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Marine-Natural-Product Development: First Discovery of Nortopsentin Alkaloids as Novel Antiviral, Anti-phytopathogenic-Fungus, and Insecticidal Agents

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Supporting Information

ABSTRACT: Nortopsentin alkaloids were found to have potent antiviral, anti-phytopathogenic-fungus, and insecticidal activities for the first time. Antiviral-activity tests revealed that these compounds were very sensitive to substituents, so a series of nortopsentin derivatives were designed, synthesized, and systematically evaluated for their antiviral activities against TMV, their fungicidal activities, and their insecticidal activities on the basis of a structural-diversity-derivation strategy. Compounds **2e** (in vivo inactivation-, curative-, and protective-activity inhibitory rates of 50, 59, and 56%, respectively, at 500 μg/mL) and **2k** (in vivo inactivation-, curative-, and protective-activity inhibitory rates of 60, 58, and 52%, respectively, at 500 μg/mL), with excellent antiviral activities and good physicochemical properties, emerged as new lead compounds for novel-antiviral-agent development. Further fungicidal-activity tests revealed that these alkaloids displayed broad-spectrum fungicidal activities. Compounds **2f**, **2h**, and **2j** emerged as new lead compounds for antifungal-activity research. Additionally, all the compounds displayed good insecticidal activities against five kinds of insects, including *Mythimna separate*, *Helicoverpa armigera*, *Ostrinia nubilalis*, *Plutella xylostella*, and *Culex pipiens pallens*.

KEYWORDS: *nortopsentin alkaloids, natural product, antiviral activity, antifungal activity, insecticidal activity*

INTRODUCTION

Natural products are substances produced by living organisms and are found in nature. Throughout history, natural products have been used in a variety of roles, including as pest-control agents,[1−3] medicines[4−6] and dyes. In addition, they can also be used as inspiration in developing novel synthetic pesticides[7,8] that possess improved efficacies beyond those of the natural products.[9−11]

Nortopsentins A−C (Figure 1), a series of bis(indolyl)-imidazoles, were isolated from the deep-water marine sponge *Spongosorites ruetzleri*.[12] Nortopsentins A−C and the debrominated derivative, nortopsentin D (Figure 1), were found to display in vitro cytotoxicity against P388 cells and antifungal activities against *Candida albicans*.[12−14] Because of the considerable activities shown, a series of nortopsentin analogues containing five-membered heterocycles, such as bis-indolylthiophenes,[15] -furans,[16] -pyrroles,[17] -isoxazoles,[16] and -thiazoles,[18] were reported. Most of these analogues displayed good antitumor activities. However, the application of these alkaloids in plant-disease prevention has not been reported.

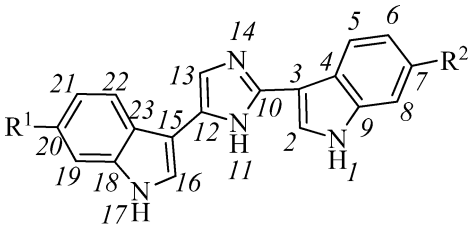

Figure 1. Structures of nortopsentin alkaloids A−D.

Plant diseases caused by viruses, fungi, bacteria, and oomycetes continuously affect people's daily lives and health. Tobacco mosaic virus (TMV), so-called because it was discovered for the first time on tobacco, was the first plant virus ever discovered. It is a well-studied plant virus and is known to infect more than 400 plant species belonging to 36 families, including tobacco, tomato, cucumber, pepper, and ornamental flowers.[19] Ribavirin, a widely used antiviral agent for preventing TMV disease, conveys an antiviral effect of less than 50% at 500 μg/mL. Therefore, the development of new, highly efficient antiviral agents is required.

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Taking into account the above findings, the discovery of novel antiviral agents from natural products was carried out by our group. A series of structurally novel natural products, such as phenanthroindolizidines,[20] harmine,[21] polycarpine,[22] and matrine,[23] have been found to have good antiviral activities. As part of our ongoing project, nortopsentin alkaloids and their derivatives were prepared and systematically evaluated for their antiviral, anti-phytopathogenic-fungus, and insecticidal activities.

MATERIALS AND METHODS

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 spectrometer with either $CDCl_3$ or DMSO-d_6 as the solvent. The chemical shifts (δ) were given in parts per million (ppm) and were measured downfield from the internal standard tetramethylsilane. High-resolution mass spectra were obtained with an FT-ICR mass spectrometer (Ionspec, 7.0 T). Compounds **10h**−**10m** were obtained from J&K Scientific.

General Procedures for the Preparation of Compounds 4a and 4b. To a solution of indole **3** (30 mmol) in CH_3CN was added 60% NaH (42 mmol) at 0 °C. The mixture was stirred for 10 min, and TsCl (33 mmol) was added. Then, the mixture was allowed to reach room temperature and was stirred for 4 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl. The resulting solution was extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to provide the corresponding compounds, **4a** and **4b**.

*N-Tosylindole (**4a**).* Brown solid; yield 99%; mp 76−78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 3.6 Hz, 1H), 7.52 (d, J = 7.6 Hz, 1H), 7.36−7.27 (m, 1H), 7.24−7.18 (m, 3H), 6.65 (d, J = 3.6 Hz, 1H), 2.33 (s, 3H).

*N-Tosyl-6-bromoindole (**4b**).* Brown solid; yield 99%; mp 131−132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 3.6 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.33 (dd, J = 8.4, 1.6 Hz, 1H), 7.25 (d, J = 8.4 Hz, 2H), 6.61 (d, J = 3.6 Hz, 1H), 2.36 (s, 3H).

General Procedures for the Preparation of Compounds 5a and 5b. To a mixture of $AlCl_3$ (300 mmol) in dichloromethane was added acetic anhydride (150 mmol) at 0 °C; then, compound **4** (50 mmol) was added, and the solution was allowed to reach room temperature and was stirred for 2 h. The resulting mixture was poured into ice water and extracted with dichloromethane (3 × 200 mL). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to provide the corresponding compounds, **5a** and **5b**.

*N-Tosyl-3-acetylindole (**5a**).* Red-brown solid; yield 99%; mp 143−145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (dd, J = 6.8, 1.6 Hz, 1H), 8.21 (s, 1H), 7.93 (dd, J = 7.0, 1.6 Hz, 1H), 7.84 (d, J = 8.4 Hz, 2H), 7.41−7.31 (m, 2H), 7.29 (d, J = 8.4 Hz, 2H), 2.58 (s, 3H), 2.37 (s, 3H).

*N-Tosyl-3-acetyl-6-bromoindole (**5b**).* Brown solid; yield 96%; mp 159−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.8 Hz, 1H), 8.16 (s, 1H), 8.10 (d, J = 1.6 Hz, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.45 (dd, J = 8.4, 1.6 Hz, 1H), 7.32 (d, J = 8.4 Hz, 2H), 2.56 (s, 3H), 2.40 (s, 3H).

General Procedures for the Preparation of Compounds 6a and 6b. To a solution of **5** (15 mmol) in ethyl acetate was added $CuBr_2$ (20 mmol). The mixture was heated at reflux for 5 h, then cooled to room temperature, and extracted with dichloromethane (3 × 200 mL). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel using petroleum ether and ethyl acetate (5:1, v/v) as the eluent to give **6a** and **6b**.

*N-Tosyl-3-(α-bromoacetyl)-indole (**6a**).* Yellow solid; yield 89%; mp 118−119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.30 (d, J = 7.6 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.85 (d, J = 8.0 Hz, 2H), 7.45−7.33 (m, 2H), 7.30 (d, J = 8.0 Hz, 2H), 4.36 (s, 2H), 2.38 (s, 3H).

*N-Tosyl-3-(α-bromoacetyl)-6-bromoindole (**6b**).* Brown solid; yield 76%; mp 162−163 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.11 (s, 1H), 7.84 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.4 Hz, 1H), 7.34 (d, J = 8.0 Hz, 2H), 4.33 (s, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.8, 146.6, 135.4, 134.0, 133.0, 130.6, 128.6, 127.2, 126.4, 124.3, 120.0, 117.8, 116.3, 31.1, 21.7.

3-Cyano-6-bromoindole (7b). To a solution of 6-bromoindole (19.4 g, 100 mmol) in DMF was added chlorosulfonamide isocyanate (10.4 mL, 12 mmol) at −50 °C in an Ar atmosphere. The temperature was raised to −10 °C, and then the solution was brought to room temperature after it was stirred for 1.5 h. The resulting solution was poured into ice water, let stand for 30 min, and filtered to give **7b**. Yellow solid (21.84 g, 99%); mp 189−190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 8.29 (d, J = 2.8 Hz, 1H), 7.76 (d, J = 1.2 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.38 (dd, J = 8.4, 1.6 Hz, 1H).

General Procedures for the Preparation of Compounds 8a and 8b. Compounds **8a** and **8b** were prepared using procedures similar to those used for the preparation of compounds **4a** and **4b**.

*N-Tosyl-3-cyanoindole (**8a**).* Brown solid; yield 99%; mp 159−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.83 (d, J = 8.0 Hz, 2H), 7.69 (d, J = 8.0 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.30 (d, J = 8.4 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.4, 134.1, 133.7, 133.2, 130.4, 128.4, 127.3, 126.6, 124.8, 120.3, 113.8, 113.5, 93.7, 21.7.

*N-Tosyl-3-cyano-6-bromoindole (**8b**).* Brown solid; yield 98%; mp 171−172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 1.2 Hz, 1H), 8.06 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 1H), 7.50 (dd, J = 8.4, 1.6 Hz, 1H), 7.35 (d, J = 8.4 Hz, 2H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.8, 134.3, 133.8, 133.5, 130.6, 128.3, 127.3, 127.2, 121.4, 120.4, 116.9, 113.0, 93.7, 21.8.

