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FULL LENGTH ARTICLE

Chemical characterization & bioactivity of diketopiperazine derivatives from the mangrove derived *Pseudonocardia endophytica*

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(3S,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione;
(3R,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione

Abstract Sediment samples from the mangrove ecosystem of Nizampatnam have been analyzed for actinomycetes as an elite source to screen for the formulation and production of antimicrobial and cytotoxic compounds. The actinomycetes strain VUK-10 has an interesting bioactivity profile and was isolated during our systematic study of mangrove actinomycetes. It was identified as *Pseudonocardia endophytica* with the aid of polyphasic taxonomy. The ethyl acetate extract of the actinobacterial culture filtrate has been purified by gel-filtration and silica gel column chromatographic purifications led to the isolation of two diketopiperazine compounds, (3S,8aS)-3-isobutyl hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**1**) and (3R,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**2**). The compounds listed, alluring cytotoxic activity against MDA-MB-231, HeLa, MCF-7 and OAW-42 cancer cell lines and also exhibited antimicrobial activities against gram-positive, gram-negative bacteria and fungi. Compound **1** recorded significant antibacterial activity against *Xanthomonas campestris* and *Escherichia coli* (8 μg/ml) and compound **2** presented highest activity against *Bacillus subtilis* (4 μg/ml). Compounds **1** and **2** were active against pathogenic fungi to plants and human beings. The activity was compared with griseofulvin and amphotericin-B, which are standard fungicides. The activity of 16 μg/ml by compound **1** was recorded against *Epidermophyton floccosum*; an anthropophilic dermatophyte responsible for tinea pedis, tinea cruris and tinea corporis. To the best of our knowledge this is the first narration on the isolation of supra said compounds from the genus *Pseudonocardia*.

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Introduction

The microbial secondary metabolites represent a boundless source of compounds with contemporary structures and impressive biological activities (Alan et al., 2015). Multiple products originating from microbial fermentation or from chemically altered products have been extensively availed, in agriculture, veterinary and pharmaceutical industries. The prospect of developing non-conventional bioactive compounds, depends on the number of strains scrutinized, their heterogeneity, distinctiveness and enabling to produce bioactive secondary metabolites (Donadio et al., 2002). The mangrove ecosystem is absolutely realistic, which is a dependable neighborhood in tropical and subtropical regions of the world for structurally complex and biologically active natural products. The saline mangrove ecosystem is fortified with organic matter and is sandwiched between terrestrial and marine environment (Kizhekkedathu and Parukuttyyamma, 2005). It is a classical habitat, studded with a divergent group of microorganisms (Arifuzzaman et al., 2010) and a hub of contemporary group of actinomycetes to experiment for new bioactive secondary metabolites.

Actinomycetes are being selected as a gifted, natural source for clinically active antibiotics. Most of them are too complicated to be blended by combinatorial chemistry. The therapeutic benefit of actinomycetes that is not only useful in the development of life saving drugs chiefly antimicrobial and cytotoxic agents, but also a profit generating podium to the pharmaceutical industry, is irrefutable (Baltz, 2007). As per the available literature about 4607 patents have been accorded on actinomycete associated products and processes. Out of 22,500 analyzed bioactive secondary metabolites, 10,100 (45%) are from actinomycetes; of which 7630 from *Streptomycetes* while 2470 from rare actinomycetes (Berdy, 2005). Based on the accredited information the *Pseudonocardia,* a rare actinomycetes genus is less scrutinized for bioactive metabolites than the other strains of the order *Actinomycetales* (Gavin et al., 2012). The genus *Pseudonocardia,* a new exclusive strain of the group actinomycetes was originally proposed by Henssen (1957), for mycolateless, nocardioform actinomycetes with a type IV cell wall. The bioactive compounds discovered from *Pseudonocardia* include azureomycins A and B (Omura et al., 1979); new quinolone compounds (Dekker et al., 1998); Pseudonocardones A, B and C, 6-deoxy-8-O-methylrabilomycin (Gavin et al., 2012); dentigerumycin (Oh et al., 2009); phenazostatin D (Maskey et al., 2003) and NPP (Lee et al., 2012) that showed potential antimicrobial and cytotoxic properties.

