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Anticonvulsant and Sedative-Hypnotic Activities of *N*-Acetyl / Methyl Isatin Derivatives

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Abstract

A series of *N*-methyl/acetyl 5-(un)-substituted isatin-3-semicarbazones were screened for anticonvulsant and sedative-hypnotic activities. The results revealed that protection was obtained in all the screens i.e., Maximal electroshock, (MES) subcutaneous pentylene tetrazole (scPTZ) and subcutaneous strychnine (scSTY) screens. Three compounds (**5a**, **5e** and **5i**) possessed anti-MES activity and all the compounds were less neurotoxic than phenytoin, carbamazepine and phenobarbital. All the compounds were completely non-toxic at 4h when compared to phenytoin, carbamazepine and phenobarbital, which were toxic at 100 and 300 mg/kg respectively. Compounds **5a**, **5b**, **5e**, **5g** and **5i** emerged as the active compounds in oral MES screen. Selected compounds were evaluated for quantification studies in MES, scPTZ and neurotoxicity screens after i. p (**5b**, **5i**) and oral administration (**5a**, **5g**) in rats. Among all the compounds **5a**, **5b** and **5g** emerged as broad-spectrum compounds as indicated by their protection in MES, scSTY and scPTZ screens. All the compounds except compound **5b** showed significant sedative-hypnotic activity.

Keywords

Isatin-3-semicarbazones • Anticonvulsant • Maximal electroshock • Sedative-hypnotic

Introduction

Epilepsy affects 1% of world's population according to the epidemiological studies. Current clinically available drugs produce satisfactory seizure control in 60–70% of patients [1]. Current drug therapy for epilepsy suffers from a number of disadvantages including the fact that the convulsions of approximately 25% epilepsies are inadequately controlled by medication. Therefore, the need for more effective and less toxic antiepileptic drugs still exists [2]. Semicarbazones have documented consistent advances in the design of novel anticonvulsant agents, through the work of Dimmock and his colleagues [3]. A number of aryl semicarbazones possessed greater protection in the MES screen [4]. If the aryl semicarbazone displaying activity in the MES screen interact at a specific binding site, it is likely that the semicarbazone group and the aryl ring align at complementary areas on a macromolecular complex in vivo; these areas have been referred to as the hydrogen bonding area and the aryl binding site, respectively [5].

It has been proposed that for activity in the MES test, a compound should have a large hydrophobic group in the close proximity to at least two electron donor atoms [6]. The semicarbazones containing a hydrophobic moiety (aryl ring) as well as two electron donor atoms in the semicarbazone group have been shown to possess activity in MES as well as scPTZ screen. Earlier, in this laboratory a number of substituted phenyl semicarbazones (4-Cl, 4-Br, 4-NO_2 etc) have been synthesized and evaluated for anticonvulsant activity. All the compounds showed anticonvulsant activity in MES, sc PTZ, sc STY and NT screens in one or more test models.

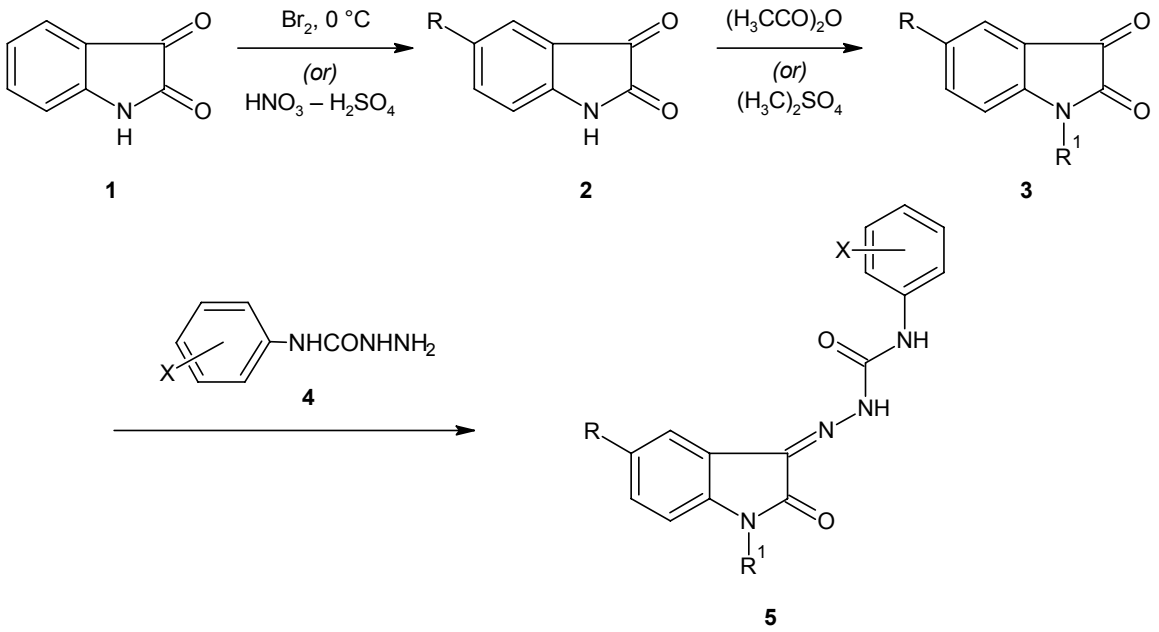
Isatin has been reported to possess anti-MES activity and it appears to have a range of actions in brain. Therefore, we have selected isatin as one hydrophobic centre. In our present research work isatin was chosen as a basic nucleus because from the earlier literature, it was clear that indole nucleus as such has got the anticonvulsant properties. Isatin also, is known to exhibit the anticonvulsant property in the sc PTZ test [7] (Mueller, 1962) substituting the hydrogen (from –NH in indole nucleus) with methyl and acetyl group enhanced the lipophilicity of the compounds.

Results and Discussion

1. Chemistry

The N-methyl/acetyl isatin (**3**) and 5-bromo/nitro-N-acetyl isatin (**2**) were prepared starting from isatin (**1**) [8]. The p-substituted phenyl semicarbazides (**4**) were prepared from appropriate anilines according to the method reported earlier [6, 9–11]. The semicarbazones (**5**) (Compounds **5a–5k**) were prepared by the condensation of N-substituted-5- (un)-substituted isatin with appropriate phenyl semicarbazides (2-Cl, 4-Cl, 4-Br, 4-NO_2 and 4-SO_2NH_2). The homogenity of the compounds was monitored by thin layer chromatography (TLC) on silica-G (Merck) coated glass plates, visualized by iodine vapour. The physical data and elemental analysis of compounds were presented in table 1 and 2.

