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Research paper

Polygala tenuifolia-Acori tatarinowii herbal pair as an inspiration for substituted cinnamic α-asaronol esters: Design, synthesis, anticonvulsant activity, and inhibition of lactate dehydrogenase study

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ABSTRACT

Inspired by the traditional Chinese herbal pair of *Polygala tenuifolia-Acori Tatarinowii* for treating epilepsy, 33 novel substituted cinnamic α-asaronol esters and analogues were designed by Combination of Traditional Chinese Medicine Molecular Chemistry (CTCMMC) strategy, synthesized and tested systematically not only for anticonvulsant activity in three mouse models but also for LDH inhibitory activity. Thereinto, **68**–**70** and **75** displayed excellent and broad spectra of anticonvulsant activities with modest ability in preventing neuropathic pain, as well as low neurotoxicity. The protective indices of these four compounds compared favorably with stiripentol, lacosamide, carbamazepine and valproic acid. **68**–**70** exhibited good LDH1 and LDH5 inhibitory activities with noncompetitive inhibition type, and were more potent than stiripentol. Notably, **70**, as a representative agent, was also shown as a moderately positive allosteric modulator at human $\alpha1\beta2\gamma2$ $GABA_A$ receptors (EC_{50} 46.3 ± 7.3 μM). Thus, **68**–**70** were promising candidates for developing into anti-epileptic drugs, especially for treatment of refractory epilepsies such as Dravet syndrome.

1. Introduction

Epilepsy is the third most common chronic brain disorder characterized by recurrent spontaneous seizures due to hyperexcitability and hypersynchrony of brain neurons [1,2]. Globally, about 10% of people will have at least one seizure in their lifetime, and about a third of them will go on to develop epilepsy [3]. Unfortunately, even with optimal anti-epileptic drug (AED) therapy, about one third of patients have poor seizure control and become medically refractory [4]. Even worse, adverse effects and drug-resistance have become leading causes of treatment failure with current AEDs, which also increase the risk of premature death [5], injury, psychosocial dysfunction, as well as lowering the quality of life [6,7]. Therefore, continuing efforts are needed to develop more effective pharmaceutical agents with less adverse effects.

The development of AEDs is closely based on the understanding of the mechanism of epileptic firing. For decades, almost all of clinically used AEDs (first-, second- and third-generation AEDs) act on the underpinnings of neuronal firing by targeting ion channels

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and/or neurotransmitter receptors on the neural cells [8]. However, in recent ten years, a different view has emerged that neuron-helper systems, such as astrocytes, the vasculature, the immune system and metabolic pathways, play a far greater role than first thought in seizure control [1,9–11]. For example, in 2015, Sada and his co-workers reported that seizures and epileptiform activity were reduced by inhibition of the metabolic pathway *via* lactate dehydrogenase (LDH), a component of the astrocyte-neuron lactate shuttle [12]. Among 20 clinical first-line antiepileptic drugs, only stiripentol (**1**, Chart 1, STP) had a partial LDH inhibitory effect [12]. Although STP exerted antiepileptic effect by two other widely recognized mechanisms, namely, inhibition of coadministered AEDs metabolism [13] and allosteric modulating of GABA receptors [14], in fact, its partial LDH inhibitory effects, just like that of ketogenic diet [12], might be the key to the treatment of Dravet syndrome, a kind of refractory epilepsy for children [15]. Thus, compounds with multi-target antiepileptic activity, especially LDH inhibitory activity, might be developed as a new generation of AEDs for the treatment of some drug-resistant or refractory epilepsies.

In the past five years, our continuous study of traditional Chinese medicine (TCM) antiepileptic herbal pair (*Polygala tenuifolia–Acori Tatarinowii* or *Yuanzhi–Shichangpu*) and *Acori Tatarinowii* lead to find α-asaronol (**3,** (*E*)-3′-hydroxyasarone, Chart 1), which showed better LDH inhibition than STP (unpublished). In addition, we found that **3** exhibited better general antiepileptic activities in maximal electroshock seizure (MES), pentylenetetrazole (PTZ) and 3-mercaptopropionic acid (3-MP)-induced mice seizures, and possessed lower acute toxicity than carbamazepine (CBZ), STP and α-asarone (**2**, Chart 1, one of key active components of *A. Tatarinowii*) [16]. Metabolic studies had proved that **3** was a metabolite of **2** [17]. In 2015, **2** was approved by China Food and Drug Administration (CFDA) for the treatment of grand and petit mal epilepsy. Nevertheless, three rare but potentially life-threatening adverse effects of **2** (carcinogenic [17,18], teratogenic [19,20] and genotoxic activity [21]) might restrict its clinical use. It has been proved that these toxic effects of **2** mainly come from its unstable epoxide metabolites (*cis* and/or *trans*-asaron-1′,2′-epoxide) by forming DNA adducts, but not metabolite **3** [22]. In addition, (*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (3,4,5-TMCA, **20**), one of active components of *Yuanzhi* also exhibited antiepileptic effects by modulating $GABA_A$ergic systems [23]. Some of its amide derivatives displayed good antiinflammatory and neuroprotective actions [24,25]. Thus, taking inspiration from old antiepileptic herbal pair of *Yuanzhi–Shichangpu* and using our drug design strategy (Combination of Traditional Chinese Medicine Molecular Chemistry, CTCMMC) [26] combined with hybrid molecules approach [27], we firstly designed a hybrid molecule **68** (Scheme 3). To our delight, **68** exhibited extraordinary antiepileptic activity in MES test. Therefore, further study the structure-activity relationship (SAR) of other analogues of **68** seems more meaningful.

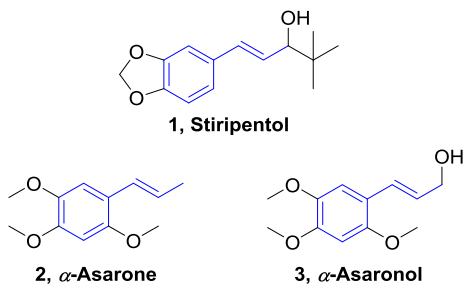


Chart 1. Compounds with subunit of propenylic phenylpropene.

In the present study, 30 new substituted cinnamic esters of α-asaronol, 3,4,5-TMCA ester of 3-(2,4,5-trimethoxyphenyl)propan-1-ol (**4**, Fig. 1), 3,4,5-TMCA amide of α-3′-aminoasarone (**5**, Figs. 1) and 3'-aminodihydroasarone (**6**, Fig. 1) were synthesized. Their anticonvulsant activities, anti-neuropathic pain, neurotoxicity, LDH inhibitory activity, and even ADME of selected compounds were systematically studied to ascertain if some of them were potential candidates for further development as new AEDs to treat refractory epilepsy. Moreover, molecular docking studies were performed to further rationalize the biological results.

2. Design rationale

The strategy of CTCMMC is founded upon two aspects: clinical effectiveness of TCM and combinatorial chemistry. Using this strategy, biologically active lead structures or fragments, stemming from different herbs in the 'composite formulae' (*fufang*), were selected or optimized without losing their functionalities, and then constructed into a set of brand-new molecules by chemosynthetic means in the light of compatibility principle of TCM (the principle of *Jun-Chen-Zuo-Shi* [28,29]). Ultimately, one or more potent molecule(s) were produced with comparable or better activity compared with the original 'composite formulae'. The goal of CTCMMC is not only to rationalized and simplify the components of TCM *fufang*, but also to fuse combination of active components by chemical means aiming to optimize the efficacy of such resultant molecules.

In this study, α-asaronol (**3**) and 3,4,5-TMCA (**20**) are the two complementary active components were selected from *Acori Tatarinowii* and *Polygala tenuifolia* respectively to serve as the parent compounds, which were then derived into two subsets of libraries: section A and B compounds (Figs. 1 and 2). Like *Polygala tenuifolia-Acori Tatarinowii* herb pair, the section A and B compounds were simply conjugated by ester or amide bond to form 33 **C** 'hybrid molecule' (**55**–**87**), which were subjected to a series of anticonvulsant evaluation to identify if some of these new compounds exhibited more potent anticonvulsant activity than their parent compounds or herbal pair.

3. Results and discussion

3.1. Chemistry

Section A compounds **3**–**6** were synthesized in a serial of classic reactions according to Scheme 1. Briefly, treatment of Meldrum's acid with methanol in toluene at reflux temperature followed by addition of 2,4,5-trimethoxybenzaldehyde **37**, pyridine and piperidine only afforded trans isomerism of cinnamic ester **38** (92.5% yield) [30], which was confirmed by H NMR analysis (see supporting information). Initially, conventional lithium aluminum hydride (LAH) selective reduction of the cinnamic ester **38** to the alcohol **3** was investigated [31], but we found an inseparable mixture of **3** and **4** (about 9:1 ratio based on HPLC analysis). **3**, however, by DIBAL-H [32] as reductant, was obtained in high yield (91%) without producing any over-reduction product **4**. Catalytic hydrogenation of mixture of **3** and **4** or pure **3** almost quantitatively led to pure **4**. It was worth mentioning that direct amination of **3** with sodium azide and 2 equivalents of triphenylphosphine to form **5** by Reddy's one pot methodology [33] was proved fruitlessly. Thus, Mitsunobu reaction condition [34,35] to give phthalimide **39** was followed by hydrazinolysis of the phthaloyl group [36] to afford primary amine **5**, which was subjected to palladium catalyzed hydrogenation condition to afford compound **6** in nearly quantitative yield.

[A] Reagents and conditions: (a) Meldrum's acid, MeOH, toluene,

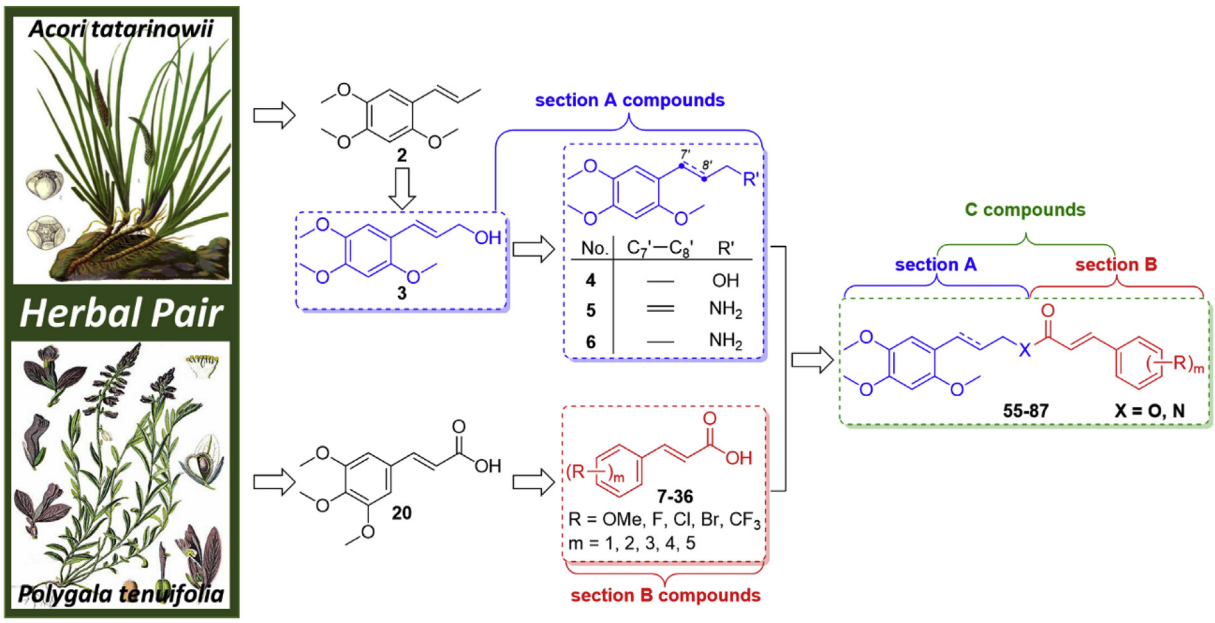
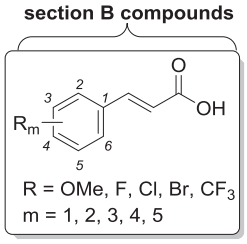


Fig. 1. Source of design ideas and the general structures of compounds designed.

reflux, 5 h, then pyridine/piperidine, rt, 24 h; (b) DIBAL-H, THF, −45 °C ~ −60 °C, 2 h; (c) Pd/C (5%, wet), H_2, rt, 20 h; (d) *o*-phthalimide, $P(Ph)_3$, DIAD, THF, 0 °C ~ rt, overnight; (e) EtOH, $NH_2NH_2 \cdot H_2O$ (80%), 76 °C, 3 h.



No.	R	m	substitution site	No.	R	m	substitution site
7	OMe	1	*2*	**22**	OMe	4	*2, 3, 4, 6*
8	OMe	1	*3*	**23**	OMe	4	*2, 3, 5, 6*
9	OMe	1	*4*	**24**	OMe	5	*2, 3, 4, 5, 6*
10	OMe	2	*2, 3*	**25**	F	1	*2*
11	OMe	2	*2, 4*	**26**	F	1	*3*
12	OMe	2	*2, 5*	**27**	F	1	*4*
13	OMe	2	*2, 6*	**28**	Cl	1	*2*
14	OMe	2	*3, 4*	**29**	Cl	1	*3*
15	OMe	2	*3, 5*	**30**	Cl	1	*4*
16	OMe	3	*2, 3, 4*	**31**	Br	1	*2*
17	OMe	3	*2, 3 ,6*	**32**	Br	1	*3*
18	OMe	3	*2, 4 ,5*	**33**	Br	1	*4*
19	OMe	3	*2, 4, 6*	**34**	CF_3	1	*2*
20	OMe	3	*3, 4, 5*	**35**	CF_3	1	*3*
21	OMe	4	*2, 3, 4, 5*	**36**	CF_3	1	*4*

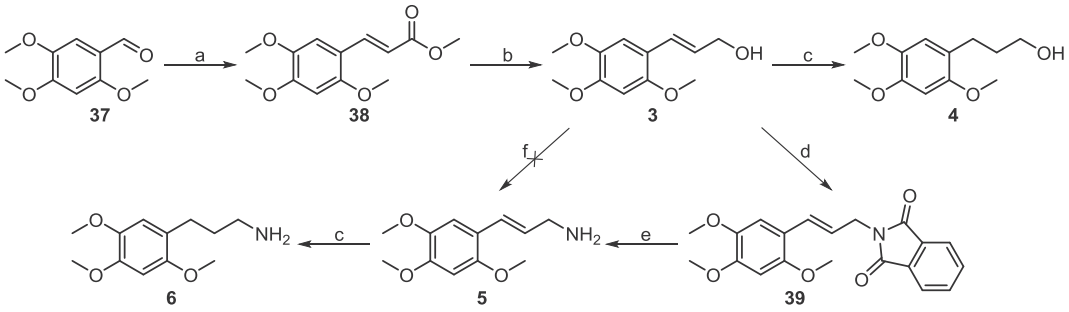
Fig. 2. Section B compounds.

Most of section B compounds could be obtained from commercial sources except some muti-methoxy-substituted cinnamic acids, such as **13, 17, 19** and **21**–**24**. Thus, these unusual cinnamic acids were synthesized in 2–10 steps using the following synthetic procedures (Scheme 2). As above mentioned (Scheme 1, condition (a)), the Meldrum's acid was treated with methanol to yield the monomethyl malonate which, in the same pot, was treated with aldehydes **40** in the presence of pyridine and piperidine to yield the desired methyl cinnamates derivatives. Subsequent hydrolysis of this methyl ester with aqueous NaOH/methanol solution furnished **13** in quantitative yield, and further purification was not required. The desired intermediates of **17**, **19** and **21**–**24** were also prepared in a similar manner from their corresponding aldehyde precursors. The olefin geometry of these cinnamic acid derivatives were assigned as (*E*) in each case, based on the H NMR coupling constant of the vinyl protons. (See supporting information).

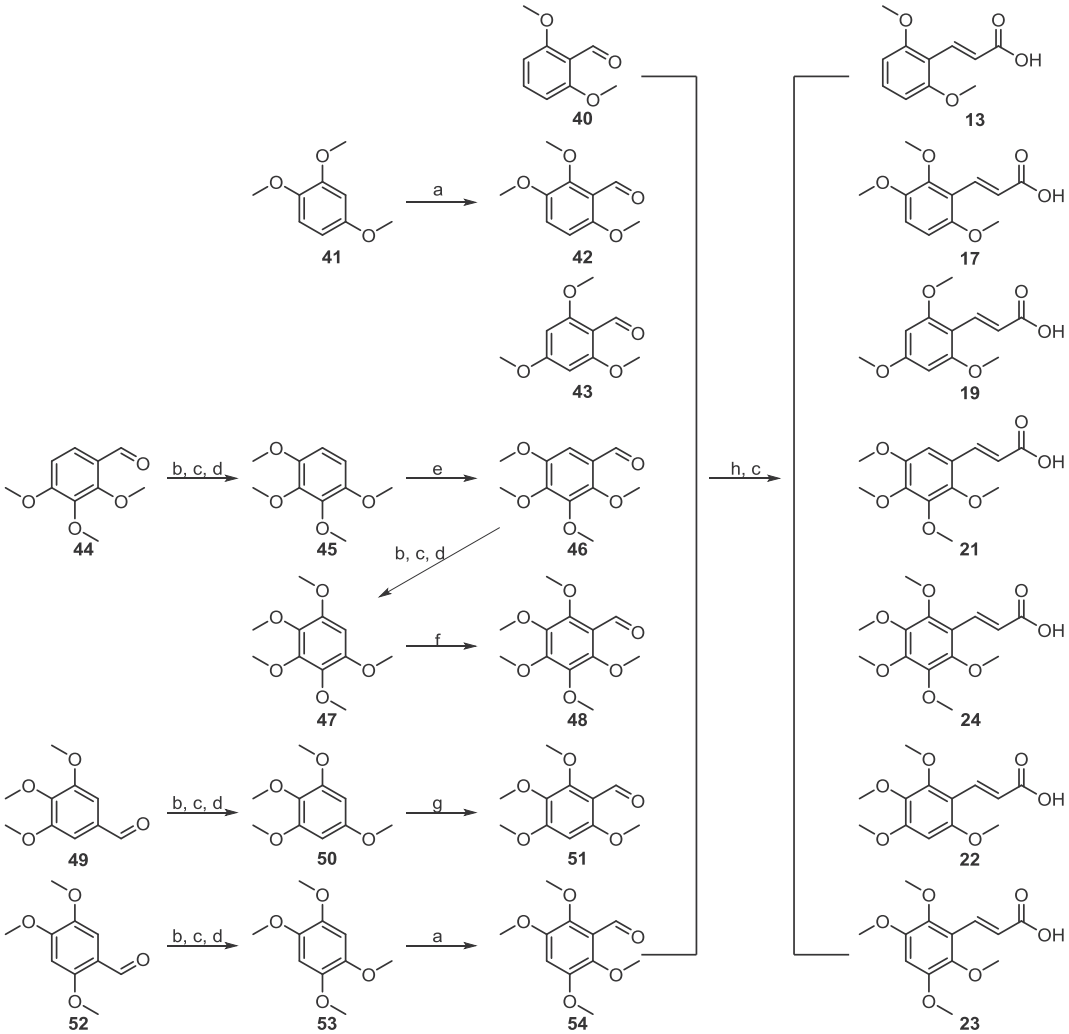
Treatment of aldehyde **44** with *m*-CPBA under Baeyer-Villiger oxidation conditions [37] gave 2,3,4-trimethoxyphenyl formate which was hydrolyzed to give desired 2,3,4-trimethoxyphenol, followed by subsequent methylation with dimethyl sulfate to obtain the 1,2,3,4-tetramethoxybenzene **45** in 70.6% yield over three steps. This procedure was also employed smoothly in the synthesis of intermediates **47**, **50** and **53**.

For the synthesis of aldehyde intermediates **42**, **46**, **48**, **51** and **54**, four different synthetic procedures were tried and evaluated (Schemes 2; conditions a, e-g), which were briefly listed in Supplemental Table 1. Initially, preparation of the aldehyde **51** was accomplished in 87% yield by treatment of compound **50** with $POCl_3$ and DMF (Scheme 2, conditions (g)) [38]. However, attempting to use this formylation method to prepare the aldehyde **46**, **48** and **54** was unsuccessful. Thus, falling back on other classical formylation methods, such as $POCl_3$/PhN(CH_3)CHO [39], TFA/HMTA [40] and *n*-BuLi/DMF [41,42], we successively achieved the compound **42**, **46**, **48** and **54** in moderate to good yield (42.9–89.4%).

The preparation of final products **55**–**85** were achieved as depicted in Scheme 3. Treatment of compound **4** with commercially available compound **20** under standard Steglich esterification conditions afforded the **85** in 71% yield after chromatography [43]. This procedure was also employed smoothly in the synthesis of



Scheme 1. Synthesis of intermediates of section A compounds (**3**–**6**)[a].



Scheme 2. Synthesis of intermediates of section B compounds (**13**, **17**, **19** and **21**–**24**)[a].
[a] Reagents and conditions: (a) THF, *n*-BuLi, DMF, −40 °C ~ −60 °C, 4 h; (b) *m*-CPBA, CH_2Cl_2, 0 °C ~ rt, 24 h; (c) MeOH, NaOH, 0 °C ~ rt, 2 h; (d) K_2CO_3, acetone, Me_2SO_4, reflux, 20 h; (e) TFA, HMTA, 70 °C, 8 h; (f) $POCl_3$, $PhN(CH_3)CHO$, rt, 18 h; (g) $POCl_3$, DCM, DMF, reflux, 4 h; (h) Meldrum's acid, MeOH, toluene, reflux, 5 h, then pyridine/piperidine, rt, 24 h.

other final products **55** to **84** with yield of 57–91%. Thereinto, the crystal structure of representative compounds **65** and **78** were confirmed by three-dimensional X-ray diffraction data (Fig. 3).

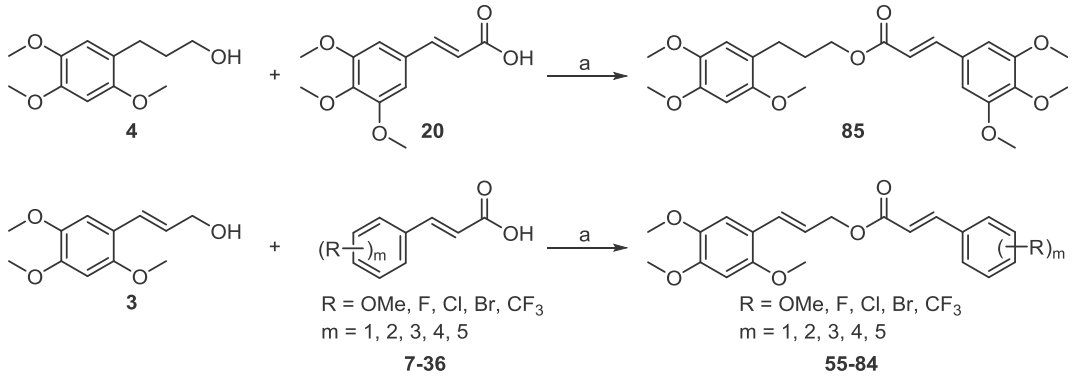
In addition, compounds **20** underwent standard EDCI-coupling conditions with saturated or unsaturated 2,3,4-trimethoxy-substituted amphetamine (**5** or **6**) to afford final amides **86** and **87** in 80.8% and 86.6% yield, respectively (Scheme 4). Much effort was not made to optimize the yields of these final products.

We reported the details (synthetic procedure, characterization and physical properties) of all the intermediates and final products in the Experimental Section. In the Supporting Information, we provided full spectroscopic data for all synthetic compounds prepared in this study.

