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Original article

Design, synthesis, antibacterial and antiviral evaluation of chalcone derivatives containing benzoxazole

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ABSTRACT

In this study, 24 chalcone derivatives containing benzoxazole were designed and synthesized, and the structures of all target compounds were confirmed by NMR and HRMS. The results of antibacterial activity showed that the median effective concentration (EC_{50}) value of **Z2** against *Xanthomonas oryzae pv.oryzae* (***Xoo***) was 8.10 μg/mL, which was better than thiodiazole copper (60.62 μg/mL), the antibacterial activity of **Z2** was verified by scanning electron microscopy (SEM). Antibacterial mechanism of **Z2** on ***Xoo*** was preliminarily explored, and the results showed concentrations were 100, 50 mg/L, which had a good cell membrane permeability to ***Xoo*** and can inhibit the EPS content and the production of extracellular enzymes, affecting the formation of biofilm, preliminary validation of the antibacterial effect of **Z2** on ***Xoo***. At 200 mg/L, **Z2** had a better protective activity on harvested potatoes, which was comparable with thiodiazole copper. The activity against tobacco mosaic virus (**TMV**) was determined with half leaf dry spot method. The EC_{50} value of curative activity of **Z15** was 101.97 μg/mL, which was superior to ningnanmycin (NNM) 294.27 μg/mL, and the EC_{50} value of protective activity of **Z16** was 104.05 μg/mL, which was superior to NNM 185.73 μg/mL. Molecular docking results showed **Z15**, **Z16** had a significantly higher affinity with tobacco mosaic virus coat protein (TMV-CP) than NNM. The above results indicated that chalcone derivatives containing benzoxazole have the potential to become antibacterial and antivirial agents.

1. Introduction

Food is the basis for the survival and development of human beings, and pesticides are the most effective means to ensure food security (Song, 2020). All the time, plant diseases caused by bacteria and viruses have resulted in serious impact on the grain yield and quality of the world every year, causing huge economic losses (Cao et al., 2023; Hu et al., 2023). According to survey data from the UN Food and Agriculture Organization, the annual yield loss caused by plant pests accounts for about 40 % of the world's total annual grain production (Godfary et al., 2010). For example, the rice bacterial leaf blight (BLB) is one of the most serious bacterial diseases caused by ***Xoo***, which can cause yield loss of the rice up to 50 % (Chen et al., 2020). Potato is the world's most important non-grain food crop and is central to global food security and is one of the important food crops in China. It is widely planted in the northwest area, and its planting area and yield account for about 1/4 of China (Xu et al., 2011; Jiang et al., 2019). However, the damaged part caused by infection with *Pectobacterium carotovorum subsp. Brasiliense* (***Pcb***) accounts for about 15 %, causing serious economic losses (Naas, H., et al., 2018; Veltman, B., et al., 2023). Nowadays, in the current stage of agricultural production, the use of chemical control led by pesticide control is still the main method of plant disease control, which has made an indelible contribution to the world food security. However, with the progress of society, the public's requirements for pesticides are

Abbreviations: ^{1}H NMR, ^{1}H nuclear magnetic resonance; ^{13}C NMR, ^{13}C nuclear magnetic resonance; ^{19}F NMR, ^{19}F nuclear magnetic resonance; HRMS, High-resolution mass spectrometry; EC_{50}, Median effective concentration; DMSO, Dimethylsulfoxide; ***Xac***, *Xanthomonas axonopodis pv. Citri*; ***Xoo***, *Xanthomonas oryzae pv. oryzae*; ***Psa***, *pseudomonas syringae pv.actinidiae*; ***Ac***, *Acidovorax citrulli*; ***Rs***, *Ralstonia solanacearum*; ***Pcb***, *Pectobacterium carotovorum* subsp. *Brasiliense*; ***Xcm***, *Xanthomonas campestris pv. mangiferae indicae*; SEM, Scanning electron microscopy; TMV, Tobacco mosaic virus; NNM, Ningnanmycin; TMV-CP, Tobacco mosaic virus coat protein; EPS, Exopolysaccharide; SAR, Structure–activity relationship.

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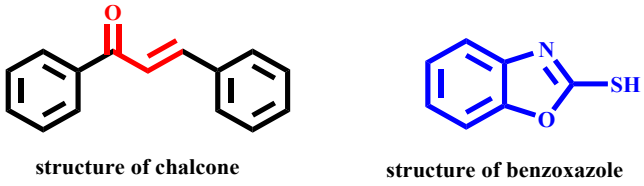


structure of chalcone **structure of benzoxazole**

Fig. 1. The structures of chalcone and benzoxazole.

becoming higher and higher, and the resistance of traditional chemical pesticides and pesticide residues are becoming increasingly prominent (Su et al., 2021; Li et al., 2019). Therefore, screening out new, efficient, safe and environmentally friendly new pesticides is nowadays an important task in the field of pesticide research (Zhou et al., 2022).

Natural products, also known as secondary metabolites, have a wide range of sources such as flavonoids, phenols, terpenoids, and terpenes (Tang et al., 2020). Natural products are widely used in medicine, pesticides and other fields because of their significant biological activities, such as antibacterial, antiviral and anti-tumor (He et al., 2021). In recent years, natural products have played a very important role in the discovery of new drugs and lead compounds. Chalcone (Fig. 1) is a significant kind of natural products, which can be obtained by aldol condensation reaction of aromatic aldehydes and ketones. It is a vitial raw material for the synthesis of spices and drugs. Its most critical functional groups are α, β-unsaturated ketone (Xia et al., 2019). It is widely distributed in plants, such as licorice, safflower, and hops (Guo et al., 2020). In previous studies, used the natural product chalcone as the lead compound, the synthesis of a series of chalcone derivatives through the principle of active splicing (Fig. 2). Such as introduction of quinoreoline (Xia et al., 2019), thiophene sulfonate (Guo et al., 2019), 1,2,4-triazine (Tang et al., 2019), thioether triazole (Chen et al., 2020), indole (Hu et al., 2023), pyrazole oxime ethers (Hu et al., 2023), quinazolone (Zhou et al., 2023) etc., these drug molecules have excellent antibacterial activity *in vitro* against ***Xoo*** and *Xanthomonas axonopodis pv citri* (***Xac***). Introduction of purine (Wang et al., 2019; Fu et al., 2020), amide (Xiao et al., 2020), piperazine (Zhou et al., 2022), [1,2,4]-triazole-[4, 3-b]-thiadiazole (Zhan et al., 2023), pyridazine (ketone) (Chen et al., 2023), pyrimidine (Zhan et al., 2023), etc., those derivatives show excellent antifungal activity *in vitro* against *Sclerotiua sclerotiorum* (*S. sclerotiorum*), *Colletotrichum gloeosporioiles* (*C. gloeosporioides*), *Botrytis cinerea* (*B. cinerea*). Futhermore, introduction of purines (Gan et al., 2017), 1,3,4-oxadiazole (Gan et al., 2017), trans ferulic acid (Gan et al., 2017), indanone (Sun et al., 2023) etc., those derivatives show excellent *in vivo* anti-TMV activity. In addition, chalcone have other biological activities, such as insecticidal (Zhang et al., 2010; Firmino et al., 2022), and anti-tumor (Saxena et al., 2007; Fathi et al., 2021), etc. Benzoxazole is a class of benzene heterocyclic compound containing O

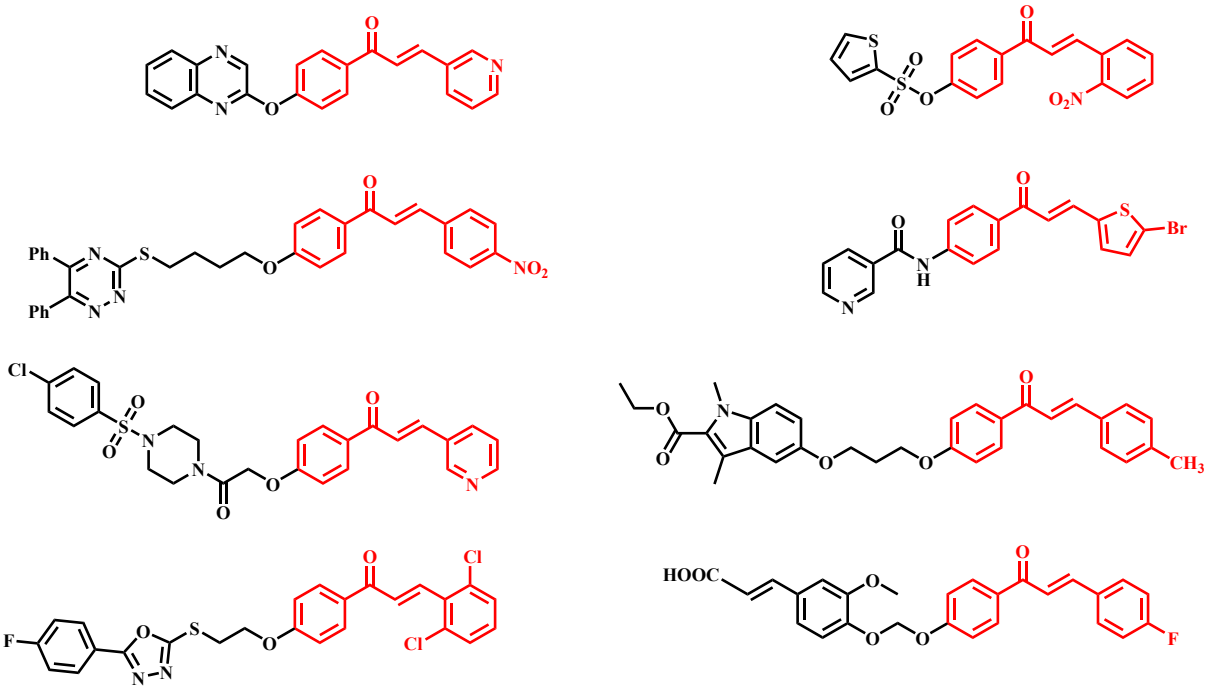


Fig. 2. The structures of some chalcone derivatives.

