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Synthetic and pharmacological studies on new simplified analogues of the potent actin-targeting Jaspamide

Stefania Terracciano [a], Ines Bruno [a,*], Elisabetta D'Amico [a], Giuseppe Bifulco [a], Angela Zampella [b], Valentina Sepe [b], Charles D. Smith [c], Raffaele Riccio [a]

[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy
[b] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Via D. Montesano 49, 80131 Napoli, Italy
[c] Hollings Cancer Center, Medical University of South Carolina, 86 Jonathan Lucas Street, Charleston, SC 29425, USA

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Dedicated to the memory of Professor Luigi Gomez-Paloma

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ABSTRACT

In the recent years, we focused our attention on the cyclodepsipeptide Jaspamide **1**, an interesting marine metabolite, possessing a potent inhibitory activity against breast and prostate cancer, as a consequence of its ability to disrupt actin cytoskeleton dynamics. Although its biological profile has been well determined, many mechanistic details are still missing in terms of molecular target identification. For this reason, we decided to synthetically modify the natural metabolite, obtaining small arrays of unnatural variants useful to illuminate the structural requirements essential for the activity. Here, we report the synthesis of seven new Jaspamide analogues **2**–**8**, containing, as the parent compound, a β-amino acid in the cyclopeptide backbone. Their biological profile is also described.

1. Introduction

As a part of our investigations on bioactive natural metabolites, which have shown to be precious tools in medicinal chemistry, recently we focused our interest on the marine metabolite Jaspamide **1**.[1] From a structural point of view, Jaspamide is a representative member of cyclodepsipeptide family that possesses an L-Ala-D-*N*-Me-2-BrTrp-L-β-Tyr tripeptide fragment, which is linked to a ω-hydroxyacid of polyketide origin, containing four methyl groups, located at 1–3 distance, as well as a trisubstituted double bond (Fig. 1). Based on its biological behaviour, it can be included in the attractive class of cytoskeleton targeting agents, which, as it has been well established, represent potential anticancer therapeutics.[2] In particular, Jaspamide showed to selectively interfere with actin microfilaments and to produce potent antiproliferative effects.[3] Although many efforts have been invested in this research area, the mechanism of action of depsipeptide-based anti-actin compounds, including chondramides,[4] and doliculide,[5] is quite unclear in terms of molecular aspects of their interaction with the biological target, and, consequently, it is quite hard to explain the similar effects of all these metabolites on actin, in absence of a receptor mapping and further structural details. Previous investigations made on Jaspamide indicated that, the two structural elements composing the molecule, the tripeptidic portion and the polyketide fragment, play a cooperative role in eliciting the bioactive shape of the compound.[6] In fact, it seems that the tripeptide moiety, in the macrocycle, is forced to adopt a preferential β-turn conformation, indicating thus that the polyketide fragment is far from accomplishing the mere function of spacer element, but it likely plays a decisive role in generating geometric constrains and, of consequence, inducing a selective conformation.

In our previous work, we synthesized a small collection of simplified analogues, all containing the common dipeptide fragment Ala-D-Trp, and, as third amino acid, replacing the unusual β-Tyr, α-Tyr, L-Val and Phg.[7] As concerning the polyketide-mimetic

Abbreviations: Boc, *tert*-butyloxycarbonyl; ClTrt-Cl, 2-chlorotrityl chloride-resin; DIEA, *N*,*N*-diisopropylethylamine; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; Fmoc-8-Aoc-OH, *N*-α-Fmoc-8-aminooctanoic acid; Fmoc-Ala-OH, *N*-α-Fmoc-L-alanine; Fmoc-Gaba, *N*-α-Fmoc-4-aminobutanoic acid; Fmoc-D-Trp(Boc)-OH, *N*-α-Fmoc-N^{in}-Boc-D-tryptophan; Fmoc-β-Ala-OH, *N*-α-Fmoc-β-L-alanine; Fmoc-β-Phe-OH, *N*-α-Fmoc-L-β-phenylalanine; Fmoc-β-Tyr-4-OMe-OH, Fmoc-L-β-tyrosine; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methyl-methanaminium hexafluorophosphate *N*-oxide; HBTU, *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; NMM, *N*-methylmorpholine; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; 3-MePea, 3-methyl-4-pentenoic acid; 4-Pea, 4-pentenoic acid; 4-Peo, 4-penten-1-ol.

* Corresponding author. Tel.: +39 089 969743; fax: +39 089 969702.
E-mail address: brunoin@unisa.it (I. Bruno).

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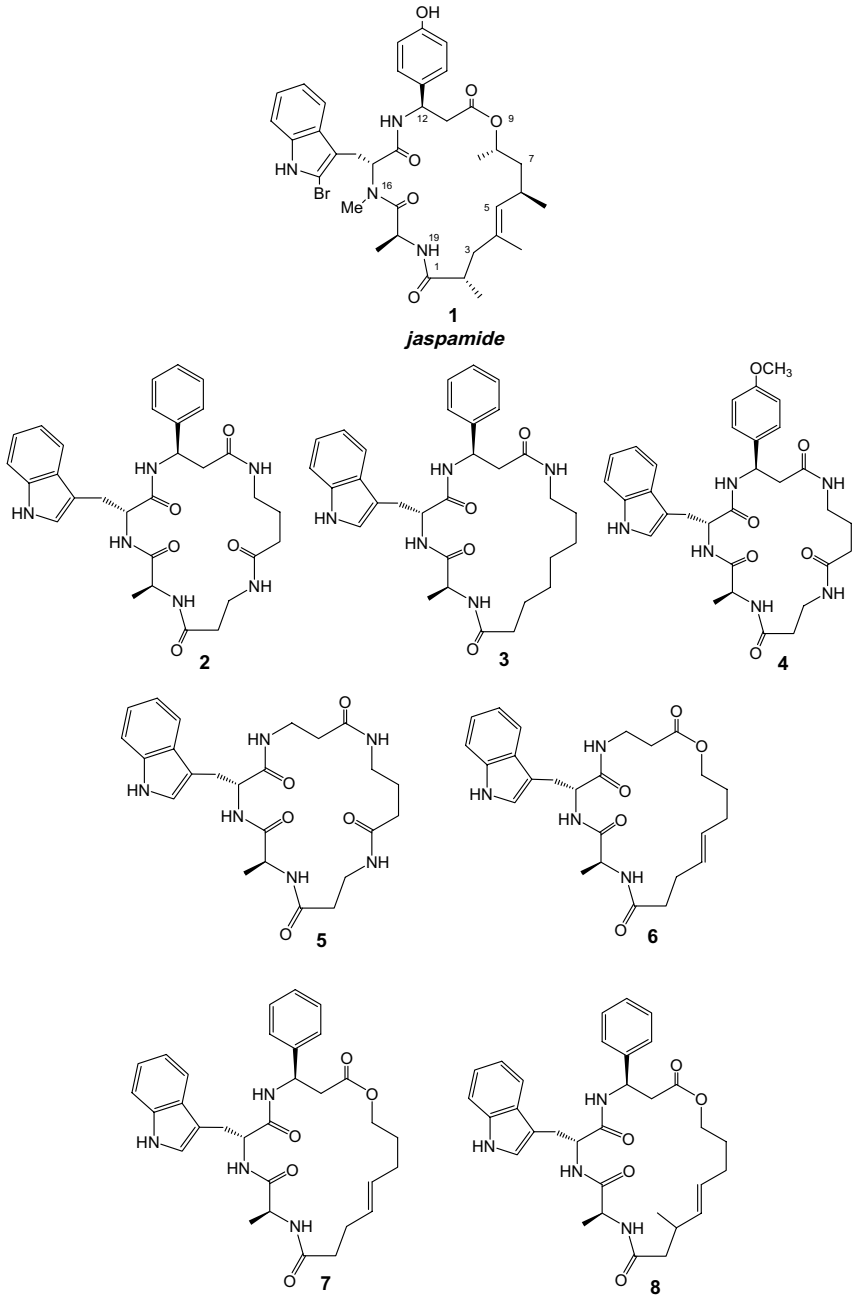


