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Functional Structure/Activity Relationships

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Design, Synthesis and Bio-activity of α-Ketoamide Derivatives Bearing a Vanillin Skeleton for Crop Diseases

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ABSTRACT: A series of novel α-ketoamide derivatives bearing vanillin skeleton was designed and synthesized. Bio-activity tests on virus and bacteria were performed. The results indicated that some compounds exhibited excellent anti-tobacco mosaic virus (TMV) activities, such as compound **34** exhibited an inactivation activity of 90.1%, curative activity of 51.8% and compound **28** exhibited curative activity of 54.8% at 500 μg/mL, which is equivalent to that of the commercial ningnanmycin (inactivation of 91.9%, curative of 51.9%). Moreover, in vitro antibacterial activity test illustrated compounds **2**, **22**, **33** showed much higher activities than commercial thiodiazole copper, which could be used as lead compounds or potential candidates. The findings of transmission electron microscopic (TEM) and molecular docking indicated that the synthesized compounds exhibited strong and significant binding affinity to TMV coat protein (CP) and could obstruct the self-assembly and increment of TMV particles. This study revealed that α-ketoamide derivatives bearing a vanillin skeleton could be used as novel potential pesticide for controlling the plant diseases.

KEYWORDS: *α-Ketoamide; Vanilline; Synthesis; Bio-activity; Molecular docking; Mechanism*.

INTRODUCTION

When it comes to plant diseases, the first thing that comes into our mind is that they can seriously affect the quality and yields of crops. Although they are normally genetically rather simple, efforts are hardly going on to prevent and control them.[1] For tobacco mosaic virus (TMV) instance, the losses caused merely by it can up to $100 million.[2] For another example, a rice bacterial disease caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*) can result in the losses up to 80% yield loss.[3] However, there is a shortage of high efficient measurements for controlling viruses and bacteria in plant**s**, as the mainstream pesticides are lower efficiency and high cost for controlling the diseases, such as ningnanmysin, bismerthiazol, thiodiazole copper.[3-5] Hence, an efficacious and environmentally-friendly pesticide is considerably demanded to control viral and bacterial diseases in crops. Vanillin (4-hydroxy-3-methoxybenzaldehyde), as one of the natural aroma molecules derived from *orchids*,[6] is a crucial component in the vanilla bean, which accounts for about 2% of the dry matter and is one of the most important widely used flavouring materials worldwide.[6,7] Besides as a flavouring agent, recent progresses of vanillin indicated that the incorporation of substructure of vanillin to some compounds exhibited excellent bio-activity as drug candidates, such as anti-bacterial, [8] anti-inflammatory,[9-11] anti-Alzheimer's,[12] anti-cancer,[13,14] anti-mutagenic [15] and anti-metastatic [16] activities. As showing two active reaction sites (Figure 1), the structure of vanillin provides a good starting point for the synthesis of new compounds with good bio-activity, which draws a great attention of many chemists. [17,18] In 2017, Zhang *et al.*[19] reported novel vanillin derivatives incorporating a bis (2-hydroxyethyl) dithhioacetal moiety (Figure 1) as antiviral agents and in 2018. Moreover, Xie *et al*. [20] designed novel quinazoline derivatives bearing a vanillin skeleton (**Fig. 1**) showing

excellent antiviral activities as well.

Amide, as one of crucial active motifs in organic synthesis, ubiquitously exists in a large number of bio-active molecules,[21] such as herbicidal [22] bactericidal [23] antiviral [24] and insecticidal molecules.[25, 26] For a specific example in 2013, the methyl thiazide bearing amide unit, as a plant activator, can be applied in many cash crops such as tobacco, rice, cucumber to mainly control viral diseases including TMV. [27] Notably, among the amide derivatives, α-ketoamide is a considerably special–amide derivative, which not only has excellent biological activity, existing in many natural products, [28 - 30] polypeptides, [31, 32] and many biologically active molecules [33, 34] but also serves as a precursor for a variety transformation in the chemical synthesis.[35, 36] Interestingly, our previous work has indicated that a series of synthesized α-ketoamide derivatives exhibited good anti-TMV activities for potential use in plant protection,[37] which is rarely reported in pesticide area.

In this work, we sought to use vanillin as the lead compound and starting material, combining the skeleton of vanillin and *α*-ketoamide to get 2-(4-hydroxy-3-methoxyphenyl)-2-oxoacetamide, then the amide structure was introduced via the site of hydroxyl, which may result in potential active molecules for controlling viruses and bacteria in crops (**Fig. 1**). Consequently, thirty-nine novel *α*-ketoamide derivatives containing the skeleton of vanillin and moiety of amide were synthesized. Bio-assays against tobacco mosaic virus (TMV) and three kinds of bacteria indicated that some of title compounds showed excellent anti-viral activities and anti-bacterial activities, which have never been reported up to now. The pot experiment, preliminary structure-activity relationship and mechanisms of action were also performed discussed.

MATERIALS AND METHODS

Instruments and Chemicals. All of the reactions were carried out by using a magnetic stirring bar under air and monitored by TLC. All synthesized compounds resolving in the $CDCl_3$ or DMSO-d6, ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a AVANCE III HD 400M NMR (Bruker Corporation, Switzerland) spectrometer operated at 20 ^{o}C. The XT-4 binocular microscope (Beijing Tech Instrument Co., China) was used to measure the melting points (uncorrected) of title compounds. HR-MS was recorded on an Orbitrap LC-MS instrument (Q-Exative, Thermo Scientific™, and American). Molecular docking was done by using BIOVIA Discovery Studio 4.5 (BIOVIA, San Diego, USA). Transmission Electron Microscopic (TEM) experiments were recorded on FEI Talos F200C (Thermo Fisher Scientific, Massachusetts, USA). The chemical materials and reagents involved in the reactions were purchased from commercial suppliers and used directly without further purification. Particularly, 2-aminopyridine and isocyanocyclohexane were purchased from TCI (Tokyo, Japan) and a series of anilines, iodobenzene acetate, and *p*-toluene sulfonic acid was purchased from Accela (Shanghai, China).

