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Isatin-Based Anticonvulsant Agents: Synthesis and Antiseizure Evaluation in Mice

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Based on the positive background of CNS activity of isatin analogs, a new series of isatin-based anticonvulsant derivatives (**3a-3m**) were designed and synthesized. According to the pharmacophoric necessities of anticonvulsant three essential parts namely aromatic ring (AR), electron donor (ED) group and hydrogen bond acceptor/donor (HAD) group were considered in the structure of designed compounds (**3a-3m**). Isatin was treated with various derivatives of aniline in the presences of glacial acetic acid under reflux condition. Two standard convulsive protocols namely maximal electroshock (MES) and pentylenetetrazole (PTZ) were utilized to investigate the anticonvulsant efficacy of these series. Besides, rotorod test was used to assess the neurotoxicity of corresponding compounds. Fortunately, the most of tested derivatives exhibited remarkable preventive effect in both applied convulsive models with low incidence of neurotoxicity in rotorod test.

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Introduction

Epilepsy is a chronic neurological disorder that caused by abnormal activity of cerebral nerve cells in CNS, which is characterized by recurrent convulsions. The disease affects more than 60 million people worldwide according to epidemiological studies [1]. It assumed that convulsions are induced due to imbalance between inhibitory and excitatory neurotransmitters. The treatment of epilepsy is always a deep challenge for researchers and clinical practitioners [2,3]. Although, various new drugs have emerged for the treatment of epilepsy in last decades, about 30% of epileptic patients are resistant to current medications and many of the accessible antiepileptic drugs [2,4]. The current antiepileptic drugs could provide satisfactory seizure control for 60-70% of patients only. These drugs also cause significant adverse reactions such as sedation, ataxia, gastrointestinal side effects, hepatotoxicity, hematotoxicity, severe dermatological side effects and etc.[5]. Based on this reason, there has been a robust motivation and efforts for development and discovery of new antiepileptic drugs [2].

Among the important pharmacophores responsible for anticonvulsant activity, the isatin scaffold is still considered a viable lead structure for the synthesis of more efficacious anticonvulsant activity [5]. Isatin is a bright orange-coloured powder with a long history and a broad range of biological and pharmacological functions. It seems that isatin may have physiological effects in some tissues like brain because of its presence in remarkable levels. It can be both anxiogenic and sedative. Brain monoamine levels may increase by isatin. It is an MAO inhibitor, especially of MAO_B. Isatin is an endogenous indole derivative vastly present in both human and other mammalian tissues and fluids. Perhaps, it occurs as a result of the tryptophan metabolic pathway [6-8]. Recent researches and reports states that many isatin derivatives exhibit a broad range of biological activities such as anticancer, antidepressant, anticonvulsant, antifungal, anti-HIV and anti-inflammatory, etc. [9-11].

Anticonvulsant activity is one of the main promising biological activities that have been demonstrated by this chemical structure [12, 13]. Hence, in the current project we decided to design a new series of anticonvulsant agents based on isatin structure (**Figure 1**). Three main pharmacophoric regions are necessary for anticonvulsant activity theoretically as illustrated for phenytoin and designed compounds **(Figure 1)** namely, (1) Aromatic ring (AR); (2) Electron donor part (ED); (3) Hydrogen bond acceptor/donor (HAD) [14, 15].