Figure 1. Structures of Jaspamide (**1**) and its new synthetic variants (**2**-**8**).

fragment, in some cases variable-length open chain linkers were used to connect the N and C termini of the tripeptide portion; in other cases an appropriately elongated *meta*-disubstituted phenyl ring was used to generate 16, 18 and 19 membered macrocycles. Unfortunately, even if all these synthetic compounds showed a moderate cytotoxicity, they were found to completely loose the target selectivity, resulting not active on actin cytoskeleton at all. Such results indicated that the extreme simplification of the parent compound was unsuccessful, by drastically affecting the biological profile. Hence, starting from the assumption that the peptide core can be considered as the pharmacophore, as first stage we worked to design a new generation of Jaspamide analogues with the intention of reproducing more accurately this structural part of the molecule, considering, of course, some crucial simplifications which can turn out useful on the synthetic plane. In more details we projected a small array of analogues, all containing as part of the peptidic core a β-amino acid residue, which, in our opinion, could elicit a β-turn conformational motif, that was supposed to be the bioactive tridimensional arrangement[7,8] As concerning the polyketide moiety, we preferred to handle, in turn, the same available linear linkers used for the previous compounds, where the macrocycle closure has been realized through an amide bond formation (**2**–**5**); furthermore, in analogues **6–8** we experienced the versatile RCM reaction to generate the cyclopeptide backbone (Fig. 1).

2. Results and discussion

2.1. Design and preliminary conformational studies

In order to synthesize molecules containing a peptidic core conformationally similar to the Jaspamide, we projected and preliminarily studied from a conformational point of view analogues **2**

and **3**. In particular, an extensive conformational analysis was performed in order to predict the geometrical features of **2** and **3** (see Section 3.3). As it is clear from Figure 2, which reports a superposition of **2** and **3**, respectively, with the X-ray structure of Jaspamide, there is a very good alignment of the peptidic core of both compounds with the natural product. Such result prompted us to synthesize these two analogues and the others of the same family in view of the expected biological activity which we hypothesized to be related to the tripeptidic central core.

2.2. Synthesis of β-aminoacid synthons

The non-racemic chiral *N*-protected β-aminoacid **12** was prepared by Michael conjugate addition of lithium (*S*)-*N*-benzyl-*N*-(methylbenzyl)amide to conjugated ester **9** (Scheme 1A) according to Davies' procedure.[9] Thus, addition of lithium amide to commercially available trans-methylcinnamate **9** afforded *N*-protected methyl ester **10** in reasonable yields and excellent stereoselectivity. N-deprotection of both *N*-benzyl protection groups was readily achieved by $Pd(OH)_2$ promoted hydrogenolysis to give the corresponding β-amino ester without compromising the stereoselectivity. Further ester hydrolysis (**11**) followed by Fmoc protection furnished the corresponding *N*-protected β-amino acid **12**, used for the solid phase syntheses of compounds **2**–**3** and **7**–**8**. For preparation of *N*-Fmoc-β-Tyr-4OMe-OH **17**, a slight different procedure was applied (Scheme 1B). Benzylation of 4-methoxy cinnamic acid **13** furnished the benzyl ester **14** in quantitative yield. Conjugate addition followed by global deprotection and *N*-Fmoc protection afforded compound **17** that was used for preparation of cyclopeptide **4**.

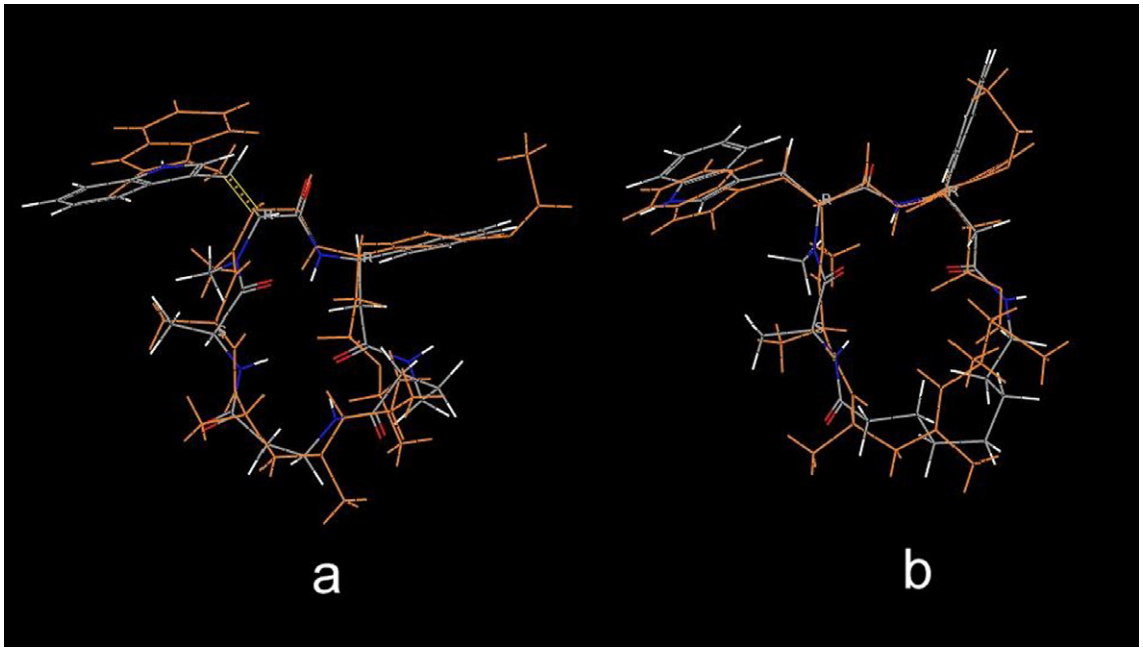
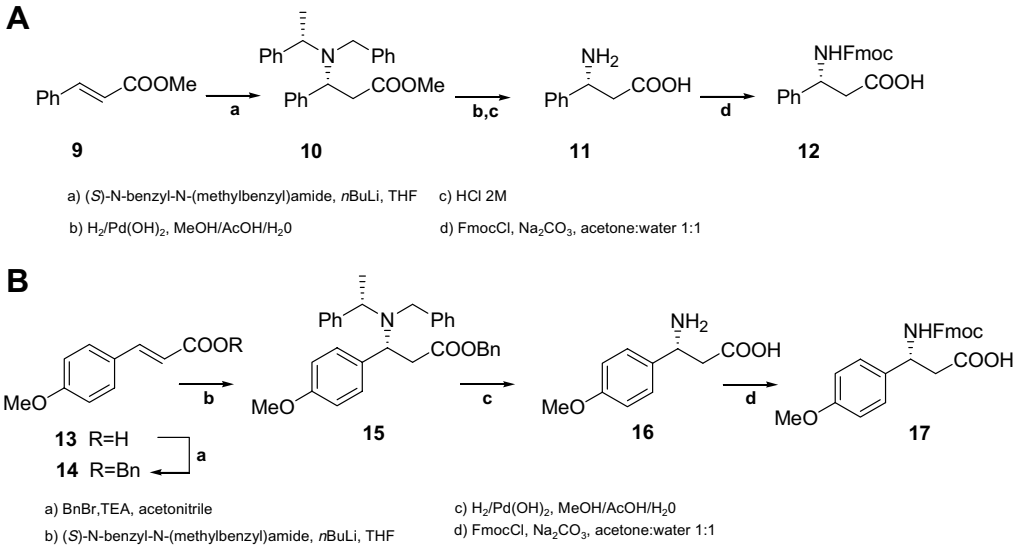


Figure 2. Superposition of **2** (a) and **3** (b), respectively, with the X-ray structure of Jaspamide. In orange are represented the synthetic compounds.



