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# Anticonvulsant Activity of Novel 1-(Substituted Benzylidene)-4-(1-(Morpholino/Piperidino Methyl)-2,3-Dioxoindolin-5-yl) Semicarbazide Derivatives in Mice and Rats Acute Seizure Models

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**A series of novel 1-(substituted benzylidene)-4-(1-(morpholino/piperidino methyl)-2,3-dioxoindolin-5-yl) semicarbazides 6a–6t was designed and synthesized on the basis of semicarbazide-based pharmacophoric model to meet the structural requirements necessary for anticonvulsant activity. The compounds were subjected to *in vivo* antiepileptic evaluation using maximal electroshock test and subcutaneous pentylenetetrazole seizure test methods. The neurotoxicity was determined by rotorod test. In the preliminary screening, compounds 6c, 6d, 6g, 6h, and 6m were found active in maximal electroshock test model, while 6g, 6i, 6m, and 6o showed significant antiepileptic activity in subcutaneous pentylenetetrazole seizure test model. Further, the compounds 6c, 6d, 6g, 6h, 6i, and 6m were administered orally to rats, of which 6c and 6g showed better activity than phenytoin. Among the synthesized compounds, 6g revealed excellent protection in both models with lower neurotoxicity.**

**Key words:** convulsions, *in vivo* studies, Indole-2,3-dione, isatin, neurotoxicity

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Epilepsy is a chronic neurological disorder, characterized by paroxysmal cerebral dysrhythmia, manifested as brief episodes (seizures) of loss or disturbance of consciousness, frequently followed by convulsions (1). About 1% of the world populations have epilepsy, with almost 90% of these people being in developing countries (2,3). Current treatment options include electrical stimulation, seizure suppression, and the use of antiepileptic drugs (AEDS). It has been estimated that 20% of the patients with epilepsy are using the first-generation AEDS like phenytoin, sodium valproate, phenobarbitone, and carbamazepine (4). Unfortunately, those drugs are associated with severe side effects (5). These limitations demand selective and lower toxicity AEDS in the field of medicinal chemistry.

Isatin, a class of heterocyclic compounds, possesses a broad spectrum of bioactivity (6–11). These properties encouraged the efforts toward the synthesis and pharmacological screening of many isatin derivatives. On the other hand, several studies have identified semicarbazides as a structurally novel class of compounds with particularly antiepileptic activity (12–14). It is well documented that the essential structural features responsible for AED is the presence of one electron donor atom (D), Hydrogen donor/acceptor unit (HAD) and a hydrophobic domain (A) (15–17). This common template was found in many AEDS (Figure 1). Motivated by the above findings and considering the wide applications of the isatin molecule in medicinal chemistry, an attempt has been made to synthesize different substituted benzylidene semicarbazide derivatives incorporating isatin moiety as a new antiepileptic agent.

## Experimental Section

### *Materials and methods*

The chemicals were obtained from Merck (Darmstadt, Germany) and SD Fine (Ahmadabad, India). For Thin Layer Chromatography (TLC), the solvent system was prepared in the following ratio: toluene/ethyl acetate/formic acid (5:4:1). UV and iodine chamber was used as visualizing methods. Melting points were determined in open glass capillary and were uncorrected. $^1$H-NMR spectral studies were carried out on a Bruker ultra shield NMR spectrometer using tetramethylsilane as internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded by Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkine Elmer model 240C analyzer.

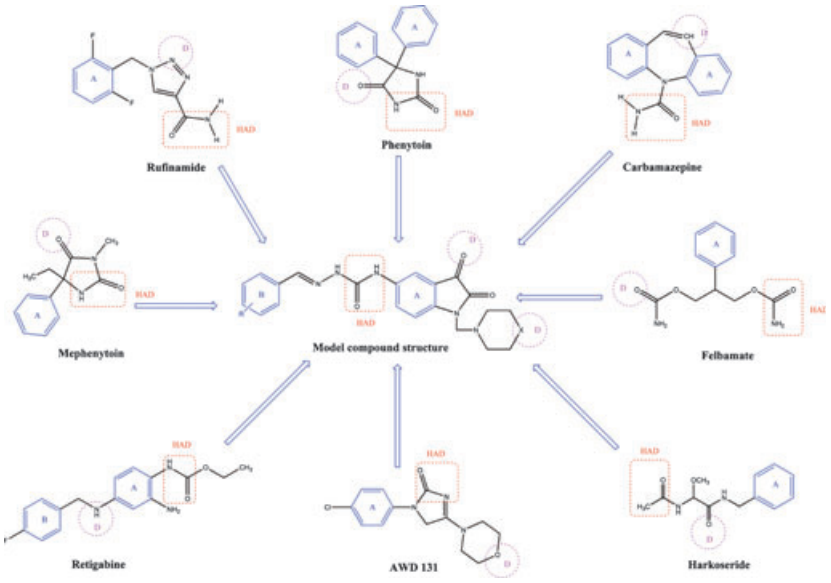



**Figure 1:** Examples of structural similarities between known antiepileptic drugs, compounds in clinical and preclinical development and the benzylidene semicarbazide incorporated isatin heterocyclic system (Model compound structure). (A) Hydrophobic aryl ring system, Hydrogen bond acceptor/donor domain (HAD), (D) Electron donor moiety and (B) Distal aryl ring.

### *Synthesis of compounds*

#### Preparation of 5-nitroisatin 1

The 5-nitroisatin **1** was prepared according to the reported literature (18).

#### 5-Nitro-1-substituted indolin-2,3-dione 2a–2b

To the solution of 5-nitro indolin-2-one **1** (0.04 mol) in 95% absolute ethanol (100 mL), aqueous formaldehyde 37% (1.0 mL) was added. Then, secondary amino compound such as morpholine/piperidine (0.04 mol) was slowly added drop-wise to the above solution under stirring. After the addition, the entire reaction mixture was stirred at room temperature for a period of 3 h and was kept aside for 48 h in a refrigerator to facilitate the formation of crystals. Finally, the crystalline form of the product was separated by filtration, washed with hexane, and vacuum dried. The desired compounds were finally recrystallized with ethanol to obtain the pure product (Scheme 1).