In the pursuit of clinically beneficial bioactive metabolites, we identified and isolated actinobacterium strain VUK-10 from unexplored mangrove sediment samples of Nizampatnam, which endorsed a broad spectrum antimicrobial activity and also probed for the active constituents of actinobacterium. Our paper vividly illustrates the process of fermentation, isolation, structural elucidation and biological assessment of purified compounds from the strain *Pseudonocardia endophytica* VUK-10.

Materials and methods

Sampling

Sediment samples from Nizampatnam mangrove ecosystem (Lat. 15°54′0N; Long. 80°40′0E) stationed along the south east coast of Andhra Pradesh were collected at bimonthly intervals from April 2010 to February 2011. Samples were collected from 6 to 10 cm depth and transported to laboratory in sterile bags and air dried at room temperature.

Isolation

The air dried sediment sample was subjected to dry heat pretreatment at 55 °C for 15 min to increase the actinobacterial population in the sample and to restrain the unwanted contaminants like fungi and bacteria. The pre-treated sample (1 g) was suspended in 100 ml of quarter strength ringer's solution, homogenized by vortexing. 100 µl of 10^{-4} dilution was spread across on asparagine glucose agar medium supplemented with nalidixic acid (25 µg/ml) and secnidazole (25 µg/ml) followed by incubation at 30 °C for one week. Morphologically distinct strains were selectively segregated and maintained by sub culturing on yeast extract–malt extract dextrose (YMD) agar medium at 4 °C for further study.

Identification

All the 55 strains isolated in this study were screened for the production of bioactive metabolites and 28 strains out of which are known for their antimicrobial activity. Of the 28 strains, 19 strains have been active against all the test microorganisms. Metabolites of the strain VUK-10 showed significant antimicrobial activity when compared to other tested ones. The strain was identified as *P. endophytica* by polyphasic taxonomy. JN087501 is the accession number of the NCBI GenBank for the 16S rRNA gene sequence of *P. endophytica*. Pure culture of actinobacteria was maintained on YMD agar slants at 4 °C for further study (Ushakiranmayi et al., 2012).

Extraction and metabolite profiling

A loopful culture of *P. endophytica* VUK-10 was cultivated in YMD broth (seed broth) and incubated on a rotary shaker (250 rpm) at 35 °C. After 48 h of incubation, the seed culture at the rate of 10% was transferred to the optimized production medium. The production medium consisted of glucose (8%), soy-peptone (1%), yeast extract (0.2%), meat extract (0.1%), $CaCO_3$ (0.3%), K_2HPO_4 (0.03%), $MgSO_4$ (0.1%), $FeSO_4$ (0.005%) and NaCl (3%) with pH adjusted to 7.0. After 48 h incubation, the seed culture at the rate of 10% was transferred to the optimized production medium.

Purification and structural elucidation of bioactive metabolites

The ethyl acetate crude extract was subjected to Sephadex LH-20 gel filtration chromatography (36 × 2.5 cm, Sephadex G-15) by using dichloromethane/methanol (1:1) as the eluent, resulting in seven fractions. Based on the ^{1}H NMR spectral data and bio-active screening, the fraction III (1.95 g) was selected for further studies and subjected to silica gel column chromatography (100–200 mesh), which afforded fractions 1–7. Based on TLC monitoring and NMR spectral data, fraction 7 was selected for further purification. Fraction 7 (360 mg) was subjected to further purification by silica gel column chromatography using dichloromethane/acetone (75:25) that afforded sub fractions A and B. The sub-fractions A and B

were further purified by silica gel column chromatography using dichloromethane/acetonitrile (85:15) and dichloromethane/isopropanol (95:5) and yielded compound **1** (18 mg) and compound **2** (51 mg), respectively. The structural elucidation of compounds **1** and **2** was carried out by detailed interpretation of NMR, mass, FTIR spectroscopic data.

