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

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

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19 **Key words:** Asenjonamides A–C, antibacterial, β -diketone, spicamycins, *Streptomyces*
20 *asenjonii*, Atacama Desert.

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22

23 Bio-guided fractionation of the culture broth extract of *Streptomyces asenjonii* strain KNN
24 42.f recovered from an extreme hyper-arid Atacama Desert soil in northern Chile led to the
25 isolation of three new bioactive β -diketones; asenjonamides A–C (**1-3**) in addition to the
26 known N-(2-(1*H*-indol-3-yl)-2-oxoethyl)acetamide (**4**), a series of bioactive acylated 4-
27 aminoheptosyl- β -N-glycosides; spicamycins A–E (**5-9**), and seven known diketopiperazines
28 (**10-16**). All isolated compounds were characterized by HRESIMS and NMR analyses and
29 tested for their antibacterial effect against a panel of bacteria.

30 INTRODUCTION

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

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

45 and very low concentrations of organic carbon.⁵ However, recent surveys indicated the
46 presence of diverse culturable bacteria in the Atacama Desert.^{6,7} The successful
47 incorporation of taxonomic information into the drug discovery process⁸ proved effective in
48 the isolation of novel filamentous actinobacteria from the desert among which the novel anti-
49 HIV-1 lentzeosides A–F were discovered.⁹ Additionally, bio-guided and genome-guided
50 screening of representatives of these actinobacteria led to the isolation of new bioactive
51 metabolites belonging to diverse structural classes such as the antimicrobial chaxamycins¹⁰
52 and chaxalactins¹¹ from *Streptomyces leeuwenhoekii* C34^{T,12} the abenquines from
53 *Streptomyces* sp. DB634,¹³ the antitumor atacamycins from *Streptomyces leeuwenhoekii*
54 C38,¹⁴ and the cell invasion inhibitor chaxapeptin from *S. leeuwenhoekii* strain C58.¹⁵ More
55 recently, co-cultivation of *S. leeuwenhoekii* with an *Aspergillus* isolate similarly led to the
56 synthesis of new luteride and pseurotin derivatives.¹⁶

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

67 RESULTS

68 When screened against a panel of bacterial isolates, only *Streptomyces asenjonii* strain KNN
69 42.f out of a collection of 10 different Atacama Desert-derived actinobacteria exhibited
70 strong antibacterial effects against Gram positive and Gram negative target microorganisms.
71 Bioactivity-guided fractionation of a large scale fermentation broth of this strain revealed the
72 CH₂Cl₂ and EtOAc fractions to be the most active. Subjecting these fractions to multiple
73 steps of medium and high pressure preparative C-18 chromatography resulted in the isolation
74 of three new and thirteen known natural products based on HRESIMS and NMR data (Figure
75 1).

76 Compound **(1)** was obtained as a white amorphous powder. Its molecular formula
77 C₁₃H₂₃NO₃ was determined by analysis of its HRESIMS quasi-molecular ion peak at *m/z*
78 264.1565 [M+Na]⁺, indicating three degrees of unsaturation. The analysis of ¹H, ¹³C (Table
79 1) and multiplicity-edited HSQC NMR spectra revealed the presence of one methyl triplet
80 (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
81 11.4/1.68, C-10), five methylenes of which one was oxygenated (δ_C/δ_H 59.5/3.36, C-2'), one
82 **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
83 three quaternary carbons, two of which were assigned to an amide carbonyl (δ_C 170.7, C-1)
84 and an α,β -unsaturated keto carbonyl (δ_C 197.4, C-3), respectively. The COSY spectrum
85 revealed distinct spin systems, comprising the one of the olefinic H-5 through H₃-9
86 consistent with a hexenyl moiety, another one of the NH through H₂-2' which indicated a
87 **hydroxyethylamino** moiety in compound **(1)** (Figure 2). The HMBC correlations H₃-11 to C-
88 1, C-2 and C-3 located this methyl doublet between 2 carbonyl moieties, while the

89 correlations of H₃-10 to C-5, C-4 and C-3 connected the hexenyl moiety to the C-3 ketone
90 (Figure 2). The HMBC correlations of NH and H₂-1' to C-1 confirmed the attachment of the
91 **hydroxyethylamino** moiety to the C-1 amide. NOESY correlations between H₃-10 and H₂-6
92 established the *E* configuration for the double bond in the hexenyl moiety. **The only**
93 **compound close to our β-diketone was siphonarienedione which was reported naturally¹⁷ and**
94 **through stereoselective total synthesis.¹⁸ Although the close similarity of siphonarienedione**
95 **¹³CNMR data, coupling patterns and optical rotation data to (1), it could not be used to**
96 **assign the stereochemistry as siphonarienedione has four additional stereocentres.** Based on
97 these findings, the structure of **(1)** was established as depicted, representing a new natural
98 product for which we propose the name asenjonamide A.

