

Novel Production of Two New Nonpolyenic Antifungal Macrolide Derivatives by *Streptomyces* Z26 Isolated from Moroccan Rhizospheric Soil

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Abstract: The aim of our study was to screen Moroccan actinomycete isolates able to produce nonpolyenic antifungal metabolites in order to contribute to limiting the problem of fungal infections emergence in particular mycotic diseases and to discover known antifungal families, especially nonpolyenic drugs. 480 isolates were tested against 5 clinically pathogenic *Candida* species. Several methods have been used to study the polyenic or non-polyenic nature of the antifungal molecules produced by Actinobacteria (i) The study of the antibacterial activity (the bacterial plasma membrane is devoid of sterols); (ii) The screening of the antimicrobial activity of resistant strains to polyenic antifungals essentially *Candida tropicalis* R2 and *Pythium irregular*; (iii) The inhibition of antifungal activity by exogenous ergosterol addition in the culture medium and (iv) The UV-Visible light spectrophotometric analysis of the crude extracts of the actinomycete isolates. Among all isolate tested, only 60 (29 %) showed an antifungal activity against all test microorganisms used. Six active isolates meet all the selection criteria and produced nonpolyenic antifungal metabolites. The taxonomic study of the promising isolate Z26, using morphological, physiological and molecular characters, showed that it has 99,43% of similarity with *Streptomyces phytohabitans* but there were some differences in morphological characteristics. In addition, the chemical study, using chromatographic and spectroscopic techniques, of the bioactive substances produced in the Z26 isolate fermentation broth in NL300 culture medium allowed the determination of two macrolide derivatives with chemical structures $C_{62}H_{100}O_{24}(m/z\ 1251.6504\ [M+Na]^+)$ and $C_{68}H_{110}O_{26}(m/z\ 1365.7174\ [M+Na]^+)$. NMR experiments revealed that the active compounds were Novonestmycin A and B. The Novonestmycins A and B are for the first time produced by an actinomycete strain purely isolated from the Moroccan ecosystems.

Keywords: New Nonpolyenic Antifungal, Clinically Pathogenic Yeasts, Moroccan Actinomycetes, Novonestmycins

Introduction

From the early 1980s, human diseases are mainly caused by the emergence of fungi, especially at the immuno-compromised and those with serious diseases. These fungal infections are essentially caused by *Candida* species. Some study revealed that these

infections have a very considerable mortality and morbidity and it is obvious that fungal diseases have appeared as the most dangerous public health problems in the world (Diekema and Pfaller, 2004; Vandeputte *et al.*, 2012).

A trend analysis of mortality due to infectious diseases reports a high increase in mortality due to

several reasons mainly to mycoses and the majority of these mycoses related deaths were attached with *Candida* species infection (McNeil *et al.*, 2001).

Numerous factors contribute to the increased fungal infections. The most important is an immune-compromised ever-expanding population because of the rupture of the mucosal or cutaneous barrier, defects in cell-mediated immunity or in the function and of number neutrophils and extremes of age (Dutta *et al.*, 2015).

Antifungal compounds are playing an important role to control the mycotic diseases but they are a very small in number but significant group among the different types of dominant drugs in the market (Atta, 2010). However, many substances, polyene ones in particular, cannot be useful in therapy because of their toxicity, while they are very interesting in agriculture, industry and animal therapy, such as amphotericin B (Laniado-Laborín and Cabrales-Vargas, 2009).

The major challenge for the pharmaceutical industry today is to find new, safe, less toxic and more effective non-polyenic antifungal compounds in order to counter the aggressive increase of opportunistic infections. Moreover, the interest in discovering new molecules has increased with the development of new ideas and approaches, such as the use the new sources of bioactive materials, like marine sponges, microorganism of soil and plants from several ecological niches (Behie *et al.*, 2016).

Actinomycetes provide many interesting bioactive substances of high commercial value and continue to be regularly screened for new bioactive molecules (Barakate *et al.*, 2002). Also, according to the pioneering work of Selman Abraham Waksman; they are known for their ability to produce medically useful antibiotics (Gebreyohannes *et al.*, 2013).

Among all the genera of actinomycetes, the genus *Streptomyces* is most found in nature with a high number of strains that can synthesize drugs and bioactive substances (Manivasagan *et al.*, 2013). In fact, 70% of the known antibiotics are produced by this genus (Procópio *et al.*, 2012) and many representatives of this group produce molecules of high commercial value in fields like as anticancer, antitumor, anti-inflammation therapy and are being widely screened for novel bioactive compounds (Manivasagan *et al.*, 2014).

Actinomycetes are used in the fermentation of active pharmaceutical compounds, such as the antifungal ones, anti-cancer, antiviral, agents of immuno-suppressor, weed killers, etc. (Hashimoto, 2007). But, exploring new ecosystems is one of the most interesting ways to isolate new strains of actinomycetes having new antimicrobial activities (Wadetwar *et al.*, 2013).

