Discovery of Novel Pimprinine and Streptochlorin Derivatives as Potential Antifungal Agents

Jing-Rui Liu 1, Jia-Mu Liu 1, Ya Gao 1, Zhan Shi 1, Ke-Rui Nie 1, Dale Guo 2, Fang Deng 2, Hai-Feng Zhang 3, Abdallah S. Ali 4, Ming-Zhi Zhang 1,*, Wei-Hua Zhang 1,† and Yu-Cheng Gu 5

Abstract: Pimprinine and streptochlorin are indole alkaloids derived from marine or soil microorganisms. In our previous study, they were promising lead compounds due to their potent bioactivity in preventing many phytopathogens, but further structural modifications are required to improve their antifungal activity. In this study, pimprinine and streptochlorin were used as parent structures with the combination strategy of their structural features. Three series of target compounds were designed and synthesized. Subsequent evaluation for antifungal activity against six common phytopathogenic fungi showed that some of thee compounds possessed excellent effects, and this is highlighted by compounds 4a and 5a, displaying 99.9% growth inhibition against Gibberella zeae and Alternaria Leaf Spot under 50 µg/mL, respectively. EC50 values indicated that compounds 4a, 5a, 8c, and 8d were even more active than Azoxystrobin and Boscalid. SAR analysis revealed the relationship between 5-(3′-indolyl)oxazole scaffold and antifungal activity, which provides useful insight into the development of new target molecules. Molecular docking models indicate that compound 4a binds with leucyl-tRNA synthetase in a similar mode as AN2690, offering a perspective on the mode of action for the study of its antifungal activity. These results suggest that compounds 4a and 5a could be regarded as novel and promising antifungal agents against phytopathogens due to their valuable potency.

Keywords: pimprinine; streptochlorin; synthesis; antifungal activity; EC50; SAR; molecular docking

1. Introduction

Natural products, small molecules isolated from biological sources, play a highly significant role in medicine and agrochemical innovation, and the repertoire of natural products offers tremendous opportunities for chemical biology and drug discovery [1–4]. Approximately two-thirds of all approved small-molecule drugs from January 1981 to September 2019 owe their origins to natural products [5]. Pimprinine is an indole alkaloid produced by many species of Streptomyces, first isolated from the filtrates of Streptomyces pimprina cultures in 1963 [6]. For decades, the marine environment has also provided a source of novel bioactive and structurally diverse natural products [7]. Streptochlorin, with a structure similar to that of pimprinine, is a bacterial metabolite originally isolated from marine Streptomyces sp. By H. Watanabe in 1988 [8]; its structure is shown in Figure 1. Members of this natural 5-(3′-indolyl)oxazole family (Figure 1), including pimprinethine, pimprinaphine, WS-30581 A and B, labradorins 1 and 2, pimprinol A, B, and C, martefragin A, deaminomartefragin A, almazole C and D, breitfussin A and B, and dipimprinine...
E and F, exhibit a wide range of potent biological activity, such as antioxidation [9,10], anticancer [11,12], antiviral [13,14], anti-angiogenesis [15], and antibiotic properties [16], anti-cell proliferation, [17] and pesticidal activity [18]. Pimprinine and streptochlorin were promising antifungal substances due to their good bioactivity in preventing many phytopathogens in our previous study [19]. Bio-screening conducted by Syngenta showed that streptochlorin displayed excellent antifungal activity against Pythium dissimile, Botrytis cinerea, Zymoseptoria tritici, Pyricularia oryzae, Fusarium culmorum, and Rhizoctonia solani in artificial media [20]. Meanwhile, pimprinine and streptochlorin lack potency under lower concentrations and are rarely extended to the next stage of study, as they are not potent enough to be used as antifungal agents, and the mode of action for their antifungal activity is still unclear. In our latest study on the structural optimizations including different modifications at the indole ring and oxazole ring [21–26], we found that the 5-(3′-indolyl)oxazole core was the essential moiety for maintaining antifungal activity.

Figure 1. Chemical structures of pimprinine, streptochlorin, and related natural products.

In this study, as a continuation of our extensive research program to discover novel bioactive lead compounds, pimprinine and streptochlorin were used as the parent structures to carry out structural optimization (Figure 2), with the structural features combination strategy of these two indole alkaloids. Three series of target compounds were designed and synthesized, aiming to discover synthetic derivatives with a modified chemical structure and improved antifungal activity. The structure–activity relationships (SAR) around pimprinine and streptochlorin were also analyzed, and the molecular docking of streptochlorin with a potential target enzyme was further performed.
Figure 2. Design strategy of target molecule.

2. Results and Discussion

2.1. Synthetic Chemistry

Novel pimprinine and streptochlorin derivatives were synthesized as depicted in Schemes 1 and 2. In this approach, we described a synthesis of 5-(3′-indolyl)oxazoles alkaloids in one pot with the reported method [27], employing 3-actylindole and amino acids as substrates to be transformed into natural products. In this reaction process, we used indole as the starting material. After the acylation of indole, which gives 3-actylindole, the common precursor indole α-keto aldehyde, which was generated by an iodination/Kornblum oxidation sequence from 3-actylindole, was trapped in situ by an amino acid via a condensation/decarboxylation/annulation/oxidation reaction sequence, to eventually approach the natural products. As reported in the literature, two equivalents of I$_2$ were used, one equivalent of I$_2$ as a halogenation reagent and the other equivalent as the oxidation reagent. In our modified synthetic process, we optimized the addition time of the reaction: 1.1 equivalents of I$_2$ were used in the initial iodination reaction, and the rest of the 0.9 equivalent I$_2$ was added after the addition of amino acid. This improved method can increase the yield by 10%. Compound data, Copies of the NMR spectra, and HR-MS (ESI) spectra can be downloaded at Supplementary Materials.

Scheme 1. Synthetic routes of pimprinine and streptochlorin derivatives.

