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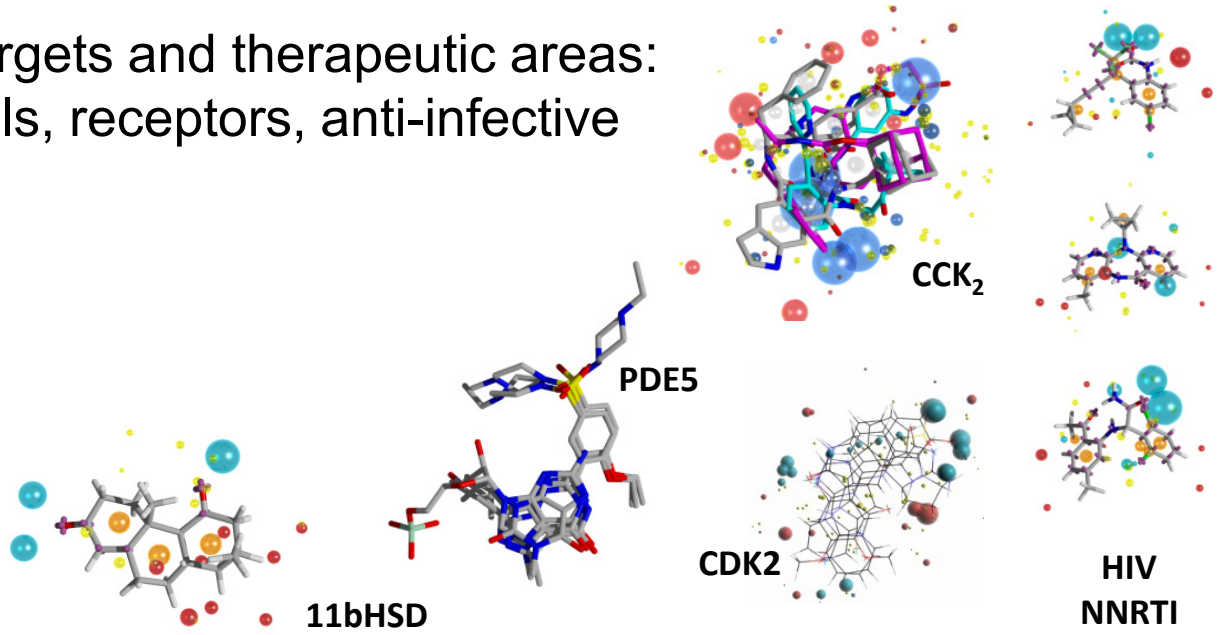
smarter chemistry | smarter decisions™

New inhibitors for a novel osteoarthritis target via a three-way collaboration with Cresset's consulting services

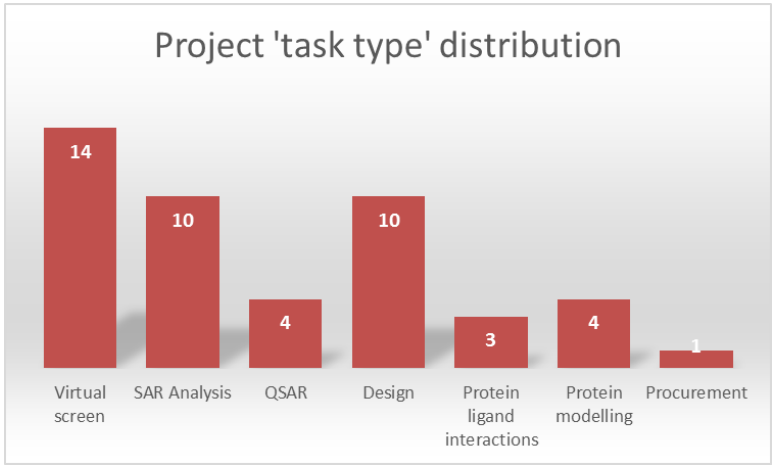
Dr Martin J. Slater

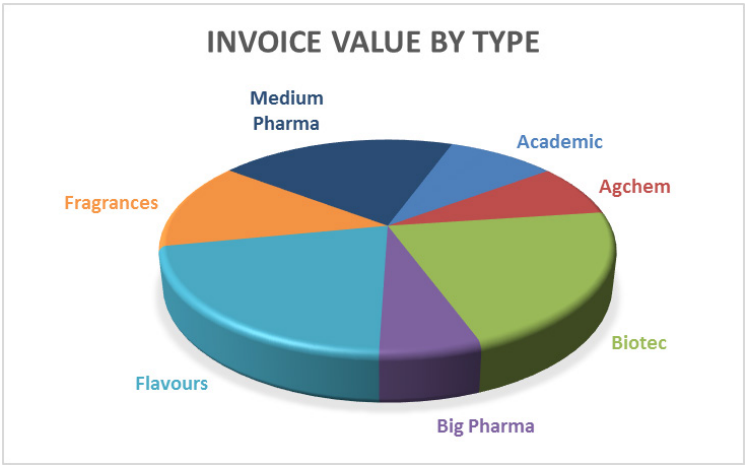
A proven track record

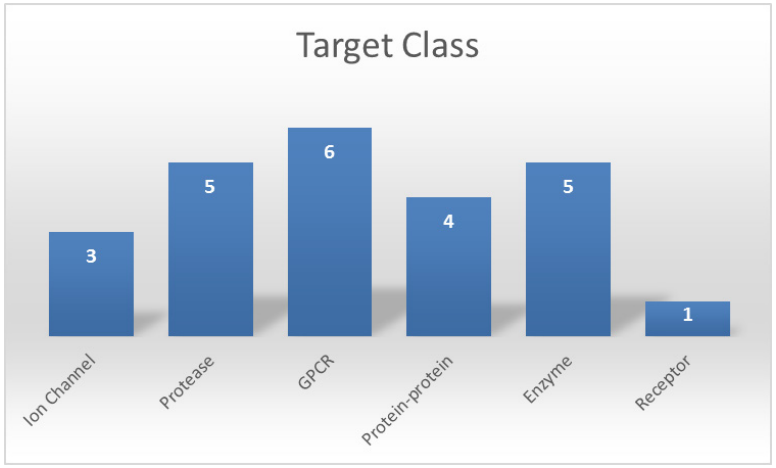
- Cresset has been offering consulting services for over a decade
- Over 160 projects completed for customers
- Scientists with over 120 years combined industrial experience
- Broad range of targets and therapeutic areas: enzymes, channels, receptors, anti-infective

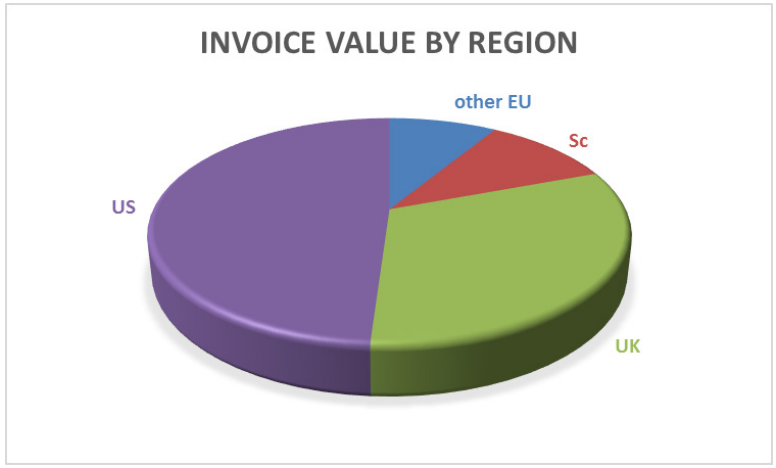


A Cresset services year: projects









As your science partner we share your goals and challenges

Cresset consulting services case study

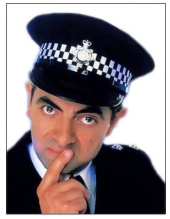
A three-way Services Collaboration on an MRC funded Osteoarthritis project:

Professor Drew Rowan
Musculoskeletal Research Group,
Institute of Cellular Medicine,
University of Newcastle

Prem Meghani and Lorna Duffy
Sygnature Discovery Ltd

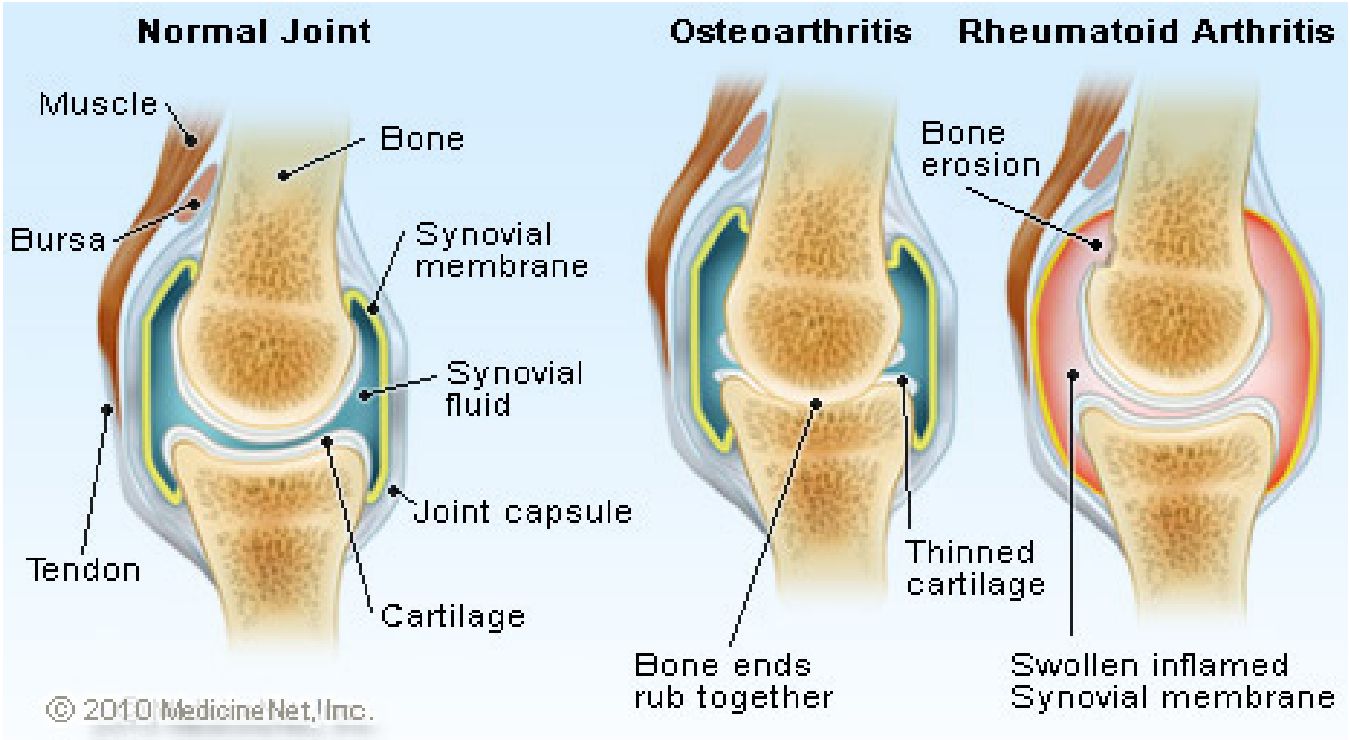
Martin Slater
Cresset

Andy Baxter
Consultant



Background: osteoarthritis & rheumatoid arthritis

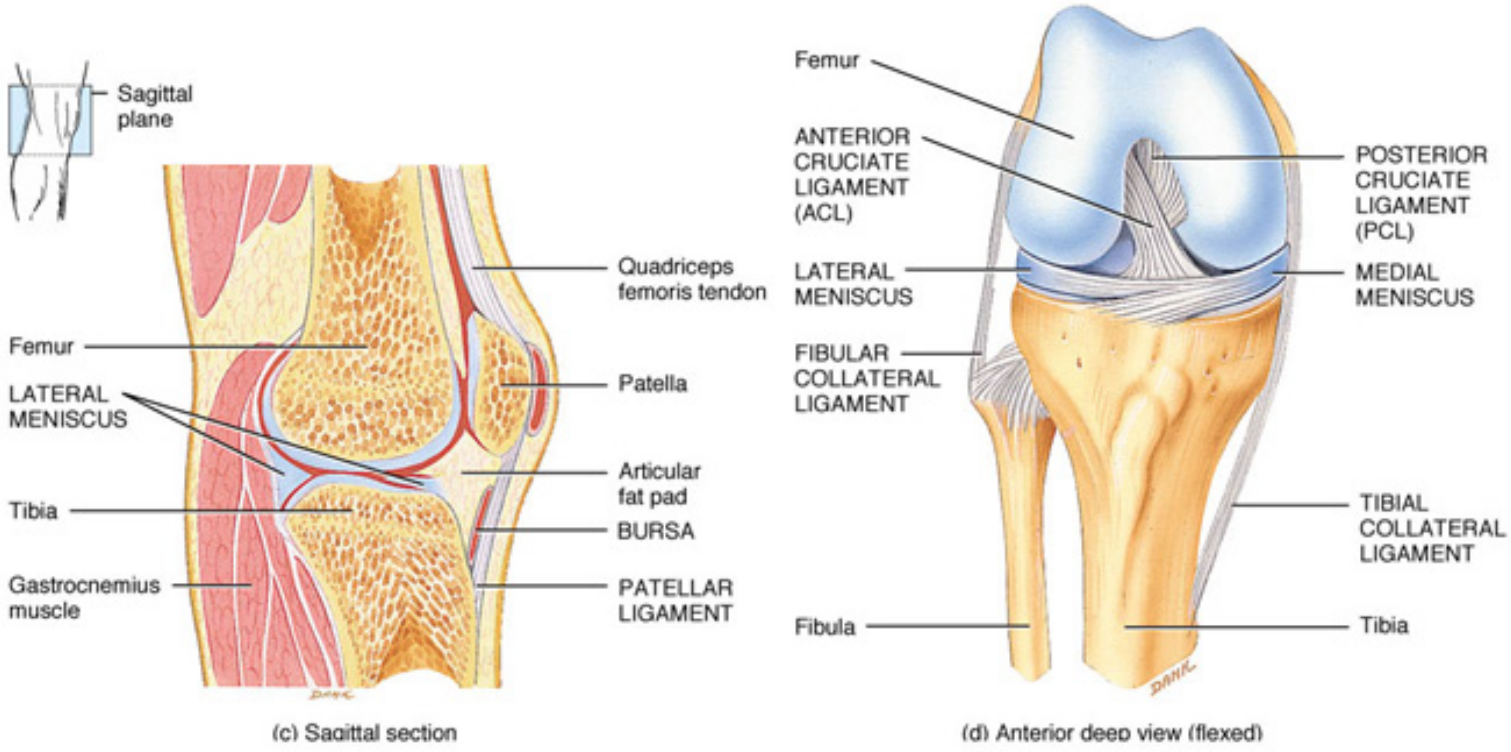
What is osteoarthritis?