The compounds were identified by elemental analysis, UV, IR, ^{1}H-NMR, ^{13}C NMR and Mass spectral data.



5a, R=H, R^1=$COCH_3$, X=4-Cl
5b, R=H, R^1=$COCH_3$, X=4-NO_2
5c, R=H, R^1=$COCH_3$, X=4-SO_2NH_2
5d, R=5-Br, R^1=$COCH_3$, X=2-Cl
5e, R=5-Br, R^1=$COCH_3$, X=4-Cl
5f, R=5-Br, R^1=$COCH_3$, X=4-NO_2
5g, R=5-Br, R^1=$COCH_3$, X=4-SO_2NH_2
5h, R=5-NO_2, R^1=$COCH_3$, X=2-Cl
5i, R=5-NO_2, R^1=$COCH_3$, X=4-Cl
5j, R=5-Br, R^1=CH_3, X=4-NO_2
5k, R=H, R^1=CH_3, X=H

Sch. 1. Synthetic protocol of N-methyl/acetyl -5-(un)-substituted isatin-3-semicarbazones

The compound 4-({[2-(1-acetyl-5-bromo-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]-carbonyl}amino)benzenesulfonamide (**5g**) when scanned between 400 to 200 nm in methanol exhibited maximum absorptions at (λ_{max}, nm) at 326.5, 279 and 271 nm. These latter two bands are characteristic of para-substituted phenyl ring. The IR spectrum showed the absorption bands at 3232 cm^{-1} and 3381 cm^{-1} of –NH stretching. The absorption band of amide C=O stretching and C=N stretching appeared at 1690 cm^{-1} and 1590 cm^{-1} respectively. The absorption bands at 1460 cm^{-1} and 1160 cm^{-1} were due to S=O stretching. The absorption band of aromatic group was observed at 804 $cm^{-1.}$

^{1}H-NMR spectra of compound **5g** revealed a singlet at δ 2.6 of 3H of the N-acetyl substituent. The singlet at δ 5.6 of 1 H was due to amidic -CONH proton, which was D_2O exchangeable. A singlet was observed at δ 9.8 for 1H of =N-NH protons that was D_2O exchangeable. A singlet at δ 10.7 of 2H confirmed the sulfamoyl group.

The mass spectra of compound **5g** showed prominent molecular ion peaks at m/z 480/482, and the doublets inferred the presence of bromine atom in the compound.

Tab. 1. Physical Data of *N*-methyl/acetyl Isatin-3-semicarbazones and their 5-Bromo/Nitro Derivatives.

Comp.	R^1	R	X	Yield (%)	MP (°C)	R_f [a]	Mol. Formula[b]
5a	$COCH_3$	H	4-Cl	72	143	0.617	$C_{17}H_{13}ClN_4O_3$
5b	$COCH_3$	H	4-NO_2	68	127	0.716	$C_{17}H_{13}N_5O_5$
5c	$COCH_3$	H	4-$SONH_2$	66	139	0.666	$C_{17}H_{15}N_5O_5S$
5d	$COCH_3$	5-Br	2-Cl	68	147	0.607	$C_{17}H_{12}BrClN_4O_3$
5e	$COCH_3$	5-Br	4-Cl	70	152	0.687	$C_{17}H_{12}BrClN_4O_3$
5f	$COCH_3$	5-Br	4-NO_2	68	117	0.632	$C_{17}H_{12}BrN_5O_5$
5g	$COCH_3$	5-Br	4-SO_2NH_2	74	173	0.469	$C_{17}H_{14}BrN_5O_5S$
5h	$COCH_3$	5-NO_2	2-Cl	62	126	0.510	$C_{17}H_{12}ClN_5O_5$
5i	$COCH_3$	5-NO_2	4-Cl	68	149	0.509	$C_{17}H_{12}ClN_5O_5$
5j	CH_3	5-Br	4-NO_2	65	97	0.529	$C_{16}H_{12}BrN_5O_4$
5k	CH_3	H	H	64	247	0.632	$C_{16}H_{14}N_4O_2$

[a] Solvent system for TLC were chloroform–methanol (9 : 1) for all the compounds.
[b] Elemental analyses for C, H, and N were within 0.4% of the theoretical values.

Tab. 2. Elemental analyses of compounds **5a–k**

Comp.	Molecular Formula	Found (%) [a], Calculated (%)		
		C	H	N
5a	$C_{17}H_{13}ClN_4O_3$	57.25 (57.32)	3.67 (3.69)	15.71 (15.68)
5b	$C_{17}H_{13}N_5O_5$	55.60 (55.58)	3.56 (3.60)	19.08 (19.12)
5c	$C_{17}H_{15}N_5O_5S$	50.91 (50.94)	3.77 (3.80)	17.46 (17.44)
5d	$C_{17}H_{12}BrClN_4O_3$	46.88 (46.92)	2.77 (2.81)	12.86 (12.82)
5e	$C_{17}H_{12}BrClN_4O_3$	46.88 (46.93)	2.77 (2.80)	15.70 (15.72)
5f	$C_{17}H_{12}BrN_5O_5$	45.77 (45.79)	2.71 (2.73)	14.59 (14.62)
5g	$C_{17}H_{14}BrN_5O_5S$	42.53 (42.55)	2.94 (2.96)	17.44 (17.46)
5h	$C_{17}H_{12}ClN_5O_5$	50.84 (50.86)	3.01 (3.03)	17.44 (17.45)
5i	$C_{17}H_{12}ClN_5O_5$	50.84 (50.85)	3.01 (3.04)	16.75 (16.78)
5j	$C_{16}H_{12}BrN_5O_4$	45.96 (45.97)	2.89 (2.91)	19.04 (19.07)
5k	$C_{16}H_{14}N_4O_2$	65.29 (65.30)	4.79 (4.81)	15.71 (15.68)

[a] Elemental analyses were determined with Perkin Elmer model 240 C analyzer

2. Pharmacology

The evaluations of the semicarbazones in the mice i.p. MES, scPTZ, scSTY and NT screens are summarized in table 3. Compounds were administered to mice by intraperitoneal route 30 min. before evaluation of the activities in these tests. Comparison with data recorded under the same conditions on phenytoin, carbamazepine and isatin, the reference prototype antiepileptic drugs.

MES and rotorod test

At doses tested (30, 100 and 300 mg/kg) compounds (**5a, 5e** and **5i**) possessed anti-MES activity at 100 mg/kg and compounds (**5a, 5d, 5f, 5g** and **5j**) were potent at 300 mg/kg.