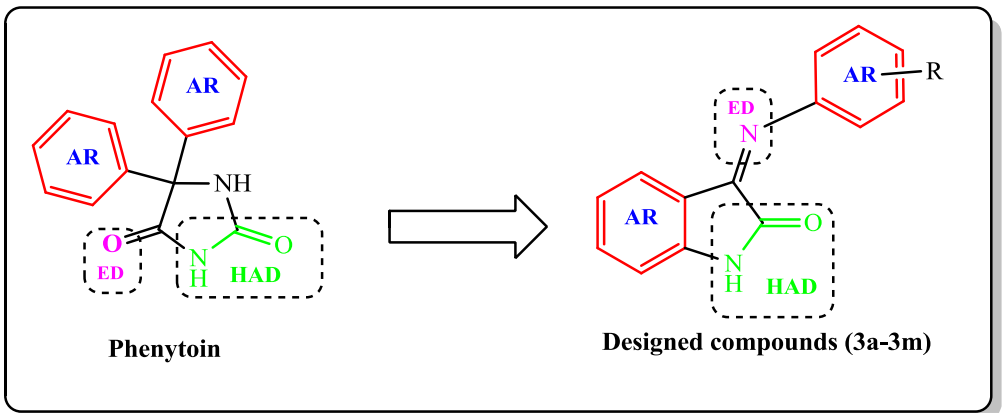

Fig. 1. Design of isatin-based anticonvulsant compounds **3a-3m**.

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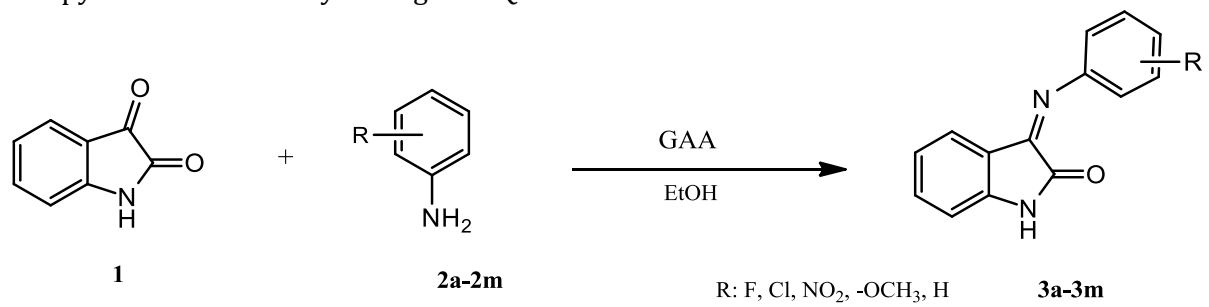
Materials and Methods

Chemistry

Reagents, solvents and starting materials were bought from the accessible commercial vendors like Merck and Sigma-Aldrich. Thin layer chromatography (TLC) on aluminium-based sheet was performed for monitoring the reaction progress. Different proportions of ethyl acetate/petroleum ether were utilized for purification of target compounds using column chromatography. All synthesized derivatives were characterized by spectroscopic techniques such as ^{1}HNMR, IR and MS. Deutrated DMSO-d_6 or chloroform ($CDCl_3$) was applied for dissolution and consequently acquisition of the corresponding ^{1}HNMR spectra by Bruker 500 MHz. The chemical shifts were reported as δ (ppm) related to the tetramethylsilane (TMS) as internal standard. IR spectra were recoded using Shimadzu 470 spectrophotometer by preparing KBr disk. Mass spectroscopy was carried out by a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV and mass of each fragment were provided with its frequency percentage. Melting points for final compounds was also obtained using melting point analyzer apparatus electrothermal 9001A model in open capillary tubes.

General procedure for synthesis of compounds 3a-3m

1 g (6.8 mmol) isatin were mixed with equimolar quantities of appropriate aniline derivative in the presence of glacial acetic acid (1 ml) in absolute ethanol (25 ml) (**Scheme 1**). The reaction mixture was refluxed overnight. TLC was done for monitoring the reaction. If completed, cold water was added to the reaction medium and the formed precipitate was filtered and washed by diethyl ether (Et_2O) and *n*-hexane. Ethyl acetate/petroleum ether was applied for purification process of obtained precipitate in column chromatography [4, 5, 16, 17].



Scheme 1. Synthetic approach for preparation of compounds **3a-3m**.

