HIGH-THROUGHPUT SCREENING STUDIES OF INHIBITION OF HUMAN CARBONIC ANHYDRASE II AND BACTERIAL FLAGELLA ANTIMICROBIAL ACTIVITY

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Carbonic anhydrase (CA) enzymes catalyze the reversible hydration of carbon dioxide to form bicarbonate and release a proton. There are currently five known CA structural families, α–, β–, γ–, δ–, and ζ–classes, as reviewed in Chapter One.

In Chapter Two, the hypothesis that a high-throughput screen (HTS) of diverse biologically active compounds could yield new structural insights into CA inhibition and identify new inhibitors for this enzyme was experimentally tested. Human CA II was screened against 960 structurally diverse, biologically active small molecules. The assay monitored CA II esterase activity against the substrate 4-nitrophenyl acetate (4-NPA) in a format allowing high-throughput screening. The assay proved to be robust and reproducible with a hit rate of <2%.

Chapter Three examines the functional mechanism of thioxolone. Analytical chemistry and biochemical methods were used to investigate the fate of thioxolone upon binding to CA II. When thioxolone binds in the active site of CA II it is cleaved and forms 4-mercaptobenzene-1,3-diol which then binds to the zinc active site via the thiol group, and is therefore the active CA inhibitor.

The preliminary development and validation of a novel methodology for the

high-throughput screening of antimicrobial compounds and inhibitors of bacterial motility is described in Chapter Four. Two basic techniques were combined to enable rapid screening for motility inhibitors; the classical bacterial swarming agar motility assay and the use of 96-well microplates common to HTS drug discovery techniques with a standard absorbance microplate reader. The feasibility of screening the *Salmonella typhimurium* SJW1103 strain, which is wild-type for flagellar motility and has peritrichously arranged flagella, was examined.

The non-physiological substrate, 4-nitrophenyl acetate, does not react with all known isozymes of α–class CAs or the γ–class CA from *M. thermophila.* Preliminary studies describing the subcloning and expression CA I and a truncated, soluble form of CA IV, are described in Chapter Five. This chapter also details the preliminary exploration of several alternative pH absorbance assays for measuring the catalytic activity of CAs. Finally, some possible directions for future research, based on the experimental results presented in Chapters 2-5, are discussed in Chapter Six.