Antifungal Amide Alkaloids from the Aerial Parts of *Piper flaviflorum* and *Piper sarmentosum*

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**Key words**
*Piper flaviflorum*, *Piper sarmentosum*, Piperaceae, amide alkaloids, antifungal activity

**ABSTRACT**
Sixty-three amide alkaloids, including three new, *piperflaviflorine A* (1), *piperflaviflorine B* (2), and *sarmentamide D* (4), and two previously synthesized ones, (1E,3S)-1-cinnamoyl-3-hydroxypyrrolidine (3) and N-[7-(4'-methoxyphenyl)ethyl]-2-methoxybenzamide (5), were isolated from the aerial parts of *Piper flaviflorum* and *Piper sarmentosum*. Their structures were elucidated by detailed spectroscopic analysis and, in case of 3, by single-crystal X-ray diffraction. Most of the isolates were tested for their antifungal and antibacterial activities. Ten amides (6–15) showed antifungal activity against *Cryptococcus neoformans* ATCC 90113 with IC₅₀ values in the range between 4.7 and 20.0 µg/mL.

**Introduction**
During the past 30 years, invasive fungal infections in humans, such as candidiasis, cryptococcosis, and aspergillosis, have become a serious public health problem [1]. These infections are major causes of mortality and morbidity, especially in patients whose immune systems are compromised by AIDS, cancer, and organ transplantation [2]. However, the development of antifungal drugs faces a serious challenge caused by toxicity, resistance, poor solubility, serious drug-drug interactions, and limited chemical scaffolds [3]. Thus, new efforts have to be devoted to the discovery of new antifungal agents with different structural scaffolds and mechanisms of action.

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The genus *Piper* belongs to the family Piperaceae and contains more than 2000 species which are distributed all over the world [4]. Phenylpropanoids, flavonoids, amide alkaloids, lignans, neo-lignans, and terpenes are common components [5–7], with amides as one class of characteristic constituents. More than 300 amide alkaloids have been identified in plants of the *Piper* genus so far, and most of them exhibit potential bioactivities, such as antifungal, antiepileptic, antidepressive, hepatoprotective, and antiplatelet aggregation activities [8,9]. *Piper flaviflorum* C. DC., a species indigenous to Southern China, has been used as an ethnomedicine by the Dai people to treat dysmenorrhea and tinea [10]. So far several cytotoxic apiophuranosides and alkaloids have been characterized from its aerial parts [10–12]. *Piper sarmentosum* Roxb. is not only edible but also possesses a variety of medicinal uses, such as alleviating cough, cold, and toothache [13]. Previously several phenols, amide alkaloids, flavones, lignans, sterols, and phenylpropanoids have been isolated from the species [14–20]. In order to explore potential antifungal lead compounds from *Piper* spp. [5,21] we investigated the amide alkaloids from the aerial parts of *P. flaviflorum* and *P. sarmentosum*, and their antifungal activities. This led to the isolation of 63 amide alkaloids, including three new amides (1, 2, and 4) and two new natural amides (3 and 5). Their structures were elucidated by detailed spectroscopic analysis and single-crystal X-ray diffraction in case of 3. Most of the isolates were tested for their antifungal and antibacterial activities.

**Results and Discussion**

Repeated column chromatography was performed over Diaion HP20SS, Sephadex LH-20, MCI-gel CHP20P, silica gel, RP-18, p-TLC, and p-HPLC to afford 43 (1–2, 5–8, 13, 15–40, 45–47, 52–55, 61–63) and 20 (3–4, 9–12, 14, 41–44, 48–51, 56–60) amides from the aerial parts of *P. flaviflorum* and *P. sarmentosum*, respectively. Among them, piperflaviflorine A (1), piperflaviflorine B (2), and sarmentamide D (4) are new compounds, while (1E,3S)-1-cinnamoyl-3-hydroxyproline (3) and N-[7-(4’-methoxyphenyl)ethyl]-2-methoxybenzamide (5) were synthetically prepared previously but isolated as a natural product for the first time.