Test microorganisms

Gram positive bacteria: *Bacillus cereus* (MTCC 430), *Streptococcus mutans* (MTCC 497), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 120), *Bacillus subtilis* (ATCC 6633), *Bacillus megaterium* (NCIM 2187); gram negative bacteria: *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus vulgaris* (MTCC 7299), *Serratia marcescens* (MTCC 118) *Xanthomonas campestris* (MTCC 2286), *Xanthomonas malvacearum* (NCIM 2954) and *Salmonella typhi* (ATCC 14028); medically important dermatophytes: *Candida albicans* (ATCC 10231) and *Epidermophyton floccosum* (MTCC 145); medically and agriculturally important filamentous fungi: *Aspergillus niger* (ATCC 1015), *Aspergillus flavus* (ATCC 9643), *Fusarium oxysporum* (MTCC 3075), *Fusarium solani* (MTCC 4634), *Penicillium citrinum* (MTCC 6489), *Verticillium alboatrum* and *Alternaria alternata* (MTCC 6572). The test microorganisms used in the present study were procured from ATCC, University Boulevard, Manassas, USA and MTCC, Chandigarh, NCIM, Pune, India and preserved at 4 °C.

Minimum inhibitory concentration (MIC) assay

The antimicrobial spectra of the bioactive compounds of the strain were determined in terms of minimum inhibitory concentration (MIC) against a wide variety of gram-positive and gram negative bacteria and fungi using the agar plate diffusion assay (Cappuccino and Sherman, 2004). Triplicate sets of the plates were maintained for each concentration of the test sample. Mueller–Hinton agar and Czapek–Dox agar media were prepared to grow the bacteria and fungi, respectively. The purified compounds were dissolved in dimethyl sulfoxide at concentrations ranging from 0 to 1000 µg/ml and used to assay against the supra mentioned test bacteria and fungi. The inoculated plates were examined after 24–48 h of incubation at 37 °C for bacteria and 48–72 h at 28 °C for fungi. The lowest concentration of the bioactive metabolites exhibiting significant antimicrobial activity against the test microorganisms was taken as the MIC of the compound.

Cell lines and culture conditions

Cell lines for testing *in vitro* cytotoxicity: human breast adeno carcinoma cell line (MDA-MB-231), human cervical cancer cell line (HeLa), human ovarian cyst adenocarcinoma cell line (OAW-42) and human breast adenocarcinoma cell lines (MCF-7) (cell lines reported to be resistant to cancer drugs). The cell lines used in the present study were obtained from National Centre for Cell Science, Pune, India. Cell lines MDA-MB-231, HeLa and OAW-42 were cultured on Dulbecco's modified Eagle's medium supplemented with fetal bovine serum (10%; (v/v)), L-glutamine (2 mM), penicillin (10 units/ml) and streptomycin (10 µg/ml), while breast cancer cell line MCF-7 was cultured on Roswell Park Memorial Institute medium 1640 supplemented with fetal bovine serum (10%; (v/v)), L-glutamine (2 mM), penicillin (10 units/ml) and streptomycin (10 µg/ml), all in a humidified atmosphere (95%) with 5% of CO_2 at 37 °C.

Cell proliferation (MTT) assay

The cytotoxicity of the compounds was assessed on the basis of the measurement of the *in vitro* growth in 96-well plates by cell mediated reduction of tetrazolium salt to water insoluble formazan crystals, as per the micro culture MTT assay (Mosmann, 1983). Cells were seeded in 96-well microtiter plates at a density of 5×10^3 per well (100 µl) containing 0.1 ml of medium. After overnight incubation, the cells were treated with different test concentrations of bioactive compounds (10, 100, 1000 and 5000 nM) at identical conditions with three replicates of each concentration. After 24 h of incubation, the cell viability was assessed by adding 20 µl of MTT (5 mg/ml in PBS) per well and the plates were incubated at 37 °C for 4 h. The formazan crystals formed in the cells were dissolved with 100 µl of 0.1% acidified isopropanol, and the rate of color development was measured at 570 nm using a microplate reader. The IC_{50} values (50% inhibitory concentration) of the compounds were calculated using Sigma Plot software with reference to that of Taxol as standard. All the experiments were carried out in triplicates.