#### 1-(Morpholinomethyl)-5-nitro indoline-2, 3-dione 2a

Yield 68%, Mp 248 °C; IR (per cm): 3018, 1734, 1565, 1352, 1070; $^1$H NMR ($CDCl_3$): 2.45 (t, $J$ = 6.0 Hz, 4H, $-CH_2$ morpholine); 3.32 (t, $J$ = 5.6 Hz, 4H, $-CH_2$ morpholine); 4.28 (s, 2H, $-CH_2$); 7.40–7.86 (m, 3H, Ar-H); MS (EI) *m/z*: 291 [$M^+$]; Anal. Calcd for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.78; H, 4.52; N, 14.38.

#### 5-Nitro-1-(piperidin-1-ylmethyl)indoline-2, 3-dione 2b

Yield 56%, Mp 221 °C; IR (per cm): 3024, 1710, 1544, 1332; $^1$H NMR ($CDCl_3$): 1.45–1.56 (m, 6H, $-CH_2$ piperidine); 2.35–2.44 (m, 4H, $-CH_2$ piperidine); 4.40 (s, 2H, $-CH_2$); 7.20–7.64 (m, 3H, Ar-H); MS (EI) *m/z*: 289 [$M^+$]; Anal. Calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53. Found: C, 58.30; H, 5.25; N, 14.58.

#### 5-Amino-1-substituted indolin-2,3-dione 3a–3b

In a round-bottomed flask containing 200 mL of absolute ethanol, compound **2a** or **2b** (0.01 mol) and iron powder (0.01 mol) was added. After the addition, the mixture was heated on an oil bath until the temperature reaches to 80–85 °C. Further, in the reaction flask, 4 mL of hydrochloric acid (1.2 M) was added, and the content was stirred in the same temperature for a period of 4 h. Finally, the slurry was filtered, and the pH of the filtrate was adjusted to 7–8 with the help of sodium bicarbonate to get the precipitate of compound **3a** or **3b**. Recrystallization was carried out with ethanol to obtain the product in the pure form.

#### 1-(Morpholinomethyl)-5-amino indoline-2, 3-dione 3a

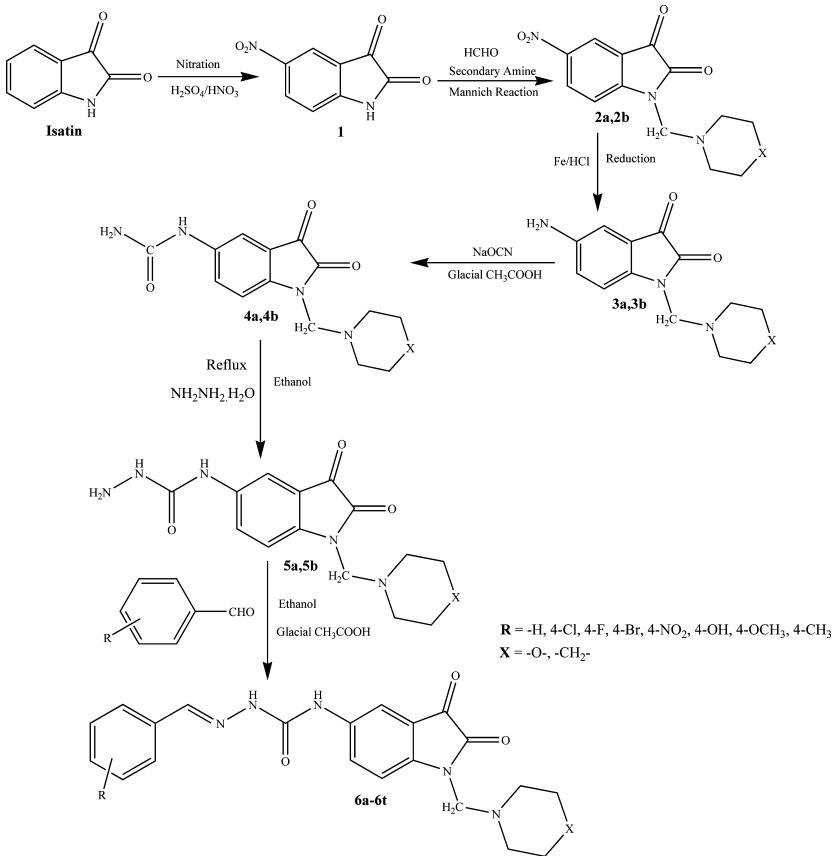
Yield 61%, Mp 226 °C; IR (per cm): 3386, 3128, 1740, 1055; $^1$H NMR ($CDCl_3$): 2.38 (t, $J$ = 6.0 Hz, 4H, $-CH_2$ morpholine); 3.38 (t, $J$ = 5.6 Hz, 4H, $-CH_2$ morpholine); 3.86 (s, 2H, $-NH_2$), 4.24 (s, 2H, $-CH_2$); 7.42–7.76 (m, 3H, Ar-H); MS (EI) *m/z*: 261 [$M^+$]; Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.94; H, 5.81; N, 16.13.

#### 5-Amino-1-(piperidin-1-ylmethyl)indoline-2, 3-dione 3b

Yield 54%, Mp 234 °C; IR (per cm): 3398, 3089, 1722; $^1$H NMR ($CDCl_3$): 1.24–1.57 (m, 6H, $-CH_2$ piperidine); 2.19–2.48 (m, 4H, $-CH_2$ piperidine); 3.74 (s, 2H, $-NH_2$), 4.35 (s, 2H, $-CH_2$); 7.36–7.68 (m, 3H, Ar-H); MS (EI) *m/z*: 259 [$M^+$]; Anal. Calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.20. Found: C, 65.05; H, 6.63; N, 16.26.

#### 1-(1-Substituted 2,3-dioxoindolin-5-yl) urea 4a–4b

Compounds **3a and 3b** (0.01 mol) were dissolved in 10 mL glacial acetic acid, and the volume was diluted to 100 mL with the same in a conical flask. A solution of NaOCN (sodium




**Scheme 1:** Synthetic protocols of intermediates and title compounds.

cyanate) (0.01 mol) and 50 mL of warm water was then added slowly with continuous stirring. Then the reaction mixture was allowed to stand for 30 min, cooled in crushed ice, and was allowed to stand for further 30 min. The product thus obtained was filtered and washed with cold water, finally dried at 100 °C. The product was recrystallized at least once from ethanol to give the pure form.

