Bioorganic Chemistry 115 (2021) 105179

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis, antiepileptic effects, and structure-activity relationships of α-asarone derivatives: In vitro and in vivo neuroprotective effect of selected derivatives

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ARTICLE INFO

Keywords:
Structure-activity relationships
α-asarone
Epilepsy
PTZ

ABSTRACT

In the present study, we compared the antiepileptic effects of α-asarone derivatives to explore their structure-activity relationships using the PTZ-induced seizure model. Our research revealed that electron-donating methoxy groups in the 3,4,5-position on phenyl ring increased antiepileptic potency but the placement of other groups at different positions decreased activity. Besides, in allyl moiety, the optimal activity was reached with either an allyl or a 1-butenyl group in conjugation with the benzene ring. The compounds **5** and **19** exerted better neuroprotective effects against epilepsy in vitro (cell) and in vivo (mouse) models. This study provides valuable data for further exploration and application of these compounds as potential anti-seizure medicines.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by spontaneous recurrent seizures with abnormal electrical discharges in the brain [1]. It is one of the most common neurological diseases affecting about 68 million people worldwide [2].

Antiepileptic drugs (AEDs) therapy is still the primary medical treatment for patients with epilepsy, of whom nearly 70% have benefit from AEDs therapy [3,4]. However, uncontrolled seizures occur in nearly one-third of epileptic patients suffering from various adverse effects or drug resistance [5,6]. Indeed, the side effects of currently available AEDs [7], including headache, depression, cognitive impairment, sedation, memory deterioration, and loss of appetite [8,9], cannot be ignored. In addition, epileptic patients, their caretakers, and society endure huge burdens owing to uncontrolled seizures and severe medication hangover [10]. Therefore, it is still urgently needed and quite challenging to develop promising AEDs with high efficacy and minimal side effects.

Natural products have attracted much attention in searching for bioactive compounds to develop new drugs in the last decades. The rhizome of Acorus Tatarinowii that belongs to Araceae Juss's family is widely used in the clinic treatment for neurologic disorders such as epilepsy, amnesia, senile dementia, etc. [11]. Alpha asarone (ASA, (E)-1-propenyl-2,4,5-trimethoxy-benzene, see Fig. 1 **A**) is one of the active components extracted from Acorus Tatarinowii, showing distinct anticonvulsant and anti-epilepsy effects [12,13]. Also, α-asaronol ((E)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-ol, see Fig. 1 **B**), which is derived from ASA and possess an anticonvulsant activity [14]. So, we expect to modify ASA's chemical structure to get new antiepileptic candidates with more powerful effects and lower toxicity.

Given this, we designed and synthesized a series of ASA derivatives with promising biological activities in the present study (Scheme 1-5, 1-30). Since more of the methoxybenzaldehyde derivatives could potentially be made from commercially available starting materials, it was decided to begin with these compounds. **2**–**22** were synthesized from methoxybenzaldehyde derivative, of which small part (**2a**, **6a**-**8a**, **10a**-**13a**) were prepared in our laboratory because they were not available commercially, and the corresponding alkyl triphenylphosphonium bromide via Wittig reaction. Besides, synthesis of **23**–**28** was achieved from commercially available starting materials methoxybenzaldehyde derivative (**6c**). The preparation of **29**–**30** was performed from compounds **5** and **19** via Pd-catalyzed hydrogenation. These structures were characterized using ^{1}H NMR and ^{13}C NMR techniques. We evaluated these derivatives' antiepileptic activity by establishing the pentylenetetrazol (PTZ)-induced seizure models, elucidating the structure-activity

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https://doi.org/10.1016/j.bioorg.2021.105179
Received 17 March 2021; Received in revised form 11 July 2021; Accepted 12 July 2021
Available online 15 July 2021

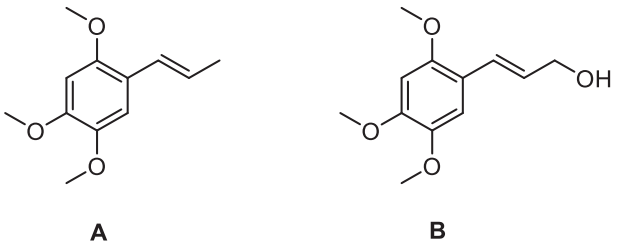


Fig. 1. Structure of α-asarone and α-asaronol.

relationships. We aimed to obtain active compounds through these procedures.

2. Results and discussion

2.1. Chemistry

The compounds **1** were synthesized according to Scheme 1. 1-(2,3,6-trimethoxyphenyl)propan-1-ol (**1a)** was synthesized by reaction of 1,2,4-trimethoxybenzene (**1b**) with propionaldehyde in the presence of *n*-butyllithium (*n*-BuLi), and N,N,N',N'-Tetramethylethylenediamine (TMEDA). Then, **1** was prepared by dehydration of **1a** by anhydrous $CuSO_4$ [15].

The synthetic routes of intermediates **2a**, **6a-8a**, **10a-13a**, and final compounds **2**–**17** were outlined in Scheme 2. 2,3,5-trimethoxybenzaldehyde (**2a)** was prepared by Dakin reaction[16], Duff reaction [17], and alkylation reaction of 2,4-dimethoxybenzaldehyde (**2d)**. The intermediate 2-ethoxy-4,5-dimethoxybenzaldehyde (**6a)** was synthesized by demethylation of 2,4,5-trimethoxybenzaldehyde (**6c)** with boron tribromide (BBr_3) [18], and ethylation of 2-hydroxy-4,5-dimethoxybenzaldehyde (**6b)** with iodoethane. Reaction of 1-(2,4-dimethoxyphenyl) ethan-1-one (**7c)** with triethylsilane (Et_3SiH) in trifluoroacetic acid (CF_3COOH) yielded 1-ethyl-2,4-dimethoxybenzene (**7b)**, followed by treatment with phosphorus oxychloride ($POCl_3$) in N,*N*-dimethylformamide (DMF) to obtain **7a** [19]. The preparation of **8a** was achieved via reaction of **2d** with ethyltriphenylphosphonium bromide in the presence of both potassium *tert*-butoxide (*t*-BuOK) and tetrabutylammonium bromide (TBAB), reduction reaction by catalyst (10% Pd/C) and formylation of **8b** formed. **10a**, **11a**, and **13a** were synthesized by alkylation of the corresponding starting material. **12b** was prepared by Duff reaction of 2,6-diisopropylphenol (**12c**), of which methylation afforded **12a** with iodomethane. **2a-17a** were converted to final compounds **2**–**17** in the reaction with ethyltriphenylphosphonium bromide in the presence of both *t*-BuOK and TBAB at 90–95 °C in DMF. The final compounds **18**–**22** were also obtained by the reaction of **18a-22a** with **5a** followed the same conditions described for the compounds **2**–**17** synthesis, which was illustrated in Scheme 3.

The total synthesis of the compounds **23**–**28** was given in Scheme 4. **23** was synthesized by the reaction of **6c** and propanedioic acid [20]. The compound **24** was prepared by the reaction of **6c** and 3-bromopropene in the presence of palladium acetate ($Pd(OAc)_2$), cuprous oxide (Cu_2O), and silver carbonate (Ag_2CO_3) at 110 °C in dimethyl sulfoxide (DMSO) and toluene, which was based on prior literature [21]. Synthesis of **25** was achieved by methyl esterification of **23**. Preparation of **26** was carried out via methyl esterification of (E)-3-(3,4,5-trimethoxyphenyl) acrylic acid (**26b**) and reduction reaction of methyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (**26a)** formed. Trifluoromethylation of **23 26b** afforded compounds **27** and **28** in the presence of copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), sodium trifluoromethanesulfinate ($NaSO_2CF_3$), and *tert*-butyl hydroperoxid (TBHP) at reflux [22], respectively. Finally, the preparation of compounds **29** and **30** was performed via reduction of **5** and **19** in the presence of 10% Pd/C and a pure hydrogen atmosphere, outlined in Scheme 5.

2.2. Structure-activity relationship

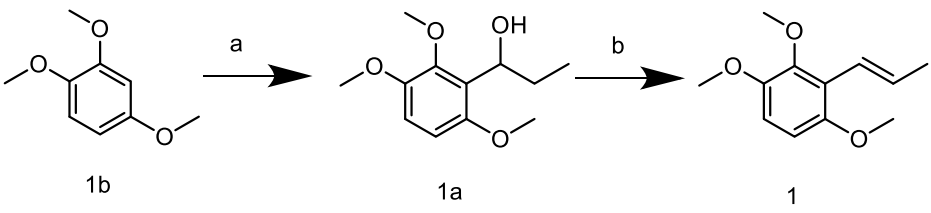
The ASA structure consists of three moieties of A, B, and C (Fig. 2). Each part structure optimization and modification was carried out, respectively. We evaluated the types, the position, and the number of the substituted groups on the phenyl ring in A and then the types of substituent R_2 in C. Next, we explored the effect of double bonds in B on the biological activity. All the synthesized compounds were screened in a pentylenetetrazole (PTZ) -induced seizure test [23]. The results were shown in Table 1. It was clear that compounds **5** and **19** had superior antiepileptic activity.

2.2.1. Effect of changing the position of trimethoxy substitution on phenyl ring in A

We first evaluated the importance of the three methoxy groups' substitution position on the phenyl ring to optimize antiepileptic activity further. Therefore, five isomers **1**–**5** with different substituted positions of methoxy groups on the phenyl ring were synthesized. From Table 1, it can be seen that compound **5** significantly prolonged the latent time of seizures when compared with the negative control (NC) group ($P < 0.05$), and this compound could also remarkably reduced Racine's sore ($P < 0.01$) and tonic seizure rate (TSR) ($P < 0.01$). Further, compared with ASA, the latent time, Racine's sore, and TSR of compound **5** performed slightly better than ASA's (Fig. 3). The structural activity analysis showed that 3,4,5-trimethoxy-substituted compound **5** exhibited great antiepileptic potency compared to the other four isomers. The fact that the change of the trimethoxy group's position on the phenyl ring significantly affected the activity suggested 3,4,5-trimethoxy substitution makes a special contribution that might be due to its ability to orient and bind with the target proteins.

2.2.2. Effect of different substituents on phenyl ring in A

After assessment of the trimethoxy substitution position on phenyl ring, the replacement of the methoxy group on the phenyl ring by other substitutions, such as ethoxy (**6**, **10**), ethyl (**7**), *n*-propyl (**8**), hydroxyl (**9**), *n*-propoxy (**11**), disubstituted isopropyl (**12**), disubstituted methyl (**13**) and bromo (**14**), was performed to explore the structure-activity relationships of these compounds. However, Table 1 shows that the difference was not statistically significant in the latent time, Racine's sore (except for compound **6**), CSR, and TSR of these compounds versus the negative control group. Although there was a statistical difference in the Racine's sore of compound **6**, no significant difference for the latent time, CSR, and TSR could be seen. These data indicated that they were inactive. Structural activity analysis showed that the enlarged molecular volume of ethoxy (**6**,**10**) and propoxyl (**11**) substituted compounds would result in steric hindrance to the target proteins binding. Alkyl



Scheme 1. Synthesis of compound **1**. Reagents and conditions: (**a**) propionaldehyde, *n*-BuLi, TMEDA, THF, 5–10 °C; (**b**) $CuSO_4$, toluene, reflux.

