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Repositioning existing drugs as modulators of PI5P 4-kinases

D. D. Yang[1], T. Rahman[2], J. H. Clarke[2], R. F. Irvine[2]. [1]University of Cambridge, Cambridge, United Kingdom, [2]Department of Pharmacology, University of Cambridge, Cambridge, United Kingdom

INTRODUCTION The functions of phosphatidylinositol 5-phosphate 4-kinases in human physiology remain elusive, although there is evidence supporting their involvement in cellular stress responses and metabolic control. Specific inhibitors for PI5P4Ks would serve as invaluable tools for studying these enzymes, and would also provide lead compounds for potential therapies. Here, we tested potential inhibitors of the PI5P4Kγ+ isoform, which were identified by *in silico* screening of a library of approved drugs using a known inhibitor, I-OMe-tyrphostin AG 538, as bait. We also tested the resistance of the +2IN(4,5) and +2IN(2,3) mutant PI5P4Kγ+ isoforms to Inhibitor 1, another known inhibitor.

METHOD Protein expression and purification were used to make recombinant PI5P4K protein. Lipid kinase assays using recombinant enzymes and [γ-^{32}P]-ATP were used to determine enzyme activity.

RESULTS No inhibitory effect on PI5P4Kγ+ activity was found with ifenprodil, carbidopa, or tolnaftate; ifenprodil showed a small stimulatory effect. +2IN(4,5) was more resistant to inhibition by Inhibitor 1 than PI5P4Kγ+. Both +2IN(4,5) and +2IN(2,3) demonstrated greater enzymatic activity than PI5P4Kγ+.

CONCLUSIONS Refinement of the screening process, screening with a different known inhibitor compound as bait, or screening of a different library of compounds, may be necessary to identify more inhibitors of PI5P4Ks. The characterisation of +2IN(4,5) and +2IN(2,3) demonstrates that mutation of the putative inhibitor-binding amino acid residues in PI5P4Kγ+ can make the enzyme more resistant to inhibition and/or increase activity. This also further validates the use of systematic, sequential mutagenesis to investigate the roles of specific residues.