SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF TRIPROPEPTIN C DERIVATIVES CONTAINING THE PYRIMIDINE RING SYSTEM

Sehei Hirosawa¹, Yoshiaki Takahashi¹*, Ayumi Murotani¹, Hideki Hashizume², Toshiaki Miyake¹, and Yuzuru Akamatsu²

¹Institute of Microbial Chemistry (BIKAKEN), Hiyoshi, Japan
²Institute of Microbial Chemistry (BIKAKEN), Tokyo, Japan

* Corresponding author: Dr Y Takahashi, Institute of Microbial Chemistry (BIKAKEN), Hiyoshi, 3-34-17 Ida, Nakahara-ku, Kawasaki, Kanagawa 211-0035, Japan.
E-mail: takashow@bikaken.or.jp

Supporting material

Index:

1. Thin-layer chromatography analysis of complex formation with GGPP S2
2. Mass analysis of complex formation with GGPP S3
1. Thin-layer chromatography (TLC) analysis of complex formation with GGPP

The binding ability of TPPC, and compounds 1, and 4 to GGPP [geranylgeranylpyrophosphate (tetraprenylpyrophosphate), a model target molecule, Sigma-Aldrich, St. Louis, MO, USA] was evaluated by TLC analysis. TPPC, 1, or 4 (each 20 µM) was mixed with GGPP (40 µM) in 80% aqueous MeOH supplemented with 20 µM calcium ions. After incubation at 37 °C for 30 minutes, the mixtures were chromatographed (chloroform/methanol/water, 8:5:1) on a silica gel 60 plate (Merck, Darmstadt, Germany). Iodide vapor and phosphomolybdic acid (PMA) color reactions were used to detect the compounds.

TPPC, 1, 4, and GGPP showed relative flow values of 0.20, 0.46, 0.53, and 0.06, respectively. TPPC, 1, and 4 were hardly detectable by PMA color reactions but were detectable by iodide vapor. When combined with GGPP, the complex mixtures of TPPC, 1, and 4 shifted to relative flow values of 0.26, 0.52, and 0.56, respectively. These results suggested that TPPC, 1, and 4 were able to bind GGPP. However, in the case of compounds 1 and 4, GGPP was not completely consumed and the intensity of the binding affinity was observed to be TPPC>1>4.

Figure S1. TLC analysis of the interaction of TPPC, 1, or 4 with GGPP

(A) I$_2$ vapor (B) PMA stain

Abbreviations: pyr-1, compound 1; pyr-4, compound 4.
2. Mass analysis of the complexes formed by TPPC or its analogs (1 or 4) with GGPP

Each complex mixture, prepared as for TLC analysis, was subjected to liquid chromatography-mass spectrometry (JMC-T100LC mass spectrometer; JEOL, Tokyo, Japan).

In all cases, the complex of TPPC (C₅₁H₈₃N₁₁O₁₉) or its analog (1 or 4) / GGPP (C₂₀H₃₆O₇P₂) / Ca²⁺ was observed at a molar ratio of 1:1:1 by time-of-flight mass spectrometry. However, the ratios of GGPP-adduct form / free form of TPPC or its analog (1 or 4) were different (TPPC>1>4).

Figure S2. MS analysis of the interaction of TPPC, 1, or 4 with GGPP

(A) TPPC + GGPP

(B) 1 + GGPP

(C) 4 + GGPP

Abbreviations: GGPP, geranylgeranylpyrophosphate (tetraprenylpyrophosphate).