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Full Paper

Design and Synthesis of 5-Substituted Benzo[*d*][1,3]dioxole Derivatives as Potent Anticonvulsant Agents

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A series of 5-substituted benzo[*d*][1,3]dioxole derivatives was designed, synthesized, and tested for anticonvulsant activity using the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens. Neurotoxicity was determined by rotarod test. In the preliminary screening, six compounds, **3a**, **3c**, **3d**, and **4d–f**, showed promising anticonvulsant activities in the MES model, and compounds **4c** and **4d** exhibited full protection against seizures at doses of 300 mg/kg in the scPTZ model. Among the synthesized compounds, **3c** as the most active compound showed high protection against the MES-induced seizures with an ED_{50} value of 9.8 mg/kg and a TD_{50} value of 229.4 mg/kg after intraperitoneal injection into mice, thus providing compound **3c** with a high protective index (TD_{50}/ED_{50}) of 23.4 comparable to those of reference antiepileptic drugs.

Keywords: Anticonvulsant activity / Benzo[*d*][1,3]dioxole derivatives / Design / Synthesis

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Introduction

Epilepsy is a chronic neurological disorder affecting 0.5–1% of the population worldwide [1]. In spite of many efforts have been made in epilepsy research, the currently available antiepileptic drugs (AEDs) still present two major limitations: Firstly, a significant group of patients (up to 30%) are resistant to the available AEDs which fail to adequately control seizures [2, 3]. Secondly, many AEDs exhibit an unfavorable side effect profile, such as headache, nausea, anorexia, ataxia, hepatotoxicity, drowsiness, gastrointestinal disturbance, gingival hyperplasia, and hirsutism [4–7]. These facts drive the ongoing search of novel AEDs, there is a substantial need to design anticonvulsants for the development of more effective and safer AEDs [8–10]. An earlier report indicated that stiripentol (STP) was effective against maximal electroshock (MES)-induced convulsions in mice with ED_{50} value of 240 mg/kg [11], and a recent study suggested that STP as a lactate dehydrogenase (LDH) inhibitor suppressed seizures *in vivo* in a mouse model of epilepsy by inhibition of the metabolic pathway via LDH. Moreover, by further modifying STP, a previously unknown LDH inhibitor (isosafrole) was identified, which shared a common skeleton of benzo[*d*][1,3]dioxole and also potently suppressed seizures *in vivo* (Fig. 1) [12].

These results hinted that the skeleton of benzo[*d*][1,3]-dioxole should play an important role in keeping anticonvulsant activity. Prompted by these observations, we herein synthesized a series of benzo[*d*][1,3]dioxole derivatives, and evaluated their anticonvulsant activities with a view to explore their potency as anticonvulsant agents.

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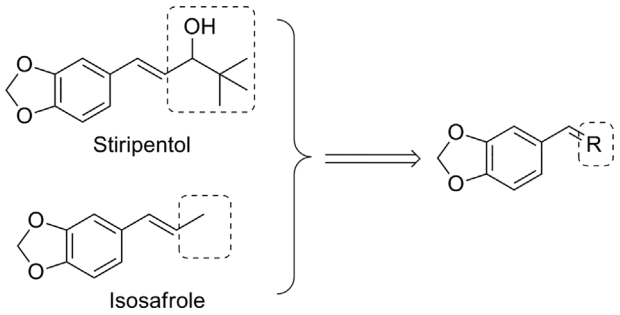


Figure 1. Reported and designed benzo[*d*][1,3]dioxole derivatives as anticonvulsants.

