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Engineered production of kitasetalic acid, a new tetrahydro-β-carboline with the ability to suppress glucose-regulated protein synthesis

Shohei Ueda[1] · Shigeru Kitani[1] · Takushi Namba[2] · Masayoshi Arai[3] · Haruo Ikeda[4] · Takuya Nihira[1,5]

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Abstract

β-Carboline alkaloids and related compounds show a broad spectrum of biological activities. We previously identified new members of the β-carboline alkaloid family by using an engineered *Kitasatospora setae* strain and a heterologous *Streptomyces* host expressing the plausible biosynthetic genes, including the hypothetical gene *kse_70640* (*kslB*). Here, we elucidated the chemical structure of a new tetrahydro-β-carboline compound (named kitasetalic acid) that appeared in a heterologous *Streptomyces* host expressing the *kslB* gene alone. Kitasetalic acid suppressed the expression of glucose-regulated protein 78 (GRP78) without inducing cell death. This is the first report to show that a tetrahydro-β-carboline compound regulates the expression of the GRP78 protein in cancer cell lines.

Introduction

β-Carboline alkaloids are a large group of indole alkaloids that are composed of a pyridine ring fused to an indole skeleton [1]. These alkaloids exhibit a broad spectrum of pharmacological and biological properties, including anti-Parkinson, anti-inflammatory, anti-methicillin-resistant *Staphylococcus aureus*, and anticancer activities [2–5]. These activities of β-carboline compounds can be attributed to the characteristic abilities of the core β-carboline structure, such as intercalation into DNA, inhibition on cyclin-dependent kinases, topoisomerase and monoamine oxidases, and interaction with receptors for serotonin, dopamine and benzodiazepine [6–10]. The structure–activity relationship of β-carboline compounds indicated that the functional groups at the C-1 and C-3 positions in the pyridine ring exert a significant effect on the biological activity [1], and that appropriate substitution could elevate the activity dramatically. Thus, expansion of the structural diversity of β-carboline compounds, especially at C-1/C-3 positions, will allow us to design new drugs with improved properties.

β-Carboline compounds are frequently isolated from higher plants, marine organisms, and mammals [11–13]. However, there are only a few studies on the β-carboline compounds from actinomycetes and filamentous fungi [14, 15]. In 2012, we isolated a new member of the β-carboline alkaloid family (kitasetaline, **1**, Fig. 1a) from the genetically modified strain *Kitasatospora setae* NBRC 14216^T [16]. The *K. setae* wild-type strain produces a negligible amount of kitasetaline, whereas precocious and abundant production of kitasetaline is observed in a disruptant of the regulatory genes involved in the secondary metabolism, enabling identification of the chemical structure of kitasetaline. Moreover, additional new β-carboline alkaloids (JBIR-133 and JBIR-134) have been identified by heterologous expression of putative kitasetaline biosynthetic genes in a *Streptomyces* host strain [17]. These findings provide us with genetic information of a possible route for

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✉ Takuya Nihira
nihira@icb.osaka-u.ac.jp

[1] International Center for Biotechnology, Osaka University, Osaka, Japan

[2] Science Research Center, Kochi University, Kochi, Japan

[3] Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

[4] Kitasato Institute for Life Sciences, Kitasato University, Kanagawa, Japan

[5] MU-OU Collaborative Research Center for Bioscience and Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand

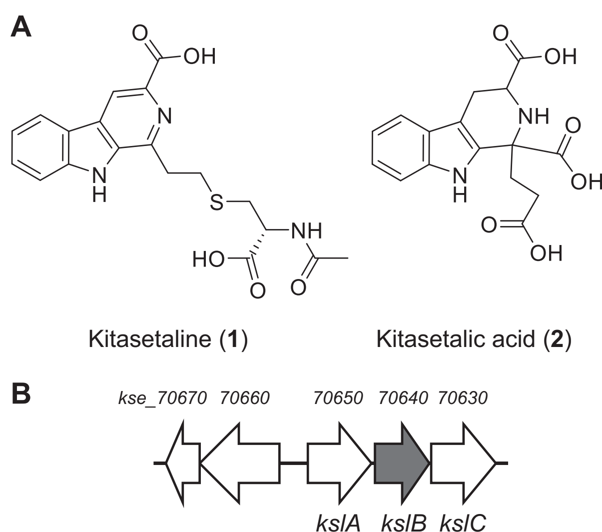

Fig. 1 Chemical structures of kitasetaline (**1**) and kitasetalic acid (**2**) **a** and organization of the *kse_70670-kse_70630* genes in *K. setae* **b**. **b** Each arrow indicates the direction of transcription and relative gene size

