FtsZ Inhibitor Analysis

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The development of multidrug resistant bacteria has created an urgent need for new antibiotics. In this study, we assess the possibility of targeting filamentous temperature-sensitive protein Z (FtsZ) as a novel drug target for Pharmaceutical Company X with the goal of developing a broad-spectrum antibiotic agent.

FtsZ is a highly conserved protein involved in cell division in all bacteria species. Although significant research in recent years has highlighted FtsZ as a promising drug target, no drugs targeting FtsZ have progressed past phase 1 clinical trials, largely due to challenges in achieving both selectivity and efficacy. Based on insights from recent research published in 2023 [1], we performed a PSI-BLAST search against *M. tuberculosis* FtsZ. The search found high similarity across gram-negative and positive-bacteria, as well as drug resistant bacterial strains. A human homolog of this protein was not found through various PSI-BLAST attempts, but the tubulin protein has long been identified as the eukaryotic homolog to FtsZ [2]. The PSI-BLAST results are summarized in Table 1. The eleven BLAST results were loaded into JalView where a Multiple Sequence Alignment (MSA) was built using ClustalO, and a phylogenetic tree was built using BLOSUM62 matrix, shown in Figure 1. The phylogenetic analysis allowed us to compare evolutionary relationships between FtsZ homologs across species.

Table 1. BLAST results of common pathogenic bacteria against *M. tuberculosis* FtsZ. No BLAST results were generated for H. sapiens, but the accepted estimated percent identity has been included [1][3][4].

Organism	Accession number	Query cover	E-value	Percent identity	Protein name
M. tuberculosis	ALB19333.1	100%	0	100%	FtsZ
B. subtilis	P17865.3	97%	2.00E-134	58%	Cell division protein FtsZ
S. aureus	P0A029.1	83%	3.00E-127	62%	Cell division protein FtsZ
E. faecalis	O08439.2	79%	4.00E-119	60%	Cell division protein FtsZ
MSSA	Q6GA26.1	83%	5.00E-112	62%	Cell division protein FtsZ
S. pneumoniae	A0A0H2ZNE0.1	76%	3.00E-104	57%	Cell division protein FtsZ
K. pneumoniae	WP_136662295.1	52%	4.00E-98	80%	Cell division protein FtsZ
E. coli	P0A9A6.1	94%	6.00E-93	45%	Cell division protein FtsZ
A. baumannii	SST03151.1	94%	8.00E-93	46%	Cell division protein FtsZ
P. aeruginosa	P47204.2	76%	2.00E-91	52%	Cell division protein FtsZ
MRSA	Q6GGB8.1	17%	4.00E-04	34%	Heat-inducible transcription repressor HrcA
H. sapiens	KAI4065760.1	N/A	N/A	<20%	Tubulin alpha 1c

Figure 1. Jalview-generated phylogenetic tree of the BLAST results.

