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Article

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Application of "hydrogen bonding interaction" in drug design Part Ⅱ: Design, synthesis, and SARs of thiophosphoramide derivatives as novel antiviral and antifungal agents

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ABSTRACT: Based on the structure of natural product harmine, lead compound **18** and the structure of compounds in part I, a series of thiophosphoramide derivatives **1–17** were designed and synthesized from various amines in one step. Their antiviral and antifungal activities were evaluated. Most of the compounds showed significantly higher antiviral activity against tobacco mosaic virus (TMV) than commercial virucide Ribavirin. Compound (*R,R*)-**17** showed the best anti-TMV activity in vitro (70%/500 μg/mL and 33%/100 μg/mL) and in vivo (inactivation effect: 68%/500 μg/mL and 30%/100 μg/mL; curative effect: 64%/500 μg/mL and 31%/100 μg/mL; protection effect: 66%/500 μg/mL and 31%/100 μg/mL) which is higher than that of Ningnanmycin and lead compound **18**. The antiviral activity of (*R,R*)-**17**·**HCl** is about similar to that of (*R,R*)-**17**. However, the antifungal activity of (*R,R*)-**17**·**HCl** against *Puccinia sorghi* is slightly lower than that of (*R,R*)-**17**. The systematic study provides compelling evidence that these simple thiophosphoramide compounds could become efficient antiviral and antifungal agents.

KEYWORDS: tobacco mosaic virus, thiophosphoramide derivatives, anti-TMV activity, *Puccinia sorghi*, antifungal activity, SARs

INTRODUCTION

Plant pathogens, including bacteria, fungi and viruses, result in substantial losses for agriculture, and are considered a threat for food security. Bacteria can infect all plant tissues during the whole plant growth cycle. More importantly, these bacteria have a strong vitality. The infective capacity of phytopathogenic fungi can be maintained over many seasons, so it is costly and difficult to for farmers control these fungal diseases.[1] In certain years, pathogenic fungi have reduced the yield of food and cash crops by nearly twenty percent. Tobacco mosaic virus (TMV) is a well-studied plant virus which can cause plant disease and creates enormous economic loss worldwide. There are about 36 plant families and more than 400 individual species which can be infected by TMV, including tobacco and some ornamental flowers. Since plants do not have the same immune system as animals, the control of plant diseases is still a challenge.[2]

Phosphoramides and their ester derivatives possess a wide range of biological activities, such as pesticidal activity, antiviral activity, herbicidal activity, and so on.[2–4] Several phosphoramides have become commercial varieties, such as acephate, cyclophosphamide, methamidophos, and so on.

Anti-plant virus agent research is not like pharmaceutical research. Only very few molecular targets are investigated and can be used in agrochemical design and discovery, [5] which increases the difficulty of discovery of antiviral molecules for plants. Hydrogen bonding interaction is an important mode of action for small molecule and biomacromolecule. In drug design and development, the applications of hydrogen

bonding take more and more attention.[6–8] Thiophosphoramide derivatives serve as catalysts through hydrogen bonding from the NH group.[9–13] Meanwhile, the TMV composing primarily with coat protein and RNA, contains many amino acid residues.[14, 15] If the thiophosphoramide derivatives can bind to the key residues of TMV CP or RNA, they may interfere with virus assembly initiation.

In our previous work, we have been devoted to synthesizing β-Carboline and tetrahydro-β-carboline alkaloids as well as their derivatives and researching their biological activity. Natural product harmine (**Figure 1**), one of the β-Carboline alkaloids isolated from P. *harmala* L., was found to have excellent anti-TMV activity (antiviral activity against TMV in vitro and inactivation, curative and protection activity in vivo were 45%, 41%, 39%, and 42%, respectively, at 500 μg mL^{-1}). Meanwhile, (1*S*,3*S*)-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carbohydrazide (**Figure 1**, Lead compound **18**), maintained high anti-TMV activity (the corresponding activity were 50%, 46%, 48%, and 50%, respectively, at 500 μg mL^{-1}). This compound also exhibited excellent stability and solubility.[16] These advantages made it an ideal lead compound for further derivation. A series of simple thiourea derivatives (**Figure 1**, Part I) were developed as a novel type antiviral agents based on the structure characteristics of harmine and lead compound **18**.[8]

Taking into account the above findings, another series of simple thiophosphoramide derivatives (**Figure 1**) were designed based on harmine, lead compound **18** and the structure of compounds in part I and synthesized as anti-TMV agents. The synthesized

compounds were characterized and systematically tested for their antiviral activity against TMV and antifungal activity against *Puccinia sorghi*.