Compounds **5b, 5d** and **5f** were potent in the MES test after 1 h at the dose of 100 mg/kg and the only compound 9 is potent after 4h at the dose of 300 mg/kg.

Sc PTZ Test

Compounds **5a** and **5e** are potent at 100 mg/kg at which they are equipotent to carbamazepine and the compound **5a** is potent at 300 mg/kg after at which carbamazepine does not exhibit activity. Compounds **5b** and **5i** are potent at 300 mg/kg for 0.5h and showed neurotoxicity equivalent to carbamazepine.

Sc STY Test

Majority of the compounds were found potent in this test at 300 mg/kg after 0.5h. Only 2 compounds **5e** and **5j** were inactive and compounds **5d**, **5g** and **5i** were potent after 4h at 300 mg/kg and compound **5c** is completely non-toxic. All the compounds except compound **5g** were toxic were equal to carbamazepine and at 0.5h they were non-toxic at 4h whereas carbamazepine and pheytoin were toxic at 300 mg/kg and 100mg/kg after 4h intervals. All the compounds were less neurotoxic than phenytoin and carbamazepine.

Mice were unable to grasp rotorod after administration of the following compounds, viz, **5a** (300, 0.5h), **5b** (300, 0.5h), **5d** (300, 0.5h), **5e** (300, 0.5h), **5g** (300, 0.5h), **5i** (300, 0.5h), and **5j** (300, 0.5)

Tab. 3. Anticonvulsant and neurotoxicity profile of the synthesized compounds.

Comp.	Intraperitoneal injection in mice[a]							
	MES screen		scPTZ screen		scSTYscreen		NT	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
5a	100	-	100	300	300	-	100	100
5b	300	100	300	-	300	-	100	-
5c	NOT	NOT	-	-	300	-	-	-
5d	300	100	-	-	300	300	100	-
5e	100	-	100	-	-	-	100	-
5f	300	100	-	-	300	-	100	-
5g	300	-	-	-	300	300	300	-
5h	100	300	300	300	100	300	100	-
5i	300	-	-	-	-	-	-	-
5j	-	-	-	-	NOT	NOT	-	-
5k	30	-	-	-	NOT	-	100	100
Carbamazepine	30	-	100	-	NOT	-	100	300
Isatin	400	-	-	-	-	-	-	-
Sodium Valproate	300	-	300	-	300	-	-	-
Phenobarbital	1100	30	30	30	-	100	100	100
Ethosuximide	-	-	300	-	300	-	-	-

[a] Doses of 10, 30, 100 and 300 mg/kg were administered.
The figures in the table reveal the minimum dose at which bioactivity was demonstrated in half or more of the animals. The lines (-) indicate the absence of activity at maximum dose administered. NOT denotes not tested.

Tab. 4. Anticonvulsant evaluation of compounds after oral administration (30 mg/kg) in rats.

Compound	15min.	30min.	1h.	2h.	4h.	Toxicity
5a	+	+	++	++	++++	-
5b	+	+++	+++	-	+++	-
5e	++++	+	-	+++	+++	-
5f	+	+	+	+	+	-
5g[a]	++	++	++	++++	++++	-
5h	++	+	++	+	-	-
5i	+++	+	+++	++	+++	-
Phenytoin	++++	-+++	+++-	++++	+	-

Symbols are as follows:
++++, activity in 75-100% of administered animals
+++, activity in 50-75% of animals
++, activity in 25-50% of animals
+, activity in 0-25% of animals
-, no activity or toxicity.
[a] Compound 5g showed the maximum activity with more duration of action until 4h

Majority of the compounds (**5a–5c**, **5e–5i**) except **5d** and **5j** were examined for activity in the rat oral MES screen and these data are presented in table 4. Initially a dose of 30 mg/kg was employed. Compounds afforded complete protection against seizures confirming their potential utility as prototypic molecules and compounds (**5a, 5b, 5e, 5g** and **5i**) emerged as the most active compounds in the rat oral MES screen. Quantification of compound **5b** and **5i** in the mice i.p MES, sc PTZ and NT screens were undertaken at the time of peak effect, namely 4h. The ED_{50} and TD_{50} figures (95% confidence intervals) in the MES, scPTZ and NT screens for **5a** and **5g** were 78.27 (66.92 - 87.34), 7150 (-), 140.38 (98.53-201.74), 64.85(49.5-87.58), 7130(-), 122.27 (101.36 - 145.13) respectively. The results are summarized in tables 6 and 7.

Sedative-hypnotic activity

All the compounds except **5i** were tested for sedative hypnotic activity at 30mg/kg for pentobarbitone-induced narcosis in rats. All the compounds except **5b** are found to potentiate the narcosis and found to have significant sedative-hypnotic activity.

The compounds were screened at 30, 100 and 300 mg/kg in the MES, scPTZ and sc STY tests. Compounds **5a, 5e** and **5i** possess anti-MES activity and have responded to anticonvulsant property in various tests and some of them have advantage over phenytoin, carbamazepine and sodium valproate in the scPTZ test also. Compound **5a** is active for 4h in the scPTZ test and found to be more potent than sodium valproate. Compounds **5a** and **5e** are equipotent to carbamazepine in the scPTZ test. They have only neurotoxicity at 100mg/kg equal to phenytoin and carbamazepine. Compounds **5a** and **5e** are more active as they are potent in MES as well in scPTZ test. There is considerable increase in anticonvulsant activity over isatin. Introduction of N-acetyl group which could be easily hydrolysed to putative =NH group and also due to the substitution of bromo and nitro group in the 5^{th} position of isatin increased the activity due to increased lipophilicity (NO_2)

π =0.11, (Br) π =0.80 Compounds **5b, 5d** and **5f** were active in the MES test after 1h. Compound **5i** is found to be the most active compound as it is potent in all the tests performed and also active when compared with phenytoin, carbamazepine, sodium valproate and ethosuximide. In mice i.p quantification the ED_{50} of compound **5b** and **5i** in MES and scPTZ are less when compared to valproate and it's more active than valproate and less active than carbamazepine and phenytoin. In rat oral quantification the ED_{50} of compound **5a** in the MES screen is less than valporate and compound **5g** was equipotent with that of phenytoin.

Tab. 5. Evaluation of compounds for sedative-hypnotic activity[a].

Compound	Mean sleeping time[b] (min.)
5a	244.27 ± 4.85
5b	39± 2
5c	69.66± 2.51
5d	53.66± 4.50
5e	243.94± 3.21
5f	183.66± 1.52
5g	237.27± 1.50
5h	114.34± 2.51
5i	202.67± 1.52
5j	182.3± 1.15

[a] Compounds were tested at a dose of 30 mg/kg (i.p.) in rats for the potentiation or antagonism of pentobarbitone induced narcosis.
[b] Each value represents the mean ± SEM of 6 rats significantly different from the control (P<0.005) in student's t-test.