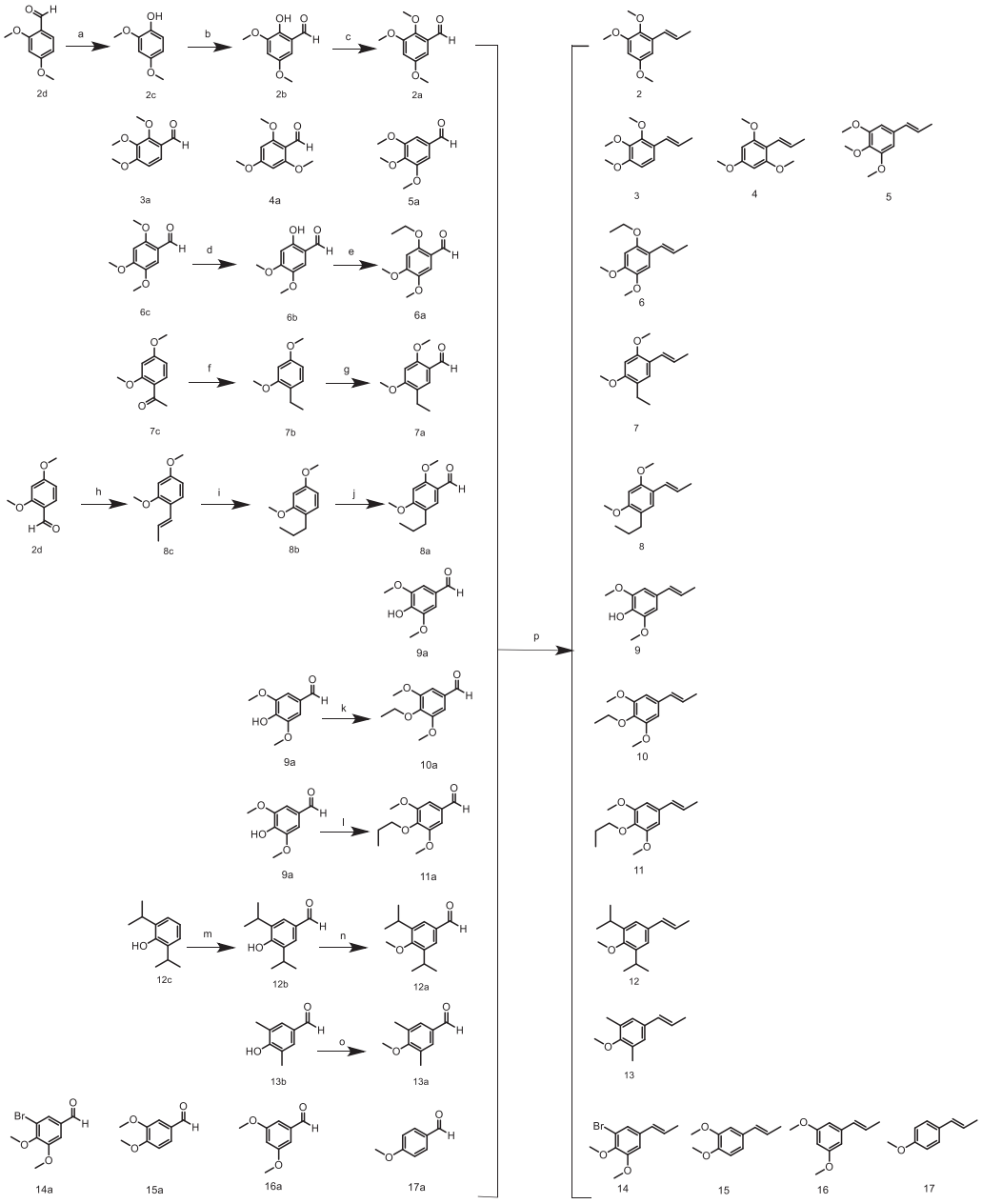
substituted compounds (**7**, **8**, **12**, and **13**) decreased the electron density of the benzene ring compared with trimethoxy substituted compounds, underscoring the importance of electron-rich substituents on the benzene ring. Interestingly, in this inference, the electron-rich hydroxyl substituted compound (**9**) should have better activity, but things go contrary to one's wishes. Since it had a low CLogP value, it showed a decreased distribution in the brain. It further confirmed our inference that the electron-deficient bromo-substituted compound (**14**) did not have any antiepileptic activity due to the further decrease of the benzene ring's electron cloud density.

2.2.3. *Effect of the number of methoxy substituents on phenyl ring in A*

To evaluate the effect of the number of methoxy substituents on the phenyl ring, we had synthesized dimethoxy-substituted compounds (**15**, **16**) and mono-methoxy substituted compounds (**17**). Although these compounds could prolong the latent time of seizures and reduce Racine's sore, these differences did not reach statistical significance (Table 1). The Racine's sore and TSR's upward trend was revealed with decreased methoxy substituents on the phenyl ring, indicating that the antiepileptic activity was also reduced. It was suggested that decreasing the number of methoxy substituents reduced the electron cloud density of benzene rings, ultimately leading to lower activity. The fact was consistent with the above conclusion.

2.2.4. *Effect of the length of the carbon chain in C*

We next evaluated the effect of the length of the R_2 carbon chain in C based on the fact that compound **5** was found optimal by studying the types, the position, and the number of the substituted groups on the phenyl ring in A. Accordingly, we synthesized a series of compounds (**18**–**21**) with different carbon chain lengths. Among these compounds, only compound **19** could prolong the latency of epileptic seizures ($P < 0.01$) and decrease Racine's sore ($P < 0.01$) and TSR ($P < 0.05$) when compared with the negative control group. Further, compared with ASA, compound **19** showed a very significant prolongation of the latent time ($P < 0.01$), and its Racine's sore also performed slightly lower than ASA's (Fig. 3). However, the other compounds' antiepileptic activities



Scheme 2. Synthesis of compound **2**–**17**. Reagents and conditions: (**a**) 30% H_2O_2, 98% H_2SO_4, MeOH, RT; (**b**) HMTA, AcOH, 90–95 °C; (**c**) K_2CO_3, CH_3I, DMF, 70–80 °C; (**d**) BBr_3, DCM, −10 °C; (**e**) K_2CO_3, CH_3CH_2I, DMF, 70–80 °C; (**f**) triethylsilane, TFA, 60–65 °C; (**g**) $POCl_3$, DMF, 60–65 °C; (**h**) ethyltriphenylphosphonium bromide, *t*-BuOK, TBAB, DMF, 90–95 °C; (**i**) 10% Pd/C, H_2, MeOH, RT; (**j**) $POCl_3$, DMF, 60–65 °C; (**k**) K_2CO_3, CH_3CH_2I, DMF, 70–80 °C; (**l**) K_2CO_3, $CH_3CH_2CH_2I$, DMF, 70–80 °C; (**m**) HMTA, AcOH, 90–95 °C; (**n**) K_2CO_3, CH_3I, DMF, 70–80 °C; (**o**) K_2CO_3, CH_3I, DMF, 70–80 °C; (**p**) ethyltriphenylphosphonium bromide, *t*-BuOK, TBAB, DMF, 90–95 °C.

were reduced. Therefore, the optimum R_2 was methyl or ethyl group in this part of the study.

2.2.5. Effect of different substituents of R_2 in C

We further evaluated the effect of different substituents of R_2 in C. Acetyl substituted compound (**22**), carboxyl substituted compound (**23**), methoxycarbonyl substituted compound (**25**), hydroxymethyl compound (**26**) and trifluoromethyl substituted compounds (**27**–**28**) were synthesized accordingly. As shown in Table 1, compounds **26** and **28** significantly prolonged the latent time of seizures (**26** $P < 0.05$, **28** $P < 0.05$), whereas only compound **28** remarkably reduced Racine's sore ($P < 0.01$). However, compound **28** did not have a statistically significant difference in TSR compared with NC group. Except for Racine's sore ($P < 0.01$), none of the other indexes are statistically different for compound **27**. On the other hand, compounds **22**, **23**, which were the metabolites of ASA [24], and **25** did not show antiepileptic activities in the tests. The result indicated that all the substituents of these compounds, except **26**, were the electron-withdrawing group, which reduced the conjugated system's electron cloud density, resulting in a decreased antiepileptic activity. Although the hydroxyl group of compound **26** was an electron-donating group, the anticonvulsant activity decreased compared to ASA. One possible reason would be that it had a low CLogP value, which caused a decreased distribution in the brain. Interestingly, the only difference of the chemical structure of **26** and α-asaronol (Fig. 1 **B**) is the position of the methoxyl group on the phenyl ring. However, there was a significant difference in antiepileptic activity. We speculate that the latter would be more prone to form electrostatic interactions and hydrophobic interactions with the target proteins. Instead, the R_2 groups of compounds **5** and **19** were methyl and ethyl groups, respectively, which increased the conjugated system's electron cloud density and thereby increased antiepileptic activities.

2.2.6. Effect of reduction and position of unsaturated double bonds unit in B

After studying a series of ASA derivatives (**1**–**23** and **25**–**28**), we planned to reduce the active derivatives' carbon–carbon double bonds (**5** and **19**). Compound **29**, which was obtained via a reduction of **5**, significantly prolonged the latent time of seizures ($P < 0.01$) and decreased Racine's sore ($P < 0.01$), but its TSR dramatically increased from 0% to 50%, meaning that its antiepileptic activity is lower than the latter's. Similarly, the reduced product (**30**) of compound **19** also proved futile. Finally, compound **24**, which was synthesized by a shift in the double bond position of the ASA side chain, had no benefit in any of the parameters. The results suggested that the carbon–carbon double bonds and their position played a critical role in these derivatives for antiepileptic activities.

2.3. The active compounds block hippocampal neuronal damage in the PTZ-induced seizure model

The hippocampus had long been a focus of epilepsy since seizures impacted neural network structure and caused hippocampal neurogenesis abnormalities [25–27]. Further, a growing body of evidence showed that a substantial number of neurons within hippocampal regions are most prone to neuronal apoptosis and loss following recurrent epileptic seizures. It is also well recognized that apoptotic cell death was a common consequence of brain injury and contributed to functional deficits following experimental seizures [28,29]. In this light, we evaluated the effects of the active compounds on PTZ-induced neuronal damage in the CA1 region of the hippocampus by using Nissl staining. As demonstrated in Fig. 4A, NC group had the fewest number of Nissl bodies in the CA1, which was also significantly reduced compared to BC group ($P < 0.01$). Both compound **5** and **19** remarkably increased the Nissl bodies' number compared with NC group (**5** $p < 0.05$, **19** $p < 0.05$). Further, the difference versus the BC group was not statistically significant. These results substantiated the idea that both compounds **5** and **19** might be capable of preventing PTZ-induced hippocampal neuronal damage.

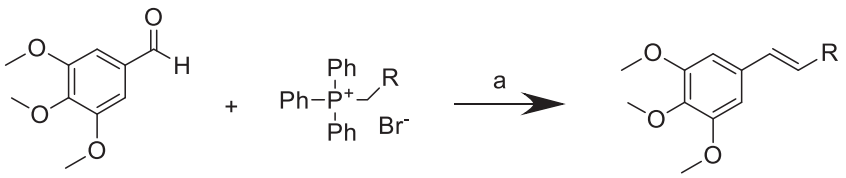
2.4. Immunohistochemical analysis of caspase-3 expression

Caspase-3, a member of cysteine proteases, played key effector roles in apoptosis in mammalian cells [30,31]. The activation and expression of caspase-3 preceded neuronal damage and led to cell apoptosis [32]. Therefore, these active compounds' effect on caspase-3 expression was also explored to test if the apoptotic responses could be interrupted after seizure-induced by PTZ. The results of the expression of caspase-3 in the hippocampus CA1 region are shown in Fig. 4**B**. The more expression of the caspase-3 protein, the severer neuronal damage in the CA1 region of the hippocampus. Compared with the BC, NC was significantly increased ($P < 0.001$). After administration, the expression of the caspase-3 protein in compounds **5** and **19** significantly decreased (**5**$P < 0.001$, **19**$P < 0.01$) when compared to NC group. Further, there was no significant difference between the administration groups and BC group. The immunohistochemical findings further confirmed that compounds **5** and **19** protected neurons from injury.

2.5. The active compound protected primary hippocampal neurons from Mg-deficient induced neurotoxicity

After confirming the effects of compounds **5** and **19** in vivo, an appropriate cell experiment was warranted to evaluate their cell-protective effect for epilepsy. It has been reported that brief exposure of hippocampal neurons in culture to Mg^{2+}-free media elicited permanently spontaneous recurrent seizures in these populations of neurons [33]. This seizure state caused the death of neurons due to excitotoxicity. Hence, the study of the neuroprotective effect of compounds **5** and **19** in the Mg^{2+}-free induced neuronal damage model was illustrative.

We firstly ensured that both compounds **5** and **19** at their effective concentrations had no significant cytotoxicity on normal neuron survival (Fig. 5A). The neuronal viability was significantly declined following treatment with Mg^{2+}-free media for 1 h compared to the control group ($P < 0.001$). In contrast, both doses of compound **5** significantly attenuated the neuron cell damage versus the model group



5a 18a-22a 18-22

18: R=H 19: R=CH_2CH_3 20: R=$CH_2CH_2CH_3$ 21: R=$CH_2CH_2CH_2CH_3$ 22: R=$COCH_3$

Scheme 3. Synthesis of compound 18–22. Reagents and conditions: (**a**) *t*-BuOK, TBAB, DMF, 90–95 °C.

(Fig. 5B, low-dose group, $P < 0.001$; high-dose group, $P < 0.001$). Also, both doses of compound **19** show very similar effects (Fig. 5B low-dose group, $P < 0.001$; high-dose group, $P < 0.01$). We observed reduced numbers of shrinking cells with synaptic reticular structure loss by compound **5** or **19** treatment (Fig. 5C). The result indicated that both **5** and **19** had a neuroprotective effect that alleviated permanently spontaneous recurrent seizures in vitro, preventing the neuron from overexcitation.

