Asenjonamides A–C, antibacterial metabolites isolated from *Streptomyces asenjonii* strain KNN 42.f from an extreme-hyper arid Atacama Desert soil

Running head: Asenjonamides A-C from *Streptomyces asenjonii*

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Bio-guided fractionation of the culture broth extract of *Streptomyces asenjonii* strain KNN 42.f recovered from an extreme hyper-arid Atacama Desert soil in northern Chile led to the isolation of three new bioactive β-diketones; asenjonamides A–C (**1**-**3**) in addition to the known N-(2-(1*H*-indol-3-yl)-2-oxoethyl)acetamide (**4**), a series of bioactive acylated 4-aminoheptosyl-β-N-glycosides; spicamycins A–E (**5**-**9**), and seven known diketopiperazines (**10**-**16**). All isolated compounds were characterized by HRESIMS and NMR analyses and tested for their antibacterial effect against a panel of bacteria.

INTRODUCTION

Natural products are considered a valuable resource for drug discovery due to their diverse chemical scaffolds which cannot be matched by any synthetic libraries. However, the discovery of new and bioactive natural products is quite challenging due to the high re-isolation rate of known metabolites. One of the main strategies to address this problem is the isolation of new metabolites through the screening of novel microorganisms from neglected and underexplored habitats, particularly the extremobiosphere that includes desert biomes, the Antarctic, and the symbionts of insects.[1-3] The incorporation of rigorous dereplication procedures into all stages of the natural product discovery process is a critical step to achieve this goal.

One such neglected habitat is the Atacama Desert in northern Chile which is known for its extreme aridity. It has been arid over at least ~15 million years and is considered to be the oldest and driest nonpolar desert on Earth.[4] Some regions in the desert were described to feature “Mars-like” soils that were deemed too extreme for life to exist owing to extreme aridity, high levels of UV radiation, the presence of inorganic oxidants, areas of high salinity,

and very low concentrations of organic carbon.[5] However, recent surveys indicated the presence of diverse culturable bacteria in the Atacama Desert.[6,7] The successful incorporation of taxonomic information into the drug discovery process [8] proved effective in the isolation of novel filamentous actinobacteria from the desert among which the novel anti-HIV-1 lentzeosides A–F were discovered.[9] Additionally, bio-guided and genome-guided screening of representatives of these actinobacteria led to the isolation of new bioactive metabolites belonging to diverse structural classes such as the antimicrobial chaxamycins[10] and chaxalactins[11] from *Streptomyces leeuwenhoekii* C34^T,[12] the abenquines from *Streptomyces* sp. DB634,[13] the antitumor atacamycins from *Streptomyces leeuwenhoekii* C38,[14] and the cell invasion inhibitor chaxapeptin from *S. leeuwenhoekii* strain C58.[15] More recently, co-cultivation of *S. leeuwenhoekii* with an *Aspergillus* isolate similarly led to the synthesis of new luteride and pseurotin derivatives.[16]

As part of our ongoing program to investigate the Atacama extremobiosphere as a source of new bioactive natural products, we have focused our attention on *Streptomyces asenjonii* strain KNN 42.f which showed strong antibacterial effects and specific UV and ^{1}H NMR pattern of secondary metabolites obtained from LCMS profile and associated NMR data. Bioactivity-guided screening of the strain led to the isolation of three new active metabolites belonging to the β-diketone family of polyketides in addition to thirteen known metabolites including the structurally unique antitumor antibiotic spicamycins, featuring different fatty acid residues, glycine, unusual amino sugars, and adenine units. Structure elucidation of these compounds was based on HRESIMS, 1D and 2D NMR analyses. The isolated compounds were screened for their antibacterial activity against a panel of bacteria.

RESULTS

When screened against a panel of bacterial isolates, only *Streptomyces asenjonii* strain KNN 42.f out of a collection of 10 different Atacama Desert-derived actinobacteria exhibited strong antibacterial effects against Gram positive and Gram negative target microorganisms. Bioactivity-guided fractionation of a large scale fermentation broth of this strain revealed the CH_2Cl_2 and EtOAc fractions to be the most active. Subjecting these fractions to multiple steps of medium and high pressure preparative C-18 chromatography resulted in the isolation of three new and thirteen known natural products based on HRESIMS and NMR data (Figure 1).

