Comp Chem in Drug Design
What Works and What Doesn't

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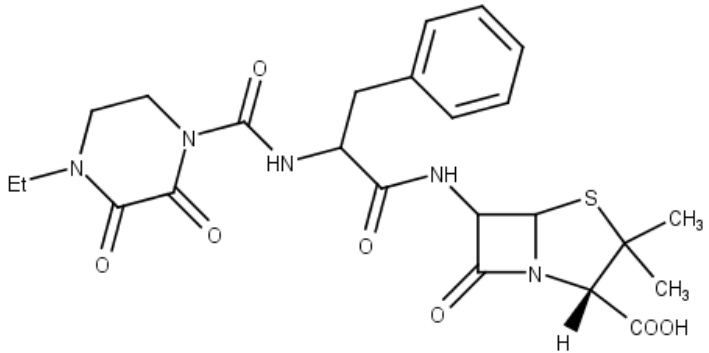
CCK-9 - 9th November, 2017

Brief Biography

- Academic
  - Biochemistry, Oxford; PhD, Manchester
- Industry
  - Beecham (SmithKline Beecham, GSK),
  - Pfizer
  - Prolifix (TopoTarget)
  - InhibOx (Oxford Drug Design)

Example 1 – Beecham - Antibiotics

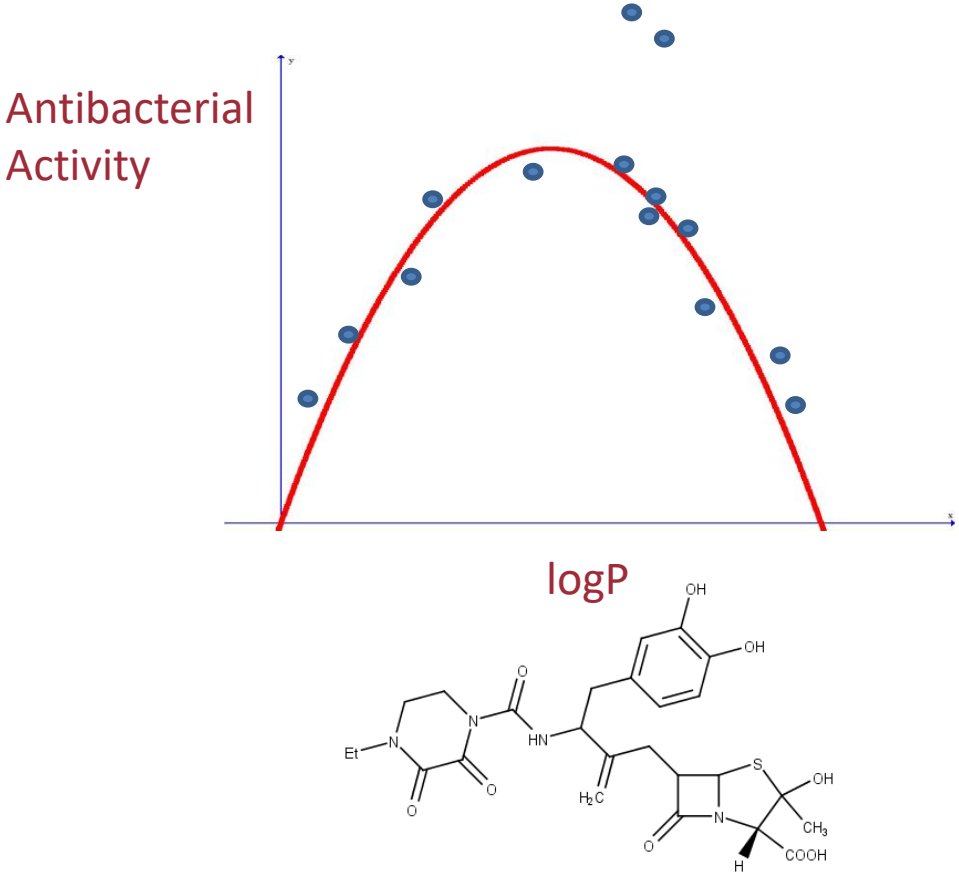
- Piperacillin – a broad-spectrum β-lactam antibiotic, first marketed in early 1980s
- Beecham programme investigated piperacillin analogues
- Modelling consisted mostly of classical QSAR
  - In 1987 there were 238 structures in the PDB!