General Procedure for Preparing the Title Compounds. As shown in **Scheme 1**, the title compounds **1-39** were provided via several steps that started from vanillin as leader compound, involving three intermediates (intermediates **A-C**)

*The preparation of Intermediate **A**.*[38] To a mixture of 2-aminopyridines (2 g, 21.12 mmol), vanillin (3.2 g, 21.12 mmol), and isocyanocyclohexane (2.3 g, 21.12 mmol), in ethanol was added β-cyclodextrin-SO_3H (2.57 g, 2.1 mmol). The reaction mixture was then allowed to stir for 1.5 h under 80 °C. After completion of this reaction, the resulting mixture was cooled, the

96 catalyst β-cyclodextrin-SO_3H was removed by filtration; the organic phase was then
97 concentrated and washed with ethyl acetate/n-hexane (1:3) and dried to give intermediate **A** in
98 82% yield (4.7 g).
99 *The preparation of Intermediate **B***.[37] To a three-necked bottle was charged with A (0.5 g, 1.48
100 mmol) and $TsOH \cdot H_2O$ (0.85 g, 4.45 mmol), followed by addition of DCE (6 mL), then adding
101 iodobenzene acetate (0.48 g, 1.48 mmol) during the stirring process. The resulting mixture was
102 then stirred at room temperature for 1 h. After completion of the reaction, the DCE was removed
103 under reduced pressure to give a crude intermediate **B**, which was then purified by column
104 chromatography on silica gel (eluting with petroleum ether/ethyl acetate = 5 : 1) to afford *N*-
105 cyclohexyl-2- (4-hydroxy-3-methoxyphenyl)-2- oxoacetamide (**B**) in 70% yield.
106 *The General Protocol for Preparation of Intermediate **C***. Based on the literature method, [39]
107 various kinds of substituted amines as raw materials (**Scheme 1**), dichloromethane (15 mL) as
108 the solvent, and triethylamine (1 mmol) were added subsequently into 50 mL round-bottomed
109 flask to stir for a while. Then under the ice condition, chloroacetyl chloride (1 mmol) diluted
110 with dichloromethane was slowly added drop-wise, the reaction was monitored by TLC and
111 completed to obtain intermediate **C**.
112 *The General Procedure for Preparation of Target Compounds **1- 39***. Taking compound **1**
113 example, resolving intermediate **B** (1 mmol) in DMF (*N*, *N*-dimethylformamide), and potassium
114 carbonate K_2CO_3 (1.2 mmol) and potassium iodide KI (0.5 mmol) were added, then adding
115 intermediate **C** (1.1 mmol) at room temperature to stir for 7 h. The reaction solution pouring
116 into water as stirring, a large amount of solid was precipitated from reaction solution and filtered
117 to give the crude products. Finally, pure products **1-39** were obtained by washing the crude

products with hexane and confirmed by the ^{1}H NMR, ^{13}C NMR and HR-MS. Physicochemical data of representative compound **1** were listed as follows. The properties for the rests of title compounds were listed in Support Information.

N-cyclohexyl-2-(3-methoxy-4-(2-oxo-2-(phenylamino)ethoxy)phenyl)-2-oxoacetamide (**1**): yield 75%; White solid; m.p. 117 – 118 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) *δ:* 8.67 (s, 1H), 8.18 (dd, *J* = 8.5, 1.8 Hz, 1H), 8.03 (d, *J* = 1.6 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 2H), 7.36 (t, *J* = 7.9 Hz, 2H), 7.16 (t, *J* = 7.4 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 4.72 (s, 2H), 4.01 (s, 3H), 3.91–3.75 (m, 1H), 1.98 (d, *J* = 12.1 Hz, 2H), 1.81 – 1.72 (m, 2H), 1.68 (d, *J* = 4.2 Hz, 1H), 1.42 (dd, *J* = 24.6, 12.3 Hz, 2H), 1.33 – 1.18 (m, 3H). **^{13}C NMR** (100 MHz, CDCl3) *δ*: 185.86, 165.68, 160.95, 151.97, 149.22, 136.91, 129.14, 128.50, 126.77, 124.91, 120.04, 113.85, 68.96, 56.12, 48.55, 32.73, 25.42, 24.78; **HR-MS** (ESI) Calculated for $C_{23}H_{26}N_5O_2$ $[M+H]^+$: 411.19145, found: 411.19037.

Antiviral Bioassay against Tobacco Mosaic Virus (TMV). Three kinds of antiviral activities (inactivation, curative and protective) for the target compounds against TMV *in vivo* were evaluated by the half-leaf spot method,[40, 41] commercial ribavirin and ningnanmycin were selected as positive controls and more operational details are shown in the supporting information. Moreover, the pot experiments were then performed using *Nicotianag lutinosa* plants that were cultivated in a greenhouse. Plants with uniform size and growth rate were selected and inoculated with TMV. After 24 h, compound **28** or ningnanmycin was sprayed on the plants. Data on the symptoms (See **Fig. S1** in Support Information) were collected three days' post-application of compound **28**.

Antibacterial Bioassays. The antibacterial activities against *Xanthomonas oryzae pv. oryzae*

(*Xoo*), *Ralstonia solanacearum* (*RS*) and *Xanthomonas axonopodis pv. Citri* (*Xac*) *in vitro* were carried out using the turbidity method (more details can be found in the supporting information).[42] The results of the antibacterial bioassays were shown in the **Table 3**.

