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Natural Products for Drug Discovery: Discovery of Gramines as Novel Agents against a Plant Virus

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ABSTRACT: Plant viral diseases seriously affect crop yield and quality. Natural product gramine (**1**) and its structure simple analogues **2**–**35** were synthesized from indoles, amines and aldehydes by one-step. The antiviral effects of these alkaloids were evaluated systematically. Most of these compounds were found to have higher antiviral effects than commercial ribavirin for the first time. Especially for compounds **22**, **30** and **31** exhibited significantly higher effects than ningnanmycin, since emerged as novel antiviral leads for further optimization. The preliminary implementation indicated that these compounds likely inhibit the assembly of TMV by crosslinking TMV CP. Gramine analogues were also found to have broad-spectrum fungicidal effects. Although gramine has been reported to have influence on germination and development of *Erysiphe graminis*, these compounds displayed no fungicidal effects against *Blum eria graminis f. sp.tritici* on wheat in our test. Some of these compounds also exhibited certain insecticidal activities.

KEYWORDS: natural product, gramine analogues, anti-TMV activity, fungicidal activity

INTRODUCTION

In the past 60 years, the population has more than doubled. The demand for more fast increasing food production is needed.[1,2] Plant diseases can cause a significant economic and environmental impact on agricultural communities. Tobacco Mosaic Virus (TMV), named after its first discovery in tobacco, is a well-studied plant virus. It is reported that TMV can infect more than 400 crops including tobacco, pepper, cucumber, banana and ornamental flowers.[3] Ningnanmycin (Figure 1), perhaps the most effective antiviral agent, only gave 50-60% control effects at 500 μg/mL. Ribavirin (Figure 1), currently widely used antiviral agent, conveys an anti-TMV effect of less than 50% at 500 μg/mL. In fact, once a plant is infected with TMV, there is no agent to cure it completely. Therefore, it is urgently needed to find agents with novel structures and unique mechanisms of action.[4]

Nitrogen-containing heterocyclic compounds often exhibit a variety of biological activities and are widely concerned in the field of medicine and pesticides. Among them, compounds containing indole skeleton have aroused great interest of chemists,[5] and have been reported to have various kinds of activities, such as antibacterial activity, anticancer activity, antiviral activity, protein kinase inhibitory effect.[6-12] Gramine (Figure 1) is an indole alkaloid widely found in raw plants and in coal tar.[12-14] This alkaloid has various kinds of activities, such as regulation of bronchial smooth muscle activity, regulation of vasodilation activity and controlling blood pressure activity.[15] It can control mitochondrial energy metabolism in rat liver and beef heart and displayed weak

acetylocholinesterase inhibitory effect.[16,17] Recent studies have shown that gramine can act as a 5-HT_{2A} receptor and thus exhibits vasodilatory activity.[15,18] In agriculture, gramine was reported to have influence on germination and development of *Erysiphe graminis*.[19] However, there is no report on the antiviral activity of gramine in plants.

In our previous work, indole alkaloid topsentin A (Figure 1) and its derivatives,[20] were found to have good anti-TMV activities. As another indole-containing natural alkaloid, gramine has more simple structure than topsentin A. As a continuation of our work, various of gramine analogues were designed (Figure 2), synthesized and systematically evaluated for their antiviral, anti-phytopathogenic fungi and insecticidal activities.

MATERIALS AND METHODS

Instruments. The melting points of the target compounds were determined on an X-4 binocular microscope (Gongyi Yuhua Instrument Co., China). NMR spectra were acquired with 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) with tetramethylsilane as internal standards. HRMS data were obtained with an FT-ICR MS spectrometer (Ionspec, 7.0 T). Analytical TLC was performed on silica gel GF 254. Column chromatographic purification was performed using silica gel. The in vitro TMV rod assembly inhibition and 20S CP Disk assembly inhibition were tested on transmission electron microscopy (Tecnai G2 F20).

General Synthesis. The synthetic route is given in Figure 3.

The detailed operation steps and experimental data of gramines **1**–**35** can be found in the Supporting Information.

Biological Assay. Each group of tests was repeated three times at 25±1 °C. Activity results were given as a percentage scale of 0−100 (0: no activity; 100: total inhibited). Detailed bioassay procedures for the anti-TMV,[21] fungicidal[22-24] and insecticidal[22] activities were described in literature, also can be seen in Supporting Information.

Mode of Action Studies.

In vitro TMV rod assembly inhibition： TMV was purified by Leberman method.[25] TMV RNA purification was performed by RNApure virus kit (CoWin Biosciences) and TMV capsid protein (TMV CP) was purified by the acetic acid method.[26] Before assembling, 20S CP Disk was obtained by hatching CP (20 mg/mL) in pH 7.0 phosphate buffer (0.1 M) at 20 °C for 12 h. Then, in vitro TMV assembly experiments were carried out by successive adding the phosphate buffer 5 μL (0.1 M, pH 7.0), 20S Disk 4 μL (2 mg/mL) and TMV RNA 1 μL (200 ng/μL). The mixture was hatched at 20 °C for 12 h and could be then transferred into the copper grid for transmission electron microscopy (TEM) assay. The assembly reaction mixture (5 μL) was mixed with 0.1 M phosphate buffer 5 μL (pH 7.0) and dropped onto the copper film waiting for 5 minutes. After the incubation, the droplet was removed by filter paper and negatively stained by 2% phosphotungstic acid (pH 7.0) for three minutes. After removing the staining agent, the copper was placed at 37 ℃ for 2 h for drying. The morphology of the reconstituted TMV rods was imaged at 200 keV on a CCD camera. For the inhibition experiments with the

target compounds, in vitro TMV reconstitution inhibition experiments were performed by successive adding phosphate buffer 4.8 μL (0.1 M, pH 7.0), 20S Disk 4 μL (2 mg/mL), TMV RNA 1 μL (200 ng/μL) and DMSO 0.2 μL or the target compound (10 μM). Repeat each experiment at least three times to ensure the reliability of the data.

