


Cite this: *RSC Adv.*, 2019, 9, 24942

## Biological activity evaluation and action mechanism of chalcone derivatives containing thiophene sulfonate†

Tao Guo, Rongjiao Xia, Mei Chen, Jun He, Shijun Su, Liwei Liu, Xiangyang Li and Wei Xue \*

A series of novel chalcone derivatives containing a thiophene sulfonate group were designed and synthesized. The structures of all title compounds were determined by  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR and HRMS. Antibacterial bioassays indicated that, compound **2l** demonstrated excellent antibacterial activities against *Xanthomonas axonopodis* pv. *citri* (Xac), with an  $\text{EC}_{50}$  value of  $11.4\ \mu\text{g mL}^{-1}$ , which is significantly superior to those of bismethiazol (BT) ( $51.6\ \mu\text{g mL}^{-1}$ ) and thiodiazole-copper (TC) ( $94.7\ \mu\text{g mL}^{-1}$ ). Meanwhile, the mechanism of action of compound **2l** was confirmed by using scanning electron microscopy (SEM). In addition, compound **2e** showed remarkable inactivation activity against *Tobacco mosaic virus* (TMV), with an  $\text{EC}_{50}$  value of  $44.3\ \mu\text{g mL}^{-1}$ , which was superior to that of ningnanmycin ( $120.6\ \mu\text{g mL}^{-1}$ ). Microscale thermophoresis (MST) also showed that the binding of compounds **2e** and **2h** to *Tobacco mosaic virus* coat protein (TMV-CP) yielded  $K_d$  values of 0.270 and  $0.301\ \mu\text{mol L}^{-1}$ , which are better than that of ningnanmycin ( $0.596\ \mu\text{mol L}^{-1}$ ). At the same time, molecular docking studies for **2e** and **2h** with TMV-CP (PDB code: 1EI7) showed that the compound was embedded well in the pocket between the two subunits of TMV-CP in each case. These results suggested that chalcone derivatives containing a thiophene sulfonate group may be considered as activators in the design of antibacterial and antiviral agents.

Received 12th July 2019  
Accepted 6th August 2019

DOI: 10.1039/c9ra05349b

rsc.li/rsc-advances

## 1. Introduction

The bacterial and virus infections of crops have become some of the world's most important agricultural problems, and the threat they pose is not only about agricultural production but also about human health.<sup>1–3</sup> Pathogens that cause bacterial diseases, such as *Xanthomonas oryzae* pv. *oryzae* (Xoo), *Ralstonia solanacearum* (Rs) and *Xanthomonas axonopodis* pv. *citri* (Xac)<sup>4–6</sup> are the world's most important and well-known plant bacteria.<sup>7–11</sup> These bacteria can actively suppress the crop yields worldwide. Besides, *Tobacco mosaic virus* (TMV), one of the world's most severe pathogenic viruses can infect a variety of crops, such as tobacco, cucumbers and other economic crops, resulting in a significant loss of crops each year.<sup>12–14</sup> To overcome these serious problems, antibacterial drugs such as bismethiazol (BT), thiodiazole-copper (TC), and ningnanmycin have been widely developed and used to reduce the infection by

bacteria and plant viruses.<sup>15–17</sup> However, prolonged use and abuse of traditional antibacterial drugs lead to increased resistance of pathogenic genes, which not only enhances the resistance of target pathogens but is also harmful to the environment and health of the plant.<sup>18–20</sup> Therefore, the development of new antibacterial and antiviral agents is an important task in pesticide science.

Recent studies have shown that chalcone readily binds to receptors of different structures to form chalcone derivatives due to its flexible structure.<sup>21–23</sup> Its derivatives have bactericidal,<sup>24,25</sup> insecticidal,<sup>26,27</sup> anticancer,<sup>28</sup> antiviral,<sup>29,30</sup> and anti-inflammatory<sup>31</sup> activities, thus demonstrating a wide range of applications in chemical and biological research. Thiophene sulfonate plays a prominent role in the studies and applications of organic chemistry.<sup>32,33</sup> As a biologically important active intermediate, it is often used to synthesize thiophene sulfonate compounds with various biological activities.<sup>34–36</sup> Therefore, thiophene sulfonate and its derivatives have a wide range of applications in industry, agriculture, pharmaceuticals and have a broad spectrum of biological activities, such as insecticidal,<sup>37,38</sup> anti-inflammatory,<sup>39,40</sup> antiviral,<sup>41,42</sup> and anticancer.<sup>43</sup>

However, to the best of our knowledge, there have been no reports on chalcone-based thiophene sulfonates so far. To find excellent biological activities and environment-friendly small organic molecules, thiophene sulfonate structure with excellent

State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Center for Research and Development of Fine Chemicals, Guizhou University, Guiyang, 550025, China. E-mail: wxue@gzu.edu.cn; Fax: +86-851-88292090; Tel: +86-851-88292090

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9ra05349b



biological activity is combined with the structure of chalcone by active splicing to design and synthesize a series of novel molecules. Through biological activity screening, it is expected to find chalcone compounds with high anti-plant viral and anti-bacterial activities, which lays a theoretical foundation for the creation of new pesticides.

## 2. Experimental

### 2.1. Instrument, chemicals and reagents

The molecular docking was performed by using DS-CDocker implemented in Discovery Studio (version 4.5). Melting points of the synthesized compounds (**2a–2v**) were measured using XT-4 Binocular Microscope (Beijing Tech. Instrument, China) without correction.  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and  $^{19}\text{F-NMR}$  spectra were obtained on a Bruker Ascend-400 spectrometer (Bruker Optics, Switzerland) and DMSO- $d_6$  or  $\text{CDCl}_3$  were used as a solvent and TMS was used as an internal standard. HRMS data were obtained using Thermo Scientific Q Exactive Hybrid Quadrupole Mass Spectrometer (Thermo Scientific Inc., St Louis, MO, USA). The microscale thermophoresis (MST) of the compounds to check the interaction with TMV-CP (*Tobacco mosaic virus* coat protein) was determined using a micro thermophoresis instrument (NanoTemper Technologies GmbH, Germany). All reagents and solvents were purchased from Chinese Chemical Reagent Company and were chemically pure analytical grade reagents. The synthetic route of chalcone derivatives containing thiophene sulfonate is shown in Scheme 1. The intermediate **1** were prepared according to the methods already reported in the literature.<sup>44</sup>

### 2.2. General procedure for the synthesis of intermediate 1

At first, aqueous sodium hydroxide solution (5% NaOH, 5 mmol) was added to a round-bottomed flask containing 1-(4-hydroxyphenyl)ethan-1-one (4 mmol) and differently substituted aldehydes (6 mmol). The mixture was stirred at ambient temperature for 12 h. The resulting dark-yellow mixture was acidified by HCl (5% HCl, 5 mmol) after the reaction was completed. Finally, the mixture was filtered under vacuum, and the residue was dried to yield intermediate **1**.

