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BRIEF COMMUNICATION

Cyclo(Pro-DOPA), a third identified bioactive metabolite produced by *Streptomyces* sp. 8812

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Abstract

A new metabolite, cyclic dipeptide, *cis*-(3*S*,8a*S*)-3-(3,4-dihydroxybenzyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione, named JS-3 was isolated from *Streptomyces* sp. 8812 fermentation broth. Its chemical structure was established by means of spectroscopic analysis. A wide-range-screening study, which included inhibition assay of DD-carboxypeptidase/transpeptidase activity, determination of antibacterial, antifungal, and antiproliferative activities as well as free-radical scavenging was performed. To authors knowledge, this is the first isolation of such compound from natural sources and the first one from bacteria, *Streptomyces*.

Introduction

In this study, we report on the isolation, identification and determination of the biological activities of *cis*-(3*S*,8a*S*)-3-(3,4-dihydroxybenzyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione. This cyclic dipeptide, named JS-3, was obtained from *Streptomyces* sp. 8812 fermentation broth as a third identified metabolite of this species [1–4]. *Streptomyces* sp. 8812 is a strain isolated from Brazilian soil. The strain is deposited in the Polish Collection of Microorganisms in Wroclaw, with an accession number B/00017. The 16S rRNA gene sequence of *Streptomyces* sp. 8812 has GenBank accession number KT951721. Previously, two bioactive metabolites with antibacterial activity were isolated and characterized. The first one is an isoquinoline alkaloid, 7-hydroxy-6-oxo-2,3,4,6-tetrahydroisoquinoline-3-carboxyl acid ($C_{10}H_9NO_4$), with molecular mass of 207.06 Da. The second *Streptomyces* sp. 8812 metabolite is a protoalkaloid, *N*-acetyl-3,4-dihydroxy-L-phenylalanine ($C_{11}H_{13}NO_5$) with molecular mass of 239.07 Da [1–4].

To evaluate biological activities of JS-3, we performed a wide-range screening study, which included inhibition assay of DD-carboxypeptidase/transpeptidase 64–575 II (DD-peptidase 64–575 II) activity [5], determination of antibacterial and antifungal activities and free-radical scavenging. Antiproliferative activity of the isolated metabolite towards cancer cell lines was also examined. *Streptomyces* sp. 8812 fermentation was conducted as previously reported [2, 4]. Isolation and purification of the JS-3 metabolite were performed based on the DD-peptidase 64–575 II assay [2, 6]. The compound was purified by chromatographic methods (anion exchange resin IRA-400; Supelco), solid phase extraction (C18 Polar; Witko), and RP-HPLC (Knauer, Germany) with C18 modified column (Atlantis, Waters) (Supplementary Materials). Its molecular formula was determined to be $C_{14}H_{16}N_2O_4$ [$(M+H)^+$ 277.11852 *m/z* (+0.24 mmu error)] on a basis of high resolution ESI-MS. The newly isolated JS-3 metabolite was identified by 1D (^{1}H NMR) and 2D NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC) spectral analysis as a diketopiperazine, cyclo(Pro-DOPA) (Fig. 1; Supplementary Figures 1S-3S). Physico-chemical properties and NMR data of the compound are listed in Table 1.

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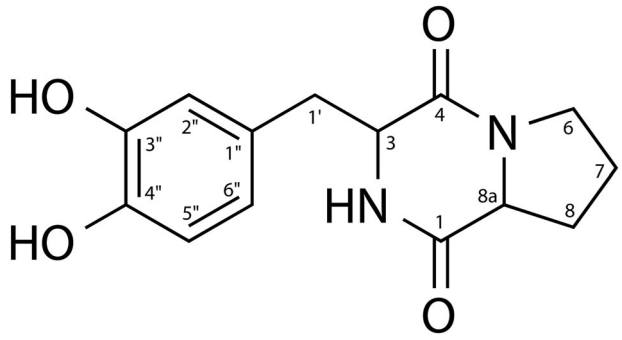

