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NOTE

New 2-(1′*H*-indole-3′-carbonyl)-thiazoles derived from the thermophilic bacterium *Thermosporothrix hazakensis* SK20-1^T

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Secondary metabolism is commonly associated with morphological development in microorganism. In fact, Actinobacteria and Myxobacteria, both of which possess relatively complex morphology, produce a number of secondary metabolites that include biomedically and industrially useful chemicals.[1–3] Thus, complex life cycles may be indicative of microorganisms with active secondary metabolism. In this context, *Thermosporothrix hazakensis* SK20-1^T, a thermophilic bacterium isolated from ripe compost produced by a field-scale composter,[4] attracted our attention because it develops aerial mycelia, which bud to form multiple exospores per mother cell.[5] This morphological differentiation is similar to that observed in *Streptomyces* species, which belong to Actinobacteria, known producers of a variety of secondary metabolites. We therefore postulated that SK20-1^T might yield novel metabolites. Indeed, we previously identified new acyloins in fermentation broth from SK20-1^T cells.[6] In the present study, we identified two new secondary metabolites derived from *T. hazakensis* SK20-1^T.

The SK20-1^T strain was grown on agar medium containing 0.1% Bacto Yeast Extract (Becton, Dickinson and Company, Sparks, NV, USA), 0.2% Bacto Tryptone, 0.1% NaCl, 0.1% $MgSO_4 \cdot 7H_2O$ and 2% agar at 50 °C for 7 days and then cultured in Difco ISP1 medium (Becton, Dickinson and Company) in a 500-ml Sakaguchi flask for 3 days to generate a seed culture. The seed culture was then transferred to a 5-l jar fermenter (Bioneer C500, B.E. Marubishi, Tokyo, Japan) containing 3 l of fermentation medium (1.0% soluble starch, 0.4% Bacto Yeast Extract and 0.2% Bacto Peptone), and SK20-1^T was cultured for 7 days at 55 °C while stirring at 300 r.p.m. The secondary metabolites produced by the bacteria were analyzed by liquid chromatography–mass spectrometry (Shimadzu UFLC/AB SCIEX TripleTOF 5600 System, Tokyo, Japan) using a C_{18} column (Capcell Pak, 2.0 × 50 mm, Shiseido, Tokyo, Japan) and a solvent gradient of 10–90% CH_3CN (containing 0.1% formic acid) over 30 min (flow rate 0.4 ml min^{-1}) at various time points over the course of the 7 days to observe metabolite production over time. Two chromatographic peaks, each thought to be a natural product unrecorded in the Dictionary of Natural Products on DVD ver. 22:2 (CRC Press) based on an investigation using the molecular formulae calculated by ESI–HRMS, were selected for further purification. After 7 days of fermentation, a crude extract was prepared by extracting the culture broth with an equal volume of water-saturated butanol. The crude extract (1.0 g) was fractionated on a Diaion HP-20 flash chromatography column (Nippon Rensui, Tokyo, Japan) using different concentrations of MeOH (20, 40, 60, 80 and 100% MeOH in water, 100 ml each) as the elution solvent. The 100% MeOH fraction was injected into a preparative HPLC system (JASCO, Tokyo, Japan) equipped with a C_{18} column (PEGASIL ODS column, 20 × 250 mm, Senshu Scientific, Tokyo, Japan) using 60% MeOH containing 0.1% trifluoroacetic acid (TFA) as the eluent at a flow rate of 8 ml min^{-1} to yield compounds **1** (5 mg) and **2** (1 mg).

