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Structural Simplification of Marine Natural Products: Discovery of Hamacanthin Derivatives Containing Indole and Piperazinone as Novel Antiviral and Anti-phytopathogenic-fungus Agents

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ABSTRACT: With the increasing severity of plant diseases and the emergence of pathogen resistance, there is an urgent need for the development of new efficient and environment-friendly pesticides. Marine natural product (MNP) resources are rich and diverse. Structural simplification based on MNPs is an important strategy to find novel pesticide candidates. In this work, the marine natural product 6″-debromohamacanthin A (**1a**) was efficiently prepared and selected as the parent structure. A series of hamacanthin derivatives were designed, synthesized, and studied on the antiviral and antifungal activities. Most of these compounds displayed higher antiviral activities than ribavirin. The antiviral activities of compounds **1a** and **13e**−**13h** are similar to or higher than that of ningnanmycin (perhaps the most efficient anti-plant-virus agent). Compound **13h** was selected for further antiviral mechanism research via transmission electron microscopy, molecular docking, and fluorescence titration. The results showed that compound **13h** could bind to TMV CP and interfere with the assembly process of TMV CP and RNA. In addition, these hamacanthin derivatives also exhibited broad-spectrum inhibitory effects against eight common agricultural pathogens. Compounds **1a**, **12b**, and **12f** with excellent fungicidal activities can be considered as new fungicidal candidates for further research. These results provide a basis for the application of hamacanthin alkaloids in crop protection.

KEYWORDS: *marine natural product, hamacanthin alkaloids, anti-TMV activity, fungicidal activity, mode of action*

INTRODUCTION

Sustainable nutrition and food security are key themes in the United Nations Sustainable Development Goals. By 2050, the world will need to feed about 9 billion people. The need to increase food production has never been so urgent.[1] Plant diseases caused by viral pathogens are seriously threatening the safety and stability of crop production. As reflected in the field of crop protection, modern chemical fungicides and antiviral drugs can be used to reduce plant diseases and increase crop yields. According to the analysis of major crops such as rice, wheat, barley, corn, etc., without crop protection, the yield will be reduced by 50%.[2] However, the emergence of pathogen resistance has made some pesticides inefficient, and after the process of long-term use, some pesticides have caused adverse effects such as environmental pollution and harm to human health. Therefore, the modern agrochemical industry needs innovative solutions to respond to current and future challenges.[3,4]

Seventy percent of the Earth's surface area is the ocean, which is rich in biological resources. The unique extreme environment of the ocean, such as high salt, high pressure, low temperature, etc., has forced marine organisms to form many marine natural products (MNPs) with unique structures and significant biological activities.[5,6] These natural products have special frameworks that can interact with biological macromolecules and have good effects in biological regulation or treatment, which provides them with great advantages for being developed into new drugs. The diversity of structure and prevalidated biological activity has made natural products valuable lead compounds for drug discovery.[7,8] However, unmodified natural products often have some shortcomings, such as limited compound availability, poor solubility, and the structural complexity of MNPs, which results in synthetic difficulties and so on.[9,10] Simplifying the complex structures of NPs without interfering with the desired biological activity provides an alternative approach for both NP lead optimization and the development of a new generation of NP-based drugs. Many successful examples have validated the effectiveness of structural simplification in the development of NP-based drugs, such as fingolimod, bicyclol, eribulin, and vorinostat.[7]

The biindole alkaloid hamacanthin was first isolated from the deep-water sponge *Hamacantha* sp. in 1994.[11] Then, Jung's group isolated hamacanthin alkaloids (Figure 1) from the sponge *Spongosorites* sp.[12,13] 6″-Debromohamacanthin A (**1a**), hamacanthin A (**1b**), hamacanthin B (**1e**), and their analogues (Figure 1) have various kinds of biological

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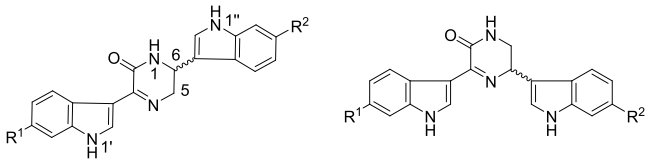

1a: R^1 = Br, R^2 = H
1b: R^1 = Br, R^2 = Br
1c: R^1 = H, R^2 = H
1d: R^1 = H, R^2 = Br
1e: R^1 = Br, R^2 = Br
1f: R^1 = H, R^2 = Br
1g: R^1 = H, R^2 = H
1h: R^1 = Br, R^2 = H

Figure 1. Hamacanthins A and B and their Congeners.

activities, including cytotoxicity,[12−14] bactericidal activity,[12,13,15] antimicrobial activity,[14] etc. The complex chemical structures of hamacanthin alkaloids complicated their total synthesis,[15−20] which limits the systematic biological activity research of these molecules. At present, the biological activity research of hamacanthin alkaloids mainly focuses on the treatment of human diseases, while there are few reports on the agricultural application of these molecules.

We have long been committed to the discovery of new pesticide candidates based on MNPs.[21,22] In this work, the marine natural product 6″-debromohamacanthin A (**1a**) was efficiently prepared and selected as the parent structure. A series of chiral hamacanthin derivatives were designed (Figure 2) and synthesized based on previous research foundation and our research experience. The antiviral and antifungal effects of these alkaloids were systematically evaluated. The antiviral mechanism of optimized compounds was further studied through transmission electron microscopy (TEM), molecular docking, or fluorescence titration.

MATERIALS AND METHODS

Materials and Equipment. *Chemicals.* The reagents were purchased from commercial sources and were used as received. All anhydrous solvents were dried and purified by standard techniques prior to use.

Instruments. The melting points of the compounds were tested on an X-4 binocular microscope (Beijing Tech Instruments Company). NMR spectra were obtained with a Bruker AV 400 MHz (100 MHz for ^{13}C) spectrometer with either $CDCl_3$ or DMSO-d_6 as the solvent. High-resolution mass spectra were obtained with an FT-ICR mass spectrometer (Ionspec, 7.0 T). The in vitro TMV rod assembly inhibition and 20S CP disk assembly inhibition were tested via transmission electron microscopy (Tecnai G2 F20). The single-crystal data were obtained via an X-ray single-crystal diffractometer (Supernova; Agilent Technologies Inc.). The photoluminescence spectra data were measured on a Hitachi F-2700 photoluminescence spectrophotometer. Compounds **4a**−**4e**[23,24] and **5a**−**5k**[25] were prepared by using reported methods, and the data are accordance with that of the literature. The detailed synthetic procedures of compounds **9**−**14** can be seen in the Supporting Information.

Preparation of (*Z*)-*N*-Benzyl-2-((*tert*-butoxycarbonyl)-amino)ethan-1-imine Oxide (2).[23] *N*-Benzylhydroxylamine (1.0 g, 8.1 mmol, 1.0 equiv) and *tert*-butyl (2-oxoethyl)carbamate (1.9 g, 12.2 mmol, 1.5 equiv) were dissolved in CH_2Cl_2 (15 mL), and then anhydrous magnesium sulfate (5.0 g) was added. The mixture was stirred at room temperature for 3 h, filtered, and concentrated in vacuum. The residue was purified by flash chromatography on a silica gel to give compound **2** as a brown oil. Yield: 37%; ^1H NMR (400 MHz, $CDCl_3$): δ 7.39 (s, 5H), 6.87 (s, 1H), 5.39 (s, 1H), 4.87 (s, 2H), 4.04 (t, J = 5.7 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.7, 129.3, 129.0, 128.8, 128.6, 128.5, 71.2, 65.6, 37.1, 28.2.

