Ahmpatinin iBu, a new HIV-1 protease inhibitor, from Streptomyces sp. CPCC 202950†

Ming-Hua Chen,‡ac Shan-Shan Chang,‡ab Biao Dong, Li-Yan Yu, Ye-Xiang Wu, Ren-Zhong Wang, Wei Jiang, Zeng-Ping Gao* and Shu-Yi Si*

Ahmpatinin iBu (1) and statinin iBu (2), two new linear peptides, a novel pyrrolidine derivative, (−)-(S)-2-[3-(6-methylheptanamido)-2-oxypyrrrolidin-1-yl]acetic acid (3), and three known peptatin derivatives (4–6) along with their corresponding methanolysis artifacts (7–9) were isolated from Streptomyces sp. CPCC 202950. Their structures were elucidated on the basis of extensive spectroscopic data using Marfey's analysis, chiral-phase HPLC, and ECD and OR calculation to determine the absolute configurations. Compound 1 contains an unusual amino acid, 4-amino-3-hydroxy-5-(4-methoxyphenyl)pentanoic acid (Ahmpa), and 3 is the first natural product with a 2-(3-amino-2-oxopyrrrolidin-1-yl)acetic acid system. Compounds 1, 2, and 4–9 are HIV-1 protease inhibitors. In particular, ahmpatinin iBu (1) exhibits significant inhibitory activity against HIV-1 protease with an IC_{50} value of 1.79 nM. A preliminary structure–activity relationship is discussed.

In our previous report, a new HIV-1 protease inhibitor named 4862F\textsuperscript{13} and 11 known compounds\textsuperscript{16} were isolated from the liquid fermentation of the strain Streptomyces sp. CPCC 202950. Unexpectedly, the crude extract of the rice culture of Streptomyces sp. CPCC 202950 also significantly inhibited HIV-1 protease activity with an IC_{50} value of 20.0 ng ml\textsuperscript{−1}. Extensive investigation of the secondary metabolites of the rice culture extract of the strain resulted in the isolation of two new linear peptides, ahmpatinin iBu (1) and statinin iBu (2), a new pyrrolidine derivative, (−)-(S)-2-[3-(6-methylheptanamido)-2-oxopyrrrolidin-1-yl]acetic acid (3), and three known peptatin derivatives along with their corresponding methanolysis artifacts (4–9) (Fig. 1). Among them, compound 1 contains an unusual amino acid, 4-amino-3-hydroxy-5-(4-methoxyphenyl)pentanoic acid. Compound 3 represents the first natural

Introduction

The most effective treatment for HIV infection, HAART (Highly Active AntiRetroviral Therapy) that including several HIV-1 protease inhibitors combination with reverse transcriptase inhibitors, and other inhibitors with integrase, membrane fusion, and viral attachment, have significantly reduced the mortality of AIDS patients and improved the quality of life of those infected with HIV.\textsuperscript{1–4} However, according to World Health Organization and Joint United Nations Program on HIV/AIDS data, in 2016, there were still 36.7 million people living with HIV, 1.8 million newly infected and 1.0 million HIV-related deaths.\textsuperscript{5–6} Therefore, AIDS is still a substantial threat to global public health. HIV-1 protease (HIV-1 PR) is an essential enzyme in the life cycle of HIV and has been used as a promising target for AIDS therapy.\textsuperscript{7–9} Although 11 HIV-1 PR inhibitors such as amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, and tipranavir have been approved by the FDA and applied extensively in clinical evaluations,\textsuperscript{10,11} drug toxicity, resistance, and drug–drug interactions remain serious problems for HIV/AIDS treatments,\textsuperscript{12–14} prompting us to discover new anti-HIV drugs.

![Fig. 1 Chemical structures of compounds 1–9](image-url)
product with a 2-(3-amino-2-oxopyrrolidin-1-yl)acetic acid system, which was synthesized in a previous study.\textsuperscript{17} Herein, we report the isolation, structural elucidation, and biological activities of these compounds.