(Z)-3-((2-Chlorophenyl)imino)indolin-2-one **(3a)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.23 (d, 1H, *J* = 10 Hz, H_7-Isatin), 6.73 (t, 1H, *J* = 10 Hz, H_5-Isatin), 6.87 (t, 1H, *J* = 10 Hz, H_4-2-Chlorophenyl), 7.05 (d, 1H, *J* = 10 Hz, H_6-Isatin), 7.24 (d, 1H, *J* = 10 Hz, H_6-2-Chlorophenyl), 7.33 (t, 1H, *J* = 10 Hz, H_5-2-Chlorophenyl), 7.39 (t, 1H, *J* = 10 Hz, H_4-Isatin), 11.01 (brs, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3213 (NH, Stretch), 3066 (C-H, Stretch, Aromatic), 1747 (C=O, Stretch). MS (*m/z*, %): 258 (M^++2, 15), 256 (M^+, 50), 230 (50), 228 (100), 111 (30), 75 (40).

(Z)-3-((3-Chlorophenyl)imino)indolin-2-one **(3b)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.32 (d, 1H, *J* = 10 Hz, H_7-Isatin), 6.73 (t, 1H, *J* = 10 Hz, H_5-Isatin), 6.86 (d, 1H, *J* = 10 Hz, H_6-Isatin), 6.94 (d, 1H, *J* = 10 Hz, H_6-3-Chlorophenyl), 7.07 (s, H_2-3-Chlorophenyl), 7.27 (t, 1H, H_5-3-Chlrophenyl), 7.33 (t, 1H, *J* = 10 Hz, H_4-Isatin), 7.45 (d, 1H, *J* = 10 Hz, H_4-3-Chlorophenyl), 10.95 (brs, NH-isatin). IR (KBr, cm^{-1}) ῡ: 3197 (NH, Stretch), 3073 (C-H, Stretch, Aromatic), 1747 (C=O, Stretch). MS (*m/z*, %): 258 (M^++2, 25), 256 (M^+, 70), 230 (15), 228 (100), 201 (20), 75 (15).

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(Z)-3-((4-Chlorophenyl)imino)indolin-2-one **(3c)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.40 (d, 1H, *J* = 10 Hz, H_7-Isatin), 6.73 (t, 1H, *J* = 10 Hz, H_5-Isatin), 6.86 (d, 1H, *J* = 10 Hz, H_6-Isatin), 7.00 (d, 1H, *J* = 10 Hz, $H_{2,6}$-4-Nitrophenyl), 7.31 (t, 1H, *J* = 10 Hz, H_4-Isatin), 10.95 (brs, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3190 (NH, Stretch), 3116 (C-H, Stretch, Aromatic), 1739 (C=O, Stretch). MS (*m/z*, %): 258 (M^++2, 35), 256 (M^+, 90), 230 (35), 228 (100), 201 (12), 166 (15), 111 (20), 75 (25).

(Z)-3-((2-Fluorophenyl)imino)indolin-2-one **(3d)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.43 (d, 1H, *J* = 10 Hz, H_7-Isatin), 6.75 (t, 1H, *J* = 10 Hz, H_5-Isatin), 6.87 (m, 1H, H_6-2-Fluorophenyl), 7.10 (m, 1H, H_4-2-Fluorophenyl), 7.26 (m, 1H, H_3-2-Fluorophenyl), 7.36 (m, 1H, H_5-2-Fluorophenyl), 7.45 (t, 1H, *J* = 10 Hz, H_6-Isatin), 7.61 (d, 1H, *J* = 10 Hz, H_4-Isatin), 11.00 (brs, 1H, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3182 (NH, Stretch), 3070 (C-H, Stretch, Aromatic), 1739 (C=O, Stretch). MS (*m/z*, %): 240 (M^+, 45), 212 (100), 185 (15), 95 (20), 75 (35).