3.2. Pharmacology

3.2.1. MES and neurotoxicity screening

Initially, we selected the mouse MES test as a basic evaluation of



No.	R	m	substitution site	No.	R	m	substitution site
55	OMe	1	*2*	**70**	OMe	4	*2, 3, 4, 6*
56	OMe	1	*3*	**71**	OMe	4	*2, 3, 5, 6*
57	OMe	1	*4*	**72**	OMe	5	*2, 3, 4, 5, 6*
58	OMe	2	*2, 3*	**73**	F	1	*2*
59	OMe	2	*2, 4*	**74**	F	1	*3*
60	OMe	2	*2, 5*	**75**	F	1	*4*
61	OMe	2	*2, 6*	**76**	Cl	1	*2*
62	OMe	2	*3, 4*	**77**	Cl	1	*3*
63	OMe	2	*3, 5*	**78**	Cl	1	*4*
64	OMe	3	*2, 3, 4*	**79**	Br	1	*2*
65	OMe	3	*2, 3, 6*	**80**	Br	1	*3*
66	OMe	3	*2, 4, 5*	**81**	Br	1	*4*
67	OMe	3	*2, 4, 6*	**82**	CF_3	1	*2*
68	OMe	3	*3, 4, 5*	**83**	CF_3	1	*3*
69	OMe	4	*2, 3, 4, 5*	**84**	CF_3	1	*4*

[a] Reagents and conditions: (a) EDCI, DMAP, 10-18 h, DCM, rt. (yield: 57–91%).

Scheme 3. Synthesis of section C compounds (**55**–**85**)[a].
[a] Reagents and conditions: (a) EDCI, DMAP, 10–18 h, DCM, rt. (yield: 57–91%).

anticonvulsant activity, because it was the most widely accepted seizure model remaining the 'valued and gold standards' in early stages of discovery of new AEDs [44,45]. The neurotoxicity of all final products was also screened by the rotorod test [46] at this stage. Table 1 presented the preliminary results of compounds **55**–**87** (dose of 100 mg/kg) at the MES test with five pretreatment times (0.5, 1, 2, 3 and 4 h) and rotorod test with three pretreatment times (0.5, 1 and 2 h). All compounds were administered intraperitoneally (ip) to mice, unless otherwise indicated. Surprisingly, our data indicated that all compounds displayed potent anticonvulsant activity and had no neurotoxicity within 2 h of drug administration (Table 1). In the whole series, 29 compounds **55**–**71**, **74**–**78**, **80**–**82**, and **84**–**87** revealed rapid onset within 0.5 h, while 26 compounds displayed long-lasting protection against convulsion showing activity up to 2 h (**56**, **59**, **62**–**66**, **70**, **77**, and **86**), 3 h (**57**, **58**, **60**, **61**, **67**–**69**, **74**–**76**, **80**–**82**, and **85**) and even 4 h (**55**, **75**), respectively. Except for weak active molecules (**72**, **73**, **79** and **83**), all other compounds showed 25–100% protection lasting from 0.5 h up to 4 h. Thereinto, 6 compounds (**55**, **64**, **65**, **68**, **69**, and **75**) exhibited most potent with maximal protection (100%) at 0.5 h, which clearly increased over that of their parent sections (**3** and **20**) and standard drug (STP). Slightly weaker efficacy was observed at the same time point, when R was 3-methoxyl (**56**), 3,4-dimethoxyl (**62**), 2,3,4,6-tetramethoxyl (**70**), 2-chlorine (**76**), 3-bromine (**80**), and 2-trifloromethyl (**82**). Additionally, we also systematically compared the effect of methoxyl group in amounts and position as well as fluoro, chloro, bromo, and trifluoromethyl group placed at benzene backbone of cinnamic acid (section B, Fig. 2) on MES activity. By and large, most of compounds with methoxyl group were better than those with halogen group in the MES test (Table 1). As to the methoxylated compounds, the number and position of methoxyl lead to different anti-MES activity, which might be associated with the electron donor and/or the steric effect of methoxyl for phenyl ring. As to the halogenated compounds, with the exclusion of highly active compound **75**, all exhibited low or modest MES activity in mice. It was worth noting that the exchange of electron-donating (ED) methoxyl in the *ortho*-position of the cinnamic acid moiety with electron-withdrawing (EW) groups, such as fluoro (**73**), chloro (**76**), bromo (**79**), and trifluoromethyl (**82**), resulted in the reduction of the MES activity. These observations suggested

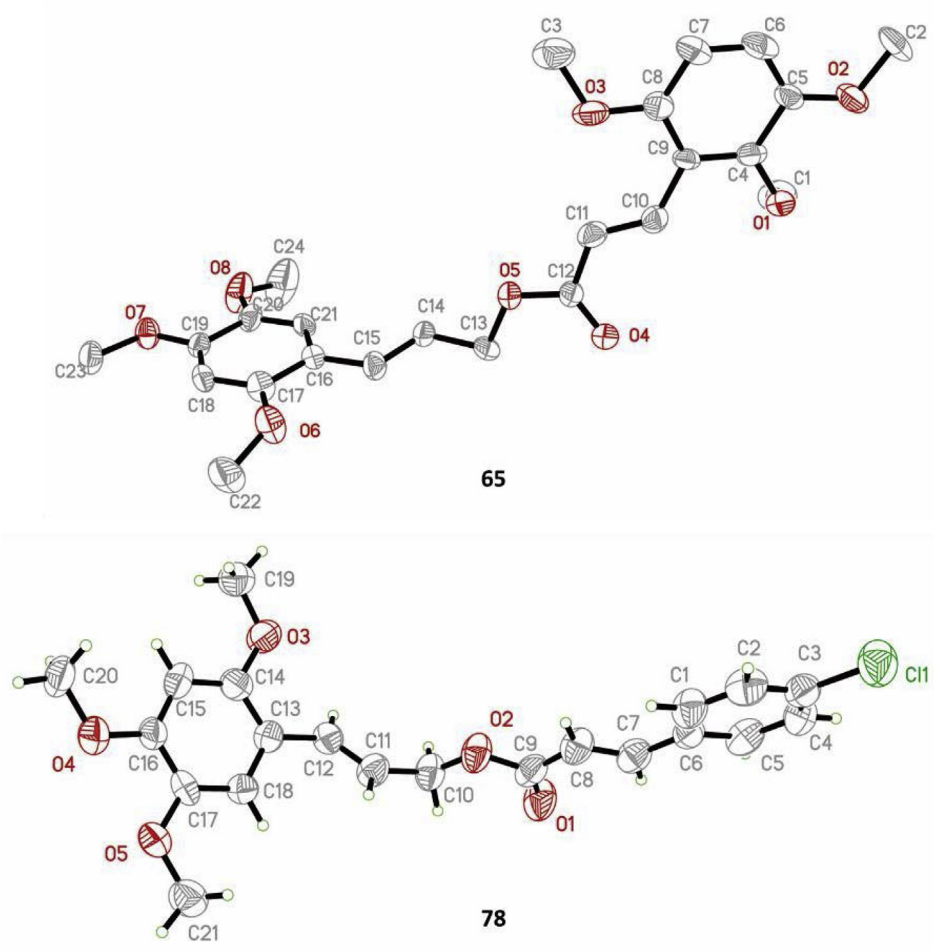
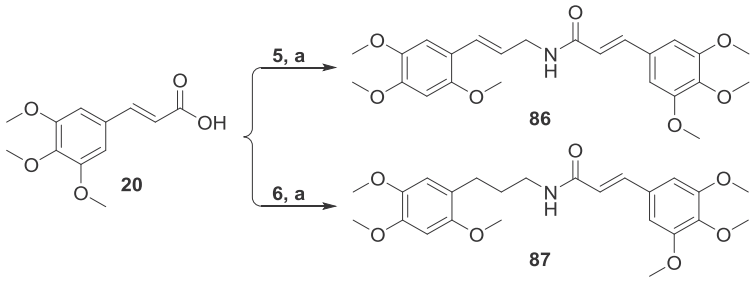


Fig. 3. Crystal structures and Oak Ridge Thermal-Ellipsoid Plot Program (ORTEP) drawing of **65** and **78**, showing the atomic numbering. Thermal ellipsoids are drawn at 50% probability.



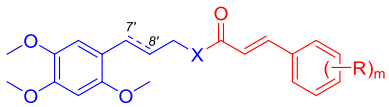
[a] Reagents and conditions: (a) EDCI, HOBt, DCM, 10 h, rt. (yield:80–86%).

Scheme 4. Synthesis of section C compounds (86 and 87)[a].
[a] Reagents and conditions: (a) EDCI, HOBt, DCM, 10 h, rt. (yield:80–86%).

that other ED groups in position 2 might potentially improve anticonvulsant properties. Finally, we briefly examined the effect of activity in section A. When R was 3,4,5-trimethoxyl, we observed that **4**, **5**, and **6** moieties provided compounds (**85**, **86**, and **87** respectively) with lower protection in the MES test compared to compound **68**. Therefore, the double bond and 3′-oxygen atom in the α-asaronol might simultaneously play a key role in anticonvulsant properties.

Next, we further evaluated 18 active compounds from Table 1 in the mouse-MES test (ip) at the dose of 50 mg/kg, at five pretreatment times 0.5, 1, 2, 3 and 4 h (Table 2). The results revealed that 14 compounds (**55**, **57**, **62**, **64**, **65**, **67**–**71**, **75**, **76**, **78**, and **81**) showed modest to good activity (at least of 50% protection in the MES test). Among these compounds, **55**, **68**–**70**, **75**, and **76** showed longer protection time starting from 0.5 h to 2 h with different maximal protective effects, i.e., 75% protection for **55**, **68**–**70**, **75**, and **76** or 50% protection for **62**, **64**, and **65**. While compounds **56**, **66**, **80**, and **82** showed short duration of anti-MES activity with weak or no protective effect at the same dose level.

Table 1
Anticonvulsant activity and acute neurotoxicity of compounds **55**–**87**: MES and rotorod test in mice (ip[a], dose of 100 mg/kg).



55-87 **X = O, N**

Compd	R	m	Sub. Site	X	$C_{7'}$-$C_{8'}$	MES[b]					TOX[c]		
						0.5 h	1 h	2 h	3 h	4 h	0.5 h	1 h	2 h
55	OMe	1	2	O	d	4[d]/4[e]	3/4	2/4	1/4	1/4	0[f]/4[e]	0/4	0/4
56	OMe	1	3	O	d	3/4	3/4	3/4	0/4	0/4	0/4	0/4	0/4
57	OMe	1	4	O	d	2/4	2/4	2/4	1/4	0/4	0/4	0/4	0/4
58	OMe	2	2, 3	O	d	2/4	2/4	1/4	1/4	0/4	0/4	0/4	0/4
59	OMe	2	2, 4	O	d	2/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
60	OMe	2	2, 5	O	d	2/4	1/4	1/4	1/4	0/4	0/4	0/4	0/4
61	OMe	2	2, 6	O	d	2/4	1/4	1/4	1/4	0/4	0/4	0/4	0/4
62	OMe	2	3, 4	O	d	3/4	2/4	2/4	0/4	0/4	0/4	0/4	0/4
63	OMe	2	3, 5	O	d	2/4	2/4	1/4	0/4	0/4	0/4	0/4	0/4
64	OMe	3	2, 3, 4	O	d	4/4	2/4	2/4	0/4	0/4	0/4	0/4	0/4
65	OMe	3	2, 3, 6	O	d	4/4	3/4	2/4	0/4	0/4	0/4	0/4	0/4
66	OMe	3	2, 4, 5	O	d	2/4	2/4	1/4	0/4	0/4	0/4	0/4	0/4
67	OMe	3	2, 4, 6	O	d	2/4	2/4	2/4	1/4	0/4	0/4	0/4	0/4
68	OMe	3	3, 4, 5	O	d	4/4	4/4	2/4	2/4	0/4	0/4	0/4	0/4
69	OMe	4	2, 3, 4, 5	O	d	4/4	3/4	2/4	1/4	0/4	0/4	0/4	0/4
70	OMe	4	2, 3, 4, 6	O	d	3/4	3/4	2/4	1/4	0/4	0/4	0/4	0/4
71	OMe	4	2, 3, 5, 6	O	d	2/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
72	OMe	5	2, 3, 4, 5, 6	O	d	1/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
73	F	1	2	O	d	1/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
74	F	1	3	O	d	2/4	2/4	1/4	1/4	0/4	0/4	0/4	0/4
75	F	1	4	O	d	4/4	4/4	3/4	2/4	1/4	0/4	0/4	0/4
76	Cl	1	2	O	d	3/4	2/4	1/4	1/4	0/4	0/4	0/4	0/4
77	Cl	1	3	O	d	2/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
78	Cl	1	4	O	d	2/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
79	Br	1	2	O	d	1/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
80	Br	1	3	O	d	3/4	3/4	3/4	1/4	0/4	0/4	0/4	0/4
81	Br	1	4	O	d	2/4	2/4	3/4	1/4	0/4	0/4	0/4	0/4
82	CF_3	1	2	O	d	3/4	2/4	2/4	1/4	0/4	0/4	0/4	0/4
83	CF_3	1	3	O	d	1/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
84	CF_3	1	4	O	d	2/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
85	OMe	3	3, 4, 5	O	s	2/4	1/4	1/4	1/4	0/4	0/4	0/4	0/4
86	OMe	3	3, 4, 5	N	d	2/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
87	OMe	3	3, 4, 5	N	s	2/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
3	–	–	–	–	–	3/4	3/4	2/4	0/4	0/4	0/4	0/4	0/4
20	–	–	–	–	–	3/4	2/4	1/4	0/4	0/4	0/4	0/4	0/4
STP[g]	–	–	–	–	–	4/4	2/4	1/4	0/4	0/4	0/4	0/4	0/4

[a] The compounds (prepared in 0.5% Tween-80 and 0.9% normal saline) were administered intraperitoneally.
[b] Maximal electroshock test (the animals were examined at five pretreatment times: 0.5, 1, 2, 3, and 4 h).
[c] Neurological toxicity (determined from the rotarod test at three pretreatment times: 0.5, 1, and 2 h).
[d] Data indicate number of mice protected.
[e] number of mice tested.
[f] the number of mice affected.
[g] STP = stiripentol.

3.2.2. *ScPTZ-induced seizures test*

Additionally, compounds emerging in Table 2 were also evaluated by *sc*PTZ seizure threshold test in mice. The results combined with corresponding clonic seizures rate (CSR), tonic seizure rate (TSR) and mortality rate (MR) profiles were presented in Table 3. All of the tested compounds exhibited satisfactory activity at dose of 300 mg/kg (at least of 50% protection), whereas compound **69**–**71** showed marginal protection at dose of 100 mg/kg (at most of 25% protection), and other compounds were completely inactive at the lower dosage. Thereinto, compound **57**, **62**, **67**, **69**, and **75** showed relatively excellent protective effect at dose of 300 mg/kg. The times to peak effect of these compounds were all at 0.5 h for 100% protection, while the points of 75% protection were found at 0.25 h and 1 h for **69**, and at 1 h for **57**, **62**, and **75**. Slightly weaker efficacy was observed for compounds **64**, **68**, **70**, **71**, **76**, **78**, **80**, and **81** with maximal peak effect for 75% protection during 0.25 h–1 h. It should be stressed, however, that compounds **69** and **70** exhibited both rapid onset and long-lasting anti-PTZ protection showing activity up to 2 h, whereas compound **62** showed shorter duration of action (within 1 h).

In general, all of the test compounds were ineffective in *sc*PTZ-induced clonic seizures at the dose of 100 mg/kg and 300 mg/kg, but exhibited satisfactory protection in *sc*PTZ-induced tonic seizure at the dose of 300 mg/kg (Table 3). Compounds **57**, **62**, **67**, **69** and **75** were the most effective with 100% inhibition of tonic seizure. Furthermore, compounds **56**, **57**, **62**, **67**, **69**, **75**, **76**, **78**, and **80**–**82** showed complete protection against *sc*PTZ-induced mortality at higher dose.

3.2.3. *Sc3-MP-induced seizures test*

Further evaluations of above selected 18 compounds were undertaken using the *sc*3-MP model, which was used to predict

Table 2
Anticonvulsant activity: MES test in mice (ip[a], dose of 50 mg/kg).

Compd	R	m	Sub. Site	X	$C_{7'}$-$C_{8'}$	MES[b]				
						0.5 h	1 h	2 h	3 h	4 h
55	OMe	1	2	O	d	3[c]/4[d]	2/4	1/4	0/4	0/4
56	OMe	1	3	O	d	1/4	0/4	0/4	0/4	0/4
57	OMe	1	4	O	d	2/4	2/4	0/4	0/4	0/4
62	OMe	2	3, 4	O	d	2/4	2/4	1/4	0/4	0/4
64	OMe	3	2, 3, 4	O	d	2/4	2/4	1/4	0/4	0/4
65	OMe	3	2, 3, 6	O	d	2/4	1/4	1/4	0/4	0/4
66	OMe	3	2, 4, 5	O	d	1/4	1/4	1/4	0/4	0/4
67	OMe	3	2, 4, 6	O	d	2/4	0/4	0/4	0/4	0/4
68	OMe	3	3, 4, 5	O	d	3/4	2/4	1/4	0/4	0/4
69	OMe	4	2, 3, 4, 5	O	d	3/4	2/4	1/4	0/4	0/4
70	OMe	4	2, 3, 4, 6	O	d	3/4	2/4	1/4	0/4	0/4
71	OMe	4	2, 3, 5, 6	O	d	2/4	1/4	0/4	0/4	0/4
75	F	1	4	O	d	3/4	1/4	1/4	0/4	0/4
76	Cl	1	2	O	d	3/4	2/4	1/4	0/4	0/4
78	Cl	1	4	O	d	2/4	2/4	0/4	0/4	0/4
80	Br	1	3	O	d	1/4	1/4	1/4	0/4	0/4
81	Br	1	4	O	d	2/4	1/4	0/4	0/4	0/4
82	CF_3	1	2	O	d	0/4	0/4	0/4	0/4	0/4

[a] The compounds (prepared in 0.5% Tween-80 and 0.9% normal saline) were administered intraperitoneally.
[b] Maximal electroshock test (the animals were examined at five pretreatment times: 0.5, 1, 2, 3, and 4 h).
[c] Data indicate number of mice protected.
[d] The total number of mice tested.

Table 3
Anticonvulsant activity: *sc*PTZ test in mice ip[a].

Compd	Dose (mg/kg)	Pretreatment time[b]				Pretreatment time (0.5 h)[c]		
		0.25 h	0.5 h	1 h	2 h	CSR %	TSR %	MR %
55	300	1[d]/4[e]	2/4	2/4	0/4	100	50	25
	100	0/4	0/4	0/4	0/4	100	100	50
56	300	1/4	2/4	1/4	0/4	100	50	0
	100	0/4	0/4	0/4	0/4	100	100	75
57	300	2/4	4/4	3/4	1/4	100	0	0
	100	0/4	0/4	0/4	0/4	100	100	50
62	300	2/4	4/4	3/4	0/4	100	0	0
	100	0/4	0/4	0/4	0/4	100	100	50
64	300	2/4	3/4	3/4	1/4	100	25	25
	100	0/4	0/4	0/4	0/4	100	100	75
65	300	2/4	2/4	2/4	0/4	100	50	50
	100	0/4	0/4	0/4	0/4	100	100	75
66	300	2/4	2/4	1/4	0/4	100	25	25
	100	0/4	0/4	0/4	0/4	100	100	75
67	300	2/4	4/4	2/4	0/4	100	0	0
	100	0/4	0/4	0/4	0/4	100	100	50
68	300	2/4	3/4	2/4	1/4	100	25	25
	100	0/4	0/4	0/4	0/4	100	100	75
69	300	3/4	4/4	3/4	2/4	100	0	0
	100	0/4	1/4	14	0/4	100	75	25
70	300	3/4	3/4	3/4	2/4	100	25	25
	100	0/4	1/4	1/4	0/4	100	25	25
71	300	2/4	3/4	2/4	2/4	100	25	25
	100	1/4	1/4	1/4	0/4	100	75	50
75	300	2/4	4/4	3/4	1/4	100	0	0
	100	0/4	0/4	0/4	0/4	100	100	75
76	300	2/4	3/4	2/4	1/4	100	25	0
	100	0/4	0/4	0/4	0/4	100	100	50
78	300	2/4	3/4	2/4	1/4	100	25	0
	100	0/4	0/4	0/4	0/4	100	100	75
80	300	2/4	3/4	2/4	0/4	100	25	0
	100	0/4	0/4	0/4	0/4	100	100	75
81	300	2/4	3/4	2/4	1/4	100	25	0
	100	0/4	0/4	0/4	0/4	100	100	25
82	300	2/4	2/4	2/4	0/4	100	50	0
	100	0/4	0/4	0/4	0/4	100	100	75

[a] The selected compounds (prepared in 0.5% Tween-80 in 0.9% normal saline) were administered intraperitoneally.
[b] Subcutaneous injection PTZ (85 mg/kg, prepared in 0.9% normal saline) at four pretreatment times after drug administration: 0.25, 0.5, 1, and 2 h.
[c] The CSR (clonic seizures rate), TSR (tonic seizure rate) and MR (mortality rate) induced by subcutaneous injection PTZ at pretreatment times 0.5 h.
[d] Numbers of mice protected.
[e] Total numbers of mice tested.

potential GABAergic efficacy of new drugs [47]. The results combining with three closely related protective parameters (CSR, TSR and MR) were simultaneously listed in Table 4. In general, compounds **68**–**70**, **76**, **78**, and **80** displayed modest activity with short durations (0.25 h–1 h). The times to peak effect of these compounds were all at 0.5 h for 50% protection. Notably, compound **55**, in spite of exhibiting excellent profile in the MES model (Tables 1 and 2), together with **57**, and **66** showed complete inactivity in this test (Table 4), while others only showed marginal activity during the same period.

Furthermore, consistent with the results in the *sc*PTZ model, none provided protection of clonic seizures at doses of 300 mg/kg. Additionally, only **69**, **70**, **76**, **78**, and **80** showed modest protection of tonic seizure. However, it appears that except compounds **55**, **57**, **81**, and **82**, all other compounds were found to be effective in *sc*3-MP-induced mortality test with at least 75% protection of mice.

3.2.4. Quantitative evaluation against MES-, scPTZ-, and sc3-MP-induced seizures

The excellent potencies of some selected compounds (**55**, **62**, **64**, **65**, **68**–**71**, and **75**) in aforementioned anticonvulsant activity tests (Tables 1–4) led us to further investigation and quantification of their pharmacological properties in mouse-MES, -PTZ, -3-MP, -Tox, and -formalin tests. The formalin test was used to determine whether active compounds could relieve the neuropathic pain [48], which was one of the most insufferable symptom accompanying seizure firing [49]. Thus, the results of the median effective doses (ED_{50}) and median toxic dose (neurological impairing dose, TD_{50}) combined with quantitative (dose range) testing in mice (ip) were summarized in Table 5. These values were calculated from dose-response curves containing 4–6 dose points (n = 8–10 per dose). Moreover, the protective indices ($PI = TD_{50}/ED_{50}$), which were the measure of the benefit-to-risk ratio for selected compounds, and partial protective profiles of four clinical AEDs: lacosamide, valproic acid, stiripentol, and carbamazepine were also listed in Table 5.

