Summary

Proteolysis-targeting chimeras (PROTACS) are heterobifunctional molecules consisting of three primary components: (1) a ligand that binds the protein of interest (POI), (2) a linker and (3) an E3 ubiquitin ligase-recruiting ligand. By bringing the POI into proximity with a naturally occurring E3-ligase, PROTACs stimulate the ubiquitination of the POI, thereby marking it for subsequent degradation by the proteasome. After promoting ubiquitination of a single target protein, the PROTAC can disengage and facilitate the degradation of additional target proteins, enabling a sub-stoichiometric mechanism of action. This property distinguishes PROTACs from classical small molecule inhibitors, which require sustained binding to achieve their therapeutic effect. As a result, PROTAC-mediated protein degradation holds significant therapeutic potential and is considered a promising new pharmaceutical paradigm.

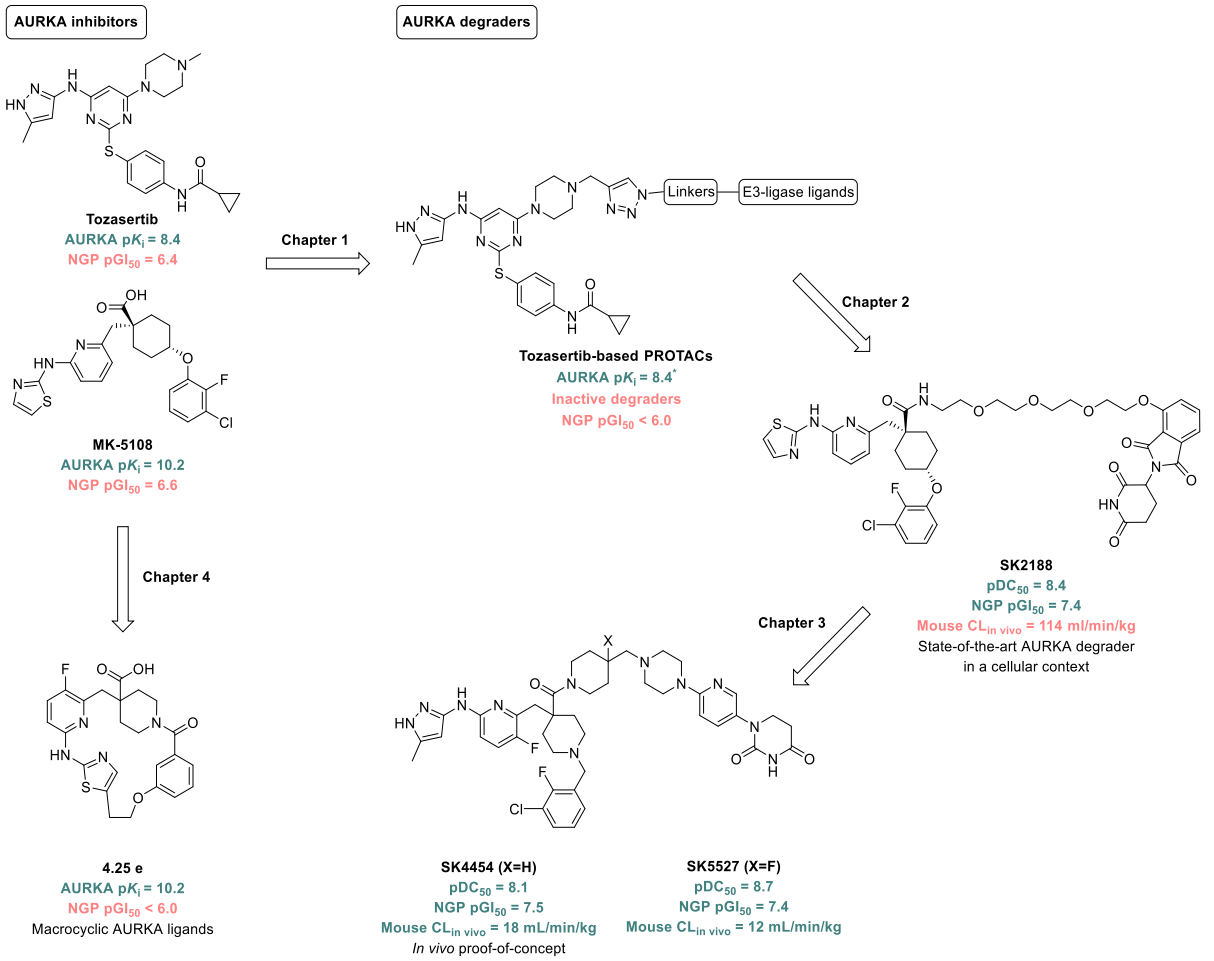
Aurora kinase A (AURKA) is a serine/threonine kinase that is often overexpressed in neuroblastoma tumours and serves as a poor prognostic indicator. It is a well-established therapeutic target in neuroblastoma due to both its kinase-dependent role during mitosis and its kinase-independent roles, such as stabilizing the MYCN oncoprotein and mediating DNA damage repair. Classical ATP-competitive kinase inhibitors primarily target AURKA's catalytic activity while triggering AURKA upregulation and have shown only minimal efficacy in clinical trials.

