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ORIGINAL ARTICLE

Anticonvulsant activity of novel 1-(morpholinomethyl)-3-substituted isatin derivatives

Govindaraj Saravanan [a,*], **Veerachamy Alagarsamy** [b], **Pandurangan Dineshkumar** [c]

[a] *Medicinal Chemistry Research Laboratory, Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, India*
[b] *Medicinal Chemistry Research Laboratory, M.N.R. College of Pharmacy, Sangareddy, Andhra Pradesh, India*
[c] *Research Laboratory, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India*

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Abstract A variety of novel isatin derivatives **5a**–**5j** and **6a**–**6j** were synthesized and characterized by spectroscopic means and elemental analysis. The title compounds were investigated for antiepileptic activity using MES and *sc*PTZ seizures tests. Neurotoxicity study was performed by the rotorod test. The relationship between the functional group variation and the biological activity of the evaluated compounds was discussed. Among the synthesized analogs, the most active one was **6f** that revealed protection in MES at a dose of 30 mg/kg (i.p.) after 0.5 h and 4 h. This molecule also provided protection in the *sc*PTZ at a dose of 100 mg/kg (0.5 h) and 300 mg/kg (4 h).

1. Introduction

Epilepsy is one of the more common brain disorders characterized by recurrent spontaneous seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness.[1] About 1% of the world population has epilepsy, with almost 90% of these people being in developing countries.[2] Epilepsy also affects about 5% of individuals over their life time. In fact, epilepsy is the 3rd most often spread neurological disorder identified in the elderly after dementia and cerebrovascular diseases.[3] Currently a significant group of patients are resistant to the available antiepileptic drugs (AEDs), hence there is a need for improved agents for the treatment of seizure disorders. Moreover, many AEDs have serious side effects such as ataxia, drowsiness, gingival hyperplasia, gastrointestinal disturbances, and megaloblastic anemia.[4,5] These limitations with current AEDs demands the necessity of the development of more selective and lower toxic AEDs in the field of medicinal chemistry.

Many studies revealed that isatin is a privileged lead molecule for scheming potential bioactive agents, and their derivatives constitute an important class of heterocyclic compounds and are shown to possess a broad spectrum of bioactivity.[6–12] These exciting properties encouraged many efforts toward the

* Corresponding author. Tel.: +91 9963023257.
E-mail address: sarachem1981@gmail.com (G. Saravanan).

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synthesis and pharmacological screening of many isatin derivatives. On the other hand, several studies have identified isoxazoles as a structurally novel class of compounds with various biological activities.[13,14]

The conformational analysis of well established AEDs (Fig. 1) showed that for anticonvulsant activity, the model must be comprised of (i) hydrogen acceptor/donor unit (HAD), (ii) one electron donor atom (D) and (iii) a hydrophobic domain (A) (aryl ring substituted/unsubstituted).[15,16] Motivated by the aforementioned findings and considering the wide applications of isatin molecule in medicinal chemistry an attempt has been made to synthesize novel 1-(morpholinomethyl)-3-substituted isatin moiety as new antiepileptic agents.

2. Materials and methods

2.1. Chemistry

All solvents used were of laboratory grade and were obtained from SD fine chemicals (Mumbai, India), and Merck (Mumbai, India). Ciprofloxacin and Ketoconazole are received as gift samples from Dr. Reddys laboratories, Hyderabad, India. Melting points were determined in open glass capillary tubes and are uncorrected. Compounds were routinely checked for their purity on Silica gel G (Merck) Thin layer chromatography (TLC) plates using ethyl acetate: methanol: toluene (40:30:30) as elevator system; iodine chamber and UV lamp were used for visualization of TLC spots. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS) FT-IR spectrophotometer. NMR spectra were recorded on Bruker Avance-300 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS) for ^1H NMR and $CDCl_3$ for ^{13}C NMR as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. Elemental analyses were performed on a Perkine Elmer model 2400 CHN analyzer and were within $\pm 0.4\%$ of the theoretical values.

2.1.1. Synthesis of isonitrosoacetanilide (*1*)

Isatin was synthesized from aniline through isonitrosoacetanilide by the reported method.[17,18] Chloral hydrate (4.5 g; 0.027 mol) was dissolved in water (60 ml). To this solution crystallized sodium sulfate (6.5 g), solution of aniline (2.3 g; 0.025 mol) in water (15 ml), and concentrated hydrochloric acid (2.56 g; 0.026 mol) were added in order to dissolve the amine. To this hydroxylamine hydrochloride (5.5 g; 0.079 mol) dissolved in water (25 ml) was added. The flask was heated over a wire gauze by a burner for about 40–45 min. After 1–2 min of vigorous boiling, the reaction was

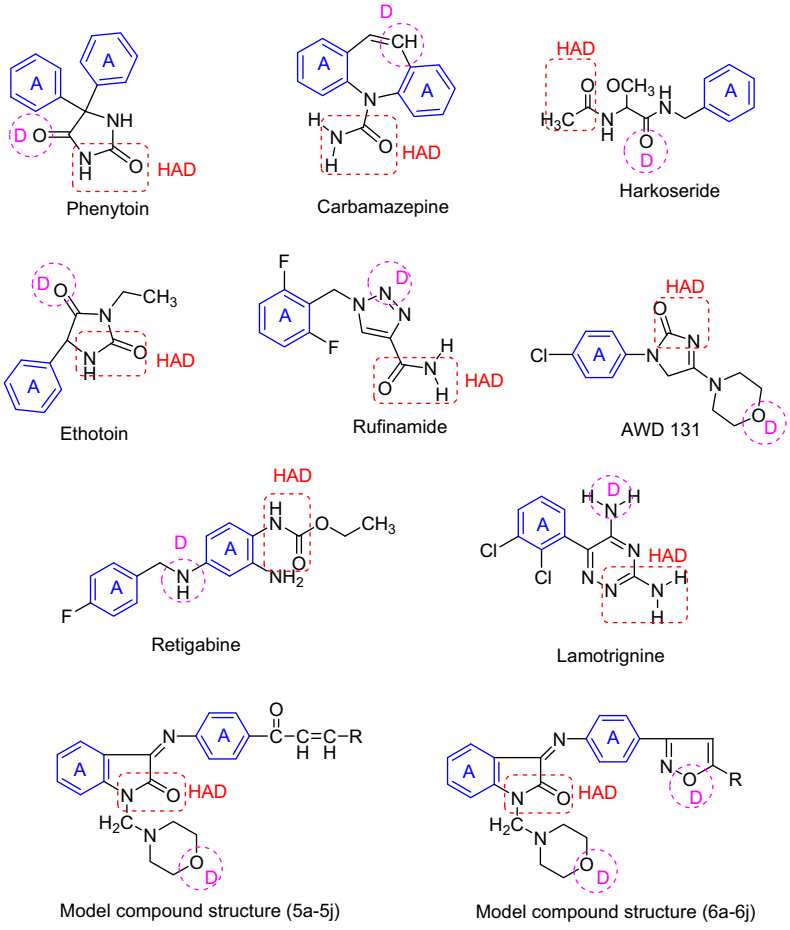


Figure 1 Pharmacophoric pattern of well known antiepileptics and model compound with its vital structural features: (A) hydrophobic aryl ring system, (HAD) hydrogen bond acceptor/donor domain, and (D) electron donor moiety.

completed. During heating some crystals of isonitrosoacetanilide **1** were separated. On cooling, the solution in running water, the remainder was crystallized. It was filtered and air dried. Yield 72%; m.p. 173–175 °C.

*2.1.2. Synthesis of isatin (**2**)*

Concentrated sulfuric acid (16.3 ml) was heated at 50 °C. To this dry isonitrosoacetanilide **1** (3.75 g; 0.023 mol) was added at such a rate as to keep the temperature between 60 and 70 °C but not higher. External cooling should be applied at this stage to carry out the reaction more rapidly. After complete addition of isonitrosoacetanilide **1** the solution was heated at 80 °C and kept at this temperature for about 10 min to complete the reaction. Then the mixture was cooled to room temperature and was poured to 10–12 times its volume of crushed ice. After standing for 2 h, the isatin was filtered with suction, washed several times with cold water to remove sulfuric acid. It was then filtered, dried and recrystallised. Yield 75%; m.p. 189–192 °C.

