Received: 25 October 2018 | Revised: 15 April 2019 | Accepted: 1 May 2019

DOI: 10.1002/ardp.201800313

DPhG ARCH PHARM
Archiv der Pharmazie

FULL PAPER

Design, synthesis, and evaluation of anticonvulsant activities of benzoxazole derivatives containing the 1,2,4-triazolone moiety

Ming-Xia Song[1] | Yuping Huang[2] | Shiben Wang[3] | Zeng-Tao Wang[4] | Xian-Qing Deng[1]

[1]Medical College, Jinggangshan University, Ji'an, China

[2]Department of Biochemistry and Molecular Biology, Gannan Medical College, Ganzhou, China

[3]School of Pharmacy, Liaocheng University, Liaocheng, China

[4]College of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang, China

Correspondence
Xian-Qing Deng, Medical College, Jinggangshan University, No. 28 Xueyuan Road, Ji'an, 343009 Jiangxi, China.
Email: dengxianqing1121@126.com

Funding information
National Natural Science Foundation of China, Grant/Award Number: 21562028; Doctoral Foundation of Jinggangshan University, Grant/Award Number: JZB1316

Abstract

A novel series of benzoxazole derivatives containing 1,2,4-triazolone (**5a–m**) was designed. These compounds were synthesized in order to screen their anticonvulsant activities by the maximal electroshock seizure (MES) model and the subcutaneous pentylenetetrazole (sc-PTZ) seizure model in mice. The rotarod test was used to evaluate their neurotoxicities. Most of the compounds showed anti-MES activities at 100 and 300 mg/kg. Compound **5f**, which showed potential anticonvulsant activity in the MES model with ED_{50} values of 22.0 mg/kg, was considered as the most promising one in this study. It exhibited greater safety than that of carbamazepine and valproate regarding neurotoxicity. The efficacy of compound **5f** in inhibiting the tonic seizures and death induced by the convulsants 3-mercaptopropionic acid and BIC was also verified. In an enzyme-linked immunosorbent assay, compound **5f** and the positive drug phenytoin significantly increased the γ-aminobutyric acid (GABA) level in the mouse brain. Further, pretreatment with an inhibitor of the GABA synthesizing enzyme dramatically raised the ED_{50} value of **5f** in the MES model. These results confirmed that the compound **5f** plays its anticonvulsive action via regulating the GABA function in the brain. Also, a docking study of the compound **5f** in the benzodiazepine (BZD) binding site of the $GABA_A$ receptor confirmed possible binding of the compound **5f** with BZD receptors.

KEYWORDS

Anticonvulsant, benzodiazepine receptors, docking, maximal electroshock seizure, sc-PTZ, triazolone

1 | INTRODUCTION

Epilepsy, one of the most common neurological disorders, affects approximately 1% of the world's population.[1,2] At present, anticonvulsant drugs have been the mainstay of therapy. However, the currently available anticonvulsants are effective in reducing the severity and number of seizures in less than 70% of patients. Moreover, their use is often associated with undesirable side effects, ranging from indisposition (naupathia) to life-threatening (hepatotoxicity or megaloblastic anemia).[3–5] Therefore, the continued search for more effective and safer anticonvulsant drugs is both an urgent and necessary task for medicinal chemistry.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the adult mammalian central nervous system. The

 © 2019 Deutsche Pharmazeutische Gesellschaft

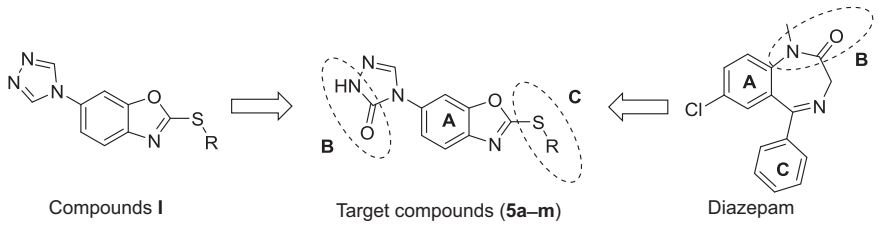


FIGURE 1 The structures of compounds **I**, diazepam, and target compounds (**5a–m**)

development of GABAergic drugs is an active area of research in the fields of medicinal chemistry and neurodegenerative disease. GABA type A receptors are valuable for their rich pharmacology as they are targets for a range of therapeutically important drugs, including the benzodiazepines (BZDs), barbiturates, neuroactive steroids, and anesthetics.[6,7] Triazole is a functionalized fragment in a variety of different compounds in medicinal chemistry and has been reported to exhibit a broad range of biological properties.[8] Since the approval and sales of triazolam and alprazolam (two of BZDs) as anticonvulsants in the 1980s, more and more medicinal chemists paid their attention to design and synthesis of the triazole-derivatives for their anticonvulsant activity.[9]

In previous work, the anticonvulsant activity of a series of benzoxazole derivatives containing triazole were reported (Figure 1; **I**).[10] Among these compounds, 2-((4-fluobenzyl)thio)-6-(4*H*-1,2,4-triazol-4-yl)benzo[*d*]oxazole was the most active one with an ED_{50} of 12.7 mg/kg and a protective index (PI) value (TD_{50}/ED_{50}) of 38.7 in the maximal electroshock seizure (MES) test. Mechanism research suggested the GABAergic system may contribute to the anticonvulsive action of this compound. As a continuation of this work, a series of benzoxazoles containing 1,2,4-triazolone moiety (**5a–m**) were designed and synthesized in the current study, in which the triazole moiety in compounds of **I** were replaced with triazolone moiety.

