# Drug Discovery Targeting GTP Metabolism for Cancer and Infectious Diseases Using X-Ray Crystallography And Cryo-EM

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GTP, an energy molecule in cells, is essential for various cellular functions, including protein synthesis. Cancer cells generally exhibit high levels of GTP due to their rapid proliferation and energy demands. Recent research suggests that cellular GTP levels are regulated independently of ATP levels and determine cellular activity, despite previous beliefs that GTP levels were regulated along with ATP levels. Our hypothesis regarding the existence of a cellular GTP sensor was confirmed through the identification of PI5P4Kβ as a GTP sensor (1, 2). PI5P4Kβ, a unique lipid kinase that prefers GTP to ATP, was found to be responsible for sensing GTP levels (1, 3, 4). Our xenograft experiment showed that cancer cells with a mutant PI5P4Kβ lacking GTP-sensing activity could not grow in mice, indicating the requirement of the GTP sensor for cancer growth. Another study showed that GTP metabolism is hijacked in cancer cells, leading to a significant increase in GTP biosynthesis to support cancer growth (5). Based on these findings, we propose that the regulation of GTP sensors and GTP biosynthetic activities could be a viable treatment option for cancer and infectious diseases that require high levels of GTP. To this end, we initiated a drug discovery study targeting PI5P4Kβ, a GTP sensor, and IMPDH2, a key enzyme in GTP biosynthesis.To identify PI5P4Kβ inhibitors, we performed a chemical screening of 1,600 FDA-approved compounds using a newly developed NMR method. Additionally, *in silico* screening was conducted using structural information on approximately 2,000,000 compounds. From these screenings, we selected several compounds and determined the crystal structures of PI5P4Kβ in complex with the compounds. Interestingly, we found two compounds that clearly bind to the PI5P4Kβ active site in different arrangements, with the two compounds sharing the position of one of the C-C bonds. This suggests that merged compounds may be more potent. We discovered two "merged" compounds with a $K_d$ of less than 1μM, and as expected, they inhibited the GTP hydrolyzing activity of PI5P4Kβ under cellular conditions. The crystal structure of PI5P4Kβ with the "merged" compound showed the compound occupying the active site. When applied to cells, these inhibitors resulted in the accumulation of the substrate of PI5P4Kβ, PI(5)P. Typical IMPDH inhibitors, such as mycophenolic acid, have been used clinically as immunosuppressive drugs due to their ability to deplete cellular GTP. However, since the cellular effect seems to be too strong, we aimed to develop allosteric inhibitors of IMPDH2 with milder effects on cells. Previous studies have shown that IMPDH2 has Bateman domains that allosterically regulate its activity. Based on the biochemical analysis, we performed an *in silico* screening of approximately 3,000,000 compounds targeting the Bateman domains to develop allosteric inhibitors. Of the 233 selected compounds, NMR screening revealed a few compounds with IMPDH inhibitory activity ($K_i$ ~ 10μM). Importantly, these compounds did not completely inhibit IMPDH2 activity, suggesting their role as allosteric inhibitors. The use of cryo-EM to determine the overall structure of the IMPDH2 inhibitor complex was necessary because the allosteric regulation of IMPDH2 changes its quaternary structure, making it difficult to determine the crystal structure of the IMPDH2 inhibitor complex by the soaking method. As expected, we obtained a structure of the inactive form (compressed form) of IMPDH2 when bound to a newly obtained compound (6). This result suggested that the compound binds to the Bateman domain, resulting in the compressed form. Cellular assays have been performed with these obtained inhibitors of PI5P4Kβ and IMPDH2. The results of the cellular assays will be presented.

*References*

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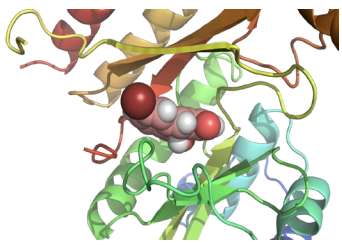

***Figure 1***