The known compounds were identified as pellitorine (6) [22], homopellitorine (7) [16], (2E)-decenoylpiperide (8) [23], 1-[(2E,4E,9E)-10-(3,4-methylenedioxyphenyl)-2,4,9-undecatrienyl]proline (9) [24], (2E,6E)-sarmentosine (10) [25], brachyamide B (11) [26], piperyline (12) [27], sarmentine (13) [23], demethoxylpipiplartine (14) [28], and pipieractam D (15) [29] (Fig. 1), respectively, by comparing their spectroscopic data with those reported previously in literature (for details on the identification of the known compounds 16–63, see Supporting Information).

Piperflaviflorine A (1), obtained as a white powder, has a molecular formula of C_{24}H_{35}NO_{3} as determined by the HREIMS (found \( m/z \) 385.2619 [M]+, calcld. for C_{24}H_{35}NO_{3}, 385.2617) and the \(^{13}\)C NMR (DEPT) spectra data, indicating eight degrees of unsaturation. The IR spectrum showed the presence of an amide functional group (3446 cm\(^{-1}\)) [30], and an aromatic and carbon-carbon double bond absorptions (1551, 1505, 1491, 1466, 1267 cm\(^{-1}\)). Characteristic signals in the \(^1\)H and \(^{13}\)C NMR spectra at \( \delta_H 5.93 \) (br s), 6.89 (d, \( J = 1.2 \) Hz), 6.73 (d, \( J = 8.0 \) Hz), and 6.75 (dd, \( J = 8.0, 1.2 \) Hz), and at \( \delta_C 100.9, 132.4, 105.3, 147.9, 146.5, 108.2, \) and 120.2, respectively, suggested a benzo[1,3]dioxol moiety [8]. Meanwhile, the presence of two trans C=C groups was indicated by signals in the \(^1\)H NMR spectrum at \( \delta_H 5.75 \) (d, \( J = 15.0 \) Hz), 6.83 (dd, \( J = 15.0, 7.5 \) Hz), 6.04 (dd, \( J = 15.6, 7.0 \) Hz), and 6.28 (dd, \( J = 15.6 \) Hz), and signals in the \(^{13}\)C NMR and DEPT spectra at \( \delta_C 123.5, 144.8, 129.5, \) and 129.2. The isobutylamine group was initially deduced from the characteristic signals at \( \delta_H 3.14 \) (t, \( J = 6.5 \) Hz), 1.79 (m), and 0.92 (d, \( J = 6.7 \) Hz) in the \(^1\)H NMR spectrum and those at \( \delta_C 166.1, 46.8, 28.6, \) and 20.1 in the \(^{13}\)C NMR spectrum (Table 1) [8]. Besides of those assigned carbon signals, there are eight methylenes (\( \delta_C 32.0, 28.2, 29.2, 29.4, 29.4, 29.4, 32.9 \)) in the molecule as deduced from the HREIMS data and \(^{13}\)C NMR spectrum. These NMR features were similar to those of the known amide alkaloid pipgulizarine from *Piper nigrum* L. [31]. The main difference between 1 and pipgulizarine is an additional methylene (\( \delta_C 28.2 \)) in 1.

The location of that additional methylene group was further determined by HMBC and COSY spectra. The HMBC correlations from OCH_{2}O (\( \delta_H 5.93 \)) to C-16 (\( \delta_C 146.5 \)), from H-19 (\( \delta_H 6.75 \) to C-17/C-15 (\( \delta_C 105.3 \)) to C-12 (\( \delta_C 129.2 \)), from H-13 (\( \delta_H 6.28 \) to C-15/C-11 (\( \delta_C 32.9 \)), and from H-12 (\( \delta_H 6.04 \) to C-14 (\( \delta_C 132.4 \), together with the correlations of H-13/H-12/H-11 (\( \delta_H 0.92 \)) in \(^1\)H\(^1\)H COSY spectrum, clearly established part A of the structural moiety (Fig. 2). The correlations from H-3’ (\( \delta_H 0.92 \)) to C-1 (\( \delta_C 46.8 \)), C-2’ (\( \delta_C 28.6 \)), from H-1’ (\( \delta_H 3.14 \)) to C-1 (\( \delta_C 166.1 \)), from H-3 (\( \delta_H 6.83 \) to C-1/C-2 (\( \delta_C 123.5 \)), and from H-2 (\( \delta_H 5.75 \) to C-1/C-4 (\( \delta_C 32.0 \)) in HMBC spectrum, together with the \(^1\)H\(^1\)H COSY correlations of NH (\( \delta_H 5.46 \))/H-1/H-2’ (\( \delta_H 1.79 \))/H-3’ and H-2/H-3/H-4 (\( \delta_H 2.16 \))/H-5 (\( \delta_H 1.43 \)), defined part B of...
the structural moiety. The remaining five overlapped CH₂ signals from δC 28 to 30 could be assigned to the linker between parts A and B as described in pipgulzarine [31]. Thus, the structure of 1 was established as shown in Fig. 1 and was named as piperflaviflorine A.

