

Full Paper

Synthesis, Antiviral and Antifungal Bioactivity of 2-Cyano-acrylate Derivatives Containing Phosphonyl Moieties

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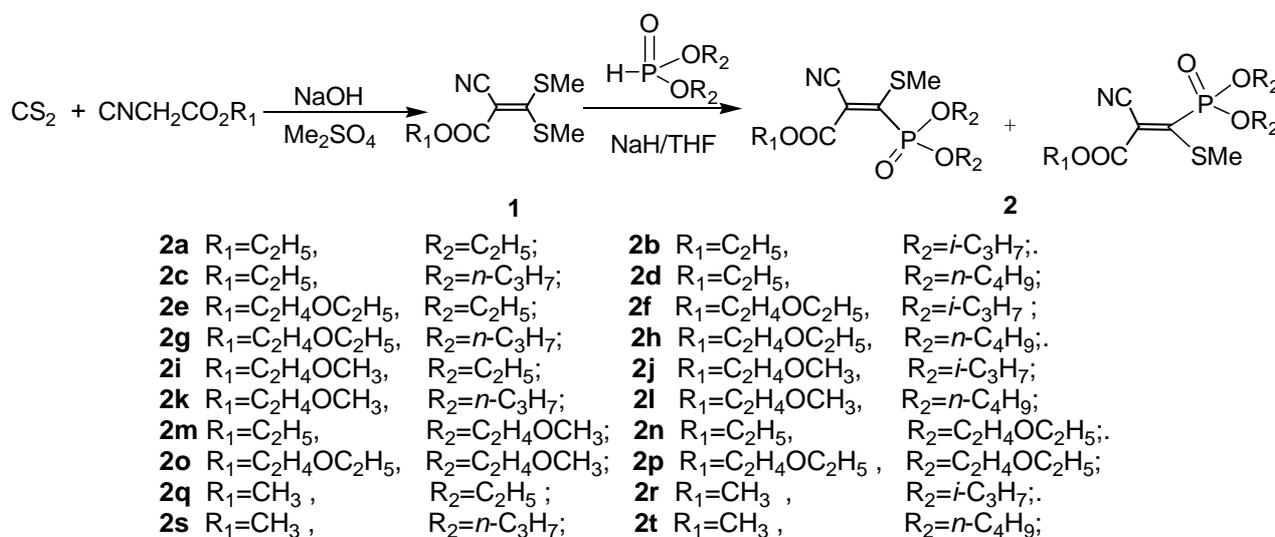
Abstract: Alkyl 2-cyano-3-methylthio-3-phosphonylacrylates were synthesized by the reaction of alkyl 2-cyano-3,3-dimethylthioacrylates with dialkyl phosphites. The structures of the new compounds were characterized by elemental analyses, IR, ^1H -, ^{13}C - and ^{31}P -NMR spectral data. These compounds were tested *in vitro* against pathogenic fungi, namely, *Fusarium graminearum*, *Cytospora mandshurica* and *Fusarium oxysporum*. Amongst all compounds, **2d** and **2t** were found to be effective against the tested fungi at 50 $\mu\text{g/mL}$. A half-leaf method was used to determine the *in vivo* protective, inactivation and curative efficacies of the title products against tobacco mosaic virus (TMV). Title compounds **2a** and **2b** were found to possess good *in vivo* curative, protection and inactivation effects against TMV with inhibitory rates at 500 mg/L of 60.0, 89.4 and 56.5 and 64.2, 84.2 and 61.2 %, respectively. To the best of our knowledge, this is the first report on the antiviral and antifungal activity of alkyl 2-cyano-3-methylthio-3-phosphonylacrylates.

Keywords: 2-Cyanoacrylate; phosphonyl moiety; antiviral activity; antifungal activity; synthesis.

Introduction

Cyanoacrylates, a class of highly potent herbicidal compounds, are known to disrupt photosynthetic electron transportation at a common binding domain on the 32 kDa polypeptide of the photosystem II (PSII) reaction center [1,2]. A large number of reports on the synthesis of cyanoacrylate derivatives exist due to their wide range of biological activities [3-6]. Some derivatives can serve not only as agrochemicals such as herbicides, insecticides, fungicides and plant virucides, but also as medicines such as antitumor agents. In our previous work, we designed and synthesized some chiral cyanoacrylates with antiviral activity by replacing the methylthio moiety of some 2-cyano-3-methylthio-3-substituted-phenylacrylates with (*R*)- or (*S*)-1-phenylethylamine groups. The (*E*) configuration of the reported chiral products was confirmed by X-ray single-crystal structure analysis. The bioassays showed that a chiral compound containing a 4-nitrophenyl moiety [(*E*)-ethyl 3[(*S*)-1-phenylethylamino]-3-(4-nitrophenylamino)-2-cyanoacrylate] exhibited good protection activity against TMV *in vivo* [7]. On the other hand, phosphonyl compounds, in general, have received wide attention in modern medicinal and pesticide chemistry. They are ideal for use in drug design due to their good bioactivity [8,9], low toxicity, and the ease of substitution with conventional heterocyclic ring groups [10-12]. In 2001 Chen *et al.* reported an efficient method for the synthesis of ethyl 2-cyano-3-methylthio-3-(diethoxyphosphonyl)acrylate under microwave irradiation conditions [13]. No reports on the fungitoxicity and antiviral activity of alkyl 2-cyano-3-methylthio-3-phosphonylacrylates have been published in the chemical or biological literature. In order to extend our research work on cyanoacrylates as antiviral agents and fungicides, we have designed and synthesized some novel cyanoacrylate derivatives **2a-2t** containing phosphonyl moieties. The synthetic route is shown in Scheme 1. Diethyl phosphite or higher homologues were employed in the reaction due to their ease of preparation. The structures of **2** were established by well defined IR, NMR and elemental analysis. The results of bioassay revealed that some compounds of the above series have good anti-TMV and antifungal activity.

Scheme 1. Synthesis of compounds **2a-2t**.