Preparation of *tert*-Butyl (2-(Benzyl(hydroxy)amino)-2-(1*H*-indol-3-yl)ethyl)carbamate (3).[23] Freshly distilled acetyl chloride (0.9 g, 11.0 mmol, 2.0 equiv) was added to the dry methanol (15 mL) solution at 0 °C under a N_2 atmosphere. The mixture was stirred for 10 min, and a mixture of indole (0.6 g, 5.5 mmol, 1.0 equiv) and compound **2** (0.7 g, 5.5 mmol, 1.0 equiv) in methanol (15 mL) was added. The mixture was stirred for further 6 h, then quenched with sodium bicarbonate solution (100 mL), and extracted with CH_2Cl_2 (50 mL × 2). The organic phases were combined and dried with anhydrous magnesium sulfate, filtrated, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel to give compound **3** as a white solid. Yield: 38%; mp: 136−140 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 11.01 (s, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.58 (s, 1H), 7.40−7.15 (m, 7H), 7.06 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 7.4 Hz, 1H), 6.46 (s, 1H), 4.17 (t, J = 6.8 Hz, 1H), 3.63−3.49 (m, 2H), 3.33 (s, 2H), 1.38 (d, J = 16.4 Hz, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.3, 137.0, 133.5, 126.4, 126.2, 125.2, 124.9, 123.8, 121.8, 118.3, 116.9, 115.8, 108.8, 75.1, 60.0, 57.8, 40.6, 25.7.

Preparation of *tert*-Butyl (2-(2-(6-Bromo-1*H*-indol-3-yl)-2-oxoacetamido)-2-(1*H*-indol-3-yl)ethyl)carbamate (6). To a stirred solution of compound **4a** (0.3 g, 1.1 mmol, 1.0 equiv) in CH_2Cl_2 (20 mL) were added Et_3N (1.3 g, 1.3 mmol, 1.2 equiv) and compound **5a** (0.2 g, 1.2 mmol, 1.1 equiv) at 0 °C. The mixture was stirred at 0 °C for 2 h and at 25 °C for 12 h, and then H_2O (50 mL) was added. The mixture was extracted with CH_2Cl_2 (30 mL × 2). The combined organic phase was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to obtain

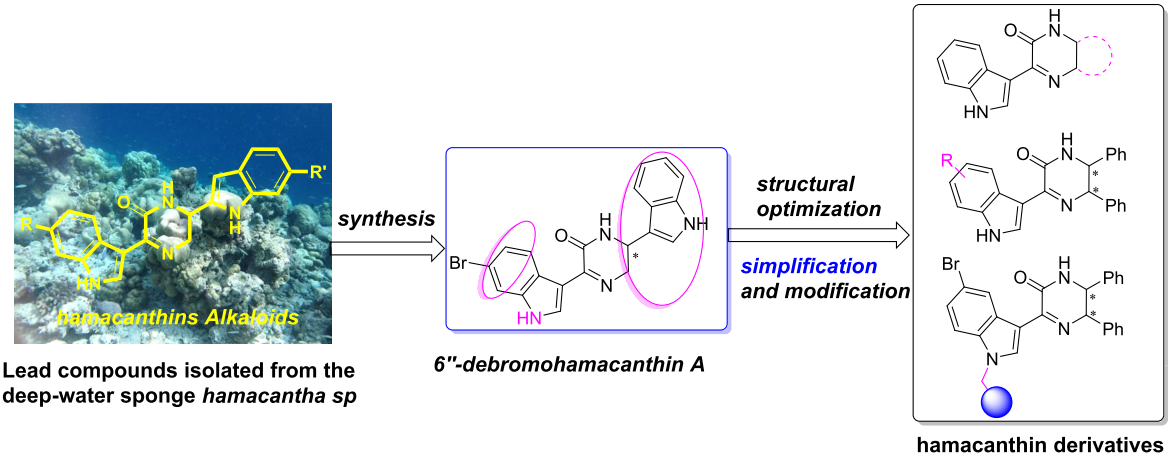

Figure 2. Design of hamacanthin analogues.

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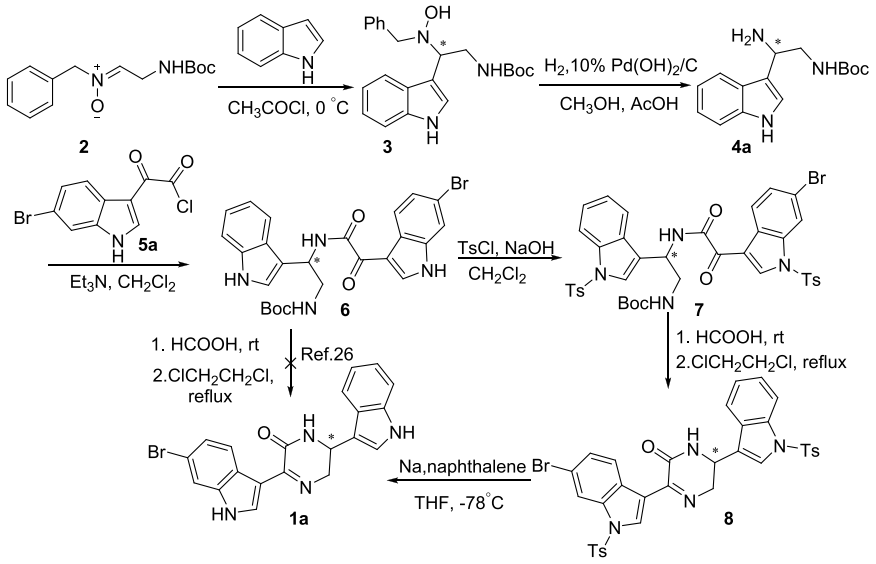

Figure 3. Synthesis of the natural product 6″-debromohamacanthin A (**1a**).

compound **6** as a brown solid, which was directly used for the next step without further purification.

Preparation of *tert*-Butyl (2-(2-(6-Bromo-1-tosyl-1*H*-indol-3-yl)-2-oxoacetamido)-2-(1-tosyl-1*H*-indol-3-yl)ethyl)-carbamate (7).[26] To a stirred solution of compound **6** (1.2 g, 2.29 mmol, 1.0 equiv) in CH_2Cl_2 (30 mL) were added 4-tosyl chloride (TsCl) (1.09 g, 5.73 mmol, 2.5 equiv), NaOH (0.14 g, 3.44 mmol, 1.5 equiv), and Et_3N (0.58 g, 5.73 mmol, 2.5 equiv). The mixture was stirred at 25 °C for 24 h, and then H_2O (50 mL) was added. The mixture was extracted with CH_2Cl_2 (50 mL × 2). The combined organic phase was dried with anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography to obtain compound **7**. Brown solid, yield: 50%; mp: 123−126 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.34 (s, 1H), 8.22−8.13 (m, 2H), 7.98 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H), 7.66−7.54 (m, 2H), 7.47 (d, J = 8.8 Hz, 1H), 7.39−7.20 (m, 7H), 5.49−5.22 (m, 1H), 4.85 (s, 1H), 3.71 (d, J = 6.3 Hz, 2H), 2.37 (d, J = 15.5 Hz, 6H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.9, 160.9, 156.7, 146.4, 145.3, 138.5, 135.3, 134.9, 134.8, 134.2, 130.5, 130.0, 128.7, 128.5, 127.3, 127.0, 126.9, 125.2, 124.0, 123.6, 123.5, 119.8, 119.7, 116.3, 115.8, 113.9, 80.3, 47.7, 43.6, 28.3, 21.7.