Piperflaviflorine B (2) was isolated as a white powder. The molecular formula of 2 was determined to be C₁₅H₂₃NO₃Na on the basis of its HRESIMS (found m/z 420.2508 [M + Na]⁺, calcd. for C₁₅H₂₃NO₃Na, 420.2508), indicating 9 degrees of unsaturation.

13C NMR and DEPT spectra (Table 1) revealed 25 carbon resonances, attributed to one carbonyl (δC 166.4), 12 alkenyl and aromatic carbons (δC 121.7, 143.1, 128.1, 129.1, 129.3, 132.4, 105.3, 147.9, 146.5, 108.2, 120.2), one dioxygenated methylene (δC 100.9), eight aliphatic methylenes (δC 32.8, 29.3, 28.7, 29.3, 29.0, 32.9, 27.0, 29.7), two methyls (δC 11.3, 17.2), and one aliphatic methine (δC 35.0). Careful comparison of the NMR data of 1 and 2 indicated that they were analogs. The differences between them were the amide substitution moiety and the aliphatic conjugated system. Instead of an isobutylamine group in compound 1, a 2-methybutylamine group is present in compound 2.

In the HMBC spectrum, correlations from H-3 (δH 3.28) to C-1 (δC 166.4)/C-5 (δC 17.2), from H-3 (δH 3.28)/H-1/b (δH 3.15) to C-1 (δC 166.4)/C-5 (δC 17.2), from H-3 (δH 3.19) to C-1/C-5 (δC 143.1), from H-2 (δH 5.74) to C-1/C-4 (δC 128.2), from H-4 (δH 6.12) to C-6 (δC 32.8), and from H-5 (δH 6.04) to C-7 (δC 29.3), together with the 1H-1H COSY correlations of H-1’/H-2’ (δH 1.60)/H-3’/H-4’, H-5’/H-2’, and H-2’/H-3’/H-4’/H-5 (δH 6.04)/H-6 (δH 2.15)/H-7 (δH 1.42) favorably supported the structural moiety part B. The structural moiety part A of 2 was identical with that of 1, which was further demonstrated by the NMR signals and HMBC correlations (Fig. 2). The absolute configuration of 2 was tentatively determined to be S by the optical rotation value ([α]D23 = −3.2) which was compared with two similar compounds, piperchabamide F ([α]D23 = 7.1) and piperchabamide E ([α]D23 = 18.7) [32, 33]. Therefore, the structure of compound 2 was established as shown in Fig. 1, and was named as piperflaviflorine B.

Compound 3 was obtained as colorless crystal. Its molecular formula was deduced as C₁₇H₁₇NO₃ from its HREIMS at m/z 217.1103 [M⁺] (calcd. for C₁₇H₁₇NO₃, 217.1103). The 1H NMR and DEPT data showed 13 carbons, including eight methines, two quaternary carbons (including one aromatic carbon and one carbonyl), and three methylenes (Table 2). The HMBC corre-
Table 2: \( ^1H \) (400 MHz) and \( ^13C \) (100 MHz) NMR spectroscopic data for compound 3 (in CDCl\(_3\)).

