Exploring Protein Stabilisation as an Approach to p53 Drug Discovery

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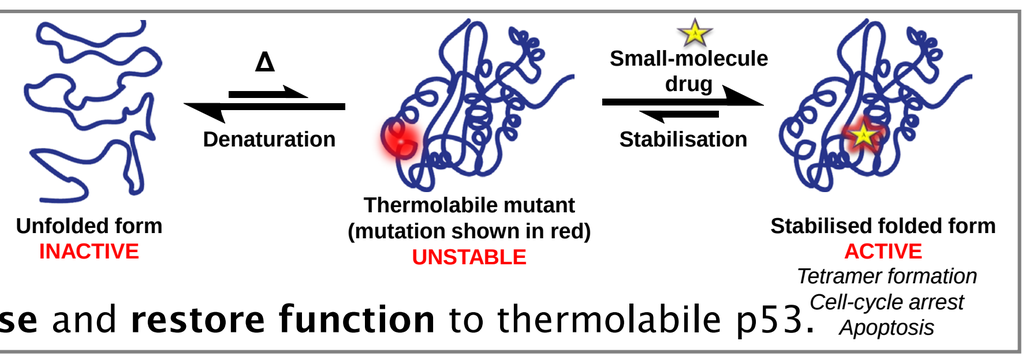
1. Introduction

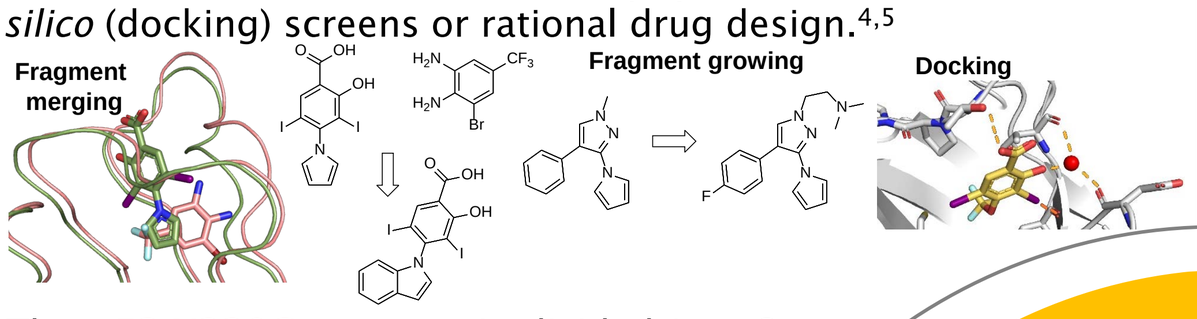
The tumour suppressor p53 is implicated in virtually all cancers.[1] Destabilising mutations can cause rapid unfolding, driving oncoprogression by attenuating function.[2] We aim to develop treatments that operate by the reactivation of thermolabile mutants using small-molecule stabilisers.



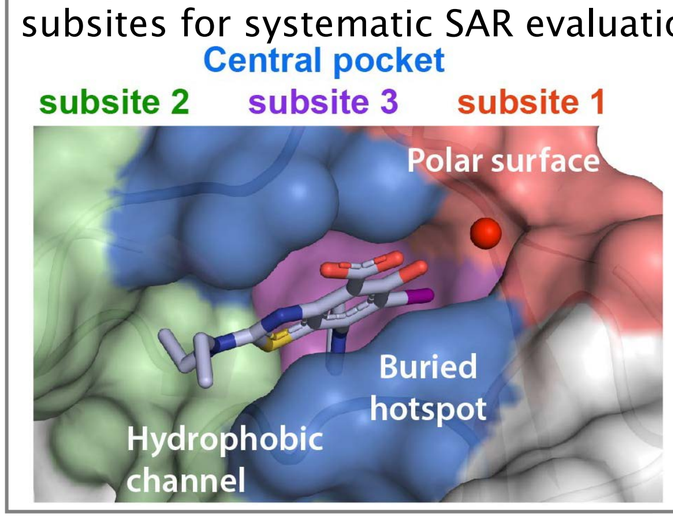
Objectives: **Identify** and **characterise** molecules that **bind**, **stabilise** and **restore function** to thermolabile p53.

3. Fragment Screening and Drug Design

Hits were found by fragment- and structure-based strategies. Novel hits were identified from biophysical (DSF, NMR) and *in silico* (docking) screens or rational drug design.[4,5]

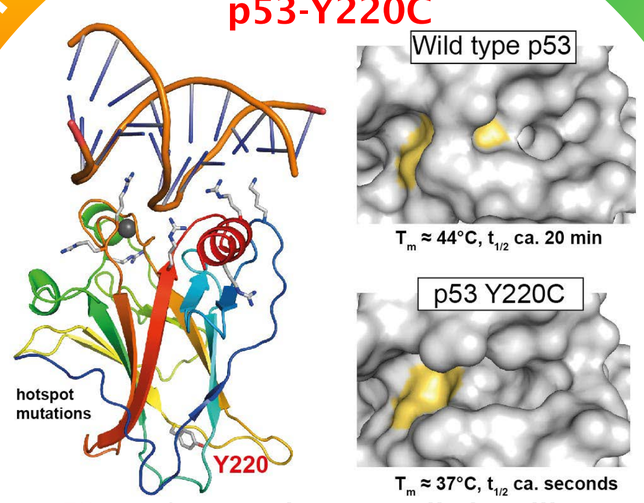


The **p53-Y220C** receptor is divided into 3 subsites for systematic SAR evaluation.



