Providing new lead series and IP position and profile for delivering in-vivo activity

M. Slater[†]

[†] Cresset, New Cambridge House, Bassingbourn Road, Litlington, Cambridgeshire, SG8 0SS, UK

Introduction

Research and development is not an easy business. Real research invariably involves starts and stops, misassumptions, poor data, ill-defined targets. The resulting path is often convoluted, peppered with roadblocks and pitfalls and at best is a fairly untidy process.

This is an account of a real project, warts and all. It is not untypical of projects conducted either in academia or the pharma industry. Ultimately, progress is made and knowledge is gained as new data is obtained from the biology, chemistry and from the modeling if it is carefully applied on the way.

In this case, despite the difficulties described below, the project enjoyed a relatively rapid progression from a patent bust to a novel and selective series with many of the problems associated with the target ultimately solved. The project is on track and Cresset's modeling has provided unique and valuable insights and will continue to add value as it is applied moving forwards.

Abstract

From 2012 to 2014 Cresset consulting services contributed computational modeling effort to a three-way collaboration with the University of Newcastle and Sygnature Discovery on an MRC funded osteoarthritis project. Strathclyde[1] and Newcastle[2] Universities conducted vital experiments that identified the protein matriptase as a key mediator in disease related pathways leading to collagen degradation in joints. This protein target[3], a member of the Serine protease family, had been crystalized with peptidomimetic inhibitors adapted from a urokinase inhibitor series by Steinmetzer[4]. These contained the well-known benzamidine P1 pocket interacting groups which are critical for potency in many of this class of protease.

The aim of the modeling exercise was to provide Newcastle University with a new lead series of inhibitors of matriptase, with both an IP position and importantly a more favorable profile for delivering in-vivo activity.

Method

Templating

Analysis of the matriptase crystal structures (PDB: 2GV6, 2GV7 and 3P8G) led to an initial binding hypothesis for the rest of the Steinmetzer SAR set. However, the best compound from the series was not crystalized and so the ability to model this into the crystal structures, using Cresset's proprietary XED force

field, required validation first. This was attempted using the 2GV6 ligand itself. This gave a surprising result (Figure 1, magenta structure), which challenged us to investigate the system in more detail incorporating additional targets and structures e.g. urokinase (PDB:2VNT) and trypsin (PDB: 1K1L).

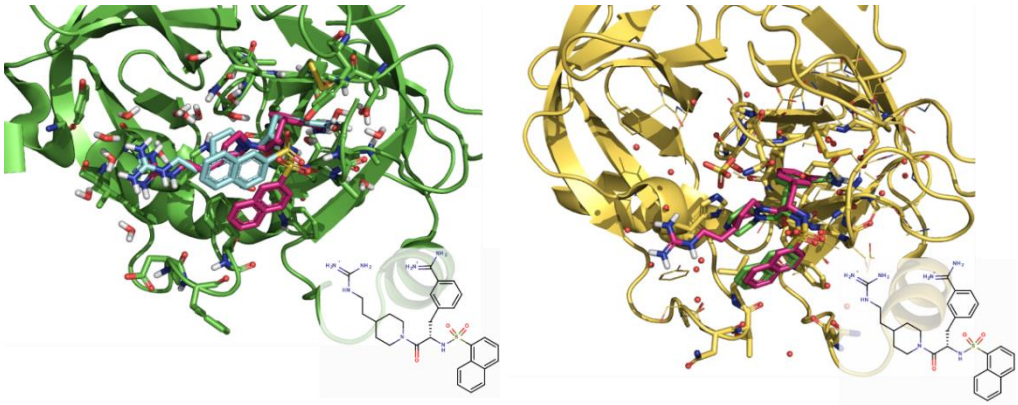

Figure 1. Dimeric matriptase (PDB: 2GV6) shown in green ribbon (one monomer shown) with sulphonamide inhibitor (cyan) and inhibitor (magenta) modeled in the monomeric form. Monomeric trypsin (PDB:1K1L) shown in yellow ribbon with inhibitor (green) and overlaid with the same modeled matriptase ligand (magenta).

The further analysis revealed that the protein ligand complex, which was actually dimeric as observed in the crystallography (cyan structure), involved significant interactions, both protein to protein and ligand to ligand, which ultimately controlled the observed binding mode. This fact resolved the conundrum of why some extremely hydrophobic parts of these ligands were putatively solvent exposed (i.e., they are not), but also highlighted the possibility that a mix of both monomeric and dimeric protein targeting had to be considered for the correct interpretation of the SAR.

Satisfyingly, the XED force field very accurately modeled the energy minimized inhibitor in the monomeric form of the protein and indeed also, as the more likely scenario for, one of the best ligand candidates (Figure 2) based on the Steinmetzer SAR.