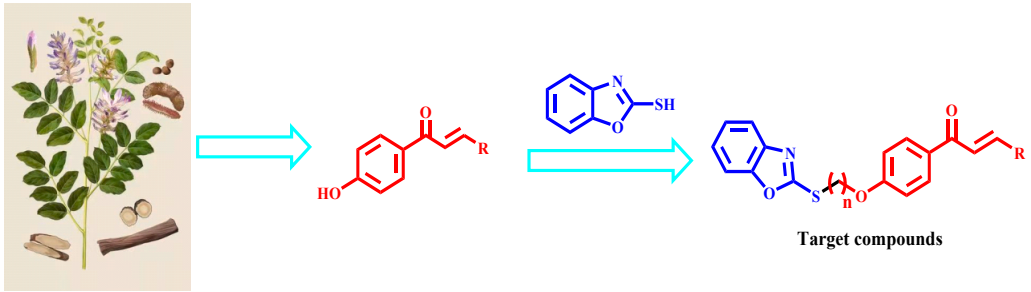
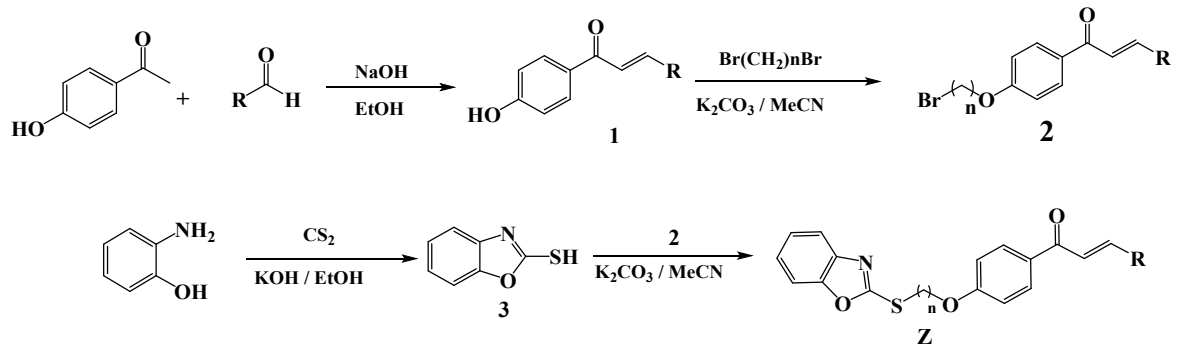


Fig. 3. Design of target compounds.



Z1: n=3, R=Ph **Z2:** n=4, R=Ph **Z3:** n=3, R-4-CH_3-Ph **Z4:** n=4, R=4-CH_3-Ph

Z5: n=3, R=4-OCH_3-Ph **Z6:** n=3, R=4-F-Ph **Z7:** n=4, R=4-F-Ph **Z-8:** n=3, R=4-Br-Ph

Z9: n=3, R=3-CH_3-Ph **Z10:** n=4, R=3-CH_3-Ph **Z11:** n=3, R=3-OCH_3-Ph **Z12:** n=4, R=3-OCH_3-Ph

Z13: n=4, R=3-F-Ph **Z14:** n=4, R=3-NO_2-Ph **Z15:** n=3, R=3-Cl-Ph **Z16:** n=4, R=3-Cl-Ph

Z17: n=3, R=3-Br-Ph **Z18:** n=4, R=3-Br-Ph **Z19:** n=3, R=2-CH_3-Ph **Z20:** n=4, R=2-CH_3-Ph

Z21: n=3, R=2-Furan **Z22:** n=4, R=2-Furan **Z23:** n=3, R=2-Thiophene **Z24:** n=4, R=2-Thiophene

Scheme 1. Synthetic route of compounds **Z1-Z24.**

and N atoms, which were found to have a wide range of biological activities, with good antibacterial (Luo et al., 2018), anticancer (Padmini et al., 2021), insecticidal (Zhi et al., 2020), and anti-tumor (Zhang et al., 2007), etc. Because benzoxazole, as a very momentous heterocyclic compound, is widely used in medicine, pesticides and other multiple research fields, so the in-depth exploration of benzoxazole compounds has been favored by many chemists. Therefore, by introducing groups with antibacterial and antiviral activities into chalcones, the design and synthesis of new green pesticides with high activity, low toxicity and has become a hot spot in pesticide research and development in recent years.

In order to obtain novel chalcone derivatives with better antiviral and bacterial inhibition, 24 chalcone derivatives containing benzoxazole were synthesized according to the principle of active sampling drug synthesis (Fig. 3 and Scheme 1). The *in vitro* antibacterial activities of all the target compounds were tested with a turbidity method, and anti-TMV activity of the target compounds was tested with the half leaf dry spot method. We found that **Z2** had a good inhibiting effect on ***Xoo***. The cell membrane permeability, exopolysaccharide, extracellular amylase and extracellular cellulase assays were performed between **Z2** and ***Xoo***, and the measurement results showed that **Z2** could inhibit the content of ***Xoo*** biofilm to achieve the effect of inhibition. Otherwise, when the drug concentration is 200 and 100 mg/L, we tested the effect of **Z2** on post-harvest potato and found that **Z2** protected the potato from being infected with ***Pcb***, which was never done in our previous work. The results of antiviral tests showed that the **Z15**, **Z16** had excellent curative, protective activities against TMV. In this study, chalcone derivatives with certain antibacterial and antiviral activity were found, which provided a certain theoretical basis for the research, development and design of new pesticides.

2. Materials and methods

2.1. Instruments and chemicals

2.1.1. Instruments

The melting points were determined by using an XT-4 binocular microscope (Beijing Taiji Instrument Co., Ltd.) and uncorrected. ^{1}H, ^{13}C, and ^{19}F nuclear magnetic resonance (**NMR**) spentra of the target compounds were determined by 500 NMR Spectrometer (Bruker Corporation, Germany). Highresolution mass spectrometry (HRMS) was conducted by using a Thermo Scientific Q Exactive (Thermo Scientific, Missouri, USA). Scanning electron microscopy (SEM) data were obtained on FEI Nova Nano 450 (Hillsboro, OR, U.S.A.).

2.1.2. Chemicals

P-hydroxyacetenone, Formaldehyde with different substituents, amino phenol, carbon disulfide, thiodiazole copper, and ningnanmycin were all purchased from Shanghai Titan Technology Co., Ltd. (Shanghai, China); the solvents such as absolute ethanol and acetonitrile are commercially available.

2.2. Synthesis

The synthetic route of the target compounds **Z1**-**Z24** is shown in Scheme 1.

2.2.1. Synthesis of intermediates *1*–*2*

The synthesis of intermediates **1**–**2** were realized according to the method of the literature (Zhan et al., 2023).

2.2.2. Synthesis of the intermediate *3*

The synthesis of intermediate **3** was realized according to the method of the literature (Niranjan, et al., 2018). 3.00 g (27.49 mmol) *o*-aminophenol was added, 1.54 g (27.49 mmol) KOH and 100 mLC_2H_5OH was added and kept for room temperature for 15 min, and then 3.31 mL (54.98 mmol) CS_2 was added and heated to 80℃, refluxed. The reaction process was monitored by TLC (PE: EA = 3:1, *v / v*). After the raw materials completely reacted and concentrated under reduced pressure. After 250 mL of ice-cold water was added, pH in the system was adjusted to 1–2 with 20 % HCl. The system was constantly stirring until a large number of solids precipitated. After it was filtered, the filtrate was discarded. The filter cake was dried to get intermediate **3**.

2.2.3. Synthesis of the target compounds *Z1-Z24*

Intermediate **3** (3.31 mmol), K_2CO_3 (9.92 mmol), and 40 mL of CH_3CN were added to a 100 mL round bottom flask and kept stirring at room temperature for 30 min. The intermediate **2** (4.96 mmol) was added to the reaction system, kept stirring the reaction for 36 h, and the