Normal and Arthritic Joints

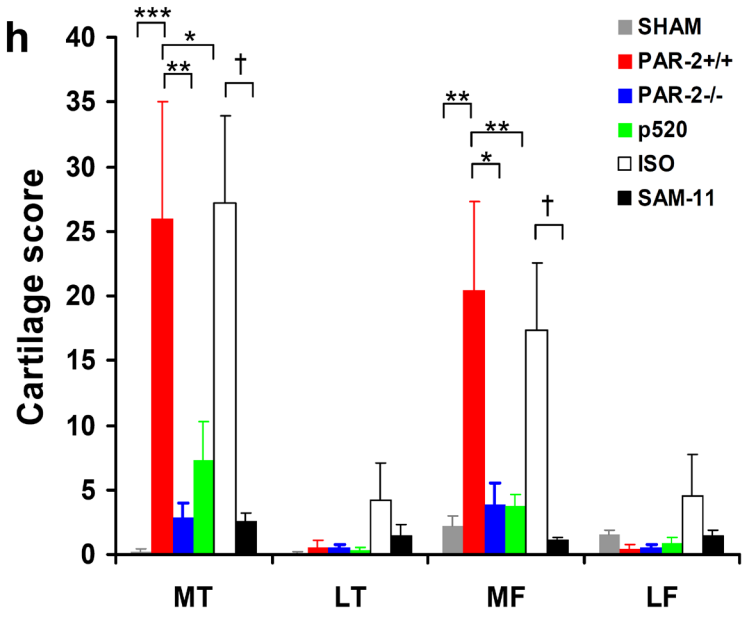
Background: *in vivo* model: DMM mouse

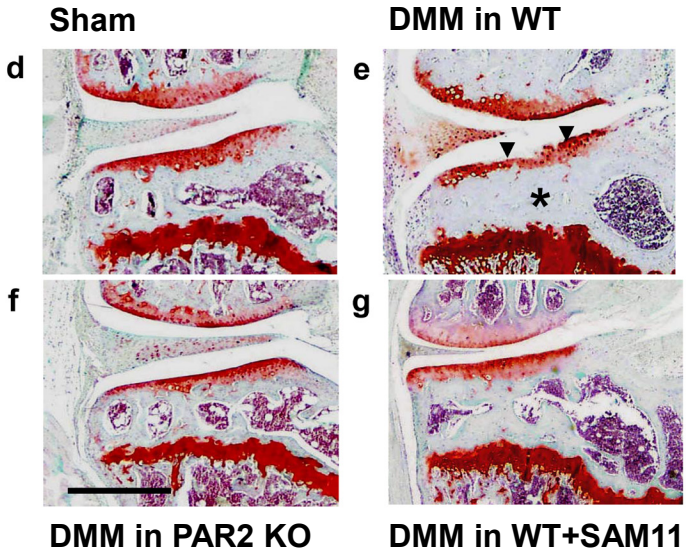
In vivo mouse model: DMM via MMTL section



Background: PAR-2 implicated

Deletion of PAR-2 prevents murine OA (4 weeks)



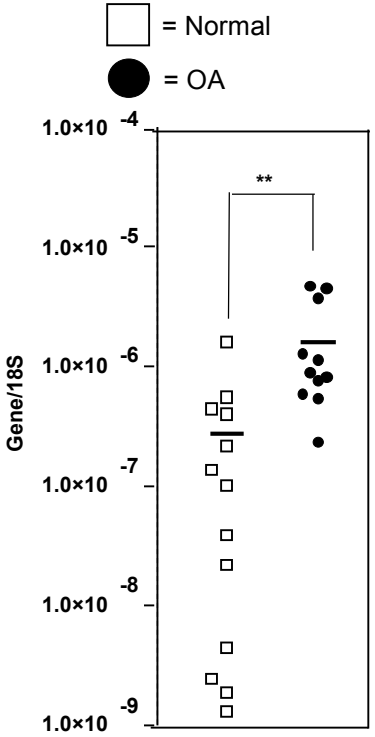


Protease-activated receptor 2: a novel pathogenic pathway in a murine model of osteoarthritis

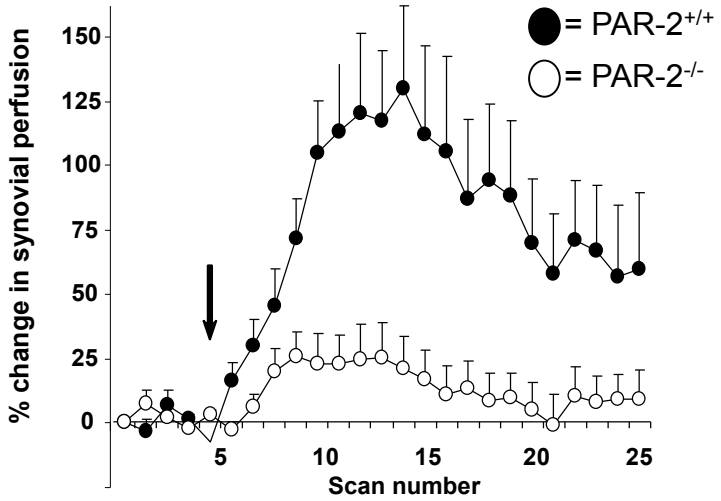
William R Ferrell,[1] Elizabeth B Kelso,[1] John C Lockhart,[2] Robin Plevin,[3] Iain B McInnes[4]

Background: Previous work at Newcastle

Matriptase identified as being up-regulated in human OA cartilage



PAR-2 is a substrate of Matriptase



in vivo, murine vasodilation assay

= addition of matriptase to exposed knee joint capsule

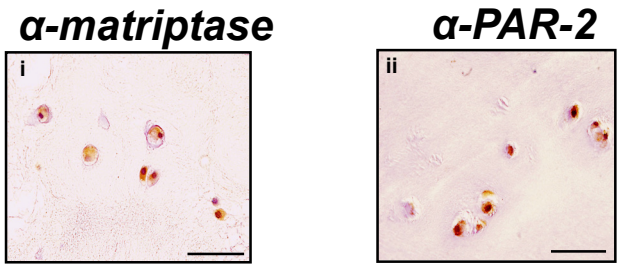
ARTHRITIS & RHEUMATISM
Vol. 62, No. 7, July 2010, pp 1955–1966

Matriptase Is a Novel Initiator of Cartilage Matrix Degradation in Osteoarthritis

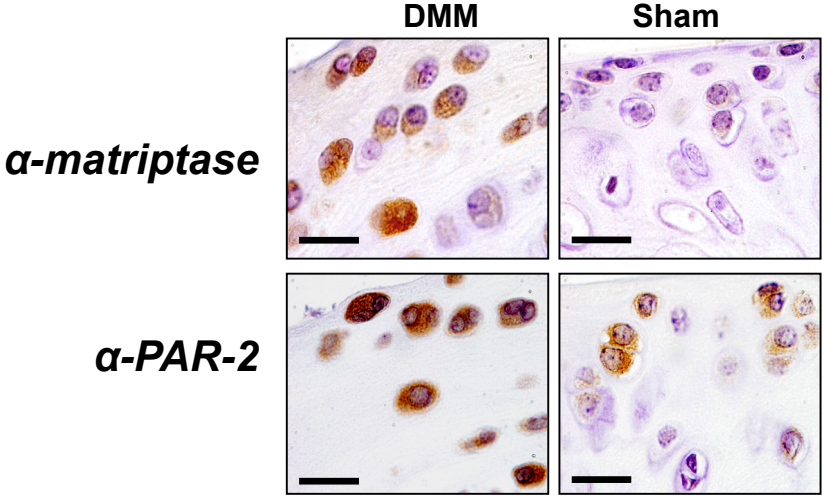
Jennifer M. Milner,[1] Amit Patel,[1] Rose K. Davidson,[2] Tracey E. Swingler,[2] Antoine Desilets,[3] David A. Young,[1] Elizabeth B. Kelso,[4] Simon T. Donell,[2] Tim E. Cawston,[1] Ian M. Clark,[2] William R. Ferrell,[4] Robin Plevin,[5] John C. Lockhart,[6] Richard Leduc,[3] and Andrew D. Rowan[1]

Background: Previous work at Newcastle

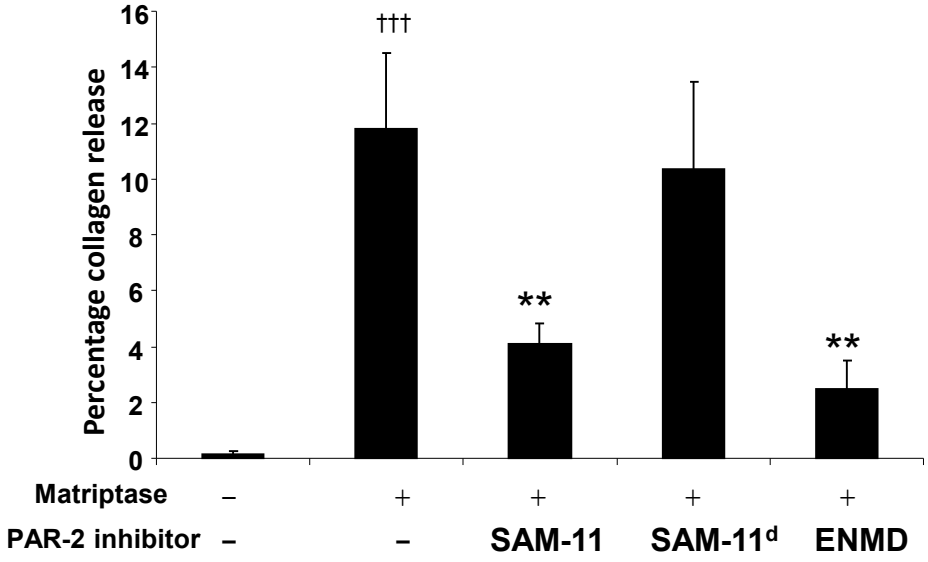
Matriptase, and its substrate PAR-2 are co-expressed in OA cartilage



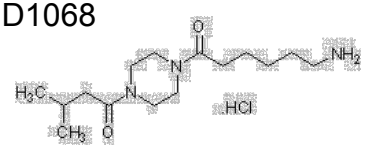
Matriptase and PAR-2 are co-expressed in murine OA



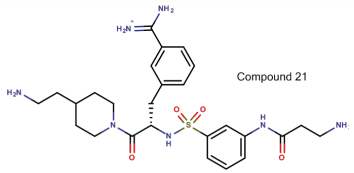
Preventing the action of Matriptase blocks OA cartilage breakdown



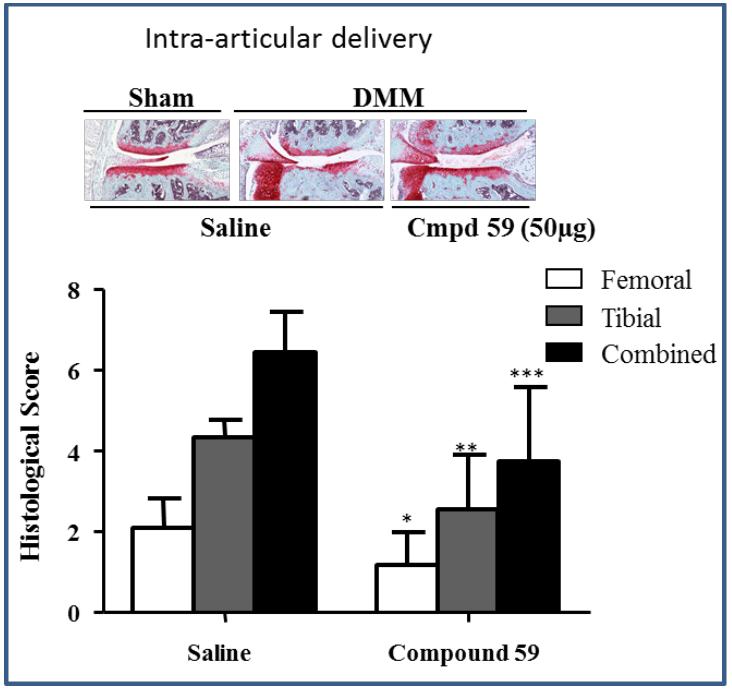
SAM-11 = neutralising Ab
SAM-11^{d} = neutralising Ab (boiled)
ENMD = ENMD1068

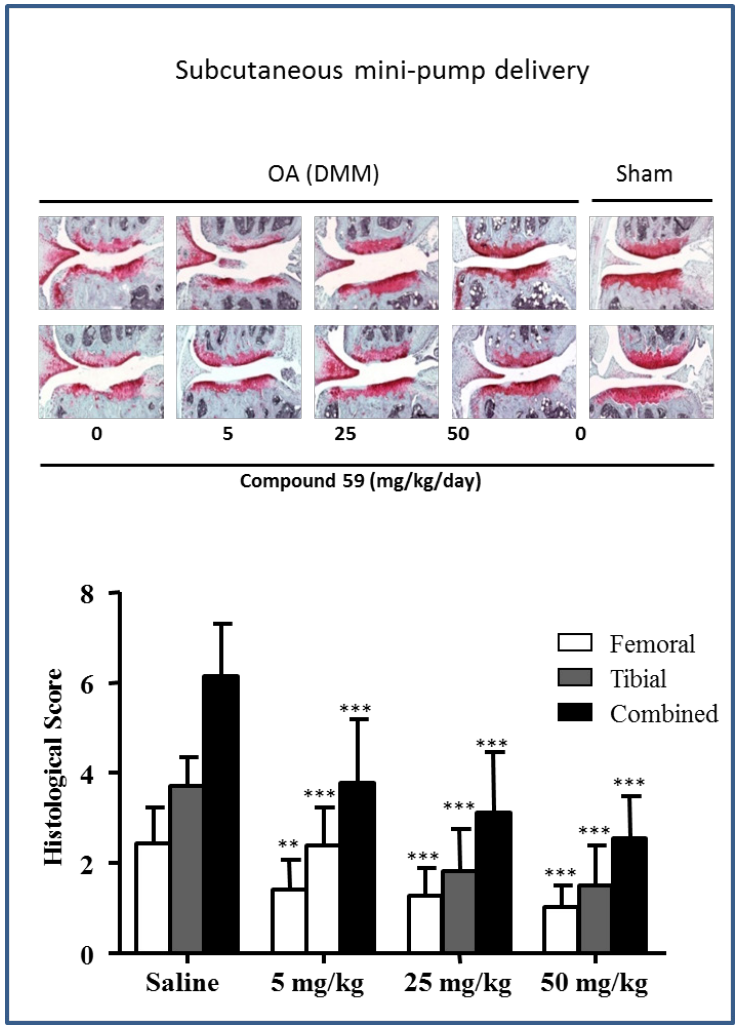


Validation: Matriptase inhibition reduces OA severity



(S)-3-amino-N-(3-(N-(1-(4-(2-aminoethyl)piperidin-1-yl)-3-(3-carbamimidoylphenyl)-1-oxopropan-2-yl)sulfamoyl)phenyl)propanamide, *tris* hydrochloride salt





