



## Original article

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

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## ARTICLE INFO

## Article history:

Received 16 December 2018

Accepted 12 June 2019

Available online 13 June 2019

## Keywords:

Antimicrobial compounds

Angucycline antibiotics

L-Tryptophan

Synthetic medium

*Streptomyces*

## ABSTRACT

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

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## 1. Introduction

Actinobacteria are Gram-positive bacteria with a genomic guanine-cytosine content higher than 55%, and most of them are mycelial. These bacteria are very interesting due to their large capacity to produce secondary metabolites with diversified chemical structures (Kemung et al., 2018; Takahashi and Nakashima, 2018). They are well-known for the production of antibacterial and antifungal antibiotics and are the source of nearly 45% of the

known molecules of microbial origin (Solecka et al., 2012) and 70% of actively marketed molecules (Solanki et al., 2008). However, they are also known for the production of diverse bioactive molecules such as antivirals, antiparasitics, immunostimulants and immunosuppressants (Solecka et al., 2012; Flatt et al., 2013; Nakae et al., 2013; Takahashi and Nakashima, 2018).

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

Considering the increasing resistance of pathogenic microorganisms to antibiotics (Messai et al., 2008; Fair and Tor, 2014; Li and Webster, 2018), and the toxicity of several antibiotic compounds (Berdy, 2005), it is essential to perpetuate research on

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Peer review under responsibility of King Saud University.



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antibiotics in the hope of finding new effective and less toxic molecules in order to control pathogenic microorganisms.

Our previous works have already demonstrated the richness and biodiversity of actinobacteria in the Saharan soils of Algeria. These studies have led to the discovery of several novel interesting antibiotics (Zitouni et al., 2004a; Yekkour et al., 2015; Khebizi et al., 2018; Lahoum et al., 2019) and several new species of actinobacteria (Aouiche et al., 2015a; Bouras et al., 2015; Chaabane Chaouch et al., 2017). The actinobacterium strain PAL114 was isolated from Saharan soil collected from Ghardaïa province, Mزاب region, south Algeria (Aouiche et al., 2014). This strain exhibited a strong antagonistic potential against several microorganisms and was found to be a producer of four bioactive molecules, saquayamycins A and C (Aouiche et al., 2014), and chaetoglobosin A and vineomycin A1 (Aouiche et al., 2015b), which were yellow and extracellular, and were produced in complex ISP2 broth medium (Shirling and Gottlieb, 1966).

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

## 2. Materials and methods

### 2.1. Actinobacterium strain and target-microorganisms

The actinobacterium strain PAL114 was isolated from a Saharan soil in Béni Isguen, Ghardaïa province, Mزاب region, southern Algeria (Aouiche et al., 2014). Based on a polyphasic study, this strain was linked to the species *Streptomyces griseoflavus* (Aouiche et al., 2015b). The strain was cultivated on ISP2 medium (Shirling and Gottlieb, 1966) composed of malt extract (10 g/l), yeast extract (4 g/l), glucose (4 g/l) and agar (20 g/l). The pH of the medium was adjusted to 7.2. The aerial and substrate mycelia were grey and brownish-yellow, respectively. In ISP2 broth, PAL114 strain grows by forming pellets that are pale brownish-yellow in color.

The target-microorganisms included Gram-positive and Gram-negative bacteria, a yeast and filamentous fungi. They are mostly pathogenic or toxigenic for humans, and many of them have multiple antibiotic resistance (Table 1). Indeed, the strains of *Staphylococcus aureus* MRSA 639c, *S. aureus* S1, *Pseudomonas aeruginosa*

**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: fosfomicin; FOX: ceftoxitin; ITR: itraconazole; GEN: gentamicin; K: kanamycin; NEO: neomycin; NYS: nystatine; LIN: lincomycin; OXA: oxacillin; PEN: penicillin G; PRL: pirlimycin; PIP: piperacillin; SPI: spiramycin; SSS: sulfamide; TE: tetracycline; TER: terbinafine; TIC: ticarcillin; TIZ: thioconazole; TOB: tobramycin; VAN: vancomycin.