Table 1
The antibacterial activity of **Z1-Z24.**

Compounds	Inhibition rates(%)[a] (100 μg/mL)						
	Xac	*Xoo*	*Psa*	*Ac*	*Rs*	*Pcb*	*Xcm*
Z1	72.3 ± 5.0	47.1 ± 2.9	44.9 ± 1.3	86.2 ± 5.6	47.9 ± 1.2	38.5 ± 4.0	14.8 ± 4.5
Z2	68.2 ± 2.7	93.0 ± 0.6	46.8 ± 2.4	67.8 ± 2.2	79.5 ± 3.4	84.6 ± 1.1	66.4 ± 3.8
Z3	23.2 ± 1.3	20.7 ± 0.7	37.0 ± 3.4	78.5 ± 2.7	68.6 ± 6.9	77.1 ± 4.5	52.0 ± 3.4
Z4	55.6 ± 4.6	21.2 ± 1.1	30.9 ± 3.6	81.2 ± 2.5	58.9 ± 4.4	72.2 ± 6.1	56.5 ± 2.3
Z5	48.5 ± 3.4	59.6 ± 3.5	8.7 ± 0.7	53.3 ± 1.9	34.1 ± 3.2	22.4 ± 3.2	36.7 ± 0.9
Z6	49.5 ± 6.4	11.3 ± 7.1	36.7 ± 1.3	75.5 ± 2.8	72.9 ± 1.7	61.6 ± 2.8	55.1 ± 1.8
Z7	30.3 ± 4.2	23.1 ± 2.6	23.4 ± 2.8	73.0 ± 2.4	73.5 ± 1.7	75.9 ± 4.3	49.6 ± 6.2
Z8	31.3 ± 6.7	24.7 ± 1.8	59.6 ± 0.9	45.1 ± 0.6	34.2 ± 3.0	43.7 ± 3.2	49.7 ± 2.2
Z9	22.9 ± 1.6	15.1 ± 1.7	30.6 ± 2.1	9.5 ± 3.6	30.5 ± 2.0	10.6 ± 3.9	57.7 ± 5.1
Z10	53.7 ± 3.4	11.0 ± 2.6	46.4 ± 6.9	81.6 ± 4.3	58.0 ± 6.6	43.7 ± 3.6	65.7 ± 2.5
Z11	56.6 ± 3.0	18.7 ± 4.6	54.5 ± 5.6	60.1 ± 3.2	62.1 ± 5.5	50.7 ± 5.8	64.0 ± 3.9
Z12	40.4 ± 3.8	33.2 ± 3.1	30.3 ± 2.6	15.7 ± 4.4	72.9 ± 5.8	41.3 ± 2.6	42.3 ± 3.2
Z13	61.2 ± 3.7	18.2 ± 2.6	47.0 ± 2.7	54.0 ± 6.3	35.8 ± 4.5	36.8 ± 2.1	38.2 ± 3.4
Z14	53.1 ± 4.6	23.7 ± 4.9	54.6 ± 2.9	60.6 ± 1.5	56.9 ± 3.5	59.7 ± 4.6	32.1 ± 5.6
Z15	42.9 ± 1.6	50.2 ± 4.1	49.1 ± 0.4	68.9 ± 3.0	49.1 ± 5.1	46.7 ± 1.7	63.3 ± 2.3
Z16	40.6 ± 4.7	19.5 ± 1.9	37.5 ± 2.5	38.8 ± 3.7	34.8 ± 2.7	41.1 ± 3.8	54.3 ± 2.6
Z17	40.2 ± 2.3	24.9 ± 5.7	18.9 ± 1.2	67.7 ± 5.7	70.9 ± 3.8	52.0 ± 5.7	78.6 ± 4.7
Z18	35.2 ± 1.8	5.2 ± 0.3	38.4 ± 1.4	37.9 ± 4.2	27.6 ± 5.3	18.2 ± 4.3	45.1 ± 4.2
Z19	60.9 ± 6.6	42.2 ± 5.6	40.5 ± 1.9	23.1 ± 4.7	54.1 ± 1.8	58.0 ± 6.2	68.8 ± 0.3
Z20	37.5 ± 2.6	49.3 ± 1.3	44.7 ± 4.2	21.1 ± 3.3	78.5 ± 2.6	55.2 ± 2.7	61.0 ± 2.3
Z21	32.0 ± 4.5	45.5 ± 1.2	10.7 ± 1.0	24.7 ± 4.7	24.6 ± 1.5	26.5 ± 2.1	1.5 ± 3.5
Z22	21.3 ± 2.0	27.4 ± 0.9	21.3 ± 3.9	9.7 ± 1.3	26.9 ± 3.3	29.1 ± 5.1	20.2 ± 1.1
Z23	15.2 ± 3.7	29.4 ± 0.2	14.9 ± 1.1	17.9 ± 2.3	23.5 ± 2.5	16.3 ± 4.1	11.7 ± 8.4
Z24	43.1 ± 9.2	9.9 ± 5.5	17.9 ± 3.0	18.6 ± 5.6	19.2 ± 2.7	10.0 ± 3.7	12.2 ± 2.7
***TC*[b]**	64.2 ± 4.2	90.7 ± 6.7	48.2 ± 1.1	47.4 ± 4.1	43.5 ± 4.2	63.0 ± 1.8	40.9 ± 3.7

[a] Average of the three replicates, [b] Thiodiazole copper.

reaction process was monitored by TLC. (PE: EA = 1:1, *v / v*). After the reaction completed, the reaction system under pressure was concentrated to remove most of the solvent. 300 mL of ice water was poured in the system, stirred until a large amount of solids, filtered. The filter cake was washed with petroleum ether 2 ~ 3 times and dried. Finally, the target compounds **Z1-Z24** were purified by column chromatography (PE: EA = 4:1, *v / v*).

3. Activity test methods

3.1. Anti-bacterial activity test

According to the method reported in the previous literature (Tang et al., 2022), *in vitro* antibacterial activities of all the target compounds against seven plant bacteria were measured at 100 μg/mL. Furthermore, EC_{50} values were screened by two-fold dilution for compounds with some antibacterial activities. Thiodiazole copper was used as a control agent and each measurement was repeated three times.

3.2. The antibacterial mechanism of *Z2* on *Xoo*

3.2.1. Effect of *Z2* on the growth rate of *Xoo*

According to the method reported in the previous literature (Feng et al., 2022), at concentrations of 6.25, 12.5, 25, 50, 100 mg/L, **Z2** to the ***Xoo*** growth rates were determined, while with 1 % DMSO as a negative control. Each concentration was repeated three times. OD_{595} was measured every 3 h for 30 h.

3.2.2. Scanning electron microscopy

SEM experiments were performed on the bacterial inhibition of **Z2**, all methods were referred to previous literature (Peng et al., 2021).

3.2.3. Cell membrane permeability assay of *Z2* to *Xoo*

The experimental method was modified with reference to the method reported in the previous literature (Wang et al., 2022; Ye et al., 2005).

3.2.4. Effect of *Z2* on the *Xoo* exopolysaccharide (*EPS*)

According to the method reported in the literature, the weighing method was employed to study the effect of **Z2** against ***Xoo*** exopolysaccharides (Pan et al., 2018), with 1 % DMSO as a negative control. Each concentration was repeated three times.

3.2.5. Effect of *Z2* on the extracellular enzyme of *Xoo*

According to the method reported previously (Li et al., 2022), the effect of **Z2** against the ***Xoo*** extracellular enzyme (extracellular amylase and extracellular cellulases) was studied after some slight modifications of the experimental procedure.

3.3. In vivo inhibitory *Pcb* activity of *Z2*

The experimental method was modified with reference to the method reported in the previous literature (Li et al., 2016), *in vivo* experiments were performed on harvested potatoes at drug concentrations of 200 and 100 mg/L, with thiodiazole copper as a control drug. These experiments were repeated 3 times.

3.4. In vivo antiviral activity assay of *Z1-Z24* against *TMV*

According to the method reported previously in the literature (Cao et al., 2023), the *in vivo* antiviral activity against TMV (curative and protective activity) was tested. The commercial drug ningnanmycin (NNM) was a control agent, and each experiment was repeated three times.

3.5. Molecular docking of the target compounds on the TMV

Molecular docking of compounds **Z15**, **Z16** and NNM with TMV-CP was performed with the method reported in the literature (Liu, et al., 2022). Molecular docking of the TMV-CP (PDB: 1EI7) with the structure of the selected compounds was performed on the Discovery Studio 2019 software.

4. Results and discussion

4.1. Chemistry

The **Z1-Z24** were synthesized according to Scheme 1. The structures of the desired target compounds were preliminarily characterized by using NMR and HNMR and the detailed data are provided for the Supporting Materials.

Table 2
The EC_{50} values of several target compounds for several plant bacteria.

Bacteria	Compounds	n	R	Toxic Regression equation	R^2	EC_{50} (μg/mL)[a]
Xac	**Z1**	3	Ph	y = 0.5744x + 0.6620	0.9731	48.24
	TC [b]	–	–	y = 2.1114x + 1.0540	0.9917	73.94
Xoo	**Z2**	4	Ph	y = 2.2563x + 2.9507	0.9884	8.10
	TC [b]	–	–	y = 2.3436x + 0.8222	0.9966	60.62
A_C	**Z1**	3	Ph	y = 2.1309x + 1.6366	0.9933	37.88
	Z3	3	4-CH_3-Ph	y = 1.8989x + 1.9037	0.9623	42.71
	Z4	4	4-CH_3-Ph	y = 1.8265x + 1.9016	0.9706	49.70
	Z6	3	4-F-Ph	y = 1.7750x + 1.8810	0.9837	57.17
	Z7	4	4-F-Ph	y = 1.8224x + 1.9062	0.9732	49.84
	Z10	4	3-CH_3-Ph	y = 1.8848x + 1.9187	0.9577	43.14
	TC [b]	–	–	y = 1.5562x + 2.0569	0.9814	77.84
R_S	**Z2**	4	Ph	y = 1.8888x + 1.9093	0.9100	43.28
	Z12	4	3-OCH_3-Ph	y = 1.7857x + 1.8605	0.9904	57.30
	Z20	4	2-CH_3-Ph	y = 1.7587x + 1.8596	0.9861	61.05
	TC [b]	–	–	y = 1.2851x + 2.0590	0.9947	194.38
Pcb	**Z2**	4	Ph	y = 2.5152x + 1.5627	0.9872	23.26
	Z3	3	4-CH_3-Ph	y = 1.8894x + 1.8137	0.9847	48.58
	Z4	4	4-CH_3-Ph	y = 1.9397x + 1.8189	0.9691	43.65
	Z7	4	4-F-Ph	y = 1.7332x + 1.9945	0.9608	54.22
	TC [b]	–	–	y = 1.1341x + 2.8194	0.9933	83.70
Xcm	**Z2**	4	Ph	y = 1.7126x + 1.9006	0.9830	64.54
	Z10	4	3-CH_3-Ph	y = 1.9402x + 1.8484	0.9779	42.11
	Z11	3	3-OCH_3-Ph	y = 1.7300x + 1.8020	0.9840	70.56
	Z15	3	3-Cl-Ph	y = 1.6907x + 1.9900	0.9689	59.21
	Z17	3	3-Br-Ph	y = 1.7532x + 1.8853	0.9814	59.78
	Z19	3	2-CH_3-Ph	y = 1.6664x + 1.9476	0.9840	67.88
	Z20	4	2-CH_3-Ph	y = 1.7303x + 1.9163	0.9852	60.56
	TC [b]	–	–	y = 1.3586x + 1.9534	0.9903	174.74

[a] Average of the three replicates, [b] Thiodiazole copper.

