



## 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

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### ABSTRACT

Epilepsy is a group of neurological disorders characterized by recurrent seizures that disturbs about 60 million people worldwide. In this article, a novel series of 3,4,5-trimethoxycinnamic acid (TMCA) ester derivatives **1–35** were designed inspired from the traditional Chinese herb pair drugs *Polygala tenuifolia* and *Gastrodia elata* and synthesized followed by *in vivo* and *in silico* evaluation of their anticonvulsant potential. All the synthesized derivatives were biologically evaluated for their anticonvulsant potential using two acute model of seizures induced in mice, the maximal electroshock (MES) and sc-pentylenetetrazole (PTZ) models. Simultaneously, the motor impairment as a surrogate of acute neurotoxicity and *in vitro* screening of cytotoxicity against HepG-2 cells line were assessed through the rotarod performance test and CCK-8 assay, respectively. In addition, the physicochemical and pharmacokinetic parameters of the active compounds were determined. Our results showed that compounds **5**, **7**, **8**, **13**, **20**, **25**, **28**, **30** and **32** exhibited preferable anticonvulsant activity in primary evaluation, with compounds **28** and **32** being the most promising anticonvulsant agents in according to results of subsequent pharmacology and toxicity evaluation. Additionally, the molecular modeling experiments predicted good binding interactions of part of the obtained active molecules with the gamma-aminobutyric acid (GABA) transferase. Therefore, it could be concluded that the synthesized derivatives **28** and **32** would represent useful lead compounds for further investigation in the development of anticonvulsant agents.

### 1. Introduction

Epilepsy is a grievous and chronic brain neurological disorder which disturbs 0.5–1.0% of population around the whole world during their lifetime [1,2]. Up to now, utilizing anti-epileptic drugs (AEDs) is still the primary therapy for the treatment of epilepsy. In spite of the fact that AEDs are general applied, side effects of using AEDs on symptoms including headache and memory deterioration should not be neglected, in addition, a significant number of epileptic patients are refractory to current pharmacological treatment. Faced with the limitation above, investigators make efforts to seek natural medicine to alleviate the side effects of epilepsy. Traditional Chinese Medicine (TCM) is an important source of kind of natural product which meets the requirement for safe and effective and alleviates the side effects of

the existing epilepsy treatment and to make it more effective [3]. TCM has been used for the treatment of epilepsy for more than two thousand years in China. Among the TCM treating for epilepsy, the pair herb drugs *Polygala tenuifolia* (Yuanzhi in Chinese) and *Gastrodia elata* (Tianma in Chinese) is a classical combination. The combining of *Polygala tenuifolia* - *Gastrodia elata* are listed in classical TCM prescription and used for curing epilepsy such as “Qian Jin Bao Ming Dan (recorded in *Yifang Dacheng*, Yuan dynasty)” and “Ding Xian Wan (recorded in *Yixue Xinwu*, Qing dynasty)” [4]. Recent years, studies about the sedative activity of *Polygala tenuifolia* [5] and the anticonvulsant effect of *Gastrodia elata* have been reported [6]. 3,4,5-Trimethoxycinnamic acid (TMCA) isolated from *Polygala tenuifolia*, gastrodin and vanillyl alcohol isolated from *Gastrodia elata* are considered as effective ingredients that play a role in anti-seizure according to the previous

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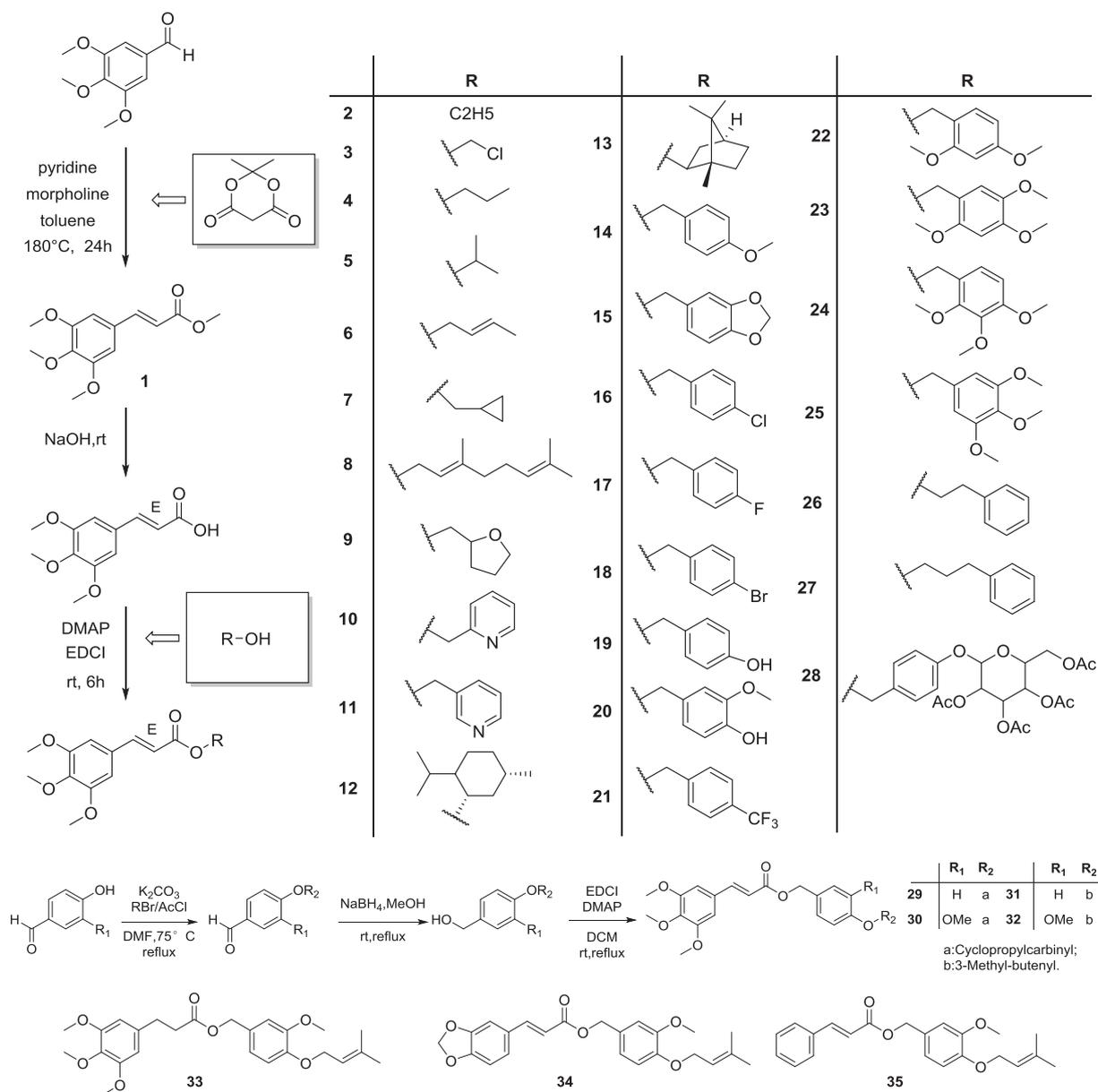
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literatures published. TMCA exhibited anticonvulsant effect in MES and PTZ model *in vivo*, with a potential mechanism of interacting with GABA<sub>A</sub>/benzodiazepine (BZD) receptor complex [7]. Gastrodin exerted anticonvulsant activity in seizure-prone gerbils via decreasing GABA transaminase, succinic semialdehyde dehydrogenase (SSADH) and succinic semialdehyde reductase (SSAR) immunoreactivity in hippocampus, suggesting that the antiepileptic effect of gastrodin may be related to increased GABA turnover [8]. Vanillol prevented injury from ferric chloride-induced seizure, meanwhile, the activity of scavenging free radical in brain was revealed [9].

Adopting the strategy of Combination of Traditional Chinese Medicine Chemistry (CMTCC), we introduce the mentioned natural product as the precursor for further investigation. Previously, we have developed promising compounds including  $\alpha$ -asaranol from *Acorus gramineus* [10], tanshinol borneol ester inspired by the pair herb drugs *Salvia miltiorrhiza* and borneol [11,12].

In previous research [13], we have reported the anticonvulsant potential of TMCA amide derivatives, which provide to us the evidence of the favorable anticonvulsant potential for TMCA derivatives. In this

study, we design a series of TMCA fatty esters and benzyl esters (Scheme 1, 1–35). Among the alcohols used for the synthesis of TMCA ester, hydroxy benzyl alcohol (19) and vanillol (20) are the constituents isolated from *Gastrodia elata*, which is correspond to the idea that creating novel anticonvulsant agents *via* the combination of effective ingredients obtaining from traditional Chinese medicines. Acegastrodine (28), which is a kind of marked sedative drug in China approved by CFDA (H20013040), is also introduced into the research to investigate the anticonvulsant effect. Besides, geraniol (8), menthol (12) and borneol (13) are derivatived from natural products as well. Furthermore, we designed compounds (29–32) to explore influence of the etherification to the anticonvulsant effect of vanillin and 4-hydroxy benzaldehyd. In China, vanillin is also used to treat epileptic petit mal approved by CFDA (H32025802). Propyl methyl group and 3-methylbutenyl group are the usual pharmacophore, thus we introduce them into our derivatives as well [14,15]. To investigate the influence of substituent group of cinnamic acid to the potential anticonvulsant effect, we designed and synthesized compounds (33–35).