### 2.3. General procedure for the synthesis of title compounds **2a–2v**

A solution of intermediate **1** (6.5 mmol) and 2-thiophenesulfonyl chloride (5.5 mmol) in acetonitrile (50 mL) was stirred until dissolved, the reaction mixture was refluxed at 90 °C for 10 h. After completion of the reaction, the whole reaction system was poured into water, extracted with ethyl acetate, the solvent was removed under depressurization, and the residue was purified by column chromatography on silica gel with a mixture (v(petroleum ether) : v(ethyl acetate) = 3 : 1) to yield the title compounds **2a–2v**.<sup>45</sup> The  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ ,  $^{19}\text{F-NMR}$  and HMRS spectra of the designated title compounds **2a–2v** are also provided in the ESI.†

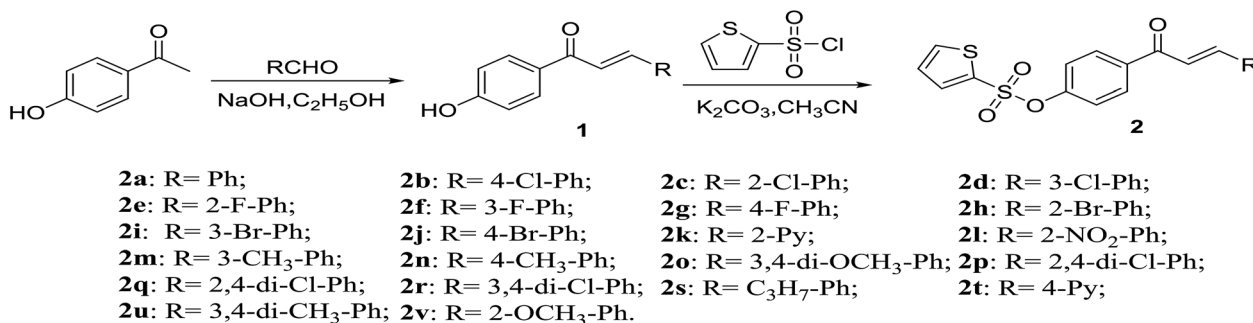
## 3. Results and discussion

### 3.1. Spectral properties

The structures of all the compounds were confirmed using  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and HRMS. In  $^1\text{H-NMR}$  spectra, multiplet signals at  $\delta$  8.27–7.26 ppm indicate the presence of protons in olefinic bonds and aromatic nuclei. The  $^{13}\text{C-NMR}$  spectra of the title compounds exhibited a characteristic (C=O) signal at  $\delta$  186.79–188.77 ppm. The  $^{13}\text{C-NMR}$  spectra of the title compounds exhibited a characteristic (thiophene-2-C) signal at  $\delta$  142.22–145.08 ppm. The strong presence of  $[\text{M} + \text{H}]^+$  ions indicates that the title compounds are in the steady state.

### 3.2. Antibacterial activity of title compounds against Xac, Xoo and Rs *in vitro*

The *in vitro* antibacterial activities of the title compounds **2a–2v** against three phytopathogenic bacteria (Xac, Xoo and Rs) were tested by using turbidimeter.<sup>46–48</sup> Commercial agricultural bactericides (TC and BT) were used as the control, as shown in Table 1, and most of the compounds exhibited significant antibacterial activities against Xac, Xoo and Rs at 100 or 50  $\mu\text{g mL}^{-1}$ . Among these, the antibacterial activities of the compounds **2e**, **2l** and **2p** against Xac at 100  $\mu\text{g mL}^{-1}$  were 77.2, 95.2 and 79.6%, respectively, which exceeded both TC (57.2%) and BT (65.3%). Compounds **2a**, **2f**, **2j**, **2l**, **2m**, **2o**, **2q** and **2u** against Xoo at 50  $\mu\text{g mL}^{-1}$  were 65.6, 60.8, 46.4, 58.0, 58.4, 58.5, 56.9 and 62.2%, respectively, which were better compared to TC (37.2%) and BT (45.2%). In particular, the antibacterial



Scheme 1 Synthetic route of title compounds **2a–2v**.



Table 1 Antibacterial activities of title compounds (2a–2v) against plant pathogens Xac, Xoo and Rs *in vitro*

Compounds	R	Xac (%)		Xoo (%)		Rs (%)	
		100 $\mu\text{g mL}^{-1}$	50 $\mu\text{g mL}^{-1}$	100 $\mu\text{g mL}^{-1}$	50 $\mu\text{g mL}^{-1}$	100 $\mu\text{g mL}^{-1}$	50 $\mu\text{g mL}^{-1}$
2a	Ph	67.5 $\pm$ 1.7	53.4 $\pm$ 1.6	72.4 $\pm$ 1.7	65.6 $\pm$ 1.4	76.6 $\pm$ 1.4	48.8 $\pm$ 1.4
2b	4-Cl-Ph	59.3 $\pm$ 2.5	22.6 $\pm$ 3.4	62.6 $\pm$ 1.3	43.8 $\pm$ 2.4	47.1 $\pm$ 3.7	40.3 $\pm$ 1.2
2c	2-Cl-Ph	45.3 $\pm$ 3.1	23.4 $\pm$ 2.9	52.9 $\pm$ 3.6	37.5 $\pm$ 2.9	53.3 $\pm$ 2.9	48.9 $\pm$ 2.5
2d	3-Cl-Ph	51.6 $\pm$ 0.9	36.7 $\pm$ 1.8	48.6 $\pm$ 2.1	29.5 $\pm$ 4.6	47.5 $\pm$ 1.0	36.8 $\pm$ 2.5
2e	2-F-Ph	77.2 $\pm$ 1.1	44.3 $\pm$ 2.1	50.5 $\pm$ 1.4	37.6 $\pm$ 1.6	71.1 $\pm$ 2.4	52.3 $\pm$ 2.4
2f	3-F-Ph	66.5 $\pm$ 1.5	44.6 $\pm$ 2.4	88.1 $\pm$ 1.7	60.8 $\pm$ 2.6	67.8 $\pm$ 0.6	55.1 $\pm$ 1.6
2g	4-F-Ph	63.5 $\pm$ 1.5	55.2 $\pm$ 1.9	43.2 $\pm$ 1.3	39.4 $\pm$ 1.6	58.3 $\pm$ 1.5	48.8 $\pm$ 1.3
2h	2-Br-Ph	52.9 $\pm$ 2.2	36.8 $\pm$ 0.9	53.3 $\pm$ 0.9	35.7 $\pm$ 3.7	52.6 $\pm$ 3.8	33.0 $\pm$ 0.8
2i	3-Br-Ph	46.5 $\pm$ 2.9	32.4 $\pm$ 3.5	36.0 $\pm$ 2.1	24.3 $\pm$ 1.4	40.8 $\pm$ 5.6	33.5 $\pm$ 1.9
2j	4-Br-Ph	52.5 $\pm$ 2.7	25.6 $\pm$ 1.7	77.3 $\pm$ 0.4	46.4 $\pm$ 2.6	56.7 $\pm$ 2.4	43.4 $\pm$ 2.5
2k	2-Py	59.7 $\pm$ 1.2	32.6 $\pm$ 1.8	43.2 $\pm$ 0.6	32.9 $\pm$ 1.2	73.7 $\pm$ 7.9	56.3 $\pm$ 2.0
2l	3-NO <sub>2</sub> -Ph	95.2 $\pm$ 0.7	72.3 $\pm$ 1.1	82.3 $\pm$ 1.1	58.0 $\pm$ 0.6	44.3 $\pm$ 3.5	21.3 $\pm$ 1.2
2m	3-CH <sub>3</sub> -Ph	44.4 $\pm$ 1.5	25.8 $\pm$ 1.7	65.3 $\pm$ 2.4	58.4 $\pm$ 1.5	91.4 $\pm$ 1.6	77.5 $\pm$ 1.3
2n	4-CH <sub>3</sub> -Ph	40.3 $\pm$ 2.8	22.4 $\pm$ 1.3	51.4 $\pm$ 2.5	22.6 $\pm$ 1.6	67.1 $\pm$ 1.9	53.3 $\pm$ 2.1
2o	3,4-di-OCH <sub>3</sub> -Ph	58.3 $\pm$ 0.5	38.7 $\pm$ 1.1	72.3 $\pm$ 1.5	58.5 $\pm$ 1.6	92.4 $\pm$ 1.2	63.3 $\pm$ 2.0
2p	2,4-di-Cl-Ph	79.6 $\pm$ 0.5	56.4 $\pm$ 1.1	44.6 $\pm$ 1.8	34.2 $\pm$ 0.9	85.2 $\pm$ 0.5	56.6 $\pm$ 2.0
2q	3,4-di-Cl-Ph	55.6 $\pm$ 2.5	18.7 $\pm$ 1.4	68.2 $\pm$ 1.1	56.9 $\pm$ 1.8	57.2 $\pm$ 1.3	47.2 $\pm$ 2.6
2r	C <sub>3</sub> H <sub>7</sub> -Ph	59.4 $\pm$ 1.4	21.4 $\pm$ 0.2	44.6 $\pm$ 1.8	34.2 $\pm$ 0.9	52.8 $\pm$ 1.6	32.2 $\pm$ 1.3
2s	2-Th	52.3 $\pm$ 2.1	38.7 $\pm$ 1.6	55.6 $\pm$ 0.4	41.8 $\pm$ 2.7	50.6 $\pm$ 1.7	42.5 $\pm$ 1.8
2t	4-Py	46.3 $\pm$ 0.8	26.1 $\pm$ 3.8	36.7 $\pm$ 1.9	25.9 $\pm$ 4.5	45.3 $\pm$ 3.2	37.6 $\pm$ 2.4
2u	3,4-di-CH <sub>3</sub> -Ph	52.4 $\pm$ 1.2	32.5 $\pm$ 1.4	83.3 $\pm$ 0.8	62.2 $\pm$ 1.0	66.3 $\pm$ 0.3	58.2 $\pm$ 0.9
2v	2-OCH <sub>3</sub> -Ph	56.2 $\pm$ 1.8	31.1 $\pm$ 1.9	56.7 $\pm$ 2.4	43.4 $\pm$ 2.5	42.3 $\pm$ 1.3	28.3 $\pm$ 2.5
TC <sup>a</sup>	—	57.2 $\pm$ 1.3	27.8 $\pm$ 3.8	50.2 $\pm$ 0.9	37.2 $\pm$ 3.2	45.2 $\pm$ 4.3	20.6 $\pm$ 2.7
BT <sup>a</sup>	—	65.3 $\pm$ 2.8	54.9 $\pm$ 5.5	64.9 $\pm$ 3.9	45.2 $\pm$ 2.0	53.7 $\pm$ 3.6	32.2 $\pm$ 2.8

