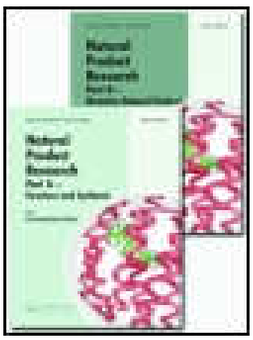


Natural Product Research

Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

An N-N linked dimeric indole alkaloid from the marine sponge-associated rare actinomycetes *Kocuria* sp. S42

Jingwei Jia, Xiaozheng Wang, Jin Sang, Zhiyong Li, Shuangjun Lin, Zixin Deng & Tingting Huang

To cite this article: Jingwei Jia, Xiaozheng Wang, Jin Sang, Zhiyong Li, Shuangjun Lin, Zixin Deng & Tingting Huang (2022): An N-N linked dimeric indole alkaloid from the marine sponge-associated rare actinomycetes *Kocuria* sp. S42, Natural Product Research, DOI: 10.1080/14786419.2022.2098496

To link to this article: https://doi.org/10.1080/14786419.2022.2098496

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2022.2098496

An N-N linked dimeric indole alkaloid from the marine sponge-associated rare actinomycetes *Kocuria* sp. S42

Jingwei Jia*, Xiaozheng Wang*, Jin Sang, Zhiyong Li, Shuangjun Lin, Zixin Deng and Tingting Huang

State Key Laboratory of Microbial Metabolism, and Joint International Research Laboratory on Metabolic & Developmental Sciences, and School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai, China

ABSTRACT

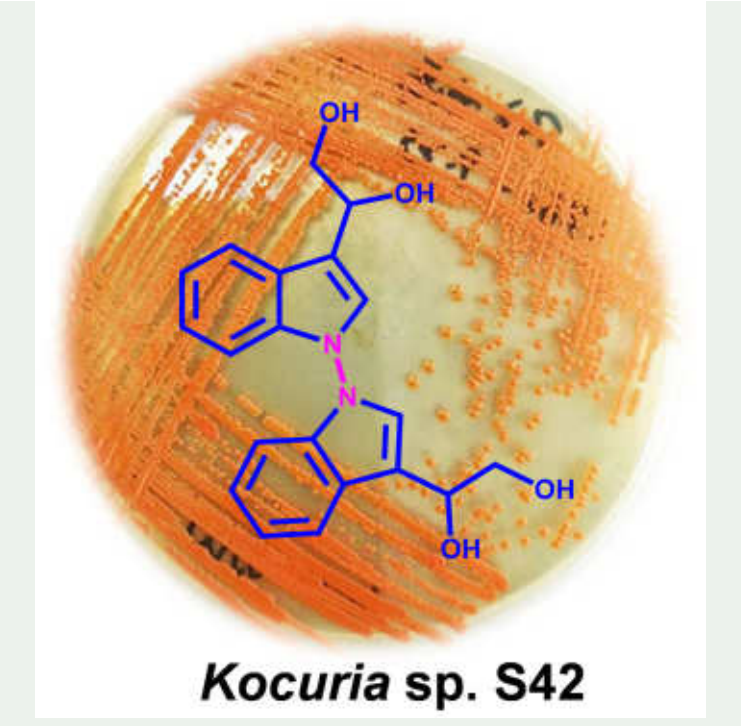
Marine derived rare actinomycetes is emerging as one of the new sources for various natural products for further drug discovery. Dimeric indole alkaloids represent a group of structurally diverse natural products and N-N linkage is a special dimerization mode. Here, we report the isolation of 1,1′-([1,1′-biindole]-3,3′-diyl) bis (ethane-1,2-diol), a new tryptophan-derived indole alkaloid from the marine sponge-derived *Kocuria* sp. S42. The structure was established based on extensive spectroscopic analyses, including nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass (HR-ESI-MS) spectrometry. The new dimeric indole alkaloid via N-N linkage exhibits moderate antimicrobial activity.

ARTICLE HISTORY
Received 23 April 2022
Accepted 27 June 2022

KEYWORDS
Rare actinomycetes; *Kocuria* sp.; N-N linked dimeric indole alkaloid; structure elucidation

CONTACT Tingting Huang tingwing82@sjtu.edu.cn
*These two authors contribute equally to this work.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2022.2098496.