Results

Purification and structural elucidation of bioactive metabolites

Compound **1** was obtained as a colorless amorphous solid, wholly soluble in dimethylsulfoxide, methanol, ethanol and chloroform, $[\alpha]_D^{25}$-78.68 (*c* 0.11, $CHCl_3$). The ^{1}H NMR spectrum of compound **1** showed signals at δ 6.98 (s, 1H); 4.06 (t, 1H, J = 6.98 Hz); 3.91 (m, 1H); 3.67–3.46 (dm, 2H); 3.28 (m, 1H); 2.08–1.67 (m, 4H); 1.62 (m, 2H); 0.98 (d, 3H, J = 6.42 Hz); and 0.92 (d, 3H, J = 6.42 Hz) (Supplementary Fig. A); while ^{13}C exhibited 10 signals at δ 169.56; 166.36; 58.00; 56.25; 45.56; 42.57; 30.86; 24.43; 22.98 and 21.36 (Supplementary Fig. B). EIMS analysis of the compound gave a molecular ion m/z at 211 (M + H); 233 (M + Na) (Supplementary Fig. C). The IR spectrum exhibited absorption bands at V_{max} 3242, 2958, 1741, 1665, 1447 and 758 cm^{-1} (Supplementary Fig. D). Based on the above spectral data, bioactive compound **1** was identified as (3S,8aS)-3-isobutylhex ahydropyrrolo[1,2-a]pyrazine-1,4-dione (Cyclo (D-Pro-D-Leu) with the molecular formula of $C_{11}H_{18}N_2O_2$ (Fig. 1).

Compound **2** was obtained as a colorless amorphous solid, freely soluble in methanol, ethanol and ethyl acetate, $[\alpha]_D^{25}$ + 33.57 (*c* 1.9, $CHCl_3$). The ^{1}H NMR spectrum of the compound showed signals at δ 6.37 (bs, 1H); 4.09 (t, 1H, J = 7.55 Hz); 3.98 (dd, 1H, J = 3.02, 9.06 Hz); 3.62–3.45 (m, 2H); 2.37–2.27 (m, 1H); 2.16–1.94 (m, 3H); 1.93–1.67 (m, 2H); 1.50 (m, 1H); 0.96 (d, 3H, J = 6.79 Hz); and 0.91 (d, 3H, J = 6.79 Hz) (Supplementary Fig. E), while ^{13}C exhibited 10 signals at δ 171.4; 167.1; 59.1; 53.4; 45.6; 36.7; 28.2; 24.8; 22.8 and 21.2 (Supplementary Fig. F). EIMS analysis of the compound gave a molecular ion m/z at 211 (M + H); 233 (M

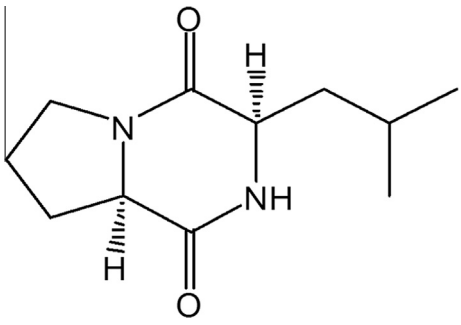

Figure 1 Molecular structure of (3S,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione **(1)** (Cyclo (D-Pro-D-Leu).

+Na) (Supplementary Fig. G). The IR spectrum displayed absorption bands at V_{max} 3260, 2953, 1672, 1635, 1432 and 709 cm^{-1} (Supplementary Fig. H). Based on the above spectral data, bioactive compound **2** was identified as (3R,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (Cyclo (L-Pro-D-Leu) with the molecular formula of $C_{11}H_{18}N_2O_2$ (Fig. 2).

Minimum inhibitory concentration (MIC) assay

Antibacterial activities of the bioactive compounds (**1** and **2**) in terms of MIC are shown in Table 1. The bioactive compounds exhibited antibacterial activity against a variety of gram-positive and gram-negative bacteria, for which the MIC values ranged from 4 to 256 μg/ml. The best activity of compound **1** was recorded against *X. campestris* and *E. coli* (8 μg/ml) followed by *X. malvacearum, B. subtilis and P. aeruginosa* (32 μg/ml). The microorganism that presented highest sensitivity toward compound **2** was *B. subtilis* (4 μg/ml) followed by *X. campestris* and *E. coli* (16 μg/ml). Tetracycline served as the positive control for the bacteria. Compared with the standard drug tetracycline, compounds **1** and **2** displayed high sensitivity against *X. campestris* and *B. subtilis*, while in some cases compound **1** (*B. subtilis*; *E. coli*) and **2** (*X. campestris*) recorded similar sensitivity like positive control (Table 1). Tetracycline, in other cases showed good antibacterial activity over the metabolites of the strain.

