The Journal of Antibiotics (2019) 72:99–105
https://doi.org/10.1038/s41429-018-0115-2

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

BRIEF COMMUNICATION

Ala-geninthiocin, a new broad spectrum thiopeptide antibiotic, produced by a marine *Streptomyces* sp. ICN19

Appadurai Muthamil Iniyan[1,2] · Enge Sudarman[3,4] · Joachim Wink[2,4] · Rajaretinam Rajesh Kannan[5] · Samuel Gnana Prakash Vincent[1]

Received: 5 July 2017 / Revised: 3 October 2018 / Accepted: 9 October 2018 / Published online: 24 October 2018

Abstract

Bioassay-guided screening of antibacterial compounds from the cultured marine *Streptomyces* sp. ICN19 provided Ala-geninthiocin (1), along with its known analogs geninthiocin (2) and Val-geninthiocin (3) and the indolocarbazole staurosporine (4). The structure of 1 was determined on the basis of 1D and 2D NMR spectra and ESI-HRMS. The absolute configurations of the amino acid residues were determined by enantioselective GC-MS analysis. Compound 1 exhibited potent activity against Gram-positive bacteria including *Staphylococcus aureus, Bacillus subtilis, Mycobacterium smegmatis*, and *Micrococcus luteus*, as well as cytotoxicity against A549 human lung carcinoma cells with an IC_{50} value of 6 nM.

The desirable biological activity of thiopeptides has stimulated renewed interest in the search for new antibiotics to find promising lead molecules. New groups of actinomycetes from unexplored habitats are pursued as sources of novel bioactive secondary metabolites. It is perhaps not surprising that marine *Streptomyces* are proving to be such a valuable source of new bioactive compounds [1–3]. As part of our continuing efforts to explore marine-derived actinomycetes, a new thiopeptide antibiotic was isolated from *Streptomyces* strain ICN19 derived from a marine sediment. The bioassay-guided chromatographic purification yielded a new compound named as Ala-geninthiocin (**1**) besides the known geninthiocin (**2**), its analog Val-geninthiocin (**3**) and staurosporine (**4**) (Figure. 1). Details of the extraction, purification, structure elucidation, and biological activity are described herein.

Strong activity was found by bioassay screening against *Staphylococcus aureus* and *Candida albicans* in the acetone extract of *Streptomyces* sp. ICN19. After extracting the active metabolites with ethyl acetate and removing the lipids with *n*-heptane, the residue of the extract was fractionated using a reversed phased Medium-pressure liquid chromatography (MPLC) column with a methanol/water gradient. Of the 11 fractions collected, 3 fractions (fr. 4, 6, and 7) exhibited antibacterial activity, i.e., fr. 4 and 7 were active against *S. aureus* and fr. 6 was active against *C. albicans*. Fr. 4 was further purified using preparative RP-HPLC to give Ala-geninthiocin (**1**) along with geninthiocin (**2**). The preparative HPLC of fr. 7 yielded Val-geninthiocin (**3**). The analytical HPLC profile of MPLC fr. 6 showed a single peak of staurosporine (**4**). The compounds were separated as Val-geninthiocin with 7.62 min retention time (RT), Ala-geninthiocin with 8.34 min RT, geninthiocin with

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41429-018-0115-2) contains supplementary material, which is available to authorized users.