Scheme 1. Preparation of β-aminoacids **12** and **17**.

2.3. Synthesis of cyclodepsipeptides

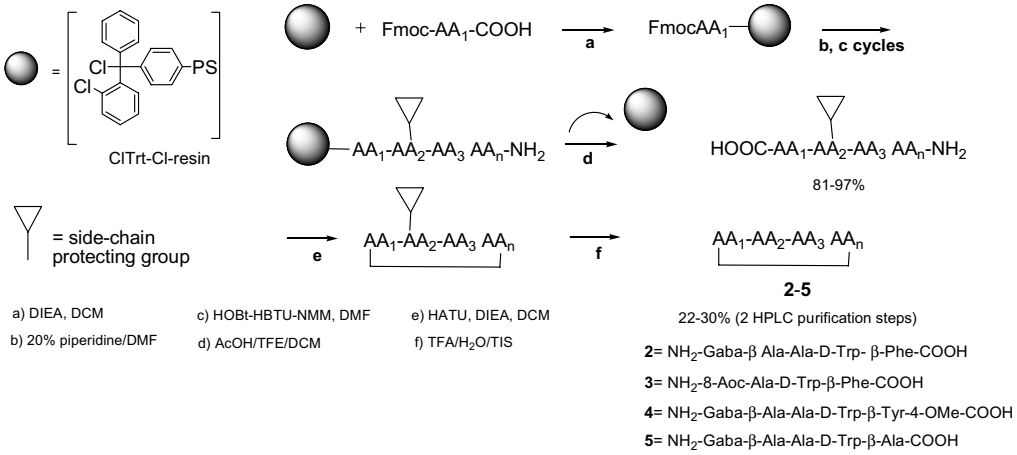
A convenient combination of both solid-phase and solution approaches was used for the synthesis of the analogues **2**–**8** (Schemes 2 and 3). Solid-phase synthesis of the linear peptide sequences, using the Fmoc/*t*Bu protection scheme, was performed on a 2-chlorotrityl chloride resin. Together with commercially available amino acids residues, also two synthetically obtained β-Phe and β-Tyr were used (see next section). Loading of the solid support was achieved, under anhydrous conditions, with the first Fmoc-protected amino acid and an excess of diisopropylethlyamine (DIEA) in DCM, followed by capping of the unreacted trityl groups with methanol, according to general procedure **a** (see Section 3).

The resulting loading degree was determined by UV spectrophotometric analysis, using the general procedure **a′** (0.17–0.53 mmol g^{-1}). The Fmoc protecting group, before each coupling step, was removed by treatment with a 20% solution of piperidine in *N*,*N*-dimethylformamide (DMF), according to general procedure **b**. The resin was then submitted to coupling-deprotection steps in order to obtain the desired linear sequences as precursors of the cyclic analogues. All the coupling steps were performed using classical SPPS conditions with hydroxybenzotriazole/*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HOBt/HBTU) in presence of *N*-methylmorpholine (NMM), as described in the general procedure **c**; the amino acid coupling degree was checked through the Kaiser and TNBS tests. After completion of peptide assembly, the Fmoc protecting group was removed from the N-terminal residue and the protected peptide was cleaved from the resin using a 2:2:6 acetic acid/2,2,2-trifluoroethanol/dicholoromethane (AcOH/TFE/DCM) solvent mixture, according to procedure **d** (81–97% overall yield with greater than 95% purity).

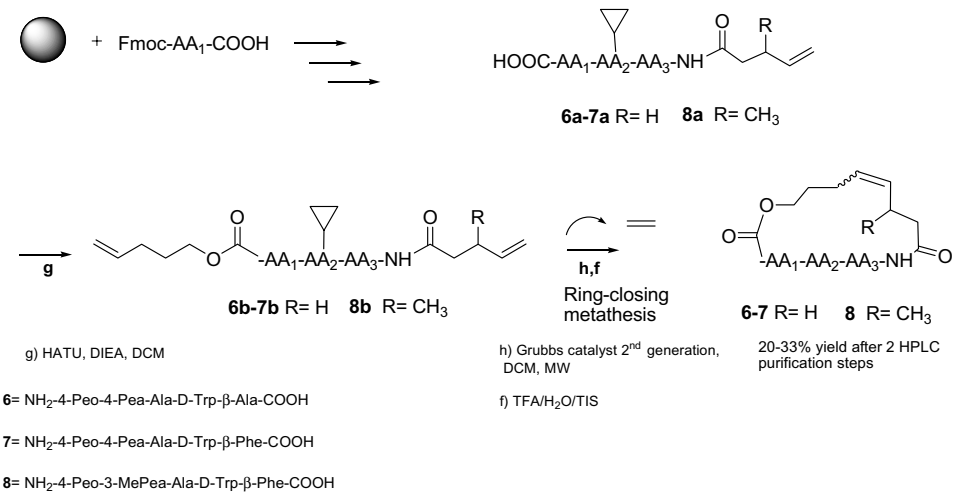
For the synthesis of analogues **2**–**5**, the intramolecular cyclization was achieved in solution under highly dilute conditions using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and DIEA in DCM/DMF, following general procedure **e** (Scheme 2).

Peptide cyclic structures were confirmed, on crude products, through analytical reversed-phase HPLC (RP-HPLC) and ESI-MS mass spectrometry analysis. Finally, after removing the protecting groups, purification on semi-preparative RP-HPLC on a C18 column, (UV–vis detection), yielded the pure cyclopeptides **2**–**5** (the cyclopeptides were obtained in 22–30% yield calculated after two HPLC purification steps). Their structures were fully elucidated by ESI-MS and NMR spectroscopic analysis.

The synthesis of the advanced intermediates **6b**–**8b** (Scheme 2) followed the SPPS protocol up to **6a**–**8a.** After cleavage from the resin, according the general procedure **d** (greater than 82% yield), ester bond formation at the C terminus of the linear peptide with 4-penten-1-ol, was performed in solution, with HATU, as coupling reagent, in basic conditions. Finally, the intramolecular cyclization



Scheme 2. General synthetic approach followed for analogues **2**-**5**.



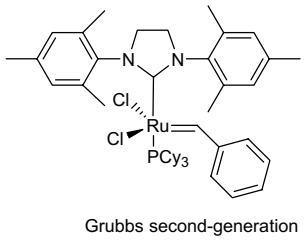
Scheme 3. General synthetic approach followed for analogues **6**-**8**.

was performed through Ring Closing Methatesis (RCM) reaction, which represents a suitable approach for the construction of variable-sized macrocyclic ring systems, especially in the synthesis of natural products.[10] As this procedure has high functional-groups tolerance, it has emerged as an efficient approach for obtaining modified cyclopeptides.

In our previous studies on cyclopeptidomimetics synthesis we experienced the efficiency of second-generation Grubbs' catalyst, which was found to be more stable and more reactive in our reaction conditions (Fig. 3).

On the basis of the reported data regarding the successful use of microwave in RCM,[11] we used MW irradiation instead of conventional heating, in order to speed up the reaction, as well as to increase reaction yield. Hence for the synthesis of analogues **6**–**8**, we used the following optimized procedure: refluxing DCM as solvent, 10 mol% of second generation Grubbs' catalyst, microwave heating at 300 W for two 40 min periods. A second addition of fresh 10 mol% catalyst was performed after the first forty minutes. In order to remove the evolving ethylene during the RCM, a gentle stream of an inert gas was employed.

To find the best cyclization conditions we performed several trials and finally we selected the very high 0.3 mM dilution. The structures of the desired compounds were confirmed by mass spectrometry and analytical RP-HPLC. After RCM, all cyclodepsipeptides were deprotected, using TFA in the presence of scavengers. Crude products were purified by semi-preparative RP-HPLC on a C18 column and the structures of cyclodepsipeptides **6**–**8** (20–33% yield after 2 purification HPLC steps) were confirmed by MS and NMR analysis.