Pharmacological Tests

The anticonvulsant evaluation [14–16] was undertaken by the National Institute of Neurological Disorders and Strokes, NIH (USA) using their reported procedures. Male albino mice (CF-1 strain, 18-25g) and male albino rats (Sprague-Dawley 100-150g) were used as experimental animals. The semicarbazones were suspended in 0.5% methyl cellulose/water mixture or in polyethylene glycol (PEG).

Anticonvulsant Screening

All of the compounds were injected intraperitoneally in a volume of 0.01ml/g body weight for mice, and evaluated in the MES, scPTZ, scSTY and NT screens, 0.004ml/g body wt. for rats of MES, scPTZ and scSTY doses of 30, 100 and 300 mg/kg at two different time intervals to 1 of 4 animals. These data are presented in table 3. Majority of the compounds were also evaluated orally in rats for activity in the MES test at several time points and presented in table 4.

Neurotoxicity (NT) screen

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay in 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

doses of 30, 100 and 300 mg/kg, Neurotoxicity was indicated by the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials.

Tab. 6. Quantification studies of selected compounds in the MES, scPTZ and neurotoxicity screens after intraperitoneal injection in mice.

Comp.	MES screen			scPTZ Screen			Neurotoxicity Screen		
	t(h)	ED_{50}[a] (mg/kg) (95%CI)[c]	Slope (SE)[b]	t(h)	ED_{50} (mg/kg) (95%CI)	Slope (SE)	t(h)	TD_{50}[d] (mg/kg) (95%CI)	Slope (SE)
5b	0.25	78.27[e] (66.92-87.34)	12.79 (5.03)	0.25	>150 (-)	-	0.25	140.38 (93.83-201.74)	5.32 (2.11)
5i	0.5	64.85 (49.5-87.58)	5.2 (1.66)	0.25	>130 (-)	-	0.25	122.27 (101.36-145.13)	8.9 (2.93)
Phenytoin	1	6.32 (5.44-7.23)	11.2 (3.52)	1	>50	-	0.5	41.2 (36.9-46.1)	14.4 (4.82)
Carbam-azepine	0.25	9.85 (8.77-10.7)	20.8 (7.15)	0.25	>50	-	0.25	47.8 (39.2-59.2)	7.98 (2.37)
Valproate	0.25	287 (237-359)	7.31 (2.48)	0.25	209 (176-249)	8.51 (2.69)	0.25	483 (412-571)	12.3 (4.01)

[a] Median Effective Dose. [b] Standard Error. [c] Confidence Interval. [d] Median Toxic dose. [e] ED_{50} significantly increased due to the presence of 4-nitro group (π=0.11) in compound **5b**.

Tab. 7. Quantification studies of selected compounds in the MES, scPTZ and neurotoxicity screens after oral administration in rats**.**

Comp.	MES screen			scPTZ Screen			Neurotoxicity Screen		
	t(h)	ED_{50}[a] (mg/kg) (95%CI)[c]	Slope (SE)[b]	t(h)	ED_{50} (mg/kg) (95%CI)	Slope (SE)	t(h)	TD_{50}[d] (mg/kg) (95%CI)	Slope (SE)
5a 4-Cl (π=0.71)	4	73.07 (34.94-143.09)	2.17 (0.77)	4	>125 (-)	-	0.25	>250	-
5g 4-Br (π=0.80)	4	26.81 (14.22-41.36)	2.89	0.9	-	-	-	-	-
Phenytoin	2	23.2 (21.4-25.4)	15.1 (4.28)	-	-	-	0.25-24	>500	-
Carbam-azepine	1	3.57 (2.41-4.72)	3.84 (1.15)	-	-	-	1	361 (319-402)	11.4 (2.96)
Valproate	0.5	395 (332-441)	8.13 (2.76)	-	-	8.51 (2.69)	0.5	859 (719-1148)	6.57 (2.17)

[a] Median Effective Dose. [b] Standard Error. [c] Confidence Intervals. [d] Median Toxic dose.

Sedative–hypnotic activity

This test was performed with the test substances in a dose of 30 mg/kg by pentobarbitone-induced narcosis in rats. The compounds in PEG were administered i.p to a group of six rats. After 30 min, rats were then placed on their back and loss of righting reflex was taken as onset of sleep. The time taken by the rats to awake was noted. A control was also performed after pretreatment with test substances vehicle (PEG) and injected pentobarbitone.

Experimental

1. Chemistry

Melting points were determined in open capillary tubes on a Thomas Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for the compounds on Jasco FT/IR 5300 (KBr).The ^{1}H-NMR and ^{13}C-NMR (300.40 MHz) spectra were recorded on JEOL–AL 300 (Fourier Transform) instruments, respectively. In proton nuclear magnetic resonance spectroscopy all exchangeable protons were confirmed by addition of D_2O. Chemical shifts are reported in ppm (δ) using tetra-methyl silane (TMS) as internal standard. Mass spectra were recorded on JEOL SX 102/DA–6000 Mass spectrometer. Elemental analyses (C, H, N) undertaken with Perkin Elmer Model 240C analyser for all the compounds and were within 0.4% of the calculated values.

N-Methyl/acetyl isatin were synthesized from isatin according to the earlier reported methods.

*1-Methyl-1*H-*indole-2,3-dione (*N-*Methylisatin) [8]*

Yield: 77% m.p. 131°C (lit. 134°C);

*1-Acetyl-1*H-*indole-2,3-dione (*N-*Acetylisatin)*

Yield: 75% m.p. 140°C (lit. 141°C).

5-Bromo/nitro N-acetyl isatin and 5-nitro N-methyl isatin were synthesized according to the earlier reported methods.

*1-Acetyl-5-bromo-1*H-*indole-2,3-dione (5-Bromo*-N-*acetylisatin) [13]*

(% Nitrogen found: 19.53%, calculated: 19.57%) Yield: 70% m.p. 171°C (lit. 170–172°C)

*1-Methyl-5-nitro-1*H-*indole-2,3-dione (5-Nitro*-N-*acetylisatin) [12]*

Yield: 62% m.p. 192°C (lit. 193–194°C)

*5-Bromo-1-methyl-1*H-*indole-2,3-dione (5-Bromo*-N-*methylisatin) [13]*

Yield: 70.7% m.p. 173°C (lit. 172–173°C) (% Nitrogen found: 17.54%, calculated: 17.57%).