General Procedures for the Preparation of Compounds 9a, 9b, and 9e−9g. To a solution of $NH_2OH{\cdot}HCl$ (30 mmol) in MeOH (100 mL) was added $NaHCO_3$ (30 mmol); the solution was stirred for 30 min, and then compound **8** (15 mmol) was added. The mixture was heated at reflux for 5 h. After being cooled to room temperature, the solution was concentrated in vacuo. Water was added to the residue, which was then filtered to provide the corresponding compounds, **9a**, **9b**, and **9e**−**9g**.

*N′-Hydroxy-N-tosylindole-3-formamidine (**9a**).* Yellow solid; yield 97%; mp 186−188 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H), 8.31 (s, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 7.39−7.32 (m, 1H), 7.27 (t, J = 7.8 Hz, 1H), 5.91 (s, 2H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.8, 146.2, 135.0, 134.3, 130.8, 128.0, 127.2, 125.8, 125.6, 124.2, 123.9, 116.7, 113.5, 21.5.

*N′-Hydroxy-N-tosyl-6-bromoindole-3-formamidine (**9b**).* Yellow solid; yield 99%; mp 178−179 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.37 (s, 1H), 8.12 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 1.2 Hz, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.49 (dd, J = 8.4, 1.2 Hz, 1H), 7.44 (d, J = 8.4 Hz, 2H), 5.97 (s, 2H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.0, 146.0, 135.1, 133.6, 130.5, 126.9, 126.7, 126.6, 125.8, 125.2, 117.8, 116.0, 115.4, 21.0.

*N-Hydeoxy-p-metoxybenzamidine (**9e**).* White crystal; yield 99%; mp 123−124 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.61 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 5.73 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.3, 151.0, 127.2, 126.2, 113.9, 55.6.

*N-Hydeoxy-thiophene-3-formamidine (**9f**).* Washy-blue crystal; yield 99%; mp 81−82 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (s, 1H), 7.81 (d, J = 2.0 Hz, 1H), 7.50 (dd, J = 4.8, 2.8 Hz, 1H), 7.38−7.30 (m, 1H), 5.79 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.4, 135.8, 126.5, 125.7, 122.8.

*N-Hydeoxy-hendecylamidine (**9g**).* White solid; yield 99%; mp 79−80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 4.54 (s, 2H), 2.13 (t, J = 7.6 Hz, 2H), 1.66−1.48 (m, 2H), 1.43−1.17 (m, 14H), 1.01−0.76 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.2, 31.9, 31.3, 29.6, 29.5, 29.3, 29.1, 26.8, 22.7, 14.1.

General Procedures for the Preparation of Compounds 10a, 10b, and 10e−10g. To a solution of **9** (3 mmol) in MeOH (40 mL) was added Raney nickel (0.5 g). The mixture was stirred for 12 h at room temperature in a H_2 atmosphere, concentrated, adjusted to pH 7 with 2 N NaOH, and then filtered to provide the corresponding compounds, **10a**, **10b**, and **10e**−**10g**.

*N-Tosylindole-3-formamidine (**10a**).* White solid; yield 92%; mp 184−185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 2H), 7.90 (s, 3H),

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7.41 (s, 2H), 7.31 (d, J = 33.2 Hz, 2H), 6.49 (s, 2H), 3.36 (s, 1H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.4, 134.6, 134.1, 130.9, 130.8, 128.6, 128.3, 127.5, 125.7, 124.3, 123.2, 117.1, 113.4, 21.5.

*N-Tosyl-6-bromoindole-3-formamidine (**10b**).* White solid; yield 92%; mp 196−197 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 2H), 8.51 (s, 1H), 8.06−7.95 (m, 3H), 7.92 (d, J = 7.6 Hz, 1H), 7.50−7.40 (m, 3H), 7.37 (t, J = 7.6 Hz, 1H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.6, 146.3, 133.9, 133.4, 130.4, 129.67, 127.2, 126.6, 125.7, 124.2, 121.7, 113.6, 113.2, 21.0.

*4-Methoxybenzimidamide acetate (**10e**).* White solid; yield 93%; mp 231−233 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.79 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 8.8 Hz, 2H), 5.02 (s, 4H), 3.90 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.3, 164.4, 129.5, 119.9, 114.3 54.9.

*Thiophene-3-carboximidamide acetate (**10f**).* Washy-blue crystal; yield 94%; mp 233−234 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.40 (s, 1H), 7.73−7.68 (m, 1H), 7.59 (d, J = 4.8 Hz, 1H), 4.96 (s, 4H), 1.95 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 160.79, 131.95, 129.40, 128.20, 125.48.

*Hendecylamidine (**10g**).* White solid; yield 99%; mp 74−75 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (t, J = 7.2 Hz, 2H), 1.91 (s, 3H), 1.57 (s, 2H), 1.39−0.99 (m, 14H), 1.02−0.68 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.4, 32.1, 31.3, 28.9, 28.8, 28.7, 28.5, 28.2, 26.2, 22.1, 13.9.

General Procedures for the Preparation of *N,N′*-Ditosylnortopsentins 2a−2m. To a solution of **6** (3 mmol) in 3:1 THF−H_2O (40 mL), $KHCO_3$ (9 mmol) was added. The mixture was stirred at reflux, and a solution of compound **10** (3 mmol) in THF was added. The mixture was heated at reflux for 4 h and then cooled to room temperature. The resulting solution was extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel using petroleum ether and ethyl acetate (3:1, v/v) as the eluent to give **2a−2m**.

*N,N′-Ditosylnortopsentin D (**2a**).* Yellow solid; yield 56%; mp 163−164 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.60 (s, 1H), 8.51 (d, J = 4.0 Hz, 1H), 8.32 (s, 1H), 8.19 (d, J = 4.4 Hz, 1H), 8.10−7.99 (m, 3H), 7.91 (d, J = 6.8 Hz, 2H), 7.88 (d, J = 6.8 Hz, 1H), 7.54−7.35 (m, 5H), 7.33 (d, J = 6.4 Hz, 2H), 7.28 (d, J = 4.8 Hz, 2H), 2.23 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 146.3, 146.0, 140.7, 135.2, 134.9, 134.3, 134.1, 130.8, 130.7, 128.3, 127.9, 127.3, 127.2, 126.2, 125.8, 124.6, 124.4, 123.0, 122.8, 121.9, 113.9, 113.7, 21.4, 21.4; HR-MS (ESI): calcd for $C_{33}H_{27}N_4O_4S_2$ [M + H]$^+$ 607.1468, found (ESI$^+$) 607.1464.

*N,N′-Ditosylnortopsentin B (**2b**).* Yellow solid; yield 66%; mp 269 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.57 (s, 1H), 8.43 (d, J = 8.4 Hz, 1H), 8.26 (s, 1H), 8.22−8.11 (m, 2H), 8.08−8.00 (m, 2H), 7.97 (d, J = 7.6 Hz, 2H), 7.91 (d, J = 7.6 Hz, 2H), 7.66 (d, J = 8.4 Hz, 1H), 7.52−7.43 (m, 3H), 7.41 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 7.6 Hz, 2H), 2.34 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 146.7, 146.0, 140.1, 135.5, 135.1, 134.4, 133.9, 131.0, 130.7, 128.2, 127.8, 127.3, 127.2, 127.0, 126.3, 125.8, 124.6, 124.4, 123.0, 121.8, 119.0, 116.2, 113.9, 112.1, 21.5, 21.4; HR-MS (ESI): calcd for $C_{33}H_{26}BrN_4O_4S_2$ [M + H]$^+$ 685.0573, found (ESI$^+$) 685.0580.

*N,N′-Ditosylnortopsentin C (**2c**).* Yellow solid; yield 67%; mp 197−198 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.56 (s, 1H), 8.45 (d, J = 6.4 Hz, 1H), 8.31 (s, 1H), 8.19 (s, 1H), 8.13 (d, J = 7.2 Hz, 1H), 8.06 (d, J = 7.2 Hz, 1H), 7.98 (s, 1H), 7.96−7.81 (m, 4H), 7.56 (d, J = 8.0 Hz, 1H), 7.53−7.41 (m, 2H), 7.41−7.31 (m, 4H), 2.29 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 146.3, 140.5, 135.8, 134.8, 134.2, 134.1, 130.6, 130.5, 127.6, 127.3, 127.2, 127.0, 126.0, 125.8, 124.4, 123.5, 123.3, 122.5, 118.5, 116.3, 113.59, 21.0; HR-MS (ESI): calcd for $C_{33}H_{26}BrN_4O_4S_2$ [M + H]$^+$ 685.0573, found (ESI$^+$) 685.0572.

*N-,N′-Ditosylnortopsentin A (**2d**).* Yellow solid; yield 59%; mp 293−294 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.52 (s, 1H), 8.44 (d, J = 8.4 Hz, 1H), 8.25 (s, 1H), 8.19−8.09 (m, 3H), 7.96 (d, J = 6.8 Hz, 2H), 7.94−7.88 (m, 3H), 7.61 (dd, J = 8.4, 1.6 Hz, 1H), 7.55 (dd, J = 8.4, 1.6 Hz, 1H), 7.42 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 2.30 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 146.7, 146.4, 140.4, 135.8, 135.5, 134.1, 133.9, 131.1, 130.9, 127.9, 127.5, 127.4, 127.3, 127.2, 127.1, 125.7, 124.7, 123.8, 123.3, 118.9, 118.5, 116.3, 116.1, 21.5, 21.5; HR-MS (ESI): calcd for $C_{33}H_{25}Br_2N_4O_4S_2$ [M + H]$^+$ 762.9678, found (ESI$^+$) 762.9683.

