Rational Design, Synthesis & Evaluation of Small Molecule Modulators of the Wnt/β-catenin Signalling Pathway

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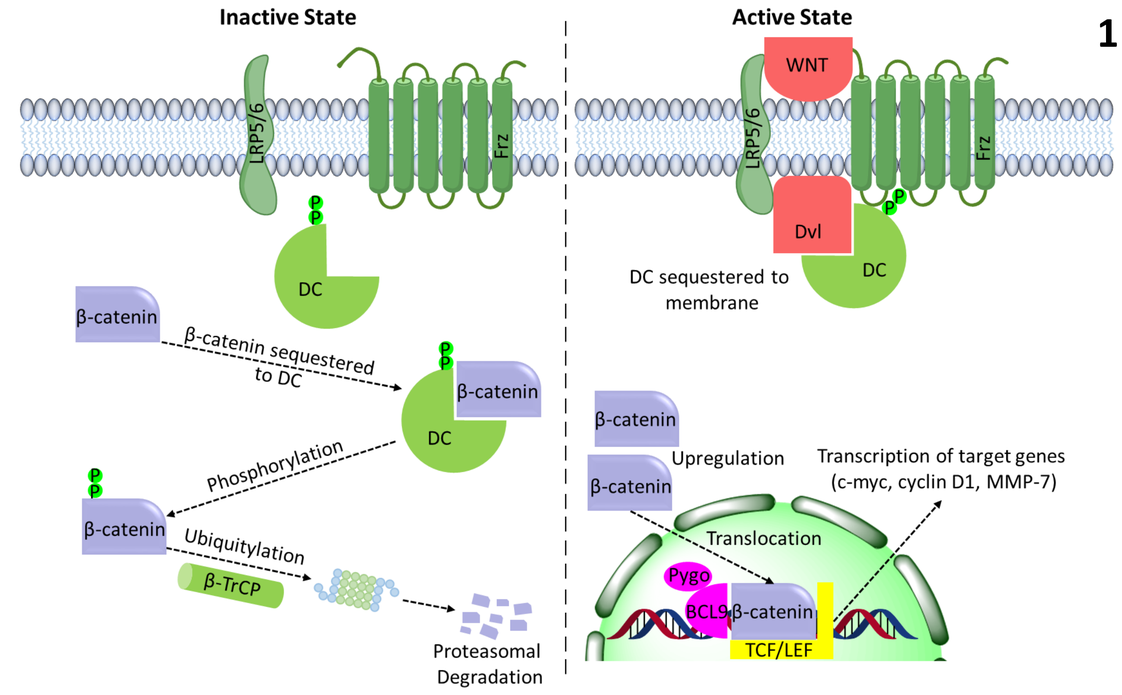


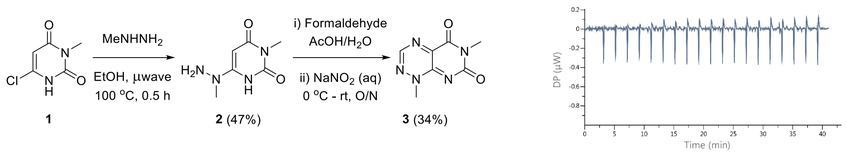
Figure 1 – Wnt signaling pathway (left) Inactive; (right) active

1 - Introduction

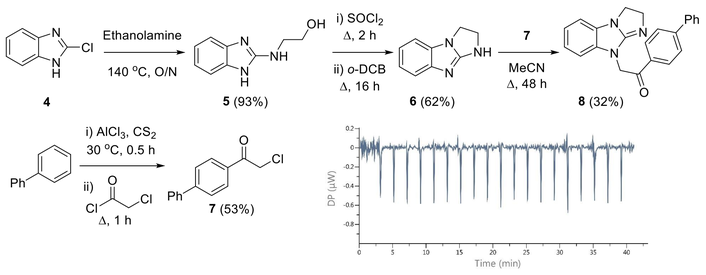
- **Wnt/β-catenin signaling** pathway is fundamental in cell differentiation, cell fate determination and tissue homeostasis amongst metazoans[1]
- Mutations in this pathway are **implicated** in various diseases including osteoporosis & **cancer**
- In particular, aberrant signaling in this pathway is linked to **85%** of **CRC cases**
- For the treatment of cancer, the signal transducer **β-catenin is a key,** but challenging **target**
- We are targeting β-catenin *via* several orthogonal approaches including probing natural product destabilisers, fragment based drug discovery and *in silico* screening

2 - Biophysical Evaluation of Reported Inhibitors

- There are multiple compounds in the literature which have been described to inhibit wnt/β-catenin signalling *via* direct targeting of β-catenin
- Whilst some compounds have been described with good biophysical validation, others have been identified from phenotypic screening assays/fluorescence based assays which can be prone to false positives
- Several inhibitors described contain PAINs motifs[2]
- One of our research objectives is to synthesis described inhibitors and validate (or not) their direct interaction with β-catenin *via* biophysical assays (DSF, STD/wLOGSY NMR, ITC)



