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Design, Synthesis, Characterization, and Biological Activities of Novel Spirooxindoles Analogues Containing Hydantoin, Thiohydantoin, Urea, and Thiourea Moieties

Linwei Chen,[1] Yanke Hao,[1] Hongjian Song,[1*] Yuxiu Liu,[1] Yongqiang Li,[1] Jingjing Zhang,[1,2*] and Qingmin Wang[1*]

[1]State Key Laboratory of Elemento-Organic Chemistry, College of Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, People's Republic of China

[2]Tianjin Agricultural University, Tianjin 300384, People's Republic of China

*To whom correspondence should be addressed. Tel: +86-(0)22-23503952; Fax:

19 +86-(0)22-23503952; E-mail: songhongjian@nankai.edu.cn; Email:

20 chemzhangjingjing@163.com; wangqm@nankai.edu.cn.

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ABSTRACT

Based on the scaffolds widely used in drug design, a series of novel spirooxindole derivatives containing hydantoin, thiohydantoin, urea, and thiourea moieties have been designed, synthesized, characterized, and first evaluated for their biological activities. The diastereoselectivity mechanism is proposed and the systematic conformational analysis is performed. The bioassays results show that the target compounds possess moderate to good antiviral activities against tobacco mosaic virus (TMV), among which compound **22** shows the highest antiviral activity *in vitro* as well as inactivation, curative, and protection activities in vivo (45±1, 47±3, 50±1, and 51±1%, 500 mg/L, respectively), higher than ribavirin (38±1, 36±1, 38±1, and 36±1%, 500 mg/L, respectively). Thus, compound **22** is a promising candidate for anti-TMV development. Most of these compounds show broad-spectrum fungicidal activities against 14 kinds of phytopathogenic fungi and selectively fungicidal activities against *Physalospora piricola*, *Sclerotinia sclerotiorum*, and *Rhizoctonia cerealis*. Additionally, some of these compounds exhibit insecticidal activity against *Culex pipiens pallens*, *Mythimna separata*, *Helicoverpa armigera*, and *Pyrausta nubilalis*. Compound **17** exhibits the highest larvicidal activity (LC_{50} was 0.32 mg/L) against *Culex pipiens pallens*.

KEYWORDS: spirooxindoles, hydantoin, thiohydantoin, urea, thiourea, characterization, antiviral activity, fungicidal activity, insecticidal activity

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INTRODUCTION

Natural products have proven to be an important source of novel compounds for drug discovery.[1,2] They posses many of the properties that make a good drug candidates, including structural diversity, specificity, and novel modes of action.[3] However, natural products also have some disadvantages such as high structural complexity, limited compound availability, and poor drug-likeness.[4] Structure modification on the core skeletons of natural products is a powerful method for overcoming these limitations and improving the efficiency of drug discovery.[5]

Spirooxindoles are a core skeleton widely existing in natural products and drugs (Figure 1). In recent years, more and more spirooxindole natural products and their derivatives have been reported to have new drug development value, and their application prospect has attracted wide attention.[6] However, these research are concentrated in the field of pharmaceuticals, few research have been done in pesticides.[7] β-Carbolines and spirooxindole alkaloids share a same biogenic precursor−tryptophan.[8, 9] In previous work, we found that β-carboline alkaloids and tryptophan showed antiviral, fungicidal, and insecticidal activities.[10, 11] So, we wonder whether spirooxindole derivatives also have similar pesticide activities.

Hydantoin, thiohydantoin, urea and thiourea are important privileged scaffolds that widely exist in natural products, medicines, and pesticides (Figure 1).[12] Part of these natural products and their derivatives exhibit wide spread biological activities, which have not only been used as antitumor,[13] anti-HIV,[14] antiarrhythmic,[15]

antiepileptic,[16] and anxiolytic agents[17] in medicinal chemistry but also been used as pesticides.[11, 18] Previous works has demonstrated that the introduction of these structures is beneficial for improving the biological activities of compounds containing these scaffolds.[19] These structures contain multiple hydrogen bond donors and receptors, which increase the probability or intensity of the interactions between small organic molecules and target proteins.[20] The inclusion of these structures into spirooxindoles would increase the molecular hydrogen bonding sites thus might improve their bioactivities.

Based on the above points, a series of spirooxindole derivatives containing hydantoin, thiohydantoin, urea, and thiourea moieties were designed, synthesized, characterized and the diastereoselectivity mechanism were studied in detail. Their anti-TMV, fungicidal, and insecticidal activities were evaluated for the first time . The structure-activity relationship study of these new spirooxindole derivatives was also discussed (Figure 2).

