[BIOINFO 2025]

AI-Guided Design of Selective Cyclic Peptide Inhibitors Against AXL Kinase for Cancer Therapy

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AXL, a member of the TAM (TYRO3, AXL, MERTK) receptor tyrosine kinase subfamily, is known as a critical driver of cancer progression and metastasis. Upon Gas6 ligand binding, AXL activation triggers epithelial-mesenchymal transition (EMT) and enhances tumor cell survival, proliferation, and metastatic potential. While Bemcentinib (BGB324), the most advanced clinical candidate, demonstrates potent AXL inhibition (IC_{50} = 14 nM), its cross-reactivity with other TAM family members–showing only 5-fold selectivity over MERTK and 10-fold over TYRO3–presents significant challenges for therapeutic application.

AXL activation occurs through ligand-induced conformational changes within the kinase domain, where key residues reposition into the ATP-binding pocket to initiate intracellular signaling cascades. This study aimed to design novel selective inhibitors that block this activation mechanism by preventing the interaction between AXL kinase and its ATP-binding pocket.

Our design strategy began with identifying functionally important hotspot residues using PyMOL structural analysis. We then generated protein binder backbones capable of targeting AXL's ATP pocket through RFdiffusion, followed by sequence optimization using ProteinMPNN. Cyclic peptide inhibitor candidates were subsequently designed using AfCycDesign. The design process utilized structural information from PDB entries of the AXL cytoplasmic kinase domain. To specifically block the active ATP pocket that forms after Gas6 binding, we employed the pocket structure itself as a targeting scaffold. Specificity was further enhanced by identifying AXL-unique hotspot residues—distinct from other TAM receptors—through BLASTP comparative analysis.

The designed peptide inhibitors underwent comprehensive in silico validation. By leveraging AI-based protein design techniques, this approach addresses the limitations of existing small-molecule drugs and demonstrates significant potential as a next-generation anticancer therapeutic combining both biocompatibility and target specificity. Furthermore, the protein–protein interaction inhibitor design strategy established in this study provides a versatile framework that can be extended to developing inhibitors against other RTK family members.