Compound **(1)** was obtained as a white amorphous powder. Its molecular formula $C_{13}H_{23}NO_3$ was determined by analysis of its HRESIMS quasi-molecular ion peak at *m/z* 264.1565 $[M+Na]^+$, indicating three degrees of unsaturation. The analysis of ^{1}H, ^{13}C (Table 1) and multiplicity-edited HSQC NMR spectra revealed the presence of one methyl triplet (δ_C/δ_H 13.6/0.88, C-9), one methyl doublet (δ_C/δ_H 14.2/1.12, C-11), one methyl singlet (δ_C/δ_H 11.4/1.68, C-10), five methylenes of which one was oxygenated (δ_C/δ_H 59.5/3.36, C-2'), one aliphatic methine (δ_C/δ_H 47.0/4.08, C-2), one olefinic methine (δ_C/δ_H 142.6/6.79, C-5) and three quaternary carbons, two of which were assigned to an amide carbonyl (δ_C 170.7, C-1) and an α,β-unsaturated keto carbonyl (δ_C 197.4, C-3), respectively. The COSY spectrum revealed distinct spin systems, comprising the one of the olefinic H-5 through H_3-9 consistent with a hexenyl moiety, another one of the NH through H_2-2' which indicated a hydroxyethylamino moiety in compound **(1)** (Figure 2). The HMBC correlations H_3-11 to C-1, C-2 and C-3 located this methyl doublet between 2 carbonyl moieties, while the

correlations of H_3-10 to C-5, C-4 and C-3 connected the hexenyl moiety to the C-3 ketone (Figure 2). The HMBC correlations of NH and H_2-1' to C-1 confirmed the attachment of the hydroxyethylamino moiety to the C-1 amide. NOESY correlations between H_3-10 and H_2-6 established the *E* configuration for the double bond in the hexenyl moiety. The only compound close to our β-diketone was siphonarienedione which was reported naturally[17] and through stereoselective total synthesis.[18] Although the close similarity of siphonarienedione ^{13}CNMR data, coupling patterns and optical rotation data to (**1**), it could not be used to assign the stereochemistry as siphonarienedione has four additional stereocentres. Based on these findings, the structure of (**1**) was established as depicted, representing a new natural product for which we propose the name asenjonamide A.

Compound (**2**) was obtained as a white amorphous powder, its molecular formula $C_{11}H_{19}NO_2$ was derived from HRESIMS analysis of its quasi-molecular ion peak at *m/z* 220.1304 $[M+Na]^+$, consistent with three degrees of unsaturation. The ^{1}H and ^{13}C NMR spectral data of (**2**) (Table 1) in addition to the NOESY correlations were almost identical to those of **1**, with the exception of the absence of resonances for the hydroxyethyl moiety which was supported by the molecular weight of (**2**) being 44 amu less than that of (**1**). This unambiguously led to the elucidation of the structure of (**2**) as shown in Figure 1 as a new natural product for which the name asenjonamide B is proposed.

Compound (**3**) was obtained as a white amorphous powder, and its molecular formula was assigned as $C_{11}H_{17}NO_2$ based on HRESIMS analysis of its quasi-molecular ion peak at *m/z* 196.1330 $[M+H]^+$ which indicated four degrees of unsaturation. The close similarity of this molecular formula to (**2**), with only 2 amu less and the absence of some proton resonances in the ^{1}H NMR indicated the same chemical class with one extra ring in the structure of (**3**).

The COSY correlations of H_2-6 through H_3-9 confirmed the *n*-butyl side chain (Figure 2). The HMBC correlations of H_3-11 to C-1, C-2 and C-3 and of H_3-10 to C-3, C-4 and C-5 established the 2-amino-2,4-dimethylcyclopent-4-ene-1,3-dione moiety. The HMBC correlations of H_2-6 to C-1, C-5 and C-4 confirmed the connectivity of the aliphatic chain to C-5. Attempts to apply Mosher's ester method failed and the compound decomposed. On that basis, compound **(3)** is considered a new natural product for which we propose the name asenjonamide C.

Compound **(4)** was identified as *N*-(2-(1*H*-indol-3-yl)-2-oxoethyl)acetamide based on comparing its accurate mass and NMR spectra with literature data.[19,20] Chemical screening of the EtOAc extract led to the isolation of a series of five acylated 4-aminoheptosyl-β-*N*-glycosides, spicamycins A–E **(5-9)** featuring an adenine base, an unusual amino sugar, and aliphatic side chains of 8-12 CH_2 groups ending in an isopropyl moiety. The structures of these compounds were elucidated by direct comparison of their HRESIMS and NMR spectroscopic data with literature data.[19] Their HRESIMS analysis (see SI) showed a characteristic pattern with quasi-molecular ion peak at *m/z* 566.3320 $[M+H]^+$ establishing the molecular formula $C_{26}H_{43}N_7O_7$ which was assigned for spicamycin A. Subsequent increases of 14 amu in the molecular ions corresponded to additional CH_2 groups giving spicamycins B–E. This was supported by ^{1}H NMR spectra which were virtually identical to those previously reported (See SI).[21] Their structure was confirmed through the first total synthesis of one of the spicamycin congeners, SPM VIII.[22] They were initially obtained as a non-separable mixture of seven compounds from the culture broth of *Streptomyces alanosinicus* 879-MT_3 and reported as potent differentiation inducer of HL-60 human promyelocytic leukemia cells.[21,23]