Results and Discussion

In order to optimize the reaction conditions for the syntheses of compounds **2a-2t**, the effects of various solvents, molar reagent ratios, reaction times and reaction temperatures on the reaction synthesis of **2b** were examined. The results are summarized in Table 1. Cyanoacrylate **2b** was obtained from **1** in poor yield (Table 1, entries 1-3) in solvents such as DMF, acetone and CH₃CN, but when tetrahydrofuran (THF) was chosen as solvent, the yield of **2b** increased from 11.2 % to 55.0 % (Table 1, entries 1, 4). Next we also examined the effect on the reaction of the molar ratios of the reactants. When the molar ratio of 2-cyano-3,3-(dimethylthio)-acrylate to *O, O'*-di-*i*-propylphosphite to sodium hydride was increased from 1:1:1 to 1:3:1, 1:3:2, 1:3:3 and 1:3:4 eq, compound **2b** was obtained in 0, 12.0, 20.0, 55.0 and 49.5 % yield, respectively (Table 1, entries 4-8). With regard to the reaction time 0, 23.0 and 55.0 % yields of **2b** were noted after 4, 8 and 16 h, respectively (Table 1, entries 9, 10, 4). When the reaction time was prolonged further to 20 h, no significant improvement was obtained (56.0 %, Table 1, entry 11), as compared to that seen after 16 h (55.0 %, Table 1, entry 4). As for the reaction temperature, a lower yield was observed at lower temperature (Table 1, entry 12). It could also be seen that the yield was significantly lower when the reaction was performed at 40-45 °C (Table 1, entry 13), compared to that seen at room temperature (Table 1, entry 4).

Table 1. Synthesis of **2b** under different reaction conditions.

Entry	Solvent	Ratio ^a	Time (h)	Reaction temperature	Yield (%) ^b
1	DMF	1:3:3	16	r.t	11.2
2	acetone	1:3:3	16	r.t	19.0
3	CH ₃ CN	1:3:3	16	r.t	34.9
4	THF	1:3:3	16	r.t	55.0
5	THF	1:3:1	16	r.t	12.0
6	THF	1:3:2	16	r.t	20.0
7	THF	1:1:1	16	r.t	0
8	THF	1:3:4	16	r.t	49.5
9	THF	1:3:3	4	r.t	0
10	THF	1:3:3	8	r.t	23.0
11	THF	1:3:3	20	r.t	56.0
12	THF	1:3:3	16	0-5°C	23.0
13	THF	1:3:3	16	40-45°C	13.0

^a Ratio of 2-cyano-3,3-dimethylthioacrylate:*O, O'*-di-*i*-propylphosphite:sodium hydride

^b Isolated yield based on 2-cyano-3,3-dimethylthioacrylate

Antifungal Bioassay: Inhibitory effects of cyanoacrylate derivatives on phytopathogenic fungi

The three fungi used in the fungicidal bioassay, *Fusarium graminearum*, *Cytospora mandshurica* and *Fusarium oxysporum*, were tested by the poison plate technique. The results of preliminary bioassays were compared with that of a commercial agricultural fungicide, hymexazol. As indicated in

Table 2, the new compounds **2d** and **2t** exhibited promising antifungal activity, inhibiting growth of *F. graminearum* at 64.5 and 49.7 %, *C. mandshurica* at 60.4 and 51.3 % and *F. oxysporum* at 65.0 and 41.1 %, respectively, which is a little lower than that of hymexazole (73.2 % against *F. graminearum*, 58.9 % against *C. mandshurica*, and 65.5 % against *F. oxysporum* at 50 µg/mL). Marked loss of activity was observed with other compounds such as **2a-2c** and **2e-2s**.

Table 2. Inhibition effects of the title compounds on fungi.

Compd.	Conc. (µg/mL)	Inhibition rate (%)		
		<i>Fusarium graminearum</i>	<i>Cytospora mandshurica</i>	<i>Fusarium oxysporum</i>
2a	50	11.14±0.21	11.00±0.25	18.21±0.11
	500	37.30±0.56	24.00±0.77	26.03±0.99
2b	50	4.94±0.49	1.34±0.65	8.08±0.98
	500	31.40±0.78	35.03±0.84	36.83±1.24
2c	50	8.31±0.57	13.10±0.78	13.47±1.24
	500	37.10±1.08	31.82±1.45	41.02±1.30
2d	50	64.50±0.40	60.43±0.63	65.03±1.22
	500	93.30±4.28	90.59±1.59	92.75±2.52
2e	50	22.04±0.90	32.01±0.78	26.71±0.90
	500	41.44±1.21	36.77±1.45	32.13±1.09
2f	50	35.22±1.78	41.09±3.21	46.11±3.34
	500	55.64±3.01	66.09±2.55	68.9±1.19
2g	50	7.79±0.57	-0.80±0.72	6.29±0.97
	500	40.0±2.15	49.47±1.01	48.80±1.33
2h	50	4.68±0.56	-3.21±0.85	5.99±0.87
	500	25.41±0.73	23.26±0.84	36.53±1.27
2i	50	5.45±0.73	-2.67±0.67	1.20±0.89
	500	16.3±0.64	8.29±0.76	20.06±0.97
2j	50	0.97±0.11	11.27±0.45	9.99±0.22
	500	20.00±0.21	26.78±0.44	30.99±1.09
2k	50	10.09±0.76	32.20±0.55	19.39±0.44
	500	31.03±0.90	36.38±1.32	40.22±1.45
2l	50	21.08±0.98	30.07±0.67	12.11±1.02
	500	34.01±1.67	29.51±1.02	32.00±0.69
2m	50	3.93±0.22	14.23±0.51	23.09±0.88
	500	27.31±0.99	16.08±0.55	41.90±2.01
2n	50	12.22±1.01	9.97±0.88	11.91±0.42
	500	24.04±0.62	36.09±0.55	35.69±1.90
2o	50	3.75±0.52	10.61±0.69	4.01±0.55
	500	21.08±0.65	48.28±0.96	29.07±0.65
2p	50	4.45±0.55	24.93±0.76	6.52±0.56
	500	28.10±0.69	47.21±0.95	35.59±0.69

Table 2. Cont.

2q	50	7.03±0.60	15.38±0.61	3.26±0.44
	500	42.15±0.79	57.82±1.11	33.58±0.68
2r	50	11.71±0.96	23.87±0.75	9.52±0.57
	500	44.03±0.81	64.72±1.26	52.13±0.84
2s	50	9.84±0.61	35.01±0.72	13.53±0.48
	500	53.40±0.92	65.78±1.29	56.64±0.90
2t	50	49.67±0.65	51.30±0.70	41.05±0.51
	500	81.97±2.91	75.33±1.71	69.42±1.23
Hymexazol ^a	50	73.2±1.41	58.89±1.16	65.51±1.76
	500	100±0.66	95.33±1.87	93.18±3.76
DMSO ^b		0	0	0

^a reference compound; ^b control.

Figure 1 shows the inhibition of mycelial growth of isolated hypha of *F. oxysporum* by compound **2d** at different concentrations (100, 50, 25, 10, and 1 µg/mL) as compared to control, when tested *in vitro*. Almost complete inhibition of mycelial growth was observed at 100 and 50 µg/mL concentrations as compared to control (full growth).