MATERIALS AND METHODS

Instruments. The melting points of the synthesized compounds were determined on an X-4 binocular microscope (Gongyi Yuhua Instrument Co., China). NMR spectra were acquired via a Bruker 400 MHz (100 MHz for ^{13}C) instrument at room temperature. Chemical shifts were measured relative to residual solvent peaks of $CDCl_3$ (^1H: δ = 7.26 ppm; ^{13}C: δ = 77.0 ppm) or d_6-DMSO (^1H: δ = 2.50 ppm; ^{13}C: δ = 39.5 ppm) with tetramethylsilane as internal standards. HRMS data were obtained via an FT-ICR MS spectrometer (Ionspec, 7.0 T). Specific rotations were measured on a Perkin-Elmer 341MC polarimeter. Symbol (±) represented trans-mixture.

General Experimental Procedures

General Procedures for the Preparation of Compounds 1–3, 14 and (*S*,*S*)-17.

To a mixture of CH_2Cl_2 (10 mL), Et_3N (5.0 mmol) and amine (5.0 mmol) was added the corresponding diphenylphosphinothioic chloride (5.0 mmol or 0.5 mmol) in CH_2Cl_2 (10–30 mL), and stirred at room temperature. When the reaction was complete, the reaction mixture was filtered or evaporated in vacuum. The pure products **1**–**3**, **14** and **(*S*,*S*)-17** were obtained by recrystallization from diethyl ether or purified by chromatography on a column of silica gel with PE-EtOAc (v/v = 3: 1).

***N*-Butyl-*P*,*P*-diphenylphosphinothioic amide** (**1**): white solid, 99% yield; mp 90−91 °C; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3H), 1.31−1.41 (m, 2H), 1.52−1.59

(m, 2H), 2.23 (br. s, 1H), 2.92 (q, J = 7.2 Hz, 2H), 7.41−7.51 (m, 6H), 7.97−8.03 (m, 4H); ^{31}P NMR ($CDCl_3$, 161.7 MHz): δ 59.24; ^{13}C NMR ($CDCl_3$, 100.6 MHz): 13.8, 20.1, 33.6 (d, $J_{C\text{-}P}$ = 9.7 Hz), 128.5 (d, $J_{C\text{-}P}$ = 12.9 Hz), 131.5, 131.7, 133.4 (d, $J_{C\text{-}P}$ = 101.8 Hz); HRMS (ESI) *m/z* calc'd for $C_{16}H_{20}NPS$ $[M+H]^+$: 290.1127, found 290.1124.

***N*-Benzyl-*P*,*P*-diphenylphosphinothioic amide** (**2**): white solid, 99% yield; mp 108−109 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.64 (br. s, 1H), 4.09 (d, J = 7.6 Hz, 2H), 7.24−7.37 (m, 5H), 7.44−7.50 (m, 6H), 8.01−8.06 (m, 4H); ^{31}P NMR ($CDCl_3$, 161.7 MHz): δ 59.82; ^{13}C NMR ($CDCl_3$, 100.6 MHz): 45.2, 127.6, 128.1, 128.5, 128.7 (d, $J_{C\text{-}P}$ = 5.4 Hz), 131.7 (d, $J_{C\text{-}P}$ = 10.9 Hz), 131.9, 133.9 (d, $J_{C\text{-}P}$ = 101.8 Hz), 139.3 (d, $J_{C\text{-}P}$ = 28.8 Hz); HRMS (ESI) *m/z* calc'd for $C_{19}H_{18}NPS$ $[M+H]^+$: 324.0970, found 324.0967.

