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Synthesis & Anticonvulsant Activity (Chemo Shock) of Schiff and Mannich bases of Isatin derivatives with 2-Amino pyridine (Mechanism of Action)

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Abstract: The treatment of 2-amino pyridine **(1)** with isatine **(2)** affords (3*Z*)-3-(pyridin-2-ylimino)-1,3-dihydro-2*H*-indol-2-one **(3)** & then treatment with formaldehyde along with secondary amine, various N-substituted of (3*Z*)-3-(pyridin-2-ylimino)-1,3-dihydro-2*H*-indol-2-one (Schiff base) in the presence of glacial acetic acid results Mannich bases **(4, 5, 6, 7, 8)**. (3*Z*)-3-(pyridin-2-ylimino)-1,3-dihydro-2*H*-indol-2-one **(3)** were synthesized by refluxing **(1,2)** in glacial acetic acid.The Mannich bases **(4, 5, 6, 7, 8)** of above condensed products were prepared by refluxing them with various secondary amines i.e., dimethylamine, diethylamine, piperazine, isopropyle amine , morpholine & with formaldehyde in the presence of glacial acetic acid in scheme 1. In scheme 2 the compound **(2)** was brominated and condensed with substituted pyridine **(1)** to give Schiff base (5-bromo-1*H*-indole-2,3-dione**)(9)** & also Mannich bases of **(9)** were synthesized**(11,12,13,14,15)** with secondary amines, in glacial acetic acid. The synthesized compounds were characterized by IR, NMR & elemental analysis. These compounds were screened for their anticonvulsant activity using different chemical induced convulsion models such as isoniazid, thiosemicarbazide & 4-aminopyridine respectively. The derivatives were also evaluated for their neurotoxicity. The result showed that some of the pyridine containing compounds were very much active against different chemo-induced convulsion models, proving their different mode of actions in the course of epileptic seizures.

Keywords: (2-amino pyridine, piperazine, isopropyl amine, morpholine, Analgesic activity**,** Isoniazid, Thiosemicarbazide, 4- aminopyridine, Anticonvulsant activity).

INTRODUCTION:

Epilepsy is a chronic neurological condition that are caused by abnormal cerebral nerve cell activity, which is characterized by recurrent seizures. The treatment of seizure are always a challenge for researchers and clinical practitioners. Several new drugs have been introduced for the treatment of epilepsy in last decades.[1] Yet about 30% of patients with epilepsy are resistant to current pharmacotherapies and many of the available antiepileptic drugs. Based on this reason, there has been a continuous attempt to find new antiepileptic drugs which increases the demand to conduct more studies in this field. There is no doubt that epilepsy

disease affects approximately 1% of the population. Around 75-80% of epileptic patients may be provided with adequate seizure control with the help of conventional antiepileptic drugs. The therapeutic failure in 20-25% of patients has stimulated intensive research on novel antiepileptic drugs.[2] All clinically effective antipsychotics (except Clozapine like) have potent post synaptic dopaminergic D2 receptor blocking action and antipsychotic potency has shown good Correlation with their capacity to bind to D2 receptor. Blockade of dopamine action in corpus striatum is responsible for the extrapyramidal symptoms (EPS) so often associated with antipsychotic drugs. In addition to dopaminergic receptor blockade, some (atypical) antipsychotics like resperidone and clozapine also block 5HT system, which helps to lessen EP reactions and is related to their usefulness in improving negative symptoms.[3] Animal models of epilepsy are most often used to investigate fundamental neuronal mechanisms of seizure as well as mechanism of potential agents. These models basically used for estimating or testing the efficacy of new antiepileptic drugs or other novel therapeutic interventions. Among various type of models, acute seizure model using chemo-convulsants is one of the most common type of model which were used effectively by early researchers and are still widely used today for studying seizure–related phenomenon. Animal models that used chemo-convulsants with specific, known neurotransmitter interactions were used to identify potential therapeutic agents. The mechanisms by which these convulsants produced seizures were thought to be important for the interpretation of a compound's mechanism of action to inhibit seizures. Some common chemo-convulsants include camphor, strychnine, isoniazid etc.[4][5] A chemical model of epilepsy is based on the application of, or withdrawal from, chemical substances with concequence appearance of epileptic sympatomatology.[6] Based on these above practical demand, in this research we try to testing chemically-induced acute seizure using isoniazid, thiosemicarbazide, then apply these model in basically testing anticonvulsive activity of different Schiff bases & Mannich bases.[7] The compound is regarded as a GABA-synthesis inhibitor. INH lowers GABA level and the activity of glutamate decarboxylase (GAD). Thiosemicarbazide can precipitate convulsions by inhibiting the GABA synthesis via cofactor antagonism through impairment of the synthesis or coenzyme action of pyridoxal phosphate [8,9,10,11,12]. The K^+ channel antagonist 4-a minopyridine 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 [13,14,15,16]. 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 act ion at different receptors or ion channels.