99 Compound **(2)** was obtained as a white amorphous powder, its molecular formula
100 C₁₁H₁₉NO₂ was derived from HRESIMS analysis of its quasi-molecular ion peak at *m/z*
101 220.1304 [M+Na]⁺, consistent with three degrees of unsaturation. The ¹H and ¹³C NMR
102 spectral data of **(2)** (Table 1) in addition to the NOESY correlations were almost identical to
103 those of **1**, with the exception of the absence of resonances for the hydroxyethyl moiety
104 which was supported by the molecular weight of **(2)** being 44 amu less than that of **(1)**. This
105 unambiguously led to the elucidation of the structure of **(2)** as shown in Figure 1 as a new
106 natural product for which the name asenjonamide B is proposed.

107 Compound **(3)** was obtained as a white amorphous powder, and its molecular formula was
108 assigned as C₁₁H₁₇NO₂ based on HRESIMS analysis of its quasi-molecular ion peak at *m/z*
109 196.1330 [M+H]⁺ which indicated four degrees of unsaturation. The close similarity of this
110 molecular formula to **(2)**, with only 2 amu less and the absence of some proton resonances in
111 the ¹H NMR indicated the same chemical class with one extra ring in the structure of **(3)**.

112 The COSY correlations of H₂-6 through H₃-9 confirmed the *n*-butyl side chain (Figure 2).
113 The HMBC correlations of H₃-11 to C-1, C-2 and C-3 and of H₃-10 to C-3, C-4 and C-5
114 established the 2-amino-2,4-dimethylcyclopent-4-ene-1,3-dione moiety. The HMBC
115 correlations of H₂-6 to C-1, C-5 and C-4 confirmed the connectivity of the aliphatic chain to
116 C-5. Attempts to apply Mosher's ester method failed and the compound decomposed. On
117 that basis, compound (3) is considered a new natural product for which we propose the name
118 asenjonamide C.

119 Compound (4) was identified as *N*-(2-(1*H*-indol-3-yl)-2-oxoethyl)acetamide based on
120 comparing its accurate mass and NMR spectra with literature data.^{19,20} Chemical screening
121 of the EtOAc extract led to the isolation of a series of five acylated 4-aminoheptosyl-β-*N*-
122 glycosides, spicamycins A–E (5-9) featuring an adenine base, an unusual amino sugar, and
123 aliphatic side chains of 8-12 CH₂ groups ending in an isopropyl moiety. The structures of
124 these compounds were elucidated by direct comparison of their HRESIMS and NMR
125 spectroscopic data with literature data.¹⁹ Their HRESIMS analysis (see SI) showed a
126 characteristic pattern with quasi-molecular ion peak at *m/z* 566.3320 [M+H]⁺ establishing the
127 molecular formula C₂₆H₄₃N₇O₇ which was assigned for spicamycin A. Subsequent increases
128 of 14 amu in the molecular ions corresponded to additional CH₂ groups giving spicamycins
129 B–E. This was supported by ¹H NMR spectra which were virtually identical to those
130 previously reported (See SI).²¹ Their structure was confirmed through the first total synthesis
131 of one of the spicamycin congeners, SPM VIII.²² They were initially obtained as a non-
132 separable mixture of seven compounds from the culture broth of *Streptomyces alanosinicus*
133 879-MT₃ and reported as potent differentiation inducer of HL-60 human promyelocytic
134 leukemia cells.^{21,23}

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

140 The preliminary bio-guided isolation revealed the CH₂Cl₂ and EtOAc fractions to possess
141 antimicrobial effects (data not shown). Asenjonamides A–C **(1-3)**, isolated from the CH₂Cl₂
142 fractions, exhibited significant antibacterial effects against Gram-positive strains with
143 asenjonamide C **(3)** showing activity comparable to that of the positive control tetracycline
144 (Table 2). Additionally, **(3)** also exhibited strong activity against Gram-negative strains in
145 relation to tetracycline and moderate effect against *M. smegmatis*. Moreover, spicamycins
146 A–E **(5-9)** exhibited weak antibacterial effects against Gram-positive strains in the MIC
147 range of 70-85 µg/mL but no effects against Gram-negative strains at the highest
148 concentration used (100 µg/mL). On the other hand, compound **(5)** and all diketopiperazine
149 compounds **(10-16)** didn't exhibit any antibacterial effects against all tested strains at the
150 highest concentration used (100 µg/mL, data not shown).

