Mapping Reactive Residues on Proteins using Electrophilic FragLites to Develop Cancer Treatments

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Objectives

1. Synthesis of a set of halogenated Michael acceptor FragLites.
2. Synthesis of the set of fluoroheterocycle and epoxide containing FragLites.
3. Soaking of compounds, crystallisation and x-ray characterisation.

Introduction

Covalent inhibition is becoming an increasingly important strategy for the development of new drug therapies for cancer. Some covalent inhibitors are a type of irreversible inhibitor; they bind strongly to an enzyme, and their action can therefore not be reversed.

In cancer treatment, irreversible inhibitors may block certain enzymes that cancer cells need to grow. This mechanism could selectively cause the death of cancer cells.

The aim of my project was to design small molecules called FragLites. Smaller fragments can more broadly sample a larger range of chemical space, making it easier for pockets in the enzyme to be identified and targeted. In effect, the smaller the molecule the more places the drug molecules bind to, making it easier to develop effective drugs.

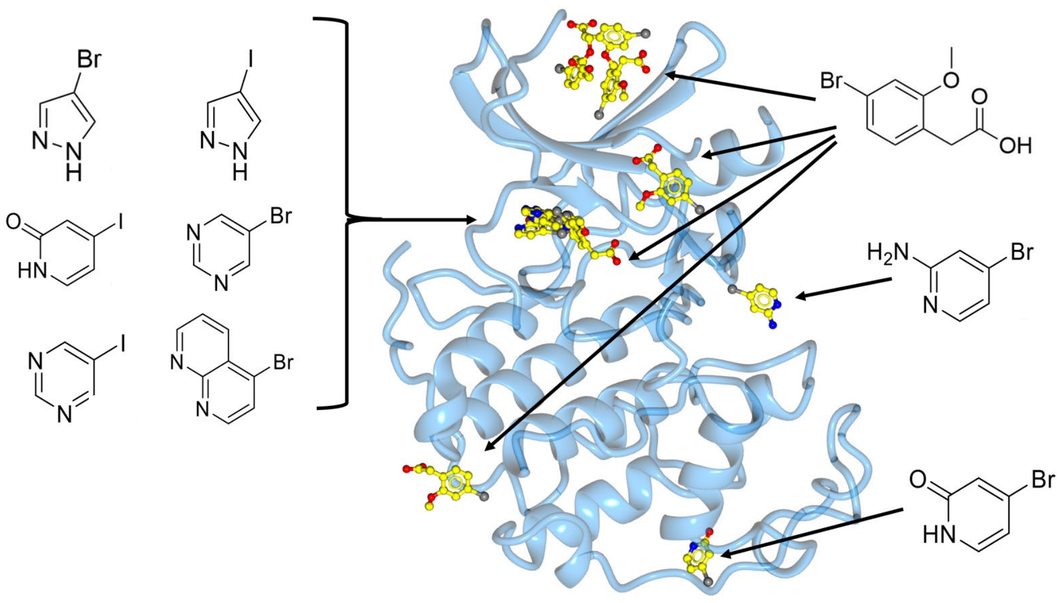

Figure 2 - FragLite compounds successfully bound non-covalently to CDK2.[3]

My Results

Although having planned >10 targets, only 5 were synthesised which were up to the standard of testing, see **Figure 1**. The compounds must have a purity of greater than 97%. This is because impurities may competitively bind to the protein leading to false positive (or negative) results.

The other targets were extremely difficult to prepare due to multiple side reactions. I tried several methods following a thorough investigation of the chemical literature. Unfortunately, the undesired products formed the bulk of the material.

My final compounds where sent off to be crystallised with different proteins. The proteins my compounds will be tested on are:

- CDK2 – involved in replication of cancer cells, if inhibited can block the cell cycle.
- BRD4 – "tumour driving" oncogenes; cancer causing genes.
- ATAD2 – promotes cell proliferation.
- Cyclin T – controls the elongation phase of RNA in cell cycle.

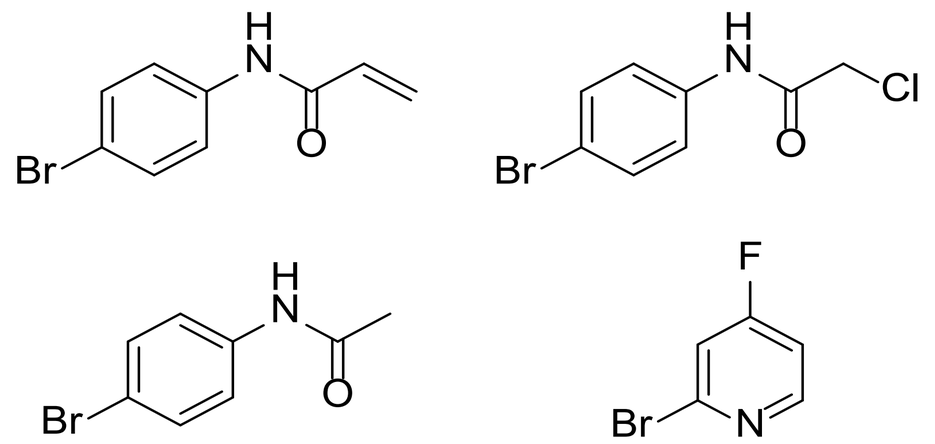

Figure 1 - FragLite compounds successfully synthesised.

Design

My project was a conceived following a recent project within the Northern Institute of Cancer Research, on non-covalent FragLites – those that bind without covalent bonds.[1] The concept was similar, with the only difference being the type of binding in which the molecules partake.

Within the different FragLites, bromine atoms were incorporated as at standard X-ray wavelengths, they generate a unique scattering of X-rays. This signal allows us to identify the binding site of the drug molecule with the enzyme.