***N*-Cyclohexyl-*P*,*P*-diphenylphosphinothioic amide** (**3**): yellow solid, 99% yield; mp 77−80 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.09−1.29 (m, 5H), 1.52−1.66 (m, 3H), 2.03−2.06 (m, 2H), 2.27 (br. s, 1 H), 3.12 (s, 1H), 7.43−7.47 (m, 6H), 7.97−8.02 (m, 4H); ^{31}P NMR ($CDCl_3$, 161.7 MHz): δ 56.88; ^{13}C NMR ($CDCl_3$, 100.6 MHz): 25.2, 25.5, 36.1 (d, $J_{C\text{-}P}$ = 4.9 Hz), 51.1, 128.4 (d, $J_{C\text{-}P}$ = 12.9 Hz), 131.5, 131.6, 135.1 (d, $J_{C\text{-}P}$ = 101.9 Hz); HRMS (ESI) *m/z* calc'd for $C_{18}H_{22}NPS$ $[M+H]^+$: 316.1283, found 316.1285.

***N*-(2-Aminoethyl)-*P*,*P*-diphenylphosphinothioic amide** (**14**): yellow solid, 95% yield; mp 94−96 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.62 (s, 2H), 1.69 (br. s, 2H), 2.84−2.88 (m, 2H), 2.93−2.98 (m, 2H), 3.16 (br. s, 1 H), 7.42−4.07 (m, 6H), 7.96−8.00 (m, 4H); ^{31}P NMR ($CDCl_3$, 161.7 MHz): δ 59.78; ^{13}C NMR ($CDCl_3$, 100.6 MHz): 42.4 (d, $J_{C\text{-}P}$ = 8.4 Hz), 43.6, 128.5 (d, $J_{C\text{-}P}$ = 12.7 Hz), 131.6 (d, $J_{C\text{-}P}$ = 11.1 Hz), 131.7, 134.2 (d, $J_{C\text{-}P}$ =

102.0 Hz); HRMS (ESI) *m/z* calc'd for $C_{14}H_{17}N_2PS$ $[M+H]^+$: 277.0923, found 277.0922.

***N*-((*1S*,*2S*)-2-Amino-1,2-diphenylethyl)-*P*,*P*-diphenylphosphinothioic amide ((*S,S*)-17)**, 80% yield, $[\alpha]_D^{24}$ +21.7 (*c* 1.0, $CHCl_3$), m.p. 103−105 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.60 (s, 2 H), 4.21 (t, *J* = 7.6 Hz, 1 H), 4.27 (d, *J* = 5.2 Hz, 1 H), 4.49−4.54 (m, 1 H), 7.11−7.18 (m, 5 H), 7.21−7.41 (m, 11 H), 7.62−7.70 (m, 4 H); ^{31}P NMR ($CDCl_3$, 161.7 MHz): δ 58.98; ^{13}C NMR ($CDCl_3$, 100.6 MHz): 61.2 (d, *J* = 7.4 Hz), 61.7, 126.9, 127.1, 127.3, 127.4, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 131.0, 131.1, 131.2, 131.3, 131.7, 131.8, 133.5, 134.5, 134.7, 140.9, 142.5; HRMS (ESI) *m/z* calc'd for $C_{26}H_{25}N_2PS$ $[M+H]^+$: 429.1549, found 429.1547.

Procedures for the Preparation of Compound (*R,R*)-17·HCl

To a solution of 1.0 mL of HCl in ethyl acetate (2 M) and 10 mL of CH_2Cl_2 was added 1.0 mmol of (*R,R*)-**17**[11] at rt. The mixture was vigorously stirred for 6 h, and then filtered to obtain the compound (*R,R*)-**17·HCl**.

***N*-((*1R*,*2R*)-2-Amino-1,2-diphenylethyl)-*P*,*P*-diphenylphosphinothioic amide hydrochloride ((*R,R*)-17·HCl)**: white solid, 99% yield; mp 155–158 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.77 (br. s, 1 H), 4.97−5.04 (m, 1 H), 6.88 (t, *J* = 8.0 Hz, 1 H), 6.97−7.11 (m, 5 H), 7.23−7.34 (m, 8 H), 7.43−7.45 (m, 2 H), 7.50−7.60 (m, 3 H), 7.74−7.79 (m, 2 H), 8.76 (s, 3 H); ^{31}P NMR ($CDCl_3$, 161.7 MHz): δ 57.61; ^{13}C NMR ($CDCl_3$, 100.6 MHz): 59.3, 59.4 (d, *J* = 19.7 Hz), 127.0, 127.5, 127.6, 127.7, 128.0, 128.03, 128.11, 128.17, 128.26, 128.31, 130.8, 130.9, 131.0, 134.8, 135.0, 135.3, 135.8, 136.0, 139.1; HRMS (ESI) *m/z* calc'd for $C_{26}H_{26}ClN_2PS$ $[M+H-HCl]^+$: 429.1549, found

429.1548.