Finally, the isolated diketopiperazine compounds were identified based on comparing their accurate mass, NMR, and optical rotation data with literature as cyclo(L-Pro-L-Val) **(10)**,[24] cyclo(L-Pro-L-Phe) **(11)**,[24] cyclo(L-Pro-L-Tyr) **(12)**,[25] brevianamide F **(13)**,[26] cyclo(3-hydroxy-L-Pro-L-Leu) **(14)**,[27] cyclo(3-hydroxy-L-Pro-L-Phe) **(15)**,[28] and cyclo(3-hydroxy-L-Pro-L-Tyr) **(16)**.[29]

The preliminary bio-guided isolation revealed the CH_2Cl_2 and EtOAc fractions to possess antimicrobial effects (data not shown). Asenjonamides A–C **(1-3)**, isolated from the CH_2Cl_2 fractions, exhibited significant antibacterial effects against Gram-positive strains with asenjonamide C **(3)** showing activity comparable to that of the positive control tetracycline (Table 2). Additionally, **(3)** also exhibited strong activity against Gram-negative strains in relation to tetracycline and moderate effect against *M. smegmatis*. Moreover, spicamycins A–E (**5-9**) exhibited weak antibacterial effects against Gram-positive strains in the MIC range of 70-85 µg/mL but no effects against Gram-negative strains at the highest concentration used (100 µg/mL). On the other hand, compound (**5**) and all diketopiperazine compounds (**10-16**) didn't exhibit any antibacterial effects against all tested strains at the highest concentration used (100 µg/mL, data not shown).

DISCUSSION

The Atacama Desert is considered to be the oldest and driest nonpolar desert on Earth, being arid since the Jurassic period and developing to hyper-aridity during the Miocene period.[6] Initially, the hyper-arid core of the Atacama Desert was considered by some to be too extreme for microbial life to exist,[4] but subsequent investigations led to the recovery of diverse cultivable microorganisms from this harsh environment indicating that it could

158 provide another unexpected resource of microbiological diversity. The discovery that a
159 representative of a recently described *Streptomyces* species isolated from an extreme hyper-
160 arid Atacama Desert soil synthesizes sixteen specialized metabolites that belong to different
161 chemical classes underlines the premise that extreme environmental conditions give rise to a
162 unique actinobacterial diversity which is the basis of novel chemistry.[7] Indeed, to date, our
163 taxonomic approach to the detection of new natural products from novel filamentous
164 actinobacteria has led to the discovery of about 50 specialized metabolites representing
165 diverse chemical classes , including alkaloids, peptides, polyketides, macrolides and terpenes
166 that exhibit a range of biological activities.[9-16] Most of these new compounds have been
167 isolated from novel streptomycetes, notably ones, like *S. asenjonii* and *S. leeuwenhoekii*, that
168 form deep rooted subclades in single and concatenated *Streptomyces* gene trees.[15,30] Since
169 our project began, numerous streptomycetes have been obtained from the complete range of
170 hyper-arid and extreme hyper-arid habitats, six of which have been taxonomically
171 characterized.[31] Such novel filamentous actinobacteria known to be present in the Atacama
172 Desert landscape are a feature of an immense untapped resource for the search and discovery
173 of the new generation of antibiotics needed for healthcare.[7,20]

174 In the current study, strong antibacterial activity was the driving force for the selection of *S.*
175 *asenjonii* isolate KNN 42.f. Chromatographic separation and spectroscopic identification of
176 active CH_2Cl_2 fractions led to the identification of asenjonamides A–C, new members of β-
177 diketone subclass of polyketides which exhibited a broad antibacterial effect against a panel
178 of different Gram-positive and Gram-negative bacteria with asenjonamide C showing a
179 comparable effect to that of the positive control, tetracycline. Based on inspection of their
180 structures, the biosynthesis of these polyketides may be similar to that of the non-peptide

part of calcaripeptide A[32] isolated from *Calcarisporium* sp. strain KF525 or the anti-HIV inhibitor aetheramide A[33] from *Aetherobacter* sp. strain SBSr003.