Fig. 1 The chemical structure of JS-3

The 1D ^{1}H and ^{13}C NMR spectra as well as 2D homonuclear and heteronuclear spectra were collected on a 700 MHz Bruker AVANCE III spectrometer, equipped with a QCI CryoProbe. The 3-mm-thin wall tubes were used with a final sample volume of 200 µl (D_2O). The residual water signal was suppressed by presaturation. Experiments were performed at 25 °C. Spectra were processed and prepared with TopSpin 3.0 Bruker Software. The assignment of proton resonances was based on the analysis of chemical shifts, proton–proton coupling pattern (Supplementary Figure 1S) and the 2D spectra (Supplementary Figure 2S). The ^{1}H NMR spectrum was found to consist of 12 signals. In the aromatic region of the ^{1}H NMR spectrum of JS-3 three signals classified as ABM system at 6.81 (d, 8.1 Hz, 1H), 6.64 (d, 2.0 Hz, 1H) and 6.55 ppm (dd, 8.1 Hz, 2.0 Hz, 1H) were observed. These signals were assigned to H5″, H2″, and H6″ protons, respectively. The remaining ten signals present in the aliphatic region were assigned as ABX and ABCD aliphatic systems. Signals at 4.45 (1H), 3.11 (dd, 14.2 Hz, 3.8 Hz, 1H), and 2.88 ppm (dd, 14.2 Hz, 4.45 Hz, 1H) were attributed to H3 and two geminal H1′ protons. The signals at 3.99 (dd, 11.7 Hz, 6.5 Hz, 1H), 1.97 (1H), 0.67 (1H), 1.71 (2H), 3.49 (1H), and 3.26 ppm (1H) were assigned to the side chain of proline. Assignment of resonances of proton-bearing carbon atoms was obtained from the ^{1}H-^{13}C HSQC spectrum (Table 1 and Supplementary Figure 3S). Both, the molecular mass and NMR data indicated that JS-3 is a cyclic dipeptide, cyclo(Pro-DOPA) (Supplementary Figures 1S-3S). IR absorption at 3265 cm^{-1} and 1636 cm^{-1} suggested the presence of hydroxyl and amide carbonyl groups in the structure. Whereas, absorption at 1474–1452 cm^{-1} and 798–646 cm^{-1} was determined to be associated with aromatic C=C stretching and bending C–H vibrations, respectively, which corresponds to the aromatic system (Supplementary Figure 4S).

For comparison analyses of JS-3 chemical structure and biological activities, two stereoisomers: *cis*-cyclo(L-Pro-L-DOPA) and *trans*-cyclo(L-Pro-D-DOPA), were synthesized. The ^{1}H and ^{13}C NMR spectra of both compounds are presented on Supplementary Figures 1S, 5S-7S. Cyclo(Pro-DOPA) was synthesized starting from *N*-Boc protected DOPA. Dipeptide Boc-DOPA-L-Pro-OMe was obtained by coupling Boc-DOPA with proline methyl ester using EDC. Cyclization proceeded smoothly at room temperature after removal of Boc protection with HCl and alkalization with Et_3N. The synthesis process was described in the Supplementary Information and the NMR spectra of intermediate products are shown on Supplementary Figures 8S-11S. The IR spectrum of *trans*-cyclo(L-Pro-D-DOPA) is presented on Supplementary Figure 12S. The ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC spectra of synthetic cyclic dipeptides, *cis*-cyclo(L-Pro-L-DOPA) and *trans*-cyclo(L-Pro-D-DOPA), are presented on Supplementary Figures 13S-15S. Both diastereoisomers of cyclo(Pro-DOPA) were obtained previously by Zhang et al. [7] by microwave irradiation of Boc-L-Pro-L-DOPA-OMe adsorbed on silica gel at 163–235 °C. Epimerization of proline resulted in formation of nearly equal amounts of *cis* and *trans* cyclo (Pro-DOPA). Comparison of JS-3 NMR spectra with respective spectra of synthetic compounds, *cis*-cyclo(L-Pro-L-DOPA) and *trans*-cyclo(L-Pro-D-DOPA), indicated that the Pro and DOPA side chain in the chemical structure of the metabolite produced by *Streptomyces* sp. 8812 are in *cis* configuration (Supplementary Figure 1S). The absolute stereochemistry of JS-3 was determined using circular dichroism spectroscopy. The CD spectrum of JS-3 reveals positive bands at 225 nm and 280 nm as well as negative at 235 nm. The same pattern is observed for *cis*-cyclo(L-Pro-L-DOPA) (Supplementary Figure 16S). Isolated metabolite JS-3 possesses an L,L configuration, similarly to majority of peptides and amino acids of natural origin. The CD spectrum for *trans*-cyclo(L-Pro-D-DOPA) was also recorded (Supplementary Figure 16S) showing negative band at 280 nm responsible for aromatic ring absorption.

