UCF76 Compounds, New Inhibitors of Farnesyltransferase Produced by *Streptomyces*

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Farnesyltransferase (FTase) catalyses the farnesylation of Ras p21 protein on a cysteine residue of a carboxyl-terminal CAAX motif. This post-translational modification is necessary for their association with plasma membranes and oncogenic activity. Therefore, inhibition of Ras farnesyltransferase presents a potential therapeutic target for novel anticancer agents[1]. Recently, several natural product inhibitors of FTase have been reported including manumycin[2], andrastin D[3], clavaric acid[4], kampanol B[5], and UCF116[6]. We have previously developed a microbial screen for inhibitors of FTase using a yeast strain with conditional deficiency in the GPA1 gene, and identified manumycin as a inhibitor of FTase. Our yeast-based microbial screening efforts have now led to the isolation of new inhibitors of FTase, UCF76-A (**1**), -B (**2**) and -C (**3**), produced by *Streptomyces* sp. In this paper, fermentation, isolation and biochemical properties of UCF76 compounds are described.

The producing organism taxonomically classified as *Streptomyces* sp. was cultivated at 28°C for 3 days in two 30-liter jar fermenters containing 16 liters of a medium consisting of glucose 25 g, glycerol 25 g, dry yeast 15 g, KH_2PO_4 0.5 g, $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.5 g, NaCl 10 g, $CaCO_3$ 0.5 g in 1 liter of water, pH 7.

Yeast assay[2] was used for detecting the active fractions in the following isolation procedure. The mycelial cake was extracted with acetone. After concentrated *in vacuo*, the extract was dissolved in $CHCl_3$, and was subjected to silica gel column chromatography (BW300, Fuji devison) developed with $CHCl_3$ - MeOH (125 : 1). The active eluates were combined and concentrated to dryness, and the residue was subjected to silica gel column chromatography (Lichroprep Si60, Merck) developed with hexane : ethyl acetate (7 : 3) to give active fractions. They are subjected to a gel filtration column chromatography (Sephadex LH20) developed with MeOH. Active fractions were combined and subjected to preparative HPLC using ODS (SH363-5 S-5 ODS, YMC) with 67% CH_3CN to give active fractions containing pure **1** (66 mg), **2** (22 mg) and **3** (295 mg).

The physico-chemical properties are summarized in Table 1. The molecular formulas of **1**, **2** and **3** were deduced to be $C_{19}H_{20}O_7$, $C_{18}H_{16}O_7$ and $C_{18}H_{16}O_6$. The structure of **1**~**3** were shown in Fig. 1. **3** is a known compound, frenolicin B[7~9]. **1** and **2** are new compounds with a structure similar to frenolicin B. The details of structure elucidation will be published elsewhere.

Yeast assay and FTase enzyme assay data of UCF76 compounds are summarized in Table 2. Data for structurally related natural products, nanaomycin A, D[10,11] and kalafungin[12,13] (Fig. 1), are also included in the Table. **1** and **3** inhibited farnesylation of viral K-Ras by bovine brain FTase[2] with the IC_{50} values of 3.7 and 1.4 μM, respectively. Those compounds are also active in yeast assay, and suppressed the lethality of *gpa1* disruption[2]. On the contrary to this, FTase inhibition by **2** is weak with the IC_{50} values of 25 μM, which is accord with the lack of activity in yeast assay. Nanaomycin A and D and kalafungin are also active both in FTase and yeast assay. FTase or GGTase-I enzyme activities were also studied by detecting the prenylation of GST-CIIS or -CIIL substrare by prenyltransferase enzyme involved in a rabbit reticulocyte lysate[14,15]. **3**, nanaomycin D and kalafungin showed selective inhibition against FTase, and they showed very weak or no inhibition against GGTase-I at the concentrations up to 100 μM.

We performed kinetic analysis of FTase inhibition by **3**. A Lineweaver-Burk plot of FTase for various concentrations of viral K-Ras protein and **3** and a constant concentration of FPP (Fig. 2A) shows that the addition of **3** changes the *Km* value for K-Ras but does not change Vmax, suggesting that **3** is a competitive inhibitor with respect to K-Ras. A secondary plot derived by plotting slopes against the concentration of **3** gave the *Ki* value of 4.3 μM. In contrast, **3** does not appear to affect the *Km* value for the FPP (Fig. 2B). The same *Km* value was observed when the Lineweaver-Burk plot was analyzed under different FPP and concentration of **3** in the presence

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Table 1. Physico-chemical properties of UCF76-A (**1**), -B (**2**) and -C (**3**).