Molecular Docking Study. The molecular docking study was carried out by using Discovery Studio 4.5. The structures of synthesized compounds were minimized to keep them in a low energy state by using the module of minimization (QM-MM). The TMV coat protein (PDB code: 1EI7) [44] was downloaded from RCSB PDB protein data bank (http://www.rcsb.org). Before docking, the protein was treated by removing water and hydrogenation. Molecular docking was then conducted on the Lib-Dock module using default conditions in Discovery Studio (DS) 4.5.

Transmission Electron Microscopic (TEM) Experiments. The samples were processed according to the methods described in the literature.[43] The solutions of active compounds and commercial ningnanmycin (positive control) were prepared at a concentration of 1000 mg L^{-1}, and mixed with TMV in equal volume to obtain a mixture with concentration of 500 mg L^{-1}. A solution containing TMV particles without the compounds was used as the blank control (CK). After mixing for 30 minutes, the mixtures were adsorbed using a 200-mesh copper grid carbon support membrane and counterstained with 1% phosphotungstic acid with a pH of 7.4. After drying, the morphology of TMV particles was observed and micrograghs were taken under TEM with a FEI Talos F200C at 200 kV.

RESULTS AND DISCUSSION

Chemistry. The synthetic route to the title compounds was showed as **Scheme 1**. The key intermediate **A** could be easily prepared by β-cyclodextrin-SO_3H catalytic one-pot process,[37] then α-ketoamide intermediate **B** was synthesized by cleavages of aromatic fused imidazoles

using iodobenzene diacetate as oxidant and the H_2O as source of the O in amide process. [37] Intermediate **C** were also easily obtained by treatment of different type of primary amine with chloroacetamide in present of trimethylamine in dichloromethane, [39] which further react with intermediate **B** in percent of K_2CO_3 and catalytic amount potassium iodide to yield the title compounds (**1-39**) in good to excellent yields.

Antiviral Activity Against TMV. The data of anti-viral activities of the targets compounds were shown in the **Table 1**, which indicated that most of target compounds exhibited anti-TMV activities in vivo from moderate to excellent at 500 μg/mL. In particular, compounds **28** and **34** were confirmed the most excellent curative activities for TMV that were equivalent to that of ningnanmycin (51.9%). The inactivation of compound **34** was also close to that of ningnanmycin. Structure-activity relationship analysis showed when R is an unsubstituted phenyl, the corresponding compound **1** did not show notable anti-virus activity. Nevertheless, when the R was a phenyl with substituents of difluoromethoxy (**28**) and trifluoromethyl (**34**) at the position **4** of benzene, the anti-TMV activity enhanced desirably. Interestingly, compound **34** also showed the best inactivation activity (90.1%), which was similar to that of ningnanmycin (91.9%). However, the curative activity sharply decreased as changing the position for the substituent of phenyl with trifluoromethoxy (compound **23**) at 3 position. Additionally, compounds **21**, **23**, **33**, **36** containing skeleton of trifluoromethyl showed the inactivation activities of 72.5%, 71.6%, 72.9%, 76.0% respectively, which were all higher than that of ribavirin (70.2%). Furthermore, in order to obtain more effective molecules with suitable substituent of phenyl, more functional groups were employed to phenyl. For instance, the bio-assay data indicated that the activities were not desirably when the phenyl was substituted by

one methyl in the position 3 (compound **5**) or substituted by one isopropyl (compound **10**) but slight promotion of curative activity and much promotion of protection activity were detected as changing the position of methyl to the position 4 (compound **8**) and curative activity would promote a lot as placing two methyl (compound **9**) at the positions 3 and 4. Moreover, considering the importance of halogen in the field of agrochemicals, such as better lipophilicity, greater metabolic stability and more excellent bio-activity compared to non-halogenated compound, [44-46] thus one, more or mixed halogen atoms substituting in phenyl were utilized. Desirably, the phenomenon of more fluorine substituent groups in phenyl higher anti-TMV activities was provided (see the data of **7**, **16**, **38** in **Table 1**). Additionally, different substituent groups in the phenyl exhibited differently activities against TMV, the order of their inactive activities concluding as follows **15** (2-CH_3-4-F) > **19** (2-Cl-5-CH_3) > **14** (2-CH_3-3-Cl) > **13** (2-CH_3-4-Br) which obviously indicated compound containing fluorine showed higher inactivation activity. Furthermore, pyridyl with trifluroumethyl substituting in the position 2 (compound **36**) exhibited much lower activity compared with compound **34**. In addition, various functional groups in pyridyl (compound **18**, **27**, **29**, **31**, **35**) or unsubstituted pyridyl (compound **2**) did not contribute to the improvement in curative activity and even showed activity much lower than the corresponding phenyl with the same substituent. To extend the scope of research, R substituent group replaced by cycloalkyl (compounds **3**, **12**), alkyl (compound **10**), benzyl (compound **4**) or *p*-chlorobenzyl (compound **20**) was considered to improve the activity. But unfortunately, with the replacement of R substituent the activity of synthesized compounds did not enhanced. The half maximal effective concentration (EC_{50}) (**Table 2**) indicated that compounds **34**, and **15** showed good inactivation activity, with EC_{50} values of 131.05 and

183.26 μg/mL respectively, which are similar to ningnanmycin (111.71 μg/mL). Compound **28** and **34** showed EC_{50} values of curative activity of 358.46 and 394.52 μg/mL, respectively. Especially, the EC_{50} value of compound **28** for curative activity is slightly lower than that of ningnanmycin (362.4 μg/mL). Moreover, a pot experiment shown in **Figure S1** (see Supporting Information) was further conducted to exhibit the anti-TMV efficiency, which indicated that after inoculating with TMV for 96 h (72 h after spraying compound **28**), the tobaccos could be infected seriously and some of the tobacco leaves infected with a large area of lesions, but the tobaccos treated by the compound **28** solution and ningnanmycin solution at 500 μg/mL still growth well **(Fig. S1**), the lesions were significantly controlled by solution of compound **28**, even slightly higher than that of commercial ninnanmycin. Moreover, we could see there is no any damage or other obvious diseases on the surface of the tobacco leaves. Preliminary results show that compound **28** has no phytotoxicity to tobacco and is safe to plant. These results demonstrated the compound **28** could be regarded as a potential anti-TMV agent or lead compound for further optimization.

