Synthesis and Fungicidal Activity of Lansiumamide A and B and Their Derivatives

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Abstract: A efficient 2-step protocol has been applied for the synthesis of Lansiumamide B (N-methyl-N-cis-styryl-cinnamamide, 2) derivatives by various substitution on the amide nitrogen with alkyl, allyl, propargyl, benzyl or ester groups. The structures of nine new compounds were characterized by HRMS, $^1$H NMR, and $^{13}$C NMR spectra. These compounds were tested in vitro against 10 strains of phytopathogenic fungi and showed a wide antifungal spectrum. The relationship between different substituents on the amide nitrogen and antifungal activity of Lansiumamide B derivatives were compared and analyzed. The result indicates that the length and steric hindrance of N-substitution have a significant impact on biological activities. It is noteworthy that the methyl or ethyl substituent on the amide nitrogen is critical for the antifungal activities.

Keywords: antifungal activity; Lansiumamide B; derivatives; SAR; chemical synthesis

1. Introduction

Lansiumamide B (N-methyl-N-cis-styryl-cinnamamide, 2) was firstly isolated from the extracts from Clausena lansium (Lour.) Skeels by Lin in 1989 [1]. Since then, Lansiumamide B has been reported to exhibit many pharmacological effects including anti-obesity [2], anti-diabetic [3], anti-necrosis [4] and anti-inflammatory [5] bioactivities. Moreover, Lansiumamide B was also evaluated as agrochemical. Intriguingly, Lansiumamide B performs a wide spectrum of efficient bioactivities, such as antifeedant activities and cytotoxicity against Spodoptera litura [6], anti-bacterial activity against Ralstonia solanacearum [7], larvicidal activity against mosquito [8], nematicidal activity against Bursaphelenchus xylophilus [9,10], insecticidal activity against Plutella xylostella and Mythimna separata, acaricidal activity against Tetramychus cinnabarinus, herbicidal activity against Echinochloa crusgalli and Abutilon theophrasti [11] and fungicidal activity against Colletotrichum gloeosporioides, Fusarium oxysporum f.sp. cubense [12] and Botrytis cinerea [11]. The biological activities of Lansiumamide B were nicely reviewed by Wan in 2012 [13].

Structurally, Lansiumamide B (2) was reported as a Z-configuration enamide, which is crucial to its biological activities [11] and might pose significant synthetic challenges. The combination of the potent bioactivities and the structural novelty has received considerable synthetic attention. The pioneering works of Taylor [14], Maier [15] and Fürstner [16] furnished the enamide unit as a mixture of E/Z isomers. In 2010, Goosßen [17] documented the stereoselective and one-step synthesis of Lansiumamide A through the use of a ruthenium-catalysed hydroamination of phenylacetylene and cinnamamide. In 2014, Marquez [18] developed another synthetic route to afford Lansiumamide A (1)
as a single double bond isomer in four steps and 20% overall yield. Then NaH-mediated methylation on the amide nitrogen simply provided the desired Lansiumamide B.

Phytopathogenic fungi causes severe losses in agricultural production. Thus, the efficient control of plant fungal disease is of great importance for food production worldwide [19]. The commercially available fungicides have been extensively used in currant agriculture and lead to the growing resistance of phytopathogenic fungi [20]. And the discovery and development of new antifungal agents would be necessary to provide new arsenals for the battle against phyto-fungal disease. Natural product derived agrochemicals have received considerable attention from researchers owing to their higher efficiency, lower mammalian toxicity, and environmental compatibility [21].

Lansiumamide B is proven to be a promising lead compound for the development of novel antifungal agents [11,12]. Some Lansiumamide B-based derivatives were designed and synthesized by Wan and coworkers [22]. However, most of them showed lower antifungal activities against Colletotrichum gloeosporioides than the lead compound (Lansiumamide B). Thus, the Z-enamide moiety was further proven to be essential for the antifungal activities [22]. These results prompted us to figure out the structure-activity relationship (SAR) of Lansiumamide B derivatives and extend the modification of Lansiumamide B with the aim of securing more potent antifungal agents. Herein, we report a new series of Lansiumamide B derivatives containing various amide nitrogen substitutions (Figure 1) and their antifungal activities. The SAR between the title compounds and their lead compound (Lansiumamide B) is also compared and discussed.