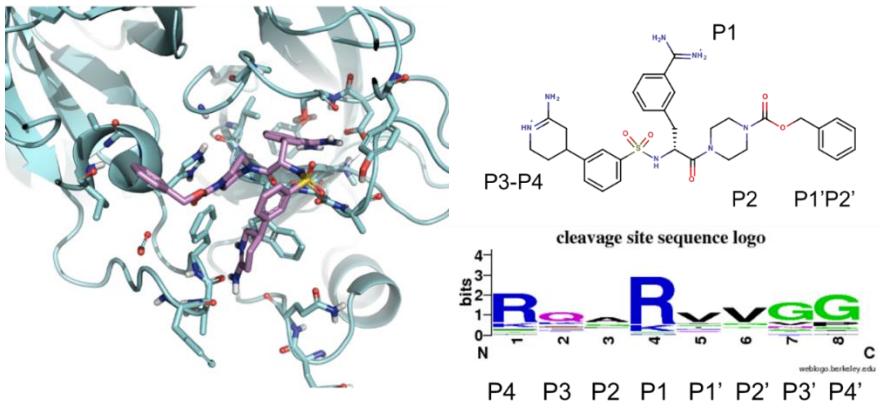

Figure 2. Matriptase binding model (PDB: 2GV7) shown in cyan ribbon with sulphonamide inhibitor (lilac) modeled in the monomeric form. Schematic binding assignment of the inhibitor's component parts to the matriptase serine protease pockets and matriptase substrate residue preferences.

Blaze virtual screening

Armed with the binding hypothesis for the most potent, but least basic, ligand example Cresset's virtual screening technology, Blaze, could be applied to find alternative scaffolds from a library of over 3 million commercially available screening compounds. Since the main liability of the reference compound was the high TPSA (> 145) and high basicity (pKa=11.6), mainly due to the presence of the benzamidine moiety, we ensured that guanidines and benzamidines were removed from the search output. This decision was not made lightly, as the benzamidine is a key determinant of potency. However, this would give the best chance of ultimately achieving the oral bioavailability target – given a willingness to optimize from a potentially weak starting point. The strategy, due to the rather large ligand, was to split the template into three appropriately sized, and more lead-like, molecules as shown in Figures 3a and 3b.

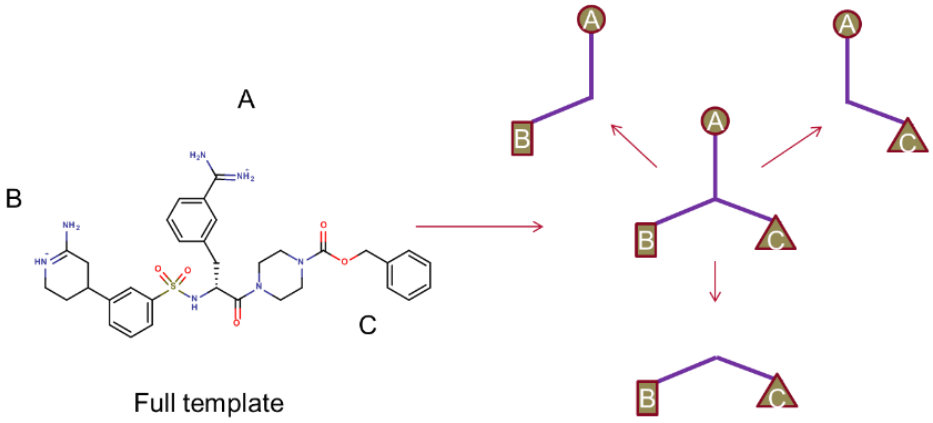

Figure 3a. Fragmentation strategy for Blaze search molecules.