In vitro 20S CP Disk assembly inhibition: For the inhibition tests with the compounds, TMV CP was first adjusted with 0.1 M phosphate buffer (pH 7.0) to 20.4 mg/mL. In vitro 20S CP Disk assembly experiments were carried out by adding 9.8 μL TMV CP (20.4 mg/mL) and 0.2 μL DMSO or the compound (10 μM). The assembly experiment was hatched at 20 °C for 12 h. The morphology of the 20S CP Disk was imaged via TEM at 200 keV on a CCD camera. Repeat each experiment at least three times to ensure the reliability of the data.

RESULTS AND DISCUSSION

Chemistry.

*Synthesis of the Compounds **1**−**35***. Indole group was selected as template for structural diversification. By introducing a series of functional groups into the side chain, we investigated the effects of electronic effect, steric hindrance, hydrophilicity and lipophilicity on the activity. The Mannich reaction in the presence of either protic or Lewis acids[14] is considered as powerful carbon-carbon bond forming processes to afford 3-substituted indoles. Compounds **1**−**35** were prepared by using the depicted procedures in Figure 3. Substituted indoles were reacted with corresponding aldehydes and amines to obtain **1**−**35** in 53%−97% yields. As the new compounds, **7**−**9**, **12**, **14**−**16**, **18** and **24**−**35**

were identified by nuclear magnetic resonance (NMR) and high-resolution mass spectrometer (HR-MS). Other known compounds were compared with literature. Compound **22** is a known compound, but no physical properties available. So the NMR and data of HR-MS of **22** were also given.

Phytotoxic Activity. The phytotoxic activities of compounds **1**−**35** were first tested by using the test plants which indicated that compounds **1**−**35** showed no phytotoxic activities at 500 μg/mL. No local lesion appear on the plant leaves. The detailed test procedures also can be seen in Supporting Information.

Antiviral Activity. The in vitro and in vivo anti-TMV activities of gramine and its analogues were listed in Tables 1 and 2. The commercial plant virucides ribavirin and ningnanmycin were selected as the controls.

In Vitro Anti-TMV Activity. Gramine analogues **1**−**35** were first tested in vitro anti-TMV activities using the conventional half-leaf method.[21] As shown in Table 1, compounds **2**, **4**, **8**−**10**, **15**, **17**, **22**, **27**, **30**, **31** and **35** displayed significantly higher antiviral activities than ribavirin. Among the compounds, **22**, **30** and **31** exhibited higher TMV inhibition effects than ningnanmycin. The mainly difference among **1**−**4** lies in the substituents in indole ring. Gramine analogues **2**−**4** displayed significantly higher antiviral activities than gramine (**1**), which showed that the introduction of substituent groups at 1-position, 5-position and 6-position of indole ring are favorable for antiviral activity. Bromination of 5-position is more favorable than 6-position (antiviral effect: **2** > **3**). Gramine analogues **5**−**12** displayed higher antiviral activities than gramine (**1**), further

revealed that the substituents of *N,N*-dimethylmethanamine region in gramine are favorable for antiviral activity. Among analogues **5**−**12**, compounds **8**, **9** and **10** exhibited much higher antiviral activities than the others, which indicated that the substituents of *N,N*-dimethylmethanamine region in gramine exhibited synergistic effect. The mainly difference among **6**, **13** and **19**−**21** lies in the replacement of *N,N*-dimethyl group in gramine. Compound **13** exhibited higher TMV inhibitory effects than the others, which showed that the introduction of 2-amine pyridine group is favorable for antiviral activity. Among derivatives **13**−**18**, compounds **15** and **17** exhibited much higher antiviral activities than the others. Based on the above structure-activity relationships, 5-bromine and 1-substituent gramine analogues **22**−**35** were further designed. Among derivatives **22**−**24**, compound **22** exhibited much higher TMV inhibitory effects than the others, which indicated that the methylation of gramine is favorable for antiviral activity. The mainly difference among **25**−**35** lies in the aryl substituents. Compounds **25**, **26**, **28**, **29**, **32**, and **34** showed similar level antiviral activities with ribavirin. Compound **33** displayed relatively lower antiviral activity than the others. Gramine analogues **27**, **30**, **31** and **35** exhibited significantly higher antiviral activities than ribavirin, especially for **30** and **31** (antiviral effect: **30** and **31** > ningnanmycin).

In Vivo Anti-TMV Activity. The in vivo antiviral tests including inactivation activity, curative activity and protection activity against TMV is carried out using reported method.[21] As depicted in Table 2, target compounds also showed higher *in vivo* TMV inhibitory effects than ribavirin. Compounds **22**, **30** and **31** showed significantly higher

TMV inhibitory effects than ningnanmycin, since emerged as novel antiviral leads for further optimization. Unlike *in vitro* anti-TMV test results, gramine analogues **1**, **4**−**6**, **14**, **15** and **18** exhibited relatively higher *in vivo* TMV inhibitory effects than *in vitro*. The protection effects of compounds **15**, **17** and **30** relatively higher than inactivation effect and curative effect, which indicated that compounds **15**, **17** and **30** may have a certain inducible antiviral activities. The other structure-activity relationships *in vivo* are similar to that of *in vitro*.