Biological Assay.

The antiviral and antifungal bioassays were performed via representative test organisms reared in the laboratory. Percentage mortalities were evaluated according to a percentage scale of 0−100, in which 0 indicates no activity and 100 indicates total kill.

Antiviral Biological Assay. The bioassays of in vitro and in vivo anti-TMV activity were carried out on *Nicotiana tabacum* L. The detailed procedure of purifying TMV and the method to test the anti-TMV activity of the synthesized compounds were the same with the literature.[17]

Antifungal Biological Assay. The detailed assay method to test the antifungal activity of the synthesized compounds was described in the literature.[18]

RESULTS AND DISCUSSION

Chemistry.

Compounds **1**−**3** , **14** and (*S,S*)**-17** were synthesized according to procedures in **Figure 2**. Amines were reacted with corresponding phosphinothioic chloride[19] to give thiophosphoramides **1**−**3**, **14** and (*S,S*)**-17** in almost quantitative to moderate yields. Compounds **4**–**13**, **15**, **16** and (*R,R*)**-17** (**Figure 3**) were obtained according to our previously reported methods. [9–12] Compound **17**·**HCl** was obtained in the presence of HCl in good yields.

Phytotoxic Activity. Firstly, compounds **1**−**17** were measured for their phytotoxic activity against tobacco.[17] The data of phytotoxic activity at 500 μg/mL indicated that all

of the compounds **1**−**17** showed no toxicity to the tested plant.

Antiviral Activity. Compounds **1**−**17** were evaluated for their antiviral activity against TMV. The commercial plant virucides Ningnanmycin, Ribavirin and lead compound **18** were used as the controls.

In Vitro Anti-TMV Activity. The results of antiviral assay in vitro were shown in Table 1. As the control, Ribavirin exhibited a 41% inhibitory effect at 500 μg/mL, whereas almost all of the compounds **1**−**17** exhibited higher antiviral activity than Ribavirin even at the concentration of 100 μg/mL. The optimal compound (*R,R*)-**17** exhibited significantly higher inhibitory effect than Ningnanmycin, which had a good chance of becoming a new lead compound for anti-TMV study. The ethylenediamine skeleton displayed important role for keeping high antiviral activity. Thiophosphoramides **1**−**3** without ethylenediamine skeleton exhibited relatively lower inhibitory effect than compounds **4**−**17** except for compound **14** (inhibitory effect: **3** ≈ **14**). Among the compounds **7**−**10**, thiophosphoramide **9** displayed the lowest inhibitory effect, which indicated that alkyl phosphate is bad for antiviral activity. The mainly difference between **11** and **12** lies in the substituent on the N atom. Thiophosphoramide **11** with stronger hydrogen donor (three N-H bond) showed higher inhibitory effect (inhibitory effect: **11** > **12**). Thiophosphoramide **11** displayed higher antiviral activity than **13**, which indicated that the S atom is favorable.

Diphenylthiophosphoramide derivatives **4**, **11**, **14**−**17** with a primary amine group displayed in vitro activity ranging from 33%−70% against TMV at 500 μg/mL. Amongst

166 them, (*R*,*R*)-**17** bearing 1,2- diphenylethylenediamine skeleton showed higher activity
167 than **11** containing 1,2-cyclohexanediamine (70% and 62%, respectively). Except for
168 compound **14,** all of primary amine-thiophosphoramide **7**−**17** exhibited much higher
169 activity than Ribavirin. The above results provided compelling evidence that a primary
170 amine on a rigid scaffold is necessary in thiophosphoramide molecule.