The objective of the present study was to search the antifungal nonpolyenic compounds produced by actinomycete isolates from different rare Moroccan ecosystems, to do the taxonomic identification of the selected isolate and to purify and elucidate the structure of the active molecules which it produces.

Materials and Methods

Selection of Actinomycete Isolates Producing Antifungal Substances

480 actinomycete isolates, originating from various Moroccan ecosystems, such as rhizospheric soil, endophytic plants, Atlas Mountains soil and Sahara sand, were used in this study. Five clinically yeast species, obtained from the Moroccan Coordinated Collections of Micro-organisms in the National Center for Scientific and Technical Research Morocco (CNRST), were used as test strains (Table 1). The selection of actinomycete strains with antifungal activity was realized by agar diffusion method in Sabouraud medium at 28°C (Ismet *et al.*, 2012).

Isolates were grown on Bennett's agar plates (10 g/L glucose (Merck), then a calibrated agar cylinder (6 mm in diameter) was cut out and placed on Sabouraud agar, which had previously been seeded with each yeast test organism. The plates were kept at 4°C for 4 h, the inhibition zones were measured at 28°C after 24-48 h of incubation (Barakate *et al.*, 2002).

Screening for Actinomycete Isolates Producing Nonpolyenic Antifungal Compounds

The selection of isolates producing only nonpolyenic antifungal metabolites was carried out using four tests:

- Antibacterial activity of actinobacteria isolates was estimated by the agar cylinders method in Mueller-Hinton media (Merck) (Lee and Hwang, 2002), against two Gram negative bacteria (*Pseudomonas aeruginosa* DSM 50090, *E. coli* ATCC 8739) and three Gram positive bacteria (*Staphylococcus aureus* 209 PCIP 53156, *Micrococcus luteus* ATCC381 and *Bacillus cereus* ATCC 14579). These strains were obtained from our laboratory collection. Plates were kept at 4°C for 2-4 h and then incubated at 37°C for 18-24 h. The Inhibition zones were then measured (Lemriss *et al.*, 2003)
- Activity against *Candida tropicalis* R2 DSM11953 and *Pythium Irregular* (CIP18), obtained from the collection of laboratory of Biology and Biotechnology of Microorganisms, Cadi Ayyad University, both are resistant to amphotericin B and nystatin (polyenic antifungals)
- Ergosterol inhibition was tested by both agar diffusion method with or without ergosterol at 50 mg/mL in the presence of *C. albicans* L4 (Ouhdouch *et al.*, 2001). The results were compared with those obtained with amphotericin B at 20 µg/well
- UV- spectra of active extracts dissolved in methanol were recorded at 220-440 nm with a UV spectrophotometer (Double Beam UV6300 PC, VWR) and compared with those of known polyenic antifungal

Table 1: Origin of the clinically yeast strains used as microorganisms test

Yeast strain	Origin
<i>Candida albicans</i> L4	Vaginal sampling
<i>Candida glabrata</i> L7	
<i>Candida krusei</i> L10	Human blood
<i>Candida parapsilosis</i> L18	Human skin
<i>Candida albicans</i> L61	Human esophagus

Morphological, Physiological and Molecular Identification of the Actinomycete Isolate

The morphological proprieties of the isolates were established at 30°C after 3 weeks of incubation on ISP media (International *Streptomyces* Project), ISP1, ISP2, ISP3 and ISP4 media and the production of melanoid pigments was tested at ISP6 and ISP7 agar after 7 days of incubation at 28°C (Ayari *et al.*, 2012).

The capacity of isolates to use various carbohydrates sources was determined on ISP basal medium 9 supplemented with 1% (w/v) of different carbon sources. Petri dishes were incubated at 28° C, the growth was determined after two weeks. The isolate's capacity to use several nitrogen sources was tested using a minimal medium; adding by filtration the sterile nitrogen source (1% w/v) (Benouagueni and Kirane, 2016).

The growth in the presence of different concentrations gradient of NaCl (1.5-30%) was determined on ISP2 agar. The ability, of actinomycete isolate, to grow at pH 5 to 10 and at a temperature range of 25 to 50°C was tested (Benouagueni and Kirane, 2016). All these biochemical and physiological tests were performed and analyzed after 2 weeks. Petri dishes were evaluated visually for little or no observable growth (-), moderate (++) , or excellent growth (+++).

The genomic DNA used for the PCR analysis was prepared from the single colonies grown on Bennett medium for 3 days. The extraction of genomic DNA was conducted using PeqGold Bacterial DNA Kit (Germany) according to the manufacturer instructions. The isolated DNA was checked for purity and quantified according to standard procedures (Kumar *et al.*, 2010).