Scheme 1. Synthesis of compounds 1–35.

## 2. Results and discussion

### 2.1. Chemistry

All titled compounds 1–35 were successfully synthesized using the synthetic protocols presented in Scheme 1. Identification of compounds 1–35 is listed as follow:

*(E)-methyl 3-(3,4,5-trimethoxyphenyl)acrylate (1)* White solid. mp: 96–97 °C. Yield: 85%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.63–7.59 (m, 1H), 6.74 (d, *J* = 2.8 Hz, 2H), 6.34 (dd, *J* = 15.8, 2.3 Hz, 1H), 3.90–3.86 (m, 9H), 3.80 (d, *J* = 2.4 Hz, 3H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.56, 153.64, 145.03, 140.38, 130.07, 117.24, 105.47, 61.16, 56.36, 51.89; HRMS(ESI) *m/z* calcd for C<sub>13</sub>H<sub>16</sub>O<sub>5</sub> ([M+Na]<sup>+</sup>): 275.0890; found: 275.0893.

*(E)-ethyl 3-(3,4,5-trimethoxyphenyl)acrylate (2)* White solid. mp: 59.7–60.1 °C. Yield: 84%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.60 (d, *J* = 15.8 Hz, 1H), 6.75 (s, 1H), 6.34 (d, *J* = 15.7 Hz, 1H), 4.26 (d, *J* = 7.2 Hz, 2H), 3.88 (d, *J* = 4.1 Hz, 9H), 1.34 (t, *J* = 7.0 Hz, 3H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.20, 153.64, 144.77, 140.42, 130.16, 117.73, 105.54, 105.42, 61.24, 60.77, 56.49, 56.39, 14.69; HRMS(ESI) *m/z* calcd for C<sub>14</sub>H<sub>18</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 267.1227; found: 267.1206; ([M+Na]<sup>+</sup>): 289.1046; found: 289.1023.

*(E)-ethyl 3-(3,4,5-trimethoxyphenyl)acrylate (3)* White solid. mp: 87–89 °C. Yield: 71%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.65 (d, *J* = 15.9 Hz, 1H), 6.76 (s, 2H), 6.38 (d, *J* = 15.9 Hz, 1H), 4.47 (t, *J* = 5.7 Hz, 2H), 3.89 (d, *J* = 4.1 Hz, 9H), 3.76 (t, *J* = 5.7 Hz, 2H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.20, 153.64, 144.76, 140.42, 130.31, 117.86, 105.49, 105.42, 61.32, 60.77, 56.39, 56.36, 29.94; HRMS(ESI) *m/z* calcd for C<sub>14</sub>H<sub>17</sub>ClO<sub>5</sub> ([M+H]<sup>+</sup>): 301.0837; found: 301.0835; ([M+Na]<sup>+</sup>): 323.0657; found: 323.0664.

*(E)-propyl 3-(3,4,5-trimethoxyphenyl)acrylate (4)* Yellow solid. mp: 72–73 °C. Yield: 78%; Rf = 0.6 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.60 (d, *J* = 15.9 Hz, 1H), 6.75 (s, 2H), 6.35 (dd, *J* = 15.9, 2.1 Hz, 1H), 4.17 (t, *J* = 6.7 Hz, 2H), 3.89 (s, 5H), 3.88 (s, 4H), 1.73 (h, *J* = 7.2 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.25, 153.64, 144.74, 140.29, 130.19, 117.74, 105.42, 66.37, 61.18, 56.37, 22.33, 10.68; HRMS(ESI) *m/z* calcd for C<sub>15</sub>H<sub>20</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 281.1384; found: 281.1382; ([M+Na]<sup>+</sup>): 303.1203; found: 303.1203.

*isopropyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (5)* White solid. mp: 68–69 °C. Yield: 70%; Rf = 0.6 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.58 (d, *J* = 15.9 Hz, 1H), 6.75 (s, 2H), 6.33 (d, *J* = 15.9 Hz, 1H), 5.14 (p, *J* = 6.3 Hz, 1H), 3.88 (d, *J* = 3.2 Hz, 9H), 1.31 (d, *J* = 6.3 Hz, 6H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.67, 153.64, 144.51, 140.23, 130.26, 118.28, 105.39, 68.03, 61.19, 56.37, 22.20; HRMS(ESI) *m/z* calcd for C<sub>15</sub>H<sub>20</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 281.1384; found: 281.1280.

*(2E)-(E)-but-2-enyl 3-(3,4,5-trimethoxyphenyl)acrylate (6)* White solid. mp: 82–84 °C. Yield: 82%; Rf = 0.6 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.60 (d, *J* = 15.9 Hz, 1H), 6.74 (s, 2H), 6.35 (d, *J* = 16.0 Hz, 1H), 5.93–5.63 (m, 1H), 4.67–4.62 (m, 2H), 3.87 (d, *J* = 1.2 Hz, 10H), 1.75 (dt, *J* = 6.6, 1.3 Hz, 3H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.88, 153.63, 144.94, 140.32, 131.76, 130.13, 125.35, 117.56, 105.42, 65.47, 61.16, 56.34, 18.01; HRMS(ESI) *m/z* calcd for C<sub>16</sub>H<sub>20</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 293.1384; found: 293.1386.

*(E)-cyclopropylmethyl 3-(3,4,5-trimethoxyphenyl)acrylate (7)* White solid. mp: 87–89 °C. Yield: 86%; Rf = 0.7 (Hexane/EtOAc = 1:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.61 (d, *J* = 15.4 Hz, 1H), 6.75 (d, *J* = 9.2 Hz, 2H), 6.38 (d, *J* = 15.8 Hz, 1H), 3.92–3.82 (m, 10H), 1.25 (s, 2H), 0.61 (d, *J* = 7.7 Hz, 2H), 0.33 (d, *J* = 4.9 Hz, 2H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.26, 153.64, 144.86, 130.19, 128.52, 117.71, 105.45, 69.58, 61.17, 56.36, 10.10, 3.50; HRMS(ESI) *m/z* calcd for C<sub>16</sub>H<sub>20</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 293.1384; found: 293.1381.

*(E)-3,7-dimethylocta-2,6-dien-1-yl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (8)* Yellow liquid. Yield: 60%; Rf = 0.7 (Hexane/EtOAc = 4:1, v/v);

<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.58 (d, *J* = 15.8 Hz, 1H), 6.73 (s, 2H), 6.34 (d, *J* = 15.9 Hz, 1H), 5.40 (s, 1H), 5.08–5.06 (m, 1H), 4.70 (d, *J* = 7.1 Hz, 2H), 3.85 (s, 9H), 1.72 (s, 3H), 1.66 (s, 5H), 1.58 (s, 4H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.08, 153.53, 144.73, 142.58, 131.93, 130.10, 124.04, 123.87, 118.42, 117.60, 105.34, 59.46, 56.23, 39.67, 26.44, 25.79, 17.81, 16.64; HRMS(ESI) *m/z* calcd for C<sub>22</sub>H<sub>30</sub>O<sub>5</sub> ([M+Na]<sup>+</sup>): 397.1998; found: 397.1998.

*(tetrahydrofuran-2-yl)methyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (9)* Pale-yellow solid. mp: 77–79 °C. Yield: 68%; Rf = 0.6 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.63 (d, *J* = 15.9 Hz, 1H), 6.75 (s, 2H), 6.41 (d, *J* = 15.9 Hz, 1H), 4.32 (dd, *J* = 11.4, 3.3 Hz, 1H), 4.23–4.06 (m, 2H), 3.88 (d, *J* = 1.5 Hz, 11H), 2.09–1.61 (m, 4H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.08, 153.64, 145.24, 130.11, 117.36, 105.47, 68.72, 66.83, 61.19, 56.37, 29.92, 28.24, 25.90; HRMS(ESI) *m/z* calcd for C<sub>17</sub>H<sub>23</sub>O<sub>6</sub> ([M+H]<sup>+</sup>): 323.1495; found: 323.1468.