2.6. Toxicity risks using in silico analysis

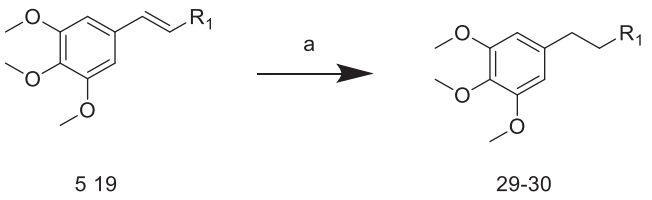
Toxicity risks were an essential factor in determining whether compounds could eventually become a drug. Osiris Property Explorer [34] was a knowledge-based algorithm that predicted mutagenic, tumorigenic, irritant, and reproductive effect of compounds, regarding chemical fragment data of existing drugs and non-drugs as published. As shown in Table 2, compounds **5** and **19** exhibited lower toxicity, which is in good agreement with in vivo and in vitro results as previously observed.

2.7. Neurotoxicity test

The results (Table 2) indicated that **5** and **19** showed lower acute neurotoxicity in the rotarod test, because their antiepileptic doses (100 mg/kg) were far less than TD_{50}. According to pharmacology and neurotoxicity, **5** and **19** were considered the most promising anti-seizure drug candidates.

3. Conclusion

In this study, a series of ASA derivatives were designed, synthesized, and assessed for antiepileptic ability. Subsequently, structure-activity relationship studies were conducted, which showed that methoxylated compounds in the 3,4,5-position on the phenyl ring had better antiepileptic activities than those replaced by other groups on phenyl ring. Further, its activities were optimal when R_2 was methyl or ethyl group. Antiepileptic activities also depended strongly on carbon–carbon double bonds and their position where the carbon–carbon double bonds formed conjugation with the benzene ring. More importantly, the active compounds **5** and **19** displayed the ability to prevent PTZ-induced hippocampal neuronal damage by Nissl staining and immunohistochemical analysis of caspase-3 expression. The MTT cell proliferation assay further demonstrated that both **5** and **19** exerted a neuroprotective effect in vitro. Due to these positive results, these compounds will be further evaluated as potential anti-seizure drug candidates.



Scheme 5. Synthesis of compound 29–30. Reagents and conditions: (**a**) 10% Pd/C, H_2, MeOH, RT. 5, 29: $R_1 = CH_3$;19, 30: $R_1 = CH_2CH_3$.

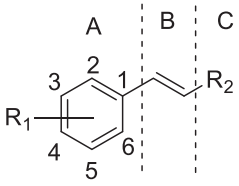


Fig. 2. Three moieties of α-asarone.

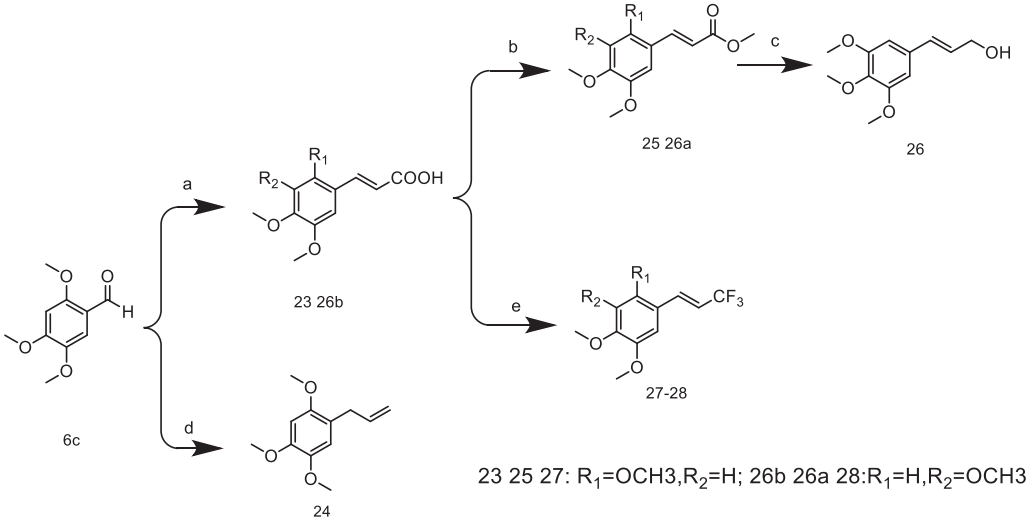
4. Experimental section

4.1. Chemistry

The chemicals and reagents were used as purchased from commercial suppliers such as J&K Scientific, Macklin and Energy Chemical. The reaction's progress was monitored by analytical thin-layer chromatography (TLC, petroleum ether: ethyl acetate-20:1 to 1:1), which was performed using silica gel G plates, and visualization of spots was accomplished with UV light (256 nm). The mixtures were purified by flash column chromatography on silica gel (Qingdao Haiyang Chemical Co., Ltd. 200–300 mesh) chromatography. ^1H NMR and ^{13}C NMR spectra were obtained by Varian UNITY INOVA-400 using $CDCl_3$ as a solvent and TMS as an internal standard. High-resolution mass spectra (HRMS) were executed on Bruker TOF Premier by the ESI method.

4.1.1. General procedure A

To a stirred mixture of phenol derivative (6.6 mmol) and potassium carbonate (K_2CO_3) (1.8 g, 13.2 mmol) in DMF (40 mL) was added the corresponding iodoalkyl derivative (9.9 mmol) under the protection of



Scheme 4. Synthesis of compound 23–28. Reagents and conditions: (**a**) propanedioic acid, pyridine, piperidine, reflux; (**b**) 98% H_2SO_4, MeOH, reflux;(**c**) DIBAL-H, THF, −40 °C; (**d**) 3-bromopropene, $Pd(OAc)_2$, Cu_2O, Ag_2CO_3, Tol, DMSO, 110 °C; (**e**) $CuSO_4{\cdot}5H_2O$, $NaSO_2CF_3$, TBHP, DCM, H_2O, reflux.

Table 1
Anti-seizure activity in the PTZ-induced seizure model (100 mg/kg) and ClogP values of compounds administered i.p. to mice.

Compound	Latent time	Racine's score	CSR%[a]	TSR%[b]	CLogP[c]
BC[d]	0	0	0	0	—
NC[e]	57.2 ± 17.0	5.0 ± 0	100.0	100.0	—
1	107.3 ± 42.1	4.7 ± 0.8	100.0	83.3	3.05
2	84.7 ± 27.0	4.5 ± 1.2	100.0	83.3	3.05
3	119.8 ± 25.8	4.8 ± 0.4	100.0	100.0	2.70
4	95.7 ± 22.2	3.7 ± 1.2*	100.0	50.0	3.40
5	290.8 ± 65.5*	1.8 ± 0.4**	100.0	0**	2.70
6	164.7 ± 72.3	3.2 ± 1.3**	100.0	50.0	3.57
7	65.2 ± 14.3	4.8 ± 0.4	100.0	100.0	4.43
8	78.8 ± 18.6	4.5 ± 1.2	100.0	83.3	4.96
9	62.7 ± 12.7	4.8 ± 0.4	100.0	100.0	2.36
10	71.7 ± 19.9	4.3 ± 1.2	100.0	66.7	3.22
11	65.7 ± 27.3	5.0 ± 0.0	100.0	100.0	3.75
12	53.0 ± 7.2	5.0 ± 0.0	100.0	100.0	5.37
13	57.8 ± 12.3	4.8 ± 0.4	100.0	100.0	4.31
14	63.2 ± 10.2	5.0 ± 0.0	100.0	100.0	3.76
15	250.0 ± 226.6	3.5 ± 1.6	100.0	50.0	3.05
16	247.0 ± 230.4	3.5 ± 1.8	100.0	50.0	3.40
17	258.7 ± 237.3	4.2 ± 2.0	83.3	83.3	3.31
18	130.2 ± 36.2	4.7 ± 0.8	100.0	83.3	2.17
19	427.8 ± 63.0**	1.5 ± 1.2**	100.0	16.7*	3.22
20	141.8 ± 35.1	2.5 ± 1.4**	100.0	33.3	3.75
21	83.8 ± 15.8	4.7 ± 0.5	100.0	100.0	4.28
22	58.7 ± 11.8	5.0 ± 0.0	100.0	100.0	1.45
23	67.5 ± 9.7	4.8 ± 0.4	100.0	100.0	1.89
24	113.0 ± 22.0	4.8 ± 0.4	100.0	100.0	2.87
25	95.3 ± 39.7	4.8 ± 0.4	100.0	100.0	2.12
26	285.7 ± 82.6*	4.8 ± 0.4	100.0	100.0	0.91
27	227.3 ± 82.9	2.3 ± 1.4**	100.0	33.3	3.01
28	112.8 ± 14.0*	3.2 ± 0.8**	100.0	33.3	2.66
29	181.3 ± 27.6**	2.7 ± 1.5**	100.0	50.0	3.00
30	80.0 ± 13.1	4.3 ± 1.2	100.0	83.3	3.53
ASA	176.8 ± 30.5**	2.0 ± 1.1**	100.0	16.7*	3.05

[a] CSR: clonic seizures rate.
[b] TSR: tonic seizure rate.
[c] ClogP was calculated by the ChemDraw Professional (v19.0) (PerkinElmer Informatics. Inc, USA).
[d] Blank control group.
[e] Negative control group.
* $P < 0.05$.
** $P < 0.01$.

nitrogen, and stirring was continued at 70–80 °C for 4 h. After the completion of the reaction (TLC monitoring), the reaction was treated with 50 mL H_2O. Then the mixture was extracted three times with 50 mL ethyl acetate (EtOAc). The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, and concentrated in reduced pressure. The residue was purified using silica gel chromatography (Pet. Ether/EtOAc, v/v = 5:1).

4.1.2. General procedure B

To a stirred mixture of alkyl triphenylphosphonium bromide (9.0 mmol), *t*-BuOK (2.7 g, 24.0 mmol), and TBAB (0.1 g, 0.3 mmol) in dry DMF (10 mL) was added the corresponding methoxybenzaldehyde derivative (6.0 mmol) under the protection of nitrogen at 0–5 °C and stirring was continued at room temperature (RT) for 0.5 h. The reaction mixture was heated at 90–95 °C for 4 h. After completing the reaction (TLC monitoring), the reaction mixture was quenched with 70 mL H_2O. Then the mixture was extracted three times with 70 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, and concentrated in reduced pressure. The residue was purified using silica gel chromatography (Pet. Ether/EtOAc, v/v = 40–20:1).

4.1.3. General procedure C

$POCl_3$ (1.7 g, 11.2 mmol) and 10 mL dry DMF were placed in a 50 mL three-mouth flask with a magnetic stirrer under the protection of nitrogen, and stirring was continued at room temperature for 30 min. To the reaction mixture was added dropwise (over 10 min) alkyldimethoxybenzene (10.2 mmol) in dry DMF (5 mL) under the protection of nitrogen at 0–5 °C. The reaction mixture was heated at 60–65 °C for 8 h. After completing the reaction (TLC monitoring), the reaction mixture was quenched with 30 mL H_2O. Then the mixture was extracted three times with 30 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, and concentrated in reduced pressure. The residue was purified using silica gel chromatography (Pet. Ether/EtOAc, v/v = 10:1).

4.1.4. General procedure D

(E)-methoxy-(propenyl)benzene analogue (16.8 mmol), 0.4 g of 10% of palladium-on-carbon (Pd/C), and methanol (MeOH) (15 mL) were placed in a 50 mL three-mouth flask with a magnetic stirrer under a hydrogen atmosphere, and stirring was continued at RT for 5 h. After completing the reaction (TLC monitoring), the reaction mixture was filtered and concentrated in reduced pressure. The residue was purified using silica gel chromatography (Pet. Ether/EtOAc, v/v = 10:1).

4.1.5. General procedure E

To a stirred mixture of phenolic compound (41.5 mmol) in acetic acid (AcOH) (130 mL) was added hexamethylenetetramine (HMTA) (11.6 g, 83.0 mmol) under the protection of nitrogen. The reaction mixture was heated at 90–95 °C for 6 h. After completing the reaction (TLC monitoring), the reaction mixture was quenched with 500 mL H_2O, added 50 mL 1 N HCl, and stirred for an additional 1 h. Then the mixture was extracted three times with 300 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, and concentrated in reduced pressure. The residue was purified using silica gel chromatography (Pet. Ether/EtOAc, v/v = 10:1).