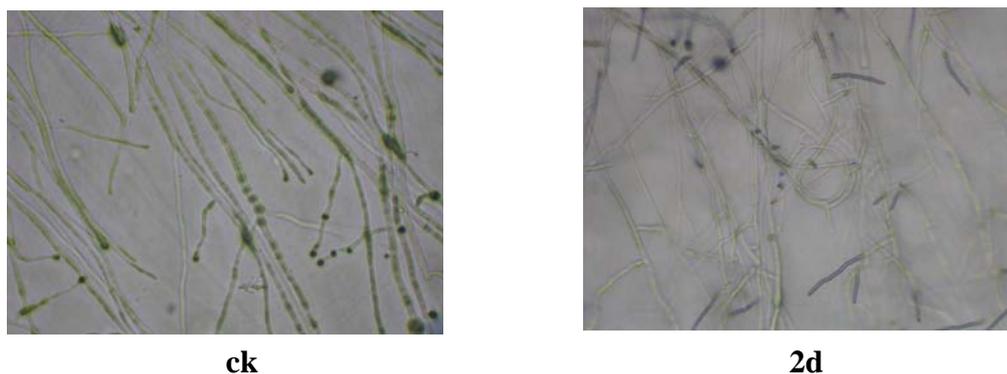
Figure 1. Effect of various concentrations of **2d** on the growth of isolated hypha of *F. oxysporum* (100, 50, 25, 5 and 1 µg/mL).



Key (µg/mL): 1, 100; 2, 50; 3, 25; 4, 5; 5, 1; and C, control.

Micro-observation results

Microphotography of the hyphal morphology of *F. oxysporum* treated with 100 µg/mL of **2d** (Figure 2) showed a series of changes, i.e. the cell of the hyphal divarication increased, hyphal knots appeared, and hypha bulged partially, compared with control.

Figure 2. Microphotograph (800×) of hyphal morphology of *F. oxysporum* treated with **2d**.*Preliminary antiviral activity assay*

The results of the *in vivo* bioassay against TMV are given in Table 3. Ningnanmycin was used as reference antiviral agent. The data provided in the table indicate that the introduction of dialkylphosphonyl moieties in cyanoacrylates might improve their protective activities. The title compounds **2a-2t** showed protection rates of 40.8–61.2 %. When R₁ and R₂ are Et, the resulting compound **2a** displayed a lower protective activity (56.5 %) than that of the reference compound (62.6 %). The highest protective activity was achieved when R₁ is Et and R₂ is *i*-Pr (compound **2b**). A protective rate of 61.2 %, equivalent to ningnanmycin, against TMV at 500 µg/mL was recorded in this case. From the data in Table 3, it may be observed that the title compounds **2a-2t** possess significant potential inactivation bioactivities, with values of 89.4, 84.7, 76.3, 80.3, 74.2, 79.6, 73.8, 70.8, 80.0, 77.9, 79.0, 78.2, 81.2, 80.2, 68.9, 67.3, 60.0, 70.0, 80.2 and 61.0 % at 500 µg/mL, respectively. Among these compounds, **2a** (R₁ and R₂ are Et) is much more active against TMV than the other ones, with an inactivation rate of 89.4 %, equivalent to ningnanmycin (92.6 %) against TMV at 500 µg/mL. The data also indicate that a change in the substituent might also affect the curative activity of the title compounds **2a-2t**. Compound **2a** (R₁ and R₂ are Et) and compound **2b** could cure TMV up to 60.0 % and 64.2 % at 500 µg/mL. The other compounds have a relatively lower curative activity than **2a** and **2b**.

Table 3. The Protective, inactivation and curative effects of the new compounds against TMV *in vivo*.

Agents	Concentration (mg/L)	Protective Effect (%)	Inactivation Effect (%)	Curative Effect(%)
2a	500	56.5*±1.4	89.4**±2.0	60.0*±0.9
2b	500	61.2*±3.2	84.7**±1.0	64.2*±1.9
2c	500	53.0*±1.8	76.3*±2.4	45.8*±1.0
2d	500	53.4*±0.7	80.3**±3.4	41.2*±2.1
2e	500	45.0*±1.1	74.2*±3.4	54.7*±2.2
2f	500	47.9*±1.7	79.6**±1.6	28.4±4.4
2g	500	40.8*±5.4	73.8*±3.0	41.8*±5.5
2h	500	46.5*±2.3	70.8*±1.4	31.4*±1.7

Table 3. Cont.

2i	500	50.1*±3.0	80.0**±2.0	36.6*±4.4
2j	500	43.2*±5.4	77.9*±0.9	42.1*±3.8
2k	500	45.7*±1.9	79.0*±0.7	44.3*±6.4
2l	500	51.2*±1.1	78.2*±0.9	42.0*±1.5
2m	500	50.0*±2.0	81.2*±1.6	50.0*±1.0
2n	500	49.7±8.0	80.2**±1.2	51.2*±1.0
2o	500	48.9*±0.9	68.9*±5.3	33.3*±4.9
2p	500	47.6*±1.0	67.3*±2.4	33.3±8.9
2q	500	45.6*±0.4	60.0*±3.1	10.9±6.0
2r	500	50.9*±1.1	70.0*±1.9	36.7*±6.7
2s	500	51.2*±0.9	80.2**±2.0	41.4*±9.9
2t	500	46.6*±5.5	61.0*±5.1	24.1±8.7
Ningnamycin	500	62.6*±2.1	92.6**±1.0	53.9*±2.3

All results are expressed as mean ± SD; $n=3$ for all groups; * $P<0.05$, ** $P<0.01$.

Conclusions

A series of novel cyanoacrylate derivatives **2a-2t** containing phosphonyl moieties were synthesized by treatment of alkyl 2-cyano-3,3-dimethylthioacrylates and dialkyl phosphites with NaH in THF solvent. This method is easy and yields the title compounds in moderate yields. The structures were verified by spectroscopic data. In the antifungal bioassay, the title compounds **2d** and **2t** were found to possess the highest activity against three kinds of fungi *in vitro*. The bioassay results showed that these title compounds exhibited moderate to good anti-TMV bioactivity. Title compounds **2a** and **2b** showed better biological activity than their structurally related analogues **2c-2t**.

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Experimental

General

The melting points of the products were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co., P.R. China) and were not corrected. The IR spectra were recorded on a Bruker VECTOR 22 spectrometer in KBr disks. ^1H -, ^{13}C - and ^{31}P -NMR spectra (solvent CDCl_3) were recorded on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Analytical TLC was performed on silica gel GF254. Column chromatographic purification was carried out using silica gel. All reagents either belonged to analytical reagent grade or were chemically pure. THF was dried,

deoxygenated and redistilled before use. Dialkyl phosphites and alkyl 2-cyano-3,3-(dimethylthio)acrylates were prepared according to literature methods [14, 15].