Scheme 2. Synthetic routes of substituted pimprinine and streptochlorin derivatives.
Therefore, we accomplished the synthesis of 5-(3-indolyl)oxazoles alkaloids (Tables 1–3), including pimprinine, pimprinethine, and labadorins 1, as well as their derivatives, directly. The subsequent NCS or NBS halogenation yielded novel 4-chloro-5-(3-indolyl)oxazoles and 4-bromo-5-(3-indolyl)oxazoles, respectively, including the marine natural product streptochlorin [11]. Particularly worth mentioning is that this is the first time that streptochlorin has been efficiently synthesized using this three-step method.

Table 1. List of the compounds 3.

| No. | Amino Acid R1 | Yield |
|-----|---------------|-------|
| 3a  | Glycine H     | 51%   |
| 3b  | Alanine Me    | 53%   |
| 3c  | 2-Aminobutyric acid Et | 49% |
| 3d  | Leucine       | 48%   |
| 3e  | Cyclohexylglycine | 53% |
| 3f  | Phenylglycine | 48%   |
| 3g  | Methionine    | 40%   |

Table 2. List of the compounds 4 and 5.

| No. | R1 = | X =  | Yield |
|-----|------|------|-------|
| 4a  | H    | Cl   | 58%   |
| 4b  | Cl   | Cl   | 65%   |
| 4c  | Cl   | Cl   | 60%   |
| 4d  | Cl   | Cl   | 76%   |
| 4e  | Cl   | Cl   | 62%   |
| 4f  | Cl   | Cl   | 57%   |
| 4g  | Cl   | /    | 50%   |

| No. | R1 = | X =  | Yield |
|-----|------|------|-------|
| 5a  | H    | Br   | 62%   |
| 5b  | Cl   | Br   | 76%   |
| 5c  | Cl   | Br   | 65%   |
| 5d  | Cl   | Br   | 74%   |
| 5e  | Cl   | Br   | 77%   |
| 5f  | Cl   | Br   | 70%   |

* The reaction system was complex, and the target compound was not obtained.

Table 3. List of the compounds 8, 9, and 10.

| No. | R2 = | R3 = | X =  | Yield |
|-----|------|------|------|-------|
| 8a  | 5-F  | Me   | H    | 35%   |
| 8b  | 5-Cl | Me   | H    | 50%   |
| 8c  | 5-Br | Me   | H    | 43%   |
| 8d  | 4-Me | Me   | H    | 32%   |
| 8e  | 6-F  | Me   | H    | 61%   |
| 8f  | 6-Cl | Me   | H    | 66%   |
| 8g  | 5-Me | Me   | H    | 39%   |
| 8h  | 6-F  | Et   | H    | 59%   |
| 8i  | 6-Cl | Et   | H    | 58%   |
| 8j  | 6-Br | Et   | H    | 36%   |
| 8k  | 5-Me | Et   | H    | 45%   |
| 9a  | 5-F  | Me   | Cl   | 30%   |
| 9b  | 5-Cl | Me   | Cl   | 35%   |
| 9c  | 5-Br | Me   | Cl   | 42%   |
| 9d  | 6-F  | Me   | Cl   | 39%   |
2.2. Antifungal Activity and Structure–Activity Relationships (SAR)

The antifungal activity of pimprineline, streptoehlorin, their derivatives, and the positive controls was evaluated with the mycelium growth rate method against six common phytopathogenic fungi, including *Alternaria Leaf Spot* (ALL), *Alternaria solani* (ALS), *Botrytis cinerea* (BOT), *Colletotrichum lagenarium* (COL), *Gibberella zeae* (GIB), and *Rhizoctonia solani* (RHI), at a concentration of 50 µg/mL. The screening results are given in Tables 4 and 5.

**Table 4.** Antifungal activity of compounds 3, 4, and 5 at the concentration of 50 µg/mL.

| No. | R = | X = | ALL | ALS | BOT | COL | GIB | RHI |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 3a  | H   | H   | 89.1 | 47.8 | 97.7 | 93.3 | 61.0 | 98.3 |
| 3b (Pimprineline) | | | 62.6 | 50.0 | 50.0 | 50.3 | 59.3 | 66.7 |
| 3c (Pimprineline) | | | 53.2 | 46.7 | 85.7 | 52.2 | 59.7 | 73.3 |
| 3d (Labradorins 1) | | | 29.3 | 20.7 | 44.3 | 46.0 | 47.4 | 61.3 |
| 3e  | H   | Cl  | 46.1 | 46.1 | 46.1 | 46.1 | 46.1 | 46.1 |
| 3f  | Cl  | H   | 71.4 | 66.0 | 60.0 | 82.7 | 85.7 | 85.7 |
| 3g  | Cl  | H   | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 |
| 4a  | Cl  | H   | 77.3 | 46.1 | 70.0 | 62.7 | 71.5 | 85.0 |
| 4b  | Cl  | H   | 77.3 | 46.1 | 70.0 | 62.7 | 71.5 | 85.0 |
| 4c  | Cl  | H   | 77.3 | 46.1 | 70.0 | 62.7 | 71.5 | 85.0 |
| 4d  | Cl  | H   | 77.3 | 46.1 | 70.0 | 62.7 | 71.5 | 85.0 |
| 4e  | Cl  | H   | 77.3 | 46.1 | 70.0 | 62.7 | 71.5 | 85.0 |
| 4f  | Cl  | H   | 77.3 | 46.1 | 70.0 | 62.7 | 71.5 | 85.0 |
| 5a  | Br  | H   | 99.9 | 69.0 | 99.9 | 88.3 | 98.7 | 96.1 |
| 5b  | Br  | H   | 99.9 | 69.0 | 99.9 | 88.3 | 98.7 | 96.1 |
| 5c  | Br  | H   | 99.9 | 69.0 | 99.9 | 88.3 | 98.7 | 96.1 |
| 5d  | Br  | H   | 99.9 | 69.0 | 99.9 | 88.3 | 98.7 | 96.1 |
| 5e  | Br  | H   | 99.9 | 69.0 | 99.9 | 88.3 | 98.7 | 96.1 |
| 5f  | Br  | H   | 99.9 | 69.0 | 99.9 | 88.3 | 98.7 | 96.1 |
| 5g  | Br  | H   | 99.9 | 69.0 | 99.9 | 88.3 | 98.7 | 96.1 |
| Osthole | | | 31.3 | 61.2 | 70.4 | 92.3 | 57.0 | 66.5 |
| Boscalid | | | 92.8 | 57.6 | 99.9 | 25.5 | 40.9 | 87.3 |
| Carbendazim | | | 6.4 | 59.6 | 99.9 | 99.9 | 99.9 | 99.9 |

