Notes

NAPYRADIOMYCINS A AND B1: NON-STEROIDAL ESTROGEN-RECEPTOR ANTAGONISTS PRODUCED BY A *Streptomyces*

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Estrogen-receptor antagonists are used in the therapy of estrogen-responsive diseases including breast cancer and endometrial cancer. Tamoxifen, the non-steroidal estrogen-receptor antagonist, is now the first choice of anti-hormonal treatment for advanced breast cancer. In spite of its effectiveness, the long-term use of tamoxifen frequently results in tumor resistance to itself and its related compounds which have the triphenylethylene moiety. Consideration mentioned above led us to screen microbial products for new non-steroidal estrogen-receptor antagonists without the triphenylethylene moiety.

In the previous papers[1~3], we reported R1128 substances as novel non-steroidal estrogen-receptor antagonists. In the course of our continuing search for non-steroidal estrogen-receptor antagonists from microbial products, strain No. 9558 was found to produce two estrogen-receptor antagonists. These compounds, named WS9558 A and B, were isolated and identified as napyradiomycins A and B1, respectively[4,5]. In this paper, we describe new biological and pharmacological properties of napyradiomycins A and B1.

Strain No. 9558 was isolated from a soil sample obtained at Kujukuri-beach, Chiba Prefecture, Japan. Characterization of the strain was performed according to the methods described previously[1]. From the results of the taxonomic studies, strain No. 9558 was considered to belong to the genus *Streptomyces* and therefore designated *Streptomyces* sp. No. 9558.

A loopful of slant culture of strain No. 9558 was inoculated into 500-ml flasks containing 160 ml of a sterile seed medium consisting of modified starch 1%, sucrose 1%, glucose 1%, Pharmamedia (Traders Protein) 1%, peptone (Kyokuto Seiyaku Co., Ltd.) 0.5%, soybean meal 0.5% and $CaCO_3$ 0.1%. The flasks were shaken on a rotary shaker (220 rpm, 7.5 cm-throw) at 30°C for 3 days. The resultant seed culture (480 ml) was inoculated into a 30-liter jar fermentor containing 20 liters of a sterile production medium consisting of soluble starch 4%, gluten meal 1%, wheat germ 0.5%, potato protein 0.5%, $CaCO_3$ 0.2%, Adekanol (LG-109, Asahi Denka Kogyo Co., Ltd.) 0.05% and Silicone (KM-70, Shin-etsu Kagaku Kogyo Co., Ltd.) 0.05% (pH 6.5). The fermentation was carried out at 30°C under aeration of 20 liters/minute and agitation of 200 rpm for 4 days.

The cultured broth (40 liters) was filtered with the aid of diatomaceous earth. The mycelial cake was extracted with acetone and filtered. The acetone extract was concentrated under reduced pressure to give an aqueous solution, adjusted to pH 7.0 and extracted with ethyl acetate. The ethyl acetate extract was concentrated under reduced pressure to give an oily residue, which was chromatographed on a Silica gel 60 column (E. Merck) with *n*-hexane - ethyl acetate (5 : 1). Active fractions were combined and chromatographed on a second Silica gel 60 column with dichloromethane. Active fractions from the second silica gel column were chromatographed on a third Silica gel 60 column with *n*-hexane - dichloromethane - acetone. WS9558 A was eluted with *n*-hexane - dichloromethane - acetone (30 : 10 : 1) and WS9558 B was eluted with *n*-hexane - dichloromethane - acetone (20 : 10 : 1). From 40 liters of the cultured broth of *Streptomyces* sp. No. 9558, 55 mg of WS9558 A and 90 mg of WS9558 B were obtained both as yellow powders.

The physico-chemical properties of WS9558 A and B are summarized in Table 1. The characteristic UV absorbances as well as both ^1H and ^{13}C NMR spectra (data not shown) revealed that WS9558 A and B were identical to napyradiomycins A and B1, respectively[4,5] (Fig. 1).

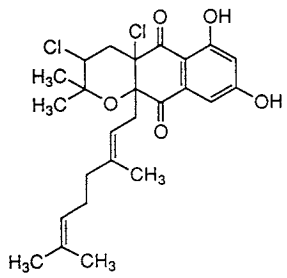
The assay of estrogen-receptor binding was examined using rat uterine cytosol as described previously[1]. Napyradiomycins A and B1 inhibited estrogen-receptor binding in a dose dependent manner, with IC_{50} values of 4.2×10^{-6} M and 3.5×10^{-7} M, respectively (Fig. 2). The activities of napyradiomycins A and B1 were less potent than that

Table 1. Physico-chemical properties of WS9558 A and B.

