

PART 1. THE DEVELOPMENT OF NON-SECOSTEROIDAL VITAMIN D RECEPTOR MODULATORS

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The vitamin D receptor is a nuclear hormone receptor that regulates cell proliferation, cell differentiation, calcium homeostasis and immunomodulation. The receptor is activated by the vitamin D metabolite, 1,25-dihydroxyvitamin D₃, which induces a cascade of events including the recruitment of coactivators that activate transcription of specific VDR target genes. Thousands of VDR agonists have been synthesized based on the secosteroid scaffold of 1,25-dihydroxyvitamin D₃. However, most of these ligands are metabolically unstable, have sub-optimal drug-like properties, and induce hypercalcemia *in vivo*. The limited numbers of VDR antagonists reported bear the same secosteroid scaffold and thus exhibit similar problems encountered by VDR agonists. VDR has been implicated with many diseases including cancer, allergies, sarcoidosis and autoimmune diseases like Crohn's disease. Therefore, the synthesis and biochemical evaluation of novel, non-secosteroidal modulators for VDR were developed and reported herein.

First, VDR inhibitors were rationally designed to directly target the interactions between VDR and coactivator SRC2. A fluorescence polarization-based assay evaluated the binding of these molecules. Next, a high throughput screening campaign with the NIH chemical and genomics center (NCGC) identified GW0742 as a novel VDR antagonist. Originally developed by GlaxoSmithKline as a selective PPAR δ agonist, GW0742 was used as a scaffold for the synthesis of VDR inhibitors with decoupled activity towards PPAR δ . Biochemical, cell-based, solubility and permeability assays determined drug-like properties of over 100 GW0742 analogs. Finally, secondary bile acids, which are known to bind VDR and modulate transcription without inducing hypercalcemia, lead to a study of phase 1 and phase 2 metabolites of lithocholic acid. In addition to biochemical and cell-based assays, a semi-quantitative real time polymerase chain reaction was used to confirm the ability of lithocholic acid derivatives to induce the transcription of VDR target genes.

PART 2. THE DEVELOPMENT OF A UNIVERSAL GTPase ASSAY

GTPases act as a molecular switches in which their "on" and "off" functions are triggered by the binding and hydrolysis of GTP. Due to their relationship to many diseases, numerous GTPase targeting drugs have been developed. One third of all drugs targeting proteins are either interacting with kinases (22% of drugs) or GTPases (15% of drugs). The growing interest in GTPase targeting drugs has promoted the development of assays that can efficiently test these compounds in a high throughput and inexpensive way. AviMed Pharmaceuticals, LLC, a local company founded by Dr. Daniel Sem, pursued the development a universal kinase/GTPase assay kit that would be affordable and commercially available for industry and research labs to test potential drug candidates. The assay designed was based on previous research conducted by the founder and relies on a beta thiol substituted ATP (GTP for GTPases) that would be enzymatically hydrolyze to produce ADP (GDP). The exposure of the thiol makes it nucleophilic and reactive towards thiol-sensitive fluorescent or calorimetric reagents such as Thiofluor 623. Herein, we report the synthesis of the assay reagents and the preliminary development of a universal, inexpensive, sensitive GTPase assay kit that directly detects the GTP β -S hydrolysis product, GDP β -Se.