Previous Results

In a previous study the hit rate for binding of non-covalent FragLites was compared with those of standard fragment libraries.[1] The hit rate, "how many molecules bind", to the protein CDK2 was considerably higher than would be expected for the hit rate in a screen of larger fragments, 29% compared to 5%. The hits can be seen in **Figure 2**.

This provides evidence that FragLites provide much more extensive coverage of chemical space within the protein than is possible with library of larger molecule. The interaction sites can be mapped, and the small FragLites can then be built up for virtual screening or to develop future library chemistry.

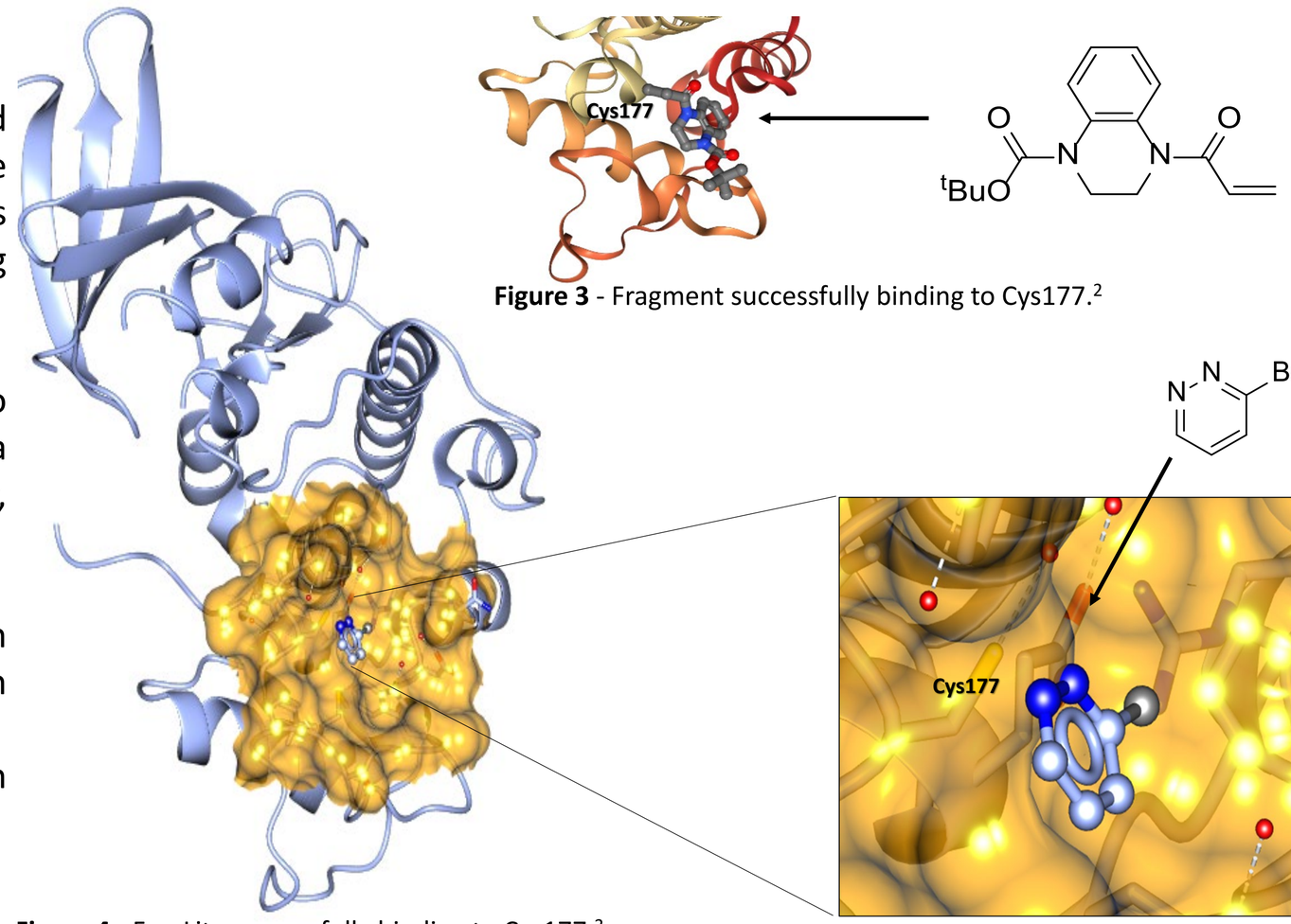

Figure 3 - Fragment successfully binding to Cys177.[2]

Figure 4 - FragLite successfully binding to Cys177.[3]

One of the compounds from a previous paper successfully covalently bound to the protein CDK2. This result was unexpected as it was thought to bind non covalently. The molecule bound to cysteine 177, and can seen by as seen in the crystal structure in **Figure 4**. When researching binding here, in a recent paper, they saw similar bonding with the molecule seen in **Figure 3**, which is bound in the same place.[2]
Screening of the remaining compounds is ongoing, as protein crystallography is labour intensive and time consuming.

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References

[1] D. J. Wood, J. D. Lopez-Fernandez, L. E. Knight, I. Al-Khawaldeh, C. Gai, S. Lin, M. P. Martin, D. C. Miller, C. Cano, J. A. Endicott, I. R. Hardcastle, M. E. M. Noble and M. J. Waring, *Journal of Medicinal Chemistry*, 2019, **62**, 3741-3752.
[2] G. B. Craven, D. P. Affron, C. E. Allen, S. Matthies, J. G. Greener, R. M. L. Morgan, E. W. Tate, A. Armstrong, D. J. Mann, *Angew. Chem. Int. Ed.* **2018**, *57*, 5257.
[3] Unpublished work by Northern Institute of Cancer Research.

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