Secondary Amides of Sulfonylated 3-Amidinophenylalanine. New Potent and Selective Inhibitors of Matriptase†

Torsten Steinmetzer,*,⊥ Andrea Schweinitz,⊥ Anne Stürzebecher,⊥ Daniel Dönnecke,⊥ Kerstin Uhland,‡ Oliver Schuster,‡ Peter Steinmetzer,§ Friedemann Müller,⊥ Rainer Friedrich,# Manuel E. Than,#,∥ Wolfram Bode,#,∥ and Jörg Stürzebecher§

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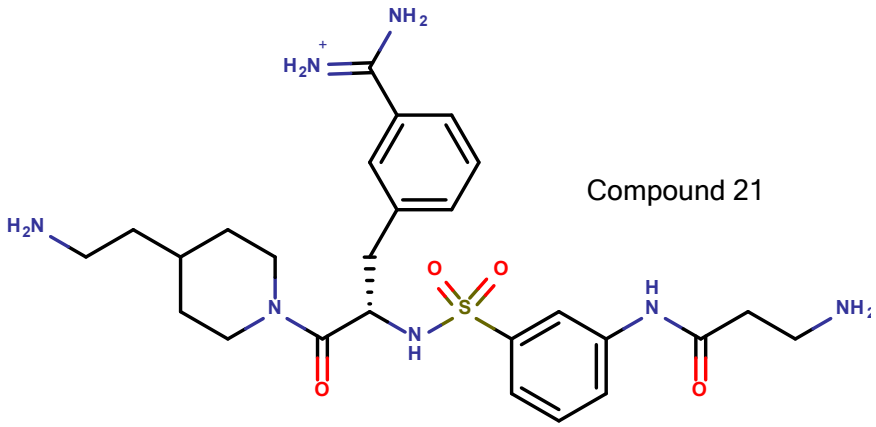
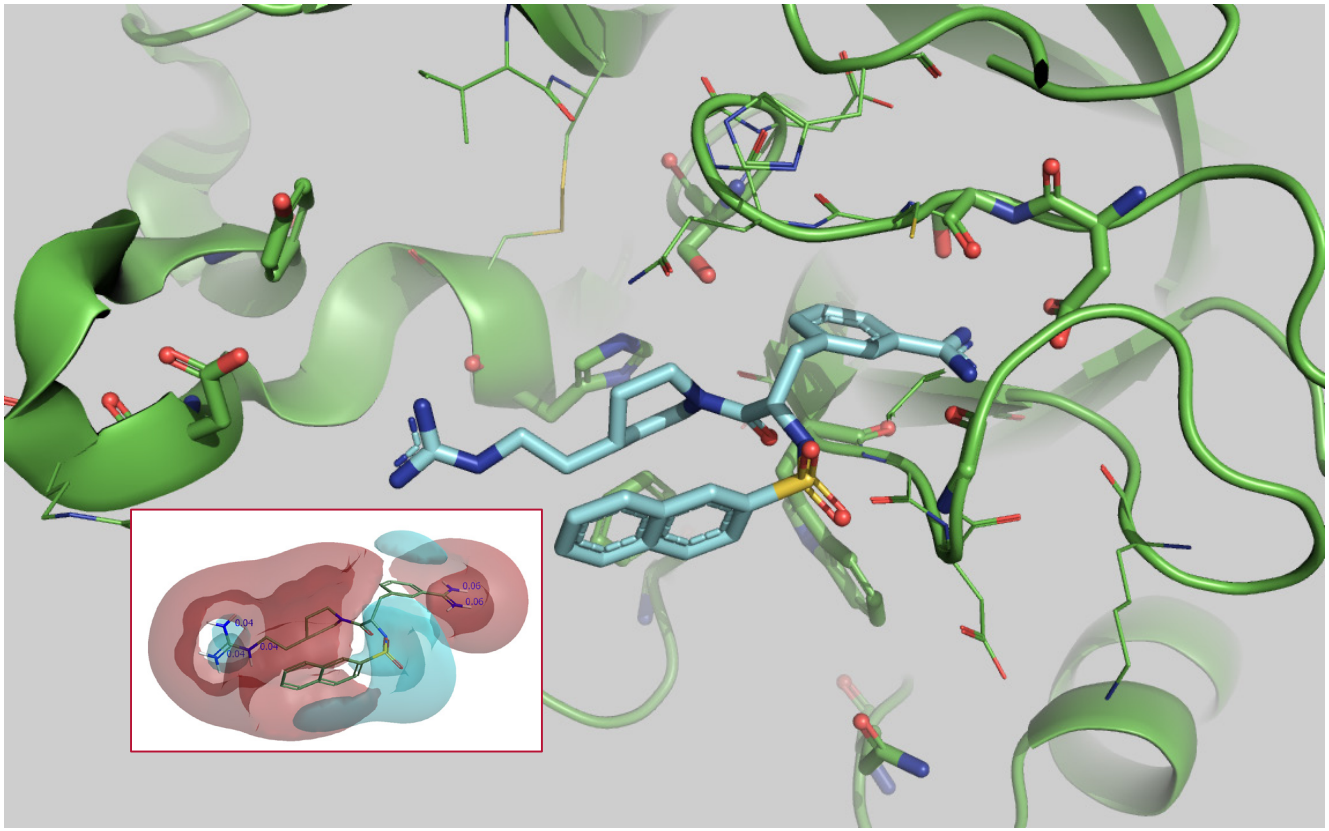
Initial goals – compound 21 SAR analysis

- > Develop suitable best (ideally monobasic) template for virtual screen from cpd 21 analogues i.e. model accurate 3D conformation
- > Retain as much selectivity information in the template – not back to square one
- > Essential to gain 3D understand cpd 21 SAR
- > Replace benzamidine if possible



Compound 21

Original reference series structure



PDB: 2GV6

Sulphonamide in Matriptase dimer.

Benzamidine in S1

Guanidine in S1’/S2’

Naphthyl at dimer interface

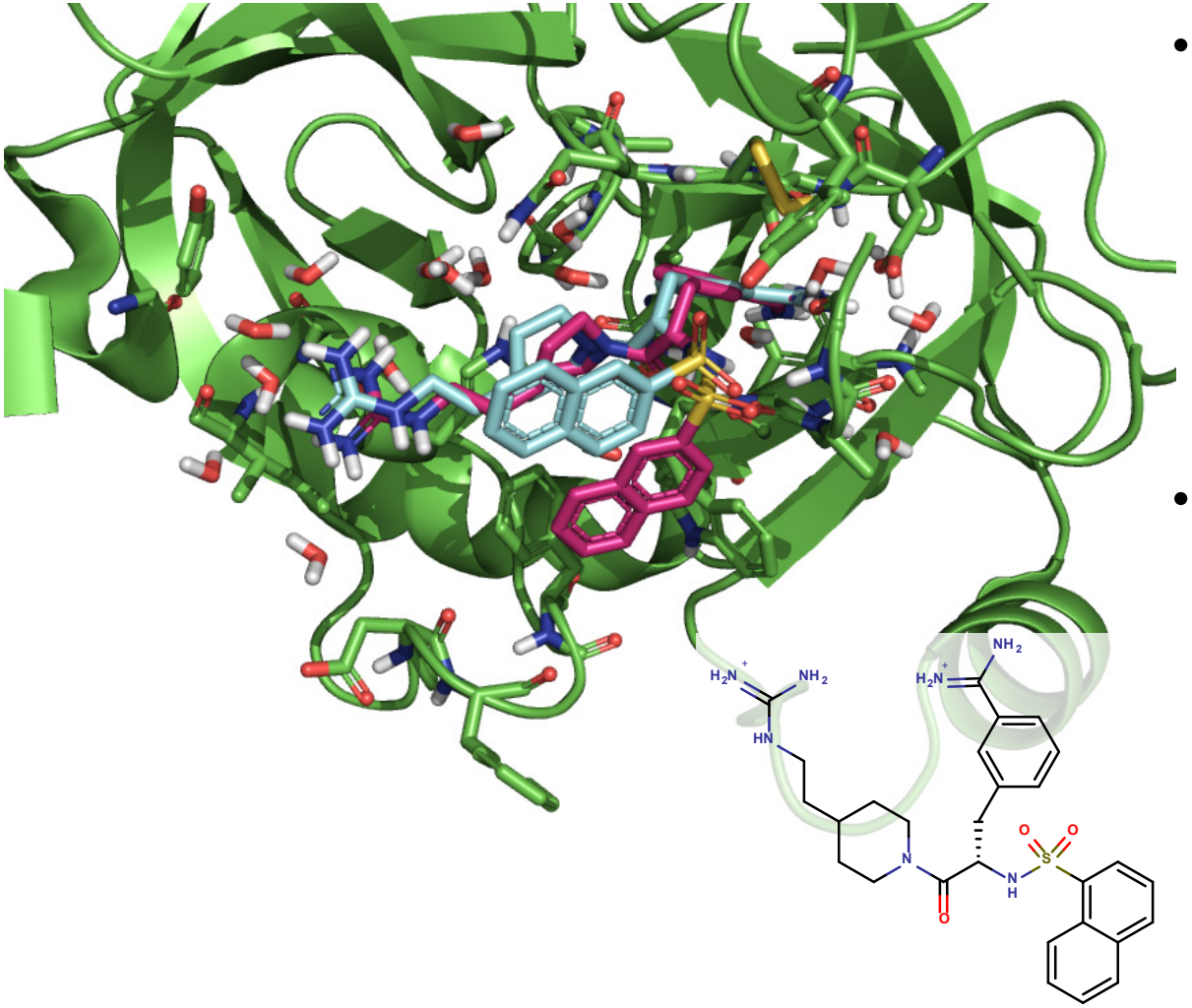
Arginine from other Matriptase protein in S3/S4

Interaction of the Asp in the S1’ 60 loop with guanidinyl piperidine of the sulphonamide series in a folded conformation. Substrate beta strand peptide mimetic H-bonding pattern reminiscent of other peptidic Matriptase inhibitors.

SAR patterns

- Initial cpd 21 series based on generic protease inhibitors hitting urokinase and trypsin
- Optimized towards matriptase potency and selectivity over this and other proteases
- Optimized systems (e.g. cpd21) are tribasic
  - A number of examples incorporate non basic side chains – would be preferred templates
- Benzamidine is critical but too basic - pushes up TPSA
  - Ph inactive, benzylamino – weakly potent

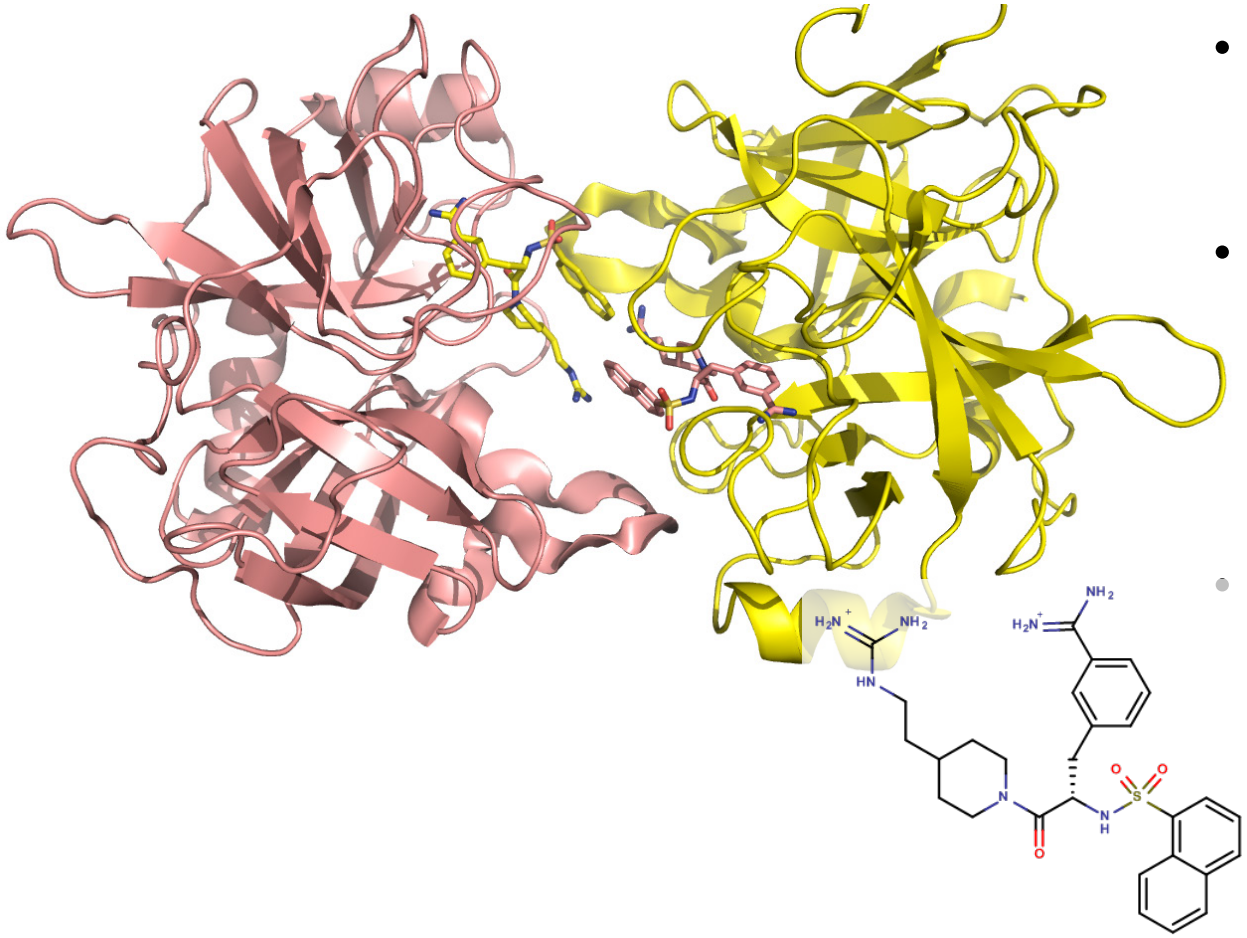
XED force field minimization of 2GV6



- Naphthyl sulphonamide (cyan) is a potential template for development of bioactive cpd21 conformation
- Significant movement observed? on minimization (magenta)