MATERIALS AND METHODS

Instruments

^{1}H, ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 400 Ultrashield NMR spectrometers. Chemical shifts (δ) were given in parts per million (ppm) and were measured downfield from internal tetramethylsilane. High-resolution mass spectrometry (HRMS) data were obtained on an FTICR-MS instrument with a q-TOF mass analyzer (Ionspec 7.0 T). Single-crystal X-ray

structure data were collected on a Rigaku Saturn 70. The melting points were determined on an X-4 microscope melting point apparatus and are uncorrected. Conversion was monitored by thin layer chromatography (TLC). Flash column chromatography was performed over silica gel (200-300 mesh).

General Synthesis

Ribavirin (Topscience Co., Ltd.), chlorothalonil (Bailing Agrochemical Co.,Ltd) and other reagents were purchased from commercial sources and were used as received. All anhydrous solvents were dried and purified according to standard techniques just before use. The synthetic routes are given in Scheme 1.

The operating steps and physical data in detail of intermediates **A−E** and target compounds **1−27** can be found in the Supporting Information.

Biological Assay

Each bioassay was repeated three times at 25 ± 1 °C. Activity results were estimated according to a percentage scale of 0-100 (0, no activity; 100, total kill). The anti-TMV,[21] fungicidal,[22] and insecticidal activities[23] of the synthesized compounds were tested using previously reported methods, The results are presented as means ± standard errors. The detail biological assay methods were given in Supporting Information.

RESULTS AND DISCUSSION

Synthesis

106 The synthesis of the key intermediate spirooxindole **E** is depicted in Scheme 1.
107 Using L-tryptophan as starting material, the tetrahydrocarboline carboxylic acid **A**
108 was constructed via Pictet−Spengler cyclization, which then reacted with thionyl
109 chloride and methanol to form methyl ester **B**. The obtained methyl ester **B** was
110 subsequently reacted with $(Boc)_2O$ to afford compound **C**. The spirooxindole **D** was
111 obtained via the spirocyclization reaction in the presence of *N*-bromosuccinimide
112 (NBS) and glacial acetic acid. It is worth mentioning that the cyclization reaction is
113 stereoselective. The NMR (nuclear magnetic resonance) spectra showed that the **E**
114 was a single isomer, indicating that the newly formed quaternary carbon was
115 chiral-specific. The possible mechanism is shown in Figure S7. In molecule **C**, the
116 ester group is axially oriented,[24] it overlaps the π orbital during the bromination step.
117 The intermediate **H** is formed with the partial charge of Br^+ distribute to ester oxygen
118 atom, which leads to the formation of *cis*-3-bromo-indolenine **I**. The following
119 addition of water to the imine group requires a before hand conformational change of
120 ring C (**J**). The stereospecific *cis*-bromohydrin (**K**) has perfectly aligned bonds for the
121 subsequent pinacol-type rearrangement that offers the observed product **D**. The
122 spirooxindole **E** is formed from the spirooxindole **D** by deprotection. Compounds
123 **1-15** containing the thiohydantoin fragment can be synthesized from the spirooxindole
124 **E** with the corresponding isothiocyanates and triethylamine (Scheme 1). In order to
125 explore the effects of substituent at 5′-position and chirality at 3- and 7a'-positions on
126 anti-TMV activities, **16** and **17** containing thiohydantoin were synthesized from L-and
127 D-tryptophan, respectively. L-and D-tryptophan went through similar reactions to

form compounds **F** and **G**,[7] respectively. Then, they reacted with phenyl isothiocyanates to form **16** and **17** (Scheme 1). Spirooxindole analogues containing hydantoin (**18-20**) were obtained by reacting **E** with the corresponding isocyanates (Scheme 1). When **E** reacted with *tert*-butyl isothiocyanates or some isocyanates, only uncyclized thiourea derivative **21** or urea derivatives **22-27** were obtained (Scheme 1). The single crystal structure of compound **6** further confirmed the chiral configuration of quaternary carbon (Figure S6 and Table S1).[25]

Configuration.

It is interesting that both thiohydantoin (**1-15**) and hydantoin (**18-20**) derivatives exhibite two sets of peaks in NMR spectra (Figure S8). However, there is only one set of peaks in the NMR spectra of thiourea (**19**), and urea (**22-27**) derivatives (Figure S9). Since the chirality of the quaternary carbon and the chirality of carbon which connected to the methyl formate is determined, both factors can be ruled out. Therefore, the only factor orients the charity of nitrogen. As we know, the lone pair occupies one sp^3 orbital of nitrogen atom. In the case of thiourea (**19**) and urea (**22-27**) derivatives, the barrier to pyramidal inversion of nitrogen is not high, and the rate of inversion in solution is too fast to be measured by NMR spectroscopy. Therefore, there is only one set of peaks for thiourea (**19**) and urea (**22-27**) derivatives (Figure S10).[26] When the fused ring has been produced, such as thiohydantoin (**1-15**) and hydantoin (**18-20**) derivatives, nitrogen atom inversion is restricted due to the high inversion barriers, thus a pair of invertomers are generated resulting in two set of peaks in NMR spectra (Figure S10).[27] When there is a methyl substitution in the

5'-position, the cyclization reaction is stereospecific due to steric hindrance, resulting in a single chiral nitrogen (**16-17**).