the supply of the β-carboline structure in bacteria. The biosynthesis of kitasetaline, JBIR-133, and JBIR-134 appears to require the enzymatic functions of KSE_70630 (KslC), KSE_70640 (KslB), and KSE_70650 (KslA) (Fig. 1b). The *kslA* gene encodes a putative flavin adenine dinucleotide-dependent oxidoreductase and the *kslC* gene encodes a putative cytochrome P450 hydroxylase, whereas the KslB protein is annotated as a hypothetical protein. The two proteins KslA and KslC appear to play roles in a tailoring step(s) for the biosynthesis of kitasetaline. However, the function of KslB remains uncertain, although we suppose that KslB is essential for the biosynthesis of β-carboline alkaloids.

Here, we report the isolation and structural elucidation of a newly appearing compound (**2**) produced by heterologous expression of the uncharacterized KslB protein. Compound **2**, named kitasetalic acid, is a new tetrahydro-β-carboline compound that has two different side chains at C-1 in the tetrahydropyridine ring, and suppresses the expression of a cell-protective endoplasmic reticulum chaperone protein in cancer cell lines.

Materials and methods

Bacterial strains, plasmids, and growth conditions

Escherichia coli DH5α was used for general DNA manipulation, *E. coli* GM2929 *hsdS*::Tn*10* was used to prepare unmethylated DNA for protoplast transformation in *Streptomyces avermitilis* SUKA22 [18], and *E. coli* F^- *dcm* Δ(*srl-recA*)*306*::Tn*10* carrying pUB307-*aph*::Tn*7* was used for *E.coli/Streptomyces* conjugation. pBluescript II SK was used for general cloning and pLT101 [19] was used for heterologous expression of the *kslB* gene. The *S. avermitilis* strains were grown at 28°C on YMS-MC medium for spore formation [17]. The media conditions and general *E. coli* and *Streptomyces* manipulations were described previously [17]. The primers used in this study are listed in Table S1.

Heterologous expression of the *kslB* gene in *S. avermitilis* SUKA22

The *kslB* gene was PCR-amplified with the primer pair kslB-Fw/kslB-Re, and was inserted into the *Eco*RV site of pBluescript II SK to yield pLT169. A 1.0 kb *Eco*RV fragment recovered from pLT169 was cloned into the blunt-ended *Bam*HI site of pLT101, resulting in pLT170. After demethylation through *E. coli* GM2929 *hsdS*::Tn*10*, pLT170 was introduced into *S. avermitilis* SUKA22 by protoplast transformation. Integration of the plasmid was confirmed by apramycin resistance and PCR analysis.

Transcriptional analysis of the *kslB* gene by semiquantitative RT-PCR

Total RNA was prepared from mycelia grown in liquid YMS medium by using an RNeasy Mini Kit (Qiagen Sciences, Germantown, MD), and treated with DNase I (Takara Bio, Shiga, Japan). The complementary DNA (cDNA) was synthesized using Superscript III RNase H^- reverse transcriptase (Invitrogen, Carlsbad, CA) and random primers (Invitrogen) according to the manufacturer's instructions. The PCR amplification was performed with Go Taq Green Master Mix (Promega KK, Tokyo). For detection of the *kslB* transcript, the conditions were 98 °C for 3 min, followed by 30 cycles of 98 °C for 30 s, 65 °C for 30 s, and 72 °C for 25 s; for the detection of the *rpoD* transcript, they were 98 °C for 3 min, followed by 30 cycles of 98 °C for 30 s, 60 °C for 30 s, and 72 °C for 35 s. The primers used for detection of the *kslB* transcript were kslB-tFw and kslB-tRe, and the primers used for detection of the *rpoD* transcript were rpoD-Fw/rpoD-Re. The absence of DNA contamination was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) without reverse transcriptase.

