Phenylthiomethyl Ketone-Based Fragments Show Selective and Irreversible Inhibition of Enteroviral 3C Proteases

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Abstract:

Lead structure discovery mainly focuses on the identification of noncovalently binding ligands. Covalent linkage, however, is an essential binding mechanism for a multitude of successfully marketed drugs, although discovered by serendipity in most cases. We present a concept for the design of fragments covalently binding to proteases. Covalent linkage enables fragment binding unrelated to affinity to shallow protein binding sites and at the same time allows differentiated targeted hit verification and binding location verification through mass spectrometry. We describe a systematic and rational computational approach for the identification of covalently binding fragments from compound collections inhibiting enteroviral 3C protease, a target with high therapeutic potential. By implementing reactive groups potentially forming covalent bonds as a chemical feature in our 3D pharmacophore methodology, covalent binders were discovered by high-throughput virtual screening. We present careful experimental validation of the virtual hits using enzymatic assays and mass spectrometry unraveling a novel, previously unknown irreversible inhibition of the 3C protease by phenylthiomethyl ketone-based fragments. Subsequent synthetic optimization through fragment growing and reactivity analysis against catalytic and noncatalytic cysteines revealed specific irreversible 3C protease inhibition.

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