	UCF76-A (1)	UCF76-B (2)	UCF76-C (3)
Appearance	yellow powder	yellow powder	orange powder
Molecular formula SI-MS(m/z)	$C_{19}H_{20}O_7$ 361 $(M+H)^+$	$C_{18}H_{16}O_7$ 345 $(M+H)^+$	$C_{18}H_{16}O_6$ 329 $(M+H)^+$
$[\alpha]_D^{24}$	+196° (c=0.18, CH_3OH)	+44° (c=0.17, CH_3OH)	+216° (c=0.44, $CHCl_3$)
UV λmax, nm in CH3OH(ε)	211 (34,700) 250 (9,500) 270 (9,900) 422 (4,300)	236 (14,800) 278 (3,300) 365 (4,900)	260 (16,641) 268 (16,776) 426 (562)
IR (KBr) cm^{-1}	3471, 1749, 1639 1614, 1458, 1284 1257, 1167, 783 710	1788, 1699, 1651 1456, 1284, 1246 1161, 1001, 928 733	3398, 1786, 1649 1621, 1456, 1286 1247, 1198, 1153 997, 923, 878, 763
Rf value*	0.31	0.24	0.30

Silica gel TLC $60F_{254}$(Merck). Hexane - EtOAc (2 : 1)

Table 2. Summary of yeast assay and prenyltransferase assay.

compound	FTase*	FTase	GGTase-I	Yeast assay**
	(bovine brain)	(reticulocyte lysate)		
1 (UCF76-A)	3.7	NT***	NT	32
2 (UCF76-B)	25	NT	NT	0
3 (UCF76-C/Frenolicin B)	1.4	1.0	> 100	39
Nanaomycin A	3.2	NT	NT	15
Nanaomycin D	1.9	2.0	> 100	31
Kalafungin	1.7	1.5	> 100	37

*IC_{50}, μM., Since DTT inactivates the naphthoquinone-type compounds, enzyme assay was done using the assay buffer without DTT.
**Halo of growth (mm) due to suppression of the lethality of *gpa1* disruption. Paper discs were soaked with 5 nmol of compound. Details of yeast assay were shown in the previous publication[2].
***not tested

of a constant concentration of K-Ras. Thus, **3** appears to act as competitive inhibitor of Ras protein and a noncompetitive inhibitor with respect to FPP. Similar results were obtained for nanaomycin A by its kinetic analysis of FTase inhibition (data not shown).

We have previously shown that *C. elegans* vulval development is a simple and effective *in vivo* system for evaluating the efficacy of FTase inhibitors against biological responses associated with ras activation[16]. According the assay methods we described before[16],

Fig. 1. Structure of UCF76 compounds, nanaomycin A and D, and kalafungin.