Compound **1** was isolated as a pale-yellow solid with the molecular formula $C_{13}H_8N_2O_3S$, indicating 11 double-bond equivalents. The UV/visible spectrum of compound **1** displayed maxima at 352 nm and 278 nm, which suggested a chromophore including an indole moiety. The ^1H NMR spectrum (600 MHz, DMSO-d_6) supported the presence of the indole moiety based on the appearance of typical chemical shifts, including a broad doublet signal (δ_H 12.32) resulting from an exchangeable NH-proton, a downfield-shifted doublet signal (δ_H 9.11), and four aromatic proton signals (δ_H 8.27, 7.55, 7.26 (2H)). ^1H-^1H COSY, HSQC and HMBC analyses of **1** revealed correlations consistent with a three-substituted indole structure (Figure 1). The product ion at *m/z* 116 in the ESI–MS spectrum, which corresponds to $C_8H_6N^+$, also supported the presence of an indole group (Figure 1). In addition, the product ion at *m/z* 144, which corresponds to $C_9H_6NO^+$, revealed the presence of a 3-carbonyl-indole group. The remaining singlet (δ_H 8.77) gave HMBC cross signals with three unassigned quaternary carbons (δ_C 170.1, 162.5 and 148.9). Along with these correlations, the presence of remaining one sulfur and two oxygen atoms in **1** suggested the presence of a thiazole ring connected to carboxylic acid. The existence of a carboxylic acid group in **1** was also suggested by the significant

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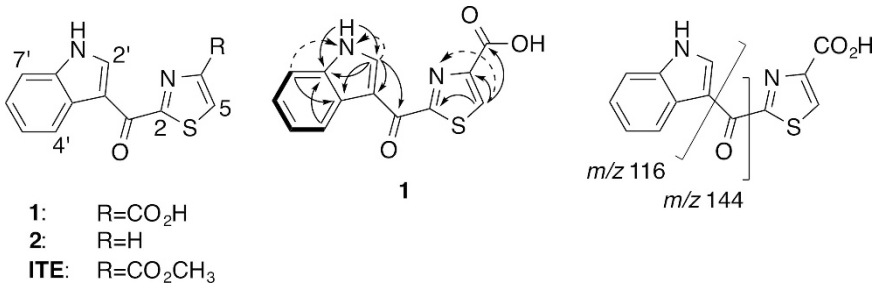

Figure 1 Structures of **1**, **2** and 2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE). ^{1}H-^{1}H COSY (bold) and HMBC (arrow) correlations and specific mass fragmentations of **1** are also shown. The correlation denoted by a dashed arrow was observed weakly in the HMBC spectrum of **1**.

shift in the HPLC retention time due to an ion-pair effect with the mobile phase containing 0.1% TFA in comparison with the mobile phase without TFA. The position of the group is most likely C-4 on the thiazole ring of **1** because the thiazole skeleton is usually generated from cysteine as described below. Although the unassigned quaternary carbon (δ_C 177.1) with a weak HMBC correlation from H′-2 (δ_H 9.11) in the indole moiety did not show any correlation with the thiazole moiety in the HMBC experiment, the overall structure of **1** was established as 2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid by comparing the NMR spectral data, mass fragmentation and UV/visible spectrum of **1** to those of the previously reported 2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) isolated from porcine lung.[7]

Compound **1**: UV-visible (MeOH) λ_{max}, nm (log ε): 272 (3.95), 278 (3.95), 352 (3.91); ^{1}H NMR and ^{13}C NMR (Table 1, Supplementary Figure S1 and S2); ESI–HRMS: *m/z* 273.0328 $[M+H]^+$; calculated for $C_{13}H_9N_2O_3S$, 273.0329.

Compound **2** was also isolated as a pale-yellow solid. The UV/visible spectrum of **2** showed maxima at 346 nm and 277 nm, similar to those of **1**. The molecular formula was determined to be $C_{12}H_8N_2OS$ based on ESI–HRMS. The molecular weight of **2** is 44 Da smaller than that of **1**, suggesting that the structures of **1** and **2** likely differ by the presence of a carboxyl group. In addition, the product ions at *m/z* 116 and *m/z* 144 in the ESI–MS spectrum also confirmed the presence of a 3-carbonyl-indole moiety. Based on the UV/visible and MS spectra, **2** was identified as the decarboxylated form of **1**. This structure was fully supported by the ^{1}H and two-dimensional NMR spectral data. In contrast to the ^{1}H NMR spectrum of **1**, two doublets (δ_H 8.13 and 8.11) were observed in the ^{1}H NMR spectrum of **2** in agreement with the decarboxylation of **1**. Furthermore, these two proton signals correlated with the quaternary carbon (δ_C 170.2) of the thiazole ring in the HMBC spectrum of **2**. Based on these spectral analyses, the complete structure of **2** was defined as 2-(1′*H*-indole-3′-carbonyl)-thiazole.