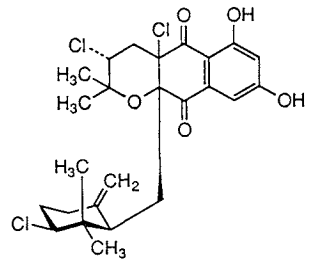
	WS9558 A	WS9558 B
Appearance:	Yellow powder	Yellow powder
FAB-MS *m/z*:	N.M.	513 $(M-H)^+$
UV λ_{max}^{EtOH} nm (log ε):	204 (4.33), 251 (4.04), 269 (4.04), 298 (sh, 3.97), 361 (3.81), 400 (sh, 3.58)	202 (4.17), 251 (4.18), 270 (sh, 4.01), 300 (3.97), 354 (3.90), 400 (sh, 3.63)
$\lambda_{max}^{EtOH-0.01\,N\,HCl}$ nm (log ε):	204 (4.32), 250 (4.10), 269 (4.11), 352 (3.82)	202 (4.15), 251 (4.26), 270 (sh, 4.04), 305 (3.84), 356 (3.88)
$\lambda_{max}^{EtOH-0.01\,N\,NH_4OH}$ nm (log ε):	204 (4.48), 260 (3.92), 303 (4.11), 387 (3.95)	203 (4.43), 245 (sh, 3.93), 259 (3.98), 299 (4.13), 384 (4.07)
TLC Rf value[a]:	0.47	0.37

[a] Plate; Silica gel 60 F_{254} (E. Merck, Art. 5715), solvent; CH_2Cl_2-acetone (20:1).
N.M.: Not measured.

Fig. 1. Structures of WS9558 A and B.



WS9558 A = Napyradiomycin A



WS9558 B = Napyradiomycin B1

Fig. 2. Inhibitory effects of WS9558 A, WS9558 B and tamoxifen on estrogen-receptor binding.
● WS9558 A, ○ WS9558 B, ■ tamoxifen.

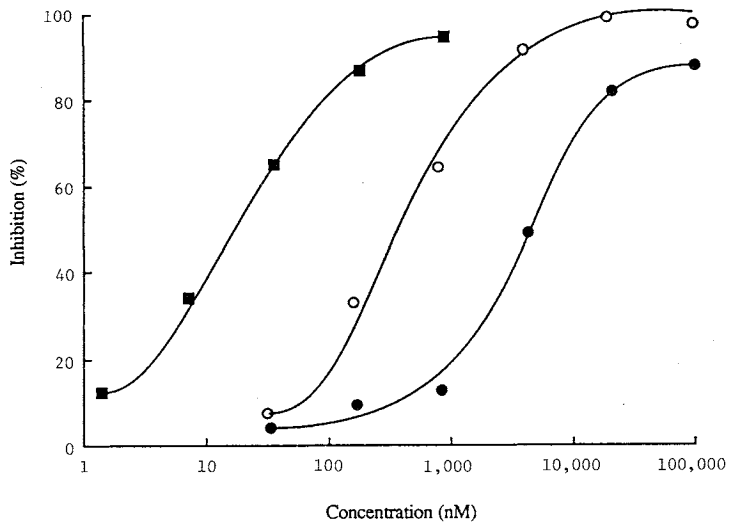


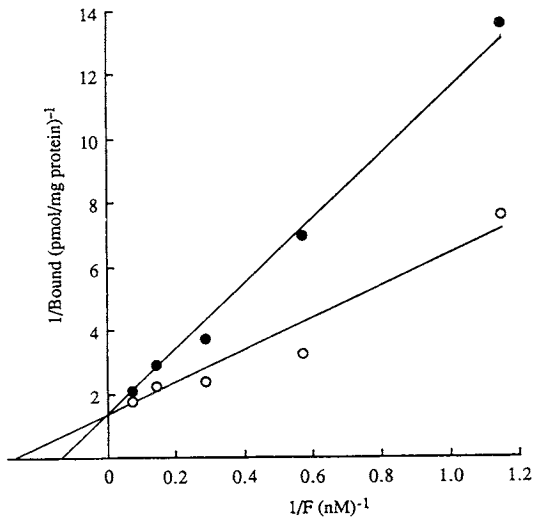
Table 2. Effects of WS9558 B and tamoxifen on the weight of rat uterus.

Drugs	Body weight gain (g)	Uterine weight (mg/100 g body)	Inhibition (%)
Control	16.5±0.7	109.5±12.6	100.0
17β-Estradiol	18.2±1.1	254.9±31.3	0.0
17β-Estradiol + WS9558 B 3.2 mg/kg	16.7±0.7	186.3± 9.1	47.2
17β-Estradiol + WS9558 B 10.0 mg/kg	17.4±0.6	196.0±13.0	40.5
17β-Estradiol + WS9558 B 32.0 mg/kg	16.2±1.6	220.4±27.6	23.7
17β-Estradiol + tamoxifen 1.0 mg/kg	16.2±1.4	191.6± 9.1	43.5
17β-Estradiol + tamoxifen 3.2 mg/kg	14.7±0.6*	164.8± 5.2*	61.9
17β-Estradiol + tamoxifen 10.0 mg/kg	13.5±0.6**	170.2± 8.3*	58.2
17β-Estradiol + tamoxifen 32.0 mg/kg	14.5±0.9*	172.4± 8.2*	56.7

For statistical significance, the STUDENT's *t*-test was analyzed against the group given 17β-estradiol alone, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Fig. 3. Lineweaver-Burk plot for inhibition of estrogen-receptor binding by WS9558 B.

