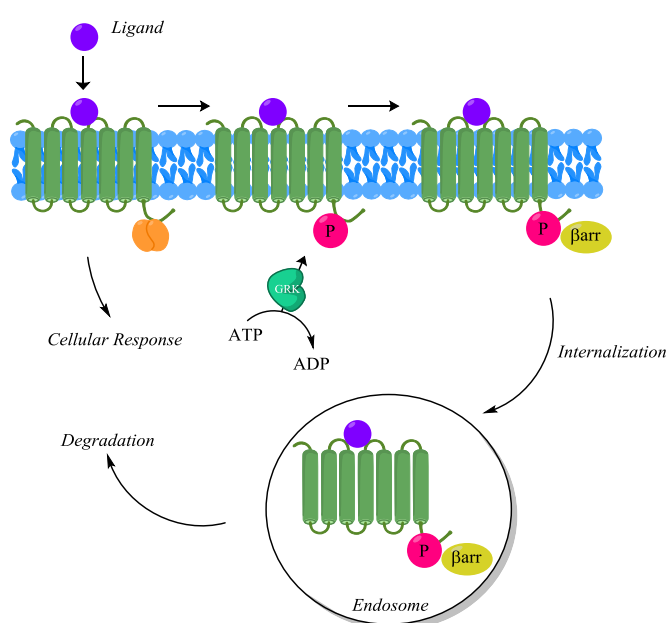


# Development of Selective, Covalent Inhibitors of GRK5 for Treatment of Heart Failure

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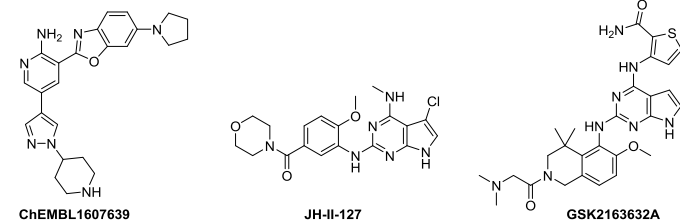
Despite the rising world-wide life-span, incidences of heart failure (HF) have markedly risen within the past ten years. According to the American Heart Association there were 915,000 new cases of HF in 2016. HF is characterized by the overstimulation of  $\beta$ -adrenergic receptors ( $\beta$ ARs) and other GPCRs by circulating catecholamines. Over-activation of  $\beta$ ARs results in their desensitization through internalization, a process initiated by GRKs. Internalization of receptors, in turn, increases the threshold of catecholamines needed to stimulate a response within the failing heart (Figure 1). Current treatments for cardiovascular diseases focus on reduction of angiotensin II production or blocking the over-activation of adrenergic receptors. However, long term use of these drugs is associated with arrhythmias. Studies suggest that inhibition of GRK2 and GRK5, the most abundant GRKs within cardiac tissue, will increase receptor responsiveness and attenuate hypertrophy in HF.

Previously, we have shown that selective inhibition of GRK2 by a small molecule inhibitor improves cardiac function. GRK5 siRNA knockout demonstrates a similar utility, but thus far there has only been one reported GRK5 selective probe (**ChEMBL1607639**, 7-fold selective for GRK5 over GRK2). A validated chemical probe selective for GRK5 would thus be an important tool for investigating the role of GRK5 in HF and other cellular processes.

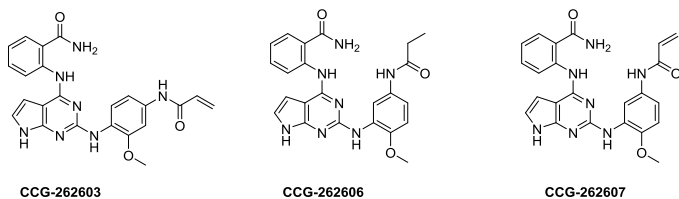


**Figure 1.** Desensitization of GPCRs by internalization catalyzed by GRKs. Internalization leads to weaker contractility of cardiomyocytes, exacerbating decline of heart function.

Initial Leads:



Previously Developed Leads from Tesmer Lab:



**Figure 2.** Initial leads for GRK5 inhibitor campaign.

In parallel, we have begun synthesis of **ChEMBL1607639** to validate its reported activity. From our initial modeling efforts, we assume that **ChEMBL1607639** can be weaponized at the terminal piperidine to interact with Cys474 on the AST. Currently, we plan to make various linkers, both flexible and rigid, to reach the optimal linker length to engage GRK5. Once weaponized, we plan to submit our compounds in complex with GRK5 to ESI-MS to determine whether these weaponized compounds can truly form the covalent interaction we desire. From this project, we expect to develop two classes of selective, covalent inhibitors for GRK5.

Targeting the non-conserved cysteine, Cys474, on the active site tether (AST), a piece of structural architecture unique to GRK5, chemical probes can be modified to form covalent interactions. We prepared a homology model of GRK5 and GRK6 to find new chemical matter that would be amenable to covalent modification. Using leads discovered in the virtual screen, two series were developed to engage GRK5 in a covalent interaction (Figure 2). Previous work on **CCG-262603** demonstrated this scaffold can be modified to interact covalently with GRK5. It was demonstrated that homologation of the warhead linker is synthetically less tractable than the direct linkage. To make this scaffold more tractable, the lead was modified to remove functionalities that introduce high lipophilicity and promote intramolecular cyclization. From these changes, we were able to prepare **CCG-264018**, a modified version of a literature compound, JH-II-127. Reduction of the terminal amide on **CCG-264018** is planned to access our previously planned homologated amine linker.