*pyridin-2-ylmethyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (10)* Pale-yellow solid. mp: 70–71 °C. Yield: 65%; Rf = 0.6 (Hexane/EtOAc = 1:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 8.69 (d, *J* = 2.2 Hz, 1H), 8.59 (d, *J* = 4.8 Hz, 1H), 7.74 (dt, *J* = 7.7, 2.0 Hz, 1H), 7.64 (d, *J* = 15.9 Hz, 1H), 7.31 (dd, *J* = 7.9, 4.9 Hz, 1H), 6.74 (s, 2H), 6.37 (d, *J* = 15.9 Hz, 1H), 5.26 (s, 2H), 3.87 (s, 9H); <sup>13</sup>C NMR (151 MHz, cdcl<sub>3</sub>) δ ppm: 166.71, 153.65, 149.96, 149.89, 145.82, 140.54, 136.21, 131.89, 129.84, 123.65, 116.77, 105.53, 63.98, 61.17, 56.36; HRMS(ESI) *m/z* calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>5</sub> ([M+H]<sup>+</sup>): 330.1341; found: 330.1343.

*pyridin-3-ylmethyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (11)* White solid. mp: 70–72 °C. Yield: 86%; Rf = 0.6 (Hexane/EtOAc = 1:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 8.69 (d, *J* = 2.2 Hz, 1H), 8.59 (d, *J* = 4.8 Hz, 1H), 7.74 (dt, *J* = 7.7, 2.0 Hz, 1H), 7.64 (d, *J* = 15.9 Hz, 1H), 7.31 (dd, *J* = 7.9, 4.9 Hz, 1H), 6.74 (s, 2H), 6.37 (d, *J* = 15.9 Hz, 1H), 5.26 (s, 2H), 3.87 (s, 9H); <sup>13</sup>C NMR (151 MHz, cdcl<sub>3</sub>) δ ppm: 166.71, 153.65, 149.96, 149.89, 145.82, 140.54, 136.21, 131.89, 129.84, 123.65, 116.77, 105.53, 63.98, 61.17, 56.36; HRMS(ESI) *m/z* calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>5</sub> ([M+H]<sup>+</sup>): 330.1341; found: 330.1350.

*2-isopropyl-5-methylcyclohexyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (12)* White solid. mp: 70–72 °C. Yield: 64%; Rf = 0.7 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.58 (d, *J* = 15.9 Hz, 1H), 6.75 (s, 2H), 6.34 (d, *J* = 15.9 Hz, 1H), 4.83 (td, *J* = 10.9, 4.4 Hz, 1H), 3.89 (s, 6H), 3.88 (s, 3H), 2.10–1.00 (m, 9H), 0.94–0.77 (m, 9H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.73, 153.62, 144.55, 140.22, 130.26, 118.20, 105.40, 74.48, 61.18, 56.37, 47.50, 41.30, 34.54, 31.66, 26.56, 23.74, 22.27, 21.02, 16.63; HRMS(ESI) *m/z* calcd for C<sub>22</sub>H<sub>32</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 377.2328; found: 377.2340; ([M+Na]<sup>+</sup>): 399.2147; found: 399.2164.

*1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (13)* White solid. mp: 69–71 °C. Yield: 55%; Rf = 0.7 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.58 (d, *J* = 15.9 Hz, 1H), 6.76 (s, 2H), 6.37 (d, *J* = 15.8 Hz, 1H), 5.02 (dt, *J* = 9.9, 2.8 Hz, 1H), 4.00 (dt, *J* = 10.0, 2.7 Hz, 1H), 3.90 (s, 6H), 3.88 (s, 3H), 2.46–2.39 (m, 1H), 2.27 (ddt, *J* = 15.0, 8.1, 2.4 Hz, 1H), 2.06 (ddd, *J* = 13.3, 9.4, 4.4 Hz, 1H), 1.89 (dd, *J* = 15.2, 10.1, 4.5 Hz, 1H), 1.71 (t, *J* = 4.7 Hz, 2H), 1.62 (s, 1H), 0.87–0.83 (m, 9H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.50, 153.63, 144.39, 140.22, 130.26, 118.25, 105.42, 80.22, 61.18, 56.39, 49.18, 48.10, 45.31, 39.25, 28.49, 26.12, 18.89, 13.53; HRMS(ESI) *m/z* calcd for C<sub>22</sub>H<sub>32</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 375.2171; found: 375.2181; ([M+Na]<sup>+</sup>): 397.1991; found: 397.1997.

*4-methoxybenzyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (14)* White solid. mp: 65–67 °C. Yield: 80%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.62 (d, *J* = 15.9 Hz, 1H), 7.38–7.33 (m, 2H), 6.94–6.70 (m, 4H), 6.37 (d, *J* = 15.8 Hz, 1H), 5.18 (s, 2H), 3.87 (d, *J* = 2.4 Hz, 9H), 3.81 (d, *J* = 6.7 Hz, 3H); <sup>13</sup>C NMR (151 MHz, cdcl<sub>3</sub>) δ ppm: 166.99, 159.90, 153.61, 145.14, 140.33, 130.37, 130.08, 128.33, 117.48, 114.20, 105.42, 66.43, 61.15, 56.33, 55.50; HRMS(ESI) *m/z* calcd for C<sub>20</sub>H<sub>22</sub>O<sub>6</sub> ([M+H]<sup>+</sup>): 359.1495; found: 359.1495; ([M+Na]<sup>+</sup>): 381.1309; found: 381.1324.

*benzo[d][1,3]dioxol-5-ylmethyl (E)-3-(3,4,5-trimethoxyphenyl)*

acrylate (**15**) White solid. mp: 88–90 °C. Yield: 76%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.62 (d, *J* = 15.9 Hz, 1H), 6.94–6.71 (m, 5H), 6.37 (d, *J* = 15.9 Hz, 1H), 5.96 (d, *J* = 11.4 Hz, 2H), 5.14 (s, 2H), 3.88 (s, 9H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.91, 153.64, 148.06, 147.90, 145.30, 140.39, 130.04, 129.98, 122.55, 117.35, 109.31, 108.50, 105.46, 101.39, 77.44, 66.58, 61.18, 56.36; HRMS(ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>20</sub>O<sub>7</sub> ([M+H]<sup>+</sup>): 373.1287; found: 373.1295.

4-chlorobenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**16**) White solid. mp: 88–91 °C. Yield: 82%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.63 (d, *J* = 15.9 Hz, 1H), 7.35 (d, *J* = 0.8 Hz, 3H), 6.75 (s, 2H), 6.38 (d, *J* = 15.9 Hz, 1H), 5.21 (s, 2H), 3.88 (s, 9H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.81, 153.67, 145.60, 140.52, 134.78, 134.41, 129.88, 129.01, 128.46, 117.04, 105.53, 65.74, 61.19, 56.38; HRMS(ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>19</sub>ClO<sub>5</sub> ([M+H]<sup>+</sup>): 363.0999; found: 363.0993.

4-fluorobenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**17**) White solid. mp: 70–72 °C. Yield: 80%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.59 (d, *J* = 15.9 Hz, 1H), 7.34–7.22 (m, 5H), 6.74 (s, 2H), 6.33 (d, *J* = 15.9 Hz, 1H), 4.44 (t, *J* = 7.1 Hz, 2H), 3.89 (s, 6H), 3.88 (s, 3H), 3.03 (t, *J* = 7.1 Hz, 2H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.07, 153.66, 145.06, 138.08, 129.12, 128.75, 126.80, 117.49, 105.49, 65.20, 61.20, 56.40; HRMS(ESI) *m/z* calcd for C<sub>19</sub>H<sub>19</sub>FO<sub>5</sub> ([M+H]<sup>+</sup>): 347.1295; found: 347.1289; ([M+Na]<sup>+</sup>): 369.1114; found: 369.1119.

4-bromobenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**18**) White solid. mp: 86–88 °C. Yield: 82%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.63 (d, *J* = 15.9 Hz, 1H), 7.54–7.48 (m, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 6.75 (s, 2H), 6.38 (d, *J* = 15.9 Hz, 1H), 5.19 (s, 2H), 3.88 (d, *J* = 0.9 Hz, 9H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.78, 153.66, 145.61, 140.52, 135.29, 131.96, 130.16, 129.93, 122.52, 117.01, 105.54, 65.75, 61.18, 56.38; HRMS(ESI) *m/z* calcd for C<sub>19</sub>H<sub>19</sub>BrO<sub>5</sub> ([M+H]<sup>+</sup>): 407.0489; found: 407.0493.

4-hydroxybenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**19**) White solid. mp: 90–92 °C. Yield: 60%; Rf = 0.4 (Hexane/EtOAc = 1:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.78 (d, *J* = 15.9 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.15 (d, *J* = 8.1 Hz, 2H), 6.81 (s, 2H), 6.62–6.44 (m, 1H), 4.78–4.66 (m, 2H), 3.90 (s, 9H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 165.64, 153.73, 150.43, 146.86, 138.68, 129.82, 128.33, 121.95, 116.59, 105.73, 65.03, 61.22, 56.42; HRMS(ESI) *m/z* calcd for C<sub>19</sub>H<sub>20</sub>O<sub>6</sub> ([M+H]<sup>+</sup>): 345.1338; found: 345.1340.

