UCF116, 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], and kampanol B[5]. Our microbial product screening efforts have now led to the isolation of new inhibitors of FTase, UCF116-A (**1**), -B (**2**) and -C (**3**), produced by *Streptomyces* sp. In this paper, fermentation, isolation and biochemical properties of UCF116 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 soluble starch 40 g, soybean meal 10 g, corn steep liquor 0.5 g, dry yeast 0.5 g, KH_2PO_4 0.5 g, $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.5 g in 1 liter of water, pH 7. Diaion HP-20 resin (10%) was added at 18 hours after inoculation. As reported previously[6], this was efficient for increasing the fermentation titer of the secondary metabolites by absorbing and thereby preventing them from the degradation during a fermentation.

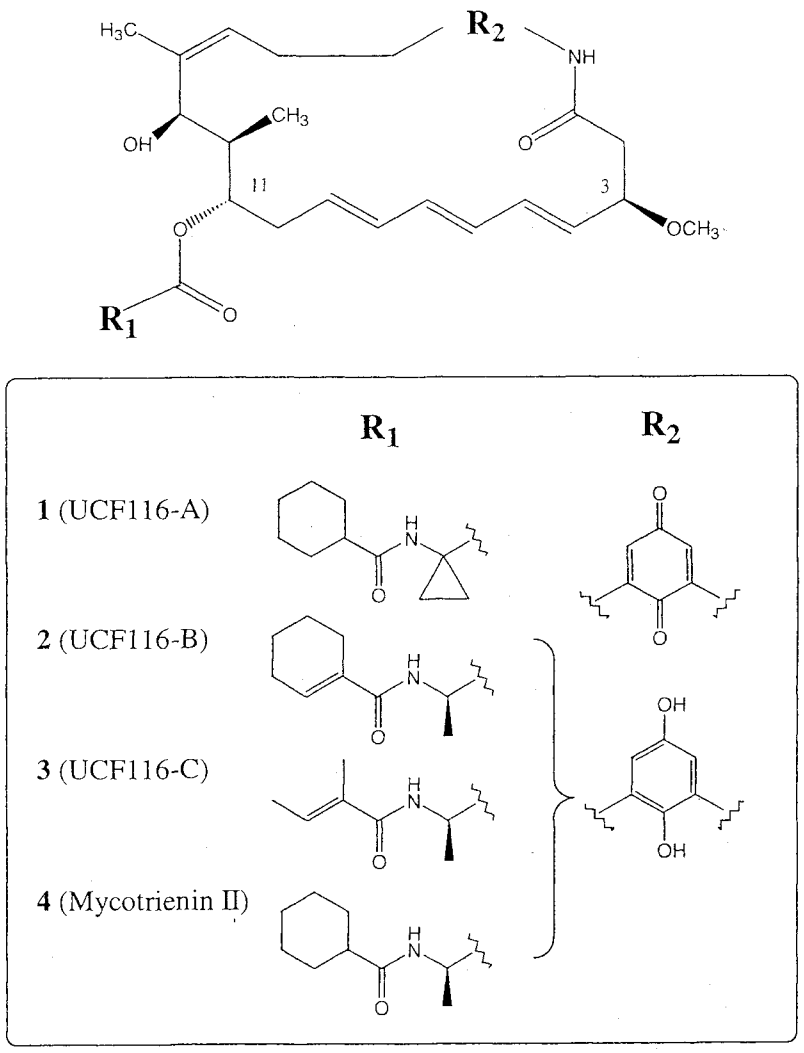
FTase enzyme assay[2] was used for detecting the active fractions in the following isolation procedure. The mycelial cake containing Diaion HP-20 resin was extracted with ethyl acetate. After concentrated *in vacuo*, the extract was dissolved in $CHCl_3$ - ethyl acetate (1 : 4), and was subjected to silica gel column chromatography (BW300, Fuji devison) developed with $CHCl_3$ - MeOH (7 : 3). 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 : MeOH (6 : 3 : 1) to give active fractions. They were combined and subjected to preparative HPLC using ODS (SH363-5 S-5 ODS, YMC) with 50% CH_3CN to give active fractions containing pure **1** (12 mg), and **2** (112 mg) and

Table 1. Physico-chemical properties of UCF116-A (**1**), -B (**2**) and -C (**3**).

	UCF116-A (**1**)	UCF116-B (**2**)	UCF116-C (**3**)
Appearance	pale yellow powder	white powder	white powder
Molecular formula HR-FAB-MS(m/z)	$C_{37}H_{48}N_2O_8$ 649.3510 $(M+H)^+$	$C_{36}H_{48}N_2O_8$ 637.3509 $(M+H)^+$	$C_{34}H_{46}N_2O_8$ 611.3356 $(M+H)^+$
$[\alpha]_D^{24}$	+31.2° (c=0.127, CH_3OH)	+327° (c=0.1334, CH_3OH)	+77° (c=0.10, CH_3OH)
UV λmax, nm in CH3OH(ε)	281 (26,200) 270 (33,400) 262 (26,800) 227 (21,500) 203 (25,900)	304 (3,300) 280 (31,300) 271 (40,900) 260 (31,600) 206 (42,100)	302 (10,900) 283 (4,100) 271 (13,300) 260 (11,200) 204 (21,900)
IR (KBr) cm^{-1}	3440, 2927, 2854 1716, 1653, 1506 1180	3367, 1732, 1653 1219, 999	3421, 1732, 1624 1537, 1454, 1213 1001
Rf value*	0.22	0.3	0.17

Silica gel TLC $60F_{254}$(Merck). Hexane - EtOAc - MeOH (6 : 3 : 1)

Fig. 1. Structure of UCF116 compounds.



inactive components **3** (10 mg) and **4** (70 mg), both of which have a similar UV absorption spectra to **1** and **2**.