4.2. Antibacterial activity of *Z1-Z24*

The inhibitory activities of the synthetic target compounds against *Xac*, ***Xoo***, *Pseudomonas syringae pv. actinidiae* (***Psa***), *Acidovorax citrulli* (***Ac***), *Ralstonia solanacearum* (***Rs***), ***Pcb***, and *Xanthomonas campestris pv. mangiferae indicae* (***Xcm***) were determined at the concentration of 100 μg/mL. The results are shown in Table 1. Some of the target compounds showed inhibitory activity against seven plant bacteria. At the concentration of 100 μg/mL, **Z1**, **Z2** showed 72.3, 68.2 % inhibition of ***Xac***, respectively, which were better than thiodiazole copper (64.2 %); **Z2** showed 93.0 % inhibition of ***Xoo***, which was superior than thiodiazole copper (90.7 %). **Z1**, **Z3**, **Z4**, **Z6**, **Z7**, **Z10** showed 86.2, 78.5, 81.2, 75.5, 73.0, 81.6 % inhibition of ***Ac***, respectively, which were better than thiodiazole copper (47.4 %). **Z2**, **Z6**, **Z7**, **Z12**, **Z17**, **Z20** showed 79.5, 72.9, 73.5, 72.9, 70.9, 78.5 % inhibition of ***Rs***, respectively, which were higher than thiodiazole copper (43.5 %). **Z2**, **Z3**, **Z4** and **Z7** showed 84.6, 77.1, 72.2 and 75.9 % inhibition of ***Pcb***, respectively, which were better than thiodiazole copper (63.0 %). **Z2**, **Z10**, **Z11**, **Z17**, **Z19** showed 66.4, 65.7, 64.0, 78.6, 68.8 % inhibition of ***Xcm***, respectively, which were superior than thiodiazole copper (40.9 %).

To further confirm the results of the preliminary screening, the EC_{50} values of some target compounds were tested, and the results are shown in Table 2. As shown in Table 2, the EC_{50} values for the compounds ranged from 8.10 to 67.88 μg/mL. Notably, the EC_{50} value of **Z2** to ***Xoo*** was 8.10 μg/mL, which was better than thiodiazole copper (60.62 μg/mL).

Table 3
Growth curves of ***Xoo*** treated with **Z2.**

Times (h)	Cell growth (OD_{595})					
	0 mg/L	6.25 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L
0	0.11 ± 0.08	0.10 ± 0.05	0.10 ± 0.04	0.10 ± 0.03	0.10 ± 0.04	0.10 ± 0.01
3	0.13 ± 0.10a	0.12 ± 0.07ab	0.12 ± 0.04ab	0.11 ± 0.05ab	0.11 ± 0.01ab	0.11 ± 0.05b
6	0.17 ± 0.06a	0.16 ± 0.09b	0.15 ± 0.05c	0.10 ± 0.04bc	0.13 ± 0.05c	0.11 ± 0.04d
9	0.21 ± 0.11a	0.19 ± 0.03b	0.17 ± 0.06c	0.14 ± 0.07 cd	0.15 ± 0.03d	0.12 ± 0.05e
12	0.28 ± 0.09a	0.25 ± 0.10b	0.21 ± 0.11c	0.18 ± 0.08d	0.16 ± 0.08d	0.11 ± 0.08e
15	0.37 ± 0.10a	0.25 ± 0.08b	0.22 ± 0.10c	0.19 ± 0.06d	0.18 ± 0.08d	0.12 ± 0.06e
18	0.43 ± 0.18a	0.29 ± 0.19b	0.23 ± 0.08c	0.22 ± 0.07c	0.19 ± 0.10d	0.14 ± 0.10e
21	0.46 ± 0.15a	0.29 ± 0.17b	0.24 ± 0.09c	0.24 ± 0.05c	0.18 ± 0.06d	0.15 ± 0.08e
24	0.55 ± 0.19a	0.31 ± 0.09b	0.26 ± 0.12c	0.25 ± 0.11d	0.19 ± 0.07e	0.15 ± 0.07f
27	0.65 ± 0.14a	0.33 ± 0.15b	0.29 ± 0.07c	0.26 ± 0.02d	0.20 ± 0.09e	0.15 ± 0.09f
30	0.70 ± 0.16a	0.38 ± 0.14b	0.34 ± 0.10c	0.28 ± 0.11d	0.19 ± 0.10e	0.15 ± 0.10f

The OD_{595} value represent average of three replicates. Different letters indicate that the means are significantly different at $p \leq 0.05$.

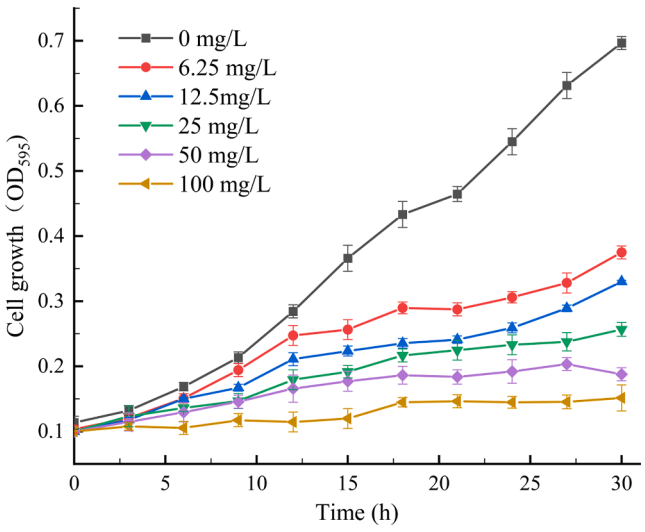


Fig. 4. Growth curves of ***Xoo*** treated with **Z2.**

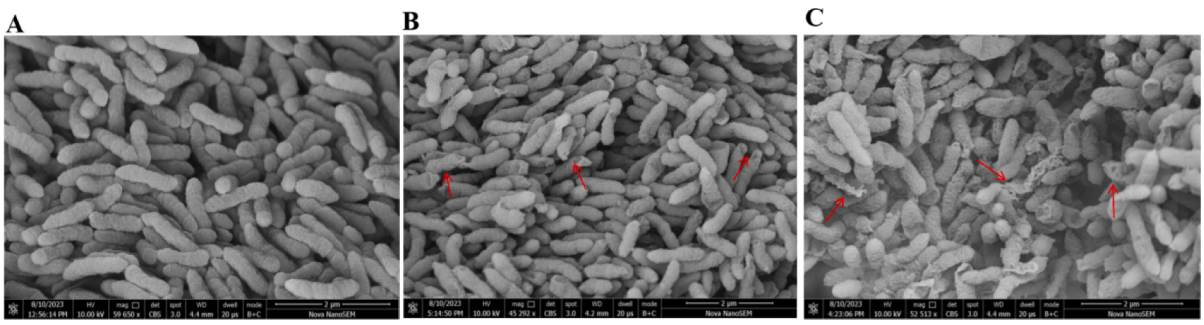


Fig. 5. SEM of the hyphae of ***Xoo*** with or without compound **Z2** treatment. (**A**) CK groups (DMSO); (**B**) **Z2** at 50 μg/mL and (**C**) **Z2** at 100 μg/mL.

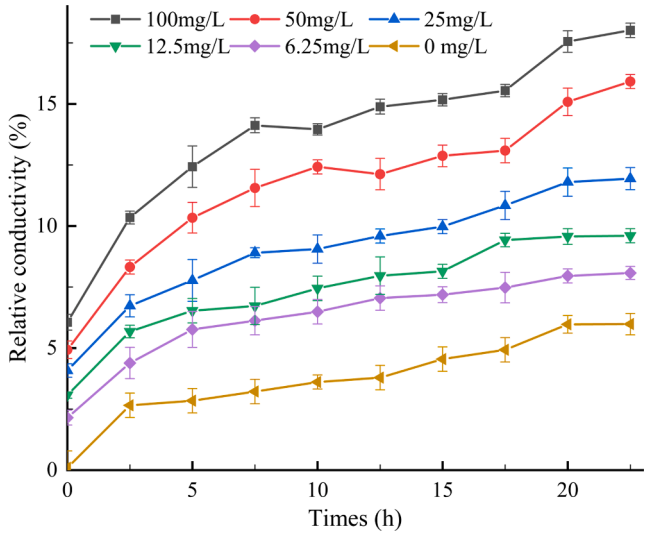


Fig. 6. Relative conductance of **Z2** treated ***Xoo*** cells at concentrations of 0, 6.25, 12.5, 25, 50, 100 mg/L.

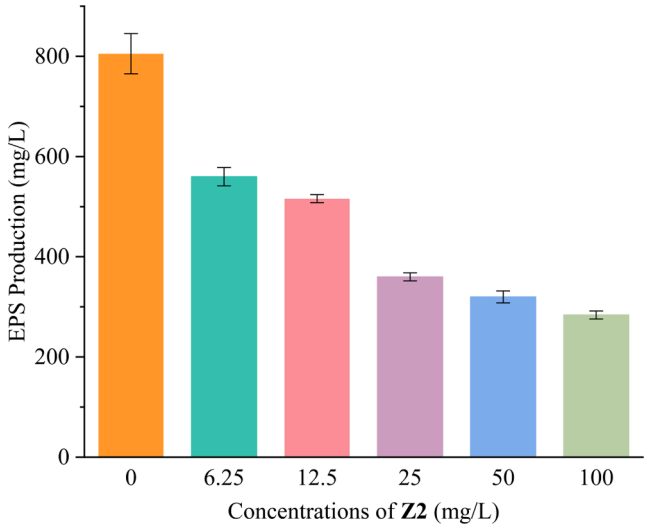


Fig. 7. Quantification of EPS production triggered by **Z2** at various concentrations ranging from 0 to 100 mg/L.