<table>
<thead>
<tr>
<th>Position</th>
<th>( \delta_{C} ), type</th>
<th>( \delta_{H} ) (mult., ( J ) in Hz)</th>
<th>Position</th>
<th>( \delta_{C} ), type</th>
<th>( \delta_{H} ) (mult., ( J ) in Hz)</th>
</tr>
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<tr>
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<td>165.0, C</td>
<td></td>
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<td>165.0, C</td>
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<tr>
<td>2</td>
<td>118.1, CH</td>
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<td>118.0, CH</td>
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<tr>
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<td>7.50 (t, 7.1)</td>
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<tr>
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<td>7.35 (m)</td>
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<td>128.5, CH</td>
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<td>129.5, CH</td>
<td>7.35 (overlap)</td>
<td>7</td>
<td>129.5, CH</td>
<td>7.35 (overlap)</td>
</tr>
<tr>
<td>1′</td>
<td>54.6, CH(_2)</td>
<td>3.66 (br d, 11.0)</td>
<td>1′</td>
<td>54.3, CH(_2)</td>
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<td>3.73 (overlap)</td>
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<td>3.66 (overlap)</td>
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<tr>
<td>2′</td>
<td>70.3, CH(_2)</td>
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<td>2′</td>
<td>68.6, CH(_2)</td>
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<td>3′</td>
<td>33.8, CH(_2)</td>
<td>2.08 (m)</td>
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<tr>
<td>4′</td>
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<td>3.72 (m)</td>
<td>4′</td>
<td>44.5, CH(_2)</td>
<td>3.82 (m)</td>
</tr>
</tbody>
</table>

Fig. 3: Key ROESY correlations of equilibriums of 3 and 4.

Fig. 4: X-ray crystallographic structure of 3.

Tinations from H-3 (\( \delta_{H} \) 2.00 or 2.08) to C-1 (\( \delta_{C} \) 54.3 or 54.6)/C-2 (\( \delta_{C} \) 68.6 or 70.3)/C-4 (\( \delta_{C} \) 44.0 or 44.5), from H-4 (\( \delta_{H} \) 3.72 or 3.82) to C-1 (\( \delta_{C} \) 165.0), and from H-1′ (\( \delta_{H} \) 3.66, 3.73 or 3.59, 3.66) to C-1 and C-2 (\( \delta_{C} \) 118.1 or 118.0), together with the \( ^1H \)-\( ^1H \) COSY correlation of H-2′ (\( \delta_{H} \) 4.51 or 4.57) with H-3′; revealed the structure fragment of a pyrrolidine ring. In addition, the HMBC correlations from H-3 (\( \delta_{H} \) 7.66 or 7.67) to C-1/C-4 (\( \delta_{C} \) 134.6)/C-5 (\( \delta_{C} \) 127.6), from H-5 (\( \delta_{H} \) 7.50) to C-7 (\( \delta_{C} \) 129.5), and from H-2 (\( \delta_{H} \) 6.65 or 6.71) to C-1/C-4, established a cinnamoyl moiety (Fig. 2). It was noted that compound 3 showed peak splitting from some protons and carbons in its NMR spectra. This phenomenon is caused by the C-N bond rotation in the solution, which frequently occurs in compounds with an amide group [34, 35]. In the ROESY spectrum, the correlations of H-2\(_{\text{trans}}\) with H-1′\(_{\text{trans}}\) and H-2\(_{\text{cis}}\) with H-4′\(_{\text{cis}}\), suggesting that both trans and cis forms exist in the solution (Fig. 3). The absolute configuration of C-2′ was determined as S by single-crystal X-ray diffraction analysis (Fig. 4). The refined Hoof parameter was 0.03(15) for 745 Bijvoet pairs with a probability of 1.000. Therefore, compound 3 was determined to be (1E,3S)-1-cinnamoyl-3-hydroxypropyridine. The Scifinder Scholar Database search indicates that (1E,3S)-1-cinnamoyl-3-hydroxypropyridine (3) prepared by chemical synthesis is available from commercial sources. However, no literature and spectroscopic data of this compound is available in literature.