N-Phenylhydrazinecarboxamide (Phenyl semicarbazide) [6]

Equimolar quantities of (0.1 mol) phenyl urea (7.4 g) and hydrazine hydrate (5 ml) in ethanol were added. The reaction mixture was made alkaline by adding sodium hydroxide (4.0 g) and is refluxed for 24 hrs and kept in ice. The precipitate formed was filtered, dried

and recrystallized from 95% ethanol to give phenyl semicarbazide. Yield: 60% m. p. 247°C. IR (KBr): 3450, 3260 (N-H), 1642 (C=O), 846 cm^{-1} ^{1}H-NMR (DMSO-d_6). δ (ppm): 5.6 (s, 2H, NH_2, D_2O exchangeable), 6.3 (s, 1H, ArNH, D_2O exchangeable), 7.2-7.8 (m, 5H, ArH), 9.7 (bs, 1H, $NHNH_2$, D_2O exchangeable)

The substituted phenyl semicarbazides (4-Cl, 4-Br, 4-NO_2, 4-SO_2NH_2) were synthesized according to earlier reported methods.

N-*(2-Chlorophenyl)hydrazinecarboxamide (*o-*Chlorophenyl semicarbazide) [6]*

Yield : 68%, m. p. 172°C (lit. 179°C);

N-*(4-Chlorophenyl)hydrazinecarboxamide (*p-*Chlorophenyl semicarbazide) [6]*

Yield : 72%, m. p. 232°C (lit. 234°C);

N-*(4-Bromophenyl)hydrazinecarboxamide (*p-*Bromophenyl semicarbazide) [9]*

Yield: 74%, m. p. 268°C (lit. 270°C);

N-*(4-Nitrophenyl)hydrazinecarboxamide (*p-*Nitrophenyl semicarbazide) [10]*

Yield: 78% m. p. 185°C (lit. 191°C);

N-*(4-Sulfamoylphenyl)hydrazinecarboxamide (*p-*Sulfamoylphenyl semicarbazide):*

Yield: 67%, m. p. 192°C (lit. 195°C).

General method for synthesis of N-substituted-5-(un)-substituted isatin-3-semicarbazones (5a–5k).

To a solution of 2/4 – substituted phenyl semicarbazides in ethanol was added an equimolar quantity (0.002 mol) of N-methyl/acetyl-5-Bromo/nitro isatin in ethanol. The pH of the reaction mixture was adjusted between 5-6 by adding glacial acetic acid. The reaction mixture was refluxed for 1-3h. The product obtained after cooling was filtered and dried. Recrystallized from 95% ethanol. The purity of the compounds was determined by TLC and the eluants used were chloroform:methanol (9:1) for all the compounds. The spectral data of the synthesized compounds are as follows (KBr) cm^{-1}.

*1-Acetyl-1*H-*indole-2,3-dione 3-[*N-*(4-chlorophenyl)semicarbazone] (5a)*

UV (methanol): (λ max) (nm) 285.5, 243. IR (KBr): 3408 (2°NH), 3213 (NH), 2922 (-CH_3), 1728 (Acetyl C=O), 1612 (amide C=O), 1589 (amide C=N), 1460, 821cm $^{-1}$ ^{1}H-NMR(DMSO-d_6): δ (ppm): 3.4 (s, 3H, N-$COCH_3$), 5.9 (s, 1H, -CONH, D_2O exchangeable), 7.01(d, indole H-5), 7.12 (m, H-6), 7.30- 7.48 (m, 2H, indole H-4 & H-7), 7.24 (d, 2H, 4-chlorophenyl,H-2, H-6, J=8.4 Hz), 7.8 (d, 2H, 4-chlorophenyl,H-3, H-5, J=8.3 Hz) 8.7 (s, 1H, -N=NH, D_2O exchangeable) ^{13}C-NMR (DMSO-d_6) δ: 20.2, 117.8, 121.7, 123.0 , 123.0 124.5, 129.1,129.1,129.4, 129.9, 131.3, 132.8, 134.0, 148.1, 157, 159, 172.1. Mass *m/z (*%): 356.0 (M^+, 20), 328.1 (10), 313.2 (60), 245.2 (30), 230.6 (30), 172.2 (60), 187.5 (20), 169.5 (15), 133.1(100), 126.1(10), 117.8 (80), 111.3 (25), 91.2 (30), 76.8 (20)

*1-Acetyl-1*H-*indole-2,3-dione 3-[*N-*(4-nitrophenyl)semicarbazone] (5b)*

UV (methanol): (λ max) (nm) 371, 247, 241. IR(KBr): 3477 (2°NH), 3368 (NH), 2924 (-

CH_3), 1745 (acetyl C=O), 1620 (C=O), 1590 (amide C=N) 837 cm^{-1} ^{1}H-NMR (DMSO-d_6): δ (ppm): 3.5 (s, 3H, N-$COCH_3$), 5.8 (s,1H, -CONH, D_2O exchangeable), 7.02(d, indole H-5), 7.14 (m, H-6), 7.32-7.50 (m, 2H, indole H-4 & H-7), 7.26 (d, 2H, 4-nitrophenyl, H-2, H-6, J=8.3 Hz), 8.0 (d, 2H, 4-nitrophenyl, H-3, H-5, J=8.3 Hz), 8.8 (s, 1H, -N=NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) δ: 20.2, 117.8, 121.3, 121.3, 121.7, 122.5, 124.5, 129.4, 129.9, 131.3, 132.8, 142.0,144.0, 148.1, 157, 159, 172.1. Mass *m/z (%)*: 367.0 (M^+, 15), 339.1 (10), 324.2 (40), 321.2 (20), 230.6 (25), 187.2 (60), 172.2 (80), 154.1(100), 137.1 (80), 122.1 (30), 110.1 (25), 107.1 (80), 93.0 (60), 77.0 (20), 65.2 (25).