General procedure for the preparation of title compounds 2a-2t.

A dry 100 mL round-bottom flask equipped with a magnetic stirrer and nitrogen inlet was charged with sodium hydride (0.66 g, 55%, 15.2 mmol) in THF (15 mL) at 0~5 °C. Dialkyl phosphite (15.2 mmol) was then slowly added through a dropping funnel into the resulting solution over a period of 30 min. Alkyl 2-cyano-3,3-(dimethylthio)acrylate (5.0 mmol) in THF (20 mL) was then slowly added to the above solution. The mixture was stirred for 16 h at room temperature. After removal of the solvent under vacuum, the residue was dissolved in ice-cold water (40 mL) and acidified with dilute hydrochloric acid (10%), extracted with ethyl acetate (3 × 20 mL) and the combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was subjected to column chromatography using a mixture of petroleum ether and ethyl acetate (4:1) as eluent to give the title compounds **2a-2t**.

Yields and product characterization

Ethyl 2-cyano-3-methylthio-3-(diethoxyphosphonyl)acrylate (2a): yellow liquid; yield 52.0%; ¹H-NMR δ: 1.37~1.45 (m, 9H, ester CH₃ + 2 × phosphonyl CH₃), 2.76 (d, *J* = 6.8 Hz, 3H, SCH₃), 4.32~4.35 (m, 6H, ester CH₂ + 2 × phosphonyl CH₂); ¹³C-NMR δ: 14.1 (ester CH₃), 16.2 (2×phosphonyl CH₃), 19.4 (SCH₃), 62.7 (ester OCH₂), 64.5 (2 × phosphonyl CH₂), 106.2 (C=C C-2), 114.5 (CN), 162.1 (C=C C-3), 165.7 (C=O); ³¹P-NMR δ: 5.1; IR ν: 2983, 2214, 1745, 1251, 1018 cm⁻¹; Anal. Calcd. for C₁₁H₁₈NO₅PS (307.5): C, 42.93; H, 5.58; N, 4.55. Found: C, 42.99; H, 5.90; N, 4.56.

*Ethyl 2-cyano-3-methylthio-3-(di-*i*-propoxyphosphonyl)acrylate (2b)*: yellow liquid; yield 55.0%; ¹H-NMR δ: 1.36~1.38 (m, 15 H, 4 × phosphonyl CH₃ + ester CH₃), 2.73 (d, *J* = 6.9 Hz, 3H, SCH₃), 4.33 (q, *J* = 7.2 Hz, 2H, ester CH₂), 4.82~4.89 (m, 2H, 2 × phosphonyl CH); ¹³C-NMR δ: 14.1 (ester CH₃), 19.4 (SCH₃), 23.9 (4 × phosphonyl CH₃), 63.1 (ester OCH₂), 64.5 (2 × phosphonyl CH₂), 74.2 (2 × phosphonyl CH), 106.9 (C=C C-2), 114.7 (CN), 162.1 (C=C C-3), 165.3 (C=O); ³¹P-NMR δ: 3.5; IR ν: 2980, 2212, 1718, 1504, 1246, 987 cm⁻¹; Anal. Calcd. for C₁₃H₂₂NO₅PS (335.1): C, 46.55; H, 6.56; N, 4.18. Found: C, 46.56; H, 6.61; N, 4.10.

*Ethyl 2-cyano-3-methylthio-3-(di-*n*-propoxyphosphonyl)acrylate (2c)*: yellow liquid; yield 50.0%; ¹H-NMR δ: 0.98~1.02 (m, 6H, 2 × phosphonyl CH₃), 1.37 (t, *J* = 7.5 Hz, 3H, ester CH₃), 1.73~1.75 (m, 4H, 2 × phosphonyl CH₂), 2.75 (d, *J* = 6.9 Hz, 3H, SCH₃), 4.09~4.19 (m, 4H, 2 × phosphonyl CH₂), 4.33 (q, *J* = 7.1 Hz, 2H, ester CH₂); ¹³C-NMR δ: 10.0 (2 × phosphonyl CH₃), 14.1 (ester CH₃), 19.5 (SCH₃), 23.7 (2 × phosphonyl CH₂), 63.3 (ester OCH₂), 69.8 (2 × phosphonyl CH₂), 106.5 (C=C C-2), 114.6 (CN), 162.1 (C=C C-3), 165.5 (C=O); ³¹P-NMR δ: 5.9; IR ν: 2970, 2214, 1735, 1463, 1251, 1006 cm⁻¹; Anal. Calcd. for C₁₃H₂₂NO₅PS (335.1): C, 46.55; H, 6.56; N, 4.18. Found: C, 46.56; H, 6.61; N, 4.18.

Ethyl 2-cyano-3-methylthio-3-(di-n-butoxyphosphonyl)acrylate (2d): yellow liquid; yield 45.6%; $^1\text{H-NMR}$ δ : 0.94~0.97 (m, 6H, 2 \times phosphonyl CH_3), 1.37 (t, $J=7.2$ Hz, 3H, ester CH_3), 1.39~1.46 (m, 4H, 2 \times phosphonyl CH_2), 1.73~1.77 (m, 4H, 2 \times phosphonyl CH_2), 2.74 (d, $J=6.9$ Hz, 3H, SCH_3), 4.23~4.34 (m, 6H, 2 \times phosphonyl CH_2 + ester CH_2); $^{13}\text{C-NMR}$ δ : 13.5 (2 \times phosphonyl CH_3), 13.6 (ester CH_3), 18.7 (2 \times phosphonyl CH_2), 18.8 (SCH_3), 32.3 (2 \times phosphonyl CH_2), 62.8 (ester CH_2), 68.0 (2 \times phosphonyl CH), 106.4 ($\text{C}=\text{C}$ C-2), 114.5 (CN), 162.1 ($\text{C}=\text{C}$ C-3), 165.6 ($\text{C}=\text{O}$); $^{31}\text{P-NMR}$ δ : 5.5; IR ν : 2960, 2214, 1735, 1465, 1259, 1028 cm^{-1} ; Anal. Calcd. for $\text{C}_{15}\text{H}_{26}\text{NO}_5\text{PS}$ (363.1): C, 49.57; H, 7.16; N, 3.86. Found: C, 49.54; H, 7.21; N, 3.69.