Preparation of 3-(6-Bromo-1-tosyl-1*H*-indol-3-yl)-6-(1-tosyl-1*H*-indol-3-yl)-5,6-dihydro-pyrazin-2(1*H*)-one (8).[19,26] To a solution of compound **7** (0.2 g, 0.24 mmol, 1.0 equiv) in CH_2Cl_2 (20 mL) was added anhydrous formic acid (20 mL). The mixture was stirred for 12 h at 25 °C. After completion, the solvent was removed in vacuum. The residue was taken into 60 mL of 1,2-dichloroethane. The mixture was treated with formic acid until pH = 4 and heated to reflux for 1 h and then concentrated. The residue was purified by column chromatography to obtain compound **8**. White solid, yield: 66%; mp: 154−158 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.25 (s, 1H), 8.84 (s, 1H), 8.21 (d, J = 8.7 Hz, 1H), 8.13 (s, 1H), 7.99 (d, J = 7.8 Hz, 2H), 7.87 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.58 (s, 1H), 7.45 (t, J = 9.7 Hz, 3H), 7.41−7.25 (m, 4H), 6.61 (d, J = 7.9 Hz, 2H), 5.12 (s, 1H), 4.21 (s, 2H), 2.19 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.6, 156.4, 146.3, 144.9, 134.7, 134.5, 133.5, 133.3, 131.9, 130.6, 129.6, 128.3, 127.4, 126.9, 126.2, 125.4, 125.2, 124.4, 123.5, 121.5, 120.5, 118.2, 115.8, 115.6, 113.3, 52.2, 45.4, 20.9, 20.7.

Preparation of 3-(6-Bromo-1*H*-indol-3-yl)-6-(1*H*-indol-3-yl)-5,6-dihydropyrazin-2(1*H*)-one (1a).[27] A solution of sodium (33 mg, 1.42 mmol, 9.0 equiv) and naphthalene (0.152 g, 1.18 mmol, 7.5 equiv) in dry THF (3 mL) was stirred for 2 h at 25 °C, then cooled to −78 °C, and added with a THF solution of compound **8** (0.113 g, 0.158 mmol, 1.0 equiv). The mixture was stirred at −78 °C for 2 h and then extracted with ethyl acetate (30 mL × 2). The combined organic phase was washed with brine, dried with anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography to give compound **1a**. Brown solid, yield: 92%; mp: 198−201 °C; $[\alpha]_D^{29}$ = +0.8° (c 1.0, THF); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.63 (d, J = 9.4 Hz, 1H), 11.05 (d, J = 9.5 Hz, 1H), 8.80 (d, J = 11.3 Hz, 1H), 8.53−8.38 (m, 1H), 8.32 (q, J = 11.4, 8.2 Hz, 1H), 7.79−7.57 (m, 2H), 7.39 (q, J = 11.2, 7.8 Hz, 1H), 7.33−7.18 (m, 2H), 7.18−6.98 (m, 2H), 5.01 (s, 1H), 4.12 (d, J = 9.3 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.6, 157.4, 137.0, 136.4, 132.7, 125.5, 125.0, 124.13, 123.4, 123.2, 121.2, 118.9, 118.7, 114.7, 114.2, 112.7, 111.7, 111.0, 53.6, 46.4; HR-MS (ESI): calcd. for $C_{20}H_{16}BrN_4O$ $[M + H]^+$: 407.0502, found (ESI$^+$): 407.0489.

Biological Assay. The anti-TMV activities and fungicidal activities of the target compounds were evaluated with three replicates at 25 ± 1 °C. Activity results were estimated according to a percentage scale of 0−100 (0: no activity; 100: totally inhibited).

The detailed procedures of anti-TMV activity and fungicidal activity were carried out applying reported methods,[28,29] which also can be found in the Supporting Information.

Mode of Action Studies. The mode of action studies of in vitro TMV and 20S CP disk assembly inhibition were carried out using the literature method,[28,29] and the detailed procedures also can be seen in the Supporting Information.

Fluorescence Spectroscopy. The binding constant was calculated by using fluorescence titration between TMV CP and compound **13h** by the literature method.[30] The detailed procedures can be found in the Supporting Information.

RESULTS AND DISCUSSION

Chemistry. Since the first report of alkaloid hamacanthin,[11] scientists have completed the synthesis of racemic and optical isomers of alkaloids hamacanthin A, hamacanthin B, and their derivatives.[16−20,31] In these synthetic methods, it is found that 1-(1*H*-indol-3-yl)ethane-1,2-diamine and its derivatives were the key intermediates. The amino alkylation of indoles at the 3-position using the nitrone **2** was selected to synthesize compound **3**.[23] The key intermediate **4a** was obtained by simultaneous reduction and debenzylation of compound **3** under mild conditions with a quantitative yield.[19] Although deprotection of **6** by formic acid to the primary amine was achieved,[19] many attempts to provide the expected 6″-debromohamacanthin A (**1a**) by ring closing

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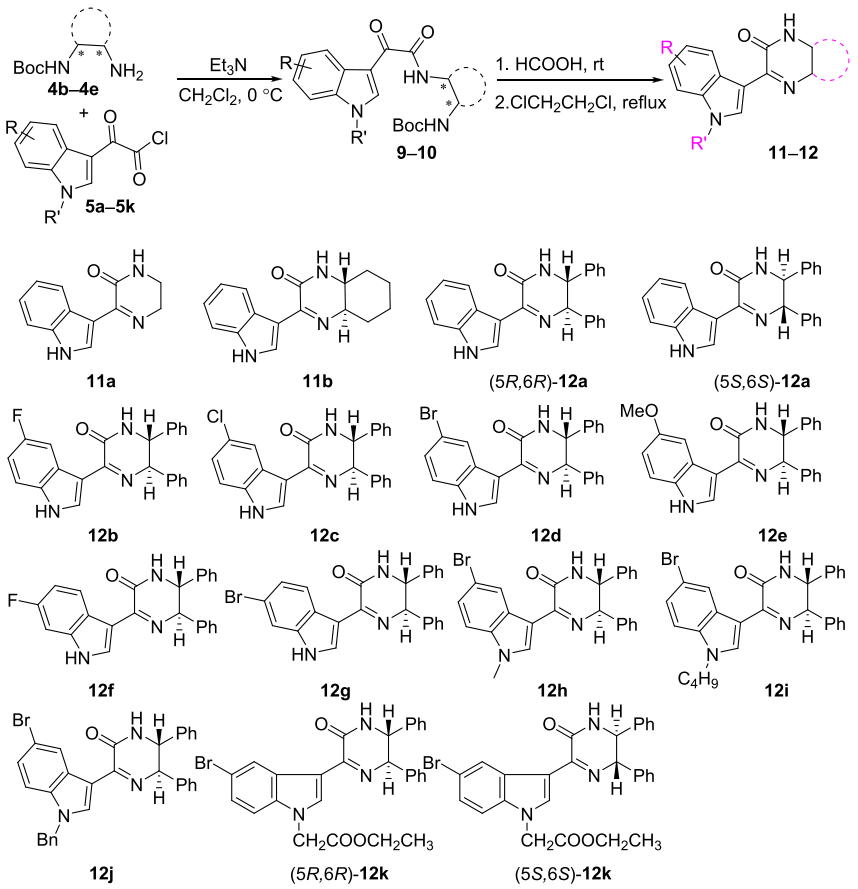

Figure 4. Synthesis of compounds **11a**, **11b**, and **12a−12k**.

failed. Fortunately, when N−H on the indole of intermediate **7** was protected by the tosyl (Ts) group, the cyclization proceeded smoothly, and compound **8** was obtained with a 98% yield. The Ts protective group of the nitrogen atom in the indole structure could be removed with sodium and naphthalene at low temperatures successfully (Figure 3). Compounds **11a**,**11b**, and **12a−12k** with different substituents of indole structures and 5,6-dihydro-1(2*H*) pyrazinone structural units were prepared by changing 1,2-diamines **4** and **5** (Figure 4).