**Results and discussion**

Ahmpatinin \textit{iBu} (1) was isolated as a white powder and was determined to be C\textsubscript{37}H\textsubscript{61}N\textsubscript{5}O\textsubscript{10} on the basis of positive HRE-SIMS, indicating 10 degrees of unsaturation. The \textit{\textsuperscript{1}H} NMR spectrum of 1 (Table 1) in DMSO-\textit{d}_\textsubscript{6} exhibited characteristics of a typical peptide, with five NH protons (\textit{\delta}_H 7.4–7.9) and five amino acid \textit{\alpha}-protons (\textit{\delta}_H 3.8–4.3). Furthermore, \textit{\textsuperscript{13}C}-disubstituted aromatic ring protons [\textit{\delta}_H 6.80 (2H, d, H-3 and H-5) and 7.12 (2H, d, H-2 and H-6)], two oxygenated methine protons [\textit{\delta}_H 3.82 and 3.85], four methylene protons, four methine protons, and a methoxyl singlet [\textit{\delta}_H (3.70, s, 3H)] along with nine additional methyl doublets (\textit{\delta}_H 0.7–1.2) were also observed in the \textit{\textsuperscript{1}H} NMR spectrum. Six carbonyl signals (\textit{\delta}_C 170–176) and five \textit{\alpha}-amino acid carbon signals (\textit{\delta}_C 48–58) were observed in the \textit{\textsuperscript{13}C} NMR data (Table 1) were measured at 600 MHz for \textit{\textsuperscript{1}H} and at 150 MHz for \textit{\textsuperscript{13}C} in DMSO-\textit{d}_\textsubscript{6}. Proton coupling constants (\textit{J} in Hz) are given in parentheses. The assignments were based on \textit{\textsuperscript{1}H–\textsuperscript{1}H COSY}, HSQC, and HMBC experiments.\textsuperscript{b} The \textit{J}-value was not determined due to overlapping signals.

| Table 1  | NMR spectroscopic data of 1 and 2\textsuperscript{a} |
|----------|-----------------------------------------------|
| 1        | \textit{\delta}_H | \textit{\delta}_C, type |
| No.      | \textit{\delta}_H | \textit{\delta}_C, type |
| Val\textsuperscript{1} | CO | 171.1, C |
|          | \textit{\alpha} | 4.14, dd(7.8, 8.4) | 57.7, CH |
|          | \textit{\beta} | 1.96, m | 30.1, CH |
|          | \textit{\gamma} | 0.81, d(6.6) | 18.4, CH\textsubscript{3} |
|          | \textit{\gamma'} | 0.81, d(6.6) | 19.8, CH\textsubscript{3} |
|          | NH | 7.79, d(8.4) | 7.78, d(9) |
| Val\textsuperscript{2} | CO | 170.6, C |
|          | \textit{\alpha} | 4.14, dd(7.8, 8.4) | 57.8, CH |
|          | \textit{\beta} | 1.96, m | 30.4, CH |
|          | \textit{\gamma} | 0.81, d(6.6) | 18.2, CH\textsubscript{3} |
|          | \textit{\gamma'} | 0.83, d(6.6) | 19.4, CH\textsubscript{3} |
|          | NH | 7.71, d(9) | 7.68, d(9) |
| Sta      | CO | 170.7, C |
|          | \textit{\alpha} | 2.11, m | 39.2, CH\textsubscript{2} |
|          | \textit{\beta} | 3.82, m | 69.0, CH |
|          | \textit{\gamma} | 3.82, m | 50.7, CH |
|          | \textit{\delta} | 1.34, m | 38.7, CH\textsubscript{2} |
|          | \textit{\epsilon} | 1.25, m | 24.2, CH |
|          | \textit{\zeta} | 0.78, d(6.6) | 21.6, CH\textsubscript{3} |
|          | \textit{\zeta'} | 0.83, d(6.6) | 23.4, CH\textsubscript{3} |
|          | NH | 7.47, d(9) | 7.50, d(9) |
| Ala      | CO | 172.1, C |
|          | \textit{\alpha} | 4.22, m | 48.2, CH |
|          | \textit{\beta} | 1.12, d(7.2) | 18.0, CH \textsubscript{3} |
|          | NH | 7.85, d(7.2) | 8.07, d(7.2) |
| Ahmppa   | CO | 172.8, C |
|          | \textit{\alpha} | 2.23, dd(15.6, 3.6) | 38.8, CH\textsubscript{2} |
|          | \textit{\beta} | 3.85, m | 67.2, CH |
|          | \textit{\gamma} | 3.83, m | 54.4, CH |
|          | \textit{\delta} | 2.75, dd(13.8, 6) | 35.4, CH\textsubscript{2} |
|          | 1 | 130.9, C |
|          | 2 | 7.12, d(8.4) | 130.0, CH |
|          | 3 | 6.80, d(8.4) | 113.5, CH |
|          | 4 | 157.5, C |
|          | 5 | 6.80, d(8.4) | 113.5, CH |
|          | 6 | 7.12, d(8.4) | 130.0, CH |
|          | NH | 7.53, d(9) | 54.9, CH\textsubscript{3} |
| Acyl     | CO | 176.2, C |
|          | \textit{\alpha} | 2.51, m | 33.6, CH |
|          | \textit{\beta} | 0.95, d(6.6) | 19.2, CH\textsubscript{3} |
|          | \textit{\beta'} | 0.98, d(6.6) | 19.3, CH\textsubscript{3} |