4-hydroxy-3-methoxybenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**20**) White solid. mp: 198–200 °C. Yield: 63%; Rf = 0.4 (Hexane/EtOAc = 1:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.79 (d, *J* = 15.9 Hz, 1H), 7.12–7.05 (m, 2H), 6.95 (dd, *J* = 8.0, 1.9 Hz, 1H), 6.82 (s, 2H), 6.58 (d, *J* = 15.9 Hz, 1H), 4.71–4.69 (m, 2H), 3.93–3.84 (m, 12H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 165.25, 153.69, 151.55, 146.82, 140.66, 139.32, 129.94, 123.09, 119.24, 116.36, 111.31, 105.71, 65.33, 61.22, 56.40; HRMS(ESI) *m/z* calcd for C<sub>20</sub>H<sub>22</sub>O<sub>7</sub> ([M+H]<sup>+</sup>): 375.1438; found: 375.1441.

4-(trifluoromethyl)benzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**21**) White solid. mp: 120–122 °C. Yield: 73%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.84–7.63 (m, 3H), 7.53 (d, *J* = 7.9 Hz, 2H), 6.76 (s, 2H), 6.40 (d, *J* = 15.9 Hz, 1H), 5.30 (s, 2H), 3.89 (d, *J* = 1.4 Hz, 9H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.74, 153.69, 145.88, 143.86, 142.32, 140.60, 132.12, 129.88, 128.43, 125.78, 116.81, 105.57, 65.56, 61.22, 56.40; HRMS(ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>19</sub>F<sub>3</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 397.1263; found: 397.1260.

2,4-dimethoxybenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**22**) White solid. mp: 74–76 °C. Yield: 67%; Rf = 0.5 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.61 (dd, *J* = 15.9, 3.3 Hz, 1H), 7.30 (d, *J* = 8.9 Hz, 1H), 6.74 (d, *J* = 10.3 Hz, 2H), 6.49 (dt, *J* = 3.9, 2.1 Hz, 1H), 6.37 (dd, *J* = 23.1, 15.9 Hz, 1H), 5.23 (s, 2H), 3.91–3.79 (m, 16H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.21, 161.56, 159.26, 153.60, 144.79, 140.22, 131.73, 130.23, 117.82,

116.96, 105.38, 104.31, 98.83, 61.99, 61.16, 56.33, 55.63; HRMS(ESI) *m/z* calcd for C<sub>21</sub>H<sub>14</sub>O<sub>7</sub> ([M+Na]<sup>+</sup>): 411.1414; found: 411.0951.

2,4,5-trimethoxybenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**23**) White solid. mp: 68–70 °C. Yield: 60%; Rf = 0.4 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.61 (d, *J* = 15.8 Hz, 1H), 6.94 (s, 1H), 6.73 (s, 2H), 6.55 (s, 1H), 6.39 (d, *J* = 16.0 Hz, 1H), 3.90 (s, 4H), 3.85 (d, *J* = 8.4 Hz, 16H); <sup>13</sup>C NMR (151 MHz, cdcl<sub>3</sub>) δ ppm: 167.18, 153.58, 152.75, 150.32, 144.90, 143.12, 140.24, 130.13, 117.65, 115.69, 114.90, 105.38, 97.74, 61.84, 61.13, 56.30; HRMS(ESI) *m/z* calcd for C<sub>22</sub>H<sub>26</sub>O<sub>8</sub> ([M+Na]<sup>+</sup>): 441.1528; found: 441.1521.

2,3,4-trimethoxybenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**24**) White solid. mp: 91–93 °C. Yield: 67%; Rf = 0.4 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, Chloroform-*d*) δ ppm: 7.61 (d, *J* = 15.7 Hz, 1H), 7.12–7.07 (m, 1H), 6.74 (d, *J* = 11.1 Hz, 2H), 6.68 (d, *J* = 8.5 Hz, 1H), 6.37 (d, *J* = 16.1 Hz, 1H), 5.22 (d, *J* = 2.3 Hz, 2H), 3.94 (d, *J* = 2.2 Hz, 3H), 3.87 (dd, *J* = 11.5, 2.0 Hz, 16H); <sup>13</sup>C NMR (151 MHz, cdcl<sub>3</sub>) δ ppm: 167.02, 154.50, 153.61, 152.86, 145.00, 142.46, 140.33, 130.11, 125.38, 122.28, 117.60, 107.36, 105.43, 62.12, 61.65, 61.14, 56.34; HRMS(ESI) *m/z* calcd for C<sub>22</sub>H<sub>26</sub>O<sub>8</sub> ([M+H]<sup>+</sup>): 419.1706; found: 419.1769.

3,4,5-trimethoxybenzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**25**) White solid. mp: 72–74 °C. Yield: 67%; Rf = 0.4 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.64 (d, *J* = 15.9 Hz, 1H), 6.75 (s, 2H), 6.64 (s, 2H), 6.40 (d, *J* = 15.8 Hz, 1H), 5.16 (s, 2H), 3.88–3.86 (m, 16H), 3.84 (d, *J* = 1.9 Hz, 3H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.90, 153.63, 145.45, 140.44, 138.26, 131.70, 129.96, 117.17, 105.91, 105.48, 66.90, 61.15, 56.36; HRMS(ESI) *m/z* calcd for C<sub>22</sub>H<sub>26</sub>O<sub>8</sub> ([M+Na]<sup>+</sup>): 441.1525; found: 441.1426.

phenethyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**26**) White solid. mp: 65–68 °C. Yield: 86%; Rf = 0.5 (Hexane/EtOAc = 2:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.59 (d, *J* = 15.9 Hz, 1H), 7.34–7.22 (m, 5H), 6.74 (s, 2H), 6.33 (d, *J* = 15.9 Hz, 1H), 4.44 (t, *J* = 7.1 Hz, 2H), 3.89 (s, 6H), 3.88 (s, 3H), 3.03 (t, *J* = 7.1 Hz, 2H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.07, 153.66, 145.06, 138.08, 129.12, 128.75, 126.80, 117.49, 105.49, 65.20, 61.20, 56.40; HRMS(ESI) *m/z* calcd for C<sub>20</sub>H<sub>22</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 343.1545; found: 343.1567.

3-phenylpropyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**27**) White solid. mp: 69–72 °C. Yield: 86%; Rf = 0.6 (Hexane/EtOAc = 2:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.59 (d, *J* = 15.9 Hz, 1H), 7.29–7.20 (m, 5H), 6.76 (s, 2H), 6.35 (d, *J* = 15.9 Hz, 1H), 4.24 (t, *J* = 6.5 Hz, 2H), 3.90 (s, 6H), 3.89 (s, 3H), 2.76 (t, *J* = 7.7 Hz, 2H), 2.09–2.01 (m, 2H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.18, 153.66, 144.91, 141.45, 140.35, 130.14, 128.64, 128.62, 126.24, 117.58, 105.47, 77.44, 64.15, 61.20, 56.40, 32.49, 30.53; HRMS(ESI) *m/z* calcd for C<sub>21</sub>H<sub>24</sub>O<sub>5</sub> ([M+H]<sup>+</sup>): 357.1702; found: 357.1698.

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-6-(4-(((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyloxy)methyl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**28**) Yellow solid. mp: 143–146 °C. Yield: 63%; Rf = 0.5 (Hexane/EtOAc = 1:2, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.62 (d, *J* = 15.9 Hz, 1H), 7.36 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.73 (s, 2H), 6.36 (d, *J* = 15.8 Hz, 1H), 5.28 (t, *J* = 8.2 Hz, 2H), 5.19 (s, 2H), 5.09 (d, *J* = 7.2 Hz, 1H), 4.23 (dd, *J* = 56.0, 3.8 Hz, 1H), 3.87 (s, 13H), 2.18–1.98 (m, 12H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 170.74, 170.43, 169.58, 169.46, 166.90, 157.04, 153.65, 145.39, 140.44, 131.29, 130.27, 129.98, 117.27, 117.23, 105.47, 99.24, 77.44, 72.89, 72.30, 71.37, 68.48, 66.06, 62.14, 61.18, 56.37; MS(ESI) *m/z* calcd for C<sub>33</sub>H<sub>38</sub>O<sub>15</sub> ([M+Na]<sup>+</sup>): 697.2103; found: 697.2099.

4-(cyclopropylmethoxy)benzyl (*E*)-3-(3,4,5-trimethoxyphenyl)acrylate (**29**) Yellow liquid. Yield: 88%; Rf = 0.6 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.59 (dd, *J* = 15.9, 3.8 Hz, 1H), 7.38–7.27 (m, 2H), 6.89 (dd, *J* = 8.2, 5.2 Hz, 2H), 6.72 (d, *J* = 3.7 Hz, 2H), 6.35 (dd, *J* = 15.9, 2.9 Hz, 1H), 5.15 (d, *J* = 4.4 Hz, 2H), 3.88–3.77 (m, 11H), 0.97–0.79 (m, 1H), 0.62 (dd, *J* = 7.9, 5.2 Hz, 2H), 0.33 (t, *J* = 5.1 Hz, 2H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.90, 159.29, 153.56, 145.04, 140.29, 130.28, 130.03, 128.20, 117.45, 114.79, 105.39, 72.94, 66.37, 56.26, 10.40, 3.33; HRMS(ESI) *m/z* calcd

**Table 1**  
Anticonvulsant activity in the MES model (100 mg/kg) and ClogP values of compounds administered i.p. to mice.