The TEM was employed to investigate the effect of compound **34** on TMV particles. The results shown in **Figure 2** revealed that compound **34** (**Fig. 2**, A) and ningnanmycin (**Fig. 2**, B) could cause the extreme impact on the self-assembles of TMV particles compared with blank control (**Fig. 2**, C). The TMV particles assembled very well. However, the external shapes of TMV particles treated by the synthesized compound were fragmented and even some of them were severe fragmented to a bending-shaped. Meanwhile, the density of virus particles is also much sparser than that of blank control. As the phenomenon mentioned above, we speculated that synthesized novel vanillin derivatives could obstruct the self-assembly and increment of TMV

228 virus particles to result in excellent inactivation activity of the compound **34**.[47] Previous work
229 has revealed the active pockets with the amino acid residues including TYR139, PHE12,
230 SER147, ALA74, VAL75, *etc.* play an important role in the self-assembly of TMV-CP.[48-50]
231 Thus, this active pocket mentioned above was chosen as the potential bonding site.[49,50] Docking
232 results (**Fig. 3**) indicated that compound **34** was embedded well in the active pocket around with
233 the amino acid residues including PHE12, TYR70, ASN73, ALA74, VAL75, TYR139, SER143,
234 VAL260. It can interact with these residues via conventional hydrogen bonds, carbon hydrogen
235 bonds, and some of the important noncovalent interactions including halogen (F), π-donor
236 hydrogen bonds and π-alkyl. These different type of interactions are very important for the
237 donation of activities. [51-53] For instance, there are six conventional hydrogen bonds between **34**
238 and TMV-CP, two of them were formed between TYR70 and fluorines in group of "-CF_3", also
239 some strong conventional hydrogen bonds were formed between the O atom and TYL 139 or
240 ALA 74 (**Fig. 3,** C), and two of them were between the O atom in vanillin skeleton and ASN
241 73. Additionally, residue PHE 12 showed a π-alkyl with "-CF_3". Bloomer has reported that
242 TYR70 and TYR 139 were parts of a cluster within the continuous hydrophobic girdle, and
243 there was a hydrophobic contact of PRO 54 with ALA 74 in a normal TMV-CP, also TYR139
244 and PHE12, which play a critical role in a continuous belt of hydrophobic interactions encircling
245 each ring of the disk assembly.[49] Moreover, compound **34** formed a strong halogen interaction
246 to TYR72, which may impact on the interaction between TYR72 and THR28 in another sub-
247 unit of TMV-CP.[54] Bhyravbhatla et al. revealed that the residues ALA74 and VAL75 showed
248 strong interactions with another subunit of TMV-CP.[48] Hence, these interactions between
249 compound **34** and the above residues may affect the self-assembly of TMV particles.[44] In

addition, compound **36** bearing the similar structure of **34** was also docked. As shown in **Figure 3** (C, F), the combination mode has changed to some extent, when transforming to pyridyl (**36**) from phenyl (**34**). There are several interactions between compound **36** and residues of SER255, VAL75, ASN73, GLN257, TYR139, VAL260, ALA74, PRO254 and including four conventional hydrogens. Although some of these residues play important roles in the interactions between the subunits of TMV-CP.[49] However, because some strong hydrogen bonds were formed among the nitrogen on the pyridine ring, fluorine and residue ASN 73, together with some other interactions, which resulted in the lowest energy conformation of molecule **36** with TMV-CP. This conformation was far different from molecule **34** with TMV-CP. And the number of hydrogen bonds is reduced from 6 to 4, which could be one of main reasons for contributing to the lower inactivity of compound **36** than compound **34**. The molecule docking results are consistent with the bio-assay data. The skeleton of benzene ring with trifluoromethyl could be a positive factor for the promotion of anti-virus inactivation activity of title compounds.

In vitro Antibacterial activity. Using the turbidity method,[42] the antibacterial activities were further screened on target compounds against *Xanthomonas oryzae pv. oryzae* (Xoo), *Ralstonia solanacearum* (RS) and *Xanthomonas axonopodis pv. Citri* (Xac). The results, listed in **Table 3**, revealed the title compounds exhibited moderate to excellent activities in vitro against three kinds of bacteria. Such as compound **22** showed the same level on *Xanthomonas oryzae pv. oryzae* as that of commercial bismerthiazol. Compounds **2**, **5**, **6**, **8**, **18**, **21**, **22**, **26**, **28**, **33** and **37** displayed excellent activities against *Ralstonia solanacearum*, which were much higher than that of commercial bismerthiazol and thiodiazole Copper. Moreover, Compounds **1**, **2**, **14**, **15**, **17**, **18**, **22**, **26**, **28**, **30**, **33**, **34** and **38** also showed good anti-bactericidal activities against

Xanthomonas axonopodis pv. Citri (*Xac*). In particular, the inhibition rates of compounds **1**, **2**, **14**, **22**, **26**, **28**, **34** were much higher than that of bismerthiazol. From the data, we can conclude compound **28** with the best anti-TMV curative activity also showed much higher antibacterial activity against *Ralstonia solanacearum*, *Xanthomonas axonopodis pv. Citri* (*Xac*) than the positive control bismerthiazol. Moreover, unlike the result of antivirus, when the R was an unsubstituted pyridyl, compound **2** showed excellent antibacterial activity of 90.3% and 90.5% at 100 μg/mL meanwhile 60.1%and 68.7% at 50 μg/mL against *Ralstonia solanacearum* and *Xanthomonas axonopodis pv. Citri* (*Xac*) respectively, which were much higher than that of bismerthiazol and thiodiazole copper. However, when the pyridyl was substituted by all kinds of functional group, the antibacterial activities all decreased comparing with compound **2**. When R is a phenyl with substituents of both thrifluoromethyl and methoxyl, compound **33** containing the skeleton of trifluoromethyl showed promising anti-bacterial activities against *R. solanacearum* and *X. axonopodis pv. Citri* (*Xac*), which was much higher than that of bismerthiazol. The EC_{50} of more active compounds **2**, **22**, **33** were also evaluated (**Table 4**). Results revealed that EC_{50} values of these compounds were much lower than that of thiodiazole copper, which means that the anti-bacterial activity was much better than thiodiazole copper and could be used as lead compounds or potential candidates.