Antiviral Activities.

In general, most of these spirooxindole analogues exhibit higher anti-TMV activities in *vitro* and inactivation, curative, and protection effects in *vivo* than compound **E** (Table 1), which suggests that the introduction of these privileged scaffolds is indeed beneficial to antiviral activities. Part of the compounds, such as compounds **4**, **16**, and **22**, show significantly higher activities than that of commercialized anti-plant virus agent ribavirin (38±1, 36±1, 38±1, and 36±1%, 500 mg/L).

For derivatives containing thiohydantoin moiety (**1-15**): When the substituent on the *N* of thiohydantoin is substituted phenyl (**1-4**), the electronic effect of the substituents on the phenyl has effect on the anti-TMV activities. The electron-donating substituent is beneficial to improving anti-TMV activities compared with electron-withdrawing substituent. For example, the structure-activity relationship shows as follows: *o*-methyl (**4**) > non-substituent (**1**) > *m*-chloro (**2**) ≈ *p*-chloro (**3**). The activities decrease obviously when the phenyl is changed to aliphatic substituents (**5-8**). The size of cyclic substituents also has an effect on the activities, expressed as the compound containing cyclopentyl (**6**) exhibits lower activities than those of cyclohexyl (**5**). But the chain length of the substituents has little effect on activities (**7** and **8**). It is adverse to the anti-TMV activities when phenyl is changed to benzyl

(**9-15**). The activities of derivative with an electron-donating substituent on the benzyl ring (**15**) are significantly better than that with electron-withdrawing substituents (**10-14**). For example, the activities of derivative with a *p*-methoxyl substituent (**15**, 35±1, 31±1, 28±2, and 29±1%, 500 mg/L) are higher than that with a *p*-chloro substituent (**11**, 0, 6±1, 0, and 0, 500 mg/L). The position of the substituents on the benzyl also affect the activities, the structure-activity relationship shows as follows: *m*-chloro substituent (**12**) ≈ *o*-chloro substituent (**13**) > dichloro substituent (**14**) > *p*-chloro substituent (**11**). When the α position of *N* (5′ position) is substituted by a methyl, compound **16** (44±1, 44±2, 48±1, and 50±1%, 500 mg/L) exhibits higher activities than that of unsubstituted one (**1**), even higher than that of ribavirin. Chiral has important influence on the anti-TMV activities, the activities of isomer **16** are significantly better than that of isomer **17**. Compounds containing hydantoin moiety (**18-20**) exhibits similar activities to the corresponding compounds containing thiohydantoin moiety.

For the derivatives containing urea moiety, they have a similar structure-activity relationship to derivatives containing thiohydantoin moiety: the activities of the compound containing aromatic substituent on *N* (**22**) are better than that of compounds containing aliphatic substituents (**21**, **23-27**). Compound **22** shows the highest activities (45±1, 47±3, 50±1, and 51±1%, 500 mg/L), which is significantly higher than that of ribavirin (Figure S11). This compound (**22**) can be used as an anti-TMV candidate for further study.

Fungicidal Activities

In general, these compounds have a broad spectrum of fungicidal activities against 14 kinds of phytopathogenic fungi (Table 2). Most of the derivatives exhibit better fungicidal activities than compound **E**. Almost all these compounds show fungicidal activities selectively against *Physalospora piricola*, *Sclerotinia sclerotiorum*, and *Rhizoctonia cerealis*. Among them, compounds **1**, **8**, and **12** show more than 60% fungicidal activities against 6 fungi. In particular, compound **21** shows more broad-spectrum fungicidal activities, with more than 60% fungicidal activities against 9 fungi. Compounds **8**, **18**, **20**, and **22** exhibit more than 90% against *Physalospora piricola* at 50 mg/L, compounds **6** and **20** show 90% and 96% fungicidal activities, respectively, against *Sclerotinia sclerotiorum* at 50 mg/L.

Insecticidal Activities

Most of these derivatives show insecticidal activities against *lepidoptera* pests such as *Mythimna separata*, *Helicoverpa armigera*, and *Pyrausta nubilalis* (Table 3). All compounds show larvicidal activity against mosquito (*Culex pipiens pallens*), and most of these derivatives show higher larvicidal activity against mosquito than that of compound **E**. Thiohydantoin, thiourea, and urea compounds exhibit higher activities than those of hydantoin compounds (Table 3). Chiral also has an important influence on the mosquito larvicidal activity, thus the activity of isomer **17** is significantly better than that of isomer **16**. Compound **17** shows 40% inhibitory effect against *Culex pipiens pallens* at 0.5 mg/L, and its LC_{50} against *Culex pipiens pallens* is 0.32 mg/L (Table S2), whereas, compound **16** exhibits only 40% mosquito larvicidal activity at 5 mg/L.

