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New jaspamide derivatives with antimicrofilament activity from the sponge *Jaspis splendans*

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Abstract—A reinvestigation of *Jaspis splendans* afforded the novel compounds jaspamide D–G plus the known compounds jaspamide and jaspamides B and C. All compounds exhibited potent cytotoxic activities and were shown to cause microfilament disruption. Biological activity and structural elucidation are reported here.
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1. Introduction

Small molecule natural products that target actin filaments with high affinity and affect the actin dynamics and cell motility are considered promising candidates for cancer chemotherapy, as well as useful tools for studying cellular systems.[1] Actin-binding toxins can be classified into two broad categories: those that primarily disrupt actin filament assembly and effectively destabilize filaments, and those that stabilize filaments and induce actin polymerization. To the former category belong a number of macrolides isolated from marine organisms such as aplyronins,[2] trioxazole macrolides,[3] swinholides,[4] sphinxolides and reidispongiolides.[5] Significant insight into the molecular basis of the activity of these macrolides has been proved by the recent X-ray solution of the toxin actin complexes by Rayment's group.[6] It has been shown that, despite the structural differences, all macrolides bind a common region on actin-G, acting as a mimic of the endogenous capping, and severing proteins that block the filament growth.[6] The same region on actin-G is involved in the binding of bistramide A, an unrelated ascidian product.[7]

The group of natural compounds that stabilize actin and induce polymerization is less rich in terms, and encompasses several cyclodepsipeptides also of marine origin.[1] One of the most studied members of this group is jaspamide (jasplakinolide, **1**), isolated from marine sponges of the *Jaspis* genus.[8] Jaspamide has many interesting biological properties including anthelminthic, catatonic, insecticidal and ichthyotoxic activities. It has been shown to be active against 36 human solid tumour cell cultures.[9] It is known that jaspamide binds to F-actin competitively with phalloidin,[10] a well-known fungal toxin affecting actin dynamics, promotes actin polymerization under non polymerizing conditions and lowers the critical concentrations of actin assembly in vitro.[11] Fluorescence and calorimetric measurements provide evidence that jaspamide enhances the rigidity and the thermal stability of actin filaments to a higher extent than phalloidin.[12] Due to the intriguing structure and remarkable biological properties, jaspamide has attracted the attention of the synthetic chemistry community and has been the subject of several total syntheses.[13] However, in recent years the attention of the organic chemists has been focused towards the rational design and synthesis of modified analogues of the natural product,[14] to reduce the structural complexity and to simplify the synthetic work, and to deeply understand the structural requirements essential to maintain the biological activities of the parent natural compound.

Apart from synthetic studies, further knowledge on the structure–activity relationship studies could arise from the chemical and biological evaluation of new natural analogues of jaspamide. Several variants of jaspamide, geodiamolides,[15] neosiphoniamolide[16] and seragamides[17] have been reported. The sponge *Jaspis splendans*, collected at the Vanuatu islands, was proved to contain large amounts of jaspamide together with minor analogues. In 1999 we reported the isolation from the Vanuatu collection of *J. splendans*, of jaspamide B (**2**) and jaspamide C (**3**) that represent the first two jaspamide analogues.[18] However, the scarcity of the material

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prevented any further pharmacological evaluation. The availability of a second batch of the sponge in our laboratory allowed us to re-examine its extracts.

This report outlines the isolation, the structural and pharmacological characterization of four novel derivatives, jaspamides D–G (**4–7**) along with the known compounds jaspamides B and C.[18]

2. Results and discussion

The lyophilized sponge was extracted with methanol, and the crude methanolic extract was subjected to a modified Kupchan's[19,20] partitioning procedure. Fractionation of the chloroform-soluble material (ca. 3.2 g) by silica gel MPLC followed by repeated reversed phase HPLC afforded pure jaspamides D–G (**4–7**).

Jaspamide D (**4**) was obtained as a white amorphous solid and its molecular formula was established by HRESIMS as $C_{37}H_{47}BrN_4O_6$. Compared with the molecular formula of **1**, there was the gain of one CH_2 group. The NMR data of **4** were very similar to that of **1**, especially for the resonances relative to the polypropionate fragment. The most striking difference was the lack of the alanine methyl doublet, which was replaced by a new diasterotopic methylene signal (δ_H 1.59 m, 1.06 m; δ_C 24.4) coupled with a high field triplet at δ_H 0.11 (3H, J=6.9 Hz, triplet). The complete structure of **4** was assigned on the basis of COSY, HSQC and HMBC data. In particular, COSY and HSQC data, by comparison with those of jaspamide, allowed us to define the presence of one residue each of β-Tyr, *N*-methylabrine and one residue of 2-aminobutyric acid (Aba). Key HMBC (Fig. 1) crosspeaks observed for jaspamide D substantiated the proposed structure as shown in Figure 1.