171 Compounds **17** exhibited best result with inhibitory rate of >60%. The optical pure
172 compounds **17** were prepared to evaluate the effect of configuration on antiviral activity.
173 (*R*,*R*)-Configuration was proven to be the optimal antiviral configuration in vitro
174 (inhibitory effect: (*R*,*R*)-**17** > (*S*,*S*)-**17**). However, the activity of (*S*,*S*)-**17** is about similar
175 to that of the mixture of (*R*,*R*)-**17** and (*S*,*S*)-**17** ((*R*,*R*)-**17**:(*S*,*S*)-**17** = 1:1). In order to
176 improve stability and water solubility, (*R*,*R*)-**17**·**HCl** was synthesized in the presence of
177 HCl. However, the result indicated that (*R*,*R*)-**17**·**HCl** exhibited about similar in vitro
178 activity with (*R*,*R*)-**17**.

179 Bioassay results of the compounds **1**−**17** were shown in Table 1. Almost all of primary
180 amine-thiophosphoramide displayed excellent anti-TMV activity in vitro. Encouraged by
181 these results, the compounds **1**−**17** were tested to evaluate their antiviral activity in vivo.

182 *In Vivo Anti-TMV Activity.* The results of anti-TMV activity in vivo were shown in
183 Table 2. Most of the compounds exhibited higher in vivo activity than that of the
184 Ribavirin. At the concentration of 500 μg/mL, (*R*,*R*)-**17** displayed the best inhibitory
185 effect of inactivation activity, curative activity and protection activity with values of 68%,
186 64% and 66%, respectively. Ribavirin as a control was studied at the same conditions

with values of 37%, 36% and 39%, respectively. By contrasting the experimental data, the results indicated that compound (*R*,*R*)-**17** was more efficient than Ribavirin in vivo activity against TMV. Under the same test conditions, Ningnanmycin as a control was also studied with values of 63%, 54% and 64%, respectively. At the concentration of 100 μg/mL, (*R*,*R*)-**17** displayed moderate activity (inactivation activity: 30%; curative activity: 31%; and protection activity: 31%), which is obviously higher than that of Ribavirin (inactivation activity: 9%; curative activity: 13%; and protection activity: 17%) and Ningnanmycin (inactivation activity: 28%; curative activity: 22%; and protection activity: 26%). (*R*,*R*)-**17**·**HCl** showed about similar in vivo anti-TMV activity with (*R*,*R*)-**17**.

The in vivo antivral activity trend is similar with in vitro. Thiophosphoramide **11** bearing cyclohexanediamine skeleton gave relatively higher activity (64%, 60%, and 65% at 500 μg/mL) than **1**−**10** and **12**−**13**. Primary amine-thiophosphoramide **14**−**17** showed moderate antiviral activity ranging from 38%−71% against TMV at 500 μg/mL (the antiviral activity in vitro: **17** >**16** > **15** > **14**). *N*-(2-aminoethyl)-*P*,*P*-diphenylphosphinothioic amide (**14**) showed relatively lower activity (inactivation activity: 44%/500 μg/mL and 11%/100 μg/mL; curative activity: 40%/500 μg/mL and 14%/100 μg/mL; and protection activity: 38%/500 μg/mL and 0%/100 μg/mL) than corresponding primary amine-thiophosphoramides and higher than **1**−**3**, which indicated that a primary amine on a rigid scaffold is necessary in thiophosphoramide molecule for maintaining high antiviral activity.

(*R*,*R*)-Configuration with higher activity than that of (*S*,*S*)-configuration is confirmed to be the optimal configuration (inhibitory effect: (*R*,*R*)-**17** > (*S*,*S*)-**17**). (*S*,*S*)-**17** showed relatively lower activity (inactivation activity: 63%/500 μg/mL and 30%/100 μg/mL; curative activity: 61%/500 μg/mL and 20%/100 μg/mL; and protection activity: 65%/500 μg/mL and 26%/100 μg/mL) than (*R*,*R*)-**17**.

In Vivo Fungicidal Activity. All the target compounds **1**−**17** were tested for their fungicidal activity. *Puccinia sorghi* which causes great damage to corn was chosen as the test object. Table 3 showed the results of inhibitory effect at different concentration against *Puccinia sorghi* on corn in vivo. Compounds **1** and **17** (inhibitory effect at 200 mg/kg: ≥ 85%) exhibited higher fungicidal activity against *Puccinia sorghi* on corn than compounds **2**−**16**, thus emerged as new antifungal lead compounds. Compound (*R*,*R*)-**17** exhibited the best fungicidal activity with 85% inhibitory effect at 100 mg/kg.

