# Development of a Fluorine Labelled Fragment Library

Steve Irving[1], Dave Brown[1,3], Siew Kuen Yeap[1], Mike Lainchbury[1], Andrew Cridland[1], Jonathan Killen[1], Simon Pearce[2], Erin Anthonyrajah[3], Michelle Rowe[3]
[1]Charles River Early Discovery, Harlow, United Kingdom; [2]Maybridge Products, Thermo Fisher Scientific; [3]Biosciences Dept, University of Kent

## Introduction

Fluorine substitution in drug molecules is frequently used to help modulate potency as well as physicochemical and pharmacokinetic properties of compounds and ~20% of known drug molecules contain a fluorine atom. In fragment screening, fluorine labels also offer a number of advantages for biophysical screening by NMR as well as crystallography enabling unambiguous orientation of fragments in observed electron density. We have developed a novel fluorine labelled fragment library from the Maybridge fluorine compound collection

## Selection of Library Compounds

**Maybridge Fluorinated compound collection**
5227 compounds

**Filter**
140 < MW <300, logP≤ 3, rotatable bonds ≤ 4, rings ≤ 4, HBA/ HBD ≥ 3
954 compounds

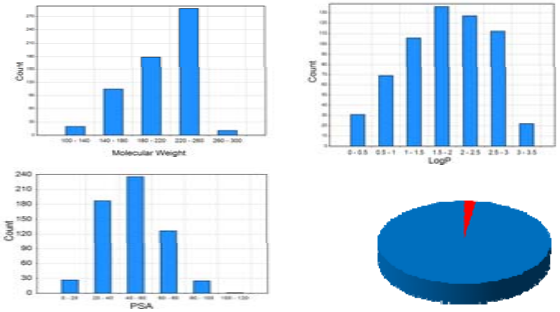
Physicochemical property thresholds were as used by Vulpetti. J. Am. Chem. Soc. **2009**, *131*, 12949–12959

**Compute Fluorine Environment Fingerprints**
317 clusters at 85% similiarity
768 clusters at 90% similarity

Fingerprints were calculated using a MOE svl script written by Andrew Henry, Chemical Computing Group. The algorithm is based on that of Vulpetti.

**Remove potential reactives, mutagenic compounds**
590 compounds

## Library Properties



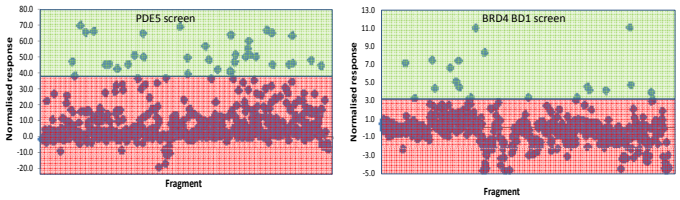

Good solubility properties were observed for the library, although a small portion showed poor DMSO solubility at 100mM and were excluded. The remaining compounds showed reasonably good aqueous solubility, with all being soluble at 100µM and the majority remaining soluble up to 1mM.



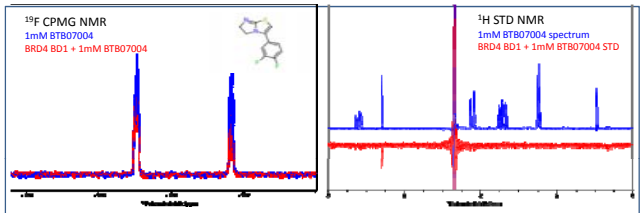


## Fragment Screening Using Fluorine Labelled Library

To test the suitability of the library for fragment screening using common biophysical screening methods, the library was screened against proteins from two different gene families, PDE5 catalytic domain and BRD4 BD1, using surface plasmon resonance (SPR). The library was screened at 100µM using biotinylated proteins, captured on a CM5 SPR chip derivatised with streptavidin.

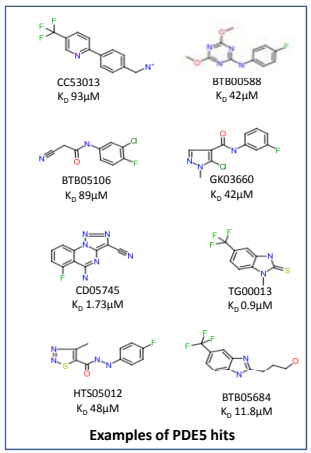


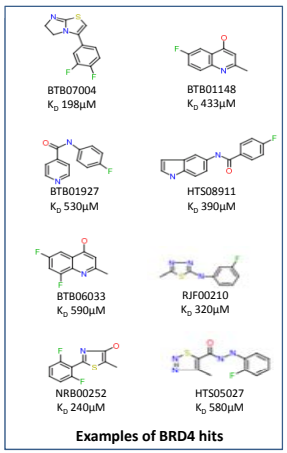

Fragments were well behaved in SPR, with most binders exhibiting 'square wave' fast on / off sensorgrams. Fragments which exhibited non-specific binding effects were rejected from the final list of hits. Hit cut off for PDE5 was set at a normalised response of 40% for PDE5 as a relatively large number of hits were obtained (giving a hit rate of 5.9%), while a lower hit rate (2.9%) was observed for BRD4.




$^{19}$F CPMG and 1H STD NMR clearly show binding of BTB07004 to BRD4BD1

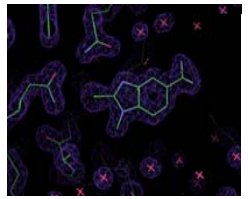
Hits were further characterised by NMR and $K_D$ values determined by SPR



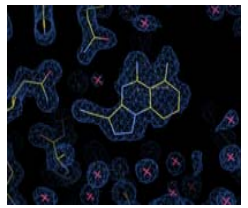




## X-Ray Crystallography

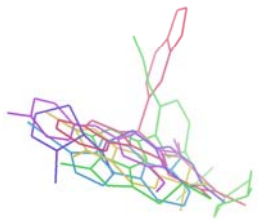
Crystal structures of a number of hits were determined with compounds selected for crystallography based on potency and novelty



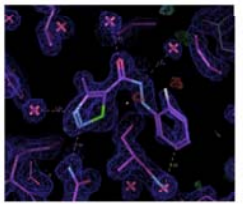
Structure of TG00013 PDE5 complex (1.7 Å)



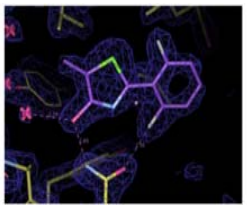
Structure of CD05745 PDE5 complex to (1.5 Å)



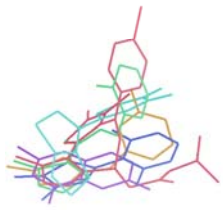
Overlay of PDE5 fragment structures with sildenafil (green) and tadalafil (red), highlights opportunities for growth of fragments



Structure of HTS05027 BRD4 BD1 complex (1.6Å)



Structure of NRB00252 BRD4 BD1 complex (1.9 Å)



Overlay of BRD4 BD1 fragment structures with literature inhibitor +JQ1 (red)

## Summary

A fluorine labelled fragment library of ~500 compounds has been designed based on the Maybridge collection of fluorinated compounds

The library has been shown to have appropriate properties for fragment screening using biophysical methods and its utility has been demonstrated against examples from two gene families.

The library compounds used in these studies are available from Maybridge and the library is currently being further developed and refined for use in NMR screening