In summary, a series of novel spirooxindole derivatives containing hydantoin, thiohydantoin, urea, and thiourea moieties were designed and synthesized based on the widely used privileged scaffolds in drug design. Firstly, we proposed a diastereoselectivity mechanism and analyzed the conformation. Then, we systematically studied the biological activity of the new compounds. The bioassays results showed that the target compounds possessed moderate to good activities against tobacco mosaic virus (TMV), among which compound **22** showed the highest antiviral activity both in vitro and in vivo. At the same time, most of these compounds exhibited broad-spectrum fungicidal activities against 14 kinds of phytopathogenic fungi and selectively fungicidal activities against *Physalospora piricola*, *Sclerotinia sclerotiorum*, and *Rhizoctonia cerealis*. Additionally, some of these compounds exhibited insecticidal activity to *Culex pipiens pallens*, *Mythimna separata*, *Helicoverpa armigera*, and *Pyrausta nubilalis*. Further investigations on structural optimization and mode of action are in progress in our laboratory.

ASSOCIATED CONTENT

Supporting Information.

The operating steps and physical data in detail of intermediates **A**−**E** and target compounds **1**−**27**. Crystal data and structure refinement of **6** (Table S1 and Figure S6). The diastereoselectivity mechanism (Figure S7). Copies of ^{1}H NMR of **15** and **27** (Figure S8 and Figure S9). Explanation of the cause of the difference in NMR spectra (Figure S10). Detailed bioassay methods for anti-TMV, fungicidal, and insecticidal

236 activities. Pictures of inactivation of **22**, ribavirin, and blank control against TMV
237 (Figure S11). LC_{50} value of compound **17** against *C. pipiens pallens* (Table S2). This
238 material is available free of charge via the Internet at http://pubs.acs.org.

239 **AUTHOR INFORMATION**

240 **Corresponding Authors**

241 *Tel: +86-(0)22-23503952. Fax: +86-(0)22-23503952.

242 E-mail: songhongjian@nankai.edu.cn (H. J. S.); chemzhangjingjing@163.com (J. J.
243 Z.); wangqm@nankai.edu.cn (Q. M. W).

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249 **Notes**

250 The authors declare no competing financial interest.

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332 These data can be obtained free of charge from The Cambridge Crystallographic Data
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342 **Figure 1**. Natural products and drugs containing spirooxindole, thiohydantoin,
343 hydantoin, thiourea, and urea scaffolds.

344 **Figure 2.** Design of target compounds.

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347 **Scheme 1**. Synthesis of spirooxindole analogues **1-27**.

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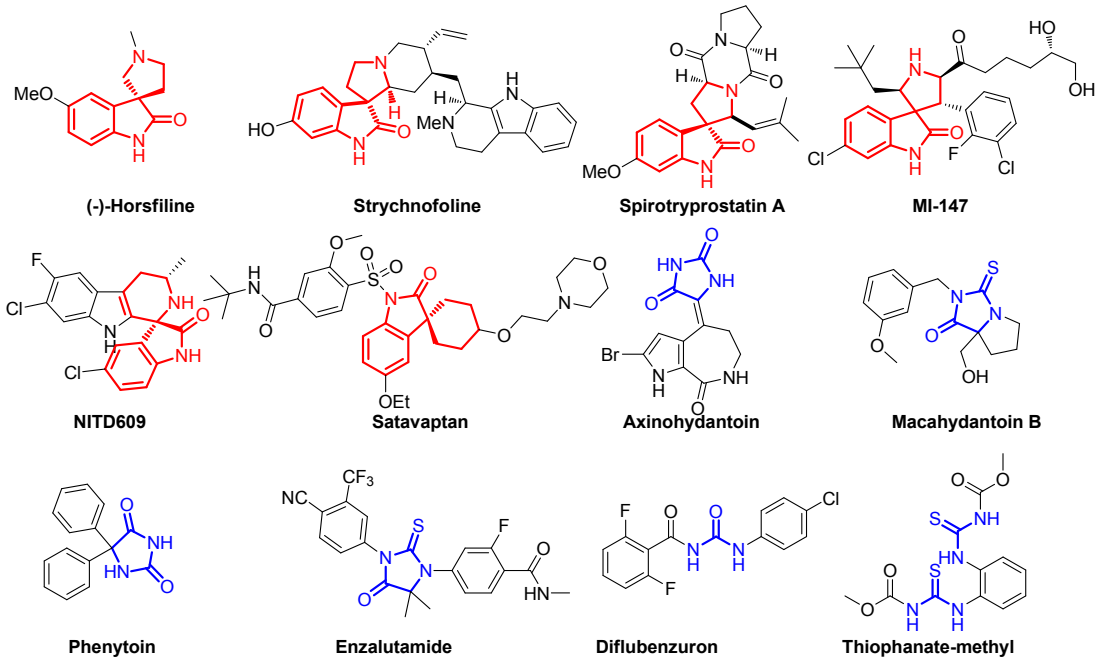

354

355 **Figure 1**.

