. 369.85, Rf value 0.66, m.p. 48-52°C, % yield 70%, IR (KBr):3330(Ar-NH),1612(C=N),1660(C=O),836(Ar-H),3300(-CONH),
^{1}HNMR(DMSO-d6, δ): 9.0(s,1H,=NNH), 6.5(s,1H,CONH),7.2(s,1H,CH=N), 7.4-7.7(m,4H,CH, phenyl),6.9-7.1(m, 5H, 3-indole),4.9(s,2H,CH_2),2.2(s,6H,2CH_3),
Elemental Analysis: Cal. C(61.70%)H(5.45%)N(18.94%),
Found C(61.74%)H(5.46%)N(18.89%).

(2E)-N-(4-chlorophenyl)-2-({1-[(diethylamino)methyl]-1H-indol-3-yl}methylidene)hydrazine carboxamide (5)
Mol. Formula $C_{21}H_{24}ClN_5O$, Mol. Wt. 397.90, Rf value 0.66, m.p. 75-78°C, % yield 66%, IR (KBr): 3355(Ar-NH), 1622(C=N),1688(C=O), 834(Ar-H),3310(-CONH)
^{1}HNMR(DMSO-d6,δ)
8.9(s,1H,=NNH),5.93(s,1H,CONH),7.77(s,1H,CH=N), 8.1-8.4(m, 4H, CH, phenyl), 6.3-7.1(m, 5H, 3-indole),4.9(s,2H,CH_2),2.4(s,4H,2CH_2), 1.3(s, 6H, 2CH_3), Elemental Analysis: Cal. C(63.39%)H(6.08%)N(17.60%),
Found C (63.41%) H (6.11%)N(17.58%).

(2E)-N-(4-chlorophenyl)-2-({1-[(morpholin-4-yl)methyl]-1H-indol-3-yl}methylidene)hydrazine carboxamide (6)

Mol. Formula $C_{21}H_{22}ClN_5O_2$, Mol. Wt. 411.88, Rf value 0.83, m.p. 50-52°C, % yield 78%, IR(KBr): 3358(Ar-NH), 1633(C=N),1684(C=O), 837(Ar-H),3330(-CONH)
^{1}HNMR(DMSO-d6,δ)8.9(s,1H,=NNH),5.9(s,1H,CONH),7.7(s,1H,CH=N),
7.3-7.6(m,5H,3-indole), 5.0(s,2H,CH_2), 7.9-8.3(m,4H, CH, phenyl), 2.3-3.6(s,8H,4CH_2)
Elemental Analysis: Cal.
C(61.24%)H(5.38%)N(17.00%)
Found C(61.33%)H(5.45%)N(17.08%)

(2E)-N-(4-bromophenyl)-2-({1-[(dimethylamino)methyl]-1H-indol-3-yl}methylidene)hydrazine carboxamide (7)

Mol. Formula $C_{19}H_{20}BrN_5O$, Mol. Wt. 414.30, Rf value 0.68, m.p. 58-60°C, % yield 82%, IR (KBr): 3348(Ar-NH), 1610(C=N),1678(C=O) 840(Ar-H),3288(-CONH),
^{1}HNMR(DMSO-d6,δ)9.2(s,1H,=NNH),6.1(s,1H,CONH),7.2(s,1H,CH=N),
7.1-7.3(m,4H,CH, phenyl),6.6-7.1(m, 5H, 3-indole) 4.9(s,2H,CH_2),2.3(s,6H,2CH_3)
Elemental Analysis: Cal. C (55.08%) H(4.87%)N(16.90%),
Found C(55.03%)H(4.88%)N(16.88%).

(2E)-N-(4-bromophenyl)-2-({1-[(diethylamino) methyl]-1H-indol-3-yl}methylidene)hydrazine carboxamide (8)

Mol. Formula $C_{21}H_{24}BrN_5O$, Mol. Wt. 442.35, Rf value 0.78, m.p. 52-55°C, % yield 75%, IR (KBr): 3360(Ar-NH), 1640(C=N),1695(C=O), 822(Ar-H),3270(-CONH),^{1}HNMR(DMSO-d6,δ)
9.1(s,1H,=NNH),5.9(s,1H,CONH),7.5(s,1H,CH=N),
7.3-7.9(m,4H,CH, phenyl),6.1-7.2(m, 5H, 3-indole) 4.9(s,2H,CH_2),2.2(s,4H,2CH_2),1.1(s,6H,2CH_3),
Elemental Analysis: Cal. C (57.02%)
H(5.47%)N(15.83%),
Found C(57.05%)H(5.49%)N(15.85%).

