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Identification and validation of small molecule modulators of the NusB-NusE interaction

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ABSTRACT

Formation of highly possessive antitermination complexes is crucial for the efficient transcription of stable RNA in all bacteria. A key step in the formation of these complexes is the protein-protein interaction (PPI) between N-utilisation substances (Nus) B and E and thus this PPI offers a novel target for a new antibiotic class. A pharmacophore developed via a secondary structure epitope approach was utilised to perform an in silico screen of the mini-Maybridge library (56,000 compounds) which identified 25 hits of which five compounds were synthetically tractable leads. Here we report the synthesis of these five leads and their biological evaluation as potential inhibitors of the NusB-NusE PPI. Two chemically diverse scaffolds were identified to be low micro molar potent PPI inhibitors, with compound (4,6-bis(2′,4′,3.4 tetramethoxyphenyl))pyrimidine-2-sulphonamido-*N*-4-acetamide **1** and *N,N′*-[1,4-butanediylbis(oxy-4,1-phenylene)]bis(*N*-ethyl)urea **3** exhibiting IC_{50} values of 6.1 μM and 19.8 μM, respectively. These inhibitors were also shown to be moderate inhibitors of Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* growth.

Antibiotic resistance has evolved against all clinically approved antibiotics.[1–5] Exacerbating this problem is the withdrawal of all major pharmaceutical companies from antibiotic research effectively severing the traditional antibiotic drug development pipeline.[6,7] Of equal concern is that the majority of new antibiotics are derivatives of existing drugs for which resistance rapidly arises or is even pre-existing.[8] Consequently, there is an urgent need to develop new antibiotic classes which are not predisposed to the development of drug resistance.[3,4,6–13]

The current arsenal of antibiotics typically target four major processes within bacteria: a) cell wall/membrane synthesis, b) translation, c) DNA replication and d) inhibition of metabolism.[1,2,14] Thus an underutilised target for antibiotic development is the critical process of transcription, with only Rifamycin and Fidaxomicin approved for limited clinical use as anti-transcription targeted drugs.[15] Transcription inhibition has the potential to offer a number of new targets for antibiotic drug development, and of particular interest are a number of critical protein-protein interactions (PPIs) which are essential for transcription regulation.[16,17]

There is a growing body of evidence suggesting that small molecule inhibitors can be used to inhibit PPIs.[18–21] These inhibitors typically target a small area generally at the centre of an interface which confers the essential binding interactions. These clusters of amino acids are termed "hot spots". The efficacy of PPI inhibition can vary from micro- to pico-molar potent.[22] We believed that this approach could be utilised to develop a new class of antibiotics which inhibit the formation of the antitermination complex. This large nucleoprotein assembly is unique to bacteria and functions to regulate the transcription of bacterial stable RNA (t- and rRNA).[23,24] An essential stage in the formation of this complex is the PPI between N-utilisation substance (Nus) B and N-utilisation substance E (NusB-NusE), which is responsible for initiation and recruitment of other Nus proteins and RNA Polymerase to form the antitermination complex.[25–27]

The NusB-NusE interface is characterised by the α1-helix of NusE which occupies a pocket of NusB (Fig. 1). This interaction is established by the amino acid residues H15, R16 and D19 of the

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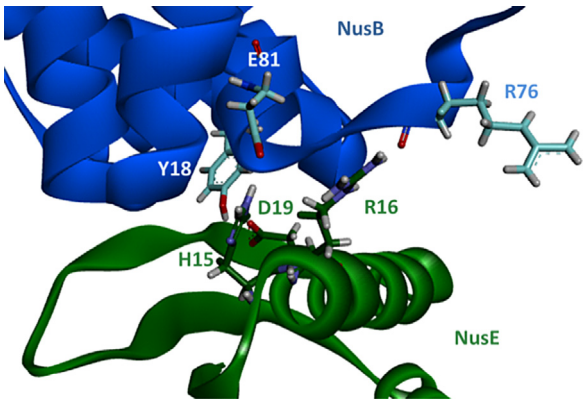

Fig. 1. The protein-protein interface of *Aquifex aeolicus* NusB-NusE (PDB ID 3R2C).[29] Depicted in blue is the protein NusB, with essential residues Y18, E81 and R76 highlighted (sticks). Shown in green is the α1-helix of NusE, with key amino acids H15, D19 and R16 (sticks) interacting with the binding pocket of NusB.

α1-helix forming electrostatic links with residues Y18, R76 and E81 of NusB. Combined, this pocket consists of eight hydrogen bond interactions, five of which are considered essential for heterodimer formation.[28,29]

Significantly, the residues identified above are conserved across many medically important bacterial strains including *Staphyloccus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenza* (Fig. 2). Moreover, the importance of this interaction was demonstrated by two point mutations in *E. coli*, nusE100 (R72G)[30] and nusB5 (Y18D);[26] these mutations are buried in the interface and directly disrupt protein-protein binding, which in turn impedes antitermination complex formation. We hypothesised that by mimicking the α1-helix of NusE, in particular H15, R16 and D19, we could competitively inhibit the NusB-NusE PPI, and develop a platform for subsequent identification of small molecule modulators of the interaction.