Analysis of secondary metabolites in the heterologous host

Spores (1.3×10^5 colony-forming unit; CFU) of the *S. avermitilis* strains were inoculated on 2.5 mL of YMS-MC medium supplemented with L-tryptophan at the final concentration of 3 mM. After incubation at 28°C for 7 days, the agar culture was diced and extracted with two volumes

Table 1 NMR data for kitasetalic acid (**2**) and in DMSO-d_6

Position	δ_C	δ_H mult (*J* in Hz)	HMBC
1	64.5, qC		
2			
3	54.8, CH	4.55, dd (11.8, 4.8)	1, 11, 4, 18
4	22.6, CH_2	3.01, dd (15.9, 11.9)	3, 10, 11, 18
		3.34, dd (15.9, 4.9)	3, 10, 11
5	119.0, CH	7.52, d (7.92)	7, 11, 13,
6	119.7, CH	7.05, m	8, 12
7	123.0, CH	7.17, ddd (1.02, 7.08, 8.16)	5, 13
8	112.1, CH	7.4, d (8.22)	6, 12
9		11.44, s	10, 11, 12, 13
10	127.6, qC		
11	107.2, qC		
12	125.4, qC		
13	137.3, qC		
14	169.6, qC		
15	31.0, CH_2	2.56, m	1, 14, 16
		2.83, m	1, 10, 16
16	28.8, CH_2	2.41, t (8.1)	1, 15, 17
17	173.7, qC		
18	170.5, qC		

of MeOH. The MeOH extract was filtrated, and then analyzed by a reversed-phase high-performance liquid chromatography (HPLC) system on an Inertsil ODS-3 column (5 µm; 4.6 i.d. × 250 mm; GL Sciences, Tokyo) with a linear gradient system (eluents: H_2O containing 0.075% trifluoroacetic acid (TFA) (A) and MeOH containing 0.01% TFA (B); 0–3 min 20% B and 3–25 min 20% B to 60% B; flow rate, 1 ml min^{-1}; UV detection at 276 nm).

Isolation and structural elucidation of compound 2

Spores (1.0×10^6 colony-forming unit; CFU) of *S. avermitilis* SUKA22 carrying pLT170 were inoculated on 20 ml of YMS-MC medium supplemented with L-tryptophan at the final concentration of 5 mM, and grown for 10 days at 28 °C. The 440 ml agar culture was diced and extracted with two volumes of MeOH, after which the MeOH extract was evaporated to dryness. The crude extract (1.5 g) was subjected to reversed-phase column chromatography using a Sep-Pak Vac C_{18} cartridge (Waters, Milford, MA) with a step-gradient system of MeOH-H_2O (0%, 10%, 20%, 30%, and 100% v/v). Compound **2** was eluted in fraction 1 (1.3 g). A part (150 mg) of the fraction was further purified using a preparative reversed-phase HPLC system on a Capcell-Pak C_{18} column (UG80; 5 µM 10 i.d. × 250 mm; Shiseido, Tokyo) with a linear gradient system (eluents: H_2O containing 0.075% TFA (A) and MeOH containing 0.01% TFA (B); 0 to 3 min 20% B, 3–3.5 min 20% B to 35% B and 3.5–15 min 35% B to 50% B; flow rate, 4 ml min^{-1}; UV detection at 276 nm), yielding 17.3 mg of pure compound **2**. HRFABMS was measured on a JEOL JMS-700 spectrometer. The UV spectrum was recorded on a JASCO V-730 spectrophotometer and the IR spectrum was recorded on an FTIR-8400S (Shimadzu, Kyoto, Japan). Optical rotation was measured on a JASCO P-2200 polarimeter. NMR spectra (^1H, 600 MHz; ^{13}C, 150 MHz) were recorded on a Bruker UltraShield 600 Plus spectrometer, and the ^1H and ^{13}C chemical shifts were referenced to the solvent signal ([DMSO]-d_6: δ_C 39.5, δ_H 2.50).

Physicochemical properties of kitasetalic acid (2)

Pale-yellow amorphous solid; UV (H_2O) λ_{max} (log ε) 218 (7.19), 272 (5.90), 279 (5.87), 289 (5.62), and 350 (4.56) nm; IR (nujol) ν_{max} 1520–1760 (br) and 3150–3600 (br) cm^{-1}; $[\alpha]^{20}_D$ −11.6 (*c* 0.004, MeOH); HRFABMS *m/z* 333.1086 $[M + H]^+$ (calculated for $C_{16}H_{17}N_2O_6$, 333.1087); ^1H and ^{13}C NMR data, see Table 1.

Antimicrobial assays

The growth inhibitory activity of **2** was evaluated in an agar diffusion susceptibility test against *Bacillus subtilis* PCI219, *E. coli* ATCC 25922, *Candida albicans* NBRC1592, *Saccharomyces cerevisiae* ATCC 9804, and *Ralstonia solanacearum* RS1000 (which is isogenic to MAFF730103) [20]. The anti-mycobacterial activity against *Mycobacterium smegmatis* mc^{2}155 and *Mycobacterium bovis* Bacille de Calmette et Guérin (BCG) Pasteur was assayed by the established 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [21].

