Development of ATX and DUSP inhibitors : inhibiting phosphate ester hydrolysis in biology

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Summary and future prospects

Autotaxin inhibitors

The first part of this thesis describes the development of inhibitors of autotaxin (ATX or ENPP2), a phosphodiesterase that is responsible for the production of the lipid lysophosphatidic acid (LPA) in the circulation. ATX is implicated in several diseases including inflammation, fibrotic disease and cancer, making it an interesting potential drug target to study. ATX inhibitors are required in the validation process of ATX as a drug target. However, at the start of this study, small molecule ATX inhibitors did not exist. In order to discover ATX inhibitors, ~40,000 small molecules were screened and several structural classes of inhibitors were identified (**Chapters 2** and **3**). After validation experiments a class of small molecules called thiazolidine-2,4-diones proved to be the most promising. This class was amenable to chemical optimization, allowing the synthesis and isolation of ~100 analogs of the confirmed thiazolidine-2,4-dione screening hit (Figure 1). In the handed optimization approach variations were introduced in a benzyl moiety (green) and a benzylidene moiety (blue), which are linked *via* a thiazolidine-2,4-dione core (Figure 1). The key step in the optimization approach was the replacement of a carboxylic acid in one of the screening hit analogs by a boronic acid. The boronic acid was introduced to target the oxygen nucleophile in threonine 210 (T210), an amino acid in the ATX active site which is essential for the activity of this enzyme. This modification resulted in a boronic acid-based inhibitor (HA130; IC_{50} = 30 nM, Figure 1) with almost a 100-fold increase in potency compared to our original screening hit (IC_{50} = 2.5 μM).

Next, the biological properties of HA130 as ATX inhibitor were explored (**Chapter 2**). It was shown that HA130 is a selective inhibitor of ATX and that it does not inhibit ENPP1, the closest family member of ATX. Finally, it was demonstrated that HA130 instantaneously lowers plasma levels of LPA in mice after intravenous administration. This implies that the production of LPA by ATX and LPA degradation by other enzymes is tightly and dynamically regulated. Thus, ATX can be targeted *in vivo* using boronic acid-based inhibitors.

The development of ATX inhibitors described in **Chapters 2** and **3** was conducted before the ATX crystal structure was resolved.

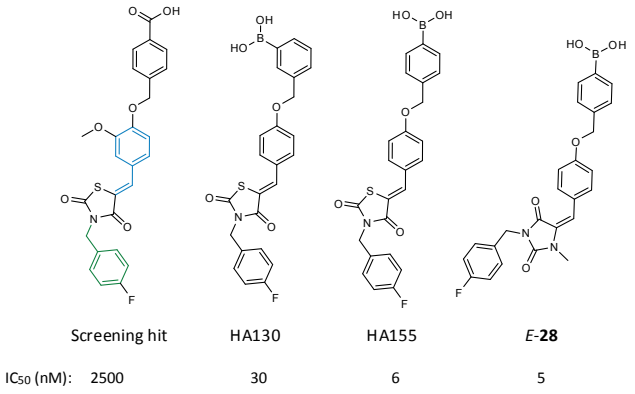

Figure 1: Selection of ATX inhibitors described in this thesis. Benzyl and benzylidene moiety in the screening hit are highlighted in green and blue, respectively.

In 2011, we obtained a crystal structure of ATX in complex with boronic acid-based inhibitor HA155 (IC_{50} = 6 nM, Figure 1). HA155 is a positional boronic acid isomer of HA130 with a higher affinity for ATX compared to HA130 (**Chapter 3**). The structure of HA155 bound to ATX proved that the boronic acid in HA155 indeed targets the threonine oxygen nucleophile in the ATX active site. Next, this structure was used to design new boronic acid-based inhibitors and replaced the thiazolidine-2,4-dione core in HA155 with other cores (**Chapter 4**) and variations of the ether linker in HA155 were investigated. This study finally led to the discovery of a highly active imidazolidine-2,4-dione analog of HA155 (*E*-**28**, IC_{50} = 5 nM) which had an *E*-configured double bond instead of a *Z*-configuration as in HA155 and all other evaluated analogs (Figure 1). To explain how *E*-**28** could bind to ATX molecular docking studies were performed. These studies suggested that *E*-**28** binds differently to the hydrophobic pocket in ATX compared to HA155 and other *Z*-configured analogs, opening possibilities for new inhibitor designs.

New inhibitor designs that could fully exploit the hydrophobic pocket in ATX are designs based on *E*-**28**. Extension from the methylene moiety in the 4-fluorobenzyl group of *E*-**28** with other aromatic moieties could target both regions in the hydrophobic pocket that are separately targeted by *E*-**28** and HA155 alone.

Since the current series of ATX inhibitors contain a fluorine atom, the introduction of the positron-emitting radionuclide fluorine-18 (F18) is an option. This will allow tracing of inhibitors *in vivo* (e.g. mice) using Positron Emission Tomography (PET) imaging to gather information about the pharmacokinetics of these inhibitors.

To validate ATX as a drug target, besides to ATX inhibitors, proper animal model systems that mimic ATX-driven diseases are needed. Few of these models exist today, especially proper models regarding cancer are lacking. Generation of such models and using them in combination with ATX inhibitors is the next step in the validation of ATX as a drug target.

DUSP inhibitors

After a short introduction on dual specificity phosphatases (DUSP, **Chapter 5**) the involvement of host phosphatases in bacterial infection of human host cells was studied. In this study a small interfering RNA (siRNA) phosphatase library was screened to learn which host phosphatases are required for the bacterial growth of *Salmonella (S.) typhimurium* in human host cells (**Chapter 6**). Among the phosphatases identified many DUSPs (DUSP3, 11 and 27) were present. In parallel, a tyrosine phosphatase-targeted small molecule library was tested in order to discover molecules that inhibit *S. typhimurium* growth in human cells. Several compounds inhibited *S. typhimurium* growth in human host cells and one of these compounds inhibited the DUSPs that were identified from the siRNA screen. The most promising compound was then further optimized to result in a potent and selective DUSP3 inhibitor (IC_{50} = 0.33 μM), with a 15-fold increased potency compared to DUSP27 (IC_{50} > 5 μM). Molecular docking of this compound in both DUSP3 and 27 suggests a hydrogen bond

between this inhibitor and a tyrosine residue in DUSP3, which cannot be formed in DUSP27, explaining the selectivity of this inhibitor. This tyrosine residue is present in the active site of DUSP3 but is not conserved in the DUSP active site consensus sequence (HCXXGXXR). Therefore, targeting non-conserved active site residues in DUSPs could be a valid strategy for the development of selective DUSP inhibitors. Developing selective phosphatase inhibitors is a challenging task due to the highly conserved active site residues in phosphatases.

For the optimization of DUSP inhibitors a similar approach was taken as for the first optimization method used for the ATX inhibitors. In this method only the outer parts (benzyl and benzylidene moieties) of the inhibitor were modified and the thiazolidine-2,4-dione core part was left untouched. For increasing affinity and/or selectivity of the DUSP inhibitors it would be useful to introduce variations in the thiazolidine-2,4-dione core of the DUSP inhibitors using, for example, modifications that are described in **Chapter 4**.

The approach taken in **Chapter 6** to inhibit host DUSP proteins that are essential for bacterial growth, seems to be a valid way to control bacterial infection. This new approach could be a useful addition to the current treatment of bacterial infections that target solely the bacteria itself, and hold promise as a new therapeutic strategy to treat bacterial infections with less chance for the development of drug resistance.