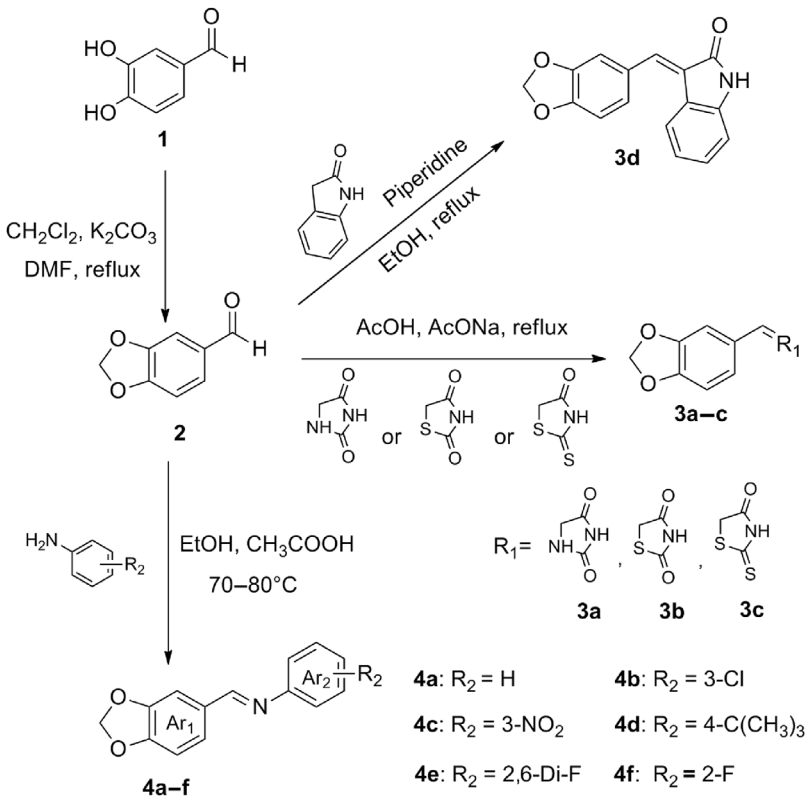
Results and discussion

Chemistry

The synthesis of compounds **3a–d** and **4a–f** was accomplished as shown in Scheme 1. Initially, 3,4-dihydroxy benzaldehyde (**1**) reacted with dichloromethane (CH_2Cl_2) in presence of dimethylformamide (DMF) according to the published method that yielded benzo[1,3]dioxole-5-carbaldehyde (**2**) [13]. Later on, the intermediate (**2**) was used as active scaffolds for the synthesis of target compounds **3a–d** and **4a–f** carrying different condensation reaction. **3a–c** were synthesized by Knoevenagel condensation reaction, that is treatment of appropriate intermediate (**2**) with five-membered heterocyclic ring containing an internal amide (such as imidazolidine-2,4-dione, thiazolidine-2,4-dione, and 2-thioxothiazolidin-4-one) at refluxing temperature in presence of sodium acetate (AcONa) and acetic acid (AcOH). Similarly, the condensation of aldehyde (**2**) with oxindole was accomplished with subsequent addition of piperidine at refluxing temperature in presence of ethanol (EtOH), which easily formed methyl 3-(benzo[*d*][1,3]dioxol-5-ylmethylene)-indolin-2-one **3d**. Finally, imines **4a–f** were obtained by the condensation of aldehyde (**2**) with different anilines, respectively. The detailed synthesis, physical data, spectral data are listed in the Experimental section.

Pharmacology

The anticonvulsant activity and neurotoxicity of all synthesized compounds **3a–d** and **4a–f** were evaluated according to the standard protocols within the Antiepileptic Drug Development (ADD) Program at the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Rockville, USA [14, 15]. The phase I evaluation included the maximal electroshock seizure (MES), the subcutaneous pentylenetetrazole (scPTZ), and neurotoxicity [16]. In our study, the test compounds were administered via *ip* injection at the doses of 30,



Scheme 1. Synthesis route of the target compounds.

Table 1. Phase I anticonvulsant screening and minimal motor impairment of the synthesized compounds (3a–d and 4a–f).

Compounds	MES screen[a]		scPTZ screen[b]		NT screen[c]	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
3a	30	30	–	–	100	100
3b	300	300	300	–	300	300
3c	30	30	–	–	300	300
3d	30	30	–	–	300	300
4a	–	–	300	–	300	300
4b	100	300	300	–	300	300
4c	–	–	300	300	100	300
4d	100	300	300	300	100	300
4e	100	300	–	–	300	300
4f	300	100	–	–	300	–
Phenytoin[d]	30	30	–	–	100	100
Phenobarbital[d]	100	30	30	300	100	300
Ethosuximide[d]	–	–	100	300	–	–

The dash (–) indicates the absence of activity at the maximum dose administered (300 mg/kg).
[a] Maximal electroshock test.
[b] Subcutaneous pentylenetetrazole test.
[c] Neurotoxocity screening (rotarod test).
[d] Data from refs. [17–19].