Compound	0.5 h	1 h	2 h	3 h	4 h	ClogP <sup>a</sup>
NS	0 <sup>b</sup> /4 <sup>c</sup>	0/4	0/4	0/4	0/4	–
CBZ	5/6	6/6	4/6	4/6	2/6	2.38
1	1/4	1/4	0/4	0/4	0/4	1.77
2	0/4	0/4	0/4	0/4	0/4	2.29
3	3/4	3/4	3/4	1/4	0/4	1.88
4	0/4	0/4	0/4	0/4	0/4	2.82
5	4/4	2/4	1/4	0/4	0/4	2.60
6	3/4	1/4	0/4	0/4	0/4	3.07
7	4/4	3/4	1/4	1/4	1/4	2.74
8	4/4	3/4	3/4	1/4	0/4	5.50
9	3/4	1/4	1/4	0/4	0/4	2.21
10	1/4	1/4	1/4	0/4	0/4	2.03
11	2/4	1/4	0/4	0/4	0/4	2.03
12	2/4	1/4	0/4	0/4	0/4	5.76
13	4/4	2/4	1/4	0/4	0/4	5.63
14	1/4	0/4	0/4	0/4	0/4	3.45
15	3/4	1/4	0/4	0/4	0/4	3.40
16	1/4	0/4	0/4	0/4	0/4	4.25
17	2/4	1/4	0/4	0/4	0/4	3.68
18	1/4	0/4	0/4	0/4	0/4	4.40
19	2/4	1/4	0/4	0/4	0/4	2.87
20	4/4	2/4	0/4	0/4	0/4	2.72
21	3/4	0/4	0/4	0/4	0/4	4.42
22	2/4	2/4	2/4	1/4	0/4	3.54
23	3/4	3/4	2/4	1/4	0/4	2.83
24	2/4	2/4	1/4	1/4	0/4	2.83
25	4/4	2/4	2/4	1/4	0/4	2.83
26	3/4	3/4	1/4	0/4	0/4	3.86
27	2/4	1/4	0/4	0/4	0/4	4.24
28	4/4	3/4	2/4	1/4	1/4	0.50
29	2/4	2/4	0/4	0/4	0/4	4.42
30	4/4	3/4	1/4	0/4	0/4	4.16
31	3/4	2/4	1/4	0/4	0/4	5.15
32	4/4	3/4	2/4	0/4	0/4	4.90
33	3/4	2/4	1/4	0/4	0/4	4.72
34	3/4	2/4	1/4	0/4	0/4	5.56
35	1/4	1/4	0/4	0/4	0/4	5.59

<sup>a</sup> Notes: ClogP was calculated by utilizing the ChemBioDraw Ultra (v13.0) (Cambridgesoft, Billerica, MA, USA).

<sup>b</sup> Protect mice.

<sup>c</sup> Total mice.

for C<sub>23</sub>H<sub>26</sub>O<sub>6</sub> ([M+Na]<sup>+</sup>): 421.1622; found: 421.1622.

**4-(cyclopropylmethoxy)-3-methoxybenzyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (30)** White solid. mp: 79–82 °C. Yield: 88%; Rf = 0.6 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 8.00 (d, J = 6.3 Hz, 1H), 7.61 (dd, J = 16.0, 6.2 Hz, 1H), 6.94 (d, J = 6.6 Hz, 2H), 6.85 (d, J = 8.2 Hz, 1H), 6.73 (d, J = 6.6 Hz, 2H), 6.41–6.34 (m, 1H), 5.15 (s, 1H), 3.87 (d, J = 15.5 Hz, 10H), 2.94 (s, 2H), 1.24 (s, 3H), 0.62 (q, J = 5.9 Hz, 2H), 0.34 (t, J = 5.2 Hz, 2H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 162.68, 153.59, 149.73, 148.89, 145.18, 140.32, 130.02, 128.79, 121.50, 117.39, 113.43, 112.56, 105.41, 74.22, 66.72, 61.12, 56.31, 10.46, 3.59; HRMS(ESI) m/z calcd for C<sub>24</sub>H<sub>28</sub>O<sub>7</sub> ([M+H]<sup>+</sup>): 429.1913; found: 429.1882.

**4-((3-methylbut-2-en-1-yl)oxy)benzyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (31)** Yellow liquid. Yield: 70%; Rf = 0.5 (Hexane/EtOAc = 5:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.62 (d, J = 15.9 Hz, 1H), 7.39–7.31 (m, 2H), 6.95–6.91 (m, 2H), 6.74 (s, 2H), 6.37 (d, J = 15.9 Hz, 1H), 5.53–5.43 (m, 1H), 5.18 (s, 2H), 4.52 (d, J = 6.7 Hz, 2H), 3.87 (d, J = 1.7 Hz, 10H), 1.80 (s, 3H), 1.74 (s, 3H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 167.01, 159.22, 153.63, 145.12, 138.53, 130.35, 130.10, 128.23, 119.75, 117.53, 114.95, 105.44, 66.48, 65.03, 61.17, 56.35, 26.03, 18.41; HRMS(ESI) m/z calcd for C<sub>24</sub>H<sub>28</sub>O<sub>6</sub> ([M+Na]<sup>+</sup>): 435.1778; found: 435.1721.

**3-methoxy-4-((3-methylbut-2-en-1-yl)oxy)benzyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (32)** White solid. mp: 47–51 °C. Yield: 77%; Rf = 0.6 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ

ppm: 7.59 (d, J = 15.9 Hz, 1H), 6.92 (d, J = 11.0 Hz, 2H), 6.84 (d, J = 8.0 Hz, 1H), 6.71 (s, 2H), 6.36 (d, J = 15.9 Hz, 1H), 5.47 (t, J = 6.7 Hz, 1H), 5.14 (s, 2H), 4.55 (d, J = 6.6 Hz, 2H), 3.85 (s, 3H), 3.83 (d, J = 2.1 Hz, 9H), 1.71 (d, J = 22.3 Hz, 6H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.93, 153.44, 149.51, 148.48, 145.12, 137.78, 129.91, 128.49, 121.35, 119.87, 117.23, 112.95, 112.22, 105.31, 77.23, 66.64, 65.82, 60.96, 56.16, 25.83, 18.24; HRMS(ESI) m/z calcd for C<sub>25</sub>H<sub>30</sub>O<sub>7</sub> ([M+Na]<sup>+</sup>): 465.1884; found: 465.1889.

**3-methoxy-4-((3-methylbut-2-en-1-yl)oxy)benzyl 3-(3,4,5-trimethoxyphenyl)propanoate (33)** Yellow liquid. Yield: 73%; Rf = 0.6 (Hexane/EtOAc = 4:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 6.81 (ddd, J = 8.8, 5.9, 2.4 Hz, 3H), 6.36 (d, J = 5.2 Hz, 2H), 5.47 (t, J = 6.6 Hz, 1H), 5.01 (s, 2H), 4.53 (d, J = 6.9 Hz, 2H), 3.83–3.74 (m, 12H), 2.87 (t, J = 7.6 Hz, 2H), 2.63 (t, J = 7.8 Hz, 2H), 1.72 (d, J = 5.8 Hz, 3H), 1.69 (d, J = 5.9 Hz, 3H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 172.65, 153.12, 149.37, 148.35, 137.65, 136.35, 136.20, 128.30, 121.09, 119.81, 112.78, 111.98, 105.17, 66.43, 65.68, 60.72, 55.83, 35.97, 31.25, 25.76, 18.16; HRMS(ESI) m/z calcd for C<sub>25</sub>H<sub>32</sub>O<sub>7</sub> ([M+Na]<sup>+</sup>): 467.204; found: 467.206.

**3-methoxy-4-((3-methylbut-2-en-1-yl)oxy)benzyl (E)-3-(benzo[d][1,3]dioxol-5-yl)acrylate (34)** Yellow liquid. Yield: 73%; Rf = 0.7 (Hexane/EtOAc = 5:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.62 (d, J = 15.9 Hz, 1H), 7.03–6.98 (m, 2H), 6.96–6.92 (m, 2H), 6.87 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.30 (d, J = 15.9 Hz, 1H), 6.00 (s, 2H), 5.56–5.49 (m, 0H), 5.16 (s, 2H), 4.59 (d, J = 6.7 Hz, 2H), 3.89 (s, 3H), 1.77 (d, J = 1.6 Hz, 3H), 1.73 (d, J = 1.4 Hz, 3H); <sup>13</sup>C NMR (151 MHz, cdcl<sub>3</sub>) δ ppm: 155.41, 153.41, 150.43, 145.96, 142.40, 130.06, 128.71, 127.20, 121.38, 120.02, 117.72, 112.98, 112.22, 110.62, 105.41, 66.77, 65.98, 56.26, 29.93, 26.06; HRMS(ESI) m/z calcd for C<sub>25</sub>H<sub>32</sub>O<sub>7</sub> ([M+Na]<sup>+</sup>): 419.1465; found: 419.1476.