Presumably, the designed compounds will act as anticonvulsant agents through BZD receptors, because they have the essential features like diazepam for binding to those receptors: an aromatic ring (A), a coplanar proton-accepting group in a suitable distance (B), and second out-of-plane aromatic ring (C). The pharmacophore moieties A and B are essential for interaction with the $GABA_A$ receptor, while the part C could potentiate binding to the receptor (Figure 1).[11,12]

Thus we report here, the synthesis, pharmacological evaluation, and docking study of 4-(2-(alkylthio)benzo[*d*]oxazol-6-yl)-2*H*-1,2,4-triazol-3(4*H*)-one derivatives (**5a–m**) as potential anticonvulsant agents.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

As seen in Scheme 1, 2-amino-4-nitrophenol (**1**) was used as the starting material, which reacted with CS_2 in the presence of $EtOH/H_2O/KOH$ to obtain 6-nitrobenzo[*d*]oxazole-2-thiol (**2**). The compound **2** reduced by $Na_2S.9H_2O$ in ethanol to give 6-aminobenzo[*d*]oxazole-2-thiol (**3**). Then, the thiol group of compound **3** was alkylated by various alkylating agents to obtain compound **4**. Finally, compound **4** was treated with methyl hydrazine carboxylate and triethyl orthoformate in the presence of sodium methoxide to provide the target compounds **5a–m**.[13] Their chemical structures were characterized using ^{1}H-NMR, ^{13}C NMR, and mass spectrometry analysis techniques. The detailed physical and analytical data of these compounds has been provided in Section 4.

2.2 | Anticonvulsant activity screens

The MES, subcutaneous pentylenetetrazole (sc-PTZ) model, and rotarod test were used to evaluate the anticonvulsant activities and neurotoxicities of all the target compounds. As shown in Table 1, most compounds showed anticonvulsant activity in the MES model at different doses. Among them, compounds **5a** and **5f** showed the best activity with the protection at the dose of 30 mg/kg, and kept active after 4 hr at the higher dose. Compounds **5b**, **5e**, **5g**, and **5m** displayed medium anticonvulsant activity at a dosage of 100 mg/kg in the MES test at 0.5 hr, and also remained active at a dose of 300 mg/kg after 4 hr. Those remaining, except **5c** and **5k**, all displayed weak activity at the dosage of 300 mg/kg in the MES test. In the sc-PTZ screens, compounds **5a–c**, **5f**, **5h**, **5j**, and **5l–m** protected the mice against sc-PTZ induced seizure at 300 mg/kg at 0.5-hr interval. Compounds **5a** and **5m** kept active after 4 hr at the same dose. In the rotarod test, none of the target compounds exhibited neurotoxicity at the maximum dose of 300 mg/kg.

It was observed that several compounds kept active at the 4-hr interval, which indicates the protective effects with long duration. In order to assess the anticonvulsant activity more accurately, the time

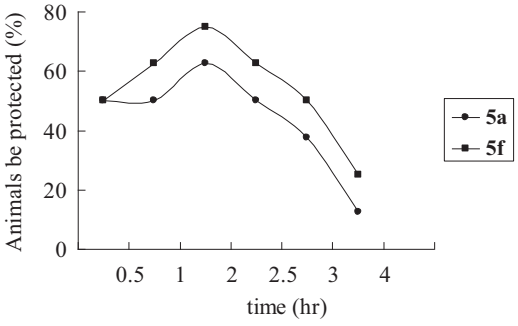
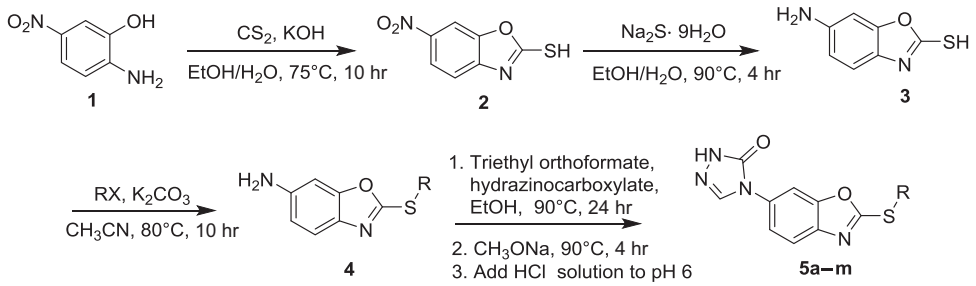


FIGURE 2 Time-course of compounds **5a** and **5f** (30 mg/kg) in the maximal electroshock seizure test (i.p.). The data illustrates % animal protected relative to animal tested (8 for each point) at various times after injection



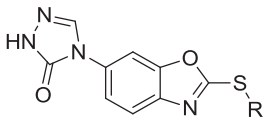
SCHEME 1 The synthesis route of target compounds (**5a–m**)

to peak effect (TPE) of compounds **5a** and **5f** was tested. As shown in Figure 2, compounds **5a** and **5f** both reached the TPE at 2-hr interval after i.p. administration. Hence, compounds **5a** and **5f** were quantitatively measured for their anticonvulsant activity at 2-hr time interval in the next assay.

Based on the notable anticonvulsant activity of **5a** and **5f** shown in the initial trials, the quantitative evaluation trials were performed to obtain the median effective dose (ED_{50}) and median toxic dose (TD_{50}). As shown in Table 2, the ED_{50} values of **5a** and **5f** were measured as 26.4 and 22.0 mg/kg in the MES model, respectively. The TD_{50} values of **5a** and **5f** were measured as 340.8 and 366.6 mg/kg, respectively, which resulted in the PI values of 12.9 and 16.7, respectively. In the aspect of safety, compounds **5a** and **5f** showed superior performance to carbamazepine and valproate.

