Case Study

Designing Small Molecule Inhibitors for Autotaxin

Structural Biology Services

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Overview

- Cayman Chemical's structural biologists provided a drug discovery platform for the generation of small molecule autotaxin (ATX) inhibitors using their gene-to-structure pipeline.
- Crystal structures of mutant human ATX in complex with four structurally distinct ATX inhibitors are presented.
- Crystal structures confirm the unique mechanisms of inhibition displayed by ATX.
- A crystal structure with arachidonic acid (AA) represents the first ATX structure containing a substrate-bound secondary lysophosphatidic acid (LPA) binding site.
- This work enhanced our client's drug discovery efforts, leading to a clinical candidate.

Background

PharmAkea, a startup biotechnology company located in San Diego, California, contacted Cayman Chemical to help determine the mode of binding for their small molecule autotaxin (ATX) inhibitors. ATX has been shown to be responsible for lysophospholipase D (lysoPLD) activity in human serum that hydrolyzes lysophosphatidylcholine (LPC) to generate lysophosphatidic acid (LPA). Elevated levels of LPA and ATX lysoPLD activity have been found in diseases ranging from renal cancer to atopic dermatitis. Thus, inhibiting ATX or antagonizing the LPA receptor makes ATX an attractive therapeutic target for many diseases.

Services Utilized

Cayman Chemical's Contract Services team provided a drug discovery platform to PharmAkea to understand the binding mechanism of small molecule ATX inhibitors using their gene to structure pipeline.

Results

A variant of full-length human ATX was generated in which the asparagine glycosylation sites at amino acid positions 54 and 411 were mutated to alanine to enhance crystal formation and maintain enzymatic activity. X-ray data from this mutant human ATX in complex with four potent, structurally distinct PharmAkea ATX inhibitors, were obtained.

These crystal structure complexes identified unique modes of ATX inhibition. Compounds binding to the catalytic domain (deep within the hydrophobic pocket of the competitive site) and partially occluding the substrate and LPA binding site displayed competitive inhibition (**Figure 1A**). Compounds binding to a second allosteric binding site adjacent to the catalytic site displayed noncompetitive inhibition with respect to lysoPLD activity (**Figure 1B**). Interestingly, in this crystal structure, LPA co-crystallized with the compound and was shown to be bound in the active site/hydrophobic pocket. This noncompetitive site is the same as a previously reported putative secondary LPA binding site in the mouse ATX crystal structure and has also been identified as a hydrophobic channel that facilitates channeling of LPA to its cognate receptors.[1] Unexpectedly, a mixed-mode inhibitor was found co-crystallized with AA, each bound in a distinct site outside of the catalytic site. This crystal structure with AA identified a novel binding mode containing a secondary LPA binding site (**Figure 1C**). An additional mixed-mode inhibitor was also identified to bind to the hydrophobic channel, which also binds AA. However, this compound did not co-crystallize with AA and instead precluded AA binding since it occupied the same hydrophobic channel where AA would bind. Since AA alone does not inhibit the lysoPLD activity of ATX, its binding to ATX is likely stabilized in the presence of the novel, noncompetitive inhibitor compound.

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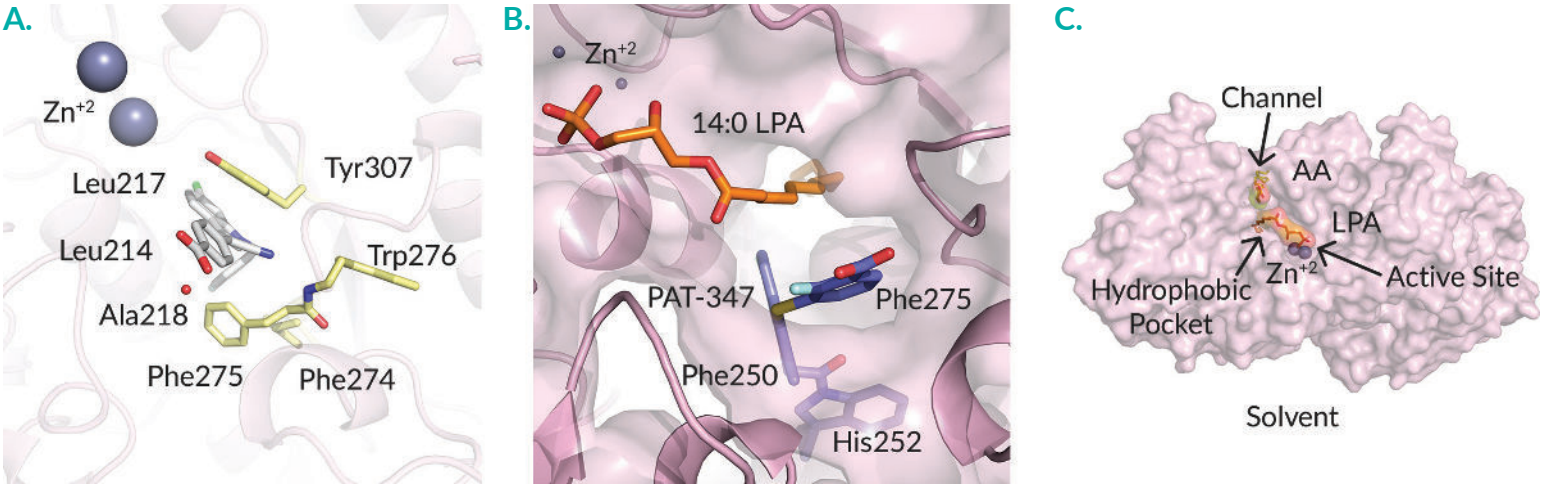

Figure 1. A. Crystal structure of PAT- 078 (grey) displaying competitive inhibition (Zn^{+2}, grey spheres). Important residues are highlighted in yellow. **B.** Crystal structure of PAT-347 (purple) displaying non-competitive inhibition (LPA, orange, Zn^{+2}, grey spheres). Important residues are highlighted in orange. **C.** Labelled surface view of ATX.

Summary

Cayman's Structural Biology Services group provided PharmAkea with crystal structures of ATX in complex with four previously unknown, structurally distinct ATX inhibitors. These X-ray structures provide insight to the structural basis for the distinct mechanisms of inhibition each compound possesses. It also revealed new information regarding the role of a secondary AA binding site on human ATX. This study advanced the discovery of more potent and selective compounds by rational design that have translated into the clinic.

Full details of this work have been published in *Molecular Pharmacology*:

Stein, A.J., Bain, G., Prodanovich. P. *et al.* Structural basis for inhibition of human autotaxin by four potent compounds with distinct modes of binding. *Mol. Pharmacol.* **88(6)**, 982–992 (2015).

Additional References

1. Nishimasu, H., Okudaira, S., Hama, K., *et al.* Crystal structure of autotaxin and insight into GPCR activation by lipid mediators. *Nat. Struct. Mol. Biol.* **18(2)**, 205–212 (2011).

Contact us to learn more about Cayman's Structural Biology Services or discuss how our team can provide solutions for your medicinal chemistry needs.

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