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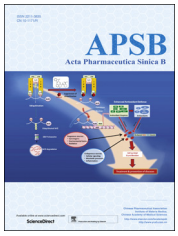

ORIGINAL ARTICLE

Synthesis, potential anticonvulsant and antidepressant effects of 2-(5-methyl-2, 3-dioxoindolin-1-yl)acetamide derivatives

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Antidepressant activity;
Pentylenetetrazole

Abstract A new series of 2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide derivatives were synthesized and evaluated for their anticonvulsive activity in a pentylenetetrazole (PTZ)-evoked convulsion model and antidepressant activity in the forced swimming test (FST) model. Eleven synthesized compounds were found to be protective against PTZ-induced seizure and showed the anticonvulsant activity. In addition, four of the synthesized compounds (**4l**, **4m**, **4p** and **4q**) showed potent antidepressant-like activity. Among these compounds, compound **4l** was found to have the most potent antidepressant-like activity, and significantly reduced the duration of immobility time at 100 mg/kg dose level when compared to the vehicle control, which is similar to the reference drug fluoxetine.

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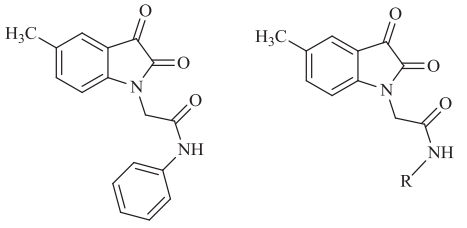
1. Introduction

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, protection against certain types of bacterial infections, antiproliferative, anti-inflammatory, antiprotozoal, proconvulsive and anticonvulsive activities[1–3].

In addition, Sridhar et al.[4] reported the anticonvulsant activity of hydrazones, the Schiff and Mannich bases of isatin, by the maximal electroshock method (MES) and metrazol-induced convulsions (MET). Li et al.[5] studied the inhibitory effect of isatin on amygdaloid kindling in rats, seizure-inducing and anticonvulsant effect in convulsion models. Pajouhesh et al.[6] synthesized a series of cyclohexane and other cyclic ketone derivatives of isatin and screened them for anticonvulsant activity. These results suggest that the researchers explored isatin as a new chemical entity with potential anticonvulsant activity. Furthermore, several researchers reported that isatin can not only evidently improve internal monoamine neurotransmitter to antagonize electric[7] and metrazol-induced seizure in mice effectively, but also decrease the epilepsy probability of audiogenic seizure in rats and enhance the anticonvulsant effect of propranolol[8–10]. In addition, in our studies[11], we synthesized a series of isatin-1-*N*-phenylacetamide derivatives and tested their anticonvulsant activity. Among these analogs, the compound **I** (Fig. 1) showed the highest anticonvulsant activity in the anti-MES and anti-PTZ tests.

As a result of our continuous effort in this area, a series of new 2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide derivatives were synthesized (Scheme 1). The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and high resolution mass spectra, and evaluated for their anticonvulsant activity against convulsions evoked by chemical substance pentylenetetrazole (PTZ) and antidepressant activity by the forced swimming test (FST), respectively.



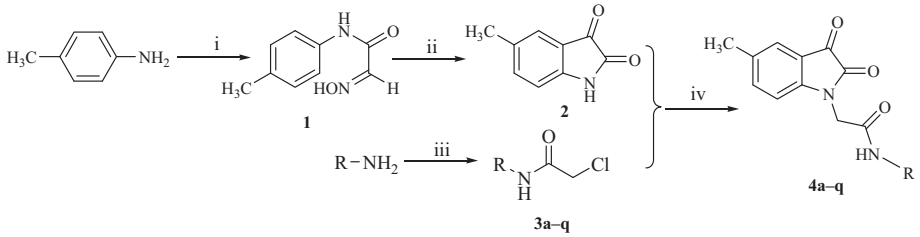
I 4a–q

Figure 1 The structures of compounds **I**, **4a–q**

2. Results and discussion

The targeted compounds **4a–q** were synthesized according to the sequence shown in Scheme 1[12]. Briefly compound **1** was prepared by the condensation of *p*-methlyphenylamine with chloral hydrate and hydroxylamine hydrochloride in 89% yield. Then, the subsequent cyclization of compound **1** in the presence of concentrated sulfuric acid at 80 °C afforded compound **2**. Compounds **3a–q** were obtained by an acylation reaction of substituted anilines using 2-chloroacetyl chloride in 80%-92% yields. Finally, compounds **4a–q** were obtained by an alkylation reaction of compounds **3a–q** with compound **2**.

