



**HAL**  
open science

## Mzabimycins A and B, novel intracellular angucycline antibiotics produced by *Streptomyces* sp. PAL114 in synthetic medium containing L-tryptophan

Samira Tata, Adel Aouiche, Christian Bijani, Nouredine Bouras, Frédéric Pont, Florence Mathieu, Nasserline Sabaou

### ► To cite this version:

Samira Tata, Adel Aouiche, Christian Bijani, Nouredine Bouras, Frédéric Pont, et al.. Mzabimycins A and B, novel intracellular angucycline antibiotics produced by *Streptomyces* sp. PAL114 in synthetic medium containing L-tryptophan. *Saudi Pharmaceutical Journal*, 2019, 27, pp.907 - 913. 10.1016/j.jsps.2019.06.004 . hal-03489018

**HAL Id: hal-03489018**

**<https://hal.science/hal-03489018v1>**

Submitted on 21 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## **Mzabimycins A and B, novel intracellular angucycline antibiotics produced by *Streptomyces* sp. PAL114 in synthetic medium containing L-tryptophan**

Samira Tata <sup>a</sup>, Adel Aouiche <sup>a</sup>, Christian Bijani <sup>b</sup>, Noureddine Bouras <sup>a,c</sup>, Frédéric Pont <sup>d</sup>, Florence Mathieu <sup>e,\*</sup>, Nasseridine Sabaou <sup>a,\*</sup>

<sup>a</sup> *Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Alger, Algeria*

<sup>b</sup> *Laboratoire de Chimie de Coordination (LCC), CNRS, Université de Toulouse, UPS, INPT, LCC, 205 Route de Narbonne, 31077 Toulouse, France*

<sup>c</sup> *Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaïa, BP 455, Ghardaïa 47000, Algeria*

<sup>d</sup> *Proteomics group, Centre de de Recherches en Cancérologie de Toulouse (CRCT), INSERM UMR1037, Toulouse, France*

<sup>e</sup> *Laboratoire de Génie Chimique, Université de Toulouse, CNRS, Toulouse, France*

\* Corresponding authors at: Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, B.P. 92, 16 050 Kouba, Algiers, Algeria (N. Sabaou).

*E-mail addresses:* [sabaou@yahoo.fr](mailto:sabaou@yahoo.fr) (N. Sabaou). [Florence.Mathieu@ensat.fr](mailto:Florence.Mathieu@ensat.fr) (F. Mathieu).

Conflict of interest. The authors declare that there is no conflict of interests regarding the publication of this paper.

1 **Mzabimycins A and B, novel intracellular angucycline antibiotics**  
2 **produced by *Streptomyces* sp. PAL114 in synthetic medium**  
3 **containing L-tryptophan**

4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23 Conflict of interest. The authors declare that there is no conflict of interests regarding the  
24 publication of this paper.  
25

26 **ABSTRACT**

27 In our previous studies, the production of four bioactive molecules by *Streptomyces* sp.  
28 PAL114 in complex ISP2 broth medium has been described. Three of these molecules belong  
29 to the angucycline family. In this study, two novel antibiotics belonging to the same family  
30 were produced by strain PAL114 on M2 synthetic medium containing L-tryptophan as  
31 precursor. These antibiotics, named mzabimycin A and B, were intracellular and produced  
32 only in the presence of L-tryptophan. After four days of culturing PAL114 in the M2 medium,  
33 the bioactive compounds were extracted from mycelium with methanol and then analyzed by  
34 HPLC on reverse phase C18 column. Two active purplish blue fractions were purified. The  
35 chemical structures of these molecules were determined on the basis of spectroscopic and  
36 spectrometric analyses (<sup>1</sup>H and <sup>13</sup>C NMR, and mass spectra). They were identified to be novel  
37 angucycline derivative antibiotics. The pure molecules showed activity against some  
38 pathogenic Gram-positive bacteria which have multiple antibiotic resistance, such as  
39 *Staphylococcus aureus* MRSA 639c and *Listeria monocytogenes* ATCC 13932.

40

41 *Keywords:*

42 Antimicrobial compounds

43 Angucycline antibiotics

44 L-Tryptophan

45 Synthetic medium

46 *Streptomyces*

47

48 **Abbreviated running headline**

49 Novel angucycline antibiotics

50

## 51 **1. Introduction**

52

53 Actinobacteria are Gram-positive bacteria with a genomic guanine-cytosine content  
54 higher than 55%, and most of them are mycelial. These bacteria are very interesting due to  
55 their large capacity to produce secondary metabolites with diversified chemical structures  
56 (Kemung et al., 2018; Takahashi and Nakashima, 2018). They are well-known for the  
57 production of antibacterial and antifungal antibiotics and are the source of nearly 45% of the  
58 known molecules of microbial origin (Solecka et al., 2012) and 70% of actively marketed  
59 molecules (Solanki et al., 2008). However, they are also known for the production of diverse  
60 bioactive molecules such as antivirals, antiparasitics, immunostimulants and  
61 immunosuppressants (Solecka et al., 2012; Flatt et al., 2013; Nakae et al., 2013; Takahashi  
62 and Nakashima, 2018).

63 The genus *Streptomyces* is known as the producer of the largest number of antibiotics. It  
64 produces about 80% of the antibiotics secreted by actinobacteria (Demain et al., 2006;  
65 Demain and Sanchez., 2009). Many of these molecules have found an important therapeutic  
66 application (Jose and Jebakumar, 2014), and some of them may have cytostatic and antitumor  
67 properties, such as urdamycins and langkocyclins (Drautz et al., 1986; Kalyon et al., 2013).

68 Considering the increasing resistance of pathogenic microorganisms to antibiotics  
69 (Messai et al., 2008; Fair and Tor, 2014; Li and Webster, 2018), and the toxicity of several  
70 antibiotic compounds (Berdy, 2005), it is essential to perpetuate research on antibiotics in the  
71 hope of finding new effective and less toxic molecules in order to control pathogenic  
72 microorganisms.

73 Our previous works have already demonstrated the richness and biodiversity of  
74 actinobacteria in the Saharan soils of Algeria. These studies have led to the discovery of  
75 several novel interesting antibiotics (Zitouni et al., 2004a; Yekkour et al., 2015 ; Khebizi et  
76 al., 2018; Lahoum et al., 2019) and several new species of actinobacteria (Aouiche et al.,

77 2015a; Bouras et al., 2015; Chaabane Chaouch et al., 2017). The actinobacterium strain  
78 PAL114 was isolated from Saharan soil collected from Ghardaïa province, Mzab region,  
79 south Algeria (Aouiche et al., 2014). This strain exhibited a strong antagonistic potential  
80 against several microorganisms and was found to be a producer of four bioactive molecules,  
81 saquayamycins A and C (Aouiche et al., 2014), chaetoglobosin A and vineomycin A1  
82 (Aouiche et al., 2015b), which were yellow extracellular and were produced in complex ISP2  
83 broth medium (Shirling and Gottlieb, 1966).