4.1.6. General procedure F

To a stirred mixture of alkyl triphenylphosphonium bromide (32.9 mmol) in dry tetrahydrofuran (THF) (60 mL) was added dropwise (over 10 min) 2.5 M *n*-BuLi in hexanes (13.1 mL, 32.9 mmol) under the protection of nitrogen at 0–5 °C and stirring was continued. After 30 min, the corresponding benzaldehyde derivative (27.4 mmol) in dry THF (30 mL) was then added dropwise (over 10 min) to the reaction mixture at

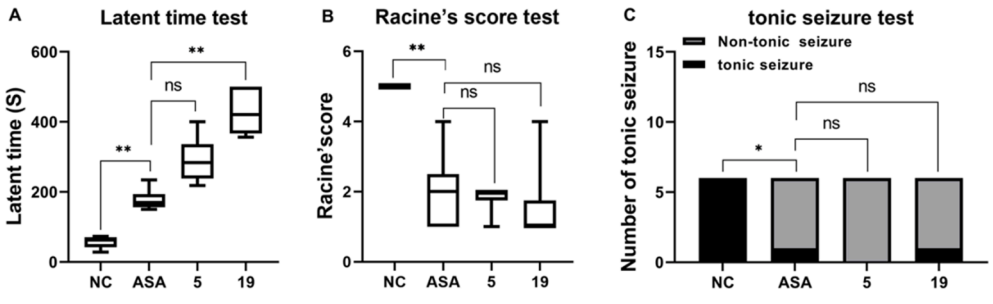


Fig. 3. Antiepileptic effects of compounds **5** and **19** in PTZ-induced seizure test. (**A**) The latency of onset time, (**B**) Racine's sore, and (**C**) tonic seizure number of seizures were shown in the PTZ test. Graphical representations of the data (**A** and **B**) were made with box-and-whisker plots, where the whiskers show the minimum and maximum, respectively (n = 6 mice in each group). Graphical representations of the data (**C**) were made with bar plots (n = 6 mice in each group). NC represents negative control group and N.S. means not significant, * $P < 0.05$, ** $P < 0.01$.

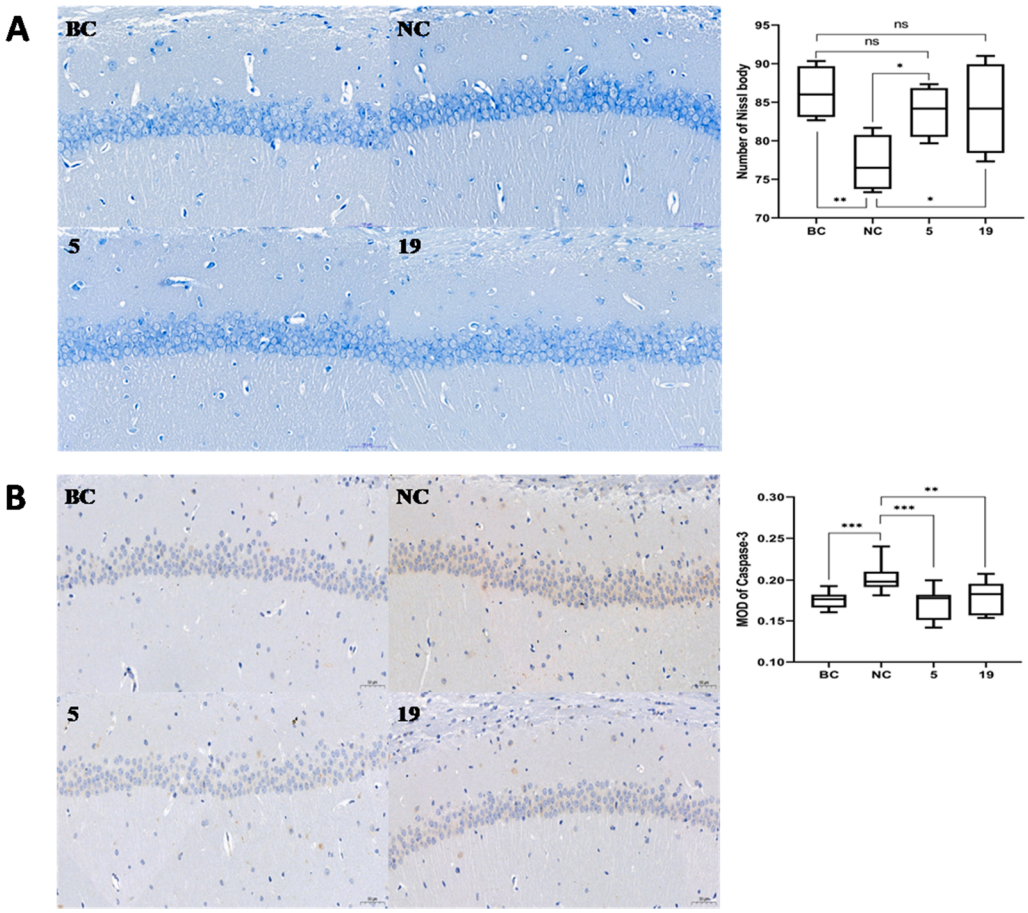


Fig. 4. Effects of compounds **5** and **19** on hippocampal neuronal damage elicited by PTZ. (**A**) Representative images of histological examination by Nissl staining showing the survival of neurons in the hippocampus CA1 region. (**B**) Representative images of immunohistochemistry showing caspase-3 protein distribution in the hippocampus CA1 region. Graphical representations of the data were made with box-and-whisker plots, where the whiskers indicate the minimum and maximum, respectively (n = 4 mice, in each group). BC, blank control group; NC, negative control group. N.S. means not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

0–5 °C. The reaction mixture was heated to RT for 4 h. After completing the reaction (TLC monitoring), the reaction mixture was quenched with 100 mL H_2O. Then the mixture was extracted three times with 100 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, and concentrated in reduced pressure. The residue was purified using silica gel chromatography (Pet. Ether/EtOAc, v/v = 10:1).

4.1.7. General procedure G

To a stirred mixture of trimethoxyphenylacrylic acid (4.2 mmol) in MeOH (20 mL) was added dropwise (over 10 min) concentrated sulfuric acid (0.6 g, 12.0 mmol) in MeOH (10 mL) at 0–5 °C. The reaction mixture was heated at reflux for 2 h. After completing the reaction (TLC monitoring), the reaction mixture was quenched with 100 mL H_2O. Then the mixture was extracted three times with 100 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, and concentrated in reduced pressure to obtain the target product.

4.1.8. General procedure H

To a stirred mixture of trimethoxyphenylacrylic acid (8.4 mmol), $CuSO_4{\cdot}5H_2O$ (0.2 g, 0.8 mmol), $NaSO_2CF_3$ (3.9 g, 25.2 mmol) in dichloromethane (DCM) (25 mL) and H_2O (10 mL) was added TBHP (5.1 mL, 42.0 mmol) at RT under the protection of nitrogen. The reaction mixture was heated at reflux for 6 h. After the completion of the reaction (TLC monitoring), the reaction mixture was quenched with sodium thiosulfate (5.3 g, 33.5 mmol) in H_2O (30 mL). Then the mixture was extracted three times with 40 mL DCM. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, and concentrated in reduced pressure. The residue was purified using silica gel chromatography (Pet. Ether/EtOAc, v/v = 30:1).

4.1.9. (E)-1,2,4-trimethoxy-3-(prop-1-en-1-yl)benzene (1)

1,2,4-trimethoxybenzene (**1b**) (3.0 g, 18 mmol) and 60 mL dry THF were placed in a 100 mL three-mouth flask with a magnetic stirrer under the nitrogen protection. After the mixture was cooled down to 5 °C, the 2.5 M solution of *n*-BuLi in hexane (19.7 mL, 49.2 mmol) was added dropwise (over 15 min), and stirring was continued at 5–10 °C. An hour later, TMEDA (2.5 mL, 16.3 mmol) and propionaldehyde (1.9 mL, 26.7 mmol) were then added dropwise (over 20 min) to the reaction mixture at 5–10 °C. After the completion of the reaction (TLC monitoring), the reaction was quenched with 3 mL H_2O. The solvent was removed in a vacuum under pressure, and the residue obtained was treated with 60 mL EtOAc and 20 mL 1 N HCl. The organic phase was collected, and the aqueous phase was extracted with 60 mL EA. Organic phases were combined, dried over anhydrous $MgSO_4$, filtered, concentrated in reduced pressure, and purified on silica gel column chromatography (Pet. Ether/EtOAc, v/v = 20:1) to obtain 2.3 g (10.2 mmol; yield: 56.7%) 1-(2,3,6-trimethoxyphenyl)propan-1-ol (**1a**).

To a stirred mixture of anhydrous copper sulfate (0.7 g, 4.4 mmol) in toluene (30 mL) was added **1a** (1.0 g, 4.4 mmol). The reaction mixture was heated at reflux for 5 h. After completing the reaction (TLC monitoring), the mixture was filtered, concentrated in reduced pressure and purified on silica gel column chromatography (Pet. Ether/EtOAc, v/v = 30:1) to obtain 0.6 g (2.9 mmol; total yield: 36.9%) (E)-1,2,4-trimethoxy-3-(prop-1-en-1-yl)benzene (**1**). ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.72–6.63 (m, 2H), 6.61–6.54 (m, 2H), 3.81 (s, 3H), 3.78 (d, J = 2.0, 6H), 1.93 (dd, J = 6.2, 1.4, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 152.10, 147.69, 147.34, 131.33, 121.40, 121.23, 110.36, 105.95, 60.22, 56.30, 55.91, 20.10; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_3$ ($[M + Na]^+$): 231.0992; found: 231.0992.

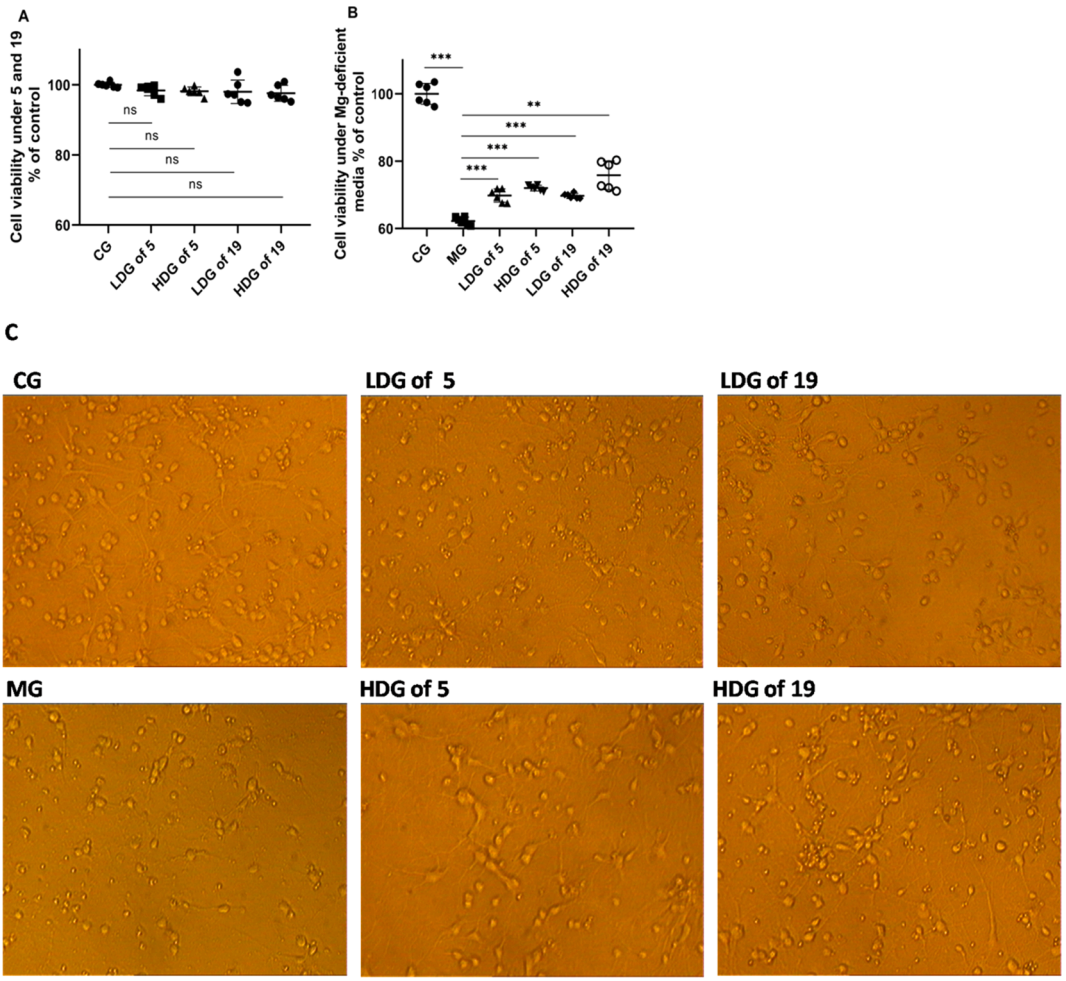


Fig. 5. The protective effects of **5** and **19** on hippocampal neuronal injury induced by Mg-deficient media. (**A**) Cell viability was assessed by MTT assay in primary hippocampal neurons. Both **5** and **19** exhibited no cytotoxicity on the normal primary hippocampal neurons (n = 6). (**B**) Both **5** and **19** treatments improved neuronal viability impaired by Mg-deficient media (n = 6). (**C**) Representative hippocampal neuron morphology was observed by an inverted optical microscope. **5** or **19** treatments rescued axonal and dendritic arbors injured by Mg-deficient media. The control group (CG), the model group (MG), the low-dose of **5** (LDG of **5**), high-dose of **5** (HDG of **5**), LDG of **19**, and HDG of **19** were treated with normal media, Mg^{2+}-free media, Mg^{2+}-free media containing 2.5 μg/mL of **5**, Mg^{2+}-free media containing 5.0 μg/mL of **5**, Mg^{2+}-free media containing 2.5 μg/mL of **19** and Mg^{2+}-free media containing 5.0 μg/mL of **19**, respectively. Graphical representations of the data were made with scatter plots. N.S. means not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2
Drug likeliness properties and toxicity risks of compounds **5** and **19** according to Osiris Property Explorer tool and their acute neurotoxicity.