Immunoblotting analysis of a glucose-regulated protein (GRP78)

The human osteosarcoma cell line U2OS, and human cervix epitheloid carcinoma cell line HeLa were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U mL^{-1} penicillin, and 100 µg mL^{-1} streptomycin. Mouse embryonic fibroblast (MEF) cells were maintained in DMEM supplemented with 10% FBS, 1% non-essential amino acids, and 0.5% 2-mercaptoethanol. All cells were maintained at 37 °C in an atmosphere containing 5% CO_2. MEF cells were obtained from Dr. Sam W Lee (Harvard University). The other cell lines were obtained from ATCC.

Immunoblotting experiments were performed as previously described, and sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed using

polyacrylamide gels. Antibody against GRP78 was purchased from Cell Signaling Technologies, and that against β-actin was purchased from Sigma. Antibodies were diluted to 1:1000 (anti-GRP78) or 1:10,000 (anti-β-actin). Secondary anti-rabbit and anti-mouse antibodies were purchased from Promega and were used at a dilution of 1:5000.

Gene expression analysis of GRP78

Quantitative RT-PCR was conducted as previously described [22]. Total RNA was normalized in each reaction using β-actin cDNA as an internal standard.

Cell viability assays

Cell viability was determined using the MTT method. After treatment with kitasetalic acid, cells were incubated with MTT solution (1 mg mL^{-1}) for 2 h. Isopropanol and HCl were then added to final concentrations of 50% (v/v) and 20 mM, respectively, and optical density at 570 nm was determined using a spectrophotometer.

Results

Heterologous expression of the *kslB* gene in *S. avermitilis* SUKA22

A homology search of the NCBI database demonstrated that the KslB homologs are widely distributed among Gram-positive bacteria and Gram-negative bacteria, such as a hypothetical protein (ALI144C_17030) (69% identity and 93% similarity) of *Actinosynnema* sp. ALI-1.44 and cucumopine synthase (44% identity and 78% similarity) of *Agrobacterium rhizogenes*. All of the proteins from actinomycetes that show moderate similarity to KslB are annotated as hypothetical proteins, but cucumopine synthases from Gram-negative bacteria have been found to catalyze a coupling between L-histidine and α-ketoglutaric acid [23]. These findings suggested that KslB is responsible for an enzymatic reaction similar to those catalyzed by cucumopine synthases.

To characterize the metabolites produced by KslB, we introduced one copy of the *kslB* gene into the genome of *S. avermitilis* SUKA22, an engineered host suitable for heterologous expression (Fig. S1). Because there is only a narrow intergenic region (74 bp) between *kslA* and *kslB*, *kslB* may form a transcriptional operon with *kslA*, with no plausible promoter-like sequences in the upstream region of *kslB*. Thus, we constructed a plasmid pLT170 that contains *kslB* under the control of the constitutive and strong *ermEp** promoter. Transcriptional analysis revealed that the introduced *kslB* gene was transcribed in *S. avermitilis* SUKA22

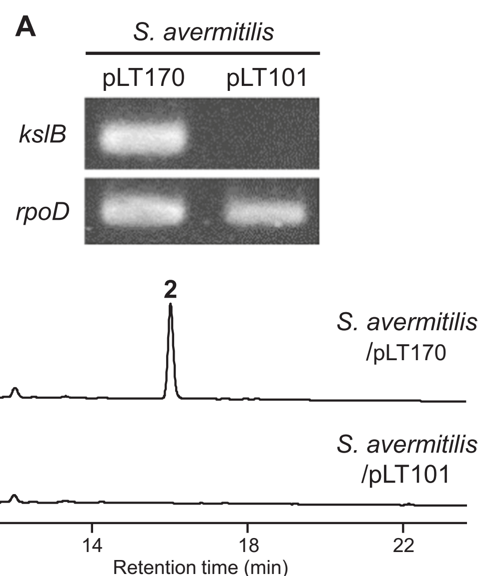

Fig. 2 Heterologous expression of the *kslB* gene in *S. avermitilis* SUKA22. *S. avermitilis*/pLT170, *S. avermitilis* carrying pLT170 harboring the *kslB* gene; *S. avermitilis*/pLT101, *S. avermitilis* carrying pLT101 (vector control). **a** Semiquantitative RT-PCR of the introduced *kslB* gene in *S. avermitilis* carrying pLT170. The *rpoD* gene was used as an internal control to normalize RNA samples. **b** HPLC analysis of the metabolite profile of *S. avermitilis* carrying pLT170. mAU, milliabsorbance units at 276 nm. Compound **2** was detected at the retention time of 16.0 min

