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SYNTHESIS AND SCREENING OF BIOLOGICALLY SIGNIFICANT INDOLE DERIVATIVES FOR ANTICONVULSANT ACTIVITY

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ABSTRACT

*In the present work, some new 5-[2(3)-dialkylamino alkoxy] Indole 2, 3-diones and **5**-Hydroxyindole 3-semicarbazone 2-ones were prepared from 5-hydroxy isatin. The structures of the products were characterized by IR, NMR, MASS Spectral studies. All the compounds were examined for anticonvulsant activity by maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) induced convulsion method. These compounds were also evaluated for their neurotoxicity study by rotorod method. Some of these compounds showed good anticonvulsant activity when compared with standard drug Phenytoin and all the compounds showed less neurotoxicity when compared with standard drug Diazepam.*

KEY WORDS

Synthesis, 5-[2(3)-dialkyl amino alkoxy] Indole 2, 3-diones, 5-Hydroxyindole 3-semicarbazone 2-ones, Anticonvulsant activity.

1. INTRODUCTION

Epilepsy, one of the common neurological disorders, is a major public health problem, affecting around 4% of individuals over their lifespan. About 20-30% of the epilepsy patients are resistant to the available medical therapies. This fact warrants the investigation for new antiepileptic drugs.

Isatin is an endogenous compound isolated in 1988 and reported [1] to possess a wide range of central nervous system activities. Surendranath pandya [2] et al. reported the synthesis and anticonvulsant activity of some novel n-methyl/acetyl, 5-(un)-substituted isatin-3-semicarbazones. In the last few years, Isatin derivatives have been discovered which show potential hypnotic [3], antibacterial [4-6] and MAO inhibitory [7] activity.

It is evident from the literature survey that Isatin derivatives, isatin semicarbazone derivatives and dialkylamino alkyl derivatives showing more promising central nervous system and anticonvulsant activities. Keeping in view of these two molecular moieties viz., 5-hydroxy isatin (Resembles serotonin) and dialkylamino alkyl (Resembles NT), it is our endeavor to bring such important moieties into a single molecular frame as a model for molecular conjunction by appropriate synthetic routes and to screen them for anticonvulsant activity and neurotoxicity.

2. MATERIALS AND METHODS

The compounds were mostly synthesized by conventional methods and described in experimental selection and also by the methods established in our laboratory.

2.1. Chemicals

Leptazole, Diazepam, Dialkylaminoalkylhalides, semicarbazidehydrochloride purchased from Sigma- Aldrich Chemicals Private Limited, Hyderabad, India. p-amino phenol, hydroxylamine hydrochloride, sodium sulfate were purchased from Merck Chemicals Private Limited, Hyderabad, India.

2.2. Chemistry

Solvents were dried or distilled before use. Melting points were obtained on a Thoshniwall melting point apparatus in open capillary tubes and are uncorrected. The purity of the compounds were ascertained by TLC on silica gel –G plates(Merck).Infrared spectra(IR) were recorded with KBR pellet on a Perkin-Elmer BX series, Infrared spectrophotometer. Mass spectra were recorded by the direct inlet method on Thadmam-mass-quantam API 400H mass spectrophotometer.^{1}H NMR spectra were recorded on Brucker spectrospin 400 MHz spectrophotometer in DMSO-d_6. 5-hydroxy Isatin was synthesized from p- amino phenol by using Sandmayer[8] method It consists in the reaction of aniline with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate to form an isonitrosoacetanilide, which after isolation, when treated with concentrated sulfuric acid, furnishes isatin in >75% overall yield.

2.3. Preparation of 5-Hydroxyindole 3-semicarbazone 2-one

5-Hydroxyisatin was heated under reflux in methanol containing two or three drops of acetic acid with semicarbazide hydrochloride for half an hour. The product thus separated was filtered and purified by recrystalization from suitable solvent. (Yield 89%, m.p.270^{o}c)