*2.1.3. Synthesis of 3-(4-acetylphenylimino)indolin-2-one (**3**)*

Isatin **2** (1.47 g, 0.01 mol) and *p*-amino acetophenone (1.33 g, 0.01 mol) was taken in round bottomed flask and dissolved in ethanol (15 ml). The above mixture was refluxed on a water bath for 6 h. The resulting solution was cooled to room temperature and poured into crushed ice. Then the solution was kept overnight in room temperature. The separated product was filtered and dried in air. The obtained product was recrystallised using alcohol. Yield 70%, m.p. 113–115 °C. IR (KBr cm^{-1}): 3359 (NH), 3066 (Ar—CH), 2918 (CH_3—CH), 1712 (C=O), 1575 (C=N), 1074 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.15 (s, 3H, $COCH_3$), 6.91–8.03 (m, 8H, Ar—CH), 8.89 (s, 1H, NH of isatin). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 32.6 (CH_3), 112.3 (C-9), 120.9 (C-7), 121.5 (C′-2 and C′-6), 125.8 (C-5), 130.2 (C-4), 131.7 (C′-3 and C′-5), 133.6 (C-6), 139.9 (C′-4), 145.4 (C-8), 159.2 (C′-1), 167.1 (C-3), 170.6 (C-2), 204.3 (C=O). MS (EI): *m*/*z* 264 [M^+]; Anal. Cald for $C_{16}H_{12}N_2O_2$: C, 72.72; H, 4.58; N, 10.60. Found: C, 72.60; H, 4.59; N, 10.63.

*2.1.4. Synthesis of 3-(4-acetylphenylimino)-1-(morpholinomethyl)indolin-2-one (**4**)*

A mixture of 3-(4-acetylphenylimino)indolin-2-one **3** (1.85 g, 0.007 mol), formaldehyde (0.3 g, 0.01 mol) and morpholine (0.6 g, 0.007 mol) in ethanol (20 ml) was stirred for the period of 1 h. The mixture was then refluxed for 4 h. Then the reaction mixture was cooled and poured in ice cold water. The obtained product was filtered and recrystallised using ethanol. Yield 78%, m.p. 96–98 °C. IR (KBr cm^{-1}): 3091 (Ar—CH), 2936 (CH_3—CH), 1747 (C=O), 1539 (C=N), 1062 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 1.92–2.87 (t, 4H, CH_2 of morpholine), 3.23 (s, 3H, $COCH_3$), 3.65–4.39 (t, 4H, CH_2 of morpholine), 4.80 (s, 2H, NCH_2N), 7.17–8.25 (m, 8H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 34.1 (CH_3), 54.8 (C″-2 and C″-6), 69.3 (C″-3 and C″-5), 72.0 (CH_2), 114.6 (C-9), 122.7 (C-7), 123.1 (C′-2 and C′-6), 127.2 (C-5), 130.5 (C-4), 132.5 (C′-3 and C′-5), 136.0 (C-6), 138.4 (C′-4), 147.8 (C-8), 155.6 (C′-1), 164.2 (C-3), 165.9 (C-2), 198.1 (C=O). MS (EI): *m*/*z* 363 [M^+]. Anal. Cald for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.55; H, 5.80; N, 11.53.

*2.1.5. Synthesis of 1-(morpholinomethyl)-3-(4-(3-substitutedacryloyl)phenylimino)indolin-2-one (**5a–5j**)*

A mixture of 3-(4-acetylphenylimino)-1-(morpholinomethyl)indolin-2-one **4** (1.09 g, 0.003 mol), different aromatic/heterocyclic aldehydes (0.003 mol) and sodium hydroxide pellets (100 mg) in ethanol (20 ml) was stirred magnetically for 1 h. Then the reaction mixture was refluxed for about 4 h. Then the resulting solution was poured into crushed ice and kept in a refrigerator overnight. The separated product was filtered, dried and crystallized from ethanol.

*2.1.5.1. 1-(Morpholinomethyl)-3-(4-(3-phenylacryloyl)phenylimino)indolin-2-one (**5a**).* Yield 73%, m.p. 193–195 °C. IR (KBr cm^{-1}): 3054 (Ar—CH), 1726 (C=O), 1550 (C=N), 1052 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.38–3.03 (t, 4H, CH_2 of morpholine), 3.10–3.77 (t, 4H, CH_2 of morpholine), 4.59 (s, 2H, NCH_2N), 7.01–7.95 (m, 13H, Ar—CH), 8.14–8.39 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 50.3 (C″-2 and C″-6), 70.7 (C″-3 and C″-5), 73.9 (CH_2), 119.1 (C-9), 124.8 (CH=CH), 125.0 (C-7), 127.6 (C′-2 and C′-6), 129.9 (C-5), 130.1 (C‴-2 and C‴-6), 132.8 (C‴-4), 133.2 (C‴-3 and C‴-5), 134.3 (C-4), 135.0 (C′-3 and C′-5), 136.5 (C-6), 137.2 (C‴-1), 138.9 (C′-4), 145.8 (CH=CH), 147.1 (C-8), 153.4 (C′-1), 160.7 (C-3), 161.9 (C-2), 192.6 (C=O). MS (EI): *m*/*z* 451 [M^+]. Anal. Cald for $C_{28}H_{25}N_3O_3$: C, 74.48; H, 5.58; N, 9.31. Found: C, 74.65; H, 5.57; N, 9.28.

*2.1.5.2. 1-(Morpholinomethyl)-3-(4-(3-p-tolylacryloyl)phenylimino)indolin-2-one (**5b**).* Yield 78%, m.p. 215–217 °C. IR (KBr cm^{-1}): 3081 (Ar—CH), 2920 (CH_3—CH), 1738 (C=O), 1563 (C=N), 1075 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 1.95–2.72 (t, 4H, CH_2 of morpholine), 3.09 (s, 3H, CH_3), 3.36–3.80 (t, 4H, CH_2 of morpholine), 4.25 (s, 2H, NCH_2N), 6.87–7.74 (m, 12H, Ar—CH), 7.91–8.27 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 26.4 (CH_3), 52.5 (C″-2 and C″-6), 68.4 (C″-3 and C″-5), 72.7 (CH_2), 116.5 (C-9), 120.2 (CH=CH), 121.6 (C-7), 124.3 (C′-2 and C′-6), 126.8 (C-5), 128.6 (C‴-2 and C‴-6), 132.0 (C‴-3 and C‴-5), 133.1 (C-4), 134.5 (C′-3 and C′-5), 136.2 (C-6), 137.0 (C‴-1), 139.5 (C′-4), 140.1 (C‴-4), 147.3 (CH=CH), 148.6 (C-8), 160.8 (C′-1), 164.5 (C-3), 165.7 (C-2), 187.4 (C=O). MS (EI): *m*/*z* 465 [M^+]. Anal. Cald for $C_{29}H_{27}N_3O_3$: C, 74.82; H, 5.85; N, 9.03. Found: C, 74.99; H, 5.86; N, 9.00.

*2.1.5.3. 1-(Morpholinomethyl)-3-(4-(3-(4-nitrophenyl)acryloyl)phenylimino)indolin-2-one (**5c**).* Yield 81%, m.p. 209–212 °C. IR (KBr cm^{-1}): 3106 (Ar—CH), 1714 (C=O), 1531 (C=N), 1506 and 1319 (NO_2), 1067 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.14–2.87 (t, 4H, CH_2 of morpholine), 3.02–3.95 (t, 4H, CH_2 of morpholine), 4.71 (s, 2H, NCH_2N), 6.65–7.83 (m, 12H, Ar—CH), 8.02–8.30 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 50.7 (C″-2 and C″-6), 64.9 (C″-3 and C″-5), 75.5 (CH_2), 120.2 (C-9), 125.8 (C‴-3 and C‴-5), 126.6 (CH=CH), 127.2 (C-7), 129.0 (C′-2 and C′-6), 130.6 (C-5), 135.3 (C‴-2 and C‴-6), 138.2 (C-4), 139.8 (C′-3 and C′-5), 140.6 (C-6), 142.1 (C′-4), 145.7 (C‴-1), 148.4 (CH=CH), 149.5 (C‴-4), 149.7 (C-8), 157.2 (C′-1), 161.8 (C-3), 162.1 (C-2), 191.3 (C=O). MS (EI): *m*/*z* 496 [M^+]. Anal. Cald for $C_{28}H_{24}N_4O_5$: C, 67.73; H, 4.87; N, 11.28. Found: C, 67.51; H, 4.88; N, 11.31.