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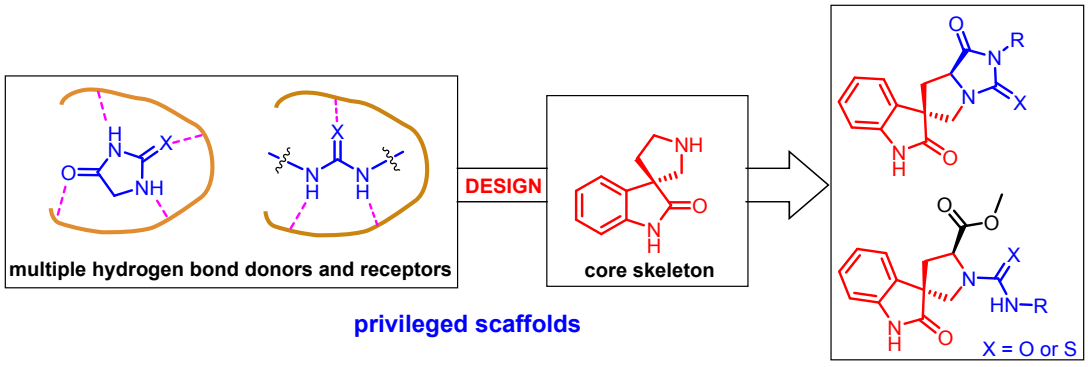

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358 **Figure 2.**

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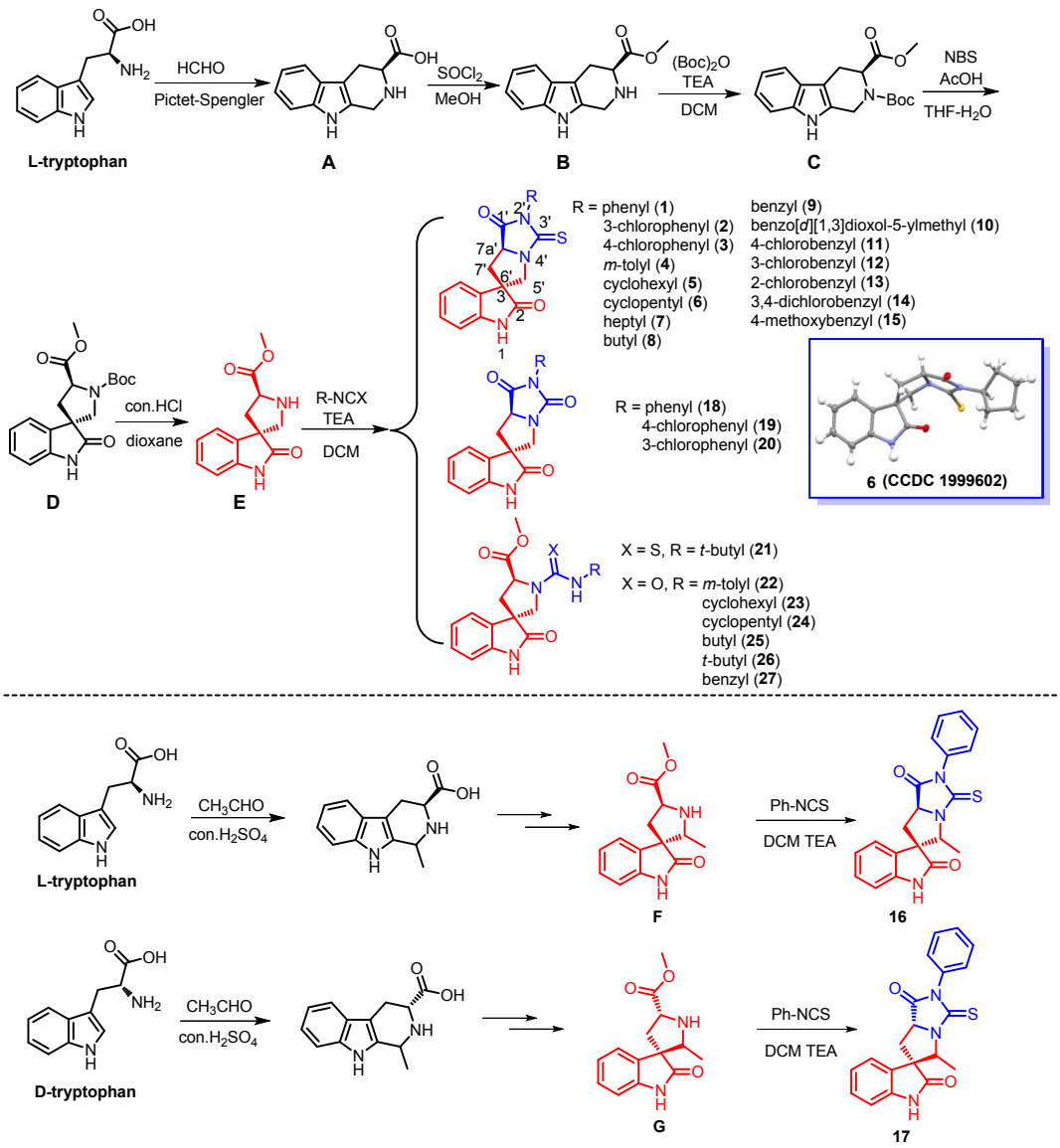

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364 **Scheme 1**.