*4-({[2-(1-Acetyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]carbonyl}amino)-benzenesulfonamide (5c)*

UV (methanol): (λ max)(nm) 325, 226. IR (KBr): 3479 (2°NH), 3375 (NH), 3275 (sulphonamido-NH), 2922(-CH_3), 1728 (Acetyl C=O), 1630 (amide C=O), 1595 (C=N), 1315, 1147 (S=O), 825 cm^{-1}. ^{1}H-NMR(DMSO-d_6): δ (ppm) 3.1 (s, 3H, N-$COCH_3$), 5.7 (s, 1H, -CONH, D_2O exchangeable), 7.01 (d, indole H-5), 7.13 (m, H-6), 7.32-7.50 (m, 2H, indole H-4 & H-7), 7.32 (d, 2H, 4-sulfamoyl, H-2, H-6), 8.2 (d, 2H, 4-sulfamoyl, H-3, H-5), 9.8 (bs, 1H, -N=NH, D_2O exchangeable), 10.5 (bs, 2H, SO_2NH_2, D_2O exchangeable) ^{13}C-NMR (DMSO-d_6) δ: 20.2, 117.8, 121.7, 121.9 ,121.9, 124.5, 127.5, 127.5, 129.4,131.3, 132.8, 135.3, 139.1, 148.1, 157, 159, 172.1. Mass *m/z (%)*: 401.4 (M^+, 10), 386.1 (20), 372.2 (15), 359.4 (40), 169.2 (60), 154.2 (80), 143.4 (100), 137.1 (80), 107.2 (60), 77.1(20).

*1-Acetyl-5-bromo-1*H*-indole-2,3-dione 3-[*N*-(2-chlorophenyl)semicarbazone] (5d)*

UV (methanol): (λ max) (nm) 302.5, 255, 244.5. IR (KBr): 3441 (2°NH), 3314 (NH), 2918 (-CH_3), 1730 (Acetyl C=O), 1653 (amide C=O), 1612 (C=N), 1464, 842, 592(C-Br) cm^{-1}.^{1}H-NMR (DMSO-d_6): δ (ppm): 3.4 (s, 3H, N-$COCH_3$), 5.8 (s, 1H, -CONH, D_2O exchangeable), 7.01-7.21 (m, 3H, indole H-4, H-6, H-7), 7.32-7.78 (m, 4H, 2-chlorophenyl, H-3 - H-6), 8.8 (s, 1H, -N=NH, D_2O exchangeable) ^{13}C-NMR (DMSO-d_6) δ: 20.2, 118.8, 120.0, 123.0 , 123.9 125.8, 127.1,129.1,130.5, 132.8, 132.9, 134.2, 134.9, 147.1, 157, 159, 172.1. Mass m/z (%) : 434.0 (M^+,15) 436.0 (M+2, 15), 391.2 (40), 323.5 (10), 306.3 (25), 245.3 (25), 219.1 (80), 169.2 (75), 137.3 (100), 126.4 (10), 111.3 (20), 92.1 (50), 80.2 (10), 77.0 (40).

*1-Acetyl-5-bromo-1*H*-indole-2,3-dione 3-[*N*-(4-chlorophenyl)semicarbazone] (5e)*

UV (methanol): (λ max) (nm) 322, 263.5. IR (KBr): 3485 (2°NH), 3317 (NH), 2935(- CH_3), 1745 (Acetyl C=O), 1650 (amide C=O), 1589 (C=N), 1460, 821 cm^{-1}.^{1}H-NMR (DMSO-d_6) δ (ppm) 3.6(s, 3H,N-$COCH_3$) 5.9 (s, 1H, -CONH, D_2O exchangeable), 7.02-7.22 (m, 3H, indole H-4, H-6, H-7), 7.25 (d, 2H, 4-chlorophenyl, H-2, H-6, J=8.4 Hz), 7.80 (d, 2H, 4-chlorophenyl, H-3, H-5, J=8.4 Hz), 8.9 (s, 1H, -N=NH, D_2O exchangeable) ^{13}C-NMR (DMSO-d_6) δ: 20.2, 118.8, 120.0, 123.0, 123.0, 123.9, 129.1,129.1, 129.9, 132.8, 132.9,134.0, 134.2, 147.1, 157, 159, 172.1. Mass *m/z (%)* : 434 (M+,15) 436 (M+2, 15), 391.3 (40), 323.2 (10), 306.1 (25), 244.8 (25), 219.0 (80), 169.3 (75),137.3 (100), 126.5 (10), 111.2 (20), 92.0 (50), 80 .1 (10), 77.1 (40).

*1-Acetyl-5-bromo-1*H*-indole-2,3-dione 3-[*N*-(4-nitrophenyl)semicarbazone] (5f)*

UV (methanol): (λ max) (nm) 369, 247. IR (KBr): 3479 (2°NH), 3324 (NH), 2926(-CH_3), 1736 (Acetyl C=O), 1630 (amide C=O), 1590 (C=N), 1460, 840 cm^{-1}.^{1}H-NMR (DMSO-d_6): δ (ppm): 3.6 (s, 3H, N-$COCH_3$), 5.8 (s, 1H, -CONH, D_2O exchangeable), 7.01-7.22 (m, 3H,

indole H-4, H-6, H-7), 7.27 (d, 2H, 4-nitrophenyl, H-2, H-6, J=8.4 Hz), 7.90 (d, 2H, 4-nitrophenyl, H-3, H-5, J=8.3 Hz), 9.1(s, 1H, -N=NH, D_2O exchangeable) ^{13}C-NMR (DMSO-d_6) δ: 20.2, 118.8, 120.0, 121.3, 121.3, 122.5, 122.5, 123.9, 132.8, 132.9, 134.2, 142.0, 144.0, 147.1, 157, 159, 172.1. Mass *m/z (%)* : 445.1 (M^+, 10) 447.1 (M+2, 10), 430.2 (10), 428.2 (10), 402.5(40), 399.2 (30), 369.0 (25), 338 (60), 323.2 (30), 217.2 (80), 154.2 (100), 137.0 (80), 123 (70), 107.0 (80), 105.1 (60), 80 .2 (10).

*4-({[2-(1-Acetyl-5-bromo-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazino]carbonyl}amino)-benzenesulfonamide (5g)*

UV (methanol): (λ max) (nm) 326.5, 279.271. IR (KBr): 3381 (2°NH), 3232 (NH), 2922 (-CH_3), 1728 (Acetyl C=O) 1690 (amide C=O) 1590 (C=N), 1160 (SO_2NH_2, S=O stretch, 840 cm^{-1} ^{1}H-NMR (DMSO-d_6): δ (ppm): 2.9 (s, 3H, N-$COCH_3$), 5.8 (s, 1H, -CONH, D_2O exchangeable), 6.80-7.12 (m, 3H, indole H-4, H-6, H-7), 7.34 (d, 2H, 4-sulfamoyl, H-2, H-6), 8.10 (d, 2H, 4-sulfamoyl, H-3, H-5), 9.9 (s, 1H, -N=NH, D_2O exchangeable), 10.6 (bs, 2H, SO_2NH_2, D_2O exchangeable) ^{13}C-NMR (DMSO-d_6) δ: 20.2, 118.8, 120.0, 121.9, 121.9, 123.9,127.5, 127.5, 132.8, 132.9, 134.2, 135.3, 139.1, 147.1, 157, 159, 172.1. Mass *m/z (%)* : 480.0 (M^+, 10), 482.0 (M+2, 10), 452.1 (15), 399.6 (10), 226.1(20), 223.1 (75), 169.1 (60), 154.2 (80), 143.1 (100), 137.1 (70), 105.2 (15), 77.2 (30)