*Compound **2e**.* Yellow solid; yield 58%; mp 151−152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 8.42−8.17 (m, 1H), 8.18−8.05 (m, 1H), 8.04−7.96 (m, 3H), 7.87 (d, J = 8.4 Hz, 2H), 7.85−7.74 (m, 1H), 7.47−7.39 (m, 1H), 7.38−7.31 (m, 3H), 7.07 (d, J = 8.8 Hz, 2H), 3.82 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.9, 146.4, 145.9, 135.2, 134.4, 130.7, 127.1, 127.0, 125.5, 124.2, 123.7, 122.3, 121.9, 114.6, 113.8, 55.7, 21.5; HR-MS (ESI): calcd for $C_{25}H_{22}N_3O_3S$ [M + H]$^+$ 444.1376, found (ESI$^+$) 444.1380.

*Compound **2f**.* Pink solid; yield 58%; mp 165−166 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (s, 1H), 8.20 (d, J = 4.4 Hz, 1H), 8.12 (s, 1H), 8.06−7.97 (m, 2H), 7.86 (d, J = 8.0 Hz, 2H), 7.80 (s, 1H), 7.76−7.70 (m, 1H), 7.69−7.62 (m, 1H), 7.46−7.39 (m, 1H), 7.39−7.34 (m, 1H), 7.32 (d, J = 8.0 Hz, 2H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 145.9, 143.5, 135.2, 134.4, 133.1, 130.7, 128.7, 127.6, 127.1, 126.3, 125.5, 124.2, 122.2, 122.2, 121.9, 113.8, 21.4; HR-MS (ESI): calcd for $C_{22}H_{18}N_3O_2S_2$ [M + H]$^+$ 420.0835, found (ESI$^+$) 420.0832.

*Compound **2g**.* Yellow solid; yield 43%; mp 195−196 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 1H), 8.07 (d, J = 7.6 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 7.84 (d, J = 8.0 Hz, 2H), 7.52 (s, 1H), 7.38 (t, J = 7.6 Hz, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.31 (t, J = 7.6 Hz, 1H), 2.65 (t, J = 7.6 Hz, 2H), 2.28 (s, 3H), 1.76−1.61 (m, 2H), 1.38−1.15 (m, 14H), 0.89−0.78 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.9, 145.9, 135.2, 134.5, 130.7, 128.7, 127.1, 125.4, 124.1, 122.1, 121.3, 113.8, 31.8, 29.4, 29.2, 29.2, 29.2, 28.5, 28.4, 22.6, 21.5, 14.4; HR-MS (ESI): calcd for $C_{28}H_{36}N_3O_2S$ [M + H]$^+$ 478.2523, found (ESI$^+$) 478.2529.

*Compound **2h**.* Yellow solid; yield 82%; mp 249−251 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.52 (s, 1H), 8.28 (s, 1H), 8.21−8.08 (m, 3H), 8.05 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 7.6 Hz, 2H), 7.77−7.58 (m, 3H), 7.53−7.47 (m, 1H), 7.47−7.35 (m, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 146.2, 145.5, 135.0, 134.2, 130.8, 129.6, 129.5, 127.9, 127.2, 127.0, 126.6, 126.0, 124.5, 124.0, 121.8, 118.1, 113.9, 113.6, 21.5; HR-MS (ESI): calcd for $C_{24}H_{20}N_3O_2S$ [M + H]$^+$ 414.1271, found (ESI$^+$) 414.1273.

*Compound **2i**.* Yellow solid; yield 77%; mp 236−237 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.43 (s, 1H), 8.40 (s, 1H), 8.36−8.32 (m, 3H), 8.23 (d, J = 7.6 Hz, 1H), 8.12 (s, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.50−7.42 (m, 2H), 7.42−7.36 (m, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 147.8, 146.2, 143.7, 135.0, 134.3, 130.8, 129.4, 128.1, 127.2, 127.0, 125.9, 124.9, 124.5, 123.9, 123.7, 122.0, 119.7, 117.6, 114.7, 113.8, 21.5; HR-MS (ESI): calcd for $C_{24}H_{19}N_4O_4S$ [M + H]$^+$ 459.1122, found (ESI$^+$) 459.1121.

*Compound **2j**.* Yellow solid; yield 53%; mp 142−144 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.94 (d, J = 6.0 Hz, 2H), 8.44 (d, J = 6.4 Hz, 2H), 8.39−8.28 (m, 2H), 8.24 (s, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.50−7.43 (m, 1H), 7.43−7.33 (m, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 146.1, 144.4, 142.8, 141.8, 135.7, 135.1, 134.3, 130.8, 128.4, 127.2, 125.8, 124.4, 123.5, 122.3, 121.0, 118.1, 115.8, 115.1, 113.8, 100.0, 21.5; HR-MS (ESI): calcd for $C_{23}H_{19}N_4O_2S$ [M + H]$^+$ 415.1223, found (ESI$^+$) 415.1225.

*Compound **2k**.* White solid; yield 49%; mp 247−248 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.18 (s, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.74 (s, 1H), 7.45−7.40 (m, 1H), 7.40−7.33 (m, 3H), 2.30 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 156.0, 146.1, 135.0, 134.3, 130.8, 128.3, 127.2, 125.7, 124.3, 122.9, 121.9, 113.8, 33.1, 29.5, 21.5; HR-MS (ESI): calcd for $C_{22}H_{24}N_3O_2S$ [M + H]$^+$ 394.1584, found (ESI$^+$) 394.1584.

*Compound **2l**.* White solid; yield 77%; mp 265−266 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.17 (s, 1H), 8.01 (d, J = 8.0 Hz, 2H), 7.94−7.81 (m, 3H), 7.49−7.41 (m, 1H), 7.41−7.31 (m, 3H), 2.52 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 145.7, 144.6, 134.5, 133.7, 130.3, 127.2, 127.0, 126.7, 125.5, 124.0, 122.7, 121.1, 115.4, 113.4, 21.0, 12.4; HR-MS (ESI): calcd for $C_{19}H_{18}N_3O_2S$ [M + H]$^+$ 352.1114, found (ESI$^+$) 352.1121.

*Compound **2m**.* Yellow solid; yield 44%; mp 243−244 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 8.42 (s, 1H), 8.14−8.09 (m, 1H),

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8.04 (d, J = 8.2 Hz, 1H), 7.97 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 8.2 Hz, 2H), 7.49 (t, J = 7.6 Hz, 1H), 746−7.38 (m, 3H), 2.33 (s, 4H), 1.35−1.25 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 150.4, 146.1, 135.0, 134.2, 130.7, 127.9, 127.8, 127.2, 125.8, 124.4, 123.0, 121.7, 115.9, 113.9, 21.5, 8.7, 8.5; HR-MS (ESI): calcd for $C_{21}H_{20}N_3O_2S$ $[M + H]^+$ 378.1271, found (ESI$^+$) 378.1274.

General Procedures for the Preparation of Compounds 1a−1d, 1f, 1g, 1l, and 1m. A solution of sodium (9.0 mmol) and naphthalene (7.5 mmol) in dry THF (20 mL) was stirred for 2 h at room temperature and then cooled to −78 °C; then, a solution of **2** (1.0 mmol) was added. The mixture was stirred at −78 °C for 2 h and then extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel using dichloromethane and methanol (20:1, v/v) as the eluent to give **1**.

*Nortopsentin D (**1a**).* Deep-red solid; yield 80%; mp 155−157 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.21−8.13 (m, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.78 (s, 1H), 7.69 (s, 1H), 7.48−7.39 (m, 2H), 7.37 (s, 1H), 7.23−7.11 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 143.3, 136.9, 136.7, 131.1, 125.1, 124.9, 124.1, 122.1, 121.8, 121.6, 120.0, 119.8, 119.4, 119.2, 116.2, 111.4, 111.3, 107.7, 106.2; HR-MS (ESI): calcd for $C_{19}H_{15}N_4$ $[M + H]^+$ 299.1291, found (ESI$^+$) 299.1293.

*Nortopsentin B (**1b**).* Purple solid; yield 97%; mp 224−225 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 11.48 (s, 1H), 11.20 (s, 1H), 8.41 (d, J = 8.4 Hz, 1H), 8.07−7.90 (m, 2H), 7.74 (s, 1H), 7.65 (s, 1H), 7.51−7.37 (m, 2H), 7.28 (d, J = 8.0 Hz, 1H), 7.19−7.06 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 142.1, 137.1, 136.4, 124.7, 124.0, 124.0, 123.1, 122.5, 121.8, 121.3, 120.0, 119.1, 114.6, 114.2, 111.6, 107.7; HR-MS (ESI): calcd for $C_{19}H_{14}BrN_4$ $[M + H]^+$ 377.0496, found (ESI$^+$) 377.0395.

*Nortopsentin C (**1c**).* Purple solid; yield 79%; mp 170−171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (s, 1H), 11.39 (s, 1H), 11.33 (s, 1H), 8.42 (d, J = 6.8 Hz, 1H), 8.01 (d, J = 4.0 Hz, 1H), 7.93 (s, 1H), 7.77 (s, 1H), 7.62 (s, 1H), 7.50−7.39 (m, 2H), 7.23 (d, J = 8.0 Hz, 1H), 7.20−7.12 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 142.6, 137.6, 136.8, 129.7, 125.7, 124.4, 123.8, 123.4, 122.7, 122.5, 120.5, 120.4, 119.2, 115.2, 114.4, 114.2, 111.6, 106.2, 103.3; HR-MS (ESI): calcd for $C_{19}H_{14}BrN_4$ $[M + H]^+$ 377.0396, found (ESI$^+$) 377.0394.