carrying pLT170 (Fig. 2a), indicating that heterologous expression of *kslB* was successfully achieved. Subsequently, the transformants of *S. avermitilis* SUKA22 were grown on the agar medium, and MeOH extracts of the agar culture were analyzed by reversed-phase HPLC. *S. avermitilis* SUKA22 carrying pLT170 was found to produce one remarkably large peak (compound **2**, 16.0 min) that was not detected in the MeOH extract of *S. avermitilis* SUKA22 carrying the empty pLT101 vector (Fig. 2b). Interestingly, external addition of L-tryptophan into the agar medium led to a remarkable increase of compound **2** production (Fig. S2). All of these results indicated that KslB biosynthesizes compound **2** by using L-tryptophan as a substrate.

Isolation and structure elucidation of compound 2 from the engineered strain expressing *kslB*

To reveal the chemical structure of compound **2**, *S. avermitilis* SUKA22 expressing *kslB* was grown on the agar medium containing 5 mM L-tryptophan. ODS column chromatography, followed by preparative reversed-phase HPLC, gave 17.3 mg of the pure compound **2** as a pale-yellow amorphous solid. The molecular formula of compound **2** was deduced to be $C_{16}H_{16}N_2O_6$ by HRFABMS analysis (positive ion mode), indicating the presence of

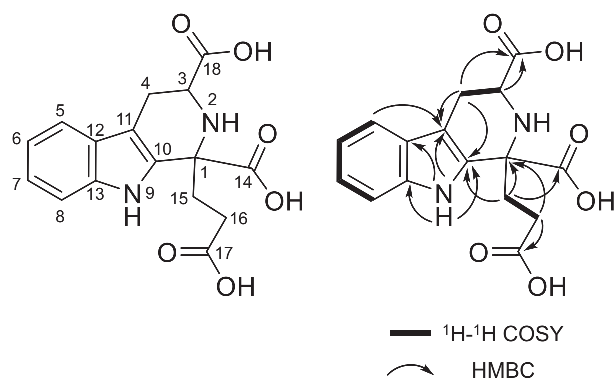

Fig. 3 Chemical structure of kitasetalic acid (**2**) (left) and the ^{1}H-^{1}H COSY and HMBC correlations (right)

7 unsaturated sites in the structure. The IR absorption bands of compound **2** suggested the presence of OH or NH groups (broad absorption bands: 3600–3150 cm^{-1}) and carbonyl groups (broad absorption bands: 1760–1520 cm^{-1}). The structure of compound **2** was established by 1D NMR (^{1}H and ^{13}C NMR) and 2D NMR (HSQC, HMBC, and ^{1}H-^{1}H COSY) analysis (Table 1).

The ^{1}H and ^{13}C NMR spectra of **2** in conjunction with DEPT-135 and the HSQC spectrum indicated the presence of four aromatic protons (δ_H 7.05, 7.17, 7.40, and 7.52), three methylene protons (δ_H 2.41, 2.56/2.83, and 3.01/3.34), one methine proton (δ_H 4.55), and eight quaternary carbons, including three carbonyl carbons (δ_C 169.6, 170.5, and 173.7). The ^{1}H–^{1}H COSY correlations revealed three partial structures: H-3 to H-4, H-5 to H-8, and H-15 to H-16 (Fig. 3). Subsequently, the HMBC correlations revealed the presence of a 1,2,3,4-tetrahydro-β-carboline structure, which includes two of the partial structures (H-3 to H-4 and H-5 to H-8). Methylene protons (δ_H 3.01 and 3.34) at C-4 and a methine proton (δ_H 4.55) at C-3 showed HMBC correlations to a carbonyl C-18 carbon, indicating that the COOH group was attached at C-3 (δ_C 54.8). The HMBC correlations from methylene protons (δ_H 2.56 and 2.83) at C-15 to two quaternary carbons at C-1 (δ_C 64.5) and C-10 (δ_C 127.6) and from methylene protons (δ_H 2.41) at C-16 to a carbonyl C-17 carbon (δ_C 173.7) revealed that the 2-carboxyethyl group, including the partial fragment (H-15 to H-16), was connected at C-1 of the tetrahydro-β-carboline structure. In addition, the ^{1}H-^{13}C long-range correlation from methylene protons at C-15 to a carbonyl C-14 carbon (δ_C 169.6) confirmed a connection between the COOH group and the tetrahydro-β-carboline structure via a quaternary C-1 carbon. Thus, these results identified the structure of compound **2** as 1-(2-carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid (Fig. 1a), which has not been isolated from any natural sources. We named compound **2** "kitasetalic acid".