Example 1 – QSAR correlations

- The modelling work uncovered a classical biphasic dependence of activity on logP
- There were, however, some outliers in the regression, with much better than expected activity
- All outliers contained a catechol sidechain
- Further investigation established that these compounds were utilizing a siderophore, iron-uptake pathway, improving penetration in Gram-negative bacteria

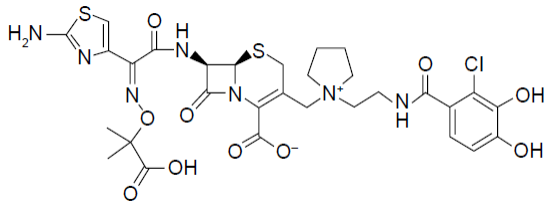
- Antibacterial activity of catecholic piperacillin analogue. Basker et al., J Antibiotics 42:1328-1330, 1989.
- Iron-regulated outer membrane proteins of Escherichia coli K-12 and mechanism of action of catechol-substituted cephalosporins. Curtis et al. Antimicrob Agents Chemother. 32:1879-86, 1988.

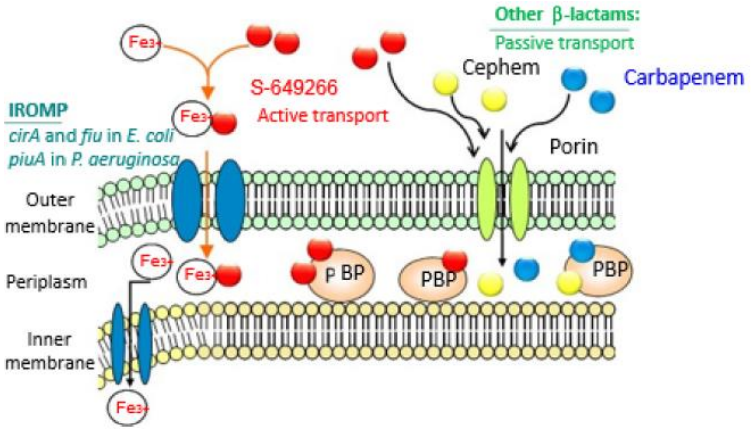


Example 1 - Conclusions

- Traditional QSAR methods have fallen out of fashion
- Despite (many) limitations they can be very informative, when applied with moderation
- Outliers are interesting – don't discard them
- The same things are discovered repeatedly

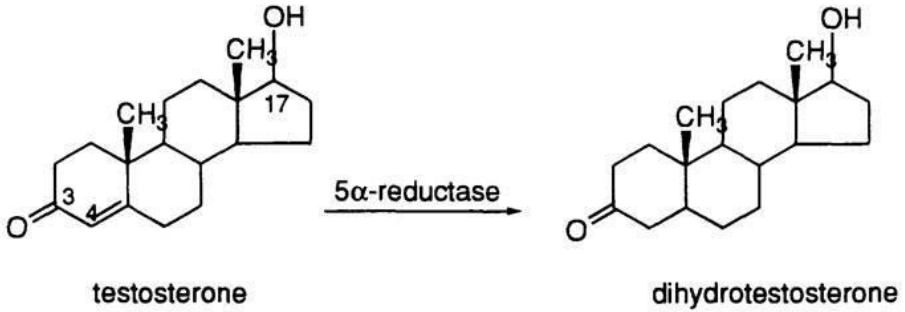
- Tillotson. Trojan Horse Antibiotics—A Novel Way to Circumvent Gram- Negative Bacterial Resistance? Infectious Diseases: Research and Treatment 2016:9 45–52 doi:10.4137/IDRT.S31567.
- In vitro antibacterial properties of cefiderocol, a novel siderophore cephalosporin, against Gram-negative bacteria. Antimicrob. Agents Chemother. doi:10.1128/AAC.01454-17





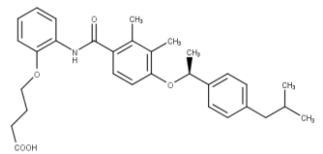
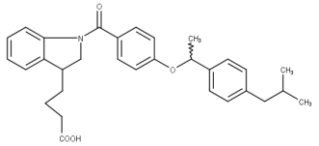
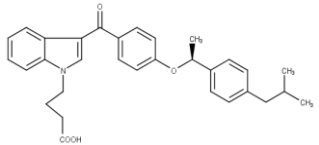
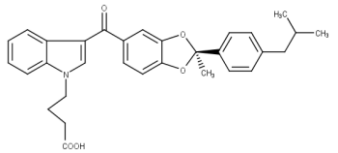
Example 2 – Pfizer - 5α-reductase inhibitors