*Nortopsentin A (**1d**).* Purple solid; yield 68%; mp 166−167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 11.49 (s, 1H), 11.33 (s, 1H), 8.41 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 6.8 Hz, 1H), 7.94 (s, 1H),

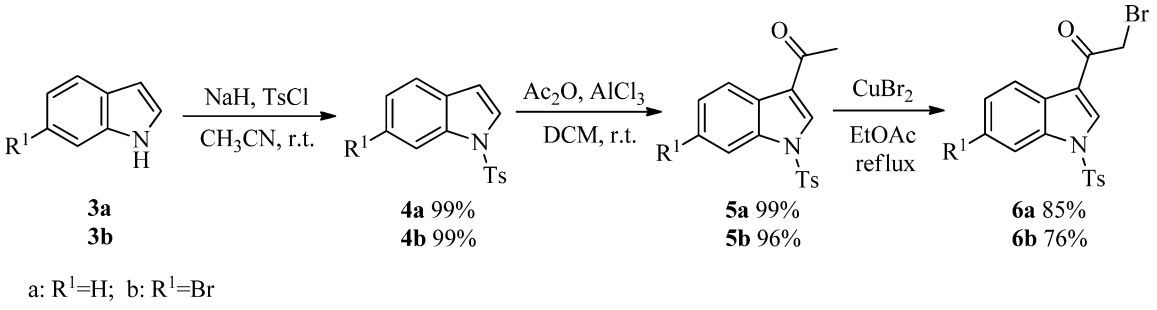

Figure 2. Synthesis of intermediate **6**.

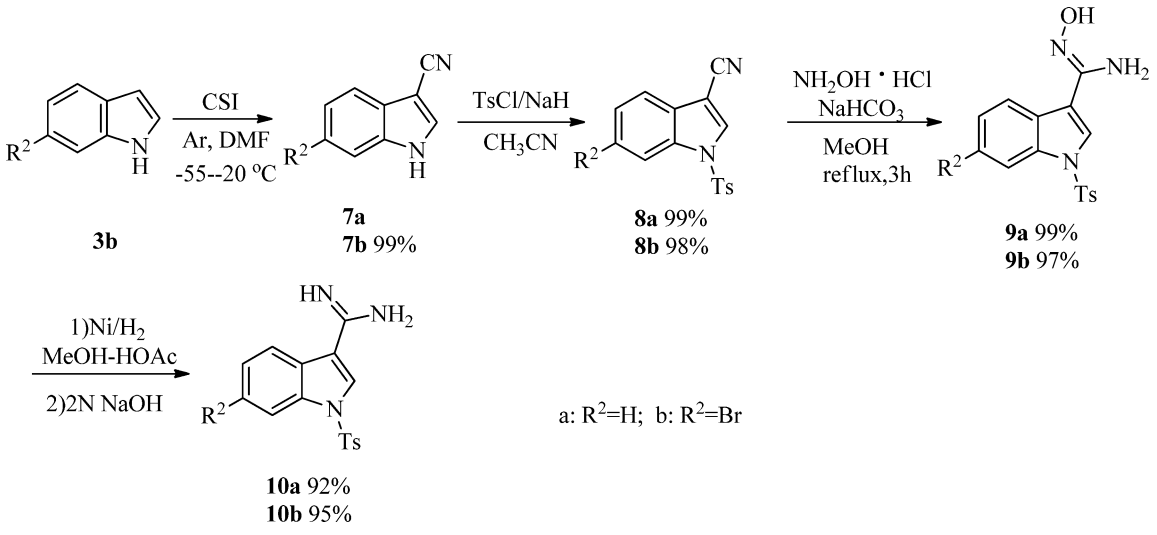

Figure 3. Synthesis of intermediates **10a** and **10b**.

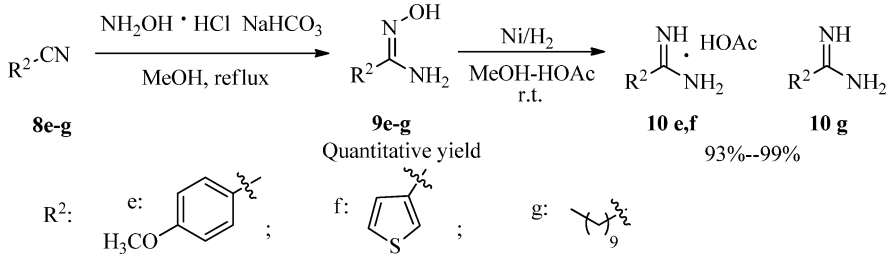

Figure 4. Synthesis of intermediates **10e−10g**.

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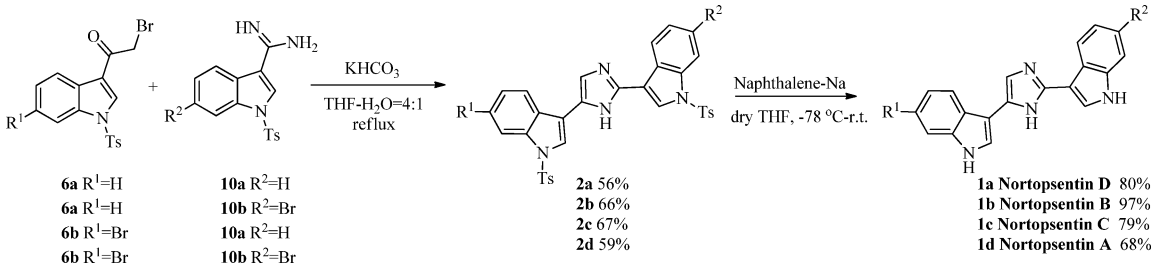
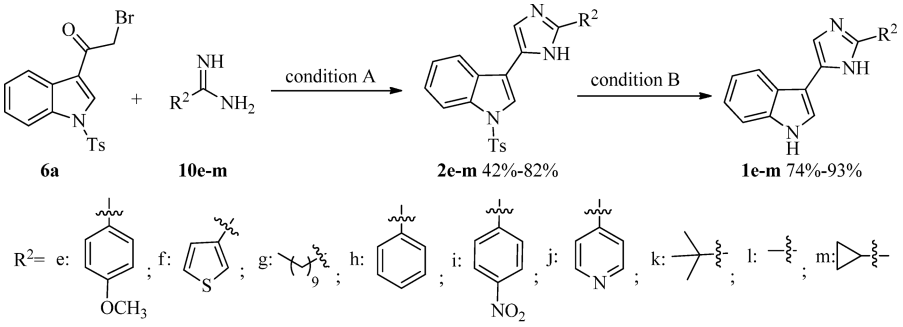


Figure 5. Synthesis of nortopsentin alkaloids A–D (**1a–1d**).



Condition A:($KHCO_3$, THF, reflux) for **2l**,($KHCO_3$, THF-H_2O=4:1, reflux) for the others
Condition B:(NaOH-MeOH-THF,reflux) for **1e, 1h, 1g, 1j, 1k;**(Na-Naphthalene,-78 °C-r.t.) for **1f, 1g, 1l, 1m.**

Figure 6. Synthesis of **1e–1m.**

Table 1. In Vitro Antiviral Activities of Ribavirin and Nortopsentins in Series 1 and 2 against TMV

compd	concn (μg/mL)	inhibition rate (%)[a]	compd	concn (μg/mL)	inhibition rate (%)[a]
1a	500	36 ± 2	**2b**	500	48 ± 2
	100	11 ± 1		100	0
1b	500	14 ± 2	**2c**	500	42 ± 1
	100	0		100	21 ± 2
1c	500	44 ± 3	**2d**	500	20 ± 2
	100	0		100	0
1d	500	35 ± 2	**2e**	500	53 ± 1
	100	17 ± 2		100	18 ± 1
1e	500	39 ± 1	**2f**	500	40 ± 2
	100	14 ± 2		100	0
1f	**500**	**58 ± 3**	**2g**	500	29 ± 1
	100	14 ± 1		100	0
1g	500	40 ± 2	**2h**	**500**	**59 ± 2**
	100	0		100	12 ± 2
1h	500	41 ± 2	**2i**	500	25 ± 1
	100	0		100	0
1i	500	34 ± 1	**2j**	500	19 ± 2
	100	0		100	0
1j	500	22 ± 1	**2k**	500	41 ± 2
	100	0		100	17 ± 1
1k	500	35 ± 3	**2l**	500	43 ± 2
	100	13 ± 1		100	25 ± 2
1l	500	51 ± 2	**2m**	**500**	**53 ± 1**
	100	19 ± 1		100	4 ± 1
1m	500	36 ± 2	ribavirin	**500**	**41 ± 1**
	100	8 ± 1		**100**	9 ± 2
2a	500	19 ± 2			
	100	0			

[a]Averages of three replicates. All the results are expressed as means ± SD.

7.76 (s, 1H), 7.64 (d, J = 13.6 Hz, 2H), 7.44 (s, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 142.8, 137.8, 137.6, 124.5, 124.4, 123.2, 123.0, 122.4, 115.1, 114.7, 114.6, 108.1; HR-MS (ESI): calcd for $C_{19}H_{13}Br_2N_4$ $[M + H]^+$ 454.9501, found (ESI$^+$) 454.9493.

General Procedures for the Preparation of Compounds 1e and 1h–1k. To a solution of compound **2** (1 mmol) in MeOH (50 mL) and THF (20 mL) was added 2 N NaOH (30 mL). The mixture was heated at reflux for 2 h in an Ar atmosphere, cooled to room

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temperature, and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel using dichloromethane and methanol (50:1, v/v) as the eluent to give **1**.

Compound ***1e***. Brown solid; yield 86%; mp 217−218 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 14.47 (s, TFA and NH) 11.73 (s, 1H), 8.16−8.06 (m, 3H), 8.05 (d, J = 2.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.30−7.23 (m, 3H), 7.22−7.15 (m, 1H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 160.3, 144.1, 136.4, 131.0, 127.3, 124.4, 123.3, 121.7, 120.2, 119.7, 119.6, 115.4, 114.4, 111.9, 105.9, 55.4; HR-MS (ESI): calcd for $C_{18}H_{16}N_3O$ $[M + H]^+$ 290.1288, found (ESI^+) 290.1294.