JS-3 belongs to diketopiperazines, which are the smallest known cyclic peptides, commonly biosynthesized from amino acids by different organisms [8]. The studied compound is structurally related to *cis*-cyclo(L-Pro-L-Tyr) (maculosin), which was previously isolated from various sources, including fungi *Alternaria alternata* [9, 10] and bacteria—*Lysobacter capsici* AZ78 [11] and *Bacillus* sp [12–14]. *Trans*-cyclo(L-Pro-D-DOPA) was also isolated from *Bacillus* sp. N strain [12, 13] *cis*-cyclo(L-Pro-L-Tyr) and *trans*-cyclo(L-Pro-D-DOPA) were also described to be isolated from *Streptomyces* sp. strain 22–4 [15]. Additionally, maculosin and its derivatives were shown by various authors to have activity against phytopathogenic bacteria [15]. Intrigued by the fact that different diketopiperazines were shown to exhibit antibacterial and antifungal activity, a series of measurements was carried out for compounds: JS-3, *cis*-cyclo(L-Pro-L-DOPA), *trans*-cyclo(L-Pro-D-DOPA) and *cis*-cyclo(L-Pro-L-Tyr), to examine this aspect

Table 1 Characteristic of evaluated compounds

(a) Physico-chemical properties

Appearance	White-cream powder
Molecular weight	276
Molecular formula	$C_{14}H_{16}N_2O_4$
HRESI-MS (*m/z*)	
Calcd	277.11828 $(M+H)^+$
Found	277.11852 $(M+H)^+$
IR (ATR) ν_{max} (cm^{-1})	3265, 1636, 1595, 1473, 1452, 1254, 1118, 798, 646
UV (MeOH) λ_{max} nm (ε)	220 (3003), 283 (1980)

(b) ^1H and ^{13}C NMR data (D_2O)

Position	δ ^1H (ppm, *J* in Hz)	δ^{13}C (ppm)
3	4.45 (m)	56.4
1′	3.11 (dd, 14.2, 3.8)	37.6
	2.88 (dd, 14.2, 4.45)	
2″	6.64 (d, 2.0)	117.4
5″	6.81 (d, 8.1)	116.0
6″	6.55 (dd, 2.0, 8.1)	122.4
8a	3.99 (dd, 11.7, 6.5)	58.4
8	1.97 (m)	27.6
	0.67 (m)	
7	1.71 (m, 2H)	20.6
6	3.49 (m)	44.6
	3.26 (m)	

(c) Biological activities of evaluated compounds

Enzyme	Inhibition, IC_{50} (mM)				
	JS-3	*Cis*-cyclo(L-Pro-L-DOPA)	*Trans*-cyclo(L-Pro-D-DOPA)	*Cis*-cyclo(L-Pro-L-Tyr)	
DD-peptidase 64–575 II	0.80 ± 0.01	0.83 ± 0.01	1.33 ± 0.01	21.05 ± 0.32	
Radical	Scavenging activity, EC_{50} (μg ml^{-1})				
	JS-3	*Cis*-cyclo(L-Pro-L-DOPA)	*Trans*-cyclo(L-Pro-D-DOPA)	*Cis*-cyclo(L-Pro-L-Tyr)	Ascorbic acid
DPPH	12.98	12.88	13.51	na	9.72
$ABTS^+$	1.71	1.59	2.13	3.60	4.89
Cell line	Antiproliferative activity[a], IC_{50} (μg ml^{-1})				
	JS-3	*Cis*-cyclo(L-Pro-L-DOPA)	*Trans*-cyclo(L-Pro-D-DOPA)	*Cis*-cyclo(L-Pro-L-Tyr)	Cisplatin
MCF-7	345.60 ± 19.40	343.61 ± 17.45	346.34 ± 15.12	719.08 ± 209.57	2.82 ± 0.55
HT-29	362.30 ± 12.50	354.40 ± 8.09	370.97 ± 30.50	na	3.47 ± 0.95
HeLa	358.40 ± 15.62	350.92 ± 12.95	369.19 ± 41.19	na	0.99 ± 0.54
BALB/3T3	380.25 ± 4.35	383.22 ± 2.78	366.33 ± 2.45	na	2.07 ± 0.04

[a] DMSO was also used as a control. It was not active towards all cell lines in concentration used.

