Novel Inhibitors of Isoleucyl-tRNA Synthetase as Potential Antibacterial Drugs

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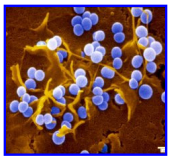
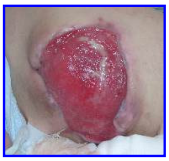
1. Bacterial Resistance

- Bacterial resistance is a constant and increasing problem.
- Each year in the UK thousands die from bacterial infections.
- 'Superbugs' like MRSA are resistant to almost all antibiotics and are extremely life-threatening.
- Development of novel antibiotics is urgent as we are rapidly running out of treatments for diseases.
- Activity in the pharmaceutical industry has dramatically declined in this area of research, so the role of academics and small biotech companies has become even more important.



Cause: Bacterial Infection

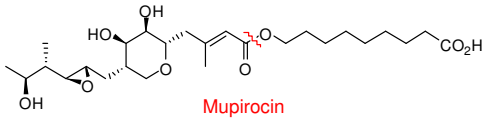


Effect: Wounds that won't heal

Solution: New Antibiotics

2. Aminoacyl-tRNA Synthetases (aaRS)

- Aminoacyl-tRNA synthetases catalyse the acylation of amino acids to tRNA molecules in the translation stage of protein biosynthesis.
- They are attractive targets as they are essential for bacterial survival[1].
- There are 20 aaRS. Focus was placed on IleRS as it is a validated drug target.
- Mupirocin (Bactroban®) is the only commercially available inhibitor of aaRS. It exhibits an 8000-fold selectivity for bacterial IleRS over human IleRS, proving selective inhibition is possible.
- Mupirocin is administered as a topical antibiotic as its ester link is cleaved *in vivo* to give inactive metabolites. There is widespread resistance to mupirocin.



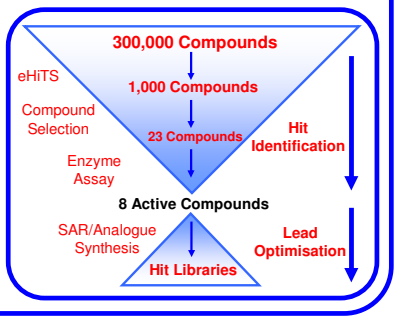
Mupirocin

3. *De Novo* Design, Virtual High Throughput Screening (VHTS) & Synthesis

SPROUT

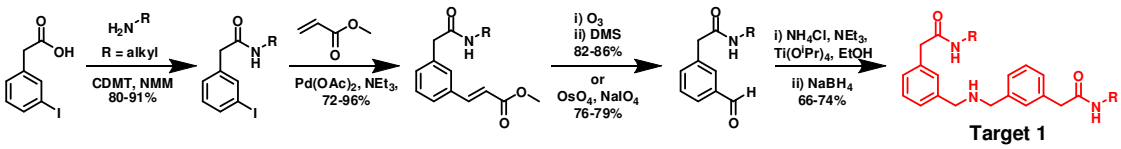
SimBioSys Inc. Your Software Partner for Rational Drug Design

- SPROUT[2] is a computer programme for *de novo* structure-based drug design which constructs templates from small molecular fragments in a stepwise manner. SPROUT was applied to a homology model of *E. coli* IleRS.
- Molecules corresponding to the designed molecular templates were synthesised and sent for biological evaluation.
- The eHiTS[3] software from SimBioSys was used to carry out VHTS. This software can rapidly dock large libraries of ligands into an active site and report the best docking pose for each ligand along with a predicted binding affinity.
- The results were progressed through various filters (e.g. predicted solubility and affinity) and the best ligands were purchased and sent for biological evaluation.
- Following identification of initial hits small focused libraries were created around active molecules to establish SAR.

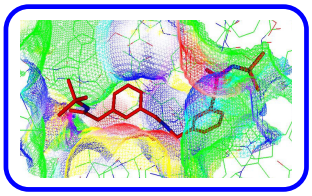


4. Synthesis

- Several molecules were chosen as targets for synthesis from designed molecular templates based on predicted binding affinity and synthetic accessibility.
- A small library based on this initial target has been synthesised successfully.



Target 1



5. Results

- Initial VHTS results were very exciting, a series of novel IleRS inhibitors have been identified.
- Small focused libraries have been synthesised to probe SAR of active molecules.
- We are currently working to lower the IC_{50} and MIC of our most active compounds.
- We hope to develop a series of lead molecules to move forward into hit to lead.

Inhibitor structures have not been disclosed as they are being considered for patenting.

Compound	Inhibition of IleRS	MIC (μg/ml) *S. aureus* 8325-4
NJP 05160137	76% (50 μM)	1
NJP 04530135	63% (50 μM)	2
NJP 02150197	100% (500 μM*)	512
NJP 04780144	100% (500 μM*)	512
NJP 01790150	100% (500 μM*)	>1024

* Awaiting testing at lower concentrations

6. Conclusions

- SPROUT design and VHTS provide a powerful tool for drug discovery. We have developed a series of novel inhibitors of IleRS.
- *De novo* designed compounds have been synthesised successfully and have been shown to exhibit high micromolar activity.
- VHTS has proved to be a very successful tool in identifying inhibitors with 35% of the compounds purchased showing at least 60% inhibition at 500 μM.

7. Acknowledgements

- We would like to thank ICAMS (Leeds, U.K.) and SimBioSys (Toronto, Canada) for technical support.

8. References

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2. Gillet, V. *et. al. J. Comput. Aided Mol. Des.* **1993**, 7*(2)*, 127-153.
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