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BENG 196

Inhibitor Design

8 May, 2023

Structure-activity Relationship based design of Inhibitor Molecules for Disease Protein Targets

Recludix Pharma is focused on finding inhibitor drugs to inhibit the STAT (Signal Transducer and Activator of Transcription) proteins. STAT proteins are exciting, relevant targets because their activation is heavily involved in many autoimmune diseases and cancers. Some of these diseases include: multiple sclerosis, rheumatoid arthritis, and eczema (Cavaleri & Scholer, 2009). These diseases are heavily impacted by the activation of the STAT proteins; thus, inhibiting them would help reduce the progression of particular diseases. This would be life changing for patients. Currently there are drugs on the market for some of these diseases. However, these drugs target the Janus Kinase (JAK) proteins. Examples of these drugs include Baricitinib, Ruxolitinib, and Abruticitinib. As seen in Figure 1, the JAK proteins are farther up the signaling cascade than the STAT proteins.

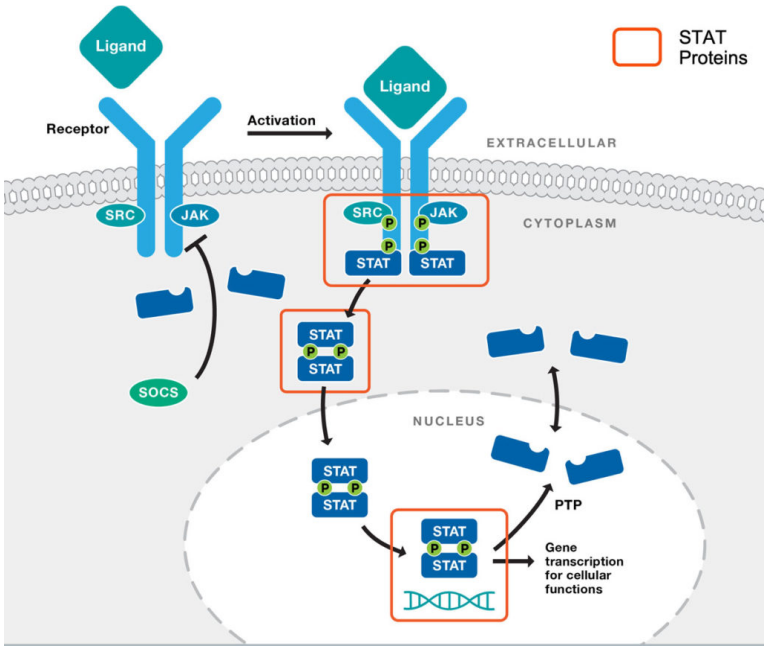

Figure 1: STAT Proteins Pathway of phosphorylation (*STAT Proteins Pathway*, Recludix)

This means that by inhibiting the JAKs all autoimmune signaling processes down the cascade will be inhibited as well. Therefore, if inhibited there is a greater risk of potential unwanted side effects. By inhibiting the JAK proteins many autoimmune functions will be eliminated which puts patients at risk for contracting another disease and having more problems fighting the disease off. Finding a drug that could instead inhibit the STATs would likely lead to less side effects because it is farther downstream. By discovering an inhibitor molecule that inhibits the STATs, Recludix would be able to bring a drug to market to treat diseases with hopes of less side effects compared to JAK inhibitors.

Clearly finding a molecule to inhibit the STATs is an exciting mission. But how does a drug discovery company go about this process? One key consideration when trying to find a potential lead chemical compound is the compound's potency. Potency refers to the ability of the compound to inhibit the target protein without needing a high concentration of said compound. It is determined by the affinity of the compound for the desired target as well as the efficacy of the drug. The more potent the compound is, the less of it will be needed in order to produce the desired inhibitory effects. It is important to find a potent inhibitor because allowing for less of the compound to be needed means that there is less risk of unwanted side effects. To aid in this process of finding a potent compound, my assigned project was to do weekly experiments, testing different compound's potency in order to determine which compounds had desirable features as well as which did not. This information is vital in the design and development of the inhibitor molecules.