Ethoxyethyl 2-cyano-3-methylthio-3-(diethoxyphosphonyl)acrylate (2e): yellow liquid; yield 49.7%; $^1\text{H-NMR}$ δ : 1.22 (t, $J=6.9$ Hz, 3H, ester O-C- CH_3), 1.38~1.45 (m, 6H, 2 \times phosphonyl CH_3), 2.75 (d, $J=8.6$ Hz, 3H, SCH_3), 3.57 (q, $J=6.3$ Hz, 2H, ester OCH_2), 3.72~3.74 (m, 2H, ester CH_2), 4.30~4.32 (m, 4H, 2 \times phosphonyl CH_2), 4.39~4.41 (m, 2H, ester CH_2); $^{13}\text{C-NMR}$ δ : 15.2 (ethoxy CH_3), 16.3 (2 \times phosphonyl CH_3), 19.6 (SCH_3), 64.6 (2 \times phosphonyl CH_2), 65.9 (ester OCH_2), 66.9 (ethoxy OCH_2), 67.8 (ester CH_2O), 106.0 ($\text{C}=\text{C}$ C-2), 114.5 (CN), 162.2 ($\text{C}=\text{C}$ C-3), 164.9 ($\text{C}=\text{O}$); $^{31}\text{P-NMR}$ δ : 5.15; IR ν : 2953, 2992, 2852, 2214, 1714, 1444, 1249, 1120, 1012 cm^{-1} ; Anal. Calcd. for $\text{C}_{13}\text{H}_{22}\text{NO}_6\text{PS}$ (351.1): C, 44.43; H, 6.27; N, 3.99. Found: C, 44.44; H, 6.31; N, 3.89.

Ethoxyethyl 2-cyano-3-methylthio-3-(di-i-propoxyphosphonyl)acrylate (2f): yellow liquid; yield 57.5%; $^1\text{H-NMR}$ δ : 1.22 (t, $J=6.9$ Hz, 3H, ester O-C- CH_3), 1.41~1.45 (m, 12H, 4 \times phosphonyl CH_3), 2.72 (d, $J=14.3$ Hz, 3H, SCH_3), 3.57 (q, $J=6.9$ Hz, 2H, ester OCH_2), 3.72~3.73 (m, 2H, ester OCH_2), 4.39~4.41 (m, 2H, ester CH_2), 4.82~4.89 (m, 2H, 2 \times phosphonyl CH); $^{13}\text{C-NMR}$ δ : 15.1 (ethoxy CH_3), 19.7 (SCH_3), 23.8 (4 \times phosphonyl CH_3), 65.7 (ester CH_2), 66.7 (ethoxy OCH_2), 67.7 (ester CH_2O), 74.2 (2 \times phosphonyl CH), 106.6 ($\text{C}=\text{C}$ C-2), 114.5 (CN), 162.0 ($\text{C}=\text{C}$ C-3), 166.8 ($\text{C}=\text{O}$); $^{31}\text{P-NMR}$ δ : 4.76; IR ν : 2978, 2933, 2214, 1732, 1454, 1251, 1118, 987 cm^{-1} ; Anal. Calcd. for $\text{C}_{15}\text{H}_{26}\text{NO}_6\text{PS}$ (379.4): C, 47.44; H, 6.85; N, 3.69. Found: C, 47.48; H, 6.91; N, 3.51.

Ethoxyethyl 2-cyano-3-methylthio-3-(di-n-propoxyphosphonyl)acrylate (2g): yellow liquid; yield 55.6%; $^1\text{H-NMR}$ δ : 0.98~1.00 (m, 6H, 2 \times phosphonyl CH_3), 1.21 (t, $J=6.9$ Hz, 3H, ester O-C- CH_3), 1.73~1.80 (m, 4H, 2 \times phosphonyl CH_2), 2.75 (d, $J=9.1$ Hz, 3H, SCH_3), 3.57 (q, $J=7.2$ Hz, 2H, ester OCH_2), 3.72~3.74 (m, 2H, ester CH_2), 4.16~4.20 (m, 4H, 2 \times phosphonyl CH_2), 4.39~4.41 (m, 2H, ester CH_2); $^{13}\text{C-NMR}$ δ : 10.1 (2 \times phosphonyl CH_3), 15.2 (ethoxy OCH_3), 19.6 (SCH_3), 23.7 (2 \times phosphonyl CH_2COP), 65.9 (ester OCH_2), 66.3 (ethoxy OCH_2), 67.8 (ester CH_2O), 70.0 (2 \times phosphonyl CH_2OP), 106.1 ($\text{C}=\text{C}$ C-2), 114.5 (CN), 162.0 ($\text{C}=\text{C}$ C-3), 164.9 ($\text{C}=\text{O}$); $^{31}\text{P-NMR}$ δ : 5.43; IR ν : 2968, 2897, 2214, 1745, 1456, 1381, 1238, 1116, 995 cm^{-1} ; Anal. Calcd. for $\text{C}_{15}\text{H}_{26}\text{NO}_6\text{PS}$ (379.4): C, 47.44; H, 6.85; N, 3.69. Found: C, 47.40; H, 6.81; N, 3.55.

Ethoxyethyl 2-cyano-3-methylthio-3-(di-n-butoxyphosphonyl)acrylate (2h): yellow liquid; yield 54.7%; $^1\text{H-NMR}$ δ : 0.94~0.96 (m, 6H, 2 \times phosphonyl CH_3), 1.22 (t, $J=6.9$ Hz, 3H, ester O-C- CH_3), 1.42~1.44 (m, 4H, 2 \times phosphonyl CH_2), 1.69~1.76 (m, 4H, 2 \times phosphonyl CH_2), 2.75 (d, $J=10.3$ Hz, 3H, SCH_3), 3.57 (q, $J=7.5$ Hz, 2H, ethoxy OCH_2), 3.72~3.74 (m, 2H, ester OCH_2), 4.23~4.25 (m, 4H, 2 \times phosphonyl CH_2), 4.39~4.41 (m, 2H, ester CH_2); $^{13}\text{C-NMR}$ (δ): 13.6 (2 \times phosphonyl CH_3), 15.2 (ethoxy OCH_3), 18.8 (2 \times phosphonyl CH_2), 19.6 (SCH_3), 32.4 (2 \times phosphonyl CH_2), 65.9 (ester

CH₂), 66.9 (ethoxy OCH₂), 67.8 (2 × phosphonyl CH₂), 68.3 (ester CH₂O), 106.1 (C=C C-2), 114.5 (CN), 162.0 (C=C C-3), 164.8 (C=O); ³¹P-NMR δ: 5.44; IR ν: 2960, 2872, 2214, 1732, 1714, 1249, 1056 cm⁻¹; Anal. Calcd. for C₁₇H₃₀NO₆PS (407.1): C, 50.11; H, 7.37; N, 3.44. Found: C, 50.13; H, 7.42; N, 3.26.