100, and 300 mg/kg in the MES screening whereas 100 and 300 mg/kg in the scPTZ screening and neurotoxicity. An observation was carried out at two different time intervals, namely 0.5 and 4 h. The screening results of anticonvulsant activity and neurotoxocity studies were summarized in Table 1.

Most of the compounds (except **4a** and **4c)** showed significant protection in MES screen which reflects the potential of these compounds against generalized tonic–clonic seizure. Compounds **3a**, **3c**, and **3d** showed protection from seizure at the lowest dose of 30 mg/kg at 0.5 and 4 h, which indicated the promising nature of the compound having quick onset and long duration of action at lower doses. The slight difference between the structures of compounds **3b** and **3c** that the oxygen atom of **3b** is replaced by sulfur isostere, resulting in compound **3c** that is more potent as anticonvulsant agents. This substitution interestingly renders about 100-fold increase in activity from **3b** to **3c**. Obtained imines derivatives **4b**, **4d, 4e, and 4f** showed moderate activity at doses of 100 mg/kg at 0.5 or 4 h post administration.

In the scPTZ screen, compounds **3b** and **4a–d** inhibited seizures, which identifies substances elevating seizure threshold. Especially, imines derivatives **4c** and **4d** exhibited full protection against seizures at doses of 300 mg/kg at 0.5 and 4 h post administration.

In the neurotoxicity screening, majority of compounds were devoid of minimal motor impairment at the doses of 30 and 100 mg/kg after 0.5 and 4 h. While **4c** and **4d** elucidated neurotoxicity at 100 mg/kg at 0.5 h followed by an increase in non-toxic potential after delayed absorption at 4 h by preventing minimal motor impairment at 300 mg/kg, and compound **3a** equalized the effect of the standard drug phenytoin.

Since the results of preliminary screening indicated the compounds that contain hydantoin or thiazolidinones ring displayed remarkable activity compared with imines derivatives in MES model, therefore, the compounds **3a–d** were further subjected to phase II screening for quantification of their anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}) in mice, and the results are presented in Table 2.

Compound **3a** with an ED_{50} value of 28.8 mg/kg in the MES model exhibited neurotoxicity at a dose of 100 mg/kg. It is worth mentioning that compound **3b** has little difference in the structure compared with **3c**, which showed especially low activity ($ED_{50} > 200$). **3c** and **3d** had ED_{50} values of 9.8 and 11.0 mg/kg in the MES model and TD_{50} values of 229.4 and 247.5 mg/kg resulting in high protective index (PI) values of $TD_{50}/ED_{50} = 23.4$ and 22.5, respectively. The PI values of the **3c** (23.4) and **3d** (22.5) were higher than the reference drugs as compared to 6.9 for phenytoin and 3.2 for phenobarbital. The PI value is considered to be an index representing the margin of safety and tolerability between anticonvulsant doses and doses of anticonvulsant drugs exerting acute adverse effects. The order of anticonvulsant activities in the MES test as judged from ED_{50} values is **3c** > **3d** > **3a** > **3b**. Actually, earlier structure–activity relationship (SAR) studies with stiripentol [21] have uncovered remarkable anticonvulsant activity for skeleton of benzo[*d*]-[1,3]dioxole. Aboul-Enein et al. [21] attached semicabazone moiety to the backbone of STP (strategy a) and cyclization of the semicarbazone (strategy b) to afford the semicarbazone **5** and racemic pyrazoline **6**,

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Table 2. Phase II quantitative anticonvulsant evaluation in MES model in mice.

Compounds	TPE (h)[a)]	ED_{50}[b)]	TD_{50}[c)]	PI[d)]
3a	1	28.8 (14.4–82.6)[e)]	nd[f)]	
3b	1	>200	nd[f)]	
3c	1	9.8 (3.0–18.1)	229.4 (197.8–264.8)	23.4
3d	3	11.0 (4.7–20.7)	247.5 (216.1–283.5)	22.5
Phenytoin[g)]	2	9.5 (8.1–10.4)	65.5 (52.5–72.9)	6.9
Phenobarbital[g)]	1	21.8 (21.8–25.5)	69 (62.8–72.9)	3.2

[a)] Time to peak effect.
[b)] ED_{50}: median effective dose affording anticonvulsant protection in 50% of animals, the dose is measured in mg/kg.
[c)] TD_{50}: median toxic dose eliciting minimal neurological toxicity in 50% of animals, the dose is measured in mg/kg.
[d)] PI: protective index (TD_{50}/ED_{50}).
[e)] 95% confidence intervals given in parentheses.
[f)] Not determined.
[g)] Data from ref. [14, 20].

respectively (Fig. 2). Interestingly, compound **5** showed a good ED_{50} value of 87 mg/kg in the MES model. In contrast to **5**, a good activity against scPTZ-induced seizures was observed in compound **6** (ED_{50} = 110 mg/kg).