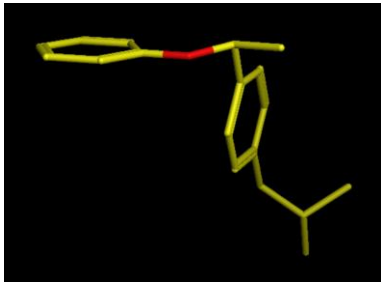
- 5α-reductase converts testosterone to the more potent androgen DHT
- Two isoforms, 5α-R1 and 5α-R2
- Inhibitors are potential treatments for benign prostatic hypertrophy, prostate cancer and male pattern baldness
- Project sought potent, non-steroidal, balanced inhibitor

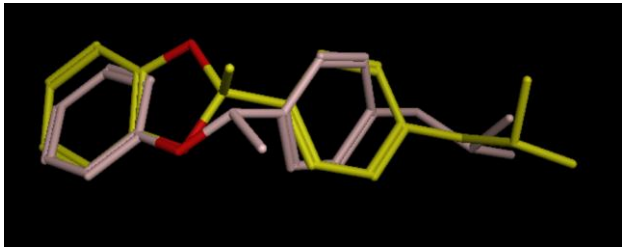


Example 2 – Conformation Analysis

- Initial weak 5α-R2 lead identified
- Objectives
  - Remove ortho-hydroxy aniline
  - Balanced isoform activity
- Approach
  - Conformational analysis
  - Seek plausible bioactive conformation hypothesis
  - Rigidify scaffold in bioactive conformation

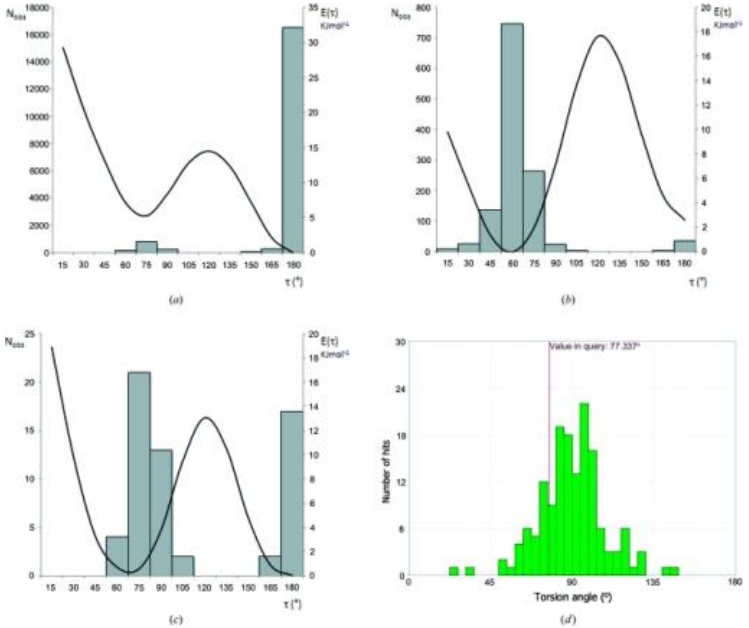
No.	Structure	Rat 5α-R (nM)	Human 5α-R1 (nM)	Human 5α-R2 (nM)
1		1.7		256
2		24	113	481
3		1	40	4
4		9	25	25





Example 2 - Conclusions

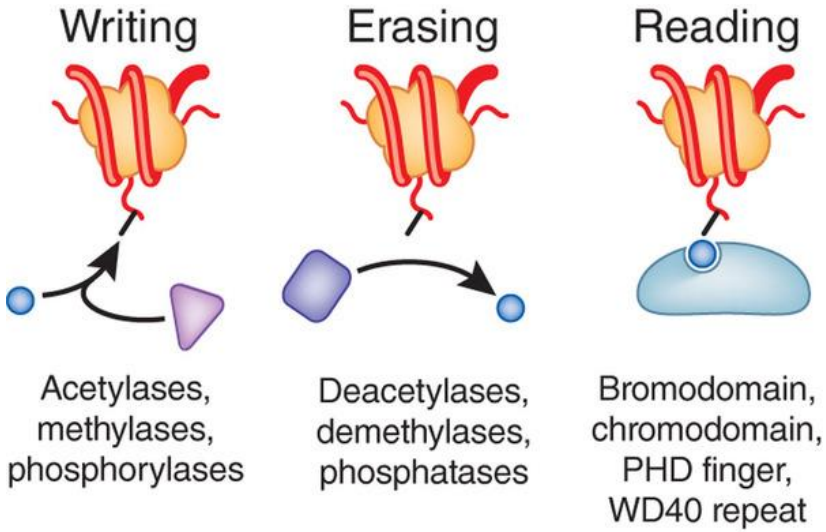
- Simple principles of conformational analysis, coupled with experimental data (CSD) can shed light on bioactive conformation
  - Significant potency gains are possible
- Outcome
  - Balanced inhibitor, long half-life, high oral bioavailability, clean tox profile, 48% reduction in prostate weight in animal model study
  - Compound from this series reached Ph-2