Antifungal activity against dermatophytes and filamentous fungi and the corresponding MIC values are recorded in Table 2. Compound **2** exhibited significant MIC value against *E. floccosum* (16 μg/ml) whereas compounds **1** and **2** recorded activity at 32 μg/ml against *C. albicans*. Among the filamentous fungi tested, *A. flavus* recorded sensitivity of 16 μg/ml toward compound **1**, and *A. alternata* that recorded no activity up to 512 μg/ml. Compound **2** was active against *F. oxysporum* at 32 μg/ml, and for this compound *V. alboatrum* recorded no activity up to 1000 μg/ml (Table 2). Both compounds recorded lower antifungal activity than the standard fungicide, Amphotericin-B against fungi.

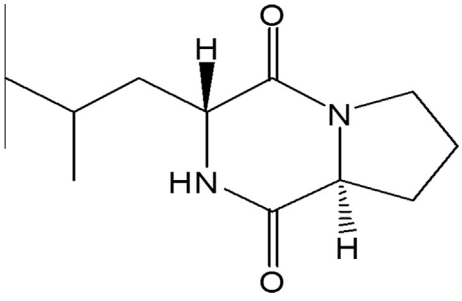

Figure 2 Molecular structure of (3R,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione **(2)** (Cyclo (L-Pro-D-Leu).

Table 1 Minimum inhibitory concentration (MIC) of bioactive compounds isolated from *Pseudonocardia endophytica* VUK-10 (MIC-(μg/ml)) against test bacteria.

Test microorganisms	Compound-**1**	Compound-**2**	Tetracycline
Staphylococcus aureus	64	128	32
Streptococcus mutans	128	256	32
S. epidermis	64	64	16
Xanthomonas campestris	8	16	16
X. malvacearum	32	64	8
Bacillus subtilis	32	4	32
B. megaterium	128	64	16
B. cereus	64	64	8
Escherichia coli	8	16	8
Pseudomonas aeruginosa	32	64	8
Serratia marcescens	64	64	32
Proteus vulgaris	128	256	16
Salmonella typhi	128	128	8

Compound-**1**: (3S,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione.
Compound-**2**: (3R, 8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione.
Data were statistically analyzed by a one way ANOVA variance and found to be significant at 0.05% ($n = 3$).

Table 2 Minimum inhibitory concentration (MIC) of bioactive compounds isolated from *Pseudonocardia endophytica* VUK-10 MIC-(μg/ml)) against dermatophytes and fungi.

Dermatophytes	Compound-**1**	Compound-**2**	Antifungal agent
Candida albicans	32	32	16
Epidermophyton floccosum	64	16	16
Fungi			
Aspergillus niger	64	128	16
Aspergillus flavus	16	128	8
Fusarium oxysporum	256	32	16
Fusarium solani	128	128	32
Penicillium citrinum	128	256	8
Verticillium alboatrum	256	ND	64
Alternaria alternata	>512	256	32

Compound-**1**: (3S,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione.
Compound-**2**: (3R,8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione.
Antifungal agent: Griseofulvin against yeast and Amphotericin-B against fungi.
ND, not detected.
Data were statistically analyzed by one way ANOVA variance and found to be significant at 0.05% ($n = 3$).

Cell proliferation (MTT) assay

The cytotoxicity of the purified compounds **1** and **2** was assayed against MDA-MB-231, HeLa, MCF-7 and OAW-42. The results exhibited that both compounds were active against the four cell lines.

The activity of compound **1** against MDA-MB-231, HeLa, MCF-7 and OAW-42 cell lines is presented in Fig. 3 (A–D). Compound **1** showed significant cytotoxicity when evaluated against MDA-MB-231, HeLa, MCF-7 and OAW-42 cell lines, displaying IC_{50} values of 100 µM (55.8%) (MDA-MB-231), 10 µM (62.8%) (HeLa), 1000 µM (55.8%) (MCF-7) and 5000 µM (53%) (OAW-42).