84 In this work, we used a synthetic medium, containing starch and L-tryptophan, in order to  
85 control the culture conditions and allow the synthesis of new molecules that we could have  
86 missed on complex ISP2 (International *Streptomyces* Project) medium. We highlight the  
87 production of novel purplish blue intracellular antibiotics. These compounds were extracted  
88 and purified, and their structure and activity were determined.

## 90 2. Materials and methods

### 92 2.1. Actinobacterium strain and target-microorganisms

94 The actinobacterium strain PAL114 was isolated from a Saharan soil in Béni Isguen,  
95 Ghardaïa province, Mzab region, southern Algeria (Aouiche et al., 2014). Based on a  
96 polyphasic study, this strain was linked to the species *Streptomyces griseoflavus* (Aouiche et  
97 al., 2015b). The strain was cultivated on ISP2 medium (Shirling and Gottlieb, 1966)  
98 composed of malt extract (10 g/l), yeast extract (4 g/l) and glucose (4 g/l). The pH of the  
99 medium was adjusted to 7.2. The aerial and substrate mycelia were grey and brownish-  
100 yellow, respectively. In ISP2 broth, PAL114 strain grows by forming pellets that are pale  
101 brownish-yellow in colour.

102 The target-microorganisms included Gram-positive and Gram-negative bacteria, a yeast  
103 and filamentous fungi. They are mostly pathogenic or toxigenic for humans, and many of  
104 them have multiple antibiotic resistance (Table 1). Indeed, the strains of *Staphylococcus*  
105 *aureus* MRSA 639c, *S. aureus* S1, *Pseudomonas aeruginosa* IPA1 and *Candida albicans* M3  
106 were isolated from sick patients in Algerian hospitals.

## 107 108 2.2. Production, extraction and purification of antibiotics

109  
110 Strain PAL114 was grown in two synthetic media, M1 and M2. Both of these media  
111 contain 10 g starch, 2 g NaCl, 0.5 g KH<sub>2</sub>PO<sub>4</sub>, 1 g K<sub>2</sub>HPO<sub>4</sub>, 0.5g MgSO<sub>4</sub>, 7 H<sub>2</sub>O and 2 g  
112 CaCO<sub>3</sub> in 1 l distilled water. However, M1 medium contains 0.25% (w/v) of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and  
113 M2 medium contains 0.05% (w/v) of L-tryptophan as nitrogen sources. The final pH of the  
114 media was adjusted to 7.2. The production of bioactive compounds was conducted in these  
115 two media. A seed culture was prepared with the same medium and used to inoculate (for  
116 each medium) sixteen 500 ml Erlenmeyer flasks, each containing 100 ml of culture media.  
117 The cultures were incubated on a rotary shaker (250 rpm) for 10 days at 30 °C. The extraction  
118 of the active compounds was carried out after centrifugation (5000 g, 20 min) of the culture  
119 broth to eliminate cells. Half of the cell-free supernatant was extracted with the same volume  
120 of dichloromethane and the other half with *n*-butanol. These two solvents were chosen  
121 because they extract the antibiotics (saquayamycins A and C, chaetoglobosin A and  
122 vineomycin A1) secreted by strain PAL114 (Aouiche et al., 2014; Aouiche et al., 2015b).  
123 Extraction of antibiotics from the mycelium was carried out according to the method of  
124 Mechliniski (1978). After centrifugation of the cultures, the mycelium was collected and  
125 washed several times with distilled water. Ten grams of wet mycelium were extracted with  
126 500 ml of methanol, stirring for 2 h at room temperature. The organic layers  
127 (dichloromethane, *n*-butanol and methanol extracts) were dehydrated with Na<sub>2</sub>SO<sub>4</sub> and

128 concentrated to dryness by a rotary evaporator under a vacuum at a temperature lower than 40  
129 °C. The residues of each extract were dissolved in 1 ml of methanol and subjected to  
130 biological assay (paper disk of 6 mm in diameter, Institute Pasteur) against the ten target-  
131 microorganisms listed in Table 1.

132 The purification of bioactive compounds was performed by Agilent reverse phase HPLC  
133 (Agilent 1260) using a C18 column (250 mm × 10 mm; 5 μm). The elution was at a flow rate  
134 of 1 ml/min with a continuous linear gradient solvent system from 20 to 100% methanol in  
135 water. The detection of products was carried out by UV at 220 nm. In order to detect the  
136 active fractions all peak fractions were collected and tested by the paper disk diffusion  
137 method against the ten target-microorganisms (Table 1). Final purification of the active  
138 fractions was achieved after the second re-injection in the HPLC under the same conditions.

139

### 140 2.3. Structure determination of the antibiotics

141

142 The structure determination of the antibiotics was made with the pure bioactive  
143 compounds. The UV spectra were determined with a Shimadzu UV 1605 spectrophotometer.  
144 The mass spectra were recorded on a LCQ ion-trap mass spectrometer (Finnigan MAT, San  
145 Jose, CA, USA) with a nanospray ion electro-spray ionization (ESI) source (positive and  
146 negative ion modes).

147 <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy were used for the characterization of compounds X3 and  
148 X4. NMR samples were prepared by dissolving 5 mg of X3 and X4 compounds in 600 μl of  
149 CD<sub>3</sub>CN. All spectra were recorded on a Bruker Avance 500 spectrometer equipped with a 5  
150 mm triple resonance inverse Z-gradient probe (TBI <sup>1</sup>H, <sup>31</sup>P, BB). All chemical shifts for <sup>1</sup>H  
151 and <sup>13</sup>C are relative to TMS using <sup>1</sup>H (residual) or <sup>13</sup>C chemical shifts of the solvent as a  
152 secondary standard. The temperature was set at 298 K. All the <sup>1</sup>H and <sup>13</sup>C signals were  
153 assigned on the basis of chemical shifts, spin-spin coupling constants, splitting patterns and



154 signal intensities, and by using  $^1\text{H}$ - $^1\text{H}$  COSY45,  $^1\text{H}$ - $^{13}\text{C}$  HSQC and  $^1\text{H}$ - $^{13}\text{C}$  HMBC  
155 experiments. Gradient-enhanced  $^1\text{H}$  COSY45 was realised included 36 scans for per  
156 increment.  $^1\text{H}$ - $^{13}\text{C}$  correlation spectra using a gradient-enhanced HSQC sequence (delay was  
157 optimised for  $^1J_{\text{CH}}$  of 145 Hz) was obtained with 200 scans per increment. Gradient-enhanced  
158 HMBC was performed allowing 62.5 ms for long-range coupling evolution (340 scans were  
159 accumulated). Typically, 2048 t2 data points were collected for 256 t1 increments.

160

#### 161 *2.4. Determination of minimum inhibitory concentrations*

162

163 Minimum inhibitory concentrations (MICs) of pure bioactive compounds were  
164 investigated using the conventional agar dilution method of [Oki et al. \(1990\)](#) against the ten  
165 target-microorganisms (Table 1). The target bacterial strains were inoculated onto Mueller  
166 Hinton medium and the fungal strains on Sabouraud medium. The media contained different  
167 concentrations of each active compound (1, 2, 3, 5, 10, 15, 20, 30, 40, 50, 60, 80 and 100  
168  $\mu\text{g/ml}$ ). After a growth period of 24-48 h at 37 °C for bacteria and 48-72 h at 28 °C for fungi,  
169 the plates were examined for growth and the lowest antibiotic concentration that inhibited the  
170 growth of each organism (MIC) was determined. Mueller Hinton and Sabouraud media,  
171 without active compound and inoculated with target organisms, were used as control  
172 treatments. All the experiments were performed in duplicate.

