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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Structure-based design, synthesis, and anticonvulsant activity of isatin-1-*N*-phenylacetamide derivatives

Chao Xie · Li-Ming Tang · Fu-Nan Li · Li-Ping Guan · Cheng-Yan Pan · Si-Hong Wang

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Abstract In an effort to develop the potent anticonvulsant agents, a series of novel isatin-1-*N*-phenylacetamide derivatives was synthesized and screened for their in vivo anticonvulsant activity against maximal electroshock test and evaluated for their neurotoxicity by the rotarod test at the same dose levels. Ten compounds exhibited the anticonvulsant activity. 2-(5-Methyl-2,3-dioxoindolin-1-yl)-*N*-phenylacetamide (**4b**) was found to be the most potent compound of the series with an ED_{50} of 91.3 mg/kg, TD_{50} of >1,000 mg/kg, a higher protective index ($PI = TD_{50}/ED_{50}$, >11) was gained than the reference drug phenobarbital and carbamazepine. The essential structural features responsible for interaction with receptor site are established within a suggested pharmacophore.

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Keywords Isatin-1-*N*-phenylacetamide · Synthesis · Anticonvulsant activity · MES

Introduction

Epilepsy is a chronic disease that is characterized by paroxysmal attacks caused by pathologic excitation of cerebral neurons. The mechanism of action of antiepileptic drugs (AEDs) consist in the blockade of voltage-dependent Na^+ channels or T-type Ca^{2+} channels, inhibition of glutamatergic transmission, and facilitation of γ-aminobutyric acid (GABA) inhibitory neurotransmission. Phamacotherapy is the mainstay treatment for epilepsy and the choice of AED for a particular patient is made according to the seizure type (Praveen *et al*., 2011). Despite the apparent success of the current discovery process, more efficacious and less toxic AEDs are of significant demand. Besides, new AEDs are needed for the patients whose seizures remain refractory to the currently available AEDs (Rémi *et al*., 2010; Deng *et al*., 2010; Rogawski and Löscher, 2004; Meldrum and Rogawski, 2007).

Isatin (2,3-dioxindole) is an endogenous compound identified in humans, and its effect has been studied in a variety of systems. Biological properties of isatin include a range of actions in the brain and offer protection against certain types of infections, such as antiproliferative, antibacterial, antiprotozoal, anti-inflammatory, proconvulsant, and anticonvulsant activities (Pandeya *et al*., 2005, 2000; Bhattacharya and Chakrabarti, 1998).

Besides, (Li *et al*., 1999) studied the inhibitory effect of isatin on amygdaloid kindling in rats, seizure and anticonvulsant effect in convulsion models. Pajouhesh *et al*., (1983) synthesized a series of cyclohexane and other cyclic ketone derivatives of isatin and screened them for anticonvulsant activity. Sridhar *et al*., (2002) reported the

anticonvulsant activity of hydrazones; Schiff and Mannich bases of isatin were evaluated by maximal electroshock test (MES) method and metrazol-induced convulsions (MET). These results suggest that researchers explore isatin as new chemical entity with the potential anticonvulsant. Furthermore, many researchers reported that isatin can not only evidently improve internal monoamine neurotransmitter to antigonize mouse or mice electric (PharmaKarali and Gursoy, 1994) and metrazol seizure effectively, but also decrease the epilepsy probability of audiogenic seizure rats and enhance the anticonvulsant effect of Propranolol (Gursoy *et al*., 1996; Kopin, 1994; Nomoto *et al*., 2001).

Our interest is in developing the new anticonvulsant compounds, in order to test this hypothesis we have synthesized a series of novel isatin-1-*N*-phenylacetamide derivative using isatin as lead compound (Fig. 1) and evaluated their anticonvulsant properties by using MES in mice. Their structures were characterized using IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis techniques. The neurotoxicity of the compounds was also evaluated at the experimental dose level.

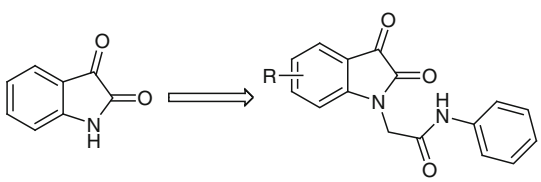
Experimental methods

Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730 (Bruker, Switzerland), ^{1}H NMR and ^{13}C NMR spectra was measured on an AV-300 (Bruker, Switzerland), and all chemical shifts were given in ppm relative to tetramethysilane. Mass spectra were measured on an HP1100LC/MS (Agilent Technologies, USA). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer (Heraeus, Hanau, Germany). The major chemicals were purchased from Alderich Chemical Corporation. All other chemicals were the analytical grade.