(Z)-3-((3-Fluorophenyl)imino)indolin-2-one **(3e)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.33 (d, 1H, *J* = 10 Hz, H_7-Isatin),), 6.72 (t, 1H, *J* = 10 Hz, H_5-Isatin), 6.78 (d, 1H, *J* = 10 Hz, 3-Fluorophenyl), 6.86 (m, 1H, *J* = 10 Hz, 3-Fluorophenyl), 7.04 (t, 1H, 3-Fluorophenyl), 7.33 (t, 1H, 3-Fluorophenyl), 7.46 (t, 1H, *J* = 10 Hz, H_6-Isatin), 7.55 (d, 1H, *J* = 10 Hz, H_4-Isatin), 10.96 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3190 (NH, Stretch), 3074 (C-H, Stretch, Aromatic), 1747 (C=O, Stretch). MS (*m/z*, %): 240 (M^+, 50), 212 (100), 185 (35), 95 (40), 75 (15).

(Z)-3-((4-Fluorophenyl)imino)indolin-2-one **(3f)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.39 (d, 1H, *J* = 10 Hz, H_7-Isatin), 6.72 (t, 1H, *J* = 10 Hz, H_5-Isatin), 6.86 (d, 1H, *J* = 10 Hz, H_4-Isatin), 7.00 (m, 2H, $H_{2,6}$-4-Fluorophenyl), 7.27 (m, 2H, $H_{3,5}$-4-Fluorophenyl), 7.32 (d, 1H, *J* = 10 Hz, H_4-Isatin), 10.94 (brs, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3159 (NH, Stretch), 3086 (C-H, Stretch, Aromatic), 1728 (C=O, Stretch). MS (*m/z*, %): 240 (M^+, 75), 212 (100), 185 (25), 95 (30), 75 (30).

(Z)-3-((2-Methoxyphenyl)imino)indolin-2-one **(3g)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 3.70 (s, 3H, $-OCH_3$), 6.40 (dd, 1H, *J* = 1.5 Hz, 10 Hz, H_7-isatin), 6.48 (dd, 1H, *J* = 2.5, 10 Hz, H_5-2-Methoxyphenyl), 6.57 (dd, 1H, *J* = 1.5, 10 Hz, H_3-3-Methoxyphenyl), 6.62 (t, 1H, H_4-2-Methoxyphenyl), 6.73 (d, 1H, *J* = 10 Hz, H_4-isatin), 6.85 (d, 1H, *J* = 10Hz, H_6-2-Methoxyphenyl), 6.93 (t, 1H, H_5-isatin), 7.15 (t, 1H, H_6-isatin), 10.42 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3387 (NH, Stretch), 3066 (C-H, Stretch, Aromatic), 1708 (C=O, Stretch). MS (*m/z*, %): 252 (M^+, 100), 224 (45), 209 (65), 181 (45).

(Z)-3-((3-Methoxyphenyl)imino)indolin-2-one **(3h)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 3.70 (s, 3H, $-OCH_3$), 6.38 (dd, 1H, *J* = 10, 2 Hz, H_3-2-Methoxyphenyl), 6.48 (dd, 1H, *J* = 10, 2.5 Hz, H_3-2-Methoxyphenyl), 6.57-6.64 (m, 2H, 2-Methoxyphenyl), 6.73 (d, *J* = 10 Hz, 1H, H_7-Isatin), 6.93 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.15 (t, 1H, *J* = 10 Hz, H_6-Isatin), 10.42 (brs, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3240 (NH, Stretch), 3089 (C-H, Stretch, Aromatic), 1708 (C=O, Stretch). MS (*m/z*, %): 252 (M^+, 100), 224 (55), 209 (40), 181 (35).

(Z)-3-((4-Methoxyphenyl)imino)indolin-2-one **(3i)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 3.76 (s, 3H, $-OCH_3$), 6.61 (d, 1H, *J* = 10 Hz, H_7-Isatin), 6.72 (t, 1H, *J* = 10 Hz, H_5-Isatin), 6.85 (d, 1H, *J* = 10 Hz, H_4-Isatin), 6.94 (d, 2H, *J* = 10 Hz, $H_{3,5}$-4-Methoxyphenyl), 7.01 (d, 2H, *J* = 10 Hz, $H_{2,6}$-4-Methoxyphenyl), 7.30 (t, 1H, *J* = 10 Hz, H_6-Isatin), 10.89 (brs, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3167 (NH, Stretch), 3113 (C-H, Stretch, Aromatic), 1739 (C=O, Stretch). MS (*m/z*, %): 252 (M^+, 100), 224 (85), 209 (60), 181 (25).

