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Semi-synthesis and biological evaluation of analogues of UK-2A, a novel antifungal antibiotic from *Streptomyces* sp. 517-02

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Abstract—Several analogues of UK-2A, a novel antifungal antibiotic isolated from *Streptomyces* sp. 517-02, were semi-synthesized for structure–activity studies. In vitro antifungal activities of these compounds against *Saccharomyces cerevisiae* IFO 0203 were evaluated by the conventional paper disk method. Several derivatives exhibited growth inhibitory activity similar to UK-2A.

UK-2A is an antifungal antibiotic produced by *Streptomyces* sp. 517-02, and is similar to antimycin A_3 (AA) in both structure and inhibitory activity toward electron transport at complex III in mitochondria.[1–4] Both UK-2A and AA consist of nine-membered dilactone rings linked via an amide bond to an aromatic acid moiety (Fig. 1): UK-2A possesses a 3-hydroxy-4-methoxypicolinic moiety, while the AAs have 3-formamidosalicylic moieties, reported to be essential for binding to complex III protein and the inhibition of electron transfer between cytochromes *b* and c_1 in the mitochondrial respiratory chain.[5] The benzyl group at the C2 position in UK-2A has not been reported in antimycins and a methyl group is lacking at the C8 position. Furthermore, UK-2A was less cytotoxic than AA against several mammalian cell lines, including murine leukemia P388, murine melanoma B16, porcine renal proximal tubule LLC-PK_1, human oral epidermoid carcinoma KB, and human colon adenocarcinoma COLO201.[6] As both UK-2A and AA inhibited mitochondrial electron transport, the difference in cytotoxicity between UK-2A and AA could be attributed to their ability to produce reactive oxygen species.[6,7] In continuing studies on UK-2A,[8] we have been interested in establishing structure–activity relations among UK-2A analogues.[9–11] This study focuses on (1) the 3′-OH group, (2) the benzene moiety, and (3) selective cleavage of the amide bond as shown in Figure 1, and reports the results of preliminary studies on the semi-synthetic preparation and biological evaluation of UK-2A analogues.

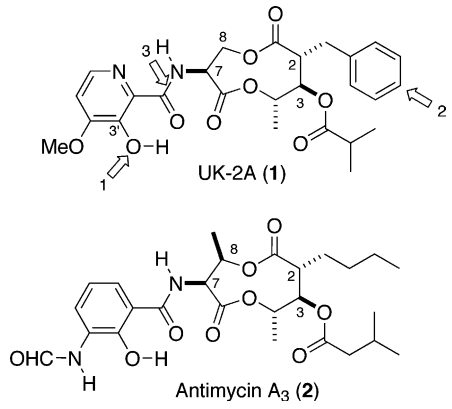

Figure 1. The structures of UK-2A and AA, showing the focus of SAR.

Keywords: Antifungal activity; Respiratory inhibition; Structure–activity relationship; Nine-membered dilactone; UK-2A.
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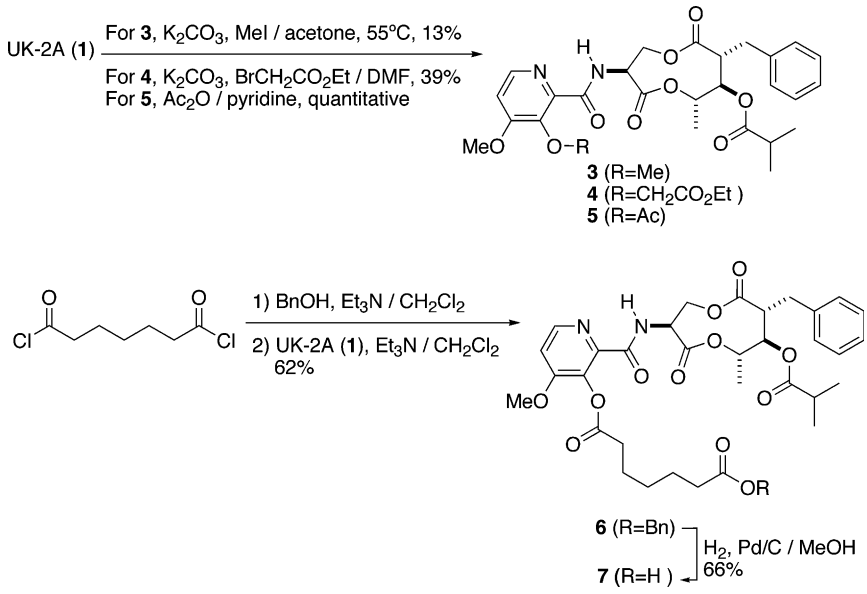