MATERIALS AND METHODS:

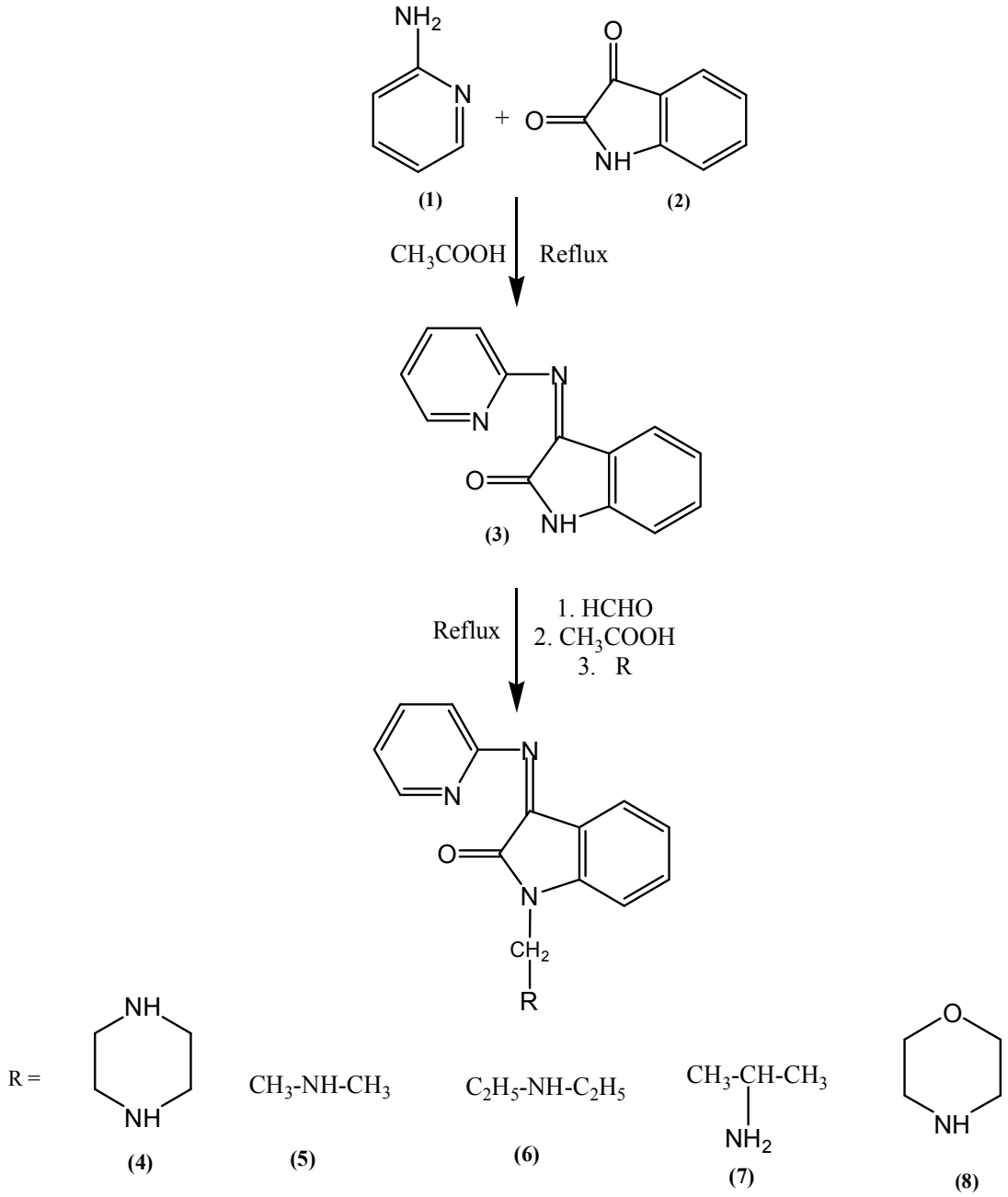
CHEMISTRY

The target compounds were synthesized as outlined in scheme-1 and scheme-2. The starting material Pyridine-2-amine**(1)** and 1H-indole-2,3-dione**(2)** were dissolved in acetic acid, and allowed to reflux(3hr.) to produce (3Z)-3-(pyridin-2-ylimino)-1,3-dihydro-2H-indol-2-one **(3)**, a Schiff base. The compounds **4,5,6,7,8** were synthesized by refluxing (3Z)-3-(pyridin-2-ylimino)-1,3-dihydro-2H-indol-2-one with piperazine, dimethyl amine, diethyl amine, isopropyl amine and morpholine respectively in acetic acid,(scheme1). The compound 5-bromo-1H-indole-2,3-dione**(9)** synthesized by bromination of compound **(2)** and the Schiff base (3Z)-5-bromo-3-(pyridin-2-ylimino)-1,3-dihydro-2H-indol-2-one **(10)** was synthesized by condensing with compound **(1).** The compounds **11, 12, 13, 14,** and **15** were synthesized by condensation of above mentioned amines respectively in presence of formaldehyde, (scheme 2).