In this study, we conducted the strategy (c) that a five-membered ring could be used to modify neopentyl alcohol based on the cyclization of the amide linker, which gave products **3a–d**. The SAR of **3a–d**, **5**, and **6** are presented in Fig. 2. Collectively, the SAR analyses indicated that the modification of neopentyl alcohol in the strategy (c) was important, and enhanced the anticonvulsant activities of STP analogs in the MES model, such as compounds **3a** (ED_{50} = 28.8 mg/kg), **3c** (ED_{50} = 9.8 mg/kg), and **3d** (ED_{50} = 11.0 mg/kg), which were more active than **5**. **3c** was the most potent of all tested and promising compounds in this work, which exhibited quick absorption with short time to peak effect (1 h).

Conclusion

In the present study, we have synthesized a series of 5-substituted benzo[*d*][1,3]dioxole derivatives as anticonvulsant agents. All the synthesized compounds were screened for their anticonvulsant activity by MES and scPTZ induced seizures test. Six compounds **3a**, **3c**, **3d**, and **4d–f** showed promising anticonvulsant activities in MES model, and imines derivatives **4c** and **4d** exhibited full protection against seizures at doses of 300 mg/kg in scPTZ model. The most active compound **3c** showed the MES-induced seizures with ED_{50} value of 9.8 mg/kg and TD_{50} value of 229.4 mg/kg, which provided compound **3c** with a high protective index (TD_{50}/ED_{50}) of 23.4 comparable to reference antiepileptic drugs. These obtained results showed that certain compounds could be useful as a template for future design, modification, and investigation to produce more active analogs.

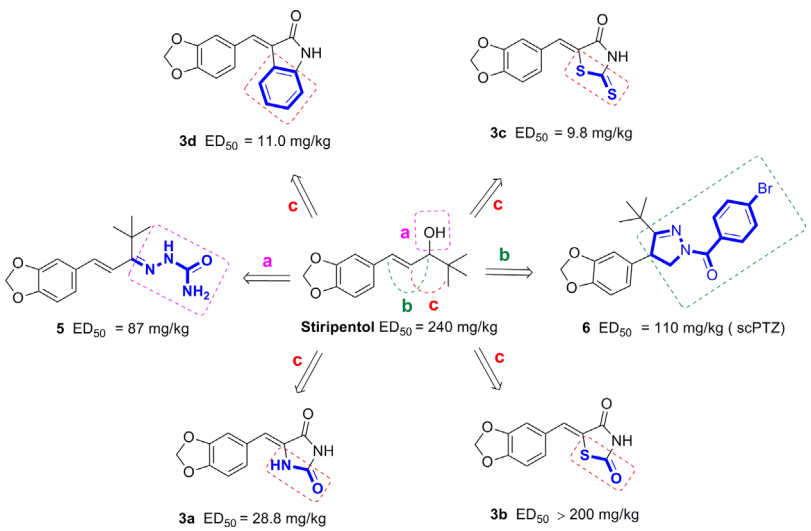

Figure 2. The SAR of **3a–d** and reported STP analogs.

Experimental

Chemistry

General

Melting points were determined in open capillary tubes and are uncorrected. Infrared spectra were measured on a PerkinElmer infrared (FTIR) spectroscopy instrument over a wave number range of 4000–450 cm^{-1}. ^{1}H-NMR spectra were measured on a Bruker Avance 600 MHz NMR spectrometer, with all chemical shifts given in ppm relative to tetramethylsilane. Chemical shift values are in hertz. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets for ^{1}H-NMR data. High resolution mass spectra were recorded on an AB SCIEX Triple TOF™ 5600 equipped with an electrospray ionization source. Dichloromethane/methanol (15:1) (**3a–d**) and ethyl acetate/*n*hexane (1:5) (**2** and **4a–f**) were used as developing solvent for TLC. The major chemicals were purchased from Xiya Reagent Co., Ltd (China).