*The synthesis of substituted 2-(phenylamino)acetaldehyde oxime(**2a–2n**)*

Sodium sulfate (8.52 g, 60 mmol) and 30 mL water were placed in a 100 mL round-bottomed flask fitted with an efficient mechanical stirrer and then the temperature was controlled at 50–55 °C. To this solution were then added, in order: chloral hydrate (1.99 g, 12 mmol), the solution of aromatic amines (0.93 g, 10 mmol) and 1.2 mL concentrated hydrochloric acid in 10 mL water, and the solution of hydroxylamine hydrochloride (2.22 g, 32 mmol) in 10 mL water. The mixture was stirred at 65–70 °C for 2–5 h, until the reaction was over which was confirmed using TLC. During the heating period, some crystals of isonitrosoacetanilide separate. The solution was cooled in running water and the product was filtered with suction, and then dried in infrared drying oven (Marvel and Hiers, 1941; Gassman *et al*., 1977; Patel *et al*., 2006).



Isatin Target compounds **4a-4n**

Fig. 1 The structure of Isatin and target compounds **4a–4n**

*The synthesis of substituted isatin (**3a–3n**)*

Concentrated sulfuric acid 30 mL was warmed to 50 °C in a 50 mL round-bottomed flask fitted with an efficient mechanical stirrer. Dry substituted compounds **2a–2n** (1.31 g, 8 mmol) are added at such a rate as to keep the temperature between 60 and 70 °C. External cooling should be applied at this stage so that the reaction can be carried out more rapidly. After the addition of the isonitroso compound is finished, the solution is heated to 80 °C and kept at this temperature for about ten to 15 min to complete the reaction. Then the reaction mixture is cooled to room temperature and poured upon ten to twelve times its volume of cracked ice. After standing for about one-half hour, the isatin is filtered with suction, washed several times with cold water to remove the sulfuric acid, and then dried by infrared. This product is pure enough for the next reaction (Marvel and Hiers, 1941; Gassman *et al*., 1977).

*The preparation of 2-chloro-N-phenylacetamide (**5**)*

Aniline (1 g, 10.7 mmol) were dissolved in a mixture of 25 mL of glacial acetic acid and 25 mL of saturated solution of sodium acetate. To this, chloroacetyl chloride (1 mL, 8.8 mmol) was added dropwise to avoid the vigorous reaction. After half an hour, product was filtered with suction, washed several times with cold water, and then infrared dried. The product was crystallized from methanol (Modi *et al*., 2011).

*General procedure for preparation of isatin-1-N-phenylacetamide(**4a–4n**)*

Compounds **3a–3n** (1 g, 6.8 mmol) were taken up in 30 mL anhydrous DMF and ice cooled with stirring. Solid K_2CO_3 (1 g, 7.2 mmol) was added in one portion, and the dark-colored suspension was raised to room temperature and stirred for a further 1 h. The appropriate 2-chloro-*N*-phenylacetamide (1 g) and KI (0.5 g, 6 mmol) were added,

and the reaction mixture was stirred at 80 °C for 2–24 h, until the reaction was over which was confirmed using TLC. The reaction mixture was poured into six times its volume of water, and then diluted hydrochloric acid was added until the pH value is between three and four stirred for a further 10 min. The crude final product was filtered with suction, washed several times with cold water, and then infrared dried. The product was crystallized from methanol (Modi *et al*., 2011). The yield, melting point, and spectral data of each compound are given below.

*2-(2,3-Dioxoindolin-1-yl)-N-phenylacetamide (**4a**)* IR (KBr) cm^{-1}: 3490, 1731, 1649, 1253. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.90 (1H, s, –NH), 7.26–7.56 (5H, m, $–C_6H_5$), 6.93–7.64 (4H, m, $–C_6H_4$), 4.57 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 211.12(C=O), 181.55(C=O), 164.33(C=O), 158.78(Ar–C), 150.87(Ar–C), 140.69(Ar–C), 138.16(Ar–C), 137.49(Ar–C), 128.81(Ar–C), 126.64(Ar–C), 124.34(Ar–C), 120.27(Ar–C), 117.34(Ar–C), 109.25(Ar–C), 98.72(Ar–C), 45.44(CH_2–C). Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; O, 17.13. Found: C, 68.46; H, 4.24; O, 17.04. MS *m/z*: 281 (M+1).

