Pterulamides I−VI, Linear Peptides from a Malaysian *Pterula* sp.

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Six new linear peptides, pterulamides I−VI (**1**−**6**), were isolated from the fruiting bodies of a Malaysian *Pterula* species. The structures were elucidated by MS and 2D NMR experiments, and the absolute configurations of the constituent amino acids established using Marfey's method. The pterulamides are mainly assembled from nonpolar *N*-methylated amino acids and, most interestingly, have non-amino-acid *N*-terminal groups, among them the unusual cinnamoyl, (*E*)-3-methylsulfinylpropenoyl, and (*E*)-3-methylthiopropenoyl groups. Furthermore, pterulamides I−V are the first natural peptides with a methylamide *C*-terminus. Pterulamides I and IV are cytotoxic against the P388 cell line with IC_{50} values of 0.55 and 0.95 μg/mL (0.79 and 1.33 μM), respectively.

Fungi of the genus *Pterula* (family Clavariaceae) are clavarioid basidiomycetes with predominantly coral-shaped fruiting bodies. A great variety of *Pterula* species occur from temperate to tropical regions,[1] but relatively little is known about the chemistry of these fungi. The only strain investigated, *Pterula* sp. 82168, was shown to produce the previously unknown natural products hydroxystrobilurin A,[2] pterulones A and B,[3,4] pterulinic acid,[3] and noroudemansin A,[5] all of which were strongly bioactive.

In this paper we describe the isolation, structural elucidation, and biological activities of six novel linear peptides, pterulamides I−VI (**1**−**6**), from the fruiting bodies of a Malaysian *Pterula* sp. These compounds are remarkable not only for their high degree of *N*-methylation but because they also contain unusual, or unprecedented, *N*-terminal groups, viz., benzoyl, cinnamoyl, (*E*)-3-methylsulfinylpropenoyl, and (*E*)-3-methylthiopropenoyl. A further structural feature hitherto unknown from natural peptides is the methylamide *C*-terminus found in pterulamides I−V.

Results and Discussion

Methanol extracts of two Malaysian *Pterula* sp. collections (A and B) exhibited strong cytotoxicity against P388 murine leukemia cells. Careful LC-MS and HPLC analyses of these two extracts indicated elements of similarity and differences between them. Six major components were identified by HPLC, and these compounds, pterulamides I−IV (**1**−**6**), were isolated using preparative HPLC. Compound **1** was present in extracts from both collections (A and B), while **2**, **3**, and **4** were found only in the extract of collection B, and **5** and **6** only in collection A. The ^{1}H NMR spectra of all six compounds (**1**−**6**) suggested *N*-methylated valyl, leucyl, or isoleucyl amino acid residues.

HRESIMS data for pterulamide I (**1**) indicated a molecular formula of $C_{34}H_{62}N_6O_7S$. The ^{1}H NMR spectrum, which displayed doubled signals for several sets of resonances, showed signals for the α-protons of five amino acids. These proved to be a convenient starting point for the identification of the individual amino acid units by COSY, TOCSY, HSQC, and HMBC experiments (see Table 1). Thus, one alanine, two valine, and two isoleucine residues were identified. Between 2.6 and 3.2 ppm in the ^{1}H NMR spectrum were six methyl groups, four of which from evaluation of the HMBC correlations could be attributed to *N*-methylation of the alanine, the two isoleucine, and one of the valine units. A fifth methyl group, a doublet at 2.69 ppm with a coupling constant of 4.0 Hz, showed a long-range *H*,*C*-coupling to the carbonyl carbon of one of the isoleucine units and *H*,*H*-coupling to an amide proton. It was concluded that this methyl group belonged to a *C*-terminal methylamide group. This was supported by the ESIMS, which showed an $[M - NHMe]^+$ ion. There remained three signals in