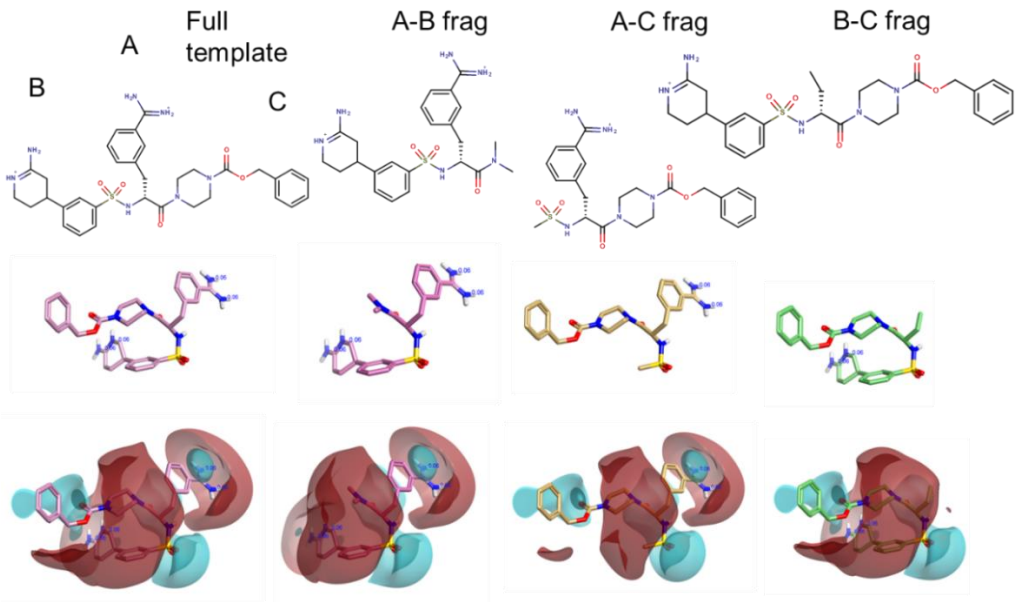

Figure 3b. Reference template fragmentation into three lead-like search molecules and their corresponding electrostatic field surfaces.

Unfortunately, only a small proportion of the virtual screening output was ultimately purchased and screened and of a 142 molecules only a single example was found (Compound 0154) which had appreciable activity at matriptase - albeit relatively weak (IC50 30μM).

This molecule was identified from the A-B fragmentation and so lacked the C limb of the template molecule. The hit was not highly basic thus it is interesting in terms of its optimization potential and because it satisfies the desired property profile we were aiming for. However, further disappointment followed from a second round of compound purchase of commercially available analogues; 29 of which did not provide any further more potent examples.

A parallel patent busting approach

At this time the chemistry partner had put in place parallel efforts on a patent busting strategy from a recently reported synthetic inhibitor. The disadvantage of this approach was that the patent bust was from a symmetric tribasic molecule and so optimization, particularly of the ADMET profile, would be problematic. Furthermore, the binding hypothesis modeling was made more complex by the inherent ambiguity provided by the symmetrical starting ligands.

However, this had provided some comparable actives to Compound 0154 (Figure 4) but via a far more expedient chemistry and with potential to design in an IP position.

	NCL 0154	NCL 0214	NCL 0215	NCL 0219	NCL 0223	NCL 0234	NCL 0235
Newcastle	31uM	15% inhibition	15% inhibition	29% inhibition	16% inhibition	20% inhibition	16% inhibition

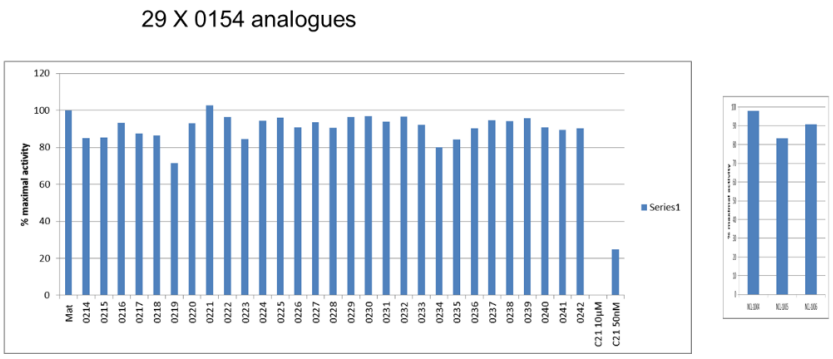

Figure 4. Activities of 29 Blaze hit analogues – and the early patent bust examples (expressed as % of activity of matriptase at 10μM inhibitor concentration).

After a systematic chemical optimization, compounds with reasonable potency began to emerge. After at least one revision of the binding hypothesis, 'on-demand' modeling support for the project finally began to reveal some key binding interactions from the SAR patterns of the new series.

A clear relationship was established between the extent of positive electrostatic field density presented towards a P1'-P2' protein feature and inhibitor affinity. This represented a significant optimization handle for adapting the series, avoiding affinity being dominated by the P1 unit and the liabilities associated with that. Figure 5 shows the electrostatic isopotential surface maps of a number of examples showing this specific effect.