2.4 Preparation of 5-[2(3)-dialkyl amino alkoxy] Indole 2,3 diones and 5-Hydroxyindole 3-semicarbazone 2-ones

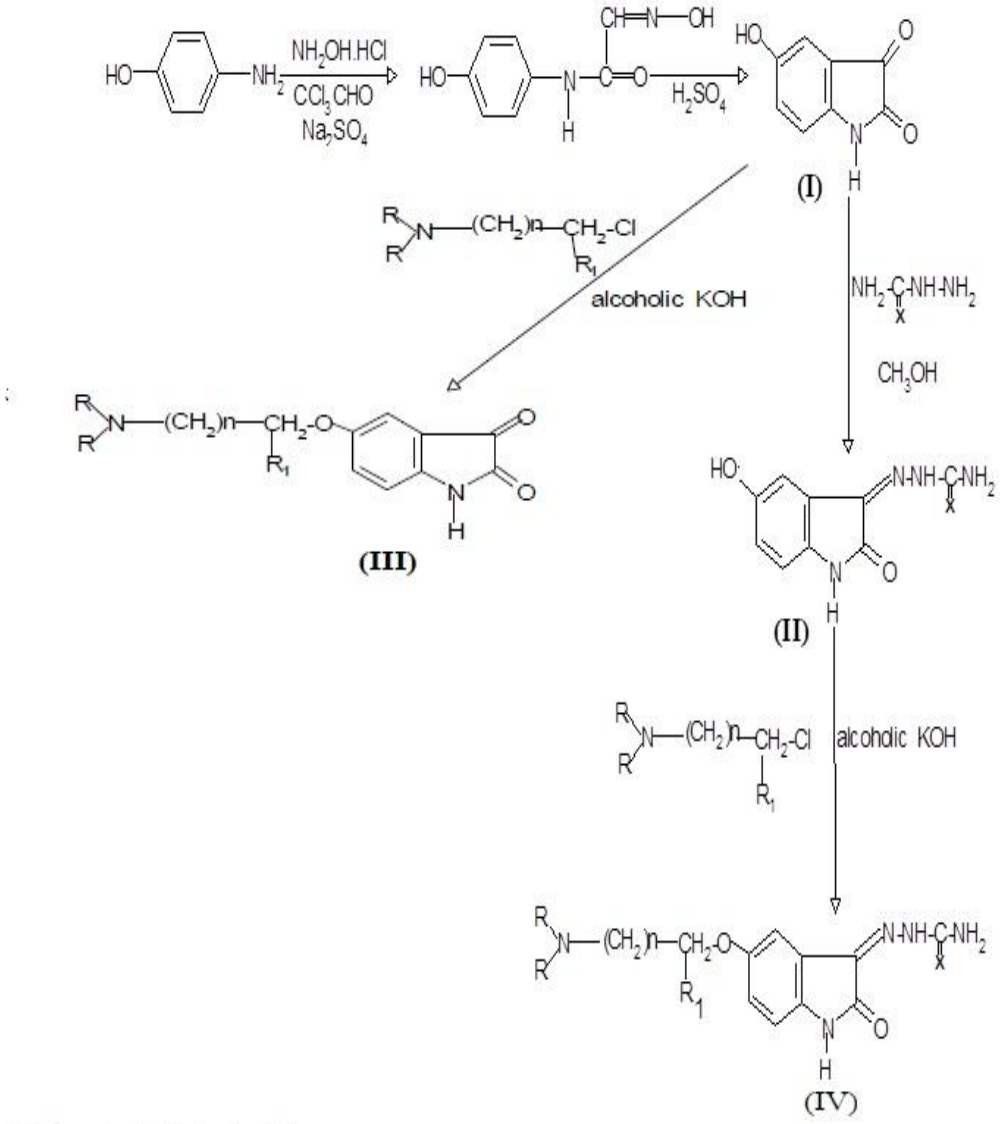
A mixture of 5-hydroxyisatin/5-Hydroxyisatin-3-semicarbazone (0.01 Moles) and dialkylamino alkylhalide (0.01 Moles) placed in 10% alcoholic potassium hydroxide and this mixture was stirred at room temperature for 6 hours .The alcohol was reduced to half of its volume and cooled. The product separated was filtered, washed with small portions of cold alcohol repeatedly and dried .It was purified by recrystalisation from hydro alcoholic mixtures to get a crystalline solid. Similarly other 5-Hydroxy Isatin derivatives as shown in **Scheme 1** were prepared and their melting points were determined in Open capillary tubes using Toshniwall melting point apparatus and are uncorrected. Purity of the compounds was checked by TLC. The physical data of the title compounds were presented in **Table 1**. The compounds were characterized by spectral data.

2.5. Spectral data

The compounds have been characterized by the spectral data IR, PMR and Mass.

IR spectrum (KBr) of compound **(III)** exhibited absorption bands (cm-1) 3421.47 (OH), 1630.08 (C = O), 1548 (Ar,C=C), 1282(C-O-C), 883.85-579.8 (Ar). ^{1}H NMR (300 MHz, DMSO-d_6): 13.3 (s, 1H, OH), 10.36(s, 1H,-CONH), 6.65-7.29(m, 3 H, Ar-H). Mass spectrum of compound III showed molecular ion (M+) base peak at m/z (164.1).