*2.1.5.4. 3-(4-(3-(4-Methoxyphenyl)acryloyl)phenylimino)-1-(morpholinomethyl)indolin-2-one (**5d**)*. Yield 74%, m.p. 180–182 °C. IR (KBr cm^{-1}): 3029 (Ar—CH), 2905 (CH_3—CH), 1752 (C=O), 1576 (C=N), 1054 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.49–2.90 (t, 4H, CH_2 of morpholine), 3.15 (s, 3H, OCH_3), 3.28–3.84 (t, 4H, CH_2 of morpholine), 4.36 (s, 2H, NCH_2N), 6.92–7.99 (m, 12H, Ar—CH), 8.15–8.41 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 54.2 (C″-2 and C″-6), 56.1 (OCH_3), 69.8 (C″-3 and C″-5), 73.3 (CH_2), 110.0 (C‴-3 and C‴-5), 119.7 (C-9), 123.2 (CH=CH), 124.0 (C-7), 124.9 (C′-2 and C′-6), 127.1 (C-5), 129.5 (C‴-2 and C‴-6), 131.4 (C‴-1), 132.0 (C-4), 132.8 (C′-3 and C′-5), 133.6 (C-6), 137.3 (C′-4), 143.9 (CH=CH), 148.2 (C-8), 155.7 (C′-1), 159.0 (C‴-4), 167.5 (C-3), 167.9 (C-2), 185.2 (C=O). MS (EI): *m/z* 481 [M^+]. Anal. Cald for $C_{29}H_{27}N_3O_4$: C, 72.33; H, 5.65; N, 8.73. Found: C, 72.12; H, 5.67; N, 8.75.

*2.1.5.5. 3-(4-(3-(4-Hydroxyphenyl)acryloyl)phenylimino)-1-(morpholinomethyl)indolin-2-one (**5e**)*. Yield 72%, m.p. 158–160 °C. IR (KBr cm^{-1}): 3513 (OH), 2997 (Ar—CH), 1734 (C=O), 1549 (C=N), 1061 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.01–2.75 (t, 4H, CH_2 of morpholine), 2.99–3.67 (t, 4H, CH_2 of morpholine), 4.16 (s, 2H, NCH_2N), 5.63 (s, 1H, OH), 6.78–7.60 (m, 12H, Ar—CH), 7.82–8.14 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 49.8 (C″-2 and C″-6), 70.4 (C″-3 and C″-5), 76.2 (CH_2), 115.5 (C‴-3 and C‴-5), 118.7 (C-9), 120.0 (CH=CH), 122.9 (C-7), 125.3 (C′-2 and C′-6), 126.2 (C-5), 130.8 (C‴-2 and C‴-6), 131.9 (C‴-1), 132.4 (C-4), 134.7 (C′-3 and C′-5), 135.3 (C-6), 139.1 (C′-4), 147.6 (CH=CH), 150.3 (C-8), 155.5 (C‴-4), 158.9 (C′-1), 161.2 (C-3), 161.6 (C-2), 192.4 (C=O). MS (EI): *m/z* 467 [M^+]. Anal. Cald for $C_{28}H_{25}N_3O_4$: C, 71.93; H, 5.39; N, 8.99. Found: C, 71.75; H, 5.41; N, 9.02.

*2.1.5.6. 3-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenylimino)-1-(morpholinomethyl) indolin-2-one (**5f**)*. Yield 79%, m.p. 173–175 °C. IR (KBr cm^{-1}): 3061 (Ar—CH), 2947 (CH_3—CH), 1749 (C=O), 1554 (C=N), 1056 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 1.87–2.59 (t, 4H, CH_2 of morpholine), 3.04 (s, 6H, $N(CH_3)_2$), 3.20–3.85 (t, 4H, CH_2 of morpholine), 4.32 (s, 2H, NCH_2N), 7.19–8.06 (m, 12H, Ar—CH), 8.20–8.47 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 43.4 ($N[CH_3]_2$), 52.7 (C″-2 and C″-6), 65.2 (C″-3 and C″-5), 72.5 (CH_2), 113.9 (C‴-3 and C‴-5), 119.1 (C-9), 122.4 (CH=CH), 123.0 (C-7), 124.8 (C′-2 and C′-6), 125.3 (C-5), 126.2 (C‴-1), 127.5 (C‴-2 and C‴-6), 130.6 (C-4), 132.9 (C′-3 and C′-5), 133.4 (C-6), 135.0 (C′-4), 143.7 (CH=CH), 146.4 (C-8), 150.1 (C‴-4), 162.8 (C′-1), 166.3 (C-3), 167.0 (C-2), 187.5 (C=O). MS (EI): *m/z* 494 [M^+]. Anal. Cald for $C_{30}H_{30}N_4O_3$: C, 72.85; H, 6.11; N, 11.33. Found: C, 73.09; H, 6.09; N, 11.30.

*2.1.5.7. 1-(Morpholinomethyl)-3-(4-(3-(3-nitrophenyl)acryloyl)phenylimino)indolin-2-one (**5g**)*. Yield 76%, m.p. 199–201 °C. IR (KBr cm^{-1}): 3093 (Ar—CH), 1716 (C=O), 1578 (C=N), 1540 and 1333 (NO_2), 1065 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.26–2.94 (t, 4H, CH_2 of morpholine), 3.27–3.90 (t, 4H, CH_2 of morpholine), 4.52 (s, 2H, NCH_2N), 6.83–7.98 (m, 12H, Ar—CH), 8.18–8.45 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 57.7 (C″-2 and C″-6), 64.1 (C″-3 and C″-5), 69.4 (CH_2), 116.6 (C-9), 123.9 (C‴-4), 125.0 (C‴-6), 125.6 (CH=CH), 126.4 (C-7), 127.1 (C′-2 and C′-6), 128.3 (C-5), 129.8 (C-4), 130.0 (C‴-3), 134.4 (C′-3 and C′-5), 135.7 (C-6), 136.5 (C‴-2), 139.1 (C‴-1), 139.8 (C′-4), 149.3 (CH=CH), 150.6 (C-8), 152.9 (C‴-5), 157.2 (C′-1), 162.8 (C-3), 163.4 (C-2), 194.7 (C=O). MS (EI): *m/z* 496 [M^+]. Anal. Cald for $C_{28}H_{24}N_4O_5$: C, 67.73; H, 4.87; N, 11.28. Found: C, 67.98; H, 4.86; N, 11.24.

*2.1.5.8. 3-(4-(3-(2-Chlorophenyl)acryloyl)phenylimino)-1-(morpholinomethyl)indolin-2-one (**5h**)*. Yield 72%, m.p. 221–223 °C. IR (KBr cm^{-1}): 3068 (Ar—CH), 1761 (C=O), 1535 (C=N), 1063 (C—O—C), 744 (Cl). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.02–2.77 (t, 4H, CH_2 of morpholine), 2.91–3.64 (t, 4H, CH_2 of morpholine), 4.09 (s, 2H, NCH_2N), 6.68–7.85 (m, 12H, Ar—CH), 7.93–8.26 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 52.4 (C″-2 and C″-6), 66.8 (C″-3 and C″-5), 70.9 (CH_2), 118.0 (C-9), 123.5 (CH=CH), 123.8 (C-7), 125.3 (C′-2 and C′-6), 126.6 (C-5), 129.1 (C‴-5), 130.7 (C‴-6), 131.5 (C‴-3), 132.2 (C‴-4), 133.0 (C-4), 135.8 (C′-3 and C′-5), 136.4 (C‴-2), 136.9 (C-6), 137.5 (C‴-1), 139.0 (C′-4), 144.3 (CH=CH), 146.1 (C-8), 161.6 (C′-1), 164.9 (C-3), 165.5 (C-2), 184.2 (C=O). MS (EI): *m/z* 487 [M^{+2}]. Anal. Cald for $C_{28}H_{24}ClN_3O_3$: C, 69.20; H, 4.98; N, 8.65. Found: C, 69.44; H, 4.97; N, 8.63.