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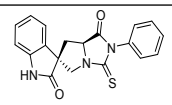
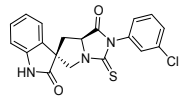
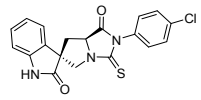
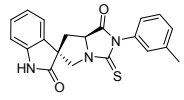
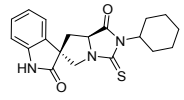
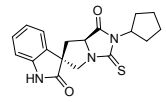
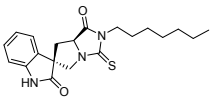
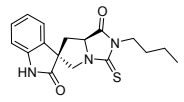
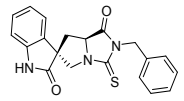
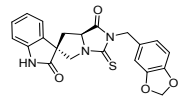
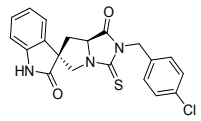
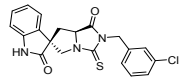
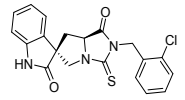
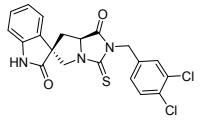
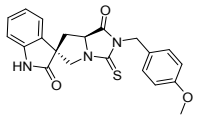
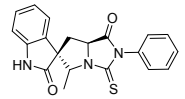
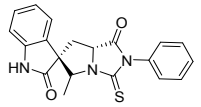
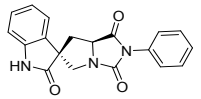
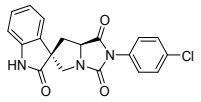
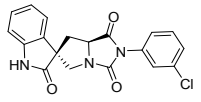
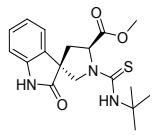
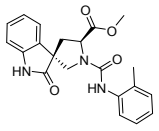
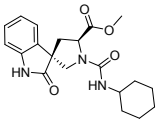
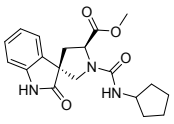
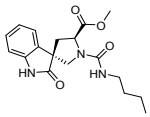
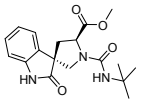
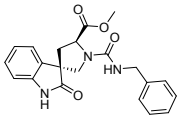
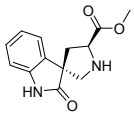
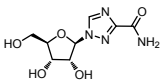
366 **Table 1**. In Vitro and in Vivo Anti-TMV Activity of Synthesized Compounds **1**−**27**.

compd	structure	inhibition rate (%)/500 mg/L			
		in vitro	inactivation	curative effect	protection effect
1		31±1	35±1	25±2	41±1
2		20±1	16±1	20±2	27±1
3		29±1	24±1	12±1	21±1
4		**45±1**	**40±1**	**50±2**	**46±2**
5		28±1	29±1	10±2	13±1
6		0	8±1	6±1	0
7		10±1	15±2	20±1	10±2
8		12±1	13±1	21±1	8±1
9		21±1	18±1	14±2	28±1
10		13±1	26±3	17±1	31±2
11		0	6±1	0	0

12		30±1	16±1	23±1	10±2
13		23±1	13±2	17±1	9±1
14		0	14±1	22±2	10±1
15		35±1	31±1	28±2	29±1
16		**44±1**	**44±2**	**48±1**	**50±1**
17		19±1	27±1	32±3	24±1
18		37±1	30±1	17±1	33±3
19		25±1	36±1	31±2	28±1
20		20±1	33±2	21±1	22±1
21		19±1	20±1	13±1	22±1
22		**45±1**	**47±3**	**50±1**	**51±1**
23		0	0	8±1	0
24		26±1	27±1	11±2	21±2

25		13±1	31±2	15±1	30±1
26		36±1	36±2	29±1	32±2
27		31±1	42±2	34±1	43±2
E		9±1	10±1	15±1	22±1
ribavirin		38±1	36±1	38±1	36±1

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368 **Table 2**. Fungicidal Activity of Compounds **1−27** against Fourteen Kinds of Phytopathogens.

