Synthesis and biological activity of 1,4-pentadien-3-one derivatives containing a triazine scaffold

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Background: Literatures revealed that 1,4-pentadien-3-one and triazine derivatives exhibited a wide variety of biological activity. In order to develop highly bioactive molecules, in this study, a series of novel 1,4-pentadien-3-one derivatives containing a triazine moiety were synthesized and investigated their antibacterial and antiviral activities. **Methods.** A series of novel 1,4-pentadien-3-one derivatives containing triazine moiety were synthesized and characterized in detail *via* ^{1}H NMR, ^{13}C NMR and HRMS spectra. The antibacterial activities against *Xanthomonas axonopodispv. citri (Xac), Xanthomonas oryzaepv. oryzae (Xoo)* and *Ralstonia solanacearum (R.s)* were evaluated at 100 and 50 *μ*g/mL using a turbidimeter, and using *N. tabacun L.* leaves under the same age as that of test subjects, the curative, protective and inactivation activities against tobacco mosaic virus (TMV) at a concentration of 500 *μ*g/mL were evaluated by the half-leaf blight spot method. **Results.** The bioassay results showed that some of the target compounds exhibited fine antibacterial activities against *Xac* and *R.s*. particularly, with half maximal effective concentration (EC_{50})values of some target compounds against *R.s* are visibly better than that of the positive control **BT**. Notably, compound **4a** showed excellent inactivation activity against TMV, the EC_{50} values of 12.5 *μ*g/mL, which was superior to that of **NNM** (13.5 *μ*g/mL). besides, molecular docking studies for **4a** with tobacco mosaic virus coat protein (TMV-CP) showed that the compound was embedded well in the pocket between the two subunits of TMV-CP. These findings indicate that 1,4-pentadien-3-one derivatives containing a triazine may be potential antiviral and antibacterial agents.

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ABSTRACT

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findings indicate that 1,4-pentadien-3-one derivatives containing a triazine may be potential antiviral and antibacterial agents.

Keywords: 1,4-pentadien-3-one, Triazine, Antiviral, Antibacterial, Molecular docking studies

INTRODUCTION

Plant pathogens have become one of the world's largest agricultural problems because they pose a significant threat not only to agricultural products but also to human health(*Li et al.,2011; Lorenzo et al.,2017*). Plant pathogens diseases, such as citrus canker, rice bacterial leaf blight and tobacco bacterial wilt, caused by *Xanthomonas axonopodispv. citri (Xac), Xanthomonas oryzaepv. oryzae (Xoo)* and *Ralstonia solanacearum (R.s)*, respectively, and difficult to control in agricultural production(*Zou et al., 2011; Li et al., 2017*). In addition, tobacco mosaic virus (TMV) can cause more than 885 plants to infected the virus, resulting in a worldwide loss of $100 million worldwide*(Su et al., 2016; Bos et al., 2000).*Therefore, the discovery and development of new antiviral and antibacterial agents with a novel mode of action are of great importance to the medical community.

1,4-pentadien-3-one derivative, derived from plant metabolic products curcumin, found to have a good range of biological activities such as antiviral *(Zhang et al., 2018),* antibacterial *(Long et al., 2015),* anticancer *(Luo et al., 2014),* anti-inflammatory *(Liu et al., 2014),* anti-oxidative *(Masuda et al., 2015),* and anti-HIV activities *(Sharma et al., 2019).* Over the past few years, the synthesis and study of pharmacological activity of 1,4-pentadien-3-one derivatives caused the attention of many chemists *(Wang et al., 2017; Zhou et al., 2017).* Further study on the structural optimization of 1,4-pentadien-3-one found that introducing benzotriazin-4(3*H*)-one *(Zhang et al., 2018),* imidazole *(Samaan et al., 2014),*thiazole *(Wang et al., 2015),* or chromone *(Chen et al., 2015)*moieties (**Figure 1. A1-A4**), could greatly enhance biological activities. Notably, Chen *et al.* verified the anti-TMV mechanism of 1,4-pentadien-3-one derivatives(**Figure 2. B**), and found 5-position of 1,4-pentadien-3-one nucleus to build a new molecular structure plays a key role in antiviral activities *(Chen et al., 2019).*

Figure 1.

Figure 2.

In addition, triazine scaffold has been associated with diversified pharmacological activities *(Irannejad et al., 2010),* such as antioxidant *(Khoshneviszadeh et al., 2016),* antithrombotic *(Tamboli et al., 2015),* antiplatelet*(Konno et al.,1993),*anticancer *(Fu et al., 2017),* thromboxane

synthetase inhibition *(Monge et al., 2010),* antimalarial *(Tamboli et al., 2015),* α-glucosidase inhibition*(Wang et al., 2016),* antiviral and antibacterial activities *(Tang et al., 2019).* Recently, chemists studies on triazine derivatives showed that the heterocyclic nitrogen had tremendous application foregrounds in the development of novel agricultural bactericides and virucides*(Zhang et al., 2018)*. Sangshetti *et al*. reported potent inhibitory effect of triazine and their derivatives against three fungals ((Candida albicans (MIC-25), Aspergillus niger (MIC-12.5) and Cryp tococcus neoformans (MIC-25)) similar to miconazole(**Figure 3. C**) *(Sangshetti et al., 2010)*.Based on these, triazine group was introduced into the 5-position of 1,4-pentadien-3-one nucleus to build a new molecular structure and the potency of which was tested in terms of biological activities (**Figure 4**).