Scheme 1 – synthesis of toxoflavin, **3** and subsequent ITC trace[3]



Scheme 2 – synthesis of CCT 03137, **8** and subsequent ITC trace[4]

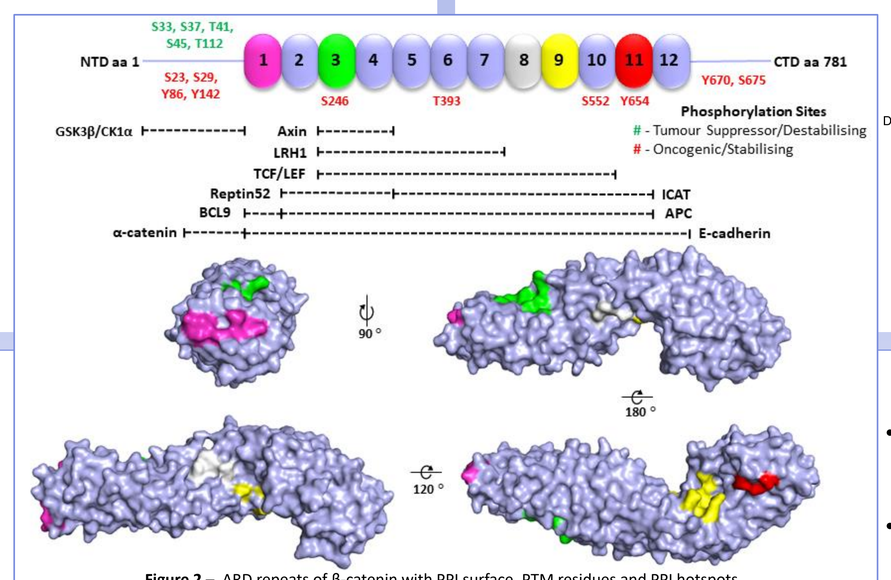
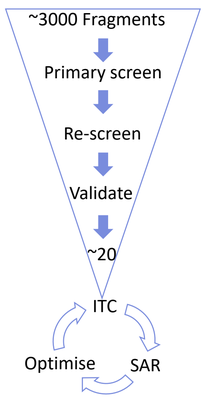


Figure 2 – ARD repeats of β-catenin with PPI surface, PTM residues and PPI hotspots

3 – High Throughput Fragment Screening

- HTS assays such as DSF have been utilised to screen fragment libraries (3k compounds) against β-catenin
- Primary screening affords a subset (10^2 compounds) which are re-screened and validated using alternate screening methods (STD NMR)
- Validated compounds *ca*. 20 are investigated using ITC
- Next generation compounds are generated *via* SAR studies as well as fragment linking/merging/growing



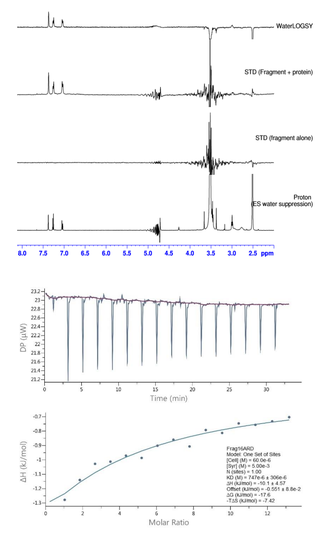
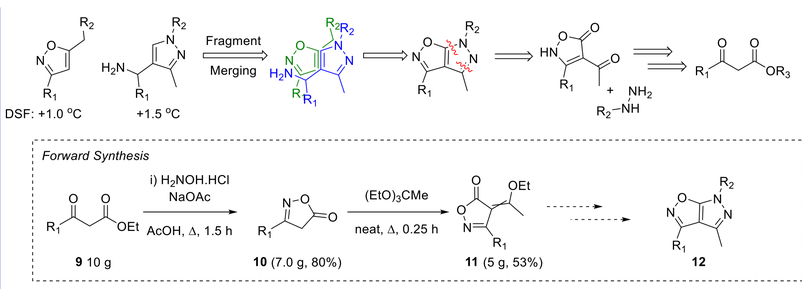


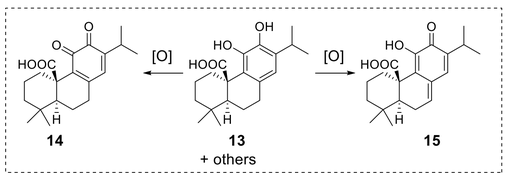
Figure 3 – (left) HTS workflow; (right, top) STD-NMR of fragment; (right, bottom) ITC of fragment