3-Mercaptopropionic acid (3-MP), as a competitive inhibitor of glutamate decarboxylase, can inhibit the synthesis of GABA, subsequently decreasing the levels of this neurotransmitter in the brain.[14] Bicuculline (BIC), a competitive $GABA_A$ receptor antagonist, is known to produce seizures in mice after subcutaneous injection at low dose.[15] To further evaluate the anticonvulsive effects of **5f**, 3-MP- and BIC-induced seizure tests were performed. As shown in Table 3, 3-MP (60 mg/kg) alone induced clonic and tonic seizures in 100% of the tested mice, while the combined treatment with **5f** led to a marketable decrease in tonic convulsions and death (from 100 to

TABLE 1 Anticonvulsant activity and neurotoxicity data of compounds **5a–m** administered intraperitoneally to mice



		Intraperitioneal injection in mice[a]					
		MES screening[b]		sc-PTZ screening[c]		NT screening[d]	
Compounds	R	0.5 hr	4 hr	0.5 hr	4 hr	0.5 hr	4 hr
5a	C_3H_7	**30**	100	300	300	–	–
5b	C_5H_9	100	300	300	–	–	–
5c	C_7H_{15}	–	–	300	–	–	–
5d	$CH_2C_6H_5$	300	300	–	–	–	–
5e	$CH_2C_6H_4$(*o-F*)	100	300	–	–	–	–
5f	$CH_2C_6H_4$(*m-F*)	**30**	100	300	–	–	–
5g	$CH_2C_6H_4$(*p-F*)	100	300	–	–	–	–
5h	$CH_2C_6H_4$(*o-Cl*)	300	–	300	–	–	–
5i	$CH_2C_6H_4$(*m-Cl*)	300	–	–	–	–	–
5j	$CH_2C_6H_4$(*p-Cl*)	300	–	300	–	–	–
5k	$CH_2C_6H_4$(*2,4-Cl_2*)	–	–	–	–	–	–
5l	$CH_2C_6H_4$(*p-CH_3*)	300	–	300	–	–	–
5m	$CH_2C_6H_4$(*p-OCH_3*)	100	300	300	300	–	–

[a]Three mice were used for each group, doses of 30, 100, and 300 mg/kg were administered. The figure in the table indicates the minimum dose whereby bioactivity was demonstrated in 2/3 or 3/3 of the mice. The animals were examined at 0.5 and 4 hr after injection was administered. A dash indicates the absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg).
[b]Maximal electroshock seizure test.
[c]Subcutaneous pentylenetetrazole test.
[d]Neurotoxicity screening (rotarod test).

TABLE 2 Quantitative anti-MES activity of 5a and 5f in mice administered intraperitoneally

Compounds	ED_{50}[a]	TD_{50}[b]	PI[c]
5a	26.4 (24.0–29.1)	340.8 (309.9–374.9)	12.9
5f	22.0 (20.1–24.1)	366.6 (333.3–403.3)	16.7
Carbamazepine	9.8 (8.9–10.8)	44.0 (40.2–48.1)	4.5
Valproate	264 (247–338)	418 (369–450)	1.6

[a]ED_{50}: median effective dose.
[b]TD_{50}: median toxic dose.
[c]PI: protective index (TD_{50}/ED_{50}).

10%, $p < 0.001$; and from 80 to 30%, $p < 0.05$, for seizures and death, respectively). As shown in Table 4, when administered with 5.4 mg/kg of BIC, the mice produced clonic seizures and tonic seizures in 100%, and death in 90%. By comparison, after combined treatment with **5f**, the tonic seizures and death of the mice were inhibited significantly (from 100 to 0%, $p < 0.001$; and from 90 to 10%, $p < 0.01$, for tonic seizures and death, respectively). The inhibitory action of compound **5f** against the tonic seizures and death induced by 3-MP and BIC further confirmed the anticonvulsant activity of **5f**. Furthermore, the suppression of **5f** on the BIC-induced seizure suggested that it may play its anticonvulsive action via acting on the $GABA_A$ active site or increasing the concentration of GABA in the brain.

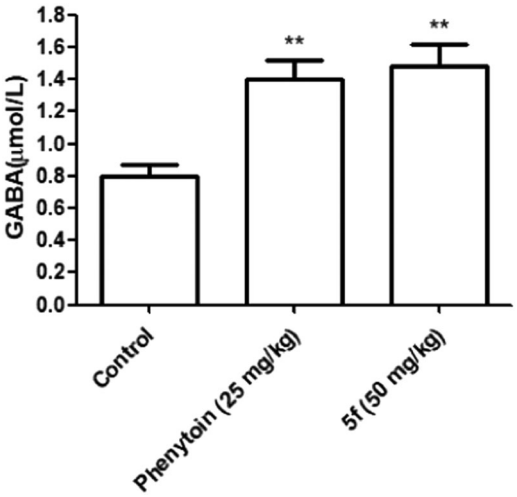


FIGURE 3 Effects of **5f** and phenytoin on whole brain γ-aminobutyric acid (GABA) concentration in mice. Data are presented as mean ± SEM. of seven animals. $^{**}p < 0.01$ versus control (animals treated with saline)

2.3 | Anticonvulsant mechanism of 5f involved in the GABAergic system

To investigate whether the concentration of GABA in the brain was involved in the anticonvulsive action of compound **5f**, an enzyme-linked immunosorbent assay (ELISA) was carried out to measure the effect of **5f** on the GABA level in mouse brain. As shown in Figure 3, when compared to the results of the control group, compounds **5f** and positive control drug phenytoin both significantly increased the level of GABA in mouse brain ($p < 0.01$).