in more detail. The activity of listed compounds towards the ATCC® collection of bacteria and fungi was evaluated by liquid microdilution method according to CLSI references. The activity towards phytopathogenic bacteria and fungi was also examined in our study. Bacterial and fungal strains, as well as the procedure are described in Supplementary Material. Only *cis*-cyclo(L-Pro-L-DOPA)/JS-3 showed moderate activity against *Staphylococcus aureus*: VISA (MIC = 256 μg ml^{-1}), MRSA (MIC = 512 μg ml^{-1}), MSSA (MIC = 512 μg ml^{-1}), and *S. epidermidis* (MIC = 256 μg ml^{-1}). All of the compounds were not active (MIC > 512 μg ml^{-1}) towards *Escherichia coli*. Only *cis*-cyclo(L-Pro-L-DOPA) and JS-3 exhibited antifungal activity (MIC = 128 μg ml^{-1}) against *Candida glabrata*. In the effect of

these studies, all tested compounds showed lack of antifungal activity (MIC > 256 μg ml^{-1}) against *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *Issatchenkia orientalis*. As already mentioned, other authors [15] reported antibacterial activity of *cis*-cyclo(L-Pro-L-Tyr) towards phytopathogenic bacteria. *Cis*-cyclo(L-Pro-L-DOPA), JS-3 and *trans*-cyclo(L-Pro-D-DOPA) exhibited moderate activity (MIC = 256 μg ml^{-1}) against *Erwinia amylovora*, whereas *cis*-cyclo(L-Pro-L-Tyr) showed no activity (MIC > 256 μg ml^{-1}). All tested compounds showed lack of activity (MIC > 256 μg ml^{-1}) towards *Pseudomonas fluorescens*, *Pseudomonas syringae* pv. *syringae*, *Agrobacterium radiobacter, Xanthomonas arboricola* pv. *Corylina*, *Pectobacterium carotovorum* and had no activity (MIC > 512 μg ml^{-1}) towards such phytopathogenic fungal species as: *Blumeriella jaapii*, *Monilinia laxa*, *Monilinia fructigena*, *Verticillium dahliae*, *Botrytis cinerea*, *Nectria galligena*, and *Neofabraea alba*.

The inhibitory activity of JS-3, *cis*-cyclo(L-Pro-L-DOPA), *trans*-cyclo(L-Pro-D-DOPA), and *cis*-cyclo(L-Pro-L-Tyr) towards DD-peptidase 64–575 II was measured under the procedures described previously (see Supplementary Materials for details) [6, 16–18]. DD-carboxypeptidases/transpeptidases (DD-peptidases) are enzymes involved in bacterial cell wall metabolism, specifically in the cross-linking during the last stage of peptidoglycan biosynthesis [5, 6]. IC_{50} values of these compounds are presented in Table 1. The isolated metabolite JS-3 showed low DD-peptidase 64–575 II inhibitory activity; however, it was more active than the previously isolated and described compound, JS-2 [2, 3]. The IC_{50} value of JS-3 was one order of magnitude lower than the IC_{50} value of JS-2 (10.97 mM).

Our study revealed, that diketopiperazines: JS-3, *cis*-cyclo(L-Pro-L-DOPA), *trans*-cyclo(L-Pro-D-DOPA), and *cis*-cyclo(L-Pro-L-Tyr) are potent free-radical scavengers. The assays were performed according to the previously described procedure (see Supplementary Materials for details). JS-3, *cis*-cyclo(L-Pro-L-DOPA) and *trans*-cyclo(L-Pro-D-DOPA) showed significant DPPH and $ABTS^{\cdot+}$ scavenging activities (Table 1). JS-3 scavenged DPPH and $ABTS^{\cdot+}$ with EC_{50} value of 12.98 and 1.71 μg ml^{-1}, respectively. In turn, *trans*-cyclo(L-Pro-D-DOPA) presented DPPH and $ABTS^{+}$ scavenging activities of 13.51 and 2.13 μg ml^{-1}, respectively. Both compounds were more potent scavengers on $ABTS^{\cdot+}$ than the reference, ascorbic acid (EC_{50} = 4.89 μg ml^{-1}). DPPH scavenging activity of *cis*-cyclo(L-Pro-L-DOPA), JS-3 and *trans*-cyclo(L-Pro-D-DOPA) was comparable to that of the ascorbic acid (EC_{50} = 9.72 μg ml^{-1}). Moreover, *cis*-cyclo(L-Pro-L-Tyr) was more potent scavenger on $ABTS^{\cdot+}$ (EC_{50} = 3.60 μg ml^{-1}) than ascorbic acid and did not show DPPH scavenging activity (Table 1).