Methoxyethyl 2-cyano-3-methylthio-3-(diethoxyphosphonyl)acrylate (2i): yellow liquid; yield 60.1%; ¹H-NMR δ: 1.36~1.45 (m, 6H, 2 × phosphonyl CH₃), 2.75 (d, *J* = 7.5 Hz, 3H, SCH₃), 3.41 (s, 3H, methoxy CH₃), 3.68~3.70 (m, 2H, ester CH₂), 4.25~4.32 (m, 4H, 2 × phosphonyl CH₂), 4.39~4.42 (m, 2H, ester CH₂); ¹³C-NMR δ: 16.2 (2 × phosphonyl CH₃), 19.5 (SCH₃), 59.2 (methoxy), 64.6 (2 × phosphonyl CH₂), 65.6 (ester CH₂), 69.8 (ester CH₂O), 105.8 (C=C C-2), 114.4 (CN), 161.9 (C=C C-3), 165.0 (C=O); ³¹P-NMR δ: 5.11; IR ν: 2916, 2214, 1732, 1444, 1249, 1122, 1012 cm⁻¹; Anal. Calcd. for C₁₂H₂₀NO₆PS (337.1): C, 42.71; H, 5.93; N, 4.15. Found: C, 42.73; H, 5.98; N, 4.05.

*Methoxyethyl 2-cyano-3-methylthio-3-(di-*i*-propoxyphosphonyl)acrylate (2j)*: yellow liquid; yield 58.1%; ¹H-NMR δ: 1.36~1.42 (m, 12H, 4 × phosphonyl CH₃), 2.72 (d, *J* = 13.2 Hz, 3H, SCH₃), 3.40 (s, 3H, ester OCH₃), 3.68~3.70 (m, 2H, ester CH₂), 4.39~4.40 (m, 2H, ester OCH₂), 4.82~4.89 (m, 2H, 2 × phosphonyl CH); ¹³C-NMR δ: 19.7 (SCH₃), 24.0 (4 × phosphonyl CH₃), 59.0 (methoxy OCH₃), 65.9 (ester CH₂), 70.0 (ester CH₂), 74.1 (2 × phosphonyl CH), 106.4 (C=C C-2), 114.4 (CN), 162.0 (C=C C-3), 165.8 (C=O); ³¹P-NMR δ: 5.10; IR ν: 2981, 2933, 2214, 1747, 1454, 1249, 1000 cm⁻¹; Anal. Calcd. for C₁₄H₂₄NO₆PS (365.1): C, 46.01; H, 6.57; N, 3.83. Found: C, 45.92; H, 6.39; N 3.83.

*Methoxyethyl 2-cyano-3-methylthio-3-(di-*n*-propoxyphosphonyl) acrylate (2k)*: yellow liquid; yield 55.3%; ¹H-NMR δ: 0.98~1.00 (m, 6H, 2 × phosphonyl CH₃), 1.73~1.80 (m, 4H, 2 × phosphonyl CH₂), 2.75 (d, *J* = 7.5 Hz, 3H, SCH₃), 3.42 (s, 3H, methoxy OCH₃), 3.69~3.70 (m, 2H, ester OCH₂), 4.13~4.21 (m, 4H, 2 × phosphonyl CH₂), 4.40~4.42 (m, 2H, ester CH₂); ¹³C-NMR δ: 10.0 (2 × phosphonyl CH₃), 19.5 (SCH₃), 23.7 (2 × phosphonyl CH₂), 59.2 (methoxy OCH₃), 65.6 (ester CH₂), 69.8 (2 × phosphonyl CH₂OP), 69.9 (ester CH₂), 105.9 (C=C C-2), 114.4 (CN), 162.1 (C=C C-3), 165.0 (C=O); ³¹P-NMR δ: 5.38; IR ν: 2968, 2897, 2214, 1724, 1465, 1247, 1000 cm⁻¹; Anal. Calcd. for C₁₄H₂₄NO₆PS (365.1): C, 46.01; H, 6.57; N, 3.83. Found: C, 45.82; H, 7.42; N 3.83.

*Methoxyethyl 2-cyano-3-methylthio-3-(di-*n*-butoxyphosphonyl)acrylate (2l)*: yellow liquid; yield 51.0%; ¹H-NMR δ: 0.94~0.97 (m, 6H, 2 × phosphonyl CH₃), 1.41~1.44 (m, 4H, 2 × CH₂), 1.69~1.76 (m, 4H, 2 × CH₂-C-O-P=O), 2.74 (d, *J* = 8.0 Hz, 3H, SCH₃), 3.41 (s, 3H, ester OCH₃), 3.68~3.70 (m, 2H, ester CH₂), 4.20~4.24 (m, 4H, 2 × phosphonyl CH₂), 4.40~4.42 (m, 2H, ester CH₂); ¹³C-NMR δ: 13.5 (2 × phosphonyl CH₃), 18.6 (2 × phosphonyl CH₂), 18.7 (SCH₃), 32.2 (2 × phosphonyl CH₂), 59.2 (methoxy OCH₃), 65.6 (ester CH₂), 68.2 (2 × phosphonyl CH₂OP), 69.8 (ester CH₂), 105.8 (C=C C-2), 114.4 (CN), 161.9 (C=C C-3), 165.0 (C=O); ³¹P-NMR δ: 5.39; IR ν: 2956, 2872, 2214, 1734, 1458, 1253, 1002 cm⁻¹; Anal. Calcd. for C₁₆H₂₈NO₆PS (393.1): C, 48.84; H, 7.12; N, 3.56. Found: C 48.80, H, 7.17; N, 3.43.

Ethyl 2-cyano-3-methylthio-3-[di-(2-methoxyethoxy)phosphonyl]acrylate (2m): yellow liquid; yield 60.0%; ¹H-NMR δ: 1.36~1.38 (m, 3H, ester CH₃), 2.78 (d, *J* = 10.9 Hz, 3H, SCH₃), 3.39 (s, 6H, 2 × phosphonyl CH₃), 3.62~3.67 (m, 4H, 2 × phosphonyl CH₂), 4.31~4.40 (m, 6H, 2 × phosphonyl CH₂ +

ester CH₂); ¹³C-NMR δ: 14.0 (ester CH₃), 19.2 (SCH₃), 58.7 (phosphonyl OCH₃), 62.7 (ester OCH₂), 66.7 (2 × phosphonyl CH₂OP), 71.0 (2 × phosphonyl CH₂), 106.0 (C=C C-2), 114.7 (CN), 162.1 (C=C C-3), 164.6 (C=O); ³¹P-NMR δ: 6.16; IR ν: 2985, 2926, 2212, 1714, 1446, 1367, 1242, 1014 cm⁻¹; Anal. Calcd. for C₁₃H₂₂NO₇PS (367.1): C, 42.50; H, 5.99; N, 3.81. Found: C, 42.45; H, 6.04; N, 3.72.

