Synthesis and Antimicrobial Activity of Calycanthaceous Derivatives

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Abstract
A series of calycanthaceous alkaloid analogs have been synthesized in excellent yields. All the target compounds were evaluated in vitro for biological activity against a broad range of plant pathogen fungi, bacteria and human pathogenic fungi, and some of the designed compounds exhibited potential activity in the primary assays. Notably, Compound b7 illustrated higher degrees of activity against Aspergillus flavus than amphotericin B, with a minimal inhibitory concentration value of 15.63 µg·mL⁻¹. Compound b7 displayed the most effective activity among the tested calycanthaceous analogs and might be a novel potential leading compound for further development of antifungal agent.

Keywords
calycanthaceous alkaloids, synthesis, plant pathogen fungi, biological activity, SAR

Introduction
The use of synthetic pesticides in agriculture worldwide is still the most widespread method for the control of plant diseases. However, the extensive application of agrochemicals over the years has led to the development of resistance in pest populations and environmental problems. Consequently, the discovery of new agrochemicals with high efficacy and selectivity against target species directly or indirectly from natural product has recently been crucial in the research and development of agrochemicals. The hexahydropyrroloindole skeletons are very important moieties that are widespread in a large family of natural products with wide range of attractive bioactivities. Calycanthaceous alkaloids1-4 (Figure 1), which contain hexahydropyrroloindole skeletons, are an important class of alkaloid that can be isolated from roots, leaves, flowers, and fruits of chimonanthus praecox.5 The Calycanthaceae plants have been used as traditional Chinese medicines for the treatment of fungal,6 hypertension, tumor, and inflammatory.7,8

Our group has recently reported the preparation and potent antimicrobial activity of calycanthaceous alkaloid derivatives.9-15 These findings inspired us to further modify the structure of calycanthaceous alkaloids with functional motifs so as to acquire potential agrochemical leads for plant disease control.

As part of ongoing efforts to discover new natural-product-based antifungal agent, 2 novel series of N-substituted calycanthaceous alkaloid derivatives were designed and synthesized, and their structures were identified on the basis of satisfactory analytical and spectral (¹H-NMR, ¹³C-NMR, and ESI-MS) data. To the best of our knowledge, the antimicrobial activities of the synthetic derivatives are reported herein for the first time.

Design and Synthesis of Calycanthaceous Alkaloids Analogs
The synthetic route to the target compounds is shown in Figure 2. A total of 36 calycanthaceous analogs were prepared from indole-3-acetonitrile via acylation at the N-position according to a previously reported procedure in our group and the spectral data were characterized by ¹H-NMR, ¹³C-NMR spectroscopy, and ESI-MS.16-25

Antimicrobial Activity
The results of the biological testing against a wide range of plant pathogen fungi, Gram-negative bacteria, Gram-positive bacteria, and human pathogenic fungi are listed in Table 1. The minimal

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inhibitory concentration (MIC) were evaluated with Carbendazim, Amphotericin B, Chlorothalonil, Gentamicin, Streptomycin, Penicillin, and Fluconazole as positive controls, to evaluate the activity of the synthesized calycanthaceous alkaloid analogs against Verticillium dahliae, Fusarium oxysporum sp. vasi infectum, Cytospora juglandis, Aspergillus flavus, Penicillium citrinum, Fusarium oxysporum, Colletotrichum orbiculare, Aspergillus niger, Beinerea pers., Curvularia lunata, Escherichia sp., Pseudomonas aeruginosa, Ralstonia solanacearum, Bacillus cereus, Staphylococcus aureus, Candida krusei, Cryptococcus neoformans, and C. tropicalis.