Cpds	Drug-likeness	Drug-score	Toxicity risks[a]				TOX-ED_{50} (mg/kg)
			M[b]	T[c]	I[d]	R[e]	
5	1.04	0.79	+	+	+	+	352.0 (253.9–489.0)
19	0.19	0.68	+	+	+	+	368.9 (285.2–477.1)

[a] (+) represent no bad effect, (–) represent bad effect.
[b] M: mutagenic effect.
[c] T: tumorigenic effect.
[d] I: irritant effect.
[e] R: reproductive effect.

4.1.10. (E)-1,2,5-trimethoxy-3-(prop-1-en-1-yl)benzene (2)

2,4-dimethoxybenzaldehyde (**2d**) (10.0 g, 60.2 mmol) and 80 mL MeOH were placed in a 100 mL three-mouth flask with magnetic stirrer. After the mixture was cooled down to 0 °C, 30% H_2O_2 (8.9 g, 78.5 mmol) in MeOH (20 mL) was added dropwise (over 15 min) at 0–5 °C. Then 1.2 mL concentrated sulfuric acid (98%) in MeOH (20 mL) was added dropwise (over 20 min) to the reaction mixture at 0–5 °C and stirring was continued at RT for 20 h. After the completion of the reaction (TLC monitoring), the reaction was quenched with sodium sulfite (2.5 g, 19.8 mmol). The reaction mixture was filtered, concentrated in reduced pressure and purified on silica gel column chromatography (Pet. Ether/EtOAc, v/v = 10:1) to obtain 6.9 g (44.8 mmol; yield: 74.4%) 2,4-dimethoxyphenol (**2c**). Finally, the synthesis of **2** was carried out via a three steps of the general procedure E, the general procedure A and the general procedure B from **2c** to obtain 0.9 g (4.3 mmol; total yield: 19.8%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.70 (dq, J = 15.9, 1.8, 1H), 6.54 (d, J = 2.8, 1H), 6.38 (d, J = 2.8, 1H), 6.24 (dq, J = 15.9, 6.7, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 3.74 (s, 3H), 1.91 (dd, J = 6.7, 1.8, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 156.06, 153.61, 140.40, 131.82, 127.12, 125.22, 100.29, 98.93, 61.01, 55.75, 55.47, 18.81; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_3$ ($[M + Na]^+$): 231.0992; found: 231.0992.

4.1.11. (E)-1,2,3-trimethoxy-4-(prop-1-en-1-yl)benzene (3)

3 was synthesized according to the general procedure B from **3a** to obtain 1.5 g (7.2 mmol; yield: 62.1%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.10 (d, J = 8.7, 1H), 6.63 (d, J = 8.7, 1H), 6.58 (dd, J = 15.8, 1.9, 1H), 6.13 (dq, J = 15.9, 6.6, 1H), 3.87 (s, 3H),

3.85 (s, 3H), 3.84 (s, 3H), 1.88 (dd, J = 6.6, 1.8, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 152.52, 150.89, 142.34, 125.16, 125.13, 124.99, 120.46, 107.72, 61.06, 60.88, 56.02, 18.84; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_3$ ([M + Na]$^+$): 231.0992; found: 231.0991.

4.1.12. (E)-1,3,5-trimethoxy-2-(prop-1-en-1-yl)benzene (4)

4 was synthesized according to the general procedure B from **4a** to obtain 1.3 g (6.2 mmol; yield: 60.7%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.55 (dd, J = 16.0, 1.5, 1H), 6.51–6.41 (m, 1H), 6.13 (s, 2H), 3.82 (s, 6H), 3.81 (s, 3H), 1.89 (dd, J = 6.2, 1.3, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 159.37, 158.76, 128.02, 120.94, 108.43, 90.76, 55.70, 55.29, 20.06; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_3$ ([M + H]$^+$): 209.1173; found: 209.1173.

4.1.13. (E)-1,2,3-trimethoxy-5-(prop-1-en-1-yl)benzene (5)

5 was synthesized according to the general procedure B from **5a** to obtain 1.1 g (5.3 mmol; yield: 69.8%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.55 (s, 2H), 6.32 (dq, J = 15.6, 1.7, 1H), 6.15 (dq, J = 15.7, 6.5, 1H), 3.86 (s, 6H), 3.83 (s, 3H), 1.87 (dd, J = 6.5, 1.6, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.27, 137.16, 133.78, 130.91, 125.31, 102.84, 60.91, 56.02, 18.37. HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_3$ ([M + Na]$^+$): 231.0992; found: 231.0992.

4.1.14. (E)-1-ethoxy-4,5-dimethoxy-2-(prop-1-en-1-yl)benzene (6)

To a stirred mixture of 2,4,5-trimethoxybenzaldehyde (**6c)** (10.0 g, 51.0 mmol) in dry DCM (150 mL) was added dropwise (over 30 min) BBr_3 (12.8 g, 51.0 mmol) in dry DCM (50 mL) under the protection of nitrogen at −10 °C and stirring was continued at RT for 4 h. After the completion of the reaction (TLC monitoring), the reaction mixture was quenched with 80 mL H_2O. Then the mixture was extracted three times with 50 mL DCM. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, concentrated in reduced pressure and purified on silica gel column chromatography (Pet. Ether/EtOAc, v/v = 5:1) to obtain 4.5 g (24.7 mmol; yield: 48.5%) 2-hydroxy-4,5-dimethoxybenzaldehyde (**6b**). Finally, the synthesis of **6** was carried out via a two steps process of the general procedure A and the general procedure B from **6b** to obtain 0.8 g (3.6 mmol; total yield: 27.8%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.94 (s, 1H), 6.66 (dq, J = 15.9, 1.8, 1H), 6.49 (s, 1H), 6.11 (dq, J = 15.9, 6.7, 1H), 4.01 (q, J = 7.0, 2H), 3.86 (d, J = 3.3, 6H), 1.89 (dd, J = 6.6, 1.8, 3H), 1.42 (t, J = 7.0, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 149.95, 148.63, 143.50, 125.16, 124.08, 119.49, 109.45, 99.52, 65.60, 56.41, 56.06, 18.82, 15.08; HRMS (ESI) *m/z* calcd for $C_{13}H_{18}O_3$ ([M + Na]$^+$): 245.1149; found: 245.1147.

4.1.15. (E)-1-ethyl-2,4-dimethoxy-5-(prop-1-en-1-yl)benzene (7)

To a stirred mixture of 1-(2,4-dimethoxyphenyl)ethan-1-one (**7c)** (3.6 g, 20.0 mmol) in CF_3COOH (20 mL) was added Et_3SiH (6.4 mL, 20.0 mmol) at RT. The reaction mixture was heated at 60–65 °C for 8 h. After the completion of the reaction (TLC monitoring), the reaction mixture was quenched with 80 mL H_2O. Then the mixture was extracted three times with 50 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, concentrated in reduced pressure and purified on silica gel column chromatography (Pet. Ether/EtOAc, v/v = 30:1) to obtain 2.0 g (12.0 mmol; yield: 60.2%) 1-ethyl-2,4-dimethoxybenzene (**7b**). Finally, the synthesis of **7** was carried out via a two steps process of the general procedure C and the general procedure B from **7b** to obtain 0.9 g (4.4 mmol; total yield: 22.2%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.18 (s, 1H), 6.64 (dq, J = 15.9, 1.8, 1H), 6.42 (s, 1H), 6.11 (dq, J = 15.9, 6.6, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 2.57 (q, J = 7.5, 2H), 1.88 (dd, J = 6.6, 1.8, 3H), 1.17 (t, J = 7.5, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 157.12, 155.21, 126.74, 125.25, 124.71, 123.97, 119.08, 95.41, 55.91, 55.53, 22.67, 18.89, 14.57; HRMS (ESI) *m/z* calcd for $C_{13}H_{18}O_2$ ([M + H]$^+$): 207.1380; found: 207.1382.

4.1.16. (E)-1,5-dimethoxy-2-(prop-1-en-1-yl)-4-propylbenzene (8)

The synthesis of **8** was carried out via a five process of the general procedure B, the general procedure D, the general procedure C and the general procedure B from **2d** to obtain 0.7 g (3.2 mmol; total yield: 21.8%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.17 (s, 1H), 6.65 (dq, J = 15.8, 1.7, 1H), 6.43 (s, 1H), 6.12 (dq, J = 15.8, 6.6, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 2.56–2.50 (m, 2H), 1.90 (dd, J = 6.6, 1.8, 3H), 1.67–1.53 (m, 2H), 0.96 (t, J = 7.4, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 157.29, 155.27, 127.64, 125.33, 123.92, 123.19, 119.02, 95.46, 55.86, 55.53, 31.68, 23.33, 18.86, 14.06; HRMS (ESI) *m/z* calcd for $C_{14}H_{20}O_2$ ([M + H]$^+$): 221.1537; found:221.1539.

4.1.17. (E)-2,6-dimethoxy-4-(prop-1-en-1-yl)phenol (9)

9 was synthesized according to the general procedure F from **9a** to obtain 0.4 g (2.1 mmol; yield: 55.6%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.57 (s, 2H), 6.31 (dq, J = 15.7, 1.8, 1H), 6.09 (dq, J = 15.5, 6.5, 1H), 5.46 (s, 1H), 3.89 (s, 6H), 1.86 (dd, J = 6.6, 1.7, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 147.08, 133.95, 130.94, 129.60, 123.86, 102.60, 56.22, 18.32; HRMS (ESI) *m/z* calcd for $C_{11}H_{14}O_3$ ([M + Na]$^+$): 217.0836; found: 217.0833.

4.1.18. (E)-2-ethoxy-1,3-dimethoxy-5-(prop-1-en-1-yl)benzene (10)

The synthesis of **10** was carried out via a two process of the general procedure A and the general procedure B from **9a** to obtain 0.8 g (3.6 mmol; total yield: 53.1%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.55 (s, 2H), 6.33 (dt, J = 15.6, 1.8, 1H), 6.15 (dq, J = 15.7, 6.5, 1H), 4.04 (q, J = 7.1, 2H), 3.85 (s, 6H), 1.87 (dd, J = 6.6, 1.7, 3H), 1.35 (t, J = 7.1, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.54, 136.12, 133.53, 130.93, 125.10, 102.91, 68.88, 56.02, 18.31, 15.47; HRMS (ESI) *m/z* calcd for $C_{13}H_{18}O_3$ ([M + Na]$^+$): 245.1149; found: 245.1148.

4.1.19. (E)-1,3-dimethoxy-5-(prop-1-en-1-yl)-2-propoxybenzene (11)

The synthesis of **11** was carried out via a two process of the general procedure A and the general procedure B from **9a** to obtain 0.7 g (3.0 mmol; total yield: 56.7%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.55 (s, 2H), 6.32 (dq, J = 15.6, 1.7, 1H), 6.14 (dq, J = 15.7, 6.5, 1H), 3.91 (t, J = 6.9, 2H), 3.84 (s, 6H), 1.87 (dd, J = 6.6, 1.6, 3H), 1.77 (h, J = 7.3, 2H), 1.00 (t, J = 7.4, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.52, 136.55, 133.47, 130.98, 125.05, 103.06, 75.18, 56.08, 23.32, 18.36, 10.36; HRMS (ESI) *m/z* calcd for $C_{14}H_{20}O_3$ ([M + Na]$^+$): 259.1305; found: 259.1307.

4.1.20. (E)-1,3-diisopropyl-2-methoxy-5-(prop-1-en-1-yl)benzene (12)

The synthesis of **12** was carried out via a three process of the general procedure E, the general procedure A and the general procedure B from **12c** to obtain 0.4 g (1.7 mmol; total yield: 29.4%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.07 (s, 2H), 6.38 (dq, J = 15.8, 1.7, 1H), 6.16 (dq, J = 15.7, 6.6, 1H), 3.73 (s, 3H), 3.32 (p, J = 6.9, 2H), 1.88 (dd, J = 6.6, 1.7, 3H), 1.25 (d, J = 7.0, 12H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.68, 141.62, 134.10, 131.08, 124.48, 121.60, 62.25, 26.51, 24.06, 18.44; HRMS (ESI) *m/z* calcd for $C_{16}H_{24}O$ ([M + H]$^+$): 233.1900; found: 233.1900.