2. Target

p53-Y220C

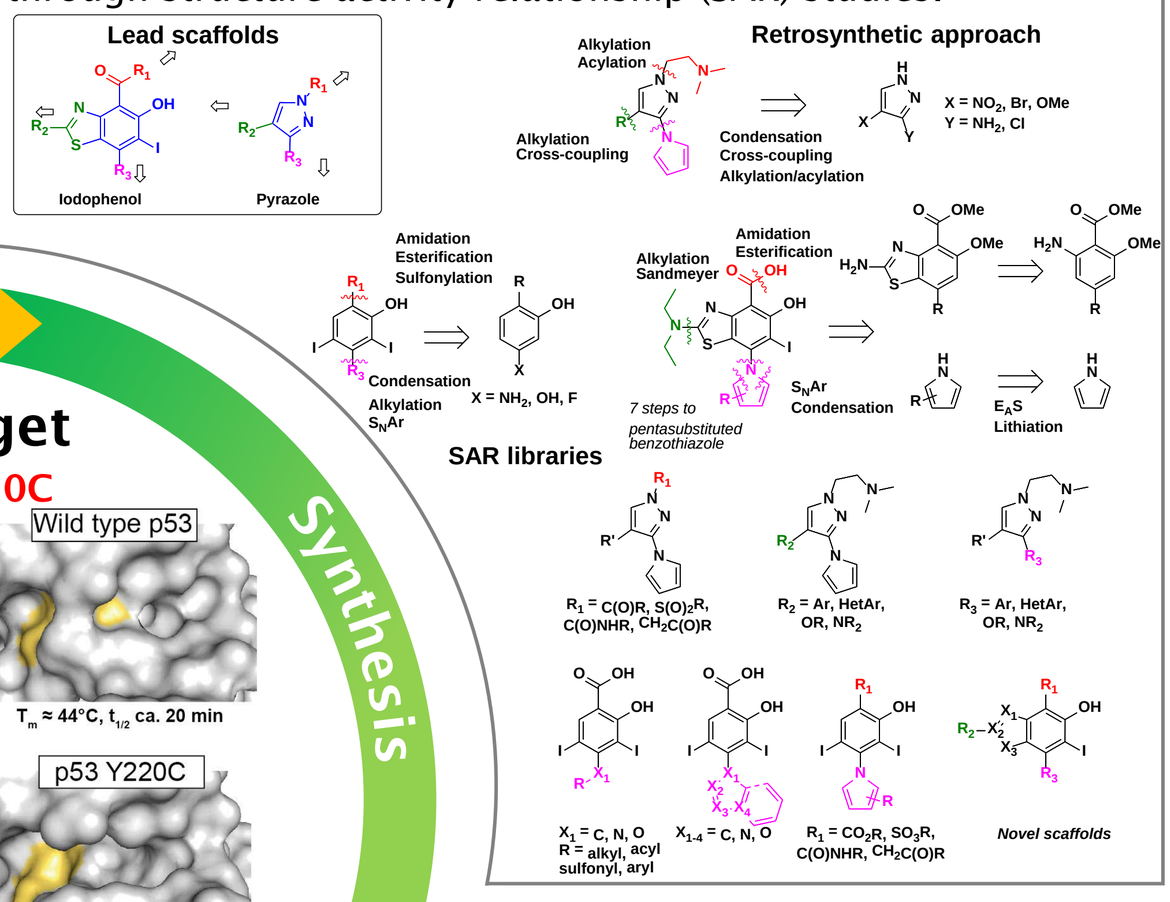


p53 regulates **anticancer cell signalling** responses. The Y220C mutation **destabilises** the core, inducing a surface pocket.[2] **Activity** at low temperatures suggests thermostabilisation could **reactivate** mutants.[3]

Design → Synthesis → Evaluation

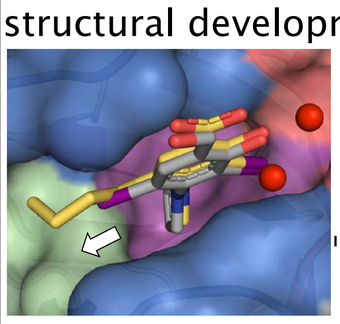
4. Synthetic Chemistry

Systematic probing of 3 vectors over 2 scaffolds was achieved through structure-activity relationship (SAR) studies.