Figure 3.

Figure 4.

MATERIALS & METHODS

Instruments and chemicals

Melting points were determined using an XT-4 digital melting-point apparatus (Beijing Tech. Instrument Co., China) and readings were uncorrected. ^{1}H NMR , ^{13}C NMR and ^{31}F NMR spectra were recorded on a 400 MHz spectrometer (Swiss Bruker) with DMSO and $CDCl_3$ as the solvent and tetramethylsilane as the internal standard. The course of the reaction was monitored by thin-layer-chromatography analysis on silica gel GF_{254} (Qingdao Haiyang Chemical Company, Ltd., Qingdao, China), and spots were visualized with ultraviolet (UV) light. High-resolution mass spectrometry (HRMS) was conducted by using a Thermo Scientific Q Exactive (Thermo Scientific, Missouri, USA). The molecular docking was performed by using DS-CDocker implemented in Discovery Studio (version 4.5). All reagents and solvents were purchased from Chinese Chemical Reagent Company and were of analytical grade reagents. The synthetic route to1,4-pentadien-3-one derivatives containing triazine moiety was shown in **Scheme 1**.

General procedure for the synthesis of intermediates

A synthetic route to 1,4-pentadien-3-one derivatives containing a triazine moiety was designed and is shown in **Scheme 1**. According to previously reported methods *(Chen et al., 2019; Tang et al., 2019;, Gan et al., 2017)*, intermediates **1** and **2** could be obtained. Using

benzyl, biacetyl and thio-semicarbazide as the initial materials in acetic acid and water was stirred at 100-110 ºC for 6-8 hours to obtain the intermediate **3** *(Tang et al., 2019).*

General procedure for the synthesis of target compounds 4a-4r

Reaction mixture was added to a solution of intermediate **2** (12mmol), intermediate **3** (10mmol) and K_2CO_3 (30 mmol) in dimethylformamide and stirred at room temperature for 6-8h. Upon completion of reaction (indicated by TLC), and ethyl acetate was used to extract three times(30 mL×3). the solvent was removed under reduced pressure, Residue was purified by silica-gel column chromatography using petroleum ether/ethyl acetate (3:1 *v/v*) to obtain target compounds **4a**–**4r**. The ^{1}H NMR, ^{13}C NMR, ^{31} F NMR and HMRS spectra of the target compounds **4a**–**4r** are also provided in the Supporting Information.

Scheme 1.

Bioactivity assay

Antibacterial activity *in vitro*

The *in vitro* antibacterial activities of target compounds **4a**–**4r** against rice bacterial leaf blight, tobacco wilt and citrus canker caused by the pathogens of *Xanthomonas axonopodispv. citri (Xac), Xanthomonas oryzaepv. oryzae (Xoo) and Ralstonia solanacearum (R.s)*, respectively, by the turbidimeter test*(Tang et al., 2019; Zhang et al., 2017).*This test method is provided in the Supporting Information.

Antiviral activities *in vivo*

The *in vivo* antibacterial activities of target compounds **4a**–**4r** against tobacco mosaic virus (TMV). by the half-leaf blight spot method*(Chen et al., 2019).*This test method is provided in the Supporting Information.

Molecular docking

Molecular docking. The molecular docking was performed by using DS-CDocker implemented in Discovery Studio (version 4.5). This test method is provided in the Supporting Information.

RESULTS

Antibacterial activities *in vitro*

The antibacterial activities of target compounds have been evaluated by the turbidimeter test *(Zhang et al.,2018; Tang et al.,2019)*. Results in **Table 1** indicated that some of synthesized compounds exhibited appreciable antibacterial activities against *Xoo*, *R.s* and *Xac* at the

concentrates of 100 and 50 μg/mL. Among these derivatives, **4n** and **4p** exhibited excellent bactericidal effect against *Xoo*, with inhibition rates of 60.5 % and 56.5 %, respectively, which were superior to bismerthiazol (**BT**, 56.1%). In addition, as **Table 1** demonstrated that the designed compounds displayed certain bactericidal effect toward *R.s*. Studies on the inhibition effect of title compounds suggested that **4a**, **4b**, **4j** and **4k** exerted the excellent inhibition effect against *R.s* with the inhibition rates of 58.2, 53.9, 53.5 and 61.9 %, respectively, which were better than that of **BT** (52.1%). It was noted that compounds **4k** (91.8 %) and **4l** (95.4 %) exposed better antibacterial activity toward *Xac* than that of **BT** (70.5 %).