*2-(5-Methyl-2,3-dioxoindolin-1-yl)-N-phenylacetamide (**4b**)* IR (KBr) cm^{-1}: 3501, 1733, 1649, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.90 (1H, s, –NH), 7.23–7.48 (5H, m, $–C_6H_5$), 6.78–7.60 (3H, m, $–C_6H_3$), 4.47 (2H, s, $–CH_2$), 2.27(3H, s, $–CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): 219.87(C=O), 183.30(C=O), 164.77(C=O), 158.78(Ar–C), 148.51(Ar–C), 138.83(Ar–C), 138.10(Ar–C), 133.62(Ar–C), 128.75(Ar–C), 125.24(Ar–C), 124.28(Ar–C), 120.32(Ar–C), 117.78(Ar–C), 115.51(Ar–C), 103.56(Ar–C), 48.00(CH_2–C), 20.71(CH_3–C). Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 69.38; H, 4.79; O, 16.31. Found: C, 69.43; H, 4.69; O, 16.20. MS *m/z*: 295 (M+1).

*2-(6-Methyl-2,3-dioxoindolin-1-yl)-N-phenylacetamide (**4c**)* IR (KBr) cm^{-1}: 3491, 1731, 1649, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.84 (1H, s, –NH), 7.22–7.45 (5H, m, $–C_6H_5$), 6.85–7.48 (3H, m, $–C_6H_3$), 4.47 (2H, s, $–CH_2$), 2.50(3H, s, $–CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): 212.32(C=O), 183.45(C=O), 164.73(C=O), 158.58(Ar–C), 151.27(Ar–C), 140.73(Ar–C), 138.00(Ar–C), 137.60(Ar–C), 128.73(Ar–C), 126.23(Ar–C), 124.31(Ar–C), 120.33(Ar–C), 116.50(Ar–C), 107.85(Ar–C), 95.71(Ar–C), 45.94(CH_2–C), 18.04(CH_3–C). Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 69.38; H, 4.79; O, 16.31. Found: C, 69.27; H, 4.59; O, 16.25. MS *m/z*: 295 (M+1).

*2-(7-Methyl-2,3-dioxoindolin-1-yl)-N-phenylacetamide (**4d**)* IR (KBr) cm^{-1}: 3507, 1732, 1651, 1252. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.99 (1H, s, –NH), 7.28–7.55 (5H, m, $–C_6H_5$), 7.02–7.64 (3H, m, $–C_6H_3$), 4.78 (2H, s, $–CH_2$), 2.45(3H, s, $–CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): 212.22(C=O), 182.49(C=O), 164.77(C=O), 158.59(Ar–C), 150.87(Ar–C), 140.58(Ar–C), 138.27(Ar–C), 136.68(Ar–C), 128.81(Ar–C), 126.27(Ar–C), 124.32(Ar–C), 120.45(Ar–C), 116.38(Ar–C), 108.97(Ar–C), 99.79(Ar–C), 44.84(CH_2–C), 18.35(CH_3–C). Anal. Calcd. for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; O, 16.31. Found: C, 69.23; H, 4.82; O, 16.24. MS *m/z*: 295 (M+1).

*2-(5-Fluoro-2,3-dioxoindolin-1-yl)-N-phenylacetamide (**4e**)* IR (KBr) cm^{-1}: 3495, 1731, 1648, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.98 (1H, s, –NH), 7.23–7.48 (5H, m, $–C_6H_5$), 6.94–7.70 (3H, m, $–C_6H_3$), 4.51 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 211.39(C=O), 182.17(C=O), 164.60(C=O), 158.79(Ar–C), 152.34(Ar–C), 142.54(Ar–C), 138.89(Ar–C), 138.11(Ar–C), 132.06(Ar–C), 128.80(Ar–C), 127.57(Ar–C), 124.52(Ar–C), 120.28(Ar–C), 118.24(Ar–C), 102.26(Ar–C), 43.55(CH_2–C). Anal. Calcd. for $C_{16}H_{11}FN_2O_3$: C, 64.43; H, 3.72; O, 16.09. Found: C, 64.35; H, 3.60; O, 15.83. MS *m/z*: 299 (M+1).

*2-(6-Fluoro-2,3-dioxoindolin-1-yl)-N-phenylacetamide (**4f**)* IR (KBr) cm^{-1}: 3490, 1732, 1649, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ10.04 (1H, s, –NH), 7.24–7.61 (5H, m, $–C_6H_5$), 6.98–7.70 (3H, m, $–C_6H_3$), 4.52 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 212.09(C=O), 181.04(C=O), 164.50(C=O), 158.85(Ar–C), 152.16(Ar–C), 142.69(Ar–C), 138.78(Ar–C), 138.19(Ar–C), 131.98(Ar–C), 128.83(Ar–C), 127.70(Ar–C), 124.27(Ar–C), 120.11(Ar–C), 118.24(Ar–C), 102.20(Ar–C), 43.64(CH_2–C). Anal. Calcd. for $C_{16}H_{11}FN_2O_3$: C, 64.43; H, 3.72; O, 16.09. Found: C, 64.38; H, 3.65; O, 15.86. MS *m/z*: 299 (M+1).

