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Utilizing a structure-based docking approach to develop potent G protein-coupled receptor kinase (GRK) 2 and 5 inhibitors

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ABSTRACT

G protein-coupled receptor (GPCR) kinases (GRKs) regulate the desensitization and internalization of GPCRs. Two of these, GRK2 and GRK5, are upregulated in heart failure and are promising targets for heart failure treatment. Although there have been several reports of potent and selective inhibitors of GRK2 there are few for GRK5. Herein, we describe a ligand docking approach utilizing the crystal structures of the GRK2–Gβγ·GSK180736A and GRK5·CCG215022 complexes to search for amide substituents predicted to confer GRK2 and/or GRK5 potency and selectivity. From this campaign, we successfully generated two new potent GRK5 inhibitors, although neither exhibited selectivity over GRK2.

G protein-coupled receptor (GPCR) kinases (GRKs) regulate the largest class of membrane receptors in the human genome via phosphorylation of receptor C-terminal tails or cytoplasmic loops, and are also implicated in several disease states.[1,2] During heart failure, levels of GRK2 and GRK5 are elevated in many tissues.[3–5] In the heart, this upregulation leads to increased desensitization and uncoupling of the GPCRs located in the heart such as the β-adrenergic and angiotensin II receptors, which regulate contractility and blood flow to the body, respectively.[6,7] Knockdown of either GRK2 or GRK5 in mice subjected to transverse aortic constriction showed cardio-protective effects.[8,9]

GRK2 and GRK5 also affect non-GPCR pathways that further mediate stress responses in the heart.[10–13] GRK2 influences cardiac glucose uptake leading to abnormal cardiac metabolism when upregulated, altering the growth of new cardiomyocytes.[10] Uniquely, GRK5 is the only GRK known to be targeted to the cell nuclei of cardiomyocytes[14,15] where it acts as a histone deacetylase (HDAC) kinase.[11] Phosphorylation of HDAC5 leads to increased expression of myocyte enhancer factor-2 which regulates the stress response in hypertrophy.[16,17] Due to these GPCR-independent roles, GRK2 and GRK5 represent promising targets that offer unique therapeutic outcomes that cannot be attained by current heart failure treatments that directly target GPCRs or angiotensin-converting enzyme.

GRK2 and GRK5 have also been implicated in other medical conditions. Elevated levels of cytosolic GRK2/5 have been implicated in Alzheimer's and Parkinson's disease.[18–20] GRK5 has additionally been shown to regulate tumor growth progression in several cancers.[20–23] Also of considerable interest are the roles of GRK2 and GRK5 in cell growth and insulin levels leading to diabetes.[24,25] Thus, chemical probes targeting either GRK2 or GRK5 (or potentially both), would be useful as tools to investigate the roles of GRK2 and GRK5 in cells and human disease.

There are several reported GRK2 and GRK5 inhibitors (Fig. 1). Compound **1o** has a GRK2 IC_{50} of 460 nM but is ~10-fold more potent for GRK5 (IC_{50} of 59 nM).[26] Thus it is one of the most potent GRK5 inhibitors reported to date and one of the few that exhibits some selectivity for GRK5 over GRK2. Limiting its usefulness is its ability to inhibit tyrosine kinases (IC_{50} for c-Met, 8 nM and IC_{50} for anaplastic lymphoma kinase, 0.3 μM).[26,27] Previously, our lab identified compound **GSK180736A** as a GRK2-selective inhibitor. Further development of this scaffold led us to **CCG215022**, which potently inhibits both GRK2 and 5 (Fig. 1).[12,13]

The parent compound **GSK180736A** was co-crystallized with GRK2–Gβγ (PDB entry 4PNK)[28] whereas **CCG215022** was co-crystallized with GRK5 (PDB entry 4WNK), allowing the use of

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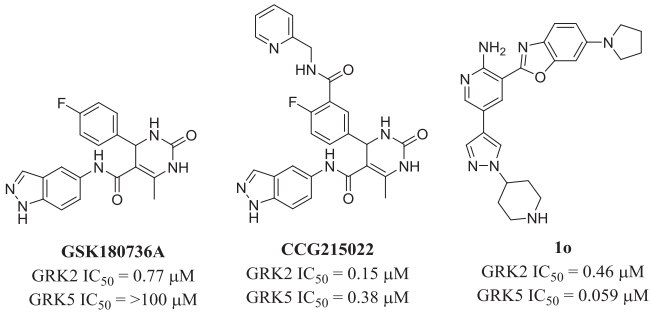

Fig. 1. Reported small molecule GRK inhibitors. GSK180736A and CCG215022 were previously reported by our lab using a radioactivity assay. Compound 1o was reported by Cho et. al. and its IC_{50} values were determined using a time-resolved fluorescence resonance energy transfer assay. Due to different assay conditions the values may not be directly comparable.

