NK372135s, NOVEL ANTIFUNGAL AGENTS PRODUCED BY *Neosartoria fischeri*

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In the course of screening program for antifungal agents, novel three congenial compounds were isolated from the cultured broth of fungus, *Neosartoria fischeri* var. *glabra* IFO9857. The congeners had a basal structure, which was di-hydrogenated form of xanthocillin[1] group and showed strong growth inhibition activity against *Candida albicans in vitro*. In this paper we report the isolation, structural elucidation and preliminary biological activity of the compounds, named NK372135A, B and C.

The antifungal activity was monitored by usual agar plate method with *Candida albicans* TIMM0144 provided by Research Center for Mycology, Teikyo Univ. The compounds were produced by rotary shaking culture of the strain in the medium containing glucose 2%, lactose 1%, sucrose 1%, glycerol 1%, Prorich (Ajinomoto Co. Ltd.) 2%, peptone 0.8%, $NaNO_3$ 0.2% and $MgSO_4$ 0.1%. The maximum productivity was attained on 5 days cultivation at 27°C. Mycelia were collected from 20 liters cultured broth by filtration, suspended in 10 liters of MeOH and removed by filtration. The resultant filtrate was applied on carbon column (0.5 liter) and elution was done with Me_2CO after washing with MeOH. The active eluate was evaporated to dryness under reduced pressure to give a brownish residue (5 g). This residue was applied on silica gel column and developed with the mixture of $CHCl_3$ - MeOH (100:1, 100:2 and 2:1, stepwisely). By this chromatography three active fractions were obtained and a putative compound in each fraction was named NK372135A, B and C in the order of the eluation. After evaporation of each fraction, the residue was subjected to Sephadex LH-20 column, developed with MeOH. The NK372135A, B and C thus obtained were concentrated to give 88 mg of A, 500 mg of B and 108 mg of C in the form of yellowish powder.

NK372135A, B and C were soluble in $CHCl_3$ and MeOH but insoluble in H_2O, and gave positive color reaction with phosphomolybdic acid sulfuric acid and iodine reagents. On silica gel TLC(Merck Art. No. 5715) developed with $CHCl_3$ - MeOH (100:2), NK372135A, B and C gave a single spot at Rf = 0.66, 0.40 and 0.13, respectively. Physico-chemical properties were as follows. A: HRFAB-MS *m/z* Found: 318.1364 (M+, $C_{20}H_{18}O_2N_2$, Calcd: 318.1369); UV λ_{max}^{MeOH} nm 225, 297; IR (KBr) cm^{-1} 3438, 2137, 2103, 1605, 1514, 1252, B: HRFAB-MS *m/z* Found: 348.1479 (M+, $C_{21}H_{20}O_3N_2$, Calcd: 348.1473); $[\alpha]_D^{20}$ +188.7 (*c* 1.0, MeOH); UV λ_{max}^{MeOH} nm 226, 290, 317; IR (KBr) cm^{-1} 3438, 2137, 2102, 1515, 1273, 1249, C: FD-MS *m/z* 334 (M+); UV λ_{max}^{MeOH} nm 224, 285, 290, 318; IR (KBr) cm^{-1} 3412, 2138, 2102, 1603, 1583, 1513, 1279, 1248.

The ^1H and ^{13}C NMR spectrometric data of NK372135A was shown in Table 1, with that of xanthocillin X dimethylether (XDE)[2]. XDE had a symmetrical structure (Fig. 1) and its assignment of NMR data was shown in Table 1. In comparison with XDE there appeared remarkablly different signals in the spectrum of NK372135A, *i.e.* δ_H 4.76 (t, 7.3), 3.13 (dd, 7.3, 13.6), 3.19 (dd 7.3, 13.6) and δ_C 61.14 (d), 40.33 (t) (Table 1). In COSY NMR analysis, strong signals were detected at δ_H 3.13, 3.19 *vs.* δ_H 4.76, at δ_H 4.76 *vs.* δ_C 61.14 and at δ_H 3.13, 3.19 *vs.* δ_C 40.33. These data gave the partial structure that one of the double bond (C_2-C_3) of XDE was hydrogenated (Fig. 1) and the signals were assigned as shown in Table 1. This idea was

Fig. 1. The structures of xanthocillin (left) and NK372135 derivatives (right).

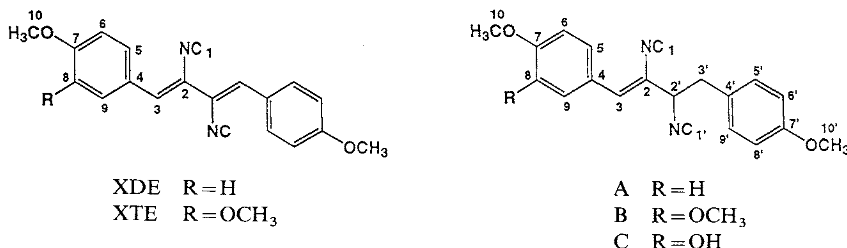

Table 1. ^{1}H and ^{13}C-NMR spectrometric data of xanthocillin X dimethylether (XDE) and NK372135A[a].