Despite the revolutionary effects of environmental metagenomics on revealing microbial diversity, there remain powerful reasons for isolating and observing the behavior of organisms in culture. Recently, a spectacular diversity of actinobacteria has been detected and described in both low and very high altitude habitats of the Atacama region that include a putative new sub-order, and several new classes, families and numerous genera.[30] The presence of such actinobacterial *dark matter* strongly supports the view that the extremobiosphere is a prime landscape for bioprospecting activities. However, while mining such metagenomic resources for novel natural products is a legitimate route for discovery, continued efforts to bring these rare and dark phylotypes into laboratory culture should not be neglected. Furthermore, culture-based studies also allow to carry out co-cultivation experiments, and we have successfully adopted this approach to include Atacama Desert microorganisms whereby many compounds were observed only in co-cultures of actinobacteria and fungi, but not in axenic cultures of the fungus or bacterium.[16,34]

Experimental

General experimental procedures. Optical rotations were measured in methanol on a Perkin Elmer 241 instrument at the sodium D line (589 nm). ^{1}H and ^{13}C NMR spectra were recorded at 25 °C with a Varian VNMRS 600 MHz NMR spectrometer. High-resolution mass spectra were acquired with a Thermo Scientific LTQ/XL Orbitrap using the following parameters: analyzer: FTMS, mass range: normal full ms 100-2000, resolution: 30,000. For LC-ESIMS, gradient separation was achieved using a Sun Fire C-18 analytical HPLC

column (5 µm, 4.6 × 150 mm, Waters) with a mobile phase of 0-100% MeOH over 30 min at a flow rate of 1 mL/min. HPLC was performed on Agilent 1260 Infinity preparative HPLC system with an Agilent Eclipse XDB-C18 column (5 µm, 10 × 250 mm, Agilent technologies, USA) monitored using an Agilent photodiode array detector. Detection was carried out at 220, 254, 280, 350, and 400 nm. MPLC separations were carried out on Biotage system using reversed-phase pre-packed columns. Detection was carried out at 220 and 280 nm. Diaion HP-20 was obtained from Resindion S.R.L., a subsidiary of Mitsubishi Chemical Co., Binasco, Italy.

Microorganism isolation and identification. *Streptomyces asenjonii* strain KNN 42.f was recovered from a plate of Gauze's No.1 agar[35] following inoculation with a suspension of an extreme hyper-arid soil collected by ATB in 2010 from the Yungay core region of the Atacama Desert (24°06'18.6"S,70°01'55.6"W at 1016 m asl).[30] Phylogenetic analysis of KNN 42.f and other isolates recovered from the same region was performed through 16S rRNA gene sequencing and showed that these strains were belonging to new species within the genus Streptomyces, and KNN 42.f was identified as *Streptomyces asenjonii* KNN 42.f and deposited in the NRRL public service collection under the accession number NRRL B-65049.[30]

Microbial fermentation, extraction and isolation. *Streptomyces asenjonii* strain KNN 42.f was fermented on modified ISP2 medium comprising malt extract (4.0 g), yeast extract (10.0 g), dextrose (10.0 g), glycerol (10.0 g) and distilled water to 1 L, pH 7.0. It was grown at a volume of 4 L by shaking at 180 rpm in an incubator shaker at 30 °C for 7 days when HP-20 resin beads were added, followed by shaking at 180 rpm for 6 h before harvest. The

harvested fermentation broth was centrifuged at 3000 rpm for 20 min, and the HP20 was washed with distilled water and then extracted with methanol (4 × 200 mL). The successive MeOH extracts were combined and concentrated *in vacuo* yielding 2.3 g of residue. The latter was suspended in distilled water (300 mL) and then successively partitioned between *n*-hexane (300 mL × 3), CH_2Cl_2 (300 mL × 3) and EtOAc (300 mL × 3). Each fraction was concentrated under reduced pressure to give *n*-hexane extract (390 mg), CH_2Cl_2 extract (310 mg), and EtOAc extract (260 mg), respectively. The CH_2Cl_2 fraction was subjected to flash chromatography on a Biotage system using a prepacked RP-18 column and a MeOH/H_2O gradient to give 5 subtractions. Sub-fraction 1 was subjected to semi-preparative HPLC using MeCN–H_2O (35–100% over 30 min, 100% for 5 min) at 2 mL/min flow rate affording compounds **10-16**. Sub-fraction 2 afforded compounds **3** (1.3 mg) and **4** (2.1 mg), while sub-fraction 3 afforded compounds **1** (1.0 mg) and **2** (5.9 mg) under the same HPLC conditions. The EtOAc fraction was subjected to flash chromatography on the Biotage system using prepacked RP-18 column chromatography using MeOH/H_2O gradient to give 4 subtractions. Sub-fraction 2 was subjected to semi-preparative HPLC and a MeCN–H_2O (15–100% over 30 min, 100% for 5 min) at a flow rate of 2 mL/min to afford compounds **5** (11 mg), **6** (3 mg), **7** (7 mg), **8** (8 mg), and **9** (4.5 mg).