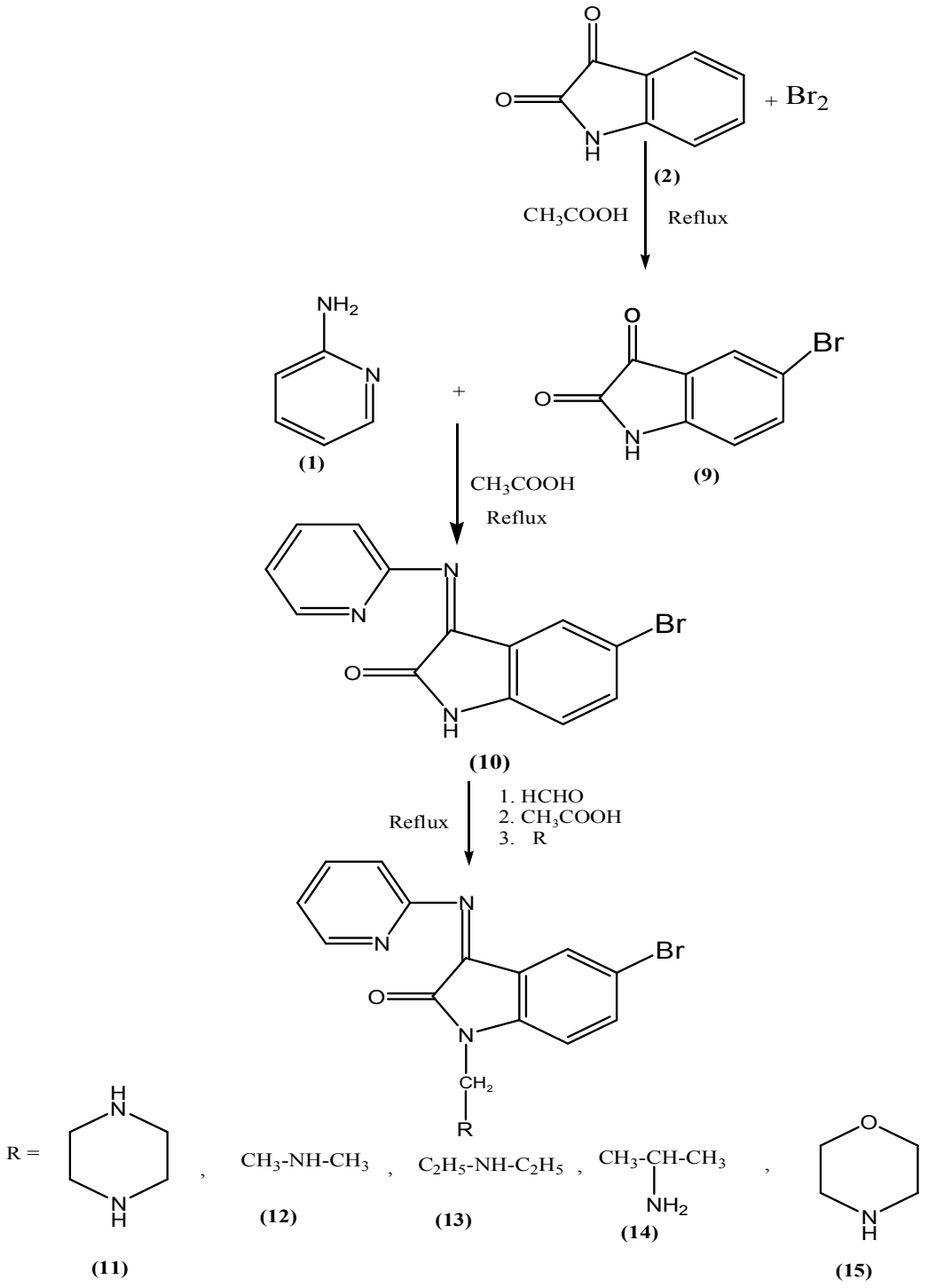
EXPERIMENTAL

All melting points are in degree centigrade and were determined on Jindal melting point apparatus and were uncorrected. The reactions were monitored and the purity of the products was checked by Thin Layer Chromatography (TLC) using Chloroform: Methanol (4:1) as solvent system. The IR spectra were recorded (KBr) on Jasco FT-IR 6100 spectro-photometer. ^{1}H NMR were recorded on Bruker Avauce II 300MHz NMR spectrometer using DMSO as a solvent. Tetramethylsilane serves as internal standard in ^{1}H NMR. Elemental analysis was done on Vario EL-III analyser.

SYNTHETIC SCHEME



Scheme-1



(Scheme-2)

SYNTHESIS OF (3Z)-3-(PYRIDIN-2-YLIMINO)-1,3-DIHYDRO-2H-INDOL-2-ONE (3)

The equimolar amount of 2-amino pyridine (0.940gm: 01 mol) & 1H-indole-2,3-dione (0.470gm: 01mol.) were taken in separate beakers and dissolved completely in acetic acid (qs). These solutions were taken in a round bottom flask. The reaction mixture was refluxed for 3 hr. on heating mental with occasional shaking. After completion of reaction, the product was then filtered and washed with water. The completion of reaction was monitored by TLC. The product was dried at room temperature & finally recrystallised with ethanol.

SYNTHESIS OF MANNICH BASES WITH DIFFERENT SEC. AMINES (4,5,6,7,8)

The equimolar amount (0.01 mol.) of (3Z)-3-(pyridin-2-ylimino)-1,3-dihydro-2H-indol-2-one and formaldehyde were taken in round bottom flask along with acetic acid and dissolved properly. Then different secondary amines were taken (for the synthesis of 4,5,6,7,8) in acetic acid. Then these reaction mixtures with different sec. amines, were refluxed for 3-4 hr. (R= Piperazine for (CP-4),dimethyle amine for (CP-5), diethylamine for (CP-6), morpholine for (CP-7), isopropylamine for (CP-8) were used respectively). After completion of reaction, the products were then filtered and recrystallised with ethanol and dried . The TLC was performed on silica gel 60 using chloroform: methanol (4:1) as a solvent system.