Two sets of primers, 27F bac (AGAGTTTGATCMTGGCTCAG) and 1492 Rni (TACGGYTACCTTGTTACGACTT), were used in this study for amplification of 16S rRNA gene fragment of the active antibiotic-producing isolates. The final 50 µL volume of reaction mixture contained 1×PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 9.0 at 25°C), DMSO 2 µL, 1 µL each dNTP, 1 µL each primer, 1µL Q5 polymerase and 1 µL of purified DNA.

PCR amplification was carried out using the thermocycler programmed as follows: A hot start of 95°C for 5 min, followed by 30 cycles of amplification at 95°C for 1 min, annealing at 54-55°C for 1 min, extension at 72°C for 2 min and extra extension at 72°C

for 10 min. Finally, the tubes were held at 4°C for direct use, or stored at -20°C until needed.

The PCR product was detected by 1% (w/v) agarose gel electrophoresis and was visualized by Ultraviolet (UV) fluorescence after staining at ethidium bromide (Kathiravan *et al.*, 2016). Electrophoresis was performed out at 110 Volts for 45 minutes. The size of the PCR products was estimated using 1 Kb DNA ladder (Promega, USA).

After sequencing, the RNA 16S sequences of the isolates were compared to those of the NCBI nucleotide database using the BLAST algorithm. The Clustal W is used to align the sequences with the highest similar nucleotide percentage and a phylogenetic tree was constructed using the neighbor-joining method with the Mega 6 software (Suardana, 2014).

Chemical Characterization of Active Compounds

Fermentation and Extraction of Active Molecules

A single colony of the strain Z26 was used as an inoculum of 500 ml Erlenmeyer flask containing 100 mL of NL300 medium (Mannitol 20g (Merck), cotton seed 20g (Pharmamedia, Hartge ingredients, Humburg, adjusted at pH 7.5). The cultures were grown at 28°C for 72 hours under constant shaking (120 rpm) using a rotatory shaker. The flasks containing the culture of Z26 were transferred to inoculate 10L fermentor and the large scale fermentation was performed at an aeration rate of 0.5 vvm and agitation of 1000 rpm for ten days (Boonlarpradab *et al.*, 2011).

About 2% of a filter aid (Hyphlo Supercel®, Johns Mansville) was mixed with the fermentation culture obtained and filtered through a filter press in order to separate the fermentation broth from the mycelium. The culture broth was adjusted to pH 5 (1N HCL) and extracted three time with ethyl acetate. After drying the combined organic phases, using a rotary evaporator, 16.28 g of crude extract could be obtained.

Fractionation, Purification and Chemical Characterization of Purified Compounds

The crude extract of the aqueous phase of the Z26 isolate has been the subject of several purification steps. In fact, precise quantities were separated in the first into several fractions by three different chromatographic methods such as, on Sephadex LH 20 column (90*2.5 cm, flow rate: 0.5 ml/min) (100% MeOH), semi-preparative reverse phase HPLC using C18 column (40g, 50µm, flow rate: 20 ml/min, Merck Germany) (100% MeOH) and Silica gel column (40g, 50µm, flow rate: 20 ml/min, Merck Germany) (100% → 80% CH₂Cl₂-MeOH). Then, an analytical HPLC analysis was performed for all fractions and the fractions obtained with the same HPLC spectrum and the same activity were merged.

The second purification step of the combined fractions was carried out by preparative HPLC using two other columns, namely the PFP column (Luna 5 μ m, 100A, 250*10 mm; flow rate: 4.5 ml/min) (acetonitril (A)/H₂O (B) : 40% (A) → 100% (A) during 50 min, Thereafter 100% (A) from 50,1 min to 55 min then 35% (A) around 60 min) and C18 column (5 μ m, 250*20 mm; flow rate: 4.5 ml/min) (acetonitril (A)/H₂O (B) : 41% (A) → 100% (A) during 60 min, Thereafter 100% (A) from 60,1 min to 65 min and 36% (A) around 70 min). Finally, we obtained 8 pure compounds but only two had a non-polyenic antifungal activity named ZD1 (6.03 mg) and ZD2 (2 mg). The compound structures were fully elucidated by 1D and 2D NMR analyses and the molecular weight was determined using masse spectroscopy (ESI-MS).

Results and Discussion

Screening for Non-Polyenic Antifungal Activity

Several actinomycetes isolates tested in this study appeared to be promising source of antifungal metabolites. Among 480 isolates, more than 28% produced molecules with antifungal activity against one or more *Candida* test strains (Fig. 1). The highest percentage of activity was noted for *Candida albicans* L4 and *C. glabrata* L7 with a percentage higher than 30%. Around 28% was recorded for *C. krusei* L10 and

C. albicans L61; and 23% for *C. parapsilosis* L18. So, the latter is the most resistant of the yeasts used (Fig. 1). However, this percentage is similar to those reported previously (between 10 and 34%) in studies on the antimicrobial activity of soil and aquatic actinomycetes (Ouhdouch *et al.*, 2001).