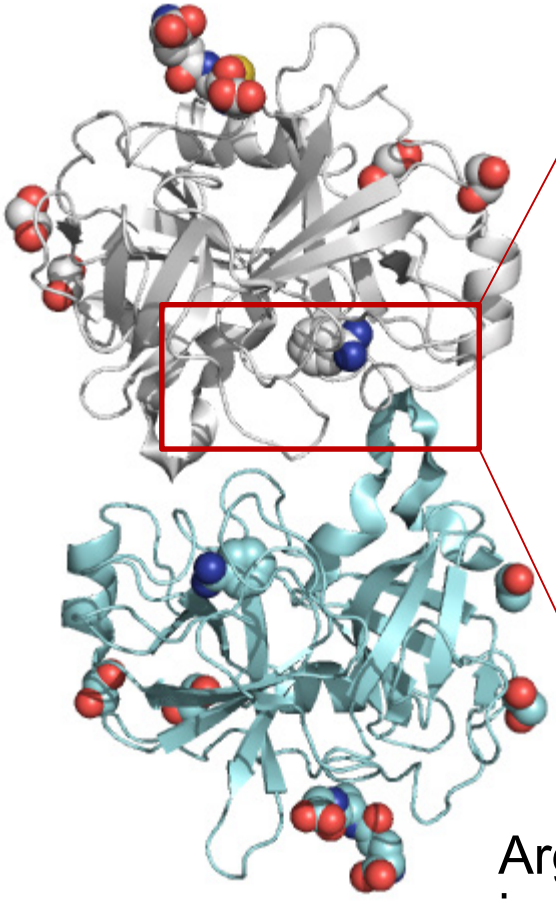
PDB:2GV6 dimer

With (45nM) naphthyl- sulphonamide

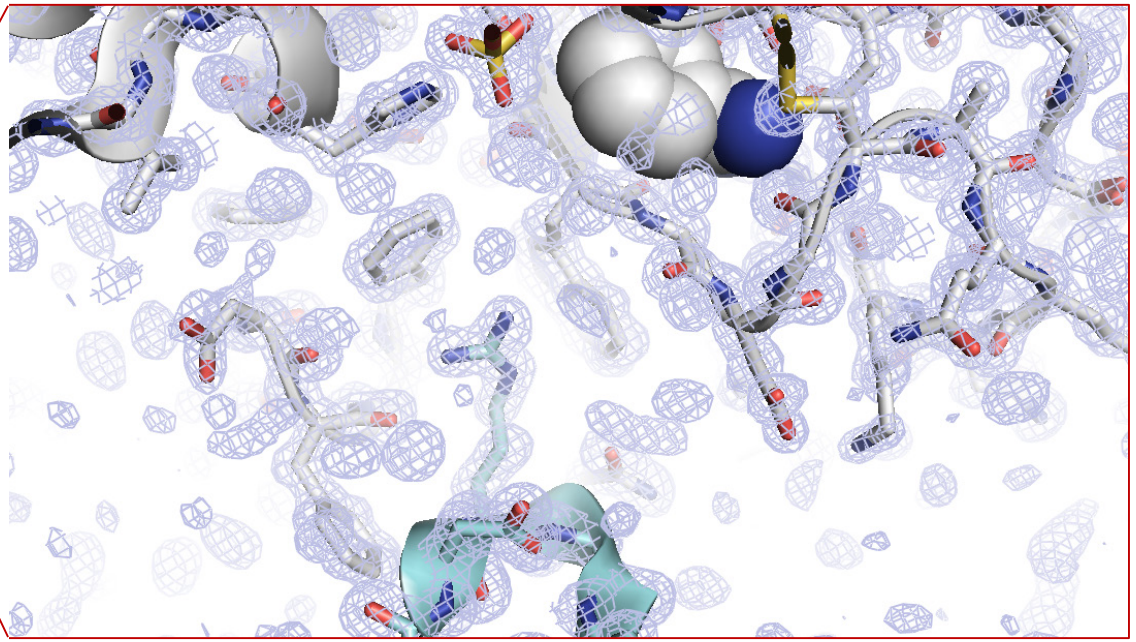


- Explained by visualizing crystal symmetry mates
- Matriptase is a dimer with close protein protein and ligand-ligand contacts
- Arginine from one unit binds below naphthyl group

PDB:3P8G dimer with electron density



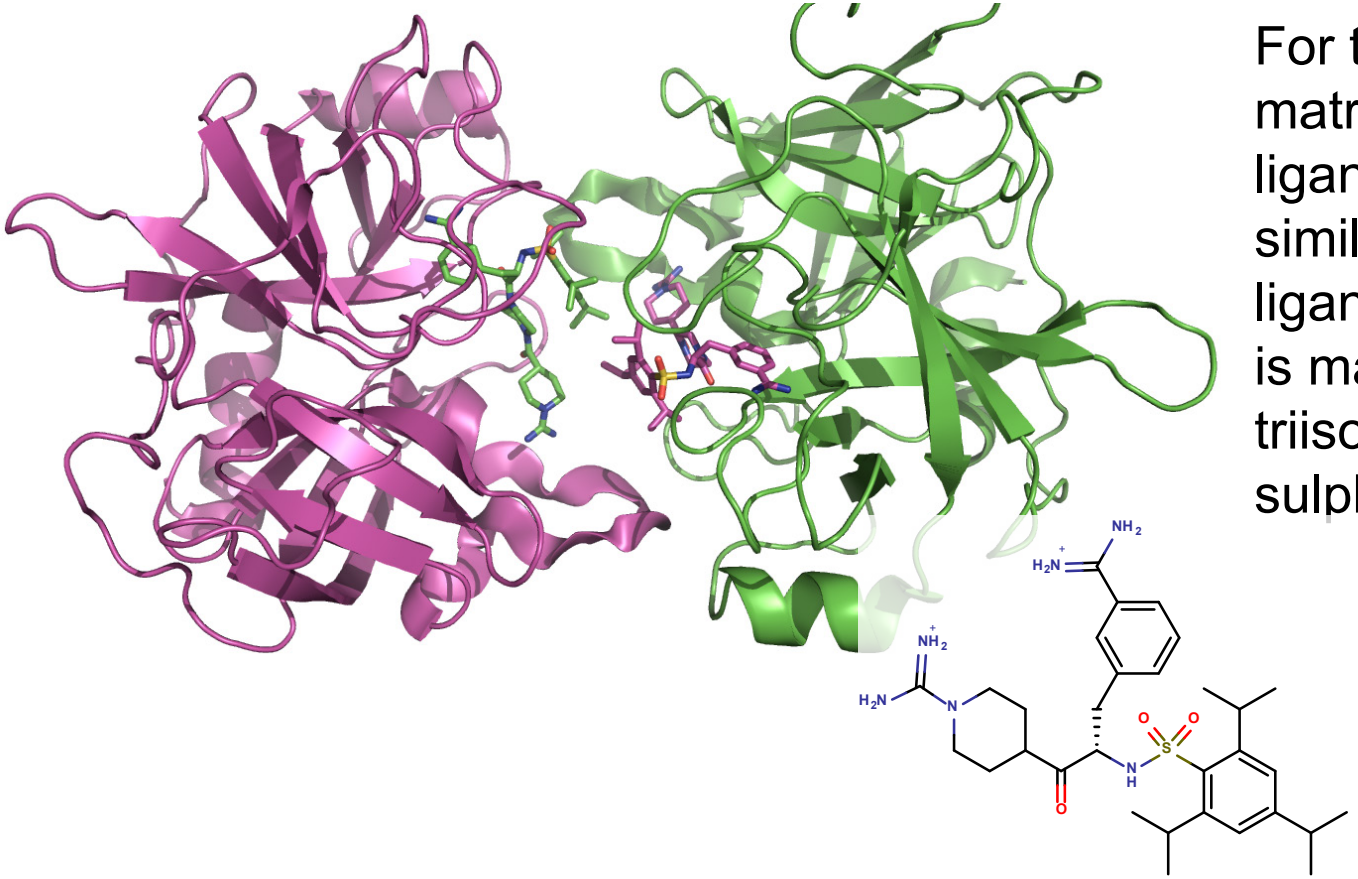
PDB:3P8G dimer with electron density real… but pharmacologically relevant?



Arginine from symmetry related monomer sits nicely in P3-P4 pocket

PDB:2GV7 dimer

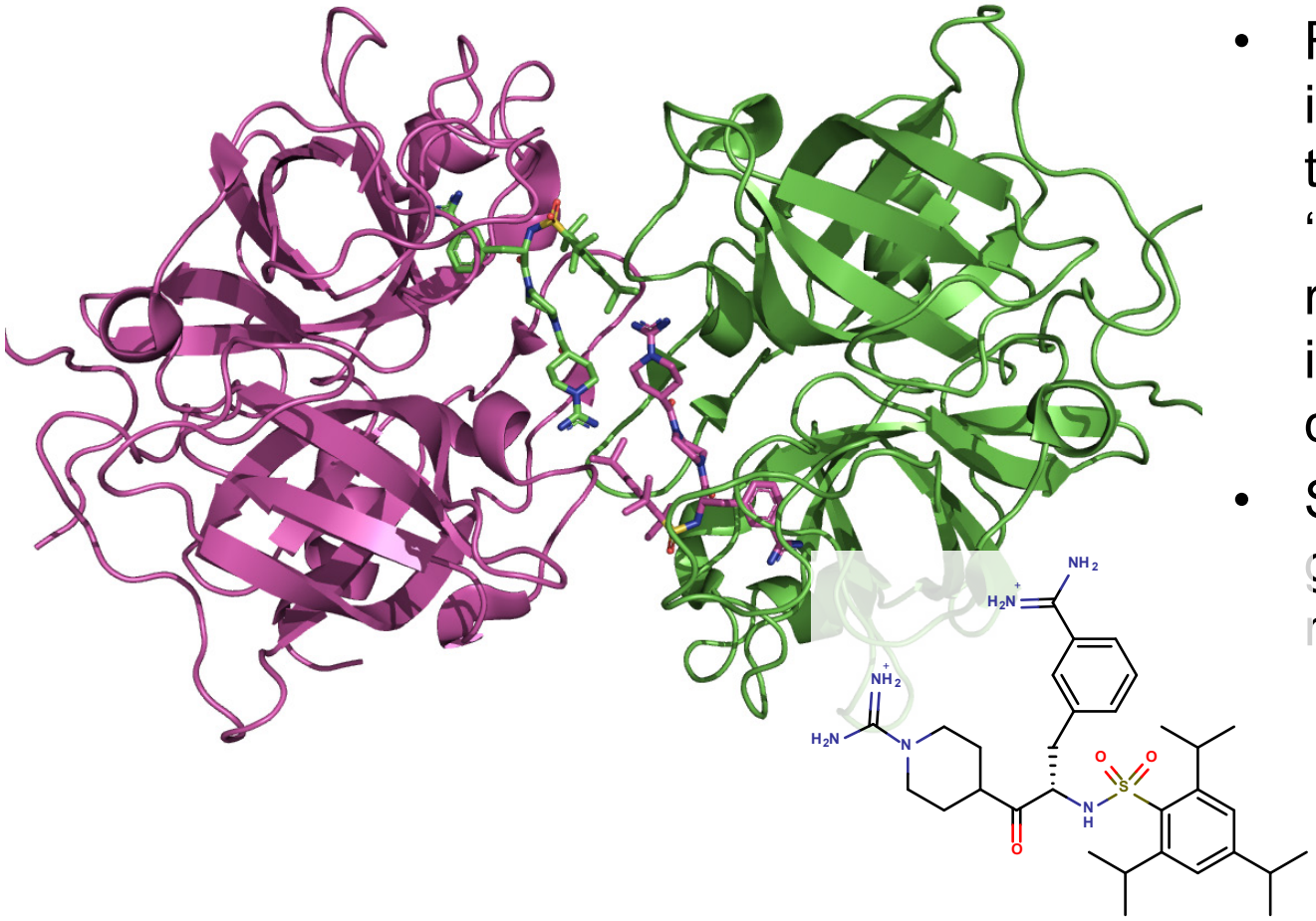
With (14nM) 2,4,6-triisopropyl-phenyl- inhibitor



For the second matriptase bound ligand example a similarly significant ligand-ligand contact is made by the triisopropyl- phenyl-sulphonamide

Urokinase PDB:2VNT

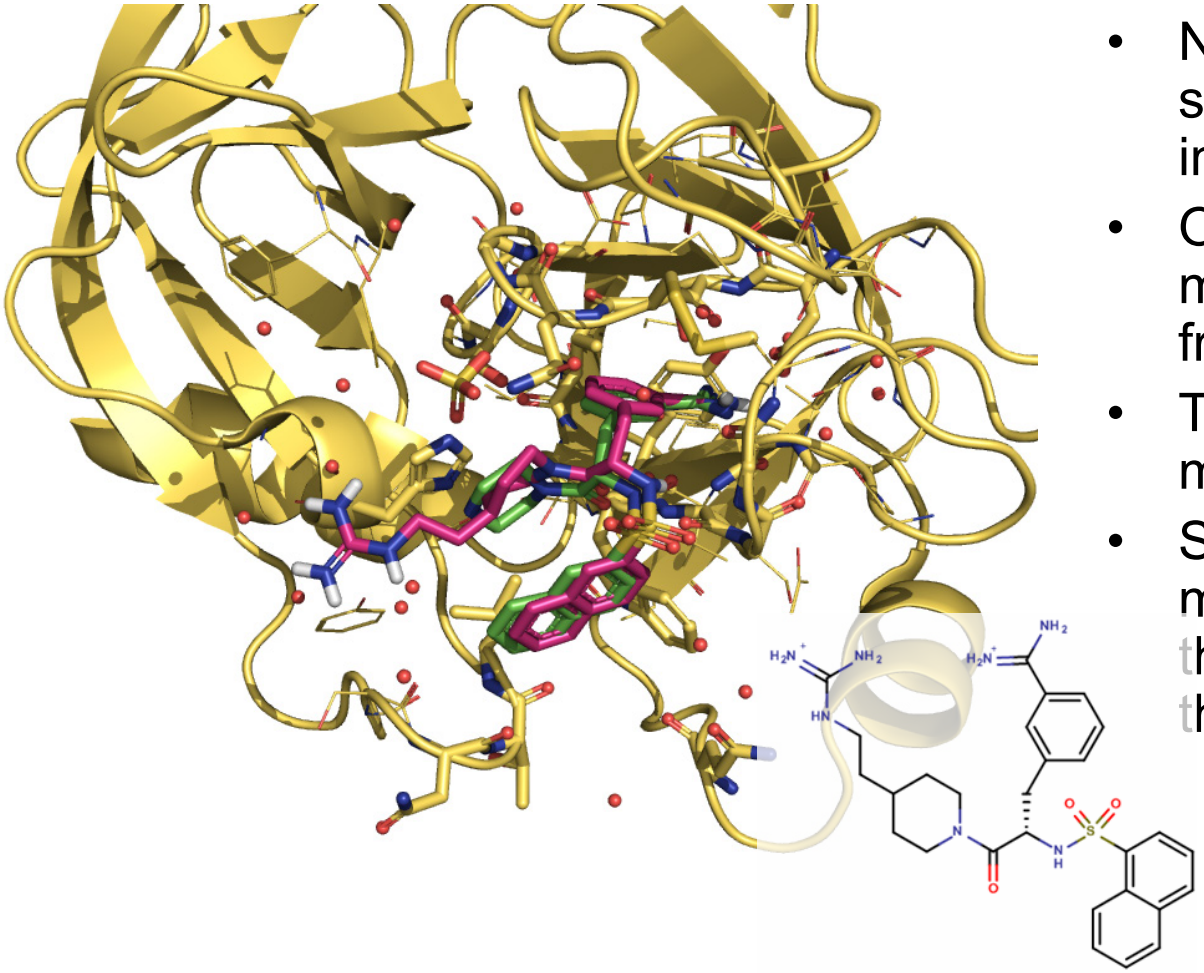
Superimposed with 2,4,6-triisopropylphenyl inhibitor



- Returning to the initial source of these ligands ‘urokinase’ again reveals the involvement of dimeric protease
- Subtly different geometry from matriptase

Trypsin ligand PDB:1K1L

With naphthyl sulphonamide inhibitor



- Napththyl sulphonamide bound into trypsin (green)
- Overlaid with XED minimized structure from 2VG6 (magenta)
- Trypsin structure is a monomer!
- Suggests we can minimize correctly into the monomeric form of the protease

Consequences of dimeric form

- Compound 21 with large and basic P3-P4 group likely to be directed towards monomer

- Small or rotatable P3-P4 analogues may hit the dimer?

