Development of inhibitors to the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO1) for cancer therapy: II. New, sensitive IDO1 assay for high throughput screening of compound libraries for novel small molecule inhibitors of IDO1

Tomek, Petr[1], Palmer, Brian D.[1], Flanagan, Jack U.[1] and Ching, Lai-Ming[1]

[1] *Auckland Cancer Society Research Centre, the University of Auckland, Auckland, New Zealand*

IDO1 has been established to suppress the immune system and promote tumour growth. It has been found in a broad range of human cancers and its overexpression is associated with poor prognosis for patients. Small-molecule inhibitors of IDO1 have demonstrated significant survival benefit in animal models of cancer and two compounds are currently in human clinical trials. We have initiated a programme at the Auckland Cancer Society Research Centre to identify and develop novel IDO1 inhibitors for the treatment of cancer.
As part of this endeavour, we firstly developed a new fluorescence assay for detection of IDO1 activity based on a chemical reaction, not previously documented, between N-formylkynurenine (NFK) and amine piperidine. The assay is 30 times more sensitive than previously described microplate assays (limit of detection 153 nM NFK), and we have subsequently miniaturized it and automated for use in JANUS robotic workstation. This enabled us to perform rapid screening of large numbers of compounds for IDO1 inhibitory activity.
The National Cancer Institute Diversity Set Ill library of 1597 compounds was screened and 4 novel IDO1 inhibitors were found. Subsequent structure-activity studies of these 4 inhibitors identified analogues exhibiting IC_{50} <500 nM. High-throughput screening of 40,000 compounds from the WEHI library identified 28 hits that inhibited IDO1. The three most potent compounds (IC_{50} 1-5 µM) were further characterized as potential hits for drug development. All three IDO1 inhibitors exhibited tight-binding, reversible mode of inhibition that has not been previously described for known IDO1 inhibitors. All three hits could inhibit IDO1 activity in cells at concentrations that had no effect on cell viability. To date, we have synthesised >100 structural analogues, some of which have greatly improved potency with IC_{50} values <10 nM for inhibition of IDO1 enzymatic activity.