The selected actinomycete isolates with antifungal activity were used in a secondary screening step for the detection of potential nonpolyenic metabolites. Four assays, which are most commonly carried out, were used.

The antifungal activity was observed in 60 isolates; 14 of them exhibited activity against both *Candida tropicalis* R2 and *Pythium irregulare*. The ability of these isolates to inhibit the growth of some Gram positive bacteria was tested. Using the antibacterial activity against bacteria lacking sterols (target of polyenes) in their cell wall, favors the selection of the non-polyenic substances (Fguira *et al.*, 2002).

According to the data in Table 2 and 3, 6 of these 14 actinomycetes isolates showed high antifungal and antibacterial activity in particular against Gram positive bacteria compared with the Gram negative ones. This different sensitivity could be attributed to morphological and chemical composition differences such as the membrane of Gram positive bacteria having lipopolysaccharides which could make the cell wall impermeable to bioactive substances (Gebreyohannes *et al.*, 2013).

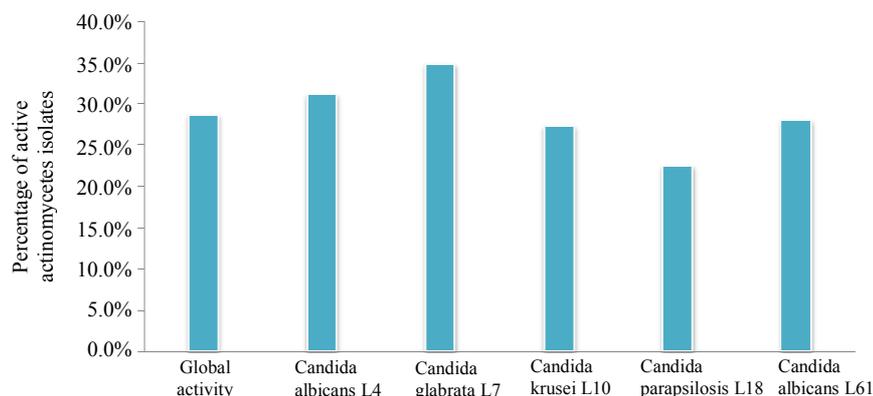


Fig. 1: Percentage of global antifungal activity against yeast test microorganism

Table 2: Antifungal activity of selected actinomycetes isolates against some pathogenic yeast strains*

Reference of isolates	Antifungal activity (in mm) against				
	<i>Candida albicans</i> L4	<i>Candida glabrata</i> L7	<i>Candida krusei</i> L10	<i>Candida parapsilosis</i> L18	<i>Candida albicans</i> L61
Z 26**	17	19	15	15	19
B 39**	19	12	14	19	12
AS25**	13	15	14	13	15
OE 25/1**	15	10	16	15	10
B 53**	15	14	11	14	13
B 19**	14	11	12	11	12

*The yeast strains were provided by the Moroccan Co-ordinated Collection of Microorganisms

**Reference number in the collection of actinomycetes of the “Laboratory of biology and Biotechnology of microorganisms, Semlalia Faculty of Sciences Marrakesh, Cadi Ayyad University Morocco”

Table 3: Results of four step screening method for potential non-polyenic antifungal actinomycete isolates

Isolates	Antibacterial activity					Antifungal activity			Ergosterol effect **		UV-visible spectra
	<i>S. aureus</i>	<i>M. luteus</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. tropicalis</i> R2	<i>P. irregulare</i>	without	with		
Z 26*	+	+	+			+	+	17	16	NP	
B 39*	+	+	+			+	+	19	19	NP	
AS 25*	+	+	+			+	+	13	12	NP	
OE 25/1*	+	+	+			+	+	15	13	NP	
B 53*	+	+	+	+		+	+	15	16	NP	
B 19*	+	+	+			+	+	14	8	NP	
Amphotericin B***	ND	ND	ND	ND	ND			16	10	P	

*Reference number in the collection of actinomycetes of the “Laboratory of biology and Biotechnology of microorganisms, Semailia Faculty of Sciences Marrakesh, Cadi Ayyad University Morocco

**Inhibition zones in mm with or without ergosterol at 50 mg/ml in Sabouraud medium

***20 µg/well or disc

NP: Non Polyenic; P: Polyenic

The plasma membrane of yeast is composed of sterols which are the targets of polyenic antifungal substances; hence, the incorporation of exogenous ergosterol in the culture medium interferes with the activity of the potential polyenic substances produced by the isolates and reduces the inhibition diameters in the second screening assay. For nonpolyenic molecules, the diameter of the inhibition zones remains constant because no interaction occurs (Thakur *et al.*, 2007). All isolates except B19 showed a reduction in the diameters of the inhibition zones (Table 3).

Spectroscopic techniques have been used to distinguish between polyenic and non-polyenic compounds (Saadoun *et al.*, 2009). The analysis of the UV spectra of the active extracts showed no polyenic absorption bands (Table 3) with a characteristic series of peaks between 260 and 405 nm (Boussaber *et al.*, 2014).