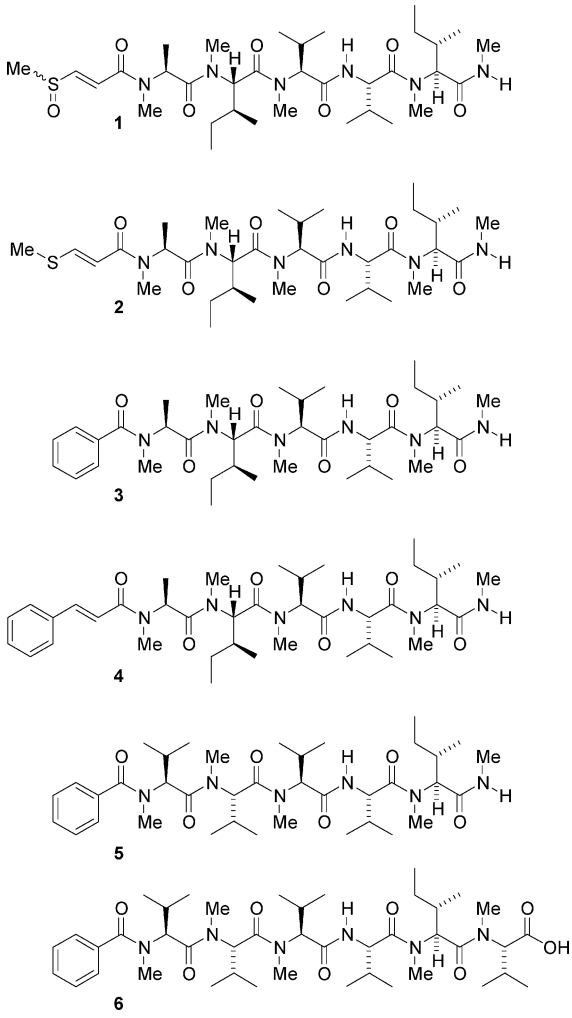

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Table 1. NMR Data of Pterulamide I (**1**) in MeOH-d_4

position	δ_C[a]	δ_H (*J* in Hz)	COSY	HMBC	TOCSY	ROESY
MSP[b]						
CO	165.7					
α	126.3	7.13 d (14.5)	*β*	CO, *β*, SO-Me	*β*	Ala-*N*Me, Ile1-α
β	149.4	7.64 d (14.5)	α	CO, α	α	SO-Me
SO-Me	39.6	2.76 s[c]		*β*	*β*	
Ala						
CO	174.1					
α	51.6	5.38 q (7.0)[c]	*β*	CO, *β*, *N*Me, *MSP*-CO	*β*	*β*, Ile1-*N*Me
β	14.5	1.34 d (7.0)[c]	α	CO, α	α	α, *N*Me
N-Me	31.7	3.15 s[c]		α, MSP-CO		*β*, MSP-α
Ile1[d]						
CO	173.0					
α	58.3	5.22 d (11.0)	*β*	CO, *β*, *β*-Me, *γ*, Ala-CO	*β*, *β*-Me, *γ*	MSP-α
β	34.2	2.16 m	α, *γ*, *β*-Me		α, *β*-Me, *γ*	Val1-*N*Me, *β*-Me, *γ*
β-Me	15.7	0.86	*β*		α, *β*, *γ*	α, *N*Me
γ	25.2	1.01 + 1.40 m	*β*	*δ*, *β*-Me	α, *β*, *β*-Me	α
δ	10.7	0.89				
N-Me	30.6	3.05 s		α, Ala-CO		*β*, Ala-α, Val1-*γ*′
Val1[d]						
CO	171.7					
α	63.4	4.63 d (11.0)	*β*	CO, *β*, *γ*, *γ*′, *N*Me, Ile1-CO	*β*, *γ*, *γ*′	*γ*, *γ*′
β	27.6	2.24 m	α, *γ*, *γ*′	α, *γ*, *γ*′	α, *γ*, *γ*′	*N*Me
γ	19.2	0.86	*β*		α, *β*, *γ*′	α, *N*Me
γ′	18.6	0.76 d (6.5)	*β*	α, *β*, *γ*	α, *β*, *γ*	α, *N*Me, Ile1-*N*Me
N-Me	31.4	3.10 s		α, Ile1-CO		*β*, *γ*, *γ*′, Ile1-α
Val2[d]						
CO	174.3					
α	55.8	4.57 m	*β*, NH	CO, *β*, *γ*/*γ*′, Val1-CO	*β*, *γ*/*γ*′	*γ*, *γ*′, Ile2-*N*Me
β	31.9	2.04 m	α, *γ*, *γ*′	α, *γ*/*γ*′	α, *γ*/*γ*′	
γ	18.7	0.90	*β*	α, *β*, *γ*′	α, *β*	α
γ′	~19	0.86	*β*		α, *β*	α
NH		8.15 d (8.0)	α			
Ile2[d]						
CO	172.7					
α	61.6	4.72 d (11.5)	*β*	CO, *β*, *β*-Me, *γ*, *N*Me, Val2-CO	*β*, *β*-Me, *γ*	*β*-Me, *γ*
β	33.0	2.07 m	α,*γ*		α, *β*-Me, *γ*	*N*Me
β-Me	15.4	0.86			α, *β*, *γ*	*N*Me
γ	25.2	0.95 + 1.35 m	*β*	*δ*, *β*-Me	α, *β*, *β*-Me	α
δ	10.5	0.84				
N-Me	31.3	3.18 s		α, Val2-CO		*β*, *β*-Me, Val2-α
CON*H*		7.93 m	CONH*Me*			
CONH*Me*	25.7	2.69 d 4.0	CON*H*	CO		