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

*Synthesis of benzo[d][1,3]dioxole-5-carbaldehyde (**2**)*

A solution of 3,4-dihydroxybenzaldehyde (2.77 g, 20 mmol) in DMF (50 mL) was added dropwise to a suspension of CH_2Cl_2 (1.9 mL, 30 mmol) and K_2CO_3 (5.5 g, 40 mmol) in DMF (50 mL). The mixture was heated under reflux for overnight, then cooled and filtered. The filtrate was washed by water and extracted with diethyl ether (3 × 100 mL). The organic layers were combined and washed by 10% NaOH, then dried (Na_2SO_4) and evaporated to give product **2** as white solid. Rf = 0.45; mp: 36–38°C; yield: 83%; FT-IR: 3081, 3000, 2918, 2852, 2793, 2752, 2722, 1673, 1600, 1490, 1443, 1250, 1032, 928, 860, 812 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 6.05 (s, 2H, CH_2), 6.90 (d, 1H, J = 8.4 Hz, Ar–H_7), 7.29 (d, 1H, J = 1.2 Hz, Ar–H_4), 7.38 (dd, 1H, J = 1.2, 8.4 Hz, Ar–H_6), 9.77 (s, 1H, CHO). ESI-HRMS calcd for $C_8H_6O_3$ ($[M+H]^+$): 151.0317; found: 151.0383.

*General procedure for the synthesis of **3a–c***

A mixture of benzo[*d*][1,3]dioxole-5-carbaldehyde **2** (0.314 g, 2.1 mmol), acetic acid glacial (15 mL), and AcONa (0.413 g, 5.0 mmol) was heated with stirring until complete dissolution occured, then hydantoin or thiazolidinones (2.3 mol) were added and refluxed for 24 h. The reaction mixture was poured into cold water and filtered, the resulted solids were recrystallized with ethanol.

*(Z)-5-Benzo[d][1,3]dioxole-5-ylmethylene-imidazolidine-2,4-dione (**3a**)*

Rf = 0.43; yield: 84%; reddish brown solid; mp: 258–260°C; FT-IR: 3400–2900 (br, NH), 3045, 2901, 1708, 1659, 1503, 1448, 1347, 1285, 1261, 1232, 1107, 1035, 1106, 927, 871 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 6.07 (s, 2H, CH_2), 6.37 (s, 1H, CH), 6.96 (d, 1H, J = 8.4 Hz, Ar–H_7), 7.13 (dd, 1H, J = 1.8, 8.4 Hz, Ar–H_6), 7.28 (d, 1H, J = 1.8 Hz, Ar–H_4), 10.46 (s, br, 1H, NH), 11.19 (s, br, 1H, NH). ^{13}C-NMR (DMSO-d_6, 600 MHz,): δ 101.88 (CH_2), 109.15 (Ar–C_7), 109.20 (Ar–C_4), 109.23 (CH), 125.29 (Ar–C_6), 126.77 (C=CH), 127.51(Ar–C_5), 148.02 (Ar–C–O), 148.28 (Ar–C–O), 156.09 (C=O), 166.05 (C=O). ESI-HRMS calcd for $C_{11}H_8N_2O_4$ ($[M+H]^+$): 233.0480; found: 233.0556.

*(Z)-5-Benzo[d][1,3]dioxole-5-ylmethylene-thiazolidine-2,4-dione (**3b**)*

Rf = 0.61 (CH_2Cl_2/CH_3OH = 15:1); yield: 85%; brown solid; mp: 256–258°C; FT-IR: 3400–2900 (br, NH), 3145, 3008, 2919, 1741, 1694, 1587, 1500, 1443, 1340, 1263, 1154, 1097, 1036, 923, 804 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 6.13 (s, 2H, CH_2), 7.08 (d, 1H, J = 7.8 Hz, Ar–H_7), 7.11 (d, 1H, J = 1.2 Hz, Ar–H_4), 7.14 (dd, 1H, J = 1.2, 7.8 Hz, Ar–H_6), 7.70 (s, 1H, CH), 12.53 (s, br, 1H, NH). ^{13}C-NMR (DMSO-d_6, 600 MHz): δ 102.48 (CH_2), 109.59 (Ar–C_7), 109.66 (Ar–C_4), 121.38 (C=CH), 126.34 (Ar–C_6), 127.63 (Ar–C_5), 132.38 (CH), 148.61 (Ar–C–O), 149.73 (Ar–C–O), 167.82 (C=O), 168.30 (C=O). ESI-HRMS calcd for $C_{11}H_7NO_4S$ ($[M+H]^+$): 250.0096; found: 250.0168.