\textsuperscript{a} NMR data (\textit{\delta}) were measured at 600 MHz for \textit{\textsuperscript{1}H} and at 150 MHz for \textit{\textsuperscript{13}C} in DMSO-\textit{d}_\textsubscript{6}. Proton coupling constants (\textit{J}) in Hz are given in parentheses. The assignments were based on \textit{\textsuperscript{1}H–\textsuperscript{1}H COSY}, HSQC, and HMBC experiments.\textsuperscript{b} The \textit{J}-value was not determined due to overlapping signals.
NMR spectrum, further indicating that 1 was a peptide. Analysis of the DEPT and $^1$C NMR spectra revealed additional 1,4-disubstituted benzene ring groups ($\delta_C$ 113–158), ten methyl groups, four methylenes, and six sp$^3$ methines.

A comparison of the NMR spectra of compounds 1 and ahapatinin ‘Bu$^{18}$ revealed that the mono-substituted benzene moiety in ahapatinin ‘Bu was replaced with a para-disubstituted aromatic ring group in 1. This was confirmed by 2D NMR (Fig. S6–S9†). HMBC correlations (Fig. 2) of H-4-OME and C-4, $\delta$-H-Ahmpa/C-1, C-2, C-6, C-$\beta$-Ahmpa and C-$\gamma$-Ahmpa, $\alpha$-H-Ahmpa/C-$\beta$-Ahmpa, C-$\gamma$-Ahmpa and C-CO-Ahmpa, $\beta$-$\alpha$-Ahmpa and C-CO-Ahmpa, and NH-Ahmpa and C-$\gamma$-Ahmpa indicated the presence of a 4-amino-3-hydroxy-5-(4-methoxyphenyl)pentanoic acid (Ahmpa) residue. Integrated 2D NMR ($^1$H NMR, COSY, HSQC, and HMBC) data were used to assign five partial structures consisting of the amino acids alanine, two valines, statine (Sta), and an isobutyl (‘Bu) group. HMBC correlations from $\alpha$-H-Ahmpa ($\delta_H$ 4.14) and NH-Ahmpa ($\delta_H$ 7.79) to ‘Bu-C=O ($\delta_C$ 176.2), $\alpha$-$\alpha$-Ahmpa ($\delta_H$ 4.14) to Val$^1$-C=O ($\delta_C$ 171.1), $\gamma$-H-Sta ($\delta_H$ 3.82) to Val$^2$-C=O ($\delta_C$ 170.6), $\alpha$-$\alpha$-Ahmpa ($\delta_H$ 4.22) to Sta-C=O ($\delta_C$ 170.7), and $\gamma$-H-Ahmpa ($\delta_H$ 3.83) to Ala-C=O ($\delta_C$ 172.1) established the sequence ‘Bu-$\alpha$-Val$^1$-$\alpha$-Val$^2$-$\gamma$-Sta-$\alpha$-Ahmpa, which was verified by NOESY correlations (Fig. 3 and S9†) from NH-Ahmpa ($\delta_H$ 7.79) to ‘Bu-$\alpha$-$\alpha$-Bu ($\delta_H$ 2.51), NH-Ahmpa ($\delta_H$ 7.71) to $\alpha$-$\alpha$-Val$^1$ ($\delta_H$ 4.14), NH-Sta ($\delta_H$ 7.47) to $\alpha$-$\alpha$-Val$^2$ ($\delta_H$ 4.14), NH-Ala ($\delta_H$ 7.85) to $\gamma$-$\beta$-Sta ($\delta_H$ 2.11), and NH-Ahmpa ($\delta_H$ 7.53) and $\alpha$-$\alpha$-Ala ($\delta_H$ 4.22).