![Figure 1. Lead compounds and design strategy for the title compounds.](image)

2. Results and Discussion

2.1. Chemical Syntheses

The synthetic route of compounds 1, 2 and 3 is outlined in Scheme 1. Ruthenium-catalyzed hydroamination of commercially available cinnamamide and phenylacetylene according to Gooßen’s protocol gave Lansiumamide A (1) in a single step and an almost quantitative yield. Final BuLi-mediated substitution on the N-H moiety of Lansiumamide A (1) afforded Lansiumamide B (2) and its derivatives 3 in good to excellent yields (73% to 89%). The synthetic route is shown in Scheme 1. The structures of 3 were firmly established by HRMS, $^1$H NMR, and $^{13}$C NMR spectra.
which could be avoided by substitution on the amide nitrogen. Secondly, increasing the substituent
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Alternaria solani
activity against antifungal activity. Noteworthy is that all of our synthetic compounds exhibit much higher inhibitory
Fusarium graminearum
and similar inhibitory rate. For example, the
descend gradually. Furthermore, we notice that as the substituent in compounds Thanatephorus cucumeris
it becomes more efficient to
from methyl to ethyl group would decrease the antifungal activity slightly. There are exceptions and
relationships can still be discovered. Firstly, the amide N-H moiety is detrimental to the bioactivities,
compounds are less active than their lead compound (Lansiumamide B), some structure-activity
activity in varying degrees against each of the test fungi. Although part or most of the synthetic
pathogenic fungi at 100 µg/mL. The results listed in Tables 1 and 2 show all the compounds display
The in vitro antifungal activities of compounds

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\text{Scheme 1. General synthetic procedure for title compounds.}
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2.2. Fungicidal Activity

The in vitro antifungal activities of compounds 1, 2, 3a to 3i were screened against ten plant
pathogenic fungi at 100 µg/mL. The results listed in Tables 1 and 2 show all the compounds display
the activity in varying degrees against each of the test fungi. Although part or most of the synthetic
compounds are less active than their lead compound (Lansiumamide B), some structure-activity
relationships can still be discovered. Firstly, the amide N-H moiety is detrimental to the bioactivities,
which could be avoided by substitution on the amide nitrogen. Secondly, increasing the substituent
from methyl to ethyl group would decrease the antifungal activity slightly. There are exceptions and
it becomes more efficient to Thanatephorus cucumeris, Fusarium solani, and Alternaria solani. As the
substituent continues to increase from the propyl to butyl group, the corresponding biological activities
descend gradually. Furthermore, we notice that as the substituent in compounds 3d, 3f, 3g, 3i
grows bulkier, the antifungal activity comes down rapidly. Thirdly, similar steric hindrance would result in
similar inhibitory rate. For example, the n-propyl and allyl group come to almost the same antifungal
activities, as does the propargyl group except that it becomes more powerful against Botrytis cinerea
and Fusarium graminearum than the ethyl group. Finally, the ester group would not help to elevate the
antifungal activity. Noteworthy is that all of our synthetic compounds exhibit much higher inhibitory
activity against Alternaria solani than the positive control carbendazim.

Table 1. Preliminary antifungal activities of all title compounds against five fungi at 100 µg/mL.

