UK-2A, B, C and D, Novel Antifungal Antibiotics from *Streptomyces* sp. 517-02 VI (2). Structure-activity Relationships of UK-2A

YOSHINOSUKE USUKI, KIMIHIKO GOTO, TETSUO KISO, KAZUNORI TANI, XU PING, KEN-ICHI FUJITA, HIDEO IIO and MAKOTO TANIGUCHI*

Graduate School of Science, Osaka City University
3-3-138 Sugimoto, Sumiyoshi-ku,
Osaka 558-8585, Japan

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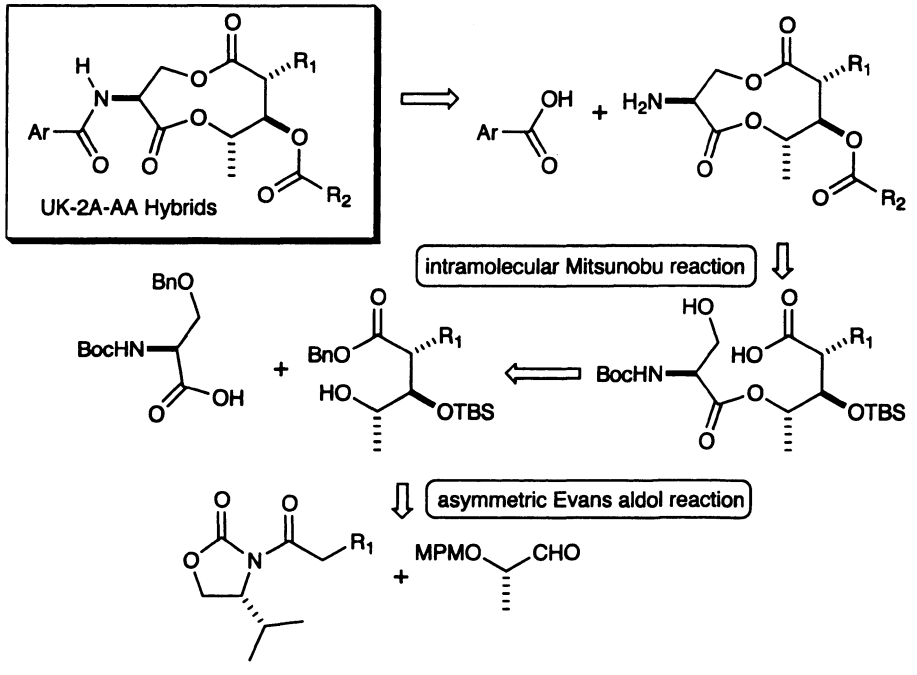
UK-2A, B, C and D (UK-2 compounds) were isolated from *Streptomyces* sp. 517-02, and are similar to antimycin A_3 (AA) in their structure and inhibitory activities towards electron transport at complex III in mitochondria[1~3]. However, the benzyl group at the C2 position in UK-2A (main component of UK-2 compounds) has never been found in known antimycins and a methyl group is lacking at the C8 position. In addition, UK-2A has a 3-hydroxy-4-methoxypicolinic moiety, while the antimycins have the 3-formamidosalicylic moiety which is essential to the inhibition of the electron transfer between cytochromes *b* and c_1 in the mitochondrial respiratory chain[4]. Furthermore, UK-2 compounds were less cytotoxic than AA against several kinds of mammalian cells: mouse leukemia P388, mouse melanoma B16, human oral epidermoid carcinoma KB and human colon adenocarcinoma COLO201. In our continuing studies on UK-2A[5~8], we have been very interested in establishing structure-activity relationships among UK-2A analogues. Recently, we reported the synthesis of UK-2A analogues where the nine-membered dilactone residue was replaced by several alkyl or isoprenyl moieties, and their biological effects[9,10]. We now like to report our preliminary studies on the synthesis of a series of hybrid molecules combining some structural features of both UK-2A and AA, and their biological properties.

The enantioselective total synthesis of UK-2A was reported by SHIMANO and co-workers[11], where the key features for the construction of the nine-membered dilactone moiety were the asymmetric Evans aldol reaction and the intramolecular Mitsunobu reaction. Their synthetic strategy (Scheme 1) was applied to the synthesis of the

Scheme 1. The synthetic strategy of UK-2A-AA hybrid derivatives.