**3-methoxy-4-((3-methylbut-2-en-1-yl)oxy)benzyl cinnamate (35)** White solid. mp: 51–52 °C. Yield: 63%; Rf = 0.7 (Hexane/EtOAc = 5:1, v/v); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ ppm: 7.49 (d, J = 16.0 Hz, 1H), 7.26 (dp, J = 7.6, 2.9, 2.4 Hz, 2H), 7.12 (h, J = 3.7, 2.9 Hz, 3H), 6.74 (d, J = 7.6 Hz, 2H), 6.65 (d, J = 7.8 Hz, 1H), 6.25 (d, J = 16.0 Hz, 1H), 5.30 (tt, J = 5.7, 2.3 Hz, 1H), 4.96 (s, 2H), 4.35 (d, J = 6.5 Hz, 2H), 3.65 (s, 3H), 1.54 (s, 3H), 1.50 (s, 3H); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ ppm: 166.74, 149.45, 148.39, 144.98, 137.58, 134.32, 130.27, 128.84, 128.04, 121.21, 119.91, 117.96, 112.83, 112.07, 66.46, 65.70, 55.84, 25.78, 18.18; HRMS(ESI) m/z calcd for C<sub>22</sub>H<sub>24</sub>O<sub>4</sub> ([M+Na]<sup>+</sup>): 375.1567; found: 375.1571.

## 2.2. Pharmacology

### 2.2.1. Maximal electroshock test (MES)

In initial MES test, all the compounds exhibited protection of the tested mice after 0.5 h except compounds **2** and **4** (Table 1), indicative of their ability to prevent seizure spread. Compounds which were significantly active at 100 mg/kg after 0.5 h in MES test including **5**, **7**, **8**, **13**, **20**, **25**, **28**, **30** and **32**, indicative of their good ability to protect from seizure. Among these compounds, only compounds **7** and **28** were effective at the same dose after 4 h. This result revealed that compounds **7** and **28** have quick onset and long duration of action at relatively higher dose. Furthermore, we evaluated the protective effect of the derivatives at lower dosage. The ED<sub>50</sub> values of potent active compounds **5**, **7**, **8**, **13**, **20**, **25**, **28**, **30** and **32** were 62.5, 12.2, 53.0, 32.9, 46.1, 40.4, 19.5, 26.0 and 21.9 mg/kg, respectively (Table 2). The therapeutic effect of the most active compounds **7** and **28** were close to CBZ (ED<sub>50</sub> = 8.8 mg/kg, Table 2) used as reference drug. As for the protective index (PI), except compound **5**, all the active compounds exhibited higher value than CBZ. Compounds **13**, **28** and **32** exerted highest value of protective index among the test compounds (> 30), suggesting a combination of good anticonvulsant effect and high safety.

### 2.2.2. Pentylentetrazole (PTZ)-induced seizure test

The anticonvulsant activity of active compounds **5**, **7**, **8**, **13**, **20**, **25**,

**Table 2**  
Further screening for some active compounds.

Com.	MES ED <sub>50</sub>	Sc-PTZ		TOX ED <sub>50</sub>	PI <sup>a</sup> (MES)
		Latent time (100 mg/kg, s)	ED <sub>50</sub>		
NS	–	150.8 ± 16.3	–	–	–
CZB <sup>b</sup>	8.8 (5.5–14.1)	–	> 100	71.6 (45.9–135)	8.1
5	62.5 (5.4–124.7)	176.3 ± 16.3	280.7 (124.9–374.0)	417.6 (306.8–471.3)	6.7
7	12.2 (7.5–18.7)	275.8 ± 12.4**	135.8 (86.4–276.5)	291.7 (253.1–349.5)	24.0
8	53.0 (26.6–113.2)	289.3 ± 18.4**	143.3 (64.9–232.9)	422.2 (345.3–514.4)	8.0
13	32.9 (17.9–61.5)	237.8 ± 11.5**	278.6 (123.5–365.1)	> 1000	30.4
20	46.1 (23.5–79.7)	263.3 ± 16.8**	208.4 (88.6–397.8)	684.3 (598.9–810.1)	14.8
25	40.4 (19.4–72.9)	251.0 ± 19.8**	223.2 (97.1–346.8)	479.5 (396.6–587.3)	11.9
28	19.5 (9.8–38.9)	274.0 ± 15.6**	178.0 (67.6–315.9)	> 1000	51.2
30	26.0 (16.6–41.3)	246.8 ± 20.5**	209.9 (148.0–394.7)	476.4 (397.3–609.4)	18.3
32	21.9 (16.7–37.2)	253.2 ± 22.3**	201.3 (149.4–275.9)	697.6 (558.1–786.8)	31.9

## Notes:

<sup>a</sup> PI: protective index (TD<sub>50</sub>/ED<sub>50</sub>).

<sup>b</sup> Reference drug, data from [16].

\*\* P < 0.01, 95% confidence intervals given in parentheses.

28, 30 and 32 were further evaluated in scPTZ-induced seizure in mice (Table 2). The result exhibited that except compound 5, all the compounds could effectively inhibit the scPTZ-induced seizure in latent time at the dose of 100 mg/kg (P < 0.01). The ED<sub>50</sub> values of potent active compounds 5, 7, 8, 13, 20, 25, 28, 30 and 32 were 280.7, 135.8, 143.3, 278.6, 208.4, 223.2, 178.0, 209.9 and 201.3 mg/kg, respectively. Compounds 7 and 8 displayed the strongest inhibitory effect in this model.

### 2.2.3. Acute neurotoxicity screening

In the neurotoxicity screen, compounds 13, 20, 28 and 32 showed weak neurotoxicity (> 500 mg/kg). Compounds 13 and 28 even possessed no obvious neurotoxicity in the maximum dose administered (1000 mg/kg) (Table 2). To our dismay, the most active compound 7 exerted marked toxicity, indicating that this compound needed further modification for more safety. Generally, substituted groups derivatived from natural product tend to exert lower neurotoxicity. According to the results of *in vivo* pharmacology and neurotoxicity, compounds 28 and 32 were regarded as the most promising compounds.

### 2.2.4. Cytotoxicity

As we can see in Table 3, except for compounds 7 and 25, most of the active compounds exerted weak cytotoxicity (< 10%) against HepG-2 cells at the dosage of 20 μM, suggesting the safety and potential for test compounds to develop as novel anticonvulsant agents.

### 2.2.5. Molecular docking analysis

Based on the pharmacology evaluation results, the active compounds 5, 7, 8, 13, 20, 25, 28, 30 and 32 were selected as ligand examples. As shown in Table 4, total scores of compounds were evaluated, and vitamin B6 phosphate was used as reference standard. When the score was more than 5 indicated that the drug could interact with the combination of domains for the protein. The docking result showed that except for compounds 13 and 28, other the tested compounds

**Table 3**  
The measured results cytotoxicity for HepG-2 cells (μM).

Com.	Inhibition rate (%)	Compound	Inhibition rate (%)
5-Fu	37.5	25	14.5
5	3.7	28	6.7
7	18.4	30	9.2
8	9.8	32	7.1
13	8.4		
20	5.7		

Notes: 5-Fu: 5-Fluorouracil is used as positive controlled drug.