| Compounds | Thaneaphorus cucumeris | Sclerotinia sclerotiorum | Botrytis cinerea | Phytophthora sojae | Fusarium graminearum |
|-----------|-------------------------|-------------------------|-----------------|-------------------|----------------------|
| No.       | R                       | Averae Inhibition Rate ± SD (%) (n = 3) |
| 1         | H                       | 53.89 ± 0.19             | 64.57 ± 0.50    | 27.09 ± 0.08      | 33.08 ± 0.61         | 18.09 ± 0.58         |
| 2         | Me                      | 91.20 ± 0.87             | 87.52 ± 2.49    | 80.60 ± 0.46      | 79.20 ± 1.76         | 76.63 ± 0.50         |
| 3a        | Et                      | 93.33 ± 0.66             | 66.55 ± 0.85    | 68.08 ± 1.14      | 62.16 ± 0.57         | 49.90 ± 0.55         |
| 3b        | n-Pr                    | 63.94 ± 1.14             | 58.23 ± 1.36    | 37.16 ± 0.96      | 49.41 ± 1.60         | 30.52 ± 1.21         |
| 3c        | n-Bu                    | 42.41 ± 0.95             | 43.61 ± 1.35    | 19.03 ± 0.76      | 36.57 ± 0.78         | 20.83 ± 1.12         |
| 3d        | allyl                   | 70.80 ± 0.94             | 62.45 ± 1.03    | 40.45 ± 0.85      | 60.19 ± 1.48         | 41.67 ± 1.82         |
| 3e        | propargyl               | 64.28 ± 0.63             | 64.90 ± 1.17    | 77.70 ± 1.43      | 47.34 ± 0.57         | 62.04 ± 0.89         |
| 3f        | 2-methylallyl           | 47.13 ± 0.49             | 35.96 ± 0.95    | 29.50 ± 0.87      | 53.94 ± 0.78         | 17.08 ± 1.39         |
| 3g        | prenyl                  | 40.02 ± 1.58             | 26.98 ± 1.28    | 32.97 ± 1.98      | 19.47 ± 1.43         | 6.74 ± 0.44          |
| 3h        | CH3CO2Et                | 27.34 ± 1.25             | 18.02 ± 0.37    | 27.23 ± 0.27      | 21.75 ± 0.62         | 26.95 ± 0.66         |
| 3i        | benzyl                  | 5.07 ± 0.81              | 25.00 ± 1.72    | 49.18 ± 0.93      | 59.01 ± 0.71         | 27.09 ± 0.76         |
| Carbendazim| 100                     | 100                      | 100             | 100               | 100                  | 100                  |
were washed with water (20 mL) and brine (20 mL), dried over Na₂SO₄. Reactions were performed with silica gel 60 (particle size 0.040–0.062 mm) supplied by Liangchen. Workup, extraction and column chromatography were used as received from commercial suppliers (400 MHz, CDCl₃ (petroleum ether/ethyl acetate = 5/1) to give the Lansiumamide A (30 mL). The resulting mixture was extracted with EtOAc (3 × 20 mL), phenylacetylene (204 mg, 2.00 mmol), and water (108 µL, 0.60 mmol) were added via syringe. The resulting solution was stirred for 6 h at 60 °C. The reaction mixtures were poured into aq NaHCO₃ (20 mL), the combined organic layers were washed with water (20 mL) and brine (20 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel using eluents (petroleum ether/ethyl acetate = 5/1) to give the Lansiumamide A (241 mg, 0.97 mmol) as a light yellow solid. Note: reaction on a larger scale (>1 mmol) resulted in lower yield. Therefore, the reaction on a 1.0 mmol scale was repeated five times to obtain 1.20 grams of Lansiumamide A (1). ¹H NMR (400 MHz, CDCl₃) δ: 7.79 (d, J = 9.2 Hz, 1H), 7.73 (d, J = 15.6 Hz, 1H), 7.38–7.30 (m, 10H), 7.13 (dd, 3.2.1. General Synthetic Procedure for Lansiumamide A (1)

Lansiumamide A (1) was synthesized according to Gooßen’s protocol [17]. An oven-dried flask was charged with the trans-cinnamamide (147 mg, 1.00 mmol), bis(2-methylallyl)-cycloocta-1,5-diene-ruthenium(II) (16.0 mg, 0.05 mmol), 1,4-bis(dicyclohexylphosphinobutane (27.0 mg, 0.06 mmol), and ytterbium triflate (24.8 mg, 0.04 mmol) and flushed with nitrogen. Subsequently, dry DMF (3 mL), phenylacetylene (204 mg, 2.00 mmol), and water (108 µL, 0.60 mmol) were added via syringe. The resulting solution was stirred for 6 h at 60 °C. The reaction mixtures were poured into aq NaHCO₃ (30 mL). The resulting mixture was extracted with EtOAc (3 × 20 mL), the combined organic layers were washed with water (20 mL) and brine (20 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel using eluents (petroleum ether/ethyl acetate = 5/1) to give the Lansiumamide A (1) (241 mg, 0.97 mmol) as a light yellow solid. Note: reaction on a larger scale (>1 mmol) resulted in lower yield. Therefore, the reaction on a 1.0 mmol scale was repeated five times to obtain 1.20 grams of Lansiumamide A (1). ¹H NMR (400 MHz, CDCl₃) δ: 7.79 (d, J = 9.2 Hz, 1H), 7.73 (d, J = 15.6 Hz, 1H), 7.38–7.30 (m, 10H), 7.13 (dd, 3.2. Synthetic Procedures