According to the reported cyclization method,[26] the transamidation−cyclization was observed in the regioisomer to result in the production of both hamacanthin A and hamacanthin B derivatives. Optically pure (1*R*,2*R*)-*N*-Boc-1,2-cyclohexane diamine (**4c**), (1*R*,2*R*)-*N*-Boc-1,2-diphenylethylene diamine (**4d**), and (1*S*,2*S*)-*N*-Boc-1,2-diphenylethylene diamine (**4e**) were selected for structural simplification of the natural product hamacanthins. It was not known whether the stereochemical structure can be maintained. A flaky crystal of **12k**, which was prepared from (1*S*,2*S*)-*N*-Boc-1,2-diphenylethylene diamine (**4e**), was obtained by recrystallization from THF:CH_3OH (v/v = 1: 1). Single-crystal X-ray diffraction analysis with Cu Kα [Flack parameter = −0.018(14), CCDC 2089734] suggested the absolute configuration of **12k** to be 5*S*,6*S* (Figure 5). Accordingly, the structure of (5*S*,6*S*)-**12k** was corroborated. By analyzing the single-crystal X-ray diffraction of compound (5*S*,6*S*)-**12k** and comparing the optical rotation values of compounds (5*R*,6*R*)-**12a**, (5*S*,6*S*)-**12a**, (5*R*,6*R*)-**12k**, and (5*S*,6*S*)-**12 k**, it can be determined that the stereochemical structure can be maintained. The structures of other chiral compounds **11b** and **12−14** were also clear demonstrations.

Compound (5*S*,6*S*)-**12l** was obtained by refluxing (5*S*,6*S*)-**12k** in the presence of hydrazine hydrate (Figure 6). Then, two series of hamacanthin derivatives were obtained as shown in Figures 6 and 7. Compound (5*S*,6*S*)-**12l** reacted with isothiocyanates or aldehydes in THF under refluxing conditions to give compounds **13a−13k** and **14a−14h** in good to moderate yields, respectively.

Phytotoxic Activity. The phytotoxic activity tests demonstrated that the hamacanthin analogues **1a**, **11a−11b**, **12a−12l**, **13a−13k**, and **14a−14h** were safe for testing on plants at 500 μg/mL. The detailed test procedures can be seen in the Supporting Information.

Antiviral Activity In Vivo. The activities of 6″-debromohamacanthin A (**1a**) and its analogues **11a**, **11b**, **12a−12l**, **13a−13k**, and **14a−14h** against TMV are listed in Table 1 with the commercial plant virucides ningnanmycin and ribavirin as the controls. Most of these compounds exhibited higher antiviral activities than ribavirin. The antiviral activities of compounds **1a** and **13e−13h** are similar to or higher than that of ningnanmycin, which may be the most effective plant viral inhibitor at present.

As shown in Table 1, the substitutes of the piperazinone ring have a great influence on antiviral activities. Removal of the indole group at the 6-position of **1a** sharply decreased the anti-TMV activity (inhibitory effect: **1a** > **11a**). Compound **11b** with a 5,6-ring structure showed the same level of biological activity as compound **11a**. The introduction of a phenyl group at the 5,6-position of 5,6-dihydro-1(2*H*)

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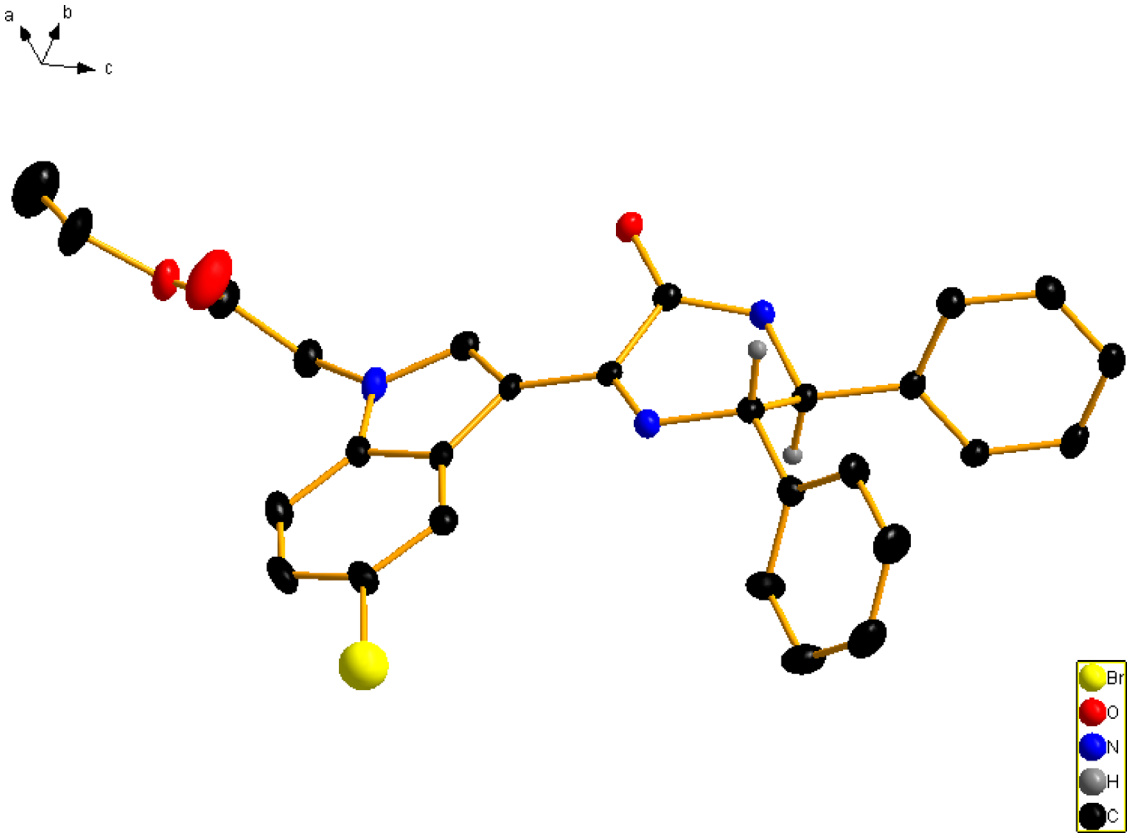

Figure 5. X-ray crystal structure of (5*S*,6*S*)-**12k**.

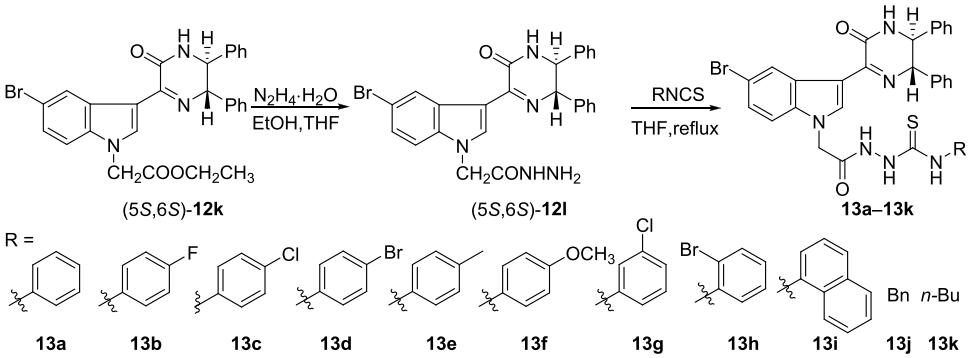

Figure 6. Synthesis of compounds **12l** and **13a**−**13k**.