Asenjonamide A (**1**). White amorphous powder; $[\alpha]^{20}_D$ +6.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) at 230 (3.8), 256 (2.5) nm; HRESIMS *m*/*z* $[M+Na]^+$ 264.1565 indicating the molecular formula $C_{13}H_{19}NO_3$ (calculated $[M+Na]^+$ ion at m/z 264.1570); NMR data: see Table 1.

Asenjonamide B (**2**). White amorphous powder; $[\alpha]^{20}_D$ +6.9 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) at 230 (3.7), 256 (2.6) nm; HRESIMS *m*/*z* $[M+Na]^+$ 220.1304 indicating the

248 molecular formula $C_{11}H_{17}NO_2$ (calculated $[M+Na]^+$ ion at m/z 220.1308); NMR data: see
249 Table 1.
250 Asenjonamide C (**3**). White amorphous powder; $[\alpha]^{20}_D$ +6.9 (*c* 0.15, MeOH); UV (MeOH)
251 λ_{max} (log ε) at 232 (3.6), 258 (2.7) nm; HRESIMS *m/z* $[M+H]^+$ 196.1330 indicating the
252 molecular formula $C_{11}H_{17}NO_2$ (calculated $[M+H]^+$ ion at m/z 196.1332); NMR data: see
253 Table 1.
254 *Antibacterial screening*. The antibacterial activity of all of the compounds was evaluated
255 against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 2116, *Escherichia coli*
256 ATCC 25922, *Enterococcus faecalis* ATCC 10541, and the acid fast strain *Mycobacterium*
257 *smegmatis* ATCC607, using the agar diffusion method and regression line analysis.[36] Filter
258 paper disks containing amoxicillin (10 μg) and tetracycline (30 μg) were used as positive
259 controls. Minimum inhibitory concentrations (MICs) against the panel of strains were
260 calculated using the method described before albeit with minor modifications.[37] In brief,
261 tested strains were grown in Müller-Hinton (MH) broth to early stationary phase and then
262 diluted to an OD600 = 0.005. The assays were performed in a 96-well microtiter plate format
263 in duplicate, with two independent cultures for each strain. All of the compounds were
264 dissolved in DMSO (Sigma) and added to the cultures in wells to give a final concentration
265 of DMSO of 10% that did not affect the growth of any of the tested strains. The effect of
266 different dilutions of the compounds (up to 100 μg/mL) on growth was assessed after 18 h
267 incubation at 37 °C using a Labsystems iEMS MF plate reader at OD_{620}. The MIC value was
268 determined as the lowest concentration showing no growth compared to the MH control.

269 **CONFLICT OF INTEREST**

270 The authors declare no conflict of interest.

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REFERENCES

1. Pidot, S. J., Coyne, S., Kloss, F., Hertweck, C. Antibiotics from neglected bacterial sources. *Int. J. Med. Microbiol.* **304**, 14−22 (2014).

2. Horikoshi, K. Antranikian, G., Bull, A. T., Robb, F. T., Stetter, K.O. (Eds.) Extremophiles Handbook, Springer: Tokyo (2011).

3. Guo, X., Liu, N., Li, X., Ding, Y., Shang, F., Gao, Y., Ruan, J., Huang, Y. Red soils harbor diverse culturable actinomycetes that are promising sources of novel secondary metabolites. *Appl. Environ. Microbiol*. **81**, 3086–3103 (2015).

4. Gómez-Silva, B., Rainey, F. A., Warren-Rhodes, K. A., McKay, C. P., Navarro-González, R. Atacama Desert soil microbiology. In Microbiology of Extreme Soils, Soil Biology; Dion, P., Nautiyal, C. S., Eds.; Springer: Berlin, **13**, 117−132 (2008).

5. Navarro-González, R. et al. Mars-Like soils in the Atacama Desert, Chile, and the dry limit of microbial Life. *Science* **302**, 1018−1021 (2003).

6. Crits-Christoph, A. et al. Colonization patterns of soil microbial communities in the Atacama Desert. *Microbiome* **1**:28. DOI:10.1186/2049-2618-1-28 (2013).

7. Bull, A. T., Asenjo, J. A., Goodfellow, M., Go´mez-Silva, B. The Atacama Desert: technical resources and the growing importance of novel microbial diversity. *Ann. Rev. Microbiol*. **70**, 215–234 (2016).