### 1-(1-(Morpholinomethyl)-2,3-dioxoindolin-5-yl)urea 4a

Yield 72%, Mp 272 °C; IR (per cm): 3364, 3096, 1746, 1648, 1030; $^1$H NMR ($CDCl_3$): 2.36 (t, $J$ = 6.0 Hz, 4H, –$CH_2$ morpholine); 3.34 (t, $J$ = 5.6 Hz, 4H, –$CH_2$ morpholine); 3.76 (s, 2H, -$NH_2$), 4.36 (s, 2H, –$CH_2$); 7.32–7.72 (m, 3H, Ar-H); 8.42 (s, 1H, Ar-NH); MS (EI) *m/z*: 304 [$M^+$]; Anal. Calcd for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.44; H, 5.32; N, 18.48.

### 1-(2,3-Dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl)urea 4b

Yield 71%, Mp 246 °C; IR (per cm): 3398, 3120, 1734, 1668; $^1$H NMR ($CDCl_3$): 1.28–1.53 (m, 6H, –$CH_2$ piperidine); 2.28–2.56 (m, 4H, –$CH_2$ piperidine); 3.88 (s, 2H, -$NH_2$), 4.24 (s, 2H, –$CH_2$); 7.20–7.68 (m, 3H, Ar-H); 8.50 (s, 1H, Ar-NH); MS (EI) *m/z*: 302 [$M^+$]; Anal. Calcd for $C_{15}H_{18}N_4O_3$: C, 59.59; H, 6.00; N, 18.53. Found: C, 60.10; H, 6.02; N, 18.60.

### 4-((1-Substituted)-2,3-dioxoindolin-5-yl) semicarbazide 5a–5b

The compounds **4a and 4b** (0.01 mol) were dissolved in 30 mL of absolute ethanol. To this, 2.5 mL of hydrazine hydrate was added, and the entire mixture was refluxed for 10–12 h with stirring (19). The contents were concentrated to half of its volume and poured onto crushed ice. The resultant precipitate was filtered, thoroughly washed with water, dried, and recrystallized from ethanol.

### 4-(1-(Morpholinomethyl)-2,3-dioxoindolin-5-yl)semicarbazide 5a

Yield 71%, Mp 275 °C; IR (per cm): 3387, 3058, 1720, 1640, 1042; $^1$H NMR ($CDCl_3$): 2.30 (t, $J$ = 6.0 Hz, 4H, –$CH_2$ morpholine); 3.42 (t, $J$ = 5.6 Hz, 4H, –$CH_2$ morpholine); 4.44 (s, 2H, –$CH_2$); 5.76 (s, 2H, -$NH_2$), 6.84–7.62 (m, 3H, Ar-H); 8.22 (s, 1H, Ar-NH); 9.84 (s, 1H, CONH); MS (EI) *m/z*: 319 [$M^+$]; Anal. Calcd for $C_{14}H_{17}N_5O_4$: C, 52.66; H, 5.37; N, 21.93. Found: C, 52.82; H, 5.39; N, 22.02.

### 4-(2,3-Dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl)semicarbazide 5b

Yield 62%, Mp 284 °C; IR (per cm): 3364, 3050, 1740, 1672; $^1$H NMR ($CDCl_3$): 1.32–1.62 (m, 6H, –$CH_2$ piperidine); 2.54–2.82 (m, 4H, –$CH_2$ piperidine); 4.22 (s, 2H, –$CH_2$); 5.88 (s, 2H, -$NH_2$); 7.12–7.58 (m, 3H, Ar-H); 8.30 (s, 1H, Ar-NH); 9.88 (s, 1H, CONH); MS (EI) *m/z*: 317 [$M^+$]; Anal. Calcd for $C_{15}H_{19}N_5O_3$: C, 56.77; H, 6.03; N, 22.07. Found: C, 56.93; H, 6.01; N, 22.14.

### *General procedure for the preparation of 1-(substituted benzylidene)-4-(1-(morpholino/piperidino methyl)-2,3-dioxoindolin-5-yl) semicarbazide derivatives 6a–6t*

Title compounds **6a–6t** were synthesized by adding **5a/5b** (0.01 mol) in fraction with the well-stirred mixture of different aromatic aldehydes (0.01 mol) in 50 mL ethanol and few drops of glacial acetic acid. Then, this mixture was refluxed for 6–8 h and kept aside. The separated product was filtered, dried, and recrystallized from ethanol.

### 1-Benzylidene-4-(1-(morpholinomethyl)-2, 3-dioxoindolin-5-yl) semicarbazide 6a

Yield 66%, Mp 262 °C; $R_f$ 0.68; IR (per cm): 3368, 2998, 1720, 1546, 1064; $^1$H NMR (DMSO-$d_6$): 2.40 (t, $J$ = 5.8 Hz, 4H, –$CH_2$ morpholine); 3.22 (t, $J$ = 5.6 Hz, 4H, –$CH_2$ morpholine); 4.45 (s, 2H, –$CH_2$); 6.90–7.45 (m, 8H, Ar-CH); 8.22 (s, 1H, Ar-NH); 8.58 (s, 1H, CH=N); 9.98 (s, 1H, CONH); MS (EI) $m/z$: 407 [$M^+$]; Anal. Calcd for $C_{21}H_{21}N_5O_4$: C, 61.91; H, 5.20; N, 17.19. Found: C, 62.08; H, 5.22; N, 17.26.

### 1-Benzylidene-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6b

Yield 664%, Mp 238 °C; $R_f$ 0.84; IR (per cm): 3392, 3024, 1728, 1548; $^1$H NMR (DMSO-$d_6$): 1.16–1.60 (m, 6H, –$CH_2$ piperidine); 2.28–2.52 (m, 4H, –$CH_2$ piperidine); 4.24 (s, 2H, –$CH_2$); 7.12–7.92 (m, 8H, Ar-H); 8.32 (s, 1H, Ar-NH); 8.46 (s, 1H, CH=N); 9.88 (s, 1H, CONH); MS (EI) $m/z$: 405 [$M^+$]; Anal. Calcd for $C_{22}H_{23}N_5O_3$: C, 65.17; H, 5.72; N, 17.27. Found: C, 65.32; H, 5.74; N, 17.33.