<sup>a</sup> The commercial agricultural antibacterial agents thiodiazole copper (TC) and bismethiazol (BT) were used as control agents.

Table 2 EC<sub>50</sub> values of the title compounds against plant pathogenic bacteria *in vitro*

Bacterial	Compounds	R	Toxic regression equation	<i>r</i>	EC <sub>50</sub> /( $\mu\text{g mL}^{-1}$ )
Xac	2e	2-F-Ph	$y = 1.2997x + 2.9498$	0.9862	46.5
	2l	3-NO <sub>2</sub> -Ph	$y = 1.5536x + 2.7744$	0.9872	11.4
	2p	2,4-di-Cl-Ph	$y = 1.1393x + 3.3968$	0.9801	27.1
	TC <sup>a</sup>	—	$y = 1.1576x + 2.7122$	0.9859	94.7
	BT <sup>a</sup>	—	$y = 1.0698x + 3.1679$	0.9831	51.6
Xoo	2a	Ph	$y = 0.8781x + 3.8623$	0.9842	19.8
	2f	3-F-Ph	$y = 1.4287x + 3.1693$	0.9869	19.1
	2j	4-Br-Ph	$y = 1.4836x + 2.5829$	0.9756	42.6
	2l	3-NO <sub>2</sub> -Ph	$y = 1.4387x + 2.9155$	0.9778	28.1
	2m	3-CH <sub>3</sub> -Ph	$y = 1.0376x + 3.3799$	0.9891	21.8
	2o	3,4-di-OCH <sub>3</sub> -Ph	$y = 1.1362x + 3.2891$	0.9981	32.0
	2q	3,4-di-Cl-Ph	$y = 1.3694x + 2.8072$	0.9876	39.9
	2u	3,4-di-CH <sub>3</sub> -Ph	$y = 1.6774x + 2.5248$	0.9928	29.8
	TC <sup>a</sup>	—	$y = 1.2058x + 2.2869$	0.9971	97.8
	BT <sup>a</sup>	—	$y = 0.9913x + 3.1606$	0.9802	71.7
Rs	2a	Ph	$y = 1.3445x + 2.8696$	0.9812	38.4
	2e	2-F-Ph	$y = 1.2021x + 3.0881$	0.9907	38.9
	2f	3-F-Ph	$y = 1.4532x + 2.6183$	0.9729	43.6
	2k	2-Py	$y = 1.4622x + 2.6783$	0.9975	38.7
	2m	3-CH <sub>3</sub> -Ph	$y = 1.3326x + 3.5835$	0.9814	11.6
	2n	4-CH <sub>3</sub> -Ph	$y = 0.9189x + 3.5445$	0.9775	38.4
	2o	3,4-di-OCH <sub>3</sub> -Ph	$y = 1.9357x + 2.2953$	0.9886	25.0
	2p	2,4-di-Cl-Ph	$y = 1.7032x + 2.4391$	0.9758	31.9
	2u	3,4-di-CH <sub>3</sub> -Ph	$y = 1.0373x + 3.3816$	0.9864	36.3
	TC <sup>a</sup>	—	$y = 1.4682x + 1.8274$	0.9758	78.8
	BT <sup>a</sup>	—	$y = 1.2581x + 2.4918$	0.9746	98.6

<sup>a</sup> The commercial agricultural antibacterial agents thiodiazole copper (TC) and bismethiazol (BT) were used as control agents.



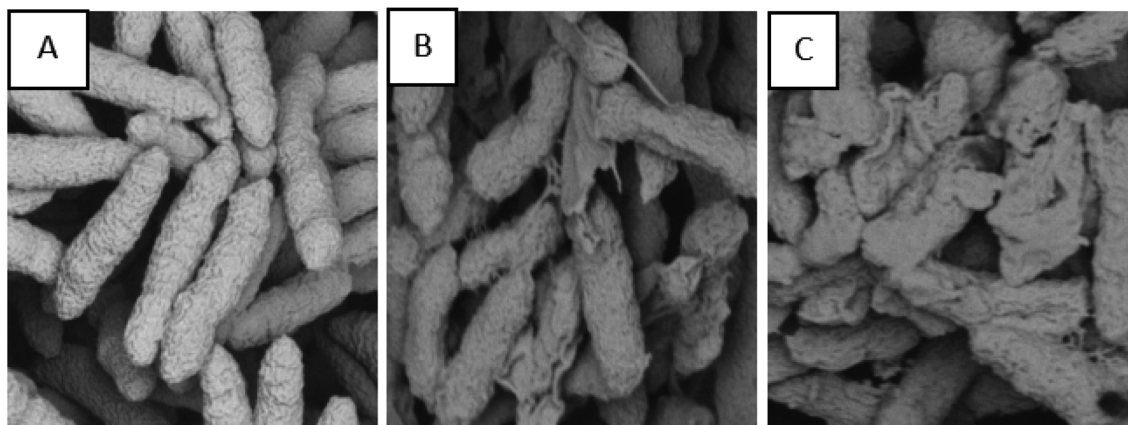


Fig. 1 SEM images for Xac after incubated using different concentrations of compound 2l, (A) 0  $\mu\text{g mL}^{-1}$ , (B) 50  $\mu\text{g mL}^{-1}$  and (C) 100  $\mu\text{g mL}^{-1}$ . Scale bar for (A), (B) and (C) are 2  $\mu\text{m}$ .

activities of the compounds **2m**, **2o** and **2p** against Rs at 100 and 50  $\mu\text{g mL}^{-1}$  were 91.4 and 77.5%, 92.4 and 63.3%, 85.2 and 56.6%, respectively, which were significantly superior to TC (45.2 and 20.6%) and BT (53.7 and 32.2%).