8. Goodfellow, M., Fiedler, H. P. A guide to successful bioprospecting: informed by actinobacterial systematics. *Antonie van Leeuwenhoek* **98**, 119–142 (2010)

9. Wichner, D., et al. Isolation and anti-HIV-1 integrase activity of lentzeosides A–F from extremotolerant *lentzea* sp. H45, a strain isolated from a high-altitude Atacama Desert soil. *J. Antibiot*. **70**, 448-453 (2017).

10. Rateb, M. E. *et al*. Chaxamycins A-D, bioactive ansamycins from a hyper-arid desert *Streptomyces* sp. *J. Nat. Prod*. **74**, 1491−1499 (2011).

11. Rateb, M. E. *et al*. Diverse metabolic profiles of a *Streptomyces* strain isolated from a hyper-arid environment. *J. Nat. Prod*. **74**, 1965−1971 (2011).

12. Busarakam, K. *et al*. *Streptomyces leeuwenhoekii* sp. nov., the producer of chaxalactins and chaxamycins, forms a distinct branch in *Streptomyces* gene trees. *Antonie van Leeuwenhoek* **105**, 849−861 (2014).

13. Schulz, D. *et al*. Abenquines A–D: aminoquinone derivatives produced by *Streptomyces* sp. strain DB634. *J. Antibiot*. **64**, 763−768 (2011).

14. Nachtigall, J. *et al*. Atacamycins A–C, 22-membered antitumor macrolactones produced by *Streptomyces* sp. C38. *J. Antibiot*, **64**, 775−780 (2011).

15. Elsayed, S. S. *et al*. Chaxapeptin, a lasso peptide from extremotolerant *Streptomyces leeuwenhoekii* strain C58 from the hyper-arid Atacama Desert. *J. Org. Chem*. **80**, 10252−10260 (2015).

16. Wakefield, J., Hassan, H. M., Jaspars, M., Ebel, R., Rateb, M. E. Dual induction of new microbial secondary metabolites by fungal bacterial co-cultivation. *Front. Microbiol*. **8**:1284 (2017) doi: 10.3389/fmicb.2017.01284.

17. Norte, M., Cataldo, F., González, A. G. Siphonarienedione and siphonarienolone, two new metabolites from *Siphonaria grisea* having a polypropionate skeleton. *Tetrahedron Lett*. **29**, 2879−2880 (1988).

18. Calter, M. A. and Liao, W. First total synthesis of a natural product containing a chiral, β-diketone: Synthesis and stereochemical reassignment of siphonarienedione and siphonarienolone. *J. Am. Chem. Soc*. **124**, 13127−13129 (2002).

19. Iakovou, K., et al. Design, synthesis and biological evaluation of novel b-substituted indol-3-yl ethylamido melatoninergic analogues. *J. Pharm. Pharmacol*., **54**, 147−156 (2002).

20. Jian, Y., Nan, W., Hai-Sheng, Y., Jiang-Chun, H., Yu-Cheng, D. A new Sesquiterpene from the medicinal fungus *Inonotus vaninii*. *Chem. Nat. Comp*. **49**, 261−263 (2013).

21. Hayakawa, Y., et al. Studies on the differentiation inducers of myeloid leukemic cells III. Spicamycin, a new inducer of differentiation of HL-60 human promyelocytic leukemia cells. *J. Antibiot*. **36**, 934−937 (1983).

22. Suzuki, T., Suzuki, S. T., Yamada, I., Koashi, Y., Yamada, K., Chida, N. Total synthesis of spicamycin. *J. Org. Chem*. **67**, 2874−2880 (2002).

23. Hayakawa, Y., et al. Spicamycin, a new differentiation inducer of mouse myeloid leukemia cells Ml and human promyelocytic leukemia cells HL-60. *Agric. Biol. Chem.* **49**, 2685−2691 (1985).

24. Chen, M.; Dewis K.; Kraut, K.; Merritt, D.; Reiber L., Trinnaman, L.; Da Costa, N. 2,5-Diketopiperazines (Cyclic Dipeptides) in Beef: Identification, synthesis, and sensory evaluation *J. Food Scienc*. **74**, 100−105 (2009).

25. Amira, R.; Yoel, K.; Yehuda, B.; Michael, S. Amino acid derivatives from the marine sponge *Jaspis digonoxea*. *J. Nat. Prod*. **57**, 829−832 (1994).

26. Muhanna, M.; Juriyati, J.; Nik, M.; Ruangelie, E.; Noraziah, M. Isolation and characterization of cyclo-(tryptophanyl-prolyl) and chloramphenicol from *Streptomyces* sp. SUK 25 with antimethicillin-resistant *S. aureus* activity. *Drug Design Develop Ther*. **10**, 1817–1827 (2016).