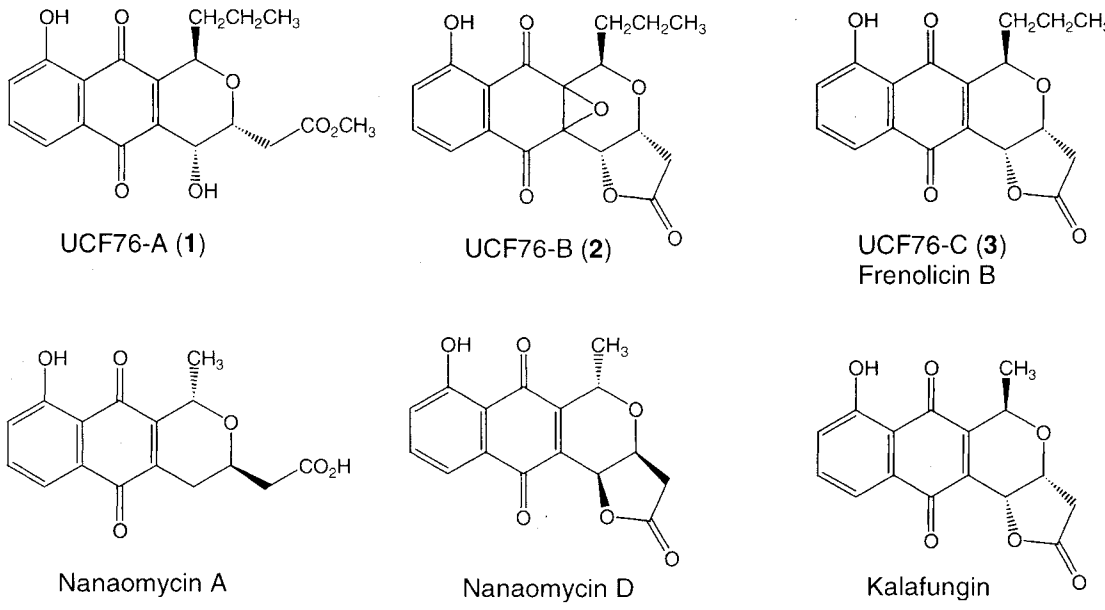
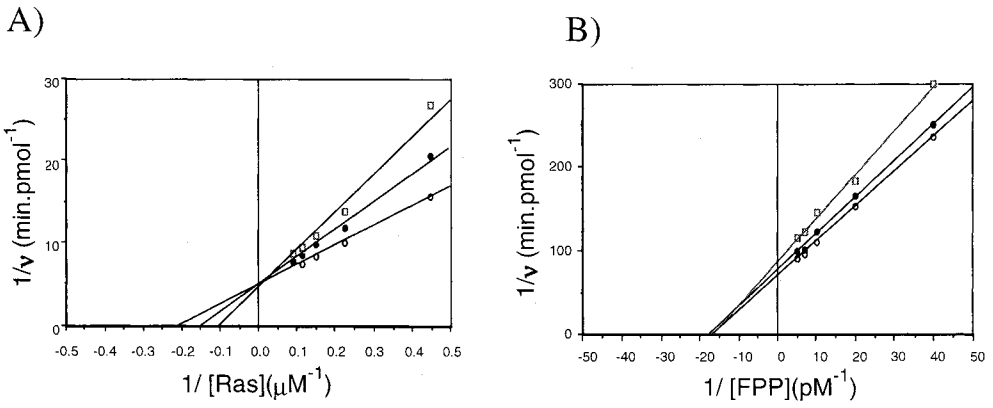


Fig. 2. Lineweaver-burk plot of FTase inhibition with UCF76-C (**3**).

Effect of **3** on the kinetics of FTase with respect to the substrate Ras (A) or FPP (B). Concentrations of **3** used were 0 (○), 1.1 (●), and 3.3 (□) μM.



compounds were added to synchronized eggs of *C. elegans*. After hatching, those worms were grown for 4 to 5 days, and the percent of adult worms with the multivulva (Muv) phenotype was determined. As shown in Table 3, **3** suppressed the Muv phenotype resulting from an activated *let-60 ras* mutation in a dose-dependent manner. Nanaomycin D also suppressed the Muv phenotype at 200 nmol. At 300 nmol of nanaomycin D, however, no eggs are hatched, indicating that it caused the embryonic lethality. Embryonic lethality was also observed for kalafungin. These results indicate that both nanaomycin D and kalafungin have some toxic effect, but **3** is less toxic so that it allow the worms to grow and eventually suppressed the activated phenotype resulting from ras mutation. Although **1** inhibited FTase, it is inactive in the *C. elegans* assay (data not shown).

Ras-competitive non-CAAX mimetics have recently been reported as inhibitors of FTase. Those include

Table 3. Effect on the Muv phenotype of *C. elegans* with activated ras mutant, *let-60* (*n1046*).

Compounds	Dose*	Muv%	n**
Control (no drug)	0	94	114
Frenolicin B (**3**)	400	24	54
	300	34	80
	200	44	88
	100	86	119
Nanaomycin D	300	EL***	0
	200	55	64
	100	94	92
Kalafungin	200	EL	3
	100	93	108

*nmol per plate

*** No of worms observed

*** embryonic lethal

synthetic compound SCH44342[17], a chembranolide diterpene type natural product[18], and UCF116[6]. The present study of UCF76 compounds revealed that frenolicin and nanaomycin family antibiotics are another example of Ras-competitive non-CAAX mimetic type FTase inhibitors. As described above, **3** inhibits FTase to the same extent as the other structurally related compounds, such as nanaomycin D and kalafungin. Despite those similar FTase inhibitory activities among those compounds, **3** is less toxic and the most effective in suppressing the activated ras phenotype in *C. elegans*. Thus, both the substituent at 9th position and the absolute configuration of lactone ring seems to be important for the suppression of an activated ras phenotype. Those intriguing SAR for those naphthoquinone type compounds suggests that the combination of the FTase enzyme assay with the simple *in vivo* assay using *C. elegans* could be useful for the discovery of more potent FTase inhibitors against Ras-activated tumors.

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