The physico-chemical properties are summarized in Table 1. The molecular formulas of **1**, **2** and **3** were deduced to be $C_{37}H_{48}N_2O_8$, $C_{36}H_{48}N_2O_8$ and $C_{34}H_{46}N_2O_8$ by HR-FAB-MS. The structure of **1**~**4** were shown in Fig. 1. **4** is a known compound, mycotrienin II[7~11]. **1**, **2**, **3** are new compounds with a structure similar to mycotrienin II. **1** has a *p*-quinone moiety but, **2** and **3** have a *p*-hydroquinone moiety. They differ from each other in the structure of a side chain at C-11. The details of structure elucidation will be published elsewhere[12].

FTase inhibition by UCF116 compounds is summarized in Table 2. **1** and **2** inhibited farnesylation of viral K-Ras by bovine brain FTase[2] with the IC_{50} values of 1.2 and 0.6 μM, respectively. However, **3** and **4** did not show FTase inhibition up to 100 μM. 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[13,14]. **1** and **2** showed selective inhibition against FTase, and they showed very weak or no inhibition against GGTase-I.

We performed kinetic analysis of FTase inhibition by **2**. Fig. 2 shows the results of an experiment in which the concentration of the viral K-Ras protein was varied in the bovine brain FTase assay while farnesyl pyrophosphate concentration was kept constant. The kinetic profile suggests that **2** is competitive with the Ras protein substrate. The apparent *Ki* was found to be 5.9 μM.

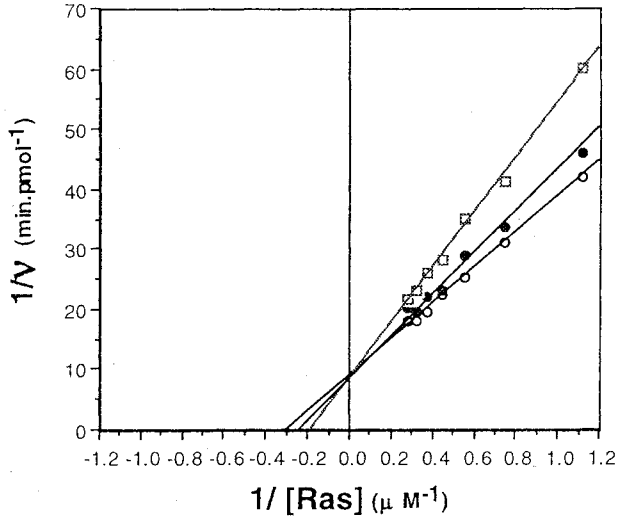
Ras-competitive non-CAAX mimetics have recently

Table 2. Inhibitory activity* against FTase and GGTase-I.

compound	FTase	FTase	GGTase-I
	(bovine brain)	(reticulocyte lysate)	
1 (UCF116-A)	1.2	4.0	68
2 (UCF116-B)	0.6	4.0	> 100
3 (UCF116-C)	> 100	NT**	NT
4 (Mycotrienin II)	> 100	NT	NT

*IC_{50}, μM., **not tested

Fig. 2. Lineweaver-Burk plot of FTase inhibition with UCF116-B (**2**).



Concentrations of UCF116-B used were 0 (○), 1.1 (●), and 3.3 (□) μM.

been reported as inhibitors of FTase. Those include synthetic compound SCH44342[15] or a chembranolide diterpene type natural product[16]. UCF116 compounds are another example of Ras-competitive non-CAAX mimetic type FTase inhibitors. It is to be noted that the substituent at the terminal amide linkage must contribute to their FTase inhibitory activity. The cyclohexenecarboxylalanine moiety of **2** seems to be essential for its FTase inhibitory activity because mycotrienin II (**4**), which has a cyclohexanecarboxylalanine, showed no FTase inhibition. This is consistent with the lack of FTase inhibition by mycotrienin I[7] and trienomycin A[17], both of which have a cyclohexanecarboxylalanine (data not shown). Although **1** has the same hexahydrobenzyl substituent as **4**, it inhibited FTase. This suggest that the cyclopropane ring in the side chain of C-11 in **1** has significantly contributed to its FTase inhibitory activity. Considering this SAR of UCF116 compounds, extensive modification of the substituent at the C-11 position of UCF116 could lead to the discovery of more potent FTase inhibitors.

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