*(Z)-5-Benzo[d][1,3]dioxole-5-ylmethylene-2-thioxo-thiazolidine-4-dione (**3c**)*

Rf = 0.67 (CH_2Cl_2/CH_3OH = 15:1); yield:87%; brown solid; mp: 301–302°C; FT-IR: 3400–2800 (br, NH), 3145, 2985, 1689, 1579, 1496, 1428 1368, 1307, 1262, 1216, 1190, 1097, 1032, 918, 802 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 6.15 (s, 2H, CH_2), 7.11 (d, 1H, J = 8.4 Hz, Ar–H_7), 7.14 (d, 1H, J = 1.8 Hz, Ar–H_4), 7.18 (dd, 1H, J = 1.8, 8.4 Hz, Ar–H_6), 7.59 (s, 1H, CH), 13.78 (s, br, 1H, NH). ^{13}C-NMR (DMSO-d_6, 600 MHz): δ 102.63 (CH_2), 109.79 (Ar–C_7), 109.99 (Ar–C_4), 123.38 (C=CH), 127.19 (Ar–C_6), 127.65 (Ar–C_5), 132.41 (CH), 148.80 (Ar–C–O), 150.17 (Ar–C–O), 169.88 (C=O), 195.88 (C=S). ESI-HRMS calcd for $C_{11}H_7NO_3S_2$ ($[M+H]^+$): 265.9867; found: 265.9946.

*(E)-3-Benzo[d][1,3]dioxol-5-ylmethylene-indolin-2-one (**3d**)*

A mixture of benzo[*d*][1,3]dioxole-5-carbaldehyde **2** (0.48 g, 3.2 mmol), piperidine, and 1,3-dihydroindol-2-one (0.425 g, 3.2 mmol) was dissolved in EtOH (20 mL), then heated at 90°C for 4 h. The reaction mixture was cooled and the solvent removed under vacuum. The resulting residue was further washed with hexane, and recrystallized by ethanol to give the product **3d**. Rf = 0.6; yield: 64%; brown solid; mp: 218–220°C; FT-IR: 3400–2700 (br, ν(N-H)), 3132, 3073, 3021, 2900, 1704, 1610, 1496, 1448, 1345, 1264, 1219, 1102, 1031, 925, 870, 812, 738 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 6.13 (s, 2H, CH_2), 6.88 (d, 1H, J = 7.8, Ar–H_7), 6.88 (t, 1H, J = 7.8, Ar′–H_5), 7.07 (d, 1H, J = 7.8, Ar′–H_7), 7.22 (t, 1H, J = 7.8, Ar′–H_6), 7.29 (s, 1H, Ar–H_4), 7.29 (d, 2H, J = 7.8 Hz, Ar–H_6), 7.54 (s, 1H, CH), 7.64 (d, 1H, J = 7.8, Ar′–H_4), 10.57 (s, br, 1H, NH). ^{13}C-NMR (DMSO-d_6, 600 MHz): δ 102.15 (CH_2), 109.13 (Ar–C_7), 109.78 (Ar′–C_7), 110.56 (Ar–C_4), 121.46 (C=CH), 121.55 (Ar–C_6), 122.69 (Ar′–C_4), 125.06 (Ar′–C_5), 126.57 (Ar′–C–C), 128.67 (Ar′–C_6), 130.30 (Ar–C_5), 136.46 (CH), 143.23 (Ar′–C–N), 148.08 (Ar–C–O), 149.13 (Ar–C–O), 169.31 (C=O). ESI-HRMS calcd for $C_{16}H_{11}NO_3$ ($[M+H]^+$): 266.0739; found: 266.0814.

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*General procedure for the synthesis of **4a–f***

A mixture of benzo[*d*][1,3]dioxole-5-carbaldehyde **2** (0.61 g, 4.0 mmol), few drops of glacial acetic acid, aniline or substituted anilines (4.0 mmol) was dissolved in dry ethanol (15 mL), then the mixture was refluxed for 4–5 h. After the reaction was completed, the reaction mixture was cooled and poured into ice water, dried, and recrystallized with absolute ethanol to give compounds **4a–f**.