[a] ^{13}C shifts are taken from the HMBC spectrum. [b] MSP = (*E*)-3-methylsulfinylpropenoyl residue. [c] Signals doubled, due to mixture of diastereomers. [d] Similar amino acids are numbered consecutively from *N*- to *C*-terminal end.

the ^{1}H NMR spectrum not accounted for by the five amino acid units and the *C*-terminal methylamide end group. These signals were the sixth methyl group (2.76 ppm) and two doublets (7.13 and 7.64 ppm). The doublet signals belonged to an *E*-alkene ($^3J_{HH}$ 14.5 Hz), with both protons showing long-range *H,C*-coupling to a carbonyl carbon at 165.7 ppm. The sixth methyl group showed long-range *H,C*-coupling to just one of the alkene carbons (149.4 ppm). The formula $C_4H_5O_2S$ for the unassigned fragment of the assumed molecular formula, the long-range *H,C*-couplings, and the chemical shift values were consistent only with an (*E*)-3-methylsulfinylpropenoyl group at the *N*-terminus of the peptide. This also explained the doubled ^{1}H NMR signals observed for the MeSO− group and all protons of the *N*-methyl alanine unit, caused by a 1:1 mixture of diastereomers differing only in the configuration of the methylsulfinyl group.

The sequence of the five amino acid units in **1** was established mainly by evaluation of long-range *H,C*-couplings of the *N*-methyl groups (Figure 1), but was also supported by ROESY correlations and the fragmentation in ESIMS. The *C*-terminal position of an *N*-methylisoleucine unit (*Ile2* in Table 1) was apparent from the coupling of the methylamide group to the carbonyl carbon of this amino acid and the *N*-methyl group of *Ile2* coupled to the carbonyl carbon of the non-*N*-methylated valine (*Val2*). From the *N*-terminus the fragment 3-methylsulfinylpropenoyl−NMe−Ala−NMe−Ile−NMe−Val was likewise established by long-range *H,C*-couplings of each *N*-methyl group to the carbonyl carbon of the respective

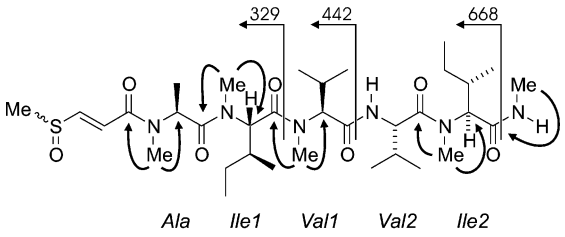

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Figure 1. Long-range *H,C*-couplings and ESIMS fragments indicative of the amino acid sequence of **1**.

next amino acid unit. The connection between the *N*-methylated and the non-methylated valine units (*Val1* and *Val2*) was not supported by NMR data but follows inevitably from the presented substructures, as it is the only possibility for joining the two fragments together. The proposed pentapeptidic structure was 3-methylsulfinylpropenoyl−NMe−Ala−NMe−Ile−NMe−Val−NH−Val−NMe−Ile−NHMe. The ESIMS was in agreement with this structure, showing the fragment ions $[M - NHMe]^+$, $[M - (NH-Val-NMe-Ile-NHMe)]^+$, and $[M - (NMe-Val-NH-Val-NMe-Ile-NHMe)]^+$.

The absolute configurations of the amino acid units were determined by acid hydrolysis followed by derivatization with Marfey's reagent (N^α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide)[6] and subsequent HPLC analysis, comparing the chromatograms with

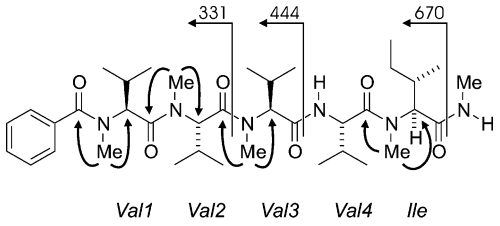

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Figure 2. Long-range *H,C*-couplings and ESIMS fragments indicative of the amino acid sequence of **5**.

those of derivatives of commercially available amino acids. All amino acids were shown to be the L-enantiomers, with isoleucine being the "normal" (2*S*,3*S*) isomer.