The anticonvulsant activity of the synthesized compounds **4a-q** was investigated in PTZ-induced model against convulsions and the results from these experiments are shown in Table 1. To explore the structure-activity relationships of 2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide derivatives, we varied the substituents on the phenyl group in the phenylacetamide ring, which contained both electron-withdrawing and electron-donating substituents. Compounds **4a–q** and the reference drug carbamazepine were administered *i.p.* into mice at a dose of 100 mg/kg. Among the synthesized compounds, eleven compounds were found to be protective against PTZ-induced seizure and showed the anticonvulsant activity. Analyzing the activity of eleven compounds (**4a**, **4d**, **4f**, **4h**, **4k–q**) led to the following structure-activity relationship. The electron-donating groups showed the following trend in anticonvulsant activity: -2-CH_3-C_6H_4 > -4-OCH_3-C_6H_4 > $CH_2C_6H_5$ ≈ compound **I**, in which the methyl group at the 3-position and 4-position on the phenyl ring did not exhibit the anticonvulsant activity against PTZ-induced seizures. The structure-activity relationships of compounds **4e–p** were analyzed. Freqently, the activity is markedly changed upon the introduction of a halogen atom. Therefore, in this paper some halogen-substituted derivatives were designed and synthesized. All halogenated compounds (**4e–p**) except **4e**, **4g**, **4i**, and **4j** displayed the anticonvulsant activity against PTZ-induced seizure, The Br analog showed higher anticonvulsant activity than F and Cl analogs and the rank of the activity order of halogen-substituted derivatives was Br > F > Cl. Bis-halogenated compounds **4n** (2,4-Cl_2) and **4o** (2,4-Br_2) also showed the anticonvulsant activity against PTZ-induced seizure. In addition, compound **4p** with an electron-



R:

4a = 2-CH_3-C_6H_4	**4b** = 3-CH_3-C_6H_4	**4c** = 4-CH_3-C_6H_4	**4d** = 4-OCH_3-C_6H_4	**4e** = 2-F-C_6H_4
4f = 3-F-C_6H_4	**4g** = 4-F-C_6H_4	**4h** = 2-Cl -C_6H_4	**4i** = 3-Cl -C_6H_4	**4j** = 4-Cl -C_6H_4
4k = 2-Br-C_6H_4	**4l** = 3-Br-C_6H_4	**4m** = 4-Br-C_6H_4	**4n** = 2,6-Cl_2-C_6H_3	**4o** = 2,4-Br_2-C_6H_3
4p = 3-CF_3-C_6H_4	**4q** = -$CH_2C_6H_5$			

Scheme 1 The synthetic pathway of titled compounds **4a–q** Reaction and condition: (i) $CCl_3CH(OH)_2$, $NH_2OH \cdot HCl$, Na_2SO_4, HCl; (ii) concentrated H_2SO_4; (iii) $ClCH_2COCl$, HOAc; (iv) DMF, KI, K_2CO_3.

Table 1 Effect of compounds **4a–q** on PTZ-induced convulsion in mice (test drug administered *i.p.*).

Compd.	Dosage (mg/kg)	PTZ			Toxicity	
		Clonic seizures	Tonic seizures	Lethality	0.5 h	4 h
I	100	1/3	2/3	2/3	0/3	0/3
4a	100	3/3	0/3	0/3	0/3	0/3
4b	100	0/3	3/3	3/3	0/3	0/3
4c	100	0/3	3/3	3/3	0/3	0/3
4d	100	2/3	1/3	1/3	0/3	0/3
4e	100	0/3	3/3	3/3	0/3	0/3
4f	100	3/3	0/3	0/3	0/3	0/3
4g	100	0/3	3/3	3/3	0/3	0/3
4h	100	1/3	2/3	2/3	0/3	0/3
4i	100	0/3	3/3	3/3	0/3	0/3
4j	100	0/3	3/3	3/3	0/3	0/3
4k	100	1/3	2/3	2/3	0/3	0/3
4l	100	1/3	2/3	2/3	0/3	0/3
4m	100	1/3	2/3	2/3	0/3	0/3
4n	100	1/3	2/3	2/3	0/3	0/3
4o	100	1/3	2/3	2/3	0/3	0/3
4p	100	3/3	0/3	0/3	0/3	0/3
4q	100	1/3	2/3	2/3	0/3	0/3
Carbamazepine	100	3/3	3/3	0/3	0/3	0/3

PTZ: subcutaneous pentylenetetrazole (metrazol) seizure test; 0/3: no activity at dose level; 3/3 (clonic seizures): noticeable activity at dose level.

withdrawing group (-CF_3) showed enhanced anticonvulsant activity. None of the compounds showed neurotoxicity at the same dose levels.