**Table 4**  
Molecular docking score for active compounds.

Compound	Score	H-boding	Residues
1OHW-ligand	11.53	4	AGR192, THR353, TRP354, GLN301,
5	5.74	2	TRP354, ASN140
7	9.43	3	SER137, GLY136, GLN301
8	7.51	3	LYS329, SER269, GLN301
13	< 0	6	SER137, GLY136, GLN301
20	7.87	4	LYS329, GLY136, SER137
25	6.51	2	GLN301, ASN352
28	< 0	11	GLU270, SER269, GLN301, TRP354, AGR100, ASN140
30	5.50	4	AGR192, SER137, LYS329
32	9.24	5	GLN301, TRP354, ASN140

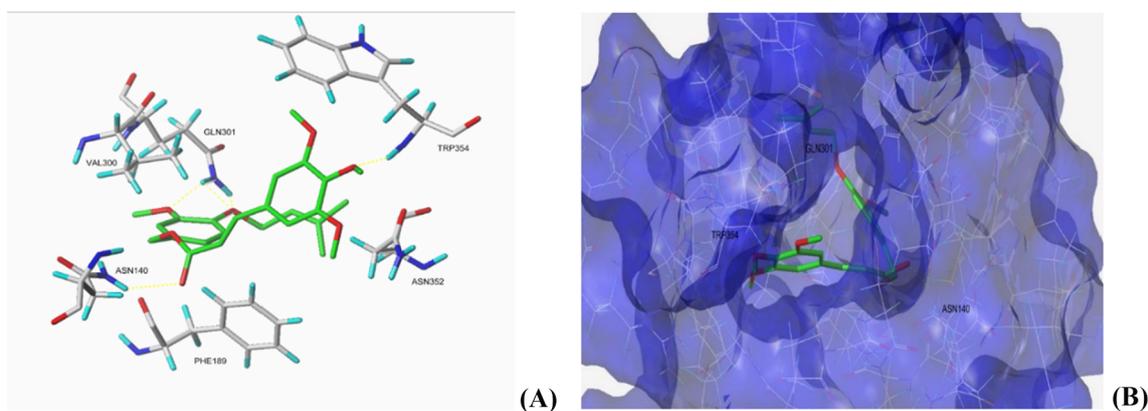
could bond to the protein with good scores. While the compound 13 and 28 did not interact with the combination of domains for the protein, suggesting that compounds 13 and 28 could possess anticonvulsant effect mediating by other targets. Based on the results of score and H-boding, compound 32 displayed the optimal interaction with the protein. The intermolecular hydrogen-bonding interactions of 32 were mainly seen with the GLN301, TRP354, ASN140 (dotted yellow line in Fig. 1), which were moderately similar to the original ligand of 1OHW. Compound 32 could be observed in the cavity of 1OHW (Fig. 1), which demonstrated the rationality of the docking study.

### 2.2.6. Predicting pharmacokinetic properties of synthesized compounds in silico

In the discovery of new CNS-active drugs including anticonvulsant agents, favorable oral bioavailability and acceptable BBB permeability of lead compounds are important factors for transduction of them into the successful drug candidates [17]. The drug-like properties of designed compounds based on Lipinski's "Rule of five" [18]. As the results presented in Table 5, except for compound 28, all active compounds follow the Lipinski's rule without causing any violation, which supposes important information about the potential druglikeness of these novel TMCA derivatives. As it was detailed in Table 5, all compounds were predicted for a certain extent transmittance for BBB, which is essential for anticonvulsant activity in brain. Results of CYP2C19 and P-gp inhibition prediction revealed that most of tested compounds could inhibit the activity of CYP2C19 and P-gp.

### 2.2.7. Structure-activity relationships

In general, the tested compounds were found to be more effective



**Fig. 1.** Docking mode of compound **32** at GABA transferas. The (A) shows the 2D interaction patterns. The legend inset represents the type of interaction between the ligand atoms and the amino acid residues of the protein. The (B) shows the 3D docking of the compound in the binding pocket.

against electrically-induced seizures (MES model) respect to the chemically-induced convulsions (PTZ test). However, compound **8** displayed stronger activity in PTZ test. As for the structure-activity relationship (Fig. 2), firstly, the ClogP values of active compounds vary widely. Ester analogues are generally low polar, and the soluble is an issue should be taken into accounts for the druggability. Ester moiety is widely distributed in the structure of marketed drugs including topiramate and felbamate, meanwhile, the reaction of ester formation is also a common formation in the drug discovery [19]. Hereinto, we introduced the active natural precursors and combined them through esterification reaction to obtain the effective anticonvulsant agents. Additionally, ester derivatives tended to be metabolized and play their therapeutic effect as metabolite *in vivo*, which was corresponding to the concept of soft drug, thus we could speculate the compound **28** active in pharmacology evaluation but pale in the docking studies follow this rule. Secondly, compared with the saturated double bond derivative **33**, compound **32** displayed superior activity in *in vivo* anticonvulsant test, suggesting that C=C bond is essential for the pharmacology property. Thirdly, 4-hydroxybenzaldehyde and 4-hydroxybenzyl alcohol analogues did not show significant inhibitory in the evaluation, revealing that the exist of methoxyl group in C-3 of vanillyl alcohol esters is important for activity. Fourth, as for the substituent groups on cinnamic acid, moieties are arranged by the activity: 3,4,5-trimethoxyl > 3,4-dioxymethyl > non-substituted. Fifth, according to the results of anticonvulsant activity and toxicity evaluation, we validate that propyl methyl and 3-methyl-butenyl etherification are helpful to promote activity and PI.

### 3. Conclusions

In summary, based on the structure of the anticonvulsant active compounds TMCA isolated from *Polygala tenuifolia* and hydroxy benzyl

alcohol and vanilloil isolated from *Gastrodia elata*, we rationally designed and successfully synthesized the derivatives of TMCA ester derivatives using the reaction of coupling reagent EDCI/DMAP as an efficient catalyst at room temperature. This method gives high yield of pure products in a short reaction time, and the reaction condition is also easy to control. All the derivatives were primarily evaluated for their anticonvulsant effect using the MES. Compounds **5**, **7**, **8**, **13**, **20**, **25**, **28**, **30** and **32** exhibited moderate activity and were tested for the activity in sPTZ model, NT and cytotoxicity model, respectively. Furthermore, the molecular modeling results also predicted good binding interactions of most active molecules with GABA transferas (10HW). Further research should focus on retaining effective groups and attenuating the toxicity of the derivatives, and definite targets are needed to prove *in vitro* as well. Above all, it could be concluded that the most promising compounds **28** and **32** are worthwhile precursors for further investigation in the development of anticonvulsant agents inspired from TCM prescription.

## 4. Experimental section

### 4.1. Chemistry

The chemicals and reagents were purchased from several chemical companies such as Alfa-Aesar, Macklin and J&K China. The progress of the reaction was monitored by thin layer chromatography (TLC- petroleum ether: ethyl acetate – 4:1 to 1:1) analysis using silica gel G plates using UV chamber in 256 nm for visualization of TLC spots. The mixtures were purified by flash column chromatography using silica gel (Qingdao Haiyang Chemical Co., Ltd. 200–300 mesh). <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were obtained by Varian Gemini 2000 DMX600 MHz FT NMR spectrometers using CDCl<sub>3</sub> as a solvent and TMS as an internal standard. The chemical shifts were expressed in ppm. Mass spectral ESI

**Table 5**  
Calculated physicochemical and pharmacokinetic parameters of the active compounds.

Compound	LogP	MW	TPSA	nON	nOHNH	BBB	Caco2	CYP2C19	P-gp
<b>5</b>	3.96	278.35	36.94	4	0	0.12	56.59	+	–
<b>7</b>	3.03	292.33	54.01	5	0	0.05	56.10	+	–
<b>8</b>	5.68	374.48	54.01	5	0	0.33	57.05	+	+
<b>13</b>	4.82	374.48	54.01	5	0	0.10	53.67	–	+
<b>20</b>	3.09	374.39	83.47	7	1	0.10	46.80	+	+
<b>25</b>	3.38	418.44	81.71	8	0	0.36	56.56	+	+
<b>28</b>	3.87	674.65	177.69	15	0	0.074	39.67	+	+
<b>30</b>	4.27	428.48	72.47	7	0	0.27	57.15	+	–
<b>32</b>	5.08	442.51	72.47	7	0	0.11	57.39	+	+

Notes: MW, molecular weight; LogP, octanol/water partition coefficient; TPSA, topological polar surface area; nON, number of hydrogen acceptors; nOHNH, number of hydrogen bond donors; BBB, blood-brain barrier; CYP2C19: Cytochrome P450 family 2 subfamily C member 19; P-gp: p glycoprotein; +: inhibition; –: non-inhibition.

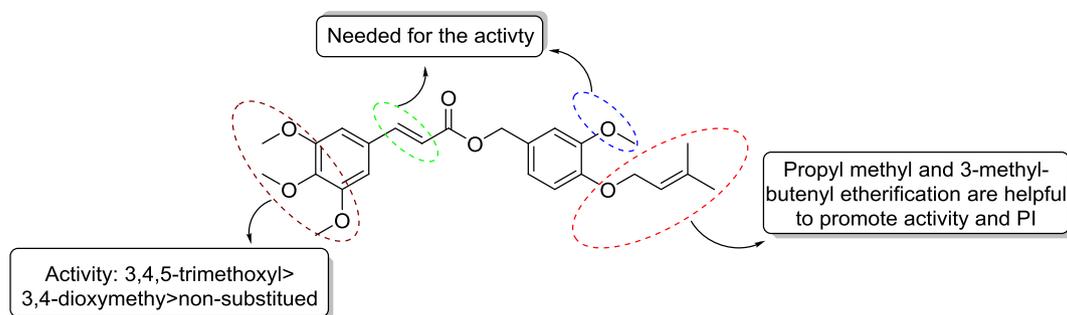


Fig. 2. Preliminary structure-activity relationship of TMCA ester derivatives.

measurements were executed on Agilent 6520 Accurate-mass Q-TOF LC/MS instruments. The spectra were performed in the positive ion mode at a declustering potential of 4000 V. Melting points were determined in a WRS-1B apparatus and are uncorrected.