3.2.1. General Synthetic Procedure for Lansiumamide A (1)

Lansiumamide A (1) was synthesized according to Gooßen’s protocol [17]. An oven-dried flask was charged with the trans-cinnamamide (147 mg, 1.00 mmol), bis(2-methylallyl)-cycloocta-1,5-diene-ruthenium(II) (16.0 mg, 0.05 mmol), 1,4-bis(dicyclohexylphosphinobutane (27.0 mg, 0.06 mmol), and ytterbium triflate (24.8 mg, 0.04 mmol) and flushed with nitrogen. Subsequently, dry DMF (3 mL), phenylacetylene (204 mg, 2.00 mmol), and water (108 µL, 0.60 mmol) were added via syringe. The resulting solution was stirred for 6 h at 60 °C. The reaction mixtures were poured into aq NaHCO₃ (30 mL). The resulting mixture was extracted with EtOAc (3 × 20 mL), the combined organic layers were washed with water (20 mL) and brine (20 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel using eluents (petroleum ether/ethyl acetate = 5/1) to give the Lansiumamide A (1) (241 mg, 0.97 mmol) as a light yellow solid. Note: reaction on a larger scale (>1 mmol) resulted in lower yield. Therefore, the reaction on a 1.0 mmol scale was repeated five times to obtain 1.20 grams of Lansiumamide A (1). ¹H NMR (400 MHz, CDCl₃) δ: 7.79 (d, J = 9.2 Hz, 1H), 7.73 (d, J = 15.6 Hz, 1H), 7.38–7.30 (m, 10H), 7.13 (dd,
3.2.2. General Synthetic Procedure for Lansiumamide B (2) the Title Compounds 3

A flame-dried round bottle under nitrogen atmosphere was charged with Lansiumamide A (100 mg, 0.4 mmol) and anhydrous tetrahydrofuran (THF, 2 mL). The solution was cooled to −78 °C and n-butyllithium (0.25 mL, 1.6 M in hexane, 0.4 mmol) was added dropwise. The reaction mixture was warmed to 0 °C, stirred for additional 30 min and then cooled to −78 °C. The corresponding iodide or bromide (0.6 mmol) was added dropwise to the cold solution. The reaction mixture was then allowed to gradually warm to room temperature and stirred overnight. Aqueous saturated NH₄Cl solution (5 mL) was added under vigorous stirring to quench the reaction. The volatiles (mainly THF) were removed under reduced pressure and the aqueous phase was extracted with diethyl ether (3 × 10 mL). The combined organic fractions were washed with water and brine, dried over anhydrous MgSO₄ and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using eluents (petroleum ether/ethyl acetate = 10/1) to afford the desired compound.

HRMS (ESI) m/z calculated for C₁₉H₂₀NO [M + H]+ 278.1545, found 278.1550.

2a. 85 mg, 81% yield; light yellow solid. ¹H NMR (400 MHz, CDCl₃) δ: 7.67 (d, J = 15.6 Hz, 1H), 7.39–7.31 (m, 10H), 6.96 (d, J = 15.6 Hz, 1H), 6.53 (d, J = 8.8 Hz, 1H), 6.27 (d, J = 8.8 Hz, 1H), 3.12 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ: 131.32, 130.56, 130.10, 129.63, 129.13, 128.76, 128.38, 127.71, 126.84, 126.25 (2 × C), 125.08, 118.30, 34.67.

3b. 91 mg, 78% yield; light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.58 (d, J = 15.6 Hz, 1H), 7.32–7.24 (m, 10H), 6.89 (d, J = 15.6 Hz, 1H), 6.34 (s, 2H), 3.65 (q, J = 7.2 Hz, 2H), 1.20 (t, J = 7.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ: 165.73, 142.44, 135.30, 134.32, 129.54, 128.67 (2 × C), 128.64 (2 × C), 125.08, 124.80, 119.49, 110.72. HRMS (ESI) m/z calculated for C₁₉H₂₀NO [M + H]+ 278.1545, found 278.1550.