The similarity in ^{1}H- and ^{13}C NMR shifts observed for jaspamide D and jaspamide implied that the stereogenic centres in the polypropionate fragment had the same relative configurations.

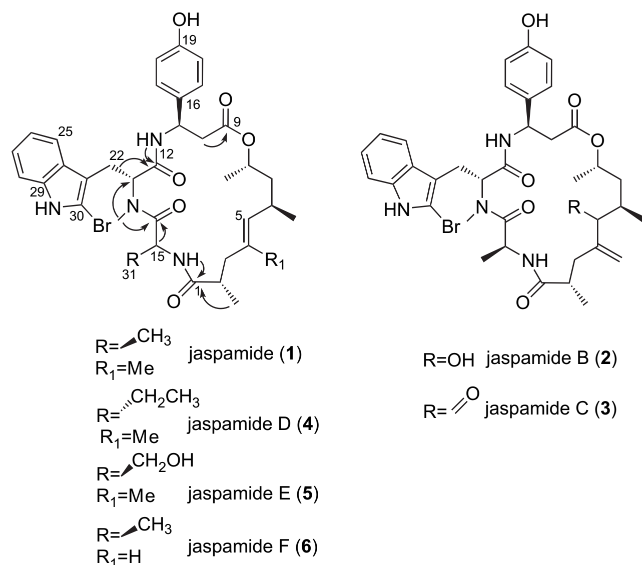

Figure 1. Jaspamide derivatives from *Jaspis splendans*.

The absolute configurations of the amino acid residues were determined by LC MS analysis of the acid hydrolyzate derivatized with Marfey's reagent ((1-fluoro-2,4-dinitrophenyl)-5-L-alaninamide; L-FDAA)[21] and comparison with appropriate amino acid standards. Both aromatic residues, β-Tyr and *N*-methylabrine were not recovered in the acid hydrolyzate of jaspamide D. Therefore, small samples of jaspamides A and D were subjected to ozonolysis followed by oxidative work-up,[22] acidic hydrolysis and Marfey's derivatization. Ion selective monitoring of FDAA-MeAsp (*m/z* 400) and of FDAA-Asp (*m/z* 386) indicated that both L-FDAA derivatives from jaspamide D coeluted with the corresponding derivatives from jaspamide. The results revealed the amino acids to be D-Aba, D-β-Tyr and D-*N*-methylabrine.

Our structural analysis of jaspamide E (**5**) began with the HRESIMS *m/z* 725.2559 $[M+H]^+$ (725.2550 calcd for $C_{36}H_{46}BrN_4O_7$). ^{1}H NMR spectra indicated that **5** didn't contain the signals assigned to the alanine unit. The presence of two additional carbinol methylene proton signals at δ_H 3.13 and δ_H 3.21, which correlated in the HSQC spectrum with a signal at δ_C 65.5, indicated that jaspamide E possesses a residue of serine rather than alanine. As inferred from a careful analysis of COSY and HSQC spectra, the resonances relative to the polypropionate portion and to the β-Tyr and *N*-Me-abrine residues were almost superimposable with that found in the parent compound. The tripeptidic sequence of **5** and placement of polypropionate fragment were assigned from the analysis of HMBC correlations reported in Figure 1. The stereochemistry of the amino acid residues was determined by Marfey's method. For the aromatic amino acids we used the procedure described for jaspamide D, although we encountered some difficulties with D- and L-serine standards, which under several chromatographic eluting conditions, showed identical retention times. The problem was solved by transforming the serine standards as well as the serine residue arising from the acidic hydrolyzate of jaspamide E into the corresponding serine methyl esters that gave well resolved peaks, even if they showed a deviation from the usual behaviour with the D-isomer eluting before the L-isomer.[23] The absolute configuration of Ser residue was determined to be L.