*2.1.5.9. 1-(Morpholinomethyl)-3-(4-(5-phenylpenta-2,4-dienoyl)phenylimino)indolin-2-one (**5i**)*. Yield 78%, m.p. 206–208 °C. IR (KBr cm^{-1}): 2993 (Ar—CH), 1720 (C=O), 1552 (C=N), 1076 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 1.98–2.60 (t, 4H, CH_2 of morpholine), 2.85–3.41 (t, 4H, CH_2 of morpholine), 4.37 (s, 2H, NCH_2N), 7.19–8.22 (m, 13H, Ar—CH), 8.33–8.98 (m, 4H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 50.2 (C″-2 and C″-6), 69.7 (C″-3 and C″-5), 71.4 (CH_2), 116.9 (C-9), 121.1 (C-7), 122.5 (C′-2 and C′-6), 123.8 (C-5), 125.3 (CH=CH-CH=CH), 126.9 (C‴-2 and C‴-6), 129.0 (C‴-4), 129.4 (C‴-3 and C‴-5), 129.7 (CH=CH—CH=CH), 130.1 (C-4), 132.5 (C′-3 and C′-5), 133.0 (CH=CH—CH=CH), 133.6 (C-6), 136.2 (C‴-1), 136.8 (C′-4), 146.5 (CH=CH—CH=CH), 149.0 (C-8), 162.7 (C′-1), 163.1 (C-3), 163.9 (C-2), 188.4 (C=O). MS (EI): *m/z* 477 [M^+]. Anal. Cald for $C_{30}H_{27}N_3O_3$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.23; H, 5.72; N, 8.83.

*2.1.5.10. 3-(4-(3-(Furan-2-yl)acryloyl)phenylimino)-1-(morpholinomethyl)indolin-2-one (**5j**)*. Yield 75%, m.p. 187–189 °C. IR (KBr cm^{-1}): 3040 (Ar—CH), 1703 (C=O), 1547 (C=N), 1055 (C—O—C). ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.45–2.94 (t, 4H, CH_2 of morpholine), 3.32–3.98 (t, 4H, CH_2 of morpholine), 4.65 (s, 2H, NCH_2N), 6.51–7.06 (m, 3H, CH of furan), 7.25–7.93 (m, 8H, Ar—CH), 8.06–8.27 (m, 2H, CH=CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 55.5 (C″-2 and C″-6), 68.2 (C″-3 and C″-5), 71.7 (CH_2), 110.4 (C‴-3), 112.9 (C‴-4), 116.6 (C-9), 120.3 (C-7), 123.0 (C′-2 and C′-6), 123.8 (C-5), 126.2 (CH=CH), 130.4 (C-4), 132.1 (C′-3 and C′-5), 133.5 (C-6), 133.9 (CH=CH), 138.2 (C′-4), 148.6 (C‴-5), 149.4 (C-8), 154.8 (C‴-2), 156.0 (C′-1), 166.4 (C-3), 166.9 (C-2), 192.3 (C=O). MS (EI): *m/z* 441 [M^+]. Anal. Cald for $C_{26}H_{23}N_3O_4$: C, 70.73; H, 5.25; N, 9.52. Found: C, 70.88; H, 5.23; N, 9.50.

*2.1.6. Synthesis of 1-(morpholinomethyl)-3-(4-(5-substitutedisoxazol-3-yl)phenylimino) indolin-2-one (**6a–6j**)*

To a mixture of 1-(morpholinomethyl)-3-(4-(3-substitutedacryloyl)phenylimino) indolin-2-one **5a–5j** (0.05 mol) in ethanol (20 ml), hydroxylamine hydrochloride (0.05 mol) was added. To this catalytic quantity of sodium acetate glacial acetic acid was added. Then the reaction mixture was stirred and refluxed for 10 h. The resulting mixture was cooled and poured in ice cold water. The obtained product was filtered, dried and recrystallised using ethanol.

*2.1.6.1. 1-(Morpholinomethyl)-3-(4-(5-phenylisoxazol-3-yl) phenylimino)indolin-2-one (**6a**).* Yield 71%, m.p. 261–263 °C. IR (KBr cm^{-1}): 3024 (Ar—CH), 1712 (C═O), 1620 (C═N), 1085 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.53–2.80 (t, 4H, CH_2 of morpholine), 2.95–3.88 (t, 4H, CH_2 of morpholine), 4.82 (s, 2H, NCH_2N), 6.39 (s, 1H, CH of isoxazole), 7.26–8.14 (m, 13H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 52.4 (C″-2 and C″-6), 65.7 (C″-3 and C″-5), 72.0 (CH_2), 94.1 (C‴-4), 118.5 (C-9), 120.9 (C-7), 124.2 (C′-2 and C′-6), 124.6 (C-5), 128.3 (C⁗-2 and C⁗-6), 129.5 (C⁗-4), 130.7 (C′-3 and C′-5), 131.0 (C⁗-3 and C⁗-5), 131.5 (C-4), 132.2 (C⁗-1), 133.1 (C-6), 131.8 (C′-4), 145.3 (C-8), 153.6 (C′-1), 163.4 (C‴-3), 164.2 (C-3), 164.9 (C-2), 172.0 (C‴-5). MS (EI): *m/z* 464 [M^+]. Anal. Cald for $C_{28}H_{24}N_4O_3$: C, 72.40; H, 5.21; N, 12.06. Found: C, 72.65; H, 5.19; N, 12.02.

*2.1.6.2. 1-(Morpholinomethyl)-3-(4-(5-p-tolylisoxazol-3-yl) phenylimino)indolin-2-one (**6b**).* Yield 79%, m.p. 289–291 °C. IR (KBr cm^{-1}): 2995 (Ar—CH), 2904 (CH_3—CH), 1758 (C═O), 1540 (C═N), 1062 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 1.91–2.74 (t, 4H, CH_2 of morpholine), 3.18 (s, 3H, CH_3), 3.40–3.96 (t, 4H, CH_2 of morpholine), 4.50 (s, 2H, NCH_2N), 6.23 (s, 1H, CH of isoxazole), 6.99–7.82 (m, 12H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 27.6 (CH_3), 50.5 (C″-2 and C″-6), 67.1 (C″-3 and C″-5), 71.9 (CH_2), 97.3 (C‴-4), 117.0 (C-9), 122.6 (C-7), 123.4 (C′-2 and C′-6), 125.8 (C-5), 126.2 (C⁗-2 and C⁗-6), 126.7 (C⁗-1), 129.3 (C′-3 and C′-5), 130.6 (C-4), 131.1 (C⁗-3 and C⁗-5), 132.5 (C-6), 132.9 (C′-4), 137.2 (C⁗-4), 148.0 (C-8), 152.4 (C′-1), 161.7 (C‴-3), 163.5 (C-3), 164.0 (C-2), 170.8 (C‴-5). MS (EI): *m/z* 478 [M^+]. Anal. Cald for $C_{29}H_{26}N_4O_3$: C, 72.79; H, 5.48; N, 11.71. Found: C, 73.00; H, 5.46; N, 11.68.

*2.1.6.3. 1-(Morpholinomethyl)-3-(4-(5-(4-nitrophenyl)isoxazol-3-yl)phenylimino)indolin-2-one (**6c**).* Yield 70%, m.p. 254–256 °C. IR (KBr cm^{-1}): 3032 (Ar—CH), 1727 (C═O), 1575 (C═N), 1526 and 1320 (NO_2), 1068 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.17–2.95 (t, 4H, CH_2 of morpholine), 3.12–3.68 (t, 4H, CH_2 of morpholine), 4.77 (s, 2H, NCH_2N), 6.56 (s, 1H, CH of isoxazole), 7.31–8.20 (m, 12H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 52.9 (C″-2 and C″-6), 68.3 (C″-3 and C″-5), 69.0 (CH_2), 99.6 (C‴-4), 120.2 (C-9), 122.8 (C⁗-3 and C⁗-5), 123.0 (C-7), 124.1 (C′-2 and C′-6), 124.7 (C-5), 129.5 (C⁗-2 and C⁗-6), 129.9 (C′-3 and C′-5), 130.2 (C-4), 131.6 (C-6), 133.0 (C′-4), 137.3 (C⁗-1), 149.1 (C-8), 150.6 (C⁗-4), 154.4 (C′-1), 163.9 (C‴-3), 164.7 (C-3), 165.1 (C-2), 172.5 (C‴-5). MS (EI): *m/z* 509 [M^+]. Anal. Cald for $C_{28}H_{23}N_5O_5$: C, 66.00; H, 4.55; N, 13.75. Found: C, 66.17; H, 4.54; N, 13.71.