The molecular formula of pterulamide II (**2**), $C_{34}H_{62}N_6O_6S$, was established by HRESIMS and contained one less oxygen atom than **1**. The NMR data of **2** were largely identical to those of **1**, with the only differences being located in the vicinity of the *N*-terminal group. ^{1}H NMR spectral data for the *N*-terminus protons again showed two one-proton alkene doublets and one methyl group, but with different chemical shifts than in the spectrum of **1**. An obvious explanation for these differences, and in accordance with the molecular formula, was the presence of a (*E*)-3-(methylthio)-propenoyl instead of an (*E*)-3-(methylsulfinyl)propenoyl group. Unlike the 3-methylsulfinyl group, the 3-methylthio group contains no stereocenter, so that, in contrast to **1** no doubled signals for the protons close to the *N*-terminus were observed in the ^{1}H NMR spectrum of **2**. Two-dimensional NMR experiments, the fragmentation pattern in ESIMS, and Marfey's analysis confirmed the rest of the structure to be identical to that of **1**. Moreover, after oxidation of **2** with $NaIO_4$ the analysis of the reaction mixture by HPLC-MS showed that **1** was formed as the sole product. The 1:1 mixture of stereoisomers observed for the methylsulfinyl group in **1** poses the question as to whether this compound is of enzymatic origin or was formed by nonenzymatic oxidation of the thioether functionality to the sulfoxide, either in the fungus or later as an artifact.

Compounds **3** and **4** were closely related to the peptides **1** and **2**. Again, evaluation of the NMR data, mass spectral fragmentation patterns, and Marfey's analysis of the constituent amino acids showed that they differed from **1** only in their *N*-terminus groups. Besides the signals for the five amino acids and the *C*-terminal methylamide group, the ^{1}H NMR spectrum of **3** contained resonances for one phenyl residue, while the spectrum of **4** showed resonances for one phenyl residue and an *E*-alkene. HMBC and COSY experiments as well as the molecular formulas $C_{37}H_{62}N_6O_6$ and $C_{39}H_{64}N_6O_6$ for **3** and **4**, respectively (HRESIMS), indicated an *N*-terminal benzoyl group for pterulamide III (**3**) and an *E*-cinnamoyl group for pterulamide IV (**4**).

The NMR data of pterulamide V (**5**) suggested a peptidic structure also for this compound, but with a different amino acid sequence from that observed for pterulamides I−IV (**1**−**4**). The ^{1}H NMR spectrum showed signals for the α-protons of five amino acids, which again served as starting points for the identification of the amino acid units. In this case this task was severely complicated by the overlapping of three of the α-proton signals. The amino acid residues identified were an *N*-methylated isoleucyl, a valyl, and three *N*-methylated valyl units. The connectivity was established to be NMe−Val−NMe−Val−NMe−Val−NH−Val−NMe−Ile using long-range *H,C*-couplings from the *N*-methyl groups and fragmentations in the ESI mass spectrum (Figure 2). At the *N*- and *C*-termini a benzoyl and a methylamide residue, respectively, were identified, in agreement with the required molecular formula, $C_{38}H_{64}N_6O_6$ (HRESIMS).

Pterulamide VI (**6**), with the molecular formula $C_{43}H_{72}N_6O_8$ (HRESIMS), was structurally closely related to **5**, with the only difference being an *N*-methylvaline at the *C*-terminus rather than a methylamide group. Thus, **6** is the only one of the six compounds isolated having a free *C*-terminus. Marfey's analysis showed that the amino acid residues in **5** and **6** are again based on the proteinogenic enantiomers L-valine and L-isoleucine.