173

### 174 **3. Results and discussion**

175

#### 176 *3.1. Production and purification of the antibiotics*

177

178 After four days of fermentation in M1 and M2 media, the culture filtrates were separated  
179 from the mycelial biomass by centrifugation, and then extracted with dichloromethane and *n*-

180 butanol. The extracts of culture filtrates from both media were brownish yellow and inactive  
181 against all target-microorganisms. The mycelial biomass and the corresponding methanolic  
182 extracts were pale brownish yellow from M1 medium, which contains NH<sub>4</sub>SO<sub>4</sub>, whereas they  
183 were dark purplish blue from M2 medium containing L-tryptophan. Furthermore, this dark  
184 purplish blue methanolic extract was active against Gram-positive bacteria (*B. subtilis* ATCC  
185 6633, *M. luteus* ATCC 9314, *L. monocytogenes* ATCC 13932, *S. aureus* MRSA 639c and *S.*  
186 *aureus* S1) but not against Gram-negative bacteria (*E. coli* E52, *P. aeruginosa* IPA1), yeast  
187 (*C. albicans* M3) and filamentous fungi (*A. carbonarius* M333 and *U. ramanniana* NRRL  
188 1829), whereas, the extract from M1 medium was found to be inactive against all target-  
189 microorganisms. The active dark purplish blue extract, obtained from M2 medium, was  
190 analyzed by HPLC. Two active fractions against Gram-positive bacteria (cited above) were  
191 detected and named X3 (retention time, 64.7 min) and X4 (retention time, 65.19 min), with  
192 the latter being predominant (Supplementary data – Fig. 1S).

193 These intracellular and antimicrobial fractions are produced only in the presence of L-  
194 tryptophan (in M2 but not in M1 medium). L-Tryptophan seems to play an essential role in  
195 the biosynthesis of the two bioactive molecules and could therefore be a precursor of the two  
196 compounds. However, we have not detected, in any extract (from supernatant and mycelium),  
197 the saquayamycins A and C, or vineomycin A1 (angucycline antibiotics), or chaetoglobosin  
198 A. These molecules, which are yellow and active against *B. subtilis* ATCC 6633 and *S.*  
199 *aureus* MRSA 639c, were detected only in the ISP2 (complex medium) culture filtrate of  
200 strain PAL114 (Aouiche et al., 2014; 2015b).

201 Through these results, it appears that strain PAL114 produces bioactive compounds with  
202 different chemical structures depending on the culture conditions. Several previous works  
203 showed the ability of strains to produce many secondary metabolites with related chemical  
204 structures depending on the available precursors. Indeed, the results of Rohr et al. (1989) on  
205 the biosynthesis of urdamycins (angucycline antibiotics) by *Streptomyces fradiae* showed that

206 this species used different labeled precursors to produce different urdamycin molecules. Thus,  
207 this species uses the 2-methyl-tryptophan as precursor to produce urdamycin D, the tyrosine  
208 to produce urdamycin C and the acetate to produce urdamycin A. All these molecules have  
209 the same central chromophore. Similar results were obtained with *Saccharothrix algeriensis*  
210 NRRL B-24137, which produces five dithiopyrrolone antibiotics in ISP2 medium (Lamari  
211 et al., 2002) and several other dithiopyrrolone molecules, in semi-synthetic medium,  
212 induced by the addition of organic acids and amino acids as precursors (Bouras et al., 2008;  
213 Merrouche et al., 2010; 2011). Lam et al. (2001) reported a similar approach of using  
214 precursor-directed biosynthesis to produce novel fluorindolocarbazoles A and B by adding  
215 DL-6-fluorotryptophan, and fluorindolocarbazole C by adding DL-5-fluorotryptophan in  
216 cultures of *Saccharothrix aerocolonigenes* ATCC 39243. This is particularly interesting  
217 research strategy for producing new antibiotic molecules.

### 218 219 3.2. Elucidation of the structure of the antibiotics

220  
221 The structure of the compounds X3 and X4 was determined by NMR and mass  
222 spectrometry. The results showed that these two compounds are novel antibiotics belonging to  
223 the angucycline family. They were named mzabimycin A (for the major compound X4) and  
224 mzabimycin B (for the minor compound X3), with reference to Mzab region, southern  
225 Algeria, the source of the soil from which *Streptomyces* strain PAL114 was isolated. The  
226 structure of mzabimycins A and B is shown in Figs. 1 and 2 respectively.

227 Mzabimycin A (X4) was obtained as a purplish blue powder. The UV-visible spectrum  
228 (Supplementary data – Fig. 2S) showed the maximal absorbance at 218, 325 and 575 nm. The  
229 ESIMS spectrum (Supplementary data – Fig. 3S) contained an ion peak at  $m/z$  1088.31 [M -  
230 H]<sup>-</sup>. Thus, the molecular weight of this compounds was  $M = 1089$ . The <sup>13</sup>C, HSQC and  
231 HMBC spectra, showed 59 carbon signals. It was possible to discern 4 ketone group

232 ( $\delta_c$  186.72 to 207.23), 3 hydroxyl group ( $\delta_c$  78.90 to 155.90), 11 ether function ( $\delta_c$  67.51 to  
233 99.05), 24  $sp^2$ -hybridized carbons ( $\delta_c$  from 107.00 to 144.00) and 11  $sp^3$ -hybridized carbons  
234 ( $\delta_c$  14.04 to 42.61). The 2D  $^1H$ - $^1H$  and  $^1H$ - $^{13}C$  experiments and especially the long range  $^1H$ -  
235  $^{13}C$  couplings observed in the HMBC spectrum (see Fig. 1) permitted to established the  
236 connectivity between all the groups of the molecule.

237 The NMR (Supplementary data – Fig. 4S) data showed that mzabimycin A represents a  
238 new antibiotic belonging to the angucycline family. This compound has a central  
239 chromophore with L-tryptophan linked to carbon number 3', and five sugars, two rhodinoses,  
240 two aculoses and one olivose.

241 Mzabimycin B was obtained as a purplish blue powder. The UV-visible spectrum  
242 (Supplementary data – Fig. 2S) showed the maximal absorbance at 218, 280, 330 and 575 nm.  
243 The ESIMS spectrum (Supplementary data – Fig. 3S) contained an ion peak at  $m/z$  1120.36  
244  $[M - H]^-$ . Thus, the molecular weight of this compound was  $M = 1121$ . The HSQC and  
245 HMBC spectra, showed 59 carbon signals. It was possible to discern 4 ketone group  
246 ( $\delta_c$  186.72 to 207.23), 3 hydroxyl group ( $\delta_c$  78.90 to 155.90), 11 ether function ( $\delta_c$  67.51 to  
247 99.05), 22  $sp^2$ -hybridized carbons ( $\delta_c$  from 107.00 to 142.89) and 13  $sp^3$ -hybridized carbons  
248 ( $\delta_c$  14.04 to 42.61). The 2D  $^1H$ - $^1H$  and  $^1H$ - $^{13}C$  experiments and especially the long range  $^1H$ -  
249  $^{13}C$  couplings observed in the HMBC spectrum (see Fig. 2) permitted to establish the  
250 connectivity between all the groups of the molecule.