The absolute configurations of Ala and Val in 1 were determined to be L using Marfey’s method (Fig. S27† and Experimental section),20,26 whereas the Ahmpa residue was established to be 3S, 4S by chiral-phase HPLC after acid hydrolysis in comparison to synthetic standards (Fig. S28† and Experimental section). In addition, the acid hydrolysis of 1 followed by HPLC purification gave statine, and its $^1$H NMR data agreed with the synthetic standard of (3S,4S)-statine. Meanwhile, the specific rotation ($[\alpha]^2_{D} = -19.5 (c 0.05, H_2O)$) of the statine from the hydrolysis of 1 was consistent with that of (3S,4S)-statine ($[\alpha]^2_{D} = -20.4 (c 0.502, H_2O)$).21 Therefore, the final structure of 1 was determined to be ‘Bu-$\alpha$-Val$^1$-$\alpha$-Val$^2$-(3S,4S)-Sta-$\alpha$-(3S,4S)-Ahmpa and was named ahapatinin ‘Bu.

Compound 2 was obtained as a white amorphous powder, and the molecular formula was assigned as C$_{25}$H$_{36}$N$_2$O$_4$ by HRESIMS based on a [M – H]$^-$ ion at 513.3275 (calc. 513.3283). The MS data for 2 indicated the absence of the Ahmpa moiety relative to compound 1. Extensive analysis of 2D NMR ($^1$H–$^1$H COSY, HSQC, HMBC, and NOESY) spectroscopic data (Fig. S15–S18†) revealed the sequence of ‘Bu-$\alpha$-Val$^1$-$\alpha$-Val$^2$-Sta-$\alpha$-Ala. The absolute configurations of the amino acid residues and the statine unit were also confirmed to be $\gamma$-$\beta$-Ala, $\gamma$-$\beta$-Val and (3S,4S)-statine using the same protocol as 1. Hence, compound 2 was deduced to be ‘Bu-$\gamma$-$\beta$-Val$^1$-$\gamma$-$\beta$-Val$^2$-(3S,4S)-Sta-$\gamma$-$\beta$-Ala and was named statinin ‘Bu.

Compound 3 was isolated as a white powder. Its molecular formula C$_{19}$H$_{22}$N$_2$O$_4$, requiring four degrees of unsaturation, was established from the HRESIMS data [m/z 285.1802 [M + H]$^+$], calculated for C$_{14}$H$_{18}$N$_2$O$_4$, 285.1808). The IR spectrum of 3 displayed the presence of hydroxy and/or amino (3287 cm$^{-1}$) and amide (1645 cm$^{-1}$) functionalities. Analysis of the $^1$H NMR, $^1$C NMR and HSQC spectra (Table 2) of 3 revealed two doublet methyls, seven sp$^3$ methylenes, two sp$^3$ methines, and three carbonyls ($\delta_C 170.0, 172.3, and 172.4$). The $^1$H–$^1$H COSY cross-peaks of H$_2$-2’/H$_2$-3’/H$_2$-4’/H$_2$-5’/H$_2$-6’/H$_2$-7’/H$_2$-8’ and HMBC correlations of H$_2$-7’/H$_2$-8’/C-6’-C and C-5’-C and C-6’-C’ and C-7’-C and C-8’-H$_2$-3’/C-3’-C and C-4’-C and C-5’-C and C-6’-H$_2$-4’/C-1’-C and C-2’-C and C-3’-C and C-4’-C and C-5’-C and C-6’-H$_2$-5’/C-1’-C’ and C-2’-C’ and C-3’-C’ and C-4’-C’ and C-5’-C’ indicated the presence of a 6-methylheptanoyl moiety in 3. Meanwhile, in conjunction with the degrees of unsaturation and the molecular composition, $^1$H–$^1$H

