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 tetrozole (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-NO2 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-NO2 and 4-SO2NH2). 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, 1H-NMR, 13C NMR and Mass spectral data.
The compound 4-({[2-(1-acetyl-5-bromo-2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazino]-carbonyl}amino)benzenesulfonamide (5g) when scanned between 400 to 200 nm in methanol exhibited maximum absorptions at $\lambda_{\text{max}}$ 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 $\delta$ 2.6 of 3H of the N-acetyl substituent. The singlet at $\delta$ 5.6 of 1 H was due to amide -CONH proton, which was D$_2$O exchangeable. A singlet was observed at $\delta$ 9.8 for 1H of =N-NH protons that was D$_2$O exchangeable. A singlet at $\delta$ 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. | R⁠¹ | R | X         | Yield (%) | MP (°C) | R_f ⁠ |
|-------|-----|---|-----------|-----------|---------|------|
| 5a    | COCH₃ | H | 4-Cl      | 72        | 143     | 0.617 |
| 5b    | COCH₃ | H | 4-NO₂     | 68        | 127     | 0.716 |
| 5c    | COCH₃ | H | 4-SONH₂   | 66        | 139     | 0.666 |
| 5d    | COCH₃ | 5-Br | 2-Cl     | 68        | 147     | 0.607 |
| 5e    | COCH₃ | 5-Br | 4-Cl     | 70        | 152     | 0.687 |
| 5f    | COCH₃ | 5-Br | 4-NO₂    | 68        | 117     | 0.632 |
| 5g    | COCH₃ | 5-Br | 4-SO₂NH₂ | 74        | 173     | 0.469 |
| 5h    | COCH₃ | 5-NO₂ | 2-Cl    | 62        | 126     | 0.510 |
| 5i    | COCH₃ | 5-NO₂ | 4-Cl    | 68        | 149     | 0.509 |
| 5j    | CH₃   | 5-Br | 4-NO₂    | 65        | 97      | 0.529 |
| 5k    | CH₃   | H | H         | 64        | 247     | 0.632 |

⁠¹Solvent system for TLC were chloroform–methanol (9 : 1) for all the compounds.

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

| Comp. | Molecular Formula | Found (%) ⁠ᵃ, Calculated (%) |
|-------|-------------------|-------------------------------|
|       |                   | C       | H       | N       |
| 5a    | C₁₇H₁₃CIN₄O₃      | 57.25 (57.32) | 3.67 (3.69) | 15.71 (15.68) |
| 5b    | C₁₇H₁₃N₅O₅        | 55.60 (55.58) | 3.56 (3.60) | 19.08 (19.12) |
| 5c    | C₁₇H₁₅N₅O₅S       | 50.91 (50.94) | 3.77 (3.80) | 17.44 (17.44) |
| 5d    | C₁₇H₁₂BrCIN₄O₃    | 46.88 (46.92) | 2.77 (2.81) | 12.86 (12.82) |
| 5e    | C₁₇H₁₂BrCIN₄O₃    | 46.88 (46.93) | 2.77 (2.80) | 15.70 (15.72) |
| 5f    | C₁₇H₁₂BrN₅O₅      | 45.77 (45.79) | 2.71 (2.73) | 14.59 (14.62) |
| 5g    | C₁₇H₁₄BrN₅O₅S     | 42.53 (42.55) | 2.94 (2.96) | 17.44 (17.46) |
| 5h    | C₁₇H₁₂CIN₅O₅      | 50.84 (50.86) | 3.01 (3.03) | 17.44 (17.45) |
| 5i    | C₁₇H₁₂CIN₅O₅      | 50.84 (50.85) | 3.01 (3.04) | 16.75 (16.78) |
| 5j    | C₁₆H₁₂BrN₅O₄      | 45.96 (45.97) | 2.89 (2.91) | 19.04 (19.07) |
| 5k    | C₁₆H₁₄N₄O₂        | 65.29 (65.30) | 4.79 (4.81) | 15.71 (15.68) |

⁠ᵃElemental 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 phenytoin 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* |
|-------|----------------------------------|
|       | MES screen | scPTZ screen | scSTY screen | 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 | - | - | - |

* 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<sup>a</sup> | ++ | ++ | ++ | ++++ | ++++ | -        |
| 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.
<sup>a</sup> 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<sub>50</sub> and TD<sub>50</sub> 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<sup>th</sup> position of isatin increased the activity due to increased lipophilicity (NO<sub>2</sub>)
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**

| Compound | Mean sleeping timeb (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) | Slope (SE) | t(h) | ED_{50}^{a} (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 |
| Carbamazepine | 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) |

* 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) | Slope (SE) | t(h) | ED_{50}^{a} (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 - | - |
| Carbamazepine | 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) |

* 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$_2$O. Chemical shifts are reported in ppm ($\delta$) 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-1H-indole-2,3-dione (N-Methylisatin) [8]
Yield: 77% m.p. 131°C (lit. 134°C);

1-Acetyl-1H-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-1H-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-1H-indole-2,3-dione (5-Nitro-N-acetylisatin) [12]
Yield: 62% m.p. 192°C (lit. 193–194°C)

5-Bromo-1-methyl-1H-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. ¹H-NMR (DMSO-d₆): δ (ppm): 5.6 (s, 2H, NH₂, D₂O exchangeable), 6.3 (s, 1H, ArNH, D₂O exchangeable), 7.2-7.8 (m, 5H, ArH), 9.7 (bs, 1H, NHNH₂, D₂O exchangeable)