Jaspamide F (**6**) was found to have a molecular formula $C_{35}H_{43}BrN_4O_6$ by HRESIMS. The NMR signals of **6**, relative to the tripeptide portion, were almost superimposable with the corresponding signals in **1**. The vinyl methyl group (δ_H 1.60, δ_C 19.0) in **1** disappeared, and an additional olefinic proton (δ_H 5.42, dt, J=15.6, 6.2 Hz) appeared instead. The complete spin system of the polyketide moiety was easily assigned as 8-hydroxy-2,6-dimethylnon-4-enoic acid by the analysis of the COSY and HMBC spectra. The *E*-configuration of the 4,5-double bond was determined by the large (J=15.6 Hz) coupling constant between H4 and H5 olefinic protons. Absolute configurations of the amino acid residues were determined by Marfey's method, as described before.

Jaspamide G (**7**) was obtained as colourless amorphous solid and showed the pseudomolecular ion peak at *m/z* 723.2398 in the HRESIMS spectrum, corresponding to the molecular formula $C_{36}H_{43}BrN_4O_7$. Compared with the molecular

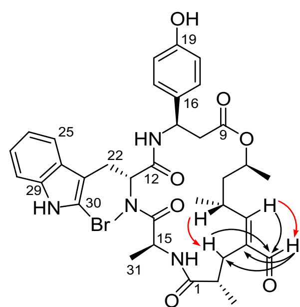

Figure 2. Jaspamide G (**7**) with HMBC (black arrows) and ROE (red arrows) correlations.

formula of **1**, there was the loss of two protons and the gain of one oxygen, as well as one additional degree of unsaturation. Inspection of 1D and 2D NMR data indicated that jaspamide shares the same tripeptide portion as **1**. A formyl group was evidenced by the presence in the ^{1}H NMR spectrum of a downfield singlet at δ 9.30, and the localization of this group at C-4 was inferred by the diagnostic downfield shift of C-5 at δ 163.7, as would be expected for the β-position in an α,β-unsaturated carbonyl, and by key HMBC correlations (Fig. 2). The *E*-configuration of the 4,5-double bond was inferred from the key ROE crosspeaks between H5 and the formyl proton, as well as between the methine proton on C6 and one of the methylene protons at C4. The assignment received further support by the upfield shift of the C3 carbon signal (δ_C 27.7), as expected for the *E*-configuration. The finding of a derivative with an inverted double bond geometry with respect to other jaspamide derivatives suggests that the origin of the formyl group in jaspamide G is not ascribable to a direct oxidation of the corresponding methyl group in jaspamide. The presence of a formyl group, and the inverted double bond geometry, determine a considerable variation of the chemical shifts of all nuclei of the polypropionate subunit. Therefore, the stereochemistry depicted in Figure 2 was tentatively assigned by analogy with the parent compound.

Table 1. Cytotoxic activity of jaspamides B–G (**2**–**7**)

Compound	Cell line IC_{50}[a] (μM)	
	MCF-7	HT-29
Jaspamide (**1**)	0.019	0.035
Jaspamide B (**2**)	3.4±1.9	3.3±0.9
Jaspamide C (**3**)	2.0	2.6±0.3
Jaspamide D (**4**)	0.05	0.08
Jaspamide E (**5**)	0.02	0.02
Jaspamide F (**6**)	30.0	Not tested
Jaspamide G (**7**)	0.60±0.07	1.66±0.07

[a] MCF-7: human breast adenocarcinoma; HT-29: colon carcinoma.

The last aspect of our study was to evaluate **2**–**7** for biological activity in a cytoskeleton morphology assay, and for cytotoxicity towards HF-29 and MCF-7 tumour cell lines. Table 1 presents a comparison of cytotoxic activity of all jaspamide derivatives so far isolated.

In Figure 3 some representative examples of microfilament assays on A-10 rat smooth-muscle cells are depicted.

The new compounds were tested at approximately the IC_{50} concentration and at 10 times the IC_{50}.