251 The NMR data (Supplementary data – Fig. 5S) showed that mzabimycin B represent a  
252 novel antibiotic belonging to the angucycline family. This compound has a central  
253 chromophore typical of angucycline compounds, but with L-tryptophan linked to carbon  
254 number 3', five sugars, two rhodinoses, one olivose, one aculose and one reduced and  
255 methoxylated aculose. These sugars were linked to the central chromophore at carbon number  
256 3 (rhodinose and aculose) and carbon number 9 (olivose, rhodinose and reduced and

257 methoxylated aculose). It differs from mzabimycin A only by the second aculose molecule  
258 that is reduced and methoxylated.

259 The structure of mzabimycins A and B do not correspond to any structure reported in the  
260 literature, notably in the [www.sciencefinder.com](http://www.sciencefinder.com) and [www.chemspider.com](http://www.chemspider.com) databases, or  
261 antibiotics described in *The Dictionary of Natural Products* ([Buckingham, 1997](#)), or in  
262 Berdy's review of bioactive microbial metabolites ([Berdy, 2005](#)). The mzabimycins A and B  
263 are, therefore, two new angucyclines that possess a chromophore containing L-tryptophan and  
264 osidic derivatives. These two compounds have the same central chromophore, which is  
265 similar to that of the urdamycin D ([Rohr et al., 1989](#)) and the langkocyclines B1 and B2  
266 ([Kalyon et al., 2013](#)). Furthermore, the urdamycin D ([Drautz et al., 1986](#)) and the  
267 langkocyclines B1 and B2 ([Kalyon et al., 2013](#)) are purple blue and have L-tryptophan linked  
268 to carbon number 3' of the central chromophore, like mzabimycins A and B. However,  
269 langkocycline B1 has four sugars (two rhodinoses and two olivoses) and langkocycline B2  
270 has five sugars (three rhodinoses and two olivoses); these sugars are linked to carbon number  
271 12b of the central chromophore. Urdamycin D contained one olivose linked to carbon number  
272 12b of the central chromophore and two olivoses and one rhodinoose linked to carbon number  
273 9 of the chromophore. Mzabimycins A and B differ from these antibiotics in the number and  
274 the composition of sugars (presence of aculose and reduced and methoxylated aculose) and in  
275 the linkage to the central chromophore. Mzabimycins A and B contain in their structure  
276 identical sugars to those of vineomycin A1, a yellow extracellular angucycline also produced  
277 by strain PAL114, not in synthetic media M1 and M2, but in complex ISP2 medium ([Aouiche  
278 et al., 2015b](#)). However, there are some differences in the central chromophore structure and  
279 the absence of L-tryptophan. These results showed some similarities between the biosynthesis  
280 processes of vineomycin A1 and mzabimycins A and B.

281 The production of antibiotics belonging to the families of angucyclines and  
282 anthracyclines (close to angucyclines) has already been demonstrated in some strains of

283 actinobacteria isolated from Saharan soils as strain PAL114. This is the case of the antibiotic  
284 R2 secreted by *Streptosporangium* sp. Sg3 (Boudjella et al., 2010) and mutactimycins C and  
285 PR secreted by *Saccharothrix* sp. SA103 (Zitouni et al., 2004b).

286

### 287 3.3. Minimum inhibitory concentrations

288

289 Minimum inhibitory concentrations (MICs) of mzabimycin A (X4) and mzabimycin B  
290 (X3), purified by HPLC, are summarized in Table 2. The results showed that these  
291 compounds have very similar with activity directed only against Gram-positive bacteria. The  
292 strains of *Micrococcus luteus* (MIC, 15 µg/ml for mzabimycin A and B) and *Listeria*  
293 *monocytogenes* ATCC 13932 (MICs, 20 µg/ml for mzabimycin B and 40 µg/ml for  
294 mzabimycin A) were the most sensitive. The other Gram-positive bacteria, including the two  
295 strains of *Staphylococcus aureus* (60-80 µg/ml) and *Bacillus subtilis* ATCC 6633 (50 µg/ml),  
296 were found to be less sensitive. All tested Gram-negative bacteria, yeasts and filamentous  
297 fungi were resistant (> 100 µg/ml).

298 It should be noted that the antimicrobial activity of urdamycin D (Drautz et al., 1986) and  
299 langkocyclins B1 and B2 (Kalyon et al., 2013), which are angucyclines close in structure to  
300 mzabimycins A and B, is also directed only against Gram-positive bacteria.

301 Angucycline antibiotics are a group of biologically active compounds with interesting  
302 activities including antibacterial, antifungal and antiviral (Kharel et al., 2012), enzyme  
303 inhibitory (Eguchi et al., 2017), and platelet aggregation inhibitory properties (Kawashima et  
304 al., 1989). Therefore, they are cytotoxic, and some molecules were used as anticancer agents  
305 in chemotherapy (Abdelfattah et al., 2008). Yu and O'Doherty (2008) showed the role of  
306 vineomycin B2 trisaccharide, consisting of aculose, rhodinose and olivose, in anticancer  
307 activity (against a panel of cancer cell lines). The same authors also showed the role of

308 antibiotic PI-080 trisaccharide, consisting of aculose and two olivoses, in anticoagulant  
309 activity.

310

### 311 **3. Conclusion**

312

313 Strain PAL114, related to *Streptomyces griseoflavus*, produced two novel intracellular  
314 antibiotics, mزابimycins A and B, in synthetic medium containing L-tryptophan as a  
315 precursor. These antibiotics, which belonged to the angucycline family, showed activity  
316 against some pathogenic Gram-positive bacteria with multiple antibiotic resistance.  
317 Considering the important biological activities of angucyclines, it would be interesting to  
318 explore other properties of these new angucyclines molecules, as for example anticancer  
319 activity, and *in vivo* evaluation studies which could prove promising.

320

### 321 **Conflict of interest**

322 The authors declare that there is no conflict of interests regarding the publication of this  
323 paper.

324

### 325 **Appendix A. Supplementary material**

326 Supplementary data associated with this article can be found, in the online version, at:

327

### 328 **References**

329

330 Abdelfattah, M.S., Kharel, M.K., Hitron, J.A., Baig, I., Rohr, J., 2008. Moromycins A and B  
331 isolation and structure elucidation of C-glycosylangucycline-type antibiotics from  
332 *Streptomyces* sp. KY002. J. Nat Prod. 71 (9), 1569–1573.

333

334 Aouiche, A., Bijani, C., Zitouni, A., Mathieu, F., Sabaou, N., 2014. Antimicrobial activity of  
335 saquayamycins produced by *Streptomyces* sp. PAL114 isolated from a Saharan soil. *J.*  
336 *Mycol.* 24 (2), e17–e23.