The FST was designed by Porsolt et al.[13] as a primary screening test for the antidepressant activity and is a behavioral test used to predict the efficacy of antidepressants. The immobility time observed in the test reflects a state of lowered mood or hopelessness in animals, thus these models are the most widely used tools for preclinical screening of the putative anti-depressive agents and have good predictive values for anti-depressive efficacy in humans[14,15]. The obtained data on the antidepressant-like activity of compounds **4a–q** and the reference drug fluoxetine (FLU) are given in Table 2 and Fig. 2 in the FST test. Except compounds **4l** (3-Br), **4m** (4-Br), and **4p** (3-CF_3), none of the halogen substituted derivatives **4e–p** showed potent antidepressant-like activity. We also investigated the influence of electron-donating groups (**4a–d** and **4q**). The results exhibited that compound **4q** significantly reduced the duration of the immobility time at 100 mg/kg, compared with the vehicle control group and were found to have the highest antidepressant-like activity. The immobility time of mice treated with compounds **4a–k**, **4n**, and **4o** did not statistically differ from that of the controls. Acute treatment with compounds **4l**, **4m**, **4p** and **4q** promoted a decrease in the immobility time in the FST, as depicted in Fig. 2. (control = 125.2 ± 26.5; **4l** = 65.7 ± 17.8; **4m** = 78.5 ± 19.1; **4p** = 75.7 ± 18.7; **4q** = 88.7 ± 18.7; FLU = 58.5 ± 9.3), and they reduced the duration of immobility time (47.5%, 37.3%, 29.2% and 39.5%, respectively) compared to the controls. Among them, compound **4l** was found to have the highest antidepressant-like activity, which is similar to the reference drug FLU (50.96%).

Some compounds that alter motor activity may give false positive/negative effects in the FST, in particular, psychomotor stimulants and drugs enhancing motor activity, which decrease immobility time by stimulating locomotor activity[16]. Thus, an additional measurement was carried out with the specific aim of observing motor activity. In this study, the effect of compound **4l** on spontaneous locomotor activity was evaluated in the open-field test, a classical animal test used to evaluate the autonomic effects of drugs and general activity of animals[17,18]. This study demonstrated

Table 2 Antidepressant activities of the compounds.

Compd.	Dose (mg/kg)	Antidepressant activity[a]
I	100	98.5 ± 23.3
4a	100	114.8 ± 6.0
4b	100	101.2 ± 18.7
4c	100	76.2 ± 18.3
4d	100	92.0 ± 19.5
4e	100	110.3 ± 22.6
4f	100	98.7 ± 16.7
4g	100	113.3 ± 8.7
4h	100	117.2 ± 16.5
4i	100	112.2 ± 26.0
4j	100	111.3 ± 24.2
4k	100	101.8 ± 12.9
4n	100	101.3 ± 18.4
4o	100	121.2 ± 15.6
Fluoxetine	10	58.5 ± 9.3***
Control	–	125.2 ± 26.5

[a]Values represent the mean ± SEM ($n = 10$).
***$P < 0.01$, significantly compared to control (Turkey's test).

that compound **4l** did not significantly change motor activity (crossing, rearing and grooming) in mice (Fig. 3). It is unlikely that the effect of compound **4l** observed in the FST was caused by the stimulation of general motor activity. This study provides evidence that compound **4l** has an antidepressant-like effect in mice.

3. Conclusions

In this study, a series of 2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide derivatives were synthesized and evaluated for their antidepressive and anticonvulsive activities. Eleven synthesized

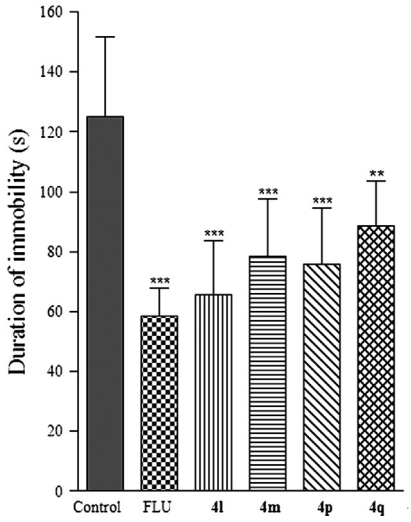


Figure 2 Immobility time of compounds **4l**, **4m**, **4p** and **4q** in mouse FST. Data expressed as mean ± SEM. ($n = 10$). Statistical analysis of data was carried out by one-way analysis of variance followed by Turkey's test. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. Control.

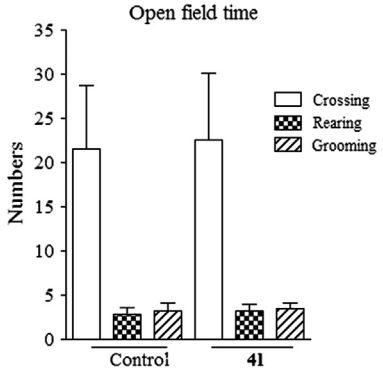


Figure 3 Exploratory activity (counts) in the open-field test. The behavioral parameters were recorded for 3 min. Crossing: number of line crossings; rearing: number of times seen standing on hind legs; grooming: number of modifications; **4l** was administered 60 min before the test. The values represent the mean ± SEM ($n = 10$).

compounds were found to have protective effects against PTZ-induced seizure and showed potent anticonvulsive activity. However, only four compounds (**4l**, **4m**, **4p** and **4q**) showed the antidepressant-like activity. Among these compounds, compound **4l** showed the highest antidepressant-like activity, and decreased immobility time by 47.5% at a dose of 100 mg/kg.