* Corresponding author: makoto@sci.osaka-cu.ac.jp

hybrid derivatives with minor modification. The structures of UK-2A-AA hybrids prepared here are represented in Fig. 1. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-LA400 spectrometer, and EI and FAB mass data were recorded on a JEOL JMS-700T mass spectrometer. The physical data thus obtained were consistent with the

Fig. 1. The structure of UK-2A, AA and their synthetic hybrids.

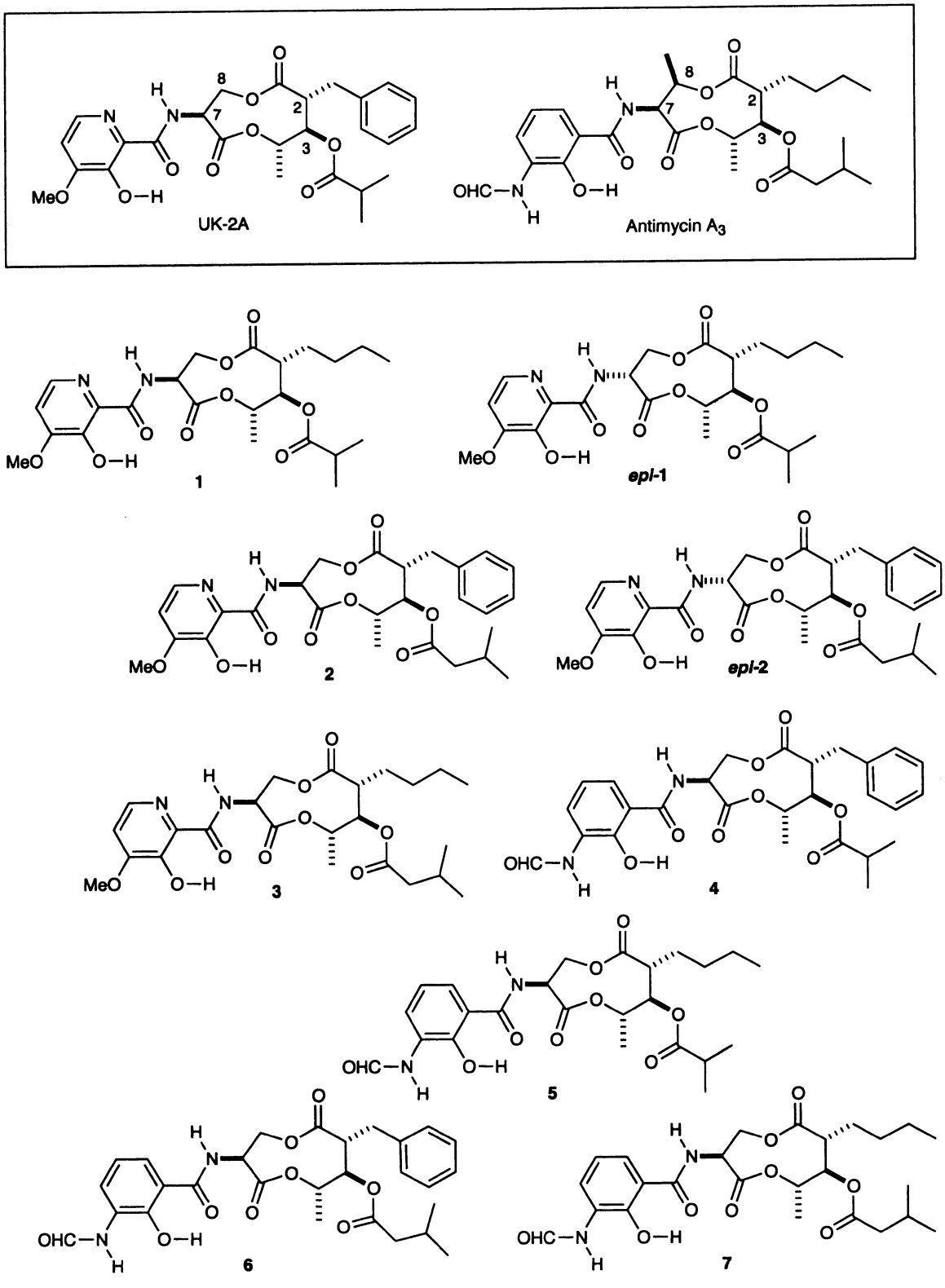


Table 1. Respiratory inhibition of UK-2A, AA, and their synthetic hybrid derivatives in bovine heart SMP.