Analysis of these data revealed that when the position of methoxyl shifted from C-2 (**55**, ED_{50} = 39.0 mg/kg, 101.4 μmol/kg) to C-3 (**56**, ED_{50} = 72.9 mg/kg, 189.6 μmol/kg), or to C-4 (**57**, ED_{50} = 70.6 mg/kg, 183.6 μmol/kg) at the cinnamic phenyl moiety, the anticonvulsant activity decreased, suggesting that methoxyl at C-2 position was favored. We also found that one of the principal active parts resided in 3, 4-dimethoxyl phenyl substructure (R = OMe, m = 2) of cinnamate moiety as seen with compound **62** (MES ED_{50} = 58.1 mg/kg, 140.2 μmol/kg), while other dimethoxyl positional isomers, including **58**, **59**, **60**, **61**, and **63**, showed similar but lower protection in the MES test (Table 1). With these two points in mind, we were not surprised to find that 2,3,4-trimethoxyl (**64**, ED_{50} = 33.4 mg/kg, 75.1 μmol/kg), 3,4,5-trimethoxyl (**68**, ED_{50} = 33.2 mg/kg, 74.7 μmol/kg), 2,3,4,5-tetramethoxyl (**69**, ED_{50} = 30.9 mg/kg, 65.1 μmol/kg) and 2,3,4,6-tetramethoxyl (**70**, ED_{50} = 33.9 mg/kg, 71.4 μmol/kg) analogues provided excellent anticonvulsant activity in the MES test, while 2,3,5,6-tetramethoxyl (**71**, ED_{50} = 57.9 mg/kg, 122.0 μmol/kg) showed dramatically lower activity profile. However, this trend was not applicable for 2,3,4,5,6-pentmethoxyl analog (**72**) which gave almost no activity in the MES test (Table 1). The reason for such discrepancy is unclear but is under investigation. Additionally, our data in MES test indicated that the electronic and hydrophobic properties of phenyl substituents might impact on compounds'

Table 4
Anticonvulsant activity: *sc*3-MP test in mice (ip[a], dose of 300 mg/kg).

compd	Pretreatment time[b]				Pretreatment time (0.5 h)[c]		
	0.25 h	0.5 h	1 h	2 h	CSR %	TSR %	MR %
55	0[d]/4[e]	0/4	0/4	0/4	100	100	50
56	1/4	1/4	1/4	0/4	100	75	25
57	0/4	0/4	0/4	0/4	100	100	50
62	0/4	1/4	0/4	0/4	100	75	25
64	0/4	1/4	0/4	0/4	100	75	25
65	1/4	1/4	0/4	0/4	100	75	25
66	0/4	0/4	0/4	0/4	100	75	25
67	0/4	1/4	0/4	0/4	100	75	25
68	0/4	2/4	0/4	0/4	100	75	25
69	0/4	2/4	1/4	0/4	100	50	25
70	1/4	2/4	0/4	0/4	100	50	25
71	1/4	1/4	0/4	0/4	100	75	25
75	0/4	1/4	0/4	0/4	100	75	0
76	0/4	2/4	1/4	0/4	100	50	0
78	1/4	2/4	0/4	0/4	100	50	25
80	1/4	2/4	1/4	0/4	100	50	0
81	0/4	1/4	0/4	0/4	100	75	50
82	0/4	1/4	0/4	0/4	100	75	50

[a] The selected compounds (prepared in 0.5% Tween-80 and 0.9% normal saline) were administered intraperitoneally.
[b] Subcutaneous injection 3-MP (60 mg/kg, prepared in 0.9% normal saline) at four pretreatment times after drug administration: 0.25, 0.5, 1, and 2 h.
[c] The CSR (clonic seizures rate), TSR (tonic seizure rate) and MR (mortality rate) induced by subcutaneous injection 3-MP at pretreatment times 0.5 h.
[d] Numbers of mice protected.
[e] Total numbers of mice tested.

anticonvulsant activity. Electron-donating groups with negative Hammett σ_p value (Supplemental Table 2.) maintained seizure protection, while electron-withdrawing groups with positive Hammett σ_p value at the same positon decreased activity. By and large, the higher value of σ_p and/or π was, the lower pharmacological activity it exhibited. All in all, **55**, **64**, **68**–**70**, and **75** were the most active compounds (ED_{50}: 30–40 mg/kg) in the MES test. Of these compounds, **69** was observed to be the most active (MES ED_{50} = 30.9 mg/kg, 65.1 μmol/kg), and it was 23.10, 3.96, and 1.17 times more potent than valproic acid, stiripentol, and carbamazepine respectively (utilizing ED_{50} as μmol/kg). Despite compound **69** showed lower protection (57.5% potency) in the MES test compared to the lacosamide, its higher safety profile in the rotorod test (TD_{50} > 1000 mg/kg) resulted in 9.0-fold (MES), 64.0-fold (*sc*PTZ), and 18.2-fold (formalin) more beneficial PI values than that of lacosamide.

Additionally, three tetramethoxyl-substituted compounds **69**–**71** and two chloro-substituted compounds **76** and **78** showed good activities in *sc*PTZ test (ED_{50} (mg/kg), [μmol/kg]: **69**, 155.3, 327.3; **70**, 154.5, 325.6; **71**, 149.2, 314.4; **75**, 43.4, 116.5). Compounds **68**–**70** exhibited the most but moderate active in *sc*3-MP test (ED_{50} (mg/kg), [μmol/kg]: **68**, >200, >450.0; **69**, >200, >421.5; **70**, 202.9, 427.6), whereas halogen-substituted compounds (**73**–**84**) lacked the activity in this test. In the formalin test, **69**–**71** and **75** displayed good activity (ED_{50} (mg/kg), [μmol/kg]: **69**, 42.2, 89.0; **70**, 46.3, 97.6; **71**, 50.8, 107.1; **75**, 43.4, 116.5). Moreover, in accordance with the results in Table 1, all selected compounds showed low neurotoxicity with TD_{50} value ~1000 or above (Table 5). In summary, it appeared that compounds **68**–**70** and **75** emerged as promising candidates with significant and broad spectrum of anticonvulsant activity.

3.2.5. Lactate dehydrogenase inhibition

Sada et al. showed that seizures might be controlled by inhibiting LDH of nervous system [12]. As mentioned above, α-asaronol (**3**) had a good LDH inhibitory activity. Thus, it was highly necessary to screen these novel α-asaronol derivatives in the LDH inhibition test.

The LDH inhibitory activities of these **C** series compounds were measured by standard enzyme kinetic experiments on human LDH1 and LDH5 purified isoforms [56]. Enzyme activity was determined by the measurement of the absorbance decrease at various wavelength (Supplemental Table 4), because the optimum detection wavelength of NADH shifted under different solution of synthetic compounds. Fig. 4 showed the inhibitory results of all the synthesized compounds against LDH1 and LDH5. Thereinto, STP served as LDH positive inhibitor while carbamazepine and valproic acid served as negative inhibitor [12]. However, unlike reported results (STP, 0.5 mM with ~30% inhibition ratio) [12], STP at the same dose level showed no activity against both LDH1 and LDH5 in our enzyme catalytic system (Fig. 4, STP_1). When the concentration of STP was increased to 2.0 mM, a smaller boost of LDH1 and LDH5 inhibition ratio (7–10%) was observed (Fig. 4, STP_2). The inconsistency between our test and literature value might be attributable to the different LDH specific activity. Compound **60** and **66** were not tested in this test because of their similar absorption spectrum with NADH (Fig. S140, Supporting Information), which seriously interfered the experimental results. With the exception of **62**, none of monomethoxyl-substituted or bismethoxyl-substituted α-asaronol cinnamate (**55**–**59**, **61**, and **63**) presented in the Fig. 4 showed inhibitory activities against LDH1 and LDH5. And **62** just exhibited marginal inhibitory activity on LDH1, while almost inactivity on LDH5 compared to the STP_2. Trimethoxyl-substituted and tetramethoxyl-substituted α-asaronol cinnamates (**64**, **65**, and **68**–**71**) showed moderate inhibitory activity (8%~27% inhibition) both on LDH1 and LDH5 without marked selectivity. Notably, the 2,4,6-trimethoxyl-substituted derivative **67** was originally shown to be the most effective for inhibition of LDH1 (33% inhibition) and LDH5 (26% inhibition) by UV-VIS spectrophotometry method (Fig. 4), but later it was quickly revised to have a weak inhibition of LDH (~5% inhibition) by mass method (Fig. 5). We thought that the higher molar absorptivity (ε) of **67** might interfere the accuracy of detection in the UV-VIS mode (Fig. S140, Supporting Information). In comparison, compounds **64** and **68**–**70** exhibited good correlations between LDH inhibition (~7%, 10%, 18%, 21% for LDH1 and ~8%, 13%, 15%, 27% for LDH5 respectively) and *in vivo* efficacy (MES ED_{50} = 75.1, 74.7, 65.1 and 71.4 μmol/kg respectively). Furthermore, the LDH inhibitory activity of **68**–**70** were at least 4 times more potent than STP. This unique pharmacological profile of **68**–**70** in LDH inhibitory test suggested they could serve as potential candidates for treatment of refractory epilepsy such as Dravet syndrome, in analogy to STP [57]. Compound **65** and **71** possessed moderate LDH inhibitory activity (Fig. 4), while exhibiting lower *in vivo* efficacy (Table 5) comparing to **68**–**70**. These relatively lower *in vivo* activity exhibited by **65** and **71** could be due to a number of factors that included oral bioavailability, biodistribution, and/or metabolic stability. Curiously, pentamethoxyl-substituted α-asaronol cinnamate (**72**) was inactive at 0.5 mM in the LDH1/LDH5 inhibitory model test. A similar trend with respect to halogen substituted α-asaronol cinnamate (**73**–**84**) and compounds **85**–**87** were also observed for the mean of LDH1/LDH5 inhibitory ratio < 5% and 9% respectively. Perhaps it was because the space steric effect or cloud density of molecule prevented binding with the LDH. In general, the LDH inhibitory profile of **C** series compounds, by and large, agreed with the above animal model results.

Although, evaluation of LDH activity by UV-VIS spectrophotometry method typically had superior simplicity and sensitivity, some substances, due to their having similar absorption wavelength with $NADH^+$, could seriously interfere the detection results. To avoid this kind of interference, we employed another more direct and sensitive method, LC-MS/MS (also termed as multiple

Table 5
Quantitative anticonvulsant data in mice (ip[a], ED_{50}, TD_{50}, and PI values).

Compd	TPE (h)[b]	MES,[c] ED_{50} (mg/kg) [μmol/kg]	*sc*PTZ,[d] ED_{50} (mg/kg) [μmol/kg]	sc3-MP,[e] ED_{50} (mg/kg) [μmol/kg]	Formalin, ED_{50} (mg/kg) [μmol/kg]	Tox,[f] TD_{50} (mg/kg) [μmol/kg]	PI[g]			
							MES	PTZ	3-MP	Formalin
55	0.5	39.0 (18.4–62.4) [101.4]	240.3 (163.5–309.2) [625.1]	>300 [780.4]	112.9 (79.9–156.5) [293.7]	>1000 [2601.3]	>25.6	>4.2	≈3.3	>8.9
56	0.5	72.9 (45.4–98.5) [189.6]	247.1 (168.4–300.1) [642.8]	248.8 (170.4–325.9) [647.2]	>100 [> 260.1]	>1000 [> 2601.3]	>13.7	>4.1	>4.0	>8.9
57	0.5	70.6 (30.6–91.6) [183.6]	243.9 (163.6–285.02) [634.4]	>300 [> 780.4]	121.8 (64.7–191.1) 316.8]	>1000 [> 2601.3]	>14.2	>4.1	≈3.3	>8.2
62	0.5	58.1 (23.6–90.5) [140.2]	173.2 (128.5–274.1) [417.9]	>300 [> 723.9]	93.3 (85.4–211.7) [225.1]	>1000 [> 2412.8]	>17.2	>5.8	≈3.3	>10.7
64	0.5	33.4 (15.5–54.3) [75.1]	202.8 (122.6–292.8) [456.3]	>300 [> 674.9]	78.4 (54.5–98.1) [176.4]	>1000 [> 2249.8]	>29.9	>4.9	≈3.3	>12.8
65	0.5	53.5 (15.0–76.6) [120.4]	211.7 (133.7–274.9) [476.3]	>300 [> 674.9]	>80.0, <100.0 [> 180.0, <225.0]	>1000 [> 2249.8]	>18.7	4.72	≈3.3	≈12.5
66	0.5	73.5 (34.5–95.6) [165.4]	>200; <300 [> 450.0; <674.9]	>300 [> 674.9]	>80.0, <100.0 [> 180.0, <225.0]	>1000 [> 2249.8]	>13.6	≈5	≈3.3	≈12.5
67	0.5	69.8 (31.0–98.9) [157.0]	211.7 (133.7–304.9) [476.3]	>300 [> 674.9]	99.0 (61.0–144.4) [222.7]	>1000 [> 2249.8]	>14.3	>4.7	≈3.3	>10.1
68	0.5	33.2 (12.9–56.0) [74.7]	203.8 (138.4–301.2) [458.5]	>200 [> 450.0]	85.5 (49.0–109.0) [192.4]	988.1 (895.3–1090.6) [2223.0]	29.80	4.9	≈3.3	11.6
69	0.5	30.9 (10.1–52.3) [65.1]	155.3 (80.1–262.2) [327.3]	>200 [> 421.5]	42.2 (21.8–94.0) [89.0]	>1000 [> 2107.4]	>32.4	>6.4	>5.3	23.7
70	0.5	33.9 (14.3–72.5) [71.4]	154.5 (87.2–256.2) [325.6]	202.9 (146.7–305.0) [427.6]	46.3 (28.2–106.2) [97.6]	936.5 (850.3–1031.5) [1973.6]	27.7	6.1	4.6	21.6
71	0.5	57.9 (25.0–92.4) [122.0]	149.2 (85.1–234.2) [314.4]	>300 [> 632.2]	50.8 (31.8–109.0) [107.1]	>1000 [> 2107.4]	>17.3	6.7	≈5	19.7
75	0.5	34.6 (17.3–54.4) [92.9]	167.2 (83.5–178.2) [449.0]	>300 [> 805.6]	43.4 (30.2–104.7) [116.5]	>1000 [> 2685.4]	>28.9	>5.9	≈5	23.0
76	0.5	58.7 (50.6–92.3) [151.0]	149.8 (45.4–298.1) [385.2]	248.3 (170.4–295.9) [638.6]	101.1 (45.0–145.9) [260.0]	>1000 [> 2571.8]	>17.0	>6.7	>4.0	>9.9
78	0.5	53.4 (46.1–91.3) [137.3]	141.7 (37.3–224.4) [364.4]	>200 [> 514.4]	107.9 (71.4–157.3) [277.5]	>1000 [> 2571.8]	>18.7	>7.1	>6.2	>9.3
80	0.5	75.7 (35.1–98.7) [174.7]	>200 [> 461.6]	215.4 (166.1–295.0) [497.1]	112.8 (76.8–157.6) [260.3]	>1000 [> 2307.9]	>13.2	≈5	>4.6	>8.9
81	0.5	61.8 (25.7–95.0) [142.6]	193.2 (114.4–274.5) [445.9]	>300 [> 692.4]	105.3 (62.4–153.0) [243.0]	1059.0 (864.6–1270.4) [2444.0]	17.1	5.5	≈5	10.1
82	0.5	85.7 (50.5–98.8) [202.9]	>200 [> 473.5]	>300 [> 710.2]	122.9 (71.4–178.3) [291.0]	>1000 [> 2367.4]	>11.7	≈5	≈3.3	>8.1
LCM	0.5	9.4 [h] (8.1–10.7) [37.6]	>500 [h] [> 1997.7]		15 [n] [0.1]	33.7 [h] (28.8–38.7) [134.6]	3.6	<0.1		2.2
VPA[i]	0.5	216.9 (207.5–226.3) [1504]	239.4 (209.2.274.1) [1660.1]			372.9 (356.0–389.8) [2585.8]	1.7	1.6		
STP[j]	0.5	277 ± 12.1 [l] [1182]	115 (99.14–133.39) [490][m]			161.7 (146.2–256.2) [690.1]	0.58	1.41		
CBZ[k]	0.5	18.07 (13.66–34.88) [76.5]	>100 [> 423.2]			71.6 (45.9–135) [303.0]	3.96	<0.7		
3	0.5	62.02 (41.72–87.6) [276.6]				689.34 (556.53–818.17) [3073.8]	11.1			

[a] The compounds were administered intraperitoneally to adult male KM mice. ED_{50} and TD_{50} values are in mg/kg (n = 6). Numbers in parentheses are 95% confidence intervals determined by Probit analysis. A dash indicates not tested.
[b] TPE: time of peak effect.
[c] MES = maximal electroshock seizure test.
[d] PTZ = pentylenetetrazole seizure test.
[e] 3-MP = 3-mercaptopropionic acid.
[f] Tox = neurological toxicity. TD_{50} value determined from the rotorod test.
[g] PI = protective indices (TD_{50}/ED_{50}).
[h] LCM = lacosamide, reference [50].
[i] VPA = valproic acid, reference [51].
[j] STP = stiripentol.
[k] CBZ = carbamazepine, reference [52].
[l] reference [53].
[m] reference [54].
[n] reference [55].

reaction monitoring (MRM)), to evaluate some representative compounds (**55**, **67**–**70**, and **75**) and reference drug (STP) for LDH inhibitory activity. The main advantage of MRM was the elimination of the effects of some uncaring substances in a complex mixture [58]. In this test, the conversion of pyruvate to lactate was selected again to evaluate the effect of LDH inhibition by monitoring the ion transition for lactic acid (m/z 89.0 → m/z 43.0) under negative ion mode (Fig. S138, Supporting Information). The limit of detection (S/N = 5) and lower limit of quantification (S/N = 10) for lactic acid were 0.25 and 0.5 ng/mL (Fig. S139, Supporting Information), respectively, showing the higher sensitivity of the MRM method. As shown in Fig. 5, the results of LDH1 and LDH5 inhibition among compounds **68**–**70** were consistent with the pattern observed from UV-VIS spectrophotometry method (Fig. 4).

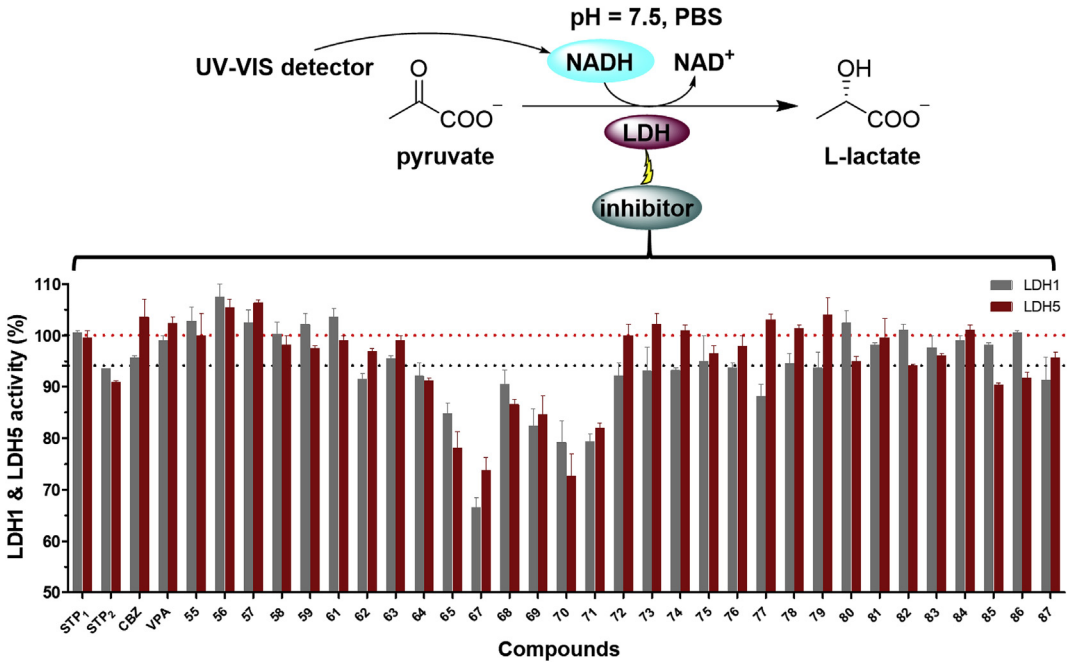


Fig. 4. Inhibition of human LDH1 and LDH5 activity by selected C series compounds (0.5 mM), measured by enzyme kinetic experiments. Values are reported as the mean (n = 2 to 6 experiments in each compound.) with SEM. LDH activity in the direction of pyruvate-to-lactate were measured by using 1.0 mM pyruvate. The positive control agent (STP) were detected in two concentrations (STP_1: 0.5 mM, STP_2: 2.0 mM) and the active level (~90%) of STP_2 is indicated as a horizontal dotted line (black), while the two negative control agents (CBZ and VPA: 0.5 mM) active level (~100%) is indicated as a horizontal dotted line (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

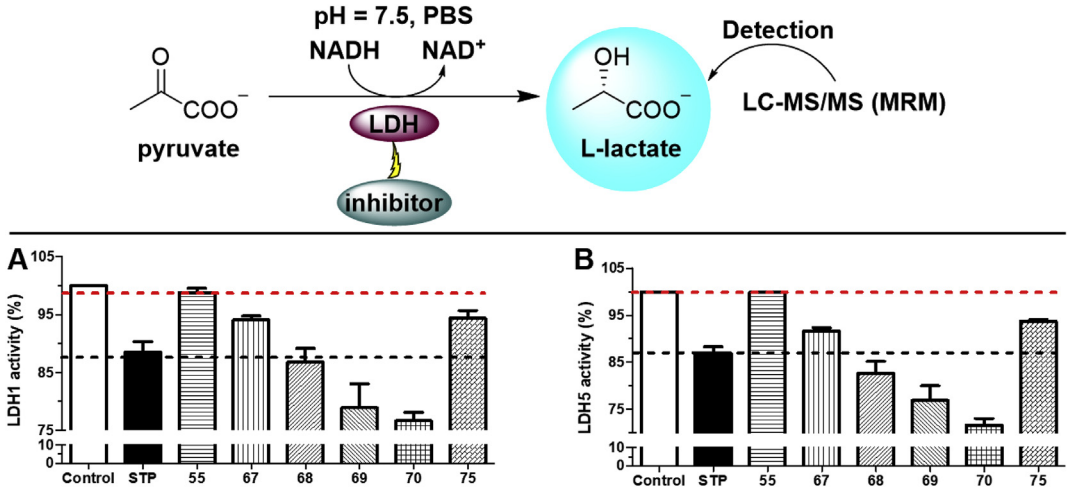


Fig. 5. Inhibition of human LDH1 (A) and LDH5 (B) activity by compound **67**–**70** (0.5 mM) and STP (2.0 mM), measured by MRM method. Values are reported as the mean (n = 4 to 6 experiments in each compound.) with SEM. LDH activity in the direction of pyruvate-to-lactate were measured by using 1.0 mM pyruvate. The positive control agent (STP) is indicated as a horizontal dotted line (black), while the blank control (without adding LDH) is indicated as a horizontal dotted line (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

However, compound **67**, in MRM test, exhibited only weak inhibitory activity for both LDH1 and LDH5, which agreed with its *in vivo* moderate effect of the MES test (Tables 1 and 5). Additionally, compounds **55** and **75** showed ~2% and ~5% inhibition for LDH1 and LDH5 respectively (Fig. 5), which were consistent with the results depicted in Fig. 4. In this regard, MRM method for detecting LDH inhibition was more objective and credible than that of spectrophotometry method in some cases.

On the basis of good LDH inhibitory activities of tri- and tetramethoxyl-substituted α-asaronol cinnamate, compounds **65**, **68** and **69** were selected for a complete enzyme kinetic analysis to verify their types of inhibition versus pyruvate. From secondary and Lineweaver-Burk plots (Fig. 6), compounds **65**, **68** and **69** were all found to be noncompetitive inhibitors of LDH1 and LDH5 in the conversion of pyruvate to lactate catalyzed by these two enzymes, which suggested that these kind of inhibitors bound to the LDHs at the different sites with pyruvate. In addition, their K_i values were also determined from Lineweaver-Burk plots and reported in Table 6.