Preliminary Mode of Action. The preliminary mode of action of compounds **15** and **22** were evaluated via TEM with RNA inhibitor antofine[27] and CP disks assembly inhibitor NK0209[28] as controls. The TMV is 300 nm length rod-shaped particle. It contains a single-stranded RNA consisting of about 6400 nucleotides. Its coat protein contains 2130 protein subunits, each of which is composed of 158 amino acids and spirally arranged around RNA molecules.[29] The test results revealed that 20S CP Disk and TMV RNA can assemble into TMV rod effectively (Figure 4, B). The use of a small amount of DMSO does not affect assembly (Figure 4, C). Compounds **15**, **22**, antofine and NK0209 can significantly inhibit the assembly of TMV rod (Figure 4, D−G). Further 20S CP Disk assembly inhibition tests were carried out to evaluate the interaction of these compounds with TMV CP. The 20S CP Disk can obtained by incubation of TMV CP at 20 °C for 12 h (Figure 5, A). The use of a small amount of DMSO has no impact on assembly of 20S CP Disk (Figure 5, B). As the TMV RNA inhibitor, antofine displayed no impact on 20S CP Disk assembly (Figure 5, C). CP disks assembly inhibitor NK0209 showed

169 significantly impact on 20S CP Disk. Although the 20S CP Disk can assemble efficiently,
170 aggregation and fusion of a large number of CP Disks was detected (Figure 5, D). Just
171 like NK0209, compounds **15** and **22** also can effectively induce CP Disks aggregation
172 and fusion (Figure 5, E, F). Compound **22** with higher in vivo TMV inhibitory effect than
173 **15** did exhibit stronger polymerization-promoting ability (As shown in Figure 5, the
174 number of 20S CP Disk in F is significantly lower than that in E). The above results
175 indicated that these compounds likely inhibit virus assembly by crosslinking TMV CP.

176 **Fungicidal Activity**. Gramines **1**−**35** were simultaneously determined for fungicidal
177 activity with chlorothalonil and azoxystrobin as controls.

178 *In Vitro Fungicidal Activity.* The in vitro fungicidal effects of gramines **1**−**35** were
179 obtained by using mycelial growth tests[22] with 14 plant pathogens as artificial media.
180 Gramines **1**−**35** exhibited broad-spectrum fungicidal activities (Table 3). What's more,
181 these compounds displayed high bioselective. Compounds **17** and **23** displayed about
182 similar level antifungal activities against *Alternaria solani* with chlorothalonil. Most of
183 these compounds showed good antifungal activities against *Phytophthora capsici* and
184 *Physalospora piricola*, especially for compounds **17** and **30** (antifungal activity against
185 *Phytophthora capsici*: **30** > 90%; antifungal activity against *Physalospora piricola*: **17**
186 and **30** ≥ 90%). Compound **8** displayed more than 60% antifungal activity against
187 *Rhizoctonia cerealis*.

188 *In Vivo Fungicidal Activity.* Gramines **1**−**35** were further tested fungicidal activities in
189 vivo.[22] The pathogens tested in this screen were *Sclerotinia sclerotiorum* on rape,

Rhizoctonia cerealis on cerealis, *Botrytis cinerea on* cucumber, *Phytophthora capsici* on capsici, *Corynespora cassiicola* on cucumber with protection and *Blum eria graminis f. sp.tritici* on wheat. The results (Table 3) showed that lots of compounds displayed more than 30% inhibitory effect. Although gramine has been reported to have influence on germination and development of *Erysiphe graminis*,[19] all compounds displayed no fungicidal activity against *Blum eria graminis f. sp.tritici* on wheat in our test.

Insecticidal Activity. Some compounds showed certain insecticidal activity. Compounds **13**, **22** and **32** exhibited broad-spectrum insecticidal activities against *Mythimna separate, Helicoverpa armigera, Ostrinia nubilalis* and *Culex pipiens pallens*.

In summary, natural product gramine and its derivatives were prepared and tested for their TMV inhibitory effects for the first time. Most of these structure simple compounds showed higher TMV inhibitory effects than ribavirin. Compounds **22**, **30** and **31** with significantly higher TMV inhibitory effects than ningnanmycin emerged as new antiviral leads for further research. The introduction of substituents at 1-position, 5-position and 6-position of indole ring are favorable for TMV inhibitory effect. Bromination of 5-position is more favorable than 6-position. Compounds **15**, **17** and **30** may have a certain inducible antiviral activities. The preliminary mode of action studies revealed that these compounds likely inhibited TMV assembly by crosslinking TMV CP. Further fungicidal activity test showed that these compounds exhibited broad-spectrum fungicidal activity. Compounds **17** and **23** displayed about similar level antifungal activities against *Alternaria solani* with chlorothalonil. Insecticidal activity bio-assay showed that gramine

211 and its derivatives also displayed a certain insecticidal activity. Current study provides
212 strong data support for the application of gramines in plant protection.

213 **ASSOCIATED CONTENT**

214 **Supporting Information**

215 The detailed bio-assay procedures. The preparation procedures and the spectra data of
216 compounds **1**−**35**. This material is available free of charge via the Internet at
217 http://pubs.acs.org.

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229 **Notes**

230 The authors declare no competing financial interest.

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Figure Captions

Figure 1. Structures of Ningnanmycin, Ribavirin, Gramine and Topsentin A.

Figure 2. Design of Gramine Analogues.

Figure 3. Synthesis of Gramine Analogues **1**–**35**.

Figure 4. TMV Rod Assembly Inhibition of Compounds **15**, **22**, NK0209 and Antofine. (A) 20S CP Disk (50 nm scale bar); (B) 20S CP Disk + RNA (100 nm scale bar); (C) 20S CP Disk + RNA + DMSO (200 nm scale bar); (D) 20S CP Disk + RNA + antofine (200 nm scale bar); (E) 20S CP Disk + RNA + NK0209 (200 nm scale bar); (F) 20S CP Disk + RNA + **15** (200 nm scale bar); (G) 20S CP Disk + RNA + **22** (100 nm scale bar).