Compound (**IIIa)** showed characteristic IR peaks at 3276(NH), 1651.96 (C=O), 1569.82 (Ar, C=C), 1276(C-O-C), 807.93(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.36(s, 1H,-CONH), 7.01-7.29(m,3 H,Ar-H),3.2 (T,2H,O-CH_2 s) ,2.9 (T,2H,N-CH_2), 1.36 (S,6H,N-$(CH_3)_2$).Mass spectrum of compound **IIIa** showed molecular ion (M+) base peak at m/z 231 (100%).It also shows peak at m/z (71) may be due to the fragmentation of the alkyl chain from the molecule ion.



5-Hydroxyisatin derivatives(III)

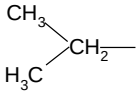
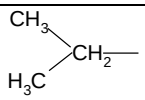
IIIa R= CH3 ; R1 = H; n=1
IIIb R=C2H5; R1= H; n=1
IIIc R= CH3; R1= H; n=2
IIId R= CH3; R1= CH3; n=1
IIIe R=CH3-CH- CH3; R1= H; n=1

5-Hydroxyisatin-3-semicarbazone(x=o) derivatives(IV)

IVa R= CH3 ; R1= H; n=1
IVb R=C2H5; R1= H; n=1
IVc R= CH3 ; R1= H; n=2
IVd R= CH3 ; R1=CH3; n=1
IVe R=CH3-CH- CH3 ; R1= H; n=1

Scheme 1: Synthetic protocol of the title compounds.

Table I: Physical data of 5-[2(3)-dialkyl amino alkoxy] Indole 2,3 diones and 5-Hydroxyindole semicarbazone 2- ones

S.No	Compound	R	R_1	N	X	M.F	% YEILD	M.P	M.Wt
1	IIIa	CH_3	H	1	O	$C_{12}H_{14}N_2O_3$	91%	<320	234
2	IIIb	C_2H_5	H	1	O	$C_{14}H_{18}N_2O_3$	86%	<320	262
3	IIIc	CH_3	H	2	O	$C_{13}H_{16}N_2O_3$	93%	<320	248
4	IIId	CH_3	CH_3	1	O	$C_{13}H_{16}N_2O_3$	85%	<320	248
5	IIIe		H	1	O	$C_{16}H_{24}N_2O_3$	81.8%	<320	292
6	IVa	CH_3	H	1	$NNHCONH_2$	$C_{13}H_{17}N_5O_3$	92%	<320	291
7	IVb	C_2H_5	H	1	$NNHCONH_2$	$C_{15}H_{21}N_5O_3$	83%	<320	319
8	IVc	CH_3	H	2	$NNHCONH_2$	$C_{14}H_{19}N_5O_3$	92%	<320	365
9	IVd	CH_3	CH_3	1	$NNHCONH_2$	$C_{14}H_{19}N_5O_3$	86%	<320	365
10	IVe		H	1	$NNHCONH_2$	$C_{17}H_{27}N_5O_3$	82%	<320	349

Compound (**IIIb**) showed characteristic IR peaks at 3274(NH), 1681.53 (C=O), 1570.21 (Ar ,C=C), 1243(C-O-C), 845.51(Ar). ^{1}H NMR (300 MHz, DMSO-d_6): 10.25(s, 1H,-CONH), 7.03-7.45(m,3 H,Ar-H),2.99 (T,2H,O-CH_2 s) ,2.72 (T,2H,N-CH_2) ,1.24 (S,10H,N-$(C_2H_5)_2$).

Mass spectrum of compound **IIIb** showed molecular ion (M+) base peak at m/z 263 (100%).It also shows peak at m/z (71) may be due to the fragmentation of the alkyl chain from the molecule ion.

Compound (**IIIc**) showed characteristic IR peaks at 3274(NH), 1651.96 (C=O), 1579.72 (Ar, C=C), 1266(C-O-C), 805.91(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.46(s, 1H,-CONH), 7.21-7.49(m,3 H,Ar-H),2.84 (T,2H,O-CH_2) , 2.51 (M,2H, CH_2),2.48 (T,2H,N-CH_2), 1.25 (S,6H,N-$(CH_3)_2$).Mass spectrum of compound **IIIc** showed molecular ion (M+) base peak at m/z 247 (100%).