Scheme 3– Fragment merging approach and forward synthesis for **X**

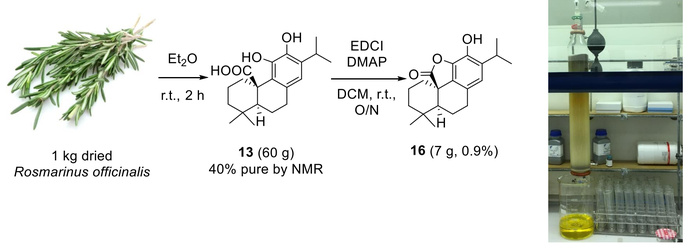
4 - Natural Product Derivatives

- Carnosic acid, **13**, has been shown to be a potent degrader of β-catenin[5]
- The mode of action of **13** is still uncertain, and its propensity for oxidation raises some questions – namely whether **13** is responsible for the biological action observed



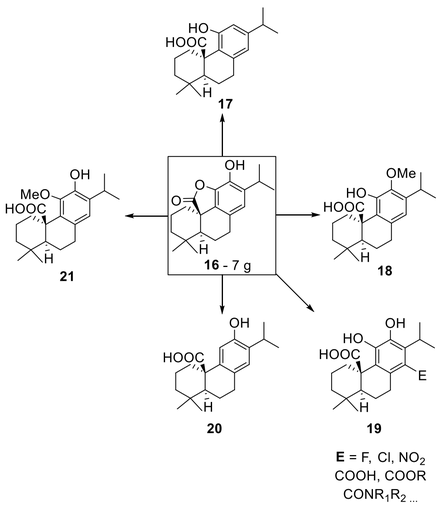
Scheme 4 – Carnosic acid, **13** and some oxidised derivatives

- We are interrogating these questions by making derivatives more stable to oxidation
- Extraction from *R. officinalis* afforded crude material for further derivatisation



Scheme 5 – Route to protected lactone and photograph of column (1.6 kg silica)

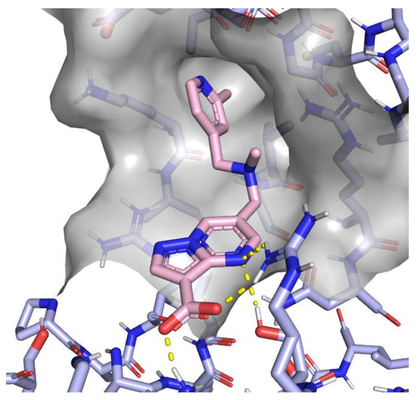
- Various functional group manipulations are envisaged to afford more oxidation resistant derivatives



Scheme 6 – Envisaged oxidation resistant derivatives

5 - Computational Screening

- The virtual screening workflow in glide was used to screen an in-house virtual library of 10^6 compounds
- Removal of reactive motifs, filtering according to Lipinski's rules and sequential higher precision docking afforded *ca*. 2k compounds for visual inspection
- MMGBSA, re-screening against different PDBs, and predicted physicochemical properties were utilised to afford a shortlist of compounds to evaluate *in vitro*



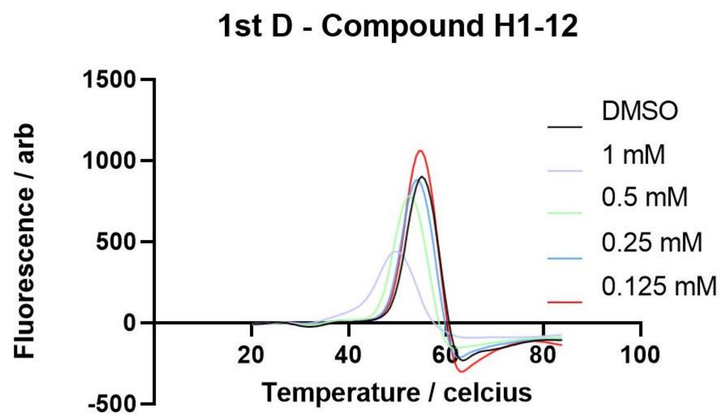


Figure 4 – (left) docking pose of compound against β-catenin (PDB – 2GL7) from HTVS; (right) DSF 1st derivative of compound H1-12 against β-catenin ARD

6 - Future Work

Section 2 - Finish biophysical evaluation of reported inhibitors (manuscript in progress)

Section 3 - Finish fragment merging approach & use HTS workflow to investigate a separate compound library

Section 4 – Finish synthesis of envisaged oxidation resistant derivatives (manuscript in progress)

Section 5 – Re-screen top HTVS compounds using DSF, STD, ITC → follow up with biological study

7 - References & Acknowledgements

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4. Ewan *et al*., *Cancer Research,* **2010**, 70, 5963-5973
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