To further understand antibacterial activity of title compounds, the EC_{50} values of some title compounds were calculated and summarized in **Table 2**. Notably, compounds **4a**, **4b**, **4j** and **4k** exerted admirable inhibition effects against *R.s*, with half maximal effective concentration (EC_{50}) values of ranging from 0.43−4.76 μg/mL, which were better than that of **BT** (EC_{50}=49.5 μg/mL). Meanwhile, compounds **4j** and **4k** showed remarkable antibacterial activities against *Xac* with the EC_{50} values of 55.53 and 129.1 μg/mL, which were better than that of **BT** (EC_{50}=153.7 μg/mL).

Table 1

Table 2

Antiviral activities against TMV *in vivo*

The antiviral activities of the title compounds **4a**–**4r** against tobacco mosaic virus (TMV) were evaluated by the half leaf method *(Chen et al., 2019)* and the results were summarized in **Table 3** and **Figure 5**. It was found that some of the title compounds exhibited good antiviral activity against TMV *in vivo*. Compounds **4f**, **4k** and **4l** showed remarkable curative activity against TMV, with values of 53.8, 66.3 and 59.9 %, respectively. which were better than that of ningnanmycin **(NNM**, 45.7%). Meanwhile, compound **4h (**61.4 %) exhibited excellent protection activity, also superior to **NNM** (53.4%). Overall, most of the compounds indicated general inactivation activity against TMV at 500 μg/mL.

Based on the previous bioassays, the EC_{50} values some of the title compounds were tested and are listed in **Table 4**. Compound **4a** exhibited excellent inactivation activity against TMV, with the EC_{50} values of 12.5 μg/mL, which was better than that of **NNM** (EC_{50}=13.5 μg/mL). Moreover, compounds **4k** and **4l** exhibited the preferably curative activity against TMV, with

154 EC_{50} values of 11.5 and 12.1 μg/mL, respectively, which were superior to that of **NNM**
155 (EC_{50}=82.2 μg/mL).
156 **Table 3**
157 **Table 4**
158 **Figure 5**
159 **Molecular docking studies**
160 Molecular docking studies (**Figure 6**) for **4a** with tobacco mosaic virus coat protein (TMV-
161 CP) (PDB code:1EI7). Molecular docking results revealed that compound **4a** was the most
162 preferred compound based on the analysis followed by **4d** and so on (**Table 3**). compound **4a**
163 binding orientation clearly is described by **Figure 6**, it forms one hydrogen bond with PHEA:12
164 with highest docking score (2.49 Å) among the designed molecules and the glide energy was also
165 less compared to others showing few hydrophobic interactions with specific residues like as
166 TYRA:139, VALA:75, LYSB:268 *etc.*
167 **Figure 6**
168 **DISCUSSION**
169 **Structure-activity relationships of antibacterial activities**
170 The antibacterial results in **Tables 1** and **2** also indicated that the different groups on R had
171 significant effects on the antibacterial activity of the title compounds. Obviously, the presence of
172 -Cl-Ph group can effectively enhance the antibacterial activity against *Xac*. As examples of this
173 phenomenon, the compounds**4k** and **4l**, which contain respectively R=4-Cl-Ph and R=2-Cl-Ph
174 groups, with the EC_{50} values of 55.53 and 129.1 μg/mL, which were better than that of **BT**
175 (EC_{50}=153.7 μg/mL). Meanwhile, when R was substituted with thiophene-2-yl and 4-Cl-Ph
176 groups, the corresponding compounds **4a**, **4b**, **4j** and **4k** exhibit remarkable antibacterial
177 activities against *R.s*, with the EC_{50} values of ranging from 0.43−4.76 μg/mL, which were better
178 than that of **BT** (EC_{50}=49.5 μg/mL).
179 **Structure-activity relationships of antiviral activities**
180 The antiviral bioassay results indicated that the title compounds showed excellent antiviral
181 activity against TMV. The preliminary SAR results were deduced on the basis of the anti-TMV
182 activity (as shown in **Table 3** and **4**). The results indicated that when R was the 4-NO_2-Ph (**4f**),
183 4-Cl-Ph (**4k**) or 2-Cl-Ph (**4l**) group, the corresponding title compounds exhibited good curative
184 activity. Furthermore, when the R was 4-OMe-Ph group, the protective activity of corresponding

compound **4h,** with the EC_{50} values of 32.1 μg/mL, which was better than that of **NNM** (EC_{50}= 82.2 μg/mL).