(2E)-N-(4-bromophenyl)-2-({1-[(morpholin-4-yl)methyl]-1H-indol-3-yl}methylidene)hydrazine carboxamide (9)

Mol. Formula $C_{21}H_{22}BrN_5O_2$, Mol. Wt. 456.33, Rf value 0.84, m.p. 50-52°C, % yield72%, IR (KBr): 3368(Ar-NH), 1670(C=N),1680(C=O), 828(Ar-H),3255(-CONH),^{1}HNMR(DMSO-d6,δ)
9.1(s,1H,=NNH),
5.9(s,1H,CONH),7.5(s,1H,CH=N),7.3-7.9(m,4H,CH,phenyl), 6.3-7.3(m,5H,3-indole) 4.8(s,2H,CH_2),2.4-3.5(m,8H,4CH_2),
Elemental Analysis: Cal. C (55.27%)
H(4.86%)N(15.35%)
Found C (54.33%) H(4.76%)N(15.56%)

Anticonvulsant activity

All the compounds were screened for anticonvulsant activities adopting the anticonvulsant drug development (ADD) program protocol. The healthy Swiss albino mice of both sexes weighing 25-30 g were taken for the study. The animals were kept in large spacious hygienic cages during the course of experimental period. The animals had free access to standard commercial diet and water ad libitum and were kept in rooms maintained at 22±1^{0}C with 12h light dark cycle.All the synthesized derivatives were dissolved in polyethylene glycol (PEG- 400). All the compounds were administered i.p. at the dose of 30 mg/kg (single dose study) to mice. The activity was established using the different chemical induced convulsion tests ie; isoniazid induced convulsion test, Thiosemicarbazide induced convulsion test and 4-aminopyridine induced convulsion in mice [18].

Chemicals induced convulsion models

Ten groups of mice (each having 6 animals) of either sex with a weight of 25-30g, were treated with the test compounds & the standards (e.g. Diazepam 10 mg/kg & Phenytoin 30mg/kg body weight) by intraperitoneal administration. Controls received the vehicle only. 30 min after i.p. treatment the animals were injected with a subcutaneous dose of 300 mg/kg body weight isoniazid (isonicotinic acid hydrazide), 20mg/kg body weight of thiosemicarbazide & 13.3 mg/kg body weight of 4-aminopyridine, respectively. During the next 120 min. the occurrence of clonic seizures, tonic seizures and death were recorded.

Neurotoxicity Screening

Rotorod test

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm^{-1}. Trained animals were given i.p. injection of the test compounds in dose of 30mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of three trials. The doses at which the animals, unable to grasp the rotorod, were determined.

Sedative- Hypnotic activity

Potentiation of Phenobarbitone induced sleeping time

This test was performed with the test substances in a dose of 30mg/kg. The compounds in PEG were

administered i.p. to group of rats. After 30 min, rats were injected i.p. with a solution of phenobarbitone (in PEG) in a dose of 40mg/kg. The rats were then placed on their back and the loss of rithing reflux was taken as onset of sleep. The time taken by rats to awake was noted. A control was also performed after pre-treatment with test substance vehicle (PEG) and injected phenobarbitone [19].

Table1. The table shows protection time (h) against seizures produced by isoniazid

Diazepam (10mg/kg) Phenytoin (30mg/kg)		Isoniazid induced convulsion model (300mg/kg)		
		Protection time (h)		
S. No.	Derivatives (30mg/kg)	0.5 h	1 h	2 h
1	2	30mg	30mg	------
2	3	30mg	30mg	30mg
3	4	30mg	30mg	------
4	5	30mg	------	------
5	6	30mg	30mg	30mg
6	7	30mg	-------	-------
7	8	30mg	30mg	30mg
8	9	30mg	-------	------
9	Diazepam	10mg	10mg	10mg
10	Phenytoin	-------	-------	------

Dose of 30mg kg^{-1} was administered *i.p.* Test compounds were suspended in polyethylene glycol (PEG). Figures in the table indicate the minimum dose with bioactivity demonstrated in half or more of the mice. The dash (–) indicates the absence of activity

Table2. The table shows protection time (h) against seizures produced by thiosemicarbazide

Diazepam (10mg/kg) Phenytoin (30mg/kg)		Thiosemicarbazide induced convulsion model (20mg/kg)		
		Protection time (h)		
S. No.	Derivatives (30mg/kg)	0.5 h	1 h	2 h
1	2	30mg	30mg	30mg
2	3	30mg	30mg	--------
3	4	30mg	30mg	--------
4	5	30mg	30mg	--------
5	6	30mg	------	--------
6	7	30mg	30mg	30mg
7	8	30mg	-------	--------
8	9	30mg	------	--------
9	Diazepam	10mg	10mg	10mg
10	Phenytoin	-------	-------	--------

Dose of 30mg kg^{-1} was administered *i.p.* Test compounds were suspended in polyethylene glycol (PEG). Figures in the table indicate the minimum dose with bioactivity demonstrated in half or more of the mice. The dash (–) indicates the absence of activity.