337

338 Aouiche, A., Bouras, N., Mokrane, S., Zitouni, A., Schumann, P., Spröer, C., Sabaou, N.,  
339 Klenk, H.P., 2015a. *Actinokineospora mzabensis* sp. nov., a novel actinomycete isolated  
340 from Saharan soil. *Antonie van Leeuwenhoek* 107 (1), 291–296.

341

342 Aouiche, A., Meklat, A., Bijani, C., Zitouni, A., Sabaou, N., Mathieu, F., 2015b. Production  
343 of vineomycin A1 and chaetoglobosin A by *Streptomyces* sp. PAL114. *Ann. Microbiol.*  
344 65 (3), 1351–1359.

345

346 Berdy, J., 2005. Bioactive microbial metabolites. *J. Antibiot.* 58 (1), 1–26.

347

348 Boudjella, H., Zitouni, A., Coppel, Y., Mathieu, F., Monje, M., Sabaou, N., 2010. Antibiotic  
349 R2, a new angucyclinone compound from *Streptosporangium* sp. Sg3. *J. Antibiot.* 63  
350 (12), 709–711.

351

352 Bouras, N., Merrouche, R., Lamari, L., Mathieu, F., Sabaou, N., Lebrihi, A., 2008. Precursor  
353 directed biosynthesis of new dithiolopyrrolone analogs by *Saccharothrix algeriensis*  
354 NRRL B-24137. *Process Biochem.* 43 (11), 1244–1252.

355

356 Bouras, N., Meklat, A., Zitouni, A., Mathieu, F., Schumann, P., Spröer, C., Sabaou, N.,  
357 Klenk, H.P., 2015. *Nocardiopsis algeriensis* sp. nov., an alkalitolerant actinomycete  
358 isolated from Saharan soil. *Antonie van Leeuwenhoek* 107 (2), 313–320.

359



360 Buckingham, J., 1997. Dictionary of natural products. UK: Chapman and Hall/CRC.

361

362 Chaabane Chaouch, F., Bouras, N., Mokrane, S., Bouznada, K., Zitouni, A., Schumann, P.,  
363 Pötter, G., Spröer, C., Klenk H.P., Sabaou, N. 2017. *Planomonospora algeriensis* sp.  
364 nov., an actinobacterium isolated from a Saharan soil of Algeria. *Antonie van*  
365 *Leeuwenhoek* 110 (2), 245–252.

366

367 Demain, A.L., 2006. From natural products discovery to commercialization: a success story.  
368 *J. Ind. Microbiol. Biotechnol.* 33 (7), 486–495.

369

370 Demain, A.L., Sanchez, S., 2009. Microbial drug discovery: 80 years of progress. *J. Antibiot.*  
371 62 (1), 5–16.

372

373 Drautz, H., Zähner, H., Rohr, J., Zeeck, A., 1986. Metabolic products of microorganisms.  
374 234. Urdamycins, new angucycline antibiotics from *Streptomyces fradiae*. I. Isolation,  
375 characterization and biological properties. *J. Antibiot.* 39 (12), 1657–1669.

376

377 Eguchi, Y., Okajima, T., Tochio, N., Inukai, Y., Shimizu, R., Ueda, S., Shinya, S., Kigawa,  
378 T., Fukamizo, T., Igarashi, M., Utsumi, R., 2017. Angucycline antibiotic waldiomycin  
379 recognizes common structural motif conserved in bacterial histidine kinases. *J. Antibiot.*  
380 70 (3), 251–258.

381

382 Fair, R.J., Tor, Y., 2014. Antibiotics and bacterial resistance in the 21st Century. *Perspect.*  
383 *Medicin. Chem.* 6 (6), 25–64.

384

385 Flatt, P., Wu, X., Perry, S., Mahmud, T., 2013. Genetic insights into pyralomicin biosynthesis  
386 in *Nonomuraea spiralis* IMC A-0156. *J. Nat Prod.* 76 (5), 939–946.  
387  
388 Jose, P.A., Jebakumar, S.R.D., 2014. Unexplored hypersaline habitats are sources of novel  
389 actinomycetes. *Front. Microbiol.* 5, 242.  
390  
391 Kawashima, A., Kishimura, Y., Tamai, M., Hanada, K., 1989. New platelet aggregation  
392 inhibitors. *Chem. Pharm. Bull.* 37 (12), 3429–3431.  
393  
394 Kalyon, B., Tan, G.Y.A., Pinto, J.M., Foo, C.Y., Wiese, J., Imhoff, J.F., Süßmuth, R.D.,  
395 Sabaratnam, V., Fiedler, H.P., 2013. Langkocyclines: novel angucycline antibiotics from  
396 *Streptomyces* sp. Acta 3034. *J. Antibiot.* 66 (10), 609–616.  
397  
398 Kemung, H.M., Tan, L.T.H., Khan, T. M., Chan K.G., Pusparajah P., Goh B.H., Lee, L.H.,  
399 2018. *Streptomyces* as a prominent resource of future anti-MRSA drugs. *Front.*  
400 *Microbiol.* 9, 2221.  
401  
402 Kharel, M.K., Pahari, P., Shepherd, M.D., Tibrewal, N., Nybo, S.E., Shaaban, K.A., Rohr, J.,  
403 2012. Angucyclines: biosynthesis, mode-of-action, new natural products, and synthesis.  
404 *Nat. Prod. Rep.* 29, 264–325.  
405  
406 Khebizi, N., Boudjella, H., Bijani, C., Bouras, N., Klenk, H.P., Pont, F., Mathieu, F., Sabaou,  
407 N., 2018. Oligomycins A and E, major bioactive secondary metabolites produced by  
408 *Streptomyces* sp. strain HG29 isolated from a Saharan soil. *J. Mycol.* 28 (1), 150–160.  
409

410 Lahoum, A., Sabaou, N., Bijani, C., Bouras, N., Pont, F., Snini, S.P., Mathieu F., 2019.  
411 Antimicrobial activities of novel bipyridine compounds produced by a new strain of  
412 *Saccharothrix* isolated from Saharan soil. Saudi Pharm. J. 27 (1), 56–65.  
413  
414 Lam, K.S., Schroeder, D.R., Veitch, J.M., Colson, K.L., Matson, J.A., Rose, W.C., Doyle,  
415 T.W., Forenza, S., 2001. Production, isolation and structure determination of novel  
416 fluoroindolocarbazoles from *Saccharothrix aerocolonigenes* ATCC39243. J. Antibiot. 54  
417 (1), 1–9.  
418  
419 Lamari, L., Zitouni, A., Boudjella, H., Badji, B., Sabaou, N., Lebrihi, A., 2002. New  
420 dithiolopyrrolone antibiotics from *Saccharothrix* sp. SA 233 I. Taxonomy, production,  
421 isolation and biological properties. J. Antibiot. 55 (8), 696–701.  
422  
423 Li, B., Webster, T.J., 2018. Bacteria antibiotic resistance: new challenges and opportunities  
424 for implant-associated orthopaedic infections. J. Orthop Res. 36 (1), 22–32.  
425  
426 Merrouche, R., Bouras, N., Coppel, Y., Mathieu, F., Monje, M.C., Sabaou, N., Lebrihi, A.,  
427 2010. Dithiolopyrrolone antibiotic formation induced by adding valeric acid to the culture  
428 broth of *Saccharothrix algeriensis*. J. Nat. Prod. 73 (6), 1164–1166.  
429  
430 Merrouche, R., Bouras, N., Coppel, Y., Mathieu, F., Sabaou, N., Lebrihi, A., 2011. New  
431 dithiolopyrrolone antibiotics induced by adding sorbic acid to the culture medium of  
432 *Saccharothrix algeriensis* NRRL B-24137. FEMS Microbiol. Lett. 318 (1), 41–46.  
433