1. Introduction

Actinomycetes are the most important group of microorganisms with a great ability to produce diverse bioactive secondary metabolites (Wang et al. 2020). The marine ecosystem covers nearly 71% of the earth's surface, harboring tremendous underexplored microbial communities. Due to the extreme environmental challenges, such as limited light, nutrients, pressure and predation, a large number of microorganisms evolve complex symbiosis to accommodate to these variations (Roue et al. 2012). These marine-derived microorganisms including actinomycetes are found to be associated with marine lives like sponges, corals, and ascidians (Blunt et al. 2012). The marine-derived actinomycetes feature special biochemical characteristics and unique metabolic pathways, leading to production of diverse secondary metabolites to assure survival in marine habitats (Montaser and Luesch 2011). Hence, actinomycetes from marine ecosystems also constitute the novel sources of diverse and potentially bioactive natural products, aside from the terrestrial actinomycetes (Subramani and Sipkema 2019; Newman and Cragg 2020).

Rare actinomycetes are specifically defined as actinomycete strains less frequently reported than that of *Streptomyces* species (Subramani and Aalbersberg 2013). Over the past three decades, a substantial number of rare actinomycetes have been isolated from the marine ecosystem and are believed to be potentially new resources of structurally diverse natural products (Subramani and Sipkema 2019). For example, from the rare actinomycete *Kocuria palustris*, a new compound named PM18110448 (Kocurin)

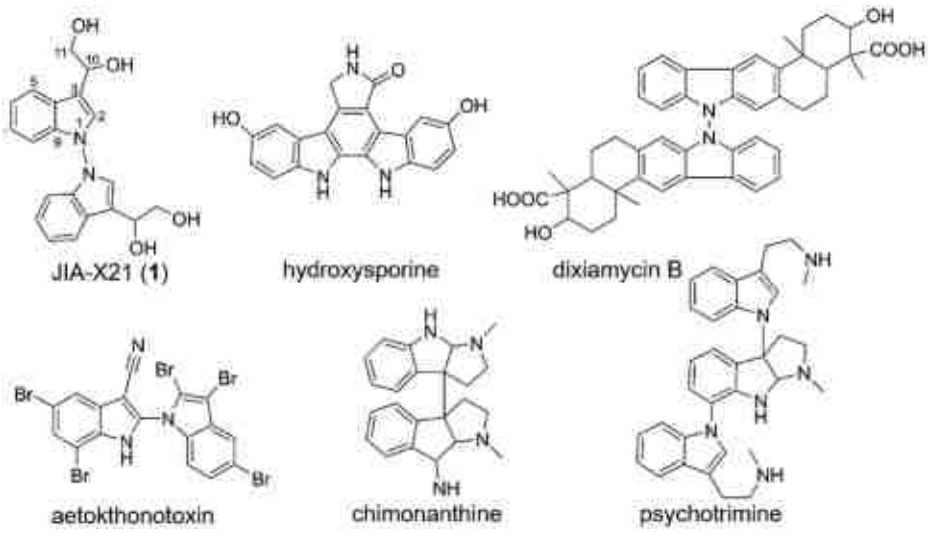


Figure 1. Representative dimeric indole alkaloids from marine natural products.

was discovered and demonstrated to have a broad spectrum of antibacterial activity (Martin et al. 2013).

Indole heterocycle alkaloids present a vital group in natural product chemistry and indole alkaloids those made up of two or more indole subunits are an important subset (Netz and Opatz 2015). Most of these alkaloids express the C-C or C-N linkage (Newhouse et al. 2010; Adak et al. 2022) while a rarity among such structures involves linkages between nitrogen atoms of the indole (Figure 1). Dixiamycin B, the pentacyclic indolesesquiterpene possesses an N-N dimeric linkage, which exhibits more potent than the monomeric xiamycin (Jin et al. 2021). In our work, we selected *Kocuria* sp. S42, a rare marine-derived actinomyces as the target strain for new natural products discovery. An N-N linked dimeric indole alkaloid has been isolated and structurally elucidated as 1,1′-([1,1′-biindole]-3,3′ diyl) bis (ethane-1,2-diol) by spectroscopic analysis. The evaluation of bioactivities of this compound (JIA-X21) exhibited moderate antibacterial activity.