The specific test I ran is known as a fluorescence polarization assay. The fluorescence polarization assay tests compounds designed by Recludix against desired STAT proteins. This assay is a competition binding assay. The fluorescent probe and small inhibitor molecule

compete to bind to the protein. If the small inhibitor molecule successfully displaces the labeled ligand molecules then the complex is small and has a faster rate of the tumbling. Fast tumbling leads to depolarized light and slow tumbling leads to polarized light (Fig. 2). The more depolarized light; the smaller the polarization (mP) value that will be reported when the plates get read under the scanner.

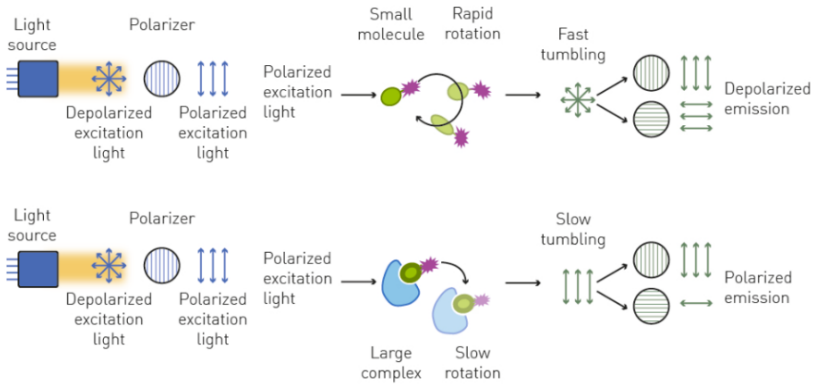

Figure 2: Polarized light will be depolarized due to presence of a small inhibitor molecule (BMG Labtech, n.d.)

The protocol for this fluorescent polarization assay is as follows: to start, I make buffers using protein and fluorescent probes. Next, I place compounds into the plate at different concentrations using a dispense machine. Then, I wait for the reaction between the fluorescent probes, protein, and compounds to take place. Finally, I put the plates through a Tecan spark which will analyze the fluorescence in the plate. Using this data, I am able to plot curves to compare potency between compounds. An example of what these curves could look like is shown below in Figure 3.

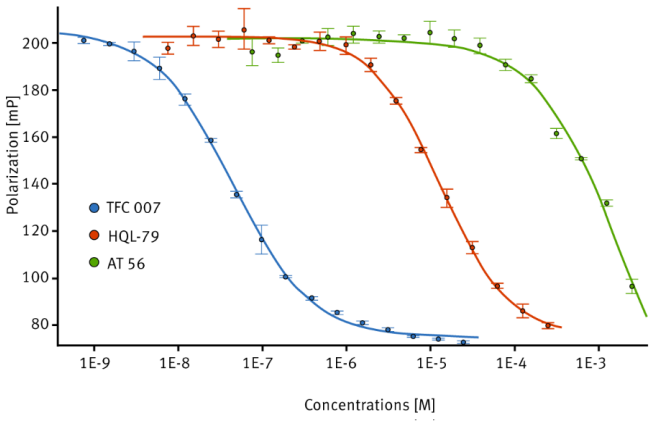

Figure 3: example plot of mP vs concentration of compound values from Fluorescence Polarization assay (BMG Labtech, n.d.)

As the concentration of the compound increases, the polarization (mP) value gets lower. Thus; the most potent compounds have lower mP values at smaller concentrations. This assay helps to determine which compounds should be scaled up to their high potency. Also; it is helpful to test lead compounds' potency against proteins that Recludix does not want it to interact with. If the compound is potent against other undesired proteins; we know that while the inhibitor molecule is potent, it is not selective. Continuing to run this assay every week with new compounds empowers the chemistry team with knowledge to inform their designing of the next set of inhibitor molecules.

References

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Cavaleri, F., & Scholer, H. (2009). Chapter 6 - Molecular Bases of Pluripotency. In *Essentials of Stem Cell Biology (Second Edition)* (second ed., 37,39-60). Science Direct. https://doi.org/10.1016/B978-0-12-374729-7.00006-8

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