CONCLUSIONS

In short, a series of 1,4-pentadien-3-one derivatives containing a triazine scaffold were synthesized. The obtained bioassay results revealed that some of the title compounds exhibited excellent antibacterial or antiviral activities and notably they were better than the commercial agents. In particular, compound **4a** showed prominent inactivation activity against TMV. Furthermore, compound **4a** had strong binding capability with TMV-CP, All these results support that the 1,4-pentadien-3-one derivatives containing a triazine scaffold possess antiviral and antibacterial agents.

ADDITIONAL INFORMATION AND DECLARATIONS

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

Supplemental information for this article can be found online.

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Table 1(on next page)

Table 1. Inhibition effect of the some title compounds against *Xoo*, *R.s* and *Xac.* [a]

Table1 indicated that some of synthesized compounds exhibited appreciable antibacterial activities against *Xoo*, *R.s* and *Xac* at the concentrates of 100 and 50 μg/mL.

Table 1. Inhibition effect of the sme title compounds against *Xoo*, *R.s* and *Xac.* [a]

Compd.	Inhibition (%)					
	Xoo		*R.s*		*Xac*	
	100μg/mL	50μg/mL	100μg/mL	50μg/mL	100μg/mL	50μg/mL
4a	19.7 ± 4.3	18.9 ± 3.5	58.2 ± 2.4	58.2 ± 3.7	43.7 ± 2.2	37.6 ± 2.4
4b	48.5 ± 5.2	33.5 ± 3.0	53.9 ± 6.5	44.6 ± 1.8	56.5 ± 1.1	41.4 ± 1.3
4c	20.9 ± 6.5	10.6 ± 1.8	15.7 ± 9.9	-	42.3 ± 2.1	44.8 ± 1.8
4d	13.2 ± 6.3	12.7 ± 2.9	38.0 ± 3.3	37.0 ± 4.3	38.2 ± 3.7	33.5 ± 3.6
4e	54.6 ± 1.8	45.0 ± 2.9	37.6 ± 4.3	28.0 ± 2.1	30.8 ± 1.0	37.2 ± 1.5
4f	12.3 ± 1.2	3.9 ± 7.5	28.5 ± 7.5	23.0 ± 3.2	47.4 ± 2.2	35.1 ± 2.7
4g	43.8 ± 2.7	43.8 ± 2.3	28.5 ± 3.1	22.6 ± 2.6	35.9 ± 13.7	29.1 ± 3.9
4h	13.9 ± 4.5	30.7 ± 6.6	28.2 ± 2.6	-	41.7 ± 4.4	43.3 ± 0.8
4i	55.6 ± 0.9	54.4 ± 2.8	18.0 ± 2.9	28.6 ± 1.3	61.6 ± 8.8	43.4 ± 2.2
4j	48.6 ± 1.1	38.9 ± 2.4	53.5 ± 2.9	45.0 ± 5.5	64.8 ± 2.9	43.0 ± 9.3
4k	10.5 ± 4.7	5.9 ± 3.7	61.9 ± 2.7	49.2 ± 2.5	91.8 ± 2.3	85.6 ± 4.7
4l	14.1 ± 2.3	21.2 ± 4.8	45.3 ± 4.4	28.6 ± 2.5	95.4 ± 9.0	68.1 ± 7.9
4m	43.6 ± 3.0	28.5 ± 4.2	18.2 ± 1.8	17.0 ± 3.7	41.2 ± 3.9	32.5 ± 5.1
4n	60.5 ± 0.9	44.3 ± 7.5	44.6 ± 8.7	32.0 ± 8.7	35.4 ± 1.3	32.3 ± 2.5
4o	41.8 ± 7.4	25.1 ± 3.0	43.5 ± 4.4	37.1 ± 3.4	41.0 ± 4.4	32.0 ± 7.6
4p	56.5 ± 3.9	27.6 ± 3.9	21.3 ± 6.2	12.7 ± 9.6	41.1 ± 1.5	28.4 ± 2.7
4q	24.0 ± 9.9	20.2 ± 2.4	18.9 ± 1.8	16.4 ± 1.80	74.6 ± 1.8	50.0 ± 2.2
4r	15.1 ± 4.8	11.0 ± 9.0	24.4 ± 7.6	11.3 ± 8.0	51.8 ± 4.4	14.8 ± 2.4
BT [b]	56.1 ± 7.3	49.3 ± 5.4	52.1 ± 3.4	44.2 ± 3.9	70.5 ± 1.5	33.6 ± 1.7

a: Average of three replicates; b: A commercial agricultural antibacterial agent Bismerthiazol was used for comparison of antibacterial activities; **BT**: Bismerthiazol.

Table 2(on next page)

Table 2. EC_{50} values of some title compounds against *Xoo*, *Xac* and *R.s.* [a]

Table 2 shown that the with half maximal effective concentration (EC_{50})values of some target compounds against Xoo, Xac *and* R.s.