✉ Samuel Gnana Prakash Vincent
prakash.vincent@msuniv.ac.in

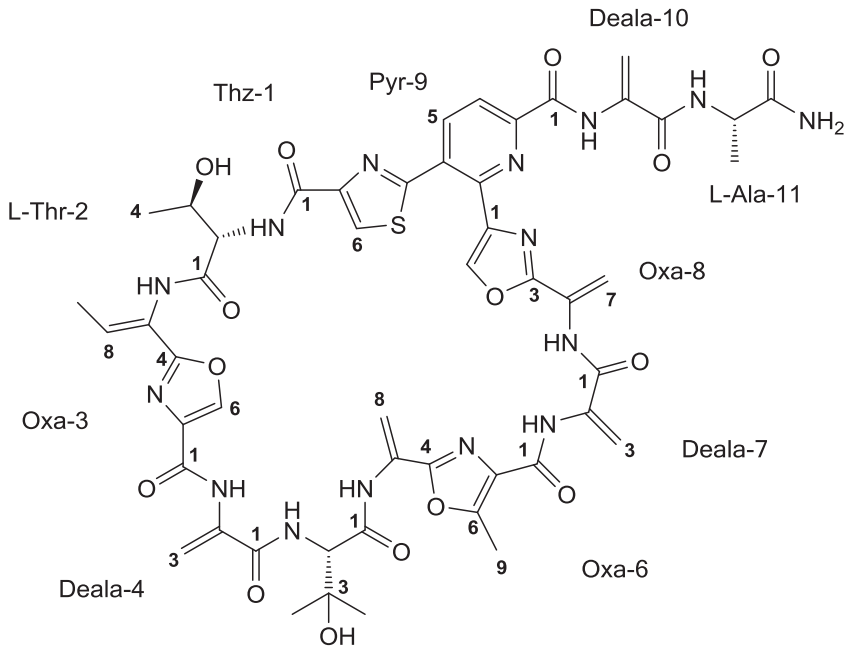
1 International Centre for Nanobiotechnology (ICN), Centre for Marine Science and Technology (CMST), Manonmaniam Sundaranar University, Rajakkamangalam-629502, Kanyakumari District, Tamil Nadu, India

2 Microbial Strain Collection, Helmholtz Centre for Infection Research GmbH (HZI), Inhoffenstrasse 7, 38124 Braunschweig, Germany

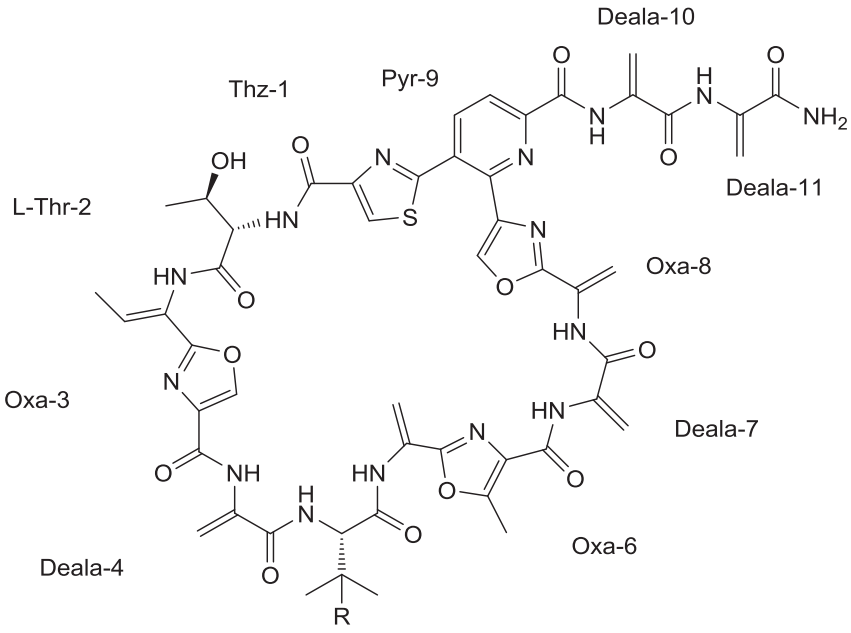
3 Department Microbial Drugs, Helmholtz Centre for Infection Research GmbH (HZI), Inhoffenstrasse 7, 38124 Braunschweig, Germany

4 German Centre for Infection Research Association (DZIF), Partner site Hannover-Braunschweig, Inhoffenstrasse 7, 38124 Braunschweig, Germany

5 Molecular and Nanomedicine Research Unit, Centre for Nanoscience and Nanotechnology (CNSNT), Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Chennai 600119 TN, India



1



2 R = OH
3 R = H

Fig. 1 Structures of isolated compounds (1–3) from *Streptomyces* sp. ICN19

9.02 min RT, and staurosporine with 21.38 min RT. The identification of the three purified metabolites was confirmed by comparing their NMR data with those reported in the literature [4–6].