The substituted phenyl semicarbazides (4-Cl, 4-Br, 4-NO₂, 4-SO₂NH₂) 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-1H-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 (-CH₃), 1728 (Acetyl C=O), 1589 (amide C=O), 1539, 1460, 821cm⁻¹ ¹H-NMR(DMSO-d₆): δ (ppm): 3.4 (s, 3H, N-COCH₂), 5.9 (s, 1H, -CONH, D₂O 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, D₂O exchangeable) 13C-NMR (DMSO-d₆) δ: 20.2, 117.8, 121.7, 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-1H-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 (-
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CH₃), 1745 (acetyl C=O), 1620 (C=O), 1590 (amide C=N) 837 cm⁻¹ 1H-NMR (DMSO-d₆): δ (ppm): 3.5 (s, 3H, N-COCH₃), 5.8 (s, 1H, -CONH, D₂O 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, D₂O exchangeable). 13C-NMR (DMSO-d₆): 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-3H-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(-CH₃), 1728 (Acetyl C=O), 1630 (amide C=O), 1595 (C=N), 1315, 1147 (S=O), 825 cm⁻¹. 1H-NMR (DMSO-d₆): δ (ppm): 3.1 (s, 3H, N-COCH₃), 5.7 (s, 1H, -CONH, D₂O 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, D₂O exchangeable), 10.5 (bs, 2H, SO₂NH₂, D₂O exchangeable) 13C-NMR (DMSO-d₆): 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), 143.4 (100), 137.1 (80), 107.2 (60), 77.1(20).

1-Acetyl-5-bromo-1H-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 (-CH₃), 1730 (Acetyl C=O), 1653 (amide C=O), 1612 (C=N), 1464, 842, 592(C-Br) cm⁻¹. 1H-NMR (DMSO-d₆): δ (ppm): 3.4 (s, 3H, N-COCH₃), 5.8 (s, 1H, -CONH, D₂O exchangeable), 7.01-7.21 (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), 8.8 (s, 1H, -N=NH, D₂O exchangeable) 13C-NMR (DMSO-d₆): 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), 391.3 (40), 323.2 (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-1H-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), 2918 (-CH₃), 1730 (Acetyl C=O), 1653 (amide C=O), 1624, 842, 592(C-Br) cm⁻¹. 1H-NMR (DMSO-d₆): δ (ppm): 3.6(s, 3H,N-COCH₃) 5.9 (s, 1H, -CONH, D₂O 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, D₂O exchangeable) 13C-NMR (DMSO-d₆): 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), 391.3 (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-1H-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(-CH₃), 1736 (Acetyl C=O), 1630 (amide C=O), 1590 (C=N), 1460, 840 cm⁻¹. 1H-NMR (DMSO-d₆): δ (ppm): 3.6 (s, 3H, N-COCH₃), 5.8 (s, 1H, -CONH, D₂O 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, D2O exchangeable) 13C-NMR (DMSO-d6) 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), 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-3H-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 (-CH3), 1728 (Acetyl C=O) 1690 (amide C=O) 1590 (C=N), 1160 (SO2NH2, S=O stretch, 840 cm-1 1H-NMR (DMSO-d6): δ (ppm): 2.9 (s, 3H, N-COCH3), 5.8 (s, 1H, -CONH, D2O 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, D2O exchangeable), 10.6 (bs, 2H, SO2NH2, D2O exchangeable) 13C-NMR (DMSO-d6) 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, 15), 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-1H-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 (-CH3), 1730 (Acetyl C=O), 1653 (amide C=O), 1585 (C=N), 1540, 1340 (C-NO2) 804 cm-1 1H-NMR (DMSO-d6): δ (ppm): 3.5 (s, 3H, N-COCH3), 5.9 (s, 1H, -CONH, D2O exchangeable), 7.10-7.30 (m, 3H, indole H-4, H-6, H-7), 7.30-7.72 (m, 4H, 2-chlorophenyl, H-3 - H-6), 8.9 (s, 1H, -N=NH, D2O exchangeable). 13C-NMR (DMSO-d6) 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-1H-indole-2,3-dione 3-[N-(4-chlorophenyl)semicarbazone] (5i)

UV (methanol): (λ max) (nm) 290, 247, 242. IR (KBr): 3427 (2°NH), 3314 (NH), 2922(-CH3), 1735 (Acetyl C=O), 1655 (amide C=O), 1548 (C=N), 1540, 1340 (C-NO2) 804 cm-1 1H-NMR (DMSO-d6): δ (ppm): 3.5 (s, 3H, N-COCH3), 5.9 (s, 1H, -CONH, D2O 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 Hz), 7.81 (d, 2H, 4-chlorophenyl, H-3, H-5, J=8.4 Hz), 8.8 (s, 1H, -N=NH, D2O exchangeable). 13C-NMR (DMSO-d6) 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), 373.2 (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-1H-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(-CH3), 1739 (Acetyl C=O), 1626 (amide C=O), 1589 (C=N), 1330 (C-NO2), 845 cm-1 1H-NMR(DMSO-d6): δ (ppm): 3.7 (s, 3H, N-CH3), 5.8 (s, 1H, -CONH, D2O 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, D2O exchangeable).
13C-NMR (DMSO-d6) 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-1H-indole-2,3-dione 3-(N-phenylisomericarbazone) (5k)

UV (methanol): (λ max) (nm) 245.5. IR (KBr): 3420 (2 °NH), 3316 (NH), 2922 (-CH₃), 1653 (Acetyl C=O) 1590 (C=N), 840 cm⁻¹. 1H-NMR (DMSO-d6): δ (ppm): 3.5 (s, 3H, N-CH₃), 5.2 (s, 1H, -CONH, D₂O 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, D₂O exchangeable). 13C-NMR (DMSO-d6) 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 tetraceole (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 moiety (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 $\pi$ value of 4-chloro group is 0.71 and bromo group is 0.86. Due to the high $\pi$ 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$_2$NH$_2$). 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.

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