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A CEMBRANOLIDE DITERPENE FARNESYL PROTEIN TRANSFERASE INHIBITOR FROM THE MARINE SOFT CORAL *LOBOPHYTUM CRISTAGALLI*

Stephen J. Coval,* Robert W. Patton, Joanne M. Petrin, Linda James, Marnie L. Rothofsky, Stanley L. Lin, Mahesh Patel, John K. Reed,[a] Andrew T. McPhail,[b] and W. Robert Bishop

Schering Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033,
[a]*Harbor Branch Oceanographic Institution, 5600 US 1 North, Fort Pierce, Florida 34946,*[1] *and*
[b]*Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, NC 27708*

Abstract: A previously described cembranolide diterpene from *Lobophytum cristagalli* was identified as a potent (IC_{50} 0.15 μM) inhibitor of farnesyl protein transferase (FPT). The compound showed selectivity for FPT as compared to the closely related enzyme geranylgeranyl protein transferase-1 (IC_{50} 5.3 μM). Kinetic evaluation suggests that this compound competes with the protein/peptide farnesyl acceptor substrate, and not with farnesyl pyrophosphate for inhibition of FPT. Copyright © 1996 Elsevier Science Ltd

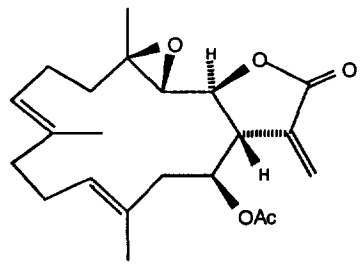
Inhibition of Ras farnesyl transferase presents a potential therapeutic target for novel anticancer agents, and several natural product inhibitors of farnesyl protein transferase (FPT) have recently been reported.[2-6] Ras proteins comprise a family of small guanine nucleotide binding proteins which participate in signal transduction and regulation of cell differentiation and proliferation.[7] Oncogenic (activated) forms of Ras are associated with a variety of human cancers, including 50% of colon and 90% of pancreatic carcinomas.[8] Ras proteins require post-translational processing in order to associate with the plasma membrane and to function in signal transduction or cellular transformation.[9] The first processing step, which is catalyzed by FPT, is addition of the isoprenoid farnesyl, to a cysteine residue near the carboxy-terminus. Herein we report that the screening of marine extracts for inhibition of FPT activity has led to the isolation of a potent inhibitor from a soft coral.

The specimen[10] of *Lobophytum cristagalli* von Marenzeller, 1886, (Order Alcyonacea, Family Alcyoniidae), was collected in the Seychelles from a depth of 15 m in January, 1989. FPT bioassay-guided fractionation of an ethanol extract by solvent partitioning (ethyl acetate/water), then silica gel column chromatography (ethyl acetate/hexane), and finally C_{18} reversed phase HPLC (methanol/water gradient), gave pure **1**.[11]

Spectroscopic analysis by NMR and mass spectrometry suggested that **1** possessed the same gross structure as a cembranolide isolated from a specimen of *L. cristagalli* collected in Sri Lanka.[12] However, there was a notable difference in their IR spectra. The compound from the Sri Lankan specimen was reported to have carbonyl/olefin absorbances at 1765, 1730, and 1660 cm^{-1}. Whereas the present sample of **1** showed only bands at 1772 and 1740 cm^{-1}; the region between 1500 and 1740 cm^{-1} was void of any absorbances. A survey

of the literature regarding related cembranolides revealed considerable variability in the reporting of an IR band in the vicinity of 1660 cm^{-1}. There did seem to be partial correlation with the reporting of 1660 cm^{-1} bands and *cis* ring fusion.

The possibility that the present compound might be stereochemically distinct, and the often significant relationship between stereochemistry and biological activity, prompted us to confirm the stereochemistry of **1** by single crystal X-ray diffraction.[13] The X-ray analysis confirmed that the current sample of **1** is identical to that reported earlier from the Sri Lankan specimen of *L. cristagalli*.



1

Compound **1** has an IC_{50} of 0.15 μM against recombinant human FPT. Additionally, it displays an IC_{50} of 5.3 μM against the closely related isoprenyl protein transferase, geranylgeranyl protein transferase-1 (human recombinant GGPT-1), indicating approximately 35-fold selectivity for FPT.

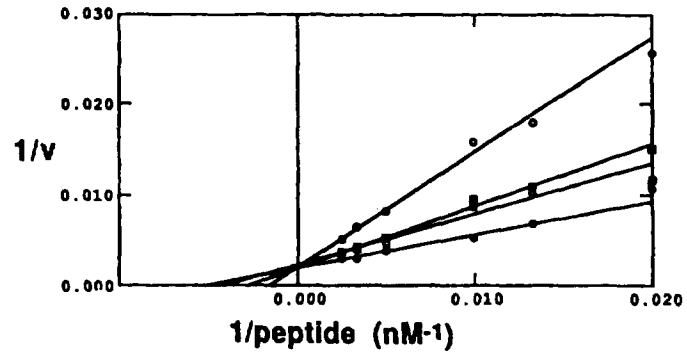
Compound **1** was also evaluated in two cell-based assays. The Cos cell assay measures processing of transiently overexpressed activated H-Ras in whole cells by immunoblot analysis of cell lysates.[14] In this assay, mature, processed H-Ras can be separated from precursor (unfarnesylated) Ras on the basis of its mobility upon SDS-polyacrylamide gel electrophoresis. At 5.3 μM compound **1** resulted in 26% inhibition of Ras processing in COS cells. Higher concentrations of **1** were cytotoxic to Cos cells, as indicated by a loss of expression of Ras protein following transfection.