Figure 5. 20S CP Disk Assembly Inhibition of Compounds **15**, **22**, NK0209 and Antofine (100 nm scale bar). (A) CP; (B) CP + DMSO; (C) CP + antofine; (D) CP + NK0209; (E) CP + **15**; (F) CP + **22**.

Table 1. In Vitro Antiviral Activities of Compounds **1**−**35**, Ribavirin and Ningnanmycin

against TMV.

Compd	Concn (μg/mL)	Inhibition rate (%)[a]	compd	Concn (μg/mL)	Inhibition rate (%)[a]
1	500	23±1	**20**	500	16±1
	100	0		100	0
2	500	51±2	**21**	500	30±2
	100	20±2		100	0
3	500	39±1	**22**	**500**	**61±2**
	100	5±1		**100**	**27±1**
4	500	48±2	**23**	500	35±3
	100	31±1		100	10±2
5	500	33±2	**24**	500	40±1
	100	4±1		100	18±2
6	500	25±2	**25**	500	45±1
	100	0		100	18±1
7	500	43±1	**26**	500	39±3
	100	16±2		100	11±2
8	500	52±2	**27**	500	54±2
	100	23±2		100	19±1
9	500	49±1	**28**	500	43±2
	100	15±1		100	16±1
10	500	51±2	**29**	500	46±2
	100	19±1		100	15±2
11	500	35±2	**30**	**500**	**62±1**
	100	0		**100**	**29±1**
12	500	46±1	**31**	**500**	**67±2**
	100	14±1		**100**	**31±2**
13	500	36±2	**32**	500	44±1
	100	0		100	18±2
14	500	35±2	**33**	500	30±1
	100	13±1		100	0
15	500	52±2	**34**	500	42±2
	100	17±1		100	16±2
16	500	36±1	**35**	500	51±1
	100	5±2		100	20±2
17	500	50±1	**Ribavirin**	500	41±1
	100	22±2		100	15±2
18	500	46±1	**Ningnanmycin**	500	58±2
	100	13±2		100	23±2
19	500	19±2	[a]*Average of three replicates; All results are*		

100	0	*expressed as mean ± SD.*

Table 2. In Vivo Antiviral Activities of Compounds **1**−**35**, Ribavirin and Ningnanmycin

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	32±1	26±1	37±2	**20**	500	21±2	18±2	26±1
	100	0	0	0		100	0	0	0
2	500	**57±1**	**53±2**	**51±1**	**21**	500	30±2	31±1	30±2
	100	**29±1**	**22±1**	**27±1**		100	0	0	6±2
3	500	42±2	47±2	41±2	**22**	**500**	**64±2**	**60±1**	**65±2**
	100	6±2	12±1	8±1		**100**	**30±2**	**31±2**	**35±1**
4	500	**59±2**	**53±2**	**54±2**	**23**	500	32±1	29±2	35±2
	100	**27±1**	**29±1**	**33±1**		100	0	0	0
5	500	46±2	42±2	39±2	**24**	500	41±2	38±1	45±2
	100	12±1	7±1	17±2		100	12±2	8±2	13±2
6	500	33±2	32±2	31±2	**25**	500	48±3	42±1	44±2
	100	0	0	0		100	18±2	14±2	16±1
7	500	52±1	45±1	41±2	**26**	500	40±2	38±1	41±2
	100	26±2	16±1	9±1		100	11±2	7±2	16±2
8	500	**57±2**	**53±1**	**61±1**	**27**	500	**57±1**	**52±2**	**56±1**
	100	**31±1**	**28±1**	**23±1**		100	**21±2**	**24±2**	**23±2**
9	500	45±4	48±2	41±2	**28**	500	47±2	44±3	46±2
	100	7±1	9±1	13±1		100	22±2	20±1	27±1
10	500	**55±1**	**51±1**	**49±2**	**29**	500	45±1	40±2	42±2
	100	**13±2**	**24±2**	**27±1**		100	18±2	12±1	16±1
11	500	43±1	36±1	36±2	**30**	**500**	**61±2**	**59±1**	**65±2**
	100	6±1	8±1	10±1		**100**	**28±1**	**27±2**	**33±1**
12	500	41±1	39±2	34±2	**31**	**500**	**68±2**	**65±1**	**66±2**
	100	6±2.0	11±2	0		**100**	**34±2**	**32±1**	**35±2**
13	500	36±1	34±2	38±2	**32**	500	44±3	45±1	40±2
	100	0	0	0		100	11±2	15±2	12±1
14	500	44±2	48±1	42±2	**33**	500	32±1	28±1	26±2
	100	10±1	21±1	15±1		100	0	0	0
15	500	**60±2**	**55±2**	**64±1**	**34**	500	41±3	37±3	42±2
	100	**13±1**	**19±2**	**32±1**		100	12±2	15±2	17±1
16	500	43±2	47±1	39±2	**35**	500	**52±2**	**50±3**	**53±2**
	100	10±2	18±1	6±1		100	**20±1**	**18±2**	**23±2**
17	500	**56±4**	**52±2**	**60±1**	**Ribavirin**	500	38±1	36±2	39±1
	100	**15±2**	**20±1**	**25±1**		100	11±1	14±1	10±1
18	500	**53±1**	**51±2**	**58±1**	**Ningnanmycin**	500	58 ± 1	56 ± 1	58 ± 2
	100	**29±2**	**31±1**	**25±1**		100	27 ± 2	25 ± 2	30 ± 1
19	500	33±2	27±2	23±2	[a] *Average of three replicates; All results are expressed as mean ± SD.*				

	100	0	0	0

Table 3. In Vitro Fungicidal Activities of Compounds **1**–**35** and Chlorothalonil against 14

Kinds of Fungi.