Grubbs second-generation

Figure 3. Grubbs' catalysts structure.

2.4. Biological activity

The antiproliferative activity of the compounds **2**–**8** was evaluated on kidney A498 adenocarcinoma cells. A moderate cytotoxicity (IC_{50} = 25 μM) was observed only for compounds **7** and **8**. The synthetic compounds **2**–**8** were also tested for their activity on the microfilaments. A498 cells were treated with either cytochalasin B as positive control for microfilament depolymerising activity, or 100 μmol amounts of compounds **2**-**8**. Whilst binucleated cells were consistently and clearly present in samples treated with cytochalasin B, no cellular aberration was observed with compounds **2**–**8**, even if a modest change on the actin morphology was observed for compound **8** (Fig. 4).

These results appeared quite unexpected, considering the good alignment found between two of our synthetic compounds and Jaspamide in our preliminary conformational studies. Presumably the extreme simplification of the polyketide moiety, proposed in order to allow more synthetic accessibility of our analogues, resulted to be detrimental for the biological activity. This hypothesis is based on the consideration that the structural variations made on the peptide portion, such as the substitution of the two rare amino acids (β-Tyr and *N*-methyl-2-brTrp) with commercially available or, at least more synthetically accessible residues, were suggested by previous investigations made on other natural actin-targeting agents[5b] which show, for instance, the absence of bromine atom on Trp residue. Our conclusion was that, even if the three peptide portions seem to play a crucial role in eliciting bioactivity, as it has emerged by several investigations, the importance of the polypropionate unit cannot be underestimated in endowing the molecule with the proper lipophilicity and in assuring the right orientation of the tripeptide backbone in the space, also in accordance with the parallel findings by Maier's group.[12] In any case, despite the disappointing biological response of our synthetic compounds, we definitely gained further information

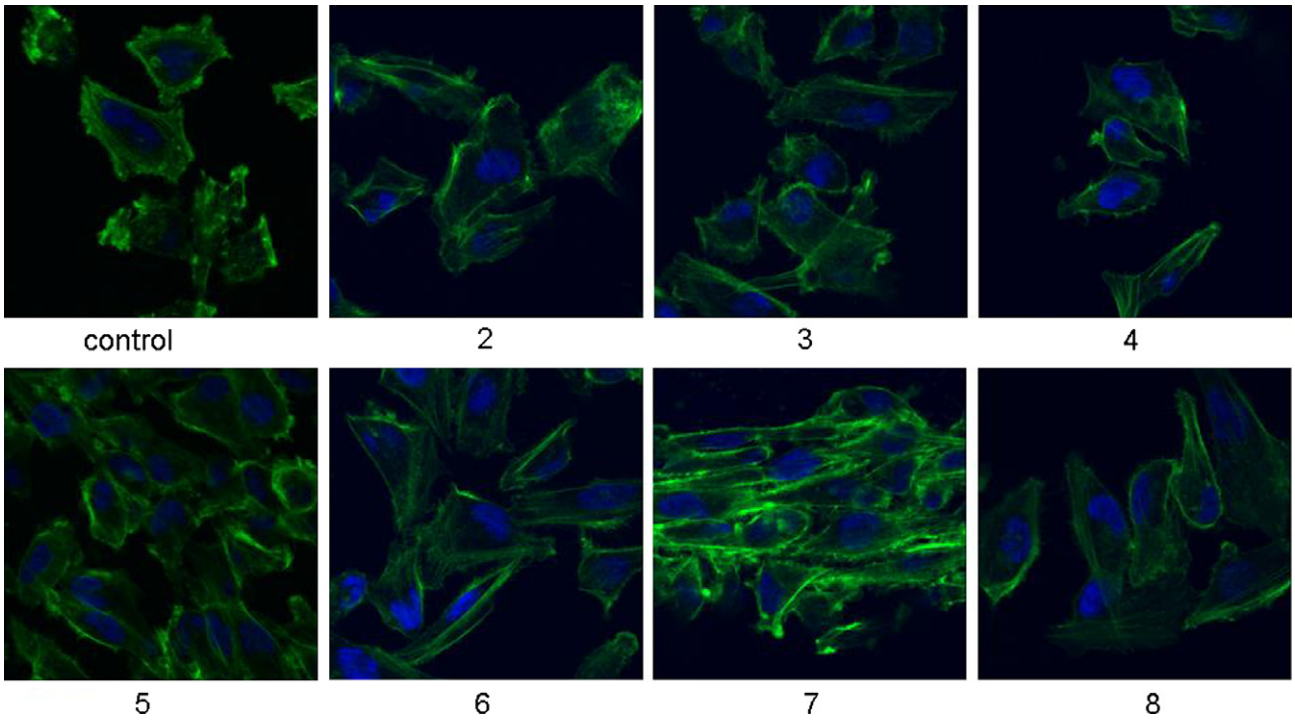


Figure 4. Microfilaments immunofluorescence assays on A-498 kidney adenocarcinoma cells of Jaspamide analogues **2**-**8**.

useful to optimize the rational design of a new generation of analogues, as these data represent the only guidelines in absence of a detailed receptor mapping and well-defined structural determinants which could clarify at molecular level the nature of interaction of Jaspamide with its biological target.

3. Experimental

3.1. General

Unless specified otherwise, all starting materials, reagents, and solvents were commercially available and used without further purification. DCM and DMF used for solid-phase reactions were synthesis grade (dried over activated 4 Å molecular sieves). Water and CH_3CN were HPLC grade. 2-Chlorotrityl chloride resin (100–200 mesh), 1% DVB, (ClTrt-Cl, loading level: 1.04 and 1.4 mmol/g), HOBt, and HBTU were purchased from Novabiochem. Fmoc-amino acids were obtained from Novabiochem, Neosystem and Fluka. HATU was purchased from Fluka. Solid-phase peptide syntheses, using the Fmoc-*t*-Bu strategy, were carried out on a polypropylene ISOLUTE SPE column on a VAC MASTER system, a manual parallel synthesis device purchased from Stepbio. All solid phase reactions were run under a nitrogen atmosphere with dry solvents. For estimation of Fmoc amino acids on the resin, absorbance at 301 nm was read employing a Shimadzu UV 2101 PC. Analytical and semipreparative reverse phase HPLC with UV detector system operating at 220 and 250 nm was performed on a Jupiter C-18 column (250 × 4.60 mm, 5μ, 300 Å; 250 × 10.00 mm, 10μ, 300 Å, respectively). The binary solvent system (A/B) was as follows: 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B).

All ring-closing metathesis (RCM) reactions were carried out under an argon atmosphere with dry, degassed solvents under anhydrous conditions. Grubbs catalyst 2nd generation was purchased from Aldrich.

All the NMR spectra (^{1}H, HMBC, HSQC, TOCSY, COSY, ROESY) were recorded on a Bruker Avance DRX600 spectrometer at T = 298 K. The compounds **2**–**8** were dissolved in 0.5 mL of 99.95% d_6-DMSO (Carlo Erba, 99.95 Atom % D) (^{1}H, δ = 2.50 ppm; ^{13}C δ = 39.5 ppm). The NMR data were processed on a Silicon Graphic Indigo 2 workstation using UXNMR software.

Electrospray mass spectrometry (ES-MS) was performed on a LCQ DECA ThermoQuest (San Josè, CA, USA) mass spectrometer. High resolution mass spectra were acquired on a Q-Tof ULTIMA (Waters, Manchester UK) calibrated with [Glu]-Fibrinopeptide B fragments using standard experimental conditions.