*2-(5-Chloro-2,3-dioxoindolin-1-yl)-N-phenylacetamide (**4g**)* IR (KBr) cm^{-1}: 3500, 1731, 1650, 1253. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.97(1H, s, –NH), 7.23–7.52 (5H, m, $–C_6H_5$), 6.93–7.67 (3H, m, $–C_6H_3$), 4.51 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 211.45(C=O), 182.12(C=O), 164.49(C=O), 158.20(Ar–C), 149.08(Ar–C), 140.37(Ar–C), 138.07(Ar–C), 137.64(Ar–C), 129.21(Ar–C), 128.80(Ar–C), 127.21(Ar–C), 124.64(Ar–C), 124.35(Ar–C), 120.20(Ar–C), 112.40(Ar–C), 43.55(CH_2–C). Anal. Calcd. for $C_{16}H_{11}ClN_2O_3$: C, 61.06; H, 3.52; O, 15.25. Found: C, 60.80; H, 3.41; O, 15.09. MS *m/z*: 315 (M+1).

*2-(6-Chloro-2,3-dioxoindolin-1-yl)-N-phenylacetamide (**4h**)* IR (KBr) cm^{-1}: 3491, 1731, 1648, 1253. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.01 (1H, s, –NH), 7.24–7.53 (5H, m, $–C_6H_5$), 6.92–7.87 (3H, m, $–C_6H_3$), 4.53 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 213.64(C=O), 180.10(C=O), 164.58(C=O), 158.66(Ar–C), 152.05(Ar–C), 143.99(Ar–C), 138.88(Ar–C), 138.25(Ar–C), 132.46(Ar–C), 128.

84(Ar–C), 126.11(Ar–C), 124.28(Ar–C), 120.25(Ar–C), 120.04(Ar–C), 109.54(Ar–C), 43.64(CH_2–C). Anal. Calcd. for $C_{16}H_{11}ClN_2O_3$: C, 61.06; H, 3.52; O, 15.25. Found: C, 60.82; H, 3.40; O, 15.05. MS *m/z*: 315 (M+1).

2-(7-Chloro-2,3-dioxoindolin-1-yl)-N-phenylacetamide ***(4i)*** IR (KBr) cm^{-1}: 3502, 1732, 1649, 1253. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.98 (1H, s, –NH), 7.23–7.55 (5H, m, $–C_6H_5$), 6.99–7.91 (3H, m, $–C_6H_3$), 4.82 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 211.32(C=O), 182.14(C=O), 165.62(C=O), 159.04(Ar–C), 146.05(Ar–C), 139.98(Ar–C), 138.42(Ar–C), 132.19(Ar–C), 128.85(Ar–C), 125.02(Ar–C), 124.20(Ar–C), 123.79(Ar–C), 120.83(Ar–C), 120.38(Ar–C), 106.99(Ar–C), 45.12(CH_2–C). Anal. Calcd. for $C_{16}H_{11}ClN_2O_3$: C, 61.06; H, 3.52; O, 15.25. Found: C, 60.87; H, 3.43; O, 15.08. MS *m/z*: 315 (M+1).

2-(5-Bromo-2,3-dioxoindolin-1-yl)-N-phenylacetamide ***(4j)*** IR (KBr) cm^{-1}: 3501, 1731, 1649, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.06 (1H, s, –NH), 7.26–7.51 (5H, m, $–C_6H_5$), 6.93–7.92 (3H, m, $–C_6H_3$), 4.52 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 199.51(C=O), 181.96(C=O), 164.57(C=O), 157.80(Ar–C), 151.83(Ar–C), 138.71(Ar–C), 138.27(Ar–C), 128.85(Ar–C), 126.02(Ar–C), 124.31(Ar–C), 124.28(Ar–C), 120.56(Ar–C), 120.31(Ar–C), 114.56(Ar–C), 102.09(Ar–C), 43.52(CH_2–C). Anal. Calcd. for $C_{16}H_{11}BrN_2O_3$: C, 53.50; H, 3.09; O, 13.36. Found: C, 53.44; H, 3.19; O, 13.22. MS *m/z*: 360 (M+1).