#### 4.1.1. General procedure for the synthesis of *trans*-TMCA and TMCA ester derivatives

To obtain *trans*-TMCA, we synthesized TMCA methyl ester (**1**) from 3,4,5-trimethoxybenzaldehyde (0.19 mol), which was treated with 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid, 0.37 mol) in methylbenzene (300 mL) catalyzed by pyridine (40 mL) and piperidine (4 mL) (Knoevenagel condensation). When the reaction was completed, it was washed with water and ethyl acetate. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. TMCA methyl ester was recrystallized from anhydrous ethanol. Consequently, recrystallized TMCA methyl ester was hydrolyzed in the presence of NaOH to acquire *trans*-TMCA.

General experimental procedure for the synthesis of TMCA ester derivatives (**1–28**): a mixture containing *trans*-3,4,5-trimethoxycinnamic acid (*trans*-TMCA, 4.0 mmol), 4-(dimethylamino)-pyridin (DMAP, 1.0 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 4.0 mmol) and alcohol (3.0 mmol) in the presence of anhydrous dichloromethane (30 mL) was thoroughly stirred at room temperature for 6–10 h till the completion of reaction as indicated by TLC analysis. The obtained product was washed with sodium bicarbonate water solution and diluted hydrochloric acid. The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness to give the crude product. The obtained residue was purified by silica gel chromatography using petroleum ether: ethyl acetate (6:1, v:v) as original eluent to give compounds **2–28** (Scheme 1).

#### 4.1.2. General procedure for the synthesis of 4-ethers- vanillyl-TMCA derivatives

Excess  $\text{K}_2\text{CO}_3$  and a small amount of KI as the catalyst were added to a solution of the vanillin or 4-hydroxy benzaldehyde (20 mmol) in dimethyl formamide (80 mL). After stirring for 30 min, cyclopropylmethyl bromide or 3-methylbutenyl bromide (22 mmol) was added, and the resulting mixture was stirred at 65 °C for 3–6 h. The reaction was monitored by TLC analysis. After completion of the reaction, the solid was filtered, and the solution was concentrated in vacuo. The crude product was purified by chromatography on silica gel using petroleum ether/ethyl acetate (5:1, v:v) as original eluent to give substituted hydroxy aldehydes. Then the substituted hydroxy aldehydes were reduced by sodium borohydride to give corresponding alcohols, and subsequently the alcohols were used to esterification with TMCA to give compounds **29–32** using EDCI-DMAP as activation system.

#### 4.1.3. General procedure for the synthesis of substituted 3-methoxy-4-((3-methylbut-2-en-1-yl)oxy)benzyl cinnamates

According to the synthetic method of compounds **1–28**, we synthesized substituted 3-methoxy-4-((3-methylbut-2-en-1-yl)oxy)benzyl cinnamates **33–35** using EDCI-DMAP as activation system.

#### 4.1.4. Computational study: calculation of psychochemical parameters

A computational study of active synthesized compounds was performed for prediction of pharmacokinetic properties such as absorption, distribution, metabolism, excretion and toxicity (ADMET), which were considered as major hallmark to confirm the efficacy of lead compounds. Theoretical calculations were performed using Molinspiration program ([www.molinspiration.com](http://www.molinspiration.com)) to predict some physicochemical and ADME parameters of the active compounds [20,21]. The values of predicted parameters, including lipophilicity, expressed as octanol/water partition coefficient (log P), molecular weight (MW), topological polar surface area (TPSA), number of hydrogen acceptors (nON), number of hydrogen bond donors (nOHNH) and molecular volume, are presented. In addition, ability of mentioned active compounds to cross the blood-brain barrier (BBB) and Caco-2 cells were also predicted by using a PreADMET program (<https://preadmet.bmdrc.kr/adme/>).

#### 4.2. Biological activity

##### 4.2.1. Animals and experimental conditions

Male KunMing-strain mice weighing 22–26 g were used in this study. All the mice were kept under controlled environmental conditions (22 ± 2 °C; 50 ± 20% humidity; 12 h light/darkcycle) with free access to pellet food and water.

##### 4.2.2. Maximal electroshock test (MES)

The MES test carried out following the method of Swinyard [22]. In the MES screen, a 60 Hz current of 50 mA intensity (instrument: YSD-4G, SN: ZH0056232, Anhui, China) was applied through corneal electrodes for a 0.25 s duration. Carbamazepine (CBZ, 53 mg/kg) was dissolved in an aqueous Tween-80 (1% v/v, 0.9% NaCl) solution as positive control group. Negative control groups composed of 1% Tween-80 solution. All the tested compounds were prepared as suspensions in aqueous Tween-80 (1% v/v, 0.9% NaCl), and intraperitoneally injected (i.p.) in a standard volume of 0.1 mL/10 g body weight. Protection against the spread of MES-induced seizures was defined as the absence of the tonic hind limb extension (hind limbs of animals outstretched 180° to the plane of the body). Mice were considered protected if they did not exhibit tonic hind. For the test groups, mice were respectively administered intraperitoneally injected with tested compounds at 100 mg/kg in preliminary evaluation and 75, 50, 25, 12.5 mg/kg in further evaluation to calculate  $\text{ED}_{50}$ . After administration of the compounds to all the mice 0.5, 1, 2, 3 and 4 h, they received an electrical stimulation in the preliminary evaluation.

##### 4.2.3. Pentylentetrazole (PTZ)-induced seizures

After mice finished to screen MES test, the anticonvulsant activities of tested compounds were also carried out using the scPTZ-induced mice seizure model. The experiments utilized 85 mg/kg pentylentetrazole that produced clonic seizures lasting for a period of at least 5 s in 97% of animals tested [23], and PTZ (85 mg/kg) was dissolved in Tween-80 (1% v/v, 0.9% NaCl) solution to subcutaneous injections to mice 30 min after the treatment of compounds. Each animal was

observed throughout the infusion period. The duration time between the start of infusion and onset of the seizure [24]. The mice were considered protectively when the compounds were in the absence of the effect of PTZ on seizure threshold. In *sc*PTZ test, all the screened compounds were injected intraperitoneally into mice at the dose of 50, 100, 150, 200 mg/kg, and number of protect animals and latent time were recorded. ED<sub>50</sub> was calculated according to the probit analysis. CBZ (200 mg/kg) was used as positive control in the screens.

#### 4.2.4. Acute neurotoxicity screening

The acute neurotoxicity of the screened compounds were measured in mice through the rotarod test [25]. The mice were trained to place on a diameter 4 cm rod that rotates at 24 rpm. Then, the trained mice were selected and random divided, and administrated intraperitoneally with the different doses of the screened compounds, as well as reference drugs so as to in order to obtain the median neurotoxic dose (TD<sub>50</sub>). 0.5 h after drug treatment, mice were placed on rotarod that rotates at 24 rpm. Neurologic toxicity is defined as the failure (drop > 3 times from rotarod in 3 min) of the mice to remain on the rod for 3 min.

#### 4.2.5. Cytotoxicity

Cells were cultured at 37 °C under a 5% CO<sub>2</sub> atmosphere. The antiproliferative ability of compounds was evaluated in HepG-2 cells by the conversion of CCK-8 to a purple formazan precipitate as previously described. Cells were seeded into 96-well plates at about five thousand cells/well. After 12 h, 20 μM of compounds was subsequently added and incubated for 48 h. The inhibition rate was calculated from plotted results using untreated cells as 100% [26,27].

#### 4.2.6. Statistical analysis

All data were presented as mean ± standard deviation. The results were analyzed by a one-way ANOVA, followed by Student's two-tailed *t*-test for the comparison between test and control, and Dunnett's test when the data involved three or more groups. The level of significance for all tests was set at *p* < 0.05. ClogP was calculated by means of ChemDraw Ultra software, version 13.0.

#### 4.2.7. Molecular modeling

For the docking studies, Surflex-Dock in Sybyl 2.0 was used. The structures of the test compounds were drawn in the Sybyl package with standard bond lengths and angles, and minimized using the conjugate gradient method. The Gasteiger-Huckel charge was applied for the minimization process, with a distance-dependent dielectric function. A preliminary docking study was carried out using the crystal structure of GABA transferase (PDB code: 1OHW). The structure was polished as follows: all water molecules were removed from the crystal structure and the ligand was extracted. The GABA transferase was then analyzed using the Protein Structure Preparation Tool in Sybyl. After adding hydrogens, the side-chain amides were also fixed and two bumping amino acids were adjusted. Stage minimization was also applied with the AMBER FF99 force field. Then the Protomol was generated.

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#### Conflict of interest statement

We declare that we have no conflict of interest.

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