The molecular formula of sarmentamidine D (4) was determined to be C\(_{15}\)H\(_{17}\)NO\(_{3}\) from its HREIMS at \( m/z \) 259.1211 [M]\(^+\) (calcd. for C\(_{15}\)H\(_{17}\)NO\(_{3}\), 259.1208). Careful analysis of NMR (1D and 2D) and MS data allowed the elucidation of 4 as an acetylated product of compound 3 (Table 3). The location of the acetyl group was determined by the HMBC correlation from H-1′\(_{\text{trans}}\) (\( \delta_{H} \) 54.3, 3.71) to COCH\(_3\) (\( \delta_{C} \) 170.7) and from H-1′\(_{\text{cis}}\) (\( \delta_{H} \) 54.3, 3.81) to COCH\(_3\) (\( \delta_{C} \) 170.4) (Fig. 2). The peak splitting was also observed in its NMR spectra. Further comparison of the optical rotation data of 4 with that of 3 [(\( \alpha \)]\(_D\) 23 + 28.5) indicated that the absolute configuration of 4 [(\( \alpha \)]\(_D\) 23 + 28.5) was 2'S. Thus, the structure of 4 was determined as shown in Fig. 1 and was named sarmentamidine D.

Compound 5, a colorless oil, possessed a molecular formula of C\(_{17}\)H\(_{19}\)NO\(_{3}\), as determined by the HREIMS at \( m/z \) 285.1373 [M]\(^+\) (calcld. for C\(_{17}\)H\(_{19}\)NO\(_{3}\), 285.1365), indicating nine degrees of unsaturation. The \( ^1H \) NMR spectrum revealed the presence of a typical para- [\( \delta_{H} \) 7.18, 6.87 (each, 2 H, d, \( J = 8.5 \) Hz, H-2′, 6′ and 3′, 5′)] and ortho- [\( \delta_{H} \) 6.91 (1 H, d, \( J = 8.3 \) Hz, H-3)], 7.41 (1 H, m, H-4), 7.05 (1 H, t, \( J = 7.7 \) Hz, H-5), and 8.21 (1 H, dd, \( J = 7.7, 1.8 \) Hz, H-6) disubstituted benzene ring. All 17 carbon resonances were well resolved in the \( ^13C \) NMR spectrum (Table 4) and further classified by DEPT as one carbonyl group (\( \delta_{C} \) 165.1), 12 aromatic carbons (\( \delta_{C} \) 111.2–158.2), two methoxyls (\( \delta_{C} \) 55.6 and 55.3), and...
two aliphatic methylenes (δC 55.6 and 55.3). The aforementioned data were similar to those of 2-hydroxybenzoic acid N-2-(4-hydroxyphenyl)ethylamide [36], and they shared the same skeleton, except for an additional methoxyl group presented in 5. The position of the additional methoxyl group in 5 was revealed to be located at C-2, based on the HMBC correlations (▶ Fig. 2) from the additional methoxyl (δH 3.77) to C-2 (δC 157.4), from H-6 (δH 8.21) to C-7 (δC 165.1), from H-8′ (δH 3.71) to C-7 (δC 165.1), from H-7′ (δH 3.86) to C-2′ (δC 129.8), and from another methoxyl (δH 3.79) to C-4′ (δC 158.2). HMBC and 1H-1H COSY correlations (see Supporting Information) favorably supported the planar structure of 5 as shown in ▶ Fig. 1. In the ROESY spectrum, correlations of δH 3.79 (MeO-4′) with δH 6.67 (H-3′) and of δH 3.77 (MeO-2) with δH 6.91 (H-3) further confirmed that the substitute positions of the two methoxyl groups were located at C-4′ and C-2. Thus, the structure of compound 5 was determined as shown in ▶ Fig. 1. Similar to 3, compound 5 was obtained as a new natural product and this was the first time to report its spectral data.

The present study led to the isolation of three new amides, piperflaviflorine A (1), piperflaviflorine B (2), and sarmentamide D (4), as well as (1E,3S)-1-cinnamoyl-3-hydroxypropyridoline (3) and N-[7′-(4′-methoxynaphthyl)ethyl]-2-methoxy-benzamide (5) that were for the first time described as natural products, along with 58 known amide alkaloids from the aerial parts of P. flaviflorum and P. sarmentosum. Forty-six isolates (3, 5–26, 28, 30–33, 35, 37, 39–40, 43–50, 52–55, 61–62) were evaluated for their antifungal (Candida albicans ATCC 90028, C. glabrata ATCC 90030, C. krusei ATCC 6258, Cryptococcus neoformans ATCC 90113, and Aspergillus fumigatus ATCC 204305) and antibacterial (Staphylococcus aureus ATCC 29213, meticillin-resistant S. aureus ATCC 33591 (MRS), Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853, and Mycobacterium intracellulare ATCC 23068) activities. Compounds 6–15 showed selective activities against C. neoformans and the results are shown in ▶ Table 5. Of these, compound 7 was the most active one with an IC50 of 4.7 µg/mL and produced a marginal minimum inhibitory concentration...
(MIC) of 20 µg/mL. The positive control amphotericin B (AMB) gave the IC50 and MIC values of 0.4 and 1.25 µg/mL, respectively.