The molecular formula $C_{50}H_{52}N_{15}O_{15}S$ of Alageninthiocin (1) was determined by ESI-HRMS analysis of the molecular ion clusters $[M + H]^+$ at *m/z* 1134.3488 and $[M + Na]^+$ at *m/z* 1156.3297. The ^1H NMR spectrum

Table 1 ^{1}H and ^{13}C NMR data of Ala-geninthiocin (1) in DMSO-d_6 (^{1}H 700.4 MHz, ^{13}C 176.1 MHz)

Unit	Pos.	δ_H, mult (*J* in Hz)	δ_C	Sequential NOEs[a]
Thz-1	1	—	159.9, C	
	2	—	149.4[c], C	
	4	—	163.1, C	
	6	8.49, s	126.9, CH	Thr-2-NH
Thr-2	NH	8.02, d (8.7)	—	Oxa-3-NH
	1	—	168.8, C	
	2	4.61, dd (8.7, 3.1)	57.8, CH	Oxa-3-NH
	3	4.29, m	67.3, CH	Oxa-3-NH
	4	1.15, d (6.1)	20.5, CH_3	
	OH	5.02, d (5.4)	—	
Oxa-3	NH	9.63, br. s	—	
	1	—	158.4, C	
	2	—	136.1, C	
	4		159.4, C	
	6	8.72, s	142.8, CH	Deala-4-NH
	7	—	123.1, C	
	8	6.55, q (7.2)	129.6, CH	
	9	1.75, d (7.2)	13.8, CH_3	
Deala-4	NH	9.39[b], br. s	—	Hyval-5-NH
	1	—	163.7, C	
	2	—	133.4, C	
	3a	5.88, s	103.7, CH_2	Hyval-5-NH
	3b	6.46, s		Hyval-5-NH
Hyval-5	NH	8.27, d (6.8)	—	Oxa-6-NH
	1	—	169.4, C	
	2	4.63, d (6.8)	61.8, CH	
	3	—	71.0, C	
	4	1.23, s	27.3, CH_3	
	5	1.21, s	26.2, CH_3	Oxa-6-NH
Oxa-6	NH	9.65, br. s	—	
	1	—	159.5, C	
	2	—	129.2, C	
	4	—	155.2, C	
	6	—	154.5, C	
	7	—	128.6, C	
	8a	5.66, s	105.7, CH_2	
	8b	6.11, s		
	9	2.62, s	11.6, CH_3	
Deala-7	NH	9.39[b]	—	
	1	—	162.7, C	
	2	—	133.8, C	
	3a	5.77 s	105.8	Oxa-8-NH
	3b	6.36, s		Oxa-8-NH
Oxa-8	NH	9.82, br. s	—	
	1	—	139.1, C	
	3	—	158.3, C	
	5	8.59, s	140.1, CH	
	6		129.2, C	
	7	5.71, s	111.3, CH_2	
Pyr-9	1	—	161.2, C	
	2	—	146.8, C	
	4	—	149.4[c], C	
	5	8.53, d (8.0)	140.9, CH	
	6	8.25, d (8.0)	121.4, CH	
Deala-10	NH	10.61, br. s	—	Ala-11-NH
	1	—	162.9, C	
	2	—	133.8, C	
	3a	5.91, s	103.8, CH_2	
	3b	6.50, s		
Ala-11	NH	8.63, br. d (7.3)		
	1	—	173.9, C	
	2	4.34, dq (7.3, 7.2)	49.0, CH	
	3	1.32, d (7.2)	17.7, CH_3	
	NH_2	7.39, s		
7.01. s				

[a]From the ROESY spectrum

[b]Overlap of ^{1}H signals

[c]Overlap of ^{13}C signals

of 1 in DMSO-d_6 displayed 6 methyls, 5 methylenes, 10 methines, and 13 exchangeable protons. The ^{13}C and DEPT NMR spectra confirmed the number of 50 carbons and revealed the presence of ten carboxylic carbons between δ_C 173.86 and 158.39, and five primary olefin carbons (δ_C 111.3, 105.8, 105.7, 103.8, and 103.8), as well as 18 quaternary olefin carbon signals. The ^{1}H,^{15}N HSQC spectrum showed $^{1}J_{NH}$ couplings of amide protons and a terminal NH_2 group. The assignment of ^{1}H and ^{13}C NMR data is presented in Table 1. Detailed analysis of the ^{1}H,^{1}H COSY, and TOCSY NMR spectra of 1 identified spin systems belonging to proteinogenic amino acids Ala and Thr, which were confirmed from the long range correlations in the ^{1}H,^{13}C HMBC spectrum. They were also compatible with the amino acid analysis of the hydrolysis products. The presence of 2 oxazoles and 1 thiazole was shown by characteristic ^{1}H and ^{13}C chemical shifts at δ_H 8.71, 8.59, and 8.49 ppm (δ_C 142.8, 140.1, and 126.9) in conjunction with the ^{1}H,^{13}C HMBC correlations to adjacent quaternary carbons (see Fig. 2) a methyl signal at 2.62 and its long range correlations indicated the existence of a methyloxazole (Oxa-3) residue [4, 7]. The Z configuration of the methyl substituted double bond of Oxa-3 was established from an intraresidueal NOE between the methyl and NH protons. Furthermore, typical shifts, COSY and HMBC correlations identified the 2,3,6-trisubstituted pyridine residue that has