Ethyl 2-cyano-3-methylthio-3-[di-(2-ethoxyethoxy)phosphonyl]acrylate (2n): yellow liquid; yield 57.6%; ¹H-NMR δ: 1.20 (q, 6H, J = 7.1 Hz, 2 × phosphonyl CH₃), 1.36~1.38 (m, 3H, ester CH₃), 2.78 (d, J=10.8 Hz, 3H, SCH₃), 3.52~3.55 (m, 4H, 2 × phosphonyl CH₂), 3.65~3.70 (m, 4H, 2 × phosphonyl CH₂), 4.31~4.40 (m, 6H, 2 × phosphonyl CH₂ + ester CH₂); ¹³C-NMR δ: 15.0 (SCH₃), 66.5 (ester OCH₂), 66.6 (2 × phosphonyl CH₂), 66.9 (2 × phosphonyl CH₂), 67.0 (2 × phosphonyl CH₂), 106.0 (C=C C-2), 114.7 (CN), 162.2 (C=C C-3), 165.0 (C=O); ³¹P-NMR δ: 5.93; IR ν: 2970, 2868, 2212, 1720, 1446, 1367, 1244, 1016 cm⁻¹; Anal. Calcd. for C₁₅H₂₆NO₇PS (395.1): C, 45.56; H, 6.58; N, 3.39. Found: C, 45.51; H, 6.63; N, 3.54.

Ethoxyethyl 2-cyano-3-methylthio-3-[di-(2-methoxyethoxy)phosphoryl]acrylate (2o): yellow liquid; yield 53.5%; ¹H-NMR δ: 1.21 (t, 3H, J = 6.9 Hz, CH₃ of ethoxy), 2.77 (d, J_{PH} = 17.0 Hz, 3H, SCH₃), 3.38 (s, 6H, 2×OCH₃), 3.55~3.74 (m, 8H, 2×CH₂C-O of phosphonyl + COOCH₂ of ester + CH₂ of ethoxy), 4.24~4.41 (m, 6H, 2×CH₂O of phosphonyl + COOCH₂ of ester); ¹³C-NMR δ: 15.1 (CH₃ of ethoxy), 19.3 (SCH₃), 58.7 (2×OCH₃), 59.0 (CH₂ of ethoxy), 66.1 (OCH₂ of ester), 66.8 (2×CH₂O of phosphonyl), 67.8 (OCCH₂ of ester), 71.1 (2×CH₂C-O of phosphonyl), 105.7 (C-2 of C=C), 114.6 (CN), 162.1 (C-3 of C=C), 165.0 (C=O); ³¹P-NMR δ: 6.11; IR ν: 2927, 2214, 1747, 1456, 1371, 1253, 1033 cm⁻¹; Anal. Calcd. for C₁₅H₂₆NO₈PS (411.1): C 43.79; H 6.37; N 3.40. Found: C 43.76, H 6.43, N 3.41.

Ethoxyethyl 2-cyano-3-methylthio-3-[di-(2-ethoxyethoxy)phosphoryl]acrylate (2p): yellow liquid; yield 56.2%; ¹H-NMR δ: 1.21~1.39 (m, 9H, 3×CH₃), 2.78 (d, J_{PH} = 17.1 Hz, 3H, SCH₃), 3.50~3.59 (m, 6H, 3 × OCH₂), 3.63~3.73 (m, 6H, 2 × phosphonyl CH₂C-O + ester COOCH₂), 4.27~4.41 (m, 6H, 2 × phosphonyl CH₂O + ester COOCH₂); ¹³C-NMR δ: 15.1 (3 × ethoxy CH₃), 19.3 (SCH₃), 66.5 (3 × ethoxy OCH₂), 66.8, 67.0 (ester OCH₂ + 2 × phosphonyl CH₂O), 68.9, 69.0 (ester OCCH₂ + 2 × phosphonyl CH₂C-O), 105.8 (C=C C-2), 114.6 (CN), 162.1 (C=C C-3), 165.1 (C=O); ³¹P-NMR δ: 6.30; IR ν: 2974, 2214, 1747, 1583, 1485, 1253, 1035 cm⁻¹; Anal. Calcd. for C₁₇H₃₀NO₈PS (439.1): C 46.46; H 6.88; N 3.19. Found: C 46.60, H 6.95, N 3.26.

Methyl 2-cyano-3-methylthio-3-(diethoxyphosphonyl)acrylate (2q): yellow liquid; yield 61.0%; ¹H-NMR δ: 1.33~1.45 (m, 6H, 2 × phosphonyl CH₃), 2.77 (d, J_{PH} = 13.7 Hz, 3H, SCH₃), 3.88 (d, J_{PH} = 7.5 Hz, 3H, OCH₃), 4.01~4.34 (m, 4H, 2 × phosphonyl CH₂); ¹³C NMR δ: 16.2 (2 × phosphonyl CH₃), 19.5 (SCH₃), 53.7 (OCH₃), 64.5 (2 × phosphonyl CH₂), 105.5 (C=C C-2), 114.6 (CN), 162.6 (C=C C-3), 165.3 (C=O); ³¹P-NMR δ: 5.0; IR ν: 2983, 2214, 1749, 1255, 1037 cm⁻¹; Anal. Calcd. for C₁₀H₁₆NO₅PS (293.1): C 40.95, H 5.50, N 4.78. Found: C 40.91, H 5.47, N 4.81.

Methyl 2-cyano-3-methylthio-3-(di-i-propoxyphosphonyl)acrylate (2r): yellow liquid; yield 58.2%; ¹H-NMR δ: 1.35~1.44 (m, 12H, 4 × phosphonyl CH₃), 2.74 (d, J_{PH} = 6.9 Hz, 3H, SCH₃), 3.87 (d, J = 8.1 Hz, 3H, OCH₃), 4.83~4.89 (m, 2H, 2 × phosphonyl CH); ¹³C-NMR δ: 19.8 (SCH₃), 23.9 (4 ×

phosphonyl CH₃), 53.7 (OCH₃), 74.4 (2 × phosphonyl CH), 106.3 (C=C C-2), 114.9 (CN), 162.7 (C=C C-3), 166.3 (C=O); ³¹P-NMR δ: 4.90; IR ν: 2980, 2214, 1753, 1454, 1251, 999 cm⁻¹; Anal. Calcd. for C₁₂H₂₀NO₅PS (335.1): C 44.85, H 6.27, N 4.36. Found: C 44.77, H 6.11, N 4.40.