3.2. Computational details

In order to allow a full exploration of the conformational space of the molecules **2** and **3**, MM/MD calculations at different temperatures (300 K, 500 K, 700 K/10 ns) were performed using the AMBER force field (MacroModel software package) All the so-obtained structures (in number of 100) were minimized using the Polak-Ribier Conjugate Gradient algorithm (PRCG, 1000 steps, maximum derivative less than 0.05 kcal/mol). This led to the selection of the lowest energy minimum conformer for the molecules; a parallel analysis was performed using the MonteCarlo Multiple Minimum (MCMM) method (50 K steps) of the MacroModel package,[13] leading to the same results obtained by MM/MD calculations.

3.3. Microwave irradiation experiments

All microwave irradiation experiments were carried out in a dedicated CEM-Discover Focused Microwave Synthesis apparatus, operating with continuous irradiation power from 0 to 300 W utilizing the standard absorbance level of 300 W maximum power. The reaction mixture was carried out in 100 mL round-bottomed flask containing a magnetic stirring and fitted with a reflux condenser.

The Discover™ system also offers controllable ramp time, hold time (reaction time) and uniform stirring. The temperature was monitored using the CEM-Discover built-in-vertically-focused IR temperature sensor. After the irradiation period, the reaction vessel was cooled rapidly (60–120 s) to ambient temperature by air jet cooling.

3.4. Representative procedure for lithium amide addition

n-BuLi (2 equiv) was added dropwise to a stirred solution of (*S*)-*N*-benzyl-*N*-(methylbenzyl)amide (2 equiv) in anhydrous THF at 0 °C and stirred for 15 min under argon. The solution was cooled at −78 °C and a solution of α,β-unsaturated ester in THF was added via cannula and stirred at −78 °C for 12 h before the addition of saturated aqueous ammonium chloride. The resulting mixture was portioned between brine and ether and the combined organic extracts dried ($MgSO_4$), filtered and concentrated in vacuo before purification on silica gel column chromatography.

3.4.1. Compound 10

Purification with hexane/ethyl acetate 996:4 (68% yield, 95% de). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (3H, d, J = 7.0 Hz), 2.70 (1H, dd, J = 9.0 e 15.2 Hz), 2.83 (1H, dd, J = 9.0 e 15.2 Hz), 3.56 (3H, s), 3.84 (2H, q, J = 14.8 Hz) 4.15 (1H, q, J = 7.07 Hz), 4.59 (1H, dd, J = 6.0 e 9.4 Hz), 7.27–7.56 (15H, ovl); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.7, 37.3, 50.6, 51.3, 56.6, 59.1, 126.5–128.2, 141.2, 141.6, 144.0, 172.0. ESI-MS: m/z 374.2 $[M+H]^+$.

3.4.2. Compound 15

Purification with hexane/ethyl acetate 99:1 (75% yield, 98% de). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (3H, d, J = 6.4 Hz), 2.64 (1H, dd, J = 9.0 e 15.0 Hz), 2.71 (1H, dd, J = 5.4 e 15.0 Hz), 3.71 (1H, m), 3.81 (3H, s), 3.84 (1H, q, J = 7.0 Hz), 4.14 (1H, q, 7.2 Hz), 4.93 (2H, m), 6.87 (2H, d, J = 8.0 Hz), 7.09–7.46 (16H, ovl); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.7, 37.3, 50.6, 51.3, 56.6, 59.1, 126.5–128.2, 141.2, 141.6, 144.0, 145.9, 159.3, 172.0.

3.5. Representative procedure for N-deprotection and hydrolysis

The *N*-protected β-amino esters arising from conjugate addition were dissolved in MeOH/acetic acid/H_20 (10:1:0.25, 2 mL/0.1 mmol) and the solution poured in the pressure bottle of a Parr hydrogenation apparatus. Pd $(OH)_2$ Degussa E101 (20 equiv) was added and the mixture shaken under 6 atm of H_2 for 12 h. The bottle was degassed, the catalyst was filtered and washed several times with MeOH. The solvent was evaporated and the crude product was immediately subjected to acidic hydrolysis without purification. β-Amino ester was dissolved in 2 M HCl (1 mL/0.2 mmol) and heated at 140 °C for 16 h. The solvent was removed *in vacuo* and the resulting material was chromatographed on silica gel eluting with DCM/MeOH 99:1.

3.5.1. Compound 11

White amorphous solid; $[\delta]_D^{25} + 6.8(c0.9, H_2O)$; ESI-MS: m/z 166.1 $[M+H]^+$.

3.5.2. Compound 16

White amorphous solid; $[\delta]_D^{25} - 0.6(c0.1, H_2O)$; ESI-MS: m/z 196.1 $[M+H]^+$.

3.6. Representative procedure for N-Fmoc protection

Na_2CO_3 (3 equiv) was added to a solution of free β-amino acids in acetone/H_2O 1:1 (2 ml/0.2 mmol) and stirred for 30 min before FmocCl was added (1.1 equiv). After stirring overnight the mixture was extracted with EtOAc for 3 times. The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. Silica gel chromatography (hexane/ethyl acetate 9:1) furnished *N*-protected β-amino acids in good yields.

3.6.1. Compound 12

Yield (75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.90 (2H, m), 3.63 (3H, s), 4.13 (1H, m), 4.21 (1H, t, J = 6.6), 4.41 (2H, d, 6.6 Hz), 7.29–7.33 (7H, m), 7.39 (2H, t, 6.4 Hz), 7.58 (2H, d, 6.4 Hz), 7.76 (2H, d, 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 40.6, 47.5, 52.1, 67.0, 120.2, 125.3, 126.4, 127.3, 127.9, 129.0, 141.5, 144.0, 155.9, 172.0. ESI-MS: m/z 388.1 $[M+H]^+$.

3.6.2. Compound 17

Yield (69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.81 (1H, dd, J = 5.9 e 15.2 Hz) 2.91 (1H, dd, J = 5.2 e 15.2 Hz), 3.80 (3H, s), 4.09 (1H, m), 4.21 (1H, t, J = 6.3 Hz), 4.39 (2H, d, 6.3 Hz), 6.87 (2H, d, J = 7.0 Hz), 7.23 (2H, d, 7.0 Hz), 7.30 (2H, t, 6.2 Hz), 7.40 (2H, t, 67.3 Hz), 7.58 (2H, d, 6.2 Hz), 7.76 (2H, d, 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.9, 41.0, 47.5, 51.4, 55.5, 61.0, 67.0, 114.3, 120.2, 125.3, 127.3, 127.6, 128.0, 141.5, 155.8, 172.0. ESI-MS: m/z 418.2 $[M+H]^+$.

3.7. General procedures for the synthesis of compounds 2–8

3.7.1. Loading of the resin

The ClTrt-Cl resin was placed in a 25 mL polypropylene ISOSOLUTE syringe on a VAC MASTER system, swollen in 3 mL of DMF for 1 h, and then washed with 2× 3 mL of DCM.

A solution of Fmoc-AA-OH (1 equiv) and DIEA (4 equiv) in 2.5 mL of dry DCM was added and the mixture was agitated for 2 h with a N_2 stream. The mixture was then removed, and the resin was washed with 3× DCM/MeOH/DIEA (17:2:1), and sequentially with the following washing/treatments: DCM 3× 3 mL, DMF 2× 3 mL, DCM 2× 3 mL (1.5 min each).

3.7.2. Estimation of the level of first residue attachment

The loading of the resin was determined by UV quantification of the Fmoc-piperidine adduct.

The assay was performed on duplicate samples: 0.4 mL of piperidine and 0.4 mL of DCM were added to two dried samples Fmoc amino acid-resin (~3.0 mg) in two volumetric flasks of 25 mL. The reaction mixture was allowed to proceed for 30 min at rt and then 1.6 mL of MeOH was added and the solutions were diluted to 25 mL volume with DCM. A reference solution was prepared in a 25 mL volumetric flask using 0.4 mL of piperidine, 1.6 mL of MeOH and DCM to volume. The solutions were shaken and the absorbance of the samples versus the reference solution was measured at 301 nm. The substitution level (expressed in mmol of amino acid/g of resin) was calculated from the equation: mmol g^{-1} = (A_{301}/7800) × (25 mL g^{-1} of resin). The obtained loading degree was 0.17–0.53 mmol g^{-1}.