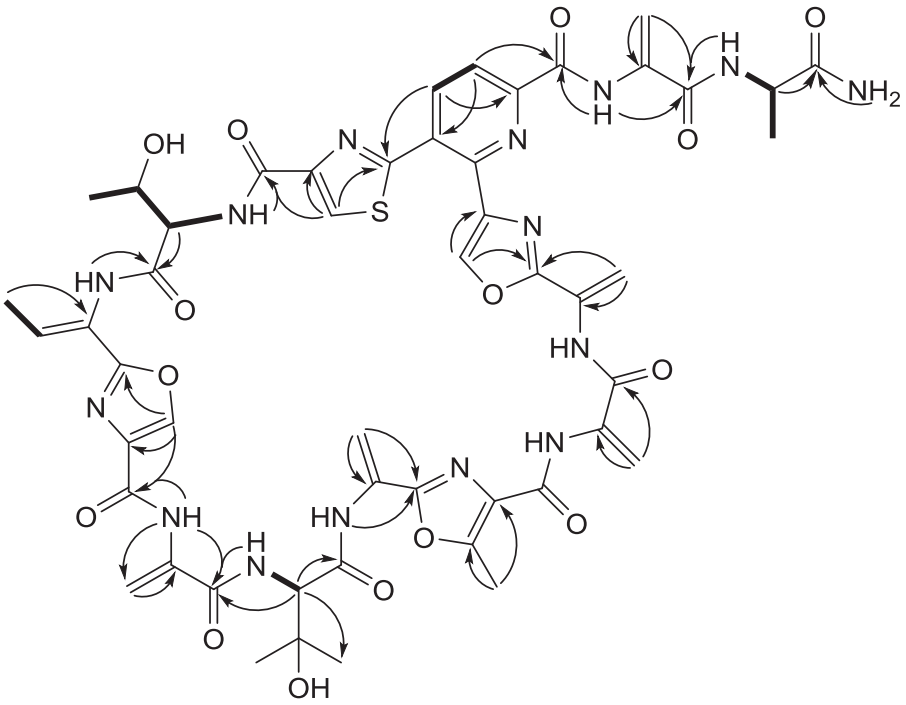


Fig. 2 Selected $^{1}H,^{1}H$ COSY (bold bonds) and $^{1}H,^{13}C$ HMBC (arrows) correlations of Ala-geninthiocin (1)

been found in other thiopeptides produced by *Streptomyces* [8, 9].

Sequential assignments were determined from the cross peaks in the $^{1}H,^{1}H$ ROESY spectrum by the NOE between H_N, H_α, H_β in residue (i) to H_N in residue (i + 1). In combination with HMBC correlations the sequential assignment method provided the complete amino acid sequence of 1 which showed that Pyr-9 was attached to Thz-1 to form a cyclic peptide. Finally the Deala-10 chain was found to be attached to Pyr-9 through long range correlations from H-6 of Pyr and NH of Deala-10 to the carbonyl carbon at 161.2 ppm. The peptide structure of 1 is largely similar to geninthiocin [4]. The only difference is the presence of an Ala residue instead of Deala at the C-terminal amide. The absolute stereochemistry of 1 was determined by enantioselective GC-MS analysis indicating the presence of L-Ala, L-Thr, and L-Hyval. Furthermore, the absolute configuration of Hyval residue from geninthiocin was assigned from the GC-MS analysis to be L as well, which can be also inferred for Val-geninthiocin as reported in the literature [5].