### 1-(4-Chlorobenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6c

Yield 78%, Mp 242 °C; $R_f$ 0.92; IR (per cm): 3384, 3092, 1718, 1552, 1084, 748; $^1$H NMR (DMSO-$d_6$): 2.40 (t, $J$ = 5.8 Hz, 4H, –$CH_2$ morpholine); 3.30 (t, $J$ = 5.6 Hz, 4H, –$CH_2$ morpholine); 4.24 (s, 2H, –$CH_2$); 6.90–7.92 (m, 7H, Ar-CH); 8.30 (s, 1H, Ar-NH); 8.54 (s, 1H, CH=N); 9.84 (s, 1H, CONH); MS (EI) $m/z$: 444 [M + 2]; Anal. Calcd for $C_{21}H_{20}ClN_5O_4$: C, 57.08; H, 4.56; N, 15.85. Found: C, 57.18; H, 4.58; N, 15.91.

### 1-(4-Chlorobenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6d

Yield 72%, Mp 287 °C; $R_f$ 0.86; IR (per cm): 3336, 2916, 1728, 1544, 778; $^1$H NMR (DMSO-$d_6$): 1.20–1.62 (m, 6H, –$CH_2$ piperidine); 2.42–2.74 (m, 4H, –$CH_2$ piperidine); 4.32 (s, 2H, –$CH_2$); 6.92–7.88 (m, 7H, Ar-H); 8.30 (s, 1H, Ar-NH); 8.60 (s, 1H, CH=N); 9.65 (s, 1H, CONH); MS (EI) $m/z$: 442 [M + 2]; Anal. Calcd for $C_{22}H_{22}ClN_5O_3$: C, 60.07; H, 5.04; N, 15.92. Found: C, 60.22; H, 5.02; N, 15.98.

### 1-(2-Chlorobenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6e

Yield 68%, Mp 218 °C; $R_f$ 0.90; IR (per cm): 3352, 3034, 1722, 1575, 1050, 755; $^1$H NMR (DMSO-$d_6$): 2.44 (t, $J$ = 5.8 Hz, 4H, –$CH_2$ morpholine); 3.32 (t, $J$ = 5.4 Hz, 4H, –$CH_2$ morpholine); 4.40 (s, 2H, –$CH_2$); 6.94–7.90 (m, 7H, Ar-CH); 8.22 (s, 1H, Ar-NH); 8.50 (s, 1H, CH=N); 9.94 (s, 1H, CONH); MS (EI) $m/z$: 444 [M + 2]; Anal. Calcd for $C_{21}H_{20}ClN_5O_4$: C, 57.08; H, 4.56; N, 15.85. Found: C, 57.22; H, 4.58; N, 15.90.

### 1-(2-Chlorobenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6f

Yield 70%, Mp 290 °C; $R_f$ 0.80; IR (per cm): 3336, 3006, 1740, 1552, 768; $^1$H NMR (DMSO-$d_6$): 1.23–1.51 (m, 6H, –$CH_2$ piperidine); 2.34–2.68 (m, 4H, –$CH_2$ piperidine); 4.23 (s, 2H, –$CH_2$); 7.10–7.92 (m, 7H, Ar-H); 8.24 (s, 1H, Ar-NH); 8.44 (s, 1H, CH=N); 9.92 (s, 1H, CONH); MS (EI) $m/z$: 442 [M + 2]; Anal. Calcd for $C_{22}H_{22}ClN_5O_3$: C, 60.07; H, 5.04; N, 15.92. Found: C, 60.24; H, 5.06; N, 15.95.

### 1-(4-Fluorobenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6g

Yield 80%, Mp 228 °C; $R_f$ 0.82; IR (per cm): 3364, 3045, 1732, 1548, 1126, 1057; $^1$H NMR (DMSO-$d_6$): 2.32 (t, $J$ = 6.0 Hz, 4H, –$CH_2$ morpholine); 3.40 (t, $J$ = 5.8 Hz, 4H, –$CH_2$ morpholine); 4.34 (s, 2H, –$CH_2$); 7.01–7.89 (m, 7H, Ar-CH); 8.14 (s, 1H, Ar-NH); 8.80 (s, 1H, CH=N); 9.96 (s, 1H, CONH); MS (EI) $m/z$: 425 [$M^+$]; Anal. Calcd for $C_{21}H_{20}FN_5O_4$: C, 59.29; H, 4.74; N, 16.46. Found: C, 59.14; H, 4.76; N, 16.52.

### 1-(4-Fluorobenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6h

Yield 76%, Mp 269 °C; $R_f$ 0.78; IR (per cm): 3335, 3054, 1728, 1541, 1148; $^1$H NMR (DMSO-$d_6$): 1.38–1.80 (m, 6H, –$CH_2$ piperidine); 2.39–3.20 (m, 4H, –$CH_2$ piperidine); 4.32 (s, 2H, –$CH_2$); 6.84–7.82 (m, 7H, Ar-H); 8.22 (s, 1H, Ar-NH); 8.68 (s, 1H, CH=N); 9.82 (s, 1H, CONH); MS (EI) $m/z$: 423 [$M^+$]; Anal. Calcd for $C_{22}H_{22}FN_5O_3$: C, 62.40; H, 5.24; N, 16.54. Found: C, 62.62; H, 5.26; N, 16.60.

### 1-(4-Nitrobenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6i

Yield 60%, Mp 278 °C; $R_f$ 0.94; IR (per cm): 3336, 3051, 1733, 1567, 1548, 1356, 1038; $^1$H NMR (DMSO-$d_6$): 2.32 (t, $J$ = 6.0 Hz, 4H, –$CH_2$ morpholine); 3.48 (t, $J$ = 5.6 Hz, 4H, –$CH_2$ morpholine); 4.32 (s, 2H, –$CH_2$); 6.76–7.84 (m, 7H, Ar-CH); 8.30 (s, 1H, Ar-NH); 8.62 (s, 1H, CH=N); 9.91 (s, 1H, CONH); MS (EI) $m/z$: 452 [$M^+$]; Anal. Calcd for $C_{21}H_{20}N_6O_6$: C, 55.75; H, 4.46; N, 18.58. Found: C, 55.94; H, 4.48; N, 18.65.