Table 2 NMR spectroscopic data of 3

| No. | $\delta_H$ | $\delta_C$, type |
|-----|-----------|----------------|
| 1   | 3.99, d(17.4) | 44.1, CH$_2$ |
| 2   | 3.89, d(17.4) | 44.1, CH$_2$ |
| 3   | 4.41, m      | 49.5, CH$_2$ |
| 4   | 2.29, m      | 26.2, CH$_2$ |
| 5   | 1.76, m      | 43.8, CH$_3$ |
| 6   | 3.33, m      | 172.3, C     |
| 7   | 2.10, t(7.2) | 35.3, CH$_2$ |
| 8   | 1.47, m      | 25.5, CH$_2$ |
| 4’  | 1.25, m      | 26.4, CH$_2$ |
| 5’  | 1.14, m      | 38.2, CH$_2$ |
| 6’  | 1.51, m      | 27.3, CH$_3$ |
| 7’  | 0.84, d(6.6) | 22.5, CH$_3$ |
| 8’  | 0.84, d(6.6) | 22.5, CH$_3$ |
| NH  | 8.13, d(8.4) |               |

a NMR data ($\delta$) were measured at 600 MHz for $^1$H and at 150 MHz for $^1$C in DMSO-$d_6$. Proton coupling constants ($\delta$) in Hz are given in parentheses. The assignments were based on $^1$H–$^1$H COSY, HSQC, and HMBC experiments.
COSY correlations of NH/H-3'/H-4'/H-5'/ along with HMBC correlations of NH/C-2', C-3' and C-4'; H-3'/C-2' and C-4'; H-2'/C-2', C-3' and C-5'; H-2'/C-2', C-3' and C-4'; and H-2'/C-1, C-2' and C-3' demonstrated the presence of a 2-(3-amino-2-oxopyrrolidin-1-yl)acetic acid moiety. Finally, the HMBC correlation from H-3' and NH to C-1” suggested that the two moieties were connected via an amide bond. Thus, compound 3 was confirmed to be 2-(3-(6-methylheptanamido)-2-oxopyrrolidin-1-yl)acetic acid. The absolute configuration at C-3' was determined by comparing the experimental circular dichroism (CD) spectrum and optical rotation (OR) data with the computed ECD and OR data. A comparison of the experimental CD spectrum with the calculated ECD spectrum of 3 (Fig. 4) demonstrated that compound 3 possessed an S configuration, which was verified by the computed OR, which was –41.5 for (S)-3. The experimental OR value was –100.5 in acetonitrile. Therefore, compound 3 was elucidated to be (−)-S)-2-(3-(6-methylheptanamido)-2-oxopyrrolidin-1-yl)acetic acid.

