RPR113228, a Novel Farnesyl-Protein Transferase Inhibitor Produced by *Chrysosporium lobatum*

Sir:

The post-translational modifications (farnesylation, proteolysis and carboxyl methylation) of $p21^{ras}$ proteins are required to promote plasma membrane association[1]. This localization is essential for the transforming activity induced by *ras* oncogenes. Inhibition of $p21^{ras}$ farnesylation has therefore been suggested as a potential strategy for antitumour chemotherapy research[2]. During our screening for farnesyl-protein transferase (FPTase) inhibitors of microbial origin, we have discovered a novel compound: RPR113228 isolated from the fermentation broth of a fungus. We herein report the production, isolation, structure elucidation and inhibitory activities of this secondary metabolite.

The producing strain was isolated from a soil sample. Aqueous soil suspension was spread on brain heart infusion agar supplemented with chloramphenicol and rose bengal (respectively, 0.2 and 0.05 g/liter). It was further identified as *Chrysosporium lobatum* on the basis of its morphological properties[3] and has been deposited in the Centraalbureau voor Schimmel Culturen, Baarn, Holland, under the number CBS12395.

Fermentation cascade for the production of active compound was carried out as following. We inoculated 2 ml of thawed stock culture from the fungus strain into two 250-ml Erlenmeyer flasks each containing 50 ml of seed medium composed of peptone 0.5%, meat extract 0.5%, glucose 1%, sodium chloride 0.5%, agar 0.1% and Tween 85 0.1%, pH 7.2 . The flasks were shaken on a rotary shaker (240 rpm) at 28°C for 5 days. The two seed flasks were combined and transferred into a 6-liter flask containing 2 liters of the seed medium. This second seed flask was stirred with a magnetic bar (600 rpm) at 23°C for 72 hours. The resulting seed culture was used for the inoculation of a 100-liter jar fermentor containing 60 liters of sterile medium consisting of malt extract 2%, agar 0.1%, Tween 85 0.1%, pH 7.2) . The fermentation was carried out for 142 hours at 23°C with agitation rate of 200 rpm, and air flow of 25 liters/minute.

RPR113228 was isolated according to the procedure indicated in Fig. 1, and displayed the physico-chemical properties summarized in Table 1. It is soluble in 1% (w/v) sodium bicarbonate solution and dimethyl sulfoxide, slightly soluble in methanol and water, insoluble in methylene chloride.

Table 1. Physico-chemical properties of RPR113228.

Appearance	Brown powder
Molecular formula	$C_{30}H_{49}O_9P$
MW	584
Isonspray-MS (*m/z*)	583 $(M-H)^-$
CI-MS (*m/z*)	486 $(M-H_3PO_4)^+$
$[\alpha]_D^{20}$	−2.3° (*c* 0.5, 0.1 N NaOH)
UV λ_{max}^{MeOH} nm (ε)	207 (3,610)
IR (KBr) cm^{-1}	3425, 2625, 2520, 1930 (OH bonded), 3040, 2950~2870 (CH aliphatic), 1710 (>C=O acid), 1260 & 1210 (>P=O and C–O acid), 1170, 1110, 1080 (C–O hydroxy), 1045 (P–OH), 930 broad (P=O)
Rf value*	0.45
Positive color reaction	Molybdenum oxide-H_2SO_4

* Silica gel TLC $60F_{254}$ (Merck). EtOAc-EtOH-H_2O (40:15:15).

Fig. 1. Isolation procedure of RPR113228.

culture broth 60 liters
- adjusted to pH 3
- extracted with EtOAc
- centrifugation

EtOAc layer
- concentrated and back-extracted with alcaline water (pH9)

Aqueous layer
- adjusted to pH 3
- extracted with EtOAc
- concentrated under reduced pressure

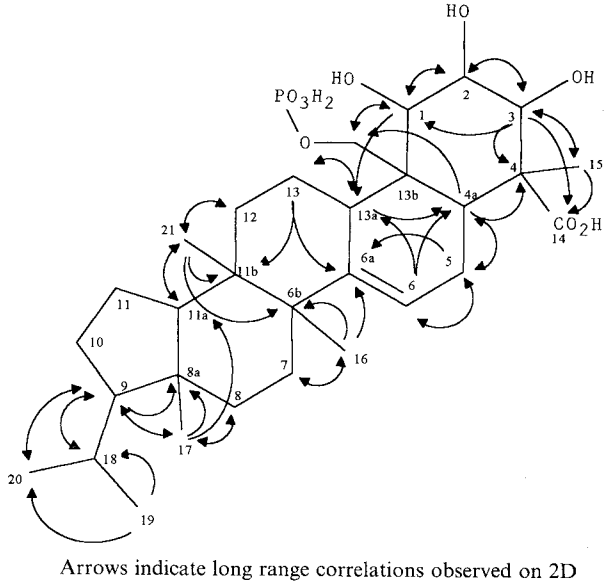
EtOAc extract
- Sephadex LH-20 chromatography (MeOH)

