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Ligand Directed Tyrosine Conjugation on Proteins using Imine Mannich Electrophiles

Cyclic imine Mannich electrophiles covalently target tyrosine and other residues for tunable covalent drug design.

Targeted covalent inhibitors (TCIs) are designed to bind and form covalent bonds with protein targets, offering benefits such as increased potency, selectivity, and prolonged modulation of target function. These advantages have enabled the inhibition of previously "undruggable" protein-protein interfaces (PPIs). Despite being an amino acid of low abundance, tyrosines are prevalent at PPIs and play crucial roles in non-covalent interactions. They are enriched in the histone binding pockets of epigenetic regulator proteins containing bromodomains and chromodomains, for which adequate inhibitor development has been challenging. Furthermore, the chemistry historically used to target tyrosines, primarily sulfonyl fluorides and fluorosulfonates, lacks selectivity and often reacts with other amino acids. No current chemistry is amenable for targeting tryptophan.

Purdue researchers have developed a strategy that utilizes two-component Mannich chemistry to modify amino acid side chains, such as tyrosines, tryptophans, and pyrrolated lysines, using various cyclic imines. This strategy has applications towards the formation of TCIs for tyrosines and other amino acids that work in vitro and within the cell. The developed cyclic imines were shown to be stable in aqueous conditions and demonstrated effectiveness as warheads in a CBX8 inhibitor model. This approach can have further applications for protein inhibition and regulation. The advantages of the strategy rely on the cyclic imine Mannich electrophile's interaction with specific amino acid side chains, inducing covalent modifications. These interactions can directly influence downstream effects that can lead to improved therapeutic outcomes.

Technology Validation:

- Liquid Chromatography-Mass Spectrometry (LC/MS) was used to monitor reactions of cyclic imine Mannich electrophiles with various aromatic groups

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within buffers and cells

- Reactions between cyclic imines and a tyrosine-containing peptides were performed

- Selectivity to other amino acids was tested via compounds mimicking amino acid side chain functional groups

- Covalent conjugation of inhibitors containing cyclic imines and the CBX8 binding proteins was confirmed by LC/MS analysis and was dependent on the presence of a particular tyrosine residue near the inhibitor binding site.

- Cellular activity of covalent, cyclic imine inhibitors to CBX8 was analyzed, monitoring gene expression in THP1 cells that treated with increasing dosages

Advantages:

- Targeted modification of aromatic amino acid sidechains

- Enhanced stability of cyclic imine derivatives in aqueous conditions

- Tunable reactivity of cyclic imine electrophiles

Applications:

- Drug development using targeted covalent inhibitors

- Modulation of protein activity within the cell

TRL: Pharmaceuticals

Intellectual Property:

Provisional-Patent, 2022-10-14, United States

PCT-Gov. Funding, 2023-10-11, WO

NATL-Patent, 2025-04-08, United States

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