Compd	Fungicidal activities (%)[a]/ 50 μg/mL													
	A.S[b]	*B.C*[b]	*B.M*[b]	*C.H*[b]	*F.C*[b]	*F.G*[b]	*F.M*[b]	*P.C*[b]	*P.I*[b]	*P.P*[b]	*R.C*[b]	*R.S*[b]	*S.S*[b]	*W.A*[b]
1	26±1	12±1	6±1	7±2	14±1	27±1	25±1	7±2	14±1	28±1	13±2	12±1	14±2	18±1
2	30±1	19±2	18±1	11±1	22±1	50±1	21±1	52±1	38±1	**69±1**	42±1	46±2	24±1	33±1
3	35±2	15±2	15±1	11±1	17±1	35±2	25±1	52±1	38±1	19±1	41±1	25±1	19±1	37±2
4	30±1	19±2	15±1	11±1	14±1	31±2	21±2	45±1	19±1	28±1	18±1	12±1	29±2	18±1
5	22±1	20±2	18±1	7±2	22±1	12±2	4±1	24±1	28±2	33±1	34±2	12±1	29±2	37±1
6	30±2	29±1	18±1	15±1	25±1	15±1	8±1	20±1	24±2	58±1	25±1	12±1	24±1	30±1
7	43±1	32±1	15±1	18±1	25±1	12±1	17±2	**62±1**	14±1	33±2	35±1	46±1	29±1	18±2
8	26±1	7±1	18±1	11±1	22±1	11±2	12±2	17±1	14±1	25±1	**63±1**	8±1	33±1	26±1
9	30±1	15±1	21±1	18±1	19±2	8±1	8±1	41±1	24±1	58±1	32±1	33±1	33±1	26±2
10	26±1	12±1	18±1	4±1	19±2	19±1	17±1	24±2	14±2	44±1	32±1	21±2	29±2	15±1
11	39±1	12±1	18±1	18±1	22±1	27±1	12±1	**62±1**	28±2	33±1	41±2	8±1	33±1	19±2
12	43±2	37±2	21±1	33±1	30±1	15±1	25±1	**62±2**	28±2	**69±1**	41±1	37±1	33±1	26±2
13	22±2	20±1	21±1	15±1	28±1	8±2	4±1	41±1	24±1	56±2	32±2	4±1	24±1	22±2
14	26±1	12±1	21±1	30±2	25±1	15±1	12±1	17±1	24±1	28±1	35±2	33±1	19±1	33±1
15	13±2	12±1	24±1	18±2	22±1	4±1	12±1	24±1	24±1	53±1	25±1	4±1	14±1	30±2
16	52±1	49±1	33±2	33±1	42±1	38±1	12±1	55±1	47±2	**64±1**	42±1	46±2	12±1	33±1
17	**61±2**	58±2	27±2	37±1	39±1	46±1	29±1	45±1	47±2	**92±1**	35±2	58±1	38±1	30±2

18	35±2	42±1	24±1	30±1	28±2	15±2	29±1	**62±1**	14±1	36±1	31±1	42±1	24±1	26±1
19	17±2	32±1	24±1	18±1	22±1	11±2	12±1	27±1	14±1	50±1	17±2	12±2	14±1	26±1
20	56±2	44±1	36±2	18±1	28±1	39±1	12±1	58±2	**62±1**	58±1	31±1	46±1	29±1	33±1
21	43±1	37±1	33±2	15±1	25±1	11±1	12±2	42±1	38±1	36±2	21±1	42±1	24±2	30±2
22	35±2	44±1	30±1	19±2	25±2	15±1	13±2	58±1	40±2	**64±2**	25±1	43±2	33±2	35±1
23	**62±2**	50±2	37±1	36±2	45±1	38±2	32±2	**82±2**	53±1	**86±2**	42±2	43±1	32±2	37±2
24	55±2	46±1	32±2	28±2	35±1	29±2	26±1	**75±2**	42±1	74±2	38±1	35±1	28±1	29±2
25	16±1	25±2	30±1	14±2	24±2	10±2	12±1	52±2	15±1	53±2	25±1	30±2	22±2	26±2
26	19±2	33±2	28±2	34±2	42±2	19±2	28±2	**62±2**	28±2	**67±1**	38±2	42±2	33±2	30±2
27	11±1	16±2	22±1	15±2	26±2	10±1	13±2	58±2	16±1	56±2	21±1	13±2	19±1	26±2
28	23±2	25±1	21±2	19±2	22±1	9±2	11±2	55±1	24±2	46±1	23±1	31±1	11±2	9±1
29	18±2	32±2	25±1	31±2	42±2	18±2	22±1	51±2	26±2	55±2	26±2	34±2	10±1	25±2
30	51±1	45±1	39±2	33±1	42±2	20±1	23±2	**92±2**	41±1	**90±1**	30±1	32±2	37±1	39±2
31	10±2	9±1	15±2	33±2	24±2	8±1	15±2	42±2	20±2	38±1	12±2	11±2	23±2	12±2
32	31±1	25±2	28±2	34±1	51±2	21±2	12±2	**72±1**	32±2	**68±2**	24±1	23±1	8±2	11±1
33	21±1	15±2	32±2	23±1	31±2	9±2	11±2	**62±1**	26±2	**63±2**	21±1	16±2	13±2	14±1
34	26±2	24±1	30±1	25±2	21±1	14±1	13±1	**65±2**	32±1	59±2	22±1	17±2	9±1	10±2
35	32±2	22±1	32±2	29±2	22±1	16±2	19±2	**76±1**	38±2	**72±2**	26±1	19±2	24±2	26±1
Chlorothalonil[c]	73±2	100	91±1	73±2	100	<50	100	100	86±1	100	100	100	<50	86±1

