Small Molecule Inhibition of *Helicobacter pylori* Glycosylation

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Abstract:
Helicobacter pylori is a ubiquitous pathogen associated with duodenal ulcers and stomach cancer. The current treatment of the disease is "triple therapy" but negative side effects and increasing antibiotic resistance have resulted in a pressing need for new, more selective therapies. The glycans that coat the surface of *H. pylori* are compelling therapeutic targets because they contain unusual monosaccharides that are absent from human cells and are linked to pathogenesis. As such, disrupting the synthesis of fully functional glycans presents a possible pathway of inhibiting the bacteria's ability to infect the host. The Dube lab previously demonstrated O-glycoside inhibitors based on rare bacterial monosaccharides to be effective in disrupting glycan biosynthesis. A recent publication by Wang et al found a novel class of metabolic inhibitors, S-glycosides, were effective at > 10-fold lower concentrations than O-glycosides in mammalian systems. We assessed S-glycosides for their ability to truncate glycan biosynthesis in *H. pylori*, as well as their downstream effects on bacterial fitness. The S-glycoside inhibitors effectively impeded glycan biosynthesis and affected bacterial fitness in a compound-dependent manner. Ultimately, selectively targeting bacterial pathogens through their unique glycans has the potential to expand our antibiotic arsenal.

Project objectives:
Our collaborator, Dr. Suvarn Kulkarni (IIT Bombay), synthesized three novel S-glycoside compounds based on bacterial monosaccharides: Bac-SBn, Dat-SBn, and Fuc-SBn. The goal of my project was to (1) assess the ability of these novel S-glycoside compounds to inhibit *H. pylori* glycosylation and (2) investigate the downstream effects of inhibited glycosylation on bacterial fitness. While Wang et al tested S-glycosides in mammalian systems, they had not yet been tested in bacteria. We queried whether they could serve as a more potent metabolic inhibitor of *H. pylori* glycans compared to previously tested O-glycosides.

Methodology:
Metabolic labeling and Western blot analysis
H. pylori were plated and grown for 3-4 days. Samples were treated with a negative control (Ac_4GlcNAc), or metabolically labeled in the absence or presence of varying concentrations (0.1-2mM) of inhibitor. Cultures of *H. pylori* were incubated for 4 days under microaerophilic conditions (14% CO_2 at 37°C). After 4 days of labeling, cells were harvested, lysed, standardized, and reacted with phos-FLAG. Reacted lysates were loaded onto SDS-page gel and separated by gel electrophoresis according to molecular weight.

Fitness assays: Growth, motility, and biofilm formation
Fitness assays measured the growth, motility, and biofilm formation of *H. pylori* with and without inhibitor. Growth curves were measured over the course of 6-10 days. *H. pylori* samples, untreated or with inhibitor, were inoculated at a starting OD_{600} of 0.1. They were grown in microaerophilic conditions and each day, the OD_{600} of each sample was measured using spectrophotometry. The motility of *H. pylori* in the absence or presence of inhibitors was monitored over the course of 10-14 days. Bacteria were standardized to an OD_{600} of 0.4. Standardized samples of bacteria were plated onto soft agar plates and incubated under microaerophilic conditions. Colony diameter was measured daily

for 10-15 days. To measure biofilm formation, *H. pylori* with and without inhibitor was standardized to an OD_{600} of 0.4 in Brucella Broth, and samples were added in triplicate to the side wells of a 96-well plate. The bacteria were incubated for 5 days under microaerophilic conditions. After incubation, media was carefully removed, and biofilm was stained with 0.15% crystal violet to visualize. Biofilm was imaged, solubilized in 30% acetic acid in water, and quantified using absorbance.

Results

All three S-glycoside inhibitors successfully impeded *H. pylori* glycan biosynthesis, as indicated by the Western blot (Fig. 1). The inhibitor Dat-SBn showed glycosylation inhibition at a lower concentration (1mM), while Bac-SBn and Fuc-SBn demonstrated inhibition only at higher concentration (2mM). I used the lowest effective concentration indicated in the Western blot to test the downstream effects of the inhibitor on bacterial fitness. All three inhibitors reduced motility relative to untreated *H. pylori* (Fig. 2). However, only Fuc-SBn showed significant differences in biofilm formation and growth relative to untreated *Helicobacter pylori*.