27. Bin, L.; Gang, C.; Jiao, B.; Yong-Kui, J.; Yue-Hu P. A bisamide and four diketopiperazines from a marine-derived *Streptomyces* sp. *J. Asian. Nat. Prod. Res*. **13**, 1146–1150 (2011).

28. Ström, K., Sjögren, J., Broberg, A. and Schnürer, J. *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid. *Appl. Environ. Microbiol*. **68**, 4322–4327 (2002).

29. Mitova, M., Giuseppina, T., Ute, H., Mueller, W. E. G., Salvatore, D-R. Exocellular cyclic dipeptides from a Ruegeria strain associated with cell cultures of *Suberites domuncula*. *Mar. Biotechnol*. **6**, 95–103 (2004).

30. Goodfellow, M., et al. *Streptomyces asenjonii* sp. nov., isolated from hyper-arid Atacama Desert soils and emended description of *Streptomyces viridosporus* Pridham et al. 1958. *Antonie van Leeuwenhoek* **110**, 1133–1148 (2017).

31. Idris, H., Goodfellow, M., Sanderson, R., Asenjo, J. A., Bull. A. T. Actinobacterial rare biospheres and dark matter revealed in habitats of the Chilean Atacama Desert. *Sci. Rep*. **7**, Article number: 8373, doi: 10.1038/s41598-017-08937-4 (2017).

32. Silber, J., Ohlendorf, B., Labes, A., Näther, C., Imhoff, J. F. Calcaripeptides A–C, cyclodepsipeptides from a *Calcarisporium* Strain. *J. Nat. Prod*. **76**, 1461–1467 (2013).

33. Plaza, A. et al., Aetheramides A and B, potent HIV-Inhibitory depsipeptides from a Myxobacterium of the new Genus "*Aetherobacter*" *Org. Lett*. **14**, 2854–2857 (2012).

34. Rateb, M. E., et al. Induction of diverse secondary metabolites in *Aspergillus fumigatus* by microbial co-culture. *RSC Adv*. **3**, 14444−14450 (2013).

35. Zakharova, O. S., Zenova, G. M., Zvyagintsey, D. G. Some approaches to the selective isolation of actinomycetes of the genus *Actinomadura* from soil. *Microbiology* **72**, 110–113 (2003).

36. Zhang, D.; Noviendri, D.; Nursid, M.; Yang, X.; Son, B. W. 12,13-dihydroxyfumitremorgin C, fumitremorgin C, and brevianamide F, antibacterial diketopiperazine alkaloids from the marine-derived fungus *Pseudallescheria* sp. *Nat. Prod. Sci*. **13**, 251–254 (2007).

37. Kronvall, G. Single-strain regression analysis for determination of interpretive breakpoints for cefoperazone disk diffusion susceptibility testing. *J. Clin. Microbiol*. **17**, 975-980 (1983).

Figure legends

Figure 1. Structures of the compounds isolated from *S. asenjonii* strain KNN 42.f.

Figure 2. Key COSY (—), HMBC (⌒) and NOESY (⌒) correlations of compounds **1** and **3**.