This is the first time that the antifungal and antibacterial activities of these 46 amide alkaloids are reported. With regard to structural requirements for activity, the α,β-unsaturated amide moiety and the unsaturated aliphatic chain seemed to be the essential for the antifungal activity, while the 3,4-methylene dioxyphenyl and phenyl groups are not the key factors for the inhibition of fungal growth. The results of the antifungal analysis of compounds 15 and 61 suggested that the aristolactam scaffold is responsible for the antifungal activity. It is noted that the substituent and their substituted position may lead to their distinct antifungal bioactivity [37]. Open chain amides, such as 6, 7, and 8, were more active than other chemotypes. This preliminary structure-activity relationship information is a basis towards further studies of this antifungal class of compounds in the future.

Materials and Methods

General experimental procedures

IR spectra were detected on a Bruker Tensor 27 spectrometer with KBr pellets. UV data were obtained on a Shimadzu UV2401PC spectrophotometer. 1D and 2D NMR spectra were recorded on Bruker DRX-500 and AV-600 spectrometers operating at 500 and 600 MHz, respectively, for 1H NMR spectra, and at 125 and 150 MHz, respectively, for 13C NMR spectra. Coupling constants are expressed in Hz and chemical shifts are given on a ppm scale with reference to the solvent signals. X-ray diffraction was done on a Bruker APEX DUO instrument. ESIMS was performed on a Waters Xevo TQ-S. HREIMS was recorded on an API Qstar Pulsar time-of-flight spectrometer and on a Waters Auto Spec Premier P776 mass spectrometer. XRPDs were recorded on an API Qstar Pulsar LC/TOF spectrometer. Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd.), Sephadex LH-20 (25–100 µm, Pharmacia Fine Chemical Co. Ltd.), MCI-gel CHP20P (75–100 µm) (Mitsubishi Chemical Co. Ltd.), LiChroprep Rp-18 gel (40–63 µm, Merck) and Diaion HP20SS (Mitsubishi Chemical Co.). P-TLC was carried out on silica gel H-precoated plates (Qingdao Haiyang Chemical Co. Ltd.). Spots were detected by spraying with Dragendorff’s reagent. HPLC was performed on a Gilson liquid chromatography with a 7 µm Zorbax SB-C18 (21.2 × 250 mm) column.

Plant material

The aerial parts of P. flaviflorum were collected from Xishuangbanna, Yunnan Province, People’s Republic of China, in June 2012 and identified by Mr. Bin Wen at Xishuangbanna Tropic Botanical Garden, Chinese Academy of Sciences (CAS). Voucher specimens (HitBTC_004858) were deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany (KIB), CAS.

The aerial parts of P. sarmentosum were collected from Hainan province, People’s Republic of China, in May 2012 and identified by Prof. Jinping Liu at Key Laboratory of Protection and Development Utilization of Tropical Crop Germplasm Resources, Hainan province, People’s Republic of China. Voucher specimens (KUN_0435270) were deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, KIB, CAS.