Compound **2**: pale-yellow solid; UV-visible (MeOH) λ_{max}, nm (log ε): 271 (3.86), 277 (3.86), 346 (3.75); ^{1}H NMR and ^{13}C NMR (Table 1, Supplementary Figure S3 and S4); ESI–HRMS: *m/z* 229.0429 $[M+H]^+$; calculated for $C_{12}H_9N_2OS$, 229.0430.

Neither **1** nor **2** exhibited antimicrobial activities against *Candida albicans* NBRC1594 at concentrations as high as 100 μM and *Micrococcus luteus* ATCC9341 at concentrations as high as 10 μM. Similarly, cytotoxicity tests against human ovarian carcinoma SKOV3 cells, mesothelioma Meso-1 cells and T lymphoma Jurkat cells revealed that high concentrations of **1** induced slight cytotoxicity against only the Jurkat cell line (approximately 30% inhibition at 50 μM, Supplementary Figure S5).

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for 1 and 2 in dimethyl sulfoxide-d6

	Compound 1		*Compound 2*	
Position	δ_H, *mult. (J in Hz)*	δ_C	δ_H, *mult. (J in Hz)*	δ_C
2	—	170.1	—	170.2
4	—	148.9	8.11 d (3.0)	126.6
5	8.77 s	134.0	8.13 d (3.0)	145.2
1′	12.32 br d	–	12.26 br s	—
2′	9.11 d (3.6)	138.7	9.07 s	138.4
3′	—	112.6	—	113.0
3a′	—	126.9	—	127.1
4′	8.27 dd (2.4, 6.0)	124.2	8.28 dd (1.8, 6.6)	123.9
5′	7.26 m	123.2	7.24 m	123.0
6′	7.26 m	122.0	7.24 m	122.0
7′	7.55 dd (1.8, 6.0)	113.3	7.53 dd (1.8, 6.0)	113.2
7a′	—	136.9	—	136.9
C=O	—	177.1	—	177.8
CO_2H	—	162.5	—	—

Natural small molecules with indole and thiazole moieties have been isolated from various biological sources, including animals,[7] plants,[8] bacteria,[9,10] fungi[11] and marine sponges.[12] However, small molecules such as **1** and **2** that contain a 3-carbonyl-indole moiety have not been explored thoroughly. In particular, the 2-(1′*H*-indole-3′-carbonyl)-thiazole carbon skeleton is rarely encountered among natural products reported in the literature; according to the Dictionary of Natural Products on DVD ver. 22:2, the only other naturally derived 2-(1′*H*-indole-3′-carbonyl)-thiazole is ITE, which was isolated from porcine lung.[7] Symbiotic or enteric bacteria are often suspected to be the biosynthetic source of secondary metabolites isolated from animals.[13,14] Therefore, we also suspect that a bacterial endosymbiont in porcine lungs might be responsible for producing ITE or a precursor of ITE. In fact, we detected ITE, as well as **1** and **2**, in the SK20-1^{T} culture (Supplementary Figure S6). Intriguingly, **2** was discovered in cultures of a myxobacterial strain 706, which was recently isolated from compost in Germany.[15]

The thiazole moieties found in natural products are usually generated through the oxidation of a thiazoline ring formed by heterocyclization between the sulfhydryl group of cysteine and the preceding carbonyl group.[16] Therefore, **1** and **2** are presumably generated through the oxidation of the thiazoline formed by the cyclization of an indole-3-glyoxylamide intermediate synthesized by condensation between cysteine and indole-3-glyoxylic acid

(Supplementary Figure S7). The elucidation of the biosynthesis of **1** and **2**, which will likely lead to the identification of novel metabolic pathways in *T. hazakensis* SK20-1^T, will be the aim of our next study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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