  These include the attractive dibasic biaryl cpd 21 analogues

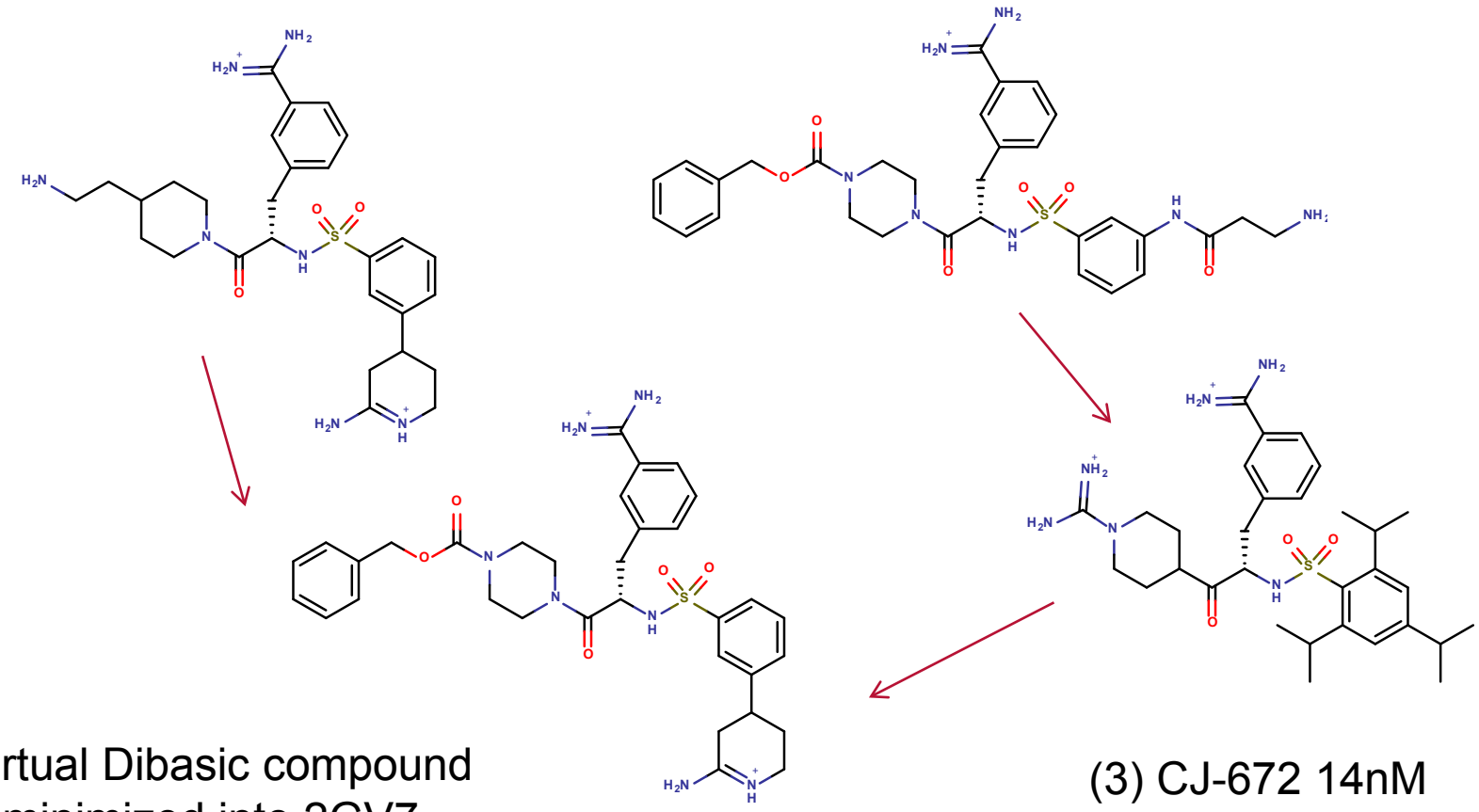
- Ligand-ligand interactions in dimer will provide non-linear SAR:

  We usually assume substitutions are independent which is certainly not true for some examples

Modeling of ‘best’ dibasic template of Cpd 21 SAR

(1) Tribasic BOMCL_19_21 0.08nM

(2) Dibasic_BOMCL19b_6 7.5nM

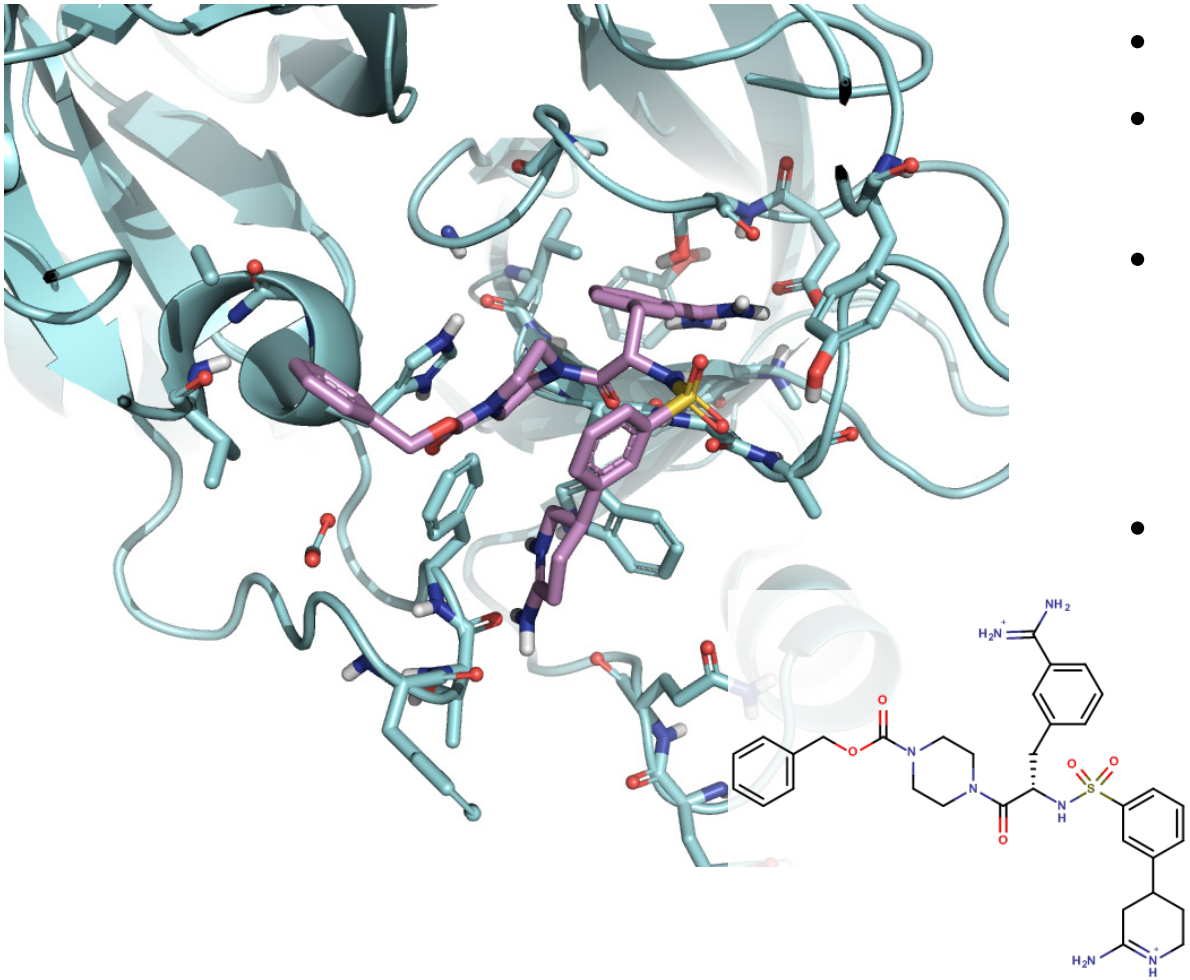


(4) Virtual Dibasic compound
Fully minimized into 2GV7
(monomer) using XED force field

(3) CJ-672 14nM
PDB:2GV7
X-ray (dimer)

Virtual ‘best’ template minimized with XED force field

Modeled into ‘monomeric’ 2GV7



- Dibasic example
- Bound well into matriptase
- Suggests sources of selectivity through P3-P4 and through P2 P1’P2’ substituents
- Removal of guanidine with neutral isosteric benzyloxycarbamate useful selectivity and potency determinant

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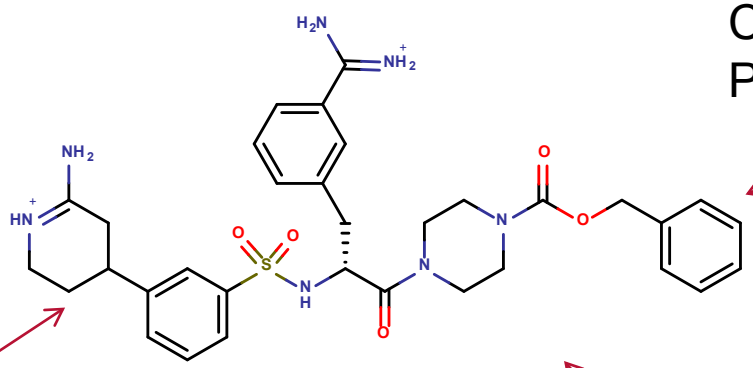
Selectivity / potency profile: best dibasic template

Potency

Potency
Selectivity

Clash thrombin Trp
Plasmin Glu

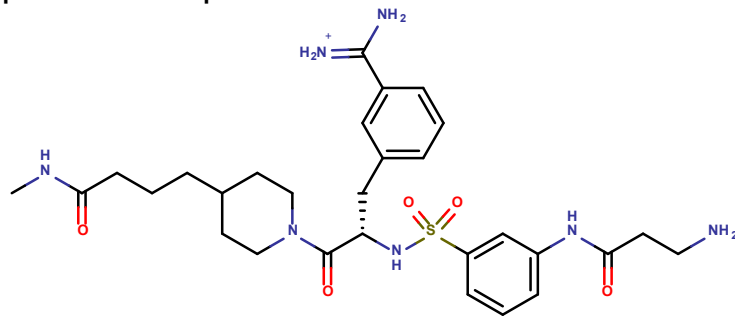


Potency
Selectivity

Clash Urokinase

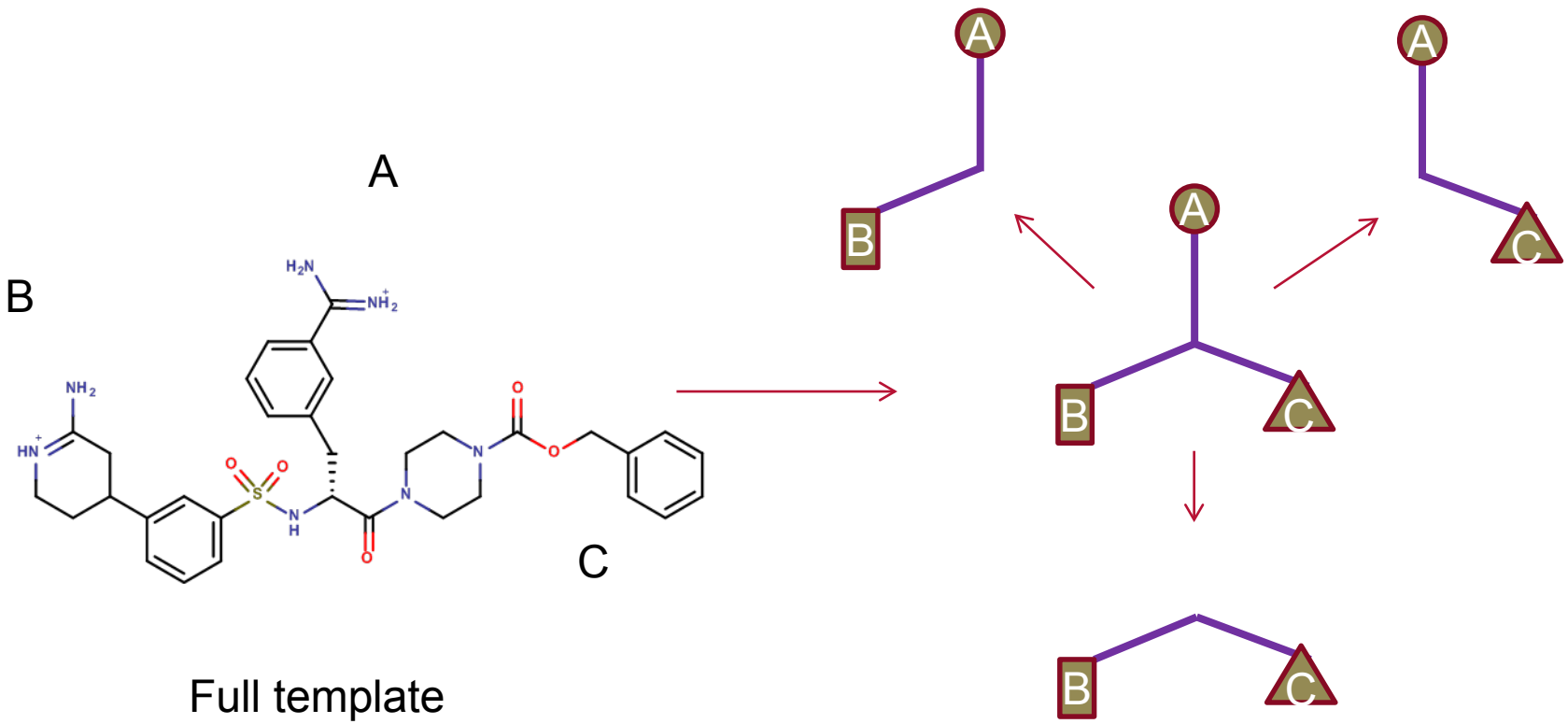
Clash Fxa Tyr

Most potent P3-P4 substitution of cpd 21 analogues less ambiguous conformation compared with cpd 21 amine



