Target Identification Studies for Two Novel Series of Rho-mediated Gene Transcription Inhibitors

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Oral Defense: March 23rd 2017; CCL 2548

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3rd year Medicinal Chemistry Departmental Seminar
Thursday, March 23rd, 2017; 4:00 PM

ABSTRACT:

Fibrosis is characterized by the excessive deposition of extracellular matrix (ECM) components. Pathological fibrosis is a devastating result of the improper regulation of the fibrotic process, and it contributes to ~45% of deaths in the developed world. Most major organs, including the heart, lungs, skin, liver, pancreas, and kidneys can be affected by fibrotic disease, ultimately contributing to its wide-spread prevalence. The build-up of collagen-rich scar tissue and the architectural distortion caused by scar retraction in epithelial organs contributes to the high mortality rate of diseases associated with fibrosis. Many of the stimuli for pathological fibrosis generation, as well as some of the underlying mechanisms that regulate fibrosis, remain unknown. A major hallmark of fibrosis is the fibroblast-to-myofibroblast transition (Fig. A). Myofibroblasts have an increased production of ECM components and impart contractile forces that cause architectural distortion to the surrounding tissue. These forces recruit signal transduction by transforming growth factor (TGF)β, lysophosphatidic acid (LPA), endothelin, and connective tissue growth factor (CTGF), ultimately activating the Rho family of GTPases and their downstream kinase, Rho-associated coiled-coil containing protein kinase (ROCK). This signaling leads to myocardin-related transcription factor (MRTF)/serum response factor (SRF)-mediate gene transcription of SRF-associated cytoskeletal genes, ultimately generating fibroblast differentiated myofibroblast cells. Since Rho/MRTF/SRF signaling is known to regulate pro-fibrotic gene expression arising from multiple extracellular signaling pathways, inhibition of this common pathway is hypothesized to be a particularly effective way to treat/prevent pathological fibrosis.

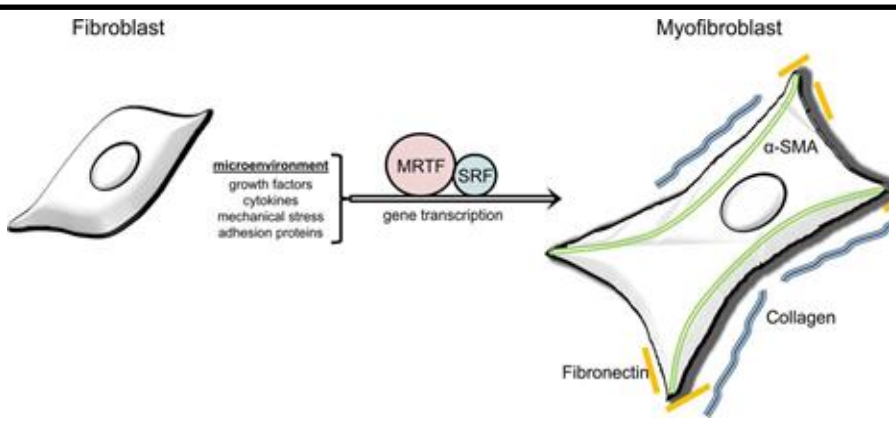

Figure A: **The fibroblast to myofibroblast transition.**

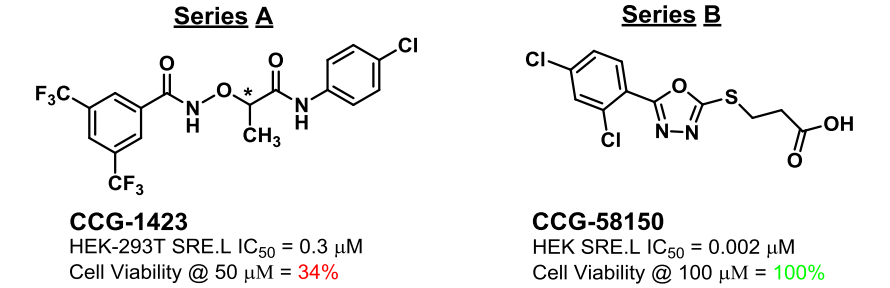

Figure B: **Novel hits from a phenotypic high-throughput screen of Rho/MRTF/SRF-mediated gene expression inhibitors**

Two distinct series of novel inhibitors of Rho/MRTF/SRF-mediated gene expression were identified by the Larsen and Neubig labs through a phenotypic high-throughput screening campaign (Fig. B). Both series have produced inhibitors that show promising results in multiple *in vitro* and *in vivo* models of fibrosis. Due to the phenotypic nature of the assay, the target(s) for the two series are unknown. Recently, potential biological targets for both series have been identified through proteomics studies using immobilized agarose resin high affinity pull-down probes. Pirin was identified for the less potent **Series A** and dCTPase for the more potent **Series B**. Validation efforts for both targets are currently underway in a collaborative effort between the Neubig, Larsen, and Martin labs. Future endeavors for this arm of the project include validating the molecular target(s) for each series in hopes to uncover potentially new targets that block mechanisms involved in the progression and maintenance of pathological fibrosis. Also, revealing the molecular targets will help facilitate clearly defining the SAR for each series, ultimately producing two novel classes of small molecule inhibitors that can potentially be used to treat fibrotic diseases. To this extent, efforts to develop derivatives of both series that improve potency, selectivity, and absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties are currently underway.