Development of a Fluorine Labelled Fragment Library

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Introduction

Fluorine substitution in drug molecules is frequently used to help modulate potency as well as physicochemical and pharmacokinetic properties of compounds with ~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 methods 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% similarity
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.

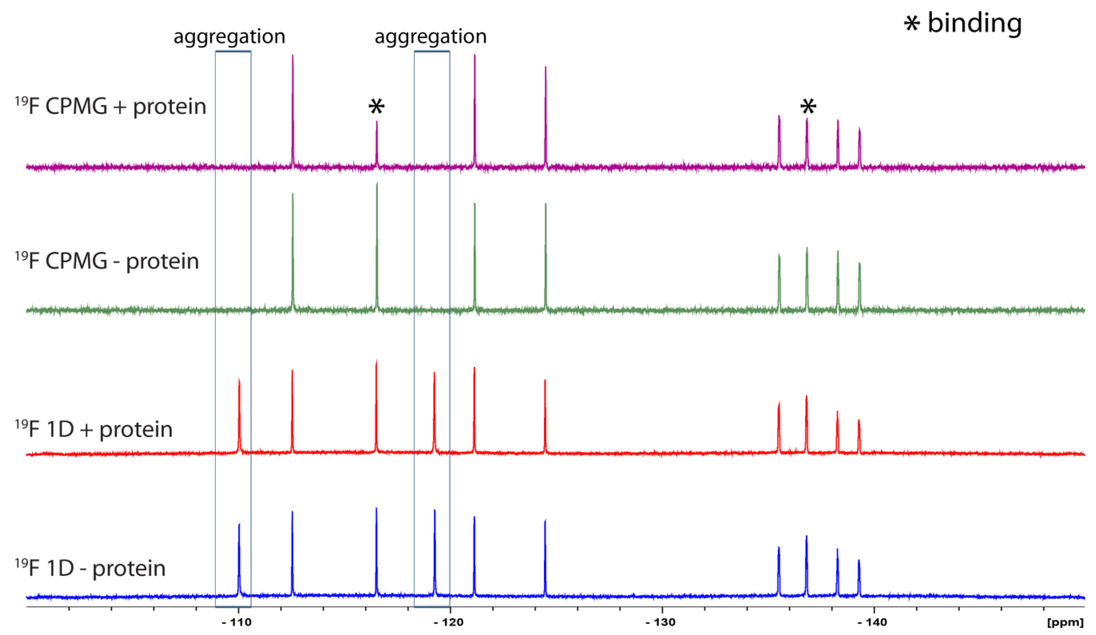
↓

Remove potential reactives, mutagenic compounds
590 compounds

Formulation of Library for ^{19}F NMR Screening

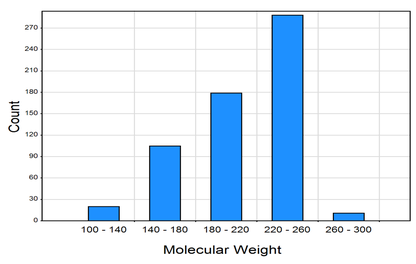
Compounds were tested for suitability for use in ^{19}F NMR screening using Carr-Purcell-Meiboom-Gill (CPMG) acquisition approaches to detect ligand binding. Hits from mixtures of up to ten compounds could be readily detected in BRD4 BD1. The CPMG method also proved to be a sensitive detector of compound aggregators in solution, highlighting compounds unsuitable for the final NMR screening library.

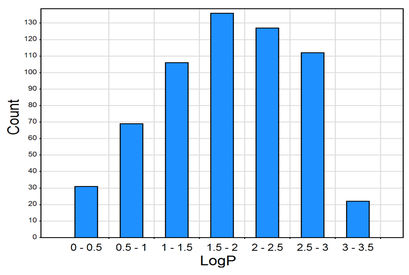
Our initial observations of ^{19}F CPMG demonstrate an improved sensitivity for registering ligand-observed interactions when compared to the ^{1}H counterpart. Additionally, the use of fluorine is further justified from the larger chemical shift range observed across ^{19}F NMR. All NMR datasets were acquired using a Bruker AV3 600 MHz with QCI-F cryoprobe.

