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NOTE

Opantimycin A, a new metabolite isolated from *Streptomyces* sp. RK88-1355

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A new metabolite containing a γ-butyrolactone and 2-hydroxy-3-formylaminobenzoic acid moieties, opantimycin A (**1**) was isolated from a microbial metabolite fraction library generated from *Streptomyces* sp. RK88-1355 by search of an LC/MS-based spectral database named NPPlot: Natural Products Plot. The structure of **1** was determined based on extensive spectroscopic methods including NMR, MS and MS/MS experiments. **1** showed moderate cytotoxicity against HL-60 cell lines and antimalarial activity against *Plasmodium falciparum* 3D7. It was speculated that **1** might be biosynthesized by a hybrid enzyme including non-ribosomal peptide synthetase and polyketide synthase, which were similar to neoantimycin biosynthetic machinery.

Microbial metabolites isolated from actinomycetes and fungi have unique and wide chemical diversity, and they are major sources in the discovery of novel drug candidates for various biological activities.[1,2] Hence they have played an important role in drug discovery and development of agrochemicals.[3] They are also used as bioprobes, which are chemical tools to investigate biological functions in chemical biology studies.[4,5] To discover and isolate such unique and important metabolites efficiently, we have constructed a microbial metabolite fraction library consisting of semi-purified metabolites by basic chromatographic techniques such as HPLC and middle pressure liquid chromatography (MPLC) coupled with LC/MS-based spectral database named NPPlot.[6,7] NPPlot is a distribution map of metabolites, which are plotted as dots in two-dimensional area by retention time and *m/z* value for *x* and *y* axes. Each metabolite has UV information of maximum absorption values for *z* axis in NPPlot, which allows us to find specific or distinctive metabolite groups easily.

In the course of screening for structurally novel metabolites from fraction libraries by NPPlot, we have discovered and isolated several new metabolites with interesting structures, such as verticilactam,[8] spirotoamides[9] and pyrrolizilactone.[10] We have recently reported new quinomycin derivatives, RK-1355A and B[11] and a new neoantimycin analogue, unantimycin A[12] from the fraction library of *Streptomyces* sp. RK88-1355 by NPPlot search, in which distinctive metabolites groups were screened by comparison of NPPlot generated from several *Streptomyces* strains. Based on our continuing search for structurally unique metabolites in the RK88-1355 fraction library, unidentified metabolites were found by NPPlot screening. They showed similar UV absorption pattern to those of neoantimycins.[12–14] However, MWs of these compounds were around 550 Da, which was less than those of neoantimycins about 100 Da. One of these metabolites, a new compound (**1**), was isolated from the related fraction by C18-HPLC. We report, herein, the isolation, structure and biological activities of **1** (Figure 1).

Opantimycin A (**1**, 1.3 mg) was isolated as pale-yellow amorphous from the fraction library generated from *Streptomyces* sp. RK88-1355 by C18-HPLC. The molecular formula of **1** was determined to be $C_{28}H_{30}N_2O_9$ by high-resolution electrospray ionization mass spectrometry (HRESIMS) (found: *m/z* 539.2026 $[M+H]^+$, calculated for $C_{28}H_{31}N_2O_9$, 539.2030). The IR spectrum implied the presence of hydroxyl (3330 cm^{-1}), carbonyl (1756 and 1729 cm^{-1}) and amide (1660 and 1525 cm^{-1}) groups (Table 1). The ^1H NMR spectrum suggested the presence of a benzene ring (δ_H 7.21 of 2H, 7.28 of 2H and 7.23) and 1,2,3-trisubstituted benzene (δ_H 6.90 dd [J=8.1, 8.1], 7.27 m and 8.54d [J=8.1]) (Table 2). It also showed a doublet signal at 8.48 ppm (J=1.2) and two exchangeable signals as broad peaks at 7.64 and 7.90 ppm, which were confirmed by the addition of D_2O and observation of disappearance of the signals in ^1H NMR spectrum (Supplementary Figures S1 and S2). These observations suggested that **1** had a related structure to neoantimycins with a benzene ring and a 2-hydroxy-3-formylaminobenzoic acid, which were representative functional groups for neoantimycins. This was also supported by unusual low-fielded chemical shift value of H-8 at 8.54 ppm and a related UV absorption spectrum with those of neoantimycins such as SW-163A.[12,13] The ^1H NMR spectrum also showed four of methyl signals including two of singlet signals at 1.16 and 1.30 ppm and two of doublet signals at 1.60 (J=6.9) and 1.88 ppm (J=6.9), which were supposed to be attached on an sp2 carbon. The ^{13}C NMR spectrum