Table 4
Effect of **Z2** on the EPS conduction of ***Xoo***.

Concentration of **Z2** (mg/L)	EPS Conduction			
	Repetition 1	Repetition 2	Repetition 3	Average content
0	848	768	800	805.3
6.25	556	580	544	560.0
12.5	524	515	508	515.7
25	360	352	369	360.3
50	308	333	320	320.3
100	284	276	290	283.3

4.3. Structure-activity relationship (SAR) analysis of the antibacterial activity

According to the bacterial inhibiting activities shown in Table 1 and Table 2, SAR analysis showed that the compounds with certain antibacterial activities have some commonalities.

(1). At the concentration of 100 μg/mL, when the substituent was Ph, the inhibiting activity was relatively best, this indicated that when R was a small steric hindrance substituent, it can enhance the antibacterial activity of the compound. Such as **Z1** (n = 3, R = Ph), **Z2** (n = 4, R = Ph) showed that the inhibitory activities against ***Xac*** were 72.3, 68.5 %, respectively. **Z2** (n = 4, R = Ph) showed the inhibitory activity against ***Xoo*** was 93.0 %. **Z1** (n = 3, R = Ph) showed the inhibitory activity against ***Ac*** was 86.2 %. **Z2** (n = 4, R = Ph) showed the inhibitory activity against ***Rs*** was 79.5 %. **Z2** (n = 4, R = Ph) showed the inhibitory activityagainst ***Pcb*** was 84.6 %, which was higher than those of other substituents.

(2). When R was electron-giving group, it can enhance the inhibiting rate of the compounds against ***Ac*** and ***Rs***, such as the inhibiting activity against ***Ac*** was **Z3** (n = 3, R = 4-CH_3-Ph, 78.5 %) > **Z6** (n = 3, R = 4-F-Ph, 75.5 %) > **Z21** (n = 3, R = 2-Furan, 24.7 %); the inhibiting activity against ***Rs*** was **Z3** (n = 3, R = 4-CH_3-Ph, 77.1 %) > **Z6** (n = 3, R = 4-F-Ph, 72.9 %) > **Z21** (n = 3, R = 2-Furan, 24.6 %).

(3). It is worth noting that when the number of carbon atoms of dibromine that replaces alkanesis was 4 and the substituent was Ph, it showed relatively significant inhibiting activity. For example, the EC_{50} values of **Z2** (n = 4, R = Ph) against ***Xoo***, ***Rs***, ***Pcb*** were 8.10, 43.28, 23.26 μg/mL, respectively, which were superior to those of other substituents.

(4). We noticed that the substituent introduced in the early stage was relatively single, so the heterocyclic substituent (such as thiophene, furan) was introduced. The preliminary screening results of the activity test showed that the four synthesized compounds containing heterocyclic substituents did not have a prominent inhibiting effect on the seven plant bacteria.

In short, this result indicated that when the number of carbon atoms of dibromine alkanesis was 4 and the substituent was Ph, it can improve the antibacterial activities of the compounds against bacteria to some extent, especially the inhibition of ***Xoo*** was obviously enhanced.

Table 5
Effect of **Z2** to extracellular amylase of ***Xoo***.

Concentration of **Z2** (mg/L)	**Halo dianeter(mm)**						
	Repetition 1–1	Repetition 1–2	Repetition 2–1	Repetition 2–2	Repetition 3–1	Repetition 3–2	Average diameter
0	26	27	25	27	27	27	26.5
25	24	25	24	25	25	26	24.8
50	22	22	22	23	22	23	22.3
100	21	20	21	21	20	19	20.3

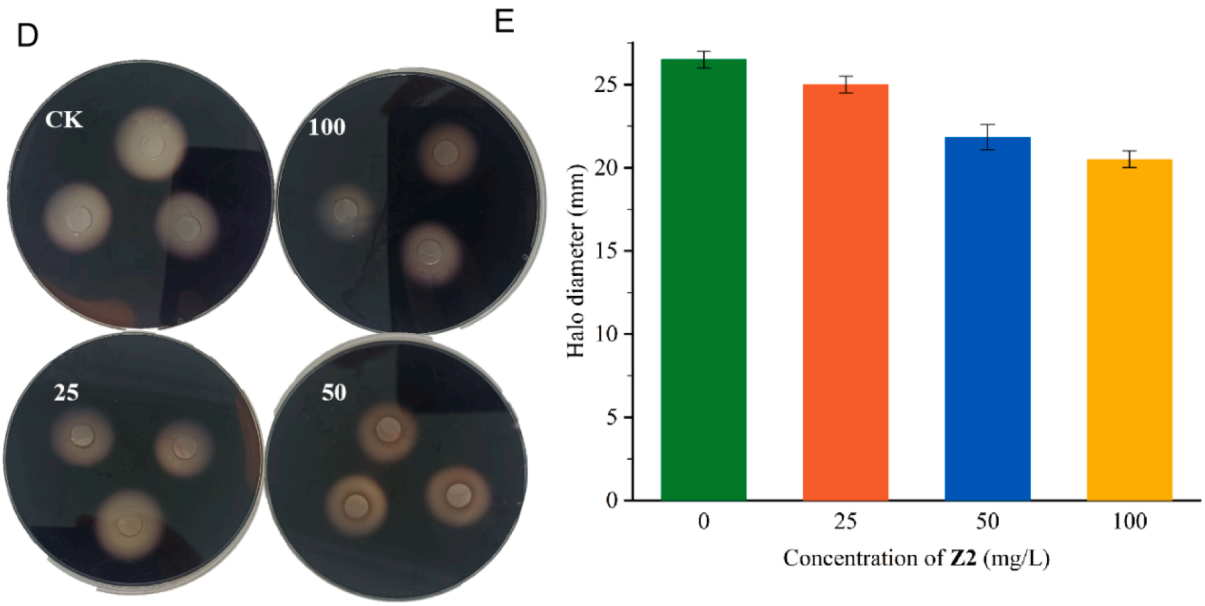


Fig. 8. Impacts of **Z2** on ***Xoo*** Extracellular cellulase (**D** and **E**).

Table 6
Effect of **Z2** to extracellular cellulases *of* ***Xoo***.

Concentration of **Z2** (mg/L)	**Halo dianeter(mm)**						
	Repetition 1–1	Repetition 1–2	Repetition 2–1	Repetition 2–2	Repetition 3–1	Repetition 3–2	Average diameter
0	24	25	25	26	25	27	25.3
25	23	22	21	21	23	24	22.3
50	18	20	19	19	19	19	19.0
100	16	17	17	17	16	17	16.7

4.4. Results of Z2 antibacterial mechanism

4.4.1. Growth rate plot of Z2 against Xoo

According to the results of the growth rate of **Z2** against ***Xoo*** shown in Table 3 and Fig. 4. The test results showed that when **Z2** concentration was 100, 50, 25 mg/L, respectively, it can effectively inhibit ***Xoo*** growth. When the concentration was 12.5, 6.25 mg/L, respectively, the inhibiting effect of ***Xoo*** is significantly worse, and the OD_{595} values increased significantly over time, which showed that the inhibition of **Z2** against ***Xoo*** in a concentration-dependent way. This result was consistent with the screening results of EC_{50} value.

4.4.2. Effect of Z2 on the hyphae morphology of Xoo

The mechanism of action of **Z2** on ***Xoo*** was initially investigated by SEM. As shown in Fig. 5, the cells in the CK group without drug treatment were homogeneous and full. After **Z2** treatment, at 50 μg/mL, some of the cells had a flattened morphology and cell membrane rupture; at 100 μg/mL, the degree of cell rupture increased significantly, showing contraction, collapse and deformation. SEM analysis showed that the greater the concentration of the drug, the greater the degree of cell membrane damage, which initially indicated that **Z2** could destroy the cell membrane.

4.4.3. Cell membrane permeability assay of Z2 to Xoo

Cell membranes are important for maintaining the integrity of cell structure and normal life activities, and the permeability of cell membranes can be evaluated by relative conductivity. To verify the above results combined with the result of screening for the SEM, the effect of **Z2** on the membrane permeability of ***Xoo*** cells was determined, and the results are shown in Fig. 6. It indicated that **Z2** increased the cell membrane permeability in a concentration-dependent manner. At the concentrations of 0, 6.25, 12.5, 25, 50 and 100 mg/L for 22.5 h, the relative conductivities of the cell membrane gradually increased, and the relative conductivities were 5.9, 8.1, 8.6, 11.9, 15.9, and 18.0 %, respectively. In particular, when the concentration of **Z2** was 50 and 100 mg/L, it can be seen that the conductivity of ***Xoo*** cells treated with **Z2** increased significantly, which indicated that when the target compound was at 50, 100 mg/L, it had obvious cell membrane permeability against ***Xoo***, which caused extensive damage to the ***Xoo*** cells membrane, leading to the rupture of the cell membrane, and the components in the

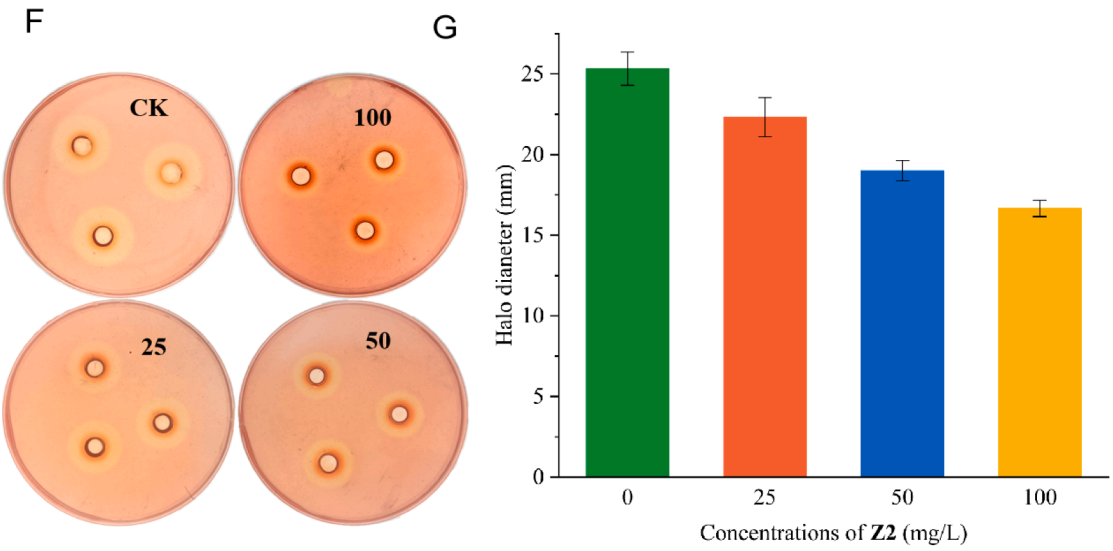


Fig. 9. Impacts of **Z2** on ***Xoo*** Extracellular amylase **(F** and **G)**.