The antibacterial activities of the compounds were further confirmed by determining their  $\text{EC}_{50}$  values, and the obtained results are shown in Table 2. Compounds **2e**, **2l** and **2p** exhibited remarkable antibacterial activities against Xac, with  $\text{EC}_{50}$  values of 46.5, 11.4, and 27.1  $\mu\text{g mL}^{-1}$ , which were much better compared to TC (94.7  $\mu\text{g mL}^{-1}$ ) and BT (51.6  $\mu\text{g mL}^{-1}$ ). Compounds **2a**, **2f**, **2j**, **2l**, **2m**, **2o**, **2q** and **2u** exhibited excellent antibacterial activities against Xoo, with  $\text{EC}_{50}$  values of 19.8, 19.1, 42.6, 28.1, 21.8, 32.0, 39.9 and 29.8  $\mu\text{g mL}^{-1}$ , respectively

which were significantly superior to TC (97.8  $\mu\text{g mL}^{-1}$ ) and BT (71.7  $\mu\text{g mL}^{-1}$ ). Compounds **2a**, **2e**, **2f**, **2k**, **2m**, **2n**, **2o**, **2p** and **2u** exhibited notable antibacterial activities against Rs, with  $\text{EC}_{50}$  values of 38.4, 38.9, 43.6, 38.7, 11.6, 38.4, 25.0, 31.9 and 36.3  $\mu\text{g mL}^{-1}$ , respectively, which were much better than TC (98.6  $\mu\text{g mL}^{-1}$ ) and BT (78.8  $\mu\text{g mL}^{-1}$ ). These results indicate that such compounds should be further studied as potential alternative templates in the search for novel antibacterial agents.

### 3.3. Structure–activity relationships of antibacterial activities

Tables 1 and 2 show that the changes in the substituted groups could significantly impact the inhibitory effects against plant

Table 3 Antiviral activities of the test compounds against TMV *in vivo* at 500  $\mu\text{g mL}^{-1}$

Compounds	R	Curative activity <sup>a</sup> (%)	Protective activity <sup>a</sup> (%)	Inactivation activity <sup>a</sup> (%)
<b>2a</b>	Ph	72.5 $\pm$ 2.5	67.0 $\pm$ 2.1	80.1 $\pm$ 1.7
<b>2b</b>	4-Cl-Ph	46.4 $\pm$ 2.6	58.3 $\pm$ 0.9	53.8 $\pm$ 2.2
<b>2c</b>	2-Cl-Ph	52.4 $\pm$ 1.2	29.9 $\pm$ 1.0	62.3 $\pm$ 1.8
<b>2d</b>	3-Cl-Ph	45.3 $\pm$ 3.8	51.9 $\pm$ 2.2	59.7 $\pm$ 2.3
<b>2e</b>	2-F-Ph	76.2 $\pm$ 1.2	70.2 $\pm$ 1.9	87.3 $\pm$ 0.7
<b>2f</b>	3-F-Ph	48.5 $\pm$ 0.9	35.3 $\pm$ 2.2	60.2 $\pm$ 1.5
<b>2g</b>	4-F-Ph	52.3 $\pm$ 1.8	58.1 $\pm$ 0.4	58.7 $\pm$ 0.6
<b>2h</b>	2-Br-Ph	73.5 $\pm$ 2.1	68.8 $\pm$ 4.9	81.1 $\pm$ 1.9
<b>2i</b>	3-Br-Ph	26.9 $\pm$ 1.8	54.6 $\pm$ 4.4	65.3 $\pm$ 3.1
<b>2j</b>	4-Br-Ph	48.4 $\pm$ 0.8	61.9 $\pm$ 2.5	58.9 $\pm$ 1.7
<b>2k</b>	2-Py	45.6 $\pm$ 1.6	42.9 $\pm$ 1.9	66.1 $\pm$ 1.8
<b>2l</b>	3-NO <sub>2</sub> -Ph	74.8 $\pm$ 1.7	69.8 $\pm$ 1.6	75.3 $\pm$ 2.4
<b>2m</b>	3-CH <sub>3</sub> -Ph	72.6 $\pm$ 1.9	52.8 $\pm$ 0.7	69.2 $\pm$ 0.7
<b>2n</b>	4-CH <sub>3</sub> -Ph	30.3 $\pm$ 1.6	35.4 $\pm$ 2.9	45.3 $\pm$ 2.7
<b>2o</b>	3,4-di-OCH <sub>3</sub> -Ph	65.3 $\pm$ 0.8	60.2 $\pm$ 1.8	79.8 $\pm$ 2.1
<b>2p</b>	2,4-di-Cl-Ph	52.8 $\pm$ 2.7	40.9 $\pm$ 1.6	35.2 $\pm$ 1.9
<b>2q</b>	3,4-di-Cl-Ph	52.1 $\pm$ 2.0	46.2 $\pm$ 2.8	55.1 $\pm$ 3.3
<b>2r</b>	C <sub>3</sub> H <sub>7</sub> -Ph	27.2 $\pm$ 1.6	41.6 $\pm$ 1.9	58.1 $\pm$ 0.9
<b>2s</b>	2-Th	48.7 $\pm$ 4.3	32.1 $\pm$ 1.9	54.2 $\pm$ 1.7
<b>2t</b>	4-Py	43.9 $\pm$ 2.8	57.6 $\pm$ 1.7	56.2 $\pm$ 3.8
<b>2u</b>	3,4-di-CH <sub>3</sub> -Ph	49.3 $\pm$ 1.9	36.3 $\pm$ 1.8	61.2 $\pm$ 0.9
<b>2v</b>	2-OCH <sub>3</sub> -Ph	53.0 $\pm$ 1.8	51.1 $\pm$ 2.6	45.2 $\pm$ 1.4
Ningnanmycin <sup>b</sup>	—	53.3 $\pm$ 1.2	62.6 $\pm$ 1.3	78.3 $\pm$ 1.5

<sup>a</sup> Average of three replicates. <sup>b</sup> The commercial antiviral agent ningnanmycin.