Table 2. EC_{50} values of some title compounds against *Xoo*, *Xac* and *R.s.* [a]

Tested bacterias	Compd.	Regression equation	r^2	EC_{50} (μg/mL)
Xoo	**4e**	y = 0.4750x + 4.0608	0.9526	94.9
	BT [b]	y = 1.5696x + 1.8988	0.9551	94.6
Xac	**4j**	y = 0.9367x + 3.3659	0.9509	55.5
	4k	y = 0.6755x + 3.5689	0.9181	129.1
	BT [b]	y = 0.3926x + 4.1415	0.9072	153.7
R.s	**4a**	y = 1.0922x + 4.2593	0.9619	4.76
	4b	y = 0.4261x + 5.1569	0.9107	0.4
	4j	y = 0.6032x + 4.8698	0.9116	1.6
	4k	y = 0.7208x + 4.8188	0.9303	1.8
	BT [b]	y = 1.0223x + 3.2674	0.9095	49.5

a: Average of three replicates; b: A commercial agricultural antibacterial agent Bismerthiazol was used for comparison of antibacterial activities; **BT**: Bismerthiazol.

Table 3(on next page)

Table 3. Antiviral activities of the target compounds against TMV *in vivo* at 500 μg/mL [a]

table 3 shown that the antiviral activities of the title compounds **4a**–**4r** against tobacco mosaic virus (TMV) at 500 μg/mL.

Table 3. Antiviral activities of the target compounds against TMV *in vivo* at 500 μg/mL [a]

Compd.	Curative activity (%)	Protective activity (%)	Inactivation activity (%)
4a	30.1 ± 0.21	16.1 ± 0.32	66.2 ± 0.02
4b	44.4 ± 0.05	54.5 ± 0.03	48.2 ± 0.02
4c	40.1 ± 0.05	35.5 ± 0.12	57.1 ± 0.02
4d	29.3 ± 0.07	53.9 ± 0.02	63.6 ± 0.03
4e	44.1 ± 0.03	14.9 ± 0.15	39.4 ± 0.07
4f	53.8 ± 0.07	39.5 ± 0.02	50.1 ± 0.02
4g	44.5 ± 0.03	44.4 ± 0.11	44.9 ± 0.07
4h	47.6 ± 0.07	61.4 ± 0.04	53.5 ± 0.05
4i	27.3 ± 0.04	33.5 ± 0.11	24.3 ± 0.07
4j	43.1 ± 0.02	26.7 ± 0.03	24.1 ± 0.11
4k	66.3 ± 0.01	24.1 ± 0.28	27.7 ± 0.01
4l	59.9 ± 0.07	18.4 ± 0.02	31.9 ± 0.09
4m	48.8 ± 0.06	28.6 ± 0.17	22.3 ± 0.09
4n	37.4 ± 0.05	27.5 ± 0.19	27.1 ± 0.07
4o	39.5 ± 0.02	22.4 ± 0.08	57.7 ± 0.01
4p	46.6 ± 0.08	41.2 ± 0.08	28.2 ± 0.09
4q	38.5 ± 0.01	31.6 ± 0.01	33.5 ± 0.02
4r	42.4 ± 0.02	34.1 ± 0.11	33.5 ± 0.02
NNM [b]	45.7 ± 2.61	53.4 ± 2.42	77.3 ± 1.60

a: Average of three replicates; *b*: A commercial agricultural antiviral agent ningnanmycin was used for comparison of antiviral activities; **NNM**: ningnanmycin.

Table 4(on next page)

Table 4 EC_{50} values of the 4a, 4d, 4h,4k and 4l against TMV *in vivo* [a]

Table 4 shown that the EC_{50} values some of the title compounds against TMV *in vivo*

Table 4 EC_{50} values of the **4a**, **4d**, **4h**,**4k** and **4l** against TMV *in vivo* [a]

Compd.	against TMV	regression equation	r^2	EC_{50}
4a	Inactivation activity	y = 0.6712x + 4.2637	0.9234	12.5
4d	Inactivation activity	y = 0.8253x + 3.7000	0.9279	37.6
4h	Protection activity	y = 0.4739x + 4.2865	0.9833	32.1
4k	Curative activity	y = 0.4261x + 4.5479	0.9382	11.5
4l	Curative activity	y = 0.6542x + 4.2925	0.9191	12.1
	Curative activity	y = 0.4415x + 4.1563	0.9720	81.4
NNM [b]	Protection activity	y = 0.4732x + 4.0939	0.9097	82.2
	Inactivation activity	y = 0.8498x + 4.0381	0.9702	13.5

a: Average of three replicates; *b*: A commercial agricultural antiviral agent ningnanmycin was used for comparison of antiviral activities; **NNM**: ningnanmycin.

Figure 1(on next page)

Figure 1. Chemical structures of bioactive molecules bearing 1,4-pentadien-3-one fragment.

Some chemical structures of bioactive molecules bearing 1,4-pentadien-3-one fragment.

