NOTE

RK-270A – C, new oxindole derivatives isolated from a microbial metabolites fraction library of *Streptomyces* sp. RK85-270

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Three new oxindole derivatives, RK-270A (**1**), B (**2**) and C (**3**) (Figure 1), were discovered and isolated from *Streptomyces* sp. RK85-270. They had an isopropylidene group at C-3 position of an oxindole skeleton, and C-1 also had a prenyl group and belonged to a class of 6-prenylated indoles. The isolation of this type of indoles was the first example as a natural product. Their cytotoxicity and antibacterial activities were evaluated.

Secondary metabolites from microorganisms are a major source of pharmaceutical leads and therapeutic agents¹ or bioprobes in a chemical biology study.² To obtain such valuable metabolites efficiently we have constructed a microbial metabolites fraction library and a spectral database based on the photodiode array detector attached LC/MS analysis.^{3,4} Through our methodology, we have identified several structurally unique metabolites, verticilactam,⁵ spirotoamides A and B,⁶ pyrrolizilactone,⁷ fraquinocins I and J⁸ and 6-dimethylallylindole (DMAI)-3-carbaldehyde.⁹ Moreover, we recently reported the advanced metabolite database Natural Products Plot (NPPlot) and discovery of new quinomycins, RK-1355A and B by the NPPlot search.¹⁰ These results have revealed the advantage of the fraction library for isolation of new metabolites.

The fraction library of *Streptomyces* sp. RK85-270, which was isolated from a soil sample collected in Java, Indonesia in 1985, was prepared from 30 l of culture broth following the scheme as described in the previous paper.^{3–6} On screening for structurally unique secondary metabolites using the database, we identified three unknown peaks with identical UV, which showed characteristic UV absorption with around 260, 265 and 300 nm indicating an indole chromophore with extended conjugation. They also showed quasi-molecular ion peaks at 242, 276 and 271 [M+H]⁺, respectively. The related fractions were purified by C₁₈-HPLC with acetonitrile/water isocratic elution to yield compounds **1** (1.2 mg), **2** (1.4 mg) and **3**

(1.3 mg) (see Supplementary Information for physicochemical properties). We report herein the structures of these three new compounds designated RK-270A (**1**), B (**2**) and C (**3**).

Compound **1** was obtained as an orange amorphous powder, and its molecular formula was determined to be C₁₆H₁₉NO by HRESIMS (*m/z* 242.1542 [M+H]⁺, calcd for C₁₆H₂₀NO, 242.1545). The IR spectrum implied the presence of an amide carbonyl group (1687 and 1617 cm⁻¹). The ¹H NMR spectrum in DMSO-*d*₆ showed four methyl signals (δ_{H} 1.67 (3H, s), 1.69 (3H, s), 2.26 (3H, s) and 2.47 (3H, s)) (Table 1). Two of them (δ_{H} 1.67 and 1.69) suggested the presence of a prenyl group with olefin and methylene signals (δ_{H} 5.26 (1H, m), 3.26 (2H, d, *J* = 7.4 Hz)). It also showed an exchangeable NH proton (δ_{H} 10.31 (1H, broad singlet; brs)) and three aromatic resonances with AB-X pattern (δ_{H} 7.40 (1H, d, *J* = 8.0 Hz), 6.74 (1H, dd, *J* = 8.0, 1.1 Hz) and 6.60 (1H, d, *J* = 1.1 Hz)) suggesting the presence of a trisubstituted benzene ring, which was supposed to be a part of an indole skeleton. The ¹H and ¹³C NMR data in conjunction with the HSQC data suggested the presence of 16 carbons, comprising four methyls, one methylene, four methines and seven quaternary carbons, which included an amide carbonyl signal at δ_{C} 168.8. In the HSQC spectrum in DMSO-*d*₆, the correlation between H-10 and C-10 was observed as a very weak signal, therefore it was confirmed by HSQC spectrum in CDCl₃. Interpretation of the 2D NMR data including DQF-COSY, HSQC and HMBC spectra led to the construction of precise structure of **1** (Figure 2). The HMBC correlations from NH signal to C-3 and C-7a, from H-4 to C-3, C-6 and C-7a, from H-5 to C-3a and C-7 and from H-7 to C-3a constructed an oxindole skeleton and substitutions at C-3 and C-6 positions in the oxindole skeleton in consideration of AB-X coupling pattern in ¹H NMR spectra, ¹³C NMR data and IR spectrum. The isopropylidene moiety and its attachment at C-3 position was confirmed by HMBC

