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NOTES

UK-2A, B, C and D, Novel Antifungal Antibiotics from *Streptomyces* sp. 517-02

VI (1). Structure-Activity Relationships of UK-2A

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Streptomyces sp. 517-02 produced several novel antifungal antibiotics, UK-2A, B, C and D (UK-2 compounds), which have similarity to antimycin A_3 (AA) in structure and inhibitory activities towards electron transport at complex III in mitochondria.[1~3] However, UK-2 compounds inhibited the growth of *Rhizopus formosaensis* IFO 4732 at 0.0125 μg/ml, while AA showed no effect on it up to 100 μg/ml. In contrast, UK-2 compounds were less active than AA against mouse leukemia P388, mouse melanoma B16, human oral epidermoid carcinoma KB and human colon adenocarcinoma COLO201 cells. In our continuing studies on UK-2A (main component of UK-2 compounds),[4~7] we have been much interested in establishing structure-activity relationships of UK-2A. Herein, we like to report our preliminary studies on the synthesis of UK-2A analogues where the nine-membered dilactone residue was replaced by several alkyl or isoprenyl moieties, and their biological effects.

The structures of UK-2A and AA derivatives prepared here are represented in Fig. 1. 3-Hydroxy-4-methoxypyridine-2-carboxylic acid was prepared from commercially available 3-hydroxypyridine in the literature procedure.[8] Prenyl amine and farnesyl amine were prepared according to the Gabriel method from the corresponding bromide.[9] Amide formation was achieved in chloroform with 1-ethyl-3-(3-dimethyl-aminopropyl)-

Fig. 1. The structures of UK-2A, AA, and their synthetic analogues.

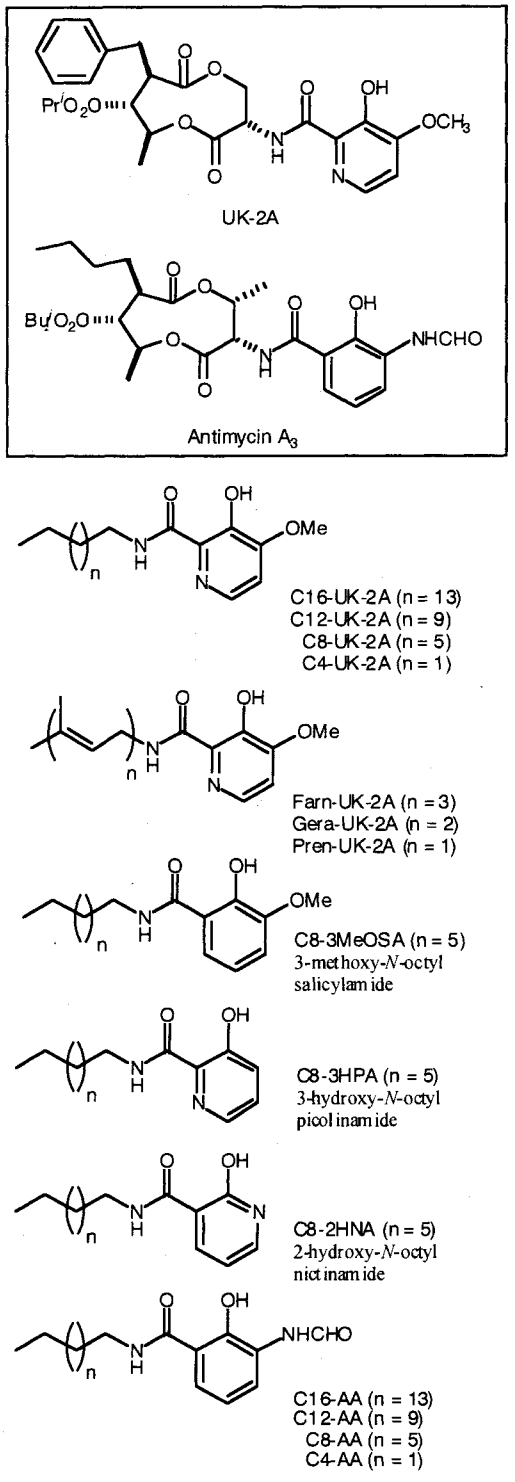

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Table 1. Antimicrobial activities (MIC) of UK-2A, AA, and their derivatives.

Compound[b]	MIC (μg/ml)[a]										
	EC	PA	BS	SA	SP	CA	RR	AN	RO	PC	MM
UK-2A	>100	>100	>100	>100	0.20	0.20	1.56	0.20	0.39	0.78	>100
C4-UK-2A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
C8-UK-2A	>100	>100	>100	>100	12.5	25	25	100	0.39	25	>100
C12-UK-2A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
C16-UK-2A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Pren-UK-2A	>100	>100	>100	>100	>100	100	>100	>100	50	>100	>100
Gera-UK-2A	>100	>100	>100	>100	100	>100	>100	>100	>100	>100	50
Farn-UK-2A	>100	>100	>100	>100	100	>100	>100	>100	0.39	>100	>100
C8-3MeOSA	>100	>100	>100	>100	>100	25	100	>100	12.5	100	>100
C8-3HPA	>100	>100	>100	>100	>100	100	>100	>100	12.5	25	>100
C8-2HNA	>100	100	100	>100	25	50	50	100	25	25	>100
AA	>100	>100	>100	>100	0.05	0.10	3.13	3.13	0.10	0.78	>100
C4-AA	>100	>100	>100	>100	50	6.25	>100	100	0.39	12.5	>100
C8-AA	>100	>100	>100	>100	25	0.39	6.25	25	0.10	1.56	12.5
C12-AA	>100	>100	>100	>100	>100	25	>100	>100	>100	>100	>100
C16-AA	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] Determined after 24-hour cultivation. [b] See Fig. 1.

