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SCREENING FOR SMALL MOLECULE REGULATORS OF THE CREATINE TRANSPORTER (SLC6A8)

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Background and aims: Impaired cardiac energetics including low creatine levels, have been implicated in the pathophysiology of ischaemia and heart failure, but are not yet a target for current therapeutics. We therefore aimed to identify novel activators of the creatine transporter (CrT) by screening an 'annotated' compound library where potency at human primary targets is already known.

Methods and Results: A high-throughput luciferase screen was established in HEK293 cells stably expressing a reporter vector consisting 3.2kb of the human CrT promoter driving the Firefly luciferase (FLuc) gene. Assay validation using min-max plates of DMSO and positive controls gave a workable RZ' of 0.47. For primary screening, 0.6x10E5 cells/well were plated onto 96-well plates containing 1uM each of 13,000 compounds and luminescence read after 24 hours. Fluorescent signal from resazurin (3.125 ug/ml) supplemented in cell media was used to normalise to cell number. False positives were eliminated if they also increased CMV-FLuc signal in a counterscreen artefact assay. We identified 436 positive hits vs DMSO (RZ'=0.599), of which, 142 passed the artefact assay and were tested in an 8-point concentration response curve (0.003 to 30 uM; RZ'=0.67). In total, 53 active compounds were identified, and gene target enrichment revealed activity at two known biological pathways.

Conclusions and future work: Our initial screen has identified compounds with activity at physiologically plausible targets. Secondary assays are ongoing to confirm transcriptional activation, cellular creatine accumulation, and to screen structurally related compounds towards a new translational tool targeting the CrT.