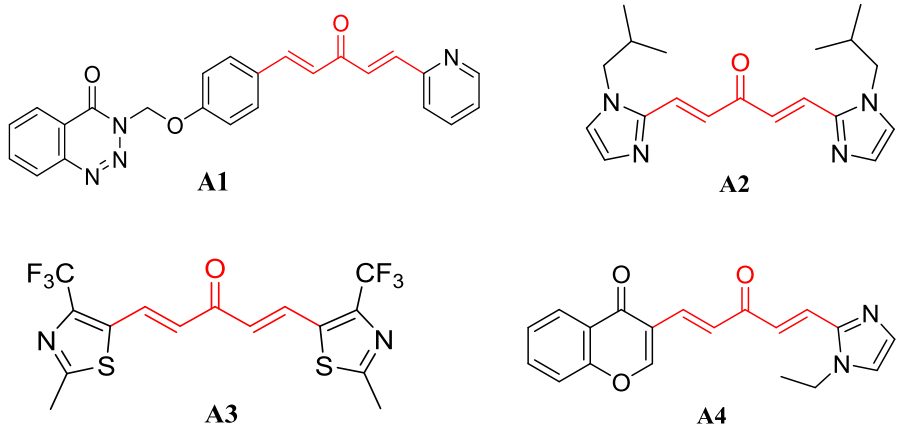

Figure 1. Chemical structures of bioactive molecules bearing 1,4-pentadien-3-one fragment.

Figure 2(on next page)

Figure 2. Figure 2. The anti-TMV mechanism of 1,4-pentadien-3-one derivatives

Chen *et al*. verified the anti-TMV mechanism of 1,4-pentadien-3-one derivatives

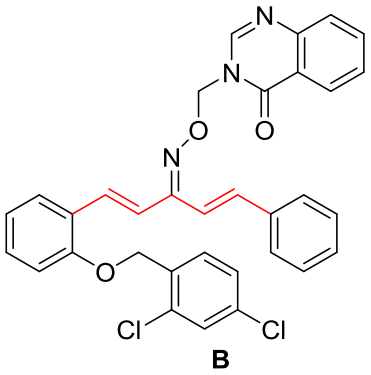

Figure 2. The anti-TMV mechanism of 1,4-pentadien-3-one derivatives

Figure 3(on next page)

Figure 3. 1,2,4-triazine fragment against three fungals (Candida albicans, Aspergillus niger and Cryp tococcus neoformans)

Sangshetti *et al*. reported potent inhibitory effect of triazine and their derivatives against three fungals ((Candida albicans (MIC-25), Aspergillus niger (MIC-12.5) and Cryp tococcus neoformans (MIC-25)) similar to miconazole

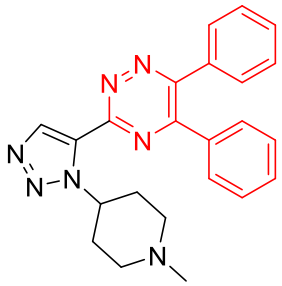

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Figure 3. 1,2,4-triazine fragment against three fungals (Candida albicans, Aspergillus niger and Cryp tococcus neoformans)

Figure 4(on next page)

Figure 4. Design strategy of title compounds.

Based on these, triazine group was introduced into the 5-position of 1,4-pentadien-3-one nucleus to build a new molecular structure and the potency of which was tested in terms of biological activities

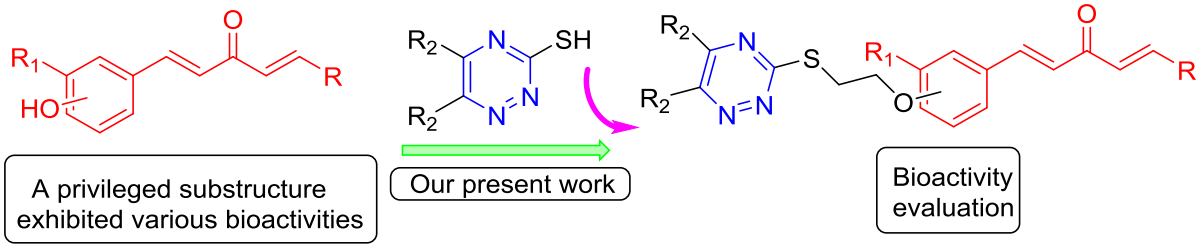


Figure 4. Design strategy of title compounds.