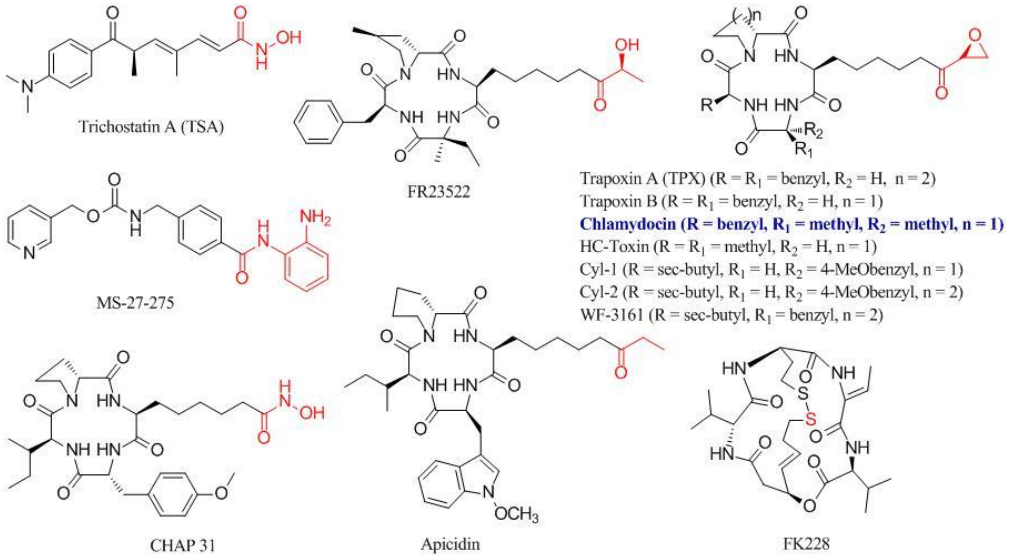
The development of non-steroidal dual inhibitors of both human 5a-reductase isoforms. Blagg et al., Bioorg Med Chem Lett 6:1517-1522, 1996.

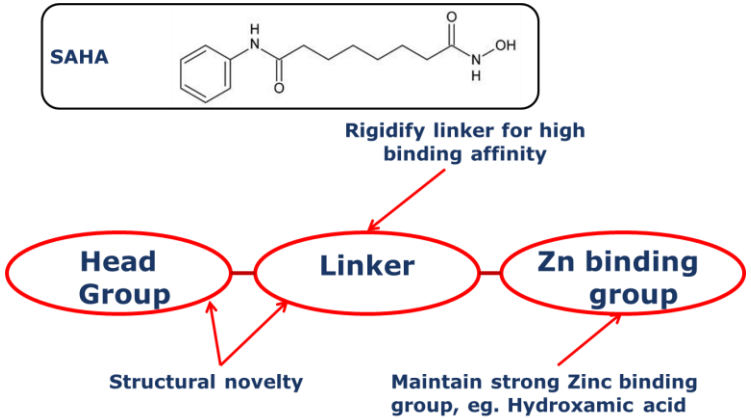
Example 3 – Prolifix – HDAC inhibitors

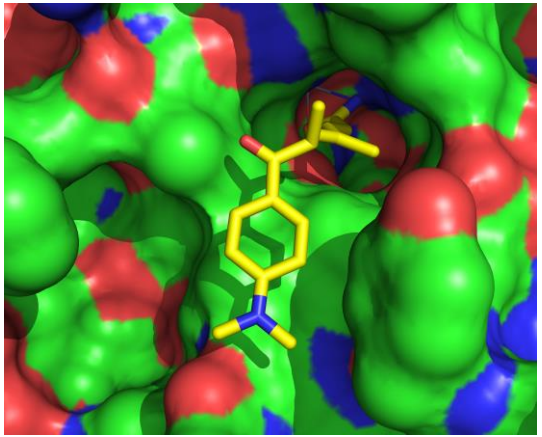
- Enzymes involved in epigenetic modifications of histones were exciting new cancer targets
- Histone deacetylase appeared an attractive target
- Multiple HDAC isoforms, limited knowledge of biological function
- Most HDAC inhibitors known at the time of project initiation were unattractive as leads