3.7.3. Fmoc deprotection

About 20% piperidine in DMF (3 mL, 1× 1.5 min), 20% piperidine in DMF (3 mL, 1× 10 min); washings in DMF 2× 3 mL, DCM 2× 3 mL, DMF 2× 3 mL, (1.5 min each).

3.7.4. Peptide coupling conditions

The coupling reaction was promoted by a HOBt/HBTU in DMF coupling protocol: Fmoc-amino acid, 4-pentenoic acid and 3-methyl-4-pentenoic acid (3–4 equiv), HOBt (3–4 equiv), HBTU (3–4 equiv) and NMM (4–5 equiv) were agitated under N_2 in 2.5 mL of DMF for 2 h. After each coupling, washings were carried out with DMF (3 mL, 3× 1.5 min), and DCM (3 mL, 3× 1.5 min).

3.7.5. Cleavage

The dried peptide resin was treated for 2 h, under stirring, with the following cleavage mixture: AcOH/TFE/DCM (2:2:6; 10 μL × 1 mg of resin). Then the resin was filtered off and washed with neat cleavage mixture (3 mL, 3× 1.5 min). After addition of hexane (15 times volume) to remove acetic acid as an azeotrope, the filtrate was concentrated and lyophilized (81–97% overall yield).

3.7.6. Cyclization

The cyclization step was performed in solution at a concentration of 7.7×10^{-5} M with HATU (2.0 equiv) and DIEA (2.5 equiv) in DCM. The solution was stirred at 4 °C for 1 h and then allowed to warm to room temperature for 1 h. The solvent was removed under reduced pressure.

3.7.7. Final deprotection

Final deprotection was carried out with TFA/H_2O/TIS (95:2.5:2.5; 100μL × 1 mg of resin) for 1 h, under stirring. The crude product was purified by semipreparative RP HPLC (22–30% yield calculated after two HPLC purification steps) and characterized by ES-MS and NMR spectra.

3.7.8. Ester bond formation

The coupling reaction was performed by using 4-penten-1-ol (1.6 equiv), HATU (3.0 equiv) and DIEA (3.8 equiv) in DCM at a concentration of 0.2 M. The solution was stirred at 4 °C for 1 h and then allowed to warm to room temperature overnight. The solvent was removed under reduced pressure. The crude product was analyzed by analytical RP HPLC and ESI-MS.

3.7.9. RCM under microwave irradiation

Optimized microwave ring closing metathesis.

To a stirred solution (0.3 mM) of the linear precursor in dry DCM was purged argon for 10 min followed by the addition of 10% mol of Grubbs catalyst 2nd. The mixture was refluxed at 60 °C under an argon atmosphere and irradiated at a maximum power (300 W) for 40 min. The reaction mixture was then degassed with N_2 to dry off any dissolved ethene, an additional 10% mol catalyst was added and the solution was stirred and subjected to MW irradiation (300 W) for a further 40 min. Finally, the solution was concentrated in vacuo and the crude cyclodepsipeptide was purified by RP-HPLC.

The crude products were purified by semipreparative reverse phase HPLC (on a Jupiter C-18 column: 250 × 10.00 mm, 10μ, 300 Å, flow rate = 4 mL/min) using the following gradient conditions (20–33% yield after 2 purification HPLC steps) and characterized by ES-MS and NMR spectra.

3.7.10. Cyclo [β-Phe-D-Trp-Ala-β-Ala-Gaba] (2)

RP-HPLC t_R = 13.35 min. from 15% B to 85% B over 35 min; 3.5 mg (27% yield) as a white solid; ES-MS, calcd for $C_{30}H_{36}N_6O_5$ 560.6; found m/z = 561.1 $[M+H]^+$, 583. 3 $[M+Na]^+$, 599. 1 $[M+K]^+$. HRMS calcd for $C_{30}H_{37}N_6O_5$ $[M+H]^+$ 561.2820; found 561.2560.

^{1}H and ^{13}C NMR data (600 MHz, DMSO-d_6): ***β*-Phe** δ_H: α 5.24, β 2.53 (2H), Ar 7.05–7.26, NH 8.46; **Trp** δ_H: α 4.58, β 2.86, 3.05, NH^{in} 10.82, **2** 7.12, **5** 7.65, **6** 6.97, **7** 7.05, **8** 7.32, NH 8.12; **Ala** δ_H: α 4.33, β 0.97, NH 8.00; ***β*-Ala** δ_H: α 2.22, 2.39, β 3.27, 3.35, NH 7.37; **Gaba** δ_H: α 2.80 (2H), β 1.49 (2H), γ 3.15 (2H); ***β*-Phe** δ_C: α 49.83, β 42.16, Ar 126.00–128.40; **Trp** δ_C, α 52.67, β 27.27, **2** 122.92, **5** 118.02, **6** 117.75, **7** 120.43, **8** 110.70; **Ala** δ_C: α 47.18, β 17.00; ***β*-Ala** δ_C: α 34.50, β 34.76; **Gaba** δ_C: α 37.18, β 24.78, γ 37.08.

3.7.11. Cyclo[β-Phe-D-Trp-Ala-8-Aoc] (3)

The RP-HPLC analysis showed a main peak at t_R = 19.70 min. from 5% B to 100% B over 45 min; 6.9 mg (23% yield) as a slightly yellow solid; ES-MS, calcd for $C_{31}H_{39}N_5O_4$ 545.6; found *m/z* = 546.2 $[M+H]^+$, 568.4 $[M+Na]^+$, 584.2 $[M+K]^+$.

HRMS calcd for $C_{31}H_{40}N_5O_4$ $[M+H]^+$ 546.3075; found 546.2985.

The minor peak eluting at t_R 20.51 min was shown to contain a diastereoisomeric compound, ES-MS, *m/z* = 546.2 $[M+H]^+$.

^1H and ^{13}C NMR data (600 MHz, DMSO-d_6): **β-Phe** δ_H: α 5.25, β 2.41 (2H), Ar 7.04–7.28, NH 8.71; **Trp** δ_H: α 4.63, β 2.80, 3.00, NH^{in} 10.75, **2** 6.93, **5** 7.57, **6** 6.96, **7** 7.04, **8** 7.30, NH 8.06; **Ala** δ_H: α 4.44, β 0.89, NH 7.86; **8-Aoc** δ_H: α 1.93, 2.13, β 1.13 (2H), γ 1.15, 1.25, δ 1.56 (2H), ε 1.35 (2H), ζ 2.98 (2H), NH 7.76; **β-Phe** δ_C: α 51.00, β 44.13, Ar 126.69–129.15; **Trp** δ_C: α 53.51, β 28.99, **2** 124.44, **5** 119.20, **6** 118.80, **7** 121.62, **8**111.85; **Ala** δ_C: α 47.62, β 19.20; **Aoc** δ_C: α 35.71, β 25.39, γ 29.00, δ 25.80, ε 28.70, ζ 39.21.

3.7.12. Cyclo [β-Tyr-4-OMe-D-Trp-Ala-β-Ala-Gaba] (4)

RP-HPLC t_R = 12.00 min. from 5% B to 100% B over 55 min; 2.9 mg (22% yield) as a slightly yellow solid; ES-MS, calcd for $C_{31}H_{38}N_6O_6$ 590.6; found *m/z* = 591.4 $[M+H]^+$, 613.2 $[M+Na]^+$.

HRMS calcd for $C_{31}H_{39}N_6O_6$ $[M+H]^+$ 591.2926; found 591.2829.