3.2.6. $GABA_A$ receptor modulating activity

The γ-aminobutyric acid receptor type A ($GABA_A$ receptor) is a

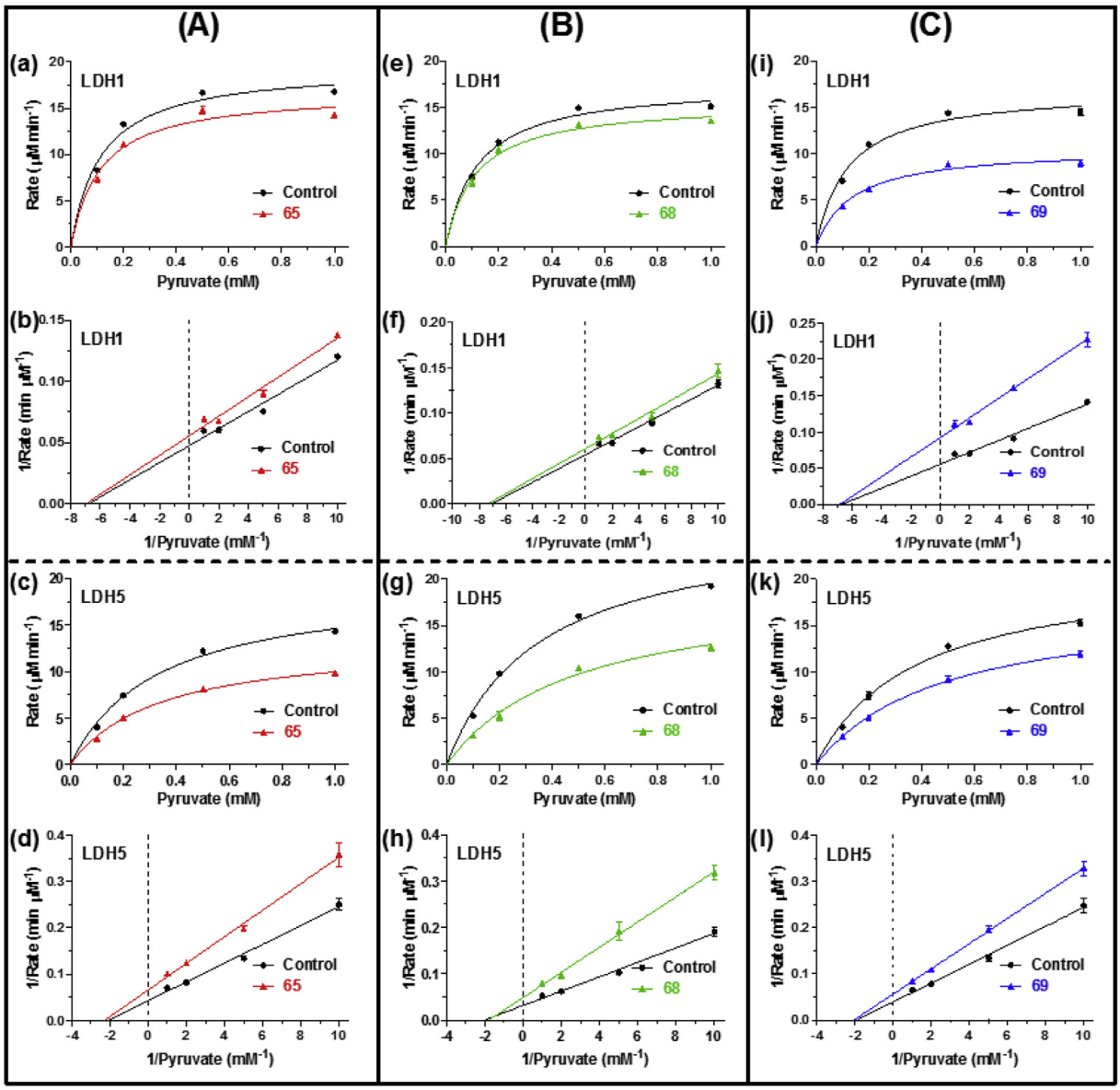


Fig. 6. Secondary plots (SP) and Lineweaver-Burk plots (LBP) of the effects of representative compounds (**65**, **68** and **69**) of two human LDHs (LDH1 and LDH5) activities. **(A)** compound **65**, (a) for LDH1, SP; (b) for LDH1, LBP; (c) for LDH5, SP; (d) for LDH5, LBP. **(B)** compound **68**, (e) for LDH1, SP; (f) for LDH1, LBP; (g) for LDH5, SP; (h) for LDH5, LBP. **(C)** compound **69**, (i) for LDH1, SP; (j) for LDH1, LBP; (k) for LDH5, SP; (l) for LDH5, LBP. Control: enzyme activity without inhibitor. Inhibitors (**65**, **68** and **69**) concentration: 1.0 mM. These plots indicate non-competitive inhibition for both human LDH1 and LDH5. The data in each point were obtained from n = 3–6. Data are mean ± SEM.

Table 6
LDH1 and LDH5 inhibition constant (K_i) of compounds **65**, **68** and **69**[a].

Entry	Comd	LDH1 (K_i, μM)	LDH5 (K_i, μM)
1	**65**	11.98 ± 1.21	3.52 ± 0.24
2	**68**	14.11 ± 1.52	3.35 ± 0.31
3	**69**	1.77 ± 0.16	4.84 ± 0.51

[a] K_i values for each single inhibitor were determined using Lineweaver-Burk plots. Values are reported as the mean (n = 3 to 6 experiments in each compound.) with SD. 100 mM sodium phosphate buffer (pH 7.5), 0.2 mM NADH, and 0.1 mM–1 mM sodium pyruvate.

ligand-gated chloride channel associated with a spectrum of refractory seizures types, such as febrile seizures (FS), childhood absence epilepsy (CAE) and Dravet syndrome etc [59–61]. A number of antiepileptic drugs have agonistic effects on $GABA_A$ receptors. Hence, modulation of $GABA_A$ receptors have been postulated to play important roles in the etiology of epilepsy. Among all known subtypes of the $GABA_A$ receptor, the $\alpha1\beta2\gamma2$ is the most abundant subtype that accounts for about 43% [62] and is localized in many regions of the brain. It was therefore important to evaluate effects of representative compound **70** on the $\alpha1\beta2\gamma2$ receptor in the human HEK-293T cell line. HEK-293T cells in culture for 12–24 h were studied with whole-cell patch-clamp recording technique. Application of 3.3–300 μM of stiripentol and compound **70** evoked $GABA_A$ currents at 3 μM (Fig. 7). Stiripentol strongly enhanced the response of $\alpha1\beta2\gamma2$ receptors to 3 μM GABA, with an average EC_{50} of 46.0 ± 9.1 μM and maximum potentiation of 310.5 ± 22.5% (n = 4) (Fig. 7A and B). Compound **70** was also a very potent modulator with similar average EC_{50} of 46.3 ± 7.3 μM (n = 4) (Fig. 7C and D). Notably, compound **70** had higher efficacy than stiripentol, with an average maximum potentiation of 355.7 ± 20.4% (n = 4).

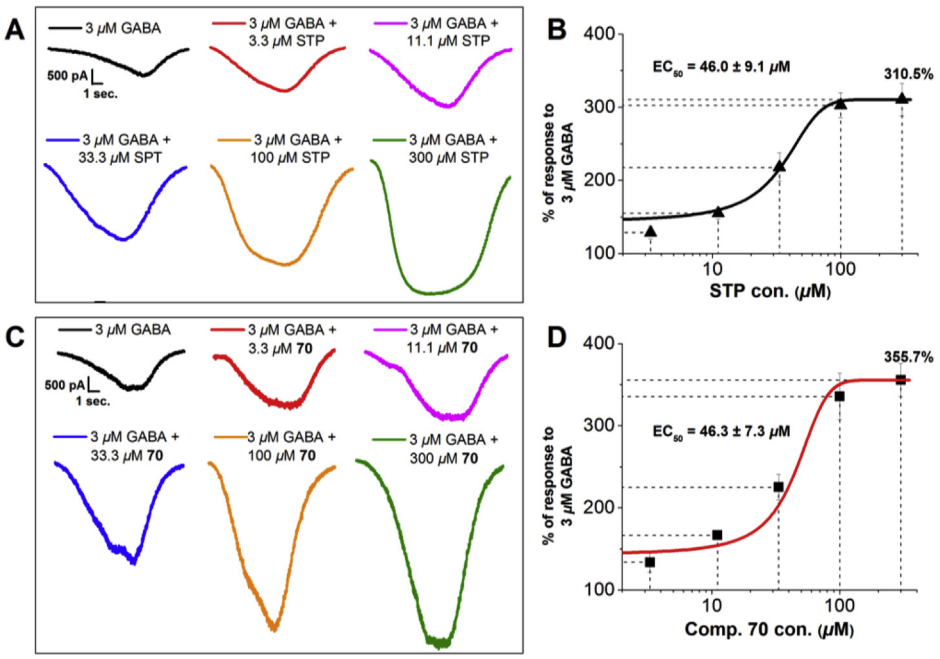


Fig. 7. Stiripentol (STP) and compound **70** increases the response to GABA in a concentration-dependent manner. HEK-293T cells were transfected with the α1 subunit along with β2 and γ2 subunits. The peak current response to 11 s applications of GABA alone or with stiripentol and compound **70** respectively were measured in cells voltage-clamped at −50 mV. The GABA concentration used represented EC_{10}~EC_{20} for $\alpha1\beta2\gamma2$ isoform. (A) and (C) Representative traces in response to GABA alone and GABA (3 μM) with increasing concentrations of stiripentol and compound **70** from 3.3 to 300 μM respectively. The amplitude of the current were increased by stiripentol and compound **70** in a concentration-dependent manner. Concentration-response relationships were constructed by measuring the peak current with concentration of stiripentol (B) and **70** (D) respectively. Symbols and bars represent the mean ± SEM. The EC_{50} of stiripentol for potentiation of the $\alpha1\beta2\gamma2$ isoform was 46.0 ± 9.1 μM (n = 4), while that for compound **70** was 46.3 ± 7.3 μM (n = 4).

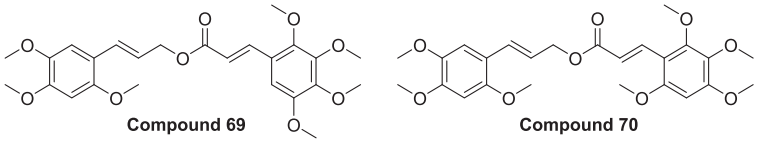
3.3. Pharmacokinetics

Following *in vitro* studies, two compounds (**69** and **70**) were selected for further studies. Their PK data (rats) were summarized in Table 7. With a 20 mg/kg intravenous (iv.) dose, **69** and **70** formulated in 2.5% poloxamer physiological saline displayed AUC of 176.3 ± 16.2 and 180.2 ± 18.2 mg·min/L in rats respectively. They possessed high *in vivo* clearance (0.16 ± 0.01 L/min/kg for **69** and 0.14 ± 0.006 L/min/kg for **70**), middle and low volume of distribution (1.98 ± 0.28 L/kg for **69** and 2.14 ± 0.28 L/kg for **70**), and short half-life ($t_{1/2}$ = 19.72 ± 4.2 min for **69** and $t_{1/2}$ = 22.80 ± 3.2 min for **70**) in rats. In addition, compound **69** and **70** dosed orally at 20, 50 and 100 mg/kg were also listed in Table 7 (n = 3). These data displayed a dose-dependent trend. For example, compound **69** exhibited rapid absorption (T_{max} = 35.56 ± 5.5 min), high C_{max} (1.89 ± 0.58 μg/mL), and AUC (138.4 ± 10.4 mg·min/L) as well as relatively moderate bioavailability (F = 31.4%) in rats after oral administration of 50 mg/kg. Compound **70** had similar but slightly higher T_{max} (38.66 ± 4.3 min), C_{max} (2.04 ± 0.24 μg/mL), AUC (156.6 ± 21.32 mg·min/L) and bioavailability (F = 34.8%) at the same oral dose.

3.4. Tissue distribution

On the basis of this promising profile, the distribution of **70** in various tissues such as heart, liver, brain, spleen, lungs and kidney was analyzed with 50 mg/kg oral dose in rats. As shown in Fig. 8, the C_{max} reached the highest value in organs such as brain, heart,

Table 7
Pharmacokinetic parameters of selected compound **69** and **70** in rat (n = 3).



Dose (mg/kg)	Compound **69**				Compound **70**			
	20 (Iv.)	20 (Po.)	50 (Po.)	100 (Po.)	20 (Iv.)	20 (Po.)	50 (Po.)	100 (Po.)
$T_{1/2}$ (min)	19.72 ± 4.2	55.83 ± 5.6	58.48 ± 6.4	56.96 ± 7.2	22.80 ± 3.2	57.88 ± 8.4	59.24 ± 7.8	58.48 ± 8.9
T_{max} (min)	N/A	35.25 ± 4.5	35.56 ± 5.5	36.16 ± 5.5	N/A	37.14 ± 6.5	38.66 ± 4.3	38.78 ± 5.9
CL (L/min/kg)	0.16 ± 0.01	0.39 ± 0.06	0.48 ± 0.06	0.56 ± 0.08	0.14 ± 0.006	0.38 ± 0.04	0.45 ± 0.06	0.52 ± 0.16
V_{ss} (L/kg)	1.98 ± 0.28	8.94 ± 0.47	9.26 ± 0.67	9.98 ± 0.49	2.14 ± 0.28	9.86 ± 0.68	10.17 ± 0.84	11.26 ± 0.87
C_{max} (μg/mL)	6.22 ± 0.76	0.88 ± 0.33	1.89 ± 0.58	3.77 ± 0.86	6.48 ± 0.46	0.98 ± 0.42	2.04 ± 0.24	3.98 ± 0.22
AUC (mg·min/L)	176.3 ± 16.2	72.80 ± 4.81	138.4 ± 10.4	306.4 ± 46.2	180.2 ± 18.2	78.22 ± 5.14	156.6 ± 21.32	328.6 ± 54.48
Oral bioavailability (% F)	N/A	41.3	31.4	34.8	N/A	43.4	34.8	36.5

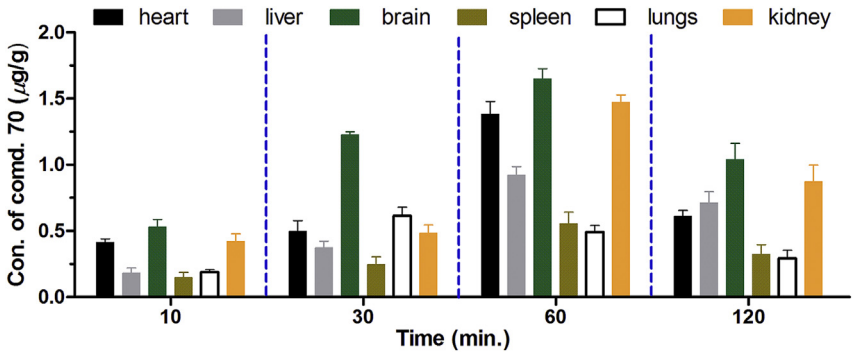


Fig. 8. Tissue distribution of compound **70** in SD rat heart, liver, brain, spleen, lungs, and kidney after a 50 mg/kg oral dose (n = 3).

and kidney at 60 min after oral administration of **70** and were about 2 times higher in the spleen and lungs. That means compound **70** could easily permeate through blood brain barrier to the brain and stayed there for a period of time.

3.5. Metabolite identification

Metabolite identification work was undertaken to establish the metabolic activity exerted upon compound **70** (Fig. 9 and Table 8). In the study, six metabolites were detected in the urine and plasma of SD rats dosed with **70**. These metabolites were named as M1 through to M6 based on their eluting time under HPLC conditions. Among the six metabolites, M6 was identified as compound **22**, M3 was identified as compound **3**, M5 was identified as compound **18**, M4 was identified as *cis*-isomer of compound **18**, and M2 and M1 were identified as glucuronic acid (GluA) ester of compound **22** and compound **18** respectively. Location of the GluA groups was established through mass spectrometry fragmentation patterns. M1 to M6 were detected both in urine and plasma samples.

3.6. Molecular modeling

Based on the inhibition results, we selected compound **70**, one of our best LDH inhibitors in our study, as a ligand example. To explore the binding modes of **70** with the active sites of LDH, molecular docking was performed using Surflex-Dock (SYBYL-X 2.0, Tripos Inc. St. Louis, USA) which was used to explore the probable binding conformation and results shown in docking scores. The crystal structure of LDH and its complexes with small molecular inhibitors were available from Protein Databank (PDB code: 4JNK [63]).

As shown in Figs. 10 and 11, **70** and ligand compound 22 ((2*R*)-2-{[5-cyano-4-(3,4-dichlorophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl]sulfanyl}2-*N*-(4-sulfamoylphenyl) propanamide) [63] of LDH could nearly overlap in the binding model. The compound **70** bound to the protein near several conserved residues involved in the catalytic processing of LDH substrates (Arg−105, Tyr−238 and Thr−247), which the Arg−105 was the key mobile residue in the enzyme's catalytic cycle [64]. The presence of **70** resulted in a relatively 'open' conformation of this region in which a fourth conserved residue required for enzyme catalysis (Arg−105) was positioned well-removed from the substrate binding site. Analysis of **70**'s binding mode in the active binding site demonstrated that the docking mode of the **70** was similar to the ligand compound with the same H-bond between Arg−105 and Thr−247. The methoxyl group of compound **70** in the α-asaronol moiety formed a hydrogen bond with NH_2 group of the LDH Arg−105 residue (−O···HN, 1.99 Å). And the methoxyl group of cinnamic acid moiety formed another hydrogen bond with NH group of LDH Thr−247 residue (−O···HN, 2.68 Å). In addition, the carbonyl present in **70** was located within H-bond distance of the LDH Tyr−238 backbone amide OH group (−O···HO, 2.53 Å). These H-bond interactions at the positions might favor the binding activity and played an important role in the inhibitory potency of **70** against LDH according to the docking results. The Surflex dock scores of **70** and ligand compound were 11.564 and 9.846 respectively, which could

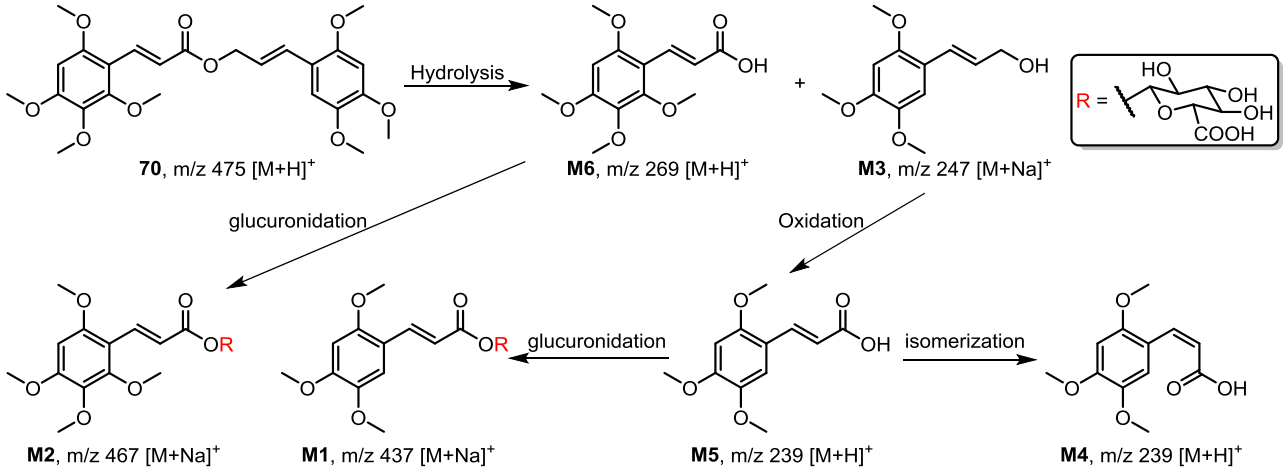


Fig. 9. Metabolic pathways of compound **70** in SD rats urine and plasma.

Table 8
Identified metabolites of compound 70 in SD rats urine and plasma (MS).

Peak ID	Mass shift	Found *m/z*	Biotransformation	RT (min)	Relative MS abundance	
					Urine	plasma
70	+1	475	parent	40.6	0.3×10^5	0.2×10^5
M1	+23	437	glucuronidation	50.1	4.9×10^5	0.1×10^5
M2	+23	467	glucuronidation	55.2	1.7×10^6	0.5×10^5
M3	+23	247	hydrolysis	67.5	0.6×10^5	0.5×10^5
M4	+1	239	isomerization	78.7	2.2×10^6	1.1×10^6
M5	+1	239	oxidation	79.8	0.9×10^7	2.9×10^7
M6	+1	269	hydrolysis	81.4	0.7×10^5	0.2×10^5

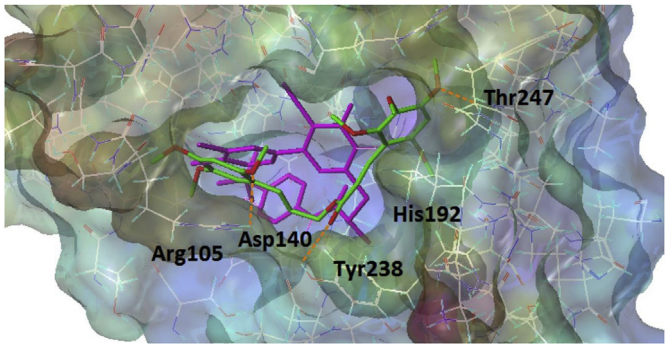


Fig. 10. Docking confirmation of compound **70** (green) and ligand compound (magenta, compound 22 in ref. [63]) with LDH. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

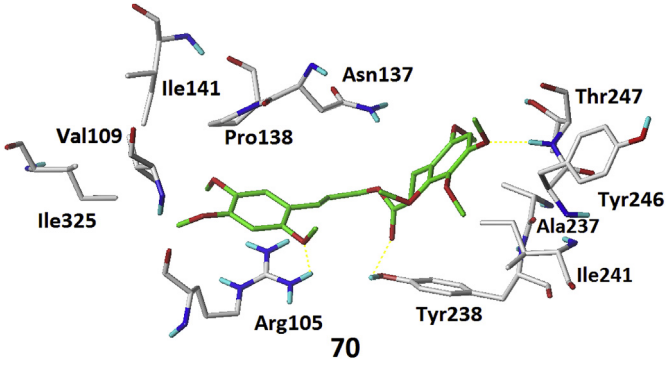


Fig. 11. Docking confirmation of compound **70** with LDH-A (Resolution: 1.9 Å, PDB code: 4JNK). Selected protein residues are shown. Hydrogen bonding interactions with the protein are indicated as dashed lines.

be inferred that the rational of the program, and the docking results were reliable.

4. Conclusions

In light of *Polygala tenuifolia-Acori Tatarinowii* herbal pair and CTCMMC strategy, we have synthesized 33 novel substituted cinnamic α-asaronol esters and analogues in this study. The structures of representative compounds **65** and **78** have been established by X-ray crystallography. Additionally, animal tests revealed that compounds **69**, **70**, and **75** possessed a better anticonvulsant activity with less neurotoxicity in comparison to that of previous lead **68**, parent compounds (**3** and **20**) and some currently known AEDs such as STP. Compound **69**–**71**, and **75** significantly attenuated neuropathic pain in the mice formalin model. Especially, compound **68**–**70** showed better inhibitory activity against LDH than that of STP. Among them, representative compound **70** exhibited good modulatory activity at human $\alpha1\beta2\gamma2$ $GABA_A$ receptors (EC_{50} 46.3 ± 7.3 μM). Thus, some of these compounds could be potentially developed as novel AEDs, especially for some of refractory epilepsies such as Dravet syndrome. In the meanwhile, further mechanistic studies concerning function of substituted cinnamic α-asaronol esters are underway in which **68**–**70** and **75** serve as important test substrates.

More importantly, the above studies once again exemplified the effectiveness and rationality of CTCMMC strategy, which not only provided a more advanced point of entry for further studies on TCM, but also allowed rapid access to design simpler structures with comparable or superior activities to that of their parent TCM.

5. Experimental section

5.1. Chemistry

5.1.1. General methods

Reactions were run using commercially available starting materials without further purification. All solvents were analytical grade, and anhydrous reactions were performed in oven-dried glassware under an atmosphere of nitrogen. The nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on Varian Gemini 2000 (600 MHz) or Bruker (500 MHz) in DMSO-d_6 or $CDCl_3$ using TMS as an internal standard. The chemical shifts δ were expressed in parts per million (ppm). The following abbreviations were used for interpreting the spectra: s, singlet; d, doublet; t, triplet; q, quadruplet; quint, quintuplet; sext, sextuplet; m, multiplet; dd, doublet of doublets; br, broad singlet. Mass spectra were obtained with Agilent 1200 series HPLC-6520 Q-TOF. Reactions were monitored by analytical thin-layer chromatography (TLC) plates (Merck 0.2 mm precoated silica gel aluminum sheets (60 F-254)) and analyzed with 254 nm UV light. The mixtures were purified by flash column chromatography using silica gel (Qingdao Haiyang Chemical Co., Ltd. 200–300 mesh). Evaporation of solvents was carried out under reduced pressure at a temperature below 50 °C. After column chromatography, appropriate fractions were pooled, evaporated, and dried at high vacuum for at least 4 h to give the desired products in high purity. The purity of the target compounds was determined by HPLC analysis, using an Agilent 1100 liquid chromatograph (UV detection at 254 or 280 nm) and a C_{18} column (250 mm × 4.6 mm, 5 μm, Zorbax). The mobile phase was a MeOH/H_2O (80/20) in 25 min; the flow rate was 0.6 mL/min. Infrared spectra were recorded with a Thermo infrared spectrometer (Nicolet 5700). Melting points were determined in open capillary tubes using a Shanghai Yice melting point apparatus (WRS-1B) and were uncorrected. All compounds tested for biological activity showed ≥95% purity by HPLC analysis (detection at 254 nm) and NMR data.