In summary, based on harmine, lead compound **18** and compounds in part I, a series of simple thiophosphoramide compounds **1**−**17** were designed and synthesized from amines in one step. Most of the compounds showed higher anti-TMV activity than commercial virucide Ribavirin. Compound (*R,R*)-**17** displayed the best anti-TMV activity in vitro and in vivo (in vitro activity: 70%/500 μg/mL and 33%/100 μg/mL; inactivation activity: 68%/500 μg/mL and 30%/100 μg/mL; curative activity: 64%/500 μg/mL and 31%/100 μg/mL; protection activity: 66%/500 μg/mL and 31%/100 μg/mL) which is higher than that of Ningnanmycin and lead compound **18**. The antiviral activity of (*R,R*)-**17**·**HCl** is about similar to that of (*R,R*)-**17**. However, the antifungal activity of (*R,R*)-**17**·**HCl**

against *Puccinia sorghi* was slightly lower than that of (*R,R*)-**17**. Present studies on antiviral and antifungal activity provide abundant support for further optimization of these simple thiophosphoramide derivatives as new potential inhibitors of plant virus. Further studies of these thiophosphoramide derivatives on structure- activity relationship and mode of action are in process in our laboratories.

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**−**3**, **14** and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Figure Captions

Figure 1. Design of Thiophosphoramide Derivatives **1**−**17**

Figure 2. Synthesis of Thiophosphoramide Derivatives **1**–**3**, **14** and (*S,S*)**-17**

Figure 3. Chemical Structures of **4**–**13**, **15**, **16**, (*R,R*)**-17** and (*R,R*)**-17**·HCl

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336 Table 1. In Vitro Antiviral Activity of Compounds Ribavirin, Ningnanmycin and **1**−**17**

337 against TMV

Compd	Concn (μg/mL)	Inhibition rate (%)[a]	compd	Concn (μg/mL)	Inhibition rate (%)[a]
1	500	27±1	**13**	500	50±2
	100	0		100	16±1
2	500	31±2	**14**	500	33±1
	100	0		100	0
3	500	34±1	**15**	500	55±1
	100	0		100	27±1
4	500	55±2	**16**	500	49±2
	100	24±1		100	0
5	500	50±1	(*R*,*R*)-**17**	**500**	**70±1**
	100	24±1		**100**	**33±2**
6	500	55±2	(*S*,*S*)-**17**	500	62±2
	100	20±1		100	24±1
7	500	63±1	(*R*,*R*)-**17**:	500	60±2
	100	28±2	(*S*,*S*)-**17** = **1**: **1**	100	25±1
8	500	60±2	(*R*,*R*)-**17**·**HCl**	**500**	**72±2**
	100	32±2		**100**	**36±1**
9	500	47±1	**Lead**	**500**	**50±2**
	100	15±1	**compound 18**	**100**	**21±1**
10	500	58±2	**Harmine**	**500**	**45±2**
	100	28±1		**100**	**20±1**
11	500	62±2	**Ribavirin**	**500**	**41±2**
	100	32±2		**100**	**10±1**
12	500	50±1	**Ningnanmycin**	**500**	**61±1**
	100	22±2		**100**	**26±1**

338 [a]*Average of three replicates; All results are expressed as mean ± SD.*

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343 Table 2. In Vivo Antiviral Activity of Compounds Ribavirin, Ningnanmycin and **1**−**17**