On the other hand, B19 isolate showed the first criteria of strain with nonpolyenic antifungal and its extract did not show polyene type UV-visible spectra. However, the size of the inhibition zones was significantly reduced after exogenous ergosterol addition. This could be explained by a coproduction of polyene and nonpolyene compounds by the same strain (Lemriss *et al.*, 2003).

After the second screening, the selected actinomycete isolate Z26 showed a strong non-polyenic antifungal activity against all test microorganisms. Thus it was further characterized based on, morphological, physiological appearance and molecular characterization.

Taxonomy of the Selected Isolate Z26

The isolate Z26 showed abundant mycelia and good growth on all media used after 2 weeks at 30°C, except in ISP 1, in which slow growth was observed (Table 4). The aerial mycelium was yellowish white on ISP 1 and grayish white on ISP 2, ISP 3, ISP 4 and Bennett. The

substrate mycelium was yellowish white on ISP1, ISP3 and Bennett, but light green on ISP2 and ISP4. Diffusible and melanoid pigments were produced only on ISP 1 and ISP 6 respectively.

The physiological properties of the isolate Z26 are shown in Table 5. Its optimal growth was observed at 28-30°C and between pH 6 and 7. It was able to grow from 1.5% of NaCl but not higher than 7%. It was able to hydrolyze D-Arabinose, Glucose, L-Rhamnose, Mannitol and L-Proline, but not Fructose and L-Asparagine.

Based on the molecular identification of 16S RNA sequence, the morphological and the chemical characteristics, the isolate Z26 belonged to the genus *Streptomyces*. Its position at the 16S rDNA *Streptomyces* tree is shown in Fig. 2. The identification level was 99.43% with *Streptomyces phytohabitans*, but strains showing a 16S RNA sequence identity of 100% do not automatically belong to the same species (Whitman *et al.*, 2012).

However, our isolate could be distinguished from *Streptomyces phytohabitans* by some phenotypic properties, for example, *Streptomyces phytohabitans* produces a pink diffusible pigment but our strain produces a light green one. There are additional differences in several morphological properties, including color of substrate and aerial mycelia (Guang-Kai *et al.*, 2012).

Structural Elucidation of the Two Molecules ZD1 and ZD2

Compound ZD1 was purified as a white amorphous powder with a molecular formula C₆₂H₁₀₀O₂₄ determined on the basis of the results of HRMS data analysis (*m/z* 1251.6504 [M+Na]⁺, calculated for C₆₂H₁₀₀O₂₄Na: 1251.6497 g/mol). It is easily soluble in MeOH and it has 10.594 min as retention time and maxima in the UV spectrum at 222, 260, 295 nm (Fig. 3 and 4).

Table 4: Differential phenotypic characteristics of *Streptomyces* sp. Z26 on different ISP media after 2 weeks of incubation at 30°C

Medium	Growth *	Aerialmycelium	Substratemycelium
Tryptone-yeast extract agar (ISP1)	+	Yellowish white	Yellowish white
Yeast extract-malt extract agar(ISP2)	+++	Grayish white	Light green
Oatmeal agar(ISP3)	+++	Grayish white	Yellowish white
Inorganic salt-starch agar(ISP4)	+++	Grayish white	Light green
Bennett agar	+++	Grayish white	Yellowish white

*: +++ Abundant, ++ moderate and + less

Table 5: Physiological properties of *Streptomyces* sp. Z26 and *S. phytohabitans* using different criteria

Characteristics	<i>Streptomyces</i> sp. Z26	<i>S. phytohabitans</i>
Growthat 25-37°C	+	+
Growthat pH 9.0	-	-
NaCl tolerance 1.5-7% (optimal)	+	+
utilisation of:		
D - Arabinose	+	+
Glucose	+	+
L-Rhamnose	+	+
Mannitol	+	+
Fructose	-	-
L-Asparagine	-	-
L-Proline	+	ND

Tests: + positive, - negative.

