Fragment Based Approaches to Targeting the CYP Enzymes from *Mycobacterium tuberculosis*

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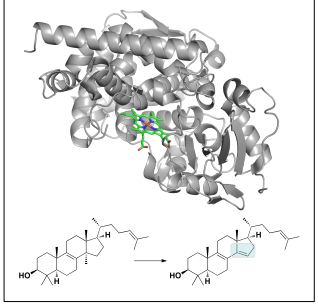
(1) CYP Enzymes from *M. tuberculosis*

- Tuberculosis is a major disease causing 1.8 million deaths per year.
- The emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* have highlighted the need for new and improved drugs to treat TB.
- *M. tuberculosis* has an unusually high number of cytochrome P450 enzymes (CYP) encoded in the *Mtb* H37Rv genome.
- Of the 20 CYP enzymes of *Mtb* only five have been structurally and functionally characterized to date.
- We are interested in targeting CYP121, 125, 142 and 144 using the fragment based approach

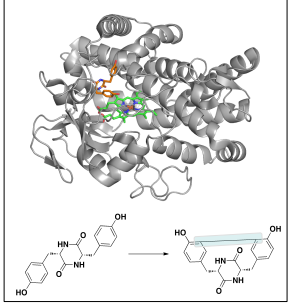
Objective

The aim of this project is to use the fragment-based approach to target the CYP enzymes of M. tuberculosis. This could offer a route to novel compounds with the potential to treat TB.

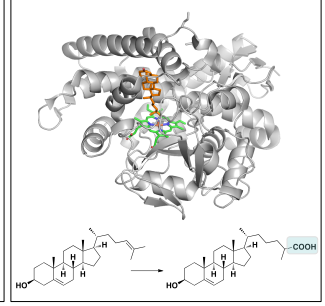
CYP51B1 (Sterol 14α-demethylase)



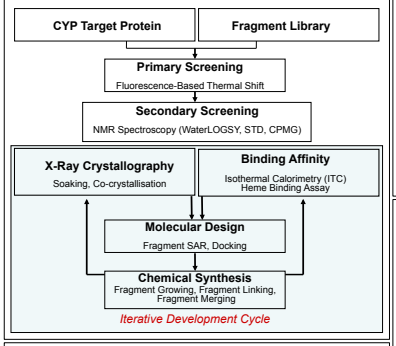
CYP121 (Mycocyclosin synthase)



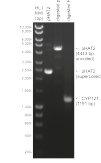
CYP124, 125, 142 (Sterol 27-monooxygenase)



(2) Fragment Screening Methodology

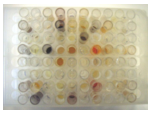


CYP Target Protein (CYP121)



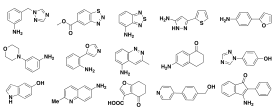
- Recombinant untagged *Mtb* CYP121 expressed and purified from the pET11a/CYP121 expression vector
- His_6-tagged CYP121 expressed and purified from the pHAT2/CYP121 vector in *E.coli* C41(DE3)
- Purification Ni column and AKTA gel filtration

Fragment Library

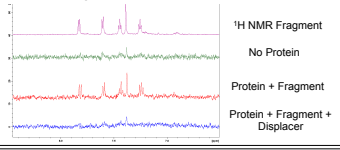


Fragment Library 'Rule of Three' compliant

MW <300 Da
cLogP = 3
H-Bond Donor ≤ 3
H Bond Acceptor ≤ 3
Rotatable Bonds ≤ 3
PSA ≤ 60 Å²

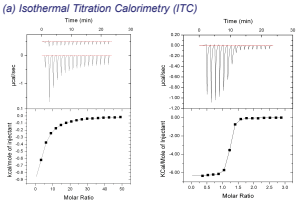


NMR Screening (STD Experiment)



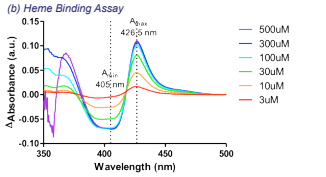
Binding Affinity Measurement

(a) Isothermal Titration Calorimetry (ITC)



Fragment (K_D = mM) *Elaborated Fragment (K_D = μM)*

(b) Heme Binding Assay



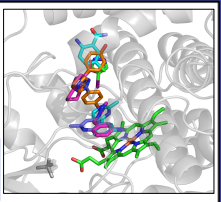
Absorbance difference spectra for CYP121 titrated with various concentrations of fragment. The shift in the Soret absorbance band is indicative of heme-iron coordination