Example 3 – Structure-based design



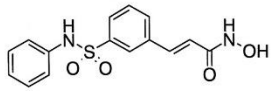
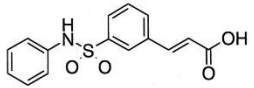
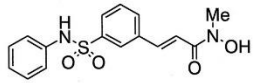
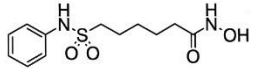
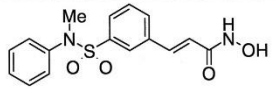
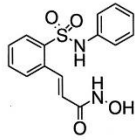
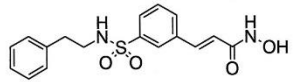




- Only X-ray data available at the time was a bacterial HDAC homologue –TSA structure

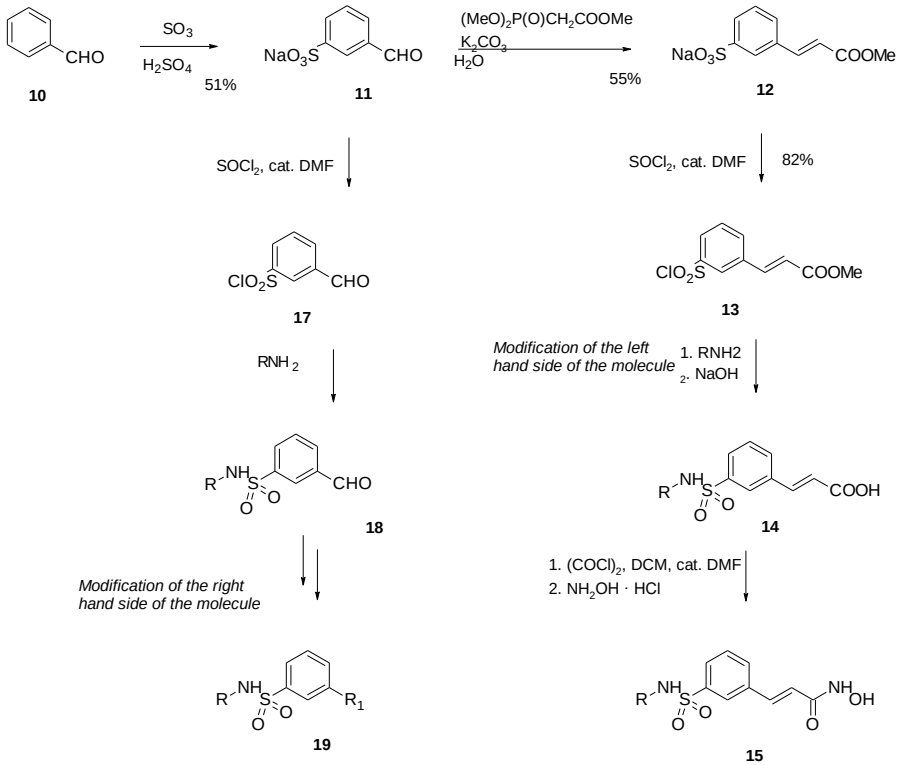
SAR for HDAC activity (HeLa cell extract)

- Zinc-binding group required
- Flexible linkers less potent
  - Short linkers inactive
- Sulphonamide direction important
- Extended configuration required
- Larger head groups well tolerated
- Many other compounds explored, but PXD101 selected for further development

Compound	Structure	HDAC inhibition IC_{50} (nM)
PXD101		27
1		>1000
2		>1000
3		300
4		238
5		>1000
6		22

Example 3 - Conclusions

- Structural input was useful at providing qualitative guidance
- Attempts were made to identify other zinc-binding groups, all unsuccessful
- Important to make the compounds you want, not just the ones that are easy – vital in gaining IP protection in this project
- Compound approved by FDA for treatment of peripheral T-cell lymphoma, July 2014

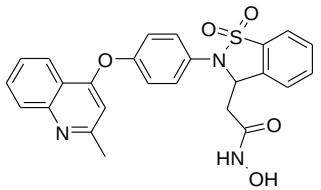


The Discovery and Development of Belinostat, Finn et al., in Successful Drug Discovery, Volume 2, Janos Fischer (Ed.), Wiley, (2016).