Compound (**IIId**) showed characteristic IR peaks at 3257(NH), 1679.64 (C=O), 1546.86 (Ar, C=C), 1245(C-O-C), 812.71(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.51(s, 1H,-CONH), 7.12-7.42(M,3 H,Ar-H),2.76 (M,2H,O-CH_2) , 2.45 (T,3H, R_1=CH_3),2.31 (M,1H,N-CH), 1.44 (S,6H,N-$(CH_3)_2$).Mass spectrum of compound **IIId** showed molecular ion (M+) base peak at m/z 247 (100%).

Compound (**IIIe**) showed characteristic IR peaks at 3257(NH), 1689.46 (C=O), 1576.34 (Ar, C=C), 1228(C-O-C), 814.53(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.26(s, 1H,-CONH), 7.34-7.51(m,3 H,Ar-H),2.96 (T,2H,O-CH_2 s) ,2.82 (T,2H,N-CH_2), 1.35 (S, 2H,N-CH) ,1.21 (D,12H,Csss -$(CH_3)_2$).Mass spectrum of compound **IIIe** showed molecular ion (M+) base peak at m/z 291 (100%).It also shows peak at m/z (71) may be due to the fragmentation of the alkyl chain from the molecule ion.

Compound (**IVa**) showed characteristic IR peaks at 3276(NH), 1651.96 (C=O), 1569.82 (Ar, C=C), 1276(C-O-C), 807.93(Ar). ^{1}H NMR (300 MHz, DMSO-d_6): 10.36(s, 1H,-CONH), 7.01-7.29(m,3 H,Ar-H),3.2 (T,2H,O-CH_2 s) ,2.9 (T,2H,N-CH_2), 1.36 (S,6H,N-$(CH_3)_2$).Mass spectrum of compound **IVa** showed molecular ion (M+) base peak at m/z 291 (100%). The mass spectrum shows its base peak at m/z 77 (100%) may be due to the fragmentation of the semicarbazone from the molecule ion.

Compound (**IVb)** showed characteristic IR peaks at 3274(NH), 1681.53 (C=O), 1570.21 (Ar ,C=C), 1243(C-O-C), 845.51(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.25(s, 1H,-CONH), 7.03-7.45(m,3 H,Ar-H),2.99 (T,2H,O-CH_2 s) ,2.72 (T,2H,N-CH_2) ,1.24 (S,10H,N-$(C_2H_5)_2$), 7.41-7.46(d,2H, NH_2) ,11.36(S,1H, NH).Mass spectrum of compound **IVb** showed molecular ion (M+) base peak at m/z 317 (100%). The mass spectrum shows its base peak at m/z 77 (100%) may be due to the fragmentation of the semicarbazone from the molecule ion.

Compound (**IVc)** showed characteristic IR peaks at 3274(NH), 1651.96 (C=O), 1579.72 (Ar, C=C), 1266(C-O-C), 805.91(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.46(s, 1H,-CONH), 7.21-7.49(m,3 H,Ar-H),2.84 (T,2H,O-CH_2) , 2.51 (M,2H, CH_2), 7.41-7.46(d,2H, NH_2) ,11.36(S,1H, NH),2.48 (T,2H,N-CH_2), 1.25 (S,6H,N-$(CH_3)_2$). Mass spectrum of compound **IVc**showed molecular ion (M+) base peak at m/z 363 (100%).

Compound (**IVd)** showed characteristic IR peaks at 3257(NH), 1679.64 (C=O), 1546.86(Ar ,C=C), 7.41-7.46(d,2H, NH_2) ,11.36(S,1H, NH) 1245(C-O-C), 812.71(Ar). ^{1}H NMR (300 MHz, DMSO-d_6): 10.51(s, 1H,-CONH), 7.12-7.42(M,3 H,Ar-H),2.76 (M,2H,O-CH_2) , 7.41-7.46(d,2H, NH_2) ,11.36(S,1H, NH), 2.45 (T,3H, R_1=CH_3),2.31 (M,1H,N-CH), 1.44 (S,6H,N-$(CH_3)_2$).Mass spectrum of compound **IVd** showed molecular ion (M+) base peak at m/z 363 (100%).