Compound [a]	pI_{50}
AA	7.1
UK-2A	6.7
1	6.6
***epi*-1**	5.0
2	6.7
***epi*-2**	5.0
3	6.7
4	7.0
5	7.0
6	7.1
7	7.0

[a] See Fig. 1.

Table 2. Cytotoxic activities (IC_{50}) of UK-2A, AA, and their synthetic hybrid derivatives against LLC-PK1 cells and partition coefficients (Log P) of the compounds.

Compound [a]	IC_{50} (μg/ml)	Log P
AA	<0.01	2.39
UK-2A	>100	2.41
1	60.5	2.06
***epi*-1**	>100	2.06
2	>100	2.59
***epi*-2**	41.0	2.59
3	46.5	2.24
4	>100	2.25
5	17.2	1.89
6	58.4	2.43
7	38.7	2.07

[a] See Fig. 1.

structures of these derivatives (Data not shown).

Inhibitory activity of these hybrid compounds for the uncoupler stimulated respiration of bovine heart submitochondrial particles (SMP) was first examined. Bovine heart SMP were prepared by the method of MATSUNO-YAGI and HATEFI[12]. The SMP respiration using 10 mM succinate as a respiratory substrate was measured with a Yanagimoto PO-100A oxygen electrode at 25°C. In Table 1, the molar concentrations of inhibitors needed to halve the 2,4-dinitrophenol-stimulated respiratory rate of bovine heart are summarized. The log of reciprocal of I_{50} is taken as the index of inhibitory.

The decrease in the inhibitory activity of C7 epimers, *i.e.* *epi*-**1** and *epi*-**2**, would be attributable to a stereochemical mismatch that interferes with their binding to the active site of complex III on SMP. Similar to UK-2A, 3-hydroxy-4-methoxypicolinic acid derivatives **1**~**3** showed relatively lower inhibitory activity than 3-formamidosalicylic acid derivatives **4**~**7**, in addition to AA. The substitution at C2, C3 or C8 position of the nine-membered ring did not affect the respiratory inhibition in the case of 3-formamidosalicylic acid derivatives; the pI_{50} values of **4**~**7** being similar to that of AA. This result suggests that the substituents on the nine-membered dilactone ring moiety themselves do not effect the respiratory inhibition in bovine heart SMP.

The cytotoxic activity of each compound was evaluated using Alamar blue assay[13]. Porcine renal proximal tubule cells (LLC-PK1) were obtained from the American Type Culture Collection (CRL 1392; Rickvill, MD). Confluent cells (1.0×10^5 cells/ml) were seeded in a 96-well plate and incubated in a medium composed of Dulbecco's modified Eagle's medium (GIBCO Laboratories; Grand Island, NY), 10% heat-inactivated bovine serum and 3.7 mg/ml of $NaHCO_3$ under a humidified atmosphere of 5% CO_2 for 24 hours at 37°C. Then cells were cultured with compounds for another 48 hours. At the end of the incubation, Alamar blue solution was added and incubation continued for another 6~9 hours. Fluorescence was detected with a Millipore Cytofluor 2300 (excitation wavelength, 530 nm; emission wavelength, 590 nm). In addition, the logarithm of partition coefficient, Log P, of each compound was estimated by CRIPPEN's method[14]. These results are summarized in Table 2.

The structural difference between **1** and UK-2A is the substituent at C2 position; **1** has a butyl group instead of a benzyl group. The IC_{50} value of **1** was smaller than that of UK-2A. The effect of the butyl group at C2 position on cytotoxicity is apparent in the other pairs: **2** and **3**, **4** and **5**, **6** and **7**. This could result from limited permeability of the cell membrane to the hybrid derivatives with a butyl group, which is less hydrophobic than a benzyl group. In the two C7 epimers which demonstrated the least inhibitory activity of SMP respiration among all derivatives tested, only *epi*-**2** showed cytotoxicity indicating cellular damage apart from the respiratory inhibition. The cytotoxic activity of C8-demethyl AA 7 was much weaker than that of AA,

while its Log P value was smaller than that of AA. The methyl group at C8-position contributes to the potency of the cytotoxic activities. Further studies are now in progress.

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