IPA1 and *Candida albicans* M3 were isolated from sick patients in Algerian hospitals.

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

Strain PAL114 was grown in two synthetic media, M1 and M2. Both of these media 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>, 0.5 g MgSO<sub>4</sub>, 7 H<sub>2</sub>O and 2 g 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 M2 medium contains 0.05% (w/v) of L-tryptophan as nitrogen sources. The final pH of the media was adjusted to 7.2. The production of bioactive compounds was conducted in these two media. A seed culture was prepared with the same medium and used to inoculate (for each medium) sixteen 500 ml Erlenmeyer flasks, each containing 100 ml of culture media. The cultures were incubated on a rotary shaker (250 rpm) for 10 days at 30 °C. The extraction of the active compounds was carried out after centrifugation (5000g, 20 min) of the culture broth to eliminate cells. Half of the cell-free supernatant was extracted with the same volume of dichloromethane and the other half with *n*-butanol. These two solvents were chosen because they extract the antibiotics (saquayamycins A and C, chaetoglobosin A and vineomycin A1) secreted by strain PAL114 (Aouiche et al., 2014; Aouiche et al., 2015b). Extraction of antibiotics from the mycelium was carried out according to the method of Mechlinski (1978). After centrifugation of the cultures, the mycelium was collected and washed several times with distilled water. Ten grams of wet mycelium were extracted with 500 ml of methanol, stirring for 2 h at room temperature. The organic layers (dichloromethane, *n*-butanol and methanol extracts) were dehydrated with Na<sub>2</sub>SO<sub>4</sub> and concentrated to dryness by a rotary evaporator under a vacuum at a temperature lower than 40 °C. The residues of each extract were dissolved in 1 ml of methanol and subjected to biological assay (paper disk of 6 mm in diameter, Institute Pasteur) against the ten target-microorganisms listed in Table 1.

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

### 2.3. Structure determination of the antibiotics

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

<sup>1</sup>H and <sup>13</sup>C NMR spectroscopy were used for the characterization of compounds X3 and X4. NMR samples were prepared by dissolving 5 mg of X3 and X4 compounds in 600 μl of CD<sub>3</sub>CN. All spectra were recorded on a Bruker Avance 500 spectrometer equipped with a 5 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 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 secondary standard. The temperature was set at 298 K. All the <sup>1</sup>H and <sup>13</sup>C signals were assigned on the basis of chemical shifts, spin-spin coupling constants, splitting patterns and signal intensities, and by using <sup>1</sup>H–<sup>1</sup>H COSY45, <sup>1</sup>H–<sup>13</sup>C HSQC and <sup>1</sup>H–<sup>13</sup>C HMBC experiments. Gradient-enhanced <sup>1</sup>H COSY45 was realized included

36 scans for per increment.  $^1\text{H}$ – $^{13}\text{C}$  correlation spectra using a gradient-enhanced HSQC sequence (delay was optimised for  $^1\text{J}_{\text{CH}}$  of 145 Hz) was obtained with 200 scans per increment. Gradient-enhanced HMBC was performed allowing 62.5 ms for long-range coupling evolution (340 scans were accumulated). Typically, 2048 t2 data points were collected for 256 t1 increments.

#### 2.4. Determination of minimum inhibitory concentrations

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

### 3. Results and discussion

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

After four days of fermentation in M1 and M2 media, the culture filtrates were separated from the mycelial biomass by centrifugation, and then extracted with dichloromethane and *n*-butanol. The extracts of culture filtrates from both media were brownish yellow and inactive against all target-microorganisms. The mycelial biomass and the corresponding methanolic extracts were pale brownish yellow from M1 medium, which contains  $\text{NH}_4\text{SO}_4$ , whereas they were dark purplish blue from M2 medium containing L-tryptophan. Furthermore, this dark purplish blue methanolic extract was active against Gram-positive bacteria (*B. subtilis* ATCC 6633, *M. luteus* ATCC 9314, *L. monocytogenes* ATCC 13932, *S. aureus* MRSA 639c and *S. aureus* S1) but not against Gram-negative bacteria (*E. coli* E52, *P. aeruginosa* IPA1), yeast (*C. albicans* M3) and filamentous fungi (*A. carbonarius* M333 and *U. ramanniana* NRRL 1829), whereas, the extract from M1 medium was found to be inactive against all target-microorganisms. The active dark purplish blue extract, obtained from M2 medium, was analyzed by HPLC. Two active fractions against Gram-positive bacteria (cited above) were detected and named X3 (retention time, 64.7 min) and X4 (retention time, 65.19 min), with the latter being predominant ([Supplementary data – Fig. 1S](#)).