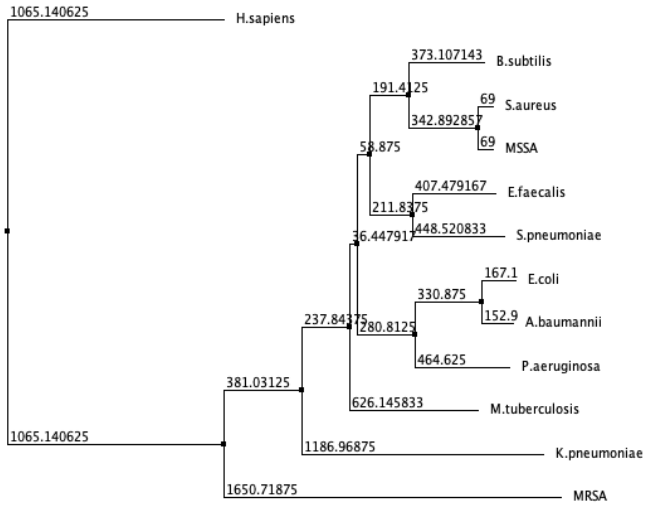
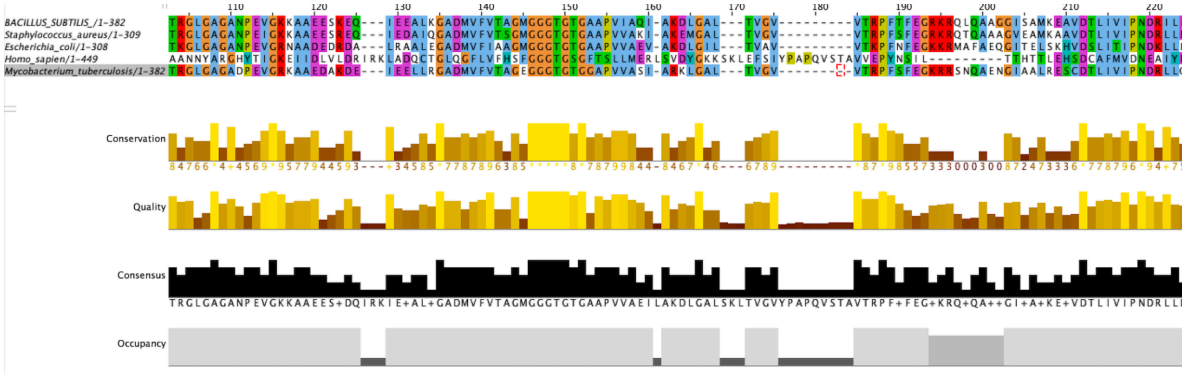


Figure 2: Multiple Sequence Alignment (MSA) of FtsZ Protein from our target *M. tuberculosis* and homologs *B. subtilis*, *S. aureus*, *E. coli*, and the human tubulin alpha-1C chain. This window focuses on the IDC binding pocket in *M. tuberculosis* roughly from residue 86-134 and 164-165. Note that residue shifting is common in Clustal Omega alignments, so these residues may experience slight positional adjustments.



For structural analysis, *M. tuberculosis* FtsZ (PDB ID: IRQ7) was compared to homologous FtsZ proteins of *B. subtilis* (PDB ID: 2VAM), *E. coli* (PDB ID: 6UMK), *S. aureus* (PDB ID: 7OJ), and human tubulin (UniProt code: Q9BQE3). PyMOL alignments revealed significant insights regarding the structural similarity and divergence. The low RMSD values with *B. subtilis* (0.785) and *E. coli* (1.291) indicate strong structural conservation, suggesting that inhibitors designed for *M. tuberculosis* FtsZ may also inhibit FtsZ in other bacterial species, making them promising candidates for broad-spectrum antibacterial agents. Although *S. aureus* shows a slightly higher RMSD (1.950), the structure remains sufficiently conserved for inhibitor design. On the other hand, the high RMSD (4.549) between *M. tuberculosis* FtsZ and human tubulin highlights significant structural differences, supporting the idea that targeting bacterial FtsZ will minimize toxicity to human cells due to low cross-reactivity with tubulin [5][6][7].

The major binding sites of FtsZ include the GDP-binding site and inter-domain cleft (IDC), and each offer different prospects for drug development. Our concern with using the

GDP-binding site, also known as the nucleotide binding domain (NBD), is the increased risk that inhibitors targeting the GDP-binding site of FtsZ could also bind to human tubulin, potentially leading to toxicity in eukaryotic cells [5][6]. The GDP-binding site shares significant homology with the GTP-binding site of eukaryotic tubulin. Both FtsZ and tubulin are part of the same protein super family, and their nucleotide-binding pockets are highly conserved [6].

The IDC of FtsZ offers a more promising target for selective drug development as this binding pocket exists solely in prokaryotic FtsZ and is not present in eukaryotic tubulin. The IDC is located between the N-terminal and C-terminal domains of FtsZ spanning the C-terminal half of H7 helix, the T7 loop, and the C-terminal domain beta sheets, and serves as a regulatory site that influences FtsZ polymerization [5][6]. The IDC binding site has been selected based on a strategic positioning for ligand interaction based on prior studies as a critical binding site for small molecule inhibitors [6]. The conservation of specific residues in the IDC is low compared to other hotspots on the protein surface. Therefore, in this iteration of the protein, the specific residues were located manually and highlighted in Figure 3 [6].