compd	fungicidal activity (%)/50 mg/L													
	F.C[a]	*C.H*	*P.P*	*A.S*	*F.G*	*F.M*	*S.S*	*P.C*	*R.C*	*B.M*	*W.A*	*P.I*	*R.S*	*B.C*
1	54±1	**63±3**	**76±1**	58±2	56±1	46±2	**86±3**	59±1	**84±1**	56±1	**64±2**	42±1	54±1	**70±2**
2	14±1	25±1	**70±2**	26±2	28±1	8±2	**80±2**	41±1	40±1	30±1	32±3	16±1	12±1	42±1
3	18±3	31±1	**76±1**	37±2	28±1	15±1	**70±1**	45±1	56±1	26±1	56±2	5±1	12±1	56±1
4	23±1	38±1	**80±2**	37±1	13±2	15±1	**70±1**	24±2	**62±1**	30±3	40±1	15±1	10±1	50±1
5	18±1	50±2	**72±1**	37±2	33±1	8±1	53±1	37±1	**62±1**	22±3	36±1	5±1	12±1	33±1
6	46±2	56±1	**72±1**	47±1	23±2	31±1	**90±1**	44±3	**73±1**	52±1	56±1	27±2	30±1	**81±1**

7	36±1	56±3	**76±1**	58±1	56±2	46±1	**76±1**	31±2	**80±1**	48±1	40±2	26±1	49±1	56±1
8	50±3	**63±1**	**93±1**	42±2	42±1	46±2	**83±1**	45±1	**82±1**	**60±3**	**60±1**	21±2	24±1	58±1
9	41±1	38±1	**80±2**	53±1	39±1	31±1	**74±2**	48±1	**60±1**	41±3	56±1	37±1	24±1	**61±1**
10	23±3	38±1	**80±2**	47±1	6±1	15±1	**84±2**	24±1	**62±1**	30±2	44±1	6±1	20±1	**78±2**
11	41±1	44±1	**80±4**	47±1	19±1	46±1	**73±2**	37±1	**73±1**	48±1	48±1	30±1	25±1	**70±2**
12	36±1	50±1	**90±3**	**63±1**	**62±1**	46±1	**88±1**	37±1	**82±1**	48±2	44±1	30±1	30±1	**64±1**
13	32±3	44±1	**80±2**	32±1	56±1	31±1	**66±1**	38±1	**70±1**	37±2	52±1	21±1	12±1	47±1
14	23±1	44±1	**62±1**	47±1	47±1	10±2	**66±1**	41±1	**62±1**	33±1	36±1	16±1	37±1	39±1
15	32±1	50±1	**72±1**	40±3	45±1	15±1	57±2	32±1	**73±1**	44±1	52±1	21±1	10±1	**67±2**
16	23±1	**63±1**	**76±1**	30±1	23±2	15±1	**84±1**	17±1	**73±4**	37±1	44±1	15±1	20±1	14±1

17	18±2	25±1	**72±2**	32±1	36±1	15±1	**72±2**	52±1	**71±2**	33±1	44±1	26±2	22±1	42±1
18	23±1	56±1	**93±1**	23±1	23±1	23±1	**77±1**	12±1	**64±1**	44±1	48±1	15±1	25±1	33±1
19	9±1	25±1	**60±3**	26±1	22±1	15±1	**72±2**	28±1	40±1	19±1	32±2	16±1	12±1	14±3
20	50±1	**75±3**	**90±3**	**63±1**	**70±1**	54±1	**96±2**	42±1	**84±1**	**63±1**	**70±2**	46±2	20±1	**75±1**
21	14±1	13±1	**62±2**	16±1	42±1	0	36±1	31±1	49±2	19±1	32±1	11±1	12±1	14±1
22	27±1	**63±2**	**90±2**	47±1	23±1	31±1	**60±1**	17±2	**62±1**	33±1	44±3	15±1	13±1	42±1
23	9±1	0	**70±1**	10±1	6±2	7±1	10±1	7±1	47±2	15±1	36±1	12±1	15±1	17±1
24	14±2	19±1	45±1	16±1	19±1	15±1	20±1	14±1	40±1	11±2	36±1	11±2	12±1	11±1
25	14±1	6±1	**62±2**	16±1	28±1	8±1	33±1	10±1	47±1	19±1	16±1	16±1	15±1	17±1
26	5±2	13±1	35±1	30±1	17±1	0	20±1	12±1	38±2	11±1	28±1	6±1	10±1	28±1

27	14±1	6±1	**62±3**	32±1	42±1	23±1	26±1	17±1	50±2	33±1	36±1	16±1	17±1	14±2
E	15±2	0	30±2	30±2	40±1	31±1	33±1	10±1	44±1	35±1	30±1	12±1	15±1	14±1
chlorothalonil	100	73±2	100	73±2	<50	100	<50	100	100	91±1	91±1	86±1	100	100

[a] *F.C*: *Fusarium oxysporiumf. sp.cucumeris*; *C.H*: *Cercospora arachidicola Hori*; *P.P*: *Physalospora piricola*; *A.S*: *Alternaria solani*; *F.G*: *Fusarium graminearum*; *F.M*: *Fusarium moniliforme*; *S.S*: *Sclerotinia sclerotiorum*; *P.C*: *Phytophthora capsici*; *R.C*: *Rhizoctonia cerealis*; *B.M*: *Bipolaris maydis*; *W.A*: *Watermelon-anthracnose*; *P.I*: *Phytophthora infestans*; *R.S*: *Rhizoctonia solani*; *B.C*: *Botrytis cinerea*.