2. Results and discussion

2.1. Cultural condition of strain S42

DNA sequencing of the housekeeping 16S rRNA gene showed significantly homologous (99.30%) to those of the *Kocuria* sp. strain SS263-23 (Accession Number: JX007975) in the GenBank database of NCBI. Phylogenetic analysis based on the 16S rRNA genes assigned species of S42 as *Kocuria* sp. (Accession Number: OM436921) (Figure S1). To explore the cultural conditions of *Kocuria* sp. S42, we carried out fermentation using selected five fermentation media according to the literature (Li and Zhang 2020), with metabolites varying in different fermentation media (Table S1 and Figure S2). The growth in M1 media is extremely well and gives the most prolific HPLC profile. We therefore chose M1 media for large-scale fermentation.

2.2. Isolation and structure elucidation of a new product from kocuria sp. S42

A special metabolite (peak ◆) in the M1 medium encouraged a large-scale fermentation of *Kocuria* sp. S42 to isolate the corresponding compound (Figure S2). Extraction of 12.0 L culture with organic solvent extraction and subsequent column chromatography resulted in the isolation of the compound JIA-X21.

JIA-X21 was isolated as a yellow solid. $[\alpha]_D^{20}$ 0 (*c* 0.05, MeOH). The melting point is 123.6 °C. High-resolution ESI-MS (HRESI-MS) analysis yielded an $[M+H]^+$ ion at *m/z* 353.1497, consistent with a molecular formula of $C_{20}H_{21}N_2O_4^+$ (calculated $[M+H]^+$ ion at *m/z* 353.1496), indicated 12 degrees of unsaturation (Figure S2).

Comprehensive analysis of the ^1H, ^{13}C, DEPT 135 and HSQC NMR spectrua acquired in methanol-d_4 revealed the presence of five aromatic methine signals [δ_C 118.2, C-5 and δ_H 7.55 (d, $J = 7.9$ Hz), H-5; δ_C 110.7, C-8 and δ_H 7.32 (d, $J = 8.0$ Hz), H-8; δ_C 118.2, C-6 and δ_H 6.99 (t, $J = 7.6$ Hz), H-6); δ_C 120.8, C-7 and δ_H 7.07 (t, $J = 7.5$ Hz), H-7; δ_C 123.0, C-2 and δ_H 7.14 (s), H-2]; one oxygenated CH_2 (δ_C 63.0, C-10 and δ_H 3.58 (1H, m), 3.51(1H, m), H-10); one oxygenated CH (δ_C 72.5, C-11 and δ_H 3.65 (1H, m), H-11) (Table S2; Figures S4–S9).

A substituted indole substructure was constructed by analysis of the COSY correlation between H-5 and H-6; H-6 and H-7; H-7 and H-8, and combined with the HMBC correlation from H-5 to C-3 (δ_C 109.0) and C-9 (δ_C 135.6); H-6 to C-4 (δ_C 127.5); H-7 to C-9 (δ_C 135.6); H-8 to C-4 (δ_C 127.5); H-2 to C-4 (δ_C 127.5) and C-9 (δ_C 135.6). Another substructure was constructed by the COSY correlation between H-10 and H-11. Finally, the HMBC correlation of H-11 to C-3 connects the two substructures together to give the partial structure **1a** (Figure S10) (Narumiya et al. 1979).

To accommodate the molecular formula and the number of observed carbon signals, JIA-X21 must have a symmetrical dimerization structure. The partial structure **1a** could be linked as a dimer through either an 11-O-O-11 ▫, 10-O-O-10 ▫, or 1N-1N ▫ linkage. The chemical shift of C-11 or C-10 is downfield about 10 ppm, supporting the location of the dimeric linkage as 1N-1N ▫ in **1a** instead of linking through 11-O-O-11 ▫ or 10-O-O-10 ▫. Finally, the structure of compound **1** was fully elucidated as 1,1′-([1,1′-biindole]-3,3′-diyl) bis (ethane-1,2-diol) and named JIA-X21.