4. Experimental

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 were measured on an AV-300 (Bruker, Switzerland), and all chemical shifts were given in ppm relative to tetramethysilane. High resolution mass spectra were measured on an MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Germany). The major chemicals were purchased from Aldrich Chemical Corporation. All other chemicals were of the analytical grade.

4.1. *Synthesis of 5-methylindoline-2,3-dione (**2**)*

Concentrated sulfuric acid 20 mL was warmed to 50 °C in a round-bottomed flask. Compound **1** (0.7 g, 4 mmol) was added to keep the temperature 50 °C. After the addition of the compound **1** was finished, the solution was heated to 80 °C for 20 min to complete the reaction. Then the reaction mixture was cooled to room temperature and poured to 5 volumes of cracked ice. The solid was filtered and washed three times with cold water to remove the sulfuric acid. The crude product was purified by recrystallization in EtOH and then dried in the air to provide compound **2**[19,20]. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.81 (s, 1H, -NH), 6.72–7.29 (m, 3H, $-C_6H_3$), 2.22 (s, 3H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.80 (C=O), 159.47 (C=O), 148.97 (Ar–C), 139.06 (Ar–C), 132.43 (Ar–C), 125.12 (Ar–C), 117.90 (Ar–C), 112.42 (Ar–C), 20.46 (CH_3–C). MS *m/z*: 162 [M+H].

4.2. *Synthesis of 2-chloro-N-substituted acetamide (**3a–q**)*

Substituted phenylamines or benzylamines (8.8 mmol) were dissolved in a mixture of glacial acetic acid (20 mL) and saturated sodium acetate solution (20 mL). And 2-chloroacetyl chloride (8.8 mmol) was added dropwise to avoid the vigorous reaction. The reaction mixture was stirred at 0 °C–5 °C for 1 h, the product was filtered and washed three times with cold water. The crude product was purified by recrystallization in EtOH[21].

4.3. *Synthesis of 2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide derivatives (**4a–q**)*

Compound **2** (1.0 g, 6.2 mmol), K_2CO_3 (0.8 g, 6.2 mmol) and 30 mL DMF were placed in a 100 mL round-bottomed flask. The mixture was stirred at room temperature for 1 h. The appropriate compounds **3a–q** (6.2 mmol) and KI (0.5 g, 3 mmol) were added and heated at 80 °C for 1–12 h. The reaction mixture was poured into water, and then adjusted pH to 3–4 using hydrochloric acid to obtain precipitation of solid (**4a–q**). The crude product was filtered, washed three times with cold water and then dried. The crude product was purified by recrystallization in MeOH[21]. The physical and spectral data of each compound are given below.

*4.3.1. 2-(5-Methyl-2,3-dioxoindolin-1-yl)-N-2-tolylacetamide (**4a**)* Mp. 233–234 °C; Yield 80%. IR (KBr, cm^{-1}): 3321, 1725, 1645, 1248. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.57 (s, 1H, -NH), 7.37–7.81 (m, 3H, C_6H_3), 6.86–7.40 (m, 4H, C_6H_4), 4.50 (s, 2H, CH_2), 2.30 (s, 3H, CH_3), 2.11 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.38 (C=O), 166.33 (C=O), 158.75 (C=O), 148.37 (Ar–C), 138.80 (Ar–C), 138.53 (Ar–C), 133.37 (Ar–C), 128.49 (Ar–C), 127.48 (Ar–C), 127.19 (Ar–C), 125.00 (Ar–C), 123.27 (Ar–C), 120.47 (Ar–C), 118.02 (Ar–C), 110.50 (Ar–C), 43.50 (CH_2–C), 24.84 (CH_3–C), 20.72 (CH_3–C). ESI-HRMS Calcd. for $C_{18}H_{16}N_2O_3^+$ [M+H]: 309.1161; Found: 309.1174.