The activity of compound **2** against MDA-MB-231, HeLa, MCF-7 and OAW-42 cell lines is presented in Fig. 4 (A–D). Compound **2** exhibited potent cytotoxicity with MDA-MB-231, HeLa, MCF-7 and OAW-42 cell lines, exhibiting IC_{50} values of 1000 µM (50%, 51.2%) (MDA-MB-231, MCF-7), 10 µM (60.4%) (HeLa) and 100 µM (50.7%) (OAW-42). Taxol, an anticancer drug used as the standard, recorded an IC_{50} value of 10 nM (59%, 60%, 57% and 63%) against the MDA-MB-231, HeLa, OAW-42 and MCF-7 cell lines.

Discussion

Diketopiperazines (DKP'S) are privileged structures for the authentication of contemporary lead compounds by combinatorial chemistry and considered exemplary for the enlightened augmentation of new therapeutic agents. They target a broad array of receptors with high affinity, dispensing an extensive spectrum of biological activities (Maristela and Ivone, 2007). DKP derivatives, originated naturally by many organisms, unfold a great number of multiple structures and biological functions, enabling them as useful chemical entities for the expedition and improvement of new drugs (Smaoui et al., 2012). Especially, some DKPs bearing proline residues isolated from marine organisms were found to have diversified bioactivities such as anti-inflammatory agents, immune modulators and tumoral cytotoxic agents (Huang et al., 2010). In the present study, the extraction and further purification of the secondary metabolites of the strain grown on glucose–soy peptone broth led to the isolation of two proline containing cyclic dipeptides, (3S,8aS)-3-isobutylhexahydropyrrolo[1,2-a] pyrazine-1,4-dione (**1**) (Cyclo (D-Pro-D-Leu) and (3R, 8aS)-3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**2**) (Cyclo (L-Pro-D-Leu) which were effective against pathogenic bacteria, fungi and cancer cell lines.