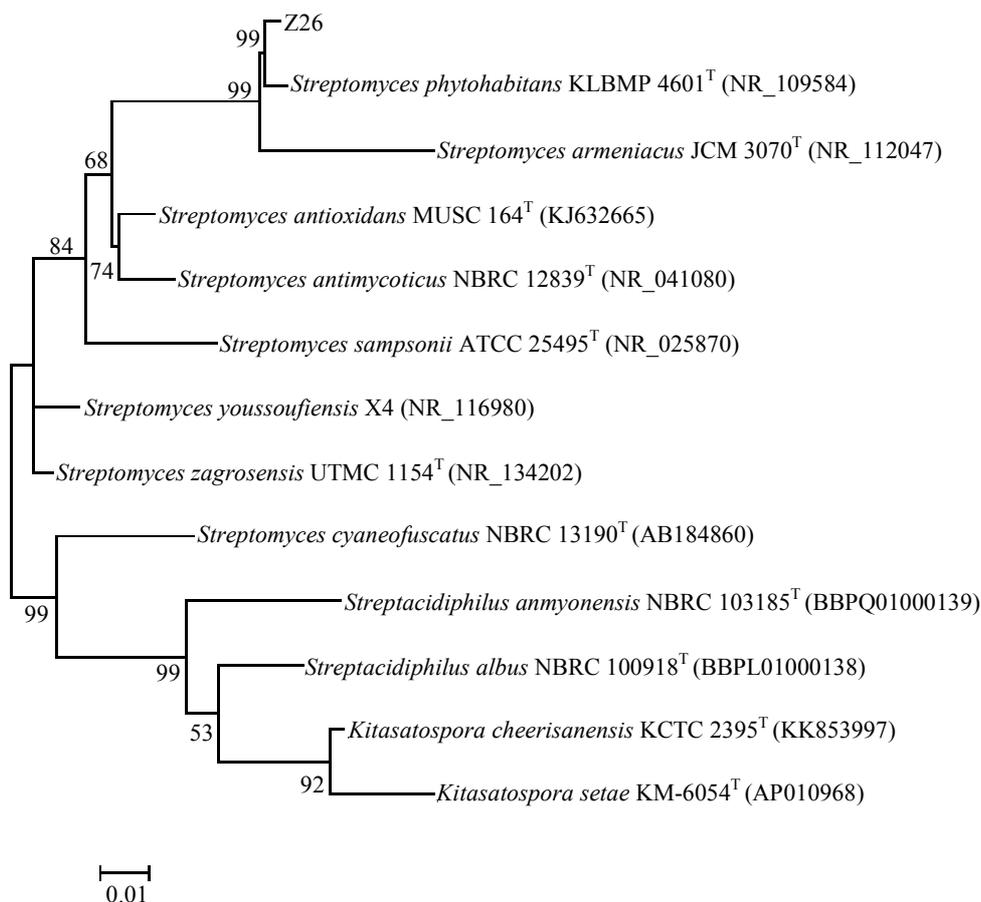


Fig. 2: Phylogenetic tree based on 16S rRNA gene sequence showing the relations between strain Z26 and type species of the genus *Streptomyces*. The numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 1000 resampled data sets (only values >50% are shown). Bar, 0.01 substitution per nucleotide position

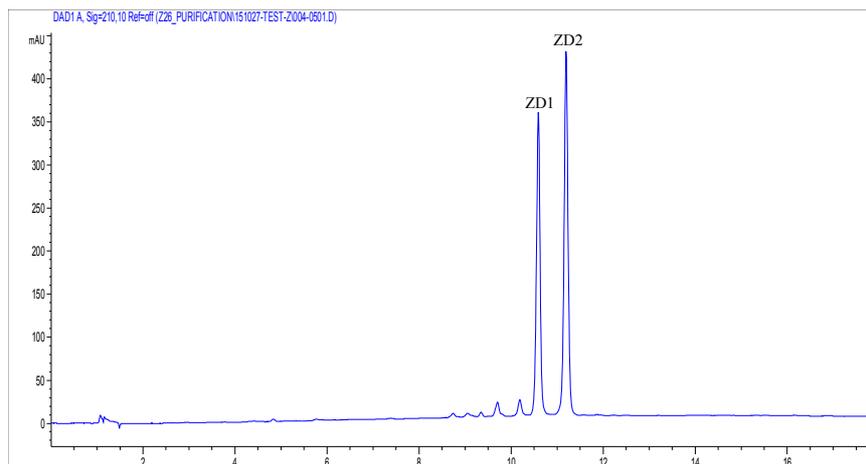


Fig. 3: Analytical HPLC Spectrum of the two derivatives of Novonestmycin A (ZD1) and Novonestmycin B (ZD2) monitored at 210 nm