Compounds 1–4 were evaluated for their antibiotic activities against a panel of human pathogenic bacteria, yeast, and fungi and all exhibited strong antibiotic activity as shown in Table 2. Compounds 2, 3, and 4 were known to exhibit antimicrobial activity [5], and compound 4 also acts as an anticancer agent targeting protein kinases [10]. Compound 1 showed inhibition of *Staphylococcus aureus, Bacillus subtilis, Mycobacterium smegmatis, Micrococcus luteus, Chromobacterium violaceum, Candida albicans, Pichia anomala*, and *Mucor hiemalis*. Compounds 1 and 2 inhibited *C. violaceum* but not compound 3. Similarly, compound 1 showed weak and compound 3 moderate activity against *C. albicans* while 2 was inactive. Thus, the antimicrobial activity of these analogs suggests that substitution of amino acids has little effect on their antimicrobial activity.

Table 2 Antibacterial and antifungal activities of compounds 1–4

	MIC (μg ml^{-1})			
	1	2	3	4
Gram positive				
Staphylococcus aureus DSM346	15	4	8	19
Bacillus subtilis DSM10	4	0.2	8	15
Mycobacterium smegmatis DSM43756	10	2	16	15
Micrococcus luteus DSM1790	1	0.2	2	5
Gram negative				
Pseudomonas aureginosa DSM1128	—	—	—	—
Chromobacterium violaceum DSM30191	19	19	—	—
E. coli DSM1116	—	—	—	—
E. coli TolC	—	—	—	8
Yeasts				
Candida albicans DSM1386	150	—	33	1
Pichia anomala DSM6766	75	150	66	1
Fungi				
Mucor hiemalis DSM63298	75	38	16	1

— no activity observed up to 150 μg ml^{-1}

Table 3 Cytotoxic activities of compounds 1–4

Cell line	IC_{50} (nM)			
	1	2	3	4
L929 murine fibrosarcoma	22	25	8	0.26
KB3.1 human cervix carcinoma	22	247	53	9
MCF-7 human breast carcinoma	32	821	108	39
A549 human lung carcinoma	6	24	12	1
PC-3 human caucasian prostate adenocarcinoma	33	194	22	10
A431 human epidermoid carcinoma	29	141	42	2
SKOV-3 human ovarian carcinoma	33	194	29	8

The thiopeptide antibiotics are inhibiting the growth of various drug-resistant pathogens, including Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant *Enterococci* (VRE) by targeting the ribosome and thus inhibiting protein synthesis [11–13]. Compound 1 showed potent antibacterial activity and weak antifungal activity. Comparatively, 3 showed better antifungal activity than its analogs. The antimicrobial activities of 2 and 3 described earlier [5] are correlating with our findings. Further, 2 has been reported as an inducer of the *tip A* promoter in streptomycetes [4].

Furthermore, these compounds showed potent cytotoxic activity against the tested cervix, breast, lung, prostate, epidermoid, and ovarian human cancer cell lines. The IC_{50} values of the analog compounds 1 and 3 were moderately higher than those of the basic compound 2 (Table 3). However, compound 4 showed significantly higher cytotoxic activity. The anticancer activity of 4 is evidenced as broad spectrum protein kinase inhibitor with mitochondrial caspase activation [14, 15]. Cumulative research findings highlight the significant role of macrocyclic peptides in various cancer therapeutic modalities. Compound 1 showed cytotoxic IC_{50} values of 22 nM against the mouse fibroblast cell line L929 and 6 nM against the human lung carcinoma cell line A549. Overall, 1 showed a higher selectivity towards mammalian cell lines than 2 in the cytotoxicity assays. It has been reported previously that the thiopeptide antibiotic thiostrepton is active against human breast cancer by inhibiting the FoxM1 transcription factor involved in tumorigenesis in the MCF7 cell line [16], which is one of the possible modes of action of the thiopeptides described above.

In conclusion, along with the new thiopeptide Ala-geninthiocin (1), three bioactive compounds were isolated from the marine *Streptomyces* sp. ICN19. All have been assayed against a panel of human pathogenic bacteria, yeasts, and fungi for their antibiotic activity and against cervix, breast, lung, prostate, epidermoid, and ovarian human cancer cell lines and a murine fibrosarcoma cell line for anticancer activity. Macrocyclic peptides provide diverse functionality with high affinity and selectivity for target proteins, while maintaining adequate bioavailability for binding. The present findings indicate that the antibiotic and anticancer activity of the thiopeptides including the new thiopeptide Ala-geninthiocin (1), geninthiocin (2), and its analog Val-geninthiocin (3) may demand further preclinical evaluations for a future therapeutic use. The possible routes of their biosynthesis and preclinical safety are currently under investigation.