Antimicrobial activity of kitasetalic acid (compound 2)

Some β-carboline compounds show antimicrobial activity against the Gram-negative and Gram-positive bacteria [24], whereas the antimicrobial activity of kitasetaline (**1**) has not been determined yet. We here attempted to investigate the antimicrobial activity of kitasetalic acid (**2**), which includes the tryptoline structure. However, there was no growth inhibitory activity of **2** against *B. subtilis*, *C. albicans*, *E. coli*, *S. cerevisiae*, or *R. solanacearum* at the 500 μg disk^{-1} level, and no growth inhibitory activity against *M. smegmatis* and *M. bovis* BCG up to 50 μM. Thus, we concluded that kitasetalic acid has no antimicrobial activity against the above-mentioned bacterial and fungal strains.

Downregulation of GRP78 in the human cell lines by kitasetalic acid

The glucose-regulated protein (GRP) 78 is involved in protein refolding in human cells and also plays an important role in maintaining the endoplasmic reticulum (ER) [25]. Because GRP78 expression is upregulated in various types of cancer cells, GRP78 has been regarded as a potential therapeutic target for cancers [26]. Thus, we investigated the ability of kitasetalic acid to downregulate GRP78 expression in three cultured cell lines: human osteosarcoma cell line U2OS, human cervix epithelial carcinoma cell line HeLa, and MEFs. The immunoblotting assays revealed that kitasetalic acid at a concentration of 100 μM selectively suppressed 50% of the GRP78 expression in both the U2OS and HeLa cell lines (Fig. 4a), whereas the GRP78 expression in MEF cells did not respond to treatment with kitasetalic acid (Fig. S3). In addition, the treatment of U2OS and HeLa cells with kitasetalic acid induced a dose-dependent decrease in the GRP78 expression. These results indicated that kitasetalic acid exhibits suppressive activity against GRP78 expression in the two cancer cell lines tested.

To further investigate whether kitasetalic acid affects the mRNA level of GRP78, we performed qRT-PCR analysis by using the U2OS and HeLa cell lines. Kitasetalic acid treatment at the concentration of 100 μM (but not at other concentrations) significantly downregulated the mRNA level of GRP78 in the U2OS and HeLa cell lines (Fig. 4b). Therefore, kitasetalic acid has the ability to control the transcriptional level of GRP78 in U2OS and HeLa cells.

Finally, we examined the effect of kitasetalic acid (**2**) on cell viability in the U2OS and HeLa cell lines. In the cell viability assays, kitasetalic acid up to 100 μM did not induce cell death in these two cell lines (Fig. 4c). Furthermore, kitasetalic acid did not affect the mRNA level of *CCAAT/*