*5.1.2. (E)-3-(2,4,5-Trimethoxyphenyl)prop-2-en-1-ol (**3**)*

To a stirred solution of methyl (*E*)-3-(2,4,5-trimethoxyphenyl) acrylate **(38)** (100 g, 0.396 mol) in anhydrous THF (800 mL) at −60 °C was added, over a 30 min period a solution of DIBAL-H (500 mL of a 1.5 M solution in hexane, 7.25 mol) in anhydrous THF (1000 mL). The reaction mixture was warmed at −45 °C and stirred for an additional 2 h. Then the reaction was quenched with saturated NH_4Cl solution and the mixture was stirred at room temperature for 30 min. The resulting precipitated inorganic salts were removed by filtration and washed with EtOAc. The aqueous layer was extracted with EtOAc (3 × 200 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 100:1−5:1) to afford 80.8 g of **3** as a light yellow solid. Yield: 91.0%. Mp 65.1−65.6 °C. TLC: R_f = 0.42 (1:1 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.97 (s, 1H), 6.86 (d, J = 16.0 Hz, 1H), 6.48 (s, 1H), 6.24 (dt, J = 6.1, 15.9 Hz, 1H), 4.30 (d, J = 6.0 Hz, 2H), 3.88 (s, 3H), 3.85 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 151.45, 149.60, 143.29, 126.96, 125.96, 117.34, 109.86, 97.49, 56.66, 56.51, 56.13. IR (KBr) 3345, 3257, 3000, 2935, 2857, 2826, 1613, 1520, 1465, 1454, 1404, 1335, 1318, 1286, 1268, 1210, 1125, 1094, 1030, 968, 869, 859, 813. HRMS (+ESI) 247.0941 $[M + Na]^+$ (calcd. for $C_{12}H_{16}O_4Na^+$ 247.0944).

*5.1.3. 3-(2,4,5-Trimethoxyphenyl)propan-1-ol (**4**)*

To an EtOH solution (15 mL) of compound **3** (1.12 g, 5.0 mmol) in a glass bottle was added Pd-C (Pd content 5%, wet, 0.20 g). The mixture was stirred at room temperature under H_2 (1 atm) for 20 h. The catalyst was filtered with the aid of a Celite pad, and the filtrate was concentrated under vacuum to obtain **4** (1.08 g) as a white solid, which could be used without further purification. Yield: 96%. Mp 44.5−44.8 °C. TLC: R_f = 0.42 (1:1 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.68 (s, 1H), 6.51 (s, 1H), 3.86 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H),3.56 (t, J = 3.8 Hz, 2H), 2.65 (t, J = 4.8 Hz, 2H), 1.86−1.74 (m, 2H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 151.40, 143.13, 121.30, 114.07, 97.67, 56.63, 56.60, 56.27, 33.28, 25.37. HRMS (+ESI) 227.1275 $[M + H]^+$, 249.1097 $[M + Na]^+$ (calcd. for $C_{12}H_{19}O_4^+$ 227.1278 and $C_{12}H_{18}O_4Na^+$ 249.1097).

*5.1.4. (E)-3-(2,4,5-Trimethoxyphenyl)prop-2-en-1-amine (**5**)*

To an EtOH solution (70 mL) of compound **39** (2.0 g, 5.6 mmol) in a glass bottle was added hydrazine hydrate (con. 80%, 1 g, 15.8 mmol). After the reaction mixture was stirred at reflux temperature for 2 h, the EtOH was evaporated. The residue was dispersed in the EtOAc (50 mL) and undissolved substance was filtered out. The filtrate was added water (50 mL) and 10% HCl solution until pH 3 was reached. The aqueous phase was removed and organic phase was dried on Na_2SO_4, then concentrated *in vacuo* to afford crude compound **5**, which was purified by flash chromatography (SiO_2; 100:0−10:1 DCM/MeOH, v/v) to give a faint yellow clear oil 1.07 g. Yield: 84.9%. TLC: R_f = 0.46 (10:1 DCM/MeOH). HRMS (+ESI) 207.1030 $[M - NH_3]^+$, (calcd. for $C_{12}H_{14}O_3^+$ 207.1016).

*5.1.5. 3-(2,4,5-Trimethoxyphenyl)propan-1-amine (**6**)*

To an EtOH solution (15 mL) of compound **5** (1.12 g, 5.0 mmol) in a glass bottle was added Pd-C (Pd content 5%, wet, 0.20 g). The mixture was stirred at room temperature under H_2 (1 atm) for 14 h. The catalyst was filtered with the aid of a Celite pad, and the filtrate was concentrated under vacuum to obtain **6** (1.06 g) as a colorless oil, which can be used in the next step without further purification. Yield: 96%. TLC: R_f = 0.52 (5:1 DCM/MeOH). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.86 (s, 1H), 6.53 (s, 1H), 6.08 (ddd, J = 5.6, 10.3, 17.2 Hz, 1H), 5.24 (dt, J = 1.6, 17.2 Hz, 1H), 5.11 (dt, J = 1.5, 10.3 Hz, 1H), 4.80 (d, J = 5.5 Hz, 1H), 3.89 (s, 1H), 3.88 (s, 3H), 3.86 (s, 1H), 3.83 (s, 3H), 3.83 (s, 4H). HRMS (+ESI) 226.1427 $[M + H]^+$ (calcd. for $C_{12}H_{20}NO_3^+$ 226.1438).

*5.1.6. (E)-2,6-Dimethoxycinnamic acid (**13**)*

Step 1. The intermediate of methyl (*E*)-3-(2,6-dimethoxyphenyl) acrylate was prepared using the same conditions as those used for the preparation of **38** except that 2,6-dimethoxybenzaldehyde **40** (3.3 g, 20.0 mmol) was used in place of 2,4,5-trimethoxybenzaldehyde **37**. White solid. Yield: 3.95 g, 89.0%. Mp 75.5−75.8 °C (lit [65]. mp 89−90 °C). TLC: R_f = 0.75 (1:2 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.14 (d, J = 16.3 Hz, 1H), 7.25 (t, J = 8.4 Hz, 1H), 6.89 (d, J = 16.4 Hz, 1H), 6.55 (d, J = 8.4 Hz, 2H), 3.87 (s, 6H), 3.79 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 169.09, 160.09, 135.71, 131.32, 120.31, 112.25, 103.73, 55.82, 51.51. HRMS (+ESI) 223.0965 $[M + H]^+$, 245.0788 $[M + Na]^+$ (calcd. for $C_{12}H_{15}O_4^+$ 223.0965 and $C_{12}H_{14}O_4Na^+$ 245.0784).

Step 2. Following a procedure similar to that described for the preparation of compound **10** (step 2) except that methyl (*E*)-3-(2,6-dimethoxyphenyl)acrylate (2.4 g, 10.8 mmol) was used in place of 2,3,4-trimethoxyphenyl formate, the title compound **13** was obtained as a white solid (2.2 g). Yield: 97.8%. Mp 135.8−136.0 °C (lit [66]. mp 79.5−81.4 °C). TLC: R_f = 0.15 (1:4 EtOAc/hexanes). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (d, J = 16.3 Hz, 1H), 7.34 (t, J = 8.4 Hz, 1H), 6.75−6.69 (m, 3H), 3.85 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.93, 159.47, 134.25, 131.68, 121.48, 111.05, 104.15, 55.93. HRMS (-ESI) 207.0661 $[M - H]^+$ (calcd. for $C_{11}H_{11}O_4^+$ 207.0663).

*5.1.7. (E)-2,3,6-Trimethoxycinnamic acid (**17**)*

Step 1. The intermediate of methyl (*E*)-3-(2,3,6-trimethoxyphenyl)acrylate was prepared using the same conditions as those used for the preparation of **38** except that 2,3,6-trimethoxybenzaldehyde **42** (3.9 g, 20.0 mmol) was used in place of 2,4,5-trimethoxybenzaldehyde **37**. Light yellow oil. Yield: 4.0 g, 80.0%. Mp 50.9−51.1 °C. TLC: R_f = 0.62 (1:5 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.01 (d, J = 16.3 Hz, 1H), 6.94 (d, J = 16.4 Hz, 1H), 6.88 (d, J = 9.0 Hz, 1H), 6.60 (d, J = 9.0 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 6H), 3.80 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 168.81, 153.75, 149.88, 147.13, 135.94, 121.88, 118.14, 114.57, 105.89, 61.01, 56.63, 56.01, 51.67. HRMS (+ESI) 253.1070 $[M + H]^+$, 275.0895 $[M + Na]^+$ (calcd. for $C_{13}H_{17}O_5^+$ 253.1071 and $C_{13}H_{16}O_5Na^+$ 275.0890).

Step 2. Following a procedure similar to that described for the preparation of compound **45** (step 2) except that methyl (*E*)-3-(2,3,6-trimethoxyphenyl)acrylate (3.0 g, 11.9 mmol) was used in place of 2,3,4-trimethoxyphenyl formate, the title compound **17** was obtained as a white solid (2.4 g). Yield: 85.7%. Mp 182.4−182.7 °C. TLC: R_f = 0.10 (1:5 EtOAc/hexanes). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.80 (d, J = 16.3 Hz, 1H), 7.09 (d, J = 9.1 Hz, 1H), 6.77 (t, J = 13.0 Hz, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.44, 152.92, 148.74, 146.53, 134.81, 122.36, 116.66, 115.21, 106.45, 60.37, 56.26, 55.98. HRMS (-ESI) 237.0765 $[M - H]^+$ (calcd. for $C_{12}H_{13}O_5^+$ 237.0768).

*5.1.8. (E)-2,4,6-Trimethoxycinnamic acid (**19**)*

Step 1. The intermediate of methyl (*E*)-3-(2,4,6-trimethoxyphenyl)acrylate was prepared using the same conditions as those used for the preparation of **38** except that 2,4,6-trimethoxybenzaldehyde **43** (3.9 g, 20.0 mmol) was used in place of 2,4,5-trimethoxybenzaldehyde **37**. White solid. Yield: 3.3 g, 66.0%. Mp 136.1−136.5 °C (lit [65]. mp 138−139 °C). TLC: R_f = 0.70 (1:2 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.08 (d, J = 16.2 Hz, 1H), 6.75 (d, J = 16.3 Hz, 1H), 6.10 (s, 2H), 3.86 (s, 6H), 3.83 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 169.54, 162.89, 161.38, 135.79, 117.23, 105.91, 90.52, 55.81, 55.48, 51.43. HRMS (+ESI) 253.1071 $[M + H]^+$, 275.0893 $[M + Na]^+$ (calcd.

for $C_{13}H_{17}O_5^+$ 253.1071 and $C_{13}H_{16}O_5Na^+$ 275.0890).

Step 2. Following a procedure similar to that described for the preparation of compound **45** (step 2) except that methyl (*E*)-3-(2,4,6-trimethoxyphenyl)acrylate (2.3 g, 9.1 mmol) was used in place of 2,3,4-trimethoxyphenyl formate, the title compound **19** was obtained as a white solid (1.8 g). Yield: 81.8%. Mp 232.8–232.9 °C. TLC: $R_f = 0.12$ (1:2 EtOAc/hexanes). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.85 (d, $J = 16.2$ Hz, 1H), 6.55 (d, $J = 16.2$ Hz, 1H), 6.28 (s, 2H), 3.85 (s, 6H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.18, 162.68, 160.75, 134.59, 117.79, 104.45, 90.95, 55.95, 55.52. HRMS (-ESI) 237.0765 $[M - H]^+$ (calcd. for $C_{12}H_{13}O_5^+$ 237.0768).

5.1.9. (E)-2,3,4,5-Tetramethoxycinnamic acid (**21**)

Step 1. The intermediate of methyl (*E*)-3-(2,3,4,5-tetramethoxyphenyl)acrylate was prepared using the same conditions as those used for the preparation of **38** except that 2,3,4,5-tetramethoxybenzaldehyde **43** (4.5 g, 20.0 mmol) was used in place of 2,4,5-trimethoxybenzaldehyde **37**. White solid. Yield: 4.4 g, 77.2%. Mp 62.3–62.6 °C. TLC: $R_f = 0.68$ (1:2 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.91 (d, $J = 16.1$ Hz, 1H), 6.78 (s, 1H), 6.40 (d, $J = 16.1$ Hz, 1H), 3.92 (s, 3H), 3.92 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.75, 149.79, 147.73, 147.34, 145.41, 139.50, 122.73, 117.81, 104.53, 61.94, 61.40, 61.33, 56.30, 51.79. HRMS (+ESI) 283.1176 $[M + H]^+$, 305.0998 $[M + Na]^+$ (calcd. for $C_{14}H_{19}O_6^+$ 283.1176 and $C_{14}H_{18}O_6Na^+$ 305.0996).

Step 2. Following a procedure similar to that described for the preparation of compound **45** (step 2) except that methyl (*E*)-3-(2,3,4,5-tetramethoxyphenyl)acrylate (2.8 g, 10.0 mmol) was used in place of 2,3,4-trimethoxyphenyl formate, the title compound **21** was obtained as a white solid (2.5 g). Yield: 89.0%. Mp 108.7–108.9 °C. TLC: $R_f = 0.88$ (100% EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.36 (s, 1H), 7.72 (d, $J = 16.1$ Hz, 1H), 7.12 (s, 1H), 6.56 (d, $J = 16.1$ Hz, 1H), 3.82 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.79, 149.60, 146.63, 146.58, 144.66, 137.78, 122.17, 119.19, 104.89, 61.62, 60.98, 60.71, 56.16. HRMS (-ESI) 267.0882 $[M - H]^+$ (calcd. for $C_{13}H_{15}O_6^+$ 267.0874).

5.1.10. (E)-2,3,4,6-Tetramethoxycinnamic acid (**22**)

Step 1. The intermediate of methyl (*E*)-3-(2,3,4,6-tetramethoxyphenyl)acrylate was prepared using the same conditions as those used for the preparation of **38** except that 2,3,4,6-tetramethoxybenzaldehyde **51** (4.5 g, 20.0 mmol) was used in place of 2,4,5-trimethoxybenzaldehyde **37**. White solid. Yield: 4.0 g, 71.2%. Mp 114.4–114.7 °C. TLC: $R_f = 0.58$ (1:2 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.97 (d, $J = 16.3$ Hz, 1H), 6.81 (d, $J = 16.2$ Hz, 1H), 6.27 (s, 1H), 3.90 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 169.22, 156.40, 155.45, 154.48, 136.45, 135.88, 118.71, 110.35, 91.99, 61.28, 61.20, 56.15, 55.98, 51.54. HRMS (+ESI) 283.1176 $[M + H]^+$, 305.0998 $[M + Na]^+$ (calcd. for $C_{14}H_{19}O_6^+$ 283.1176 and $C_{14}H_{18}O_6Na^+$ 305.0996).

Step 2. Following a procedure similar to that described for the preparation of compound **45** (step 2) except that methyl (*E*)-3-(2,3,4,6-tetramethoxyphenyl)acrylate (2.8 g, 10.0 mmol) was used in place of 2,3,4-trimethoxyphenyl formate, the title compound **22** was obtained as a white solid (2.6 g). Yield: 92.2%. Mp 191.6–192.0 °C. TLC: $R_f = 0.67$ (100% EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.07 (s, 1H), 7.76 (d, $J = 16.2$ Hz, 1H), 6.62 (d, $J = 16.2$ Hz, 1H), 6.54 (s, 2H), 3.88 (s, 9H), 3.80 (s, 4H), 3.69 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.82, 155.88, 155.38, 153.37, 135.64, 134.90, 118.93, 108.68, 93.03, 60.96, 60.57, 56.11, 56.05. HRMS (-ESI) 267.0882 $[M - H]^+$ (calcd. for $C_{13}H_{15}O_6^+$ 267.0874).

5.1.11. (E)-2,3,5,6-Tetramethoxycinnamic acid (**23**)

Step 1. The intermediate of methyl (*E*)-3-(2,3,5,6-tetramethoxyphenyl)acrylate was prepared using the same conditions as those used for the preparation of **38** except that 2,3,5,6-tetramethoxybenzaldehyde **54** (4.52 g, 20.0 mmol) was used in place of 2,4,5-trimethoxybenzaldehyde **37**. White solid. Yield: 3.96 g, 70.1%. Mp 77.3–77.5 °C. TLC: $R_f = 0.72$ (1:2 EtOAc/hexanes).

Step 2. Following a procedure similar to that described for the preparation of compound **45** (step 2) except that methyl (*E*)-3-(2,3,5,6-tetramethoxyphenyl)acrylate (2.82 g, 10.0 mmol) was used in place of 2,3,4-trimethoxyphenyl formate, the title compound **23** was obtained as a light kelly solid (2.45 g). Yield: 91.3%. Mp 182.2–182.4 °C. TLC: $R_f = 0.65$ (100% EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.71 (d, $J = 16.3$ Hz, 1H), 6.88 (s, 1H), 6.77 (d, $J = 16.3$ Hz, 1H), 3.83 (s, 6H), 3.66 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.14, 148.79, 141.17, 135.03, 123.10, 121.17, 101.70, 60.23, 56.35. HRMS (-ESI) 267.0878 $[M - H]^+$ (calcd. for $C_{13}H_{15}O_6^+$ 267.0874).

5.1.12. (E)-2,3,4,5,6-Pentamethoxycinnamic acid (**24**)

Step 1. The intermediate of methyl (*E*)-3-(2,3,4,5,6-pentamethoxyphenyl)acrylate was prepared using the same conditions as those used for the preparation of **38** except that 2,3,4,5,6-pentamethoxybenzaldehyde **48** (5.13 g, 20.0 mmol) was used in place of 2,4,5-trimethoxybenzaldehyde **37**. Colorless oil. Yield: 4.53 g, 72.6%. TLC: $R_f = 0.55$ (1:2 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.84 (d, $J = 16.3$ Hz, 1H), 6.85 (d, $J = 16.3$ Hz, 1H), 3.95 (s, 3H), 3.84 (s, 6H), 3.81 (s, 6H), 3.77 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 149.51, 149.34, 143.21, 135.70, 120.72, 61.53, 61.31, 61.12, 51.63.HRMS (+ESI) 313.1282 $[M + H]^+$, 335.1107 $[M + Na]^+$ (calcd. for $C_{15}H_{21}O_7^+$ 313.1282 and $C_{15}H_{20}O_7Na^+$ 335.1101).

Step 2. Following a procedure similar to that described for the preparation of compound **45** (step 2) except that methyl (*E*)-3-(2,3,4,5,6-pentamethoxyphenyl)acrylate (3.12 g, 10.0 mmol) was used in place of 2,3,4-trimethoxyphenyl formate, the title compound **24** was obtained as a white solid (2.87 g). Yield: 96.2%. Mp 141.1–141.2 °C (lit [67]. mp 138–139 °C) TLC: $R_f = 0.62$ (100% EtOAc). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.67 (d, $J = 16.3$ Hz, 1H), 6.69 (d, $J = 16.3$ Hz, 1H), 3.90 (s, 3H), 3.79 (s, 6H), 3.76 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.29, 149.01, 148.73, 142.90, 134.53, 121.59, 116.38, 61.15, 61.00, 60.85. HRMS (-ESI) 297.0980 $[M - H]^+$ (calcd. for $C_{14}H_{17}O_7^+$ 297.0980).

5.1.13. Methyl (E)-3-(2,4,5-trimethoxyphenyl)acrylate (**38**)

The Meldrum's acid (54.0 g, 0.37 mol) was dissolved in toluene (500 mL), and then methanol (20.0 mL, 0.49 mol) was added and the mixture was stirred at reflux temperature for 5 h. The reaction was cooled to room temperature, added with 2,4,5-trimethoxybenzaldehyde (37.3 g, 0.19 mol), pyridine (40.0 mL) and piperidine (4.0 mL). The mixture was stirred at room temperature for another 24 h and monitored by TLC. The volatile materials were removed *in vacuo*. Water was added, and the mixture was extracted with EtOAc (3 × 200 mL). The combined organic solution was dried ($MgSO_4$) and concentrated to give light yellow crude solid, which was recrystallized from a mixture of EtOAc/petroleum ether (1:9) to afford pure product 44.3 g. The procedure was adapted from Xia et al. [30]. Light yellow solid. Yield: 92.5%. Mp 105.2–105.4 °C (lit [65]. mp 97–98 °C). TLC: $R_f = 0.69$ (1:1 EtOAc/hexanes). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, $J = 16.1$ Hz, 1H), 7.00 (s, 1H), 6.49 (s, 1H), 6.37 (d, $J = 16.1$ Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.29, 154.01, 152.23, 143.36, 139.84, 115.51, 115.03, 111.00, 96.98, 56.57, 56.49, 56.20, 51.65. HRMS (+ESI) 253.1086 $[M + H]^+$, 275.0895 $[M + Na]^+$ (calcd. for $C_{13}H_{17}O_5^+$ 253.1071 and $C_{13}H_{16}O_5Na^+$ 275.0890).

*5.1.14. (E)-2-(3-(2,4,5-Trimethoxyphenyl)allyl)isoindoline-1,3-dione (**39**)*

To a 100 mL glass bottle compound **3** (1.0 g, 4.44 mmol) was added and dissolved in THF. Phthalimide (0.98 g, 6.66 mmol) and triphenylphosphine (1.51 g, 5.77 mmol) were then added and the reaction was cooled to 0 °C. Diisopropyl azodicarboxylate (1.17 g, 5.77 mmol) was then added dropwise over 2 min. The reaction was allowed to warm to room temperature and stirred overnight under nitrogen. When the reaction was complete by TLC the reaction was added water (50 mL) and EtOAc (50 mL). The organic phase was separated and dried on Na_2SO_4, then concentrated *in vacuo* to afford semisolid, which was purified by flash chromatography (SiO_2; 5:1–3:1 petroleum ether/ethyl acetate, v/v) to give a white solid 1.40 g [68]. Yield: 89.2%. Mp 149.6–150.0 °C. TLC: $R_f = 0.68$ (1:2 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.84 (dd, $J = 3.0$, 5.4 Hz, 2H), 7.70 (dd, $J = 3.0$, 5.4 Hz, 2H), 6.95 (d, $J = 15.9$ Hz, 1H), 6.90 (s, 1H), 6.45 (s, 1H), 6.13 (dt, $J = 6.7$, 15.9 Hz, 1H), 4.43 (d, $J = 6.7$ Hz, 2H), 3.87 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 168.17, 151.56, 149.66, 143.19, 134.02, 132.29, 128.54, 123.34, 120.92, 116.88, 109.68, 97.41, 56.67, 56.43, 56.08, 40.40. HRMS (+ESI) 354.1319 $[M + H]^+$, 376.1135 $[M + Na]^+$ (calcd. for $C_{20}H_{20}NO_5^+$ 354.1336 and $C_{20}H_{19}NO_5Na^+$ 376.1155).