^1H and ^{13}C NMR data (600 MHz, DMSO-d_6): **β-Tyr-4-OMe** δ_H: α 5.19, β 2.40 (2H), Ar 6.77–7.00, 4-OMe 3.72, NH 8.65; **Trp** δ_H: α 4.57, β 2.85, 3.03, NH^{in} 10.77, **2** 6.95, **5** 7.64, **6** 6.97, **7** 7.02, **8** 7.30, NH 8.07;**Ala** δ_H: α 4.32, β 0.97, NH 7.97; **β-Ala** δ_H: α 2.38 (2H), β 3.12, 3.37, NH 7.50; **Gaba** δ_H: α 1.90 (2H), β 1.20 (2H), γ 2.93, 3.08, NH 7.78; **β-Tyr-4-OMe** δ_C: α 49.50, β 43.27, Ar 113.20–130.00, 4-OMe 54.85; **Trp** δ_C: α 52.72, β 27.75, **2** 123.26, **5** 118.32, **6** 118.06, **7** 120.60, **8** 110.92; **Ala** δ_C: α 47.46, β 17.00; **β-Ala** δ_C: α 34.73, β 35.21; **Gaba** δ_C: α 33.00, β 28.25, γ 37.53.

3.7.13. Cyclo [β-Ala-D-Trp-Ala-β-Ala-Gaba] (5)

RP-HPLC t_R = 13.84 min from 5% B to 100% B over 50 min; 3.0 mg (30% yield) as a slightly yellow solid; ES-MS, calcd for $C_{24}H_{32}N_6O_5$ 484.5; found *m/z* = 485.2 $[M+H]^+$, 507.3 $[M+Na]^+$, 523.2 $[M+K]^+$.

HRMS calcd for $C_{24}H_{33}N_6O_5$ $[M+H]^+$ 485.2507; found 485.2587.

^1H and ^{13}C NMR data (600 MHz, DMSO-d_6): **β-Ala** δ_H: α 1.96 (2H), β 3.37 (2H), NH 7.99; **Trp** δ_H: α 4.42, β 2.90, 3.14, NH^{in} 10.78, **2** 7.13, **5** 7.60, **6** 6.95, **7** 7.03, **8** 7.29, NH 8.11; **Ala** δ_H: α 4.29, β 0.90, NH 7.89; **β-Ala** δ_H: α 2.16, 2.34, β 3.20, 3.35, NH 7.59; **Gaba** δ_H: α 1.96 (2H), β 1.55 (2H), γ 3.00 (2H), NH 7.66; **β-Ala** δ_C: α 32.60, β 35.33; **Trp** δ_C: α 53.07, β 27.10, **2** 123.50, **5** 118.27, **6** 117.94, **7** 120.55, **8** 110.91; **Ala** δ_C: α 47.33, β17.41; **β-Ala** δ_C: α 34.98, β 35.02; **Gaba** δ_C: α 32.60, β 25.16, γ 37.25.

3.7.14. Cyclo [β-Ala-D-Trp-Ala-4-Pea-4-Peo] (6)

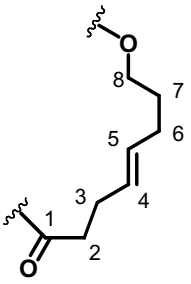
RP-HPLC t_R = 31.05 min. from 10% B to 60% B over 65 min; 1.7 mg (23% yield) as a white solid; ES-MS, calcd for $C_{25}H_{32}N_4O_5$ 468.5; found *m/z* = 469.3 $[M+H]^+$, 491.2 $[M+Na]^+$.

HRMS calcd for $C_{25}H_{33}N_4O_5$ $[M+H]^+$ 469.2445; found 469.2338.

The *E* isomer was obtained with 90% selectivity as determined by HPLC.

RP-HPLC of the *Z* isomer t_R = 32.15 min. from 10% B to 60% B over 65 min.

^1H and ^{13}C NMR data (600 MHz, DMSO-d_6): **β-Ala** δ_H: α 2.43, 2.53, β 3.16, 3.48, NH 8.03, **Trp** δ_H: α 4.48, β 2.87, 3.08, NH^{in}, **2** 7.09, **5** 7.59, **6** 6.95, **7** 7.03, **8** 7.29, NH 8.06; **Ala** δ_H: α 4.33, β 0.90, NH 7.79; **4-Pea-4-Peo** δ_H: **2** 2.05, 2.23, **3** 2.06, 2.25, **4-CH** 5.31 (overlapping signals), **5-CH** 5.31 (overlapping signals), **6** 1.88, **7** 1.51, **8** 3.88, 3.98; **β-Ala** δ_C: α 34.22, β 35.50; **Trp** δ_C: α 53.23, β 28.29, **2** 123.99, **5** 118.88, **6** 118.42, **7** 121.20, **8** 111.43; **4-Pea-4-Peo** δ_C: **2** 34.94, **3** 27.92, **4-CH** 129.55, **5-CH** 129.55, **6** 28.43, **7** 27.79, **8** 63.39.



4-Pea-4-Peo

3.7.15. Cyclo[β-Phe-D-Trp-Ala-4-Pea-4-Peo] (7)

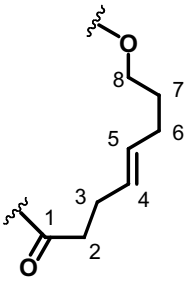
RP-HPLC t_R = 26.50 min. from 5% B to 100% B over 60 min; 1.9 mg (20% yield) as a slightly brown oil; ES-MS, calcd for $C_{31}H_{36}N_4O_5$ 544.6; found *m/z* = 545.2 $[M+H]^+$.

HRMS calcd for $C_{31}H_{37}N_4O_5$ $[M+H]^+$ 454.2758; found 545.2527.

The *E* isomer was obtained with 83% selectivity as determined by HPLC.

RP-HPLC of the Z isomer t_R = 27.56 min. from 5% B to 100% B over 60 min.

^1H and ^{13}C NMR data (600 MHz, DMSO-d_6): **β-Phe** δ_H: δ 5.28, β 2.69, 2.76, Ar 7.24–7.70, NH 8.89; **Trp** δ_H: δ 4.61, β 2.95 (2H), NH^{in} 10.74, **2** 7.00, **5** 7.62, **6** 6.95, **7** 7.03, **8** 7.31, NH 8.09; **Ala** δ_H: δ 4.44, β 0.83, NH 7.77; **4-Pea-4-Peo** δ_H:**2** 2.01, 2.30, **3** 2.04, 2.24, **4-CH** 5.30 (overlapping signals), **5-CH** 5.30 (overlapping signals), **6** 1.87, **7** 1.50, **8** 3.90, 4.05; **β-Phe** δ_C: δ 49.84, β 42.50, Ar 126.66–128.01; **Trp** δ_C: δ 52.72, β 28.00, **2** 123.54, **5** 118.70, **6** 117.92, **7** 120.84, **8** 112.90; **Ala** δ_C: δ 47.37, β 19.36; **4-Pea-4-Peo** δ_C: **2** 33.68, **3** 27.00, **4-CH** 128.47, **5-CH** 128.47, **6** 27.97, **7** 27.48, **8** 63.40.



4-Pea-4-Peo

3.7.16. Cyclo[β-Phe-D-Trp-Ala-3-MePea-4-Peo] (8)

RP-HPLC t_R = 38.56 min. from 5% B to 20% B over 5 min and then from 20% B to 60% B over 50 min; slightly brown oil; ES-MS, calcd for $C_{32}H_{38}N_4O_5$ 558.6; found *m/z* = 559.2 $[M+H]^+$, 581.3 $[M+Na]^+$.

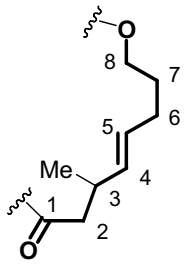
HRMS calcd for $C_{32}H_{39}N_4O_5$ $[M+H]^+$ 559.2915; found 559.2603.

The *E* isomer was obtained with 90% selectivity as determined by HPLC.

RP-HPLC of the *Z* isomer t_R = 39.80 min. from 5% B to 20% B over 5 min and then from 20% B to 60% B over 50 min.