### 1-(4-Nitrobenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6j

Yield 64%, Mp 258 °C;$R_f$ 0.74; IR (per cm): 3354, 3023, 1740, 1574, 1566, 1344; $^1$H NMR (DMSO-$d_6$): 1.32–1.69 (m, 6H, –$CH_2$ piperidine); 2.28–2.72 (m, 4H, –$CH_2$ piperidine); 4.34 (s, 2H, –$CH_2$);

6.74–7.82 (m, 7H, Ar-H); 8.30 (s, 1H, Ar-NH); 8.52 (s, 1H, CH=N); 9.92 (s, 1H, CONH); MS (EI) *m/z*: 450 [$M^+$]; Anal. Calcd for $C_{22}H_{22}N_6O_5$: C, 58.66; H, 4.92; N, 18.66. Found: C, 58.84; H, 4.94; N, 18.73.

### 1-(3-Nitrobenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6k

Yield 72%, Mp 287 °C; $R_f$ 0.70; IR (per cm): 3358, 3042, 1726, 1572, 1560, 1382, 1047; $^1$H NMR (DMSO-$d_6$): 2.48 (t, *J* = 5.8 Hz, 4H, –$CH_2$ morpholine); 3.32 (t, *J* = 5.6 Hz, 4H, –$CH_2$ morpholine); 4.34 (s, 2H, –$CH_2$); 7.12–7.87 (m, 7H, Ar-CH); 8.36 (s, 1H, Ar-NH); 8.68 (s, 1H, CH=N); 9.96 (s, 1H, CONH); MS (EI) *m/z*: 452 [$M^+$]; Anal. Calcd for $C_{21}H_{20}N_6O_6$: C, 55.75; H, 4.46; N, 18.58. Found: C, 55.90; H, 4.44; N, 18.64.

### 1-(3-Nitrobenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6l

Yield 70%, Mp 296 °C; $R_f$ 0.88; IR (per cm): 3367, 2994, 1730, 1564, 1556, 1372; $^1$H NMR (DMSO-$d_6$): 1.29–1.60 (m, 6H, –$CH_2$ piperidine); 2.44–2.86 (m, 4H, –$CH_2$ piperidine); 4.44 (s, 2H, –$CH_2$); 6.85–7.86 (m, 7H, Ar-H); 8.22 (s, 1H, Ar-NH); 8.62 (s, 1H, CH=N); 9.84 (s, 1H, CONH); MS (EI) *m/z*: 450 [$M^+$]; Anal. Calcd for $C_{22}H_{22}N_6O_5$: C, 58.66; H, 4.92; N, 18.66. Found: C, 58.83; H, 4.94; N, 18.70.

### 1-(4-Bromobenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6m

Yield 62%, Mp 222 °C; $R_f$ 0.64; IR (per cm): 3341, 3036, 1729, 1550, 1046, 682; $^1$H NMR (DMSO-$d_6$): 2.44 (t, *J* = 6.0 Hz, 4H, –$CH_2$ morpholine); 3.26 (t, *J* = 5.8 Hz, 4H, –$CH_2$ morpholine); 4.50 (s, 2H, –$CH_2$); 7.04–7.76 (m, 7H, Ar-CH); 8.40 (s, 1H, Ar-NH); 8.75 (s, 1H, CH=N); 9.90 (s, 1H, CONH); MS (EI) *m/z*: 488 [M + 2]; Anal. Calcd for $C_{21}H_{20}BrN_5O_4$: C, 51.86; H, 4.15; N, 14.40. Found: C, 51.98; H, 4.17; N, 14.46.

### 1-(4-Bromobenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6n

Yield 76%, Mp 237 °C; $R_f$ 0.62; IR (per cm): 3356, 3043, 1736, 1546, 658; $^1$H NMR (DMSO-$d_6$): 1.20–1.58 (m, 6H, –$CH_2$ piperidine); 2.26–2.72 (m, 4H, –$CH_2$ piperidine); 4.24 (s, 2H, –$CH_2$); 6.88–7.74 (m, 7H, Ar-H); 8.28 (s, 1H, Ar-NH); 8.51 (s, 1H, CH=N); 9.90 (s, 1H, CONH); MS (EI) *m/z*: 486 [M + 2]; Anal. Calcd for $C_{22}H_{22}BrN_5O_3$: C, 54.56; H, 4.58; N, 14.46. Found: C, 54.68; H, 4.60; N, 14.51.

### 1-(4-Hydroxybenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6o

Yield 60%, Mp 208 °C; $R_f$ 0.75; IR (per cm): 3498, 3351, 3048, 1739, 1538, 1034; $^1$H NMR (DMSO-$d_6$): 2.44 (t, *J* = 6.2 Hz, 4H, –$CH_2$ morpholine); 3.26 (t, *J* = 5.8 Hz, 4H, –$CH_2$ morpholine); 4.38 (s, 2H, –$CH_2$); 5.14 (s, 1H, OH); 6.87–7.88 (m, 7H, Ar-CH); 8.42 (s, 1H, Ar-NH); 8.69 (s, 1H, CH=N); 9.92 (s, 1H, CONH); MS (EI) *m/z*: 423 [$M^+$]; Anal. Calcd for $C_{21}H_{21}N_5O_5$: C, 59.57; H, 5.00; N, 16.54. Found: C, 59.78; H, 5.02; N, 16.59.

### 1-(4-Hydroxybenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6p

Yield 72%, Mp 192 °C; $R_f$ 0.81; IR (per cm): 3516, 3366, 2984, 1750, 1555; $^1$H NMR (DMSO-$d_6$): 1.29–1.71 (m, 6H, –$CH_2$ piperidine); 2.52–2.94 (m, 4H, –$CH_2$ piperidine); 4.54 (s, 2H, –$CH_2$); 5.02 (s, 1H, OH); 7.12–7.98 (m, 7H, Ar-H); 8.36 (s, 1H, Ar-NH); 8.62 (s, 1H, CH=N); 9.88 (s, 1H, CONH); MS (EI) *m/z*: 421 [$M^+$]; Anal. Calcd for $C_{22}H_{23}N_5O_4$: C, 62.70; H, 5.50; N, 16.62. Found: C, 62.92; H, 5.48; N, 16.56.