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Figure 1 Structures of compounds **1**, **2** and **3**.Table 1 NMR spectroscopic data (500 MHz) for compounds **1**, **2** and **3**

Position	1 ^a		2 ^a		3 ^a	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1-NH	—	10.31, brs ^b	—	10.34, brs	—	10.37, brs
2	168.8	—	168.9	—	169.3 ^c	—
3	122.7	—	122.8	—	123.1	—
3a	121.4	—	121.2	—	122.3	—
4	123.5	7.40, d (8.0)	123.1	7.38, d (8.0)	124.1	7.43, d (8.0)
5	120.8	6.74, dd (8.0, 1.1)	122.0	6.79, dd (8.0, 1.1)	121.5	6.79, dd (8.0, 1.2)
6	141.2	—	141.4	—	140.0	—
7	108.8	6.60, d (1.1)	110.0	6.71, d (1.1)	109.6	6.63, d (1.2)
7a	140.6	—	140.2	—	141.2	—
8	152.5	—	152.0	—	153.5	—
9	24.7	2.26, s	24.7	2.27, s	25.3	2.28, s
10	22.1	2.47, s	22.0	2.48, s	22.7	2.48, brs
1'	33.8	3.26, d (7.4)	37.6	2.88, d (13.2)	34.5	3.43, d (7.5)
				2.31, dd (13.2, 10.3)		
2'	123.1	5.26, m	79.1	3.27, dd (10.3, 1.2)	134.0	6.42, ddd (7.5, 7.5, 1.2)
3'	131.9	—	71.7	—	132.6	—
4'	25.5	1.69, s	24.0	1.05, s	170.5 ^b	—
5'	17.7	1.67, s	26.9	1.10, s	13.3	1.82, d (1.2)
2'-OH	—	—	—	4.44, brs	—	—
3'-OH	—	—	—	4.26, brs	—	—
4'-NH ₂	—	—	—	—	—	6.87, brs
						7.32, brs

^aRecorded in DMSO-*d*₆.^bbrs: broad singlet.^cIndicated carbons showed only weak resonances in the ¹³C NMR spectrum, but their presence and connectivity were clearly evidenced by all conducted 2D NMR experiments. Figure 2 Key 2D NMR correlations of **1**, **2** and **3**.

correlations from both H-9 and H-10 to C-8, from H-9 to C-10, and from both H-9 and H-10 to C-3. The assignments of C-9 and C-10 signals were established by the low field chemical shift value of H-10 rather than that of H-9. The prenyl group was constructed by HMBC correlations from H-1' to C-3', from H-2' to both C-4' and C-5', from both H-4' and H-5' to C-3 and from H-5' to C-4'. The attachment of the prenyl group was established by HMBC correlation from both H-5 and H-7 to C-1' and from H-1' to C-6. The

assignment of C-4' and C-5' signals were performed owing to their chemical shifts (δ_C 17.7 (C-5') and 25.5 (C-4')). Thus, the structure of **1** was designated as RK-270A.

The molecular formula of compound **2** was determined to be C₁₆H₂₁NO₃ by HRESIMS. The IR absorption at 1683 and 1622 cm⁻¹ and identical UV spectrum with that of **1** suggested that **2** had the same oxindole skeleton as **1**. However, the IR spectrum showed an additional absorption at 3388 cm⁻¹, indicating the presence of a