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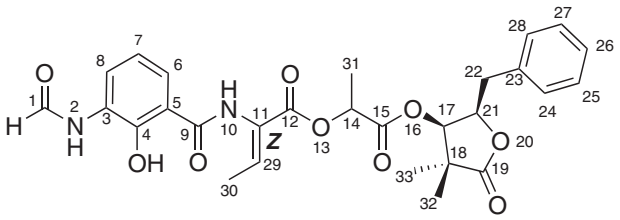

Figure 1 Structure of compound **1**.

Table 1 Physicochemical properties of compound 1

Appearance	*Pale yellow amorphous*
Optical rotation (MeOH)	$[\alpha]_{589}^{23}$ +60 (*c* 0.03)
Molecular formula	$C_{28}H_{30}N_2O_9$
UV (MeOH) λ_{max} (log ε) (nm)	226 (4.40), 327 (3.54)
IR (ATR) ν_{max} (cm^{-1})	3330, 2917, 1756, 1729, 1660, 1525, 1434, 1259,1209, 1130 and 1097
ESIMS (*m/z*)	539 $[M+H]^+$
HRESIMS (*m/z*)	Found 539.2026 $[M+H]^+$
	Calcd $C_{28}H_{31}N_2O_9$, 539.2030

Abbreviations: ATR, attenuated total reflection; ESIMS, electrospray mass spectrometry.

showed 26 signals, two of which were observed as a double intensity supporting the presence of a benzene ring (Supplementary Figure S3). It also contained four carbonyl carbon signals at 163.6, 168.7, 169.9 and 179.2 ppm, which were confirmed by ^{13}C DEPT experiment (Supplementary Figure S4). The planar structure was investigated by the detailed interpretation of 2D NMR spectra including HSQC, DQF-COSY, HSQC-TOCSY, HMBC and phase-sensitive NOESY (Figure 2 and Supplementary Figures S5–S9). The connections between proton and carbon were confirmed by the correlations observed in the HSQC spectrum. The proton spin networks between H-1 and NH-2, H-6 to H-8, H-14 and Me-31, H-17 to H-22, H-24 to H-28, and H-29 and H-30 were confirmed by the correlations observed in DQF-COSY and HSQC-TOCSY (Figure 2a). A 2-hydroxy-3-formylaminobenzoic acid moiety was confirmed by the HMBC correlations from H-1 to C-3, H-6 to C-4 and C-9, H-7 to C-3 and C-5, and H-8 to C-3 and C-4. A benzene moiety was confirmed by an HMBC correlation from H-25 and 27 to C-23 and attached to C-21 through a methylene carbon of C-22 assigned by HMBC correlations from H-22 to C-23 and C-24 and 28. A partial structure from C-30 to C-22 were constructed by combination of consideration of ^{13}C NMR chemical shift values of oxygenated carbons at C-14 and C-17 and HMBC correlations from H-30 to C-11, H-29 to C-11 and C-12, H-14 to C-12, H-31 to C-15, H-17 to C-15 and C-19, and Me-32 and 33 correlating each other, to C-17, C-18 and C-19. A γ-butyrolactone was constructed by the consideration of ^{13}C NMR chemical shift value of C-19 at 179.2 ppm, C-21 at 80.3 ppm, ^1H NMR chemical shift value of H-21 at 4.76 ppm (ddd, $J=9.1$, 4.6, 3.4 Hz) and the index of hydrogen deficiency of 15. The connection between C-9 and N-10 was constructed by the NOESY correlation between H-6 and NH-10. C-11 was considered connecting to N-10 by the ^{13}C NMR chemical shift value of 124.9 ppm although no HMBC correlation was observed from NH-10 to C-11. To confirm the connection, MS/MS experiment was carried out, and it showed fragment ions at *m/z* 375, 247 and 164 in ESI-positive mode (Figure 2c). These observations supported the