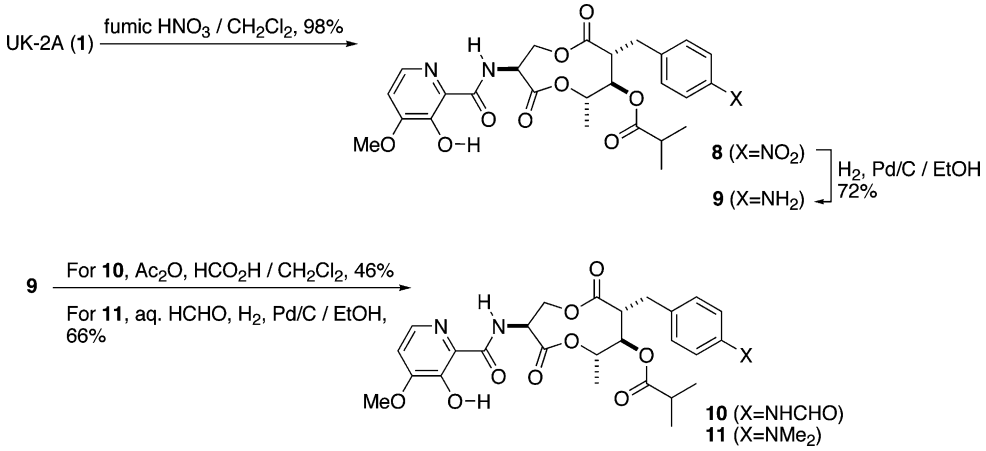
Scheme 1. Alkylation and acylation of the 3′-OH group of UK-2A.

To examine the role of the 3′-OH group in analogue activity, the chemically modified analogues **3**–**7** were prepared as shown in Scheme 1. Treatment of UK-2A with iodomethane and ethyl bromoacetate under alkaline conditions afforded the corresponding 3′-*O*-alkylated compounds **3** and **4**, respectively, in modest yields. Acylation of UK-2A with acetic anhydride and pyridine proceeded smoothly to afford compound **5** in quantitative yield. Heptanedioyl dichloride was treated with an equimolar amount of benzyl alcohol and triethyl amine in dichloromethane; addition of a UK-2A solution in dichloromethane afforded the corresponding diester derivative **6** in 62% yield. Hydrogenation of **6** over Pd/C in MeOH gave monoacid **7** in 66% yield.

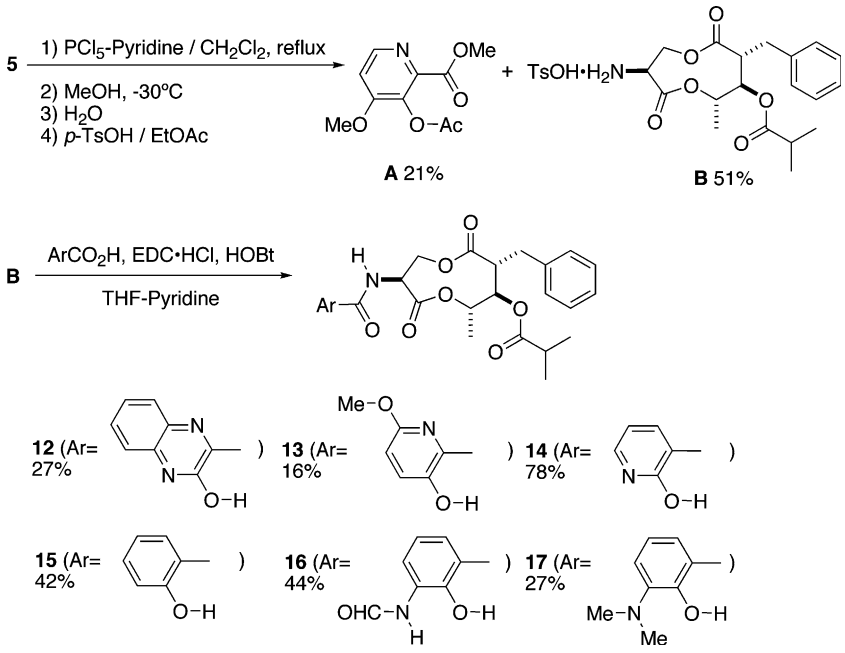
Then we were interested in introducing nitrogen functionality into the benzene moiety of UK-2A (Scheme 2). Treatment of UK-2A with fumic HNO_3 in dichloromethane at −20 °C achieved *para*-selective nitration of the benzene ring in good yield. Compound **8** thus obtained was hydrogenated over Pd/C in EtOH to give amine **9** in 72% yield. Treatment of **9** with Ac_2O and formic acid in dichloromethane afforded *N*-formated **10**. In the presence of aqueous HCHO, **9** was hydrogenated over Pd/C in EtOH to give *N*,*N*-dimethyl amine **11** in 66% yield.