*5.1.15. 2,3,6-Trimethoxybenzaldehyde (**42**)*

n-Butyl lithium in hexane 45 mL (2.5 M, 0.11 mol) was added dropwise to a solution of 1,2,4-trimethoxybenzene **41** (16.8 g, 0.10 mol) in anhydrous THF (160 mL) at −60 °C under a nitrogen atmosphere (about 30 min). A white precipitate was formed and temperature raised to −50 °C. After completion of adding *n*-Butyl lithium, the reaction mixture was stirred additionally for 1.5 h between −40 °C and -60 °C. Then, anhydrous DMF (8.7g, 0.12 mol) was added dropwise at −60 °C. During this addition, white precipitate was disappear. The mixture was allowed to stir at −40 °C for 2 h (monitored by TLC), then cautiously quenched with saturated NH_4Cl solution and followed by stirring at room temperature for another 30 min. The reaction was extracted with EtOAc (3 × 150 mL) and saturated NH_4Cl solution (250 mL). The total organic layer was washed with brine (3 × 150 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a crude residue, which was subjected to flash chromatography on silica gel (petroleum ether/dichloromethane/ethyl acetate, 9:1:1–7:1:1–6:1:1) to afford 8.4 g of **42** as a light yellow solid. Yield: 42.9%. Mp 28.2–28.5 °C (lit [69]. mp 20 °C). TLC: $R_f = 0.55$ (1:1 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) [41] δ 10.42 (s, 1H), 7.06 (d, $J = 9.1$ Hz, 1H), 6.62 (d, $J = 9.1$ Hz, 1H), 3.89 (s, 3H), 3.82 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 189.80, 154.93, 152.14, 146.87, 119.74, 119.21, 106.46, 62.08, 56.79, 56.28. HRMS (+ESI) 197.0809 $[M + H]^+$, 219.0627 $[M + Na]^+$ (calcd. for $C_{10}H_{13}O_4^+$ 197.0808 and $C_{10}H_{12}O_4Na^+$ 219.0628).

*5.1.16. 1,2,3,4-Tetramethoxybenzene (**45**)*

Step 1. In a 2 L three-necks flask was added with 2,3,4-trimethoxybenzaldehyde **44** (58.9 g, 0.3 mol) followed by anhydrous DCM (1.2 L) and cooled to 0–5 °C. To this solution, *m*-CPBA (75%, 89.8 g, 0.39 mol) was then added portion-wise whilst maintaining temperature of the reaction mixture in the range 0–5 °C. The reaction mixture was allowed to warm to room temperature and stirred for 24 h to yield a white slurry. The precipitate was filtered and washed with 200 mL of anhydrous DCM, then the filtrate was washed with 10% (w/w) sodium bisufite (2 × 200 mL/each time for 20 min), 5% aqueous Na_2CO_3 (2 × 200 mL/each time for 20 min), water (2 × 200 mL), brine (2 × 200 mL) until the starch iodide paper showed no oxidant remained in organic phase. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a crude 2,3,4-trimethoxyphenyl formate (73 g) as colorless oil, which was used in the next step without further purification. TLC: $R_f = 0.60$ (1:5 EtOAc/hexanes).

Step 2. Crude 2,3,4-trimethoxyphenyl formate (73 g) was added to methanol (120 mL) and treated with aq. 10% NaOH (300 mL) at 0 °C. The resulting homogeneous solution was stirred and allowed to warm to 40 °C for 2 h and determined to be complete by TLC. Methanol was distilled out completely from the reaction mixture under reduced pressure. The residue was added to H_2O (200 mL), acidified with 3 N HCl and extracted with DCM (3 × 150 mL). The organic layer was washed with H_2O (2 × 200 mL), dried over Na_2SO_4, filtered and concentrated completely under reduced pressure to yield 2,3,4-trimethoxyphenol (71 g) as a white solid (wet). TLC: $R_f = 0.50$ (1:5 EtOAc/hexanes). This material was used for the subsequent reaction without further purification.

Step 3. To a solution of 2,3,4-trimethoxyphenol (71 g, 0.38 mol), K_2CO_3 (50 g, 0.36 mol) in acetone (400 mL) at room temperature was added dimethyl sulfate (53 g, 0.42 mol) in 15 min. The reaction mixture was stirred and refluxed for 20 h, and the solvent was evaporated followed by the addition of water (400 mL). The mixture was simply stirred for 15 min and allowed to stand at room temperature for 4 h. The crude crystalline product formed and was collected, washed with water and purified by recrystallization from MeOH to afford 1,2,3,4-tetramethoxybenzene **45** (42 g) as white solid. Total yield (from step 1 to 3): 70.6%. Mp 85.8–86.1 °C (lit [70]. mp 87–87.5 °C). TLC: $R_f = 0.75$ (1:5 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.58 (s, 2H), 3.90 (s, 6H), 3.82 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 147.91, 143.52, 106.53, 61.33, 56.52. HRMS (+ESI) 199.0968 $[M + H]^+$, 221.0788 $[M + Na]^+$ (calcd. for $C_{10}H_{15}O_4^+$ 199.0965 and $C_{10}H_{14}O_4Na^+$ 221.0784).

*5.1.17. 2,3,4,5-Tetramethoxybenzaldehyde (**46**)*

1,2,3,4-tetramethoxybenzene **45** (8.2 g, 41.4 mmol) and hexamethylenetetramine (7.0 g, 50.0 mmol) were dissolved in trifluoroacetic acid (110 mL). The mixture was stirred and heated to 70 °C for 8 h. After the completion of reaction monitored by TLC, the reaction mixture was condensed under reduced pressure and neutralized with cold saturated $NaHCO_3$ solution to pH ~7. The desired product was extracted with DCM (2 × 150 mL), washed with brine (2 × 150 mL), dried over anhydrous sodium sulfate, concentrated, and purified by silica-gel flash chromatography (petroleum ether/dichloromethane/ethyl acetate, 9:1:1) to afford 2,3,4,5-tetramethoxybenzaldehyde (8.4 g) as light yellow solid. Yield: 89.4%. Mp 35.2–35.5 °C (lit [71]. mp 37–39 °C) TLC: $R_f = 0.62$ (1:5 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) [72] δ 10.27 (s, 1H), 7.09 (s, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 3.92 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 188.89, 152.42, 149.92, 149.42, 146.72, 124.11, 103.82, 62.97, 61.39, 61.37, 56.23. HRMS (+ESI) 227.0914 $[M + H]^+$, 249.0734 $[M + Na]^+$ (calcd. for $C_{11}H_{15}O_5^+$ 227.0914 and $C_{11}H_{14}O_5Na^+$ 249.0733).

*5.1.18. 1,2,3,4,5-Pentamethoxybenzene (**47**)*

Following the three steps similar to that described for the preparation of compound **45** except that an equivalent amount of 2,3,4,5-tetramethoxybenzaldehyde **46** (67.9 g, 0.3 mol) was used in place of 2,3,4-trimethoxybenzaldehyde **44**, the title compound **47** was obtained as a colorless oil, which solidified to a white solid on prolonged standing (46.5 g). Total yield: 67.8%. Mp 56.4–56.6 °C (lit [73]. mp 59–61 °C). TLC: $R_f = 0.70$ (1:5 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.29 (s, 1H), 3.94 (s, 3H), 3.84 (s, 6H), 3.82 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 149.17, 147.95, 136.62, 93.40, 61.51, 56.50. HRMS (+ESI) 229.1076 $[M + H]^+$, 251.0888 $[M + Na]^+$ (calcd. for $C_{11}H_{17}O_5^+$ 229.1071 and $C_{11}H_{16}O_5Na^+$ 251.0890).

*5.1.19. 2,3,4,5,6-Pentamethoxybenzaldehyde (**48**)*

Following a procedure similar to that described for the preparation of compound **46** except that an equivalent amount of 1,2,3,4,5-pentamethoxybenzene **47** (9.4 g, 41.4 mmol) was used in place of 1,2,3,4-tetramethoxybenzene **45**, the title compound **48** was obtained as a colorless oil (9.5 g). Yield: 89.6%. TLC: $R_f = 0.60$ (1:4 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) [74] δ 10.28 (s, 1H), 4.03 (s, 3H), 3.89 (s, 6H), 3.85 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 188.69, 153.41, 151.86, 142.94, 118.97, 62.41, 61.58, 61.54. HRMS (+ESI) 257.1042 $[M + H]^+$, 279.0837 $[M + Na]^+$ (calcd. for $C_{12}H_{17}O_6^+$ 257.1020 and $C_{12}H_{16}O_6Na^+$ 279.0839).

*5.1.20. 1,2,3,5-Tetramethoxybenzene (**50**)*

Following the three steps similar to that described for the preparation of compound **45** except that an equivalent amount of 3,4,5-trimethoxybenzaldehyde **49** (58.9 g, 0.3 mol) was used in place of 2,3,4-trimethoxybenzaldehyde **44**, the title compound **50** was obtained as a colorless oil, which solidified to a white solid on prolonged standing (40.2 g). Total yield: 67.6%. Mp 69.3–69.4 °C (lit [75]. mp 46 °C). TLC: $R_f = 0.78$ (1:4 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.14 (s, 2H), 3.83 (s, 6H), 3.77 (d, $J = 2.5$ Hz, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 156.33, 153.80, 132.33, 91.71, 61.10, 56.15, 55.61. HRMS (+ESI) 199.0970 $[M + H]^+$, 221.0786 $[M + Na]^+$ (calcd. for $C_{10}H_{15}O_4^+$ 199.0965 and $C_{10}H_{14}O_4Na^+$ 221.0784).

*5.1.21. 2,3,4,6-Tetramethoxybenzaldehyde (**51**)*

Phosphorus oxychloride (13.9 g, 90 mmol) was added to anhydrous DMF (100 mL) and stirred at a temperature of 0 °C for about 15 min under nitrogen atmosphere, followed by addition of 1,2,3,5-Tetramethoxybenzene **50** (9.0 g, 45 mmol) dropwise over a period of 30 min. The reaction mixture was stirred for 1 h at 0 °C, then allowed to warm to room temperature and stirred overnight. The mixture was poured over crushed ice followed by saturated sodium carbonate solution. The aqueous solution was extracted with dichloromethane (2 × 150 mL). The combined organic layers were washed with brine (2 × 150 mL). The organic layer was dried over anhydrous sodium sulfate, and the sodium sulfate was removed by filtration. The solvent was removed by rotary evaporation to obtain the light yellow oil which was purified by silica-gel flash chromatography (eluent with petroleum ether/ethyl acetate, 10:1–5:1–2:1) to afford pure 2,3,4,6-tetramethoxybenzaldehyde (9.0 g) as a white solid. Yield: 87.4%. Mp 86.0–86.3 °C (lit [39]. mp 88.5–89 °C). TLC: $R_f = 0.35$ (1:3 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 10.29 (s, 1H), 6.25 (s, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 188.13, 159.30, 158.99, 156.86, 136.02, 112.68, 91.68, 62.23, 61.33, 56.32, 56.21. HRMS (+ESI) 227.0914 $[M + H]^+$, 249.0732 $[M + Na]^+$ (calcd. for $C_{11}H_{15}O_5^+$ 227.0914 and $C_{11}H_{14}O_5Na^+$ 249.0733).

*5.1.22. 1,2,4,5-Tetramethoxybenzene (**53**)*

Following the three steps similar to that described for the preparation of compound **45** except that an equivalent amount of 2,4,5-trimethoxybenzaldehyde **52** (58.9 g, 0.3 mol) was used in place of 2,3,4-trimethoxybenzaldehyde **44**, the title compound **53** was obtained as a white solid (38.8 g). Total yield: 65.2%. Mp 100.4–100.7 °C (lit [66]. mp 99.7–101.8 °C). TLC: $R_f = 0.50$ (1:1 EtOAc/hexanes). ^{1}H NMR (600 MHz) δ 6.60 (s, 2H), 3.84 (s, 12H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 143.25, 100.76, 57.14. HRMS (+ESI) 199.0963 $[M + H]^+$, 221.0786 $[M + Na]^+$ (calcd. for $C_{10}H_{15}O_4^+$ 199.0965 and $C_{10}H_{14}O_4Na^+$ 221.0784).

*5.1.23. 2,3,5,6-Tetramethoxybenzaldehyde (**54**)*

Following a procedure similar to that described for the preparation of compound **46** except that an equivalent amount of 1,2,4,5-tetramethoxybenzene **53** was used in place of 1,2,3,4-tetramethoxybenzene **45**, the title compound **54** was obtained as a white solid (8.8 g). Yield: 93.6%. Mp 72.0–72.3 °C (lit [66]. mp 79.5–81.4 °C). TLC: $R_f = 0.50$ (1:4 EtOAc/hexanes). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 10.40 (s, 1H), 6.78 (s, 1H), 3.88 (s, 6H), 3.85 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 190.14, 149.38, 143.86, 143.02, 124.38, 104.85, 100.29, 62.33, 56.85. HRMS (+ESI) 227.0913 $[M + H]^+$, 249.0736 $[M + Na]^+$ (calcd. for $C_{11}H_{15}O_5^+$ 227.0914 and $C_{11}H_{14}O_5Na^+$ 249.0733).

*5.1.24. General method for the preparation of cinnamic acid ester derivatives (**55**–**85**)*

A mixture of the compound **3** or **4** (5 mmol), cinnamic acid derivatives (6 mmol), and DMAP (1 mmol) in DCM (15 mL) was stirred for 10 min at room temperature. Successively, EDCI (6–10 mmol) were added quickly in one portion. The reaction mixture was continually stirred at room temperature for 10–18 h under nitrogen and monitored by TLC. Then the clear solution treated with an aqueous saturated $NaHCO_3$ solution containing ice (60 mL) for 5 min. The aqueous reaction mixture was extracted with DCM (3 × 20 mL). The combined extracts were dried with dry Na_2SO_4 and concentrated *in vacuo*. The crude compound was purified by flash chromatography (SiO_2; 100:0–50:50–0:100 hexanes/DCM, v/v). The final esters were obtained as solid substances after concentration of organic solvents under reduced pressure.

*5.1.24.1. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2-methoxyphenyl)acrylate (**55**).* Light yellow solid. Yield: 87.5%. Mp 96.4–96.8 °C. TLC: $R_f = 0.56$ (1:1 EtOAc/hexanes). HPLC (purity 97.7%): $t_R = 7.62$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.03 (d, $J = 16.2$ Hz, 1H), 7.50 (d, $J = 7.7$ Hz, 1H), 7.34 (t, $J = 7.8$ Hz, 1H), 7.00 (s, 1H), 6.99–6.93 (m, 2H), 6.90 (d, $J = 8.3$ Hz, 1H), 6.57 (d, $J = 16.1$ Hz, 1H), 6.50 (s, 1H), 6.26 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.86 (d, $J = 6.7$ Hz, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ167.47, 158.46, 151.76, 149.97, 143.40, 140.46, 131.57, 129.08, 129.04, 123.52, 121.76, 120.79, 118.72, 117.11, 111.23, 110.08, 97.62, 65.94, 56.71, 56.59, 56.17, 55.56. IR (KBr) 3398, 2929, 2828, 1705, 1634, 1599, 1513, 1489, 1465, 1438, 1408, 1373, 1319, 1249, 1211, 1108, 1032, 984, 969, 949, 856, 835, 789, 754, 592, 562, 460 cm^{-1}; HRMS (+ESI) 407.1464 $[M + Na]^+$ (calcd. for $C_{22}H_{24}O_6Na^+$ 407.1465).

*5.1.24.2. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(3-methoxyphenyl)acrylate (**56**).* Light yellow solid. Yield: 91.3%. Mp 65.0–65.2 °C. TLC: $R_f = 0.60$ (1:1 EtOAc/hexanes). HPLC (purity 96.6%): $t_R = 8.24$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.69 (d, $J = 16.0$ Hz, 1H), 7.29 (t, $J = 7.9$ Hz, 1H), 7.11 (d, $J = 7.6$ Hz, 1H), 7.04 (s, 1H), 7.00 (s, 1H), 6.99 (d, $J = 16.0$ Hz, 1H), 6.93 (dd, $J = 8.2$, 1.7 Hz, 1H), 6.50 (s, 1H), 6.47 (d, $J = 16.0$ Hz, 1H), 6.30–6.22 (m, 1H), 4.87 (d, $J = 6.7$ Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ166.81, 159.92, 151.75, 149.99, 144.87, 143.34, 135.82, 129.93, 129.22, 121.38, 120.82, 118.43, 116.93, 116.20, 112.97, 110.07, 97.52, 66.04, 56.61, 56.53, 56.10, 55.32. IR (KBr) 3404, 3048, 2991, 2945, 2836, 1707, 1642, 1593, 1517, 1492, 1468, 1452, 1436, 1408, 1311, 1292, 1252, 1224, 1212, 1175, 1037, 1010, 990, 969, 927, 853, 839, 784, 677 cm^{-1}. HRMS (+ESI) 407.1465 $[M + Na]^+$ (calcd. for $C_{22}H_{24}O_6Na^+$ 407.1465).

*5.1.24.3. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(4-methoxyphenyl)acrylate (**57**).* White solid. Yield: 90.6%. Mp 95.5–95.8 °C. TLC: $R_f = 0.55$ (1:1 EtOAc/hexanes). HPLC (purity 99.6%): $t_R = 9.25$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.99 (d, $J = 16.1$ Hz, 1H), 6.98 (t, $J = 10.4$ Hz, 3H), 6.48 (d, $J = 2.3$ Hz, 2H), 6.41 (d, $J = 16.0$ Hz, 1H), 6.26 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.85 (d, $J = 6.6$ Hz, 2H), 3.91 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H),

3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.31, 161.50, 151.80, 150.01, 144.71, 143.43, 129.86, 129.11, 127.30, 121.69, 117.10, 115.68, 114.45, 110.11, 97.63, 65.94, 56.74, 56.61, 56.20, 55.51. IR (KBr) 2932, 2837, 1705, 1640, 1604, 1577, 1514, 1456, 1425, 1404, 1327, 1254, 1162, 1029, 983, 923, 873, 825, 767, 749, 656, 555, 517, 474 cm^{-1}; HRMS (+ESI) 407.1465 [M + Na]$^+$ (calcd. for $C_{22}H_{24}O_6Na^+$ 407.1465).

*5.1.24.4. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,3-dimethoxyphenyl)acrylate (**58**).* White solid. Yield: 90.6%. Mp 81.0–81.3 °C. TLC: R_f = 0.60 (1:1 EtOAc/hexanes). HPLC (purity 96.3%): t_R = 9.25 min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.02 (d, *J* = 16.2 Hz, 1H), 7.14 (d, *J* = 7.9 Hz, 1H), 7.04 (t, *J* = 8.0 Hz, 1H), 6.99 (d, *J* = 15.5 Hz, 1H), 6.99 (s, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 6.52 (d, *J* = 16.2 Hz, 1H), 6.49 (s, 1H), 6.26 (dt, *J* = 15.8, 6.7 Hz, 1H), 4.87 (d, *J* = 6.6 Hz, 2H), 3.89 (s, 3H), 3.86 (d, *J* = 2.4 Hz, 9H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.11, 153.20, 151.75, 149.97, 148.53, 143.37, 139.73, 129.07, 128.69, 124.26, 121.59, 119.50, 119.35, 117.03, 114.01, 110.07, 97.57, 66.01, 61.41, 56.67, 56.57, 56.14, 55.94. IR (KBr) 3449, 2935, 2836, 1715, 1639, 1608, 1581, 1520, 1466, 1404, 1309, 1270, 1218, 1180, 1092, 1066, 1033, 989, 943, 873, 817, 783, 751 cm^{-1}; HRMS (+ESI) 415.1705 [M + H]$^+$, 437.1571 [M + Na]$^+$ (calcd. for $C_{23}H_{27}O_7^+$ 415.1751 and $C_{23}H_{26}O_7Na^+$ 437.1571).

*5.1.24.5. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,4-dimethoxyphenyl)acrylate (**59**).* White solid. Yield: 75.3%. Mp 120.0–120.3 °C. TLC: R_f = 0.52 (1:1 EtOAc/hexanes). HPLC (purity 96.3%): t_R = 10.25 min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.92 (s, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 6.98 (d, *J* = 18.7 Hz, 2H), 6.51–6.43 (m, 4H), 6.26 (dt, *J* = 15.9, 6.7 Hz, 1H), 4.85 (d, *J* = 6.6 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.89, 162.81, 159.99, 151.75, 149.93, 143.41, 140.42, 130.61, 128.89, 121.96, 117.19, 116.72, 116.06, 110.07, 105.32, 98.52, 97.64, 65.78, 56.74, 56.59, 56.18, 55.58. IR (KBr) 3399, 3000, 2949, 2887, 2826, 1708, 1625, 1600, 1518, 1468, 1439, 1421, 1407, 1305, 1282, 1211, 1163, 1107, 1033, 1002, 973, 938, 876, 842, 817, 600, 535, 447 cm^{-1}. HRMS (+ESI) 437.1571 [M + Na]$^+$ (calcd. for $C_{23}H_{26}O_7Na^+$ 437.1571).

*5.1.24.6. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,5-dimethoxyphenyl)acrylate (**60**).* White solid. Yield: 87.6%. Mp 114.1–114.5 °C. TLC: R_f = 0.58 (1:1 EtOAc/hexanes). HPLC (purity 97.9%): t_R = 8.84 min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.00 (d, *J* = 16.1 Hz, 1H), 7.04 (d, *J* = 3.0 Hz, 1H), 6.98 (d, *J* = 13.6 Hz, 2H), 6.89 (dd, *J* = 9.0, 3.0 Hz, 1H), 6.83 (d, *J* = 9.0 Hz, 1H), 6.53 (d, *J* = 16.1 Hz, 1H), 6.49 (s, 1H), 6.26 (dt, *J* = 15.9, 6.7 Hz, 1H), 4.86 (dd, *J* = 6.7, 1.0 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.83 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.31, 153.55, 152.92, 151.75, 149.96, 143.38, 140.19, 129.08, 124.02, 121.67, 118.87, 117.21, 117.06, 113.35, 112.51, 110.06, 97.58, 65.96, 56.69, 56.57, 56.14, 55.85. IR (KBr) 3426, 2999, 2961, 2935, 2830, 1698, 1655, 1629, 1609, 1579, 1502, 1463, 1404, 1376, 1256, 1168, 1109, 1041, 969, 856, 834, 808, 745, 714, 640, 574 cm^{-1}. HRMS (+ESI) 437.1571 [M + Na]$^+$ (calcd. for $C_{23}H_{26}O_7Na^+$ 437.1571).

*5.1.24.7. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,6-dimethoxyphenyl)acrylate (**61**).* White solid. Yield: 70.3%. Mp 121.9–122.3 °C. TLC: R_f = 0.62 (1:1 EtOAc/hexanes). HPLC (purity 97.1%): t_R = 9.49 min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.18 (d, *J* = 16.3 Hz, 1H), 7.25 (t, *J* = 8.5 Hz, 1H), 6.98 (d, *J* = 17.8 Hz, 2H), 6.93 (d, *J* = 16.3 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 2H), 6.49 (s, 1H), 6.28 (dt, *J* = 15.9, 6.7 Hz, 1H), 4.87 (d, *J* = 6.7 Hz, 2H), 3.89 (s, 3H), 3.86 (d, *J* = 1.1 Hz, 9H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 168.55, 160.10, 151.69, 149.86, 143.35, 135.80, 131.30, 128.78, 122.06, 120.54, 117.19, 112.31, 110.03, 103.71, 97.60, 65.72, 56.69, 56.54, 56.12, 55.81. IR (KBr) 3445, 1702, 1619, 1596, 1518, 1478, 1439, 1406, 1311, 1283, 1258, 1213, 1184, 1158, 1111, 1028, 993, 976, 873, 827, 783, 743 cm^{-1}; HRMS (+ESI) 437.1571 [M + Na]$^+$ (calcd. for $C_{23}H_{26}O_7Na^+$ 437.1571).

*5.1.24.8. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(3,4-dimethoxyphenyl)acrylate (**62**).* Gray-yellow solid. Yield: 84.2%. Mp 79.4–79.8 °C. TLC: R_f = 0.62 (1:1 EtOAc/hexanes). HPLC (purity 96.7%): t_R = 7.04 min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.66 (d, *J* = 15.9 Hz, 1H), 7.10 (d, *J* = 8.2 Hz, 1H), 7.05 (s, 1H), 6.99 (s, 1H), 6.98 (d, *J* = 15.4 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.49 (s, 1H), 6.35 (d, *J* = 15.9 Hz, 1H), 6.25 (dt, *J* = 15.9, 6.7 Hz, 1H), 4.85 (d, *J* = 6.6 Hz, 2H), 3.92–3.87 (m, 9H), 3.85 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.15, 151.77, 151.20, 150.00, 149.27, 144.92, 143.38, 129.17, 127.49, 122.72, 121.57, 117.02, 115.86, 111.11, 110.09, 109.67, 97.58, 65.94, 56.69, 56.58, 56.15, 56.06, 55.96. IR (KBr) 3452, 2937, 2836, 1711, 1637, 1600, 1518, 1454, 1403, 1337, 1315, 1257, 1232, 1208, 1173, 1152, 1135, 1034, 1022, 993, 978, 869, 845, 810, 798, 751, 573 cm^{-1}; HRMS (+ESI) 437.1572 [M + Na]$^+$ (calcd. for $C_{23}H_{26}O_7Na^+$ 437.1571).