(Z)-3-((2-Nitrophenyl)imino)indolin-2-one **(3j)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.57 (t, 1H, *J* = 10 Hz, H_4-2-Nitrophenyl), 6.86 (d, 1H, *J* = 10 Hz, H_7-Isatin), 6.96 (d, 1H, *J* = 10 Hz, H_6-2-

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Nitrophenyl), 7.02 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.35 (t, 1H, *J* = 10 Hz, H_5-2-Nitrophenyl), 7.45 (d, 1H, *J* = 10 Hz, H_6-Isatin), 7.53 (t, 1H, *J* = 10 Hz, H_4-Isatin), 7.91 (d, 1H, *J* = 10 Hz, H_6-2-Nitrophenyl). IR (KBr, cm^{-1}) ῡ: 3174 (NH, Stretch), 3109 (C-H, Stretch, Aromatic), 1732 (C=O, Stretch), 1504, 1342 (NO_2, Stretch). MS (*m/z*, %): 267 (M^+, 20), 147 (45), 138 (45), 119 (100), 108 (10), 92 (85), 64 (40).

(Z)-3-((3-Nitrophenyl)imino)indolin-2-one **(3k)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.32 (d, 1H, *J* = 10 Hz, H_7-Isatin), 7.72 (t, 1H, *J* = 10 Hz, H_5-3-Nitrophenyl), 7.82 (s, 1H, 3-Nitrophenyl), 7.92 (d, 1H, *J* = 10 Hz, H_6-3-Nitrophenyl), 8.07 (d, 1H, *J* = 10 Hz, H_4-3-Nitrophenyl), 6.69 (t, 1H, *J* = 10 Hz, H_5-Isatin), 6.86 (d, 1H, *J* = 10 Hz, H_6-Isatin), 7.33 (t, 1H, *J* = 10 Hz, H_4-Isatin), 10.94 (brs, 1H, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3197 (NH, Stretch), 3089 (C-H, Stretch, Aromatic), 1716 (C=O, Stretch), 1527, 1346 (NO_2, Stretch). MS (*m/z*, %): 267 (M^+, 25), 239 (15), 193 (20), 147 (65), 138 (40), 119 (100), 92 (60), 64 (15).

(Z)-3-((4-Nitrophenyl)imino)indolin-2-one **(3l)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.55 (d, 1H, *J* = 10 Hz, $H_{2,6}$-4-Nitrophenyl), 6.87 (d, 1H, *J* = 10 Hz, H_7-Isatin), 7.02 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.46 (d, 1H, *J* = 10 Hz, H_4-Isatin), 7.54 (t, 1H, *J* = 10 Hz, H_6-Isatin), 7.90 (d, 1H, *J* = 10 Hz, $H_{2,6}$-4-Nitrophenyl), 10.95 (brs, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3232 (NH, Stretch), 3113 (C-H, Stretch, Aromatic), 1743 (C=O, Stretch), 1597, 1315 (NO_2, Stretch). MS (*m/z*, %): 267 (M^+, 15), 239 (20), 193 (10), 147 (80), 138 (35), 119 (100), 108 (20), 92 (90), 76 (15), 64 (30).