Diketopiperazines were also shown before by other authors to have antiproliferative activity towards cancer cells [19]. In our screening study, we evaluated in vitro antiproliferative activity of *cis*-cyclo(L-Pro-L-DOPA), JS-3, *trans*-cyclo(L-Pro-D-DOPA), and *cis*-cyclo(L-Pro-L-Tyr) on human cancer cell lines: cervical cancer HeLa, breast cancer MCF-7, colon cancer HT-29, and normal murine fibroblasts BALB/3T3 cell line. The applied method is described in Supplementary Information and results are included in Table 1. All tested compounds, except *cis*-cyclo(L-Pro-L-Tyr) exhibited low antiproliferative activity towards the mentioned cancer cell lines (IC_{50} in range of 343.61–370.97 μg ml^{-1}).

Genotoxicity of JS-3, *cis*-cyclo(L-Pro-L-DOPA), *trans*-cyclo(L-Pro-D-DOPA), and *cis*-cyclo(L-Pro-L-Tyr) was also examined by applying the disc-diffusion method according to Kada's procedure [20]. For this purpose, two genetically modified *Bacillus subtilis* strains, M45 rec^{-} and H17 rec^{+} (obtained from Dr Yoshito Sadaie from the Department of Induced Mutation, National Institute of Genetics, Shizuoka, Japan), were used [21]. In effect of these assays, the studied compounds were found not to inhibit the growth of the tested *B. subtilis* strains and, hence, were determined as nongenotoxic.