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

Through these results, it appears that strain PAL114 produces bioactive compounds with different chemical structures depending on the culture conditions. Several previous works showed the ability of strains to produce many secondary metabolites with related chemical structures depending on the available precursors. Indeed,

the results of [Rohr et al. \(1989\)](#) on the biosynthesis of urdamycins (angucycline antibiotics) by *Streptomyces fradiae* showed that this species used different labeled precursors to produce different urdamycin molecules. Thus, this species uses the 2-methyl-tryptophan as precursor to produce urdamycin D, the tyrosine to produce urdamycin C and the acetate to produce urdamycin A. All these molecules have the same central chromophore. Similar results were obtained with *Saccharothrix algeriensis* NRRL B-24137, which produces five dithiolopyrrolone antibiotics in ISP2 medium ([Lamari et al., 2002](#)) and several other dithiolopyrrolone molecules, in semi-synthetic medium, induced by the addition of organic acids and amino acids as precursors ([Bouras et al., 2008; Merrouche et al., 2010; 2011](#)). [Lam et al. \(2001\)](#) reported a similar approach of using precursor-directed biosynthesis to produce novel fluoroindolocarbazoles A and B by adding DL-6-fluorotryptophan, and fluoroindolocarbazole C by adding DL-5-fluorotryptophan in cultures of *Saccharothrix aerocolonigenes* ATCC 39243. This is particularly interesting research strategy for producing new antibiotic molecules.

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

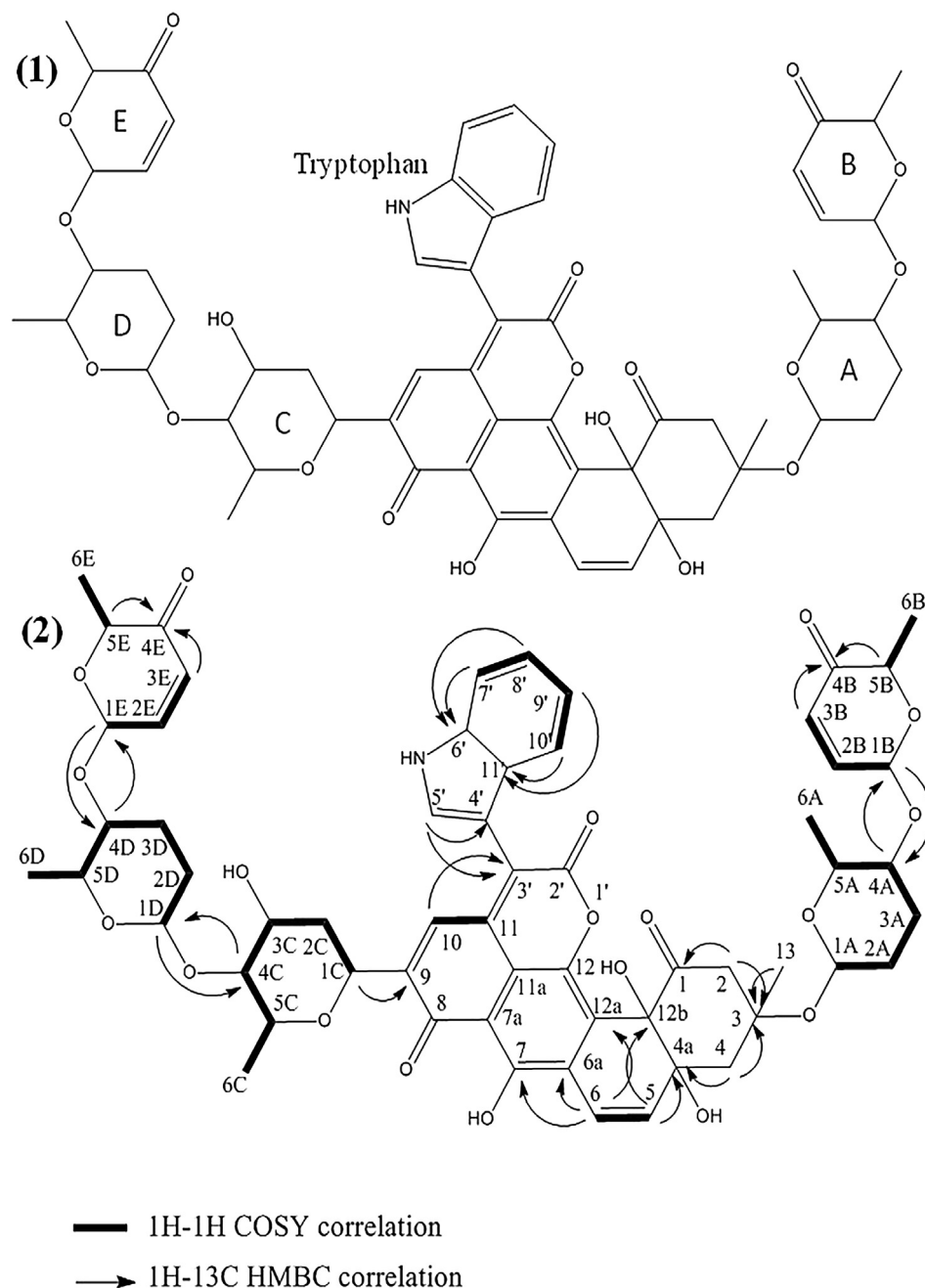
The structure of the compounds X3 and X4 was determined by NMR and mass spectrometry. The results showed that these two compounds are novel antibiotics belonging to the angucycline family. They were named mzabimycin A (for the major compound X4) and mzabimycin B (for the minor compound X3), with reference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