Table 4 EC<sub>50</sub> values of some compounds against TMV

	Compounds	R	Toxic regression equation	<i>r</i>	EC <sub>50</sub> <sup>a</sup> /(μg mL <sup>-1</sup> )
Curative	2a	Ph	$y = 1.5764x + 1.2033$	0.9948	256.1
	2e	2-F-Ph	$y = 1.2516x + 2.1833$	0.9797	178.0
	2h	2-Br-Ph	$y = 1.6378x + 0.9941$	0.9981	279.2
	2l	3-NO <sub>2</sub> -Ph	$y = 1.5526x + 1.2543$	0.9958	258.5
	2m	3-CH <sub>3</sub> -Ph	$y = 1.3411x + 1.6219$	0.9740	330.3
	2o	3,4-di-OCH <sub>3</sub> -Ph	$y = 1.2168x + 1.9170$	0.9829	341.7
Protection	Ningnanmycin <sup>b</sup>		$y = 1.1975x + 1.8793$	0.9956	403.7
	2a	Ph	$y = 1.3994x + 1.4998$	0.9745	317.1
	2e	2-F-Ph	$y = 1.3560x + 1.7053$	0.9767	269.0
	2h	2-Br-Ph	$y = 1.2375x + 1.9576$	0.9913	287.4
	2l	3-NO <sub>2</sub> -Ph	$y = 1.5374x + 1.2315$	0.9901	282.6
	Ningnanmycin <sup>b</sup>		$y = 1.2081x + 1.9784$	0.9804	317.0
Inactivation	2a	Ph	$y = 1.0219x + 3.0517$	0.9985	80.6
	2e	2-F-Ph	$y = 1.0379x + 3.2916$	0.9894	44.3
	2h	2-Br-Ph	$y = 0.9258x + 3.3392$	0.9973	62.2
	2o	3,4-di-OCH <sub>3</sub> -Ph	$y = 0.9448x + 3.2055$	0.9935	79.3
	Ningnanmycin <sup>b</sup>		$y = 1.0209x + 2.8752$	0.9818	120.6

<sup>a</sup> Average of three replicates. <sup>b</sup> The commercial antiviral agent ningnanmycin.

bacteria, and analysis on the structure–activity relationships is discussed below. For instance, the designated compounds **2e** (R = 2-F-Ph), **2l** (R = 3-NO<sub>2</sub>-Ph) and **2p** (R = 2,4-di-Cl-Ph) exhibited significant anti Xac at 100 μg mL<sup>-1</sup>, with the inhibition rates of 77.2, 95.2 and 79.6%, respectively. These compounds were found to be more active compared to other tested compounds. However, when R was substituted with Ph, 3-F-Ph, 4-Br-Ph, 3-NO<sub>2</sub>-Ph, 3,4-di-OCH<sub>3</sub>-Ph, and 3,4-di-CH<sub>3</sub>-Ph groups, the activities of the corresponding compounds **2a**, **2f**, **2j**, **2l**, **2o** and **2u**, against Xoo at 100 μg mL<sup>-1</sup> were 72.4, 88.1, 77.3, 82.3, 72.3 and 83.3%, respectively, which were higher than that of bismerthiazol (64.9%) and thiadiazole-copper (50.2%). Notably, when R groups was Ph (**2a**), 3-F-Ph (**2e**), 2-Py (**2k**), 3-CH<sub>3</sub>-Ph (**2m**), 3,4-di-OCH<sub>3</sub>-Ph (**2o**) and 2,4-di-Cl-Ph (**2p**) excellent biological activity was found anti Rs at 100 μg mL<sup>-1</sup>, inhibition rates of 76.6, 71.1, 73.7, 91.4, 92.4 and 85.2%, respectively.

#### 3.4. Scanning electron microscopy (SEM) study

Following the antibacterial activities of the compounds, as shown in Tables 1 and 2, the antibacterial mechanism of Xac was further studied through SEM analysis.<sup>49</sup> It can be seen that the compound destroys the cell membrane, and more importantly, as the concentration of the compound increases, the damage of the cell membrane becomes more serious. For example, when the concentration was 50 μg mL<sup>-1</sup>, a part of the cell membrane begins to destroy (Fig. 1B). However, when the concentration was further increased to 100 μg mL<sup>-1</sup>, most of the cell membrane was damaged, and only a small number of cells was present (Fig. 1C). In contrast, in the absence of treatment with compound **2l**, the cells were smooth and well stacked with an intact membrane (Fig. 1A). These SEM images further confirm that compound **2l** destroys bacterial cells and may eventually kill the cells.

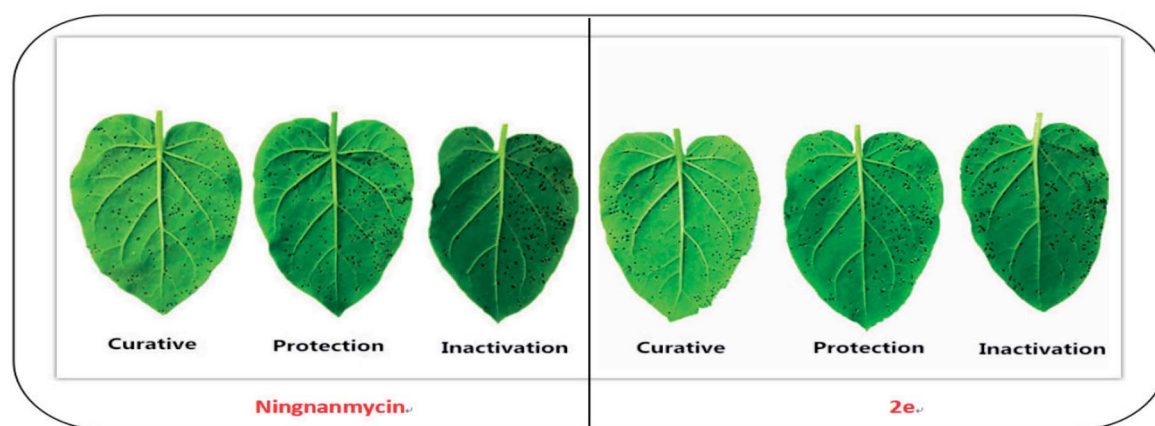


Fig. 2 Tobacco leaf morphology effects of the ningnanmycin and **2e** against TMV *in vivo*. (Right leaf: not treated with compound; left leaf: smeared with compound).



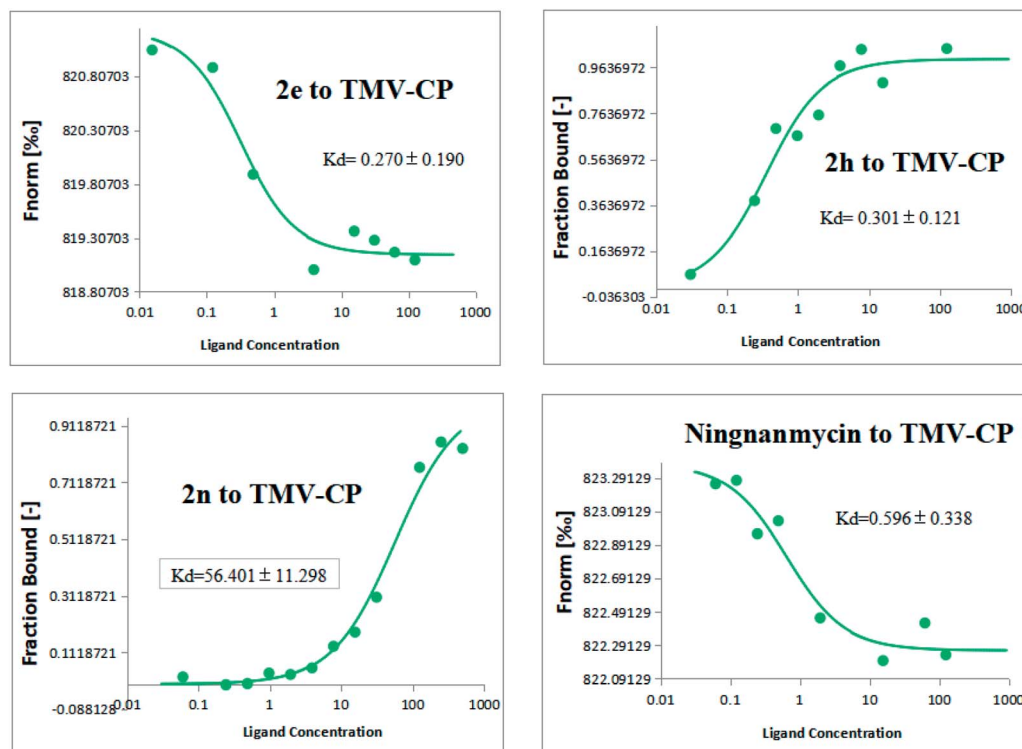


Fig. 3 Microscale thermophoresis (MST) results of compounds 2e, 2h, 2n and ningnanmycin.