For all of the jaspamide compounds at the IC_{50} dose, there were extensive changes in microfilament morphology, while microtubule structure was essentially unaltered. There were

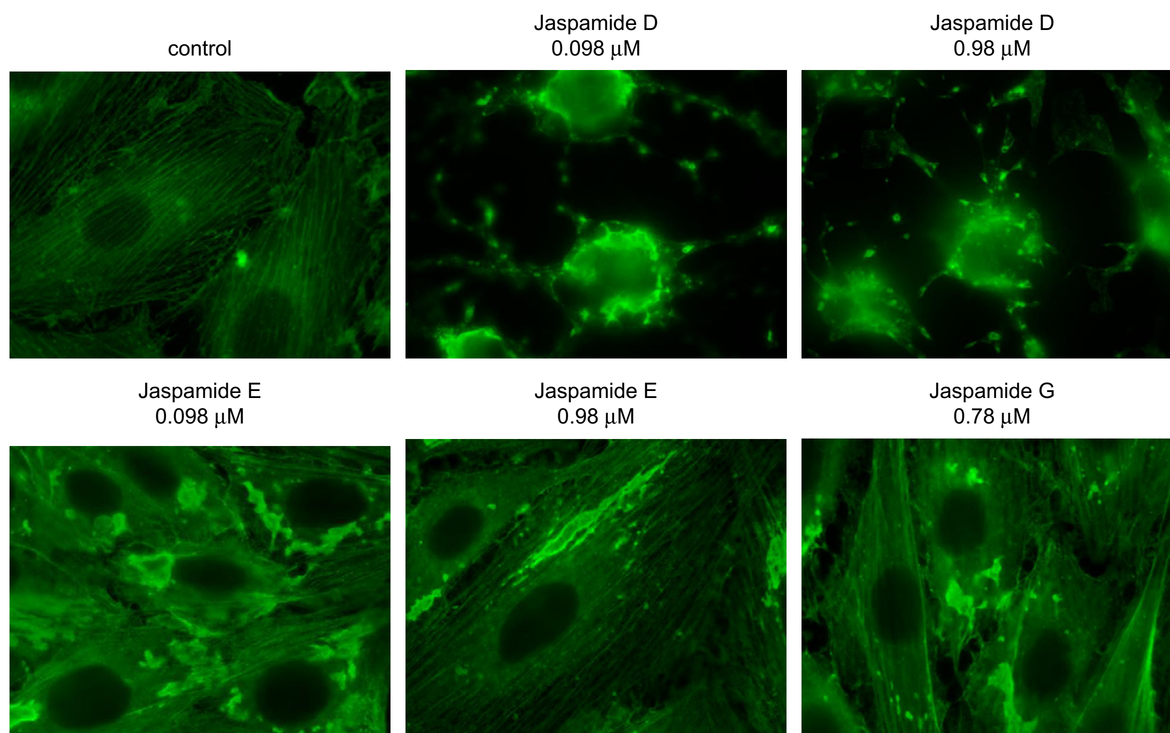

Figure 3. Microfilament disruption induced by jaspamides.

many loci of actin aggregation and loss of organization in the stress fibres.

For all of the jaspamide compounds, the $10\times IC_{50}$ concentration killed the cells, manifested as pronounced contraction, highly fluorescent aggregations of actin and frequent multinucleated cells.

The biological data obtained on these jaspamide derivatives allow us to draw some conclusions on the structural features required for the cytotoxic and antimicrofilament activities. It was previously proposed that the conserved β-turn folding characteristic of the D-Trp-β-Tyr region of natural jaspamide is responsible for the antimicrofilament activity.[14b] Indeed, all the new compounds feature unperturbed D-Trp-β-Tyr, and show a biological activity similar to that displayed by the parent compound, confirming the importance of this subunit in the interaction with the actin system.

As for the cytotoxicity, a broad range of cytotoxicity (0.02–30 μM) was observed. A comparative analysis of the structural modifications, and the observed cytotoxicity suggested that the cell growth inhibitory activity is more sensitive to the geometrical/conformational features of the entire macrocycle portion rather than to the punctual variation of the functional groups.

It has been suggested that jaspamide is a secondary metabolite of microbial origin.[24] The finding of new jaspamide derivatives arising from the incorporation of different amino acid units and of acetate instead of propionate units indicates a great variability of the domains of the PKS/NRPS multienzymatic systems of the bacteria inhabiting the sponge, an aspect that deserves further investigation.