Alternative dibasic analogue BOMCL19b_4 (6.3nM) ambiguous P2 –P1' P2' substituent and ambiguous P3-P4 substituent conformation not selective against FXa

Search molecule strategy



Full template

Search molecule/fragment strategy

Full template

A-B frag

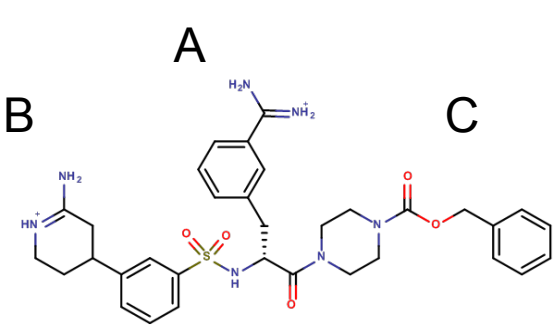
A-C frag

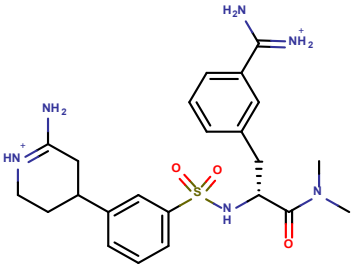
B-C frag

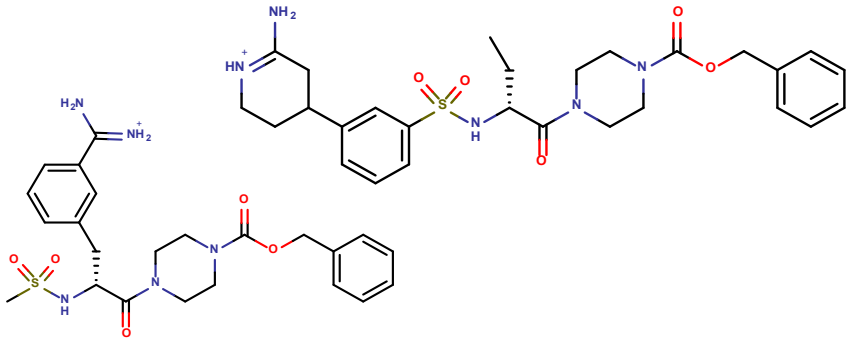
A

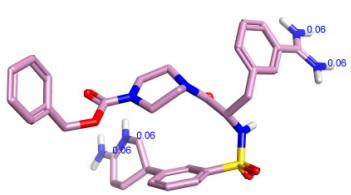
B

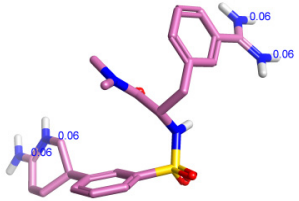
C

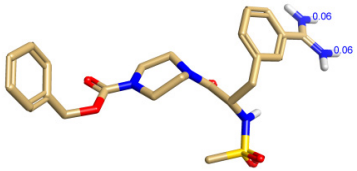


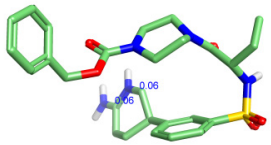


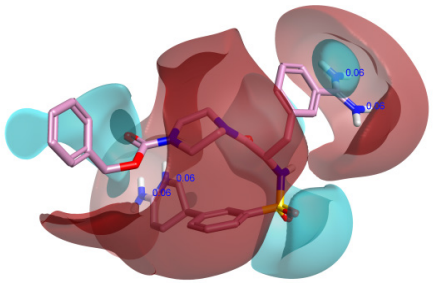


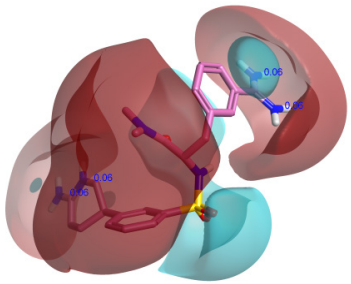


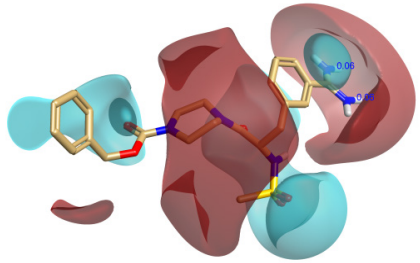


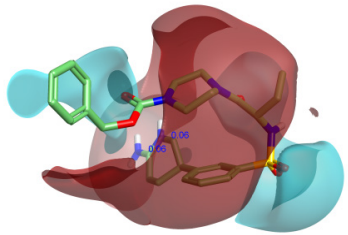












P1 pocket targeting

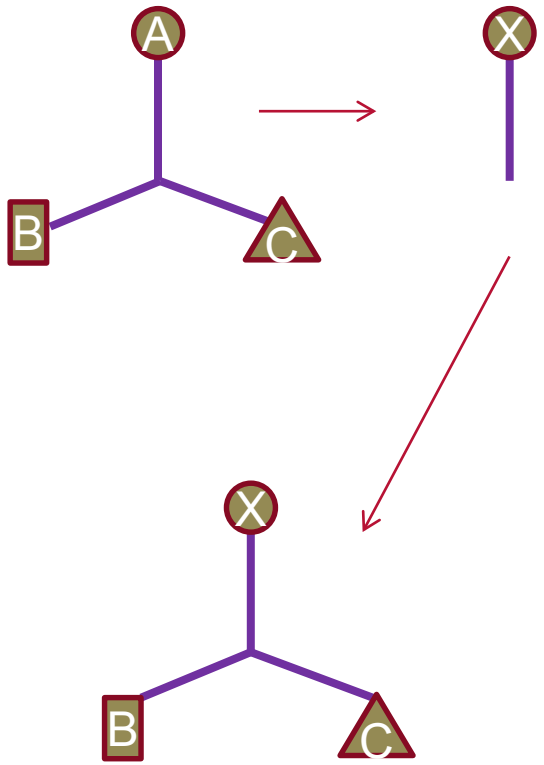
> Removal of Benzamidine is Key for providing a drug-like compound

> Analysis of the x-rays of related proteases suggested alternatives to the benzamidine would be an excellent way to proceed:

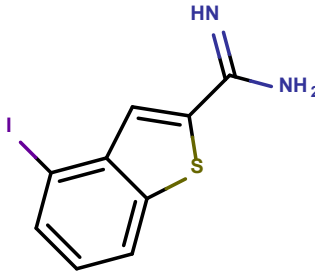
Tricks from Thrombin, Fxa

Tricks from Urokinase

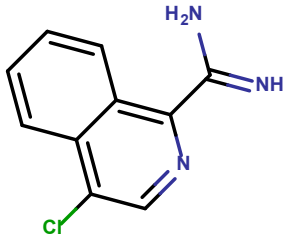
> Care required as these systems are not necessarily interchangable



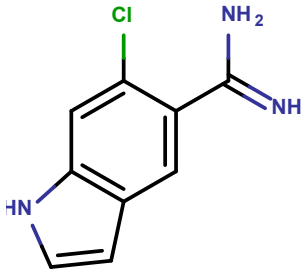
Example alternative P1 fragments



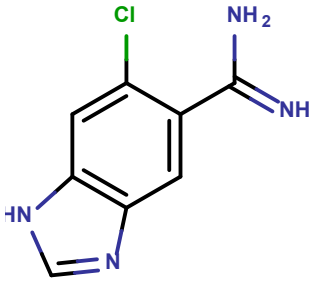
200nM Urokinase
PDB:1C5W
440nM Trypsin
PDB:1C5Q



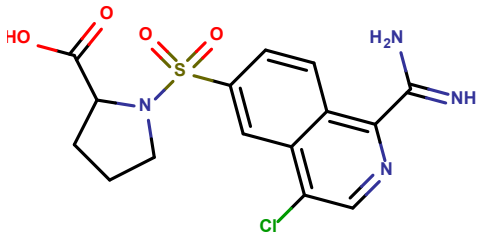
Urokinase
of a 9nM ligand
PDB:2VNT

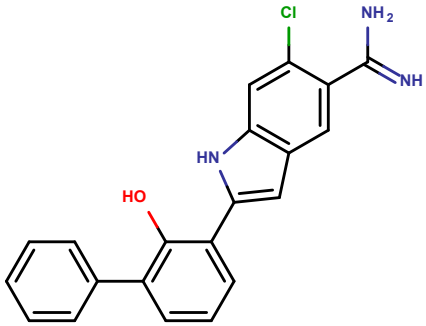


Beta trypsin
of a 100nM
ligand
PDB:1GJ6

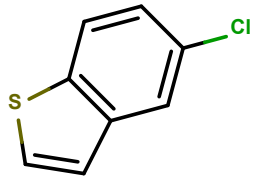


Beta trypsin
of a 800nM
ligand
PDB:1O2L

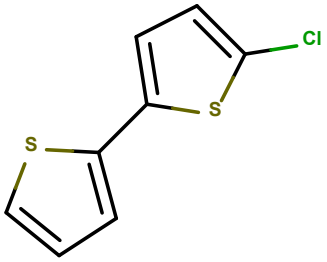




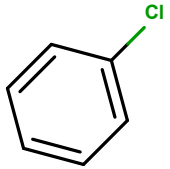
Example alternative P1 fragments



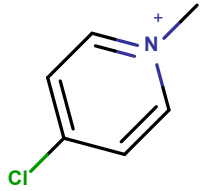
FXa
of a 47nM
PDB:2J38



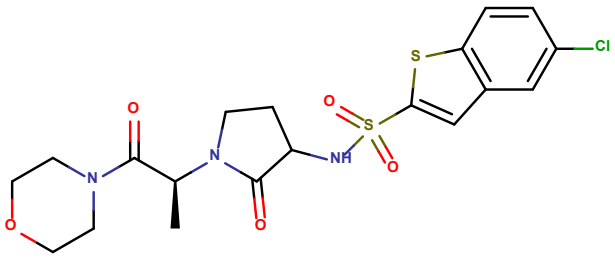
FXa
of a 4nM ligand
PDB:2Jn5

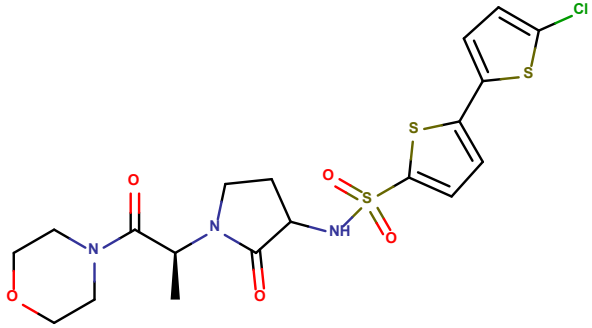


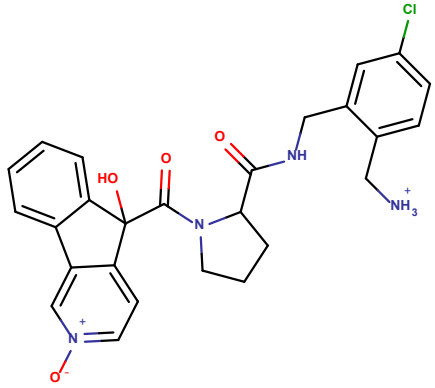
Thrombin
of a 770pM
ligand
PDB:1ZRB



Thrombin
ligand
PDB:3QX5



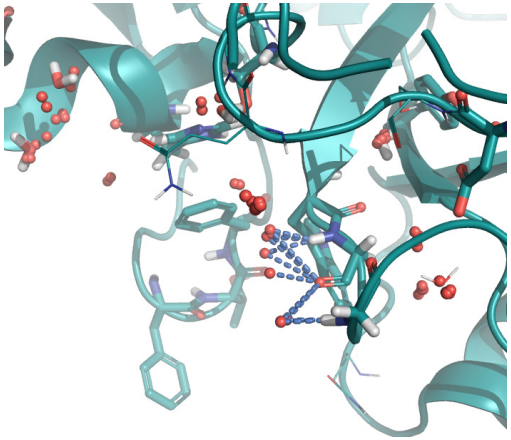


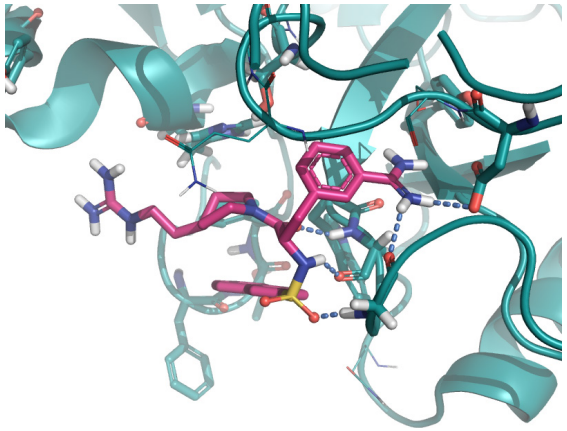


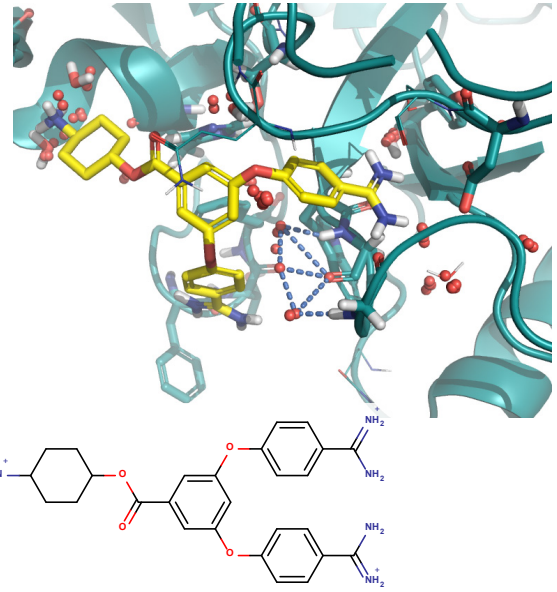
Selected first 3 urokinase and trypsin fragments as a template to look for field similars

Parallel strategy: Patent busting approach

> Literature cpd and water







- Water in matriptase apo structures is stable at the lip of the s1 pocket
- Cpd 21 displaces some of these waters to make similar interactions
- Patent compounds may stabilize the water rather than displacing it

Patent busting approach and suggestions

- A proposed hetero system as potentially suitable replacement for the literature scaffold which will provide rapid synthetic evaluation