### 3.5. Antiviral activity of title compounds against TMV *in vivo*

Using the same age tobacco leaves as the test subjects, the *in vivo* healing and protective activities of TMV at  $500 \mu\text{g mL}^{-1}$  were assessed by the half-blight spot method.<sup>50–52</sup> For this, a commercial agricultural antiviral agent ningnanmycin was used as a control, and the obtained preliminary bioassay results are shown in Table 3. The compounds exhibited significant antiviral activity against TMV. Among them, compounds 2a, 2e, 2h, 2l, 2m and 2o showed excellent curative activities against TMV, with the inhibition of 72.5, 84.2, 73.5, 81.8, 72.6 and 75.3%, which were better than ningnanmycin (53.3%). The protective activities of 2a, 2e, 2h and 2l (70.0, 75.2, 71.8 and 72.8%, respectively) against TMV were more effective than ningnanmycin (62.6%). In particularly, compounds 2a, 2e, 2h and 2o showed excellent inactivation activities against TMV, with the inhibition of 80.1, 87.3, 81.1 and 79.8%, which were better than ningnanmycin (78.3%).

To compare the antiviral activity of the synthesized compounds (2a–2v),  $\text{EC}_{50}$  values of 2a, 2e, 2h, 2l, 2m and 2o were calculated and have been summarized in Table 4. It could be seen that compounds 2a, 2e, 2h and 2l exhibited higher curative activity than ningnanmycin ( $403.7 \mu\text{g mL}^{-1}$ ), with  $\text{EC}_{50}$  values of 256.1, 178.0, 279.2 and  $258.5 \mu\text{g mL}^{-1}$ , respectively. Compounds 2e and 2l exhibited the best protective activity against TMV, with  $\text{EC}_{50}$  values of 269.0 and  $282.6 \mu\text{g mL}^{-1}$ , respectively, which were better than that of ningnanmycin ( $317.0 \mu\text{g mL}^{-1}$ ). Among them, compounds 2e and 2h exhibited the best inactivation activity against TMV, with  $\text{EC}_{50}$  values of 44.3 and  $62.2 \mu\text{g mL}^{-1}$ , respectively, which were better than that of ningnanmycin ( $120.6 \mu\text{g mL}^{-1}$ ). It can be indicated from these

results that most of these novel chalcone derivatives containing thiophene sulfonate could be further studied as a potential alternative template in the search for novel antiviral agents (Fig. 2).

### 3.6. Structure–activity relationships of antiviral activities

As indicated in Tables 3 and 4, most of the thiophene sulfonate chalcone derivatives showed significant antiviral activities against TMV, and some of the structure–activity relationships can be analyzed and summarized. First, the Ph, 2-F-Ph, 2-Br-Ph, 3- $\text{NO}_2$ -Ph and 3- $\text{CH}_3$ -Ph groups at –R position greatly improved the curative activities of the title compounds against TMV. For instance, the curative activities of the target compounds 2a, 2e, 2h, 2l and 2m, which were better than that of other substituent groups. In addition, when –R was Ph, 2-F-Ph, 2-Br-Ph and 3- $\text{NO}_2$ -Ph groups, the protective activities of the corresponding compounds 2a, 2e, 2h and 2l at  $500 \mu\text{g mL}^{-1}$  were better than that of ningnanmycin. When R was with Ph (2a), 2-F-Ph (2e), 2-Br-Ph (2h) and 3,4-di- $\text{OCH}_3$ -Ph (2o) groups, the corresponding compounds presented excellent inactivation activity against TMV. For instance, some target compounds the curative, protection and inactivation effects which were better than that of ningnanmycin following the order of 2e (R = 2-F-Ph) > 2h (R = 2-Br-Ph) > 2a (R = Ph). The detailed values corresponding to these results can be seen in Table 3.

### 3.7. Binding sites of 2e, 2h, 2n and ningnanmycin to TMV-CP

To further analyze the interactions between the title compounds and TMV coat protein (TMV-CP), the microscale



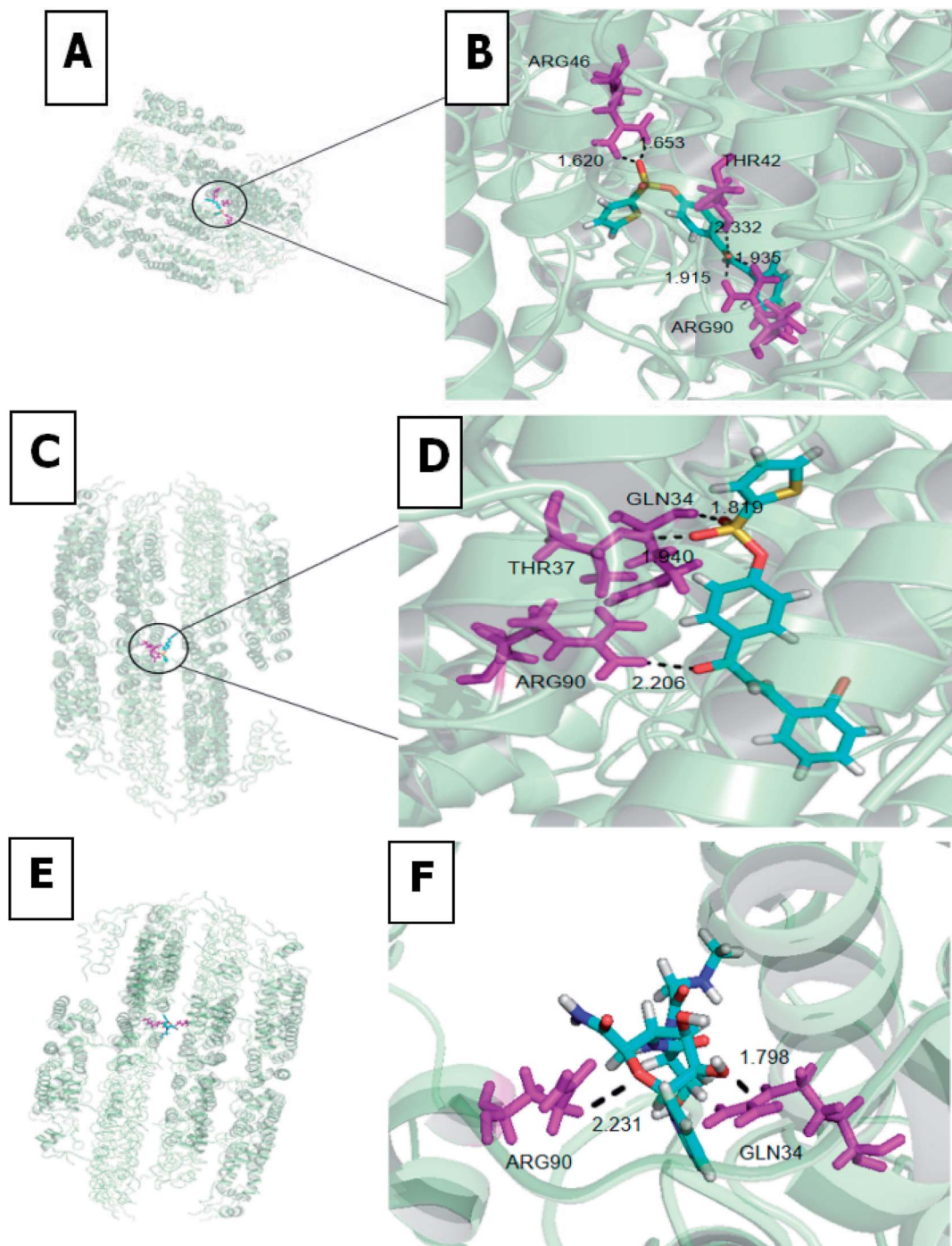


Fig. 4 Molecular docking studies of compounds **2e** (A and B), **2h** (C and D) and ningnanmycin (E and F).