In summary, the authors report on the isolation of a *cis*-cyclo(L-Pro-L-DOPA) from *Streptomyces* sp. 8812, termed JS-3. To our knowledge, this is the first isolation of this compound from natural sources and the first one from bacteria, *Streptomyces*. Our screening study was aimed at elucidating the biological properties of cyclo(Pro-DOPA). It revealed a variety of biological activities of this cyclic dipeptide, including potent free-radical scavenging activity and moderate antibacterial properties. The isolated JS-3 diketopiperazine is active towards VISA strains, which are currently a global threat in therapy of bacterial infections. The chemical scaffold of the compound provides a new, potential antibiotic template, unrecognized so far among the existent antibiotic classes. Biological activities of compounds: *cis*-cyclo(L-Pro-L-DOPA), JS-3, *trans*-cyclo(L-Pro-D-DOPA), and *cis*-cyclo(L-Pro-L-Tyr)) were compared. The presence of a hydroxyl group in compounds *cis*-cyclo(L-Pro-L-DOPA) and *trans*-cyclo(L-Pro-D-DOPA) was shown to have substantial influence on their biological activity in comparison to *cis*-cyclo(L-Pro-L-Tyr), while configuration of Tyr and Pro seems not to render any impact. The discovery of JS-3, a novel metabolite, is anticipated to enrich the existing collection of natural compounds and broaden the knowledge about the wide diversity of actinomycetes secondary metabolites. Taking into account the valuable biological properties and lack of genotoxicity, JS-3 may serve as model structure for further investigations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Solecka J, Rajnisz A, Postek M, Zajko J, Kawecki R, Havlicek V, Bednarek E, Kozerski L. N-acetyl-3,4-dihydroxy-l-phenylalanine, a second identified bioactive metabolite produced by *Streptomyces* sp. 8812. J Antibiot. 2012;65:219–21.
2. Solecka J, Rajnisz A, Laudy AE. A novel isoquinoline alkaloid, DD-carboxypeptidase inhibitor, with antibacterial activity isolated from *Streptomyces* sp. 8812. Part I: taxonomy, fermentation, isolation and biological activities. J Antibiot. 2009;62:575–80.
3. Solecka J, Sitkowski J, Bocian W, Bednarek E, Kawecki R, Kozerski L. A novel isoquinoline alkaloid, DD-carboxypeptidase inhibitor, with antibacterial activity isolated from *Streptomyces* sp. 8812. Part II: physicochemical properties and structure elucidation. J Antibiot. 2009;62:581–5.
4. Rajnisz A, Guśpiel A, Postek M, Ziemska J, Laskowska A, Rabczenko D, Solecka J. Characterization and optimization of biosynthesis of bioactive secondary metabolites produced by *Streptomyces* sp. 8812. Pol J Microbiol. 2016;65:51–61.
5. Solecka J, Kurzatkowski W. Affinity of exocellular DD-carboxypeptidase/ transpeptidase from *Saccharopolyspora erythraea* PZH TZ-575 to beta-lactam compounds. Med Dosw Mikrobiol. 1999;51:151–65.
6. Frere JM, Leyh-Bouille M, Ghuysen JM, Nieto M, Perkins HR. Exocellular DD-carboxypeptidases-transpeptidases from *Streptomyces*. Methods Enzymol. 1976;45:610–36.
7. Zhang ChJ, Zhang Z-H, Xu B-L. Synthesis of cis- and trans-3-(3,4-dihydroxybenzyl)-hexahydropyrrolo[1,2-a]piperazine-1,4-dione under microwave Irradiation by solid-phase. Chin J Synth Chem. 2008;16:398–401.
8. Martins MB, Carvalho I. Diketopiperazines: biological activity and synthesis. Tetrahedron. 2007;63:9923–32.
9. Stierle AC, Cardellina JH, Strobel GA. Maculosin, a host-specific phytotoxin for spotted knapweed from *Alternaria alternata*. Proc Natl Acad Sci USA. 1988;85:8008–11.
10. Bobylev MM, Bobyleva LI, Strobel GA. Synthesis and bioactivity of analogs of maculosin, a host-specific phytotoxin produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*). J Agric Food Chem. 1996;44:3960–4.
11. Puopolo G, Cimmino A, Palmieri MC, Giovannini O, Evidente A, Pertot I. Lysobacter capsici AZ78 produces cyclo(l-Pro-l-Tyr), a 2,5-diketopiperazine with toxic activity against sporangia of *Phytophthora infestans* and *Plasmopara viticola*. J Appl Microbiol. 2014;117:1168–80.
12. Nishanth KS, Mohandas C, Siji JV, Rajasekharan KN, Nambisan B. Identification of antimicrobial compound, diketopiperazines, from a Bacillus sp. N strain associated with a rhabditid entomopathogenic nematode against major plant pathogenic fungi. J Appl Microbiol. 2012;113:914–24.
13. Kumar N, Mohandas C, Nambisan B, Kumar DR, Lankalapalli RS. Isolation of proline-based cyclic dipeptides from *Bacillus* sp. N strain associated with rhabditid [corrected] entomopathogenic nematode and its antimicrobial properties. World J Microbiol Biotechnol. 2013;29:355–64.
14. Yonezawa K, Yamada K, Kouno I. New diketopiperazine derivatives isolated from sea urchin-derived *Bacillus* sp. Chem Pharm Bull. 2011;59:106–8.
15. Wattana-Amorn P, Charoenwongsa W, Williams C, Crump MP, Apichaisataienchote B. Antibacterial activity of cyclo(l-Pro-l-Tyr) and cyclo(d-Pro-l-Tyr) from *Streptomyces* sp. strain 22-4 against phytopathogenic bacteria. Nat Prod Res. 2016;30:1980–3.
16. Adam M, Damblon C, Plaitin B, Christiaens L, Frére JM. Chromogenic depsipeptide substrates for beta-lactamases and penicillin-sensitive DD-peptidases. Biochem J. 1990;270:525–9.
17. Adam M, Damblon C, Jamin M, Zorzi W, Dusart V, Galleni M, el Kharroubi A, Piras G, Spratt BG, Keck W. Acyltransferase activities of the high-molecular-mass essential penicillin-binding proteins. Biochem J. 1991;279:601–4.
18. Solecka J, Lysek R, Furman B, Chmielewski M, Kurzatkowski W. Practical use of DD-peptidase 64-575 for the assay of inhibition activity of natural and synthetic beta-lactam compounds. Acta Pol Pharm. 2003;60:115–8.
19. van der Merwe E, Huang D, Peterson D, Kilian G, Milne PJ, Van dV, Frost C. The synthesis and anticancer activity of selected diketopiperazines. Peptides. 2008;29:1305–11.
20. Kada T, Moriya M, Shirasu Y. Screening of pesticides for DNA interactions by rec-assay and mutagenesis testing, and frameshift mutagens detected. Mutat Res. 1974;26:243–8.
21. Sadaie T, Kada T. Recombination-deficient mutants of *Bacillus subtilis*. J Bacteriol. 1976;125:489–500.