Table3. The table shows protection time (h) against seizures produced by 4-aminopyridine

Diazepam (10mg/kg) Phenytoin (30mg/kg)		4-aminopyridine induced convulsion model (13.3mg/kg)		
		Protection time (h)		
S. No.	Derivatives (30mg/kg)	0.5 h	1 h	2 h
1	2	Proconvulsion	--------	---------
2	3	Proconvulsion	-------	---------
3	4	Proconvulsion	-------	---------
4	5	Proconvulsion	-------	---------
5	6	Proconvulsion	-------	---------
6	7	Proconvulsion	-------	---------
7	8	Proconvulsion	-------	---------
8	9	Proconvulsion	-------	---------
9	Diazepam	--------	--------	---------
10	Phenytoin	30mg	30mg	30mg

Dose of 30mg kg^{-1} was administered *i.p.* Test compounds were suspended in polyethylene glycol (PEG). Figures in the table indicate the minimum dose with bioactivity demonstrated in half or more of the mice. The dash (–) indicates the absence of activity.

Table4. The table shows result of neurotoxicity screening of tested compounds

Neurotoxicity Testing at 0.5h & 4h		Neurotoxicity screening using rotorod model (No. of neurotoxic mice / No. of mice tested)	
S. No.	Derivatives (30mg/kg)	0.5 h	4 h
1	2	(0/6)	(0/6)
2	3	(0/6)	(0/6)
3	4	(0/6)	(0/6)
4	5	(0/6)	(0/6)
5	6	(0/6)	(0/6)
6	7	(0/6)	(0/6)
7	8	(0/6)	(0/6)
8	9	(0/6)	(0/6)

Results &Discussion

All the synthesized derivatives were evaluated at the dose of 30mg/kg body weight & have shown good anticonvulsant activity & the compounds 3, 6 & 7 (table 1) were found to be most active amongst all the screened compounds using isoniazid induced model, 2 & 6 (table 2) against thiosemicarbazide induced model & all the tested compounds were found to potentiate convulsions (table3) produced by 4-Aminopyridine, respectively. Activity of the drugs interfering with motor coordination was checked by the rotorod test. None of the compound was found to be neurotoxic (table 4) at a dose of 30mg/kg body weight amongst all the tested compounds.The sedation properties of synthesized derivatives were investigated using Phenobarbitone induced sleeping time. The compounds 6 & 8 (table 5) were found to cause sedation.

Table5. The table shows sedative property of tested compounds

Sedative-Hypnotic testing		Sedative- hypnotic screening using Phenobarbitone induced sleeping time model	
S. No.	Derivatives (30mg/kg)	Sleeping time (Mean ± SEM)	
1	2	65±9.00	NS
2	3	68±12.6	NS
3	4	59±10.53	NS
4	5	60±11.92	NS
5	6	141±11.21**	
6	7	63±9.05	NS
7	8	148±12.15**	
8	9	64±10.12	NS
9	Phenobarbitone	56±11.47	

Values represent the mean ± SEM of six animals for each *Significant at $p<0.05$, **Significant at $p<0.01$ (Dunnett's test),Test drug (30mg/kg), Phenobarbitone (40mg/kg), NS denotes not significant at $p<0.01$(Dunnett's test.

The results showed that most of the derivatives were active anticonvulsant against hydrazides used as convulsants & they share a common action namely, through facilitation of GABA synthesis which was prevented by the hydrazides via inhibition of glutamic acid decarboxylase. The derivatives were found to act as convulsants also when screened against 4-Aminopyridine producing convulsion via K+ channel antagonism. Phenyl ring was substituted with halo substituents e.g. Cl and Br, because they are known to increase anticonvulsant activity. Between Cl & Br substituents, bromo series compounds were found to be more active than chloro series.

Conclusion

The research concludes that the drugs acting as anticonvulsant via GABA receptor could also potentiate the convulsion through K^+ channels. The present studies revealed that indole derivatives could be used to synthesize the compounds having potent anticonvulsant activity with different mechanisms of actions.

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