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Structural Analysis of SET7/9 in Complex with Cyproheptadine and its Derivative

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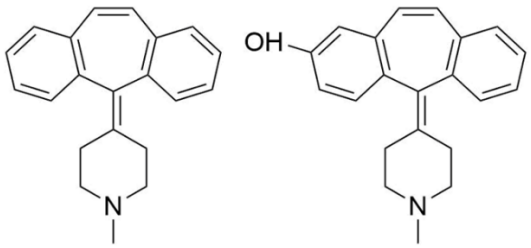
1 Introduction

SET7/9 is a lysine methyltransferase that transfers the methyl group of the coenzyme *S*-adenosylmethionine (SAM) to a specific lysine residue in a substrate protein. The substrate proteins of SET7/9 include histone H3 and other non-histone proteins, such as estrogen receptor α (ERα). SET7/9 is implicated in diverse biological processes. Specific SET7/9 inhibitors are regarded as a useful chemical probe to investigate the biological function of protein methylation. In addition, because ERα is overexpressed in many breast cancers, small-molecule SET7/9 inhibitors are expected to become potential anticancer agents.

Prof. Minoru Yoshida (the University of Tokyo and RIKEN) and Prof. Akihiro Ito (Tokyo University of Pharmacy and Life Sciences and RIKEN) performed a chemical library screening for SET7/9 inhibitors and identified cyproheptadine (Fig. 1; hereafter referred to as **1**) as an inhibitor. Compound **1** is an antagonist of the histamine receptor (H_1) and serotonin receptor (5-HT2A), and has already been used as an anti-allergic agent. Compound **1** inhibited enzymatic activity of SET7/9 with an IC_{50} value of 1.0 μM *in vitro*. It also destabilized ERα and suppressed the estrogen-dependent breast cancer cell (MCF7) growth [1]. We determined the crystal structure of SET7/9 in complex with **1** and elucidated its binding mode [1].

Based on the structure, Prof. Tomoya Hirano (Tokyo Medical and Dental University) and his colleagues developed a more potent SET7/9 inhibitor, 2-hydroxycyproheptadine (Fig. 1; hereafter referred to as **2**), which inhibited SET7/9 with an IC_{50} value of 0.41 μM. We determine the structure of SET7/9 in complex with **2** [2].

In this short report, we summarize the structural features of SET7/9 in complex with **1** and **2**.



1: Cyproheptadine **2**: 2-Hydroxycyproheptadine

Fig. 1: Chemical structures of the inhibitors

2 Experiment

The binary complex of SET7/9–SAM were crystallized by the handing-drop method at 277K with a reservoir solution containing 0.1M Tris-HCl (pH 8.5) and 31% PEG 6000. The crystals of the ternary complex of SET7/9–SAM–**1** were obtained by soaking the SET7/9–SAM crystals into a crystallization drop containing 3 mM of **1** for overnight. The crystals of the ternary complex of SET7/9–sinefungin–**2** were also obtained by the soaking method, where sinefungin was used as a SAM analogue. The crystals of the SET7/9–sinefungin complex, grown under similar conditions to those of the SET7/9–SAM complex, were soaked into a crystallization drop containing 3 mM of **2** for ten days. The diffraction data from the SET7/9–SAM-**1** and SET7/9–sinefungin–**2** crystals were collected at AR-NW12A and BL-17A of Photon Factory, respectively. Data processing, molecular replacement, and structure modelling and refinement were carried out similarly for the both complexes, using HKL2000, CCP4 programs, Phaser, Coot and Phenix. The apo form structure of SET7/9 (PDB: 1N6A) was used for the search model in the molecular replacement for SET7/9–SAM–**1**, while the refined SET7/9–SAM–**1** structure was used as the starting model for SET7/9–sinefungin–**2**. The initial coordinates and parameters for the compounds were generated using eLBOW in the Phenix suite. The final coordinates and structure factors have been deposited in PDB with the accession codes 5AYF for SET7/9–SAM–**1** and 5YLT for SET7/9–sinefungin–**2**.

3 Results and Discussion

The crystal structure of SET7/9 in complex with SAM and **1** was determined at 2.01 Å resolution. The bound **1** molecule was unambiguously identified in the electron density map. Compound **1** binds to the peptide-binding site in the SET domain of the protein, which is on the opposite side of the domain from the SAM-binding site (Fig. 2). The methylpiperidine ring of **1** is accommodated in the lysine access channel. The methylpiperidine nitrogen atom forms a 2.7 Å hydrogen bond with the carbonyl oxygen of Thr266. The methylpiperidine ring makes hydrophobic contacts with Tyr335 by stacking its ring to that of Tyr335 (Fig. 3A). The dibenzocycloheptene ring of **1** occupies the position of the Tyr337 aromatic ring in a substrate-lysine bound SET7/9 structure. The electron density of Tyr337 is absent in the compound **1**-bound structure, suggesting the conformation of the Tyr337 side chain is not fixed. A stretch of weak electron density was observed above the dibenzocycloheptene rings, to which the main chain of six