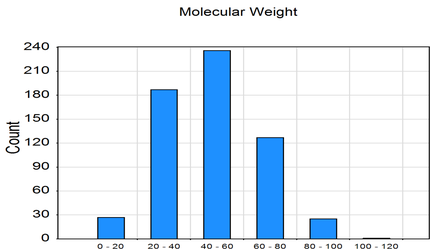


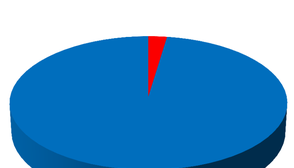
^{19}F Spectra of compound mixture showing BRD4 BD1 fragment binders, non-binders and aggregator

Library Properties

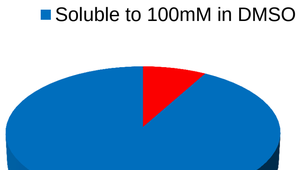






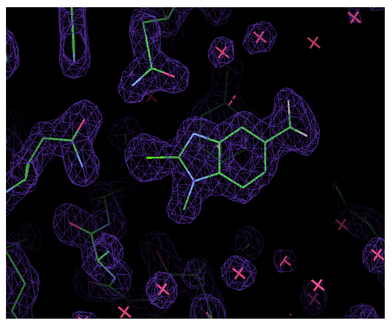


Good solubility properties were observed for the library, although a small portion showed poor DMSO solubility at 100mM and were excluded. The remaining compounds showed reasonably good aqueous solubility, with all being soluble at 100µM and the majority remaining soluble up to 1mM.

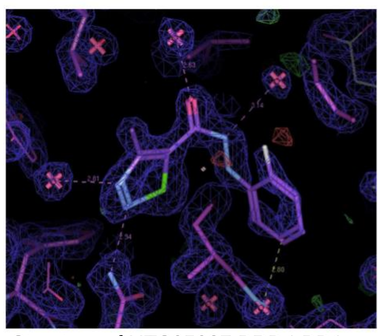


X-Ray Crystallography

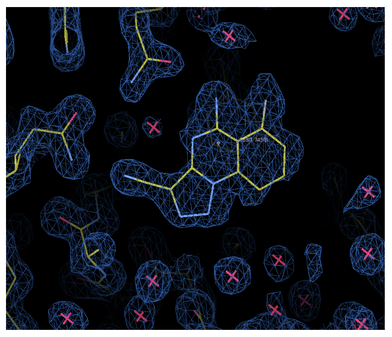
Crystal structures of a number of hits were determined with compounds selected for crystallography based on potency and novelty.



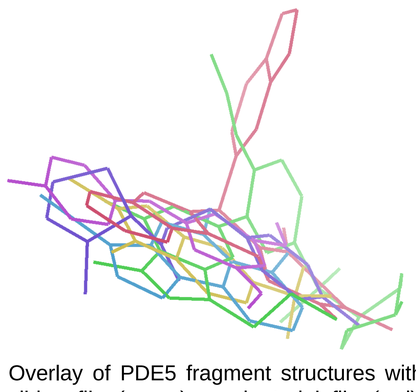
Structure of TG00013 PDE5 complex (1.7 Å)



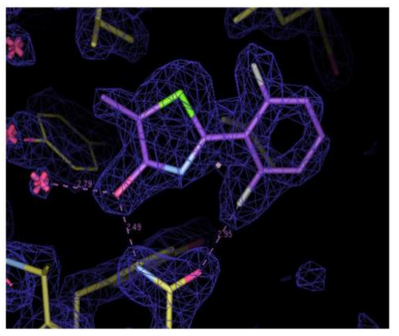
Structure of HTS05027 BRD4 BD1 complex (1.6Å)