3. Experimental

3.1. General experimental procedures

Specific rotations were measured on a Perkin–Elmer 243 B polarimeter. High-resolution ESIMS spectra were performed with a Micromass QTOF Micro mass spectrometer. ESIMS experiments were performed on an Applied Biosystem API 2000 triple–quadrupole mass spectrometer. NMR spectra were obtained on Varian Inova 500 and Varian Inova 700 NMR spectrometers (^{1}H at 500 and 700 MHz, ^{13}C at 125 and 175 MHz, respectively) equipped with a Sun hardware, δ (ppm), J in hertz, spectra referred to $CDCl_3$ as internal standard (δ_H=7.26, δ_C=77.0). HPLC was performed using a Waters Model 510 pump equipped with Waters Rheodine injector and a differential refractometer, model 401.

All reagents were commercially obtained (Aldrich, Fluka) at the highest commercial quality and used without further purification except where noted. All reactions were monitored by TLC on silica gel plates (Macherey-Nagel).

3.2. Sponge material and separation of individual peptides

J. splendans (order Choristidae, family Jaspidae) was collected at a depth of 15–20 m at Tonga, in the Vanuatu Island, in June 1996. The samples were frozen immediately after collection and lyophilized to yield 360 g of dry mass. Taxonomic identification was performed by Prof. John Hooper of Queensland Museum, Brisbane, Australia, and reference specimens are on file (R1646) at the ORSTOM Centre of Noumea.

The lyophilized material (360 g) was extracted with methanol (4×2.5 L) at room temperature and the crude methanolic extract (90 g) was subjected to a modified Kupchan's partitioning procedure as follows. The methanol extract was dissolved in a mixture of MeOH/H_2O containing 10% H_2O and partitioned against *n*-hexane. The water content (% v/v) of the MeOH extract was adjusted to 30% and partitioned against $CHCl_3$. The aqueous phase was concentrated to remove MeOH and then extracted with *n*-BuOH. The chloroform-soluble material (ca. 3.2 g) was chromatographed by silica gel MPLC (Macherey-Nagel 200–400 mesh, eluting with CH_2Cl_2/MeOH 0–10%), the fractions were collected on the basis of their TLC retention times.

Fraction 4 was purified by HPLC on a μ-Bondapack C18 column (10 μm, 300×7.8 mm, 4.0 mL/min) with 65% MeOH/H_2O as eluent to give 96.4 mg of jaspamide (t_R=20 min). The baseline was collected and further purified on a Phenomenex Luna C18 column (3 μm, 150×4.6 mm, 1.0 mL/min) with 65% MeOH/H_2O as eluent to give 0.9 mg of jaspamide D (t_R=63 min).

Fraction 5 was purified by HPLC on a μ-Bondapack C18 column (10 μm, 300×7.8 mm, 4.0 mL/min) with 65% MeOH/H_2O as eluent to give 66.7 mg of jaspamide. The baseline was collected and further purified on a Phenomenex Luna C18 column (3 μm, 150×4.6 mm, 1.0 mL/min) with 65% MeOH/H_2O as eluent to give 0.8 mg of jaspamide G (t_R=31 min).

Fraction 6 was purified by HPLC on a Phenomenex Luna C18 column (3 μm, 150×4.6 mm, 1.0 mL/min) with 65% MeOH/H_2O as eluent to give 5.0 mg of jaspamide E (t_R=25 min) and 5.1 mg of jaspamide F (t_R=16 min).

3.3. Characteristic data for each compound

Jaspamide D: white amorphous solid; $[\alpha]_D^{25}$ +20.1 (*c* 0.05, chloroform); ^{1}H and ^{13}C NMR data in $CDCl_3$ given in Table 2; ESIMS: *m/z* (%) 723.5–725.5 (20) $[M+H]^+$, 745.6–747.4 (100) $[M+Na]^+$. HRMS (ESI): calcd for $C_{37}H_{48}BrN_4O_6$: 723.2757–725.2737; found: 723.2766 $[M+H]^+$.

Jaspamide E: white amorphous solid; $[\alpha]_D^{25}$ +42.2 (*c* 0.05, chloroform); ^{1}H and ^{13}C NMR data in $CDCl_3$ given in Table 2; ESIMS: *m/z* (%) 725.6–727.5 (20) $[M+H]^+$, 747.4–749.4 (100) $[M+Na]^+$. HRMS (ESI): calcd for $C_{36}H_{46}BrN_4O_7$: 725.2550–727.2529; found: 725.2559 $[M+H]^+$.