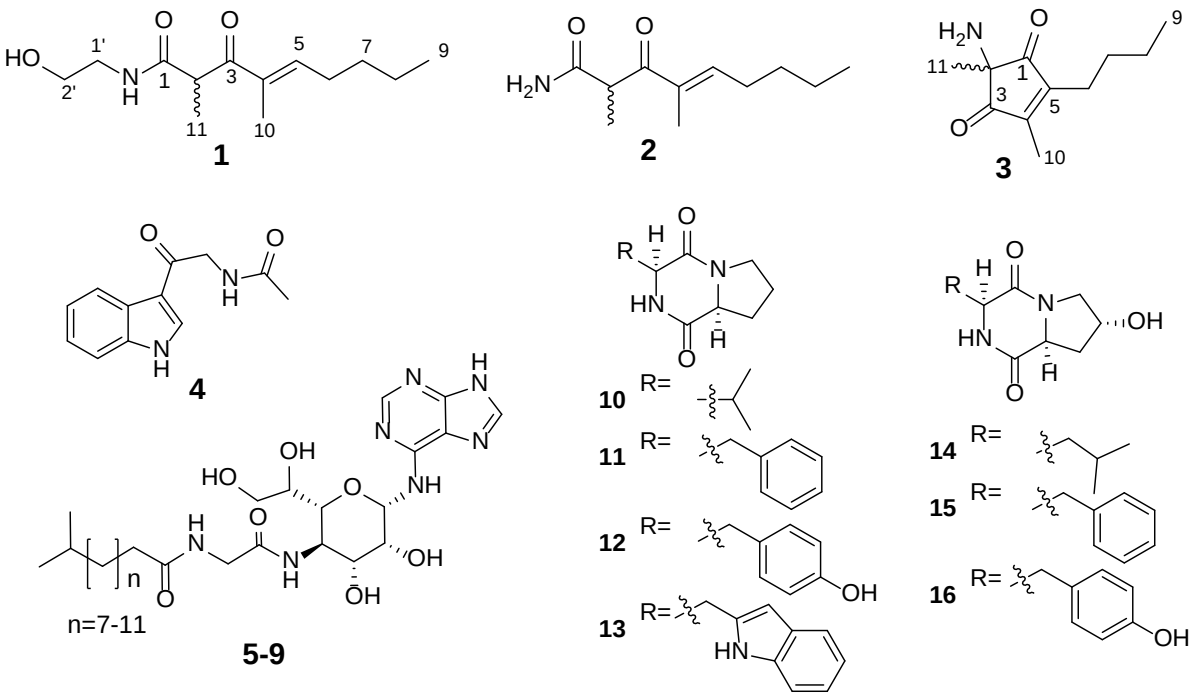

Figure 1. Structures of the compounds isolated from *S. asenjonii* strain KNN 42.f.

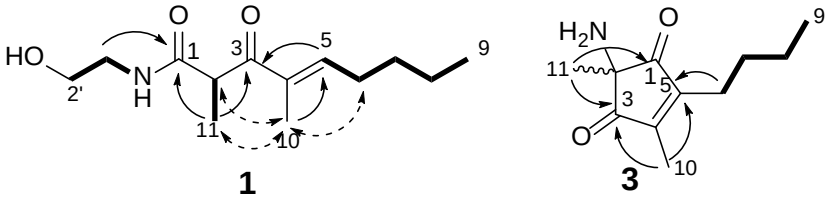

Figure 2. Key COSY (—), HMBC ( ) and NOESY ( ) correlations of compounds **1** and **3**.

385 **Table 1**. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data of **1**-**3** (298 K, DMSO-

386 d_6).

	1		**2**		**3**	
No.	δ_C, mult.*	δ_H (mult, *J* in Hz)	δ_C, mult.	δ_H (mult, *J* in Hz)	δ_C, mult.*	δ_H (mult, *J* in Hz)
1	170.7, C	-	172.6, C	-	203.8, C	-
2	47.0, CH	4.09 (q)	47.0, CH	4.08 (q)	69.9, C	-
3	197.4, C	-	197.6, C	-	203.8, C	-
4	135.5, C	-	135.6, C	-	156.0, C	-
5	142.6, CH	6.76 (t, 7.1)	142.7, CH	6.78 t (7.1)	152.7, C	-
6	28.0, CH_2	2.20 (q)	28.1, CH_2	2.21 (q)	23.1, CH_2	2.41 (t, 7.6)
7	30.0, CH_2	1.40 (m)	30.2, CH_2	1.41 (m)	29.1, CH_2	1.41 (m)
8	21.9, CH_2	1.30 (m)	21.8, CH_2	1.32 (m)	22.1, CH_2	1.29 (m)
9	13.7, CH_3	0.88 (t, 7.3)	13.8, CH_3	0.89 (t, 7.3)	13.7, CH_3	0.88 (t, 7.3)
10	11.4, CH_3	1.66 (s)	11.5, CH_3	1.67 (s)	9.1, CH_3	1.96 (s)
11	14.2, CH_3	1.12 (d, 7.0)	14.3, CH_3	1.13 (d, 7.0)	20.0, CH_3	1.14 (s)
1'	41.2, CH_2	3.08 (m)	-	-	-	-
2'	59.5, CH_2	3.36 (m)	-	-	-	-
NH_2		-	-	6.95 (bs)	-	5.98 (bs)
NH		8.11 (bs)	-	-	-	-

387 *^{13}C assignments were based on HSQC and HMBC spectra.

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Table 2. Antibacterial Activity of compounds **1-3** and **5-9**.

Compound	Average MIC (μg/mL)[a]				
	S. aureus	*B. subtilis*	*E. coli*	*E. faecalis*	*M. smegmatis*
1	3.6	3.9	16.8	12.2	18.6
2	3.1	3.3	17.3	13.7	19.1
3	1.8	1.7	5.4	3.9	10.3
5	77.0	72.0	>100	>100	>100
6	72.0	68.0	>100	>100	>100
7	74.0	69.0	>100	>100	>100
8	79.0	75.0	>100	>100	>100
9	84.0	77.0	>100	>100	>100
Tetracycline	1.5	1.2	4.1	2.9	3.8
Amoxicillin	0.05	0.03	0.8	0.3	0.9

[a] average of two independent replicates.