[a]Average of three replicates; All results are expressed as mean ± SD. [b]A.S, Alternaria solani; *B.C, Botrytis cinerea.*;

B.M, Bipolaris maydis; *C.H, Cercospora arachidicola Hori*; *F.C, Fusarium oxysporium f. sp. cucumeris*; *F.G, Fusarium graminearum*; *F.M, Fusarium moniliforme*; *P.C, Phytophthora capsici*; *P.I, Phytophthora infestans*; *P.P, Physalospora piricola*; *R.C, Rhizoctonia cerealis*; *R.S, Rhizoctonia solani*; *S.S, Sclerotinia sclerotiorum*; *W.A, watermelon anthracnose*. [c] *The commercial agricultural fungicide was used for comparison of antifungal activity.*

Table 4. In Vivo Fungicidal Activities of Compounds **1**–**35** and Azoxystrobin against 6 Kinds of Fungi.

compd	Inhibition rate (%)[b]/ 200 μg/mL					
	S.S[a]	*R.C*	*B.C*	*P.C*	*C.C*	*B.G*
1	20±2	22±1	28±2	26±2	**32±1**	0
2	18±2	22±2	11±2	21±1	25±2	0
3	16±1	19±2	23±2	15±2	**37±2**	0
4	16±2	15±2	16±2	27±1	13±2	0
5	15±2	18±1	13±2	**31±2**	28±2	0
6	16±2	22±2	19±1	23±2	**35±1**	0
7	11±1	**30±2**	16±2	17±2	**36±2**	0
8	9±2	13±1	19±2	16±2	18±1	0
9	9±2	12±2	28±2	23±1	16±2	0
10	11±2	16±1	25±2	17±2	15±2	0
11	29±2	22±2	8±1	**35±2**	12±2	0
12	26±1	19±2	20±1	**39±2**	**40±2**	0
13	12±2	8±2	12±2	11±1	16±2	0
14	28±1	8±2	23±1	9±2	28±2	0
15	25±2	27±2	19±2	15±2	17±1	0
16	9±2	**32±1**	27±2	**35±2**	**41±2**	0
17	18±2	21±2	19±2	**45±2**	**41±2**	0
18	29±2	27±1	28±2	11±1	12±2	0
19	11±1	12±2	19±1	21±2	28±1	0
20	18±2	23±1	19±2	11±1	15±2	0
21	9±1	11±2	19±1	13±2	21±1	0
22	20±2	15±1	19±2	20±1	12±2	0
23	27±1	**33±2**	20±1	**43±2**	**35±1**	0
24	11±2	22±1	15±2	26±1	26±2	0
25	25±1	22±2	17±1	25±2	26±1	0
26	21±2	**32±1**	25±2	**36±1**	**39±2**	0
27	15±1	22±2	13±1	15±2	22±1	0

28	11±2	20±1	25±2	22±1	21±2	0
29	26±1	12±2	27±1	23±2	16±1	0
30	21±2	12±1	25±2	**36±1**	**46±2**	0
31	9±1	12±2	17±1	15±2	16±1	0
32	21±2	29±1	25±2	**36±1**	**36±2**	0
33	25±1	23±2	27±1	**35±2**	**38±1**	0
34	16±2	22±1	18±2	26±1	16±2	0
35	15±1	22±2	19±1	15±2	29±1	0
azoxystrobin[c]	100	100	100	85±2	80±1	81±2

[a] *S.S, Sclerotinia sclerotiorum* (rape-protection); *R.C, Rhizoctonia cerealis*; *B.C, Botrytis cinerea.* (cucumber-protection); *P.C, Phytophthora capsici; C.C*, *Corynespora cassiicola* (cucumber-protection); *B. G, Blum eria graminis f. sp.tritici* (wheat-protection). [b] *Average of five replicates*. [c] The dilution of azoxystrobin is 1000 times.

Table 5. Larvacidal Activities of Compounds **1**–**35** and Rotenone against Oriental Armyworm (*Mythimna Separate*), Cotton Bollworm (*Helicoverpa Armigera*), Corn Borer (*Ostrinia Nubilalis*) and Mosquito (*Culex Pipiens Pallens*).

	Larvacidal activities (motality %) at concn (μg/mL)			
compd	M. Separat	H. armigera	O. Nubilalis	C. Pipiens Pallens
	600	600	600	10
1	0	0	0	0
2	0	0	0	0
3	0	5	20	20
4	20	35	35	40
5	20	15	25	20
6	0	20	25	80
7	0	10	25	40
8	15	15	30	20
9	0	0	0	20
10	0	20	20	60
11	0	0	5	20
12	20	20	10	20
13	**30**	**45**	**50**	**80**
14	0	15	10	40

15	0	5	0	20
16	10	15	15	20
17	0	10	10	40
18	20	10	15	20
19	0	5	0	20
20	20	10	25	20
21	0	5	10	30
22	**20**	**30**	**35**	**35**
23	30	15	25	40
24	15	10	15	30
25	0	0	0	20
26	0	30	10	30
27	20	10	15	35
28	10	15	15	30
29	0	0	0	10
30	20	30	45	60
31	10	20	30	45
32	**30**	**45**	**30**	**60**
33	20	10	0	0
34	0	0	0	0

35	20	30	10	50
Rotenone	100	75	60	100

Figure 1.