Jaspamide F: white amorphous solid; $[\alpha]_D^{25}$ −15.7 (*c* 0.07, chloroform); ^{1}H and ^{13}C NMR data in $CDCl_3$ given in Table 3; ESIMS: *m/z* (%) 695.5–697.5 (20) $[M+H]^+$, 717.3–719.3 (100) $[M+Na]^+$. HRMS (ESI): calcd for $C_{35}H_{44}BrN_4O_6$: 695.2444–697.2424; found: 695.2450 $[M+H]^+$.

Table 2. ^{1}H and ^{13}C NMR data (700 MHz, $CDCl_3$) for compounds **4** and **5**

Position	Jaspamide D (**4**)		Position	Jaspamide E (**5**)	
	δ_H	δ_C		δ_H	δ_C
1	—	174.8	1	—	177.6
2	2.50 m	39.5	2	2.58 m	40.2
3a	1.83 d (16.2)[a]	40.8	3a	1.94 d (16.0)	41.1
3b	2.38 dd (16.2, 11.4)		3b	2.33 dd (16.0, 11.6)	
4	—	133.9	4	—	134.0
5	4.75 d (9.5)	127.9	5	4.77 d (9.8)	128.6
6	2.26 m	29.4	6	2.27 m	29.4
7a	1.11 m	43.9	7a	1.19 m	43.8
7b	1.25 m		7b	1.32 m	
8	4.59 m	71.6	8	4.64 m	70.8
9	—	170.1	9	—	171.1
10a	2.62 dd (5.2, 14.9)	40.5	10a	2.60 dd (4.9, 12.2)	40.4
10b	2.65 dd (4.8, 14.9)		10b	2.70 dd (6.8, 15.0)	
11	5.30 m	49.5	11	5.31 m	49.2
12	—	170.0	12	—	169.8
13	5.86 dd (6.8, 11.3)	56.0	13	5.77	55.7
14	—	173.3	14	—	171.3
15	4.79 m	51.5	15	4.76 m	54.4
16	—	132.0	16	—	132.1
17	7.07 d (8.2)	127.9	17	7.01 d (8.3)	127.8
18	6.75 d (8.2)	115.9	18	6.71 d (8.3)	116.0
19	—	155.1	19	—	155.4
20	6.75 d (8.2)	115.9	20	6.71 d (8.3)	116.0
21	7.07 d (8.2)	127.9	21	7.01 d (8.3)	127.8
22a	3.12 dd (11.3, 15.3)	23.9	22a	3.30 ovl	23.4
22b	3.33 dd (6.8, 15.3)		22b	3.30 ovl	
23	—	110.7	23	—	109.4
24	—	127.3	24	—	127.5
25	7.25 ovl	110.9	25	7.24 ovl	111.5
26	7.12 t (7.3)	120.8	26	7.09 t (7.7)	120.6
27	7.15 t (7.6)	123.0	27	7.15 t (7.3)	129.0
28	7.56 d (7.9)	118.7	28	7.51 d (8.1)	118.4
29	—	136.2	29	—	136.3
30	—	108.4	30	—	110.4
31a	1.59 m	24.4	31a	3.21 m	65.5
31b	1.06 m		31b	3.13 m	
32	0.11 t (6.9)	7.07	—	—	—
Me-2	1.12 d (7.1)	21.6	Me-2	1.15 d (6.9)	20.8
Me-4	1.60 s	19.1	Me-4	1.58 s	18.8
Me-6	0.83 d (7.0)	22.6	Me-6	0.84 d (6.8)	22.6
Me-8	1.06 d (6.9)	19.7	Me-8	1.08 d (6.1)	19.3
Me-N	3.0 s	31.5	Me-N	2.98 s	31.3
NH-Tyr	7.61 d (8.7)		NH-Tyr	7.40 d (8.0)	
NH-Trp	8.02 s		NH-Trp	8.02 s	
NH-Aba	6.53 d (6.6)		NH-Ser	6.87 d (6.4)	

Ovl: overlapped.
[a] Coupling constants are in parentheses and given in hertz. ^{1}H and ^{13}C assignments aided by COSY, TOCSY, HMQC and HMBC experiments.

Jaspamide G: white amorphous solid; $[\alpha]_D^{25}$ −6.7 (*c* 0.06, chloroform); ^{1}H and ^{13}C NMR data in $CDCl_3$ given in Table 3; ESIMS: *m/z* (%) 723.7–725.8 (20) [M+H]+, 745.9–747.6 (100) [M+Na]+. HRMS (ESI): calcd for $C_{36}H_{44}BrN_4O_7$: 723.2393–725.2373; found: 723.2398 [M+H]+.