*a* ALL, Alternaria Leaf Spot; ALS, Alternaria solani; BOT, Botrytis cinerea; COL, Colletotrichum lagenarium; GIB, Gibberella zeae; RHI, Rhizoctonia solani. *b* The data were the average value of three replications; the bold indicates data equal to or above 55% control.
Table 5. Antifungal activity of compounds 8, 9, and 10 at a concentration of 50 μg/mL.

| No.  | R² = | R³ = | X = | ALL  | ALS  | BOT  | COL  | GIB  | RHI  |
|------|------|------|-----|------|------|------|------|------|------|
| 8a   | 5-F  | Me   | H   | 53.2 | 24.4 | 12.1 | 24.1 | 38.3 | 47.5 |
| 8b   | 5-Cl | Me   | H   | 44.9 | 12.7 | 25.8 | 23.4 | 40.6 | 45.1 |
| 8c   | 5-Br | Me   | H   | 37.8 | 24.8 | 35.9 | 90.1 | 37.5 | 46.4 |
| 8d   | 4-Me | Me   | H   | 79.0 | 37.0 | 86.0 | 91.2 | 75.3 | 68.5 |
| 8e   | 6-F  | Me   | H   | 46.0 | 18.5 | 34.6 | 41.4 | 37.5 | 61.7 |
| 8f   | 6-Cl | Me   | H   | 43.1 | 21.7 | 31.8 | 42.4 | 43.9 | 75.5 |
| 8g   | 5-Me | Me   | H   | 60.3 | 33.9 | 80.9 | 92.8 | 64.7 | 69.0 |
| 8h   | 6-F  | Et   | H   | 57.1 | 41.5 | 42.6 | 68.5 | 55.8 | 79.2 |
| 8i   | 6-Cl | Et   | H   | 28.7 | 11.4 | 26.7 | 32.6 | 43.1 | 61.2 |
| 8j   | 6-Br | Et   | H   | 28.2 | 17.2 | 21.4 | 27.5 | 35.1 | 62.5 |
| 8k   | 5-Me | Et   | H   | 39.7 | 14.2 | 65.5 | 87.2 | 55.9 | 73.4 |
| 9a   | 5-F  | Me   | Cl  | 26.2 | 31.5 | 60.5 | 30.9 | 37.8 | 61.0 |
| 9b   | 5-Cl | Me   | Cl  | 31.7 | 30.4 | 34.4 | 32.6 | 35.2 | 42.8 |
| 9c   | 5-Br | Me   | Cl  | 18.7 | 1.9  | 40.9 | 6.8  | 53.4 | 35.2 |
| 9d   | 6-F  | Me   | Cl  | 15.1 | 0.0  | 26.9 | 14.8 | 37.5 | 55.8 |
| 9e   | 6-F  | Et   | Cl  | 13.2 | 14.3 | 10.8 | 11.2 | 10.0 | 29.2 |
| 9f   | 6-Cl | Me   | Cl  | 24.6 | 26.7 | 31.5 | 33.1 | 37.5 | 68.1 |
| 9g   | 6-Cl | Et   | Cl  | 12.4 | 12.2 | 10.5 | 6.5  | 7.5  | 61.4 |
| 9h   | 6-Br | Et   | Cl  | 17.0 | 12.5 | 1.8  | 11.8 | 13.2 | 21.6 |
| 9i   | 5-Me | Et   | Cl  | 21.9 | 11.7 | 35.2 | 35.7 | 29.1 | 56.8 |
| 10a  | 4-Me | Me   | Br  | 30.6 | 29.6 | 36.7 | 29.8 | 31.6 | 58.9 |
| 10b  | 5-F  | Me   | Br  | 22.2 | 22.8 | 33.4 | 26.2 | 26.4 | 45.8 |
| 10c  | 5-Cl | Me   | Br  | 10.6 | 7.0  | 16.8 | 0.0  | 29.4 | 45.8 |
| 10d  | 5-Br | Me   | Br  | 63.5 | 33.1 | 87.6 | 71.9 | 72.2 | 74.2 |
| 10e  | 6-F  | Me   | Br  | 23.8 | 14.0 | 20.5 | 27.9 | 22.1 | 58.2 |
| 10f  | 6-F  | Et   | Br  | 35.9 | 28.6 | 25.9 | 30.7 | 31.7 | 64.1 |
| 10g  | 6-Cl | Me   | Br  | 30.7 | 25.0 | 32.8 | 33.6 | 40.3 | 71.4 |
| 10h  | 6-Cl | Et   | Br  | 25.1 | 28.8 | 18.2 | 25.3 | 22.5 | 63.7 |
| 10i  | 6-Br | Et   | Br  | 27.6 | 21.9 | 1.75 | 24.2 | 22.3 | 48.4 |

**Osthole** / / / 31.3 61.2 70.4 92.3 57.0 66.5

**Boscalid** / / / 92.8 57.6 99.9 25.5 40.9 87.3

**Carbendazim** / / / 6.4 59.6 99.9 99.9 99.9

---

*All data were the average value of three replications; the bold indicates data equal to or above 55% control.*

It was observed that compounds 3a, 4a, 5a, 8d, and 8g showed significant antifungal activity against four kinds of fungi during the primary screening. The antifungal activity ranged from 60.3% to 99.9% at 50 μg/mL, and this was highlighted by the inhibition rates of these four molecules against *Colletotrichum lagenarium*, ranging from 88.3% to 94.6%. The most active compounds, 4a (streptochlorin) and 5a, were also compared with commercial fungicides in radar charts shown in Figure 3, and this indicates that 4a and 5a showed more effective or equivalent control against the four kinds of fungi than the positive controls.
It was observed that compounds 3a, 4a, 5a, 8d, and 8g showed significant antifungal activity against Gibberella zeae, some of which showed high inhibitory effects against Botrytis cinerea and Rhizoctonia solani, respectively.

