THE DESIGN AND SYNTHESIS OF ENZYME INHIBITORS TO INCREASE SENSITIVITY TO CHEMOTHERAPY

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1. INTRODUCTION AND PROJECT BACKGROUND

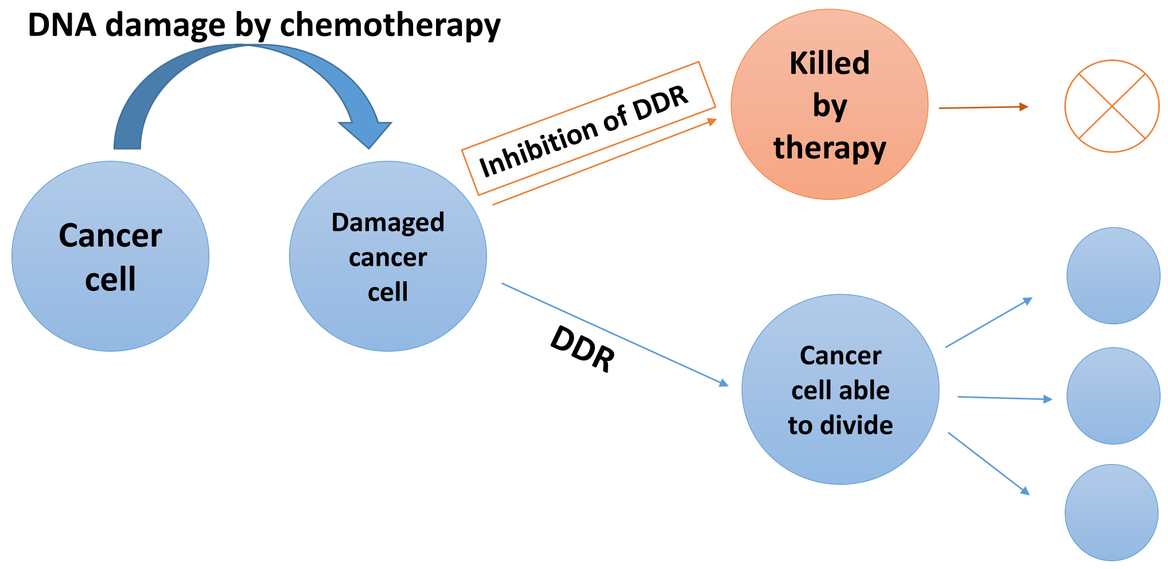
Evotec (Founded, 1993) is a multinational contract research organisation. It provides services to clients along the entire drug-discovery timeline. I have been working to synthesise potential drug molecules on a small scale to be tested for their ability to inhibit a target enzyme.

- I synthesised a total of 75 final compounds. The project focused on the development of a drug candidate to overcome chemotherapy drug-resistance.

The molecular target for the project was an enzyme which allows cancer cells to proliferate by repairing damaged DNA, this enzyme is upregulated in cancer cells.

1. Design enzyme inhibitors for combination with chemotherapy drugs
2. Increase sensitivity to chemotherapy
3. Overcome resistance to chemotherapy

- Most failures of chemotherapy are related to drug-resistance [1].
- This resistance may occur by a number of mechanisms including DNA-damage repair (**DDR**).
- DDR is common to DNA-damaging chemotherapeutic agents (eg. cisplatin).

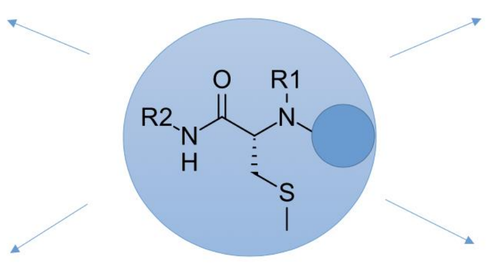


2. ORGANIC SYNTHESIS OF POTENTIAL DDR INHIBITORS

Two different clusters (groups of compounds) identified from a HTS were investigated.

- Both clusters target the DDR enzyme.
- The aim was to inhibit the enzyme's mechanism of action.