151

152 DISCUSSION

153 The Atacama Desert is considered to be the oldest and driest nonpolar desert on Earth, being
154 arid since the Jurassic period and developing to hyper-aridity during the Miocene period.⁶
155 Initially, the hyper-arid core of the Atacama Desert was considered by some to be too
156 extreme for microbial life to exist,⁴ but subsequent investigations led to the recovery of
157 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 CH₂Cl₂ 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

181 part of calcaripeptide A³² isolated from *Calcarisporium* sp. strain KF525 or the anti-HIV
182 inhibitor aetheramide A³³ from *Aetherobacter* sp. strain SBSr003.

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

196 **Experimental**

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

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

211 *Microorganism isolation and identification.* *Streptomyces asenjonii* strain KNN 42.f was
212 recovered from a plate of Gauze's No.1 agar³⁵ following inoculation with a suspension of an
213 extreme hyper-arid soil collected by ATB in 2010 from the Yungay core region of the
214 Atacama Desert (24°06'18.6"S,70°01'55.6"W at 1016 m asl).³⁰ Phylogenetic analysis of
215 KNN 42.f and other isolates recovered from the same region was performed through 16S
216 rRNA gene sequencing and showed that these strains were belonging to new species within
217 the genus *Streptomyces*, and KNN 42.f was identified as *Streptomyces asenjonii* KNN 42.f
218 and deposited in the NRRL public service collection under the accession number NRRL B-
219 65049.³⁰

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

225 harvested fermentation broth was centrifuged at 3000 rpm for 20 min, and the HP20 was
226 washed with distilled water and then extracted with methanol (4×200 mL). The successive
227 MeOH extracts were combined and concentrated *in vacuo* yielding 2.3 g of residue. The
228 latter was suspended in distilled water (300 mL) and then successively partitioned between
229 *n*-hexane (300 mL \times 3), CH₂Cl₂ (300 mL \times 3) and EtOAc (300 mL \times 3). Each fraction was
230 concentrated under reduced pressure to give *n*-hexane extract (390 mg), CH₂Cl₂ extract (310
231 mg), and EtOAc extract (260 mg), respectively. The CH₂Cl₂ fraction was subjected to flash
232 chromatography on a Biotage system using a prepacked RP-18 column and a MeOH/H₂O
233 gradient to give 5 subfractions. Sub-fraction 1 was subjected to semi-preparative HPLC
234 using MeCN–H₂O (35–100% over 30 min, 100% for 5 min) at 2 mL/min flow rate affording
235 compounds **10-16**. Sub-fraction 2 afforded compounds **3** (1.3 mg) and **4** (2.1 mg), while sub-
236 fraction 3 afforded compounds **1** (1.0 mg) and **2** (5.9 mg) under the same HPLC conditions.
237 The EtOAc fraction was subjected to flash chromatography on the Biotage system using
238 prepacked RP-18 column chromatography using MeOH/H₂O gradient to give 4 subfractions.
239 Sub-fraction 2 was subjected to semi-preparative HPLC and a MeCN–H₂O (15–100% over
240 30 min, 100% for 5 min) at a flow rate of 2 mL/min to afford compounds **5** (11 mg), **6** (3
241 mg), **7** (7 mg), **8** (8 mg), and **9** (4.5 mg).

242 Asenjonamide A (**1**). White amorphous powder; $[\alpha]_D^{20} +6.7$ (*c* 0.1, MeOH); UV (MeOH)
243 λ_{\max} (log ϵ) at 230 (3.8), 256 (2.5) nm; HRESIMS *m/z* [M+Na]⁺ 264.1565 indicating the
244 molecular formula C₁₃H₁₉NO₃ (calculated [M+Na]⁺ ion at *m/z* 264.1570); NMR data: see
245 Table 1.