In order to compare the antifungal activity of the synthesized target compounds with that of the most frequently used commercial fungicides Boscalid, Azoxystrrobin, and Carbendazim, EC$_{50}$ values of the highly active compounds (4a, 5a, 8c, 8d) were further measured, as these compounds showed equivalent or even better performance than the positive controls. As shown in Table 6, it was noticed that the EC$_{50}$ value of 4a against Botrytis cinerea was as low as 0.3613 µg/mL, which is more effective than Boscalid (5.2606 µg/mL) and Azoxystrrobin (4.3516 µg/mL), and compound 5a exhibited better activity against Alternaria leaf spot (3.4086 µg/mL) and Colletotrichum lagenarium (8.1215 µg/mL) than their corresponding controls. Moreover, the antifungal activity of 5a was equivalent to that of Carbendazim and Boscalid against Gibberella zeae and Rhizoctonia solani, respectively.

In spite of the difficulties in finding clear structure–activity relationships from the biological data, some broad conclusions can still be drawn.

First, the halogenated compounds (compounds 4 and 5) generally displayed more potent activity and a broader antifungal spectrum in the artificial media assays compared with their unhalogenated counterparts (compounds 3). On the whole, the compound whose 4-position of the oxazole ring was substituted by a halogen atom (Cl, Br) showed better antifungal activity than those that were not halogenated, though compound 3a also demonstrated 97.7% and 98.3% inhibition against Botrytis cinerea and Rhizoctonia solani, respectively. This was equivalent to or even more active than the halogenated counterparts.

Second, bio-screening data of the antifungal activity indicated that the compound with H, Me, or Et substituted at the 2-position of the oxazole ring exhibited more potent antifungal activity than those with other substituents. This is highlighted by compounds 3a, 3b, and 3c and their corresponding halogenated counterparts 4a, 4b, and 4c and 5a, 5b, and 5c, which showed more effective control than the compound with a larger substituent. Therefore, we kept the substituent at the 2-position of oxazole as methyl or ethyl and introduced various substituents at the indole ring, such as methyl and halogen, and these modifications resulted in a number of highly active compounds (8d, 8g, 8k, and 10d), some of which showed high inhibitory effects against Colletotrichum lagenarium, such as 8d (91.2%) and 8g (92.8%).
| Pathogen                  | Compound | Toxic Regression       | R          | EC50 (µg/mL) | 95% Confidence Interval |
|--------------------------|----------|------------------------|------------|--------------|-------------------------|
| **Alternaria leaf spot** | 4a       | Y = 2.7969 + 1.7139X   | 0.9802     | 19.2928      | 10.2574–36.2873         |
|                          | 5a       | Y = 3.9921 + 1.8926X   | 0.9974     | 3.4068       | 3.1301–3.7119           |
|                          | 8d       | Y = 1.9984 + 2.0046X   | 0.9904     | 31.4399      | 24.7935–39.8527         |
|                          | Boscalid | Y = 5.1084 + 1.0376X   | 0.9935     | 0.7862       | 0.6462–0.9566           |
|                          | Carbendazim | Y = -1.8843 + 5.7567X | 0.9265     | 15.6994      | 5.6810–43.3849          |
| **Alternaria solani**    | 5a       | Y = 3.8271 + 1.0526X   | 0.9977     | 13.0099      | 11.8507–14.2825         |
|                          | Boscalid | Y = 4.3437 + 0.4903X   | 0.9806     | 21.8016      | 12.7424–37.3012         |
|                          | Carbendazim | Y = 3.2290 + 2.5855X | 0.9688     | 4.8412       | 3.3993–6.8947           |
| **Botrytis cinerea**     | 4a       | Y = 5.5662 + 1.2805X   | 0.9413     | 0.3613       | 0.0753–1.7329           |
|                          | 5a       | Y = 4.6370 + 6.9223X   | 0.9167     | 1.1283       | 0.4899–2.5988           |
|                          | 8d       | Y = 0.7267 + 3.0369X   | 0.9837     | 25.5341      | 19.8748–32.8049         |
|                          | Boscalid | Y = 5.2263 + 0.7489X   | 0.9810     | 0.4986       | 0.3268–0.7608           |
|                          | Azoxytrobin | Y = 4.4507 + 0.8502X | 0.9921     | 4.3516       | 3.4330–5.5160           |
| **Colletotrichum lagenarium** | 4a         | Y = 4.1129 + 1.5735X   | 0.9967     | 3.6625       | 3.2373–4.1435           |
|                          | 5a       | Y = 3.6409 + 1.494X    | 0.9232     | 8.1215       | 4.8507–13.5978          |
|                          | 8c       | Y = 4.8309 + 1.5299X   | 0.9954     | 1.2899       | 1.0572–1.5739           |
|                          | 8d       | Y = 2.8392 + 2.2448X   | 0.9997     | 9.1740       | 8.8047–9.5588           |
|                          | Azoxytrobin | Y = 4.2298 + 0.4299X | 0.9968     | 61.8611      | 49.2272–77.7376         |
|                          | Boscalid | Y = 2.9242 + 1.351X    | 0.9673     | 34.3930      | 18.9576–62.3960         |
| **Gibberella zeae**      | 5a       | Y = 5.1780 + 1.0649X   | 0.9090     | 0.6805       | 0.2148–2.1553           |
|                          | Carbendazim | Y = 6.1001 + 8.7644X | 0.9653     | 0.7490       | 0.4996–1.1228           |
| **Rhizoctonia solani**   | 5a       | Y = 5.1533 + 0.7519X   | 0.9603     | 0.6215       | 0.1817–2.1250           |
|                          | Boscalid | Y = 5.1182 + 0.5510X   | 0.9942     | 0.6103       | 0.4943–0.7535           |
|                          | Carbendazim | Y = 5.2412 + 4.4774X | 0.9993     | 0.8833       | 0.8410–0.9279           |