Table 2. ^{1}H and ^{13}C Data of the *N*-Terminal Groups of Pterulamides II to IV (**2**−**4**) in $CDCl_3$[a]

position	δ_C[b]	δ_H (*J* in Hz)
pterulamide II: MTP[c]		
CO	165.9	
α	111.1	6.05 d (14.6)
β	147.9	7.80 d (14.6)
S-Me	15.0	2.37 *s*
pterulamide III: benzoyl		
CO	171.7	
1	135.7	
2,6	126.8	7.36 m
3,5	128.6	7.41 m
pterulamide IV: cinnamoyl		
CO	166.8	
α	116.4	6.87 d (15.5)
β	143.7	7.72 d (15.5)
C-1	137.7	
C-2,6	127.7	7.53 m
C-3,5	128.7	7.37 m
C-4	129.7	7.37 m

[a] Chemical shifts differed significantly from those of **1** only in the *N*-terminal groups; see Supporting Information for full NMR data of all compounds. [b] ^{13}C shifts are taken from the HMBC spectra. [c] MTP = (*E*)-3-methylthiopropenoyl residue.

Co-occurring with the linear pterulamide peptides in the same *Pterula* sp. extracts was a series of cyclic peptides that have been named the pteratides.[7] In a cytotoxicity assay two of the pterulamides, **1** and **4**, exhibited strong activity against P388 murine leukaemia cells with IC_{50}'s of 0.55 μg/mL (0.79 μM) and 0.95 μg/mL (1.33 μM), respectively (pterulamide VI was not tested, due to contamination with small amounts of the strongly bioactive pteratides[7]). None of the pterulamides tested positive in antimicrobial assays against a range of Gram-positive and Gram-negative bacteria and fungi.

From several perspectives the pterulamides constitute a group of structurally unusual peptides. Besides the high degree of *N*-methylation, the pterulamides show an intriguing range of rare or unprecedented terminal groups. The 3-(methylsulfinyl)propenoyl group of **1** and the 3-(methylthio)propenoyl group of **2** have never been reported as *N*-terminal groups of natural peptides, nor are they part of any known fungal natural product. The 3-(methylthio)-propenoyl group is known only from the metabolites of certain plant species, e.g., *Petasites formosanus*,[8] and from one *Streptomyces* species,[9] while the 3-(methylsulfinyl)propenoyl moiety is known only from plant metabolites.[8,10] The benzoyl *N*-terminus (as observed in **3**, **5**, and **6**) is a structural feature that has, to our knowledge, never been described before as part of a linear fungal peptide, but is known from fungal cyclodepsipeptides.[11,12] Additionally, the cinnamoyl group is part of only one group of cyclic plant peptides,[13] but not of any natural linear peptides. A methylamide on the *C*-terminus (as in **1**−**5**) is, to our knowledge, unprecedented in a natural peptide.

Other fungal peptides formed from the nonpolar amino acids valine and isoleucine and also highly *N*-methylated are the dictyonamides from a marine alga-derived fungus[14] and MHR1 and MHR2, from a marine sponge-derived *Acremonium* sp.[15] However, these examples differ significantly from the pterulamides in their respective *N*- and *C*-terminal groups.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341. NMR spectra were recorded on a Varian (UNITY INOVA) AS-500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons

