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A Novel Metal-Chelating Inhibitor of Protein Farnesyltransferase

Akiyuki Hamasaki,[a] Hayato Naka,[b] Fuyuhiko Tamanoi,[c] Kazuo Umezawa[b] and Masami Otsuka[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan*
[b]*Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-0061, Japan*
[c]*Department of Microbiology and Molecular Genetics, Molecular Biology Institute, University of California, Los Angeles, CA 90095-1489, USA*

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Abstract—A novel metal chelator comprising a 4-(naphthalen-1-yl)pyridine and 2-aminoethanethiol was synthesized. This showed inhibitory activity against human protein farnesyltransferase with IC_{50} 1.9 μM, induced morphological change in K-ras-NRK cells at 0.5 μg/mL and showed growth inhibition of K-ras-NRK cells with IC_{50} 0.32 μg/mL.

Farnesyltransferase (FTase) is an enzyme that catalyzes the post-translational modification of protein having carboxy-terminal CAAX (C, cysteine; A, an aliphatic amino acid; X, C-terminal amino acid) sequence by farnesyl pyrophosphate.[1–5] Substrates of FTase include Ras-related GTP-binding proteins, nuclear lamins, γ-subunits of heterodimeric G-proteins, and proteins involved in visual signal transduction.[5–7] It has been reported that mammalian FTase is a heterodimer of α and β subunits and the β subunit contains a zinc at the CAAX motif binding site, coordinated by Asp297 β, Cys299 β, His362 β and a water molecule.[8–11] As the farnesylation of Ras protein is critical for its transforming activity, Ras FTase is an important target for cancer therapy. Many inhibitors of FTase have been reported, for example CAAX motif analogues, farnesyl pyrophosphate derivatives, bisubstrate analogues, natural products and library compounds.[1,2] However, there has been no report of inhibitors that target the zinc site of the FTase β subunit except our zinc chelator **1** (namely HPH-5 in ref 12) that showed modest inhibitory activity against FTase (IC_{50} 620 μM).[12] Herein, we describe remarkable improvement of FTase inhibitory activity by the structural modification of compound **1**.

Compound **1** was originally designed in our continuing effort to explore novel metal chelators that could inhibit zinc proteins including zinc finger proteins HIV-EP1 and Sp1 by symmetrically placing metal-chelating appendages on 4-dimethylaminopyridine.[13,14] The finding that compound **1** also inhibit FTase relatively effectively prompted us to optimize its FTase-specificity by structural modification. Our molecular design was based on the information of the crystal structure of FTase. The β subunit of FTase contains a zinc near to a hydrophobic cleft lined with 10 highly conserved aromatic residues Trp102 β, Trp106 β, Trp303 β, Phe253 β, Phe302 β, Tyr105 β, Tyr154 β, Try205 β, Tyr361 β and Tyr365 β.[8] Thus, we attempted the introduction of an aromatic substituent into the pyridine ring of compound **1** and designed naphthyl derivative **2** (Fig. 1).

Synthesis of Compound

Synthesis of compound **2** was carried out as shown in Scheme 1. Thus, introduction of a naphthyl group into a pyridine ring was achieved by the Suzuki coupling between dimethyl 4-chloropyridine-2,6-dicarboxylate **3**[15] and commercially available naphthalene-1-boronic acid **4** using $Pd(PPh_3)_4$, KBr, K_3PO_4 to afford 4-(naphthalen-1-yl)pyridine derivative **5** in 62% yield. Diester **5** was then converted into the corresponding dialdehyde **7** by borohydride reduction (83% yield) and subsequent oxidation (61% yield). The dialdehyde **7** was transformed into dithiol **2**[16] in 65% overall yield according to the procedure of Zhang et al., that is condensation with 2-aminoethanethiol followed by cyanoborohydride reduction.[17]

*Corresponding author. Tel.: +81-96-371-4620; fax: +81-96-371-4620; e-mail: motsuka@gpo.kumamoto-u.ac.jp

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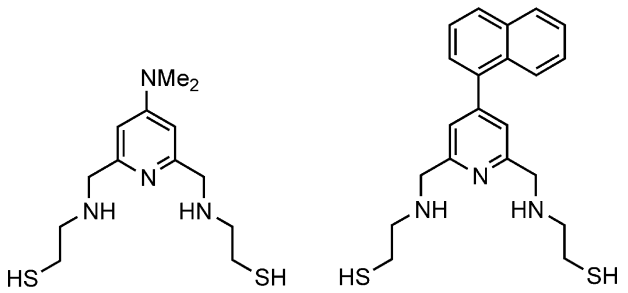


Figure 1. Structure of metal-chelating inhibitors of farnesyltransferase.