*4.3.2. 2-(5-Methyl-2,3-dioxoindolin-1-yl)-N-3-tolylacetamide (**4b**)* Mp. 242–245 °C; Yield 84%. IR (KBr, cm^{-1}): 3320, 1726, 1645, 1249. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.91 (s, 1H, NH), 7.36–7.91 (m, 3H, C_6H_3), 6.81–7.64 (m, 4H, C_6H_4), 4.47 (s, 2H, CH_2), 2.28 (s, 3H, CH_3), 2.23 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz):

183.24 (C=O), 165.15 (C=O), 158.79 (C=O), 148.38 (Ar–C), 138.57 (Ar–C), 136.29 (Ar–C), 133.51 (Ar–C), 130.61 (Ar–C), 127.09 (Ar–C), 126.33 (Ar–C), 125.98 (Ar–C), 122.21 (Ar–C), 118.01 (Ar–C), 117.48 (Ar–C), 110.46 (Ar–C), 43.88 (CH_2–C), 21.54 (CH_3–C), 18.16 (CH_3–C). ESI-HRMS Calcd. for $C_{18}H_{16}N_2O_3^+$ [M+H]: 309.1161; Found: 309.1160.

*4.3.3. 2-(5-Methyl-2,3-dioxoindolin-1-yl)-N-4-tolylacetamide (**4c**)*
Mp. 258–259 °C; Yield 87%. IR (KBr, cm^{-1}): 3324, 1730, 1647, 1246. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.79 (s, 1H, NH), 7.33–7.97 (m, 3H, C_6H_3), 6.78–7.54 (m, 4H, C_6H_4), 4.46 (s, 2H, CH_2), 2.26 (s, 3H, CH_3), 2.21 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.33 (C=O), 164.76 (C=O), 158.76 (C=O), 148.66 (Ar–C), 138.84 (Ar–C), 138.62 (Ar–C), 138.15 (Ar–C), 133.49 (Ar–C), 128.62 (Ar–C), 125.15 (Ar–C), 124.96 (Ar–C), 120.83 (Ar–C), 117.79 (Ar–C), 117.36 (Ar–C), 110.62 (Ar–C), 43.51 (CH_2–C), 21.52 (CH_3–C), 20.70 (CH_3–C). ESI-HRMS Calcd. for $C_{18}H_{16}N_2O_3^+$ [M+H]: 309.1161; Found: 309.1169.

*4.3.4. N-(4-Methoxyphenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4d**)*
Mp. 241–243 °C; Yield 79%. IR (KBr, cm^{-1}): 3322, 1731, 1646, 1245. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.27 (s, 1H, NH), 7.47–8.14 (m, 3H, C_6H_3), 6.96–7.52 (m, 4H, C_6H_4), 4.52 (s, 2H, CH_2), 3.34 (s, 3H, OCH_3), 2.29 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.30 (C=O), 164.57 (C=O), 158.80 (C=O), 148.46 (Ar–C), 138.79 (Ar–C), 134.42 (Ar–C), 133.77 (Ar–C), 133.63 (Ar–C), 129.22 (Ar–C), 125.24 (Ar–C), 124.56 (Ar–C), 122.67 (Ar–C), 120.43 (Ar–C), 117.80 (Ar–C), 110.45 (Ar–C), 69.59 (OCH_3–C), 43.53 (CH_2–C), 20.71 (CH_3–C). ESI-HRMS Calcd. for $C_{18}H_{16}N_2O_4^+$ [M+H]: 325.1110; Found: 325.1119.

*4.3.5. N-(2-Fluorophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4e**)*
Mp. 216–217 °C; Yield 60%. IR (KBr, cm^{-1}): 3341, 1731, 1648, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.18 (s, 1H, NH), 7.36–7.80 (m, 3H, C_6H_3), 6.89–7.33 (m, 4H, C_6H_4), 4.49 (s, 2H, CH_2), 2.27 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.50 (C=O), 169.52 (C=O), 157.86 (C=O), 144.57 (Ar–C), 138.57 (Ar–C), 136.98 (Ar–C), 134.23 (Ar–C), 133.78 (Ar–C), 130.16 (Ar–C), 127.03 (Ar–C), 125.08 (Ar–C), 123.41 (Ar–C), 120.21 (Ar–C), 117.54 (Ar–C), 116.71 (Ar–C), 43.59 (CH_2–C), 20.96 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{13}FN_2O_3^+$ [M+H]: 313.0910; Found: 313.0917.

*4.3.6. N-(3-Fluorophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4f**)*
Mp. 238–240 °C; Yield 67%. IR (KBr, cm^{-1}): 3343, 1732, 1648, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.21 (s, 1H, NH), 7.48–7.74 (m, 3H, C_6H_3), 6.78–7.46 (m, 4H, C_6H_4), 4.50 (s, 2H, CH_2), 2.26 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.40 (C=O), 165.58 (C=O), 164.20 (C=O), 158.73 (Ar–C), 148.94 (Ar–C), 140.09 (Ar–C), 138.92 (Ar–C), 136.24 (Ar–C), 133.32 (Ar–C), 130.46 (Ar–C), 117.76 (Ar–C), 115.48 (Ar–C), 110.71 (Ar–C), 107.39 (Ar–C), 43.48 (CH_2–C), 20.68 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{13}FN_2O_3^+$ [M+H]: 313.0910; Found: 313.0915.