3.4. Determination of absolute stereochemistry

3.4.1. Peptide hydrolysis. Peptide samples (200 μg) were dissolved in degassed 6 M HCl (0.5 mL) in an evacuated glass tube and heated at 160 °C for 16 h. The solvent was removed in vacuo and the resulting material was subjected to further derivatization.

3.4.2. LC–MS analysis of Marfey's (FDAA) derivatives. A portion of the hydrolyzate mixture (800 μg) or the amino acid standard (500 μg) was dissolved in 80 μL of a 2:3 solution of TEA/MeCN and treated with 75 μL of 1% 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) in 1:2 MeCN/acetone. The vials were heated at 70 °C for 1 h, and the contents were neutralized with 0.2 M HCl (50 μL) after cooling to room temperature. An aliquot of the L-FDAA (or D-FDAA) derivative was dried under vacuum, diluted with MeCN/5% HCOOH in H_2O (1:1) and separated on a Vydac C18 (25×1.8 mm i.d.) column by means of a linear gradient from 10% to 50% aqueous acetonitrile containing 5% formic acid and 0.05% trifluoroacetic acid, over 45 min at 1 mL/min. The RP-HPLC system was connected to the electrospray ion source by inserting a splitter valve and the flow going into the mass spectrometer source was set at a value of 100 μL/min. Mass spectra were acquired in positive ion detection mode (*m/z* interval of 320–900) and the data were analyzed using the suite of programs Xcalibur (ThermoQuest, San José, California); all masses were

Table 3. ^{1}H and ^{13}C NMR data (700 MHz, $CDCl_3$) for compounds **6** and **7**

Position	Jaspamide F (**6**)		Position	Jaspamide G (**7**)	
	δ_H	δ_C		δ_H	δ_C
1	—	174.5	1	—	175.6
2	2.29 m	42.0	2	2.57 m	39.8
3a	1.92 m	35.8	3a	2.45 d (11.2)	27.7
3b	2.36 m		3b	2.60 m	
4	5.42 dt (6.2, 15.6)[a]	126.9	4	—	139.3
5	5.22 dd (7.6, 15.6)	135.1	5	6.37 d (10.0)	163.7
6	1.90 m	33.6	6	2.95 m	31.0
7a	1.12 m	41.8	7a	1.40 m	43.5
7b	1.44 m		7b	1.54 m	
8	4.74 m	70.5	8	4.88 m	69.4
9	—	170.2	9	—	170.2
10a	2.60 dd (7.0, 15.0)	40.7	10a	2.63 dd (3.5, 16.7)	40.7
10b	2.68 dd (4.5, 15.0)		10b	2.82 dd (10.9, 16.7)	
11	5.22 m	48.9	11	5.16 m	49.8
12	—	168.9	12	—	175.2
13	5.76 dd (6.6, 9.8)	55.6	13	5.70 dd (5.5, 10.7)	55.8
14	—	174.0	14	—	174.1
15	4.74 m	45.7	15	4.36 m	45.9
16	—	132.2	16	—	134.5
17	7.00 d (8.3)	127.5	17	7.05 d (7.3)	127.7
18	6.70 d (8.3)	115.5	18	6.70 d (8.3)	115.7
19	—	154.6	19	—	156.3
20	6.70 d (8.3)	115.5	20	6.70 d (8.3)	115.7
21	7.00 d (8.3)	127.5	21	7.05 d (7.3)	127.7
22a	3.21 dd (9.8, 15.3)	23.6	22a	3.15 dd (10.3, 15.0)	23.4
22b	3.35 dd (6.6, 15.3)		22b	3.35 dd (5.5, 15.0)	
23	—	110.4	23	—	111.9
24	—	127.1	24	—	136.5
25	7.25 ovl	110.7	25	7.20 d (7.3)	110.7
26	7.09 t (7.4)	120.6	26	7.05 t (7.3)	120.3
27	7.15 t (7.2)	122.7	27	7.10 t (7.6)	122.4
28	7.54 d (8.4)	118.4	28	7.49 d (8.0)	118.5
29	—	135.8	29	—	137.9
30	—	108.8	30	—	111.9
31	0.78 d (6.7)	18.1	31	0.74 d (6.75)	16.7
Me-2	1.10 d (6.8)	19.7	Me-2	1.14 d (6.3)	19.1
Me-6	0.87 m	22.3	HCO-4	9.30	196.2
Me-8	1.04 d (6.3)	19.3	Me-6	0.97 d (6.6)	20.2
Me-N	3.0 s	31.0	Me-8	1.03 d (6.4)	21.3
NH-Tyr	7.68 m		Me-N	3.0 s	31.5
NH-Trp	8.05 s		NH-Tyr	7.26 d (7.8)	
NH-Ala	6.58 d (6.4)		NH-Trp	8.16 s	
			NH-Ala	6.06 d (6.2)	