Farnesyltransferase Assays

FTase assay was carried out for 15 min at 37 °C using human FTase, a peptide substrate containing a C-terminal CAAX motif (glutathione *S*-transferase fused with Cys-Ile-Ile-Ser) and [^{3}H]farnesyl pyrophosphate substrate as described.[12] Figure 2 shows the influence of compound **2** on the activity of FTase. Compound **2** strongly inhibited FTase with an IC_{50} of 1.9 μM, comparable to the known FTase inhibitors manumycin and SCH44342 (Table 1). As the IC_{50} value of the dimethylamino compound **1** is 620, the prominent effect of the introduction of the naphthyl group is evident.

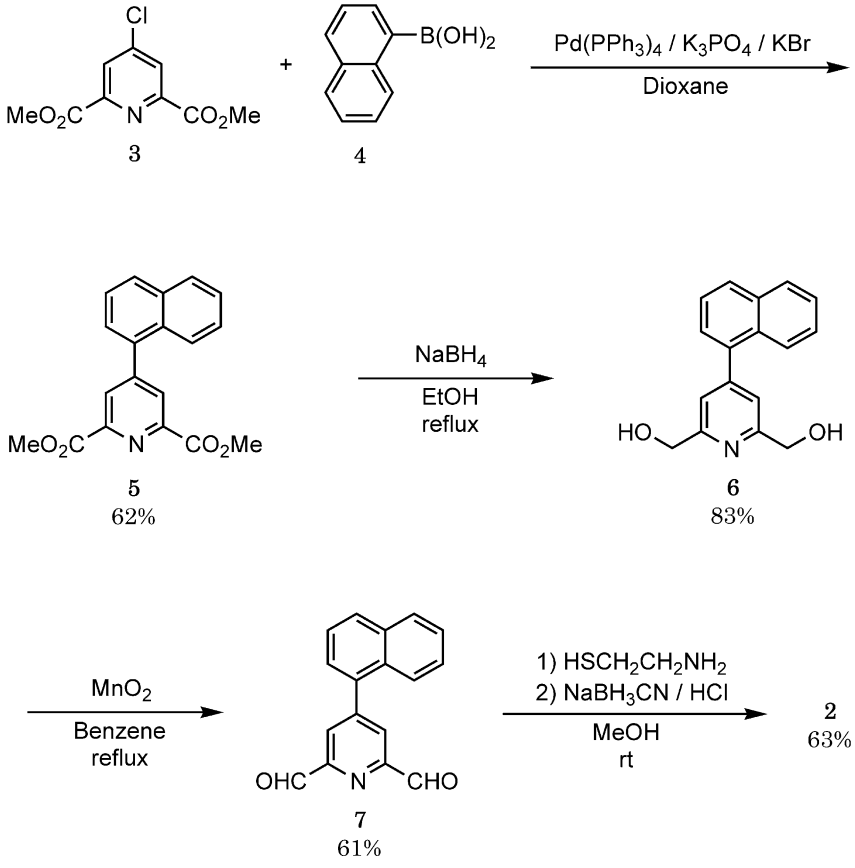
Figure 3 shows the effect of $ZnCl_2$ on the FTase inhibition of compound **2**. The inhibitory effect of compound **2** (10 μM) (b) was completely reversed when 20 μM of $ZnCl_2$ was added together with compound **2** (d), demonstrating that the effect of compound **2** is related to the zinc ion involved in the FTase catalysis. When $ZnCl_2$ was added after 5 min incubation of FTase with compound **2**, the inhibition was not reversed (e). This could be explained by invoking that compound **2** bound the zinc site of the β subunit of FTase during the initial 5 min incubation and the subsequent supply of external zinc did not affect the tight complex of compound **2**–FTase.