In conclusion, a series of novel α-ketoamide derivatives bearing vanillin skeleton was designed, synthesized by using the vanillin as a starting material and lead compound. Results of bio-assays indicated some of the synthesized compounds showed excellent anti-viral and anti-bacterial activity. In particular, EC_{50} value of compound **28** for curative activity is slightly lower than ningnanmycin (362.4 μg/mL). The pot experiment indicated that compound **28** could be

regarded as a potential anti-TMV agent and lead compound. TEM results and molecular docking indicated compound **34** could obstruct the self-assembly of TMV virus particles. Moreover, EC_{50} values of compounds **2**, **22**, **33** revealed that these compounds showed much higher activity than that of commercial thiodiazole copper, which could be used as a lead compound or potential candidate. These results indicated vanillin skeleton could be used as a lead structure for innovation of pesticide for crop disease, further bio-assays of these active compounds and structure optimization based on the structure of vanillin are under way in our group.

ASSOCIATED CONTENT

The Supporting Information is available free of charge at http://pubs.acs.org.

Supporting Information − Protocols for the antiviral bioassay against TMV; the pictures of pot experiment for compound **28**; protocols for antibacterial bioassays; physical, chemical properties data and the copies of the NMR spectra for compounds **1** to **39** (PDF).

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Notes

The authors declare no competing financial interest.

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482

Figure Captions

Scheme 1. Synthetic route to target compounds **1**-**39**.

Fig. 1. The design of the title compounds.

Fig. 2. The results of TEM. compound **34** (A); ningnanmycin (B); blank control (C).

Fig. 3. Molecular docking results of compounds **34** (A, B, C) and **36** (D, E, F).

489 **Table 1.** Inhibitory effects of the title compounds against TMV at 500 μg/mL.

Compd	Inactivation effect(%)[a]	Curative effect(%)[a]	Protection effect(%)[a]	Compd	Inactivation effect(%)[a]	Curative effect(%)[a]	Protection effect(%)[a]
1	62.1±0.7	38.0±1.5	34.8±0.1	**22**	64.1±2.5	24.7±0.9	35.1±1.7
2	66.1±2.1	44.2±2.5	30.1±3.0	**23**	71.6±2.8	43.8±4.2	26.4±2.2
3	67.1±4.2	42.2±0.8	22.6±1.0	**24**	56.7±4.7	25.7±0.08	29.1±0.5
4	33.2±5.0	39.0±3.3	33.9±2.2	**25**	63.6±1.6	4.10±0.4	46.1±2.9
5	44.6±1.4	17.4±0.7	25.2±2.4	**26**	68.2±2.3	26.7±4.7	19.5±1.4
6	43.7±0.2	49.7±1.1	36.3±0.4	**27**	64.5±0.3	34.4±1.7	29.3±2.9
7	34.0±2.9	31.9±1.3	42.8±1.1	**28**	53.4±1.0	**54.8±1.9**	29.9±4.6
8	30.4±3.8	27.1±2.6	51.3±1.2	**29**	59.9±4.3	18.1±2.9	31.0±0.7
9	34.3±1.3	44.1±1.6	22.3±0.2	**30**	64.1±4.5	22.7±1.8	39.4±1.7
10	37.6±0.3	38.4±3.5	29.5±2.3	**31**	47.0±3.0	30.8±3.6	48.5±0.8
11	26.7±1.4	22.8±2.6	25.6±0.9	**32**	28.5±2.8	43.2±2.1	21.8±2.1
12	22.8±2.1	39.3±0.4	14.7±0.5	**33**	72.9±4.2	48.7±4.2	37.0±0.7
13	29.3±1.5	32.3±2.0	51.5±1.1	**34**	**90.1±4.4**	**51.8±1.9**	**61.5±3.8**
14	40.0±4.3	46.2±0.5	36.7±3.3	**35**	52.6±0.2	25.6±5.1	13.1±0.7
15	**79.9±2.6**	**38.3±2.5**	**47.9±4.7**	**36**	76.0±1.2	24.6±3.6	34.7±1.6
16	29.4±3.9	42.1±0.4	43.3±1.2	**37**	49.5±0.1	47.1±0.9	34.2±4.2
17	38.2±2.0	25.4±2.2	48.3±4.0	**38**	56.5±2.6	48.1±3.4	47.2±2.7
18	37.3±2.2	16.5±0.9	55.3±4.7	**39**	49.5±0.1	36.6±1.2	52.1±1.4
19	60.7±1.6	38.8±0.9	31.6±0.5	Ribavirin	70.2±3.5	46.9±3.2	44.5±1.8
20	25.8±3.0	24.3±1.3	34.7±4.5	Ningnanmycin	91.9±4.3	51.9±0.3	68.0±3.0
21	72.5±2.5	29.9±0.5	57.3±0.6				

490 [a]Average of three replicates.

