High-throughput screening for IRF4 inhibitors

S. Stevens, J. Lopez Fernandez. Newcastle University, Newcastle upon Tyne, United Kingdom.

Introduction Multiple myeloma currently has no cure.[1] If the expression of interferon regulatory factor 4 (IRF4) is switched off, using small hairpin RNAs (shRNAs), then this is toxic for the cells of MM.[1] Furthermore, a study showed that there is a therapeutic window when inhibiting IRF4 for the treatment of MM.[2] As a result of these findings, an inhibitor of IRF4 has the potential to cure MM with minimal side effects.

Method Starting from a high throughput screen, 150 000 compounds were screened resulting in 280 hits targeting Interferon Regulatory Factor 4 (IRF4). These hit compounds were filtered down and the most promising hit, pyridazinone **1** (Figure 1) was chosen for synthetic studies. Analogues of **1** were synthesised that would provide information regarding the Structure- Activity Relationships (SARs) around this scaffold. Work focussed on either the aromatic system bound to the N1 of the pyridazinone heterocycle mainly by a deletion approach or by changing the pyridazinone heterocycle itself. Due to the poor solubility of the compounds even in DMSO, work also focused on improving the solubility as well as the potency of these compounds. A further set of analogues included the acid functionality in the *para* position and to put groups in the *ortho* or *meta* position. Various validation experiments were carried out for **1** in addition to the FP assay including isothermal titration calorimetry (ITC) experiments, NMR studies and protein crystallography. Fig. 1: Schematic of the FP HTS.

Results Analogues of **1** showed, at most, the same level of potency in the Fluoresence Polarisation (FP) assay. The ITC experiments, NMR studies and protein crystallography all carried out were either inconclusive or did not showing any binding of **1** to the IRF4 protein or IRF4 DBD.

Conclusions The lack of conclusive biophysical data for **1** and without being able to gain potency through synthesis of analogues, further investigation into **1** as an IRF4 DBD inhibitor has been stopped. Funding by Cancer Research UK, Astex Pharmaceuticals and the School of Chemistry at Newcastle University is gratefully acknowledged.

References

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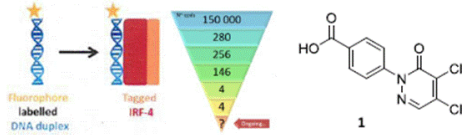

Fig. 1: Schematic of the FP HTS.