2-(6-Bromo-2,3-dioxoindolin-1-yl)-N-phenylacetamide ***(4k)*** IR (KBr) cm^{-1}: 3494, 1731, 1650, 1250. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.93 (1H, s, –NH), 7.24–7.56 (5H, m, $–C_6H_5$), 6.98–7.75 (3H, m, $–C_6H_3$), 4.88 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 215.26(C=O), 182.17(C=O), 165.59(C=O), 159.19(Ar–C), 147.52(Ar–C), 143.33(Ar–C), 138.31(Ar–C), 128.79(Ar–C), 125.30(Ar–C), 124.28(Ar–C), 124.23(Ar–C), 121.10(Ar–C), 120.48(Ar–C), 112.57(Ar–C), 104.38(Ar–C), 44.83(CH_2–C). Anal. Calcd. for $C_{16}H_{11}BrN_2O_3$: C, 53.50; H, 3.09; O, 13.36. Found: C, 53.41; H, 3.12; O, 13.28. MS *m/z*: 360 (M+1).

2-(7-Bromo-2,3-dioxoindolin-1-yl)-N-phenylacetamide ***(4l)*** IR (KBr) cm^{-1}: 3492, 1731, 1649, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.06 (1H, s, –NH), 7.24–7.65 (5H, m, $–C_6H_5$), 6.93–7.80 (3H, m, $–C_6H_3$), 4.53 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 212.11(C=O), 181.98(C=O), 164.52(C=O), 158.05(Ar–C), 149.63(Ar–C), 140.45(Ar–C), 138.22(Ar–C), 128.80(Ar–C), 127.38(Ar–C), 124.27(Ar–C), 121.14(Ar–C), 120.21(Ar–C), 119.16(Ar–C), 112.99(Ar–C), 102.35(Ar–C), 55.63(CH_2–C). Anal. Calcd. for $C_{16}H_{11}BrN_2O_3$: C, 53.50; H, 3.09; O, 13.36. Found: C, 53.46; H, 3.15; O, 13.24. MS *m/z*: 360 (M+1).

2-(5,7-Dibromo-2,3-dioxoindolin-1-yl)-N-phenylacetamide ***(4m)*** IR (KBr) cm^{-1}: 3497, 1732, 1649, 1250. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.89 (1H, s, –NH), 7.24–7.64 (5H, m, $–C_6H_5$), 6.99–7.88 (2H, m, $–C_6H_2$), 4.86 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 212.45(C=O), 181.05(C=O), 165.42(C=O), 156.79(Ar–C), 150.98(Ar–C), 144.24(Ar–C), 138.31(Ar–C), 128.80(Ar–C), 126.82(Ar–C), 124.33(Ar–C), 124.15(Ar–C), 120.54(Ar–C), 120.23(Ar–C), 116.54(Ar–C), 105.29(Ar–C), 49.58(CH_2–C). Anal. Calcd. for $C_{16}H_{10}Br_2N_2O_3$: C, 43.87; H, 2.30; O, 10.96. Found: C, 43.68; H, 2.19; O, 10.78. MS *m/z*: 439 (M+1).

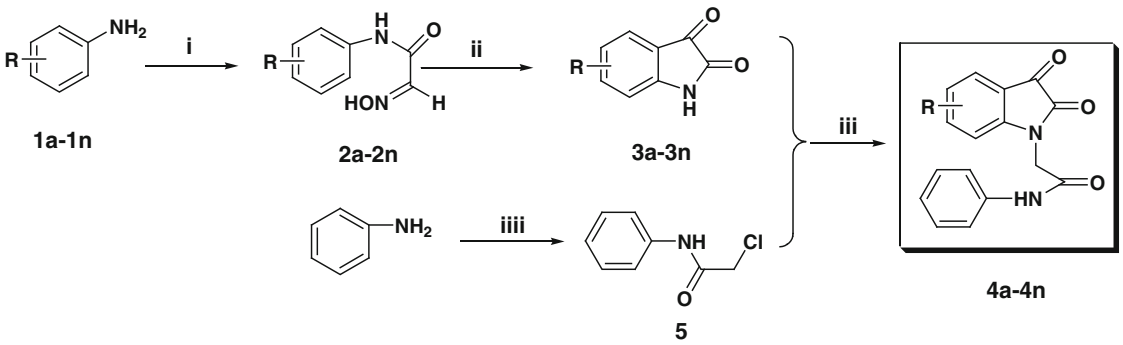
2-(5-Nitro-2,3-dioxoindolin-1-yl)-N-phenylacetamide ***(4n)*** IR (KBr) cm^{-1}: 3496, 1731, 1649, 1253. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.72 (1H, s, –NH), 7.27–7.58 (5H, m, $–C_6H_5$), 7.05–8.03 (3H, m, $–C_6H_3$), 4.55 (2H, s, $–CH_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 210.12(C=O), 182.09(C=O), 164.97(C=O), 156.80(Ar–C), 151.08(Ar–C), 143.74(Ar–C), 138.34(Ar–C), 128.67(Ar–C), 126.69(Ar–C), 124.31(Ar–C), 124.19(Ar–C), 120.36(Ar–C), 120.19(Ar–C), 115.54(Ar–C), 104.78(Ar–C), 48.48(CH_2–C). Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 59.08; H, 3.41; O, 24.59. Found: C, 60.18; H, 3.34; O, 24.47. MS *m/z*: 326 (M+1).