491

492 **Table 2** The EC_{50} (μg/mL) of inactivation and curative activities of the compounds **15**, **28** and **34** against TMV. [a]

Compd	Inactivation Effect			Curative Effect		
	Regression equation	R^2	EC_{50}	Regression equation[a]	R^2	EC_{50}
15	y=0.40x+4.09	0.96	\	\	\	\
28	\	\	\	y=0.89x+2.73	0.98	358.46
34	y=1.04x+2.79	0.96	131.05	y=0.86x+2.77	0.99	394.52
Ningnanmycin	y=1.33x+2.27	0.97	111.71	y=0.99x+2.54	0.98	362.4

493 [a]Average of three replicates.

494

495 **Table 3** The antibacterial activities against bacteria. [a]

Compd	*Xanthomonas oryzae pv. oryzae*		*Ralstonia solanacearum*		*Xanthomonas axonopodis pv. Citri*	
	100 *μg/mL*	50 *μg/mL*	100 *μg/mL*	50 *μg/mL*	100 *μg/mL*	50 *μg/mL*
1	32.7±0.8	23.4±2.4	58.7±2.6	48.3±2.6	79.0±2.9	71.0±2.6
2	29.2±2.4	90.3±3.6	90.5±3.7	24.3±1.5	60.1±2.9	68.7±2.9
3	26.9±4.4	64.3±1.0	46.3±2.1	14.2±1.4	54.5±0.8	29.9±0.1
4	33.7±0.6	72.2±5.0	49.4±4.6	21.4±4.3	51.2±4.2	36.3±1.7
5	17.9±3.9	75.7±2.8	44.1±1.0	16.8±3.2	47.1±2.9	38.6±1.4
6	45.3±1.7	84.4±3.6	55.9±4.4	20.8±2.7	46.4±3.1	46.0±0.9
7	33.0±3.6	68.5±1.9	64.0±4.1	18.6±2.9	53.5±3.8	49.4±0.8
8	25.6±3.0	73.9±2.4	51.8±0.4	15.8±3.8	48.4±2.6	31.7±1.7
9	44.9±1.4	63.3±0.4	55.3±1.5	28.9±4.3	52.4±0.3	37.1±2.6
10	38.4±3.0	70.3±1.4	48.0±1.5	23.4±3.9	64.5±2.8	42.5±1.4
11	28.0±4.2	63.7±1.7	43.4±0.9	7.6±3.0	46.4±2.1	35.7±2.1
12	43.3±3.8	40.2±1.1	50.0±1.2	30.2±0.2	18.4±3.4	46.6±0.9
13	19.9±0.6	53.1±0.8	47.9±2.0	11.3±3.1	43.1±0.6	28.5±2.8
14	30.1±0.9	65.4±1.8	86.7±3.3	10.0±0.8	48.0±4.6	44.5±1.9
15	33.8±3.3	46.1±3.2	76.3±2.7	27.4±1.1	39.8±3.2	52.7±3.2
16	44.6±1.7	70.5±1.6	59.4±4.2	18.1±2.8	53.2±3.2	37.7±2.8
17	47.6±2.6	56.0±2.4	72.1±2.5	17.9±1.5	43.5±2.9	59.9±1.3
18	43.0±4.5	76.4±2.0	75.4±2.0	19.9±1.5	66.7±4.9	36.5±2.0
19	31.9±1.7	63.4±2.0	33.6±2.1	24.9±3.0	49.9±3.1	22.5±1.3
20	36.7±4.5	69.8±2.7	47.2±1.0	23.0±4.0	46.4±0.8	28.9±3.7
21	43.7±1.4	77.6±2.7	52.4±3.3	26.5±2.5	50.5±0.4	37.1±0.9
22	58.9±2.0	90.3±3.5	77.9±1.5	46.0±1.5	60.3±3.0	37.1±2.6
23	37.7±0.1	39.3±1.5	52.4±3.7	29.9±1.1	36.3±2.6	35.4±1.8
24	23.2±1.7	56.5±2.0	56.8±0.5	14.5±3.8	43.2±2.1	37.5±3.2
25	38.3±2.8	61.6±4.7	61.2±1.1	19.4±3.5	36.5±3.4	40.1±1.1
26	44.1±2.1	85.2±2.3	72.1±1.5	19.0±1.0	58.4±4.4	62.1±4.0
27	35.2±0.9	35.7±0.5	45.0±2.8	22.7±1.5	31.1±0.5	29.5±3.3
28	43.7±3.4	76.7±3.7	81.5±5.3	33.0±2.3	52.5±3.6	32.0±2.6
29	27.8±2.5	51.1±2.6	49.3±3.5	12.0±4.2	36.2±4.1	39.6±1.4
20	31.0±3.1	72.9±2.0	72.9±1.2	23.2±3.0	31.9±2.0	41.2±2.6
31	50.9±1.7	50.0±3.1	65.1±3.4	29.0±2.3	27.5±0.6	33.2±2.9
32	25.4±1.9	47.6±4.3	46.3±2.1	11.1±3.3	32.9±2.3	32.7±4.3
33	42.1±2.4	95.7±2.8	79.7±4.1	28.0±0.2	69.7±4.6	54.3±2.8
34	54.6±2.5	65.11±4.6	83.4±1.8	26.5±0.8	38.3±1.5	32.1±0.9
35	26.0±2.6	48.4±1.0	48.4±1.8	12.9±1.5	33.4±4.3	46.7±0.4
36	32.6±2.5	49.7±4.0	67.5±1.5	13.0±2.0	34.6±2.6	61.7±1.8
37	53.2±1.0	77.8±1.7	60.8±2.8	37.1±1.1	40.4±1.6	56.7±0.4
38	41.9±3.8	43.1±4.4	75.8±1.3	25.8±0.6	33.5±4.1	47.9±0.7
39	41.9±3.8	47.1±3.4	43.2±2.3	25.8±0.6	33.5±4.1	28.1±2.3
Bismerthiazol	60.4±2.2	43.0±4.7	73.2±1.3	55.3±2.0	/	/
Thiodiazole Copper	/		64.5±2.5	40.4±3.5	67.5±2.3	50.5±2.9