*(E)-N-(Benzo[d][1,3]dioxol-5-ylmethylene)aniline (**4a**)*

Rf = 0.50; yield: 56.4%; pale yellow solid; mp: 64–66°C; FT-IR: 3062, 2785, 1623, 1601, 1495, 1448, 1261, 1210, 1104, 1038, 934, 812, 767, 697 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 6.07 (s, 2H, CH_2), 6.91 (d, 1H, J = 7.8 Hz, Ar_1–H_7), 7.20–7.25 (m, 3H, Ar_2–H), 7.30 (dd, 1H, J = 1.2 Hz, 7.8 Hz, Ar_1–H_6), 7.39–7.42 (m, 2H, Ar_2–H), 7.56 (d, 1H, J = 1.2 Hz, Ar_1–H_4), 8.36 (s, 1H, CH). ESI-HRMS calcd for $C_{14}H_{11}NO_2$ ($[M+H]^+$): 226.0790; found: 226.0861.

*(E)-N-(Benzo[d][1,3]dioxol-5-ylmethylene)-3-chloroaniline (**4b**)*

Rf = 0.64; yield: 62.0%; white solid; mp: 60–61°C; FT-IR: 3060, 3011, 2887, 1629, 1601, 1501, 1440, 1254, 1210, 1089, 1032, 929, 862, 821, 775, 683 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 6.07 (s, 2H, CH_2), 6.91 (d, 1H, J = 7.8 Hz, Ar_1–H_7), 7.08–7.10 (m, 1H, Ar_2–H), 7.19–7.22 (m, 2H, Ar_2–H), 7.29 (dd, 2H, J = 1.2 Hz, 7.8 Hz, Ar_1–H_6), 7.32 (t, 1H, J = 7.8 Hz, Ar_2–H_5), 7.53 (d, 1H, J = 1.2 Hz, Ar_1–H_4), 8.32 (s, 1H, CH). ESI-HRMS calcd for $C_{14}H_{10}ClNO_2$ ($[M+H]^+$): 260.0400; found: 260.0472.

*(E)-N-(Benzo[d][1,3]dioxol-5-ylmethylene)-3-nitroaniline (**4c**)*

Rf = 0.45; yield: 79.9%; pale yellow solid; mp: 116–118°C; FT-IR: 3076, 2783, 1628, 1596, 1498, 1447, 1523, 1344, 1261, 1211, 1081, 1036, 932, 816, 800, 689 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 6.10 (s, 2H, CH_2), 6.94 (d, 2H, J = 7.8 Hz, Ar_1–H_7), 7.33 (d, 2H, J = 1.2, 7.8 Hz, Ar_1–H_6), 7.52–7.54 (m, 1H, Ar_2–H_6), 7.56 (d, 1H, J = 1.2 Hz, Ar_1–H_4), 7.57 (t, 1H, J = 7.8 Hz, Ar_2–H_5), 8.04 (t, 1H, J = 1.8 Hz, Ar_2–H_2), 8.09–8.10 (m, 1H, Ar_2–H_4), 8.39 (s, 1H, CH). ESI-HRMS calcd for $C_{14}H_{10}N_2O_4$ ($[M+H]^+$): 270.0641; found: 271.0713.

*(E)-N-(Benzo[d][1,3]dioxol-5-ylmethylene)-4-(tert-butyl)-aniline (**4d**)*

Rf = 0.70; yield: 91.8%; pale yellow solid; mp: 92–93°C; FT-IR: 3084, 2962, 2863, 2776, 1624, 1589, 1501, 1442, 1252, 1214, 1100, 1038, 932, 875, 830, 812 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 1.37 (s, 9H, $3CH_3$), 6.06 (s, 2H, CH_2), 6.90 (d, 1H, J = 7.8 Hz, Ar_1–H_7), 7.17 (dt, 2H, J = 2.4, 8.4 Hz, Ar_2–H_2, and Ar_2–H_6), 7.29 (dd, 1H, J = 1.2, 7.8 Hz, Ar_1–H_6), 7.43 (dt, 2H, J = 2.4, 8.4 Hz, Ar_2–H_3, and Ar_2–H_5), 7.56 (d, 1H, J = 1.2 Hz, Ar_1–H_4), 8.38 (s, 1H, CH). ESI-HRMS calcd for $C_{18}H_{19}NO_2$ ($[M+H]^+$): 282.1416; found: 282.1486.