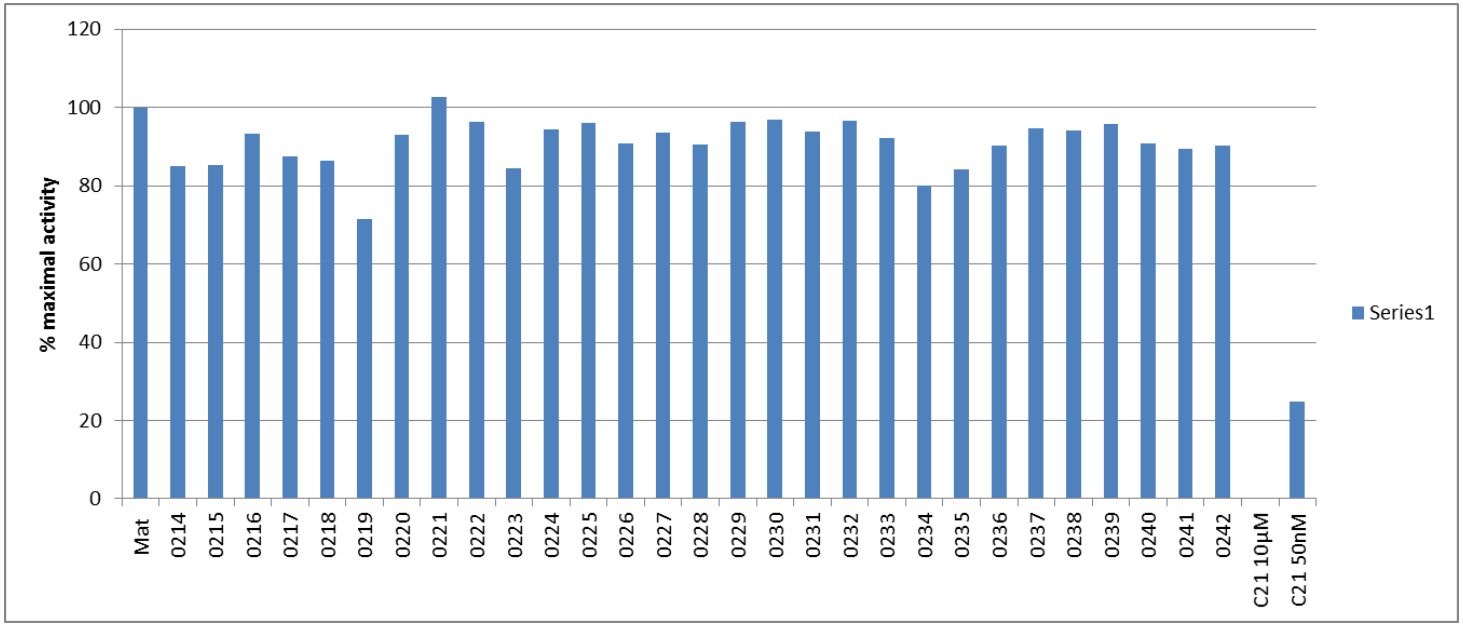
- Main concerns are:
  - (1) require evidence that new core active
  - (2) linking chemistry and reproduction of the Literature cpd geometry
  - (3) Aryl ring electronics?
  - (4) Activity determined by decoration thus moving forwards without benzamidine may be tricky?
  - (5) Symmetry – binding mode prediction not trivial

- Benefits
  - Probably the most facile approach synthetically

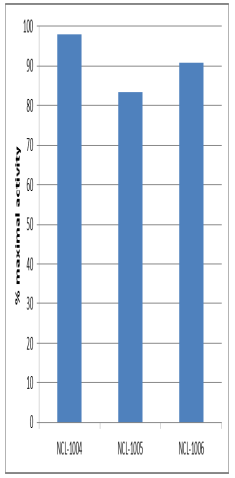
Results of 142 virtual screening hits purchased

	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

29 X 0154 analogues

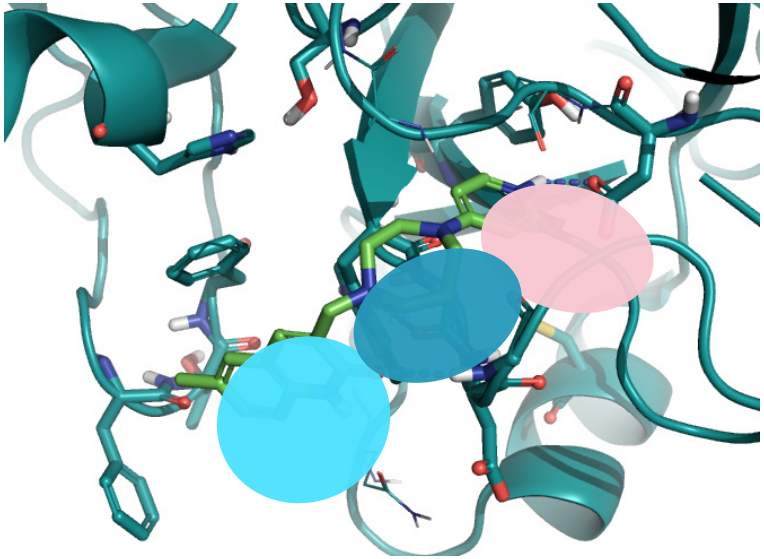


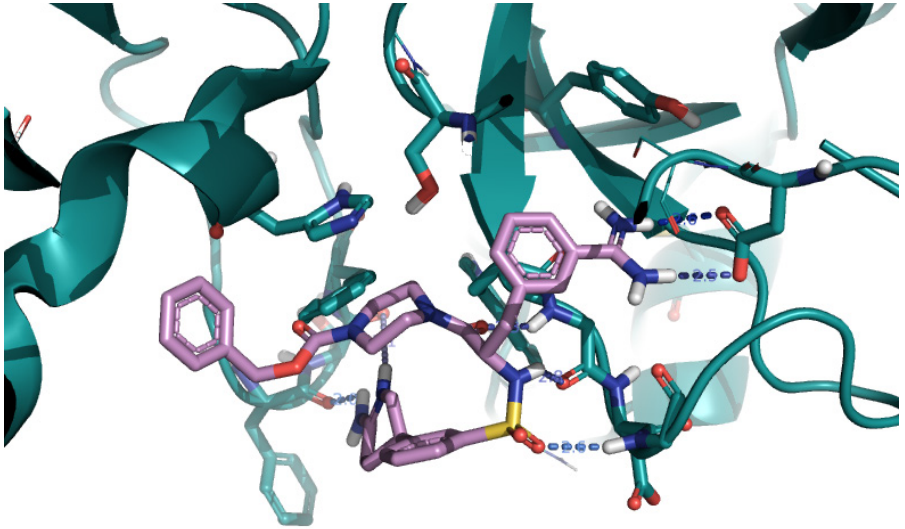
First novel patent bust attempts



Virtual screening hit analysis

> Screening hits 154, 234 and 119 were analysed for 3D alignment with the cpd 21 binding model





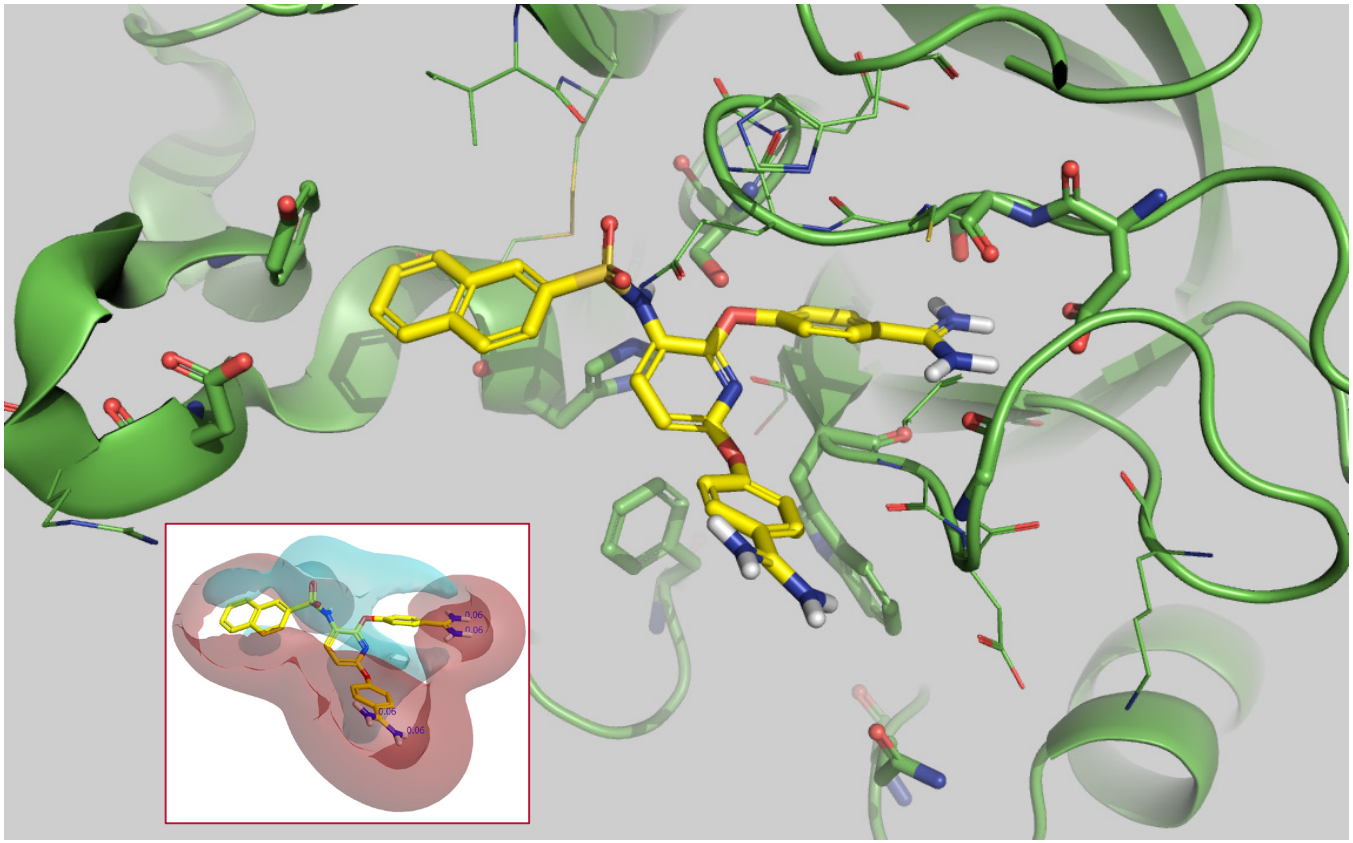
> Key features present in cpd 21 are missing in 154 but primarily low activity likely due to an inferior S1 binding fragment

> 119 is devoid of the sulphonamide portion but has a basic tail reminiscent of the template – suggesting possible fusion of 154 and 119 features.

Conclusions: virtual screening hits

- Main issue with the virtual screening hits is likely to have been an absence of a suitable ‘active’ mimetic of the critical benzamidine
- Some SAR from the hit analogues confirming that this 154 is an active series albeit weakly active 30 microM.
- We know from cpd 21 SAR that this S1 fragment is important
  - Greatest chance meeting the threshold hit criteria using these VS hits is manipulation of the S1 pocket
- Much scope for engineering cpd 21-like features
- **Greater synthetic expediency via the patent bust**

New Matriptase structure data



Lit. paper:

Matriptase inhibitor modeled into 2GV6

possibly a dimer

Benzamidines S1

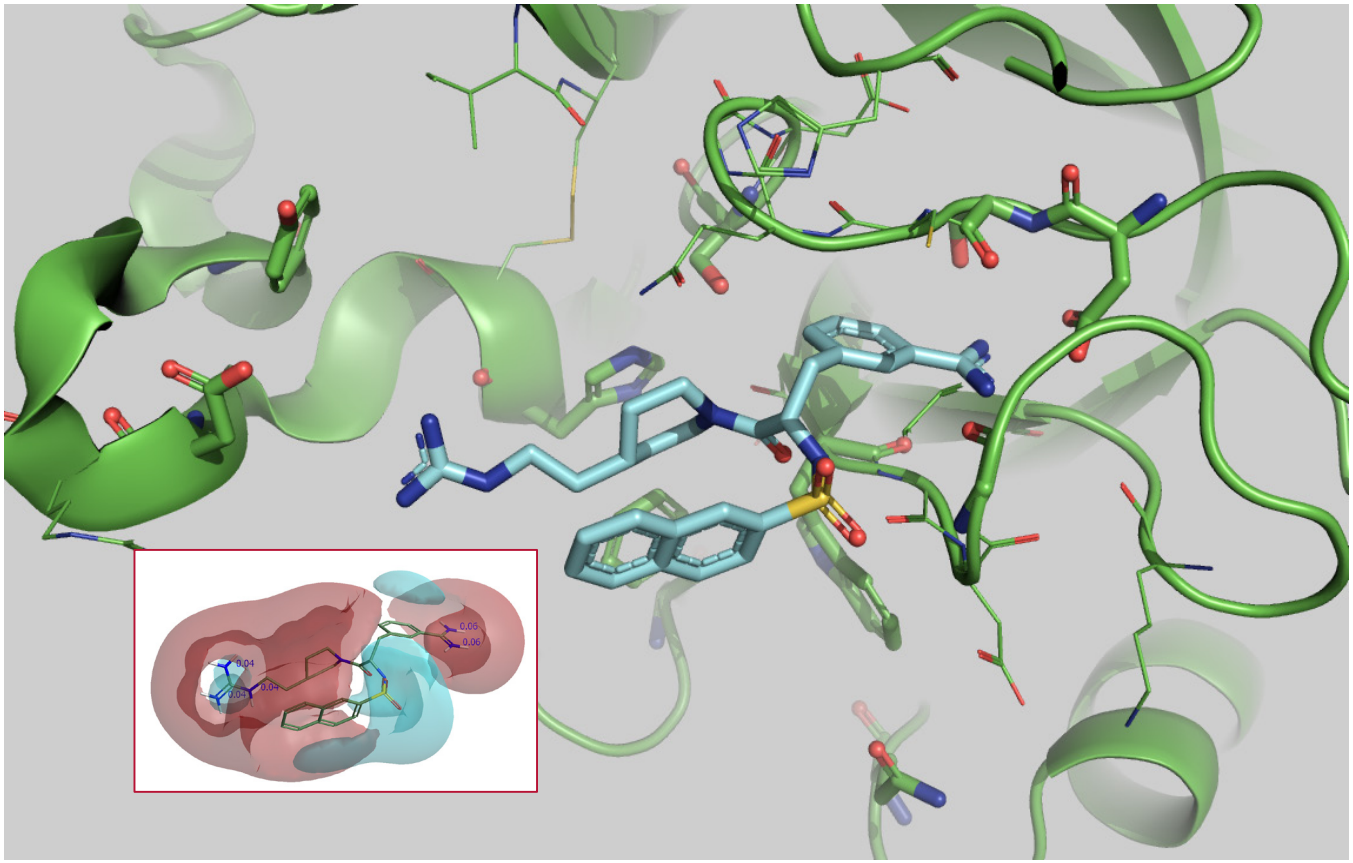
Naphthyl in S1’ Sulphonamide near catalytic triad

Second benzamidine towards S4

S3 free for dimer arginine as in 2GV6.