Thiosemicarbazide (TSC), a competitive inhibitor of the GABA synthesis enzyme, can inhibit the synthesis of GABA, resulting in a decrease of GABA level in the brain.[16] To further confirm the anticonvulsive mechanism of **5f** involved in the GABAergic system, the influence of TSC on the anti-MES activity of **5f** was investigated. As shown in Figure 4, the ED_{50} value of **5f** in the normal mice in MES model was 22.0 mg/kg, while the ED_{50} value of **5f** in the treated mice was 273.86 mg/kg. Obviously, pretreatment with TSC (25 mg/kg/day for 3 days) significantly increased the ED_{50} value of **5f** in the MES model ($p < 0.001$). This result further suggested that compound **5f** plays its anticonvulsive action via regulating the GABA function in the brain.

2.4 | Docking study

To confirm whether the anticonvulsant activity of the synthesized compounds mediates through BZD receptors, the molecular docking of representative **5f** with the BZD-binding site of $GABA_A$ receptor (α1β2γ2) was performed by Discovery Studio 4.5 version. Interactions of the compound **5f** with amino acids of BZD-binding pocket of $GABA_A$ receptor were illustrated and shown in Figure 5. As can be seen in Figure 5, the most important residues in the binding mode of compound **5f** are Thr206, Tyr 209, Phe77, His101, Tyr159, Heu140, Val190, and Glu189. The carbonyl group of compound **5f**, as a H-bond acceptor, was involved in an interaction with the hydroxy group of Thr206, while the trizolone ring was responsible for π-π interaction with Tyr209 and Heu140. In addition to above, the benzoxazole and terminal phenyl moiety also showed π-π interactions with some critical amino acid residues (Phe77, Tyr159, His101, and Val190). Moreover, the hydrogen bonding between the F atom of the compound **5f** and

TABLE 3 Effects of compound **5f** on 3-MP-induced seizures in mice

Compound	Doses (mg/kg)	Test time (hr)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO	–	0.5	100	100	80
Carbamazepine	30	0.5	100	0***	0**
5f	30	0.5	100	10***	30*

Note: Results are expressed as a percentage of animals that showed clonic and tonic convulsions and death among all animals tested. Ten mice were used in each group. Significance determined with Fisher's exact test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control group (3-MP, 60 mg/kg).
Abbreviation: DMSO, dimethyl sulfoxide

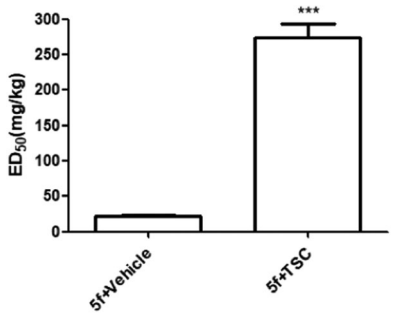


FIGURE 4 Effects of the thiosemicarbazide on the anticonvulsant activity of **5f** against maximal electroshock-induced seizures in mice. All data are presented as median effective dose (ED_{50}) ± SEM. All drugs were administered i.p. in a single injection, 30 minutes before maximal electroshock-induced seizures; ***$p < 0.001$ versus control group (pretreatment with saline)

residue Glu189 may help to explain the best activity of **5f** among this series of compounds. According to the reports of Richter and Mohammadi-Khanaposhtani,[17,18] Thr206, Tyr209, Phe77, His101, and Tyr159 are the critical residues in the binding mode of diazepam. Therefore, the binding mode of compound **5f** in the BZD-binding pocket of $GABA_A$ receptor resembled to that of diazepam, and the synthesized compounds may well play its anticonvulsant activity via mediating BZD receptors.

3 | CONCLUSION

In summary, we have synthesized a series of 4-(2-(alkylthio)benzo[*d*]-oxazol-6-yl)-2*H*-1,2,4-triazol-3(4*H*)-one (**5a–m**) and investigated their anticonvulsant activities using MES and PTZ models. In the current work, compound **5f** was considered as the most promising one, which displayed outstanding anticonvulsant activity in the MES model with an ED_{50} value of 22.0 mg/kg. The protective action of **5f** in 3-MP- and BIC-induced seizure models further confirmed its anticonvulsant activity. Furthermore, experiments investigating the anticonvulsant mechanism of **5f** suggested that the compound **5f** plays its anticonvulsive action by increasing the GABA content or regulating the GABA function in brain. The docking of **5f** in the BZD-binding site of $GABA_A$ receptor demonstrated that this compound has a similar binding to diazepam, thus it may well play its anticonvulsant activity via mediating BZD receptors.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Melting points were measured by a capillary tube. Infrared spectroscopy was determined (in KBr using an FTIR1730 spectrometer [Perkin Elmer, Waltham, MA]). ^{1}H NMR and ^{13}C NMR spectroscopies were measured on an AV-300 spectrometer (Bruker, Switzerland) using tetramethylsilane as the internal standard. An MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Germany) was used to measure high resolution mass spectroscopy. The major chemicals were bought from Aldrich Chemical Corporation (St. Louis).

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

4.1.2 | Procedures for the synthesis of compounds 2, 3, and 4

Compounds **2**, **3**, and **4** were previously reported.[10]

4.1.3 | General procedure for the synthesis of 4-(2-(alkylthio)benzo[*d*]oxazol-5-yl)-2,4-dihydro-3*H*-1,2,4-triazol-3-ones (5a–m)

Compounds **4** (10 mmol), methyl hydrazinocarboxylate (50 mmol), and triethyl orthoformate (50 mmol) were placed into a round-bottomed flask containing 30 ml anhydrous alcohol and refluxed for 24 hr. After cooling, sodium methylate (50 mmol) was added and refluxed. After the reaction was completed (the mixture went pink as the sign), the mixture was cooled down to room temperature and diluted by 120 ml water followed the neutralizing with HCl solution to pH 6. The precipitate formed was filtered and washed with water, which was then purified by recrystallization with ethanol to obtain compounds **5a–m**. The yield, melting point, analytical data, and the spectral data of each compound are given below.