*(E)-N-(Benzo[d][1,3]dioxol-5-ylmethylene) -2,6-difluoroaniline (**4e**)*

Rf = 0.64; yield: 57.9%; white solid; mp: 89–90°C; FT-IR: 3076, 2913, 2797, 1631, 1600, 1500, 1451, 1262, 1236, 1212, 1040, 927, 874, 771, 706 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 6.08 (s, 2H, CH_2), 6.91 (d, 1H, J = 7.8 Hz, Ar_1–H_7), 6.95–7.00 (m, 2H, Ar_2–H_3, and Ar_2–H_5), 7.04–7.09 (m, 1H, Ar_2–H_4), 7.31 (dd, 1H, J = 1.2, 7.8 Hz, Ar_1–H_6) 7.60 (d, 1H, J = 1.2 Hz, Ar_1–H_4), 8.50 (s, 1H, CH). ESI-HRMS calcd for $C_{14}H_9F_2NO_2$ ($[M+H]^+$): 262.0601; found: 262.0672.

*(E)-N-(Benzo[d][1,3]dioxol-5-ylmethylene)-2-fluoroaniline (**4f**)*

Rf = 0.62; yield: 74.3%; pale yellow solid; mp: 72–74°C; FT-IR: 3020, 2907, 2788, 1623, 1585, 1496, 1450, 1262, 1228, 1103, 1030, 927, 822, 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 6.07 (s, 2H, CH_2), 6.91 (d, 1H, J = 7.8 Hz, Ar_1–H_7), 7.13–7.18 (m, 4H, Ar_2H), 7.31 (dd, 1H, J = 1.2, 7.8 Hz, Ar_1–H_6), 7.59 (d, 1H, J = 1.2 Hz, Ar_1–H_4), 8.42 (s, 1H, CH). ESI-HRMS calcd for $C_{14}H_{10}FNO_2$ ($[M+H]^+$): 244.0696; found: 244.0772.

Pharmacology

Maximal electroshock test (MES)

The maximal electroshock seizure test was carried out according to the standard protocol [14]. Male mice (Kunming, China), weighing 20–25 g, were used as experimental animals. Mice were housed under temperature-controlled conditions (25–30°C) and a 12-h light/dark cycle. They were allowed to acclimatize with free access to food and water for a 24-h period before testing except during the experiment. Abolition of hind limb tonic extension spasm was recorded as anticonvulsant activity. The test compounds were dissolved in an aqueous solution of 50% polyethylene glycol. In preliminary screening, each compound was administered as an *ip* injection at three dose levels (30, 100, 300 mg/kg body mass) and the anticonvulsant activity was assessed after 0.5 and 4 h intervals of administration.

Subcutaneous pentylenetetrazole seizure test (scPTZ)

This test involved treating the mice with metrazol (pentylenetetrazole, 85 mg/kg in mice). This produced clonic seizures lasting for a period of at least 5 s in 97% of the animals tested. At the anticipated time of testing, the convulsant was subcutaneously administered. The test compound was intraperitoneally administered in mice and the animals were observed over a 30-min period. Mice were tested 0.5 and 4 h after doses of 100 and 300 mg/kg of the test compound were administered. The absence of clonic spasms over the period of observation indicated the compound's ability to abolish the effect of pentylenetetrazol on the seizure threshold.

Neurotoxicity screening

The rotarod test was used to evaluate neurotoxicity. The mice were trained to stay on an accelerating rotarod that rotated at six revolutions per minute. The rod diameter was 3.2 cm. Trained animals were given *ip* injection of the test compounds in doses of 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials.

Quantification studies
The ED_{50} was calculated using the Bliss method. The ED_{50} values were presented as the mean with 95% confidence intervals. Neurotoxicity was expressed as the median toxic dose (TD_{50} in mg/kg) eliciting minimal neurological toxicity in 50% of animals. The quantitative determinations of ED_{50} and TD_{50} values were performed at the previously estimated time of peak effect after *ip* injection into mice. Groups of six animals received various doses of compounds **3a–d** until at least three points were established in the range of 10–90% seizure protection. The results are shown in Table 2.

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