Compound (**IVe)** showed characteristic IR peaks at 3257(NH), 1689.46 (C=O), 1576.34 (Ar, C=C), 1228(C-O-C), 814.53(Ar). ^{1}H NMR (300 MHz, DMSO-d_6): 10.26(s, 1H,-CONH), 7.34-7.51(m,3 H,Ar-H),2.96 (T,2H,O-CH_2 s) , 7.41-7.46(d,2H, NH_2) ,11.36(S,1H, NH),2.82 (T,2H,N-CH_2), 1.35 (S, 2H,N-CH) ,1.21 (D,12H,Csss -$(CH_3)_2$).Mass spectrum of compound **IVe** showed molecular ion (M+) base peak at m/z 347 (100%). The mass spectrum shows its base peak at m/z 77 (100%) may be due to the fragmentation of the semicarbazone from the molecule ion.

3. Pharmacology

3.1. Anticonvulsant Activity

Materials: Normal saline, test compounds, Leptazole, stop watch, phenytoin.

Animals: Swiss mice.

3.1.1. Maximal electroshock seizure (MES) method [9]

Method: The anticonvulsant activity was studied by Maximal Electroshock Induced Convulsion method by using electro-convulsometer. Healthy male mice weighing between 20-25g were fasted for overnight and divided into groups of six animals each. The test compounds suspended in normal saline were administered at a dose of 100 mg/kg body weight i.p. The control group animals received only vehicle (Normal saline). The test started 30 min after i.p. injection. Maximal seizures were induced by the application of electrical current to the brain via corneal electrodes. The stimulus parameter for mice was 50 mA in a pulse of 60 Hz for 200 ms. Abolition of the hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity; results are presented in **Table 2**.

Table 2: Anticonvulsant activity and neurotoxicity study of 5-[2(3)-dialkylamino alkoxy] Indole 2,3-diones

S.No	Compound	Animals protected in %		Neurotoxicity (%)
		MES induced Convulsions	Chemically induced convulsions	
1	IIIa	65.54	60.9	7
2	IIIb	58.61	64.6	18
3	IIIc	48.42	56.6	6.7
4	IIId	41.5	49.8	3.4
5	IIIe	37.46	44.83	4.6
6	IVa	65.18	71.2	7
7	IVb	66.68	65.77	5
8	IVc	54.76	44.3	6.2
9	IVd	38.18	40.3	7.8
10	IVe	37.44	33.9	7
11	Phenytoin	100	89.36	-
12	Control	0	0	2
13	Diazepam	-	-	88

Number of animals n=6, The compounds were tested at adose of 100mg/kg (b.w)

3.1.2. Pentylenetetrazole (PTZ) method [10]

Materials: Normal saline, test compounds, Leptazole, stop watch, phenytoin.

Animals: Swiss mice.

Method: The anticonvulsant activity was studied by using Leptazole (Pentylene tetrazole) as a chemical convulsion inducer. Healthy male mice weighing between 20-25g were fasted for overnight and divided into groups of six animals each. The animals were injected with Leptazole (80mg/kg) given intraperitonially. Those animals which show convulsions were selected for the experiment. The test compounds suspended in normal saline were administered at a dose of 100 mg/kg body weight i.p. The control group animals received only vehicle (Normal saline). The Leptazole is again given in the same dose and the time taken for convulsions to start was noted, results are presented in **Table 2** and **Figure 1**.

3.1.3. Neurotoxicity study [11]

Healthy male mice weighing between 20-25gm were fasted for overnight and divided into groups of six animals each. Turn of the rotating rod, select an appropriate speed (25 rpm), and place the animal one by one on the rotating rod. A normal mouse (untreated) generally falls off within 3-5 min. Test compound dissolved in saline were administered, intraperitonially in a dose of 100 mg/kg. The control group received saline only. One group of animals was administered diazepam as a standard (i.p 4

mg/kg). After 30 minutes, repeated the experiment as done earlier noted the fall of time of animals before and after test compounds and diazepam treatment respectively, results are presented in **Table 2 & Figure 2**.