434 Messai, Y., Iabadence, H., Benhassine, T., Alouache, S., Tazir, M., Guatier, V., Arlet, G.,  
435 Bakour, R., 2008. Prevalence and characterization of extended-spectrum  $\beta$ -lactamases in  
436 *Klebsiella pneumoniae* in Algiers hospitals (Algeria). *Pathol. Biol.* 56 (5), 319–325.  
437  
438 Mechlinski, W., 1978. The polyene antifungal antibiotics. In: Laskin, A.I., Lechevalier, H.A.,  
439 eds. *Handbook of microbiology*. Vol. III. CRC Press, 93–107.  
440  
441 Nakae, K., Kurata, I., Kojima, F., Igarashi, M., Hatano, M., Sawa, R., 2013. Sacchathridine,  
442 A, a prostaglandin release inhibitor from *Saccharothrix* sp. *J. Nat. Prod.* 76 (4), 720–722.  
443  
444 Oki, T., Tenmyo, O., Tomatsu, K., Kamei, H., 1990. Pradimicins A, B and C: new antifungal  
445 antibiotics. II. *In vitro* and *in vivo* biological activities. *J. Antibiot.* 43 (7), 763–770.  
446  
447 Rohr, J., Beale J.M., Floss H.G., 1989. Urdamycins new angucycline antibiotics from  
448 *Streptomyces fradiae*. IV. Biosynthetic studies of urdamycins A ~ D. *J. Antibiot.* 41 (7),  
449 1151–1157.  
450 Shirling, B., Gottlieb, D., 1966. Methods for characterization of *Streptomyces* species. *Int. J.*  
451 *Syst. Bacteriol.* 16 (3), 3313–3340.  
452  
453 Solanki, R., Kahanna, M., 2008. Bioactive compounds from marine actinomycetes. *Indian J.*  
454 *Microbiol.* 48 (4), 410–431.  
455 Solecka, J., Zajko, J., Postek, M., Rajnisz, A., 2012. Biologically active secondary  
456 metabolites from actinomycetes. *Cent Eur. J. Biol.* 7 (3), 373–390.  
457  
458 Takahashi, Y., Nakashima, T., 2018. Actinomycetes, an inexhaustible source of naturally  
459 occurring antibiotics. **Antibiotics 7 (3), 74.**

460

461 Yekkour, A., Meklat, A., Bijani, A., Toumatia, O., Errakhi, R., Lebrihi, A., Mathieu, F.,

462 Zitouni, A., Sabaou, N., A novel hydroxamic acid-containing antibiotic produced by a

463 Saharan soil-living *Streptomyces* strain. *Lett. Appl. Microbiol.* 60 (6), 589–596.

464

465 Yu, X., O’Doherty, A., 2008. De novo asymmetric synthesis and biological evaluation of the

466 trisaccharide portion of PI-080 and vineomycin B2. *Org. Lett.* 10 (20), 4529–4532.

467

468 Zitouni, A., Boudjella, H., Mathieu, F., Sabaou, N., Lebrihi, A., 2004a. Mutactimycin PR, a

469 new anthracycline antibiotic from *Saccharothrix* sp. SA 103. I. Taxonomy, fermentation,

470 isolation and biological activities. *J. Antibiot.* 57 (6), 367–372.

471

472 Zitouni, A., Lamari, L., Boudjella, H., Badji, B., Sabaou, N., Gaouar, A., Mathieu, F., Lebrihi,

473 A., Labeda, D.P., 2004b. *Saccharothrix algeriensis* sp. nov., isolated from Saharan soil.

474 *Int. J. Syst. Evol. Microbiol.* 54 (4), 1377–1381.