*5.1.24.9. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(3,5-dimethoxyphenyl)acrylate (**63**).* White solid. Yield: 80.8%. Mp 133.3–133.5 °C. TLC: R_f = 0.65 (1:1 EtOAc/hexanes). HPLC (purity 97.9%): t_R = 9.73 min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.64 (d, *J* = 15.9 Hz, 1H), 6.99 (d, *J* = 15.9 Hz, 1H), 6.99 (s, 1H), 6.66 (d, *J* = 2.0 Hz, 2H), 6.52–6.47 (m, 2H), 6.44 (d, *J* = 15.9 Hz, 1H), 6.25 (dt, *J* = 15.9, 6.7 Hz, 1H), 4.86 (d, *J* = 6.7 Hz, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.80 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.84, 161.11, 151.81, 150.05, 145.01, 143.41, 136.41, 129.32, 121.41, 118.73, 116.99, 110.11, 106.06, 102.69, 97.59, 66.12, 56.70, 56.60, 56.18, 55.53. IR (KBr) 3448, 2996, 2946, 2838, 1698, 1634, 1599, 1521, 1467, 1441, 1428, 1404, 1359, 1326, 1293, 1276, 1241, 1214, 1166, 1127, 1109, 1068, 1058, 1031, 1012, 997, 972, 919, 858, 835, 752, 674, 650 cm^{-1}; HRMS (+ESI) 437.1572 [M + Na]$^+$ (calcd. for $C_{23}H_{26}O_7Na^+$ 437.1571).

*5.1.24.10. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,3,4-trimethoxyphenyl)acrylate (**64**).* White solid. Yield: 80.8%. Mp 75.2–75.5 °C. TLC: R_f = 0.56 (1:1 EtOAc/hexanes). HPLC (purity 99.1%): t_R = 7.67 min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.91 (d, *J* = 16.1 Hz, 1H), 7.26 (d, *J* = 8.8 Hz, 1H), 6.98 (d, *J* = 15.4 Hz, 2H), 6.69 (d, *J* = 8.8 Hz, 1H), 6.50 (s, 1H), 6.46 (d, *J* = 16.1 Hz, 1H), 6.26 (dt, *J* = 6.7, 16.0 Hz, 1H), 4.86 (d, *J* = 6.7 Hz, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.47, 155.61, 153.41, 151.75, 149.96, 143.40, 142.44, 139.97, 128.96, 123.32, 121.77, 121.60, 117.11, 117.07, 110.08, 107.68, 97.60, 65.88, 61.53, 60.99, 56.70, 56.58, 56.16. IR (KBr) 2948, 1715, 1639, 1611, 1520, 1469, 1404, 1317, 1209, 1182, 1110, 1032, 979, 870, 852, 819, 770, 652, 593, 464 cm^{-1}; HRMS (+ESI) 467.1676 [M + Na]$^+$ (calcd. for $C_{24}H_{28}O_8Na^+$ 467.1676).

*5.1.24.11. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,3,6-trimethoxyphenyl)acrylate (**65**).* Light yellow solid. Yield: 76.4%. Mp 91.9–92.3 °C. TLC: R_f = 0.50 (1:1 EtOAc/hexanes). HPLC (purity 98.5%): t_R = 7.75 min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.05 (d, *J* = 16.3 Hz, 1H), 7.00 (d, *J* = 3.5 Hz, 2H), 6.97 (d, *J* = 3.8 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 6.59 (d, *J* = 9.1 Hz, 1H), 6.50 (s, 1H), 6.27 (dt, *J* = 6.7, 16.0 Hz, 1H), 4.87 (dd, *J* = 0.6, 6.7 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.83 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 168.27, 153.76, 151.75, 149.94, 147.11, 143.42, 136.02, 128.90, 122.11, 121.96, 118.18, 117.21, 114.55, 110.08, 105.86, 97.65, 65.89, 61.03, 56.74, 56.61, 56.60, 56.18, 55.99. IR (KBr) 3396, 3043, 3002, 2964, 2935, 2838, 1706, 1633, 1608, 1582, 1515, 1486, 1466, 1406, 1325, 1256, 1201, 1159, 1104, 1068, 1042, 1028, 1008, 995,

968, 941, 874, 853, 794, 722 cm^{-1}. HRMS (+ESI) 467.1676 $[M + Na]^+$ (calcd. for $C_{24}H_{28}O_8Na^+$ 467.1676).

*5.1.24.12. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,4,5-trimethoxyphenyl)acrylate (**66**).* Light yellow solid. Yield: 86.8%. Mp 114.8–115.2 °C. TLC: $R_f = 0.60$ (1:1 EtOAc/hexanes). HPLC (purity 95.9%): $t_R = 6.94$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.99 (d, $J = 16.1$ Hz, 1H), 7.02–6.96 (m, 3H), 6.48 (d, $J = 2.3$ Hz, 2H), 6.41 (d, $J = 16.0$ Hz, 1H), 6.26 (dt, $J = 6.7$, 15.9 Hz, 1H), 4.85 (d, $J = 6.6$ Hz, 2H), 3.91 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.69, 153.95, 152.11, 151.67, 149.86, 143.29, 143.22, 139.89, 128.95, 121.77, 116.99, 115.66, 114.92, 110.78, 109.87, 97.43, 96.79, 65.82, 56.67, 56.51, 56.44, 56.39, 56.13. IR (KBr) 3409, 2935, 2830, 1699, 1609, 1515, 1468, 1445, 1406, 1301, 1277, 1209, 1167, 1127, 1031, 998, 969, 869, 809, 541 cm^{-1}. HRMS (+ESI) 467.1676 $[M + Na]^+$ (calcd. for $C_{24}H_{28}O_8Na^+$ 467.1676).

*5.1.24.13. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,4,6-trimethoxyphenyl)acrylate (**67**).* White solid. Yield: 57.5%. Mp 150.2–150.5 °C. TLC: $R_f = 0.72$ (1:1 EtOAc/hexanes). HPLC (purity 98.8%): $t_R = 8.14$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.12 (d, $J = 16.2$ Hz, 1H), 6.99 (s, 1H), 6.96 (d, $J = 16.0$ Hz, 1H), 6.79 (d, $J = 16.2$ Hz, 1H), 6.48 (s, 1H), 6.27 (dt, $J = 15.9$, 6.7 Hz, 1H), 6.09 (s, 2H), 4.84 (d, $J = 6.7$ Hz, 2H), 3.88 (s, 3H), 3.85 (s, 3H), 3.85 (s, 6H), 3.82 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 168.98, 162.86, 161.36, 151.68, 149.82, 143.37, 135.87, 128.63, 122.29, 117.40, 117.28, 110.03, 105.93, 97.64, 90.47, 65.57, 56.72, 56.55, 56.14, 55.77, 55.45. IR (KBr) 3442, 2936, 1700, 1604, 1519, 1493, 1468, 1439, 1404, 1309, 1288, 1233, 1214, 1159, 1123, 1032, 995, 973, 954, 875, 811, 524 cm^{-1}; HRMS (+ESI) 467.1676 $[M + Na]^+$ (calcd. for $C_{24}H_{28}O_8Na^+$ 467.1676).

*5.1.24.14. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (**68**).* Light yellow solid. Yield: 71.2%. Mp 72.0–72.3 °C. TLC: $R_f = 0.62$ (1:1 EtOAc/hexanes). HPLC (purity 95.1%): $t_R = 6.66$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.64 (d, $J = 15.9$ Hz, 1H), 7.00 (d, $J = 15.9$ Hz, 1H), 7.00 (s, 1H), 6.76 (s, 2H), 6.51 (s, 1H), 6.39 (d, $J = 15.9$ Hz, 1H), 6.26 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.87 (d, $J = 6.7$ Hz, 2H), 3.91 (s, 3H), 3.88 (s, 6H), 3.88 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.93, 153.56, 151.84, 150.10, 145.02, 143.44, 140.22, 130.07, 129.38, 121.44, 117.48, 117.01, 110.15, 105.33, 97.61, 66.12, 61.12, 56.74, 56.65, 56.28, 56.21. IR (KBr) 3513, 2925, 1709, 1636, 1583, 1508, 1464, 1245, 1128, 1031, 976, 835, 632 cm^{-1}; HRMS (+ESI) 467.1678 $[M + Na]^+$ (calcd. for $C_{24}H_{28}O_8Na^+$ 467.1676).

*5.1.24.15. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,3,4,5-tetramethoxyphenyl)acrylate (**69**).* Light yellow solid. Yield: 70.4%. Mp 68.3–69.7 °C. TLC: $R_f = 0.73$ (1:1 EtOAc/hexanes). HPLC (purity 99.3%): $t_R = 9.06$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.95 (d, $J = 16.1$ Hz, 1H), 7.00 (s, 1H), 6.99 (d, $J = 15.9$ Hz, 1H), 6.79 (s, 1H), 6.50 (s, 1H), 6.44 (d, $J = 16.1$ Hz, 1H), 6.26 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.87 (dd, $J = 6.7$, 0.8 Hz, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.18, 151.81, 150.04, 149.78, 147.75, 147.34, 145.40, 143.43, 139.58, 129.17, 122.80, 121.62, 118.09, 117.08, 110.12, 104.52, 97.63, 66.04, 61.98, 61.41, 61.33, 56.73, 56.63, 56.28, 56.20. IR (KBr) 3426, 3056, 2996, 2943, 2833, 1709, 1628, 1606, 1591, 1520, 1490, 1468, 1435, 1414, 1353, 1322, 1287, 1247, 1207, 1165, 1132, 1088, 1038, 1008, 990, 970, 934, 884, 861, 846, 814 cm^{-1}. HRMS (+ESI) 497.1783 $[M + Na]^+$ (calcd. for $C_{25}H_{30}O_9Na^+$ 497.1782).

*5.1.24.16. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,3,4,6-tetramethoxyphenyl)acrylate (**70**).* White solid. Yield: 63.5%. Mp 121.3–121.6 °C. TLC: $R_f = 0.50$ (1:1 EtOAc/hexanes). HPLC (purity 99.5%): $t_R = 8.58$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.01 (d, $J = 16.3$ Hz, 1H), 7.00 (s, 1H), 6.98 (d, $J = 16.0$ Hz, 1H), 6.84 (d, $J = 16.3$ Hz, 1H), 6.49 (s, 1H), 6.30–6.24 (m, 2H), 4.85 (dd, $J = 6.7$, 0.9 Hz, 2H), 3.90 (s, 6H), 3.89 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 168.68, 156.42, 155.43, 154.50, 151.74, 149.90, 143.43, 136.43, 135.97, 128.73, 122.17, 118.94, 117.28, 110.41, 110.08, 97.67, 91.97, 65.72, 61.31, 61.19, 56.76, 56.60, 56.19, 56.14, 55.97. IR (KBr) 3439, 2996, 2937, 2838, 1699, 1623, 1600, 1572, 1498, 1463, 1439, 1404, 1344, 1299, 1288, 1237, 1209, 1157, 1110, 1043, 1030, 1015, 983, 923, 864, 827, 810 cm^{-1}. HRMS (+ESI) 497.1783 $[M + Na]^+$ (calcd. for $C_{25}H_{30}O_9Na^+$ 497.1782).

*5.1.24.17. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2,3,5,6-tetramethoxyphenyl)acrylate (**71**).* Light yellow solid. Yield: 73.2%. Mp 117.2–117.6 °C. TLC: $R_f = 0.52$ (1:1 EtOAc/hexanes). HPLC (purity 100.0%): $t_R = 8.33$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.96 (d, $J = 16.3$ Hz, 1H), 7.01 (d, $J = 16.3$ Hz, 1H), 7.00 (s, 1H), 7.99 (d, $J = 16.3$ Hz, 1H), 6.59 (s, 1H), 6.49 (s, 1H), 6.27 (dt, $J = 6.7$, 15.9 Hz, 1H), 4.87 (d, $J = 6.2$ Hz, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.86 (s, 6H), 3.83 (s, 3H), 3.76 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 168.02, 151.67, 149.86, 149.17, 143.29, 142.32, 136.17, 128.94, 122.97, 122.69, 121.72, 116.96, 109.82, 97.38, 66.04, 60.98, 56.68, 56.58, 56.52, 56.13. IR (KBr) 3435, 2935, 2835, 1711, 1628, 1587, 1517, 1487, 1467, 1404, 1349, 1290, 1245, 1209, 1167, 1095, 1063, 1033, 1009, 972, 873, 821 cm^{-1}. HRMS (+ESI) 497.1783 $[M + Na]^+$ (calcd. for $C_{25}H_{30}O_9Na^+$ 497.1782).

*5.1.24.18. (E)-3-(2,4,5-trimethoxyphenyl)allyl(E)-3-(2,3,4,5,6-pentamethoxyphenyl) acrylate (**72**).* White solid. Yield: 64.5%. Mp 102.0–102.5 °C. TLC: $R_f = 0.67$ (1:1 EtOAc/hexanes). HPLC (purity 99.1%): $t_R = 10.15$. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.90 (d, $J = 16.3$ Hz, 1H), 6.99 (s, 1H), 6.98 (d, $J = 18.0$ Hz, 1H), 6.91 (d, $J = 16.3$ Hz, 1H), 6.49 (s, 1H), 6.26 (dt, $J = 6.7$, 15.9 Hz, 1H), 4.86 (d, $J = 6.6$ Hz, 2H), 3.98 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.86 (s, 6H), 3.84 (s, 6H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 151.65, 149.84, 149.58, 149.36, 143.27, 143.24, 135.83, 128.83, 121.80, 121.03, 117.37, 116.97, 109.81, 97.37, 65.92, 65.90, 61.60, 61.38, 61.22, 56.66, 56.51, 56.12. IR (KBr) 3400, 2974, 2941, 2839, 1705, 1624, 1589, 1513, 1467, 1408, 1323, 1301, 1267, 1211, 1171, 1126, 1062, 1034, 1001, 976, 957, 937, 868 cm^{-1}. HRMS (+ESI) 527.1889 $[M + Na]^+$ (calcd. for $C_{26}H_{32}O_{10}Na^+$ 527.1888).

*5.1.24.19. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2-fluorophenyl) acrylate (**73**).* Light yellow solid. Yield: 72.9%. Mp 73.5–73.8 °C. TLC: $R_f = 0.50$ (1:1 EtOAc/hexanes). HPLC (purity 100.0%): $t_R = 9.17$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.85 (d, $J = 16.2$ Hz, 1H), 7.54 (t, $J = 7.5$ Hz, 1H), 7.38–7.32 (m, 1H), 7.16 (t, $J = 7.5$ Hz, 1H), 7.13–7.06 (m, 1H), 7.00 (d, $J = 15.9$ Hz, 1H), 7.00 (s, 1H) 6.58 (d, $J = 16.2$ Hz, 1H), 6.50 (s, 1H), 6.26 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.88 (d, $J = 6.7$ Hz, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.74, 162.27, 160.58, 151.79, 150.02, 143.38, 137.56, 131.75, 129.29, 124.51, 122.53, 121.35, 120.75, 116.96, 116.20, 110.08, 97.56, 66.17, 56.66, 56.57, 56.14. IR (KBr) 3416, 1709, 1640, 1608, 1513, 1466, 1403, 1318, 1276, 1212, 1030, 974, 944, 860, 839, 805, 767, 651, 460, 411 cm^{-1}; HRMS (+ESI) 395.1265 $[M + Na]^+$ (calcd. for $C_{21}H_{21}FO_5Na^+$ 395.1265).

*5.1.24.20. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(3-fluorophenyl) acrylate (**74**).* White solid. Yield: 75.4%. Mp 89.9–90.2 °C. TLC: $R_f = 0.66$ (1:1 EtOAc/hexanes). HPLC (purity 100.0%): $t_R = 10.41$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.67 (d, $J = 16.0$ Hz, 1H), 7.39–7.31 (m, 1H), 7.29 (d, $J = 7.7$ Hz, 1H), 7.21 (d, $J = 9.6$ Hz, 1H), 7.07 (td, $J = 8.3$, 1.7 Hz, 1H), 7.00 (d, $J = 15.9$ Hz, 1H), 6.99 (s, 1H), 6.50

(s, 1H), 6.47 (d, $J = 16.0$ Hz, 1H), 6.25 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.87 (dd, $J = 6.7$, 0.8 Hz, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ166.53, 163.89, 162.25, 151.81, 150.07, 143.55, 143.39, 136.78, 130.56, 129.40, 124.14, 121.26, 119.61, 117.15, 114.32, 110.11, 97.55, 66.22, 56.65, 56.58, 56.15. IR (KBr) 1711, 1643, 1612, 1519, 1440, 1405, 1337, 1272, 1211, 1168, 1119, 1079, 1029, 970, 861, 824, 803, 728, 694, 657 cm^{-1}; HRMS (+ESI) 395.1266 [M + Na]$^+$ (calcd. for $C_{21}H_{21}FO_5Na^+$ 395.1265).

*5.1.24.21. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(4-fluorophenyl) acrylate (**75**).* White solid. Yield: 71.6%. Mp 92.9–93.3 °C. TLC: $R_f = 0.55$ (1:1 EtOAc/hexanes). HPLC (purity 98.8%): $t_R = 8.59$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.67 (d, $J = 16.0$ Hz, 1H), 7.50 (dd, $J = 8.5$, 5.5 Hz, 2H), 7.06 (t, $J = 8.6$ Hz, 2H), 6.99 (s, 1H), 6.98 (d, $J = 16.0$ Hz, 1H), 6.49 (s, 1H), 6.39 (d, $J = 16.0$ Hz, 1H), 6.25 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.86 (d, $J = 6.7$ Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.80, 164.82, 163.15, 151.81, 150.06, 143.68, 143.40, 130.77, 130.01, 129.32, 121.39, 117.95, 116.96, 116.22, 116.07, 110.11, 97.57, 66.12, 56.68, 56.59, 56.16. IR (KBr) 2937, 1705, 1640, 1607, 1590, 1517, 1461, 1401, 1374, 1310, 1174, 1129, 1110, 1033, 975, 937, 867, 823, 810, 746, 650, 501, 476 cm^{-1}; HRMS (+ESI) 395.1265 [M + Na]$^+$ (calcd. for $C_{21}H_{21}FO_5Na^+$ 395.1265).

*5.1.24.22. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2-chlorophenyl) acrylate (**76**).* Light yellow solid. Yield: 90.3%. Mp 65.2–65.5 °C. TLC: $R_f = 0.56$ (1:1 EtOAc/hexanes). HPLC (purity 99.2%): $t_R = 10.75$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.13 (d, $J = 16.0$ Hz, 1H), 7.62 (d, $J = 7.5$ Hz, 1H), 7.41 (d, $J = 7.6$ Hz, 1H), 7.34–7.24 (m, 2H), 7.00 (d, $J = 15.9$ Hz, 1H), 7.0 (s, 1H), 6.50 (s, 1H), 6.47 (d, $J = 16.0$ Hz, 1H), 6.26 (dt, $J = 15.8$, 6.7 Hz, 1H), 4.89 (d, $J = 6.7$ Hz, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.47, 151.81, 150.05, 143.41, 140.79, 135.06, 132.82, 131.15, 130.28, 129.30, 127.74, 127.19, 121.35, 120.87, 116.99, 110.11, 97.59, 66.25, 56.71, 56.61, 56.18. IR (KBr) 1708, 1636, 1606, 1586, 1513, 1468, 1403, 1369, 1315, 1267, 1230, 1211, 1174, 1135, 1030, 973, 941, 859, 835, 767, 748, 681, 585, 448 cm^{-1}; HRMS (+ESI) 411.0971 [M + Na]$^+$ (calcd. for $C_{21}H_{21}ClO_5Na^+$ 411.0970).

5.1.24.23. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(3-chlorophenyl) acrylate (77). Light yellow solid. Yield: 82.4%. Mp 78.2–78.4 °C. TLC: $R_f = 0.60$ (1:1 EtOAc/hexanes). HPLC (purity 97.4%): $t_R = 11.66$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.64 (d, $J = 16.0$ Hz, 1H), 7.50 (s, 1H), 7.39 (d, $J = 7.4$ Hz, 1H), 7.36–7.28 (m, 2H), 6.99 (d, $J = 15.9$ Hz, 1H), 6.99 (s, 1H), 6.50 (s, 1H), 6.47 (d, $J = 16.0$ Hz, 1H), 6.25 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.87 (d, $J = 6.7$ Hz, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.51, 151.82, 150.08, 143.40, 143.36, 136.35, 135.01, 130.24, 129.41, 127.92, 126.32, 121.27, 119.70, 116.93, 110.12, 97.56, 66.25, 56.68, 56.60, 56.17. IR (KBr) 3430, 2930, 1721, 1639, 1611, 1564, 1519, 1476, 1405, 1378, 1345, 1295, 1272, 1209, 1169, 1124, 1076, 1029, 1003, 970, 886, 858, 822, 784, 735, 671, 577 cm^{-1}; HRMS (+ESI) 411.0972 [M + Na]$^+$ (calcd. for $C_{21}H_{21}ClO_5Na^+$ 411.0970).

*5.1.24.24. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(4-chlorophenyl) acrylate (**78**).* Light yellow solid. Yield: 85.1%. Mp 92.6–92.9 °C. TLC: $R_f = 0.56$ (1:1 EtOAc/hexanes). HPLC (purity 98.1%): $t_R = 9.27$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.66 (d, $J = 16.0$ Hz, 1H), 7.44 (d, $J = 8.5$ Hz, 2H), 7.35 (d, $J = 8.5$ Hz, 2H), 6.99 (d, $J = 16.0$ Hz, 1H), 6.99 (s, H), 6.49 (s, 1H), 6.44 (d, $J = 16.0$ Hz, 1H), 6.29–6.21 (m, 1H), 4.86 (dd, $J = 6.7$, 1.0 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.70, 151.83, 150.09, 143.56, 143.42, 136.30, 133.03, 129.40, 129.35, 129.30, 121.33, 118.81, 116.96, 110.13, 97.58, 66.21, 56.70, 56.62, 56.19. IR (KBr) 2937, 2833, 1705, 1640, 1607, 1590, 1517, 1461, 1401, 1374, 1310, 1213, 1130, 1111, 1033, 976, 937, 868, 847, 823, 810, 746, 650, 501, 476 cm^{-1}; HRMS (+ESI) 411.0972 [M + Na]$^+$ (calcd. for $C_{21}H_{21}ClO_5Na^+$ 411.0970).

*5.1.24.25. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2-bromophenyl)acrylate (**79**).* Light yellow solid. Yield: 79.3%. Mp 87.3–87.6 °C. TLC: $R_f = 0.60$ (1:1 EtOAc/hexanes). HPLC (purity 97.2%): $t_R = 9.03$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.09 (d, $J = 15.9$ Hz, 1H), 7.60 (ddd, $J = 7.6$, 4.3, 1.0 Hz, 2H), 7.32 (t, $J = 7.5$ Hz, 1H), 7.22 (t, $J = 7.7$ Hz, 1H), 7.00 (d, $J = 15.9$ Hz, 1H), 6.99 (s, 1H), 6.50 (s, 1H), 6.43 (d, $J = 15.9$ Hz, 1H), 6.26 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.88 (d, $J = 6.7$ Hz, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ166.38, 151.83, 150.07, 143.43, 143.36, 134.64, 133.56, 131.32, 129.30, 127.90, 127.83, 125.46, 121.37, 121.08, 117.01, 110.13, 97.61, 66.26, 56.73, 56.63, 56.20. IR (KBr) 3449, 1712, 1636, 1609, 1513, 1469, 1439, 1407, 1314, 1263, 1208, 1178, 1032, 973, 855, 762 cm^{-1}; HRMS (+ESI) 455.0464 [M + Na]$^+$ (calcd. for $C_{21}H_{21}BrO_5Na^+$ 455.0465).