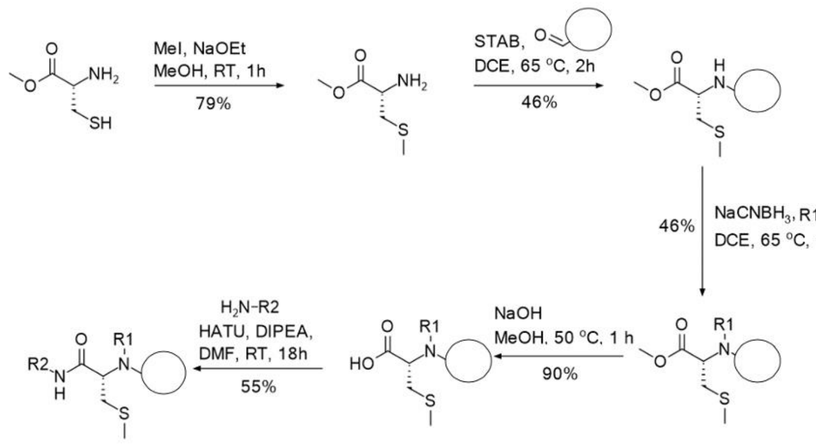
Compounds were designed using structure-activity relationships (**SARs**) based on the hit compound which showed activity in biochemical assays which tested enzyme inhibition.

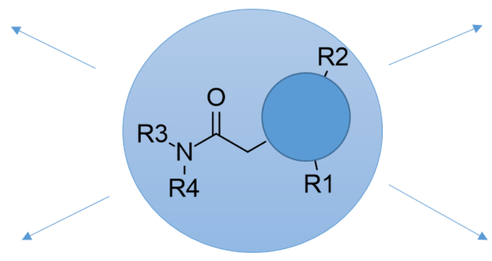


An example synthesis for cluster 1 compounds is shown below.

The steps common to each total synthesis are:

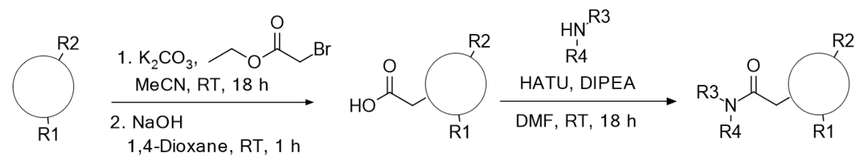
- Reductive amination (Step 2 and step 3)
- HATU amide coupling (Step 5)



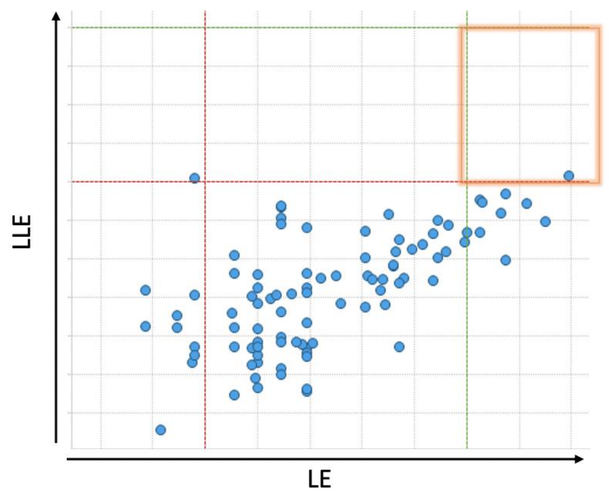


The second cluster had a shorter synthetic route. The carboxylic acid derivative was synthesised as a building block on a 5 g scale. Final products were synthesised via HATU amide coupling.

Total synthesis was required when varying R1/R2 groups to probe the structure-activity relationships (SARs) on the right-hand side of the molecule.