Naphthyl group much higher than the previous sulphonamide S1’ interacting group Naphthyl probably not optimal wrt pi face density. Unusual position for S4 benzamidine moiety likely to be a function of multimeric state of inhibited Matriptase.

Original reference series structure



PDB: 2GV6

Sulphonamide in Matriptase dimer.

Benzamidine in S1

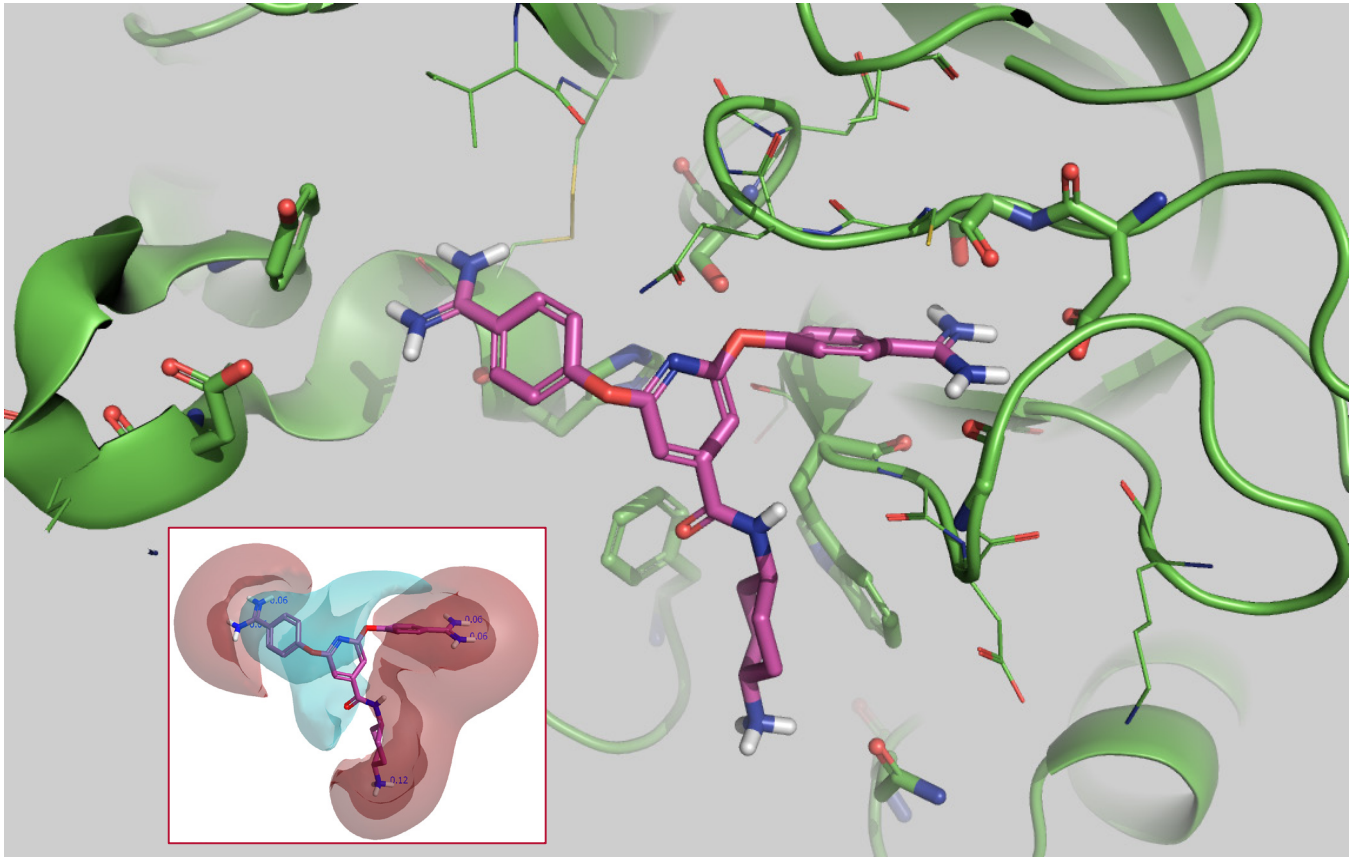
Guanidine in S1’/S2’

Naphthyl at dimer interface

Arginine from other Matriptase protein in S3/S4.

Interaction of the Asp in the S1’ 60 loop with guanidinyl piperidine of the sulphonamide series in a folded conformation. Substrate beta strand peptide mimetic H-bonding pattern reminiscent of other peptidic Matriptase inhibitors.

New Matriptase structural data



Lit. paper:

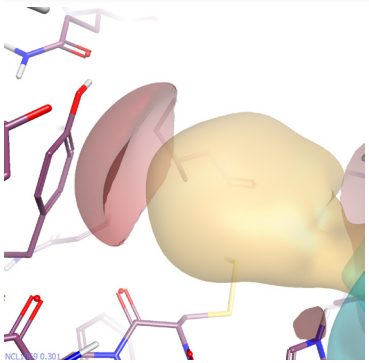
Matriptase inhibitor modeled into 2GV6

Benzamidines in S1 and S1’

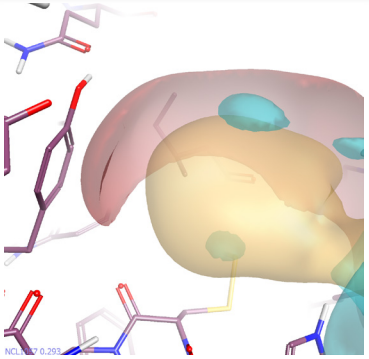
Amino piperidine amide in S3/S4.

Benzamidine makes beautiful interactions with Tyr in the 60-loop electrostatically optimized for this interaction although pi face electron density onto the disulphide may not be optimal.

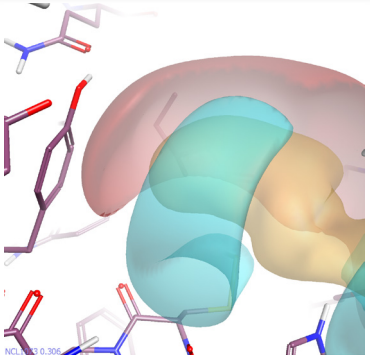
P1'-P2' pocket electrostatics and SAR



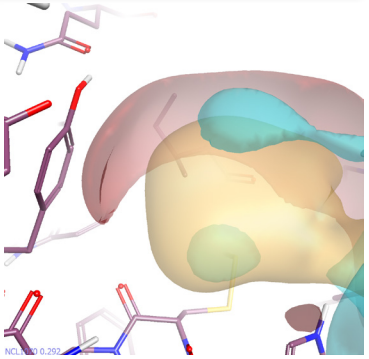
NCL1069 (149 nM)



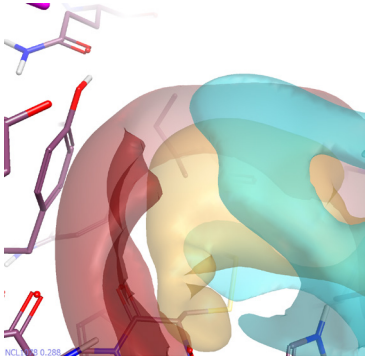
NCL1057 (315 nM)



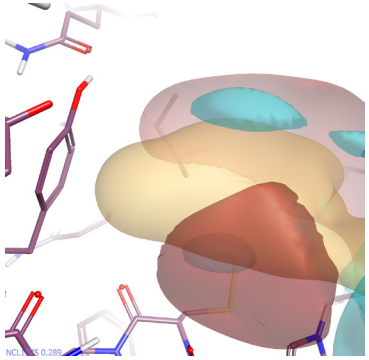
NCL 1073 (647 nM)



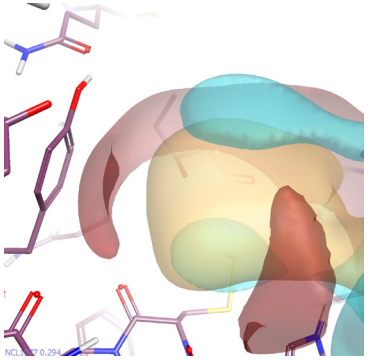
NCL 1070 (2074 nM)



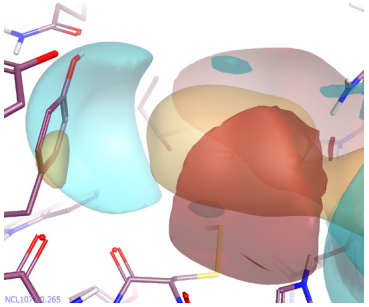
NCL1078 (2075 nM)



NCL1075 (2237 nM)



NCL 1077 (2284 nM)

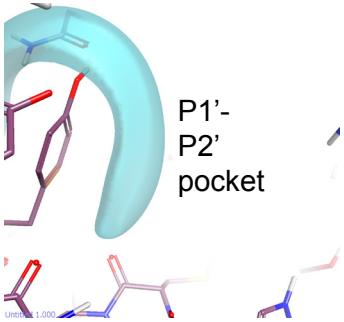


NCL 1071 (3663 nM)

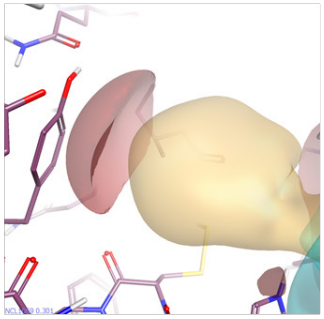
Electrostatics an be seen very nicely in Cressets Forge software – all current example SAR tracks very well indeed – balance of (1) Pi face negative density suppression (2) Largest 3-4 edge positive field and (3) Sensitivity to steric bulk in 4-5 positions

cresset

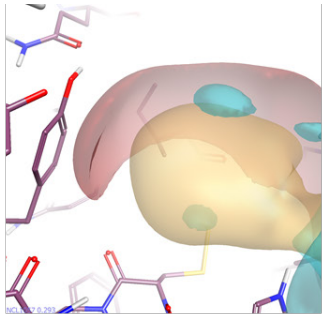
P1’-P2’ pocket electrostatics and SAR



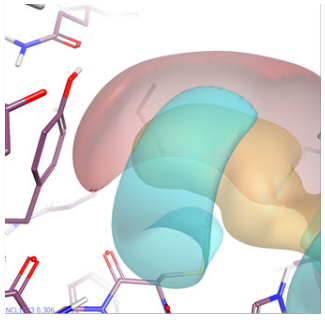
Tyrosine (negative)



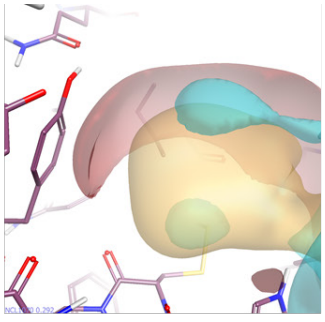
NCL1069 (149 nM)



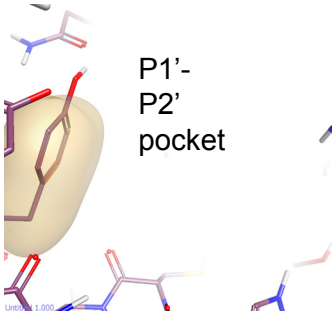
NCL1057 (315 nM



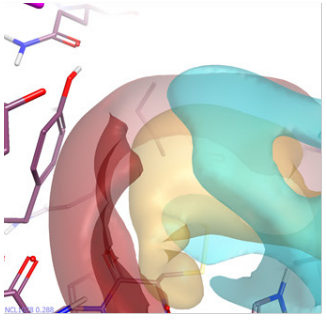
NCL 1073 (647 nM)



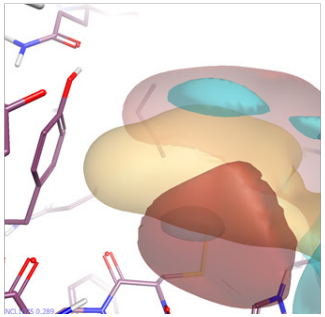
NCL 1070 (2074 nM)



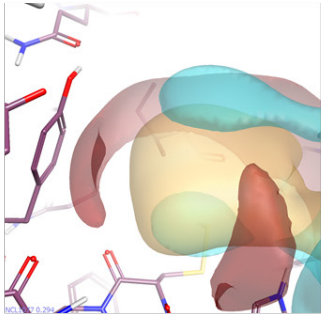
Tyrosine (hydrophobic)



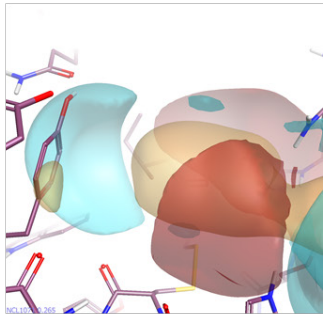
NCL1078 (2075 nM)



NCL1075 (2237 nM)



NCL 1077 (2284 nM)



NCL 1071 (3663 nM)

Results: Matriptase inhibition reduces OA severity - NCE

Ncl	K_i	select	Ncl	K_i	select
1010	13.7	635	1065	12.4	15
1011	442	6.3	**1066**	3.7	14
1014	256	376	1069	35.5	14
1036	1582	18	1070	494	9
1038	258	5.3	1071	872	1
1047	312	31	1072	276	7.2
1057	75	19	1073	154	80

Table 1: Potency data of novel MIs. Using Boc-QAR-NHMec as substrate, IC_{50} values were first determined experimentally for matriptase and trypsin. The fold selectivity was then calculated, whilst Ki values (all nM) for matriptase were calculated using the Cheng-Prusoff equation. **Green** indicates within target criteria; **Amber** indicates within acceptable criteria; **Red** not aligned with success criteria. Comparative cmpd 59 data (used to validate target in MS1) are: Ki = 3.8nM with 11-fold selectivity vs trypsin.

Newcastle experiments performed by: W Hui, DJ Wilkinson, A Destrument, S Watson

New NCE Matriptase inhibitor

> Proposed hetero system provided relatively rapid synthetic evaluation and systematic optimisation

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 cyctotoxicity, PBMC's	EC_{50} = 54.8µM MEC = 39µM
Novelty *(patentable)*	Novel IP

> Still one problem to solve…….but almost there

Conclusions

- Virtual screening is not always easy
  - Need to screen enough examples?
- Patent busting can be synthetically expedient
  - But still hard slog
- Suggestions from the modeling were valuable to both instances
- Still fundamental problems remain to be solved

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martin@cresset-group.com



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