496 [a]Average of three replicates

497

499 **Table 4.** The EC_{50} (μg/mL) of antibacterial activity of compounds **2**, **22**, **33** against *RS* and *Xac*.[a]

Compd	Regression equation	R^2	EC_{50} for RS	Regression equation	R^2	EC_{50} for Xac
2	y=2.59x+0.984	0.98	35.25	y=0.81x+3.818	0.90	28.23
22	y=2.97x+0.275	0.99	38.90	\	\	\
33	y=2.93x+0.645	0.93	30.43	\	\	\
Thiodiazole-copper	y =1.03x+2.940	0.99	99.10	y =2.15x+0.938	0.98	77.10

500 [a]Average of three replicates; *RS: Ralstonia solanacearum; Xac*: *Xanthomonas axonopodis pv. Citri.*

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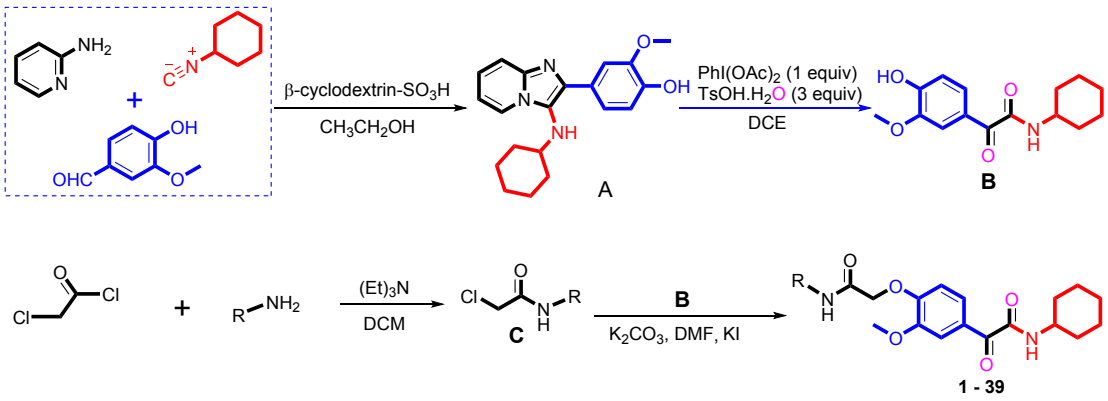
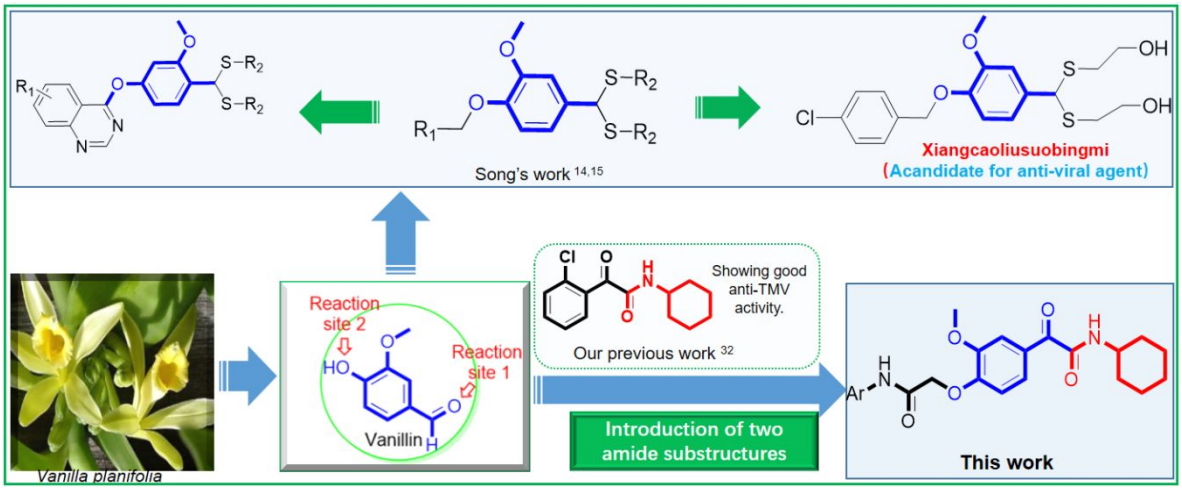


1. R=-C_6H_5; **2**. R= -pyridyl; **3**. R=-cyclopropyl; **4.** R= benzyl;
5. R=3-CH_3-C_6H_4; **6**. R=4-Cl-C_6H_4; **7**. R=2-F-C_6H_4; **8**. R=4-CH_3-C_6H_4;
9. R=3,4-$(CH_3)_2$-C_6H_3; **10**. R=-$CH(CH_3)_2$; **11**. R=3-isopropylphenyl; **12**. R=-cyclohexyl;
13. R=4-Br-2-CH_3-C_6H_3; **14**. R=3-Cl-2-CH_3-C_6H_3; **15**. R=4-F-2-CH_3-C_6H_3; **16**. R=2,4-$(F)_2$-C_6H_3;
17. R=2-Cl-4-F-C_6H_3; **18**. R=5-Br-6-CH_3-pyrididyl; **19**. R=2-Cl-5-CH_3-C_6H_3; **20**. R=4-Cl-benzyl;
21. R=4-F-3-CH_3-C_6H_3; **22**. R=4-Cl-3-F-C_6H_3; **23**. R=3-OCF_3-C_6H_4; **24**. R=3-Cl-2-F-C_6H_4;
25. R=2-CH_3-4-OCH_3-C_6H_3; **26**. R=3,4-$(OCH_3)_2$-C_6H_3; **27**. R=5,6-$(Cl)_2$-pyrididyl; **28**. R=2-$OCHF_2$-C_6H_4;
29. R=6-CH_3-pyrididyl; **30**. R=4-F-3-NO_2-C_6H_3; **31**. R=2-CH_3-pyrididyl; **32**. R=4-Br-2-Cl-C_6H_3;
33. R=3-OCH_3-5-CF_3-C_6H_3; **34**. R=4-CF_3-C_6H_4; **35**. R=2,6-$(Cl)_2$-pyrididyl; **36**. R=6-CF_3-C_6H_4;
37. R=4-Br-3-F-C_6H_3; **38**. R=2,4,5-$(F)_3$-C_6H_2; **39**. R=2-F-4-I-C_6H_3.