Third, the synthesized derivatives of pimprinine and streptochlorin seemed more active in inhibiting the growth of *Alternaria leaf* spot, *Colletotrichum lagenarium*, *Gibberella zeae*, and *Rhizoctonia solani*, in particular for *Rhizoctonia solani*, the soilborne pathogen that caused rice sheath blight, resulting in annual severe losses in yield and quality in many rice production areas worldwide. Further, 13 of the 49 target molecules showed growth inhibition above 70%, and this was highlighted by compounds 3a and 5a, which displayed 98.3% and 96.1% growth inhibition, respectively—even more active than that of Osthole and Boscalid.

2.3. Molecular Modeling

The mode of action for the antifungal activity of pimprinine and streptochlorin derivatives is still not clear, though it has been reported that pimprinine is a potent inhibitor of monoamine oxidase [28,29]. Molecular docking in our previous study indicated that a pimprinine and streptochlorin derivative binds with leucyl-tRNA synthetase in a similar mode as AN2690, offering a perspective on the potential target for the antifungal activity of this series of indole natural products [26].

We performed molecular modeling studies using the X-ray structure of *Thermus thermophiles* LeuRS (PDB ID: 2V0C). The protein was downloaded in high resolution solved at 1.85 Å from [https://www.rcsb.org/](https://www.rcsb.org/) (accessed on 13 January 2021). The protein crystal structure of fLeuRS [30] and the selected ligand 4a were prepared by Discovery Studio 2.5, and the subsequent docking study was performed using MOE. After the molecular docking, the best binding mode of 4a (yellow in Figure 4) was selected according to the results of the docking energy, as compared with the AN2690-AMP in the fLeuRS (Figure 4).
was stirred at 110 °C for 2 h. When TLC monitoring showed that the reaction was complete, it was quenched with water and extracted with CH$_2$Cl$_2$ three times (3 × 50 mL). The organic layer was washed with water and brine and dried over anhydrous Na$_2$SO$_4$. After rotary evaporation, the residue was purified by column chromatography over silica gel (eluent: petroleum ether/acetone = 10:1) to give the pure compound 2.

Further, 3-acetylindoles (2 and 7) was synthesized using the reported methods [31] or purchased through Nanjing Crystal Chemicals Technology Co., Ltd (Nanjing, China). All the reaction yields were not optimized.

3.1.1. Preparation of 1-(1H-indol-3-yl)ethan-1-one (2)

Compound 1 (3.51 g, 30.00 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (20 mL) and cooled to 0–5 °C under a N$_2$ atmosphere. SnCl$_4$ (4.2 mL, 36.0 mmol) was added dropwise, then warmed to room temperature and allowed to react for 0.5 h, followed by the dropwise addition of 2.1 mL (30.0 mmol) CH$_3$COCl. The mixture was left to react for about another 2 h. When TLC monitoring showed that the reaction was complete, it was quenched with water and extracted with CH$_2$Cl$_2$ three times (3 × 50 mL). The organic layer was washed with water and brine and dried over anhydrous Na$_2$SO$_4$. After rotary evaporation, the residue was purified by column chromatography over silica gel (eluent: petroleum ether/acetone = 10:1) to give the pure compound 2.

3.1.2. Preparation of 2-substituted-5-(1H-indol-3-yl)-oxazole (3)

A mixture of compound 2 (0.64 g, 4.0 mmol), I$_2$ (0.66 g, 4.4 mmol) in DMSO (3.0 mL), was stirred at 110 °C for 1 h, until almost full conversion of the substrates was indicated by

Figure 4. Molecular modeling: (a) proposed interaction between compound 4a and tLeuRS; (b) superposition diagram of 4a and AN2690-AMP in the editing pocket of tLeuRS.

The simulated models and scores indicated that compound 4a putatively binds with tLeuRS in a similar mode as AN2690. It formed two weak hydrogen bonds with residues Thr248 and Thr252, a C–H⋯π interaction with residue Asp344, cation–π interactions with residue Arg346, and a halogen bond between Cl and Arg346 (Figure 4).

3. Materials and Methods

3.1. Chemistry

All general reagents and substrates commercially available were purchased from Alfa Aesar (Beijing, China) or through Nanjing JG-Chemicals (Nanjing, China) and were used without further purification. All solvents and liquid reagents were dried by standard methods in advance and distilled before use. Column chromatography was performed using silica gel (200–300 mesh). Melting points were determined using a Büchi M-560 melting point apparatus. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (Rheinstetten, Germany) in a DMSO-$d_6$, CD$_3$OD-$d_4$ or Acetone-$d_6$ solution. The chemical shifts (δ), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, q = quadruplet), coupling constants (Hz), and coupling constants (J) relative to tetramethylsilane are given in parts per million (ppm) and Hertz (Hz), respectively. HR-MS (ESI) spectra were obtained on an Agilent Technologies 6540 UHD Q-TOF LC-MS (Palo Alto, CA, USA).