Table 2 ^1H and ^{13}C NMR chemical shifts of compound 1 in $CDCl_3$

Position	δ_C	δ_H	*Multiplicity, (J in Hz)*
1	159.1	8.48	d (1.2)
2-NH	–	7.90	brs
3	127.7	–	
4	151.0	–	
5	113.4	–	
6	120.7	7.27	m
7	119.3	6.90	dd (8.1, 8.1)
8	125.0	8.54	d (8.1)
9	168.7	–	
10-NH	–	7.64	brs
11	124.9	–	
12	163.6	–	
14	69.6	5.27	q (6.9)
15	169.9	–	
17	79.0	5.28	d (3.4)
18	45.0	–	
19	179.2	–	
21	80.3	4.76	ddd (9.1, 4.6, 3.4)
22			
a	35.4	2.88	dd (14.9, 4.6)
b		3.06	dd (14.9, 9.1)
23	136.5	–	
24, 28	129.4	7.21	m (2H)
25, 27	129.0	7.28	m (2H)
26	127.3	7.23	m
29	137.4	7.10	q (6.9)
30	15.3	1.88	d (3H, 6.9)
31	17.3	1.60	d (3H, 6.9)
32	18.3	1.16	s (3H)
33	22.9	1.30	s (3H)

^1H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively.

connection between NH-10 and C-11 and also C-9 and NH-10, and the planar structure of **1** was confirmed. The geometry at Δ^{11} was deduced as *Z*-configuration by the comparison of ^{13}C NMR chemical shift value of Me-30 at 15.3 ppm with those reported for phomalide and isophomalide,[15] which had identical dehydrobutyrine units of *E*- and *Z*-configuration (14.4 and 15.3 ppm in chloroform-*d* for *E*- and *Z*-configuration) with that of **1**. It was also supported by the NOESY correlation between H-29 and Me-31 (Figure 2a). The relative stereochemistry on the γ-butyrolactone was assigned to have *cis* configuration at C-17 and C-21 by the NOESY correlation between H-21 to both of H-17 and Me-33 (Figure 2b). As the result, the structure of **1** was determined as shown in Figure 1, designated as opantimycin A.

Cytotoxicities against human cervix epidermoid carcinoma cell line, HeLa, human promyelocytic leukemia cell line, HL-60, and rat kidney cells infected with ts25, a T-class mutant of *Rous sarcoma* virus Prague strain, *src*^{ts}-NRK, antimicrobial activities against *Staphylococcus aureus* 209, *Escherichia coli* HO141, *Aspergillus fumigatus* Af293, *Pyricularia oryzae* kita-1, and *Candida albicans* JCM1542 and antimalarial activity against *P. falciparum* 3D7 for **1** were tested. **1** showed moderate cytotoxicity against HL-60 cell lines with IC_{50} value of 4.4 μg ml^{-1}. It also showed weak antimalarial activity with IC_{50} value of 13 μg ml^{-1}, but did not show any effects on other microbes used for the assay up to IC_{50} values of 30 μg ml^{-1}. Neoantimycins and antimycins, both of which have a 2-hydroxy-3-formylaminobenzoic acid as a representative functional group, showed potent antimicrobial activity