In addition to compounds 1–3, the known pepstatin Ac (4), pepstatin Pr (5), pepstatin methyl ester (7), pepstatin Pr methyl ester (8), and pepsinostreptin methyl ester (9) were also isolated and identified from the strain Streptomyces sp. CPCC 202950. Compounds 4–6 incubated with methanol showed the presence of corresponding methyl esters 7–9, respectively. These results indicated that 7–9 were indeed artifacts derived from methanolysis during the isolation process. Furthermore, a new benzamide analog and seven known compounds were isolated from the inactive fractions of the crude extract in our previous report.23

Pepstatin and ahmppain derivatives have been shown to exhibit significant inhibitory activity against aspartic protease.18-24-28 HIV-1 PR is a 22 kDa dimeric aspartyl protease. As expected, compounds 1 and 2 along with pepstatin derivatives 4–9 from the active fractions showed significant inhibitory activity against HIV-1 protease with IC50 values of 1.79 nM to 3.19 μM, while compound 3 was inactive (the positive control indinavir gave an IC50 value of 1.82 nM) in preliminary in vitro assays (Table 3). A comparison of the activities of 4–6 and 7–9 demonstrated that the exposed terminal carboxyl group was very important for the HIV-1 protease inhibitory activity. Analysis of the inhibitory abilities of 1 and 4–9 indicated that the Ahmppa unit was better than the statine moiety. Compound 2 showed weaker inhibitory ability than 1 and 4–9, suggesting that the presence of the statine or Ahmppa unit could enhance the HIV-1 protease inhibitory activity. In addition, all of the compounds were not active at toward Hela, HepG2, and U2OS tumor cells at 100 μM in in vitro tests.

### Experimental

#### General experimental methods

ORs were measured using a Rudolph Research Autopol III automatic polarimeter. UV, CD, and IR spectra were recorded using a Cary 300 spectrometer, a JASCO J-815 CD spectrometer, and a Nicolet 5700 FT-IR spectrometer (FT-IR microscope transmission), respectively. 1H and 13C NMR spectra were obtained at 600 and 150 MHz, respectively, using a Bruker AVIIIHD-600 spectrometer with solvent peaks used as references. HR-ESIMS data were measured using a Micromass Autospec-Ultima ETOF spectrometer and Thermo LTQ Orbitrap XL mass spectrometer. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China) and MCI gel CHP20P (Mitsubishi Chemical Corporation, Tokyo, Japan). HPLC separation was performed using an Agilent 1200 series (quaternary pump, autosampler, diode array detector) with a Shiseido Capcell-Pak C18 MGII column (5 μm, 250 × 10 mm). TLC was performed using glass-precoated silica gel GF254 plates (Qingdao Marine Chemical Inc., Qingdao, China). Cervical cancer (Hela), hepatocellular carcinoma (HepG2), and human osteosarcoma (U2OS) cell lines were obtained from the National Infrastructure of Cell Line Resource (Beijing, China).

#### Microorganism and fermentation

Strain CPCC 202950 was identified as a member of the genus Streptomyces on the basis of 16S rRNA sequence analysis and deposited at the China Pharmaceutical Culture Collection (Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, no. CPCC 202950).

The strain was cultured on slants of YM (0.4% yeast extract, 1% malt extract, 0.4% glucose, and 1.2% agar) at 28 °C for 7 d. Agar plugs were cut into small pieces (about 0.5 × 0.5 × 0.5 cm3) under aseptic conditions and 5 of these pieces were used to inoculate in three Erlenmeyer flasks (500 ml), each containing 100 ml of media (glucose 0.5%, yeast extract 0.5%, soluble starch 2.0%, soybean meal 1%, peptone 0.5%, beef extract 0.5%, corn steep liquor 0.4%, CaCO3 0.4%, and CoCl2·6H2O 0.002%), and the final pH was adjusted to 7.2. After

Fig. 4 Experimental ECD spectrum of 3 (blue) and the calculated ECD spectra of (R)-3 (red) and (S)-3 (black).
sterilization, three flasks of the inoculated media were incubated at 28 °C on a rotary shaker at 220 rpm for two days to prepare the seed culture. Spore inoculum was prepared by suspending the seed culture in sterile, distilled H2O to give a final spore/cell suspension of 1 x 10^8/ml. Fermentation was carried out in 30 Fernbach flasks (500 ml), each containing 80 g of rice. Distilled H2O (120 ml) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 10.0 ml of the spore inoculum and incubated at 28 °C for 30 day.