To support our hypothesis that targeting the NusB-NusE PPI would prevent the assembly of this complex a 9-mer peptide, H-YDHRLLDQS-NH_2, was synthesised and screened as a potential inhibitor. The 9-mer returned an IC_{50} of 71 ± 6.2 μM, confirming the potential to inhibit this PPI and supporting the NusB-NusE PPI interface as a potentially druggable target. Building on this we used a secondary structure epitope approach comprising a single face of the α1-helix of NusE with critical hot spot residues H15, R16 and D19 of the *A. aeolicus* NusB-NusE-*boxA* structure (PDB: 3R2C)[29] to develop a NusB-NusE interaction pharmacophore (Supplementary Data).

Our pharmacophore was developed from the partial sequence alignment of α1-helix NusE and two sequences which comprise the NusB binding pocket. Sequence alignment of NusE and NusB from *Aquifex aeolicus*, *Bacillus subtilis*, *Escherichia coli*, *Haemophilus influenza*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Streptococcus pneumoniae* highlighted high sequence homology and conservation across a range of bacterial species supportive of a broad spectrum antibacterial target. Of particular note, the NusE α1-helix showed minimal sequence deviation, suggesting its essential role in NusB-NusE PPI binding (Fig. 3A).

Having identified the conserved nature of these proteins, we next compared the protein alignment with published NMR and X-ray crystal structures of the NusB-NusE heterodimer in *E. coli* (PDB: 3D3B) and *A. aeolicus* (PDB: 3R2C) to determine key amino acid interactions.[28,29,31] Our analysis identified the major hydrogen bonding contributions as NusB E81 (*E. coli* E81)-NusE H15 (*E. coli* H15), NusB Y16 (*E. coli* Y18)-NusE D19 (*E. coli* D19) and NusB R76 (*E. coli* E75)-NusE R16 (*E. coli* R16). These data were consistent with the hotspot mutation identified by Friedman et al.[27] These three key amino acid interactions were then superimposed as hydrogen bond acceptor or hydrogen bond donor query features, according to the characteristics of each amino acid, over a single face of α1-helix of NusE using Discovery Studio (BIOVIA) (Fig. 2b). This gave rise to a hydrogen bond acceptor (x = 18.197; y = −30.736; z = 47.491) and two hydrogen bond donors (x = 27.296; y = −21.920; z = 42.021 and x = 25.296; y = −23.413; z = 48.000) regions. The features were used to define a central point on the residue and a location constraint sphere with a radius of 1.6 Å (based on a strong hydrogen bond interaction). In an effort to minimise potential steric clashes, a total of 21 exclusion spheres of 1.2 Å radius were installed and ultimately defined the shallow binding groove of NusB.

In total, the NusB-NusE pharmacophore comprised a hydrogen bond acceptor, two hydrogen bond donors and 21 exclusion zones.

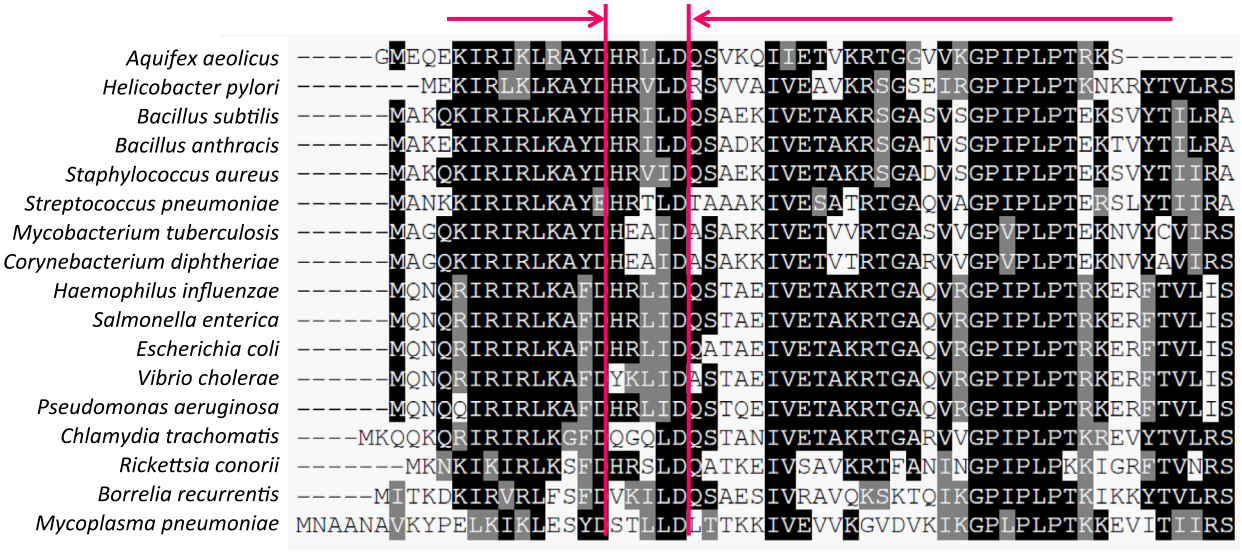

Fig. 2. Protein sequence alignment of NusE spanning 17 species of bacteria. Black regions indicate conserved residues; grey regions, partial conservation; white region, no residue conservation across species. The region highlighted by the red boundary represents the key NusE residues involved in binding to NusB: H15, R16 and D19.