Significance and Interpretation of Results

I found that the S-glycosides successfully inhibited *Helicobacter pylori* glycosylation and impacted bacterial fitness in a compound-dependent manner. The results indicate that S-glycosides are an effective method of inhibiting glycoprotein biosynthesis, and in turn are a promising pathway for therapeutics. Their efficacy relative to O-glycoside inhibitors requires more experimentation. In the future, we would also like to test S-glycoside inhibitors on other bacteria species to see if they elicit different defects and therefore act in a bacteria-selective manner. Small molecule inhibitors have the potential to expand our antibiotic arsenal through selective targeting of bacterial pathogens.

Figures

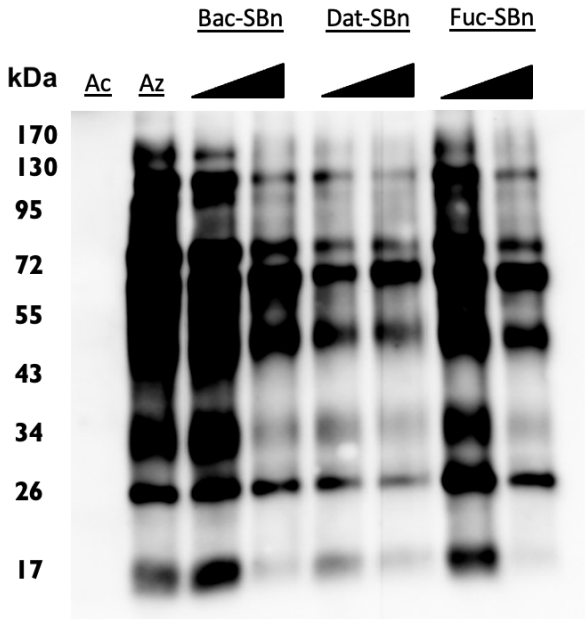
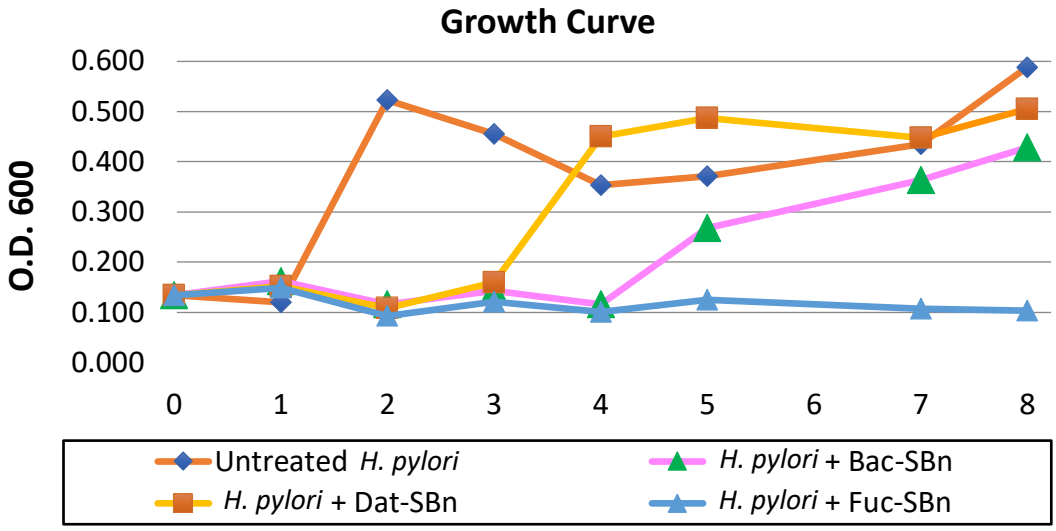