In striking contrast to compound **2**, conventional metal chelating agents 1,10-phenanthroline, dipyridyl and ethylenediamine showed virtually no inhibitory activity (Table 1), suggesting that, intriguingly, the inhibition was not only due to the metal binding power of the compound **2** but also due to the affinity to the aromatic pocket in the β subunit of FTase by virtue of the naphthyl group.

Table 1. Inhibition of farnesyltransferase by various compounds

Compd	IC_{50}, μM
1	620[a]
2	1.9
Manumycin	11.9
SCH44342	2.5[a]
1,10-Phenanthroline	4000[a]
Dipyridyl	100,000[a]
Ethylenediamine	15,500[a]

[a]Values reported in ref 12.



Scheme 1. Synthesis of compound **2**.

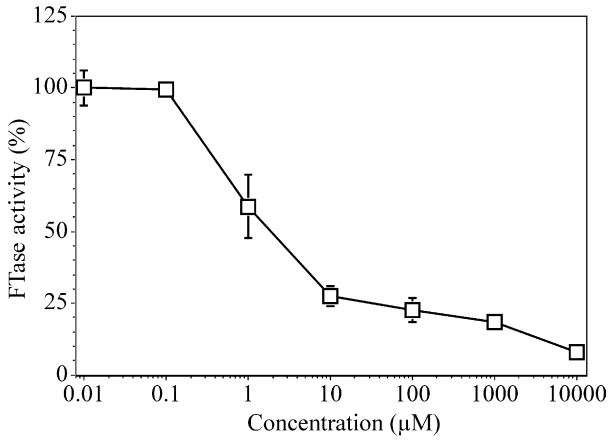


Figure 2. Inhibition of human farnesyltransferase by compound **2**. The reaction mixture contained 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$, 5 mM dithiothreitol, [^{3}H]farnesyl pyrophosphate, 8 μM glutathione *S*-transferase-Cys-Ile-Ile-Ser, 94 nM human farnesyltransferase. Compound **2** was dissolved in ethanol and added to the reaction mixture. After incubation for 15 min at 37 °C, incorporation of ^{3}H into the peptide substrate was measured by a scintillation counter.

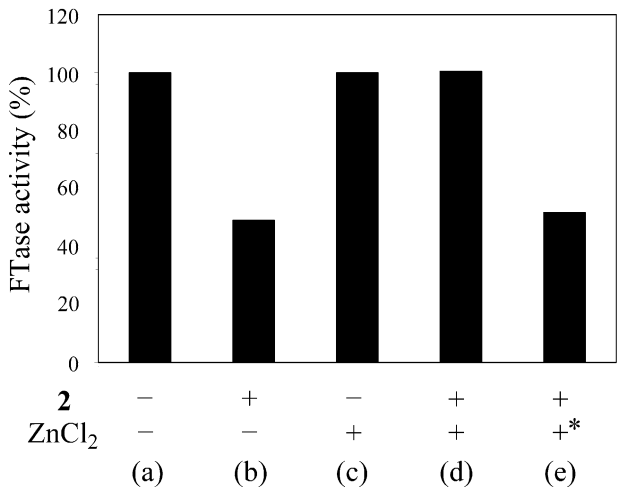


Figure 3. Influence of $ZnCl_2$ (20 μM) in the inhibition of farnesyltransferase by compound **2** (10 μM). Assays were carried out without compound **2** and $ZnCl_2$ (a), with compound **2** (b), without compound **2** with $ZnCl_2$ (c), with compound **2** and $ZnCl_2$ (d). Farnesyltransferase was pre-incubated with compound **2** for 5 min at 37 °C prior to the addition of $ZnCl_2$ (e).

Effect on K-ras-NRK Cells

Compound **2** effectively induced morphological change in K-ras-NRK cells, transformed rat kidney cells as shown in Figure 4. K-ras-NRK cells were treated with compound **2** (0.5 μg/mL, approximately 2 μM), incubated for 24 h and observed under phase contrast microscopy. Induction of normal flat morphology in K-ras-NRK cells was observed (b). This morphological change was the same as that induced by 1.0 μg/mL of conophylline, a vinca alkaloid isolated as a ras function inhibitor (c).[18,19] Thus, compound **2** was suggested to inhibit K-ras functions in cultured cells.