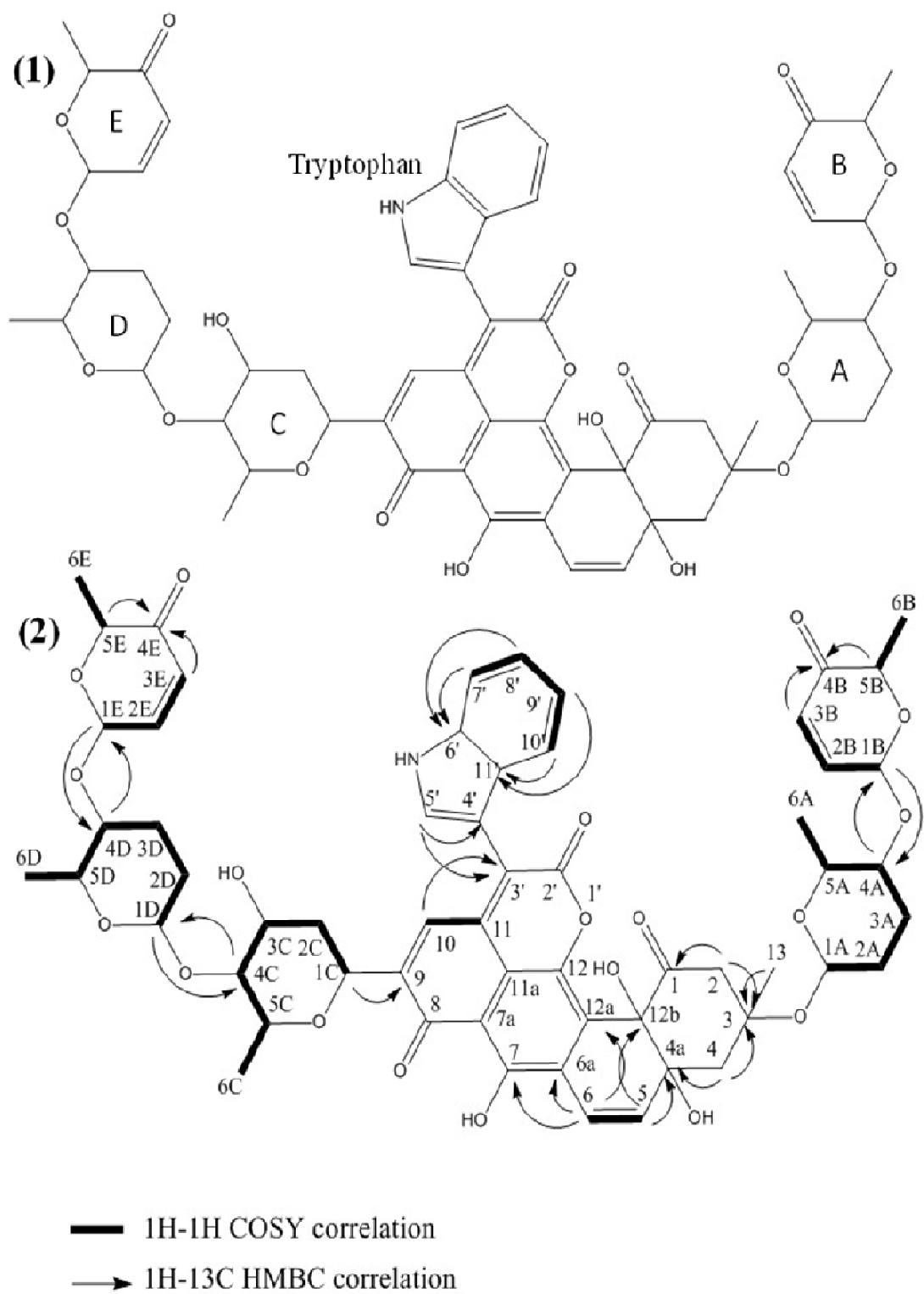
## Legends of figures

**Fig. 1.** Structure of mzabimycin A (X4 compound) (1) and HMBC and COSY correlations (2).

A and D, rhodnose; B and E, aculose; C, olivose.

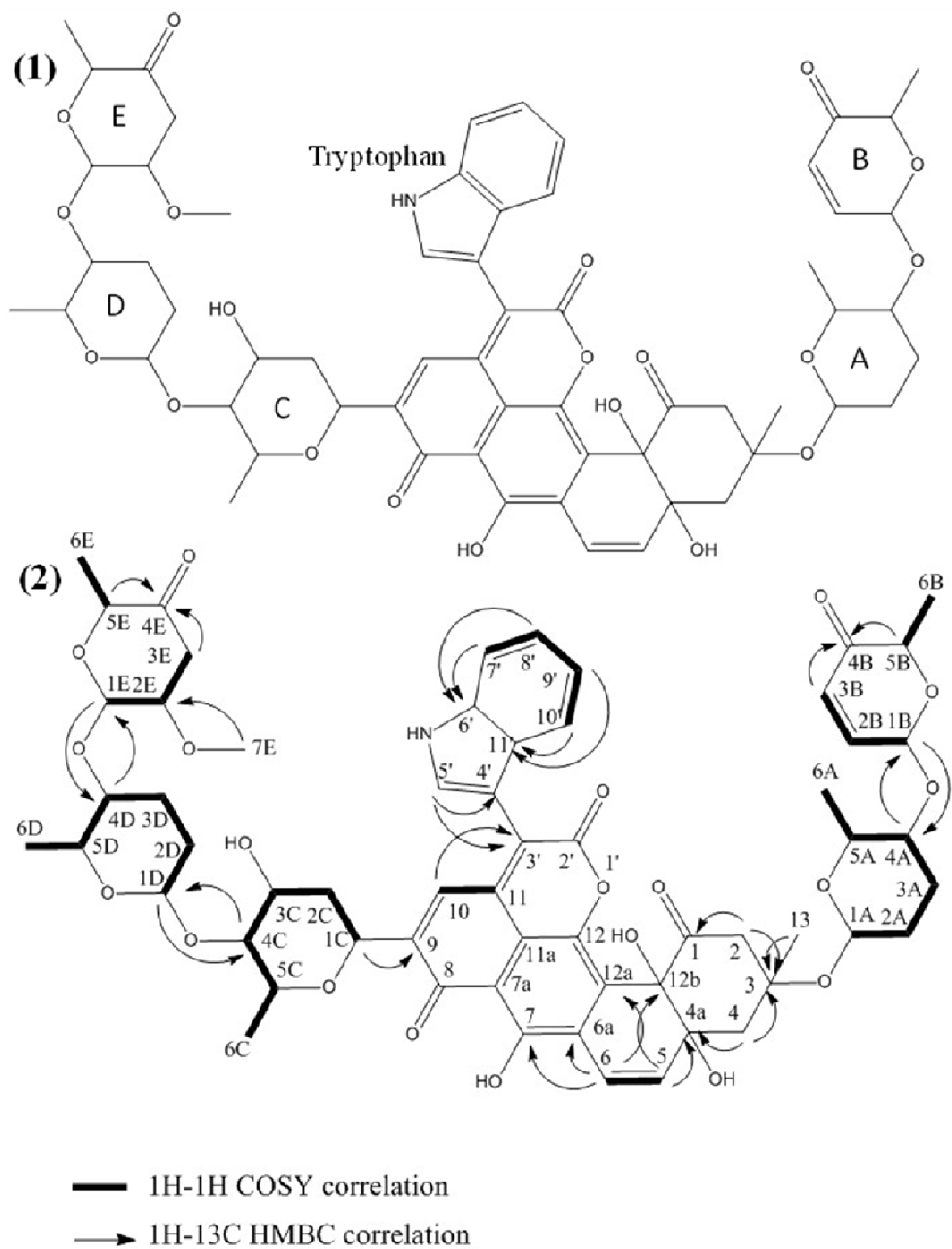
**Fig. 2.** Structure of mzabimycin B (X3 compound) (1) and HMBC and COSY correlations (2).

A and D, rhodnose; B, aculose; C, olivose; E, reduced and methoxylated aculose.



**Fig. 1.** Structure of mzabimycin A (X4 compound) (1) and HMBC and COSY correlations (2).

A and D, rhodinose; B and E, aculose; C, olive.



**Fig. 2.** Structure of mzabimycin B (X3 compound) (1) and HMBC and COSY correlations (2).

A and D, rhodnose; B, aculose; C, olivose; E, reduced and methoxylated aculose.



**Table 1**

Resistance patterns of target-microorganisms.

Microorganisms	Resistance to
<i>Bacillus subtilis</i> ATCC 6633	NEO
<i>Micrococcus luteus</i> ATCC 9314	NEO
<i>Escherichia coli</i> E52	ATM, CAZ, CTX, FEP, GEN, PIP, TIC, TOB
<i>Pseudomonas aeruginosa</i> IPA1	AMX, CAR, ERY, GEN, NEO, SPI, SSS, VAN
<i>Staphylococcus aureus</i> S1	CLD, GEN, K, PEN, VAN
<i>Staphylococcus aureus</i> MRSA 639c	FA, K, OXA, PEN, TE
<i>Listeria monocytogenes</i> ATCC 13932	OXA, FOS, CAZ, CTX, CXC, FEP, FOX, LIN, CLD, PRL, CIP
<i>Candida albicans</i> M3	CHX, ITR, NYS, TER, TIZ
<i>Umbelopsis ramanniana</i> NRRL 1829	CHX, ITR, TER, TIZ
<i>Aspergillus carbonarius</i> M333	CHX, NYS

AMX: amoxicillin; ATM: aztreonam; CAR: carbenicillin; CAZ: ceftazidim; CHX: cycloheximide; CIP: ciprofloxacin; CLD: clindamycin; CTX: cefotaxime; CXC: cefotaxime + clavulanic acid; ERY: erythromycin; FEP: cefepime; FA: fusidic acid; FOS: fosfomycin; FOX: cefoxitin; ITR: itraconazole; GEN: gentamicin; K: kanamycin; NEO: neomycin; NYS: nystatine; LIN: lincomycin; OXA: oxacillin; PEN: penicillin; PRL: pirlimycin; PIP: piperacillin; SPI: spiramycin; SSS: sulfamide; TE: tetracycline; TER: terbinafine; TIC: ticarcillin; TIZ: thioconazole; TOB: tobramycin; VAN: vancomycin.

