Synthesis of PF-06928215 and analogues

Les Dakin

Abstract

Discovery of PF-06928215 as a high affinity inhibitor of cGAS enabled by a novel fluorescence polarization assay

Cyclic GMP-AMP synthase (cGAS) initiates the innate immune system in response to cytosolic dsDNA. After binding and activation from dsDNA, cGAS uses ATP and GTP to synthesize 2′, 3′-cGAMP (cGAMP), a cyclic dinucleotide second messenger with mixed 2′-5′ and 3′-5′ phosphodiester bonds. Inappropriate stimulation of cGAS has been implicated in autoimmune disease such as systemic lupus erythematosus, thus inhibition of cGAS may be of therapeutic benefit in some diseases; however, the size and polarity of the cGAS active site makes it a challenging target for the development of conventional substrate-competitive inhibitors. We report here the development of a high affinity (K_D = 200 nM) inhibitor from a low affinity fragment hit with supporting biochemical and structural data showing these molecules bind to the cGAS active site. We also report a new high throughput cGAS fluorescence polarization (FP)-based assay to enable the rapid identification and optimization of cGAS inhibitors. This FP assay uses Cy5-labelled cGAMP in combination with a novel high affinity monoclonal antibody that specifically recognizes cGAMP with no cross reactivity to cAMP, cGMP, ATP, or GTP. Given its role in the innate immune response, cGAS is a promising therapeutic target for autoinflammatory disease. Our results demonstrate its druggability, provide a high affinity tool compound, and establish a high throughput assay for the identification of next generation cGAS inhibitors.

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