Example 3a – InhibOx - TACE

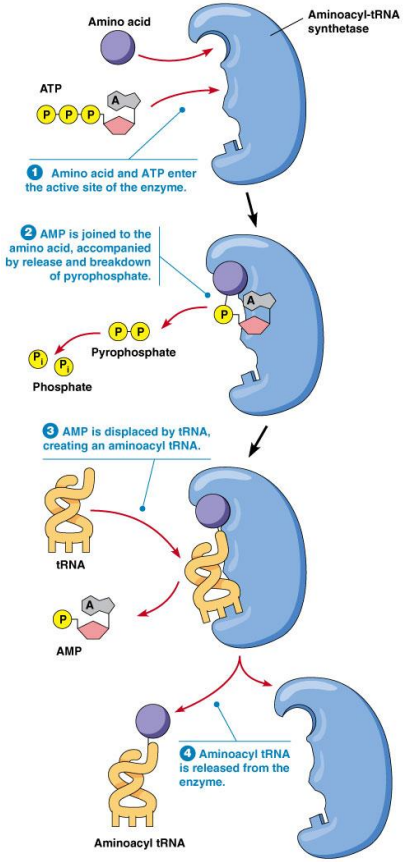
- Opportunistic collaboration with TopoTarget exploring hydroxamate compounds in another Zn-enzyme
- Leads identified (not active against HDAC)
- Optimization challenge was selectivity over other ADAM/MMP family members
- Achieved through discovery of novel hydroxamate-bearing scaffold with other known TACE-selective functionality
- Conclusion – build on what you know and have

Enzyme	IC_{50} (nM)	
	Apratastat	(-)-IX3496024
TACE	2	1
MMP 1	26	*>10000*
MMP 2		-3%@5μM
MMP 3	4	>10000
MMP 7		31%@5μM
MMP 8		-13%@5μM
MMP 9	40	>10000
MMP 10		9%@5μM
MMP 12	1	5300
MMP 13		-10%@5μM
MMP 14		4%@5μM
ADAM 9	92	>10000
ADAM 10	79	1400

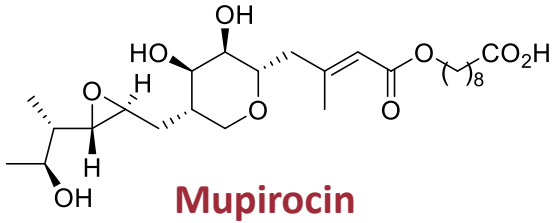


Example 4 – Oxford Drug Design - Antibacterials

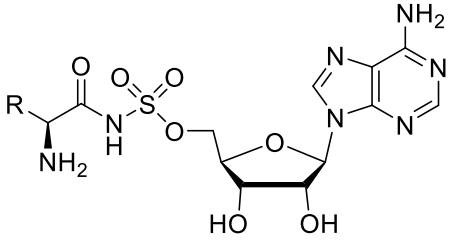
- Target – aminoacyl-tRNA Synthetases
- Essential enzymes – generate aminoacyl-tRNA for ribosomal protein synthesis
- Individual family members, e.g. Leucyl-tRNA (LeuRS) synthetase are conserved across bacteria, but significantly different to human enzymes
- Clinically validated mechanism – mupirocin – IleRS inhibitor – natural product - topical only – Gram-positive spectrum
- Aim: aaRS inhibitors with systemic activity and Gram-negative spectrum