*2.1.6.4. 3-(4-(5-(4-Methoxyphenyl)isoxazol-3-yl)phenylimino)-1-(morpholinomethyl) indolin-2-one (**6d**).* Yield 73%, m.p. 282–284 °C. IR (KBr cm^{-1}): 3058 (Ar—CH), 2937 (CH_3—CH), 1765 (C═O), 1562 (C═N), 1056 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.37–3.25 (t, 4H, CH_2 of morpholine), 3.31 (s, 3H, OCH_3), 3.53–3.98 (t, 4H, CH_2 of morpholine), 4.94 (s, 2H, NCH_2N), 6.32 (s, 1H, CH of isoxazole), 7.07–7.96 (m, 12H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 49.7 (C″-2 and C″-6), 57.0 (OCH_3), 67.4 (C″-3 and C″-5), 71.9 (CH_2), 96.5 (C‴-4), 110.3 (C⁗-3 and C⁗-5), 114.8 (C-9), 120.6 (C-7), 122.0 (C⁗-1), 122.4 (C′-2 and C′-6), 123.9 (C-5), 130.2 (C⁗-2 and C⁗-6), 130.7 (C′-3 and C′-5), 131.2 (C-4), 131.9 (C-6), 132.5 (C′-4), 145.3 (C-8), 153.0 (C′-1), 158.8 (C⁗-4), 161.4 (C‴-3), 163.5 (C-3), 163.9 (C-2), 169.0 (C‴-5). MS (EI): *m/z* 494 [M^+]. Anal. Cald for $C_{29}H_{26}N_4O_4$: C, 70.43; H, 5.30; N, 11.33. Found: C, 70.61; H, 5.32; N, 11.29.

*2.1.6.5. 3-(4-(5-(4-Hydroxyphenyl)isoxazol-3-yl)phenylimino)-1-(morpholinomethyl) indolin-2-one (**6e**).* Yield 80%, m.p. 268–270 °C. IR (KBr cm^{-1}): 3502 (OH), 3013 (Ar—CH), 1719 (C═O), 1537 (C═N), 1054 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.03–2.83 (t, 4H, CH_2 of morpholine), 3.15-3.74 (t, 4H, CH_2 of morpholine), 4.46 (s, 2H, NCH_2-N), 5.49 (s, 1H, OH), 6.70 (s, 1H, CH of isoxazole), 7.18–8.03 (m, 12H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 51.5 (C″-2 and C″-6), 67.1 (C″-3 and C″-5), 70.8 (CH_2), 98.0 (C‴-4), 117.2 (C⁗-3 and C⁗-5), 118.7 (C-9), 122.1 (C-7), 122.8 (C′-2 and C′-6), 123.9 (C⁗-1), 125.3 (C-5), 127.3 (C′-3 and C′-5), 127.6 (C⁗-2 and C⁗-6), 130.0 (C-4), 132.4 (C-6), 132.9 (C′-4), 148.1 (C-8), 155.6 (C′-1), 157.4 (C⁗-4), 162.5 (C‴-3), 163.1 (C-3), 163.7 (C-2), 170.2 (C‴-5). MS (EI): *m/z* 480 [M^+]. Anal. Cald for $C_{28}H_{24}N_4O_4$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.81; H, 5.05; N, 11.68.

*2.1.6.6. 3-(4-(5-(4-(Dimethylamino)phenyl)isoxazol-3-yl)phenylimino)-1-(morpholino methyl)indolin-2-one (**6f**).* Yield 72%, m.p. 239–241 °C. IR (KBr cm^{-1}): 3075 (Ar—CH), 2913 (CH_3—CH), 1731 (C═O), 1558 (C═N), 1070 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.25–3.19 (t, 4H, CH_2 of morpholine), 3.27 (s, 6H, $N(CH_3)_2$), 3.42–3.97 (t, 4H, CH_2 of morpholine), 4.61 (s, 2H, NCH_2N), 6.45 (s, 1H, CH of isoxazole), 6.80–7.91 (m, 12H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 36.8 ($N(CH_3)_2$), 53.0 (C″-2 and C″-6), 68.3 (C″-3 and C″-5), 71.6 (CH_2), 96.2 (C‴-4), 113.5 (C⁗-3 and C⁗-5), 118.4 (C-9), 120.7 (C⁗-1), 123.1 (C-7), 123.9 (C′-2 and C′-6), 125.1 (C-5), 129.8 (C⁗-2 and C⁗-6), 130.4 (C′-3 and C′-5), 130.7 (C-4), 132.1 (C-6), 132.5 (C′-4), 146.3 (C-8), 149.0 (C⁗-4), 152.8 (C′-1), 161.6 (C‴-3), 163.5 (C-3), 163.8 (C-2), 174.3 (C‴-5). MS (EI): *m/z* 507 [M^+]. Anal. Cald for $C_{30}H_{29}N_5O_3$: C, 70.99; H, 5.76; N, 13.80. Found: C, 70.75; H, 5.78; N, 13.84.

*2.1.6.7. 1-(Morpholinomethyl)-3-(4-(5-(3-nitrophenyl)isoxazol-3-yl)phenylimino)indolin-2-one (**6g**).* Yield 74%, m.p. 246–248 °C. IR (KBr cm^{-1}): 3023 (Ar—CH), 1711 (C═O), 1641 (C═N), 1514 and 1337 (NO_2), 1094 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.19–2.72 (t, 4H, CH_2 of morpholine), 2.90–3.62 (t, 4H, CH_2 of morpholine), 4.33 (s, 2H, NCH_2N), 6.50 (s, 1H, CH of isoxazole), 7.28–8.05 (m, 12H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 51.4 (C″-2 and C″-6), 67.2

(C″-3 and C″-5), 70.9 (CH_2), 100.6 (C‴-4), 115.3 (C-9), 118.1 (C⁗-4), 118.9 (C-7), 119.5 (C⁗-2), 123.0 (C′-2 and C′-6), 126.2 (C-5), 128.7 (C′-3 and C′-5), 130.0 (C-4), 131.4 (C⁗-5), 132.8 (C-6), 133.0 (C⁗-1), 133.7 (C′-4), 135.3 (C⁗-6), 149.6 (C-8), 150.1 (C⁗-3), 154.5 (C′-1), 160.0 (C‴-3), 163.9 (C-3), 164.2 (C-2), 167.8 (C‴-5). MS (EI): *m*/*z* 509 [M^+]. Anal. Cald for $C_{28}H_{23}N_5O_5$: C, 66.00; H, 4.55; N, 13.75. Found: C, 65.79; H, 4.56; N, 13.80.

*2.1.6.8. 3-(4-(5-(2-Chlorophenyl)isoxazol-3-yl)phenylimino)-1-(morpholinomethyl)indolin-2-one (**6h**).* Yield 78%, m.p. 293–295 °C. IR (KBr cm^{-1}): 3080 (Ar—CH), 1747 (C═O), 1542 (C═N), 1076 (C—O—C), 739 (Cl). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.16–2.90 (t, 4H, CH_2 of morpholine), 3.18–3.71 (t, 4H, CH_2 of morpholine), 4.58 (s, 2H, NCH_2N), 6.34 (s, 1H, CH of isoxazole), 6.95–7.99 (m, 12H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 52.0 (C″-2 and C″-6), 65.7 (C″-3 and C″-5), 71.2 (CH_2), 99.4 (C‴-4), 116.9 (C-9), 121.5 (C-7), 123.8 (C′-2 and C′-6), 125.1 (C-5), 128.6 (C⁗-5), 129.3 (C′-3 and C′-5), 129.8 (C⁗-6), 130.2 (C-4), 130.7 (C⁗-3), 131.4 (C⁗-4), 131.9 (C-6), 132.0 (C′-4), 132.6 (C⁗-2), 137.3 (C⁗-1), 146.1 (C-8), 151.8 (C′-1), 163.5 (C‴-3), 165.2 (C-3), 165.7 (C-2), 170.3 (C‴-5). MS (EI): *m*/*z* 500 [M^{+2}]. Anal. Cald for $C_{28}H_{23}ClN_4O_3$: C, 67.40; H, 4.65; N, 11.23. Found: C, 67.62; H, 4.66; N, 11.19.