hydroxyl group. The ^1H NMR spectrum in $\text{DMSO}-d_6$ was also similar to that of **1**, except for the disappearance of the olefin proton and appearance of two exchangeable signals at δ_{H} 4.44 and 4.26 as broad signals and an oxymethine signal at δ_{H} 3.27, which were confirmed by HSQC spectrum. The ^{13}C NMR spectrum in $\text{DMSO}-d_6$ showed 16 signals including identical signals for the oxindole skeleton with those of **1**. However, the olefin signals were disappeared and two oxygenated signals at δ_{C} 71.7 as a quaternary one and δ_{C} 79.1 as a methine were observed. On the basis of the above observation, **2** was supposed to be a dihydroxylated derivative of **1** at the Δ^2 . The planner structure of **2** was established by the same manner as **1**. The assignments of C-9 and C-10 were established by their ^1H NMR chemical shift values and confirmed by NOESY correlation between H-4 and H-9 (Figure 2). To determine the absolute configuration for C-2', preparation of the ester of **2** using α -methoxy- α -trifluoromethylphenylacetic acid (MTPA) or α -methoxy- α -trifluoromethylphenylacetyl chloride (MTPACL) were carried out by application of the modified Mosher's method (see Supplementary Information for detail).¹¹ However, all of the approaches employed did not yield the desired product. Therefore, the optical rotation value of **2** was compared with those of (R)-6-(2,3-dihydroxy-3-methylbutyl)indole and (R)-6-(2,3-dihydroxy-3-methylbutyl)indolin-2-one.¹² Compound **2** displayed a negative optical rotation as same as the literature. Thus, the absolute configuration at C-2' was supposed to be R-configuration, and the structure of **2** was designated as RK-270B.

Compound **3** had a molecular formula of $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_2$ determined by HRESIMS. The ^1H and ^{13}C NMR spectra in $\text{DMSO}-d_6$ were similar to those of **1** for the oxindole skeleton with isopropylidene group at C-3, which was also supported by the identical UV spectrum and similar IR spectrum with those of **1**. However, one methyl signal was disappeared and two exchangeable signals assigned as NH_2 protons (δ_{H} 7.32 (1H, brs) and 6.87 (1H, brs)) were observed in the ^1H NMR spectrum of **3**. In addition, the ^{13}C NMR spectrum showed the additional carbonyl carbon at δ_{C} 170.5 as a weak signal, which was clearly observed by the HMBC correlations and confirmed by ^{13}C NMR data obtained in CD_3OD , and the missing of one methyl signal. These observation suggested that one of the methyl groups at C-3' of **1** was replaced by an amide group. The detailed structure was determined by the same manner as **1** and **2** (Figure 2) and designated as RK-270C.

Compounds **1**, **2** and **3** were evaluated for cytotoxic activity against human cervical cancer cells (HeLa), human promyelocytic leukemia cells (HL-60), mouse temperature-sensitive cdc2 mutant cells (tsFT210) and rat kidney cells that were infected with ts25 (*src*^{ts}-NRK) and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus fumigatus*, *Magnaporthe oryzae* and *Candida albicans*. Compound **1** showed moderate cytotoxicity against all of four cell lines with IC_{50} values of 6.6, 5.5, 10.9 and $15.3\ \mu\text{g ml}^{-1}$, respectively. Compound **1** also showed weak antifungal activity against *M. oryzae* with IC_{50} value of $8.7\ \mu\text{g ml}^{-1}$. In contrast, **2** and **3** did not show any effects up to $30\ \mu\text{g ml}^{-1}$. These results suggest that the prenyl group is important for the activities, which will be an important knowledge for future SAR study.

Three new oxindole derivatives, RK-270A (**1**), B (**2**) and C (**3**) were isolated from *Streptomyces* sp. RK85-270 based on our methodology constructing the fraction library with spectral database. They had an

isopropylidene group at C-3 position and prenyl group or its related side chains at C-6 position. Even though prenylated indole derivatives are widely distributed in nature,¹³ the isolation of the prenylated oxindole with the isopropylidene group at C-3 position is the first example as a natural product. We have reported the isolation of the new prenylated indole, 6-DMAI-3-carbaldehyde from *Streptomyces* sp. SN-593 and identified the key enzyme (IptA) for the prenylation at C-6 position.⁹ Recently, Satou *et al.*¹⁴ have reported the isolation of 3-hydroxy-6-dimethylallylindolin-2-one from *Actinoplanes missouriensis* and its biosynthetic pathway. However, a gene responsible for oxindole formation still remains unsolved. In addition, conversion of **1** into **2** and **3** requires successive hydroxylation and carboxamide formation. Identification of the gene cluster of **1**–**3** is indispensable to address the mechanism of biosynthesis and future derivatization of oxindoles which might have a strong biological activity.

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