(3) Fragment Hits

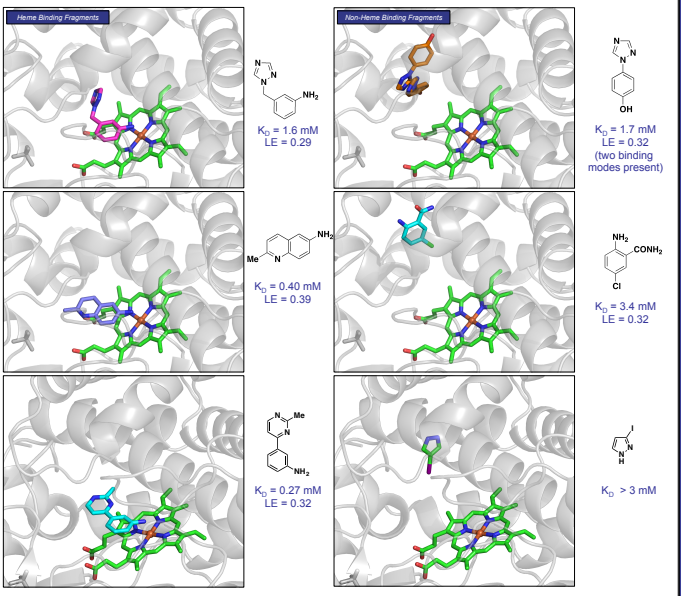
CYP121

	Number of Fragments	Hit Rate
Fragments Screened by Thermal Shift	665	
Thermal Shift hits (ΔTm >0.8ºC)	66	9.9%
Fragments rescreened by ^{1}H NMR	56	
cYY displaced hits	26	3.9%

Overlay of X-Ray crystal structures showing differing binding modes of each of the fragments

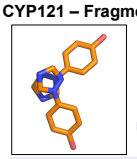


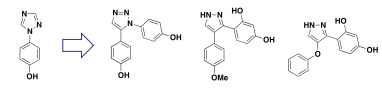
Ligand Efficiency
LE = ΔG / N
(N = number of non-hydrogen atoms)



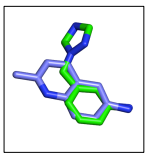
(4) Fragment Elaboration through synthesis

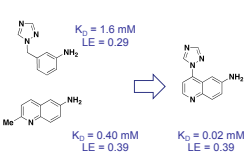
CYP121 – Fragment Merging





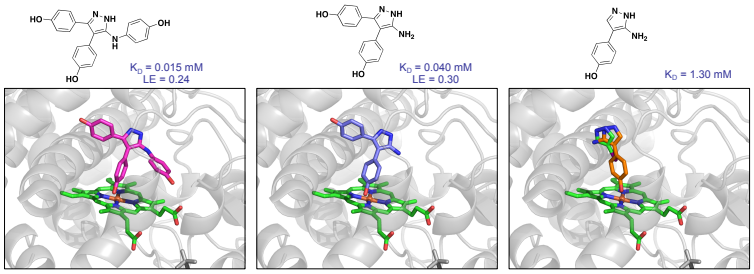
In the initial X-ray crystal structure of the fragment in CYP121 two binding modes were observed. A number of molecules were synthesised to see whether these two binding modes could be incorporated into one molecule, of which the best had a K_D of 0.50 mM.





- Merging of the two fragments showed a significant increase in potency to 0.02 mM and the ligand efficiency is maintained at 0.39.
- Introduction of other azole heterocycles instead of the 1,2,4-triazole had a detrimental effect on the potency and ligand efficiency

CYP121 – Fragment Growing



- This elaborated compound was chosen based on the 1,2,4-triazole fragment. This was obtained through a search of the DuPont compound collection.
- Key interactions are formed through the aminopyrazole ring.

- Removal of phenol ring attached to NH_2 gave a small drop in potency. However ligand efficiency is increased.
- NH_2 is a good vector for further synthesis to pick up further interactions with protein backbone and conserved waters.

- Removal of second phenol ring shows a significant drop in potency and ligand efficiency
- Binding mode is significantly different and a similar binding pose to iodopyrazole (in green) was observed.

(5) Future Work

CYP121

- CYP121: Explore further fragment merging and fragment linking options to develop compounds that will bind to CYP121 with nanomolar potency.
- CYP125: Measure potency of fragments and explore fragment growing to increase potency.
- Examine selectivity of developed compounds against other CYP's (Human and *M. tuberculosis*)
- Measure MIC's of elaborated compounds against *M. tuberculosis* in collaboration with researchers at the National Institute of Health (NIH)

Other CYP's

- Protein expression and purification of other CYP's in collaboration with the University of Manchester
- Fragment screen against CYP 142, 141, 126, 124 and 144
- Develop 96-well plate heme assay for screening of focused fragment library to discover heme-iron binding fragments.
- Examine selectivity of developed compounds against other CYP's (Human and *M. tuberculosis*)

(6) References and Acknowledgements

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