This pocket is defined by a combination of hydrophobic, polar, and charged residues providing strong ligand binding potential. Hydrophobic residues such as LEU 119 and 124, and ILE 86 create a stable non-polar environment, ideal for the core of a ligand to interact through van der Waals forces which can stabilize the ligand within the pocket. Charged polar residues like ARG 123, GLU 88, and ASP 120 provide points for electrostatic and hydrogen bonding interactions which are essential for binding affinity and specificity. These interactions have been demonstrated to stabilize ligands in the IDC region of FtsZ [6]. Additionally, GLY 122 and GLY 134 introduce flexibility within the pocket allowing for conformational adjustments to accommodate ligands of varying sizes and shapes which permits us a broader range of ligand design increasing our chances for finding effective drug candidates [5]. Figure 4 demonstrates docking results of previously identified potential inhibitors of FtsZ at this binding site. The strong docking scores recorded for these inhibitors provides significant encouragement to the potential druggability of this binding site.

Figure 3. Identified residues of the IDC binding site of *M. tuberculosis* FtsZ.

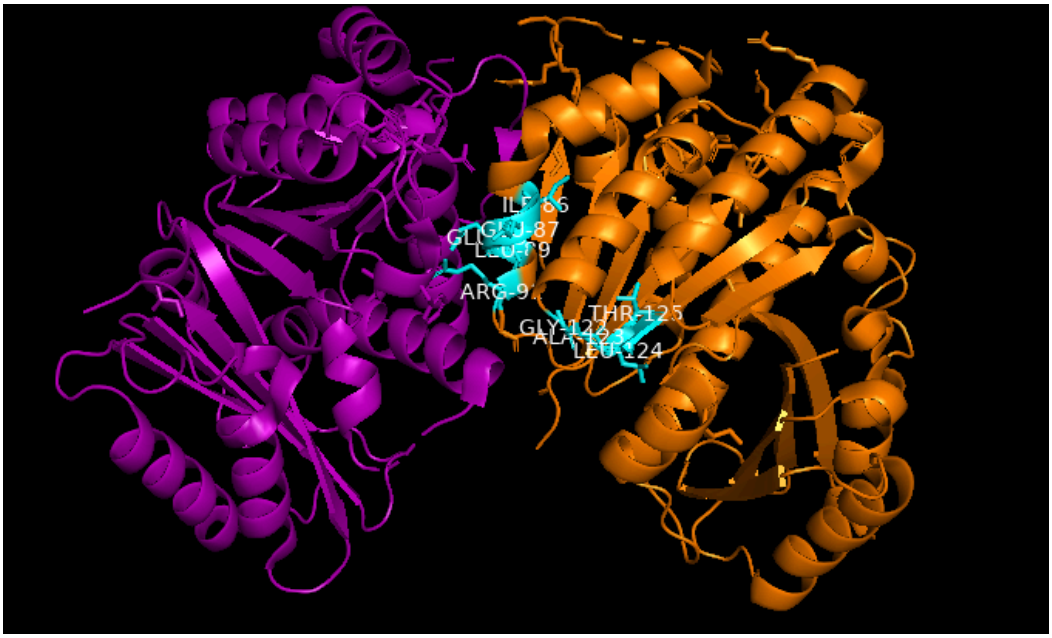
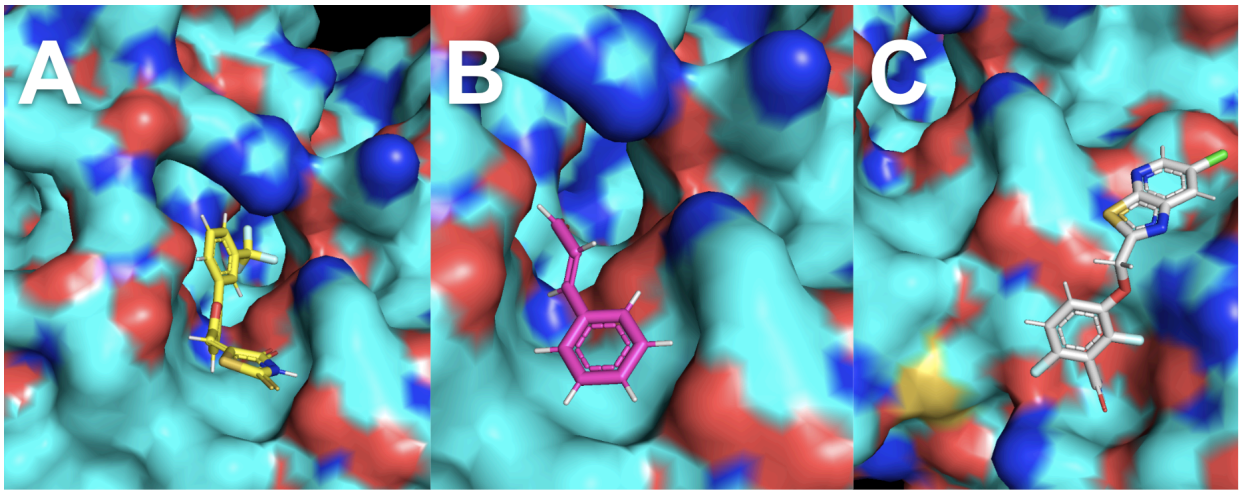