*4.3.7. N-(4-Fluorophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4g**)*
Mp. 257–259 °C; Yield 70%. IR (KBr, cm^{-1}): 3341, 1731, 1648, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.18 (s, 1H, NH), 7.36–7.80 (m, 3H, C_6H_3), 6.89–7.33 (m, 4H, C_6H_4), 4.49 (s, 2H, CH_2), 2.27 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.28 (C=O), 165.12 (C=O), 158.74 (C=O), 148.63 (Ar–C), 138.91 (Ar–C), 133.55 (Ar–C), 130.12 (Ar–C), 125.18 (Ar–C), 130.46 (Ar–C), 127.32 (Ar–C), 125.02 (Ar–C), 124.64 (Ar–C), 117.79 (Ar–C), 115.82 (Ar–C), 110.92 (Ar–C), 43.31 (CH_2–C), 20.66 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{13}FN_2O_3^+$ [M+H]: 313.0910; Found: 313.0921.

*4.3.8. N-(2-Chlorophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4h**)*
Mp. 198–199 °C; Yield 82%. IR (KBr, cm^{-1}): 3334, 1730, 1648, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.23 (s, 1H, NH), 7.37–7.89 (m, 3H, C_6H_3), 6.85–7.34 (m, 4H, C_6H_4), 4.50 (s, 2H, CH_2), 2.27 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.33 (C=O), 164.46 (C=O), 158.78 (C=O), 156.25 (Ar–C), 148.51 (Ar–C), 138.79 (Ar–C), 133.57 (Ar–C), 131.16 (Ar–C), 125.18 (Ar–C), 124.78 (Ar–C), 122.02 (Ar–C), 120.79 (Ar–C), 117.82 (Ar–C), 113.85 (Ar–C), 110.51 (Ar–C), 43.46 (CH_2–C), 20.70 (CH_3–C). ESI–HRMS Calcd. for $C_{17}H_{13}ClN_2O_3^+$ [M+H]: 329.0615; Found: 329.0619.

*4.3.9. N-(3-Chlorophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4i**)*
Mp. 251–252 °C; Yield 80%. IR (KBr, cm^{-1}): 3330, 1732, 1649, 1250. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.83 (s, 1H, NH), 7.43–8.02 (m, 3H, C_6H_3), 6.93–7.40 (m, 4H, C_6H_4), 4.59 (s, 2H, CH_2), 2.30 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.26 (C=O), 165.21 (C=O), 158.76 (C=O), 148.67 (Ar–C), 139.77 (Ar–C), 138.92 (Ar–C), 133.88 (Ar–C), 133.50 (Ar–C), 130.11 (Ar–C), 125.16 (Ar–C), 123.88 (Ar–C), 119.84 (Ar–C), 118.13 (Ar–C), 117.76 (Ar–C), 110.75 (Ar–C), 43.50 (CH_2–C), 20.69 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{13}ClN_2O_3^+$ [M+H]: 329.0615; Found: 329.0620.

*4.3.10. N-(4-Chlorophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4j**)*
Mp. 258.5 °C; Yield 87 %. IR (KBr, cm^{-1}): 3342, 1734, 1648, 1249. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.04 (s, 1H, NH), 7.46–7.61 (m, 3H, C_6H_3), 6.77–7.34 (m, 4H, C_6H_4), 4.47 (s, 2H, CH_2), 2.26 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.34 (C=O), 165.62 (C=O), 158.72 (C=O), 148.73 (Ar–C), 138.80 (Ar–C), 134.51 (Ar–C), 133.37 (Ar–C), 129.77 (Ar–C), 127.63 (Ar–C), 125.04 (Ar–C), 123.88 (Ar–C), 119.84 (Ar–C), 118.13 (Ar–C), 117.69 (Ar–C), 110.94 (Ar–C), 43.27 (CH_2–C), 20.69 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{13}ClN_2O_3^+$ [M+H]: 329.0615; Found: 329.0911.

*4.3.11. N-(2-Bromophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4k**)*
Mp. 201–202 °C; Yield 81 %. IR (KBr, cm^{-1}): 3342, 1733, 1649, 1252. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.58 (s, 1H, NH), 7.53–7.63 (m, 3H, C_6H_3), 6.86–7.49 (m, 4H, C_6H_4), 4.55 (s, 2H, CH_2), 2.28 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.21 (C=O), 165.51 (C=O), 158.66 (C=O), 148.22 (Ar–C), 138.67 (Ar–C), 136.27 (Ar–C), 134.22 (Ar–C), 133.51 (Ar–C), 129.23 (Ar–C), 128.52 (Ar–C), 127.07 (Ar–C), 125.00 (Ar–C), 123.88 (Ar–C), 117.46 (Ar–C), 110.94 (Ar–C), 42.86 (CH_2–C), 21.06 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{13}BrN_2O_3^+$([M +H]$^+$): 373.0110; Found: 373.0121.