Compound ***1f***. Brown oil; yield 93%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (d, J = 2.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H), 7.67 (d, J = 5.2 Hz, 1H), 7.47 (dd, J = 4.8, 2.8 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.36 (s, 1H), 7.21−7.15 (m, 1H), 7.15−7.08 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 142.6, 136.9, 131.6, 131.6, 126.4, 125.4, 125.1, 122.0, 121.8, 121.7, 119.5, 119.2, 117.4, 111.4, 107.3; HR-MS (ESI): calcd for $C_{15}H_{12}N_3S$ $[M + H]^+$ 266.0746, found (ESI^+) 266.0748.

Compound ***1g***. Brown oil; yield 84%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.68−7.57 (m, 1H), 7.44 (d, J = 2.8 Hz, 1H), 7.30−7.21 (m, 1H), 7.04 (d, J = 3.2 Hz, 1H), 7.02−6.88 (m, 2H), 2.55 (s, 2H), 1.54 (s, 2H), 1.05 (s, 14H), 0.76−0.66 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 148.0, 136.9, 130.7, 124.9, 121.5, 119.3, 119.2, 115.1, 111.3, 107.7, 31.8, 29.4, 29.4, 29.2, 29.1, 29.1, 28.5, 27.6, 22.5, 13.3; HR-MS (ESI): calcd for $C_{21}H_{30}N_3$ $[M + H]^+$ 324.2434, found (ESI^+) 324.2436.

Compound ***1h***. White solid; yield 82%; mp 205 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 11.79 (s, 1H), 8.17 (s, 1H), 8.16−8.12 (m, 2H), 8.10 (d, J = 2.0 Hz, 1H), 7.97 (d, J = 7.6 Hz, 1H), 7.80−7.64 (m, 3H), 7.55 (d, J = 7.6 Hz, 1H), 7.31−7.24 (m, 1H), 7.24−7.18 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 143.5, 136.9, 132.3, 129.9, 127.4, 125.6, 124.5, 123.7, 122.8, 120.7, 119.7, 117.4, 114.6, 112.7, 102.7; HR-MS (ESI): calcd for $C_{17}H_{14}N_3$ $[M + H]^+$ 260.1182, found (ESI^+) 260.1184.

Compound ***1i***. Red solid; yield 90%; mp 244−246 °C; ^{1}H NMR (400 MHz, DMSO-d_6 + 1% TFA) δ 11.55 (s, 1H), 8.42 (d, J = 8.4 Hz, 2H), 8.32 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 7.6 Hz, 1H), 7.94 (s, 1H), 7.92 (s, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.29−7.19 (m, 1H), 7.19−7.11 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6 + 1% TFA) δ 147.5, 142.4, 136.9, 134.1, 133.7, 126.8, 124.9, 124.2, 122.4, 120.3, 120.2, 112.5, 106.1, 100.0; HR-MS (ESI): calcd for $C_{17}H_{13}N_4O_2$ $[M + H]^+$ 305.1033, found (ESI^+) 305.1035.

Compound ***1j***. Orange-yellow solid; yield 74%; mp 244−246 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 11.31 (s, 1H), 8.88−8.50 (m, 2H), 8.03 (s, 1H), 8.00−7.90 (m, 2H), 7.81 (s, 1H), 7.64 (s, 1H), 7.46 (d, J = 7.6 Hz, 1H), 7.22−7.15 (m, 1H), 7.15−7.08 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.1, 142.3, 137.3, 136.4, 124.7, 122.6, 121.5, 120.0, 119.4, 118.7, 111.7; HR-MS (ESI): calcd for $C_{16}H_{13}N_4$ $[M + H]^+$ 261.1135, found (ESI^+) 261.1137.

Compound ***1k***. Orange-yellow solid; yield 77%; mp 106−108 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 7.91 (d, J = 7.6 Hz, 1H), 7.64 (s, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.22 (s, 1H), 7.14 (t, J = 7.2 Hz, 1H), 7.08 (t, J = 7.2 Hz, 1H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.5, 136.4, 124.8, 121.6, 121.1, 119.9, 118.9, 111.5, 32.5, 29.6; HR-MS (ESI): calcd for $C_{15}H_{18}N_3$ $[M + H]^+$ 240.1595, found (ESI^+) 240.1496.

Compound ***1l***. Brown solid; yield 87%; mp 96−98 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 11.43 (s, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.73 (s, 1H), 7.52 (s, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.23−7.14 (m, 1H), 7.14−7.06 (m, 1H), 2.49 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 143.7, 136.9, 130.0, 124.7, 123.1, 122.2, 120.0, 114.0, 112.3, 106.5, 13.0; HR-MS (ESI): calcd for $C_{12}H_{12}N_3$ $[M + H]^+$ 198.1026, found (ESI^+) 198.1026.

Table 2. In Vivo Antiviral Activities of Ribavirin and Nortopsentins in Series 1 and 2 against TMV

compd	concn (μg/mL)	inactivation effect (%)[a]	curative effect (%)[a]	protective effect (%)[a]	compd	concn (μg/mL)	inactivation effect (%)[a]	curative effect (%)[a]	protective effect (%)[a]
1a	500	54 ± 3	47 ± 4	49 ± 1	**2b**	500	43 ± 2	35 ± 5	47 ± 3
	100	26 ± 3	18 ± 1	13 ± 2		100	7 ± 1	8 ± 2	16 ± 1
1b	500	32 ± 2	25 ± 3	37 ± 1	**2c**	500	58 ± 4	56 ± 2	50 ± 3
	100	0	0	5 ± 1		100	15 ± 4	21 ± 1	12 ± 1
1c	500	35 ± 1	31 ± 2	37 ± 2	**2d**	500	28 ± 3	23 ± 5	33 ± 1
	100	0	0	9 ± 1		100	0	0	0
1d	500	48 ± 3	51 ± 2	44 ± 4	**2e**	**500**	**50 ± 3**	**59 ± 4**	**56 ± 2**
	100	16 ± 1	12 ± 1	10 ± 3		**100**	**18 ± 1**	**21 ± 2**	**30 ± 1**
1e	500	49 ± 3	45 ± 5	51 ± 2	**2f**	500	44 ± 3	41 ± 4	50 ± 5
	100	9 ± 1	12 ± 2	19 ± 3		100	0	15 ± 3	17 ± 1
1f	500	58 ± 3	51 ± 1	49 ± 2	**2g**	500	42 ± 3	39 ± 1	45 ± 2
	100	22 ± 2	30 ± 1	16 ± 1		100	4 ± 1	0	0
1g	500	54 ± 1	48 ± 3	46 ± 2	**2h**	500	58 ± 3	50 ± 4	54 ± 4
	100	20 ± 1	10 ± 4	11 ± 5		100	20 ± 1	17 ± 1	27 ± 3
1h	500	54 ± 4	46 ± 3	43 ± 5	**2i**	500	35 ± 2	32 ± 2	40 ± 3
	100	27 ± 2	18 ± 1	24 ± 1		100	6 ± 2	0	0
1i	500	43 ± 1	37 ± 4	41 ± 5	**2j**	500	29 ± 5	34 ± 3	26 ± 3
	100	0	5 ± 1	0		100	0	0	0
1j	500	41 ± 4	33 ± 1	34 ± 2	**2k**	**500**	**60 ± 3**	**58 ± 3**	**52 ± 4**
	100	15 ± 1	9 ± 2	0		**100**	**26 ± 1**	**18 ± 2**	**29 ± 2**
1k	500	51 ± 4	44 ± 2	48 ± 3	**2l**	500	53 ± 3	49 ± 2	57 ± 1
	100	17 ± 1	15 ± 3	23 ± 3		100	8 ± 4	17 ± 1	19 ± 1
1l	500	51 ± 3	55 ± 1	59 ± 4	**2m**	500	40 ± 5	48 ± 3	40 ± 2
	100	19 ± 1	17 ± 2	25 ± 4		100	7 ± 1	13 ± 1	0
1m	500	36 ± 5	41 ± 4	39 ± 3	ribavirin	**500**	**41 ± 1**	**37 ± 1**	**38 ± 2**
	100	8 ± 2	0	11 ± 1		**100**	**9 ± 2**	**13 ± 1**	**15 ± 2**
2a	500	31 ± 1	25 ± 3	29 ± 3					
	100	0	4 ± 1	0					

[a]Averages of three replicates. All the results are expressed as means ± SD.