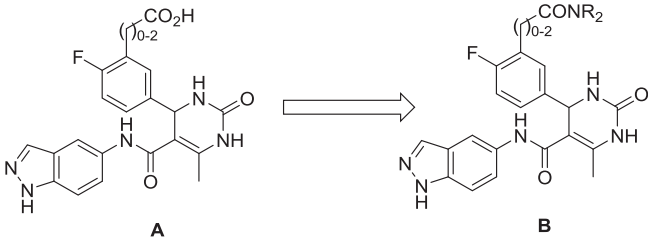

Fig. 3. Virtual library of amides generated from GSK180736A carboxylic acid homologs.

structure-guided drug design to develop potent GRK5 inhibitors.[29] The two compounds bind similarly in the kinase active sites of GRK2 and GRK5, respectively (Fig. 2). The indazole forms two hydrogen bonds to backbone atoms in the hinge of the kinase, the dihydropyrimidinone sits in the ribose subsite, and the fluorophenyl ring packs under the P-loop. The amide-linked pyridine extension of **CCG215022** forms additional hydrogen bonds via its amide with the P-loop and via the pyridine nitrogen with Lys220 and Asp329.[28,29] We hypothesized that we could use these two crystal structures and molecular modeling to design new compounds *in silico* with improved selectivity and potency for either GRK2 or GRK5.

Enumeration of virtual compounds and docking of those compounds was conducted using the computational chemistry package MOE.[30] Our campaign began with an extensive virtual screen using a library of commercially available amines from Sigma Aldrich. Virtual compounds (amides **B**, Fig. 3) were enumerated based on the **GSK180736A** template bearing homologous carboxylic acids off the fluorophenyl ring (**A)** (Fig. 3). The three acid scaffolds **A** were combined with primary and secondary amines (R_2NH) with molecular weights less than 215 g/mol. Initially nearly 15,000 amine structures were retrieved. After removal of amines containing expected reactive or mutagenic chemical motifs by MOE, the number of structures dropped to just over 11,000. The resulting amide compounds **B** were then filtered by a molecular-weight cut off of 550 to give 9183 virtual unique amide-linked structures. We chose a slightly higher molecular weight cut-off of 550 to allow for more diversity of appendages in our larger ethylene linked scaffold which already had a molecular weight of 437 g/mol.

A pharmacophore model was generated based on the ligand orientations in the GRK2–Gβγ·**GSK180736A** and GRK5·**CCG215022** crystal structures. The model restricted the docked ligands to be in an orientation similar to what was seen in the respective crystal structures. As represented in Fig. 4, the center of the indazole and the fluorophenyl rings were constrained by a spherical volume indicated by the green circles in Fig. 4 (radii: 1.8 Å for the indazole rings and 2.5 Å for the fluorophenyl). The two nitrogens of the indazole that form hydrogen bonds with the hinge of the kinase domain were constrained to a spherical volume with a radii of 1.8 Å (purple and cyan circles in Fig. 4). The goal was to allow these motifs to move within a constrained volume of the active site such that new amide substituents would necessarily be projected into the hydrophobic subsite where they could pick up additional interactions to increase potency of the molecules.

The virtual compounds were then docked into ligand-free crystal structures of GRK2–Gβγ and GRK5 using a rigid model of the protein and a flexible ligand model that obeyed the constraints of the pharmacophore model. The results were sorted by highest docking scores (S), where a lower number correlates with tighter binding, and then further divided into three groups. The first group contained compounds predicted to be both potent and selective for GRK2 (S score for GRK2 2 units lower than that of GRK5). The second group contained compounds that were predicted to be both potent and selective for GRK5 (S score for GRK5 2 units lower than that of GRK2). The third group encompasses compounds that were predicted to be equipotent for both GRK2 and GRK5 (having S scores within 2 units of each other) and that had good docking scores. After filtering out any diamines, carboxylic acids, or other reactive, unstable, or toxic motifs that were missed in the

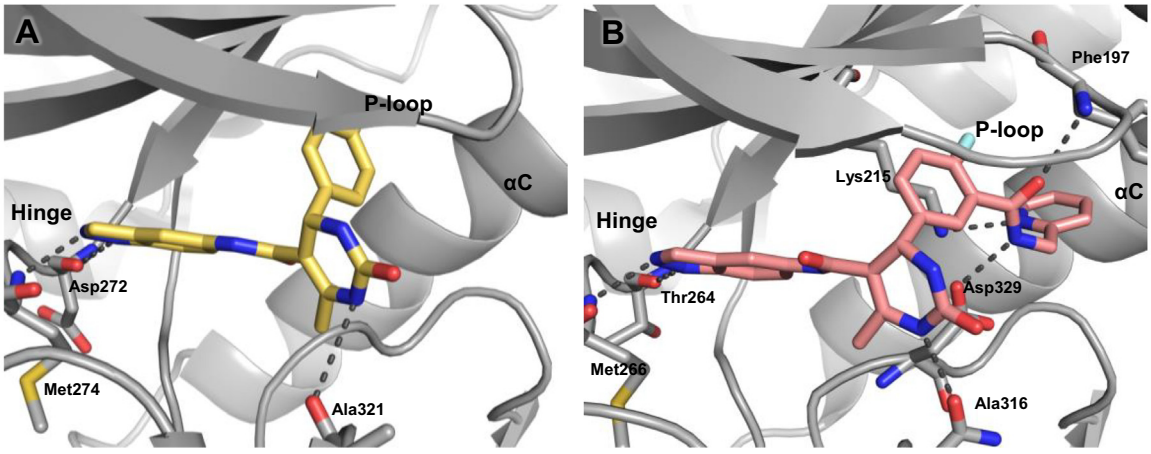

Fig. 2. Comparison of the A) GRK2–Gβγ·GSK180736A (4PNK) and B) GRK5·CCG215022 (4WNK) crystal structures utilized in the docking campaign. GSK180736A is drawn with yellow carbons, CCG215022 is drawn with salmon carbons, and H-bonds are shown as dark grey dashed lines.

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