*4.3.12. N-(3-Bromophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4l**)*

Mp. 240–241 °C; Yield 77 %. IR (KBr, cm^{-1}): 3342, 1733, 1649, 1252. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.93 (s, 1H, NH), 7.60–7.70 (m, 3H, C_6H_3), 6.73–7.46 (m, 4H, C_6H_4), 4.46 (s, 2H, CH_2), 2.26 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.19 (C=O), 165.39 (C=O), 158.72 (C=O), 148.36 (Ar–C), 138.76 (Ar–C), 135.48 (Ar–C), 133.66 (Ar–C), 132.86 (Ar–C), 128.01 (Ar–C), 127.42 (Ar–C), 126.97 (Ar–C), 125.22 (Ar–C), 118.29 (Ar–C), 117.82 (Ar–C), 110.70 (Ar–C), 43.33 (CH_2–C), 20.72 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{13}BrN_2O_3^+$ [M+H]: 373.0110; Found: 373.0119.

*4.3.13. N-(4-Bromophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl) acetamide (**4m**)*

Mp. 234–235 °C; Yield 83 %. IR (KBr, cm^{-1}): 3340, 1732, 1647, 1250. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.00 (s, 1H, NH), 7.55–8.03 (m, 3H, C_6H_3), 7.00–7.55 (m, 4H, C_6H_4), 4.56 (s, 2H, CH_2), 2.30 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.14 (C=O), 165.00 (C=O), 158.79 (C=O), 148.23 (Ar–C), 138.81 (Ar–C), 133.81 (Ar–C), 130.13 (Ar–C), 127.08 (Ar–C), 126.14 (Ar–C), 125.39 (Ar–C), 123.06 (Ar–C), 122.15 (Ar–C), 118.37 (Ar–C), 117.81 (Ar–C), 110.30 (Ar–C), 43.58 (CH_2–C), 20.69 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{13}BrN_2O_3^+$ [M+H]: 373.0110; Found: 373.0123.

*4.3.14. N-(2,6-Dichlorophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide (**4n**)*

Mp. 268–270 °C; Yield 73 %. IR (KBr, cm^{-1}): 3342, 1730, 1649, 1253. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.08 (s, 1H, NH), 7.55–7.85 (m, 3H, C_6H_3), 6.85–7.43 (m, 3H, C_6H_3), 4.52 (s, 2H, CH_2), 2.29 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.24 (C=O), 164.90 (C=O), 158.75 (C=O), 148.46 (Ar–C), 138.86 (Ar–C), 136.90 (Ar–C), 133.67 (Ar–C), 130.56 (Ar–C), 128.66 (Ar–C), 127.48 (Ar–C), 125.27 (Ar–C), 121.55 (Ar–C), 120.34 (Ar–C), 117.76 (Ar–C), 110.49 (Ar–C), 43.48 (CH_2–C), 20.70 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{12}Cl_2N_2O_3^+$ [M+H]: 363.0225; Found: 363.0229.

*4.3.15. N-(2,6-Dibromophenyl)-2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide (**4o**)*

Mp. 272–274 °C; Yield 70 %. IR (KBr, cm^{-1}): 3340, 1736, 1652, 1250. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.06 (s, 1H, NH), 7.42–8.03 (m, 3H, C_6H_3), 6.93–7.39 (m, 3H, C_6H_3), 4.60 (s, 2H, CH_2), 2.29 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.41 (C=O), 165.25 (C=O), 158.81 (C=O), 148.95 (Ar–C), 138.96 (Ar–C), 137.99 (Ar–C), 133.32 (Ar–C), 131.87 (Ar–C), 128.56 (Ar–C), 127.35 (Ar–C), 125.07 (Ar–C), 121.82 (Ar–C), 117.83 (Ar–C), 116.00 (Ar–C), 111.07 (Ar–C), 43.57 (CH_2–C), 20.64 (CH_3–C). ESI-HRMS Calcd. for $C_{17}H_{12}Br_2N_2O_3^+$ [M+H]: 451.9215; Found: 451.9197.

*4.3.16. 2-(5-Methyl-2,3-dioxoindolin-1-yl)-N-(3-(trifluoromethyl)phenyl)acetamide (**4p**)*

Mp. 234–235 °C; Yield 65 %. IR (KBr, cm^{-1}): 3341, 1731, 1648, 1251. ^1H NMR ($CDCl_3$, 300 MHz): δ 10.12 (s, 1H, NH), 7.52–7.60 (m, 3H, C_6H_3), 6.82–7.50 (m, 4H, C_6H_4), 4.53 (s, 2H, CH_2), 2.34 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.90 (C=O), 169.50 (C=O), 165.54 (C=O), 163.55 (Ar–C), 153.08 (Ar–C), 143.55 (Ar–C), 138.82 (Ar–C), 138.50 (Ar–C), 130.07 (Ar–C), 126.94 (Ar–C), 126.84 (Ar–C), 122.56 (Ar–C), 120.17 (Ar–C), 119.18 (Ar–C), 113.13 (Ar–C), 99.00 (Ar–C), 48.25 (CH_2–C), 25.44 (CH_3–C). ESI-HRMS Calcd. for $C_{18}H_{13}F_3N_2O_3^+$ [M+H]: 363.0878; Found: 363.0871.

