# Investigation of the binding mode of stylissatin A on lysosomal PPCA and development of its analogs for anti-obesity agents

○Dewi Luthfiana,[1] Yiting Sun,[1] Ayumi Dakiiwa,[1] Takahiro Shibata,[1] Masaki Kita[1]

[1]*Graduate School of Bioagricultural Sciences, Nagoya University*

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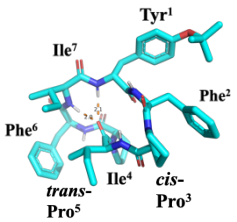
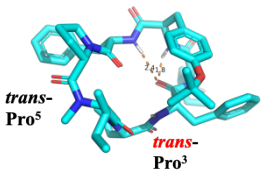
Stylissatin A (SA) is an anti-inflammatory cyclic heptapeptide of marine sponge *Stylissa massa*.[1] SA inhibited nitric oxide production in LPS-stimulated murine macrophage RAW264.7 cells with low cytotoxicity. SA and its derivatives (SAs), especially D-Tyr[1]-*t*BuSA potently inhibit adipocyte differentiation in the murine 3T3-L1 preadipocyte.[2] SAs also inhibit the interaction between lysosomal protective protein cathepsin A (PPCA) and neuraminidase 1 (Neu1), which causes lipid droplet degradation.[3] Understanding SA-PPCA interaction is essential for the discovery and development of novel anti-obesity drugs. Therefore, to investigate the binding mode of SAs on PPCA, dmpy-NASA-DACN probe was designed. By the Huisgen reaction, dmpy-NASA-ligand conjugates can be easily prepared for in-situ labeling and binding position analysis of unknown targets.

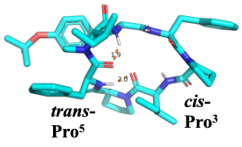
Additionally, conformational study revealed that *N*-methylation of amide moiety on isoleucine residue induced significant conformational change on SAs. While introducing *N*-methyl group can alter the conformation and intramolecular hydrogen-bond potential, it may also impact the target selectivity and biological activity of the peptide. To synthesize these peptides, solid-phase peptide synthesis (SPPS) followed by macrocyclization in solution were employed. This study will elucidate the impact of *N*-methyl group incorporation on target selectivity and bioactivity of SA analogs through in-vitro and in-vivo evaluations.



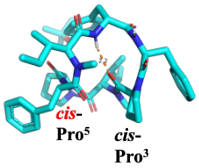

D-Tyr[1]-*t*BuSA




D-Tyr[1]-*t*BuSA-(*N*Me-Ile[4])




D-Tyr[1]-*t*BuSA-(*N*Me-Ile[7])




D-Tyr[1]-*t*BuSA-(*N*Me-Ile[4,7])

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