3c. 92 mg, 75% yield; light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.59 (d, J = 15.6 Hz, 1H), 7.34–7.26 (m, 10H), 6.90 (d, J = 15.6 Hz, 1H), 6.36 (d, J = 8.8 Hz, 1H), 6.31 (d, J = 8.8 Hz, 1H), 3.58 (t, J = 7.6 Hz, 1H), 1.61 (dt, J = 14.8, 7.6 Hz, 2H), 1.32 (dq, J = 14.8, 7.2 Hz, 2H), 0.90 (t, J = 7.2 Hz, 3H). ¹³C NMR (400 MHz, CDCl₃) δ: 165.82, 142.48, 135.31 (2 × C), 129.54, 128.68 (2 × C), 128.65 (2 × C), 128.61 (2 × C), 128.22, 127.86 (2 × C), 127.14, 118.84, 41.92, 13.15. HRMS (ESI) m/z calculated for C₂₀H₂₂NO [M + H]+ 292.1701, found 292.1697.

3d. 103 mg, 89% yield; light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.61 (d, J = 15.6 Hz, 1H), 7.34–7.26 (m, 10H), 6.91 (d, J = 15.6 Hz, 1H), 6.37 (d, J = 10.0 Hz, 1H), 6.32 (d, J = 10.0 Hz, 1H), 5.87 (ddt, J = 10.0, 8.0, 5.6, 1H), 5.14 (dd, J = 10.0, 8.0 Hz, 2H), 4.21 (d, J = 5.6 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ: 165.81, 142.93, 135.20, 134.29, 132.78, 129.67, 128.72 (2 × C), 128.69 (2 × C), 128.65 (2 × C), 128.60 (2 × C), 128.21, 127.87 (2 × C), 127.68, 126.84, 118.79, 46.96, 30.12, 20.36, 13.84. HRMS (ESI) m/z calculated for C₂₁H₂₄NO [M + H]+ 306.1858, found 306.1855.

3e. 95 mg, 83% yield; light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.61 (d, J = 15.6 Hz, 1H), 7.33–7.24 (m, 10H), 6.86 (d, J = 15.6 Hz, 1H), 6.42 (s, 2H), 4.39 (s, 2H), 2.23 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ: 165.75, 143.53, 135.03, 134.04, 129.83, 128.80 (2 × C), 128.71 (2 × C), 128.54 (2 × C), 128.46, 128.03.
127.98 (2 × C), 126.48, 117.91, 78.73, 71.98, 35.82. HRMS (ESI) m/z calculated for C_{20}H_{18}NO [M + H]^+ 288.1388, found 288.1383.

3f. 99 mg, 82% yield; light yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ: 7.64 (d, J = 15.6 Hz, 1H), 7.35–7.26 (m, 10H), 6.96 (d, J = 15.6 Hz, 1H), 6.37 (d, J = 8.8 Hz, 1H), 6.32 (d, J = 8.8 Hz, 1H), 4.87 (s, 1H), 4.74 (s, 1H), 4.18 (s, 2H), 1.71 (s, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) δ: 165.97, 143.12, 140.38, 135.19, 134.29, 129.70, 128.72 (2 × C), 128.70 (2 × C), 128.64 (2 × C), 128.27, 127.94 (2 × C), 127.20, 126.93, 118.30, 112.68, 52.05, 20.40. HRMS (ESI) m/z calculated for C_{21}H_{22}NO [M + H]^+ 304.1701, found 304.1704.

3g. 100 mg, 79% yield; light yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ: 7.58 (d, J = 15.2 Hz, 1H), 7.34–7.25 (m, 10H), 6.88 (d, J = 15.2 Hz, 1H), 6.32 (s, 2H), 5.28 (t, J = 7.2 Hz, 1H), 4.21 (d, J = 7.2 Hz, 2H), 1.69 (s, 3H), 1.62 (s, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) δ: 165.70, 142.47, 136.54, 135.32, 134.43, 129.53, 128.65 (4 × C), 128.58 (2 × C), 128.18, 127.86 (2 × C), 127.56, 127.03, 119.31, 118.75, 44.56, 25.73, 17.95. HRMS (ESI) m/z calculated for C_{22}H_{24}NO [M + H]^+ 318.1858, found 318.1859.