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Table 3. Fungicidal Activities of Nortopsentins in Series 1 and 2 against 14 Kinds of Fungi

	fungicidal activity (%)[a] at 50 mg/kg													
compd	*Fc*[c]	*CH*	*Pp*	*Rc*	*Bm*	wa	*Fm*	*As*	*Fg*	*Pi*	*Pc*	*Ss*	*Rs*	*Bc*
1a	26 ± 1	29 ± 2	**58 ± 1**	26 ± 2	21 ± 1	42 ± 2	9 ± 1	33 ± 1	10 ± 1	16 ± 2	3 ± 1	39 ± 2	14 ± 1	7 ± 1
1b	15 ± 2	**52 ± 1**	47 ± 1	35 ± 3	11 ± 1	28 ± 2	12 ± 1	33 ± 3	10 ± 1	16 ± 1	7 ± 2	45 ± 1	27 ± 2	9 ± 1
1c	28 ± 1	32 ± 3	34 ± 1	11 ± 2	21 ± 1	**58 ± 1**	21 ± 3	27 ± 1	0	26 ± 2	7 ± 1	32 ± 1	17 ± 3	9 ± 3
1d	21 ± 1	13 ± 2	36 ± 1	7 ± 1	13 ± 2	26 ± 1	30 ± 2	13 ± 2	5 ± 1	16 ± 2	3 ± 1	12 ± 1	24 ± 2	22 ± 1
1e	21 ± 1	23 ± 1	**58 ± 1**	**66 ± 1**	23 ± 1	40 ± 1	21 ± 1	27 ± 1	10 ± 2	11 ± 1	10 ± 1	19 ± 1	14 ± 1	6 ± 1
1f	13 ± 3	19 ± 1	31 ± 2	13 ± 1	6 ± 2	0	18 ± 2	7 ± 1	5 ± 1	11 ± 1	7 ± 2	12 ± 2	16 ± 1	33 ± 2
1g	15 ± 1	16 ± 1	39 ± 2	38 ± 2	4 ± 1	26 ± 2	21 ± 1	33 ± 3	16 ± 1	21 ± 2	3 ± 1	40 ± 1	36 ± 2	31 ± 1
1h	19 ± 2	16 ± 1	**56 ± 1**	**54 ± 1**	21 ± 1	35 ± 1	18 ± 1	40 ± 1	33 ± 1	16 ± 1	16 ± 1	17 ± 1	9 ± 1	16 ± 2
1i	4 ± 1	7 ± 1	23 ± 3	6 ± 1	6 ± 1	33 ± 2	24 ± 1	20 ± 2	14 ± 1	11 ± 1	10 ± 1	17 ± 1	9 ± 1	4 ± 1
1j	17 ± 1	10 ± 1	36 ± 1	18 ± 2	4 ± 1	37 ± 1	12 ± 1	27 ± 1	33 ± 3	11 ± 1	16 ± 2	15 ± 1	5 ± 1	6 ± 1
1k	21 ± 1	23 ± 2	48 ± 1	4 ± 1	9 ± 1	35 ± 2	21 ± 1	40 ± 1	19 ± 1	11 ± 1	13 ± 1	32 ± 3	0	12 ± 1
1l	9 ± 1	16 ± 1	33 ± 2	7 ± 1	9 ± 2	35 ± 1	24 ± 2	33 ± 2	5 ± 1	11 ± 2	7 ± 1	23 ± 1	16 ± 1	14 ± 2
1m	11 ± 2	32 ± 1	31 ± 1	6 ± 1	4 ± 1	16 ± 1	30 ± 1	53 ± 1	5 ± 1	5 ± 1	10 ± 2	12 ± 1	20 ± 1	14 ± 1
2a	9 ± 1	3 ± 1	25 ± 1	0	6 ± 1	26 ± 2	18 ± 1	7 ± 1	14 ± 1	11 ± 2	7 ± 1	12 ± 2	14 ± 2	6 ± 1
2b	4 ± 1	7 ± 1	14 ± 2	0	6 ± 1	30 ± 1	21 ± 2	13 ± 1	33 ± 3	5 ± 1	7 ± 1	9 ± 1	5 ± 1	3 ± 1
2c	9 ± 1	7 ± 1	4 ± 1	7 ± 1	6 ± 1	33 ± 2	21 ± 1	7 ± 1	19 ± 1	5 ± 1	16 ± 1	12 ± 1	5 ± 1	6 ± 1
2d	15 ± 1	13 ± 1	11 ± 1	7 ± 1	21 ± 2	28 ± 1	15 ± 1	7 ± 1	14 ± 1	5 ± 1	7 ± 1	19 ± 1	9 ± 1	6 ± 2
2e	17 ± 1	10 ± 2	28 ± 1	4 ± 1	9 ± 1	28 ± 1	15 ± 2	20 ± 2	10 ± 2	5 ± 1	13 ± 2	12 ± 2	27 ± 3	6 ± 1
2f	13 ± 1	10 ± 1	25 ± 2	**90 ± 2**	13 ± 1	33 ± 3	24 ± 1	7 ± 1	24 ± 2	11 ± 1	7 ± 1	21 ± 1	5 ± 1	19 ± 2
2g	21 ± 3	0	27 ± 1	4 ± 1	9 ± 2	26 ± 1	21 ± 1	7 ± 1	5 ± 1	11 ± 2	3 ± 1	12 ± 1	14 ± 1	12 ± 1
2h	11 ± 1	16 ± 1	30 ± 1	5 ± 1	15 ± 1	30 ± 1	24 ± 2	27 ± 2	10 ± 1	11 ± 1	10 ± 1	**95 ± 2**	9 ± 1	6 ± 1
2i	15 ± 3	7 ± 1	44 ± 1	10 ± 1	15 ± 1	19 ± 2	27 ± 1	7 ± 1	19 ± 1	21 ± 1	10 ± 2	19 ± 1	23 ± 3	6 ± 1
2j	23 ± 1	32 ± 1	50 ± 3	**89 ± 1**	19 ± 2	26 ± 1	18 ± 1	40 ± 1	14 ± 1	21 ± 1	16 ± 2	**59 ± 1**	32 ± 1	6 ± 1
2k	15 ± 1	16 ± 2	42 ± 1	18 ± 1	11 ± 1	26 ± 2	21 ± 1	20 ± 2	5 ± 1	16 ± 1	7 ± 1	6 ± 1	36 ± 1	14 ± 2
2l	28 ± 2	32 ± 1	42 ± 1	31 ± 1	2 ± 1	26 ± 1	33 ± 1	7 ± 1	42 ± 3	11 ± 1	17 ± 1	12 ± 2	36 ± 1	14 ± 1
2m	19 ± 1	0	39 ± 2	50 ± 1	15 ± 2	33 ± 1	27 ± 2	27 ± 1	10 ± 1	11 ± 1	7 ± 1	32 ± 1	5 ± 1	6 ± 1
chlorothalonil[b]	100	70 ± 2	100	75 ± 1	<50	100	<50	100	100	92 ± 1	92 ± 2	87 ± 3	100	100
carbendazim[b]	<50	<50	<50	<50	100	<50	100	<50	100	100	100	100	100	<50

[a]Averages of three replicates. [b]The commercial agricultural fungicides chlorothalonil and carbendazim were used for the comparisons of the antifungal activities. [c]*Fc, Fusarium oxysporum* f. sp. *cucumeris*; *CH, Cercospora arachidicola Hori*; *Pp, Physalospora piricola*; *Rc, Rhizoctonia cerealis*; *Bm, Bipolaris maydis*; wa, watermelon anthracnose; *Fm, Fusarium moniliforme*; *As, Alternaria solani*; *Fg, Fusarium graminearum*; *Pi, Phytophthora infestans*; *Pc, Phytophthora capsici*; *Ss, Sclerotinia sclerotiorum*; *Rs, Rhizoctonia solani*; *Bc, Botrytis cinerea*.

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Compound ***1m***. Brown solid; yield 92%; mp 96−98 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (d, J = 8.0 Hz, 1H), 7.56 (s, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.18−7.12 (m, 2H), 7.1−7.07 (m, 1H), 2.06−1.96 (m, 1H), 0.97−0.92 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.5, 136.8, 130.5, 127.4, 125.4, 125.0, 121.5, 121.4, 119.3, 119.1, 115.6, 111.2, 107.9, 8.3, 6.5; HR-MS (ESI): calcd for $C_{14}H_{14}N_3$ $[M + H]^+$ 224.1182, found (ESI^+) 224.1187.

Biological Assay. Each bioassay was repeated three times at 25 ± 1 °C. The activity results were estimated according to a percentage scale of 0−100 (0 indicating no activity and 100 indicating total mortality).

Detailed bioassay procedures for the anti-TMV,[20] fungicidal,[24] and insecticidal[24] activities are described in our published literature.

RESULTS AND DISCUSSION

Chemistry. Although nortopsentins have been synthesized by the palladium-catalyzed coupling of 3-indolylboronic acids with halogenated imidazoles,[14,25] and the corresponding bis-(indolyl)thiazole analogues have been prepared,[26] the reported methods cannot be used for structure−activity-relationship studies because they result in low yields and harsh conditions, complex materials, and many steps are required. A novel route for the preparation of nortopsentin derivatives is needed. As shown in Figure 2, commercially available indoles (**3**) were reacted with tosyl chloride to give the protected indoles (**4**), which were treated with acetic anhydride and brominated to give the key intermediate α-bromoketones (**6**). As depicted in Figures 3 and 4, treatment of the indoles (**3**) with chlorosulfonyl isocyanate (CSI) and protection with tosyl chloride gave the 3-cyano indoles (**8**), which were reacted with hydroxylamine and then reduced with hydrogen to give the key intermediate amidines (**10**). As depicted in Figure 5, condensation of the α-bromoketones (**6**) with the amidines (**10**) gave protected nortopsentins **2a**−**2d**. Experiments on various conditions, such as CH_3ONa, KOH, SmI_2, and Cs_2CO_3, were carried out for the removal of the Ts group. Only Na/naphthalene could successfully give nortopsentins **1a**−**1d**. To investigate the influence of the indole unit on bioactivity, indole-imidazole compounds **2e**−**2m** and **1e**−**1m**, in which one indole unit per compound was replaced with another substituent, were designed and synthesized. As shown in Figure 6, a similar procedure for the preparation of the nortopsentins was used. The removal of the Ts group was carried out under refluxing conditions in a solution of 2 N NaOH or in Na/naphthalene conditions. As mentioned in the literature,[27] the imidazole rings of nortopsentins have two types of mutual variations. Nortopsentins in series **1** existed as tautomers. ^{1}H NMR spectra of the nortopsentins in series **1** showed splitting of all the signals, which could be suppressed by the addition of 1% CF_3COOH to the deuterated solvent.

Phytotoxic Activity. The phytotoxic-activity tests showed that the nortopsentins in series **1** and their derivatives in series **2** were safe for testing on plants at 500 μg/mL.

Antiviral Activity. The results of the anti-TMV activities of the nortopsentins in series **1** and **2** are listed in Tables 1 and 2 with the commercial plant virucide ribavirin as the control.