Extraction and isolation

The fermented material was ultrasonicated with 95% EtoH (2 x 8.0 l x 40 min), and the EtoH extracts were combined and evaporated under reduced pressure to yield an aqueous suspension (2.0 l). The suspension was partitioned with EtoAc (4 x 2.0 l). The EtOAc extract (15.0 g) was chromatographed over a silica column using a gradient elution of increasing MeOH (0–100%) in CH2Cl2 to give 10 fractions (F1–F10). The fraction of F6 was also subjected to silica gel CC using a gradient elution of increasing MeOH (0–100%) in CH2Cl2 to give six parts (F6-1–F6-6). Further purification of F6-1 and F6-3 with reversed-phase semi-preparative HPLC (Capcell Pak C18-MGII 5 μm, 10 mm x 250 mm, 1.5 ml min^-1, 37% MeCN in 0.1% trifluoroacetic acid) yielded 2 (13.5 mg) and 1 (8.6 mg), respectively. The aqueous phase was chromatographed over MCI gel (CHP20P, 1 l) with successive elution using H2O, 10% EtoH, 20% EtoH, 35% EtoH, 50% EtoH, and 70% EtoH to afford fractions M1–M5. M4 was further separated by reversed-phase semi-preparative HPLC (Capcell Pak C18-MGII 5 μm, 10 mm x 250 mm, 1.5 ml min^-1, 35% MeCN in 0.1% trifluoroacetic acid) to afford 3 (102.0 mg).

Physical–chemical properties of 1–3

Ahmpatin 'Bu (1). White amorphous powder; [α]D^20 = −75.0 (c 0.5, CH3CN : H2O 1 : 1); UV (MeOH) [max (log e)] 223 (4.44), 275 (3.56) nm; IR νmax 3285, 2961, 2925, 2873, 2851, 1635, 1547, 1515, 1468, 1390, 1249, 1183, 1148, 1099, 1038, 722 cm^-1; 1H NMR (DMSO-d6, 600 MHz) data and 13C NMR (DMSO-d6, 150 MHz) data, see Table 1. (+)-HR-ESIMS m/z 736.4474 [M + H]^+ (calcd for C25H45N4O7, 736.4479).

Statinin 'Bu (2). White amorphous powder; [α]D^20 = −85.5 (c 0.6, CH3CN : H2O 1 : 1); IR νmax 3285, 3080, 2964, 2874, 1727, 1636, 1545, 1466, 1389, 1284, 1229, 1150, 1098, 1053, 721 cm^-1; 1H NMR (DMSO-d6, 600 MHz) data and 13C NMR (DMSO-d6, 150 MHz) data, see Table 1. (+)-HR-ESIMS m/z 513.3275 [M − H]^− (calcd for C21H25NO5, 513.3283).

(+)-(−)-S-2-[3-(6-Methylheptanamido)-2-oxopyrrolidin-1-yl] acetic acid (3). White amorphous powder; [α]D^20 = −100.5 (c 1.0, MeCN); CD (MeCN) 216 (Δε = −15.88) nm; IR νmax 3287, 3066, 2947, 2871, 1700, 1644, 1551, 1465, 1439, 1397, 1300, 1263, 1188, 1148, 926, 721, 636 cm^-1; 1H NMR (DMSO-d6, 600 MHz) data and 13C NMR (DMSO-d6, 150 MHz) data, see Table 1. (+)-HR-ESIMS m/z 285.1802 [M + H]^+ (calcd for C14H22NO4, 285.1800).
(3R,4R), and (3S,4R)-Ahmppa were analyzed with a chiral-phase column (MCI GEL CRS10W, 4.6 × 50 mm, Mitsubishi Chemical Corporation) using 2 mM CuSO4/H2O solution as the mobile phase with flow rate at 0.8 ml min−1 and UV detection at 254 nm on a Shimadzu LC-20A HPLC instrument. Ahmppa residue (tR = 12.07 min) in ahmpatinin 1Bu (1) was found to correspond to the (3S,4S)-configuration by comparison of the retention times (tR) with those of standard Ahmppa units: (3S,4S)-Ahmppa (11.96), (3R,4S)-Ahmppa (10.63), (3R,4R)-Ahmppa (10.06), and (3S,4R)-Ahmppa (10.36).