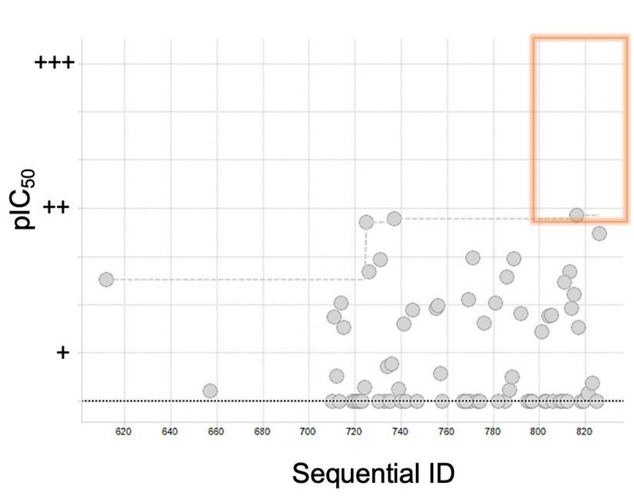


3. RESULTS



LE is the ligand efficiency, a measure of the binding energy of non-H atoms of a ligand to the enzyme active site. **LLE** is the lipophilic ligand efficiency which measures lipophilicity versus *in vitro* potency of a molecule.

- This plot is used to identify compounds that contain the minimum structural features required for potency, i.e. the pharmacophore.
- The goal is to move towards high LLE and LE values where binding levels are high, and the lipophilicity is at a minimum.

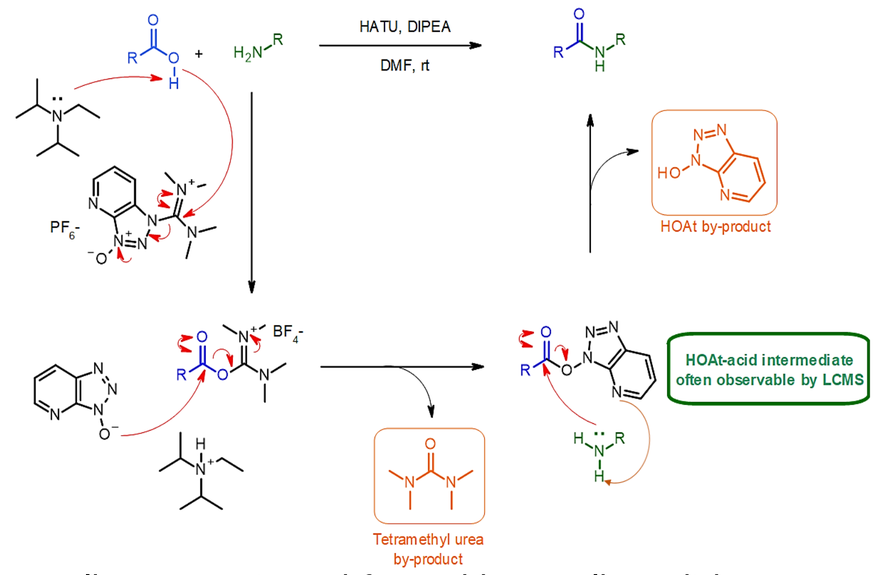


IC_{50} = the concentration of compound needed to inhibit 50% of enzyme action.

pIC_{50} = the negative log of the molar IC_{50} value. The aim is to move to the nanomolar range which is above 7-8 on the logarithmic scale.

The positive trend shown by the dotted line shows the sequential increase in potency as more compounds were synthesised.

4. MECHANISM OF A COMMON REACTION



HATU is a coupling reagent used for amide couplings, it is commonly used in peptide synthesis since the alpha stereocentre is not affected by this mechanism. Amide coupling is a common synthetic step to the formation of compounds of both clusters. The mechanism of this coupling is shown above.

5. CONCLUSIONS

Cluster 1: the most potent compound was optimised to improve ADME properties (absorption, distribution, metabolism, excretion).

- The next step would be to implement functional changes to the lead compound. The aim is to reduce lipophilicity, improve solubility and increase metabolic stability whilst maintaining potency.

Cluster 2: SAR exploration led to an increased potency via the introduction of functional changes.

- The next step would be to use SARs to improve potency from micromolar IC_{50} values (++) to the nanomolar range (+++).

The project: Chemistry work on this project began in 2018 and is continuing in the hit to lead and lead-optimisation stages of the drug discovery process.

6. REFERENCES

1. B. Mansoori, A. Mohammadi, S. Davudian, S. Shirjang and B. Baradaran, *Adv. Pharm. Bull*., 2017, **7**, 339–348.