thermophoresis (MST) analysis was used.<sup>53,54</sup> The MST results as summarized in Fig. 3 show that the binding of compounds **2e**, **2h**, **2n** and ningnanmycin to TMV-CP protein yielded  $K_d$  values of  $0.270 \pm 0.190 \mu\text{mol L}^{-1}$ ,  $0.301 \pm 0.121 \mu\text{mol L}^{-1}$ ,  $56.401 \pm 11.298 \mu\text{mol L}^{-1}$  and  $0.596 \pm 0.338 \mu\text{mol L}^{-1}$ , respectively. As indicated in MST, **2e** or **2h** with ningnanmycin share strong affinity, which is contrary to **2n** as it shares weak affinity. These results exhibited that the combining capacity in the following order of **2e** > **2h** > ningnanmycin > **2n** is consistent with the

trends of the screening of antiviral activities. Based on the experimental results it can be predicted that compounds **2e** and **2h** may interact with TMV-CP. As depicted in Fig. 3 the bioactivity was mainly determined by the electrostatic interactions, and the activity could have been enhanced by the presence of aromatic ring which is rich in electrons for absorption. For example, when R was substituted with 2-F-Ph and 2-Br-Ph groups, the corresponding compounds **2e** and **2h** exhibited a stronger combining capacity, with  $K_d$  values of  $0.270 \pm 0.190$





$\mu\text{mol L}^{-1}$  and  $0.301 \pm 0.121 \mu\text{mol L}^{-1}$ , as compared to that of compound **2h** ( $56.401 \pm 11.298 \mu\text{mol L}^{-1}$ ), which was substituted with a 4-CH<sub>3</sub>-Ph.

### 3.8. Molecular docking of **2e** or **2h** and TMV-CP

To identify the **2e** and **2h** recognition sites in TMV-CP (PDB code: 1EI7), we performed molecular docking using the Gold method with 200 cycles. As depicted in Fig. 4, the three compounds were well-embedded in the activity pocket (ARG-46, THR-42, ARG-90, GLN-34, THR-37, etc.) between the two subunits of TMV-CP. Among them, ARG-46 had strong hydrogen bond with **2e** (1.620 Å and 1.653 Å), C=O (**2e**) demonstrates three hydrogens with the THR-42 (O–H = 2.332 Å), ARG-90 (O–H = 1.915 Å and 1.935 Å). Moreover, thiophene sulfonate demonstrates strong hydrogen bond with GLN-34 (O–H = 1.819 Å), THR-37 showed one hydrogen bond **2h** (1.940 Å), there was also one hydrogen bond between the C=O and the residue ARG-90 (O–H = 2.206 Å). In addition, GLN-34 had strong hydrogen bond with ningnanmycin (1.798 Å), C=O (ningnanmycin) demonstrates one hydrogens with the ARG-90 (2.231 Å). It can be seen that the combination of **2e** and **2h** with TMV-CP has several more stable hydrogen bonds than ningnanmycin and TMV-CP, thus indicating that **2e** and **2h** have better antiviral activity than ningnanmycin. These interactions between molecules and TMV-CP are likely to weaken the interaction of two subunits of TMV-CP, thereby preventing the self-assembly of TMV particle, as well as the binding capability with TMV-CP. The results of molecular docking studies were consistent with the experimental results (protection and curative activities) and support that the chalcone derivatives containing thiophene sulfonate may be potential lead structures for developing novel anti-TMV agents.

## 4. Conclusions

In summary, with the aim of developing a novel, highly efficient, and environmentally benign virucide, in this study a thiophene sulfonate group was introduced into chalcone derivatives to synthesize 22 derivatives. Also, their *in vitro* antibacterial activities against Xac, Xoo, and Rs and their *in vivo* antiviral activity against TMV were evaluated. Bioassay results showed that several of the title compounds exhibited good antibacterial and antiviral activities. In particular, compound **2l** showed remarkable activity against Xac. Among them, compounds **2a**, **2e**, **2h**, **2l**, **2m** and **2o** showed excellent curative activities against TMV. Based on the above results, these novel thiophene sulfonate chalcone derivatives should be further studied as potential alternative templates in the search for novel antibacterial and antiviral agents.

## Conflicts of interest

The authors declare that they have no competing interests.

## Acknowledgements

This research was funded by the National Key Research and Development Program of China (No. 2017YFD0200506) and the Science and Technology Project of Guizhou Province (No. 20185781).

## References

- 1 Z. F. Li, S. L. Wu, X. F. Bai, Y. Liu, J. F. Lu, Y. Liu, B. G. Xiao, X. P. Lu and L. Fan, *J. Bacteriol.*, 2011, **193**, 6088–6089.
- 2 F. D. Lorenzo, A. Palmigiano, A. Silipo, Y. Desaki, D. Garozzo, R. Lanzetta, N. Shibuya and A. Molinaro, *Carbohydr. Res.*, 2016, **427**, 38–43.
- 3 B. Li, B. P. Liu, C. L. Shan, M. Ibrahim, Y. S. Lou, Y. L. Wang, G. L. Xie, H. Y. Li and G. H. Sun, *Pest Manage. Sci.*, 2013, **69**, 312–320.
- 4 S. Perumalsamy, M. Bharani, M. Sudha, P. Nagarajan, L. Arul, R. Saraswathi, P. Balasubramanian and J. Ramalingam, *Plant Breed.*, 2010, **129**, 400–406.
- 5 P. Y. Wang, L. Zhou, J. Zhou, Z. B. Wu, W. Xue, B. A. Song and S. Yang, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 1214–1217.
- 6 A. G. De Oliveira, L. S. Murate, F. R. Spago, L. D. Lopes, J. P. D. Beranger, J. A. B. S. Martin, M. A. Nogueira, J. C. P. Mello, C. G. T. D. Andrade and G. Andrade, *Biol. Control*, 2011, **56**, 125–131.
- 7 T. R. Gottwald, X. Sun, T. Riley, J. H. Graham, F. Ferrandino and E. L. Taylor, *Phytopathology*, 2002, **92**, 361–377.
- 8 T. B. Adhikari, R. C. Basnyat and T. W. Mew, *Plant Dis.*, 1999, **83**, 46–50.
- 9 Z. Ji, C. Ji, B. Liu, L. Zou, G. Chen and B. Yang, *Nat. Commun.*, 2016, **7**, 13435–13444.
- 10 L. F. Zou, Y. R. Li and G. Y. Chen, *Agric. Sci. China*, 2011, **10**, 1139–1150.
- 11 L. F. Zou, X. P. Wang, Y. Xiang, B. Zhang, Y. R. Li and J. S. Wang, *Appl. Environ. Microbiol.*, 2006, **72**, 6212–6224.
- 12 M. H. Chen, Z. Chen, B. A. Song, P. S. Bhadury, S. Yang, X. J. Cai, D. Y. Hu, W. Xue and S. Zeng, *J. Agric. Food Chem.*, 2009, **57**, 1383–1388.
- 13 X. H. Gan, D. Y. Hu, P. Li, J. Wu, X. W. Chen, W. Xue and B. A. Song, *Pest Manage. Sci.*, 2016, **72**, 534–543.
- 14 X. H. Gan, D. Y. Hu, Y. J. Wang, L. Yu and B. A. Song, *J. Agric. Food Chem.*, 2017, **35**, 665–672.
- 15 P. Y. Wang, L. Zhou, J. Zhou, Z. B. Wu, W. Xue, B. A. Song and S. Yang, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 1214–1217.
- 16 X. Wang, P. Li, Z. Li, J. Yin, M. He, W. Xue, Z. Chen and B. A. Song, *J. Agric. Food Chem.*, 2013, **61**, 9575–9582.
- 17 X. M. Zhong, X. B. Wang, L. J. Chen and W. Xue, *Chem. Cent. J.*, 2017, **11**, 106.
- 18 S. Perumalsamy, M. Bharani, M. Sudha, P. Nagarajan, L. Arul, R. Saraswathi, P. Balasubramanian and J. Ramalingam, *Plant Breed.*, 2010, **129**, 400–406.
- 19 Y. Q. Hu, S. Zhang, Z. Xu, Z. S. Lv, M. L. Liu and L. S. Feng, *Eur. J. Med. Chem.*, 2017, **141**, 335–345.
- 20 Y. J. Lin, Z. L. He, E. N. Roskopf, K. L. Conn, C. A. Powell and G. Lazarovits, *Plant Dis.*, 2010, **94**, 201–206.





