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

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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**
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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 (^1H and ^{13}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 KH₂PO₄, 1 g K₂HPO₄, 0.5g MgSO₄, 7 H₂O and 2 g
112 CaCO₃ in 1 l distilled water. However, M1 medium contains 0.25% (w/v) of (NH₄)₂SO₄ 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 Na₂SO₄ 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 ¹H and ¹³C 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 CD₃CN. All spectra were recorded on a Bruker Avance 500 spectrometer equipped with a 5
150 mm triple resonance inverse Z-gradient probe (TBI ¹H, ³¹P, BB). All chemical shifts for ¹H
151 and ¹³C are relative to TMS using ¹H (residual) or ¹³C chemical shifts of the solvent as a
152 secondary standard. The temperature was set at 298 K. All the ¹H and ¹³C signals were
153 assigned on the basis of chemical shifts, spin-spin coupling constants, splitting patterns and

154 signal intensities, and by using ^1H - ^1H COSY45, ^1H - ^{13}C HSQC and ^1H - ^{13}C HMBC
155 experiments. Gradient-enhanced ^1H COSY45 was realised included 36 scans for per
156 increment. ^1H - ^{13}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 NH₄SO₄, 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]⁻. Thus, the molecular weight of this compounds was $M = 1089$. The ¹³C, HSQC and
231 HMBC spectra, showed 59 carbon signals. It was possible to discern 4 ketone group

232 (δ_c 186.72 to 207.23), 3 hydroxyl group (δ_c 78.90 to 155.90), 11 ether function (δ_c 67.51 to
233 99.05), 24 sp^2 -hybridized carbons (δ_c from 107.00 to 144.00) and 11 sp^3 -hybridized carbons
234 (δ_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 (δ_c 186.72 to 207.23), 3 hydroxyl group (δ_c 78.90 to 155.90), 11 ether function (δ_c 67.51 to
247 99.05), 22 sp^2 -hybridized carbons (δ_c from 107.00 to 142.89) and 13 sp^3 -hybridized carbons
248 (δ_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 and 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

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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.