### 1-(4-Methoxybenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6q

Yield 76%, Mp 216 °C; $R_f$ 0.79; IR (per cm): 3384, 3028, 1744, 1546, 1032; $^1$H NMR (DMSO-$d_6$): 2.50 (t, *J* = 6.0 Hz, 4H, –$CH_2$ morpholine); 2.84 (s, 3H, $OCH_3$); 3.42 (t, *J* = 5.6 Hz, 4H, –$CH_2$ morpholine); 4.16 (s, 2H, –$CH_2$); 6.86–7.90 (m, 7H, Ar-CH); 8.29 (s, 1H, Ar-NH); 8.52 (s, 1H, CH=N); 9.82 (s, 1H, CONH); MS (EI) *m/z*: 437 [$M^+$]; Anal. Calcd for $C_{22}H_{23}N_5O_5$: C, 60.40; H, 5.30; N, 16.01. Found: C, 60.24; H, 5.28; N, 16.07.

### 1-(4-Methoxybenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6r

Yield 69%, Mp 235 °C; $R_f$ 0.83; IR (per cm): 3346, 2986, 1718, 1552; $^1$H NMR (DMSO-$d_6$): 1.29–1.62 (m, 6H, –$CH_2$ piperidine); 2.22–2.50 (m, 4H, –$CH_2$ piperidine); 2.72 (s, 3H, $OCH_3$); 4.38 (s, 2H, –$CH_2$); 6.89–7.76 (m, 7H, Ar-H); 8.22 (s, 1H, Ar-NH); 8.52 (s, 1H, CH=N); 9.88 (s, 1H, CONH); MS (EI) *m/z*: 435 [$M^+$]; Anal. Calcd for $C_{23}H_{25}N_5O_4$: C, 63.44; H, 5.79; N, 16.08. Found: C, 63.62; H, 5.80; N, 16.13.

### 1-(4-Methylbenzylidene)-4-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) semicarbazide 6s

Yield 71%, Mp 198 °C; $R_f$ 0.77; IR (per cm): 3372, 3036, 1740, 1548, 1036; $^1$H NMR (DMSO-$d_6$): 2.32 (s, 3H, $CH_3$); 2.63 (t, *J* = 5.8 Hz, 4H, –$CH_2$ morpholine); 3.42 (t, *J* = 5.6 Hz, 4H, –$CH_2$ morpholine); 4.20 (s, 2H, –$CH_2$); 6.82–7.84 (m, 7H, Ar-CH); 8.18 (s, 1H, Ar-NH); 8.38 (s, 1H, CH=N); 9.98 (s, 1H, CONH); MS (EI) *m/z*: 421 [$M^+$]; Anal. Calcd for $C_{22}H_{23}N_5O_4$: C, 62.70; H, 5.50; N, 16.62. Found: C, 62.48; H, 5.48; N, 16.67.

### 1-(4-Methylbenzylidene)-4-(2,3-dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl) semicarbazide 6t

Yield 66%, Mp 210 °C; $R_f$ 0.71; IR (per cm): 3368, 3031, 1741, 1564; $^1$H NMR (DMSO-$d_6$): 1.34–1.69 (m, 6H, –$CH_2$ piperidine); 2.24 (s, 3H, $CH_3$); 2.64–2.98 (m, 4H, –$CH_2$ piperidine); 4.46 (s, 2H, –$CH_2$); 7.08–7.94 (m, 7H, Ar-H); 8.14 (s, 1H, Ar-NH); 8.47 (s, 1H, CH=N); 9.94 (s, 1H, CONH); MS (EI) *m/z*: 419 [$M^+$]; Anal. Calcd for $C_{23}H_{25}N_5O_3$: C, 65.85; H, 6.01; N, 16.70. Found: C, 66.04; H, 5.99; N, 16.77.

## *Pharmacology*

All the compounds' antiepileptic activity was screened by maximal electroshock test (MES) and subcutaneous pentylenetetrazole

seizure test (*sc*PTZ) method. Maximal electroshock test model employs an electrical stimulus, whereas *sc*PTZ model utilizes chemically induced myoclonic seizures and is capable of recognizing the agents that act by raising the seizure threshold (20). All the animal experiments were approved by Institutional Animal Ethical Committee (IAEC/III/22/BCOP/2011). Compounds are administered by *i.p* injection in mice at dose levels of 30, 100, and 300 mg/kg at 0.5 and 4 h. The acute neurological toxicity (NT) was determined by rotorod model. To further differentiate the activity between rodent species, the most active compounds were tested in rats.

### *The maximal electroshock test*

For the MES, a drop of anesthetic and electrolyte solution was applied to the eyes of the animal before placement of the corneal electrodes. The electrical stimulus in the MES test was 50 mA, 60 Hz, for mice and 150 mA, 60 Hz, for rats was applied for 0.2 seconds by an apparatus similar to that described by Woodbury and Davenport (21). For the abolition of the hindleg, tonic extensor component of the seizure was used as the endpoint. Mice were initially tested with different doses of 30, 100, and 300 mg/kg, while rats were initially screened at a fixed dose of 30 mg/kg by oral route.

### *The subcutaneous pentylenetetrazole seizure test*

Subcutaneous injection of the convulsant pentylenetetrazole produces clonic seizures in laboratory animals. Animals were pretreated with various doses of the test compound by *i.p.* injection. Pentylenetetrazole is injected in the midline of the neck. The animals were placed in isolation cages to minimize stress and were observed for the next 30 min for the presence or absence of a seizure. An episode of clonic spasms, approximately 3–5 seconds, of the fore and/or hindlimbs, jaws, or vibrissae is taken as the endpoint. Animals that do not meet this criterion are considered protected.

### *Acute toxicity-minimal motor impairment*

To assess a compound's undesirable side effects (toxicity), animals were monitored for overt signs of impaired neurological or muscular function. In mice, the rotorod (22) procedure is used to disclose minimal muscular (MMI) or neurological impairment. When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for long periods of time. The animal is considered toxic if it falls off this rotating rod three times during a 1-min period. In addition to MMI, animals may exhibit a circular or zigzag gait, abnormal body posture and spread of the legs, tremors, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy, loss of placing response, and changes in muscle tone.