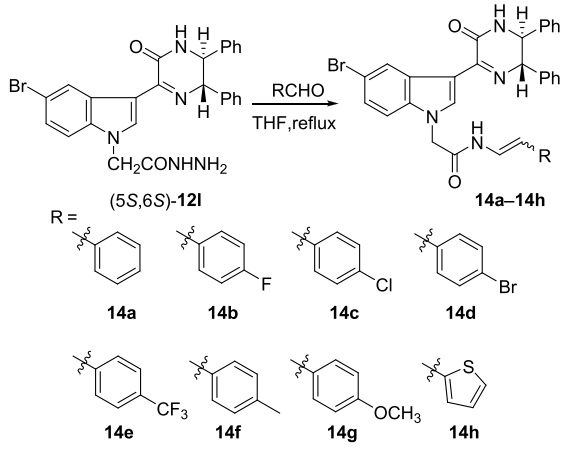

Figure 7. Synthesis of compounds **14a**−**14h**.

pyrazinone can improve the anti-TMV activity, and the chirality at the 5,6-position has little effect on the activity (inhibitory effect: (5*R*,6*R*)-**12a** ≈ (5*S*,6*S*)-**12a** > **11a**). Compounds **12a**−**12d** exhibited about a similar level of anti-TMV activities, which indicated that the introduction of halogen at the 5-position of the indole ring had little effect on the activity. However, the introduction of a methoxy group at the 5-position, a halogen group at the 6-position, and an alkyl or benzyl group at the 1-position of the indole ring was not conducive to the improvement of antiviral activity (inhibitory effect: **12a** > **12e**−**12 k**). (5*S*,6*S*)-**12k** with relatively high antiviral activity was further modified. To our delight, the introduction of a hydrazide group at the 1-position of the indole ring significantly improved the biological activity (inhibitory effect: **12l** > (5*S*,6*S*)-**12k**).

Thiourea is a kind of pharmacophore widely existing in drug molecules. The introduction of thiourea can increase the

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Table 1. In Vivo Antiviral Activities of 1a, 11a, 11b, 12a−12l, 13a−13k, 14a−14h, Ribavirin, and Ningnanmycin against TMV

compd	concn (μg/mL)	inactive effect (%)[a]	curative effect (%)[a]	protective effect (%)[a]	compd	concn (μg/mL)	inactive effect (%)[a]	curative effect (%)[a]	protective effect (%)[a]
1a	500	53 ± 1	51 ± 1	56 ± 1	**13c**	500	49 ± 2	46 ± 2	48 ± 4
	100	30 ± 1	25 ± 1	29 ± 1		100	23 ± 3	23 ± 1	23 ± 2
11a	500	44 ± 2	39 ± 2	32 ± 2	**13d**	500	45 ± 1	45 ± 3	47 ± 3
	100	9 ± 1	15 ± 1	11 ± 1		100	28 ± 1	23 ± 4	26 ± 2
11b	500	36 ± 2	32 ± 3	40 ± 3	**13e**	500	58 ± 2	58 ± 2	61 ± 2
	100	18 ± 3	12 ± 1	15 ± 2		100	25 ± 2	23 ± 4	28 ± 4
(5*R*,6*R*)-**12a**	500	48 ± 3	44 ± 2	50 ± 3	**13f**	500	57 ± 4	55 ± 4	59 ± 3
	100	19 ± 2	16 ± 3	17 ± 2		100	25 ± 3	26 ± 2	29 ± 2
(5*S*,6*S*)-**12a**	500	47 ± 4	45 ± 2	51 ± 3	**13g**	500	55 ± 3	53 ± 2	57 ± 3
	100	16 ± 1	19 ± 1	20 ± 1		100	27 ± 1	25 ± 3	27 ± 3
12b	500	47 ± 3	45 ± 4	49 ± 1	**13h**	500	60 ± 1	59 ± 1	63 ± 1
	100	22 ± 2	17 ± 3	23 ± 3		100	34 ± 2	31 ± 2	37 ± 2
12c	500	43 ± 1	42 ± 1	45 ± 1	**13i**	500	45 ± 3	43 ± 2	47 ± 3
	100	22 ± 1	15 ± 1	17 ± 1		100	17 ± 1	18 ± 3	21 ± 1
12d	500	48 ± 2	46 ± 2	53 ± 2	**13j**	500	46 ± 3	47 ± 4	48 ± 4
	100	26 ± 3	19 ± 1	24 ± 2		100	21 ± 2	21 ± 3	21 ± 4
12e	500	25 ± 2	21 ± 3	23 ± 1	**13k**	500	52 ± 3	50 ± 4	55 ± 2
	100	0	0	0		100	25 ± 1	21 ± 3	29 ± 4
12f	500	34 ± 3	36 ± 4	39 ± 2	**14a**	500	30 ± 3	28 ± 2	30 ± 4
	100	17 ± 1	13 ± 2	23 ± 3		100	11 ± 3	12 ± 1	11 ± 3
12g	500	27 ± 2	22 ± 3	25 ± 3	**14b**	500	38 ± 2	35 ± 3	38 ± 3
	100	0	0	0		100	26 ± 4	21 ± 3	26 ± 2
12h	500	39 ± 2	38 ± 2	40 ± 2	**14c**	500	32 ± 1	32 ± 2	35 ± 4
	100	19 ± 2	17 ± 2	21 ± 2		100	10 ± 2	9 ± 3	13 ± 2
12i	500	15 ± 2	11 ± 3	12 ± 2	**14d**	500	36 ± 2	37 ± 4	39 ± 3
	100	0	0	0		100	29 ± 1	23 ± 1	25 ± 3
12j	500	35 ± 1	33 ± 3	36 ± 1	**14e**	500	23 ± 3	19 ± 3	23 ± 2
	100	12 ± 2	10 ± 3	14 ± 2		100	9 ± 2	9 ± 3	11 ± 2
(5*R*,6*R*)-**12k**	500	39 ± 1	37 ± 2	42 ± 3	**14f**	500	41 ± 4	39 ± 4	43 ± 3
	100	17 ± 2	16 ± 1	19 ± 2		100	16 ± 1	17 ± 1	19 ± 2
(5*S*,6*S*)-**12k**	500	40 ± 1	38 ± 4	44 ± 3	**14g**	500	44 ± 1	45 ± 2	47 ± 4
	100	20 ± 2	18 ± 1	20 ± 2		100	24 ± 3	21 ± 1	24 ± 4
12l	500	51 ± 4	49 ± 1	53 ± 1	**14h**	500	50 ± 1	48 ± 2	53 ± 3
	100	22 ± 2	20 ± 1	26 ± 1		100	26 ± 2	25 ± 3	28 ± 3
13a	500	48 ± 1	45 ± 2	49 ± 3	ribavirin	500	39 ± 1	37 ± 3	41 ± 1
	100	17 ± 3	19 ± 1	23 ± 1		100	13 ± 2	13 ± 1	15 ± 1
13b	500	44 ± 3	45 ± 2	47 ± 2	ningnanmycin	500	54 ± 1	53 ± 3	59 ± 2
	100	25 ± 3	21 ± 4	23 ± 1		100	27 ± 2	25 ± 2	27 ± 3