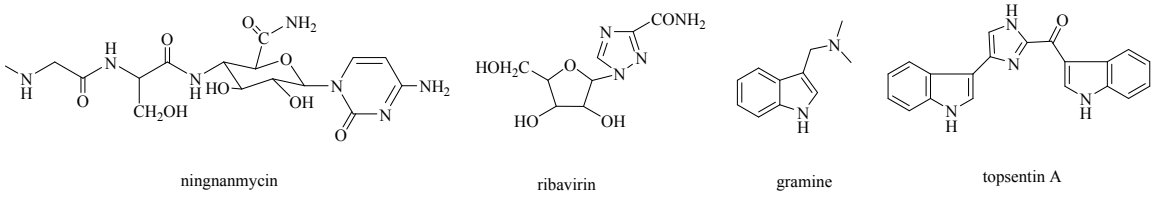

Figure 2.

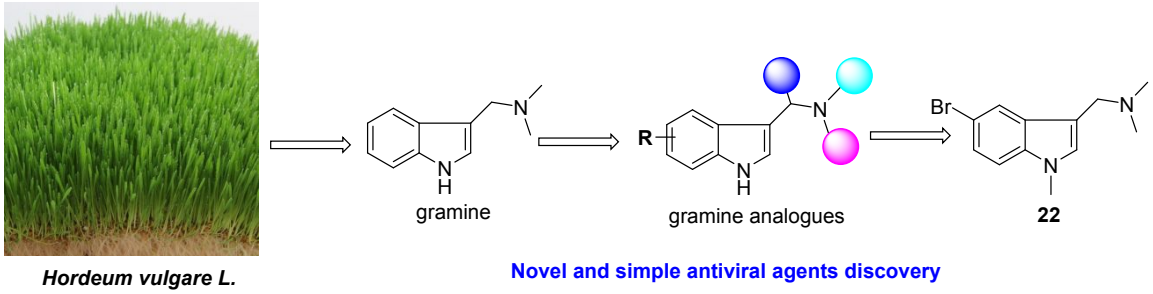

Figure 3.

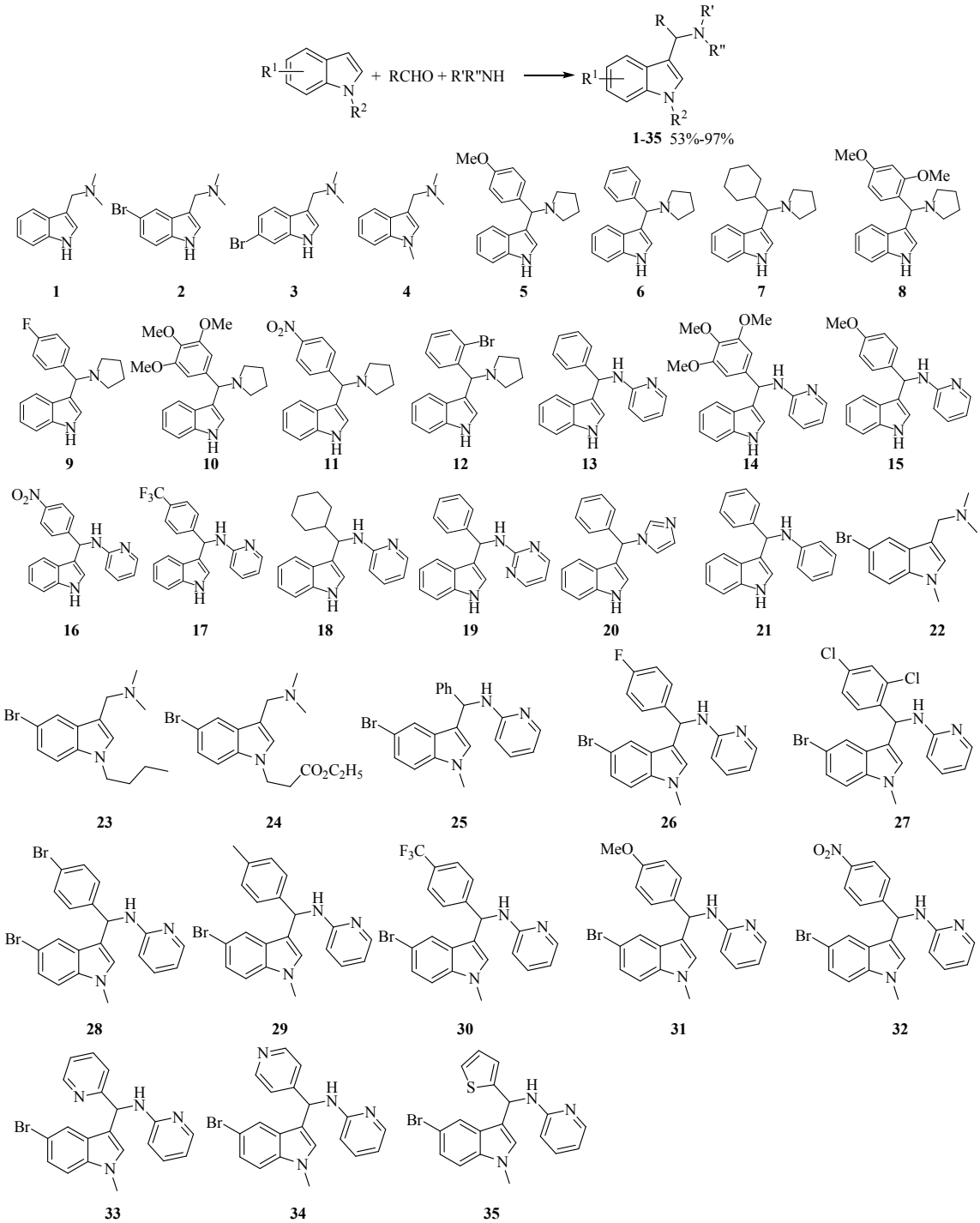
+ RCHO + R'R"NH
1-35 53%-97%
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Figure 4.