It is manifested that compounds a1, a6, a7, a8, generally exhibit more effective antimicrobial activity than the positive control. Compounds a6 and a7 illustrated improved activity against C. lunata compared with the positive control carbendazol, both with the same MIC value of 125 µg mL⁻¹. Compound a1 manifested better activity against V. dahliae than Chlorothalonil, with a MIC value of 31.25 µg mL⁻¹. The activity of Compound a17 is more potent than carbendazol, amphotericin B, and Chlorothalonil against F. oxysporum, all with the same MIC value of 31.25 µg mL⁻¹. Compound a1 manifested much more activity against A. flavus than amphotericin B, with a MIC value of 31.25 µg mL⁻¹. Compound a8 showed comparable control efficacy against F. oxysporum to carbendazol, with a MIC value of 125 µg mL⁻¹. Compounds a6 and a7 revealed comparable control efficacy against C. lunata to carbendazol, both with the same MIC value of 125 µg mL⁻¹. Compound b1 showed comparable control efficacy against A. flavus to amphotericin B, with a MIC value of 31.25 µg mL⁻¹. The activity of compound b7 is more potent than amphotericin B against A. flavus, with a MIC value of 15.63 µg mL⁻¹. Compounds b6 and b7 revealed improved activity against C. juglandis compared with the positive control carbendazol and Chlorothalonil, both with the same MIC value of 125 µg mL⁻¹. Compounds b6, b7, and b8 illustrated greater activity against C. juglandis than amphotericin B, all with the same MIC value of 250 µg mL⁻¹. Compounds b6, b7, and b8 manifested much more activity against C. orbiculare than amphotericin B and Chlorothalonil, all with the same MIC value of 250 µg mL⁻¹. Compounds b2, b3, and b6 showed more effective activity against C. tropicalis than carbendazol and Fluconazole, all with the same MIC value of 250 µg mL⁻¹.

**Materials and Methods**

**Instruments and Chemicals**

All reagents and solvents were reagent grade or purified according to standard methods before use. Analytical thin-layer chromatography was performed with silica gel plates using silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd.). Melting points were measured on an Electrothermal digital apparatus and

![Figure 1. Structures of calycanthaceous alkaloids.](image1)

![Figure 2. Synthetic route to the title compounds a1 to a18 and b1 to b18.](image2)
were uncorrected. The $^1$H-NMR (500 MHz), and $^{13}$C-NMR (125 MHz) were obtained on an AM-500 FT-NMR spectrometer (Bruker Corporation) with CDCl$_3$ as the solvent and tetramethylsilane as the internal standard. Mass spectrum (MS) were recorded under electrospray ionization (ESI) conditions using a LCQ Fleet instrument (Thermo Fisher). Yields were not optimized. The title compounds were synthesized under a nitrogen atmosphere.

### Synthesis

**Intermediates 1 to 3** were synthesized according to our previously reported procedure.

**Synthesis of Compounds a1 to a18 and b1 to b18.** Compound 3 was dissolved in pyridine (10 mL), then, the corresponding desired reagent was added at 0°C. After refluxing for 2 h, the mixture was warmed to room temperature. Then, the
resulting mixture were reacted for 1.5 h. At last, the resulting mixture was quenched with methanol (2 mL), and extracted with ethyl acetate. The organic extracts were combined, washed with brine, dried over Na₂SO₄, and concentrated. Purification by flash chromatography on silica gel afforded the compounds a₁ to a₁₈ and b₁ to b₁₈ in yields from 80% to 96% (for characterization data see Supplemental Materials).

**Biological Activity**

The antimicrobial activity of calycanthaceous alkaloids analogs was measured according to the previously reported method.

The tested compounds dissolved in 5% dimethyl sulfoxide, to a concentration of 1.02 mg/mL, 100 µL of the solutions were added to the first well and serially diluted from first well by taking 100 µL into second. This 2-fold dilution was continued down the plate and 100 µL from the eighth column of the plated discarded. The ninth column of the plate was reserved for negative control wells (without inocula) and the tenth column, for the positive growth control wells (without antibacterial agent). The antibacterial concentrations were 256, 128, 64, 32, 16, 8, 4, and 2 µg mL⁻¹ respectively. The antibacterial test plates were incubated aerobically at 37 °C for 24 h, the antifungal test plates were incubated aerobically at 28 °C for 48 h. The MICs were examined. All tests were performed in triplicate and repeated if the results differed.

**Conclusions**

A total of 36 novel tetrahydropyrroloindole-based calycanthaceous alkaloid analogs were prepared using indole-3-acetonitrile as the starting material via acylation at the N-position and the activity against a wide range of plant pathogen fungi were screened. Notably, compound b₇ illustrated potent activity against A. flavus than that of amphotericin B, with MIC value of 15.63 µg mL⁻¹. Compound b₇ displayed a remarkably activity among the tested calycanthaceous analogs and might be a novel potential leading compound for further development of antifungal agents. Further structural optimization of calycanthaceous alkaloid is well under way, alongside more detailed biological testing of the most active compounds, aiming to improve their levels of antifungal activity.

**Author Contributions**

SZ, HB and JZ contributed to designed research; CY KH and YG contributed to performed research; YC and RZ contributed to performed statistical analysis; SZ wrote the paper; and HB and JZ reviewed the manuscript.

**Declaration of Conflicting Interests**

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**Supplemental Material**

Supplemental material for this article is available online.

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