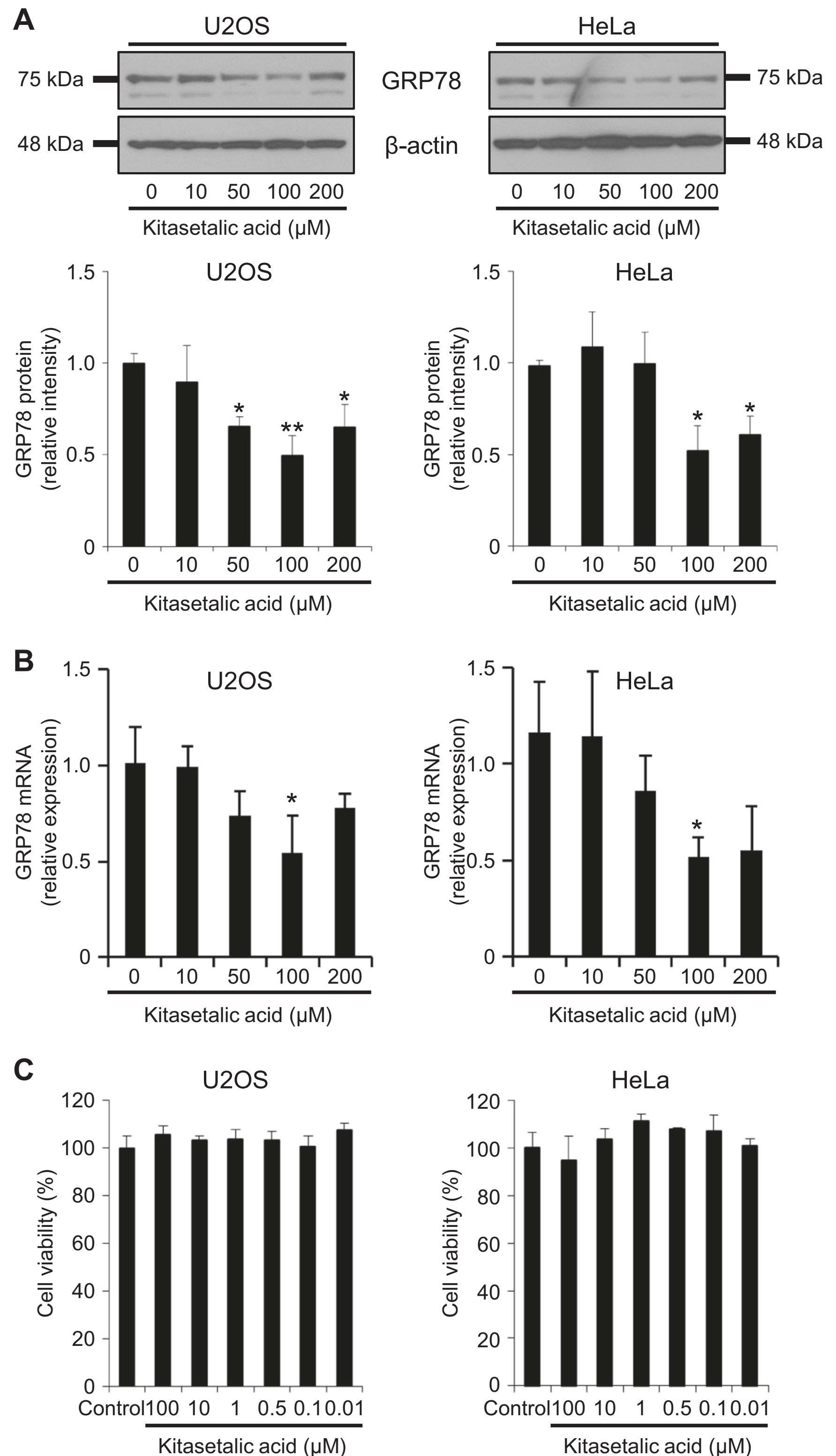

Fig. 4 Inhibition of GRP78 expression by kitasetalic acid in the U2OS and HeLa cell lines. U2OS and HeLa cells were incubated with kitasetalic acid at the indicated concentrations for 24 h. Data are presented as the mean ± standard deviation (SD) from three different experiments. **a** Immunoblotting analysis of GRP78 protein. Cell lysates were subjected to immunoblotting with anti-GRP78 and anti-β-actin antibodies (upper panels). The band intensity of GRP78 and β-actin was processed by densitometric scanning using imageJ software (NIH image) (lower panels). P values were calculated using two-way ANOVA followed by Tukey's test; $*P < 0.05$ and $**P < 0.01$. (*, vs 0 μM of kitasetalic acid) **b** Expression of GRP78 mRNA in U2OS and HeLa cells treated with kitasetalic acid. Total RNA was subjected to qRT-PCR analysis using the primer pair for *GRP78* and β-actin. Data were normalized to β-actin expression. P values were calculated using two-way ANOVA followed by Tukey's test; $*P < 0.05$ (*, vs 0 μM of kitasetalic acid). **c** Cytotoxicity assay of kitasetalic acid. Cell viability was determined by MTT assay

enhancer-binding protein homologous protein (CHOP), which has an important role in ER stress-induced cell death (Fig. S4) [27]. All of these results indicated that kitasetalic acid has no cytotoxicity against these cancer cells.