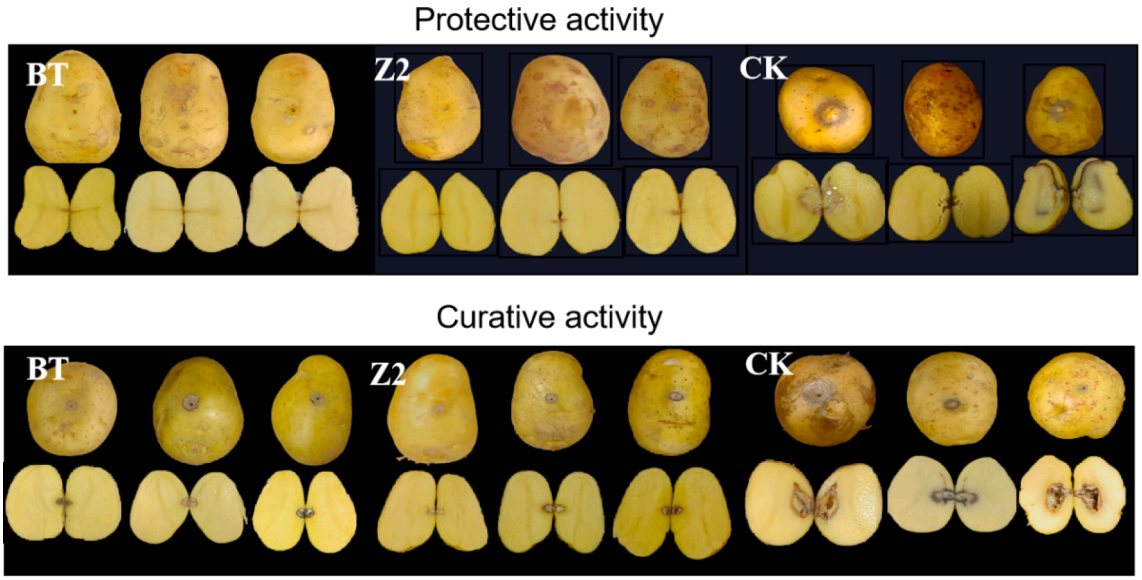


Fig. 10. Effect of BT, **Z2**, CK on post-harvest potato at a concentration of 200 mg/L.

membrane were released, affecting the integrity of the ***Xoo*** cell membrane, causing cells death, resulting in an increased relative conductivity. When the drug concentration was 6.25, 12.5, 25 mg/L, it can be seen that the relative conductivity does not change much, indicating that the permeability of the three concentrations weakens with the lower concentration, and the permeability of the ***Xoo*** cell membrane was significantly worse, so the destruction of the ***Xoo*** cell membrane was reduced to inhibit ***Xoo***, which was consistent with the EC_{50} value determination results.

4.4.4. Effect of Z2 on the Xoo exopolysaccharide (EPS)

During the establishment of bacterial colonies and the formation of biofilms, bacteria secrete extracellular polysaccharides (EPS) to protect themselves from adverse environments(Yun et al., 2006). Bacterial EPS is not only the primary substrate required for biofilm formation but also helps bacteria to resist host plant defense responses (Milling et al., 2011). At the same time, it also creates resistance to some antibiotics, which leads to the deterioration of drug resistance problems (Kakkar et al., 2015). Therefore, the effect of **Z2** on ***Xoo*** EPS production was investigated at drug concentrations of 0, 6.25, 12.5, 25, 50, 100 mg/L, respectively. As shown in Table 4 and Fig. 7. The result showed that when the drug concentrations were 0, 6.25, 12.5, 25, 50 and 100 mg/L, respectively, the EPS contents of ***Xoo*** were 805.3, 560.0, 515.7, 360.3, 320.3, 283.3 mg/L, respectively, which suggested that the **Z2** affects EPS production in a concentration-dependent manner, when EPS production decreased significantly with increasing compound concentration, especially the EPS content was at 100 and 50 mg/L. Thus, **Z2** can significantly reduce bacterial biofilm formation and EPS production.

4.4.5. Effect of Z2 on the extracellular enzyme of Xoo

Most bacteria will produce and secrete various extracellular proteolytic enzymes during the growth stage, such as amylase, cellulase, pectinase, etc. These enzymes help to degrade extracellular macromolecules and obtain nutrients to meet nutritional needs of bacteria, improving adaptability of bacteria to the environment, and facilitating the colonization and infection in the plant body (Sinha et al., 2021; Huang et al., 2022). Therefore, the effects of **Z2** on ***Xoo*** extracellular amylase and extracellular cellulase were explored at the concentrations

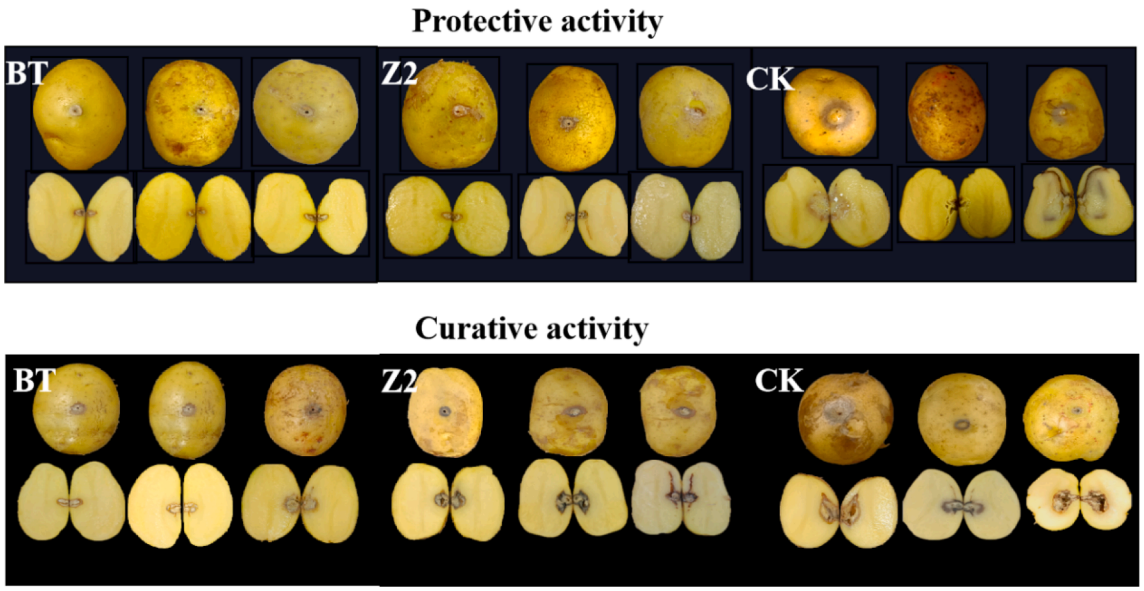


Fig. 11. Effect of BT, **Z2**, CK on post-harvest potato at a concentration of 100 mg/L.

Table 7
Antivial activity of the target compounds against TMV at 500 mg/L.

Compound.	n	R	Curation (%)[a]	Protection(%)[a]
Z1	3	Ph	60.0 ± 3.9	73.0 ± 3.9
Z2	4	Ph	76.1 ± 0.7	69.2 ± 4.9
Z3	3	4-CH_3-Ph	59.9 ± 0.4	62.7 ± 3.1
Z4	4	4-CH_3-Ph	76.0 ± 0.8	72.8 ± 3.6
Z5	3	4-OCH_3-Ph	74.2 ± 4.9	77.2 ± 1.5
Z6	3	4-F-Ph	78.0 ± 3.5	71.8 ± 1.8
Z7	4	4-F-Ph	64.1 ± 3.0	72.7 ± 3.8
Z8	3	4-Br-Ph	48.8 ± 2.8	55.7 ± 3.8
Z9	3	3-CH_3-Ph	62.3 ± 4.5	52.0 ± 1.7
Z10	4	3-CH_3-Ph	57.0 ± 5.6	60.1 ± 0.7
Z11	3	3-OCH_3-Ph	55.1 ± 1.8	63.4 ± 1.4
Z12	4	3-OCH_3-Ph	67.4 ± 3.3	63.1 ± 1.2
Z13	4	3-F-Ph	68.0 ± 4.9	66.3 ± 4.9
Z14	4	3-NO_2-Ph	66.5 ± 3.2	70.7 ± 4.0
Z15	3	3-Cl-Ph	84.0 ± 2.3	76.8 ± 4.2
Z16	4	3-Cl-Ph	73.7 ± 4.1	75.6 ± 2.4
Z17	3	3-Br-Ph	58.3 ± 1.4	62.9 ± 1.8
Z18	4	3-Br-Ph	80.4 ± 4.7	73.0 ± 5.1
Z19	3	2-CH_3-Ph	73.7 ± 3.8	66.2 ± 1.0
Z20	4	2-CH_3-Ph	51.0 ± 4.4	52.5 ± 2.0
Z21	3	2-Fruan	56.3 ± 4.0	60.1 ± 6.2
Z22	4	2-Fruan	56.1 ± 5.0	54.5 ± 3.9
Z23	3	2-Thioen	62.2 ± 3.0	62.3 ± 2.8
Z24	4	2-Thioen	39.4 ± 3.6	48.9 ± 0.8
NNM[b]	–	–	57.4 ± 1.8	65.0 ± 5.1

[a] Average of three replicates, [b] Ningnanmycin (NNM).

of 25, 50, 100 mg/L, respectively, and the experimental results are as follows.