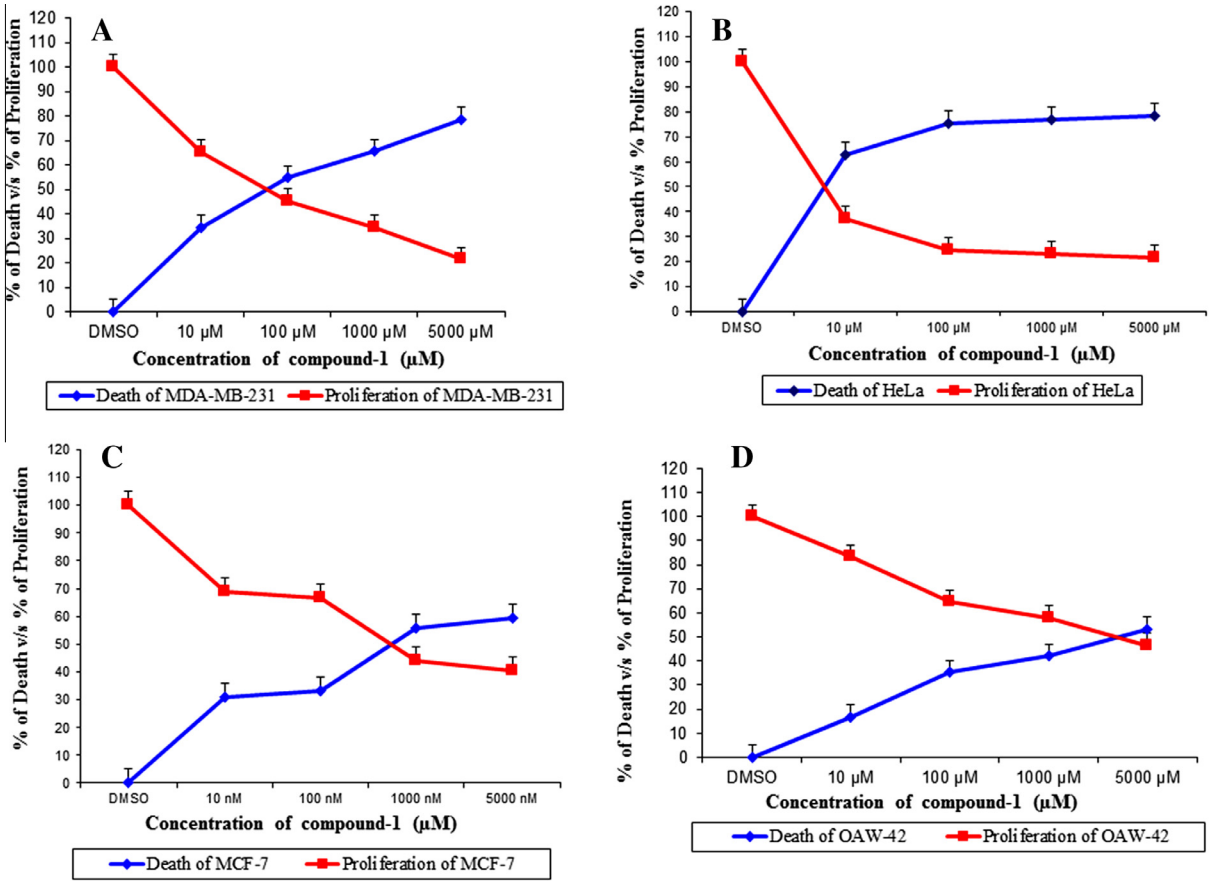

Figure 3 Dose response curve of Compound-**1** on the growth of (A) MDB-MB-231 (B) HeLa (C) MCF-7 (D) OAW-42 cell lines. Data of the percentage of the cell viability was statistically analyzed by a one-way ANOVA. The results from the three independent experiments were expressed as mean ± deviation and were considered statistically significant for a *p*-value lower than 0.05% ($p < 0.05$).

