Fragment-based discovery of the first nonpeptidyl inhibitor of dipeptidyl peptidase 11 from *Porphyromonas gingivalis*

N. Tanaka\(^1,2\), Y. Suzuki\(^3\), A. Nakamura\(^3\), Y. Watanabe\(^1\), M. Sekiya\(^4\), S. Roppongi\(^5\), C. Kushibiki\(^4\), Ippei Iizuka\(^4\), O. Tani\(^6\), H. Sakashita\(^6\), K. Inaka\(^7\), H. Tanaka\(^8\), M. Yamada\(^9\), K. Ohta\(^9\), N. Honma\(^3\), Y. Shida\(^3\), W. Ogawawara\(^3\), M. Nakanishi-Matsui\(^4\), T. Nonaka\(^4\), H. Gouda\(^1\), Y. Sakamoto\(^4\)

\(^1\)School of Pharmacy, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan, \(^2\)School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan, \(^3\)Department of Bioengineering, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan, \(^4\)School of Pharmacy, Iwate Medical University, 1-1-1 Iida-dori, Yahaba, Iwate 028-3694, Japan, \(^5\)School of Medicine, Iwate Medical University, 1-1-1 Iida-dori, Yahaba, Iwate 028-3694, Japan, \(^6\)Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan, \(^7\)Maruwa Foods and Biosciences Inc., 170-1 Tsutsui-cho, Yamatokoriyama, Nara 639-1123, Japan, \(^8\)Confocal Science Inc., 5-14-15 Fukasawa, Setagaya-ku, Tokyo 158-0081, Japan, \(^9\)Japan Aerospace Exploration Agency (JAXA), 2-1-1 Sengen, Tsukuba, Ibaraki 305-8505, Japan

tanakan@pharm.kitasato-u.ac.jp, sakamoto@stbio.org

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Dipeptidyl peptidase 11 from *Porphyromonas gingivalis* (PgDPP11) belongs to a new class of serine peptidases, clan PA family S46, and preferentially cleaves substrate peptides with Asp/Glu at the P1 position (NH\(_2\)-P2-P1-P1'-P2'-... where the P1-P1' bond is the scissile bond). Because S46 peptidases are not found in mammals, these enzymes are attractive targets for novel antibiotics. However, potent and selective inhibitors of these peptidases have not been developed to date.

In this study, we determined a crystal structure of PgDPP11 in complex with citrate ions at a 1.50 Å resolution using a space-grown crystal. The bound citrate ion, a potassium ion, and a water molecule in the S1 subsite of PgDPP11 were regarded to mimic the binding of an acidic amino acid and were utilized as a pharmacophore for an *in silico* inhibitor screening. The screening resulted in the first nonpeptidyl inhibitor of S46 peptidases, SH-5 (2-[(2-aminoethyl)amino]-5-nitrobenzoic acid, C\(_9\)H\(_{11}\)N\(_3\)O\(_4\)). The binding mode of SH-5 was confirmed by crystal structure analysis at a 2.39 Å resolution. SH-5 showed a dose-dependent inhibitory effect against the growth of *P. gingivalis*. Thus, this hit compound will be a good starting point for the design of potent and specific inhibitors of DPP11s.

The process of inhibitor discovery in the present study has two unique features. The first is the use of a space-grown crystal-derived high-resolution structure as the template for pharmacophore setting. The crystal was obtained under a microgravity environment in the Japanese experimental module “Kibo” at the International Space Station. The second unique feature is the incorporation of ion and solvent molecules in the pharmacophore that we used (cyan and magenta spheres in Fig. 1), while in most cases of pharmacophore-based *in silico* screening, pharmacophore features are set on the atoms of the bound fragment molecule.

Figure 1. A 3D pharmacophore model for the first-stage screening. Hydrogen bond donor and acceptor features are shown as cyan and magenta spheres, respectively.