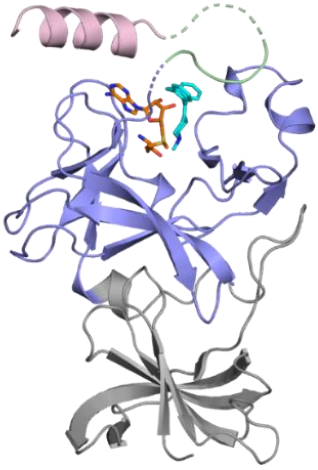


Fig. 2: Overall structure of SET7/9 with bound **1** (cyan) and SAM (orange).

residues were modelled as a part of the flexible loop before the C-terminal α helix (Fig. 3A).

The crystal structure of SET7/9 in complex with sinefungin and **2** was determined at 1.69 Å resolution. The electron density map showed clearly the molecule of **2** with a bulge that corresponds to the 2-hydroxy group (Fig. 3B). Compound **2** binds to SET7/9 at the same position and with the same orientation as **1**. As in the SET7/9–SAM–**1** complex, the methylpiperidine nitrogen atom forms a 2.6 Å hydrogen bond with the carbonyl oxygen atom of Thr266, and the methylpiperidine ring makes hydrophobic contacts with the side chain of Tyr335 (Fig. 3B).

Importantly, the hydroxy group of **2** forms 2.6 and 3.0 Å hydrogen bonds with the side-chain oxygen atom and main-chain nitrogen atom of Asp338, respectively, which contribute to the increased affinity for SET7/9. The side chain of Tyr337 was found completely flipped outward. The dibenzosuberene moiety is surrounded by hydrophobic residues Pro341, Pro342, Pro350, Trp352, and Tyr353. The residues from Tyr337 to Ser345 were visible in the electron density map of SET7/9–sinefungin–**2**, with only the residues 346–348 just before the C-terminal helix lacking enough electron density for modelling. The interactions between **2** and Asp338 seem to restrain the conformation of the flexible loop, and accordingly Tyr337 and the subsequent residues, which was not clearly visible in the SET7/9–SAM–**1** structure, became visible in the electron density map (Fig. 3B).

In summary, we determined the crystal structure of SET7/9–SAM–**1** and elucidated how compound **1** binds to the substrate-binding site of SET7/9. Based on the structure, we developed a more potent **2** and determined the crystal structure of SET7/9–sinefungin–**2**. The hydroxy group of **2** forms hydrogen bonds with Asp338 and restrain the conformation of the flexible loop before the C-terminal helix.

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References

[1] Y. Takemoto *et al*., *J. Med. Chem.* **59**, 3650 (2016).
[2] T. Hirano *et al*., *ChemMedChem* **13**, 1530 (2018).

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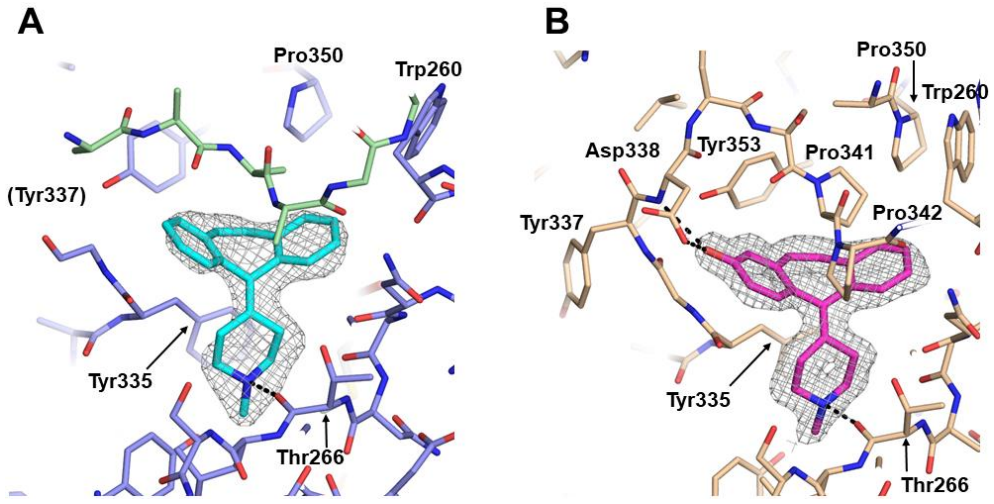

Fig. 3: The binding modes of the inhibitors. (A) Compound **1** is shown in cyan, SET7/9 residues are in blue, and the main-chain only residues in the weak electron density are in green. The position of missing Tyr337 is indicated with the label in parentheses. (B) Compound **2** is shown in magenta and SET7/9 residues in orange. In the both figures, electron density for the inhibitors are shown in gray meshes.