Materials and methods

General experimental procedures

Optical rotation was measured with a Perkin-Elmer 241 MC instrument, UV data recorded on a Shimadzu UV-Vis spectrophotometer UV-2450 using methanol (UVASOL, Merck). ^{1}H NMR, ^{13}C, and ^{15}N NMR spectra were recorded on a Bruker AVANCE DMX- 700 (^{1}H 700 MHz, ^{13}C 176 MHz) spectrometer. ESI-HRMS mass spectra were obtained with a Maxis ESI-TOF mass spectrometer (Bruker Daltonics) attached to an Agilent 1200 series HPLC system: column 100 × 2.1 mm, C_{18} XBridge TM, 3.5 µm (Waters), solvent A: 5% acetonitrile in water, 5 mmol L^{-1} NH4Ac, 0.04 ml L^{-1} acetic acid; solvent B: 95% acetonitrile in water, 5 mmol L^{-1}, 0.04 ml L^{-1} acetic acid, gradient 10% B increasing to 100% B in 30 min, and maintaining 100% B for 10 min, flow rate 0.3 ml min^{-1}; UV detection 200–500 nm. Analytical RP-HPLC was carried out with an Agilent 1260 HPLC system equipped with a UV diode-array detector and a Corona Ultra detector (Dionex); column 125 × 2 mm, Nucleodur 5 µm C18 (Macherey Nagel), solvent A: 5% acetonitrile in water, 5 mmol L^{-1} NH_4Ac, 0.04 ml L^{-1} acetic acid, solvent B: 95% acetonitrile, 5 mmol L^{-1} NH_4Ac, 0.04 ml L^{-1} acetic acid, gradient from 10% B to 100% B in 30 min, 10 min 100% B, flow rate 0.3 ml min^{-1}. GC/MS analysis was performed on an automated Applied Biosystems ABI-420-A amino acid analyser.

Producer strain

The strain *Streptomyces* sp. ICN19 was isolated from subtidal marine sediment collected at two feet depth at Chinnamuttam coast of Kanyakumari, India, and isolated using standard dilution-plating with the modified Gause's inorganic agar media [17] containing 20 g soluble starch, 1 g KNO_3, 0.5 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 18 g agar in 1 L of 50% sea water (pH 7.2–7.4). A pure culture was maintained in a glycerol suspension (20%, w/v) at −20 °C. 16S rRNA gene sequencing was carried out and the aligned sequence was identified against closely

related sequences of representative *Streptomyces* sp. retrieved from the GenBank and EzTaxon databases. The sequence was deposited in GenBank with the accession number KU738607.

Screening, extraction, and Isolation

GYM broth medium containing glucose 4 g, yeast extract 4 g, malt extract 10 g, $CaCO_3$ 2 g, and distilled water 1 L adjusted at pH 7.2 using KOH was inoculated with a 1 cm^2 agar plug of a well sporulated mycelium from *Streptomyces* sp. ICN19 and incubated in a shaker at 30 °C up to 7 days. After 7 days 5% by volume of this inoculum was transferred into 10 L shake flasks (100 × 250 ml flask with 100 ml broth) of medium HZI 5254 containing glucose 15 g, soy-meal 15 g, corn steep 5 g, $CaCO_3$ 2 g, NaCl 5 g, and distilled water 1 L at pH 7.0 and incubated in a shaker at 30 °C up to 5 days. The production broth was harvested by centrifuging at 7000 rpm. Mycelial biomass and cell free supernatant were collected separately. The mycelial cake was extracted three times with acetone (900 ml). After filtration and evaporation, the residual water phase was partitioned with ethyl acetate six times and dried in vacuo. The residue (1.94 g) of the ethyl acetate extract was further dissolved in methanol (+ 10% water) and extracted six times against *n*-heptane to remove the liphophilic components. The methanol phase was dried to afford 996 mg crude extract. The extract was separated by preparative RP-MPLC chromatography [column 480 × 30 mm, ODS AQ, 120 Å, 16 µm (Kronlab); solvent A: water/methanol 1/1, solvent B: methanol, gradient: 20% B isocratic 4 min, from 20% B to 80% B in 260 min, from 80% B to 100% B in 30 min; flow rate 30 ml min^{-1}, UV detection 210 nm]. Totally 11 fractions were collected and tested against *S. aureus* and *C. albicans*. The analytical HPLC profile of MPLC Fr. 6 containing 41 mg of staurosporine (4) was found to be pure and was active against *C. albicans*. Fr. 4 (285 mg) with strong activity against *S. aureus* was separated by preparative RP-HPLC [column: 250 × 21.2 mm, Nucleodur 100-10 C_{18} (Macherey-Nagel); solvent A: water, solvent B: acetonitrile, gradient from 30% B to 60% B in 60 min, 10 min 100% B, flow rate 20 ml min^{-1}, UV detection 220 nm] and delivered 13 mg of Ala-geninthiocin (1) and 73 mg of geninthiocin (2) as the main compound. Fr. 7 (23 mg) was purified using 35% B to 55% B in 60 min and 100% B for 10 min to yield 2.6 mg of Val-geninthiocin (3).