(Z)-3-(Phenylimino)indolin-2-one **(3m)**

^{1}HNMR (500 MHz, DMSO-d_6) δ (ppm): 6.30 (d, 1H, *J* = 10 Hz, H_7-Isatin), 6.94 (d, 1H, *J* = 10 Hz, H_6-Isatin), 7.04 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.54 (t, 1H, *J* = 10 Hz, H_6-Isatin), 6.67 (t, 1H, *J* = 10 Hz, Phenyl), 6.86 (t, 1H, *J* = 10 Hz, Phenyl), 7.21 (t, 1H, *J* = 10 Hz, Phenyl), 7.29 (t, 1H, *J* = 10 Hz, Phenyl), 7.43 (t, 1H, *J* = 10 Hz, Phenyl), 10.94 (brs, NH-Isatin). IR (KBr, cm^{-1}) ῡ: 3186 (NH, Stretch), 3113 (C-H, Stretch, Aromatic), 1732 (C=O, Stretch). MS (*m/z*, %): 222 (M^+, 12), 179 (25), 145 (35), 131 (60), 91 (100), 77 (20), 43 (15).

Anticonvulsant and neurotoxicity identification tests

The evaluation of the anticonvulsant activity was in accordance with the NIH Anticonvulsant Drug Development (ADD) Program [18]. Adult male NMRI mice (20-25 g) were used as experimental animals. Screening was performed by using of pentylenetetrazole (PTZ), and maximal electroshock seizure (MES) model of seizures. Drugs were dissolved in 0.5% methylcellulose and administered intraperitoneally (ip) in a volume of 0.01 mL/g body weight. The identification procedure is briefly discussed below. Sixteen mice were divided into three groups of four, eight, and four mice. Drugs are then given in 30, 100, or 300 mg/kg to the groups respectively. Thirty minutes later a rotorod test was performed on all animals. After the rotorod test one animal in each group was subjected to the PTZ test and the MES test was performed on one animal in the 30 and 300 mg/kg group and three animals in the 100 mg/kg group. In the PTZ test PTZ, 85 mg/kg dissolved in 0.9% saline, was injected subcutaneously as the convulsive agent. In the MES test, 60-Hz current (50 mA) was delivered for 0.2 second through corneal electrodes in each mice. After four hours of drug injection the rotorod test was performed on the remaining animals in each group and then they were subjected to the MES and PTZ tests as discussed above.

In the MES test after the shock was delivered animals were observed to see if they show a tonic extension in their hind limbs. A tonic extension is defined as the full extension (180°) of the hind limbs with the plane of the body. Absence of this component meant that the test compound was able to prevent the spreading of seizure discharge in the brain.

In the PTZ test animals were observed for 30 minutes after the PTZ injection and absence of a clonic spasm persisting for at least 5 seconds meant that the test substance has the ability to raise the seizure threshold.

In the rotorod test the speed of the rod was set at 6 rpm. If the animal was unable to maintain its

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balance in a 1 minute challenge test the substance was considered neurotoxic.

Identification of median effective dose (ED_{50}) and median neurotoxic dose (TD_{50})

For ED_{50} determination, groups of six mice were injected with different doses of each compound and either PTZ or MES test was done at the time of peak effect (determined previously). We used the method of Litchfield and Wilcoxon for the computation of ED_{50} [18]. TD_{50} was determined with the rotorod test. Groups of animals (n=6) were injected with different doses of test compounds and minimal neurotoxicity were recorded. Protective index (PI)=TD_{50}/ED_{50} was calculated for the MES test. When there was a solubility limitation the results were reported as being greater than the last meaningful PI (**Table 2**).

Table 1. Physicochemical properties of compounds **3a-3m**.

Compound	(R)	Closed Formula	MW(g/mol)	m.p (^{o}C)	Yield (%)
3a	2-Cl	$C_{14}H_9ClN_2O$	256	137	62
3b	3-Cl	$C_{14}H_9ClN_2O$	256	225	91
3c	4-Cl	$C_{14}H_9ClN_2O$	256	256	93
3d	2-F	$C_{14}H_9FN_2O$	240	195	72
3e	3-F	$C_{14}H_9FN_2O$	240	215	64
3f	4-F	$C_{14}H_9FN_2O$	240	211	70
3g	2-OCH_3	$C_{15}H_{12}N_2O_2$	252	239	35
3h	3-OCH_3	$C_{15}H_{12}N_2O_2$	252	>300(decomposed)	42
3i	4-OCH_3	$C_{15}H_{12}N_2O_2$	252	232	41
3j	2-NO_2	$C_{14}H_9N_3O_3$	267	190	39
3k	3-NO_2	$C_{14}H_9N_3O_3$	267	191	37
3l	4-NO_2	$C_{14}H_9N_3O_3$	267	189	41
3m	H	$C_{14}H_{10}N_2O$	222	176	57

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Table 2. Anticonvulsant activity of compounds **3a-3m**.

