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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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

89 correlations of H$_3$-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 H$_2$-1' to C-1 confirmed the attachment of the

91 hydroxyethylamino moiety to the C-1 amide. NOESY correlations between H$_3$-10 and H$_2$-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 naturally[17] and

94 through stereoselective total synthesis.[18] Although the close similarity of siphonarienedione

95 ^{13}CNMR 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 C$_{11}$H$_{19}$NO$_2$ 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 ^{1}H and ^{13}C 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 C$_{11}$H$_{17}$NO$_2$ 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 ^{1}H NMR indicated the same chemical class with one extra ring in the structure of (**3**).

112   The COSY correlations of H_2-6 through H_3-9 confirmed the *n*-butyl side chain (Figure 2).

113   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

114   established the 2-amino-2,4-dimethylcyclopent-4-ene-1,3-dione moiety. The HMBC

115   correlations of H_2-6 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 CH_2 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 $C_{26}H_{43}N_7O_7$ which was assigned for spicamycin A. Subsequent increases

128   of 14 amu in the molecular ions corresponded to additional CH_2 groups giving spicamycins

129   B–E. This was supported by ^{1}H 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-MT_3 and reported as potent differentiation inducer of HL-60 human promyelocytic

134   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

181   part of calcaripeptide A[32] isolated from *Calcarisporium* sp. strain KF525 or the anti-HIV

182   inhibitor aetheramide A[33] 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). ^{1}H and ^{13}C 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 × 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 × 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 agar[35] 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

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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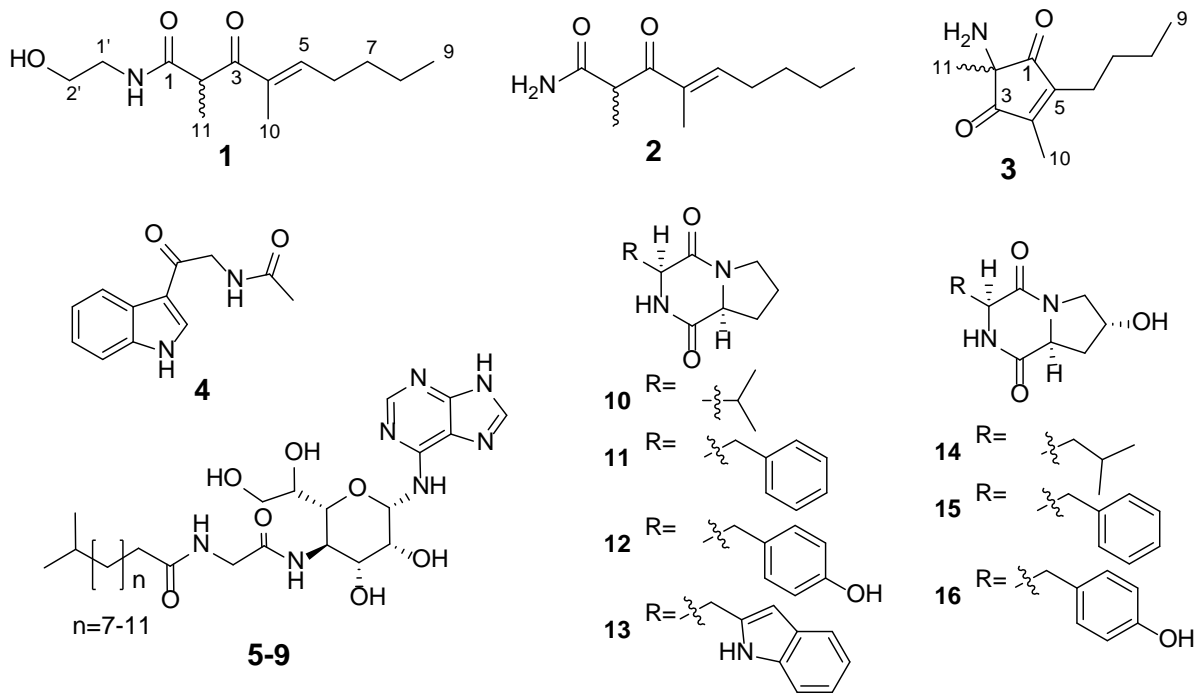369   cefoperazone disk diffusion susceptibility testing. *J. Clin. Microbiol*. **17**, 975-980 (1983).

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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
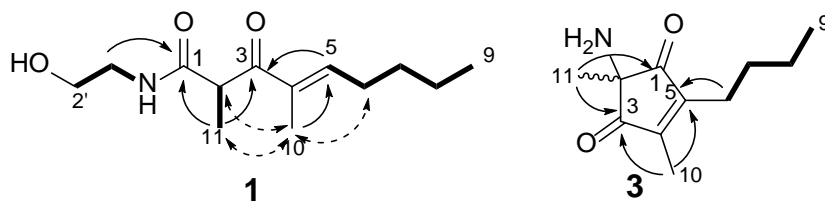
374   compounds **1** and **3**.

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

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Figure 2. Key COSY ($\longrightarrow$), HMBC ($\frown$) and NOESY ($\cdots$) correlations of compounds **1** and **3**.

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385   **Table 1**. ^{1}H (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 (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

394   [a] average of two independent replicates.

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