Figure 4. 1-Click Docking results of three identified small molecule inhibitors to *M. tuberculosis* FtsZ. (A) PC190723, docking score: -5.9, (B) Cinnamaldehyde, docking score: -4.4, (C) CCR-11, docking score: -6.4.



In our research, we found relatively high sequence and structural similarity of *M. tuberculosis* FtsZ with FtsZ from both gram-negative and gram-positive bacteria, as seen in our BLAST search, MSA, and low RMSD values from PyMOL alignments. In contrast, the eukaryotic (human) homolog, tubulin, had a significantly higher RMSD score highlighting key structural differences that reduce the risk of cross-reactivity. In the analysis of the binding regions of the proteins, the GDP binding region was highly conserved for all species FtsZ and human tubulin. However, the IDC differed significantly between FtsZ and human tubulin, marking it as a potential binding site for both a specific and selective drug. FtsZ has long been established as a druggable target, and many small molecules have been identified to date as inhibitors of FtsZ, and those small molecules have been shown to inhibit multiple pathogenic bacterial species in vitro [1] [6] [8]. Docking experiments were successful for multiple previously identified inhibitors,

for the IDC, and other minor binding sites of this protein [6]. Between our investigation and previous research, we think this is a great choice of protein for specificity and selectivity, being broadly similar amongst bacterial species, while differing significantly from the human homolog, reducing potential for toxicity.

We also found some limitations of this protein that may cause issues in the development of an FtsZ inhibitor. In our investigation of the IDC binding site, significant differences in residue composition were found between the gram-positive and negative species. This has also been noted in literature, and is likely a reason FtsZ inhibitors have not yet entered the market [9]. Additionally, the GDP-bound, GTP-bound, monomer, and polymer forms of FtsZ all create slight differences in the conformation of the IDC, making it difficult to predict the exact shape of the binding site [6]. Additionally, the disorganized c-terminal end of FtsZ has not been fully characterized, leaving some questions to the involvement of this portion of the protein to its function or binding abilities [8].

Our recommendation to Company X is to target the poorly saturated multidrug resistant tuberculosis drug market by developing a small molecule drug that specifically inhibits *M. tuberculosis* FtsZ, and evaluate the identified molecule's ability to act as a broad-spectrum antibiotic. With our investigation, and the wealth of previous and current research, we believe that with careful design and optimization, that immediate development by Company X could produce the first marketable product targeting the FtsZ protein.

References:

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