Mzabimycin A (X4) was obtained as a purplish blue powder. The UV-visible spectrum ([Supplementary data – Fig. 2S](#)) showed the maximal absorbance at 218, 325 and 575 nm. The ESIMS spectrum ([Supplementary data – Fig. 3S](#)) contained an ion peak at  $m/z$  1088.31  $[\text{M} - \text{H}]^-$ . Thus, the molecular weight of this compounds was  $M = 1089$ . The  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts are given in [Table 2](#). The  $^{13}\text{C}$ , HSQC and HMBC spectra, showed 59 carbon signals. It was possible to discern 4 ketone group ( $\delta_c$  186.72 to 207.23), 3 hydroxyl group ( $\delta_c$  78.90 to 155.90), 11 ether function ( $\delta_c$  67.51 to 99.05), 24  $\text{sp}^2$ -hybridized carbons ( $\delta_c$  from 107.00 to 144.00) and 11  $\text{sp}^3$ -hybridized carbons ( $\delta_c$  14.04 to 42.61). The 2D  $^1\text{H}$ – $^1\text{H}$  and  $^1\text{H}$ – $^{13}\text{C}$  experiments and especially the long range  $^1\text{H}$ – $^{13}\text{C}$  couplings observed in the HMBC spectrum (see [Fig. 1](#)) permitted to establish the connectivity between all the groups of the molecule.

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

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

The NMR data ([Supplementary data – Fig. 5S](#)) showed that mzabimycin B represent a novel antibiotic belonging to the angucycline family. This compound has a central chromophore typical of