*4-(2-(Propylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5a**)*

Mp 168–169°C, yield 59%. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.12 (t, 3H, J = 7.4 Hz, CH_2CH_3), 1.87–1.94 (m, 2H, CH_2CH_3), 3.33 (t, 2H, J = 7.2 Hz, SCH_2), 7.41 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.9 Hz, Ph-H), 7.70 (d, 1H, J = 8.5 Hz, Ph-H), 7.76 (s, 1H, CH=N), 7.79 (d, 1H, J = 1.9 Hz,

TABLE 4 Effects of compound **5f** on BIC-induced seizures in mice

Compound	Doses (mg/kg)	Test time (hr)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
DMSO	–	0.5	100	100	90
Carbamazepine	30	0.5	100	0***	20**
5f	30	0.5	100	0***	10**

Note: Results are expressed as a percentage of animals that showing clonic and tonic convulsions and death among all animals tested. The number of animals tested in each group was 10. Significance determined with Fisher's exact test. **$p < 0.01$, ***$p < 0.001$ when versus control group (BIC, 5.4 mg/kg). Abbreviation: DMSO, dimethyl sulfoxide

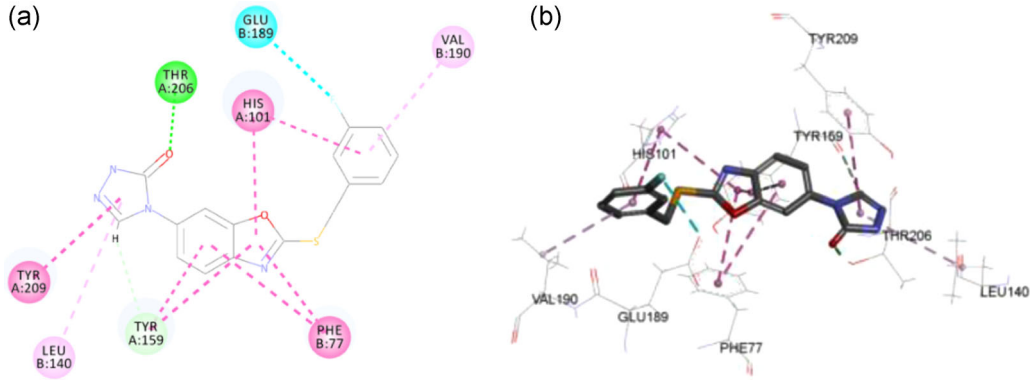


FIGURE 5 The binding modes of compound **5f** within the benzodiazepine-binding pocket of $GABA_A$ (A for 2D model; B for 3D model). GABA, γ-aminobutyric acid

Ph-H), 10.82 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$, 75 MHz): δ 167.4, 154.1, 151.8, 141.4, 136.0, 129.3, 118.9, 118.8, 105.2, 34.3, 22.7, 13.2. IR (KBr) cm^{-1}: 3211 (N-H), 1710 (C=O). ESI-HRMS calcd for $C_{12}H_{13}N_4O_2S^+$ ($[M+H]^+$): 277.0754; found: 277.0758.

*4-(2-(Pentylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5b**)*

Mp 160–162°C, yield 66%. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.95 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.32–1.59 (m, 4H, $(CH_2)_2CH_3$), 1.82–1.91 (m, 2H, SCH_2CH_2), 3.34 (t, 2H, J = 7.3 Hz, SCH_2), 7.40 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.7 Hz, Ph-H), 7.70 (d, 1H, J = 8.4 Hz, Ph-H), 7.76 (s, 1H, CH=N), 7.79 (d, 1H, J = 1.7 Hz, Ph-H), 10.63 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$, 75 MHz): δ 167.3, 154.0, 151.8, 141.5, 135.8, 129.2, 118.9, 118.8, 105.2, 32.4, 30.8, 28.9, 22.1, 13.9. IR (KBr) cm^{-1}: 3213 (N-H), 1711 (C=O). ESI-HRMS calcd for $C_{14}H_{17}N_4O_2S^+$ ($[M+H]^+$): 305.1067; found: 305.1075.

*4-(2-(Heptylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5c**)*

Mp 167–168°C, yield 67%. ^{1}H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 0.83 (t, 3H, J = 6.7 Hz, CH_2CH_3), 1.18–1.46 (m, 8H, $(CH_2)_4CH_3$), 1.68–1.85 (m, 2H, SCH_2CH_2), 3.25 (t, 2H, J = 7.3 Hz, SCH_2), 7.50 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.9 Hz, Ph-H), 7.57 (d, 1H, J = 8.5 Hz, Ph-H), 7.87 (d, 1H, J = 1.9 Hz, Ph-H), 8.09 (s, 1H, CH=N), 11.87 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 166.3, 153.5, 151.6, 140.6, 136.1, 130.4, 118.4, 118.4, 104.4, 32.3, 31.6, 29.3, 28.6, 28.4, 22.5, 14.2. IR (KBr) cm^{-1}: 3215 (N-H), 1712 (C=O). ESI-HRMS calcd for $C_{16}H_{21}N_4O_2S^+$ ($[M+H]^+$): 333.1380; found: 333.1373.