Extraction and isolation

The air-dried and powdered aerial parts of P. flaviflorum (15.0 kg) were extracted with MeOH (3 × 40 L) at 60°C (8 h × 3). After removal of the solvent under reduced pressure, the crude extract (1.1 kg) was suspended in H2O (10 L) and partitioned with CHCl3 (3 × 20 L). The CHCl3 extract (315 g) was subjected to Diaion HP20SS, silica gel, Rp-18 CC, p-TLC, p-HPLC, and recrystallization in MeOH to afford compounds 1–2, 5–8, 13, 15–40, 45–57, 52–55, and 61–63. The aerial part of P. sarmentosum (11 kg) was extracted with MeOH (3 × 30 L) at 60°C (8 h × 3). The solvent was evaporated under vacuum to give a residue (975 g) which was dispersed in H2O (1 L) and then extracted with petroleum ether (3 × 3 L). The petroleum ether extract (423 g) was subjected to MCI-gel CHP20P, silica gel, Rp-18 CC, p-TLC, and p-HPLC to give compounds 3–4, 9–12, 14, 41–44, 48–51, and 56. The aqueous portion (550 g) was subjected to Diaion HP20SS, Sephadex LH-20, MCI-gel CHP20P, and silica gel CC to yield 57–60.

For details on the isolation and purification of these compounds, see Supporting Information. The purities of these compounds were > 95%, as determined by HPLC.

Characterization

Piperflaviflorine A (1): White powder; UV (CHCl3) λmax (log ε): 305 (2.99), 264 (3.32), 239 (3.20), 229 (3.15), 208 (3.07), 197 (3.03) nm; IR (KBr) νmax: 3446, 1666, 1629, 1551, 1506, 1492, 1467, 1257 cm−1; 1H and 13C NMR data, see Table 1; positive ESIMS: m/z 408 [M + Na]+; HREIMS: m/z 385.2619 [M]+ (calcd. for C24H35NO3, 385.2617).

Piperflaviflorine B (2): White powder; [α]D20 = −3.2 (c 0.13, MeOH); UV (MeOH) λmax (log ε): 260 (3.95), 208 (3.73), 192 (3.67) nm; IR (KBr) νmax: 3441, 1657, 1628, 1615, 1550, 1504, 1493, 1445, 1255 cm−1; 1H and 13C NMR data, see Table 1; positive ESIMS: m/z 420 [M + Na]+; HREIMS: m/z 420.2508 [M + Na]+ (calcd. for C24H35NO3Na, 420.2508).

(1S,3S)-1-Cinnamoyl-3-hydroxypyrrolidine (3): Colorless crystal; [α]D20 + 28.5 (c 0.11, MeOH); UV (MeOH) λmax (log ε): 281 (3.72), 217 (3.54), 204 (3.51) nm; IR (KBr) νmax: 3420, 3295, 1647, 1550, 1504, 1493, 1445, 1255 cm−1; 1H and 13C NMR data, see Table 1; positive ESIMS: m/z 420 [M + Na]+; HREIMS: m/z 420.2508 [M + Na]+ (calcd. for C24H35NO3Na, 420.2508).

Table 5 Antifungal activity against C. neoformans of compounds 6–15.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 (µg/mL)</th>
<th>Compounds</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>7.7</td>
<td>12</td>
<td>15.9</td>
</tr>
<tr>
<td>7</td>
<td>4.7</td>
<td>13</td>
<td>10.4</td>
</tr>
<tr>
<td>8</td>
<td>7.5</td>
<td>14</td>
<td>18.1</td>
</tr>
<tr>
<td>9</td>
<td>18.5</td>
<td>15</td>
<td>13.2</td>
</tr>
<tr>
<td>10</td>
<td>20.0</td>
<td>AMB*</td>
<td>0.4</td>
</tr>
<tr>
<td>11</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*AMB (Amphotericin B) was used as a positive control.
under room temperature from MeOH solution. Crystal data for \( S \)-\([\text{a}]\)^{+} 28.0 ± 2.0 (c 0.15, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε): 281 (3.71), 217 (3.47), 204 (3.51) nm; IR (KBr) \( \nu_{\text{max}} \): 3425, 1713, 1649, 1596, 1543, 1431, 1255 cm\(^{-1}\); \(^1\)H and \(^13\)C NMR data, see ▶ Table 2: positive ESIMS: \( m/z \) 240 \([\text{M} + \text{Na}]^+\); HREIMS: \( m/z \) 217.1103 \([\text{M}]^+\) (calcld. for \( \text{C}_{13}\text{H}_{12}\text{N}_{2}\text{O}_{2}, 217.1103\)).