*4.4.5.1. Effect of **Z2** on extracellular amylase of **Xoo**.* The results of the effect of **Z2** on the extracellular amylase activity of ***Xoo*** are shown in Table 5 and Fig. 8. In the starch plate of incubation after 3 d, treatment with I_2/KI solution and 70 % ethanol decorating treatment, a clear hydrolysis coil was observed in the control group with an average diameter of 26.5 mm. After treatment with **Z2** at the concentrations of 25, 50, 100 mg/L, the average diameter of the transparent hydrolysis circle treated was 24.8, 22.3 20.3 mm, respectively and the diameter of the hydrolysis circle was reduced by 6.4, 15.7, and 23.3 %, respectively. The results showed that **Z2** has some inhibiting activity against ***Xoo*** extracellular amylase.

*4.4.5.2. Effect of **Z2** on the extracellular cellulases of **Xoo**.* The effect of **Z2** on extracellular cellulase of ***Xoo*** was obtained as shown in Table 6 and Fig. 9, and the test results of **Z2** on extracellular cellulase were the same as that of extracellular amylase. The cellulose plates had been incubated for 3 d, stained with 0.1 % Congo red solution and decolorized with the 1 mol/L NaCl solution. A transparent circle was clearly visible in the control group, with a mean diameter of 25.3 mm. In group treated with **Z2** at the concentration of 25, 50, 100 mg/L, respectively. The average diameters of the hydrolysis circles were 22.3, 19.0 and 16.7 mm, which result in reducing the corresponding extracellular cellulases by 11.8, 25.0 and 34.2 %, respectively. The test results showed **Z2** has some inhibiting effect on the extracellular cellulase of ***Xoo***.

In conclusion, at the concentration of 100 mg/L of **Z2**, on the one hand, **Z2** can change the permeability of ***Xoo*** cell membrane and destroy the integrity of the cell membrane. On the other hand, it can affect the content of EPS and extracellular amylase and extracellular cellulase of ***Xoo*** cell, and block ***Xoo*** biofilm to obtain external nutrients, thus affecting the growth rate of ***Xoo*** cells and achieving the purpose of inhibiting ***Xoo***.

*4.5. Effect of **Z2** on harvested potato*

The antibacterial activity (protection and curation) effect on harvested potato of **Z2** was tested at the drug concentration of 200 and 100 mg/L, respectively, and the test results are shown in Figs. 10, 11. According to Fig. 10, at the concentration of 200 mg/L, as a negative control, the CK group inoculated with ***Pcb*** after only water, the potato turned black and soft. After incision, the interior was almost soft and decayed, with numerous water stains, and **Z2** showed better results of protection and curation of the potato. Especially, the protective activity was even more significant, there were few lesions of the epidermis and interior of the potato (with an effect close to thiodiazole copper). According to Fig. 11, at 100 mg/L, the protective and curative effect of **Z2** was significantly reduced, the reduction in the curative effect was even more distinct, the potato was partially decomposed, but the lesion was still less than that of CK. This result indicated that **Z2** has a certain protective effect on the potato. However, it can be seen from Fig. 11 that **Z2** has a poor curative effect on potato at the drug concentration of 100 mg/L, and the extent of the lesion was comparable to the CK.

In conclusion, at high concentration, **Z2** showed better protective activity of potatoes, but showed less curative activity of potatoes infected with ***Pcb***.

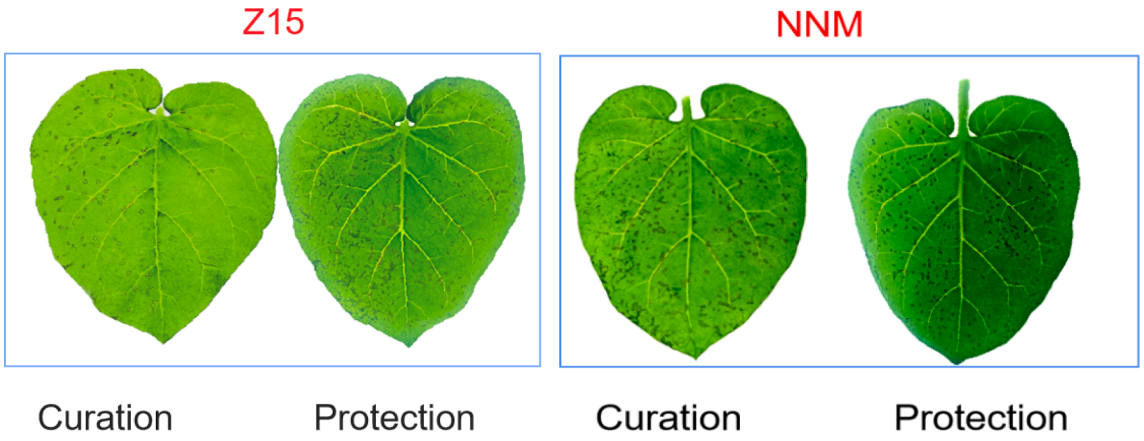


Fig. 12. Anti-TMV activities of **Z15** and NNM *in vivo*.

Table 8
EC_{50} values of some of the target compounds against TMV *in vivo*.

	Compounds	n	R	Toxic Regression	R^2	EC_{50} (μg/mL)[a]
Curation	**Z4**	4	4-CH_3-Ph	y = 1.3716x + 1.8734	0.9735	190.34
	Z5	4	4-OCH_3-Ph	y = 1.1376x + 24749	0.9781	165.83
	Z6	3	4-F-Ph	y = 1.7490x + 2.5044	0.9824	133.07
	Z15	3	3-Cl-Ph	y = 1.3837x + 2.2209	0.9725	101.97
	Z16	4	3-Cl-Ph	y = 1.1810x + 2.6054	0.9707	106.56
	Z18	4	3-Br-Ph	y = 1.1934x + 2.5723	0.9874	108.21
	NNM[b]	–	–	y = 1.1888x + 2.0648	0.9974	294.27
Protection	**Z4**	4	4-CH_3-Ph	y = 1.0554x + 2.7497	0.9979	135.57
	Z5	4	4-OCH_3-Ph	y = 1.8142x + 0.9455	0.9840	171.74
	Z6	3	4-F-Ph	y = 1.402x + 2.6069	0.9639	125.56
	Z15	3	3-Cl-Ph	y = 1.2312x + 2.5102	0.9912	105.26
	Z16	4	3-Cl-Ph	y = 1.2984x + 2.3808	0.9881	104.05
	Z18	4	3-Br-Ph	y = 1.2205x + 2.4632	0.9917	119.81
	NNM[b]	–	–	y = 0.9067x + 2.9428	0.9821	185.73

[a] Average of three replicates, [b] Ningnanmycin (NNM).

4.6. *Antiviral activity of **Z1**-**Z24** against **TMV** in vivo*

At a drug concentration of 500 μg/mL, the anti-TMV activities of the target compounds **Z1**-**Z24** were tested by the half-leaf dry spot method, and the test results are shown in Table 7, most of the compounds showed some anti-TMV activities. It is worth noting that the curative activities of **Z2**, **Z4**, **Z5**, **Z6**, **Z15**, **Z16**, **Z18** and **Z19** were 76.1, 76.0, 74.2, 78.0, 84.0, 73.7, 80.4, 73.7 %, respectively, which were superior to NNM (57.4 %); the protective activities of **Z1**, **Z4**, **Z5**, **Z6**, **Z7**, **Z14**, **Z15**, **Z18** were 73.0, 72.8, 77.2, 71.8, 72.7, 70.7, 76.8, 73.0 %, respectively, which were higher than NNM (65.0 %). Among them, the tobacco leaf morphology of **Z15** and NNM against TMV *in vivo* is shown in Fig. 12.

Based on the results of the initial screening, the EC_{50} values of some compounds against TMV were further tested and the results are shown in Table 8. The EC_{50} values of curative activities of **Z15**, **Z16**, and **Z18** were 101.97,106.56, and 108.21 μg/mL, respectively, which were better than NNM which was 294.27 μg/mL. The EC_{50} values of protective activities of **Z15**, **Z16**, and **Z18** were 105.26, 104.05 and 119.81 μg/mL, respectively, which were superior than NNM (185.73 μg/mL).

The structure–activity relationship (SAR) was analyzed according to the anti-TMV activities shown in Table 7 and Table 8.

When the number of C-atoms of brominated alkane was 3 and R was an electron-withdrawing phenyl group with a better activity against TMV. For example, the curative activity situations were **Z15** (n = 3, R = 3-Cl-Ph, 84.0 %) > **Z9** (n = 3, R = 3-CH_3-Ph, 62.3 %) > **Z23** (n = 3, R = 2-Thiophene, 62.2 %) > **Z21** (n = 3, R = 2-Furan, 56.3 %) > **Z11** (n = 3, R = 3-OCH_3-Ph, 55.1 %). The protective activity situations were **Z15** (n = 3, R = 3-Cl-Ph, 76.8 %) > **Z11** (n = 3, R = 3-OCH_3-Ph, 63.4 %) > **Z23** (n = 3, R = 2-Thiophene, 62.3 %) > **Z21** (n = 3, R = 2-Furan, 60.1 %) > **Z9** (n = 3, R = 3-CH_3-Ph, 52.0 %).