## Results and Discussion

### *Synthesis*

The structures of the synthesized compounds were confirmed by spectral (IR, $^1$H-NMR, and mass) and elemental analyses data. The introduction of nitro group in compound **1** was confirmed by the appearance of two strong bands in its IR spectrum at 1570 and 1348 per cm, which arises because of asymmetrical and symmetrical stretching vibrations. The formation of compounds **2a** and **2b** was confirmed by the appearance of singlet between $\delta$ 4.28 and $\delta$ 4.40 respectively for two protons in its $^1$H-NMR spectra, which might be accountable for $CH_2$ group connecting the isatin core with secondary amines through Mannich reaction. The conversion of nitro to amino group in compounds **3a** and **3b** can be acknowledged by strong absorption peak at 3386 and 3398 per cm in IR because of N-H Stretching. The IR spectrum of title compound **6g** over the 3045, 1732, and 1548 per cm, which was assigned to Ar-H, carbonyl, and azomethine (CH=N) stretching. It is the $^1$H-NMR spectrum that showed a singlet at $\delta$ 8.54 ppm because of the proton attached to the imine carbon. A group of signals appeared between $\delta$ 6.87 and 7.89 ppm corresponds to Ar-H protons.

### *Biological result and discussions*

#### The maximal electroshock test

The antiepileptic activity of all the synthesized compounds has been shown in Table 1. Compounds **6c**, **6d**, **6g**, **6h**, and **6m** showed protection at 30 mg/kg, and compounds **6e**, **6f**, **6j**, **6k,** and **6n** showed protection at 100 mg/kg, while compounds **6o**, **6p**, **6r**, and **6s** exhibited protection at 300 mg/kg after 0.5 h. Compounds **6c**, **6g**, and **6i** showed protection against the MES screen at 30 mg/kg, and compounds **6a**, **6d**, **6h**, **6j**, **6l**, and **6m** showed protection at a dose level of 100 mg/kg, while compounds **6e**, **6f**, **6n–6r**, and **6t** showed protection at a dose level of 300 mg/kg after 4 h. The most active compounds **6c** and **6g** showed activity both at 0.5 and 4 h at dose level of 30 mg/kg indicating that they have rapid onset, more effective and long acting. Similarly, compounds **6d**, **6h**, and **6m** were also found to be more effective but short acting, which was evidenced by the requirement of higher dose (100 mg/kg) at 4 h. The compound **6j** showed activity both at 0.5 and 4 h period at a dose level of 100 mg/kg, indicating that compound was effective and long acting. Compounds **6e**, **6f**, and **6n** demonstrated protection in mice at 100 mg/kg and 300 mg/kg after 0.5 and 4.0 h, respectively. The compounds **6o**, **6p**, and **6r** showed activity both at 0.5 and 4 h period at a of dose of 300 mg/kg, indicating that compounds are less potent and long acting. Compound **6i** showed activity at 30 mg/kg, and **6a and 6l** showed activity at 100 mg/kg only at 4 h, indicating that compounds have sustained activity. Compound **6k** showed activity only at 0.5 h at a dose level of 100 mg/kg, indicating that compound has rapid onset and shorter duration of action. Compounds **6q**, **6s**, and **6t** exhibited some protection in either of the tested timepoints, but a poor onset was observed at a higher dose (300 mg/kg). In contrast, compound **6b** was not active at both the tested dose levels.

#### The subcutaneous pentylenetetrazole seizure test

In the chemoshock investigation, most of the compounds showed moderate to good antiepileptic activity. The compounds **6g**, **6i**,

**Table 1:** Antiepileptic activity and neurotoxicity of compounds **6a–6t** administered intraperitoneally to mice

| | MES[a] screening | | *sc*PTZ[b] screening | | NT[c] screening | |
|---|---|---|---|---|---|---|
| Compound | 0.5 h[d] | 4.0 h[d] | 0.5 h[d] | 4.0 h[d] | 0.5 h[d] | 4.0 h[d] |
| **6a** | – | 100 | – | – | 30 | 30 |
| **6b** | – | – | 300 | – | ND | ND |
| **6c** | 30 | 30 | – | – | – | – |
| **6d** | 30 | 100 | 300 | – | – | – |
| **6e** | 100 | 300 | – | – | 300 | – |
| **6f** | 100 | 300 | – | – | – | – |
| **6g** | 30 | 30 | 100 | 300 | – | – |
| **6h** | 30 | 100 | 300 | 300 | – | 300 |
| **6i** | – | 30 | 100 | 300 | – | – |
| **6j** | 100 | 100 | 300 | 300 | 300 | 300 |
| **6k** | 100 | – | – | – | 300 | 300 |
| **6l** | – | 100 | – | – | – | – |
| **6m** | 30 | 100 | 100 | 300 | 100 | 300 |
| **6n** | 100 | 300 | 300 | – | 100 | 300 |
| **6o** | 300 | 300 | 100 | 300 | – | 300 |
| **6p** | 300 | 300 | 300 | – | ND | ND |
| **6q** | – | 300 | – | – | ND | ND |
| **6r** | 300 | 300 | – | – | ND | ND |
| **6s** | 300 | – | – | – | ND | ND |
| **6t** | – | 300 | – | – | ND | ND |
| Phenytoin | 30 | 30 | – | – | 100 | 100 |
| Ethosuximide | – | – | 100 | 300 | – | – |

MES, maximal electroshock test; *sc*PTZ, subcutaneous pentylenetetrazole seizure test; ND, not determined.
[a]Maximal electroshock test (administered intraperitoneally to mice at doses ranging from 30 to 300 mg/kg).
[b]Subcutaneous pentylenetetrazole test (administered intraperitoneally to mice at doses ranging from 30 to 300 mg/kg).
[c]Neurotoxicity (administered intraperitoneally to mice at doses ranging from 30 to 300 mg/kg).
[d]Time of test after drug administration.
The sign '–' (endash) represents an absence of activity at maximum dose administered (300 mg/kg).

**6m**, and **6o** showed activity at a dose level of 100 mg/kg, while compounds **6b**, **6d**, **6h**, **6j**, **6n**, and **6p** showed activity at dose of 300 mg/kg at 0.5 h. Only compounds **6g**, **6h**, **6i**, **6j**, **6m**, and **6o** showed protection at dose of 300 mg/kg even at 4 h. Compounds **6h** and **6j** have shown activity at dose of 300 mg/kg at 0.5 and 4 h, indicating that these compounds have moderate activity. The compounds **6g**, **6i**, **6m**, and **6o** were found to be most potent with rapid onset and intermediate duration of action. None of the compounds were found active at dose level of 30 mg/kg. Compounds **6a**, **6c**, **6e**, **6f**, **6k**, **6l**, **6q**, **6r**, **6s**, and **6t** showed no response at both tested dose levels, while compounds **6b**, **6d**, **6n**, and **6p** were found to be effective with short duration of action.