Further, 3-acetylindoles (2 and 7) was synthesized using the reported methods [31] or purchased through Nanjing Crystal Chemicals Technology Co., Ltd (Nanjing, China). All the reaction yields were not optimized.
TLC analysis, then α-amino acid (8.0 mmol) and I₂ (0.54 g, 3.6 mmol) were added, and the mixture was stirred at 110 °C for 10-15 min. Then, 50 mL water and 30 mL saturated brine solution were added to the mixture and extracted with CH₂Cl₂ three times (3 × 50 mL). The extract was washed with 10% Na₂S₂O₃ solution (3 × 50 mL), dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (eluent: petroleum ether/acetone = 8:1) to afford the product 3. Information for the compounds is shown in Table 1.

3.1.2. Preparation of 2-substituted-5-(1H-indol-3-yl)-oxazole (3)

A mixture of compound 3 (0.50 mmol) in THF-CCl₄ (10 mL, 1:1 in v/v), NCS or NBS (0.55 mmol) was added, and the resulting mixture was heated at 45 °C for about 8 h, then allowed to cool down. The solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography (eluent: petroleum ether/acetone = 8:1), in order to give the desired intermediate compounds 4 or 5, respectively. Information for the compounds is shown in Table 2.

3.1.3. General Procedure for the Synthesis of 2-substituted-4-halogen-5-(1H-indol-3-yl)oxazole (4 or 5)

To a stirred solution of 3 (0.50 mmol) in THF-CCl₄ (10 mL, 1:1 in v/v), NCS or NBS (0.55 mmol) was added, and the resulting mixture was heated at 45 °C for about 8 h, then allowed to cool down. The solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography (eluent: petroleum ether/acetone = 8:1), in order to give the desired intermediate compounds 4 or 5, respectively. Information for the compounds is shown in Table 2.

3.1.4. Synthesis of Substituted 5-(1H-indol-3-yl)-2-methyloxazoles (8–10)

The synthetic procedures for compounds 8–10 were the same as those described in the general procedure for the synthesis of compounds 3–5. The synthetic route is shown in Scheme 2, and information for the compounds is shown in Table 3.

3.2. Biological Assays

Antifungal activity testing of the target compounds was carried out using mycelia growth-inhibitory rate methods (Figure 5). The samples were tested at a concentration of 50 µg/mL. Boscalid, Carbendazim, Osthole, and Azoxystrobin were used as positive controls. The tested fungi were provided by the Laboratory of Plant Disease Control, Nanjing Agricultural University, and the experimental procedure for the antifungal activity was performed according to the paper from Department of Plant Pathology, Nanjing Agricultural University [32]. The strains were activated in PDA at 25 °C for 2–15 days to obtain new mycelia, and the edge of the mycelia was punched before the antifungal activity assay. The results of the testing on target compounds against Alternaria leaf spot, Alternaria solani, Botrytis cinerea, Colletotrichum lagenarium, Gibberella zeae, and Rhizoctonia solani are listed in Tables 4 and 5.

Figure 5. Antifungal activity test with mycelia growth-inhibitory rate methods: (a) blank control; (b) testing sample; (c) positive control.

3.3. Molecular Modeling Strategy

Discovery Studio 2.5 was used for the preparation of the protein and ligand. We deleted the water molecules in the protein, supplemented incomplete amino acid residues, and hydrotreated the protein. For ligand molecules, we used the software to draw small molecules, optimize the three-dimensional structure, conduct hydrotreatment, and complete energy minimization. The corresponding parameter setting panel was opened, and generally, the default value was set. Then, we clicked ‘Run’ to obtain the processed ligand molecule. Subsequent semi-flexible docking was performed using MOE. The number of
placement poses was 50. After the molecular docking, the best binding mode was selected for analysis.

4. Conclusions

In conclusion, the natural products pimprinine and streptochlorin were used as the parent structures with the combination strategy of their structural features. Three series of derivatives were effectively synthesized from the starting material indole, using Vilsmeier–Haack acetylation, iodination/Kornblum oxidation, and oxazole annulation in a sequential order. The antifungal activity of 49 designed derivatives against six common phytopathogenic fungi was evaluated at a concentration of 50 µg/mL, and the results showed some of the target molecules possessed excellent antifungal activity, such as compounds 3a, 4a, 5a, 8c, 8d, and 8g, displaying more than 90% growth inhibition against at least one of the tested fungi. The compounds showed antifungal activity equivalent to or even more effective than the positive controls, and this was highlighted by compounds 3a, 4a, and 5a, which displayed over 90% growth inhibition against three kinds of fungi, showing a very broad antifungal spectrum. Especially for the compounds 4a and 5a, EC_{50} values against Botrytis cinerea were as low as 0.3613, and 1.1283 µg/mL, respectively, which represents better antifungal activity than that of the commonly used fungicides Azoxystrobin and Boscadil. The SAR study revealed the relationship between the 5-(3'-indolyl)oxazole scaffold and antifungal activity, which gives a useful insight into the development of new target molecules. Molecular docking models indicate that 4a binded with leucyl-tRNA synthetase in a similar mode as AN2690, offering a perspective on the study of the mode of action of the antifungal activity of pimprinine and streptochlorin derivatives. These results therefore suggest that compounds 4a and 5a could be regarded as novel and promising antifungal agents against phytopathogens due to their valuable potency.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/md20120740/s1, Compound data, Copies of the NMR spectra, and HR-MS (ESI) spectra.

Author Contributions: M.-Z.Z.: writing—original draft preparation, writing—review and editing, project administration, funding acquisition; J.-R.L.: writing—review and editing, methodology, data curation; J.-M.L.: investigation, data curation; Y.G.: methodology; Z.S.: methodology; K.-R.N.: methodology; D.G.: resources; F.D.: resources; H.-F.Z.: supervision; Y.-C.G.: supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (22177051, 32061143045), the Sichuan Science and Technology Program (2022YFN0068, 2021YFN0134), and the College Student Research Training Program (202110307002T).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the manuscript and in the Supplementary Materials.