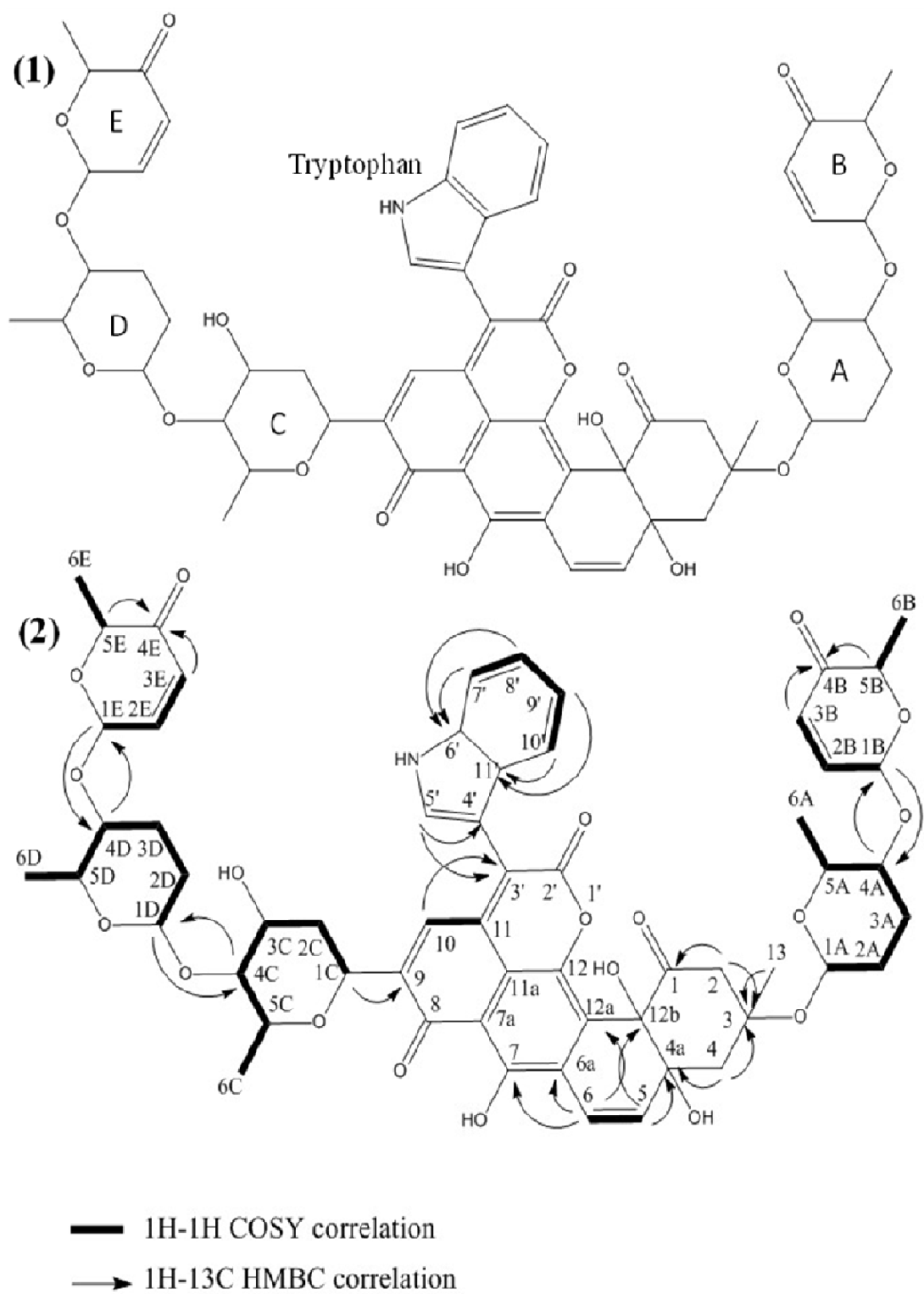


Fig. 1. Structure of mzabimycin A (X4 compound) (1) and HMBC and COSY correlations (2).

A and D, rhodinosose; B and E, aculosose; C, olivose.

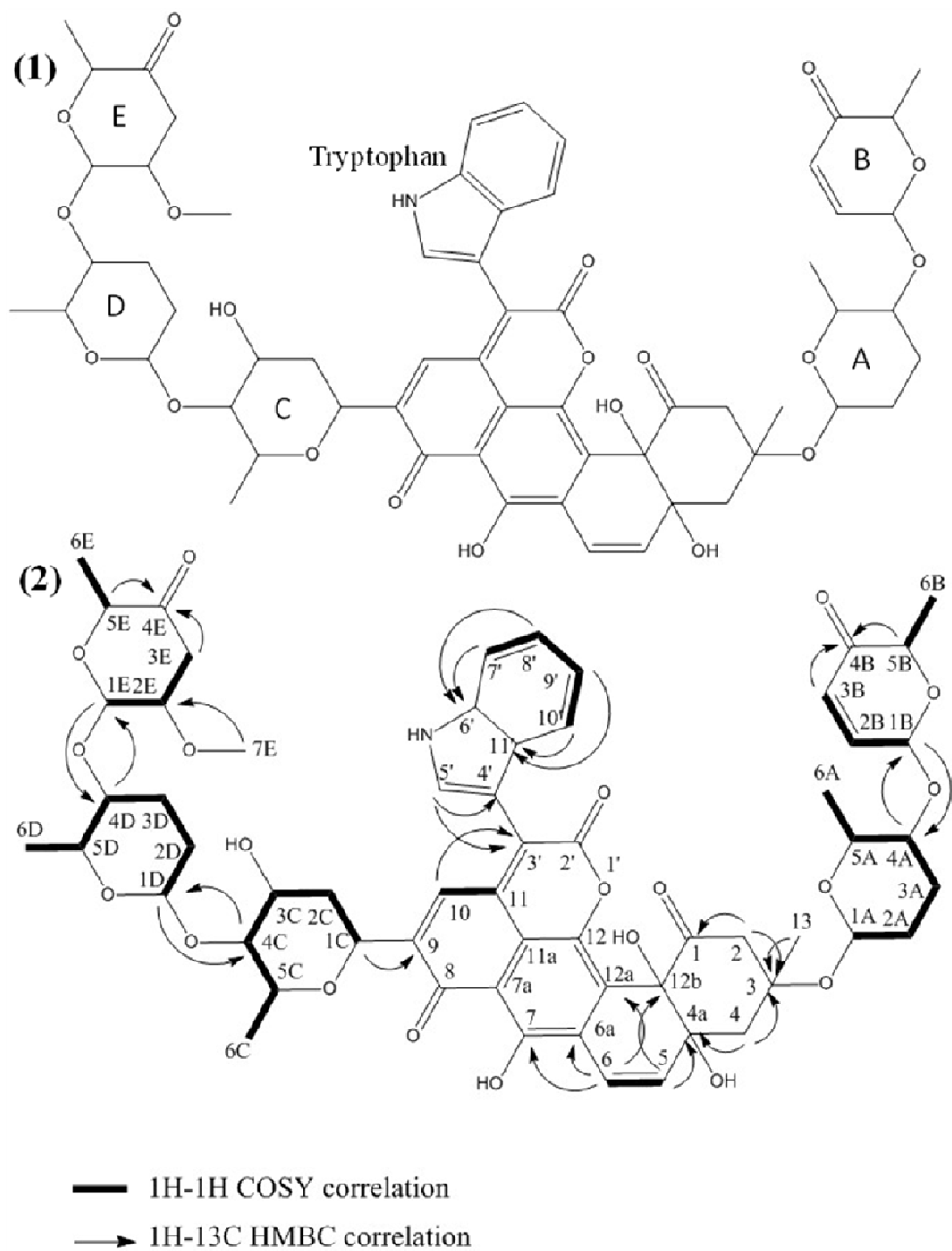


Fig. 2. Structure of mzabimycin B (X3 compound) (1) and HMBC and COSY correlations (2).

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

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

^1H and ^{13}C NMR data assignments of X3 (mzabimycin B) and X4 (mzabimycin A) in CD_3CN at 298 K. See Figs. 1 and 2 for numbering of hydrogen and carbon atoms.

^1H and ^{13}C number	^1H chemical shift, ppm				^{13}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;