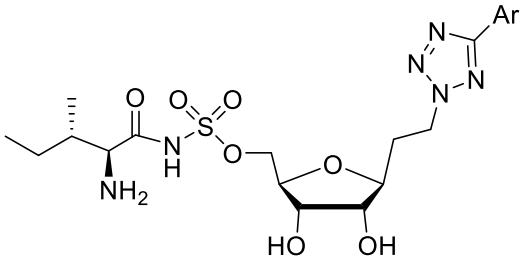
tRNA Synthetase – Rational Design



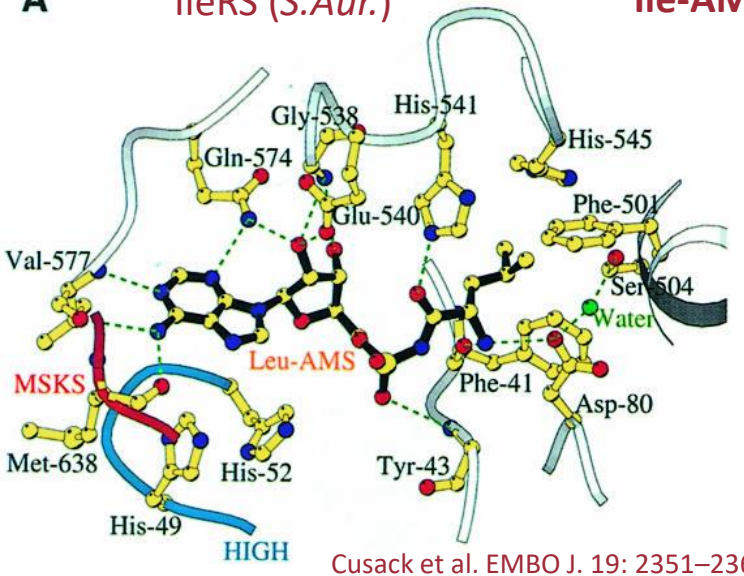
Mupirocin
IleRS (*S.Aur.*)



Ile-AMS (Ile, LeuRS)



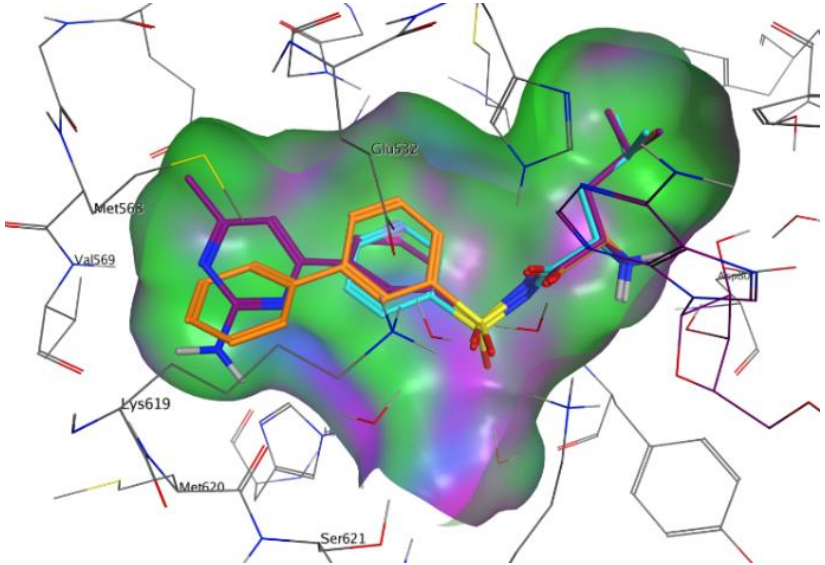
Cubist Pharmaceuticals (IleuRS)

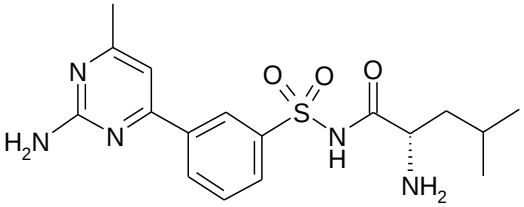


Cusack et al. EMBO J. 19: 2351–2361, 2000

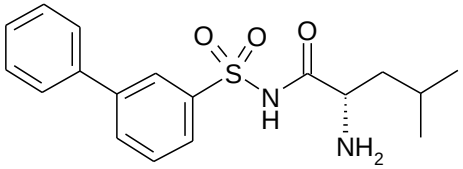
- Challenges
  - Prior art compounds have little, if any, Gram-negative activity
  - Natural product and reaction intermediate analogues unattractive as leads

tRNA Synthetases – Surprising SAR

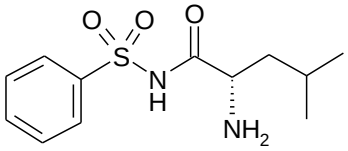




IC_{50}EcoLeuRS = 41 nM
K_D EcoLeuRS = 10 nM (ITC)
ΔH = -12.5 kcal/mol;
-TΔS = 1.6 kcal/mol