4.1.21. (E)-2-methoxy-1,3-dimethyl-5-(prop-1-en-1-yl)benzene (13)

The synthesis of **13** was carried out via a two process of the general procedure A and the general procedure B from **13b** to obtain 0.9 g (5.1 mmol; total yield: 55.6%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.00 (s, 2H), 6.31 (dd, J = 15.8, 2.0, 1H), 6.13 (dq, J = 15.7, 6.5, 1H), 3.72 (s, 3H), 2.28 (s, 6H), 1.87 (dd, J = 6.6, 1.7, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 156.02, 133.58, 130.73, 130.50, 126.25, 124.53, 59.73, 18.47, 16.12; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O$ ([M + H]$^+$): 177.1274; found: 177.1277.

4.1.22. (E)-1-bromo-2,3-dimethoxy-5-(prop-1-en-1-yl)benzene (14)

14 was synthesized according to the general procedure B from **14a** to

obtain 0.6 g (2.3 mmol; yield: 54.8%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.09 (s, 1H), 6.80 (s, 1H), 6.26 (dt, J = 15.6, 1.8, 1H), 6.21–6.07 (m, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 1.86 (dt, J = 6.4, 1.5, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.57, 145.28, 135.31, 129.49, 126.55, 122.16, 117.73, 109.01, 60.63, 56.00, 18.40; HRMS (ESI) *m/z* calcd for $C_{11}H_{13}BrO_2$ ($[M + H]^+$): 257.0172; found: 257.0183.

4.1.23. (E)-1,2-dimethoxy-4-(prop-1-en-1-yl)benzene (15)

15 was synthesized according to the general procedure B from **15a** to obtain 0.7 g (3.9 mmol; yield: 73.5%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.91–6.81 (m, 2H), 6.78 (d, J = 8.2, 1H), 6.33 (dq, J = 15.6, 1.7, 1H), 6.10 (dq, J = 15.7, 6.6, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 1.86 (dd, J = 6.6, 1.7, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 148.97, 148.16, 131.14, 130.64, 123.73, 118.67, 111.18, 108.46, 55.87, 55.74, 18.38; HRMS (ESI) *m/z* calcd for $C_{11}H_{14}O_2$ ($[M + Na]^+$): 201.0886; found: 201.0887.

4.1.24. (E)-1,3-dimethoxy-5-(prop-1-en-1-yl)benzene (16)

16 was synthesized according to the general procedure B from **16a** to obtain 0.9 g (5.0 mmol; yield: 72.1%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.50 (d, J = 2.3, 2H), 6.39–6.30 (m, 2H), 6.23 (dq, J = 15.7, 6.4, 1H), 3.79 (s, 6H), 1.88 (dd, J = 6.5, 1.5, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 160.88, 140.02, 131.01, 126.34, 103.95, 99.08, 55.29, 18.43; HRMS (ESI) *m/z* calcd for $C_{11}H_{14}O_2$ ($[M + H]^+$):179.1067; found:179.1073.

4.1.25. (E)-1-methoxy-4-(prop-1-en-1-yl)benzene (17)

17 was synthesized according to the general procedure B from **17a** to obtain 1.0 g (6.7 mmol; yield: 67.2%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.27–7.19 (m, 2H), 6.85–6.76 (m, 2H), 6.32 (dq, J = 15.7, 1.8, 1H), 6.06 (dq, J = 15.7, 6.6, 1H), 3.75 (s, 3H), 1.83 (dd, J = 6.6, 1.7, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 158.66, 130.86, 130.44, 126.95, 123.47, 113.95, 55.26, 18.48; HRMS (ESI) *m/z* calcd for $C_{10}H_{12}O$ ($[M + H]^+$): 149.0961; found: 149.0963.

4.1.26. 1,2,3-trimethoxy-5-vinylbenzene (18)

18 was synthesized according to the general procedure F from **18a** to obtain 0.5 g (2.6 mmol; yield: 53.2%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.69–6.58 (m, 3H), 5.66 (dd, J = 17.5, 0.8, 1H), 5.21 (dd, J = 10.8, 0.8, 1H), 3.88 (s, 6H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.27, 137.99, 136.73, 133.28, 113.22, 103.26, 60.88, 56.04; HRMS (ESI) *m/z* calcd for $C_{11}H_{14}O_3$ ($[M + Na]^+$): 217.0836; found: 217.0835.

4.1.27. (E)-5-(but-1-en-1-yl)-1,2,3-trimethoxybenzene (19)

19 was synthesized according to the general procedure B from **19a** to obtain 1.1 g (4.9 mmol; yield: 75.9%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.57 (s, 2H), 6.31 (dt, J = 15.7, 1.5, 1H), 6.18 (dt, J = 15.7, 6.4, 1H), 3.87 (s, 6H), 3.83 (s, 3H), 2.29–2.17 (m, 2H), 1.09 (t, J = 7.4, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.28, 137.19, 133.76, 132.26, 128.70, 102.91, 60.93, 56.04, 25.98, 13.69; HRMS (ESI) *m/z* calcd for $C_{13}H_{18}O_3$ ($[M + Na]^+$): 245.1149; found: 245.1149.

4.1.28. (E)-1,2,3-trimethoxy-5-(pent-1-en-1-yl)benzene (20)

20 was synthesized according to the general procedure B from **20a** to obtain 2.1 g (8.9 mmol; yield: 55.6%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.57 (s, 2H), 6.31 (dt, J = 15.8, 1.5, 1H), 6.14 (dt, J = 15.7, 6.9, 1H), 3.87 (s, 6H), 3.83 (s, 3H), 2.18 (qd, J = 7.1, 1.4, 2H), 1.50 (h, J = 7.4, 2H), 0.96 (t, J = 7.4, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.28, 137.21, 133.75, 130.56, 129.80, 102.92, 60.93, 56.05, 35.05, 22.56, 13.77; HRMS (ESI) *m/z* calcd for $C_{14}H_{20}O_3$ ($[M + Na]^+$): 259.1305; found: 259.1306.

4.1.29. (E)-5-(hex-1-en-1-yl)-1,2,3-trimethoxybenzene (21)

21 was synthesized according to the general procedure B from **21a** to obtain 2.2 g (8.8 mmol; yield: 59.1%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.57 (s, 2H), 6.30 (d, J = 15.9, 1H), 6.14 (dt, J = 15.6, 6.8, 1H), 3.87 (s, 6H), 3.83 (s, 3H), 2.26–2.15 (m, 2H), 1.52–1.31 (m, 4H), 0.93 (t, J = 7.1, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.27, 137.18, 133.77, 130.78, 129.60, 102.90, 60.92, 56.04, 32.63, 31.54, 22.28, 13.97; HRMS (ESI) *m/z* calcd for $C_{15}H_{22}O_3$ ($[M + Na]^+$): 273.1462; found: 273.1464.

4.1.30. (E)-4-(3,4,5-trimethoxyphenyl)but-3-en-2-one (22)

22 was synthesized according to the general procedure B from **22a** to obtain 3.1 g (13.1 mmol; yield: 50.4%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.43 (d, J = 16.2, 1H), 6.77 (s, 2H), 6.63 (d, J = 16.2, 1H), 3.89 (s, 6H), 3.88 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 198.27, 153.49, 143.51, 140.31, 129.88, 126.55, 105.39, 60.99, 56.17, 27.44; HRMS (ESI) *m/z* calcd for $C_{13}H_{16}O_4$ ($[M + Na]^+$): 259.0941; found: 259.0942.

4.1.31. (E)-3-(2,4,5-trimethoxyphenyl)acrylic acid (23)

To a stirred mixture of **6c** (1.2 g, 5.5 mmol) and propanedioic acid (1.1 g, 10.9 mmol) in pyridine (20 mL) was added piperidine **(**0.2 mL, 2.2 mmol) at RT. The reaction mixture was heated at reflux for 5 h. After the completion of the reaction (TLC monitoring), the reaction mixture pH was adjusted to 3.0 using 1 N HCI. Then the mixture was extracted three times with 100 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered and concentrated in reduced pressure to obtain 1.0 g (4.2 mmol; yield: 68.6%) **23**. ^{1}H NMR (400 MHz, Chloroform-d) δ = 8.07 (d, J = 16.0, 1H), 7.03 (s, 1H), 6.50 (s, 1H), 6.38 (d, J = 16.0, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 173.16, 154.26, 152.57, 143.28, 141.88, 114.67, 114.59, 110.98, 96.72, 56.43, 56.33, 56.08; HRMS (ESI) *m/z* calcd for $C_{12}H_{14}O_5$ ($[M + Na]^+$): 261.0734; found: 261.0735.

4.1.32. 1-allyl-2,4,5-trimethoxybenzene (24)

To a stirred mixture of 3-bromopropene (4.6 g, 37.7 mmol), Pd $(OAc)_2$ (0.8 g, 3.7 mmol), Cu_2O (0.03 g, 0.2 mmol) and Ag_2CO_3 (16.0 g, 56.6 mmol) in toluene (360 mL) and DMSO (18 mL) was added **6c (**4.0 g, 18.9 mmol) at RT. The reaction mixture was heated at 110 °C for 6 h. After the completion of the reaction (TLC monitoring), the reaction mixture was quenched with 100 mL H_2O. Then the mixture was extracted three times with 100 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, concentrated in reduced pressure and purified on silica gel column chromatography (Pet. Ether/EtOAc, v/v = 20:1) to obtain 0.2 g (1.0 mmol; yield: 4.7%) **24**. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.69 (s, 1H), 6.53 (s, 1H), 5.96 (ddt, J = 15.7, 10.8, 6.5, 1H), 5.09–4.99 (m, 2H), 3.88 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.33 (dt, J = 6.5, 1.6, 2H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 151.32, 147.90, 143.01, 137.33, 120.02, 115.20, 113.96, 98.01, 56.62, 56.60, 56.25, 33.67; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_3$ ($[M + H]^+$): 209.1173; found: 209.1177.

4.1.33. Methyl (E)-3-(2,4,5-trimethoxyphenyl)acrylate (25)

25 was synthesized according to the general procedure G from **23** to obtain 0.7 g (2.8 mmol; yield: 91.1%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.96 (d, J = 16.1, 1H), 7.00 (s, 1H), 6.49 (s, 1H), 6.36 (d, J = 16.1, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 168.21, 153.90, 152.12, 143.24, 139.74, 115.37, 114.89, 110.88, 96.86, 56.43, 56.35, 56.06, 51.52; HRMS (ESI) *m/z* calcd for $C_{13}H_{16}O_5$ ($[M + Na]^+$): 275.0890; found: 275.0886.

4.1.34. (E)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-ol (26)

Methyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (**26a)** was synthesized according to the general procedure G from (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid (**26b)** to obtain 3.0 g (11.9 mmol; yield:

90.8%) of the target product.

To a stirred mixture of **26a** (2.3 g, 9.1 mmol) in dry THF (30 mL) was added dropwise (over 20 min) 1.5 M solution of diisobutyl aluminium hydride (DIBAL-H) in hexanes (14.0 mL, 21.0 mmol) kept at −40 °C under the protection of nitrogen and stirring was continued for 10 min. After the completion of the reaction (TLC monitoring), the reaction mixture was quenched with 200 mL H_2O. The pH of the mixture was adjusted to 3.0 by dilute hydrochloric acid. Then the mixture was extracted three times with 200 mL EtOAc. The organic phase was collected, dried over anhydrous $MgSO_4$, filtered, concentrated in reduced pressure and purified on silica gel column chromatography (Pet. Ether/EtOAc, v/v = 20:1) to obtain 1.3 g (5.8 mmol; total yield: 57.7%) **26**. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.59 (s, 2H), 6.51 (dt, J = 15.9, 1.6, 1H), 6.26 (dt, J = 15.7, 5.7, 1H), 4.30 (dd, J = 5.7, 1.5, 2H), 3.84 (s, 6H), 3.82 (s, 3H), 1.86 (s, 1H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.32, 137.88, 132.46, 131.08, 128.09, 103.53, 63.61, 60.95, 56.08; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}O_4$ ($[M + Na]^+$): 247.0941; found: 247.0942.