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

hydrogen bond interaction between drug molecules and biomacromolecules. Based on this, a series of thiourea derivatives **13a−13k** were designed and synthesized, and their antiviral activity was evaluated. As shown in Table 1, all the thiourea-containing compounds **13a−13 k** demonstrated higher antiviral activities than ribavirin. The difference between compounds **13a−13h** is the substituted group on the benzene ring. Compounds **13b−13d** with an electron-drawing group on the 4-position of the benzene ring showed similar antiviral activities with **13a**, which is slightly lower than that of **12l**. Compounds **13e−13h** exhibited significantly higher antiviral activities than **13a**, which indicated that the electron-donating group in the para position and the halogen-substituting group in ortho and meta positions of the benzene ring are beneficial to improve the activity. Compound **13h**, with better activity than ningnanmycin and **1a**, emerged as a new antiviral agent. Compared with phenyl, naphthalene and benzyl have little effect on biological activity, while alkanes are more beneficial to virus activity (inhibitory effect: **13k** > **13a** ≈ **13j** ≈ **13i**).

Acylhydrazone is also a good pharmacophore. We also prepared a series of acylhydrazone derivatives **14a−14e**. The anti-TMV activities of **14a−14e** are similar to or higher than that of ribavirin. Compound **14h** showed the same level of antiviral activity with **12l** and **1a**.

Through systematic structure−activity relationship studies, we found a highly effective antiviral candidate and a variety of effective structural skeletons.

Preliminary Mode of Action. The optimized compound **13h** was further investigated for its antiviral mechanism according to the reported method.[28] As shown in Figure 8, the 20S CP disk can be assembled with the RNA, forming a rod-like length of approximately 300 nm TMV virus particles (Figure 8A). A small amount of dimethylsulfoxide (DMSO) does not inhibit the assembly of viral particles (Figure 8B). Compound **13h** can significantly inhibit the assembly process

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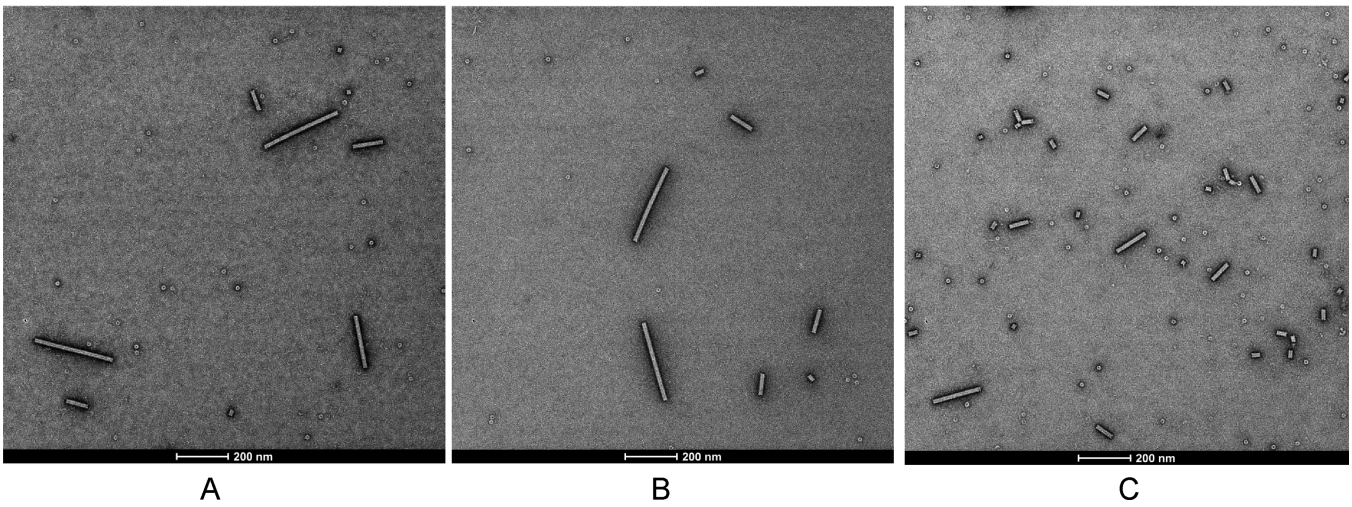

Figure 8. TMV rod assembly inhibition of compound **13h** (200 nm scale bar): (A) 20S CP disk + RNA, (B) 20S CP disk + RNA + 1/100 DMSO, and (C) 20S CP disk + RNA +10 μM **13h**.

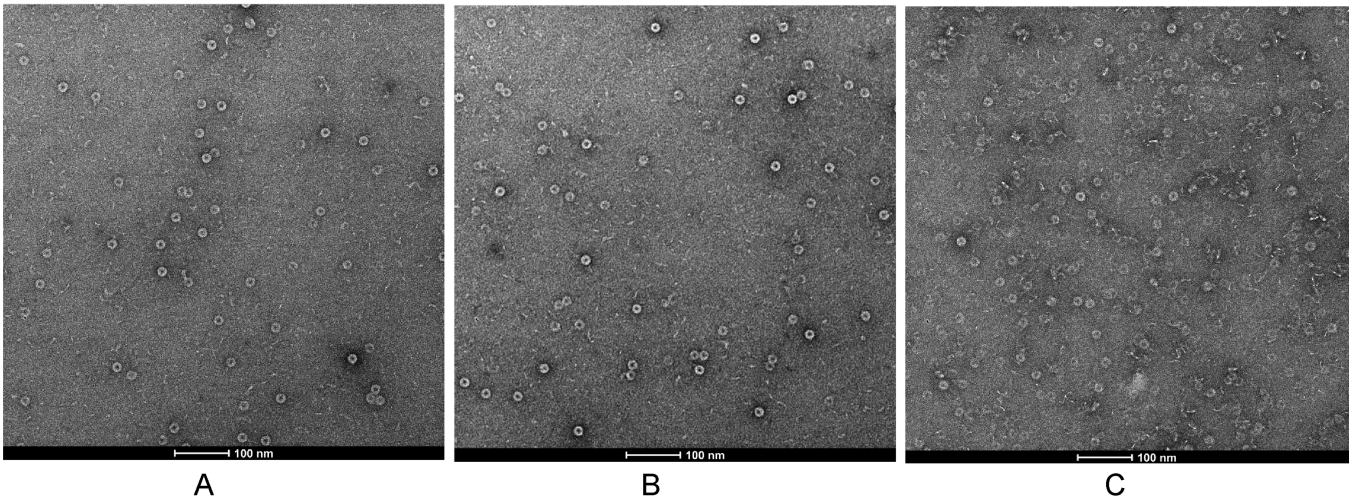

Figure 9. 20S CP disk assembly inhibition of compound **13h** (100 nm scale bar): (A) CP, (B) CP + 1/100 DMSO (100 nm scale bar), and (C) CP + 10 μM **13h**.