Compound	R	Class	Dose (mg/kg)	Activity MES time (h)		TOX time (h)		Activity PTZ time (h)	
		1, 2, 3, 4		0.5	4	0.5	4	0.5	4
			30	0/1	0/1	0/4	0/2	1/1	1/1
3a	2-Cl	1	100	2/3	2/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	0/4	0/2	1/1	1/1
			30	1/1	0/1	1/4	0/2	0/1	0/1
3b	3-Cl	3	100	3/3	2/3	5/8	3/4	0/1	0/1
			300	1/1	1/1	4/4	2/2	1/1	1/1
			30	1/1	0/1	0/4	0/2	0/1	0/1
3c	4-Cl	1	100	2/3	1/3	4/8	3/4	0/1	0/1
			300	1/1	1/1	4/4	2/2	0/1	0/1
			30	1/1	1/1	0/4	0/2	1/1	1/1
3d	2-F		100	3/3	2/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	1/4	0/2	1/1	1/1
			30	0/1	0/1	0/4	0/2	0/1	0/1
3e	3-F	1	100	2/3	3/3	7/8	4/4	0/1	0/1
			300	1/1	1/1	4/4	2/2	1/1	1/1
			30	1/1	0/1	1/4	0/2	0/1	0/1
3f	4-F	3	100	2/3	2/3	8/8	4/4	0/1	0/1
			300	1/1	1/1	4/4	2/2	0/1	0/1
			30	1/1	1/1	0/4	0/2	1/1	0/1
3g	$2\text{-}OCH_3$		100	3/3	3/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	2/4	2/2	1/1	1/1
			30	1/1	1/1	0/4	0/2	0/1	0/1
3h	$3\text{-}OCH_3$	1	100	3/3	3/3	3/8	0/4	1/1	0/1
			300	1/1	1/1	4/4	1/2	1/1	1/1
			30	1/1	1/1	0/4	0/2	1/1	1/1
3i	$4\text{-}OCH_3$		100	3/3	2/3	2/8	2/4	1/1	1/1
			300	1/1	1/1	4/4	2/2	1/1	1/1
			30	1/1	0/1	0/4	0/2	1/1	0/1
3j	$2\text{-}NO_2$		100	3/3	3/3	1/8	0/4	1/1	1/1
			300	1/1	1/1	0/4	1/2	1/1	1/1
			30	1/1	0/1	0/4	0/2	1/1	1/1
3k	$3\text{-}NO_2$	1	100	3/3	0/3	2/8	1/4	1/1	1/1
			300	1/1	1/1	1/4	0/2	1/1	1/1
			30	0/1	0/1	1/4	0/2	0/1	0/1
3l	$4\text{-}NO_2$	1	100	2/3	2/3	6/8	4/4	1/1	0/1
			300	1/1	1/1	4/4	2/2	1/1	1/1
			30	1/1	0/1	0/4	0/2	1/1	0/1
3m	H	1	100	3/3	3/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	0/4	0/2	1/1	1/1

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Table 3. Anticonvulsant and neurotoxicity data of some potent derivatives.