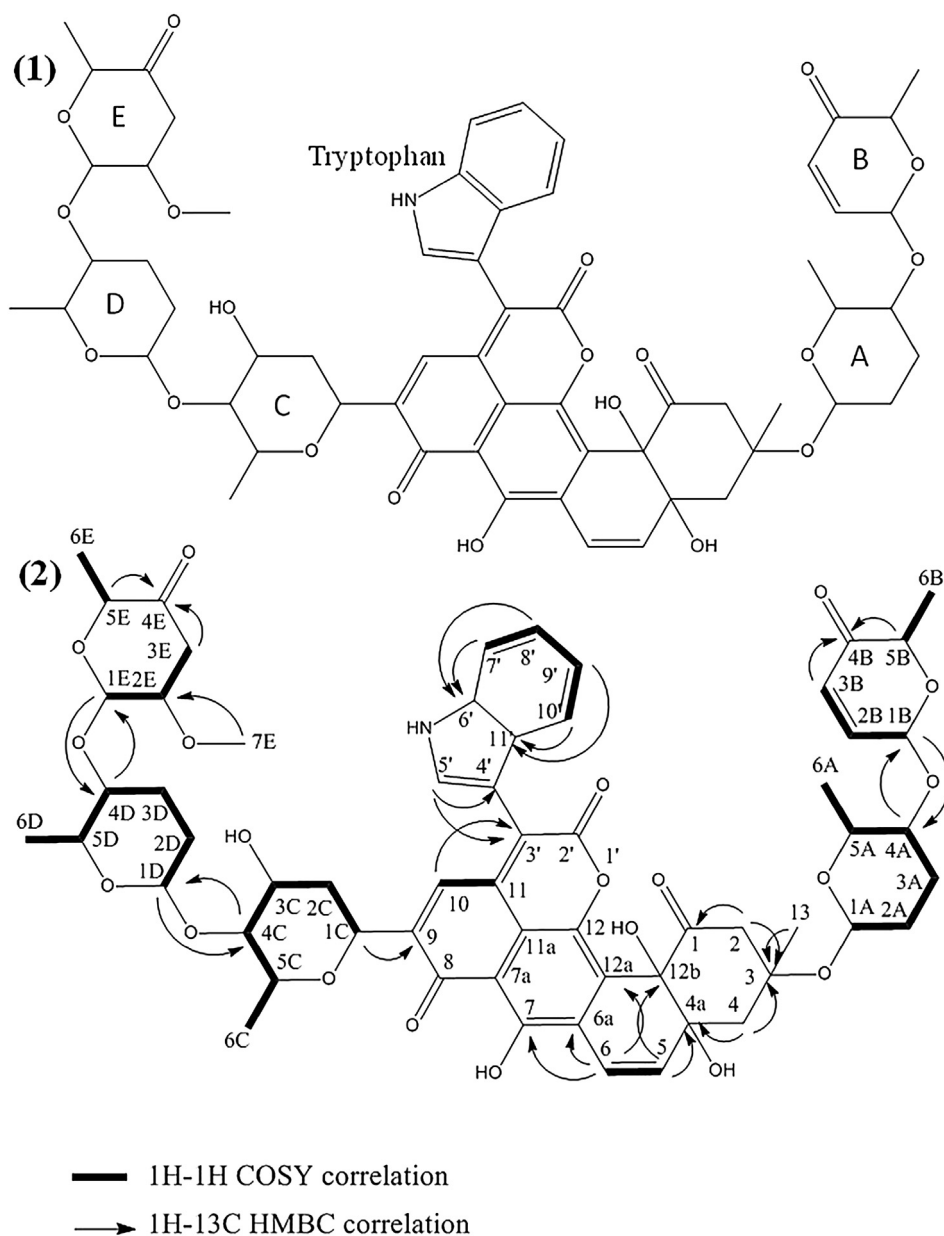


**Fig. 1.** Structure of mzabimycin A (X4 compound) (1) and HMBC and COSY correlations (2). A and D, rhodinose; B and E, aculose; C, olivose.

angucycline compounds, but with L-tryptophan linked to carbon number 3', five sugars, two rhodinoses, one olivose, one aculose and one reduced and methoxylated aculose. These sugars were linked to the central chromophore at carbon number 3 (rhodinose and aculose) and carbon number 9 (olivose, rhodinose and reduced and methoxylated aculose). It differs from mzabimycin A only by the second aculose molecule that is reduced and methoxylated.