2.3. Antibacterial activity

The isolated JIA-X21 was tested for antimicrobial activity *in vitro* against *E. coli*, *B. subtilis*, *P. aeruginosa*, and *S. aureus*. The compound JIA-X21 inhibits *E. coli*, *B. subtilis*, *P. aeruginosa* at minimum inhibitory concentration (MIC) values of 31 μg/mL, 31 to 63 μg/mL, and 63 to 125 μg/mL, respectively. It didn't show any antibacterial activity against *S. aureus*.

3. Experimental

3.1. General experimental procedures

LC-QTOF-MS analysis was performed on an Agilent 1290 series LC system in tandem with a 6545 Accurate-Mass Q-TOF MASS Spectrometer with an ESI source (50–1000 *m/*

z mass range, positive mode). Chromatography was carried out on Sephadex LH-20 (GE Healthcare Bio-Science AB, Pittsburgh, USA), reversed-phase silica gel (YMC*GEL, 12 nm S-50 μm, AAG12S50), and reversed-phase HPLC using ZORBAX Eclipse-XDB-C18 column (250 × 4.6 mm, 5 μm, Agilent), respectively. The melting point was determined by differential scanning calorimeter DSC2A-01130. All ^{1}H (700 MHz) and ^{13}C NMR (175 MHz) and 2D NMR (HMBC, HSQC) spectra were recorded on Bruker AVANCE NEO 700 instruments.

The 16S rRNA gene sequence of strain S42 was proofread using Chromas (version 2.6.5), and compared with the sequences available in NCBI (http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST). Multiple sequence alignment was performed using CLUSTALX. Phylogenetic tree was constructed using MEGA 11.

3.2. Bacterial strain, culture conditions and fermentation

Kocuria sp. S42 was isolated from a marine sponge *Agelas cathodes* sample, Yongxing Island, South China Sea (May 2011). Five fermentation media were tested for *Kocuria* sp. S42 (Table S1). All media were supplemented with sea salt (38.0 g L^{-1}) to simulate the conductivity and salinity conditions of the sea water. *Kocuria* sp. S42 was grown on solid ISP2 (glucose 4.0 g L^{-1}, yeast extract 4.0 g L^{-1}, malt extract 10.0 g L^{-1}, sea salt 38.0 g L^{-1}, adjusted to pH 7.2 before autoclave) agar plate for 3 days at 30 °C. *Kocuria* sp. S42 mycelium (about 1 cm^2) was inoculated into 250 mL Erlenmeyer flask containing 50 mL of ISP2 as seed medium and incubated at 30 °C and 220 rpm for 2 days. 500 mL M1 medium (starch 30.0 g L^{-1}, soybean powder 10.0 g L^{-1}, yeast extract 2.5 g L^{-1}, $CaCO_3$ 3 g L^{-1}, sea salt 38.0 g L^{-1}, pH 7.0) in 2000 mL Erlenmeyer flasks was inoculated with 10% (v/v) seed culture and incubated at 30 °C, 220 rpm for 5 days.

3.3. Extraction and isolation

After 5-day fermentation, 12.0 L of *Kocuria* sp. S42 fermentation broth were harvested by centrifugation (6500 rpm, 20 min). The separated supernatant was extracted three times with the same volume of ethyl acetate. The ethyl acetate extracts were combined and removed under the reduced pressure, and the crude extract (5.42 g) was subjected to Sephadex LH-20 column and eluted with methanol as the mobile phase to yield eight fractions (Frs. A-Frs. H). Fr. A was further chromatographed on semi-preparative reversed HPLC (XBridge C18,10 × 250 mm, 5 μm, Fisher Wharton) using a gradient elution system of 55 to 60% acetonitrile in H_2O containing 0.1% formic acid (t_R = 25.0 min). Each sub-fraction was tracked by HPLC analysis to afford a pure compound JIA-X21 (3.0 mg).

3.4. Antimicrobial assay

Antimicrobial assays were carried out using *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus* as the indicators. Stock solutions of the compound were prepared in methanol,

and the antimicrobial assays were carried out in 96-well plates against the microbial strains (5×10^5 CFU/mL) using a modification of the published method (Yang et al. 2006; Malapaka et al. 2007). After incubation for 14-16 h at 37 °C, the absorbance at 600 nm was measured using a microplate reader (BioTek, Winooski, VT, USA). Kanamycin was used as the reference compound.