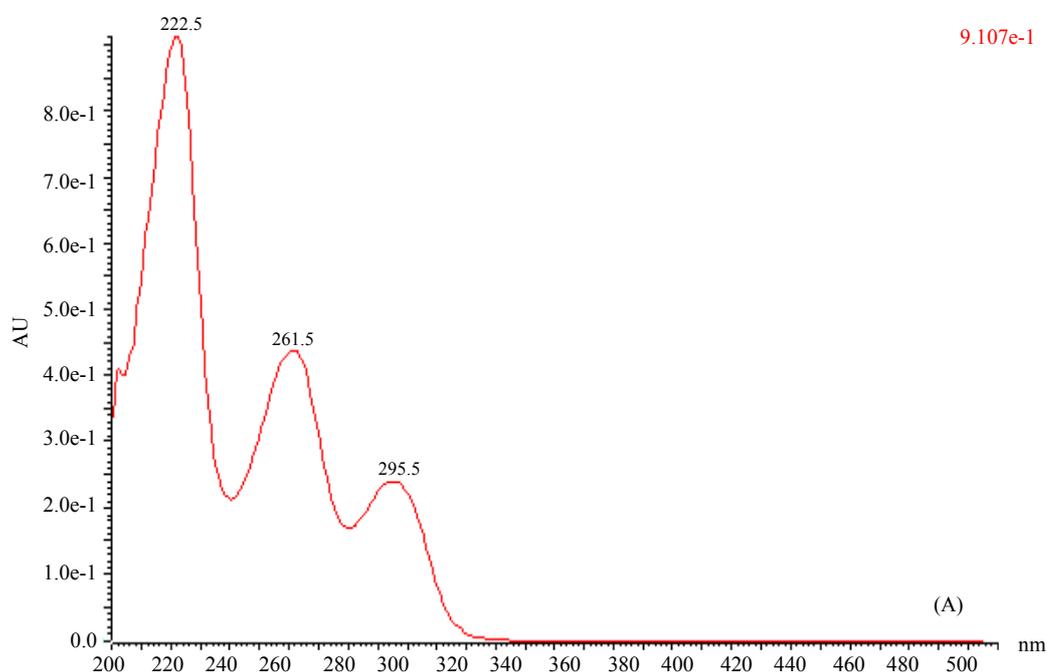


Fig. 4: UV-visible spectrum of NovonestmycinA (ZD1)

The ^{13}C NMR data shows the presence of height quaternary carbons (including a hemiacetal carbon around δ_c 99.9 and four carbonyl carbons at δ_c 208.5, δ_c 173.9, δ_c 168.9 and δ_c 167.8), eight aromatic carbons, seven aliphatic methines, eighteen oxygenated methines with an anomeric carbon at δ_c 98.5, twelve methylenes and eleven methyl carbons with methoxycarbon at δ_c 61.4 (Fig. 6).

The ^1H NMR spectrum shows three aromatic protons at δ_H 6.82 (1H, d, $J = 9.0$ Hz, H-6''), δ_H 7.46 (1H, d, $J = 2.0$ Hz, H-3'') and 7.46 (1H, dd, $J = 9.0, 2.0$ Hz, H-7''), This implies that the structure of ZD1 contains a 1,3,4-tri-substituted benzene. The COSY ^1H - ^1H spectrum presents a series of correlations of three partial structural units called: H-16 to H-39, H-16 to H-39 and H-1' to H-6'.

Similarly, compound ZD2 was purified as a white amorphous powder with the formula $\text{C}_{68}\text{H}_{110}\text{O}_{26}$ and molecular weight m/z 1365.7174 $[\text{M}+\text{Na}]^+$ (calculated for $\text{C}_{68}\text{H}_{110}\text{O}_{26}\text{Na}$: 1365.7178 g/mol). It is also soluble in Methanol; his retention time is 11.200 min and 222, 260, 290 as picks on the UV spectrum (Fig. 3 and 5).

Comparing the NMR ^{13}C spectrum of ZD1 and ZD2, The evident differences were the addition of six carbons including one methyl carbon, one anomeric carbon, two methylenes carbons and two oxygenated carbon methines (Fig. 6). The COSY ^1H - ^1H spectrum indicates the existence of a correlation between H-1''' and C-5''' which reveals the presence of one fragment of 2,3,6-trideoxyhexose which was linked to C-4'' by a correlation between H-1''' to C-4''.