**Table 2**

$^1\text{H}$  and  $^{13}\text{C}$  NMR data assignments of X3 (mzabimycin B) and X4 (mzabimycin A) in  $\text{CD}_3\text{CN}$  at 298 K. See Figs. 1 and 2 for numbering of hydrogen and carbon atoms.

$^1\text{H}$ and $^{13}\text{C}$ number	$^1\text{H}$ chemical shift, ppm				$^{13}\text{C}$ chemical shift, ppm	
	X3		X4		X3	X4
1	-	-	-	-	206.90	206.00
2	3.03	(m,2H)	3.03	(m,2H)	49.87	49.82
3	-	-	-	-	81.00	81.46
4	1.97-2.31	(d,15.6,2H)	1.97-2.30	(d,15.6,2H)	43.43	43.50
4a	-	-	-	-	79.70	79.80
5	6.09	(d,10.0,1H)	6.09	(d,10.0,1H)	137.30	138.00
6	7.03	(d,10.0,1H)	7.03	(d,10.0,1H)	118.00	118.00
6a	-	-	-	-	123.30	123.50
7	-	-	-	-	155.90	155.90
7a	-	-	-	-	114.9	114.8
8	-	-	-	-	186.27	186.84
9	-	-	-	-	142.80	148.89
10	8.05	(s,1H)	8.05	(s,1H)	134.93	134.93
11	-	-	-	-	118.60	116.60
11a	-	-	-	-	127.25	127.00
12	-	-	-	-	142.8	142.6
12a	-	-	-	-	128.10	127.00
12b	-	-	-	-	78.90	79.80
13	1.33	(m,3H)	1.32	(m,3H)	24.83	24.80
1'	-	-	-	-	-	-
2'	-	-	-	-	159.20	159.20
3'	-	-	-	-	131.00	131.00
4'	-	-	-	-	107.00	107.00
5'	7.83	(s,1H)	7.83	(s,1H)	131.24	131.24
6'	-	-	-	-	136.00	136.00
7'	7.59	(d,7.8,1H)	7.59	(d,7.8,1H)	112.00	112.00
8'	7.29	(t,7.8,1H)	7.29	(t,7.8,1H)	123.00	123.00
9'	7.19	(t,7.8,1H)	7.19	(t,7.8,1H)	121.00	121.00
10'	7.53	(d,7.8,1H)	7.53	(d,7.8,1H)	120.20	120.20

11'	-	-	-	-	127.00	127.00
1A	5.26	(m,1H)	5.25	(m,1H)	91.88	92.00
2A	1.98	(m,2H)	1.98	(m,2H)	24.04	24.04
3A	1.94-1.98	(m,2H)	1.94-1.98	(m,2H)	24.25	24.25
4A	3.72	(m,1H)	3.71	(m,1H)	76.18	76.00
5A	4.14	(m,1H)	4.14	(m,1H)	66.56	66.72
6A	1.24	(d,6.5,3H)	1.24	(d,6.5,3H)	16.52	16.47
1B	5.30	(dd,9.0-3.4,1H)	5.30	(dd,9.0-3.4,1H)	95.00	95.00
2B	6.07	(d,9.0,1H)	6.07	(d,9.0,1H)	126.48	128.48
3B	7.00	(d,9.0,1H)	7.00	(d,9.0,1H)	144.00	144.00
4B	-	-	-	-	197.00	197.00
5B	4.60	(d,4.0, 1H)	4.60	(d,4.0, 1H)	70.14	70.14
6B	1.31	(d,4.0, 3H)	1.31	(d,4.0, 3H)	14.43	14.43
1C	4.73	(d,11.0,1H)	4.73	(d,11.0,1H)	70.65	70.65
2C	1.26-2.41	(m,2H)	1.72-2.41	(m,2H)	39.18	39.18
3C	3.74	(m,1H)	3.74	(m,1H)	70.96	70.78
4C	2.85	(m,1H)	2.86	(m,1H)	87.81	87.78
5C	3.43	(m,1H)	3.43	(m,1H)	74.50	74.55
6C	1.07	(dd,6.0-1.8,3H)	1.06	(dd,6.0-1.8,3H)	17.86	17.59
1D	4.89	(m,1H)	4.88	(m,1H)	99.05	99.18
2D	1.60-1.94	(m,2H)	1.60-1.99	(m,2H)	24.50	24.50
3D	1.90-1.93	(m,2H)	1.90-1.93	(m,2H)	24.25	24.25
4D	3.75	(m,1H)	3.72	(m,1H)	75.42	76.00
5D	4.23	(m,1H)	4.22	(m,1H)	67.51	67.44
6D	1.93	(dd,8.7-2.0,3H)	1.71	(dd,7.9-3.5,1H)	16.16	16.18
1E	4.96	(m,1H)	5.30	(m,1H)	99.40	95.00
2E	3.81	(m,1H)	6.07	(d,9.0,1H)	79.00	126.48
3E	2.54-2.83	(dt,15.4-4.8,2H)	7.00	(d,9.0,1H)	40.13	144.00
4E	-	-	-	-	207.23	197.00
5E	4.29	(m,1H)	4.60	(m,1H)	71.53	70.14
6E	1.19	(d,3.6,3H)	1.30	(m,3H)	13.94	14.43
7E	1.45	(d,5.5,3H)	-	-	15.50	-

The numbering of the atoms was made like that of antibiotics urdamycin D (Rohr et al., 1989) and langkocyclines B1 and B2 (Kalyon et al., 2013), which are closely related to the structures of mزابimycins A and B.

**Table 3**

Minimum inhibitory concentrations (MICs) of X3 (mzabimycin B) and X4 (mzabimycin A) produced by *Streptomyces* sp. PAL114 against several target microorganisms.

Target microorganisms	MICs ( $\mu\text{g/ml}$ )*	
	X3	X4
<i>Bacillus subtilis</i> ATCC 6633	50	50
<i>Micrococcus luteus</i> ATCC 9314	15	15
<i>Staphylococcus aureus</i> MRSA 639c	60	60
<i>Staphylococcus aureus</i> S1	80	80
<i>Listeria monocytogenes</i> ATCC 13932	20	40
<i>Escherichia coli</i> E52	> 100	> 100
<i>Pseudomonas aeruginosa</i> IPA1	> 100	> 100
<i>Candida albicans</i> M3	> 100	> 100
<i>Umbelopsis ramanniana</i> NRRL 1829	> 100	> 100
<i>Aspergillus carbonarius</i> M333	> 100	> 100

\* MIC values represent the mean of two replicates;