Table 3. NMR Data of Pterulamide V (**5**) in $CDCl_3$

position	δ_C	δ_H (*J* in Hz)	COSY	HMBC	ROESY	TOCSY
benzoyl						
CO	171.9					
1	136.4					
2/6	126.4	7.30 m	3/5	CO, 2/6, 4	Val1-*N*Me	3/5
3/5	128.6	7.40	2/6	1, 2/6, 3/5		2/6
4	129.6	7.40		1, 2/6, 3/5		
Val1[a]						
CO	170.40					
α	58.3	5.33 d (11.0)	*β*	CO, *β*, *γ*, *γ′*, *N*Me, *Bz*-CO	*γ*, *γ′*, *N*Me, Val2-*N*Me	*β*, *γ*, *γ′*
β	26.8	2.43 m	α, *γ*, *γ′*		*N*Me, *γ*, *γ′*	α, *γ*, *γ′*
γ	19.5	0.98 d (7.0)	*β*	α, *β*, *γ′*	α, *β*	α, *β*
γ′	18.1	0.93	*β*	α, *β*, *γ*	α, *β*, *N*Me	α, *β*
N-Me	32.9	2.87 s		α, *Bz*-CO	α, *β*, *γ′*, *Bz*-1	
Val2[a]						
CO	171.0					
α	58.4	5.23 d (11.0)	*β*	CO, *β*, *γ*, *γ′*, *N*Me, Val1-CO	*γ*, *γ′*, Val3-*N*Me	*β*, *γ*, *γ′*
β	27.2	2.43 m	α, *γ*, *γ′*		*N*Me, *γ*, *γ′*	α, *γ*, *γ′*
γ	19.6	0.90	*β*	α, *β*, *γ′*	α, *β*	α, *β*
γ′	17.8	0.83	*β*	α, *β*, *γ*	α, *β*	α, *β*
N-Me	30.5	3.11 s		α, Val1-CO	*β*, Val1-α	
Val3[a]						
CO	169.8					
α	62.4	4.63	*β*	*γ′*, *N*Me		*β*, *γ*, *γ′*
β	26.3	2.26 m	α, *γ*, *γ′*		*N*Me, *γ*, *γ′*	α, *γ*, *γ′*
γ	19.2	0.85	*β*	α, *β*, *γ′*	*β*	α, *β*
γ′	18.3	0.74 d (6.5)	*β*	α, *β*, *γ*	*β*	α, *β*
N-Me	30.8	3.07 s		α, Val2-CO	*β*, Val2-α	
Val4[a]						
CO	173.1					
α	53.8	4.69	*β*, NH	CO	*Ile-N*Me	NH, *β*, *γ*, *γ′*
β	31.2	2.00 m	α, *γ*, *γ′*			NH, α, *γ*, *γ′*
γ	18.9	0.83	*β*	α, *β*, *γ′*		NH, α, *β*
γ′	18.09	0.83	*β*	α, *β*, *γ*		NH, α, *β*
NH		6.98 bs	α			α, *β*, *γ*, *γ′*
Ile						
CO	170.41					
α	60.9	4.66	*β*	CO, *β*-Me, *N*Me		*β*, *γ*(1.30)
β	31.5	2.11 m	α, *β*-Me		*N*Me	α, *γ*, *β*-Me
β-Me	15.4	0.96	*β*	α, *β*, *γ*		α, *β*
γ	24.3	0.91 + 1.30 m				
δ	10.5	0.81 t (7.5)		*β*, *γ*		
N-Me	31.0	3.12 s		α, Val4-CO	*β*, *Val4*-α	
CON-H		6.45 bs	*CON*-Me			*CON*-Me
CON-Me	25.8	2.74 d (4.7)	*CON*-H			*CON*-H

[a] Similar amino acids are numbered consecutively from *N*- to *C*-terminal end.

Table 4. ^1H and ^{13}C Data of the *C*-Terminal Isoleucyl−Valyl Part of Pterulamide VI (**6**) in $CDCl_3$[a]

position	δ_C[b]	δ_H (*J* in Hz)
Ile		
CO	171.8	
α	56.5	5.28 d (11.0)
β	33.5	2.14 m
β-Me	15.3	0.87
γ	24.1	0.94 + 1.25 m
δ	10.7	0.82
N-Me	30.7	3.10 s
Val		
CO	171.4	
α	63.9	4.65
β	26.9	2.30
γ	18.9	0.79
γ′	19.5	1.05 d (6.0)
N-Me	33.1	3.10 s

[a] Chemical shifts did not differ significantly from those of **5** for the remaining part of the structure; see Supporting Information for full NMR data of all compounds. [b] ^{13}C shifts are taken from the HMBC spectra.

(*δ* 3.30 and 7.25 for MeOH-d_4 and $CDCl_3$, respectively) and the solvent carbons (*δ* 49.30 and 77.01 for MeOH-d_4 and $CDCl_3$, respectively) as internal references. HRESIMS were acquired using a Micromass LCT TOF mass spectrometer. Solvents used for extraction and isolation were distilled prior to use. Cytotoxicities against P388 murine leukemia cells were measured using a standard protocol.[16]

Fungal Material. Fruiting bodies of an unidentified *Pterula* species were collected in the Sungkai Wildlife Forest, Perak, Malaysia (collection A), and in the Urban Nature Centre, Kuala Lumpur, Malaysia (collection B). Voucher specimens have been deposited in the collection at the School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia (collection A; UKM-F4794), and Institute of Biological Sciences, University of Malaya (collection B; UM-F4872). Identification of the fungal material was made by one of the authors (A.L.J.C.) based on the characteristic ageotropic, multifid, brown fruit bodies growing on dead sticks. Fungal thalli from the two separate collections were cut into pieces and extracted with MeOH.