Methyl 2-cyano-3-methylthio-3-(di-n-propoxyphosphonyl)acrylate (2s): yellow liquid; yield 56.3%; ¹H-NMR δ: 0.98~1.00 (m, 6H, 2 × phosphonyl CH₃), 1.72~1.80 (m, 4H, 2 × phosphonyl CH₂C-O), 2.76 (d, *J*_{PH} = 4.6 Hz, 3H, SCH₃), 3.87 (d, *J* = 10.3 Hz, 3H, OCH₃), 3.99~4.21 (m, 4H, 2 × phosphonyl CH₂O); ¹³C-NMR δ: 10.1 (2 × phosphonyl CH₃), 19.6 (SCH₃), 23.8 (2 × phosphonyl CH₂C-O), 53.8 (OCH₃), 70.0 (2 × phosphonyl CH₂O), 105.7 (C=C C-2), 114.6 (CN), 162.5 (C=C C-3), 165.4 (C=O); ³¹P-NMR δ: 5.3; IR ν: 2968, 2214, 1751, 1462, 1253, 1002 cm⁻¹; Anal. Calcd. for C₁₂H₂₀NO₅PS (321.3): C 44.85, H 6.27, N 4.36. Found: C 45.01, H 6.18, N 4.38.

Methyl 2-cyano-3-methylthio-3-(di-n-butoxyphosphonyl)acrylate (2t): yellow liquid; yield 57.3%; ¹H-NMR δ: 0.94~0.98 (m, 6H, 2 × phosphonyl CH₃), 1.38-1.47 (m, 4H, 2 × phosphonyl CH₂CCO), 1.65-1.77 (m, 4H, 2 × phosphonyl CH₂CO), 2.75 (d, *J*_{PH} = 4.6 Hz, 3H, SCH₃), 3.87 (d, *J* = 10.3 Hz, 3H, OCH₃), 4.08~4.25 (m, 2H, 4H, 2 × phosphonyl CH₂O); ¹³C-NMR δ: 13.6 (2 × phosphonyl CH₃), 18.7 (2 × phosphonyl CH₂CCO), 18.8 (SCH₃), 32.2 (2 × phosphonyl CH₂C-O), 53.8 (OCH₃), 68.2 (2 × phosphonyl CH₂O), 105.6 (C=C C-2), 114.6 (CN), 162.6 (C=C C-3), 165.3 (C=O); ³¹P-NMR δ: 5.3; IR ν: 2960, 2214, 1743, 1462, 1255, 1020 cm⁻¹; Anal. Calcd. for C₁₄H₂₄NO₅PS (349.1): C 48.13, H 6.92, N 4.01. Found: C 48.46, H 7.04, N 3.91.

Antifungal bioassay

The antifungal activity of all synthesized compounds were tested against three pathogenic fungi, namely *Fusarium graminearum*, *Cytospora mandshurica* and *Fusarium oxysporum*, by the poison plate technique [16]. Compounds were dissolved in DMSO (1 mL) before mixing with potato dextrose agar (PDA, 90 mL). The final concentration of compounds in the medium were fixed at 50 and 500 µg/mL. All types of fungi were incubated in PDA at 25±1 °C for 4 days to get new mycelium for the antifungal assays, then a mycelia disk of approximately 4 mm diameter cut from culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25±1 °C for 5 days. DMSO in sterile distilled water served as control, while hymexazole served as positive control. For each treatment, three replicates were conducted. The radial growth of the fungal colonies were measured and the data were statistically analyzed. The inhibitory effects of the test compounds on these fungi *in vitro* were calculated by the formula $I = (C-T/C) \times 100$, where C represents the diameter of fungi growth on untreated PDA, and T represents the diameter of fungi on treated PDA while I represents the inhibition rate.

Hyphal morphology of *F. oxysporum*

Compound **2d** (final conc. 100 µg/mL) was added to sterilized Czapek media (0.2 % NaNO₃, 0.131 % K₂HPO₄·3H₂O, 0.05 % KCl, 0.05 % MgSO₄·7H₂O, 0.00183 % FeSO₄·7H₂O, 3 % sucrose, pH 6.8) [17] in which *F. oxysporum* had incubated for a few days. After incubating together at 27 °C for 24 h, it was observed under microscope (Olympus 800 ×). Acetone (0.5 mL) served as the control.

Antiviral Biological Assays

Purification of tobacco mosaic virus: Using Gooding's method [18], the upper leaves of *Nicotiana tabacum* L inoculated with TMV were selected and were ground in phosphate buffer, then filtered through double layer pledget. The filtrate was centrifuged at 10,000 g, treated twice with PEG and centrifuged again. The whole experiment was carried out at 4 °C. Absorbance values were estimated at 260 nm using an ultraviolet spectrophotometer. Virus concn = $(A_{260} \times \text{dilution ratio}) / E^{1\text{cm}}$ ^{0.1%, 260nm}

Protective effects of compounds on TMV in vivo: The compound solution was smeared on the left side while solvent was served as control on the right side of growing *Nicotiana tabacum*. L leaves of the same ages. The leaves were then inoculated with the virus after 12 hours. A brush was dipped in tobacco mosaic virus of 6×10^{-3} mg/mL to inoculate the leaves, which were previously scattered with silicon carbide. The leaves were then washed with water and rubbed softly along the nervature once or twice. The local lesion numbers appearing 3-4 days after inoculation were counted [7]. Three repetitions were conducted for each compound.

Inactivation effect of compounds on TMV in vivo: The virus was inhibited by mixing with the compound solution at the same volume for 30 minutes. The mixture was then inoculated on the left side of the leaves of *Nicotiana tabacum*. L., while the right side of the leaves was inoculated with the mixture of solvent and the virus for control. The local lesion numbers were recorded 3-4 days after inoculation [7]. Three repetitions were conducted for each compound.

Curative effect of compounds on TMV in vivo: Growing leaves of *Nicotiana tabacum*. L of the same ages were selected. The tobacco mosaic virus (concentration of 6×10^{-3} mg/mL) was dipped and inoculated on the whole leaves. Then the leaves were washed with water and dried. The compound solution was smeared on the left side and the solvent was smeared on the right side for control. The local lesion numbers were then recorded 3-4 days after inoculation [7]. For each compound, three repetitions were conducted to ensure the reliability of the results, which were measured according to the following formula:

$$\text{Inhibition rate (\%)} = \frac{\text{av local lesion numbers of control (not treated with compound)} - \text{av local lesion numbers smeared with drugs}}{\text{av local lesion numbers without drugs}}$$

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Sample Availability: Samples of the compounds are available from authors.