*4-(2-(Benzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5d**)*

Mp 199°C, yield 59%. ^{1}H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.50 (s, 2H, SCH_2), 7.21–7.41 (m, 5H, benzyl-H), 7.44 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.9 Hz, Ph-H), 7.58 (d, 1H, J = 8.5 Hz, Ph-H), 7.82 (d, 1H, J = 1.9 Hz, Ph-H), 7.92 (s, 1H, CH=N), 11.82 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.6, 153.6, 151.8, 140.6, 135.9, 135.8, 130.3, 129.1, 128.8, 128.0, 118.6, 118.5, 104.6, 36.4. IR (KBr) cm^{-1}: 3206 (N-H), 1711 (C=O). ESI-HRMS calcd for $C_{16}H_{13}N_4O_2S^+$ ($[M+H]^+$): 325.0754; found: 325.0753.

*4-(2-(2-Fluorobenzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5e**)*

Mp 215–216°C, yield 57%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.52 (s, 2H, SCH_2), 6.98–7.27 (m, 3H, benzyl-H), 7.42 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.0 Hz, Ph-H), 7.45–7.51 (m, 1H, benzyl-H), 7.59 (d, 1H, J = 8.5 Hz, Ph-H), 7.80 (d, 1H, J = 2.0 Hz, Ph-H), 7.86 (s, 1H, CH=N), 11.79 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.4, 160.9 (d, $^1J_{c\text{-}f}$ = 246.3 Hz), 153.6, 151.8, 140.6, 135.7, 131.3, 131.2, 130.3, 130.1(d, $^3J_{c\text{-}f}$ = 8.2 Hz), 124.5 (d, $^4J_{c\text{-}f}$ = 3.6 Hz), 123.2 (d, $^2J_{c\text{-}f}$ = 14.5 Hz), 118.6 (d, $^3J_{c\text{-}f}$ = 11.0 Hz), 115.6 (d, $^2J_{c\text{-}f}$ = 20.0 Hz), 104.7, 29.8. IR (KBr) cm^{-1}: 3200 (N-H), 1711 (C=O). ESI-HRMS calcd for $C_{16}H_{12}FN_4O_2S^+$ ($[M+H]^+$): 343.0660; found: 343.0665.

*4-(2-(3-Fluorobenzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5f**)*

Mp 209°C, yield 69%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.47 (s, 2H, SCH_2), 6.88–7.28 (m, 4H, benzyl-H), 7.40 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.9 Hz, Ph-H), 7.58 (d, 1H, J = 8.5 Hz, Ph-H), 7.78 (d, 1H, J = 1.9 Hz, Ph-H), 7.82 (s, 1H, CH=N), 11.76 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.3, 162.6 (d, $^1J_{c\text{-}f}$ = 244.6 Hz), 153.6, 151.8, 140.5, 138.8 (d, $^3J_{c\text{-}f}$ = 7.6 Hz), 135.8, 130.5, 130.4, 125.0, 124.9, 118.6 (d, $^3J_{c\text{-}f}$ = 10.5 Hz), 116.0 (d, $^2J_{c\text{-}f}$ = 21.9 Hz), 114.8 (d, $^2J_{c\text{-}f}$ = 20.9 Hz), 104.7, 35.8. IR (KBr) cm^{-1}: 3198 (N-H), 1710 (C=O). ESI-HRMS calcd for $C_{16}H_{12}FN_4O_2S^+$ ($[M+H]^+$): 343.0660; found: 343.0662.

*4-(2-(4-Fluorobenzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5g**)*

Mp 219–221°C, yield 61%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.53 (s, 2H, SCH_2), 6.98–7.04 (m, 2H, benzyl-H), 7.45–7.50 (m, 2H, benzyl-H), 7.56 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.8 Hz, Ph-H), 7.62 (d, 1H, J = 8.5 Hz, Ph-H), 7.92 (d, 1H, J = 1.8 Hz, Ph-H), 8.17 (s, 1H, CH=N), 11.91 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.4, 162.1 (d, $^1J_{c\text{-}f}$ = 244.0 Hz), 153.5, 151.7, 140.3, 136.2, 132.6

(d, $^{4}J_{c\text{-}f}$ = 3.2 Hz), 131.2 (d, $^{3}J_{c\text{-}f}$ = 8.2 Hz), 130.7, 118.6, 118.5, 115.6 (d, $^{2}J_{c\text{-}f}$ = 21.4 Hz), 104.4, 35.5. IR (KBr) cm^{-1}: 3144 (N-H), 1711 (C=O). ESI-HRMS calcd for $C_{16}H_{12}FN_4O_2S^+$ ($[M+H]^+$): 343.0660; found: 343.0660.

*4-(2-(2-Chlorobenzylthio)benzo[d]oxazol-6-yl)-2H-1,2, 4-triazol-3(4H)-one (**5h**)*

Mp 201–203°C, yield 63%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.59 (s, 2H, SCH_2), 7.18–7.23 (m, 2H, benzyl-H), 7.33 (d, 1H, J = 7.5 Hz, benzyl-H), 7.44 (d, 1H, J = 7.6 Hz, benzyl-H), 7.57 (dd, 1H, J_1 = 8.6 Hz, J_2 = 2.2 Hz, Ph-H), 7.60 (d, 1H, J = 8.6 Hz, Ph-H), 7.81 (d, 1H, J = 2.2 Hz, Ph-H), 7.93 (s, 1H, CH=N), 11.89 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.5, 153.6, 151.8, 140.6, 135.9, 134.1, 133.8, 133.2, 131.4, 130.3, 129.7, 127.3, 118.7, 118.7, 104.7, 34.3. IR (KBr) cm^{-1}: 3205 (N-H), 1711 (C=O). ESI-HRMS calcd for $C_{16}H_{12}ClN_4O_2S^+$ ($[M+H]^+$): 359.0364; found: 359.0369.