The structure of mzabimycins A and B do not correspond to any structure reported in the literature, notably in the [www.sciencefinder.com](http://www.sciencefinder.com) and [www.chemspider.com](http://www.chemspider.com) databases, or antibiotics described in The Dictionary of Natural Products (Buckingham, 1997), or in Berdy's review of bioactive microbial metabolites (Berdy, 2005). The mzabimycins A and B are, therefore, two new angucyclines that possess a chromophore containing

L-tryptophan and osidic derivatives. These two compounds have the same central chromophore, which is similar to that of the urdamycin D (Rohr et al., 1989) and the langkocyclines B1 and B2 (Kalyon et al., 2013). Furthermore, the urdamycin D (Drautz et al., 1986) and the langkocyclines B1 and B2 (Kalyon et al., 2013) are purple blue and have L-tryptophan linked to carbon number 3' of the central chromophore, like mzabimycins A and B. However, langkocycline B1 has four sugars (two rhodinoses and two olivoses) and langkocycline B2 has five sugars (three rhodinoses and two olivoses); these sugars are linked to carbon number 12b of the central chromophore. Urdamycin D contained one olivose linked to carbon number 12b of the central chromophore and two olivoses and one rhodinose linked to carbon number 9 of the chromophore. Mzabimycins A and B differ from



**Fig. 2.** Structure of mzabimycin B (X3 compound) (1) and HMBC and COSY correlations (2). A and D, rhodinose; B, aculose; C, olivose; E, reduced and methoxylated aculose.

these antibiotics in the number and the composition of sugars (presence of aculose and reduced and methoxylated aculose) and in the linkage to the central chromophore. Mzabimycins A and B contain in their structure identical sugars to those of vineomycin A1, a yellow extracellular angucycline also produced by strain PAL114, not in synthetic media M1 and M2, but in complex ISP2 medium (Aouiche et al., 2015b). However, there are some differences in the central chromophore structure and the absence of L-tryptophan. These results showed some similarities between the biosynthesis processes of vineomycin A1 and mzabimycins A and B.

The production of antibiotics belonging to the families of angucyclines and anthracyclines (close to angucyclines) has already been demonstrated in some strains of actinobacteria isolated from Saharan soils as strain PAL114. This is the case of the antibiotic R2 secreted by *Streptosporangium* sp. Sg3 (Boudjella et al., 2010) and mutactimycins C and PR secreted by *Saccharothrix* sp. SA103 (Zitouni et al., 2004b).

### 3.3. Minimum inhibitory concentrations

Minimum inhibitory concentrations (MICs) of mzabimycin A (X4) and mzabimycin B (X3), purified by HPLC, are summarized in Table 3. The results showed that these compounds have very similar activity directed only against Gram-positive bacteria. The strains of *Micrococcus luteus* (MIC, 15 µg/ml for mzabimycin A and B) and *Listeria monocytogenes* ATCC 13932 (MICs, 20 µg/ml for mzabimycin B and 40 µg/ml for mzabimycin A) were the most sensitive. The other Gram-positive bacteria, including the two strains of *Staphylococcus aureus* (60–80 µg/ml) and *Bacillus subtilis* ATCC 6633 (50 µg/ml), were found to be less sensitive. All tested Gram-negative bacteria, yeasts and filamentous fungi were resistant (>100 µg/ml).

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

**Table 2**  
<sup>1</sup>H and <sup>13</sup>C NMR data assignments of X3 (mzabimycin B) and X4 (mzabimycin A) in CD<sub>3</sub>CN at 298 K. See Figs. 1 and 2 for numbering of hydrogen and carbon atoms.