In Vitro Anti-TMV Activity. As shown in Table 1, most of these compounds displayed good antiviral activities, especially compounds **1e**, **1g**, **1h**, **2f**, and **2k**, which exhibited the same level of antiviral activity as the plant virucide ribavirin (41% in vitro activity at 500 μg/mL). Compounds **1f**, **1l**, **2b**, **2e**, **2h**, **2l**, and **2m** exhibited higher antiviral activities than the plant virucide ribavirin at 500 μg/mL. Among the compounds, **1f**, **2h**, and **2m** exhibited much higher antiviral activities than ribavirin. The main differences among **1a**−**1d** lie in the bromine. Nortopsentin B (**1b**) displayed significantly lower antiviral activity than the others, which indicated that the substituents at the 7-position and 20-position exhibited synergistic effects. When the 7-position is bromine, and the 20-position is hydrogen, the structure is bad for antiviral activity. However, the introduction of Ts groups at the 1-position and 17-position changed this activity law (antiviral effects: **1a** > **2a**, **1b** < **2b**, **1c** ≈ **2c**, **1d** > **2d**), which indicated that these compounds were very sensitive to substituents, thus increasing the molecular regulation. The above results also showed that the influence of indole unit at the 10-position is more significant than that at the 12-position. A series of 10-position-indole-unit-replaced derivatives, **1e**−**1m**, were prepared and evaluated for their antiviral activities. Compounds **1e** and **1h** showed activities similar to that of **1a** and slightly higher than that of **1i**. Compounds **2e** and **2h** showed activities significantly higher than those of **2a** and **2i**. The electron-deficient aryl group is bad for antiviral activity (antiviral effects: **1i** < **1e** and **1h**, **2i** < **2e** and **2h**). The replacement of the indole unit with thiophene is favorable (antiviral effect: **1f** > **1a**), but its replacement with pyridine is unfavorable (antiviral effect: **1j** < **1a**). The introduction of an alkyl at the 10-position is favorable for activity (antiviral effects: **1g** and **1l** > **1a**, **2g** and **2k**−**2m** > **2a**).

In Vivo Anti-TMV Activity. As depicted in Table 2, the nortopsentins in series **1** and their derivatives in series **2** also showed good in vivo antiviral activities. Most of the compounds had activities similar to that of the plant virucide ribavirin. Among

Table 4. In Vivo Fungicidal Activities of Nortopsentins in Series 1 and 2 against Five Kinds of Fungi

	inhibition rate (%)[a] at 200 mg/kg				
compd	*Cc*[b]	*Pc*	*Ss*	*Rs*	*Bc*
1a	13 ± 2	0	13 ± 1	3 ± 1	6 ± 1
1b	20 ± 1	0	20 ± 2	4 ± 1	13 ± 3
1c	15 ± 1	0	15 ± 1	10 ± 1	8 ± 1
1d	10 ± 2	0	8 ± 2	13 ± 1	9 ± 1
1e	11 ± 1	2	15 ± 1	0	8 ± 3
1f	18 ± 2	6 ± 1	3 ± 1	10 ± 1	12 ± 1
1g	20 ± 2	7 ± 1	20 ± 2	11 ± 1	13 ± 1
1h	13 ± 1	11 ± 1	13 ± 1	11 ± 2	6 ± 1
1i	18 ± 3	7 ± 1	10 ± 1	0	12 ± 2
1j	10 ± 1	7 ± 1	10 ± 2	3 ± 1	2 ± 1
1k	10 ± 2	8 ± 1	18 ± 2	8 ± 1	3 ± 1
1l	16 ± 1	5 ± 1	16 ± 1	6 ± 1	9 ± 1
1m	18 ± 1	9 ± 1	13 ± 2	3 ± 1	12 ± 1
2a	16 ± 2	2 ± 1	16 ± 1	10 ± 1	9 ± 2
2b	12 ± 1	8 ± 2	7 ± 1	0	9 ± 1
2c	11 ± 1	12 ± 1	11 ± 1	6 ± 1	4 ± 1
2d	10 ± 2	8 ± 1	10 ± 2	8 ± 1	3 ± 1
2e	18 ± 1	9 ± 1	9 ± 1	6 ± 1	12 ± 2
2f	16 ± 1	5 ± 1	16 ± 2	6 ± 1	9 ± 1
2g	12 ± 2	0	12 ± 1	4 ± 1	4 ± 1
2h	13 ± 1	8 ± 1	33 ± 2	8 ± 1	6 ± 1
2i	16 ± 1	14 ± 2	8 ± 1	6 ± 1	9 ± 1
2j	14 ± 1	12 ± 1	14 ± 1	6 ± 1	7 ± 1
2k	7 ± 2	0	4 ± 1	8 ± 1	12 ± 1
2l	16 ± 1	13 ± 1	13 ± 1	6 ± 2	12 ± 1
2m	12 ± 1	10 ± 2	12 ± 1	11 ± 1	5 ± 1
azoxystrobin[c]	**100**	**100**	**83 ± 2**	**81 ± 1**	**82 ± 2**

[a]Averages of five replicates. [b]*Cc*, *Corynespora cassiicola* (cucumber protection); *Pc*, *Phytophthora capsici* (pepper protection); *Ss*, *Sclerotinia sclerotiorum* (rape protection); *Rs*, *Rhizoctonia solani* (rice protection); *Bc*, *Botrytis cinerea* (cucumber protection). [c]The dilution of azoxystrobin is 1000×.

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Table 5. Larvicidal Activities of Nortopsentins in Series 1 and 2 against Oriental Armyworm (*Mythimna separate*), Cotton Bollworm (*Helicoverpa armigera*), Corn Borer (*Ostrinia nubilalis*), Diamond Back Moth (*Plutella xylostella*), and Mosquito (*Culex pipiens pallens*)

	larvicidal activities (% mortality) at concn (μg/mL)								
	M. separat		*H. armigera*	*O. nubilalis*	*P. xylostella*		*C. pipiens pallens*		
compd	600 μg/mL	200 μg/mL	600 μg/mL	600 μg/mL	600 μg/mL	200 μg/mL	10 μg/mL	5 μg/mL	2 μg/mL
1a	45	—	25	30	50	—	35	—	—
1b	20	—	25	30	0	—	**100**	45	—
1c	0	—	0	0	0	—	70	—	—
1d	15	—	5	20	**100**	**40**	**100**	**100**	**65**
1e	0	—	0	0	0	—	40	—	—
1f	20	—	10	10	**100**	**50**	20	—	—
1g	15	—	10	15	0	—	35	—	—
1h	15	—	10	20	75	—	35	—	—
1i	20	—	20	25	0	—	35	—	—
1j	15	—	10	25	0	—	60	—	—
1k	**100**	**20**	**80**	**75**	0	—	55	—	—
1l	45	—	30	25	0	—	5	—	—
1m	0	—	0	0	**100**	**70**	15	—	—
2a	0	—	0	0	70	—	**100**	**45**	—
2b	45	—	20	30	0	—	20	—	—
2c	15	—	10	20	0	—	35	—	—
2d	5	—	5	5	50	—	40	—	—
2e	15	—	10	10	0	—	55	—	—
2f	25	—	15	15	0	—	15	—	—
2g	20	—	5	10	0	—	20	—	—
2h	5	—	5	5	**90**	**40**	35	—	—
2i	0	—	0	0	0	—	35	—	—
2j	**100**	0	**75**	**85**	**100**	**50**	**100**	**100**	**40**
2k	15	—	10	15	80	30	45	—	—
2l	0	—	0	0	**90**	**30**	**100**	**45**	—
2m	0	—	0	0	40	—	**100**	**65**	—
rotenone	65	—	45	35	100	100	100	60	35

the compounds, **1f**, **1l**, **2c**, **2e**, **2h**, **2k**, and **2l** exhibited antiviral activities much higher than that of ribavirin (the inactivation, curative, and protective effects in vivo were 41, 37, and 38%, respectively). Compounds **2e** and **2k**, with excellent activities and good physicochemical properties, emerged as new lead compounds for the development of novel antiviral agents. The in vivo antiviral activities of most of the compounds were higher than those in vitro, which indicated that these alkaloids might be able to induce disease resistance in plants. The natural product nortopsentin A and its analogue nortopsentin D exhibited higher activities than nortopsentin B and nortopsentin C. However, the introduction of Ts groups reverses this (antiviral effects: **2b** and **2c** > **2a** and **2d**). The 10-position-indole-unit-replaced derivatives, **1e**−**1m**, displayed activities similar to that of **1a**. However, the replacement of the indole unit at the 10-position of **2a** significantly increased the antiviral activity (antiviral effects: **2e**−**2m** > **2a**). The other structure−activity relationships in vivo were similar to those in vitro.

Fungicidal Activity. The nortopsentins in series **1** and their derivatives were also evaluated for their fungicidal activities with the commercial fungicides chlorothalonil and carbendazim as the controls.

In Vitro Fungicidal Activity. First, the in vitro antifungal-activity tests showed that the nortopsentins in series **1** and their derivatives in series **2** exhibited broad-spectrum fungicidal activities against 14 kinds of phytopathogenic fungi at 50 mg/kg (Table 3). Some of the compounds showed levels of antifungal activity similar to that of the commercial fungicide carbendazim. Nortopsentin D showed higher activity than carbendazim against *Physalospora piricola*. Compounds **2f** and **2j** exhibited excellent fungicidal activities against *Rhizoctonia cerealis* (fungicidal activities of 90 and 89%, respectively), which were higher than those of the commercial fungicides, chlorothalonil and carbendazim. Compound **2h** exhibited excellent fungicidal activity (fungicidal activity of 95%) against *Sclerotinia sclerotiorum*, which was higher than those of the commercial fungicides, chlorothalonil and carbendazim. Compounds **2f**, **2h**, and **2j** emerged as new lead compounds for antifungal-activity research.