○ No WS9558 B, ● WS9558 B 3.9×10^{-7} M.



of tamoxifen. Lineweaver-Burk plot analysis for inhibition of estrogen-receptor binding by napyradiomycin B1 suggested that it was a competitive inhibitor (Fig. 3). In an attempt to determine whether napyradiomycin B1 was estrogen-receptor antagonist or agonist, the colony formation assay of estrogen-responsive human mammary adenocarcinoma MCF-7 cells in soft agar was performed according to the method described previously[3]. Napyradiomycin B1 did not stimulate the growth of MCF-7 cells, but inhibited colony formation in the absence of 17β-estradiol (MIC 7.6×10^{-7} M). However, the inhibitory activity of napyradiomycin B1 against the colony formation of MCF-7 cells was reversed in the presence of estradiol (data not shown). These results suggest that napyradiomycin B1 was an estrogen-receptor antagonist just as tamoxifen and R1128 substances. In the colony formation assay, the inhibitory activity of napyradiomycin B1 was less potent than that of tamoxifen[3].

Further, the estrogen-receptor antagonistic activity of napyradiomycin B1 was evaluated *in vivo*. Injection of 17β-estradiol caused the hypertrophy of uterus in immature female rats[6]. Female Sprague-Dawley rats (3 weeks old) were given a subcutaneous injection of estradiol (2 mg/kg). At the same time, napyradiomycin B1 dissolved in sesame oil was administered subcutaneously to the rats. The administration of estradiol and napyradiomycin B1 was repeated once a day for 3 consecutive days. On the fourth day, uteri were excised and weighed. When napyradiomycin B1 was subcutaneously administered, the estradiol-induced increase in the weight of uterus was reduced slightly (Table 2). No decrease in the body weight of the rats was observed at this time. These results suggest that the estrogen-receptor antagonistic activity of napyradiomycin B1 is shown *in vivo*. In this experiment, the inhibitions of both napyradiomycin B1 and tamoxifen were not dose dependent. Tamoxifen is known to show partial estrogen-receptor agonistic activity in this model[6], and thus napyradiomycin B1 might also exhibit partial estrogen-receptor agonistic activity in this model.

Napyradiomycins A and B1 have thus far only been noted for their antimicrobial activities[4]. In the present study, it was revealed that they also had the properties of estrogen-receptor antagonist.

References

1) HORI, Y.; Y. ABE, M. EZAKI, T. GOTO, M. OKUHARA & M. KOHSAKA: R1128 substances, novel non-

steroidal estrogen-receptor antagonists produced by a *Streptomyces*. I. Taxonomy, fermentation, isolation and biological properties. J. Antibiotics 46: 1055~1062, 1993

2) HORI, Y.; S. TAKASE, N. SHIGEMATSU, T. GOTO, M. OKUHARA & M. KOHSAKA: R1128 substances, novel non-steroidal estrogen-receptor antagonists produced by a *Streptomyces*. II. Physico-chemical properties and structure determination. J. Antibiotics 46: 1063~1068, 1993

3) HORI, Y.; Y. ABE, M. NISHIMURA, T. GOTO, M. OKUHARA & M. KOHSAKA: R1128 substances, novel non-steroidal estrogen-receptor antagonists produced by a *Streptomyces*. III. Pharmacological properties and antitumor activities. J. Antibiotics 46: 1069~1075, 1993

4) SHIOMI, K.; H. IINUMA, M. HAMADA, H. NAGANAWA, M. MANABE, C. MATSUKI, T. TAKEUCHI & H. UMEZAWA: Novel antibiotics napyradiomycins. Production, isolation, physico-chemical properties and biological activity. J. Antibiotics 39: 487~493, 1986

5) SHIOMI, K.; H. NAKAMURA, H. IINUMA, H. NAGANAWA, K. ISSHIKI, T. TAKEUCHI & H. UMEZAWA: Structures of new antibiotics napyradiomycins. J. Antibiotics 39: 494~501, 1986

6) PASQUALINI, J. R.; C. SUMIDA & N. GIAMBIAGI: Pharmacodynamic and biological effects of antiestrogens in different models. J. Steroid. Biochem. 31: 613~643, 1988