Atom No.[b]	XDE		NK372135A	
	δ_C (m)	δ_H (m, J Hz)	δ_C (m)	δ_H (m, J Hz)
1	173.30 (s, 2C)		172.98 (s)	
1′			159.78 (br s)	
2	116.18 (br s, 2C)		117.74 (br s)	
2′			61.14 (br d)	4.76 (t, 7.3)
3	127.46 (d, 2C)	7.01 (s, 2H)	131.92 (d)	6.50 (s)
3′			40.33 (t)	3.13 (dd, 7.3, 13.6) 3.19 (dd, 7.3, 13.6)
4	124.83 (s, 2C)		128.19 (s)	
4′			125.73 (s)	
5, 9	131.74 (d, 4C)	7.78 (d, 8.8, 4H)	132.26 (d, 2C)	7.61 (d, 8.8, 2H)
5′, 9′			131.60 (d, 2C)	7.21 (d, 8.8, 2H)
6, 8	114.47 (d, 4C)	6.98 (d, 8.8, 4H)	115.33 (d, 2C)	6.97 (d, 8.8, 2H)
6′, 8′			115.12 (d, 2C)	6.86 (d, 8.8, 2H)
7	161.14 (s, 2C)		162.69 (s)	
7′			160.69 (s)	
10	55.43 (q, 2C)	3.87 (s, 6H)	55.94 (q)	3.83 (s, 3H)
10′			55.71 (q)	3.75 (s, 3H)

[a] Recorded in $CDCl_3$.
[b] Refered to Fig. 1.

supported by two proton couplings at $H_{2'}$ *vs.* $2H_{3'}$ [$J=7.3$] and $H_{3'}$ (δ 3.13) *vs.* $H_{3'}$, (δ 3.19) [$J=13.6$] and long range couplings at $H_{2'}$ *vs.* H_3 and at $H_{3'}$ *vs.* $H_{5'}$, $H_{9'}$ (Data not shown). However, it was not clear whether $C_{1'}$ (δ_C 159.78) was attributed to isonitril ($-N{\equiv}C_{1'}$) or nitril ($-C_{1'}{\equiv}N$) group. According to HMBC NMR analysis a signal at $C_{1'}$ (δ_C 159.78) *vs.* $H_{3'}$ (δ_H 3.13, 3.19) was not observed. Therefore, isonitril group was reasonable as shown in Fig. 1.

NK372135B and C were considered to be in the same fundamental structure, since NMR spectrometric data of $C_{1'}$, $C_{2'}$, $C_{3'}$, carbons, $H_{2'}$, $H_{3'}$ protons and IR spectrum on isonitril groups (around 2102 and 2137) were quite identical. In comparison with the data of NK372135A, B and C lost a signal of H_8, suggesting a substitution had occured. In NK372135B there were additional signals of δ_C 55.9 [q] and δ_H 3.29 [s, 3H] and a chemical shift of C_8 altered to 148.95 ppm. Therefore it was concluded that a H_8 proton was substituted with $-OCH_3$ group (Fig. 1). In NK372135C an additional signal of proton was detected at δ_H 5.63 (s, 1H) and chemical shift of C_8 altered to 145.68 ppm. Therefore it was concluded that H_8 proton was substituted with $-OH$ group (Fig. 1). These structural elucidations on the NK372135s were consistent with the data of molecular formula deduced.

The growth inhibition activity of NK372135s and xanthocillins against *Candida albicans* was examined. As shown in Table 2 NK372135A had stronger activity than XDE. Similarly NK372135B was stronger than methoxy-xanthocillin X dimethylether (XTE)[2] which was dehydrogenated at C_2-C_3 bond of NK372135B (Fig 1). These results suggested that the hydrogenated structrue of NK372135 at C_2-C_3 bond must be needed for high anti-*Candida* activity. Among NK372135s the substitution of H_8 also affected the anti-*Candida* activity of NK372135 (Table 2) and NK372135C showed the most potent activity against *Candida albicans*. Our preliminary data showed that NK372135s had an activity against other clinically troublesome fungus, *i.e. Cryptococcus neoformans* and so on. The precise biological

Table 2. Anti-*Candida albicans* activity of NK3721325s.

Compound	IC_{50} (μg/ml)[a]
Nystatin	0.8
NK372135A	2.12
NK372135B	0.53
NK372135C	0.27
XDE	12.1
XTE	8.2

[a] IC_{50} value was determined with micro-liquid dilution method. *Candida albicans* strain was inoculated in a buillon medium (Meat extract 1%, Peptone 1% and NaCl 0.5% [pH=7.2]) and cultured at 30°C for 16 hours. The growth was monitored by OD_{600} and IC_{50} value was estimated.

properties and antifungal activity *in vivo* are now under the examination and will be reported elsewhere.

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