4.1.35. (E)-1,2,4-trimethoxy-5-(3,3,3-trifluoroprop-1-en-1-yl)benzene (27)

27 was synthesized according to the general procedure H from **23** to obtain 1.0 g (3.8 mmol; yield: 46.9%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.37 (dq, J = 16.1, 2.2, 1H), 6.92 (s, 1H), 6.51 (s, 1H), 6.17 (dq, J = 16.2, 6.7, 1H), 3.92 (s, 3H), 3.87 (s, 6H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.21, 151.58, 143.23, 132.53, 132.46, 132.39, 132.32, 128.25, 125.58, 122.91, 120.24, 114.25, 113.92, 113.78, 113.59, 113.26, 110.99, 96.98, 56.53, 56.32, 56.06; HRMS (ESI) *m/z* calcd for $C_{12}H_{13}F_3O_3$ ($[M + Na]^+$): 285.0709; found: 285.0712.

4.1.36. (E)-1,2,3-trimethoxy-5-(3,3,3-trifluoroprop-1-en-1-yl)benzene (28)

28 was synthesized according to the general procedure H from **26b** to obtain 1.1 g (4.2 mmol; yield: 43.1%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 7.06 (dq, J = 16.1, 2.2, 1H), 6.66 (s, 2H), 6.11 (dq, J = 15.9, 6.5, 1H), 3.88 (s, 6H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.50, 139.73, 137.76, 137.69, 137.63, 137.56, 128.95, 127.68, 125.01, 122.34, 119.66, 115.64, 115.30, 114.97, 114.63, 104.69, 60.93, 56.13; HRMS (ESI) *m/z* calcd for $C_{12}H_{13}F_3O_3$ ($[M + Na]^+$): 285.0709; found: 285.0702.

4.1.37. 1,2,3-trimethoxy-5-propylbenzene (29)

29 was synthesized according to the general procedure D from **5** to obtain 1.9 g (9.0 mmol; yield: 81.2%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.39 (s, 2H), 3.84 (s, 6H), 3.81 (s, 3H), 2.56–2.48 (m, 2H), 1.70–1.56 (m, 2H), 0.94 (t, J = 7.3, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 152.99, 138.53, 135.92, 105.27, 60.81, 55.99, 38.50, 24.66, 13.88; HRMS (ESI) *m/z* calcd for $C_{12}H_{18}O_3$ ($[M + Na]^+$): 233.1149; found: 233.1148.

4.1.38. 5-butyl-1,2,3-trimethoxybenzene (30)

30 was synthesized according to the general procedure D from **19** to obtain 1.8 g (8.0 mmol; yield: 85.6%) of the target product. ^{1}H NMR (400 MHz, Chloroform-d) δ = 6.39 (s, 2H), 3.84 (s, 6H), 3.82 (s, 3H), 2.59–2.50 (m, 2H), 1.59 (tt, J = 9.3, 6.9, 2H), 1.37 (h, J = 7.3, 2H), 0.93 (t, J = 7.4, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ = 153.01, 138.76, 135.88, 105.22, 60.85, 56.02, 36.15, 33.77, 22.47, 13.98; HRMS (ESI) *m/z* calcd for $C_{13}H_{20}O_3$ ($[M + Na]^+$): 247.1305; found: 247.1304.

4.2. Pentylenetetrazole (PTZ)-induced seizure test

In this study, all experiment procedures for the use and care of the animals were approved by the Sichuan University Ethics Committee for the Use of Laboratory Animals with a license number as SYXK (Chuan) 2018–113 and were strictly conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All male KunMing mice (weight 25–30 g) and newborn SD rats (weight 5–8 g) were purchased from Chengdu Dossy Experimental Animals Co., Ltd. (Sichuan, China). In the PTZ-induced seizure test, a dose of PTZ 75 mg/kg was used to induce tonic seizures (Racine's score ≥ 4) lasting for at least 5 s in all mice tested. PTZ was dissolved in saline, and all the tested compounds were prepared with 5–10% Solutol HS-15 and 90–95% saline, which was intraperitoneally (i.p.) injected in a standard volume of 0.1 mL/10 g body weight [35,36]. In the PTZ test, all the compounds were injected i.p. into mice at the dose of 100 mg/kg body weight, and then PTZ was injected i.p. into mice at 3 min after the treatment of compounds. The antiepileptic activities were evaluated using the average Racine's score at the endpoint of 0.5 h after injection of PTZ [37]. The Racine grading criteria was stage 0 (normal non-epileptic activity), stage 1 (facial movements, wet dog shaking, or scratching), stage 2 (head nodding or tail flicking), stage 3 (one forelimb extension or one limb clonus), stage 4 (multiple limb clonus or tonic) and stage 5 (falling, tumbling and generalized tonic-clonic seizures). The latency of onset time of seizure (Racine's score ≥ 1) were also recorded. The clonic seizures rate (CSR) and the tonic seizure rate (TSR) were calculated according to the seizure severity that Racine's score (1–3) were classified as the clonic seizures and Racine's score (4–5) as the tonic seizure. Besides synthesized compounds, the ASA group (dissolved in 10% Solutol HS-15 and 90% saline) and the solvent group were included as a clinical drug control and the negative control group (NC). The solvent was injected i.p. into mice in a volume of 0.1 mL/10 g body weight, and then saline was injected i.p. into mice at 3 min after the treatment of solvent, which was used as a blank control group (BC).

4.3. Brain tissue preparation

Five days after conducting the behavioral tests, each group randomly selected four mice in BC, NC, compounds **5**, and **19** groups. The selected mouse was deeply anesthetised by injecting 10% chloral hydrate (4 mL/kg) i.p. and then perfused through the left ventricle with 20 mL saline, followed by 20 mL 4% paraformaldehyde (PFA). The brain tissues were carefully removed and then were fixed with 4% PFA for 72 h. The fixed tissues were dehydrated sequentially in 70%, 80%, 90% alcohol for 90 min. The tissue blocks were paraffin-embedded, and serial coronal sections were cut with a thickness of 5 um for later use [38].

4.4. Nissl staining

Nissl staining was carried out according to the standard practice of pathological examination. In brief, the coronal sections were immersed in 3-aminopropyl-3-ethylylsilane (APES) to make the brain tissues closely packed to the slides. After dewaxed and rehydrated, the sections were stained with a toluidine blue solution at 50 °C for 20 min, washed with distilled water, soaked in 70% alcohol, and differentiated in 95% alcohol. After rapidly dehydrated with anhydrous alcohol, the sections were cleaned with xylene until transparent and sealed tightly with neutral gum. Nissl staining images were acquired by a Pannoramic 250 digital slice scanner (Danjier, China) and analyzed by Image-Pro Plus 6.0 (Media Cybernetics, USA). The mean density of neurons in the CA1 region of the hippocampus was calculated from up to three non-overlapping and representative images obtained from each mouse's brain slice in different groups.

4.5. Caspase-3 activity determination

The devoted coronal sections were dewaxed, treated with 3% hydrogen peroxide in methanol for 10 min, and then washed with PBS three times for 5 min each at RT. Subsequently, the sections were immersed in 0.01 M citrate solution (pH 6.0), heated to boiling status in a microwave oven, and then cooled at room temperature. After 5 min, the heating–cooling operation was repeated once. When the sections

were cooled to room temperature, sections were washed with PBS twice for 5 min each at RT. The sections were rinsed and added with normal goat serum (ZSGB-BIO, Beijing, China) in drops for blocking at RT for 20 min, then incubated with primary antibody against caspase-3 (rabbit polyclonal antibody, 1:50 dilution, abcam, UK) overnight at 4 °C. The secondary antibody with biotin-labelled goat anti-rabbit (ZSGB-BIO, Beijing, China) was added dropwise, incubated at 37 °C for 30 min, and then washed with PBS three times for 5 min each time. The sections were immediately developed with DAB chromogen kit (ZSGB-BIO, Beijing, China) for 2 min at RT and then washed with distilled water. Finally, the sections were slightly re-dyed with hematoxylin, dehydrated, transparently treated, and sealed with neutral gum.

Images were collected by a Pannoramic 250 digital slice scanner (Danjier, China) and analyzed by Image-Pro Plus 6.0 (Media Cybernetics, USA). Integrated optical density (IOD) and the positive area of all images in the CA1 region of the hippocampus were measured from up to three non-overlapping and representative images obtained from the brain slice of each mouse in different groups. The mean optical density (MOD) of each image was also calculated by dividing IOD by the positive area.

4.6. Culture of primary hippocampal neurons

Primary hippocampal neuron was isolated from the cerebral hippocampi of newborn SD rats according to the literature[39] with minor modifications. Briefly, after dissection, the hippocampi were digested by treatment with phosphate-buffered saline (PBS) containing 0.25% (m/v) trypsin (Gibco, USA) for 1 h at 37 °C. After the digestion was terminated by adding 1 mL fetal bovine serum (Gibco, USA), the cell suspension was filtered through a 70 μm cell strainer (BD Biosciences, USA) to remove chunks of undissociated tissue. The collected lysate containing hippocampal neurons was centrifugated at 1000 rpm/min for 10 min at 4 °C to remove enzymes and cellular debris. The resulting cell pellet was resuspended in DMEM/F12 (Gibco, USA) supplemented with 1% (v/v) streptomycin-penicillin (Hyclone, USA) and 20% (v/v) fetal bovine serum. The resuspended neurons (5.0×10^5 cells/well) were seeded into a 24-well plastic culture dish pre-coated with 0.1 mg/mL poly-lysine (Solarbio, Beijing, China), and incubated for 4 h at 37 °C. The culture medium was then replaced by a serum-free Neurobasal™-A (Gibco, USA) medium supplemented 2% (v/v) B27 (Gibco, USA) and 1% (v/v) streptomycin-penicillin. Medium change was performed by replacing one-half of the volume with a fresh medium every day. Within a few days of incubation, the primary hippocampal cultures form a dense network of neurites, and the neurons were usually used for the experiments 8 days after seeding.

4.7. Cell viability assay

The cells viability was measured to evaluate the neuroprotective effect of compound **5** and **19** in Mg^{2+}-free induced neuronal damage model by quantitative colorimetric assay with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma- Aldrich, USA), which is an indicator of the mitochondrial activity of living cells [40]. Morphological characteristics of primary neurons were observed using an inverted microscope (Changfang optical, Shanghai, China). In brief, primary hippocampal neurons were incubated with **5** or **19** (dissolved in Mg^{2+}-free media to the final concentration of 2.5 μg/mL or 5.0 μg/mL) for 1 h and then returned to Mg^{2+} containing media for 24 h at 37 °C. The control group (CG) and the model group (MG) were treated with normal media and Mg^{2+}-free media, respectively. Following neurons exposure to 100 μL MTT (5 mg/mL) for 4 h, the supernatants were carefully removed, and 500 μL dimethyl sulfoxide (Solarbio, Beijing, China) was added to dissolve the purple formazan. Finally, cell viability was measured by a microplate reader (Bio-Rad, Richmond, CA, USA) at the wavelength of 570 nm.

4.8. Neurotoxicity test

The rotarod test's acute neurotoxicity of compounds **5** and **19** was measured in mice [41]. The mice were trained to place on a diameter 4 cm rod rotating at a speed of 24 rpm. The trained mice were selected and randomly grouped, one day later and administrated the testing compounds through the intraperitoneal route to obtain TD_{50} (the median neurotoxic dose). 3, 30, and 60 min after drug treatment, each mouse was placed on rotarod rotating at a speed of 24 rpm. Neurologic toxicity is defined as the mouse not being kept on the rod for 3 min test (Falling more than three times from the rotarod).

4.9. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0 software, and the graphical representations were carried out in GraphPad Prism 8.0. If the data were normally distributed and groups had equal variances, multiple comparisons among groups were evaluated by one-way analysis of variance (ANOVA) followed by LSD post hoc analysis. If the data were normally distributed and groups had unequal variances, multiple comparisons among groups were performed using one-way ANOVA with Dunnett's T3 test. If data were not normally distributed (Racine's score), the test group and NC group comparisons were carried out by the Mann-Whitney *U* test (nonparametric test). The CSR and TSR were analyzed using fisher's exact test between the test group and NC group. The values of $p < 0.05$ were considered statistically significant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81473168 and No. 81602954); Key Research and Development Projects of Sichuan Department of Science and Technology, China (No. 2019YFS0328); Sichuan Youth Science and Technology Innovation Research Team Funding, China (No.2016TD0001); Sichuan Science and Technology Program, China (No.2019YJ0036); and the Fundamental Research Funds of the Central Universities, China (No. YJ201561).