Isolation. The crude extract of collection A (1.7 g) was partitioned between MeOH−H_2O (19:1) and petroleum ether. The MeOH phase was dried and partitioned between EtOAc and H_2O. Drying of the EtOAc phase gave a desalted and defatted extract (91 mg), which was fractionated by preparative HPLC (RP18, 19 × 300 mm; solvents: H_2O + 0.05% TFA (A), MeCN (B); gradient: 0 min, 75% B; 15 min, 100% B; 25 min, 100% B; 11 mL min^{-1}; UV detection at 210 nm). Fractions A, B, and C were collected at 5−8, 12−13, and 13−14 min, respectively. In all following purification steps the same solvents and detection wavelengths were used. Fraction A was submitted to preparative HPLC (Phenomenex Luna C18, 10 × 250 mm, 5 *μ*m); gradient elution from 10% to 80% MeCN over 25 min; 5 mL min^{-1}. Pterulamide I (**1**) was eluted after 15.5 min (1.7 mg). Fraction B was likewise subjected to preparative HPLC (Phenomenex Luna C18, 10 × 250 mm, 5 *μ*m; two columns in series) with isocratic elution (75%

MeCN; 4 mL min^{-1}). Pterulamide V (**5**) was eluted after 17.0 min (2.5 mg). Fraction C was purified by preparative HPLC under the same conditions as fraction B. Pterulamide VI (**6**) was eluted after 19.5 min (1.0 mg).

The crude extract of collection B (336 mg) was defatted and desalted as described above. The resultant extract (97 mg) was fractionated on an open RP18 column (LiChroprep RP18, 40−63 μm, Merck) eluting with H_2O−MeOH (1:1), MeOH, and MeOH−CH_2Cl_2 (3:1). The combined MeOH and MeOH−CH_2Cl_2 fractions were further purified by preparative HPLC (Phenomenex Luna C18, 10 × 250 mm, 5 μm; gradient elution from 40% to 80% MeCN over 20 min; 5 mL min^{-1}). This yielded the following new peptides: pterulamide II (**2**; 21.0 min; 3.4 mg), pterulamide III (**3**; 21.4 min; 1.3 mg), and pterulamide IV (**4**; 23.5 min; 0.5 mg).

Pterulamide I (1): white solid; $[\alpha]^{20}_D$ −92 (*c* 0.1, MeOH); for ^1H, ^{13}C, COSY, HMBC, ROESY, and TOCSY NMR data, see Table 1; ESIMS *m*/*z* 721.4 (100) $[M + Na]^+$, 699.5 (23) $[M + H]^+$, 668.5 (3) $[M - NHMe]^+$, 442.3 (27) [668 − (NHVal−NMeIle)]$^+$, 369.2 (10), 353.3 (5), 329.2 (5) [442 − NMeVal]$^+$, 301.2 (9); HRESIMS *m*/*z* 699.4484 $[M + H]^+$ (calcd for $C_{34}H_{63}N_6O_7S$, 699.4479).

Pterulamide II (2): white solid; $[\alpha]^{20}_D$ −169 (*c* 0.22, MeOH); for NMR data, see Table 2 and Supporting Information (Table S1); ESIMS *m*/*z* 705.4 (100) $[M + Na]^+$, 683.4 (88) $[M + H]^+$, 652.4 (5) $[M - NHMe]^+$, 525.3 (5) [652 − NMeIle]$^+$, 498.4 (100) [M − (MeSCH═CHCO−NMeAla)]$^+$, 426.3 (30) [525 − NHVal]$^+$, 313.2 (20) [426 − NMeVal]$^+$, 186.0 (20) [313 − NMeIle]$^+$; HRESIMS *m*/*z* 683.4526 $[M + H]^+$ (calcd for $C_{34}H_{63}N_6O_6S$, 683.4530).

Pterulamide III (3): white solid; $[\alpha]^{20}_D$ −159 (*c* 0.16, MeOH); for NMR data, see Table 2 and Supporting Information (Table S2); ESIMS *m*/*z* 709.5 (100) $[M + Na]^+$, 687.5 (70) $[M + H]^+$, 656.5 (15) $[M - NHMe]^+$, 529.4 (10) [656 − NMeIle]$^+$, 496.2 (10), 430.3 (30) [529 − NHVal]$^+$, 317.2 (10) [430 − NMeVal]$^+$, 190.1 (10) [317 − NMeIle]$^+$; HRESIMS *m*/*z* 687.4819 $[M + H]^+$ (calcd for $C_{37}H_{63}N_6O_6$, 687.4809).