344 against TMV

Compd	Concn (μg/mL)	Inactivation effect (%)[a]	Curative effect (%)[a]	Protection effect (%)[a]	Compd	Concn (μg/mL)	Inactivation effect (%)[a]	Curative effect (%)[a]	Protection effect (%)[a]
1	500	22±1	31±2	36±1	**13**	500	56±2	53±1	49±2
	100	0	0	0		100	25±1	20±1	12±1
2	500	35±1	28±3	37±1	**14**	500	44±2	40±2	38±1
	100	0	0	0		100	11±2	14±2	0
3	500	26±2	32±1	22±2	**15**	500	52±1	53±1	46±1
	100	0	0	0		100	17±2	17±2	20±2
4	500	56±1	54±2	54±1	**16**	500	49±1	43±1	44±2
	100	25±1	27±1	27±2		100	13±2	19±2	15±2
5	500	56±1	54±3	58±1	(*R*,*R*)-**17**	500	**68±2**	**64±2**	**66±1**
	100	15±1	26±1	27±1		100	**30±2**	**31±2**	**31±2**
6	500	58±2	52±1	54±2	(*S*,*S*)-**17**	500	63±2	61±1	65±2
	100	28±1	19±1	22±1		100	30±1	20±2	26±1
7	500	61±2	59±2	62±1	(*R*,*R*)-**17**:	500	64±2	60±1	65±2
	100	21±1	30±1	25±1	(*S*,*S*)-**17** = **1**: **1**	100	32±1	29±2	34±1
8	500	**64**±1	**62**±1	**58**±1	(*R*,*R*)-**17**·**HCl**	**500**	**71±2**	**68±1**	**67±1**
	100	22±2	26±2	21±1		**100**	**39±1**	**35±2**	**30±2**
9	500	52±1	51±1	53±2	**Lead**	500	**46±2**	**48±2**	**50±1**
	100	23±1	20±1	23±1	**compound 18**	100	**17±1**	**20±2**	**23±1**
10	500	62±2	59±1	57±2	**Harmine**	500	**41±1**	**39±1**	**42±2**
	100	21±2	24±1	21±1		100	**11±1**	**16±2**	**16±1**
11	500	64±1	60±2	65±1	**Ribavirin**	500	37±2	36±2	39±1
	100	31±1	20±1	26±1		100	9±1	13±2	17±1
12	500	55±1	53±3	47±1	**Ningnanmycin**	500	63±2	54±1	64±2
	100	23±1	16±1	18±1		100	28±1	22±1	26±1

345 [a] *Average of three replicates; All results are expressed as mean ± SD.*

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351 Table 3. In Vivo Fungicidal Activity of the Selected Compounds against *Puccinia sorghi*

352 on corn

compd	Inhibition rate[a](%)		
Concn (mg/kg)	200	100	50
1	85±1	75±2	20±2
2	70±2	NT	NT
3	40±3	NT	NT
4	50±1	NT	NT
5	45±2	NT	NT
6	40±3	NT	NT
7	30±1	NT	NT
8	35±2	NT	NT
9	30±3	NT	NT
10	20±1	NT	NT
11	50±2	NT	NT
12	60±2	NT	NT
13	40±3	NT	NT
14	20±1	NT	NT
15	30±3	NT	NT
16	10±1	NT	NT
(*R*,*R*)**-17**	98±2	85±1	30±2
(*S*,*S*)**-17**	95±1	70±2	20±1
(*R*,*R*)**-17**·**HCl**	98±2	60±2	10±1
Azoxystrobin	100±1	100±1	99±1

353 [a] *Average of three replicates; All results are expressed as mean ± SD.*

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Figure 1.

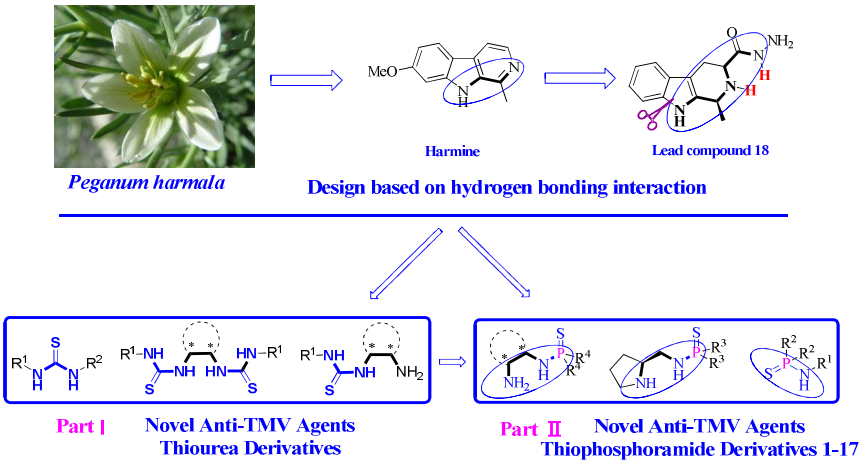


Figure 2.