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

248 molecular formula C₁₁H₁₇NO₂ (calculated [M+Na]⁺ ion at m/z 220.1308); NMR data: see
249 Table 1.

250 Asenjonamide C (**3**). White amorphous powder; [α]_D²⁰ +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 C₁₁H₁₇NO₂ (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 μ g) and tetracycline (30 μ g) 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 μ g/mL) on growth was assessed after 18 h
267 incubation at 37 °C using a Labsystems iEMS MF plate reader at OD₆₂₀. 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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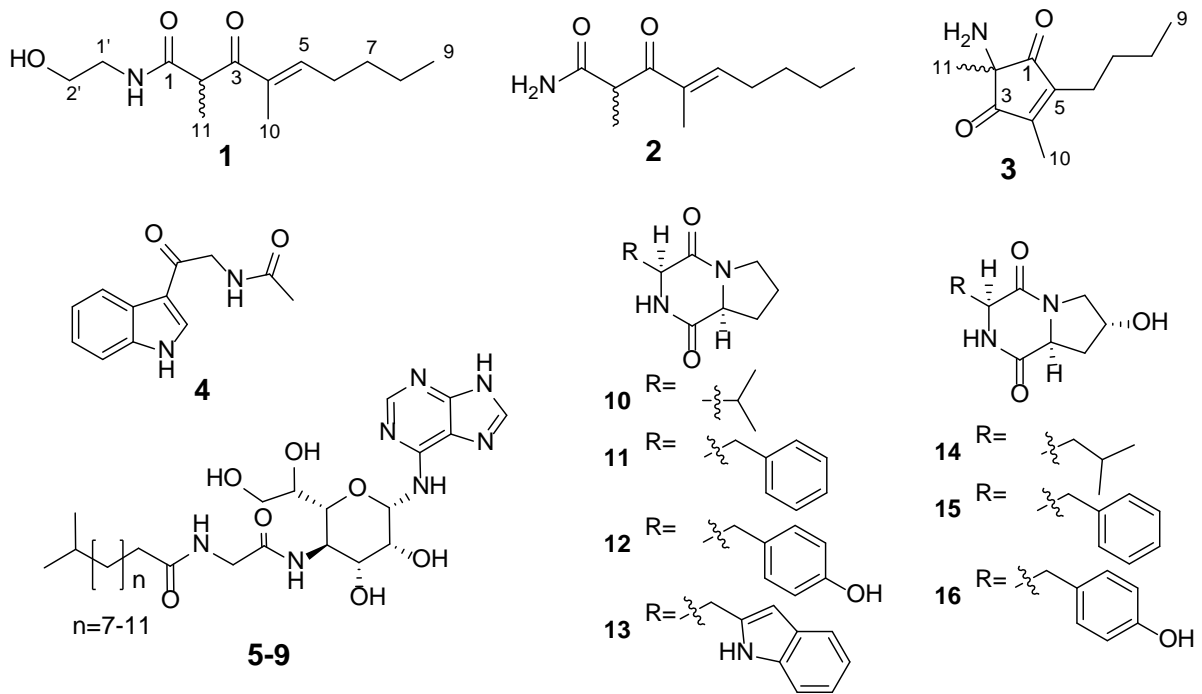
371 **Figure legends**

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

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

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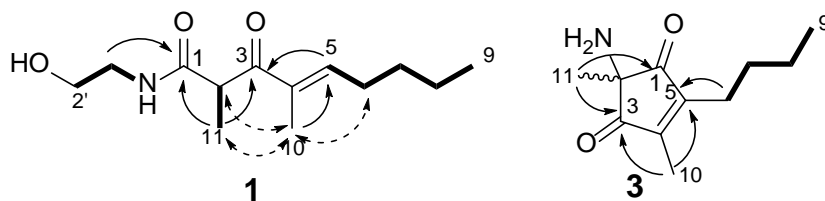


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378 **Figure 1.** Structures of the compounds isolated from *S. asenjonii* strain KNN 42.f.

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

384

385 **Table 1.** ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data of **1-3** (298 K, DMSO-
 386 d_6).

No.	1		2		3	
	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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393 **Table 2.** Antibacterial Activity of compounds **1-3** and **5-9**.

Compound	Average MIC ($\mu\text{g/mL}$) ^a				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>M. smegmatis</i>
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

394 ^a average of two independent replicates.

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