Pharmacology

The MES test and rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program of the National Institutes of Health following previously described testing procedures (USA) (Krall *et al*., 1978; Porter *et al*., 1984). All compounds, which were dissolved in DMSO, were evaluated for the anticonvulsant activity with Balb/e mice in the 20–25 g weight range. Groups of 10 mice were given a range of intraperitoneal (*i.p.*) doses of the test drug until at least three points were established in the range of 10–90 % seizure protection or minimal observed neurotoxicity. The control drug carbamazepine was tested by the same procedure. From the plots of these data, the respective ED_{50} and TD_{50} values, 95 % confidence intervals, slopes of the regression line, and the standard error of the slopes were calculated by means of a computer program written by the National Institute of Neurological Disorders and Stroke.

The MES test

Seizures were elicited with a 60-Hz alternating current of 50 mA intensity in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. At 30 min after the administration of the compounds, the activities were evaluated in MES test.



R:

4a=H 4b=*o*-CH_3 4c=*m*-CH_3 4d=*p*-CH_3 4e=*m*-F
4f=*p*-F 4g=*o*-Cl 4h=*m*-Cl 4i=*p*-Cl 4j=*o*-Br
4k=*m*-Br 4l=*p*-Br 4m=2,4-Br_2 4n=*p*-NO_2

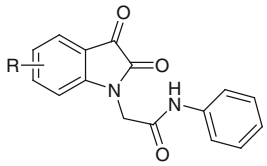
Reaction and condition:

(i) $CCl_3CH(OH)_2$, $NH_2OH{\cdot}HCl$, Na_2SO_4, HCl, H_2O, 65–70℃; (ii) concentrated H_2SO_4, 80℃, H_2O, H_2SO_4;

(iii)$ClCH_2COCl$, HAc/satirated NaAc, 0–5℃; (iiii)DMF, KI, K_2CO_3, 80℃

Scheme 1 The synthetic pathway of titled compounds **4a**–**4n**

Table 1 Physical data of Isatin-1-*N*-phenylacetamide of compounds **4a**–**4n**



4a-4n

Compound	R	m.p. (°C)	Yield (%)	Formula	Recrystn solvent
4a	–H	228–229	73	$C_{16}H_{12}N_2O_3$	EtOH
4b	*o*-CH_3	249–250	84	$C_{17}H_{14}N_2O_3$	EtOH
4c	*m*-CH_3	268–269	75	$C_{17}H_{14}N_2O_3$	EtOH
4d	*p*-CH_3	243–245	70	$C_{17}H_{14}N_2O_3$	EtOH
4e	*o*-F	220.1–220.6	80	$C_{16}H_{11}FN_2O_3$	EtOH
4f	*m*-F	224.3–224.9	66	$C_{16}H_{11}FN_2O_3$	EtOH
4g	*o*-Cl	236–239	85	$C_{16}H_{11}ClN_2O_3$	EtOH
4h	*m*-Cl	234–236	83	$C_{16}H_{11}ClN_2O_3$	EtOH
4i	*p*-Cl	243–244	78	$C_{16}H_{11}ClN_2O_3$	EtOH
4j	*o*-Br	248–249	81	$C_{16}H_{11}BrN_2O_3$	EtOH
4k	*m*-Br	255–256	77	$C_{16}H_{11}BrN_2O_3$	EtOH
4l	*p*-Br	242–243	71	$C_{16}H_{11}BrN_2O_3$	EtOH
4m	2,4-Br_2	208–210	54	$C_{16}H_{10}Br_2N_2O_3$	EtOH
4n	*p*-NO_2	172.2–172.7	65	$C_{16}H_{11}N_3O_5$	EtOH

The rotarod test

At 30 min after the administration of the compounds, the animals were tested on a 1-in. diameter; knurled plastic rod rotating at 6 rpm for 1 min. Neurotoxicity was indicated by the inability of an animal to maintain equilibrium in each of three trials (Sun *et al*., 2006).