Acknowledgments: We thank the Bayer Grants4Ag Initiative for their support. We thank Ge-Fei Hao and Chen-Yang Jia from Central China Normal University for their kind help with the molecular modeling. We also thank Vincent W.-F. Tai from Antiviral DPU GlaxoSmithKline (RTP, NC, US) for his kind suggestions and helpful discussion nine years ago.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

| Abbreviation | Full Name                                      |
|--------------|-----------------------------------------------|
| aaRS         | aminoacyl-tRNA synthetase                     |
| AMP          | adenosine monophosphate                       |
| DME          | 1,2-dimethoxyethane                           |
DMF  N,N-dimethylformamide
DMSO  dimethylsulfoxide
HRMS  high-resolution mass spectra
LeuRS  leucyl-tRNA synthetase
m.p.  melting point
NBS  N-bromosuccinimide
NCS  N-chlorosuccinimide
NMR  nuclear magnetic resonance
THF  tetrahydrofuran
TLC  thin layer chromatography
\( v/v \)  ratio by volume

References

1. De Rop, A.S.; Rombaut, J.; Willems, T.; De Graeve, M.; Vanhaecke, L.; Hulpiau, P.; De Maeseneire, S.L.; De Mol, M.L.; Soetaert, W.K. Novel Alkaloids from Marine Actinobacteria: Discovery and Characterization. *Mar. Drugs* **2022**, *20*, 6. [CrossRef] [PubMed]

2. Atanasov, A.G.; Zotchev, S.B.; Dirsch, V.M.; Supuran, C.T. Natural products in drug discovery: Advances and opportunities. *Nat. Rev. Drug Discov.* **2021**, *20*, 200–216. [CrossRef]

3. El-Hossary, E.M.; Cheng, C.; Hamed, M.M.; El-Sayed Hamed, A.N.; Ohsien, K.; Hentschel, U.; Abdelmohsen, U.R. Antifungal potential of marine natural products. *Eur. J. Med. Chem.* **2017**, *126*, 631–651. [CrossRef]

4. Dai, J.K.; Dan, W.J.; Wan, J.B. Natural and synthetic beta-carboline as a privileged antifungal scaffolds. *J. Nat. Prod.* **2022**, *85*, 229, 114057. [CrossRef] [PubMed]

5. Newman, D.J.; Cragg, G.M. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, *83*, 770–803. [CrossRef] [PubMed]

6. Joshi, B.S.; Taylor, W.I.; Bhate, D.S.; Karmarkar, S.S. The structure and synthesis of pimprinine. *Tetrahedron* **1963**, *19*, 1437–1439. [CrossRef]

7. Lever, J.; Kreuder, F.; Henry, J.; Hung, A.; Allard, P.M.; Brkljaca, R.; Rix, C.; Taki, A.C.; Gasser, R.B.; Kaslin, J.; et al. Targeted Isolation of Antibiotic Brominated Alkaloids from the Marine Sponge Pseudoceratina durissima Using Virtual Screening and Molecular Networking. *Mar. Drugs* **2022**, *20*, 554. [CrossRef]

8. Watanabe, H.; Amano, S.; Yoshida, J.; Takase, Y.; Miyadoh, S.; Sasaki, T.; Hatsu, M.; Takeuchi, Y.; Kodama, Y. A new antibiotic SF2583A, 4-chloro-5-(3′-indolyl) oxazole, produced by Streptomyces. *Meiji Seika Kenkyu Nenpo* **1988**, *27*, 55–62.

9. Nishida, A.; Fuwa, M.; Naruto, S.; Sugano, Y.; Saito, H.; Nakagawa, M. Solid-phase synthesis of 5-(3-indolyl)oxazoles that inhibit lipid peroxidation. *Tetrahedron Lett.* **2000**, *41*, 4791–4794. [CrossRef]

10. Takahashi, S.; Matsunaga, T.; Hasegawa, C.; Saito, H.; Fujita, D.; Kiuchi, F.; Tsuda, Y. Martefragin A, a novel antineoplastic agent, in cholangiocarcinoma. *J. Antibiot.* **2007**, *60*, 200–216. [CrossRef]

11. Kwak, T.W.; Shin, H.J.; Jeong, Y.I.; Han, M.E.; Oh, S.O.; Kim, H.J.; Kim, D.H.; Kang, D.H. Anticancer activity of streptochlorin, a novel antineoplastic agent, in cholangiocarcinoma. *Drug Des. Dev. Ther.* **2015**, *9*, 2201–2214. [CrossRef] [PubMed]

12. Liu, M.; Wan, Z.; Yang, S.; Liu, F.; Yang, X.; Wu, Z.; Zhang, Y.; Wang, K.; Fang, W. Two new dipimprinine alkaloids from soil-derived *Streptomyces* sp. 44414B. *Antibiot.* **2021**, *74*, 474–476. [CrossRef]

13. Wei, Y.; Fang, W.; Wan, Z.; Wang, K.; Yang, Q.; Cai, X.; Shi, L.; Yang, Z. Antiviral effects against EV71 of pimprinine and its derivatives isolated from *Streptomyces* sp. *Virol. J.* **2014**, *11*, 15. [CrossRef] [PubMed]

14. Liu, B.; Li, R.; Li, Y.; Li, S.; Yu, J.; Zhao, B.; Liao, A.; Wang, Y.; Wang, Z.; Lu, A.; et al. Discovery of Pimprinine Alkaloids as Novel Agents against a Plant Virus. *J. Agric. Food Chem.* **2019**, *67*, 1795–1806. [CrossRef]

15. Choi, I.K.; Shin, H.J.; Lee, H.S.; Kwon, H.J. Streptochlorin, a marine natural product, inhibits NF-κB activation and suppresses angiogenesis in vitro. *J. Microbiol. Biotechnol.* **2007**, *17*, 1336–1343.