Figure 5(on next page)

Figure 5. Tobacco leaf morphology effects of the NNM and 4k, 4h and 4a against TMV *in vivo* (Right leaf: not treated with compound,Left leaf: smeared with compound)

Tobacco leaf morphology effects of the NNM and 4k, 4h and 4a against TMV *in vivo* (Right leaf: not treated with compound,Left leaf: smeared with compound)

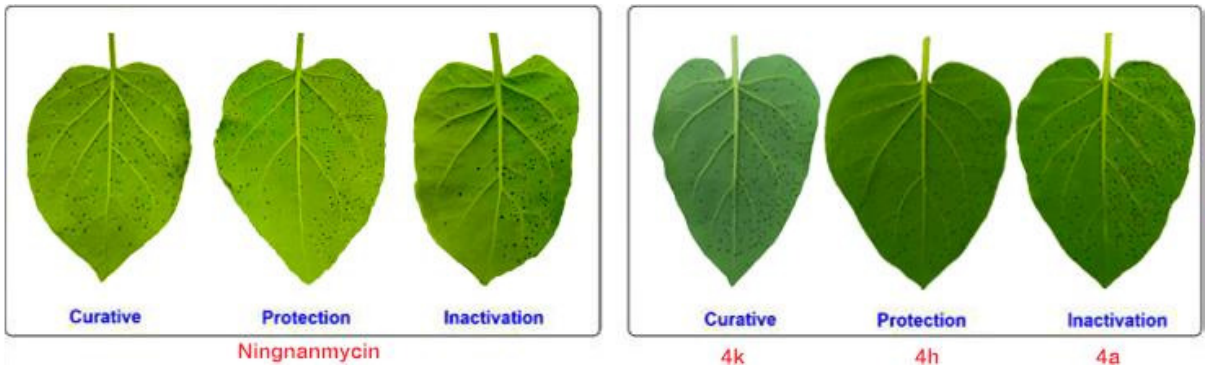


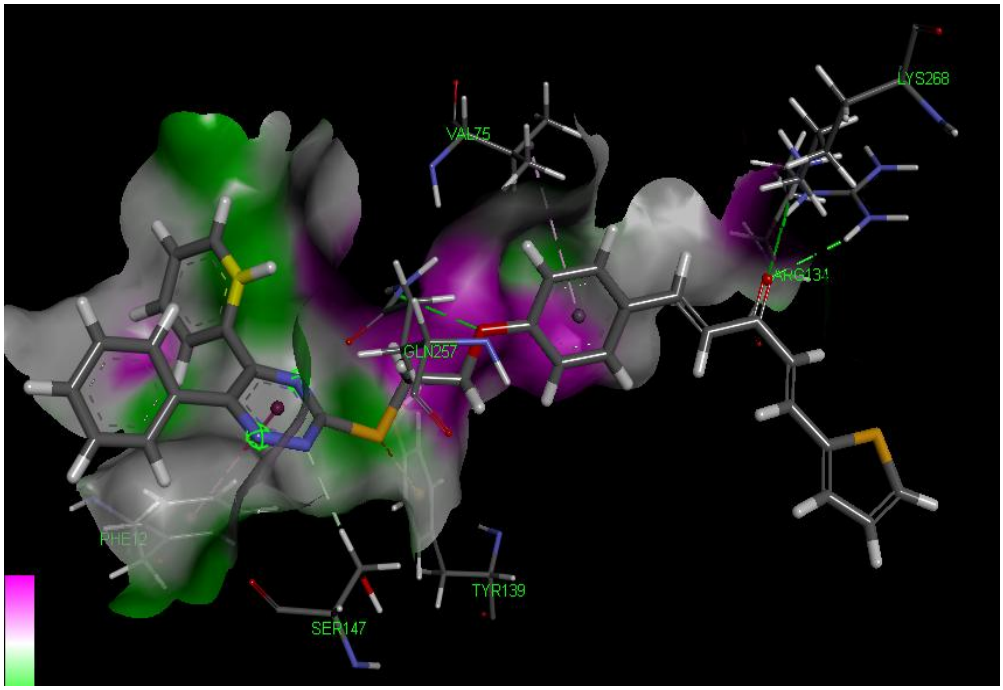
Figure 5. Tobacco leaf morphology effects of the NNM and **4k**, **4h** and **4a** against TMV *in vivo*

(Right leaf: not treated with compound,Left leaf: smeared with compound)

Figure 6(on next page)

Figure 6. The binding mode of compound 4a docked with TMV-CP.

Molecular docking studies for **4a** with tobacco mosaic virus coat protein (TMV-CP) (PDB code:1EI7).