^1H and ^{13}C NMR data (600 MHz, DMSO-d_6): **β-Phe** δ_H: δ 5.30, β 2.68, 2.78, Ar 7.22–7.34, NH 8.90; **Trp** δ_H: δ 4.63, β 2.77, 2.95, NH^{in} 10.75., **2** 7.02,**5** 7.66, **6** 6.97, **7** 7.05, **8** 7.30, NH 8.14; **Ala** δ_H: δ 4.47, β 0.80, NH 7.75; **3-MePea-4-Peo** δ_H:**2** 1.78, 2.11,**3** 2.46, **3-Me** 0.94,**4-CH** 5.23,**5-CH** 5.31,**6** 1.87 (2H), **7** 1.52 (2H), **8** 3.93, 4.09; **β-Phe** δ_C: δ 49.80, β 42.27, Ar 126.37–128.89; **Trp** δ_C: δ 53.00, β 28.68, **2** 121.10, **5** 118.96, **6** 118.36, **7** 121.24, **8** 111.41; **Ala** δ_C: δ 47.07,

β 47.07; **3-MePea-4-Peo** δ_C: **2** 43.82,**3** 33.96, **3-Me** 21.82,**4-CH** 134.70,**5-CH** 128.06,**6** 28.66, **7** 28.20, **8** 64.20.



3-MePea-4-Peo

3.8. Biological tests

3.8.1. Cytotoxicity assay

A498 kidney adenocarcinoma cells were maintained in a R-MEM medium containing 10% fetal bovine serum and 50 μg/mL gentamycin at 37 °C in an atmosphere of 5% CO_2 and 95% air. To determine the cytotoxicities of the test compounds, cells were plated into 96-well tissue culture plates at approximately 15% confluency and were allowed to attach and recover for 24 h. The cells were then treated in triplicate with varying concentrations of the test compound for 48 h, and cell survival was assayed using the sulforhodamine B (SRB) binding assay.[14] The percentage of cell survival was calculated as the percentage of SRB binding as compared with control cultures.

3.8.2. Immunofluorescence assays

A-498 cells were grown to near-confluency on glass coverslips and treated with the indicated compounds for 16 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 as previously described.[15]

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References and notes

1. (a) Zabriskie, T. M.; Klocke, J. A.; Ireland, C. M.; Marcus, A. H.; Molinski, T. F.; Faulkner, D. J.; Xu, C.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 3123–3124; (b) Crews, P.; Manes, L. V.; Boehler, M. *Tetrahedron Lett.* **1986**, *27*, 2797–2800; (c) Braekman, J. C.; Daloze, D.; Moussiaux, B.; Riccio, R. *J. Nat. Prod.* **1987**, *50*, 994–995.
2. (a) Fenteany, G.; Zhu, S. *Curr. Top. Med. Chem.* **2003**, *3*, 593–616; (b) Giganti, A.; Friederich, E. *Prog. Cell Cycle Res.* **2003**, *5*, 511–525; (c) Allingham, J. S.; Klenchin, V. A.; Rayment, I. *Cell. Mol. Life Sci.* **2006**, *63*, 2119–2134.
3. (a) Senderowicz, A. M.; Kaur, G.; Sainz, E.; Laing, C.; Inman, W. D.; Rodriguez, J.; Crews, P.; Malspeis, L.; Grever, M. R.; Sausville, E. A.; Duncan, K. L. *J. Natl. Cancer Inst.* **1995**, *87*, 46–51; (b) Bubb, M. R.; Senderowicz, A. M.; Dunkan, K. L.; Korn, E. D. *J. Biol. Chem* **1994**, *269*, 14869–14871; (c) Spector, I.; Breat, F.; Shochet, N.; Bubb, M. *Microsc. Res. Tech.* **1999**, *47*, 18–37; (d) Bubb, M. R.; Spector, I.; Beyer, B.; Fosen, K. A. *J. Biol. Chem.* **2000**, *275*, 5163–5170; (e) Visegràdy, B.; Lorinczy, D.; Hild, G.; Somogyi, B.; Nyitrai, M. *FEBS Lett.* **2004**, *565*, 163–166; (f) Visegràdy, B.; Lorinczy, D.; Hild, G.; Somogyi, B.; Nyitrai, M. *FEBS Lett.* **2005**, *579*, 6–10.
4. (a) Kunze, B.; Jansen, R.; Sasse, F.; Hofle, G.; Reichenbach, H. *J. Antibiot.* **1995**, 1262; (b) Holzinger, A.; Lutz-Meindl, U. *Cell. Motil. Cytoskeleton* **2001**, *48*, 87–95.
5. (a) Ishiwata, H.; Nemoto, T.; Ojija, M.; Yamada, K. *J.Org. Chem.* **1994**, *59*, 4710–4711; (b) Bai, R.; Covell, D. G.; Chunfeng, L.; Gosh, A. K.; Hamel, E. *J. Biol. Chem.* **2002**, *277*, 32165–32171.
6. (a) Marimganti, S.; Yasmeen, S.; Fischer, D.; Maier, M. E. *Chem. Eur. J.* **2005**, *11*, 6687–6700; (b) Hu, T-S.; Tannert, R.; Arndt, H. D.; Waldmann, H. *Chem. Commun.* **2007**, 3942–3944.
7. (a) Terracciano, S.; Bruno, I.; Bifulco, G.; Smith, C. D.; Gomez-Paloma, L.; Riccio, R. *J. Nat. Prod.* **2004**, *67*, 1325–1331; (b) Terracciano, S.; Bruno, I.; Bifulco, G.; Avallone, E.; Smith, C. D.; Gomez-Paloma, L.; Riccio, R. *Bioorg. Med. Chem.* **2005**, *13*, 5225–5239.
8. Kahn, M.; Nakanishi, H.; Su, T.; Lee, J. H.; Johnson, M. *Int. J. Pept. Protein Res.* **1991**, *38*, 324–334.
9. Davies, S. G.; Smith, A. D.; Price, P. D. *Tetrahedron Asymmetry* **2005**, 2833–2891.
10. (a) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413–4450; (b) Grubbs et al *Org. Lett.* **1999**, *1*, 953–956; (c) Fustner, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 3012–3043; (d) Reichwein, J. F.; Versluis, C.; Liskamp, R. M. J. *J.Org. Chem.* **2000**, *65*, 6187–6195; (e) Kotha, S.; Sreenivasachary, N.; Mohanraja, K.; Durani, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1421–1423;; (f) Wels, B.; Kruijtzer, J. A. W.; Garner, K.; Nijenhuis, W. A. J.; Gispen, W. H.; Adan, R. A. H.; Liskamp, R. M. J. *Bioorg. Med. Chem.* **2005**, *13*, 4221–4227; (g) Kazmaier, U.; Hebach, C.; Watzke, A.; Maier, S.; Mues, H.; Huch, V. *Org. Biomol. Chem.* **2005**, *3*, 136–145; (h) Gradillas, A.; Pérez-Castells, J. *Angew. Chem. Int. Ed.* **2006**, *45*, 6086–6101.
11. (a) Appukkuttan, P.; Dehaen, W.; Van der Eycken, E. *Org. Lett.* **2005**, *7*, 2723–2726; (b) Coquerel, Y.; Rodriguez, J. Eur. *J. Org. Chem.* **2008**, 1125–1132. and references reported therein.
12. Marimganti, S.; Wieneke, R.; Geyer, A.; Maier, M. E. *Eur. J. Org. Chem.* **2007**, 2779–2790.
13. Mohamadi, F.; Richards, N. G.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.
14. (a) Skehan, P.; Storeng, P.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112; (b) Vichai, V.; Kirtikara, K. *Nat. Protocols* **2006**, *1*, 1112–1116.
15. Smith, C. D.; Zhang, X.; Mooberry, S. L.; Patterson, G. M.; Moore, R. E. *Cancer Res.* **1994**, *54*, 3779–3784.