Developing Metalloenzyme Inhibitor Library Compounds

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Background

- **Metalloenzymes:** A class of proteins containing metals in structural and catalytic roles
- Activation or misregulation can lead to various disease states: cancer, cardiovascular disease, infection, etc
- **Metal binding groups (MBGs):** Small organic molecules that can bind to metal ions in catalytic site
- **Objective:** Make better Metalloenzyme Inhibitors

Enzymes of Interest-

- HIV-Integrase
- Matrix Metalloproteinases
- Histone Deacetylaces
- Carbonic Anhydrase
- DNA Nicking and Religating Enzyme (NES)
- New-Delhi-beta-lactamase-1 (NDM-1)

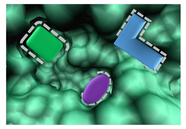
Fragment Based Lead Design

Fragment Library

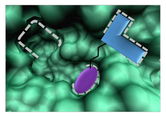
Biological Assays

Hits

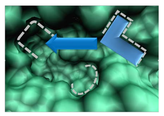
Structural Determination



Fragment Development



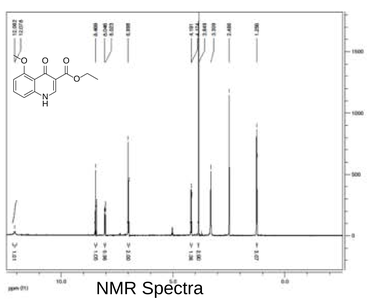
Linking



Growing/Expanding

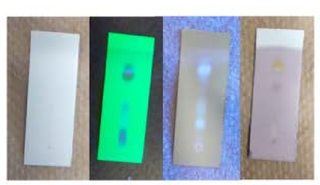
Merging

Instruments and Techniques



NMR Spectra

Auto Column: Silica Purification Column system

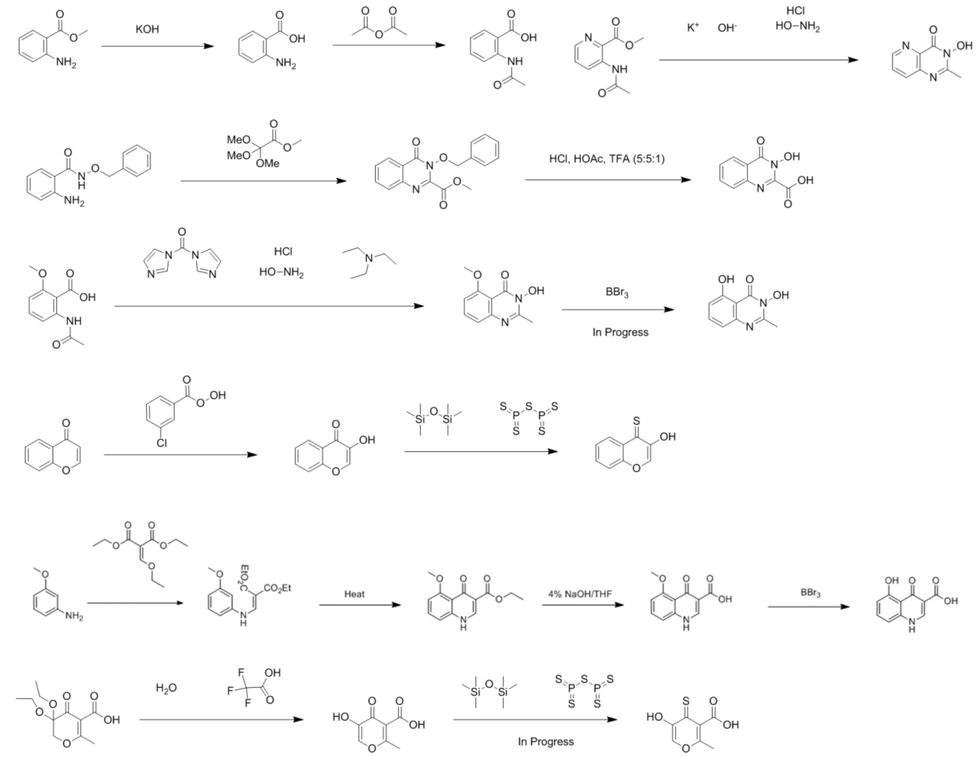


TLC Plates: Before, UV light, Black Light, Vanillin Stain

Rotovap: Rotary Vacuum Evaporation System

NMR Machine

Reactions We Did



Process

As we start the reactions, we measure out specific quantities of each molecule needed. Then we combine them and stir the reaction between several hours to overnight. To speed up some reactions, we put it in a heat bath or we put it in the microwave.

After it is done reacting, we check if the reaction is complete by doing a TLC (thin-layer chromatography). TLC is a chromatography technique used to separate polar substances from nonpolar substances. We can see by using TLC if the reaction is complete by spotting a little of the starting material and of the reaction on the plate. If the reaction is complete, we can do a silica purification column to separate the different molecules.

Before we do a column, we have to dry our reaction with silica on the rotovap. Rotovaps (rotary-evaporators) spin and heat reactions under vacuum to evaporate off solvents and leave solids. We load the dried reaction solids into the column and flush the column with different solvents. After the column, we combine the solvent that contains our product molecule and dry it. We then perform several molecular analysis techniques to check if we actually made our desired molecule, such as NMR and mass spectroscopy.

A NMR is a physical phenomenon in which hydrogen nuclei in a magnetic field absorb and re-emit electromagnetic radiation. Each different hydrogen in a molecule gives a characteristic signal peak, and tells us about the structure of our product molecule. A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio, and tells us the molecular weight of our product molecule.