3h. 98 mg, 73% yield; light yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ: 7.71 (d, J = 15.6 Hz, 1H), 7.41–7.32 (m, 10H), 6.96 (d, J = 15.6 Hz, 1H), 6.61 (d, J = 8.8 Hz, 1H), 6.29 (d, J = 8.8 Hz, 1H), 4.23 (s, 2H), 4.18 (q, J = 7.2 Hz, 2H), 1.25 (t, J = 7.2 Hz, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) δ: 169.02, 166.94, 144.01, 134.94, 134.02, 129.98, 128.79 (6 × C), 128.42, 128.10 (2 × C), 127.76, 125.69, 117.52, 61.30, 48.97, 14.16. HRMS (ESI) m/z calculated for C_{22}H_{24}NO [M + H]^+ 336.1600, found 336.1606.

3i. 115 mg, 85% yield; light yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ: 7.64 (d, J = 15.6 Hz, 1H), 7.35–7.23 (m, 15H), 6.92 (d, J = 15.6 Hz, 1H), 6.31 (d, J = 8.8 Hz, 1H), 6.28 (d, J = 8.8 Hz, 1H), 4.78 (s, 2H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) δ: 165.99, 143.12, 137.14, 135.20, 134.24, 129.10, 128.75 (4 × C), 128.70 (2 × C), 128.52 (4 × C), 128.34, 127.94 (2 × C), 127.51, 127.41, 127.26, 118.41, 50.03. HRMS (ESI) m/z calculated for C_{24}H_{22}NO_{3} [M + H]^+ 340.1701, found 340.1728.

3.3. Bioassays

The antifungal activity of all synthesized compounds 1–3 was tested against ten plant pathogenic fungi, namely Phytophthora sojae, Sclerotinia sclerotiorum, Thanatephorus cucumeris, Fusarium graminearum, Pyricularia oryzae, Fusarium solani, Rhizoctonia solani Kuehn, Botrytis cinerea, Gloeosporium piperatum and Alternaria solani by the plate technique. Compounds were dissolved in DMSO (10 mL) before mixing with Potato Dextrose Agar (PDA 90 mL) medium. The final concentration of compounds 1–3 in the medium were fixed at 100 μg/mL. Ten kinds of fungi were incubated in PDA at 25 °C for five days to get a new mycelium for the antifungal assay, then a mycelia disk of an approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. The inoculated plates were incubated at 25 °C for five days. DMSO in sterilized distilled water served as the control, while carbendazim was used as a positive control for each treatment, three replicates were carried out. The radial growth of the fungal colonies was measured on the sixth day and the data were statistically analyzed. The relative control efficacy of compounds compared to the blank assay was calculated via the following equation: I (%) = [(CK − PT/CK)] × 100%, where I is the relative control efficacy, CK is the average disease index during the blank assay, and PT is the average disease index after treatment during testing. The in vitro inhibiting effects of the test compounds on the fungi were calculated by the formula CV = (A − B)/A, where A represents the diameter of fungi growth on untreated PDA, B represents the diameter of fungi on treated PDA, and CV represents the rate of inhibition. All of the strains were conserved in the Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University (Fuzhou, China).
4. Conclusions

In summary, we report the synthesis of a new series of Lansiumamide B derivatives in a simple and efficient way and the evaluation of their in vitro antifungal activity against ten plant pathogenic fungi. Most of the synthetic compounds exhibit a potential inhibition activity against all the tested fungi and some of them are more effective than carbendazim. Some of the compounds, especially 2 and 3a, are identified as the most active and therefore of great potential to be developed as new antifungal agents. SAR analysis shows that the activity is highly sensitive to the substituent on the amide nitrogen. The introduction of methyl or ethyl group might enhance the activity, whereas the presence of N-H or group with larger steric hindrance causes decrease of activity. Therefore, these results are of great significance for the design, synthesis, and development of novel antifungal reagents containing Z-enamides. Further structural modification is ongoing in our laboratory, with the aim to secure compounds with improved antifungal activity.

Supplementary Materials: Copies of 1H, 13C, spectra of new compounds. This material is available free of charge online.

Author Contributions: H.X. supervised the synthesis steps; T.C., L.H. and Q.S. conducted the synthetic experiments; Z.L. and Y.S. performed the antifungal experiments; M.-A.O. supervised the antifungal experiments; H.X. and L.S. wrote the manuscript; L.S. designed the target compound and supervised all part of the work and approved the final manuscript.

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**Sample Availability:** Samples of the compounds **1, 2, 3a–i** are available from the authors.

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