Scheme1. Synthetic route to target compounds **1**-**39**.



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507 **Fig. 1.** The design of the title compounds

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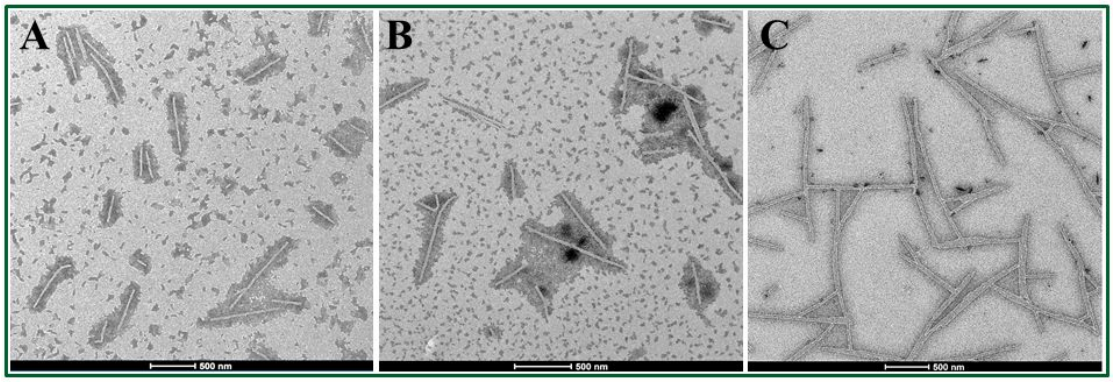

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511 **Fig. 2.** The results of TEM. compound **34** (A); ningnanmycin (B); blank control (C).

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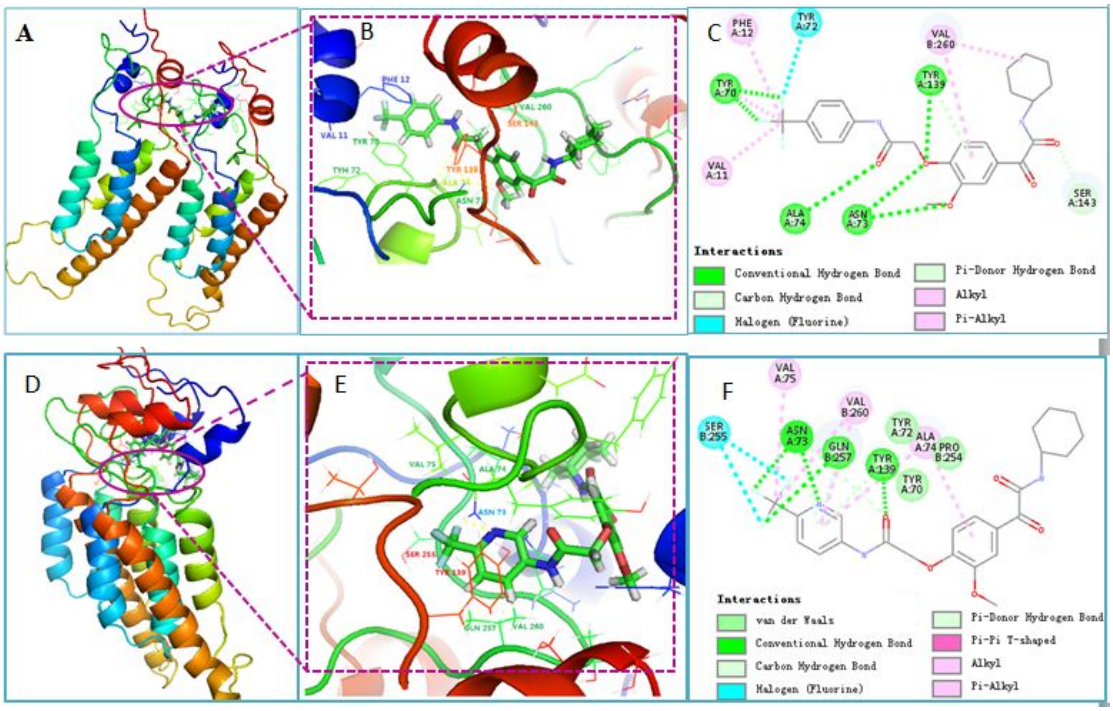

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515 **Fig. 3.** Molecular docking results of compounds **34** (A, B, C) and **36** (D, E, F).

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519 **Table of Contents Graphic**
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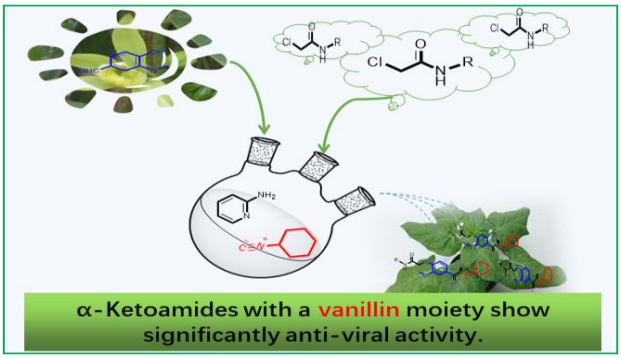

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