## Neurotoxicity screen

All the compounds evaluated for its neurotoxicity except **6b** and **6p–6t** because of its poor response in antiepileptic activity. In neurotoxic screening, compounds **6c**, **6d**, **6f**, **6g**, **6i**, and **6l** were not found to be neurotoxic at the maximum dose administered (300 mg/kg). The compound **6a** showed neurotoxicity at 30 mg/kg. Compounds **6m** and **6n** showed neurotoxicity at a dose of 100 mg/kg at 0.5 h and 300 mg/kg at 4 h, while the remaining compounds **6e**, **6h**, **6j**, **6k**, and **6o** showed neurotoxicity at a dose level of 300 mg/kg at 0.5 h and/or 4 h period.

**Table 2:** Antiepileptic activity and toxicity of compounds **6c**, **6d**, **6g**, **6h**, **6i**, and **6m** administered orally (30 mg/kg) to rats

| | MES[a] | | | | | |
|---|---|---|---|---|---|---|
| Compound | 0.25 h[c] | 0.5 h[c] | 1 h[c] | 2 h[c] | 4 h[c] | TOX[b] |
| **6c** | 1/4 | 2/4 | 2/4 | 4/4 | 4/4 | 0/4 (–)[d] |
| **6d** | 1/4 | 2/4 | 2/4 | 3/4 | 3/4 | 0/4 (–)[d] |
| **6g** | 1/4 | 2/4 | 2/4 | 4/4 | 4/4 | 0/4 (–)[d] |
| **6h** | 1/4 | 2/4 | 2/4 | 3/4 | 3/4 | 0/4 (–)[d] |
| **6i** | 0/4 | 0/4 | 0/4 | 0/4 | 2/4 | 0/4 (–)[d] |
| **6m** | 0/4 | 2/4 | 1/4 | 1/4 | 2/4 | 0/4 (–)[d] |
| Phenytoin | 1/4 | 4/4 | 3/4 | 3/4 | 3/4 | 0/4 (–)[d] |

MES, maximal electroshock test.
[a]Maximal electroshock test (dose of 30 mg/kg was administrated; the data indicate number of rats protected/number of rats tested).
[b]Neurotoxicity (number of rats protected/number of rats tested).
[c]Time after drug administration.
[d](–) No neurotoxicity at dose tested.

## Antiepileptic activity of selected compounds on rats by oral administration

As can be seen from these data in Table 2, the most active compounds are **6c** and **6g**, which protected 100% (4/4) of rats at time-points 2 and 4 h, 50% (2/4) at 0.5 and 1 h, and 25% (1/4) at 0.25 h. This molecule was more active and showed longer duration of satisfactory action than phenytoin. The other compounds **6d** and **6h** were moderately effective in rat MES oral screen and protected 75% (3/4) at 2 and 4 h, 50% (2/4) at 0.5 and 1 h, while 25% (1/4) at 0.25 h. Compounds **6i** and **6m** showed 50% (2/4) protection at 4 h. The compound **6m** also showed 50% (2/4) protection at 0.5 h and 25% (1/4) of protection at 1 and 2 h. All derivatives tested were non-neurotoxic when given orally. The inhibition of electrically induced seizures that is characteristic for phenytoin and phenytoin-like drugs may indicate the influence of compounds on voltage depended $Na^+$ channels as the most reasonable mechanism of antiepileptic action.

### *Structure activity relationships analysis*

With the results of the preliminary anticonvulsant screening and on correlating the structures of the sample candidates **6a–6t** with their biological activity, it has been observed that most active were the morpholine substituted derivatives. When morpholine ring was replaced by piperidine, the anticonvulsant activity was altered in both the models and has exhibited less-significant effect. The increases in antiepileptic activity of test candidates with morpholine derivatives may be attributed to the presence of extra one electronegative oxygen atom on morpholine (which is absent in piperidine) ring that might be accountable for additional hydrogen bonding with the binding site. Taking into consideration the influence of the substituents at the distal phenyl ring appeared to greatly influence the

antiepileptic activity. From the results, it seems that the most favorable was the presence of highly electro negative chloro, fluoro, or bromo atoms at *para* position of the distal phenyl ring (**6c, 6d**, **6g**, **6h**, and **6m**). The *ortho* substituted derivatives (**6e** and **6f**) were less active. Presence of nitro substituent at *para* position (**6i** and **6j**) offered some protection, while *meta* substituents (**6k** and **6l**) did not greatly influence the antiepileptic activity. Three electron-releasing derivatives containing *p*-OH, *p*-$CH_3$, and *p*-$OCH_3$ (**6o**–**6t**) were also prepared. However, these compounds exhibited reduced anticonvulsant activities in MES screening than the corresponding halogen-substituted derivatives. Introduction of the –OH (**6o**) was found to show better activity only in *sc*PTZ. The other substituents like methoxy (**6q** and **6r**) and methyl (**6s** and **6t**) showed very poor activity in MES and completely devoid of activity in *sc*PTZ.

## Conclusions and Future Directions

By choosing appropriate experimental conditions, we were able to synthesize 1-(substituted benzylidene)-4-(1-(morpholino/piperidino methyl)-2,3-dioxoindolin-5-yl) semicarbazide derivatives **6a**–**6t** in good yields, and the same was evaluated in animal models of epilepsy and neurotoxicity. All the title compounds satisfied the needed pharmacophoric structural requirement that existed in the well-established AEDs. The most active compounds **6c**, **6d**, **6g**, **6h**, and **6m** revealed protection against the MES at a dose of 30 mg/kg in 0.5 h after *i.p* administration, while compound **6g**, **6i**, **6m**, and **6o** showed significant antiepileptic activity in *sc*PTZ model. Further, compounds **6c**, **6d**, **6g**, **6h**, **6i**, and **6m** were given orally to rats at a dose of 30 mg/kg. Only compounds **6c** and **6g** showed better antiepileptic activity in oral dose than phenytoin. Among the twenty derivatives, **6g** was found to be the most potent in the different models of epilepsy, suggesting a potential broad-spectrum anticonvulsant activity. It also showed marked lower neurotoxicity, and therefore offered a higher protective index. Therefore, such compounds would represent a fruitful matrix for the development of a new class of antiepileptic agents and would deserve further investigation and derivatization as a promising scaffold.

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## Conflict of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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