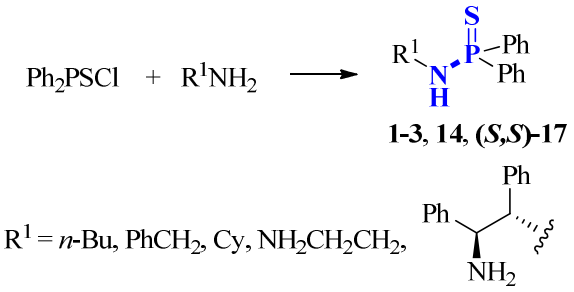
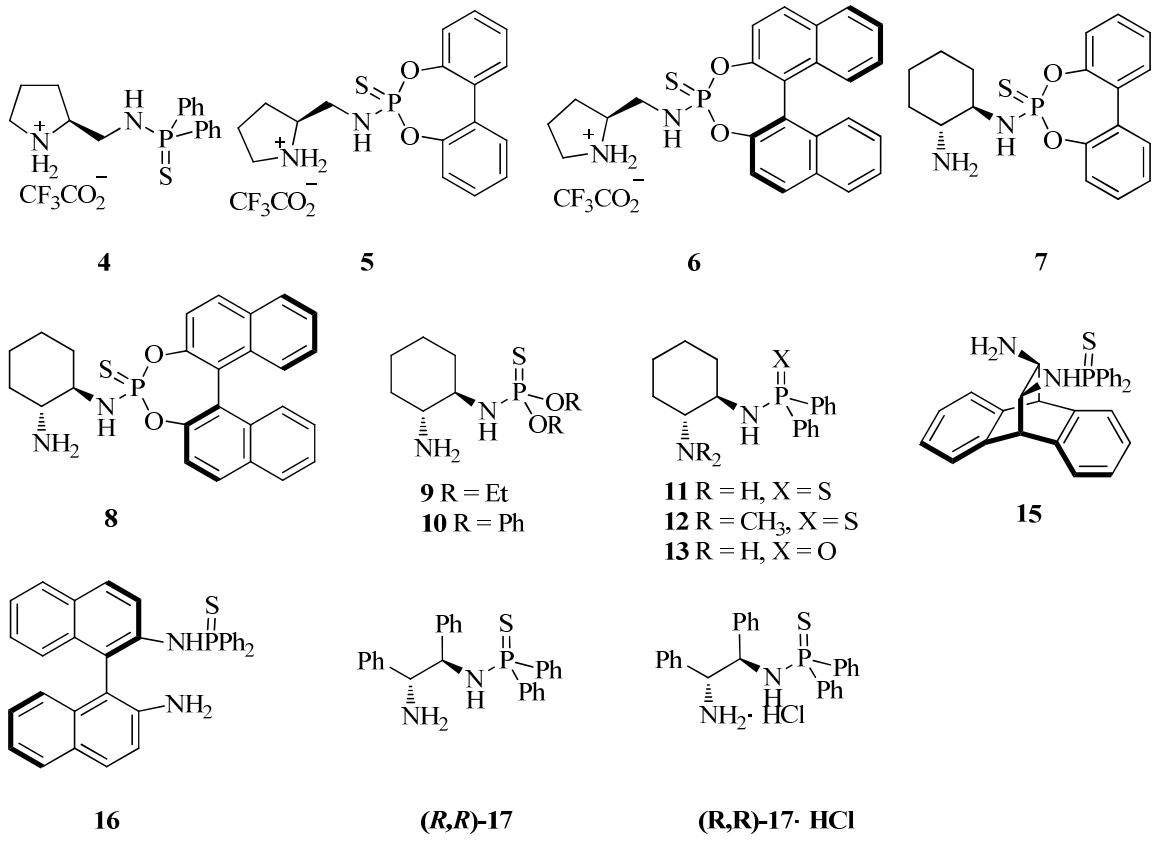


Figure 3.



TOC ***graphic***

Agrochemical Bioregulators