Pterulamide IV (4): white solid; $[\alpha]^{20}_D$ −270 (*c* 0.02, MeOH); for NMR data, see Table 2 and Supporting Information (Table S3); ESIMS *m*/*z* 735.5 (95) $[M + Na]^+$, 713.5 (100) $[M + H]^+$, 682.5 (5) $[M - NHMe]^+$, 456.3 (30) [M − (NHMe−NMeIle−NHVal)]$^+$, 368.3 (20), 343.2 (20) [456 − NMeVal]$^+$, 216.40 (40) [343 − NMeIle]$^+$; HRESIMS *m*/*z* 713.4944 $[M + H]^+$ (calcd for $C_{39}H_{65}N_6O_6$, 713.4965).

Pterulamide V (5): white solid; $[\alpha]^{20}_D$ −85 (*c* 0.1, MeOH); for ^1H, ^{13}C, COSY, HMBC, ROESY, and TOCSY NMR data, see Table 3; ESIMS *m*/*z* 724.4 (40) $[M + Na]^+$, 701.4 (100) $[M + H]^+$, 670.4 (6) $[M - NHMe]^+$, 444.3 (25) [670 − (NHVal−NMeIle)]$^+$, 370.2 (25), 331.2 (10) [444 − NMeVal]$^+$; HRESIMS *m*/*z* 701.4993 $[M + H]^+$ (calcd for $C_{38}H_{65}N_6O_6$, 701.4965).

Pterulamide VI (6): white solid; $[\alpha]^{20}_D$ −232 (*c* 0.1, MeOH); for NMR data see Table 4 and Supporting Information (Table S4); ESIMS *m*/*z* 823.5 (95) $[M + Na]^+$, 801.5 (100) $[M + H]^+$, 670.6 (10) $[M - NMeValOH]^+$, 625.5 (15), 620.6 (20), 584.6 (10) [M − (Bz−NMeVal)]$^+$, 576.6 (10), 444.4 (15) [670 − (NHVal−NMeIle)]$^+$, 396.4 (10), 331.3 (10) [444 − NMeVal]$^+$, 301.2 (10), 218.2 (20) [331 − NMeVal]$^+$, 157.1 (32); HRESIMS *m*/*z* 801.5483 $[M + H]^+$ (calcd for $C_{43}H_{73}N_6O_8$, 801.5490).

Preparation and Analysis of Marfey Derivatives. Pterulamides I−VI (**1**−**6**) were each hydrolyzed by heating the respective peptide (0.3 mg) in HCl (1 mL; 6 M) at 110 °C for 24 h. After cooling, the solution was evaporated to dryness and redissolved in H_2O (50 μL). To each of these acid hydrolyzate solutions, or to a solution of the reference amino acid (50 μL; 50 mM), was added a solution of FDAA (Marfey's reagent, N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide)[6] in acetone (100 μL of 1% (w/v) solution). After addition of $NaHCO_3$ solution (20 μL; 1 M), the mixture was incubated for 1 h at 40 °C. The reaction was stopped by addition of HCl (10 μL; 2 M), the solvents were evaporated to dryness, and the residue was redissolved in MeOH−H_2O (1 mL; 1:1). An aliquot of this solution (10 μL) was analyzed by HPLC (Phenomenex Luna C18, 250 4.6 mm, 5 μm; solvents: A is H_2O + 0.05% TFA, B is MeCN; linear gradient: 0 min 35% B, 30 min 45% B; 25 C; 1 mL min^{-1}). Retention times (min) of the amino acid derivatives were as follows: L-Val (11.4), D-Val (16.2), NMe-L-Val (15.2), NMe-D-Val (18.5), NMe-L-Ile (20.4), NMe-D-Ile (25.0), NMe-L-*allo*-Ile (20.9), and NMe-D-*allo*-Ile (22.5). For the separation of the NMe-Ala derivatives different HPLC conditions were used (Phenomenex Prodigy ODS, 250 × 4.6 mm, 5 μm; solvents: A is H_2O + 0.05% TFA, B is MeCN; linear gradient: 0 min 45% B, 30 min 65% B; 24 °C; 1 mL min^{-1}), giving retention times of 13.6 and 13.1 min for the NMe-L-Ala and the NMe-D-Ala derivatives, respectively.

Oxidation of Pterulamide II (2). To a solution of pterulamide II (50 μL; 10 mM in MeOH) was added an aqueous $NaIO_4$ solution (450 μL; 56 mM). This mixture was left for 24 h at room temperature and then directly analyzed by HPLC-MS.

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Supporting Information Available: NMR data tables for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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