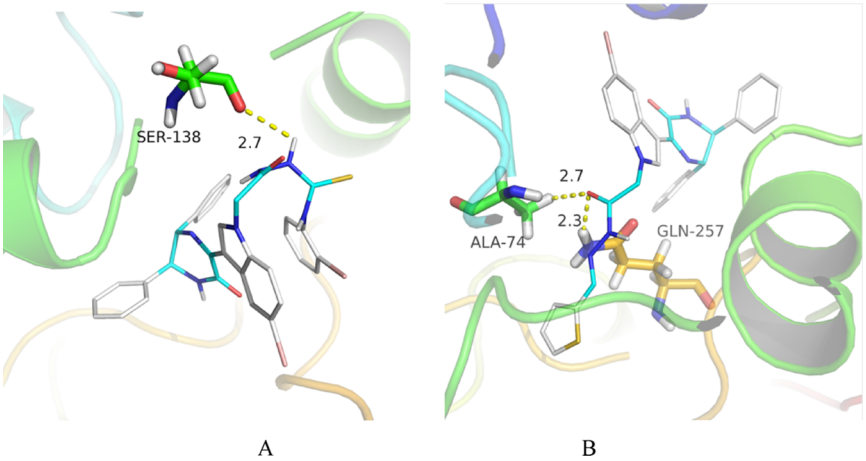

Figure 10. Molecule docking results of compounds **13h** and **14h** with TMV CP.

of TMV virus particles and result in no intact virus particles (Figure 8C). The 20S CP disk assembly experiment was further designed to study the interaction between **13h** and the 20S CP disk. 20S CP disks have a disk-like structure (Figure 9A), and a small amount of DMSO does not affect the formation of the 20S CP disk (Figure 9B). Compound **13h** can interact with the 20S CP disk and cause depolymerization of the 20S CP disk (Figure 9C), which

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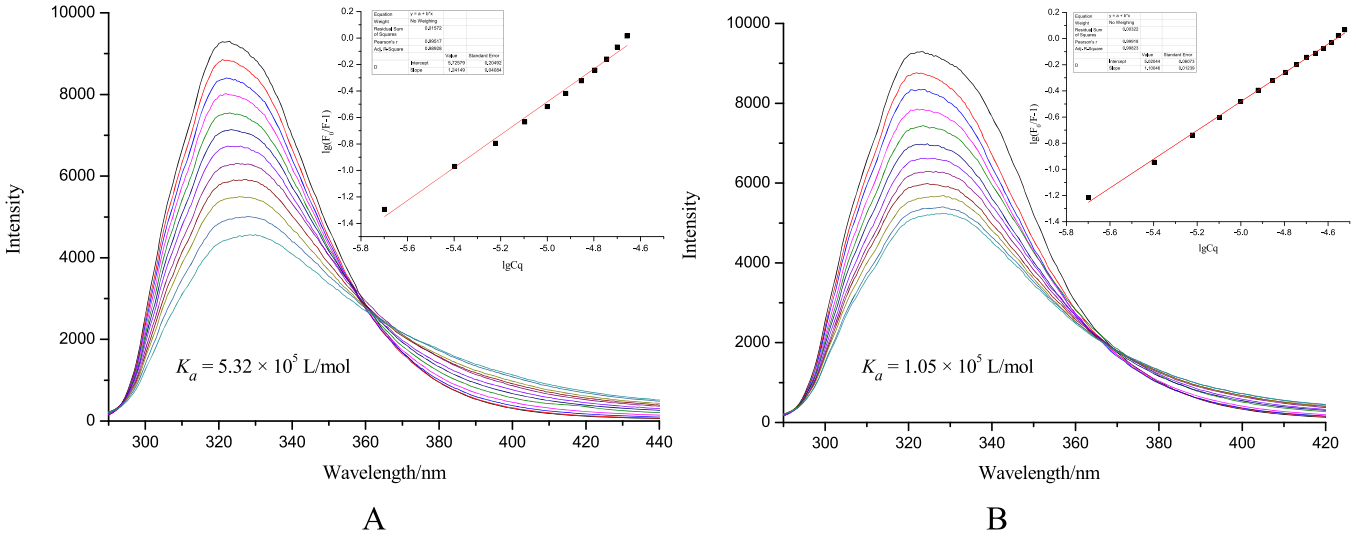

Figure 11. Fluorescence titration. Fluorescence emission spectra of TMV CP in the presence of (A) **13h** and (B) **14h** with different concentrations (λ_{ex} = 278 nm). Inset: the linear relationship for quenching TMV CP by **13h** and **14h**, respectively.

indicates that inhibition of virus assembly by interaction with CP was the main reason for the antiviral activity of these compounds. How these compounds affect the assembly process of virus particles needs to be further studied.

Molecular Docking. To further disclose the binding modes between hamacanthin analogues and TMV CP (PDB code: 1EI7), a molecular docking study was carried out using AutoDock-vina 1.1.2.[32] Combined with anti-TMV activity and molecular structure characteristics, compounds **13h** and **14h** were chosen for molecular docking research (Figure 10). As shown in Figure 10A, compound **13h** interacted with TMV CP through one conventional hydrogen bond. In this interaction, N−H of **13h** interacted with the oxygen atoms of the SER-138 residue by hydrogen bonding: N−H···O═C = 2.7 Å. Compound **14h** forms two hydrogen bonds with the amino acid residue ALA-74 (2.7 Å) and GLN-257 (2.3 Å) (Figure 10B). The results of molecular docking showed that hamacanthin analogues had a strong interaction with TMV CP, and the interaction sites and strength were greatly affected by the molecular structure.

Binding Constant of Compounds 13h and 14h to TMV CP. TMV CP is an important functional protein for TMV to infect the host and an essential protein for virus assembly initiation and elongation.[33] To further verify the interaction of 6″-debromohamacanthin A (**1a**) and its derivatives with TMV CP, the interaction of compounds **13h** and **14h** with TMV CP was measured with FT according to the reported literature.[34] The results of fluorescence spectroscopy analysis are exhibited in Figure 11 Compound **13h** has a good affinity to TMV CP, with a K_a value of 5.32×10^5 L/mol (Figure 11A), which is better than compound **14h** ($K_a = 1.05 \times 10^5$ L/mol, Figure 11B). This result confirms that compounds **13h** and **14h** can combine with TMV CP and the binding ability is consistent with the antiviral activity.

Fungicidal Activity. The fungicidal activities of 6″-debromohamacanthin A (**1a**) and its derivatives **11a**, **11b**, **12a**−**12l**, **13a**−**13k**, and **14a**−**14 h** were also evaluated, and the commercial fungicides chlorothalonil and carbendazim were used as controls (Table 2). At a concentration of 50 μg/mL, 6″-debromohamacanthin A (**1a**) and its derivatives have broad-spectrum inhibitory activities against all eight common agricultural pathogens. 6″-Debromohamacanthin A (**1a**), **12b**, and **12f** exhibited moderate to good antifungal activities against all the tested fungi. Compounds **12b**, **12f**, **12k**, **13h**, and **14a** not only gave higher inhibitory effects against *Alternaria solani* than chlorothalonil and carbendazim but also displayed more than 80% inhibitory effects on three pathogens including *Sclerotinia sclerotiorum*, *Physalospora piricola*, and *Rhizoctonia cerealis*. For *P. piricola*, some compounds of each series showed more than 80% inhibitory activities, such as **1a**, **12b**, **12c**, **13h**, and **14a**. The structure−activity relationship was different from that of antiviral activity. Compounds **12b** and **12c** with poor antiviral activities showed good fungicidal effects. Therefore, hamacanthin alkaloids also can be considered as novel fungicide candidates for further research.