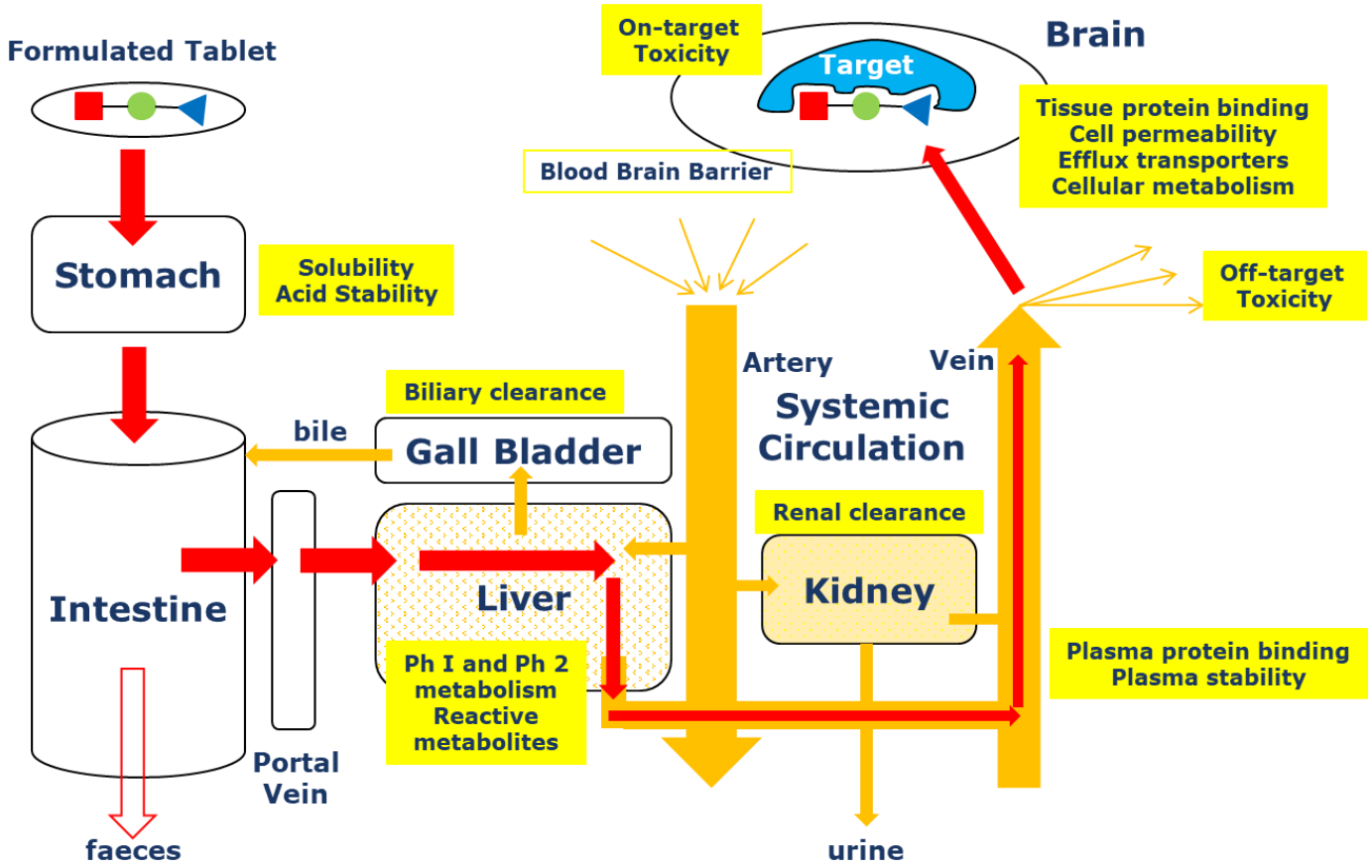
IC_{50}EcoLeuRS = 32 nM
K_D EcoLeuRS = 10 nM (ITC)
ΔH = -8.1 kcal/mol;
-TΔS = -2.8 kcal/mol



IC_{50}Eco LeuRS = 49 nM;
K_D EcoLeuRS = 1 nM (ITC)
ΔH = -13.1 kcal/mol;
-TΔS = 1.1 kcal/mol

- Enzymes show great conformation shifts on ligand binding
- Quantitative correlations with IC_{50} and thermodynamic data challenging

NB – Drug Design is Mostly Not About Potency



Conclusions – What Works and What Doesn't

- Use the simplest possible model
  - E.g. Simple QSAR, combined with interpretable descriptors
- Compare/validate with experimental data
- Don't believe the model, even if it appears to be working well
  - Always make compounds to challenge your assumptions
- Make the compounds you should, even if they are hard (within reason)
- The search for quantitative models usually fails, and they are generally not crucial to project progress
- Have a good memory – experience is valuable – but remain open-minded

Acknowledgements

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Dr. Colin Greengrass
Dr. Graham Maw

Innovate UK

NABARSI
fighting antibiotic resistance