Compound	R	MES test	Rotorod test	PI	PTZ test
		ED_{50} (µmol/Kg)	TD_{50} (µmol/Kg)		ED_{50} (µmol/Kg)
3j	2-NO_2	133.7	1104.2	8.25	194.7
3a	2-Cl	271.09	2245.7	8.28	80.85
3g	2-OCH_3	95.2	1029.36	10.8	103.17
3d	2-F	85.29	1812.91	21.25	73.3
3m	H	160.8	2243.2	13.9	154.05
	Diazepam	7.541	5.2	0.68	1.756

Results and Discussion

All synthesized compounds **3a-3m** were tested by two anticonvulsant models. Pentylenetetrazole (PTZ) and maximal electroshock (MES) protocols were utilized for anticonvulsant assay in mice and obtained data were reported as **Tables 1-3**. Neurotoxicity was also assessed by rotorod model in mice. According to **Table 1**, the most of evaluated derivatives demonstrated remarkable antiseizure activity at 30 mg/Kg dose in both MES and PTZ model. Interestingly, the most of them were also nontoxic at this dose in rotorod model.

Structure activity relationship (SAR)

Different derivatives containing electron withdrawing as well as electron donating moieties were synthesized to explore the role of electronic effect on the phenyl residue. Therefore, halogens like chlorine and fluorine and also nitro group were applied as electron withdrawing moieties. On the other hand, methoxy substituent was introduced on the phenyl residue as electron donating moiety. Fluorine as electron withdrawing moiety caused a better enhancement in anticonvulsant activity in comparison with chlorine. Fluorine when substituted at positions *meta* and *para* prevented the convulsive effect both in PTZ and MES model. Movement of fluorine to the *ortho* position declined the preventive effect and was detrimental for anticonvulsant activity. Chlorinated derivatives (**3a-3c**) were effective at dose 30 mg/Kg in MES as well as PTZ model. Only, compound **3a** with *ortho* positioning of the chlorine did not render effectiveness in PTZ model. Compounds **3b** and **3c** did not exhibited rapid onset of action after 0.5 h but showed preventive effect after 4 h. Derivatives that substituted with nitro moiety (**3j, 3k, 3l**) were also effective anticonvulsant agents especially at dose 30 mg/Kg in MES model. Amongst them, compound **3l** exerted faster initiation in anticonvulsant activity compared to compounds **3j** and **3k**. Namely, 0.5 h after injection of compound **3l** with 30mg/Kg anticonvulsant activity was observed but this evident was not seen for compounds **3j** and **3k**. But, compounds **3j** and **3k**, had long duration of action and provided anticonvulsant effect 4 h after dose injection. Nitro containing derivatives were also potent anticonvulsant agents in PTZ model while nitro group substituted at *ortho* and *para* positions. Substitution of nitro moiety at position *meta* of the phenyl residue did not cause a beneficial anticonvulsant activity at all tested doses 30, 100 and 300 mg/Kg. Comparison of compounds **3j** (2-NO_2) and **3l** (3-NO_2) in PTZ model display that *para* positioning of the nitro moiety improves the onset of action. Compounds **3g**, **3h** and **3i** as methoxylated derivatives did not possess significant and acceptable anticonvulsant activity in MES model at 30-300 mg/Kg doses. Probably electron donating property and hydrophilicity are not positive factors on the phenyl residue to enhance the potency. Hydrophilicity may decrease the BBB passing and CNS penetration. But in PTZ model, methoxylated derivatives were so effective at 30 mg/Kg dose especially at positions *ortho* and *meta*. Fortunately, these derivatives did not demonstrate any neurotoxicity in rotorod model at 30 mg/Kg. Testing of compound **3m** as substituent-free derivative on the phenyl residue demonstrated a favorable antiseizure potency at 30 mg/Kg dose in MES as well as PTZ model. High lipophilicity and consequently high CNS

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penetration and bioavailability may be the responsible parameters for this evident. This compound did not exert any neurotoxicity up to 300 mg/Kg dose in investigated mice.

Conclusion

A new series of isatin-based anticonvulsant agents were synthesized and their antiseizure activity was investigated. Overall, the synthesized compounds demonstrated potent anticonvulsant effects in MES as well as PTZ convulsive models in mice.

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Conflict of Interests

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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