The chemo-selective amide bond cleavage of UK-2A was expected to afford the nine-membered dilactone amine **B**, which is a useful building block for UK-2A derivatives. However, the acidic or basic hydrolysis of amide bonds requires prolonged heating that could decompose UK-2A. A survey of mild hydrolysis reagents led to the choice of phosphorus pentachloride (PCl_5), which has been applied to the removal of amide side chains in the industrial scale manufacture of semisynthetic cephalosporins[12] and penicillins.[13]

Treatment of 3′-*O*-acetylated UK-2A (**5**) with PCl_5–pyridine (2 equiv of each) in dichloromethane, followed by



Scheme 2. Introduction of nitrogen functionality of the benzene ring of UK-2A.



Scheme 3. Chemo-selective cleavage of the amide bond and condensation with *o*-hydroxyl aromatic acids.

heating under reflux for 1.5 h, afforded the imidoyl chloride intermediate, which was then converted to the imino ether with MeOH. Further aqueous work-up gave **B** as a *p*-toluenesulfonic acid salt in 51% yield along with methyl picolinate derivative **A** in 21% yield. Amide formation of **B** with various *o*-hydroxyl aromatic acids was achieved in THF–pyridine with EDC·HCl and HOBt in modest to good yields. The structures of UK-2A derivatives (**12**–**17**) prepared according to this method are presented in Scheme 3.

Compounds **3**–**17**[14] were then evaluated for in vitro antifungal activity against *Saccharomyces cerevisiae* IFO 0203 by the conventional paper disk method using a test medium composed of 1% yeast extract, 2% polypeptone, 3% glycerol, and 2% Bacto-agar. Results are summarized in Table 1.

O-Alkylation products (**3** and **4**) did not inhibit the growth of *S. cerevisiae*, while the *O*-acylated derivatives **5**–**7** showed inhibitory activity less than that exhibited by UK-2A, suggesting that in vivo hydrolysis of **5**–**7** regenerates UK-2A, and the carboxylic group in **7** facilitated smooth reaction. The ether bonds in **3** and **4** are chemically and enzymatically stable and may prevent the regeneration of UK-2A in vivo. The free 3′-OH group is thus essential for the growth inhibitory activity as reported earlier for AA.[5] Among derivatives with a substituent at the *para* position of the benzene ring (**8**–**11**), the *p*-nitro derivative **8** showed the strongest inhibitory activity; no relation between the electronegativity or basicity of substituents and growth inhibition was found. The replacement of a picolinic acid moiety by salicylic acid derivatives resulted in a slight decrease in the inhibition. 3-Hydroxy-2-quinoxalinecarboxylic acid derivative **12** and 3-hydroxy-6-methoxypicolinic acid derivative **13** showed inhibitory activity similar to UK-2A, suggesting that substitutions at the 5′- and/or 6′-position of picolinic acid are not effective promoters of growth inhibitory activity.

In summary, chemical modifications of the 3′-OH group revealed that the free hydroxyl group at the 3′ position is necessary for the inhibitory activity in UK-2A as well as AAs. Nitration at the *para* position of the benzene moiety and chemo-selective cleavage of the amide bond were achieved in good yields without decomposition of the nine-membered dilactone ring of UK-2A. *p*-Amino derivative **9** is a suitable precursor for photoaffinity-labeled

Table 1. Antifungal activity of UK-2A, AA, and their derivatives (**3**–**17**)[a]

Compounds	Dose (μg/disc)			
	0.025	0.05	0.125	0.25
UK-2A (**1**)	19	22	26	26
AA (**2**)	16	18	19	26
3	0	0	0	0
4	0	0	0	0
5	10	12	14	18
6	8	10	15	18
7	12	16	21	24
8	12	15	18	20
9	0	0	8	10
10	0	0	0	0
11	0	11	15	19
12	14	18	20	24
13	16	19	24	27
14	0	12	16	17
15	8	8	11	12
16	8	12	16	17
17	8	8	12	14

[a] Values are stated in diameter of inhibitory zone around the discs (mm).

analogues designed for elucidating the UK-2A binding site on mitochondria.

Acknowledgements

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