*5.1.24.26. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(3-bromophenyl)acrylate (**80**).* White solid. Yield: 86.3%. Mp 77.5–77.8 °C. TLC: $R_f = 0.58$ (1:1 EtOAc/hexanes). HPLC (purity 98.5%): $t_R = 9.49$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.71 (s, 1H), 7.68 (d, $J = 16.0$ Hz, 1H), 7.54 (d, $J = 8.0$ Hz, 1H), 7.48 (d, $J = 7.8$ Hz, 1H), 7.30 (dd, $J = 12.7$, 4.7 Hz, 1H), 7.04 (d, $J = 16.0$ Hz, 1H), 7.04 (s, 1H), 6.55 (s, 1H), 6.51 (d, $J = 16.0$ Hz, 1H), 6.30 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.92 (d, $J = 6.7$ Hz, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.89 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.47, 151.82, 150.08, 143.40, 143.25, 136.63, 133.14, 130.87, 130.50, 129.41, 126.75, 123.12, 121.26, 119.72, 116.93, 110.12, 97.56, 66.24, 56.68, 56.60, 56.17. IR (KBr) 2927, 1720, 1638, 1611, 1559, 1518, 1473, 1404, 1377, 1296, 1209, 1029, 1002, 969, 883, 859, 821, 782, 729, 669, 575, 533 cm^{-1}; HRMS (+ESI) 455.0465 [M + Na]$^+$ (calcd. for $C_{21}H_{21}BrO_5Na^+$ 455.0465).

*5.1.24.27. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(4-bromophenyl)acrylate (**81**).* White solid. Yield: 81.6%. Mp 105.3–105.6 °C. TLC: $R_f = 0.56$ (1:1 EtOAc/hexanes). HPLC (purity 97.0%): $t_R = 9.61$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.64 (d, $J = 16.0$ Hz, 1H), 7.51 (d, $J = 8.4$ Hz, 2H), 7.38 (d, $J = 8.4$ Hz, 2H), 6.99 (d, $J = 15.9$ Hz, 1H), 6.99 (s, 1H), 6.50 (s, 1H), 6.46 (d, $J = 16.0$ Hz, 1H), 6.24 (dt, $J = 15.9$, 6.7 Hz, 1H), 4.86 (d, $J = 6.7$ Hz, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ166.68, 151.83, 150.09, 143.62, 143.42, 133.46, 132.26, 129.56, 129.41, 124.65, 121.31, 118.93, 116.95, 110.13, 97.58, 66.22, 56.70, 56.62, 56.19. IR (KBr) 3437, 2936, 2837, 1705, 1640, 1607, 1586, 1517, 1487, 1462, 1450, 1399, 1311, 1291, 1276, 1213, 1176, 1129, 1110, 1071, 1043, 1033, 1008, 974, 937, 870, 820, 496 cm^{-1}; HRMS (+ESI) 455.0463 [M + Na]$^+$ (calcd. for $C_{21}H_{21}BrO_5Na^+$ 455.0465).

*5.1.24.28. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(2-(trifluoromethyl)phenyl)acrylate (**82**).* White solid. Yield: 74.2%. Mp 84.8–85.1 °C. TLC: $R_f = 0.65$ (1:1 EtOAc/hexanes). HPLC (purity 99.0%): $t_R = 7.77$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.10 (dd, $J = 15.8$, 1.7 Hz, 1H), 7.69 (d, $J = 8.0$ Hz, 2H), 7.55 (t, $J = 7.6$ Hz, 1H), 7.47 (t, $J = 7.6$ Hz, 1H), 7.00 (d, $J = 15.7$ Hz, 1H), 6.99 (s, 1H), 6.50 (s, 1H), 6.44 (d, $J = 15.8$ Hz, 1H), 6.26 (dt, $J = 16.0$, 6.6 Hz, 1H), 4.88 (dd, $J = 6.7$, 1.0 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.06, 151.84, 150.07, 143.42, 140.45, 133.52, 132.21, 129.69, 129.30, 128.02, 126.25, 124.94, 123.12, 122.58, 121.26, 116.98, 110.14, 97.60, 66.31, 56.69, 56.61, 56.18. IR (KBr) 2948, 1715, 1639, 1611, 1520, 1469, 1404, 1317, 1209, 1182, 1110, 1032, 979, 870, 852, 819, 770, 652, 593, 464 cm^{-1}; HRMS (+ESI) 445.1236 [M + Na]$^+$ (calcd. for $C_{22}H_{21}F_3O_5Na^+$ 445.1233).

*5.1.24.29. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(3-(trifluoromethyl)phenyl)acrylate (**83**).* White solid. Yield: 77.7%. Mp 126.4–126.6 °C. TLC: $R_f = 0.56$ (1:1 EtOAc/hexanes). HPLC (purity 100.0%): $t_R = 10.31$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.75 (s, 1H), 7.72 (d, $J = 16.0$ Hz, 1H), 7.68 (d, $J = 7.7$ Hz, 1H), 7.62 (d, $J = 7.6$ Hz, 1H), 7.50 (t, $J = 7.7$ Hz, 1H), 6.99 (d, $J = 15.7$ Hz, 1H), 6.99 (s, 1H), 6.53 (d, $J = 16.0$ Hz, 1H), 6.50 (s, 1H), 6.25 (dt, $J = 6.7$, 15.8 Hz, 1H), 4.88 (d, $J = 6.6$ Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.36, 151.83, 150.10, 143.40, 143.13, 135.32, 131.64, 131.13, 129.56, 129.49, 126.73, 124.69, 122.97, 121.17, 120.20, 116.89, 110.12, 97.54, 66.30, 56.65, 56.59, 56.15. IR (KBr) 2936, 1715, 1643, 1624, 1518, 1492, 1468, 1436, 1402, 1382, 1337, 1163, 1035, 983, 860, 804, 774, 761, 743, 711, 692, 659, 636, 603, 577, 559, 513, 449, 416 cm^{-1}; HRMS (+ESI) 445.1236 $[M + Na]^+$ (calcd. for $C_{22}H_{24}O_6Na^+$ 445.1233).

*5.1.24.30. (E)-3-(2,4,5-trimethoxyphenyl)allyl (E)-3-(4-(trifluoromethyl)phenyl)acrylate (**84**).* Light yellow solid. Yield: 84.8%. Mp 109.5–109.7 °C. TLC: $R_f = 0.66$ (1:1 EtOAc/hexanes). HPLC (purity 98.6%): $t_R = 10.38$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.72 (d, $J = 16.0$ Hz, 1H), 7.63 (q, $J = 8.6$ Hz, 4H), 7.00 (d, $J = 15.6$ Hz, 1H), 6.99 (s, 1H), 6.54 (d, $J = 16.0$ Hz, 1H), 6.50 (s, 1H), 6.25 (dt, $J = 6.8$, 15.9 Hz, 1H), 4.88 (d, $J = 6.7$ Hz, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.38, 151.87, 150.15, 143.43, 143.12, 137.92, 131.76, 129.59, 128.30, 125.98, 124.84, 123.03, 121.15, 120.82, 116.89, 110.16, 97.57, 66.38, 56.69, 56.63, 56.19. IR (KBr) 3449, 2943, 2838, 1704, 1612, 1519, 1469, 1448, 1405, 1325, 1289, 1248, 1211, 1157, 1118, 1065, 1048, 1028, 1010, 983, 963, 866, 832, 753, 656, 591, 502, 418 cm^{-1}; HRMS (+ESI) 445.1235 $[M + Na]^+$ (calcd. for $C_{22}H_{21}F_3O_5Na^+$ 445.1233).

*5.1.24.31. 3-(2,4,5-Trimethoxyphenyl)propyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (**85**).* White solid. Yield: 71.2%. Mp 50.0–50.4 °C. TLC: $R_f = 0.58$ (1:1 EtOAc/hexanes). HPLC (purity 98.5%): $t_R = 7.19$ min ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.58 (d, $J = 15.9$ Hz, 1H), 6.76 (s, 2H), 6.71 (s, 1H), 6.51 (s, 1H), 6.36 (d, $J = 15.9$ Hz, 1H), 4.22 (t, $J = 6.5$ Hz, 2H), 3.89 (s, 6H), 3.88 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 2.68 (t, $J = 7.4$ Hz, 2H), 1.98 (p, $J = 6.7$ Hz, 2H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.14, 153.56, 151.69, 148.01, 144.68, 142.93, 140.18, 130.08, 121.19, 117.63, 114.42, 105.31, 97.86, 64.43, 61.11, 56.85, 56.39, 56.36, 56.29, 29.30, 26.42. IR (KBr) 3448, 2942, 2839, 1704, 1634, 1582, 1522, 1510, 1461, 1423, 1397, 1339, 1311, 1261, 1245, 1229, 1229, 1207, 1154, 1129, 1065, 1037, 1004, 975, 854, 822 cm^{-1}. HRMS (+ESI) 447.2015 $[M + H]^+$, 469.1833 $[M + Na]^+$ (calcd. for $C_{24}H_{31}O_8^+$ 447.2013 and $C_{24}H_{30}O_8Na^+$ 469.1833).

*5.1.25. General method for the preparation of cinnamic acid amide derivatives (**86** and **87**)*

A mixture of the compound **5** or **6** (5.0 mmol), cinnamic acid derivatives (6 mmol), and HOBt (6.0 mmol) in DCM (15.0 mL) was stirred for 10 min at room temperature. Successively, EDCI (8.0 mmol) were added quickly in one portion. The reaction mixture was continually stirred at room temperature for 10 h under nitrogen and monitored by TLC. After workup and purification as same as the procedures of cinnamic acid esters, the final amides were obtained as solid substances in good yield.

*5.1.25.1. (E)-3-(3,4,5-trimethoxyphenyl)-N-((E)-3-(2,4,5-trimethoxyphenyl)allyl) acrylamide (**86**).* Light yellow solid. Yield: 80.8%. Mp 68.1–68.4 °C. TLC: $R_f = 0.30$ (1:1 EtOAc/hexanes). HPLC (purity 95.4%): $t_R = 5.12$ min ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.55 (d, $J = 15.5$ Hz, 1H), 6.81 (s, 1H), 6.72 (s, 2H), 6.56 (s, 1H), 6.54 (d, $J = 9.2$ Hz, 1H), 6.32 (d, $J = 15.5$ Hz, 1H), 6.06 (ddd, $J = 5.1$, 10.3, 17.1 Hz, 1H), 5.81 (ddt, $J = 1.6$, 4.9, 8.6 Hz, 1H), 5.18–5.12 (m, 2H), 3.88 (s, 3H), 3.87 (s, 6H), 3.86 (s, 6H), 3.84 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 164.75, 153.53, 151.69, 149.40, 143.35, 141.31, 139.72, 137.89, 130.63, 120.37, 120.06, 114.85, 113.43, 105.14, 98.54, 61.08, 56.77, 56.70, 56.39, 56.30, 53.24. IR (KBr) 3361, 3283, 2987, 2939, 2836, 1659, 1618, 1580, 1512, 1462, 1415, 1403, 1325, 1277, 1248, 1209, 1152, 1127, 1034, 1007, 828, 608 cm^{-1}. HRMS (+ESI) 444.2000 $[M + H]^+$ (calcd. for $C_{24}H_{30}NO_7^+$ 444.2017).

*5.1.25.2. (E)-3-(3,4,5-trimethoxyphenyl)-N-(3-(2,4,5-trimethoxyphenyl)propyl)acrylamide (**87**).* White solid. Yield: 86.6%. Mp 159.6–159.8 °C. TLC: $R_f = 0.27$ (1:1 EtOAc/hexanes). HPLC (purity 100.0%): $t_R = 6.22$ min ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.52 (d, $J = 15.5$ Hz, 1H), 6.78 (s, 1H), 6.72 (s, 2H), 6.59 (d, $J = 9.2$ Hz, 1H), 6.55 (s, 1H), 6.29 (d, $J = 15.5$ Hz, 1H), 5.09–5.02 (m, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.87 (s, 6H), 3.86 (s, 3H), 3.84 (s, 3H), 1.93–1.80 (m, 2H), 0.88 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 164.74, 153.39, 151.47, 148.75, 142.97, 140.78, 130.62, 121.21, 120.66, 113.64, 104.98, 98.25, 60.95, 56.66, 56.35, 56.27, 56.17, 53.84, 28.63, 11.24. IR (KBr) 3422, 3291, 2957, 2937, 2839, 1658, 1616, 1584, 1510, 1461, 1421, 1324, 1278, 1240, 1209, 1152, 1128, 1035, 1001, 829, 610 cm^{-1}. HRMS (+ESI) 446.2151 $[M + H]^+$ (calcd. for $C_{24}H_{32}NO_7^+$ 446.2173).

5.2. Biological evaluation

5.2.1. Animals and drugs

All animals experiments were performed with the use of male Kunming mice (Experimental Animal Center, Xi'an Jiaotong University, Xi'an, China) weighing 25–30 g. All the mice were kept under controlled environmental conditions (22 ± 2 °C; 50 ± 20% humidity; 12/12 h light/dark cycle) with free access to pellet food and water. Experiments were performed following approval of Committee on Ethics of Experimental Animal Administration of the Northwest University and were in accordance with the National Institutes of Health guidelines [76]. Each experiment consisted of several groups of 4–6 mice, one group received the vehicle control (normal saline (NS) containing 0.5% Tween-80) and the other groups received different doses of tested compounds. All tested compounds were dissolved in 0.5% Tween-80 and injected ip to mice (10 mL/kg volume) 30 min before testing. In *sc*PTZ and *sc*3-MP tests, the time-effect relationship was determined at 0.25, 0.5, 1.0 and 2.0 h after drugs treatment. PTZ was purchased from Alfa Aesar, Shanghai, China. Lot: 10180463; 3-MP was purchased from J&K Scientific Ltd., Beijing, China. Lot: LD50Q10; CBZ (J&K Scientific Ltd., Beijing, China. Lot: LLA0P07), STP (TCI, Shanghai, China. Lot: YDNLC-CP) and VPA (J&K Scientific Ltd., Beijing, China. Lot: LV10N57) were used as reference anticonvulsant drugs for comparison.

5.2.2. Maximal electroshock seizure (MES) test

The MES test were carried out according to previously described method [45]. In experiments with mice, a current of 50 mA with a pulse frequency of 50 Hz for 0.2 s was applied *via* ear-clip electrodes by an electronic stimulator (SEN-3201, Nihon Konden). 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). For the vehicle group, stimulation with this current caused 100% tonic hind limb extension in mice. For the test groups, 0.5, 1, 2, 3 and 4 h after administration of the compounds all the mice were treated with current electrical stimulation. An effective dose protecting 50% of the mice (ED_{50}) of the most active compounds against the tonic hind limb extension and its associated 95% confidence interval were calculated by Probit analysis.

5.2.3. scPentylenetetrazole (scPTZ)-induced seizure test

The anticonvulsant activity of the test compounds was determined with *sc*PTZ-induced seizure test. In this model of induced seizure, 85 mg/kg of freshly prepared solution of PTZ was administered subcutaneously to all the mice. This produces tonic seizures could last at least 5 s in 97% of animals tested. Each group of 4 mice were tested at 0.25, 0.5, 1.0 and 2.0 h following doses of 100 and 300 mg/kg of compounds. The mice were considered to be protective when the compounds were in the absence of the effect of PTZ on seizure threshold (last at least 5 s). The percentage of incidence of tonic seizures, clonic seizures (jerking, twitching, etc.) as well as the mortality rate were observed for 30 min after PTZ injection. Additionally, the median effective dose (ED_{50}) and 95% confidence intervals of some most active compounds were calculated by Probit-regression method in SPSS software (Version 13.0).

5.2.4. 3-Mercaptopropionic acid (3-MP)-induced seizure test

The anticonvulsant activity of the selected compounds was determined with *sc*3-MP-induced seizure test. In this model of induced seizure, 60 mg/kg of freshly prepared solution of 3-MP was administered subcutaneously to all the mice. It produced tonic seizures that last at least 5 s in 97% of animals tested. Each group of 4 mice were tested at 0.25, 0.5, 1.0 and 2.0 h following doses of 100 and 300 mg/kg of each compound. The mice were considered protected when the compound in the absence or presence of the effect of 3-MP on seizure threshold (a 5 s episode of tonic convulsions). Meanwhile, the percentage of incidence of tonic seizures, clonic seizures as well as the mortality rate were observed for 30 min after 3-MP injection. The median effective dose (ED_{50}) and 95% confidence intervals of the most active compounds were calculated by Probit-regression method in SPSS software.

5.2.5. Neurotoxicity screening

The neurotoxicity of compounds **55**–**87** was assessed by evaluating motor coordination (minimal motor impairment) in mice using rotarod test. The mice were trained to position on a 4 cm diameter rod that rotated at a constant speed of 24 rpm. Then, trained mice were administrated ip. with test compounds at the doses of 50, and 100 mg/kg 0.5, 1, and 2 h after compounds treatment, each animal was placed on rotarod that rotated at 24 rpm. Neurologic toxicity was defined as the failure (drop more than 3 times from rod in 3 min) of the mice to remain on the rod for 3 min. TD_{50} for the most active compounds (doses of 200, 400, 600, 800 and 1000 mg/kg, ip) at 0.5 h were also calculated based on Probit-regression method in SPSS software.

5.2.6. Formalin-induced paw licking test

Formalin-induced tonic pain were carried out according to Hunskaar and Hole [77]. The selected compounds or vehicle were administered by intraperitoneal injection to each mouse. Thirty minutes after pretreatment, an intrapaw injection of 2.5% formalin solution (25 μL) was given to the right sub-plantar surface of the mice, and then the mice were returned to the observation cages. The compound's efficacy in the 2nd phase (15–30 min after formalin injection) was viewed as predictive of efficacy in animal models of neuropathic pain [78]. Thus, in our study, the duration time that the mice spent licking or biting its right hindpaw was determined and recorded for each consecutive 5 min time binned for 30 min after the injection of formalin in the 2nd-phase of the test. The selected compound's main outcome measure was the 2nd-phase value at which 50% of mice showed a paw lick duration of nociceptive response less than 80 s (ED_{50}), which corresponds to a 50% reduction of the nociceptive response compared with the mean of vehicle-treated (normal saline) mice. Then, the ED_{50} values, with 95% confidence intervals, were calculated using Probit-regression method in SPSS software.

5.2.7. LDH inhibition assay

5.2.7.1. UV-VIS spectrophotometry method. This assay was carried out using, with slight modification, the procedure described by Carlotta [56] and Sada [12] respectively. The effect of compounds **55** to **87** on LDH enzyme activity was measured using purified human lactate dehydrogenase isoforms 1 and 5 (Lee Biosolution, Inc. LDH1, Lot No. MS7381; LDH5, Lot No: 04B2913). LDH activity was determined at 37 °C by measuring the oxidation of NADH spectrophotometrically at various wavelengths (supporting Information Table 4) in the direction of pyruvate to lactate. The apparent Michaelis-Menten constants (K_m) of LDH1 and LDH5 for NADH and pyruvate were measured from Lineweaver-Burk plots and determined in saturating conditions as described below. In 100 mM sodium phosphate buffer (pH 7.5), 0.015 units of LDH1 or LDH5 were combined with 1.0 mM sodium pyruvate and 0.2 mM NADH. This condition was used for the evaluation of the percent inhibition ratio of the compounds. The reaction velocity of LDH1 or LDH5 was determined spectrophotometrically (Agilent Cary 8453) by a decrease in absorbance at the optimum detection wavelength ($\varepsilon = 6.2\ mM^{-1}\ cm^{-1}$) of NADH, and the value of optical density was determined at 30 s intervals for 4.5 min. K_m values for substrate were determined from initial rate measurements at 37 °C by nonlinear regression analysis with the GraphPad Prism 5.0. Since all of **C** series of compounds were poorly soluble in water, 0.5% DMSO and 0.5% Tween-80 as cosolvent were added to reaction mixture without reducing the LDH activity. In addition, inhibition mode and K_i values for the representative compounds (**65**, **68**, and **69**) were determined by the following conditions. In 100 mM sodium phosphate buffer (pH 7.5), 0.015 units of LDH1 or LDH5 were combined with 0.2 mM NADH and 0.1 mM–1.0 mM sodium pyruvate. Then, the inhibition mode and K_i values for each single inhibitor were determined using Lineweaver-Burk plots.

5.2.7.2. Multiple reaction monitoring (MRM) method. The reaction velocity of purified human LDH1 and LDH5 was determined by an increase of lactic acid. The concentration of lactic acid was determined at 60 s intervals for 5 min. In the test, acetonitrile (100 μL) was added to the portion of reaction mixture (100 μL) to cease the reaction, which was subjected to vortex (30 s), centrifugation (9000 rpm), and water dilution (800 μL) procedures. Then the mixture was detected by MRM conditions (supporting information).

5.2.8. Cells expressing GABAa receptors

A stable HEK293T-GABAa cell line expressing GABAa receptor subunits $\alpha1\beta2\gamma2$ (kindly provided by PharmaCore Labs (PCL) Co., Ltd) was used for the study. Cells were maintained in cell culture medium (DMEM, 11% FBS, 100 μg/ml G418, 40 μg/ml Hygromycin B, 80 μg/ml Zeocin) and incubated at 37 °C in a humidified incubator with 5% CO_2 and 95% air. 24–48 h prior to electrophysiological recordings, cells were split and seeded onto round glass cover slips placed in Petri dishes and maintained under the same incubation and media conditions.

5.2.9. Electrophysiological recordings

The electrophysiological characterization of stiripentol and compound **70** at $GABA_A$Rs was performed essentially as described by Christian et al. [79]. Cover slips with cells were transferred to the stage of a TI-S-FLU microscope (Nikon, Japan), and were perfused with external solution at room temperature (22–25 °C). The external solution consisted of (in mM): 140 NaCl, 4.7 KCl, 1.0 $MgCl_2$, 2.0 $CaCl_2$, 11 glucose, and 10 HEPES (4-(2-hydroxyethyl)-1-piperazineethanesul fonic acid) with pH = 7.4 and osmolarity

adjusted to 295–305 mOsm. Recording electrodes of 2–4 MΩ tip resistance were made with P97 micropipette puller (Sutter Instrument Company, One Digital Drive, Novato, CA 94949) from borosilicate glass tubings with filament (BF150-86-10, Sutter Instrument Company) and filled with an internal solution of (in mM): 110 CsCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$, 10 HEPES, 10 EGTA (Ethylene glycol-bis(2-aminoethylether)- *N*,*N*,*N*′,*N*′-tetraacetic acid), 2.0 ATP-Na_2 and 25 TEA·Cl with pH = 7.2 and osmolarity adjusted to 300–310 mOsm. Patch-clamp techniques in voltage clamp mode were used to record from HEK293T-GABAa cells in the whole-cell configuration using an EPC-10 amplifier (HEKA, Germany). Cells were held at −50 mV during the recordings. Whole-cell currents were analyzed using Patchmaster software (HEKA, Germany). To allow quick switch and exchange of the test compounds, perfusion during the recordings was achieved through an 8 Channel Bath Perfusion System (VC^3-8PG, ALA Scientific Instruments, Inc., USA). All experiments were performed at room temperature.

5.3. Pains

All of the active compounds were electronically filtered for structural attributes consistent with classification as pan-assay interference compounds (PAINS) and were found to be negative [80,81].

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111650.

Abbreviations used

ADME	Absorption, Distribution, Metabolism, Excretion
AED	antiepileptic drug
CBZ	carbamazepine
CFDA	China Food and Drug Administration
m-CPBA	*m*-chloroperbenzoic acid
CTCMMC	Combination of traditional Chinese medicine molecular chemistry
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutyl aluminum hydride
DMAP	4-dimethylaminopyridine
ED	electron-donating
EW	electron-withdrawing
EC_{50}	effective concentration (50%)
ED_{50}	effective dose (50%)
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HMTA	hexamethylenetetramine
ip	intraperitoneal
LAH	lithium aluminum hydride
LDH	lactate dehydrogenase
NADH	β-nicotinamide adenine dinucleotide disodium salt
S/N	Signal-to-Noise
ORTEP	oak ridge thermal-ellipsoid plot program
PK	pharmacokinetics
SAR	structure-activity relationship
*sc*PTZ	subcutaneous injection-pentylenetetrazole
*sc*3-MP	3-mercaptopropionic acid
TCM	Traditional Chinese medicine
TD_{50}	neurological impairment (toxicity, 50%)
TFA	trifluoroacetic acid
3,4,5-TMCA	3,4,5-trimethoxycinnamic acid

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