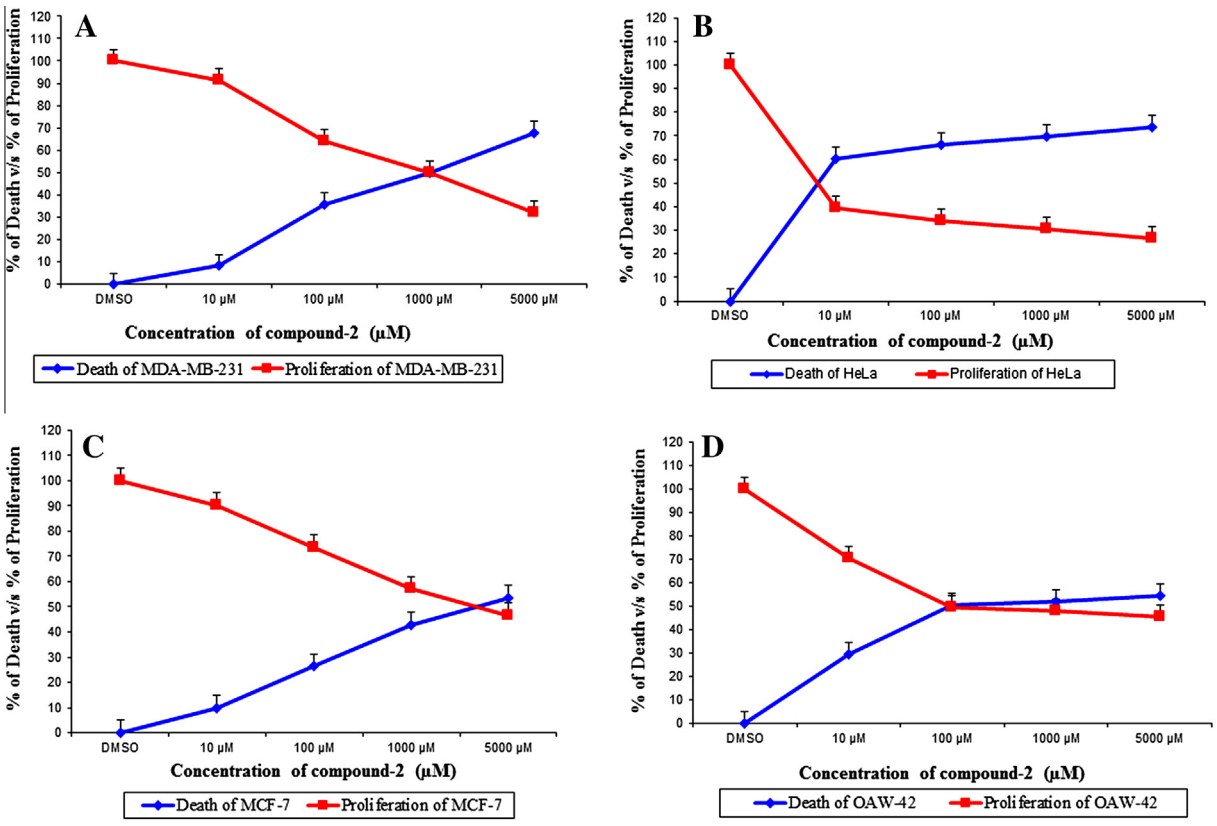

Figure 4 Dose response curve of Compound-**2** on the growth of (A) MDB-MB-231 (B) HeLa (C) MCF-7 (D) OAW-42 cell lines. Data of the percentage of the cell viability was statistically analyzed by one-way ANOVA analysis. The results from the three independent experiments were expressed as mean ± deviation and were considered statistically significant for a *p*-value lower than 0.05% ($p < 0.05$).

The isolation of DKPs **1** and **2** from *P. endophytica* VUK 10 is the first communication in literature with complete structural characterization. The two DKPs acknowledged in this study were marginally diverse from the other compounds. Compound **1** has S-Leu and compound **2** has R-Leu. The NMR spectral data and optical rotation values obtained for compounds indicated that compound **1** is a diastereomer of compound **2**. Compound **1** was previously reported as a natural product from bacteria (Yang et al., 2002) and marine sponges (Wegerski et al., 2004). The production of compound **2** was previously reported from a marine derived *Streptomyces* sp. (Bin et al., 2011). Antibacterial activity of Cyclo (Leu-Pro) was reported on vancomycin resistant *Enterococcus faecalis* strains (Rhee et al., 2001).

Our results emphasize that compound **1** is an exceptionally potential inhibitor against *X. campestris* (causes bacterial leaf spot on peppers and tomatoes), *E. coli* (causes cholecystitis, bacteremia, cholangitis and urinary tract infection) *C. albicans* and *A. flavus* (causes aspergillosis). It was demonstrated that Cyclo (L-Leu-L-Pro) was shown to possess MIC of 16 µg/ml against pathogenic bacteria *E. coli* (Rhee, 2004). We observed a greater potency against same bacteria with inhibition at 8 µg/ml, for compound **1**. Similarly anti fungal activity of Cyclo (L-Leu-L-Pro) was previously reported against *Aspergillus parasiticus* with inhibition at a range of 0.2 mg/ml (Yan et al., 2004), whereas compound **1** displayed superior activity with 16 µg/ml against a similar variety of fungus, *A. flavus*. This might be due to the chirality variation in the amino acids causing an effect on topography of the molecule and thus resulting in biased inhibition. Compound **2** results indicate antimicrobial activity against *B. subtilis* (causes disease in severely immune compromised patients), *E. floccosum* (causes tinea pedis, tinea cruris and tinea corporis), *C. albicans* (causes oral thrush and vaginal infection) and *F. oxysporum* (causes fusarium wilt, fungal keratitis and onychomycosis). Anti microbial studies of compound **2** were less explored; however, the present study recorded superior antibacterial activity against *B. subtilis* than the positive control tetracycline. Anti cancer activity of Cyclo (Leu-Pro) was previously documented against HeLa and MCF-7 cell lines (Brauns et al., 2004). The results in the present study registered phenomenal potency across similar cell lines. In addition, anti cancer activity of compounds **1** and **2** against MDA-MB-231 and OAW-42 cell lines Germana et al. (2015), reported in the study for the first time. The data cataloged in this paper apparently justified that our compounds illustrated convincing anti cancer activities at nanomolar concentrations.

The bioactive compounds of the current work purified from strain VUK-10 showed significant antimicrobial activity against gram positive, gram negative bacteria, dermatophytes and filamentous fungi. On the other hand potent cytotoxic activity recorded against MDA-MB-231, HeLa, OAW-42 and MCF-7 cell lines. Hence, the strain is an important and encouraging microorganism enable to produce antimicrobial and cytotoxic compounds. This is the first report on isolation and characterization of compounds, (3S,8aS)-3-isobutylhexa hydropyrrolo[1,2-a]pyrazine-1,4-dione (**1**) and (3R,8aS)-3-iso

butylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**2**) from genus *Pseudonocardia*.

Conflict of interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejar.2016.03.001.

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