*2.1.6.9. 1-(Morpholinomethyl)-3-(4-(5-styrylisoxazol-3-yl)-phenylimino)indolin-2-one (**6i**).* Yield 75%, m.p. 230–232 °C. IR (KBr cm^{-1}): 3024 (Ar—CH), 1711 (C═O), 1596 (C═N), 1083 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.00–2.67 (t, 4H, CH_2 of morpholine), 2.85–3.41 (t, 4H, CH_2 of morpholine), 5.09 (s, 2H, NCH_2N), 6.83 (s, 1H, CH of isoxazole), 7.52–8.37 (m, 13H, Ar—CH), 8.56–8.92 (m, 2H, CH═CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 50.9 (C″-2 and C″-6), 68.2 (C″-3 and C″-5), 72.6 (CH_2), 103.1 (C‴-4), 119.8 (C-9), 122.4 (C-7), 122.9 (CH═CH), 123.5 (C′-2 and C′-6), 124.2 (C-5), 128.0 (C⁗-2 and C⁗-6), 129.3 (C⁗-4), 130.6 (C⁗-3 and C⁗-5), 130.9 (C′-3 and C′-5), 131.5 (C-4), 132.3 (C-6), 132.8 (C′-4), 134.0 (CH═CH), 136.2 (C⁗-1), 149.7 (C-8), 152.5 (C′-1), 160.4 (C‴-3), 163.9 (C-3), 164.2 (C-2), 167.6 (C‴-5). MS (EI): *m*/*z* 490 [M^+]. Anal. Cald for $C_{30}H_{26}N_4O_3$: C, 73.45; H, 5.34; N, 11.42. Found: C, 73.67; H, 5.32; N, 11.39.

*2.1.6.10. 3-(4-(5-(Furan-2-yl)isoxazol-3-yl)phenylimino)-1-(morpholinomethyl)indolin-2-one (**6j**).* Yield 81%, m.p. 272–274 °C. IR (KBr cm^{-1}): 3092 (Ar—CH), 1715 (C═O), 1539 (C═N), 1072 (C—O—C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 2.06–2.81 (t, 4H, CH_2 of morpholine), 2.97–3.63 (t, 4H, CH_2 of morpholine), 4.42 (s, 2H, NCH_2N), 6.57 (s, 1H, CH of isoxazole), 6.80–7.19 (m, 3H, CH of furan), 7.33–8.28 (m, 8H, Ar—CH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ ppm: 52.4 (C″-2 and C″-6), 67.1 (C″-3 and C″-5), 69.6 (CH_2), 99.0 (C‴-4), 104.7 (C⁗-4), 106.2 (C⁗-3), 116.5 (C-9), 122.3 (C-7), 124.1 (C′-2 and C′-6), 125.2 (C-5), 127.6 (C′-3 and C′-5), 130.3 (C-4), 131.8 (C-6), 132.1 (C′-4), 141.3 (C⁗-5), 148.5 (C-8), 154.0 (C′-1), 154.7 (C⁗-2), 160.4 (C‴-5), 163.2 (C‴-3), 164.5 (C-3), 164.9 (C-2). MS (EI): *m*/*z* 454 [M^+]. Anal. Cald for $C_{26}H_{22}N_4O_4$: C, 68.71; H, 4.88; N, 12.33. Found: C, 68.49; H, 4.87; N, 12.37.

2.2. Pharmacology

All the synthesized compounds were evaluated for their antiepileptic effects using male albino mice (Swiss, 18–25 g) and rat (Wistar 100–150 g). The primary qualitative evaluations performed in mice involved two epilepsy tests (MES: Maximal Electroshock Seizure test and *sc*PTZ: Subcutaneous pentylenetetrazole). Acute neurological toxicity induced by the compounds in mice was assessed through a standardized rotorod test. In the initial screening, candidate compounds were screened for their antiepileptic potential through MES and *sc*PTZ models in mice at a dose level of 30, 100 and 300 mg/kg by intraperitoneal (i.p.) route and the groups of mice are tested at different time points (i.e., 0.5 and 4 h) post administration of the test candidate. It is generally acknowledged that the MES model, which uses an electrical stimulus, induces generalized tonic–clonic seizures. Through electrical induction, it is used to help recognize those compounds which prevent seizure spread. The *sc*PTZ is a model where the myoclonic seizures were induced by chemical induction. It helps in identifying those compounds that might act by increasing the seizure threshold. Each group consisted of six animals. The animals maintained in colony cages at 25 ± 2 °C, relative humidity of 45–55%, under a 12 h light and dark cycle; were fed standard animal feed.[19] All the animals were acclimatized for a week before use.

2.2.1. The maximal electroshock test (MES)

The MES is a model for generalized tonic–clonic seizures and provides a hint of a compound's ability to stop seizure spread when all neuronal circuits in the brain are maximally active. These seizures are extremely reproducible and are electro physiologically reliable with human seizures. For the MES, a drop of anesthetic and electrolyte solution (tetracaine hydrochloride (0.5%) in saline (0.9%)) was applied to the eyes of individual animal before to 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 delivered for 0.2 s by an apparatus similar to a previously reported method.[20,21] Abolition of the hindleg tonic extensor component of the seizure was used as the endpoint. Mice are initially tested with different doses of 30, 100 and 300 mg/kg of test compound given by i.p. injection at various intervals while rats are initially screened at a fixed dose of 30 mg/kg given by oral route.

2.2.2. The subcutaneous pentylenetetrazole seizure test (scPTZ)

Subcutaneous injection of the convulsant Pentylenetetrazole produces clonic seizures in laboratory animals. The scPTZ test detects the ability of test compounds to raise the seizure threshold of an animal and thus protect it from exhibiting a clonic seizure. Animals are pretreated with various doses of the test compound given by i.p. injection. The dose of Pentylenetetrazole which induces convulsions in 97% of animals (CD_{97}: 85 mg/kg mice) is injected into a loose fold of skin in the midline of the neck. The animals are placed in isolation cages to minimize stress[22] and observed for the next 30 min for the presence or absence of a seizure. An episode of clonic spasms, approximately 3–5 s, of the fore and/or hindlimbs, jaws, or vibrissae is taken as the endpoint. Animals which do not meet this criterion are considered protected.