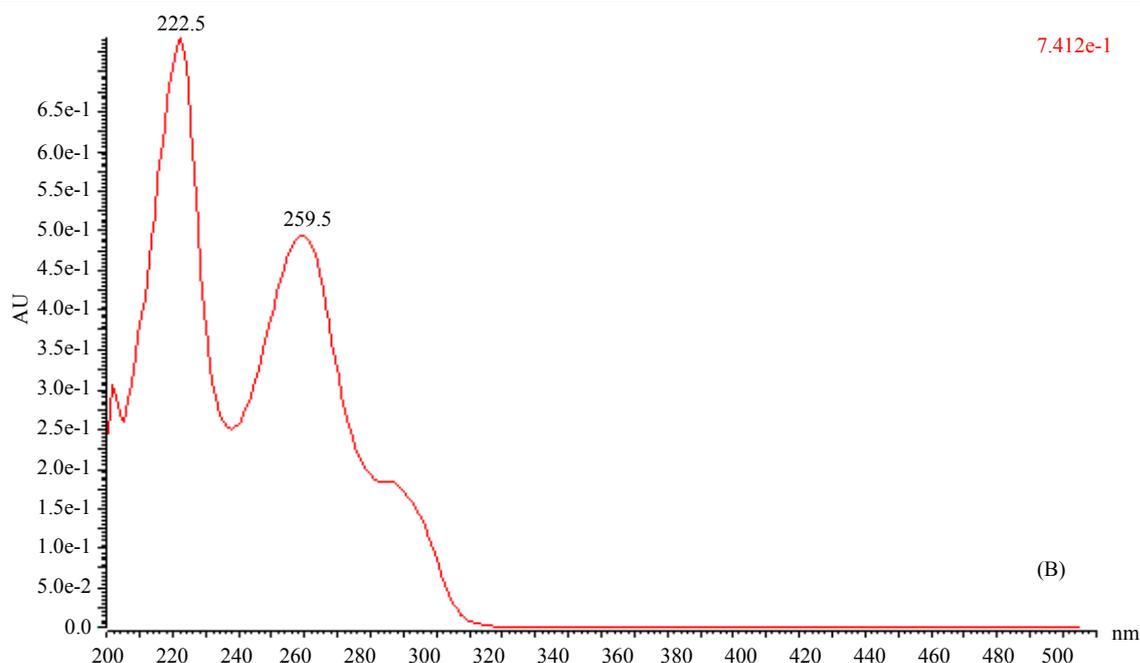


Fig. 5: UV-visible spectrum of Novonestmycin B (ZD2)

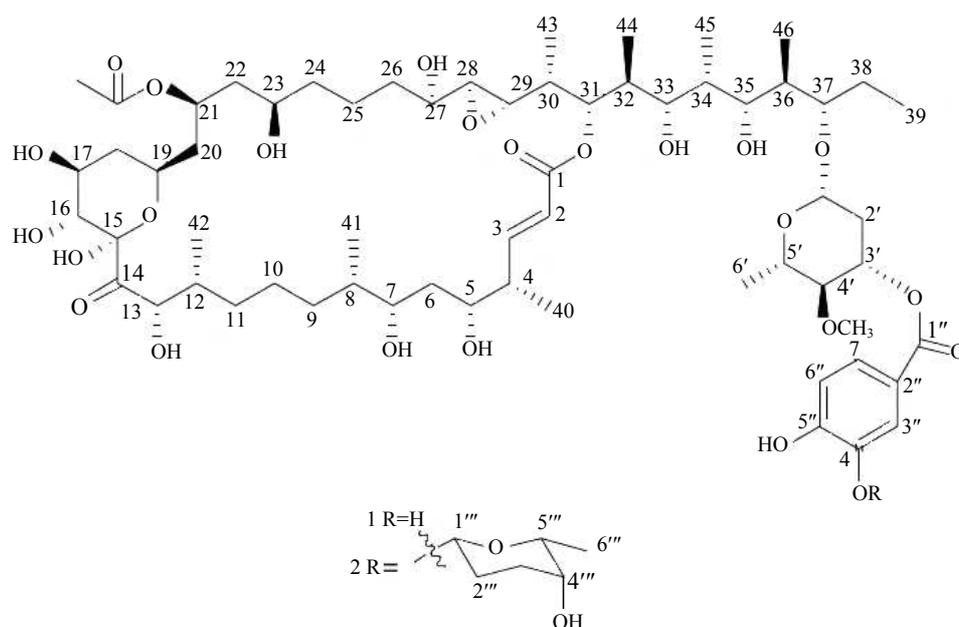


Fig. 6: Chemical structures of the NovonestmycinA (ZD1) and Novonestmycin B (ZD2)

The two compounds ZD1 and ZD2 were newly discovered and whose names are Novonestmycins A and B respectively (Wan *et al.*, 2015). Novonestomycins A and B have a closer structure related to the structure of Notonesomycin A, the 32-member macrolide antibiotics produced by *Notonesogenes* 647-AV1 (Sasaki *et al.*, 1986). Novonestomycin B (ZD2) and Notonésomycin A have the same skeleton and the difference between these two compounds is the substitution in the macrolide and the benzene nucleus.

Moreover, the structure of Novonestmycin A (ZD1) was similar to that of Notonesomycin A3, which was elucidated on the basis of HMBC data analysis by revealing the connectivities of the structural units: HMBC correlations from H-13 to C-14 and H-16 to C-14 which indicates that the ketone group was located at position C-14 (Kihara *et al.*, 1995).

Novonestomycins A and B have a strong antifungal activity against some fungi strains which is similar to that of a structurally related compound named

liposidolide A, the 36-member macrolide antibiotics produced by *Streptomyces* sp. RS-28 but Notonomycin A has only a moderate activity (Kihara *et al.*, 1995).

Brasinolide A, another compound with a similar structure, showed only a moderate antifungal activity against *Aspergillus niger* (Tanaka *et al.*, 1997). These results suggest that 14-ketone groups may be important for the antifungal activity.

Conclusion

The findings confirm that the Moroccan ecosystems (little or not exploitable) are very rich in microorganisms that are able to produce bioactive molecules which might play a role in future therapeutics. This encourages us to better exploit them in order to discover other new substances.

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Author's Contributions

All authors equally contributed in this work.

Conflict of Interest Statement

The authors have declared no conflict of interest.

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