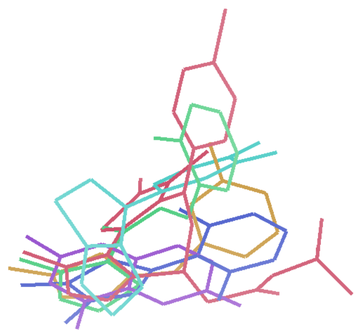
Structure of CD05745 PDE5 complex to (1.5 Å)



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



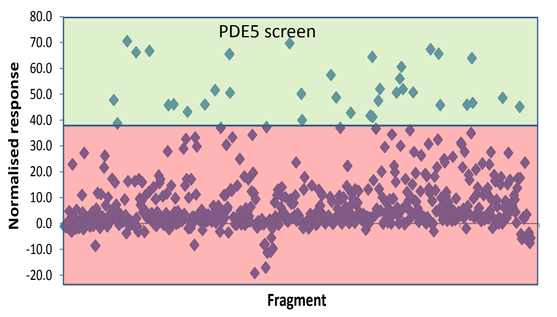
Structure of NRB00252 BRD4 BD1 complex (1.9 Å)

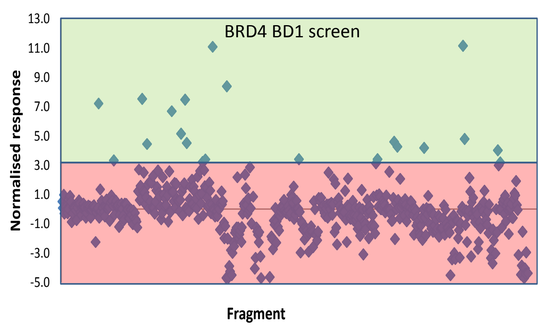


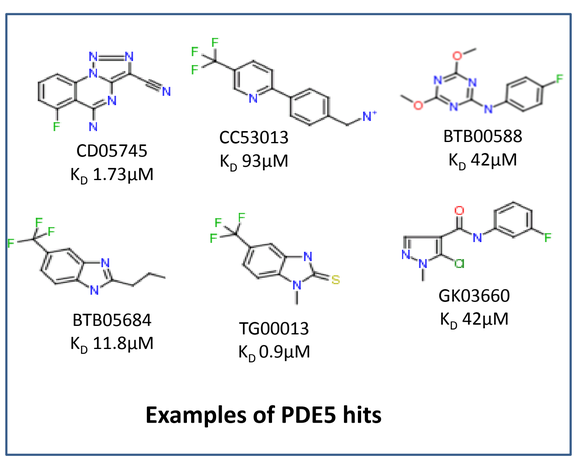
Overlay of BRD4 BD1 fragment structures with literature inhibitor +JQ1 (red)

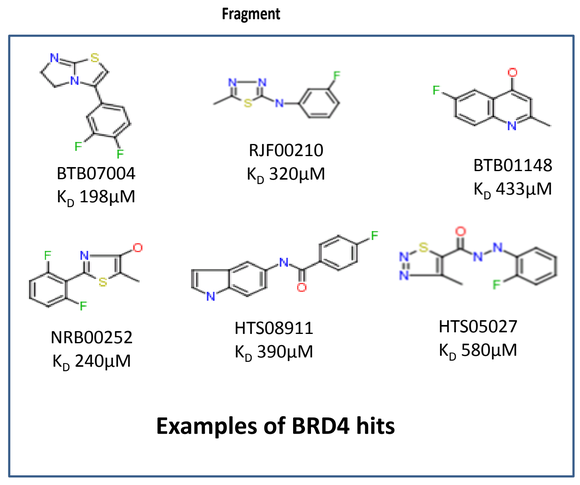
Fragment Screening Using Fluorine Labelled Library

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.









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 formulation is currently being finalised for use in NMR screening.

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