Novel covalent modulators of plasma kallikrein activity

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Kallikreins (KLKs) are a family of 15 serine proteases that play crucial roles in inflammation and vascular permeability. From them, plasma kallikrein (pKLK) uniquely occurs in plasma and it contributes to the production of bradykinin (BK) via cleavage of high-molecular-weight kininogen (HMWK), leading to vasodilatation, but also edema, and inflammation. The pKLK activity dysregulation incites edema formation, particularly in Hereditary Angioedema (HAE), a genetic and potentially life-threatening disease resulting from underexpression of C1-inhibitor (C1-INH) and resulting pKLK overactivity.

Existing therapies such as Berotralstat (Orladeyo), Lanadelumab (Takhzyro), and C1-INH supplement (Berinert, Haegarda) target pKLK through non-covalent mechanisms. Lunadelumab and C1-INH are administered only in HAE acute attacks in medical facilities, while Berotralstat requires frequent administration to sustain the therapeutic and prophylaxis effects. Taking this into account, these therapies are very cost-intensive and, moreover, in most cases, they cannot be delivered orally. Only Berotralstat is orally available, but due to its reversible inhibition, it requires daily intake to maintain efficacy.

A major limitation of current drugs is their reliance on reversible binding, which permits relatively rapid enzyme reactivation along with the drug elimination. Covalent inhibitors represent a highly promising alternative to overcome this**.** Covalent modulators form high-energy, irreversible bonds with the target, leading to sustained enzyme inactivation until protein resynthesis. That mechanism of action provides a potential for reduced dosing frequency, improved pharmacodynamics, and lower treatment costs. Despite growing interest in covalent inhibition for serine proteases, no covalent inhibitors of pKLK have been reported to date. However, evidence from related enzymes (e.g., KLK6, KLK5) suggests that carefully designed electrophilic warheads can engage catalytic serine residues selectively and effectively.

Although KLK active sites are generally conserved and often feature neutral or negatively charged surfaces, the pKLK binding pocket is unique in its positively charged region near the catalytic triad. In this project, we propose a new class of covalent inhibitors, designed to exploit this distinctive environment using covalent warheads tailored specifically for pKLK.

In the context of serine proteases, various covalent warheads have been investigated, especially groups based on boron or phosphorus atoms as electrophilic centers. Using innovative phosphorus- and silicon-based warheads designed by our team, we aim to introduce highly potent covalent inhibitors that selectively target pKLK by leveraging its specific microenvironment.

This project aims to provide a new class of compounds that may be used as therapeutics for HAE. Our innovative compounds offer sustained activity, reduced dosing frequency, and ultimately lower treatment costs. Moreover, the small-molecule nature of our inhibitors supports the development of orally available agents, offering more accessible options for prophylactic treatment.