SYNTHESIS OF 5-BROMO-1H-INDOLE-2,3-DIONE (9)

Eequimolar amount of 1H-indole-2,3-dione (5 gm., 0.01 mol.) & bromine was taken in round bottom flask along with acetic acid. The temperature was maintained up to 0-5^{0}C on ice bath throughout the reaction. This condition was maintained about 1 hr. After completion of reaction, the reaction mixture was poured in cold water & filtered, and washed with distilled water. The product was then dried at room temperature and its TLC was performed by using chloroform : methanol (4:1) as mobile phase.

SYNTHESIS OF (3Z)-5-BROMO-3-(PYRIDIN-2-YLIMINO)-1,3-DIHYDRO-2H-INDOL-2-ONE (10)

The equimolar amount of 2-amino pyridine &5-bromo-1H-indole-2,3-dione**(CP-9)** was taken in acetic acid in a round bottom flask and dissolved properly. This reaction mixture was refluxed for about 3-4 hr. After completion of reaction these mixture was poured in distilled water and filtered then washed properly. The completion of reaction was monitored by TLC analysis for several times in chloroform: methanol (4:1).

SYNTHESIS OF VARIOUS MANNICH BASES OF (3Z)-5-BROMO-3-(PYRIDIN-2-YLIMINO)-1,3DIHYDRO-2H-INDOL-2-ONE :(11,12,13,14,15.)

The equimolar amount of (3Z)-5-bromo-3-(pyridin-2-ylimino)-1,3-dihydro-2H-indol-2-one**(CP-10)** (0.2gm, .01 mol) different secondary amines [R= Piperazine for (CP-11),dimethyle amine for (CP-12), diethylamine for (CP-13), morpholine for (CP-14), isopropylamine for (CP-15)] were taken in around bottom and dissolved in acetic acid. A small quantity of formaldehyde was added to this reaction mixture. These reaction mixtures were refluxed for 3-4 hr. on heating mental. The completion of reaction was monitored by TLC analysis for several times in chloroform: methanol (4:1). Then product was evaporated on water bath and dried.

PHYSICO-CHEMICAL PROPERTIES

S.N.	Compound code	Molecular formula	Molecular weight	m.p. (°C)	Rf-value	Solubility in DMSO
1.	**3**	$C_{13}H_9N_3O$	223.23	178-180	0.82	Soluble
2.	**4**	$C_{18}H_{19}N_5O$	321.38	118-120	0.85	Soluble
3.	**5**	$C_{16}H_{16}N_4O$	280.32	72-74	0.76	Soluble
4.	**6**	$C_{18}H_{20}N_4O$	308.38	118-120	0.80	Soluble
5.	**7**	$C_{18}H_{18}N_4O_2$	322.36	70-72	0.78	Soluble
6.	**8**	$C_{17}H_{18}N_4O$	294.35	84-86	0.70	Soluble
7.	**9**	$C_8H_4BrNO_2$	226.03	222-224	0.73	Soluble
8.	**10**	$C_{13}H_8BrN_3O$	302.13	184-186	0.81	Soluble
9.	**11**	$C_{18}H_{18}BrN_5O$	400.27	128-130	0.85	Soluble
10.	**12**	$C_{16}H_{15}BrN_4O$	359.22	75-77	0.82	Soluble
11.	**13**	$C_{18}H_{19}BrN_4O$	387.27	79-81	0.84	Soluble
12	**14**	$C_{18}H_{17}BrN_4O_2$	401.26	60-62	0.84	Soluble
13.	**15**	$C_{17}H_{17}BrN_4O$	373.25	80-82	0.87	Soluble