In Vivo Fungicidal Activity. The nortopsentins in series **1** and their derivatives in series **2** were further evaluated for their in vivo fungicidal activities against a series of plant pathogens. Their abilities to protect cucumbers from *Corynespora cassiicola*, peppers from *Phytophthora capsici*, rape from *Sclerotinia sclerotiorum*, rice from *Rhizoctonia solani*, and cucumbers from *Botrytis cinerea* were determined, and the results (Table 4) revealed that the nortopsentins in series **1** and their derivatives in series **2** also displayed good in vivo fungicidal activities.

Insecticidal Activity. The nortopsentins in series **1** and their derivatives were also evaluated for their insecticidal activities with the commercial insecticide rotenone as the control. Some compounds exhibited excellent insecticidal activities (Table 5). Compounds **1k** and **2j** exhibited broad-spectrum insecticidal activities that induced >70% mortality in *Mythimna separate*, *Helicoverpa armigera Hubner*, and *Ostrinia nubilalis* at 600 mg/kg. Compounds **1d**, **1f**, **1m**, **2h**, **2j**, and **2l** exhibited >90% insecticidal activities against *Plutella xylostella* at 600 mg/kg, and some

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mortalities reached 100%. Compounds **1b**, **1d**, **2a**, **2j**, **2l**, and **2m** exhibited good insecticidal activities against mosquito larvae, especially compounds **1d** and **2j**, which exhibited 100% mortality at 5 mg/kg.

In summary, nortopsentins A–D and a series of derivatives were designed, synthesized, and evaluated for their antiviral, anti-phytopathogenic-fungus, and insecticidal activities for the first time. Most of these compounds displayed good to excellent antiviral activities. The structure–activity-relationship study revealed that these compounds were very sensitive to substituents. Compounds **2e** and **2k**, which had excellent antiviral activities and good physicochemical properties, emerged as new lead compounds for the development of novel antiviral agents. Further fungicidal-activity tests revealed that these alkaloids displayed broad-spectrum fungicidal activities. Compounds **2f**, **2h**, and **2j** emerged as new lead compounds for antifungal-activity research. Insecticidal-activity tests revealed that these alkaloids also displayed good insecticidal activities. The above results provide fundamental support for the development of nortopsentins as potential agrochemical bioregulators.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b00507.

^{1}H and ^{13}C NMR and HRMS spectra of nortopsentins in series **1** and **2** and ^{1}H NMR spectra of compounds **4**–**10** (PDF)

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Godfrey, C. R. A. Fungicides and bactericides. In *Agrochemicals from Natural Products*; Godfrey, C. R. A., Ed.; Marcel Dekker: NewYork, 1995; pp 311–339.

(2) Dayan, F. E.; Cantrell, C. L.; Duke, S. O. Natural products in crop protection. *Bioorg. Med. Chem.* **2009**, *17*, 4022–4034.

(3) Cantrell, C. L.; Dayan, F. E.; Duke, S. O. Natural products as source for new pesticides. *J. Nat. Prod.* **2012**, *75*, 1231–1242.

(4) Dias, D. A.; Urban, S.; Roessner, U. A historical overview of natural products in drug discovery. *Metabolites* **2012**, *2*, 303–336.

(5) Newman, D. J.; Cragg, G. M. Natural products as drugs and leads to drugs: the historical perspective. In *Natural Product Chemistry for Drug Discovery*; Buss, A. D., Butler, M. S., Eds.; Royal Society of Chemistry Publishing: Cambridge, U.K., 2010; pp 3–27.

(6) Newman, D. J.; Cragg, G. M. Natural products as sources for new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* **2012**, *75*, 311–335.

(7) Song, B. A.; Yang, S.; Jin, L. H.; Bhadury, P. S. *Environment-friendly anti-plant viral agents*; Chemical Industry Press: Beijing, China, 2009; pp 1–305.

(8) Rimando, A.; Duke, S. O. Natural products for pest management. In *Natural Products for Pest Management*; Rimando, A., Duke, S. O., Eds.; American Chemical Society: Washington, DC, 2006; Vol. *927*, pp 2–21.

(9) Zhang, G. P.; Hao, G. F.; Pan, J. K.; Zhang, J.; Hu, D. Y.; Song, B. A. Asymmetric synthesis and bioselective activities of α-aminophosphonates based on the dufulin motif. *J. Agric. Food Chem.* **2016**, *64*, 4207–4213.

(10) Gan, X. H.; Hu, D. Y.; Wang, Y. J.; Yu, L.; Song, B. A. Novel *trans*-ferulic acid derivatives containing a chalcone moiety as potential activator for plant resistance induction. *J. Agric. Food Chem.* **2017**, *65*, 4367–4377.

(11) Zhang, J.; Zhao, L.; Zhu, C.; Wu, Z. X.; Zhang, G. P.; Gan, X. H.; Liu, D. Y.; Pan, J. K.; Hu, D. Y.; Song, B. A. Facile synthesis of novel vanillin derivatives incorporating a bis(2-hydroxyethyl)dithioacetal moiety as antiviral agents. *J. Agric. Food Chem.* **2017**, *65*, 4582–4588.

(12) Sakemi, S.; Sun, H. H. Nortopsentins A, B, and C. cytotoxic and antifungal imidazolediylbis[indoles] from the Sponge *Spongosorites ruetzleri*. *J. Org. Chem.* **1991**, *56*, 4304–4307.

(13) Sun, H. H.; Sakemi, S., Gunasekera, S.; Kashman, Y.; Lui, M.; Burres, N.; McCarthy, P. Isolation of nortopsentin A, B, and C from marine sponges as antitumor and antimicrobial agents. U.S. Patent US4970226, 1990; *Chem. Abstr.* **1991**, *115*, 35701z.

(14) Kawasaki, I.; Yamashita, M.; Ohta, S. Total synthesis of nortopsentins A–D, marine alkaloids. *Chem. Pharm. Bull.* **1996**, *44*, 1831–1839.

(15) Diana, P.; Carbone, A.; Barraja, P.; Montalbano, A.; Martorana, A.; Dattolo, G.; Gia, O.; Dalla Via, L.; Cirrincione, G. Synthesis and antitumor properties of 2,5-bis(3′-indolyl)thiophenes: Analogues of marine alkaloid nortopsentin. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2342–2346.

(16) Diana, P.; Carbone, A.; Barraja, P.; Kelter, G.; Fiebig, H. H.; Cirrincione, G. Synthesis and antitumor activity of 2,5-bis(3′-indolyl)-furans and 3,5-bis(3′-indolyl)-isoxazoles, nortopsentin analogues. *Bioorg. Med. Chem.* **2010**, *18*, 4524–4529.

(17) Carbone, A.; Parrino, B.; Barraja, P.; Spanò, V.; Cirrincione, G.; Diana, P.; Maier, A.; Kelter, G.; Fiebig, H. H. Synthesis and antiproliferative activity of 2,5-bis(3′-indolyl)pyrroles, analogues of the marine alkaloid nortopsentin. *Mar. Drugs* **2013**, *11*, 643–654.

(18) Spanò, V.; Attanzio, A.; Cascioferro, S.; Carbone, A.; Montalbano, A.; Barraja, P.; Tesoriere, L.; Cirrincione, G.; Diana, P.; Parrino, B. Synthesis and antitumor activity of new thiazole nortopsentin analogs. *Mar. Drugs* **2016**, *14*, No. e226.

(19) Liu, L. R. *The control of disease and pests of tobacco*; Science Press: Beijing, China, 1998.

(20) Wang, Z. W.; Wei, P.; Wang, L. Z.; Wang, Q. M. Design, synthesis, and anti-tobacco mosaic virus (TMV) activity of phenanthroindolizidines and their analogues. *J. Agric. Food Chem.* **2012**, *60*, 10212–10219.

(21) Song, H. J.; Liu, Y. X.; Liu, Y. X.; Wang, L. Z.; Wang, Q. M. Synthesis and antiviral and fungicidal activity evaluation of β-carboline, dihydro-β-carboline, tetrahydro-β-carboline alkaloids, and their derivatives. *J. Agric. Food Chem.* **2014**, *62*, 1010–1018.

(22) Guo, P. B.; Wang, Z. W.; Li, G.; Liu, Y. X.; Xie, Y. F.; Wang, Q. M. First discovery of polycarpine, polycarpaurines A and C, and their derivatives as novel antiviral and anti-phytopathogenic fungi agents. *J. Agric. Food Chem.* **2016**, *64*, 4264–4272.

(23) Ni, W. J.; Li, C. J.; Liu, Y. X.; Song, H. J.; Wang, L. Z.; Song, H. B.; Wang, Q. M. Various bioactivity and relationship of structure–activity of matrine analogues. *J. Agric. Food Chem.* **2017**, *65*, 2039–2047.

(24) Zhao, H. P.; Liu, Y. X.; Cui, Z. P.; Beattie, D.; Gu, Y. C.; Wang, Q. M. Design, synthesis, and biological activities of arylmethylamine

DOI: 10.1021/acs.jafc.8b00507
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

substituted chlorotriazine and methylthiotriazine compounds. *J. Agric. Food Chem.* **2011**, *59*, 11711−11717.
(25) Tan, J.; Chen, Y.; Li, H.; Yasuda, N. Suzuki-Miyaura cross-coupling reactions of unprotected haloimidazoles. *J. Org. Chem.* **2014**, *79*, 8871−8876.
(26) Gu, X. H.; Wan, X. Z.; Jiang, B. Syntheses and biological activities of bis(3-indolyl)thiazoles, analogues of marine bis(indole)alkaloid nortopsentins. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 569−572.
(27) Guinchard, X.; Vallée, Y.; Denis, J. N. Total synthesis of marine sponge bis(indole) alkaloids of the topsentin class. *J. Org. Chem.* **2007**, *72*, 3972−3975.

DOI: 10.1021/acs.jafc.8b00507
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