Enantioselective analysis of amino acids by GC/MS

Sample was hydrolyzed using 4 N TFA at 110 °C for 24 h. After drying, the resulting free amino acids were derivatized with 4 N HCl/propan-2-ol (1 h, 110 °C) and, after removal of reagents, the amino acid isopropyl esters were then acylated with pentafluoropropionic acid anhydride in CH_2Cl_2 (150 °C, 12 min). Excess reagents were again removed and the amino acid derivates analyzed on a Chirasilval column (50 m) connected to a GCQ ion trap mass spectrometer. The constituent amino acids were identified by their characteristic mass spectra and their enantiomerity was determined by comparison to standard D and L amino acids. D-Ala 10.42, L-Ala 10.86, D-Thr 11.93, L-Thr 12.13, D-Hyval 12.35, and L-Hyval 12.47 min were used as commercial reference standard.

Ala-geninthiocin (1): $C_{50}H_{51}N_{15}O_{15}S$, white amorphous solid; $[\alpha]_D^{20} = +92.8$ (*c* 1.38, CH_3OH); UV/Vis (CH_3OH): λ_{max} (log ε_{max}) = 237 nm (4.67); NMR spectroscopic data (^1H: 700 MHz, ^{13}C: 176 MHz, $CDCl_3$) Table 1; ESI-HRMS: $C_{50}H_{52}N_{15}O_{15}S$ $[M + H]^+$ *m/z* calcd 1134.3483, found 1134.3488; $C_{50}H_{51}N_{15}O_{15}SNa$ $[M + Na]^+$ *m/z* calcd 1156.3302, found 1156.3297.

Biological assays

Antimicrobial assay: Minimum inhibitory concentrations (MIC) in µg ml^{-1} were determined in 96-well microtiter plates with EBS medium (0.5% peptone, 0.5% glucose, 0.1% meat extract, 0.1% yeast extract, 50 mM HEPES for bacteria, and MYC medium for yeast and fungi, respectively. Twenty microliter aliquots (150 µg ml^{-1} from 1 mg ml^{-1} concentration) of compounds were tested against four different Gram-positive (*Staphylococcus aureus* DSM 346, *Bacillus subtilis* DSM 10, *Mycobacterium smegmatis* DSM 43756, *Micrococcus luteus* DSM 1790) and three Gram-negative bacteria (*Chromobacterium violaceum* DSM 30191, *Pseudomonas aeruginosa* DSM 1128, *Escherichia coli* DSM 1116, *Escherichia coli* TolC), two yeasts (*Candida albicans* DSM 1386, *Pichia anomala* DSM 6766), and a fungal strain (*Mucor hiemalis* DSM 63298). Negative control wells were left blank. Compounds were dissolved in methanol. Cell density was adjusted to about 5×10^6 ml^{-1}.