2.2.3. Acute toxicity-minimal motor impairment

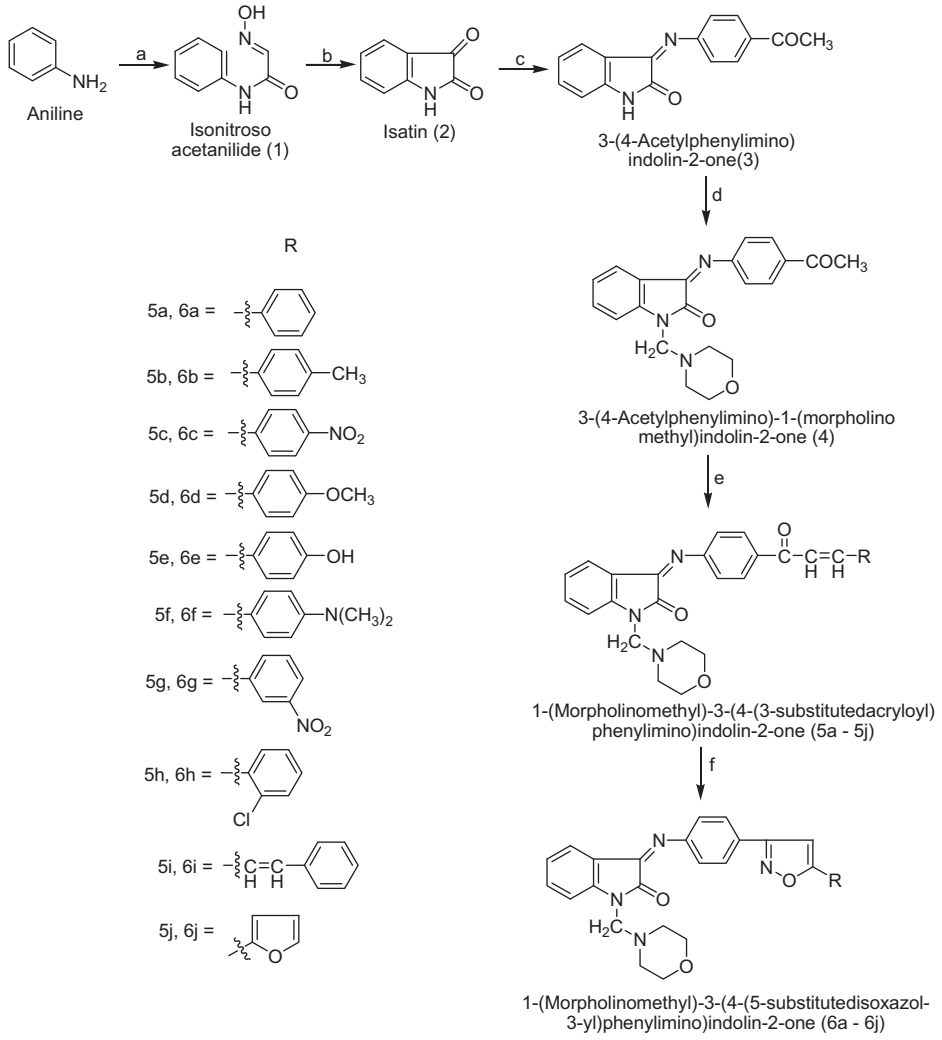
To assess a compound's undesirable side effects (toxicity), animals are monitored for overt signs of impaired neurological or muscular function. In mice, the rotorod[23] procedure is used to disclose minimal muscular impairment (MMI) or minimal neurological impairment (MNI). When a mouse is placed on 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.

3. Results and discussion

3.1. Chemistry

The reaction sequence leading to the synthesis of novel isatin derivatives **5a–5j** and **6a–6j** is outlined in Scheme 1. Initially aniline was treated with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate to form an isonitrosoacetanilide **1** with a loss of 3 mol of hydrochloric acid and one mole of water. In the subsequent step isatin **2** was synthesized through cyclization reaction by treating isonitrosoacetanilide **1** with concentrated sulfuric acid. Isatin **2** was treated with *p*-amino acetophenone to produce 3-(4-acetylphenylimino)indolin-2-one **3** by a condensation reaction, which proceeds selectively on the carbonyl group in position 3 of the isatin ring. Further compound **3** was treated with formaldehyde and secondary amines (morpholine) to synthesize 3-(4-acetylphenylimino)-1-(morpholinomethyl)indolin-2-one **4** by the Mannich reaction. In the pre final step isatin Mannich base analog was treated with different aromatic/heterocyclic aldehydes in the presence of sodium hydroxide to isolate a corresponding Schiff base derivatives **5a–5j**. Finally the Schiff base analogs **5a–5j** were treated with hydroxylamine hydrochloride in the presence of sodium acetate and glacial acetic acid to obtain **6a–6j** through simple ring closure reaction. TLC was performed throughout the reactions to optimize the reactions for purity and completion.

The structures of the synthesized compounds were confirmed by spectral (IR, ^{1}H NMR, mass and elemental analysis)



Scheme 1 Reagents and conditions: (a) $CCl_3CH(OH)_2$, NH_2OH. HCl, 45 min heating; (b) H_2SO_4, heating at 80 °C for 10 min; (c) 4-$NH_2C_6H_4COCH_3$, C_2H_5OH, reflux 6 h; (d) HCHO, morpholine, C_2H_5OH, 1 h stirring, 4 h reflux; (e) RCHO, C_2H_5OH, NaOH, 1 h stirring, 4 h reflux; (f) NH_2OH. HCl, C_2H_5OH, CH_3COOH, CH_3COONa, 10 h reflux.

analyses data. IR, ^{1}H NMR, mass and elemental analyses data of the synthesized compounds are in accordance with the assigned structures. The IR spectrum of **5f** displayed absorption bands at 3061, 2947, 1749, 1554 and 1056 cm^{-1} which was assigned to Ar—CH, CH_3—CH, C=O, C=N and C—O-C vibrations respectively. Also, its ^{1}H NMR spectrum showed double doublet at δ 8.20–8.47 ppm for two protons of CH=CH which confirms the formation of **5f**. It also showed the following other signals in its ^{1}H NMR spectrum at δ ppm 1.87–2.59 (t, 4H, CH_2 of morpholine), 3.04 (s, 6H, $N(CH_3)_2$), 3.20–3.85 (t, 4H, CH_2 of morpholine), 4.32 (s, 2H, NCH_2N) and 7.19–8.06 (m, 12H, Ar—CH) which supports the allotted structure of **5f**. Similarly IR spectrum of **6f** displayed absorption bands at 3075, 2913, 1731, 1558 and 1070 cm^{-1} which were assigned to Ar—CH, CH_3—CH, C=O, C=N and C—O—C vibrations respectively. Appearance of a singlet for one proton of isoxazole (C-4) at δ 6.45 ppm confirms the formation of compound **6f**. In addition the ^{1}H NMR spectrum of compound **6f** also showed signals at following δ ppm 2.25–3.19 (t, 4H, CH_2 of morpholine), 3.27 (s, 6H, $N(CH_3)_2$), 3.42–3.97 (t, 4H, CH_2 of morpholine), 4.61 (s, 2H, NCH_2N), 6.80–7.91 (m, 12H, Ar—CH) which further confirms the assigned structure of **6f**.

3.2. Pharmacology

For the identification of antiepileptic activity in mice, test compounds **5a**–**5j** and **6a**–**6j** were administered i.p. and challenged by maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizure test. Compounds found to be active in these seizure challenges are generally regarded to be significantly useful candidates in the treatment of partial, generalized and even absence seizures. The data regarding the antiepileptic screening of all the compounds are reported in Table 1.

3.2.1. The maximal electroshock test (MES)

Most of the isoxazole coupled isatin derivatives **6a**–**6j** exhibited moderate to good antiepileptic activity in the MES screening; whereas acryloylphenylimino attached isatin derivatives **5a**–**5j** exhibited poor activity. Out of several tested compounds in the electroshock investigation, four compounds **6b**, **6d**, **6e** and **6f** were found to be significantly active. Out of these four compounds, **6e** and **6f** showed protection at the lowest dose of 30 mg/kg after 0.5 h and 4.0 h. Whereas, compounds **6b** and **6d** displayed protection at the lowest dose of 30 mg/kg after 0.5 h. But at a higher dose (300 mg/kg) compounds **6b** and **6d** continued to show the activity after 4.0 h indicating the fast onset as well as long duration of action of these compounds. The hopeful activity of the compounds may be attributed to the substitutions at C-5 of isoxazole. These compounds contain electron donating groups such as methyl, methoxy, hydroxy and dimethylamino group at the distal aryl ring attached to isoxazole. At a dose of 100 mg/kg, after 0.5 h compounds **5e**, **5f**, **6a**, **6i** and **6j** showed protection indicating the ability of these

Table 1 Antiepileptic activity and neurotoxicity of compounds **5a**–**5j** and **6a**–**6j** administered intraperitoneally to mice.