*4-(2-(3-Chlorobenzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5i**)*

Mp 196–197°C, yield 72%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.45 (s, 2H, SCH_2), 7.18–7.31 (m, 3H, benzyl-H), 7.41 (s, 1H, benzyl-H), 7.40 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.9 Hz, Ph-H), 7.57 (d, 1H, J = 8.5 Hz, Ph-H), 7.78 (d, 1H, J = 1.9 Hz, Ph-H), 7.82 (s, 1H, CH=N), 11.76 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.3, 153.6, 151.8, 140.6, 138.2, 135.6, 134.2, 130.3, 130.1, 129.0, 128.0, 127.4, 118.7, 118.6, 104.7, 35.7. IR (KBr) cm^{-1}: 3206 (N-H), 1710 (C=O). ESI-HRMS calcd for $C_{16}H_{12}ClN_4O_2S^+$ ($[M+H]^+$): 359.0364; found: 359.0361.

*4-(2-(4-Chlorobenzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5j**)*

Mp 235–237°C, yield 63%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.46 (s, 2H, SCH_2), 6.79 (d, 2H, J = 8.4 Hz, benzyl-H), 7.32 (d, 2H, J = 8.4 Hz, benzyl-H), 7.47 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.9 Hz, Ph-H), 7.58 (d, 1H, J = 8.5 Hz, Ph-H), 7.77 (d, 1H, J = 1.9 Hz, Ph-H), 7.99 (s, 1H, CH=N), 11.85 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.7, 159.3, 153.6, 151.7, 140.6, 135.9, 130.4, 130.3, 127.8, 118.6, 118.5, 114.2, 104.5, 36.1. IR (KBr) cm^{-1}: 3208 (N-H), 1711 (C=O). ESI-HRMS calcd for $C_{16}H_{12}ClN_4O_2S^+$ ($[M+H]^+$): 359.0364; found: 359.0360.

*4-(2-(2,4-Dichlorobenzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5k**)*

Mp 190–194°C, yield 70%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.55 (s, 2H, SCH_2), 7.17 (dd, 1H, J_1 = 8.3 Hz, J_2 = 1.5 Hz, benzyl-H), 7.36 (d, 1H, J = 1.5 Hz, benzyl-H), 7.45 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.7 Hz, Ph-H), 7.57 (d, 1H, J = 8.3 Hz, benzyl-H), 7.58 (d, 1H, J = 8.5 Hz, Ph-H), 7.77 (d, 1H, J = 1.7 Hz, Ph-H), 7.93 (s, 1H, CH=N), 11.83 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.1, 153.6, 151.9, 140.5, 135.7, 134.9, 134.4, 132.8, 132.4, 130.4, 129.4, 127.4, 118.7, 118.5, 104.6, 33.7. IR (KBr) cm^{-1}: 3212 (N-H), 1714 (C=O). ESI-HRMS calcd for $C_{16}H_{11}Cl_2N_4O_2S^+$ ($[M+H]^+$): 392.9974; found: 392.9982.

*4-(2-(4-Methylbenzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5l**)*

Mp 224°C, yield 75%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 2.25 (s, 3H, Ar-CH_3), 4.46 (s, 2H, SCH_2), 7.07 (d, 2H, J = 7.9 Hz, benzyl-H), 7.28 (d, 2H, J = 7.9 Hz, benzyl-H), 7.44 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.0 Hz, Ph-H), 7.57 (d, 1H, J = 8.5 Hz, Ph-H), 7.81 (d, 1H, J = 2.0 Hz, Ph-H), 7.93 (s, 1H, CH=N), 11.82 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.7, 153.6, 151.7, 140.5, 137.0, 135.8, 132.8, 130.3, 129.4, 129.0, 118.6, 118.5, 104.6, 36.3, 21.2. IR (KBr) cm^{-1}: 3199 (N-H), 1713 (C=O). ESI-HRMS calcd for $C_{17}H_{15}N_4O_2S^+$ ($[M+H]^+$): 339.0910; found: 339.0906.

*4-(2-(4-Methoxybenzylthio)benzo[d]oxazol-6-yl)-2H-1,2,4-triazol-3(4H)-one (**5m**)*

Mp 202–206°C, yield 73%. H-NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 1.17 (s, 3H, Ar-OCH_3), 4.47 (s, 2H, SCH_2), 7.23 (d, 2H, J = 8.2 Hz, benzyl-H), 7.38 (d, 2H, J = 8.2 Hz, benzyl-H), 7.45 (d, 1H, J = 8.5 Hz, Ph-H), 7.57 (d, 1H, J = 8.5 Hz, Ph-H), 7.82 (s, 1H, Ph-H), 7.94 (s, 1H, CH=N), 11.83 (s, 1H, CO-NH). ^{13}H-NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 165.3, 153.5, 151.8, 140.4, 135.9, 135.2, 133.4, 130.7, 130.5, 128.8, 118.7, 118.5, 104.5, 35.5, 29.6. IR (KBr) cm^{-1}: 3200 (N-H), 1713 (C=O). ESI-HRMS calcd for $C_{17}H_{15}N_4O_3S^+$ ($[M+H]^+$): 355.0859; found: 355.0859.

