De Novo Design & Synthesis of Novel RNA Polymerase Inhibitors as Potential Anti-Tuberculosis Agents

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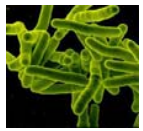
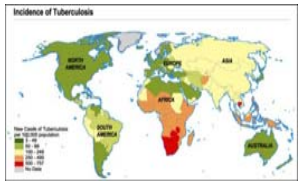
1. Tuberculosis (TB)

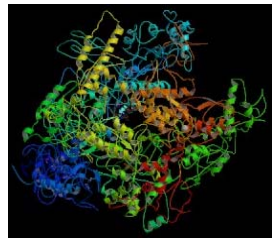


- Despite advances in chemotherapy and the BCG vaccine, TB is one of the world's most serious bacterial infectious diseases.[1]
- The WHO declared TB a global public health emergency due to a rapid increase in MDR strains of *Mycobacterium tuberculosis*.
- TB kills 8000 people a day, 2-3 million each year & another 8-10 million new individuals get infected.
- TB treatment relies on drugs up to fifty years old & takes 6-9 months to complete.

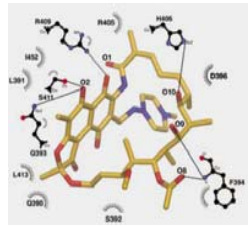


There is an urgent need to develop novel anti-TB drugs to combat drug resistant TB & shorten the length of treatment.

2. RNA Polymerase (RNAP) - The Target



- RNAP is the essential enzyme involved in bacterial gene transcription.
- RNAP is the target of rifampicin (Rif), the most potent drug to date for the treatment of TB.
- An X-ray crystal structure of RNAP complexed with rifampicin is available.



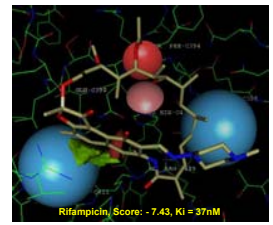
- Unfortunately, bacteria develop resistance to rifampicin with high frequency.
- Novel RNAP inhibitors are needed to overcome the resistance problems.

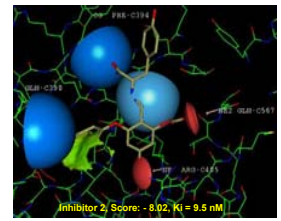
De novo design of novel inhibitors of RNAP is an attractive way forward.

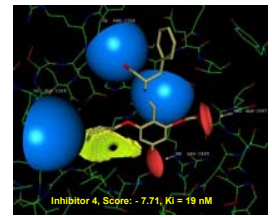
3. *De Novo* Drug Design

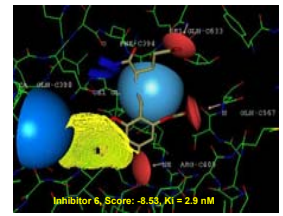
SPROUT

- SPROUT[2-3] is a powerful suite of software modules which takes any protein structure, identifies binding regions & generates small structures suitable as ligands.
- SPROUT has been used to produce small inhibitors of RNAP designed to contact the same target sites as rifampicin excluding the most resistance-prone residues.



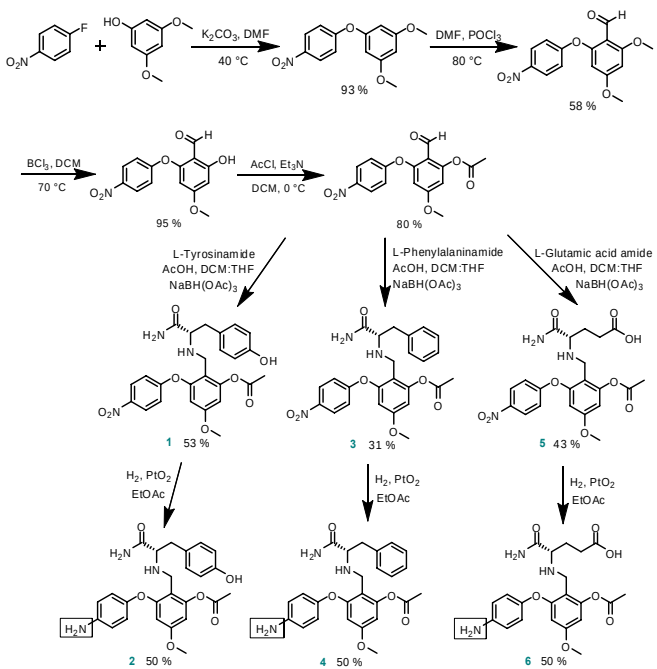






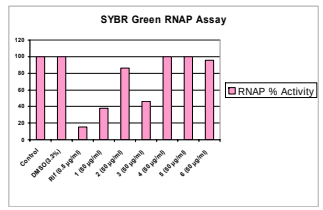
- All designed inhibitors show hydrogen bonding interactions with essential residues (shown above) & a strong hydrophobic interaction at the rifampicin binding site.

4. Synthesis



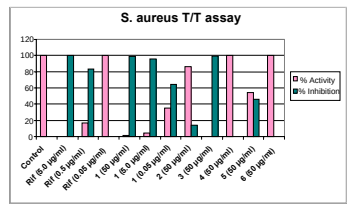
5. Biological Activity

- *In vitro* RNAP activity against *Escherichia coli* RNAP using SYBR Green assay[4]



Samples (μg/ml)	RNAP % Activity
Rif (0.5)	15.4
1 (50)	38
3 (50)	46

- *Staphylococcus aureus* transcription/translation (T/T) assay[5]



Samples (μg/ml)	% Activity	% Inhibition
Rif (0.05)	100	0
1 (0.05)	35.4	64.6
3 (50)	0.16	99.84
5 (50)	54.12	45.88

6. Orientation of GLN390

- Biological data indicates that compounds 1 & 3 with nitro functionalities are more active than the corresponding amines 2 & 4.
- Results suggest that the positions of the O & N atoms within the side chain of GLN390 are the reverse of those shown in the RNAP crystal structure.
- This would allow the nitro groups in inhibitors 1 & 3 to act as H - bond acceptors via contact with the NH_2 of GLN390, thus accounting for the higher activity of 1 & 3.

7. Conclusions - A More Potent Inhibitor than Rifampicin[6]

- We have produced the first ever *de novo* designed small molecule inhibitors of bacterial RNAP using SPROUT.
- The designed inhibitors have been synthesised efficiently in 5-6 steps as single enantiomers.
- Compounds 1 & 3 are the most active with 62 % and 54 % inhibition of *E. coli* RNAP activity at 50 μg/ml respectively.
- In particular, compound 1 shows 64 % inhibition of protein synthesis in the *S. aureus* T/T assay at 0.05 μg/ml whereas rifampicin is inactive at this concentration.

This work paves the way for the development of novel TB drugs.

8. References

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9. Acknowledgements

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http://www.rcuk.ac.uk/