Compound	MES[a] screening		*sc*PTZ[b] screening		NT[c] screening	
	0.5 h[d]	4.0 h[d]	0.5 h[d]	4.0 h[d]	0.5 h[d]	4.0 h[d]
5a	300	—	300	—	300	—
5b	300	300	—	300	—	300
5c	—	—	—	—	ND	ND
5d	300	300	300	—	300	—
5e	100	—	300	300	—	—
5f	100	300	300	300	—	—
5g	—	—	—	—	ND	ND
5h	—	—	—	300	ND	ND
5i	300	—	—	—	ND	ND
5j	—	300	300	—	—	300
6a	100	300	300	—	—	—
6b	30	300	300	300	—	300
6c	—	—	—	300	ND	ND
6d	30	300	100	300	—	—
6e	30	30	100	300	300	300
6f	30	30	100	300	—	—
6g	—	—	300	—	ND	ND
6h	300	—	—	—	ND	ND
6i	100	300	—	300	100	—
6j	100	—	300	—	—	300
Phenytoin[e]	30	30	ND	ND	100	100
Ethosuximide[f]	ND	ND	100	300	—	—

The sign — (mdash) represents an absence of activity at maximum dose administered (300 mg/kg).
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.
[e] Reference drug, data for phenytoin.[15]
[f] Reference drug, data for ethosuximide.[16]

compounds to protect from seizures at a relatively lower dose. These compounds except **5e** and **6j** were also active after 4.0 h at 300 mg/kg dose.

3.2.2. The subcutaneous pentylenetetrazole seizure test (*sc*PTZ)

Compounds that revealed protection in the *sc*PTZ test representing the ability of a substance to increasing seizure thresholds. Among the entire tested compounds in *sc*PTZ test, **6d**, **6e** and **6f** were found to be significantly active at a dose of 100 mg/kg after 0.5 h and these compounds continued to show activity after 4.0 h at 300 mg/kg dose. The above results were equal to results obtained for ethosuximide which is recognized as reference AEDs for this screen. Except **5c**, **5g**, **5i** and **6h**, rest of the compounds **5a**, **5b**, **5d**–**5f**, **5h**, **5j**, **6a**–**6c**, **6g**, **6i** and **6j** were active at 300 mg/kg after 0.5 h and/or 4.0 h. It was observed that in this method, the most active compound has an electron releasing group substituted distal aryl ring at C-5 of isoxazole resulting in increased antiepileptic activity.

Numerous compounds that are, **5a**, **5b**, **5d**–**5f**, **5i**, **5j**, **6a**, **6b** and **6d**–**6j** showed activity in either MES or *sc*PTZ model at any one of the tested doses after 0.5 h. The study reveals that 75% of the compounds showed activity in MES screening, whereas in the *sc*PTZ test 80% of the compounds were active at any one of the tested doses. This information revealed that most of compounds possessed some *sc*PTZ selectivity. In general it was observed that the isoxazole coupled isatin derivatives exhibited better activity than the corresponding acryloylphenylimino attached isatin derivatives. This may be because of the fact that isoxazole coupled isatin derivatives are better fitted into the receptor site.

3.2.3. Acute toxicity-minimal motor impairment

The neurotoxicity study revealed that the majority of the candidate compounds exhibited neurotoxicity at doses higher than commonly prescribed drugs Phenytoin or Carbamazepine [15]. But while evaluating antiepileptic compounds, separation between the antiepileptic and neurotoxic doses is desirable. Due to its poor response in antiepileptic activity, apart from **5c**, **5g**–**5i**, **6c**, **6g** and **6h**, rest of all compounds were screened for the neurotoxicity study. At 100 mg/kg dose **6i** was found to be neurotoxic after 0.5 h. Compounds **5a**, **5b**, **5d**, **5j**, **6b**, **6e**, and **6j** showed neurotoxicity at 300 mg/kg after 0.5 h and/or 4.0 h, whereas all other compounds **5e**, **5f**, **6a**, **6d** and **6f** were not found to be neurotoxic at the maximum administered dose.

3.2.4. Antiepileptic activity and toxicity of compounds **6b**, **6d**, **6e** and **6f** administered orally (30 mg/kg) to rats

Ability to inhibit epilepsy when given by the oral route is a valuable property of candidate antiepilepsy. This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of antiepileptic activity or neurotoxicity. We identified four compounds **6b**, **6d**, **6e** and **6f** from the initial screen that were further evaluated for oral availability using the MES acute seizure model and neurotoxicity in rats at a dose of 30 mg/kg. The results obtained are presented in Table 2.

From this information it was observed that the most active compound is **6f** which protected 100% (4/4) of rats at time points 2 h and 4 h, 75% (3/4) at 1 h, 50% (2/4) at 1 h, and 25% (1/4) at 0.25 h. These molecules were more active and showed a longer duration of satisfactory action than Phenytoin. The other compound **6e** was moderately effective in rat MES oral screening and protected only 75% (3/4) of the tested animals at the time points 2 h and 4 h respectively. Moreover this compound protected 50% (2/4) of the tested animals at 0.5 h and 1 h respectively and 25% (1/4) of tested animals at 0.25 h. Compounds **6b** and **6d** were found to be least effective. Compound **6b** protected 50% (2/4) of the rats at 4 h and 25% (1/4) of the rats at 2 h; whereas compound **6d** protected 50% (2/4) of the rats at 0.5 h and 25% (1/4) of the rats at 1 h. All derivatives tested were non-neurotoxic when given orally. The *in vivo* data in rats confirmed absorption of compounds from the gastrointestinal tract and also their penetration into central nervous system. The inhibition of electrically induced seizures that is characteristic for Phenytoin and Phenytoin-like drugs may point out the influence of compounds on voltage dependent Na^+ channels as the most probable mechanism of antiepileptic action.

3.2.5. Structural activity relationship

On correlating the structures of the title compounds with their biological activity, it has been observed that, out of several tested compounds isoxazole substituted isatin derivatives **6a**–**6j** showed better activity than the corresponding acryloylphenylimino substituted isatin derivatives **5a**–**5j**. The increases in antiepileptic activity of isoxazole substituted isatin derivatives may be attributed to the presence of an extra electron donor atom on the isoxazole ring (which is absent in acryloylphenylimino substituted isatin derivatives) which might be accountable for additional bonding with the binding site. Within the isoxazole

Table 2 Antiepileptic activity and toxicity of compounds **6b**, **6d**, **6e** and **6f** administered orally (30 mg/kg) to rats.

Compound	MES[a]					TOX[b]
	0.25 h[c]	0.5 h[c]	1 h[c]	2 h[c]	4 h[c]	
6b	0/4	0/4	0/4	1/4	2/4	0/4 (-)[d]
6d	0/4	2/4	1/4	0/4	0/4	0/4 (-)[d]
6e	1/4	2/4	2/4	3/4	3/4	0/4 (-)[d]
6f	1/4	2/4	3/4	4/4	4/4	0/4 (-)[d]
Phenytoin[e]	1/4	4/4	3/4	3/4	3/4	0/4 (-)[d]

[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.
[e] Reference drug, data for phenytoin.[15]

derivatives compounds possessing electron releasing groups exhibited higher antiepileptic activity than the compounds containing an electron withdrawing group. However, the unsubstituted derivative exhibits moderate antiepileptic activity.

4. Conclusion

In summary, remembering the pharmacophoric pattern of clinically active AEDs we have designed and synthesized novel indole-2,3-dione derivatives. The structure of synthesized compounds **5a**–**5j** and **6a**–**6j** satisfied all the pharmacophoric structural requirements of AEDs. Entire title compounds were screened for their antiepileptic activity by MES and *sc*PTZ model. The active compounds were also tested for its neurotoxicity. Generally in this study, isoxazole substituted isatin derivatives showed better activity than the corresponding acryloylphenylimino substituted isatin derivatives. In addition electron donating groups containing the compound exhibited higher antiepileptic activity than the electron withdrawing groups containing compound. Out of several tested compounds the most active one was 3-(4-(5-(4-(dimethylamino)phenyl)isoxazol-3-yl)phenylimino)-1-(morpholinomethyl)indolin-2-one **6f** that revealed protection in the electrically induced seizures at a dose of 30 mg/kg (i.p.) after 0.5 h and 4 h. This molecule also provided protection in the *sc*PTZ at a dose of 100 mg/kg and 300 mg/kg after 0.5 h and 4 h respectively. Moreover this compound also exhibited better antiepileptic activity in the oral dose than the standard drug Phenytoin. As a result the compound **6f** emerged out as the pilot molecule with a broad spectrum of antiepileptic activity without any neurotoxicity.

5. Conflict of interest

None.

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