4.2 | Pharmacology

4.2.1 | Animals and experimental conditions

Experiments were carried out on Kunming mice (half male and female) weighing 18–24 g. The mice were housed collectively in polycarbonate cages in groups of 10, where they were maintained on a 12-hr light/dark cycle in a temperature-controlled (25 ± 2°C) laboratory with free access to food and water. Each animal was used only once. Procedures involving animals and their care were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*, 8th Edition, National Academies Press, Washington, DC. Local ethical committee approval was also obtained (No. 20170707). In addition, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Electroconvulsions were produced by an electric stimulation generator (JTC-1, Chengdu, China). The following drugs were used in this study: pentylenetetrazole, 3-MP, TSC, and BIC (these drugs were obtained from Aladdin Industrial Inc., Shanghai, China), valproate sodium, carbamazepine, and phenytoin (these drugs were obtained from Melongpharma, Dalian, China).

4.2.2 | Experimental method

The animal experiments, including the MES test, subcutaneous pentylenetetrazole-induced seizure test (sc-PTZ), the rotarod test, 3-MP-induced seizure test, BIC-induced seizures test, determination of brain GABA concentrations by ELISA, and test for the effects of TSC on the anti-MES action of **5f** were carried out according to the previous report.[13]

4.3 | Docking studies

The homology model of the diazepam-bound $GABA_A$ receptor developed by Ernst et al. was retrieved from the Supporting Information of their published paper.[18] The three-dimensional structures of **5f** was constructed using Chem3D Ultra 12.0 software (Chemical Structure Drawing Standard; Cambridge Soft Corporation [2010]), then it was energetically minimized by using MMFF94 with 5000 iterations and minimum RMS gradient of 0.10. For protein preparation, the hydrogen atoms were added, and water and impurities were removed. The 3D structure of **5f** was placed during the molecular docking procedure. Types of interactions of the docked protein with **5f** were analyzed after the end of molecular docking. Compound would retain 10 poses, and were ranked and selected by CDOCKER_INTERACTION_ENERGY. The lowest energy conformation of ligand-enzyme complex was evaluated by Discovery Studio 4.5 Client.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (No. 21562028) and the Doctoral Foundation of Jinggangshan University (No. JZB1316).

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Xian-Qing Deng http://orcid.org/0000-0001-8180-610X

REFERENCES

[1] E. Beghi, *Pharmacol. Res.* **2016**, *107*, 79.
[2] S. P. Singh, R. S. Ankaraneni, A. R. Antony, *Neurol. India* **2017**, *65*(*Suppl*), S6.
[3] A. Gaitatzis, J. W. Sander, *CNS Drugs* **2013**, *27*, 435.
[4] B. Błaszczyk, W. Lasoń, S. J. Czuczwar, *Pharmacol. Rep.* **2015**, *67*, 426.
[5] P. Ferrer, E. Ballarín, M. Sabaté, X. Vidal, M. Rottenkolber, J. Amelio, J. Hasford, S. Schmiedl, L. Ibáñez, *Neuroepidemiology* **2014**, *42*, 107.
[6] M. Mula, *Future Med. Chem.* **2011**, *3*, 177.
[7] P. V. Ramachandran, A. Shekhar, *Future Med. Chem.* **2011**, *3*, 139.
[8] A. Ayati, S. Emami, A. Foroumadi, *Eur. J. Med. Chem.* **2016**, *109*, 380.
[9] M. X. Song, X. Q. Deng, *J. Enzyme Inhib. Med. Chem.* **2018**, *33*, 453.
[10] M. X. Song, B. Q. Rao, B. B. Cheng, Y. Wu, H. Zeng, Y. G. Luo, X. Q. Deng, *CNS Neurol. Disord. Drug Target.* **2017**, *16*, 187.
[11] T. Akbarzadeh, S. A. Tabatabai, M. J. Khoshnoud, B. Shafaghi, A. Shafiee, *Bioorg. Med. Chem.* **2003**, *11*, 769.
[12] M. Faizi, S. Dabirian, H. Tajali, F. Ahmadi, E. R. Zavareh, S. Shahhosseini, S. A. Tabatabai, *Bioorg. Med. Chem.* **2015**, *23*, 480.
[13] M. X. Song, Z. Y. Wang, S. H. He, S. W. Yu, S. L. Chen, D. F. Guo, W. H. Zhao, X. Q. Deng, *Molecules* **2018**, *23*, 756.
[14] E. W. Crick, I. Osorio, M. Frei, A. P. Mayer, C. E. Lunte, *Eur. J. Pharm. Sci.* **2014**, *57*, 25.
[15] C. Rubio, M. Rubio-Osornio, S. Retana-Marquez, M. Lopez, V. Custodio, C. Paz, *Cent. Nerv. Syst. Agents Med. Chem.* **2010**, *10*, 298.
[16] G. S. Collins, *Biochem. Pharmacol.* **1973**, *22*, 101.
[17] M. Mohammadi-Khanaposhtani, M. Shabani, M. Faizi, I. Aghaei, R. Jahani, Z. Sharafi, N. Shamsaei, M. Zafarghandi, M. Mahdavi, T. Akbarzadeh, S. Emami, T. Akbarzadeh, S. Emami, A. Shafieem, A. Foroumadi, *Eur. J. Med. Chem.* **2016**, *112*, 91.
[18] L. Richter, C. de Graaf, W. Sieghart, Z. Varagic, M. Mörzinger, I. J. P. de Esch, G. F. Ecker, M. Ernst, *Nat. Chem. Biol.* **2012**, *8*, 455.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Song M-X, Huang Y, Wang S, Wang Z-T, Deng X-Q. Design, synthesis, and evaluation of anticonvulsant activities of benzoxazole derivatives containing the 1,2,4-triazolone moiety. *Arch Pharm Chem Life Sci*. 2019;e1800313.
https://doi.org/10.1002/ardp.201800313