16. Kroiss, J.; Kaltenpother, M.; Schneider, B.; Schwing, M.G.; Hertweck, C.; Maddula, R.K.; Strohm, E.; Svatos, A. Symbiotic Streptomyces provide antibiotic combination prophylaxis for wasp offspring. *Nat. Chem. Biol.* **2010**, *6*, 261–263. [CrossRef] [PubMed]

17. Park, C.; Shin, H.J.; Kim, G.Y.; Kwon, T.K.; Nam, T.J.; Kim, S.K.; Cheong, J.; Choi, I.W.; Choi, Y.H. Induction of apoptosis by streptochlorin isolated from *Streptomyces* sp. in human leukemic U937 cells. *Toxicon* **2008**, *22*, 1573–1581. [CrossRef] [PubMed]

18. Zhang, M.Z.; Mulholland, N.; Seville, A.; Hough, G.; Smith, N.; Dong, H.Q.; Zhang, W.H.; Gu, Y.C. First discovery of pimprinine derivatives and analogs as novel potential herbicidal, insecticidal and nematicidal agents. *Tetrahedron* **2021**, *79*, 131835. [CrossRef]

19. Kumari, A.; Singh, R.K. Medicinal chemistry of indole derivatives: Current to future therapeutic perspectives. *Bioorg. Chem.* **2019**, *89*, 103021. [CrossRef] [PubMed]

20. Zhang, M.Z.; Chen, Q.; Mulholland, N.; Beattie, D.; Irwin, D.; Gu, Y.C.; Yang, G.F.; Clough, J. Synthesis and fungicidal activity of novel pimprinine analogues. *Eur. J. Med. Chem.* **2012**, *53*, 283–291. [CrossRef] [PubMed]

21. Zhang, M.Z.; Mulholland, N.; Beattie, D.; Irwin, D.; Gu, Y.C.; Chen, Q.; Yang, G.F.; Clough, J. Synthesis and antifungal activity of 3-(1,3,4-oxadiazol-5-yl)-indoles and 3-(1,3,4-oxadiazol-5-yl)methyl-indoles. *Eur. J. Med. Chem.* **2013**, *63*, 22–32. [CrossRef]
22. Zhang, M.Z.; Chen, Q.; Xie, C.H.; Mulholland, N.; Turner, S.; Irwin, D.; Gu, Y.C.; Yang, G.F.; Clough, J. Synthesis and antifungal activity of novel streptochlorin analogues. *Eur. J. Med. Chem.* **2015**, *92*, 776–783. [CrossRef]

23. Zhang, M.Z.; Jia, C.Y.; Gu, Y.C.; Mulholland, N.; Turner, S.; Beattie, D.; Zhang, W.H.; Yang, G.F.; Clough, J. Synthesis and antifungal activity of novel indole-replaced streptochlorin analogues. *Eur. J. Med. Chem.* **2017**, *92*, 669–674. [CrossRef] [PubMed]

24. Jia, C.Y.; Xu, L.Y.; Yu, X.; Ding, Y.B.; Jin, B.; Zhang, M.Z.; Zhang, W.H.; Yang, G.F. An efficient synthesis and antifungal evaluation of natural product streptochlorin and its analogues. *Fitoterapia* **2018**, *126*, 106–110. [CrossRef] [PubMed]

25. Song, Z.L.; Zhu, Y.; Liu, J.R.; Guo, S.K.; Gu, Y.C.; Han, X.Y.; Dong, H.Q.; Sun, Q.; Zhang, W.H.; Zhang, M.Z. Diversity-oriented synthesis and antifungal activities of novel pimprinine derivative bearing a 1,3,4-oxadiazole-5-thioether moiety. *Mol. Divers.* **2021**, *25*, 205–221. [CrossRef]

26. Gao, Y.; Huang, D.C.; Liu, C.; Song, Z.L.; Liu, J.R.; Guo, S.K.; Tan, J.Y.; Qiu, R.L.; Jin, B.; Zhang, H.F.; et al. Streptochlorin analogues as potential antifungal agents: Design, synthesis, antifungal activity and molecular docking study. *Bioorg. Med. Chem.* **2021**, *35*, 116073. [CrossRef]

27. Xiang, J.C.; Wang, J.G.; Wang, M.; Meng, X.G.; Wu, A.X. One-pot total synthesis: The first total synthesis of chiral alkaloid pimprinol A and the facile construction of its natural congeners from amino acids. *Tetrahedron* **2014**, *70*, 7470–7475. [CrossRef]

28. Naik, S.R.; Harindran, J.; Varde, A.B. Pimprinine, an extracellular alkaloid produced by Streptomyces CDRIL-312: Fermentation, isolation and pharmacological activity. *J. Biotechnol.* **2001**, *88*, 1–10. [CrossRef] [PubMed]

29. Takeuchi, T.; Ogawa, K.; Inuma, H.; Suda, H.; Ukita, K. Monoamine oxidase inhibitors isolated from fermented broths. *J. Antibiot.* **1973**, *26*, 162–167. [CrossRef] [PubMed]

30. Rock, F.L.; Mao, W.M.; Yaremchuk, A.; Tukalo, M.; Crepin, T.; Zhou, H.C.; Zhang, Y.K.; Hernandez, V.; Akama, T.; Baker, S.J.; et al. An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site. *Science* **2007**, *316*, 1759–1761. [CrossRef] [PubMed]

31. Ottoni, O.; Neder, A.D.; Dias, A.K.; Cruz, R.P.; Aquino, L.B. Acylation of indole under Friedel-Crafts conditions—an improved method to obtain 3-acylindoles regioselectively. *Org. Lett.* **2001**, *3*, 1005–1007. [CrossRef] [PubMed]

32. Liu, Y.; Chen, Z.; Ng, T.B.; Zhang, J.; Zhou, M.; Song, F.; Lu, F.; Liu, Y. Bacisubin, an antifungal protein with ribonuclease and hemagglutinating activities from Bacillus subtilis strain B-916. *Peptides* **2007**, *28*, 553–559. [CrossRef] [PubMed]