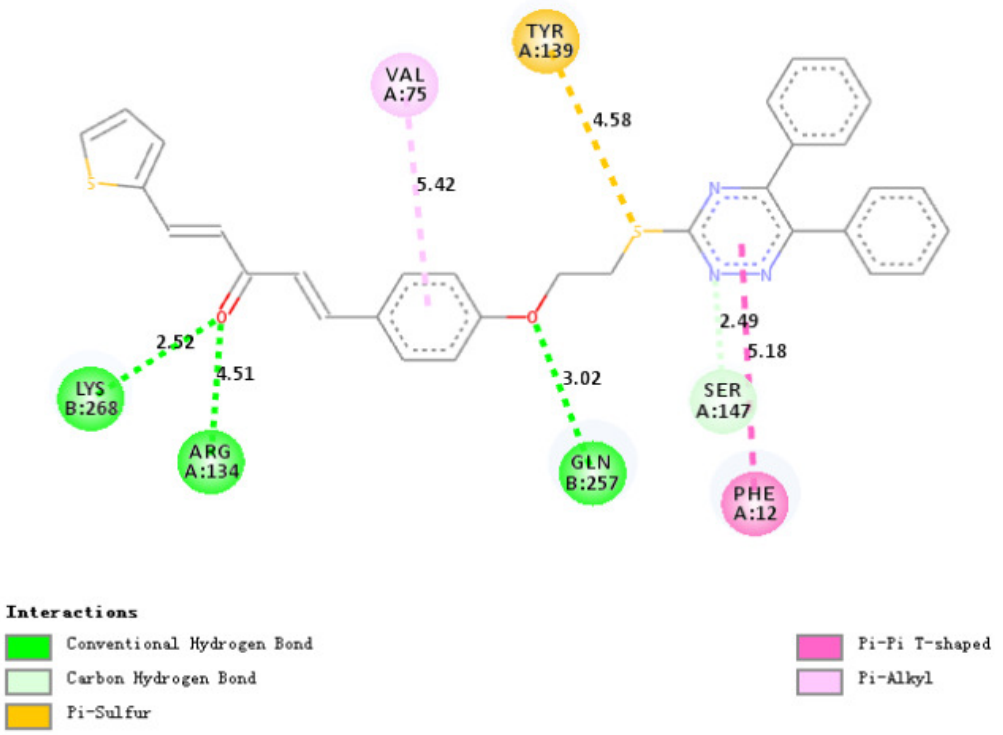
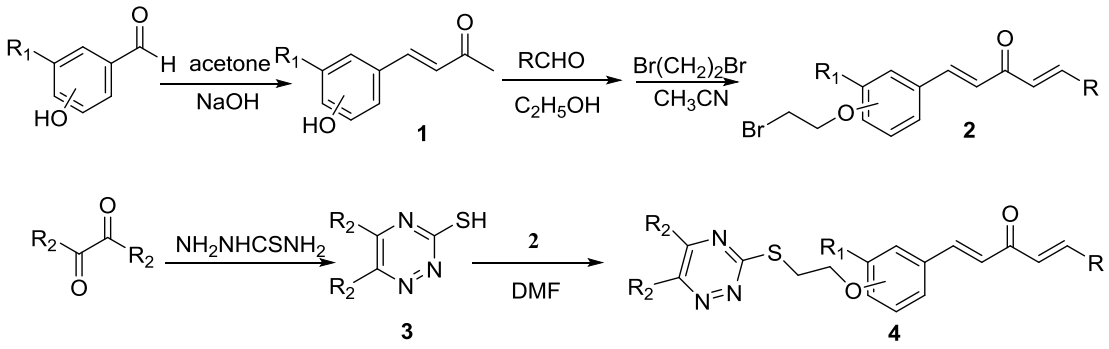


Figure 6. The binding mode of compound **4a** docked with TMV-CP.

Figure 7(on next page)

Scheme 1. Synthesis route for the target compounds

The synthetic route to1,4-pentadien-3-one derivatives containing triazine moiety was shown in **Scheme 1**.



4a: O=4, R=2-thiophene, R_1=H; R_2= -Ph;
4b: O=2, R=4-Cl-Ph, R_1=H; R_2= -Ph;
4c: O=2, R=4-NO_2-Ph, R_1=H; R_2= -Ph;
4d: O=4, R=4-Cl-Ph, R_1=OCH_3; R_2= -Ph;
4e: O=4, R=3,4-di-OMe-Ph, R_1=H; R_2= -Ph;
4f: O=4, R=4-NO_2-Ph, R_1=H; R_2= -Ph;
4g: O=4, R=2-pyridine, R_1=H; R_2= -Ph;
4h: O=4, R=4-OMe-Ph, R_1=-OMe; R_2= -Ph;
4i: O=4, R=2,4-di-OMe-Ph, R_1=OMe; R_2= -Ph;
4j: O=4, R=2-thiophene, R_1=H; R_2= -Me;
4k: O=4, R=4-Cl-Ph, R_1=H; R_2= -Ph;
4l: O=4, R=2-Cl-Ph, R_1=H; R_2= -Ph;
4m:O=2, R=3,4-di-OMe-Ph, R_1=H; R_2= -Ph;
4n: O=2, R=2-pyridine, R_1=H; R_2= -Ph;
4o: O=2, R=3-Me-Ph, R_1=H; R_2= -Ph;
4p: O=2, R=2-F-Ph, R_1=H; R_2= -Ph;
4q: O=2, R=2-furan R_1=H; R_2= -Ph;
4r: O=2, R=4-Br-Ph, R_1=H; R_2= -Ph;

Scheme 1. Synthesis route for the target compounds