*4.3.17. N-Benzyl-2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide (**4q**)*

Mp. 192–194 °C; Yield 75 %. IR (KBr, cm^{-1}): 3343, 1733, 1649, 1253. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.68 (s, 1H, NH), 7.33–7.89 (m, 3H, C_6H_3), 6.77–7.25 (m, 5H, C_6H_5), 4.34 (s, 2H, CH_2), 4.29 (s, 2H, CH_2), 2.29 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 183.19 (C=O), 169.56 (C=O), 165.30 (C=O), 163.34 (Ar–C), 158.77 (Ar–C), 148.37 (Ar–C), 141.41 (Ar–C), 138.87 (Ar–C), 133.79 (Ar–C), 125.91 (Ar–C), 125.37 (Ar–C), 119.87 (Ar–C), 117.74 (Ar–C), 110.42 (Ar–C), 86.59 (Ar–C), 51.43 (Ar-CH_2), 43.54 (CH_2–C), 20.67 (CH_3–C). ESI-HRMS Calcd. for $C_{18}H_{16}N_2O_3^+$: 309.1161; Found: 309.1176.

4.4. PTZ-induced seizure

PTZ-induced seizure test was carried out by the standard procedures described in the Antiepileptic Drug Development Program of the National Institutes of Health (USA)[22,23]. All compounds, which were dissolved in DMSO, were evaluated for the anticonvulsive activity in BALB/e mice in the 18–25 g weight range. At 30 min after the administration of the test compounds, 85 mg/kg of PTZ dissolved in saline was administered intraperitoneally (*i.p.*). The animals were placed in individual cages and observed for 1 h. The number of clonic, tonic seizures, and lethality as well as the number of deaths was noted.

4.5. Forced swimming test (FST)

The FST used was described in detail elsewhere by Porsolt et al.[24,25]. The synthesized compounds were screened for their anti-depressive activity. Local breed, male BALB/e mice (18–25 g) were used in the FST under standard conditions with free access to food and water. They were housed in groups of six. On the testing day, mice were dropped one at a time into a plexiglass cylinder (height 25 cm, diameter 10 cm) containing 10 cm of water at 22 ± 2 °C. During the testing, mice were assigned into different groups ($n=8$ for each group). The synthesized compounds (100 mg/kg) and fluoxetine as a reference antidepressant (10 mg/kg) were dissolved in DMSO and injected intraperitoneally (*i.p.*) in a standard volume of 0.1 mL/20 g body weight, 30 min prior to the test. Then, the mice were dropped individually into the pelxiglass cylinder and left in the water for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. A mouse was judged immobile if it floated in the water in an upright position and made only slight movements in order to prevent sinking. The duration of immobility was recorded during the last 4 min of the 6-min-test. Immobility period was regarded as the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above the water. Following swimming sessions, they were then towel dried and returned to their housing condition. The animals were used only once in this test. All FSTs were performed between 11:00 and 17:00.

4.6. 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[26].

4.7. Open-field test

Open-field tests were used to evaluate the exploratory activity of the animals[27]. The investigated compound **4l** suspended in aqueous Tween 80 was administered 60 min before the experiment. The study was carried out in mice according to the Archer's method[28], with slight modifications. Each mouse was placed individually in the center of an open-field apparatus, and the locomotor activity was assessed. The open-field apparatus was a non-transparent plastic container (80 cm × 60 cm × 30 cm), with the underside divided into 48 units of size 10 cm × 10 cm, without walls. The animals were gently placed in the center of the platform and were allowed to explore their surroundings. Hand-operated counters were used to score locomotion (ambulation, numbers of crossing lines with all four paws) and rearing frequencies (number of times an animal stood on its hind legs) for 3 min. The researchers, who did not know which groups had been treated, scored the behaviors in the open-field. The experiments were performed in a dark room, and the apparatus was illuminated by a 60-W bulb giving a yellowish light, positioned 1 m above the center of the apparatus.

4.8. Statistical analysis

Results are expressed as mean ± SEM; *n* represents the number of animals. Data obtained from pharmacological experiments were analyzed with Turkey's multiple comparison tests, using the GraphPad Prism program (GraphPad Software Inc., San Diego, USA). *P* value of less than 0.05 was considered statistically significant.

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