Active fractions
- C_{18} HPLC (BioSil HL90-10), CH_3CN-H_2O (76:24) with 0.1% TFA (v/v) 3 ml/minute, UV 210nm

eluate
- desalted on Mega Bond Elut C_{18} (Varian)

RPR113228 (980 mg)

Fig. 2. Structure of RPR113228.



Arrows indicate long range correlations observed on 2D HMBC experiment.

This article is a special contribution in honour of Professor SATOSHI ŌMURA's 60th birthday.

Table 2. NMR data for compound RPR113228 in DMSO-d_6 (T=300 K).

Position No	δ_C (150 MHz)	δ_H (600 MHz)
1	82	2.98 (1H, d, 6 Hz)
2	74	3.70 (1H, t, 7 Hz)
3	77.5	3.50 (1H, d, 7 Hz)
4	52.7	
4a	47	2.05 (1H, m)
5	27	2.10 (1H, m)
		1.77 (1H, m)
6	117	5.28 (1H, m)
6a	147	
6b	42.6	
7	31.3	1.35 (1H, m)
		1.50 (1H, m)
8	37	1.64 (1H, m)
		1.40 (1H, m)
8a	43.5	
9	60	0.94 (1H, m)
10	29	1.15 (1H, m)
		1.74 (1H, m)
11	20.7	1.20 (1H, m)
		1.35 (1H, m)
11a	55	1.35 (1H, m)
11b	36.3	
12	33.7	1.29 (2H, t, 3Hz)
13	20.7	2.19 (1H, m)
		1.75 (1H, m)
13a	50.5	2.63 (1H, m)
13b	45.7	
14	180	
15	11.8	1.17 (3H, s)
16	25	0.99 (3H, s)
17	15	0.67 (3H, s)
18	31.4	1.40 (3H, m)
19	23	0.83 (3H, d, 6 Hz)
20	24	0.79 (3H, d, 6 Hz)
21	22	0.81 (3H, s)
22	60.5	3.60 (1H, d, 6 Hz)
		4.02 (1H, t, 6 Hz)

The molecular formula was established as $C_{30}H_{49}O_9P$ by negative ionspray MS (m/z 583 $(M-H)^-$) and NMR analysis. The ^1H NMR spectrum shows numerous aliphatic signals, six methyl and three hydroxyl groups, suggesting that this compound is related to the triterpenes family. The singulet at 5.28 ppm indicates an ethylenic bond. The presence of a phosphate group is suggested by FT-IR. spectrum (1045 and 930 cm^{-1}) and CI-MS (m/z 486 $(M-H_3PO_4)^+$). This was confirmed using ^{31}P NMR (142 MHz), with a single signal observed at 1.2 ppm. A ^1H-^{31}P coupling is observed between phosphorus and protons of methylene at 60.5 ppm, showing the phosphate group location. FT-IR spectrum also indicates the presence of one carboxylic group on the molecule (1710 cm^{-1}). The analysis of 2D NMR TOCSY, HMQC and HMBC spectra of RPR113228 revealed an unusual skeleton for this molecule. The arrows on structure show the long range ^1H-^{13}C couplings detected by HMBC experiment, which have been used for structure elucidation (Fig. 2). The ^1H and ^{13}C assignments deducted from 2D NMR experiments are given in Table 2. The proposed structure is consistent with all above spectroscopic evidence.

Inhibition of human FPTase by RPR113228 was examined with two different farnesyl acceptor substrates: lamin B terminal sequence peptide using scintillation proximity assay technology[4] or recombinant $p21^{H\text{-}ras}$ protein using TCA precipitation methodology as previously described[5]; the respective IC_{50} values obtained were 0.83 and 2.1 μM. Furthermore, inhibition of FPTase by RPR113228 was competitive with respect to farnesyl pyrophosphate ($Ki=0.4\,\mu M$). RPR113228 showed selective inhibition of FPTase since it exhibited an IC_{50} of 59 μM toward human geranyl-geranyl protein transferase and no inhibition was found toward rat liver squalene synthase.

This secondary metabolite represents a novel potent inhibitor of Ras post-translational processing that might be useful as anticancer agent particularly in colon and pancreatic carcinomas.

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