*1-Acetyl-5-nitro-1*H*-indole-2,3-dione 3-[*N*-(2-chlorophenyl)semicarbazone] (5h)*

UV (methanol): (λ max) (nm) 294, 246, 241. IR (KBr): 3427 (2°NH), 3314 (NH), 2922(-CH_3), 1730 (Acetyl C=O), 1653 (amide C=O), 1585 (C=N), 1540, 1340 (C-NO_2) 804 cm^{-1} ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.5 (s, 3H, N-$COCH_3$), 5.9 (s, 1H, -CONH, D_2O exchangeable), 7.10-7.30 (m, 3H, indole H-4, H-6, H-7), 7.30-7.74 (m, 4H, 2-chlorophenyl, H-3 - H-6), 8.9 (s, 1H, -N=NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) δ: 20.2, 118.7, 122.6, 123.0, 123.6, 124.8, 125.8, 127.1, 129.1, 130.5, 132.8, 134.9, 144.1, 154.2, 157, 159, 172.1. Mass *m/z (%)*: 401.0 (M^+, 15), 403.0 (M+2,15), 373.1 (40), 245.2 (30), 189.2 (100), 183.0 (80), 176.1 (60), 172.1 (60), 169.1(15), 126.1(10), 111.2 (25), 80.8 (15), 77.0 (30)

*1-Acetyl-5-nitro-1*H*-indole-2,3-dione 3-[*N*-(4-chlorophenyl)semicarbazone] (5i)*

UV (methanol): (λ max) (nm) 290, 247, 242. IR (KBr): 3429 (2°NH), 3314 (NH), 2922(-CH_3), 1735 (Acetyl C=O), 1655 (amide C=O), 1548 (C=N), 1332 (C-NO_2), 821cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ (ppm): 3.5 (s, 3H, N-$COCH_3$), 5.9 (s, 1H, -CONH, D_2O exchangeable), 7.05-7.26 (m, 3H, indole H-4, H-6, H-7), 7.24 (d, 2H, 4-chlorophenyl, H-2, H-6, J=8.3 Hz), 7.81 (d, 2H, 4-chlorophenyl, H-3, H-5, J=8.4 Hz), 8.8 (s, 1H, -N=NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) δ: 20.2, 118.7, 122.6, 123.0, 123.0, 123.6, 124.3, 129.1, 129.1, 129.9, 132.8, 134.0, 144.1, 154.2, 157, 159, 172.1. Mass *m/z (%)*: 401.0 (M^+, 15), 403.0 (M+2,15), 373.2 (30), 245.0 (30), 189.1 (100), 183.0 (75), 176.1 (60), 172.0 (60), 168.9 (15), 126.0 (10), 111.0 (25), 80.5 (15), 77.0 (30)

*5-Bromo-1-methyl-1*H*-indole-2,3-dione 3-[*N*-(4-nitrophenyl)semicarbazone] (5j)*

UV (methanol): (λ max) (nm) 369.5, 245.5, IR (KBr): 3470 (2°NH), 3360 (NH), 2959(-CH_3), 1739 (Acetyl C=O), 1626 (amide C=O), 1589 (C=N), 1330 (C-NO_2), 845 cm^{-1} ^{1}H-NMR(DMSO-d_6): δ (ppm): 3.7 (s, 3H, N-CH_3), 5.8 (s, 1H, -CONH, D_2O exchangeable), 6.90 -7.21 (m, 3H, indole H-4, H-6, H-7), 7.28 (d, 2H, 4-nitrophenyl, H-2, H-6, J=8.3 Hz), 7.92 (d, 2H, 4-nitrophenyl, H-3, H-5, J=8.3 Hz), 9.8 (s, 1H, -N=NH, D_2O exchangeable).

^{13}C-NMR (DMSO-d_6) δ: 30.2, 118.8, 120.0, 121.3, 121.3, 122.5, 122.5, 123.9, 132.8, 132.9, 134.2, 142.0, 144.0, 146.4, 157, 163.5. Mass *m/z (%):* 418 (M^+, 30), 420 (M+2, 30), 403 (20),388.2 (15), 380.1 (40), 372.0 (20), 199.2 (60), 181.0 (55), 144.3 (100), 138.2 (80), 123.2 (80), 107.2 (70), 105.2 (60), 80.1 (15).

*1-Methyl-1*H-*indole-2,3-dione 3-(*N-*phenylsemicarbazone) (5k)*

UV (methanol): (λ max) (nm) 245.5. IR (KBr): 3420 (2°NH), 3316 (NH), 2922(-CH_3), 1653 (Acetyl C=O) 1590 (C=N), 840 cm^{-1}.^{1}H-NMR (DMSO-d_6): δ (ppm): 3.5 (s, 3H, N-CH_3), 5.2 (s, 1H, -CONH, D_2O exchangeable), 7.01(d, indole H-5), 7.13 (m, H-6), 7.31- 7.48 (m, 2H, indole H-4 & H-7), 7.35-7.90 (m, 5H, phenyl H-2-H-6), 8.5 (s, 1H, -N=NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6) δ: 30.2, 117.8, 121.6, 121.6, 121.7, 124.4, 124.5, 129.0, 129.0, 129.4, 131.3, 132.8, 135.9, 147.4, 157, 163.5. Mass *m/z (%)*: 294 (M^+,10), 279.0 (200, 266.1 (30), 215.2 (60), 202.1 (40), 137.2 (70), 112.1 (60), 107.3 (100), 76.8 (30).

2. Pharmacological Evaluations

All studies were performed under protocols approved by the Institutional Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke (NINDS), NIH in strict compliance with the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Academy Press, Washington, D.C.; The animal facilities were fully accredited by the American Association for the Accreditation of Laboratory Animal Care).

The initial anticonvulsant evaluation of N-methyl/acetyl isatin and 5-nitro/bromo N-acetyl/ methyl isatin-3-semicarbazones was undertaken by following the Anticonvulsant Drug Development (ADD) program protocol. [14–16]. The profile of anticonvulsant activity was established after intraperitoneal (i.p) injections by one electrical and two chemical tests. The electrical test employed was the maximal electroshock seizure (MES) pattern test. The chemical tests employed were the subcutaneous pentylene tetrazole (scPTZ) seizure threshold test and subcutaneous strychnine (scSTY) seizure threshold test. Minimal motor impairment was measured by the rotorod (neurotoxicity, NT) test.