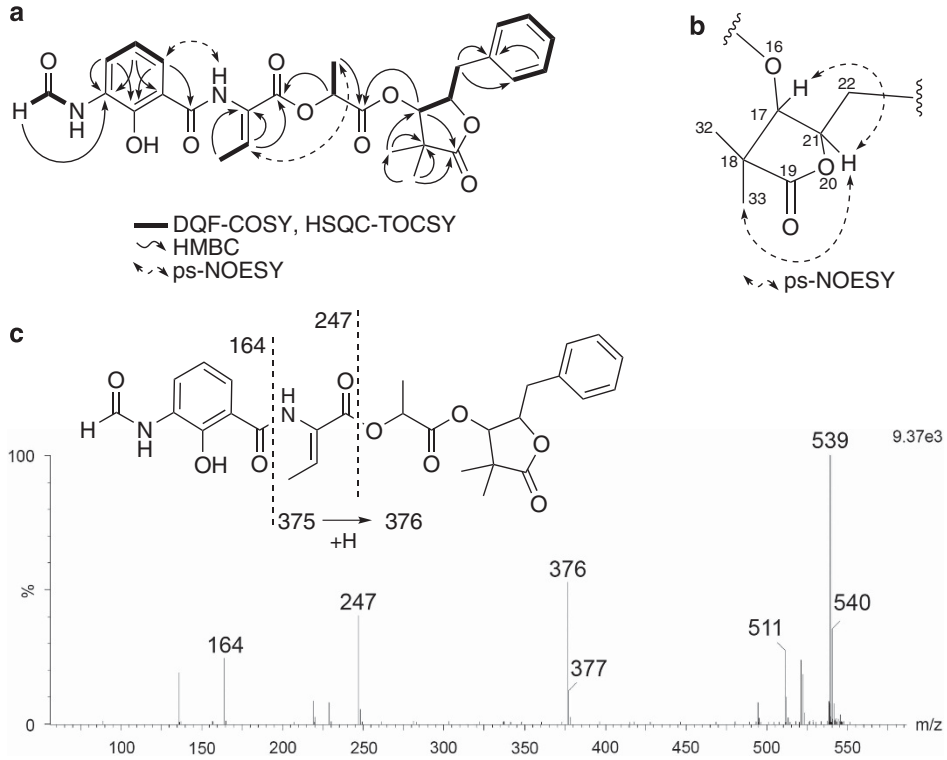

Figure 2 Key 2D NMR correlations and MS/MS fragmentation pattern observed for **1**. (**a**) 2D NMR correlations for determination of the planar structure. (**b**) phase-sensitive NOESY correlations for γ-butyrolactone moiety. (**c**) MS/MS experiment was carried out on a Waters Synapt G2 (Milford, MA, USA). The target ion was *m/z* 539 for **1** $[M+H]^+$ and the spectrum was recorded with collision energy of 15 eV by ESI-positive mode.

against *C. albicans*,[14,16] and it is reported that the 2-hydroxy-3-formylaminobenzoic acid is essential for the activity.[17] However, **1** did not show any effects against *C. albicans*, suggesting that the macrolide core structure might be also important for the antifungal activity.

Compound **1** had the 2-hydroxy-3-formylaminobenzoic acid and phenyl group, which were representative groups for the class of neoantimycins, and was speculated to have the related biosynthesis pathway with those of neoantimycins. The macrolide core is also the exemplary structure for neoantimycins. However, **1** has the γ-butyrolactone instead of the macrolide core. Isoneoantimycin is the only metabolite reported as a natural product[18] containing above three functional groups without the macrolide core, but is composed of the identical units with those of neoantimycin. **1** on the other hand does not contain the 2-hydroxy-3-methyl-valeric acid unit found in isoneoantimycin and neoantimycin, and the hydroxyl group derived from the threonine is dehydrated to form dehydrobutyrine. This type of compound was isolated and reported for the first time as a metabolite from *Streptomyces* sp., and analogous metabolites were detected in the fraction library. It is speculated that **1** uses a 2-hydroxy-3-formylaminobenzoic acid as the starter unit using biosynthetic machinery similar to neoantimycin[19] and an unidentified dehydratase catalyzing the formation of dehydrobutyrine. Through the NPPlot screening, we have found some related metabolites to **1**, which show identical UV absorption pattern with slightly different *m/z* values, and will report isolation and structures of these metabolites in the near future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)