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ABSTRACT
CHARACTERIZING SUBSTITUTED IMIDAZOLIDINETRIONES AS A NOVEL CLASS OF PYRUVATE CARBOXYLASE INHIBITORS

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Pyruvate carboxylase (PC) is implicated in a variety of diseases, including type-2 diabetes, cancer, and bacterial/viral infections. However, there are currently no molecular tools capable of precisely manipulating PC activity both *in vitro* and *in vivo*. This dissertation describes the identification and characterization of 1,3-disubstituted imidazolidinetriones as a novel class of potent, selective, and permeable allosteric inhibitors of *Staphylococcus aureus* PC. Based on kinetic, structural, and biophysical data, this class of inhibitors is hypothesized to bind at the noncatalytic "exo binding" site on PC. This exo binding site is reportedly important for catalysis, but it had not previously been considered a druggable site. This dissertation also demonstrates that allosterically activated PC is significantly less sensitive to small molecule inhibition compared to nonactivated PC. This discovery raises an important new consideration for the development of small molecule inhibitors targeting human PC; since human PC requires activation by acetyl-CoA for catalytic activity, future drug discovery efforts must be performed against allosterically activated forms of PC. Finally, *in vitro* evidence is presented to refute the recent claims that two natural products, erianin and anemoside B4, are inhibitors of human PC. This dissertation presents a strategic framework to advance drug discovery targeting human PC. It outlines optimized screening procedures and explores possible avenues for the identification of inhibitors of activated human PC. Overall, this work significantly advances the development of chemical probes that target human PC, and ultimately helps to expand the available toolkit used to study the role of PC in disease.

In Progress