ABSTRACT

Nitric Oxide (NO) is a gaseous signalling molecule that is produced by virtually all organisms, from bacteria to plants and animal cells. NO plays a crucial role in many biological processes. However, an excessive production of this molecule can be detrimental, contributing to the development of many pathological conditions. NO is synthesised from L-arginine via a reaction catalysed by the enzyme nitric oxide synthase (NOS). The activity of NOS is inhibited by the methylated arginines asymmetric dimethylarginine (ADMA) and monomethyl arginine (L-NMMA), that act as endogenous competitive inhibitors of all three isoforms of NOS (nNOS, eNOS, iNOS). Both ADMA and L-NMMA are metabolised by the cytosolic enzyme dimethylarginine dimethylaminohydrolase 1 (*h*DDAH1) into L-citrulline (CIT) and dimethylamine (DMA) or monomethylamine (MMA), respectively. Two isoforms of DDAH have been reported in humans, identified as DDAH1 and dimethylarginine dimethylaminohydrolase 2 (DDAH2), but DDAH1 appears to be the major player in methylated arginine metabolism. Thus, *h*DDAH1 is a crucial NO modulator and inhibition of *h*DDAH1 activity results in the accumulation of ADMA and L-NMMA and the consequent inhibition of NOS. Furthermore, enhanced *h*DDAH1 expression has been linked to several pathophysiological conditions and there is increasing evidence demonstrating its role as an emerging therapeutic target to excessive NO production.

In this research project, the potential of seven novel compounds to act as *h*DDAH1 inhibitors was measured; six of these molecules identified through Artificial Intelligence (AI) and one compound specifically designed within our lab using the known *h*DDAH1 inhibitor ZST316 as a template. A robust Ultra-Performance Liquid Chromatography – coupled to Mass Spectrometry (UPLC-MS) based *h*DDAH1 activity assay was also developed and used to measure *h*DDAH1 catalysed CIT formation. Method validation included the determination of instrument and method precision, intra- and inter-day variability and accuracy, robustness,

linearity, and lower limit of detection (LLOD) and quantification (LLOQ). The assay was also investigated for protein and time linearity and the model that best describe the enzyme kinetics identified, followed by screening of the inhibitory potential of seven novel compounds. This research proposal hypothesised that at least one of the seven screened compounds would act as a *h*DDAH1 inhibitor with a constant of inhibition (K_i) <10 μM and/or half maximal inhibitory concentration (IC_{50}) <20 μM. The method developed was robust, reproducible, precise (%CV < 11%), and accurate (accuracy between 101 and 105 %). LLOD was 0.4 μM, LLOQ was 1 μM and calibration curves were always linear. A linear relationship was observed between the concentration of CIT formed and total protein concentration and incubation time and the Michaelis Menten model best fit the enzymatic system, with K_m of 112 ± 9 μM and V_{max} of 839 ± 23 pmol*min^{-1}*mg^{-1}. According to the findings, none of the screened compounds designed by AI acted as a *h*DDAH1 inhibitor, however the compound designed within the Clinical Pharmacology lab, ZIR26, has exhibited an estimated IC_{50} comprised between 1 and 10 μM. This supports the hypothesis and should be explored further in future experiments. Future perspectives include the screening of the remaining 64 potential inhibitor identified by AI, full kinetic characterisation of novel inhibitors and investigation of *h*DDAH1 inhibition in cellular and animal models of diseases characterised by excessive NO synthesis.