Table 3 lists the results obtained from the initial anti-convulsant evaluation compared to the clinically proven antiepileptics like phenytoin, carbamazepine and isatin. Some compounds were administered orally to rats and examined in the MES screen and the data are presented in table 4. The compounds were also evaluated for the sedative-hypnotic activity by using pentobarbitone induced narcosis in rats [6] and presented in table 5.

Conclusions

The results of the investigations indicate that from our earlier studies the three essential structural features to interact at the binding site are

- a lipophilic moeity (4-bromophenyl, 4-chlorophenyl or 4-nitrophenyl).
- a hydrogen bonding domain (amide function -NH-CO-NH-)
- a distal aryl ring at the carbimino terminal benzylidene ring for controlling the pharmacokinetic properties of the compounds.

The π value of 4-chloro group is 0.71 and bromo group is 0.86. Due to the high π value the lipophilicity is increased. Considering this paradigm, we have substituted isatin with 5-bromo and 5-nitro groups and have also substituted with ortho and para substitution in semicarbazides with these groups (2-Cl, 4-Cl, 4-Br, 4-NO_2, 4-SO_2NH_2), Due to high lipophilicity it has exhibited anticonvulsant activity in MES, scPTZ, scSTY and NT screens. The compounds **5a, 5b, 5e, 5g** and **5i** were the most active compounds and emerged as lead compounds in further modification of semicarbazones as anticonvulsants.

Due to hydrophobic nature of bromo group in phenyl moiety of isatin the molecule is more active, further introduction of acetyl group has also increased the lipophilicity of the compound, which will enhance the absorption of the molecule. The acetyl group can also be easily hydrolysed to give a free N-H containing compound necessary for hydrogen bonding which may be responsible for the bioactivity, further acyl groups are needed to explore the structural activity relationship.

In conclusion compound **5a, 5e** and **5i** could be the lead compounds for further beneficial modification in the design of semicarbazones as anticonvulsants.

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Authors' Statements

Competing Interests

The authors declare no conflict of interest.

Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details.

References

[1] Perucen E.
The new generation of antiepileptic drugs: Advantages and disadvantages.
Br J Clin Pharmacol. 1996; 42: 531–543.
doi:10.1111/j.1365-2125.1996.tb00107.x

[2] Mattson RH.
Introduction and Symposium overview.
Epilepsia. 1996; 37: S1–S3.
doi:10.1111/j.1528-1157.1996.tb06033.x

[3] Dimmock JR, Sidhu KK, Thayer RS, Mack P, Dutty MJ, Reid RS, Quail JW.
Anticonvulsant activities of some aryl semicarbazones displaying potent oral activity in the maximal electroshock screen in rats accompanied by high protection indices.
J Med Chem. 1993; 36:2243–2252.
doi:10.1021/jm00068a001

[4] Dimmock JR, Puthucode RN, Smith JM, Hetherington M, Quail JW, Pugazhenthi U, Lechler, Stables JP.
(Aryloxy) Aryl semicarbazones and related compounds: A novel class of anticonvulsants possessing high activity in maximal electroshock screen.
J Med Chem. 1996; 39: 3984–3997.
doi:10.1021/jm9603025

[5] Dimmock JR, Pandeya SN, Quail JW, Pugazhenthi U, Allen TM, Kao GY, Balzarini JD, De Clercq E.
Evaluation of the semicarbazones, thio semicarbazones and biscarbhydrazones of some aryl alicylic ketones for anticonvulsant and other biological properties.
Eur J Med Chem. 1995; 39: 303–314.
doi:10.1016/0223-5234(96)88238-9

[6] Pandeya SN, Ponnilavarasan I, Pandey A, Lakhan R, Stables JP.
Evaluation of p-nitrophenyl substituted semicarbazones for anticonvulsant properties.
Pharmazie. 1999; 54: 923–925.
PMid:10631757

[7] Müller M.
Vergleichende pharmakologische Studien mit Indol und Isatin
[Comparative pharmacologic studies with indole and isatin].
Med Exp. 1962; 7: 155–160.
doi:10.1159/000135214

[8] Borsche W, Meyer R.
Untersuchungen über Isatin und verwandte Verbindungen, IV.: Über die Einwirkung von Hydrazin auf Isatin und N-Methyl-isatin.
[Isatin and related compounds (IV) Action of Hydrazine on isatin and N-methyl isatin].
Chem Ber. 1921; 54: 2844–2853.
doi:10.1002/cber.19210541036

[9] Pandeya SN, Yogeeswari P, Stables JP.
Synthesis and anticonvulsant activity of 4-bromophenyl substituted aryl semicarbazones.
Eur J Med Chem. 2000; 35: 879–886.
doi:10.1016/S0223-5234(00)01169-7

[10] Pandeya SN, Mishra V, Ponnilavarasan I, Stables JP.
Anticonvulsant activity of p-chlorophenyl substituted aryl semicarbazones. The role of terminal primary amino group.
Pol J Pharmacol. 2000; 52: 283-290.
PMid:8720804

[11] Pandeya SN, Manjula H, Stables JP.
Design of semicarbazones and their bioisosteric analogues as potential anticonvulsants.
Pharmazie. 2001; 56: 121–124.
PMid:11234338

[12] Calvery HO, Noller CR, Adams R.
Arsonophenyl - cinchonic and (Arsonocinchophen) and Derivatives II.
J Am Chem Soc. 1925; 47: 3059–3067.
doi:10.1021/ja01689a035

[13] Pandeya SN, Smitha S, Stables JP.
Anticonvulsant and sedative-hypnotic activities of N-substituted isatin-3-semicarbazones.
Arch Pharm. 2002; 4:129-134.
doi:10.1002/1521-4184(200204)335:4<129::AID-ARDP129>3.0.CO;2-W

[14] Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA.
Antiepileptic drug Development: II Anticonvulsant and drug screening.
Epilepsia. 1978; 19: 404–428.
doi:10.1111/j.1528-1157.1978.tb04507.x

[15] Kupferberg HJ.
Strategies for identifying and developing new anticonvulsant drugs.
Pharm Weekbl Sci. 1992; 14: 132–138.
doi:10.1007/BF01962704

[16] Porter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferberg HJ, Scotville B, White B.
Antiepileptic drug development program.
Cleveland Clin Quart. 1984; 51: 293–305.