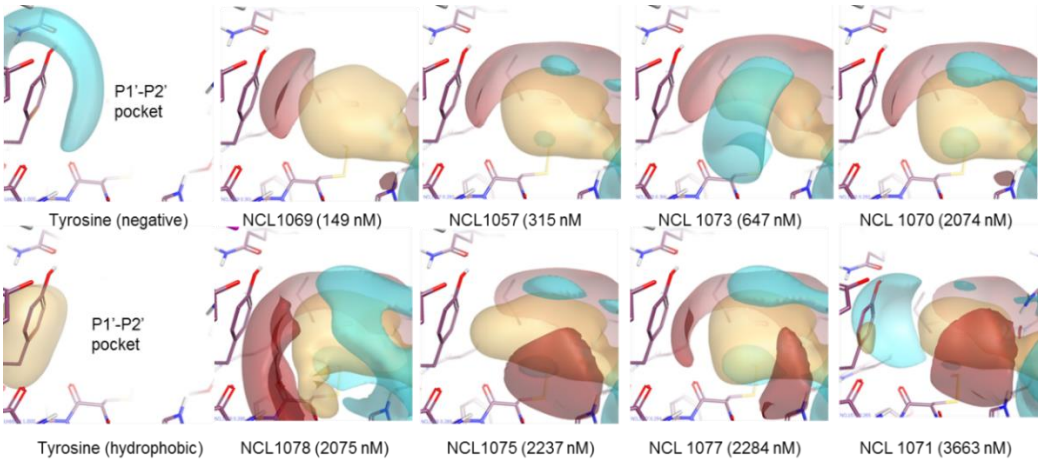

Figure 5. Hydrophobic (brown) and negative (blue) isopotential surface of the tyrosine residue (left). Positive isopotential surface (red), hydrophobic potential (brown) and negative potential (blue) of the P1'-P2' interacting group of a series of novel matriptase inhibitors (in order of decreasing IC50; left to right).

The above figure shows graphically how the activity is related to a combination of the thickness of the ligands positive isopotential surface (i.e., the strength of the electrostatic complementarity between this feature and the face of the tyrosine residue) and steric interactions. The face of the tyrosine aryl ring has a similarly prominent electrostatic surface potential but with an opposite sign (negative).

At the conclusion of the current funding round the compound with the most favored profile was NCL 1066. Details of its profile are shown in Table 1.

Table 1. Compound NCL 1066 profile.

Property (Criteria)	NCL 1066
IC_{50} *(<0.1μM)* Ki *(<1μM)*	16nM 7.8nM
Selectivity (fold over matriptase): (Hepsin, Thrombin, Matriptase-2, Trypsin) (>10 fold)	149x, 173x, 426x, 13x
MW *(<500)*	502
cLogP *(<5)*	2.8
HBA *(<10)*	9
HBD *(<5)*	7
tPSA *(<150)*	145

Property (Criteria)	NCL 1066
H plasma stability *(>50% rem/ 2h)*	100% rem/ 2h
HLM	<1μL/ml/mg protein
Caco A-B flux (P_{app}) *(no significant efflux observed)*	0.32 x10^{-6} cm/s (ER ~1.33)
CyP_{450} inhibition: *(3A4, 2C9, 2C19, 2D6 and 1A1) (all >10uM)*	All IC_{50} >25uM
Herg IC_{50}	>5μM
Acute cytotoxicity, PBMC's	EC_{50} = 54.8μM MEC = 39μM
Novelty *(patentable)*	Novel IP

Conclusion

The project enjoyed a relatively rapid progression from a patent bust to a novel and selective series with many of the problems associated with the target ultimately solved. One exception was the main liability issue of 'membrane penetration' which was present from the outset and remains to be resolved. But, with one potent series well established, and a second series from the virtual screen with potential to be explored, the project is on track to provide what was promised.

Cresset's modeling provided unique and valuable insights to the project and will continue to add value as it is applied moving forwards towards the development of series 1 and 2.

Acknowledgements

Sygnature (chemistry): L. Duffy, P. Meghani; University of Newcastle (biology): Prof. Drew Rowan, W. Hui, D. J. Wilkinson, A. Destrument, S. Watson; Cresset (modeling): A. Vinter.

References and Links

1. Ferral, W. R. et al, Protease-activated receptor-2: a novel pathogenic pathway in a murine model of osteoarthritis, *J. Arthritis & Rheumatism,* **2010** (http://strathprints.strath.ac.uk/20137/).
2. Rowan, A. D. et al, *Matriptase Is a Novel Initiator of Cartilage Matrix Degradation in Osteoarthritis, Arthritis & Rheumatism*, **2010**, Vol. 62, No. 7, pp 1955–1966.
3. http://merops.sanger.ac.uk/; search: ID = S01.302: Name = matriptase.
4. Steinmetzer, T. et al, Secondary Amides of Sulfonylated 3-Amidinophenylalanine. New Potent and Selective Inhibitors of Matriptase, *J. Med. Chem.* **2006,** *49,* 4116-4126.