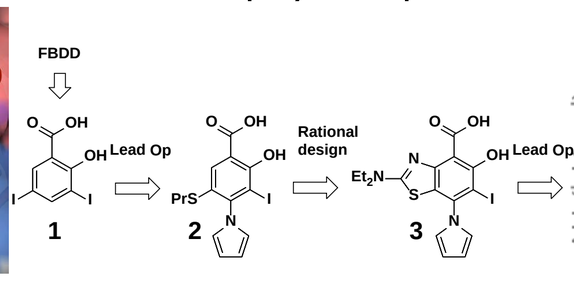


5. Biophysical Evaluation

Compounds were studied for thermal shift (**TS**) using differential scanning fluorimetry (**DSF**) and binding constant (**Kd**) by isothermal titration calorimetry (**ITC**). X-ray crystallography allowed correlation between structural development and biophysical profiles.

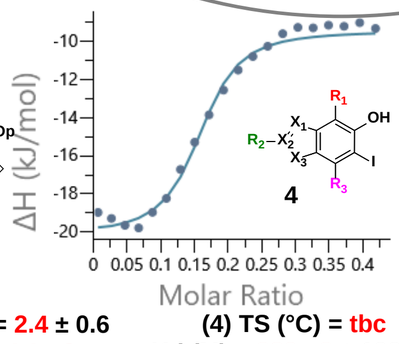


Grow into channel
→ Increase affinity

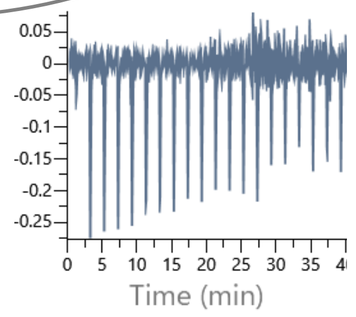


(1) TS (°C) = n.d.
Kd (M) = 820e-6

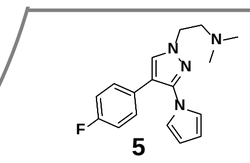
(2) TS (°C) = 2.2 ± 0.6
Kd (M) = 14e-6



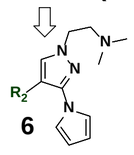
(3) TS (°C) = 2.4 ± 0.6
Kd (M) = 4.1e-6



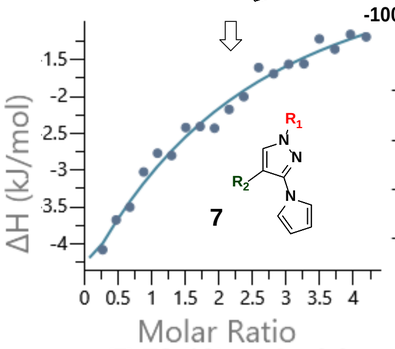
(4) TS (°C) = tbc
Kd (M) = 930e-9 ± 190e-9



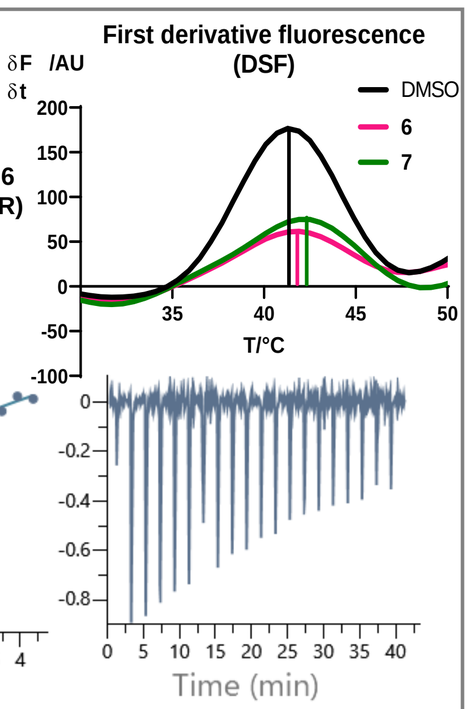
(5) TS (°C) = 0.8 ± 0.6
Kd (M) = 140e-6 (NMR)



(6) TS (°C) = 1.2 ± 0.6
Kd (M) = 250e-6 ± 120e-6



(7) TS (°C) = 1.8 ± 0.6
Kd (M) = 210e-6 ± 78e-6



6. Conclusions and Future Work

Through iterative design, synthesis and biophysical evaluation, we report the discovery and optimisation of novel ligand series targeting cancer mutant p53-Y220C. Three sites of the pocket were probed extensively in SAR studies culminating in advanced ligands that bound in the low μM to nM range, stabilised the protein by up to 2.4 ˚C and had precise binding modes elucidated. We thus present well-characterised ligands for further study (cell biology, animal models) and lead development. Validation of this approach paves the way towards first-in-class cancer therapeutics targeting p53 by protein stabilisation.

7. References

1. *Hum Mutat*, 2002, **19**, 607-14; 2. *Eur J Med Chem*, 2018, **152**, 101-14; 3. *Carcinogenesis*, 2007, **28**, 289-98; 4. *J Am Chem Soc*, 2012, **134**, 6810-8; 5. *Nucl Acids Res*, 2013, **41**, 6034-44.

EPSRC Engineering and Physical Sciences Research Council
DKTK Deutsches Konsortium für Translationale Krebsforschung
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