- 21 R. Schobert, B. Biersack and A. Dietrich, *J. Med. Chem.*, 2009, **52**, 241–246.
- 22 S. Vogel, M. Barbic and J. Guido, *Eur. J. Med. Chem.*, 2010, **45**, 2206–2213.
- 23 V. Tomar and G. Bhattacharjee, *Eur. J. Med. Chem.*, 2010, **45**, 745–751.
- 24 P. L. Zhao, C. L. Liu, W. Huang, Y. Z. Wang and G. F. Yang, *J. Agric. Food Chem.*, 2007, **55**, 5697–5700.
- 25 R. Sribalan, G. Banupriya, M. Kirubavathi, A. Jayachitra and G. Padmini, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 5624–5630.
- 26 G. Pasquale, G. P. Romanelli, J. C. Autino, J. García, V. O. Erlinda and P. R. Duchowicz, *J. Agric. Food Chem.*, 2012, **60**, 692–697.
- 27 S. Attar, Z. O'Brien, H. Alhaddad, M. L. Golden and A. G. Urrea, *Bioorg. Med. Chem.*, 2011, **19**, 2055–2073.
- 28 D. Coskun, M. Erkisa, E. Ulukaya, M. F. Coskun and F. Ari, *Eur. J. Med. Chem.*, 2017, **136**, 212–222.
- 29 X. H. Gan, D. Y. Hu, Y. J. Wang, L. Yu and B. A. Song, *J. Agric. Food Chem.*, 2017, **65**, 4367–4377.
- 30 A. L. Cole, S. Hossain and A. M. Cole, *Bioorg. Med. Chem.*, 2016, **24**, 2768–2776.
- 31 J. F. Li, D. Li, Y. M. Xu, Z. B. Guo, X. Liu, H. Yang, L. C. Wu and L. S. Wang, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 602–606.
- 32 S. J. William, J. H. Melanie, A. S. Stacey and E. M. Rockafellow, *J. Org. Chem.*, 2009, **74**, 2765–2770.
- 33 M. K. Parai, G. Panda, V. Chaturvedi, Y. K. Manju and S. Sinha, *Bioorg. Med. Chem. Lett.*, 2007, **18**, 289–292.
- 34 A. M. Isloor, B. Kalluraya and K. Sridhar, *Eur. J. Med. Chem.*, 2010, **45**, 825–830.
- 35 M. B. Félix, E. R. Souza, M. de Lima, D. K. G. Frade, V. d. L. Serafim and M. R. de Oliveira, *Bioorg. Med. Chem. Lett.*, 2016, **24**, 3972–3977.
- 36 D. H. Pandya, J. A. Sharma, H. B. Jalani, A. N. Pandya, V. Sudarsanam, S. Kachler and K. K. Vasu, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 1306–1309.
- 37 R. Wang, X. Y. Zhi, J. Li and H. Xu, *J. Agric. Food Chem.*, 2015, **63**, 6668–6674.
- 38 R. F. Sun, Z. W. Wang, Y. Q. Li, L. X. Xiong, Y. X. Liu and Q. M. Wang, *J. Agric. Food Chem.*, 2013, **61**, 517–522.
- 39 K. A. K. Musa and L. A. Eriksson, *J. Phys. Chem. B*, 2009, **113**, 11306–11313.
- 40 R. M. Mohareb, M. Y. Zaki and N. S. Abbas, *Steroids*, 2015, **98**, 80–91.
- 41 S. G. Gallego, M. J. Serramía, E. Arnaiz, L. Díaz, M. A. Muñoz-Fernández, P. Gómez-Sal, M. F. Ottaviani and R. Gómez, *Eur. J. Med. Chem.*, 2011, **10**, 1657–1665.
- 42 Z. Wang, D. W. Kang, M. Chen, G. C. Wu, D. Feng, T. Zhao, Z. X. Zhou, Z. P. Huo and X. Y. Liu, *Chem. Biol. Drug Des.*, 2018, **92**, 2009–2021.
- 43 N. K. Duddukuri, S. Thatikonda, C. Godugu, R. A. Kumar and N. Doijad, *ChemistrySelect*, 2018, **3**, 6859–6864.
- 44 J. Zhang, F. Yang, Z. T. Qiao, M. Y. Zhu and H. C. Zhou, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 5797–5801.
- 45 B. Prasad, R. Adepu, S. Sandra, D. Rambabu, G. Rama Krishna, C. M. Reddy, G. S. Deora, P. Misra and M. Pal, *Chem. Commun.*, 2012, **48**, 10434–10436.
- 46 X. Wang, P. Li, Z. N. Li, J. Yin, M. He, W. Xue, Z. W. Chen and B. A. Song, *J. Agric. Food Chem.*, 2013, **61**, 9575–9582.
- 47 P. Li, D. Y. Hu, D. D. Xie, J. X. Chen, L. H. Jin and B. A. Song, *J. Agric. Food Chem.*, 2018, **66**, 3093–3100.
- 48 X. H. Ruan, C. Zhang, S. C. Jiang, T. Guo, R. J. Xia, Y. Chen, X. Tang and W. Xue, *Molecules*, 2018, **23**, 3132–3142.
- 49 J. Chen, J. Shi, L. Yu, D. Liu, X. H. Gan, B. A. Song and D. Y. Hu, *J. Agric. Food Chem.*, 2018, **66**, 5335–5345.
- 50 Z. X. Wu, J. Zhang, J. X. Chen, J. K. Pan, L. Zhao, D. Y. Liu, A. W. Zhang, J. Chen, D. Y. Hu and B. A. Song, *Pest Manage. Sci.*, 2017, **73**, 2079–2089.
- 51 L. J. Chen, T. Guo, R. J. Xia, X. Tang, Y. Chen, C. Zhang and W. Xue, *Molecules*, 2019, **24**, 925–937.
- 52 J. Zhou, Q. Q. Tao, P. Y. Wang, W. B. Shao, Z. B. Wu, Z. Li and S. Yang, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 1742–1746.
- 53 X. Y. Li, J. Liu, X. Yang, Y. Ding, J. Wu, D. Y. Hu and B. A. Song, *Bioorg. Med. Chem.*, 2015, **23**, 3629–3637.
- 54 C. J. Wienken, P. Baaske, U. Rothbauer, D. Braun and S. Duhr, *Nat. Commun.*, 2010, **1**, 100–107.