Results and discussion

Chemistry

The isatin and substituted isatins **4a**–**4n** were prepared by following the procedure reported earlier (Marvel and Hiers, 1941; Gassman *et al*., 1977; Patel *et al*., 2006) (Scheme 1).

Briefly, the starting material aromatic amines reacted with chloral hydrate and sodium sulfate to yield the intermediates **2a**–**2n**. Then, substituted 2-(phenylamino)acetaldehyde oxime (**2a**–**2n**) was stirred with concentrated sulfuric acid to give the substituted isatin (**3a**–**3n**). The latter compounds (**3a**–**3n**) reacted with compound **5** to obtain isatin-1-*N*-phenylacetamide derivatives **(4a**–**4n)**, the physical data of isatin-1-*N*-phenylacetamide of compounds **4a**–**4n** are shown in Table 1. All spectral data are in accordance with the assumed structures. The IR spectra of the compounds afforded the phenylacetamide C=O stretching (1,731–1,733 cm^{-1}) and NH stretching (3,490–3,507 cm^{-1}). In the ^1H NMR spectra, the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra showed the NH– proton as a singlet at 9.72–10.06 ppm.

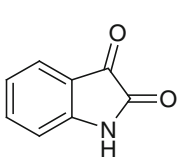
Pharmacology

The results of pharmacology test of all synthesized compounds and the reference drugs are shown in Table 2. Ten compounds showed the anticonvulsant activity. The lead compound Isatin exhibited anti-MES effect only at a dose level of 300 mg/kg, nine compounds (**4a**–**4e**, **4g**, **4i**, **4j**, and **4n**) exhibited anti-MES effect at a dose of 100 mg/kg, the compound 4**m** showed at a dose level of 300 mg/kg anti-MES effect. Whereas four compounds (**4f**, **4h**, **4k**, and **4l**) do not exhibit the anticonvulsant activity at a dose level of 300 mg/kg. In which, all compounds did not show the neurotoxicity at the same dose levels.

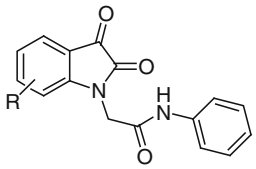
On the basis of phase I screening, six compounds which exhibited the better anti-MES activity were then subjected to phase II trials for quantification of their anticonvulsant activity and neurotoxicity in mice. The data were also compared with the marketed anticonvulsant drugs phenobarbital and carbamazepine. The results are shown in Table 3. Analyzing the activities of six compounds (**4a**–**4e** and **4g**), the following structure–activity relationship was gained. For the electron donor-substituted phenyl derivatives **(4b**–**4d)**, the potency anticonvulsant activity order was *o*-CH_3 > *p*-CH_3 > *m*-CH_3, in which the compound **4b** was the most anticonvulsant activity with an ED_{50} of 91.3 mg/kg and TD_{50} of >1,000 mg/kg, and a higher protective index (PI = TD_{50}/ED_{50}, >11) was gained than the reference drug phenobarbital and carbamazepine. Comparison of the halogen-substituted derivatives indicated that the F atom had a larger contribution to the anticonvulsant activity than the Cl and Br atom; in these halogen derivatives, the activity order was *o*-F > *o*-Cl > *o*-Br; however, the compound **4e** was the most anticonvulsant activity with an ED_{50} of 88.0 mg/kg and TD_{50} of >800 mg/kg, and a higher protective index (PI = TD_{50}/ED_{50}, >9.1) was gained than the reference drug phenobarbital.

The bio-evaluation lead to an understanding of the importance of the interaction of the chemical moieties with the receptors which mediate anticonvulsant activity. The structural features of the series of compounds possess essential pharmacophores present in the standard drugs such as phenobarbitone, phenytoin, and carbamazepine as reported (Pandeya and Raja, 2002). A scrutiny for certain selected structures for active anticonvulsant has been shown to possess a hydrogen donor acceptor unit (HBD), hydrophobic unit (R), and an electron donor group (D). In our present series of isatin derivatives, the most active compound **4b** possesses all the requirements essential for the anticonvulsant activity as proposed by Dimmock and others, as shown in Fig. 2.