Further, compound **2** showed an inhibition in the growth of K-ras-NRK cells. Cells were incubated with

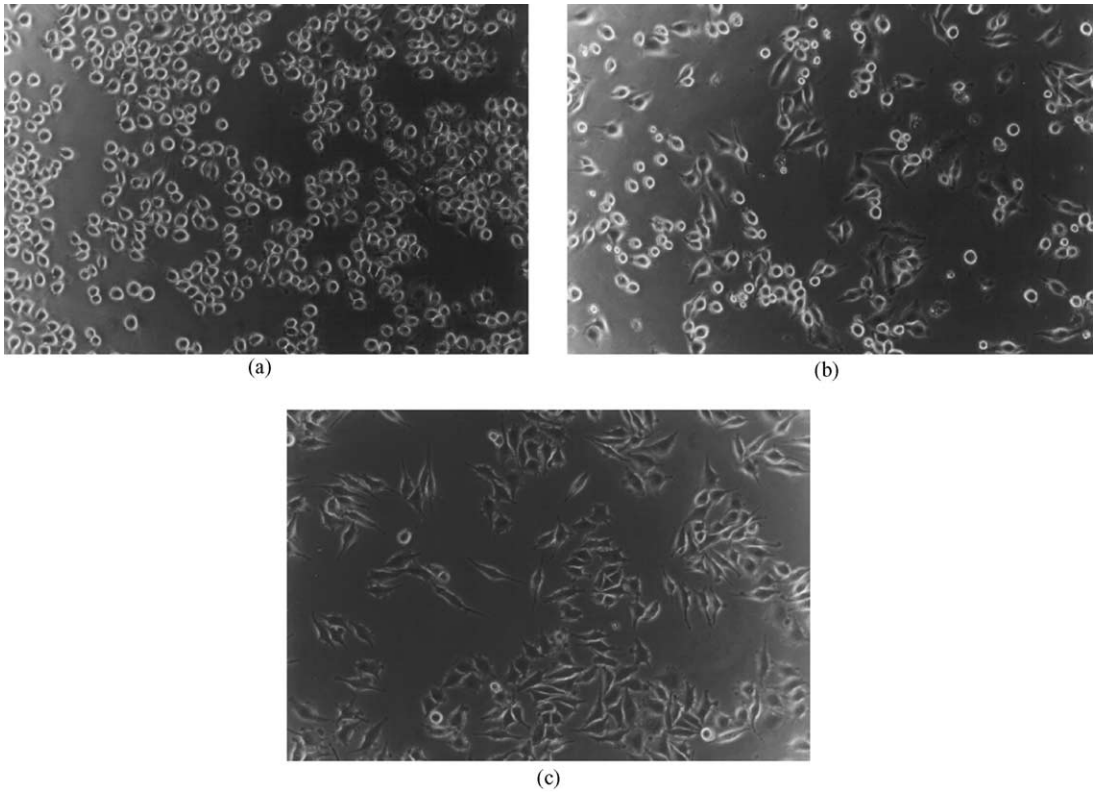


Figure 4. Morphological change of K-ras-NRK cells induced by chemicals. K-ras-NRK cells were seeded in a 48-well chamber slide. After 24 h, compound was added and the cells were further incubated for 24 h and observed under phase contrast microscopy: (a) control (no chemical), (b) compound **2** 0.5 μg/mL, (c) conophylline 1.0 μg/mL.

various concentrations of compound **2** for 1, 2 and 3 days and the cell numbers were counted. The IC_{50} value was 0.32 μg/mL.

Conclusion

Since Ras FTase is regarded as an important molecular target in cancer therapy, there have been many reports on the classes of inhibitors including the CAAX motif analogues, farnesyl pyrophosphate analogues, natural and synthetic inhibitors. However, metal chelators have not been explored as a class of FTase inhibitors. Our inhibitor design is based on the combination of a Zn-chelating core and a substituent to recognize the aromatic residues surrounding the Zn site of FTase. We were successful in improving the FTase inhibitory activity of the previously reported Zn-chelating compound **1** by introducing a naphthyl group to fit in the substrate binding cleft of FTase to attain IC_{50} of 1.9 μM.

Compound **2** targeted the zinc site as supported by the $ZnCl_2$ addition experiments. Our finding is in consistent with the report of Qian et al. that aromatic substitution of the CAAX peptidomimetics resulted in the enhancement of the potency in FTase inhibition.[20] The further structural modification study is currently under way and will be published elsewhere.

Acknowledgements

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