Identification of natural products as inhibitors of bacterial histidine kinases

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Histidine kinases play a key role as regulator enzymes in two-component systems for bacterial signaling. By targeting the conserved ATP-binding domain of histidine kinases for inhibition, we hope to prevent these ubiquitous proteins from initiating crucial signaling cascades, ultimately limiting an organism's ability to cause infection.

We used a fluorescence polarization-based high-throughput screen to assess over 25,000 natural product extracts from marine-derived strains of *Streptomyces*. From this screen, 37 extracts demonstrated inhibition of the HK protein. Sixteen of those extracts had dose dependent activity and were chosen as viable leads. Currently, we are focused on the isolation and structure elucidation of active compounds from one of these strains. Due to the complexity of natural product extracts, extensive purification is required before studies to identify the compounds can begin. The isolation workflow involves resin-based extraction, fractionation on a C18 silica column, and reverse phase chromatography. A fluorescence activity assay is used to track the active compounds through each step.

Preliminary findings from this activity assay and mass spectrometry analysis indicate that there is a putative family of compounds that inhibit bacterial histidine kinases. Here, we present initial results focused on one of the active compounds, including accurate mass studies for molecular formula determination and MS/MS fragmentation and NMR studies for structure elucidation.

Further characterization will be required, including dose dependent studies of the active compound. With bacterial resistance to current antibiotics ever rising, inhibition of histidine kinases is a vital step towards the development of a new class of antibiotics with a novel mechanism of action.