Cytotoxicity assay: In vitro cytotoxicity (IC_{50}) was determined against seven cancer cell lines. A 60 µl amount of serial dilutions from an initial stock of 1 mg ml^{-1} in Methanol of the test compounds was added to 120 µl aliquots of a cell suspension (50,000 ml^{-1}) in 96-well microplates. After 5 days of incubation, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay was performed, and the absorbance measured at 590 nm using an ELISA plate reader (Victor). The concentration, at which the growth of cells was inhibited to 50% of the control (IC_{50}), was obtained from the dose-response curves. The negative control was methanol.

Acknowledgements The support of the Department of Science and Technology, Science and Engineering Research Board (DST-SERB), Govt. of India [F.No. SR/SO/HS-104/2012] to SGPV is gratefully

acknowledged. The European Molecular Biology Organization (EMBO) is gratefully acknowledged for the Short term fellowship grant to AMI [ASTF No. 90 – 2016]. We thank Manfred Nimtz for the GC-MS analysis of amino acids. We thank Christel Kakosche, Romy Schade, and Wera Collisi for their excellent technical assistance.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Raju R, Andrew MP, Zeinab K, Bernhardt PV, Capon RJ. Heronamycin A: a new benzothiazine ansamycin from an Australian marine-derived *Streptomyces* sp [J]. Tetrahedr Lett. 2012;53:1063–5.
2. Mullowney MW, et al. Diazaquinomycins E-G, novel diazaanthracene analogs from a marine derived *Streptomyces* sp. Mar Drugs. 2014;12:3574–86.
3. Vicente J, et al. Monacyclinones, new angucyclinone metabolites isolated from *Streptomyces* sp. M7-15 associated with the Puerto Rican sponge *Scopalina ruetzleri*. Mar Drugs. 2015;13:4682–700.
4. Yun BS, Hidaka T, Furihata K, Seto H. Microbial metabolites with tipA promoter inducing activity. II. Geninthiocin, a novel thiopeptide produced by *Streptomyces* sp. DD84. J Antibiot. 1994;47:969–75.
5. Sajid I, Shaaban KA, Frauendorf H, Hasnain S, Laatsch HZ. Val-Geninthiocin: structure elucidation and MSn fragmentation of thiopeptide antibiotics produced by *Streptomyces* sp. RSF18. Z Naturforsch. 2008;B63:1223–30.
6. Schupp P, Proksch P, Wray V. Further new staurosporine derivatives from the ascidian *Eudistoma toealensis* and its predatory flatworm *Pseudoceros* sp. J Nat Prod. 2002;65:295–8.
7. Yun BS, Seto HPromoinducin. a novel thiopeptide produced by *Streptomyces* sp. SF2741. Biosci Biotech Biochem. 1995;59:876–80.
8. Abe H, Kushida K, Shiobara Y, Kodama M. The sutructures of sulfomycin I and berninamycin A. Tetrahedr Lett. 1988;29: 1401–4.
9. Bowers AA, Waslch CT. Genetic interception and structural characterization of thiopeptide cyclization precursors from *Bacillus cereus*. J Am Chem Soc. 2010;132:12182–4.
10. Gescher A. Analogs of staurosporine: potential anticancer drugs? Gen Pharmac. 1998;31:721–8.
11. Porse BT, Leviev I, Mankin A, Garrett RA. The antibiotic thiostrepton inhibits a functional transition within protein L11 at the ribosomal GTPase centre. J Mol Biol. 1998;276:391–404.
12. Harms JM, et al. Translational regulation via L11: molecular switches on the ribosome turned on and off by thiostrepton and micrococcin. Mol Cell. 2008;30:26–38.
13. Zhang Q, Liu W. Biosynthesis of thiopeptide antibiotics and their pathway engineering. Nat Prod Rep. 2013;30:218–26.
14. Tamaoki T, et al. Staurosporine, a potent inhibitor of phospholipid/Ca kinase. Biochem Biophys Res Commun. 1986;135:397–402.
15. Bertrand R, Solary E, O' Connor P, Kohn KW, Pommier Y. Induction of a common pathway of apoptosis by staurosporine. Exp Cell Res. 1994;211:314–21.
16. Hegde NS, Sanders DA, Rodriguez R, Balasubramanian S. The transcription factor FOXM1 is a cellular target of the natural product thiostrepton. Nat Chem. 2011;3:725–31.
17. Gause GF, Preobrazhenskaya TP, Sveshnikova GV, Terekhova LP, Maksimova TS. A guide for determination of actinomycetes. Moscow: Nauka (in Russian); 1983.