Table 2 The primary evaluation of compounds **4a**–**4n** in anticonvulsant activity (test drug administered *i.p.*)



Isatin



4a-4n

Compound	R	Dosage (mg/kg)	MES[a]		Rotarod[b]	
			0.5 h	4 h	0.5 h	4 h
Isatin	–	300	1/5	0/5	0/5	0/5[c]
4a	–H	100	2/5	0/5	0/5	0/5
4b	*o*-CH_3	100	3/5	0/5	0/5	0/5
4c	*m*-CH_3	100	1/5	0/5	0/5	0/5
4d	*p*-CH_3	100	2/5	0/5	0/5	0/5
4e	*o*-F	100	3/5	0/5	0/5	0/5
4f	*m*-F	300	0/5	0/5	0/5	0/5
4g	*o*-Cl	100	3/5	0/5	0/5	0/5
4h	*m*-Cl	300	0/5	0/5	0/5	0/5
4i	*p*-Cl	100	1/5	0/5	0/5	0/5
4j	*o*-Br	100	1/5	0/5	0/5	0/5
4k	*m*-Br	300	0/5	0/5	0/5	0/5
4l	*p*-Br	300	0/5	0/5	0/5	0/5
4m	2,4-Br_2	300	2/5	0/5	0/5	0/5
4n	*p*-NO_2	100	1/5	0/5	0/5	0/5

[a] MES seizure test (number of animals protected/number of animals tested)

[b] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested)

[c] Compounds are metabolized/excreted at 4 h

Table 3 Phase-II quantitative anticonvulsant data in mice (test drug administered *i.p.*)

Compound	R	MES, ED_{50}^{a}	TD_{50}^{b}	PI (TD_{50}/ED_{50})[c]
4a	–H	94.7 (86.2–104.0)[d]	>1,000	>10.6
4b	*o*-CH_3	91.3 (75.9–109.8)	>1,000	>11
4c	*m*-CH_3	136.3 (113.4–164.0)	>1,000	>7.3
4d	*p*-CH_3	113.6 (94.5–136.7)	>1,000	>8.8
4e	*o*-F	88.0 (73.2–105.9)	>800	>9.1
4g	*o*-Cl	109.5 (93.4–128.5)	>800	>7.3
Phenobarbital	–	21.8 (21.8–25.5)	69 (62.8–72.9)	3.2
Carbamazepine	–	8.8 (5.5–14.1)	71.6 (45.9–135)	8.1

[a] ED_{50} median effective dose affording anticonvulsant protection in 50 % of animals, the dose is measured in mg/kg

[b] TD_{50} median toxic dose eliciting minimal neurological toxicity in 50 % of animals, the dose is measured in mg/kg

[c] *PI* protective index (TD_{50}/ED_{50})

[d] 95 % confidence intervals given in parentheses

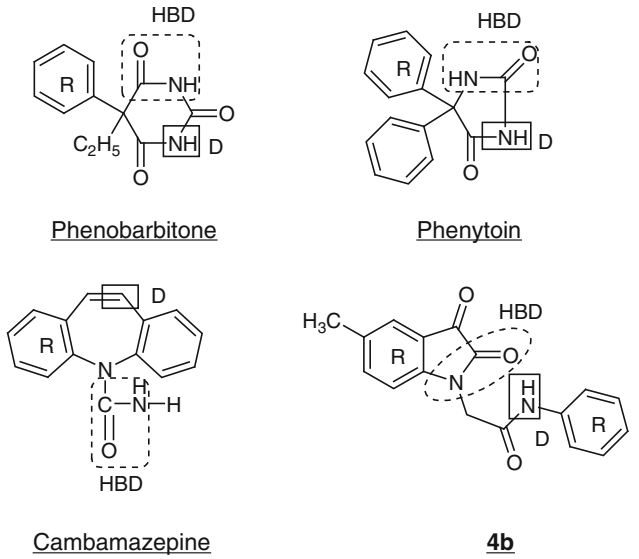


Fig. 2 Development of a pharmacophore model. *HBD* hydrogen bonding domain, *D* electron donor, *R* hydrophobic unit

Conclusion

In summary, we have synthesized 14 compounds of isatin-1-*N*-phenylacetamide and screened for their in vivo anticonvulsant activity. Ten compounds showed the anticonvulsant activity and lower neurotoxicity. Among, the compound **4b** was found to be the most potent compound of the series with an ED_{50} of 91.3 mg/kg and TD_{50} of >1,000 mg/kg, and a higher protective index (PI = TD_{50}/ED_{50}, >11) was gained than the reference drug phenobarbital and carbamazepine. The essential structural features responsible for interaction with receptor site are established within a suggested pharmacophore.

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