4. Conclusions

In summary, we isolated and identified a new N-N linked dimeric indole alkaloid from *Kocuria* sp. S42, a marine sponge-associate rare actinomycetes from the South China Sea. Until now, very few compounds have been reported from *Kocuria* sp. and our work provides an opportunity to investigate the metabolic potential of *Kocuria* sp. Besides, this is the first report of a dimeric indole alkaloid via N-N linkage from rare actinomycetes. The discovery of JIA-X21 from the strain would expand the family of dimeric indole alkaloids and further explore the N-N dimerization mode from genomic landscape.

Acknowledgment

We would like to thank the Instrumental Analysis Center of Shanghai Jiao Tong University for determined the melting point and obtaining the NMR data.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the grants from the National Key Research and Development Program of China (2018YFA0901900, 2019YFC0312501) and Shanghai Subject Chief Scientist (21XD1401300), respectively.

References

Adak S, Lukowski AL, Schafer RJB, Moore BS. 2022. From tryptophan to toxin: nature's convergent biosynthetic strategy to aetokthonotoxin. J Am Chem Soc. 144(7):2861–2866.

Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. 2012. Marine natural products. Nat Prod Rep. 29(2):144–222.

Jin E, Li H, Liu Z, Xiao F, Li W. 2021. Antibiotic dixiamycins from a cold-seep-derived *Streptomyces olivaceus*. J Nat Prod. 84(9):2606–2611.

Li J, Zhang S. 2020. *Kocuria coralli* sp. nov., a novel actinobacterium isolated from coral reef seawater. Int J Syst Evol Microbiol. 70(2):785–789.

Malapaka VR, Barrese AA, Tripp BC, Tripp BC. 2007. High-throughput screening for antimicrobial compounds using a 96-well format bacterial motility absorbance assay. J Biomol Screen. 12(6):849–854.

Martin J, da SST, Crespo G, Palomo S, Gonzalez I, Tormo JR, de la Cruz M, Anderson M, Hill RT, Vicente F, et al. 2013. Kocurin, the true structure of PM181104, an anti-methicillin-resistant

Staphylococcus aureus (MRSA) thiazolyl peptide from the marine-derived bacterium *Kocuria palustris*. Mar Drugs. 11(2):387–398.

Montaser R, Luesch H. 2011. Marine natural products: a new wave of drugs? Future Med Chem. 3(12):1475–1489.

Narumiya S, Takai K, Tokuyama T, Noda Y, Ushiro H, Hayaishi O. 1979. A new metabolic pathway of tryptophan initiated by tryptophan side chain oxidase. J Biol Chem. 254(15):7007–7015.

Netz N, Opatz T. 2015. Marine indole alkaloids. Mar Drugs. 13(8):4814–4914.

Newhouse T, Lewis CA, Eastman KJ, Baran PS. 2010. Scalable total syntheses of N-linked tryptamine dimers by direct indole-aniline coupling: psychotrimine and kapakahines B and F. J Am Chem Soc. 132(20):7119–7137.

Newman DJ, Cragg GM. 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 83(3):770–803.

Roue M, Quevrain E, Domart-Coulon I, Bourguet-Kondracki ML. 2012. Assessing calcareous sponges and their associated bacteria for the discovery of new bioactive natural products. Nat Prod Rep. 29(7):739–751.

Subramani R, Aalbersberg W. 2013. Culturable rare Actinomycetes: diversity, isolation and marine natural product discovery. Appl Microbiol Biotechnol. 97(21):9291–9321.

Subramani R, Sipkema D. 2019. Marine rare actinomycetes: A promising source of structurally diverse and unique novel natural products. Mar Drugs. 17(5):249.

Wang C, Lu Y, Cao S. 2020. Antimicrobial compounds from marine actinomycetes. Arch Pharm Res. 43(7):677–704.

Yang L, Portugal F, Bentley WE. 2006. Conditioned medium from *Listeria innocua* stimulates emergence from a resting state: not a response to *E. coli* quorum sensing autoinducer AI-2. Biotechnol Prog. 22(2):387–393.