The second cell-based assay measures the ability of FPT inhibitors to selectively block the growth of T24 human bladder carcinoma cells (containing an activated H-Ras oncogene) on top of a confluent monolayer of normal human fibroblasts. Potent, non-cytotoxic FPT inhibitors block the growth of the carcinoma cells in this assay while leaving the untransformed monolayer intact. Compound **1** at 4.0 μM in this assay displayed cytotoxicity against both the fibroblast monolayer and the tumor cells, indicative of nonspecific cytotoxicity.

The terpenoid nature of **1** suggests that it might compete with farnesyl pyrophosphate (Fpp) for binding to FPT, rather than with the Ras protein/peptide isoprene acceptor. Since Fpp is a critical intermediate in the biosynthesis of a number of isoprene-derived cellular metabolites, an Fpp competitive compound could impact on a number of metabolic pathways perhaps leading to cytotoxicity. A series of kinetic studies were carried out to determine whether **1** competes with Ras protein or with Fpp to inhibit FPT.

The kinetic profile suggests that **1** is competitive with the Ras protein/peptide substrate. Figure 1 shows double-reciprocal plots of 1/reaction velocity vs. 1/peptide substrate concentration at different concentrations of **1**. The peptide substrate in this experiment is a biotinylated peptide with a sequence derived from the C-terminus of the K-Ras4B protein and assays were run as described previously.[14] The common y-intercept indicates that V_{max} is largely unchanged by **1**. The K_i(app) in this experiment was 0.17 μM.

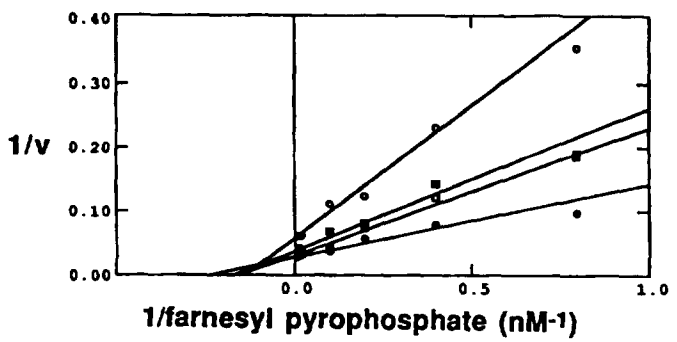
Figure 1. Compound **1** is competitive with respect to the peptide/protein substrate of FPT.



Scintillation proximity assays were conducted as previously described.[14] The peptide substrate concentration was varied (50 to 400 nM) while the farnesyl pyrophosphate concentration was held constant (90 nM). Concentrations of **1** were 0 (●); 40 nM (❑); 120 nM (■); and 400 nM (○). Data were analyzed by non-linear regression analysis using the program k.Cat (BioMetallics, Inc.).

Figure 2 shows data from a similar experiment in which the peptide substrate concentration was held constant and the concentration of the isoprene donor, farnesyl pyrophosphate, was varied. The results indicate that **1** has little effect on the apparent Km for farnesyl pyrophosphate (4 nM in this experiment), as indicated by the near common intercept on the x-axis, suggesting that this compound is non-competitive with respect to the isoprenyl pyrophosphate.

Figure 2. Compound **1** is non-competitive with respect to the farnesyl pyrophosphate substrate of FPT.



Scintillation proximity assays were conducted as previously described.[14] The farnesyl pyrophosphate substrate concentration was varied (2.5 to 100 nM) while the peptide substrate concentration was held constant (100 nM). Concentrations of **1** were 0 (●); 40 nM (❑); 120 nM (■); and 400 nM (○). Data were analyzed by non-linear regression analysis using the program k.Cat (BioMetallics, Inc.).

The discovery of farnesyl protein transferase inhibition by **1**, a cembrane diterpene, brings a new class of compounds into the realm of FPT inhibitors. The cembranolide skeleton of **1** is structurally distinct from

other compound classes that have been identified as potent FPT inhibitors. The general cytotoxicity of **1** diminishes its potential as an anti cancer agent. However, the full biological activity profile, especially the kinetics, suggests that FPT active cembranoids with less cytotoxicity may exist.

References and Notes

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10. The sample was a 15 cm diameter, flattened cup on a short stalk, with low radial ridges on the upper surface of the capitulum, and tan in color. Spicules and gross morphology fit the species description in Verseveltd, J. *Zool. Verhadelingen Leidin* **1971**, *117*, 1, but lacked the finger-like lobes. A taxonomic voucher sample is deposited in the Harbor Branch Oceanographic Museum (Catalog Number 012:00780; HBOI/DBMR Number 19-I-89-1-008).
11. **1** : (SCH56421): $[\alpha]^D$ -344° (MeOH), IR (film) 2925, 1772, 1740, 1229 cm^{-1}; HREIMS observed 374.2092, calcd 374.2093 for $C_{22}H_{30}O_5$, ^{13}C NMR ($CDCl_3$) δ 170.6 s, 169.6 s, 136.3 s, 133.6 s, 130.4s, 129.2 d, 125.4 t, 123.6 d, 80.7 d, 72.9 d, 65.6 d, 59.9 s, 41.6 d, 39.7 t, 39.7 t, 37.3 t, 24.8 t, 24.4 t, 21 q, 17 q, 16.7 q, 15.7 q. ^1H NMR ($CDCl_3$) δ 6.48 s and 5.79 s (H_2-16), 5.16 t (H-7), 5.08 dt (H-14), 4.93 br d (H-11), 4.10 dd (H-2), 3.40 br s (H-1), 2.68 d (H-3), 2.05 s (OAc methyl), 1.75 br s (H-18), 1.73 br s (H-19), 1.35 s (H-20). Yield: 0.02% of wet soft coral.
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