HPLC analysis of the acid hydrolysates of 1 and 2 using Marfey’s method. Approximately 0.5 mg of 1 and 2 were hydrolyzed with 1 ml of 6 N HCl at 110 °C for 16 h. The hydrolysate was evaporated to dryness and redissolved in H2O (200 µl). To one portion (100 µl), 100 µl of a 1% solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) in acetone and 20 µl of 1 M NaHCO3 were added. The reaction mixture was heated at 40 °C for 1 h, cooled to room temperature, neutralized with 2 M HCl (10 µl), and diluted with MeCN (100 µl). Similarly, the standard d-Ala, l-Ala, d-Leu, and l-Leu were derivatized separately. The Marfey’s derivatives of the hydrolysate and standards were analyzed by HPLC using the following conditions. Column, Agilent ZORBAX SB-Aq C18 column (5 µm, 4.6 mm × 150 mm); flow rate, 1.0 ml min−1; solvent A, 0.1% trifluoroacetic acid in an aqueous solution; solvent B, MeCN; elution, 20–50% B in A over 60 min; UV detection at 340 nm; column temperature, 30 °C. The retention times for the FDA derivatives of the standards d-Ala, l-Ala, d-Leu, and l-Leu were 39.93, 36.26, 49.43, and 44.10 min, respectively. The FDA derivatives of the amino acids from the hydrolysate of 1 and 2 showed peaks at 36.30 (l-Ala) and 44.16 (l-Leu) min, respectively, and the amino acids were assigned to be L-Ala and L-Leu in 1 and 2, respectively.

ECD and OR calculations of compound 3. Conformational analysis of the S-enantiomer was carried out via Monte Carlo searching with the MMF94s molecular mechanics force field using the Spartan 10 software. Thirty-two geometries having relative energies within 6 kcal mol−1 were optimized using DFT at the B3LYP/6-31G (d) level in vacuum with the Gaussian 09 program. The 52 B3LYP/6-31G(d)-optimized conformers with relative energies ranging from 0 to 4.0 kcal mol−1 were then reoptimized at the wB97XD/DGDZVP level in acetonitrile. ECD and OR computations for all wB97XD/DGDZVP-optimized conformers were carried out at the CAM-B3LYP/DGDZVP and B3LYP/DGDZVP levels in acetonitrile, respectively. Boltzmann statistics were computed for ECD simulations with a standard deviation of σ 0.3 eV. The ECD spectra were then simulated using the GaussSum 2.25 program. The final ECD spectrum of (S)-3 was obtained according to the Boltzmann distribution theory and the relative Gibbs free energy (∆G).

Analysis of the inhibition of HIV-1 protease activity. This was measured using the previously described protocol. The positive control indinavir gave an IC50 value of 1.82 nM.

Cytotoxic activity assay. This was measured using the previously described protocol. The positive control doxorubicin showed in vitro cytotoxicity against cervical cancer (Hela), hepatocellular carcinoma (HepG2), and human osteosarcoma (U2OS) cell lines with IC50 values of 1.42, 1.56, and 2.78 µM, respectively.

Conclusions
In summary, we have isolated two new linear peptides, ahmpatinin 1Bu (1) and statinin 1Bu (2), and a novel pyrrolidine analog, (−)-(S)-2-[3-(6-methylheptanamido)-2-oxopyrrolidin-1-yl]acetic acid (3), from a Streptomyces sp. strain. The unusual amino acid, 4-amino-3-hydroxy-5-(4-methoxyphenyl)pentanoic acid (Ahmppa) of ahmpatinin 1Bu was identified for the first time from a natural source, and 3 represents the first reported instance of a natural pyrrolidine possessing a 2-(3-amino-2-oxopyrrolidin-1-yl)acetic acid system. In addition, ahmpatinin 1Bu (1) displayed significant inhibitory activity against HIV-1 protease.

Conflicts of interest
There are no conflicts to declare.

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