**Sarmentamide D (4):** Colorless gum; \([\alpha]\)^{+} 20.8 (c 0.15, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log ε): 281 (3.71), 217 (3.47), 204 (3.51) nm; IR (KBr) \( \nu_{\text{max}} \): 3425, 1713, 1649, 1596, 1543, 1431, 1255 cm\(^{-1}\); \(^1\)H and \(^13\)C NMR data, see ▶ Table 2: positive ESIMS: \( m/z \) 282 \([\text{M} + \text{Na}]^+\); HREIMS: \( m/z \) 259.1211 \([\text{M}]^+\) (calcld. for \( \text{C}_{13}\text{H}_{12}\text{N}_{2}\text{O}_{2}, 259.1208\)).

\( N\)-\([\text{N}^\text{4}-\text{methoxyethyl}]-\text{2-methoxybenzamide} \) (5): Colorless oil; UV (MeOH) \( \lambda_{\text{max}} \) (log ε): 284 (2.9), 224 (3.6), 203 (3.92) nm; IR (KBr) \( \nu_{\text{max}} \): 3391, 1652, 1601, 1513, 1484, 1465 cm\(^{-1}\); \(^1\)H and \(^13\)C NMR data, see ▶ Table 4: positive ESIMS: \( m/z \) 308 \([\text{M} + \text{Na}]^+\); HREIMS: \( m/z \) 285.1373 \([\text{M}]^+\) (calcld. for \( \text{C}_{17}\text{H}_{19}\text{NO}_{3}, 285.1365\)).

**X-ray crystallography of 3**

\( (1\E,3S)-1\text{cinnamoyl-3-hydroxypyrrolidine} \) was crystallized under room temperature from MeOH solution. Crystal data for 3: \( \text{C}_{15}\text{H}_{17}\text{NO}_{3}, 259.1208\). The final \( R \) values were 0.0906 \([I > 2\sigma(I)]\). The final \( \sigma \) values were 0.2536 \([I > 2\sigma(I)]\). The final \( R \) values were 0.2546 (all data). The goodness of fit on \( F^2 \) was 1.113. Flack parameter = -0.1(6). The Hooft parameter is 0.03(15) for 745 Bijvoet pairs. The structure of 3 was solved by method (SHELXS97), expanded using difference Fourier techniques, and refined by the program and full-matrix least-squares calculations. The nonhydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at the calculated positions. Crystallographic data for the structure of 3 have been deposited at the Cambridge Crystallographic Data Centre (CCDC number 1408477). Copies of the data can be obtained free of charge from the CCDC via http://www.ccdc.cam.ac.uk/services/structures?access=referee&searchdepnums=1408477&searchauthor=Shi.

**Antifungal and antibacterial bioassays**

All the organisms were obtained from the American Type Culture Collection (Manassas, VA) and included Candida albicans ATCC 90028, Candida glabrata ATCC 90030, Candida kruusei ATCC 6258, Cryptococcus neoformans ATCC 90113, and Aspergillus fumigatus ATCC 204305, and the bacteria Staphylococcus aureus ATCC 29213, methicillin-resistant Staphylococcus aureus ATCC 33591 (MRS), Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853, and Mycobacterium intracellulare ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI (formerly NCCLS) methods [38, 39]. \( M. \) intracellulare was tested using a modified method of Franzblau et al. [40]. All samples were serially diluted in 20% DMSO/saline and transferred in duplicate to 96-well flat bottom microplates, with the highest test concentration at 20 \( \mu \)g/mL. Microbial inocula were prepared by correcting the OD630 of the microbe suspensions in incubation broth to afford final target inocula after addition to the samples. Amphotericin B (88.4% of purity, MP Biomedicals) was used as a pure positive control (100% of purity) by calculating its percentage. In other words, 1 mg of the sample was treated as 0.884 mg of pure amphotericin B. The detailed protocol has been described in a previous article [41].

**Supporting information**

Details on the identification of the known compounds 16–63, the isolation and purification of 1–63, 1D and 2D NMR and MS spectra for compounds 1–5, and X-ray crystal structure (CIF) for compound 3 are available as Supporting Information.

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**Conflict of Interest**

There are no conflicts of interest among the authors.

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