Discussion

The majority of β-carboline alkaloids have been found in plants, whereas only a few β-carboline compounds have been

reported so far from bacteria [11, 28]. Identification and engineering of a biosynthetic pathway of bacterial β-carboline compounds might enable us to generate β-carboline derivatives with intriguing biological activities. However, our current poor understanding of the β-carboline biosynthetic pathway in bacteria has hindered the engineered production of β-carboline analogs. We previously demonstrated that three new β-carboline compounds (kitasetaline, JBIR-133, and JBIR-134) are produced from a heterologous host expressing the putative biosynthetic genes, which included *kslB* annotated as a hypothetical gene [17]. In the present study, we have shown that kitasetalic acid, a new tetrahydro-β-carboline compound (also known as tryptoline), was produced by the heterologous expression of the *kslB* gene alone in the versatile model host *S. avermitilis* SUKA22, and that kitasetalic acid has the ability to downregulate the expression of GRP78 in cancer cells. A few indolotryptolines, such as BE-54017, cladoniamide A and borregomycin A, have been identified as tetrahydro-β-carboline compounds from actinomycetes strains or metagenome [29–31]. However, these compounds have a more complicated structure than kitasetalic acid, and their biosynthesis requires an oxytryptophan dimerization enzyme for the core structure [31], which is quite different from the structure of kitasetalic acid. To our knowledge, this is the first report on the identification of a simple tetrahydro-β-carboline compound in bacteria. Moreover, the heterologous expression of the *kslB* gene allowed us not only to generate a new tetrahydro-β-carboline compound, but also to better understand the kitasetaline (**1**) biosynthetic pathway, in which KslB presumably catalyzes the condensation between L-tryptophan and α-ketoglutaric acid to generate kitasetalic acid as a putative intermediate of kitasetaline. Interestingly, another KslB homolog (KSE_75260), in addition to KslB, is also present in *K. setae*, and KslB homologs are widespread among Gram-positive and Gram-negative bacteria. Therefore, heterologous expression of these KslB homologs in a suitable host could expand the structural diversity of β-carboline alkaloids and related compounds, and may lead to the discovery of new biosynthetic enzymes to catalyze unprecedented reactions.

The β-carboline compounds (kitasetaline (**1**), JBIR-133 and JBIR-134) have neither antimicrobial activity nor nematocidal activity, and no effect on the cell cycle status of Jurkat cells [16]. Recently, Savi et al. [32] demonstrated that 1-vinyl-β-carboline-3-carboxylic acid displays growth inhibitory activity against Gram-positive bacteria and fungi, suggesting that the vinyl side chain at C-1 of the β-carboline structure is crucial for the physiological activity of this compound. On the other hand, kitasetalic acid (**2**) has two different side chains (a carboxylic group and a carboxyethyl group) at C-1 of the tetrahydro-β-carboline structure, and also has no antibacterial and antifungal activities. However, we revealed that kitasetalic acid (**2**) has the suppression activity against GRP78 expression in the cancer cell lines, but not against normal-like MEF cells (Fig. 4). GRP78 protein allows cancer cells to adapt to chronic stress in the tumor microenvironment, which promotes tumor proliferation, survival, metastasis, and resistance to a wide variety of therapies [26]. Therefore, several researches have strongly suggested that the suppression of GRP78 protein expression is a promising target for anticancer drugs in various types of cancer [26]. Although some tetrahydro-β-carbolines show anticancer activity [33], there has been no report that they inhibit the expression of the GRP78 protein in human cell lines. The expression of GRP78 protein is well known to be upregulated by ER stress [25]. Interestingly, kitasetalic acid suppressed tunicamycin-induced GRP78 expression, but not thapsigargin-induced GRP78 expression (Fig. S5), suggesting that kitasetalic acid has an ability to suppress ER stress in part. Furthermore, we found that kitasetalic acid (**2**) has no cytotoxicity against the cancer cells examined in this study, suggesting that this compound could be a new lead compound for downregulating GRP78 protein expression without inducing cell death. Liu et al. [34] reported that tetrahydro-β-carboline derivatives containing an acylhydrazone moiety could be chemically synthesized by using 1-methyl-3-carboxylic tetrahydro-β-carboline, and that some of these derivatives exhibited new biological activities. Further structural modification of kitasetalic acid (**2**) by chemical synthesis or genetic engineering would improve the physiological properties for the generation of novel therapeutic agents for cancer.

In conclusion, we have shown that kitasetalic acid (**2**) is generated by the heterologous expression of the *kslB* gene, and that it is a new member of the tetrahydro-β-carboline compounds. Moreover, this compound shows interesting biological activity, and has a unique chemical structure compared with those of known downregulators of GRP78 protein expression. Kitasetalic acid (**2**) could thus become a lead inhibitory molecule for cotreatment with other anticancer drugs as a novel therapeutic strategy.

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Compliance with ethical standards

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

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