We hypothesized that targeted AURKA degradation using PROTACs could overcome AURKA upregulation and enhance the therapeutic efficacy by targeting both AURKA's kinase-dependent and scaffolding functions. Therefore, the primary aim of this PhD thesis is to design, synthesize and identify potent and selective AURKA-targeting PROTACs and to compare their effect with that of established AURKA inhibitors in neuroblastoma cells. A secondary aim is to optimize such an initial PROTAC hit as to investigate their therapeutic potential *in vivo*.

In **chapter I**, we describe our efforts in the design and synthesis of AURKA-targeting degraders based on the AURKA ligand tozasertib. Despite exploring multiple linkers and E3-ligase ligands, none of these constructs proved to be effective degraders. Consequently, we explored a different AURKA ligand, MK-5108 for the construction of a series of Cereblon-recruiting bivalent ligands (**chapter II**). Unlike tozasertib-based PROTACs, MK-5108-based analogues, were able to reduce AURKA levels at low nM concentrations. Among these, PROTAC **SK2188**, induced potent, rapid, profound and selective degradation of AURKA. Compared to its inhibitor, **SK2188**, showed 10 times more potent cell growth inhibition in neuroblastoma cell lines and patient-derived organoids, highlighting the therapeutic potential of MK-5108-based PROTACs. However, **SK2188** exhibited high *in vivo* clearance, limiting its potential for *in vivo* evaluation. In **Chapter III**, we therefore optimized the structure of **SK2188** by exploring various linkers, CRBN- and AURKA ligands, ultimately leading to the discovery of the potent AURKA degraders **SK4454** and **SK5527**, both of which displayed improved *in vivo* pharmacokinetic profiles and even modest oral bioavailability. Pharmacodynamic experiments revealed that **SK4454** significantly reduced AURKA levels in tumour tissues from neuroblastoma IMR-32 xenograft-bearing mice, providing proof-of-concept for *in vivo* AURKA degradation. Further studies on tumour growth inhibition, following multiple administrations, will be necessary to fully compare its therapeutic potential with that of established AURKA inhibitors. In **chapter IV**, we explored the potential to transform known AURKA inhibitors into macrocyclic analogues that are fixed in a conformation similar to

that adopted by the acyclic analogues upon binding to AURKA. These macrocyclic AURKA ligands were shown to retain excellent affinity. Although these macrocycles failed to enter NB cells, they might serve as new ligands for future AURKA-targeting degraders.

Overall, we successfully developed potent, fast, and selective Aurora Kinase A degraders, such as **SK2188.** Further structural optimization efforts led to the discovery of high-quality degrader **SK4454**, which demonstrated *in vivo* AURKA degradation activity. These findings lay a strong foundation for continued validation and for advancing AURKA degraders as a therapeutic option for neuroblastoma.



Schematic overview of the development of AURKA degraders and macrocyclic AURKA inhibitors described in this thesis.

** pK_i -value of PROTAC 1.58d*