ELEMENTAL ANALYSIS

S.N.	Compound code	Elemental analysis	
		Calculated	found
1.	**3**	C(69.95),H(4.06),N(18.82).	C(69.90),H(4.00),N(18.0).
2.	**4**	C(67.27),H(5.96),N(21.79).	C(67.20),H(5.90),N(21.70).
3.	**5**	C(68.55),H(5.75),N(19.99).	C(68.50),H(5.70),N(19.92).
4.	**6**	C(70.11),H(6.54),N(18.17).	C(70.15),H(6.62),N(18.11).
5.	**7**	C(67.07),H(5.63),N(17.38).	C(67.10),H(5.62),N(17.31).
6.	**8**	C(69.37),H(6.16),N(19.03).	C(69.35),H(6.15),N(19.10).
7.	**9**	C(42.51),H(1.78),N(6.20).	C(42.49),H(1.76),N(6.25).
8.	**10**	C(51.68),H(2.67),N(13.91).	C(51.66),H(2.65),N(13.92).
9.	**11**	C(54.01),H(4.53),N(17.50).	C(54.00),H(4.59),N(17.48).
10.	**12**	C(53.50),H(4.21),N(15.60).	C(53.42),H(4.19),N(15.58).
11.	**13**	C(55.82),H(4.95),N(14.47).	C(55.80),H(4.93),N(14.49).
12.	**14**	C(53.88),H(4.22),N(13.96).	C(53.76),H(4.20),N(13.99).
13.	**15**	C(54.70),H(4.59),N(15.01).	C(54.64),H(4.57),N(15.10).

SPECTRAL ANALYSIS

S.N.	Comp. code	IR Spectra	NMR Spectra
1.	3	3038(C-H), 1697(C=O), 1604(C=N), 1580(C=C).	7.0-7.5(4H,m,Ar.),7.8-8.0(4H,m,Pyri.), 9.5(1H,s,NH).
2.	4	3038(C-H), 1697(C=O), 1604(C=N), 1580(C=C).	7.0-7.5(4H, m, Ar.), 7.8-8.0 (4H,m,Pyri.), 4.03 (2H,s,-CH_2), 2.4-3.0 (8H,t,Piperi.), 9.5 (1H,s,-NH).
3.	5	3037(CH), 1696(C=O), 1605(C=N),1340(>N-)	7.0-7.5(4H, m, Ar.),7.8-8.0 (4H, m, Pyri.), 4.03(2H,s,-CH_2),2.2-2.4(6H,t,-CH_3).
4.	6	3038(C-H), 1695(C=O), 1605(C=N),1342(>N-)	7.0-7.5(4H, m, Ar.), 7.8-8.0 (4H, m, Pyri.), 4.03 (2H,s,-CH_2),2.2-2.4(4H,q,-CH_2),1.0-1.2(6H,t,-CH_3).
5.	7	3039(C-H), 1698(C=O), 1280(-O-)1605(C=N)	7.0-7.5(4H, m, Ar.), 7.8-8.0 (4H,m, Pyri.), 4.03 (2H,s,-CH_2),2.4-2.6(8H,t,-CH_2).
6.	8	1310(NH), 1697(C=O), 1605(C=N)	7.0-7.5(4H, m, Ar.), 7.8-8.0 (4H, m, Pyri.), 4.03 (2H,s,-CH_2), 9.4(1H,s,-NH), 3.8(1H,s,-CH), 1.0-1.2(6H,t,-CH_3).
7.	10	1710(C=O), 1560(>N-H),660(C-Br)	7.5-7.8(3H,m,Ar.),7.4-8.0(4H,m,Pyri.),9.5(1H,s,-NH).
8.	12	1705(C=O),3037(C-H), 1610(C=N),1335(>N-)	7.5-7.8(3H,m,Ar.),7.4-8.0 (4H,m,Pyri.), 4.03(2H,s,-CH_2-),1.0-1.2(6H, t,-CH_3).
9.	13	1703(C=O),3040(C-H), 1610(C=N),1337(>N-)	7.5-7.8(3H,m,Ar.), 7.48.0(4H,m,Pyri.), 4.03(2H,s,-CH_2-),2.4(4H,q,-CH_2),1.0-1.2(6H,t,-CH_3).
10.	14	3036(C-H), 1698(C=O),1280(-O-) 1585(C=C), 1600(C=N)	7.5-7.8(3H,m,Ar.), 7.48.0(4H,m,Pyri.), 4.03(2H,s,-CH_2-),2.4-3.8(8H,m,Morpho.).