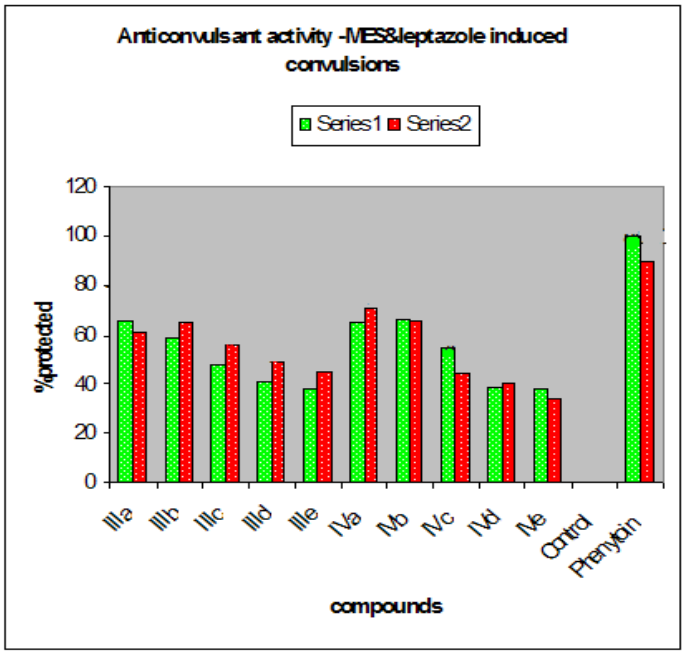


Figure 1: Anticonvulsant activity of 5-[2(3)-dialkylamino alkoxy] Indole 2, 3-dions and 5-Hydroxyindole 3-semicarbazone 2-ones.

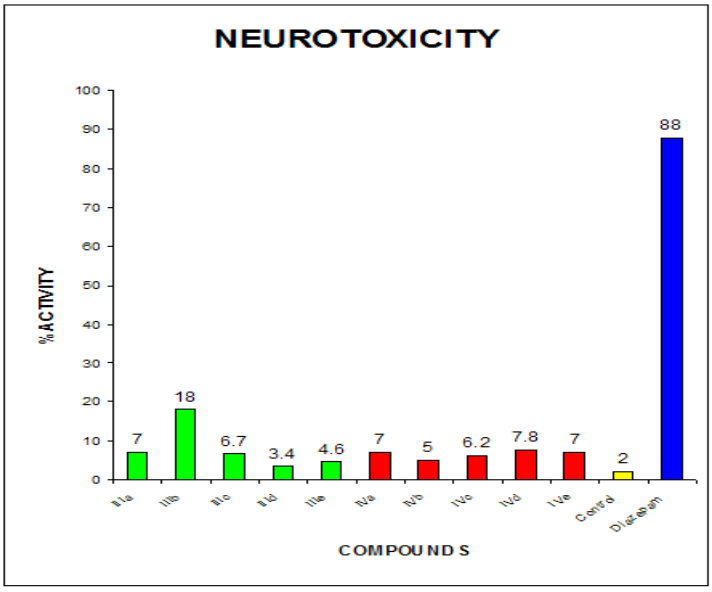


Figure 2: Neurotoxicity study of the 5-[2(3)-dialkylamino alkoxy] Indole 2, 3-diones and 5-Hydroxyindole 3- semicarbazone 2-ones

4. RESULTS AND DISCUSSIONS

Physical data TLC, IR, ^{1}H NMR and mass spectra confirmed the structures and purity of the synthesized compounds. All the title compounds decomposed before melting. All the synthesized compounds were evaluated for their in vivo anticonvulsant and skeletal muscle relaxant activity. It was observed that compounds **IVa, IIIa, IVb, IIIb,** exhibited more promising anticonvulsant activity. Among the test compounds **IIIc, IVc, IIId, IVd, IIIe, IVe** were found to be next in the order of reducing the duration of convulsions. Compounds with dimethyl amino and diethyl amino ethyloxy group at c5 of Isatin showed more protection against Maximal Electroshock Seizure (MES) induced convulsions where as compounds **IIIa, IIIb and IVa, IVb** exhibited more protection activity against chemically (Leptazole)induced convulsions. 5-[2(3)-dialkylamino alkoxy] isatin -3-semicarbazones showing more activity compared to 5-[2(3)-dialkylamino alkoxy] isatins .All the test compounds showed less (<20%) neurotoxicity (Skeletal muscle relaxant activity) when compared with Diazepam.

5. CONCLUSION

A new series of five 5-[2(3)-dialkyl amino alkoxy] Indole 2,3 dione derivatives were synthesized by reacting 5-hydroxyindole 2,3 dione with 2-N,N di alkylamino alkyl halides.Evaluation of these compounds as anticonvulsant and skeletal muscle relaxant activity revealed that the compounds **IVa**($R=CH_3$)**, IVb**($R=C_2H_5$)**, IIIa**($R=CH_3$) **and IIIb**($R=C_2H_5$) with a dimethyl and diethyl amino ethyl chain derivatives was found to be relatively superior in anticonvulsant activity and other compounds(**IIIc, IVc, IIId, IVd, IIIe, IVe**) are next in the order of activity.All the compounds showed less neurotoxicity compared to Diazepam.

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