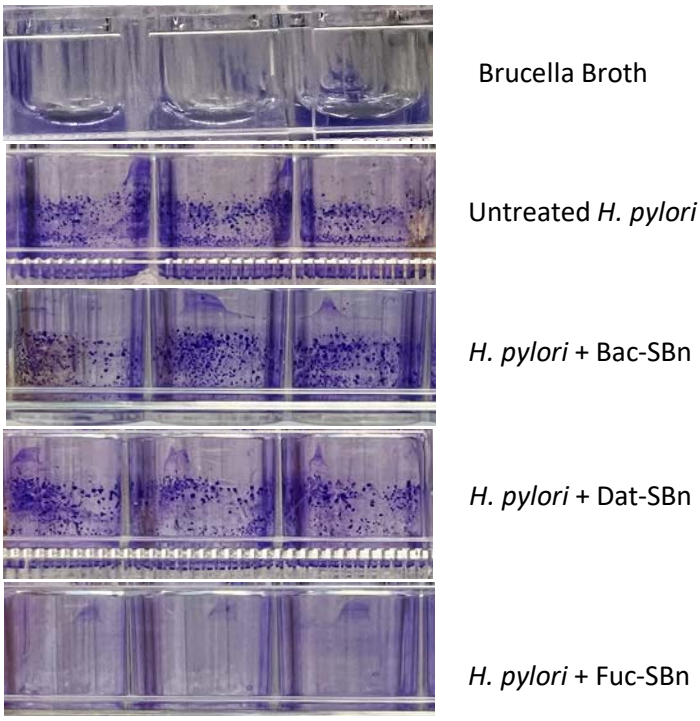
Figure 1. Western blot analysis indicates that S-glycosides inhibit *H. pylori* glycan biosynthesis in a concentration-dependent manner. *H. pylori* was treated with $Ac_4GlcNAc$ (- control), $Ac_4GlcNAz$ (+ control), or with increasing concentrations of inhibitor as indicated by the shaded triangle. The shortest

point represents 1mM concentrations and the tallest point represents 2mM. Reduced protein banding relative to Az (+ control) indicates inhibited glycan biosynthesis.

A)



B)



C)

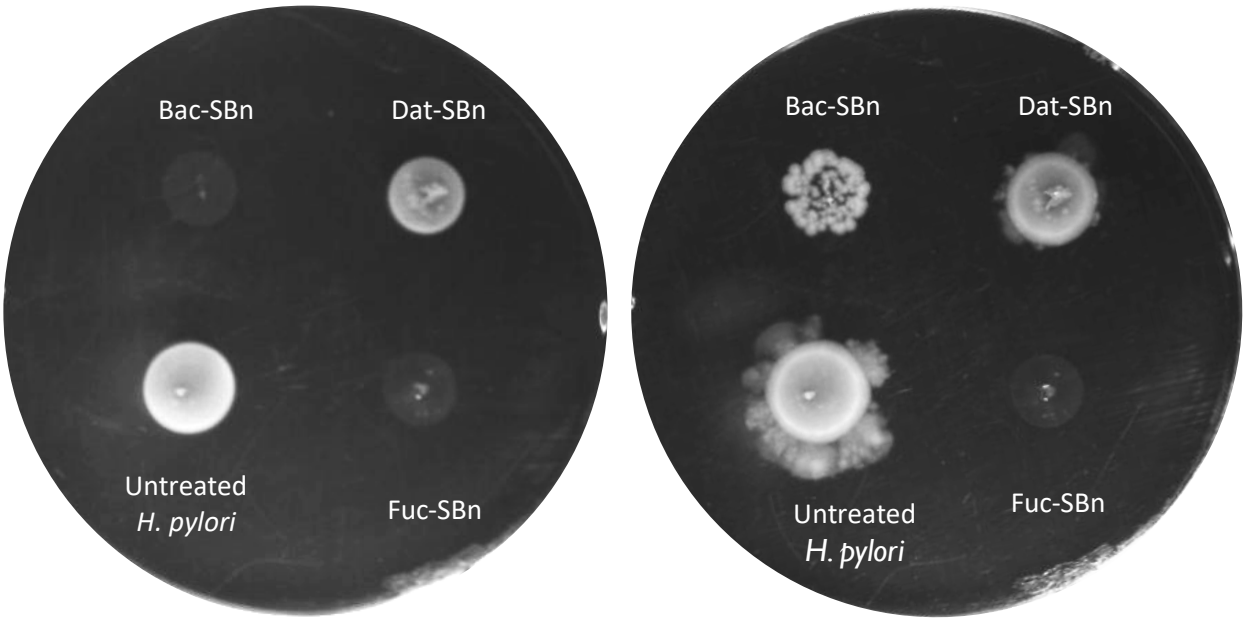


Figure 2. *H. pylori* were treated with 1 or 2 mM of S-glycosides and scored for (A) growth by monitoring optical density at 600 nm, (B) biofilm formation using a crystal violet assay, and (C) motility on soft agar. All three inhibitors reduced motility relative to untreated *H. pylori.* However, only Fuc-SBn showed significant differences in biofilm formation and growth relative to untreated *H. pylori*.

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