<sup>1</sup> H and <sup>13</sup> C number	<sup>1</sup> H chemical shift, ppm		<sup>13</sup> C chemical shift, ppm	
	X3	X4	X3	X4
1	–	–	–	–
2	3.03	(m,2H)	3.03	(m,2H)
3	–	–	–	–
4	1.97–2.31	(d,15.6,2H)	1.97–2.30	(d,15.6,2H)
4a	–	–	–	–
5	6.09	(d,10.0,1H)	6.09	(d,10.0,1H)
6	7.03	(d,10.0,1H)	7.03	(d,10.0,1H)
6a	–	–	–	–
7	–	–	–	–
7a	–	–	–	–
8	–	–	–	–
9	–	–	–	–
10	8.05	(s,1H)	8.05	(s,1H)
11	–	–	–	–
11a	–	–	–	–
12	–	–	–	–
12a	–	–	–	–
12b	–	–	–	–
13	1.33	(m,3H)	1.32	(m,3H)
1'	–	–	–	–
2'	–	–	–	–
3'	–	–	–	–
4'	–	–	–	–
5'	7.83	(s,1H)	7.83	(s,1H)
6'	–	–	–	–
7'	7.59	(d,7.8,1H)	7.59	(d,7.8,1H)
8'	7.29	(t,7.8,1H)	7.29	(t,7.8,1H)
9'	7.19	(t,7.8,1H)	7.19	(t,7.8,1H)
10'	7.53	(d,7.8,1H)	7.53	(d,7.8,1H)
11'	–	–	–	–
1A	5.26	(m,1H)	5.25	(m,1H)
2A	1.98	(m,2H)	1.98	(m,2H)
3A	1.94–1.98	(m,2H)	1.94–1.98	(m,2H)
4A	3.72	(m,1H)	3.71	(m,1H)
5A	4.14	(m,1H)	4.14	(m,1H)
6A	1.24	(d,6.5,3H)	1.24	(d,6.5,3H)
1B	5.30	(dd,9.0–3.4,1H)	5.30	(dd,9.0–3.4,1H)
2B	6.07	(d,9.0,1H)	6.07	(d,9.0,1H)
3B	7.00	(d,9.0,1H)	7.00	(d,9.0,1H)
4B	–	–	–	–
5B	4.60	(d,4.0, 1H)	4.60	(d,4.0, 1H)
6B	1.31	(d,4.0, 3H)	1.31	(d,4.0, 3H)
1C	4.73	(d,11.0,1H)	4.73	(d,11.0,1H)
2C	1.26–2.41	(m,2H)	1.72–2.41	(m,2H)
3C	3.74	(m,1H)	3.74	(m,1H)
4C	2.85	(m,1H)	2.86	(m,1H)
5C	3.43	(m,1H)	3.43	(m,1H)
6C	1.07	(dd,6.0–1.8,3H)	1.06	(dd,6.0–1.8,3H)
1D	4.89	(m,1H)	4.88	(m,1H)
2D	1.60–1.94	(m,2H)	1.60–1.99	(m,2H)
3D	1.90–1.93	(m,2H)	1.90–1.93	(m,2H)
4D	3.75	(m,1H)	3.72	(m,1H)
5D	4.23	(m,1H)	4.22	(m,1H)
6D	1.93	(dd,8.7–2.0,3H)	1.71	(dd,7.9–3.5,1H)
1E	4.96	(m,1H)	5.30	(m,1H)
2E	3.81	(m,1H)	6.07	(d,9.0,1H)
3E	2.54–2.83	(dt,15.4–4.8,2H)	7.00	(d,9.0,1H)
4E	–	–	–	–
5E	4.29	(m,1H)	4.60	(m,1H)
6E	1.19	(d,3.6,3H)	1.30	(m,3H)
7E	1.45	(d,5.5,3H)	–	–

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 mzabimycins A and B.

Angucycline antibiotics are a group of biologically active compounds with interesting activities including antibacterial, antifungal and antiviral (Kharel et al., 2012), enzyme inhibitory (Eguchi et al., 2017), and platelet aggregation inhibitory properties (Kawashima et al., 1989). Therefore, they are cytotoxic, and some molecules were used as anticancer agents in chemotherapy

(Abdelfattah et al., 2008). Yu and O'Doherty (2008) showed the role of vineomycin B2 trisaccharide, consisting of aculose, rhodnose and olivose, in anticancer activity (against a panel of cancer cell lines). The same authors also showed the role of antibiotic PI-080 trisaccharide, consisting of aculose and two olivoses, in anticoagulant activity.

**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 (µg/ml) <sup>*</sup>	
	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

<sup>\*</sup> MIC values represent the mean of two replicates;

#### 4. Conclusion

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

#### Declaration of Competing Interest

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

#### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jsps.2019.06.004>.

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