References

[1] S.D. Reddy, I. Younus, V. Sridhar, D.S. Reddy, Neuroimaging Biomarkers of Experimental Epileptogenesis and Refractory Epilepsy, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20010220.
[2] C. Zhang, P. Kwan, The Concept of Drug-Resistant Epileptogenic Zone, Front. Neurol. 10 (2019) 558, https://doi.org/10.3389/fneur.2019.00558.
[3] A. Golyala, P. Kwan, Drug development for refractory epilepsy: The past 25 years and beyond, Seizure 44 (2017) 147–156, https://doi.org/10.1016/j.seizure.2016.11.022.
[4] M. Bialer, S.I. Johannessen, R.H. Levy, E. Perucca, T. Tomson, H.S. White, Progress report on new antiepileptic drugs: A summary of the Thirteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XIII), Epilepsia 58 (2017) 181–221. http://doi.org/10.1111/epi.13634.
[5] Z. Zhao, Y. Bai, J. Xie, X. Chen, X. He, Y. Sun, Y. Bai, Y. Zhang, S. Wu, X. Zheng, Excavating precursors from the traditional Chinese herb Polygala tenuifolia and Gastrodia elata: Synthesis, anticonvulsant activity evaluation of 3,4,5-trimethoxycinnamic acid (TMCA) ester derivatives, Bioorg. Chem. 88 (2019), 102832, https://doi.org/10.1016/j.bioorg.2019.03.006.
[6] V. Franco, J.A. French, E. Perucca, Challenges in the clinical development of new antiepileptic drugs, Pharmacol. Res. 103 (2016) 95–104, https://doi.org/10.1016/j.phrs.2015.11.007.
[7] A. Verrotti, A. Scaparrotta, M. Cofini, F. Chiarelli, G.M. Tiboni, Developmental neurotoxicity and anticonvulsant drugs: a possible link, Reprod. Toxicol. (Elmsford, N.Y.) 48 (2014) 72–80, https://doi.org/10.1016/j.reprotox.2014.04.005.

[8] A. Easter, M.E. Bell, J.R. Damewood, W.S. Redfern, J.-P. Valentin, M.J. Winter, C. Fonck, R.A. Bialecki, Approaches to seizure risk assessment in preclinical drug discovery, Drug Discovery Today 14 (2009) 876–884, https://doi.org/10.1016/j.drudis.2009.06.003.
[9] W. Löscher, H. Klitgaard, R.E. Twyman, D. Schmidt, New avenues for anti-epileptic drug discovery and development, Nat. Rev. Drug Discovery 12 (2013) 757–776, https://doi.org/10.1038/nrd4126.
[10] A.K. Ngugi, C. Bottomley, I. Kleinschmidt, J.W. Sander, C.R. Newton, Estimation of the burden of active and life-time epilepsy: a meta-analytic approach, Epilepsia 51 (2010) 883–890, https://doi.org/10.1111/j.1528-1167.2009.02481.x.
[11] Q.-X. Chen, J.-K. Miao, C. Li, X.-W. Li, X.-M. Wu, X.-P. Zhang, Anticonvulsant activity of acute and chronic treatment with a-asarone from Acorus gramineus in seizure models, Biol. Pharm. Bull. 36 (2013) 23–30, https://doi.org/10.1248/bpb.b12-00376.
[12] C. Huang, W.-G. Li, X.-B. Zhang, L. Wang, T.-L. Xu, D. Wu, Y. Li, α-asarone from Acorus gramineus alleviates epilepsy by modulating A-type GABA receptors, Neuropharmacology 65 (2013) 1–11, https://doi.org/10.1016/j.neuropharm.2012.09.001.
[13] Z.-J. Wang, S.R. Levinson, L. Sun, T. Heinbockel, Identification of both GABAA receptors and voltage-activated Na(+) channels as molecular targets of anticonvulsant α-asarone, Front. Pharmacol. 5 (2014) 40, https://doi.org/10.3389/fphar.2014.00040.
[14] X. He, Y. Bai, M. Zeng, Z. Zhao, Q. Zhang, N. Xu, F. Qin, X. Wei, M. Zhao, N. Wu, Z. Li, Y. Zhang, T.-P. Fan, X. Zheng, Anticonvulsant activities of α-asaronol ((E)-3'-hydroxyasarone), an active constituent derived from α-asarone, Pharmacol. Reports PR 70 (2018) 69–74, https://doi.org/10.1016/j.pharep.2017.08.004.
[15] J. Poplawski, B. Lozowicka, A.T. Dubis, B. Lachowska, S. Witkowski, D. Siluk, J. Petrusewicz, R. Kaliszan, J. Cybulski, M. Strzałkowska, Z. Chilmonczyk, Synthesis and hypolipidemic and antiplatelet activities of alpha-asarone isomers in humans (in vitro), mice (in vivo), and rats (in vivo), J. Med. Chem. 43 (2000) 3671–3676, https://doi.org/10.1021/jm000905n.
[16] C.M.G. Azevedo, C.M.M. Afonso, J.X. Soares, S. Reis, D. Sousa, R.T. Lima, M. H. Vasconcelos, M. Pedro, J. Barbosa, L. Gales, M.M.M. Pinto, Pyranoxanthones: Synthesis, growth inhibitory activity on human tumor cell lines and determination of their lipophilicity in two membrane models, Eur. J. Med. Chem. 69 (2013) 798–816, https://doi.org/10.1016/j.ejmech.2013.09.012.
[17] S. Rahmani-Nezhad, M. Safavi, M. Pordeli, S.K. Ardestani, L. Khosravani, Y. Pourshojaei, M. Mahdavi, S. Emami, A. Foroumadi, A. Shafiee, Synthesis, in vitro cytotoxicity and apoptosis inducing study of 2-aryl-3-nitro-2H-chromene derivatives as potent anti-breast cancer agents, Eur. J. Med. Chem. 86 (2014) 562–569, https://doi.org/10.1016/j.ejmech.2014.09.017.
[18] C. Schultze, B. Schmidt, Prenylcoumarins in One or Two Steps by a Microwave-Promoted Tandem Claisen Rearrangement/Wittig Olefination/Cyclization Sequence, J. Organic Chem. 83 (2018) 5210–5224, https://doi.org/10.1021/acs.joc.8b00667.
[19] T. de Paulis, Y. Kumar, L. Johansson, S. Rämsby, H. Hall, M. Sällemark, K. Angeby-Möller, S.O. Ogren, Potential neuroleptic agents. 4. Chemistry, behavioral pharmacology, and inhibition of 3Hspiperone binding of 3,5-disubstituted N-(1-ethyl-2-pyrrolidinyl)methyl-6-methoxysalicylamides, J. Med. Chem. 29 (1986) 61–69, https://doi.org/10.1021/jm00151a010.
[20] K. Yang, Y. Li, Q. Tang, L. Zheng, D. He, Synthesis, mitochondrial localization of fluorescent derivatives of cinnamamide as anticancer agents, Eur. J. Med. Chem. 170 (2019) 45–54, https://doi.org/10.1016/j.ejmech.2019.03.001.
[21] J. Wang, Z. Cui, Y. Zhang, H. Li, L.-M. Wu, Z. Liu, Pd(II)-catalyzed decarboxylative allylation and Heck-coupling of arene carboxylates with allylic halides and esters, Org. Biomol. Chem. 9 (2011) 663–666, https://doi.org/10.1039/c0ob00696c.
[22] Z. Li, Z. Cui, Z.-Q. Liu, Copper- and iron-catalyzed decarboxylative tri- and difluoromethylation of α, β-unsaturated carboxylic acids with CF3SO2Na and (CF2HSO2)2Zn via a radical process, Org. Lett. 15 (2013) 406–409, https://doi.org/10.1021/ol3034059.
[23] W. Löscher, D. Hönack, C.P. Fassbender, B. Nolting, The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs III. Pentylenetetrazole seizure models, Epilepsy Res. 8 (1991) 171–189, https://doi.org/10.1016/0920-1211(91)90062-K.
[24] K. Björnstad, A. Helander, P. Hultén, O. Beck, Bioanalytical investigation of asarone in connection with Acorus calamus oil intoxications, J. Anal. Toxicol. 33 (2009) 604–609, https://doi.org/10.1093/jat/33.9.604.
[25] K.-O. Cho, Z.R. Lybrand, N. Ito, R. Brulet, F. Tafacory, L. Zhang, L. Good, K. Ure, S. G. Kernie, S.G. Birnbaum, H.E. Scharfman, A.J. Eisch, J. Hsieh, Aberrant hippocampal neurogenesis contributes to epilepsy and associated cognitive decline, Nat. Commun. 6 (2015) 6606, https://doi.org/10.1038/ncomms7606.
[26] A.M. Dam, Epilepsy and neuron loss in the hippocampus, Epilepsia 21 (1980) 617–629, https://doi.org/10.1111/j.1528-1157.1980.tb04315.x.
[27] K. Tsunashima, C. Schwarzer, E. Kirchmair, W. Sieghart, G. Sperk, GABAA receptor subunits in the rat hippocampus III: altered messenger RNA expression in kainic acid-induced epilepsy, Neuroscience 80 (1997) 1019–1032, https://doi.org/10.1016/S0306-4522(97)00144-9.
[28] Y. Huang, X. Liu, Y. Liao, C. Luo, D. Zou, X. Wei, Q. Huang, Y. Wu, MiR-181a influences the cognitive function of epileptic rats induced by pentylenetetrazol, Int. J. Clin. Exp. Path. 8 (2015) 12861–12868.
[29] J. Yue, J. He, Y. Wei, K. Shen, K. Wu, X. Yang, S. Liu, C. Zhang, H. Yang, Decreased expression of Rev-Erbα in the epileptic foci of temporal lobe epilepsy and activation of Rev-Erbα have anti-inflammatory and neuroprotective effects in the pilocarpine model, J. Neuroinflammat. 17 (2020) 43, https://doi.org/10.1186/s12974-020-1718-7.
[30] M. Krajewska, H.G. Wang, S. Krajewski, J.M. Zapata, A. Shabaik, R. Gascoyne, J. C. Reed, Immunohistochemical analysis of in vivo patterns of expression of CPP32 (Caspase-3), a cell death protease, Cancer Res. 57 (1997) 1605–1613.
[31] D.W. Nicholson, A. Ali, N.A. Thornberry, J.P. Vaillancourt, C.K. Ding, M. Gallant, Y. Gareau, P.R. Griffin, M. Labelle, Y.A. Lazebnik, Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis, Nature 376 (1995) 37–43, https://doi.org/10.1038/376037a0.
[32] M. Sakurai, T. Nagata, K. Abe, T. Horinouchi, Y. Itoyama, K. Tabayashi, Survival and death-promoting events after transient spinal cord ischemia in rabbits: induction of Akt and caspase3 in motor neurons, J. Thoracic Cardiovasc. Surg. 125 (2003) 370–377, https://doi.org/10.1067/mtc.2003.112.
[33] S. Sombati, R.J. Delorenzo, Recurrent spontaneous seizure activity in hippocampal neuronal networks in culture, J. Neurophysiol. 73 (1995) 1706–1711, https://doi.org/10.1152/jn.1995.73.4.1706.
[34] https://www.organic-chemistry.org/prog/peo/.
[35] T. Hanada, Y. Hashizume, N. Tokuhara, O. Takenaka, N. Kohmura, A. Ogasawara, S. Hatakeyama, M. Ohgoh, M. Ueno, Y. Nishizawa, Perampanel: a novel, orally active, noncompetitive AMPA-receptor antagonist that reduces seizure activity in rodent models of epilepsy, Epilepsia 52 (2011) 1331–1340, https://doi.org/10.1111/j.1528-1167.2011.03109.x.
[36] J. Vamecq, D. Lambert, J.H. Poupaert, B. Masereel, J.P. Stables, Anticonvulsant activity and interactions with neuronal voltage-dependent sodium channel of analogues of ameltolide, J. Med. Chem. 41 (1998) 3307–3313, https://doi.org/10.1021/jm9608772.
[37] R.J. Racine, Modification of seizure activity by electrical stimulation: Cortical areas, Electroencephalogr. Clin. Neurophysiol. 38 (1975) 1–12, https://doi.org/10.1016/0013-4694(75)90204-7.
[38] S. Khamse, S.S. Sadr, M. Roghani, G. Hasanzadeh, M. Mohammadian, Rosmarinic acid exerts a neuroprotective effect in the kainate rat model of temporal lobe epilepsy: Underlying mechanisms, Pharm. Biol. 53 (2015) 1818–1825, https://doi.org/10.3109/13880209.2015.1010738.
[39] B. Ahlemeyer, E. Baumgart-Vogt, Optimized protocols for the simultaneous preparation of primary neuronal cultures of the neocortex, hippocampus and cerebellum from individual newborn (P0.5) C57Bl/6J mice, J. Neurosci. Methods 149 (2005) 110–120, https://doi.org/10.1016/j.jneumeth.2005.05.022.
[40] F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival, J. Immunol. Methods 89 (1986) 271–277, https://doi.org/10.1016/0022-1759(86)90368-6.
[41] Z. Zhao, Y. Bai, X. Chen, S. Wu, X. He, Y. Bai, Y. Sun, X. Zheng, Design, synthesis and biological evaluation of (E)-3-(3,4,5-trimethoxyphenyl) acrylic acid (TMCA) amide derivatives as anticonvulsant and sedative agents, Med. Chem. Res. 27 (2018) 2387–2396, https://doi.org/10.1007/s00044-018-2228-3.