ANTICONVULSANT ACTIVITY

All the compounds were screened for anticonvulsant activities adopting the anticonvulsant drug development (ADD) program protocol. The investigations were conducted on Swiss albino mice of either sex (22-25gm). Food and water were withdrawn prior to the experiments. 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 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.[17]

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 dose at which the animals unable to grasp the rotorod, was determined.

RESULTS & DISCUSSION

All the synthesized derivatives were evaluated at the dose of 30mg/kg body weight & have shown good anticonvulsant activity. The compounds 3, 4 & 10, 15 (table 1) were found to be most active amongst all the screened compounds using isoniazid induced model, as well as against thiosemicarbazide induced model. All the tested compounds were found to potentiate convulsions (table2) 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 2) at a dose of 30mg/kg body weight amongst all the tested compounds. On the basis of above results, 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. All 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. Br, because they are known to increase anticonvulsant activity.

Table 1. The table shows the result of anticonvulsant screening using chemical induced convulsion models:-

Compound Code	**Isoniazid induced**(300mg/kg)			**Thiosemicarbazide induced**(20mg/kg)		
	0.5 hr	1.0 hr	2.0 hr	0.5 hr	1.0 hr	2.0 hr
Control	Protected	Protected	Protected	Protected	Protected	Protected
3	30	30	NP	30	30	30
4	30	30	NP	30	30	30
5	30	30	NP	30	NP	NP
6	30	30	NP	30	30	NP
7	30	NP	NP	30	30	NP
8	30	NP	NP	30	NP	NP
9	30	30	NP	30	30	NP
10	30	30	30	30	30	30
11	30	30	NP	30	30	NP
12	30	NP	NP	30	NP	NP
13	30	30	NP	30	30	NP
14	30	NP	NP	30	30	NP
15	30	NP	NP	30	30	30
Diazepam Phenytoin	10mg NP	10mg NP	10mg NP	10mg NP	10mg NP	10mg NP

Dose of 30mg/kg. body wt. 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. "NP" denots not protected at 30 mg. dose.

Table 2. Anticonvulsant activity & Neurotoxicity testing:

Compound code	4-aminopyridine induced (13.3mg/kg)	Neurotoxicity testing (30mg/kg)	
	0.5 hr	0.5 hr	4.0 hr
Control	Protected	NN	NN
3	Proconv.	NN	NN
4	Proconv.	NN	NN
5	Proconv.	NN	NN
6	Proconv.	NN	NN
7	Proconv.	NN	NN
8	Proconv.	NN	NN
9	Proconv.	NN	NN
10	Proconv.	NN	NN
11	Proconv.	NN	NN
12	Proconv.	NN	NN
13	Proconv.	NN	NN
14	Proconv.	NN	NN
15	Proconv.	NN	NT
Diazepam	Proconv.	NN	NN
Phenytoin	30 mg	NN	NN

Dose of 30mg/kg. body wt. 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. "NP" denots not protected at 30 mg. dose.
"NN"=Non Neurotoxic up to the dose 30 mg/kg b.wt. "NT"= Neurotoxic.

CONCLUSION

The research work concludes that the drugs acting as anticonvulsant via GABA receptor could also potentiate the convulsion through K^+ channels. The present studies revealed that pyridine containing compounds could be used to synthesize the potent anticonvulsant activity with different mechanisms of actions & these compounds will be fruitful in the synthesis of antiepileptic agents.

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