Ovl: overlapped.
[a] Coupling constants are in parentheses and given in hertz. ^{1}H and ^{13}C assignments aided by COSY, TOCSY, HMQC and HMBC experiments.

reported as average values. Capillary temperature was set at 280 °C, capillary voltage at 37 V, tube lens offset at 50 V and ion spray voltage at 5 V.

Retention times of authentic FDAA-amino acids: L-Aba (20.1 min), D-Aba (25.6 min); L-Ala (16.6 min), D-Ala (20.5 min).

The hydrolyzate of jaspamide D contained D-Aba (25.6 min). The hydrolyzate of Jaspamides F–G contained L-Ala (16.6 min).

3.4.3. Determination of the absolute stereochemistry of serine residue in jaspamide E. Jaspamide E (0.7 mg) was dissolved in degassed 6 M HCl (0.5 mL) in an evacuated glass tube and heated at 160 °C for 16 h. The solvent was removed in vacuo and the resulting material was subjected to Marfey's derivatization with L-FDAA. The product obtained was dissolved in CH_2Cl_2, diazomethane was added dropwise and the yellow mixture was stirred under argon for 10 min. The obtained FDAA-serine methyl ester was subjected to LC–MS analysis.

Retention times of authentic FDAA-serine methyl esters: L-Ser methyl ester (18.1 min); D-Ser methyl ester (14.7 min). The hydrolyzate of Jaspamide E contained L-Ser methyl ester (18.1 min).

3.4.4. Determination of the absolute stereochemistry of aromatic amino acids in jaspamides D–G. To determine the absolute configuration of NMe-abrine and β-Tyr, an authentic sample of jaspamide was used as standard.

A stream of ozone in O_2 was bubbled through cooled solutions of jaspamides D–G (0.2 mg) or of jaspamide (0.5 mg) in MeOH (0.5 mL) at −78 °C for 1 h. Hydrogen peroxide (35%, 10 drops) was added to the reaction mixture, which was then allowed to stand at room temperature overnight. The solvent was removed under a stream of N_2. The ozonolysis products of jaspamides D–G and jaspamide were then

dissolved in degassed 6 M HCl (0.5 mL) in an evacuated glass tube and heated at 160 °C for 16 h. The solvent was removed in vacuo. The resulting material from jaspamide was subjected to Marfey's derivatization with L- and D-FDAA and LC–MS analysis.

Retention times of authentic FDAA-amino acids from jaspamide: L-MeAsp (14.2 min), D-MeAsp (16.3 min), L-Asp (15.6 min), D-Asp (16.8 min).

The hydrolyzate of jaspamides D–G contained D-Asp (16.8 min) and D-MeAsp (16.3 min).

3.5. Cytotoxicity assays

The human tumour cell lines MCF-7 (breast adenocarcinoma) and HT-29 (colon carcinoma) were obtained from the American Type Culture Collection and grown in DMEM medium containing 10% foetal bovine serum and 50 μg/mL gentamycin following standard techniques. To determine the effects of the test compounds on proliferation, cells were plated into 96-well microtiter plates and allowed to attach for 24 h. Varying concentrations (0.05–50 μM) of the test compounds were added to individual wells and the cells were incubated for an additional 72 h. At the end of this period, the number of viable cells was determined using the sulforhodamine-binding assay.

3.6. Immunofluorescence Assays

A-10 cells were grown to near-confluency on glass coverslips and treated with the indicated compounds for 24 h. Microtubules and microfilaments were stained with monoclonal anti-β-tubulin and monoclonal anti-actin antibodies, respectively, and visualized with fluorescein-conjugated anti-mouse IgG.

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