EC: *Escherichia coli* IFO 3992. PA: *Pseudomonas aeruginosa* IFO 3080. BS: *Bacillus subtilis* IFO 3007.
SA: *Staphylococcus aureus* NCTC 8530. SP: *Schizosaccharomyces pombe* IFO 0342.
CA: *Candida albicans* IFO 1061. RR: *Rhodotorula rubra* IFO 0001. AN: *Aspergillus niger* ATCC 6275.
RO: *Rhizopus oryzae* IFO 4766. PC: *Penicillium chrysogenum* IFO 4626. MM: *Mucor mucedo* IFO 7684.

carbodiimide and 1-hydroxy-benzotriazole. Analogues having 3-formylamino-salicylyl group, *i.e.* AA derivatives were prepared from 2-hydoxy-3-nitrobenzamides in 2 steps: hydrogenation over 10% palladium on carbon in ethanol and *N*-formylation with *N*,*N*-dicyclohexyl-carbodiimide in toluene. The crude products were purified by preparative thin-layer chromatography with methanol - chloroform or ethyl acetate - hexane. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-LA400 spectrometer, and EI mass data were recorded on a JEOL JMS-700T mass spectrometer. The physical data thus obtained were consistent with the structures of these derivatives (Data not shown).

In the antimicrobial assay, each compound was first dissolved in DMF. The MIC values were measured by the serial 2-fold agar dilution method[10] after a 24-hour cultivation in 3% nutrient agar at 30°C for bacteria and in Sabouraud dextrose agar at 25°C for yeasts and filamentous fungi. The results are summarized in Table 1. All tested compounds did not show any growth inhibitory activity against both Gram-negative and Gram-positive bacteria up to 100 μg/ml. However, C8-UK-2A, C8-2HNA, C4-AA, and C8-AA showed antifungal activity. These results indicate salicylic acid moiety or pyridinecarboxylic acid moiety plus a hydrophobic structure is at least necessary for expression of antifungal action. Therefore, these derivatives act on unique functions or organella such as mitochondria in eucaryotes including fungi and mammalian cells.

C8-UK-2A inhibited the growth of various yeasts and filamentous fungi, and the MIC values of this derivative were generally smaller than those of other UK-2A derivatives and C8-3HPA were. Moreover, among C4-, C8-, C12-, and C16-UK-2A, only C8-UK-2A showed antifungal activities. The attribution of C8-alkyl group to the antimicrobial activity could be applied to the case of AA derivatives with various alkyl chains. This result suggests that 9-membered dilactone ring moiety itself is not essential for the activity, and C8-alkyl group is flexible and hydrophobic that makes C8-UK-2A interact the binding domain to prevent yeasts and filamentous fungi from growing. Therefore, decreases in the activity of isoprenylated UK-2A derivatives would be attributed to a loss of flexibility, which interferes in their taking active conformations. C8-3HPA differs from C8-UK-2A in having

no methoxy group attached to the picolinic acid moiety. The methoxy group may play an important role in the growth inhibitions.

Compared to UK-2A, AA had strong cytotoxicity against porcine renal proximal tubule LLC-PK1 cells as well as other types of cultured cells.[5] Instead of evaluating cytotoxicities of derivatives of UK-2A and AA, inhibitory activity of these compounds for the uncoupler stimulated respiration of bovine heart submitochondrial particles (SMP) was examined. Bovine heart SMP were prepared by the method of MATSUNO-YAGI and HATEFI.[11] The SMP respiration using 10 mM succinate as the respiratory substrate was measured with a Yanagimoto PO-100A oxygen electrode at 25°C. In Table 2 are summarized the molar concentrations of inhibitors needed to halve the 2,4-dinitrophenol-stimulated respiratory rate of bovine heart. The log of reciprocal of I_{50} is taken as the index of inhibitory.

Interestingly, C8-3MeOSA has shown comparably high inhibitory activity similar to C8-AA and AA, although its antimicrobial activities were weaker than those were. This could be resulted from limited permeability of the cell membrane to C8-3MeOSA, which has a methoxy group on benzene ring instead of formylamino group. The activity of C8-UK-2A was about hundredth of that of UK-2A, while the activity of C8-AA was about third of that of AA. This suggests that mode of action of C8-UK-2A would be different from that of UK-2A. Further studies are now in progress.

Table 2. Respiratory inhibition of UK-2A, AA, and their synthetic derivatives in bovine heart SMP.

Compound [a]	pI_{50}
AA	7.1
UK-2A	6.7
C8-UK-2A	4.7
C8-3MeOSA	6.5
C8-AA	6.6
C8-3HPA	4.4
C8-2HNA	4.1

[a] See Fig. 1.

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