When the number of C-atoms of brominated alkanes was n = 4 and R was an electron-withdrawing phenyl group with a better activity against TMV. For example, the curative activity situations were **Z16** (n = 4, R = 3-Cl-Ph, 73.7 %) > **Z12** (n = 4, R = 3-OCH_3-Ph, 67.4 %) > **Z10** (n = 4, R = 3-CH_3-Ph, 57.0 %) > **Z22** (n = 4, R = 2-Furan, 56.1 %) > **Z24** (n = 4, R = 2-Thiophene, 39.4 %). The protective activity situations were **Z16** (n = 4, R = 3-Cl-Ph, 75.6 %) > **Z12** (n = 4, R = 3-OCH_3-Ph, 63.1 %) > **Z10** (n = 4, R = 3-CH_3-Ph, 60.1 %) > **Z22** (n = 4, R = 2-Furan, 54.5 %) > **Z24** (n = 4, R = 2-Thiophene, 48.9 %).

We noticed that the substituent groups introduced in the early stage were relatively single, so we introduced heterocyclic substituents (thiophene, furan) and tested these target compounds for their activities against TMV, but the test results showed that the activities of the target compounds did not change significantly after the introduction of the heterocyclic substituents. For example, the curative activity situations were **Z21** (n = 3, R = 2-Furan, 56.3 %), **Z22** (n = 4, R = 2-Furan, 56.1 %), **Z23** (n = 3, R = 2-Thiophene, 62.2 %), **Z24** (n = 4, R = 2-Thiophene, 39.4 %); the protective activity situations were **Z21** (n = 3, R = 2-Furan, 60.1 %), **Z22** (n = 4, R = 2-Furan, 54.5 %), **Z23** (n = 3, R = 2-Thiophene, 62.3 %), **Z24** (n = 4, R = 2-Thiophene, 48.9 %).

In summary, **Z15** (n = 3, R = 4-Cl-Ph) and **Z16** (n = 4, R = 4-Cl-Ph) showed significant inhibiting activities against TMV in terms of curative, protective and inactivated activities, when n = 3, R was halogen-substituted phenyl, the protective and curative activities of the target compound against TMV were enhanced.

4.7. *Molecular docking of compounds **Z15, Z16** and **NNM** with TMV-CP*

According to the screening results of the activities of some compounds against TMV, in Table 7, **Z15 and Z16** have some curative and protective activities against TMV. Therefore, we explored the

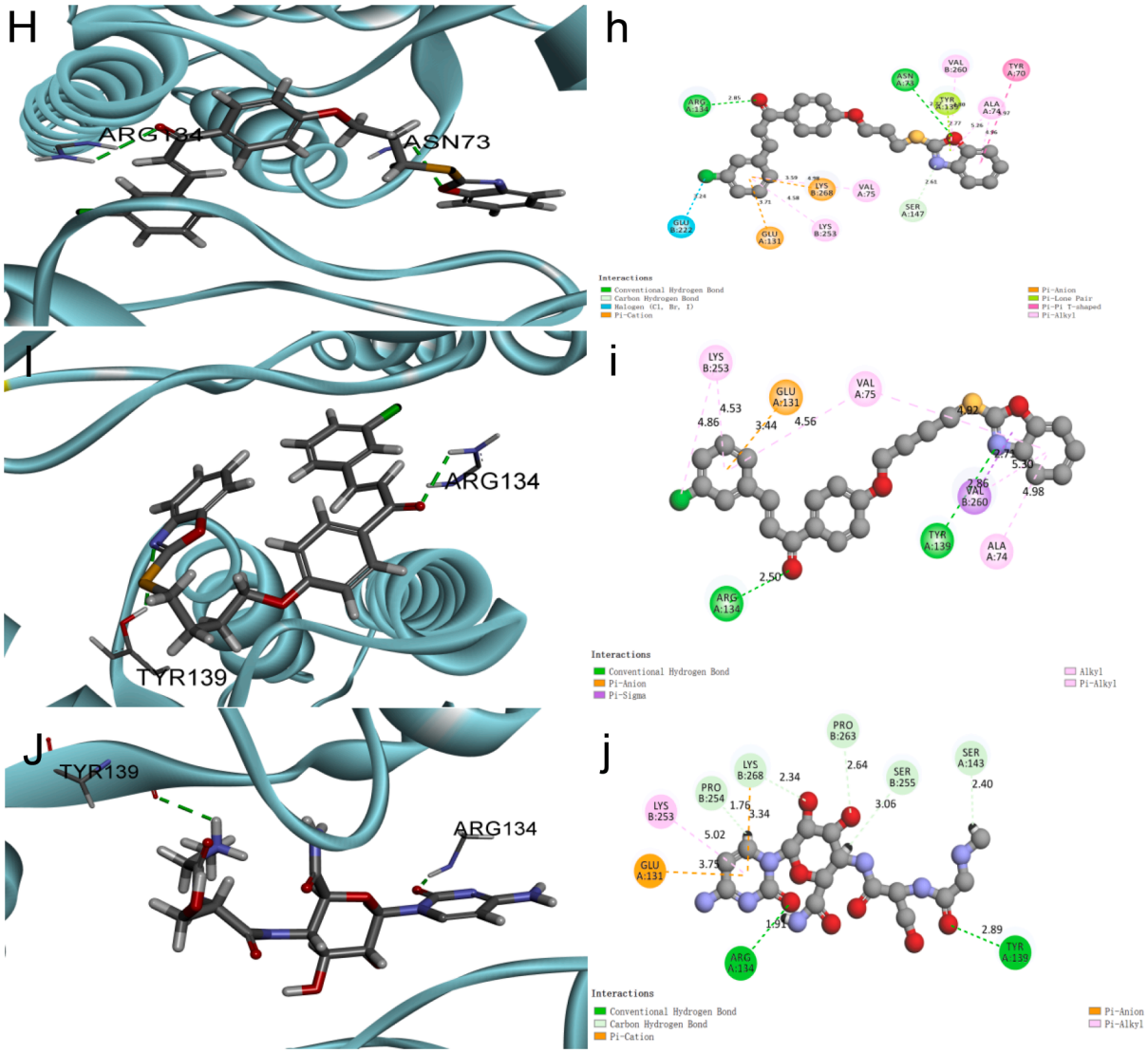


Fig. 13. Molecular docking results of **Z15** (**H**, **h**), **Z16** (**I**, **i**), **NNM** (**J**, **j**).

similarities and differences of binding modes of **Z15**, **Z16**, NNM and TMV-CP by molecular docking and the results are shown in Fig. 13. Molecular docking results revealed that **Z15**, **Z16** and NNM are capable of hydrogen bonding with TMV-CP, and the common amino acid residues between **Z15**, **Z16** and TMV-CP are ARG-A-134, TYR-A-139. **Z15** formed two hydrogen bonds with TMV-CP, in which the benzoxazole nitrogen atom of **Z15** formed a hydrogen bond with residue TYR-A-73 (2.77 Å) of TMV-CP, and the chalcone-based oxygen atom formed a hydrogen bond with residue ARG-A-134 (2.85 Å). **Z16** formed two hydrogen bonds with TMV-CP, in which the benzoxazole nitrogen atom of **Z16** formed a hydrogen bond with residue TYR-A-139 (2.85 Å) of TMV-CP. The chalcone-based oxygen atom formed a hydrogen bond with residue ARG-A-134 (2.50 Å). Similarly, NNM forms hydrogen bonds with amino acid residues ARG-A-134 (1.91 Å) and TYR-A-139 (2.89 Å). Through molecular docking, the results showed that the better anti-TMV activities of **Z15** and **Z16** may be related to the amino acid residue TYR-A-139, the strength of hydrogen bonds of **Z15**, **Z16**, and NNM with the amino acid residue TYR-A-139 are as follows, **Z15** > **Z16** > NNM.

In addition, **Z15**, **Z16**, and NNM formed 10, 8, 8 hydrophobic bonds with other amino residues of TMV-CP, respectively. Such as Pi-Alkyal, Pi-Pi, Pi-amion, Pi-sigma, and Alkyal. Due to the formation of these hydrophobic bonds provides a certain intermolecular force, it is likely to weaken the interaction of two subunits of TMV-CP, and prevents the self-assembly of TMV particle, as well as the binding capability with TMV-CP (Tang et al., 2019). Thus, the effect against TMV was achieved. The molecular docking results are that **Z15** and **Z16** have some anti-TMV activity, and **Z15** > **Z16** > NNM, which is in line with the data obtained from the preliminary screening data.

5. Conclusions

In this study, a series of chalcone derivatives containing benzoxazole were synthesized. The results of biological assays showed that some of the target compounds had excellent antibacterial and antiviral activities. The inhibiting activity of the **Z2** against ***Xoo*** was tested with turbidity method, and the test results showed that **Z2** had excellent inhibiting effect on ***Xoo***. To study the mechanism of its inhibition, the cell membrane permeability, the content of EPS, the content of extracellular amylase and extracellular cellulase assays were performed between **Z2** and ***Xoo***, and the measurement results showed that **Z2** could inhibit the content of ***Xoo*** biofilm to achieve the effect of inhibition. Moreover, when the drug concentrations were at of 200 and 100 mg/L, we tested the effect of **Z2** on post-harvest potato and found that **Z2** have a good protective activity for potatoes. Finally, **Z15** and **Z16** surpassed the commercial drug NNM in terms of both curative and protective

activities. Molecular docking assay of **Z15** and **Z16** was performed, and the results of mechanism of action were consistent with the preliminary screening data. Consequently, this study lays the foundation for the application of chalcone derivatives in pesticides.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

The Supporting Information includes the characterization data, HNMR and HRMS spectrogram for the target compounds. Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2023.105368.

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