In summary, using the natural product 6″-debromohamacanthin (**1a**) as the parent structure, three series of hamacanthin derivatives were designed, synthesized, and investigated for the antiviral and antifungal activities. Starting from (1*R*,2*R*)-*N*-Boc-1,2-diphenylethylene diamines or (1*S*,2*S*)-*N*-Boc-1,2-diphenylethylene diamines can efficiently achieve the structure-simplified hamacanthin derivatives. Most of these compounds exhibited higher antiviral activities than ribavirin. Compounds **1a** and **13e**−**13 h** displayed similar or higher anti-TMV activities than ningnanmycin at 500 μg/mL. Compound **13h** was selected for further anti-TMV mechanism research by TEM, molecular docking, and fluorescence titration. The results revealed that compound **13h** could bind to TMV CP with an excellent affinity (K_a = 5.32×10^5 L/mol), leading to inhibition of viral assembly. Furthermore, these hamacanthin derivatives also exhibited broad-spectrum fungicidal activities. Compounds **12b**, **12f**, **12k**, **13h**, and **14a** exhibited moderate to good antifungal activities, with an activity of more than 80% against some fungi, such as *S. sclerotiorum*, *P. piricola*, and *R. cerealis*. This work involves leader selection, design and synthesis, structure−activity relationship, and mechanism of action, which lays a foundation for the application of these compounds as plant disease protective agents.

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Table 2. Fungicidal Activities of 1a, 11a, 11b, 12a−12l, 13a−13k, 14a−14h against Eight Kinds of Fungi

	fungicidal activities (%)[a] at 50 μg/mL							
compd	S.S[b]	C.H[b]	F.C[b]	P.C[b]	A.S[b]	P.P[b]	R.C[b]	P.G[b]
1a	83 ± 1	61 ± 2	48 ± 1	85 ± 2	81 ± 1	80 ± 2	94 ± 1	64 ± 2
11a	12 ± 2	22 ± 3	14 ± 1	3 ± 1	35 ± 2	32 ± 1	22 ± 1	0
11b	28 ± 1	15 ± 2	4 ± 1	9 ± 1	13 ± 3	6 ± 1	7 ± 1	18 ± 1
(5*R*,6*R*)-**12a**	50 ± 2	37 ± 1	26 ± 1	27 ± 1	42 ± 1	55 ± 3	53 ± 1	0
(5*S*,6*S*)-**12a**	33 ± 1	26 ± 1	36 ± 2	20 ± 1	24 ± 2	58 ± 1	49 ± 1	0
12b	80 ± 2	58 ± 1	42 ± 1	41 ± 1	56 ± 2	91 ± 1	80 ± 1	91 ± 1
12c	56 ± 1	55 ± 2	37 ± 1	24 ± 2	38 ± 1	80 ± 2	61 ± 1	64 ± 2
12d	9 ± 1	21 ± 2	18 ± 1	28 ± 1	29 ± 3	46 ± 2	25 ± 3	0
12e	44 ± 2	37 ± 3	36 ± 2	40 ± 3	6 ± 1	54 ± 1	44 ± 1	0
12f	81 ± 1	70 ± 2	58 ± 1	41 ± 1	50 ± 1	80 ± 2	88 ± 1	82 ± 2
12g	30 ± 1	26 ± 2	11 ± 1	16 ± 1	30 ± 1	39 ± 2	37 ± 2	0
12h	23 ± 1	5 ± 1	14 ± 1	12 ± 1	0	31 ± 1	16 ± 3	0
12i	15 ± 1	0	25 ± 3	12 ± 1	18 ± 2	31 ± 2	37 ± 1	0
12j	14 ± 1	0	14 ± 1	12 ± 1	24 ± 1	46 ± 2	30 ± 2	0
(5*R*,6*R*)-**12k**	40 ± 1	18 ± 1	21 ± 1	12 ± 1	13 ± 1	80 ± 1	12 ± 1	18 ± 1
(5*S*,6*S*)-**12k**	40 ± 1	18 ±	21 ± 1	12 ± 1	13 ± 1	80 ± 1	12 ± 1	18 ± 1
12l	36 ± 1	9 ± 1	8 ± 1	24 ± 2	31 ± 1	0	30 ± 1	77 ± 1
13a	42 ± 1	15 ± 2	19 ± 1	6 ± 2	6 ± 1	30 ± 2	24 ± 1	9 ± 1
13b	36 ± 1	3 ± 1	19 ± 2	9 ± 1	6 ± 1	9 ± 1	8 ± 1	9 ± 1
13c	42 ± 3	15 ± 1	17 ± 1	6 ± 1	6 ± 1	0	17 ± 1	9 ± 1
13d	22 ± 1	9 ± 1	15 ± 1	6 ± 1	13 ± 3	12 ± 1	11 ± 1	9 ± 1
13e	39 ± 1	12 ± 1	12 ± 1	9 ± 1	10 ± 1	6 ± 1	27 ± 2	5 ± 1
13f	64 ± 1	10 ± 1	12 ± 1	3 ± 1	50 ± 1	6 ± 1	17 ± 1	55 ± 2
13g	28 ± 1	9 ± 1	14 ± 1	6 ± 1	10 ± 1	21 ± 1	11 ± 1	9 ± 1
13h	28 ± 2	3 ± 1	12 ± 1	15 ± 1	10 ± 2	80 ± 2	16 ± 1	10 ± 2
13i	36 ± 1	3 ± 1	17 ± 1	6 ± 1	9 ± 1	6 ± 1	18 ± 2	5 ± 1
13j	50 ± 1	12 ± 2	21 ± 1	6 ± 1	31 ± 1	21 ± 1	17 ± 1	9 ± 1
13k	50 ± 1	18 ± 1	21 ± 3	7 ± 2	13 ± 1	36 ± 2	15 ± 1	9 ± 1
14a	51 ± 2	9 ± 1	31 ± 1	9 ± 1	9 ± 1	80 ± 1	12 ± 1	5 ± 1
14b	4 ± 2	6 ± 1	15 ± 1	9 ± 1	38 ± 1	50 ± 1	12 ± 2	9 ± 1
14c	33 ± 3	6 ± 1	12 ± 1	9 ± 1	31 ± 2	51 ± 2	17 ± 1	5 ± 1
14d	11 ± 1	6 ± 1	10 ± 1	3 ± 1	31 ± 3	51 ± 1	17 ± 3	18 ± 2
14e	83 ± 1	18 ± 3	23 ± 1	6 ± 1	38 ± 2	35 ± 1	24 ± 1	27 ± 3
14f	19 ± 3	15 ± 1	15 ± 1	3 ± 1	31 ± 1	35 ± 1	27 ± 1	9 ± 1
14g	42 ± 2	21 ± 1	17 ± 1	6 ± 1	44 ± 1	21 ± 1	35 ± 1	9 ± 1
14h	11 ± 1	15 ± 1	12 ± 1	6 ± 1	31 ± 1	6 ± 1	16 ± 1	10 ± 1
chlorothalonil[c]	100	74 ± 2	100	91 ± 1	<50	89 ± 1	100	100
carbendazim[c]	92 ± 1	52 ± 1	100	90 ± 2	<50	100	100	100

[a]Average of three replicates; all results are expressed as mean ± SD. [b]F.C, *Fusarium oxysporum* f. sp. *cucumeris*; C.H, *Cercospora arachidicola* Hori; P.P, *Physalospora piricola*; R.C, *Rhizoctonia cerealis*; A.S, *Alternaria solani*; P.G, *Pyricularia grisea*; P.C, *Phytophthora capsici*; S.S, *Sclerotinia sclerotiorum*. [c]The commercial agricultural fungicides were used for comparison of antifungal activity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c04098.

Detailed bio-assay procedures, detailed procedures for fluorescence titration, detailed synthesis of compounds **9**−**14**, and spectra data of compounds **1**−**14** (PDF)

Crystallographic data of (5*S*,6*S*)-**12k** (CIF)

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Notes

The authors declare no competing financial interest.

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