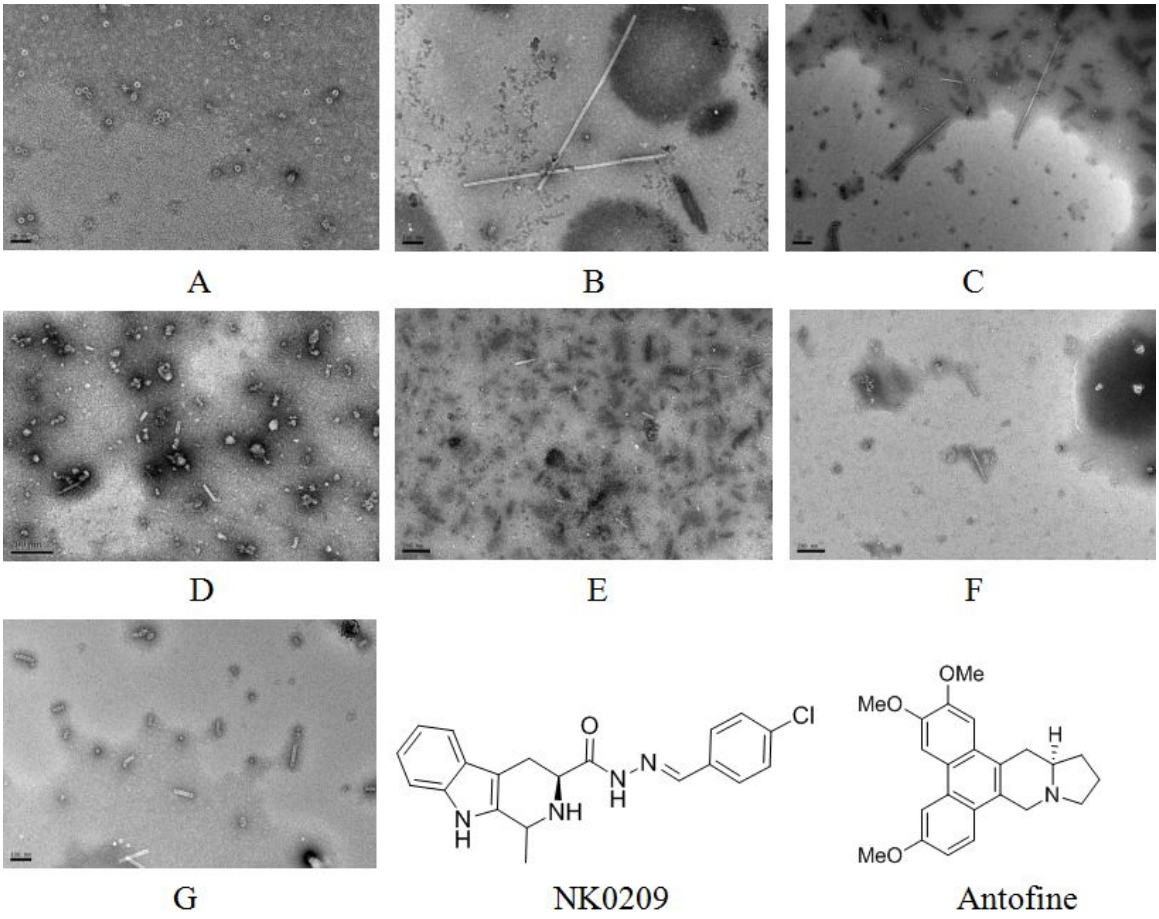
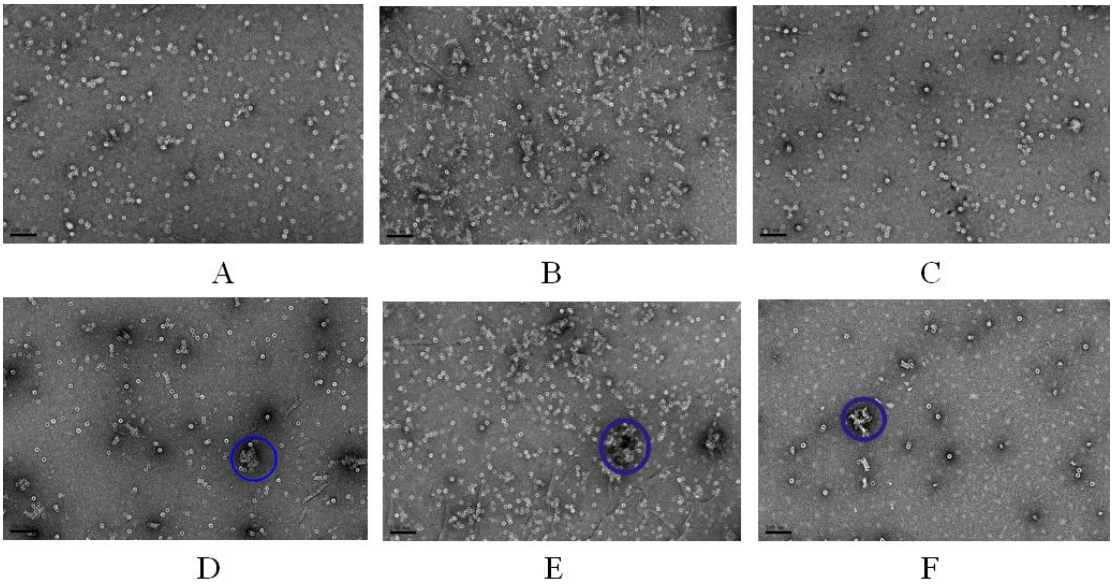


Figure 5.



TOC ***graphic***

Agrochemical Bioregulators