372 **Table 3**. Insecticidal Activity of Compounds **1**−**27** against *Culex pipiens pallens*,

373 *Mythimna separata*, *Helicoverpa armigera*, and *Pyrausta nubilalis.*

compd	larvicidal activity (%) at concn (mg/L)							
	C. pipiens pallens					*M.separata*	*H. armigera*	*P. nubilalis*
	10	5	2	1	0.5	600	600	600
1	100	80±0	40±0	−	−	20±0	30±0	20±0
2	100	80±0	40±0	−	−	60±0	30±0	40±0
3	100	90±0	50±0	20±0	−	20±0	40±0	30±0
4	100	60±0	33±6	−	−	46±6	20±0	20±0
5	40±0	−	−	−	−	50±0	36±6	20±0
6	100	70±0	40±0	−	−	26±6	10±0	30±0
7	100	60±0	23±6	−	−	40±0	20±0	60±0
8	60±0	20±0	−	−	−	70±0	10±0	16±6
9	100	80±0	60±0	40±0	−	26±6	0	0
10	100	80±0	46±6	−	−	60±0	20±0	26±6
11	100	20±0	−	−	−	40±0	20±0	20±0
12	100	40±0	−	−	−	20±0	40±0	16±6

13	70±0	20±0	–	–	–	76±6	20±0	20±0
14	40±0	–	–	–	–	30±0	10±0	60±0
15	100	60±0	40±0	–	–	0	0	0
16	100	70±0	20±0	–	–	30±0	20±0	40±0
17	**100**	**100**	**96±6**	**80±0**	**46±6**	70±0	30±0	40±0
18	80±0	40±0	–	–	–	20±0	16±6	20±0
19	60±0	20±0	–	–	–	40±0	30±0	10±0
20	56±6	–	–	–	–	5±0	0	0
21	100	80±0	20±0	–	–	10±0	20±0	10±0
22	100	60±0	40±0	–	–	60±0	40±0	30±0
23	100	40±0	–	–	–	30±0	50±0	60±0
24	100	46±6	–	–	–	50±0	30±0	16±6
25	100	100	60±0	20±0	–	70±0	30±0	40±0
26	100	60±0	20±0	–	–	23±6	20±0	20±0
27	100	100	60±0	40±0	–	10±0	10±0	16±6
E	100	40±0	–	–	–	10±0	30±0	10±0

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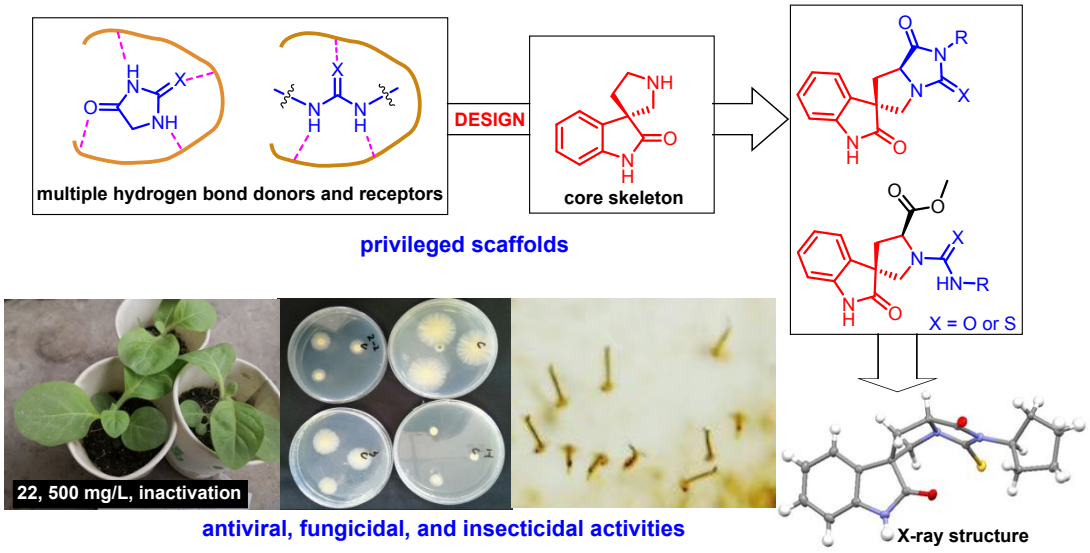
382 ***TOC graphic***



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